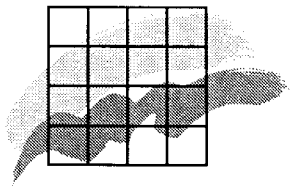

Chemical substances and chemical preparations

Identification of organic colourants in cosmetics by HPLC-photodiode array detection

NERI Technical Report No. 183

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Data sheet

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Contents

Preface

Colourants are commonly used in the formulation of cosmetic products. Some of the colourants may be skin irritant and/or harmful. The colourants in the cosmetic products are washed off into the wastestream. However, this has not yet been considered as a major environmental problem.

The colourants in the cosmetic products are regulated by the Statutory Order from the Danish Ministry of Environment and Energy on cosmetic products. Present investigation was undertaken to check the compliance of the cosmetic products with the cosmetic legislation. Financial support for the investigation was provided by the Danish Environmental Protection Agency (DEPA). Ms. Lone Mikkelsen from Danish EPA closely followed the progress of the study.

The study required a number of colourants which were not available through the suppliers of laboratory chemicals. Ellis & Everard (England), BASF (Germany) and Keuringsdienst Van Waren, Enschede (Netherlands) provided important cosmetic colourants to make this study possible. We are grateful for their generous support.

Resumé

Anvendelsen af farvestoffer til formulering af kosmetiske produkter er reguleret af Miljø- og Energiministeriets bekendtgørelse no. 805 af 30. maj 1996 (no. 456 af 2. juni 1995 ved start tidspunktet af nærværende projektet) kosmetiske produkter. Formålet med nærværende studie var at udvikle en analysemetode til undersøgelse af organiske farvestoffer i kosmetiske produkter, samt at undersøge om, at kosmetiske produkter på det danske marked overholder Miljø- og Energiministeriets bestemmelser med hensyn til indhold af farvestoffer.

I nærværende arbejde er den til EU Kommissionen forslået analysemetode videre udviklet og optimeret til rutineanalyser af organiske farvestoffer i kosmetiske produkter. Reference farvestofferne opløst i egnede opløsningsmidler samt farvestofekstrakt af kosmetiske produkter analyseres ved højtryks væskekromatografi (HPLC) ved anvendelse af en analytisk kolonne pakket med polymert materiale og en mobil fase bestående af citrat buffer, ion-par reagens tetrabutylammoniumhydroxid, acetonitril og tetrahydrofuran (gradient eluering). Farvestofferne detekteres ved deres ultraviolet-synlig (UV-Vis) spektra i bølgelængdeområdet 275 nm - 760 nm med anvendelse af en fotodiodarray detektor. Millennium Software anvendes til opsamling af kromatografiske data samt til den videre behandling af de opsamlede data. Identifikationen af farvestofferne udføres ved sammenligning af retentionstider (t_R) og UV-Vis spektra af kromatografiske toppe i maxplot kromatogrammer fra HPLC-analyse af prøverne med UV-Vis spektra af reference farvestofferne. Til dette formål er ved anvendelse af Millennium Software opbygget et spektralbibliotek, "COSCOLOR", der indeholder t_R og UV-Vis spektra af de fleste af de tilladte organiske farvestoffer samt t_R og spektra af en række relevante forbudte farvestoffer. Spektralbiblioteket blev anvendt ved rutineanalyser af farvestoffer i kosmetiske produkter.

Der er udviklet prøvetilberedningsmetoder til rutineanalyser af organiske farvestoffer i forskellige typer af kosmetiske produkter. Fastfase ekstraktion med anvendelse af C-18 silica og amino-silica er anvendt til prøvetilberdning. Farvestoffer kunne ikke ekstraheres fra følgende type kosmetiske produkter: øjenskygge, øjenblyant, eyeliner og mascara. Der anvendes muligvis uorganiske farvestoffer i formulering af disse produkter.

Ved anvendelse af de udviklede metoder er der undersøgt i alt 139 kosmetiske produkter for indhold af farvestoffer. Analyserne kunne uden prolemer udføres rutinemæssigt, undtagen for identifikation af farvestoffer i shampooer med svag farve. Der er blevet i rapporten forslået flere måder til at løse de opstående analyseproblemer.

Undersøgelsen viste at 26 af de 128 tilladte organiske farvestoffer var tilstede i de undersøgte produkter. Dette tyder på, at en stor del af de tilladte farvestofferne anvendes ikke i kosmetiske produkter. En revision af listen over tilladte farvestoffer (bilag 4 til kosmetik bekendtgørelse) bør derfor overvejes. Syv af undersøgte produkter (5%) indeholdt 4 ikke-tilladte farvestoffer.

Undersøgelsen er gennemført i samarbejde med Miljøstyrelsen.

Summary

The use of colourants in the formulation of cosmetic products is regulated by the EU's Cosmetic Directive 76/768/EC [Statutory order form the Danish Ministry of Environment and Energy, No. 805 of August 30, 1996 (No. 456 of June 2, 1995, when present study was started)] on cosmetic products. The aims of the present study were to develop an analytical method for the identification of organic colourants in the cosmetic products and to check the compliance of these products with the Cosmetic Directive, with respect to content of these colourants.

In the present study we have improved and optimized an analytical method, recently proposed to EU Commission, for the analysis of organic colourants. The reference colourants dissolved in appropriate solvents and the colour extracts from cosmetic products are analysed by high performance liquid chromatography employing analytical coloum with polymer packing and a mobile phase consisting of citrate buffer, ion-pairing reagent tetrabutylammonium hydroxide, acetonitrile and tetrahydrofuran (gradient elution) followed by detection across the wavelength range 275 nm -760 nm employing a photodiode array detector. Millennium Software has been used for data acquisition and data processing. The identification of the colourants in the samples is performed by comparing the retention times (t_R) and the UV-visible spectra of the the chromatographic peaks in the max-plot chromatograms obtained by the analysis of the samples, with the t_R and spectra of the reference colourants. A spectral library "COSCOLOR" consisting of HPLC retention times and the spectra of 129 organic colourants (most of the permitted and some relevant non-permitted) has been build using the Millennium Software. The spectral library has been used for the identification of colourants in the cosmetic products.

The sample preparation methods for various types of cosmetics, with a few exceptions, have also been developed to perform routine analysis of organic colourants in these products. Solid phase extraction employing C-18 silica as well as aminobonded silica have been used for the extraction of colourants from the cosmetic products. It was not possible to extract colourants from eye liner, eye shadow, eye pencil and mascara. It seems that mainly inorganic colourants are used in the formulation of these products.

Identification of organic colourants has been performed in 139 cosmetic products employing the methods developed in the present study. Problems were encountered for the analysis of colourants in some samples, especially faint-coloured shampoos. A number of ways to deal with the identification of colourants in such samples are described.

The study revealed that only 26 of the 128 permitted organic colourants were present in the cosmetic products investigated. This observation may indicate that only a samll number of the permitted organic colourants are used in the formulation of cosmetic products. Thus, it should be considered whether a revision of the Annex IV of the Cosmetic Directive is necessary. Four non-permitted colourants were identified in 5% (n = 7) of the investigated samples.

The study has been performed in cooperation with the Danish Environmental Protection Agency.

1 Introduction

Colourants are dyes and pigments derived from natural materials or prepared as synthetic chemicals that are used as colouring agent for consumer and industrial products, or they may be used as such (for example in laboratory investigations). Over 10,000 chemicals, both organic and inorganic compounds, are in use as colourants in the formulation of various types of consumer and industrial products: food, cosmetics, medicine, toys, plastics, printing inks, paints, textiles, etc. Colourants are even added in gasoline to discriminate, for example, leaded gasoline from the unleaded gasoline. Keeping in pace with the modern technology, new dyes and pigments have been synthesized for specific application areas, for example, dyes for various types of synthetic fibers and polymers, dyes for displays, dyes for optical storage, dyes for non-linear optics, dyes as organic photoconductors and laser dyes (see References 1 & 2 for the details and synthesis and application areas of the dyes and pigments).

The colourants can be classified according to their chemical structures: inorganic pigments, azo dyes, nitro- and nitroso dyes, anthraquinone dyes, indigoid dyes, natural colours (anthocyanins, carotenoids), etc. Apart from the chemical nomenclature, colourants have also been described in several other ways. For example, colourants have been described as the visual colour with the prefix referring to their application area(s): FD&C (Food Drug & Cosmetics) or D&C or F&D, Vat and so on. But for the unequivocal understanding of a colourant by the industries, scientists, administrators, etc., a colourant should be described by its CAS No. (Chemical Abstract Registry Number) or by its CI No. (Colour Index Number). CI Nos. are 5 digit numbers and they are basically based on the chemical structures of the colourants. For example CI 10... refers to a nitroso dye, and CI 77... refers to an inorganic pigment.

Annual global production and use of dyes and pigments in 1988 was >800,000 tons (1). About 15% of the total world production, corresponding to approximately 128 tons per day, is lost during the synthesis and processing of colourants. Azo dyes constitute the largest class of dyes (>50 %) used in industries, textile industry in particular.

Not all colourants are safe for the humans and/or the environment. Thus, several dyes have been considered as mutagenic, carcinogenic, skin sensitizer, etc. (3, 4). The environmental toxicology of the dyes and pigments has been poorly investigated by now. It is estimated that 10-40% of the dyes used in textile industries are lost during the dyeing process and are released as effluent. Also the colourants in the cosmetic products are washed off into the wastestream. Azo dyes are resistant to aerobic degradation (5, 6), and under anaerobic conditions they can be reduced to potentially carcinogenic amines (7, 8).

There are no standard methods for the elimination of dyes from wastestream. The commonly used methods for the removal of dyes from wastestream are flocculation with lime, activated charcoal adsorption and biotreatment. The lime and charcoal treatment generate solid wastes which require costly disposal methods. Biotreatment process rely on microorganism to degrade dyes and pigment. Since the synthetic dyes are resistant to aerobic biodegradation, this process may be insufficient. Other possible ways to remove azo dyes from wastestream are degradation by UV-enhanced oxidation of the dyes and hydroxy radical mediated degradation of these compounds (9, 10). However, one of the major product of the hydroxy radical mediated degradation of colourants is benzene (11), which is a well known carcinogen. Although attention has been focused on textile dyes, cosmetic and food colours have also been detected in wastewater (12).

The use of colourants in food, drug and cosmetics is regulated in most of the countries. In Member States of the European Union (EU), the colourants in cosmetics are regulated by the EU's Cosmetic Directive 76/768/EC/Statutory Order No. 805 of August 30, 1996 (No.456 of June 2, 1995, when present project was started) of the Ministry of Environment and Energy in Denmark (13). The Directive restricts the use of the colourants in cosmetic products in 4 ways: i) permitted colourants, ii) application areas of the colourants and iii) maximum allowed concentration of certain permitted colourants according to Annex IV of the Directive; and iv) by listing certain non-permitted colourants in Annex II of the Directive (see Appendix I of the present report). Thus, the Directive allows 157 colourants together with insoluble Ba-, Sr- and Zr-lakes, salts and pigments of 16 of the permitted colourants in cosmetic formulations. 96 of the permitted colourants belong to Application Area 1 (all products), 5 colourants for Application Area 2 (all cosmetics except the products to be used around the eyes), 24 colourants for Application Area 3 (specifically permitted for the use in the formulation of cosmetic products which do not come in contact with mucous membranes) and finally 32 colourants for the Application Area 4 (specifically permitted for the cosmetic products which come in contact with skin for a short period). Restrictions concerning maximum concentration of the permitted colourants concern only CI 12085, CI 15630 an CI 42520. Besides above mentioned, purity criteria of some of the colourants have also been defined in the Cosmetic Directive.

Among the permitted colourants 29 are inorganic pigments, the remaining 128 being organic dyes and pigments. Of the permitted organic colourants 34% (n = 53) belong to the class of azo dyes/pigments, 8% (n = 12) are triarylmethane dyes, and 7% (n = 11) belong to xanthene and 7% (n=11) to the anthraquinone classes of dyes/pigments. Remaining permitted colourants belong to following classes of dyes/pigments: nitro-, nitroso-, azin, oxazin, stilbene, pyrene, quinolin, phthalocyanin, indigoid, carotenoids, and natural colours (anthocyanins, carotenoid, capsanthin, and so on).

The responsible authority for the implementation of Cosmetic Directive in Denmark, Danish Environmental Protection Agency, required the control of colourants in cosmetic products in Denmark. To check the compliance of the cosmetic products with the cosmetic legislation, an analytical method for the routine analysis of colourants in cosmetics was required. Several methods of analyses of colourants have been described in the literature (12, 14-30). The methods described have been applied to specific dyes/classes of dyes or for the analysis of dyes in specific matrices or for checking the impurities in the dyes. The described analytical methods are based mainly on high performance liquid chromatography (HPLC)/capillary zone electrophoresis followed by multiwavelength UV-visible detection/photodiode array (PDA) detection/mass spectrometric detection. But none of the methods can be directly applied for the analysis of all types of colourants in cosmetics, as regulated by the Cosmetic Directive. Gagliardi et al. (31) reported a reversed phase HPLC method for the analysis of cosmetic dyes followed by detection at 4 wavelengths. They further demonstrated the application of the method for the analysis of xanthene dyes in lipstick samples (32). However, to ascertain the identification of the colourants a photodiode array detection across the wavelength range 275 nm - 750 nm is required. Furthermore, the application of the method (31) has neither been demonstrated for the analysis of dyes other than xanthene dyes, nor for the analysis of colourants in cosmetic products other than lipsticks. Also more than 15 years of efforts of the EU's Working Party on the Methods of Chemical Analysis has not resulted in a standard method for the analysis of the colourants in cosmetics. However, a HPLC-PDA method (SPC/1327/93) for the analysis of organic colourants in cosmetics, proposed recently to EU Commission (33), appeared as promising (34). This method, based on a previously described method for the analysis of acidic dyes (35), employs gradient elution with ion-pairing and an analytical HPLC column with a polymeric packing for the chromatography. A collaborative trial for the identification of a limited number of colourants in lipsticks and nail varnishes revealed that the method (34) needed improvements for the routine analysis of colourants in cosmetics. In cooperation with Danish Environmental Protection Agency, the present study was started to develop a method for the routine analysis of organic colourants in various types of cosmetic products and to check the compliance of the colourants present in the cosmetic products, available in the Danish Market, with the Cosmetic Directive.

In the present work, cosmetic products have been investigated only for the identification of organic colourants present in these products. No attempt has been made to analyse dye intermediates (oxidation hair dyes), which are regulated by the Annex III of the Cosmetic Directive. Efforts were made to obtain all of the permitted colourants through chemical suppliers, suppliers of colourants to cosmetic industry and through the laboratories involved in the analyses of cosmetic colourants. However, of the permitted organic colourants (n = 128), a few could not be obtained (Table 1).

On the other hand, it was possible to get the non-permitted colourants according to Annex II of the Cosmetic Directive as well as some other similar colourants (Table 1). It should be noted that some of the non-permitted colourants have previously been permitted.

Table 1: List of the non-available permitted colourants and the investigated non-permitted colurants

Colourants could not be obtained (CI No.)	Investigated non-permitted colourants (CI No.)
11725, 18736, 21230, 24790, 40820, 50325, 60724, 73385, 73900, 75135, 75170, Acid red 195, caramel, capsorubin	12075, 12100, 12140, 13065, 15585, 18950, 26105, 42535, 42555, 42640, 42650, 45170, 45170:1 46500, 61554, 75660

2 Samples

For the analysis of colourants, 139 cosmetic products were purchased from the Danish retail market. The products represented 52 cosmetic manufacturers from Denmark, Germany, France, Italy, England, Belgium, Ireland, Netherlands, Austria, Switzerland, Sweden, Spain and USA. The products selected were from various categories of cosmetics: 48 lipsticks and related products (Table 2), 19 nail varnishes (Table 3), 17 mascara/eye liner/eye pencil/eye shadow (Table 4) and 55 diverse cosmetics (shampoo, bath gel, cream, body lotion, deo roll-on, skin-tonic, after-shave, toothpaste, mouthwash, beauty toner, etc., Table 5). In general, various faint and bright coloured cosmetics, except for lipsticks and related products, were selected for the study. Lipsticks and related products selected for the study were mainly red (various shades), because an additional aim of the investigation of the colourants in these products was to investigate the possible association of relatively high Ba content in red lipsticks (36) with the presence of Ba-lakes of certain permitted colours.

Table 2: Identification of the investigated lipsticks/lipliners/lip gloss.

NERI reg. no.	Lipstick/lipliner/lip gloss	Manufacturer/Importer
5-1288	Creamy lip gloss	
5-1289	Nature calling 02 , lipstick	Hennes & Mauritz (H&M) cosmetic
5-1290	Nature calling 06, lipstick	H &M Cosmetic
5-1291	Wet´n´Wild 553, lipstick	H & M cosmetic
5-1292	Color shop 138, lipstick	H & M cosmetic
5-1293	Isadora 49, lipstick	H & M cosmetic
5-1294	Aesthetic 122, lipstick	H & M cosmetic
5-1295	Aesthetic 103, lipstick	H & M cosmetic
5-1296	Wet´n´Wild 529A, lipstick	H & M cosmetic
5-1297	Colourings 1246, lipstick	The Body Shop
5-1298	Colourings 635, lipstick	The Body Shop
5-1305	H.R., rouge glorious 14, lipstick	Helena Rubinstein
5-1306	Chanel, rouge a levers 38, lipstick	Chanel
5-1307	YSL, fard a levres transparentrent 9, lipstick	Yves Saint Laurent
5-1308	Christian Dior, rouge a levres 863, lipstick	Christian Dior
5-1309	Lancome, rose absolut 16, lipstick	Lancome
5-1310	Clinique, different 65, lipstick	Clinique
5-1311	E.Lauder, re-nutriv all-day R87, lipstick	Estée Lauder
5-1312	E.Arden, luxury matte 04, lipstick	Elisabeth Arden
5-1313	E.Arden, lip SPA 80, lipstick	Elisabeth Arden
5-1314	Clarins, rouge eclat 103, lipstick	Clarins
5-1315	Chicogo, colour intensiv 105, lipstick	Chicogo (A)
5-1316	Rimmel 06, lipstick	Rimmel (UK)
5-1317	Rimmel 05, lipstick	Rimmel (UK)
5-1348	Juvena lipstylo pink 300	Juvena
5-1351	Lobello rosé, læbepomade	BDF Beirsdorf
5-1358	Jade lipliner intensive	Jade (A)
5-1359	Max Factor lipliner, rich red	Max Factor
5-1360	Gosh lipliner	Gosh International

Table 2: Continued.

NERI reg. no.	Lipstick/lipliner/lip gloss	Manufacturer/Importer
5-1369	Max Factor, lasting colour 829, lipstick	Max Factor
5-1370	Max Factor, high definition 135, lipstick	Max Factor
5-1371	L'Oreal, Brilliant grottie 01, lipstick	L'Oreal
5-1372	Four Seasons, Premiere 36, lipstick	F. Cosmetics (E)
5-1373	Pierre Robert 15, lipstick	Pierre Robert
5-1374	P.Robert, luxury lip colour 33	Pierre Robert
5-1375	Revlon, superlative lipstick 48,	Revlon
5-1376	Gosh, lambada 60, lipstick	Gosh International
5-1377	For You 208, lipstick	Cottan (D)
5-1378	Jade, lip gloss med lanolin 38	Jade (A)
5-1379	Jade long lasting 10, lipstick	Jade (A)
5-1380	Juvena, super lip colour 45	Juvena
5-1381	Oriflame 12, lipstick	Oriflame international
5-1382	Oriflame 41, lipstick	Oriflame international
5-1383	Collection 2000, 70, lipstick	Collection 2000
5-1384	Collection 2000, 73, lipstick	Collection 2000
5-1385	Vevay 8702, lipstick	Vevay international
5-1386	Vevay 03, lipstick	Vevay international
5-1414	Yves Rocher 01, lipstick	Yves Rocher
5-1415	Yves Rocher 03, lipstick	Yves Rocher

Table 3. Identification of the investigated nail varnishes.

NERI reg. no.	Nail varnish	Manufacturer/Importer
5-1281	Nature Calling 04	H&M Cosmetic
5-1282	Wet 'n' Wild 414	Pavon Ltd./H&M Cosmetic
5-1283	Isa Dora 53	H&M Cosmetic
5-1346	Revlon 27	Revlon
5-1349	Four Seasons confetti colors 36	F. Cosmetics (E)
5-1361	Pierre Robert 58	Pierre Robert
5-1362	Gosh 66	Gosh International
5-1363	Jade 11	Jade (A)
5-1364	For You 37	Cottan (D)
5-1365	L'Oreal Perfection 106	L'Oreal
5-1366	Pierre Robert 22	Pierre Robert
5-1387	Vevay Colour Silks 08	Vevay International
5-1388	Vevay Colour Silks 01	Vevay International
5-1390	Oriflame 12	Oriflame International
5-1391	Oriflame 41	Oriflame International
5-1393	Collection 2000, 44	Collection 2000 Ltd.
5-1394	Collection 2000, 106	Collection 2000 Ltd.
5-1416	Yves Rocher 05	Yves Rocher
5-1417	Yves Rocher 47	Yves Rocher

Table 4. Identification of the investigated mascara (M), eye liner (EL)/eye pencil (EP) and eye shadow (ES) products.

NERI reg. no.	Product identification	Manufacturer/importer
5-1279	M Nature Calling, 03 blue	H&M Cosmeteic
5-1280	M Nature Calling, 02 brown	H&M Cosmetic
5-1318	M Chicogo, 1 endless brown	Chicogo (A)
5-1347	M Revlon, 02 black/brown	Revlon
5-1350	M Four Seasons Premiere, 112 black	F. Cosmetics (E)
5-1353	M Max Factor 2000, 3 black/brown	Max Factor
5-1354	M L'Oreal Perfection, Ultra long, blue	L'Oreal
5-1389	M Vevay Colour Silks, 8834 mocha	Vevay International
5-1392	M Oriflame, 02 deep brown	Oriflame International
5-1395	M Collection 2000, 5 electric blue	Collection 2000 Ltd. (UK)
5-1418	M Yves Rocher, Douceur Naturelle, 03 brown	Yves Rocher
5-1345	ES Jade, 06 ombre a' paupiéres	Jade (A)
5-1352	ES Pierre Robert 27 glacier blue (light + dark)	Pierre Robert
5-1355	EP Gosh, green	Gosh International
5-1356	EP Gosh, hot blue	Gosh International
5-1357	EL Jade, blue	Jade (A)
5-1396	EL Collection 2000, regal blue 8	Collection 2000 Ltd.

Table 5. Diverse cosmetic products investigated for the identification of colourants.

NERI reg. no.	Product identification	Manufacturer/Importer
5-1278	Gosh Woman, a case with 4 shower and bath gel: Spring (green), Summer (light red), Autumn (orange), and Winter (violet)	E. Tjellesen A/S
5-1284	Nature Calling, duschschampoo, aloe vera, masculin, green	H&M Cosmetic
5-1285	Nature Calling, shampoo, Boysensenbär, deep violet/red	H&M Cosmetic
5-1286	Liquid soap, violet	Hennes & Mauritz
5-1287	Johnson's, bubble bath, green	Johnson & Johnson Ltd.
5-1299	Camomile shampoo, yellow	The Body Shop
5-1300	Facial wash, tea tree oil, green	The Body SHOP
5-1301	Elder Flower Water, orange	The Body Shop
5-1302A	The Natural Choice, Papaya, 2-i-1 shampoo & balsam, orange	Demeter & Beauty Products Ltd. (UK)/ The Body Shop (DK)
5-1302B	The Natural Choice, Annanas, bath oil, yellow	Demeter & Beauty Prodcts Ltd. (UK)
5-1303	Matas After Shave, yellow/green	MATAS
5-1304	Matas Mild skin-tonic, pink	MATAS
5-1319	Rexona gradual deo classic, light red	Elida Robert
5-1320	Extase deo roll-on, violet ???	Royal Sanders (NL)
5-1321	Boston, man, bath & shower gel, green	Antonio Puig SA (E)
5-1322	Body & Nature body lotion, vild brombær, light red/light	FDB
5-1323	Vaselin Intensive Care, lotion, light red	Elida Robert
5-1324	Fyrrenåle skumbad, green	Nordisk parfumerivarefabrik A/S
5-1325	Mild shampoo, pink/light red	Dansk Supermarked A/S
5-1326	Active intensive shampoo, blue	Henkel Barnängen A/S
5-1327	Date conditioning shower creme, blue	Mölnlycke A/S

Table 5. Continued.

NERI reg. no.	Product identification	Manufacturer/Importer
5-1328	Respons shampoo, yellow	Colgate Palmolive A/S
5-1329	Actibrush mouth wash, blue	Colgate Palmolive A/S
5-1330	Zendium mouth wash, blue	Blumøller
5-1331	On line cream soap, wild rose, red	Forte (S)
5-1332	Elida Elegant, shampoo, violet	Elida Robert A/S
5-1333	Sanex intensive moisturizing shampoo, yellow	Blumøller
5-1334	VO5 Hair & Body shampoo, blue	Cederroth A/S
5-1335	VO5 shampoo for normal hair, orange	Cederroth A/S
5-1336	Swiss Formula, Hair repair, shampoo, blue	St. Ives Laboratories, Inc.
5-1337	Oil of Ulay Beauty Toner, blue	Dan-Salg A/S
5-1338	Soft cremetvål, yellow	Nordisk Parfumerivarefabrik A/S
5-1339	Thera-med, liquid 2-in 1, tandpasta & mund-vand, green	Henkel Barnängen A/S
5-1340	Swiss Formula, Papaya Shampoo	St. Ives Laboratories, Inc.
5-1341	Karex, håndvask	Cussons, UK
5-1342	Nivea Visage, gentle eye make-up remover	BDF, Beiersdorf
5-1343	Cutex neglelakfjerner	CMP Monaco, Spain
5-1344	Aqua-fresh, triple protection, fresh mint, tooth paste	SmithKline Beecham
5-1367	Montagne Jennesse, Orchid oil foam bath	Montagne Jennesse, UK
5-1368	Montagne Jennesse, Seaweed Mineral body scrub	Montagne Jennesse, UK
5-1397	Kolibri shampoo med balsam effekt, yellow	Cutisan Laboratorium
5-1398	Kyrell hair shampoo til daglig brug, blue/green	E. Kiessling & Cie. GmbH & Co.(D)
5-1399	Man shampoo for body & hair, yellow	E. Kiessling &Cie. GmbH & Co. (D)
5-1400	MilDeen protein shampoo, orange	Gallon Kosmetik (D)

Table 5. Continued.

NERI reg. no.	Product identification	Manufacturer/Importer
5-1401	Waterlilly kamille shampoo, orange	FDB
5-1402	Alea Olie shampoo	Nordisk Syntese
5-1404	Lux beauty elegance, showergel, light red	Lever
5-1405	Waterlilly body shampoo, bruse- og karbad, blue	FDB
5-1407	Revlon Aquamarine, body lotion, yellow/orange	Revelon
5-1408	Rexona creme deo, faint red/pink	Elida Robert
5-1409	Man Paris, aftershave, green	MV Marken-Vertrieb Kosmetik GmbH Cologne Co. (D)
5-1410	Clearasil, tonic, blue	Dan-Salg A/S
5-1411	L'Oreal Plénitude, extra mild tonic lotion, faint red	L'Oreal
5-1412	Waterlilly gele spray, violet	FDB
5-2773	Matas Mild skin-tonic, pink	Matas

3 Experimental

An ion-pair HPLC method followed by photodiode array detection (Appendix II) has been used for the analysis of the colourants in the present investigation. The HPLC method used is a modification of the method SPC/1327/93 described earlier (33). The details of the method are described in Appendix II of the present report. In brief, the HPLC method employs a PLRP-S 100Å HPLC column and gradient elution with a mobile phase consisting of citrate buffer containing ion-pairing reagent tetrabutylammonium hydroxide, acetonitrile and tetrahydrofuran. The spectra of the eluted substances were recorded between 275 nm - 760 nm. A spectral library, i.e. COSCOLOR consisting of the HPLC retention times (t_R) and spectra of 129 organic cosmetic colourants, including most of the permitted organic colourants and some relevant non-permitted colourants, has been build (Appendix III). The spectral library thus prepared has been used for the identification of the colourants in cosmetic products.

Depending upon the product (matrix) categories, a number of sample preparation methods have been developed for the extraction of colourants from various types of cosmetics (Appendix II). Liquid cosmetics with relatively low matrix, for example, mouth wash, skin tonic, etc. were analysed either directly or after concentration. Extraction of colourants from cosmetics with heavy matrix was performed employing a solid phase extraction (SPE) technique. Thus, colourants from lipsticks (and related products) and nail varnishes were extracted employing aminopropyl bonded silica SPE cartridges, and the extraction of colourants from various other types of cosmetics was performed employing C-18 silica SPE cartridges. The sample preparation methods have been optimized for the routine analysis of colourants in various types of cosmetic products. The colourants form mascara, eye pencil, eyeliner and eye shadow products colud not be extracted.

4 Results and Discussion

The aims of the study were to develop a method for the analysis of the colourants in various types of cosmetic products and to check the compliance of these products with the EU's Cosmetic Directive with respect to content of colourant(s). As there are >10,000 dyes and pigments available for colouring various types of products/materials, it was agreed to concentrate on the development of an analytical method for permitted colourants (and some relevant non-permitted colourants) according to the Cosmetic Directive. 29 inorganic and 128 organic colourants are permitted for the use in cosmetic formulations. 13 colourants are banned in cosmetic formulations according the Cosmetic Directive. Despite continuous efforts in the last 15 years, the EU Working Party on the Methods of Cosmetic Analysis has not been able to present a standard method for the analysis of all permitted/non-permitted colourants. The UK Group of the Working Party worked for nearly 5 years on organic cosmetic colourants, and they proposed in 1993 a HPLC method for the analysis of organic colourants in cosmetics (SPC/1327/93) to EU Commission (33). With the participation of 7 laboratories in 1994/1995, the method was subjected to a collaborative trial for the identification of 40 colourants in 12 samples of lipsticks and nail varnishes and in 5 solutions containing mixtures of up to 7 different reference colourants. The results of the trial revealed laboratory scores for the identification of the colourants in lipstick samples as 68% - 94%. For nail varnish samples laboratory scores were 33% - 78% and for reference colourant mixtures 68% - 84% (34).
It was concluded by the report of the collaborative trial (34) that the proposed method can be applied for the analysis of organic colour- ants in lipsticks and nail varnishes, but the method needed further improvements at several stages. Thus, we decided to improve the above mentioned method for the analysis of organic colourants in cosmetics rather than starting from scratch.

The principle of the method SPC/1327/93 is a chromatographic separation of the colourants by HPLC employing ion-paring and gradient elution followed by the identification of the colourants on the basis of their HPLC-t_R and UV-visible spectra. The colourants from cosmetic products were extracted either by dissolving the sample in an appropriate solvent followed by filtration or by solid phase extraction (SPE). As described in following paragraphs, we employed basically the same method, but we improved and optimized the method for the identification of colourants in various types of cosmetic products. Finally, we applied the method for the identification of the colourants in 139 cosmetic products for daily use.

A gradient mobile phase consisting of acetonitrile (ACN), tetrahydrofuran, and an aqueous buffer containing citric acid and the ion-pairing reagent tetrabutyl ammonium hydroxide (TBAH) was used for the HPLC of the colourants. The three components of the mobile phase were degassed separately before mixing them to prepare the gradient mobile phase. Thus, an uneven vaporisation of THF and ACN from an 1:1 mixture of these solvents (as used in the original method) was avoided. The mobile phase composition was thus slightly different compared to that used in the original method, but day to day variation in the HPLC-t_R of the reference colourants was restricted to <5% and the separation of various colourants from each other was improved. The solvent delivery pump (Waters 616) used in the present study made it possible to mix the three isolated components of the mobile phase to prepare the gradient mobile phase.

The pre-column and the analytical column as well as the flow of the mobile phase in the present study are the same as described in the original method. Both the pre-column and the analytical column were maintained at 25±1°C. The t_R of the reference colourants by HPLC analysis performed at 25°C are relatively longer than those described in the original method which employs 40°C for HPLC analysis. However, in the present investigation, the separation of the colourants from each other was improved without peak broadning. The relative standard deviation of t_R of various colourants analysed in the present investigation was 0.50% - 5.0%.

In general, 1 - 2 mg of the reference colourants were dissolved in the suitable solvents (see Appendix III) and 10 µl of the solutions were analysed by the HPLC method as described in Appendix II. The aim was to obtain 0.05 - 0.80 AU (absorbance units) absorbance for each colourant at their λ_{max}. It was, therefore, necessary to adjust the concentrations of some of the colourants accordingly. CI 46500 & CI 73915 showed a high signal at 290 nm & 295 nm respectively, but the signals at 450 nm - 550 nm (characteristic spectra) were relatively low (see Appendix III). The reference colourant solutions, except CI 75130, were stored in closed vials and they were found to be stable (qualitatively) for at least 1 month. CI 75130 should be analysed immediately after the substance is dissolved in the proper solvent (see Appendix III). This solution was stable for less than an hour at room temperature in day light.

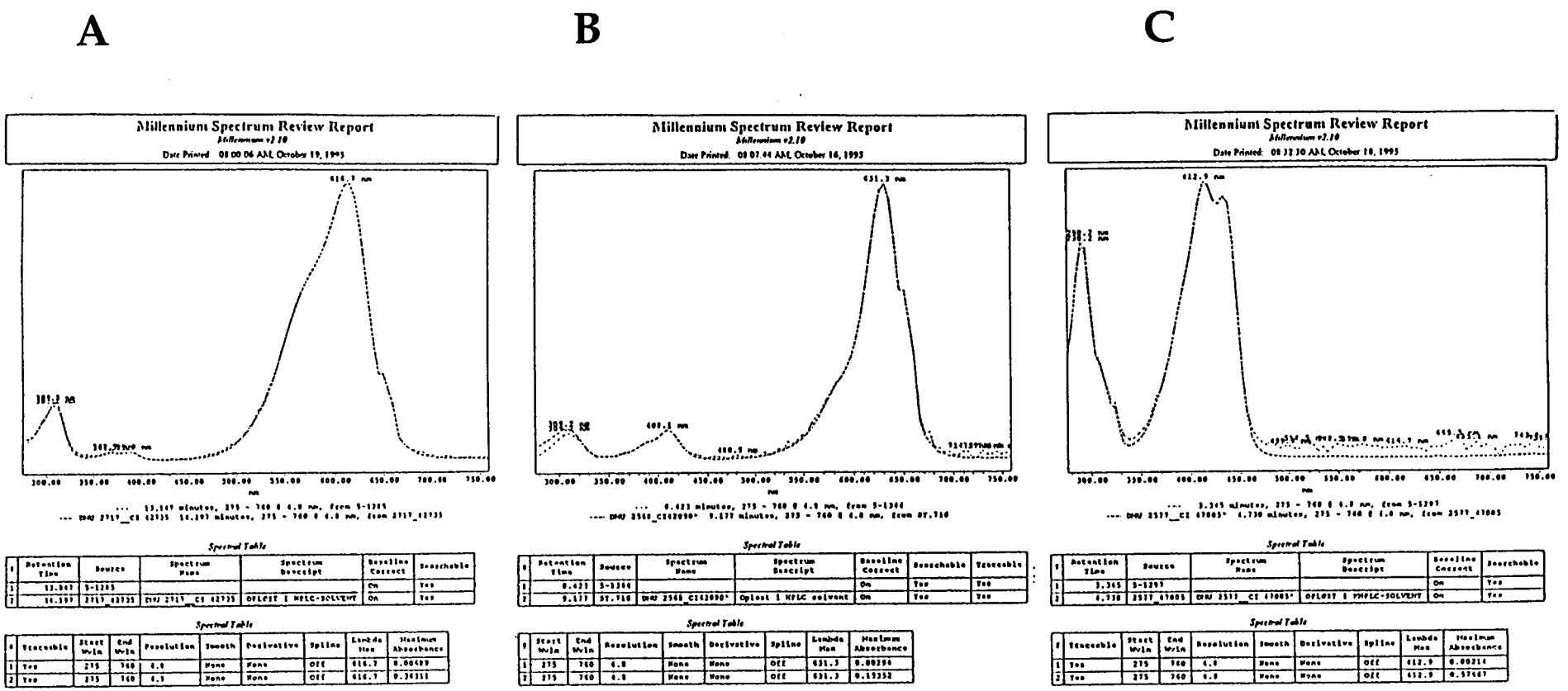
Some of the colourants could not be dissolved in organic solvents and some others were partially soluble. It was not possible to obtain a signal corresponding to ≥ 0.05 AU for some of the partially soluble colourants (for example CI 73360). Finally, some of the colorants (for example, CI 20040) although soluble in organic solvents, they did not show any spectrum after HPLC analysis. This may be due to irreversible adsorption of the colourants in the HPLC column. The factors responsible for this may be an unsuitable column packing or pH and composition of the mobile phase or the ion-pairing reagent used for the HPLC analysis of these colourants.

Among the organic colourants in our stock which could not be analysed for one or the other reason are:
CI 20040, CI 21100, CI 21108, CI 51319, CI 69800, CI 69825, CI 71105, CI 74100, CI 74160, CI 74260, betain and betanin.

The spectra of the colourants, passing through the photodiode array detector (PDA), were recorded across the wavelength range 275 nm-760 nm, which covers the λ_{max} as well as other features of the UV-visible spectra of the permitted/non-permitted colourants investigated in the present study. The data was acquired continuously with the acquisition rate of 1 spectrum/sec through out the HPLC analysis. Continues data acquisition across the complete wavelength range is necessary to ascertain the detection of all colourants, even those in low concentration. In the original method, spectra of the substances eluting from the analytical column were recorded across the complete wavelength range only when the signal (absorbance) at 265 nm or 590 nm increased a predefined value. Thus, the spectrum of the colourants, not having significant absorbance at the predefined wavelengths or below the predefined signal threshold, will not be recorded. This may influence the detection limits of the colourants, and certain colourants present in a sample may not be identified. The detection limit (corresponding to an absorbance of approximately 0.01 AU at λ_{max}) for the colourants investigated in the present study were 50 ng - 500 ng, when 10 µl of the colourant solutions were analysed. It was, however, possible to detect still lower concentration of the colourants, because up to 50 µl of the solutions of the reference colourants can be analysed without any problem. Examples of the identification of some of the colourants present in low concentration in cosmetic products (absorbance down to 0.002 AU for the analysis of 10 µl of the sample extracts) are shown in Fig. 1. It should be noted that to identify substances in low concentration, area rejection parameter for the data processing should be kept as low as possible. We used 1000 counts as area rejection.

The suggestions and recommendations for the data acquisition according to Millennium Software Package for the Waters 996 PDA, except for the resolution, were followed to achieve the maximum sensitivity and selectivity for the identification of the colourants. A resolution of 4.8 nm was used for the data acquisition. With 4.8 nm resolution, every HPLC analysis occupied approximately 1.2 mb space in the hard-disc. For the resolution 1.2 nm, approximately 5 mb disc-space will be required for each HPLC analysis. Thus, for the analysis of a sample with 12 fractions with some blanks, 70-80 mb hard-disc space will be required. For making situation even more complex, a stream cartridge tape of capacity 80 mb will be required for each sample. With the experiences from the present study, data acquisition using 4.8 nm resolution has been found to be suitable for the routine analysis of identification of colourants in cosmetic products. The amount of data/disc-space may be restricted by limiting the wavelength range for the data acquisition. However, in doing this, one should be absolutely sure about that important features of the UV-visible spectrum of an analyte(s) under investigation will not be missed.

Figure 1: Identification of colourants with low concentration in cosmetic products A: shampoo 5-1285, B: toothpaste 5-1344 and C: lipstick 5-1297. In all cases, 10 µl of the sample extract was analysed.



A higher resolution (1.2 nm) may be useful for the differentiation between the colourants with identical spectra and similar HPLC-t_R, for example , CI 45405/CI 45410, and CI 45425/CI 45430. The UV-visible spectra of CI 45405 and CI 45410 recorded employing 1.2 nm resolution, however, were no different from the respective-spectra recorded with 4.8 nm resolution (results not shown). Another approach to differentiate between such pairs of co-lourants may be the use of a different gradient of the mobile phase. However, even the extension of the mobile phase gradient (between 10 - 30 min) by 10 min revealed that these pairs of colourants still had similar t_R.

The acquired HPLC-PDA data was processed to obtain max-plot chromatogram of the substances, where a max-plot chromatogram of a substance is defined as the chromatographic peak of the substance at its λ_{max}. The max-plot chromatogram of a sample (extract) will show chromatographic peaks of all the substances which pass through the PDA detector and absorb within the predefined wavelength range (Fig 2). The advantage of a max-plot chromatogram is that colorants even at low concentration (as described above) can be identified. The disadvantage with the max-plot chromatogram is that the chromatogram may show many chromatographic peaks of non-target substances, see for example max-plot chromatogram of the analysis of colour extract from a nail varnish (Fig 3A). Yet another disadvantage of max-plot chromatogram may be observed when two substances coelute, resulting in an additive spectrum. However, this problem can be solved in several ways, for example, the sample extract may be fractionated (prior to analysis by HPLC) to separate the two substances. Thus, HPLC analysis of a nail varnish extract (Sample No. 5-1417, Fig. 4A) revealed that the product contained CI 15850 (t_R 13.037 min) and the possible presence of another colourant colluding with a matrix component (Fig. 5A) in one of the major chromatographic peaks, t_R 14.270 min. Fractionation of the sample extract employing amino bonded silica-SPE followed by HPLC-analysis of the fractions revealed that an unidentified substance (the matrix component, a non-colourant), correspon-ding to the above mentioned major peak, was separated (fraction 3, t_R 14.317 min, Figs. 4C & 5B) from the colourant. The colourant was eluted in fraction 9 (t_R 14.245) and that was identified as CI 15880:1 (Figs. 4E, & 5C). The CI 15850 was eluted in fraction 7. Another way to solve the problem of identification of colluding substances is to subtract the spectrum of the non-target substance (if that is a known substance) from the additive spectrum. Yet another possible way to get rid of non-target substance spectrum is to restrict the wavelength range for data acquisition. For example, the spectrum (absorbance at λ_{max} specifically) of most of the surfactant (which have λ_{max} at 270 nm - 290 nm) will vanish when the data is acquired from 310 nm. Although a little but insignificant loss in fine structures of some of the colourant spectra may be expected (see further).

Figure 2: Max-plot chromatogram of SPE-fraction 1 of the sample extract from lipstick 5-1375.

Millennium Results Report			September 13, 1995		Page: 1 of 2
Report Method:	PDA_GITTE		Version:	2.10	
For Sample:	5-1375-1	Vial: 5	Injection:	1	Channel: 996
Proc Chan:	pda_275_760		Processed:	09/13/95 05:52:41 PM	
Channel Descr:	PDA MaxPlot (275.0 nm to 760.0 nm)				

Millennium Sample Information

Project Name:	SCR_farver		
Sample Name:	5-1375-1		
Vial:	5	Sample Type:	Unknown
Injection:	1	Volume:	10.00
Channel:	996	Run Time:	40.0 min
Date Acquired:	09/13/95 05:11:46 PM	Date Processed:	09/13/95 05:52:41 PM
SampleWeight:	1.00000	Dilution:	1.00000
Acq Meth Set:	scr_farve_ms		
Processing Method:	scr_farve_pm		

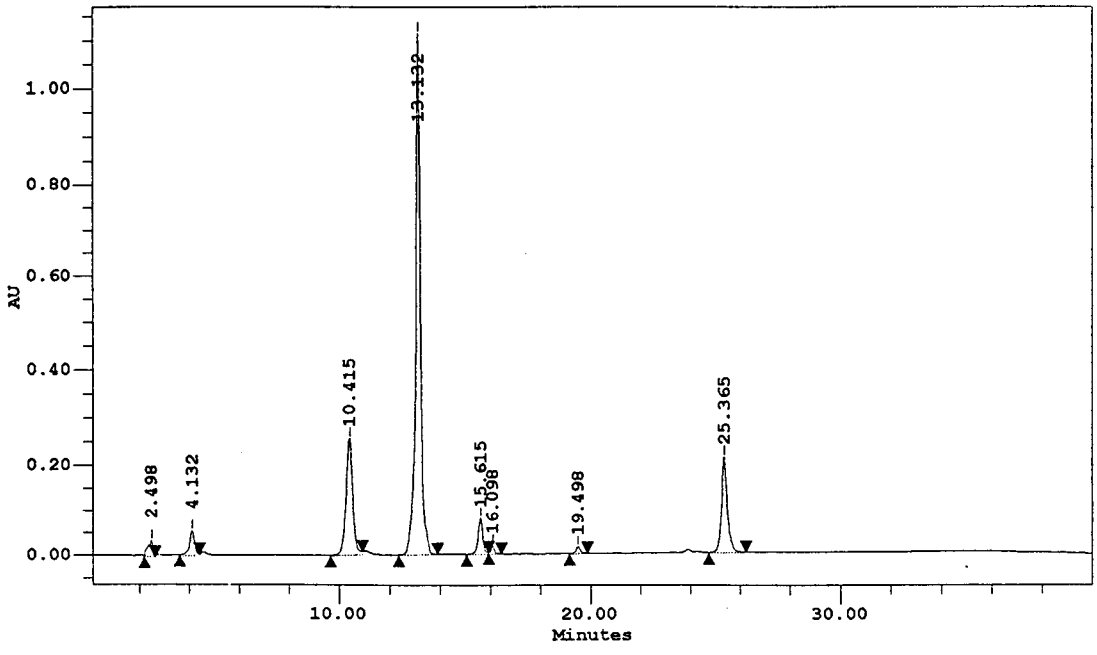
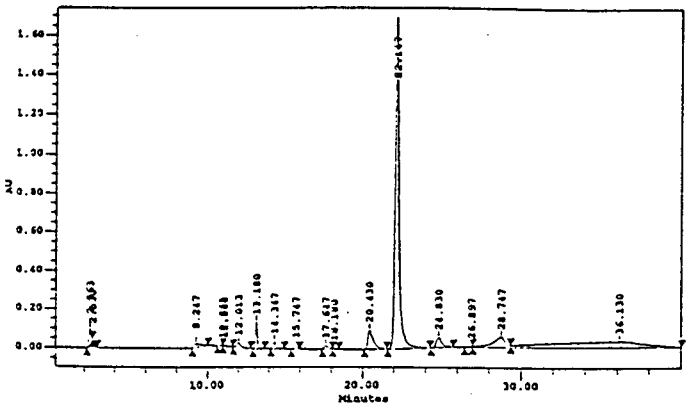
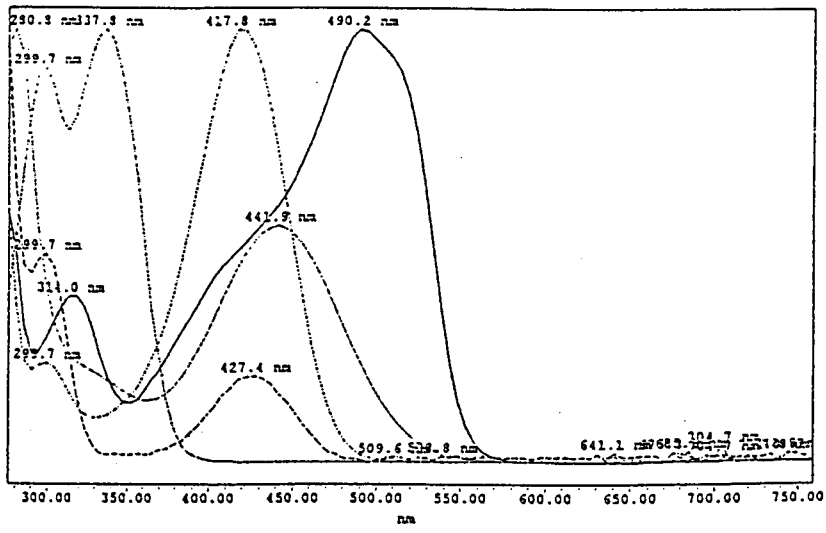


Figure 3: Max-plot chromatogram (A) of a nail varnish (Sample No. 5-1393) extract. The chromatogram contains many peaks. Only the chromatographic peaks showing a characteristic UV-visible spectrum (possible colourants) are included in the spectrum review (B). Spectrum with λ_{max} 490.2 was identified as CI 15850. Remaining spectra could not be related to specific compounds.

A



B



---- 10.963 minutes, 275 - 760 @ 4.8 nm, from 5-1393_DIREKTE
---- 11.080 minutes, 275 - 760 @ 4.8 nm, from 5-1393_DIREKTE
—— 13.180 minutes, 275 - 760 @ 4.8 nm, from 5-1393_DIREKTE
—— 14.347 minutes, 275 - 760 @ 4.8 nm, from 5-1393_DIREKTE
—— 24.830 minutes, 275 - 760 @ 4.8 nm, from 5-1393_DIREKTE

Spectral Table

#	Retention Time	Source	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution	Smooth
1	10.963	5-1393_DIREKTE	On	Yes	Yes	275	760	4.8	None
2	11.080	5-1393_DIREKTE	On	Yes	Yes	275	760	4.8	None
3	13.180	5-1393_DIREKTE	On	Yes	Yes	275	760	4.8	None
4	14.347	5-1393_DIREKTE	On	Yes	Yes	275	760	4.8	None
5	24.830	5-1393_DIREKTE	On	Yes	Yes	275	760	4.8	None

Spectral Table

#	Derivative	Spline	Lambda Max	Maximum Absorbance
1	None	Off	417.8	0.00609
2	None	Off	276.0	0.00464
3	None	Off	490.2	0.13943
4	None	Off	290.8	0.04273
5	None	Off	337.5	0.06432

Figure 4: Max-plot chromatogram of sample extract (Sample No. 5-1417) analysed prior to fractionation (A), SPE-fractions 1, 3, 7 & 9 (B, C, D & E respectively). CI 15850 was identified in A (t_R 13.037 min) and D (t_R 13.065 min), one of the major peaks in A (t_R 22.253 min) was eluted in SPE-fraction 1 (B, t_R 22.115 min), the other major peak in A (t_R 14.270 min) was partly eluted in fraction 3 (C, t_R 14.317 min) and partly eluted in fraction 9 (E, t_R 14.245 min). The peak with t_R 14.245 min (E) was identified as CI 15880:1 (see Figure 5).

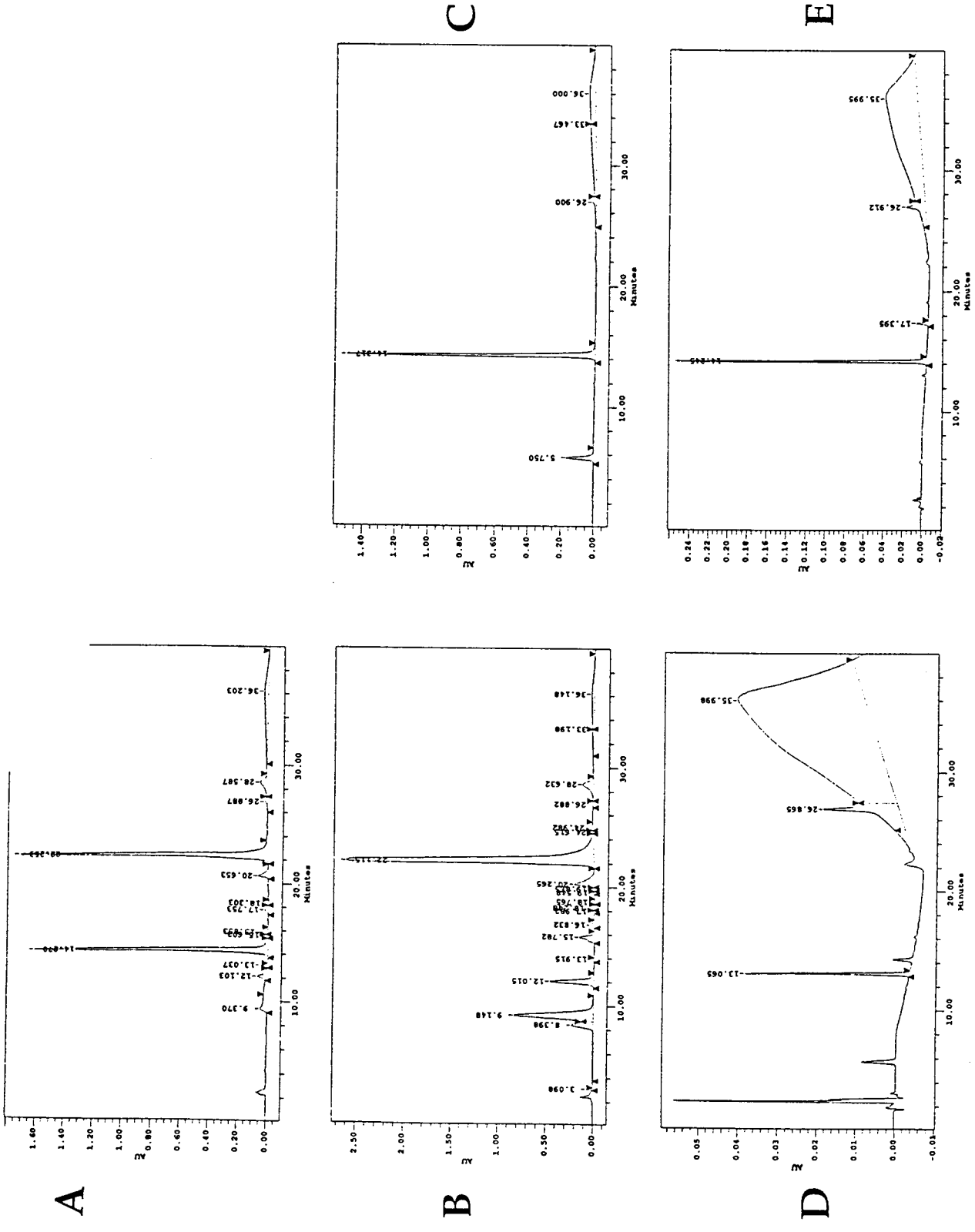
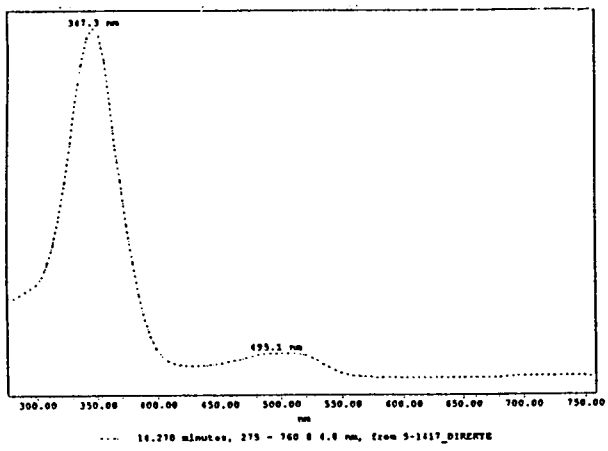
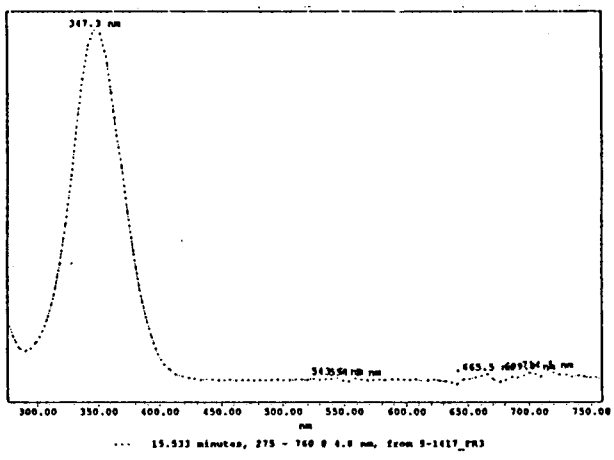


Figure 5: Spectra of A: coleuting substances (chromatographic peak with t_R 14.270 min, Fig. 4A), and its fractions B: chromatographic peak with t_R 14.317 (Fig. 4C) and C: chromatographic peak with t_R 14.245 (Fig. 4E). C was identified as CI 15880:1.

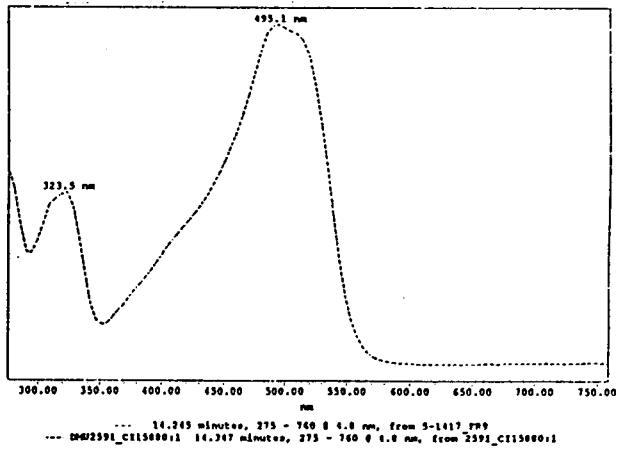
A



B



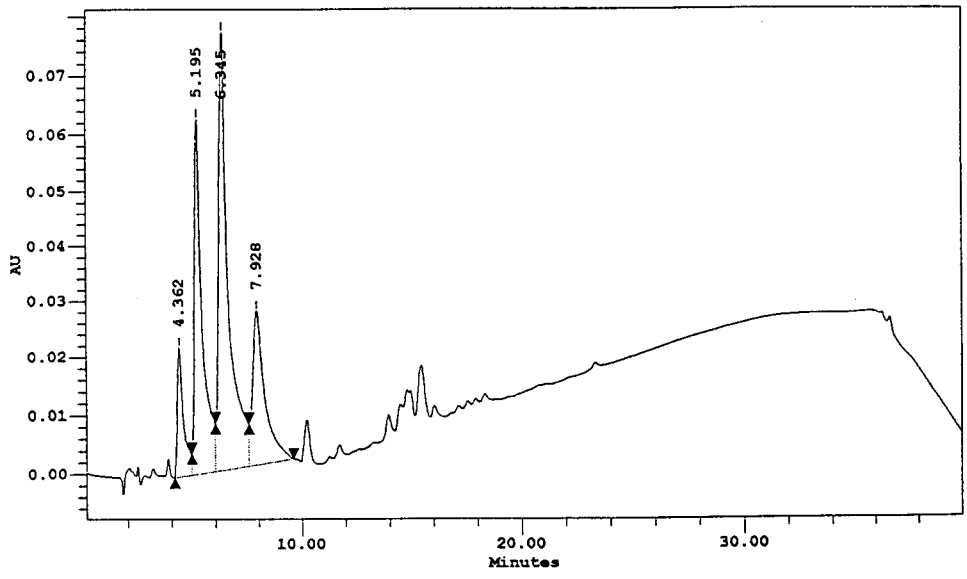
C

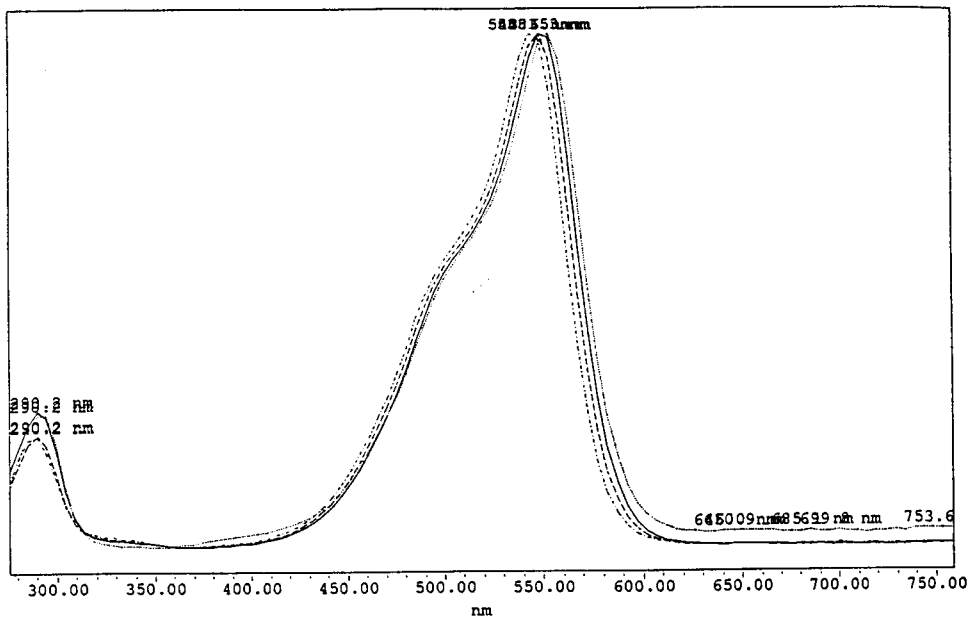


By the use of Millennium Software Package, a spectral library "COSCOLOR" of the analysed reference colourants as well as the significant impurities in the reference colourants has been build (Appendix III). The library COSCOLOR contains the spectra, and the retention times of the investigated colourants as well as impurities in these. The spectra of a colourant having impuritiesare marked with *, **, *** and **** . The spectrum with * represents the max-plot peak with the maximum absorbance (the main substance), the spectrum marked with ** represents max-plot peak with the next highest absorbance, and so on. Maximum 4 spectra, corresponding to 4 max-plot peaks of a colourant are included, eventhrough its max-plot chromatogram contained more than 4 chromatographic peaks, (For example, CI 42510, Fig. 6). Furthermore, for the reasons discussed earlier, the spectra of CI 46500, CI 73360 and CI 73915 are not included in the library COSCOLR, they are only shown in the Appendix III.

The identification of the colourants in the various categories of cosmetics was performed after the proper pretreatment of the samples to partially or totally remove the matrix, followed by the HPLC analysis as described for the reference colourants. If a colourant extract was faint, up to 50 µl of the extract was analysed. The data processing and the identification of the colourants was performed automatically employing the Millennium Software and spectral library COSCOLOR. The match parameters included 10% retention time window (2 x RSD of t_R) and 10 nm spectrum window (2 x resolution). The identification of the doubtful cases, matchangle >2, were checked manually. An example of the routine analysis for the identification of colourants is shown in Figs. 7 - 10. Thus, for the analysis of a lipstick sample (Sample No. 5-1375, SPE fraction 1), max-plot chromatogram, peak results and PDA results are shown in Fig 7. The spectrum index of the max-plot chromatogram revealed the presence of 3 possible colourants in this fraction (Fig. 8). However, only 2 of them were identified by automatic library search as shown in PDA results (Fig. 7). Eventhrough, the matchangle for one of the identified substances (Fig. 7, PDA results, peak with t_R 4.132 min) was >2, the manual search revealed that the spectrum of this peak satisfactorily matched with that of CI 19140:1 (Fig. 9). The manual library search also revealed that the spectrum (Fig. 10) and t_R of the unidentified max-plot peak did not match with any of the entries in COSCOLOR. A matchangle >2 for CI 19140:1 is possibly due to much lower concentration of the colourant in the sample (absorbance 0.0550 AU) compared to that of the reference colourant (absorbance 0.8035 AU) employed for building the spectral library (Fig. 9). This is supported by the fact that identification of some of the colourants in several sample was possible only when the larger volumes (> 10 µl) of the sample extracts were analysed (Figs. 11 & 12). However, in some cases it may not be appropriate to analyse volumes >10 µl of a sample extract because the concentrations of the possible interfering substances will also be increased. In such cases, either the reference substance should be added in the sample extract or the spectrum of the reference colourant with concentration similar to that in the sample should be analysed for the spectrum match.

Figure 6: Max-plot chromatogram and spectra of the chromatographic peaks of the reference colourant CI 42510.





4.362 minutes, 275 - 760 @ 4.8 nm, from 2653_42510
5.195 minutes, 275 - 760 @ 4.8 nm, from 2653_42510
6.345 minutes, 275 - 760 @ 4.8 nm, from 2653_42510
7.928 minutes, 275 - 760 @ 4.8 nm, from 2653_42510

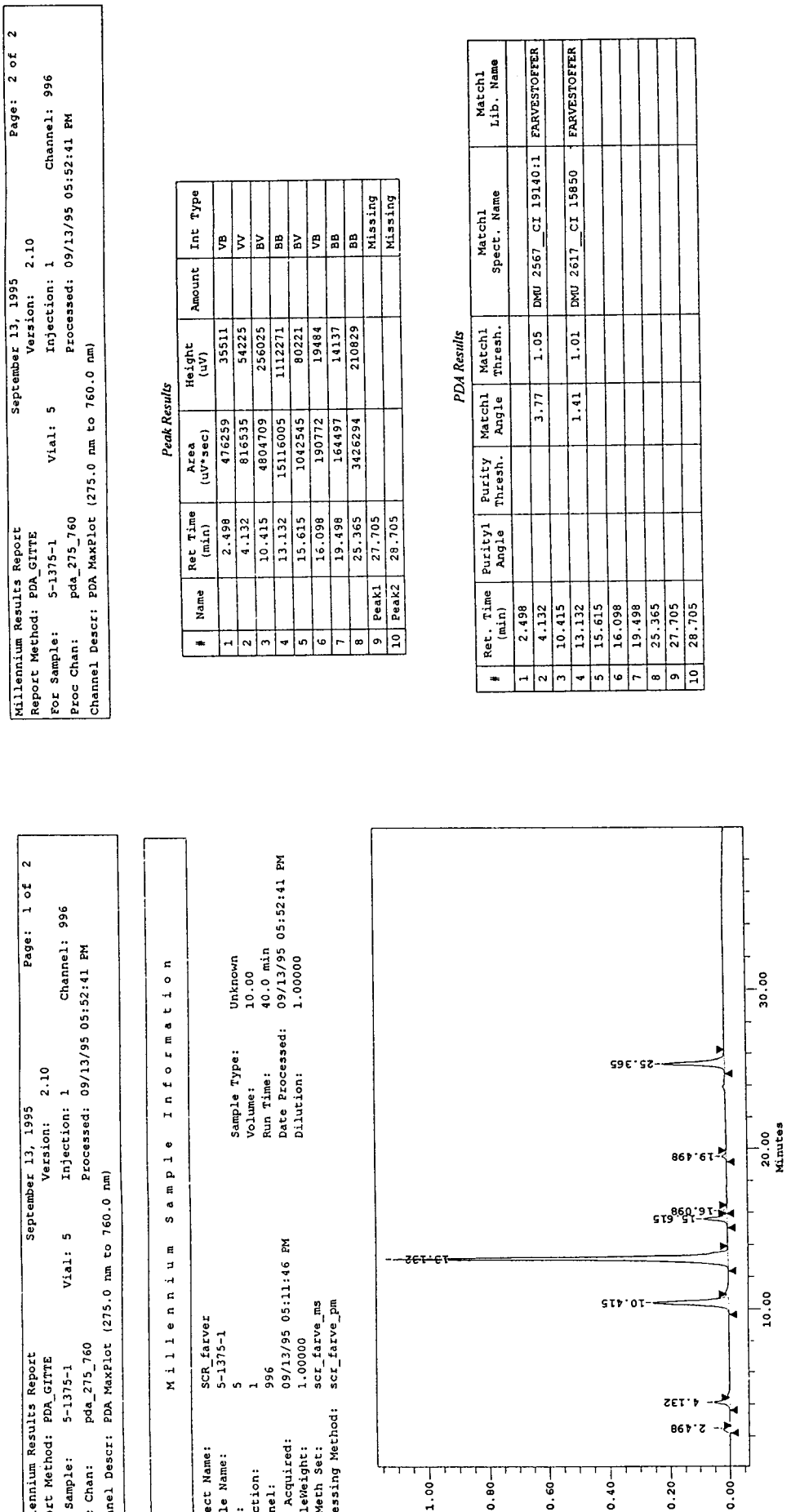
Spectral Table

#	Retention Time	Source	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution	Smooth
1	4.362	2653_42510	On	Yes	Yes	275	760	4.8	None
2	5.195	2653_42510	On	Yes	Yes	275	760	4.8	None
3	6.345	2653_42510	On	Yes	Yes	275	760	4.8	None
4	7.928	2653_42510	On	Yes	Yes	275	760	4.8	None

Spectral Table

#	Derivative	Spline	Lambda Max	Maximum Absorbance
1	None	Off	543.6	0.02228
2	None	Off	548.5	0.06269
3	None	Off	548.5	0.07675
4	None	Off	553.3	0.02671

Figure 7: Results of analysis of a sample extract, Sample No. 5-1375, SPE fraction 1.



Millennium Results Report — September 13, 1995 — Page: 2 of 2
Report Method: PDA_GITTE — Version: 2.10
For Sample: 5-1375-1 — Vial: 5 — Injection: 1 — Channel: 996
Proc Chan: pda_275_760 — Processed: 09/13/95 05:52:41 PM
Channel Descr: PDA MaxPlot (275.0 nm to 760.0 nm)

Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1		2.498	476259	35511		VB
2		4.132	816535	54225		VV
3		10.415	4804709	256025		BV
4		13.132	15116005	1112271		BB
5		15.615	1042545	80221		BV
6		16.098	190772	19484		VB
7		19.498	164497	14137		BB
8		25.365	3426294	210829		BB
9	Peak1	27.705				Missing
10	Peak2	28.705				Missing

PDA Results

#	Ret. Time (min)	Purity1 Angle	Purity Thresh.	Match1 Angle	Match1 Thresh.	Match1 Spect. Name	Match1 Lib. Name
1	2.498						
2	4.132			3.77	1.05	DMU 2567_CI 19140:1	FARVESTOFFER
3	10.415						
4	13.132			1.41	1.01	DMU 2617_CI 15850	FARVESTOFFER
5	15.615						
6	16.098						
7	19.498						
8	25.365						
9	27.705						
10	28.705						

Millennium Sample Information

Project Name:	SCR_farver		
Sample Name:	5-1375-1	Sample Type:	Unknown
Vial:	5	Volume:	10.00
Injection:	1	Run Time:	40.0 min
Channel:	996	Date Processed:	09/13/95 05:52:41 PM
Date Acquired:	09/13/95 05:11:46 PM	Dilution:	1.00000
SampleWeight:	1.00000		
Acq Meth Set:	scr_farve_ms		
Processing Method:	scr_farve_pm		

Millennium Results Report — September 13, 1995 — Page: 1 of 2
Report Method: PDA_GITTE — Version: 2.10
For Sample: 5-1375-1 — Vial: 5 — Injection: 1 — Channel: 996
Proc Chan: pda_275_760 — Processed: 09/13/95 05:52:41 PM
Channel Descr: PDA MaxPlot (275.0 nm to 760.0 nm)

Figure 8: Spectrum index of the max-plot chromatogram of 5-1375-1 (Fig. 7).

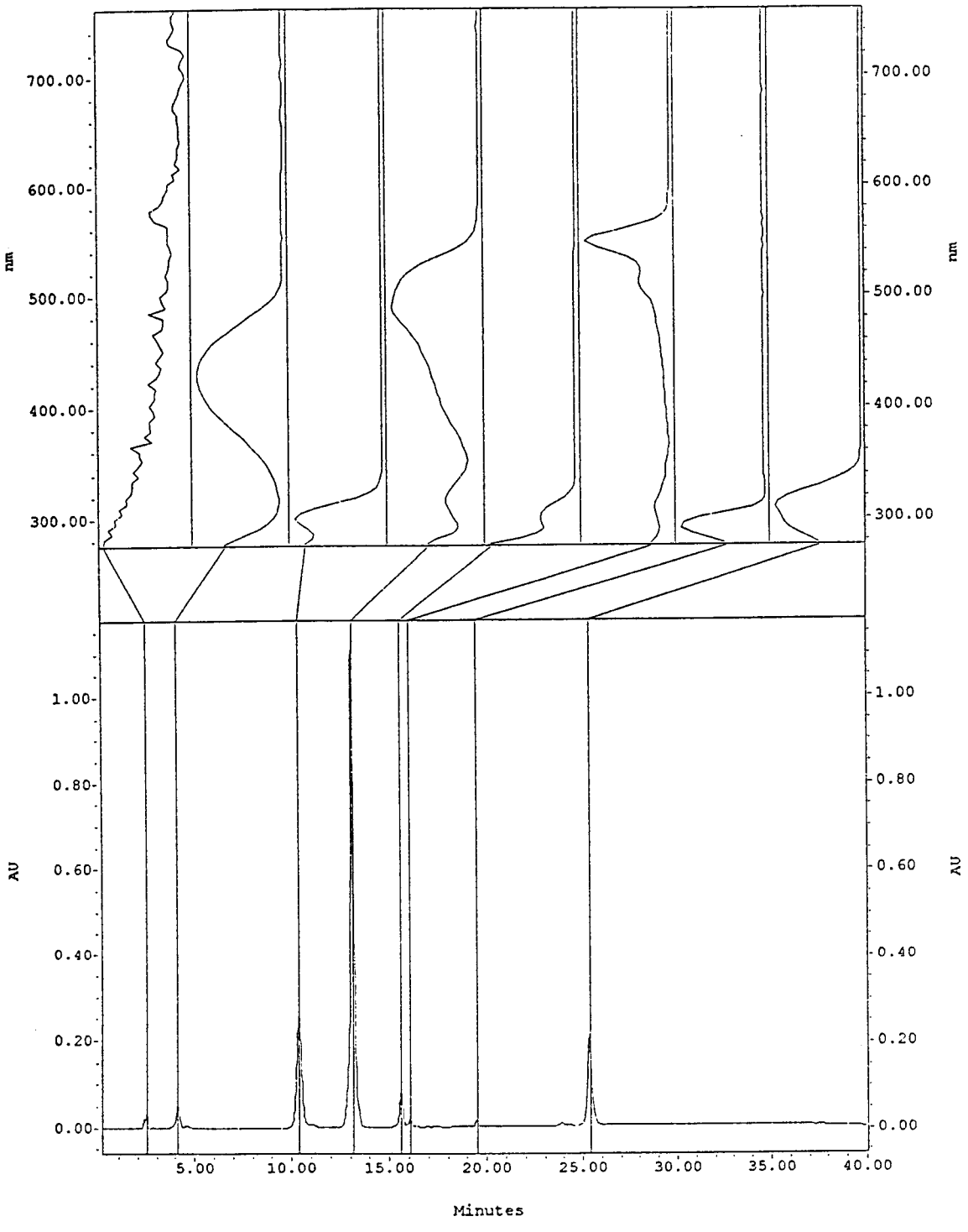
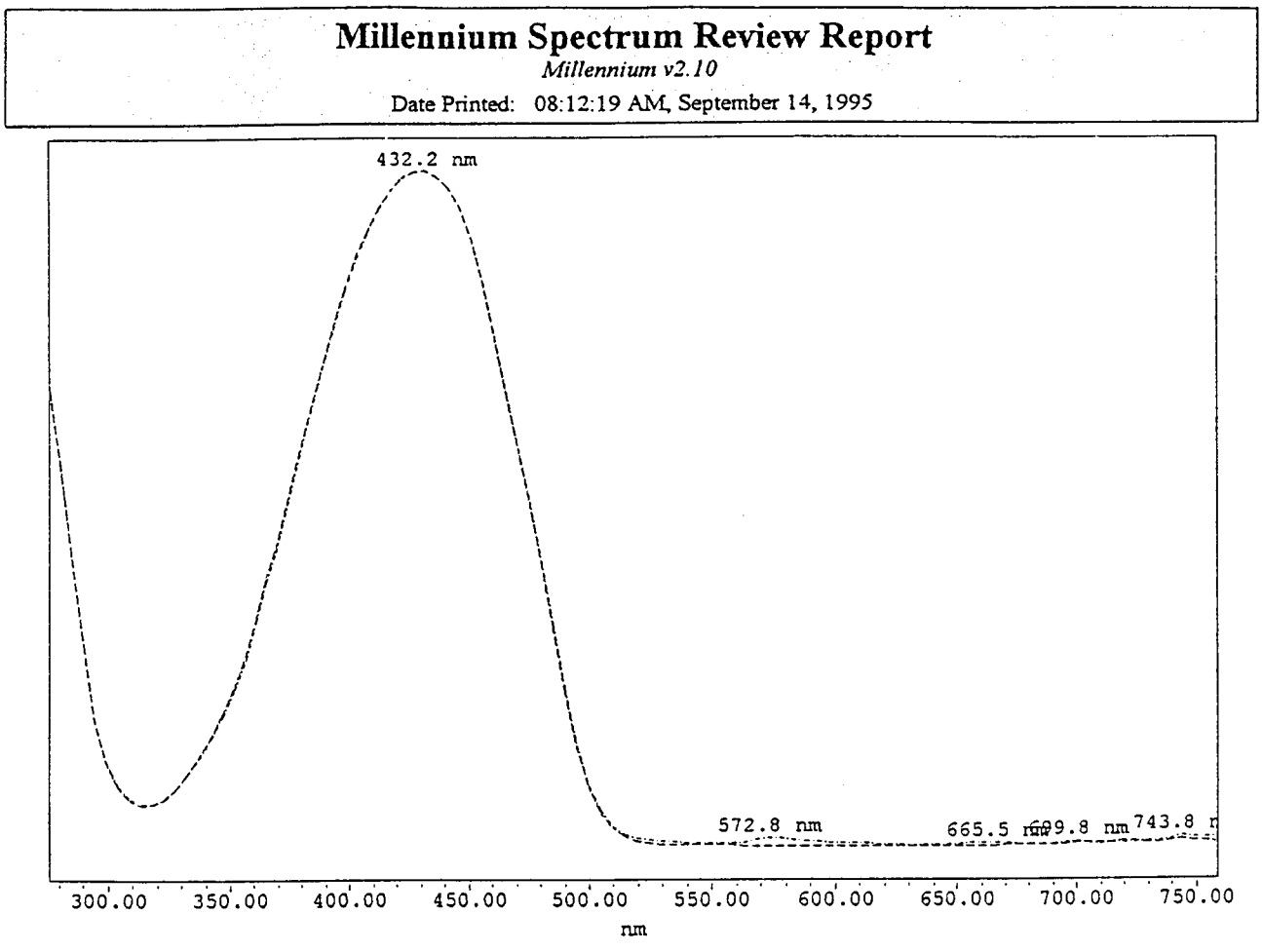


Figure 9: Spectrum match of the chromatographic peak with t_R 4.132 min (Fig. 7) with the spectrum of the reference colourant CI 19140:1.



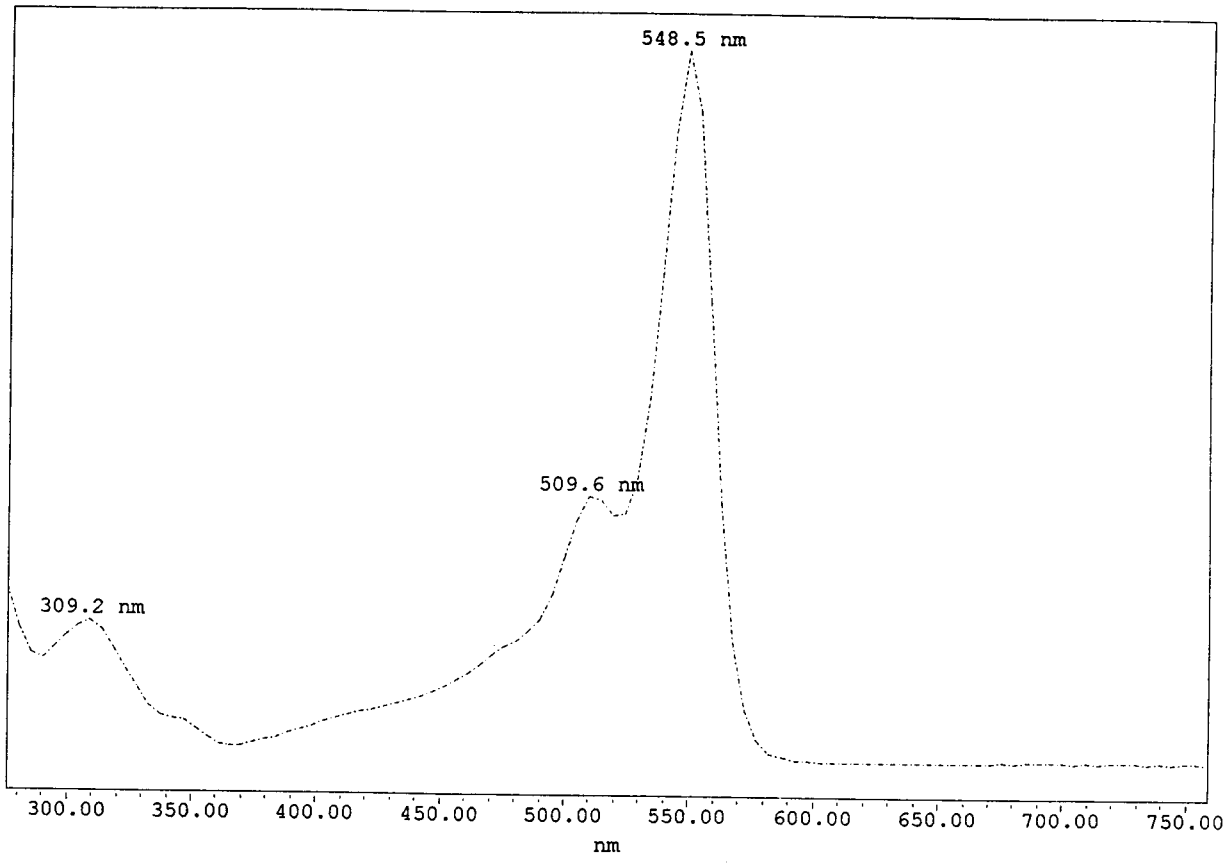
Spectral Table

#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	4.132	5-1375-1			On	Yes
2	4.222	2567_19140:1	DMU 2567__CI 19140:1	OPLØST I HPLC-SOLVENT	On	Yes

Spectral Table

#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	275	760	4.8	None	None	Off	432.2	0.05509
2	Yes	275	760	4.8	None	None	Off	432.2	0.80356

Figure 10: The spectrum of the unidentified max-plot peak from the sample extract 5-1375-1 (Figs. 7 & 8).



Spectral Table

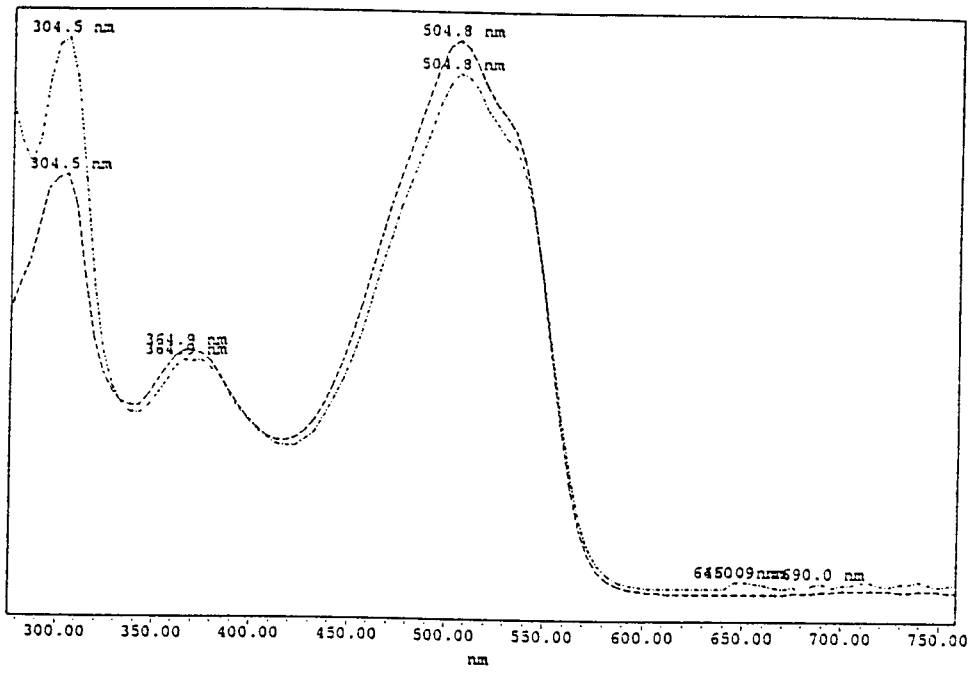
#	Retention Time	Source	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution	Smooth
1	16.098	5-1375-1	On	Yes	Yes	275	760	4.8	None

Spectral Table

#	Derivative	Spline	Lambda Max	Maximum Absorbance
1	None	Off	548.5	0.02388

Figure 11: Identification of CI 14700 in Sample No. 5-1323. A: analysis of 10 µl of the sample extract, absorbance at λ_{max} (504.8 nm) for the sample extract and the reference colourant are 0.00752 AU and 0.65165 AU respectively. B: analysis of 25 µl of the sample extract, absorbance for the sample extract and the reference colourant are 0.02046 AU and 0.65165, respectively.

A



B

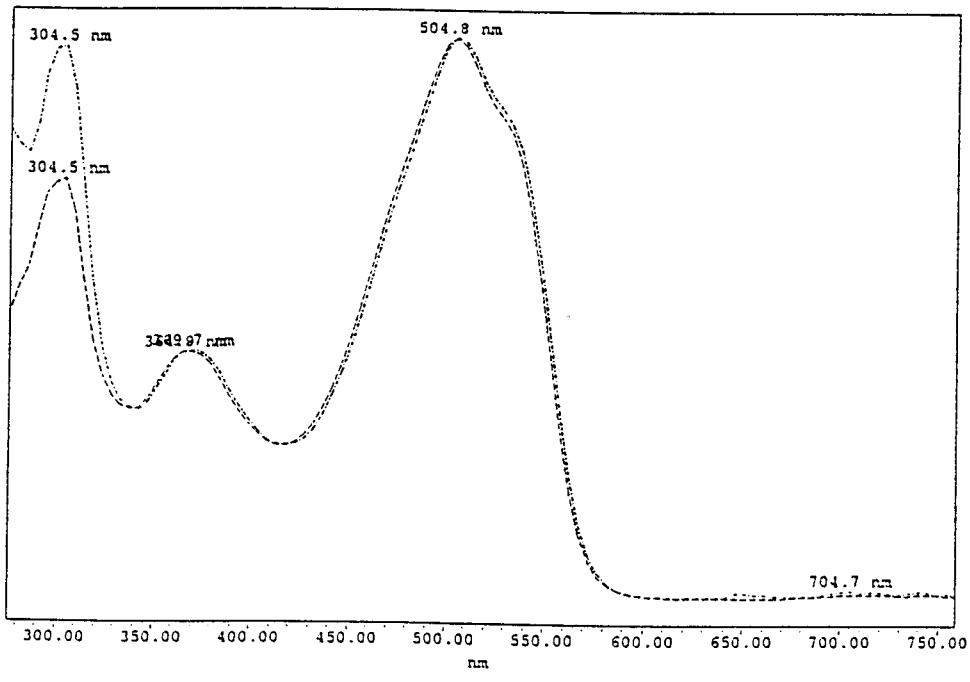
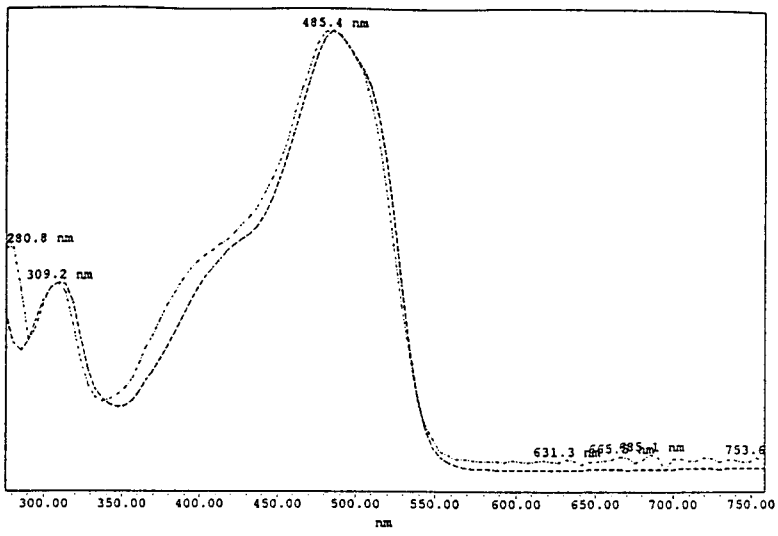
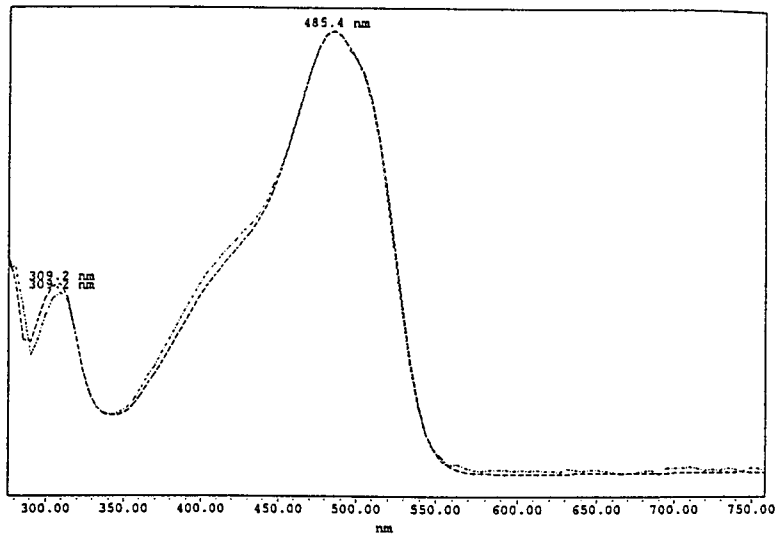


Figure 12: Identification of CI 15585 in the Sample No. 5-1349 (SPE fraction 12). A, B & C represent analysis of 10 µl, 25 µl and 50 µl of the sample extract respectively. A better match of the sample spectrum with that of CI 15585 was observed with the increasing volume of the sample extract analysed.

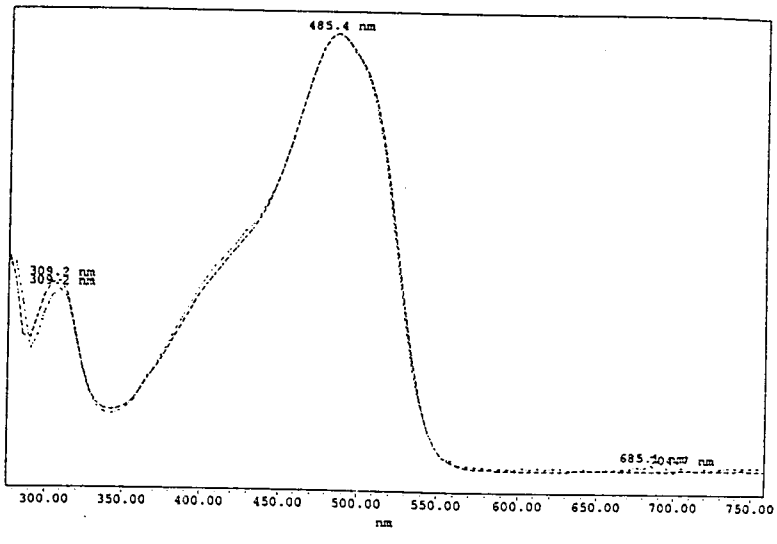
A



B



C



Finally, during the routine analysis, it was also controlled that at least the visible colour of a product was identified. For example, when a violet coloured product was not found to contain a violet colourant but it contained a red colourant, then the product must contain a blue colourant as well.
Among the decoration cosmetics, methods for the sample preparation for the routine analysis of colourants in lipsticks and nail varnishes have been optimized. The colourants from the lipstick samples were extracted in acidified dimethylformamide as described previously (33). However, a new scheme for the solid phase extraction (SPE) of the colourants from the total colour extract, employing aminobonded silica, has been developed. The method developed for the sample preparation of lipsticks (Appendix II) has been optimized and that has been used routinely without any problem.

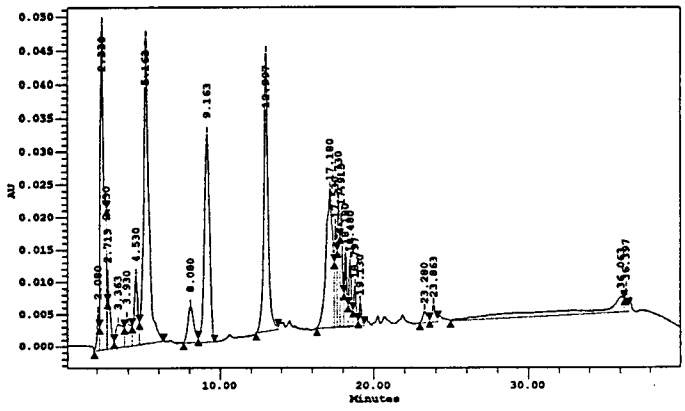
The sample preparation method for the analysis of the colourants in nail varnishes (Appendix II) is a major revision of the previously described method (33). This method is similar to the method developed for the lipsticks, except that the colourants were extracted by ultrasonication of the suspensions of nail varnishes in methanol followed by centrifugation of the extract. It was necessary to analyse both the total extracts of nail varnishes and the fractions obtained by SPE of the total extracts for the complete identification of the colourants in these products. (Figs. 4 & 5). As demonstrated in these figures, the identification of the CI 15580:1 in the sample 5-1417 was possible only when the SPE-fractions of the colour extract were analysed. The example also demonstrates that when a matrix component and a colourant present in a sample coelute under the experimental conditions, an additive spectrum (colourant+ matrix component) will be obtained. Identification of colourants in such cases is not difficult but certainly need some experience applying the present analytical method. The method for the analysis of colourants in nail varnishes has also been used routinely without any problem.

Analysis of colourants in other decoration cosmetics (17 samples of eye shadow, mascara, eye pencil and eye liner, Table 4) was not possible, except for one sample of eye shadow (Sample No. 5-1352). The colurants from these products could not be extracted in organic solvents despite various efforts (see further).

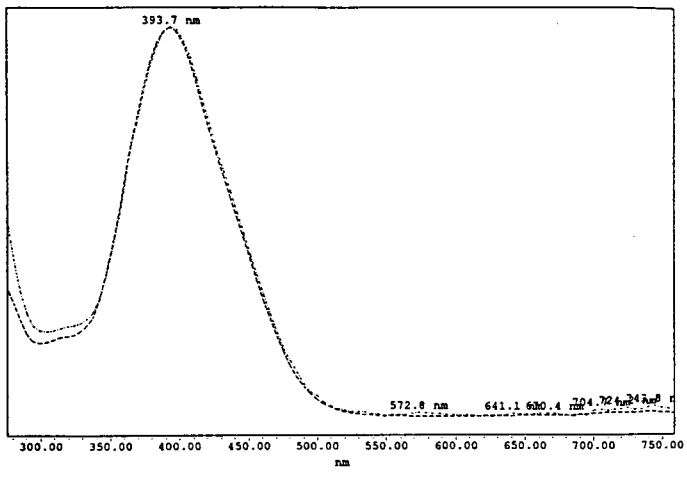
Depending upon the product category (shampoo, bath gel, skin tonic, body lotion, cream, deo roll-on, aftershave lotion, etc.), a number of methods for the analysis of colourants in these products have been developed. Most of these products contain surfactant and many of the products are only faintly coloured. The strongly coloured products with relatively high amounts of surfactant (thick consistency, for example, shampoos and bath gels) were analysed after diluting them appropriately (Appendix II, 6.3.2). Thus, in a green shampoo (Sample No. 5-1284) CI 13015 and CI 42090 (Fig. 13) and in another green shampoo (Sample No. 5-1321) CI 19140:1 and CI 42090 were identified (Fig. 14).

Figure 13: Identification of colourants in a green shampoo (Sample No. 5-1284), 10 µl of the sample solution was analysed (or sample preparation see Appendix II, 6.3.2. A: max-plot chromatogram, B: spectrum match of chromatographic peak with t_R 4.530 min with the spectrum of the reference colourant CI 13015, and C: spectrum match of chromatographic peak with t_R 9.163 min with the spectrum of the reference colourant CI 42090.

A



B



C

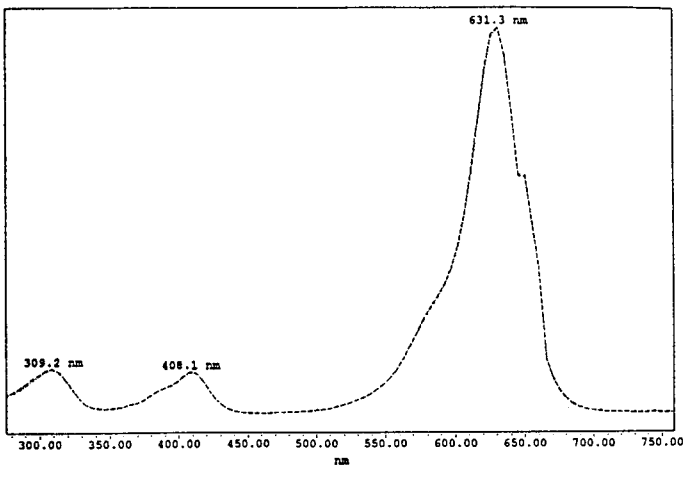
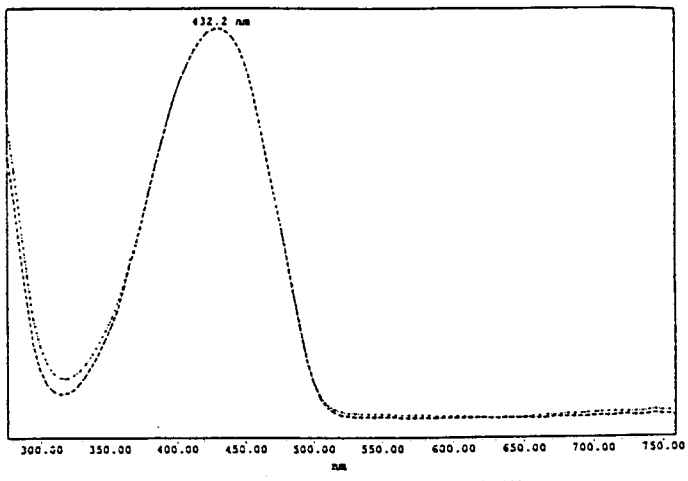


Figure 14: Identification of CI 19140:1 (A) and CI 42090 (B) in the green shampoo 5-1321. As described in the spectral tables, the t_R of the colourants in the HPLC analysis of the shampoo solution were altered dramatically. The spectral tables also show the differences in the absorbance of the colourants in the sample solution and in the solution of reference colourant.

A



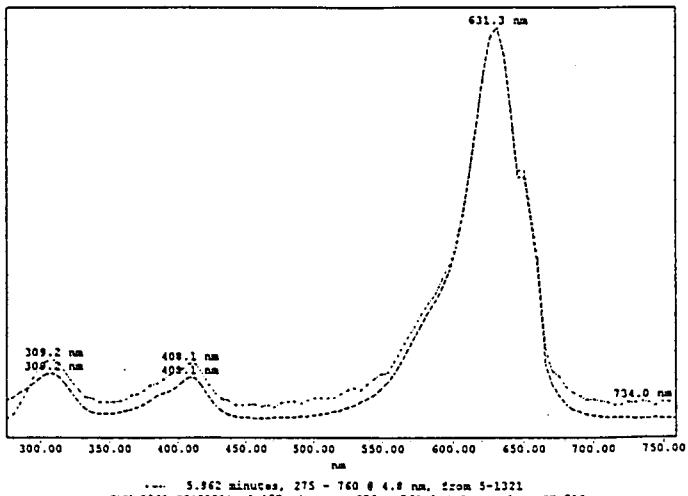
Spectral Table

#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	3.129	5-1321			On	Yes
2	4.222	2567_19140:1	DMU 2567_CI 19140:1	OPLSST I HPLC-SOLVENT	On	Yes

Spectral Table

#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	275	760	4.8	None	None	Off	432.2	0.14972
2	Yes	275	760	4.8	None	None	Off	432.2	0.80356

B



Spectral Table

#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable	Traceable
1	5.862	5-1321			On	Yes	Yes
2	9.177	ST.710	DMU 2568_CI42090*	Oplest 1 HPLC solvent	On	Yes	Yes

Spectral Table

#	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	275	760	4.8	None	None	Off	631.3	0.00403
2	275	760	4.8	None	None	Off	631.3	0.15352

As shown in Fig. 14, the t_R of the colourants in the HPLC of shampoos was altered, possibly due to interference of the surfactant in ion-pairing. Furthermore, the results also show large differences in the concentrations of the substances present in the sample compared to those of the reference colourants. The faintly coloured product with relatively low surfactant content (for example, skin tonic and mouth wash) were analysed after approximately 5x concentration. It was, however, also possible to identify colourants in some of the faint coloured samples by analysing upto 50 µl of the unconcentrated sample.
Thus, CI 45170 was identified in a pink skin tonic both when 10 µl of the 5x concentrated sample was analysed (Fig. 15) and also when 50 µl of the unconcentrated sample was analysed (Fig. 16). However, a method was required for the extraction/enrichment of the colours from the products with faint colours and thick consistency. A SPE method has been developed for the extraction of colourants from these products. As most of the commonly used surfactant can be adsorbed on C-18 silica, attempts were made to extract colourants from these products by SPE employing C-18 silica cartridges. From practical point of view, application of a sample solution (5 g sample suspended in 25 ml methanol) on 10 g C-18 silica was found to be suitable for the routine analysis. This procedure removed most, but not all, of the surfactant present in the sample. The colour extract, obtained by SPE was concentrated to approximately 4 ml, and that was analysed as reference colourants.

The presence of (small) amounts of surfactant in the concentrated samples (skin tonic, mouth washes) or concentrated colour extracts (from shampoos, etc.) may create problems with the analyses of the colourants by the present HPLC method which involves ion-pairing with TBAH. It appeared that the surfactant present in the sample extracts interfered with the ion-pairing of the colurants with TBAH. Thus, the colourants eluted from the coloumn had an altered t_R, and they also showed an additive spectrum with some matrix component (Fig. 17), similar to that described earlier in the case of a nail varnish. Even an additive spectrum of two colourants was observed in one case (Sample No. 5-1325, Fig. 18), In such cases, a manual search for the match of sample spectrum with the reference spectra in the spectral library was required. In Sample No. 5-1325 (Fig. 18) CI 18050 was identified by manual library search, but the identification of another possible colourant (λ_{max} 632.6 nm, possibly CI 42090) in the sample could not be performed.

In several cases of analysis of shampoo extracts, an additive spectrum of a (possible) surfactant and a colourant was observed, for example, Sample No. 5-1367 (Fig. 19). No attempt was made to deduct the spectrum of the surfactant from their additive spectra with the colourants, because many different types of surfactant are used in cosmetics and the identification of the surfactant used in a specific product will be an additional project.

Figure 15: Spectrum index plot obtained by the analysis of 10 µl of 5x concentrated skin tonic 5-1304. The spectrum of the samll peak with t_R 13.387 min matched with that of the reference colourant CI 45170.

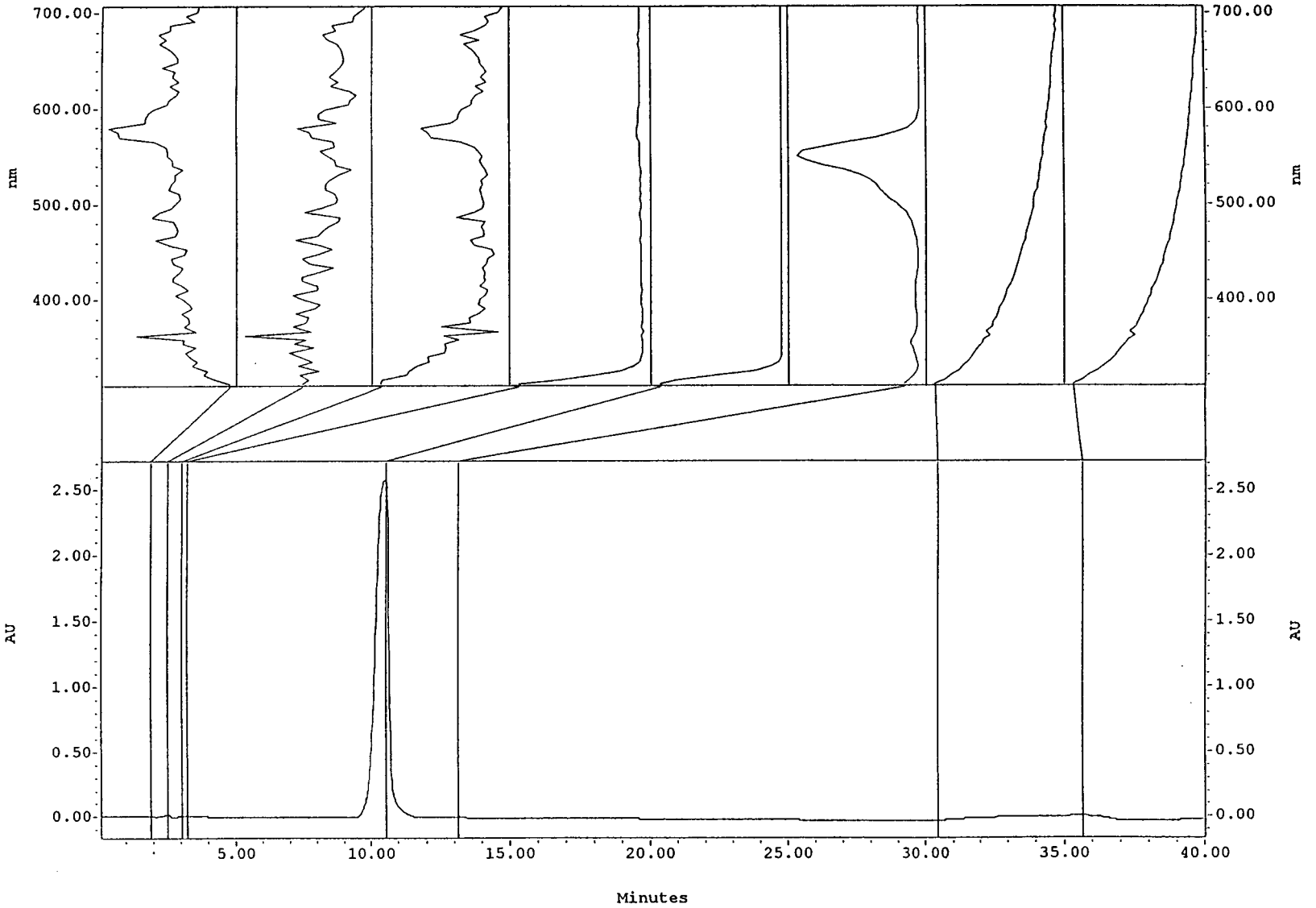


Figure 16: Spectrum index plot obtained by the analysis of 50 µl of unconcentrated skin tonic 5-1304. The spectrum of the samll peak with t_R 13.387 min matched with that of the reference colourant CI 45170.

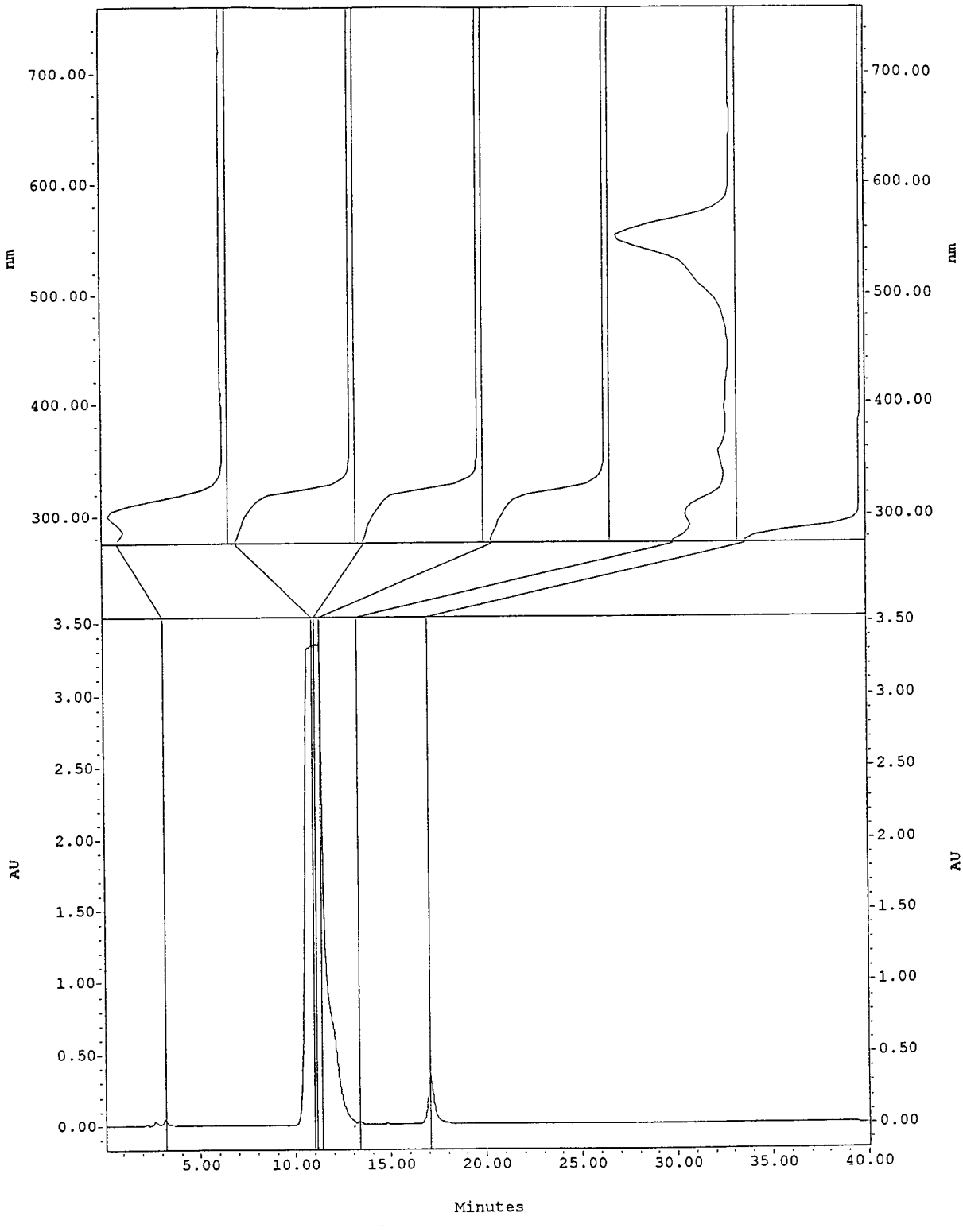


Figure 17: Analysis of the shampoo 5-1405. The product was labelled to contain CI 42090. The data acquisition was started from 310 nm to cut off the spectrum (λ_{max}) of the surfactant present in the sample. But still an additive spectrum of CI 42090, with some other matrix component(s), was obtained.

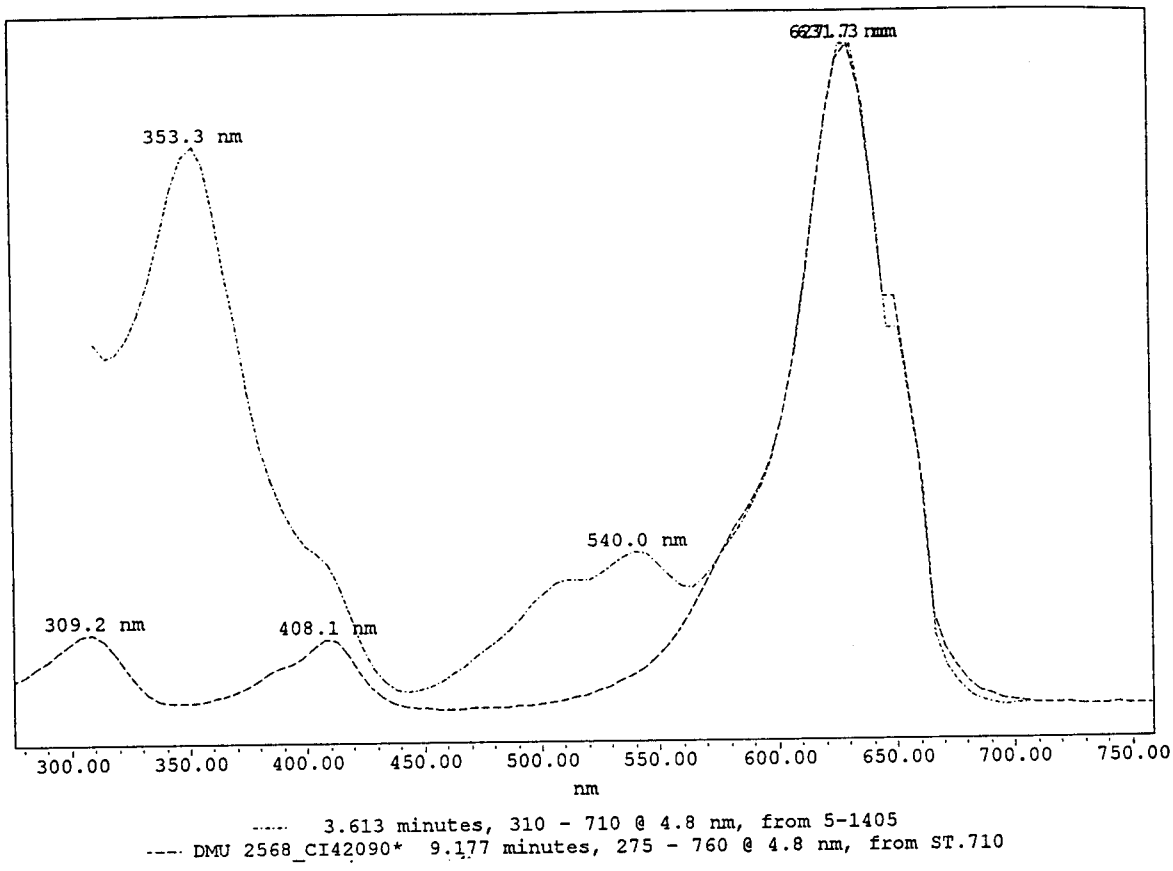
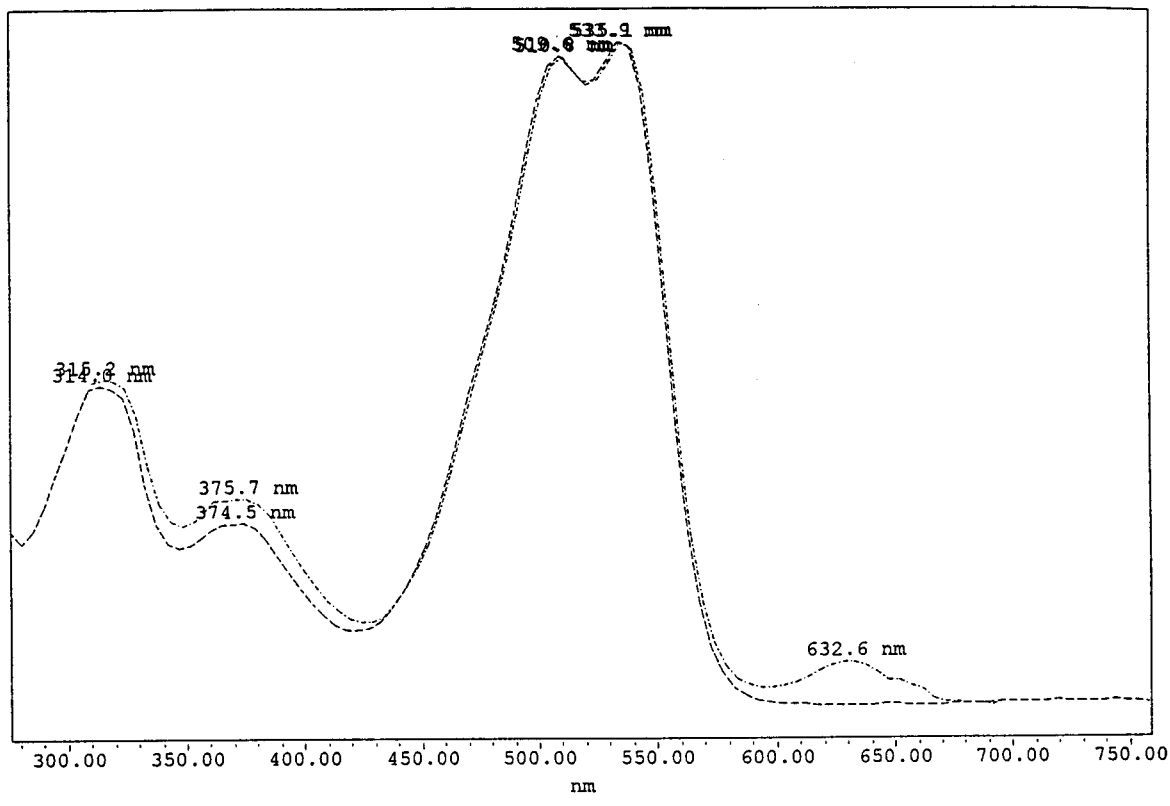


Figure 18: Analysis of a shampoo extract (Sample No. 5-1325) showing an additive spectrum of CI 18050 and (possibly) CI 42090. Only the identification of CI 18050 was confirmed.



Spectral Table

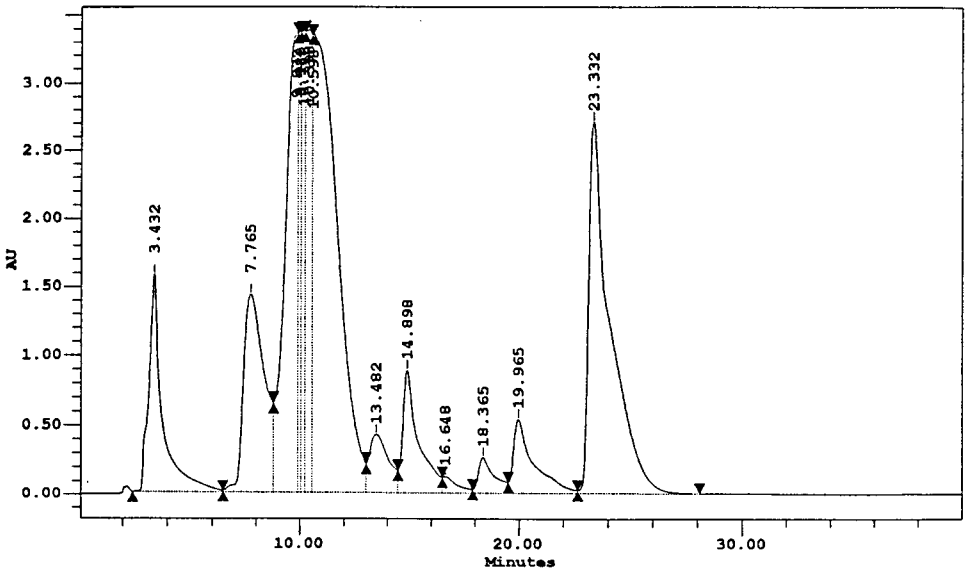
#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	3.752	5-1325			On	Yes
2	10.843	2639_18050	DMU 2639__CI 18050	OPLØST I HPLC-SOLVENT	On	Yes

Spectral Table

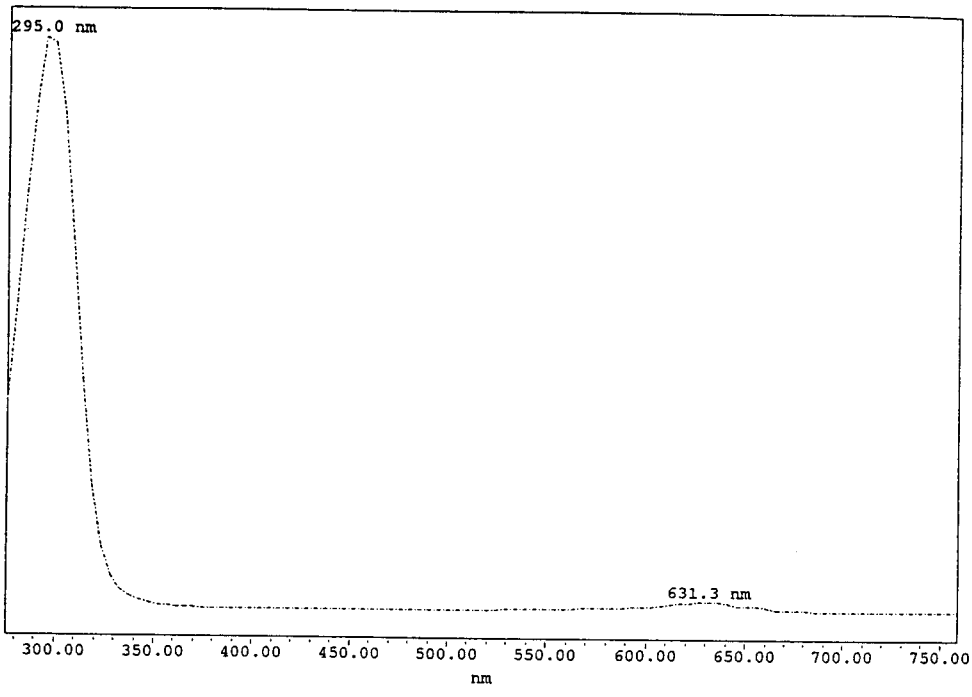
#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	310	710	4.8	None	None	Off	535.1	0.04734
2	Yes	275	760	4.8	None	None	Off	533.9	0.32930

Figure 19: Analysis of a blue shampoo (Sample No. 5-1367). The possible colourant in this sample, CI 42090, colluded with a surfactant (?) in the sample. A: max-plot chromatogram of the sample, B: spectrum of the substance(s) eluted in the chromatographic peak with t_R 3.432 min.

A



B



Spectral Table

#	Retention Time	Source	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution	Smooth
1	3.432	5-1367	On	Yes	Yes	275	760	4.8	None

Spectral Table

#	Derivative	Spline	Lambda Max	Maximum Absorbance
1	None	Off	295.0	1.57626

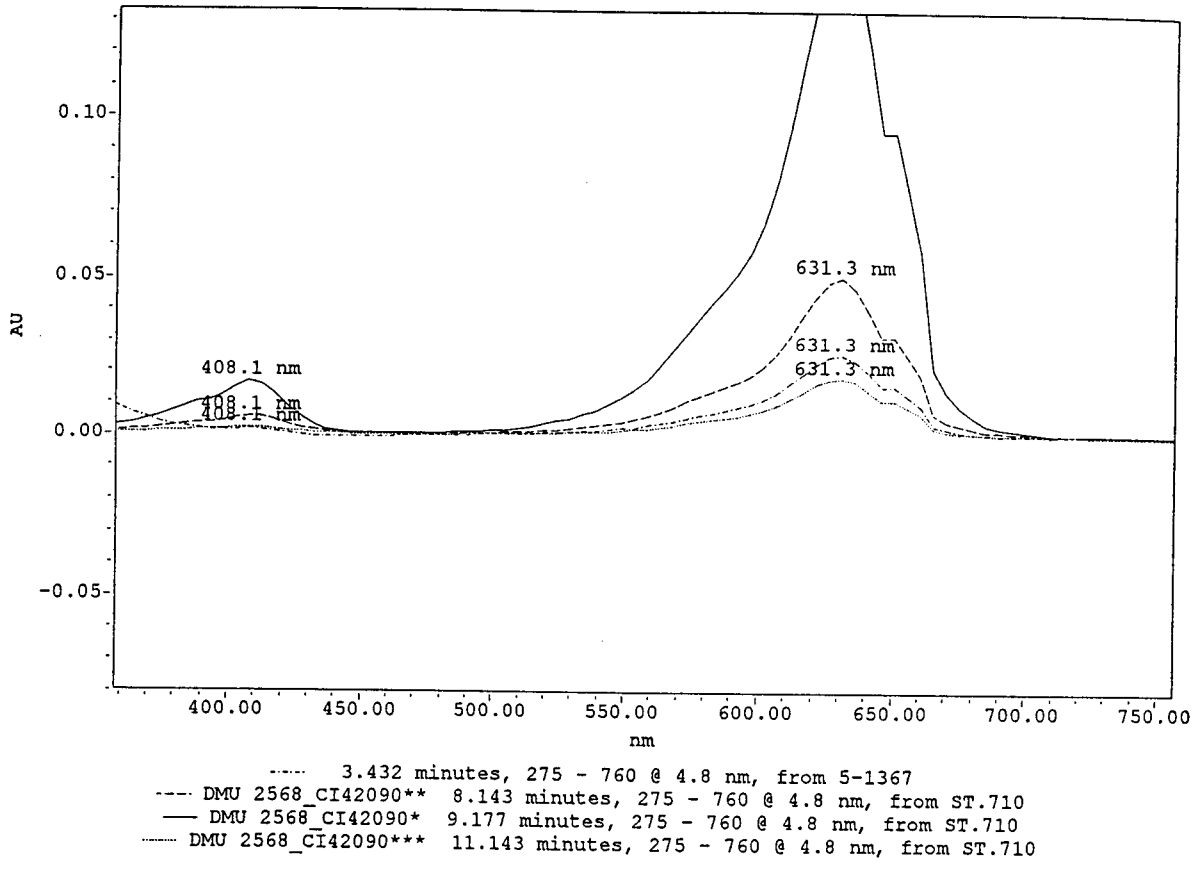
In a few cases, it was possible to remove some of the surfactant present in the colour extract by an additional SPE employing 0.5g C-18 silica cartridges during the sample preparation. Other ways for the identification of the colourants in these cases were: 1) limiting the wavelength range for the comparison of the spectrum of the colourant (+ the surfactant) with the reference spectrum in the library (Fig. 20), or 2) by setting the start wavelength for the data acquisition at 310 nm. However, the last mentioned is not always helpful, especially when a matrix component(s), besides a surfactant, also coelutes with a colourant (+ surfactant) (Fig. 21).

Besides the above mentioned, a few other practical problems may be encountered during the routine analysis of the colourants. One of the problems may be to discriminate between the colourants with similar/identical spectra as well as similar t_R, for example, CI 45425/CI 45430 and CI 45405/CI 45410. As all of these colours are permitted in cosmetics, no additional exercise may be required to check the compliance of a product with the Cosmetic Directive, with regard to colour content. Similarly, additional experiments may not be required to differentiate between various salts/lakes of specific colourants (for example, CI 15850:1/ CI 15850:2), unless a specific salt/lake of the colourant is not permitted. It was, however, possible by the present HPLC method to differentiate among salts/lakes of some of the colourants on the basis of their t_R, for example, CI 19140/CI19140:1 and CI 45170/CI 45170:1, CI 47005/CI 47005:1, and so on (see Appendix III).

Analysis of colourants in 48 lipstick and related products revealed that 93% of these products (lipsticks with various shades of red colour) contained CI 15850 (Table 6). Other frequently present colourants in the investigated samples were CI 15880, CI 15985, CI 19140/19140:1, CI 45380 and CI 45405/45410. Besides above mentioned, CI 17200, CI 45370, and CI 47005 were also identified in a few products. None of the investigated colourants were detected in the skin-colour lipstick. Three of the samples (Sample Nos. 5-1294, 5-1295, and 5-1380) were found to contain non-permitted colourants 45170/45170:1 and 15585. In the max-plot chromatograms of colour extracts of several lipstick samples, spectra of some chromatographic peaks (λ_{max} 417 nm - 558 nm) did not match with any of the spectra in the spectral library COSCOLOR (Fig 22). These peaks may represent certain colourants present in the products, but not investigated in the present study. These are described as other possible colourants in Table 6. For obvious reasons, it could not be decided whether they are the permitted colours.

The study included mainly red lipsticks of various shades because they are commonly used and also because we wished to investigate a possible association between a relatively high soluble Ba content with the presence of Ba-lakes of permitted colourants in these products. In Table 6 the content of soluble Ba in the investigated products are described together with the identified collourants.

Figure 20: Spectrum review demonstrating identification of CI 42090 in the sample 5-1367 by restricting the wavelength range for the spectrum match. The sample spectrum (chromatographic peak with t_R 3.432 min, Fig. 19) matched with the spectrum of the reference colourant CI 42090.



