

Short communication

Analysis of impurities of isopropyl myristate by gas–liquid chromatography

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Abstract

Isopropyl myristate (tetradecanoic acid isopropyl ester) is commercially available in different qualities with specifications for content as low as 90%. Analysis by gas–liquid chromatography with flame ionization detection revealed up to four impurities exceeding the 0.1% level. Impurities identified by gas chromatography–mass spectrometry were the isopropyl esters of C₁₂ and C₁₆ fatty acids (dodecanoic acid and hexadecanoic acid, respectively) and the respective ethyl and methyl ester of C₁₄ fatty acid (tetradecanoic acid). A relatively high level of impurities was also found in the standard material specified by German pharmacopoeia DAB 10 for calibration of response factors for internal standard quantitation. Therefore, results of quantitative analysis were found to be significantly different depending on the method used for quantitation.

Keywords: Pharmaceutical analysis; Isopropyl myristate

1. Introduction

Isopropyl myristate (IPM), the isopropyl ester of tetradecanoic acid, is used in cosmetics as a substitute for natural oils because it is absorbed easily into the skin and has excellent spreading properties [1]. Additionally, IPM can be used as an emulsifier [2]. In topical pharmaceutical creams and transdermal pharmaceutical preparations, IPM is also used as a co-solvent with skin penetration enhancement properties [3] for actives.

Specifications of IPM for use in pharmaceuticals are published in various pharmacopoeias, e.g., the United States Pharmacopoeia [4], the British Pharmacopoeia [5] or the Deutsches Arzneimittelbuch [6]. These specifications are rather similar with

assayed purity required as greater than 90%. The remaining impurities are not specified in any monograph, leaving up to 10% of the product composition as unknowns.

Possible by-products are ester of hexadecanoic acid and other fatty acids [7]. The impact of those impurities on the properties of IPM, especially skin penetration enhancement is currently investigated in our laboratory.

All three pharmacopoeia describe use of GLC employing packed columns as well as capillary columns. However, no specifications regarding chromatographic separation are given, except for USP [4] where peaks of hexadecanoic acid isopropyl ester and IPM must have a resolution of not less than 6.

The present paper describes a GLC method with flame ionization detection (GC–FID) for separation of IPM from related compounds. Results for IPM

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batches from different manufacturers and different qualities are reported.

2. Experimental

2.1. Reagents and materials

Official reference materials of IPM (IPM USP-CRS, IPM DAB-CRS) were obtained from Promochem (Selze, Germany).

Batches of IPM were purchased from Henkel (Düsseldorf, Germany). Quality was according to DAB 10 [6]. Additionally, samples of IPM were obtained from Synopharm (Barsbüttel, Germany) and Akzo Nobel (Düren, Germany). Quality of these samples was for synthesis and for use in cosmetics, respectively.

1-Decanol was supplied by Merck (Darmstadt, Germany). Ethyl acetate analytical-reagent grade was purchased from J.T. Baker (Deventer, Netherlands). Dodecanoic acid isopropyl ester was supplied by Fluka (Basel, Switzerland). Tetradecanoic acid ethyl ester was purchased from Merck.

All materials were used as received.

2.2. Sample preparation

Internal standard stock solution: 6 g 1-decanol, accurately weighed, is placed in a 100 ml volumetric flask and dissolved in ethyl acetate. Solution is made up to volume using the same solvent.

Internal standard solution: 20.0 ml aliquot of internal standard stock solution is diluted to 2000 ml using ethyl acetate.

IPM sample solution: Place 100 mg of IPM in a 100 ml volumetric flask and dissolve the material in internal standard solution. The solution is made up to volume using internal standard solution.

GC calibration solution: 500 mg IPM reference standard, accurately weighed, is placed in a 50 ml volumetric flask and dissolved in internal standard solution. The solution is made up to volume using internal standard solution. By diluting this solution eight different concentrations are prepared covering the range from 0.1 to 4.0 mg/ml. Use internal standard solution for dilution.

2.3. GC-FID parameters

All GC-FID experiments were performed using a HP 5890 series II GC system equipped with split/splitless injector, flame ionization detector, and HP 7673 autosampler. A 30 m×0.32 mm I.D. DB-Wax (J&W Scientific, Folsom, CA, USA) coated with a 0.25 µm film was used. Column temperature was programmed from 150°C to 190°C with a rate of 5°C/min and then heated to 230°C with a rate of 60°C/min. Final temperature was kept for 2 min. Injector and detector temperatures were 250°C each. Nitrogen with a flow of 1.2 ml/min was used as carrier gas. Split flow and purge were adjusted to 15 ml/min and 3 ml/min, respectively. A 1 µl sample solution was injected in split mode. FID gases were air and hydrogen running at 400 ml/min and 30 ml/min, respectively. Nitrogen was used as make-up gas with a flow of 29 ml/min.

Data acquisition and processing were performed using the HP ChemStation software running on a HP Vectra 486 personal computer. Data were collected at a rate of 10 Hz.

