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Supercritical fluid extraction of 13-*cis* retinoic acid and its photoisomers from selected pharmaceutical dosage forms

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Abstract

13-*Cis* retinoic acid (Accutane®) was extracted from a cream, gel, capsule and beadlet dosage form using supercritical carbon dioxide modified with 5% methanol as the mobile phase. The pump pressure and the extraction chamber and restrictor temperature were experimentally optimized at 325 atm and 45°C, respectively. A 2.5-min static and 5-min dynamic extraction time were used. The supercritical fluid extraction (SFE) eluent was trapped in methanol, injected into the high-performance liquid chromatographic (HPLC) system, and quantitated by ultraviolet detection at 360 nm. Application of the SFE method to spiked placebo dosage forms gave 13-*cis* retinoic acid recoveries of 98.8, 98.9, 98.8 and 100% for the cream, gel, capsule and beadlet, respectively, with R.S.D.s in the range 0.6–0.9% ($n = 4$). Inter-day percent error and precision of the extraction were 1.1–2.0 and 0.2–2.4% ($n = 3$), respectively, and intra-day percent error and precision were 1.0–3.0 and 0.3–2.1% ($n = 8$), respectively. Percent error and precision data for spiked celite samples in the 0.05–1.0 μg ml^{-1} range were 0.59–4.75 and 1.8–2.1% ($n = 3$), respectively. The extraction method was applied to commercial 13-*cis* retinoic acid dosage forms and the results compared to unextracted samples. Linear regression analysis of concentration versus peak height gave a correlation coefficient of 0.9991 with a slope of 7.468 and a y-intercept of 0.1923. The percent error and precision data were 1.3–5.3 and 0.2–1.5% ($n = 4$), respectively. The photoisomers of 13-*cis* retinoic acid were also extracted with the method and recoveries of 90.4–92.4% with R.S.D.s of 1.5–3.4% were obtained ($n = 4$). © 1997 Elsevier Science B.V.

Keywords: 13-*Cis* retinoic acid; Dosage forms; Photoisomers; Supercritical fluid extraction

1. Introduction

13-*Cis* retinoic acid, Accutane®, is indicated for the treatment of severe recalcitrant cystic acne. The mechanism of action is believed to involve the inhibition of sebaceous gland function and follicular keratinization. 13-*Cis* retinoic acid is known to have adverse effects typical of chronic hypervitaminosis A and teratogenic effects; therefore, the use of the drug should be restricted to non-pregnant females and patients who are unresponsive to conventional acne therapies [1].

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Supercritical fluid extraction (SFE) is a relatively new technique in the field of analytical chemistry. It has evolved in the last decade as an alternative method of sample preparation prior to analysis [2]. SFE has been applied to the determination of pharmaceuticals in plasma by Liu and Wehmeyer [3]. The use of SFE for sample preparation in the analysis of benzodiazepines in solid dosage forms was investigated by Lawrence et al. [4]. Another application of SFE to a dosage form was reported by Howard et al. who isolated felodipine from sustained released tablets [5].

In this paper, the extraction of 13-*cis* retinoic acid and its photoisomers is described for cream, gel, capsule and beadlet dosage forms, using supercritical fluid carbon dioxide containing 5% methanol. Fig. 1 shows the chemical structures of 13-*cis* retinoic acid and all-*trans*-retinoic acid derivatives which are also degradation products of 13-*cis* retinoic acid [6]. Various retinoic acid derivatives including 13-*cis* retinoic acid have been analyzed using high-performance liquid chromatography (HPLC) with programmed gradient elution [7]. Electrochemical detection of retinoids using normal phase HPLC was studied by Bryan et al. [8]. The isolation of 13-*cis* retinoic acid and other retinoids from biological fluids has been extensively reviewed by various authors [9–14]. Kril et al. developed a method to determine tretinoin, a photodegradation product of 13-*cis* retinoic acid, in creams and DePaolis determined various retinoids in creams and gels by thin layer chromatography (TLC) and HPLC [15,16]. Advantages of applying supercritical fluid extraction to the isolation of 13-*cis* retinoic acid from its various dosage forms are the lack of sample exposure to light during the extraction, the speed of isolation, and the lack of solvent waste which may accompany other classical techniques such as liquid/liquid extraction. In addition, use of carbon dioxide as the supercritical fluid eliminates the environmental limitations of conventional solvents. This study was conducted to serve as a model system for demonstrating the potential applications of SFE to medications in dosage forms more complex than tablets or capsules

