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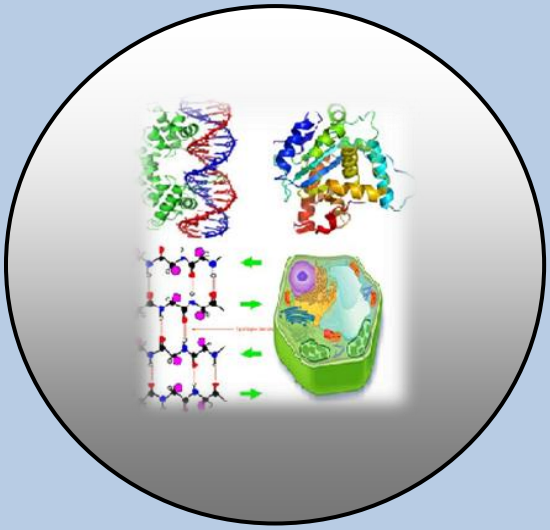

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RESEARCH PAPER

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ABSTRACT

A highly selective matrix assisted solid phase dispersion (MSPD) extraction purification method for the pre concentration of azimsulfuron in buffallow milk. A C18 sorbent based MSPD column was used for extraction of the analyte and extracted the azimsulfuron residues from the sample using acidified ethyl acetate and methylene chloride mixture. The extracts were further purified by passing through the disposable silica mega bond – elute ® cartridges prior to quantification by HPLC on an agilent SB-phenyl column (150mm length x 4.6mm id x 5µm). The HPLC detection was studied with UV detector at 220 nm. Acetonitrile and 0.1% ortho phosphoric acid (40:60 v/v) was used as mobile phase at a flow rate of 1.0 mL min^{-1}. The linearity of azimsulfuron was observed over the concentration range 0.05 to 5 µg/mL and the regression coefficient (r^2) 0.9999. The mean recoveries of azimsulfuron form milk at 0.05 and 0.5 µg g^{-1} fortification levels were in the range of 85-91%.The limit of quantification and detection was established as 0.05 and 0.02 µg g^{-1} respectively. The proposed method was successfully applied for the determination of residues in buffallow milk.

Key words: Azimsulfuron, MSPD, HPLC, UV and Buffallow milk.

INTRODUCTION

Sulfonylurea herbicides are a relatively new class of compounds whose herbicidal activity was discovered in the mid 1970s (Anna Laura Capriotti et al., 2010). They are widely used all over the world for controlling weeds in several crops, e.g., rice, wheat, maize, barley, sugar beet, and tomato. Their rapid and good acceptance was due to the high efficacy at low application rates (10-15 g/ha) and very low acute and chronic mammalian toxicities. Azimsulfuron affects sensitive weeds through inhibition of the enzyme acetolactate synthase (ALS). Inhibition of ALS leads to the cessation of cell division and subsequent growth processes in plants. Azimsulfuron is taken up mainly by leaves and shoots and, to a lesser extent roots. Once taken up, it is translocated via both xylem and phloem.

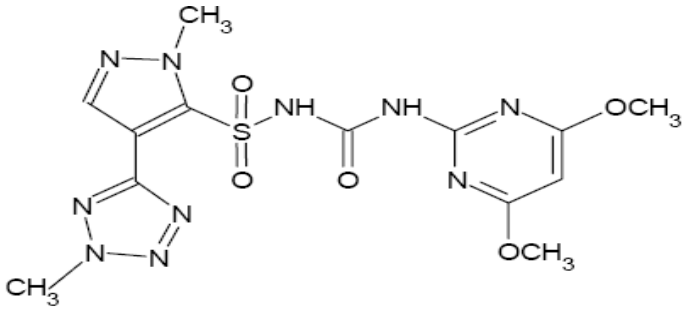

Figure 1. Structure of azimsulfuron.

