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Solid-phase extraction and liquid chromatographic quantitation of insect repellent N,N-Diethyl-*m*-toluamide in plasma

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Abstract

A sensitive and reliable method based on solid-phase extraction and reversed-phase liquid chromatography was developed and validated for the quantitation of insect repellent *N,N*-diethyl-*m*-toluamide (DEET) in plasma. *N,N*-diethyl-2-phenylacetamide was used as internal standard in the extraction which employed C_{18} solid-phase extraction cartridges. The wash solvent was 3 ml acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (10:90, v/v), and the eluting solvent was 1 ml acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (40:60, v/v). The eluent obtained from the extraction cartridge was directly analyzed on a reversed-phase C_8 column with UV detection at 220 nm. A clean chromatogram and high sensitivity were achieved at this wavelength. The mobile phase was acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (36:64 v/v). The retention time was 7.9 min for the internal standard and 9.6 min for DEET when the mobile phase was delivered at 1.0 ml min^{-1}. The overall absolute recovery was 97.7% with a standard deviation (SD) of 3.9 ($n = 9$) for DEET and 100.2% with a SD of 3.4 ($n = 3$) for the internal standard. The limit of quantitation was found to be 15 ng ml^{-1} with a relative standard deviation of 12%. For the analyses of DEET-spiked plasma samples with five replicates each at 50, 500 and 1500 ng ml^{-1}, the overall intra- and inter-day precisions were 5.7% and 5.5% respectively, and the overall intra- and inter-day accuracies were 2.0% and 2.4% respectively. The correlation coefficient for calibration plots in the concentration range 15–1500 ng ml^{-1} was typically 0.999. The method was applicable to both dog and human plasma samples and was successfully used in pharmacokinetic studies of DEET in beagle dogs after intravenous bolus and topical routes of administration.

Keywords: Insect repellent; *N,N*-diethyl-*m*-toluamide; Plasma assay; Reversed-phase liquid chromatography; Solid-phase extraction

1. Introduction

N,N-diethyl-*m*-toluamide (DEET) is the most widely used all-purpose insect repellent worldwide [1,2]. Its repellent activity has been demonstrated in more than 20 genera of insects [3]. In protection-time tests, DEET was found to be highly effective against yellow-fever mosquito (*Aedes aegypti*), salt-marsh mosquito (*Aedes taeniarhynchus*) and common malaria mosquito (*Anopheles*

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quadrimaculatus). It was also highly effective against flies such as stable fly (*Stomoxys calcitrans*), deer fly (*Chrysops atlanticus*) and sand fly (*Ulicoides canithorax*) [4,5]. It is estimated that about 40 million packages of DEET product are sold in the United States every year and over 30% of the United States population use DEET-containing insect repellents annually [1].

Although the general safety of DEET has been established by extensive animal testing and about 40 years of human experience with commercial formulations containing DEET at levels as high as 100% [1,2], the toxicological characterization of DEET remains incomplete as evidenced by incidences of both local and systemic toxic reactions. Cases of toxic encephalopathy [6–8], acute manic psychosis [9], and adverse skin reactions [10–12], which were caused by extensive dermal and transdermal absorption of DEET, have been reported. In some studies, DEET was shown to have an enhancing effect on the transdermal absorption of drugs, insecticides and herbicides [13–15].

A liquid chromatographic method for the quantitation of DEET in serum using solid-phase extraction in sample preparation was described [16]. In this method, C_{18} solid-phase extraction cartridges were preconditioned with 5 ml of methanol and 30 ml of water. 2 ml of serum samples were diluted with 10 ml of saline and passed through the cartridges. The cartridges were rinsed twice with 30 ml of deionized water before they were eluted with 5 ml of methanol. The eluents were reduced to 2 ml with a solvent evaporator and analyzed by reversed-phase liquid chromatography on a C_8 column with UV detection at 240 nm. The limit of quantitation was reported as 180 ng ml^{-1}.

