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Short communication

Quantitation of the organic solvent extractables (OSE) of petrolatum and analysis by capillary gas chromatography

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1. Introduction

Petrolatum-containing pharmaceutical creams and ointments are often extracted with organic solvents in the preparation of sample solutions for the assay of an active ingredient. The extraction of large amounts of the nonpolar petrolatum base into these polar organic solvents has not been reported, nor has their interference on sample preparation and analysis been previously demonstrated.

Organic extracts of petrolatum-based creams and ointments haze or gel when combined with aqueous solutions owing to organic solvent extractables (OSE) of petrolatum carried into sample preparations. This hazing hinders accurate dilution by coating glassware and blocking complete drainage of pipettes. Membrane filters clog after passing only 4–5 ml of these gelled OSE in solution, and a deterioration of reversed-phase HPLC performance has been seen when these OSE are injected on-column. Petrolatum samples were extracted with organic solvents to model several sample preparation procedures used for the analysis of petrolatum-containing formulations. The OSE of these samples were quantitated and characterized to identify techniques that reduce extractables carried into solution.

Petrolatum is a complex mixture of predominantly saturated hydrocarbons with considerable lot-to-lot variability in its normal, isoparafffin and cyclic paraffin content [1,2]. Unsaturated and aromatic hydrocarbons have been separated from the parent petrolatum by π-complexation between silver ions and carbon–carbon double and aromatic bonds [3]. Various investigators have explored the correlation between the chemical composition and the rheological quality of petrolatums [4–9].

Thirteen petrolatum samples were extracted with heated methanol and acetonitrile. Selected samples were similarly prepared with heated methanol followed by cold filtration, extraction with heated methanol–water (95:5, v/v) and by emulsification and resolidification with tetrahydrofuran and methanol, respectively. The solubil-

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ity of saturated hydrocarbons is influenced by the molecular weight and degree of compound ramification (brancing) and also the temperature and polarity of the solvent.

Petrolatum extractables were examined by gas chromatography (GC) on a single megabore, thin-film, capillary column [10]. The order of elution from the column was correlated with component boiling point and carbon number. The GC elution profile of petrolatums can produce a unique fingerprint suitable for the rigors of forensic evidence [11,12].

2. Experimental

2.1. Materials

Pharmaceutical-grade petrolatum samples (Table 1) were provided by PENRECO (Karns City, PA) and by the Sonneborn Division of Witco (New York, NY). Mineral oil was purchased from Ruger Chemicals (Hillside, NJ). Normal paraffin standards (C5–44), were obtained from ChemService (West Chester, PA). Methanol, acetonitrile, tetrahydrofuran and cyclohexane were purchased from Fisher Scientific (Fair Lawn, NJ). All solvents were of chromatographic purity.

2.2. Extractions

Ten gram petrolatum samples were combined with 200 ml of methanol or acetonitrile in a stoppered flask, heated at 70°C for 10 min, vigorously shaken for 5 min and allowed to equilibrate to room temperature. Petrolatum samples from each supplier producing the highest and lowest yields of OSE were subjected to additional extractions. These selected samples were extracted with 200 ml of heated methanol–water (95:5, v/v), using the same weights and extraction conditions. These four samples were also extracted with 200 ml of heated methanol with the cooled extraction solutions further chilled for 15 min in an ice–methanol bath. Precipitates were removed from the chilled solution by filtration through a Whatman GD/X filter. These selected samples were also emulsified with 50 ml of tetrahydrofuran at room temperature with shaking. The emulsion was resolidified with the addition of 150 ml of methanol and 5 min of vigorous shaking. The extraction solutions were decanted and the remaining solids rinsed with their respective extraction solvent. The combined solutions were reduced to oily residues by rotary evaporation. The residues were dried at 55°C for 30 min and subjected to reduced pressure for an additional 30 min at ambient temperatre. The percentages of OSE were determined gravimetrically.

2.3. Gas chromatography

A Hewlett-Packard (San Fernando, CA) Model 5890 Series II chromatograph was equipped with a cool on-column injector, a Hamilton (Reno, NV) 10 μl tapered needle syringe and a flame ionization detector. An Rtx-1 (dimethylpolysiloxane) fused-silica capillary column (30 m × 0.53 mm i.d., 0.1 μm film thickness) from Restek (Bellefonte, PA) was installed with helium as the carrier gas and the constant flow control set at 5 ml min^{-1}. The initial oven temperature was 50°C. Five minutes after injection, the temperature was increased to 360°C at a constant rate of 3°C min^{-1}, and held at 360°C for 30 min. The injector temperature was controlled to 3°C above the oven temperature and the detector was set to 380°C. Chromatographic data were collected using Turbochrom, Version 4, a data acquisition system from PE-Nelson (San Jose, CA). On-column injections of 2 μl were made of 1 mg ml^{-1} mineral oil, petrolatum and petrolatum OSE dissolved in cyclohexane. Chromatograms were baseline corrected against a blank injection.