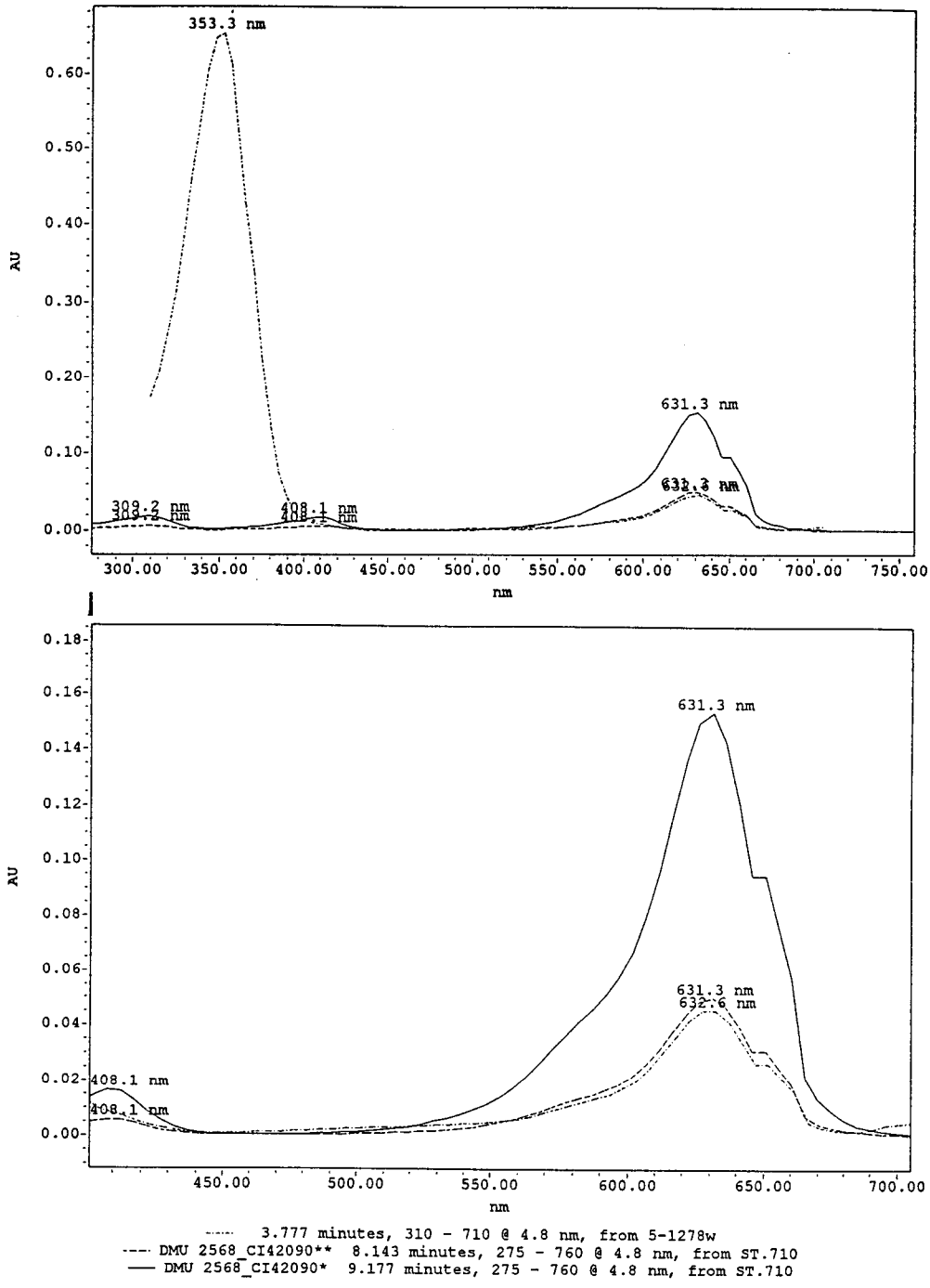
Spectral Table

#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	3.432	5-1367			On	Yes
2	8.143	ST.710	DMU 2568_CI42090**	Opløst i HPLC solvent	On	Yes
3	9.177	ST.710	DMU 2568_CI42090*	Opløst i HPLC solvent	On	Yes
4	11.143	ST.710	DMU 2568_CI42090***	Opløst i HPLC solvent	On	Yes

Spectral Table

#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	275	760	4.8	None	None	Off	295.0	1.58665
2	Yes	275	760	4.8	None	None	Off	631.3	0.05013
3	Yes	275	760	4.8	None	None	Off	631.3	0.15352
4	Yes	275	760	4.8	None	None	Off	631.3	0.01809

Figure 21: Identification of CI 42090 in a shampoo (Sample No. 5-1278W). To cut off the possible interference with the surfactant present in the sample, the data was-acquired from 310 nm. The colourant, however, colluded with a matrix component (λ_{max} 353.3 nm).



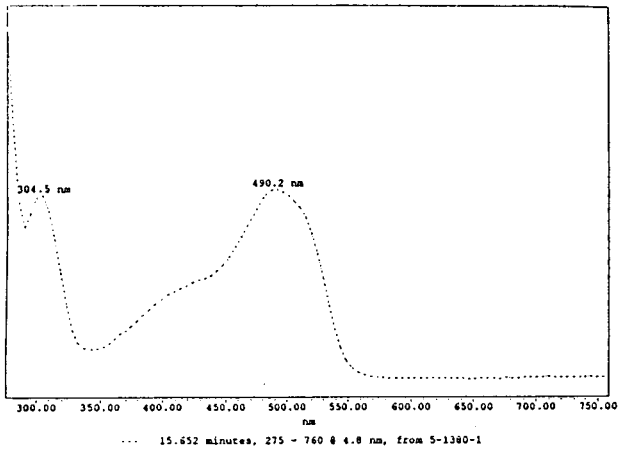
Spectral Table

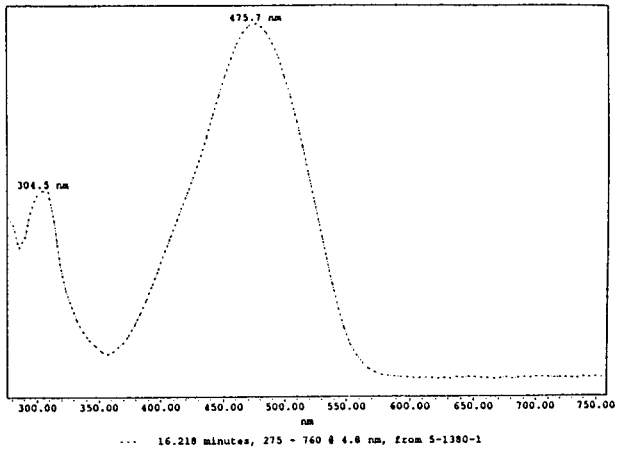
#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	3.777	5-1278w			On	Yes
2	8.143	ST.710	DMU 2568_CI42090**	Opløst i HPLC solvent	On	Yes
3	9.177	ST.710	DMU 2568_CI42090*	Opløst i HPLC solvent	On	Yes

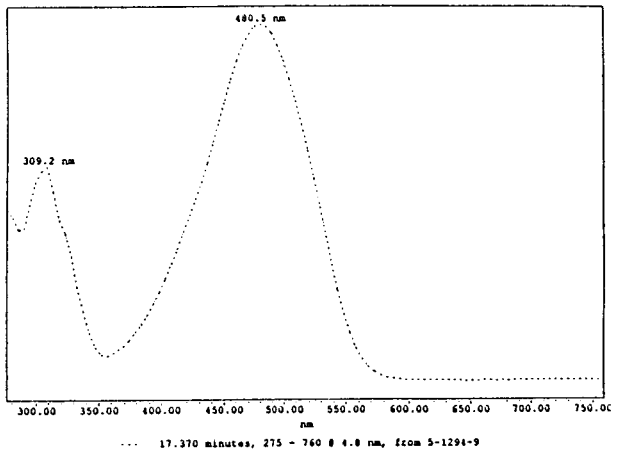
Spectral Table

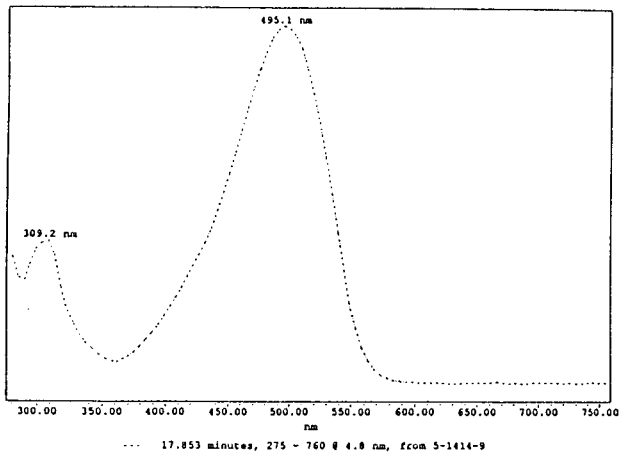
#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	310	710	4.8	None	None	Off	353.3	0.65271
2	Yes	275	760	4.8	None	None	Off	631.3	0.05013
3	Yes	275	760	4.8	None	None	Off	631.3	0.15352

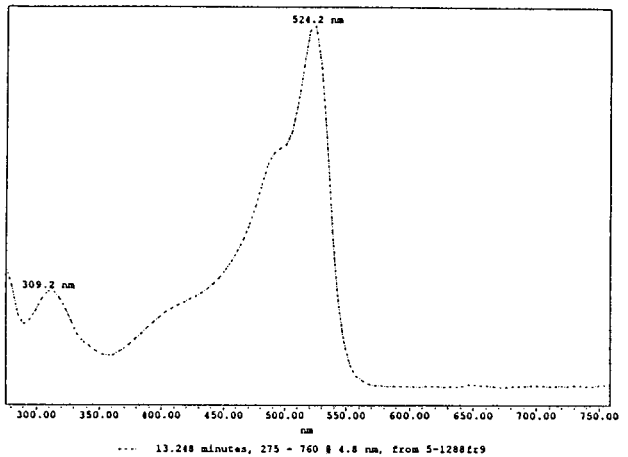
Figure 22: Spectra of some of the unidentified substances in lipsticks and related products, referred as possible colourants in Table 6.











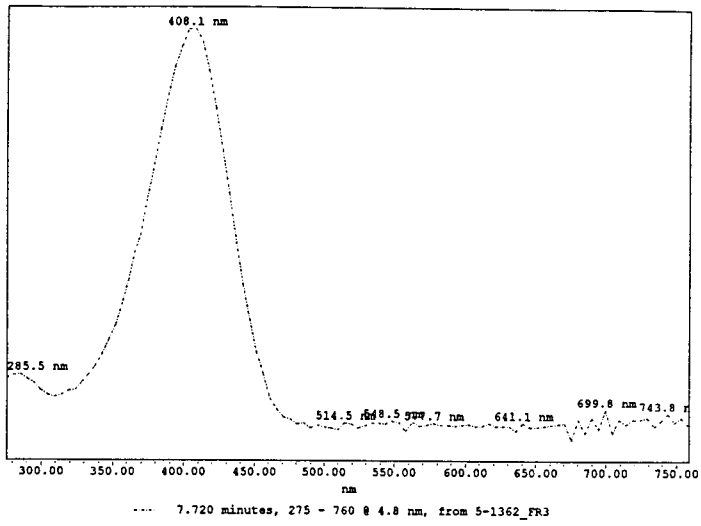
Soluble Ba content (analysed in an earlier study, see Ref. 36) in 28% of the investigated products was < 250 ppm, 250 ppm - 500 ppm soluble Ba in 28% of the products, 500 ppm - 1000 ppm soluble Ba in 8% of the products and >1000 ppm soluble Ba was present in 11% of the products. In general, pink and violet samples contained minimum soluble Ba. Apart from the skin colour lipstick, relatively low soluble Ba content were found in the samples which did not contain CI 15850: 8 ppm, 56 ppm and 62 ppm respectively in the samples 5-1379, 5-1378 and 5-1383. This may indicate an association between low content of soluble Ba and the absence of Ba-lake of CI 15850 in these products. In other words, relatively high concentration of soluble Ba in lipsticks may be associated with the presence of Ba-lakes of the colurants in these products. Relatively low soluble Ba in two of the products containing CI 15850, 47 ppm and 79 ppm respectively in the samples 5-1309 (pink) and 5-1314 (violet), may be due to presence of either low concentration of Ba-lake of the colourant or it may be due to the use of Ca-salt of the colourant in the formulation of these products. It was not possible to differentiate Ba-lake of CI 15850 from the Ca-salt of CI 15850 in the present investigation.

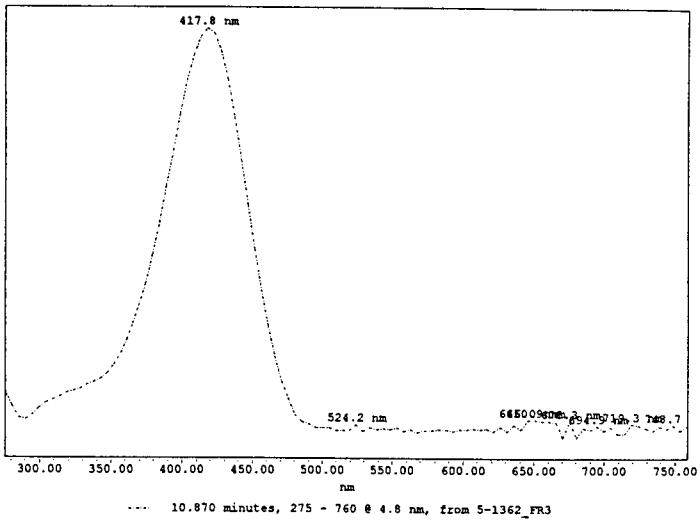
The colourants identified in nail varnishes (red, pink and violet) were CI 15850, CI 15880, CI 15585, CI 15865, CI 15630 and CI 19140/CI 19140:1 (Table 7). One of the products (Sample No. 5-1349) was found to contain a non-permitted colourant CI 15585. In the max-plot chromatogram of 79% (n = 15) of the investigated nail varnish samples (79%), spectra of some of the chromatographic peaks (λ_{max} 408nm - 495nm) did not match with the spectra of reference colourants in the spectral library COSCOLOR (Fig. 23). They are described as other possible colourants in Table 7.

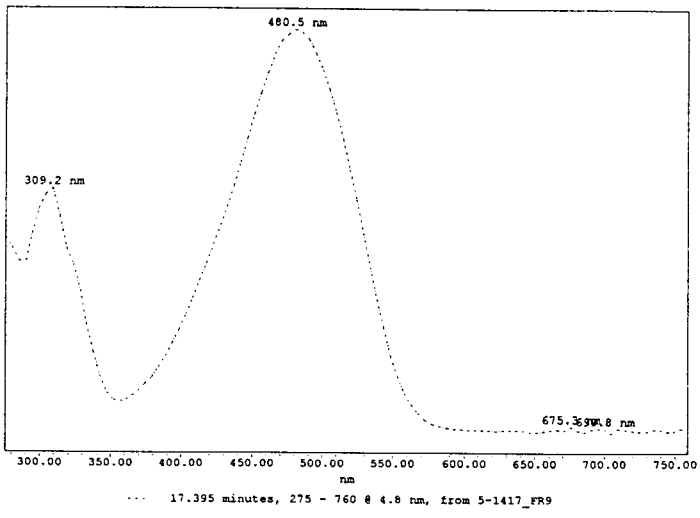
Among other investigated decoration cosmetics, a sample of dark blue eye shadow (Sample No. 5-1352) was found to contain CI 15850 (Table 8). The blue colour could not be extracted from the sample in organic solvents contain acid (sulphuric, hydrochloric up to 4 M) or base (sodium hydroxide 2 M). It was also not possible to extract colourant(s) from other samples of eye shadow, eye liner, eye pencil and mascara. in organic solvents containing acid/base Thus, it appears that inorganic pigments are used for the coloration of these decoration cosmetics.

As described above, a number of methods for the sample preparation were developed for the analysis of colourants in diverse cosmetics (shampoos, skin tonic, mouth wash etc). For routine analysis of colourants in these products, it was easy to decide the method that may be suitable for the analysis of colourants in a given product (see Appendix III). As described before, the application of two methods for the analysis of colourants in certain samples, for example, analysis of 10 µl of 5x concentrated a skin tonic (Sample No. 5-1304) or analysis of 50 µl of the same unconcentrated skin tonic revealed the presence of CI 45170 in the product. The same was true for the analysis of a concentrated or unconcentrated mouth wash product (Sample No. 5-1330); and for

Figure 23: Spectra of some of the unidentified substances in nail varnishes, referred as possible colourants in Table 7.







the analysis of 2 samples of strongly coloured shampoos (Sample Nos. 5-1284 & 5-1285) analysed after proper dilution or after extraction of colourants employing SPE. However, problems were encountered for the analysis of colourants in one of the 2 samples of aftershave investigated. Thus, the analysis of 10 µl of 5x concentrated aftershave (Sample No. 5-1303, yellow/green aftershave) revealed the presence of several chromatographic peaks with additive spectra (Fig. 24). As shown in Fig. 25, some of the possible colourants present in this sample may be CI 47005 and CI 42045 (Fig. 25). Identification of any colourant in this sample was not possible. In a red/blue toothpaste sample (Sample No. 5-1344, consisting of separate blue and red stribes), it was possible to identify only blue colour (Table 8). The red colour was not soluble under experimental conditions. It may be an inorganic pigment. A yellow bath oil product (Sample No. 5-1302B) was analysed without any treatment of the sample, because the sample could not concentrated to less than approximately 90%, and SPE methods for the extraction of the colourant from this sample did not work properly. None of the chromatographic peaks in the max-plot chromatogram of the sample (Fig. 26) revealed a spectrum that could be matched with the reference spectra in the library COSCOLOR. The yellow colourant present in this sample may be one of those which could not be analysed by the present method, or that we do not have in our stock.

The colourants identified in the investigated cosmetics as well as the colourants labelled on these products (only some of the products) are described in Table 8. There was good agreement between the results of the study and the labelled colourants. Some products were labelled to (may) contain several colours, but not all colours were identified in the respective samples. This is possible that cosmetic industry use the same label for a product formulation with various colours. In one of the samples, (faint blue shampoo, Sample No. 5-1327), the labelling identified the presence of the blue colourant CI 74100. However, as described before, this colour could not be analysed by the analytical method described in the present study. Three of the investigated samples of diverse cosmetics (Sample Nos. 5-1286, 5-1304 & 5-1402 were found to contain non-permitted colourants CI 42650/CI 45170/ CI 42555.

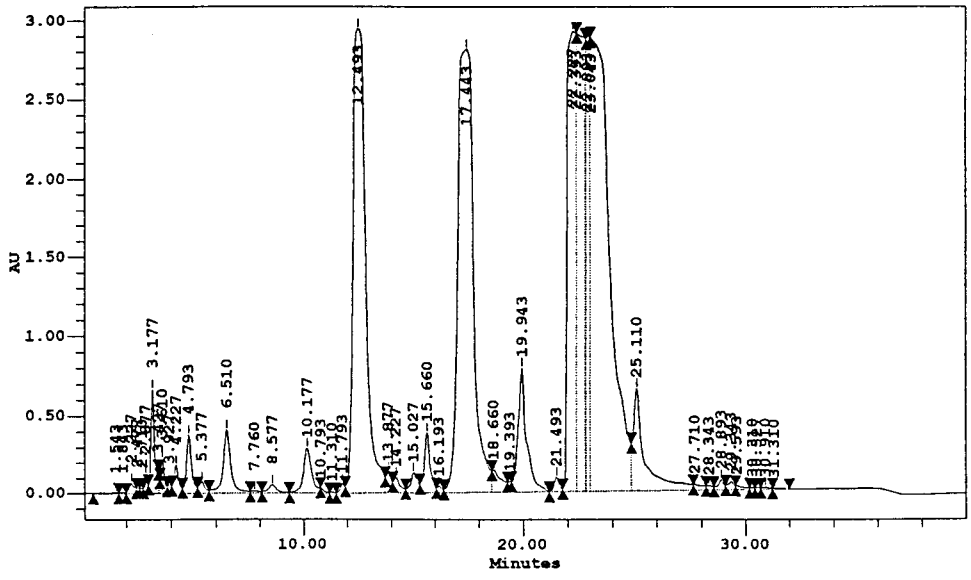
All of the natural ingredients based cosmetic products analysed in the present study (Sample Nos. 5-1281, 5-1284, 5-1285,5-1289, 5-1290, 5-1297, 5-1298 5-1299, 5-1300, 5-1301, 5-1302A, 5-1322 & 5-1324) were found to contain synthetic colours (Table 8). The worst cases of deception may be the bright green coloured products containing a synthetic blue and a synthetic yellow colour. In a previous study, we have shown that 35% of the natural ingredients based cosmetics contained synthetic perfumes (37). Synthetic chemicals, for example, surfactant, preservatives, sunscreens are also commonly used in the formulation of cosmetic products including the so called natural ingredients based cosmetics. These observations may raise the question about the designation of a cosmetic product as "based on natural ingredients".

All in all 30 organic colourants (26 permitted and 4 non-permitted) were identified in the 139 cosmetic products investigated in the present study.
Although, the study represents only 139 cosmetic samples, it is surprising that only 26 of the 128 permitted organic colourants were used in the formulation of these products. Therefore, attention should be paid to the list of permitted colourants, so that the colourants which are not used by the cosmetic industry any more should be removed from Annex IV of the Cosmetic Directive.

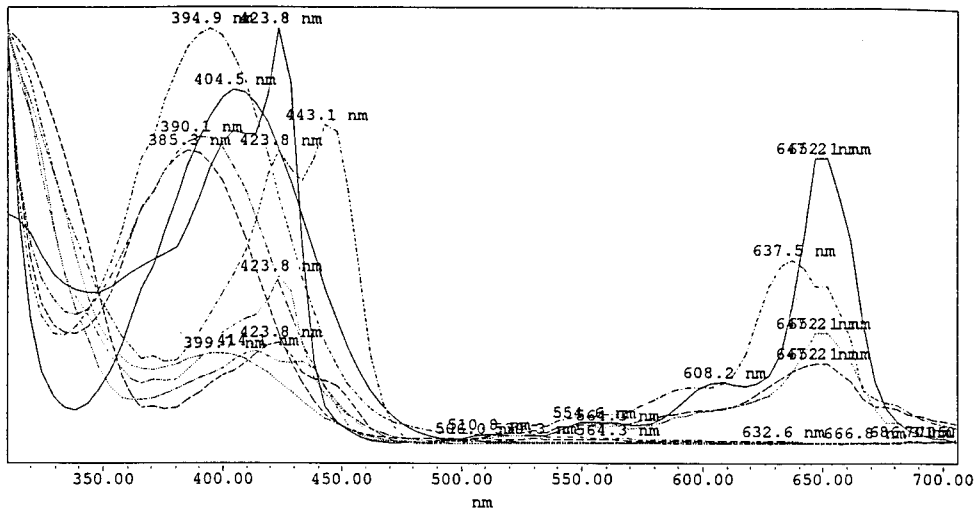
Seven of the investigated products (5%) contained non-permitted colourants. The Danish Environmental Protection Agency complained to the manufactures/importers of these 7 products. These products have been removed them from Danish Market.

Figure 24: Analysis of 10 µl of the 5x concentrated aftershave 5-1303. A: max-plot chromatogram, and B: spectrum review of some of the selected chromatographic peaks .

A

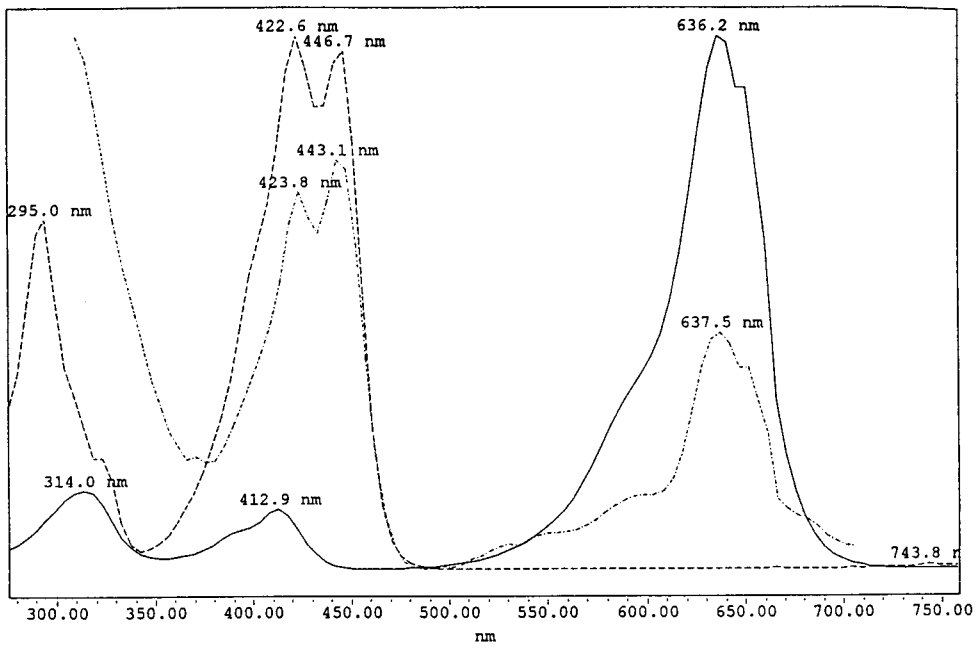


B



8.577 minutes, 310 - 710 @ 4.8 nm, from 5-1303
10.793 minutes, 310 - 710 @ 4.8 nm, from 5-1303
11.793 minutes, 310 - 710 @ 4.8 nm, from 5-1303
13.877 minutes, 310 - 710 @ 4.8 nm, from 5-1303
15.027 minutes, 310 - 710 @ 4.8 nm, from 5-1303
19.393 minutes, 310 - 710 @ 4.8 nm, from 5-1303
30.310 minutes, 310 - 710 @ 4.8 nm, from 5-1303
30.527 minutes, 310 - 710 @ 4.8 nm, from 5-1303
30.910 minutes, 310 - 710 @ 4.8 nm, from 5-1303
31.310 minutes, 310 - 710 @ 4.8 nm, from 5-1303

Figure 25: The possible colourant(s) in the sample 5-1303.



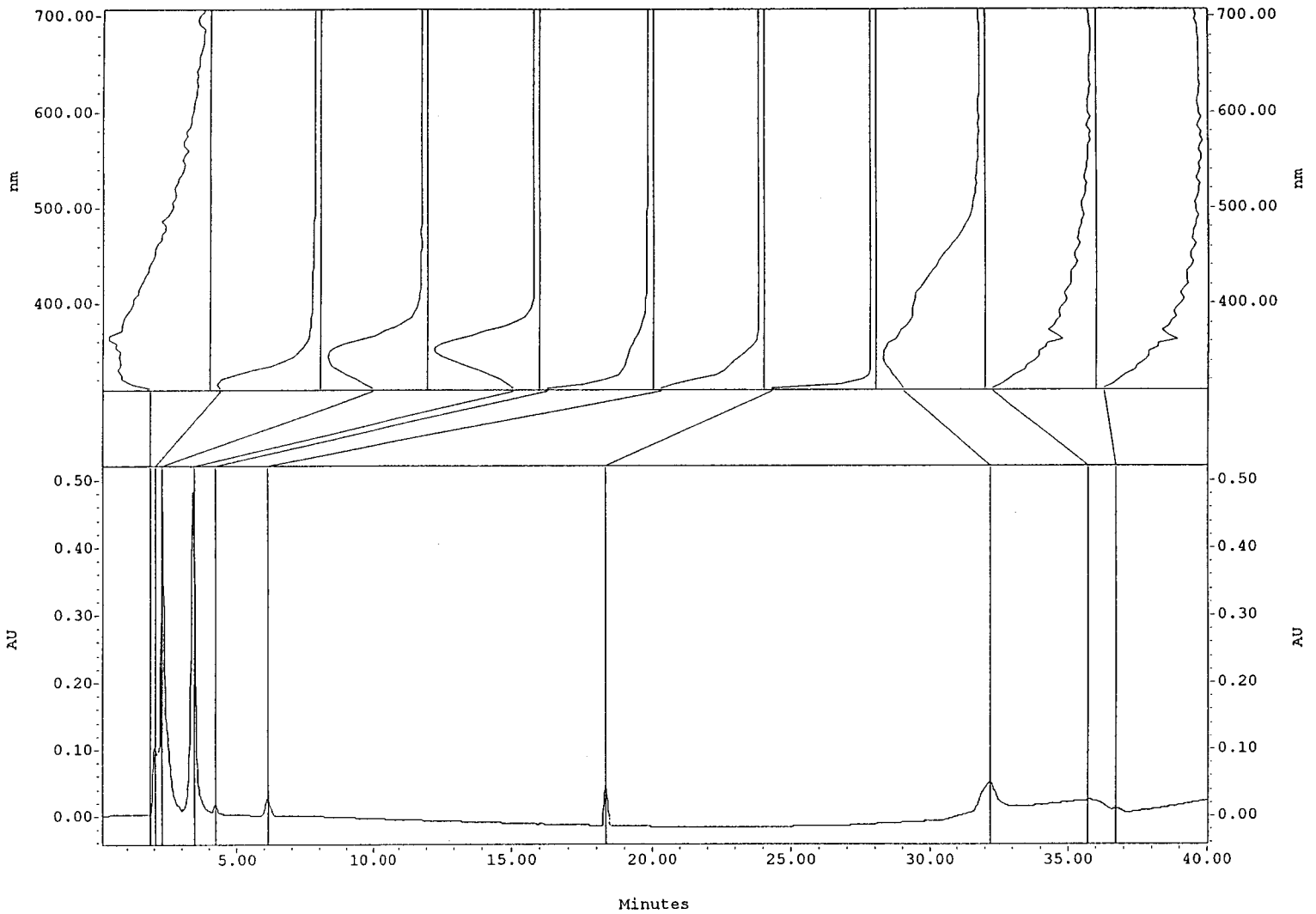
Spectral Table

#	Retention Time	Source	Spectrum Name	Spectrum Descript	Baseline Correct	Searchable
1	30.310	5-1303			On	Yes
2	6.130	2577_47005	DMU 2577__CI 47005***	OPLØST I HPLC-SOLVENT	On	Yes
3	7.885	2652_42045	DMU 2652__CI 42045	OPLØST I HPLC-SOLVENT	On	Yes

Spectral Table

#	Traceable	Start Wvln	End Wvln	Resolution	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	Yes	310	710	4.8	None	None	Off	310.4	0.01124
2	Yes	275	760	4.8	None	None	Off	422.6	0.05865
3	Yes	275	760	4.8	None	None	Off	636.2	0.33730

Figure 26: Spectrum index of the bath oil sample (Sample No. 5-1320B) analysed.



Millennium PDA Spectrum Index Plot - SampleName 5-1302b, maxplot_275_760 - PDA MaxPlot (275.0 nm to 760.0 nm)

Table 6: The identified/unidentified colourants and the content of soluble Ba in lipsticks/lipliners/lip gloss.

NERI Reg. no.	Colour of the product	Identified colourants	Other possible colourants t_R min	λ_{max} nm	Soluble Ba content (ppm)
5-1288	Red	C.I. 15985, C.I. 45370:1	13.242	524.2a	not determined
5-1289	Red	C.I. 15850, C.I. 19140, C.I. 19140:1	-		1283
5-1290	Red/brown	C.I. 15850, C.I. 45410:1/45405	-		718
5-1291	Red	C.I. 15850, C.I. 19140:1	-		1978
5-1292	Red	C.I. 15850	-		546
5-1293	Red	C.I. 15850	-		397
5-1294	Red	C.I. 15850, C.I. 15880:1, C.I. 19140:1, C.I. 45170, C.I. 45380	17.370	480.5a	378
5-1295	Red	C.I. 15850, C.I. 15585, C.I. 45170, C.I. 45380, C.I. 45410	-		427
5-1296	Red/brown	C.I. 15850	-		494
5-1297	Red/brown	C.I. 15850, C.I. 47005	-		680
5-1298	Red	C.I. 15850, C.I. 45410:1	-		1576
5-1305	Red	C.I. 15850	-		174
5-1306	Red	C.I. 15850, C.I. 15985	-		839
5-1307	Violet	C.I. 15850	-		142
5-1308	Red	C.I. 15850	-		417
5-1309	Red/Pink	C.I. 15850, C.I. 45410:1	-		47
5-1310	Skin colour	-	-		not detected
5-1311	Red/brown	C.I. 15850, C.I. 19140:1	-		416
5-1312	Red	C.I. 15850, C.I. 15985, C.I. 45410:1	-		383
5-1313	Red	C.I. 15850, C.I. 19140	-		1084
5-1314	Violet	C.I. 15850, C.I. 17200	-		79

t_R : HPLC-retention time
a : significant peak, absobance >0.02 AU
b : minor peak, absorbance <0.02 AU

Tabel 6: Continued.

NERI reg. no.	Colour of the product	Identified colourants	Other possible colourants t_R min	λ_{max} nm	Soluble Ba content (ppm)
5-1315	Red/brown	C.I. 15850, C.I. 19140:1	-		373
5-1316	Red	C.I. 15850	-		1088
5-1317	Brown	C.I. 15850	-		389
5-1351	Faint pink	C.I. 45380	-		not determined
5-1358	Red	C.I. 15850	15.712 16.262 18.878	490.2b 475.7a 543.6b	not determined
5-1359	Red	C.I. 15850, C.I. 19140, C.I. 19140:1	15.682 16.215	490.2b 475.7a	not determined
5-1360	Red	C.I. 15850, C.I. 15985, C.I. 19140, C.I 19140:1	15.680 16.213	490.2b 480.5a	not determined
5-1369	Red	C.I. 15985, C.I. 19140:1, C.I. 45410	13.238 19.000	519.3a 558.2a	964
5-1370	Red	C.I. 15850, C.I. 45370:1, C.I. 45410:1/45405	-		1159
5-1371	Red/pink	C.I. 15850, C.I. 15985	-		101
5-1372	Red	C.I. 15850, C.I. 19140 C.I. 45410:1/45405	18.967	558.2a	519
5-1373	Red/orange	C.I. 15850, C.I. 15985	-		640
5-1374	Skin colour/red	C.I. 15850, C.I. 19140	-		421
5-1375	Red	C.I. 15850, C.I. 19140:1, C.I. 45410:1/45405	16.098	548.5a	715
5-1376	Red	C.I. 15510, C.I. 15850, C.I.15880:1, C.I. 19140:1	-		1934
5-1377	Red/pink	C.I. 15850, C.I. 45410:1	-		202
5-1378	Violet	C.I. 45380, C.I. 45405	-		56
5-1379	Red/pink	C.I. 19140:1, C.I. 45380, C.I. 45410:1	-		8
5-1380	Red	C.I. 15585, C.I.15850, C.I. 15985	15.652 16.218	490.2a 475.7a	2104
5-1381	Red/pink	C.I. 15850, C.I.17200, C.I. 45410:1	-		339
5-1382	Red	C.I. 15850, C.I. 19140:1	-		1540

t_R : HPLC-retention time
a : significant peak, absorbance >0.02 AU
b : minor peak, absorbance <0.02 AU

Tabel 6: Continued.

NERI reg. no.	Colour of the product	Identified colourants	Other possible colourants t_R min	λ_{max} nm	Soluble Ba content (ppm)
5-1383	Violet	C.I. 15880:1, C.I. 45410:1	-		62
5-1384	Red	C.I. 15850, C.I. 15985, C.I. 19140:1	17.870	495.1a	1764
5-1385	Red	C.I. 15850, C.I. 45405, C.I. 47005	12.083	417.8b	1868
5-1386	Violet	C.I. 15850,	-		315
5-1414	Red	C.I. 15850, C.I. 45405/45410:1	17.853	495.1a	243
5-1415	Red	C.I. 15850	-		148

t_R : HPLC-retention time
a : significant peak, absobance >0.02 AU
b : minor peak, absorbance <0.02 AU

Table 7: Identification of colourants in nail varnishes.

NERI reg. nr.	Colour of the product	Identified colourants	Other possible colourants $t_{R\,(min)}$	λ_{max} (nm)
5-1281	Faint violet	C.I. 15850	7.643 10.876	408.1 417.8
5-1282	Red	C.I. 15850	-	
5-1283	Violet	C.I. 15880:1	10.710 16.705	417.8 432.2
5-1346	Red	C.I. 15850	-	
5-1349	Red/violet	C.I. 15585, C.I. 15630, C.I. 15850, C.I. 15865:3	10.668 17.645	417.8 475.7
5-1361	Pink	C.I. 15850, C.I. 15880:1	10.598	417.8
5-1362	Violet	C.I. 15880:1	7.724 10.870 17.390	408.1 417.8 480.5
5-1363	Red	C.I. 15850, C.I. 19140:1	-	
5-1364	Faint violet	C.I. 15850, C.I. 15985	10.877	417.8
5-1365	Red/violet	C.I. 15850, 15880:1	10.712 17.428	417.8 480.5
5-1366	Red/orange	C.I. 15850	15.033 15.683 16.267	475.7 485.4 495.1
5-1387	Orange	C.I. 15850, C.I. 19140, C.I. 19140:1	10.585	417.8
5-1388	Pink	C.I. 15850, C.I. 15880:1	10.742 14.006 17.423	417.8 432.2 480.5
5-1390	Pink	C.I. 15850, C.I. 15880:1	10.900	417.8
5-1391	Orange	C.I. 15850, C.I. 19140, C.I. 19140:1	7.573 17.673 19.79	408.1 480.5 441.9
5-1393	Dark violet/-brown	C.I. 15850	10.963 11.080 14.347	417.8 427.4 441.9
5-1394	Orange	C.I. 15850, C.I. 19140	-	
5-1416	Pink	C.I. 15850, C.I. 19140:1	10.738	417.8
5-1417	Faint violet	C.I. 15850, C.I 15880:1	17.395	480.5

Table 8: Identification of colourants in diverse cosmetics and toiletries.

NERI reg. no.	Product category	Product-Colour	SP* Method	Colourants Identified	Colourants labled	Remarks
5-1278 Spring	Shampoo	Green	6.3.1	C.I. 15510 C.I. 42090	C.I. 15510 C.I. 42090	-
5-1278 Summer	Shampoo	Faint red	6.3.1	C.I. 16035	C.I. 16035	-
5-1278 Autumn	Shampoo	Orange	6.3.1	C.I. 15510 C.I. 16035	C.I. 15510 C.I. 16035	-
5-1278 Winter	Shampoo	Violet	6.3.1	C.I. 16035 C.I. 42090	C.I. 16035 C.I. 42090	-
5-1284	Shampoo	Dark green	6.3.1 & 6.3.2	C.I. 13015 C.I. 42090	None	-
5-1285	Shampoo	Dark red/ violet	6.3.1 & 6.3.2	C.I. 42735, C.I. 13015	None	+ Unknown (red?)
5-1286	Liquid soap	Violet	6.3.1	C.I. 42650	none	-
5-1287	Shampoo	Green	6.3.1	C.I. 47005 C.I. 42090	C.I. 47005 C.I. 42090	-
5-1299	Shampoo	Yellow	6.3.1	C.I. 19140	FD & C yellow 5	-
5-1300	Shampoo	Green	6.3.1	C.I. 19140 C.I. 42090	FD&C yellow 5, FD & C blue 1	-
5-1301	Skin tonic	Orange	6.3.4	C.I. 19140 C.I. 14700	none	-
5-1302A	Shampoo	Orange	6.3.1	C.I. 19140	none	-
5-1302B	Bath oil	Yellow	6.3.1 & 6.3.4	-	none	Could not be eluted from SEP-Pak column
5-1303	After-shave	Yellow	6.3.4	-	none	C.I. 47005? C.I. 42045?
5-1304	Skin tonic	Pink/ faint red	6.3.1 & 6.3.4	C.I. 45170	none	-
5-1319	Roll-on	Pink/ faint red	6.3.1 & 6.3.2	C.I. 14700 C.I. 28440	none	-
5-1320	Roll-on	Faint Violet	6.3.6	C.I. 42090 C.I. 45100	none	-
5-1321	Shampoo	Green	6.3.1	C.I. 19140:1 C.I. 42090	none	-
5-1322	Cream	Pink/ violet	6.3.3	C.I. 16255 C.I. 42090	none	-
5-1323	Cream	Light red	6.3.3	C.I. 14700	C.I. 14700 C.I. 47005	47005 in another product?
5-1324	Shampoo	Green	6.3.1	C.I. 19140 C.I. 42090	none	-

* SP method: sample preparation method (cf. Appendix II)

Table 8: Continued.

NERI reg. no.	Product category	Colour	SP* Method	Colourants Identified	Colourants labled	Remarks
5-1325	Shampoo	Faint red/ pink	6.3.1	C.I. 18050	none	+ C.I. 42090?
5-1326	Shampoo	Blue	6.3.1	C.I. 42090	C.I. 42090	-
5-1327	Shampoo	Blue	6.3.1	Could not be eluted from Sep-Pak column	C.I. 12490 C.I. 74100 C.I. 18050	Faint colour, SP* method 6.3.2 not applicable
5-1328	Shampoo	Yellow	6.3.1	C.I. 47005	none	-
5-1329	Mouth wash	Blue	6.3.4	C.I. 42090	none	-
5-1330	Mouth wash	Blue	6.1 & 6.3.1	C.I. 42051	none	-
5-1331	Shampoo	Red/ orange	6.3.1 & 6.3.2	C.I. 13015	none	+ Unknown (red?)
5-1332	Shampoo	Violet	6.3.1	C.I. 16255 C.I. 42090	none	-
5-1333	Shampoo	Yellow	6.3.1	C.I. 15985	none	-
5-1334	Shampoo	Blue	6.3.2	C.I. 42090	C.I. 42090 C.I. 47005	C.I. 47005 in another product?
5-1335	Shampoo	Orange	6.3.1 & 6.3.2	C.I. 15510	C.I. 15510	-
5-1336	Shampoo	Blue	6.3.1	C.I. 42090	Blue 1	-
5-1337	Skin tonic	Blue	6.3.1	C.I. 42090	none	-
5-1338	Shampoo	Yellow	6.3.1	C.I. 19140	none	-
5-1339	Toothpaste and mouth wash	Green	6.3.1	C.I. 42090 C.I. 47005	none	-
5-1340	Shampoo	Orange	6.3.1	C.I. 15510	none	-
5-1341	Shampoo	Blue	6.3.1	C.I. 42051	none	-
5-1342	Skin tonic	Blue	6.3.4	C.I. 42045 C.I. 42051	none	-
5-1343	Skin tonic	Faint red	6.3.4 6.3.4	C.I. 17200	none	-
5-1344	Toothpaste	Blue, red and white stribes	6.3.4	C.I. 42090	none	Insoluble red
5-1352	Eye shadow	Deep blue	6.2	C.I. 15850	none	Insoluble blue

* SP: sample preparation method (cf. Appendix II)

Table 8: Continued.

NERI reg. no.	Product category	Colour	SP* Method	Colourants Identified	Colourants labled	Remarks
5-1367	Shampoo	Blue	6.3.1	C.I. 42090	none	-
5-1368	Shampoo	Green	6.3.1	C.I. 15985 C.I. 42090	none	-
5-1397	Shampoo	Yellow	6.3.1 & 6.3.2	C.I. 47005	C.I. 47005	-
5-1398	Shampoo	Blue/ green	6.3.1	C.I. 42053	C.I. 42053	-
5-1399	Shampoo	Yellow	6.3.1	C.I. 15510	C.I. 15510	-
5-1400	Shampoo	Orange	6.3.1	C.I. 15985	C.I. 15985 C.I. 42051	C.I. 42051 in another product?
5-1401	Shampoo	Orange	6.3.1	C.I. 19140 C.I. 16255	none	-
5-1402	Shampoo	Violet	6.3.1	C.I. 42555	none	-
5-1404	Shampoo	Light red	6.3.1	C.I. 45100	none	-
5-1405	Shampoo	Blue	6.3.1	C.I. 42090	C.I. 42090 C.I. 18050	C.I. 18050 in another product?
5-1407	Cream	Yellow/ orange	6.3.3	C.I. 19140 C.I. 14700	none	-
5-1408	Roll-on	Faint red	6.3.3	C.I. 16035	none	-
5-1409	After-shave	Green	6.3.4	C.I. 20170 C.I. 42090	none	-
5-1410	Skin tonic	Blue	6.3.4	C.I. 42051	C.I. 42051	-
5-1411	Skin tonic	Light red	6.3.4	C.I. 16035	none	-
5-1412	Gele Spray	Violet	6.3.4	C.I. 18050 C.I. 42735	C.I. 18050 C.I. 42735	-
5-2773	Skin tonic	Faint red/ pink	6.3.4	C.I. 17200	none	-

* SP: sample preparation method (cf. Appendix II)

6 References

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Appendix I

Colourants which are permitted in the formulation of cosmetic products according to Annex IV of the Cosmetic Directive

Anvendelsesområde:
Kolonne 1: Farvestoffer tilladt i alle kosmetiske produkter.
Kolonne 2: Farvestoffer tilladt i alle kosmetiske produkter med undtagelse af kosmetiske produkter til anvendelse omkring øjnene, navnlig øjenmake-up og rensemidler hertil.
Kolonne 3: Farvestoffer, der udelukkende er tilladt i kosmetiske produkter, som ikke er bestemt til at komme i berøring med slimhinderne.
Kolonne 4: Farvestoffer, der udelukkende er tilladt i kosmetiske produkter, som er bestemt til kun at komme i kortvarig berøring med huden.

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
10006	16143-80-9	grøn				x	
10020	19381-50-1	grøn			x		
10316[4]	846-70-8	gul		x			
11680	2512-29-0	gul			x		
11710	6486-23-3	gul			x		
11725	6371-96-6	orange				x	
11920	2051-85-6	orange	x				
12010	6535-42-8	rød			x		
12085[4]	2814-77-9	rød	x				maksimalt 3% i det færdige produkt
12120	2425-85-6	rød				x	
12370	6535-46-2	rød				x	
12420	6471-51-8	rød				x	
12480	6410-40-8	brun				x	
12490	6410-41-9	rød	x				
12700	4314-14-1	gul				x	
13015	2706-28-7	gul	x				E 105
14270	547-57-9	orange	x				E 103
14700	4548-53-2	rød	x				
14720	3567-69-9	rød	x				E 122
14815	3257-28-1	rød	x				E 125
15510[4]	633-96-5	orange		x			
15525	5850-80-6	rød	x				

Farve: Colourant, Farveindeksnummer: Colour Index No.,
Anvendelsesområde: Application Area (see also Introduction).

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
15580	5850-87-3	rød	x				
15620	1568-56-0	rød			x		
15630[4]	1248-18-6	rød	x				maksimalt 3% i det færdige produkt
15800	6371-76-2	rød			x		
15850[4]	5858-81-1	rød	x				
15865[4]	15782-05-5	rød	x				
15880	6417-83-1	rød	x				
15980	2347-72-1	orange	x				E 111
15985[4]	15790-07-5	gul	x				E 110
16035	25956-17-6	rød	x				
16185	915-67-3	rød	x				E 123
16230	1936-15-8	orange			x		
16255[4]	2611-82-7	rød	x				E 124
16290	5840-44-2	rød	x				E 126
17200[4]	3567-66-6	x					
18050	3734-67-6		x				
18130	10236-37-0	rød			x		
18690	5601-29-6	gul			x		
18736	6408-26-0	rød			x		
18820	6359-82-6	gul			x		
18965	6359-98-4	gul	x				
19140[4]	12225-21-7	gul	x				E 102
20040	5979-28-2	gul				x	maksimalt 5 ppm 3,3'-dimethylbenzidin i farvestoffet
20170	1320-07-6	orange			x		
20470	1064-48-8	sort				x	
21100	5102-83-0	gul				x	maksimalt 5 ppm 3,3'-dichlorbenzidin i farvestoffet
21108	5567-15-7	gul				x	do
21230	6706-82-7	gul			x		
24790	13421-53-9	rød				x	

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
26100	*85-86-9*	*rød*			*x*		Renhedskriterier: anilin ≤ 0,2% 2-naphtol ≤ 0,2% 4-aminoazobenzen ≤ 0,1% 1-(phenylazo)-2-naphtol ≤ 3% 1-(2-(phenylazo)phenylazo)-2-naphtol ≤ 2%
27290[4]	5413-75-2	rød				x	
27755	2118-39-0	sort	x				E 152
28440	2519-30-4	sort	x				E 151
40215	1325-54-8	orange				x	
40800	7235-40-7	orange	x				
40820	12676-20-9	orange	x				E 160 e
40825	11091-11-1	orange	x				E 160 f
40850	514-78-3	orange	x				E 161 g
42045	129-17-9	blå			x		
42051[4]	3536-49-0	blå	x				E 131
42053	2353-45-9	grøn	x				
42080	3486-30-4	blå				x	
42090	3844-45-9	blå	x				
42100	4857-81-2	grøn				x	
42170	5863-51-4	grøn				x	
42510	632-99-5	violet			x		
42520	3248-91-7	violet				x	maksimalt 5 ppm i det færdige produkt
42735	6505-30-2	blå			x		
44045	2580-56-5	blå			x		
44090	3087-16-9	grøn	x				E 142
45100	3520-42-1	rød				x	
45190	6252-76-2	violet				x	
45220	5873-16-5	rød				x	
45350	2321-07-5	gul	x				maksimalt 6% i det færdige produkt
45370[4]	596-03-2	orange	x				maksimalt 1% fluorescein og 2% monobromfluorescein
45380[4]	17372-87-1	rød	x				do

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
45396		orange	x				I læbestifter må farvestoffet kun anvendes i form af fri syre med en koncentration på højst 1%
45405	6441-77-6	rød		x			maksimalt 1% fluorescein og 2% monobromfluorescein
45410[4]	13473-26-2	rød	x				do
45425	38577-97-8	rød	x				maksimalt 1% fluorescein og 3% monojodfluorescein
45430[4]	16423-68-0	rød	x				E 127 do
47000	8003-22-3	gul			x		
47005	8004-92-0	gul	x				E 104
50325	6837-46-3	violet				x	
50420	8005-03-6	sort			x		
51319	6358-30-1	violet				x	
58000	72-48-0	rød	x				
59040	6358-69-5	grøn			x		
60724	19286-75-0	violet				x	
60725	81-48-1	violet	x				
60730	4430-18-16	violet			x		
61565	128-80-3	grøn	x				
61570	4403-90-1	grøn	x				
61585	4474-24-2	blå				x	
62045	4368-56-3	blå				x	
69800	81-77-6	blå	x				E 130
69825	130-20-1	blå	x				
71105	4424-06-0	orange			x		
73000	482-89-3	blå	x				
73015	16521-38-3	blå	x				E 132
73360	2379-74-0	rød	x				
73385	5462-29-3	violet	x				
73900	1047-16-1	violet				x	
73915	980-26-7	rød				x	

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
74100	574-93-6	blå				x	
74160	147-14-8	blå	x				
74180	1330-38-7	blå				x	
74260	1328-53-6	grøn		x			
75100	42553-65-1	gul	x				
75120	6983-79-5	orange	x				E 160 b
75125	502-65-8	gul	x				E 160 d
75130	7488-99-5 7235-40-7 472-93-5 7488-99-5	orange	x				E 160 a
75135	79-75-4	gul	x				E 161 d
75170	73-40-5	hvid	x				
75300	458-37-7	gul	x				E 100
75470	1260-17-9	rød	x				E 120
75810	8049-84-1	grøn	x				E 140 og E 141
77000	7429-90-5	hvid	x				E 173
77002	1332-73-6 21645-51-2	hvid	x				
77004	8047-76-5	hvid	x				
77007	57455-37-5	blå	x				
77015	1309-37-1	rød	x				
77120	7727-43-7	hvid	x				
77163	7787-59-9	hvid	x				
77220	471-34-1	hvid	x				E 170
77231	10101-41-4	hvid	x				
77266	1333-86-4 7440-44-0	sort	x				
77267	8021-99-6	sort	x				
77268:1	1345-12-6	sort	x				E 153
77288	1308-38-9	grøn	x				fri for chromation
77289	1324-77-2	grøn	x				fri for chromation
77346	1345-16-0	grøn	x				
77400	7440-50-8	brun	x				
77480	7440-57-5	brun	x				E 175
77489	1345-25-1	orange	x				E 172
77491	1309-37-1	rød	x				E 172

Løbenummer Farveindeksnummer benævnelse [3]	CAS-nummer	Farve	Anvendelsesområde				Øvrige begrænsninger og krav[2]
			1	2	3	4	
77492	20344-49-4	gul	x				E 172
77499	12227-89-3	sort	x				E 172
77510	14038-43-8	blå	x				fri for cyanodion
77713	546-93-0	hvid	x				
77742	10101-66-3	violet	x				
77745	10124-54-6	rød	x				
77820	7740-22-4	hvid	x				E 174
77891 (og præparater heraf med glimmer)	13463-67-7	hvid	x				E 171
77947	1314-13-2	hvid	x				
Lactoflavin	33-88-5	gul	x				E 101
Karamel	8028-89-5	brun	x				E 150
Capsanthin capsorubin	465-42-9 470-38-2	orange	x				E 160 c
Rødbedefarve betaniner	107-43-7	rød	x				E 162
Anthocyaner		rød	x				E 163
Aluminium, zink-, magnesium- og calsiumstearater							
Bromthymolblåt	76-59-5	blå				x	
Bromcresolgrønt	76-60-8	grøn				x	
Acid red 195	12220-24-5	rød		x			

(1) Ligeledes tillades de nævnte farvestoffers lakker eller salte, som indeholder stoffer, hvis anvendelse ikke er forbudt i medfør af bilag 2.

(2) Når bogstavet "E" i medfør af bestemmelserne i EØF-direktiverne af 1962 om levnedsmidler og farvestoffer er anbragt foran farvestoffets nummer, skal farvestoffet opfylde de i disse direktiver anførte renhedskrav. Hvor bogstavet "E" udgår i denne bekendtgørelse, gælder fortsat de almindelige kriterier i bilag III til direktiv af 1962 om farvestoffer.

(3) Farveindeksnummeret refererer til værket Rowe Index, 3. udgave, Society of Dyers and Colourists, Bradford, England, 1979.

(4) Ligeledes tillades uopløselige barium-, strontium- og zirkoniumlakker, -pigmenter og -salte af disse farvestoffer, såfremt de består en uopløselighedsprøve baseret på en i EF vedtaget metode.

The colourants which are not permitted for the use in cosmetic formulations, according to the Annex II of the Cosmetic Directive

CI 12140
CI 13065
CI 15585
CI 26105
CI 42535
CI 42555
CI 42555-1
CI 42555-2
CI 42640
CI 45170
CI 45170:1
CI 61554

Appendix II

Analytical method for the identification of colourants in cosmetic products

1 Materials

1.1 Reference colourants

A number of the colourants used in the present study were kindly donated by BASF, Germany; Ellis & Everard, England and from Keuringsdienst Van Waren, Enschede, Holland. Other reference colourants were purchased through Aldrich-Chemie., Germany; Fluka, Switzerland; Extrasynthese, France; Sigma Chemical Company, USA and Tokyo Kasei Organic Chemicals, Japan.

1.2 Reagents

All the reagents were of analytical grade and suitable for HPLC where appropriate. Buffers and solvents used as mobile phase for HPLC were filtered through a 45 µm membrane filter before use and they were degassed by helium during HPLC.

1.2.1 Methanol gradient grade (for chromatography, Lichrosolv, E. Merck)
1.2.2 Acetonitril (ACN) (for chromatography, Lichrosolv, E.Merck)
1.2.3 Tetrahydrofuran (THF) HPLC grade (Rathburn, Scotland)
1.2.4 Dimethylformamide (DMF) (Proanalysi, E. Merck)
1.2.5 Orthophosphoric acid, 85%, HPLC grade (BDH)
1.2.6 Acidified dimethylformamide (DMF/H^+): DMF (1.2.4)/orthophosphoric acid (1.2.5) (95:5, v/v)
1.2.7 Tetrabutylammonium hydroxide (TBAH), 40% (E.Merck)
1.2.8 0.1 mol/L tetrabutylammonium hydroxide solution (TBAH solution): dilute 0.5 mol/L TBAH (E.Merck) to 0.1 mol/L with water
1.2.9 Sodium hydroxide solution, 0.2 mol/L in water
1.2.10 Concentrated ammonia solution (d_{20} = 0.88 g/l)
1.2.11 Citric acid,H_2o (M&B)
1.2.12 HPLC buffer: In a 1 l measuring flsak, dissolve 1.4 g citric acid (1.2.11) and 6.8 g TBAH (1.2.7) in water and adjust pH of the solution to 9.0 with ammonia (1.2.10)
1.2.13 HPLC solvent: Buffer (1.2.12)/ACN/THF (75:12.5/12.5, v/v/v)
1.2.14 Anti-boiling granules (BDH)

Safety

a) The organic solvents used for the analysis are highly flammable and/or harmful/toxic.
b) Peroxides are formed in THF in contact wit, thus creating the danger of explosion. THF should be stored under nitrogen: flush nitrogen over THF before closing the bottle.
c) Ammonia, sodium hydroxide, orthophosphoric acid and TBAH are corrosive.
d) Colourants should be handled carefully, some of the organic colourants may be skin irritant, harmful or toxic.

1.3 Glassware, Filters and Solid Phase Extraction (SPE) Cartridges

1.3.1 Normal laboratory glass- and plasticware

1.3.2 0.45 µm hydrophilic filters: Sartorious Minisart N or NML

1.3.3 0.45 µm hydrophobic membrane filters: Sartorious Minisart SRP 25

1.3.4 Filter paper: Whatman No. 2

1.3.5 SPE cartridges Varian Megabondelut (1225-6020), amino bonded, size 12 cc/2 g

1.3.6 SPE cartridges Waters (WAT036925), C-18 silica, size 10 g/20cc

2 Apparatus

2.1 Waterbath with thermostat

2.2 Centrifuge

2.3 HPLC instrument consisting of a solvent delivery pump with the possibility to mix up to 4 solvents (Waters 616), an autosampler (Waters 717), a photodiode array detector (Waters 996) with possibility for recording the spectra from 275 nm - 760 nm. Millennium version 2.10 software was used for the control of HPLC analysis, data collection, data processing, building spectral library and library search etc.

2.4 HPLC Column: Gaurd column PLRP-S Gaurd Cartridge, 5 mm x 3 mm and analytical column PLRP-S 100 Å, 5 µm, 150 mm x 4.6 mm from Polymer Laboratories, or equivalent.

2.5 Column oven

2.6 Ultrasonic-bath

2.7 Rotary evaporator

3 Chromatographic Procedure

3.1 Set up the solvent delivery system as described in the following table:

Gradient time table

Time min	Flow ml/min	%Buffer 3.1.2.12	%ACN	%THF	%Water	Curve
0.0	0.8	75.0	12.0	13.0	0.0	-
0.1	0.8	75.0	12.0	13.0	0.0	Linear
2.5	0.8	75.0	12.0	13.0	0.0	Linear
25.0	0.8	5.0	47.0	48.0	0.0	Linear
30.0	0.8	5.0	47.0	48.0	0.0	Linear
35.0	0.8	75.0	12.0	13.0	0.0	Linear
50.0	0.8	75.0	12.0	13.0	0.0	Linear
60.0	0.1	0.0	12.0	13.0	75.0	Linear

3.2 Set the column oven temperature to 25°C.

3.3 Set the photodiode array detector to monitor and record the spectra of all eluted peaks across the wavelength range 275 nm - 760 nm. Other conditions: resolution 4.8 nm, 1 spectrum per second and no smoothing.

Note 1: Under the defined conditions, data from each HPLC run occupies 1.2 mb in the hard disc. If 1.2 nm resolution is employed, approximately 5 mb disc-space will be required for each HPLC analysis.

Note 2: Store the column in ACN/THF (70:300), when not in use. Before switching over to ACN/THF from mobile phase containing buffer, column must be washed with at least 30 ml water/ACN/THF (75:12:13). Similarly, before switching over to buffer/ACN/THF from ACN/THF, the column must be washed with at least 30 ml water/ACN/THF (75:12:13).

4 Analysis of Reference Colourants

4.1 Prepare solution of the reference colourants with the concentration of 1 - 2 mg/10 ml in appropriate solvent (for some substances a higher concentration may be necessary, the aim should be to get an absorbance 0.05-0.80 AU at λ_{max} of the colourant). Appendix III lists the solvents which should be used for each colourant. Some of the substances are only partially soluble and stirring (over night) may be necessary for dissolving sufficient amount of the colourant to give absorbance >0.05 AU. Filter the solution through an appropriate membrane filter (hydrophilic filter 1.3.2 when the solution is in water or HPLC solvent 1.1.13, hydrophobic filter 1.3.3 when the solution contains > 25% organic solvent). Transfer the filtered solution of the colourants in HPLC vials and close the vials. Store the solutions in dark at 4°C.

4.2 Condition the HPLC column with the mobile phase as described in the gradient time table (3.1), for 40 min.

4.3 Inject 10 µl of each reference solution prepared as above (6.1) and run HPLC employing the gradient time table (5.1), analysis time 40 min for each solution. Record the retention time (t_R) and UV/Visible spectrum of each colourant peak. Ideally, the absorbance for each colourant should be 0.05-0.80 AU at its λ_{max}. If the absorbance is below 0.05 AU, a concentrated solution of the respective colourant should be analysed, or upto 50 µl of the colourant solution may be analysed. In case of absorbance is higher than 0.8 AU, the solution should be diluted appropriately.

4.4 With the use of the Millennium software, process the data to create a max-plot chromatogram, and build a spectral library consisting of the t_R and spectrum of each of the reference colourant and of the significant impurities in the reference colourants. The spectral library will be used for the identification of colourants in the cosmetic products.

5 Analysis of colourants in cosmetic products

5.1 Lipsticks and related products

5.1.1 Weigh approximately 1 g sample in a 50 ml dark glass bottle with screw cap, add 8 ml DMF/H^+ (1.2.6) and some anti-boiling granules (1.2.14), and heat the mixture at 90°C for 15 min. Cool the mixture to room temperature, add 4 ml 0.1 mol/L TBAH (1.2.8), mix and centrifuge at 3000 rpm for 5 min or filter through a filter paper (1.3.4). Collect the sample colour extract (supernatant/filterate) and fractionate it employing SPE technique as described below (6.1.2).

5.1.2 Wash an amino bonded SPE cartridge (1.3.5) with 5 ml buffer/ACN/THF (1.2.13). Positive N_2 pressure should be used for the SPE. Load 2.3 ml of the sample colour extract (supernatant/filterate as obtained above, 5.1.1) on the washed SPE cartridge, elute exactly the same volume (the colour front reaches the bottom of the SPE cartridge) and discard the eluate. Apply solvents and collect the eluates (fractions) as described in the following table.

Solid phase extraction of colourants

Elution step	Solvent applied	Fractions eluted	Fraction No.
1	Buffer/ACN/THF (3.1.2.13) 5 x 4 ml	5 x 4 ml	1 - 5
2	0.2 mol/L NaOH (3.1.2.9) 3 x 5 ml	3 x 5 ml	6 - 8
3	Buffer/ACN/THF (3.1.2.13) 2 x 5 ml	2 x 5 ml	9, 10
4	ACN/THF (50:50) 2 x 5 ml	2 x 5 ml	11, 12

Filter the fractions through appropriate membrane filters and store the filterates in closed vials for HPLC analysis.

Note 3: The table describes general guidelines for SPE. If during the fractionation a sharp and narrow colour band is observed, it should be collected as a samller fraction rather than diluting it with excess solvent. The volume of the eluant already in use should be increased until a coloured fraction is completely eluted, before changing to the next eluant. In the elution steps 3 & 4, the volume of the eluants may be reduced when no more colour is eluted. However, the volume of the eluants in steps 1 & 2 must not be reduced. Otherwise, some matrix components of the product will elute in the next fractions and they may interfere with the analysis of the colourants.

5.1.3 Analyse 10 µl of each of the fractions obtained in 5.1.2 by HPLC as for reference colourants and record the t_R and spectrum of each max-plot peak.

5.2 Nail varnishes

5.2.1 Weigh approximately 1 g sample in a beaker, add 5 ml mehanol and sonicate the sample for 15 min. Centrifuge the mixture at 3000 rpm for 5 min. Fractionate 2.3 ml of the sample colour extract employing an amino bonded SPE cartridge as described in 5.1.2, and store the remaining unfractionated extract in a vial for HPLC analysis.

5.2.2 Analyse 10 µl of total colour extract and each of the fractions obtained in 5.1.2 by HPLC as for reference colourants and record the t_R and spectrum of each peak.

Note 4: In general, some colourants in a nail varnish product were identified only when total colour extracts were analysed. However, SPE of the colour extracts followed by the analysis of SPE fractions was found to be necessary for the complete identification of the colourants present in these product.

5.3 Diverse cosmetics

Sample preparation method for the analysis of colourants in diverse cosmetics depends upon the product category as well as the consistency of the products. One of the following methods should be used as a guideline for the analysis of colourants in some commonly used cosmetic products.

5.3.1 Shampoos

5.3.1.1 Dissolve approximately 5 g sample in 25 ml methanol by gentle stirring.

5.3.1.2 Condition a Sep Pak C18 SPE cartridge (1.3.6) by washing it with 20 ml methanol. Load the sample solution (6.3.1.1) and apply vacuum. Collect the eluate, discarding first 5 ml. Concentrate the eluate to approximately 4 ml using rotary evaporator (50°-60°C, vacuum 250-300 mbar, formation of foam by the sample must be avoided). Collect the concentrated colour extract in a closed vial.

5.3.1.3 Depending upon the strength of the concentrated colour extract (5.3.1.2), analyse 10 µl-50 µl of the extract by HPLC as for the reference colours.

5.3.2 Shampoos with strong colours

5.3.2.1 Dissolve approximately 2 g shampoo in 5 ml methanol by gentle stirring. Filter the solution through a membrane filter 1.3.3. Store the filterate in a closed vial, at room temperature.

5.3.2.2 Analyse 10 µl of the filterate by HPLC as for the reference colourants.

5.3.3 Cream and cream deodorants

5.3.3.1 In a 100 ml screw cap bottle, mix approximately 5 g sample with 25 ml methanol. Add some anti-boiling granules and heat the mixture for 15 min at 60°C. Cool the solution.

5.3.3.2 Condition a Sep Pak C18 SPE cartridge (1.3.6) by washing it with 20 ml methanol. Load the sample solution (6.3.1.1) and apply vacuum. Collect the eluate, discarding first 5 ml. Concentrate the eluate to approximately 4 ml using rotary evaporator (50°-60°C, vacuum 250-300 mbar, formation of foam by the sample must be avoided). Collect the concentrated colour extract in a closed vial.

5.3.3.3 Depending upon the strength of the concentrated colour extract (5.3.3.2), analyse 10 µl-50 µl of the extract by HPLC as for the reference colours.

5.3.4 Skin-tonics, make-up removers, mouthwashes, and similar other fluids
5.3.4.1 Filter approximately 3 ml sample through a membrane filter 1.3.2.
6.3.4.2 Analyse 50 µl of the filtrate by HPLC as for the reference colourants.

Note 5: If the colour of the product is very faint, the sample should be concentrated 5x, and the analysis of up to 50 µl of the concentrated sample may be performed.

5.3.5 Deodorants (roll-on)
Follow the procedure described for shampoos (5.3.1).

5.3.6 Eye shadows
Follow the procedure described for nail varnishes (5.2).

Note 6: It appears that mainly inorganic colourants are used in these products. Thus, no colourant may be eluted in the extraction solvent.

5.3.7 Eye liners, Eye pencils and Mascara
The procedure described for the analysis of colourants in lipsticks (5.1) is recommended. However, by the application of this method, no colour was extracted from these samples in the present investigation (it appears that only inorganic colourants are used in the formulation of these products).

6. Identification

Compare the t_R and spectrum of each peak in the chromatogram of a sample with those of reference colourants in the spectral library prepared in 3.5.4. The t_R window 10% and the spectrum window of 10 nm should be set for library search.

Note 7: In geneneral spectrum window of 5 nm, for data recorded at 4.8 nm resolution, has been found to be suitable for the identification of the unknown colourants. However, matrix components of the cosmetic products present in the colour extract of a sample, the solvent used for the extraction of colourant(s) as well as the volume of the extract analysed may influence the composition of the mobile phase, pH of the mobile phase, the concentration of ion-pairing reagent (TABH) in the mobile phase, etc. These factors may have significant influence in the t_R & λ_{max} of a colourant. In practice, λ_{max} of only a few of the colourants in the samples were found to deviate, by >1 resolution factor, i.e. ±4.8 nm. The setting the spectrum window at 10 nm, for the library search of unknowns, will resolve the above mentioned problem.

The cosmetic matrix components, surfactants in particular, were fond to have a significant influence in the t_R of the colourants. Sometimes colourants coeluted with a matrix component(s), thus leading to an additive spectrum. Furthermore, the spectrum of a colourant in low concentration may not be visible in the "normalized" additive spectrum of the colourant and a surfactant(present in relatively high concentration). The identification of unknown colourants under these situations can be performed satisfactorily, in most cases, by manual library search and by the use of facilities available by the software - se Results and Discussion for some examples.

7. Report

Report the colourant(s) identified in a cosmetic products as colour index numbers (CI .....) or common name if the colourant is not classified as a CI No.

Appendix III

Chromatogram and spectra of the reference colourants analysed by the ion-pair gradient HPLC method described in Appendix II

Description of the spectral library "COSCOLOR" of reference colourants

The spectral library "COSCOLOR" (FARVESTOFFER) has been build by the use of Millennium Software, Version 2.1. The spectral library contains the retention times and the spectra of the colourants as well as the significant impurities in these. The solvents used to dissolve the colourants are described as "opløst i.....". The colourants are described both as CI No. as well as the DMU (NERI) Standard-Chemical Registry Number. The colourants are described in the order of increasing HPLC retention time of these substances (they can also be arranged by DMU No. or by their λmax, but not with the CI No.). The max-plot chromatogram and spectra of the colourants are shown in the order of increasing CI No.

The spectra of a colourant having impurities are marked with *, **, *** and **** . The spectrum with * represents the chromatographic peak with the maximum absorbance (the main substance), the spectrum marked with ** represents chromatographic peak with the next highest absorbance, and so on. Maximum 4 spectra, corresponding to 4 chromatographic peaks of a colourant are included, eventhough its maxplot chromatogram contained more than 4 chromatographic peaks. The spectra of impurities are not shown here, but they are included in the spectral library.

The spectral library "COSCOLOR" in a floppy disc can be provided to non-commercial organizations upon written request to NERI or DEPA.

PDA Spectra Table

#	Retention Time	Source	Library Name	Spectrum Name
1	2.512	2673_RIBOFLAVI	FARVESTOFFER	DMU 2673_RIBOFLAVIN
2	2.675	2690_cyan_anth	FARVESTOFFER	DMU 2690_CYANIDINCL_ANTHOCY*
3	2.875	2690_cyan_anth	FARVESTOFFER	DMU 2690_CYANIDINCL_ANTHOCY**
4	3.077	2636_73000	FARVESTOFFER	DMU 2636_CI 73000**
5	3.222	2638_CI44090	FARVESTOFFER	DMU 2638_CI 44090*
6	3.222	2689_oen_antho	FARVESTOFFER	DMU 2689_OENINCL_ANTHOCYAN*
7	3.387	2691_DELPHIDIN	FARVESTOFFER	DMU 2691_DELPHIDIN_ANTHOC***
8	3.405	2689_oen_antho	FARVESTOFFER	DMU 2689_OENINCL_ANTHOCYAN**
9	3.500	2640_75470	FARVESTOFFER	DMU 2640_CI 75470
10	3.512	2607_CI45350	FARVESTOFFER	DMU 2607_CI 45350
11	3.667	2620_CI73015:1	FARVESTOFFER	DMU 2620_CI 73015:1
12	3.748	2605_73015	FARVESTOFFER	DMU 2605_CI 73015*
13	3.787	2677_CI45220	FARVESTOFFER	DMU 2677_CI 45220**
14	3.962	2564_14270	FARVESTOFFER	DMU 2564_CI 14270*
15	4.218	2567_CI19140:1	FARVESTOFFER	DMU 2567_CI 19140:1
16	4.288	2689_oen_antho	FARVESTOFFER	DMU 2689_OENINCL_ANTHOCYAN***
17	4.362	2653_42510	FARVESTOFFER	DMU 2653_CI 42510****
18	4.390	2583_59040	FARVESTOFFER	DMU 2583_CI 59040*
19	4.427	2623_19140	FARVESTOFFER	DMU 2623_CI 19140
20	4.538	2638_CI44090	FARVESTOFFER	DMU 2638_CI 44090**
21	4.730	2577_47005	FARVESTOFFER	DMU 2577_CI 47005*
22	4.915	2614_CI47005:1	FARVESTOFFER	DMU 2614_CI 47005:1*
23	4.938	2688_CI45396	FARVESTOFFER	DMU 2688_CI 45396**
24	5.120	2656_13015	FARVESTOFFER	DMU 2656_CI 13015
25	5.122	2654_42520	FARVESTOFFER	DMU 2654_CI 42520***
26	5.195	2653_42510	FARVESTOFFER	DMU 2653_CI 42510**
27	5.290	2583_59040	FARVESTOFFER	DMU 2583_CI 59040***
28	5.295	2678_75100	FARVESTOFFER	DMU 2678_CI 75100*
29	5.453	2571_16185	FARVESTOFFER	DMU 2571_CI 16185*
30	5.805	2683_16290	FARVESTOFFER	DMU 2683_CI 16290
31	5.987	2691_DELPHIDIN	FARVESTOFFER	DMU 2691_DELPHIDIN_ANTHOC*
32	6.070	2677_CI45220	FARVESTOFFER	DMU 2677_CI 45220*
33	6.130	2577_47005	FARVESTOFFER	DMU 2577_CI 47005***
34	6.188	2654_42520	FARVESTOFFER	DMU 2654_CI 42520**
35	6.345	2653_42510	FARVESTOFFER	DMU 2653_CI 42510*
36	6.412	2580_45190	FARVESTOFFER	DMU 2580_CI 45190***
37	6.552	2579_45370:1	FARVESTOFFER	DMU 2579_CI 45370:1*
38	6.553	2595_15985	FARVESTOFFER	DMU 2595_CI 15985
39	6.612	2678_75100	FARVESTOFFER	DMU 2678_CI 75100***
40	6.932	2605_73015	FARVESTOFFER	DMU 2605_CI 73015**
41	6.995	2564_14270	FARVESTOFFER	DMU 2564_CI 14270**
42	7.113	2632_42053	FARVESTOFFER	DMU 2632_CI 42053**
43	7.140	2714_CI15980	FARVESTOFFER	DMU 2714_CI 15980
44	7.218	2601_CI45100	FARVESTOFFER	DMU 2601_CI 45100**
45	7.253	2691_DELPHIDIN	FARVESTOFFER	DMU 2691_DELPHIDIN_ANTHOC**
46	7.638	2654_42520	FARVESTOFFER	DMU 2654_CI 42520*
47	7.863	2632_42053	FARVESTOFFER	DMU 2632_CI 42053*

PDA Spectra Table

#	Retention Time	Source	Library Name	Spectrum Name
48	7.885	2652_42045	FARVESTOFFER	DMU 2652_CI 42045
49	7.928	2653_42510	FARVESTOFFER	DMU 2653_CI 42510***
50	8.162	2568_CI42090	FARVESTOFFER	DMU 2568_CI 42090**
51	8.230	2577_47005	FARVESTOFFER	DMU 2577_CI 47005**
52	8.277	2589_16035	FARVESTOFFER	DMU 2589_CI 16035
53	8.448	2614_CI47005:1	FARVESTOFFER	DMU 2614_CI 47005:1**
54	8.853	2563_18965	FARVESTOFFER	DMU 2563_CI 18965
55	9.145	2568_CI42090	FARVESTOFFER	DMU 2568_CI 42090*
56	9.345	2678_75100	FARVESTOFFER	DMU 2678_CI 75100**
57	9.372	2723_27755	FARVESTOFFER	DMU 2723_CI 27755
58	9.480	2597_75660	FARVESTOFFER	DMU 2597_CI 75660
59	9.618	2601_CI45100	FARVESTOFFER	DMU 2601_CI 45100*
60	9.752	2604_CI16255:1	FARVESTOFFER	DMU 2604_CI 16255:1
61	9.922	2688_CI45396	FARVESTOFFER	DMU 2688_CI 45396*
62	9.975	2584_15525	FARVESTOFFER	DMU 2584_CI 15525
63	10.230	2610_CI10020	FARVESTOFFER	DMU 2610_CI 10020
64	10.317	2629_CI16230	FARVESTOFFER	DMU 2629_CI 16230
65	10.438	2688_CI45396	FARVESTOFFER	DMU 2688_CI 45396***
66	10.558	2622_CI16255	FARVESTOFFER	DMU 2622_CI 16255
67	10.715	2613_42051	FARVESTOFFER	DMU 2613_CI 42051
68	10.728	2603_28440	FARVESTOFFER	DMU 2603_CI 28440
69	10.807	2594_CI17200	FARVESTOFFER	DMU 2594_CI 17200
70	10.843	2639_18050	FARVESTOFFER	DMU 2639_CI 18050
71	11.178	2568_CI42090	FARVESTOFFER	DMU 2568_CI 42090***
72	11.368	2579_45370:1	FARVESTOFFER	DMU 2579_CI 45370:1**
73	11.387	2598_45380	FARVESTOFFER	DMU 2598_CI 45380**
74	11.460	2694_ME VIOL2B	FARVESTOFFER	DMU 2694_CI 42535***
75	11.558	2633_58000	FARVESTOFFER	DMU 2633_CI 58000*
76	11.595	2678_75100	FARVESTOFFER	DMU 2678_CI 75100****
77	11.903	2571_16185	FARVESTOFFER	DMU 2571_CI 16185**
78	12.003	2600_18950	FARVESTOFFER	DMU 2600_CI 18950**
79	12.103	2575_14720	FARVESTOFFER	DMU 2575_CI 14720
80	12.107	2702_14815	FARVESTOFFER	DMU 2702_CI 14815
81	12.110	2694_ME VIOL2B	FARVESTOFFER	DMU 2694_CI 42535**
82	12.118	2627_CI14720:1	FARVESTOFFER	DMU 2627_CI 14720:1
83	12.222	2657_CI42080	FARVESTOFFER	DMU 2657_CI 42080**
84	12.365	2614_CI47005:1	FARVESTOFFER	DMU 2614_CI 47005:1***
85	12.420	2695_CI42555	FARVESTOFFER	DMU 2695_CI 42555**
86	12.467	2664_CI42100	FARVESTOFFER	DMU 2664_CI 42100**
87	12.583	2576_45410:1	FARVESTOFFER	DMU 2576_CI 45410:1**
88	12.593	2694_ME VIOL2B	FARVESTOFFER	DMU 2694_CI 42535*
89	12.675	3542_CI42650	FARVESTOFFER	DMU 3542_CI 42650***
90	12.733	2664_CI42100	FARVESTOFFER	DMU 2664_CI 42100*
91	12.752	2672_75810	FARVESTOFFER	DMU 2672_CI 75810**
92	12.760	2700_42640	FARVESTOFFER	DMU 2700_CI 42640**
93	12.838	2586_14700	FARVESTOFFER	DMU 2586_CI 14700
94	12.845	2578_CI45430	FARVESTOFFER	DMU 2578_CI 45430**

PDA Spectra Table

#	Retention Time	Source	Library Name	Spectrum Name
95	12.853	2695_CI42555	FARVESTOFFER	DMU 2695_CI 42555*
96	13.075	3542_CI42650	FARVESTOFFER	DMU 3542_CI 42650*
97	13.105	2710_42170	FARVESTOFFER	DMU 2710_CI 42170**
98	13.143	2700_42640	FARVESTOFFER	DMU 2700_CI 42640*
99	13.203	2592_CI45405	FARVESTOFFER	DMU 2592_CI 45405**
100	13.207	2617_CI15850:1	FARVESTOFFER	DMU 2617_CI 15850:1
101	13.277	2590_15850	FARVESTOFFER	DMU 2590_CI 15850
102	13.338	2710_42170	FARVESTOFFER	DMU 2710_CI 42170*
103	13.353	2598_45380	FARVESTOFFER	DMU 2598_CI 45380*
104	13.428	2580_45190	FARVESTOFFER	DMU 2580_CI 45190**
105	13.463	2581_CI45170	FARVESTOFFER	DMU 2581_CI 45170
106	13.512	2698_SOL RED49	FARVESTOFFER	DMU 2698_CI 45170:1
107	13.520	2574_CI10316	FARVESTOFFER	DMU 2574_CI 10316
108	13.590	2583_59040	FARVESTOFFER	DMU 2583_CI 59040**
109	13.660	2700_42640	FARVESTOFFER	DMU 2700_CI 42640***
110	13.875	2633_58000	FARVESTOFFER	DMU 2633_CI 58000**
111	13.880	2646_bromocre	FARVESTOFFER	DMU 2646_BROMOCREOSOL****
112	13.963	2667_CI75120	FARVESTOFFER	DMU 2667_CI 75120
113	14.137	2649_CI11920	FARVESTOFFER	DMU 2649_CI 11920*
114	14.147	2646_bromocre	FARVESTOFFER	DMU 2646_BROMOCREOSOL**
115	14.158	3542_CI42650	FARVESTOFFER	DMU 3542_CI 42650**
116	14.197	2717_42735	FARVESTOFFER	DMU 2717_CI 42735
117	14.285	2672_75810	FARVESTOFFER	DMU 2672_CI 75810*
118	14.312	2578_CI45430	FARVESTOFFER	DMU 2578_CI 45430***
119	14.322	2718_40215	FARVESTOFFER	DMU 2718_CI 40215*
120	14.327	2573_CI15510	FARVESTOFFER	DMU 2573_CI 15510
121	14.347	2591_CI15880:1	FARVESTOFFER	DMU 2591_CI 15880:1
122	14.620	2659_61570	FARVESTOFFER	DMU 2659_CI 61570**
123	14.655	2725_CI15865:3	FARVESTOFFER	DMU 2725_CI 15865:3
124	14.795	2619-45430:1	FARVESTOFFER	DMU 2619_CI 45430:1
125	14.795	2578_CI45430	FARVESTOFFER	DMU 2578_CI 45430*
126	14.825	2588-CI45425:1	FARVESTOFFER	DMU 2588_CI 45425:1
127	14.870	2592_CI45405	FARVESTOFFER	DMU 2592_CI 45405***
128	14.887	2659_61570	FARVESTOFFER	DMU 2659_CI 61570*
129	14.888	2709_15580	FARVESTOFFER	DMU 2709_CI 15580
130	14.922	2718_40215	FARVESTOFFER	DMU 2718_CI 40215**
131	15.272	2657_CI42080	FARVESTOFFER	DMU 2657_CI 42080*
132	15.397	2646_bromocre	FARVESTOFFER	DMU 2646_BROMOCREOSOL*
133	15.408	2666_15800	FARVESTOFFER	DMU 2666_CI 15800
134	15.568	2655_27290	FARVESTOFFER	DMU 2655_CI 27290
135	15.657	2682_18690	FARVESTOFFER	DMU 2682_CI 18690***
136	15.928	2569_18820	FARVESTOFFER	DMU 2569_CI 18820
137	15.935	2662_74180	FARVESTOFFER	DMU 2662_CI 74180*
138	15.947	2593_20170	FARVESTOFFER	DMU 2593_CI 20170
139	16.068	2647_61585	FARVESTOFFER	DMU 2647_CI 61585
140	16.103	2592_CI45405	FARVESTOFFER	DMU 2592_CI 45405*
141	16.177	2634_20470	FARVESTOFFER	DMU 2634_CI 20470

PDA Spectra Table

#	Retention Time	Source	Library Name	Spectrum Name
142	16.222	2699_13065	FARVESTOFFER	DMU 2699_CI 13065
143	16.250	2576_45410:1	FARVESTOFFER	DMU 2576_CI 45410:1*
144	16.375	2570_15620	FARVESTOFFER	DMU 2570_CI 15620*
145	16.562	2651_50420	FARVESTOFFER	DMU 2651_CI 50420
146	16.675	2570_15620	FARVESTOFFER	DMU 2570_CI 15620**
147	16.680	2668_60730	FARVESTOFFER	DMU 2668_CI 60730
148	16.742	2720_15630	FARVESTOFFER	DMU 2720_CI 15630
149	16.987	2600_18950	FARVESTOFFER	DMU 2600_CI 18950*
150	17.163	2646_bromocre	FARVESTOFFER	DMU 2646_BROMOCREOSOL***
151	17.202	2662_74180	FARVESTOFFER	DMU 2662_CI 74180***
152	17.277	2685_Brthymobl	FARVESTOFFER	DMU 2685_BROMOTHMOL BLUE
153	17.298	2585_15585	FARVESTOFFER	DMU 2585_CI 15585
154	17.405	2716_18130	FARVESTOFFER	DMU 2716_CI 18130
155	17.495	2580_45190	FARVESTOFFER	DMU 2580_CI 45190*
156	17.512	2707_62045	FARVESTOFFER	DMU 2707_CI 62045
157	18.062	2630_CI44045	FARVESTOFFER	DMU 2630_CI 44045**
158	18.085	2662_74180	FARVESTOFFER	DMU 2662_CI 74180****
159	18.690	2682_18690	FARVESTOFFER	DMU 2682_CI 18690**
160	18.797	2648_CI75300	FARVESTOFFER	DMU 2648_CI 75300
161	18.845	2630_CI44045	FARVESTOFFER	DMU 2630_CI 44045*
162	19.473	2682_18690	FARVESTOFFER	DMU 2682_CI 18690*
163	19.585	2631_CI47000	FARVESTOFFER	DMU 2631_CI 47000
164	19.695	2712_12420	FARVESTOFFER	DMU 2712_CI 12420**
165	19.827	2636_73000	FARVESTOFFER	DMU 2636_CI 73000*
166	20.195	2704_10006	FARVESTOFFER	DMU 2704_CI 10006
167	20.502	2662_74180	FARVESTOFFER	DMU 2662_CI 74180**
168	22.087	2637_12010	FARVESTOFFER	DMU 2637_CI 12010*
169	23.813	2565_CI12120	FARVESTOFFER	DMU 2565_CI 12120
170	23.987	2649_CI11920	FARVESTOFFER	DMU 2649_CI 11920**
171	24.315	2722_CI12490	FARVESTOFFER	DMU 2722_CI 12490
172	24.555	2660_12150	FARVESTOFFER	DMU 2660_CI 12150
173	24.737	2596_12075	FARVESTOFFER	DMU 2596_CI 12075*
174	24.742	2587_11680	FARVESTOFFER	DMU 2587_CI 11680
175	25.153	2705_12700	FARVESTOFFER	DMU 2705_CI 12700
176	26.503	2696_61554	FARVESTOFFER	DMU 2696_CI 61554
177	26.550	2572_12100	FARVESTOFFER	DMU 2572_CI 12100
178	26.618	2582_11710	FARVESTOFFER	DMU 2582_CI 11710
179	26.677	2599_12085	FARVESTOFFER	DMU 2599_CI 12085
180	26.787	2596_12075	FARVESTOFFER	DMU 2596_CI 12075**
181	27.193	2697_12140	FARVESTOFFER	DMU 2697_CI 12140
182	27.518	2713_12480	FARVESTOFFER	DMU 2713_CI 12480
183	27.718	2686_CI40850	FARVESTOFFER	DMU 2686_CI 40850
184	28.083	2663_60725	FARVESTOFFER	DMU 2663_CI 60725
185	28.350	2566_26100	FARVESTOFFER	DMU 2566_CI 26100
186	28.587	2637_12010	FARVESTOFFER	DMU 2637_CI 12010**
187	29.190	2721_12370	FARVESTOFFER	DMU 2721_CI 12370
188	29.462	2712_12420	FARVESTOFFER	DMU 2712_CI 12420*

PDA Spectra Table

#	Retention Time	Source	Library Name	Spectrum Name
189	29.528	2692_26105	FARVESTOFFER	DMU 2692_CI 26105
190	29.563	2650_61565	FARVESTOFFER	DMU 2650_CI 61565
191	29.772	2676_40825	FARVESTOFFER	DMU 2676_CI 40825
192	29.888	2684-capsan	FARVESTOFFER	DMU 2684_CAPSANTHIN****
193	30.768	2628_CI40800	FARVESTOFFER	DMU 2628_CI 40800**
194	31.168	2635_75125	FARVESTOFFER	DMU 2635_CI 75125
195	31.405	2684-capsan	FARVESTOFFER	DMU 2684_CAPSANTHIN***
196	31.585	2628_CI40800	FARVESTOFFER	DMU 2628_CI 40800*
197	31.643	2679_CI75130	FARVESTOFFER	DMU 2679_CI 75130
198	31.672	2684-capsan	FARVESTOFFER	DMU 2684_CAPSANTHIN**
199	31.872	2684-capsan	FARVESTOFFER	DMU 2684_CAPSANTHIN*
200	31.988	CI 21230	FARVESTOFFER	DMU XXX_CI 21230

PDA Spectra Table

#	Spectrum Descript	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution
1	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
2	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
3	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
4	OPLØST I THF	On	Yes	Yes	275	760	4.8
5	OPLØST I THF/BUFFER (50:50)	On	Yes	Yes	275	760	4.8
6	OPLØST I VAND	On	Yes	Yes	275	760	4.8
7	OPLØST I HPLC-SOLVENT (5 TOPPE)	On	Yes	Yes	275	760	4.8
8	OPLØST I VAND	On	Yes	Yes	275	760	4.8
9	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
10	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
11	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
12	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
13	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
14	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
15	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
16	OPLØST I VAND	On	Yes	Yes	275	760	4.8
17	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
18	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
19	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
20	OPLØST I THF/BUFFER (50:50)	On	Yes	Yes	275	760	4.8
21	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
22	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
23	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
24	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
25	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
26	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
27	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
28	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
29	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
30	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
31	OPLØST I HPLC-SOLVENT (5 TOPPE)	On	Yes	Yes	275	760	4.8
32	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
33	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
34	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
35	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
36	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
37	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
38	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
39	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
40	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
41	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
42	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
43	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
44	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
45	OPLØST I HPLC-SOLVENT (5 TOPPE)	On	Yes	Yes	275	760	4.8
46	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
47	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8

PDA Spectra Table

#	Spectrum Descript	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution
48	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
49	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
50	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
51	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
52	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
53	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
54	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
55	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
56	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
57	OPLØST I THF/VAND (50:50)	On	Yes	Yes	275	760	4.8
58	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
59	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
60	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
61	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
62	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
63	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
64	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
65	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
66	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
67	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
68	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
69	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
70	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
71	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
72	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
73	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
74	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
75	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
76	OPLØST I ACN/VAND (50:50)	On	Yes	Yes	275	760	4.8
77	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
78	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
79	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
80	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
81	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
82	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
83	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
84	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
85	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
86	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
87	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
88	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
89	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
90	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
91	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
92	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
93	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
94	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8

PDA Spectra Table

#	Spectrum Descript	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution
95	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
96	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
97	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
98	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
99	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
100	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
101	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
102	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
103	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
104	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
105	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
106	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
107	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
108	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
109	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
110	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
111	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
112	OPLØST I ETHANOL	On	Yes	Yes	275	760	4.8
113	OPLØST I THF/VAND (50:50)	On	Yes	Yes	275	760	4.8
114	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
115	OPLØST I HPLC-SOLVENT (6 TOPPE)	On	Yes	Yes	275	760	4.8
116	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
117	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
118	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
119	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
120	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
121	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
122	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
123	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
124	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
125	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
126	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
127	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
128	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
129	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
130	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
131	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
132	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
133	OPLØST I THF	On	Yes	Yes	275	760	4.8
134	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
135	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
136	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
137	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
138	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
139	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
140	OPLØST I HPLC-SOLVENT (4 TOPPE)	On	Yes	Yes	275	760	4.8
141	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8

PDA Spectra Table

#	Spectrum Descript	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution
142	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
143	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
144	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
145	OPLØST VAND/HPLC-SOLVENT*	On	Yes	Yes	275	760	4.8
146	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
147	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
148	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
149	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
150	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
151	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
152	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
153	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
154	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
155	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
156	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
157	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
158	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
159	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
160	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
161	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
162	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
163	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
164	OPLØST I THF	On	Yes	Yes	275	760	4.8
165	OPLØST I THF	On	Yes	Yes	275	760	4.8
166	OPLØST I HPLC-SOLVENT/THF (50:50)	On	Yes	Yes	275	760	4.8
167	OPLØST I HPLC-SOLVENT	On	Yes	Yes	275	760	4.8
168	OPLØST I THF	On	Yes	Yes	275	760	4.8
169	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
170	OPLØST I THF/VAND (50:50)	On	Yes	Yes	275	760	4.8
171	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
172	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
173	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
174	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
175	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
176	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
177	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
178	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
179	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
180	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
181	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
182	OPLØST I THF	On	Yes	Yes	275	760	4.8
183	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
184	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
185	OPLØST I THF	On	Yes	Yes	275	760	4.8
186	OPLØST I THF	On	Yes	Yes	275	760	4.8
187	OPLØST I THF	On	Yes	Yes	275	760	4.8
188	OPLØST I THF	On	Yes	Yes	275	760	4.8

PDA Spectra Table

#	Spectrum Descript	Baseline Correct	Searchable	Traceable	Start Wvln	End Wvln	Resolution
189	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
190	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
191	OPLØST I HPLC-SOLVENT/THF (50:50)	On	Yes	Yes	275	760	4.8
192	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
193	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
194	OPLØST I THF	On	Yes	Yes	275	760	4.8
195	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
196	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
197	OPLØST I HPLC-SOLVENT/THF (50:50)	On	Yes	Yes	275	760	4.8
198	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
199	OPLØST I THF/ACN (50:50)	On	Yes	Yes	275	760	4.8
200	OPLØST I ACN/THF	On	Yes	Yes	275	760	4.8

PDA Spectra Table

#	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
1	None	None	Off	276.0	0.51238
2	None	None	Off	611.8	0.21745
3	None	None	Off	276.0	0.22980
4	None	None	Off	309.2	0.07296
5	None	None	Off	631.3	0.21960
6	None	None	Off	572.8	0.05490
7	None	None	Off	568.0	0.00451
8	None	None	Off	276.0	0.03009
9	None	None	Off	280.8	0.26882
10	None	None	Off	495.1	0.26704
11	None	None	Off	290.2	0.06230
12	None	None	Off	285.5	0.86141
13	None	None	Off	524.2	0.04210
14	None	None	Off	432.2	0.89824
15	None	None	Off	432.2	0.46847
16	None	None	Off	276.0	0.02029
17	None	None	Off	543.6	0.02228
18	None	None	Off	456.4	0.33268
19	None	None	Off	427.4	0.42092
20	None	None	Off	631.3	0.01630
21	None	None	Off	412.9	0.57667
22	None	None	Off	412.9	0.13694
23	None	None	Off	495.1	0.15441
24	None	None	Off	393.7	0.52895
25	None	None	Off	548.5	0.05604
26	None	None	Off	548.5	0.06269
27	None	None	Off	280.8	0.00766
28	None	None	Off	441.9	0.30981
29	None	None	Off	524.2	0.56566
30	None	None	Off	519.3	0.23502
31	None	None	Off	616.7	0.03195
32	None	None	Off	533.9	0.32588
33	None	None	Off	422.6	0.05865
34	None	None	Off	548.5	0.36661
35	None	None	Off	548.5	0.07675
36	None	None	Off	509.6	0.00604
37	None	None	Off	509.6	0.19924
38	None	None	Off	485.4	0.36020
39	None	None	Off	432.2	0.06043
40	None	None	Off	290.2	0.01591
41	None	None	Off	432.2	0.20303
42	None	None	Off	611.8	0.05988
43	None	None	Off	480.5	0.04976
44	None	None	Off	548.5	0.01097
45	None	None	Off	384.1	0.01616
46	None	None	Off	553.3	0.81044
47	None	None	Off	611.8	0.11210

PDA Spectra Table

#	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
48	None	None	Off	636.2	0.33730
49	None	None	Off	553.3	0.02671
50	None	None	Off	631.3	0.13432
51	None	None	Off	417.8	0.12940
52	None	None	Off	509.6	0.24750
53	None	None	Off	417.8	0.03106
54	None	None	Off	403.3	0.52169
55	None	None	Off	631.3	0.39941
56	None	None	Off	441.9	0.12300
57	None	None	Off	592.3	0.16796
58	None	None	Off	403.3	0.15905
59	None	None	Off	563.1	0.25572
60	None	None	Off	509.6	0.04484
61	None	None	Off	495.1	0.58205
62	None	None	Off	485.4	0.32778
63	None	None	Off	290.2	0.17923
64	None	None	Off	495.1	0.17328
65	None	None	Off	509.6	0.05878
66	None	None	Off	509.6	0.30242
67	None	None	Off	636.2	0.44738
68	None	None	Off	577.7	0.66217
69	None	None	Off	533.9	0.33385
70	None	None	Off	533.9	0.32930
71	None	None	Off	631.3	0.04914
72	None	None	Off	514.5	0.06303
73	None	None	Off	514.5	0.01899
74	None	None	Off	577.7	0.13330
75	None	None	Off	276.0	0.62670
76	None	None	Off	441.9	0.04136
77	None	None	Off	514.5	0.01639
78	None	None	Off	342.5	0.10114
79	None	None	Off	519.3	0.61406
80	None	None	Off	304.5	0.39441
81	None	None	Off	587.4	0.48504
82	None	None	Off	519.3	0.18336
83	None	None	Off	621.6	0.01360
84	None	None	Off	417.8	0.01131
85	None	None	Off	587.4	0.01220
86	None	None	Off	641.1	0.04126
87	None	None	Off	379.3	0.01202
88	None	None	Off	592.3	0.65532
89	None	None	Off	587.4	0.04639
90	None	None	Off	641.1	0.13080
91	None	None	Off	611.8	0.03054
92	None	None	Off	587.4	0.11971
93	None	None	Off	504.8	0.65165
94	None	None	Off	524.2	0.03874

PDA Spectra Table

#	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
95	None	None	Off	592.3	0.18935
96	None	None	Off	597.2	0.25062
97	None	None	Off	670.4	0.16035
98	None	None	Off	592.3	0.48467
99	None	None	Off	524.2	0.06387
100	None	None	Off	490.2	0.37130
101	None	None	Off	490.2	0.58933
102	None	None	Off	670.4	0.35166
103	None	None	Off	524.2	0.22116
104	None	None	Off	314.0	0.04458
105	None	None	Off	553.3	0.26107
106	None	None	Off	553.3	0.38651
107	None	None	Off	437.0	0.62868
108	None	None	Off	548.5	0.02148
109	None	None	Off	587.4	0.04124
110	None	None	Off	276.0	0.04276
111	None	None	Off	441.9	0.02173
112	None	None	Off	451.5	0.06122
113	None	None	Off	427.4	0.23741
114	None	None	Off	446.7	0.03602
115	None	None	Off	597.2	0.05952
116	None	None	Off	616.7	0.36311
117	None	None	Off	621.6	0.60058
118	None	None	Off	529.0	0.01655
119	None	None	Off	417.8	0.10787
120	None	None	Off	485.4	0.42128
121	None	None	Off	495.1	0.21744
122	None	None	Off	285.5	0.06503
123	None	None	Off	485.4	0.17925
124	None	None	Off	533.9	0.44330
125	None	None	Off	533.9	0.73877
126	None	None	Off	533.9	0.45458
127	None	None	Off	543.6	0.03759
128	None	None	Off	285.5	0.18823
129	None	None	Off	485.4	0.77099
130	None	None	Off	432.2	0.06225
131	None	None	Off	636.2	0.32356
132	None	None	Off	408.1	0.32711
133	None	None	Off	276.0	0.16644
134	None	None	Off	509.6	0.66922
135	None	None	Off	398.5	0.05016
136	None	None	Off	398.5	0.30731
137	None	None	Off	665.5	0.31814
138	None	None	Off	456.4	0.52420
139	None	None	Off	276.0	0.12915
140	None	None	Off	548.5	0.55777
141	None	None	Off	621.6	0.82226

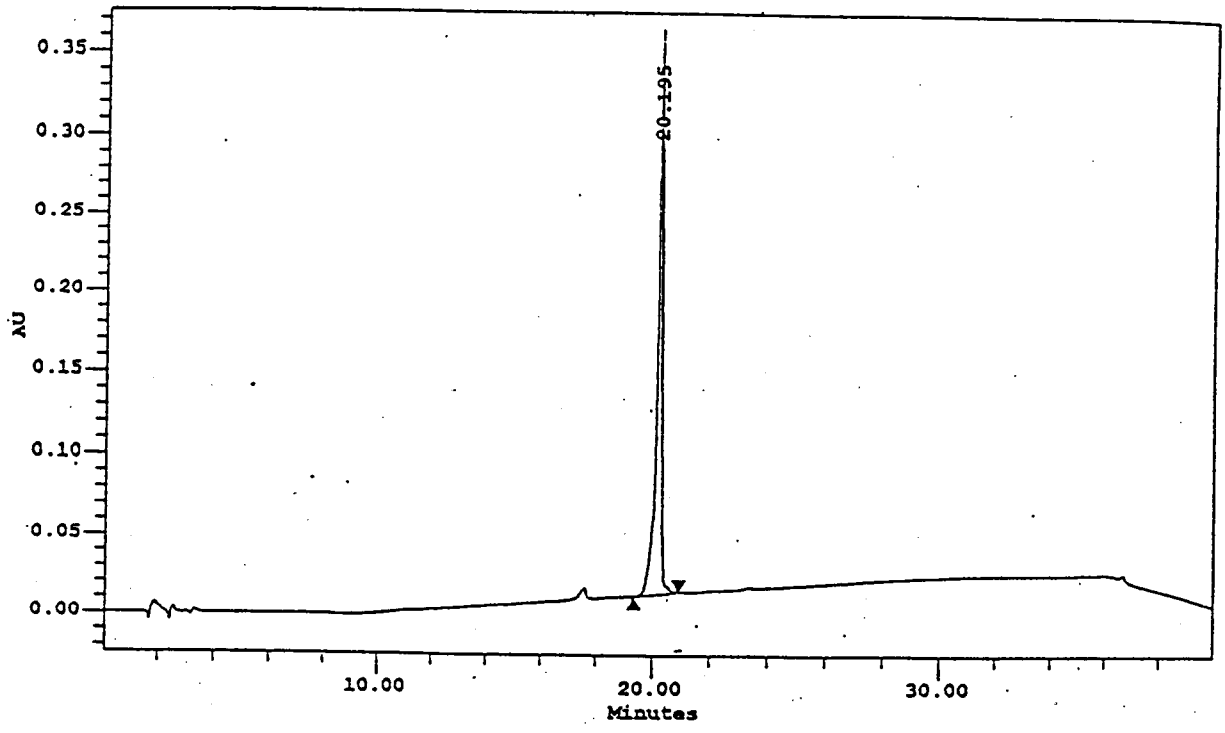
PDA Spectra Table

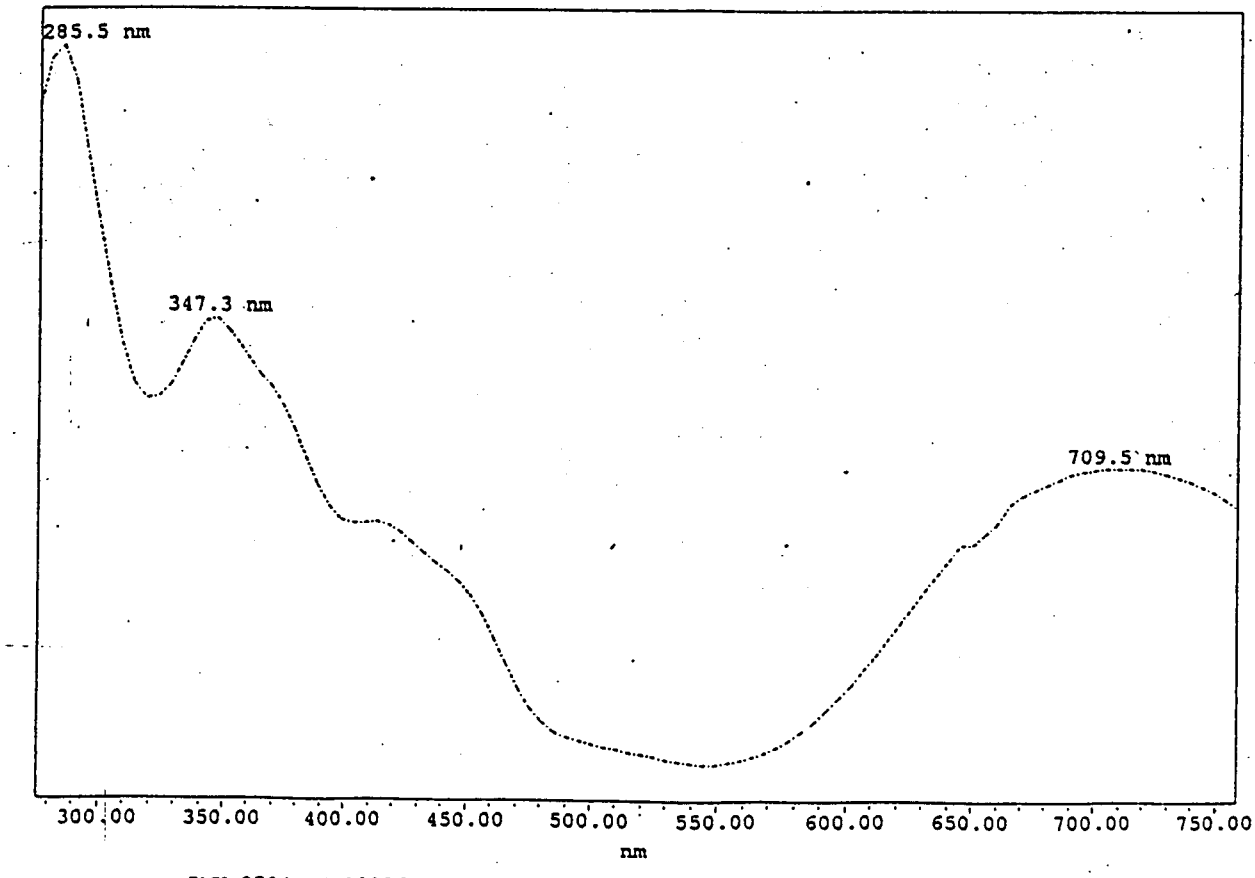
#	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
142	None	None	Off	422.6	0.78163
143	None	None	Off	548.5	0.39431
144	None	None	Off	514.5	0.20880
145	None	None	Off	299.7	0.14257
146	None	None	Off	509.6	0.10392
147	None	None	Off	276.0	0.57308
148	None	None	Off	495.1	0.54006
149	None	None	Off	398.5	0.15412
150	None	None	Off	412.9	0.02860
151	None	None	Off	670.4	0.10465
152	None	None	Off	626.5	0.13478
153	None	None	Off	485.4	0.17890
154	None	None	Off	563.1	0.45439
155	None	None	Off	295.0	0.15815
156	None	None	Off	636.2	0.25251
157	None	None	Off	602.1	0.02186
158	None	None	Off	665.5	0.05344
159	None	None	Off	393.7	0.08482
160	None	None	Off	427.4	0.56599
161	None	None	Off	616.7	0.50056
162	None	None	Off	441.9	0.14047
163	None	None	Off	412.9	0.34444
164	None	None	Off	276.0	0.03960
165	None	None	Off	285.5	0.09877
166	None	None	Off	285.5	0.34982
167	None	None	Off	670.4	0.15162
168	None	None	Off	412.9	0.33760
169	None	None	Off	509.6	0.26062
170	None	None	Off	432.2	0.01156
171	None	None	Off	509.6	0.03954
172	None	None	Off	499.9	0.44185
173	None	None	Off	480.5	0.25875
174	None	None	Off	408.1	0.43141
175	None	None	Off	393.7	0.37657
176	None	None	Off	641.1	0.27458
177	None	None	Off	485.4	0.12989
178	None	None	Off	408.1	0.29171
179	None	None	Off	485.4	0.28684
180	None	None	Off	485.4	0.01657
181	None	None	Off	495.1	0.22212
182	None	None	Off	495.1	0.25195
183	None	None	Off	480.5	0.21786
184	None	None	Off	276.0	0.36199
185	None	None	Off	509.6	0.33406
186	None	None	Off	374.5	0.05176
187	None	None	Off	276.0	0.11469
188	None	None	Off	276.0	0.25787

PDA Spectra Table

#	Smooth	Derivative	Spline	Lambda Max	Maximum Absorbance
189	None	None	Off	514.5	0.24789
190	None	None	Off	280.8	0.75686
191	None	None	Off	446.7	0.17034
192	None	None	Off	470.9	0.01675
193	None	None	Off	446.7	0.03390
194	None	None	Off	475.7	0.36854
195	None	None	Off	480.5	0.01992
196	None	None	Off	456.4	0.58375
197	None	None	Off	451.5	0.02550
198	None	None	Off	475.7	0.03333
199	None	None	Off	470.9	0.07500
200	None	None	Off	347.3	0.26514

