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Rapid determination of the synthetic pyrethroid insecticide, deltamethrin, in rat plasma and tissues by HPLC

Kyu Bong Kim [a,b], Michael G. Bartlett [a], Sathanandam S. Anand [a], James V. Bruckner [a,*], Hyo Jung Kim [a]

[a] *Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA 30602, USA*
[b] *Pharmacology Department, National Institute of Toxicological Research, Korea Food and Drug Administration, 5-Nokbun-dong, Eunpyung-gu, Seoul 122-704, South Korea*

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Abstract

Deltamethrin (DLM), [(*S*)-α-cyano-d-phenoxybenzyl-(1*R*,3*R*)-e-(2,2 dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate], is a pyrethroid insecticide widely used in agriculture and households. There are several methods for analysis of DLM in biological fluids and tissues, but these methods are time consuming. They generally involve the extraction of DLM with lipid-soluble solvents such as *n*-pentane, *n*-hexane, diethylether or acetone, and subsequent evaporation of the solvent. A more rapid and sensitive high-performance liquid chromatography (HPLC) method to analyze DLM in plasma and tissues (liver, kidney, and brain) was developed and validated according to U.S. Food and Drug Administration (U.S. FDA) and International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The limit of detection (S/N of 3/1) for DLM was 0.01 μg/ml for plasma, liver, kidney and brain. The method performances were shown to be selective for DLM and linear over the concentration range 0.01–20.0 μg/ml. For five replications of samples at 0.05, 0.1, 0.2, 1.5 and 4.0 μg/ml, intraday precision and accuracy values were in the range of 0.7–13.1% relative standard deviation (%R.S.D.) and 1.8–14.1%Error, respectively. Interday ($n = 15$) precision and accuracy values at 0.05, 0.1, 0.2, 1.5, and 4.0 μg/ml were in the range of 3.2–15.2% (%R.S.D.) and 3.7–14.8%Error, respectively. The absolute recoveries of DLM ranged from 93 to 103% for plasma, 95 to 114% for liver, 97 to 108% for kidney, and 95 to 108% for brain. This method can be quite useful for DLM pharmacokinetic and tissue distribution studies, for which multiple plasma and tissue samples have to be analyzed quickly with high reproducibility.

Keywords: Deltamethrin; HPLC; Pyrethroid insecticide; Plasma; Tissues

1. Introduction

Pyrethroid insecticides are widely used in agriculture to protect crops, in the household to control pests, and in public health to control diseases caused by vectors or intermediate hosts [1,2]. Pyrethroids accounted for about 25% of the worldwide insecticide market in 1998 [3]. That percentage share has increased substantially over the last few years in the U.S. [4], as a result of the U.S. Environmental Protection Agency's (EPA's) restrictions on household and agricultural use of organophosphates. Pyrethroids' popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals.

There have been a variety of approaches developed since the 1970s to quantify pyrethroids in biological samples. These approaches can be categorized as biological, immunological and chemical. Biological assays such as the LC50 (i.e., concentration required to kill 50% of an insect population) were carried out by entomologists [5,6]. Immunoassays were developed to rapidly detect trace levels of pyrethroids in environmental and food samples [7,8]. Chemical assays have included gas chromatography [9,10], thin-layer chromatography [11,12], and high-performance liquid chromatography (HPLC) with ultraviolet detection. HPLC methods have been utilized to determine concentrations of the pyrethroid, deltamethrin (DLM) in milk, foods, environmental specimens, blood and various tissues [13–17]. Isolation and concentration of DLM in samples

* Corresponding author. Tel.: +1 706 542 5405; fax: +1 706 542 5358.
E-mail address: bruckner@rx.uga.edu (J.V. Bruckner).

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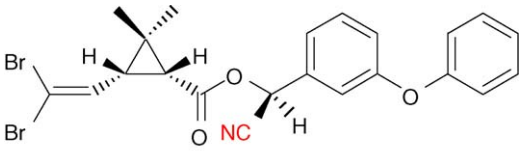

Deltamethrin

Fig. 1. Chemical structure of deltamethrin.

has involved a combination of liquid–liquid and solid phase extraction.