The generally low limit of quantitation for DEET achieved by the liquid chromatographic method could be attributed to the spectrophotometric property of DEET on which the detection depended. DEET is a neutral compound with a poor UV absorption profile and does not fluoresce. Its UV absorbance falls sharply on increasing the wavelength from 200 nm to 225 nm, becomes weak at wavelengths greater than 225 nm, and approaches zero beyond 250 nm. The strategy to detect it sensitively by a spectrophotometric method is to use as short a wavelength as possible. However, the challenge imposed by this approach is that many of the endogenous components in plasma or serum exhibit strong UV absorption in this wavelength region, which could cause strong interference. In order to monitor the eluent at a short wavelength to achieve high sensitivity in the liquid chromatographic analysis, a highly effective extraction procedure is required.

The primary objective of this study was aimed at achieving a high limit of quantitation for the assay and making it useful in the pharmacokinetic studies of DEET after low dose administration. An effective solid-phase extraction procedure which could selectively recover the analytes from the plasma matrix and enable the employment of UV detection at 220 nm in liquid chromatographic analysis was developed.

2. Experimental

2.1. Materials and instrumentation

DEET was obtained from Sigma Chemical Co. (St. Louis, MO). Ammonium acetate and the internal standard *N,N*-diethyl-2-phenylacetamide were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Glacial acetic acid and HPLC-grade acetonitrile were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). Water used in the sample preparation and liquid chromatographic analysis was doubly-distilled and further purified by a Millipore water purification system (Continental Water Systems Corp., El Paso, TX). Millipore filter membranes (Type GS, 0.22 μm) for buffer and mobile phase filtration were obtained from Millipore Corp. (Bedford, MA). All the chemicals were of the highest purity commercially available. The Vac-Elut™ manifold and the Bond-Elut® cartridges (CN, C_8, and C_{18}; 100 mg, 1 ml) used in the solid phase-extraction were obtained from Varian Analytical Supplies Co. (Harbor City, CA).

The liquid chromatographic system consisted of a pump (Beckman 112 Solvent Delivery Module, Beckman Instruments, Inc., Fullerton, CA), a single-loading injector (Rheodyne model 7125,

Rheodyne Inc., Cotati, CA) with a 50 μl sample loop, a guard column (Perisorb® RP-18, P.J. Cobert Associates, Inc., St. Louis, MO), a reversed-phase C_8 column (Microsorb-MV™, 150 mm × 3.9 mm i.d., Rainin Instrument Co., Woburn, MA), a variable wavelength UV detector (Spectro Monitor III Model 1204A, Laboratory Data Control, Riviera Beach, FL) and an integrator (HP3396B, Hewlett-Packard, Wilmington, DE).

2.2. Preparation of standard solution

Concentrated stock solutions of DEET and the internal standard were prepared at 500 μg ml^{-1} in acetonitrile. Dilute stock solutions were prepared from the concentrated solutions with water at 5 μg ml^{-1} and 500 ng ml^{-1} for DEET, and 5 μg ml^{-1} for the internal standard. They were stored in a refrigerator at 4°C. Reference standards of DEET for the recovery study were prepared with the mobile phase to obtain concentrations of 50, 500 and 1500 ng ml^{-1}. For calibration and recovery study, spiked plasma samples of 15–50 ng ml^{-1} and 100–1500 ng ml^{-1} were prepared using the 500 ng ml^{-1} and 5 μg ml^{-1} dilute stock solutions respectively. Acetate buffer (pH 4.5; 0.03 M) was prepared by mixing equal volumes of acetic acid (0.06 M) and ammonium acetate (0.06 M), and the pH was adjusted accordingly with the addition of ammonium acetate (0.03 M) or acetic acid (0.03 M). It was filtered through a 0.22 μm Millipore membrane before use.