2.4. Validation results

Validation was performed according to ICH guideline Q2B [8]. Results were range 100–4000 µg/ml, correlation coefficient 0.9999, limit of quantification 100 µg/ml, repeatability 0.37% ($n=7$), accuracy 98.8% ($n=7$).

3. Results and discussion

3.1. IPM standard material

For the assay according to DAB 10 internal standard methodology is specified [6]. Calibration of response factor is performed using official IPM reference material (DAB 10 CRS). When analyzing this reference material using the GC conditions described, a number of peaks were observed (Fig. 1). All peaks eluting with retention times shorter than the internal standard were found to be impurities of 1-decanol and are therefore not related to IPM. These peaks are marked with an asterisk in Fig. 1. Apart from those impurities, several other peaks

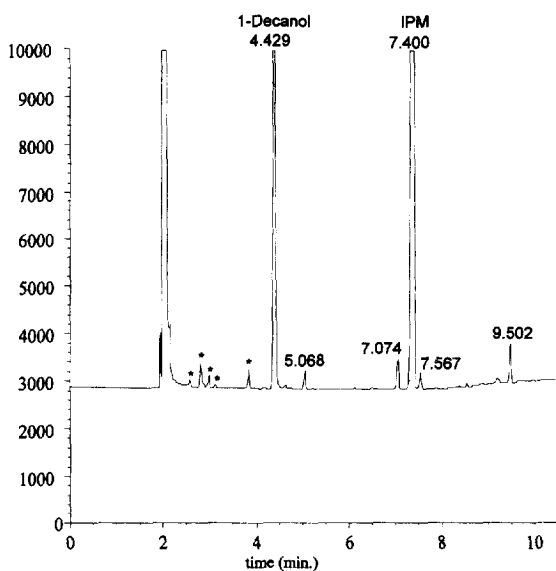


Fig. 1. Chromatogram of IPM reference material (DAB 10 CRS).

were detected. The relative amounts of those with relative retention times (RRT, IPM=1.0) of 0.685, 0.956, 1.023 and 1.284 exceeded 0.1% calculated as IPM.

GC-MS was applied for identification of these 4 peaks. The peak at RRT 0.685 was tentatively identified as dodecanoic acid isopropyl ester ($C_{15}H_{30}O_2$). Library search in the Wiley library gave a match quality of 87.

At RRT 1.284, hexadecanoic acid isopropyl ester ($C_{19}H_{38}O_2$) was found. Library search match quality for isopropyl hexadecanoate was 76 (Wiley library).

These two peaks represent the isopropyl ester of the C_{12} and C_{16} fatty acid analogues of IPM. Both compounds have previously been described to be the most prevalent impurities of IPM [9].

The peak at RRT 0.956 was identified by library search in the Wiley library as tetradecanoic acid methyl ester with a fit of 98.

Separation of the peak at RRT 1.023 from IPM was difficult with the GC-MS system. Despite optimization of initial temperature and rate, no separation could be achieved. Examination of reconstructed ion chromatograms (RIC) for some significant ions of IPM (m/z 270, 228, 102) showed a difference to the TIC indicating co-elution. Examination of mass spectra of the tailing edge of that peak

revealed a signal at m/z 88 as significant difference to those acquired at the leading edge of that peak. Anticipation of McLafferty rearrangement indicates formation of $C_4H_8O_2^+$ (m/z 88) from tetradecanoic acid ethyl ester. Other ions indicating the ethyl ester analogue of IPM, e.g., M^+ at m/z 256 were also present. However, co-elution and high concentration of IPM prevented extraction of a mass spectrum useful for library search. Tetradecanoic acid ethyl ester was therefore obtained as pure material and analyzed by GC-FID and GC-MS. Using the GC-FID system, retention time was identical to that of the peak under consideration. When co-injecting sample solution and solution of tetradecanoic acid ethyl ester, the peak area was increased and no separation was observed. GC-MS analysis of this material revealed a mass spectrum showing the expected ions at m/z 256 and 88, respectively.

USP also specifies an official standard material. However, USP assay is performed using % total peak area method. Therefore, no standard for quantitative analysis is required. The standard material is consequently certified for identity testing only. When analyzing that standard using the GC parameters described only one impurity was detected. This peak was tentatively identified as dodecanoic acid isopropyl ester. Concentration of IPM was calculated as 99.7%.

3.2. IPM raw material

IPM samples of various qualities were obtained from several suppliers. Qualities were "according to DAB 10", "for synthesis" and "for cosmetics". DAB 10 material was specified as stated in the respective monograph. Synthesis and cosmetic material was less thoroughly specified, with the main parameter being assay $\geq 95\%$.