Deltamethrin (DLM), [(*S*)-α-cyano-d-phenoxybenzyl-(1*R*, 3*R*)-e-(2,2 dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate] (Fig. 1), is one of the most neurotoxic pyrethroids. It acts by delaying closure of sodium channels, resulting in a tail current that is characterized by a slow influx of sodium during the end of neuronal depolarization [18,19]. Typical signs and symptoms of acute poisoning of laboratory animals and humans by DLM and other Type II pyrethroids include salivation, hyperexcitability and choreoathetosis. Immature rats are much more susceptible to acute DLM neurotoxicity than adults [20,21], due in large measure to inefficient metabolic detoxification of the parent compound [22]. DLM is hydrolyzed by esterases and hydroxylated by cytochrome P450s [23–25]. At present, there is concern that DLM and possibly other pyrethroids, like certain organophosphates, may exhibit potential to be developmental neurotoxicants in infants and children [26,27].

The EPA has been given responsibility in the U.S. for assessing pesticides and other chemicals that may pose risks to the health of children and adults. The degree and duration of toxicity are largely dependent upon the concentration of toxicant in the target tissue and how long it remains there. Physiologically-based pharmacokinetic (PBPK) models are a useful tool in risk assessment, in that they can be used to predict concentrations of toxic forms of chemicals in target organs (e.g., brain) over time following a variety of exposure scenarios [28,29]. Construction of a PBPK model for a toxic chemical requires data from toxicology and PK studies in animals. Mature and immature rodents are commonly used as surrogates for adults and children. Inherent limitations of such studies include, among others, small biological sample size and limitation of the dose of chemical that can be given, due to toxicity. Few PK data for DLM are therefore available. Anadon et al. [16] did conduct an experiment with adult rats, but had to administer a neurotoxic dose [i.e., 26 mg DLM/kg body weight (bw)] in order to characterize a relatively complete time-course of DLM in the blood and brain.

Immature rats succumb to this and lower doses. Therefore, analytical sensitivity is a key consideration in development of an analytical technique suitable for DLM kinetics time-course studies in small animals and their offspring. Detection limits as low as 0.001 and 0.005 μg/ml of sample have been reported [13,16], but these HPLC procedures were not adequately validated, were time consuming and required large sample volumes. Serial blood sampling and sacrifices of groups of animals in PK time-course experiments produce a myriad of samples to be analyzed. To our knowledge, only Ding et al. [17,30] reported a fully-validated HPLC analytical method for DLM. Its limit of quantitation was 0.1 μg/ml plasma. The method utilized protein precipitation with acetonitrile rather than liquid–liquid or solid-phase extraction for sample preparation. It was not applied to liver, kidney or brain and was somewhat time consuming, in that it required solvent extraction and evaporation to dryness.

The objective of the current effort was to develop a more rapid and sensitive HPLC technique to quantify DLM in the large numbers of low-volume blood and tissue samples generated in PK studies in small animals. An important aim was to validate the procedure by U.S. FDA [31] and ICH [32] guidelines for analytical method validation including accuracy, precision, linearity, limit of detection, limit of quantitation, recovery and stability.

2. Experimental

2.1. Chemicals and animals

Standard DLM (purity, 98.8%) was kindly provided by Bayer CropScience AG (Monheim, Germany). Acetonitrile (HPLC-grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, sulfuric acid and deionized water (HPLC-grade) were obtained from J.T. Baker (Phillipsburg, NJ, USA).

Male Sprague–Dawley (SD) rats (Charles River Laboratories, Raleigh, NC, USA) were used to provide blank biological matrices and for PK studies with DLM. The protocol for this study was approved by the University of Georgia Animal Care and Use Committee. Each rat was housed in a cage with a 12 h light/12 h dark cycle at ambient temperature (22 °C) and relative humidity (55 ± 5%). Food (5001 Rodent Diet, PMI Nutrition International LLC, Brentwood, MO, USA) and tap water were provided ad libitum.