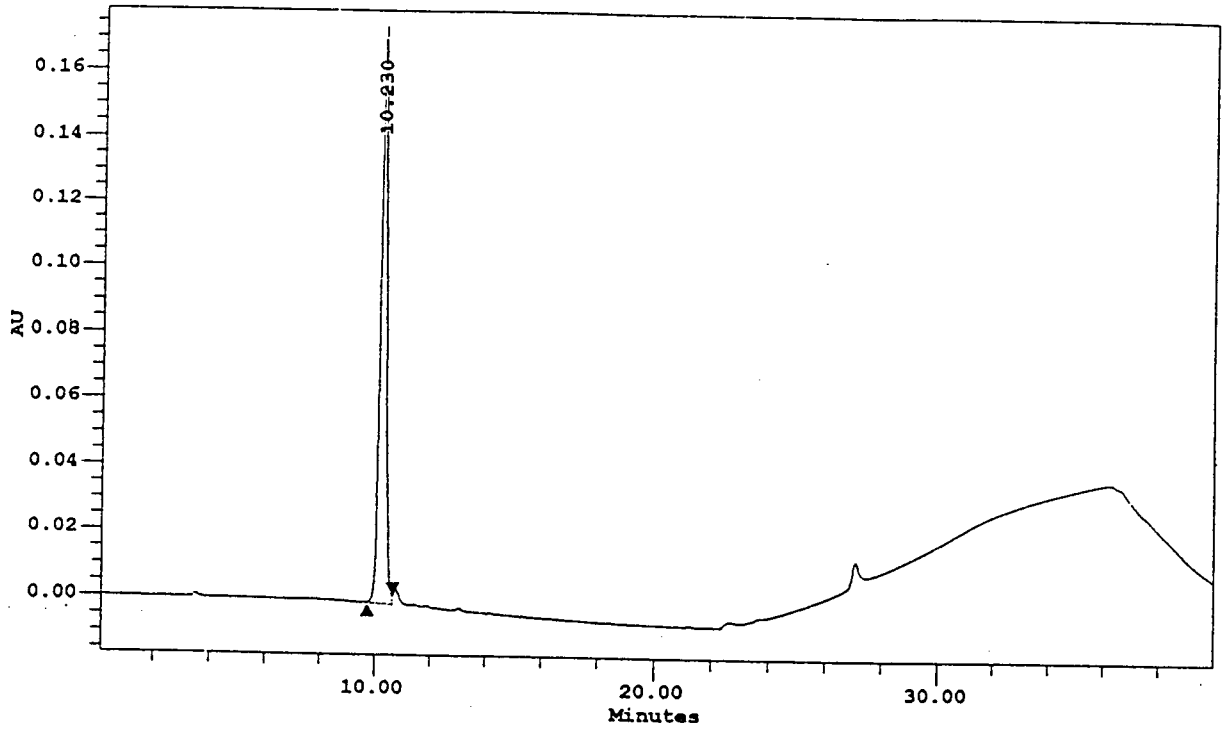
DMU 2704_C.I. 10006

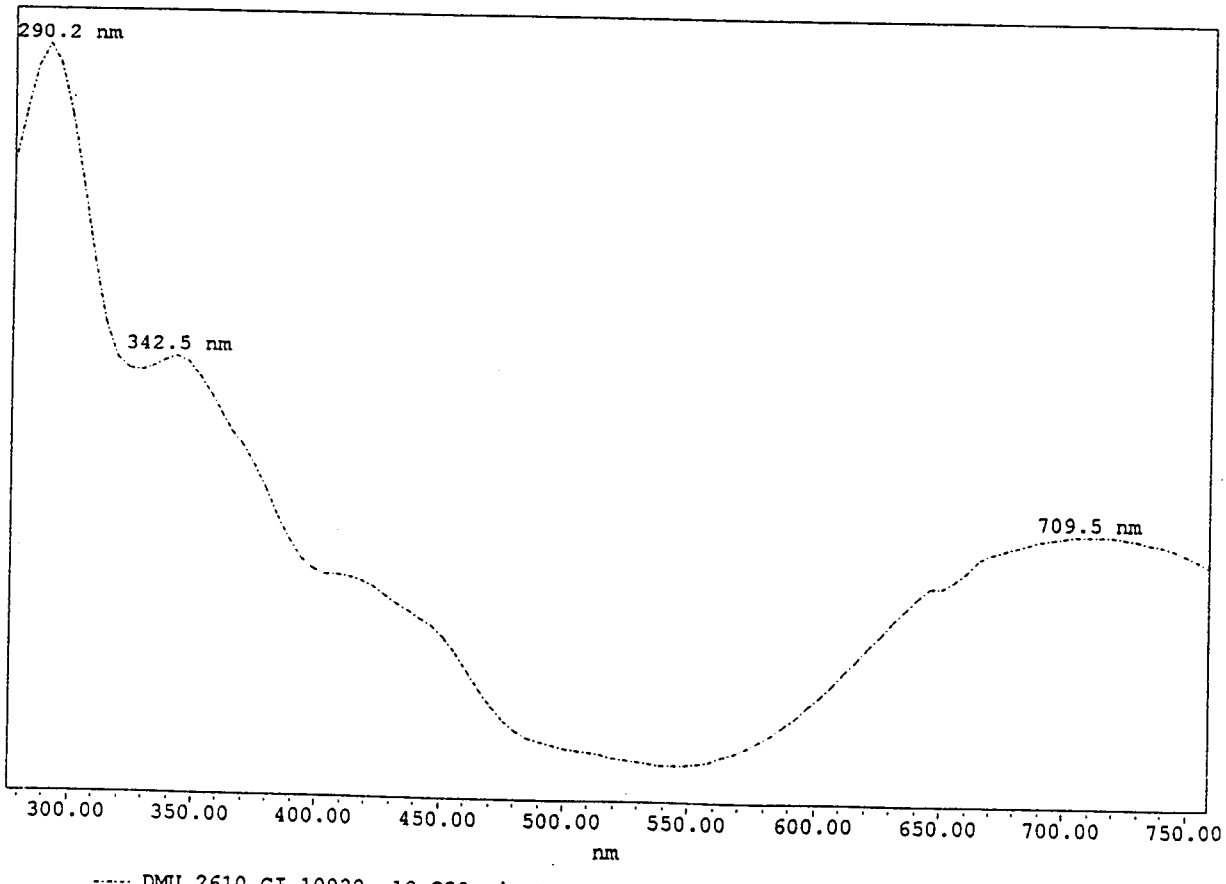




DMU 2704_CI 10006 20.195 minutes, 275 - 760 @ 4.8 nm, from 2704_10006

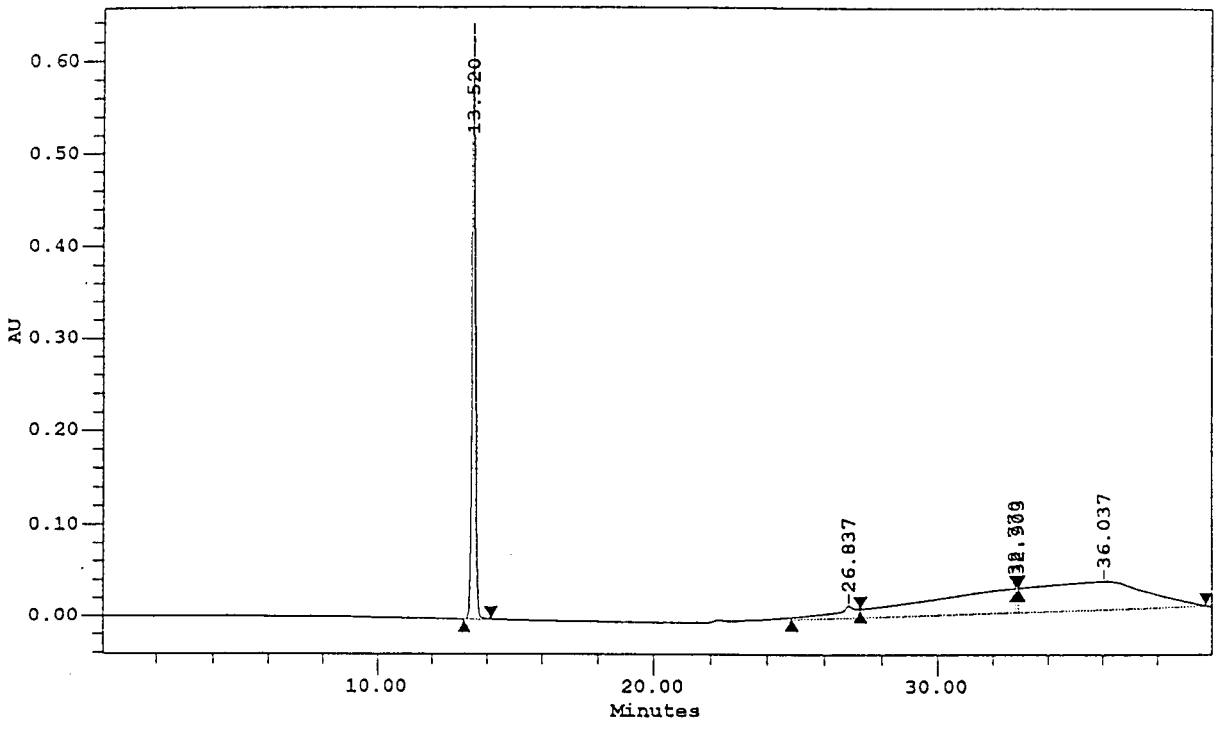
DMU 2610_C.I. 10020

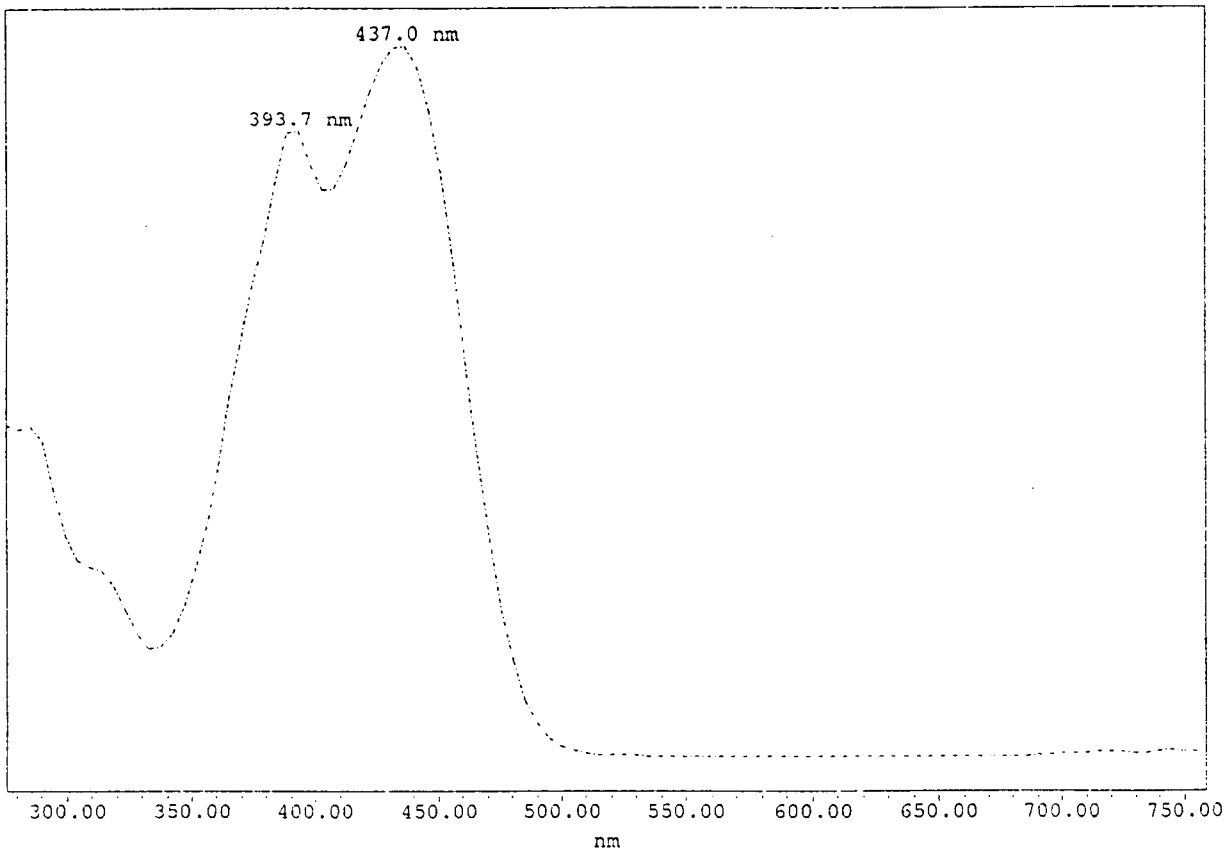




DMU 2610_CI 10020 10.230 minutes, 275 - 760 @ 4.8 nm, from 2610_CI10020

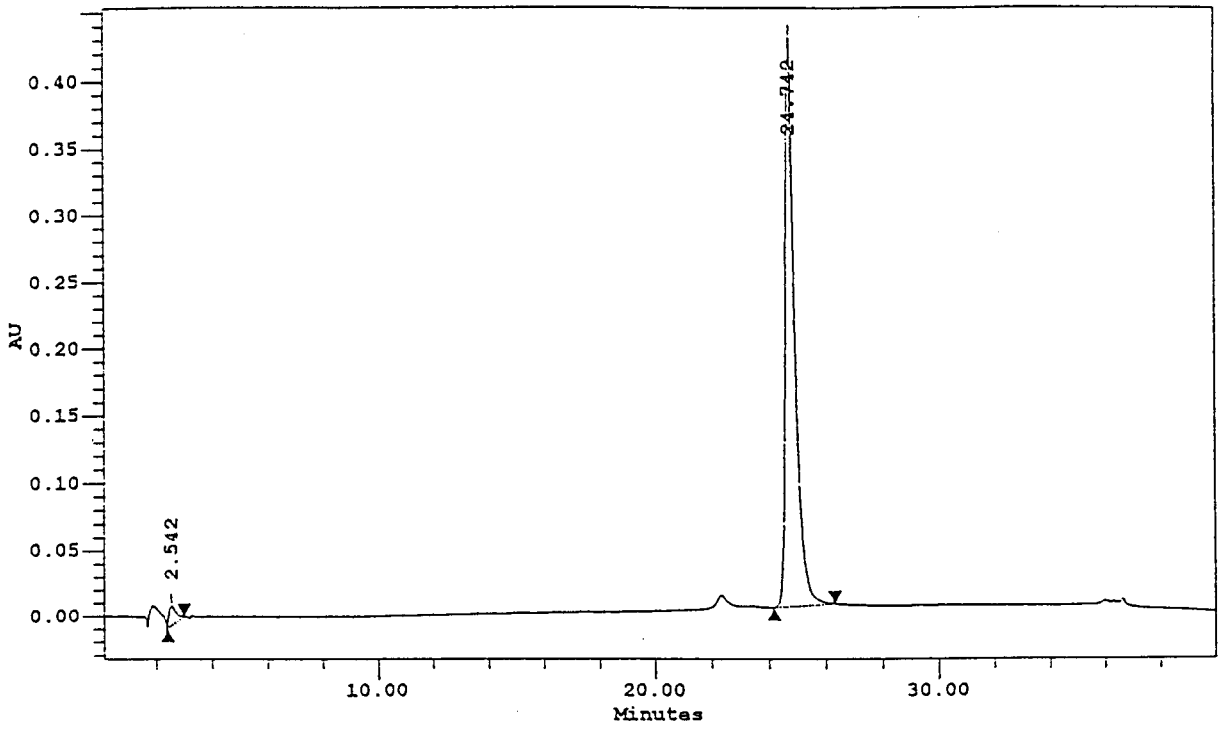
DMU 2574_C.I. 10316

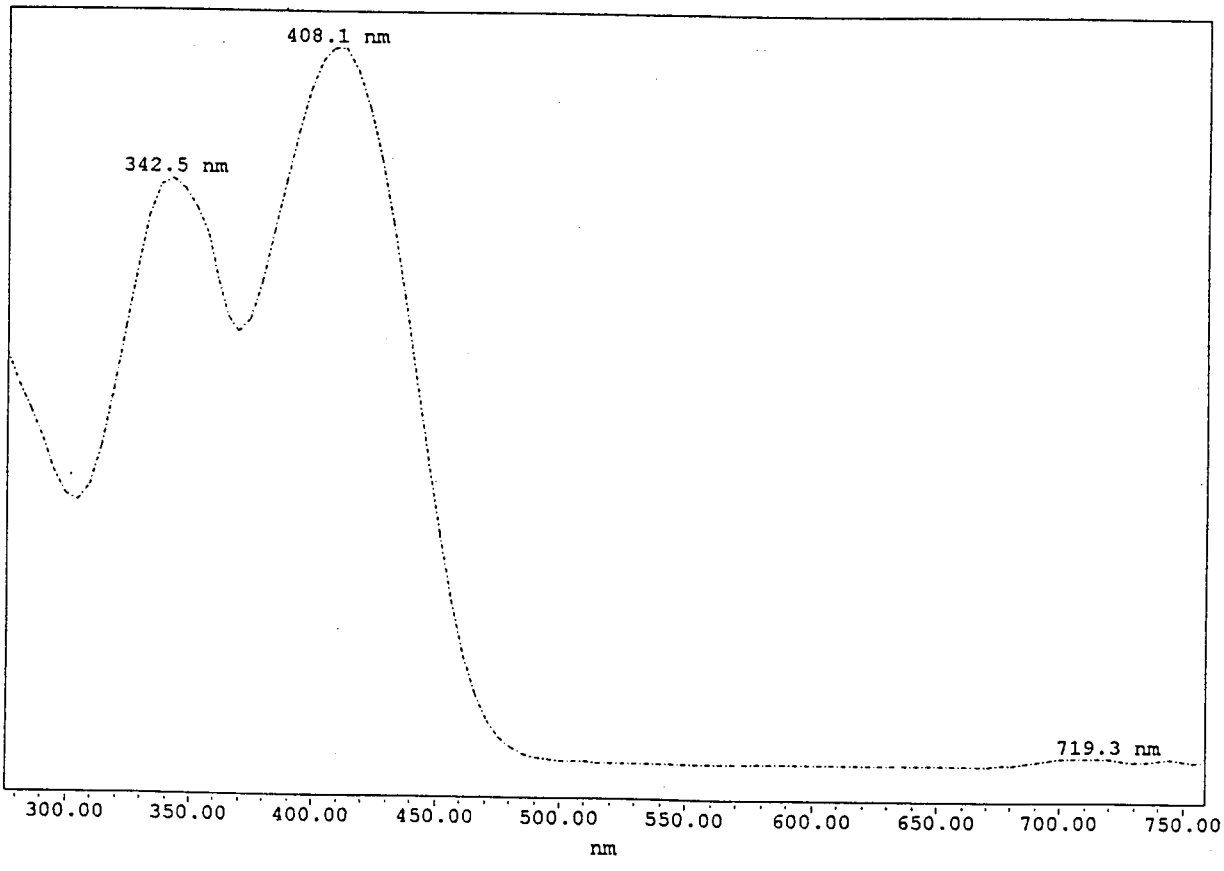




---- DMU 2574_CI 10316 13.520 minutes, 275 - 760 @ 4.8 nm, from 2574_CI10316

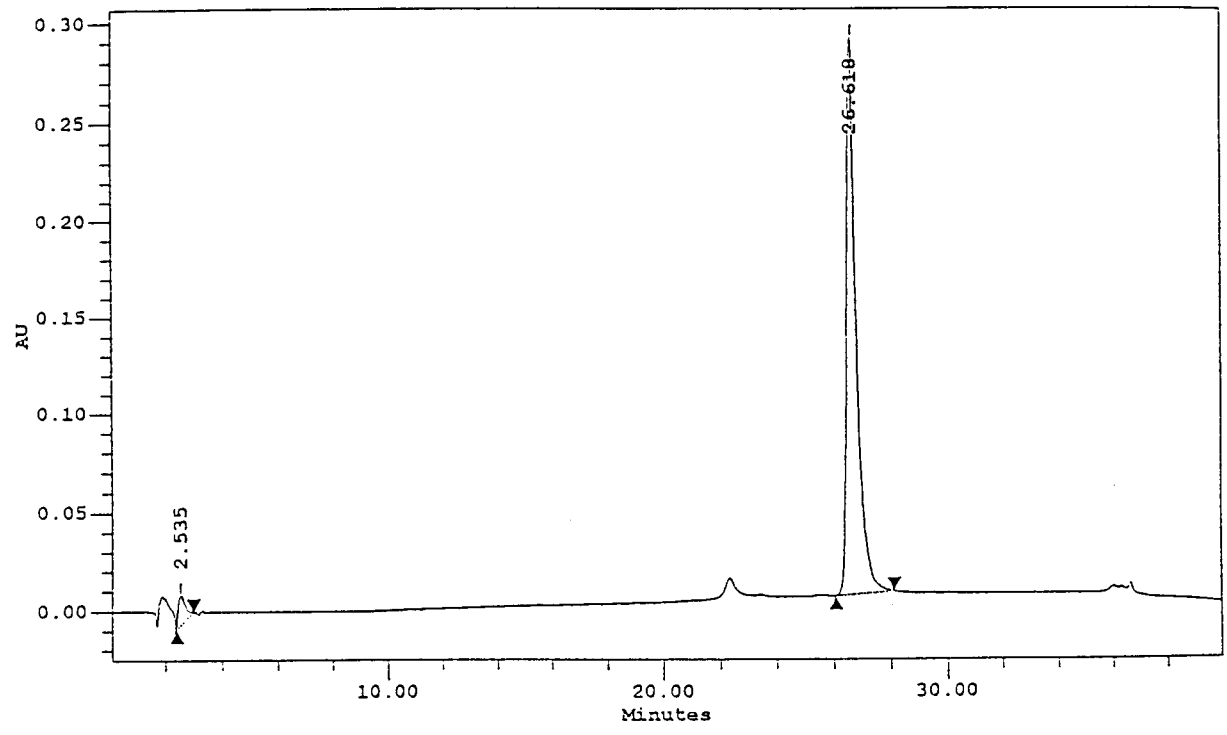
DMU 2587_C.I. 11680

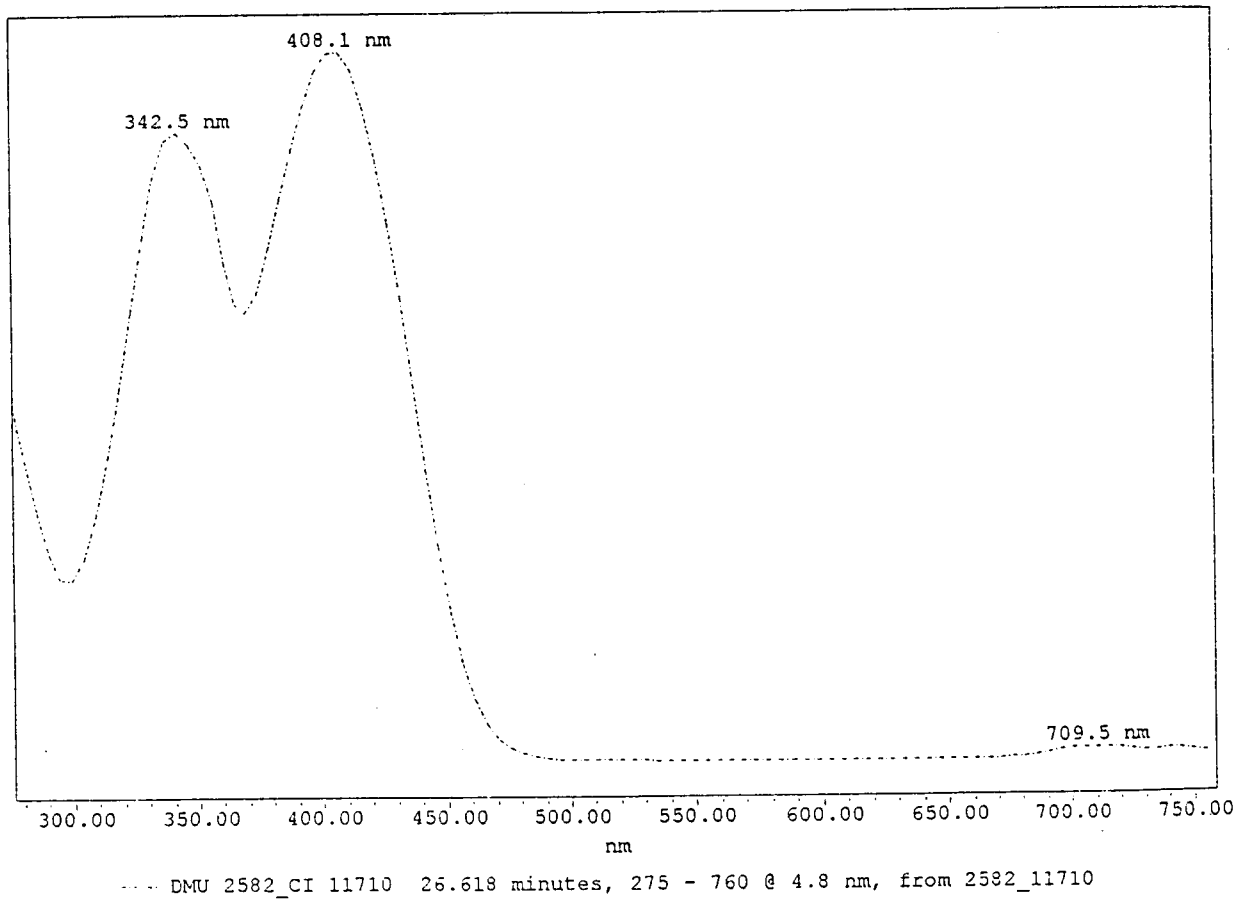




DMU 2587_CI 11680 24.742 minutes, 275 - 760 @ 4.8 nm, from 2587_11680

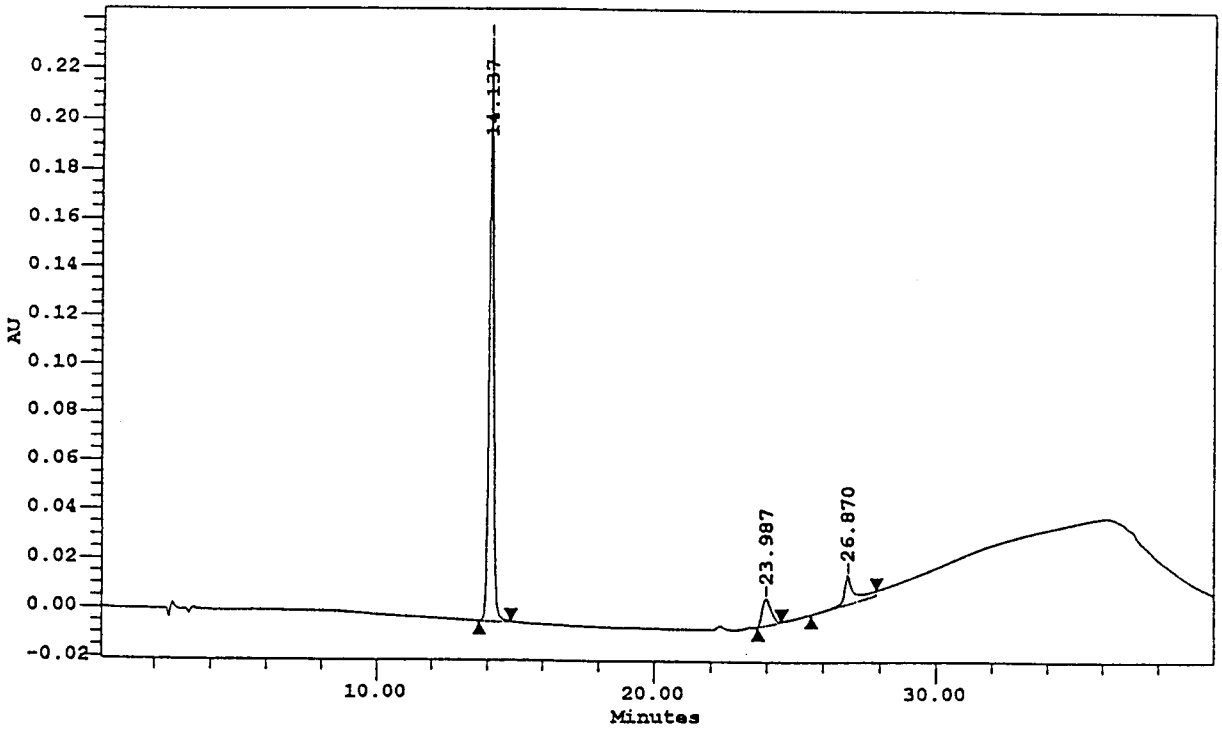
DMU 2582_C.I. 11710

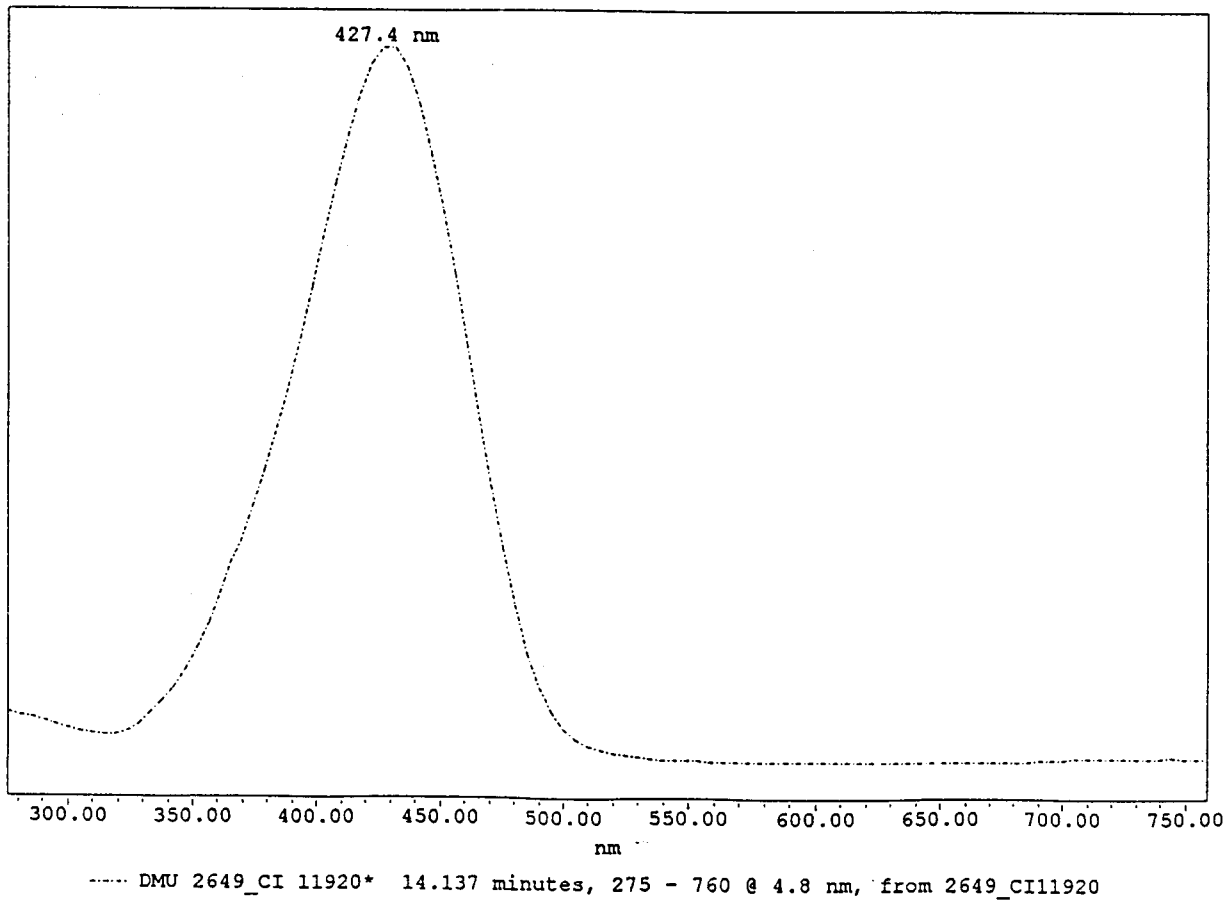




DMU 2582_CI 11710 26.618 minutes, 275 - 760 @ 4.8 nm, from 2582_11710

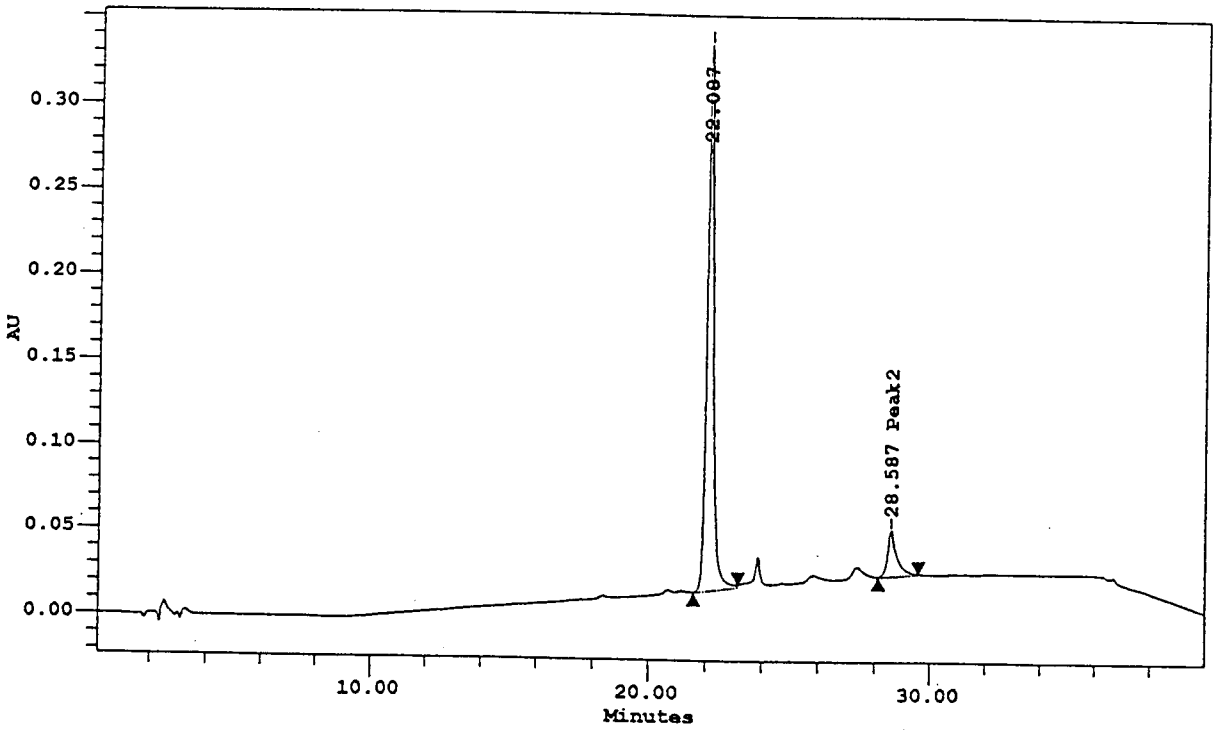
DMU 2649_C.I. 11920

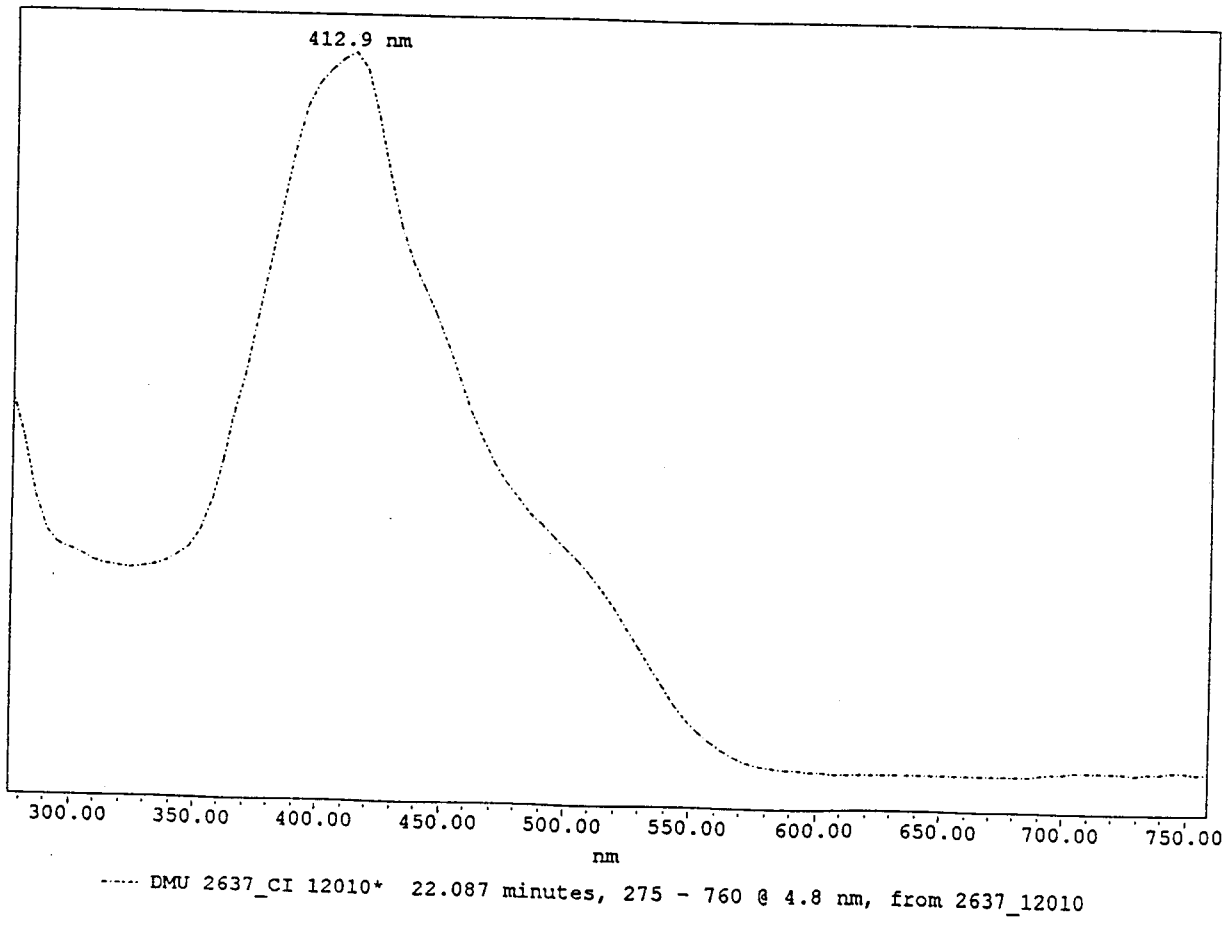




DMU 2649_CI 11920* 14.137 minutes, 275 - 760 @ 4.8 nm, from 2649_CI11920

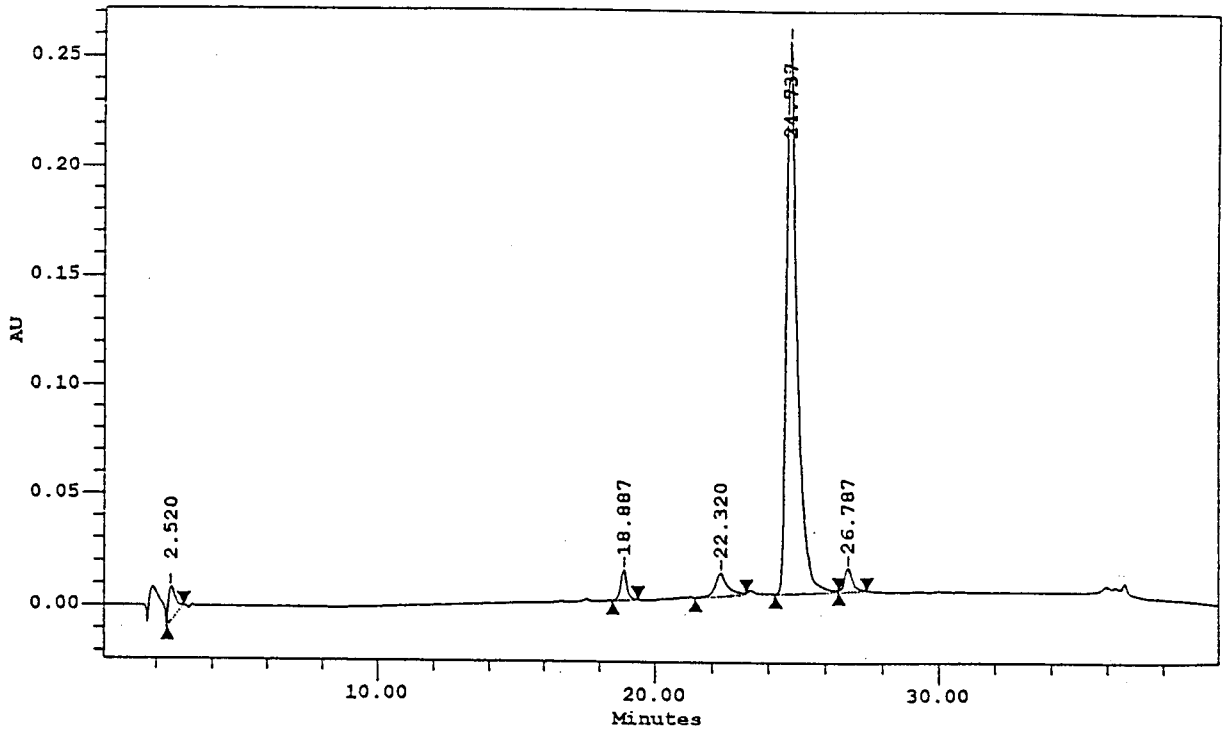
DMU 2637_C.I. 12010

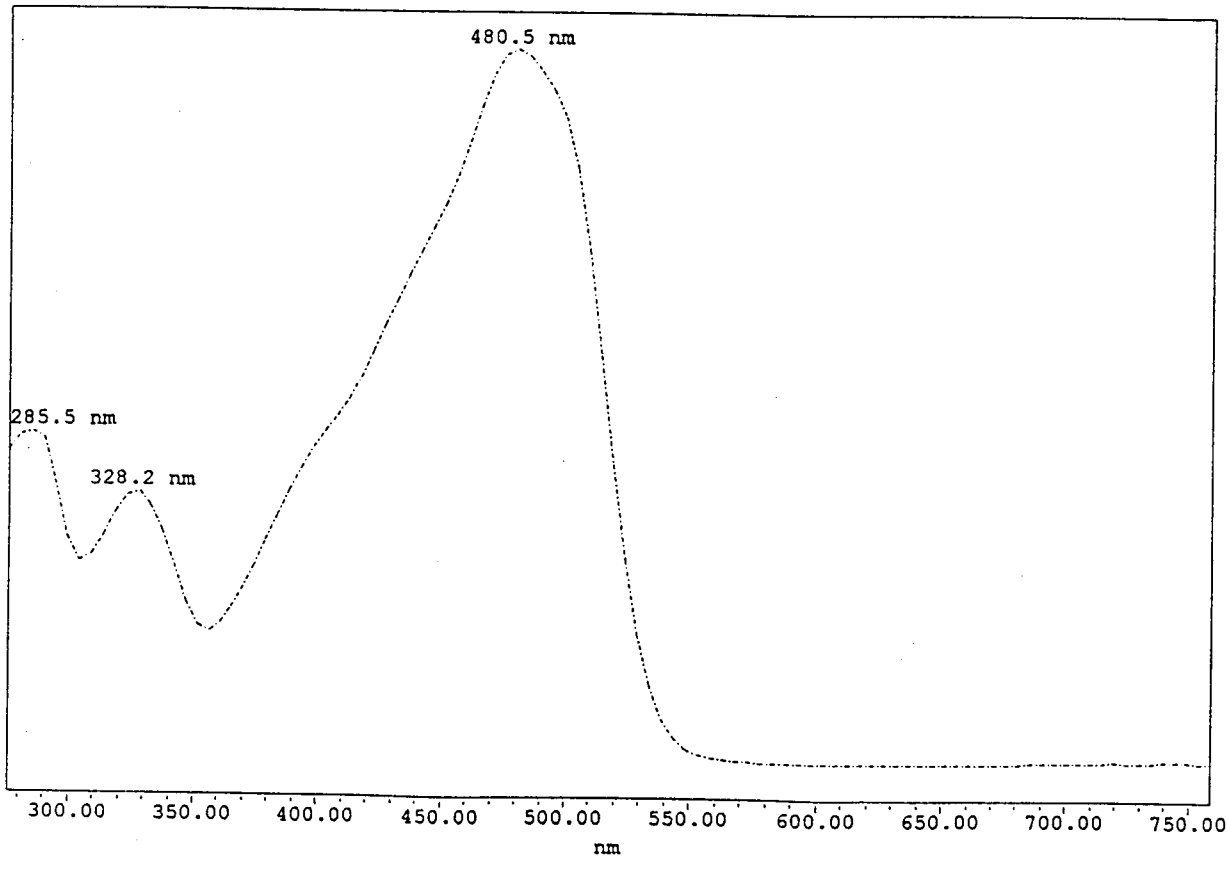




DMU 2637_CI 12010* 22.087 minutes, 275 - 760 @ 4.8 nm, from 2637_12010

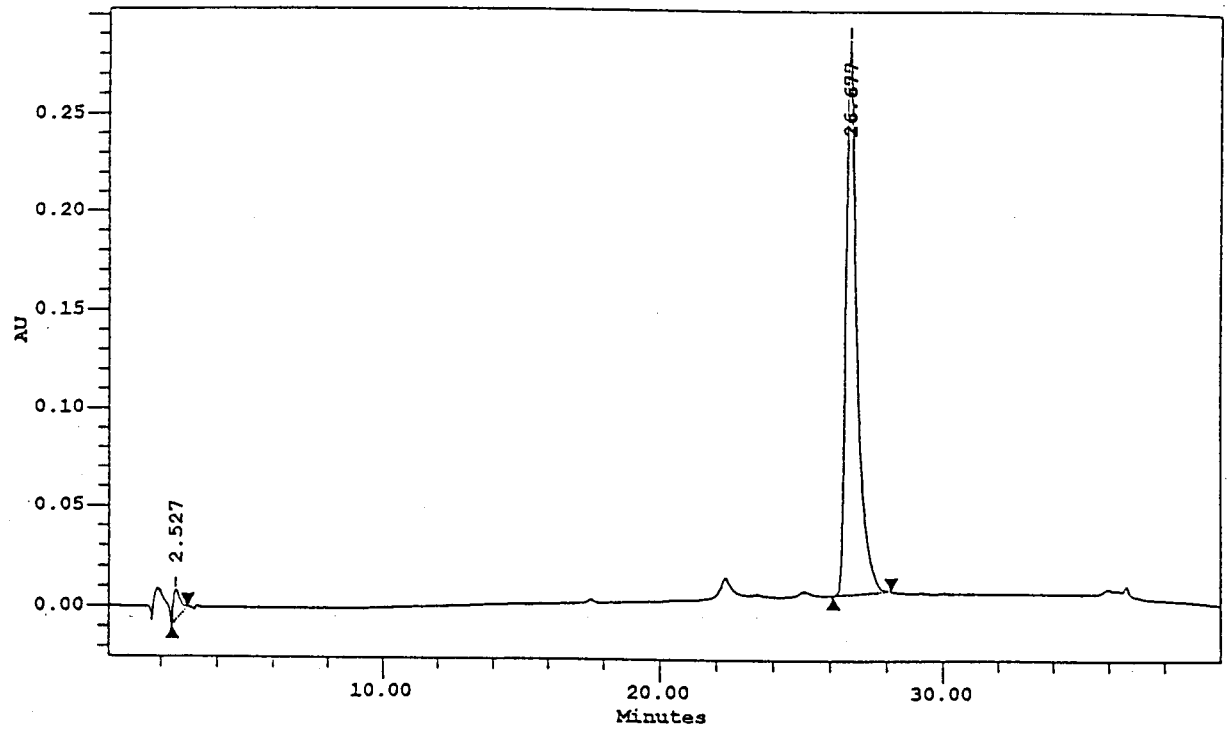
DMU 2596_C.I. 12075

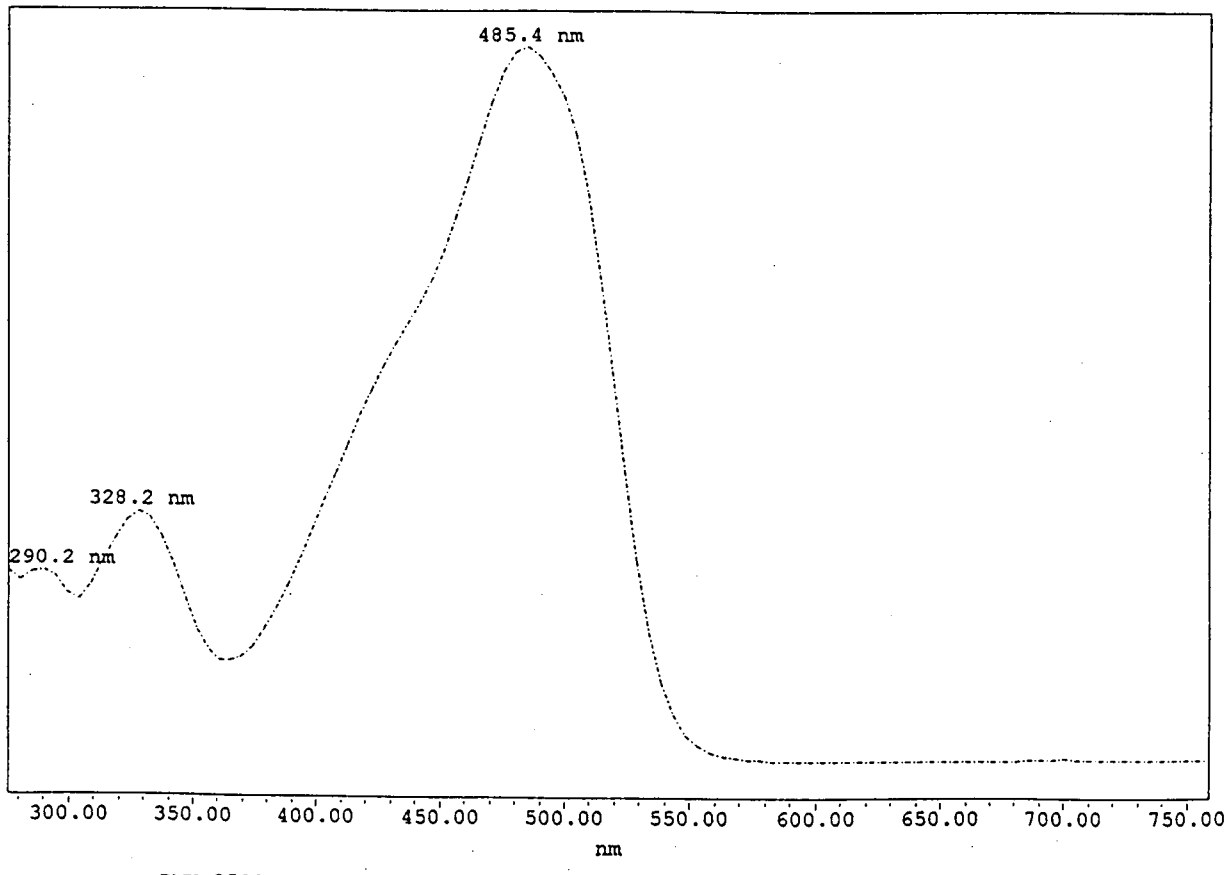




----- DMU 2596_CI 12075* 24.737 minutes, 275 - 760 @ 4.8 nm, from 2596_12075

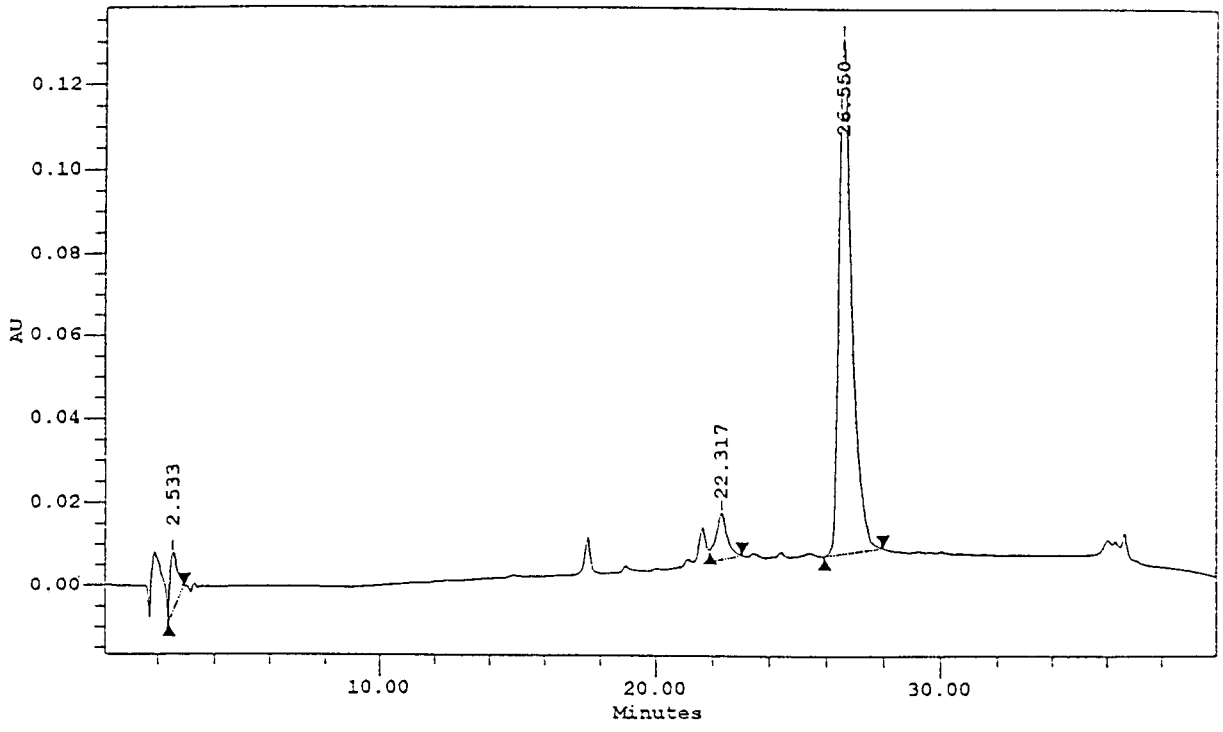
DMU 2599_C.I. 12085

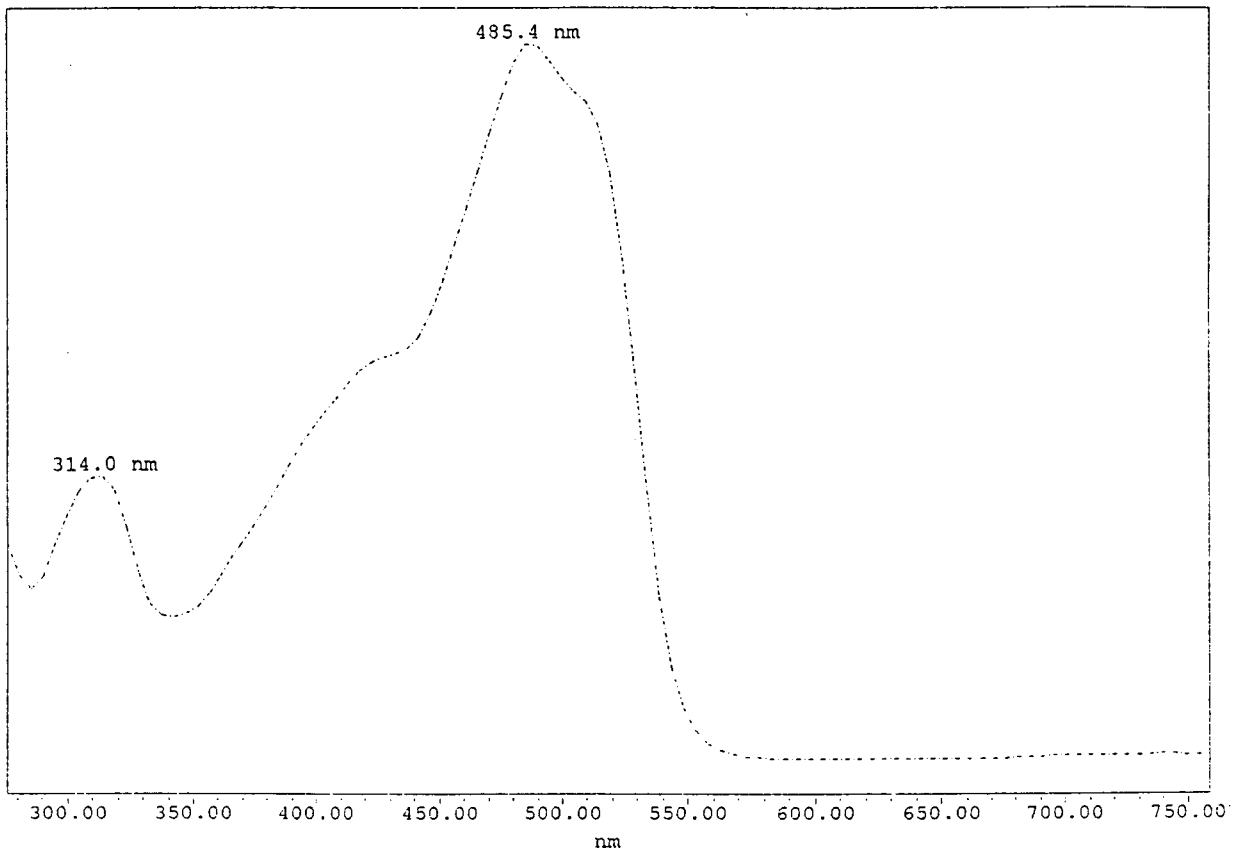




----- DMU 2599_CI 12085 26.677 minutes, 275 - 760 @ 4.8 nm, from 2599_12085

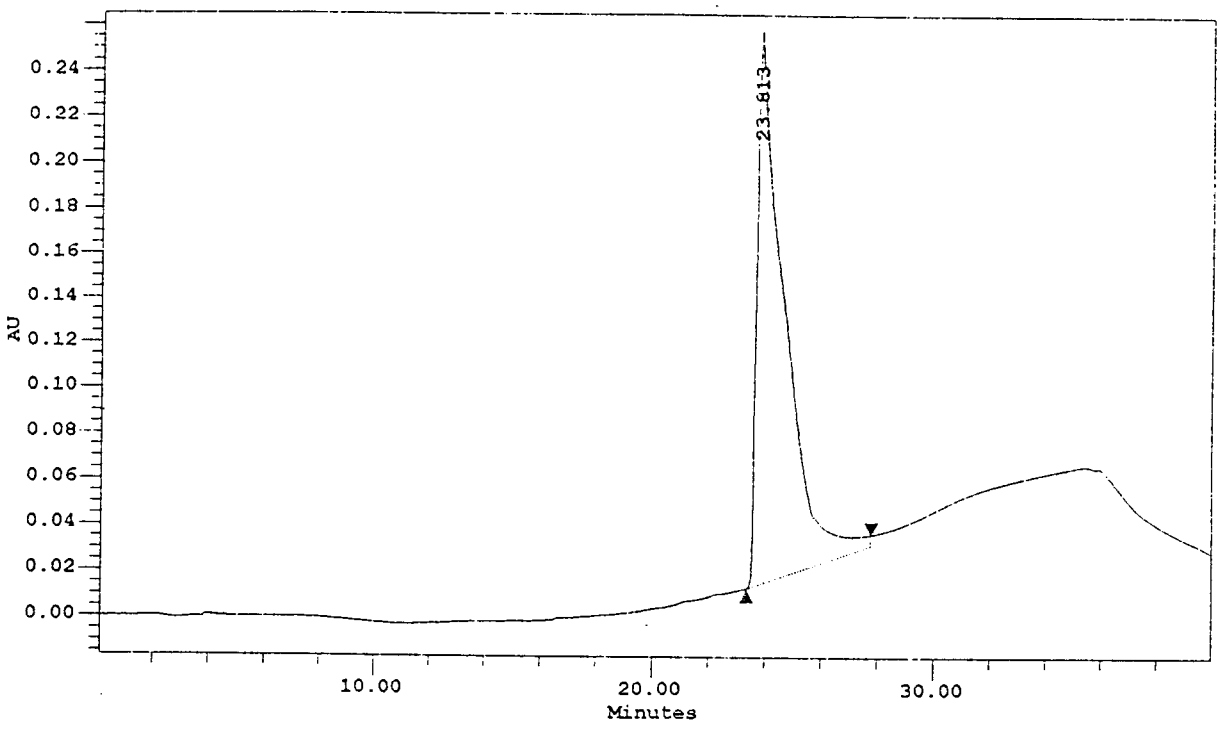
DMU 2572_C.I. 12100

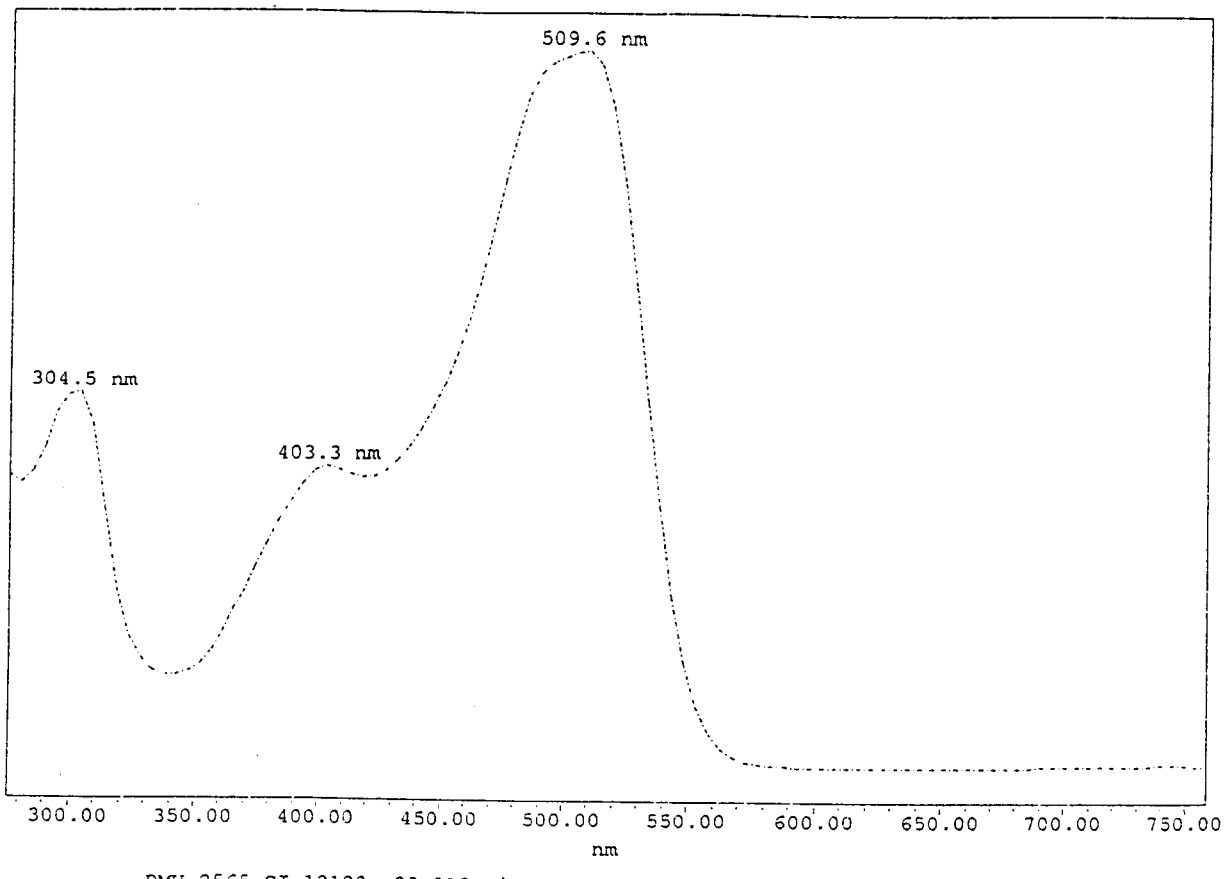




DMU 2572_CI 12100 26.550 minutes, 275 - 760 @ 4.8 nm, from 2572_12100

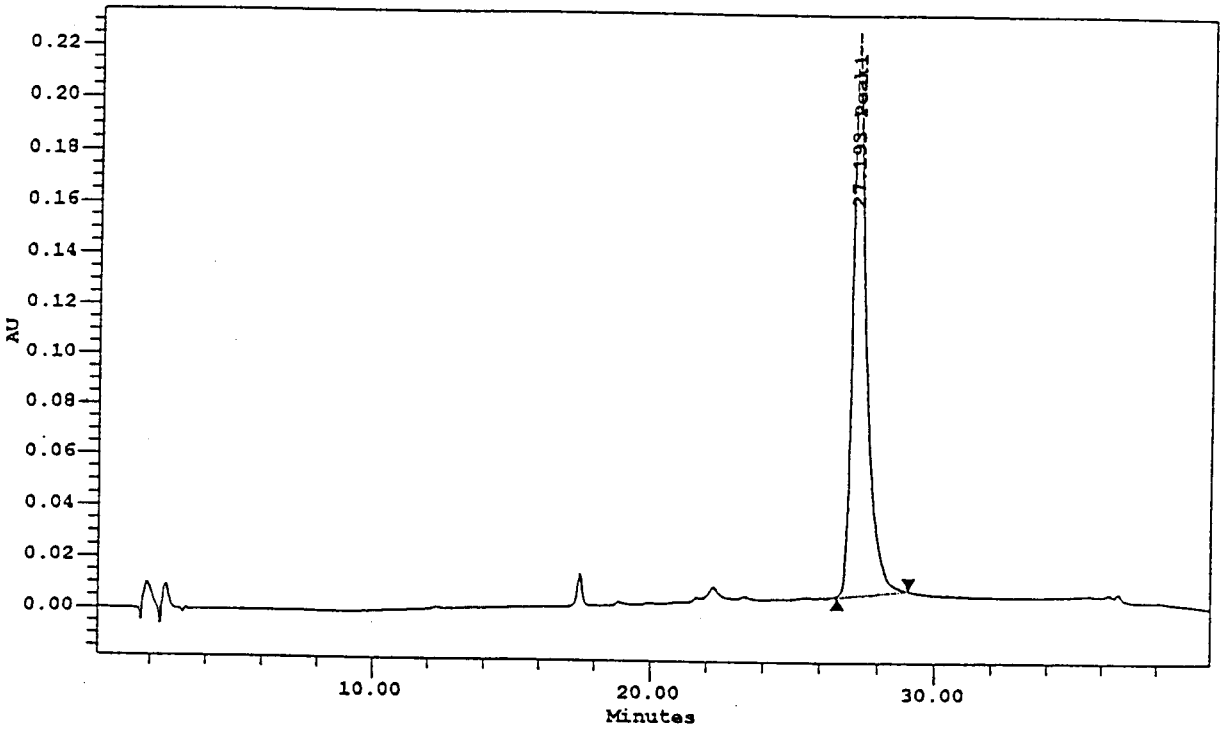
DMU 2565_C.I. 12120

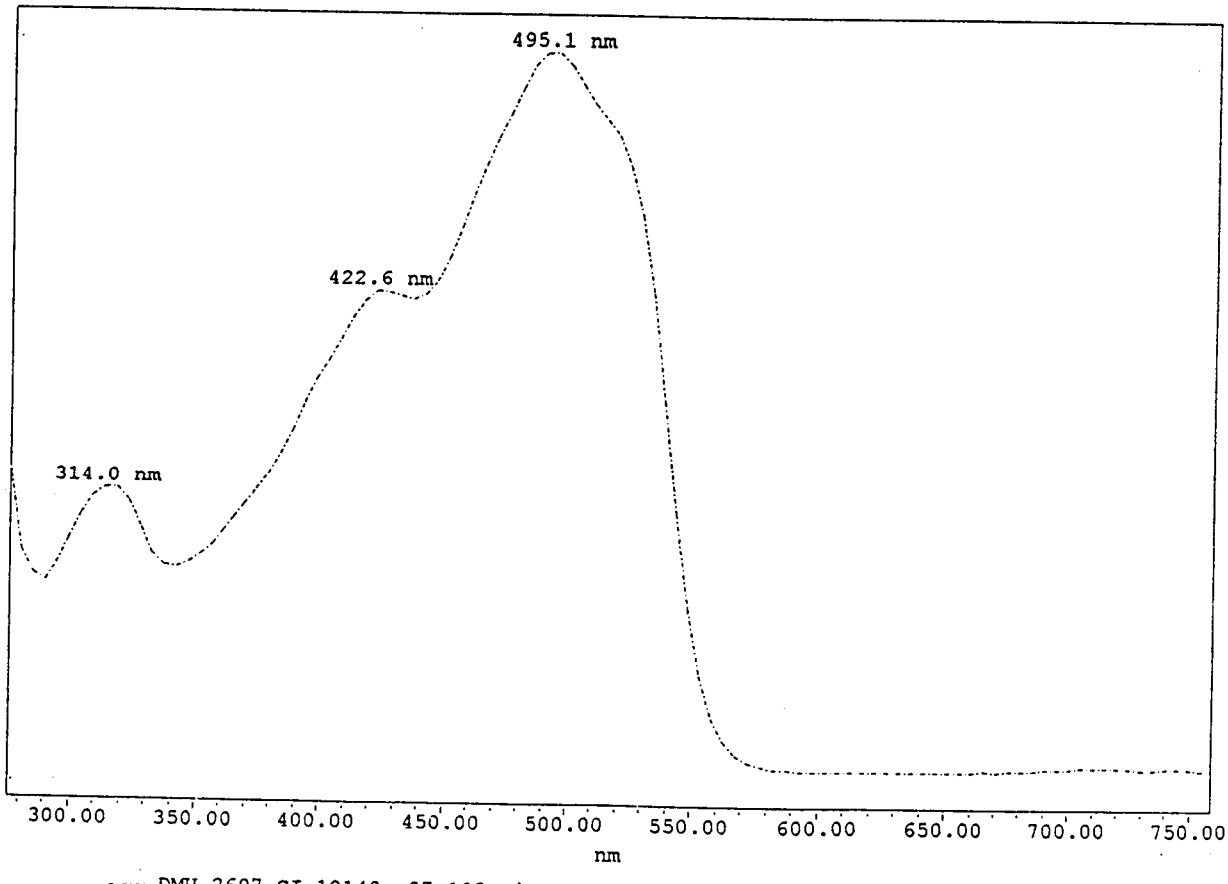




DMU 2565_CI 12120 23.813 minutes, 275 - 760 @ 4.8 nm, from 2565_CI12120

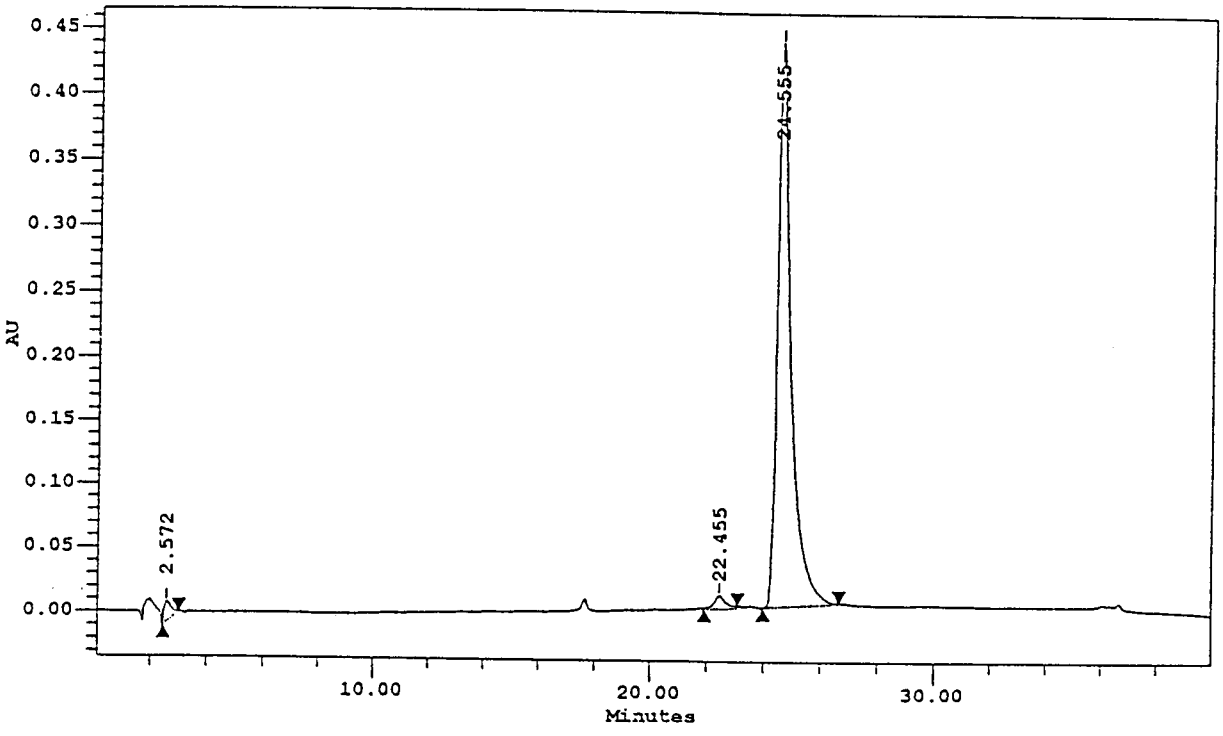
DMU 2697_C.I. 12140

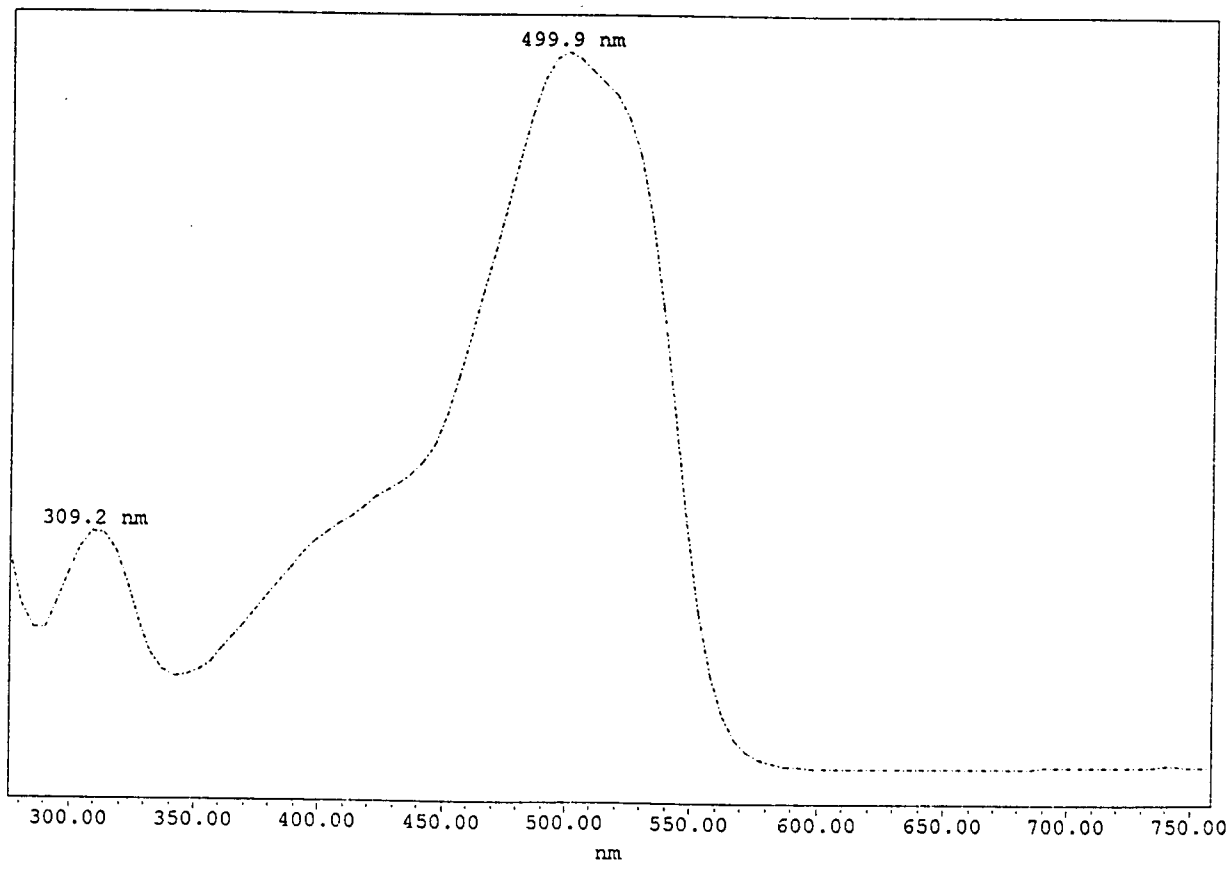




DMU 2697_CI 12140 27.193 minutes, 275 - 760 @ 4.8 nm, from 2697_12140

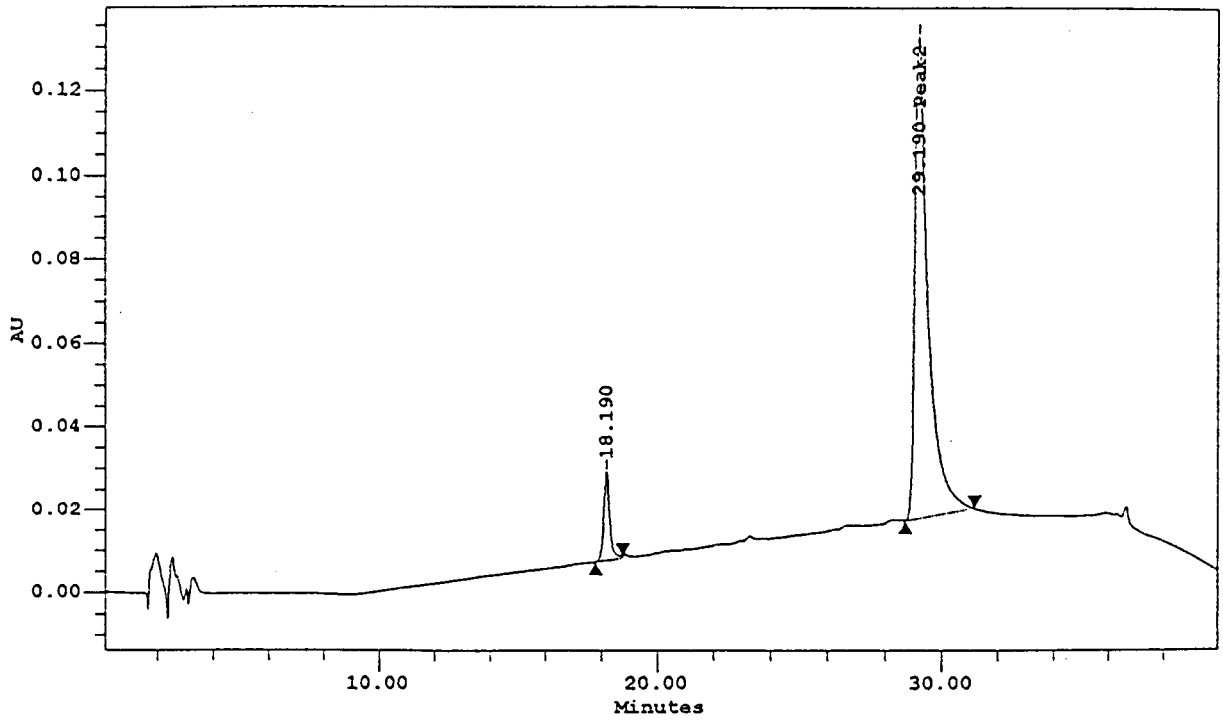
DMU 2660_C.I. 12150

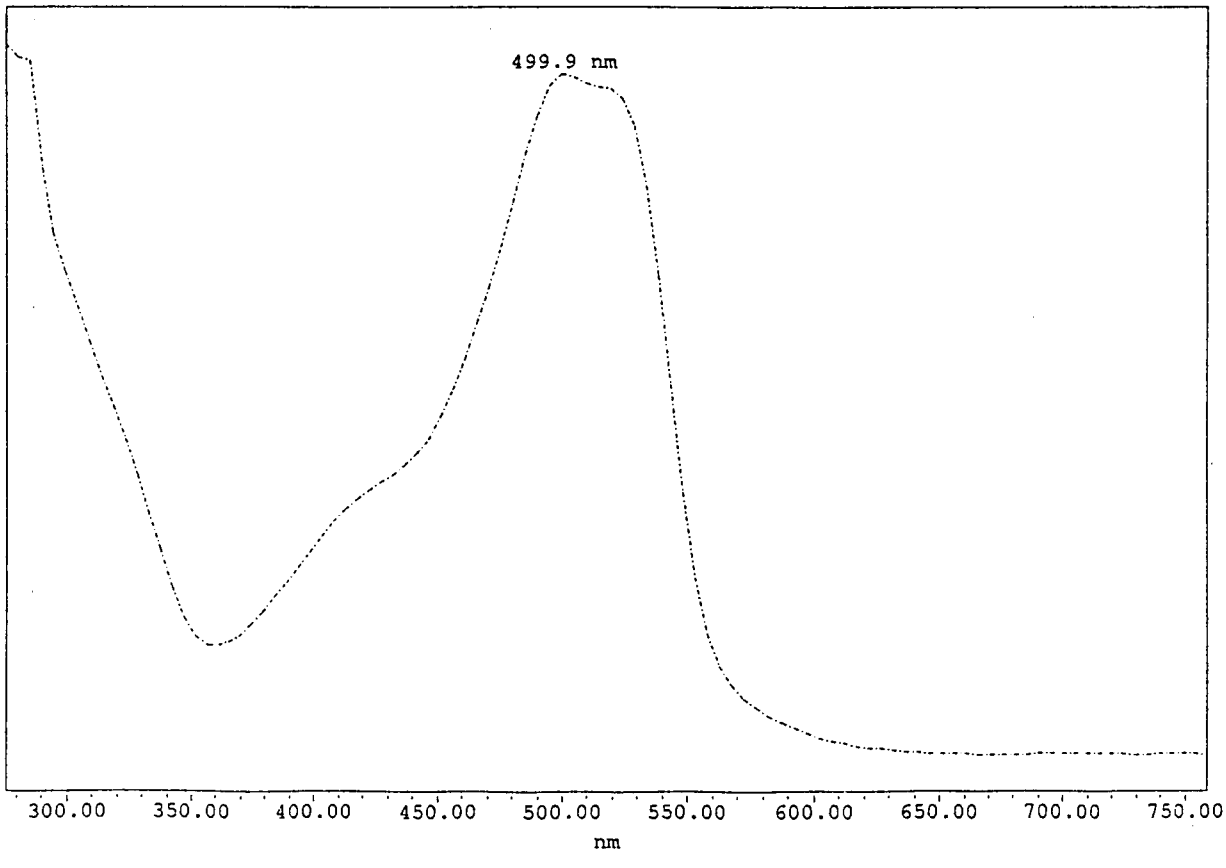




DMU 2660_CI 12150 24.555 minutes, 275 - 760 @ 4.8 nm, from 2660_12150

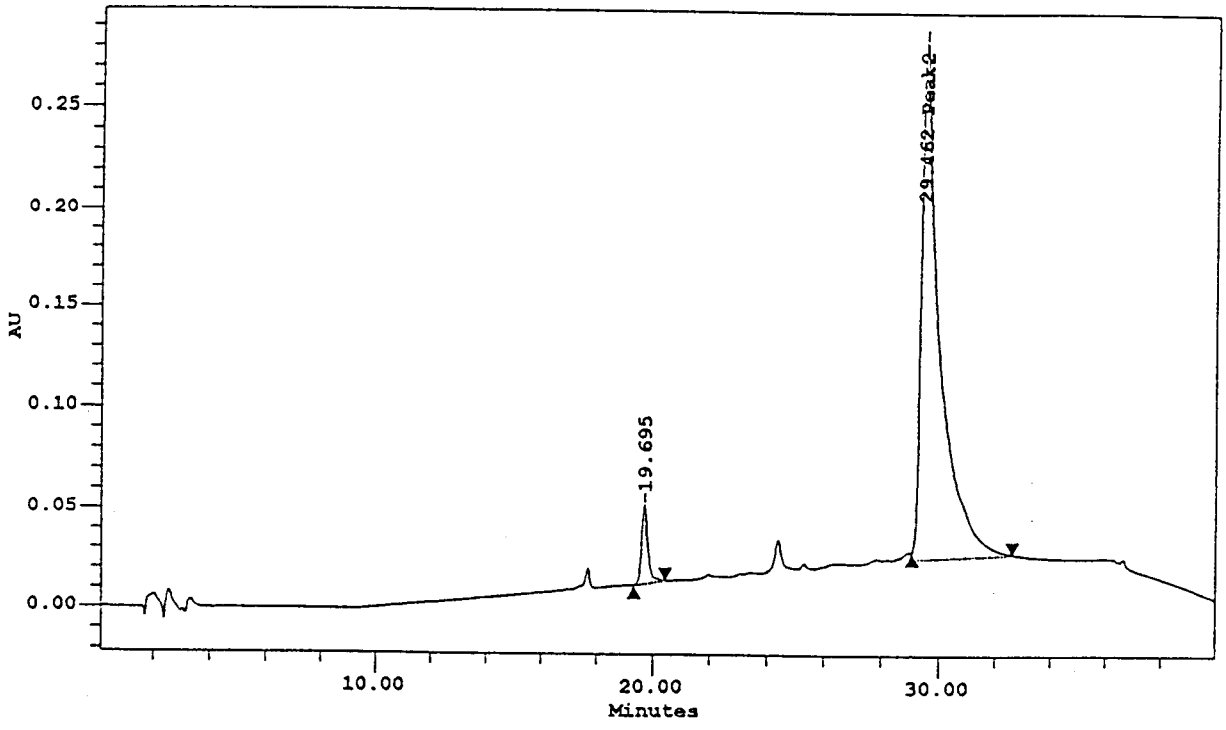
DMU 2721_C.I. 12370

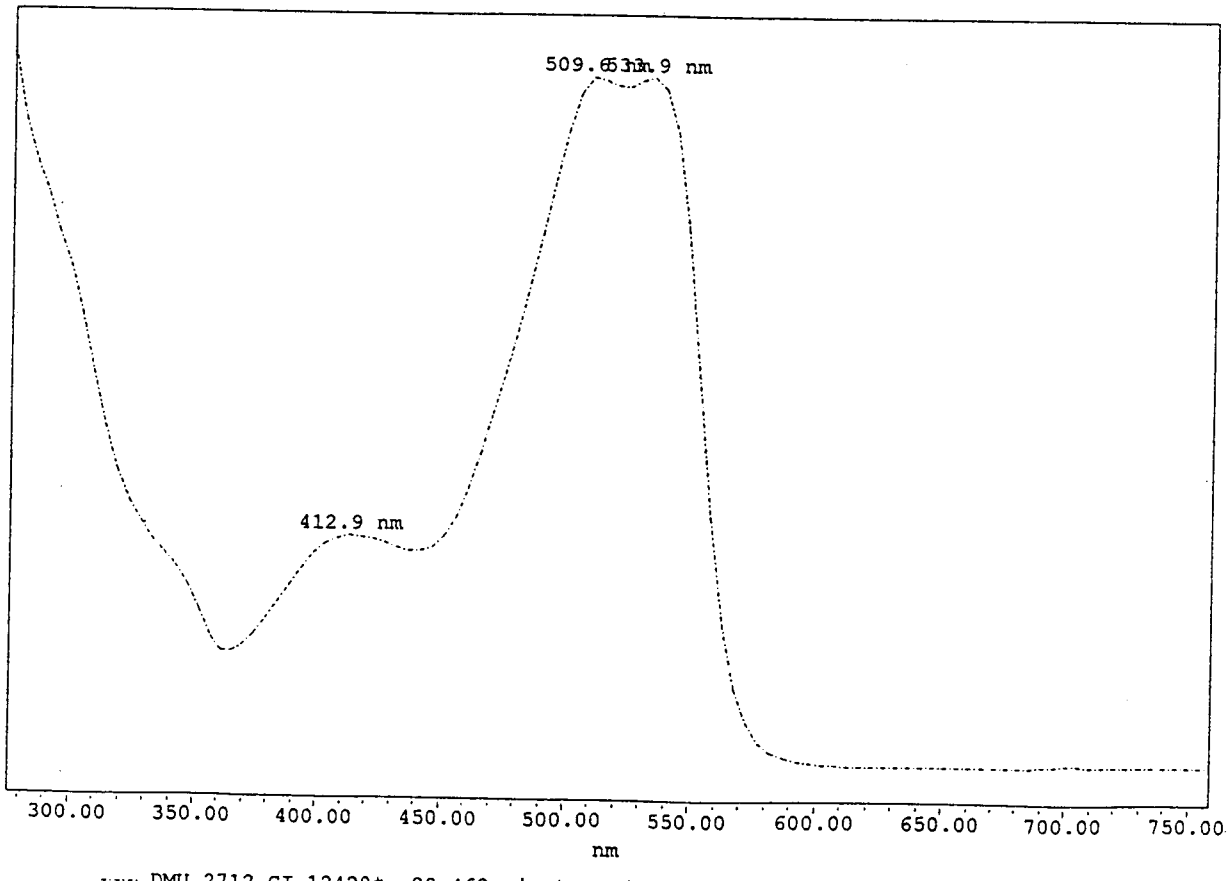




DMU 2721_CI 12370 29.190 minutes, 275 - 760 @ 4.8 nm, from 2721_12370

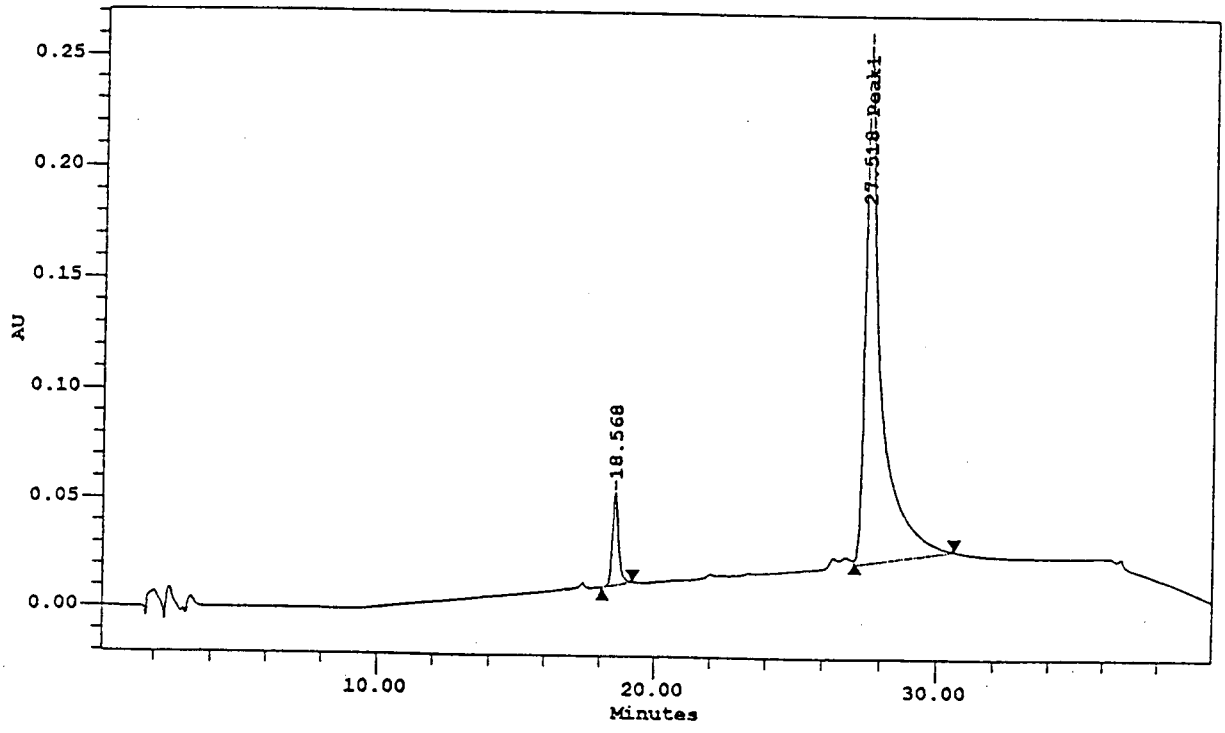
DMU 2712_C.I. 12420

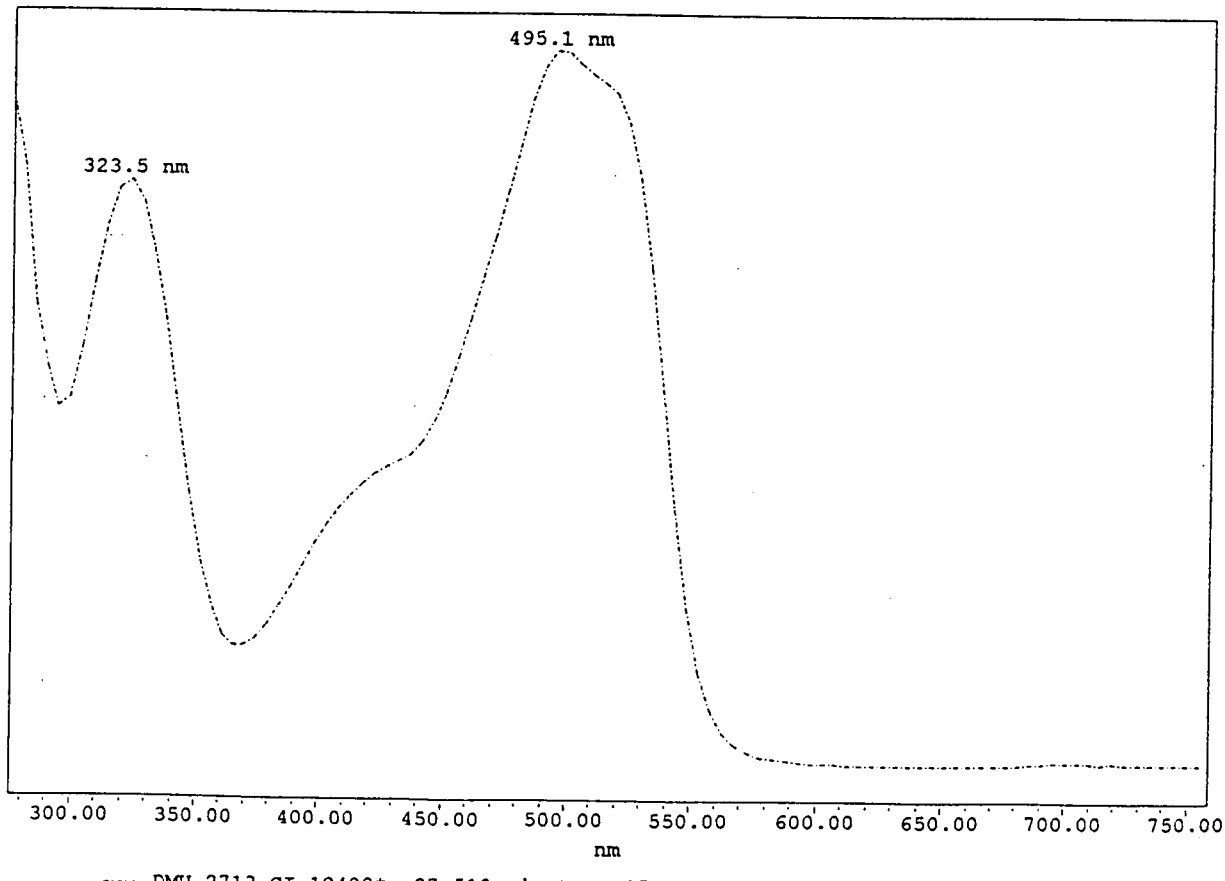




DMU 2712_CI 12420* 29.462 minutes, 275 - 760 @ 4.8 nm, from 2712_12420

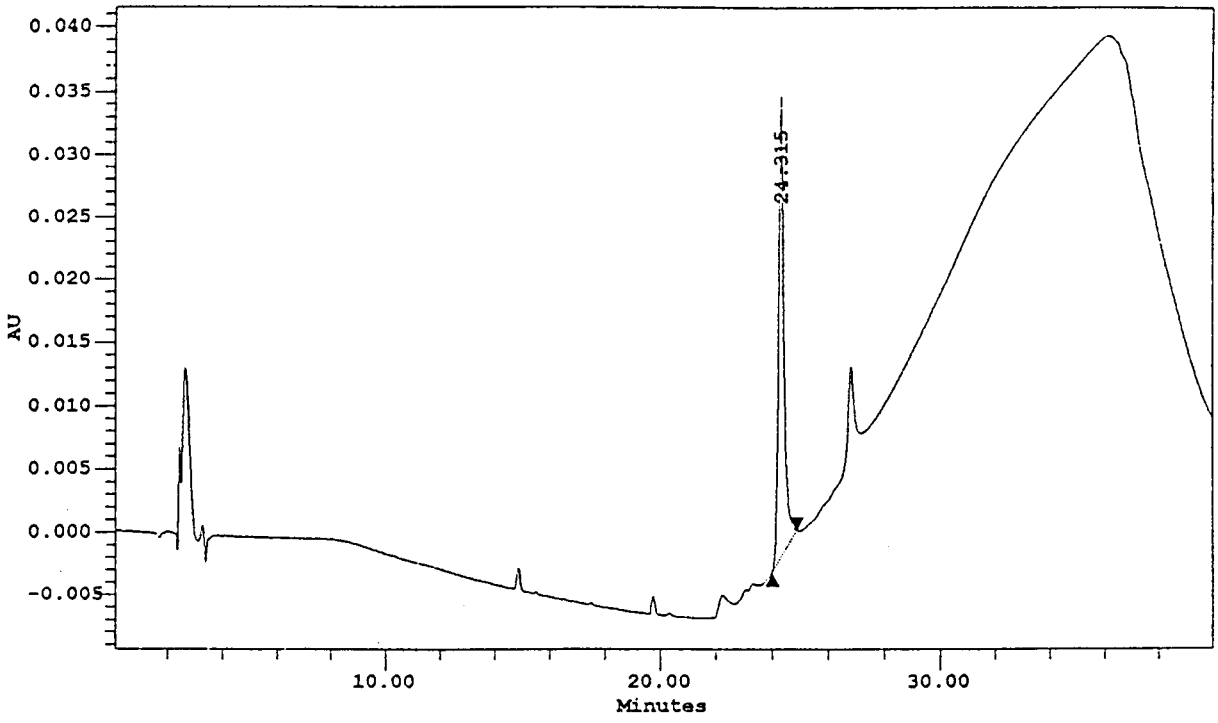
DMU 2713_C.I. 12480

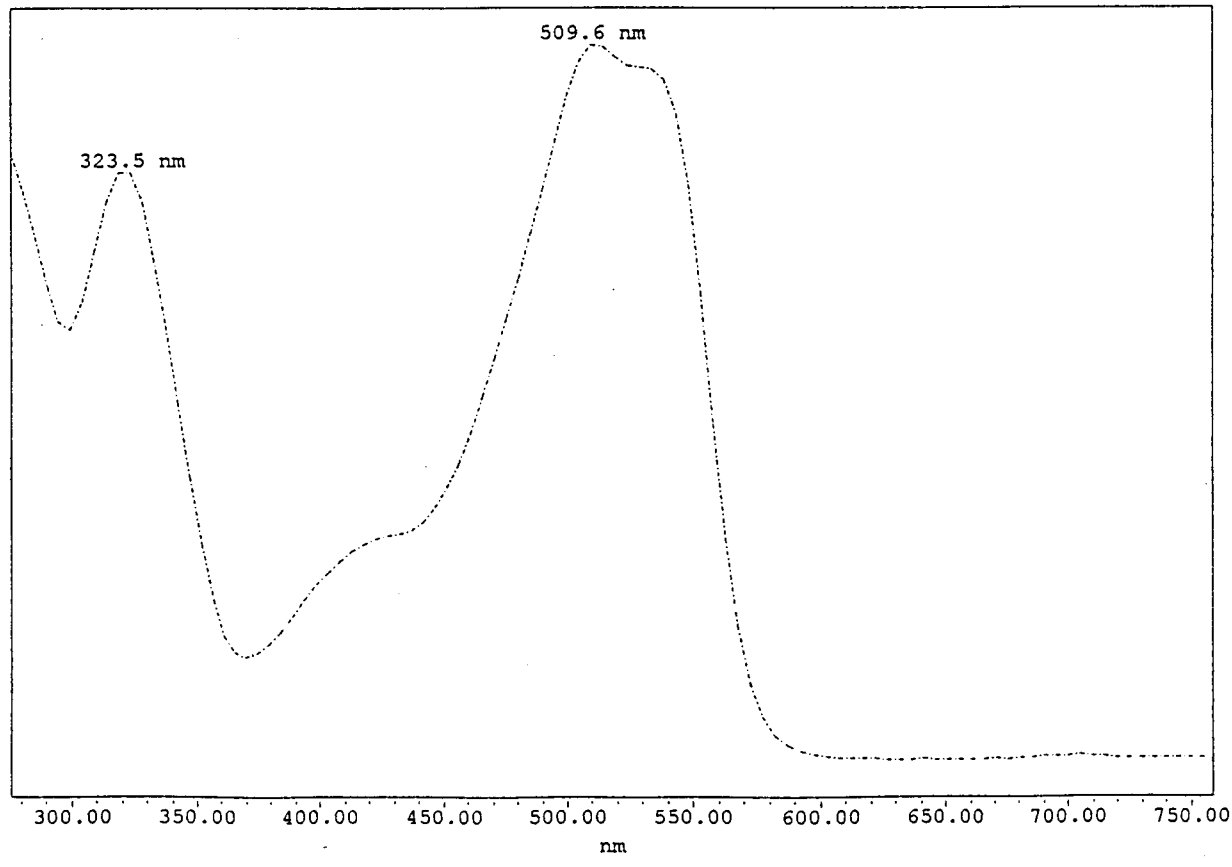




DMU 2713_CI 12480* 27.518 minutes, 275 - 760 @ 4.8 nm, from 2713_12480

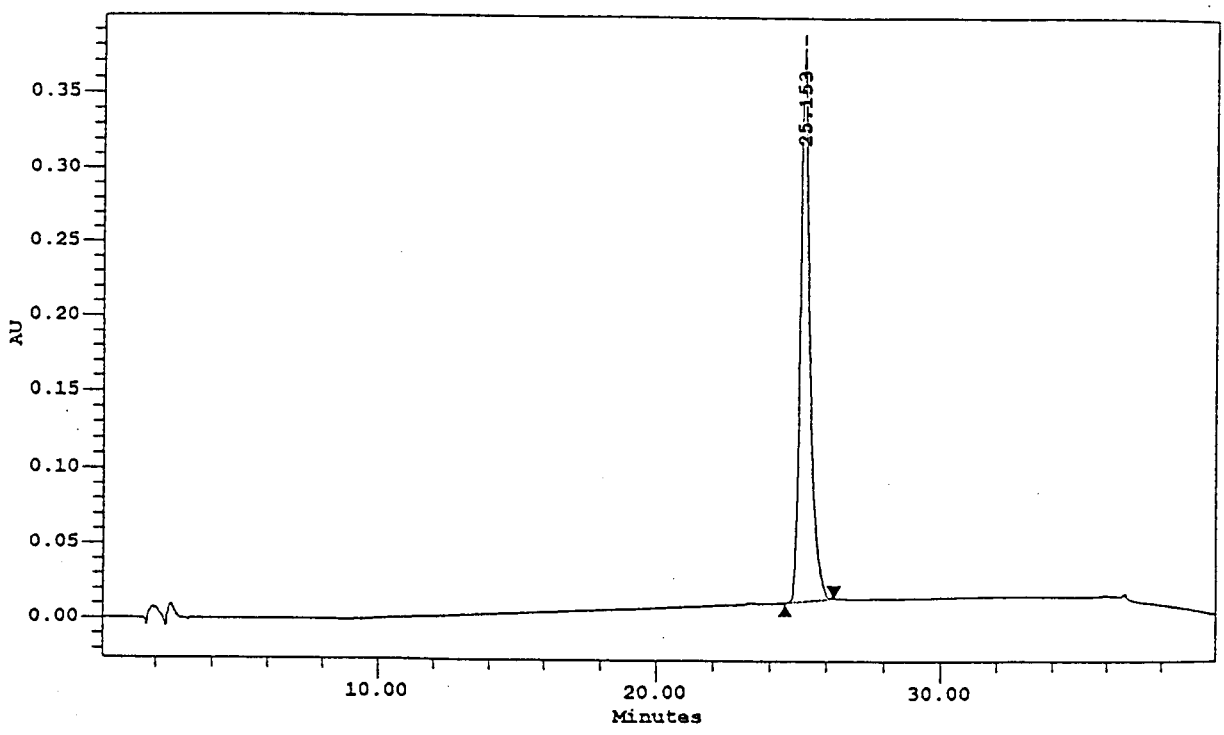
DMU 2722_C.I. 12490

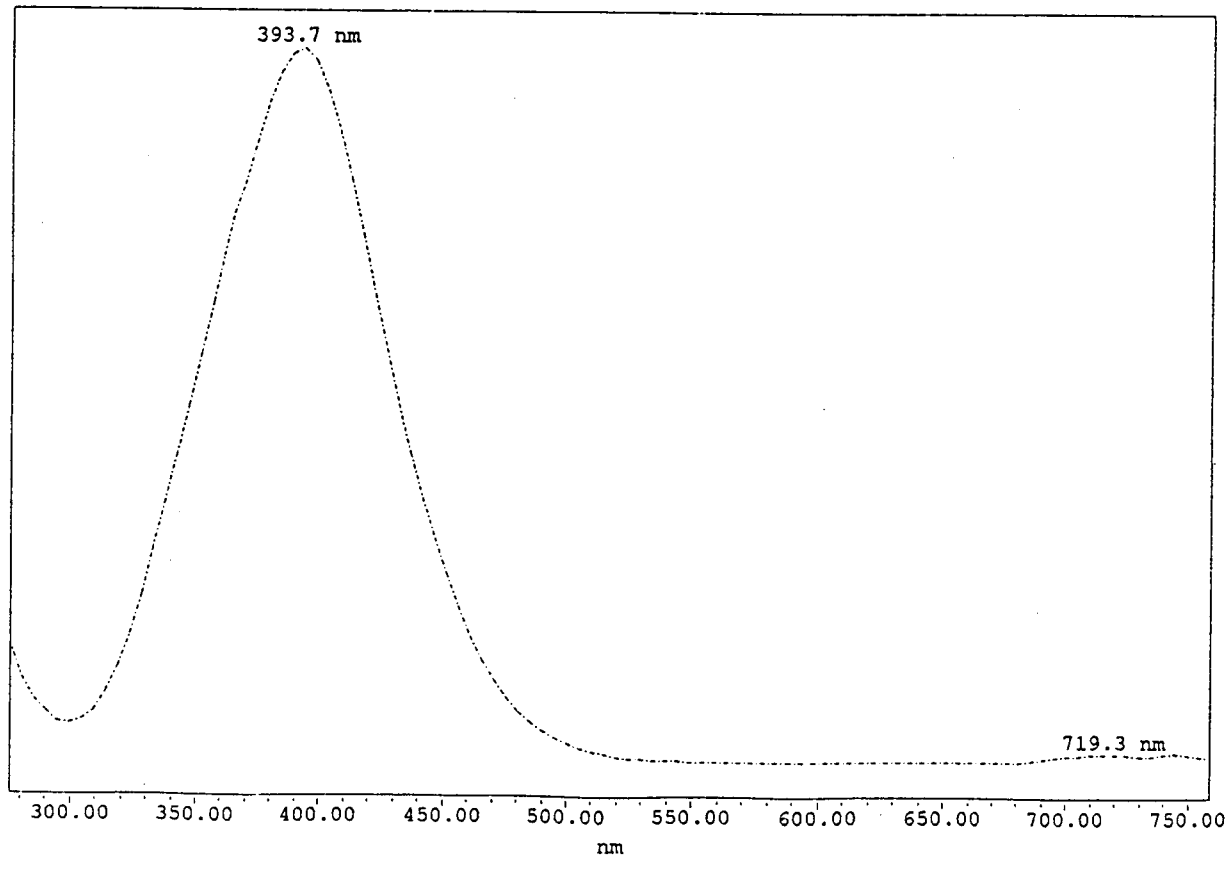




DMU 2722_CI 12490 24.315 minutes, 275 - 760 @ 4.8 nm, from 2722_CI12490

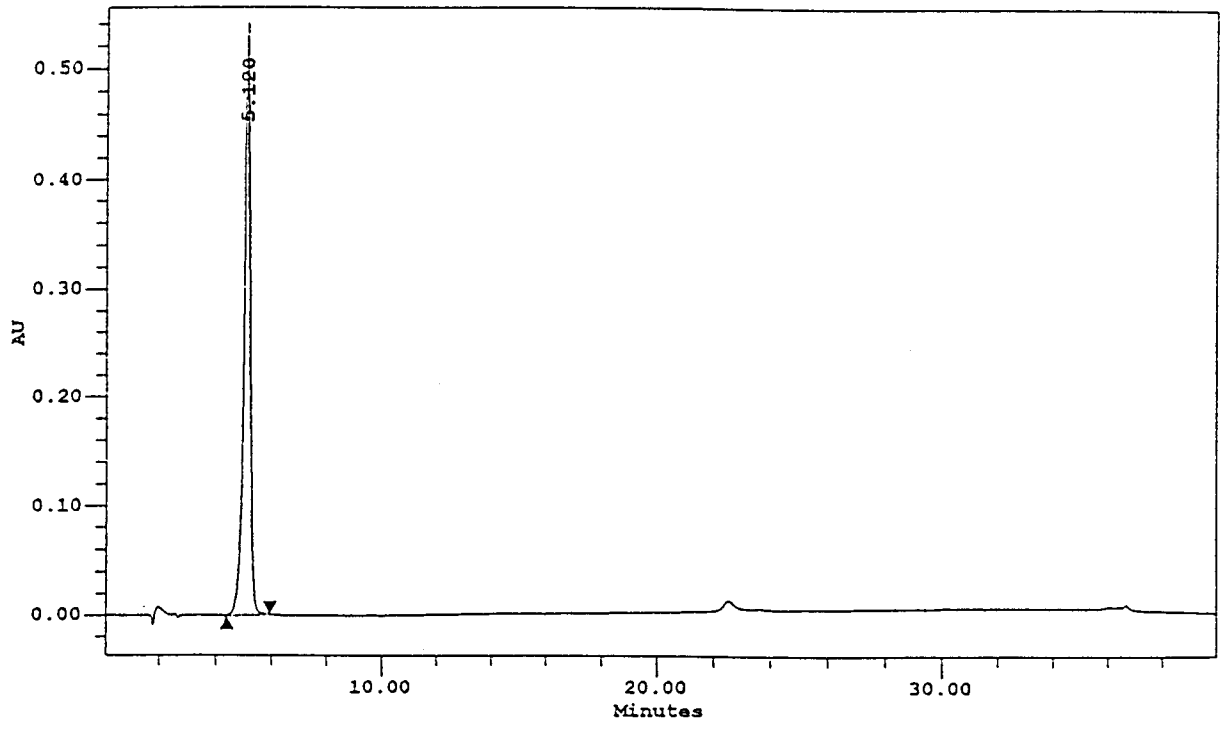
DMU 2705_C.I. 12700

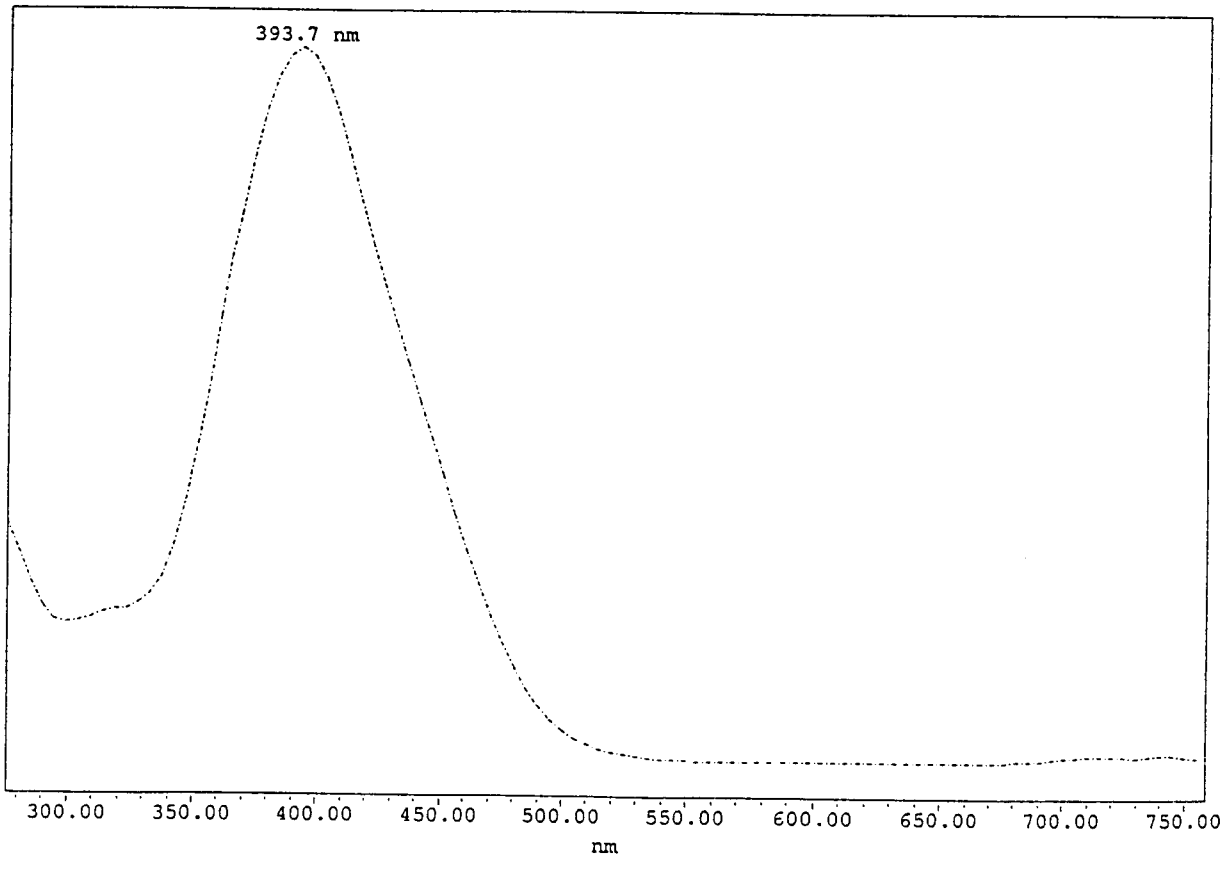




DMU 2705_CI 12700 25.153 minutes, 275 - 760 @ 4.8 nm, from 2705_12700

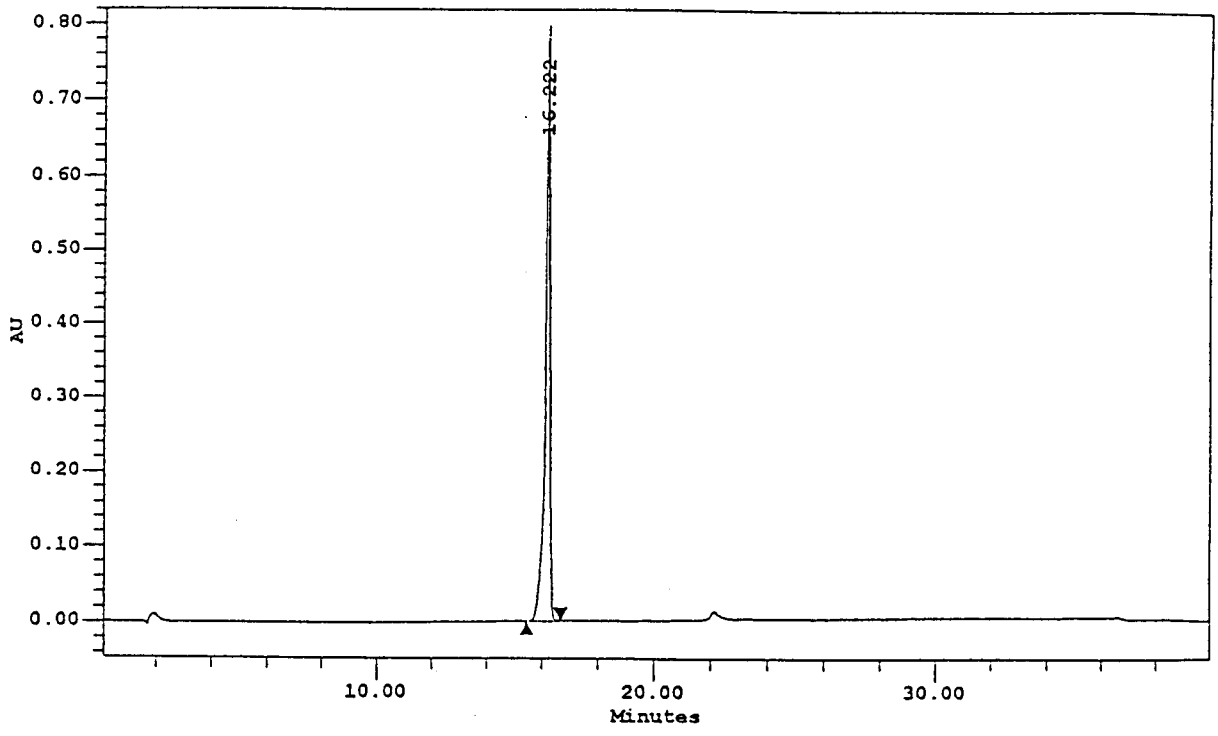
DMU 2656_C.I. 13015

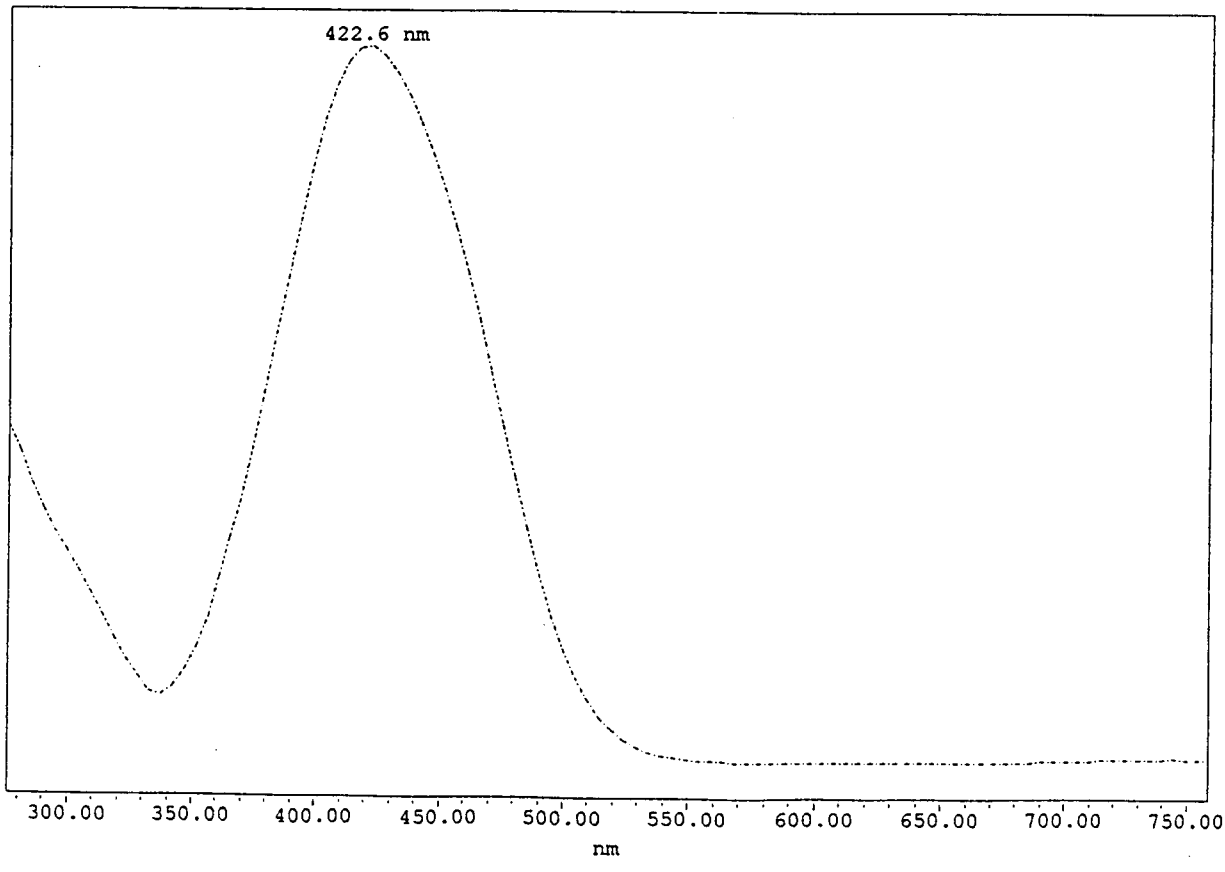




DMU 2656_CI 13015 5.120 minutes, 275 - 760 @ 4.8 nm, from 2656_13015

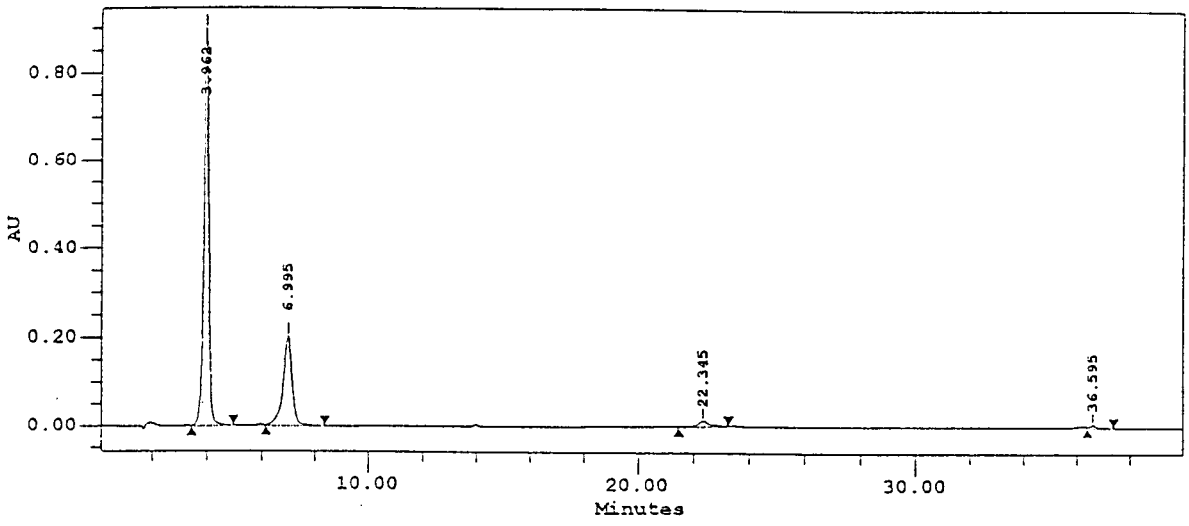
DMU 2699_C.I. 13065

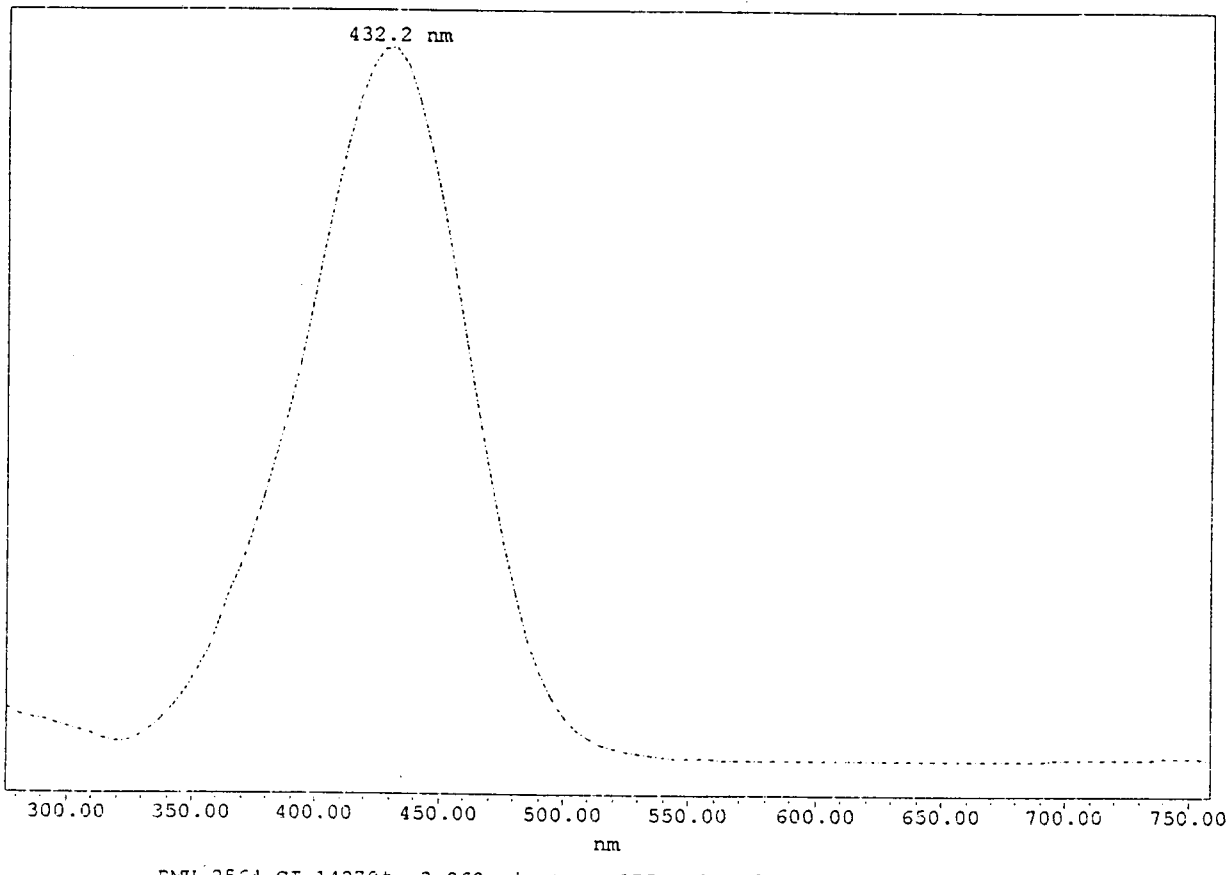




DMU 2699_CI 13065 16.222 minutes, 275 - 760 @ 4.8 nm, from 2699_13065

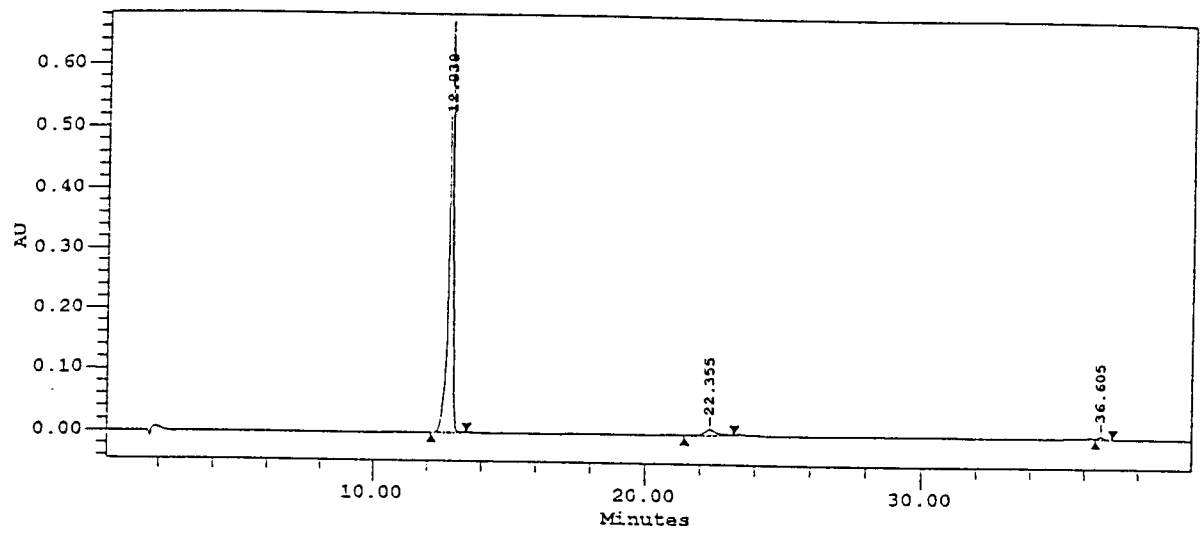
DMU 2564_C.I. 14270

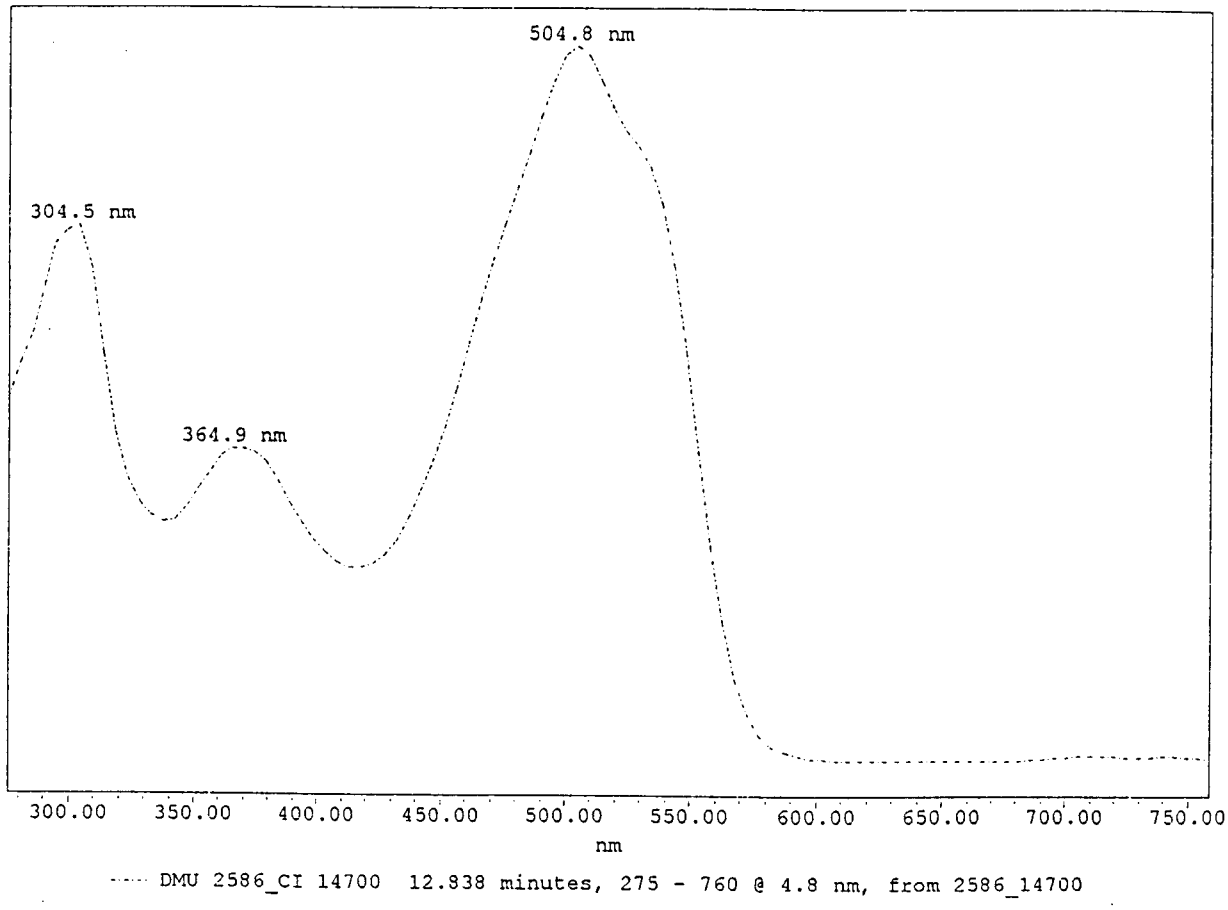




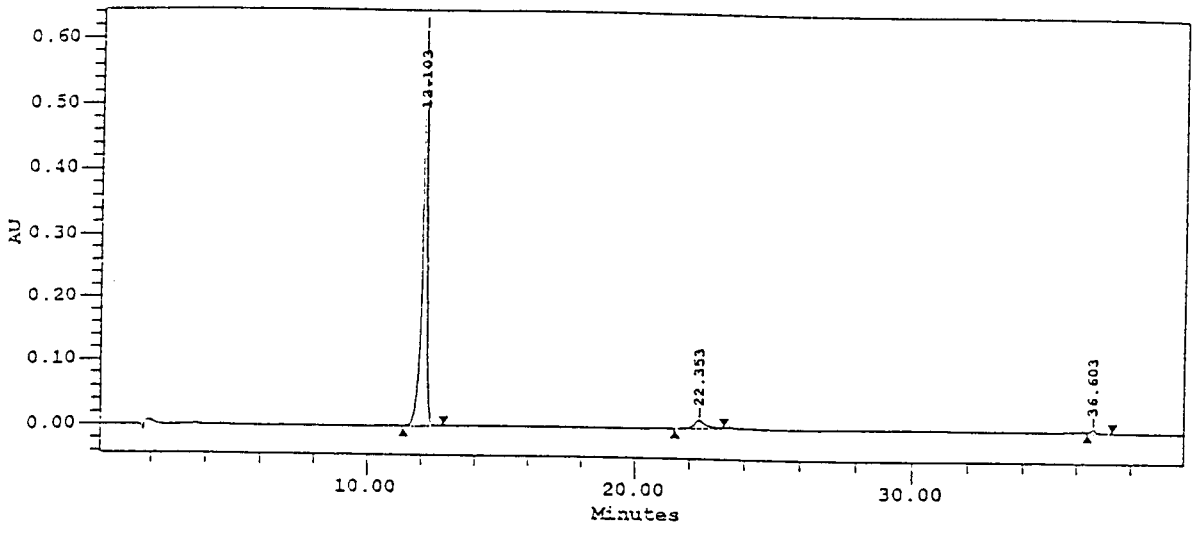
--- DMU 2564_CI 14270* 3.962 minutes, 275 - 760 @ 4.8 nm, from 2564_14270

