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Analytical Methods

Analysis of pesticide residues in sugarcane juice using QuEChERS sample preparation and gas chromatography with electron capture detection

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ABSTRACT

QuEChERS sample preparation was used for the determination of 7 pesticides residues in 80 samples of sugarcane juice collected from two Brazilian cities, in two different periods. The method involved extraction with acetonitrile, liquid–liquid partition with addition of $MgSO_4$ and NaCl followed by dispersive SPE cleanup with PSA sorbent and the analyses were carried out with a GC–ECD equipment. The method was validated using sugarcane juice spiked at 0.025, 0.10 and 0.20 mg/L and the average recovery by the method varied from 62.9% to 107.5% with RSDs < 18%. The method showed good linearity and the LODs for the pesticides studied ranged from 0.003 to 0.04 mg/L. No pesticide residue was detected (>LOD) amongst the 80 samples analysed.

1. Introduction

Brazil is the world's largest sugarcane producer: in the last 2008/2009 season the production was 569 million tons and the yield area was 6.7 million hectares (UNICA, 2010). São Paulo (SP) state is responsible for 60% of the production, and the harvesting season goes from May to November. Sugar and alcohol are the most important industrial products from sugarcane but there are others derived products such as sugarcane juice, which is a very popular and common beverage in many Brazilian cities. Sugarcane juice is usually commercialised by street vendors who extract the juice from the cane by using mills.

Recent research in Brazil indicates that sugarcane juice can be used as nutritional supplement by athletes (Fava, 2004) and this study points to an increase in sugarcane juice consumption. Consumers have always wanted food quality and safety. In this manner, information and studies regarding pesticide residue has become a usual practice.

The application of herbicides is a routine for controlling harmful grass in sugarcane crops, and also other types of pesticides are applied for pest and disease control. Systemic pesticides applied on crops are absorbed either by the plant roots or foliar parts and are incorporated into the tissues, and in the case of sugarcane it can result in the presence of their residues in the juice.

Due to pesticides toxicity, several countries, the European Union and the Codex Alimentarius have established maximum residue levels (MRLs) in water, ground and food for a large number of pesticides. In Brazil, the MRLs are established by the National Health Surveillance Agency (ANVISA) (ANVISA, 2009). In sugarcane crops ANVISA authorises the use of 65 pesticides.

There are several studies regarding development and validation of analytical methodologies for pesticide residues analysis in juices and, in some cases, the method was applied to samples and the pesticide residues levels was reported. The most common fruit juices analysed are: orange, grape, apple and tomato and, in general, the pesticide levels detected and reported in the studies are considered low (Albero, Sánchez-Brunete, & Tadeo, 2003, 2005; Gilbert-López, García-Reyes, Mezcua, Molina-Díaz, & Fernández-Alba, 2007; Picó & Kozmutza, 2007; Rawn, Roscoe, Krakalovich, & Hanson, 2004; Tadeo, Sánchez-Brunete, Albero, & González, 2004).

QuEChERS (standing for Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation was introduced by Anastassiades, Lehotay, Stajnbaher, and Schenck (2003) and it has been used around the world in many studies for pesticide residue analysis in different matrix samples (Aysal, Ambrus, Lehotay, & Cannavan, 2007; Húšková, Matisová, & Kirchner, 2008; Lehotay, 2007; Lehotay, de Kok, Hiemstra, & Van Bodegraven, 2005b; Lehotay, Mastovská, & Yun, 2005a; Lesueur, Knittl, Gartner, Mentler, & Fuerhacker, 2008; Looser, Kostelac, Scherbaum, Anastassiades, & Zipper, 2006; Mastovská & Lehotay, 2004; Nguyen, Lee, Lee, Lee, & Lee, 2007; Nguyen, Yu, Lee, & Lee, 2008; Payá et al., 2007).

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For this study 7 pesticides with their use authorised by ANVISA in sugarcane crops were selected for analysis. The selection of these compounds considered some aspects such as (a) their mode of action (systemic), (b) the amenability with the ECD detector and (c) a study by Armas, Monteiro, Amâncio, Correa, and Guercio (2005) that reported the 24 pesticides more likely to be used in sugarcane crops in a region of the Sate of São Paulo.

The objective of the present study was to apply QuEChERS sample preparation for determination of 7 pesticides in sugarcane juice samples collected in two Brazilian cities, Ribeirão Preto, SP and Campinas, SP, during harvest time and in the season between harvest cycles. The results will help to verify if seasonal and geographical variations may influence the pesticide levels in the samples.

2. Material and methods

2.1. Standards and reagents

Pesticides standards (trifluralin, atrazine, acetochlor, alachlor, endosulfan-alpha, endosulfan-beta and endosulfan-sulphate), with a minimum of 98% purity, were purchased from ChemService, USA.

