Determination of deltamethrin and its metabolite 3-phenoxybenzoic acid in male rat plasma by high-performance liquid chromatography

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Abstract

The pyrethroid insecticide—deltamethrin ([S]-α-cyano-3-phenoxybenzyl-(1R,cis)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate) is widely used throughout the world in agricultural applications. It is also used to treat humans, thereby creating a possible public health concern. The toxic effects of deltamethrin in mammals include choreoathetosis, hyperexcitability and salivation. The principal mechanisms of metabolism for deltamethrin are ester hydrolysis and oxidation at the 4′ position of the terminal aromatic ring. Few studies have been conducted on the toxicokinetics of deltamethrin or its metabolites, mainly due to the lack of quick, sensitive, and validated analytical methods. In this study, we describe the first method to simultaneously determine deltamethrin and its major metabolite 3-phenoxybenzoic acid (3-PBAcid). This method utilizes protein precipitation and high-performance liquid chromatography to support a toxicokinetic study in the rat. The limit of quantitation for both deltamethrin and 3-PBAcid was 0.1 μg/ml. The recoveries for deltamethrin and 3-PBAcid were approximately 91 and 94%, respectively. Intra-day (n = 5) precision (relative standard deviation, %R.S.D.) and accuracy (%Error) for deltamethrin ranged from 1.5 to 12.3% and from 4.0 to 10.6%, respectively. The inter-day (n = 15) precision (%R.S.D.) and accuracy (%Error) for deltamethrin ranged from 7.1 to 11.6% and from 6.3 to 8.2%, respectively. Intra-day (n = 5) precision (%R.S.D.) and accuracy (%Error) for 3-PBAcid ranged from 1.7 to 13.1% and from 1.3 to 14.5%, respectively. The inter-day (n = 15) precision (%R.S.D.) and accuracy (%Error) for 3-PBAcid ranged from 5.4 to 14.5% and from 5.1 to 12.8%, respectively. Finally, this method was applied to a toxicokinetic study of deltamethrin in the rat.

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1. Introduction

Deltamethrin (DLM) ([S]-α-cyano-3-phenoxybenzyl-(1R,cis)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate) is a synthetic type II pyrethroid insecticide [1]. It is one of the most potent insecticides known and is widely used in veterinary products to control lice, flies, and ticks on cattle, sheep, and pigs, as well as in agricultural formulations to control numerous insect pests on fruits, vegetables, and field crops. In humans, deltamethrin is effective as a pediculocide [2]. The major toxic effects of deltamethrin include choreoathetosis, hyperexcitability and salivation [3]. These effects are generally rapid in onset and brief in duration [4]. However, there is one reported human fatality involving a 30-year-old male who died 2 days after consuming about 30 ml of deltamethrin [5]. Another possible adverse effect of deltamethrin is teratogenesis, making studies involving pregnant animals another important issue [6].

Previous studies of deltamethrin metabolism in rats revealed that the principal mechanisms of metabolism are ester hydrolysis and oxidation at the 4′ position of the terminal aromatic ring [7]. Though it is generally accepted that metabolism of deltamethrin forms metabolites that have little or no demonstrable toxicity [8,9], obtaining information on the stoichiometry of deltamethrin and its metabolites will provide a basis for understanding the susceptibilities of...
different ages and species of mammals to this chemical. In this research, 3-phenoxypybenzoic acid (3-PBAcid) was selected as the metabolite to be studied, because it is the major metabolite of the aromatic portion of the alcohol moiety of deltamethrin [7]. The other major metabolite, resulting from the ester hydrolysis of deltamethrin, is \( \text{cis}-3-(2,2\text{-dibromovinyl})-2,2\text{-dimethylcyclopropane-1-carboxylic acid (Br}_2\text{CA)} \). Unfortunately, this molecule only possesses one double bond and it is not a viable candidate for determination using ultraviolet detection.

