Assessment of matrix effect in quantitative milk analysis for pyrethroids based on GC-MS/MS

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ABSTRACT

In chromatographic analysis of complex samples, the chromatographic responses attributed to pesticides may undergo changes caused by matrix components. These variations can be due by the phenomenon named matrix effect. Cyfluthrin (CYF), cypermethrin (CYP), deltamethrin (DEL), and permethrin (PER) were extracted from raw milk and analysis carried out using a Gas Chromatography coupled to mass spectrometry (GC-MS/MS) technique. The aim of the present work was to investigate the variations of chromatographic response when milk samples are diluted with different solvents (acetone, acetonitrile or hexane) in final dilution immediately after the extraction. Results showed differences between solvents and acetonitrile showed more appropriate results for all pyrethroids.

KEYWORDS: pesticides; matrix effect; raw milk.

INTRODUCTION

Currently, pesticides for cattle have been considered very important to provide the extensive livestock, particularly in tropical regions where the environmental conditions, such as high temperatures and humidity have an important role on both cattle production and the spread of parasites in farming animals. Brazil is one of the most important world producers of animal origin foods and because weather conditions the parasitic diseases are by far the most important factor responsible for losses in livestock production (FEIJÓ et al. 2013). Tick has been considered as one of the main responsible for substantial reductions in the production of food such as milk (GRAF et al. 2004). Nowadays, the control of parasitic diseases of veterinary importance still relies on the use of chemicals, being pyrethroid insecticides (PYR; Fig. 1), especially cypermethrin, widely used for this purpose (GRAF et al. 2004; SASSINE et al. 2004; MATISOVÁ & HROUZKOVÁ, 2012). Although most of parasites have a well-defined life cycle, which lead to the application of antiparasitic drugs only in specific periods along the year, ticks infestation may occur at any time and relies on the continuous usage of PYR in dairy cattle, which may lead to the occurrence of its residues in milk or meat (BISSACOT & VASSILIEFF, 1997; FEO et al. 2010).

There are several ways through which these compounds reach the milk including improper use of the drug, contamination of animal feeding stuffs, environmental contamination and animal-to-animal drugs transfer. Moreover, considering the diverse nature of PYR contamination, this group has been studied in both environmental and food contamination cycles (BISSACOT & VASSILIEFF, 1997; FEO et al. 2010; MATISOVÁ & HROUZKOVÁ, 2012).

Considering the analytical scenario, mass spectrometry (MS) associated with separation technique has been considered the technology of choice for the quantitative analysis when high sensibility and specificity are required. This aspect is mainly critical for analyses in low levels and complex matrices (FEO et al. 2010). Although extensive clean-up of extracts are employed to reduce analytical variability in results, sometimes associated with high cost procedures using sorbents, gas chromatography (GC) remains significantly influenced by the injection system parameters (STĚPÁN et al. 2004; KENDE et al. 2006; POOLE, 2007).

According to the classical concept of split and splitless injection, the liquid sample leaving the syringe needle must be evaporated before it reaches the column entrance. Then the vapor is either split or transferred into the column in splitless mode. However, vaporization process involves many variables associated with intrinsic factors of solvent and analytes (vapor pressure, boiling point, heat capacity), injection parameters (mode, temperature, volume), residual matrix co-extractive and interactions with each other (FAJGELJ & AMBRUS, 2000; KENDE et al. 2006; POOLE, 2007). Considering the system high complexity, practical approaches to understand its influence on methodologies in development is a usefully strategy (FAJGELJ & AMBRUS, 2000; KENDE et al. 2006; POOLE, 2007).