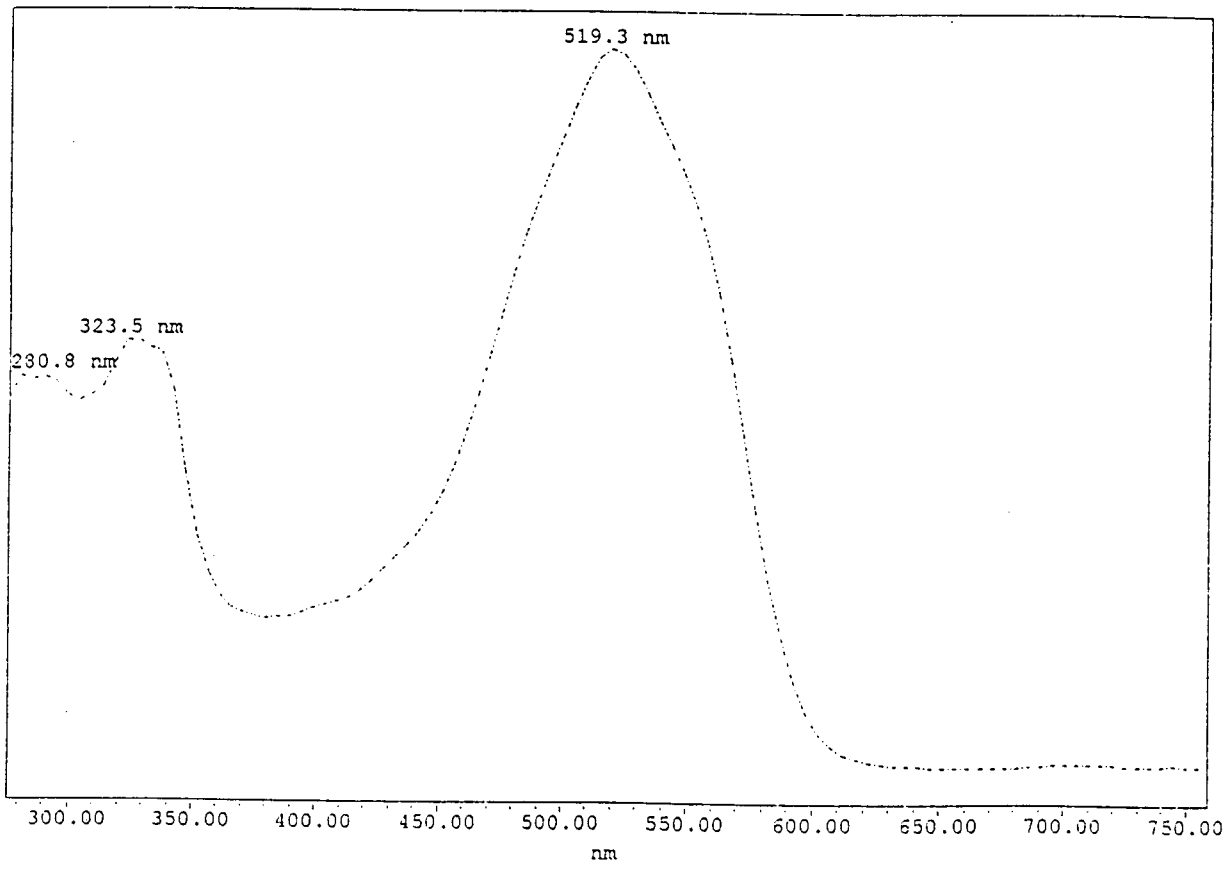
DMU 2586_C.I. 14700





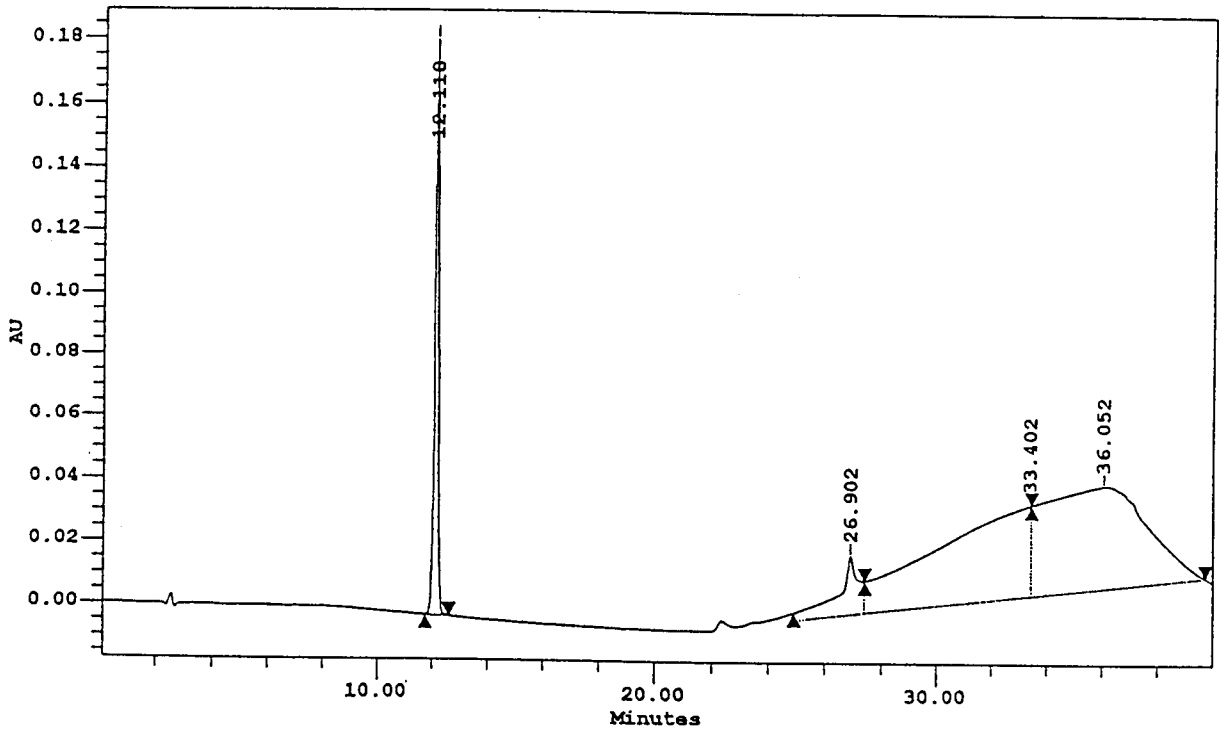
DMU 2575_C.I. 14720

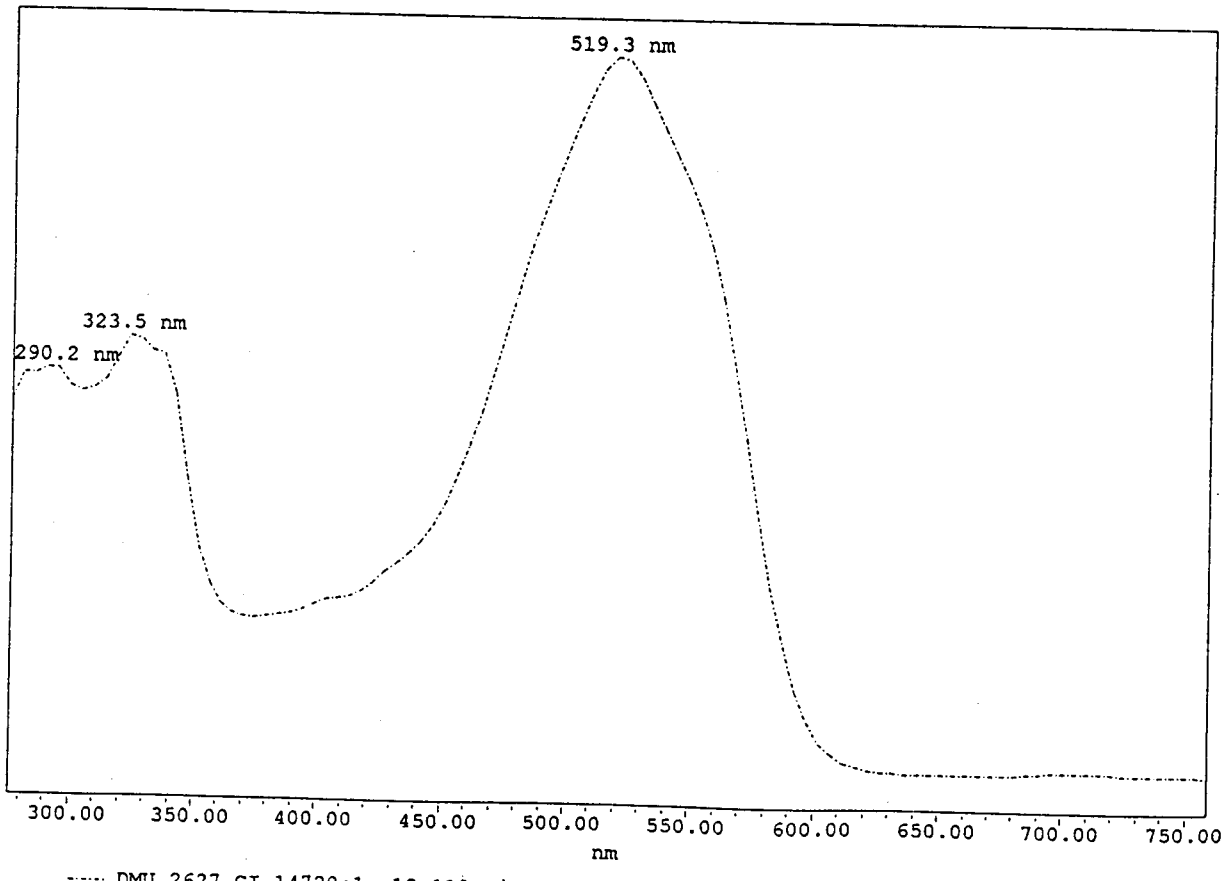




--- DMU 2575_CI 14720 12.103 minutes, 275 - 760 @ 4.8 nm, from 2575_14720

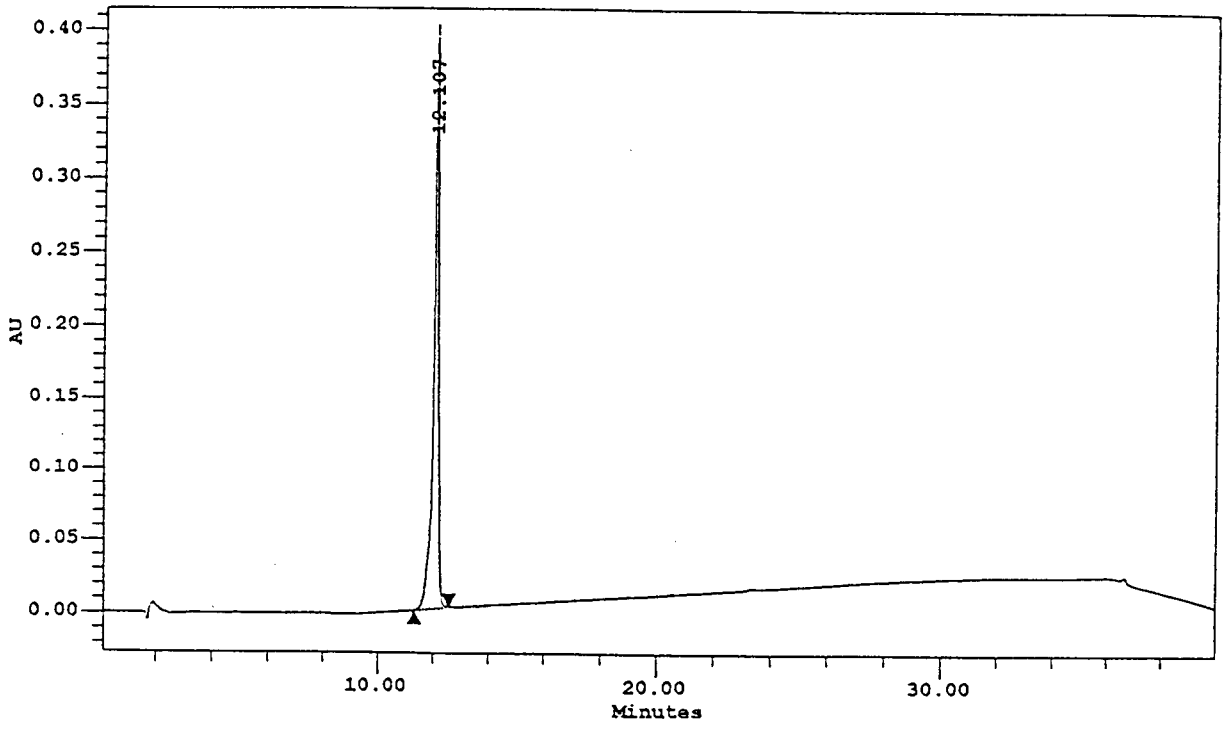
DMU 2627_C.I. 14720:1

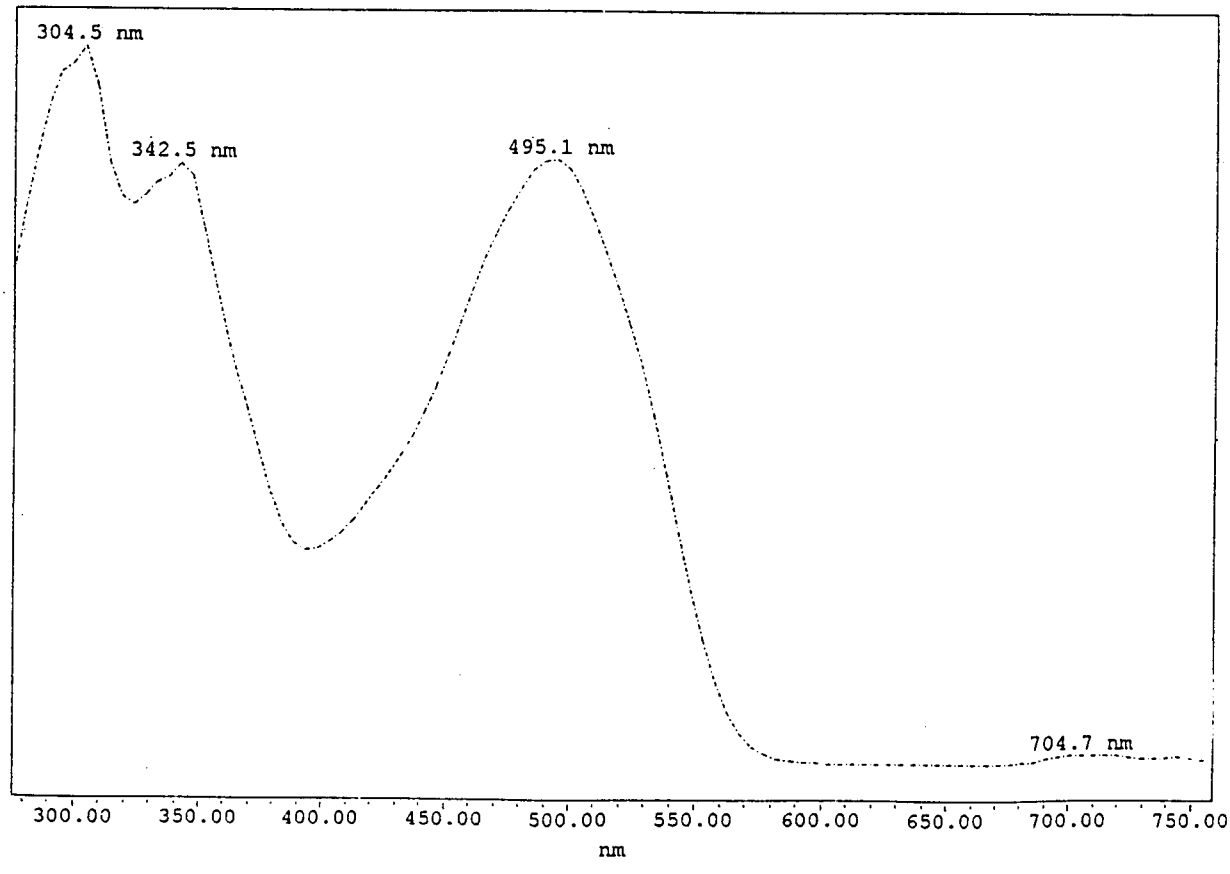




DMU 2627_CI 14720:1 12.118 minutes, 275 - 760 @ 4.8 nm, from 2627_CI14720:1

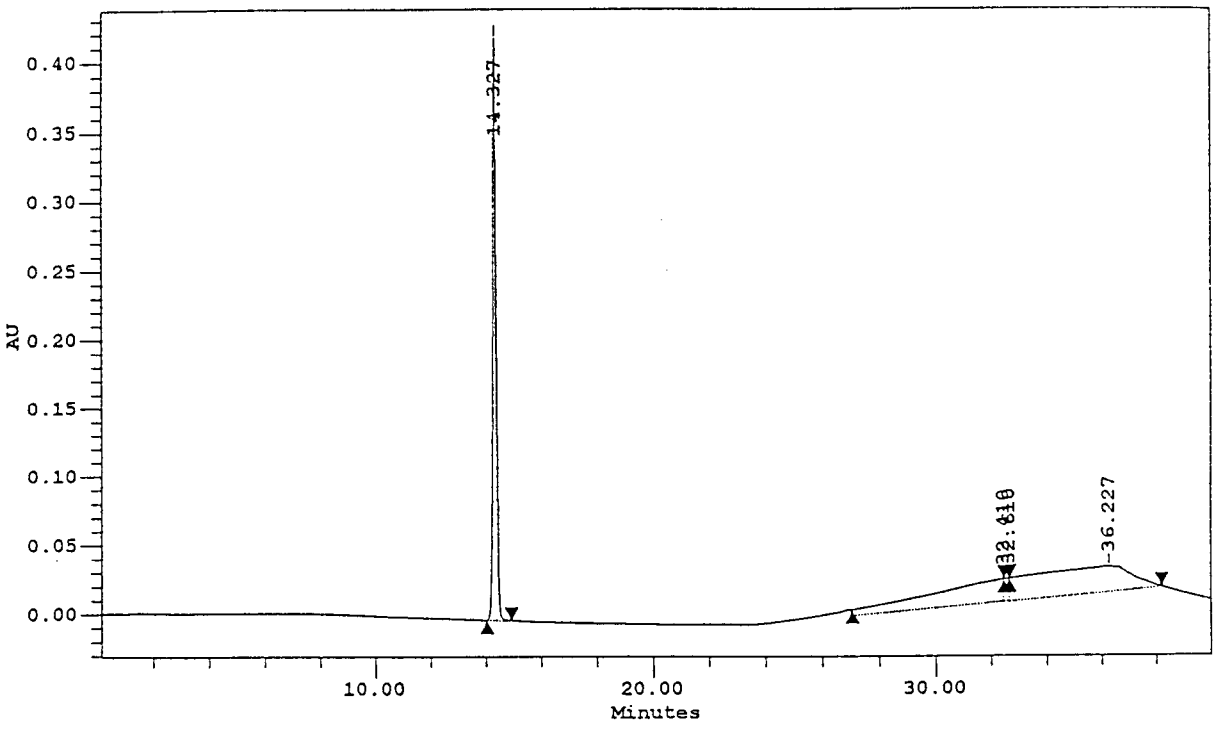
DMU 2702_C.I. 14815

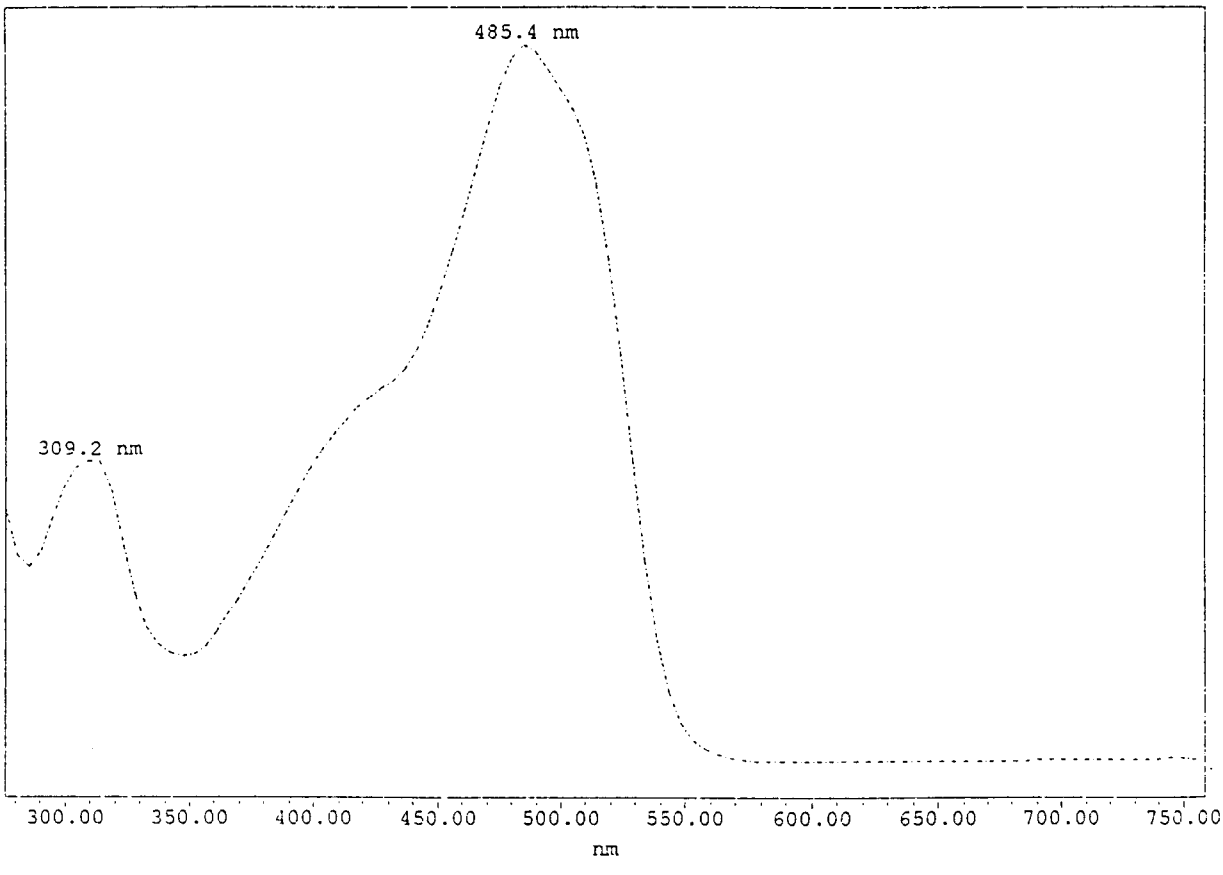




---- DMU 2702_CI 14815 12.107 minutes, 275 - 760 @ 4.8 nm, from 2702_14815

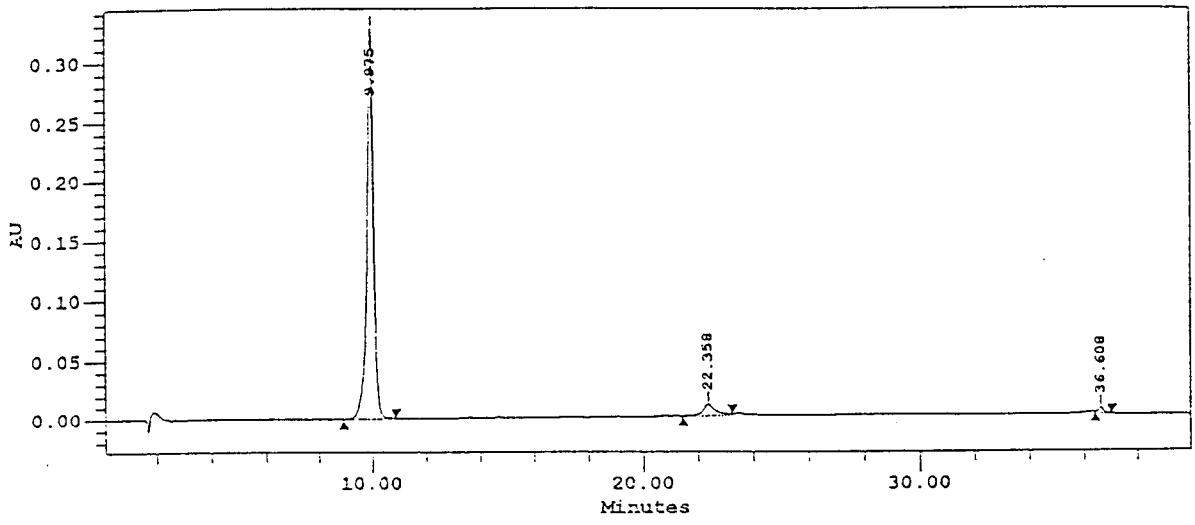
DMU 2573_C.I. 15510

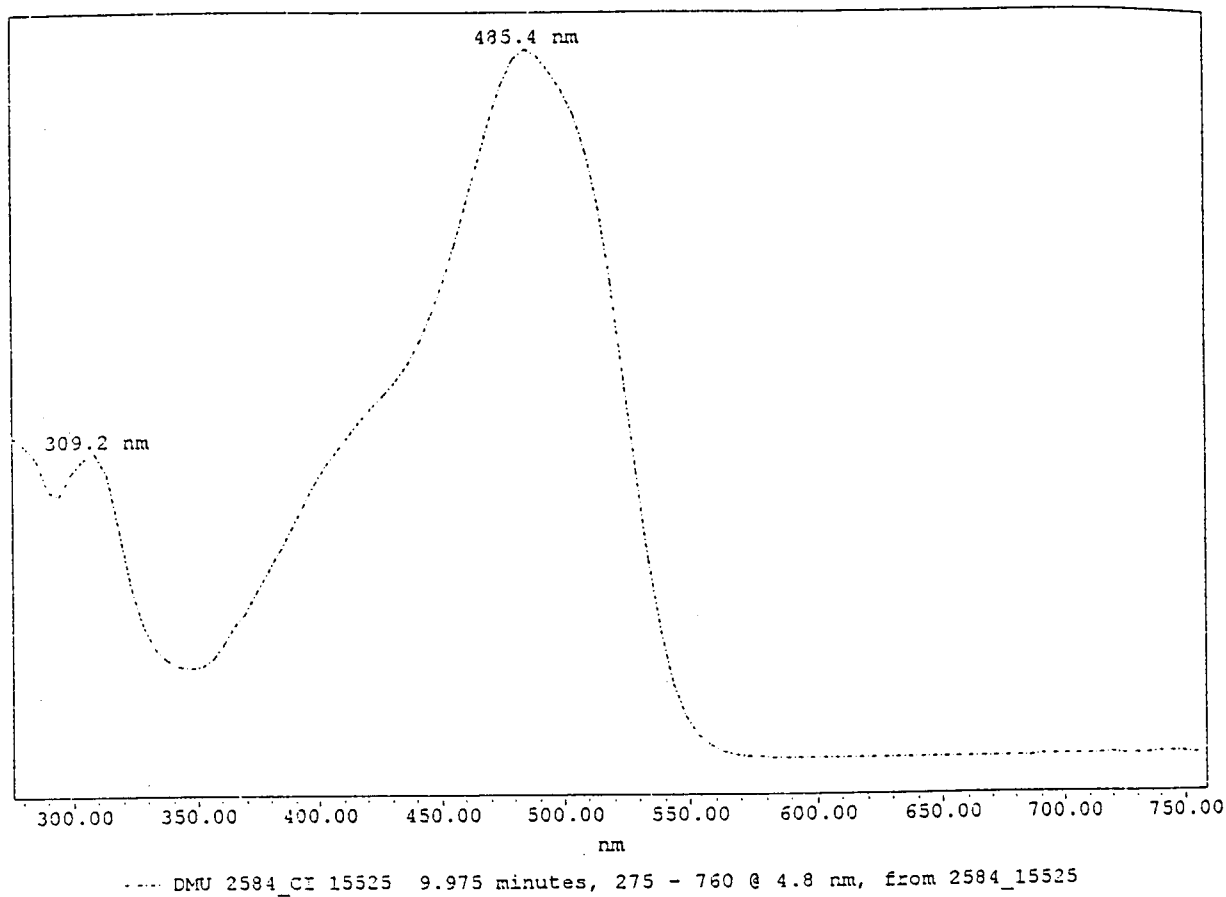




---- DMU 2573_CI 15510 14.327 minutes, 275 - 760 @ 4.8 nm, from 2573_CI15510

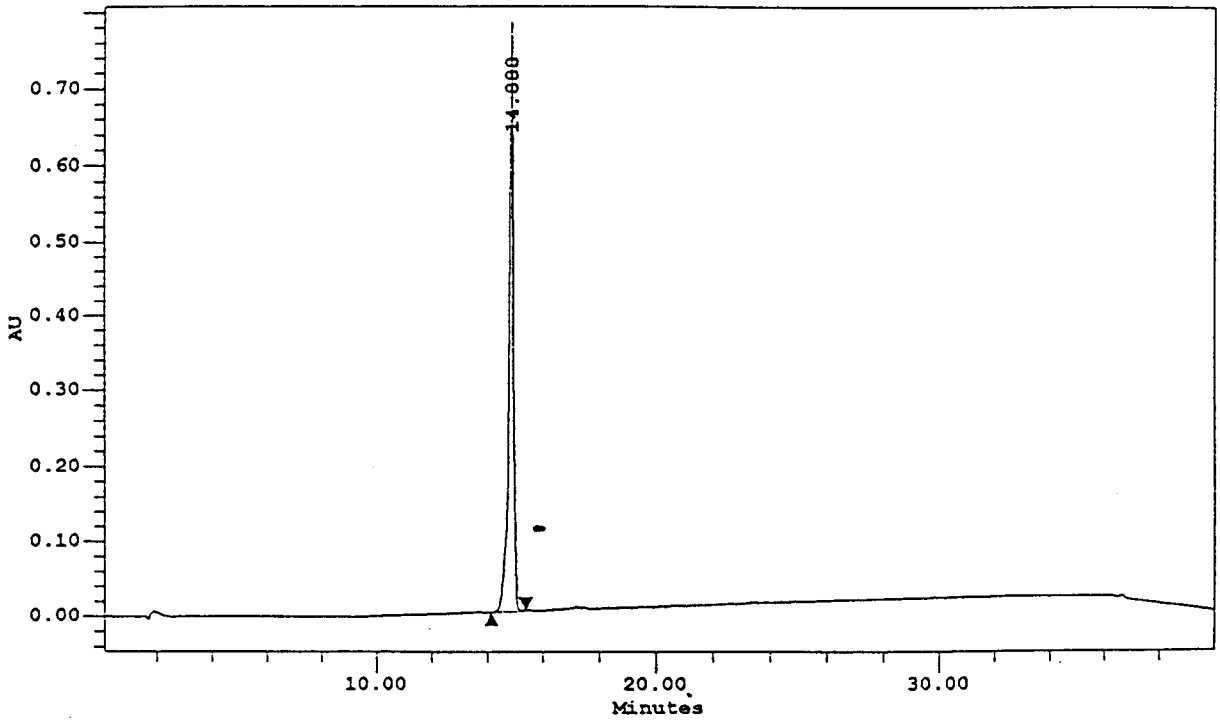
DMU 2584_C.I. 15525

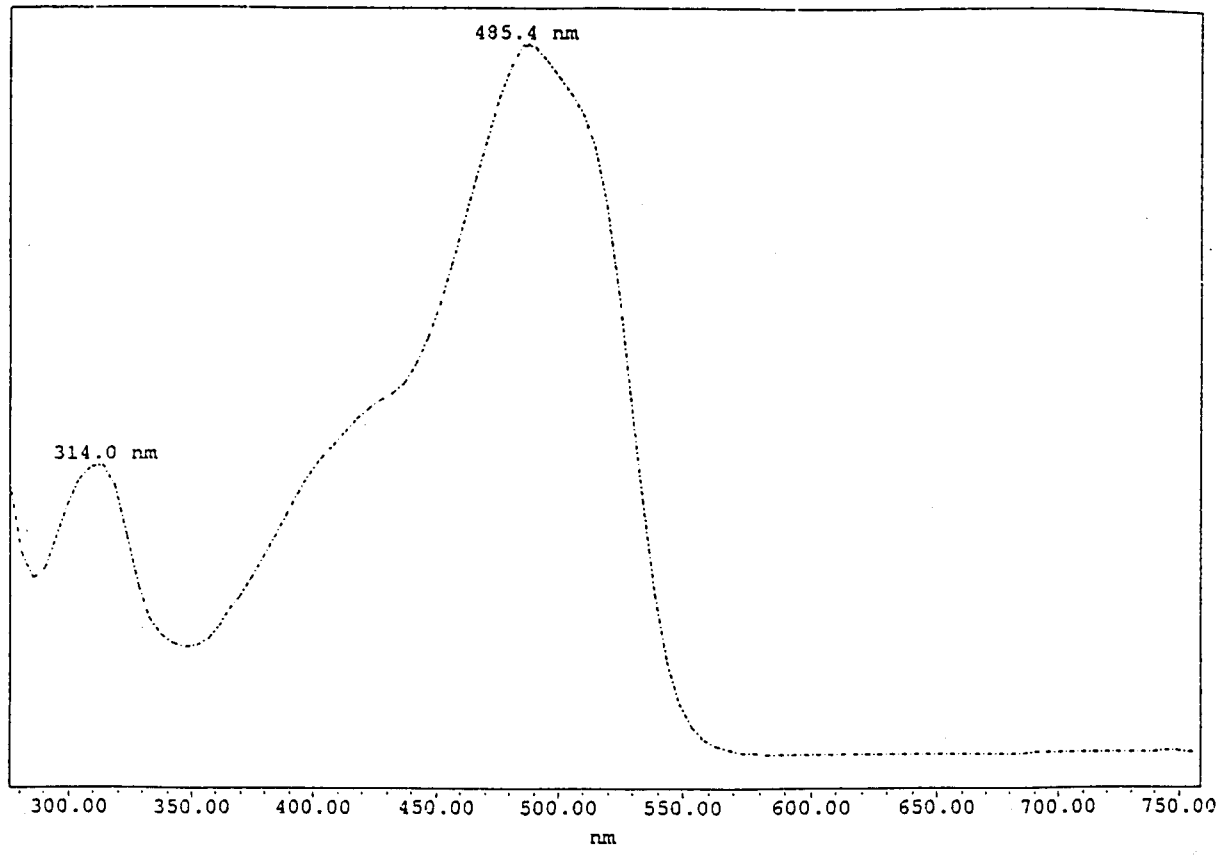




DMU 2584_CI 15525 9.975 minutes, 275 - 760 @ 4.8 nm, from 2584_15525

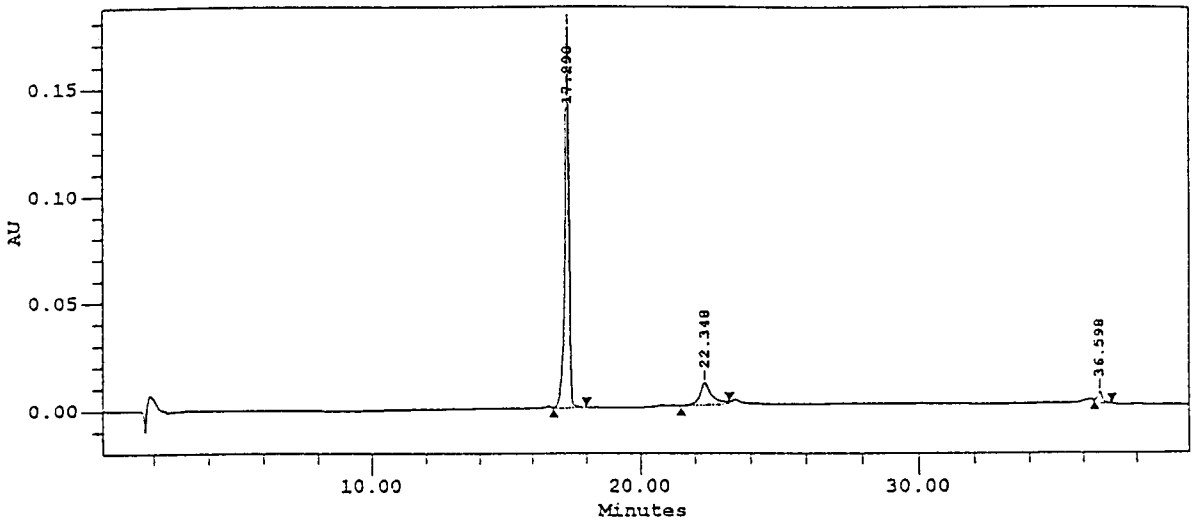
DMU 2709_C.I. 15580

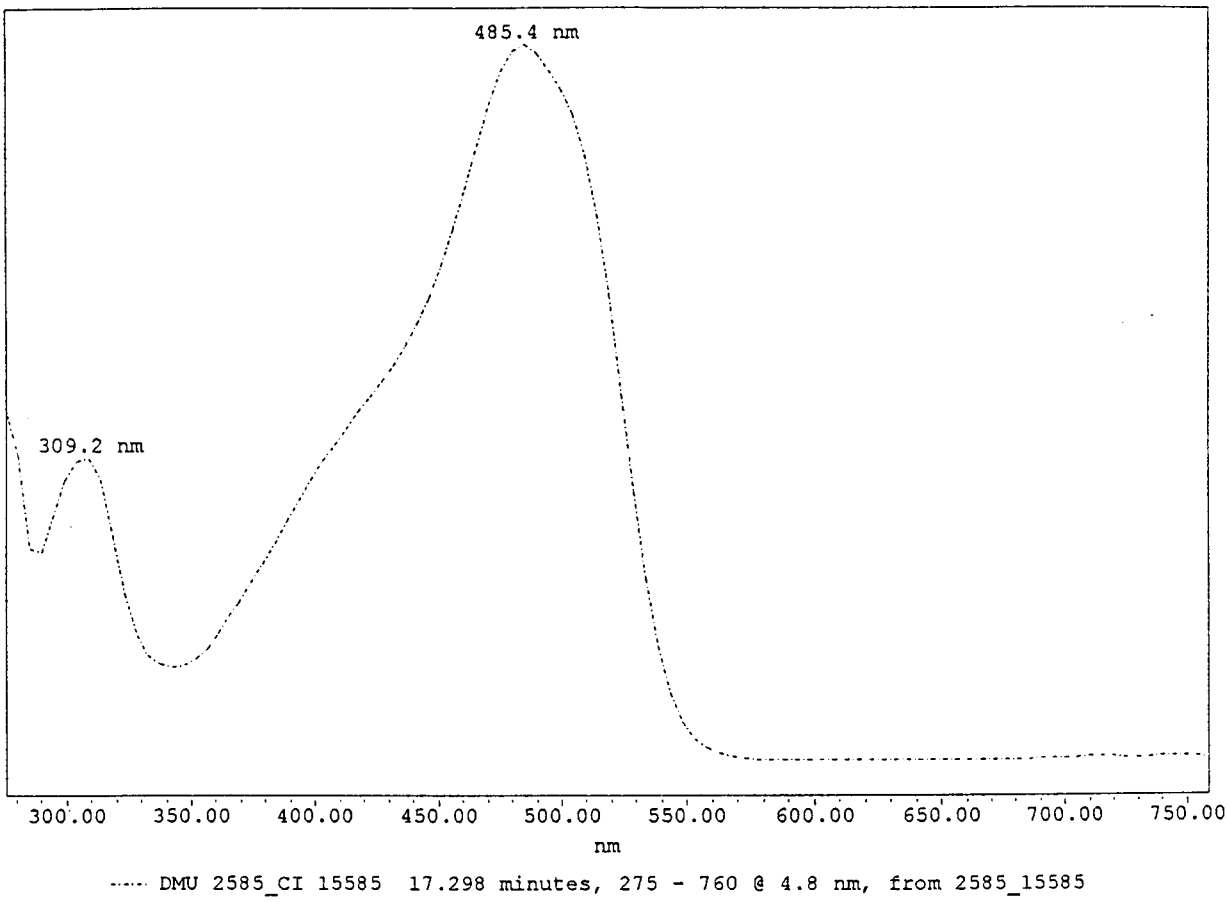




DMU 2709_CI 15580 14.888 minutes, 275 - 760 @ 4.8 nm, from 2709_15580

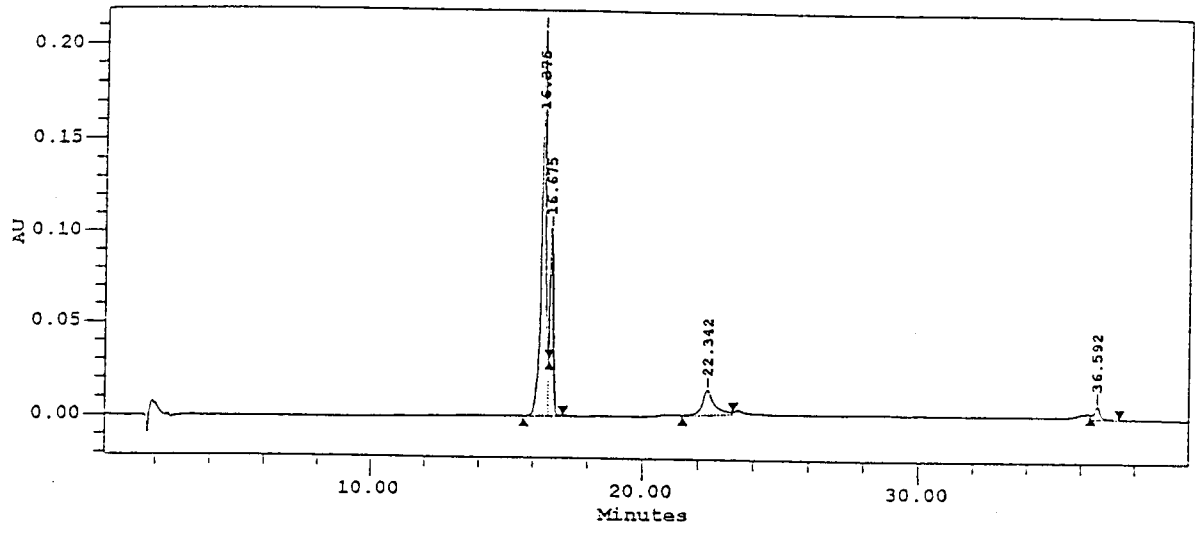
DMU 2585_C.I. 15585

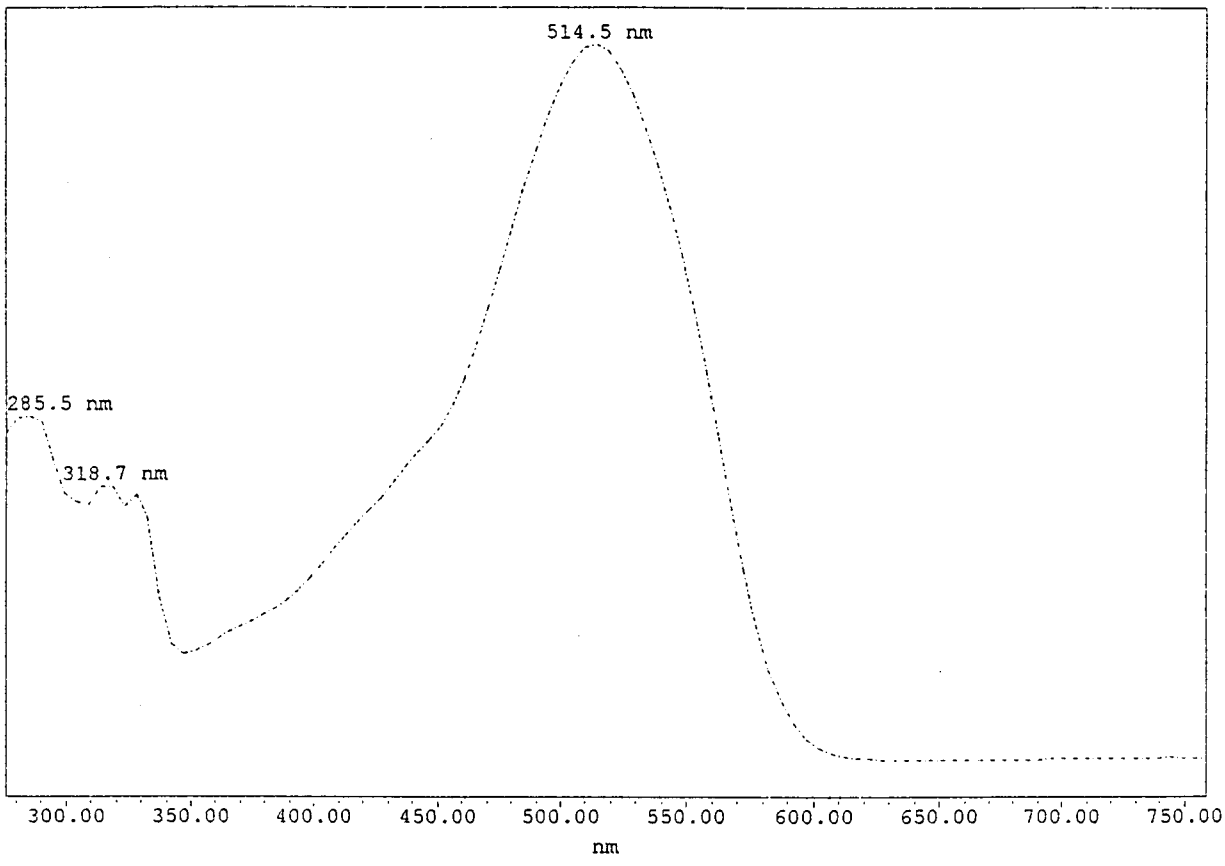




DMU 2585_CI 15585 17.298 minutes, 275 - 760 @ 4.8 nm, from 2585_15585

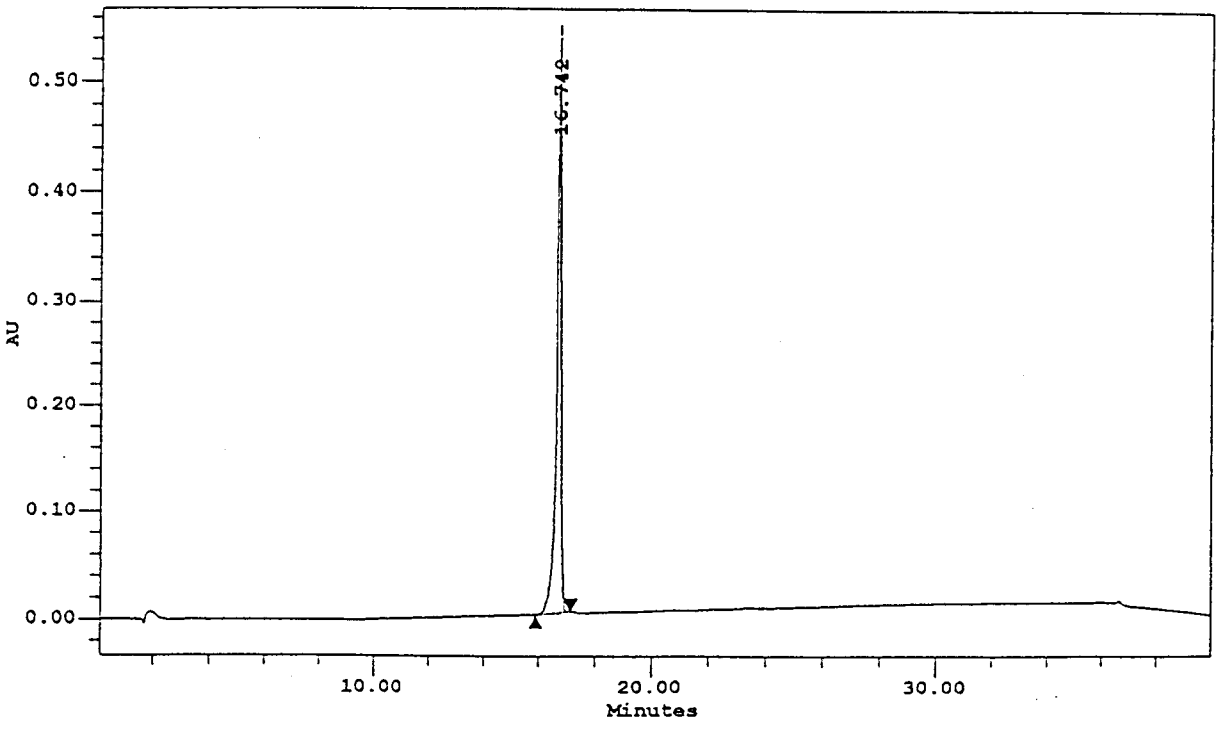
DMU 2570_C.I. 15620

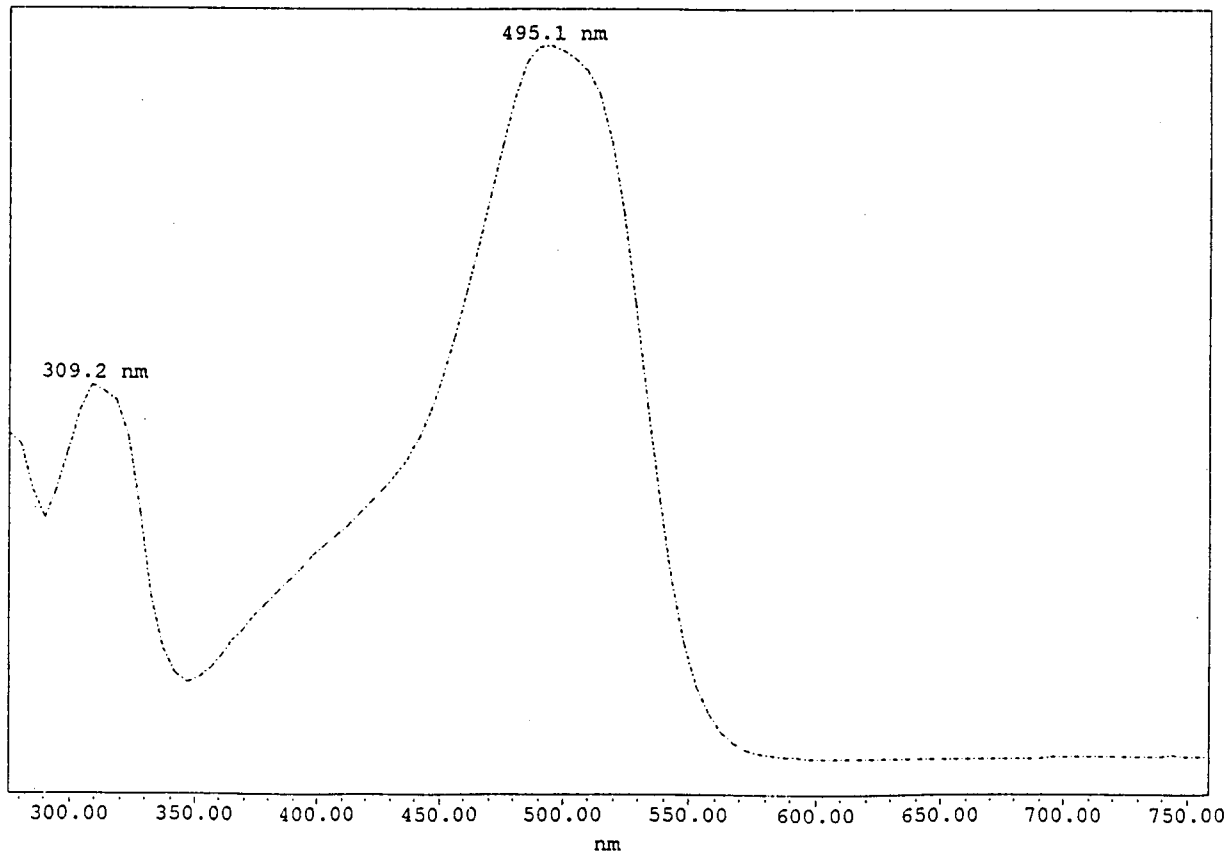




DMU 2570_CI 15620* 16.375 minutes, 275 - 760 @ 4.8 nm, from 2570_15620

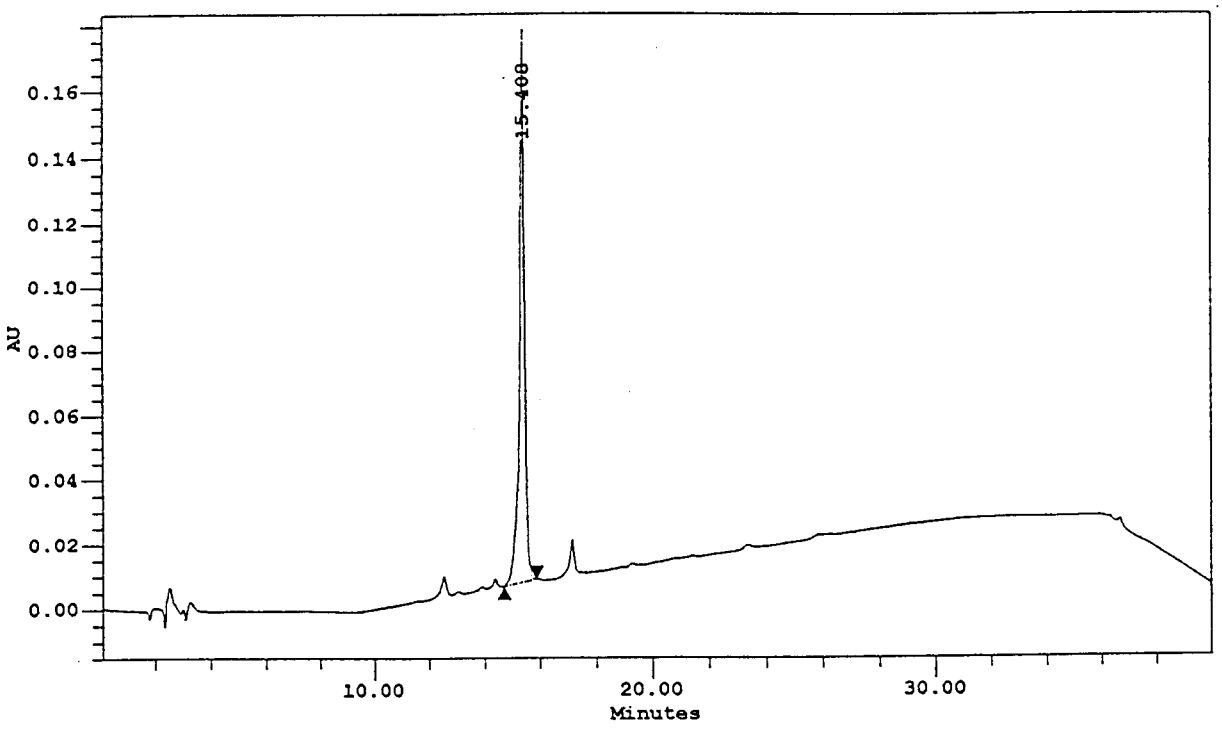
DMU 2720_C.I. 15630

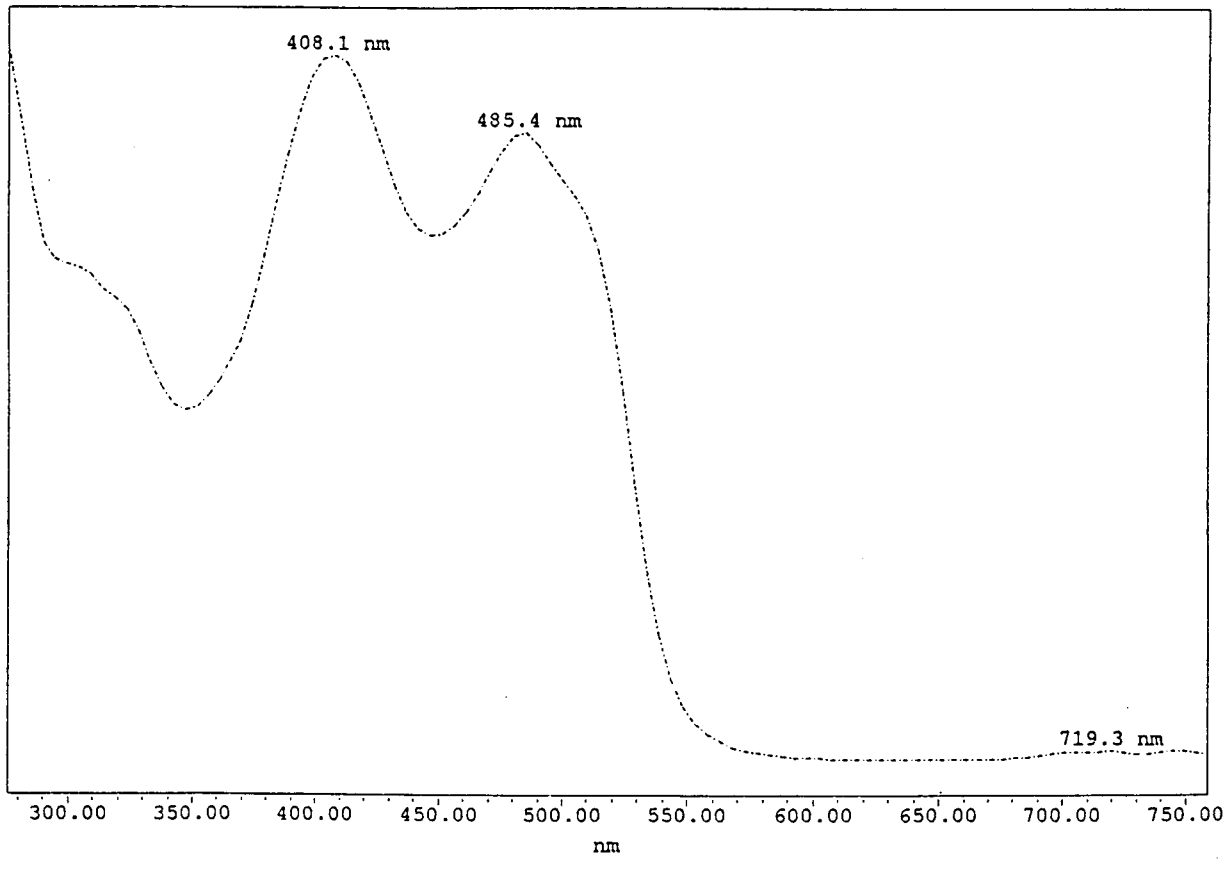




DMU 2720_CI 15630 16.742 minutes, 275 - 760 @ 4.8 nm, from 2720_15630

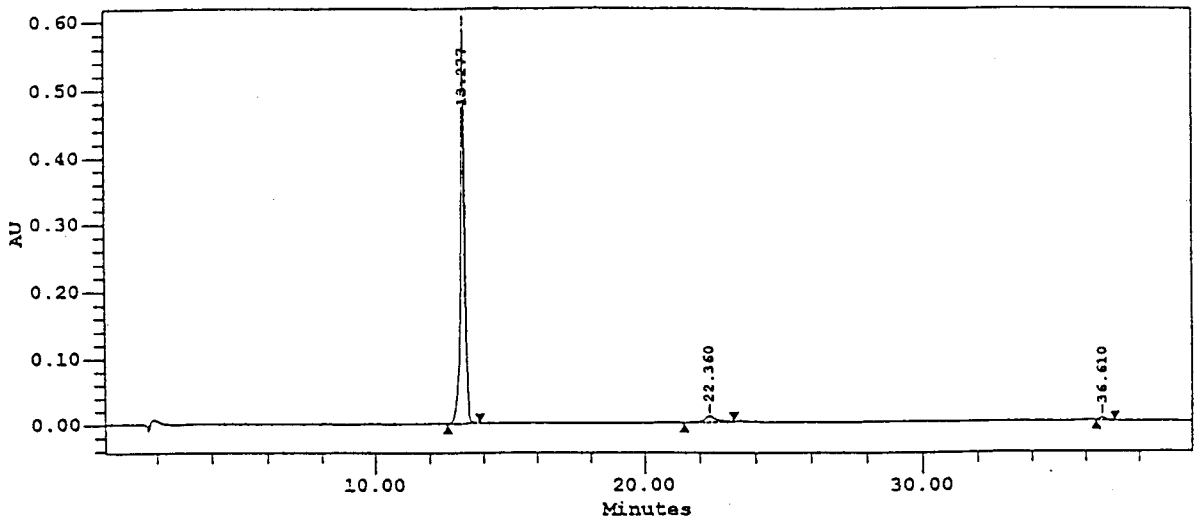
DMU 2666_C.I. 15800

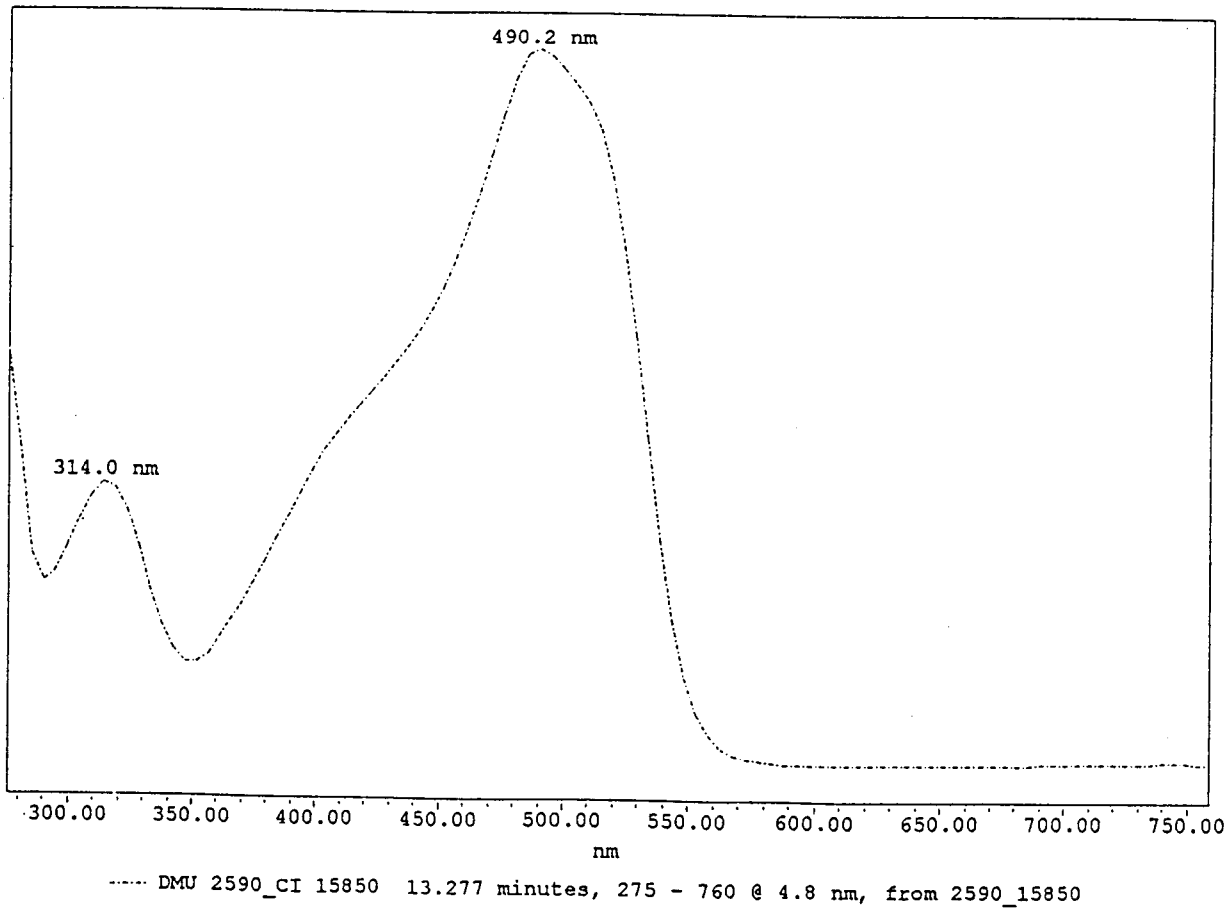




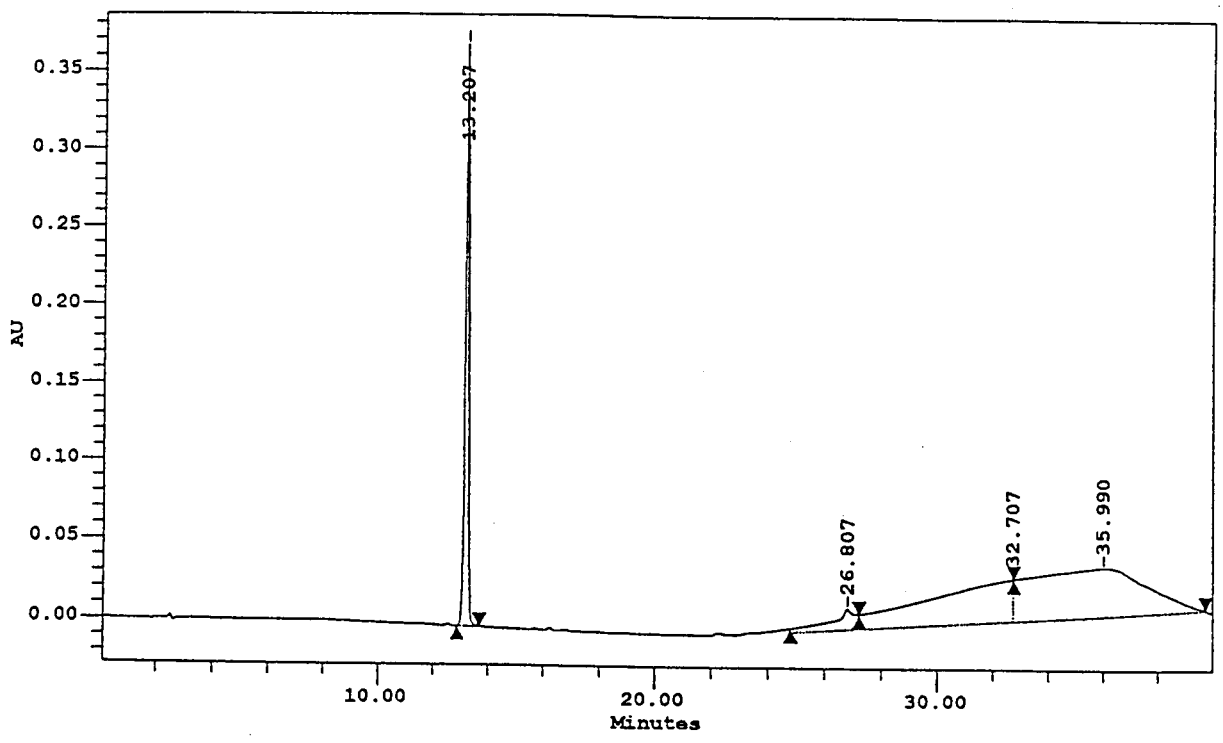
DMU 2666_CI 15800 15.408 minutes, 275 - 760 @ 4.8 nm, from 2666_15800

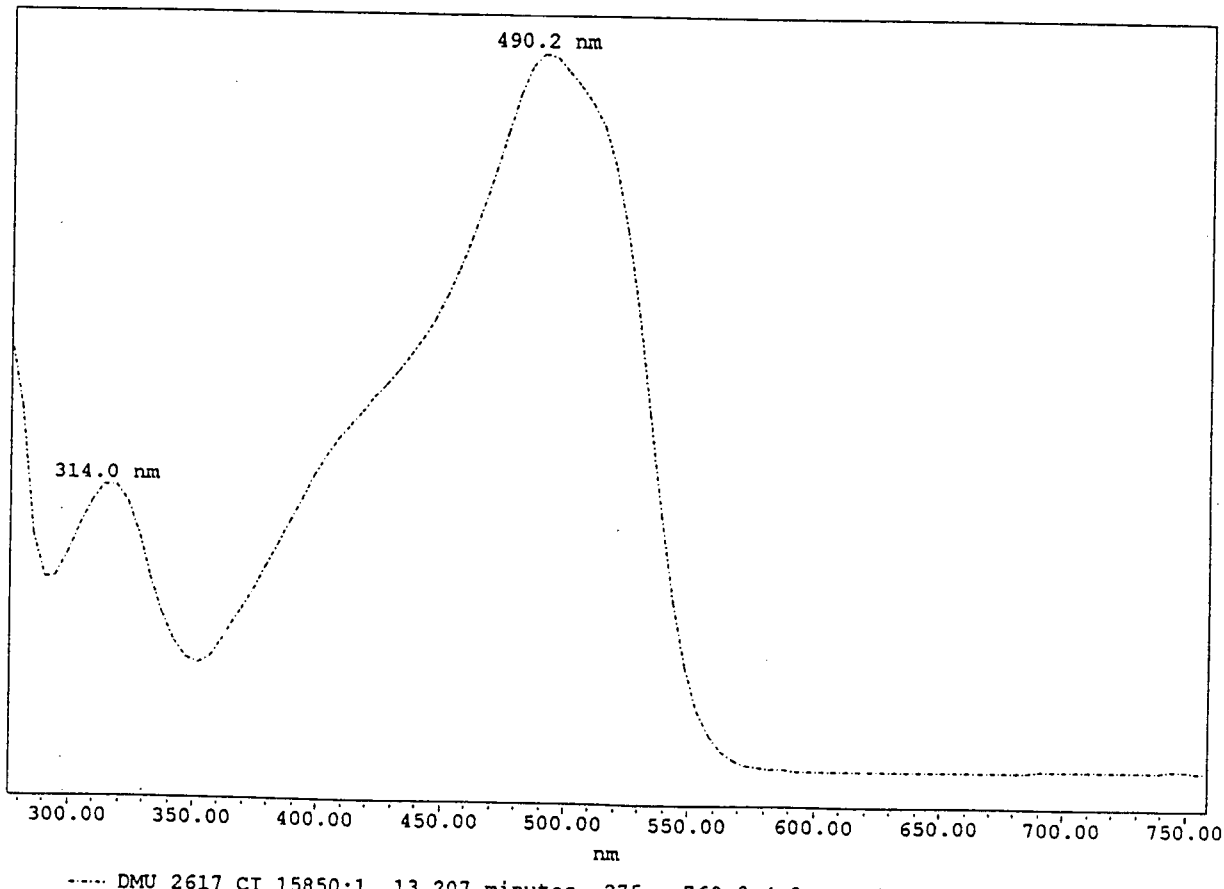
DMU 2590_C.I. 15850





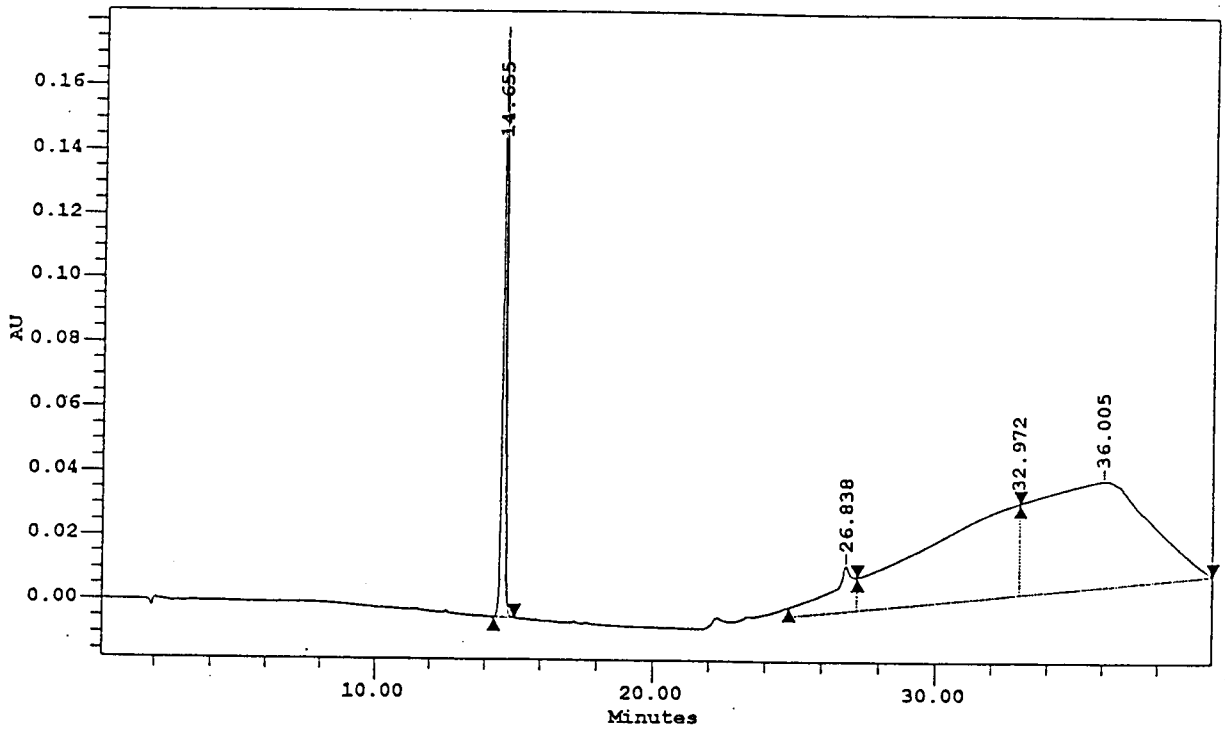
DMU 2617_C.I. 15850:1

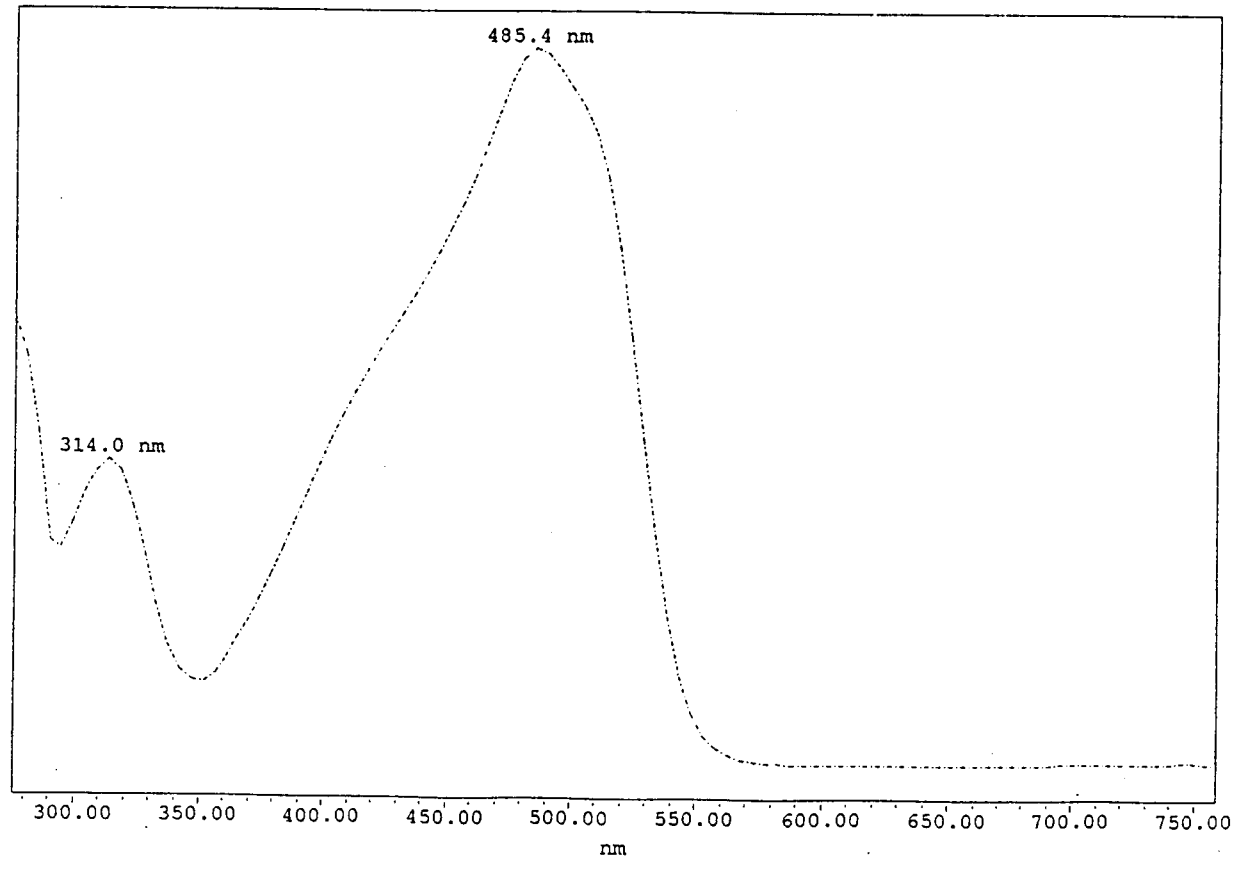




DMU 2617_CI 15850:1 13.207 minutes, 275 - 760 @ 4.8 nm, from 2617_CI15850:1

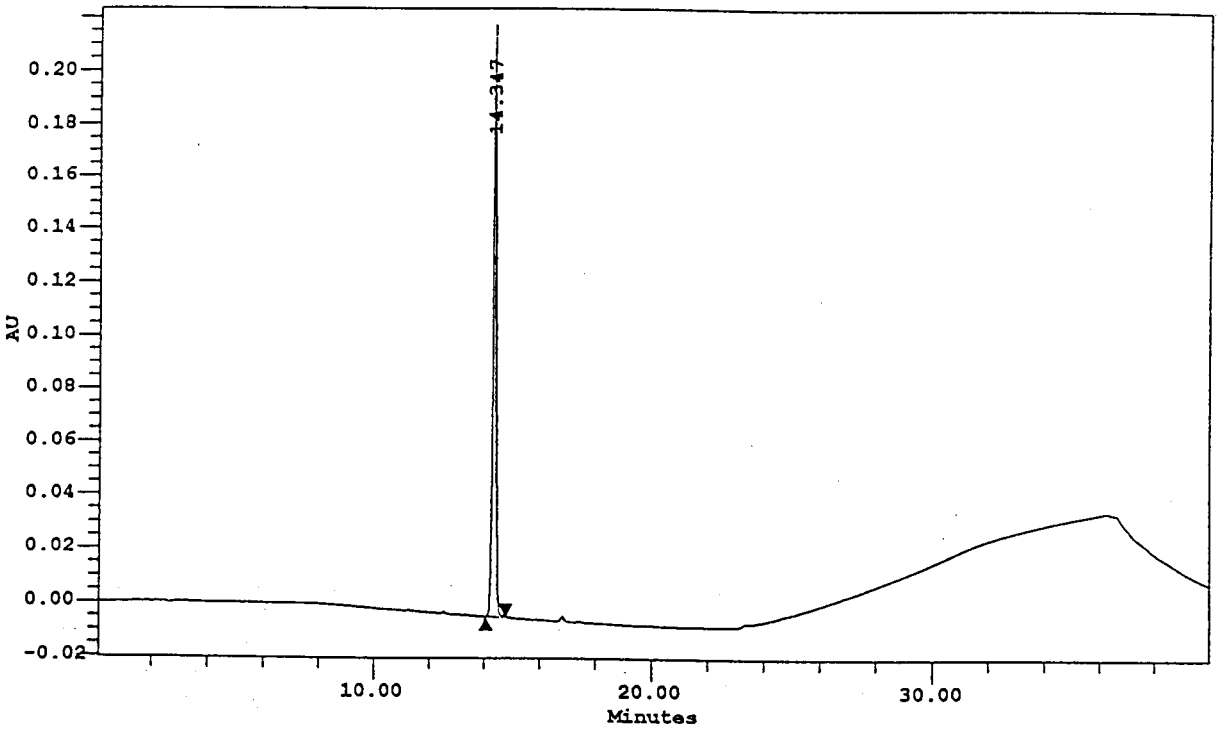
DMU 2725_C.I. 15865:3

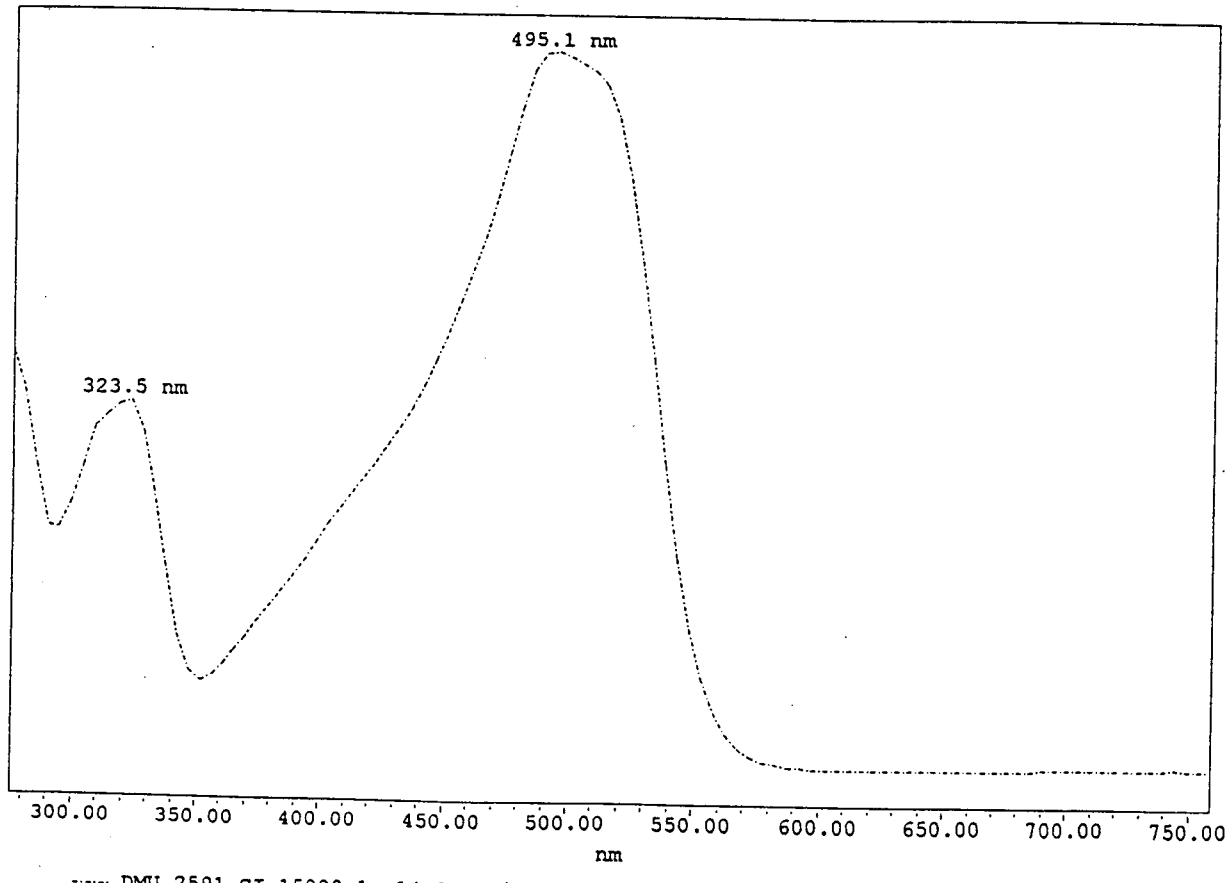




DMU 2725_CI 15865:3 14.655 minutes, 275 - 760 @ 4.8 nm, from 2725_CI15865:3

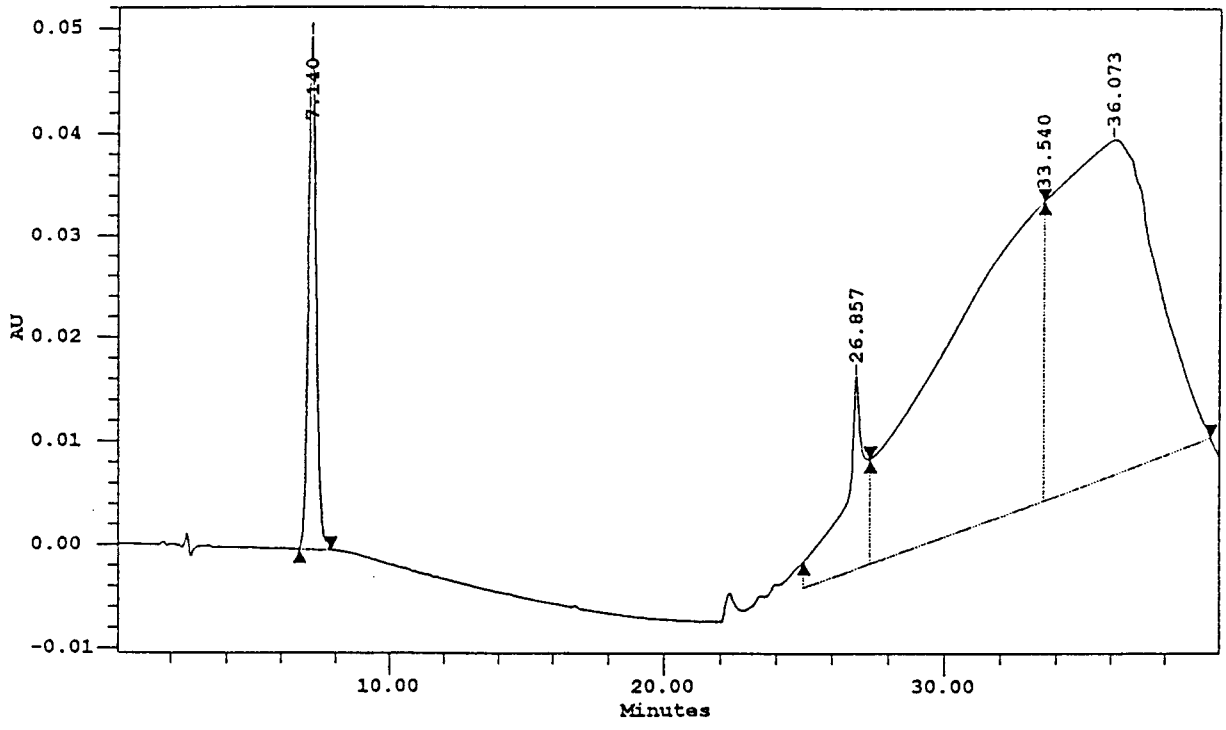
DMU 2591_C.I. 15880:1

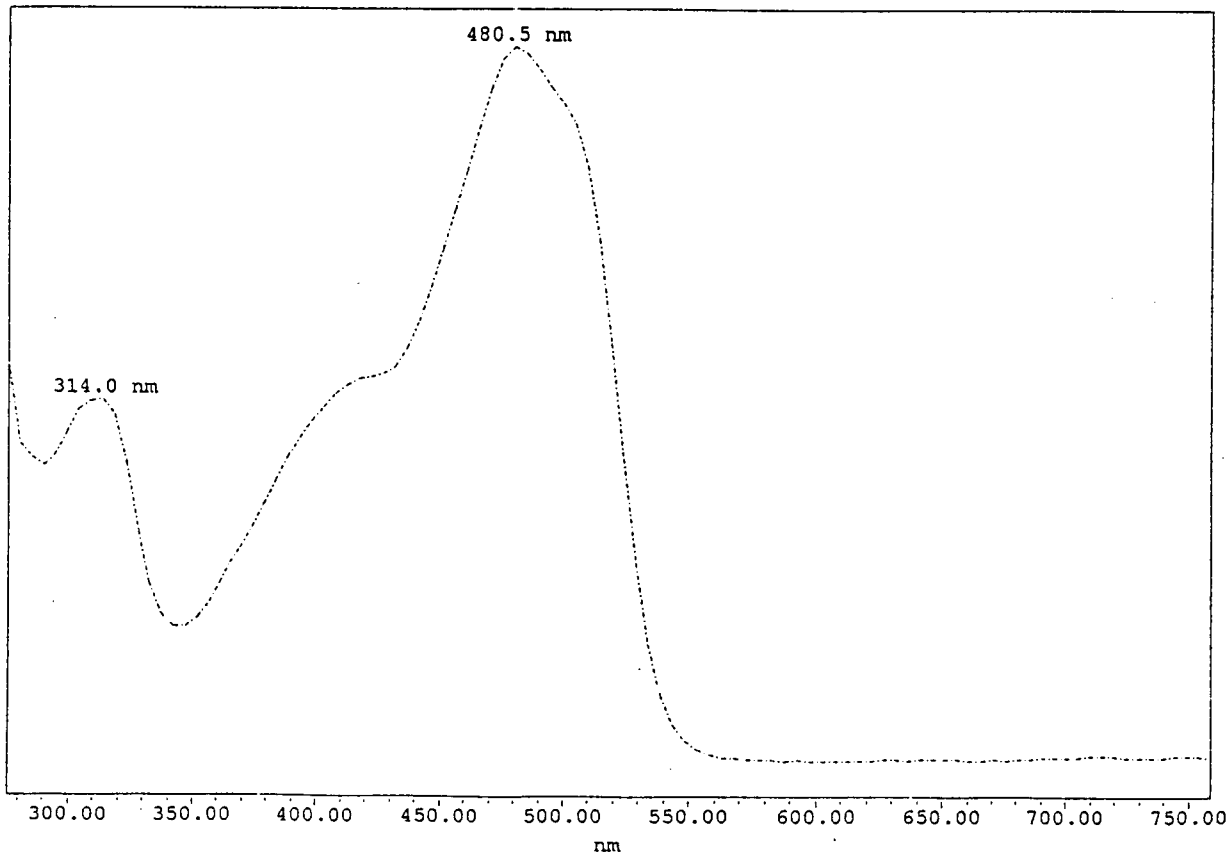




DMU 2591_CI 15880:1 14.347 minutes, 275 - 760 @ 4.8 nm, from 2591_CI15880:1

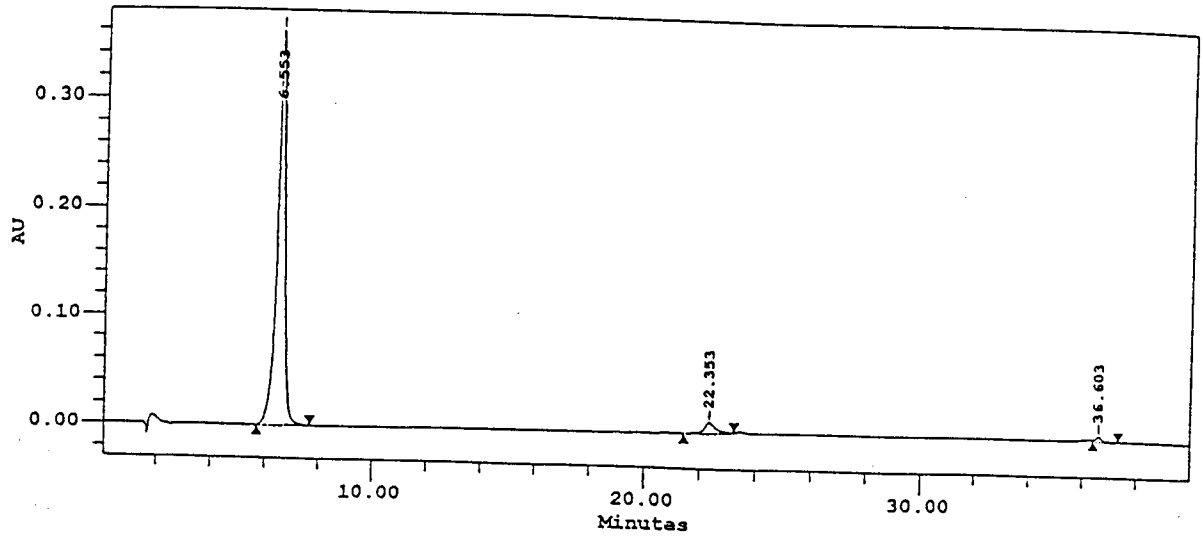
DMU 2714_C.I. 15980

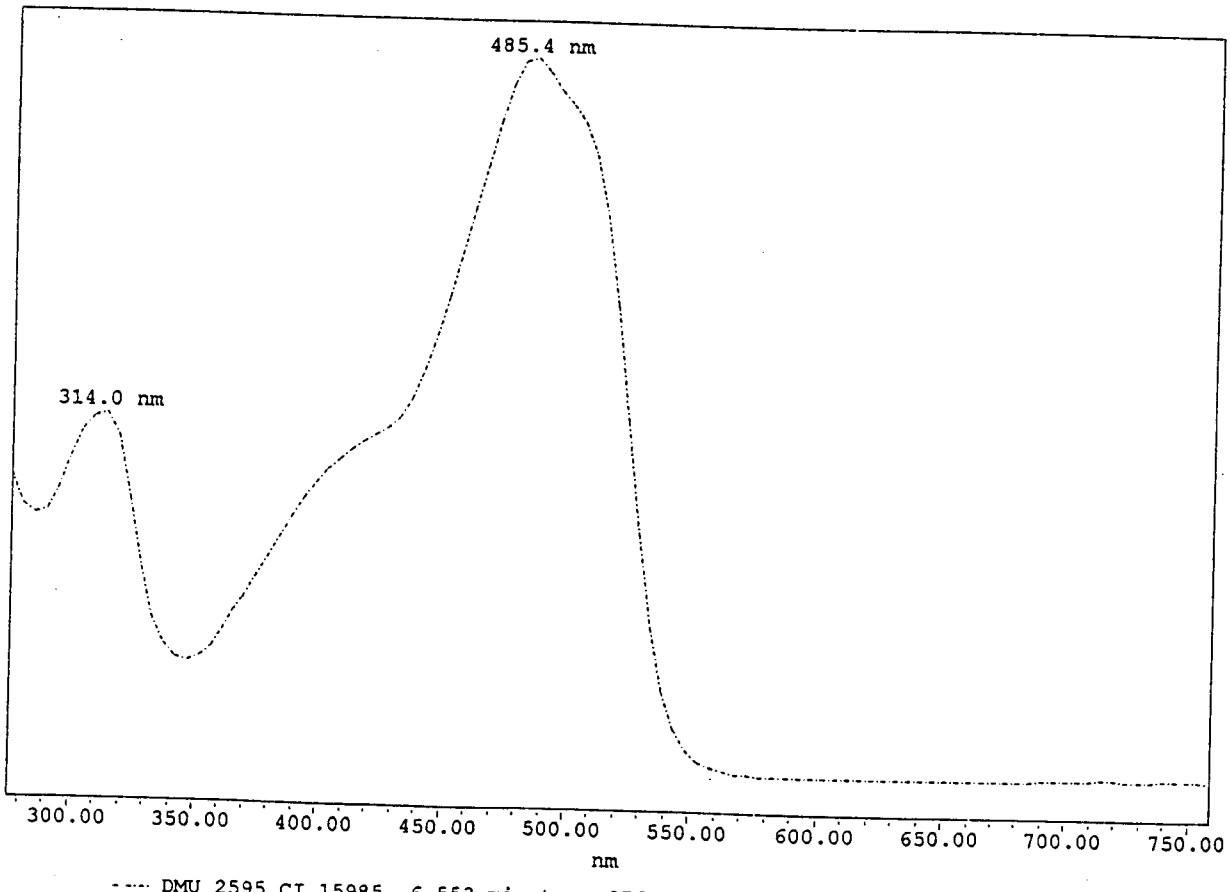




DMU 2714_CI 15980 7.140 minutes, 275 - 760 @ 4.8 nm, from 2714_CI15980

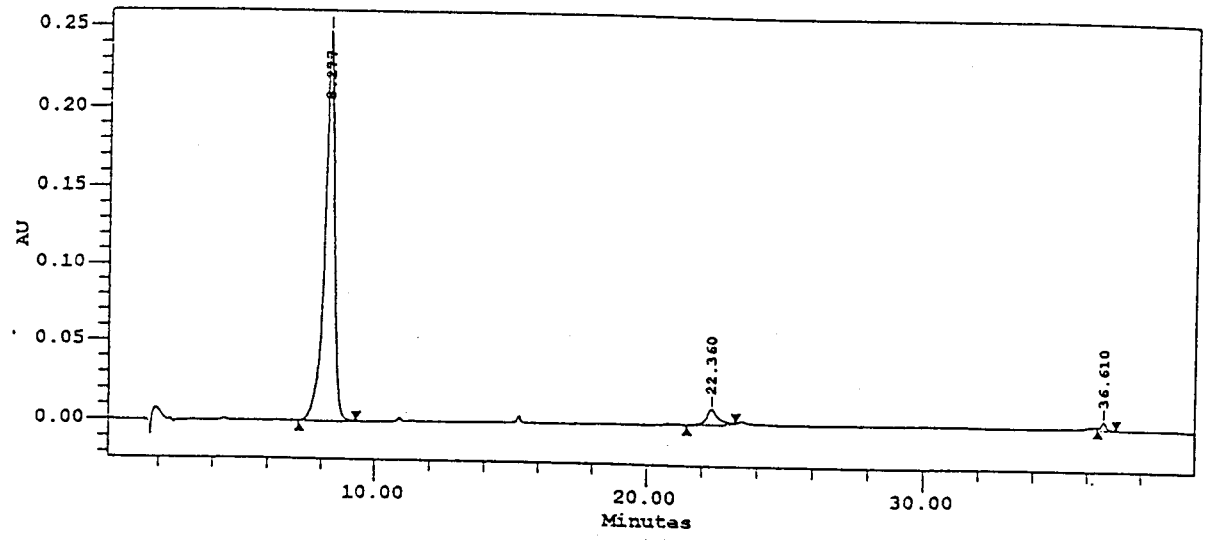
DMU 2595_C.I. 15985

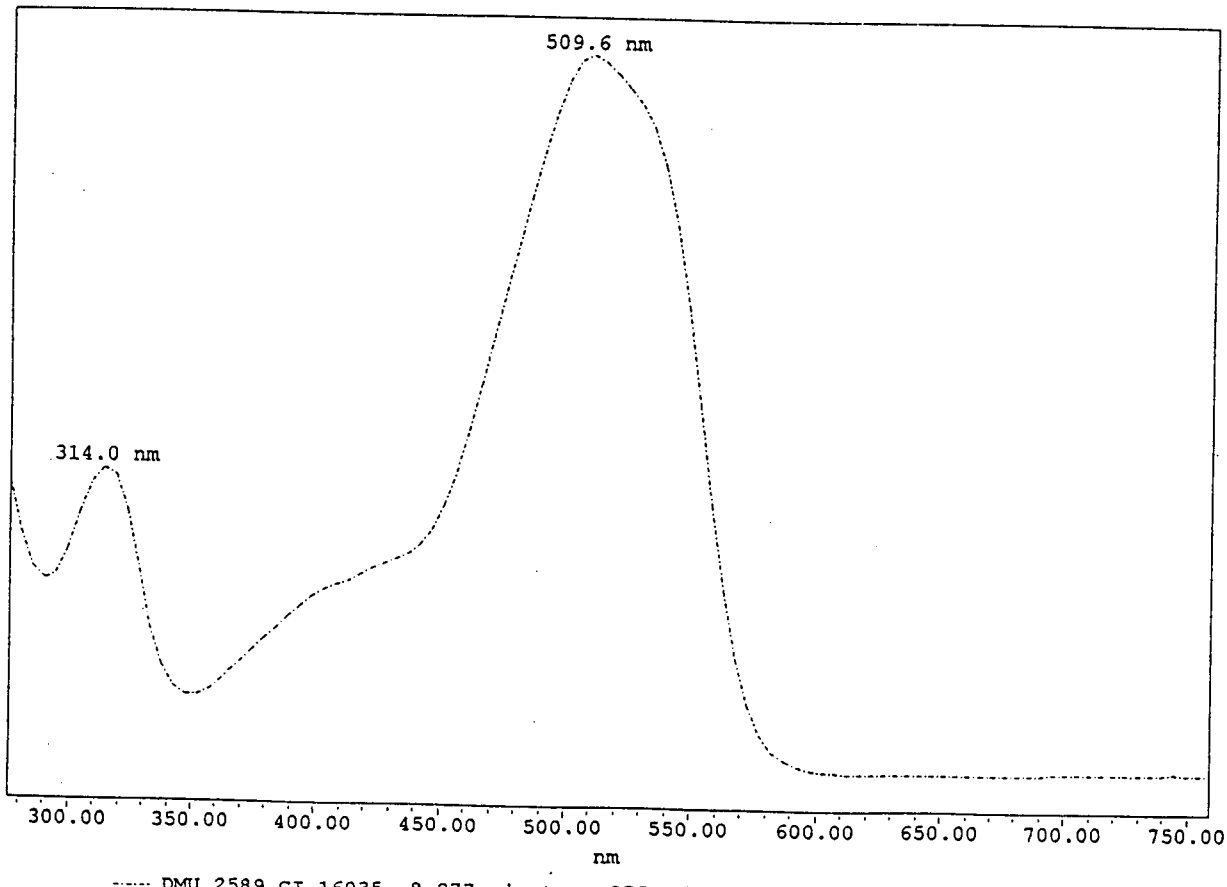




---- DMU 2595_CI 15985 6.553 minutes, 275 - 760 @ 4.8 nm, from 2595_15985

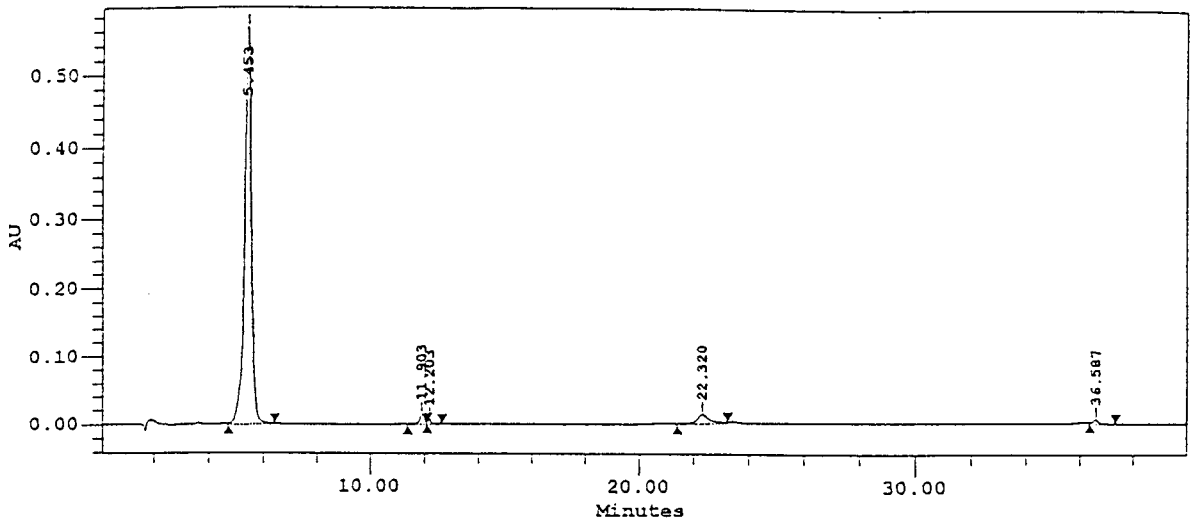
DMU 2589_C.I. 16035

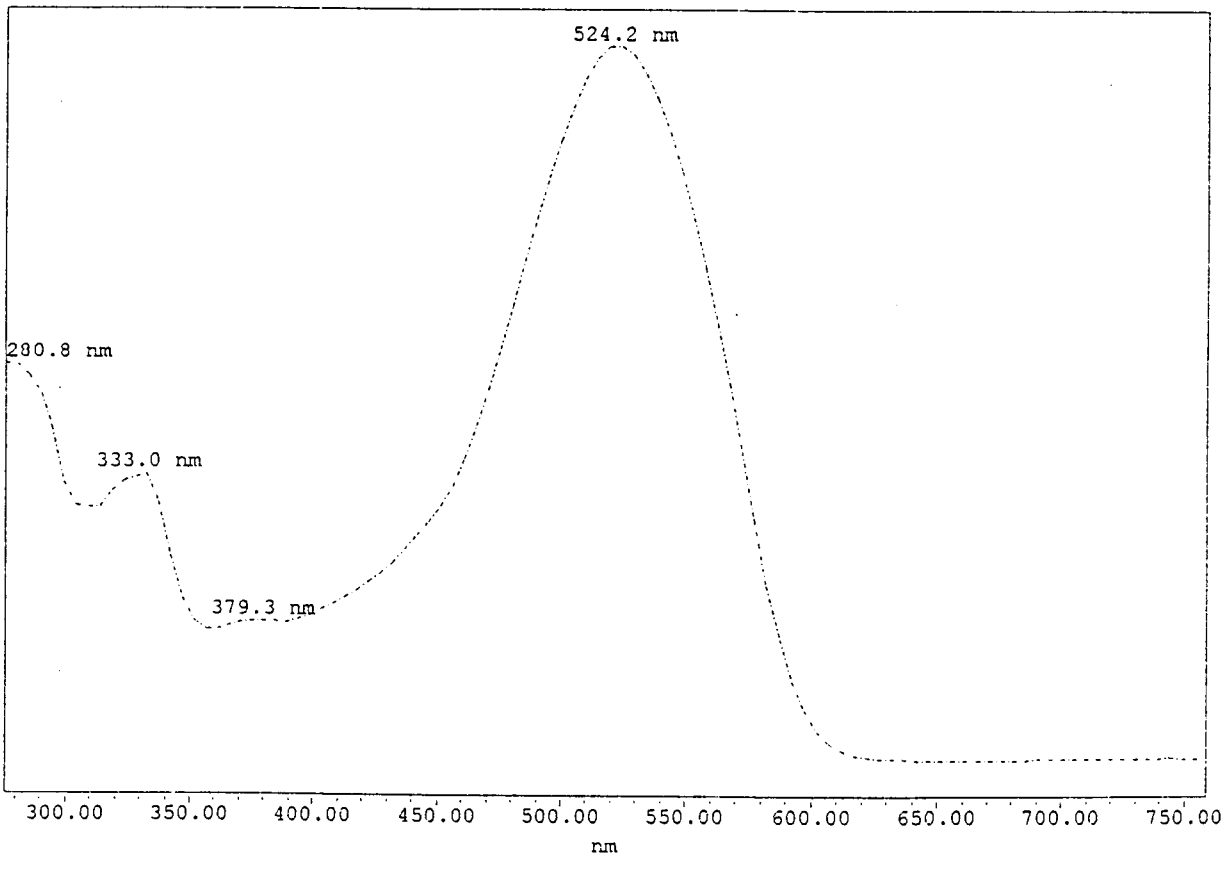




DMU 2589_CI 16035 8.277 minutes, 275 - 760 @ 4.8 nm, from 2589_16035

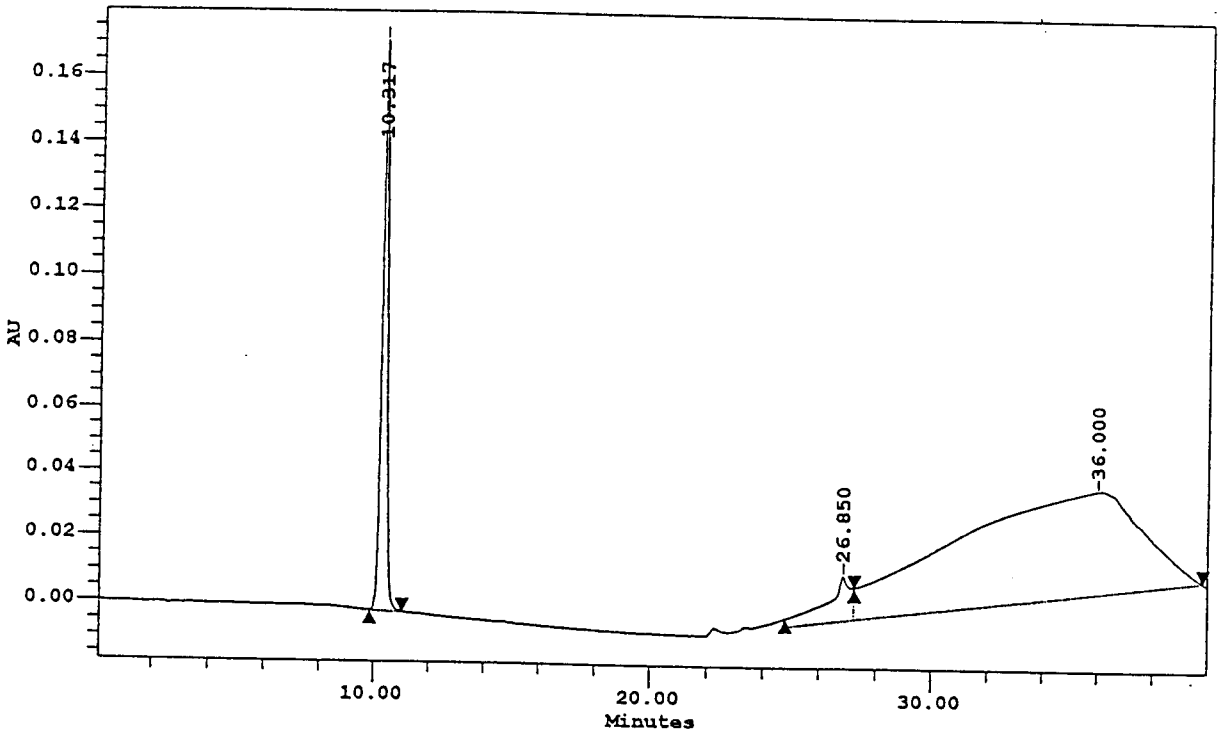
DMU 2571_C.I. 16185

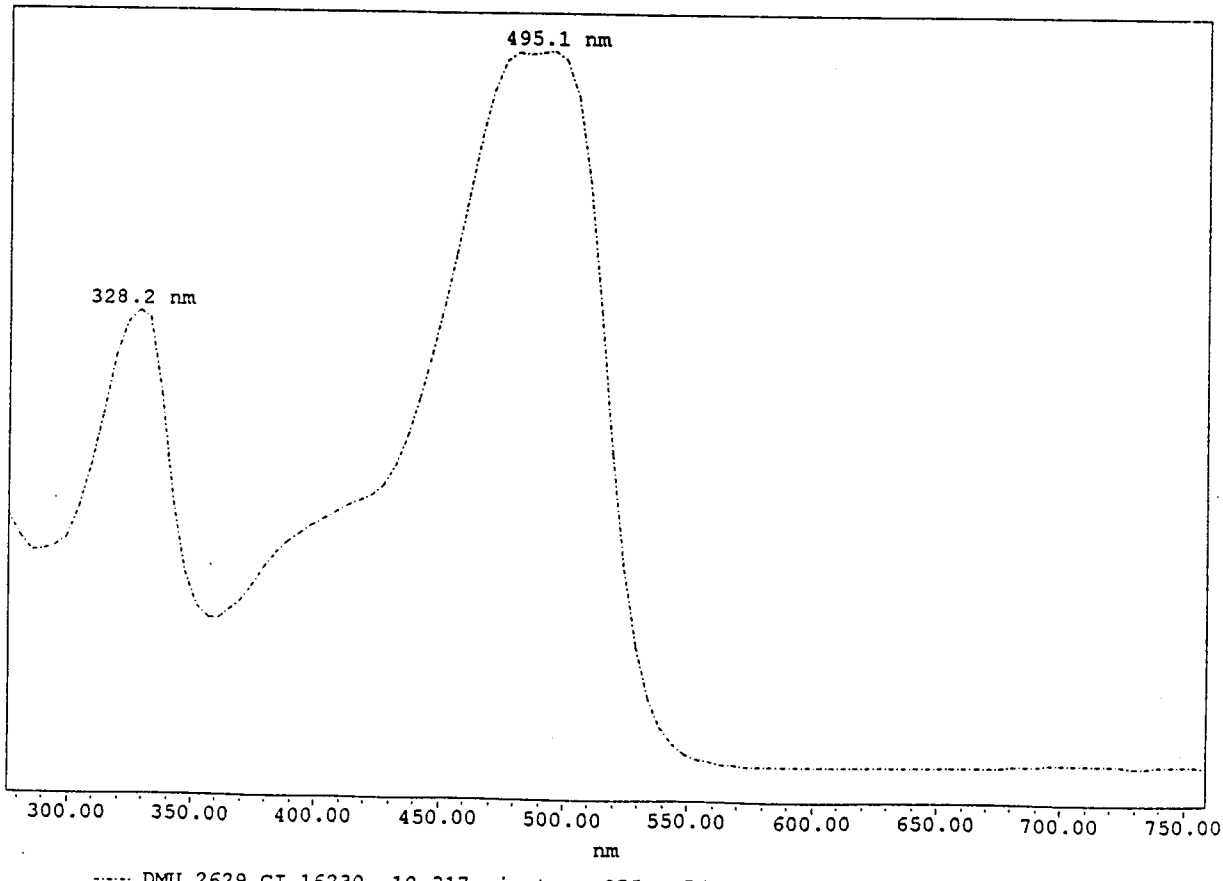




DMU 2571_CI 16185* 5.453 minutes, 275 - 760 @ 4.8 nm, from 2571_16185

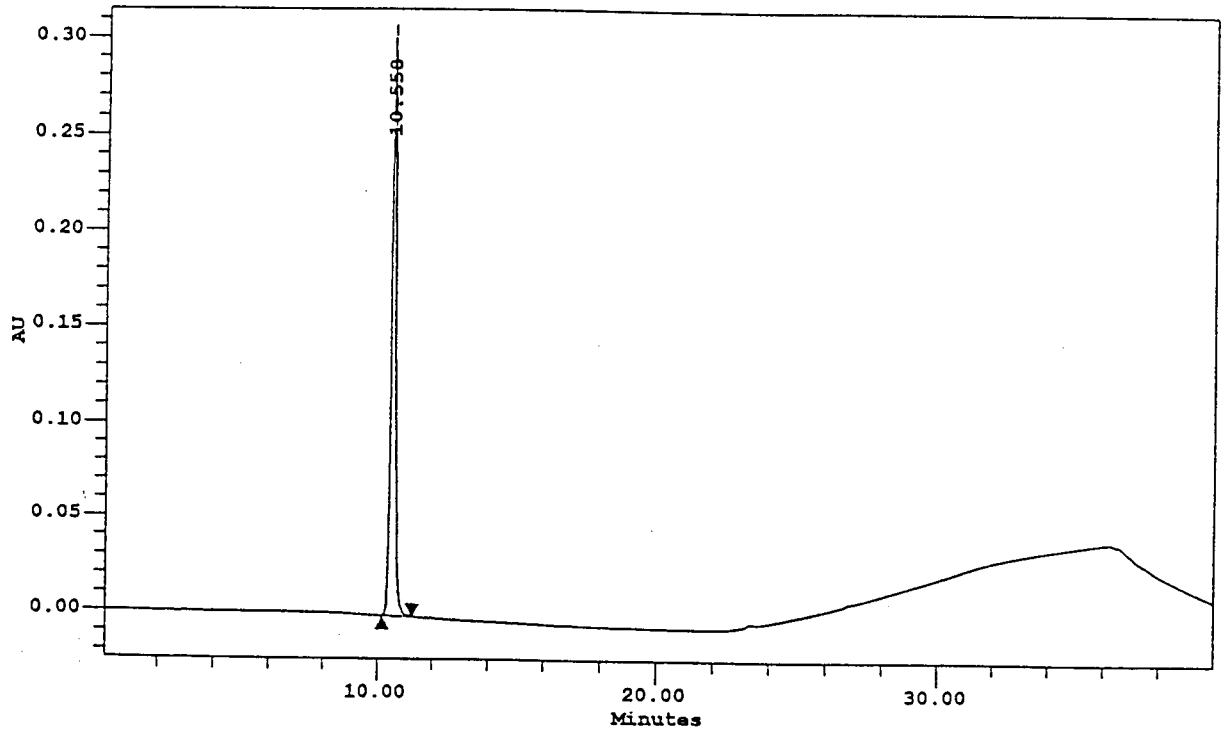
DMU 2629_C.I. 16230

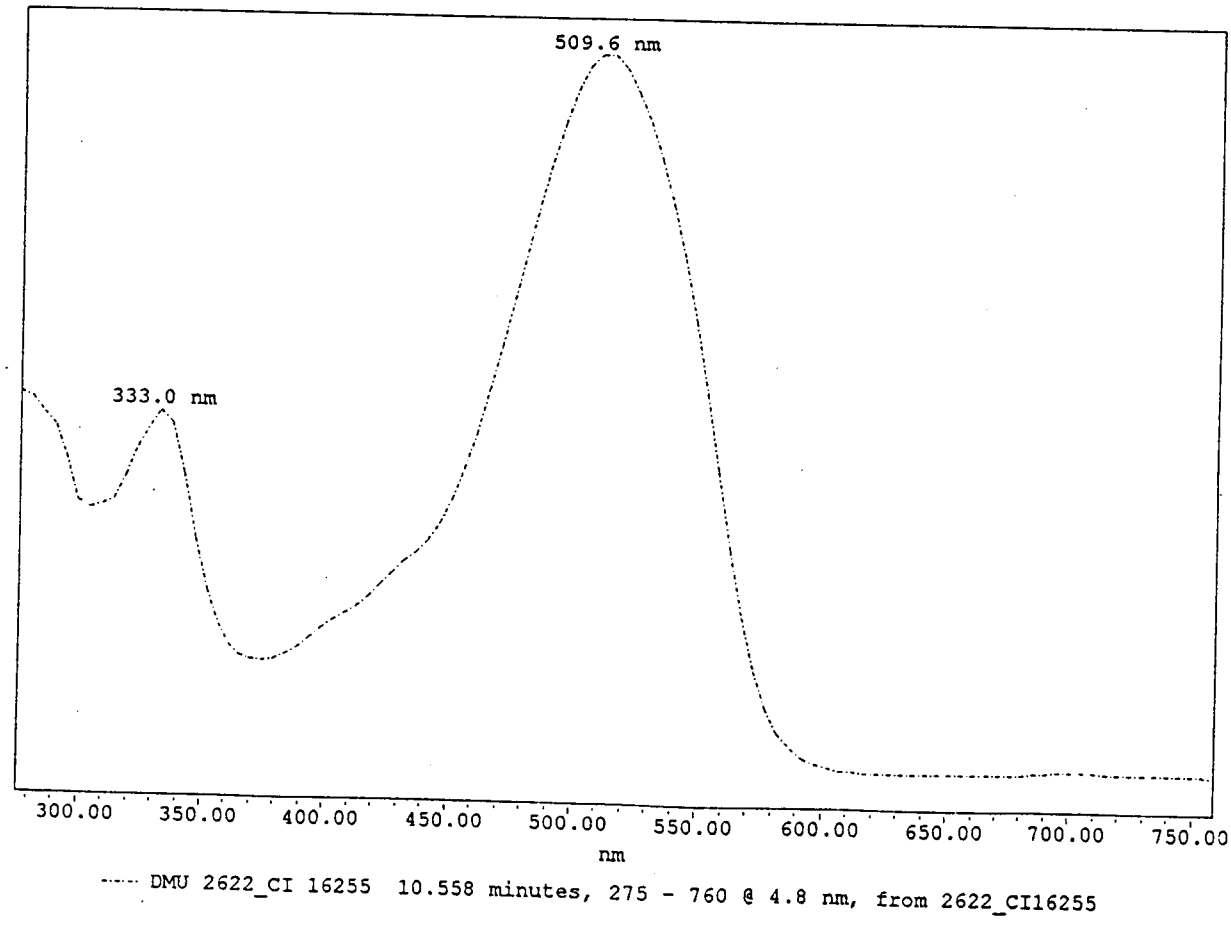




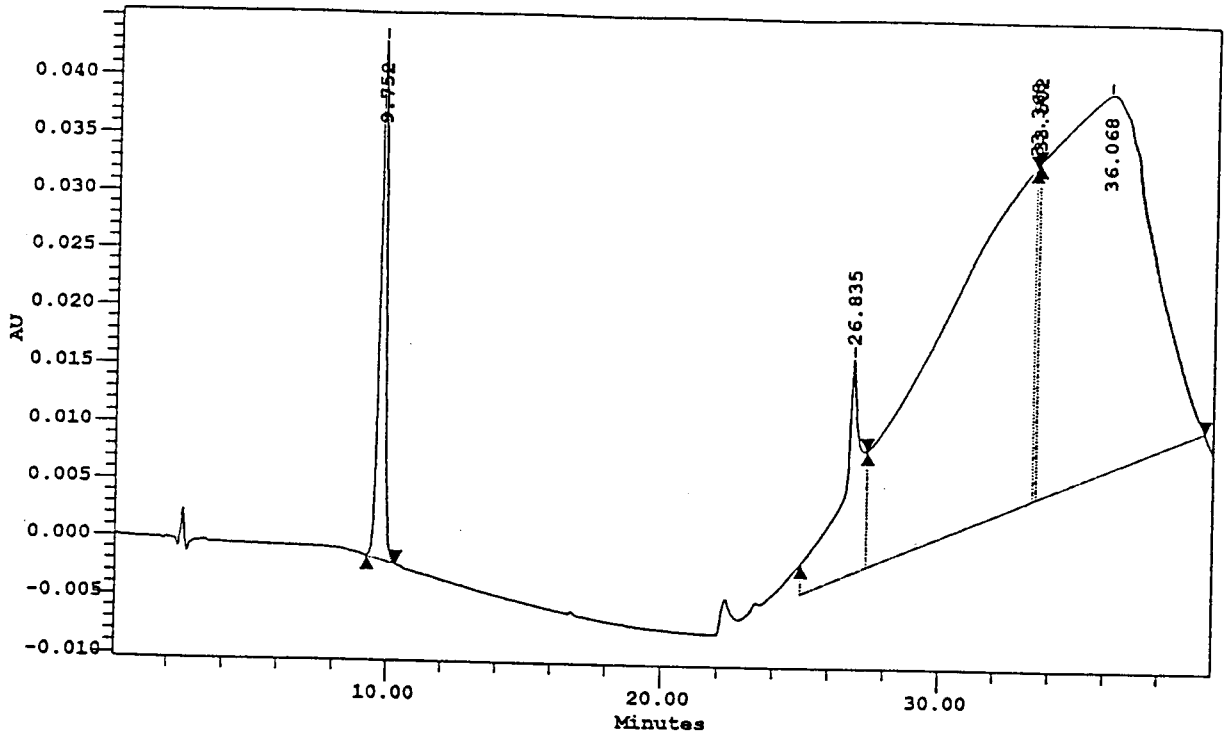
DMU 2629_CI 16230 10.317 minutes, 275 - 760 @ 4.8 nm, from 2629_CI16230

