International Journal of Research in Chemistry and Environment
Vol. 2 Issue 3 July 2012(100-105)
ISSN 2248-9649

Research Paper

Matrix Solid Phase Dispersion Extraction and High-Performance Liquid Chromatography Determination of Bispyribac-Sodium in Goat Tissue Samples

***Rao Nageswara T., Attamkuru Ramesh and Parvathamma T.**
Department of Analytical Chemistry, International Institute of Bio-technology and Toxicology (IIBAT), Padappai, Chennai 601 301, Tamil Nadu, INDIA

Available online at: www.ijrce.org

(Received 29th April 2012, Accepted 20th June 2012)

Abstract: *A highly selective matrix solid phase dispersion (MSPD) extraction purification method for the preconcentration of bispyribac-sodium in goat tissues (liver, kidney, muscle and fat) was developed. A C18 sorbent based MSPD column was used for extraction of the analyte and extracted the bispyribac-sodium 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 –elut ® cartridges prior to quantification by HPLC on an Agilent Zorbax column (150mm length x 4.6mm id x 3.5µm). The HPLC detection was studied with UV detector at 246 nm. Acetonitrile and water (0.1% formic acid) 55:45 v/v was used as mobile phase at a flow rate of 0.7 ml min^{-1}. The linearity of bispyribac-sodium was observed over the concentration range 0.01 to 2 µg/mL and the regression coefficient (R^2) 0.9997. The mean recoveries of bispyribac-sodium form tissues at 0.03 and 0.3 µg g^{-1} fortification levels were in the range of 86-97%.The limit of quantification and detection was established as 0.03 and 0.01 µg g^{-1} respectively. The proposed method was successfully applied for the determination of residues in goat liver, kidney, and muscle and fat samples.*

Keywords: bispyribac-sodium, MSPD, HPLC, UV, Goat Tissue.

Introduction

Bispyribac-sodium, sodium 2,6-bis [(4,6-dimethoxy-2-pyrimidinyl) oxy] benzoate (Figure 1). Which was first developed by Japan Kumiai Chemical, belongs to the pyrimidinyl oxybenzoic acid group. 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 (40-50 g/ha) and very low acute and chronic mammalian toxicities. Bispyribac-sodium 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. Bispyribac-sodium 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.

In general matrix solid phase dispersion (MSPD) involves the blending of chemically modified solid supports with the tissue 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.

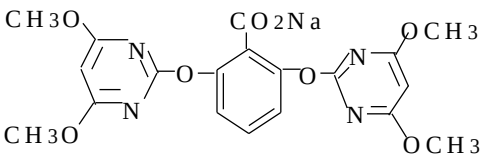

Figure 1: Structure of Bispyribac-sodium

In this manner the tissue 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 have been explained in Figure 2.

In this present study MSPD has been successfully used to extract Bispyribac-sodium from biological samples considerably reducing analytical time, the sample size and solvent consumption. However, the use of a MSPD for extraction of bispyribac-sodium residues form goat tissues, such as liver, kidney, muscle and fat, 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.

Material and Methods

Chemicals: Bispyribac-sodium analytical reference standard (Purity 99.5%) obtained from Sigma Aldrich. 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-Elut®, 1g/6mL) from varian associates, CA.

Instrumentation and Chromatography: A HPLC shimadzu prominence system (Japan) consisting of LC-20 AT pump, CT0-20A column oven, SIL-20A auto sampler and UV detector was used for this experiment. The auto sampler was set at 10º C. the absorbance was measured at 246 nm. An Agilent Zorbax column (150mm length x 4.6mm id x 3.5µm) 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 water (0.1% formic acid) 55:45, v/v. the flow rate was 0.7 ml/min.

Preparation of sample extract

Analyte extraction procedure: The harvested tissue sample (liver, kidney, muscle and fat) was cut in to pieces and homogenized using a high speed blender. 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 tissue 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 eluate 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.

