ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 291–299

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Simultaneous determination of malathion, permethrin, DEET (*N*,*N*-diethyl-*m*-toluamide), and their metabolites in rat plasma and urine using high performance liquid chromatography

Aqel W. Abu-Qare, Mohamed B. Abou-Donia *

Department of Pharmacology and Cancer Biology, Duke University Medical Center, PO Box 3813, Durham, NC 27710 USA

Received 31 October 2000; received in revised form 30 January 2001; accepted 9 February 2001

Abstract

A method was developed for the separation and quantification of the insecticide malathion (*O*,*O*-dimethyl-*S*-(1,2-carbethoxyethyl) phosphorodithioate), its metabolite malaoxon (*O*,*O*-dimethyl-*S*-(1,2-carbethoxyethyl) phosphorothioate), the insecticide permethrin (3-(2,2-dichloro-ethenyl)-2,2-dimethylcyclopropanecarboxylic acid(3-phenoxyphenyl)methylester), two of its metabolites *m*-phenoxybenzyl alcohol and *m*-phenoxybenzoic acid, the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET), and its metabolites *m*-toluamide and *m*-toluic acid in rat plasma and urine. The method used high performance liquid chromatography (HPLC) with reversed phase C_{18} column, and UV detection at 210 nm. The compounds were separated using gradient of 45–99% acetonitrile in water (pH 3.5) at a flow rate ranging between 0.5 and 2 ml/min in a period of 15 min. The retention times ranged from 7.4 to 12.3 min. The limits of detection ranged between 20 and 100 ng/ml, while limits of quantitation were 50–150 ng/ml. Average percentage recovery of five spiked plasma samples were 80.1 ± 4.2, 75.2 ± 4.6, 84.5 ± 4.0, 84.3 ± 3.4, 82.8 ± 3.9, 83.9 ± 5.5, 82.2 ± 6.0, 83.1 ± 4.3, and from urine 78.8 ± 3.9, 76.4 ± 4.9, 82.3 ± 4.5, 82.5 ± 3.9, 81.4 ± 4.0, 83.9 ± 4.3, 81.5 ± 5.0, and 84.5 ± 3.8 for, malathion, malaoxon, DEET, *m*-toluamide, *m*-toluic acid, permethrin, *m*-phenoxybenzyl alcohol, and *m*-phenoxybenzoic acid, respectively. The method was reproducible and linear over range between 100 and 1000 ng/ml. This method was applied to analyze the above chemicals and metabolites following combined dermal administration in rats.

Keywords: West Nile Virus; Reversed phase HPLC; Combined exposure; Malathion; DEET; Permethrin

1. Introduction

Malathion is a widely used organophosphate insecticide in agriculture [1] and in public health programs for controlling mosquito born-disease [2]. Malathion disrupts the nervous system by

* Corresponding author. Tel.: +1-919-6842221; fax: +1-919-6818224.
E-mail address: donia@acpub.duke.edu (M.B. Abou-Donia).

0731-7085/01/$ - see front matter
PII: S0731-7085(01)00407-1

inhibiting cholinesterase enzymes [3]. Toxic symptoms resulting from human exposure to malathion, include breathing problems, headache, nausea and dizziness, while high exposure can produce fatal poisoning [1,3]. Furthermore, malathion, has found to cause oxidative stress in rats and mice [4,5], and it is a suspected hormone disrupter [6]. Permethrin is a pyrethroide insecticide applied inside homes and in public places [7]. Pyrethroides modify sodium channel to open longer during a depolarization pulse [8], and act as a weak hormone mimic in test tube studies [9]. *N*,*N*-diethyl-*m*-toluamide (DEET) was applied as insect repellent on the skin against mosquitoes and other biting insects [10]. DEET had direct effect on the nervous system in laboratory animals resulting in spongiform myelinopathy in the brain stem with signs include ataxia, seizures, and death [11]. In other study, extensive and repeated topical application of DEET resulted in human poisoning including two deaths [12].

Absorption, disposition, metabolism, and excretion of malathion has been studied in animals and humans [3,4,13]. Permethrin has been reported to be absorbed into plasma, metabolized, and excreted as metabolites in the urine following oral or intravenous dose in rats [14], and in rabbits [15]. Absorption and excretion of DEET and metabolites were rapid after dermal application in human [16,17], in rats [18], and in dogs [19].

