Cypermethrin Residues Determination in the Milk of a Lactating Dairy Cow by Gas Chromatography–Ion Trap Mass Spectrometry

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Abstract
The aim of this work was to detect and quantitate residues of the pyrethroid insecticide cypermethrin in the milk of a lactating dairy cow. The efficiency of the method was settled through recovery experiments in which certain amounts of the pyrethroid cypermethrin were spiked up to milk. For this purpose, the matrix solid-phase dispersion method was applied followed by clean-up with silica-gel column. The extracts obtained were analyzed by gas chromatography–ion trap mass spectrometry. With the aid of the selected ion storage technique, it was feasible to select specific ions from the analyte of interest, which could be separated from the organic matrix. After the establishment of optimum conditions for the detection of pyrethroid residues of cypermethrin, samples of milk were collected from a cow submitted to a veterinary treatment for elimination of parasites using an insecticide with 50 g/L of active ingredient in its formula. The data obtained showed milk contamination with a maximum concentration of 0.168 mg/kg 24 h after insecticide treatment. Considering the maximum residual limit for cypermethrin in milk of 0.010 mg/kg, samples collected 15 and 24 h after treatment showed concentrations about 16 times above this value, and samples collected 11 days after treatment showed concentrations about 7 times above this value. Seventeen days after treatment, cypermethrin was not detected in the milk.

Introduction
During the investigations about changes of chemical structures of natural pyrethrins, synthetic pyrethroids were produced with increased stability and insecticidal activity. Among them, cypermethrin, which was first described in 1974, is a highly active pyrethroid used against a variety of agriculture pests, in public health and animal breeding (1).

In Brazil, pyrethroids are widely used to treat lactating dairy cows, mainly in the battle against ticks, flies, lice, and dermatobia. An investigation accomplished after dermal application of pyrethroids showed insecticidal residues in the milk of the treated animals. The analysis performed by high-performance liquid chromatography (HPLC) with ultraviolet detection, showed a cypermethrin concentration of 0.36 mg/kg 24 h after application, and 0.50 mg/kg 24 h after application (2,3). Other analyses performed with gas chromatography with an electron capture detector (GC–ECD) were carried out through recovery experiments in which pyrethroid quantities, ranging from 0.04 to 0.41 mg/kg, were spiked in milk (4).

GC–mass spectrometry (GC–MS) has been largely used to determine pyrethroids, not only to confirm results of other techniques, but also as an independent method, mainly because of its selectivity and unambiguous and simultaneous characterization of the insecticides to be analyzed. This is clear when compared with purely chromatographic methods that depend only on retention time comparison. In contrast, the GC–ion trap (IT) MS technique determines the ionic fragments originated by the target molecule and compares it with data of masses stored on a computer and in some cases determines the molecular ion.

Methods using bidimensional (mass filters) and three-dimensional (ion-trap) GC–MS devices have been employed in a variety of environmental matrix and food to evaluate pyrethroid residues. Among these methods, an interlaboratory study identified and quantified multiresidue pyrethroid in agricultural products (5). Cypermethrin and its degradation products were analyzed by GC–MS with electron impact ionization after forensic applications (6). Also the monitoring of pyrethroid metabolites in human urine was performed by GC–tandem ITMS (7).

The purpose of this work is to evaluate the efficiency of a pyrethroid extraction methodology in milk through recovery experiments and to apply it to the detection of cypermethrin residues originated by topical application on a lactating cow submitted to a veterinary treatment with a commercial insecticide that contains an active ingredient in its formulation. This work also aims to establish optimum conditions to detect and quantitate cypermethrin by GC–ITMS.
Experimental

Extraction methodology
The extraction method used on this work is based on the adsorption of solutes over the column material (adsorption chromatography). The solutes are eluted by a mobile phase where the less adsorbed components are eluted first and the more adsorbed components are eluted more slowly, so that the desired separation occurs (8,9).