2.3. Solid-phase extraction

1 ml of plasma sample was dispensed into a 5 ml disposable glass culture tube. 200 μl of the internal standard dilute stock solution and 3 ml of water were added. The mixture was briefly vortexed prior to the solid-phase extraction.

C_{18} solid-phase extraction cartridges (100 mg, 1 ml) were placed on the Vac-Elut™ manifold which was connected to a vacuum source. The preconditioning was accomplished by washing the cartridges with 2 ml of acetonitrile followed by 2 ml of water. Only a low vacuum (< 0.5 in. Hg) was applied to moderate the speed so that the C_{18} sorbent in the cartridges could be fully activated by this procedure. A small volume of water was left in the cartridge to prevent the sorbent from drying before the loading of a sample.

The plasma sample which had been diluted and spiked as described was transferred into a preconditioned C_{18} cartridge using a disposable glass pipet ≈ 1 ml at a time. Low vacuum (< 0.5 in. Hg) was used to moderate the extraction speed at ≈ 0.5 ml min^{-1}. Drawing and dispensing of the sample were performed slowly with caution in order to avoid the formation of air bubbles in the cartridge which could hinder the plasma sample from passing through the sorbent bed. With the tip of the glass pipet placed against the inner wall of the cartridge, the formation of air bubbles was greatly reduced. After all the plasma sample was passed through the cartridge, the culture tube was rinsed twice with 0.5 ml of water which was also loaded into the cartridge.

The optimized wash solvent was 3 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (10:90, v/v). The wash solvent was introduced into the cartridge ≈ 1 ml at a time while rinsing the inner wall. Washing in this manner achieved maximum elimination of the endogenous plasma components adsorbed on the inner wall of a cartridge and prevented them from being present in the eluent and thus causing interfering peaks in the chromatogram. The wash speed was also adjusted to ≈ 0.5 ml min^{-1} by applying a low vacuum (< 0.5 in. Hg). Higher vacuum (≈ 5 in. Hg) was applied to eliminate the excess wash solvent remaining in the sorbent at the end of the wash. The cartridge was then removed from the Vac-Elut™ manifold. The housing hole for the cartridge was rinsed with 0.5 ml of water followed by 0.5 ml of acetonitrile and then dried by applying a high vacuum (≈ 10 in. Hg).

The washed cartridge was reseated on the Vac-Elut™ manifold and eluted with 1 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (40:60, v/v). The elution was performed under low vacuum (< 0.5 in. Hg) so as to moderate the elution speed at ≈ 0.5 ml min^{-1}. Slow elution was critical to high recovery since it facilitated sufficient interactions among the analytes, sorbent and eluting

solvent. The eluting solvent retained in the sorbent was recovered by using a high vacuum ($\approx$ 10 in. Hg) at the end of the elution. The eluent from the cartridge was then vortexed and directly injected onto the column for quantitation. Up to eight samples could be processed simultaneously on the Vac-Elut™ manifold by an operator.

2.4. Chromatographic condition

The mobile phase for the liquid chromatographic analysis was acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (36:64, v/v) and was delivered at 1.0 ml min^{-1}. It was filtered through a 0.22 μm Millipore membrane, degassed sonically and kept overnight before use. The eluent was monitored by UV detection at 220 nm with the sensitivity of the detector set at 0.01 AUFS. The attenuation of the integrator was set at 4, and the chart speed was 0.2 cm min^{-1}. The cleanliness of the liquid chromatographic system was maintained every working day by rinsing it with methanol, methanol–water (50:50, v/v) and water for 30 min in sequence at a flow rate of 0.5 ml min^{-1}. The chromatographic analysis was performed at ambient temperature.

3. Results and discussion

3.1. Selection of internal standard

Effort was devoted to the selection of an internal standard which has a similar chromatographic property to DEET. Since the sample was to be processed by solid-phase extraction, the internal standard should have simlar adsorption and desorption properties to those of DEET during the extraction. To achieve these goals, several DEET structure-similar amides were screened. *N*,*N*-diethyl-2-phenylacetamide, an isomer of DEET, was found to be an excellent internal standard since it turned out to have similar extraction recovery and chromatographic retention time to those of DEET under various extraction and chromatographic conditions.