2.2. Preparation of stock, standard solutions, and calibration standards

A DLM stock solution was prepared in acetonitrile at a final concentration of 1.0 mg/ml. The stock solution was stored in a freezer at −20 °C, though DLM is generally believed to be stable at least 6 months at room temperature [2]. Working standard solutions with concentrations of 0.05, 0.1, 0.5, 1.25, 1.5, 2.5, 3.75, 5.0, 10.0, 15.0, 25.0, and 100 μg/ml were prepared by appropriate dilution of the 1.0 mg/ml stock solution with acetonitrile. Plasma, liver, kidney and brain specimens were collected from SD rats for blank matrices. Mixtures of appropriate volume (30 μl) of a working solution and blank matrix (120 μl) were prepared for calibration standards. The final calibration standards were 0.01, 0.02, 0.1, 0.25, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0 and 20.0 μg DLM/ml.

2.3. Analysis system

The analysis was conducted with a HPLC system consisting of a Shimadzu HPLC (Shimadzu, Canby, OR, USA) equipped with a pump (LC-10AT), degasser (DGU-14A), auto-sampler (SIL-HT), detector (SPD-10AV) and computer with an EZStart

7.2 SP1 Rev B. The analytical column was an Ultracarb 5 ODS (20) column (250 mm × 4.6 mm; 5 μm particle) (Phenomenex, Torrance, CA, USA), and the guard column was a Phenomenex fusion RP 4 mm × 3 mm (Torrance, CA). The mobile phase was 80% acetonitrile and 20% sulfuric acid (1%, v/v) (v/v). The flow rate was set at 1.0 ml/min. The eluate was monitored at 230 nm. Under these chromatographic conditions, DLM eluted at approximately 14.5 min.

2.4. Extraction procedure

2.4.1. Plasma

To generate plasma, blood samples collected in heparinized tubes were centrifuged for 5 min at 13,000 rpm using a microcentrifuge (Microfuge 22R Centrifuge, Beckman Coulter, Fullerton, CA, USA). 65 μl of plasma were added to microcentrifuge tubes containing 130 μl of acetonitrile. These were vigorously agitated on a vortex mixer (Mini Vortexer, VWR, West Chester, PA, USA) for 30 s. The tubes were then centrifuged for 5 min at 13,000 rpm in the microcentrifuge, and 50 μl of the clear supernatant injected onto the column.

2.4.2. Liver, kidney, and brain

Liver, kidney and brain were isolated from the rats and homogenized in four volumes of 50% (v/v) acetonitrile in distilled water with a Tissumizer (Tekmar, Cincinnati, OH, USA). 65 μl of the tissue homogenates were added to microcentrifuge tubes containing 130 μl of acetonitrile. These tubes were vigorously mixed with a vortex mixer for 30 s and centrifuged for 5 min at 13,000 rpm in the microcentrifuge. 50 μl of the clear supernatant were injected onto the column.

2.5. Method validation

2.5.1. Preparation of samples

30 μl of DLM standard solutions were added to 120 μl of blank plasma or tissue homogenate in a 1.5-ml microcentrifuge tube. The final concentrations of samples were 0.05, 0.1, 0.2, 1.5, and 4.0 μg/ml, respectively. This mixture was vortexed for 10 s and then used.

2.5.2. Specificity

Blank samples were analyzed as described above, and the chromatograms were visually evaluated for occurrence of substances that might interfere with the DLM peak.

2.5.3. Absolute recovery, accuracy and precision

Absolute recovery was calculated from the peak areas of DLM in plasma, liver, kidney and brain compared with those of standard solutions. For intraday accuracy and precision, the samples ($n = 5$) spiked at concentrations of 0.05, 0.1, 0.2, 1.5, and 4.0 μg/ml were analyzed. The accuracy was expressed as the absolute error percentage and calculated from (|mean of measured concentration − added concentration|/added concentration) × 100. The precision was expressed as the relative standard deviation (%R.S.D.) and calculated from the standard deviation divided by the mean of the detected concentration. Interday accuracy and precision were determined in five replicates of the biological sample spiked at concentrations of 0.05, 0.1, 0.2, 1.5, and 4.0 μg/ml and performed on three different days.

2.5.4. Limit of detection and limit of quantification

The limit of detection (LOD) was defined as the lowest concentration of DLM (S/N = 3), and the limit of quantitation (LOQ) was set at the lowest validation point.

2.5.5. Linearity of calibration curve

The calibration curves were obtained by peak area versus DLM concentration.

