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Determination of Residues of Metribuzin in Soil and Sugarcane by QuEChERS

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This paper aims to apply a modified QuEChERS method of mertribuzin extraction from sugarcane plant parts, soils. The quantification of metribuzin was carried out by high performance liquid chromatography coupled with diode array detector. The method was validated by evaluating the analytical curve, linearity, limits of detection and limits of quantification, precision (repeatability) and accuracy (recovery), after the optimization of extraction parameters for the determination of metribuzin. The method provided limits of quantification in the range of 0.05-0.10 mg kg^{-1} for different matrices with the method detection limit of 0.03 mg kg^{-1} for all matrices. The developed method was applied to study the metribuzin persistence in sugarcane plant parts and soil from the field experimentation which received two different doses of metribuzin along with control. It was found that the residue of metribuzin in soil and sugarcane parts were below the detection limit (0.03 mg kg^{-1}) except at the higher dose of application.

Keywords: Metribuzin, Sugarcane, Soil, QuEChERS, Liquid chromatography.

INTRODUCTION

Herbicide use in modern agriculture is necessary to reduce the pressure of weeds and insects in monoculture cropping systems. Large quantities of these compounds are applied directly to the soil and the extensive and inappropriate use of these products in agriculture unfavourably affects the whole ecosystem by entering into the food chain and polluting the soil, air, ground and surface water. Hence, the monitoring of herbicide residues in soil environment is essential in the interest of public health safety. For this, the extraction and analysis method should be appropriate and easy to have quick, continuous monitoring of its residues in different matrices.

Sample preparation is a very important part of the analytical method. The development of an appropriate sample preparation procedure includes a number of steps, such as extraction and cleanup, to obtain a final extract concentrate of target analytes as free as possible of matrix compounds. Due to the low levels of herbicides that may be found in soil, an enrichment of the analyte concentration must be achieved before its instrumental determination. Herbicides in foods are usually extracted by liquid-liquid extraction (LLE), matrix solid-phase dispersion (MSPD), solid-phase micro extraction (SPME) and QuEChERS (quick, easy, cheap, effective, robust and safe) which was proposed first time by Anastassiades and Lehotay[1]. The QuEChERS sample treatment method has mainly been used for the extraction of different pesticides from food matrices with high water content[2]. The QuEChERS approach is flexible and serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the lab. However, the use of QuEChERS in soils[3] and sugarcane is limited for analyzing the herbicides especially metribuzin.

So far analysis of metribuzin and its metabolites has mainly been accomplished by different chromatographic methods using either solid phase extraction or liquid-liquid partitioning[4-6]. Microwave-assisted water extraction method (MAWE) was developed for the analysis of metribuzin with its major conversion products in soil was analyzed by HPLC-DAD using 10 mM phosphate buffer, pH 7.0 as extracting solvent and aqueous extracts[7,8]. Perez *et al.*[9] extracted metribuzin from soil samples in an ultrasonic bath using methanol and obtained 86.7 to 104.2 % recovery in micellar electrokinetic chromatography (MEKC). Niell *et al.*[10] compared two extraction solvents and conditions for three sulfonylurea herbicides residues in milled rice with liquid chromatography/diode array detection analysis. Moreover, the QuEChERS sample preparation is applied mostly for the LC/MS or GC/MS which are not affordable for all.

Sugarcane (*Saccharum officinarum* L.) cultivation is one of the most important agricultural activities in India and worldwide where its main end products are sugar, alcohol and derived foods. Continuous use of same group of herbicides causes shift in weed flora and develop herbicide resistant

weeds. Also the bio accumulation and biomagnifications of the herbicide residues in soil and crops may takes place. Metribuzin (4-amino-6-*tert*-butyl-3-methylthio-1,2,4-triazin-5-one), a triazine herbicide used for pre and post emergence control of annuals grasses and broad leaf weeds in sugarcane, soybean, wheat *etc.*[11]. It is necessary to develop simple extraction and analytical methodologies to monitor triazines mainly metribuzin in the environment, to study fate and transport, modeling, ecotoxicology risk assessment and to develop management strategies.