3.2. Selection of sorbent for solid-phase extraction

DEET is a neutral amide with an aromatic ring structure and is practically insoluble in water [17]; nonpolar interaction was thus considered as the primary mechanism in solid-phase extraction. Among the non-polar sorbents commercially available, cyanopropyl (CN), octyl (C_8) and octadecyl (C_{18}) were evaluated for DEET in terms of adsorption and desorption properties.

Of the endogenous plasma components adsorbed on the sorbent along with DEET and the internal standard, some are more lipophilic than others. Among the lipophilic components, some are stronger and some weaker than the analytes in terms of lipophilicity. The strategy undertaken was to wash off the hydrophilic components by using a polar wash solvent, and then elute the analytes selectively with a less polar eluting solvent. Therefore, the wash solvent should have the strength to wash off only the plasma components which were more hydrophilic than the analytes in order to achieve high recovery. Similarly, the eluting solvent should be just strong enough to elute down the analytes while keeping the plasma components which were more lipophilic than the analytes retained on the sorbent so as to obtain a clean chromatogram without late-eluting peaks. This strategy required the sorbent to have a moderate affinity for the analytes.

The use of a CN cartridge led to the cleanest extraction but low recovery, which was probably due to the weak lipophilicity of the CN sorbent. The C_{18} cartridge was demonstrated to be a better choice than the C_8 cartridge for the analytes since the nonpolar interactions between C_{18} sorbent and the analytes were stronger. The analytes could be more firmly adsorbed on the sorbent and thus allowed the use of a stronger wash solvent, which in turn resulted in a more efficient removal of the polar plasma components while enabling DEET to be eluted selectively with high recoveries using an eluting solvent of suitable strength.

3.3. Optimization of wash and eluting solvent

Effects of pH, solvent modifier and amount of wash solvent on wash efficiency were scrutinized. The wash solvents tested included hydrochloric acid (0.01 M), ammonium acetate buffer (pH 4.5; 0.03 M), sodium phosphate buffer (pH 5.9; 0.03 M), pure water and potassium chloride (0.01 M). Methanol and acetonitrile, the solvent modifiers, were examined in the wash solvents at various concentrations (5%, 10%, 15%, 20% and 30%, v/v). The amount of wash solvent was tested in an incremental fashion (0.5, 1, 1.5 and 2 ml) when methanol or acetonitrile was used as the eluting solvent. The eluent was evaporated under a nitrogen stream to dryness and reconstituted with 1 ml of the mobile phase before being injected onto the column for quantitation. Direct injections of the eluents were also tried. However, these tests all yielded unsatisfactory chromatograms in which one or more of the following problems occurred: (1) unstable baseline; (2) peaks of DEET and the internal standard overlapping with the interfering peaks of plasma origin; and (3) peaks of plasma origin with retention times as long as 90 min present in the chromatogram. The baseline problem was exacerbated when a higher level of methanol was involved in the procedure. It is suspected that substantial UV absorption of methanol at 220 nm was responsible for this phenomenon. Methanol was excluded from further tests for this reason.

