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Analysis of the Herbicide Bispyribac-sodium in Rice by Solid Phase Extraction and High Performance Liquid Chromatography

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Abstract Bispyribac-sodium is a commonly used herbicide. An analytical method employing HPLC with a diode array detector was developed to determine bispyribac-sodium residues in rice. The liquid–liquid partition and anion exchange solid phase procedures that were developed provide effective extraction and cleanup methods for analysis feasibility. Recoveries ranged from 83.98 to 98.51% with a relative standard deviation of 0.56–6.36% and sensitivity of 0.01 mg/kg. Bispyribac-sodium residues in rice were further confirmed by LC–MS. The proposed method was successfully applied to the analysis of bispyribac-sodium residues from a rice field in Jiangxi Province that had been treated using bispyribac-sodium.

Keywords Bispyribac-sodium · Rice · Solid phase extraction · HPLC–DAD

Agriculture, especially grain planting, plays a very important role in China where the 2008 rice production was 192 million tons. However, grain planting is labor intensive and requires weed control. The use of bispyribac-sodium could assist farmers in handling the latter problem.

Bispyribac-sodium, sodium 2,6-bis [(4, 6- dimethoxy-2-pyrimidinyl) oxy] benzoate (Fig. 1), which was first developed by Japan Kumiai Chemical, belongs to the pyrimidinyl oxybenzoic acid group. In rice planting, bispyribac-sodium is absorbed through the leaf surface and translocated throughout the plant to inhibit the activity of acetolactate synthase, which results in death of the weeds (Ding et al. 2009). Bispyribac-sodium is used as a post-emergence contacting herbicide to control *E. phyllopogon*, *C. diffformis* and some dicotyledons in rice fields (Fischer et al. 2000). It can be used alone or together with bensulfuron-methyl, quinclorac and others (Osuna et al. 2002). Bispyribac-sodium has a broad herbicide spectrum and low dosage (with a recommended application rate of 40–50 g active ingredient ha^{-1}) (Braverman and Jordan 1996). It is a faintly acidic compound, with a pKa of 3.05, and two forms of different molecular weight (452 and 430 g mol^{-1}). Bispyribac-sodium is highly soluble in water (73.3 g/L, 25°C) and methanol (26.3 g/L, 25°C) and slightly soluble in acetone (United States of America (USA) Environmental Protection Agency (EPA) 2001).

Bispyribac-sodium presents a low risk to wild mammals, birds, earthworms, bees, aquatic invertebrates and fish. The toxicology reports for bispyribac-sodium have not yet been reviewed by the EPA and toxic endpoints have not yet been established, specifically chronic and acute oral toxicity endpoints. Many countries or regions have developed a series of standards for the maximum residue limits (MARLs) of bispyribac-sodium. Japan demands that this be $\leq$0.1 mg/kg in rice. The USA demands a level $\leq$0.02 mg/kg in rice and processed products. The EU demands a level $\leq$0.01 mg/kg in agricultural products except rice which is at a level $\leq$0.02 mg/kg.

Bispyribac-sodium cannot be analyzed in rice matrices without appropriate sample preparation. Liquid–liquid extraction has been employed for this purpose, but results in the inclusion of too many impurities to satisfy testing requirements. Various cleanup techniques have been developed to overcome these shortcomings (Rodrigues et al. 2007). Solid phase extraction (SPE) is the most commonly used. In SPE, the choice of appropriate sorbent

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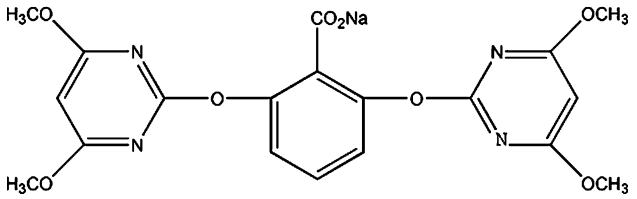

Fig. 1 Structure of bispyribac-sodium

is critical to obtain full recovery and high enrichment (Hadjmohammadi et al. 2010).