2. Experimental

2.1. Reagents and chemicals

HPLC-grade methanol, acetonitrile and glacial acetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Supercritical fluid chromatography grade carbon dioxide containing 5% methanol was obtained from Scott Specialty Gases (Plumsteadville, PA, USA). SFE wet support (Celite, Cat. No. 68-3867-010) was purchased from Isco (Lincoln, NE, USA). Deionized–distilled water was obtained by a filtration system

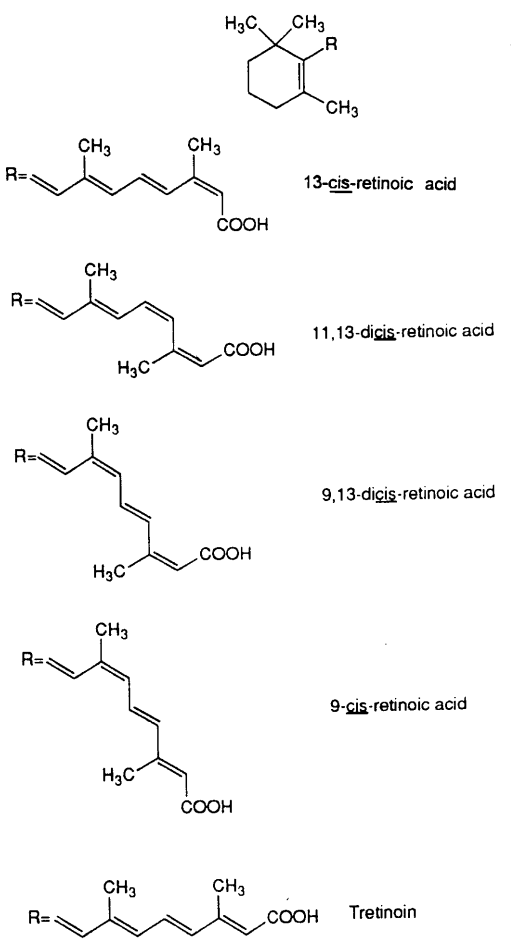

Fig. 1. Structures of 13-*cis* retinoic acid and its photoisomers.

from Continental Water Systems (Roswell, GA, USA). The following 13-*cis* retinoic acid placebo and actual dosage forms were obtained from Hoffman–LaRoche (Nutley, NJ): 13-*cis* retinoic acid cream 0.1% w/w or 30 mg g^{-1}—placebo, lot C167031, cream, lot 167021; 13-*cis* retinoic acid gel 0.05% w/w or 15 mg/g^{-1}— placebo, lot 178424, gel, lot L178414; Accutane® capsules 10 mg— placebo, lot C166551-01, 10 mg capsules, lot C166511; and Accutane® beadlets 10% w/w or 100 mg g^{-1}—placebo, lot 175663, beadlet, lot L175653. The following reference standards were also supplied by Hoffman–LaRoche: 13-*cis* retinoic acid, lot 19545-17-3; 11,13-di-*cis*-retinoic acid, lot 15386-5; 9,13-di-*cis*-retinoic acid, lot 19986-134B; 9-*cis*-retinoic acid, lot 14100-14; and tretinoin, lot 702006.

2.2. Instrumentation

Supercritical fluid extraction was performed on an Isco Model SFX 2-10 Supercritical Fluid Extractor (Cat. No. 67-9000-040) equipped with 1.5 ml min^{-1} heated capillary restrictors and a temperature controller (Cat No. 68-3960-001). Stainless steel extraction cartridges (Isco 0.5 ml, Cat No. 68-3867-001) were used to load the sample into the extraction chamber. Low actinic volumetric flasks were used to prepare all solutions in the assay.

Chromatography was performed on an isocratic HPLC system consisting of a Micromeritics Model 760 HPLC pump and a Model 728 autosampler (Norcross, GA, USA) and a Kratos Spectroflow Model 757 UV-VIS variable wavelength detector (Ramsey, NJ, USA) set at 360 nm. Data acquisition was performed on a Hewlett-Packard Model HP-3395 Integrator (Palo Alto, CA, USA). Separations were accomplished on an Alltech/Applied Science 5-μm Spherisorb ODS-2 column (250 × 4.6 mm i.d.) (Deerfield, IL, USA) maintained at ambient temperature (23 ± 1°C) The mobile phase used for the HPLC separation was acetonitrile:methanol:0.05% glacial acetic acid in deionized–distilled water (42.5:32.5:25 v/v/v). The sample loop of the autosampler was 20 μl and the flow rate was 1.2 ml min^{-1}.