3. Results and discussion

Thirteen petrolatum samples were extracted with organic solvents to model several sample preparation procedures used for the analysis of petrolatum-containing formulations. The organic solvent, the aqueous content and the sample preparation technique were all important factors in determining the percentage of petrolatum extractables carried into the sample solution.

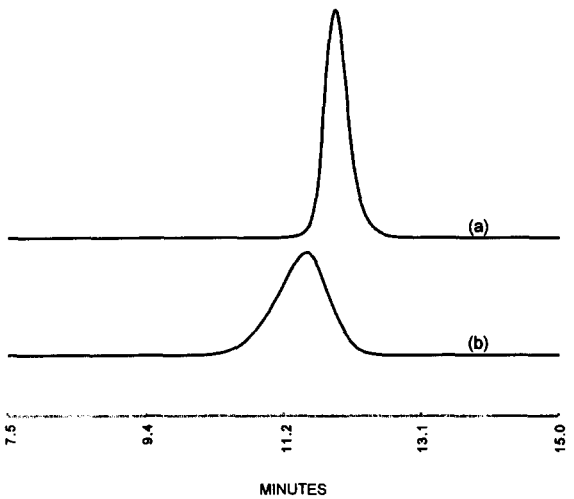

Fig. 1. Effect of OSE on HPLC performance. System: Mometasone Furoate, Assay, USP 23, Supplement 2. (a) Elution of 0.4 μg mometasone furoate in methanol; (b) following a neat injection of 100 μl of petrolatum OSE.

Organic solvent extractables of petrolatum samples were clear to slightly translucent oils, similar to mineral oil in appearance, viscosity and flow characteristics. When OSE were injected directly on to the stationary phase of a reversed-phase HPLC system (Fig. 1), the chromatographic performance deteriorated. Stripping the OSE from the column with a tetrahydrofuran rinse and re-equilibrating with mobile phase restored the column's performance and the original peak shape returned.

Methanol extracts 1.4–2.4 times more OSE by weight from petrolatum than does acetonitrile (Table 1). Methanol extractables are reduced 28–43% by precipitation, using an ice–methanol bath and subsequent filtration. The inclusion of 5% water in methanol extractions is effective (but less consistent) in reducing OSE, yielding 11–73% levels compared with extraction with 100% methanol. Multiple methanol extractions of a petrolatum sample continue to extract OSE, with each subsequent extraction removing less material from the parent petrolatum (Fig. 2). Tetrahydrofuran forms an emulsion with petrolatum at room temperature, but despite resolidification by addition of methanol, 227–346% more OSE are brought into the extraction solvent than by methanol alone.

GC elution of OSE and their parent petrolatums is a simulated distillation, producing a unique GC profile. Two petrolatum samples of similar rheology can each produce individual

Table 1
Percentage organic solvent extractables of petrolatum

Sample	MeOH	MeOH cold-filtered	MeOH–H_2O (95:5, v/v)	ACN	THF–MeOH
PENRECO					
Ultima	5.1	–	–	2.6	–
Super white	3.5	2.3	1.1	2.1	12.1
Snow White	4.9	–	–	3.1	–
Regent	5.8	4.2	1.7	3.7	17.3
Lily White	3.8	–	–	2.5	–
Cream White	4.3	–	–	3.0	–
Witco					
Super White[a]	4.4	–	–	2.2	–
White 1S[a]	3.3	1.9	2.4	1.7	10.8
Yellow 2A[a]	3.3	–	–	1.7	–
Perfecta	8.2	4.7	0.9	3.8	18.6
Super White[b]	4.1	–	–	1.9	–
White[b]	4.5	–	–	1.9	–
Yellow[b]	5.4	–	–	2.8	–

[a] Protopet grade petrolatum.
[b] Fonoline grade petrolatum.

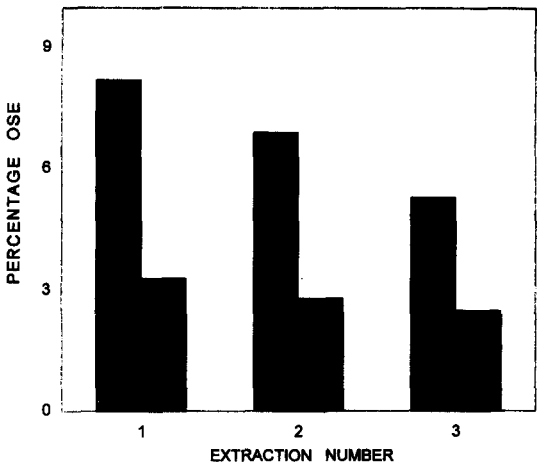

Fig. 2. Perecntage of OSE removed by successive methanol extractions. Light boxes, Perfecta Petrolatum; dark boxes, White Protopet 1S.

chromatographic fingerprints (Fig. 3). The chromatographic "hump(s)" [12] is (are) composed of isoparaffins and cyclanes. These components are sometimes referred to as the isofraction. The normal paraffins are clearly visible as distinct peaks. GC profiles of methanol and acetonitrile OSE samples from the same parent petrolatum were nearly identical.