2.6. Application to pharmacokinetic studies

Pharmacokinetic tissue distribution experiments with adult (∼90-day-old) and immature (10-day-old) male SD rats were conducted. Blood was serially collected from the same adult animals to decrease intersubject variability in the pharmacokinetic profiles. Each adult rat was anesthetized by IM injection of 0.1 ml/100 g bw of a "cocktail" consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine hydrochloride (20 mg/ml) (3:2:1, v/v/v). A cannula (PE50 polyethylene tubing) was surgically inserted into the right carotid artery and securely ligated. The cannula was passed under the skin and exteriorized at the nape of the neck, so the animals could move about freely following their recovery. Water was provided, but food was withheld during the 24-h post-surgical recovery period before dosing. The cannulated adult rats were given a single oral dosage of 10 mg DLM/kg bw (in 2 ml glycerol formal/kg bw). Arterial blood samples of 150 μl were collected from the indwelling cannula from four animals (0.25, 0.5, 1, 2, 4, 6, 9, 12, and 24 h post dosing).

Plasma samples were processed by the procedure previously described in Section 2.4.1. In order to study DLM deposition in brain, adult rats ($n = 5$) per time-point were given 10 mg DLM/kg bw orally (in 2 ml glycerol formal/kg bw) and euthanized by CO_2 asphyxiation 0.5, 1, 2, 6, 12 and 24 h after dosing. Ten-day-old immature rats ($n = 3$ per time-point) were given 2 mg DLM/kg bw orally (in 2 ml glycerol formal/kg bw) for determination of plasma, brain, liver and kidney deposition. They were decapitated 0.5, 1, 2, 6, 12 and 24 h after dosing. Tissue sample preparation was as described previously in Section 2.4.2.

3. Results and discussion

3.1. Analytical method

This HPLC method proved to be rapid and sensitive for quantitation of DLM in blood and tissues. Other published HPLC methods [13–17] are laborious, in that they require day-long evaporation to dryness and have longer column retention times. With the current method, DLM was extracted from plasma by vortexing it in twice its volume of acetonitrile for 30 s. Acetonitrile in water (50%, v/v) was initially used to homogenize tissues