Over the past 30 years, a number of methods have been developed to measure deltamethrin. These methods can be divided into three different categories, high-performance liquid chromatography methods, gas chromatography methods, and radiometric methods. One of the earliest methods for measuring deltamethrin involved HPLC [10]. This method used both normal and reverse phase separations for deltamethrin from agricultural formulations. Other HPLC methods involve the determination of deltamethrin from cattle dipping baths, blood and milk from cows, and recently honey [11–13]. There is no sample preparation, other than diluting the sample into the linear range of the method, for testing cattle dipping baths and formulations. The methods using blood, milk and honey use a combination of liquid–liquid extraction (LLE) and solid-phase extraction (SPE) for the isolation and concentration of deltamethrin.

The first gas chromatography methods for the determination of deltamethrin from biological matrices, including urine, feces, breast milk and several tissues, occurred in 1982 [14]. This method used liquid–liquid extraction to isolate the deltamethrin followed by determination using packed column GC and an electron capture detector. More recently there have been several papers describing the use of GC–MS and GC–MS–MS for the determination of deltamethrin from human urine and blood [15–17]. The GC methods are quite sensitive, however, they involve large sample sizes (3–15 ml), extensive sample preparation (LLE followed by SPE, and derivatization for metabolites), and have long run times (60–70 min). In addition, the GC methods will detect either the parent compound or the 3-phenoxypybenzoic acid and Br\(_2\)CA metabolites, but not both. A recent paper by Valverde et al. has pointed out an additional concern for the use of gas chromatography for the determination of deltamethrin [18]. This paper shows that the pyrethroid pesticide tralomethrin is thermally degraded to deltamethrin in the injection port of a gas chromatograph leading to concerns about the ability of GC methods to accurately quantitate deltamethrin in the presence of tralomethrin.

To date, the only study to simultaneously measure deltamethrin and any of its metabolites was performed by Anadon et al., who studied the parent compound and the minor metabolite 4′-HO-deltamethrin in rat plasma and tissues [2]. The Anadon method used liquid–liquid extraction with pentane followed by HPLC–UV determination. There is some validation data shown but it is unclear how the precision, accuracy, and recovery data were obtained. It is also unclear what sample size is required for this method, although the fact that animals are sacrificed for each data point and the extraction requires 24 ml of pentane, lead to the conclusion that the sample size is quite large.

Hence, the objective of this research was to develop a quick, sensitive, and fully validated HPLC method to determine deltamethrin and its major metabolite 3-PBAcid in rat plasma. The ability to use protein precipitation instead of liquid–liquid or solid-phase extraction simplifies this method with respect to other literature methods. The current method facilitates serial sampling from a single animal assisting with toxicokinetic studies of deltamethrin. The ability to perform serial sampling from the same animal decreases errors in the toxicokinetic profiles caused by differences between animals with respect to absorption, distribution, metabolism, and excretion (ADME).

2. Experimental

2.1. Reagents and chemicals

Analytical standards of deltamethrin and its metabolite 3-phenoxypybenzoic acid (3-PBAcid) were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). The chemical structures of deltamethrin and 3-PBAcid are shown in Fig. 1. HPLC-grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Reagent-grade phosphoric acid was purchased from J.T. Baker (Phillipsburg, NJ, USA). Deionized water used was generated from a Continental Deionized Water System (Natick, MA, USA). Blank male rat plasma was purchased from Harlan Bioproducts for Science Inc. (Indianapolis, IN, USA).

2.2. Preparation of stock and standard solutions

Individual deltamethrin and 3-PBAcid stock solutions were prepared in acetonitrile to give a final concentration of 1.0 mg/ml. Individual standard solutions with concentrations of 0.5, 1.25, 1.5, 2.5, 3.75, 5.0, 10.0, 15.0, and 25.0 μg/ml were prepared by serial dilution of deltamethrin and 3-PBAcid stock solutions with acetonitrile. Precision and accuracy standards (0.5, 1.0, 7.5, and 20.0 μg/ml) were also prepared in the same manner. All stock and standard solutions were made fresh for each day of analysis or validation.