Several analytical methods have been used for identification and quantification of the above chemicals and their metabolites, when applied as individual, in plasma and urine samples. These methods used high performance liquid chromatography (HPLC) [14–25], high performance liquid chromatography-mass spectrometry (HPLC-MS) [27], gas chromatography (GC) [15,28,29], gas chromatography-mass spectrometry (GC-MS) [26], and high performance thin-layer chromatography (HPTLC) [30].

Recently malathion, permethrin and DEET have been simultaneously used to protect against West Nile Virus by killing adult mosquitoes in some parts of the United States [9,31]. As a result, thousands of people could be exposed to malalthion, permethrin and DEET inside homes and in public places. To examine their possible pharmacokinetics interactions, we developed a reliable method for simultaneous analysis of malathion, permethrin, DEET and their metabolites in rat plasma and urine using solid phase extraction coupled with reversed phase-HPLC.

2. Experimental

2.1. Chemicals and materials

Malathion (99% *O*,*O*-dimethyl-*S*-(1,2-carbethoxyethyl) phosphorodithioate), malaoxon (98% *0,0*-dimethyl-*S*-(1,2-carbethoxyethyl) phosphorothioate), *m*-phenoxybenzoic acid, and *m*-phenoxybenzyl alcohol (Fig. 1) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Permethrin (99% 3-(2,2-dichloro-ethenyl)-2,2-dimethylcyclopropanecarboxylicacid(3-phenoxyphenyl) methylester) was obtained from Chem Service, Inc. (West Chester, PA, USA). DEET (98% *N*,*N*-Diethyl-*m*-toluamide) (Fig. 1) was obtained from Aldrich Chem Co., Inc. (Milwakee, WI, USA). *m*-Toluamide and *m*-toluic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Water (HPLC grade) and acetonitrile were obtained from Mallinckrodt Baker, Inc. (Paris, Kentucky, USA). C_{18} Sep-Pak® cartridges were obtained from Waters Corporation (Waters Corporation, Milford, MA, USA).

2.2. Animals

Rats (Sprague–Dawley) were purchased from Zivic Miller (Zelienople, PA, USA). The animals were kept in plastic metabolic cages. Five rats were treated with a combined dermal dose of 10 mg/kg of malathion, a 200 mg/mg of DEET, and a 1.3 mg/kg of permethrin. The doses were selected to represent real-life exposure: DEET and permethrin doses were determined by US Department of Defense (personal communication), while the dose of malathion is approximately 1% of its dermal LD_{50} in rats. Five untreated control rats were treated with dermal dose of ethanol. The animals were held in metabolic cages as to allow collection of urine samples. Urine samples were collected from treated and controls 12 h after dosing. The animals were

anesthetized with halothane and sacrified by heart exsanguinations at 12 h, blood was collected via heart puncture with a heparinized syringe and centrifuged at 2400 rpm for 15 min at 5°C to separate plasma. Urine and plasma samples were stored at −20°C prior to analysis.

2.3. Instrumentation

The liquid chromatographic system (Waters 2690 Separation Module), consisted of a Waters 600E Multisolvent delivery system pumps, a Waters Ultra WISP 715 autoinjector, and a Waters 2487 Dual λ absorbance detector (Waters Corporation, Milford, MA). A guard column (Supelco, 2 cm × 4.0 mm, 5μm (Supelco Park, Bellefonte, PA), and a reversed-phase C_{18} column μBondapak™ C_{18} 125Å 10 μm, 3.9 × 300 mm were used, (Waters Corporation, Milford, MA).

2.4. Sample preparation

A 0.5 ml plasma and urine samples from untreated rats were spiked with concentrations ranging between 100 and 1000 ng/ml of each of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid. Spiked and treated samples were acidified with 1 N acetic acid (pH 3.5). Disposable C_{18} Sep-Pak Vac 3cc (500 mg) cartridges (Waters Corporation, Milford, MA) were conditioned with 3 ml of acetonitrile, then equilibrated using 3 ml of water prior use. The spiked urine and plasma samples were vortexed for 30 s, centrifuged for 5 min at 1000 × *g*, and the supernatant was loaded into the disposable cartridges, then washed with 3 ml of water, and eluted two times by 2 ml of acetonitrile, then by 2 ml of methanol, and reduced to 500 μl using stream of nitrogen, prior to analysis by HPLC.

