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Analytical Methods

A simple and sensitive single-step method for gas chromatography–mass spectrometric determination of fipronil and its metabolites in sugarcane juice, jaggery and sugar

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ABSTRACT

A simple and sensitive single-step method for gas chromatography–mass spectrometric determination of fipronil and its metabolites viz., fipronil desulfinyl, fipronil sulphide and fipronil sulphone in sugarcane juice, jaggery and sugar has been developed. Acetonitrile was superior to ethyl acetate in terms of selectivity, though they were on par with each other in terms of recoveries. This method does not require any cleanup as the PSA-based cleanup was on par with no-cleanup treatment. Interestingly, the recoveries of fipronil and its metabolites decreased with increased amounts of C_{18} from 10 to 50 mg/g of matrix. Matrix effects were insignificant and the limit of quantification was 0.005 μg/g. The recoveries of fipronil and its metabolites varied between 87.5% and 108.5% with the RSD of 0.2–5.3% for all the three matrices studied. This method has also been validated by monitoring fipronil and its metabolites in the retail outlet samples of sugarcane juice, jaggery and sugar.

1. Introduction

India is the world's second largest producer of sugarcane. In 2011, it produced 355 million tonnes of sugarcane from 5.07 million hectares with an average productivity of 68 t/ha (Solomon, 2011). About 60–70% of the cane produced in the country is utilised for the production of sugar (Nair, 2011) and 25–30% is being utilised for the production of jaggery and *khandsari* (Singh, Singh, Anwar, & Solomon, 2011). Sugarcane juice is also a very popular beverage among the rural poor and is usually commercialised by street vendors. Though the experimental maximum yield of sugarcane is 325 t/ha, the national average hovers between 66 and 70 t/ha (Solomon, 2011). Insect pests are among the few important constraints which limit the productivity of sugarcane to a considerable extent across the Indian sub-continent. The early shoot borer, *Chilo infuscatellus* Snellen and the root borer, *Emmalocera depresella* Swinhoe are among the 20 major pests causing 22–33% and 35% loss in yield, respectively (Directorate of Sugarcane Development, 2012). Fipronil is one of the important broad-spectrum insecticides recommended for the management of *C. infuscatellus* and *E. depresella* across the country to achieve the economic yield (Central Insecticide Board & Registration Committee, 2009).

Fipronil, the phenylpyrazole insecticide [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile] blocks $GABA_A$-gated chloride channels in the central nervous system of insects. Disruption of the $GABA_A$ receptors by fipronil prevents the uptake of chloride ions resulting in excess neuronal stimulation and death of the target insect (Cole, Nicholson, & Casida, 1993). Fipronil and its metabolites exhibit differential binding affinity for $GABA_A$ receptor subunits of insects and mammals. Fipronil has higher binding affinity for insect receptor complexes compared to mammalian complexes. The lower binding affinity for mammalian receptors enhances selectivity for insects and increases the margin of safety for humans and animals (Cole et al., 1993; Hainzl, Cole, & Casida, 1998; Ratra & Casida, 2001; Ratra, Kamita, & Casida, 2001). However, fipronil sulphone, the primary biological metabolite of fipronil has been reported to be 20 times more active at mammalian chloride channels than at insect chloride channels (Zhao, Yeh, Salgado, & Narahashi, 2005). Fipronil desulfinyl, the primary environmental metabolite (photo product) of fipronil is 9–10 times more active at the mammalian chloride channel than the parent compound and thus, reducing the selectivity between insects and humans (Hainzl & Casida, 1996; Hainzl et al., 1998). The residues of fipronil and its

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metabolites may be expected in the sugarcane juice and its downstream commercial products such as jaggery and sugar. Hence, development of methods for determination of residues of fipronil and its metabolites in sugarcane juice, jaggery and sugar deserves importance to ensure safety to people across the world and India in particular, as it is the largest consumer of sugar in the world.