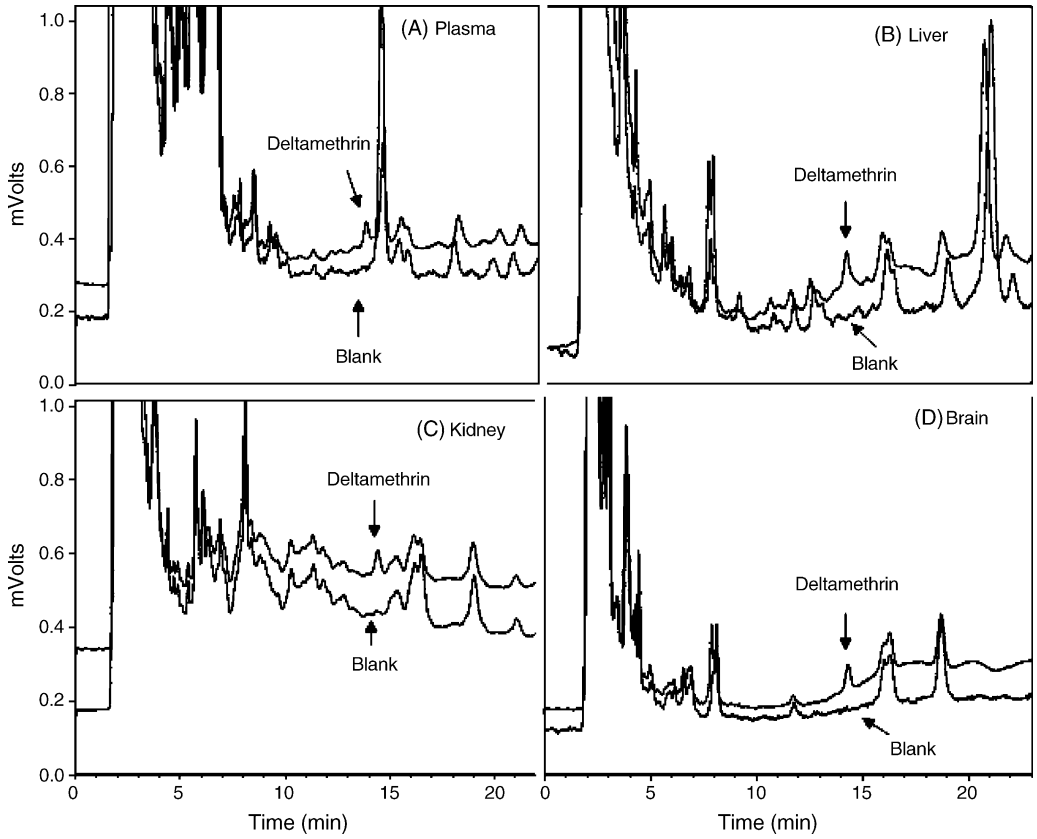

Fig. 2. Chromatograms of blank and DLM (0.05 μg/ml)-spiked plasma (A), liver (B), kidney (C) and brain (D) on a C18 Ultracarb 5 ODS (20) (4.6 mm × 250 mm, 5 μm) analytical column.

to achieve better extraction. DLM was extracted by adding this homogenate to twice the volume of acetonitrile. An aliquot of supernatant was injected directly onto the column without taking the samples through an evaporation process. Eliminating acetonitrile volatilization enabled us to analyze many more samples in a day. This is essential for PK studies, as described in the Introduction. DLM is a highly lipophilic compound with an octanol–water partition coefficient (K_{ow}) of 4.53 [33]. This value means that DLM can be transferred much more efficiently into the lipid-soluble solvent, acetonitrile, than water. Thus, acetonitrile acts not only to precipitate proteins but to solubilize the highly lipophilic DLM. Evaporation of solvent and reconstitution are thereby avoided, and acetonitrile containing DLM can be injected directly into the HPLC.

The chemical structure and chromatographic separation of DLM are shown in Figs. 1 and 2, respectively. The retention time was 14.5 min when a C18 column was used. With a C8 column, the retention time was 9.1 min. Baseline resolution was achieved using the chromatographic conditions described in the Experimental section. DLM was completely separated, as no interfering or co-eluting peaks with similar retention times were found in the chromatograms of blank biological samples (Fig. 2).

3.2. Method validation for plasma, liver, kidney, and brain

The calibration curves for plasma, liver, kidney, and brain showed good linearity over the range from 0.01 to 20.0 μg/ml for DLM ($r^2 > 0.998$ in all cases). The LOD for DLM was determined by analysis of standard-spiked samples of gradually decreasing concentration. The LOD was defined as the concentration at which the signal/noise ratio was ∼3. The LOD and LOQ were found to be approximately 0.01 and 0.05 μg/ml, respectively. This LOQ is lower than that reported by Ding et al. [17], and the present method's LOD is three-fold lower. Although other investigators have reported even lower LODs (0.001 μg/ml by Bissacot and Vassilieff [13] and 0.005 μg/ml by Anadon et al. [16]), their methods' applications to pharmacokinetic studies have the following limitations: (1) both methods utilized time-consuming solvent evaporation, and Bissacot and Vassilieff [13] also used a complex extraction procedure; (2) both methods required very large volumes of blood, which is a limiting factor if these methods are to be applied to mice, young rats or serial sampling from an adult rat or larger animal; and (3) neither method was fully validated.

As shown in Table 1, absolute recoveries of DLM from spiked plasma, liver, kidney and brain were in the range of 93–114%. The present extraction efficiencies (93–103% recovery from rat plasma) were higher than those (91 and 83–89%) for HPLC methods previously developed by our laboratory [17,30]. This observation implies that the evaporation and reconstitution steps formerly used may be responsible for the lower recoveries of DLM. Recoveries of DLM from rat liver (95–114%), kidney (97–108%), and brain (95–108%) were also higher than from those achieved from placenta or fetal tissues [30].