2.3. Preparation of stock and standard solutions of 13-cis retinoic acid

Weighed aliquots of 13-*cis* retinoic acid reference standard were placed in individual 10 ml volumetric flasks and methanol was added to volume to obtain stock solutions of 0.125, 0.625, 1.25 and 2.0 mg ml^{-1}. Dilutions of the stock solutions were used to prepare standard solutions for the 13-*cis* retinoic acid calibration curve. In addition, standard solutions (0.5 μg ml^{-1}) of 11,13-di-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid, 9-*cis*-retinoic acid, and tretinoin were prepared in methanol in individual 10 ml volumetric flasks for the photoisomer study.

2.4. Sample preparation of spiked placebo dosage forms

Placebo 13-*cis* retinoic acid cream weighing 1.25 g was placed in a 10 ml volumetric flask and 1 ml of the 1.25 mg ml^{-1} 13-*cis* retinoic acid stock solution in methanol was added. The flask was vortexed for 15 min and methanol was added to volume.

Placebo 13-*cis* retinoic acid gel weighing 1.25 g was added to a 10 ml volumetric flask and 1 ml of a 0.625 mg ml^{-1} 13-*cis* retinoic acid stock solution in methanol was added. The sample was vortexed for 15 min and methanol was added to volume.

Two placebo Accutane capsules were cut open in the bottom of a 50 ml beaker with a scalpel and tweezers, and the tools were rinsed with approximately 40 ml of methanol into the beaker. The mixture was sonicated for 10 min, the contents were filtered through a sintered funnel using an applied vacuum and the filtrate was added to an actinic 100 ml volumetric flask. The beaker, capsule shells and funnel walls were rinsed with 50 ml of methanol, the washing transferred into the flask, and methanol was added to volume. This placebo capsule solution was then added to a 10 ml volumetric flask to which had been placed a 1 ml aliquot of the 2 mg ml^{-1} 13-*cis* retinoic acid stock solution in methanol.

Placebo Accutane beadlets were ground in a mortar with a pestle and 1.25 mg of the powder

placed in a 0.5 ml extraction cell partially filled with celite. To the extraction cell was added 100 μl of the 0.125 mg ml^{-1} 13-*cis* retinoic acid stock solution in methanol. The extraction cell was then completely filled with celite.

2.5. Sample preparation of actual 13-cis retinoic acid dosage forms

Aliquots (1.25 g) of 0.1% w/w 13-*cis* retinoic acid cream and 0.05% w/w 13-*cis* retinoic acid gel were individually weighed into tared 10 ml volumetric flasks. Methanol was added to volume and each mixture was vortexed for 15 min.

Two 10 mg 13-*cis* retinoic acid capsules were cut open as described above and the filtered samples and washings were transferred to an actinic 100 ml volumetric flask with methanol added to volume.

A quantity of 10% w/w 13-*cis* retinoic acid beadlets was ground to a powder with a mortar and pestle and a weighed 1.25 mg aliquot placed in a 0.5 ml extraction cell partially filled with celite. The extraction cell was then completely filled with celite.

2.6. General SFE extraction method

A 100 μl aliquot of each methanolic solution prepared from cream, gel and capsule placebo and actual dosage forms were added to a 0.5 ml extraction cartridge partially filled with celite. For the beadlet placebo and dosage form, weighed aliquots of the powdered sample were added directly to the extraction cell as described above. The extraction cell was then loaded into the extraction chamber and the samples were extracted at a chamber and restrictor temperature of 45°C at a pressure of 325 atm. The static and dynamic extraction times were 2.5 and 5 min, respectively. The mobile phase used in the extraction was SFE/SFC grade carbon dioxide containing 5% methanol. The analytes were captured in a solvent trap containing 17 ml of methanol. After each extraction, the methanolic extract was transferred from the trap to a 25 ml actinic volumetric flask where the extract was allowed to equilibrate to ambient temperature for 10 min before adding methanol to volume. Aliquots (20 μl) of the extracts were then injected into the liquid chromatograph.

