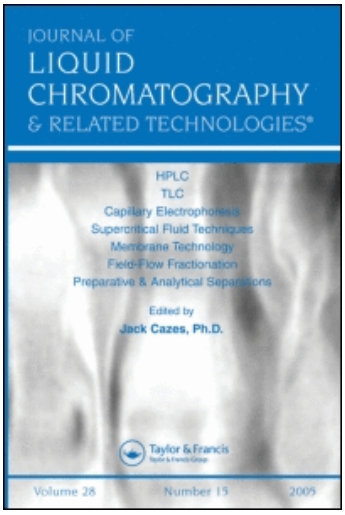


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Deltamethrin and Its Metabolites, 3-Phenoxybenzoic Acid and 3-Phenoxybenzyl Alcohol, in Maternal Plasma, Amniotic Fluid, and Placental and Fetal Tissues by HPLC

Yan Ding[a]; Catherine A. White[a]; James V. Bruckner[a]; Michael G. Bartlett[a]
[a] Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, Georgia, USA

Online publication date: 27 May 2004

To cite this Article Ding, Yan , White, Catherine A. , Bruckner, James V. and Bartlett, Michael G.(2004) 'Determination of Deltamethrin and Its Metabolites, 3-Phenoxybenzoic Acid and 3-Phenoxybenzyl Alcohol, in Maternal Plasma, Amniotic Fluid, and Placental and Fetal Tissues by HPLC', Journal of Liquid Chromatography & Related Technologies, 27: 12, 1875 — 1892

To link to this Article: DOI: 10.1081/JLC-120038774
URL: http://dx.doi.org/10.1081/JLC-120038774

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 12, pp. 1875–1892, 2004

Determination of Deltamethrin and Its Metabolites, 3-Phenoxybenzoic Acid and 3-Phenoxybenzyl Alcohol, in Maternal Plasma, Amniotic Fluid, and Placental and Fetal Tissues by HPLC

Yan Ding, Catherine A. White, James V. Bruckner, and Michael G. Bartlett*

Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, Georgia, USA

ABSTRACT

Pyrethroid insecticide deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate] is widely used throughout the world on cotton, crops, and stored products. It is also used to treat livestock and is, thus, a public health concern. The toxic effects of deltamethrin in mammals include tremors and salivation. Few studies have been performed to characterize the toxicokinetics of deltamethrin, mainly due to the lack of a sensitive and reliable analytical method. An analytical method was, thus, developed and validated for the

*Correspondence: Michael G. Bartlett, Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA 30602, USA; E-mail: bartlett@mail.rx.uga.edu.

DOI: 10.1081/JLC-120038774 1082-6076 (Print); 1520-572X (Online)

determination of deltamethrin concentrations in pregnant rat maternal plasma, amniotic fluid, placenta, and fetus using protein precipitation and reversed-phase high performance liquid chromatography. The limits of quantitation for deltamethrin were 100 ng/mL for amniotic fluid and 200 ng/mL for plasma, placental and fetal tissues. Recoveries ranged from 83.0% to 98.1% for deltamethrin, 90.5% to 99.7% for 3-phenoxybenzoic acid (3-PB Acid), and 82.4% to 92.3% for 3-phenoxybenzyl alcohol (3-PB Alc). The intra-day ($n = 5$) precision and accuracy for deltamethrin were in the range of 0.2–7.8 (relative standard deviation, RSD) and 0.4–11.2% (% error). The intra-day ($n = 5$) precision and accuracy for 3-PB Acid were in the range of 1.1–13.6% and 1.1–13.0%. The intra-day ($n = 5$) precision and accuracy for 3-PB Alc were in the range of 0.6–11.5% and 1.9–13.7%. Inter-day ($n = 15$) precision and accuracy for deltamethrin ranged from 3.1% to 12.0% (% RSD) and 2.5% to 8.8% (% error). Inter-day ($n = 15$) precision and accuracy for 3-PB Acid ranged from 1.8% to 12.5% and 2.1% to 10.0%. Inter-day ($n = 15$) precision and accuracy for 3-PB Alc ranged from 2.4% to 11.3% and 3.1% to 10.5%.

Key Words: Deltamethrin; HPLC; Toxicokinetics; Pregnant rat.

INTRODUCTION

Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl-(1*R*)-*cis*-3-(2,2-dibromovinyl)-2, 2-dimethylcyclopropane carboxylate] is a synthetic type II pyrethroid insecticide.[1] It is one of the most potent pyrethroids known and is widely used in veterinary products to control lice, flies, and ticks on cattle, sheep, and pigs. It is also used in agricultural formulations to control numerous insect pests on fruit, vegetables, and field crops. In human medicine, deltamethrin is effective as a pediculocide.[2] Its toxic effects include choreoathetosis and salivation. Those effects are generally rapid in onset and brief in duration.[3] Substantial oral and dermal exposures of humans typically produce GI upset and parasthesias, respectively. There is a report, however, of a 30-year-old male who died 2 days after consuming about 30 mL of deltamethrin.[4] There do not appear to be reports of developmental effects in humans.

Investigation of developmental effects of deltamethrin in animal models has yielded conflicting results.[3,5,6] In one of the studies, no serious signs of fetotoxicity or teratogenicity were observed in fetuses of rats given deltamethrin in oral doses of 1.0, 2.5, or 5.0 mg/kg/day, during gestation days 6 through 15, although, the highest dose level resulted in the death of 4/20 treated dams.[5] In another study, Kavlock et al. observed no significant signs of fetotoxicity or teratogenicity in fetuses of mouse or rat dams

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

administered deltamethrin during major stages of organogenesis, at oral dose levels up to, and including, those eliciting overt signs of maternal toxicity (up to 5 and 12 mg/kg/day in mice and rats, respectively).[3] Abdel-Khalik et al. reported significant dose-dependent postimplantation loss and retarded growth of fetuses of rat dams administered oral doses of deltamethrin of 1.0, 2.5, or 5.0 mg/kg/day, from gestation days 6 through 15.[6] The investigators noted that the developmental effects may have resulted, at least in part, from compromised placental tissues in treated dams, since placental weight was increased in all treatment groups.[6] It is not clear whether pyrethroids cross the placenta and reach the fetus in toxicologically significant amounts.[7–9] Findings in a limited number of animal studies indicate that in utero exposure to certain pyrethroids can result in persistent effects on neurotransmitters,[10,11] and on the immune system[12] of immature rats.