2.5. Chromatographic conditions

A 10 μl solution of plasma or urine solutions was injected into HPLC. The mobile phase was water (adjusted to pH 3.50 using 1 N acetic acid): acetonitrile gradient at flow rate programmed from 0.5 ml/min from zero-9 min, increased to 2

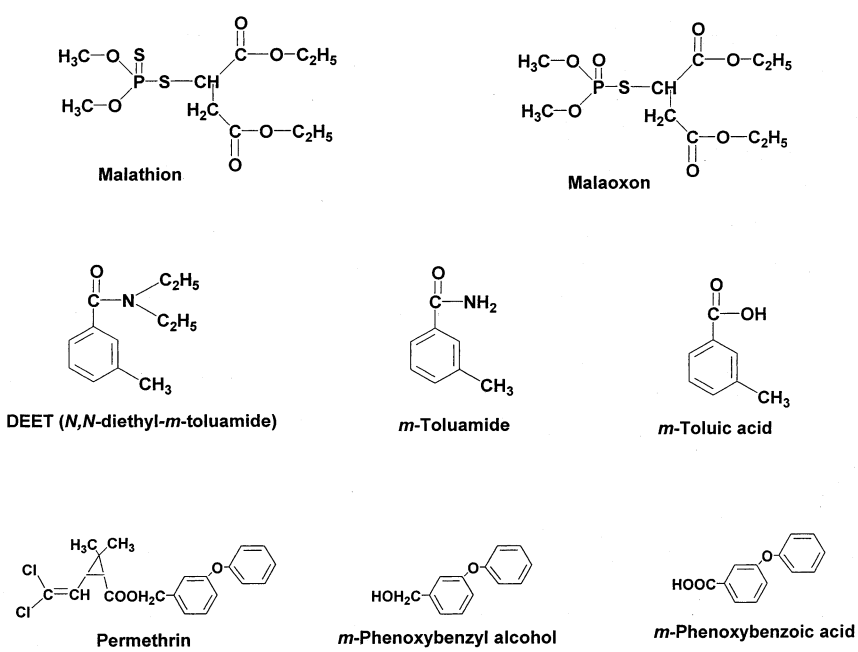

Fig. 1. Structures of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid.

ml/min by 10 min, then returned to 0.5 ml/min at 13 min. The gradient started at 45% acetonitrile until 9 min, increased to 90% acetonitrile by 10 min. Then the system returned to 45% acetonitrile at 13 min where it was kept under this condition for 2 min to re-equilibrate. The eluents were monitored by UV detection of wavelength of 210 nm. The chromatographic analysis was performed at ambient temperature.

2.6. Calibration procedures

Five different calibration standards of a mixture of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid were prepared in acetonitrile. Their concentrations ranged from 100 to 1000 ng/ml. Linear calibration curves were obtained by plotting the peak areas of the individual chemicals as a function of the concentration using GraphPad Prism program for windows (GraphPad Software, Inc., San Diego, CA, USA). The standard curves were used to determine recovery of the chemicals from plasma and urine samples.

2.7. Limits of detection (LOD) and limits of quantitation (LOQ)

Limits of detection and quantitation were determined at the lowest concentration to be detected or quantify, taking into consideration a 1:3 and 1:10 baseline noise: calibration point ratio, respectively. The LOQ was repeated five times for confirmation.

3. Results

3.1. Standard calibration curves

The standard calibration curves of peak area against concentration of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid are shown in Fig. 2. Linearity of the calibration curves for the three compounds was achieved at concentrations ranging from 100 to 1000 ng/ml.

3.2. Chromatogram

Chromatographic profiles were obtained for rat plasma and urine samples after solid phase extraction using C_{18} Sep Pak® cartridges under HPLC conditions as described above (Fig. 3 Fig. 4). Retention times were 7.4, 8.3, 9.1, 9.7, 10.2, 10.7, 11.4 and 12.3 min for *m*-toluamide, *m*-toluic acid, malaoxon, DEET, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, malathion, and permethrin, respectively. The total run time was 15 min. Clean chromatogram shows no interference from endogenous substances in plasma and urine samples and proves the selectivity of the method.