In general matrix solid phase dispersion (MSPD) involves the blending of chemically modified solid supports with the milk sample in a mortar and pestle. The MSPD process incorporates the classical methods of the use of abrasives to disrupt sample architecture and the use of a solvent or detergent to disrupt cellular membranes and components, with the solvent now being bound to the abrasive solid support (Hicks and Watrous, 1999, Garcia-Lopez et al., 2008, Degelmann et al., 2004 and Barker et al., 1989). In this manner the milk can be completely disrupted and distributed over the surface of the solid support so as to maximize the interactions of the solid support and bonded liquid phase chemistry and interactions with the individual cellular components and their subsequent interactions with one another. The contents are transferred to a syringe – barrel column and the compounds of interest are eluted, in many cases, for direct analysis. This technique has been found to be useful for the extraction of a variety of organic compounds in a wide variety of solid sample matrices. In some cases co-columns of material, such as alumina, silica, florisil or additional C18 or some other absorption or solid-phase extraction (SPE) supports have been placed at the bottom of the syringe-barrel column prior to addition of the MSPD blend or have been post elution as a second column, in order to further assist the extraction process and remove co-eluting interferences. The collected elution solvent(s) may then be processed for appropriate analysis. The steps involved in general matrix solid phase dispersion extraction has been explained in **Figure 2**.

Azimsulfruon is a very polar compound (log Poct = -2.08) and has high solubility in water (72.3 g/L). These physicochemical properties make the use of classical organic solvents extractions very difficult and require cleanup procedure with suitable solid adsorbent (Barker, 2007).

For this reason, the determination of azimsulfuron in biological matrixes is a challenging task. Various methods have been described in literature for the determination of azimsulfuron in surface water, aqueous solutions, soil, grains, seeds, vegetables and fruits. In this present study MSPD has been successfully used to extract sulfonylurea herbicide azimsulfuron from biological samples considerably reducing analytical time, the sample size and solvent consumption (Ishimitsu et al., 2002 and Akiyama et al., 2002). However, the use of a MSPD for extraction of azimsulfuron residues form buffallow milk has not been reported. This work is focused on the development and evaluation of a simple sample preparation strategy based on MSPD coupled with HPLC-UV determination.

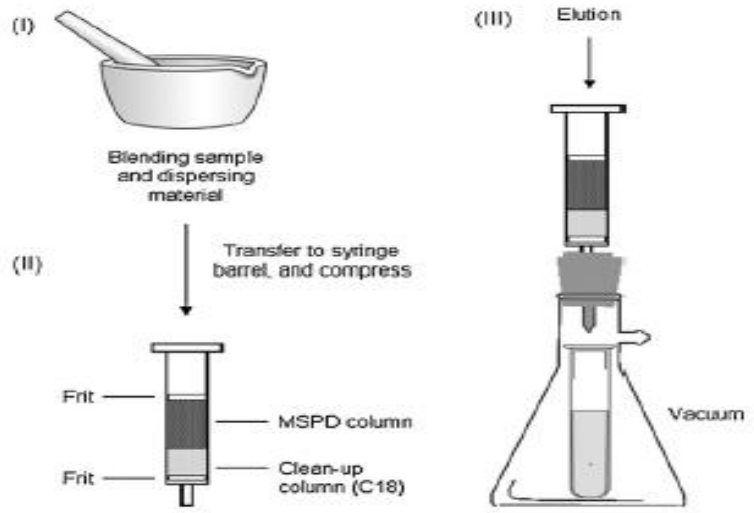

Figure 2. Matrix solid-phase dispersion procedure. Main steps of the matrix solid-phase dispersion extraction procedure: (I) The sample is blended with the dispersant material in a mortar with a pestle; (II) The homogenized powder is transferred in a solid-phase extraction cartridge, and compressed; (III) Elution with a suitable solvent or solvent mixture is performed by the aid of vacuum pump.