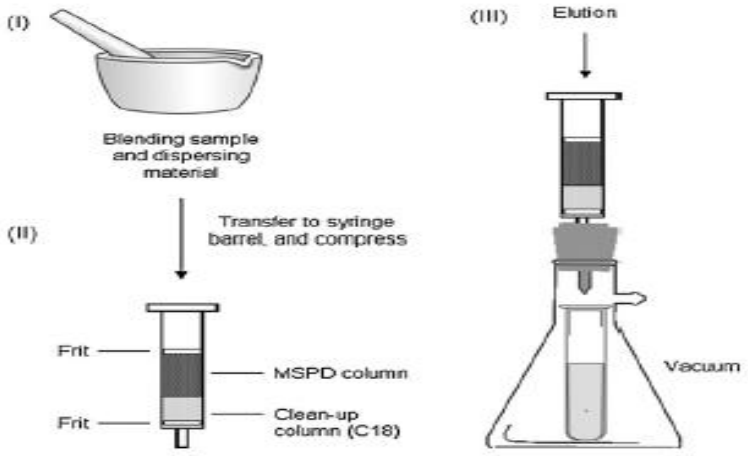

Figure 2: Matrix solid-phase dispersion procedure. Main steps of the matrix solid-phase dispersion extraction procedure: (I) the sample is blended with the dispersant material in a mortar with a pestle, (II) The homogenized powder is transferred in a solid-phase extraction cartridge, and compressed,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

Analyte purification procedure: The above eluate 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 elut® 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 Elut® , 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 Elut® 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 Elut®. 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 eluate in a 15 mL graduated centrifuge tube. Evaporated to dryness under a gentle stream of nitrogen at 40-45 ºC. finally the sample was reconstituted with 2 mL of acetonitrile and water pH 2.5 mixture (40:60, v/v). All the samples were subjected to HPLC-UV analysis.

Accuracy and precision studies: Accuracy and precision studies were carried out on goat tissue samples (2 g) spiked with suitable volume of the working standard solutions and left to stand at room temperature for 15 minutes. Samples were spiked with bispyribac-sodium standard at 0.03 and 0.3 µg g-1. Three replicate samples were analyzed for each concentration level to evaluate the relative standard deviation (RSD).

Results and Discussion

HPLC performances

Linearity and repeatability

The calibration curve (Figure 3) was established by analyzing standard solutions of seven different concentrations in the range of 0.01-2 µg/mL by HPLC-UV analysis. The relationships between the analyte concentration (X) and peak area of measured signal (Y) are noted as regression equation Y= 100.9 + 31541X (R^2 = 0.9997). The standard solutions were used to determine the intra-day (three replicates at each concentration, 1 day) and inter-day (three replicates at each concentration, 3days) repeatability by assaying liver samples under the selected optimal conditions. The results of intra-day and inter-day repeatability (for three levels) are shown in Table 1. Relative standard deviations (RSD) were between 1.52% and 3.22% for the intra-day and between 3.12% and 5.69% for inter-day analysis.

Table 1
Intra-day and inter-day repeatability of the assay

Concentration injected (µg mL^{-1})	Intra-day repeatability RSD (n=3) %	Inter-day repeatability RSD (n=9) %
0.1	3.22	5.69
1	2.67	3.47
10	1.52	3.12

Detection and quantification limits

The limit of quantification of bispyribac-sodium was determined to be 0.03 mg/kg. This quantification limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (86-97%) 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.

Table 2
Percentage recoveries of bispyribac-sodium in, liver, muscle, kidney and fat samples with two different spiked levels (n=3)

Sample	Spiked level (µg /kg)	Bispyribac-sodium	
		Recovery (%)	RSD (%)
Liver	0.3	90.5	2.98
	0.03	87.6	3.85
Muscle	0.3	91.3	3.98
	0.03	88.1	4.57
Kidney	0.3	89.6	3.54
	0.03	86.8	5.02
Fat	0.3	88.0	4.29
	0.03	86.6	6.02

Accuracy and precision

The recovery was studied at two levels (0.03µg g-1 and 0.3 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 bispyribac-sodium of two levels as can be seen from Table 2. The results of mean recoveries were in the range between 86-97 % with the RSD between 2.51% and 6.02%.

The HPLC-UV analysis using the optimal extraction and detection conditions described shows the target analyte in the tissue samples were free from the interference Representative chromatogram of bispyribac sodium obtained from standard, blank goat Tissue sample and higher fortification in goat Tissue were given in (Figure 3-5).