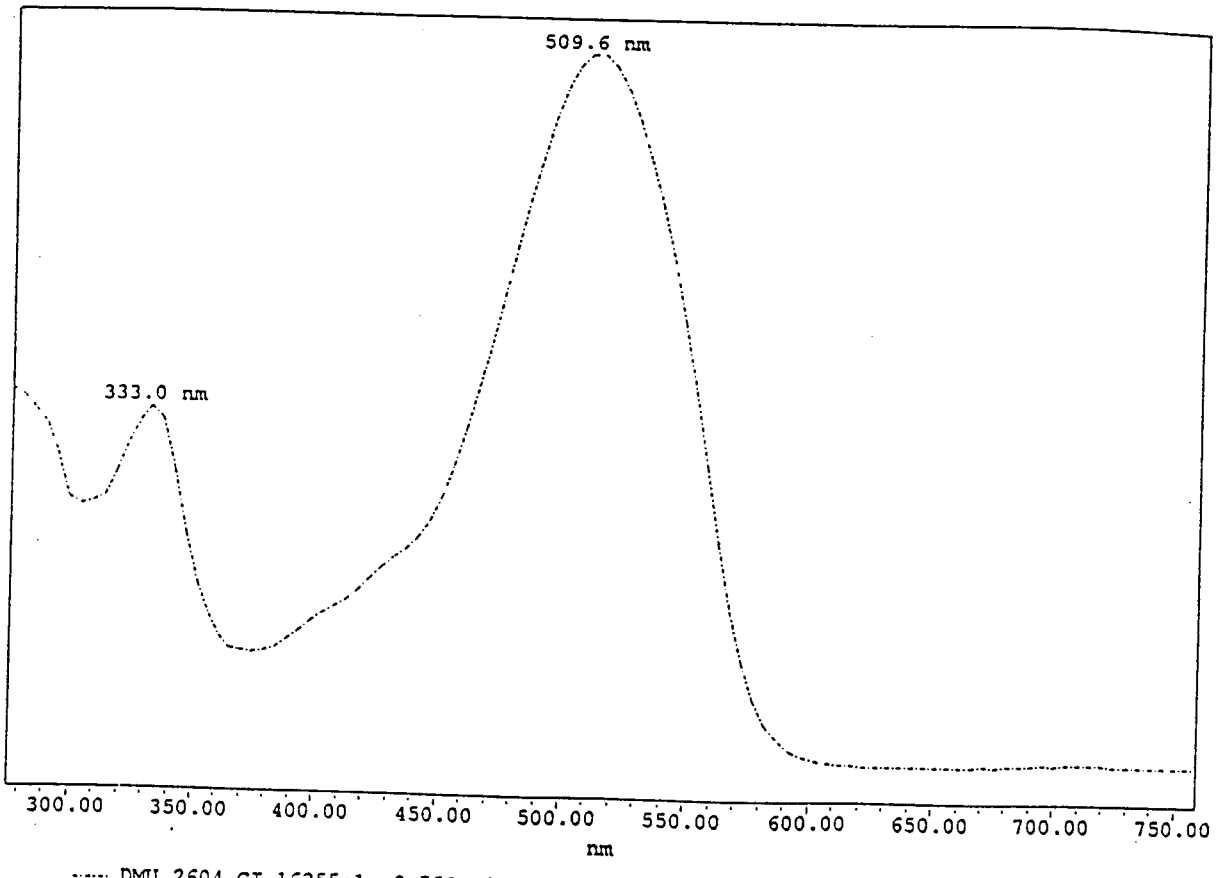
DMU 2622_C.I. 16255





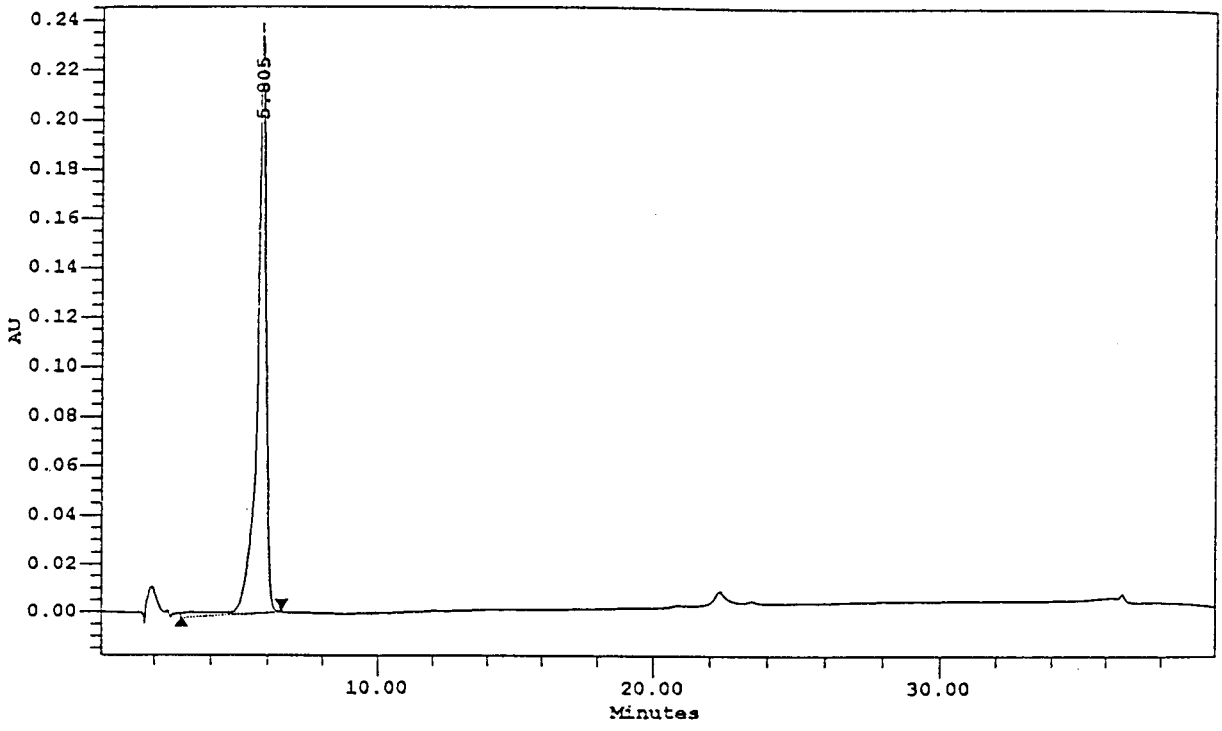
DMU 2604_C.I. 16255:1

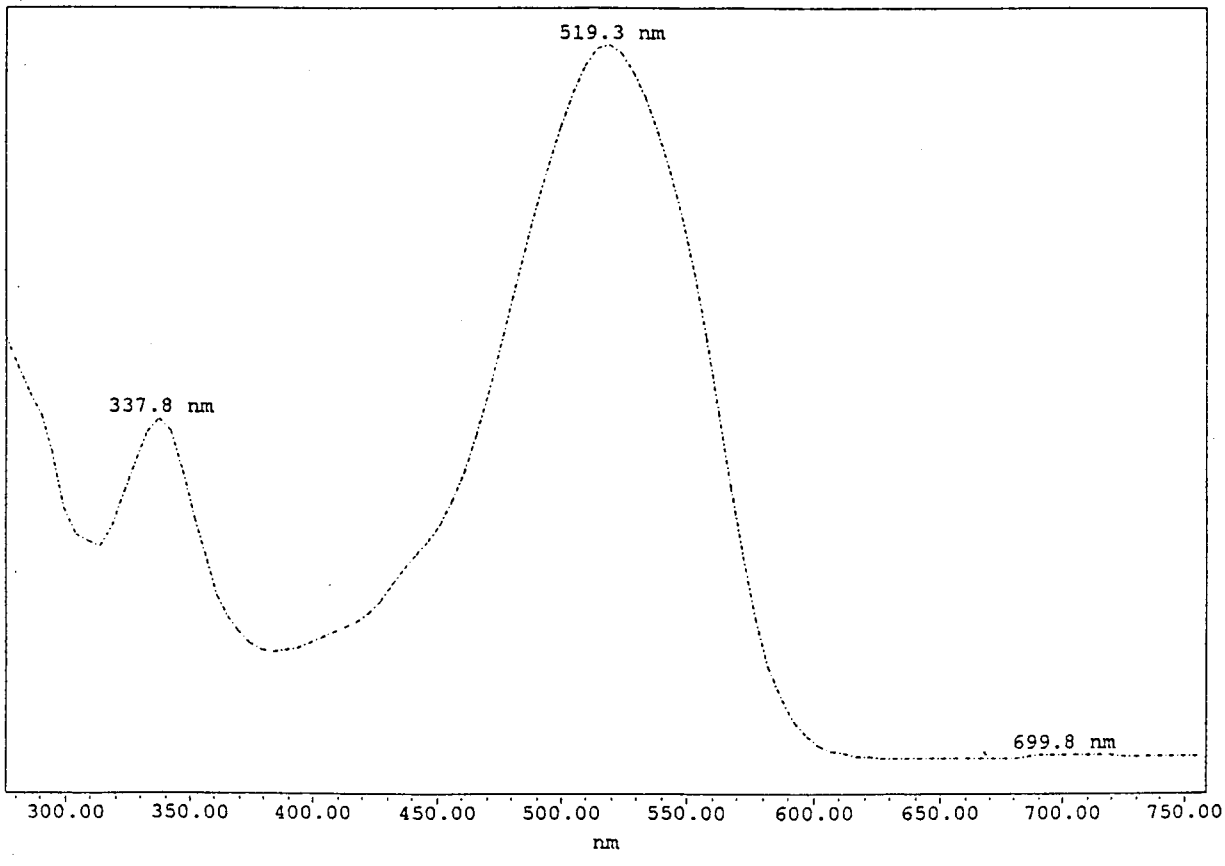




DMU 2604_CI 16255:1 9.752 minutes, 275 - 760 @ 4.8 nm, from 2604_CI16255:1

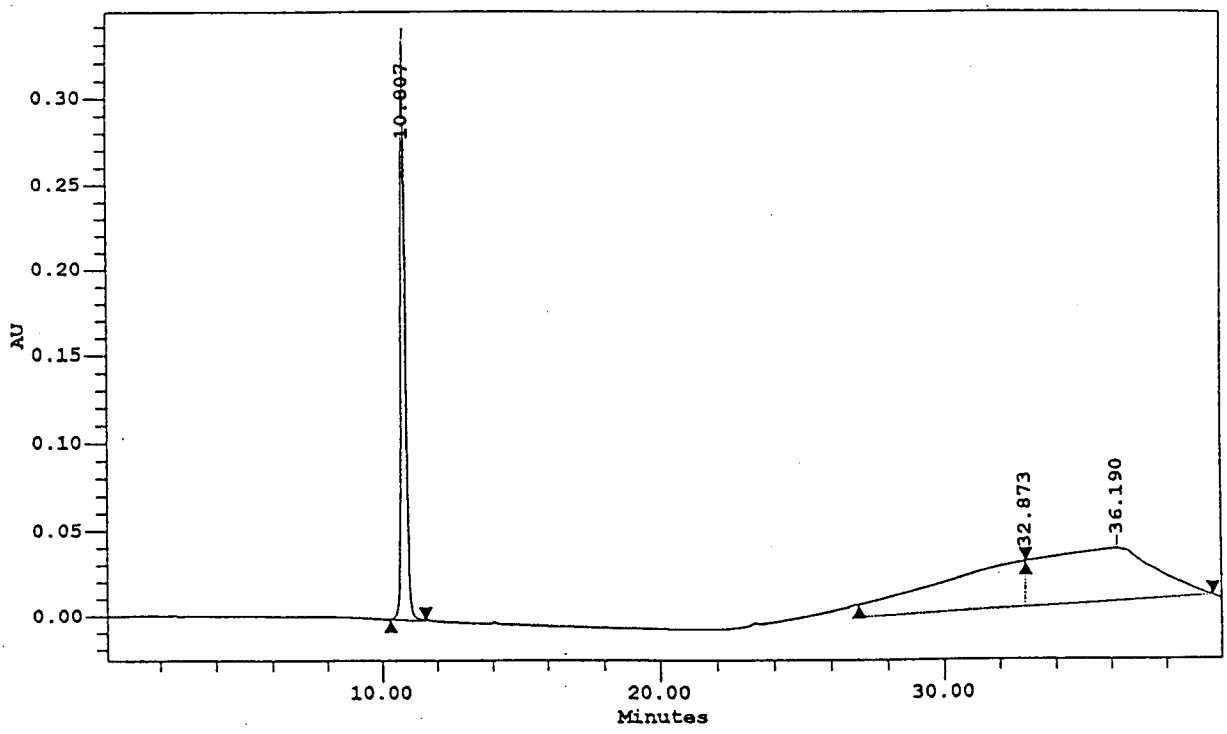
DMU 2683_C.I. 16290

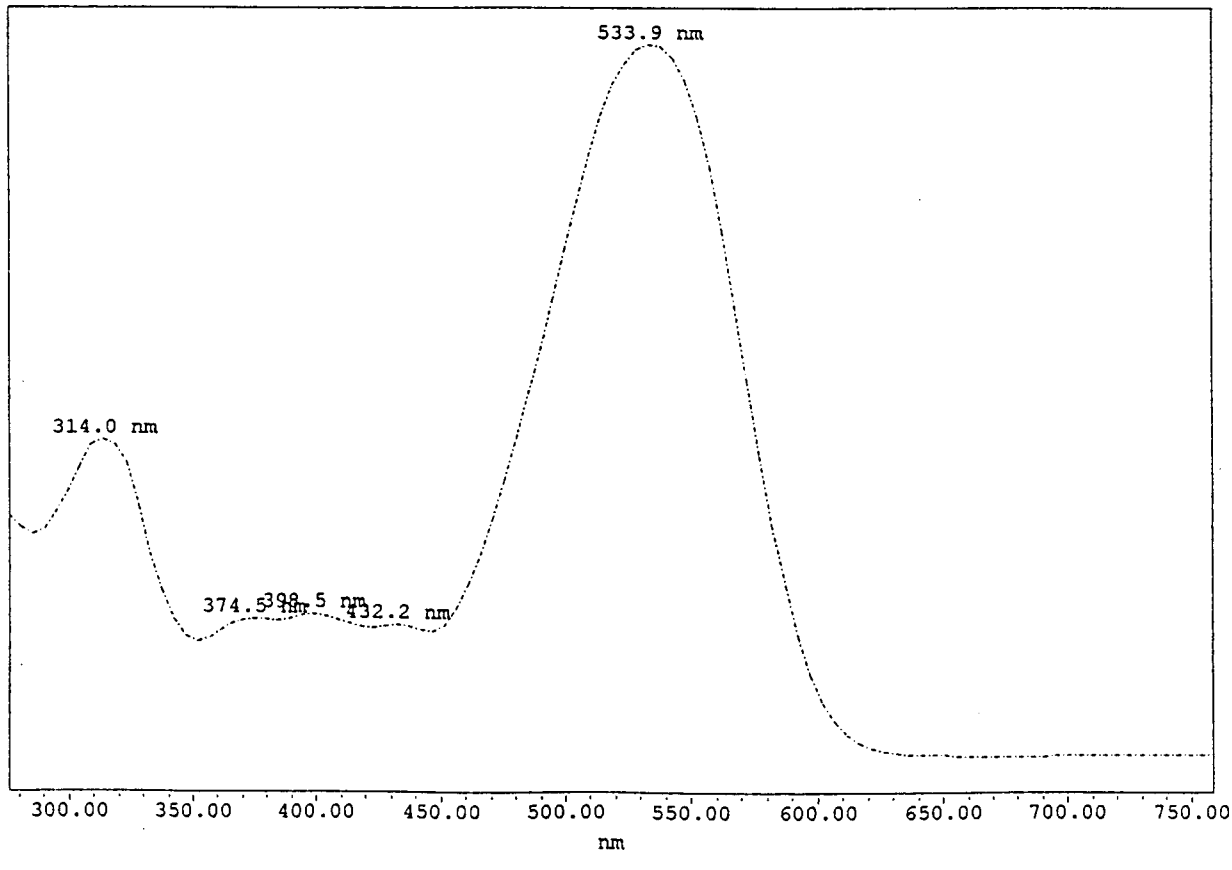




DMU 2683_CI 16290 5.805 minutes, 275 - 760 @ 4.8 nm, from 2683_16290

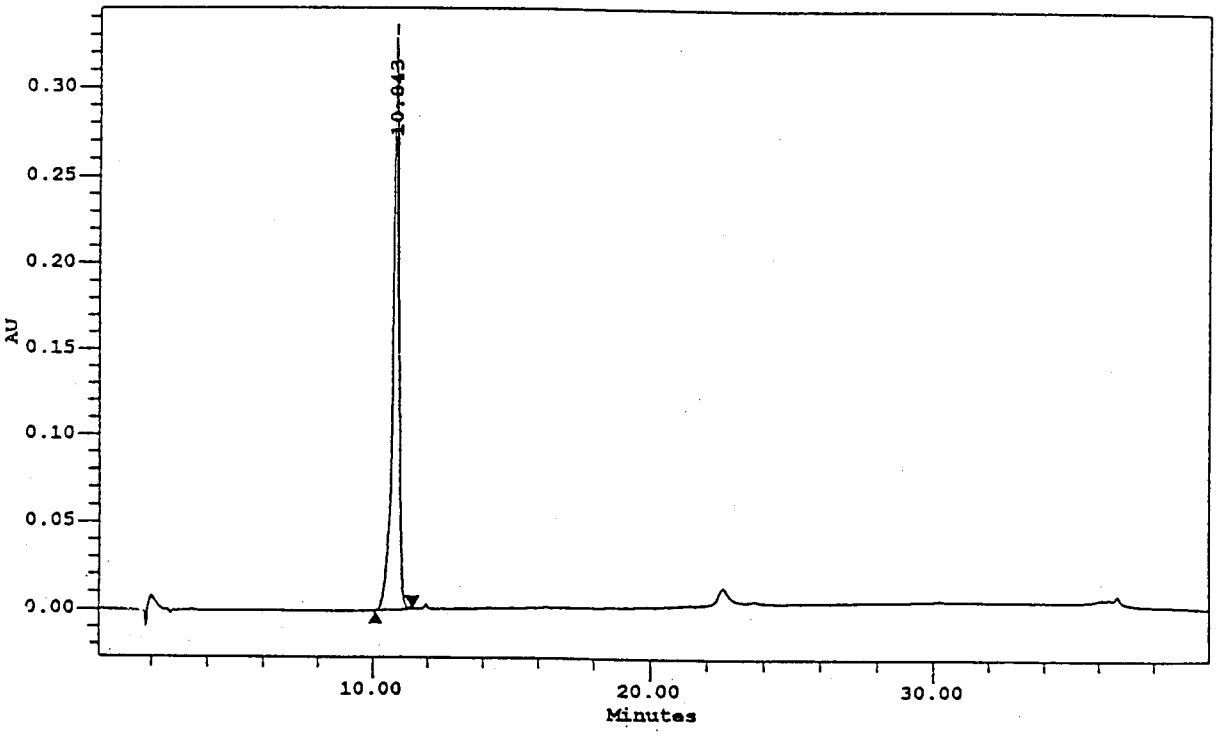
DMU 2594_C.I. 17200

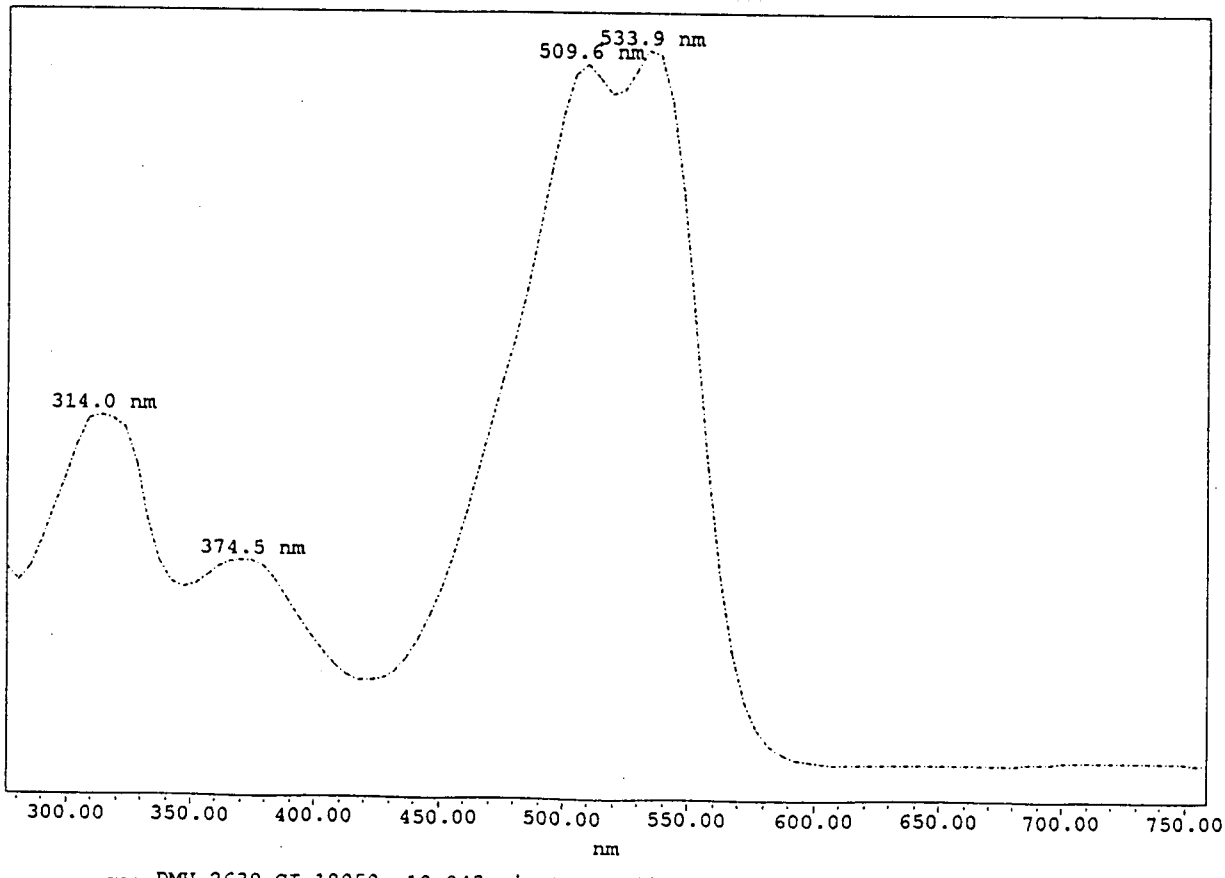




DMU 2594_CI 17200 10.807 minutes, 275 - 760 @ 4.8 nm, from 2594_CI17200

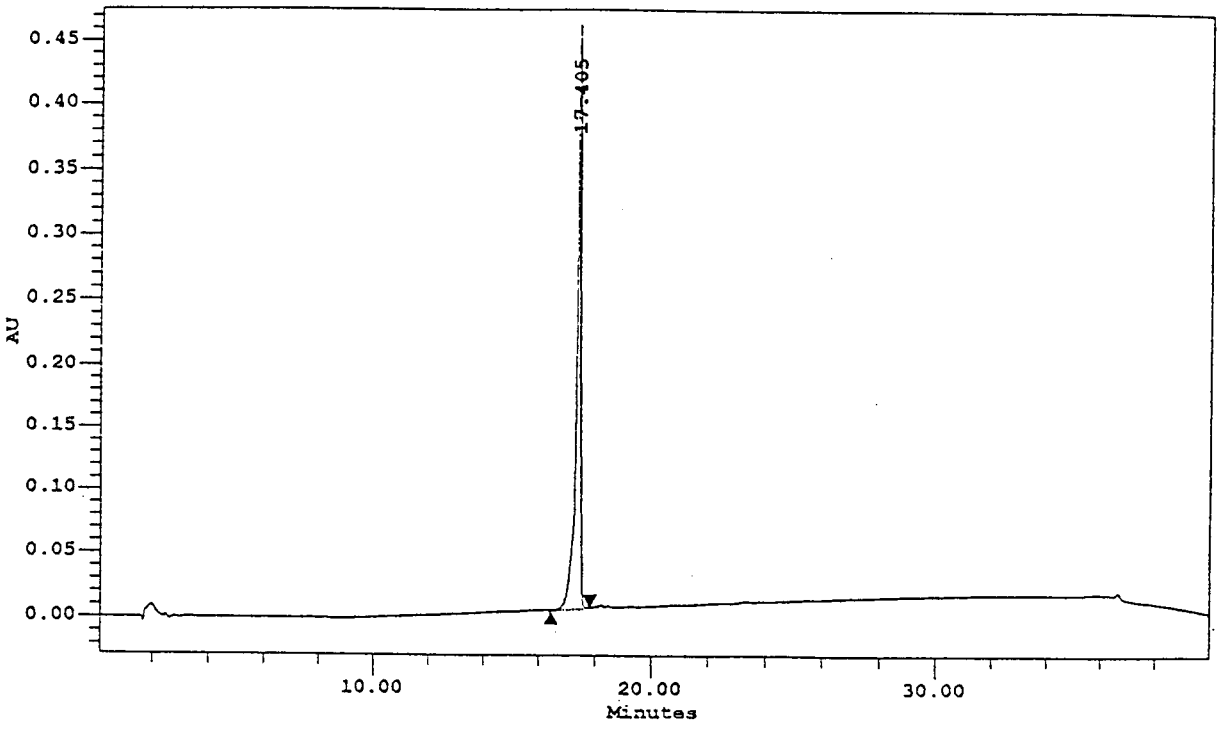
DMU 2639_C.I. 18050

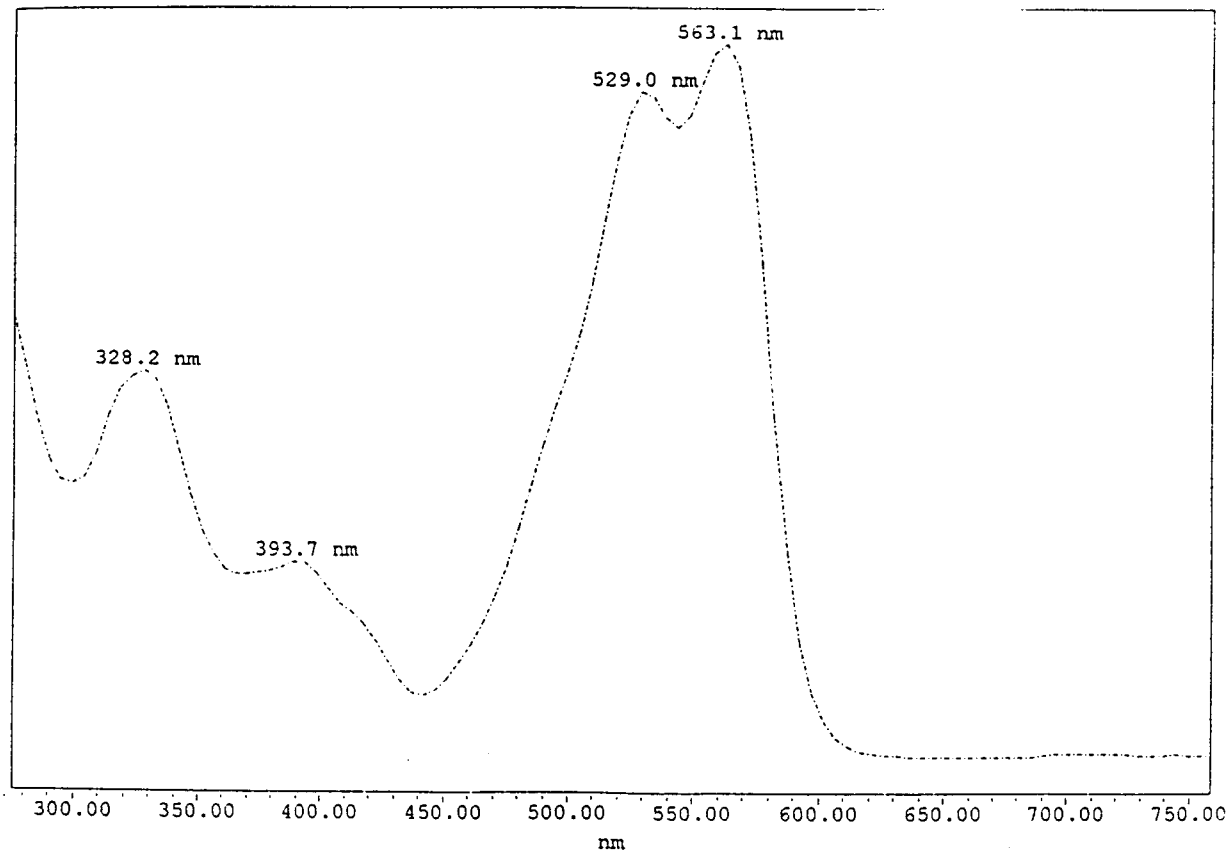




DMU 2639_CI 18050 10.843 minutes, 275 - 760 @ 4.8 nm, from 2639_18050

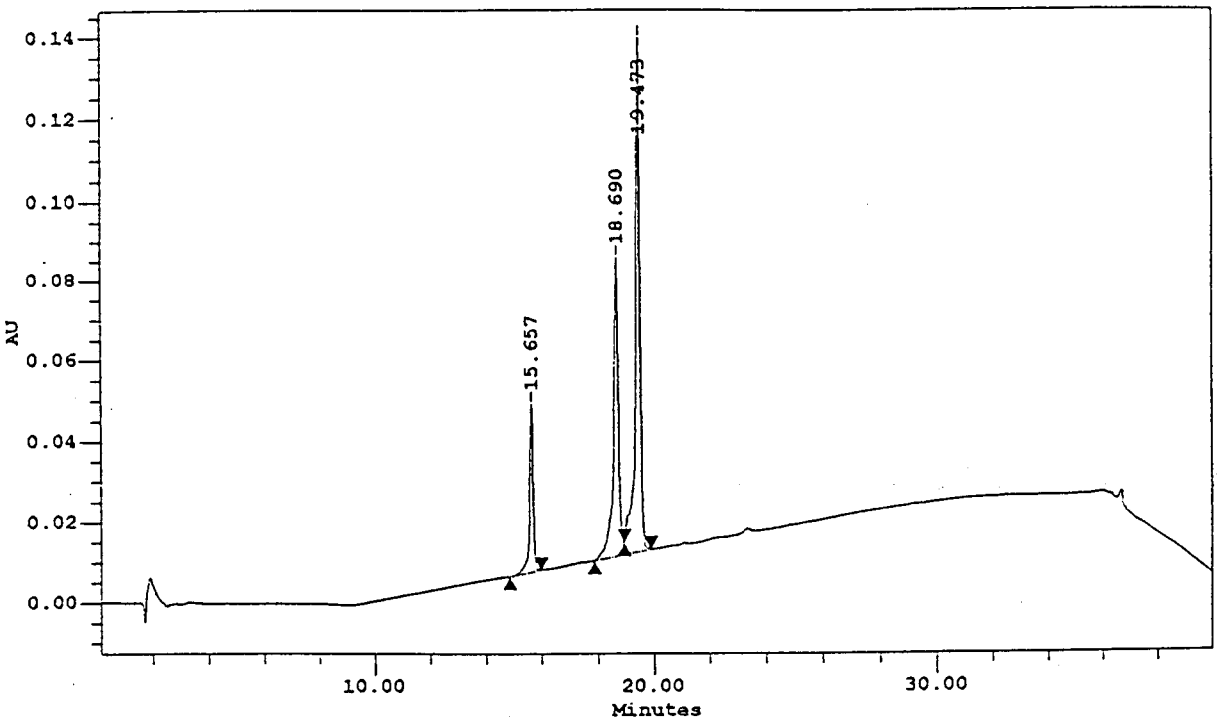
DMU 2716_C.I. 18130

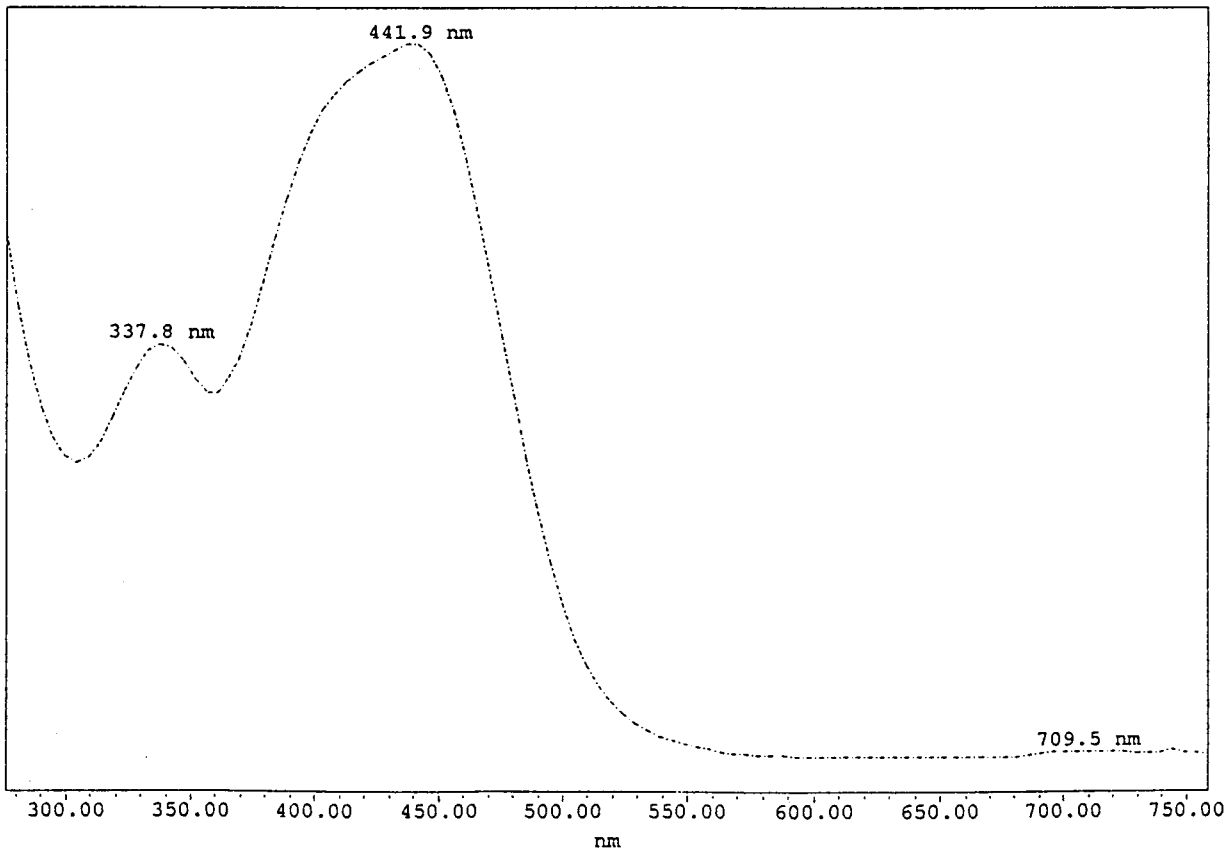




DMU 2716_CI 18130 17.405 minutes, 275 - 760 @ 4.8 nm, from 2716_18130

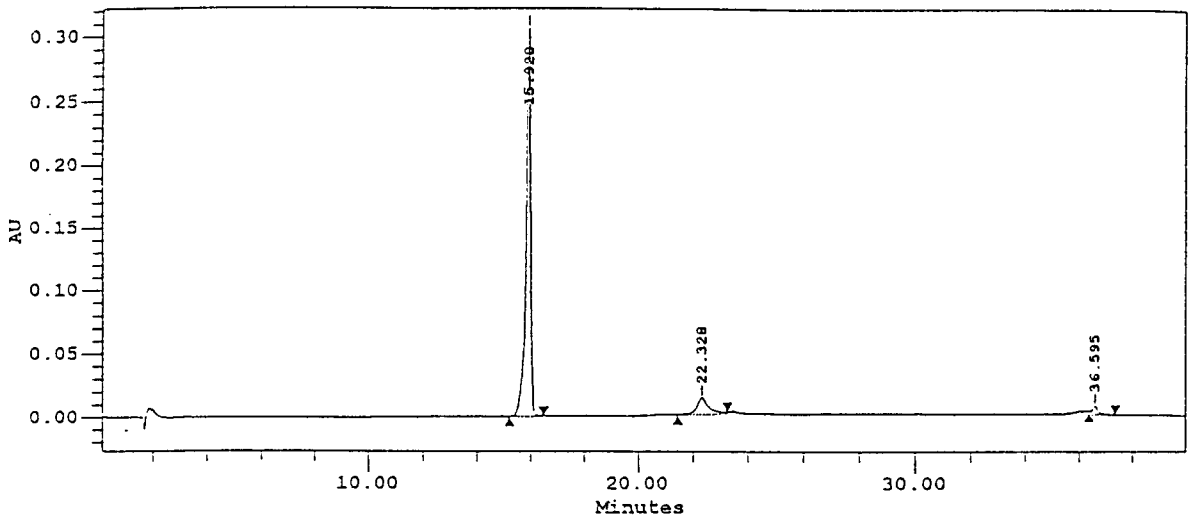
DMU 2682_C.I. 18690

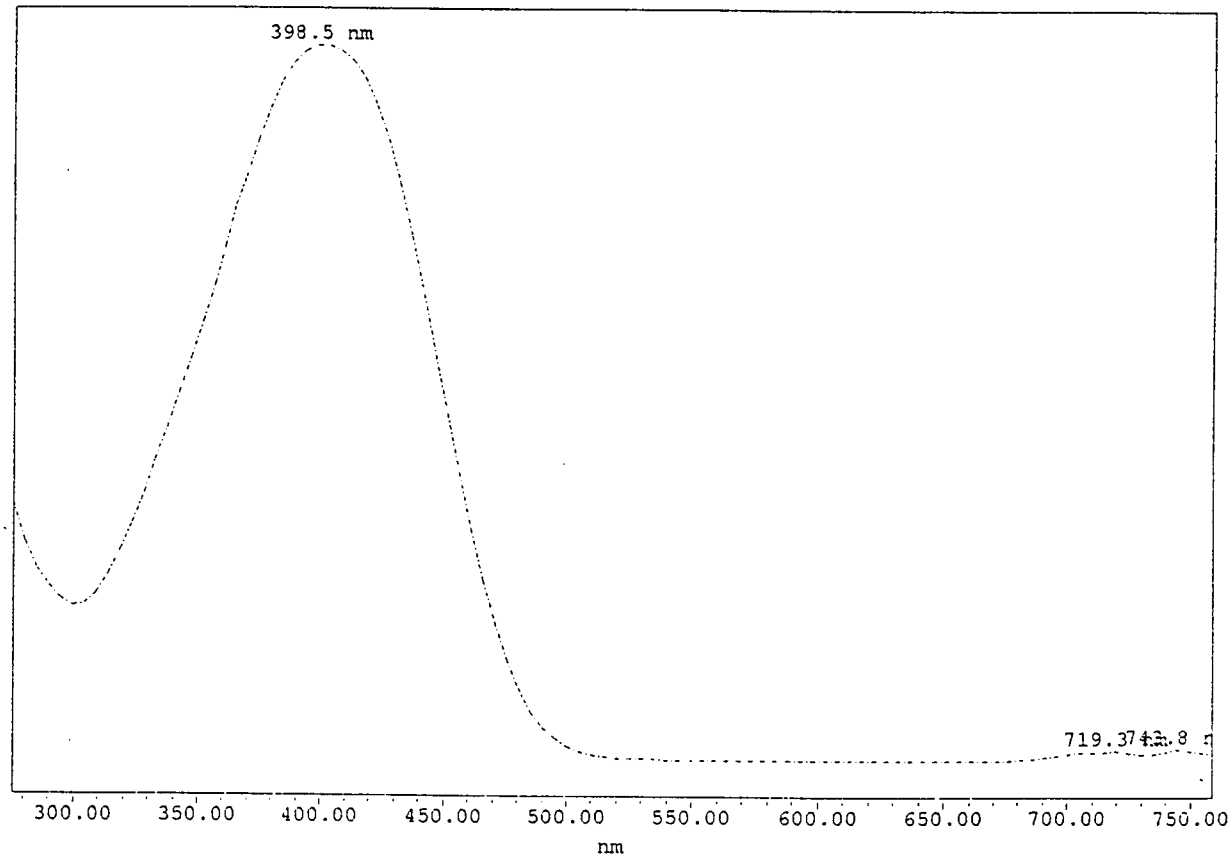




DMU 2682_CI 18690* 19.473 minutes, 275 - 760 @ 4.8 nm, from 2682_18690

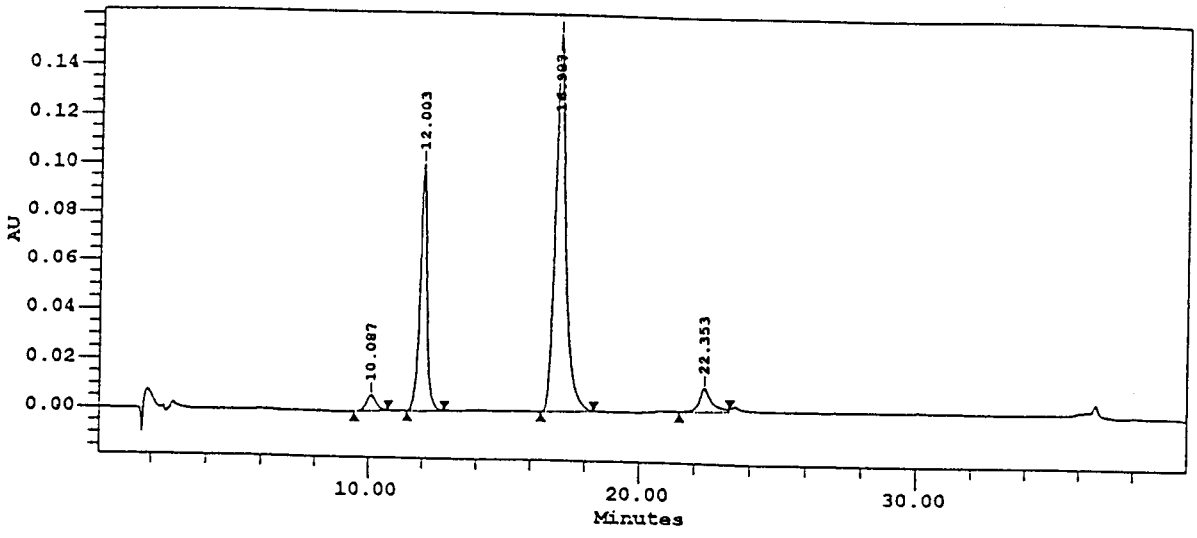
DMU 2569_C.I. 18820

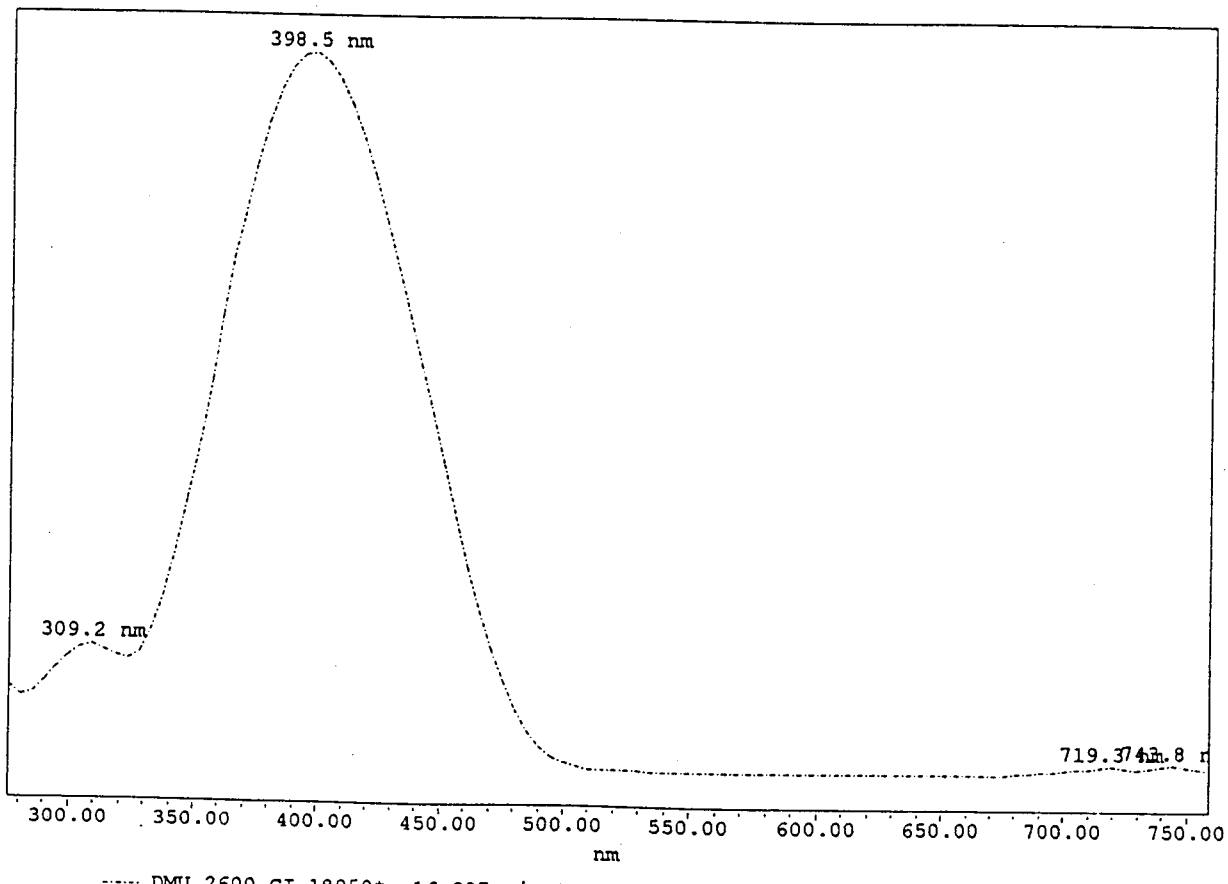




---- DMU 2569_CI 18820 15.928 minutes, 275 - 760 @ 4.8 nm, from 2569_18820

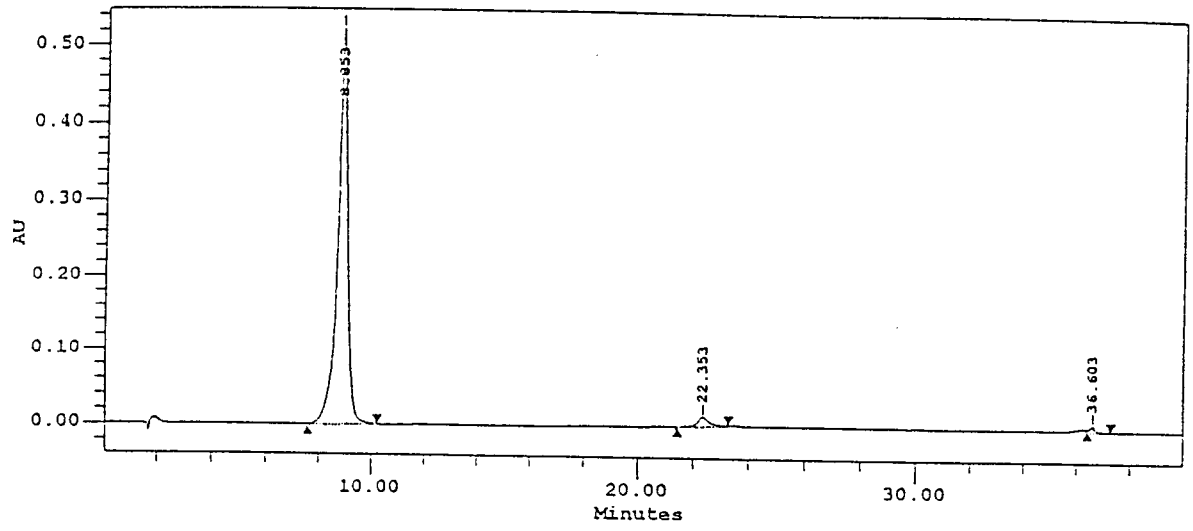
DMU 2600_C.I. 18950

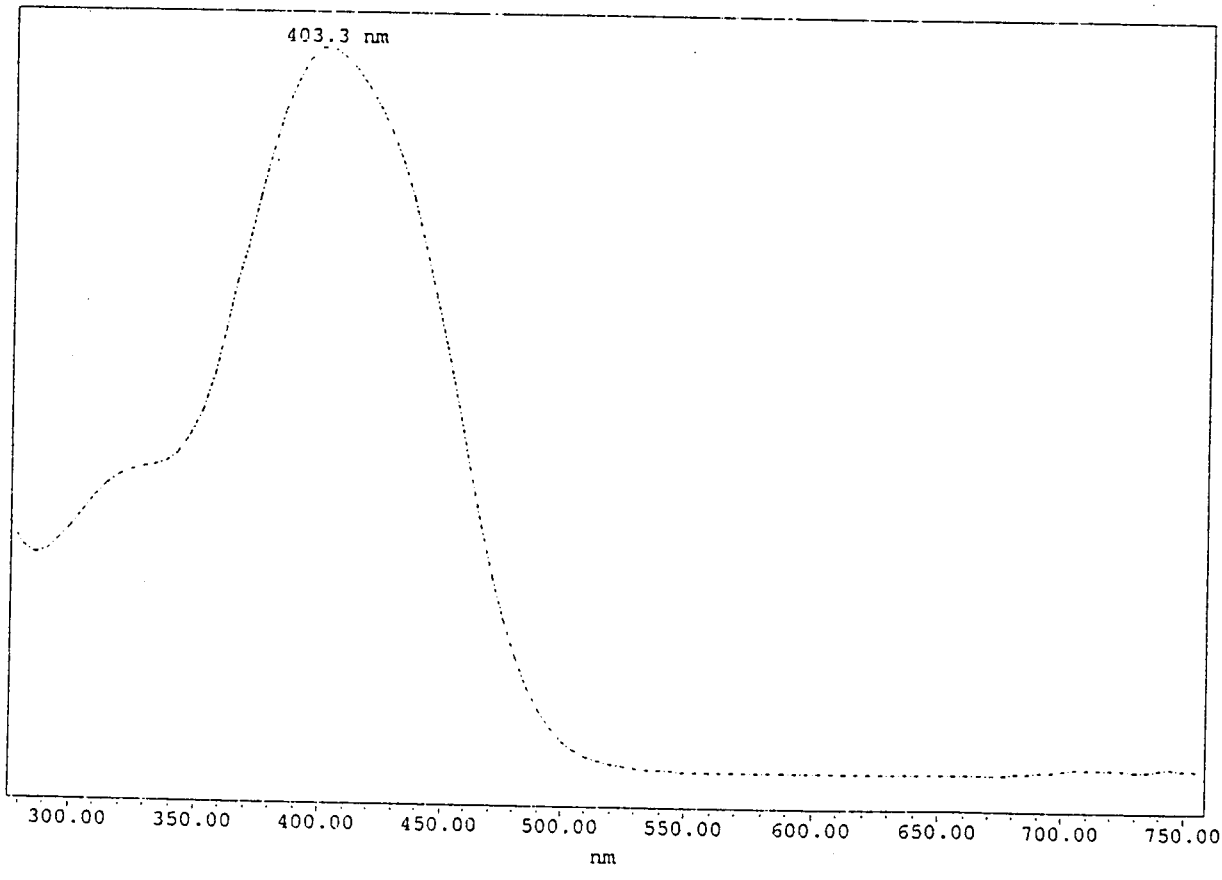




DMU 2600_CI 18950* 16.987 minutes, 275 - 760 @ 4.8 nm, from 2600_18950

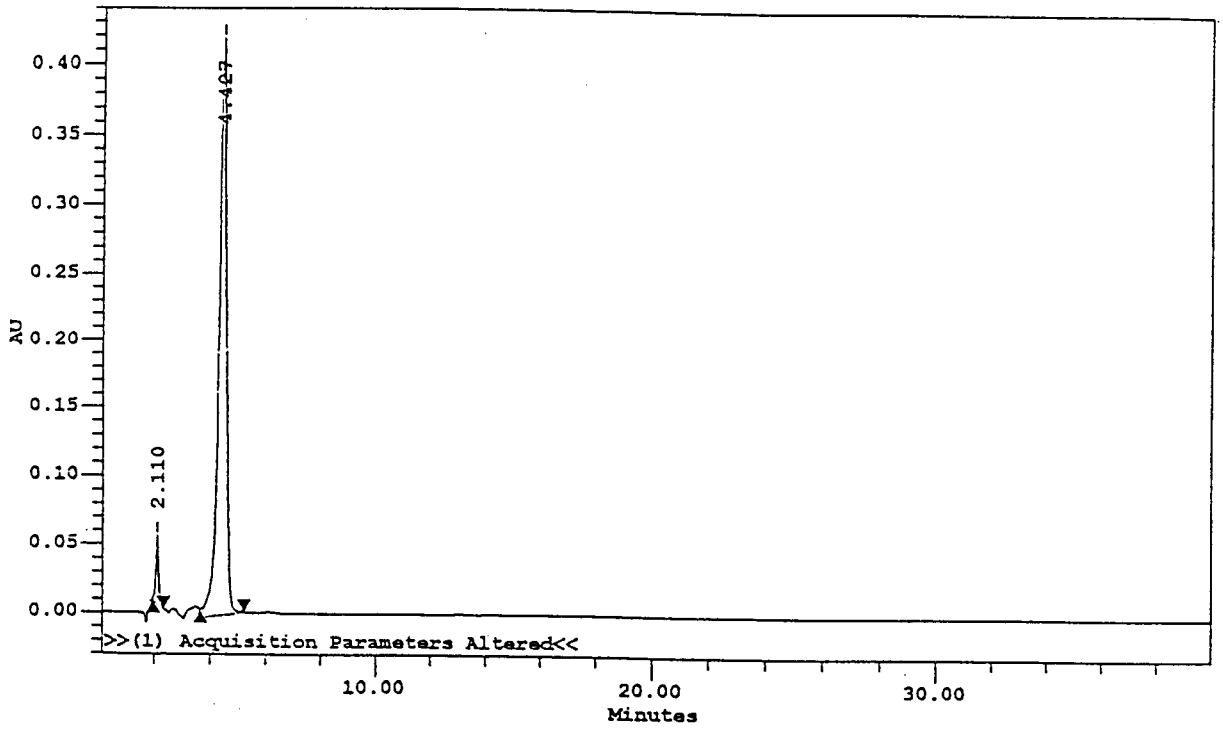
DMU 2563_C.I. 18965

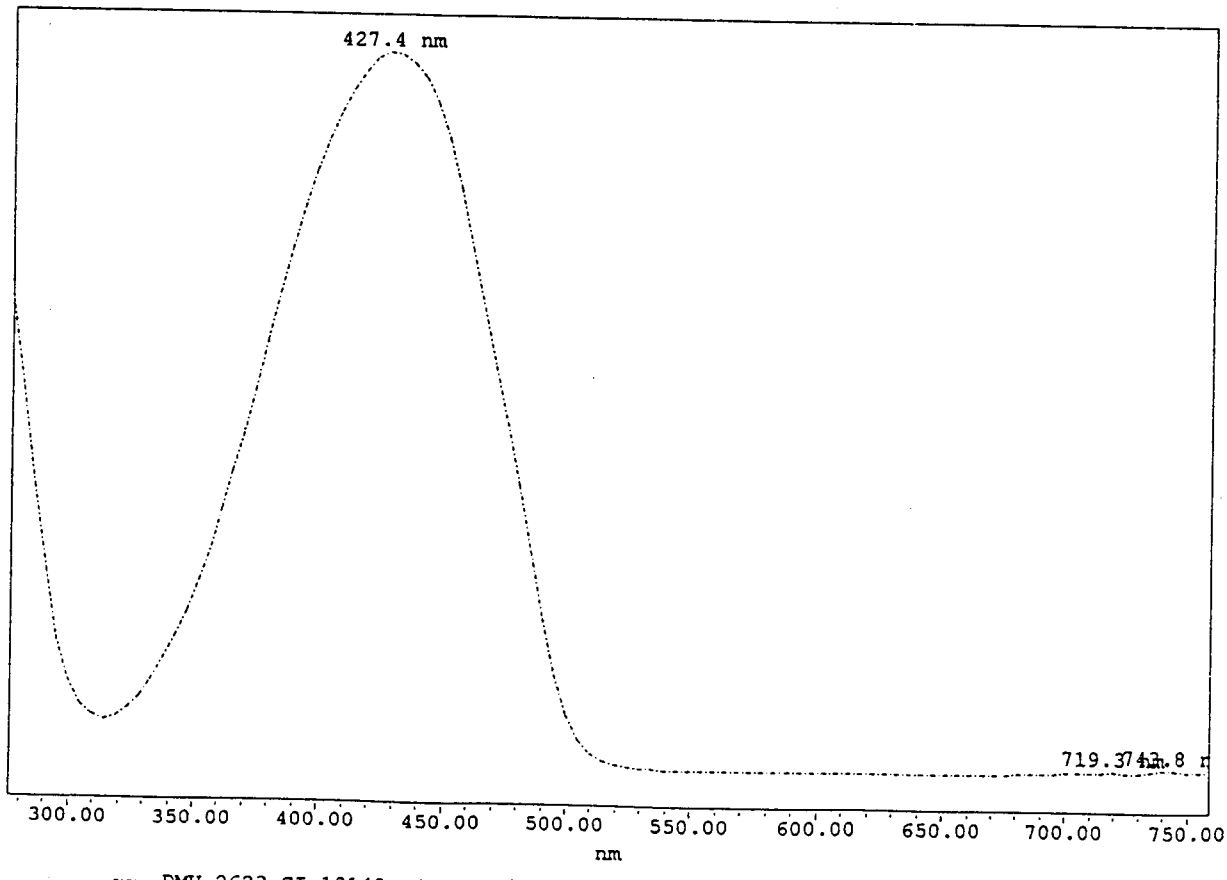




DMU 2563_CI 18965 8.853 minutes, 275 - 760 @ 4.8 nm, from 2563_18965

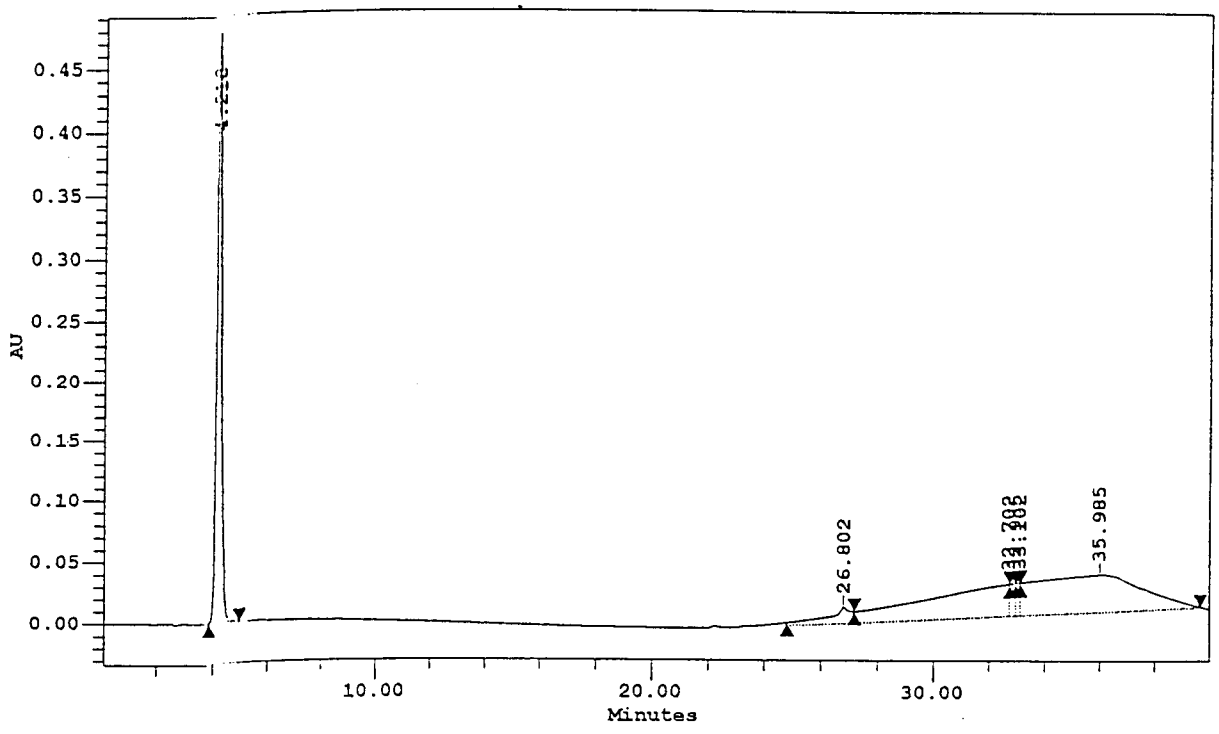
DMU 2623_C.I. 19140

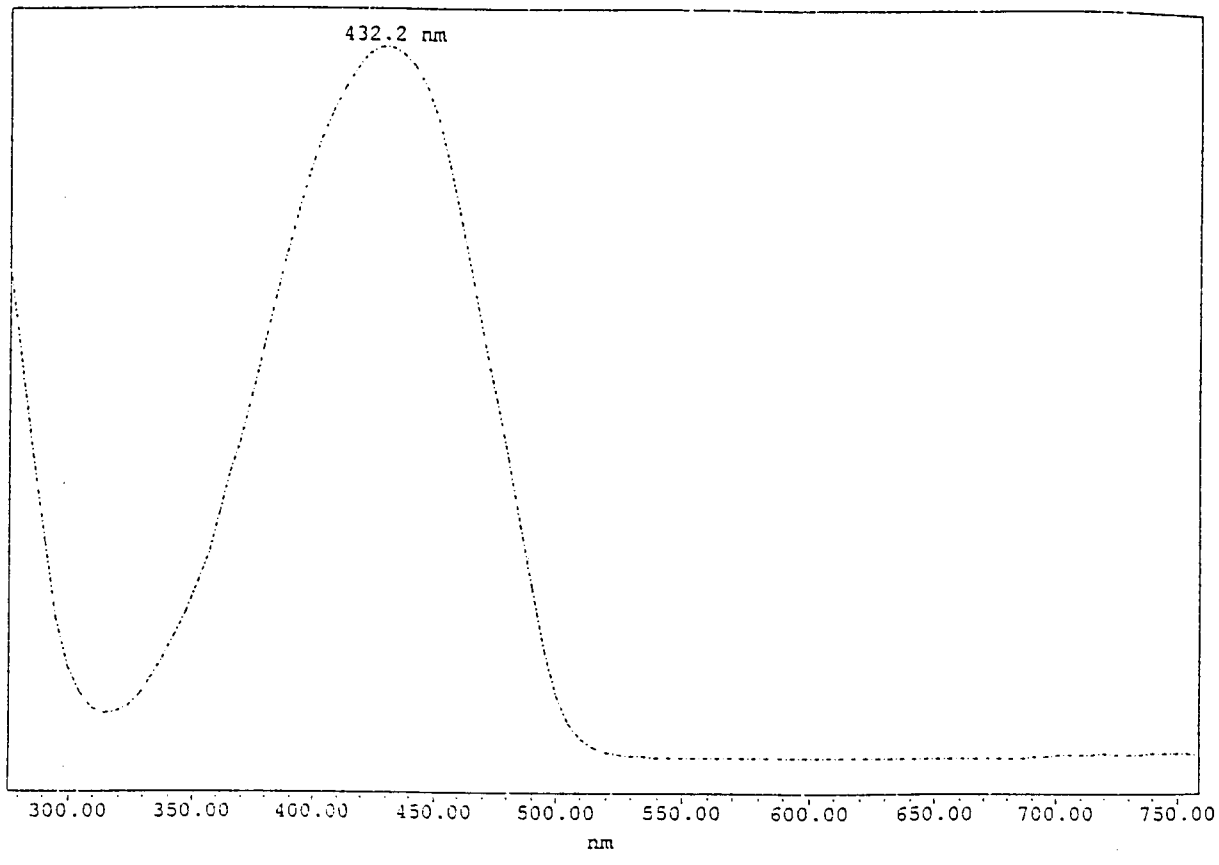




DMU 2623_CI 19140 4.427 minutes, 275 - 760 @ 4.8 nm, from 2623_19140

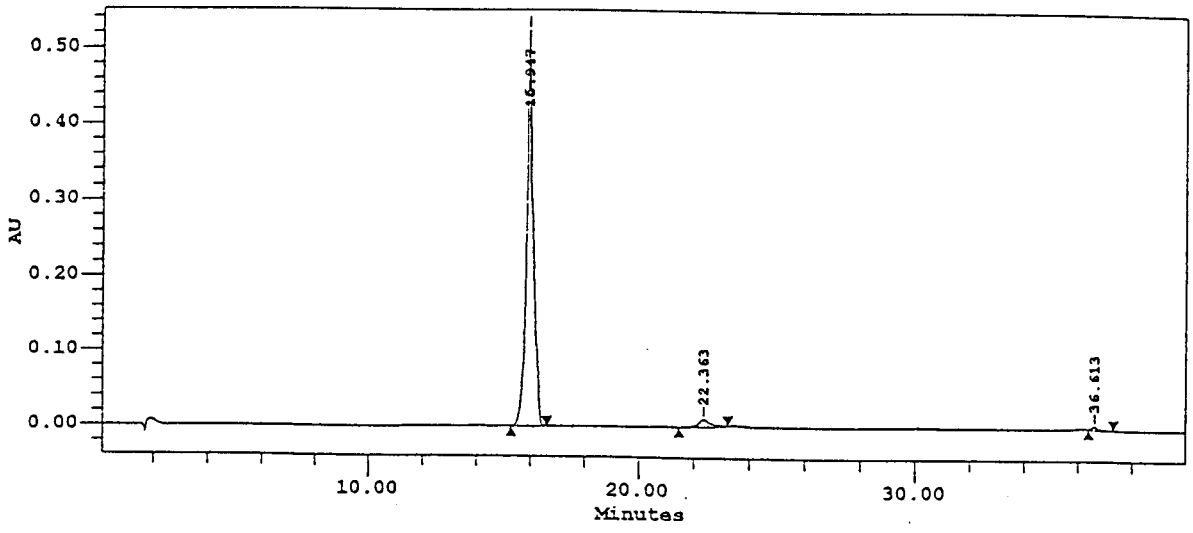
DMU 2567_C.I. 19140:1

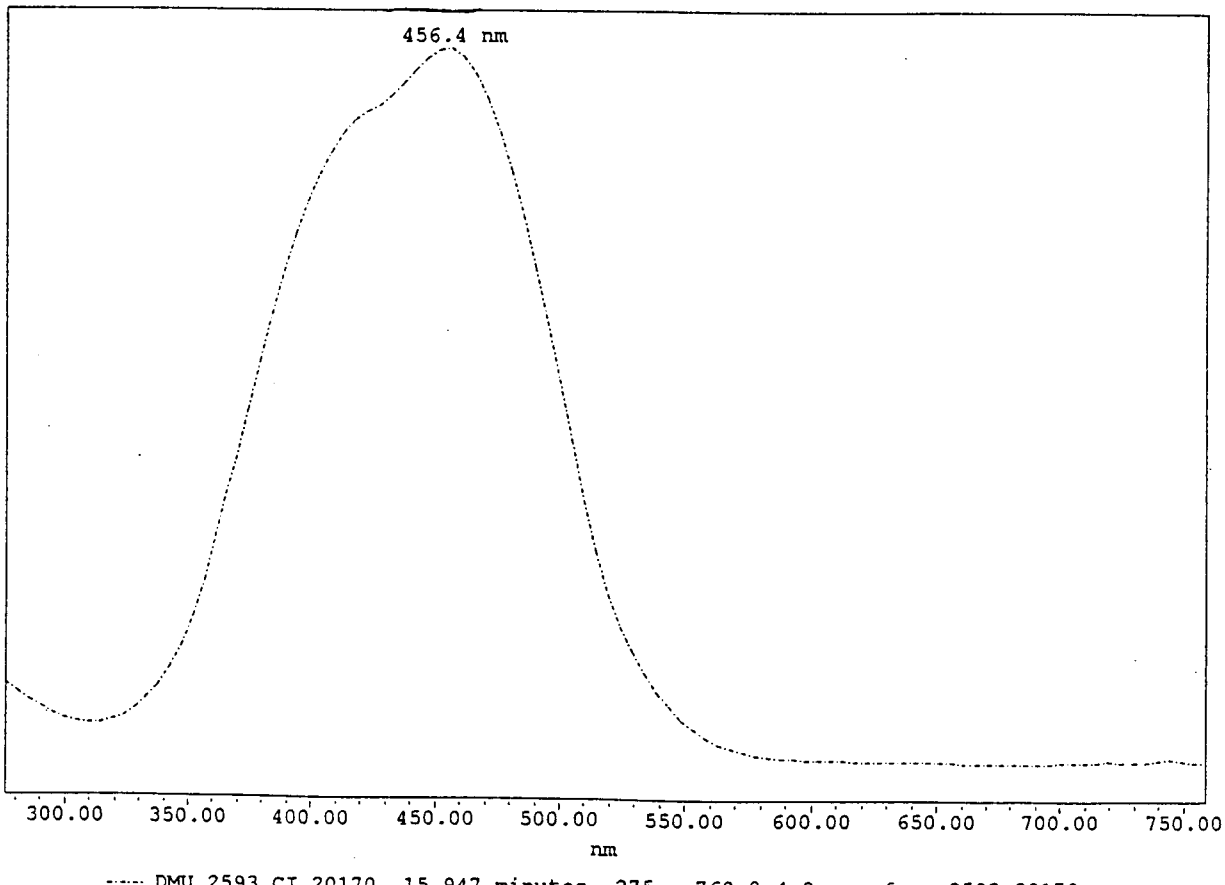




DMU 2567_CI 19140:1 4.218 minutes, 275 - 760 @ 4.8 nm, from 2567_CI19140:1

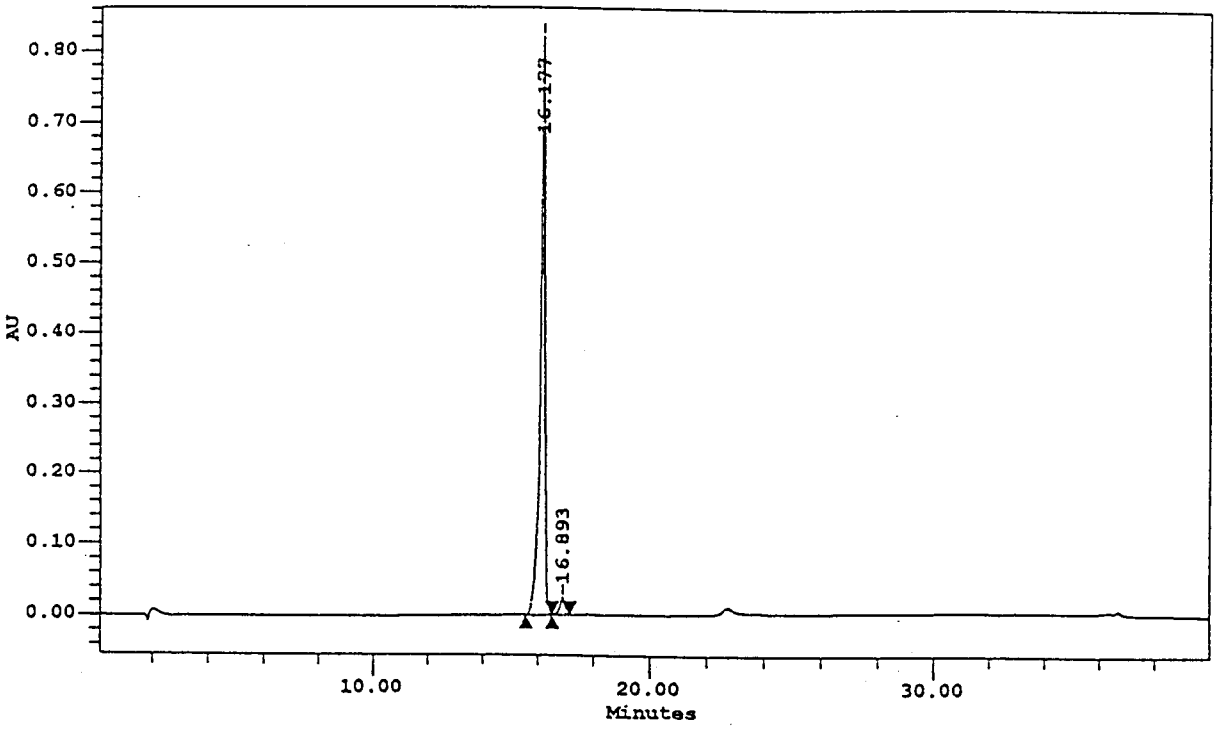
DMU 2593_C.I. 20170

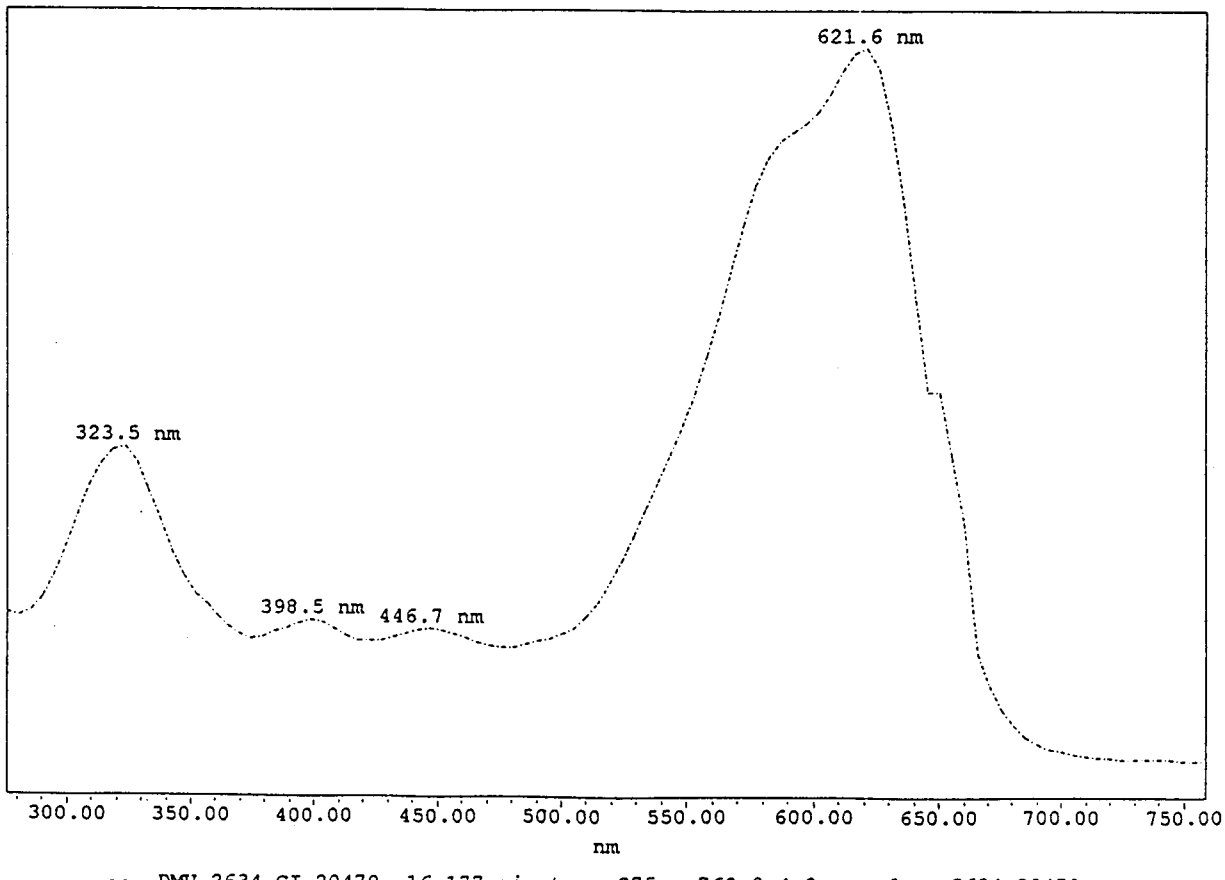




DMU 2593_CI 20170 15.947 minutes, 275 - 760 @ 4.8 nm, from 2593_20170

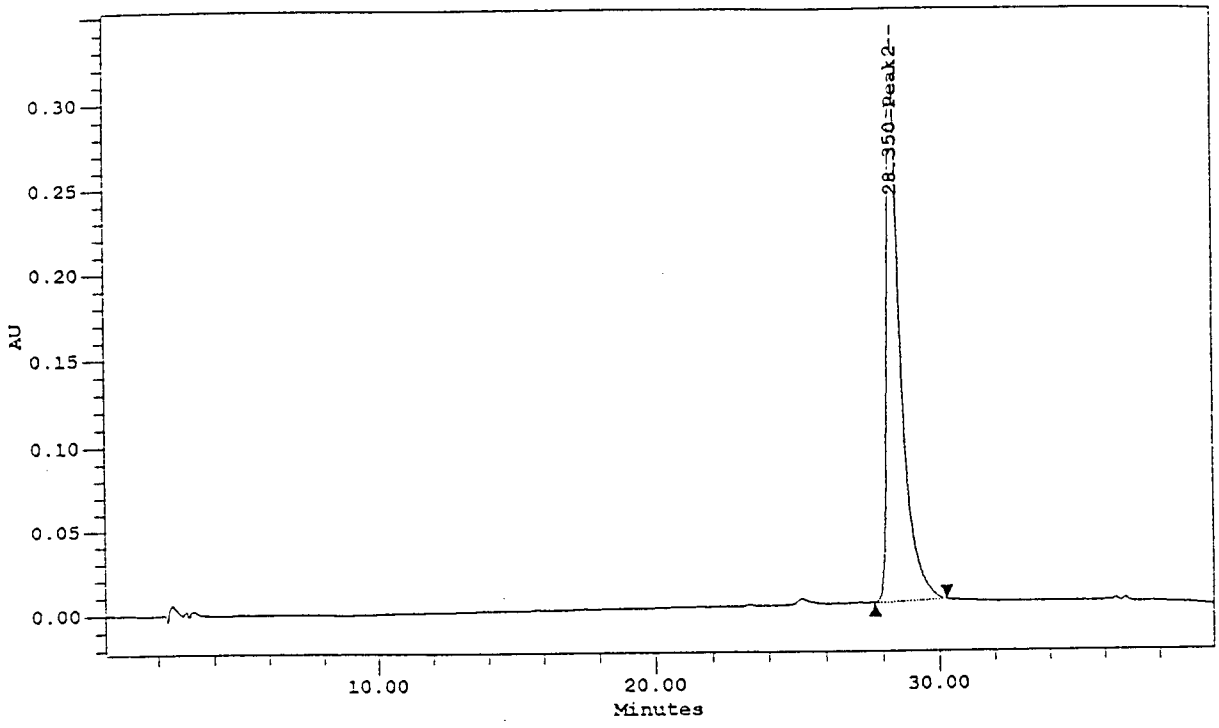
DMU 2634_C.I. 20470

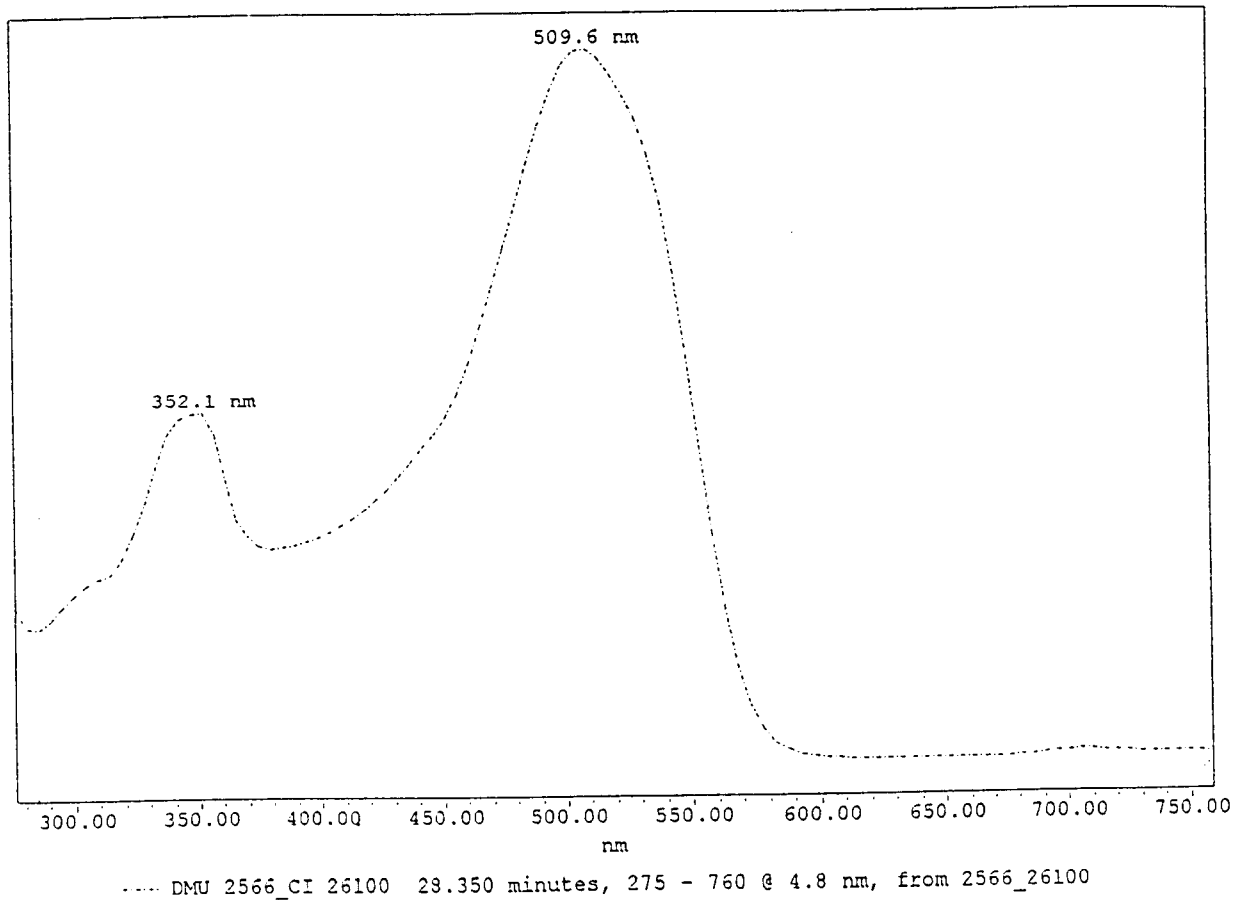




DMU 2634_CI 20470 16.177 minutes, 275 - 760 @ 4.8 nm, from 2634_20470

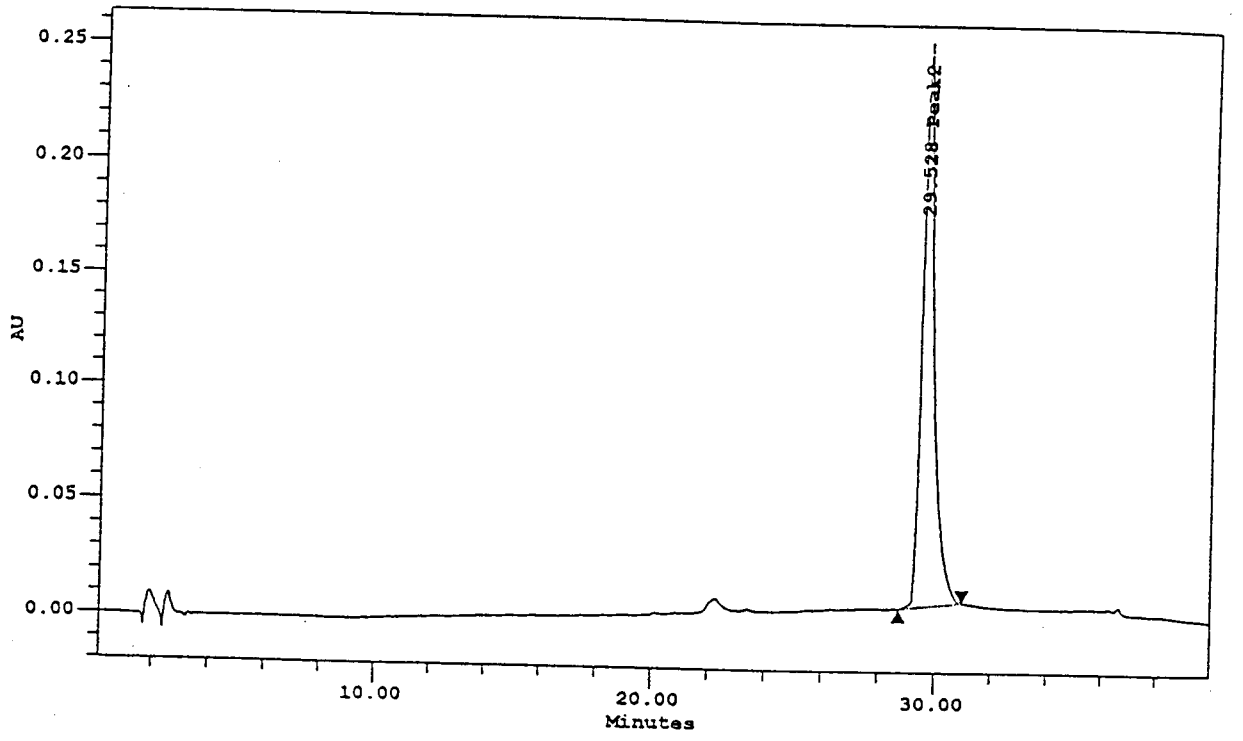
DMU 2566_C.I. 26100

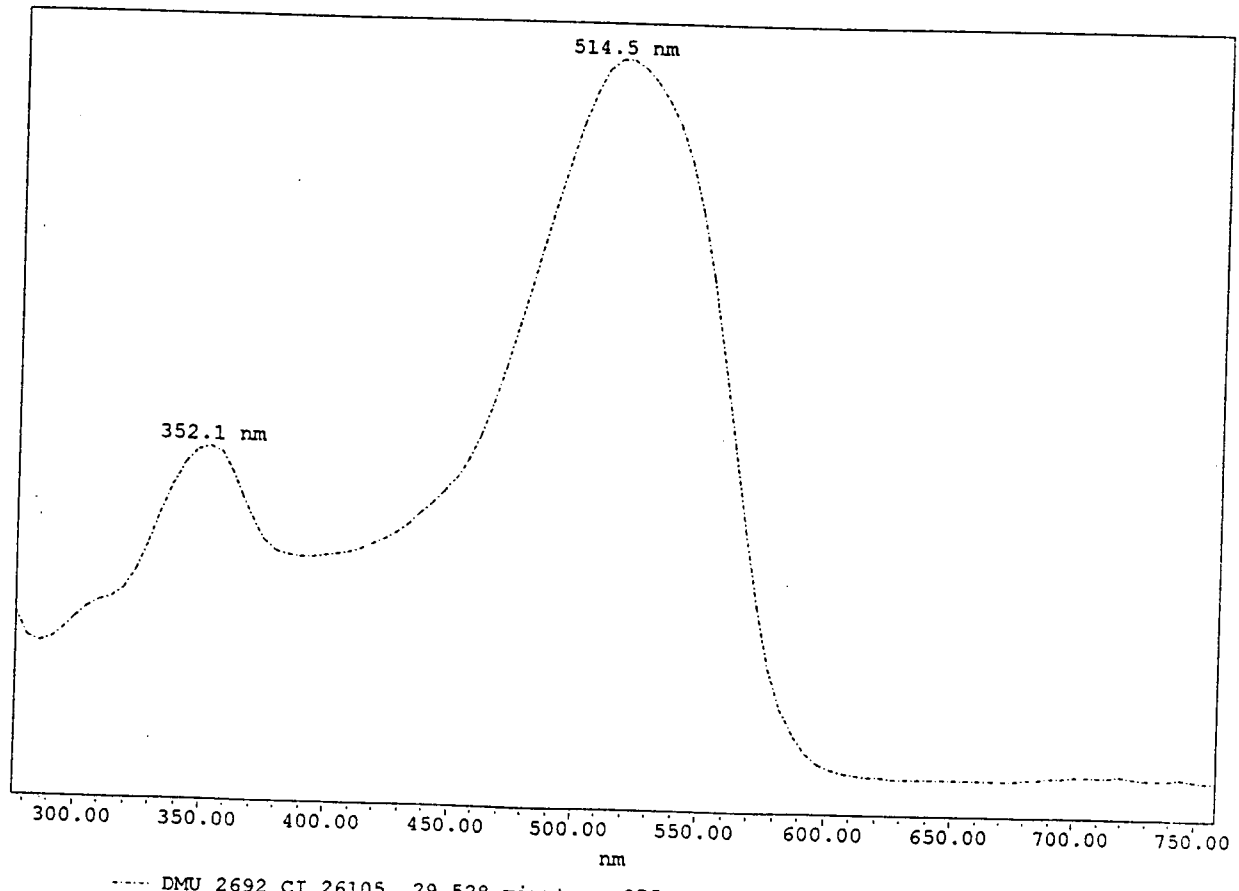




---- DMU 2566_CI 26100 28.350 minutes, 275 - 760 @ 4.8 nm, from 2566_26100

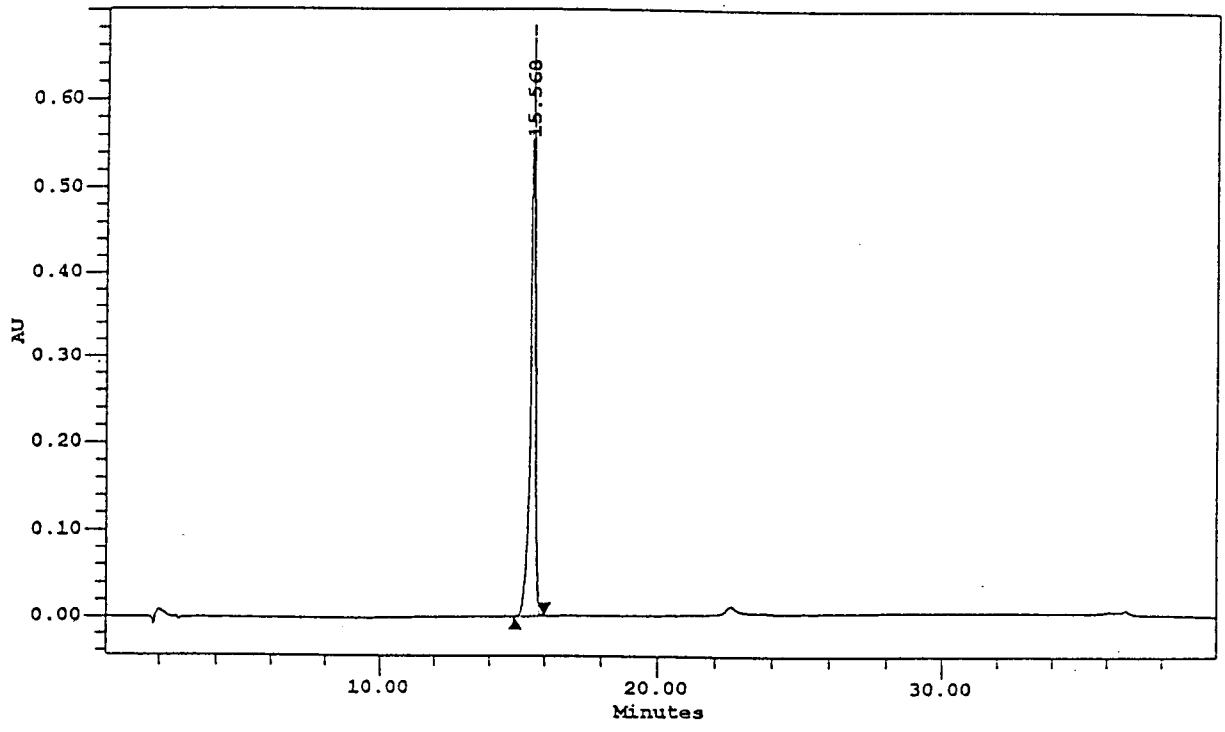
DMU 2692_C.I. 26105

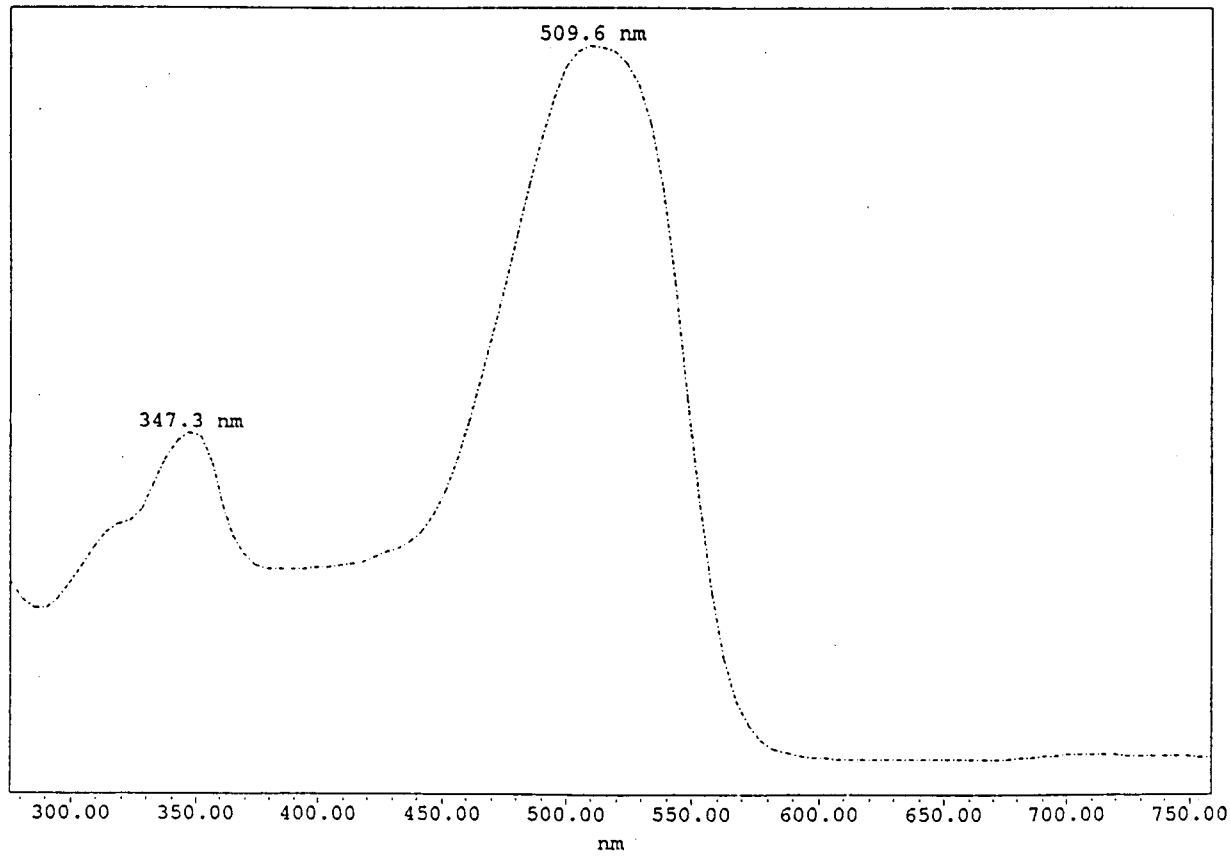




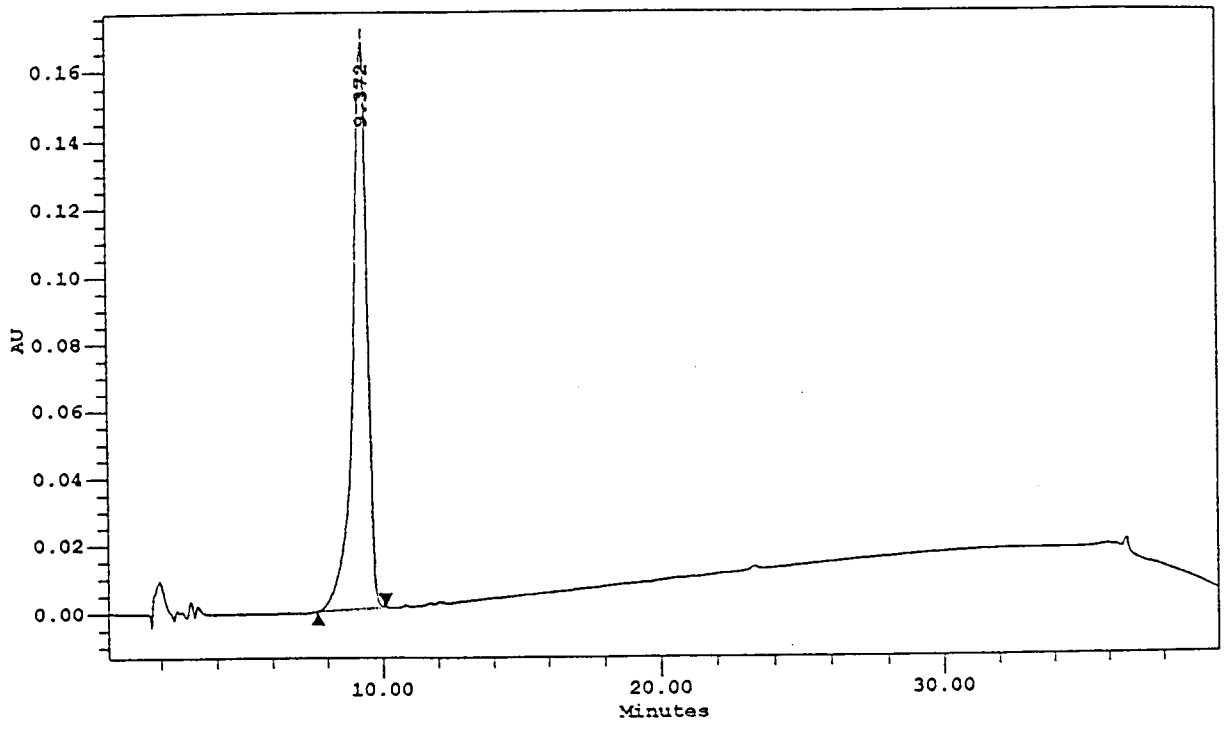
DMU 2692_CI 26105 29.528 minutes, 275 - 760 @ 4.8 nm, from 2692_26105

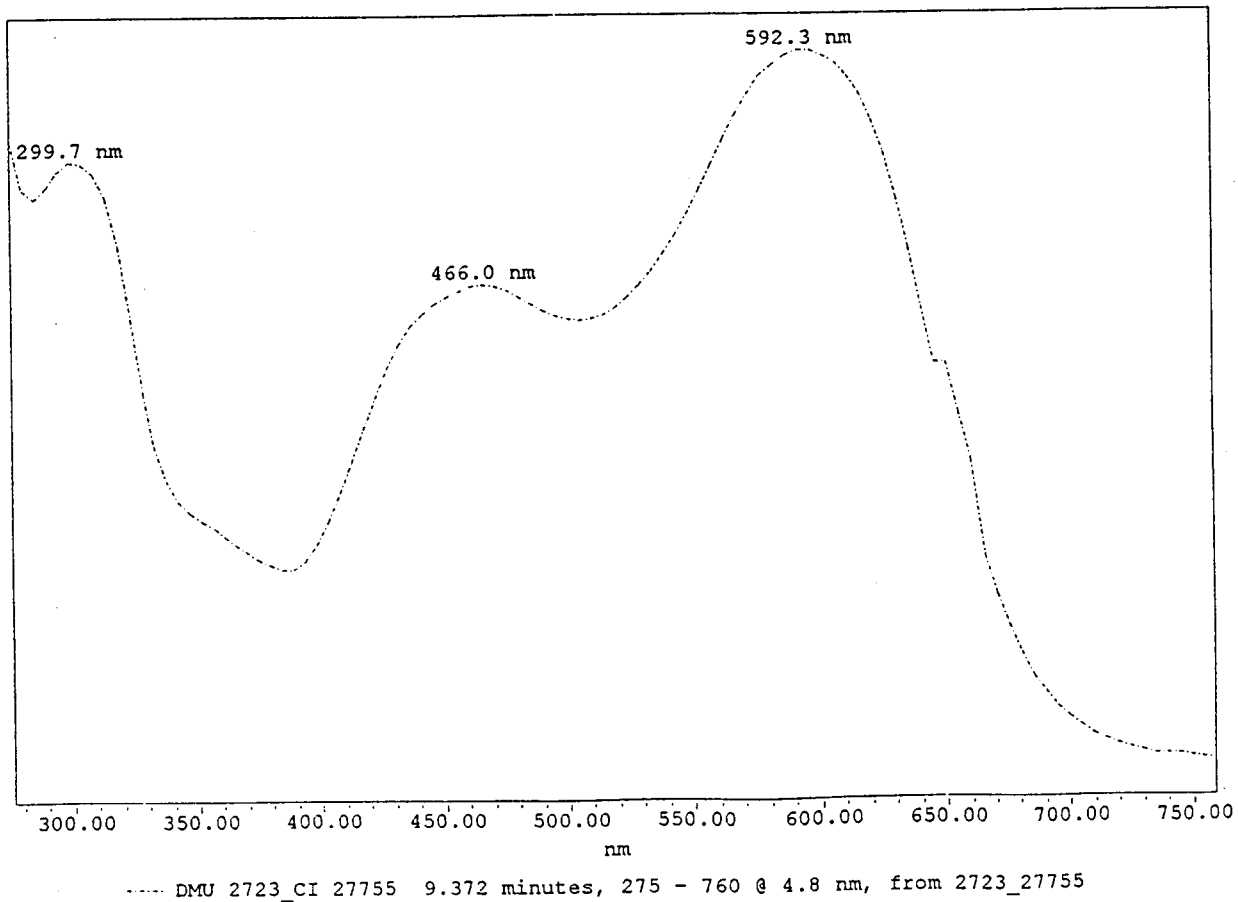
DMU 2655_C.I. 27290





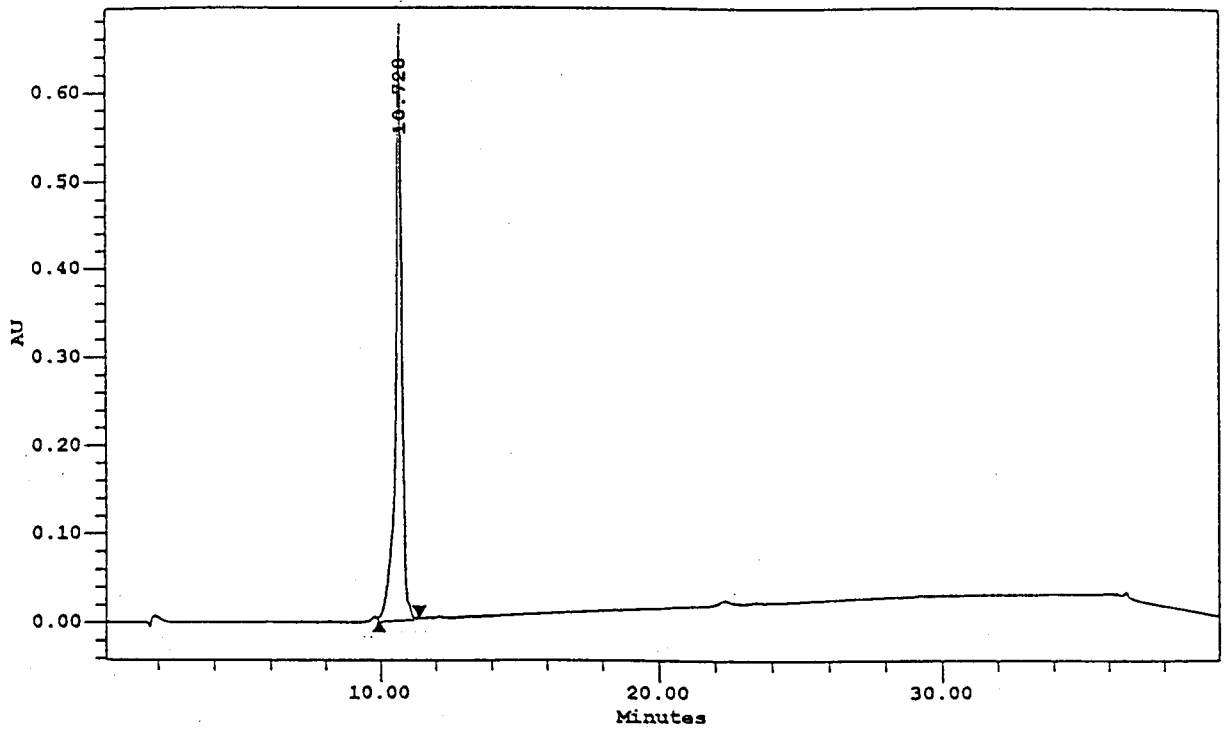
DMU 2723_C.I. 27755

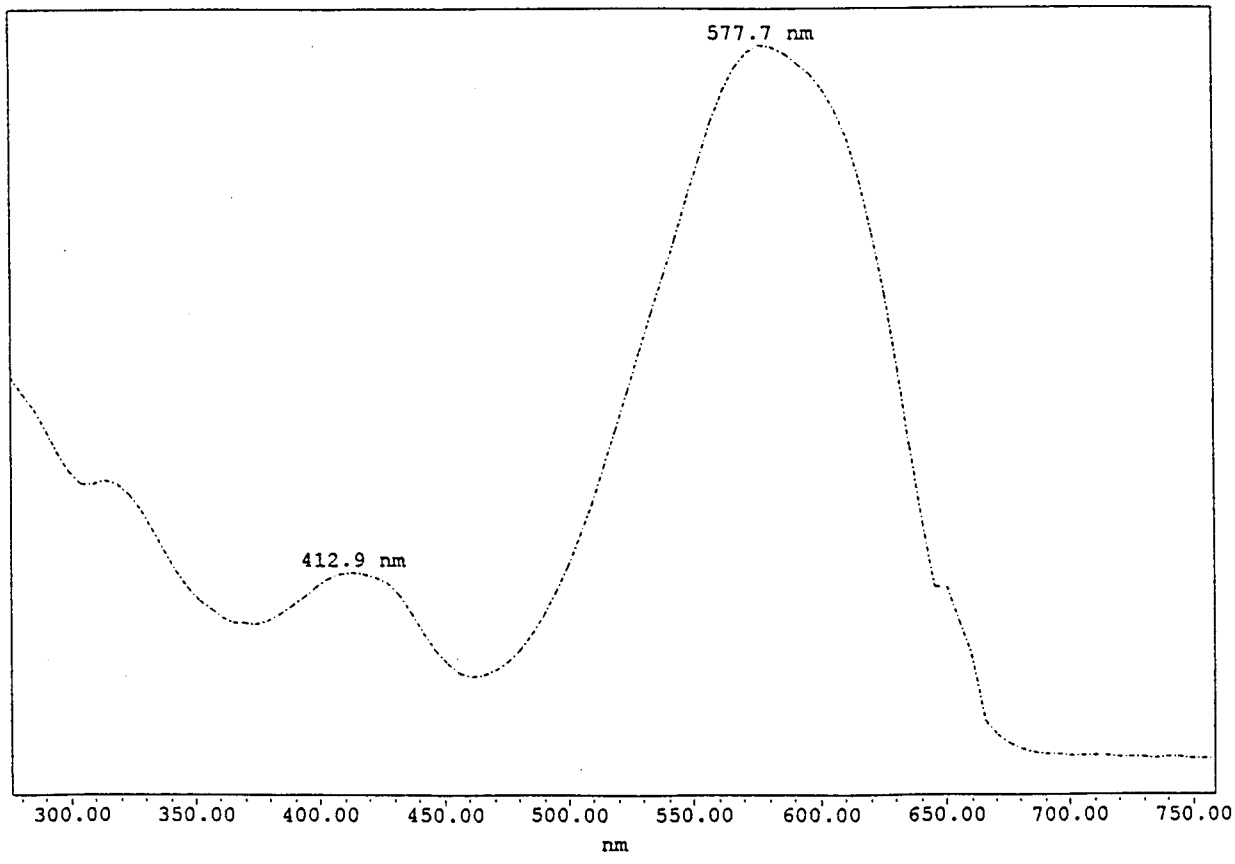




DMU 2723_CI 27755 9.372 minutes, 275 - 760 @ 4.8 nm, from 2723_27755

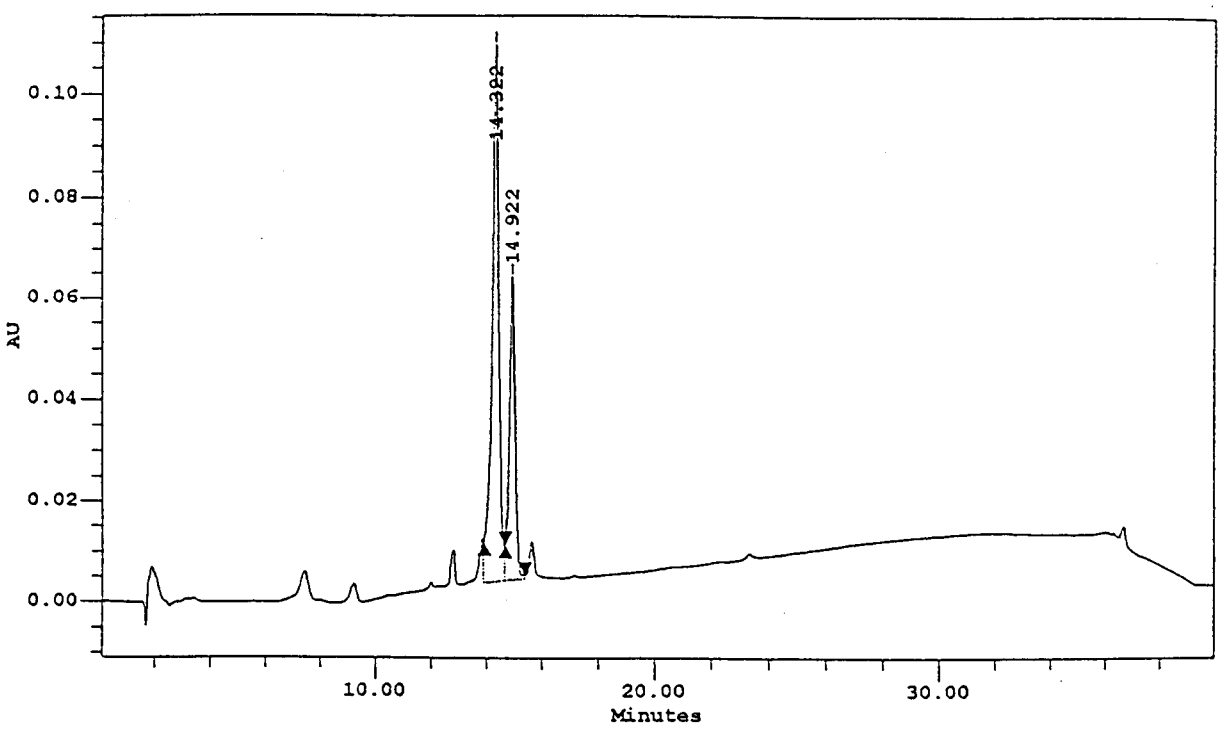
DMU 2603_C.I. 28440

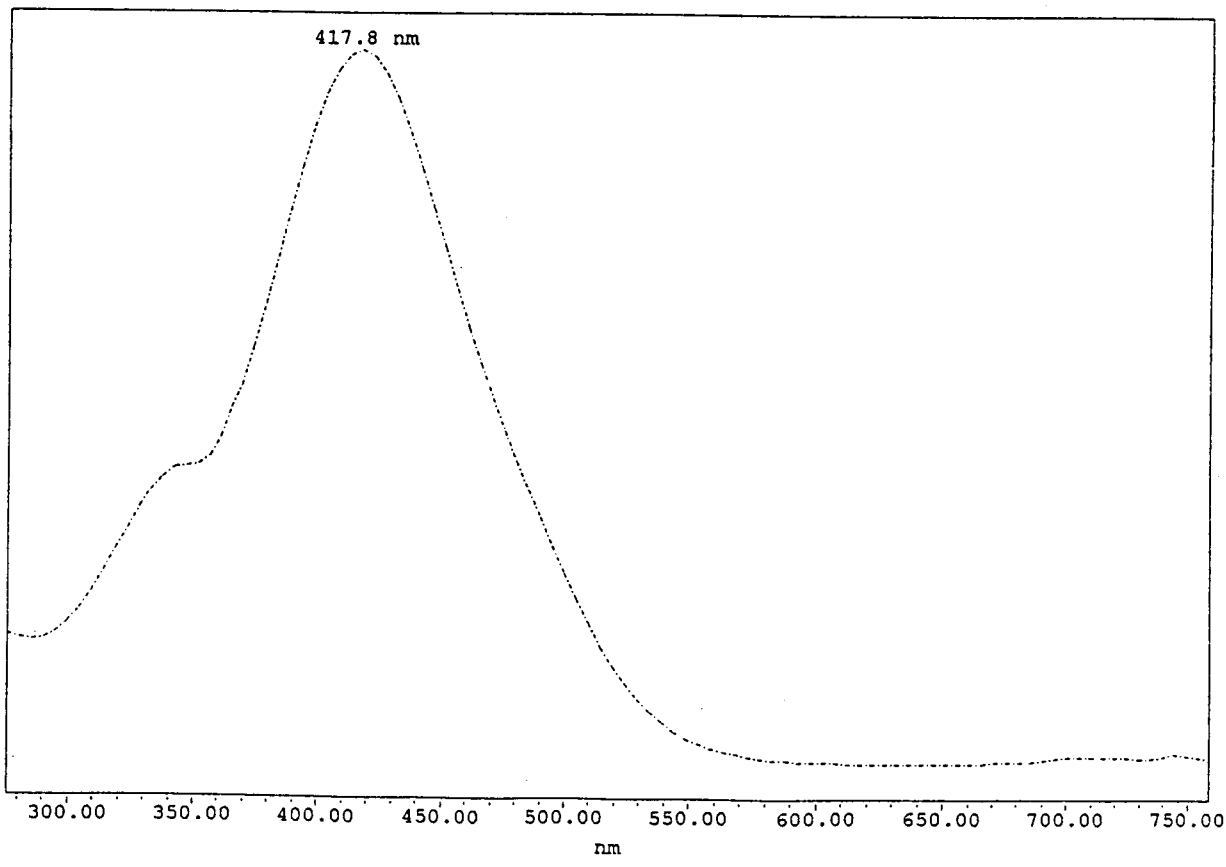




DMU 2603_CI 28440 10.728 minutes, 275 - 760 @ 4.8 nm, from 2603_28440

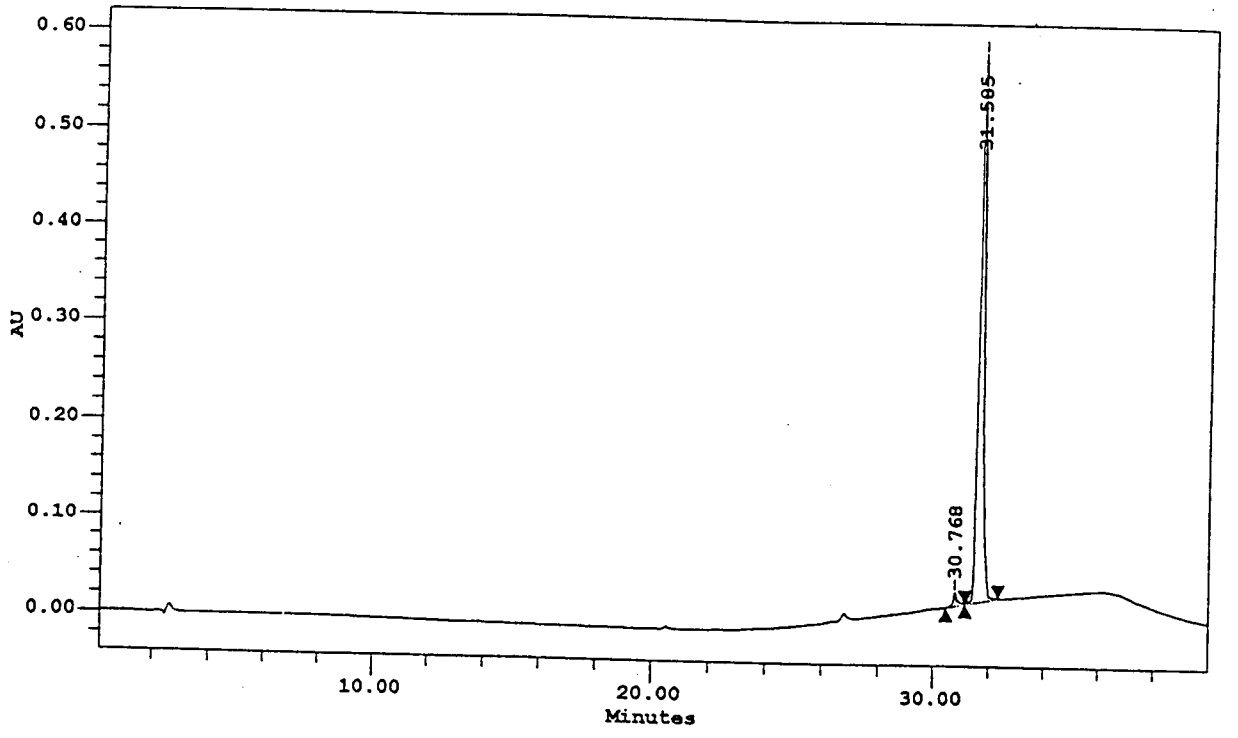
DMU 2718_C.I. 40215

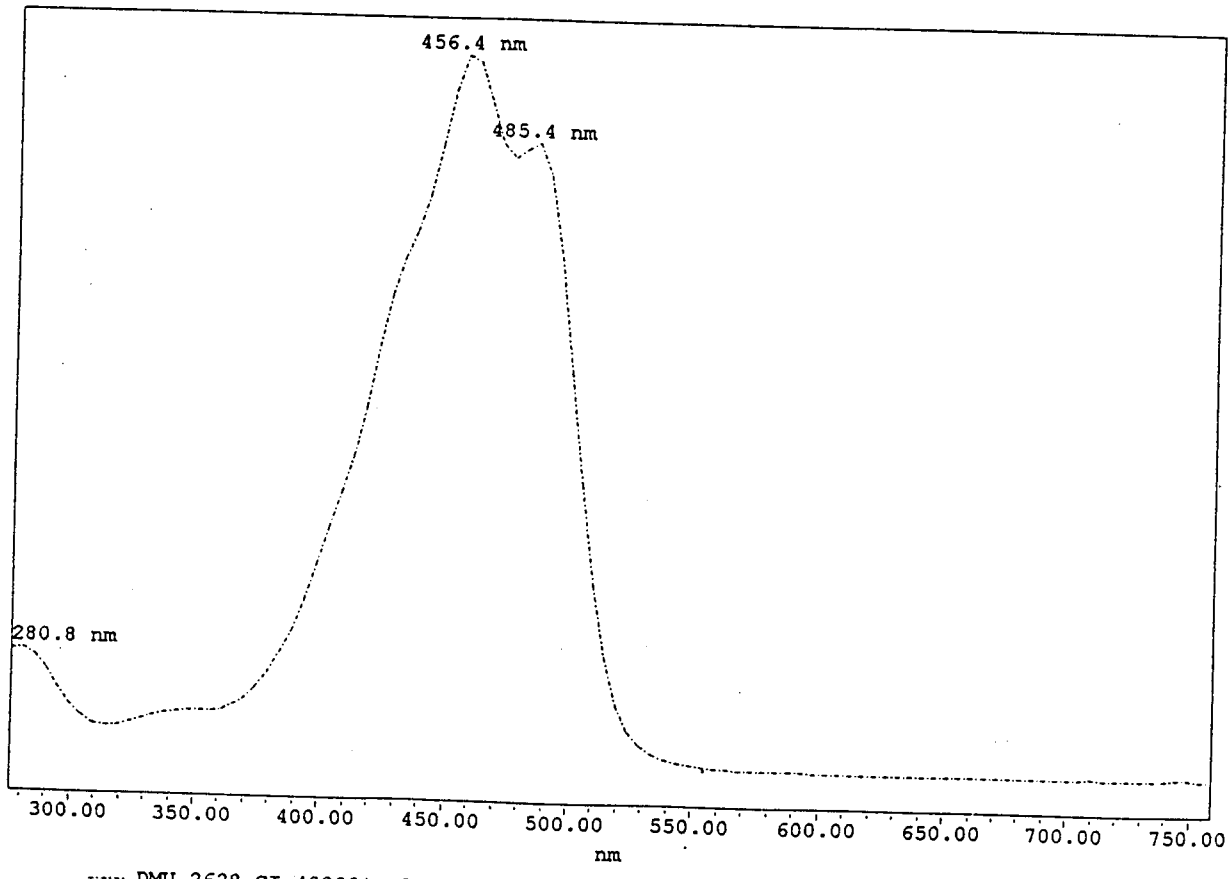




DMU 2718_CI 40215* 14.322 minutes, 275 - 760 @ 4.8 nm, from 2718_40215

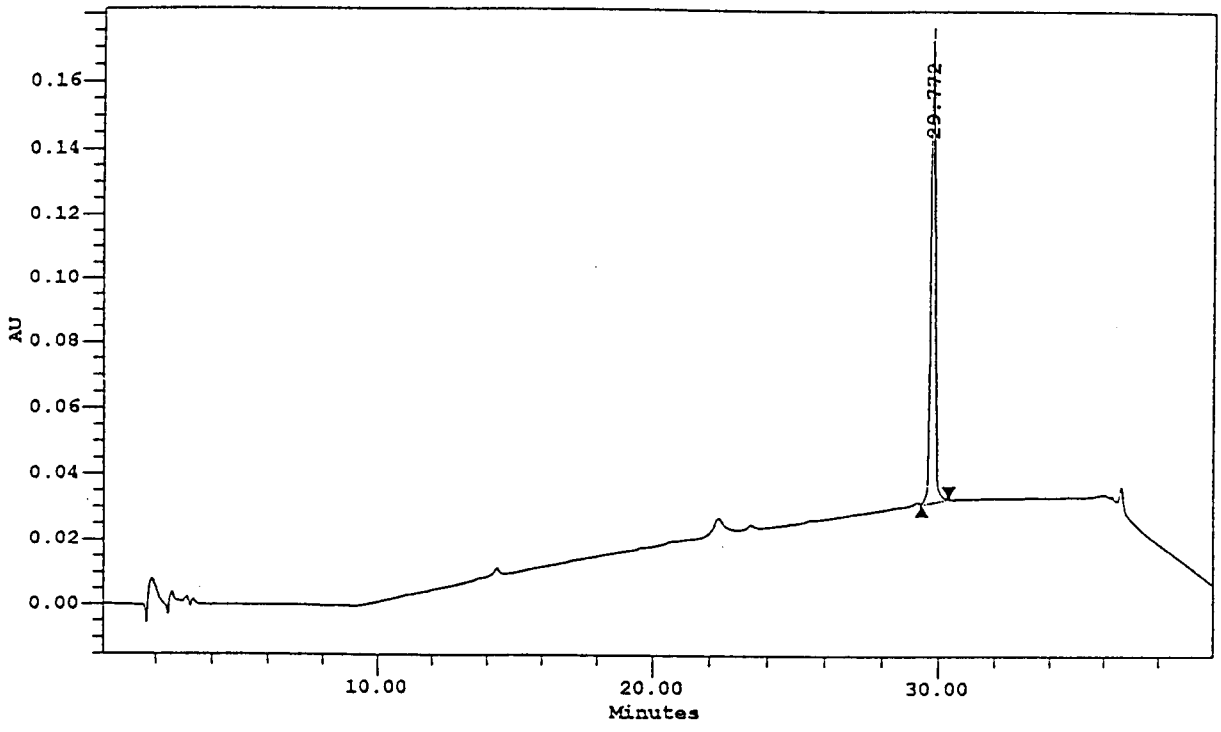
DMU 2628_C.I. 40800

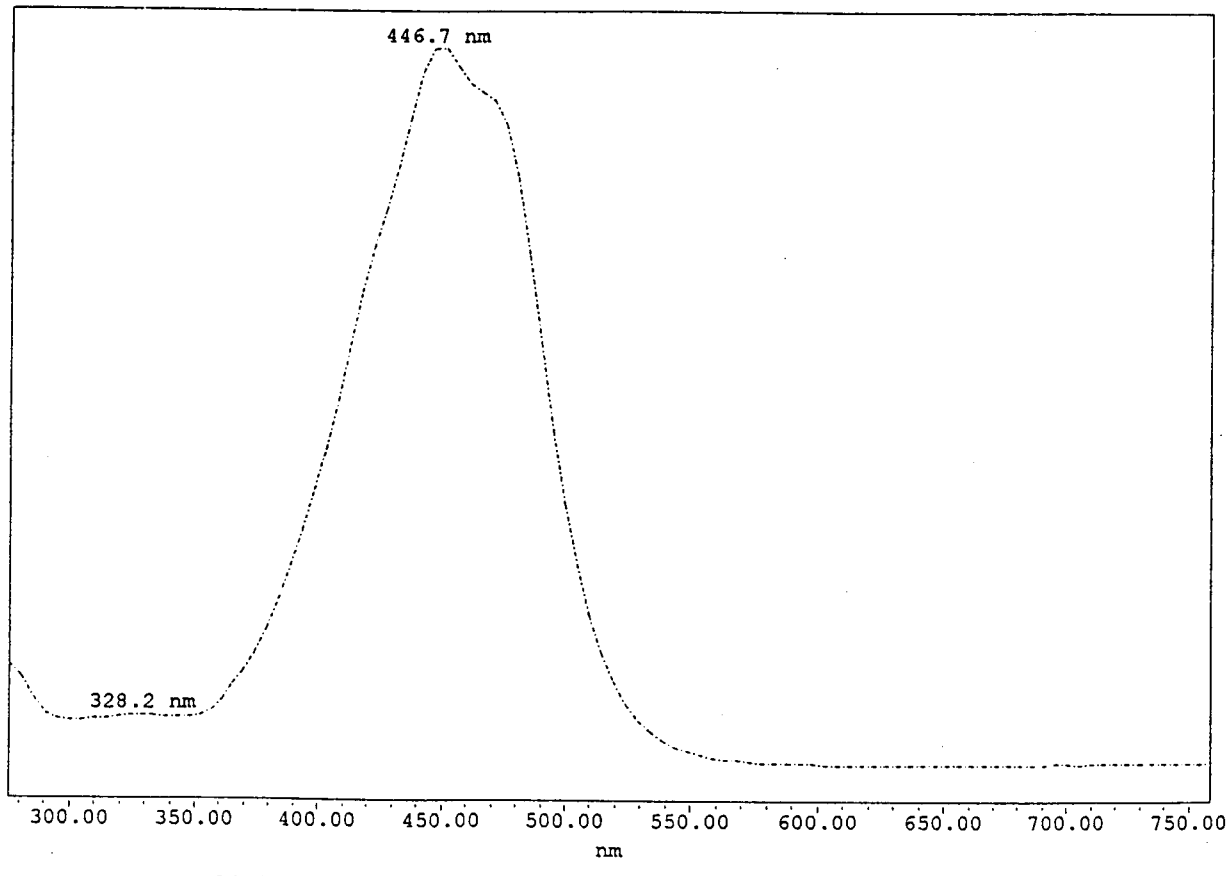




DMU 2628_CI 40800* 31.585 minutes, 275 - 760 @ 4.8 nm, from 2628_CI40800

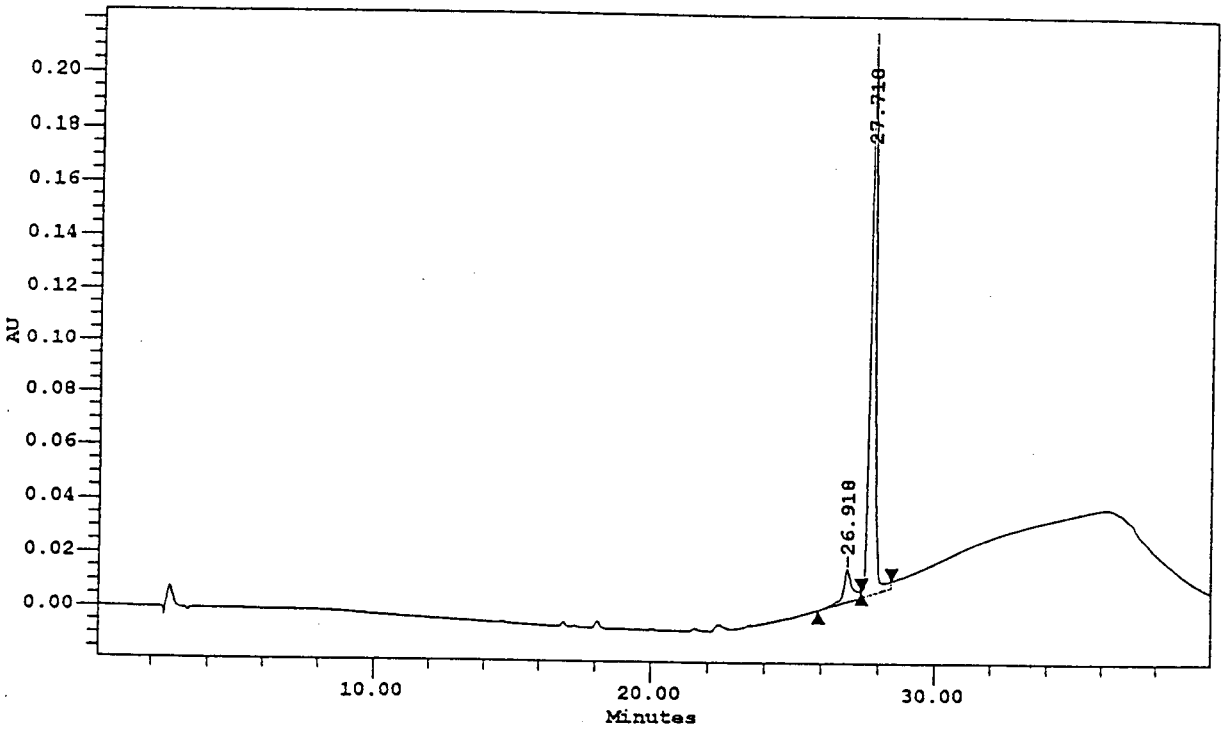
DMU 2676_C.I. 40825

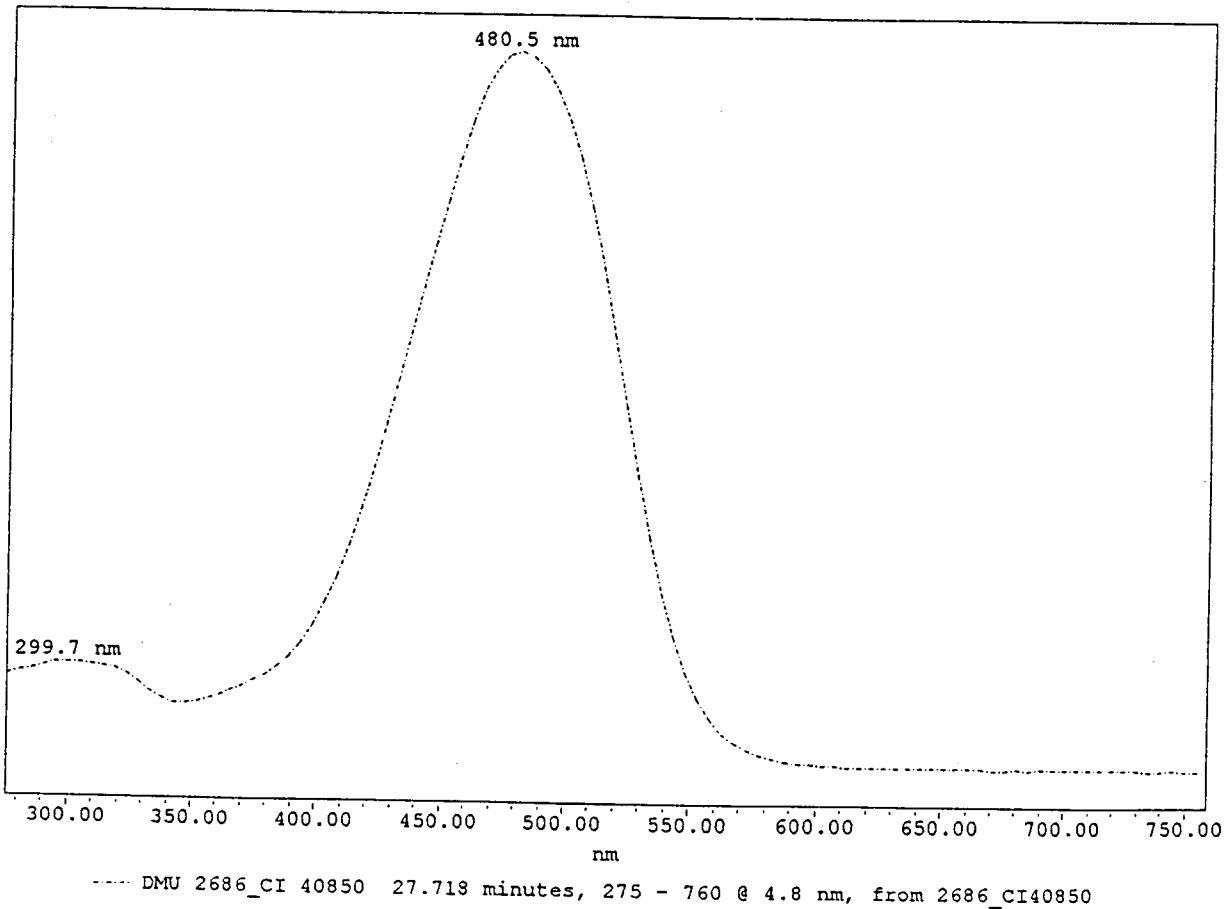




DMU 2676_CI 40825 29.772 minutes, 275 - 760 @ 4.8 nm, from 2676_40825

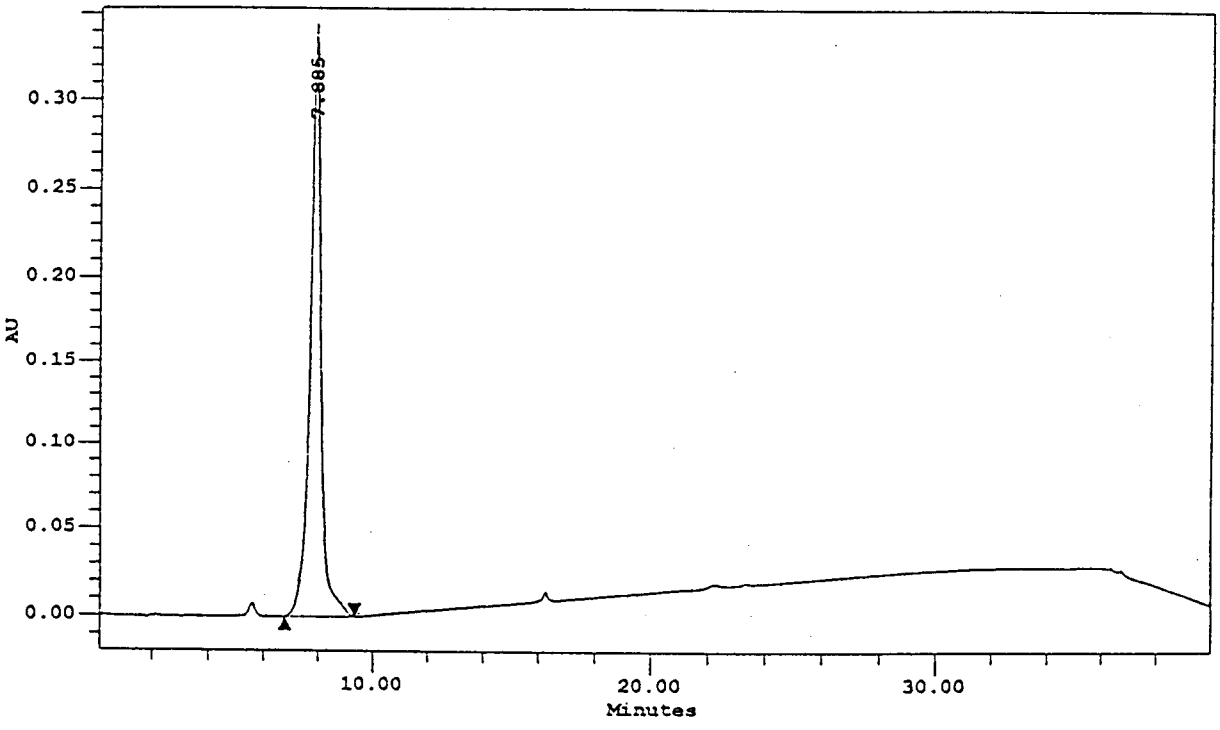
DMU 2686_C.I. 40850

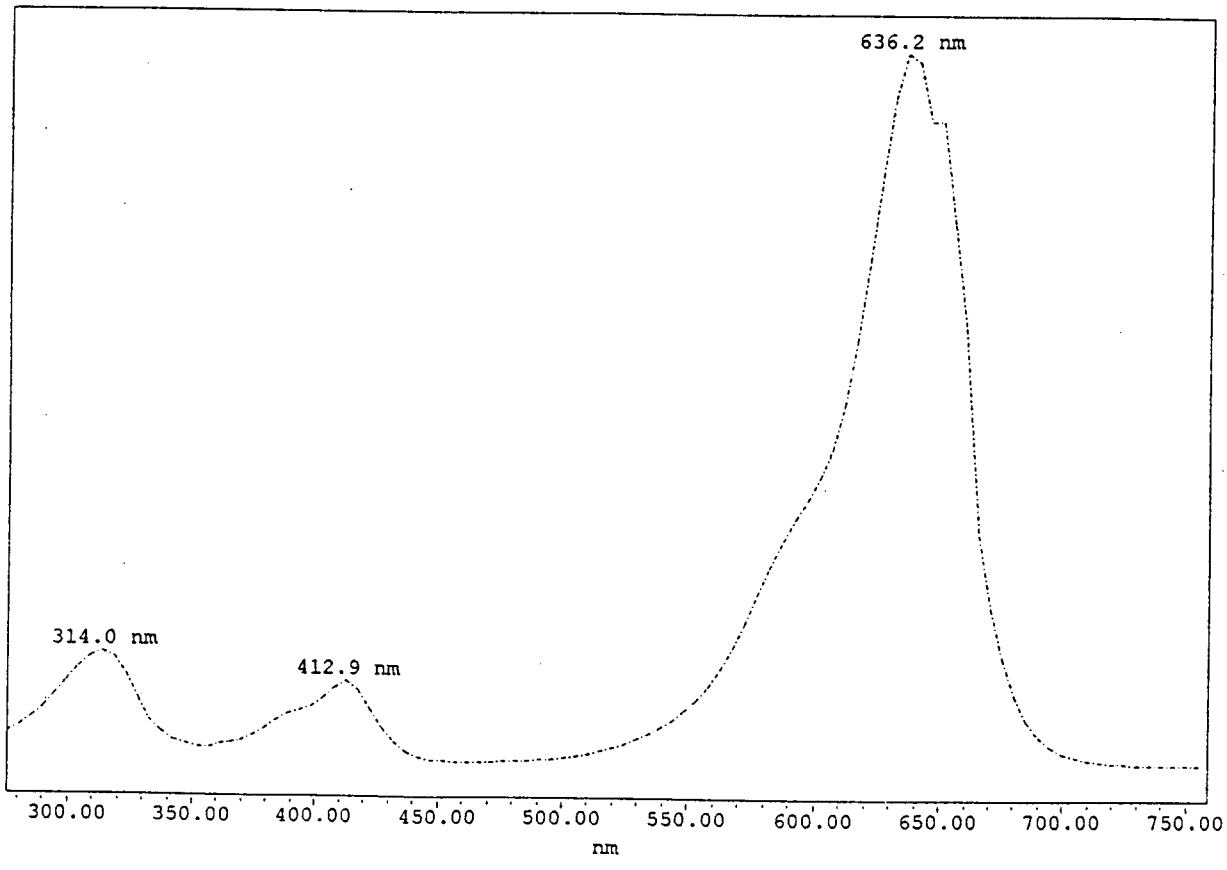




DMU 2686_CI 40850 27.718 minutes, 275 - 760 @ 4.8 nm, from 2686_CI40850

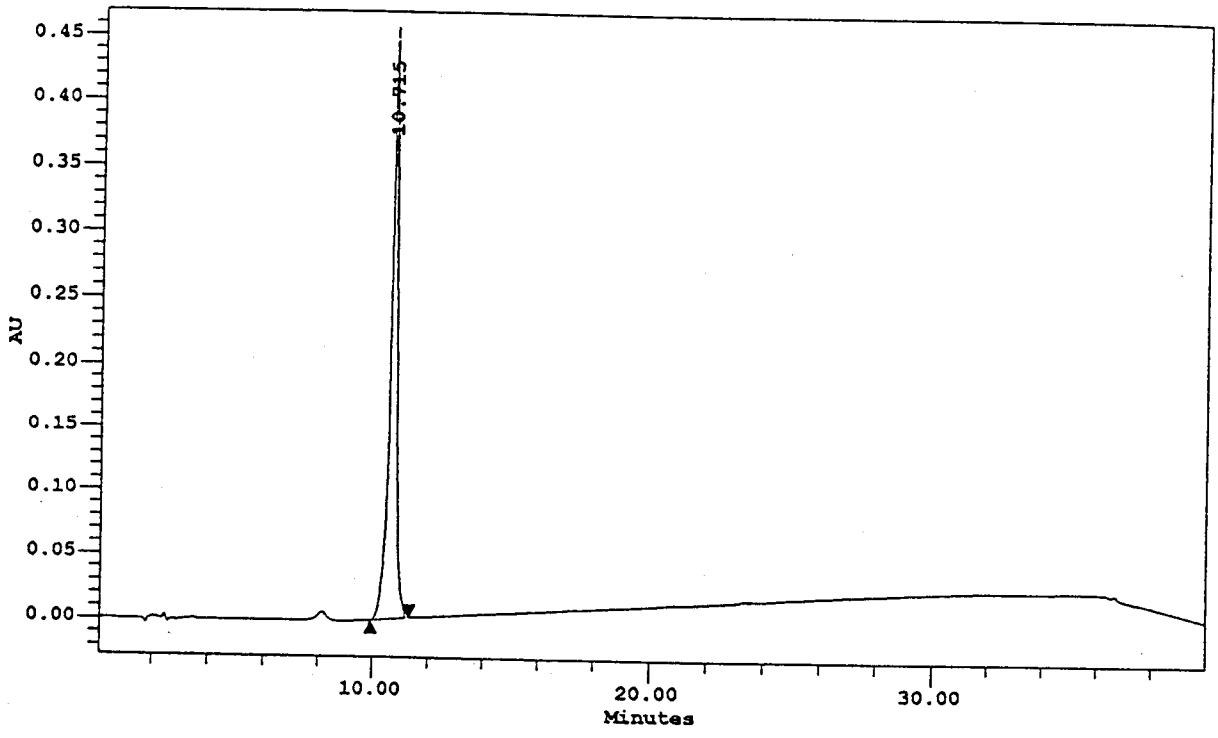
DMU 2652_C.I. 42045

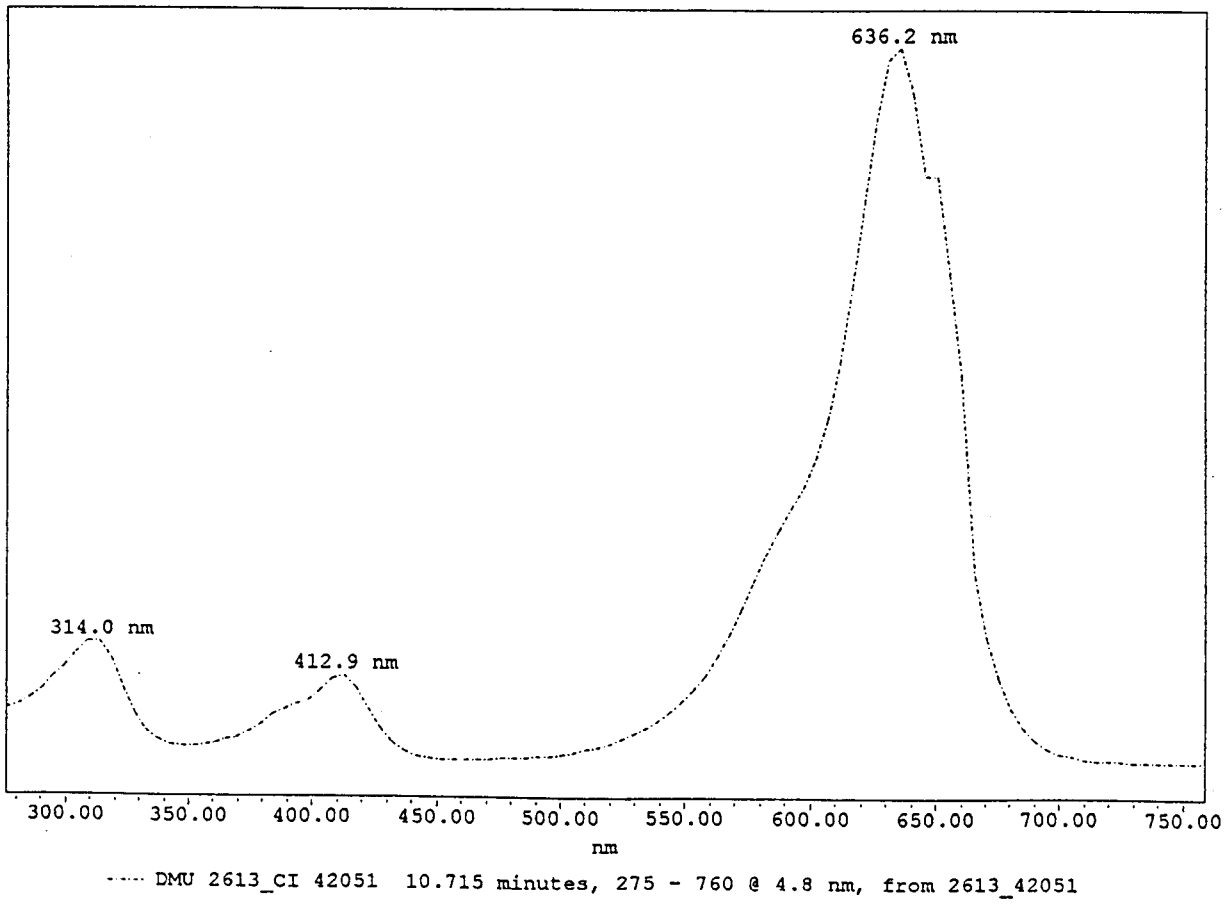




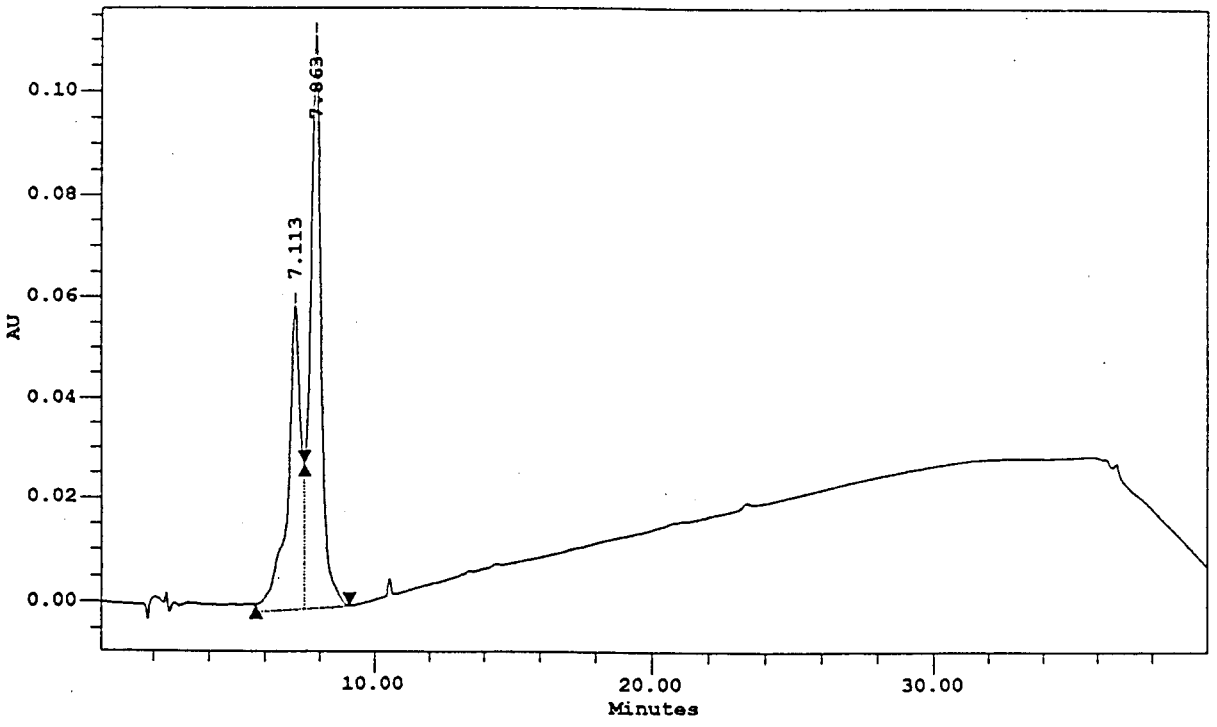
DMU 2652_CI 42045 7.885 minutes, 275 - 760 @ 4.8 nm, from 2652_42045