Among the unsatisfactory chromatograms obtained from testing of the wash solvents, one with good recovery and baseline-resolution of the analyte peaks was yielded when acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (10:90, v/v) was used as the wash solvent. However, the chromatogram was far from satisfactory because of the presence of several late-eluting peaks. This problem was later eliminated in the optimization of eluting solvent where a series of acetonitrile–ammonium acetate solvents (pH 4.5; 0.03 M) (60:40; 50:50; 40:60 and 30:70, v/v) was tested. It was found that the use of 1 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (40:60, v/v) resulted in a clean chromatogram without late-eluting peaks. The lipophilic components in plasma which accounted for late-eluting peaks were effectively retained on the sorbent during elution. Interestingly, the level of acetonitrile in the eluting solvent was very critical for successful DEET elution as evidenced by the following two observations: (1) the use of 1 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (35:65, v/v) as the eluting solvent resulted in a mean extraction recovery of 72.3% ($n = 3$); (2) using 1 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (45:55, v/v) led to the presence of late-eluting peaks in the chromatogram; and (3) the number of late-eluting peaks increased when a higher level of acetonitrile was included in the eluting solvent. It was also found that the extraction reproducibility was undermined, reflected by the occasional appearance of small interfering peaks in the chromatogram, if ammonium acetate buffer (0.03 M) was not incorporated in the wash and eluting solvents. This can be explained by the fact that the adsorption and desorption properties of some of the plasma components were pH-sensitive, and the adsorption and desorption became consistent when the solvents were buffered.

Taking the resolution, recovery and cleanliness of the chromatogram into consideration, the solvent conditions for the solid-phase extraction were finalized: 3 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (10:90, v/v) as the wash solvent, and 1 ml of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (40:60, v/v) as the eluting solvent. The combined use of these wash and eluting solvents yielded clean eluents which were directly injected in liquid chromatographic analysis. No condensation or reconstitution was required due to the facts that the baseline was stable, and the limit of quantitation provided by this procedure was satisfactory. Direct injection of the eluent greatly accelerated the entire analysis.

3.4. Selection of column and mobile phase

Both reversed-phase C_8 and C_{18} columns were used in the method development. They all provided satisfactory results for the samples processed as described. Peaks of DEET, the internal standard and the residual plasma components were baseline-resolved using acetonitrile–ammo-

nium acetate (pH 4.5; 0.03 M) (36:64, v/v) as the mobile phase at a flow rate of 1.0 ml min^{-1}. Nevertheless, the C_8 column was preferred because peaks of the internal standard and DEET were eluted ≈ 6 min faster than when a C_{18} column was used. The advantage of employing a C_8 column is particularly prominent when a large number of samples are to be analyzed.

Acetonitrile was selected as the organic modifier for the mobile phase because of its low absorbance at 220 nm. It was finalized at 36% (v/v) since this was the highest level at which the peaks of DEET and the internal standard has short retention times whilst still being baseline-resolved. It was observed that the retention time for DEET or the internal standard did not change significantly as the pH of the mobile phase varied. This is probably due to the neutral property of DEET and the internal standard. However, ammonium acetate (pH 4.5; 0.03 M) was used in the mobile phase because it was effective at stabilizing the baseline.

3.5. Chromatogram

Good chromatographic profiles were obtained for the dog plasma samples when they were processed under the final solid-phase extraction and liquid chromatographic conditions. It was also observed that human plasma spiked with DEET and the internal standard yielded good chromatograms when subjected to the same procedures. Fig. 1 shows sample chromatograms of dog and human plasma and a chromatogram obtained using the reported extraction method. The retention time was 7.9 min for the internal standard and 9.6 min for DEET with the use of acetonitrile–ammonium acetate (pH 4.5; 0.03 M) (36:64, v/v) as the mobile phase at a flow rate of 1.0 ml min^{-1}.

3.6. Calibration and linearity

The standard calibration plot of peak area ratio (DEET/internal standard) against DEET concentration was constructed by analyzing 1 ml plasma samples spiked with the internal standard at 1000 ng ml^{-1} and DEET at 15, 50, 100, 200, 300, 500, 1000 and 1500 ng ml^{-1}. 50 μl of the 1 ml of eluent obtained from the extraction cartridge was injected in the chromatographic analysis. As shown in Table 1, the concentration–peak area ratio relationship was found to be linear in the concentration range 15–1500 ng ml^{-1}. Using regression analysis, the following equation was obtained from three calibration plots prepared on three separate days within 1 week:

$$R = 0.009926 + 0.001330C$$

where C (ng ml^{-1}) is the concentration of DEET in plasma, and R is the peak area ratio (DEET/internal standard). The intercept and slope were mean values.