The reliability of these data is uncertain, due to limitations in the study designs. One problem, was the length of time (i.e., 1–8 days) between administration of the last dose and measurement of tissue levels of test compounds. More definitive studies of placental transport of major pyrethroids would be desirable.[10]

A pregnant rat model can be used to investigate the placental transfer of deltamethrin, since such studies cannot be conducted in humans. This model is relevant because of the similar changes seen in the hemochordial placenta and the hemodynamic pregnancy of rats and humans.[13,14] The containment of each rat pup in an individual fetal sack and the large litter size, also make it a useful model for serial sampling of maternal plasma, amniotic fluid, fetus and placenta in toxicokinetic studies.[15]

Previous studies of deltamethrin metabolism in rats, have revealed that the principal mechanisms of metabolism are ester cleavage and oxidation at the 4′ position of the aromatic ring.[16] Though it is generally accepted that metabolism of deltamethrin results in the formation of compounds that have little or no demonstrable toxicity,[17,18] incorporation of metabolite information into the toxicokinetic study of deltamethrin would help to understand the stoichiometry of absorption, distribution, metabolism, and excretion (ADME) of deltamethrin. In this research project, 3-phenoxybenzoic acid (3-PB Acid) and 3-phenoxybenzyl alcohol (3-PB Alc) were selected for study. 3-PB Acid is a major metabolite of the aromatic portion of deltamethrin. 3-PB Alc was not observed in an earlier study of deltamethrin metabolism in the rat.[16] Our purpose was to verify this earlier observation and to provide a method that could be transferred to other species, including humans, in whom this metabolite has been reported.

Several chromatographic methods have been developed to determine deltamethrin and some of its metabolites including 4′-HO-deltamethrin, from different matrices such as a cattle dip, rat plasma, and cow blood and

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

milk.[2,19–21] Most of the HPLC methods for deltamethrin analysis were not validated.[19–21] For the method that was validated, its animal experiment procedure requires the sacrifice of animals at each individual time point of sample collection postdose.[2] This procedure will incur inherent errors in the toxicokinetic profiles caused by ADME differences between each animal.

Hence, the objective of this research was to develop a quick, sensitive, and validated HPLC method to determine deltamethrin and its metabolites, 3-PB Acid and 3-PB Alc, in pregnant rat maternal plasma, amniotic fluid, placental and fetal tissues. This will also facilitate serial sampling from a single animal to assist in the placental transfer study of deltamethrin.

EXPERIMENTAL

Reagents and Chemicals

Analytical standards of deltamethrin and its metabolites 3-PB Acid and 3-PB Alc were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). HPLC-grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ). Reagent grade phosphoric acid was purchased from J. T. Baker (Phillipsburg, NJ). Deionized water was generated from a Continental Deionized Water System (Natick, MA).

Preparation of Stock and Standard Solutions

Individual deltamethrin, 3-PB Acid, and 3-PB Alc stock solutions were prepared in acetonitrile to give a final concentration of 1.0 mg/mL. Individual standard solutions with concentrations of 1.0, 1.5, 2.5, 3.75, 5.0, 10.0, 15.0, and 25.0 μg/mL for maternal plasma, placental, and fetal tissues, 0.5, 1.25, 1.5, 2.5, 3.75, 5.0, 10.0, 15.0, and 25.0 μg/mL for amniotic fluid were prepared by serial dilution of deltamethrin, 3-PB Acid, and 3-PB Alc stock solutions with acetonitrile. Precision and accuracy standards (1.0, 2.0, 7.5, and 20.0 μg/mL for maternal plasma, placental and fetal tissues, 0.5, 1.0, 7.5, and 20.0 μg/mL for amniotic fluid) were prepared in the same manner. All stock and standard solutions were made fresh for each day of analysis or validation.

Chromatographic System

The HPLC experiment was performed on a Hewlett-Packard (Agilent) Series II 1090 Liquid Chromatographic System (Palo Alto, CA). The detector

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

used was a Waters Lambda-Max Model 481 LC Spectrophotometer (Milford, MA). The data processing device was a Hewlett-Packard (Agilent) 3395 Integrator. The software used was Hewlett-Packard (Agilent) ChemStation for LC Rev. A.04.01. Chromatographic separation was achieved on a Zorbax 80SB-CN column ($4.6 \times 250\,mm^2$, $5\,\mu m$) from MAC-MOD Analytical, Inc. (Chadds Ford, PA), equipped with a Phenomenex C-18 4 mm L × 2 mm I.D. guard column (Torrance, CA).

Chromatographic Conditions

The mobile phase used was acetonitrile and deionized water (adjusted to pH 2.4 using phosphoric acid). The gradient used for maternal plasma and amniotic fluid was 20% acetonitrile at time 0, 60% acetonitrile at 15 min, 80% acetonitrile at 18 min, 80% acetonitrile at 20 min, 20% acetonitrile at 22 min, and 20% acetonitrile at 24 min. The gradient used for placental and fetal tissues was 20% acetonitrile at time 0, 60% acetonitrile at 15 min, 80% acetonitrile at 22 min, 80% acetonitrile at 24 min, 20% acetonitrile at 26 min, and 20% acetonitrile at 28 min. The flow rate was 0.8 mL/min, and the detection wavelength was 210 nm for maternal plasma and amniotic fluid, and 220 nm for placental and fetal tissues. Under the chromatographic conditions described, deltamethrin eluted at approximately 22.3 min for maternal plasma and amniotic fluid, and 24.0 min for placental and fetal tissues. 3-PB Acid and 3-PB Alc eluted at approximately 16.3 and 15.5 min for all four matrices.