With this background the present work was carried out to extract the residues of metribuzin from soils and sugarcane plant parts using QuEChERS method by HPLC. Developed method was validated and also used to assess the persistence of metribuzin in post harvest soils and sugarcane plant which treated with metribuzin.

EXPERIMENTAL

Technical standard of metribuzin (98.2 % purity) was obtained from M/s. Crystal Crop Protection Pvt. Ltd., New Delhi. Methanol and acetonitrlie HPLC grade was purchased from S.D. Fine Chemicals, India. All other chemicals used in extraction and clean up were obtained from Sisco Research Laboratory, Mumbai, India. Primary secondary amine (Bondesil-PSA, 40 μm) was obtained from Agilent Technologies, USA.

Preparation of solutions: The stock solution of metribuzin containing 1000 mg L^{-1} was prepared in methanol HPLC grade and stored at -18 °C. Intermediate working standard of 100 mg L^{-1} was prepared in methanol HPLC and was used to prepare the working standard solutions from 0.001 to 5.0 mg L^{-1}. Working standards were used for spiking the samples of different matrices and preparing the analytical curves in methanol HPLC.

Chromatographic conditions: To determine the optimum chromatographic conditions, an Agilent C18 column (XDB 150 × 4.6 mm i.d., 5 μm particle size) with different mobile phases comprising several combinations of methanol/acetonitrile and Milli-Q water were tested to provide better separation. The pH of the Milli-Q water was adjusted by a thermo pH meter (model ORION 5 STAR). The mobile phases were degassed for 0.5 h in an ultrasonic bath before use. Separation was performed using an Agilent 1200 series HPLC equipped with DAD detector and auto sampler, Rheodyne 20 μL loop injector, connected to EZChrom Elite software (Agilent, USA) for data acquisition. The analytical column was conditioned by passing the mobile phase for 30 min at a flow rate of 1.0 mL min^{-1} and operated at 30 °C. The flow rate was set to 0.5 mL min^{-1} for detection and quantification of the metribuzin. The response of detector was recorded from 190 to 400 nm to find out the lambda maximum and minimum for metribuzin with the injection volume of 20 μL. The identification of the herbicides in the samples was accomplished on the basis of their retention time and by comparison between the DAD spectrum of the standard solutions and samples.

QUEChERS sample preparation: A modified QuEChERS method was used for the preparation of sample extracts. According to this method, required quantity (Table-1) of the finely ground sub-sample was placed in a polypropylene centrifuge tube (50 mL) and ultrapure water (milli-Q) was added (Table-1) and mixed using a vortex mixer for 1 min. Subsequently, 20 mL of different extractants (MeOH alone, MeCN and MeOH + MeCN) were added to each set of replication and the mixture was shaken vigorously for 2 min and then sonicated for 30 min at 40 °C. To this, 1.8 g of anhydrousmagnesium sulfatge and 2 g of sodium acetate was added, vortexed for 2 min and then centrifuged at 5000 rpm for 5 min. The extract was then separated from sediments by simple decantation.

TABLE-1
SAMPLE PREPARATION DETAILS FOR METRIBUZIN EXTRACTION

Sample preparation	Soil	Sugarcane stem/leaf	Juice
Sample size	10 g	5 g	10 mL
Water added	5 mL	3 mL	5 mL

Clean up: A 10 mL aliquot of the extract was transferred into a polypropylene centrifuge tube containing 100 mg anhydrous magnesium sulphate, per mL acetonitrile extract. The tube was vortexed for 0.5 min and centrifuged at 5000 rpm for 2 min. An aliquot (upper layer) of 5 mL was evaporated under a stream of nitrogen and the residue was re-dissolved in acetonitrile for LC analysis after filtering it with 0.2 μm Paul nylon membrane filter. An aliquot of 5 mL (upper layer) without concentration under stream of nitrogen also injected after filtering for the comparison of results. The optimization procedure was performed in triplicate and injected three times (n = 9) and the determination were carried out in HPLC-DAD. Clean up was also conducted with the use of 0.3 mg PSA/mL of extract for comparison.