Stock solutions of individual standards (10 mg/L) were prepared in hexane and stored in the dark at −18 °C. The calibration standards solutions contained the 7 pesticides in concentrations ranging from 0.05 to 2 mg/L and were prepared in toluene. Standard solutions prepared in acetonitrile were used for spiking sugarcane juices samples at 0.025, 0.1 and 0.2 mg/L levels.

All organic solvents used in the study were pesticide grade or HPLC grade and purchased from J.T. Baker, USA. Reagent grade NaCl (Merck, Germany) and anhydrous $MgSO_4$, purity > 98% (Synth, Brazil) were used. The $MgSO_4$ was heated in a muffle furnace for 5 h at 500 °C for phthalates and moisture removal. Primary secondary amine (PSA) sorbent (40 μm particle size) was obtained from Varian®.

2.2. Samples

Samples were collected as usually sold for consumption: sugarcane was crushed at the moment of purchase and the juices were stored in glass flasks with screw caps and kept frozen at −18 °C until analyses, which were performed within a month.

Samples were collected from 20 different suppliers in the cities of Ribeirão Preto and Campinas, State of São Paulo, Brazil. Sampling was done in two periods of 2007: during harvest time (September/October 2007) and in the period between harvests (March/April 2007).

The total of 80 samples were analysed in duplicate for the presence of 7 pesticides (trifluralin, atrazine, acetochlor, alachlor, endosulfan-alpha, endosulfan-beta and endosulfan-sulphate).

Analytical methodology was validated using a blank sugarcane juice, from an organic crop, acquired from a supplier in Campinas, SP, Brazil.

Fortified samples were prepared by spiking 10 mL of blank sugarcane juice sample with different volumes of standard solution in acetonitrile. The sugarcane juice was then homogenised by vortex mixing for 30 s.

2.3. GC–ECD analysis

Analyses were carried out with a HP-6890 Series GC gas chromatograph equipped with an electron-capture detector (μECD-Ni63) (Hewlett–Packard, Avondale, PA, USA). The system was equipped with split–splitless injection inlet and 3.0 μL of the sample was injected in splitless mode at 240 °C. A HP-5 fused silica capillary column (Agilent 19091 J-413, 30 m × 0.25 mm × 0.25 μm, 5%-phenyl-methylpolysiloxane) was used with nitrogen as carrier gas at a constant flow (1.0 mL/min). The GC oven was operated with the following temperature program: initial temperature 80 °C held for 1 min, ramped at 10 °C/min to 220 °C not held, followed by a ramp of 3 °C/min to 280 °C. Temperature of the ECD detector was at 320 °C. The total run time was 35 min and Agilent ChemStation chromatography data system was used for instrument control and data analysis. Quantification of the pesticides was by peak area using the external standard method.

2.4. QuEChERS sample preparation

The sugarcane juice samples were prepared with QuEChERS method. For extraction, 10 mL sample were transferred into a polypropylene centrifuge tube, 10 mL acetonitrile were added and the solution was mixed using a Vortex mixer for 1 min then, 4 g anhydrous $MgSO_4$ and 1 g NaCl were added and solution was mixed again for 1 min. The tube was centrifuged for 10 min at 1200 rpm (Anastassiades et al., 2003).

Cleanup was performed according to Lehotay (2007). Four millilitre aliquot of the upper layer was transferred to a polypropylene centrifuge tube containing 200 mg PSA and 600 mg anhydrous $MgSO_4$. The aliquot taken was different from the original version, nevertheless the salts proportion was preserved. The extract was mixed using a vortex for 30 s and then centrifuged for 3 min at 3500 rpm. Two millilitre of the upper layer were transferred into a glass flask and the extract was evaporated in a water bath at 40 °C under nitrogen flow until total dryness. The extract was diluted in 500 μL toluene. This procedure resulted in an amount of sample in the final extract of 4 mL/mL.

2.5. Method validation

The validation of the analytical method was performed by the following parameters: linearity, precision and accuracy, limits of detection and quantification, and repeatability. All the analyses were carried out using the same blank sample of sugarcane juice.

Linearity was determined by constructing calibration curves with standard solutions, in toluene, containing all pesticides in the range of 0.05–2.0 mg/L. Three injections were made at each of the 8 concentration levels.

The limits of detection (LODs) and quantification (LOQs) were calculated in accordance with Taylor (1987) and INMETRO (2007) guidelines. For this purpose, 7 independent analyses of a sugarcane juice sample spiked with pesticides at a level of 0.025 mg/L (0.1 mg/L for atrazine) were performed. The LOD and LOQ were calculated from the standard deviation of these determinations.

Accuracy and precision data were obtained with recovery studies carried out by spiking samples with pesticide standards at levels of 0.025, 0.10 and 0.20 mg/L. The spiked samples as well as the unspiked controls were analysed in seven replicates. Repeatability of the method was evaluated through the relative standard deviation (RSD, %) associated to measurements of the pesticide performed during recovery analyses.