3.3. Extraction efficiency and recovery

The average extraction recoveries of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid were determined at concentrations ranging between 100 and 1000 ng/ml (Table 1 Table 2). Spiked plasma and urine samples were extracted and analyzed for each concentration in five replicates. Average percentage recovery of five spiked plasma samples were 80.1 ± 4.2, 75.2 ± 4.6, 84.5 ± 4.0, 84.3 ± 3.4, 82.8 ± 3.9, 83.9 ± 5.5, 82.2 ± 6.0, 83.1 ± 4.3, and from urine 78.8 ± 3.9, 76.4 ± 4.9, 82.3 ± 4.5, 82.5 ± 3.9, 81.4 ± 4.0, 83.9 ± 4.3, 81.5 ± 5.0, and 84.5 ± 3.8 for, malathion, malaoxon, DEET, *m*-toluamide, and *m*-toluic acid, permethrin, *m*-phenoxybenzyl alcohol, and *m*-phenoxybenzoic acid, respectively.

3.4. Limits of detection

Blank plasma and urine samples from untreated rats were used as references for plasma and urine collections. Limits of detection were calculated from a peak signal to noise ratio of 3:1. The resulting detection limits were 50, 100, 50, 20, 20, 50, 50, and 50 ng/ml for malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid, respectively.

3.5. Limits of quantitation (LOQ)

Limits of quantitation in control plasma sam-

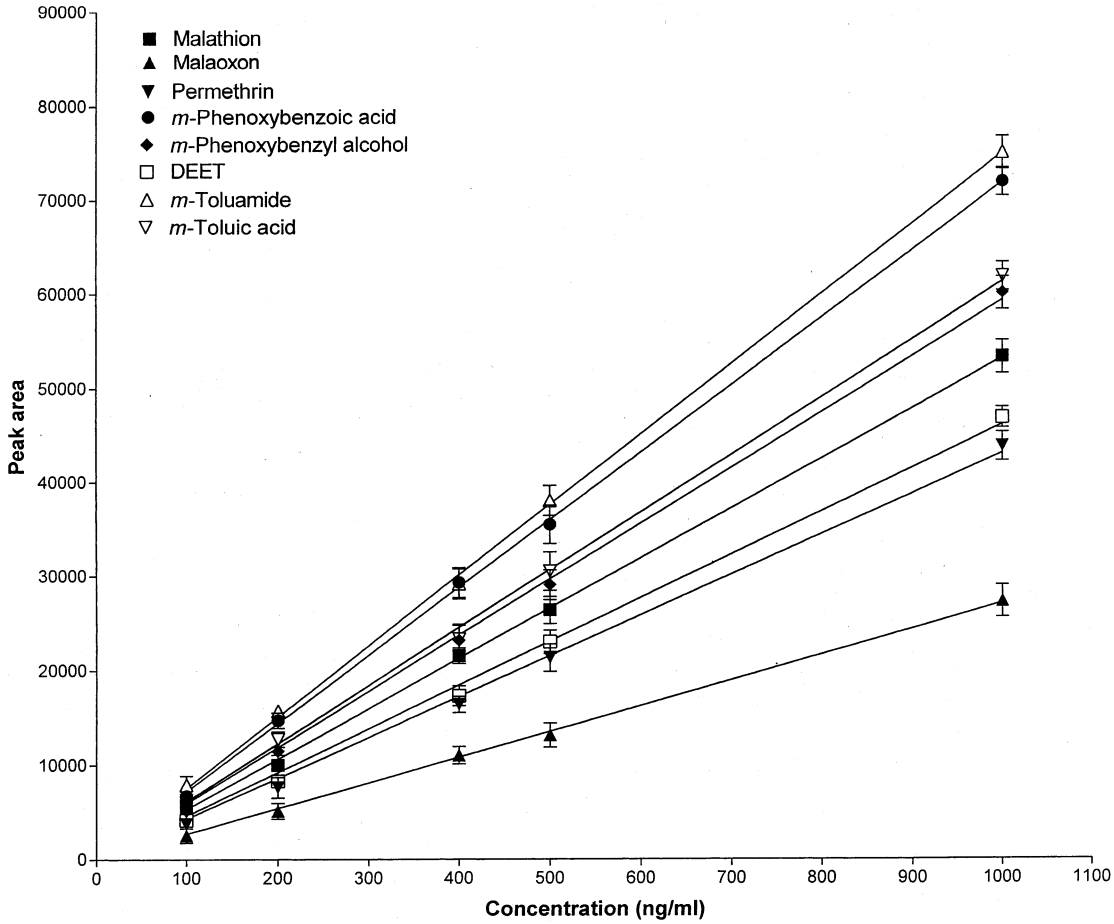

Fig. 2. Standard calibration curves of malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid.

ples were determined to be 100, 150, 100, 50, 50, 100, 100, and 100 ng/ml, while from spiked urine samples were 100, 150, 150, 100, 100, 100, 100, and 100 ng/ml for malathion, malaoxon, permethrin, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, DEET, *m*-toluamide, and *m*-toluic acid, respectively.