MATERIAL AND METHODS

Azimsulfuron analytical reference standard (Purity 99.6%) obtained from Sigma Aldrich, India. Acetonitrile and hexane were HPLC grade (Merck). Ethyl acetate, methylene chloride, potassium phosphate monobasic, glacial acetic acid, phosphoric acid and ammonium hydroxide were AR grade (Merck). Milli-Q water was obtained from milli-Q systems India. SPME C18 (Derivatized silica packing, 40µm particle size, 60 Aº pore size) obtained from J. T. Baker, Phillipsburg, NJ and silica SPE cartridges (SI Mega Bond-Elute®, 1g/6mL) from varian associates, CA.

HPLC-PDA separation parameters

A HPLC shimadzu prominence system (Japan) consisting of LC-20 AT pump, CT0-20A column oven, SIL-20A auto sampler and photo diode array (PDA) detector was used for this experiment. The auto sampler was set at 10º C. the absorbance was measured at 220 nm. An agilent SB-phenyl column (150mm length x 4.6mm id x 5µm) was used as the analysis column.

The column was maintained at a temperature 40ºC.The chromatographic data were collected by LC-Solutions software. The mobile phase consisted of acetonitrile and 0.1% Ortho phosphoric acid 40:60, v/v. the flow rate was 1 mL/min.

LC-MS/MS separation parameters

A mass spectrum was recorded on an Agilent 6490 triple quadruple mass spectrometer equipped with an ESI source. System control and data acquisition were controlled by Agilent mass hunter software. Aliquots of 10 µL were injected to the LC-MS/MS system using Agilent SB-phenyl column (5µm particle size, 4.6 mm i.d. ,150 mm length) with flow rate of 1.0 1 mL/min having acetonitrile as mobile phase A (40%) and 0.1% Ortho phosphoric acid in HPLC grade water (60%) as mobile phase B were used. The nebulizer gas (nitrogen) flow was fixed to 10 L/min. MS/MS mode operation was done with helium as collision gas, with a pressure of 4 x 10-4 milli bar. A capillary voltage of 4.5 kV was used in positive ionization mode. The interface temperature was set at 360 °C. The scan range was 50 - 450 m/Z.

Preparation of sample extracts

Analyte extraction procedure

A 2 g of sample was transferred into a glass mortar and a suitable amount of intermediate standard solution prepared in acetonitrile was added to the sample. Air dried the samples for about 15 minutes to allow the acetonitrile to evaporate from the milk samples before proceeding. 6g of washed C18 packing material (dispersion adsorbent) was mixed with the sample. Allowed the sample/packing mixture to air dry for one hour. Transferred the packing mixture to a 75 mL reservoir containing a frit at the bottom. Connected the reservoir to a solid-phase extraction vacuum manifold and applied vacuum to draw air through the sample mixture for about 30 minutes. Added 30 mL of hexane to the reservoir and drawn using light vacuum through the packed bed at a flow rate of 10-15 mL/min. Allowed the packed bed to go dry after all the hexane has passed through. Added 30 mL of C18 Eluting solution (ethyl acetate acidified with 1.0% glacial acetic acid) to the packed reservoir and drawn through the packed bed at a flow rate of 10-15 mL/min. collected the elute in a 50 mL tube and evaporated the sample to 5-10 mL under a gentle stream of nitrogen, with the water bath temperature set at 40-45 º C.