Calibration Curves

Blank plasma, amniotic fluid, placenta, and fetal tissues were collected from untreated, anesthetized pregnant Sprague–Dawley rats (Harlan, Indianapolis, IN). Blank placental and fetal tissue homogenates were prepared by homogenization with two volumes of deionized water (w/v) in a Tekmar tissue grinder (model SDT-1810, Cincinnati, OH). Calibration standards for maternal plasma, placental and fetal tissues were prepared by spiking 120 μL of the tissue homogenate or biological fluid with 30 μL of deltamethrin, 3-PB Acid, and 3-PB Alc standard solution to obtain deltamethrin, 3-PB Acid, and 3-PB Alc concentrations of 0.2, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, and 5.0 μg/mL. Calibration standards for amniotic fluid were prepared by spiking 80 μL of the biological fluid with 20 μL of deltamethrin, 3-PB Acid, and 3-PB Alc standard solutions to obtain deltamethrin, 3-PB Acid, and 3-PB Alc concentrations of 0.1, 0.25, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, and

5.0 μg/mL. All the spiked tissue homogenate or biological fluid standards were then extracted from the biological matrices using the procedures described below. All standards were prepared on the day of analysis.

Extraction Procedures

Maternal plasma, amniotic fluid, placental and fetal tissue samples were all subjected to protein precipitation. In a 1.5 mL microcentrifuge tube, 30 μL of deltamethrin, 3-PB Acid, and 3-PB Alc solution were added to 120 μL of plasma. The tube was vortexed briefly before the addition of 540 μL of cold acetonitrile. The tubes were votexed again for 60 sec and centrifuged at 13,000 rpm for 10 min. The supernatant was evaporated under a gentle stream of nitrogen until reaching dryness. The pellet was reconstituted in 100 μL of 50% acetonitrile in water. The reconstituted solution was votexed for 60 sec and sonicated for 5 min, vortexed again for 30 sec and centrifuged for 5 min at 13,000 rpm, using the same microcentrifuge. The resulting solution was then transferred to an injection vial, where 25 μL of sample was injected onto the HPLC column.

In a 1.5 mL microcentrifuge tube, 20 μL of deltamethrin, 3-PB Acid, and 3-PB Alc solution were added to 80 μL of amniotic fluid. The tube was vortexed briefly before the addition of 450 μL of cold acetonitrile. The remaining extraction procedure was the same as plasma extraction. The resulting solution was then transferred to an injection vial, where 25 μL of sample was injected onto the HPLC column.

In a 1.5 mL centrifuge tube 120 μL of the tissue homogenate, 30 μL of deltamethrin, 3-PB Acid, and 3-PB Alc standard solution were combined and vortexed for 10 sec. To precipitate proteins, 700 μL of ice-cold acetonitrile was added to the tube. The remaining extraction procedure was the same as with the plasma extraction. The resulting solution was then transferred to an injection vial where 30 μL of sample was injected onto the HPLC column.

RESULTS AND DISCUSSION

The structures of deltamethrin, 3-PB Acid, and 3-PB Alc are shown in Fig 1. Separation of deltamethrin, 3-PB Acid, and 3-PB Alc from interfering matrix peaks was explored using different kinds of columns, mobile phases, and gradient. Baseline resolution of deltamethrin, 3-PB Acid, and 3-PB Alc was achieved using the chromatographic conditions described in the Experimental section. Fig 2(a–d) show chromatograms of spiked deltamethrin,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

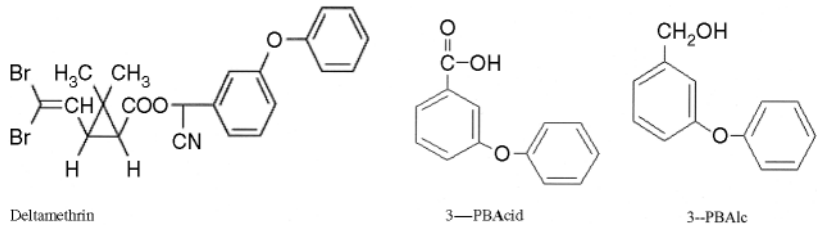


Figure 1. Chemical structures of deltamethrin, 3-PB Acid, and 3-PB Alc.

3-PB Acid, and 3-PB Alc (3.0 μg/mL) in rat maternal plasma, amniotic fluid, placental and fetal tissues.