3.6. Application of the method to biological samples

In order to test its applicability, the method was applied for the analysis of the three chemicals and their metabolites following combined dermal administration in rats. The rats were sacrificed at 12 h following dosing. In plasma, malathion, DEET, *m*-toluamide, permethrin, and *m*-phenoxybenzyl alcohol were detected. Their levels were 286 ± 53, 702 ± 186, 273 ± 94, 186 ± 32, and 212 ± 38 ng/ml, respectively. Also, traces of malaoxon were detected in plasma samples. In urine, DEET, and *m*-phenoxybenzyl alcohol have been detected 12 h after treatment. Their levels were 425 ± 142 and 183 ± 36 ng/ml, respectively. The results were corrected based on the percentage recoveries of the above chemicals from plasma and urine samples.

4. Discussion

In this study, we developed an HPLC method for the separation and quantification of malalthion, permethrin, DEET and their metabolites in rat plasma and urine. The method is significant at this time where the three chemicals are in use against West Nile Virus, and possible interactions between the three chemicals could lead to more toxicity. The method could be used in studying pharmacokinetic interactions between the compounds. The chromatogram obtained following solid phase extraction and HPLC analysis shows no interference from plasma and urine endogenous substances, indicating an efficient clean up method used and proving selectivity of the method. Simultaneous determination of the parent compounds and their metabolites are cost efficient and save time for sample preparation and clean up.

Linearity of standard calibration curves for the chemicals in this method was obtained over a range between 100 and 1000 ng/ml. This range is in agreement with earlier studies using similar ranges. Eilln et al. [20] reported linear range between 40 and 500 ng/ml for DEET in plasma using HPLC, while Taylor et al. [32] reported linearity over a range between 19 and 1910 ng/ml for DEET using gas chromatography.

Recoveries of the anlaytes were suitable for application of the method for the determination of treated samples for parent compounds and their metabolites following real-life exposure. The selected dose of malathion represent less than 1% of its dermal LD_{50} in rats [1], while the dose of permethrin and DEET was determined by US Department of Defense (personal communications). Recoveries of the analytes in this method were between 75 and 91%. This range lies within

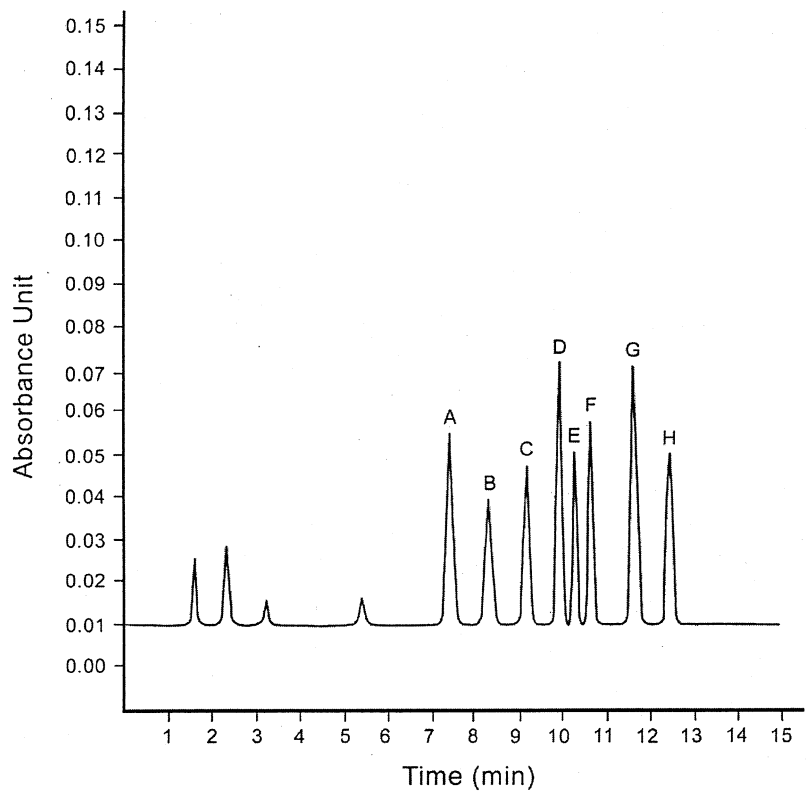

Fig. 3. Chromatogram of spiked plasma sample with 500 ng/ml of (A) *m*-toluamide, (B) *m*-toluic acid, (C) malaoxon, (D) DEET, (E) *m*-phenoxybenzyl alcohol, (F) *m*-phenoxybenzoic acid, (G) malathion, and (H) permethrin under established HPLC conditions.