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![Chemical structures of deltamethrin (A) and 3-PBAcid (B).](image-url)
2.3. Chromatographic conditions

The mobile phase used for the male rat plasma was acetonitrile and deionized water (adjusted to pH 2.4 with phosphoric acid). The percentage of acetonitrile was linearly increased from 20% at time 0 to 60% at 18 min and then to 80% at 21 min, remained at 80% for 2 min, and linearly decreased to 20% acetonitrile at 25 min. The column was then equilibrated at 20% acetonitrile for 2 min prior to the next injection. The flow rate was 0.8 ml/min, and the detection wavelength was 210 nm. Under the chromatographic conditions described, deltamethrin and 3-PBAcid eluted at approximately 25 and 18 min, respectively.

2.5. Calibration curve

Calibration standards for male rat plasma were prepared by spiking 120 μl of blank plasma with 30 μl of a deltamethrin and 3-PBAcid standard solution to obtain deltamethrin and 3-PBAcid concentrations of 0.1, 0.25, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, and 5.0 μg/ml. Five replicates of blanks spiked with deltamethrin and 3-PBAcid concentrations of 0.1 μg/ml (limit of quantitation, LOQ), 0.2, 1.5, and 4.0 μg/ml were prepared for each validation day to test the precision (relative standard deviation, %R.S.D.) and accuracy (%Error). The limit of quantitation was defined as the lowest concentration where data could be obtained with precision and accuracy of 15% or better.

2.7. Validation procedure

Assay precision and accuracy for deltamethrin and 3-PBAcid were calculated for the male rat plasma over a range of 3 days. Blank plasma was spiked with deltamethrin and 3-PBAcid to yield final concentrations corresponding with those used in the calibration curve (0.1, 0.25, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0, and 5.0 μg/ml). Five replicates of blanks spiked with deltamethrin and 3-PBAcid concentrations of 0.1 μg/ml (limit of quantitation, LOQ), 0.2, 1.5, and 4.0 μg/ml were prepared for each validation day to test the precision (relative standard deviation, %R.S.D.) and accuracy (%Error). The limit of quantitation was defined as the lowest concentration where data could be obtained with precision and accuracy of 15% or better.

2.8. Sample collection

The protocol for this study was approved by the University of Georgia Animal Use and Care Committee. The rat was housed one animal per cage in the College of Pharmacy animal facility (AALAC accredited). The environment was controlled (20–22°C, 14 h of light per day) with daily feedings of standard chow pellets and water ad libitum.

2.3. Chromatographic system

The HPLC work was performed on a Hewlett-Packard (Agilent) Series II 1090 Liquid Chromatography (Palo Alto, CA, USA), with a Waters Lambda-Max Model 481 LC spectrophotometric detector (Milford, MA, USA). A Hewlett-Packard (Agilent) 3395 Integrator and a Hewlett-Packard (Agilent) ChemStation for LC Rev. A 04.01 software were utilized. Chromatographic separation was achieved on a Zorbax 80 Å SB-CN column (4.6 mm × 250 mm, 5 μm) from MAC-MOD Analytical Inc. (Chadds Ford, PA, USA) equipped with a Phenomenex C-18 4 mm length × 2 mm i.d. guard column (Torrance, CA, USA).

2.6. Extraction procedure

In a 1.5 ml centrifuge tube, 120 μl of the male rat plasma, 30 μl of deltamethrin and 3-PBAcid standard solution were combined and vortexed for 10 s. To precipitate proteins, 540 μl of ice-cold acetonitrile was added to the tube. The tube was then vortexed for 60 s and centrifuged at 13,000 rpm for 10 min in a microcentrifuge (Biofuge pico, Heraeus Instruments, Hanau, Germany). The supernatant was evaporated under a gentle stream of nitrogen until reaching dryness. The pellet was reconstituted in 150 μl of 50% acetonitrile in deionized water. The reconstituted solution was vortexed for 60 s and sonicated for 5 min, vortexed again for 30 s and centrifuged for 5 min at 13,000 rpm using the same microcentrifuge. The resulting solution was then transferred to an injection vial from which 25 μl of sample was injected onto the HPLC column.
the formation of 3-PBAcid. It was difficult to even determine the identity of the analytes, especially 3-PBAcid. Perchloric acid also precipitates the analytes, but caused significant distortion of the peak shapes. However, for the types of toxicokinetic studies needed for deltamethrin, the increase in sensitivity was believed to be worth the difficulty associated with working with a greater intensity of background peaks. 210 nm is very close to the UV cut-off wavelength for most organic solvents and pH-adjusting reagents. Therefore, phosphoric acid and acetonitrile were selected for the mobile phase because they have the lowest contribution to the background when compared to other chemicals.