The extraction of cypermethrin was based on the Steinwander (10) method in which extraction and purification are performed simultaneously. Milk or aqueous milky products, 10 g, are mixed with silica-gel (70 to 230 mesh) and homogenized with mortar and pestle. After that, the milk dispersed on the solid matrix is placed in the column previously filled with silica-gel (10% distilled water), necessary to clean-up. A mixture of the solvents dichloromethane and n-hexane (50:50, v/v) is poured in the column. Cypermethrin moves with the solvent from the upper silica-gel to the lower 10% water column where it is purified. Subsequently, the eluted is evaporated with a roto-vapor until it is reduced in quantity from 5 to 8 mL.

Recovery experiments
In order to evaluate the feasibility of the extraction procedure, certain amounts of cypermethrin were added to 2 g of milk powder with different quantities of water. It was verified that the best condition for the extraction occurred with 7 g water content. This value presented 31% of the sum of silica-gel (15 g) plus water. The efficiency of the extraction dropped to 4% when water content was up to 40% (10 g). In this value the column reached saturation in terms of adsorption capacity. Recoveries up to 70% occurred only with concentrations lower than 44 mg/L. The limit of detection (LOD) of pyrethroid cypermethrin, considering chromatographic and spectrometric conditions, is 33 mg/L or, considering 1-mL sample injection, 33 ng.

Instrumental analysis
A Saturn-3/Varian (Varian Instruments) ITMS associated with a Varian model 3400 CX GC equipped with a 30-m DB-5 capillary column (J&W Scientific, Folsom, CA), with an internal diameter of 0.25-mm and 0.25-µm film thickness was used. Helium was used as carrier gas and gave a column head pressure of 12 p.s.i. (1 p.s.i. = 6894.76 Pa) and an average flux of 1 mL/min.

The temperature program for the GC column consisted of a 2-min hold at 50°C, a 23.70°C/min ramp to 240°C, a 6.20°C/min ramp to 260°C and an 8-min hold at 260°C. The total run time was 30.23 min. The injector, transfer line, and ion trap temperature were set at 260°C, 270°C, and 170°C, respectively.

The mass range used was m/e 65 to 400 with a scan time of 500 ms in the electron impact mode with electrons of 70 eV. The electron multiplier voltage was set as 1500 V, and the storage radio frequency was 1.1 MHz. The selected ion storage (SIS), which is a resonant ejection mode, was used. This technique consists of an application of multi-frequency waveforms imposed to the end-cap electrodes so that undesired ions are ejected and characteristic ions are stored. For this purpose, a waveform was built in order to store cypermethrin ions at m/e 127, 152, and 181 and eject unwanted ones. The 93% cypermethrin standard was provided by Bayer S.A. (Sao Paulo, Brazil).

Results and Discussion
A lactating diary cow was treated against parasites with a commercial insecticide applied topically. The insecticide is a concentrate containing 50 g/L of cypermethrin (3-(2,2-dichloroethenyl)-2,2-dimethylcyclopentanecarboxylic acid cyano(3-phenoxophenyl) methyl) ester in its formulation. It was administered according to product-use instructions. 10 mL of the reported commercial insecticide diluted in 4 L of water giving a concentration of 125 mg/L of the active ingredient. The insecticide containing 0.5 g of cypermethrin was sprayed on the animal’s body on the back from shoulder to sacrum.

After the treatment the milk was collected, extracted, and concentrated at 1 mL of n-hexane. In order to acquire each chromatogram, 1 mL of the extract were injected and an-
alyzed by GC–ITMS. Four chromatograms for each extract were obtained, referring to the extraction of milk samples collected 15 h, 24 h, 11 days, and 17 days after the commercial insecticide application. The reason for choosing the days specified in this work was the weather condition. Care should be taken in collecting samples on rainy days, because this would represent loss of the analyte originally received by the animal.

There were four peaks in each chromatogram, corresponding to cis-1, cis-2, trans-1, and trans-2 (A, B, C, and D, respectively) diastereomers (Figure 1). The GC separation technique has the ability of distinguishing just four of the eight cypermethrin optical isomers (diastereomers). However, each diastereomer represents two enantiomers that can be separated by a chiral HPLC method or by using a cellulose-based phase (11). The exactness of the four isomers can be verified by the precise agreement of the retention times, ionic assignments, and resolutions between the standard analyte and the extracted analyte.

Each diastereomer was formed, point-by-point, by a mass spectrum of the cypermethrin characteristic ionic fragments (Figure 2).