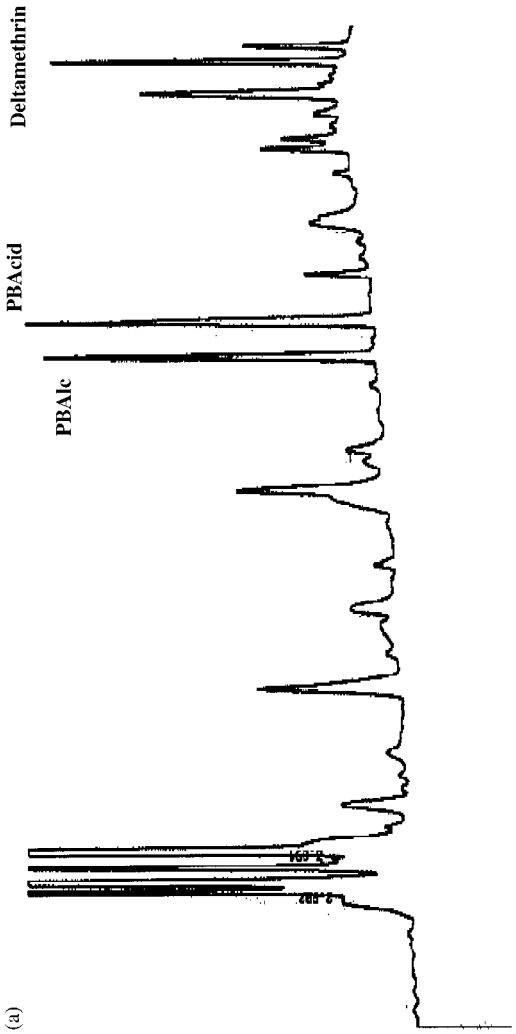
The calibration curves for each day of validation and analysis showed good linearity over the concentration range from 0.1 to 5.0 μg/mL for amniotic fluid and 0.2 to 5.0 μg/mL for plasma, placental and fetal tissues. Microsoft Excel and JMP statistical software were used to generate linear regression equations for all calibration curves. The r^2 values of the calibration curves for deltamethrin, 3-PB Acid, and 3-PB Alc in all four matrices, are summarized in Table 1.

The limits of detection (LOD) for deltamethrin, 3-PB Acid, and 3-PB Alc in biological matrices, were determined by analysis of standard-spiked samples of gradually decreasing concentration. The LODs of deltamethrin, 3-PB Acid, and 3-PB Alc were determined as a concentration at which the signal/noise ratio was ~3. The LOD for each compound was approximately 0.05 μg/mL for all four biological matrices.

To investigate the extraction efficiencies of deltamethrin, 3-PB Acid, and 3-PB Alc from the various biological matrices (maternal plasma, amniotic fluid, fetal and placental tissues), standard-spiked matrix samples at the 0.8 and 4.0 μg/mL concentrations were subjected to extraction and analysis. For each standard concentration, five replicates were investigated. The resulting peak areas were compared to peak areas of samples containing equal amounts of analytes in corresponding blank biological matrices. The recoveries for deltamethrin, 3-PB Acid, and 3-PB Alc from the four individual matrices are shown in Table 2.

Assay precision and accuracy were calculated for each matrix over 3 days. Blanks from each matrix were spiked with deltamethrin, 3-PB Acid, and 3-PB Alc to yield final concentrations corresponding with those in the calibration curves. Five replicates of blank maternal plasma, placental and fetal tissues spiked with deltamethrin, 3-PB Acid, and 3-PB Alc concentrations of 0.2 μg/mL (limit of quantitation, LOQ), 0.4, 1.5, and 4.0 μg/mL, were prepared for each validation day to test the precision (relative standard

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

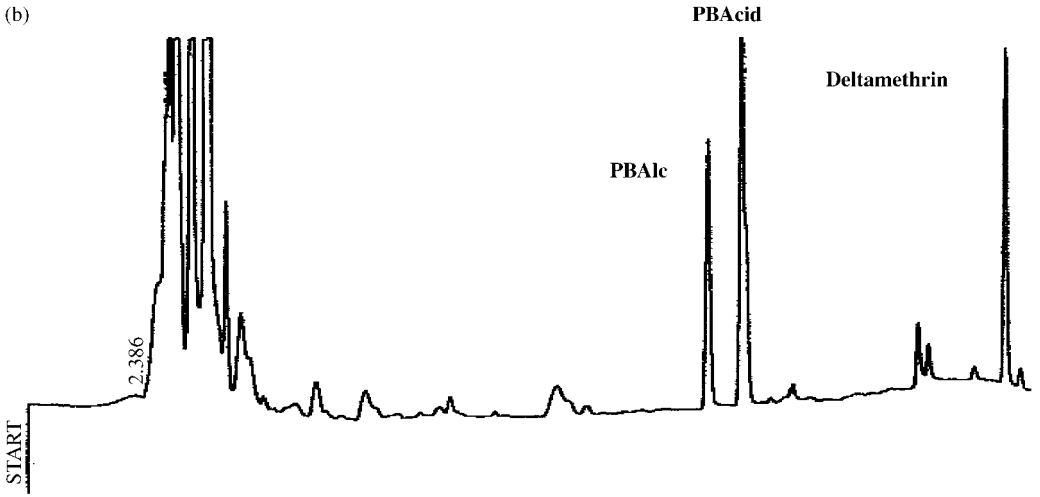
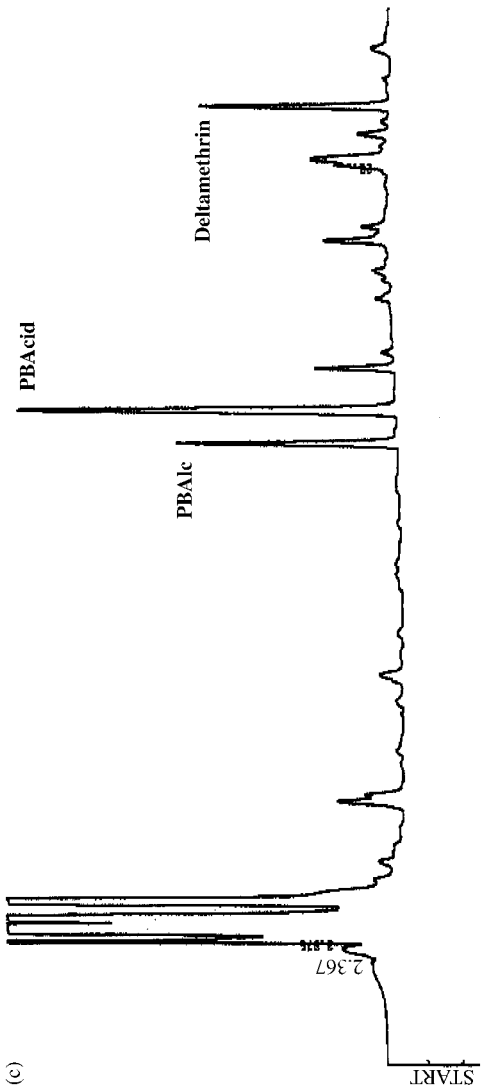