Several columns were explored to separate deltamethrin and 3-PBAcid from matrix peaks. The columns can be considered in two categories, pure hydrocarbon stationary phases (C8 and C3) and cyano columns. The hydrocarbon columns were unable to resolve endogenous peaks from the analytes (especially 3-PBAcid) under the restrictive mobile phase requirements that were needed to operate at 210 nm. We attempted to use ion-pairing agents to change the retention behavior of 3-PBAcid, but were unable to find one that could be used at 210 nm. The retention behavior of deltamethrin and 3-PBAcid on the cyano columns was such that we could obtain resolution of the analytes from the endogenous peaks. There were two different columns with identical cyano stationary phases that were tested. The columns differed in column diameter and pore size. Both columns were 150 mm in length. The column with a smaller internal diameter (2.1 mm versus 4.6 mm) also had a larger pore size (300 Å versus 80 Å). Normally, columns with a smaller i.d. provide improved separations due to greater efficiency. We also felt that the column pore size might be important because of the high molecular weight of deltamethrin (505 Da). Both columns were able to provide separation of endogenous components from the two analytes. However, the run time required for the larger pore size column was more than 20 min longer and it was difficult to separate 3-PBAcid from the endogenous peaks. The longer retention time decreased sample throughput and caused excessive band broadening, which decreased the ultimate utility of the assay. The use of an 80 Å pore size column appears to be quite important for this application and was therefore used for all further experiments.

For the extraction of deltamethrin and 3-PBAcid, different extraction schemes were used including, protein precipitation, solid-phase extraction, and liquid–liquid extraction. Protein precipitation was attempted using 2 M perchloric acid, acetonitrile, and ethanol. The perchloric acid was effective in removing most of the endogenous compounds but caused significant distortion of the peak shapes of the analytes, especially 3-PBAcid. Perchloric acid also caused hydrolysis of the deltamethrin ester bond resulting in the formation of 3-PBAcid. It was difficult to even determine the actual recovery because of the poor chromatography and hydrolysis of one of the analytes. Ethanol had a low recovery (approximately 50%) and higher background levels due to co-extracted endogenous compounds when compared to the other types of precipitation. Acetonitrile had the highest levels of recovery of the precipitation methods and did not cause chromatographic and recovery issues like perchloric acid.

Liquid–liquid extraction was also difficult because 3-PBAcid required neutralization prior to extraction using either pentane or ethyl acetate. We noticed the loss of deltamethrin to ester hydrolysis from either formic or trifluoroacetic acid addition to assist with the extraction of 3-PBAcid. Solid-phase extraction using either C-18 or polymeric mixed phase cartridges also provided unsatisfactory results for the recovery of the analytes. The recovery of 3-PBAcid required pH modification or the use of an ion-pairing agent to prevent analyte break through when loading the sample on the SPE cartridge. Even when using these additional reagents, the recovery of 3-PBAcid was never better than 70%. The recovery of deltamethrin was greater than 85% when using acetonitrile protein precipitation, liquid–liquid extraction, or solid-phase extraction. However, the metabolite 3-PBAcid was more difficult to isolate without using acidic conditions that would result in degradation of deltamethrin. Therefore, due to stability conditions we pursued protein precipitation using acetonitrile as the method for preparing samples for analysis. The combination of using protein precipitation and a low wavelength for detection required a gradient method for separation from endogenous components in the sample. However, the resulting method provided good recovery of both analytes, no alterations of chromatographic peak shape, and baseline separation from endogenous components.

3.2. Validation results

Separation of deltamethrin and 3-PBAcid from interfering matrix peaks was explored using different columns, mobile phases and gradients. Baseline resolution of deltamethrin and 3-PBAcid was achieved using the chromatographic conditions described in the experimental section. Fig. 2 shows chromatograms of blank plasma and plasma spiked with deltamethrin and 3-PBAcid (3 µg/ml).