3. Results and discussion

3.1. Spiked matrix SFE

The initial SFE method development for the extraction of 13-*cis* retinoic acid from spiked celite and cream and beadlet dosage forms used hexane in the solvent trap. Hexane was selected since it was compatible with the normal-phase HPLC mobile phase containing approximately 98% hexane and there were no plans at that time to evaporate the trapping solvent to dryness. It was reported that solubility of 13-*cis* retinoic acid in hexane was 0.5 mg ml^{-1} and it appeared reasonable that the hexane would satisfactorily trap the analyte. The extraction data later indicated that this was an incorrect assumption. However, the selection of methanol as spiking solvent for the retinoic acid was a good choice since it showed excellent solubility for the analyte and the volatility of the methanol would have been an advantage if an evaporation step was utilized.

The initial conditions chosen for the extraction chamber and restrictors were 300 atm and 55°C with static and dynamic extraction times arbitrarily set at 5 and 15 min, respectively. The times for static and dynamic extraction were comparable to other studies reported in the literature [2–5]. The solvent trap contained approximately 17 ml of hexane which, after extraction, was brought to volume at 25 ml. An aliquot was then injected into the HPLC system for quantitation. The 13-*cis* retinoic acid was spiked onto celite in the extraction cell as a dioxane solution of the reference standard. Similarly, the 13-*cis* retinoic acid cream was dissolved in dioxane and applied to the celite and a weighed quantity of the 13-*cis* retinoic acid beadlets were added to the celite in the extraction cell. The 13-*cis* retinoic acid extraction for all three samples was monitored with a reported normal-phase HPLC method using a silica column and a hexane–ethyl acetate (98:2 v/v) mobile phase containing 0.1% glacial acetic acid

Table 1
Inter- and intra-day accuracy and precision data from SFE/HPLC assay of 13-*cis* retinoic acid on spiked celite

	Conc. added (μg ml^{-1})	Conc. found (μg ml^{-1})	Percent error (%)	R.S.D. (%)	n[a]
Inter-day	0.100	0.098 ± 0.003[b]	2.0	2.4	3
	0.250	0.247 ± 0.002	1.2	0.7	3
	0.800	0.791 ± 0.002	1.1	0.2	3
Intra-day	0.100	0.097 ± 0.002	3.0	2.1	8
	0.250	0.247 ± 0.002	1.2	0.8	12
	0.800	0.792 ± 0.003	1.0	0.3	8

[a] The n-value for Inter-day was the number of samples per day for 3 days and for intra-day was the number of samples assayed on the same day.
[b] Mean $\pm$ S.D. based on $n = 4$.

at a flow rate of 1 ml min^{-1} with detection at 360 nm [17]. The absolute recoveries calculated from a comparison of peak heights of extracted to unextracted analyte were 17–18% for all three types of samples. Increasing the pressure to 325 atm and lowering the temperature to 45°C increased the recovery slightly to 19–21%. Poor recoveries were obviously due to the lack of sufficient solubility of the analyte in hexane.

The next phase of the SFE method development involved the use of a more polar solvent such as an alcohol in the solvent trap. Alcohols may serve as more efficient solvent traps due to their higher solubility of 13-*cis* retinoic acid (7.2 mg ml^{-1} in methanol). In this case, a reversed-phase HPLC system was established to monitor the 13-*cis* retinoic acid recovery; methanol, ethanol, 1-propanol, and 1-butanol were used in the solvent trap at 325 atm and 45°C with 5 min static/15 min dynamic extraction times. Recoveries of 98.6 ± 0.9, 98.0 ± 0.5, 100.2 ± 0.9 and $101.0 \pm 0.9\%$ ($n = 6$) were calculated for methanol, ethanol, 1-propanol and 1-butanol, respectively. Methanol was chosen as the best solvent for the solvent trap based on its compatibility with the HPLC mobile phase and its solubility of 13-*cis* retinoic acid and its photoisomers. In addition, split peaks appeared in the chromatograms obtained from the longer chain alcohols such as propanol and butanol. It was postulated that longer chain alcohols may interact more strongly with the analyte than the mass transfer process of the analyte to the stationary phase. Hence, there would be a lag in mass transfer between the analyte plug and the stationary phase resulting in peak splitting. The peaks for 13-*cis* retinoic acid were much sharper comparing methanol to ethanol and this might be of benefit in assays of the drug and its photoisomers.