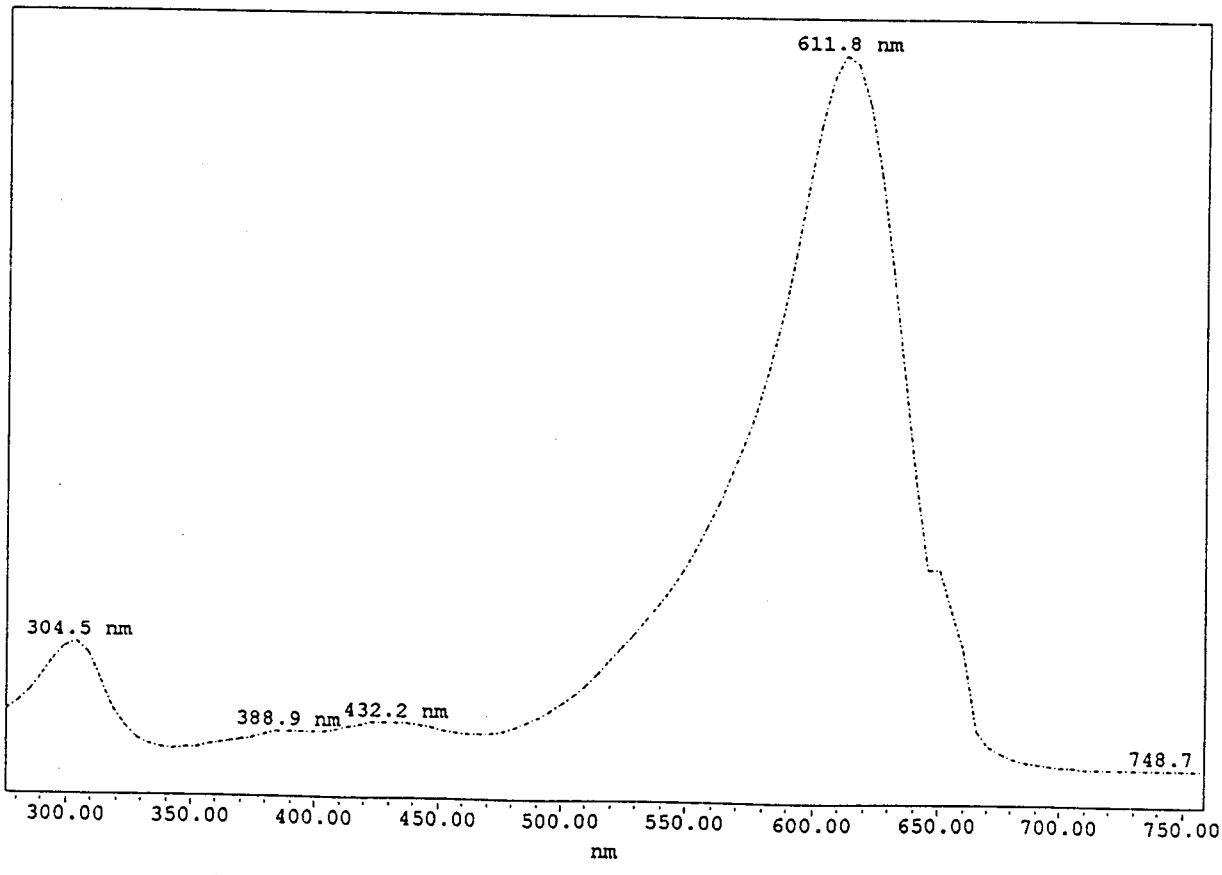
DMU 2613_C.I. 42051





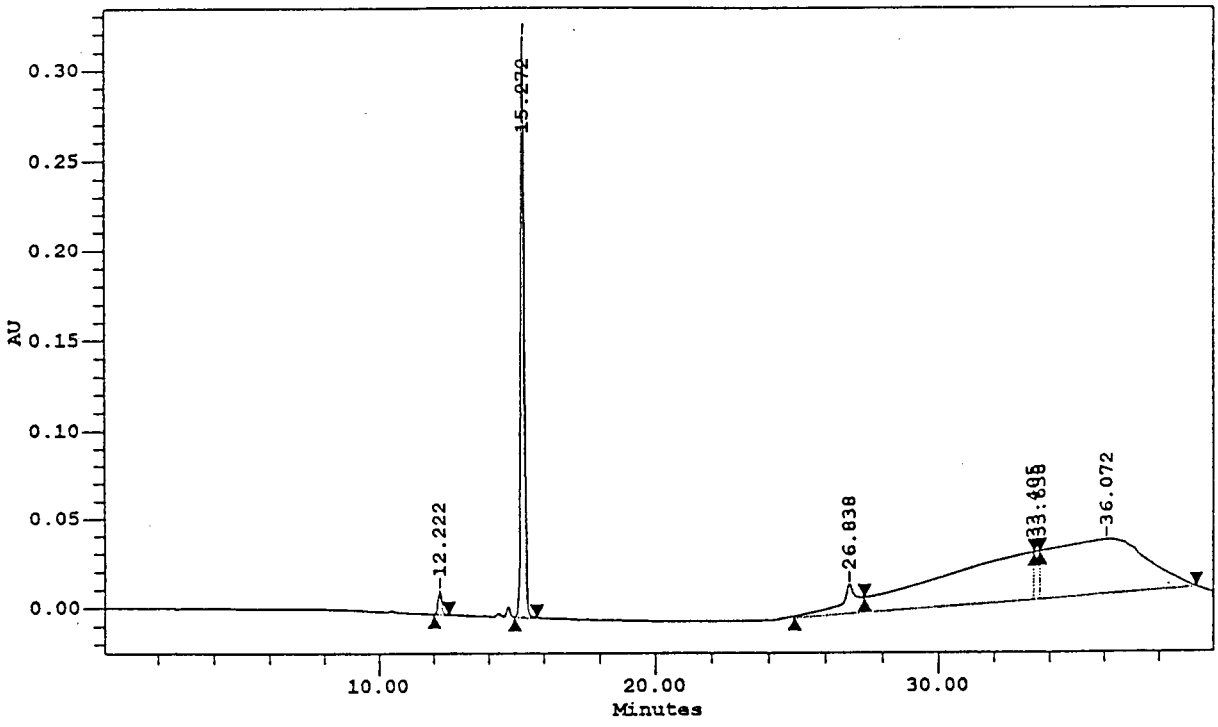
DMU 2632_C.I. 42053

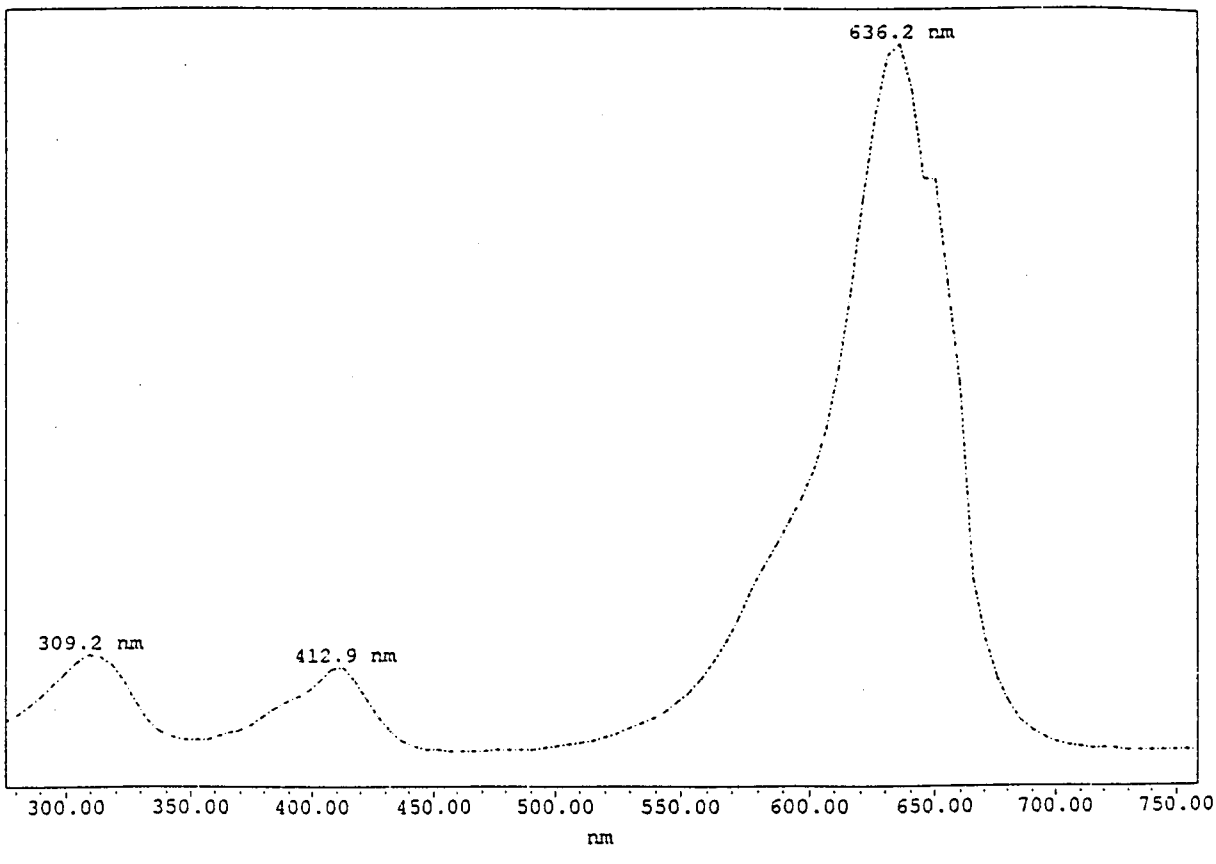




DMU 2632_CI 42053* 7.863 minutes, 275 - 760 @ 4.8 nm, from 2632_42053

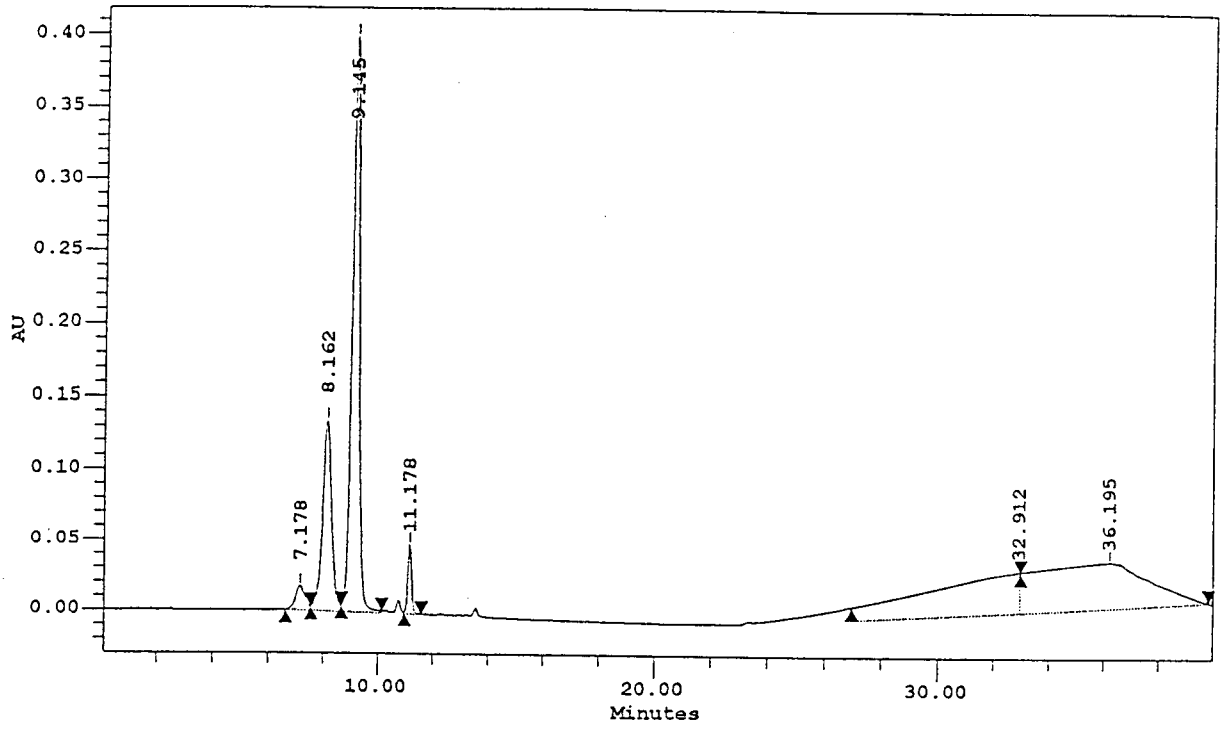
DMU 2657_C.I. 42080

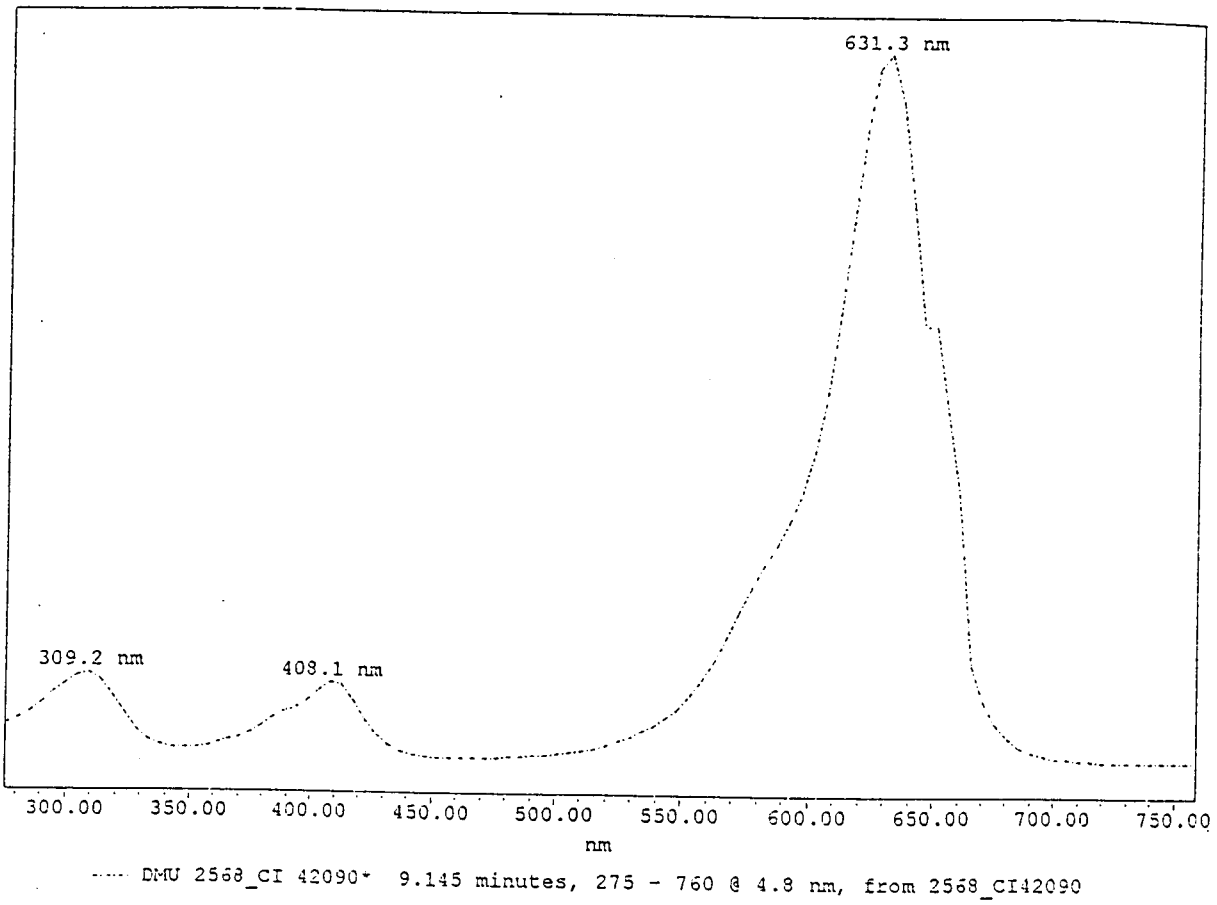




DMU 2657_CI 42080* 15.272 minutes, 275 - 760 @ 4.8 nm, from 2657_CI42080

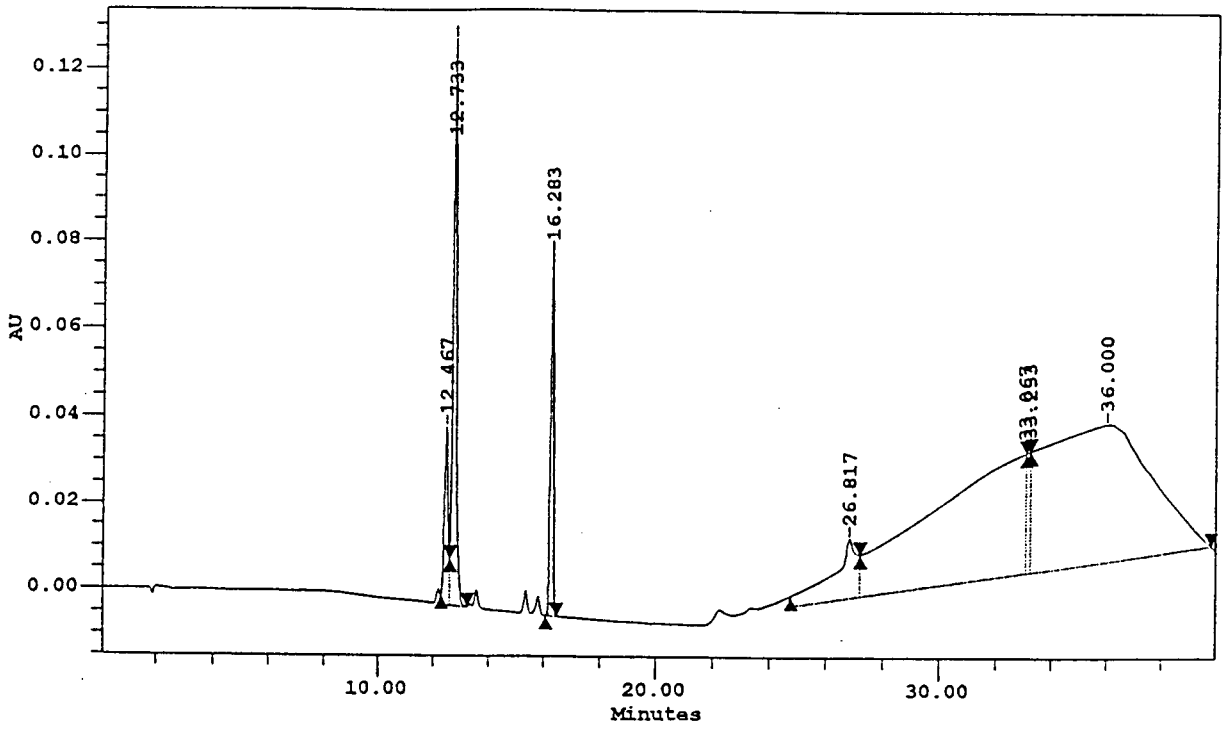
DMU 2568_C.I. 42090

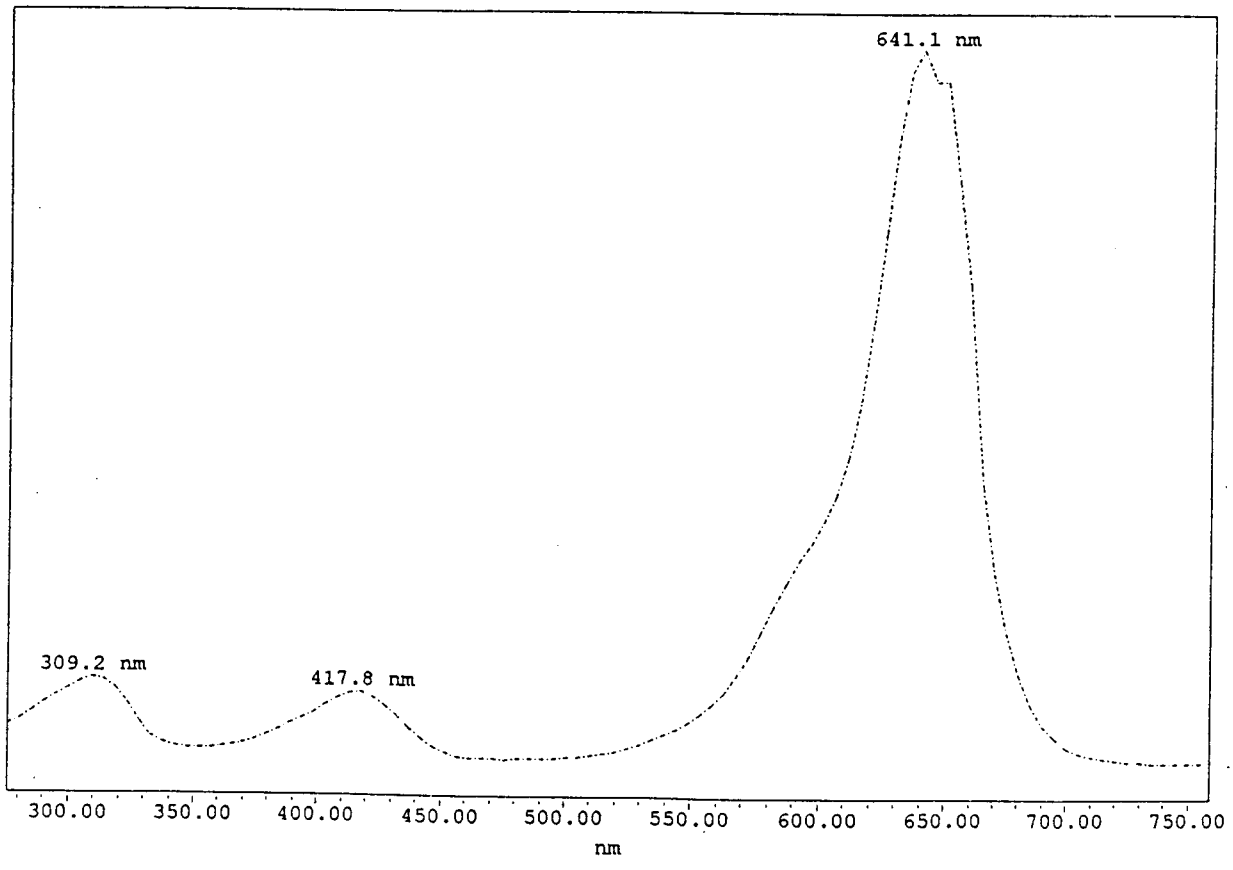




DMU 2568_CI 42090* 9.145 minutes, 275 - 760 @ 4.8 nm, from 2568_CI42090

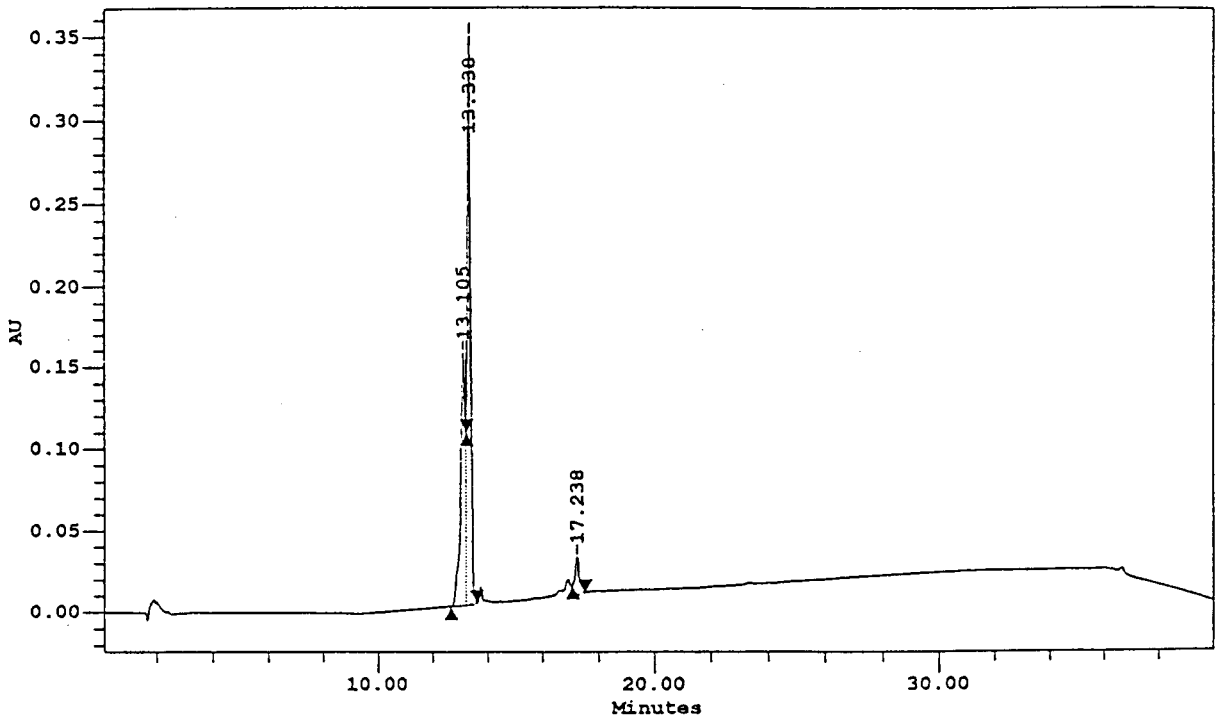
DMU 2664_C.I. 42100

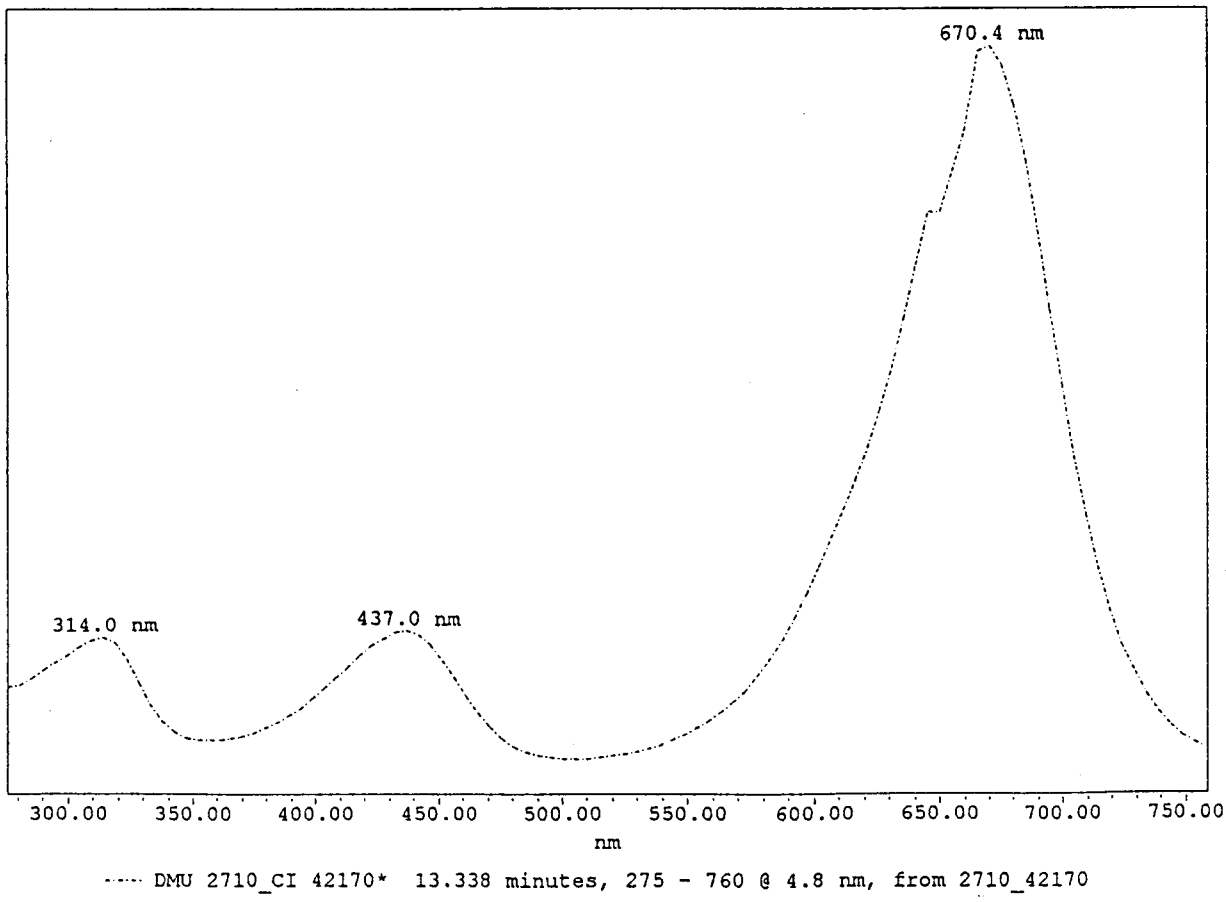




DMU 2664_CI 42100* 12.733 minutes, 275 - 760 @ 4.8 nm, from 2664_CI42100

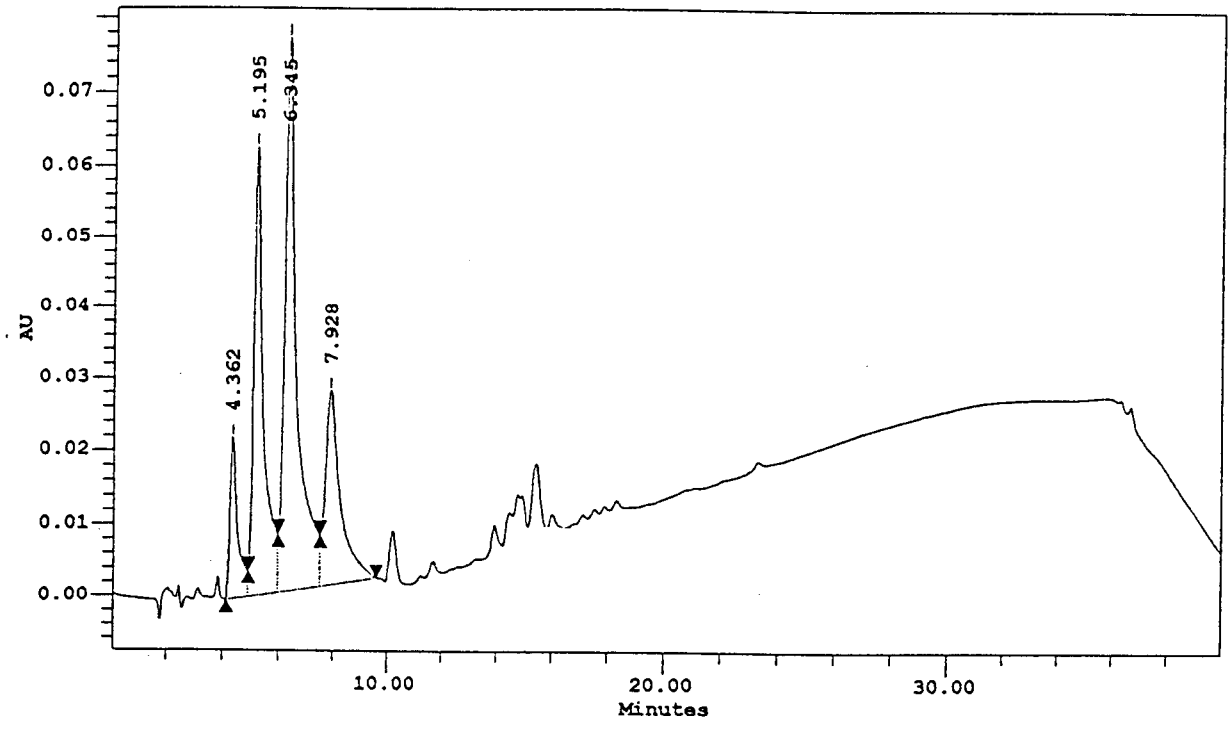
DMU 2710_C.I. 42170

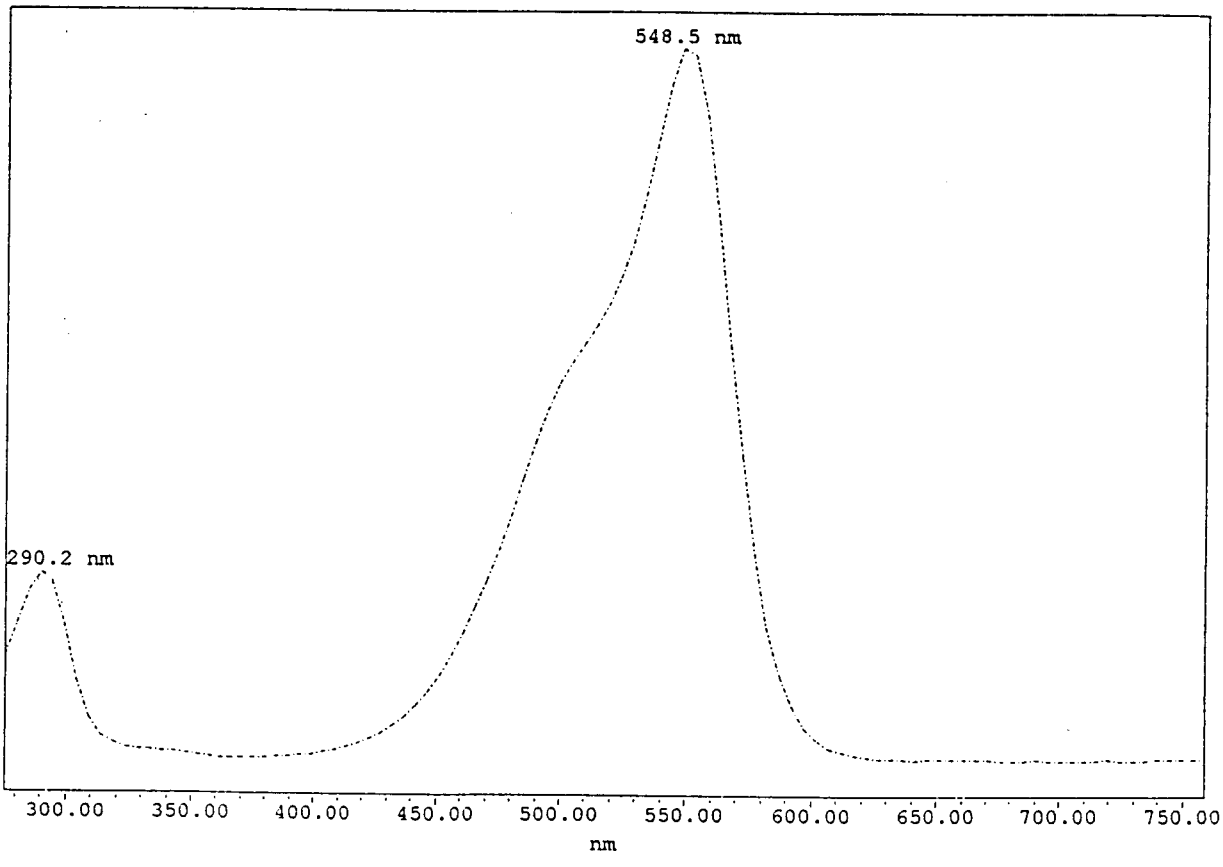




····· DMU 2710_CI 42170* 13.338 minutes, 275 - 760 @ 4.8 nm, from 2710_42170

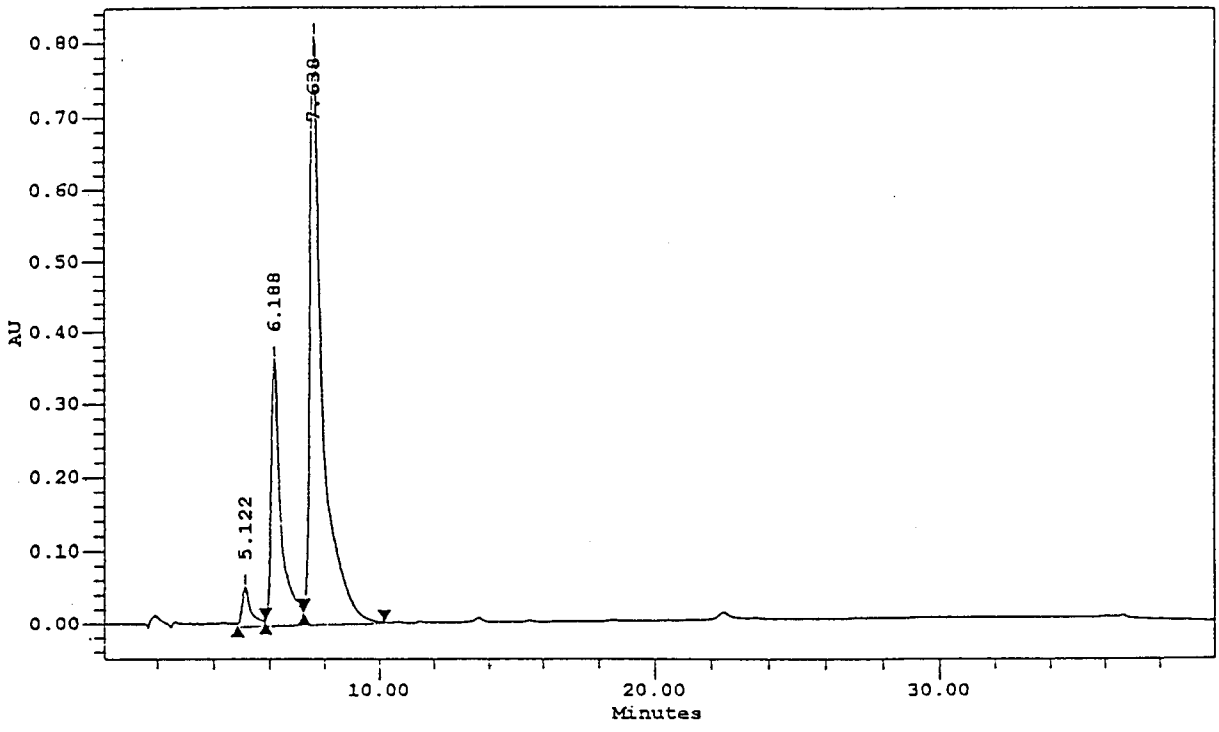
DMU 2653_C.I. 42510

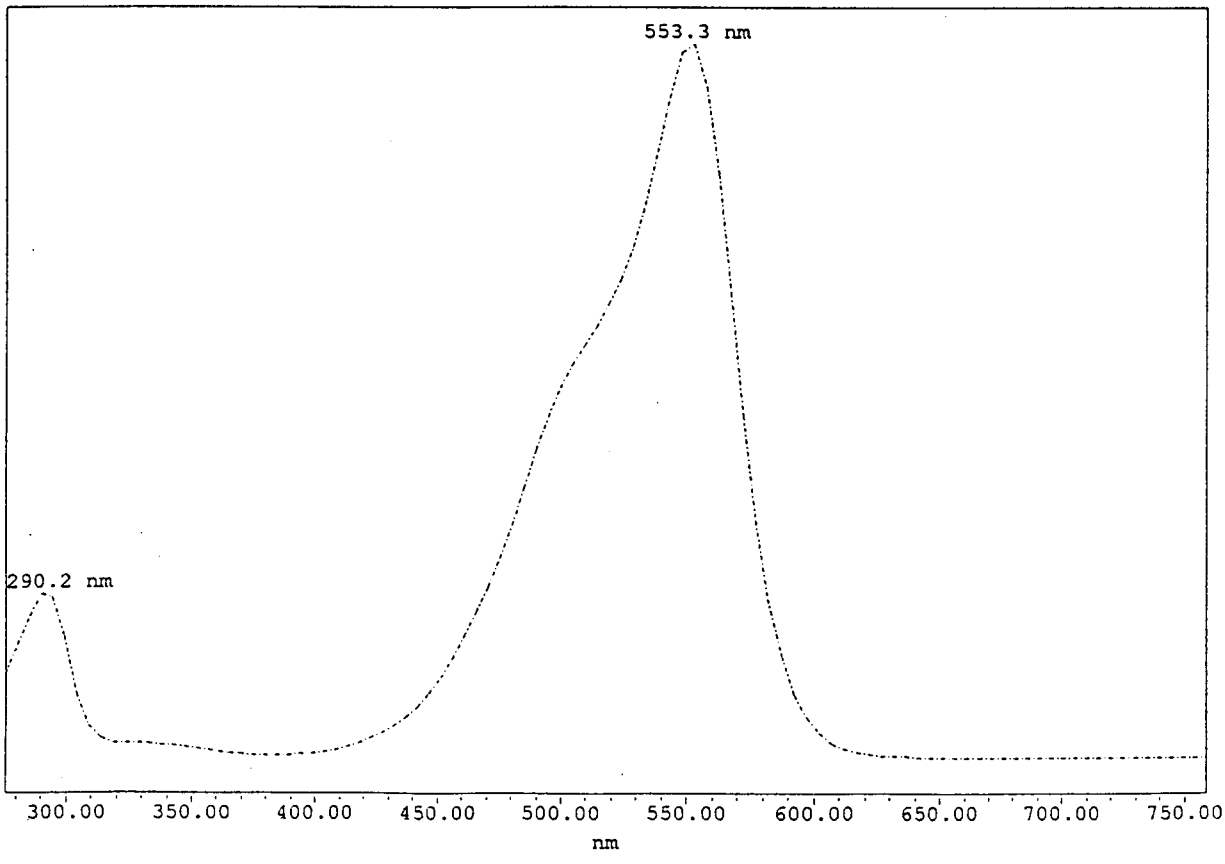




DMU 2653_CI 42510* 6.345 minutes, 275 - 760 @ 4.8 nm, from 2653_42510

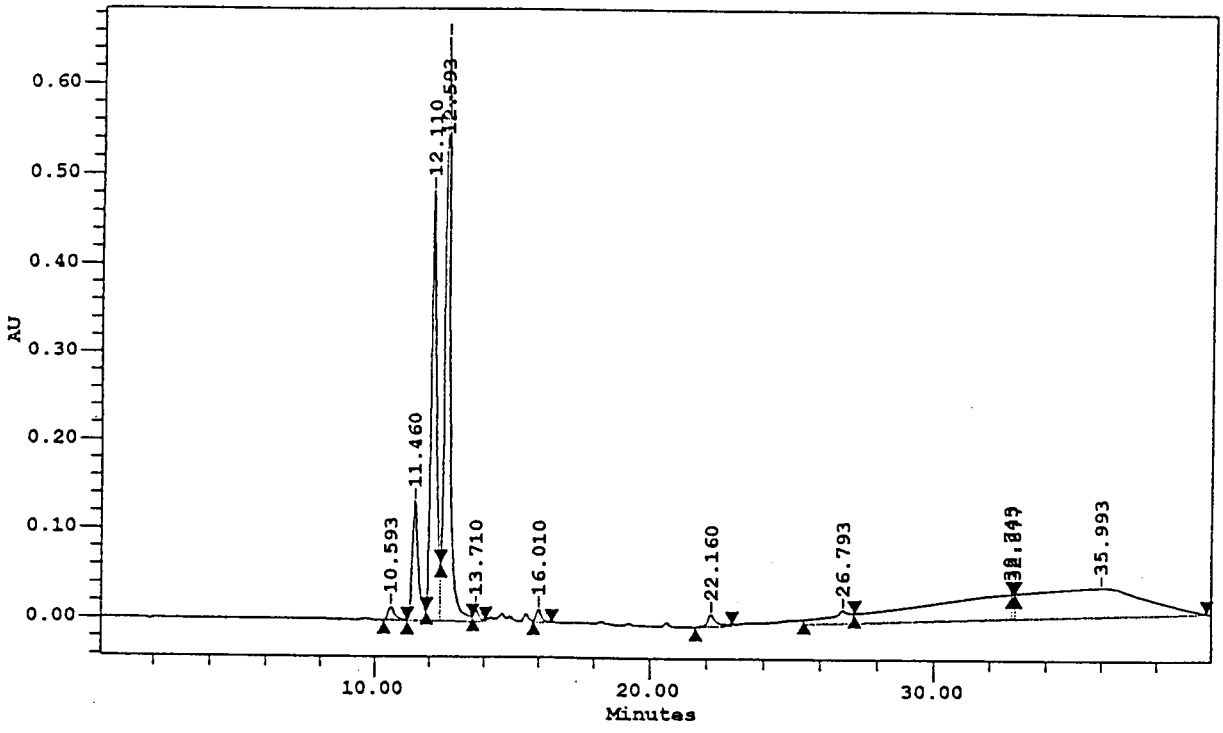
DMU 2654_C.I. 42520

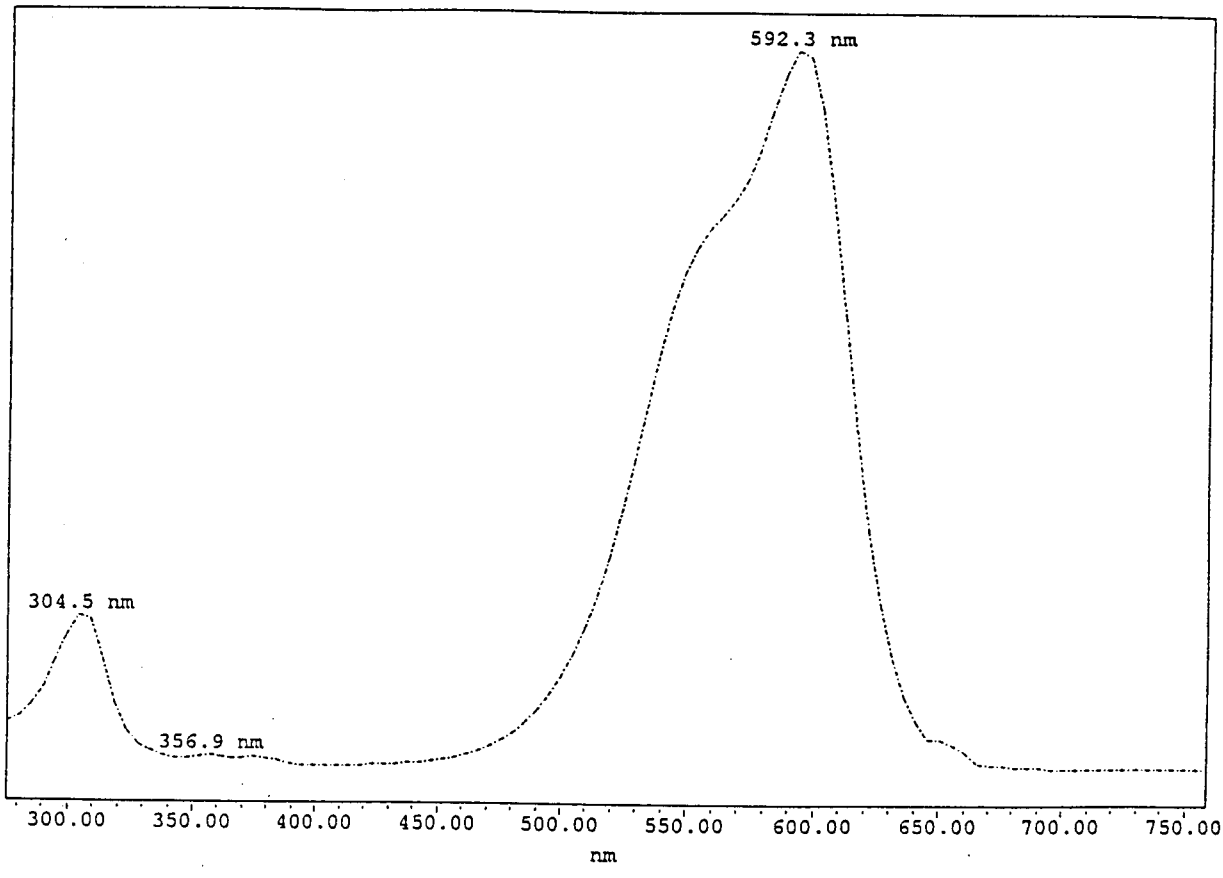




DMU 2654_CI 42520* 7.638 minutes, 275 - 760 @ 4.8 nm, from 2654_42520

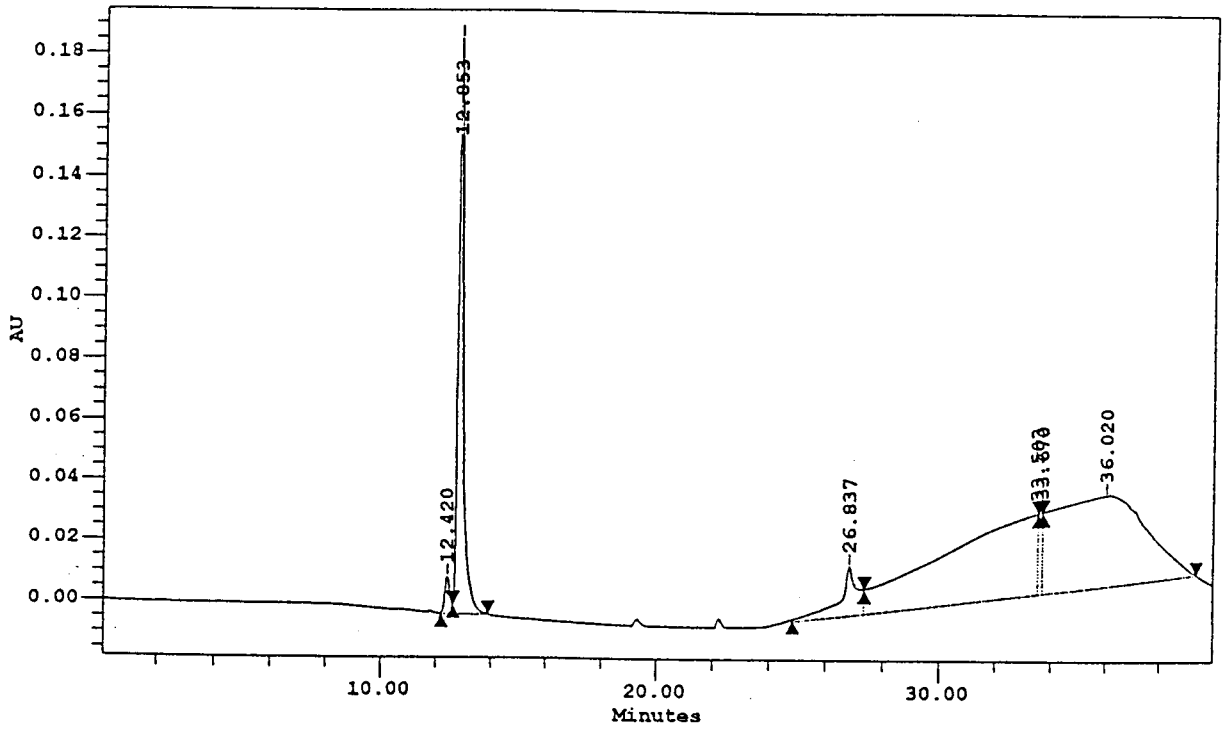
DMU 2694_C.I. 42535

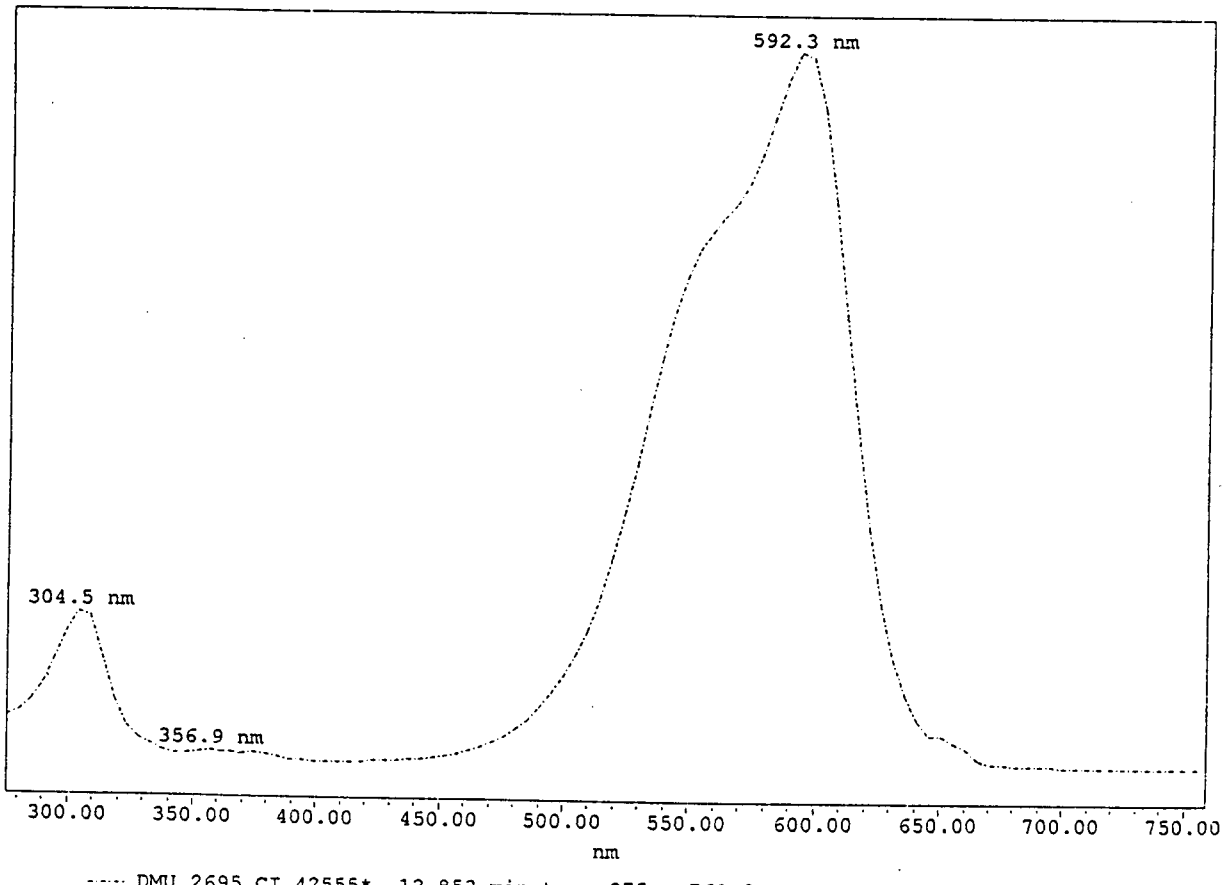




DMU 2694_CI 42535* 12.593 minutes, 275 - 760 @ 4.8 nm, from 2694_ME VIOL2B

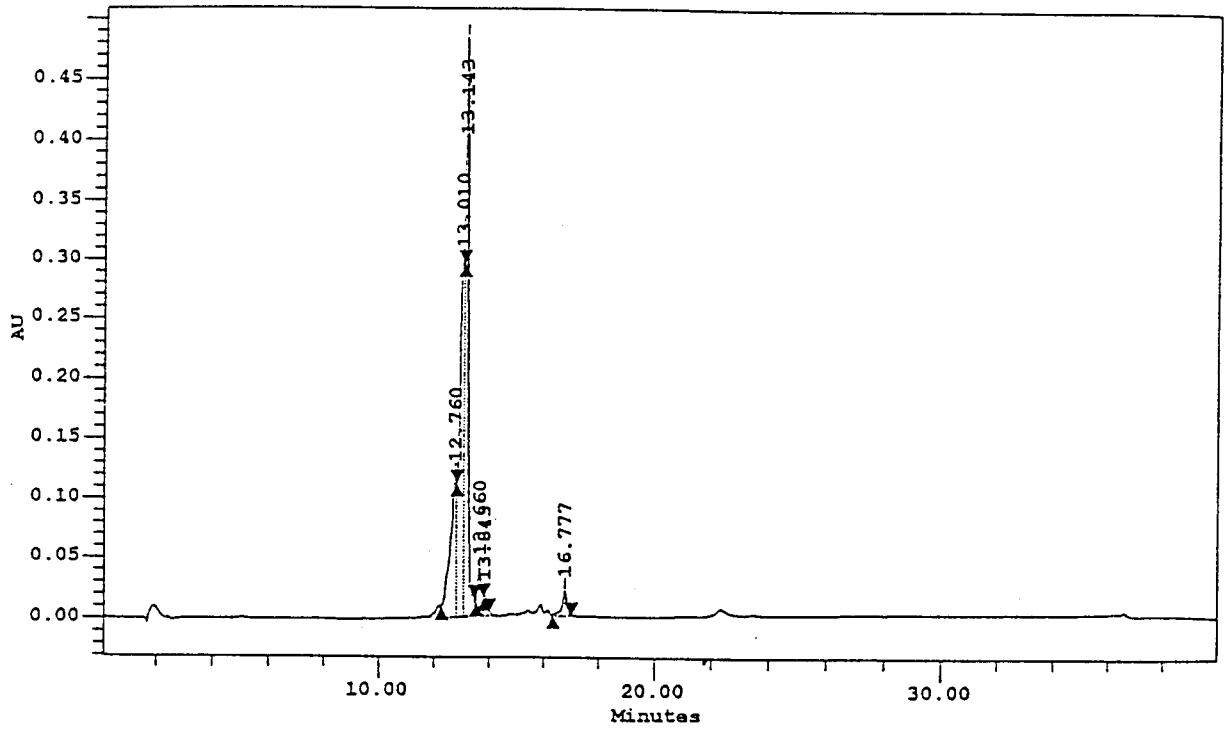
DMU 2695_C.I. 42555

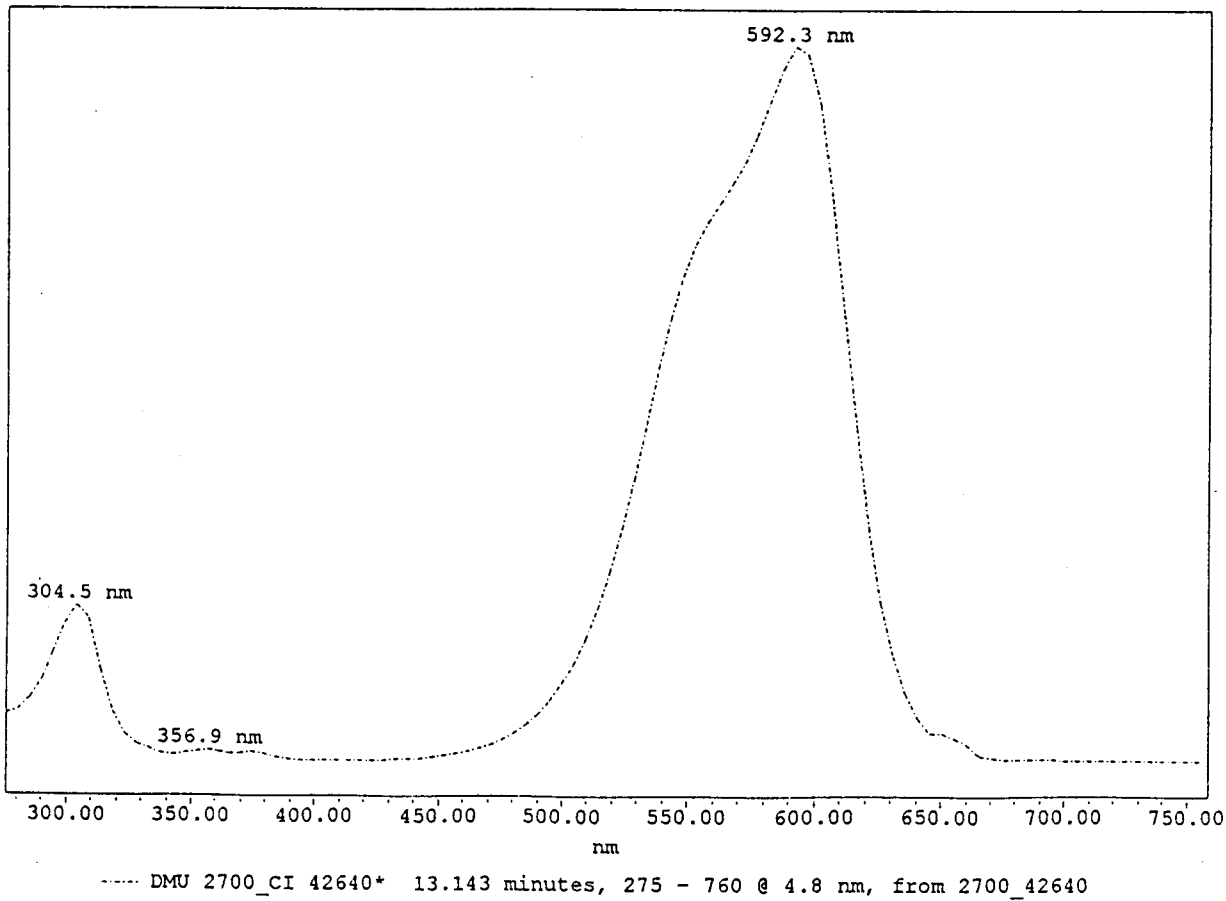




DMU 2695_CI 42555* 12.853 minutes, 275 - 760 @ 4.8 nm, from 2695_CI42555

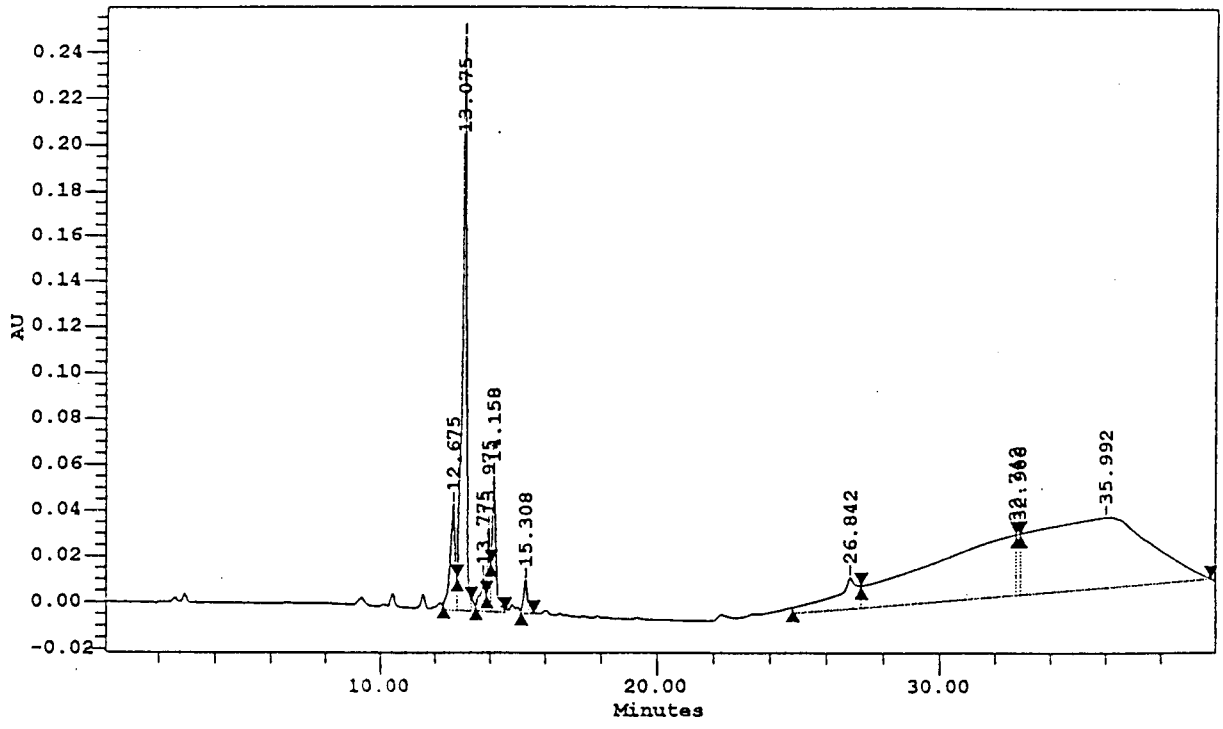
DMU 2700_C.I. 42640

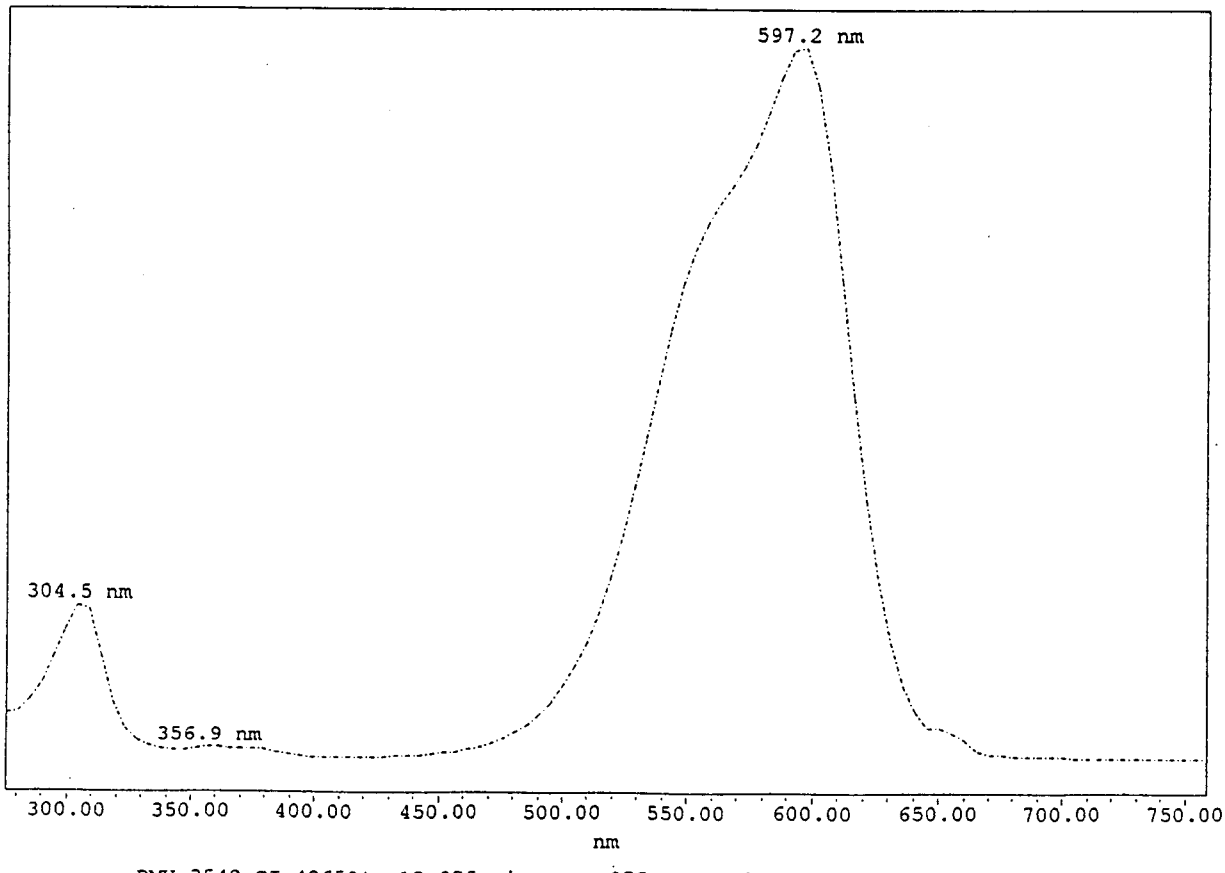




DMU 2700_CI 42640* 13.143 minutes, 275 - 760 @ 4.8 nm, from 2700_42640

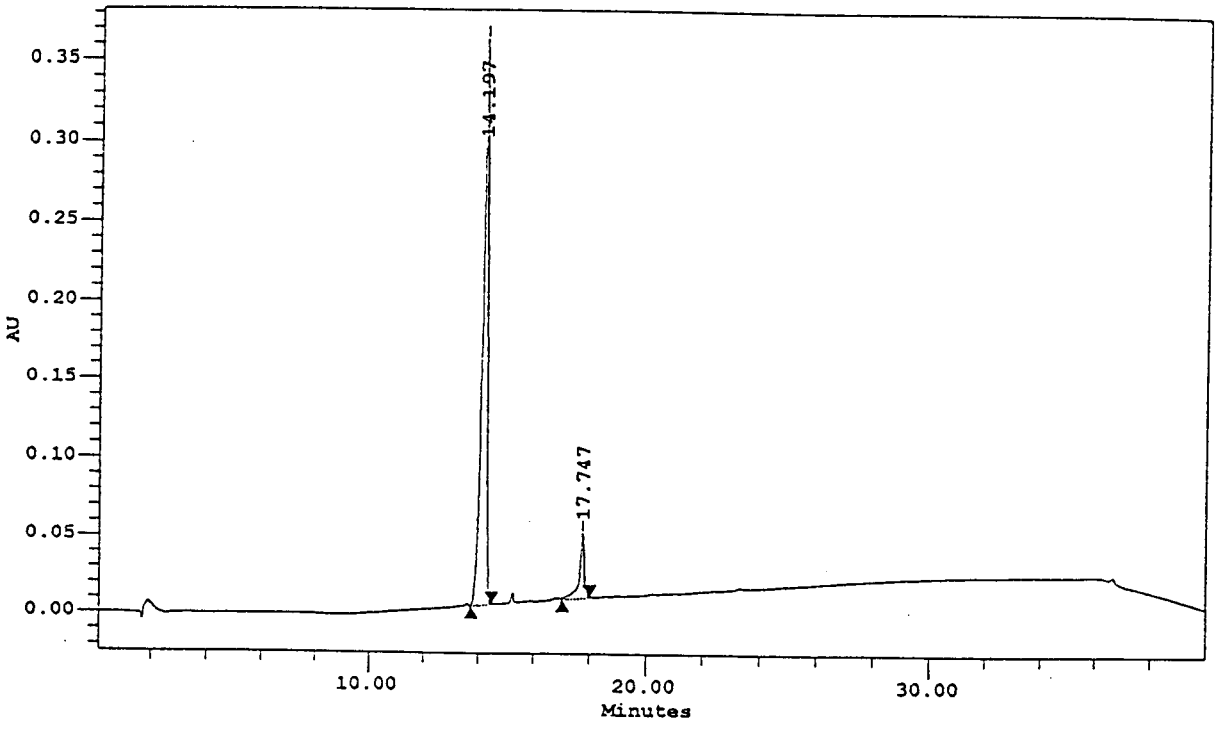
DMU 3542_C.I. 42650

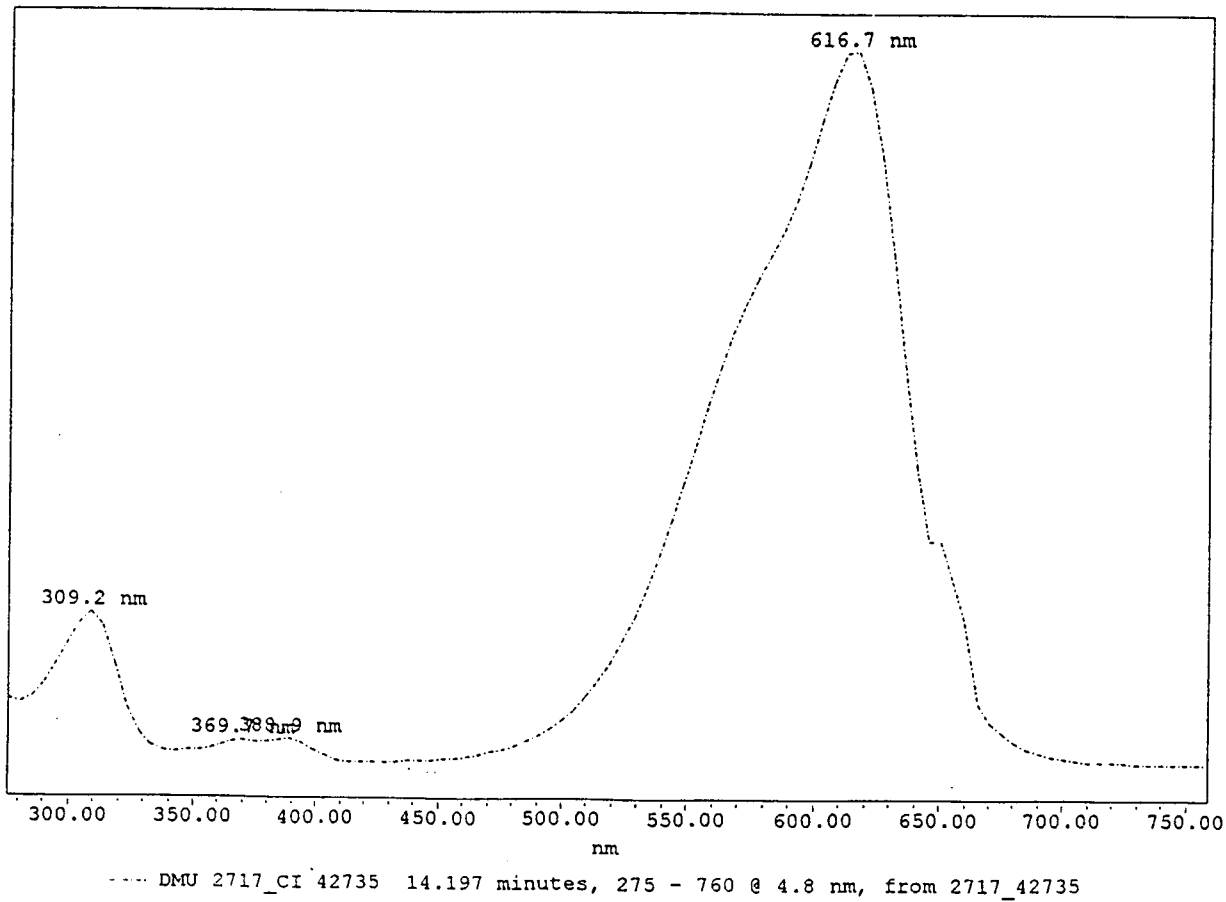




DMU 3542_CI 42650* 13.075 minutes, 275 - 760 @ 4.8 nm, from 3542_CI42650

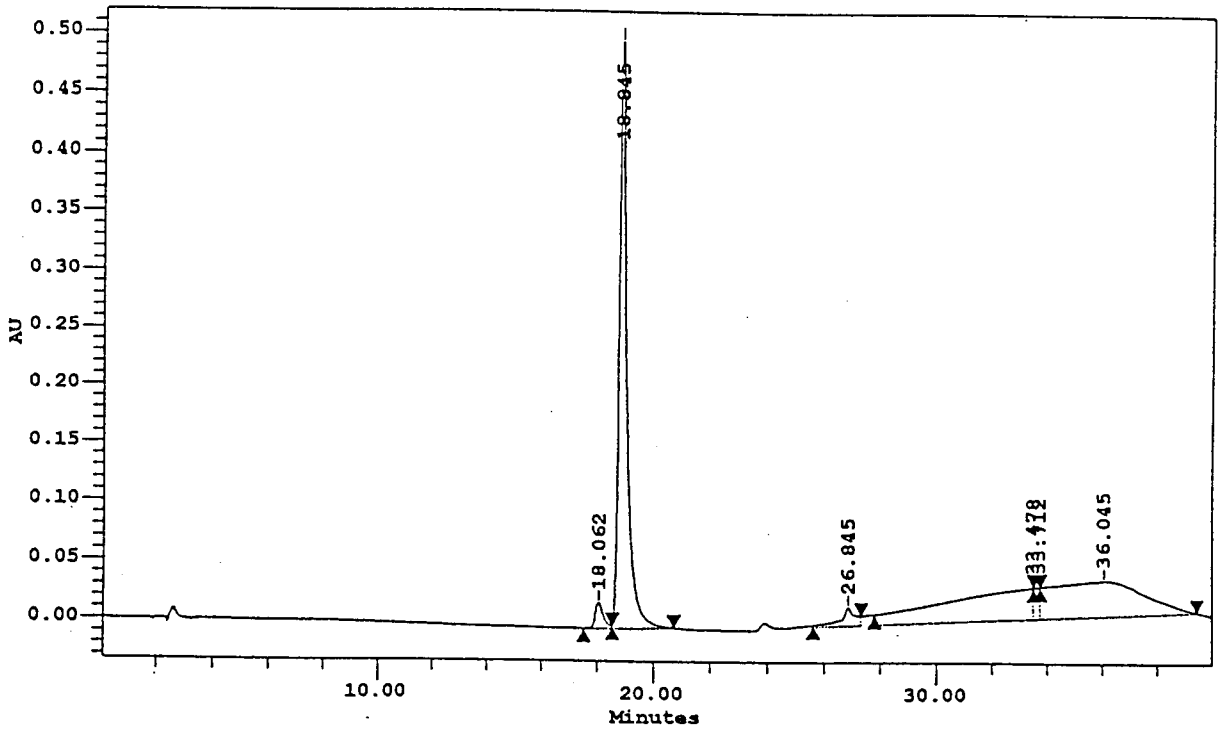
DMU 2717_C.I. 42735

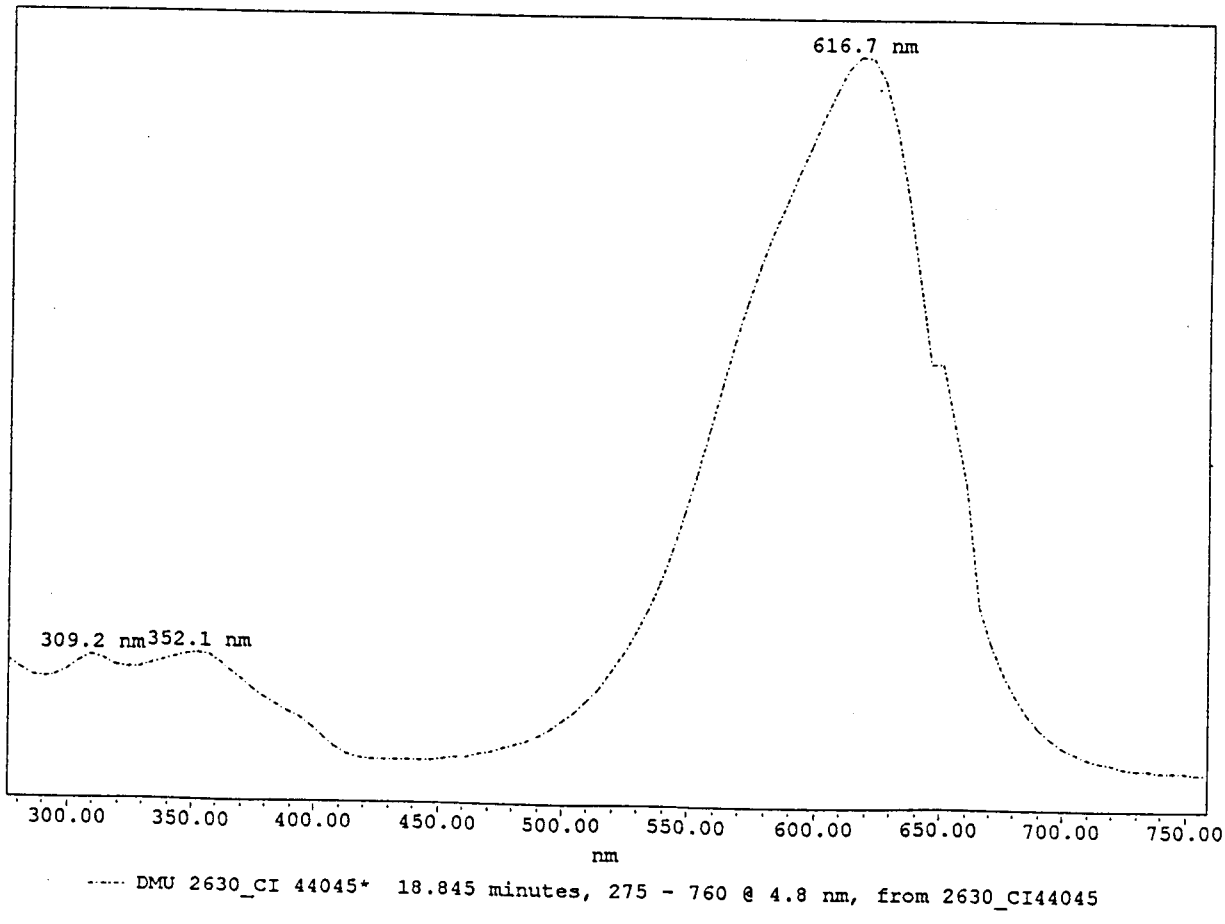




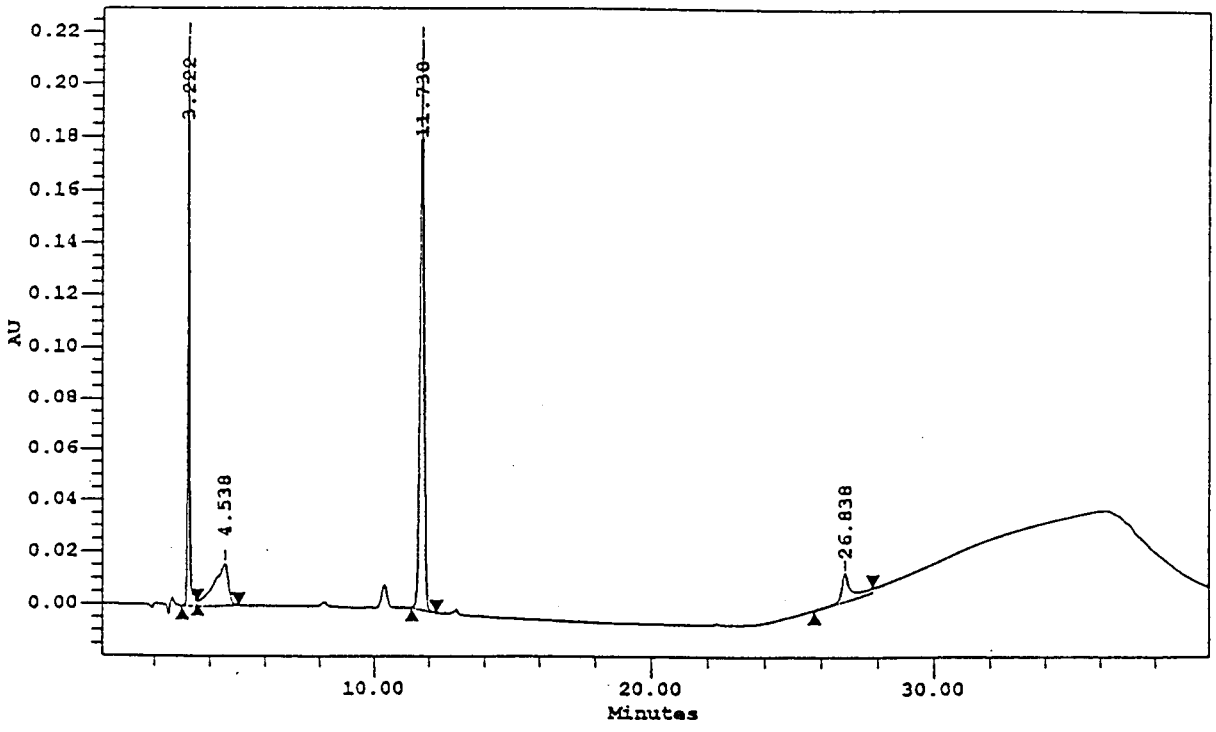
DMU 2717_CI 42735 14.197 minutes, 275 - 760 @ 4.8 nm, from 2717_42735

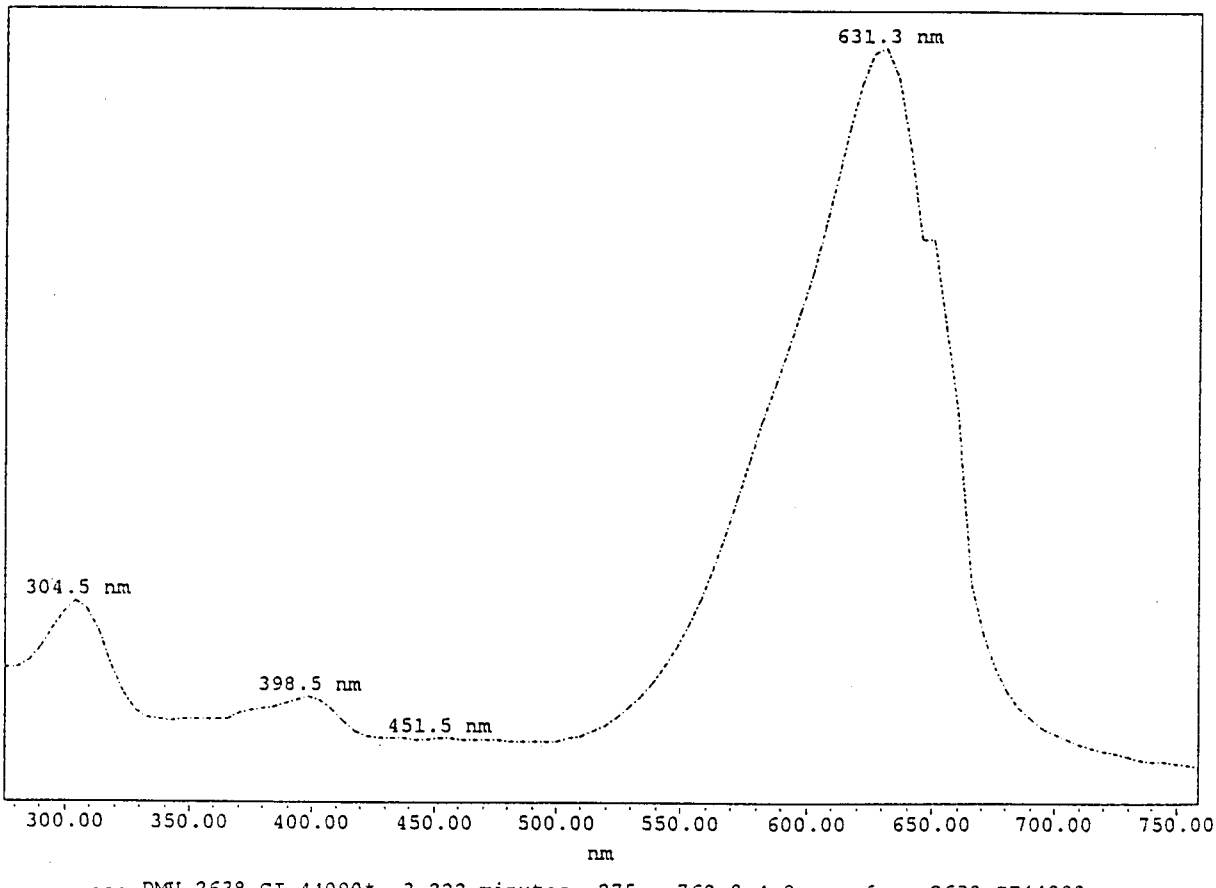
DMU 2630_C.I. 44045





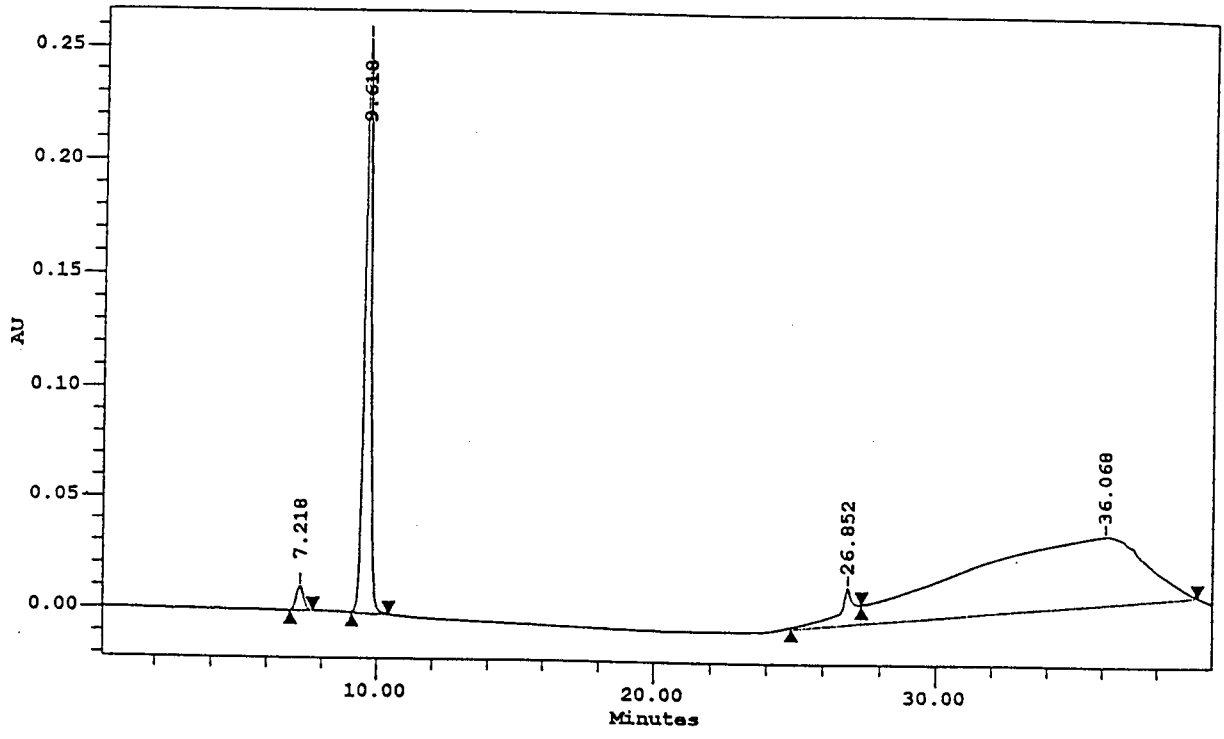
DMU 2638_C.I. 44090

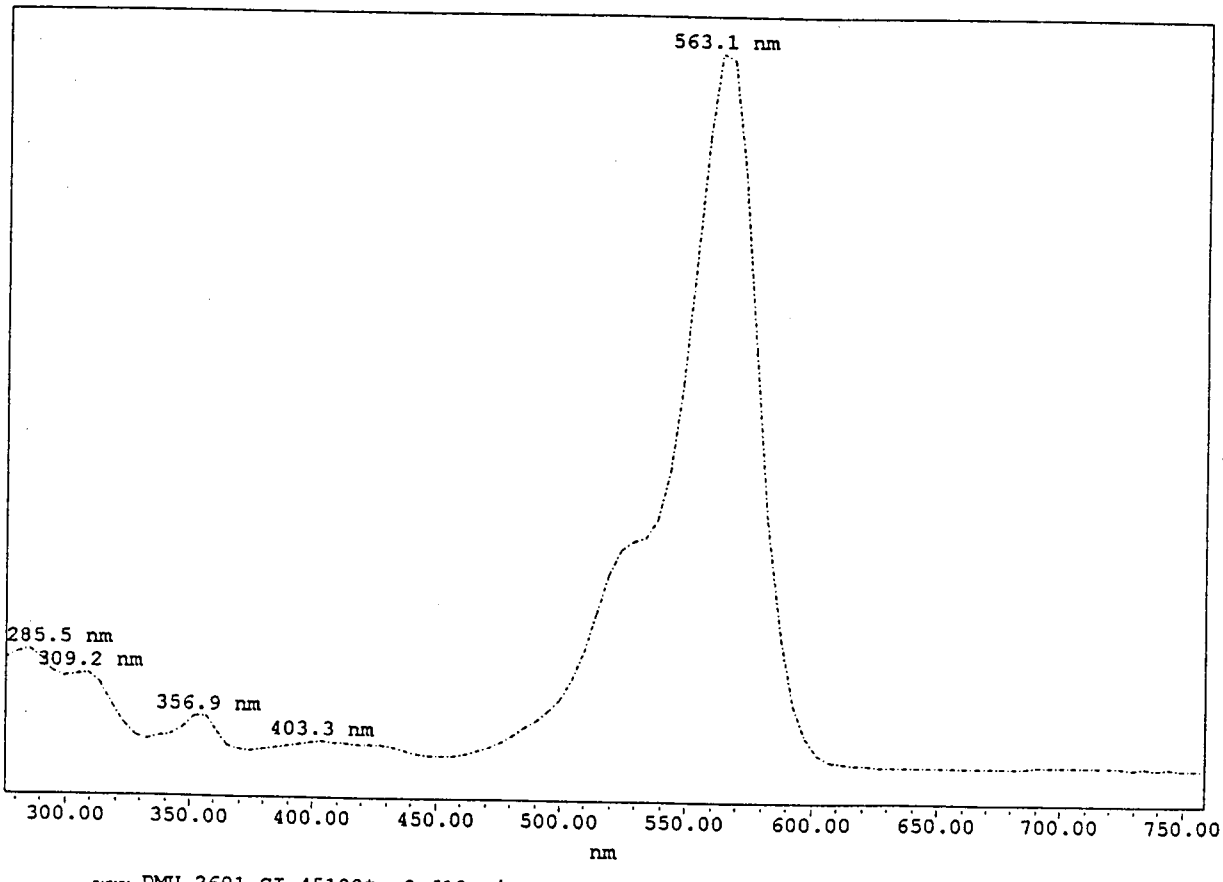




DMU 2638_CI 44090* 3.222 minutes, 275 - 760 @ 4.8 nm, from 2638_CI44090

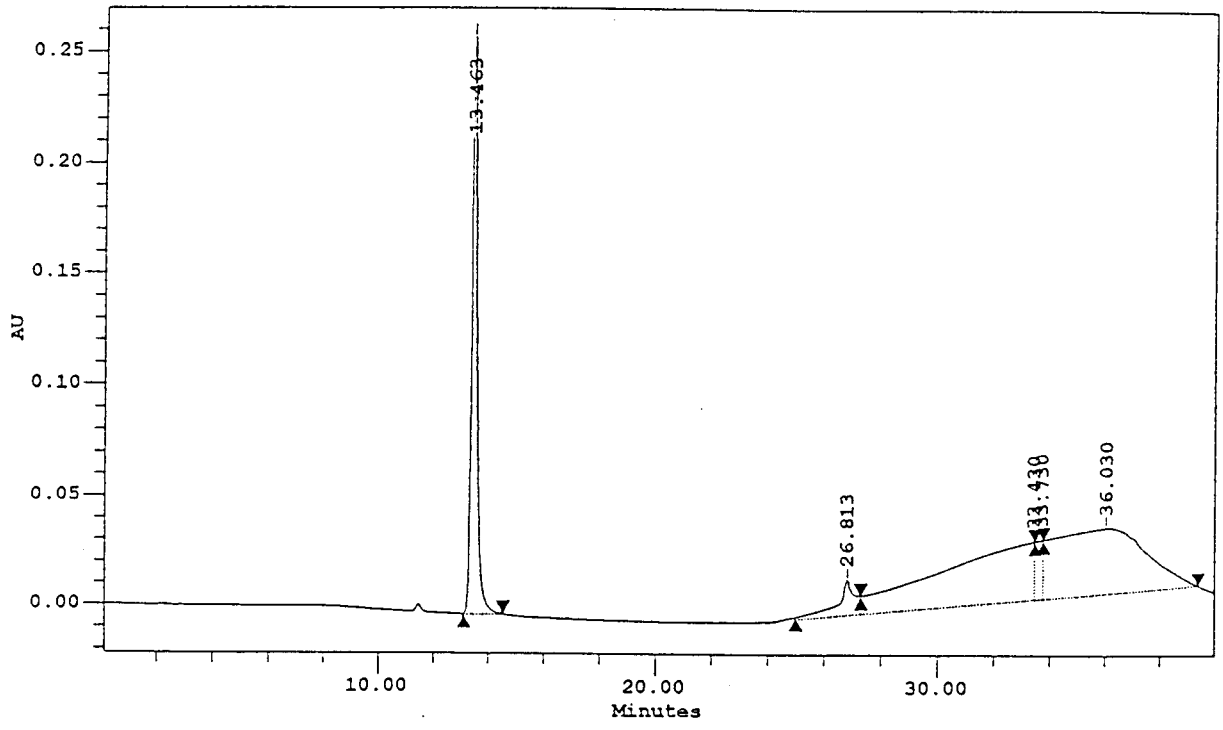
DMU 2601_C.I. 45100

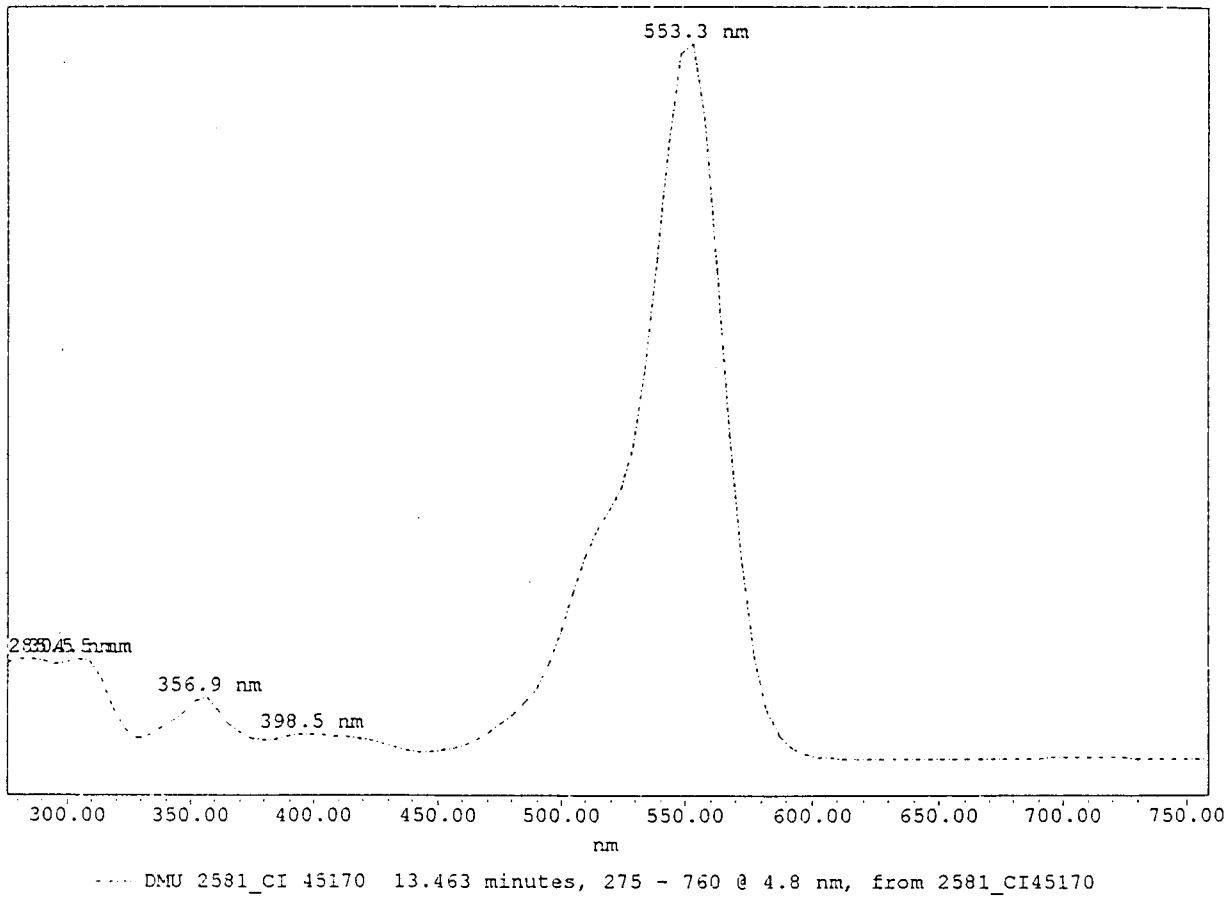




DMU 2601_CI 45100* 9.618 minutes, 275 - 760 @ 4.8 nm, from 2601_CI45100

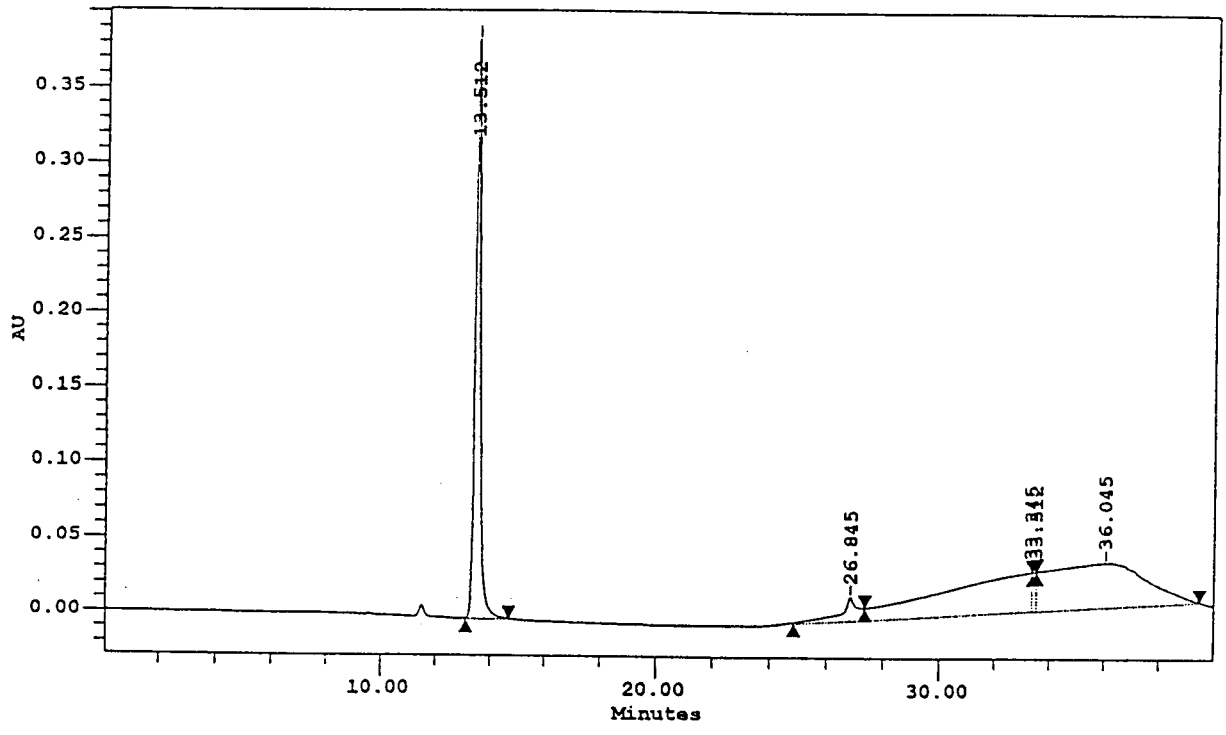
DMU 2581_C.I. 45170

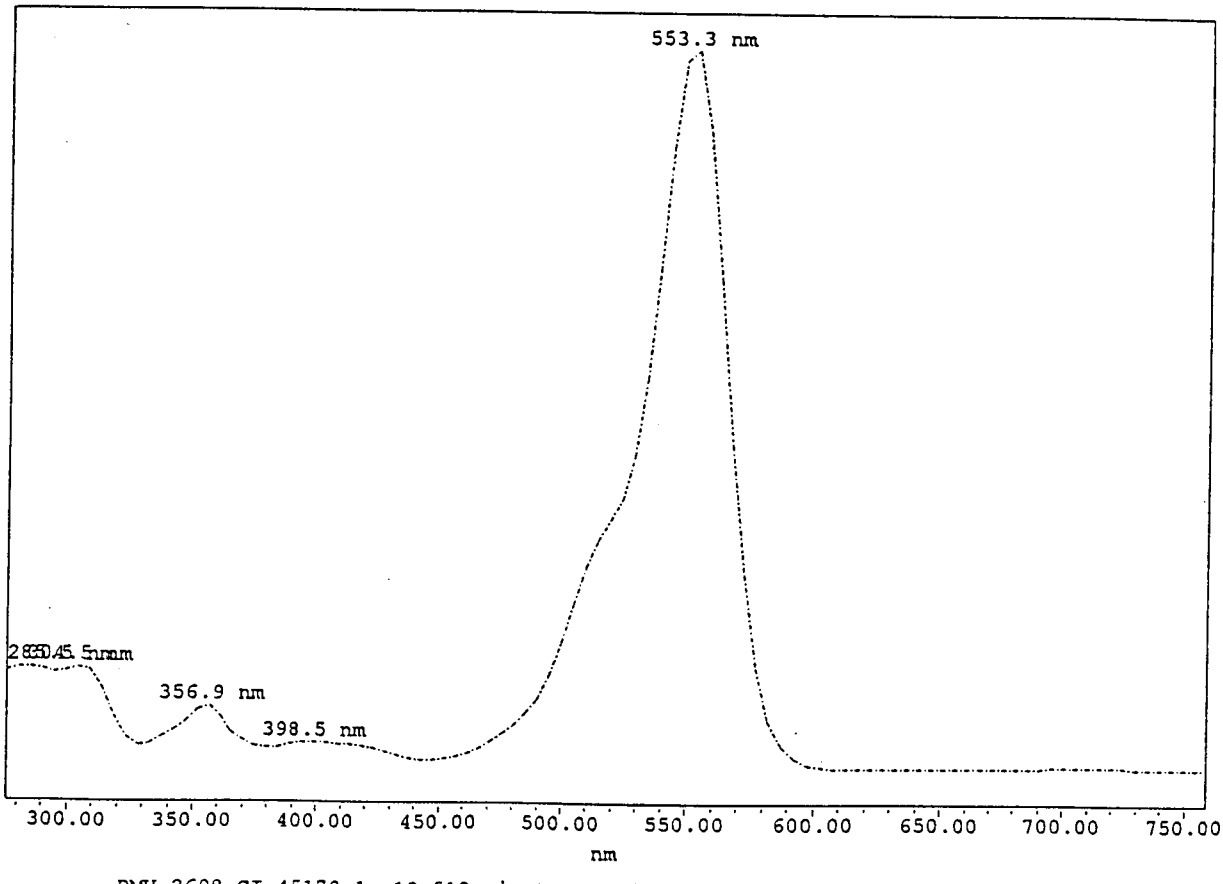




DMU 2581_CI 45170 13.463 minutes, 275 - 760 @ 4.8 nm, from 2581_CI45170

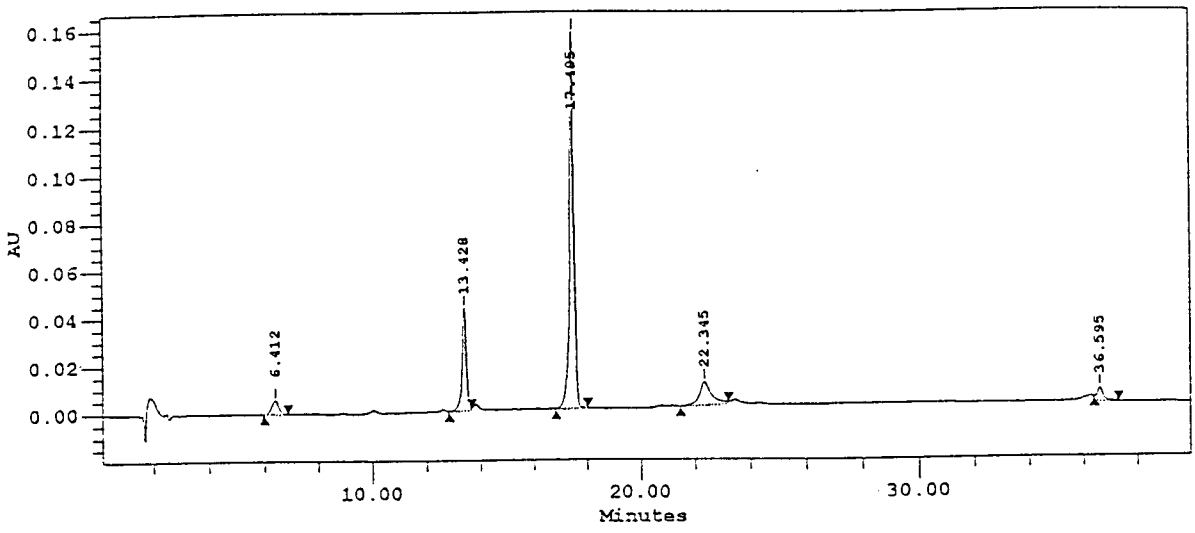
DMU 2698_C.I. 45170:1

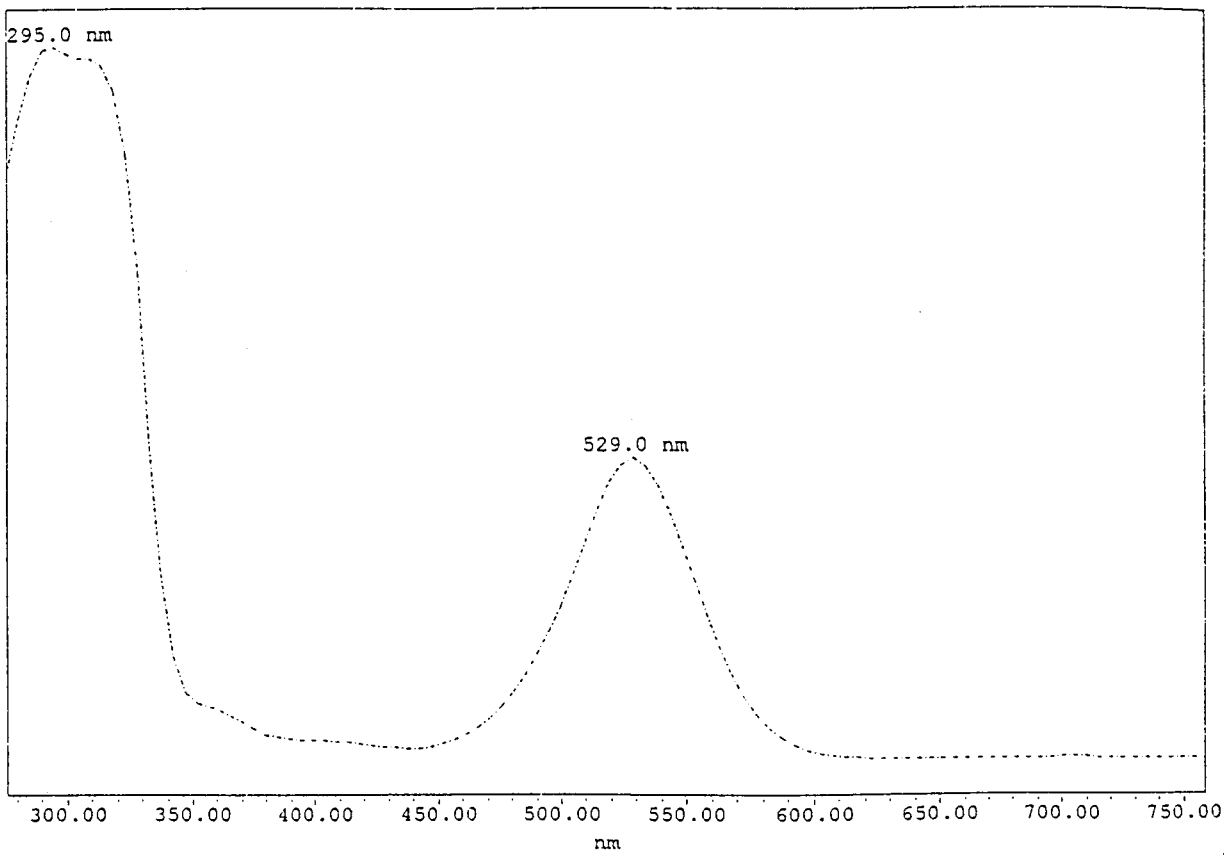




DMU 2698_CI 45170:1 13.512 minutes, 275 - 760 @ 4.8 nm, from 2698_SOL RED49

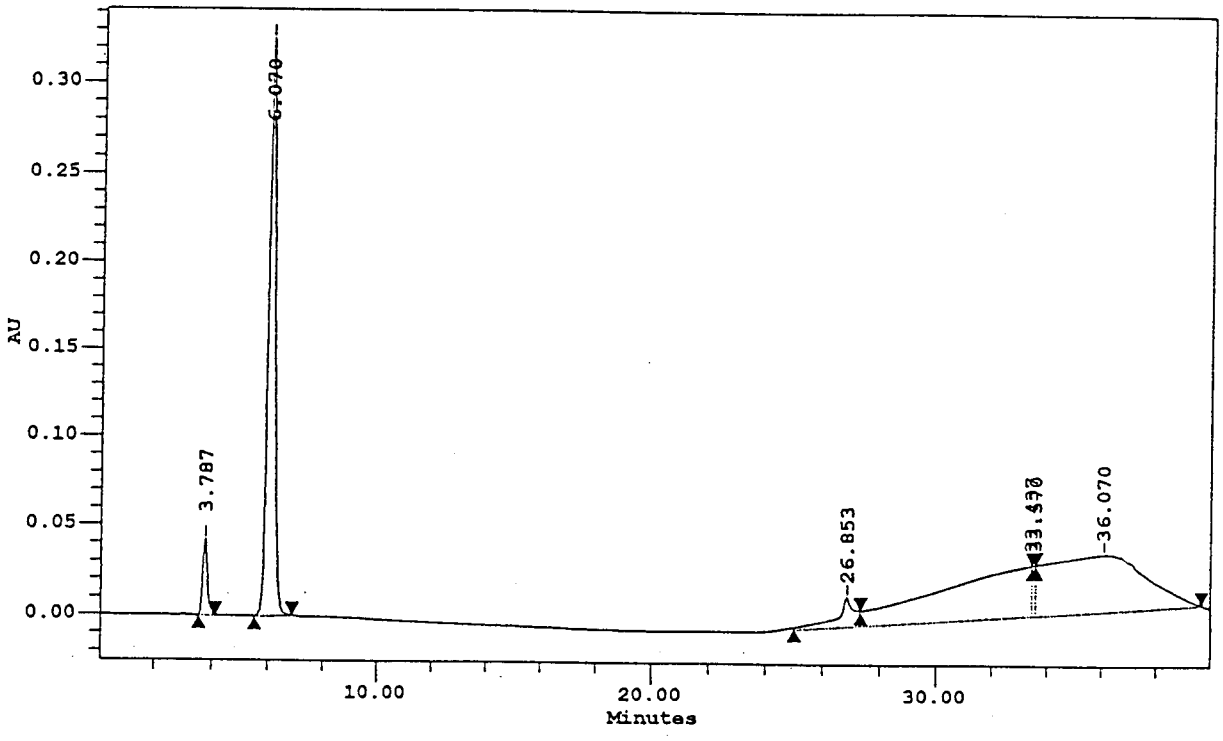
DMU 2580_C.I. 45190

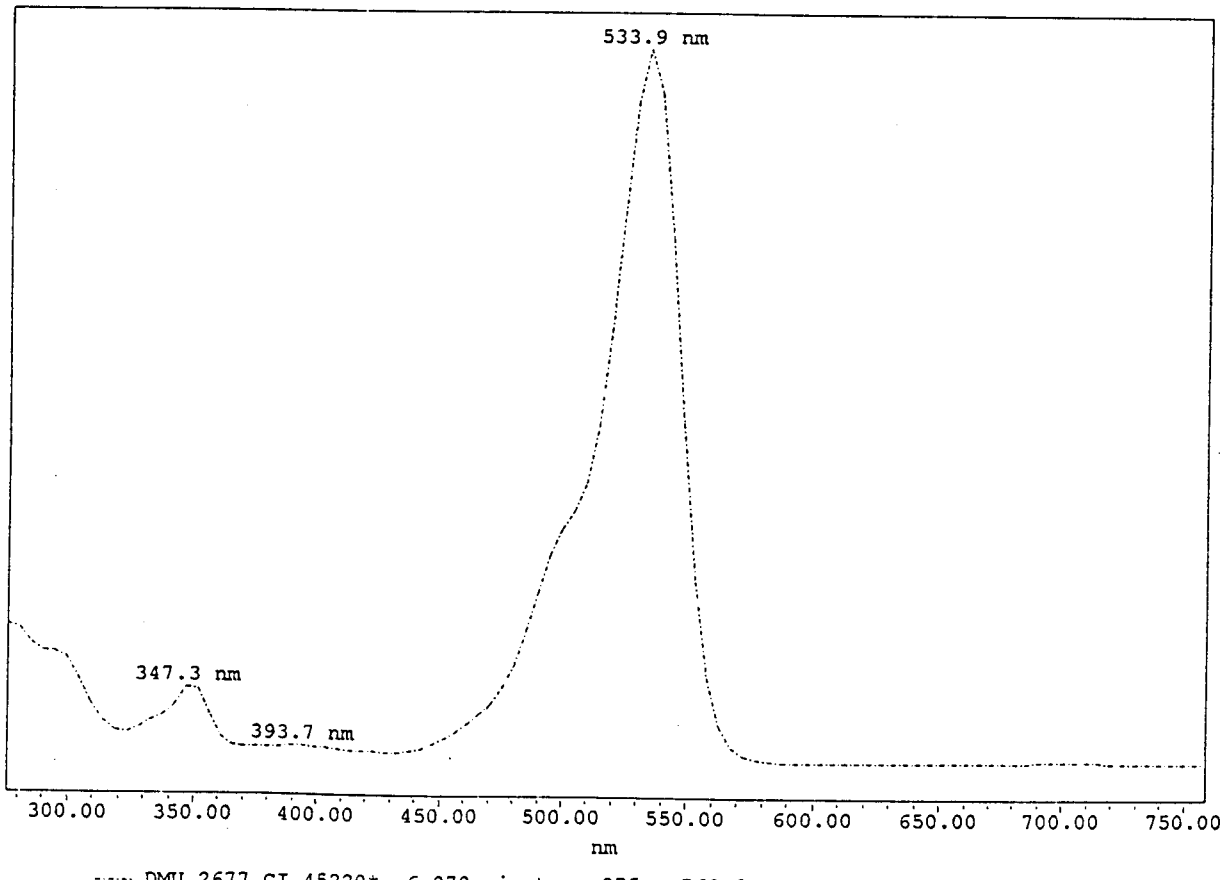




DMU 2580_CI 45190* 17.495 minutes, 275 - 760 @ 4.8 nm, from 2580_45190

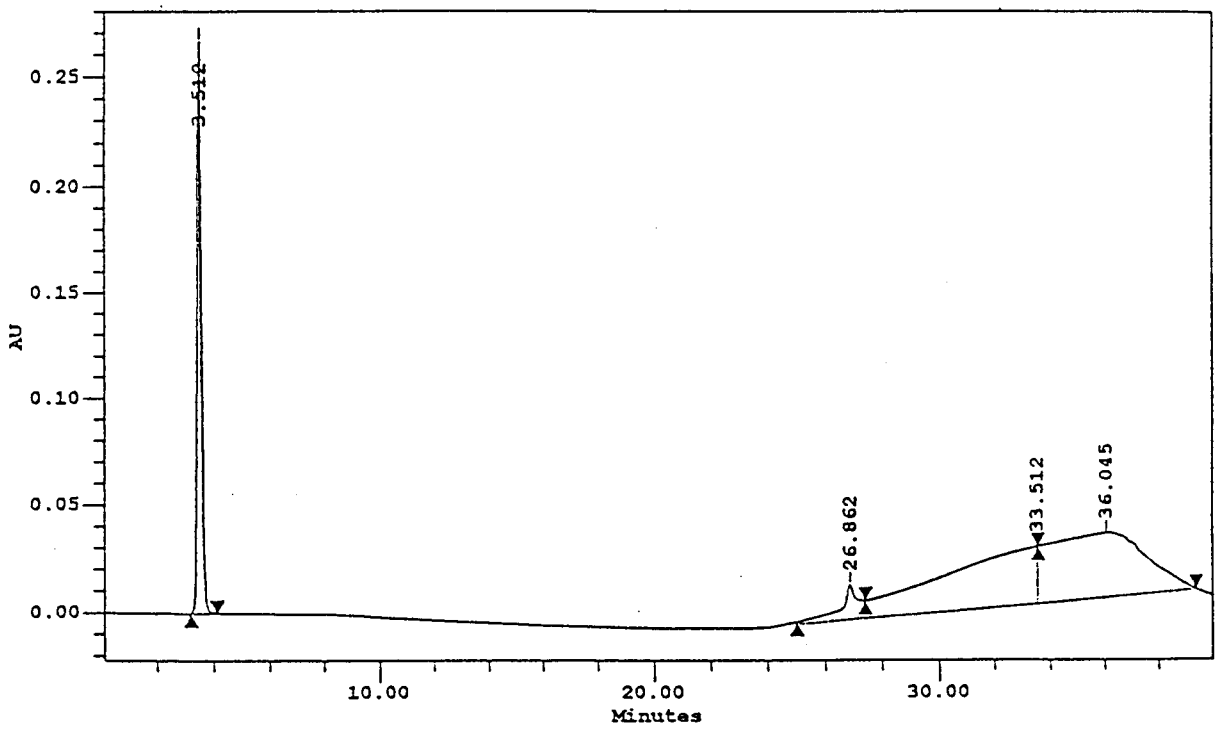
DMU 2677_C.I. 45220

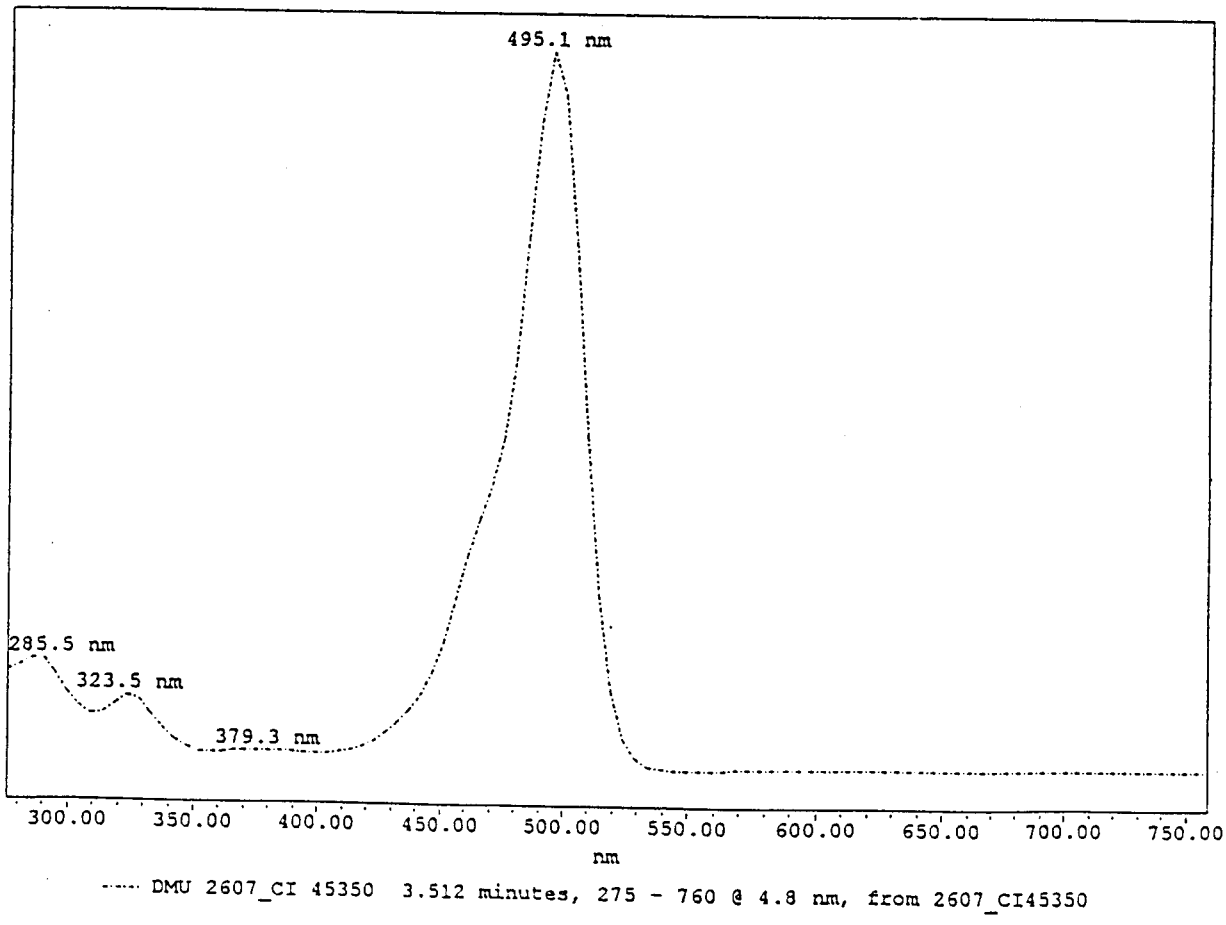




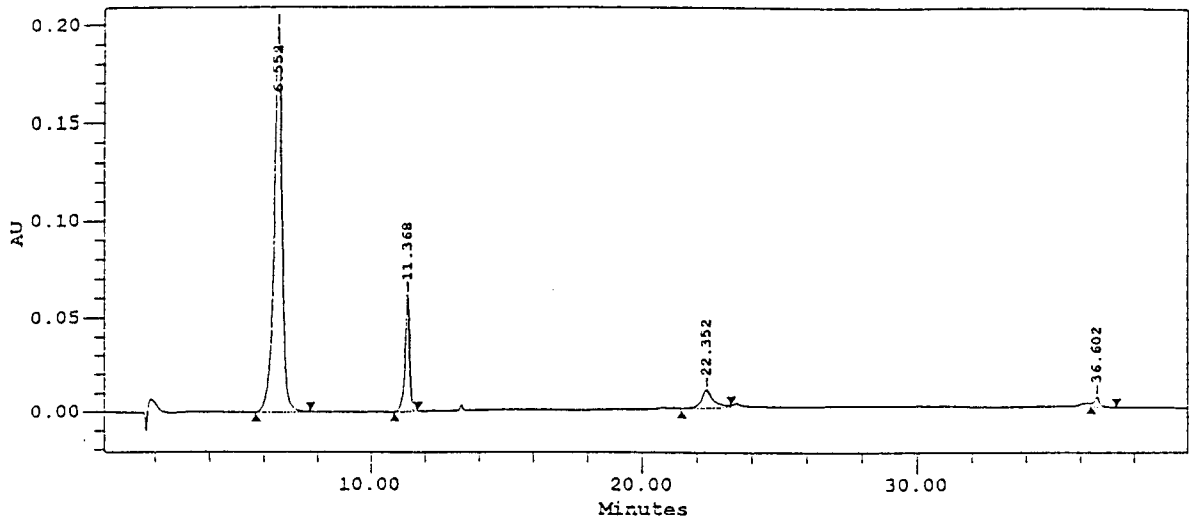
----- DMU 2677_CI 45220* 6.070 minutes, 275 - 760 @ 4.8 nm, from 2677_CI45220