Figure 2. (a) Chromatographs of 3-PB Alc (3.0 μg/mL, 15.524 min), 3-PB Acid (3.0 μg/mL, 16.315 min), and deltamethrin (3.0 μg/mL, 22.354 min) spiked maternal plasma on a Zorbax 80SB-CN (4.6 × 250 mm^2, 5 μm) analytical column. (b) Chromatographs of 3-PB Alc (3.0 μg/mL, 15.540 min), 3-PB Acid (3.0 μg/mL, 16.295 min), and deltamethrin (3.0 μg/mL, 22.370 min) spiked amniotic fluid on a Zorbax 80SB-CN (4.6 × 250 mm^2, 5 μm) analytical column. (c) Chromatographs of 3-PB Alc (3.0 μg/mL, 15.593 min), 3-PB Acid (3.0 μg/mL, 16.439 min), and deltamethrin (3.0 μg/mL, 24.078 min) spiked placenta on a Zorbax 80SB-CN (4.6 × 250 mm^2, 5 μm) analytical column. (d) Chromatographs of 3-PB Alc (3.0 μg/mL, 15.606 min), 3-PB Acid (3.0 μg/mL, 16.453 min), and deltamethrin (3.0 μg/mL, 24.082 min) spiked fetus on a Zorbax 80SB-CN (4.6 × 250 mm^2, 5 μm) analytical column.

(continued)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



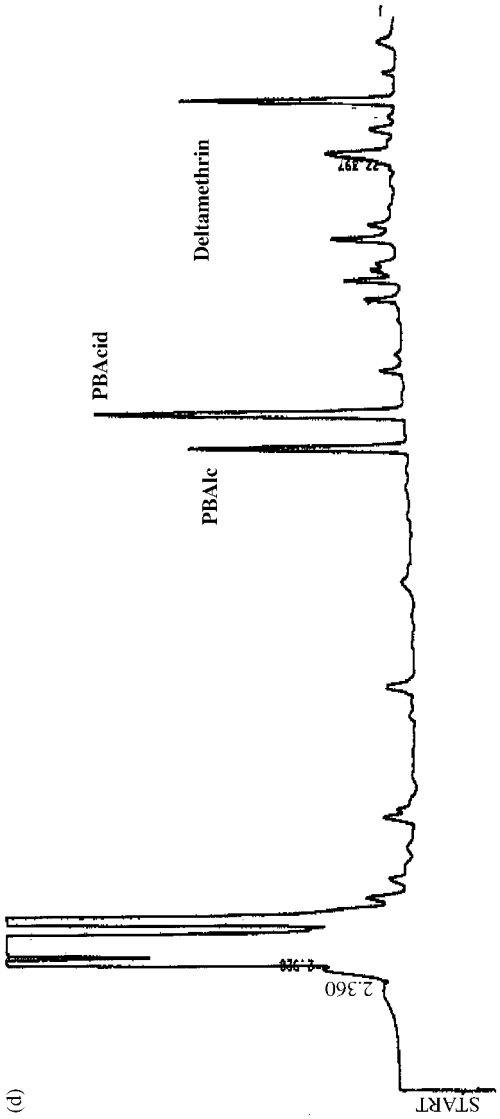


Figure 2. Continued.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. The r^2 values of calibration curves for deltamethrin, 3-PB Acid, and 3-PB Alc from maternal plasma, amniotic fluid, placental and fetal tissues ($n = 3$).

	r^2 Range
Maternal plasma	
Deltamethrin	0.99930–0.99982
PB Acid	0.99964–0.99998
PB Alc	0.99917–0.99999
Amniotic fluid	
Deltamethrin	0.99821–0.99950
PB Acid	0.99729–0.99978
PB Alc	0.99739–0.99904
Placental tissue	
Deltamethrin	0.99878–0.99993
PB Acid	0.99945–0.99992
PB Alc	0.99941–0.99987
Fetal tissue	
Deltamethrin	0.99937–0.99953
PB Acid	0.99955–0.99969
PB Alc	0.99881–0.99951

deviation, % RSD) and accuracy (% error). Five replicates of blank amniotic fluid spiked with deltamethrin, 3-PB Acid, and 3-PB Alc concentrations of 0.1 μg/mL (LOQ), 0.2, 1.5, and 4.0 μg/mL, were prepared for each validation day to test the precision and accuracy. Intra day ($n = 5$) and inter day ($n = 15$) precision and accuracy were calculated from standard curves constructed from each of the four biological matrices studied, and are shown in Tables 3 and 4, respectively. The intra day ($n = 5$) precision and accuracy for deltamethrin were in the range of 0.2–7.8 (% RSD) and 0.4–11.2 (% error) for the four matrices. The intra day ($n = 5$) precision and accuracy for 3-PB Acid were in the range of 1.1–13.6 (% RSD) and 1.1–13.0 (% error) for all four matrices. The intra day ($n = 5$) precision and accuracy for 3-PB Alc were in the range of 0.6–11.5 (% RSD) and 1.9–13.7 (% error) for all four matrices. Inter day ($n = 15$) precision and accuracy for deltamethrin ranged from 3.1% to 12.0% (% RSD) and 2.5% to 8.8% (% error), respectively, for the four matrices. Inter-day ($n = 15$) precision and accuracy for 3-PB Acid ranged from 1.8% to 12.5% (% RSD) and 2.1% to 10.0% (% error), respectively, for all four matrices. The inter day ($n = 15$) precision and accuracy for 3-PB Alc ranged from 2.4% to 11.3% (% RSD) and 3.1% to 10.5% (% error).