Method performance: The linearity of the calibration curve was studied at a concentration ranged between 0.005 and 0.5 μg mL^{-1} with triplicate injections of seven calibration standards prepared in blank matrix extract in methanol. The accuracy and precision of the method was assessed using spiked samples of different matrices. Recovery of metribuzin from different matrices were determined for four replicates at four spiking levels of 0.01, 0.05, 0.1,0.5 and 1.0 mg kg^{-1} to evaluate the efficiency of the extraction and clean-up methodology. Limit of quantification was defined as the lowest spiking level, at which the validation was achieved and was determined based on the accuracy and precision data obtained through the recovery studies.

Experimental details: The soil and sugarcane plant samples were collected from sugarcane fields which have not received metribuzin previously and were used for conducting the recovery study. The properties of soils used for the recovery study are given in Table-2.

TABLE-2
PROPERTIES OF THE SOILS

Properties	Soil 1	Soil 2
Texture	Sandy clay loam	Silty clay
Soil pH	8.21	6.60
EC (dS m^{-1})	0.56	0.31
Organic carbon (%)	0.51	0.82

Field experiments were laid out to study the persistence of metribuzin 70 % WP applied to the sugarcane field as early post emergence weed control. The experiments were conducted at Eastern Block Farm of Tamil Nadu Agricultural University, Coimbatore during *rabi* (Oct.-Nov.) season 2011-12 and late (April-May) season 2012-13. Metribuzin was applied at two levels (500 and 1000 g ha^{-1}) along with control and replicated thrice. Sugarcane variety CO 86032 was grown during both the years. Calculated quantity of metribuzin was applied as early post emergence on 20 days after planting the cane by maintaining the optimum moisture in the field. The knapsack sprayer with flat fan nozzle was used for spraying metribuzin with the spray volume of 750 L of water ha^{-1}. Soil and plant samples were collected from the experimental plots at the time of harvest and stored at -15 °C for residue analysis. The soil of the experimental fields was sandy clay loam and has the electrical conductivity 0.52 dS m^{-1}, soil reaction 8.18 and organic carbon 0.53 %.

RESULTS AND DISCUSSION

Analytical performance and HPLC-DAD conditions optimization: Several mixtures of mobile phases with acetonitrile and methanol were tested in the binary mode to find a shorter run time with good separation and identification of the metribuzin. The mixture of methanol: 0.1 % formic acid in milliQ water (55:45 v/v) was found to be satisfactory and the matrix interferences was less; besides the run time was short with good resolution. When mixture of acetonitrile: water (70/30, v/v) was used, matrix interferences of plant sample was observed, though the run time was shorter and the substances were detected within 15 min. The use of gradient elution of the mobile phases was not satisfactory. The effective separation of the peaks in the chromatogram was also achieved when the methanol: 0.1 % formic acid in milliQ water (55:45 v/v) mobile phase was used at the flow rate of 0.5 mL min^{-1}. The response of the detector from 190 to 400 nm ranges showed that the metribuzin have two lambda max *viz*., 230 and 296 nm (Fig. 1a) and the molecule was resolved at 5.4 min ± 0.15 min. The chromatogram and spectrum of the metribuzin in methanol solvent is shown in Fig. 1a and 1b, respectively. Since the interference was less and also the resolution was good at 296 nm, the entire analysis was done at this wavelength. The purity of the metribuzin peak was also excellent at 296 nm (Fig. 2).

