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Quantitative and Qualitative Determination of Dimethyl Phthalate and N, N-Diethyl-m-Toluamide in Repellents Commercial Formulations by High Performance Thin Layer Chromatography

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Abstract: In this study HPTLC was used for simultaneous quantitative and qualitative determination of N, N-diethyl meta toluamide (DEET) and dimethyl phthalate (DMP), which are the main elements and active ingredients in current chemical repellents. Some defined amounts of commercial form of 3 repellents included trench pomade, stick insect repellent (SIR), which is containing 33% of DEET and DMP60 (dimethyl phthalate 60%) dissolved in ethyl acetate solvent, separately. The method employed TLC aluminum plate precoated with silica gel plates (SiO_4) $60F_{245}$ as the stationary phase. The solvent system consisted of benzene-diethyl ether-hexane (5:3:2, v/v/v) as mobile phase. The multiple level method used for spotting. Densitometric analysis of repellents was carried out using TLC scanner 3 and CATS4 software in the absorption/reflection mode at 230 nm. According to the results, the type and amount of active ingredients in DMP60 lotion was 61.8 g (SE = ±1.6) per 100 cc and in SIR, 31.3 g (SE = ±0.8) diethyl meta toluamide per 100 g of repellents raw materials. Also the active ingredients in trench pomade were determined as a combination of DMP and DEET by rates of 5.5 g (SE = ±0.2) and 25 g (SE = ±1) per 100 g repellents commercial formulations, respectively. In this study, the value of R_f for DMP and DEET was calculated 0.71±0.2 and 0.32±0.2, respectively. HPTLC is a suitable method to quantitatively and quantitatively determine repellents which have DMP and DEET active ingredients. Since most of commercial chemical repellents have this active ingredient, adjusting and setting HPTLC up can be important.

Key words: Thin layer chromatography, repellents, dimethyl phthalate, diethyl tuloamide

INTRODUCTION

Repellents mostly contain chemicals and are used on skin and clothes to protect human against insects biting and insect born disease (Frances and Writz, 2005; Fradin and Day, 2002). N, N-diethyl meta toluamide (DEET) and dimethyl phthalate (DMP) are most common chemicals included in repellent, which can be formulated alone or in combination with each other and other chemical repellents (Debboun *et al*., 2005; Khoobdel *et al*., 2007). In addition to using them as repellents, they have many other applications in chemical, plastics, cosmetics and other industries (Spurr and McGregor, 2003). Like most of chemicals and insecticides, quantitative and qualitative repellent analysis is mostly done by Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) (Selim *et al*., 1995; Taylor *et al*., 1994; Chen and Wang, 1996; Cherstniakova *et al*., 2006; Kasichayanula *et al*., 2005).

There is only one report of simultaneous assay of DEET and DMP by Thin Layer Chromatography (TLC) in literature (Markovic *et al*., 1999).

Since HPTLC is inexpensive, faster and easier than HPLC and GC (Denistrop, 2000). Adjusting and using this method to determine repellents quantitatively and qualitatively can be very effective.

In this study, it was preferred to firstly adjust HPTLC for simultaneous quantitative and qualitative determination of DEET and DMP which are the active ingredients of many current chemical repellents.

MATERIALS AND METHODS

Chemicals: The trench pomade repellent was obtained from Tolid Daru Co. This repellent is provided in metal tube with cream formulation with a pure weight of 25 g. DMP60 lotion was synthesized in an academic center in Iran. It was formulated in the form of solution and lotion

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in 50 cc glass container with isopropyl alcohol solvent and Towin 80 propellant.

The SIR, which was made with gel stick formulation in faculty of pharmacology of Shiraz University, was bought from Iran pharmacies. This repellent is in the form of cylinder with pure weight of 8.5 g.

All solvents which were used in this study were bought from Merck Co. DEET and DMP standards were obtained from Switzerland Accustandard Ltd.

Silica gel HPTLC plates (SiO_4, $60F_{254}$) which were used as stationary phase, were bought from Merck Co in 20×20 cm dimensions. Capillary tubes with 1, 2 and 5 μL capacity which was used for spotting purchased from CAMAG Ltd.

This study was conducted during 2004-2006 in Chemistry and Biochemistry of Pesticides Laboratory in Tehran University of Medical Sciences in Iran.

Standard and sample preparation: Standard materials were diluted in ethyl acetate (extra pure) so that each 1 μL of the solution contained 1 μg of the standard material.

A quantity of 200 mg of trench pomade, 200 mg of SIR gel stick and 200 μL of DMP60 lotion were separately transferred to three 20 mL volumetric flasks and dissolved up to the mark with ethyl acetate. Decanter was used to separate solvents included in the solution. Then, 5 cc of the each pure solution repellents was picked and dissolved again in 10 cc of ethyl acetate solvent and used to spotting on silica gel plates. To evaluate performance of this method in determining low limitation (LOD), the produced solution was diluted again.