Results from the analysis of the three materials using the method described in the experimental section (Section 2.3) are shown in Fig. 2. Quantitative results are given in Table 1. Fig. 2a shows a typical chromatogram obtained from IPM quality "according to DAB 10". The peaks were tentatively identified as isopropyl ester of C_{12} and C_{16} fatty acids and methyl and ethyl ester of C_{14} fatty acid. Total sum of impurities was 5.4% calculated as % total peak area (Table 1). Results of quantitation of

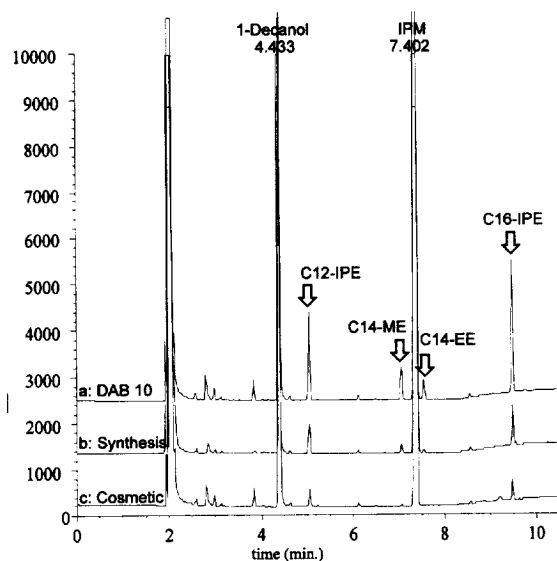


Fig. 2. GC-FID chromatograms of IPM of different qualities. (a) "according to DAB 10"; (b) "for synthesis" and (c) "for cosmetic". C₁₂-IPE: dodecanoic acid isopropyl ester; C₁₄-ME: tetradecanoic acid methyl ester; C₁₄-EE: tetradecanoic acid ethyl ester; C₁₆-IPE: hexadecanoic acid isopropyl ester.

IPM show significant differences when using I.S. or % total peak area method for quantitation (Table 1). These differences are due to the high degree of impurities in the standard material used for calculation of response factors.

The chromatogram in Fig. 2b, for IPM quality

"for synthesis", looks much cleaner. No new impurities could be identified. Total sum of impurities was 1.7% (Table 1). Again, different results for assay were obtained using either I.S. or 100% method for quantitation with I.S. method resulting in considerably higher values (Table 1).

The chromatogram in Fig. 2c shows the results obtained by the analysis of IPM quality "for cosmetic". A number of additional peaks were observed which have been identified before. Total sum of those impurities was 0.7% (Table 1). Results of assay were again different depending on the method used for quantitation (Table 1).

4. Conclusion

Commercially available isopropyl myristate is a mixture of different esters of several fatty acids. The main component (94.8–99.1%) is the isopropyl ester of C₁₄ fatty acid. Up to four impurities were identified in the material. The by-products identified were the isopropyl ester of C₁₂ and C₁₆ fatty acid and the methyl ester and ethyl ester of C₁₄ fatty acids. Chromatographic parameters have to be optimized thoroughly for separation of the methyl and ethyl ester analogues of IPM from the main component.

A high degree of impurities was found in the standard material recommended by DAB 10. When

Table 1
Results of analysis of different IPM qualities

Quality	IPM assay (%)			Impurities (%) (based on % total peak area)				
	I.S. ^c	100% ^b	Certified ^a	C ₁₂ -IPE	C ₁₄ -ME	C ₁₄ -EE	C ₁₆ -IPE	Other
According to DAB 10	96.4	94.5	92.6	1.6	0.7	0.6	2.1	0.4
According to DAB 10	98.8	97.8	98.9	0.4	0.6	0.6	0.2	0.2
According to DAB 10	98.7	97.5	97.1	0.8	0.6	0.5	0.6	–
Cosmetic	101.5	99.3	>95	0.3	–	–	0.4	–
Cosmetic	101.2	99.4	98.5	0.1	–	–	0.5	–
Cosmetic	101.7	99.3	>95	0.3	–	–	0.4	–
Synthesis	99.8	98.3	>99	0.8	0.1	–	0.8	–

^a Supplier certificate.

^b Percent total peak area method.

^c Internal standard quantification, DAB 10 CRS used for calibration.

C₁₂-IPE: Dodecanoic acid isopropyl ester.

C₁₄-ME: Tetradecanoic acid methyl ester.

C₁₄-EE: Tetradecanoic acid ethyl ester.

C₁₆-IPE: Hexadecanoic acid isopropyl ester.

using this standard for calculation of response factors for quantitative analysis by I.S. method, the results were significantly higher than with % total peak area method. When analyzing raw materials, the method of analysis, particularly quantification method and also the standard material must be harmonized between supplier and customer. Due to the problems with standard materials, the % total peak area method appears to be best suited to analysis of IPM for use as pharmaceutical raw material.

System suitability specification should include not only C₁₆ fatty acid isopropyl ester but also the respective methyl and ethyl ester of C₁₄ fatty acid.

All materials tested met the minimum requirements of assayed content ($\geq 90\%$), as specified in the three pharmacopoeias mentioned. However, results indicate that commercially available material often shows significantly higher values. In terms of pricing the cosmetic quality can be purchased at competitive prices.

Therefore, tougher specifications (e.g., $\geq 95\%$) should be applied to ensure maximum product safety.

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