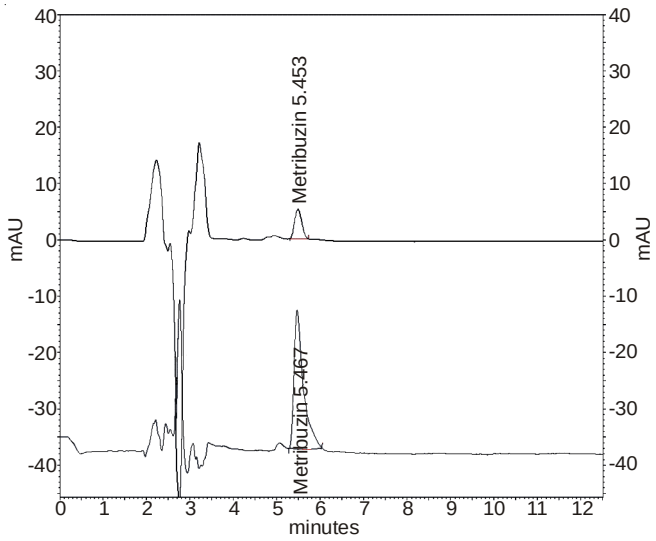

Fig. 1a. Chromatogram of metribuzin detected at 230 and 296 nm by HPLC-DAD

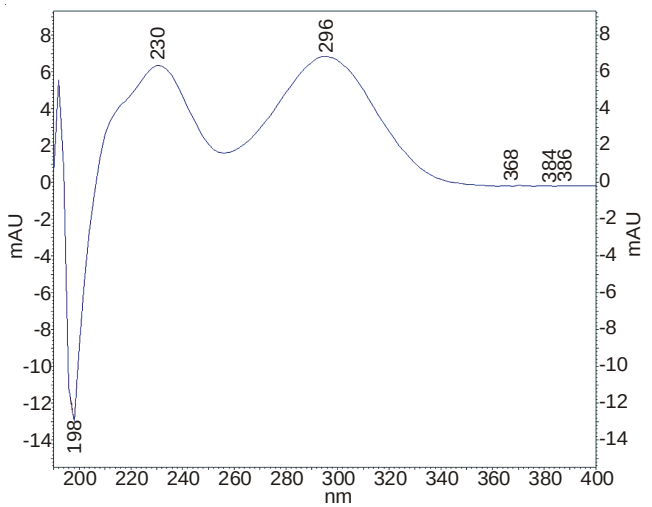

Fig. 1b. Spectrum of metribuzin standard 0.5 µg mL^{-1} detected by HPLC-DAD

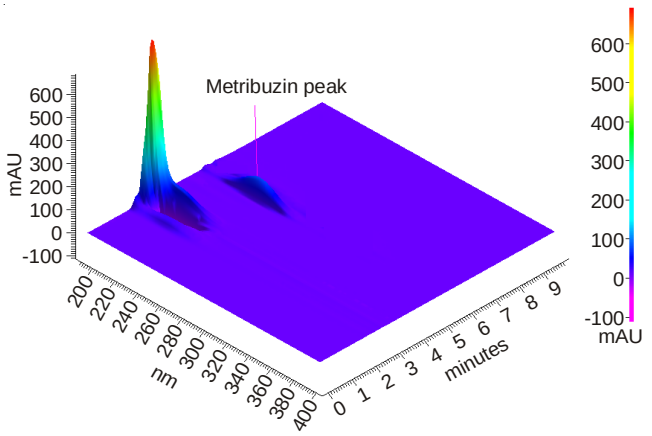

Fig. 2. 3D plot of metribuzin peak detected by HPLC-DAD

The standard calibration curve of metribuzin detected by HPLC/DAD was constructed by plotting the analyte concentration *versus* peak area. The regression equation of the standard calibration curve was y = 10666 x + 2407 (R^2 = 0.996) and the calibration curve showed excellent linearity (Fig. 3) in the concentration ranges of 0.01-1.0 µg mL^{-1}.

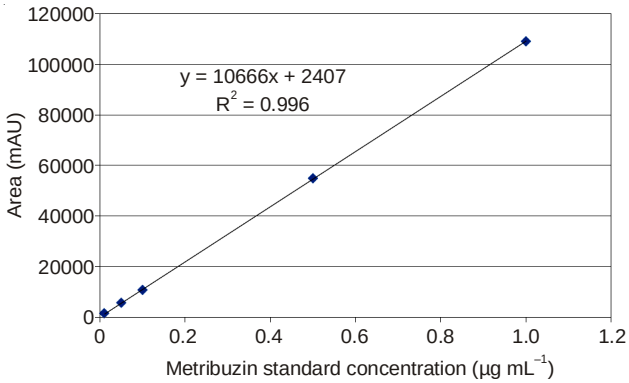

Fig. 3. Calibration curve of metribuzin standards detected by HPLC-DAD

The minimum concentration of herbicide molecule that was detected with acceptable certainty called the limit of detection (LOD) by the instrument was assessed by the repeated injection of the lowest concentration for 7 times. The limit of detection for metribuzin was found to be 0.01 μg mL^{-1}. The limits of detection of the proposed method were determined at a signal-to-noise (S/N) ratio of 3 for the metribuzin. Detector showed good sensitivity for the metribuzin standard up to 0.001 μg mL^{-1} but did not follow the linearity.

