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Short communication

Determination of some insect repellents in cosmetic products by high-performance thin-layer chromatography

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Abstract

A simple and reliable thin-layer chromatographic method for the determination of N,N-diethyl-*m*-toluamide (DEET) and dimethyl phthalate (DMP) in raw material and cosmetic products was developed and validated. A benzene-diethyl ether-cyclohexane (5:3:2, v/v/v) solvent system was used for quantitative evaluation of chromatograms. The chromatographic zones corresponding to the spots of DEET and DMP on the silica gel plates were scanned in the reflectance/absorbance mode at 230 nm.

The method was found to be reproducible and convenient for the quantitative analysis of DEET and DMF in raw material and cosmetic products.

Keywords: Insect repellents; Cosmetics; Diethyltoluamide; Dimethyl phthalate; Densitometry

1. Introduction

Insect repellents represent the oldest and for many years most widely used chemical substances, which discourage insect attack, and as a result offer protection from insect bites [1]. Moskitox® is the mixture of two repellents N,N-diethyl-*m*-toluamide (DEET) and dimethyl phthalate (DMP) in hexylene glycol.

Different analytical methods have were used for identification and the determination of DEET and DMP in cosmetic formulations, biological fluids and postmortem specimens: ion mobility spectrometry [2], polarography [3], IR spectometry [4], titrimetry [5] and chromatography [6–16].

Chromatographic techniques mostly HPLC [6–11] and GC [12–15] were used for their determination. Conventional TLC was used only for identification of DMP [12] and DEET [16].

There are no reports of the simultaneous assay of DEET and DMP in literature using planar chromatography. Instrumental planar chromatography, equipped with automatic application devices and a computer-controlled system for the video evaluation and quantification of chromatograms has been considered a reliable method for quantitative drug analysis [17].

The advantage of instrumental planar chromatography, such as the ability to utilize a low volume of mobile phase as well as to utilize solvents unsuitable for HPLC, speed of separation and low cost, have long been recognized [17].

Therefore this paper focuses on the development of a simple, accurate, and rapid quantitative HPTLC method for simultaneous determination of DEET and DMP.

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2. Experimental

2.1. Chemicals

DEET and DMP were purchased from Merck (Darmstadt, Germany). Methyl-*p*-hydroxybenzoate and propyl-*p*-hydroxybenzoate were obtained from Fluka (Switzerland). Moskitox raw material was obtained from Dragoco (Vienna, Austria).

Cosmetic emulsion *Sole efecta 6+R* contains 5% (w/w) of Moskitox was obtained from ICN Yugoslavia (Belgrade, Yugoslavia). Gel stick *Autmic* contains 20% (w/w) of Moskitox was obtained from Sanitaria (Novi Sad, Yugoslavia). All other chemicals and solvents were of analytical grade.

2.2. Instruments

A TLC Scanner II with a computer system and Cats Software (V.3.15) were provided by Camag (Muttenz, Switzerland).The radiation source was a deuterium lamp. A Nanomat III was used as the application device (Camag). Chromatoplates HPTLC 20×10 cm Silica gel $60F_{254}$ were purchased from Merck.

2.3. Sample preparation

2.3.1. Standard solutions

A stock solution of 1.82 mg/ml of DEET and 1.60 mg/ml of DMP was prepared in ethyl acetate; calibration solutions were prepared by diluting the stock solution, such that the application of 1 μl aliquots covering the ranges 180–900 ng per spot of DEET and 150–800 ng per spot mg of DMP.

2.3.2. Sample solutions

A quantity of 43 mg of Moskitox raw material was transferred to 25 ml calibrated flask and dissolved up to the mark with ethyl acetate. Four milliliters of this solution was then diluted to the mark with ethyl acetate in 10 ml calibrated flask.

A quantity of 400 mg of cosmetic emulsion was transferred to 10 ml calibrated flask and dissolved up to the mark with ethyl acetate.

A quantity of 6o mg of gel stick was transferred to 10 ml calibrated flask and dissolved to the mark with ethyl acetate.

2.4. Chromatography

1 μl loading of each standard and sample solution was spotted on the HPTLC plate. Ascending chromatography was performed in a twin-trough TLC chamber using benzene-diethyl ether-cyclohexane (5:3:2, v/v/v) as a solvent. The chromatographic zones corresponding to the spots of DEET and DMP were scanned at 230 nm in reflectance/absorbance mode. Each plate could accommodate 12 sample spots and 6 standards. The running time was 15 min.

3. Results and discussion

Scanned profiles of HPTLC chromatograms of cosmetic preparations are presented in Fig. 1. Migration distances of DEET and DMP were 32.9±1.5 mm and 67.9±1.4 mm, respectively. Relatively low values of RSD for migration distances of DEET of 4.5% and DMP of 2.0% calculated for 36 spots and applied on three different plates, show high reproducibility of chromatographic system.

The calibration functions for DEET and DMP were tested over the range 180–900 ng per spot for DEET, and 150–800 ng per spot for DMP. The best fit for the calibration lines was found when the calibration data were analyzed using a second-degree polynomial regression. The regression equations. were: $y=114.7+5.6x\pm5.7\cdot10^{-4}x^2$ for DEET and $y=-23.6+2.9x\pm7.0\cdot10^{-4}x^2$ for DMP . The correlation coefficient was >0.997. The standard deviations of calibration curves were 3.7% and 2.3% for DEET and DMP, respectively.