The effects of temperature and heated restrictors were investigated. The SFE conditions of 325 atm and 5 min static/15 min dynamic extraction times were kept constant. Both the extraction chamber and heated restrictor were maintained at the same temperature (which was varied between 40 and 65°C). The data revealed that recoveries of 95.1 ± 0.7, 98.6 ± 0.9, 91.7 ± 1.3 and $87.3 \pm 1.5\%$ ($n = 6$) were obtained for 40, 45, 55 and 65°C, respectively. Based on the data, the extraction chamber and heated restrictor were maintained at 45°C.

The effect of the extraction chamber pressure on the percent recovery of 13-*cis* retinoic acid was investigated. Recoveries of the drug using conditions of 45°C and 5 min static/15 min dynamic extraction times were 91.4 ± 0.8, 98.6 ± 0.9, 90.4 ± 1.7 and $87.8 \pm 0.5\%$ ($n = 6$) for 300, 325, 350 and 375 atm, respectively. The data indicated that 325 atm gave the highest extraction efficiency and was selected for the assay. The SFE parameters of 325 atm and 45°C appeared to yield a supercritical fluid that had the correct solvating properties that allowed the efficient transport of 13-*cis* retinoic acid from the solid celite matrix to the methanol solvent trap. The sharp analyte recovery versus pressure is typical of SFE extraction of a solid matrix. Density of the supercritical fluid is greatly affected by pressure changes and this

Table 2
SFE/HPLC assay of spiked placebo dosage forms containing 13-*cis* retinoic acid

Dosage form	Conc. added (μg ml^{-1})	Conc. found (μg ml^{-1})	Percent recovery (%)	R.S.D. (%)
Cream	0.500	0.494 ± 0.005[a]	98.1	0.9
Gel	0.250	0.247 ± 0.0002	98.9	0.6
Capsule	0.800	0.790 ± 0.006	98.8	0.7
Beadlet	0.500	0.500 ± 0.004	100.0	0.8

[a] Mean ± S.D. based on $n = 4$.

relates to the pressure curve which is the ultimate working force matching solubility parameters of the analyte.

The dynamic extraction time was the next parameter to be optimized. With 325 atm, 45°C and 5 min static extraction time, recoveries of 98.9 ± 1.7, 94.9 ± 1.2 and 91.9 ± 0.2% ($n = 6$) were obtained for dynamic times of 5, 10 and 20 min, respectively. This behavior was opposite of what was expected. It was theorized that bubbling the carbon dioxide into the solvent trap may have caused aerosol formation that could transport the drug out of the solvent trap mechanically, thus decreasing extraction efficiency. A value of 5 min was chosen for the dynamic extraction time. Unfortunately, no data was obtained during the study in which a known amount of 13-*cis* retinoic acid would have been added to the solvent trap and extracted with an equivalent volume of carbon dioxide to determine if analyte loss were indeed due to some mechanical transport and not due to decreased extraction of the analyte from the solid matrix.

The static extraction time was then varied from 0 to 7.5 min. The SFE conditions of 325 atm, 45°C and a 5 min dynamic extraction time were kept constant. The results showed recoveries of 97.0 ± 1.7, 98.4 ± 0.9 and 98.2 ± 1.1% for 0, 2.5 and 7.5 min, respectively. The equilibration period allowed by the static extraction time can be important. No static extraction time gave a lower percent extraction compared to increases in static extraction time. When 2.5 min was compared to 7.5 min, there was no significant difference in extraction efficiency; the 2.5 min static extraction time was chosen for 13-*cis* retinoic acid.

The final parameters selected for the SFE extraction of 13-*cis* retinoic acid from the four dosage forms were a pressure of 325 atm, an extraction chamber and restrictor temperature of 45°C, a dynamic extraction time of 5 min, and a static extraction time of 2.5 min. The solvent trap contained approximately 17 ml of methanol which was bought to a volume of 25 ml after the extraction was completed. Using these parameters, a 13-*cis* retinoic acid standard curve was then established with a concentration range which encompassed the expected concentrations of the samples from each of the four dosage forms. The standard curve was plotted using concentrations of 0.05, 0.20, 0.40 and 1.0 g ml^{-1}. Linear regression analysis of concentration versus peak height (mm) showed a correlation coefficient from spiked celite of $r = 0.9991$ with a slope of 7.468 and a y-intercept of 0.1923. Two spiked 13-*cis* retinoic acid samples at 0.10 and 0.80 μg ml^{-1} gave a percent error of 4.75% (R.S.D. = 1.8%) and 0.59% (R.S.D. = 2.1%), respectively, when the predicted concentration from the standard curves were compared to the actual spiked concentrations ($n = 3$). These data showed that the extraction of 13-*cis* retinoic acid was linear in the 0.05–1.00 μg ml^{-1} concentration range in which the extraction of the dosage forms would be performed.