The ion at m/z 163 was not used on detection and quantitation because there was an interfering compound eluting with the same retention time and yields the same ion associated with cypermethrin. In this case ions at m/z 127, 152, and 181 were chosen (Figure 3). Consequently, the ionic matrix effect could be minimized in a way that extraction and clean-up procedure could not.

The B isomer observed in Figure 1 was chosen for quantitation because A isomer had the co-elution compound squalene (C30H50) with a retention time difference of 0.02 min. Thus, it was very difficult to separate the two compounds by means of chromatography because the resolution necessary to separate two adjacent peaks is 0.08 min.

Cypermethrin concentrations in milk were achieved with an external calibration curve built with standard solution which concentration varied from 0.04 to 0.44 mg/L. The areas of in-

![Figure 3](image-url) Cypermethrin chromatogram of a 24-h milk sample extraction. In detail is shown a selected ion storage (SIS) mass spectrum of the cis-2 isomer.

![Figure 4](image-url) Calibration curve of cypermethrin using standard solutions ranging from 0.04 to 0.44 mg/L.

<p>| Table 1. Cypermethrin Concentration in Milk for Distinct Times of Collected Samples |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Collection Time</th>
<th>Average Concentration (mg/kg)</th>
<th>Standard Deviation (mg/kg)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 hours</td>
<td>0.165 ± 0.008</td>
<td></td>
<td>4.85</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.168 ± 0.036</td>
<td></td>
<td>21.43</td>
</tr>
<tr>
<td>11 days</td>
<td>0.074 ± 0.009</td>
<td></td>
<td>12.16</td>
</tr>
<tr>
<td>17 days</td>
<td>Not detected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 5](image-url) Electron impact ionization fragments of the cypermethrin mass spectra.
individual isomer-B peaks were obtained on triplicate and the data were adjusted by the minimum square method (Figure 4). The correlation coefficient obtained by the linear regression of the calibration has the value of 0.948 and the regression equation is:

\[ RIC = 622.12 + (7475.34)C \]  \hspace{1cm} \text{Eq. 1} 

Where RIC is the relative ionic current and C is the cypermethrin concentration. Considering Equation 1, it was feasible to calculate the cypermethrin concentration of the samples collected after 15 h, 24 h, 11 days, and 17 days after the treatment (Table I).

Considering the starting concentration of the insecticides applied (125 mg/L) and the data obtained on Table I, the quantities secreted relative to the applied were 0.00132%, 0.00134%, and 0.00045% at 15 h, 24 h, and 11 days, respectively, after treatment. The low concentrations achieved basically occur because of the strong metabolism that takes place in the animal’s organism. This fact can be attributed to the weakness of the ester bond yielding the main metabolites by hydrolytic cleavage followed by oxidation. These reactions yield the compounds 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-oxycarbonyl acid and 3-phenoxycarbonyl acid. Both metabolites are partly conjugated to, for example, glucuronic acid and finally eliminated renally (12).

The absence of the molecular peak (415 m. u.) and the presence of the 181, 207, and 209 ions (considering the isotopic contribution of 37Cl) on mass spectra from Figure 2 are strong evidences of the molecular fragility of cypermethrin and pyrethroids in general, which possess the same ester bond (Figure 5).

Conclusions

This work confirms the evidence of contamination in milk samples by cypermethrin as determined by Bissacco and Vassileff (2,3). Moreover, with a GC–ITMS apparatus, the presence of the characteristic ions of the analyte can be assigned accurately, the four isomers can be satisfactorily separated and they can be distinguished from coelucent interfering peaks.

Data provided by the Codex Alimentarius Commission (USA) set up the maximum residual limit (MRL) for cypermethrin in milk as 0.010 mg/kg (13). The data obtained on this study indicates that the cypermethrin residues in the milk are about 16 times superior of this limit on samples collected 15 h and 24 h after application, and about 7 times higher than this limit for the sample collected 11 days after application. After 17 days of treatment, cypermethrin in the milk was undetected, considering the LOD 33 mg/L for this pyrethroid. Based on the presented data, consumers should avoid ingestion of milk until at least the 11th day after the animal has been treated for parasites with a commercial insecticide.

References


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