Most petrolatum OSE components elute the GC column before *n*-tetracontane (Fig. 4c), indicating component boiling points below 510°C and carbon numbers below 40. GC profiles of OSE are similar to the profile of mineral oil [13], with the

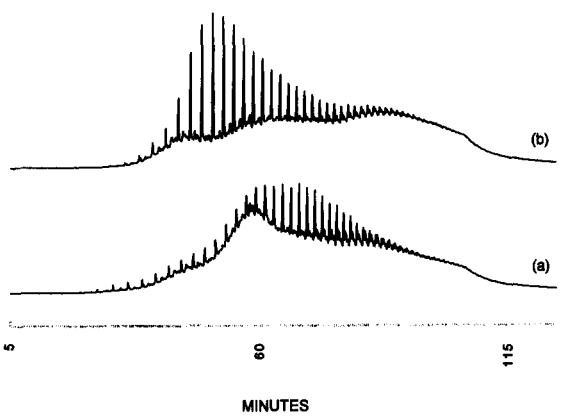

Fig. 3. GC profiles of petrolatums with similar physical properties. (a) Regent; (b) Yellow Fonoline.

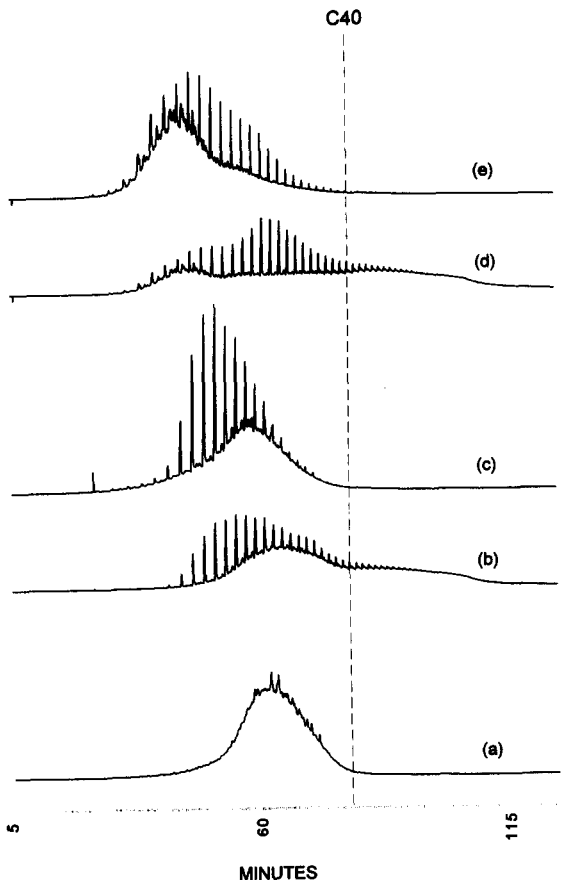

Fig. 4. GC profiles of (a) mineral oil; (b) Super White Fonoline; (c) methanol OSE of Super White Fonoline; (d) Perfecta; (e) methanol OSE of Perfecta. The dashed line marks the elution of *n*-tetracontane.

notable absence of normal paraffins in the mineral oil chromatogram. Normal paraffins longer than C24 (mp. 54°C) were precipitated by chilling. When a parent petrolatum contains components in the C16–25 elution range, these compounds are observed in the OSE profile (Fig 4d and e) and the yield of extractables carried into the solvent is increased.

4. Conclusions

Extraction of petrolatum-containing formulations with methanol, acetonitrile or tetrahydrofuran will carry hydrocarbons into the solvent.

These preparations can haze or gel when combined with aqueous solutions, interfering with sample dilutions and making filtration difficult. When these extractables are carried into a sample solution used for HPLC analysis, they are injected on to the stationary phase and the chromatographic performance can be degraded.

The complete extraction of the acitve ingredient is the flrst priority in the analysis of petrolatum-containing formulations. After this is assured, sample preparation procedures should minimize organic solvent extractables of petrolatum. When the sample preparation requires extraction with a pure organic solvent, the solutions should be subsequently chilled and filtered. Precipitating the normal paraffins and other components reduces OSE by up to a third. An aqueous content as small as 5% reduces the methanol extraction of offending hydrocarbons up to tenfold. Using acetonitrile also reduces the level of extractables carried into the sample preparation compared with methanol extractions. The use of a single extraction, with the addition of an internal standard to account for any volume variation, is recommended since multiple extractions of petrolatum continue to partition extractables into solution. Tetrahydrofuran allows the formation of a petrolatum emulsion and intimate mixing without the use of heated solvents. While it is the most efficient solvent examined for extracting a petrolatum base, tetrahydrofuran also transports the greatest percentage of OSE into solution. Sample preparations using tetrahydrofuran should account for this handicap as well as increased safety concerns.

Acknowledgements

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