The next phase of the extraction method development for 13-*cis* retinoic acid was to establish the amount of inter/intra variability of the method. These studies were run over a 3 day period at three different 13-*cis* retinoic acid concentrations (0.100, 0.250 and 0.800 μg ml^{-1}; see Table 1). At 0.100 μg ml^{-1} concentration of analyte, inter/intra-day precision was less than desirable. It was decided to work at slightly higher

Table 3
Comparison of SFE/HPLC vs. HPLC assay of 13-*cis* retinoic acid dosage forms

Dosage form	Labeled amount	SFE percent recovery (%)	SFE R.S.D. (%)	HPLC percent recovery (%)	HPLC R.S.D. (%)
Cream	0.1% w/w	98.7 ± 0.2[a]	0.2	99.0 ± 0.1[a]	1.0
Gel	0.05% w/w	105.3 ± 0.2	0.2	107.6 ± 0.1	0.5
Capsule	10 mg	101.4 ± 1.6	1.5	100.9 ± 0.2	1.5
Beadlet	10% v/v	101.5 ± 1.2	1.2	104.0 ± 0.1	0.8

[a] Mean $\pm$ S.D. based on $n = 4$.

13-*cis* retinoic acid concentrations (> 0.250 μg ml^{-1}), since better accuracy and precision data were obtained for both inter and intra-day variabilities.

3.2. Actual sample SFE

The first step toward the extraction of actual dosage forms using the established SFE conditions was to spike the four placebo dosage forms with known amounts of 13-*cis* retinoic acid. Four placebo cream samples were initially spiked with 13-*cis* retinoic acid and the samples were extracted and compared to an unextracted standard. The assay results showed an extraction efficiency of $96.84 \pm 0.54\%$ with an R.S.D. of 0.56% ($n = 12$). It was thought that recovery might be increased slightly by also extracting the standard prepared in methanol. This method was attempted with a spiked placebo cream and a 1.8% rise in the extraction efficiency was obtained. The absolute recovery of the SFE extraction did not change, but the relative recovery of the analyte was improved by treating the standard in an identical manner as the sample. Therefore, each spiked extracted placebo sample was compared to an extracted standard. The recovery data for the spiked placebo cream, gel, capsule and beadlet samples are summarized in Table 2. Extraction efficiencies for the cream, gel and capsules were very similar, all falling within 98.8–100.0%. The beadlets showed a quantitative recovery compared to the other dosage forms. These high extraction efficiencies indicated that the SFE method removed virtually all of the analyte from the spiked placebo matrix. Next, actual dosage forms were analyzed. The SFE data was compared to that obtained from a non-extraction HPLC method. As shown in Table 3, the percent recoveries of the four dosage forms using SFE were comparable to HPLC assays [17] and clearly show that SFE may be applied as an alternate method for sample preparation of these retinoic acid dosage forms.

A preliminary study was performed on 13-*cis* retinoic acid cream to determine if a quantitative recovery of the medication would be obtained by applying the cream sample directly to celite in the extraction cartridge rather than as a spiked methanol solution onto celite. In the study, a 2.5 ml extraction cartridge was employed, and the weighed amount of celite needed to completely fill