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 2. The percentage recovery ±SD ($n = 5$) of deltamethrin, 3-PB Acid, and 3-PB Alc from rat maternal plasma, amniotic fluid, placental and fetal tissues.

Analyte concentration (μg/mL)	Maternal plasma	Amniotic fluid	Placental tissue	Fetal tissue
Deltamethrin				
0.8	83.0 ± 9.4	90.5 ± 11.8	88.0 ± 2.0	90.3 ± 5.2
4.0	88.8 ± 1.1	98.1 ± 1.8	89.0 ± 1.8	93.7 ± 2.7
3-PB Acid				
0.8	91.2 ± 4.9	99.7 ± 4.0	91.3 ± 2.0	92.7 ± 3.5
4.0	90.5 ± 2.2	97.0 ± 7.6	91.9 ± 1.8	95.3 ± 2.8
3-PB Alc				
0.8	86.9 ± 9.3	82.4 ± 3.5	90.4 ± 3.4	87.8 ± 3.6
4.0	86.2 ± 2.5	86.5 ± 7.3	92.3 ± 1.1	89.4 ± 2.2

Stability testing was performed for deltamethrin, 3-PB Acid, and 3-PB Alc. Spiked biological fluids, or tissue homogenate samples (3.0 μg/mL), were exposed to three consecutive freeze/thaw cycles over a period of 4 days. On day 1, 20 blank biological matrices samples were spiked with deltamethrin, 3-PB Acid, and 3-PB Alc, to give a final concentration of 3.0 μg/mL, and five of them were extracted and analyzed as described above. The remaining 15 spiked plasma samples were stored at −20°C. On the following three consecutive days, the spiked plasma samples were thawed, and five more extracted and analyzed. The day-to-day measured peak areas of deltamethrin, 3-PB Acid, and 3-PB Alc were compared and the results listed in Table 5. The % RSD between the average peak area of deltamethrin, 3-PB Acid, and 3-PB Alc each day, was less than 9.6%. There was no distinctive decline in peak areas for either deltamethrin, 3-PB Acid, or 3-PB Alc over three consecutive freeze/thaw cycles. The stability of extracted biological fluid, or tissue homogenate samples in the autosampler, was also tested for deltamethrin, 3-PB Acid, and 3-PB Alc. Six extracted biological matrice samples containing 3.0 μg/mL of deltamethrin, 3-PB Acid, and 3-PB Alc, were put into the autosampler for a 25-hr period. At time 0, one sample was injected onto the HPLC column and analyzed. Over the following 25 hr, one additional sample was injected and analyzed approximately every 5 hr. The peak areas for deltamethrin, 3-PB Acid, and 3-PB Alc in each injection, were compared. The % RSD between each sample was <11.2% for all three compounds. There was no obvious decline in peak areas between each injection (Table 6).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 3. The intra day precision (% RSD) and accuracy (% error) of deltamethrin, 3-PB Acid, and 3-PB Alc in rat maternal plasma, amniotic fluid, placental and fetal tissues.

Concentration added (μg/mL)	Deltamethrin found (μg/mL)	RSD (%)	Error (%)	3-PB Acid found (μg/mL)	RSD (%)	Error (%)	3-PB Alc found (μg/mL)	RSD (%)	Error (%)
Maternal plasma									
0.2	0.186 ± 0.009	4.961	7.201	0.226 ± 0.009	4.153	13.036	0.225 ± 0.014	6.034	12.276
0.4	0.386 ± 0.018	4.675	4.534	0.415 ± 0.016	3.750	4.100	0.421 ± 0.039	9.305	5.886
1.5	1.519 ± 0.054	3.539	3.280	1.573 ± 0.066	4.194	5.515	1.600 ± 0.071	4.461	6.951
4.0	4.000 ± 0.188	4.705	3.536	4.141 ± 0.327	7.904	5.167	4.016 ± 0.245	6.096	4.121
Amniotic fluid									
0.1	0.111 ± 0.006	5.770	11.153	0.111 ± 0.006	4.976	11.145	0.102 ± 0.012	11.470	8.051
0.2	0.215 ± 0.017	7.694	9.476	0.218 ± 0.008	3.770	9.238	0.191 ± 0.014	7.570	6.636
1.5	1.540 ± 0.088	5.722	5.785	1.561 ± 0.113	7.241	6.995	1.705 ± 0.089	5.192	13.664
4.0	4.014 ± 0.220	5.475	4.382	4.088 ± 0.195	4.769	4.431	4.428 ± 0.257	5.815	10.693
Placental tissue									
0.2	0.217 ± 0.004	1.676	8.256	0.208 ± 0.002	1.176	4.019	0.223 ± 0.012	5.487	11.558
0.4	0.422 ± 0.005	1.289	5.598	0.421 ± 0.004	1.069	5.175	0.408 ± 0.011	2.858	2.662
1.5	1.465 ± 0.018	1.210	2.361	1.494 ± 0.020	1.353	1.133	1.478 ± 0.029	1.971	1.921
4.0	4.017 ± 0.009	0.225	0.413	4.107 ± 0.154	3.745	2.675	4.071 ± 0.063	1.555	1.987
Fetal tissue									
0.2	0.199 ± 0.015	7.784	4.974	0.212 ± 0.029	13.563	11.349	0.208 ± 0.020	9.679	6.149
0.4	0.392 ± 0.014	3.456	2.491	0.410 ± 0.011	2.777	2.748	0.417 ± 0.017	4.057	4.863
1.5	1.498 ± 0.025	1.674	1.165	1.546 ± 0.024	1.541	3.064	1.555 ± 0.045	2.921	3.678
4.0	3.995 ± 0.030	0.747	0.549	4.245 ± 0.288	6.791	6.126	4.188 ± 0.027	0.637	4.689

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 4. The inter day precision (% RSD) and accuracy (% error) of deltamethrin, 3-PB Acid, and 3-PB Alc in rat maternal plasma, amniotic fluid, placental and fetal tissues.