The present study was intended to further investigate about the relation between the choice of solvents used in final dilution and chromatographic response on the reliability of quantitative results. Pyrethroids (Fig. 1) cyfluthrin (CYF), cypermethrin (CYP), deltamethrin (DEL), and permethrin (PER) in raw milk matrix using GC-MS/MS were evaluated in splitless mode.
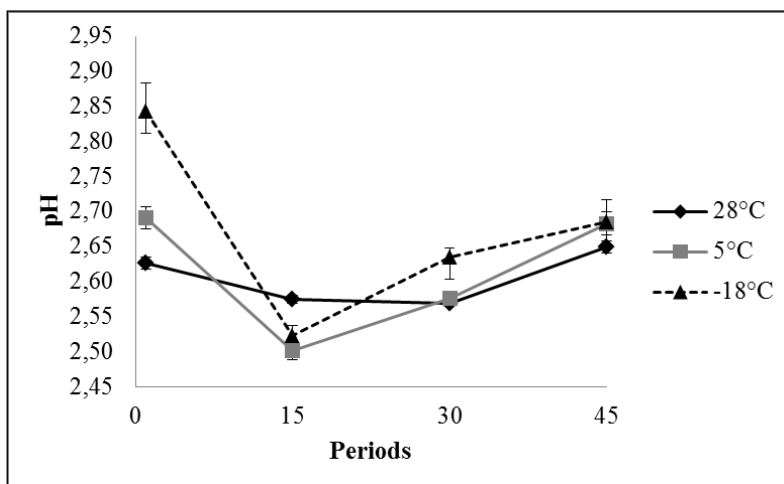
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Figure 1. Pyrethroids used in the present work considering molecular weight (a) and respective substituents (R1, R2, R3 and R4) in the general structure.

MATERIALS AND METHODS

MATERIALS

Individual PYR standards with purity between 96.7 and 99.8% (CYF, CYP, DEL and PER) were obtained from Restek (U.S.A, Bellefonte). CYF, CYP, FEV and PER were a mixture of isomers. A stock solution of each pesticide was prepared individually in acetonitrile (ACN) to obtain the primary calibration solution (1000 mg L-1; stored at -20°C) from which the intermediate standard solutions were prepared by dilution in Hexane (HEX), acetone (ACO), acetonitrile (ACN) at 100 mg L-1 and stored in a refrigerator at 5°C. HEX, ACO and ACN were purchased from Mallinckrodt Baker (Phillipsburg, USA). Sodium chloride was purchased from Merck® (Darmstadt, Germany). Blank milk samples were obtained from certified producer.

CHROMATOGRAPHIC ANALYSIS

GC-MS/MS analysis was achieved using an auto sampler 7890A equipped with MMI inlet at pulsed splitless mode, ultra-inert liner with temperature program 70 °C at 0.1 min, 400 °C min^{-1} until 240 °C and pulse of the 35 psi for 3 min; 3 μL. Mass spectrometry analysis was carried out through multiple reaction mode (MRM) monitoring 2 transitions (qualitative and quantitative). Chromatographic column was a DB-5ms (30 m × 0.25 mm × 0.25 μm film thickness). The temperature program was 100 °C (1 min) to 250 °C at 20 °C min^{-1}, to 260 °C (3 min) at 5 °C min-1 and to 330 °C (5 min) at 20 °C min^{-1}. Helium was used as carrier gas with the flow of 1.2 mL min^{-1} and N2 was used as collision gas (MENEGHINI et al., 2014).

SAMPLE PREPARATION

Sample extraction was carried out as described elsewhere (RÜBENSAM et al., 2013). A 5 mL of sample aliquot of was placed into a 50 mL polypropylene tube. After homogenization four aliquots of ACN (2.5 mL) were added successively to the samples with 10 s of mixing on a vortex between each solvent addition. These

mixtures were shaken on a shaking table, at 240 rotations per minute, for 15 min. Approximately 2 g of sodium chloride were added to each sample, followed by homogenization for 5 min, and subsequent centrifugation for 10 min at 2200 G. The top layer was transferred to a 15 mL polypropylene centrifuge tube and stored in the freezer at -20 °C for 6 h. After this time, the liquid extract was transferred to a 50 mL centrifuge tube, evaporated to dryness at 35 °C under nitrogen flow and fortified in the range from 20 to 200 ng.mL^{-1} for each PYR. Finally, 0.3 mL of ACN, HEX or ACO was added prior GC-MS/MS analysis. Each matrix-matched curve war performed in triplicate and all curves were analyzed at random order.