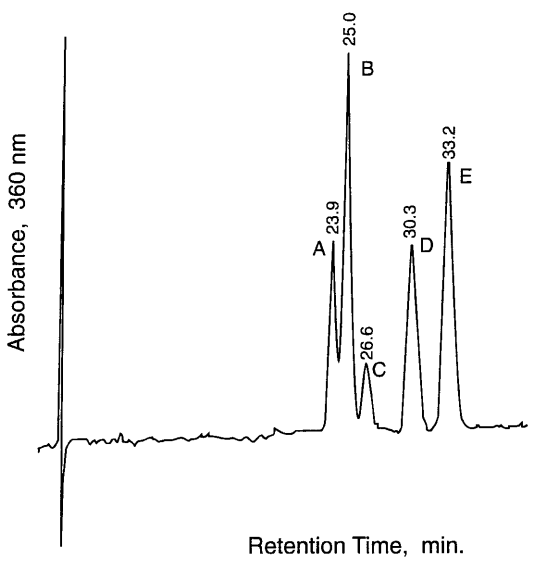

Fig. 2. Typical HPLC chromatogram of (A) 11,13-di-*cis*-retinoic acid, (B) 13-*cis* retinoic acid, (C) 9,13-di-*cis*-retinoic acid, (D) 9-*cis*-retinoic acid, and (E) tretinoin.

Table 4
SFE/HPLC assay of 13-*cis* retinoic acid photoisomers on spiked celite

Analyte	Conc. added (μg ml^{-1})	Conc. found (μg ml^{-1})	Percent recovery (%)	R.S.D. (%)
11,13-Di-*cis*-retinoic acid	0.500	0.459 ± 0.001[a]	91.8	2.5
9,13-Di-*cis*-retinoic acid	0.500	0.452 ± 0.02	90.4	3.4
9-*Cis*-Retinoic acid	0.500	0.456 ± 0.002	91.2	3.3
Tretinoin	0.500	0.462 ± 0.001	92.4	1.5

[a] Mean ± S.D. based on $n = 4$.

the cartridge was previously determined. A 0.25 g quantity of the cream was added to a small porcelain evaporating dish containing approximately 2/3 of the celite needed to fill the cartridge. The cream and celite were then carefully mixed with a glass stirring rod and the mixture was placed in the extraction cartridge with the aid of a spatula. The remaining 1/3 of the celite was mixed with the remaining residue in the evaporating dish and the mixture added carefully to the cartridge. A recovery value of 13-*cis* retinoic acid of 92.6 ± 0.6% (R.S.D. = 0.7%, n = 3) was obtained comparing the extracted cream sample to an unextracted 13-*cis* retinoic acid standard solution. The methanol in the collection trap was initially turbid, but the turbidity largely disappeared upon standing at ambient temperature for 15 min prior to injection into the liquid chromatograph.

The 13-*cis* retinoic acid gel was also added directly to the celite in a likewise manner. A 2.5 ml volume extraction cartridge was also used for the extraction of a weighed quantity of 0.25 g of the gel. The comparison of the extracted gel sample to an unextracted 13-*cis* retinoic acid standard solution showed a recovery of 97.2 ± 2.1 (R.S.D. = 2.2%, $n = 3$). The methanol solvent trap for the gel extraction lacked the turbidity observed previously for the cream.

The percent recoveries of 13-*cis* retinoic acid obtained by direct addition of the cream and gel to the celite were about 4–6% less than those obtained by applying a methanolic solution of the same dosage form to celite prior to extraction. The extraction of the cream added directly to the celite showed a precision comparable to the extraction of the spiked methanol solution, but the gel mixed with celite showed a slightly higher precision that did the sample prepared in methanol. The direct addition of these dosage forms to the extraction cartridge is efficient and may save solvent and sample preparation time.

3.3. Photoisomer SFE

As a concurrent study, the four photoisomers of 13-*cis* retinoic acid were extracted under the same conditions used for 13-*cis* retinoic acid, Fig. 2 shows the chromatographic separation of 13-*cis* retinoic acid from these related compounds on the reversed-phase HPLC system. These included 11,13-di-*cis* retinoic acid, 9,13-di-*cis* retinoic acid, 9-*cis* retinoic acid and tretinoin. The results shown in Table 4 indicated that percent recoveries of 90–92.5% were obtained. These data imply that these compounds can be co-extracted with 13-*cis* retinoic acid from dosage forms and quantitated.

4. Conclusions

SFE has been shown to be directly applicable to the extraction of 13-*cis* retinoic acid from spiked celite and from placebo and commercial dosage forms onto celite. Using 13-*cis* retinoic acid under optimized SFE conditions, co-extraction of the photoisomers was obtained in the 90–95% range. A comparison of the SFE/HPLC method with an unextracted sample/HPLC assay method showed similar results, indicating that SFE could be applied as a sample preparation technique for 13-*cis* retinoic acid dosage forms.

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