In order to maintain analytical quality control, for each sample batch analysed a spiked sample (similar to the ones used in the recovery study) was analysed simultaneously. Batch results were considered unsatisfactory when the sample used as quality control had low recovery.

3. Results and discussion

Fig. 1 presents representative chromatograms for a standard pesticide mixture solution, a spiked and a blank sugarcane juice sample. Adequate separation of the 7 pesticides was achieved.

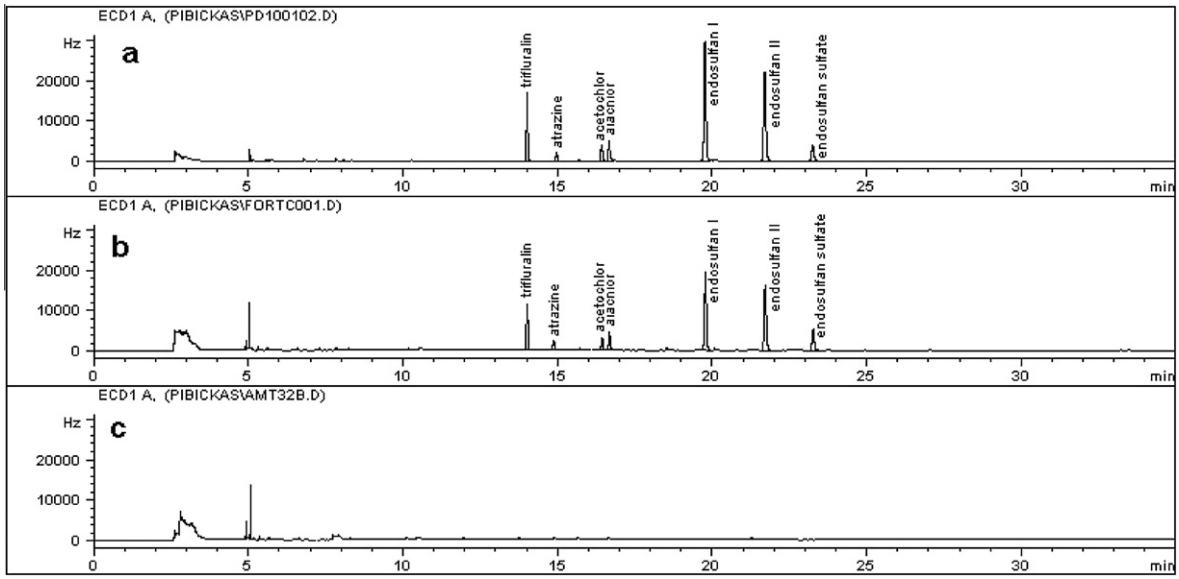

Fig. 1. GC–ECD chromatograms of (a) standard solution of 7 pesticides (0.4 mg/L), (b) spiked sugarcane juice sample (0.1 mg/L) and (c) blank sugarcane juice sample.

The blank sample chromatogram shows no interference peaks in the retention times of the target compounds.

All pesticides showed linearity in the concentration range of 0.05–2.0 mg/L, with correlation coefficients (r) higher than 0.995. Relative standard deviations (RSD) of the three replicate injections ranged from 3.7% to 9.3% showing good repeatability.

Table 1 presents recovery data and repeatability (RSD) for the seven pesticides analysed in 3 different spiking levels.

The recoveries and RSD ranged from 62.9% to 107.5% and 5.1% to 17.6%, respectively. The lowest spike level (0.025 mg/L) presented recovery for 6 pesticides in the range of 87.4–99.9% as recommended by SANCO Guidelines (European Commission, 2007). Table 1 does not present recovery results for atrazine at 0.025 mg/L level, as this level is lower than the LOD of the compound. Therefore the ECD detector was shown to be not as sensible for this molecule as it is for the others.

As shown in Table 1, recoveries were lower than 70% in some cases (levels of 0.2 mg/L for endosulfan α and 0.1 mg/L for endosulfan α and endosulfan β), however these recoveries may be acceptable due to RSD values (11.1, 5.9 and 9.4, respectively) (Mastovská & Lehotay, 2004).

The repeatability of the method was satisfactory for all pesticides, since the RSD was below 20% (European Commission, 2007).

Table 1 also presents the LODs and LOQs for the 7 pesticides analysed. LODs ranged from 0.003 (endosulfan α) to 0.040 mg/L (atrazine). According to the table, for all 7 pesticides analysed the LODs presented are lower than the respective maximum residue levels (MRLs) established by Brazilian regulation for sugarcane. Brazilian regulation does not establish MRLs for sugarcane juice.