Extraction and clean up evaluation: The results of the modified QuEChERS method applied to the extraction of metribuzin from different matrices were obtained by injections into the HPLC-DAD. Acidified acetonitrile, methanol and the mixture of acetonitrile and methanol were tested as extraction solvents. It was observed that the mixture of acetonitrile and methanol was found to be best for the metribuzin extraction from soil and sugarcane with the recovery of more than 80 % (Fig. 4). The increasing proportion of methanol as extractant might enhanced the metribuzin recovery as reported by Locke *et al.*[12]. In the present study, the metribuzin recovery from soils ranged from 43 to 48, 57 to 63 and 79 to 82 % respectively by different extractants *viz.*, acidified MeCN, MeOH and mixture of both. Similar results were also obtained for the sugarcane plant parts; however the recovery was lower than that obtained for soil. Advantage of the buffered QuEChERS modification has also been reported by the Wang *et al.*[13] for the extraction of pyrazosulfuron ethyl from different soils with good recovery and RSD. The acidified MeCN and MeOH mixture along with the sodium acetate for extraction might maintain consistent pH throughout the extraction and yielded maximum metribuzin recovery.

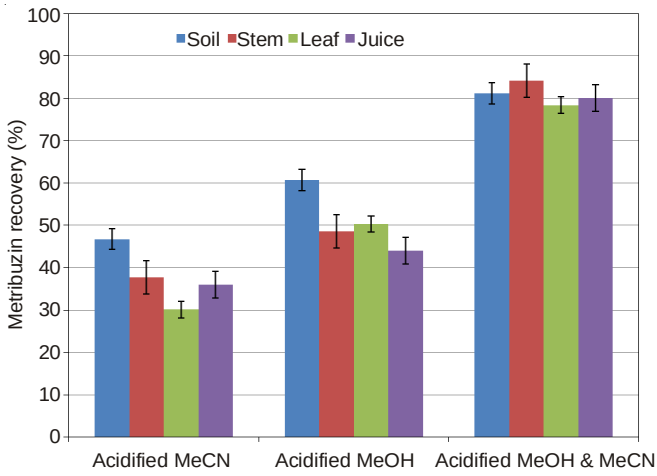

Fig. 4. Comparison of different solvent extraction of metribuzin from the soil and sugarcane plant parts fortified at 0.5 mg kg^{-1}. Error bars indicates the standard deviation (n = 7)

Use of primary secondary amine for the cleanup reduced the recovery to less than 40 % (data not given) irrespective of matrices and might be due to the reaction of the metribuzin with the primary secondary amine[10]. Similar results were reported by Wang *et al.*[13] that the addition of 1 % acetic acid in acetonitrile as a modification of QuEChERS method without primary secondary amine and C18 sorbent gave good recovery of pyrazosulfuron ethyl from soil. Sampaio *et al.*[14] also observed lower recoveries (35.4 % with RSD < 5.11 %) for 2,4-D from sugarcane honey with primary secondary amine clean up. Injection of the upper layer from the centrifuge without concentration under stream of nitrogen was not good since the method detection limit is more than 0.1 mL kg^{-1} of the sample matrices.

Modified QuEChERS method validation: The developed modified QuEChERS extraction and clean up methodology (mixture of 1 % acidified MeOH and MeCN and without primary secondary amine) was validated using two different soils and different parts of sugarcane *viz.*, juice, stem and leaf.

The estimated method detection limit (EMDL) for metribuzin was found to be 0.03 mg kg^{-1} for all matrices with the signal to noise ratio of 3:1 as determined by HPLC/DAD and no substrate interferences were observed at this detection limit as evidenced by the control sample analysis. Whereas the limits of quantification (LOQ) were obtained as the lowest spiked level with acceptable recovery and RSD (Table-3). The limits of quantification was estimated to be 0.05 mg Kg^{-1} for soils and juice and 0.10 mg Kg^{-1} for the sugarcane leaf and stem corresponding to the lowest spiking level in which more than 70 % recovery was obtained with the RSD of less 10 %. The average recovery of metribuzin from soil and plant parts was given in Table-3.