STATISTICAL PROCEDURES

Response comparison between ACO, ACN and HEX was performed using analysis of variance (ANOVA) using Minitab® version 17.1.0 for the calibration curves. When significant difference was noted, a t-test was carried out. Analysis was assessed considering a $p < 0.05$ as significant.

RESULTS AND DISCUSSION

MASS SPECTROMETRY OPTIMIZATION

Table 1 shows final parameters for MS/MS. In order to maximize the response of the instrument for each compound the choice of precursor ion, product ion and collision energy were optimized. Initially a full scan was carried out for each PYR using only single quadrupole (electronic impact fragmentation).

Table 1. Multiple reaction monitoring (MRM) final conditions.

Analyte	*(Q) Precursor ion/ Product ion (m/z)	** (q) Precursor ion/ Product ion (m/z)	+CE (eV)+
PER	183.3/165.1	183.3/168.1	10/15
CYF	181.1/152.1	181.1/127.1	5/5
CYP	163.0/127.1	163.0/91.1	5/5
DEL	166.9/125.0	124.9/89.0	5/5

NOTE: *Qualitative transition; **Quantitative transition; + Collision energies used to qualitative and quantitative transitions, respectively.

After, fragments with major abundance (pattern- ion) were acquired in product-ion scan mode to generate daughter-ions (originated by gas collision in the second quadrupole). Energies or transitions were optimized using Mass Hunter Optimizer® tool and two of them were used as qualitative and quantitative transitions in final MRM method.

SOLVENT RESPONSE COMPARISON

Besides extraction efficiency of residues from matrix, the performance characteristics of clean-up step are closely related to the quality of generated data. The absence of matrix components in purified sample, and thus elimination of matrix-induced errors is generally a way to obtain appropriate responses (ŠTĚPÁN et al. 2004). However, some compounds have high affinity by surfaces like

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pyrethroids. They have a hydrophobic nature and adsorb onto glass surfaces or solids with which they come in contact (ALBASEER, NAGESWARA & MUKKANI, 2010). This way, rapid extractions procedures are indicated to avoid losses. Besides, this trend for adsorption is amplified in gas state which occurs during injection transference. Figure 2 and 3 show a summary for differences when different solvents are used in final dilution stage.
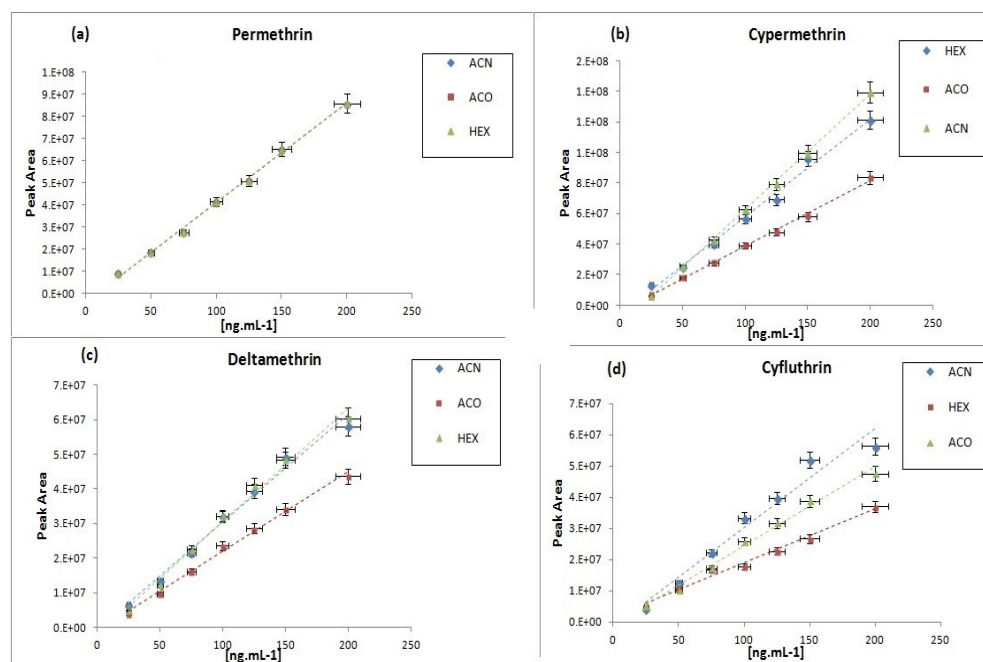