Spotting: One to five microliter loading of each standard and sample solution was spotted on the HPTLC plate, such that the application covering 0.1-20 μg per each spot. In each plate, 16 spot were performed which contained 4 standard spots and 12 samples (from the solution which were prepared with repellents). Distance between tracks was 1 cm. In this study, the multiple level spotting method was used (Fig. 1).

Development: Plates were developed using a mobile phase consisting of benzene-diethyl ether-hexane (5:3:2, v/v/v). Liner ascending was carried out in a twin-trough glass chamber (CAMAG, Switzerland) equilibrated with mobile phase.

The optimized chamber saturation time for mobile phase was 30 min at room temperature. The running time was 22-25 min.

Plate scanning: The developed plate was air-dried for 10 min and then the spots were seen in UV cabinet with 254 nm. The slit dimension was set at 10.0×0.40 mm. The monochromator bandwidth was set at 20 nm and a

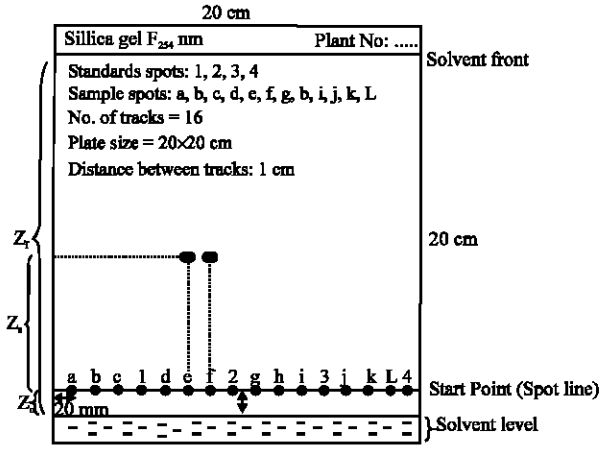

Fig. 1: Schematic of the spotted silica gel TLC plate

scanning speed 20 mm sec^{-1} was employed. Densitometric scanning was performed on CAMAG TLC scanner 3 (V.1.14 S/N: 080320) in absorption/reflection measurement mode at 230 nm and operated by CATS4 software (version4.06, S/N: 805A007). The source of radiation utilized was the deuterium lamp.

TLC parameters calculation: The spot Migration Distance (MD) for the developed spots was defined by the distance between the solvent front and the starting line during the developing period. Spots developing rate or R_f values (Retardation factor) were determined. The position of a substance zone (spot) in a thin layer chromatogram can be described by R_f (Denistrop, 2000, 1991).

The flow constant or velocity constant (K) and mean velocity (V) are measurement of the migration rate of the solvent front. They are important parameters for TLC users. The flow constant and mean velocities are given by the following equation:

$$V = Z_f/t$$
$$K = Z_f^2/t$$
$$R_f = Z_S/Z_f - Z_O$$

Where:

Z_S = Distance of the substance zone from the starting line (mm)
Z_f = Distance of the solvent front from the solvent line (mm)
Z_O = Distance between the solvent level and the starting line (mm)
t = The development time (min)

The Limit Of Detection (LOD) was determined by spotting using solution containing 10-200 ng μL^{-1}. one

microliter from each solution was spotted on the TLC plate to obtain LOD range of 50-100 ng per spot. Each concentration was spotted six times on the TLC plate.

The sensitivity of the method was determined with respect to LOD, linearity range and correlation coefficient. The LOD was calculated as 5 times the noise level (Nitin and Mangal, 2007).

Data analysis which included average amount of materials in each spot was automatically done by CATS4 software. In addition, the amount of DEET and DMP in the performed solution was calculated by a simple proportion in volume or weight of each repellent. The variance analysis (ANOVA) was used to compare the average of DEET and DMP by HPTLC method for the 3 studied repellents and the GC analysis values reported by producer companies.

RESULTS

Spots scans and analysis on silica gel plates showed that the type of active ingredients of repellents in lotion and SIR were just DMP and DEET, respectively. The active ingredient in trench pomade is a combination of DMP and DEET. Scanned profiles of HPTLC chromatograms of trench pomade in integration phase are presented in Fig. 2.

According to the densitograms of repellents raw materials, DMP60 and SIR have a pick in their curves, but in trench pomade there were 2 picks which were, respectively compatible with DEET and DMP standard picks (Fig. 3).