3.7. Extraction efficiency

The absolute extraction recovery of DEET by this method was determined at concentrations of 50, 500 and 1000 ng ml. The absolute recovery of the internal standard at 1000 ng ml^{-1} was also assessed in order to confirm the reliability of *N,N*-diethyl-2-phenylacetamide as internal standard. Spiked plasma samples at each concentration were extracted and analyzed under the described conditions in three replicates. The peak area was compared to the mean peak area resulting from the analyses of three reference standards containing corresponding levels of DEET or the internal standard. As shown in Table 2, high recoveries were obtained at low (50 ng ml^{-1}), medium (500 ng ml^{-1}) and high (1000 ng ml^{-1}) concentrations. The overall absolute recovery was 98.7% with a SD of 3.6 ($n = 9$) for DEET, and 98.3% with a SD of 3.7 ($n = 3$) for the internal standard. *N,N*-diethyl-2-phenylacetamide, an isomer of DEET, proved to be an excellent internal standard for this method owing to the fact that high and similar absolute recoveries were obtained for the two analytes.

3.8. Limit of quantitation

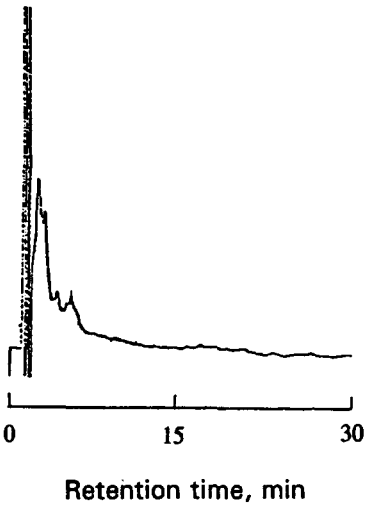
The limit of quantitation was determined by analyzing spiked plasma samples at the presumed lowest quantifiable level in five replicates. At 15 ng ml^{-1}, the relative standard deviation (RSD)

was 12% when 1 ml of plasma was used and 50 μl of the 1 ml of eluent obtained from the extraction cartridge was analyzed. Thus, the limit of quantitation was accordingly set as 15 ng ml^{-1} for this method.

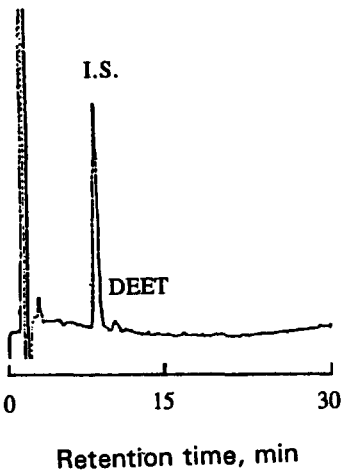
3.9. Precision and accuracy

The study of intra- and inter-day variations used blank plasma samples spiked with the internal standard at 1000 ng ml^{-1} and DEET at 50, 500, and 1000 ng ml^{-1}. In the evaluation of intra-day variation, five replicates were prepared at each concentration and analyzed on one single day. The inter-day variation was evaluated on five consecutive days by analyzing one sample prepared at each concentration every day. As shown in Table 3, precision, defined as RSD (%), ranged from 2.6% to 11.1%; accuracy, defined as the relative deviation (%) of the measured from the theoretical, was not greater than 5.1%. The overall intra- and inter-day precisions were 5.7% and 5.5% respectively, and the overall intra- and inter-day accuracies were 2.0% and 2.4% respectively.