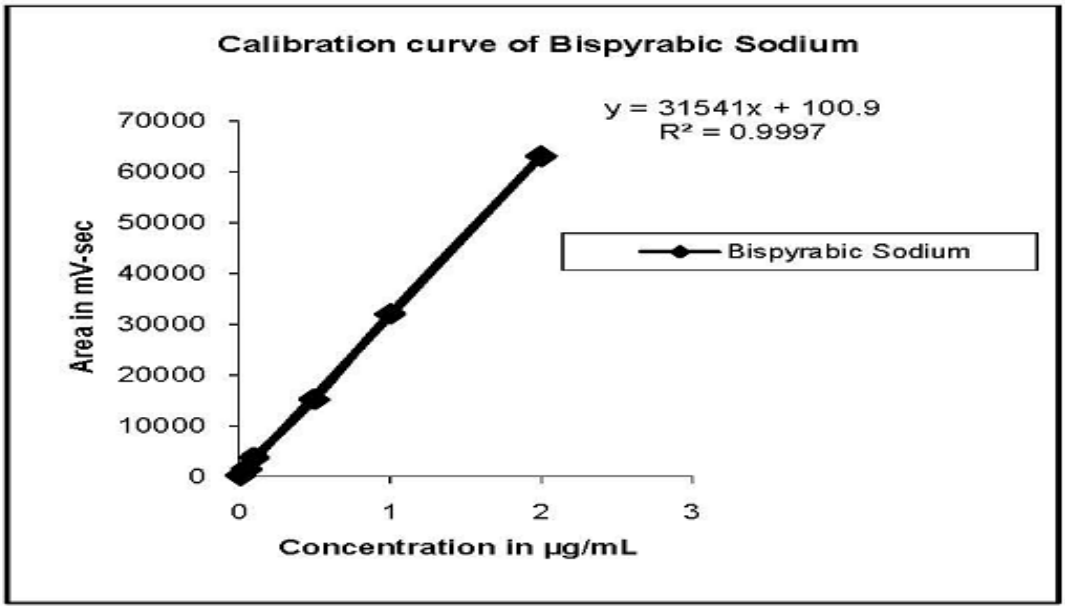

Figure 3: Representative calibration curve of bispyrabic sodium

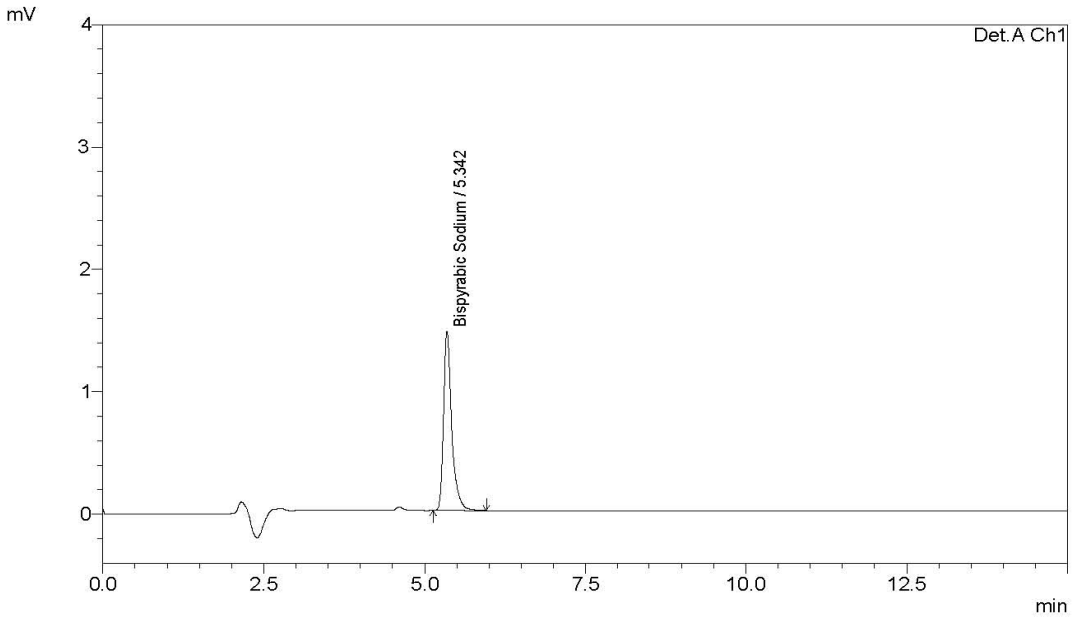

Figure 4: A representative chromatogram obtained from bispyribac-sodium standard

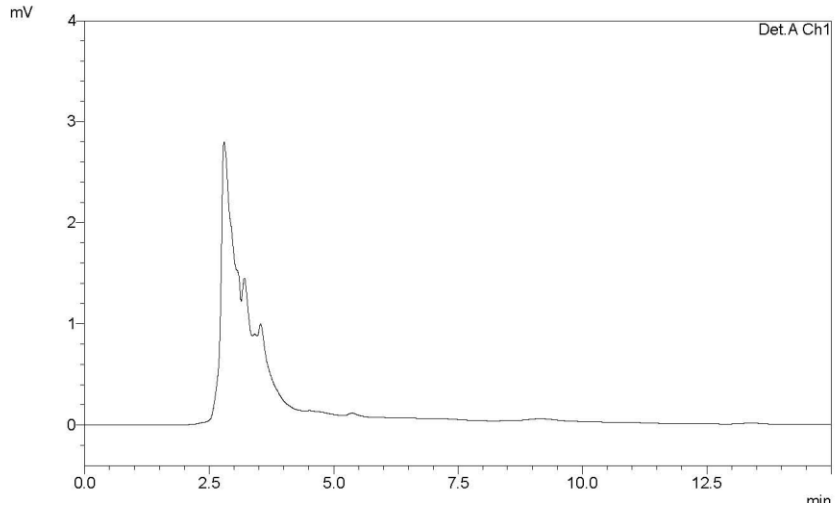

Figure 5: A representative chromatogram obtained from blank goat Tissue sample

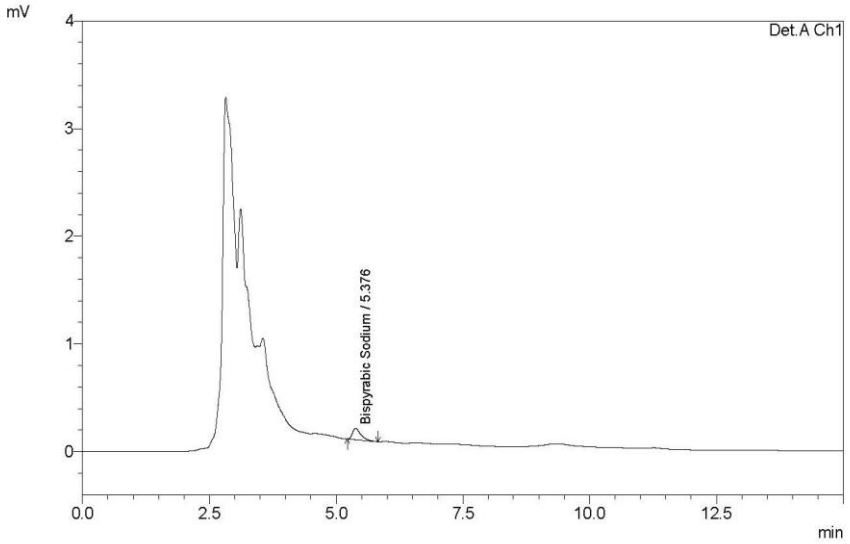

Figure 6: A representative chromatogram obtained from goat Tissue sample spiked at 0.3 µg g^{-1}

MSPD extraction

The MSPD extraction procedure of the described method is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the matrix. Whole animal tissues extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method.

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.

Conclusion

The proposed MSPD method can be readily applied to the extraction of bispyribac-sodium in 2g of tissue sample (liver, kidney, muscle and fat). This analytical method is suitable for the quantification of bispyribac sodium in tissues (liver, kidney, muscle and fat) at levels down to 0.03 mg/kg (ppm).Average recoveries at the limit of quantification ranged from 86 to 97 % with standard deviations in the range of 2.31 to 5.79. Good repeatability was demonstrated for all matrices. The method was demonstrated to have no interferences from the matrix.

Acknowledgement

The authors are thankful to the Management, and Dr. P. Balakrishnamurthy, Director, IIBAT, for providing necessary facility to conduct the experiment.

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