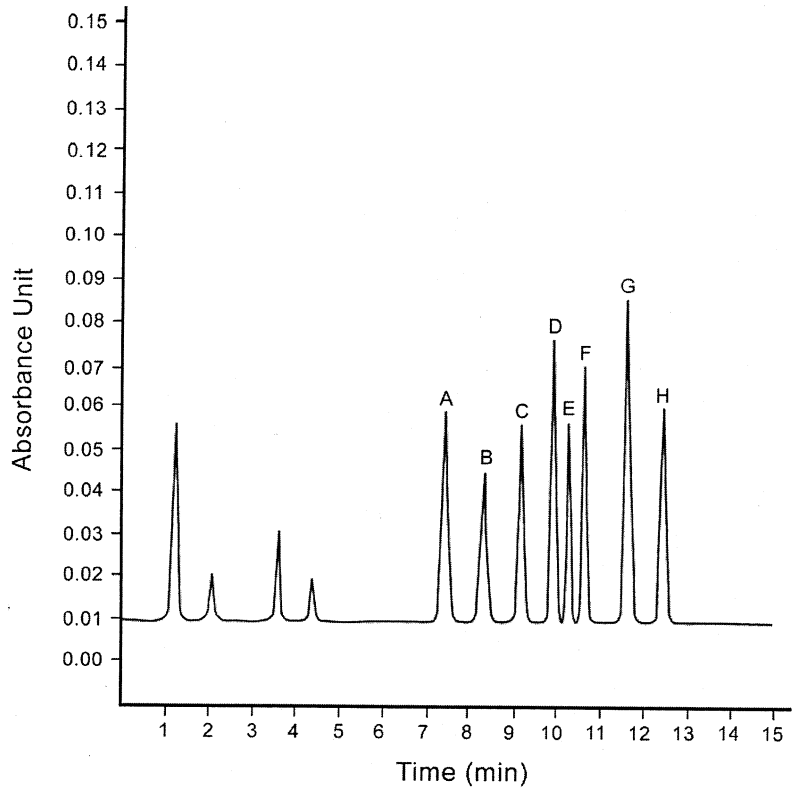

Fig. 4. Chromatogram of spiked urine sample with 500 ng/ml of (A) *m*-toluamide, (B) *m*-toluic acid, (C) malaoxon, (D) DEET, (E) *m*-phenoxybenzyl alcohol, (F) *m*-phenoxybenzoic acid, (G) malathion, and (H) permethrin under established HPLC conditions.

Table 1
Percentage (%) recovery[a] of malathion, DEET, permethrin, and their metabolites from rat plasma

Concentration (ng/ml)	Malathion	Malaoxon	DEET	*m*-Toluamide	*m*-Toluic acid	Permethrin	*m*-Phenoxybenzyl alcohol	*m*-Phenoxybenzoic acid
1000	83.2 ± 4.6	79.2 ± 3.8	91.1 ± 2.7	88.7 ± 4.2	88.5 ± 3.8	85.7 ± 5.8	84.9 ± 5.1	88.2 ± 2.6
500	83.9 ± 3.0	78.0 ± 4.0	84.2 ± 5.2	86.3 ± 2.8	85.9 ± 2.8	88.3 ± 5.2	82.3 ± 4.8	84.2 ± 4.9
400	80.5 ± 5.7	76.1 ± 2.9	83.7 ± 4.2	85.1 ± 4.3	82.6 ± 3.2	85.2 ± 4.9	84.7 ± 8.3	82.9 ± 5.6
200	78.2 ± 4.8	70.1 ± 6.9	82.9 ± 3.8	81.5 ± 3.9	80.7 ± 4.3	81.9 ± 5.6	82.1 ± 6.2	83.7 ± 4.7
100	75.2 ± 3.1	72.4 ± 4.7	80.5 ± 4.2	79.8 ± 1.9	76.5 ± 5.2	78.2 ± 6.2	77.1 ± 5.7	76.5 ± 3.8

[a] Values are expressed as mean ± S.D. of five replicates.