Analyte purification procedure

The above elute transferred to a 15 mL centrifuge tube and evaporated to dryness under a gentle stream of nitrogen, again with a 40-45 º C water bath. Added 5 mL ethyl acetate to the sample and sonicated for about 5 minutes, then mixed well on a vortex mixer. 5 mL of hexane was added and mixed well. Connected a 1g silica mega bond elute® to the solid-phase extraction vacuum manifold. Preconditioned the column by passing 10 mL of acetonitrile through the cartridge at a flow rate of 5 to 15 mL/min, followed by 15 mL of SPE wash solution(50% hexane/50% ethyl acetate, v/v).Transferred the sample to the preconditioned silica Mega Bond Elute® , applied light vacuum and allowed the sample to pass through at a flow rate of 1-5 mL/min. Rinsed the centrifuge tube with 5 mL of SPE wash solution and passed through Bond Elute® at a flow rate of 1- 5 mL/min and allowed the cartridge packing to dry only after all of the SPE wash solution has gone through the Bond Elute®. Eluted the sample with 5 mL of SPE eluting solution under light vacuum as before at a flow rate of 5-10 mL/min. Collected the elute in a 15 mL graduated centrifuge tube. Evaporated to dryness under a gentle stream of nitrogen at 40-45 º C. finally the sample was reconstituted with 2 mL of acetonitrile and 0.1% ortho phosphoric acid (40:60, v/v). All the samples were subjected to HPLC-UV analysis.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered (Nageswara Rao et al., 2014). The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 µg/mL. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

Calculations

The concentration of acetaminophen in the samples analyzed by HPLC was determined directly from the standard curve.

$Y = mx + c$

Where,

Y = peak area of standard (mAU*sec)

m = the slope of the line from the calibration curve

x = concentration of injected sample (mg/L)

c = 'y' intercept of the calibration curve

The recovered concentration or Dose concentration was calculated by using the formula:

$$\text{Recovered concentration or Dose concentration} = \frac{(x-c)\ X\ D\ X\ 100}{m\ X\ P}$$

Where,

m = the slope of the line from the calibration curve

x = sample area of injected sample (µV*sec)

c = 'y' intercept of the calibration curve

D = Dilution Factor

P = Purity of Test item

$$\%\ \text{Recovery} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

RESULTS AND DISCUSSION

Specificity

Aliquots of azimsulfruon, control sample solution, extracted solvents and mobile phase solvents were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in **Figure 3, 4 and 5**. Furthermore, the retention time of azimsulfruon was 7.2 min (Approximately) and the mass fragment selected for evaluation was m/z of base peak 181.5 and parent peak of m/z 425. Showed in **Figure 6**.

Linearity

10.04 mg of azimsulfuron reference standard was taken into 10 mL volumetric flask and dissolved in acetonitrile, sonicated and made up to the mark with the same solvent.

The concentration of the stock solution was 1000 µg/mL. From this stock solution prepared by different known concentrations of standard solutions (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared into a different 10 mL volumetric flasks and made upto the mark with acetonitrile. The serial dilution details were presented in **Table 1.** These standard solutions were directly injected into a HPLC. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of standards were used to calculate linear regression equations. These were Y=6495.87X + 29.28 with correlation coefficient of 0.9999 azimsulfuron respectively. A calibration curve showed in **Figure 7**.

Table 1. Serial dilutions of linearity standard solutions.

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
1000	1.000	10	100
100	0.500	10	5
100	0.200	10	2
100	0.100	10	1
10	0.5	10	0.5
10	0.1	10	0.1
1	0.5	10	0.05

Table 2. Recoveries of the azimsulfuron from fortified sugarcane juice control sample (n=6).

Fortification Concentration in µg/mL	Replication	Recovery (%)
0.05	R1	86
	R2	84
	R3	86
	R4	85
	R5	87
	R6	84
	Mean	**85.33**
	STDEV	**1.21**
	RSD in %	**1.42**
0.5	R1	90
	R2	92
	R3	90
	R4	89
	R5	92
	R6	93
	Mean	**91.00**
	STDEV	**1.55**
	RSD in %	**1.70**