Table 1
Absolute recovery (%) of DLM in rat plasma and tissues

Sample	*n*	DLM concentration added (μg/ml)	Absolute recovery (%)[a]
Plasma	5	4.0	93 ± 7
		1.5	101 ± 7
		0.2	102 ± 2
		0.1	102 ± 5
		0.05	103 ± 6
Liver	5	4.0	103 ± 3
		1.5	95 ± 2
		0.2	107 ± 2
		0.1	114 ± 7
		0.05	100 ± 10
Kidney	5	4.0	104 ± 4
		1.5	97 ± 4
		0.2	97 ± 1
		0.1	102 ± 6
		0.05	108 ± 4
Brain	5	4.0	108 ± 5
		1.5	104 ± 4
		0.2	95 ± 3
		0.1	106 ± 1
		0.05	103 ± 9

All the values are expressed as mean ± S.D.
[a] Absolute recovery was calculated from the peak areas of DLM in plasma, liver, kidney and brain compared with standard solutions.

Intraday and interday accuracy and precision were determined to evaluate the reliability of the current analytical method. The intraday and interday accuracy and precision were evaluated using 0.05, 0.1, 0.2, 1.5 and 4.0 μg DLM/ml (Tables 2 and 3). These concentrations were similar to those employed previously by Ding et al. [17]. Both intraday and interday accuracy and precision for DLM in plasma and tissues were between 1.8 and 14.8 (%Error), and 0.7 and 15.2 (%R.S.D.), respectively. The interday and intraday accuracy and precision for DLM in plasma (4.7–11.2%Error and 1.6–13.5%R.S.D.) and in brain (3.3–14.8%Error and 0.9–14.8%R.S.D.) are in the range reported previously [16,17]. Ding et al. [30] found good intraday and interday accuracy and precision for DLM analyses of amniotic fluid, placenta and fetal tissue. The currently observed inter- and intraday accuracy and precision for DLM in liver (1.8–11.7%Error and 1.9–15.2%R.S.D.) and in kidney (2.0–13.1%Error and 0.7–10.5%R.S.D.) were comparable to those tissues and acceptable according to the criteria of the U.S. FDA [31]. Stability tests were not performed, because DLM has been shown to be a very stable compound in plasma by Ding et al. [17].

Like that of Ding et al. [17], the present method can also measure 3-phenoxybenzoic acid (PBA), one of DLM's major hydrolytic metabolites. Its retention time is at 3.9 min (data not shown). We, however, have not validated PBA quantitation, because the parent compound is the primary moiety of interest in toxicokinetic studies, as it is the proximate neurotoxicant. The cytochrome P450-mediated oxidative metabolites are not commercially available. Synthesis of hydroxy metabolites is

Table 2
The intraday precision (%R.S.D.) and accuracy (%Error) of DLM analyses

Sample	*n*	DLM concentration added (μg/ml)	DLM		
			Mean conc.[a] ± S.D.	Accuracy[b]	Precision[c]
Plasma	5	4.0	4.03 ± 0.29	4.7	7.2
		1.5	1.57 ± 0.10	5.0	6.4
		0.2	0.19 ± 0.00	4.8	1.6
		0.1	0.09 ± 0.01	7.4	5.5
		0.05	0.047 ± 0.004	9.1	8.2
Liver	5	4.0	4.07 ± 0.12	2.6	2.8
		1.5	1.51 ± 0.03	1.8	1.9
		0.2	0.21 ± 0.01	3.9	4.2
		0.1	0.09 ± 0.01	11.7	7.6
		0.05	0.047 ± 0.002	7.0	3.6
Kidney	5	4.0	4.02 ± 0.17	3.3	4.1
		1.5	1.52 ± 0.06	3.7	4.1
		0.2	0.20 ± 0.00	2.0	0.7
		0.1	0.09 ± 0.01	7.5	6.2
		0.05	0.056 ± 0.005	13.1	8.1
Brain	5	4.0	4.17 ± 0.18	4.7	4.3
		1.5	1.64 ± 0.06	9.1	3.5
		0.2	0.21 ± 0.01	3.3	3.2
		0.1	0.11 ± 0.00	4.8	0.9
		0.05	0.057 ± 0.007	14.1	13.1

[a] Mean conc., mean concentration (μg/ml) was determined from calibration curve.
[b] The absolute error percentage was calculated from the formula of (|mean of found concentration − added concentration|/added concentration) × 100 (%).
[c] The precision was evaluated as the %R.S.D.