The limit of detection (LOD) was determined by fitting the interday, back-calculated standard deviations of each calibration standard. The *y*-intercept was then equal to SD_0 (the estimated standard deviation at a concentration of zero). The LOD was defined as 3SD. The LOD for DEET and DMP was found to be 37 and 25 ng,which is near experimental value of 30 ng.

Suitability of HPTLC method for quantitative determination of DEET and DMP was further approved through next validation specifications: precision and accuracy. The precision of the method was determined by running replicate samples, each containing 490 and 98.8 ng of DEET and 516 and 103

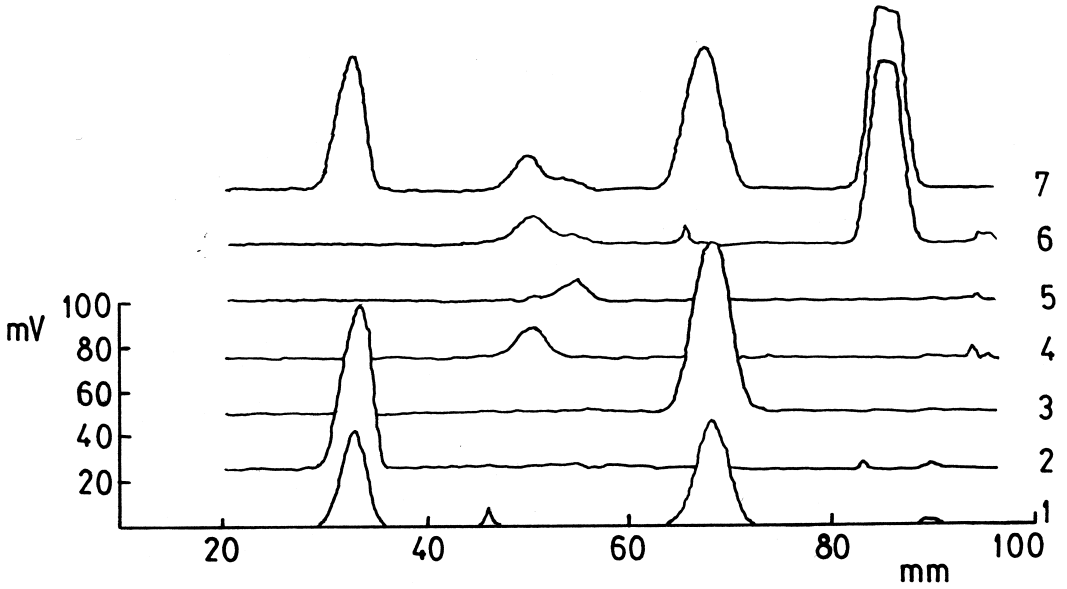

Fig. 1. Densitograms of Moskitox raw material (1); standards of DEET and DMP (2 and 3, respectively); preservatives methyl-*p*-hydroxybenzoate and propyl-*p*-hydroxybenzoate (4 and 5, respectively); mixture of preservatives and sunscreen substances (parsols) (6); and cosmetic emulsion (7).

ng of DMP; the relative standard deviation were 2.6% and 0.75% for DEET and 2.05% and 3% for DMP, respectively. The accuracy of the densitometric method was proved by determination of DEET and DMP from the laboratory-prepared cosmetic emulsion and stick gel spiked with 6.6 mg of DEET and DMP and 4.2 mg DEET and DMP, respectively; Recoveries obtained for DEET were 103.16% and 98.5% and for DMP were 104.7% and 100.8% .

The selectivity of the method was tested and it was found that excipients, preservatives (methyl- and propyl-*p*-hydroxybenzoate), and sunscreen substances (parsols) presented in formulations do not interfere the determination of DEET and DMP, which is shown in Fig.1. Hexylene glycol as an constituent of Moskitox raw material was detected after post chromatographic derivatization with 20% sulfuric acid; the migration distance of the fluorescent zones correspond to hexylene glycol observed at 366 nm was about 25 mm.

The results of quantitative assay of DEET and DMP in raw material, cosmetic emulsion and gel stick are presented in the Table 1.

4. Conclusion

The application of HPTLC with UV scanning densitometry provides a simple, rapid, and reliable system for the assay of DEET and DMP. The instrumental planar chromatography can be used as an alternative chromatographic technique for the determination of insect repellents.

Table 1
Assay results for the determination of DEET and DMP

Sample	DEET		DMP	
	Expected[a] (mg)	Found (mg)	Expected (mg)	Found (mg)
Moskitox Raw material	12.85	12.24±1.6[b]	12.85	12.64±1.3
Cosmetic Emulsion	6.0	5.63±4.1	6.0	6.10±1.6
Gel stick	3.70	3.83±6.0	3.70	3.55±7.3

[a] Calculated according to declaration for contents of DEET of 30% and of DMP of 30% in Moskitox raw material.
[b] Relative standard deviation.

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