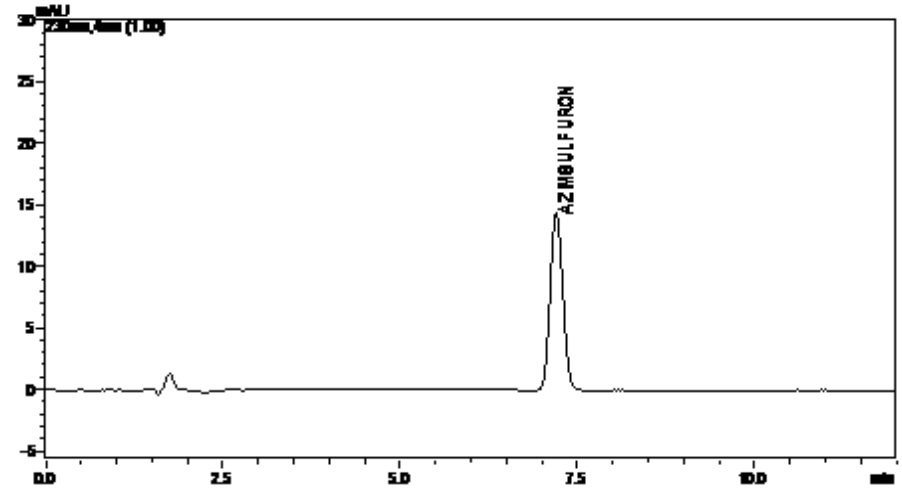

Figure 3. A representative chromatogram obtained from azimsulfuron standard.

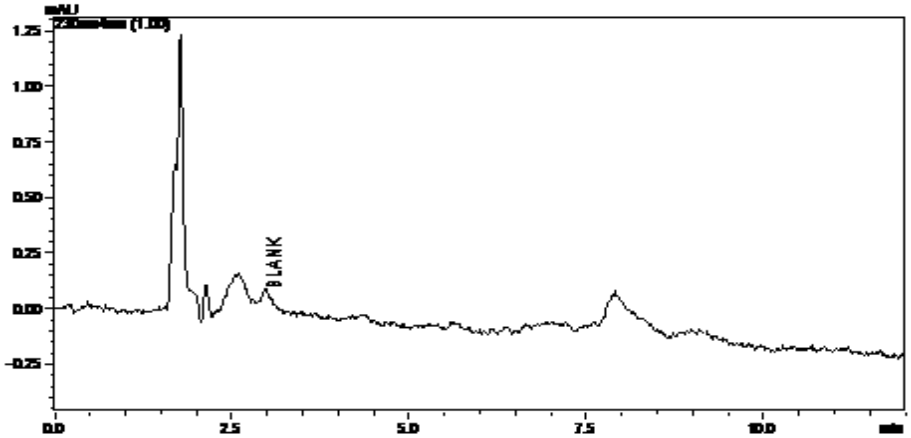

Figure 4. A representative chromatogram obtained from blank milk sample.

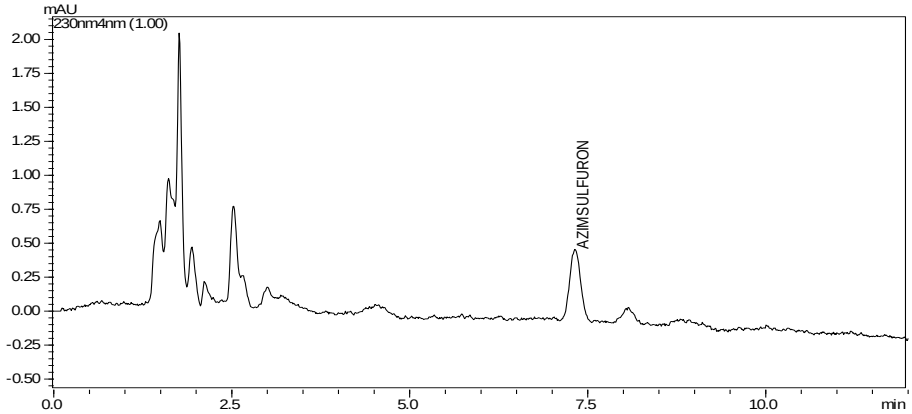

Figure 5. A representative chromatogram obtained from milk sample spiked at 0.5 µg g^{-1}.