Figure 2. Calibration curves obtained for PYR (a,b,c,d) when different solvents (ACN, HEX, ACO) are used in final dilution.

The energy (heat form) consumed in inlet device is provided by the heating block of the injector. Transference from the heating cartridges to the vaporizing chamber is too slow and only the liner wall is important as a heat reservoir. Because the used solvents have differences about heat capacity, vapour pressure and boiling point, some changes in chromatographic profiles can occur. Considering a qualitative perspective it is possible to note a distortion in peak shape when ACO is used (Figure 3, b) for PER (+) but the signal intensity is maintained. In the other hand, for CYP (++) minor intensity is observed when ACO is used. Because the difference is not constant during all acquisition time, a statistical evaluation is necessary. Table 2 shows the differences for calibration curves. Two features can be observed: response is different for each PYR and for two compounds (CYP and CYF) ACN produced an increment in response.

The limiting factor in rapid evaporation is the transfer of heat to the liquid sample, usually primarily consisting of solvent and co-extractive material when matrix is present. Common perception could lead a statement that faster evaporation of the solvent, improves peak shape.

However, taking in account the temperature program used in the present work, ACN has a heat capacity larger than ACO and HEX. Besides, boiling point of ACN is higher too. Thus, considering these unfavourable characteristics, parameters like peak shape should be worst when ACN was used because heat

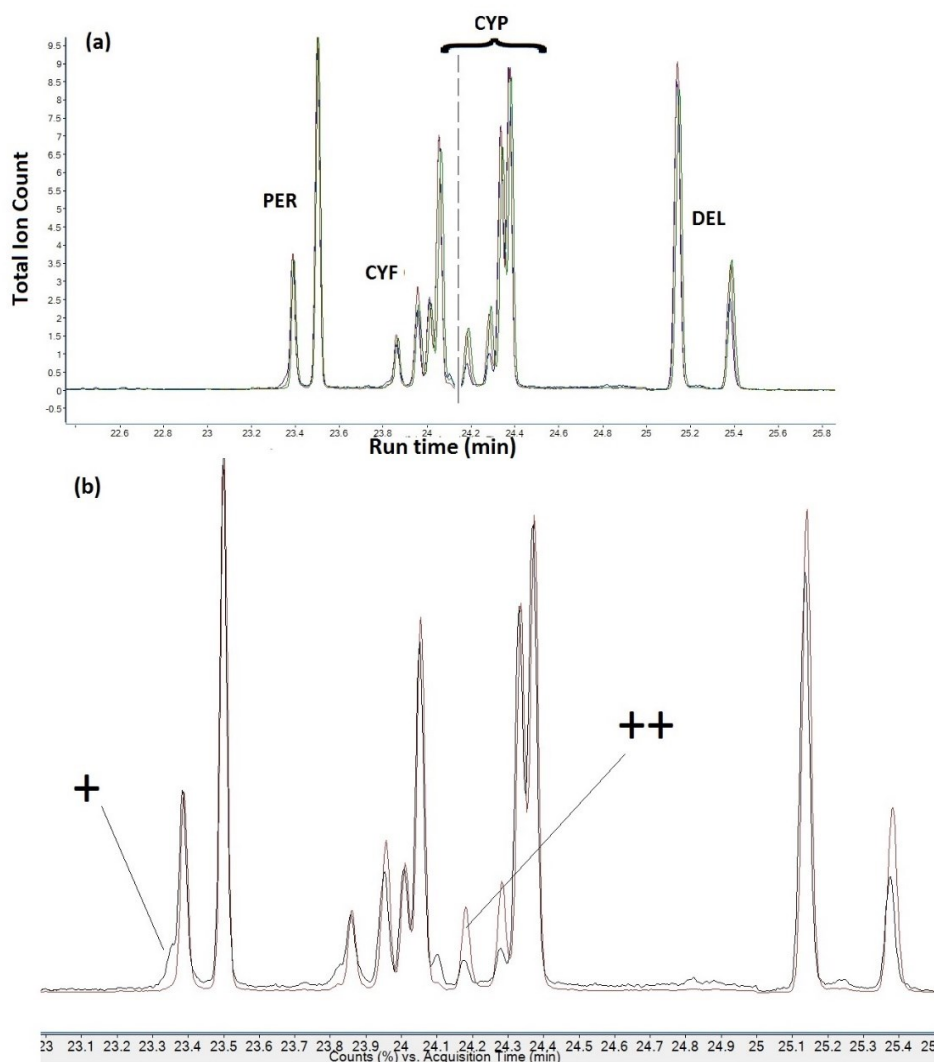transfer became slower and consequently the transference to column could be less efficient.