In the present study, there was a need of a concentration step as well as the substitution of the solvent prior to injection, since toluene is better suited than acetonitrile for GC–ECD analysis.

Table 1
Fortification experiments of pesticide residue from spiked sugarcane juice at different levels (recovery and repeatability), limit of detection (LOD), limit of quantification (LOQ) and maximum residue limit (MRL).

Pesticide	Spiked level (mg/L)	Recovery (%)[a]	RSD%	LOD (mg/L)	LOQ (mg/L)	MRL (mg/kg)[b]
Trifluralin	0.025	99.9	12.7	0.010	0.019	0.05
	0.1	107.5	9.7			
	0.2	86.4	10.3			
Atrazine	0.025	–	–	0.040	0.080	0.25
	0.1	98.2	13.6			
	0.2	73.8	10.6			
Acetochlor	0.025	94.7	5.2	0.004	0.007	0.1
	0.1	85.6	6.2			
	0.2	81.4	13.7			
Alachlor	0.025	93.9	15.3	0.011	0.022	0.1
	0.1	88.5	5.9			
	0.2	78.4	12.2			
Endosulfan α	0.025	90.2	5.1	0.003	0.007	0.01
	0.1	67.2	5.9			
	0.2	65.4	11.1			
Endosulfan β	0.025	87.4	9.3	0.006	0.012	
	0.1	62.9	9.4			
	0.2	83.1	15.4			
Endosulfan SO_4	0.025	99.8	6.6	0.005	0.010	
	0.1	89.1	10.6			
	0.2	82.2	17.6			

RSD = relative standard deviation.
[a] $n = 7$.
[b] Source: ANVISA (2009).

There are only a few studies that made use of QuEChERS sample preparation, modified or not, for pesticide analysis when using a GC coupled with an ECD detector (Aysal et al., 2007; Barakat, Badawy, Salama, Attallah, & Maatook, 2007). As in the present study, Barakat et al. (2007) also made use of a concentration step and a change in the solvent (hexane/acetone) prior to injection in the GC–ECD system.

The results of the method validation indicate that the QuEChERS sample preparation coupled with the GC–ECD analysis is suitable for the determination of trifluralin, atrazine, acetochlor, alachlor, endosulfan-alpha, endosulfan-beta and endosulfan-sulphate in sugarcane juice. In addition, as sugarcane juice contains from 14.5% to 23.5% of sucrose (Prati & Camargo, 2008); these results indicate that the QuEChERS sample preparation can be successfully used for analysing pesticide residues in samples with high sugar content. Barakat et al. (2007) also reported successful use of QuEChERS when analysing pesticide residues in honey.

One drawback would be that, when using GC–ECD for analysis, an alternative method, such as MS detection, would be needed for identity peak confirmation in case any compound is detected in the samples.

As for the 80 sugarcane juice samples analysed in the present study, no pesticide residue was detected (>LOD) in any of the samples, in this manner there was no need for confirming the identity of the compounds.

There are only few data on the literature regarding the presence of pesticide residues in sugarcane juice.

Results obtained in the present study are similar to the ones reported by Zuin et al. (2006) who analysed 6 samples of sugarcane juice collected in the city of São Carlos, SP, Brazil, for the presence of 17 pesticides (including alachlor and atrazine) using gas chromatography coupled with a mass spectrometry detector. As a result, alachlor was not detected in none of the samples while atrazine was detected in 2 sugarcane juice samples. However, the levels of atrazine detected were lower than the LOD established in the present study (0.04 mg/L).

As stated before, there are no MRLs established for pesticides in sugarcane juice and no pesticide residue was detected (>LOD) in any of the 80 analysed samples. So, when using the MRLs established for sugarcane for comparison, the samples can be considered as in accordance with Brazilian regulation, since the LODs are lower than the MRLs for the 7 pesticides studied. Considering that the production of 1 L of juice requires the use of approximate 2 kg of sugarcane the MRLs would still not be violated.

The results of the present study are similar to the ones reported in previous studies where low levels of pesticide residues were determined in different types of fruit juices (Albero et al., 2003,2005; Gilbert-López et al., 2007; Picó & Kozmutza, 2007; Rawn et al., 2004; Tadeo et al., 2004; Zuin et al., 2006).

4. Conclusion

The QuEChERS sample preparation is suitable for determination of the 7 analysed pesticides in sugarcane juice, demonstrating the great versatility of QuEChERS method that can be used for pesticide residue analysis in matrices with high sugar content and can be used with GC–ECD analysis as well.

None of the 7 pesticides analysed was detected in the samples. Therefore results indicate that there is no seasonal or geographical variation in the levels of these pesticides. In this manner, one can assume that there is no apparent risk to the consumers of Campinas and Ribeirão Preto regarding sugarcane juice intake, taking into account the presence of the 7 pesticides studied.

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