Table 3
The interday precision (%R.S.D.) and accuracy (%Error) of DLM

Sample	*n*	DLM concentration added (μg/ml)	DLM		
			Mean conc.[a] ± SD	Accuracy[b]	Precision[c]
Plasma	15	4.0	3.80 ± 0.23	5.7	6.0
		1.5	1.58 ± 0.14	8.1	8.9
		0.2	0.20 ± 0.02	5.4	7.8
		0.1	0.10 ± 0.01	6.6	7.3
		0.05	0.047 ± 0.006	11.2	13.5
Liver	15	4.0	3.96 ± 0.25	5.1	6.3
		1.5	1.48 ± 0.06	3.7	4.4
		0.2	0.20 ± 0.01	4.2	5.2
		0.1	0.10 ± 0.01	6.4	6.4
		0.05	0.047 ± 0.007	10.1	15.2
Kidney	15	4.0	4.08 ± 0.20	4.5	4.9
		1.5	1.53 ± 0.08	4.5	5.1
		0.2	0.19 ± 0.01	4.6	4.2
		0.1	0.10 ± 0.01	7.4	8.1
		0.05	0.054 ± 0.006	9.9	10.5
Brain	15	4.0	4.11 ± 0.26	6.1	6.4
		1.5	1.54 ± 0.11	6.7	7.3
		0.2	0.21 ± 0.01	6.7	3.2
		0.1	0.10 ± 0.01	4.7	4.6
		0.05	0.056 ± 0.008	14.8	14.8

[a] Mean conc., mean concentration (μg/ml) was determined from calibration curve.
[b] The absolute error percentage was calculated from the formula of (|mean of found concentration − added concentration|/added concentration) × 100 (%).
[c] The precision was evaluated as the %R.S.D.

currently underway in our laboratory. A method for analysis of hydroxy and hydrolytic metabolites of DLM is planned for the future.

3.3. Application of DLM analysis to PK studies in rats

The most recent analytical method was applied to tissue disposition experiments with 10-day-old and adult SD rats. Due to DLM's greater toxicity in young rats, they were gavaged with just 2 mg DLM/kg bw. The adults received 10 mg/kg orally. Plasma and tissue samples were collected and analyzed for DLM as described in the Experimental section. Plasma and tissue DLM concentration versus time profiles for the mature and immature animals are shown in Figs. 3 and 4, respectively. All animals exhibited transient salivation. The maximum DLM concentrations (C_{maxs}) of 0.95 μg/ml plasma and 0.21 μg/g brain in adult rats were observed 1 and 2 h, respectively, after dosing (Fig. 3). The finding of lower DLM levels in brain than in plasma is in agreement with a report by Rickard and Brodie [34]. C_{maxs} of 0.8 μg/ml plasma and 0.1 μg/g brain were measured in the 10-day-old rats, although they received only 20% of the dose given the adults. This disproportionately high internal exposure to the neurotoxic parent compound is consistent with reports of the immature rats' limited detoxification capacity and increased susceptibility [20–22]. DLM concentrations decreased slowly in plasma and tissues of immature and adult rats during the 24-h monitoring period (Fig. 4). Previously, this insecticide could be detected for just 8.3 h in the plasma of an adult SD rat dosed orally with 20 mg DLM/kg [17]. A preliminary elimination half-life of 5.17 h was calculated from this abbreviated dataset, though kineticists prefer that chemical concentrations be monitored for 4–5 half-lives to yield accurate parameters. The currently-described analytical method is sensitive enough to allow this