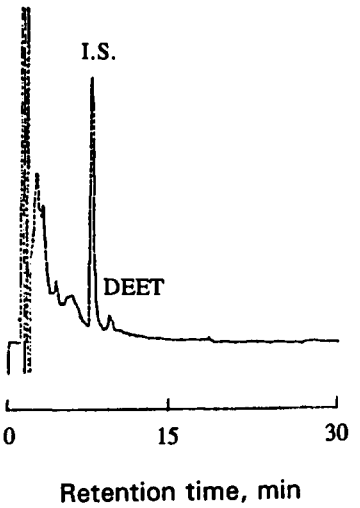
(A)



(C)



(B)



(D)

Fig. 1(A–D)

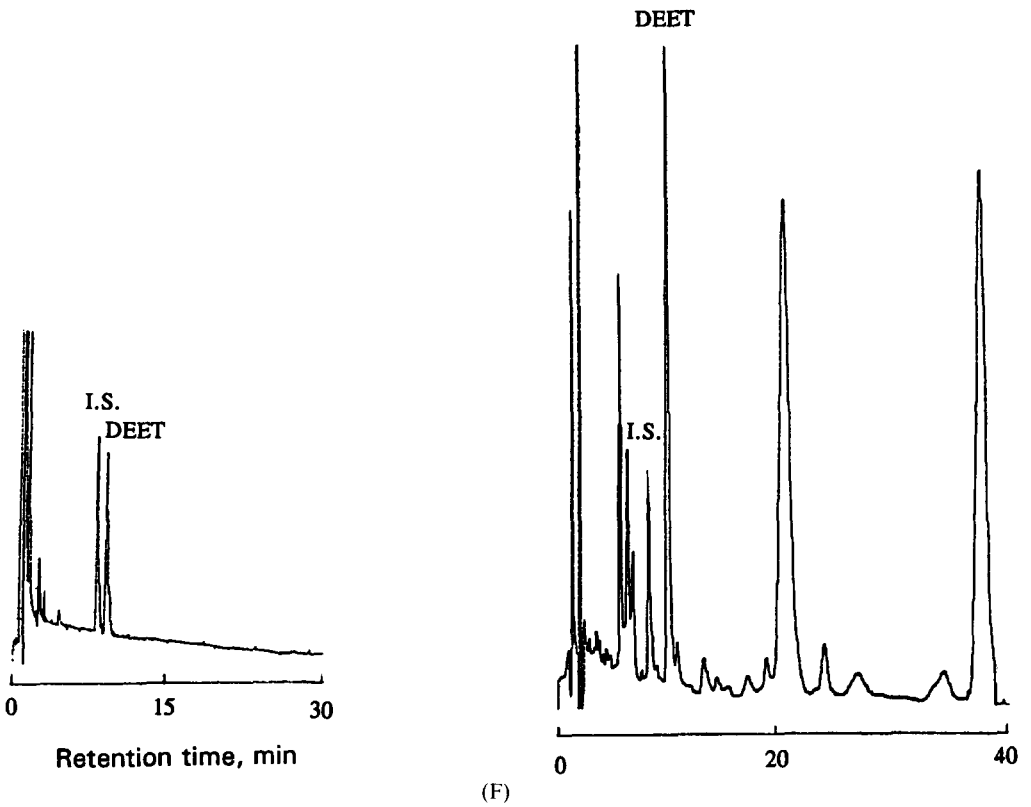


Fig. 1. Sample chromatograms of dog and human plasma: (A) blank dog plasma; (B) dog plasma spiked with DEET (50 ng ml^{-1}); (C) blank human plasma; (D) human plasma spiked with DEET (50 ng ml^{-1}); (E) dog plasma taken 45 min after intravenous bolus administration of 4 mg DEET kg^{-1}. DEET concentration was 794 ng ml^{-1}; (F) dog plasma spiked with DE241–250 ng ml^{-1}) processed following the published solid-phase extraction method.