Concentration added (μg/mL)	Deltamethrin found (μg/mL)	RSD (%)	Error (%)	3-PB Acid found (μg/mL)	RSD (%)	Error (%)	3-PB Alc found (μg/mL)	RSD (%)	Error (%)
Maternal plasma									
0.2	0.188 ± 0.017	9.114	8.330	0.200 ± 0.025	12.529	9.968	0.215 ± 0.018	8.165	8.801
0.4	0.388 ± 0.030	7.760	6.590	0.410 ± 0.023	5.498	4.875	0.393 ± 0.044	11.315	7.785
1.5	1.472 ± 0.054	3.699	3.378	1.515 ± 0.084	5.519	4.846	1.545 ± 0.092	5.934	5.652
4.0	3.963 ± 0.151	3.800	2.777	4.056 ± 0.217	5.348	3.408	4.028 ± 0.222	5.510	4.096
Amniotic fluid									
0.1	0.105 ± 0.008	7.319	7.065	0.104 ± 0.009	8.799	8.731	0.105 ± 0.012	11.033	10.499
0.2	0.198 ± 0.016	8.101	6.471	0.208 ± 0.020	9.686	9.269	0.195 ± 0.016	8.318	7.197
1.5	1.542 ± 0.073	4.724	4.779	1.571 ± 0.113	7.217	6.478	1.631 ± 0.101	6.222	9.674
4.0	4.143 ± 0.250	6.037	4.989	4.151 ± 0.238	5.724	5.018	4.385 ± 0.242	5.530	9.630
Placental tissue									
0.2	0.214 ± 0.018	8.358	8.264	0.214 ± 0.018	8.406	7.187	0.213 ± 0.014	6.601	7.773
0.4	0.412 ± 0.013	3.117	3.798	0.412 ± 0.021	5.123	5.256	0.403 ± 0.015	3.734	3.141
1.5	1.538 ± 0.064	4.173	4.121	1.523 ± 0.046	3.042	2.085	1.544 ± 0.057	3.690	4.045
4.0	4.185 ± 0.246	5.881	4.690	4.107 ± 0.112	2.737	2.752	4.191 ± 0.162	3.863	4.844
Fetal tissue									
0.2	0.197 ± 0.024	12.037	8.781	0.203 ± 0.018	8.715	5.781	0.206 ± 0.021	10.024	7.560
0.4	0.409 ± 0.024	5.971	4.822	0.408 ± 0.010	2.436	2.598	0.405 ± 0.025	6.145	4.734
1.5	1.556 ± 0.072	4.645	4.177	1.554 ± 0.028	1.777	3.616	1.553 ± 0.037	2.403	3.628
4.0	4.086 ± 0.204	4.987	2.534	4.199 ± 0.273	6.507	4.980	4.113 ± 0.282	6.869	4.321

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 5. Results of freeze/thaw stability study of deltamethrin, 3-PBA, and 3-PA in plasma, amniotic fluid, placental and fetal tissues.

	Peak area ± SD		
	Deltamethrin	3-PB Acid	3-PB Alc
Maternal plasma			
Day 1	61379.4 ± 3838.6	127875.8 ± 3696.0	86063.6 ± 879.1
Day 2	65308.2 ± 1193.4	126191.2 ± 2151.9	85280.0 ± 3022.2
Day 3	66736.4 ± 1000.2	127522.2 ± 1446.0	89690.4 ± 798.4
Day 4	65268.6 ± 1311.6	128783.6 ± 3128.0	87767.4 ± 2278.9
% RSD	3.6	0.8	2.2
Amniotic fluid			
Day 1	73014 ± 3069.3	133151.2 ± 5556.1	77122.8 ± 5509.0
Day 2	72379 ± 1887.8	134009.6 ± 2069.1	77954.0 ± 1748.0
Day 3	77257.2 ± 3777.4	143238.6 ± 4273.6	89192.2 ± 4800.3
Day 4	65212.4 ± 5754.9	124664.6 ± 8388.1	71173.6 ± 6956.3
% RSD	6.9	5.7	9.5
Placental tissue			
Day 1	47736.4 ± 1754.9	101931.2 ± 10525.5	55532.8 ± 2030.3
Day 2	47875 ± 1773.1	98165.2 ± 4367.6	53838.4 ± 2778.7
Day 3	46371 ± 2587.4	96591.6 ± 5480.9	49898.6 ± 3769.2
Day 4	46997 ± 902.9	97773.2 ± 2358.3	52610.0 ± 1310.5
% RSD	1.5	2.3	4.5
Fetal tissue			
Day 1	50553.4 ± 1059.5	100485.4 ± 1934.2	55391.2 ± 1755.7
Day 2	50398.6 ± 1209.6	101309.6 ± 1509.5	55259.6 ± 1293.2
Day 3	48252.2 ± 1187.9	99237.2 ± 1794.6	50696.0 ± 1837.1
Day 4	48606.2 ± 1352.5	100510 ± 2878.4	54442.8 ± 2500.8
% RSD	2.4	0.9	4.1

Table 6. Percentage RSD of autosampler stability samples for deltamethrin, 3-PB Acid, and 3-PB Alc in rat maternal plasma, amniotic fluid, placental and fetal tissues ($n = 6$).