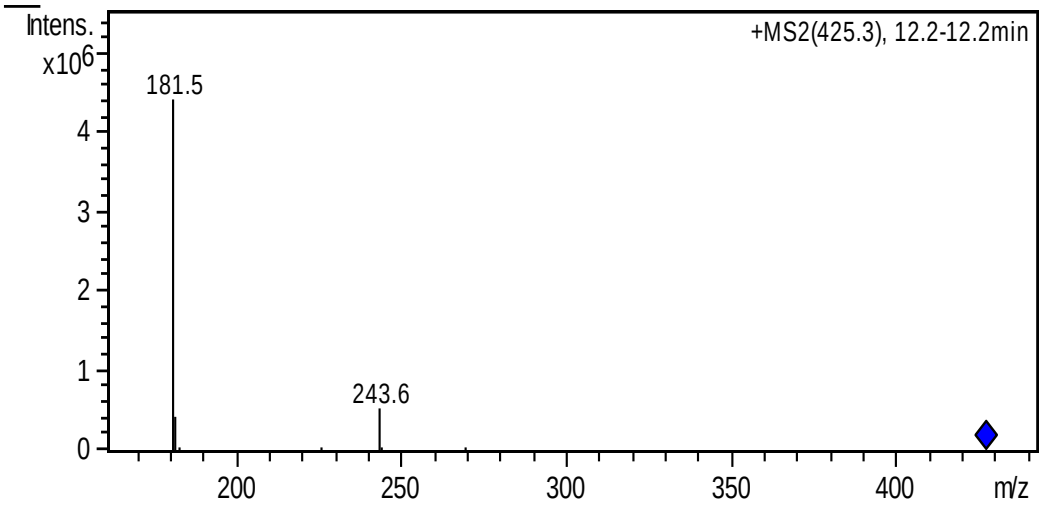

Figure 6. LC-ESI-MS2 Spectra of azimsulfuron.

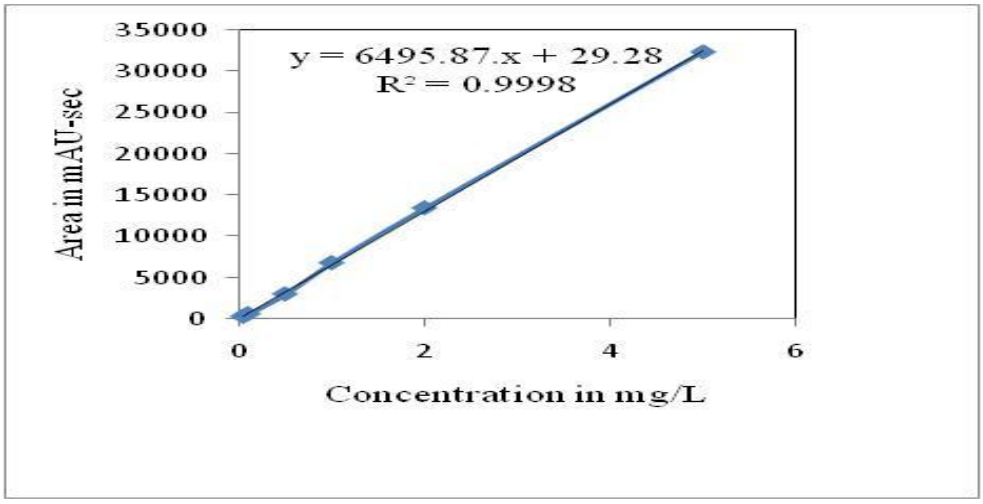

Figure7. Representative Calibration curve of azimsulfuron.

Detection and quantification limits

The limit of quantification of azimsulfuron was determined to be 0.05 mg/kg. This quantification limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (84-86%) were achieved. This quantification limit also reflects the fortification level at which an analyte peak is consistently generated at a level approximately 10 times the baseline noise in the chromatogram. The limit of detection is considered to be a level of approximately three times of the peak of interest. It must be recognized that the limit of detection will vary between matrices and from day to day.