the reported values in the literature, taking into consideration simultaneous determination of the three chemicals and their metabolites. Recoveries of DEET from serum and urine were reported to be 93–95%, and 65–70%, respectively, using GC-MS as an analytical technique [33], while recovery of DEET from water samples was 45.6% using Micllar kinetic chromatography method [34]. In earlier studies, recovery of pyrethroids and metabolites from rat urine ranged between 90 and 98% using GC-MS [26]. Bissacot and Vassilieff [35] reported recoveries between 78 and 91% of four pyrethroids from milk and blood of lactating dairy cows using HPLC. Futagami et al. [30]

Table 2
Percentage (%) recovery [a] of malathion, DEET, permethrin, and their metabolites from rat urine

Concentration (ng/ml)	Malathion	Malaoxon	DEET	*m*-Toluamide	*m*-Toluic acid	Permethrin	*m*-Phenoxybenzyl alcohol	*m*-Phenoxybenzoic acid
1000	83.0 ± 5.1	78.0 ± 4.8	85.2 ± 3.8	90.1 ± 3.0	82.1 ± 2.8	85.3 ± 4.8	83.1 ± 4.9	90.1 ± 3.6
500	81.9 ± 2.0	75.3 ± 3.8	82.0 ± 6.1	84.7 ± 3.6	83.1 ± 3.7	87.0 ± 4.2	80.6 ± 5.4	87.2 ± 4.2
400	77.2 ± 3.7	79.5 ± 5.9	80.3 ± 5.6	80.2 ± 5.1	83.2 ± 4.0	88.2 ± 3.9	84.3 ± 6.1	82.6 ± 4.9
200	76.1 ± 4.8	74.3 ± 6.5	83.2 ± 4.2	78.1 ± 4.1	78.2 ± 3.2	80.1 ± 3.6	81.1 ± 4.0	83.5 ± 3.7
100	76.0 ± 4.1	74.9 ± 3.7	80.6 ± 2.9	79.2 ± 3.6	80.5 ± 6.3	78.3 ± 5.2	76.2 ± 4.7	79.1 ± 2.8

[a] Values are expressed as mean ± S.D. of five replicates.

reported a recovery of 84.5 and 95.8% for malathion from urine samples using solid phase and liquid-liquid extraction, respectively, Malathion recovery from blood sample was between 85 and 97% [22].

The limits of detection reported in the described method allow to determination of samples from treated animals following doses resemble real-life exposure. The ability to detect parent compounds and metabolites in plasma after 12 h of dosing is an evidence of the method suitability. Only traces of malaoxon have been detected in plasma. This could be due to its rapid degradation and elimination. The failure to detect DEET metabolites *m*-toluamide and *m*-toluic acid, permethrin and its metabolite *m*-phenoxybenzoic acid in the urine might due to rapid hydrolysis and conjugation of permethrin, DEET and the targeted metabolites. The reported limits of detection in the literature are consistent with our results for the simultaneous determination of the analytes that ranged between 20 and 100 ng/ml. In earlier study, limits of detection of malathion using HPTLC were 120 ng/ml [30]. Detection limits of permethrin in urine samples were 0.3–0.5 μg/l using GC-MS technique [26]. Bissacot and Vassilieff [35] reported detection limit of 1 μg/g of four pyrethroids in milk and blood of lactating dairy cows using HPLC. The detection limit of DEET was 90 ng/ml and 90 ng/g from urine and serum, respectively, using HPLC-UV method [16], and 15 ng/ml for DEET in human and dog plasma using HPLC [36].

A reliable, rapid and simple HPLC method was developed for separation and quantification of malathion, permethrin and selected metabolites in rat spiked and treated plasma and urine samples. Solid phase extraction was used which selectively extracted the above chemicals from plasma and urine samples without interference of an expected mixture of metabolites and endogenous compounds. The method could be applied routinely for monitoring of these compounds in human plasma and urine samples of people exposed to the compounds in some areas where these chemicals are used to control West Nile Virus. Also this method could be used to study the pharmacokinetic profiles of these compounds, alone and in combination.