The calibration curves for each day of validation and analysis showed good linearity over the range from 0.1 to 5.0 µg/ml for both deltamethrin (r² = 0.9979–0.9998) and 3-PBAcid (r² = 0.9980–0.9999). The range of concentrations encompassed the estimated range of post oral dose (20 mg/kg body weight) concentrations in the plasma.

The limits of detection (LOD) for deltamethrin and 3-PBAcid were 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 LODs were found to be approximately 0.03 µg/ml for both deltamethrin and 3-PBAcid.
concentrations were extracted and analyzed \((n = 5)\). An equal Standard-spiked plasma samples at the 0.8 and 4.0 \(g/ml\) from plasma were expressed in terms of relative recovery.

Fig. 2. Chromatograms of blank, 3-PBAcid (3 \(g/ml\), 17.8 min) (A) and deltamethrin (3 \(g/ml\), 25.4 min) (B) spiked male rat plasma on a Zorbax 80 \(\AA\) SB-CN (4.6 mm × 250 mm, 5 \(\mu m\)) analytical column.

The extraction efficiencies for deltamethrin and 3-PBAcid from plasma were expressed in terms of relative recovery. Standard-spiked plasma samples at the 0.8 and 4.0 \(g/ml\) concentrations were extracted and analyzed \((n = 5)\). An equal number of plasma blanks were extracted and spiked post-extraction. The peak areas of these two sample sets were compared. The recovery of deltamethrin was 90.8 ± 3.0 at the 0.8 \(g/ml\) level and 91.0 ± 4.8 at the 4.0 \(g/ml\) level. The recovery for 3-PBAcid was 94.8 ± 3.0 at the 0.8 \(g/ml\) level and 94.4 ± 2.4 at the 4.0 \(g/ml\) level.

The intra-day \((n = 5)\) precision and accuracy for deltamethrin (spiked concentrations 0.1, 0.2, 1.5, and 4.0 \(g/ml\)) were in the range of 1.5–12.3\% (±R.S.D.) and 4.0–10.6\% (±Error), respectively. The inter-day \((n = 15)\) precision and accuracy for 3-PBAcid (spiked concentrations 0.1, 0.2, 1.5, and 4.0 \(g/ml\)) ranged from 7.1 to 11.6\% (±R.S.D.) and 5.1 to 12.8\% (±Error), respectively. The intra- and inter-day precision (%R.S.D.) and accuracy (%Error) of deltamethrin in male rat plasma are listed in Table 3. The %R.S.D. between the average peak area of deltamethrin and 3-PBAcid each day was less than 4.0%. There was no distinctive decline in peak areas for either deltamethrin or 3-PBAcid over three consecutive freeze/thaw cycles at 3.0 \(g/ml\) level. The stability of extracted male rat plasma samples in the autosampler was also evaluated. Six extracted male rat plasma samples containing 3.0 \(g/ml\) of deltamethrin and 3-PBAcid were put in the autosampler for a 25 h period. At time 0, one plasma sample was injected onto the HPLC column and analyzed. In the following 25 h, one plasma sample was injected and analyzed approximately every 5 h. The peak areas for deltamethrin and 3-PBAcid in each injection were compared. The %R.S.D. between each sample was <15.0\% for both compounds and there was no obvious decline in peak areas between each injection.

### 3.3. Stability testing results

Stability testing was performed for deltamethrin and 3-PBAcid. Spiked male rat plasma (3.0 \(g/ml\)) samples were subjected to three consecutive freeze/thaw cycles over the period of 4 days. On day 1, 20 blank plasma samples were spiked with deltamethrin and 3-PBAcid to give a final concentration of 3.0 \(g/ml\). Five of these were extracted and analyzed as described earlier. The remaining 15 spiked plasma samples were stored at −20°C. Each of 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 and 3-PBAcid were compared and the results listed in Table 3. The %R.S.D. between the average peak area of deltamethrin and 3-PBAcid each day was less than 4.0%. To demonstrate the utility of this assay, one male rat was dosed with 20 mg deltamethrin per kg body weight. Plasma samples were collected, extracted, and analyzed as described earlier. Sample peak areas were used to determine deltamethrin and 3-PBAcid concentrations from the regression equation obtained from standard-spiked samples prepared in blank male rat plasma. Fig. 3 shows the plasma concentration versus time profiles for deltamethrin and 3-PBAcid. By use of WinNonlin (Pharsight, Mountain View, CA, USA), it was possible to calculate a time to peak \(T_{\text{max}}\) of 72 min and a peak concentration \(C_{\text{max}}\) of 0.19 \(g/ml\) for