Persistence of metribuzin in soil and sugarcane parts: The modified QuEChERS method developed was used for the extraction of metribuzin from the real field samples collected at the time of sugarcane harvest which received different doses of metribuzin and analyzed by HPLC-DAD. Results (Table-4) indicated that the herbicide metribuzin was below detectable limit in the soil (0.05 mg kg^{-1}) and sugarcane plant parts (0.1 mg kg^{-1}) at the dose of 500 g ha^{-1}. However metribuzin residue was detected in the soil at the higher rate of 1000 g ha^{-1}. Though it was detected, the quantity was within the limits of phyo-toxicity concentrations to the sensitive crops suggested for atrazine (0.0005-0.8 ppm) in soil which is also belongs to a triazine family[15]. Since the metribuzin has high mobility with the Koc value of 24.3-106.0 mL/g and DT_{50} value of 5.2 to 22.4 days[16], it was not persist in the experimental soil at the recommended lower rate of application. Previous researchers[16-20] have indicated that soil organic carbon content is largest single

TABLE-3
RECOVERY OF METRIBUZIN FROM SOILS AND SUGAR CANE PLANT PARTS

Fortified concentration (μg g^{-1})	Recovery (%)* ± Standard deviation				
	Soil 1	Soil 2	Stem	Leaf	Juice
0.05	75.0 ± 3.30	79.8 ± 3.70	43.23 ± 9.40	18.86 ± 4.63	89.36 ± 4.58
0.10	82.6 ± 2.60	94.7 ± 2.89	80.31 ± 7.53	71.86 ± 7.77	95.25 ± 2.37
0.50	83.3 ± 0.71	90.2 ± 5.01	84.23 ± 9.94	78.46 ± 6.51	92.19 ± 3.46
1.00	84.3 ± 0.82	97.4 ± 1.30	84.11 ± 2.18	88.30 ± 8.97	102.92 ± 5.36

*Average of four replications

TABLE-4
RESIDUE OF METRIBUZIN (mg kg^{-1}) IN SOIL AND PLANT PARTS AT HARVEST FROM FIELD EXPERIMENTS (MEAN OF TWO SEASONS)

Treatments	Soil	Cane juice	Stem	Leaf
Metribuzin 70 % WP 500 g ha^{-1}	BDL*	BDL	BDL	BDL
Metribuzin 70 % WP 1000 g ha^{-1}	0.017	BDL	BDL	BDL
Untreated control	BDL	BDL	BDL	BDL

*BDL: Below detectable level

factor responsible for metribuzin sorption in soils. Though, very small quantity of metribuzin residue was detected in the present field experimental soil at a higher rate of application, the continuous and in appropriate use of this herbicide might cause pollution of water bodies due to its high mobility[6] in light textured soils like sandy soils as it can readily leach[21]. Janaki *et al*.[22] and Tandon and Singh[23] also reported the detection of atrazine residues in the soils grown with maize when it was applied at higher rates than the recommended level.

The residue of metribuzin in the sugarcane plant parts *viz*., stem, juice and leaves were below detectable limit (Table-4) which was well under the MRL suggested by the PMRA[24] for the sugarcane (0.1 ppm) and its molasses (2.0 ppm). EFSA[16] reported that after 100 days, less than 10 % of metribuzin residues were present in plant and also confirmed that the residues were not accumulated in the plant. It is therefore, concluded that the significant residues of metribuzin will be it is unlikely present in the sugarcane plant parts at present levels of application (500 g or 1000 g ha^{-1}).

Conclusion

The proposed method offers good accuracy and precision to determine metribuzin residues in soil and sugarcane plant parts. The modified QuEChERS method is validated through recovery studies using different soils and sugarcane plant parts. It was found that the method is rapid and selective, with a simple sample preparation procedure that could be used for the detection and quantification of metribuzin residues in soil and sugarcane plant parts using the HPLC-DAD. Modified QuEChERS method was also applied to study the persistence of metribuzin in soil and sugarcane plant parts from the experimental fields which received two different doses of metribuzin. It was found that the residues of metribuzin in soil and sugarcane parts were below the detection limit (0.01 mg kg^{-1}) except in the soil at the higher dose of application.

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