3.10. Application

The method was successfully applied in pharmacokinetic studies of DEET in beagle dogs following intravenous bolus and topical routes of administration. More than 250 plasma samples were analyzed using this method, and the results appeared to be satisfactory. Fig. 2 shows a mean concentration–time profile of DEET in plasma after an intravenous bolus dose of 4.0 mg DEET kg^{-1} in four beagle dogs.

Table 1
Linearity of calibration plots obtained on three separate days within 1 week

Day	Slope[a]	Intercept	r^2
1	0.001408	0.013297	0.999
2	0.001253	0.006981	0.999
3	0.001330	0.009499	0.999
Mean	0.001330	0.009926	0.999
SD	0.000075	0.002591	0.000

[a] In units of ml ng^{-1}.

Table 2
Absolute recoveries of DEET and the internal standard

Compound	Concentration (ng ml^{-1})	Recovery (mean ± SD, $n = 3$)
DEET	50	98.9 ± 2.9
	500	96.9 ± 2.9
	1000	100.2 ± 4.9
Internal standard	1000	98.3 ± 3.7

Table 3
Precision[a] and accuracy[b] data

Concentration (ng ml^{-1})	Intra-day ($n = 5$)		Intra-day ($n = 5$)	
	Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)
50	5.8	+1.2	11.1	+1.5
500	7.6	+3.3	2.7	+5.1
1000	3.7	−1.4	2.6	+0.7

[a] Precision = SD/mean × 100%.
[b] (measured − theoretical)/theoretical × 100%.

4. Conclusions

A rapid and reliable HPLC method was developed and validated for the quantitation of DEET in plasma with the establishment of an effective solid-phase extraction procedure in which DEET and the internal standard, *N,N*-diethyl-2-phenylacetamide, were selectively extracted at high recoveries from the plasma matrix. The method was successfully applied in pharmacokinetic studies of DEET in beagle dogs after intravenous bolus and topical routes of administration.

Compared with the recently published liquid chromatographic method which also employed solid-phase extraction in the sample preparation [9], the current method had advantages such as: (1) an internal standard was used to achieve greater reliability; (2) the limit of quantitation was significantly improved; (3) the time required for the sample preparation was greatly reduced due to the use of much smaller amounts of preconditioning, wash and eluting solvents; and (4) the eluent obtained from the extraction cartridge was directly injectable for liquid chromatographic analysis where the eluent was monitored by UV-detection at 220 nm.

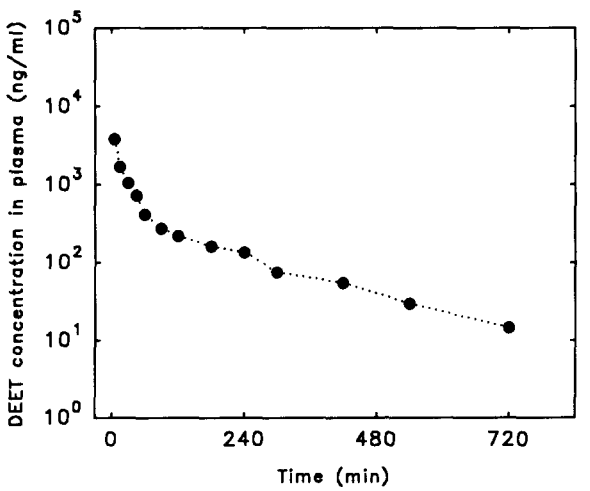


Fig. 2. Mean concentration–time profile of DEET in plasma after i.v. bolus administration of 4 mg DEET kg^{-1} in four beagle dogs.

The current method also exhibited advantages over the published GC method for the quantitation of DEET in plasma [18,19]. While offering a similar limit of quantitation, the current method employed a solid-phase extraction procedure which was a considerable time-saver compared to the liquid–liquid extraction used in the GC method. Also, the current method was environment-friendly since it used only a small volume of acetonitrile instead of large amounts of strong organic solvents in the extraction. The method is recommended for further analytical studies of DEET in plasma.

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