Few analytical methods have been reported for the determination of fipronil and its metabolites in different matrices such as soil, water, human urine (Vílchez, Prieto, Araujo, & Navalón, 2001), honeybees (Morzycka, 2002), honey (García-Chao et al., 2010), pollen (Kadar & Faucon, 2006) milk, cattle feed (Le Faouder et al., 2007), ovine plasma (Bichon, Richard, & Le Bizec, 2008), grape leaves, berries (Mohapatra et al., 2010), cotton lint and seed (Chopra, Chauhan, Kumari, & Dahiya, 2011). These methods have their own merits and demerits. Matrix solid-phase dispersion (MSPD) method developed by Morzycka (2002) was rapidly given up as it has been proved effective only for solid matrices such as honeybees. Kadar and Faucon (2006) developed a liquid chromatography–tandem mass spectrometry method characterised by cumbersome sample preparation steps for highly complex matrices like pollen. Le Faouder et al. (2007) developed a lengthy and laborious protocol for the determination of fipronil and its metabolites in milk and cattle feed. It involved extraction of target analytes with comparatively larger volume of solvents followed by a delipidation step and solid-phase extraction (SPE) cleanup. The SPE cleanup was performed in a single-step with atoll XC alone (for milk samples), or in two-steps (an additional step with florisil) for plant samples. This was followed by the highest level of concentration aimed at drastically reduce the limit of detection of the method. The SPE procedure of García-Chao et al. (2010) involved the costly vacuum manifold system for extraction of fipronil and its metabolites from honey and pollen matrices. The method developed by Vílchez et al. (2001) employed a specially designed solid-phase micro extraction (SPME) device made of fused-silica fibre coated with polymeric stationary phase (85 μm polyacrylate) for extraction of fipronil from very low volume of samples (0.5 g for soil, ⩽4 mL for human urine and water samples). Bichon et al. (2008) developed a method meant for ultra-low volume of matrix (200 μL plasma) as the quantities of plasma available are often limited. Despite their good sensitivity, the suitability of these two methods only for low to ultra-low volume of matrices precludes their use for agricultural commodities, which require relatively larger sample size for adequate representation. The analytical methods developed for grape leaves, berries (Mohapatra et al., 2010), cotton lint and seed (Chopra et al., 2011) involved extraction of analytes with larger volume of solvents followed by the traditional liquid–liquid partitioning and column cleanup with adsorbents like florisil alone or with neutral alumina for final determination in GC–ECD. The limit of quantification of these two methods is comparatively higher (0.01 μg/g) besides the involvement of larger volume of organic solvents. Therefore, we first had to develop a rapid, simple and sensitive method for the determination of fipronil and its metabolites in sugarcane juice, jaggery and sugar.

2. Materials and methods

2.1. Chemicals and reagents

Certified reference standards of fipronil (97.5%) and its metabolites viz., fipronil desulfinyl (97.8%), fipronil sulphide (98.8%) and fipronil sulphone (99.7%) were obtained from Bayer CropScience Ltd., India. The SPE sorbents primary secondary amine (Bondesil-PSA, 40 μm particle size) and Bondesil-C_{18} (40 μm particle size) were procured from Agilent Technologies, USA. The organic solvents used in the study were of HPLC grade and purchased from Thomas Baker (Chemicals) Ltd., Mumbai, India. Analytical reagent grade anhydrous $MgSO_4$ and NaCl were purchased from Merck India Ltd., Mumbai.

2.2. Preparation of standard solutions

The stock solutions (1000 μg/mL) of fipronil and its metabolites were prepared by accurately weighing 10 mg of each analyte in volumetric flasks (certified A class) and dissolving in 10 mL of n-hexane. These were stored in dark vials at 4 °C. A working standard mixture of 100 μg/mL was prepared by appropriate dilution of the stock solutions, from which the calibration standards (0.003–1.0 μg/mL) were prepared by serial dilution with n-hexane.

2.3. Sample preparation

Sugarcane juice was extracted immediately after harvesting the 10 months-old crop (variety Co-86032) grown at Sugarcane Breeding Institute (Indian Council of Agricultural Research), Coimbatore (Tamil Nadu, India) using motorised sugarcane crusher. The sugarcane juice was filtered through Whatman No. 1 filter paper under mild-suction and used for analyses. Jaggery was also prepared from the same variety used for the extraction of juice, while the refined sugar was purchased from the retail market. Jaggery and sugar samples were ground to fine powder in mortar and then used for analyses.

2.3.1. Extraction

A portion (10 g) of well-homogenised sample was placed into a 50 mL screw-capped oak ridge tube. The target compounds were extracted with 20 mL of the solvent (acetonitrile/ethyl acetate) as 10 mL of the extraction solvent was insufficient to get enough volume of organic phase from jaggery and sugar samples for downstream cleanup process. The oak ridge tube was closed tightly and shaken vigorously for 1 min to ensure better interaction between the solvent and sample. Then 1 g of NaCl and 4 g of $MgSO_4$ (anhydrous) were added to the sample, vortexed for 1 min and centrifuged (Superspin R-V/FA; Plasto Crafts, Mumbai, India) at 5000 rpm for 10 min at room temperature. The supernatant (4 mL) was either concentrated under gentle stream of nitrogen (15 psi) in the Turbovap LV (Caliper Life Sciences, Russelsheim, Germany) at 40 °C and reconstituted in 1 mL of hexane for analysis in the GC–MS without cleanup or subjected to cleanup with the sorbents as detailed below.

2.3.2. Cleanup

Dispersive solid phase extraction (d-SPE) cleanup with PSA or C_{18} was compared with no-cleanup (no sorbent) in terms of analyte recovery and interference from the study matrices (sugarcane juice, jaggery and sugar). After centrifugation at 5000 rpm for 10 min as described in the extraction, 8 mL of supernatant was transferred to 15 mL centrifuge tubes containing varied levels of PSA (10, 25 and 50 mg/g of matrix) and anhydrous $MgSO_4$ (150 mg/g of matrix). Simultaneously, C_{18} was also evaluated at varied levels (10, 25 and 50 mg/mL) along with $MgSO_4$ (150 mg/g of matrix). The extract was vortexed for 30 s and then centrifuged at 3000 rpm for 10 min. The supernatant (4 mL) was transferred to turbo tube and concentrated to near dryness under a gentle stream of nitrogen in the Turbovap LV as described earlier. The residue was reconstituted in 1 mL of hexane and thus, the amount of sample in the final extract was equivalent to 2 g/mL.