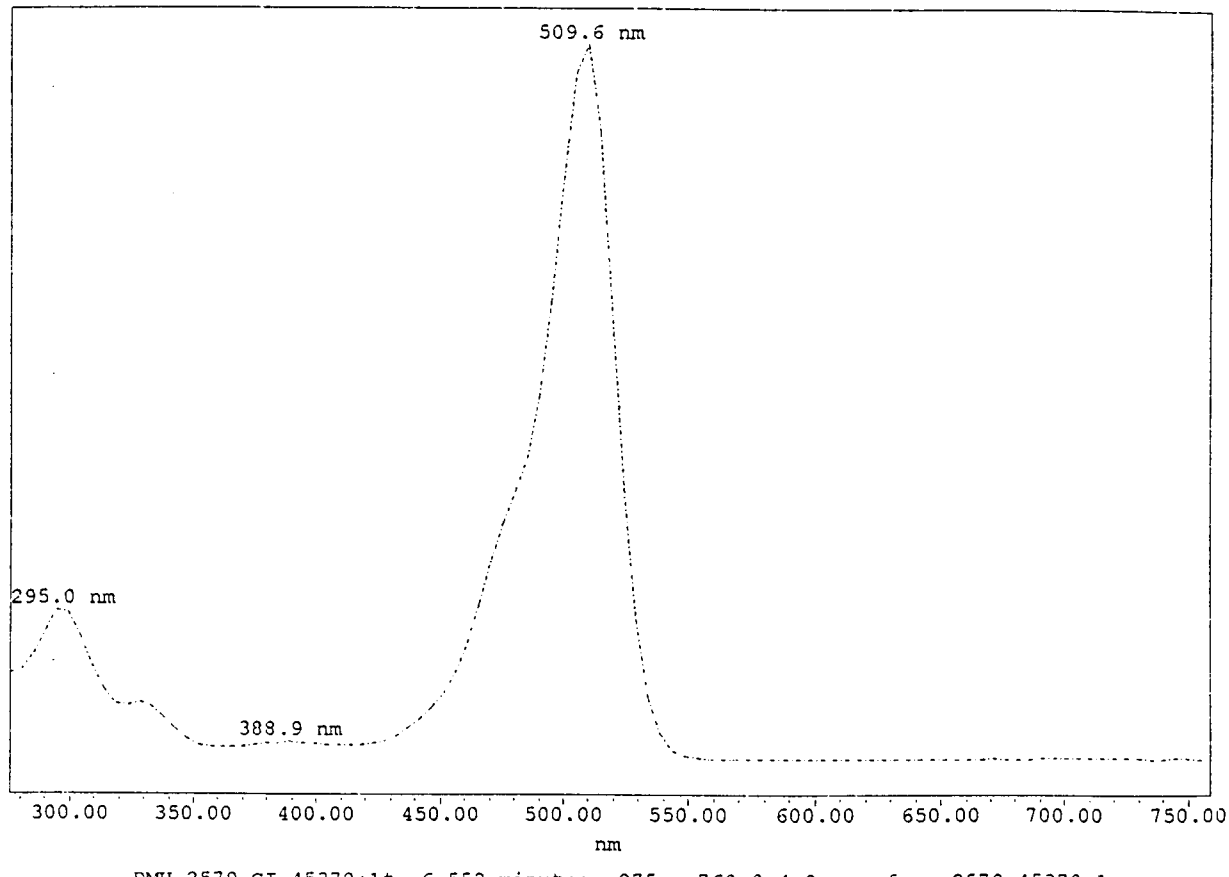
DMU 2607_C.I.45350





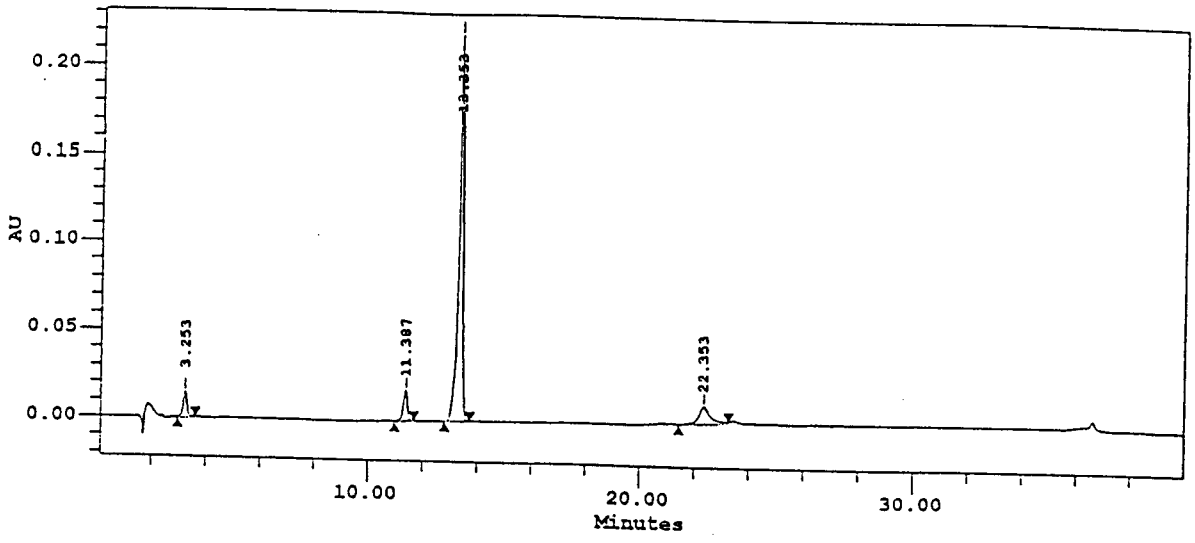
DMU 2579_C.I. 45370:1

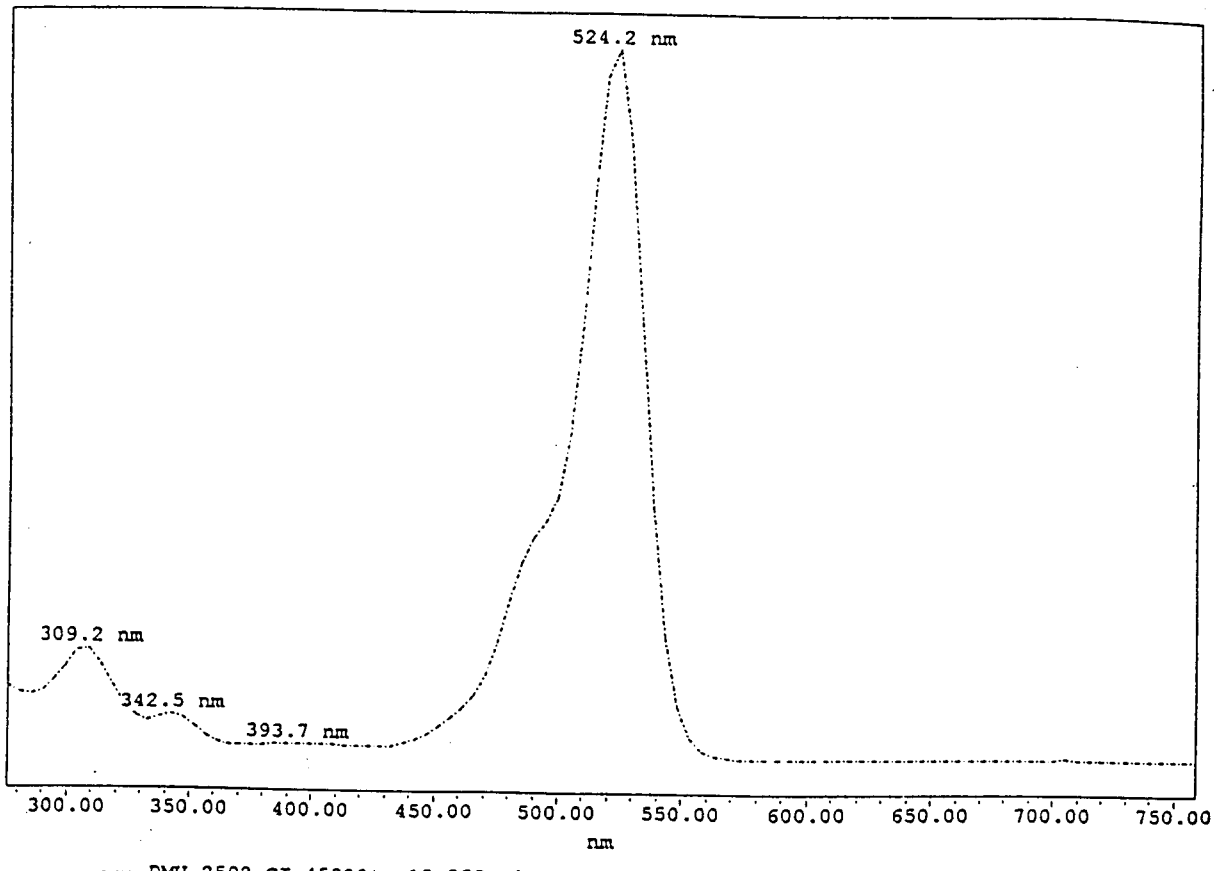




DMU 2579_CI 45370:1* 6.552 minutes, 275 - 760 @ 4.8 nm, from 2579_45370:1

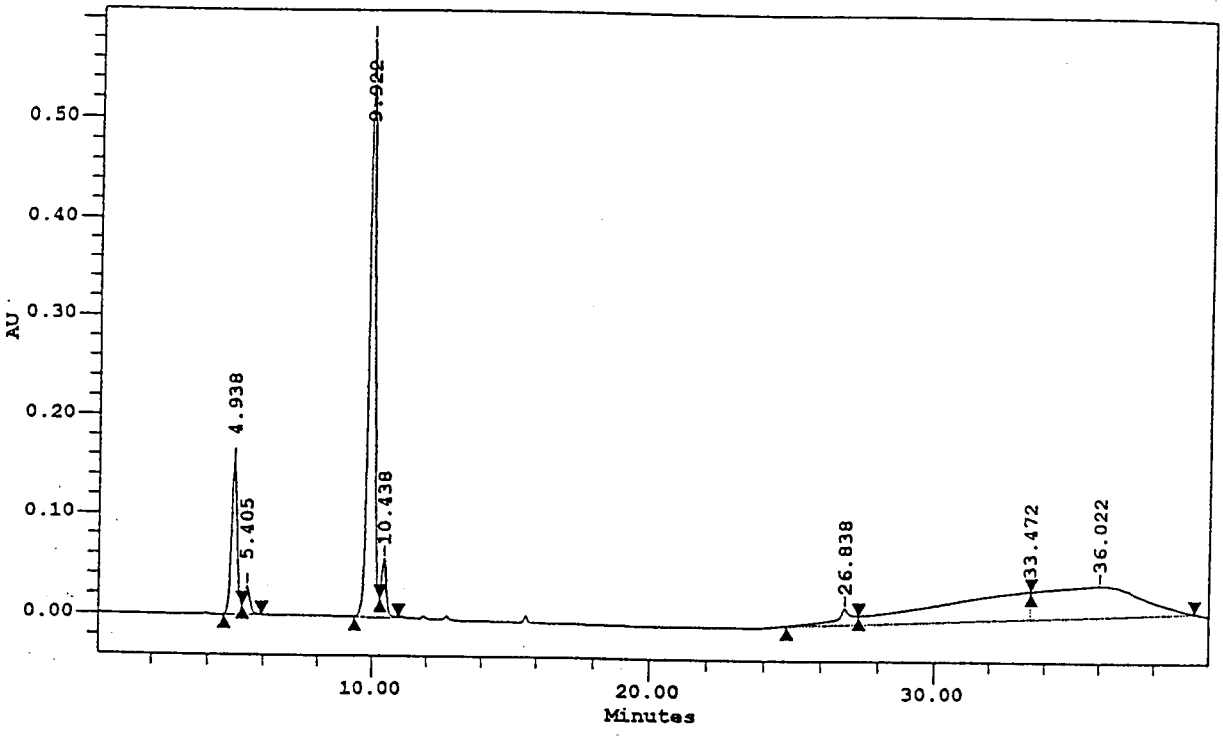
DMU 2598_C.I. 45380

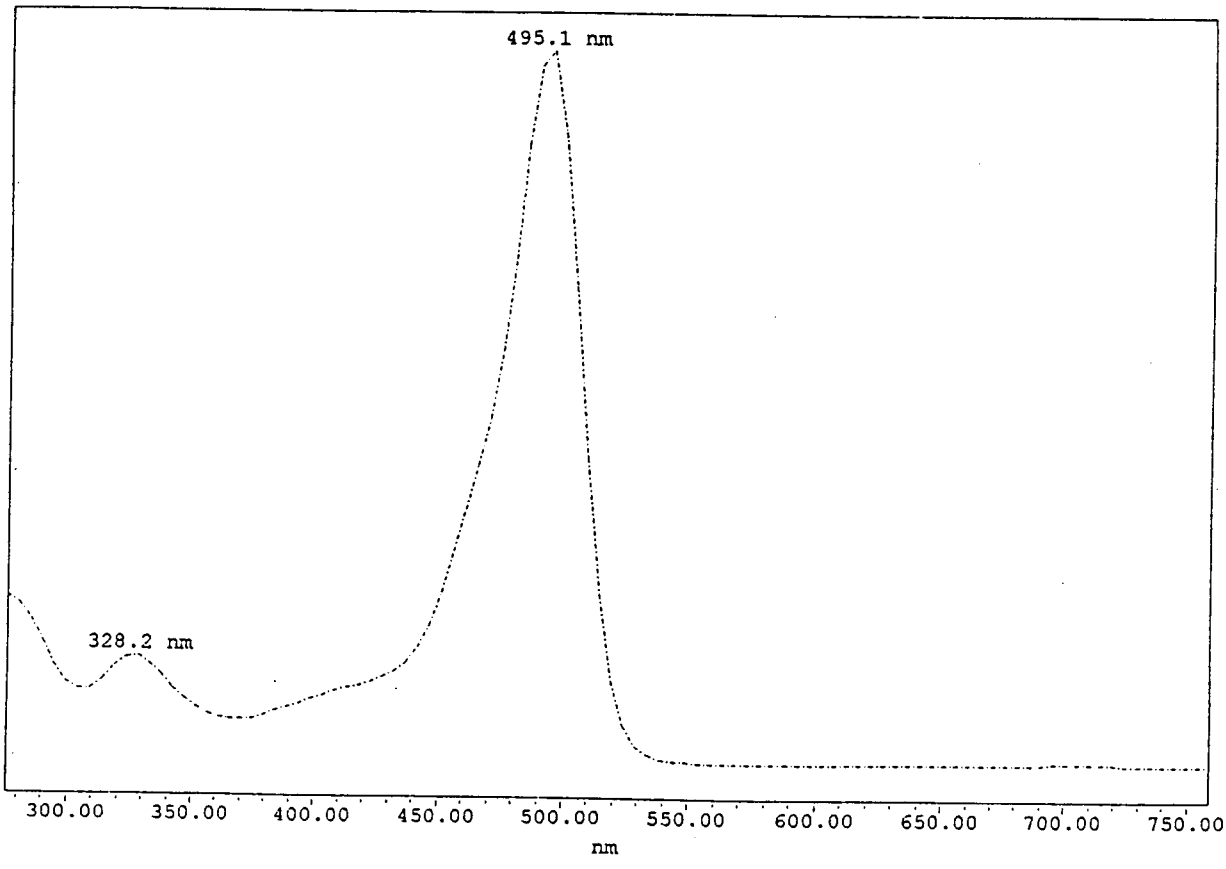




DMU 2598_CI 45380* 13.353 minutes, 275 - 760 @ 4.8 nm, from 2598_45380

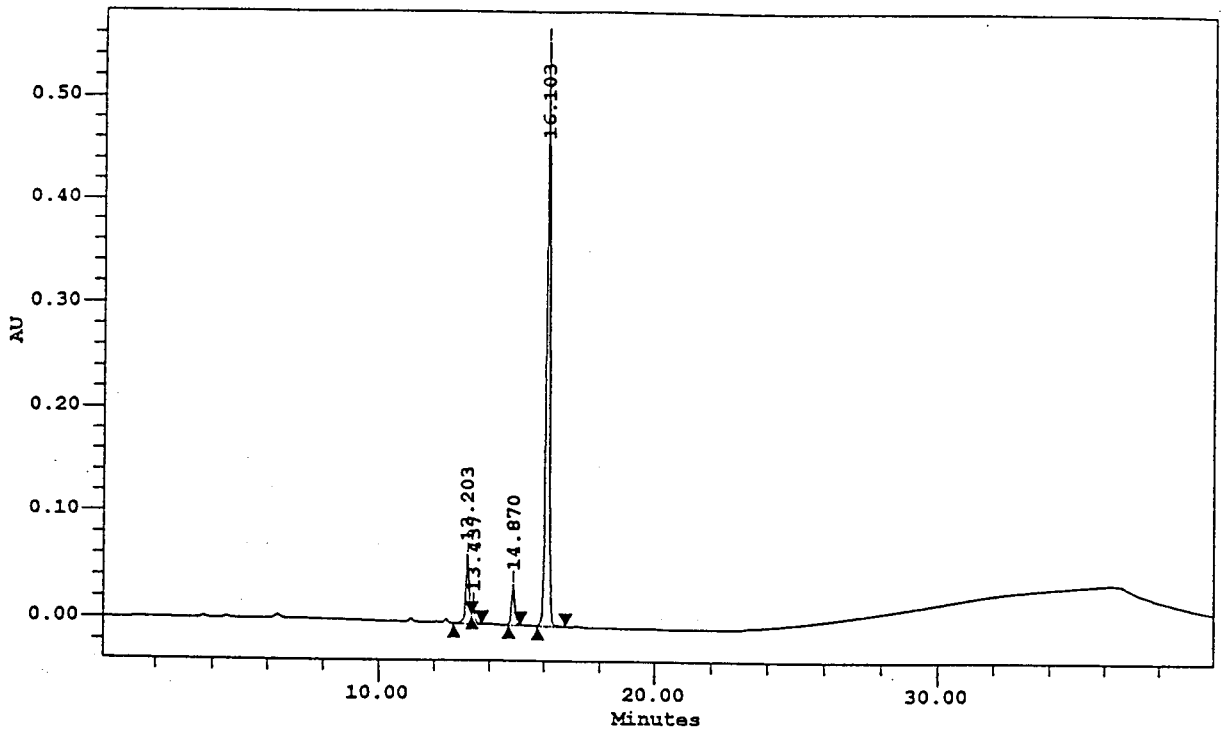
DMU 2688_C.I. 45396

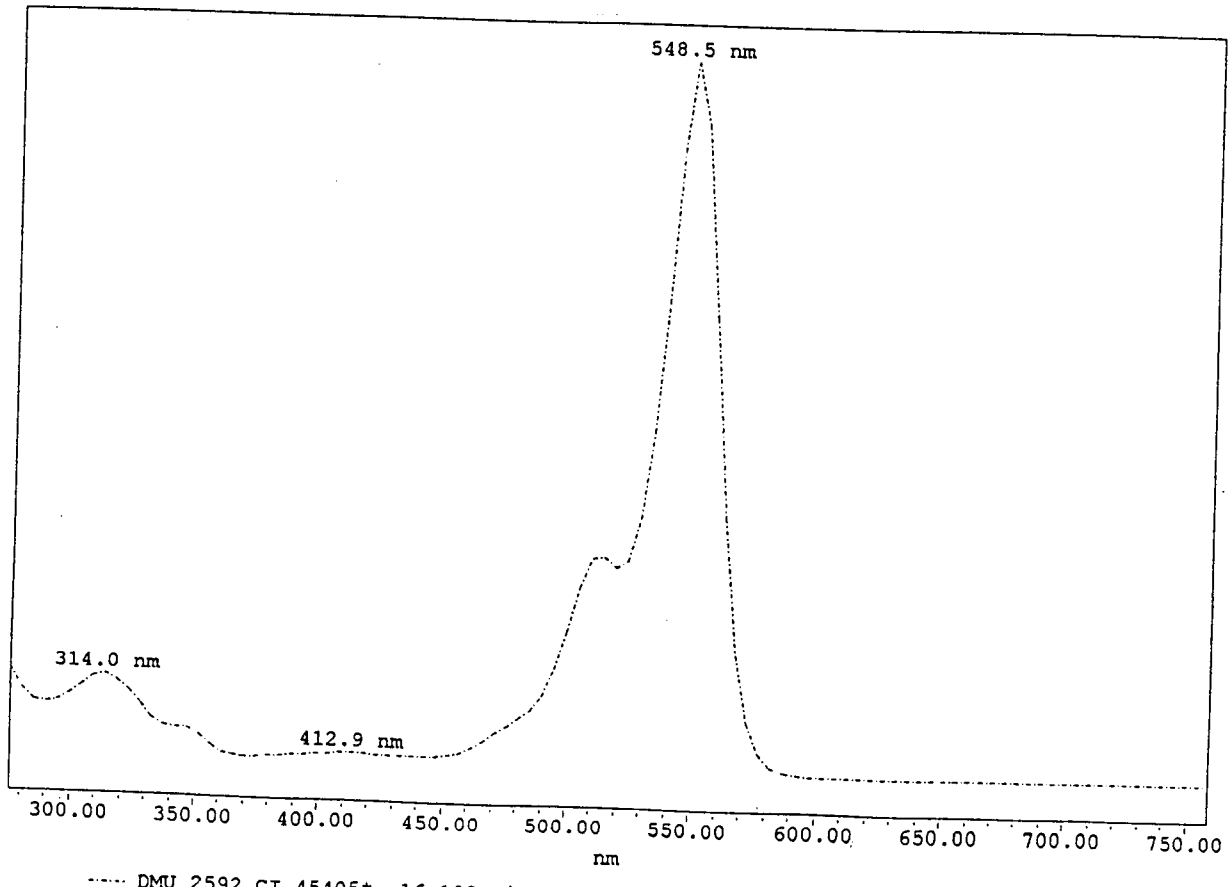




DMU 2688_CI 45396* 9.922 minutes, 275 - 760 @ 4.8 nm, from 2688_CI45396

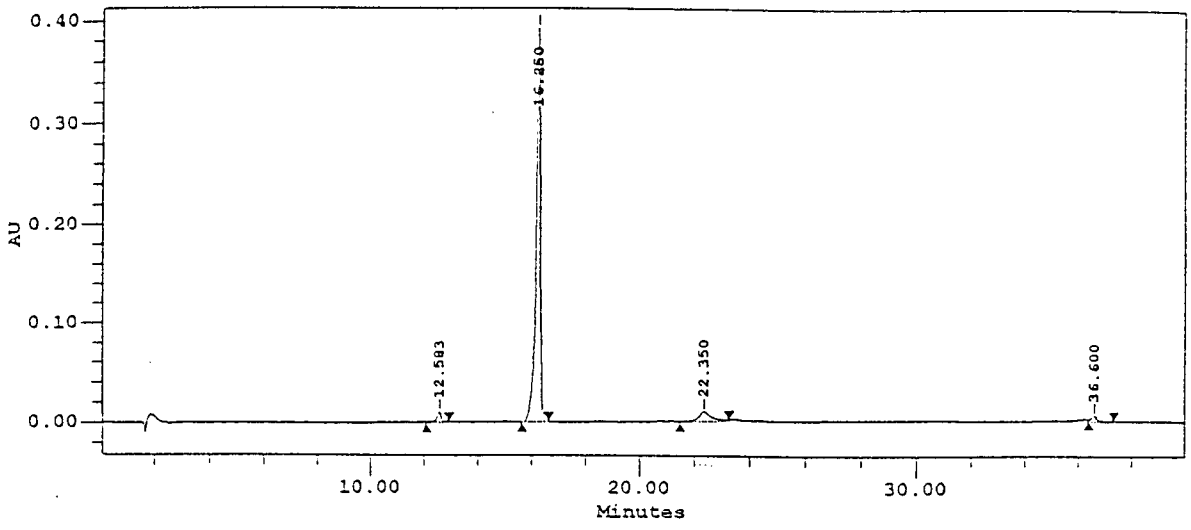
DMU 2592_C.I. 45405

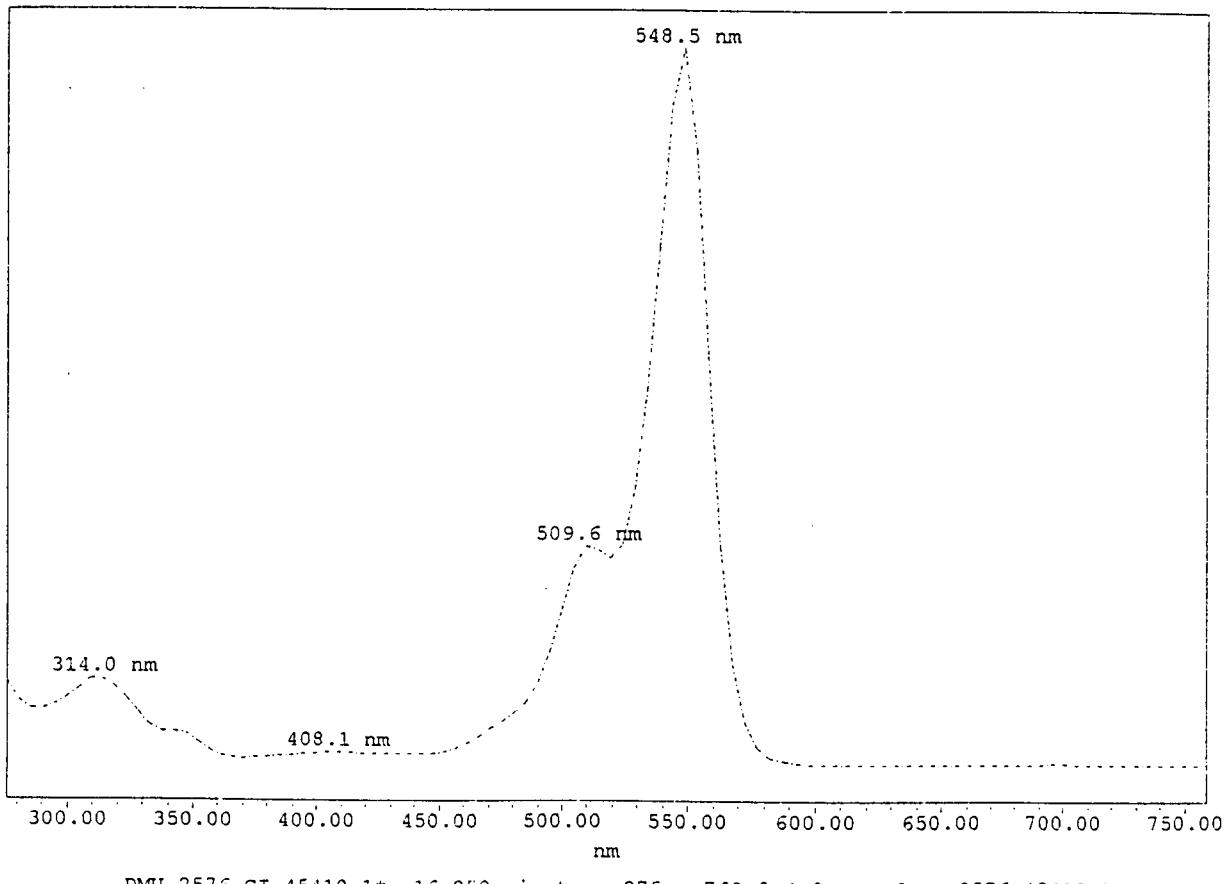




DMU 2592_CI 45405* 16.103 minutes, 275 - 760 @ 4.8 nm, from 2592_CI45405

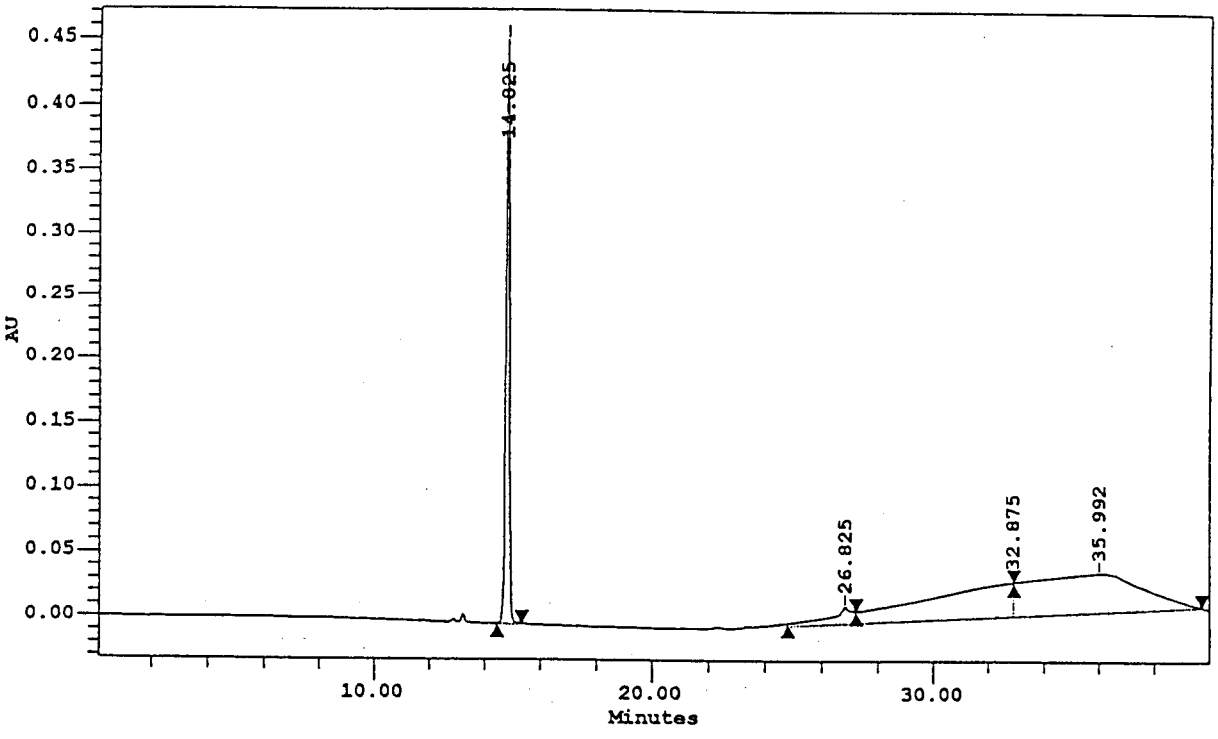
DMU 2576_C.I. 45410:1

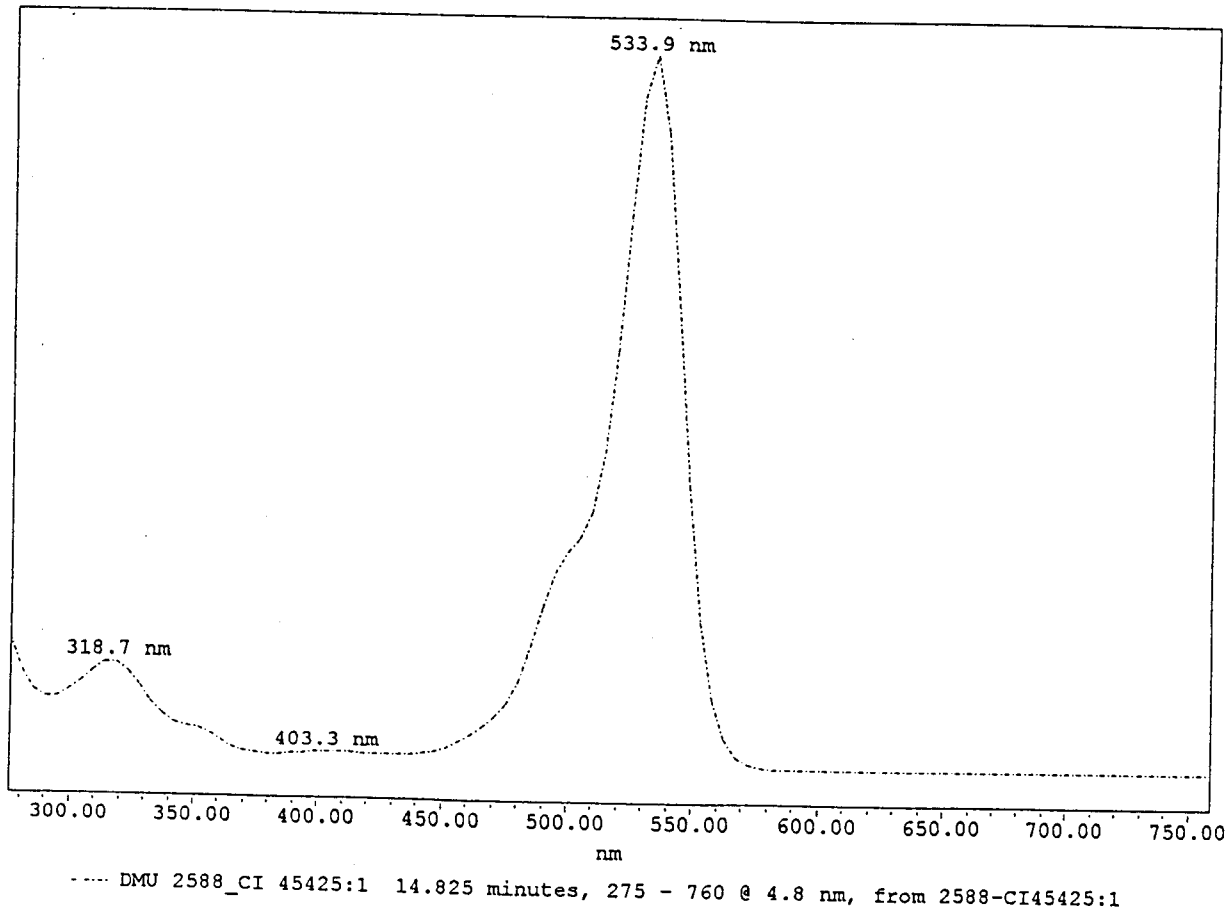




DMU 2576_CI 45410:1* 16.250 minutes, 275 - 760 @ 4.8 nm, from 2576_45410:1

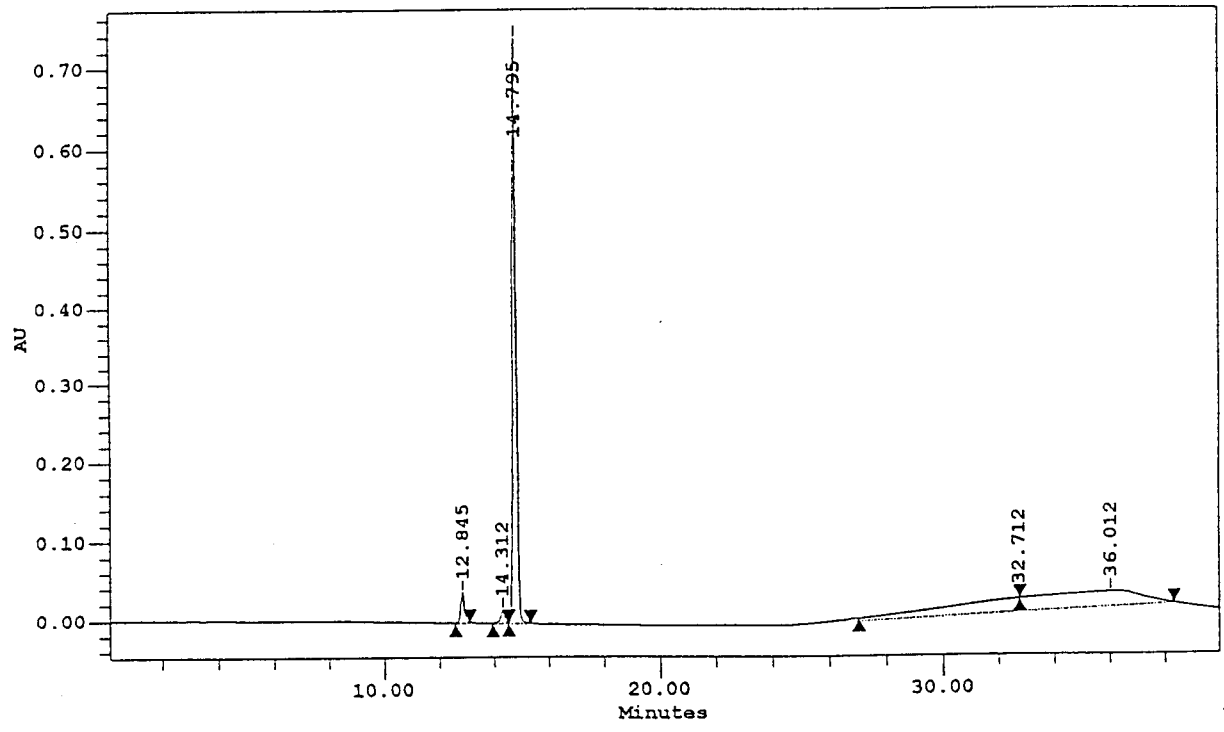
DMU 2588_C.I. 45425:1

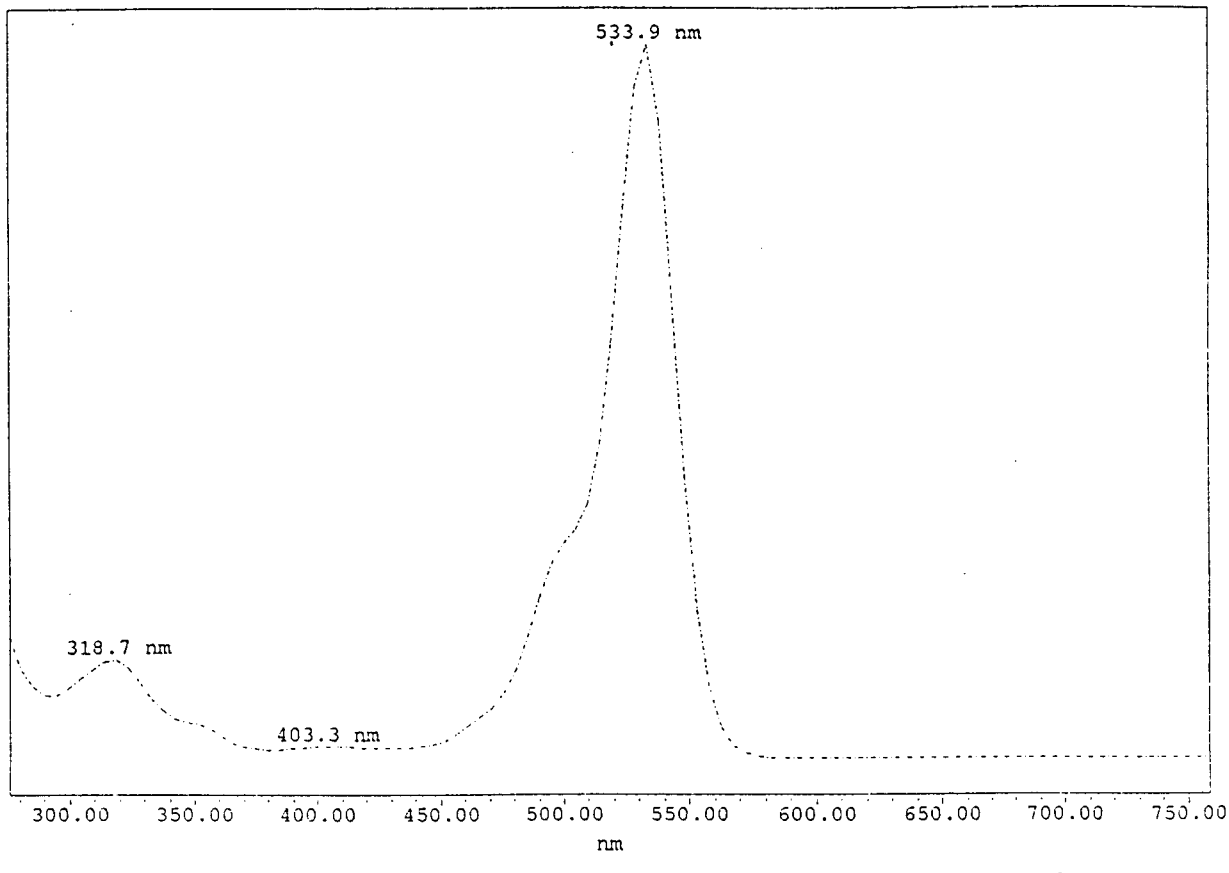




DMU 2588_CI 45425:1 14.825 minutes, 275 - 760 @ 4.8 nm, from 2588-CI45425:1

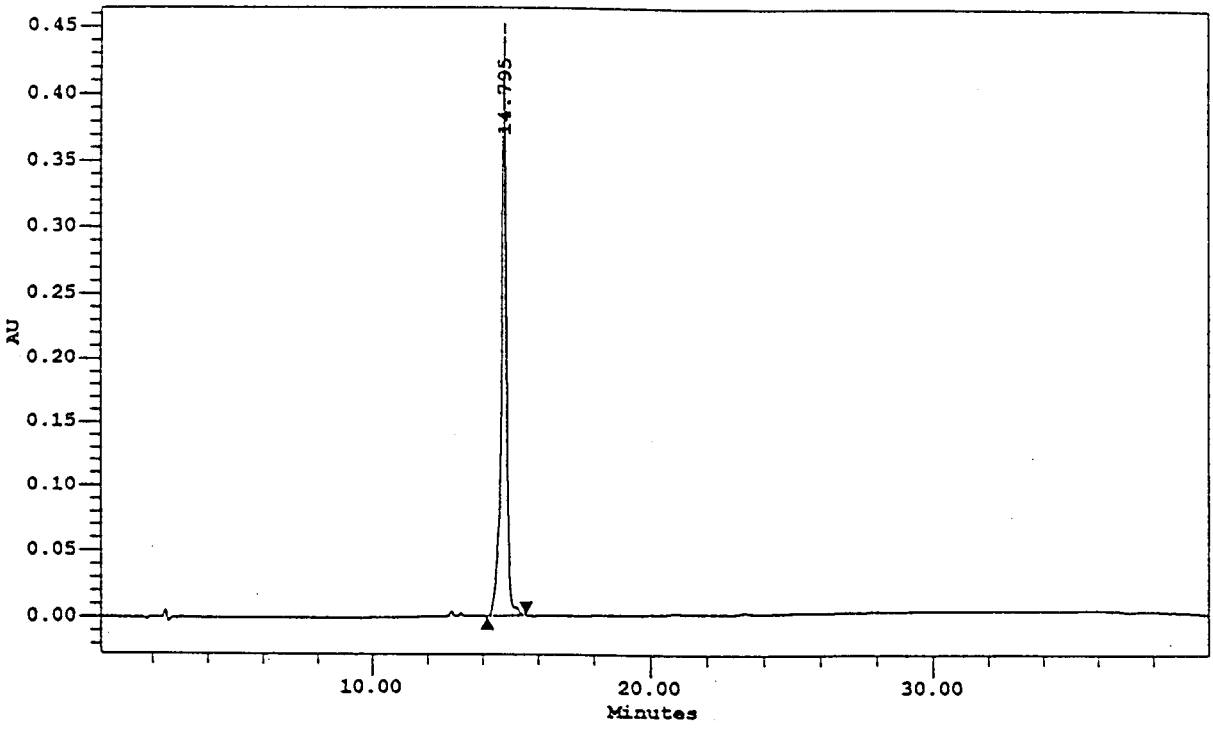
DMU 2578_C.I. 45430

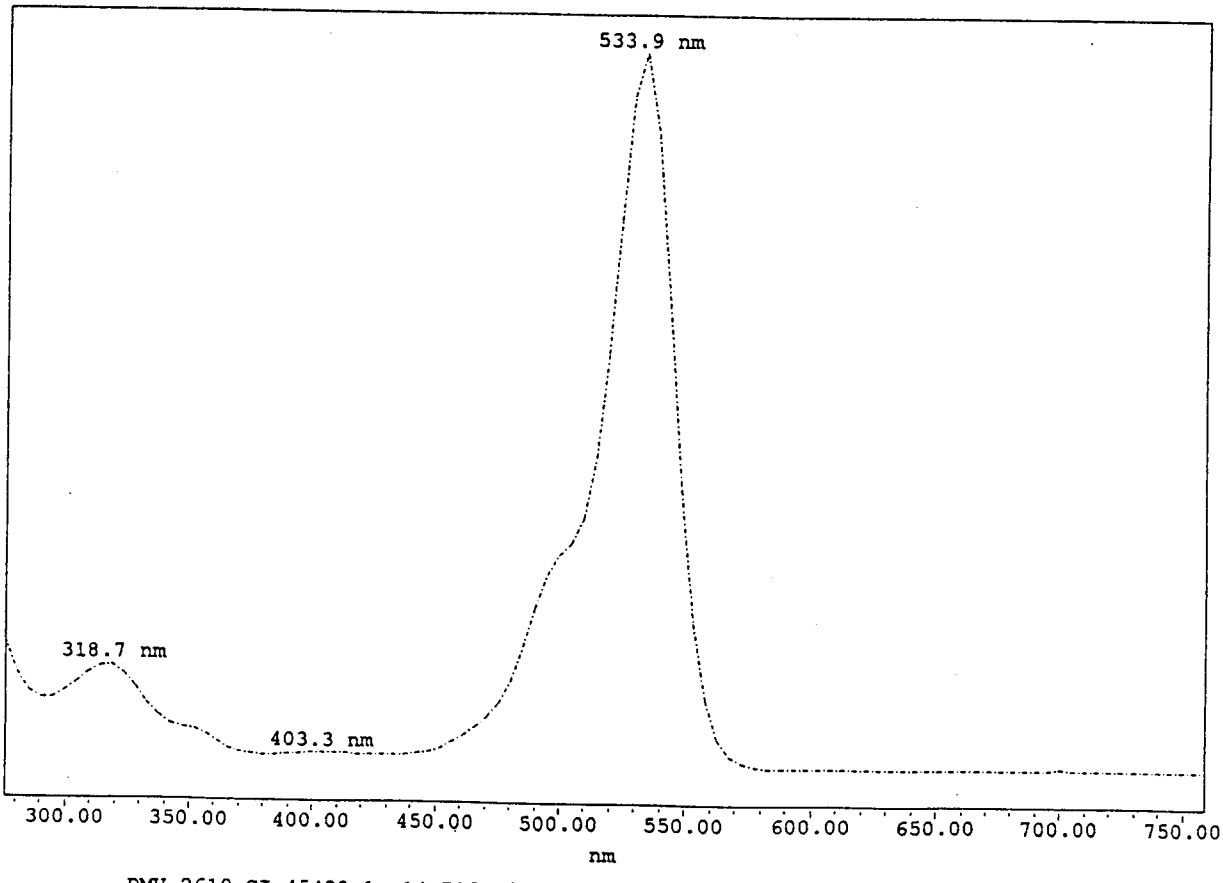




DMU 2578_CI 45430* 14.795 minutes, 275 - 760 @ 4.8 nm, from 2578_CI45430

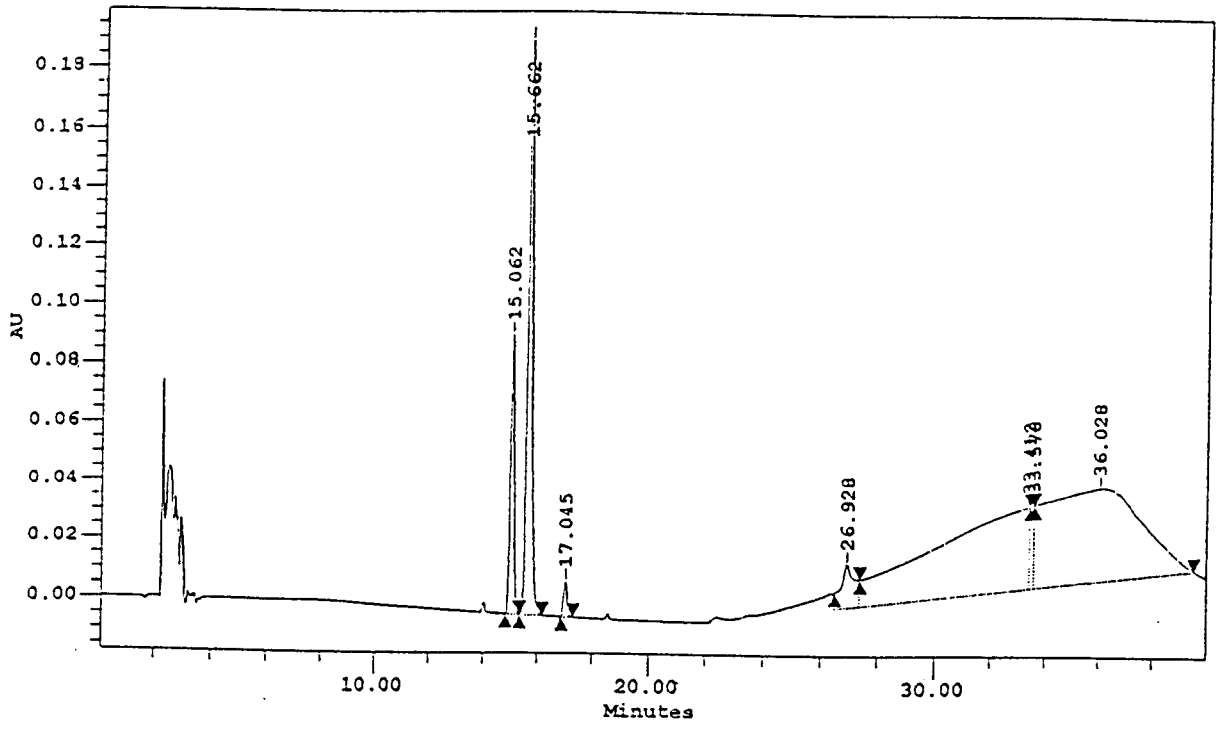
DMU 2619_C.I. 45430:1

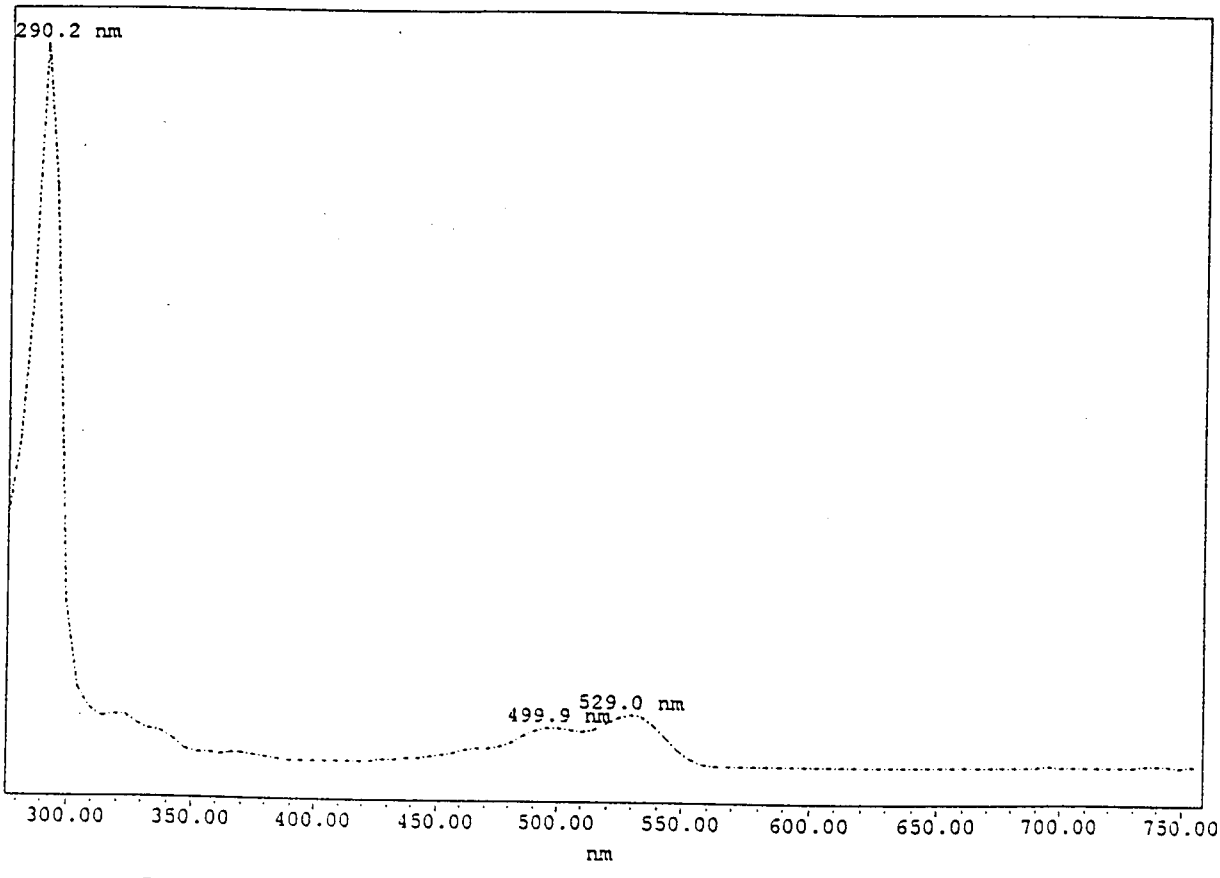




DMU 2619_CI 45430:1 14.795 minutes, 275 - 760 @ 4.8 nm, from 2619-45430:1

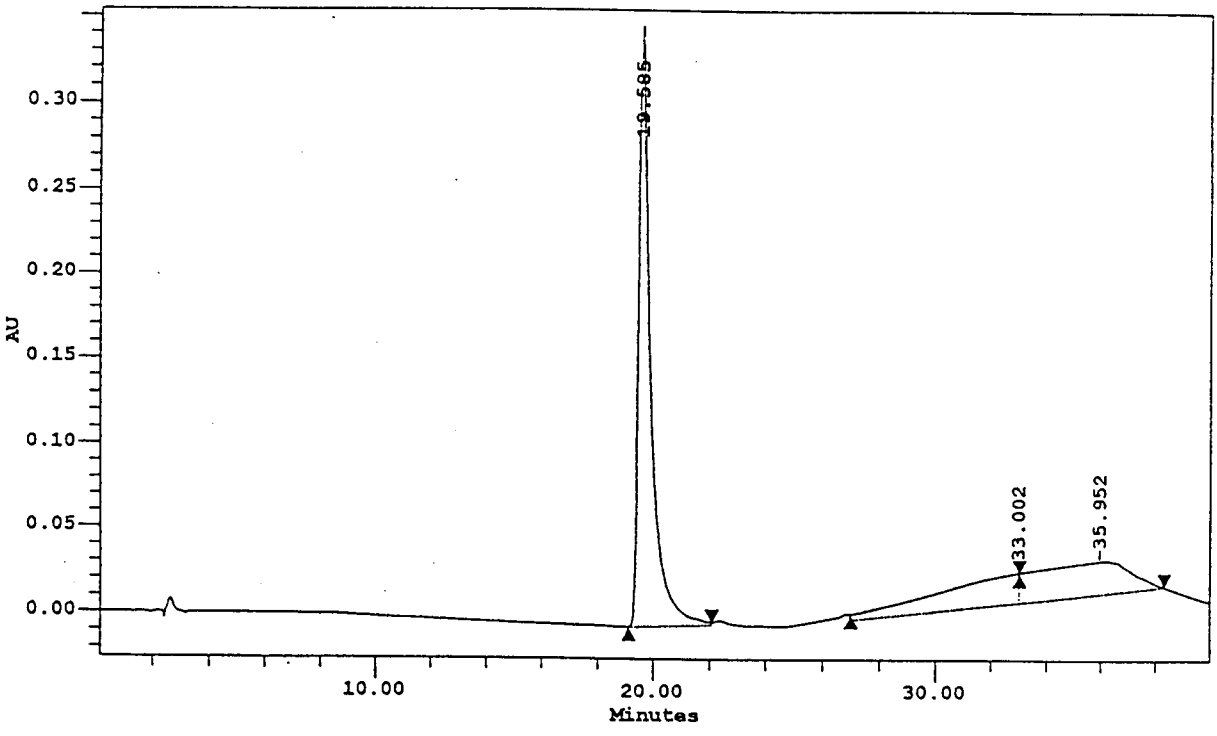
DMU 2693_C.I. 46500

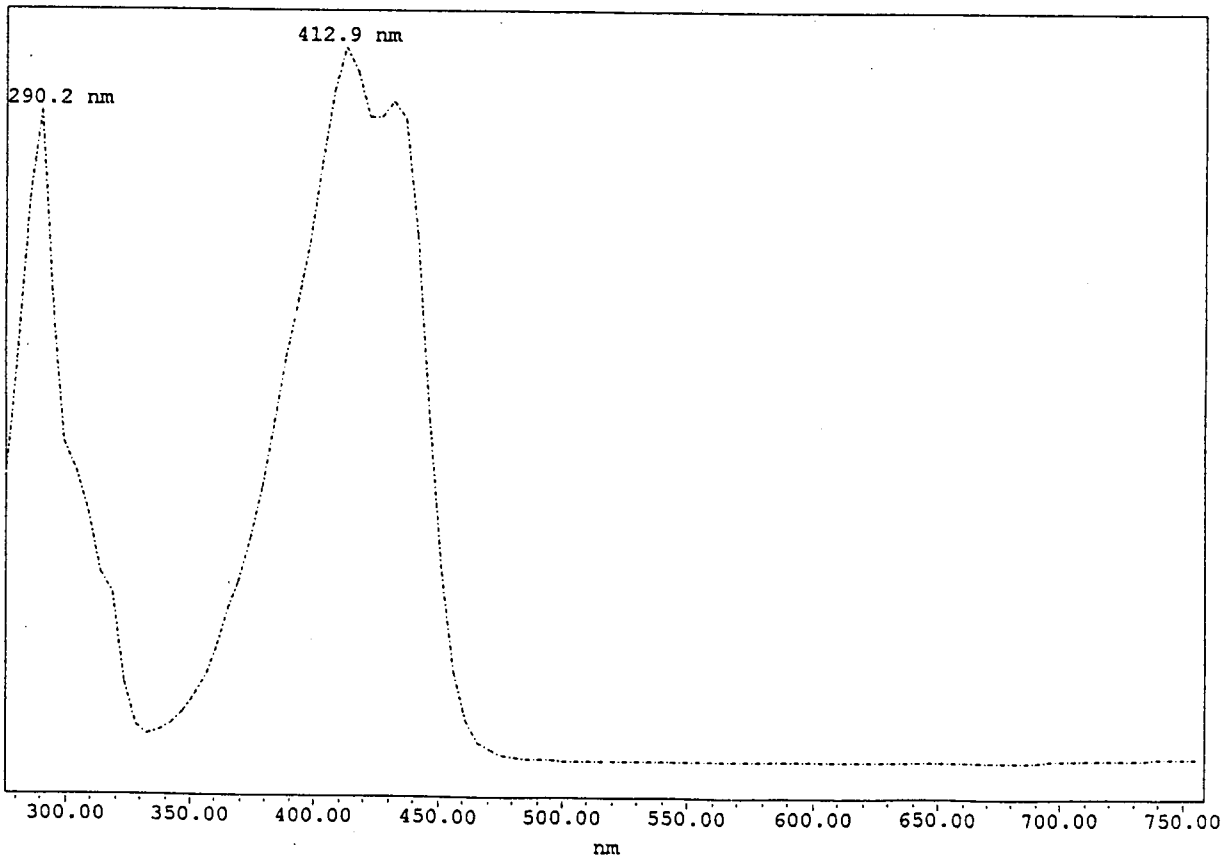




DMU 2693_CI46500* 15.662 minutes, 275 - 760 @ 4.8 nm, from 2693_CI46500

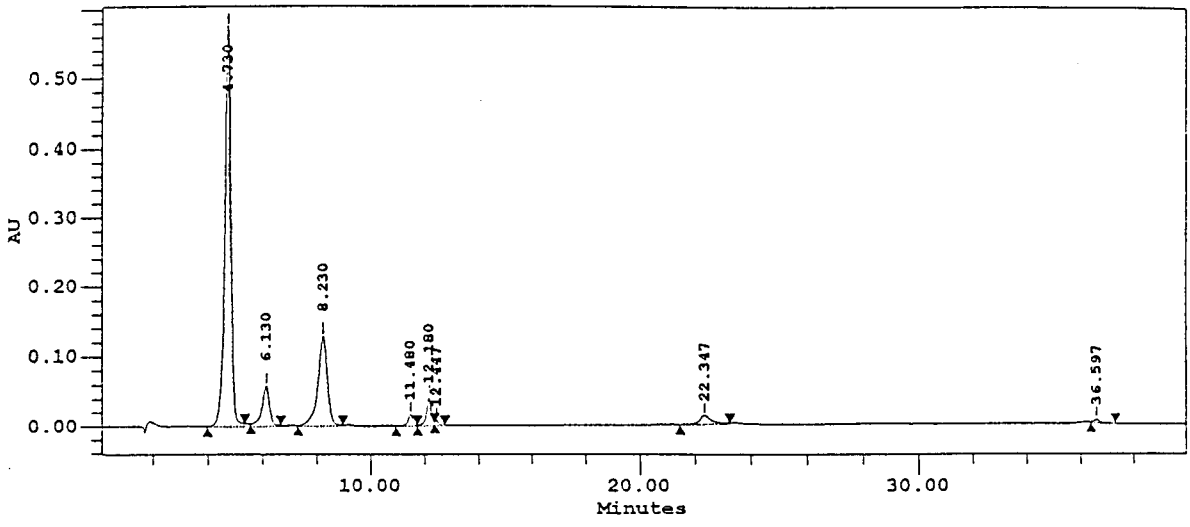
DMU 2631_C.I. 47000

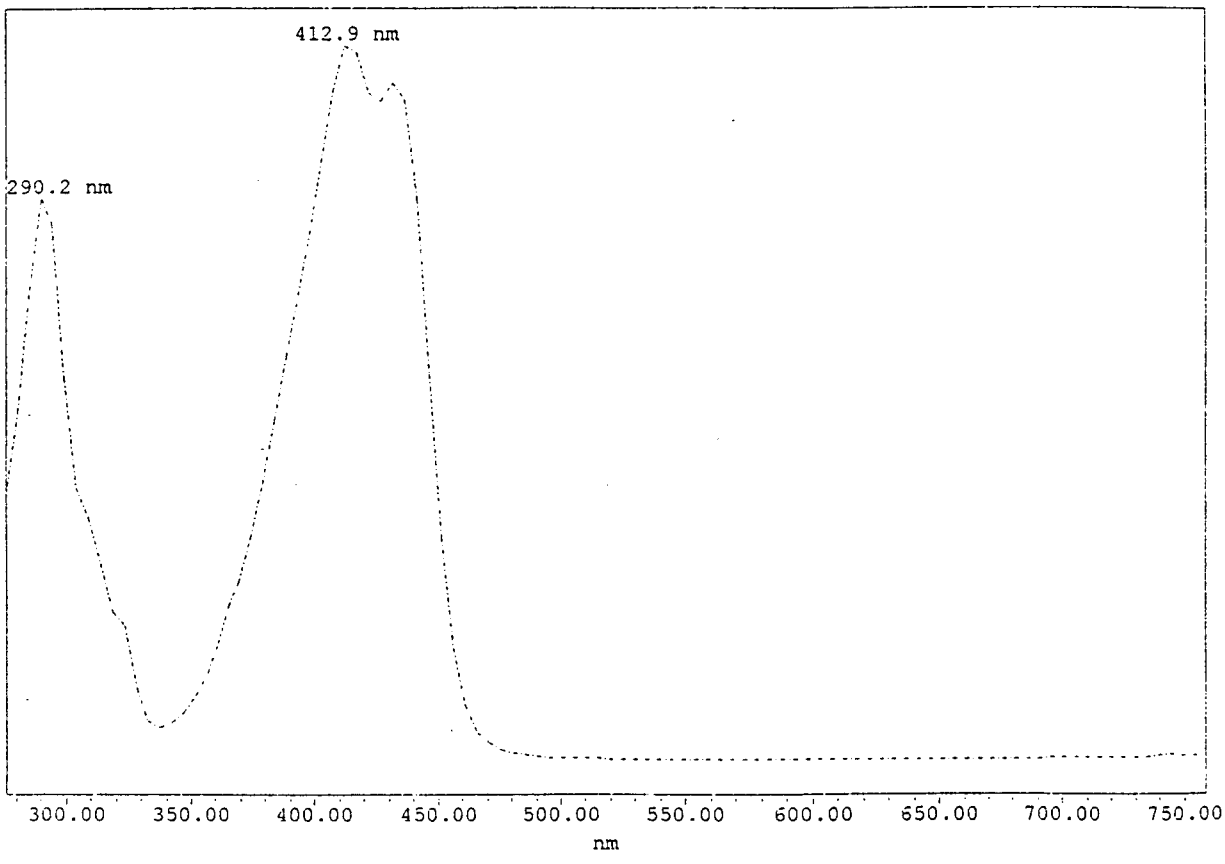




DMU 2631_CI 47000 19.585 minutes, 275 - 760 @ 4.8 nm, from 2631_CI47000

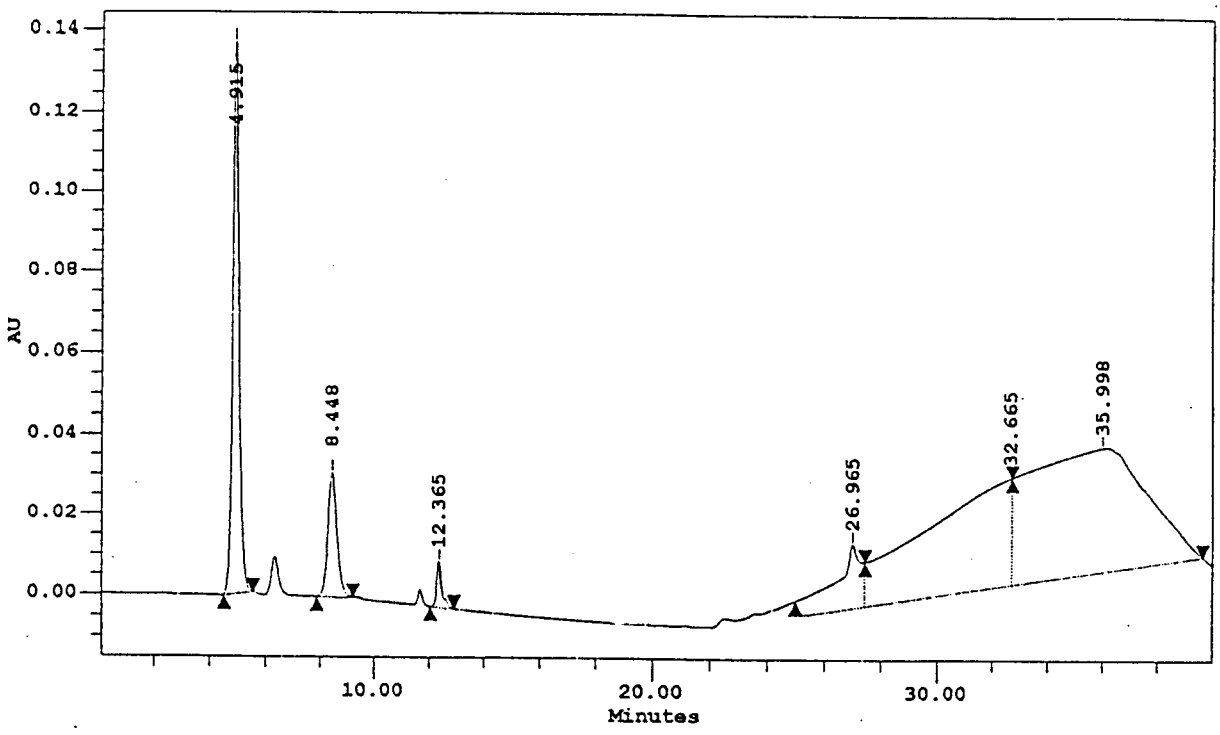
DMU 2577_C.I. 47005

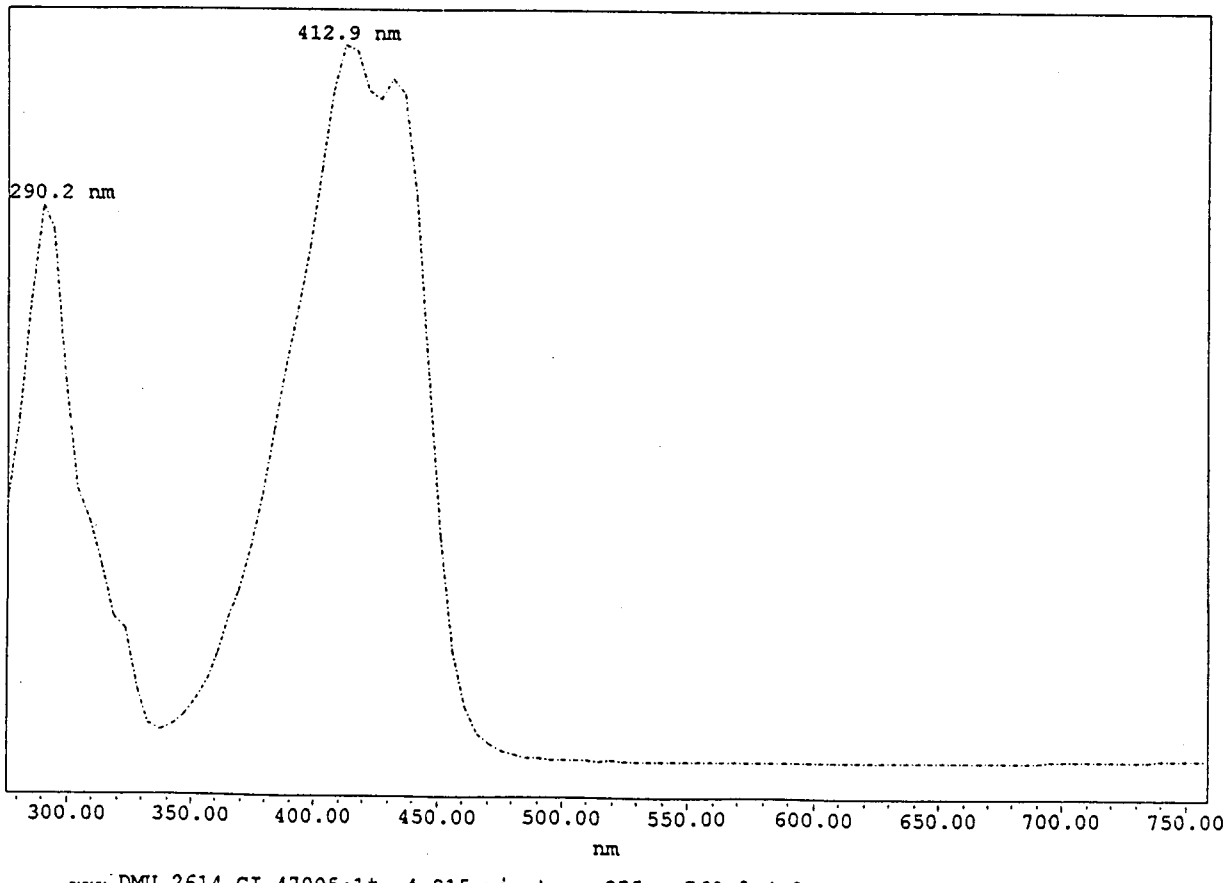




DMU 2577_CI 47005* 4.730 minutes, 275 - 760 @ 4.8 nm, from 2577_47005

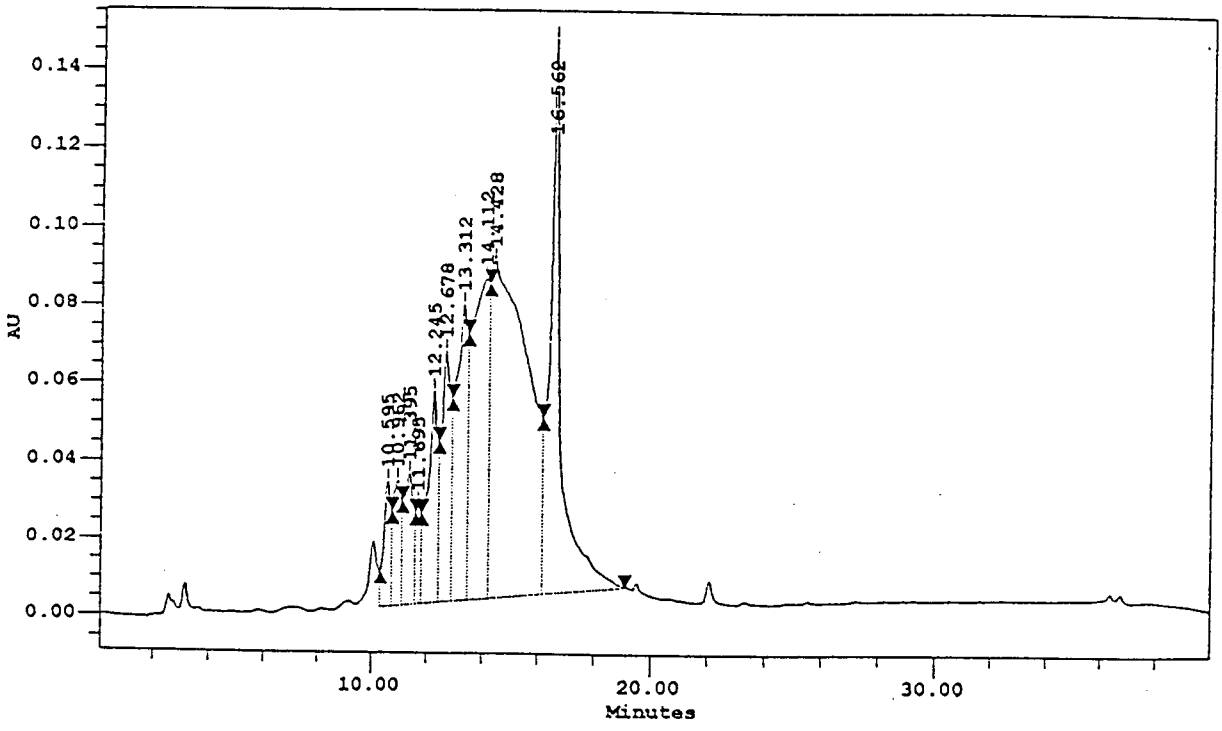
DMU 2614_C.I. 47005:1

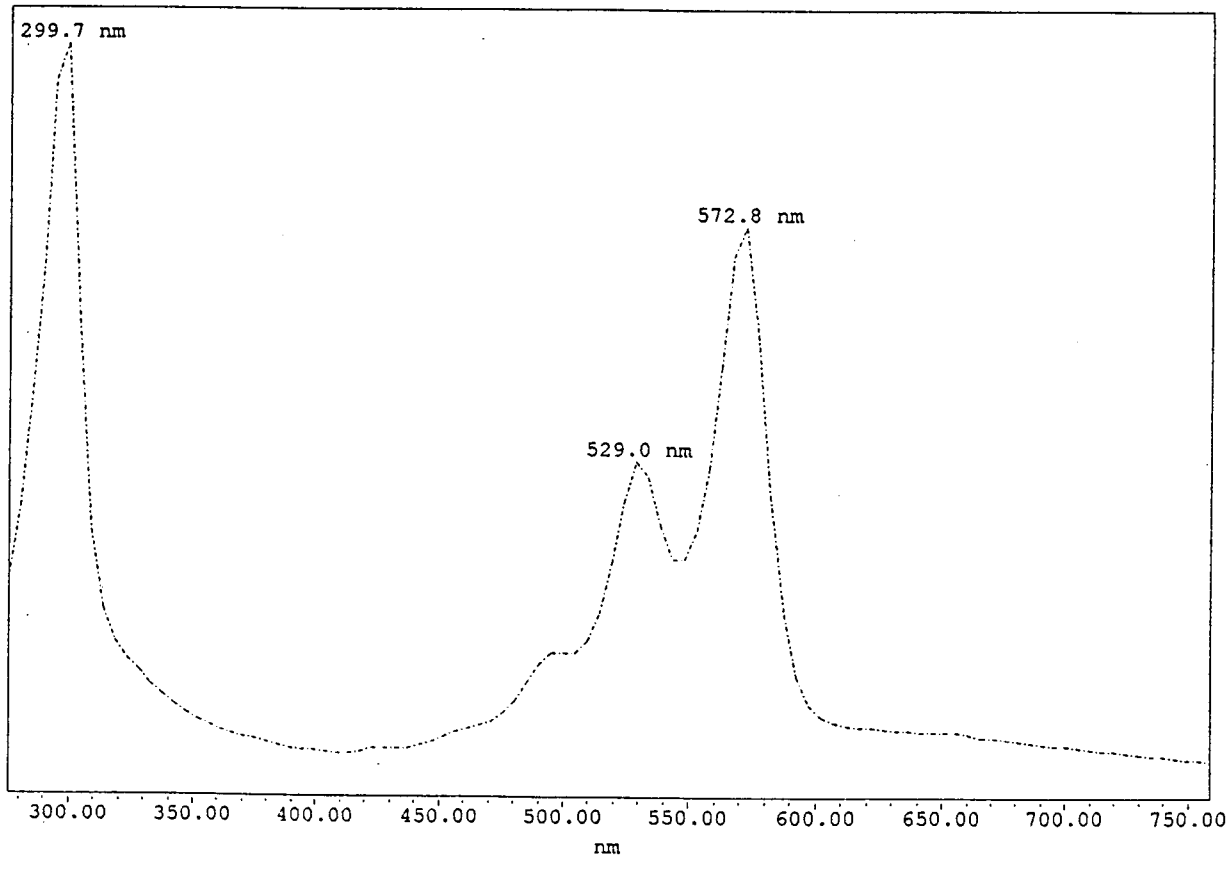




DMU 2614_CI 47005:1* 4.915 minutes, 275 - 760 @ 4.8 nm, from 2614_CI47005:1

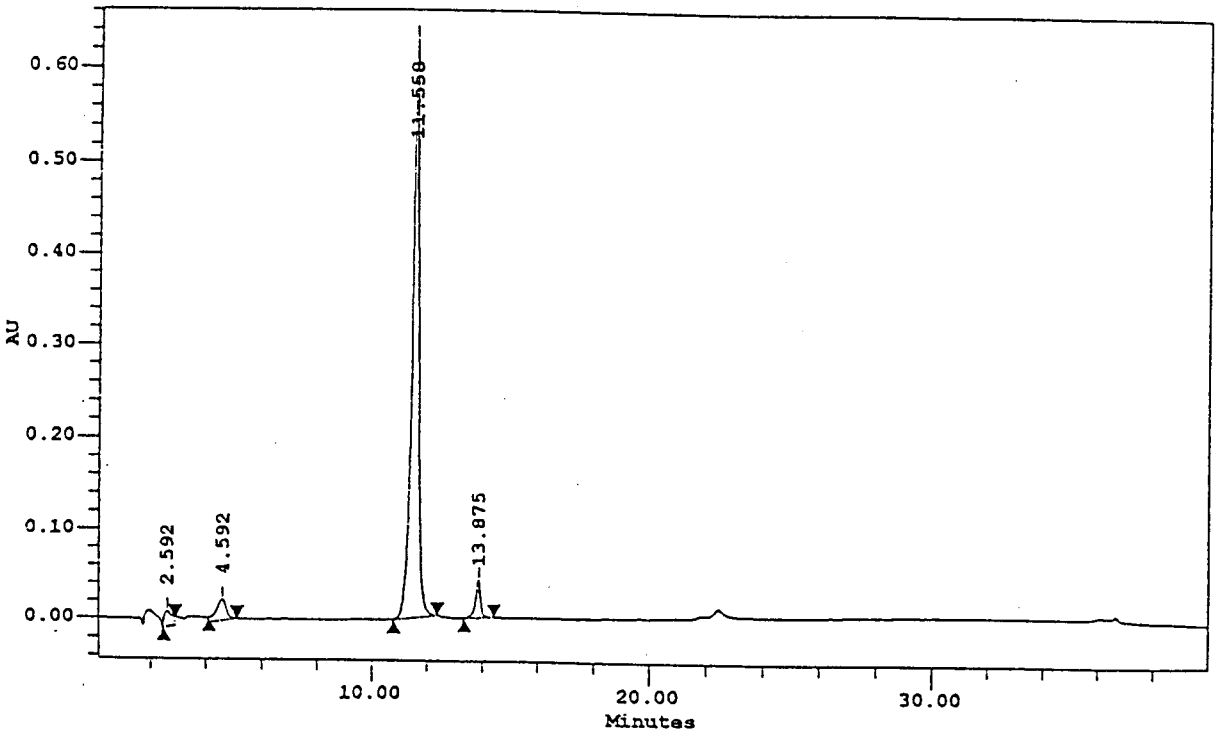
DMU 2651_C.I. 50420

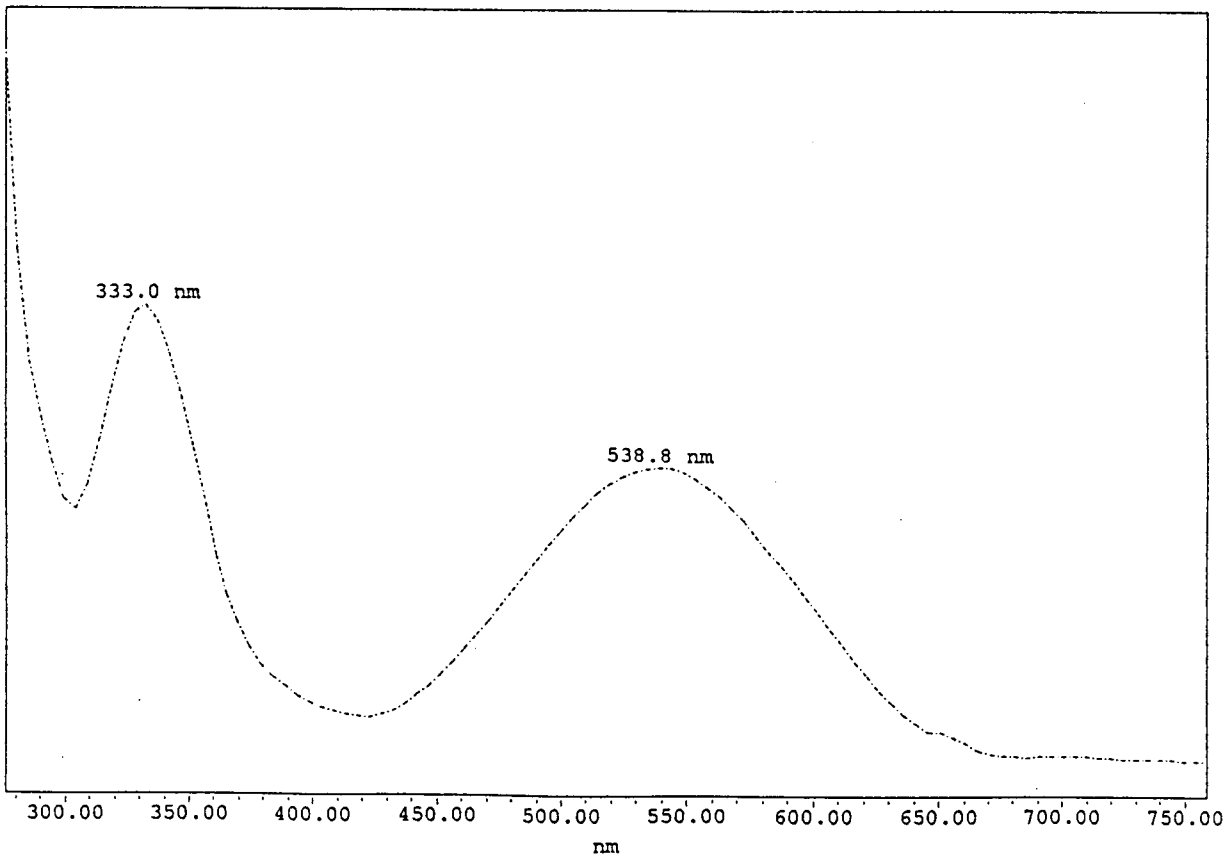




DMU 2651_CI 50420 16.562 minutes, 275 - 760 @ 4.8 nm, from 2651_50420

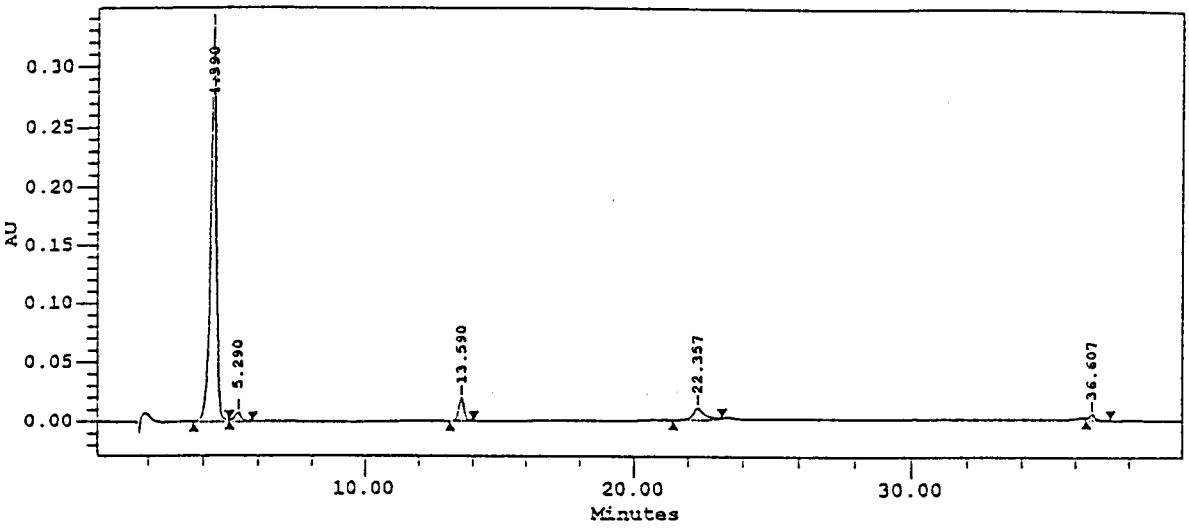
DMU 2633_C.I. 58000

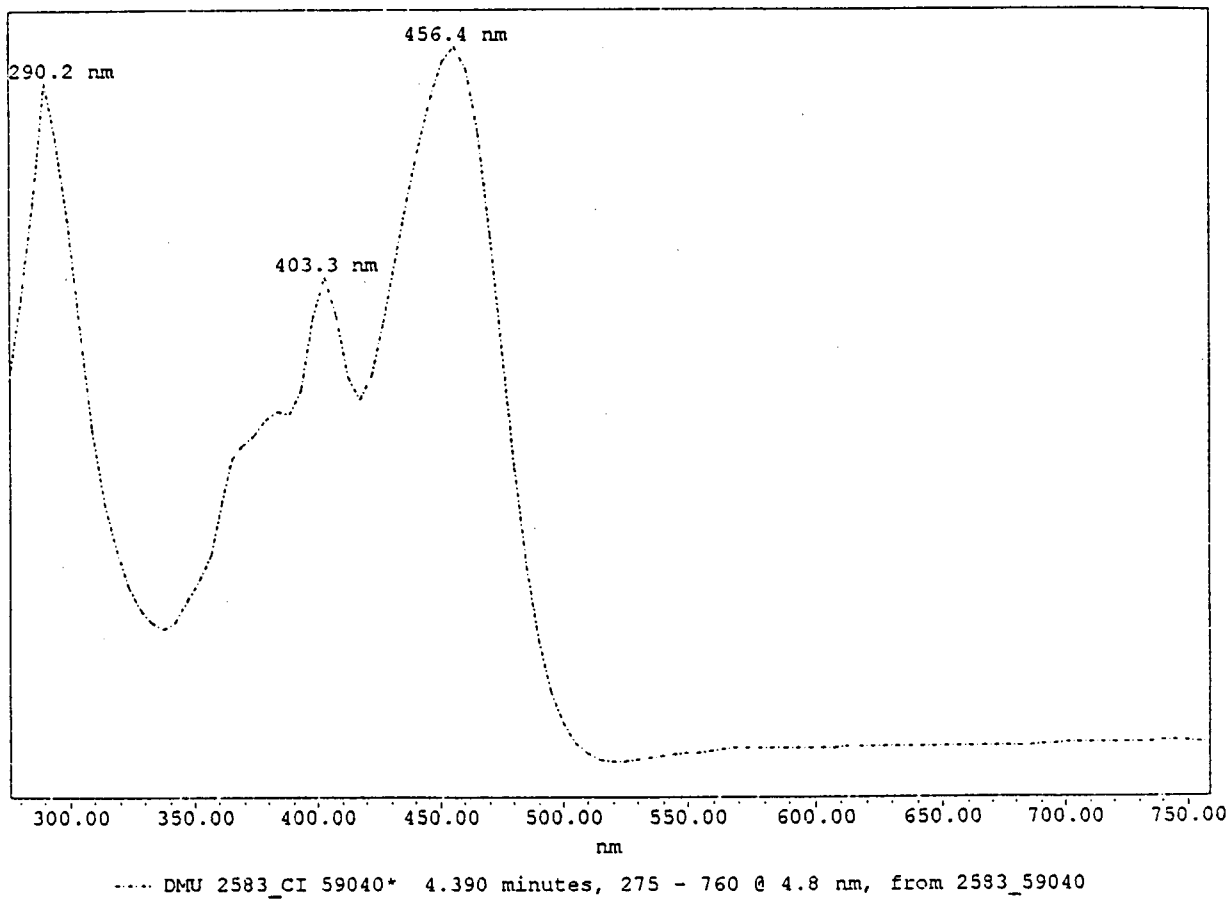




DMU 2633_CI 58000* 11.558 minutes, 275 - 760 @ 4.8 nm, from 2633_58000

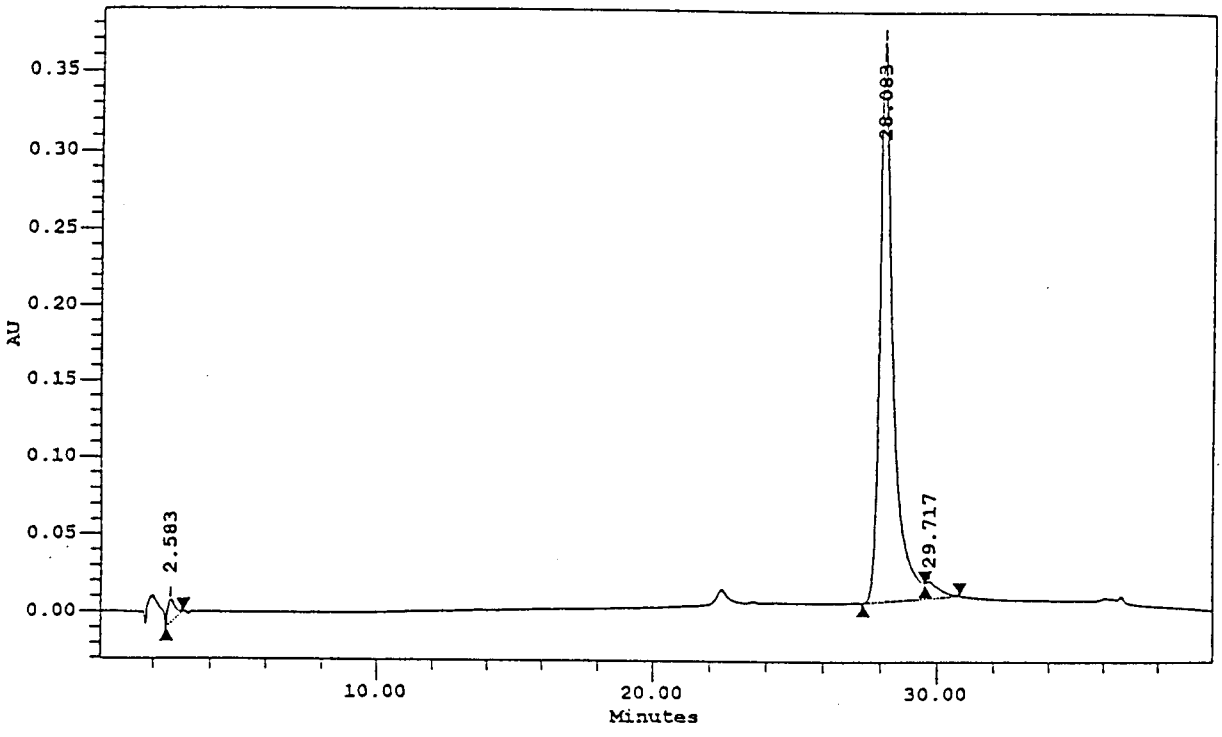
DMU 2583_C.I. 59040

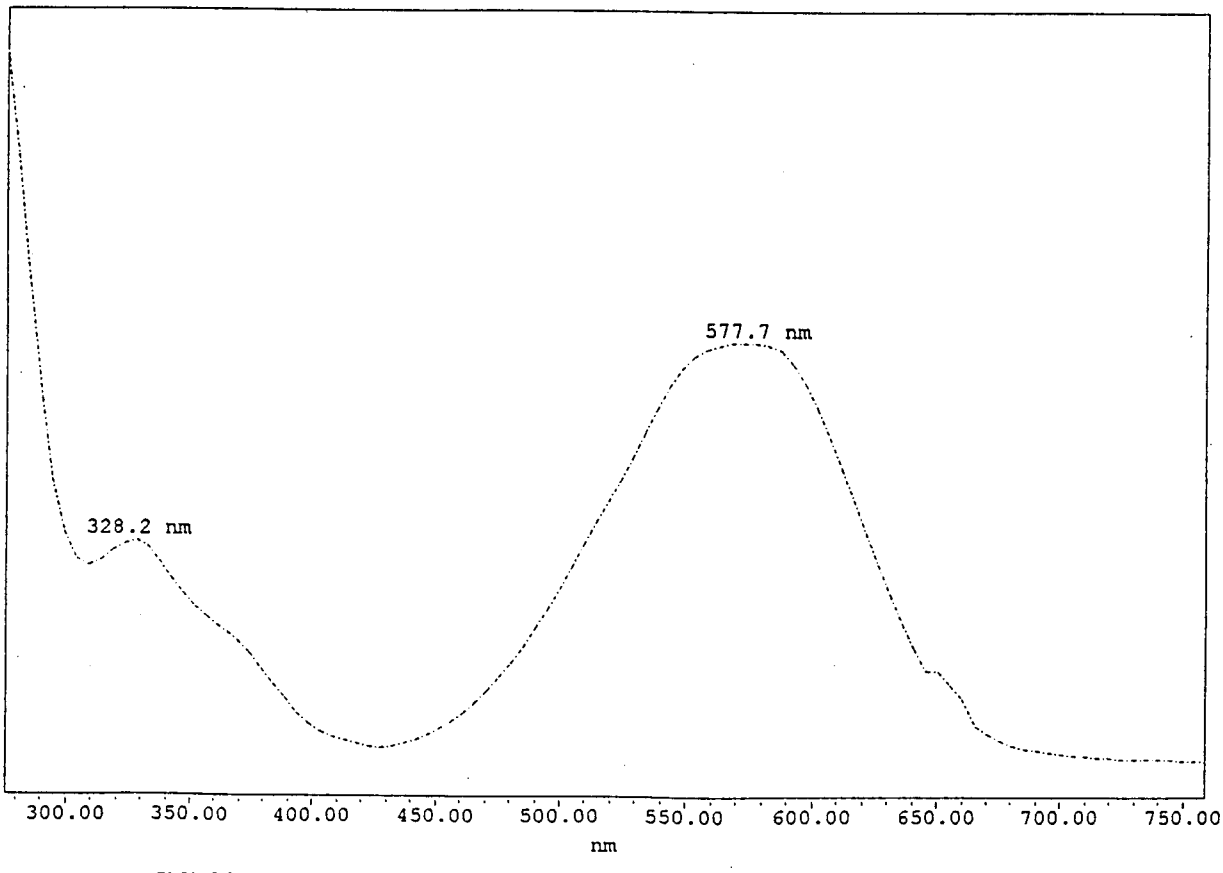




----- DMU 2583_CI 59040* 4.390 minutes, 275 - 760 @ 4.8 nm, from 2583_59040

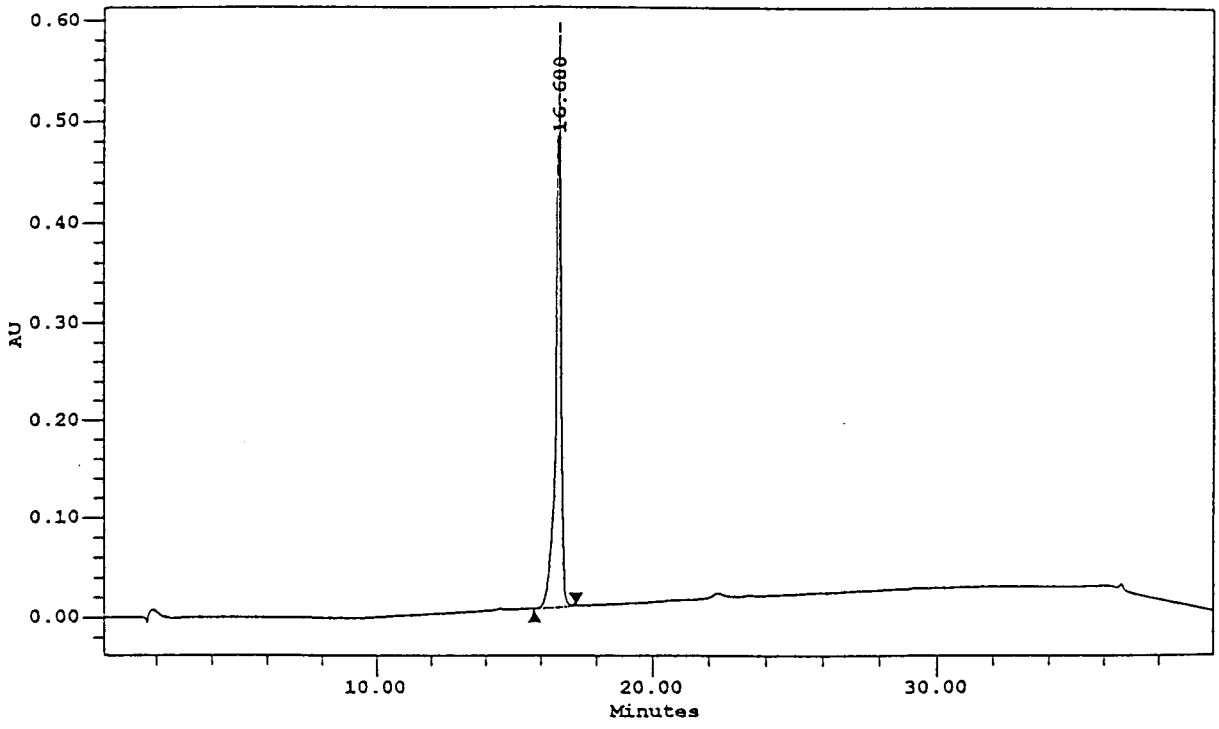
DMU 2663_C.I. 60725

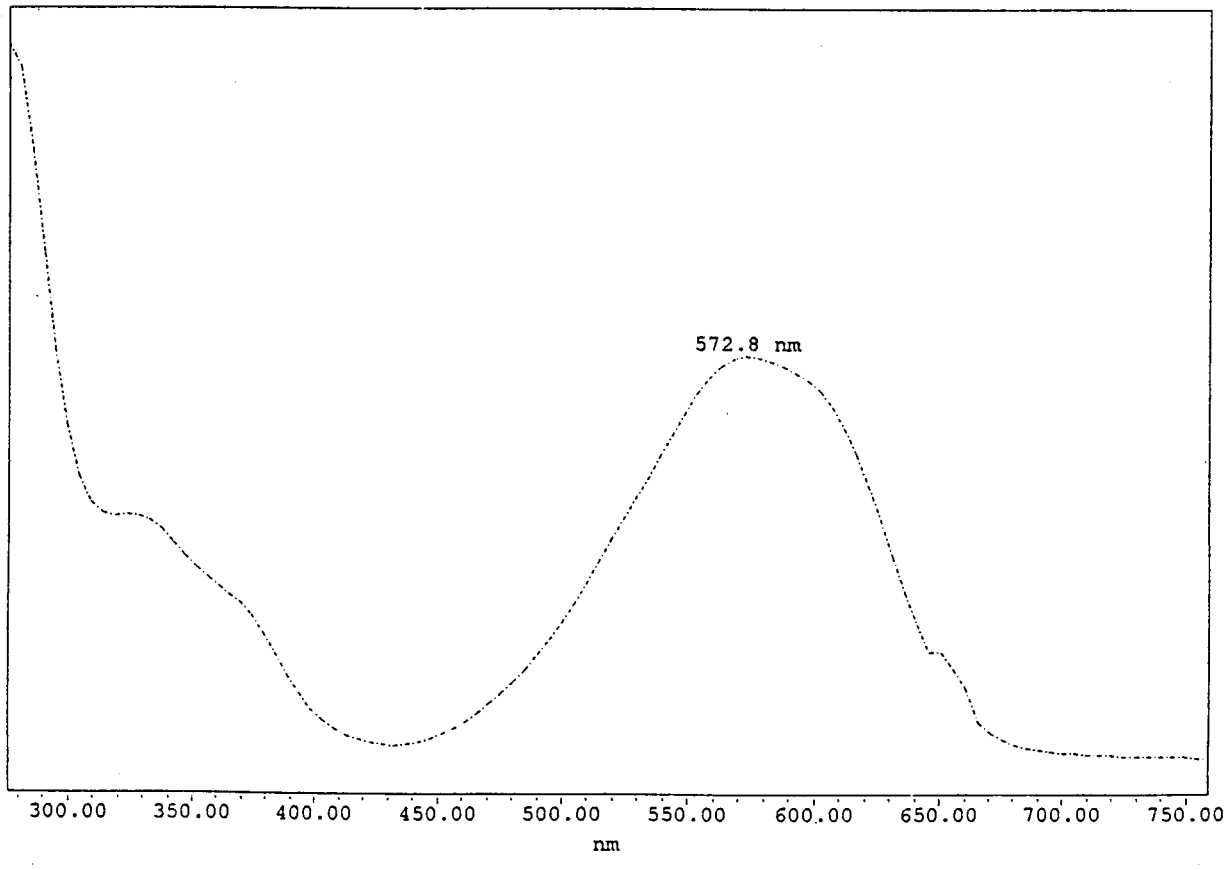




DMU 2663_CI 60725 28.083 minutes, 275 - 760 @ 4.8 nm, from 2663_60725

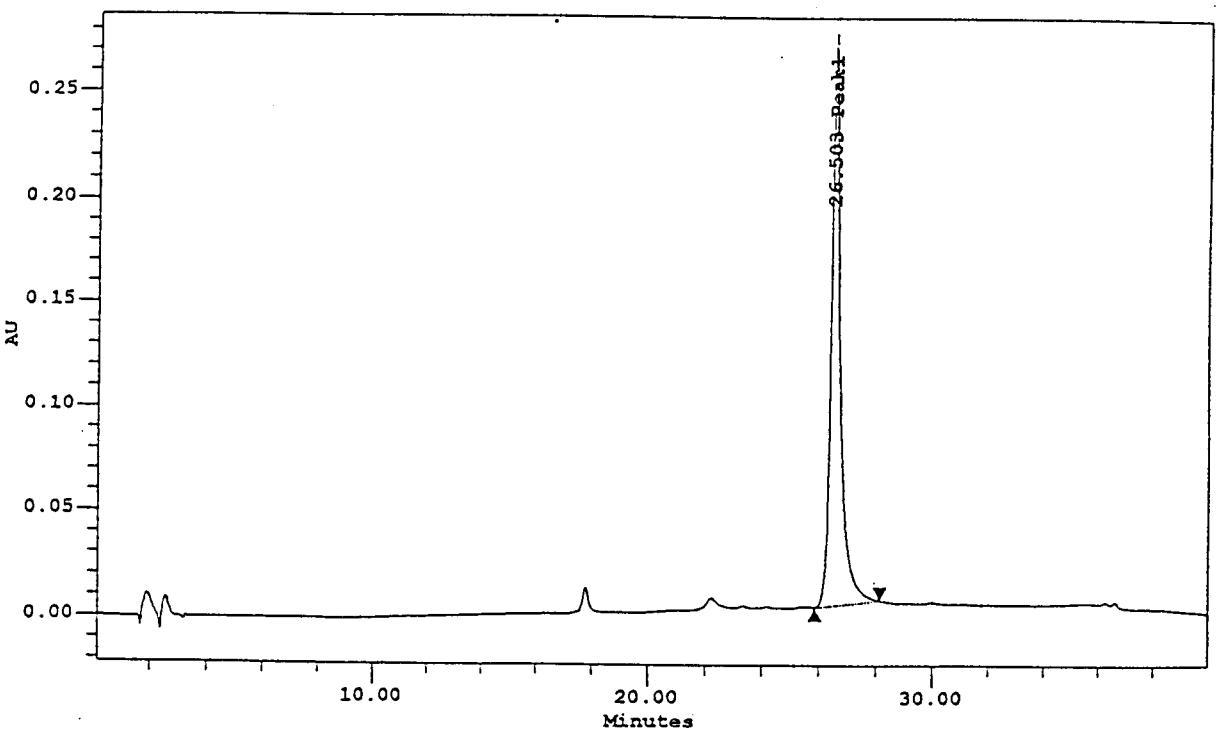
DMU 2668_C.I. 60730

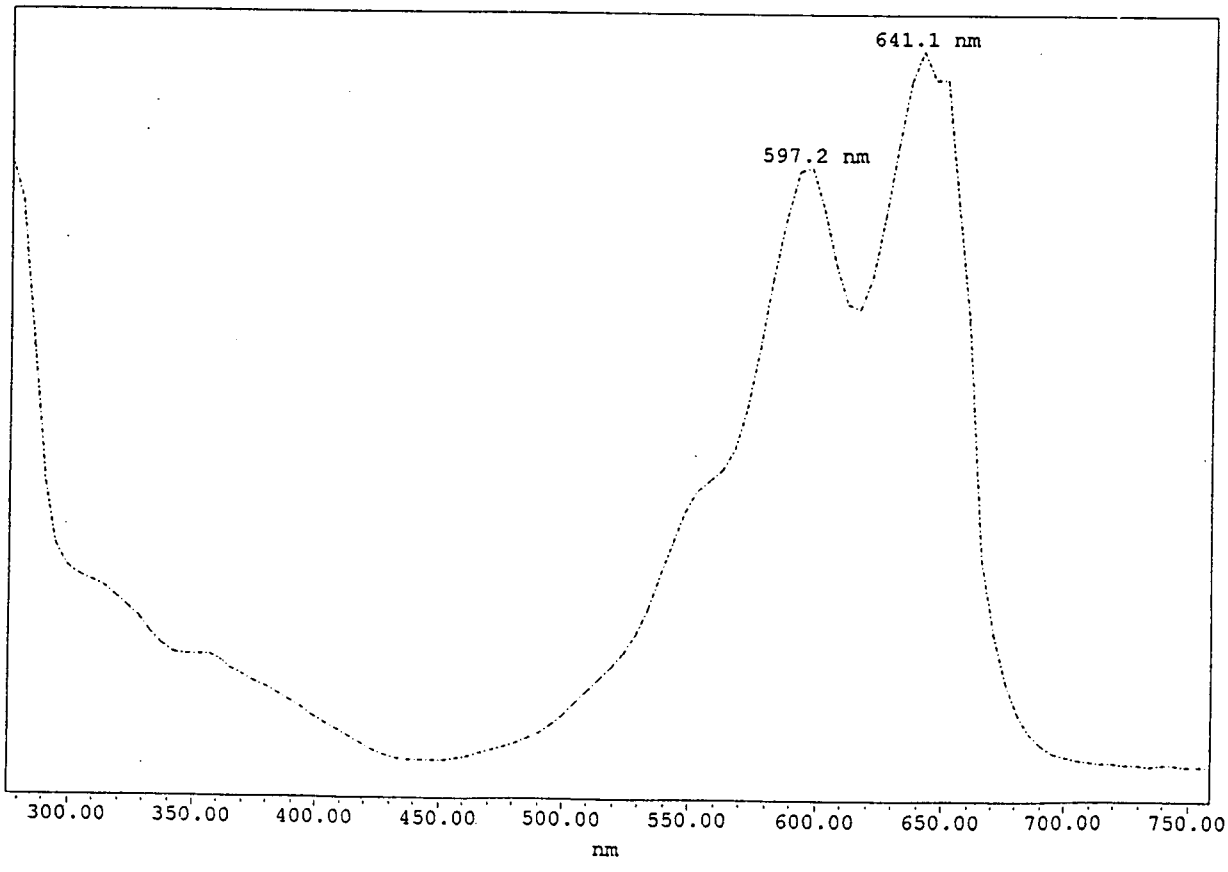




DMU 2668_CI 60730 16.680 minutes, 275 - 760 @ 4.8 nm, from 2668_60730

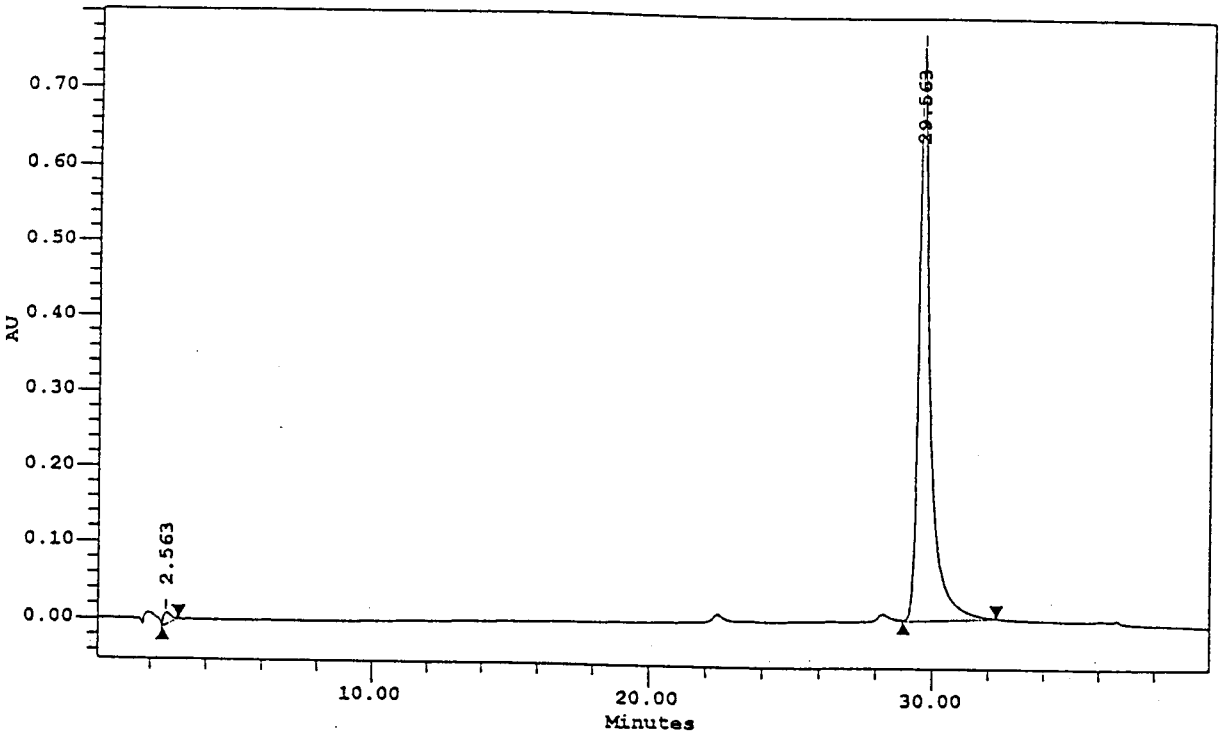
DMU 2696_C.I. 61554

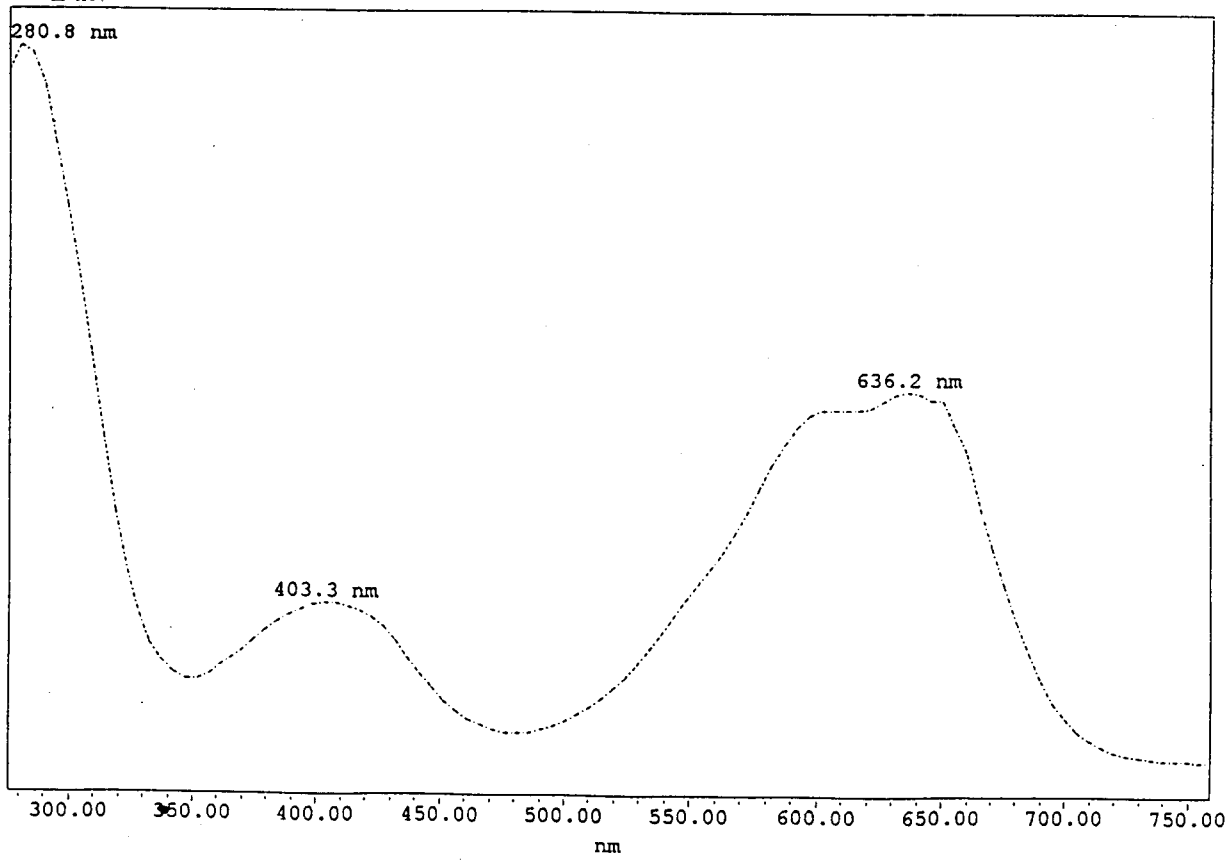




DMU 2696_CI 61554 26.503 minutes, 275 - 760 @ 4.8 nm, from 2696_61554

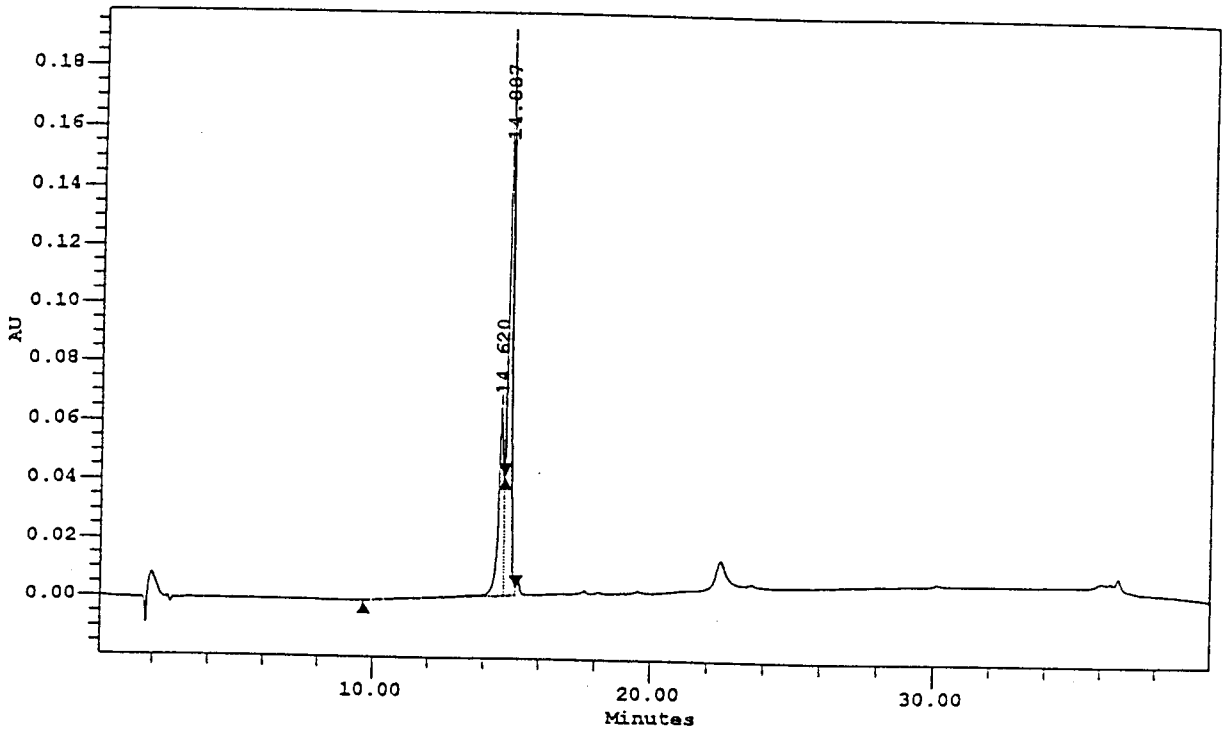
DMU 2650_C.I. 61565

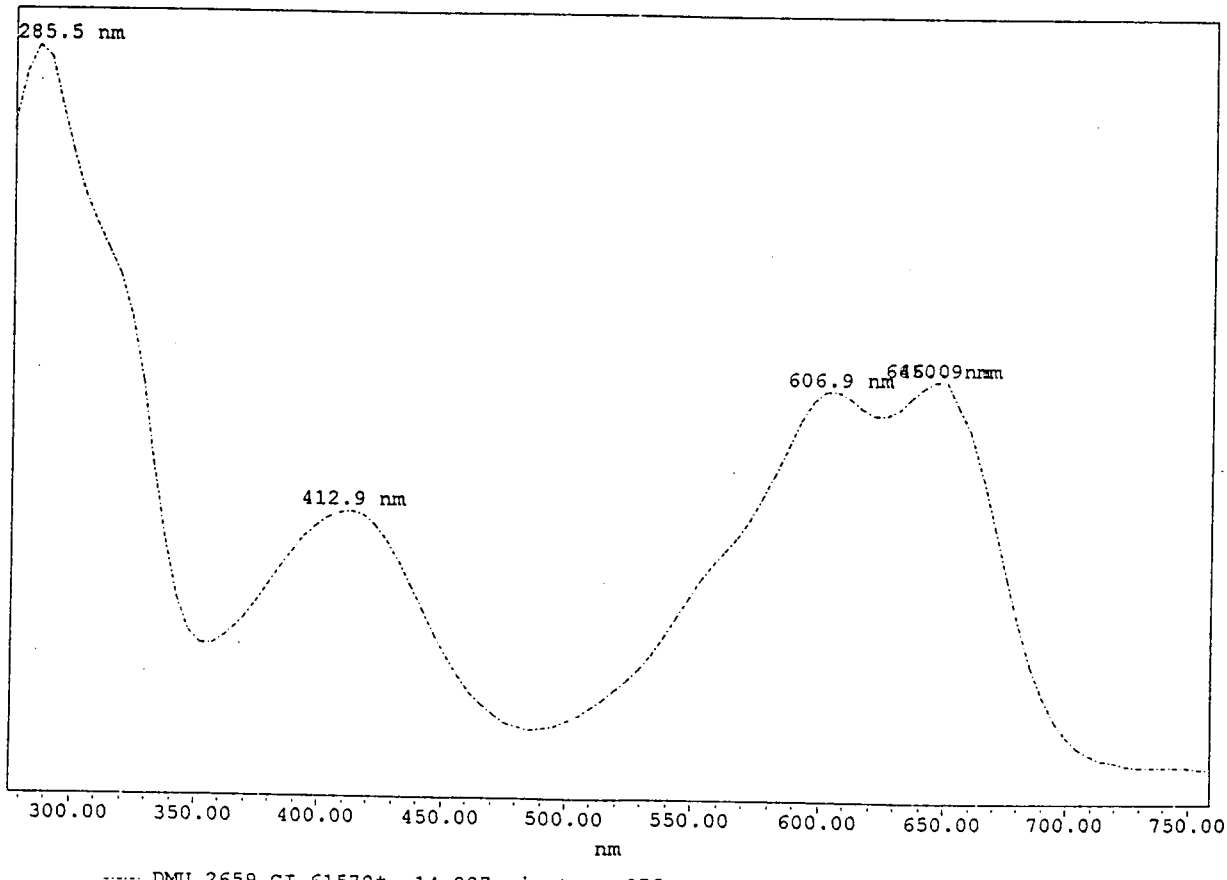




DMU 2650_CI 61565 29.563 minutes, 275 - 760 @ 4.8 nm, from 2650_61565

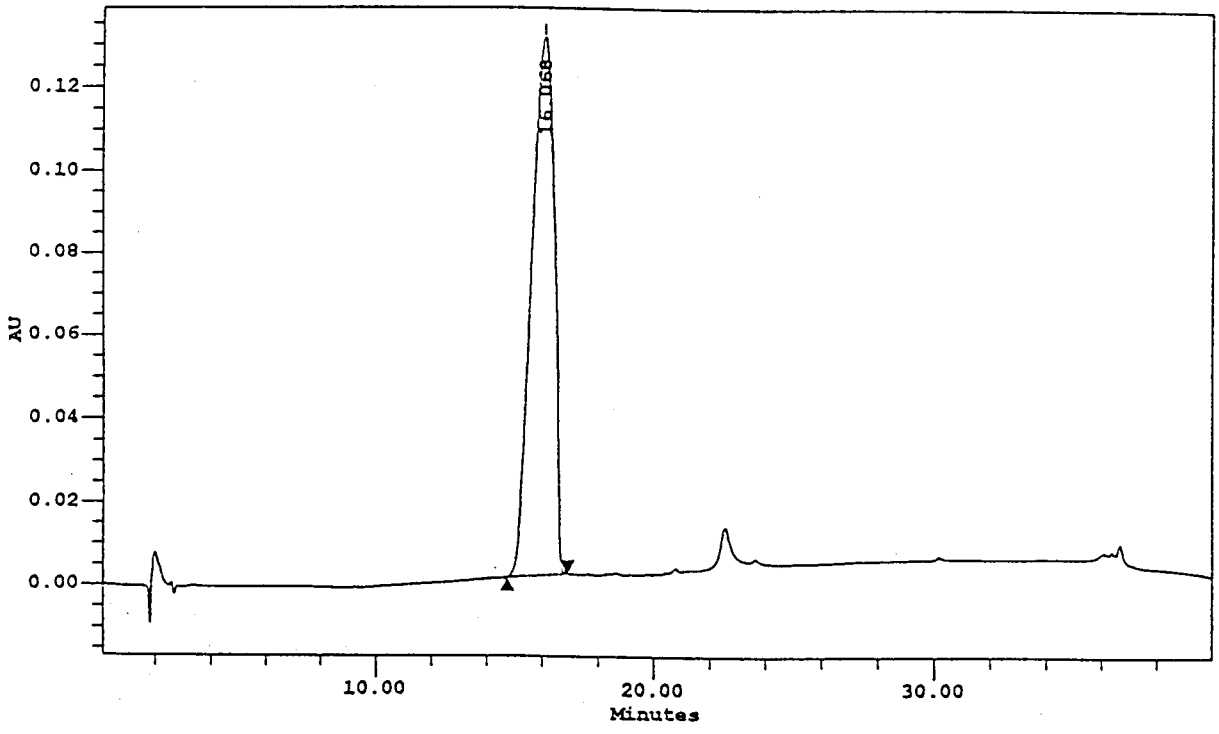
DMU 2659_C.I. 61570

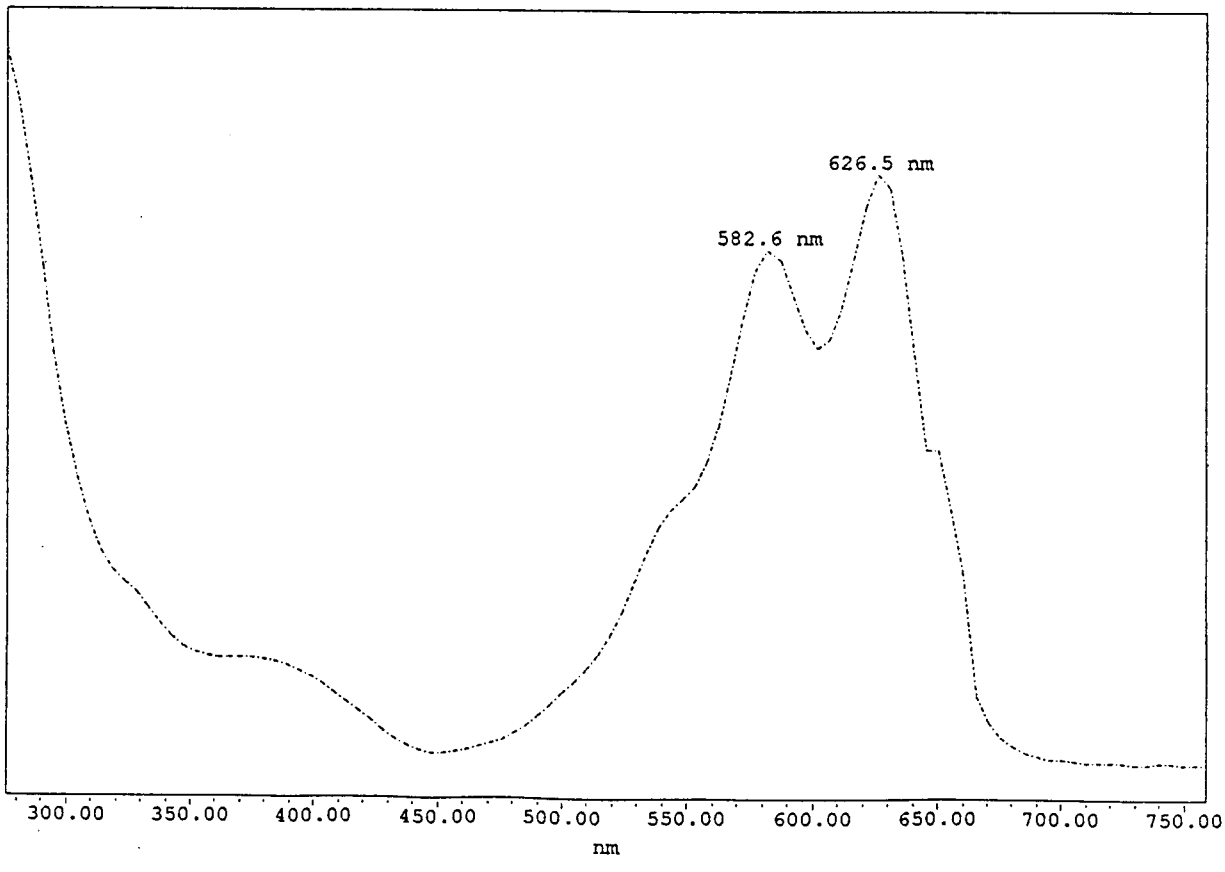




DMU 2659_CI 61570* 14.887 minutes, 275 ~ 760 @ 4.8 nm, from 2659_61570

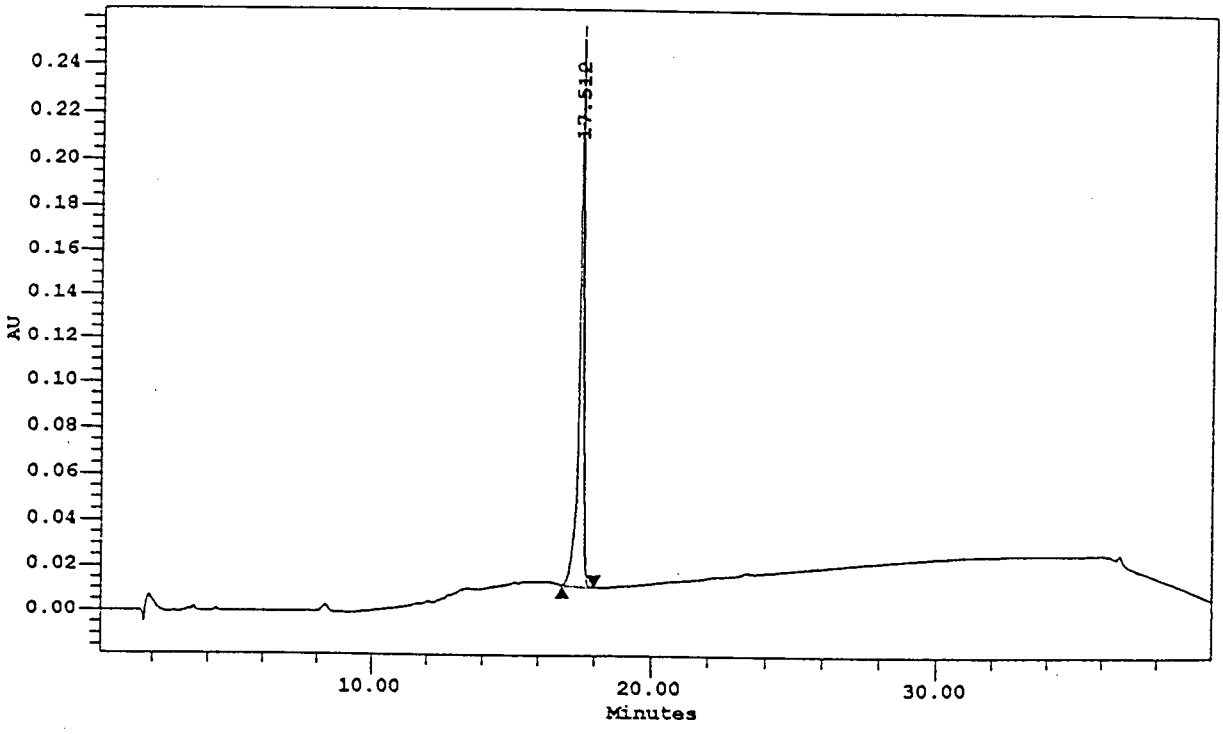
DMU 2647_C.I. 61585

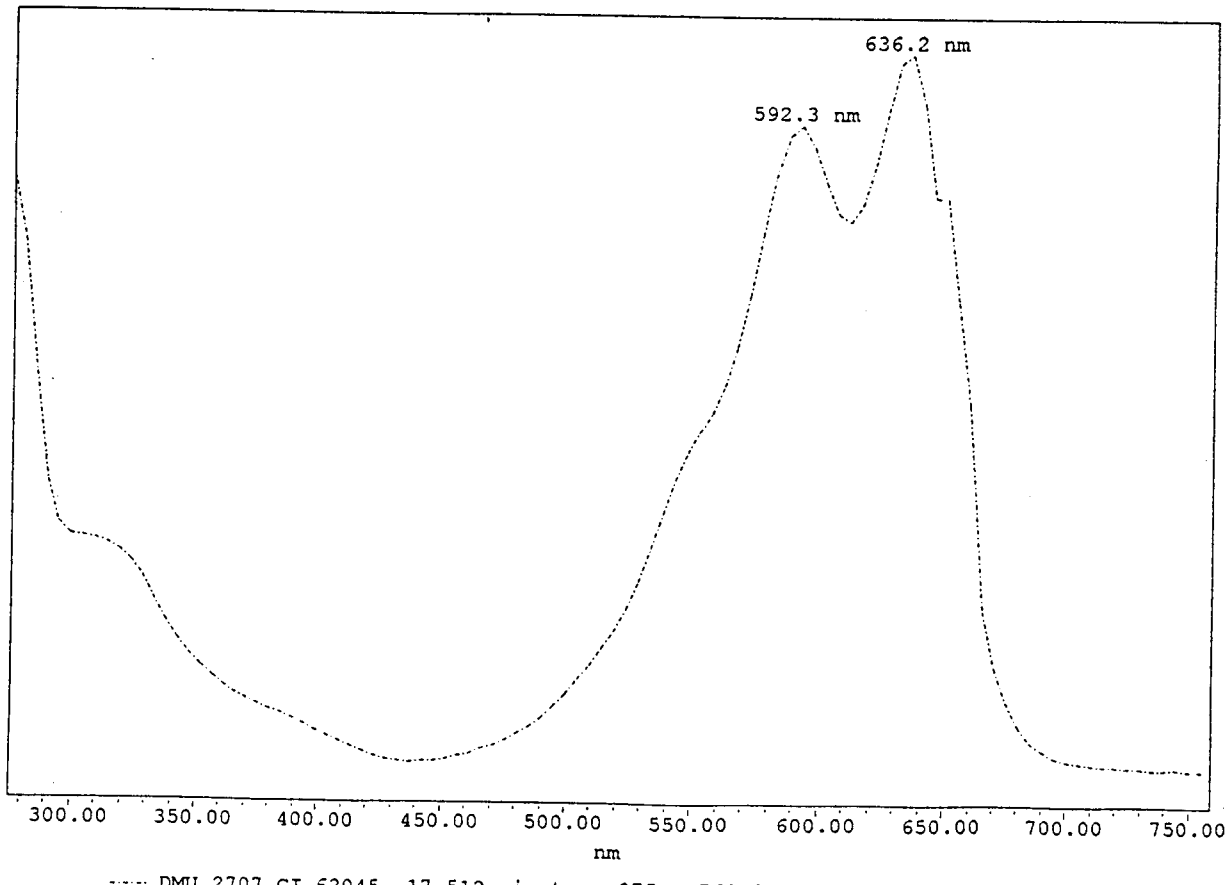




DMU 2647_CI 61585 16.068 minutes, 275 - 760 @ 4.8 nm, from 2647_61585

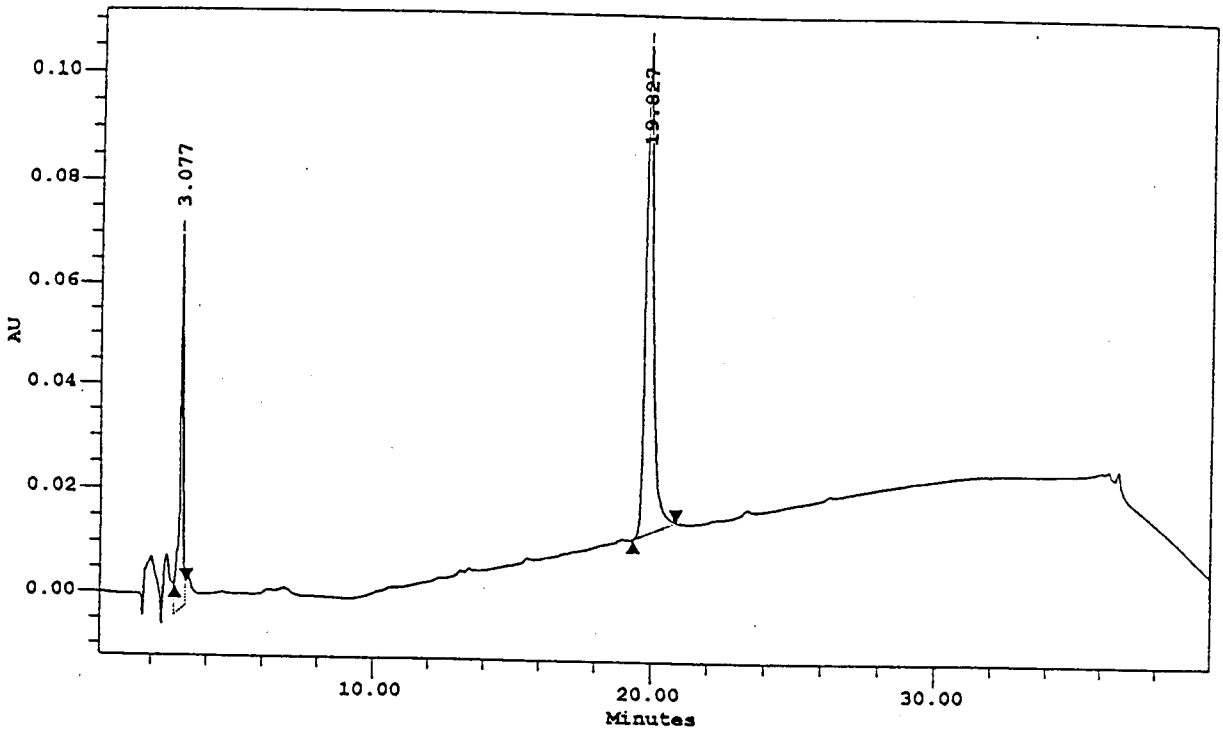
DMU 2707_C.I. 62045