Figure 3. Chromatographic profile when ACN, HEX or ACO are used in final dilution (a); Difference between ACO and ACN for PER (+) and CYP (++) in the peak shape.

Table 2. Summary of evaluation for chromatographic response obtained with ACN, ACO and HEX curves considering a linear regression model.

Compound	Recovery (%)[+]	CV (%)[++]	R^2 (ACN)*	R^2 (ACO)	R^2 (HEX)	ANOVA (p-value)	t-test**
PER	75	12.0	0.98	0.98	0.97	>0.05	-
CYF	80	6.5	0.97	0.98	0.96	<0.05	ACN>ACO>HEX
CYP	82	7.2	0.97	0.97	0.97	<0.05	ACN>ACO>HEX
DEL	68	11.5	0.98	0.98	0.98	<0.05	ACO

NOTE: + Value calculated using peak area ratio between sample fortified before the extraction and after the extraction; ++ Coefficient of variation calculated for triplicate recovery; *Determination coefficient obtained with calibration curve for each solvent, ** Order of magnitude when difference between responses was significant.

As were afore mentioned, phenomena in injector can be attributed to many factors and in some cases a good prediction without experimental approach is not possible. In the present work ACN showed good response for analyzed compounds and we suggest that this effect could be related with favourable interaction between co-extractive in liner and CAN, which are more pronounced than physical properties of ACN.

CONCLUSIONS

Extraction of PYR from bovine milk showed to be applicable using liquid-liquid extraction with low temperature purification. The proposed extraction presented good perspective for application because it is simple, easy and suitable for processing a large volume of samples with low consumption of organic solvent. Careful assessment to choice the solvent for final dilution deserves attention. The precision and accuracy of the method should be assessed using the final procedure of extraction intended to be validated with the respective solvent. Matrix effect may be eliminated or minimized changing and improving sample extraction procedure, but, in some cases as PYR, co-extractives can work as a protector.

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Avaliação do efeito da matriz na análise quantitativa do leite para piretróides com base em GC-MS/MS

RESUMO

Na análise cromatográfica de amostras complexas, as respostas cromatográficas atribuídas aos pesticidas podem sofrer alterações causadas pelos componentes da matriz. Essas variações podem ser devidas ao fenômeno denominado efeito de matriz. Ciflutrina (CYF), cipermetrina (CYP), deltametrina (DEL) e permetrina (PER) foram extraídas do leite cru e as análises realizadas por cromatografia gasosa acoplada a espectrometria de massa (GC-MS/MS). O objetivo do presente trabalho foi investigar as variações da resposta cromatográfica quando amostras de leite são diluídas com diferentes solventes (acetona, acetonitrila ou hexano) na diluição final imediatamente após a extração. Os resultados mostraram diferenças entre os solventes e a acetonitrila mostrou resultados mais adequados para todos os piretróides.

PALAVRAS-CHAVE: pesticidas; efeito matriz; leite cru.

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