The Migration Distance (MD) and R_f for DMP and DEET were calculated as:

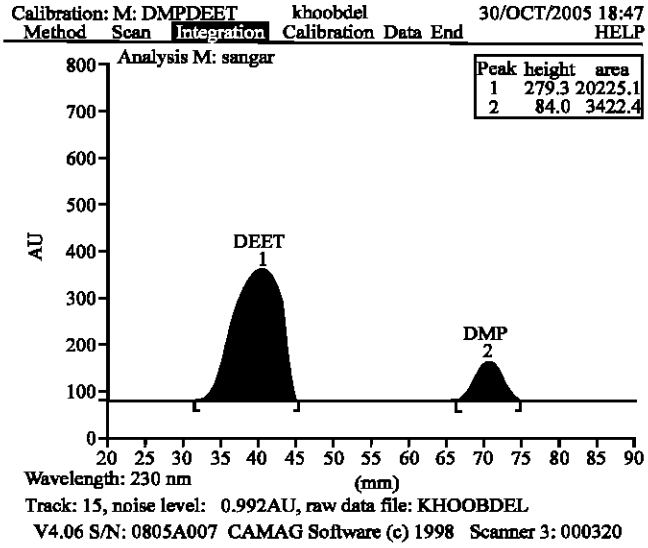

Fig. 2: The integration of trench pomade raw material (the area under curves is the amount of DEET and DMP in trench pomade, respectively)

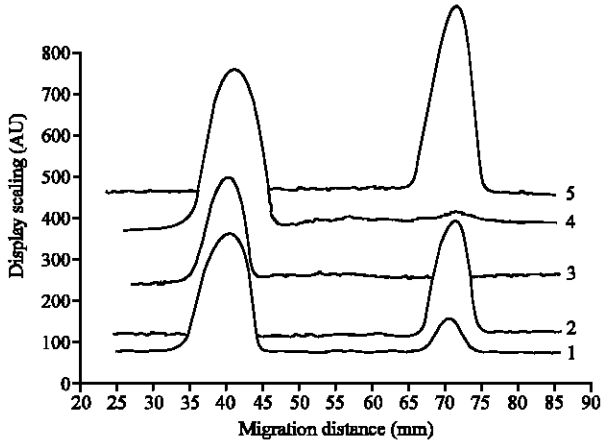

Fig. 3: Densitograms of repellents raw materials; (1) Trench pomade, (2) Standard of DMP, (3) Standard of DEET, (4) Stick Insect Repellent (SIR) or DEET 33% and (5) DMP60

Table 1: The amount of DEET and DMP in three repellents (active ingredient (g) of repellents per each 100 g or 100 cc of the repellent commercial form)

	Active ingredients (g) (± SE)	
Repellents	DEET	DMP
DMP60(lotion)	-	61.8 (± 1.6)
SIR	31.3 (± 0.8)	-
Trench pomade	25 (± 1)	5.5 (± 0.2)

SIR: Stick Insect Repellent

MD_{DMP} (±SD) = 56.7±0.66 mm
MD_{DEET} (±SD) = 25.9±1.05 mm
$R_{f\,DMP}$ = 56.7/80 = 0.70±0.2
$R_{f\,DEET}$ = 25.9/80 = 0.32±0.2

The flow constant (K) and solvent mean velocity (V) were also calculated as:

$K = (85)^2/23.5 = 307.5\ mm^2\ min^{-1}$
$V = 85/23.5 = 3.62\ mm\ min^{-1}$

To calculate the amount of DMP and DEET in each spot, CATS4 software was used and after that, the amount and percentage of these materials determined in each repellent (Table 1).

DISCUSSION

In this study, the low limitation of HPTLC for both DEET and DMP was determined 50-100 ng.

In this study, the limit of detection (LOD) for both DEET and DMP was determined 50-100 ng.

The combination of trench pomade, DMP60 lotion and SIR which was quantitatively and qualitatively determined by HPTLC in this study, is compatible with the characteristics presented by companies and producers of repellents. Comparison between determined amount of DEET and DMP in studied repellent using HPTLC and presented values by producers using GC showed that there was not a significant difference in determination of repellents type and amount $p>0.05$ (Table 2). Therefore, HPTLC is more suitable and easier to analyze repellents which have DEET and DMP active ingredients than other methods. Although it had been tested and confirmed by Morkovic *et al.* (1999) in Serbia to determine repellents in cosmetic products, but the difference is that they had used Benzene-Diethyl ether-cyclohexane solvent to develop and appear spots.

In this study because of cyclohexan toxicity, hexane was used instead. With this solvent combination, the LOD of DEET and DMP was acceptable and determined 50-100 ng.

Table 2: Comparison between HPTLC analysis of repellents and GC results by the producer company (type and amount)

	Analysis methods (%) (± SE)	
Repellents	GC	HPTLC
DMP60 (lotion)	DMP 60%	DMP 61.8% (± 1.6)
SIR	DEET 33%	DEET 31.3% (± 0.8)
Trench pomade	DEET(24-25)% + DMP (4-5)%	DEET 25.0% (± 1.0) + DMP 5.5% (± 0.2)

SIR: Stick Insect Repellent

In Morkovic *et al.* (1999) study, the LOD for DEET and DMP was determined 37 and 25 ng, respectively, which is near to present finding. It is possible to correct that by precise adjusting of the method. The replacement of Cyclohexane with hexane in our mobile phase was effective in spot development and appearance.

In conclusion HPTLC is a fast, reliable, simple, inexpensive and suitable method for quantitative and qualitative simultaneously determination of DEET and DMP.

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