	Deltamethrin	3-PB Acid	3-PB Alc
Maternal plasma	11.1	9.0	10.2
Amniotic fluid	4.7	7.4	8.4
Placental tissue	1.4	2.7	3.2
Fetal tissue	7.4	2.2	1.7

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

CONCLUSIONS

A sensitive and efficient method for the extraction and analysis of deltamethrin, its metabolites 3-PB Acid and 3-PB Alc, in pregnant rat maternal plasma, amniotic fluid, fetal and placental tissues was developed and validated. This method yields high recoveries, shows good linearity, precision and accuracy within the range of 0.1–5.0 μg/mL for amniotic fluid, and 0.2–5.0 μg/mL for the other biological matrices. This method will facilitate toxicokinetic investigations of placental transport and disposition of deltamethrin.

ACKNOWLEDGMENTS

The authors wish to thank the University of Georgia Research Foundation and the US Environmental Protection Agency Grant #R830800-01 for support of this work.

REFERENCES

1. Elliot, M.; Farnham, A.W.; Janes, N.F.; Needham, P.H.; Pulman, D.A. Synthetic insecticide with a new order of activity. Nature **1974**, *248*, 710–711.
2. Anadon, A.; Martinez-Larranaga, M.R.; Fernandez-Cruz, M.L.; Diaz, M.J.; Fernandez, M.C.; Martinez, M.A. Toxicokinetics of deltamethrin and its 4′-HO-metabolite in the rat. Toxicol. Appl. Pharmacol. **1996**, *141*, 8–16.
3. Kavlock, R.; Chernoff, N.; Baron, R.; Linder, R.; Rogers, E.; Carver, B. Toxicity studies with decamethrin, a synthetic pyrethroid insecticide. J. Environ. Pathol. Toxicol. **1979**, *2*, 751–765.
4. Peter, J.V.; John, G.; Cherian, A.M. Pyrethroid poisoning. J. Assoc. Physicians India **1996**, *44*, 343–344.
5. Bhaumik, A.; Gupta, P.K. Teratogenicity of decamethrin in rats. Indian Vet. J. **1990**, *67*, 213–219.
6. Abdel-Khalik, M.M.; Hanafy, M.S.M.; Abdel-Aziz, M.I. Studies on the teratogenic effects of deltamethrin in rats. Dtsch. Tieraztl. Wschr. **1993**, *100*, 142–143.
7. Kaneko, H.; Izumi, T.; Ueda, Y. Metabolism and placental transfer of stereoisomers of tetramethrin isomers in pregnant rat. J. Pestic. Sci. **1984**, *9*, 249–258.
8. Quistad, G.B.; Staiger, L.E.; Janieson, G.C. Metabolism of fluvalinate by a lactating dairy cow. J. Agric. Food Chem. **1982**, *30*, 895–901.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

9. Shiba, K.; Kaneko, H.; Kakuta, N. Placental transfer of esfenvalerate in pregnant rats. J. Pestic. Sci. **1990**, *15*, 169–174.
10. Malaviya, M.; Husain, R.; Seth, P.K. Perinatal effect of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rat. Vet. Hum. Toxicol. **1993**, *35*, 119–122.
11. Santoni, G.; Cantalamessa, F.; Spreghini, E. Alterations of T cell distribution and functions in prenatally cypermethrin-exposed rats: possible involvement of catecholamines. Toxicology **1999**, *138*, 175–187.
12. Santoni, G.; Cantalamessa, F.; Mazzucca, L. Prenatal exposure to cypermethrin modulates rat NK cell cytotoxic functions. Toxicology **1997**, *120*, 231–242.
13. Boike, G.M.; Deppe, G.; Young, J.D.; Gove, N.L.; Bottoms, S.F.; Malone, J.M.; Malviya, V.K.; Sokol, R.J. Chemotherapy in a pregnant rat model 1. Mitomycin C pregnancy specific kinetics and placement transfer. Gynecol. Oncol. **1989**, *34*, 187.
14. Faber, J.J.; Thomburg, K.L. *Placental Physiology: Structure and Function of Fetomaternal Exchange*; Raven: New York, 1983.
15. Brown, S.D.; White, C.A.; Chu, C.K.; Bartlett, M.G. Determination of acyclovir in maternal plasma, amniotic fluid, fetal and placental tissues by HPLC. J. Chromatogr. B **2002**, *772*, 327–334.
16. Ruzo, L.O.; Unai, T.; Casida, J.E. Decamethrin metabolism in rats. J. Agric. Food Chem. **1978**, *26*, 918–925.
17. Gray, A.J.; Soderlund, D.M. Mammalian toxicology of pyrethroids. In *Progress in Pesticide Biochemistry and Toxicology*; Hutson, D.H., Roberts, T.R., Eds.; John Wiley and Sons Ltd.: New York, 1985; Vol. 21, 193–248.
18. Hutson, D.H. The metabolic fate of synthetic pyrethroid insecticides in mammals. Prog. Drug Metab. **1979**, *5*, 215–252.
19. Pavan, F.A.; Dallago, R.M.; Zanella, R.; Martins, A.F. Determination of deltamethrin in cattle dipping baths by high-performance liquid chromatography. J. Agric. Food Chem. **1999**, *47*, 174–176.
20. Mourot, D.; Delepine, B.; Boisseau, J.; Gayot, G. High-performance liquid chromatography of decamethrin. J. Chromatogr. **1979**, *173*, 412–414.
21. Bissacot, D.Z.; Vassilieff, I. HPLC determination of flumethrin, deltamethrin, cypermethrin, and cyhalothrin residues in the milk and blood of lactating dairy cows. J. Anal. Toxicol. **1997**, *21*, 397–402.

Received January 12, 2004
Accepted February 4, 2004
Manuscript 6315

Request Permission or Order Reprints Instantly!