References

[1] (WHO/FAO) Data Sheets on Pesticides. 29 (1977).
[2] L. Mccarroll, M.G. Paton, S.H. Karunaratne, H.T. Jayasuryia, K.S. Kalpage, J. Hemingway, Nature 407 (2000) 407961.
[3] M.B. Abou-Donia, Pesticides, in: M.B. Abou-Donia (Ed.), Neurotoxicology, CRC Press Publications, 1992, p. 437.
[4] R.S. Ahmed, V. Seth, S.T. Pasha, B.D. Banerjee, Food Chem. Toxicol. 38 (2000) 443.
[5] E. Yarsan, M. Tanyuksel, S. Celik, A. Aydin, Bull. Environ. Contam. Toxicol. 63 (1999) 575.
[6] S. Orme, S. Kegley, Pesticide Action Network, (2000).
[7] World Health Organization, Permethrin, Environ. Health Criteria, 94 (1990).
[8] T. Narahashi, Neurotoxicology 6 (1985) 3.
[9] Rachel's Environment and Health Biweekly, 709 (2000): October 12.
[10] M. Brown, A.A. Hebert, J. Am. Acad. Dermatol. 36 (1997) 243–249.
[11] R.D. Verschoyle, A.W. Brown, C. Nolan, D.E. Ray, T. Lister, Fundam. Appl. Toxicol. 18 (1990) 79–88.
[12] E.H. Roland, J.E. Jan, J.M. Rigg, Can. Med. Assoc. J. 132 (1985) 155–156.

[13] R.I. Krieger, T.M. Dinoff, Arch. Environ. Contam. Toxicol. 38 (2000) 546.
[14] A. Anadon, M.R. Martinez-Larranaga, M.L. Diaz, P. Bringas, Toxicol. Appl. Pharmacol. 110 (1991) 1.
[15] G. Leng, K.H. Kuehn, U. Idel, Sci. Total Environ. 199 (1997) 173.
[16] A.W. Smallwood, K.E. DeBord, L.K. Lowry, J. Anal. Toxicol. 16 (1992) 10–13.
[17] S. Selim, R.E. Hartnagel, T.G. Osimitz, K.L. Gabriel, G.P. Schoenig, Fundam. Appl. Toxicol. 25 (1995) 95–100.
[18] G.P. Schoenig, R.E. Hartnagel, T.G. Osimitz, S. Llanso, Drug Metab. Dispos. 24 (1996) 156–163.
[19] H. Qiu, H.W. Jun, J. Tao, J. Pharm. Sci. 86 (1997) 514–516.
[20] R.I. Ellin, P. Zvirbis, M.R. Wilson, J. Chromatogr. 228 (1982) 235–244.
[21] I. Kaur, R.P. Mathur, S.N. Tandon, Biomed. Chromatogr. 11 (1997) 22.
[22] Y. Cho, N. Matsuoka, A. Kamiya, Chem. Pharm. Bull. 45 (1997) 737.
[23] R. Kumar, Biomed. Chromatogr. 3 (1989) 272.
[24] V.K. Sharma, R.K. Jadhav, G.J. Rao, A.K. Saraf, H. Chandra, Forensic Sci. Int. 48 (1990) 21.
[25] R.K. Jadhav, V.K. Sharma, G.J. Rao, A.K. Saraf, H. Chandra, Forensic Sci. Int. 52 (1992) 223.
[26] J. Angerer, A. Ritter, J. Chromatogr. B 695 (1997) 217–226.
[27] M.D. Beeson, W.J. Driskell, D.B. Barr, Anal. Chem. 71 (1999) 3526.
[28] H.L. Sondgrass, J. Toxicol. Environ. Health 35 (1992) 91.
[29] T. Schettler, Generations at Risk: Reproductive Health and the Environment, MIT Press, Cambridge, MA, 1999, p. 186.
[30] K. Futagami, C. Narazaki, Y. Kataoka, H. Shuto, R. Oishi, J. Chromatogr. B 704 (1997) 369.
[31] New York City department of Health, (2000) West Nile Virus fact sheets, May 2000.
[32] W.G. Taylor, T.J. Danielson, R.W. Spooner, R. Lorriane, L.R. Golsteyn, Drug Metab. Dispos. 22 (1994) 106–112.
[33] A.D. Fraser, M. MacNeil, M. Theriault, W. Morzycki, J. Anal. Toxicol. 19 (1995) 197–199.
[34] Y. He, H.K. Lee, Electrophoresis 18 (1997) 2036–2041.
[35] D.Z. Bissacot, I. Vassilieff, J. Anal. Toxicol. 21 (1997) 397.
[36] H. Qiu, H.W. Jun, J. Pharam. Biomed. Anal. 15 (1996) 241–250.