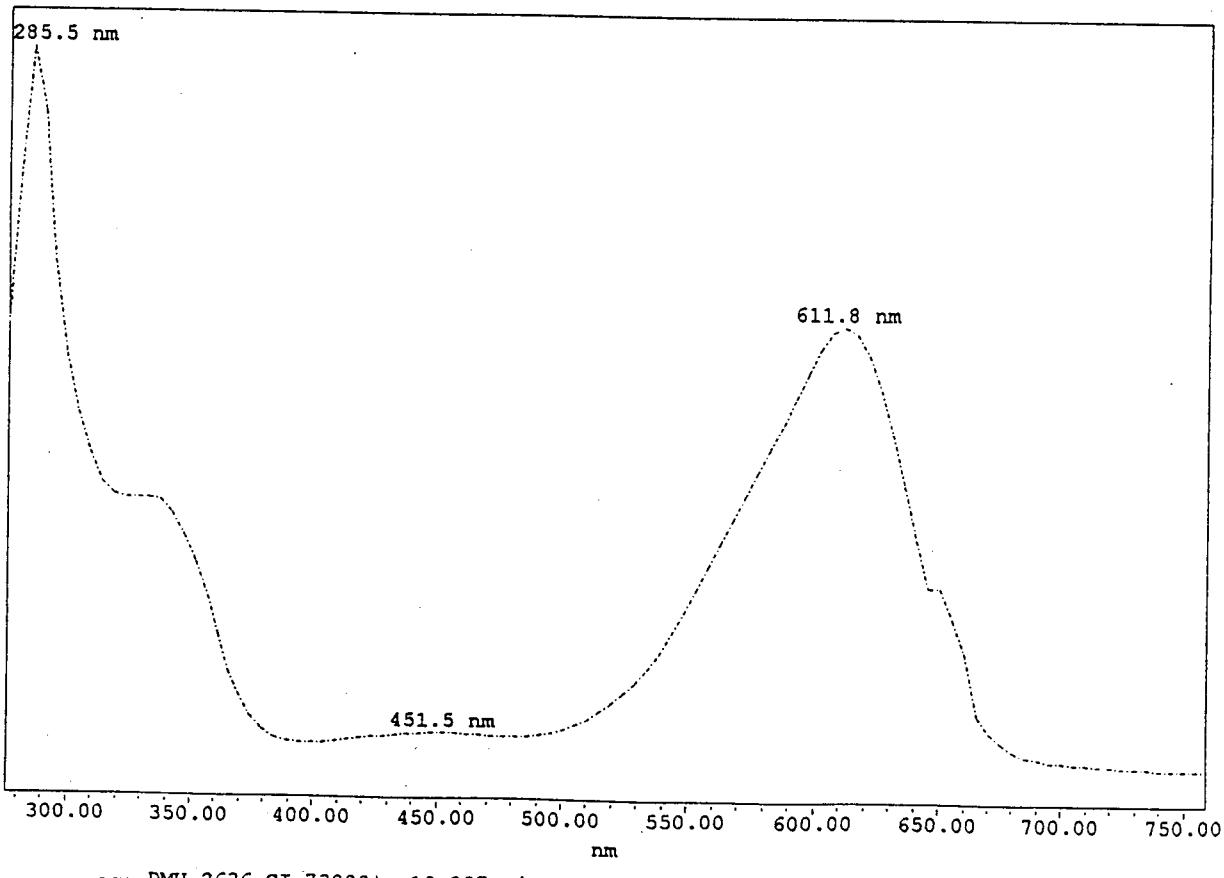




DMU 2707_CI 62045 17.512 minutes, 275 - 760 @ 4.8 nm, from 2707_62045

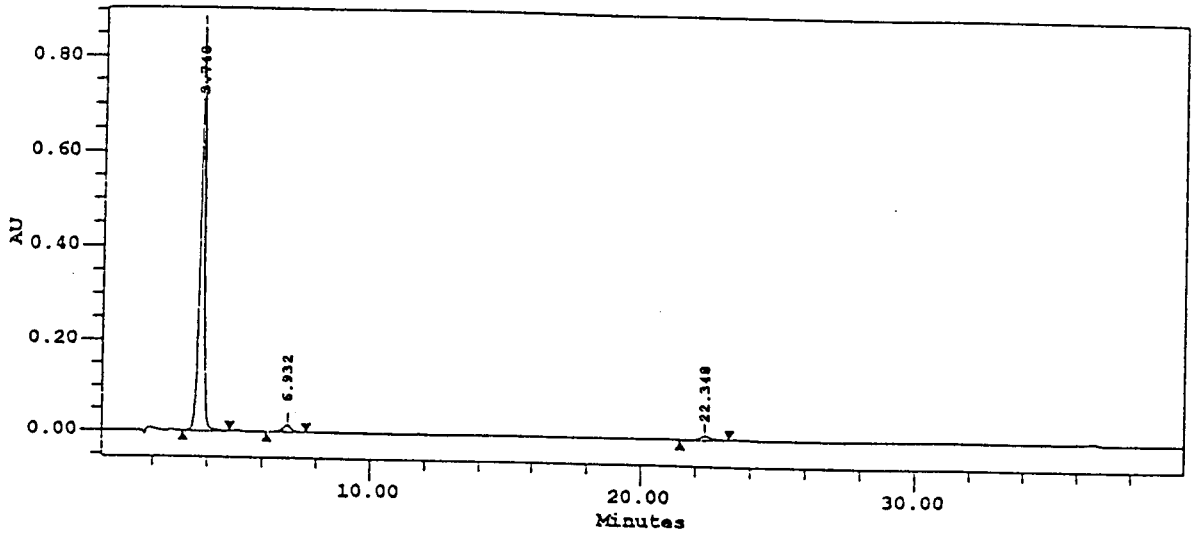
DMU 2636_C.I. 73000

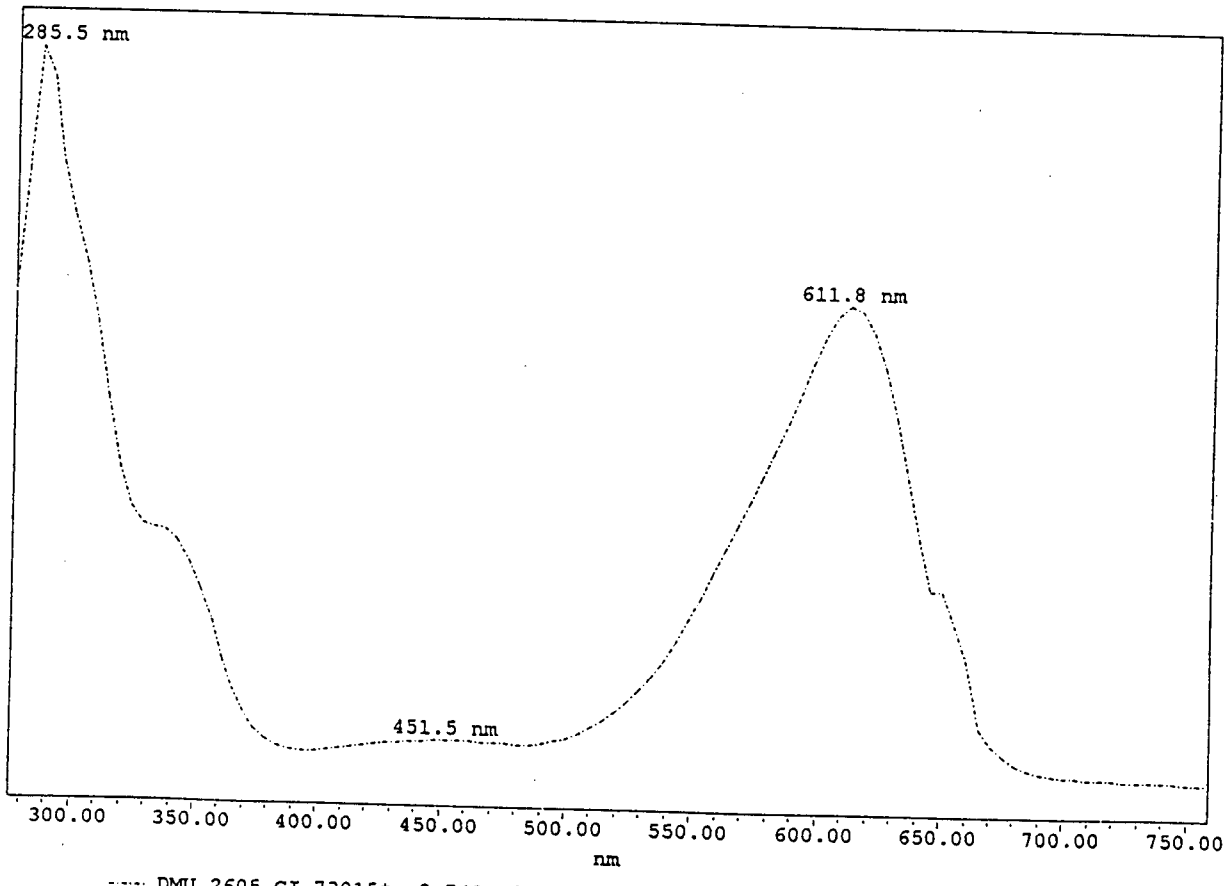




DMU 2636_CI 73000* 19.827 minutes, 275 - 760 @ 4.8 nm, from 2636_73000

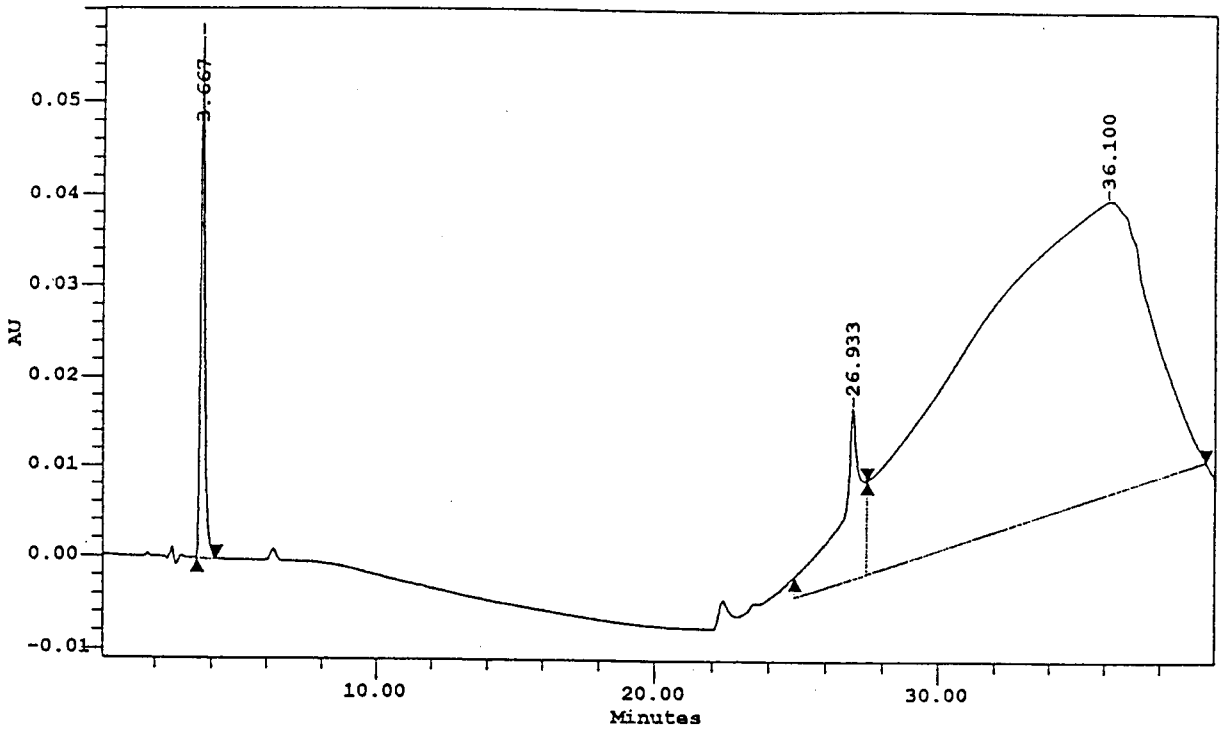
DMU 2605_C.I. 73015

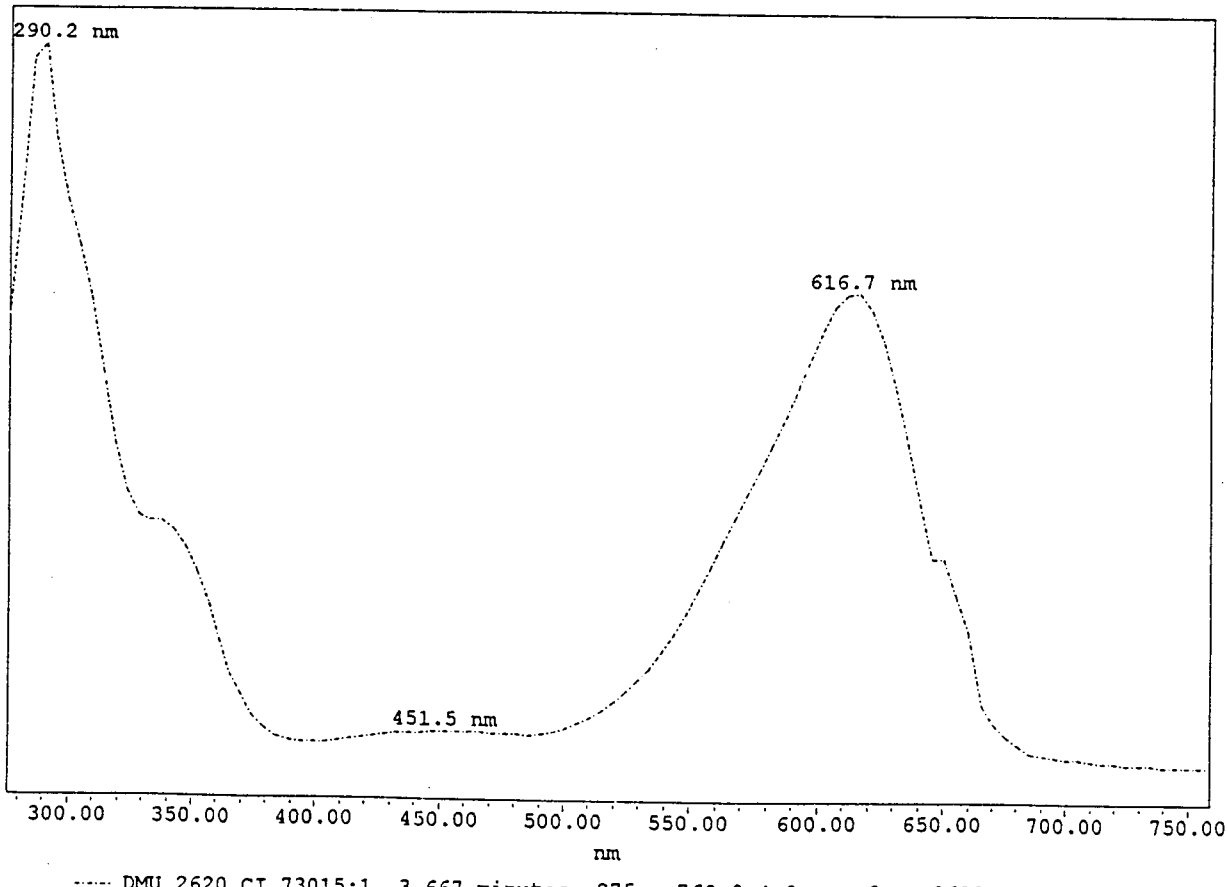




DMU 2605_CI 73015* 3.748 minutes, 275 - 760 @ 4.8 nm, from 2605_73015

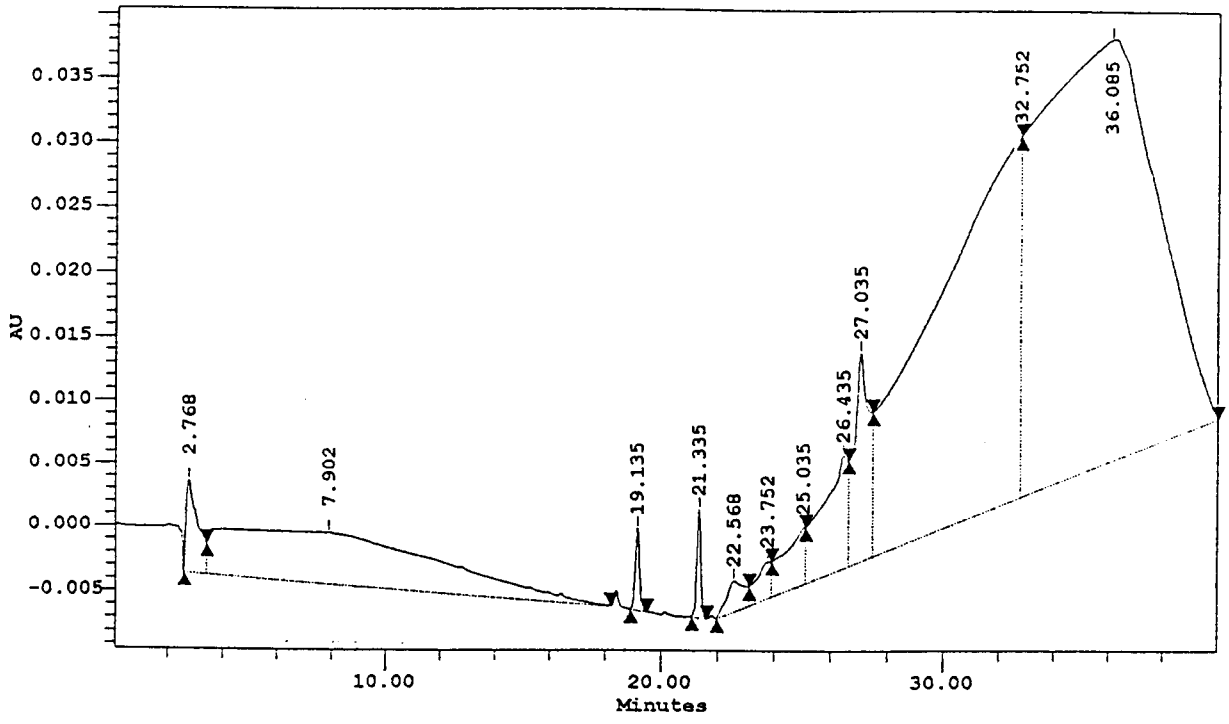
DMU 2620_C.I. 73015:1

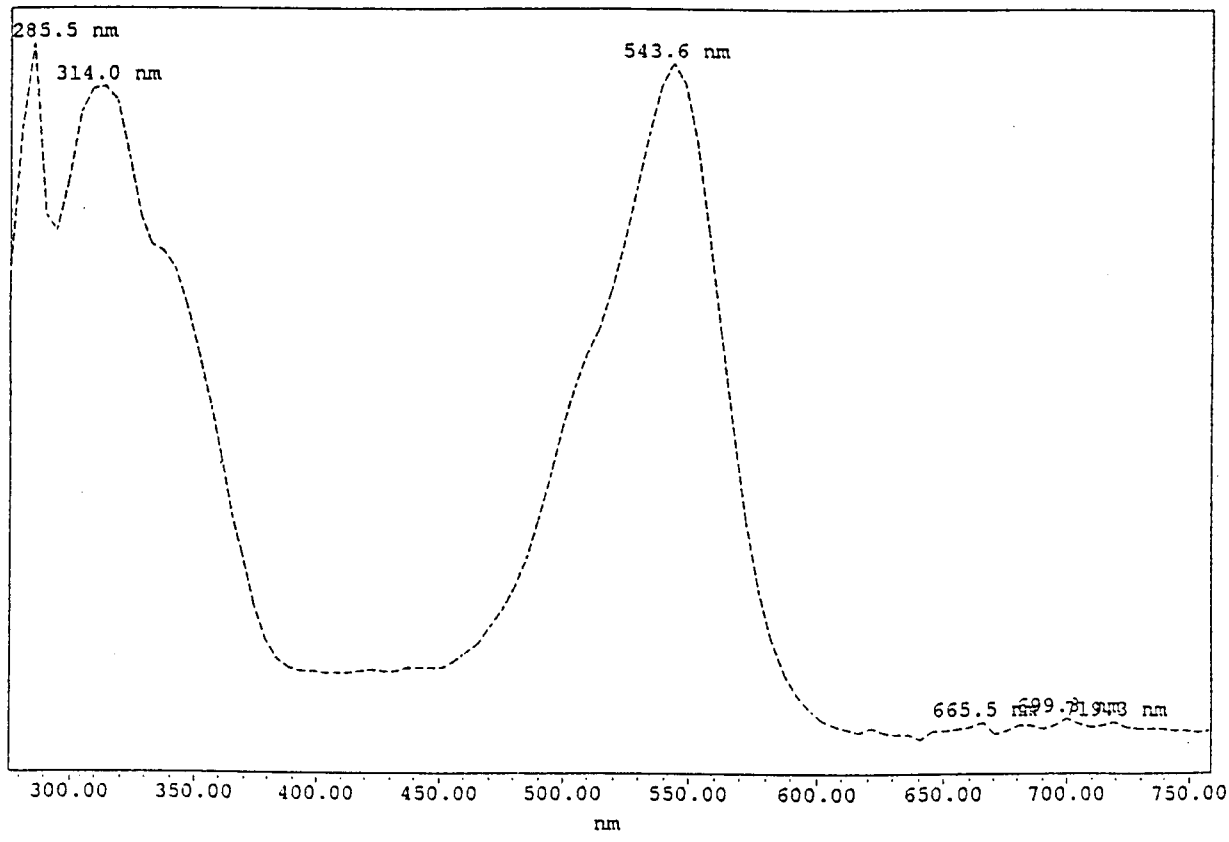




DMU 2620_CI 73015:1 3.667 minutes, 275 - 760 @ 4.8 nm, from 2620_CI73015:1

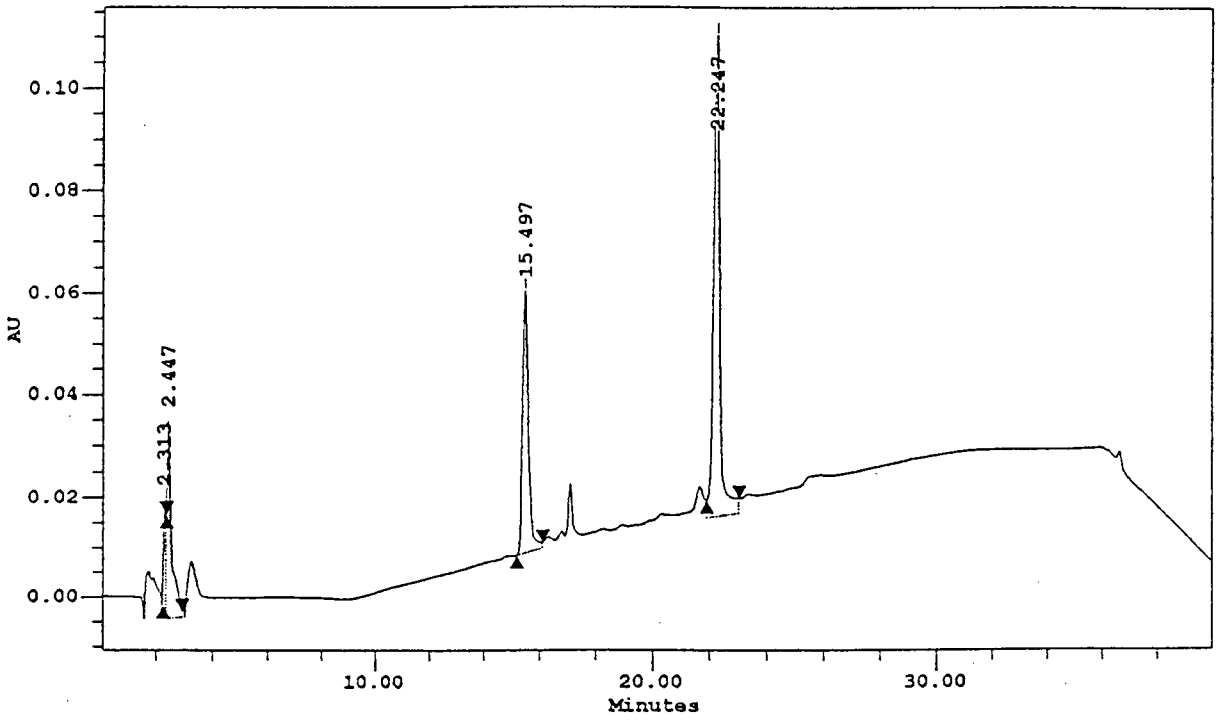
DMU 2661_C.I. 73360

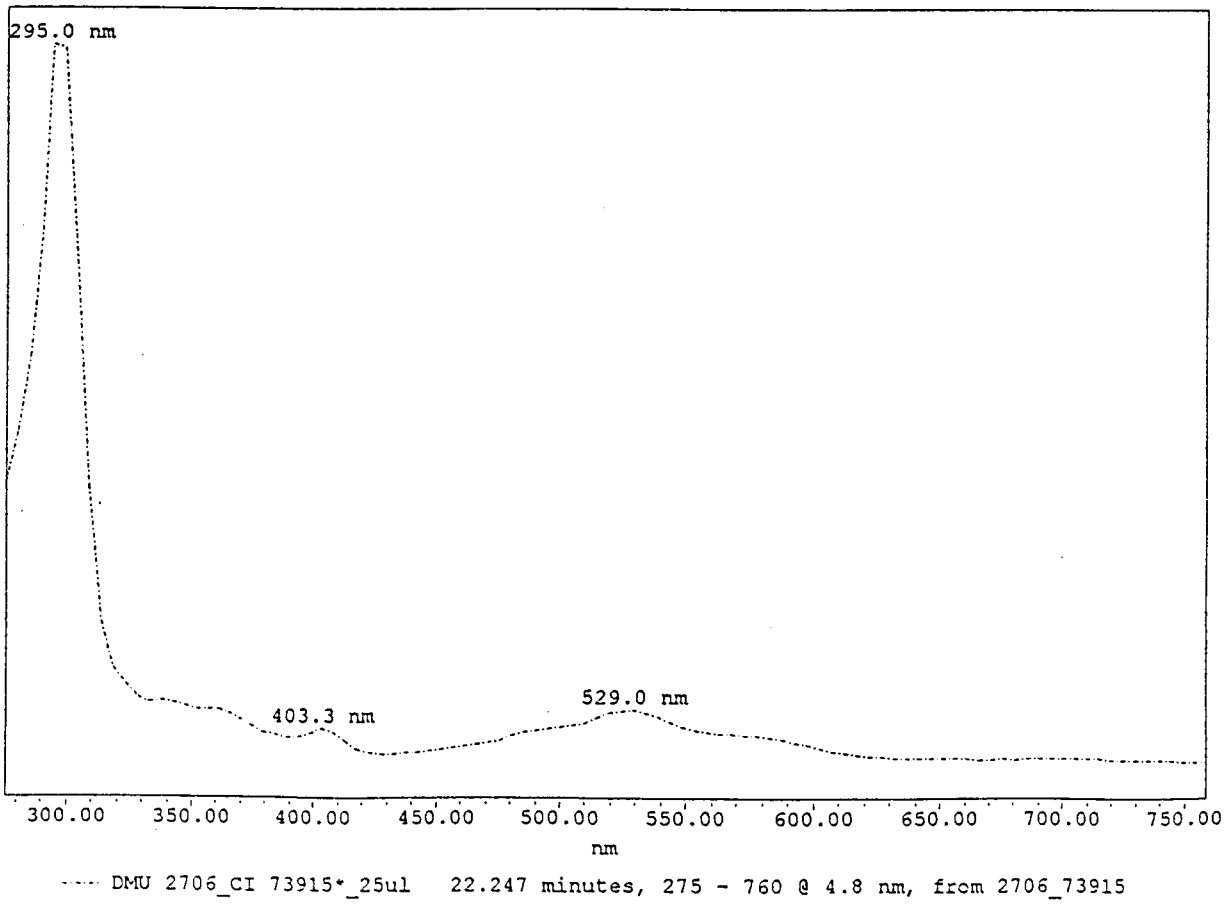




----- 19.135 minutes, 275 - 760 @ 4.8 nm, from 2661_73360 T/A
---- DMU 2661_CI73360 19.135 minutes, 275 - 760 @ 4.8 nm, from 2661_73360 T/A

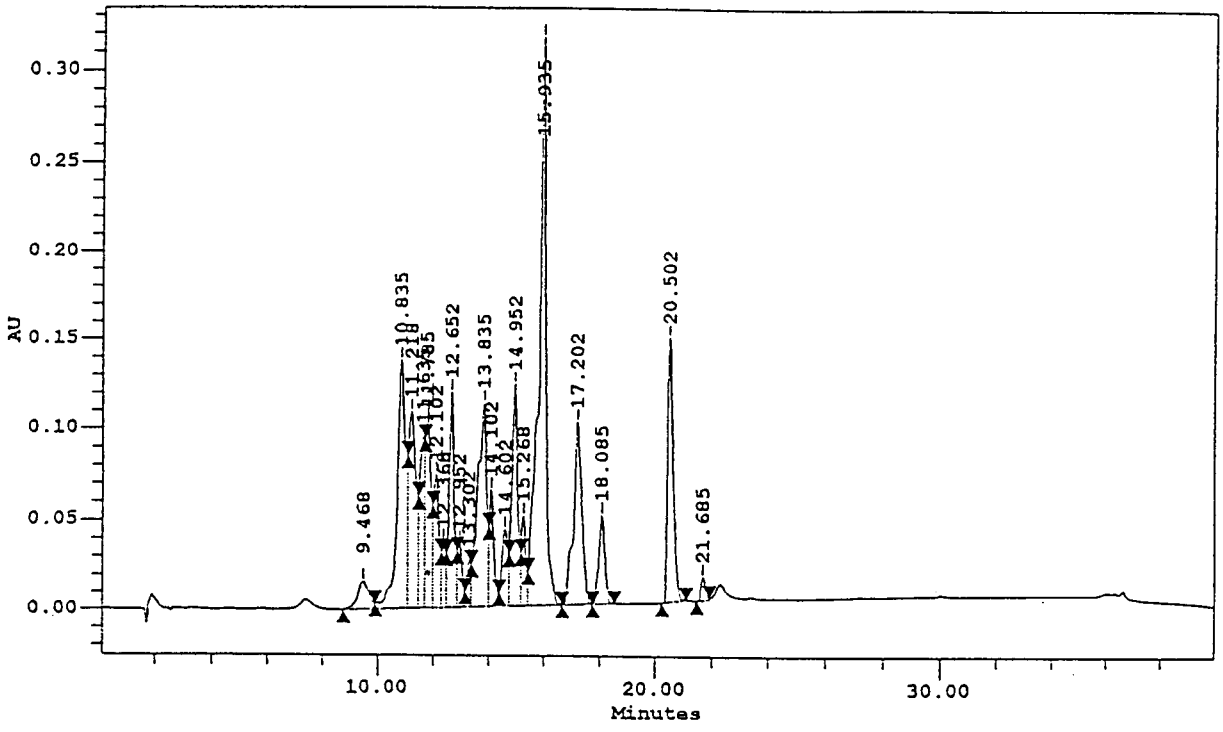
DMU 2706_C.I. 73915

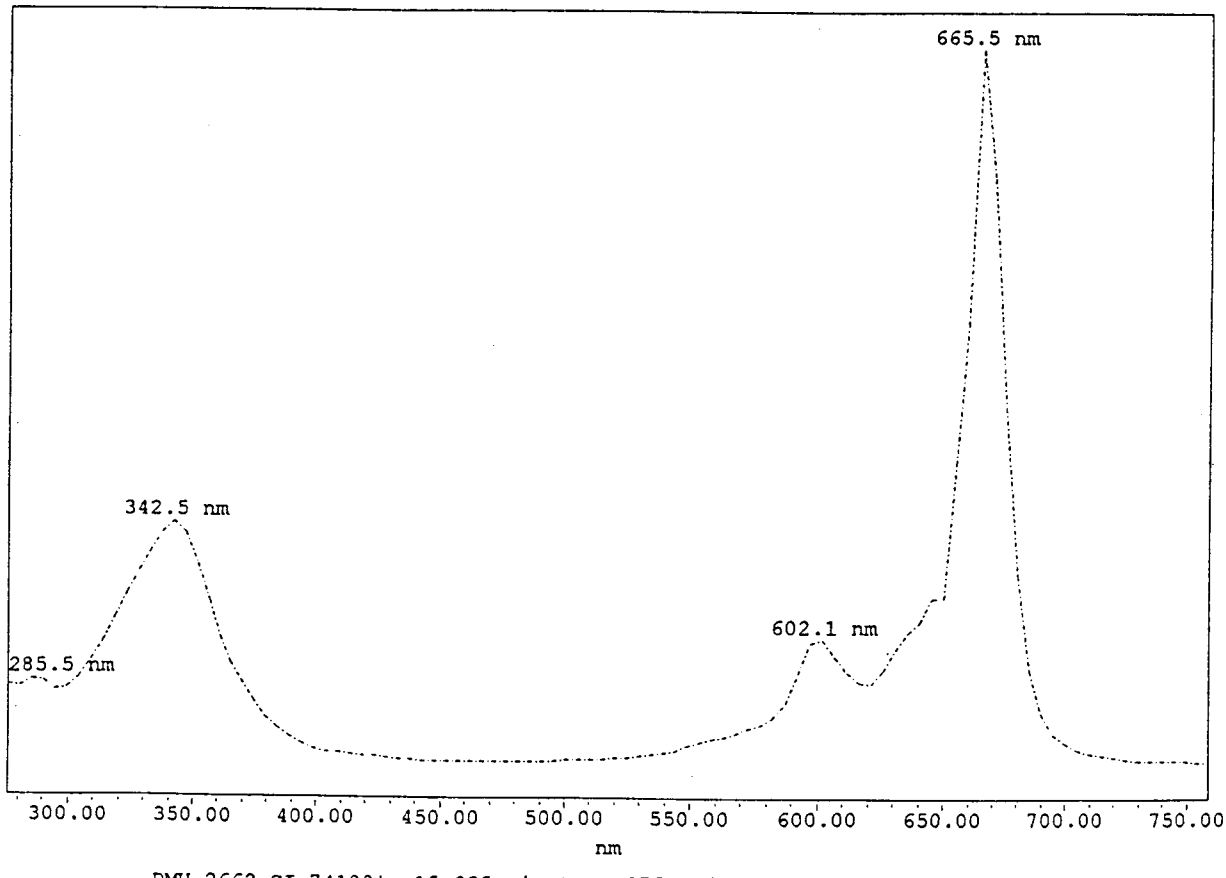




DMU 2706_CI 73915*_25ul 22.247 minutes, 275 - 760 @ 4.8 nm, from 2706_73915

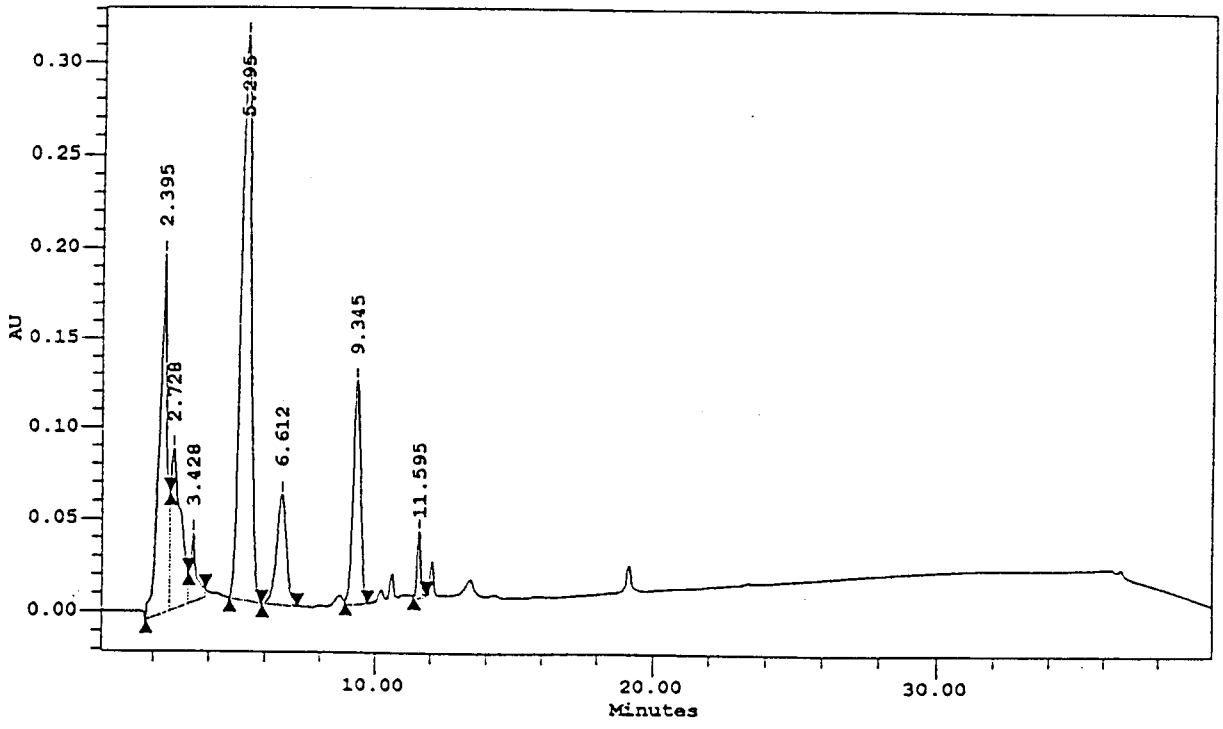
DMU 2662_C.I. 74180

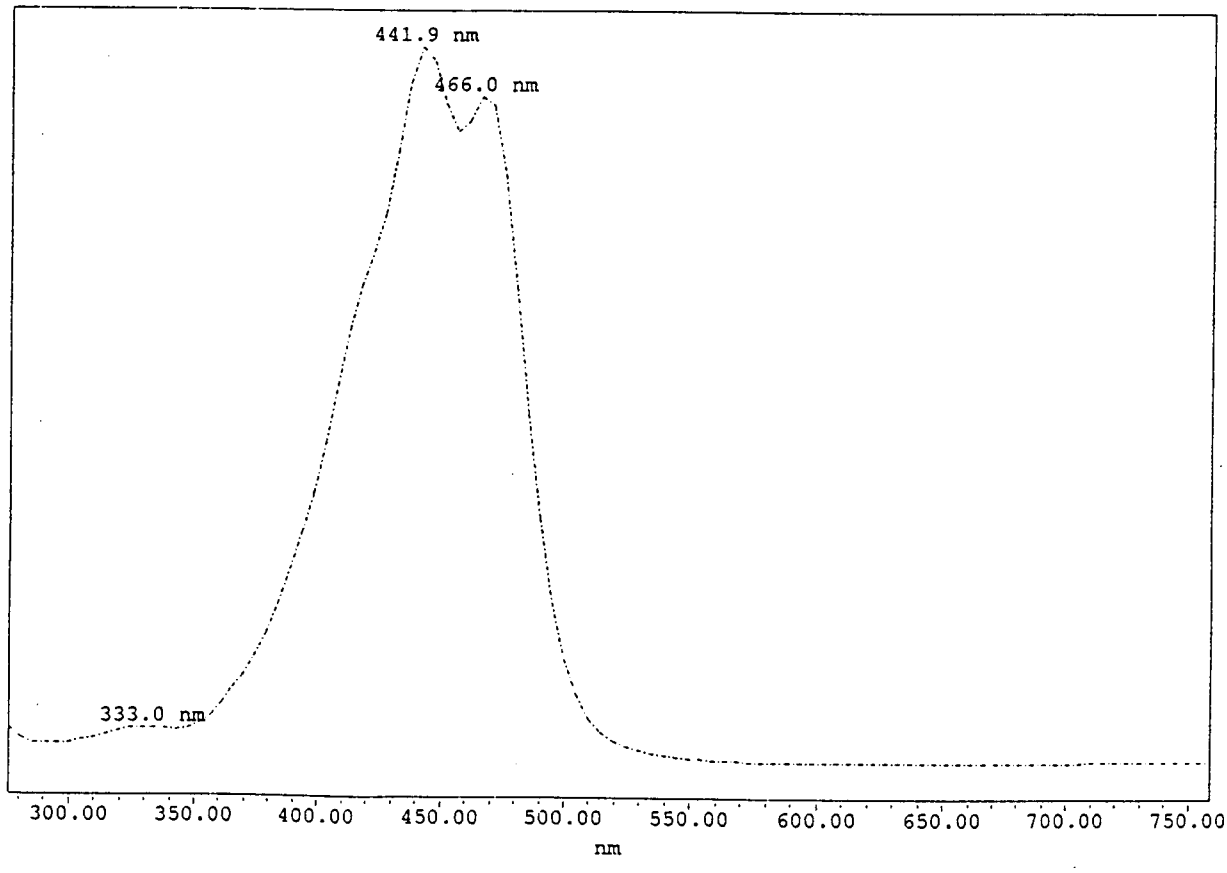




DMU 2662_CI 74180* 15.935 minutes, 275 - 760 @ 4.8 nm, from 2662_74180

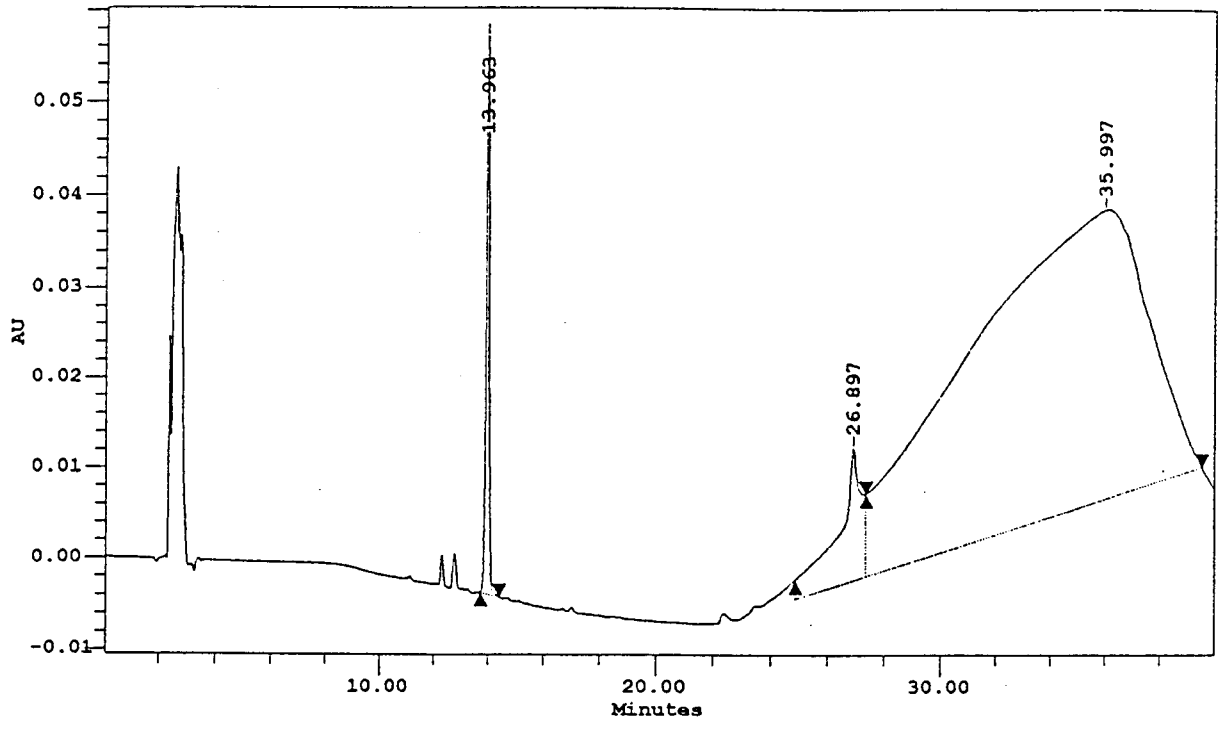
DMU 2678_C.I. 75100

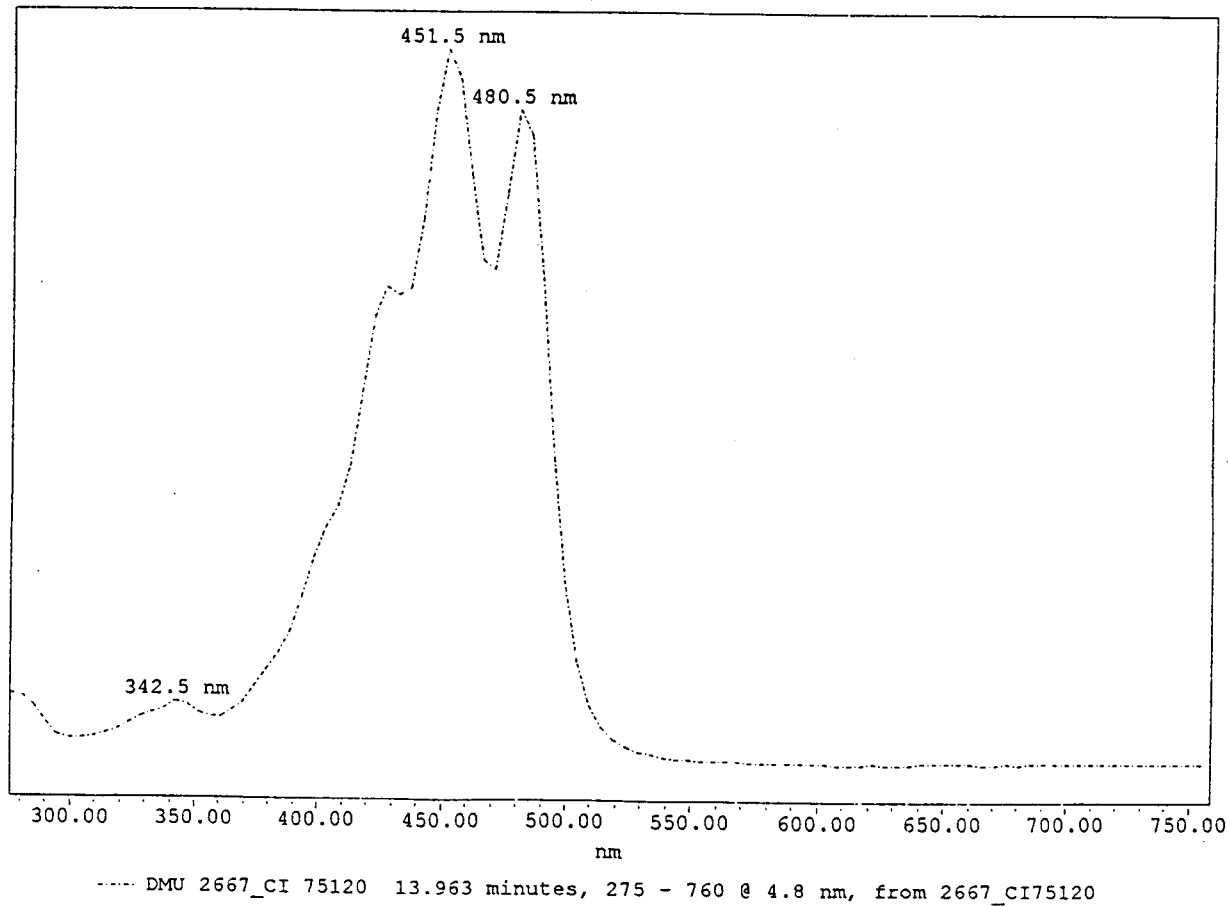




DMU 2678_CI 75100* 5.295 minutes, 275 - 760 @ 4.8 nm, from 2678_75100

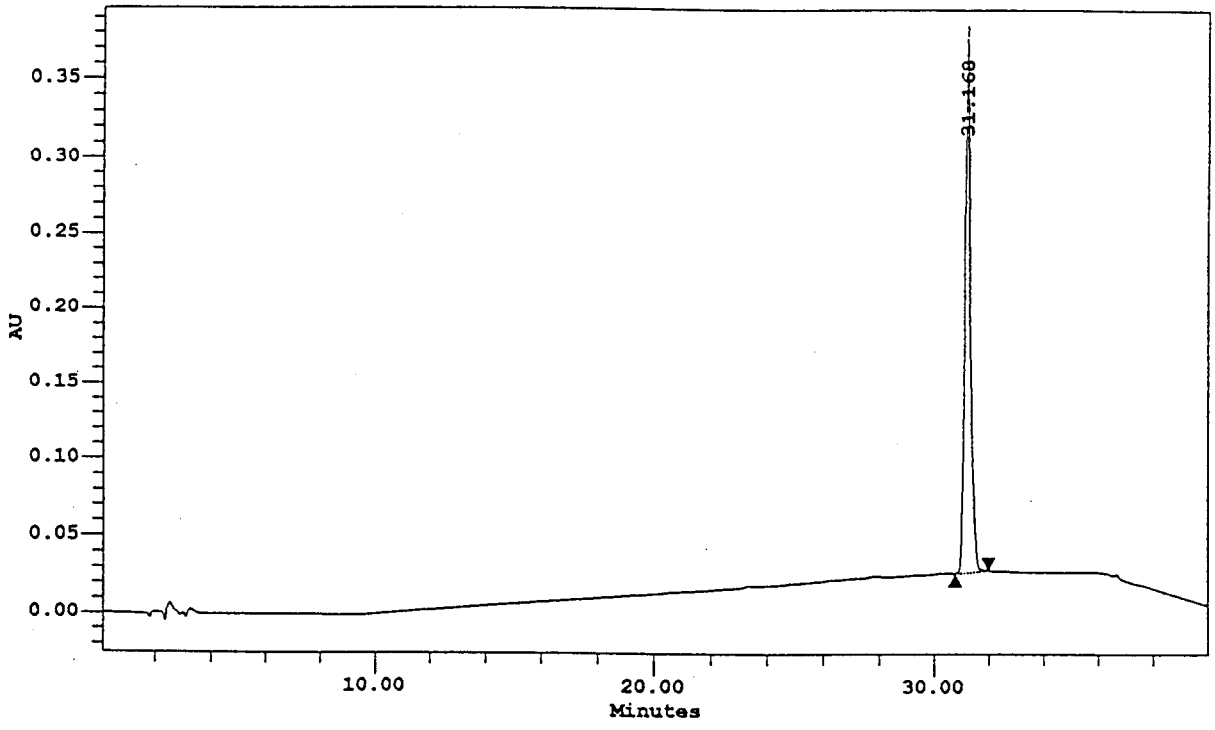
DMU 2667_C.I. 75120

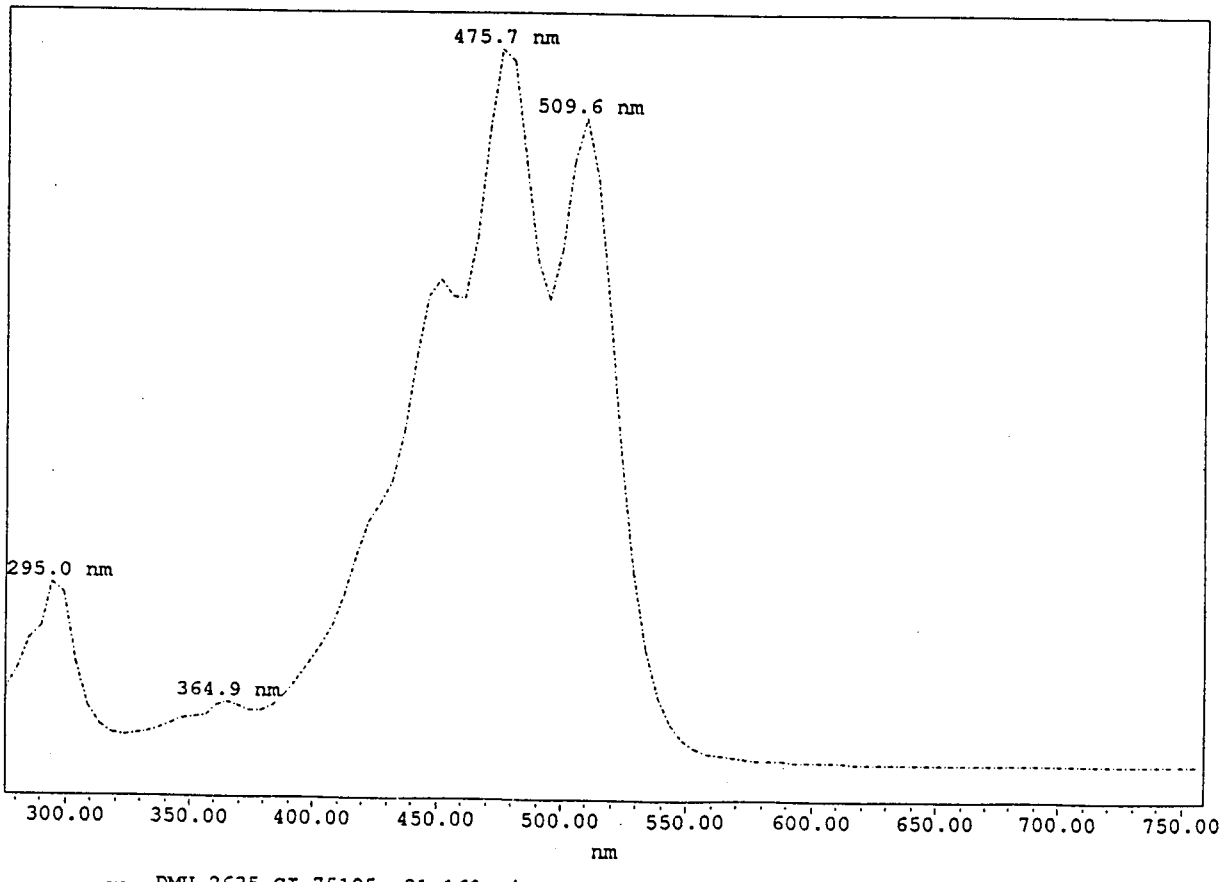




DMU 2667_CI 75120 13.963 minutes, 275 - 760 @ 4.8 nm, from 2667_CI75120

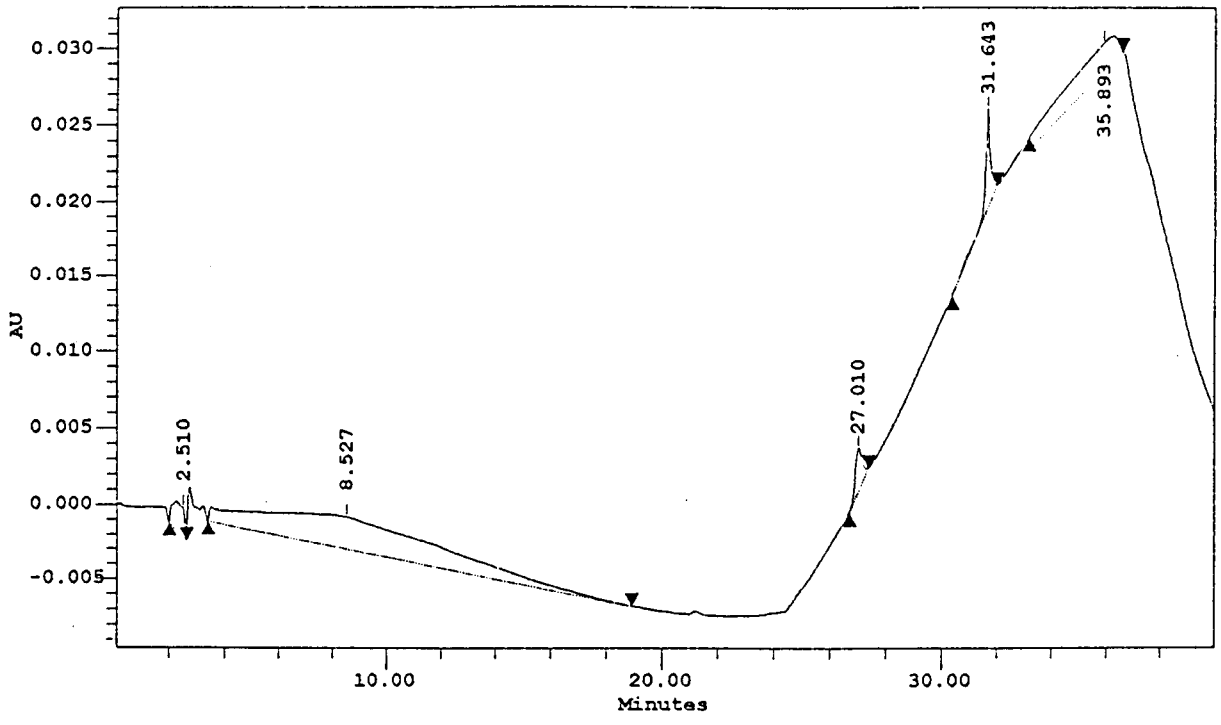
DMU 2635_C.I. 75125

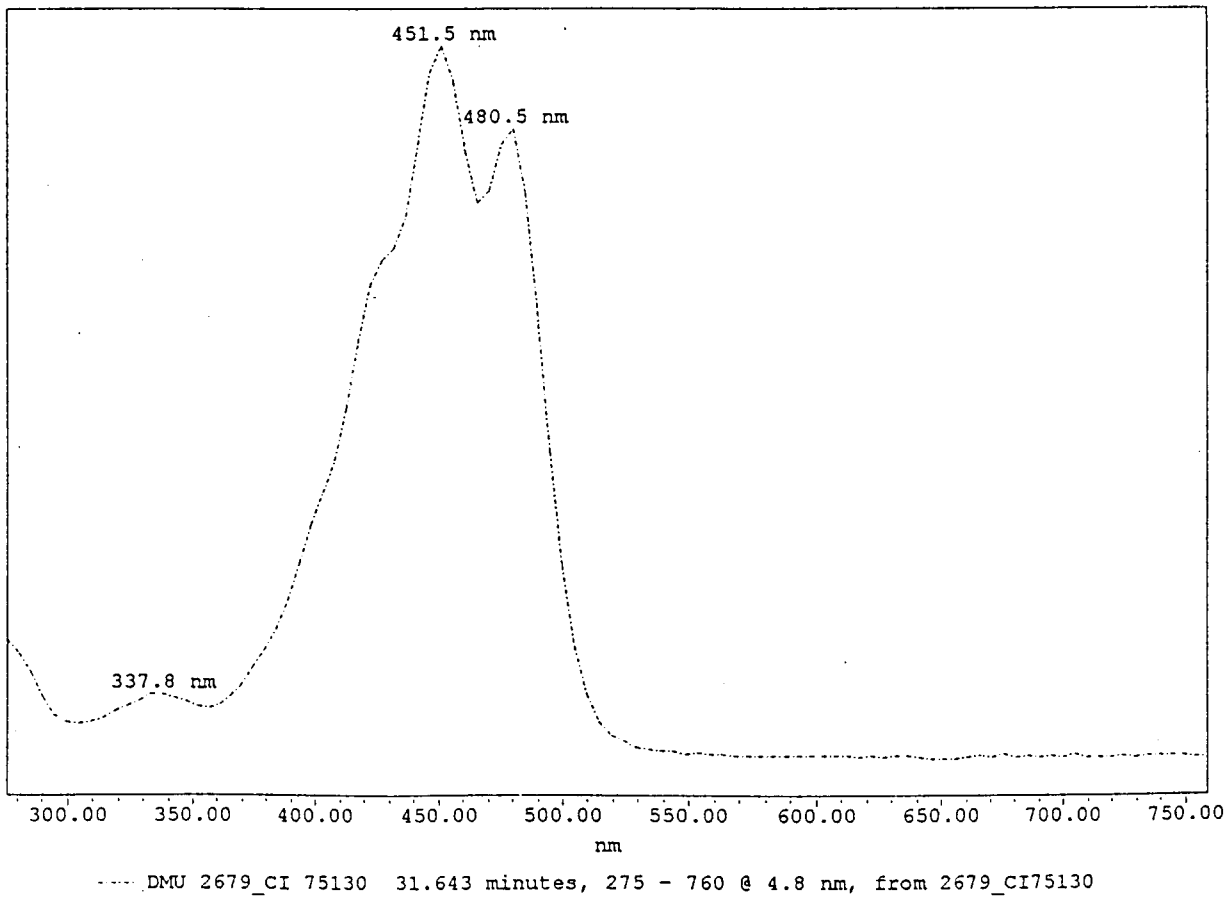




DMU 2635_CI 75125 31.168 minutes, 275 - 760 @ 4.8 nm, from 2635_75125

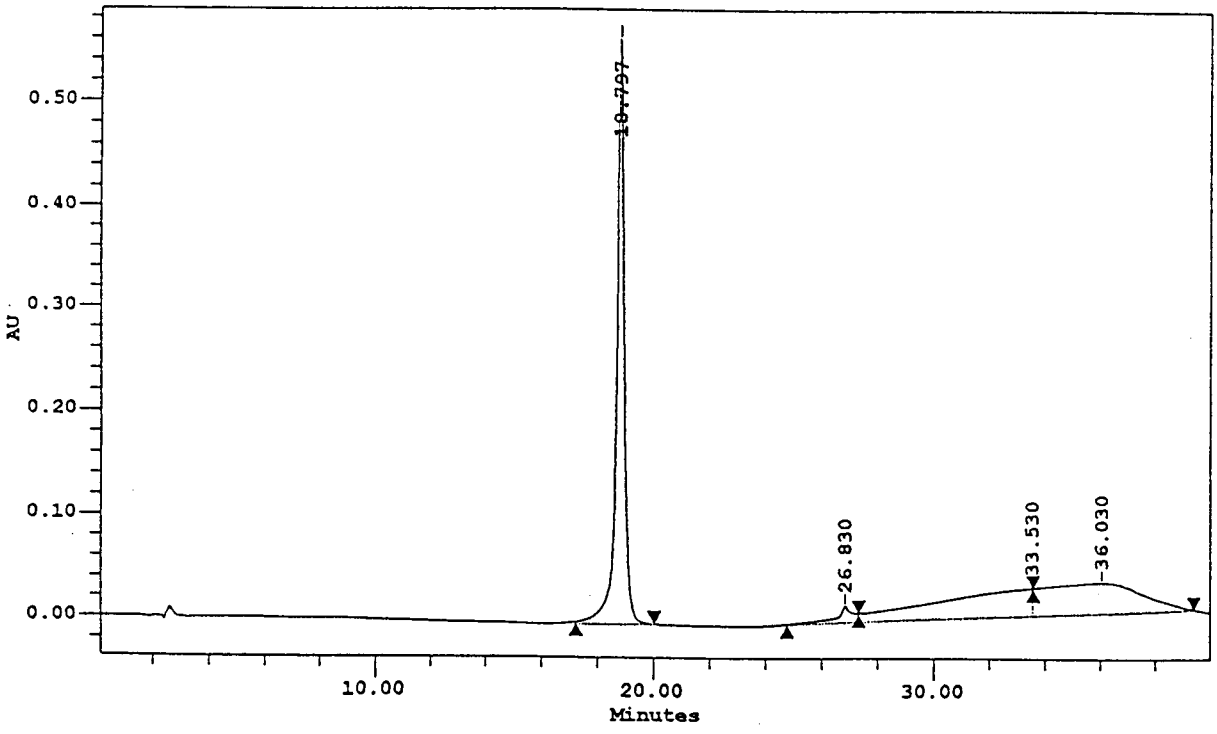
DMU 2679_C.I. 75130

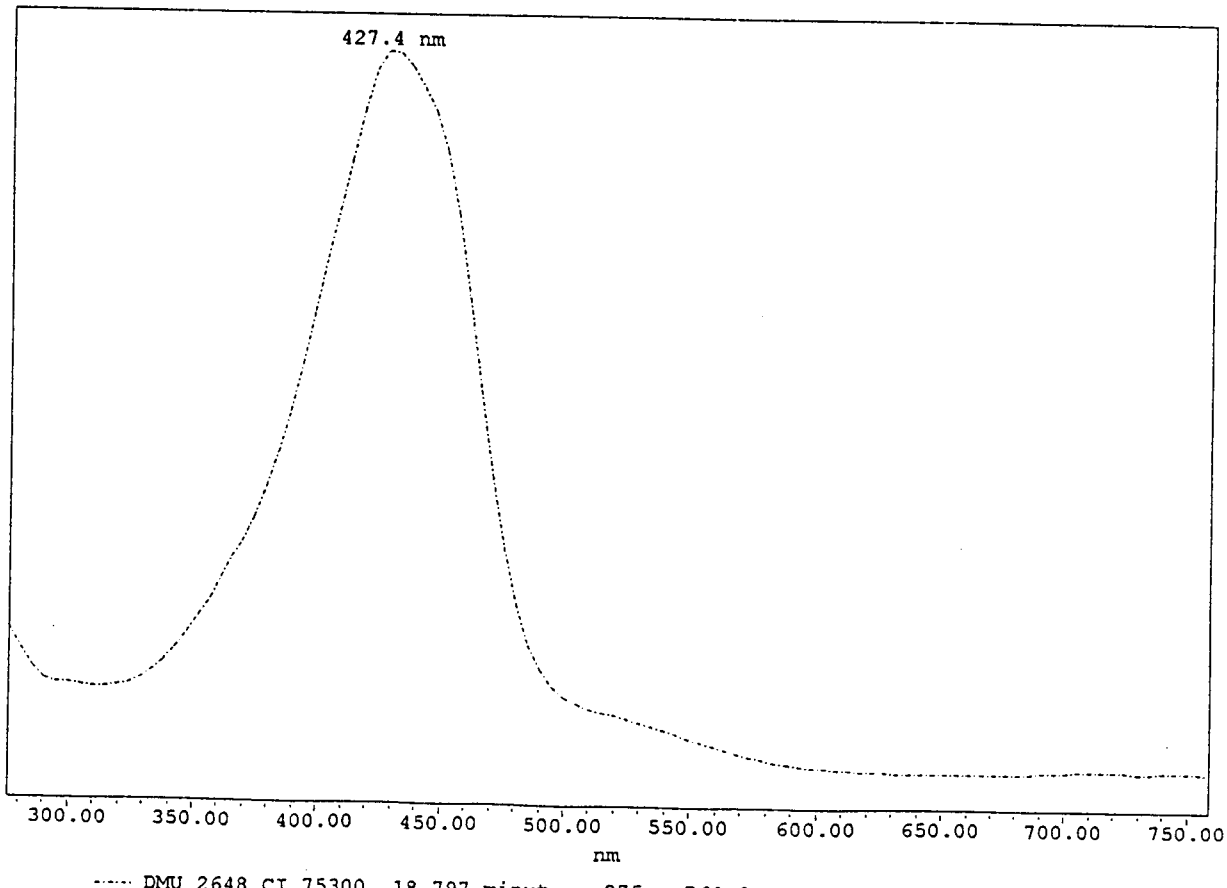




DMU 2679_CI 75130 31.643 minutes, 275 - 760 @ 4.8 nm, from 2679_CI75130

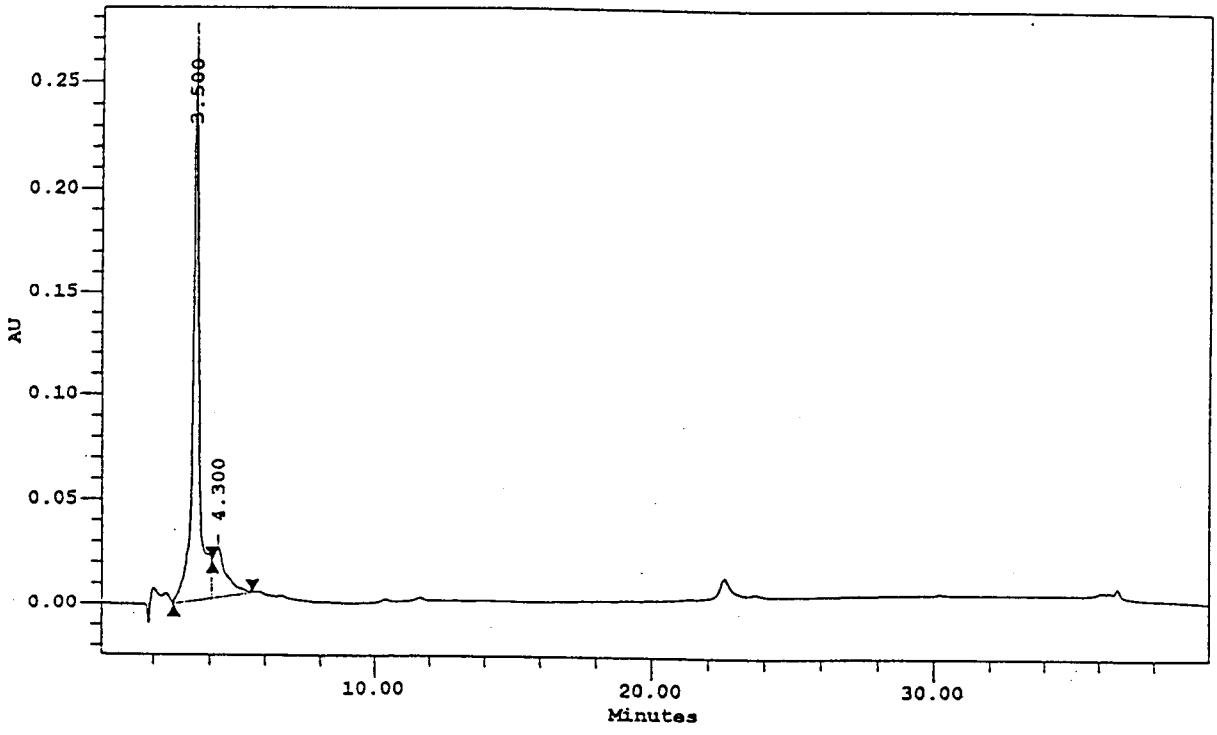
DMU 2648_C.I. 75300

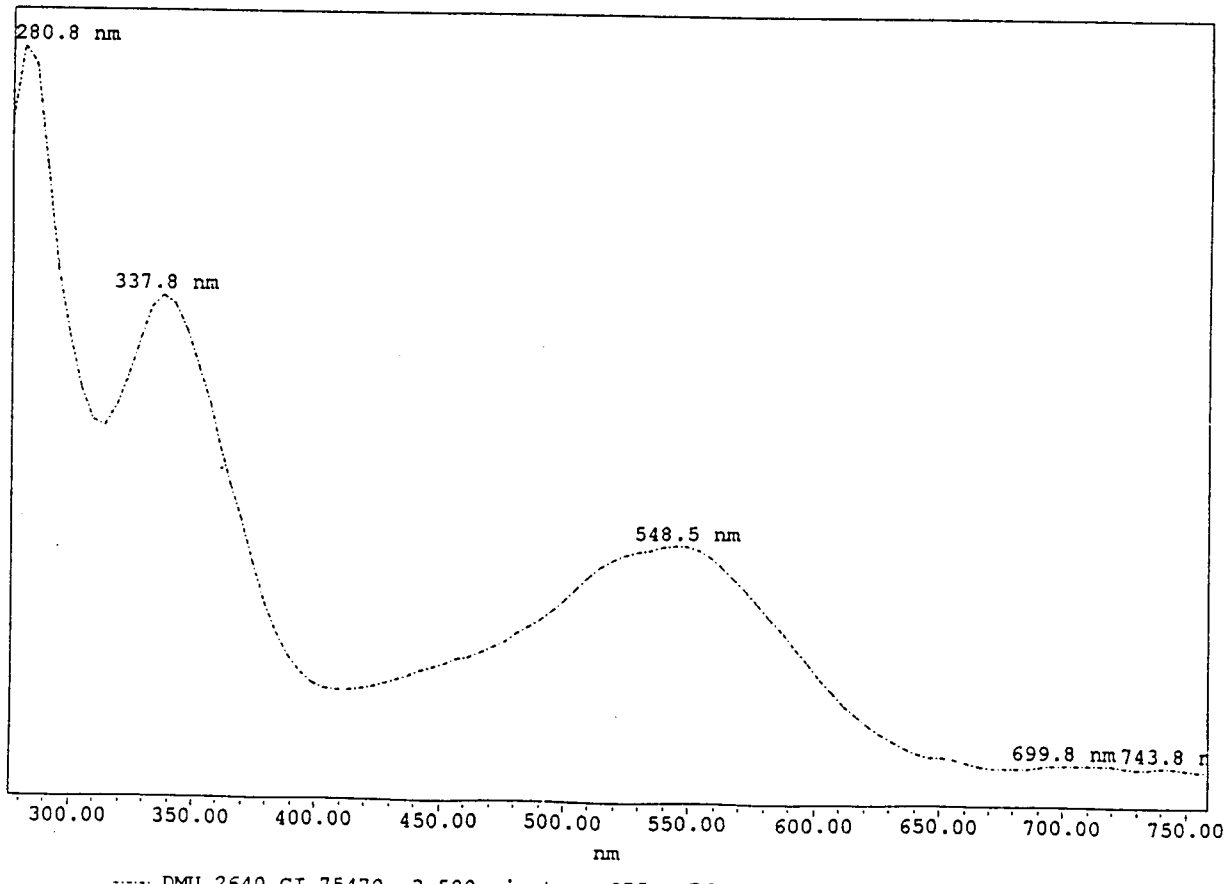




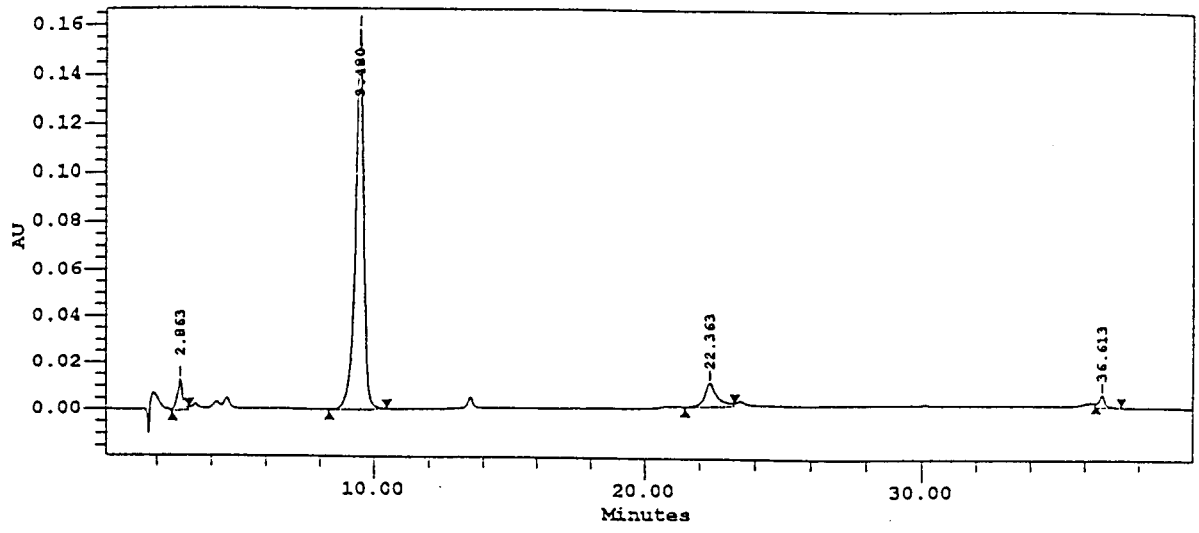
DMU 2648_CI 75300 18.797 minutes, 275 - 760 @ 4.8 nm, from 2648_CI75300

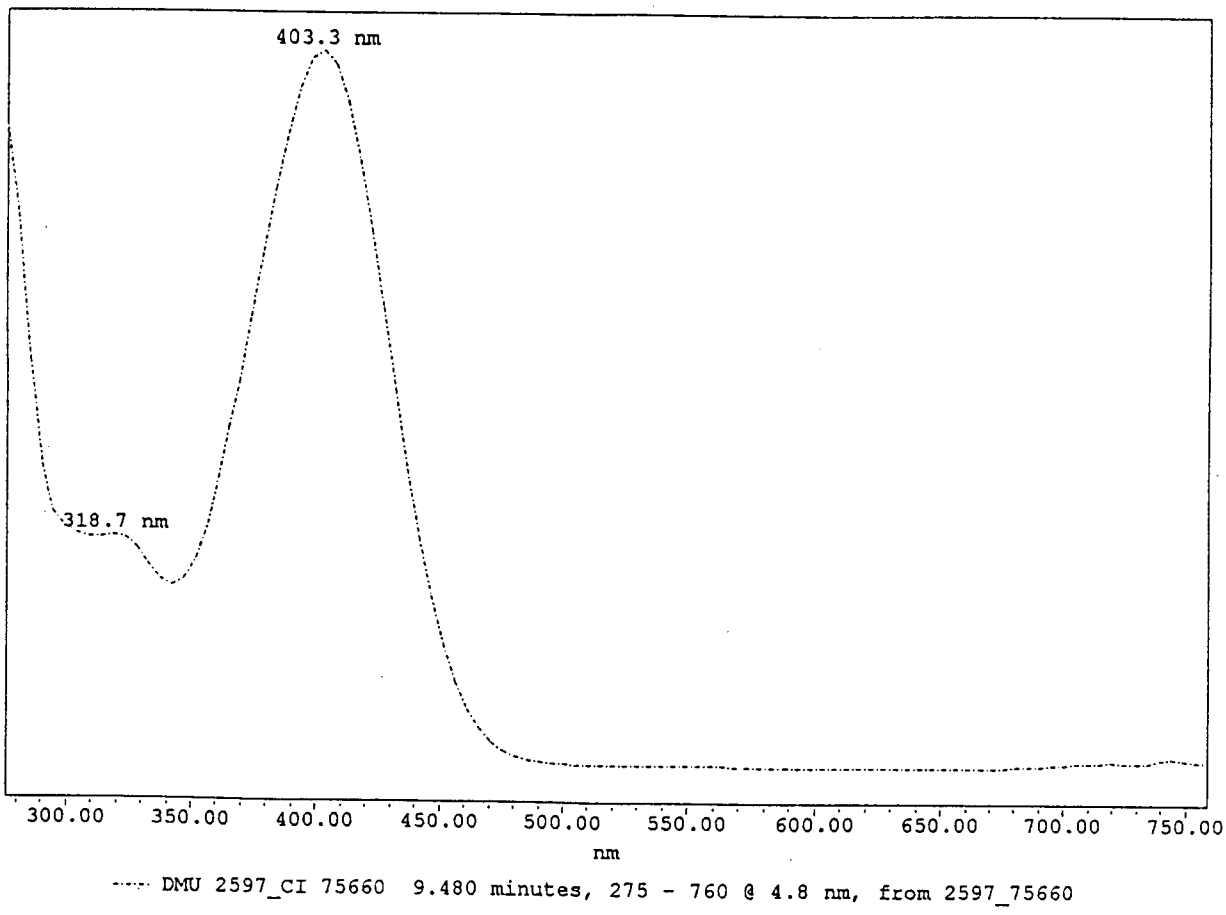
DMU 2640_C.I. 75470



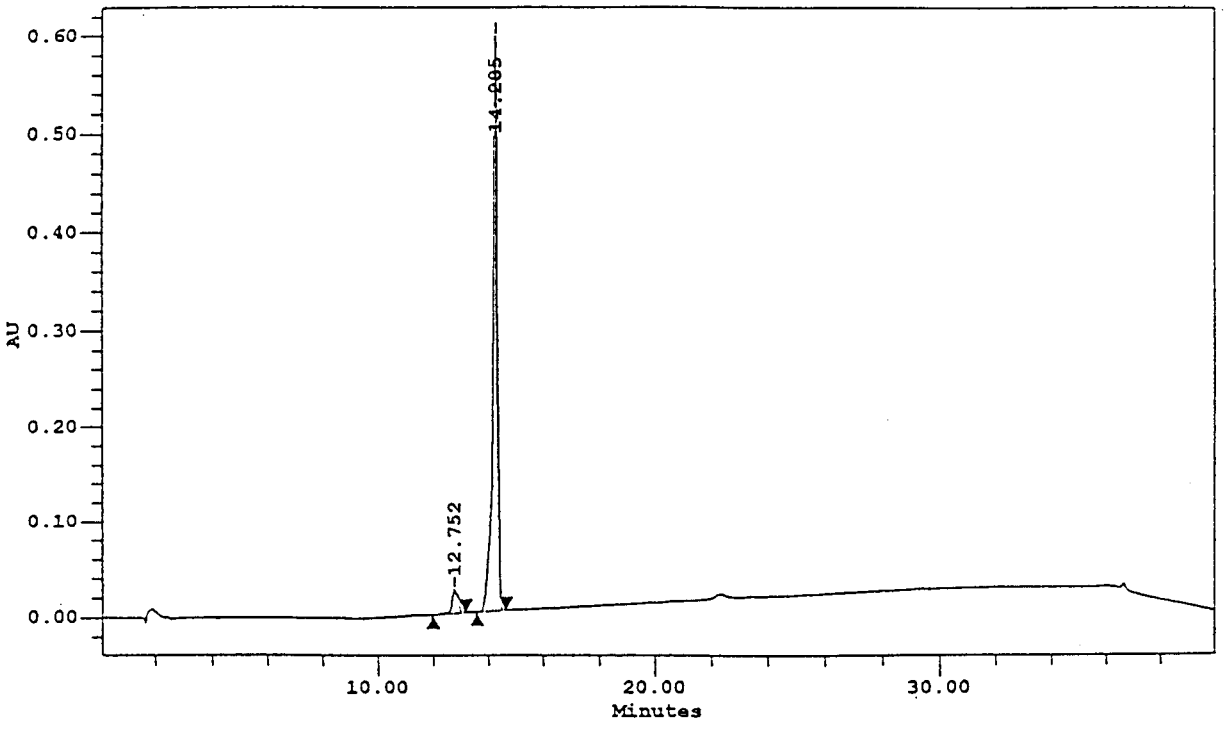


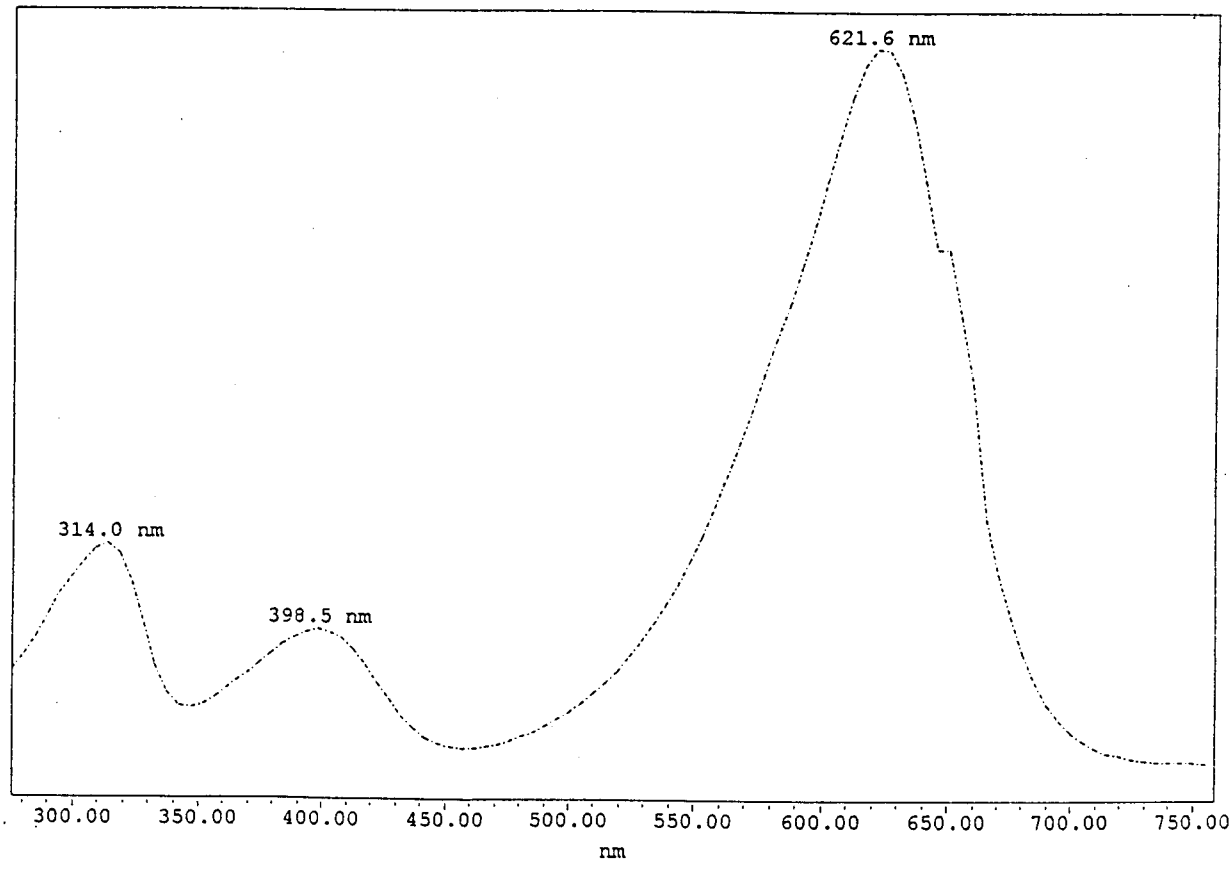
DMU 2597_C.I. 75660





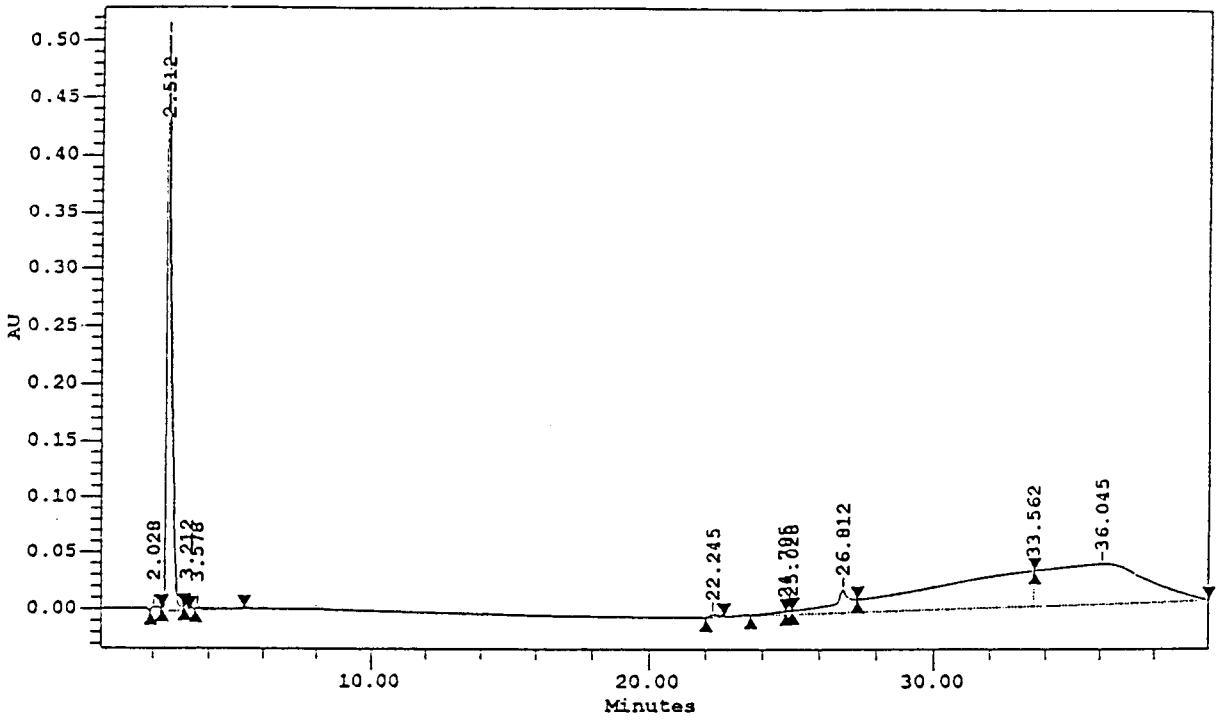
DMU 2672_C.I. 75810

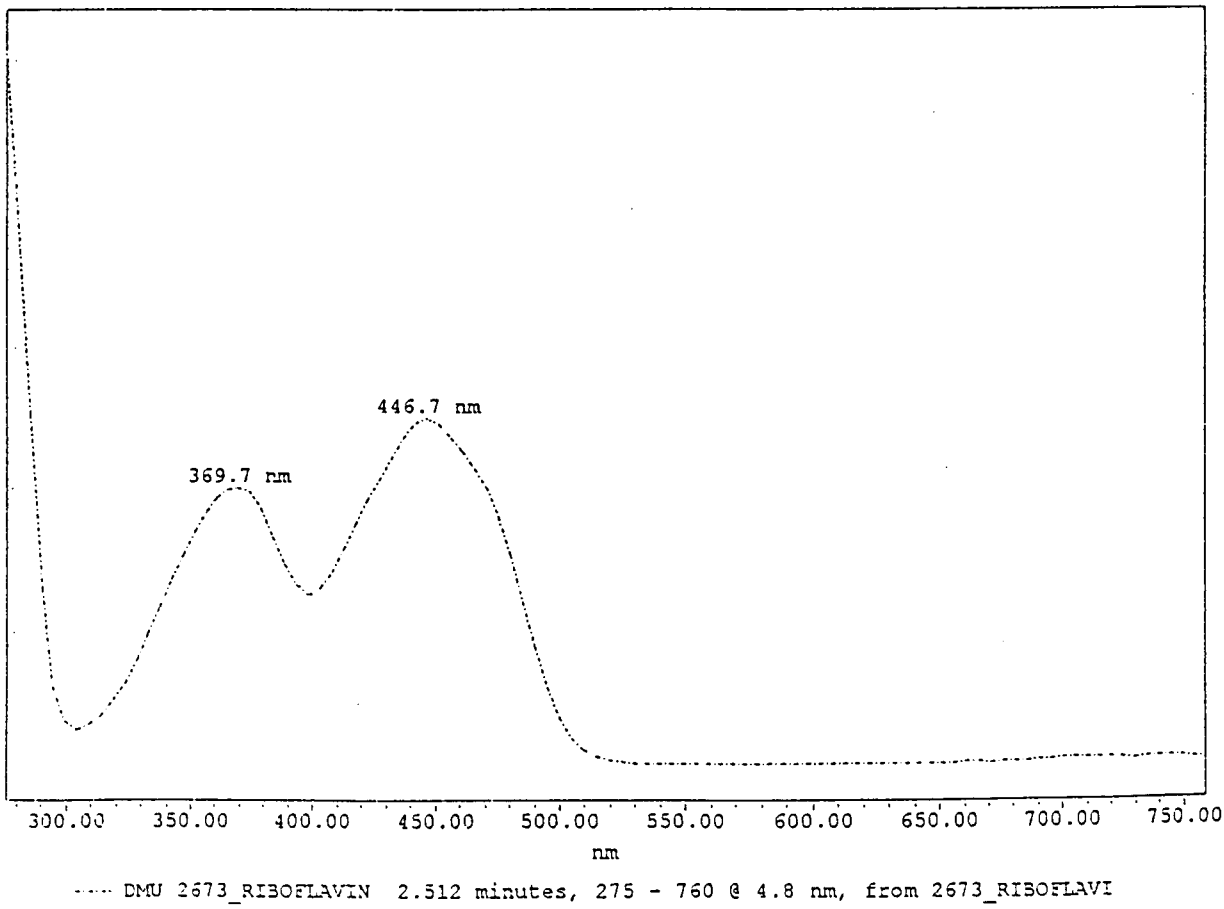




DMU 2672_CI 75810* 14.285 minutes, 275 - 760 @ 4.8 nm, from 2672_75810

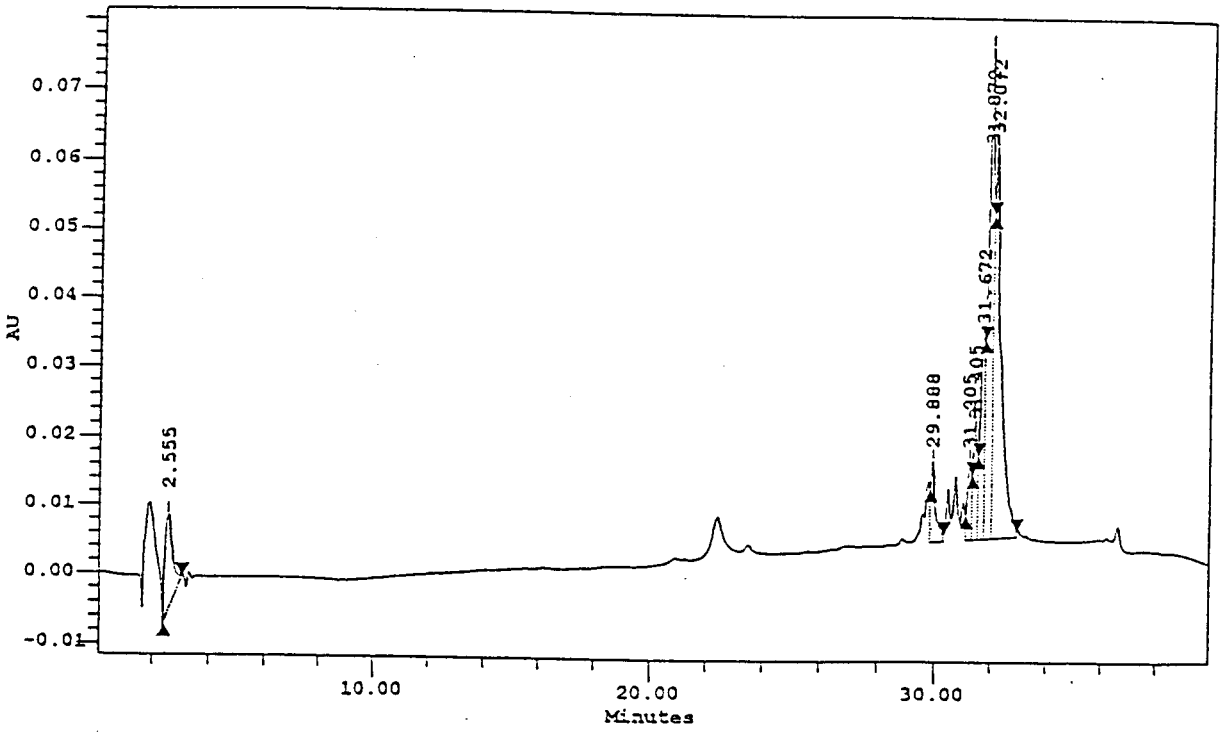
DMU 2673_Lactoflavine

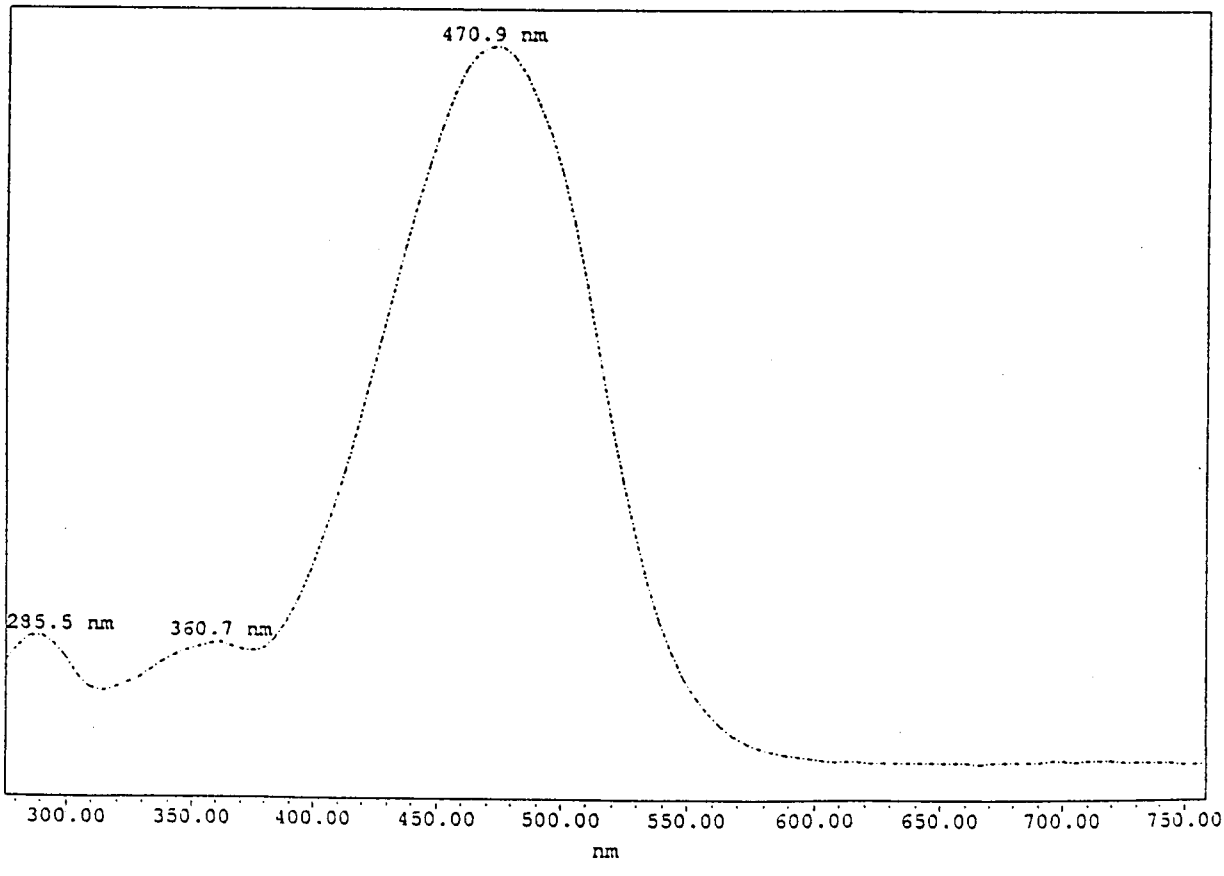




DMU 2673_RIBOFLAVIN 2.512 minutes, 275 - 760 @ 4.8 nm, from 2673_RIBOFLAVI

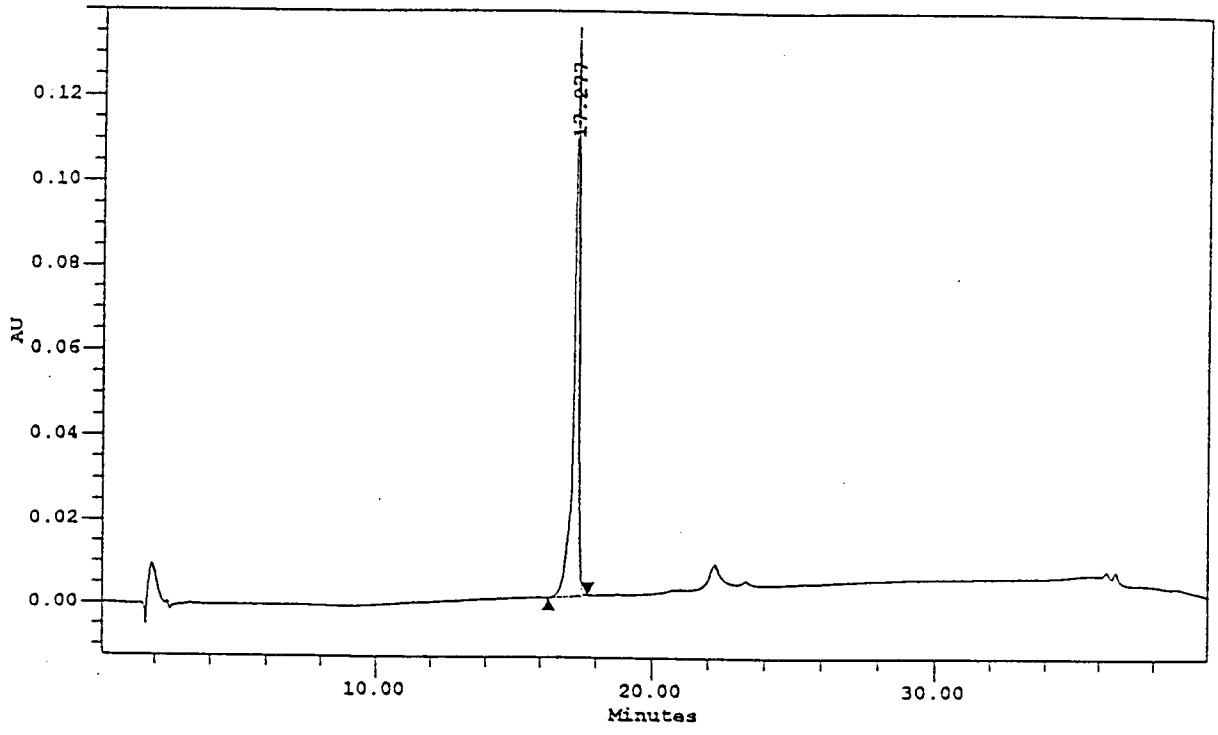
DMU 2684_Capsanthin

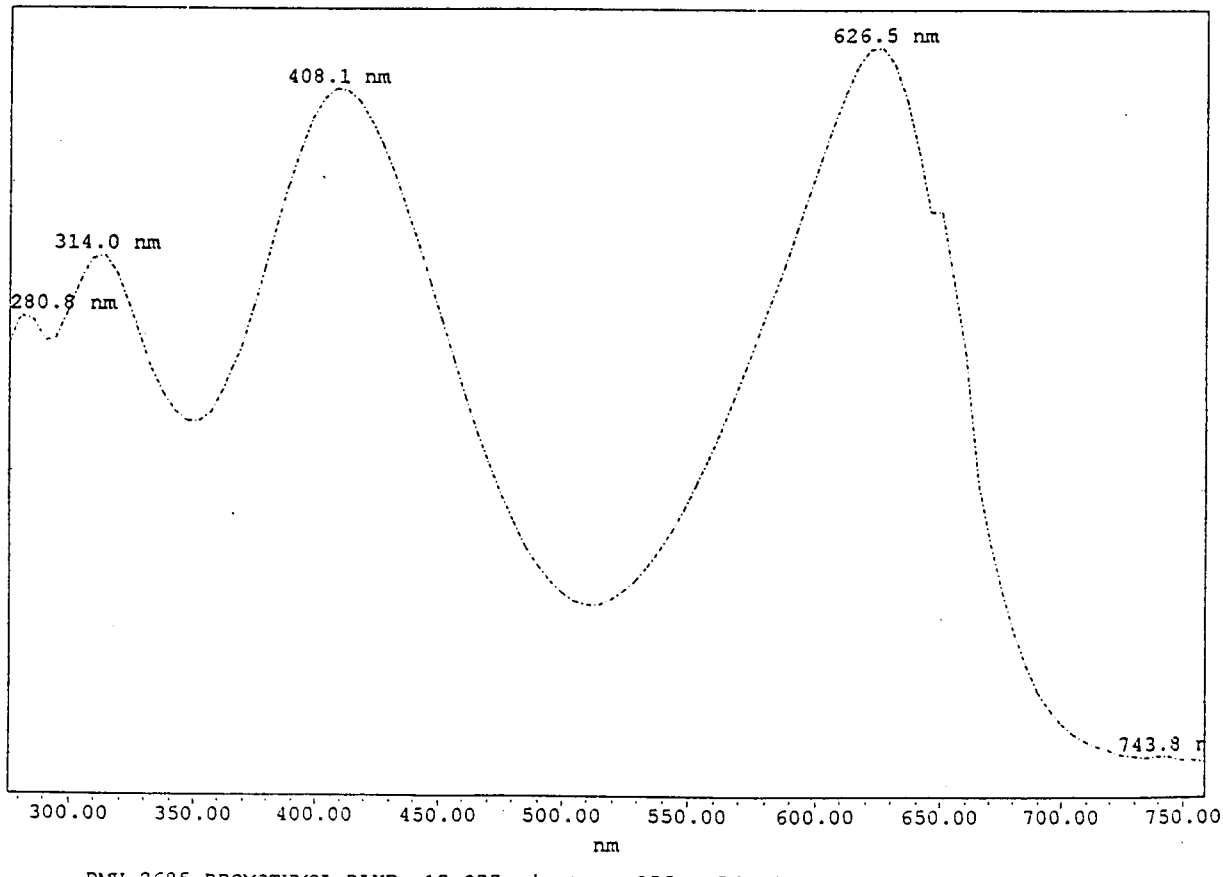




DMU 2684_CAPSANTHIN* 31.872 minutes, 275 - 760 @ 4.8 nm, from 2684-capsan

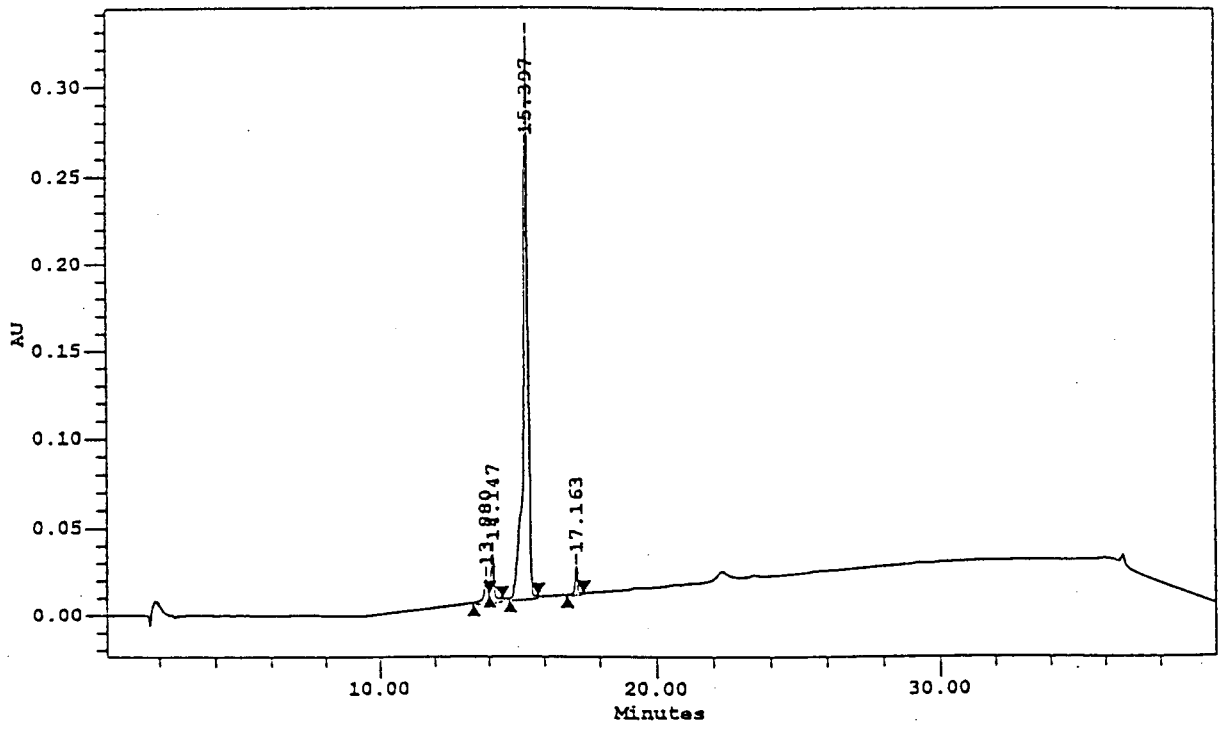
DMU 2685_ Bromothymol blue

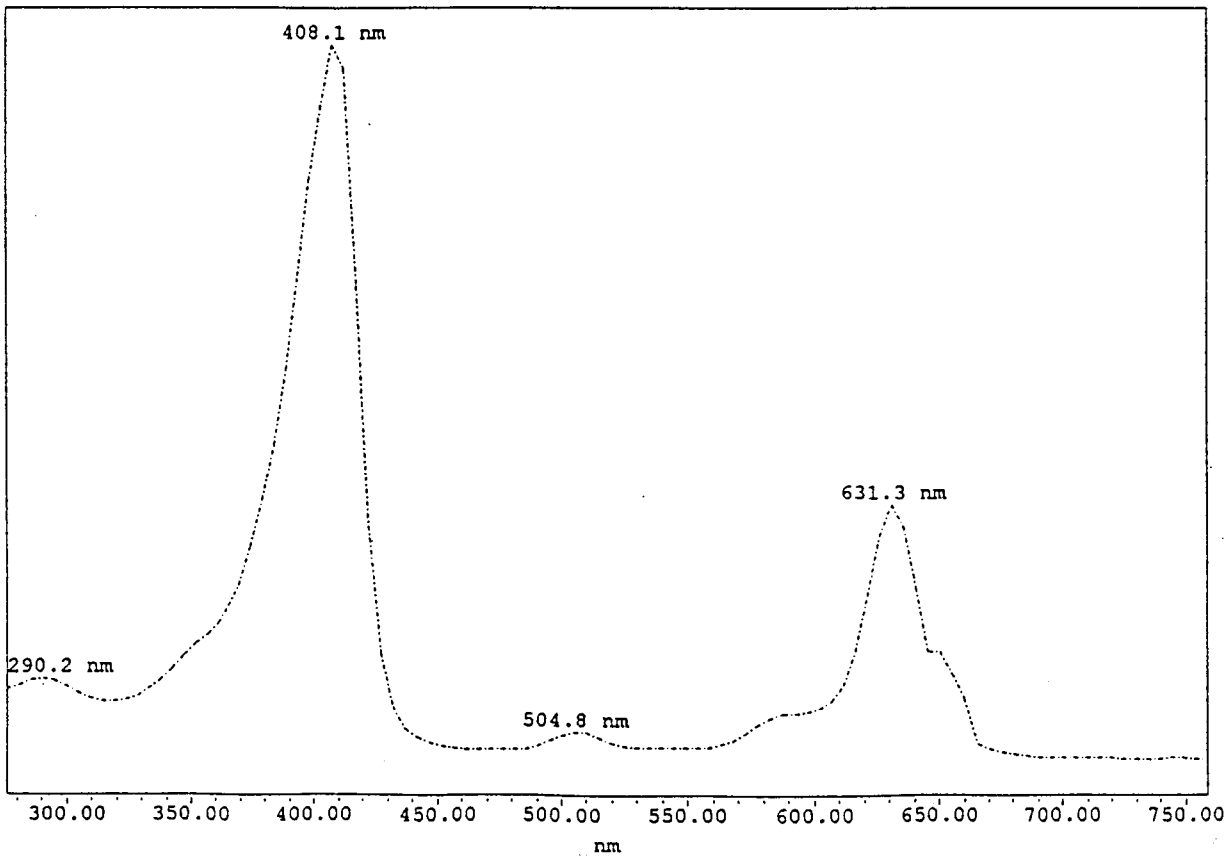




DMU 2685_BROMOTHMOL BLUE 17.277 minutes, 275 - 760 @ 4.8 nm, from 2685_Brthymobl

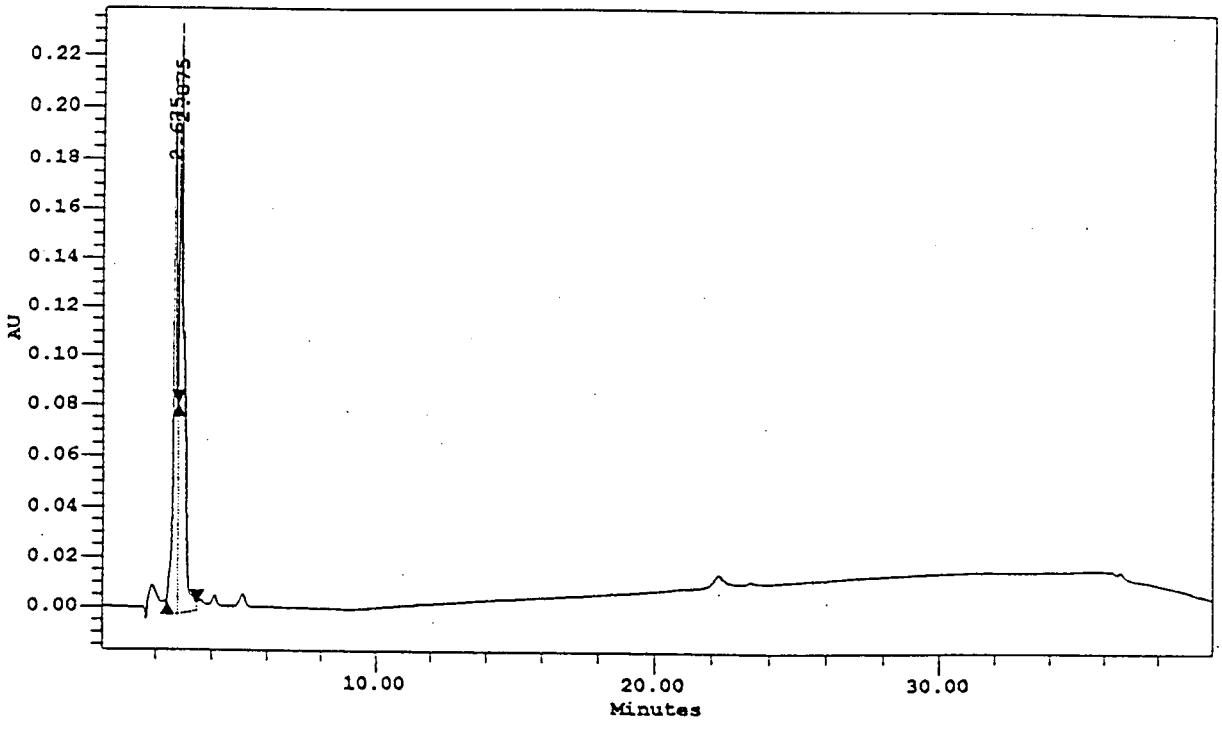
DMU 2646_Bromocresol green

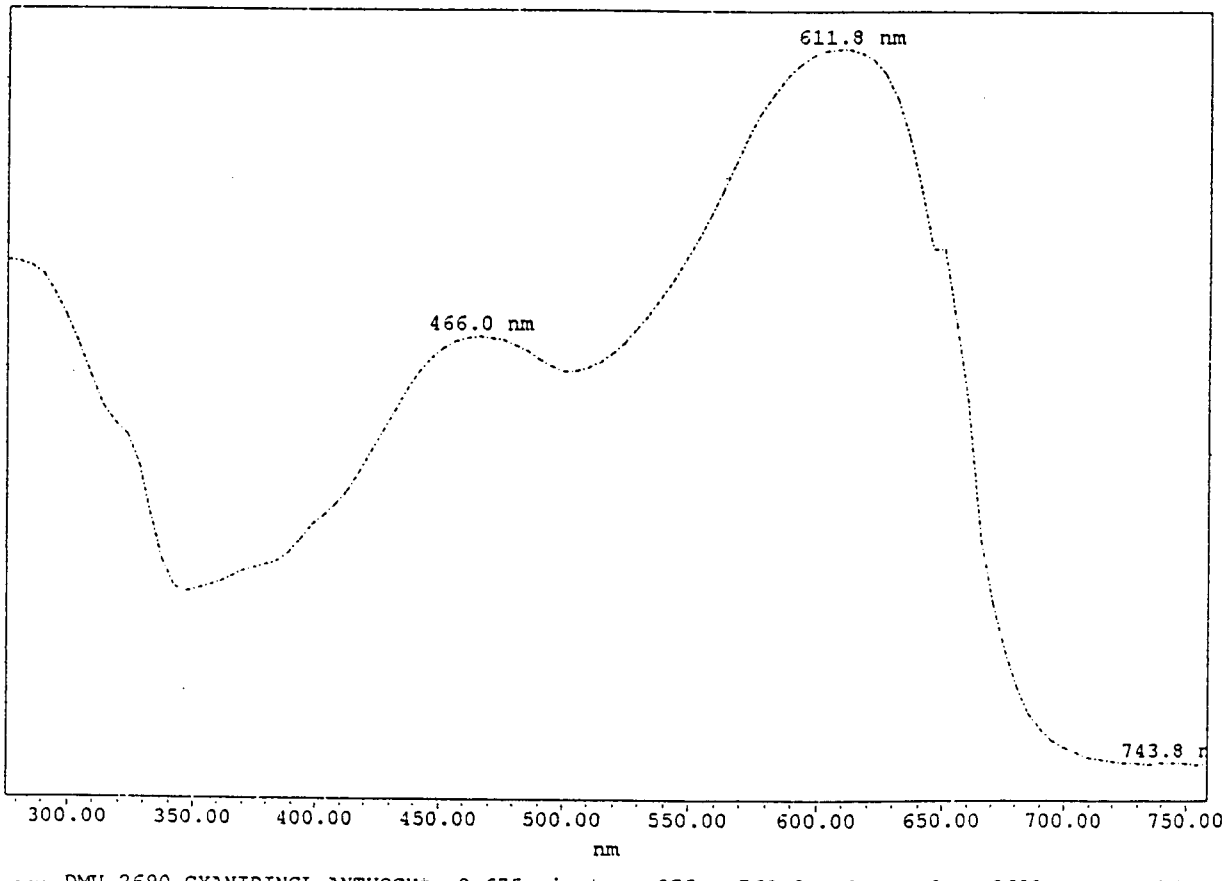




DMU 2646_BROMOCREOSOL* 15.397 minutes, 275 - 760 @ 4.8 nm, from 2646_bromocre

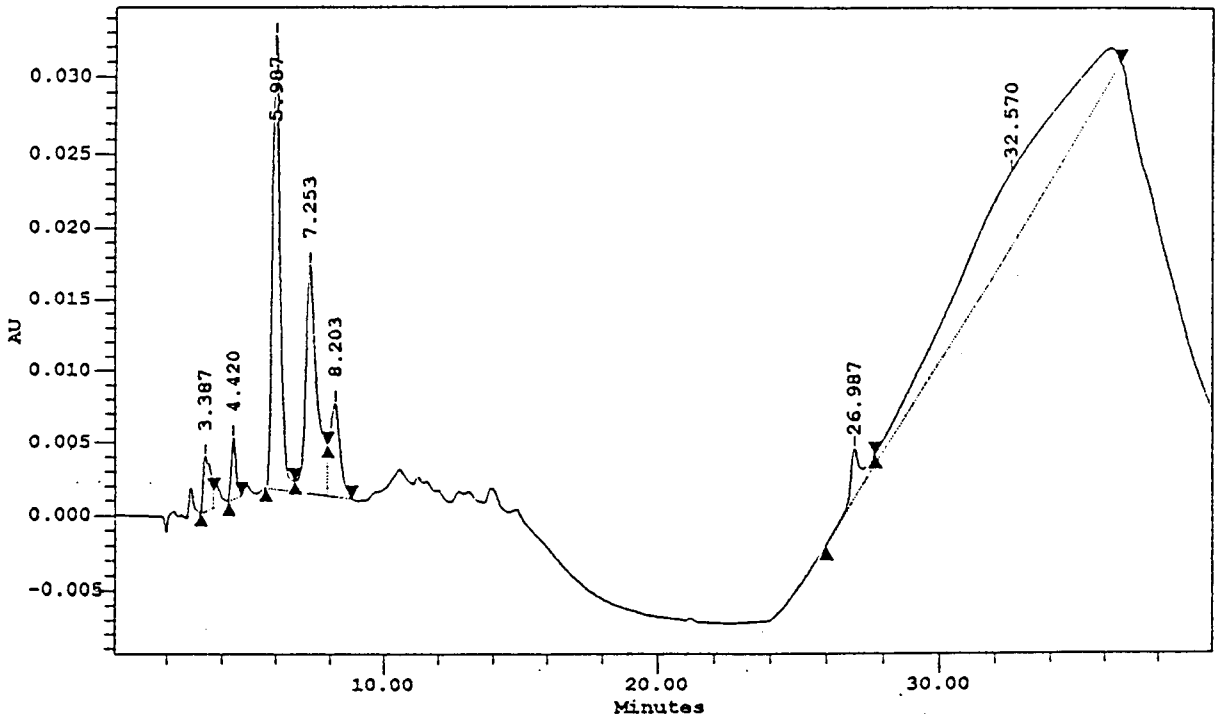
DMU 2690_Cyanidin_Anthocyanin

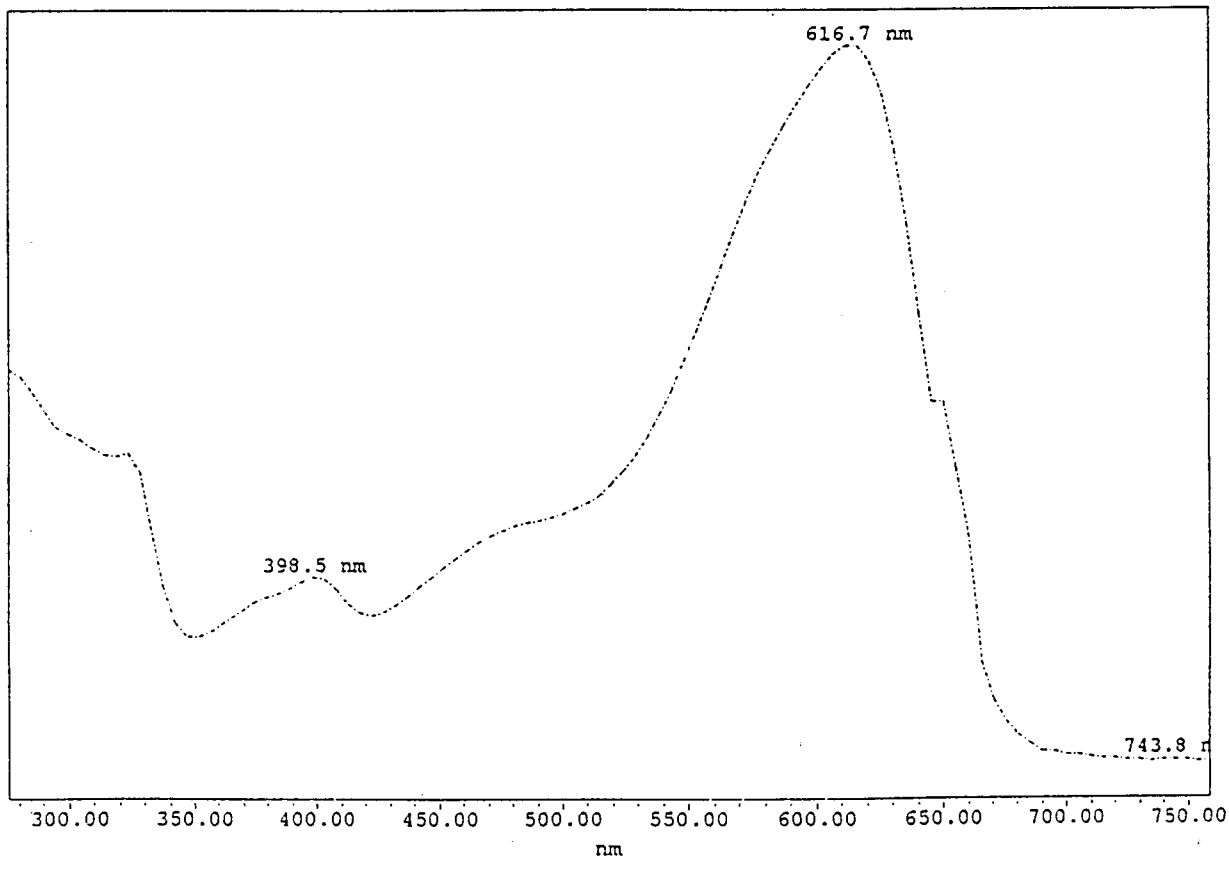




····· DMU 2690_CYANIDINCL_ANTHOCY* 2.675 minutes, 275 - 760 @ 4.8 nm, from 2690_cyan_anth

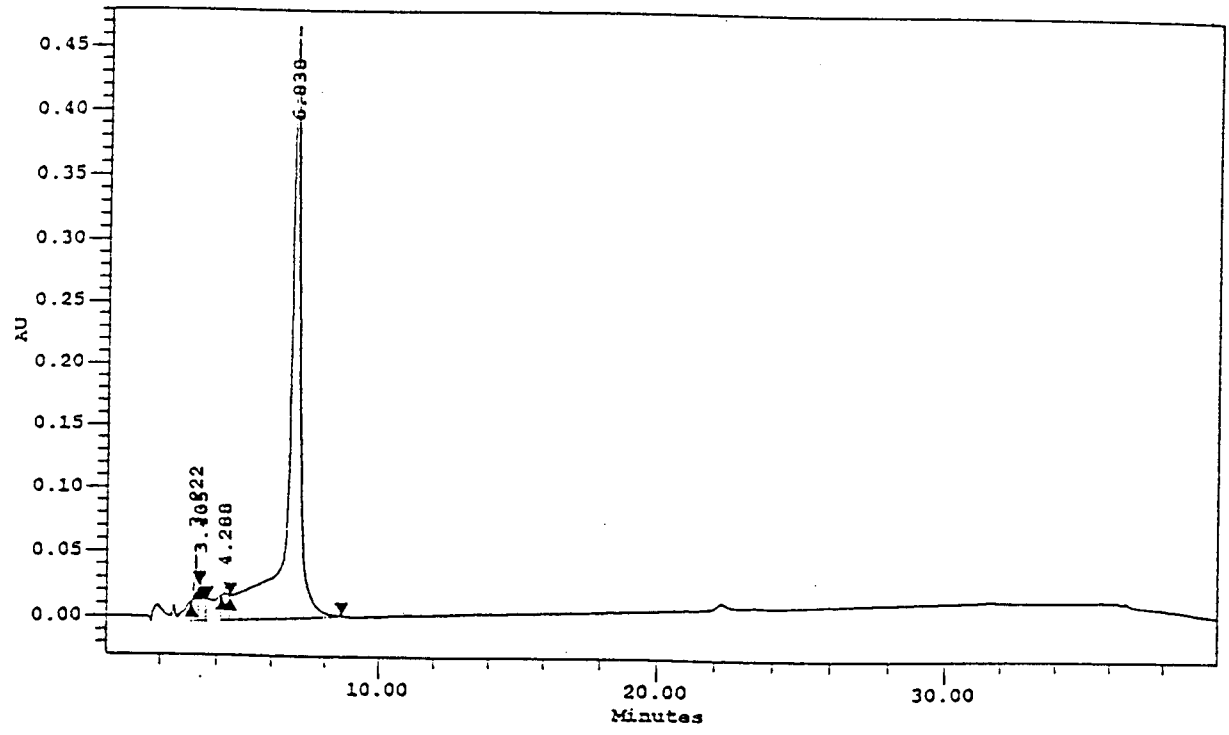
DMU 2691_Delphidin_Anthocyanin

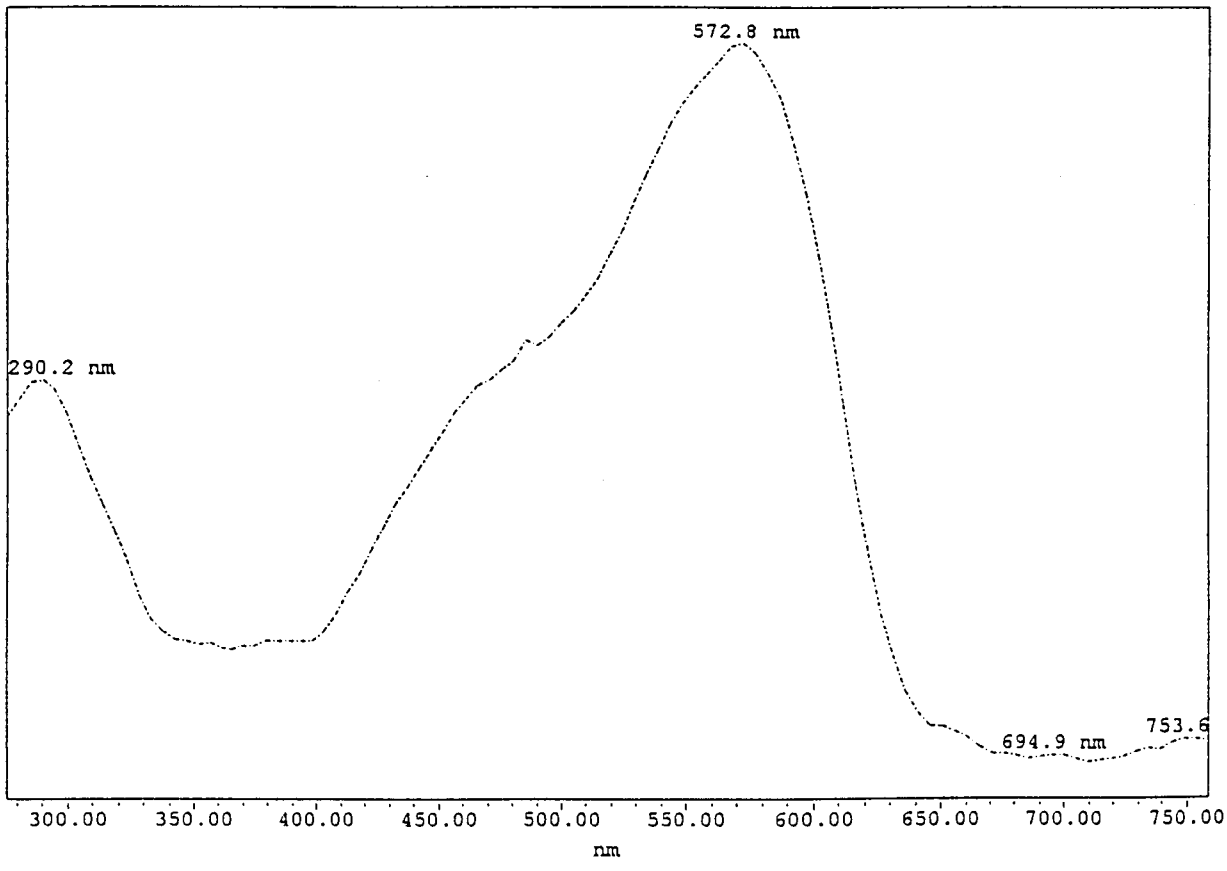




···· DMU 2691_DELPHIDIN_ANTHOC* 5.987 minutes, 275 - 760 @ 4.8 nm, from 2691_DELPHIDIN

DMU 2689_Oenin_Anthocyanin





----- DMU 2689_OENINCL_ANTHOCYAN* 3.222 minutes, 275 - 760 @ 4.8 nm, from 2689_oen_antho

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