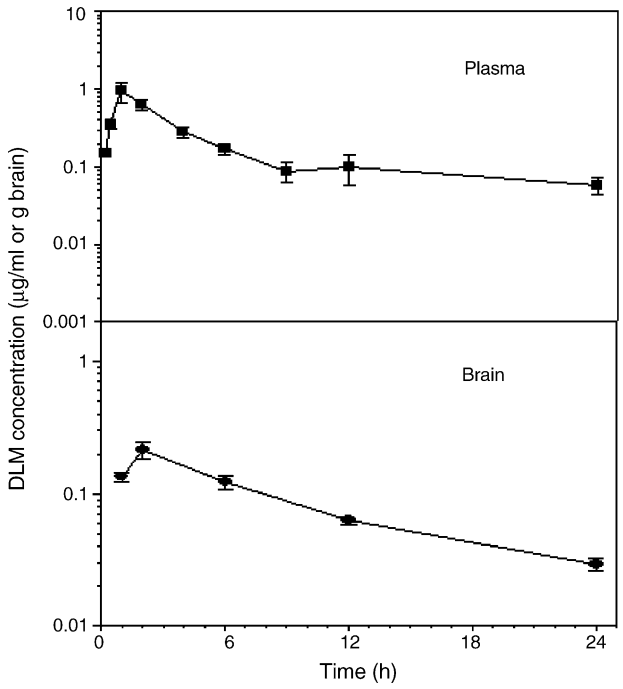

Fig. 3. Plasma and brain concentration vs. time profiles of deltamethrin (DLM) after administration of an oral dose of 10 mg DLM/kg to adult rats ($n = 4$ for plasma and $n = 5$ for brain DLM analyses at each time-point). Values represent mean ± S.D.

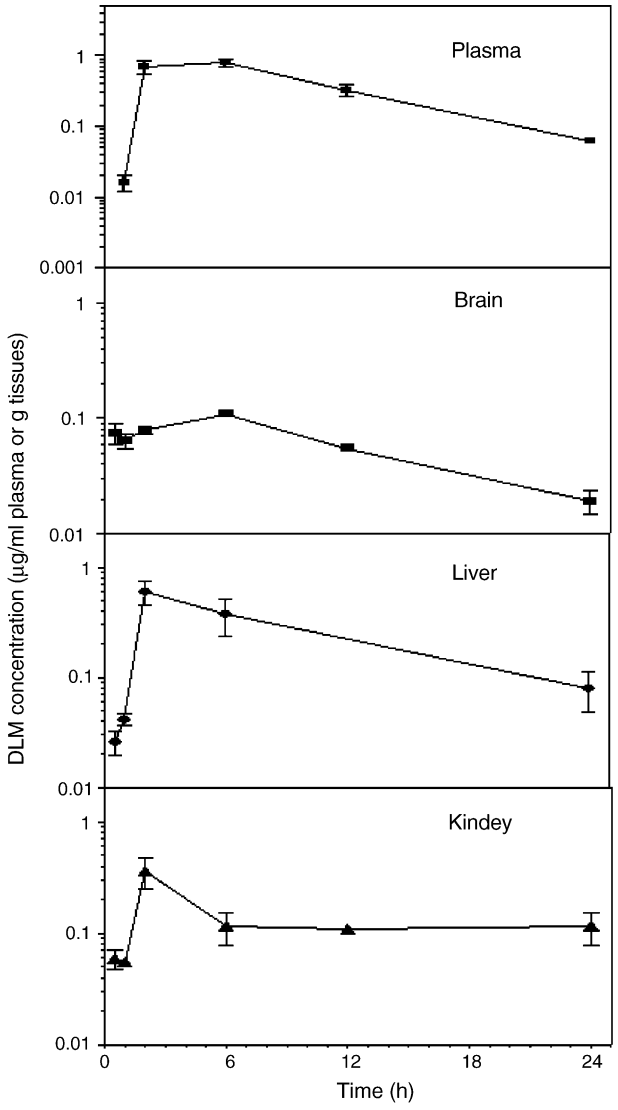

Fig. 4. Plasma and tissues concentration vs. time profiles of deltamethrin (DLM) after oral administration of 2 mg DLM/kg to 10-day-old rats ($n = 3$ at each time-point). Values represent mean ± S.D.

with the small sample volumes that are available from immature rodents.

4. Conclusion

Although the acute toxicity of DLM is well characterized, there has been a lack of published simple and reliable analytical methods to support pharmacokinetic studies of this compound. Therefore, such a method for quantitation of DLM in plasma and tissues was developed and validated following U.S. FDA and ICH guidelines. The present procedure does not include the time-consuming solvent evaporation step used previously. Our procedure yielded high recoveries, showed good linearity, precision and accuracy within the range of 0.05–4.0 μg/ml. It should be quite useful for pharmacokinetic studies of low doses of toxic pyrethroids, where complete blood and tissue concentration time-profiles are required for accurate calculation of key pharmacokinetic parameters. This method is currently being used in our laboratory to investigate the plasma and tissue disposition of DLM in developing and mature rats.

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