Accuracy and precision

The recovery was studied at two levels (0.05µg g-1 and 0.5 µg g-1) using the spiked samples. The method validation of spiked samples indicted that the present method provides good recoveries and reasonable precision for sulfonylurea herbicide azimsulfuron of two levels as can be seen from **Table 2**. The results of mean recoveries were in the range between 85-91 % with the RSD between 1.21% and 1.70%.

MSPD extraction

MSPD conditions were carefully selected to yield extracts with the highest recovery for the pesticide and the lowest amount of matrix compounds. C18- bonded silica was chosen as the dispersion sorbent because its non-polar character provides the best affinity, and because it Causes the complete disruption and distribution of the lipophilic entities commonly existing in animal tissues such as liver or muscle. [18-20]

Typically, a sample to sorbent ratio of 1:3 was used in this study. Preliminary assays for the optimization of the extraction method were made with 100 mg of sample and 400 mg of C18.Optimisation of the final protocol was performed with 2 g of sample to enhance sensitivity and 6g of C18. Elution solvents were studied in order to obtain perfect recoveries. Finally optimization of the elution sequence was performed using ethyl acetate acidified with 1.0% glacial acetic acid.

CONCLUSIONS

The proposed MSPD method can be readily applied to the extraction of azimsulfuron in 2g of milk. This analytical method is suitable for the quantification of azimsulfruon in milk at levels down to 0.05 mg/kg. Average recoveries at the limit of quantification ranged from 85 to 91 % with standard deviations in the range of 1.21 to 1.70. Good repeatability was demonstrated for milk matrices. The method was demonstrated to have no interferences from the matrix.

Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO guidelines). Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the azimsulfruon residues in different commodities (Food, oil, fruit, and water and soil samples.

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REFERENCES

Anna Laura Capriotti, Chiara Cavaliere, Piero Giansanti, Riccardo Gubbiotti, Roberto Samperi and Aldo Lagana 2010. Recent developments in matrix solid phase dispersion extraction. *J. Chromtogr. A*, 1217: 2521-2532.

Hickes H. and M. Watrous 1999. Multiresidue method for determination of sulfonylurea herbicides in water by liquid chromatography with confirmation by capillary electrophoresis. *J. AOAC Int.,* 82:1523-1533.

Garcia-Lopez, M., P. Canosa and I. Rodriguez. 2008. Trends and recent applications of matrix solid phase dispersion. *Annal. Bioanal. Chem.,* 391: 963-974.

Degelmann, P., J. Wenger, R. Niessner and D. Knopp 2004. Development of a class-specific ELISA for sulfonylurea herbicides (Sulfuron Screen). *Environ. Sci. Technol.*, 38: 6795-67802.

Barker, S. A., Long, A. R. and C. R. Short 1989. Isolation of drug residues from tissues by solid phase dispersion. *J. Chromatogr.,* 475: 353-361.

Barker, S. A. 2007. Matrix solid phase dispersion (MSPD). *Biophys. Methods, J. Biochem.* 70: 151-162.

SANCO Guidelines 2009. Method validation and quality control procedures for pesticides residues analysis in food and feed. *Document NO.SANCO/10684/2009*.

Ishimitsu, S., A. Kaihara, K. Yoshii, Y. Tsumura, Y. Nakamura and Y. Tonogai 2002. Simultaneous Determination of azimsulfuron, flazasulfuron and halosulfuron- methyl in grains, seeds, Vegetables and fruits by HPLC. *J. Health Sci.,* 48: 335-340.

Tentu, Nageswara Rao, D. Sreenivasulu, E. G. Sreenivasula Reddy and Kappari, Sharadha Devi. 2014. A novel method for determination of tetracycline and its metabolite residues in cow milk. *Research Journal of Pharmacy and Technology,* 7: 513-516.

Akiyama, Y., N. Yoshioka and M. Tsuji 2002. Determination of ten sulfonyl urea herbicides in unpolished rice by solid phase extraction cleanup and LC-Diode array detection. *Shokuhin Eiseigaku Zasshi,* 43 :99-103.

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