### Table 1

The intra- and inter-day precision (%R.S.D.) and accuracy (%Error) of deltamethrin in male rat plasma

<table>
<thead>
<tr>
<th>Concentration added ((\mu g/ml))</th>
<th>Intra-day ((n = 5))</th>
<th>Inter-day ((n = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration found ((\mu g/ml))</td>
<td>±R.S.D.</td>
</tr>
<tr>
<td>0.1</td>
<td>0.056 ± 0.001</td>
<td>11.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.107 ± 0.023</td>
<td>12.3</td>
</tr>
<tr>
<td>1.5</td>
<td>1.407 ± 0.120</td>
<td>7.5</td>
</tr>
<tr>
<td>4.0</td>
<td>4.162 ± 0.061</td>
<td>1.5</td>
</tr>
</tbody>
</table>
The intra- and inter-day precision (%R.S.D.) and accuracy (%Error) of 3-PBAcid in male rat plasma

<table>
<thead>
<tr>
<th>Concentration added (µg/ml)</th>
<th>Intra-day (n = 5)</th>
<th>Inter-day (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration found (µg/ml)</td>
<td>%R.S.D.</td>
</tr>
<tr>
<td>0.1</td>
<td>0.088 ± 0.006</td>
<td>6.8</td>
</tr>
<tr>
<td>0.2</td>
<td>0.173 ± 0.017</td>
<td>9.8</td>
</tr>
<tr>
<td>4.0</td>
<td>4.041 ± 0.068</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 3 Results of freeze/thaw stability testing of deltamethrin and 3-PBAcid in male rat plasma

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Deltamethrin</th>
<th>3-PBAcid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>66583 ± 1802</td>
<td>126568 ± 2340</td>
</tr>
<tr>
<td>Day 2</td>
<td>69091 ± 934</td>
<td>116530 ± 4117</td>
</tr>
<tr>
<td>Day 3</td>
<td>69669 ± 973</td>
<td>118924 ± 664</td>
</tr>
<tr>
<td>Day 4</td>
<td>65610 ± 1402</td>
<td>120025 ± 3596</td>
</tr>
<tr>
<td>%R.S.D.</td>
<td>2.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Fig. 3. Representative plasma concentration–time profile of deltamethrin and 3-PBAcid in a male rat after an oral dose of 20 mg/kg body weight.

deltamethrin. A half-life of 310 min (5.17 h), volume of distribution of 40.3 l/kg, and total clearance of 90.0 ml/min/kg were also calculated from these data. These values were in close agreement with previously reported values for deltamethrin toxicokinetics in rats [2,20]. For 3-PBAcid, the T\text{max} was 180 min, and the C\text{max} was 0.318 µg/ml. A 3-PBAcid half-life of 236 min (3.93 h) was also calculated from the data. Some of the data points for deltamethrin were below the LOQ (100 ng/ml) but were above the LOD (30 ng/ml). This may lead to more errors in the calculation of the toxicokinetic parameters for deltamethrin. For the metabolite 3-PBAcid, all the data points reported were above the LOQ (100 ng/ml).

4. Conclusions

A sensitive and efficient method for the extraction and analysis of deltamethrin and its metabolite, 3-PBAcid, in male rat plasma was developed and validated. This method yielded high recoveries, showed good linearity, precision and accuracy within the range of 0.1–5.0 µg/ml. The estimated toxicokinetic parameters from the analysis of collected male rat plasma samples were comparable to literature data, further validating the reliability of this method for the determination of deltamethrin and 3-PBAcid in male rat plasma. This analytical method can be utilized in toxicokinetic investigations of deltamethrin and could be useful for environmental monitoring of this important pesticide.

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