Some researchers have done much work to seek a simple and rapid method to determine the bispyribac-sodium residue in agricultural products. However, results have not been feasible, with problems experienced including low extraction ratios and cleanup conditions (Kurz et al. 2009; Niell et al. 2010). This paper presents a simple, rapid and sensitive method for the quantification of bispyribac-sodium residues in rice based on liquid–liquid partition combined with SPE and the subsequent determination by high performance liquid chromatography utilizing a diode array detector (HPLC–DAD).

Materials and Methods

Bispyribac-sodium standards (99.9% minimum purity) were obtained from Fluka (Germany), Dikma (USA) and its commercial formulation (30% aqueous solution) was provided by Xiwang Jintan (China). The stock solution was prepared in an acetonitrile/water mixture (65:35 v/v) and kept at 4°C in a refrigerator. All working solutions were prepared daily by diluting this solution and the stock solution was renewed each week. HPLC grade acetonitrile, methanol and formic acid were supplied by Tedia (USA). HPLC grade water was produced using a Milli-Q water purification system (Millipore Co. Milford, USA). All other reagents were analytical grade and were purchased from Sinopharm Chemical Reagent (China).

A high performance liquid chromatograph (Waters 2690) equipped with a C_{18} reverse phase chromatographic column (250 × 4.6 mm I.D., particle size 5 μm, Dikma, China) was attached to a DAD. The HPLC determination of analytes was performed using a mobile phase consisting of acetonitrile/water (65:35 v/v) with a flow rate of 1.0 mL/min at 30°C. All mobile phases were passed through a 0.45 μm membrane filter and were degassed under vacuum. The sample injection volume was 20 μl and analytes were monitored at 248 nm. Total analysis time was 20 min. The LC–MS (Agilent 1100 series) was applied under the following conditions: Mass Range Mode, Std/Normal; Ion Source Type, ESI; Ion Polarity, Positive; Dry temperature (set), 325°C; Scan Range, 100–1,000 m/z. The analytical column was a C_{18} column (150 × 4.6 mm I.D. particle size 5 μm, Agilent, USA) and other chromatographic conditions were the same as used in HPLC analysis. The SPE cartridges, ProElut PXA, 60 mg/3 mL (Dikma, China), Oasis MAX 6 cc (Waters, USA), and Supelco LC-Florisil (USA) were evaluated.

In this work, five pieces of each type of shelled rice were purchased randomly from Jing'an County, Jiangxi Province; Xiaogan County, Hubei Province and Yongfeng Country, Jiangxi Province. The samples were stored in a dry, cool place. All were crushed and sieved through a 420 μm mesh sieve. The solvent extraction method was applied as described in Fig. 2.

ProElut PXA and Oasis cartridges were conditioned using 3 mLmethanol and 3 mL ultra-pure water. For the Supelco LC-Florisil cartridge, conditioning was carried out with 3 mL acetonitrile and 3 mL ultra-pure water before an appropriate aqueous solution sample volume was passed through each cartridge at the optimized flow rate by a vacuum pump. The ProElut PXA and Oasis cartridges were then washed with 3 mL 0.05 M sodium acetate solution to remove the co-adsorbed matrix materials from the cartridges. Subsequently, bispyribac-sodium retained on the cartridges was eluted with 3 mL 2% formic acid methanol solution. For the Supelco LC-Florisil cartridge, 3 mL 0.1 M acetic acid was used to remove the unadsorbed sample and 3 mL iso-pentane to elute the bispyribac-sodium. The eluate was blown to near dryness using a rotary vacuum evaporator. Finally, the residue was reconstituted in 1 mL of mobile phase and analyzed by HPLC–DAD.

Studies were carried out to validate the method, sensitivity, repeatability, reproducibility and recovery. Fortified samples were used for precision and accuracy studies. Similarly, purified extracts were used for the sensitivity study to prevent matrix effects. For the sensitivity study, the absolute detection limit, detection limit and quantification limit were determined. For the repeatability study, five samples were analyzed by the same operator, with the same equipment, in the same laboratory on the same day. Conditions for the reproducibility study were: analysis of three samples by the same operator, the same equipment, in the same laboratory, on three different days within 2 weeks. Finally, for the recovery study, ten samples were analyzed. Studies of repeatability, reproducibility and recovery were conducted by spiking extracts at three levels. All analyses were performed in duplicate.

Results and Discussion

The calibration curve of each compound was established using nine concentration levels (10, 4, 2, 1, 0.5, 0.25, 0.12,

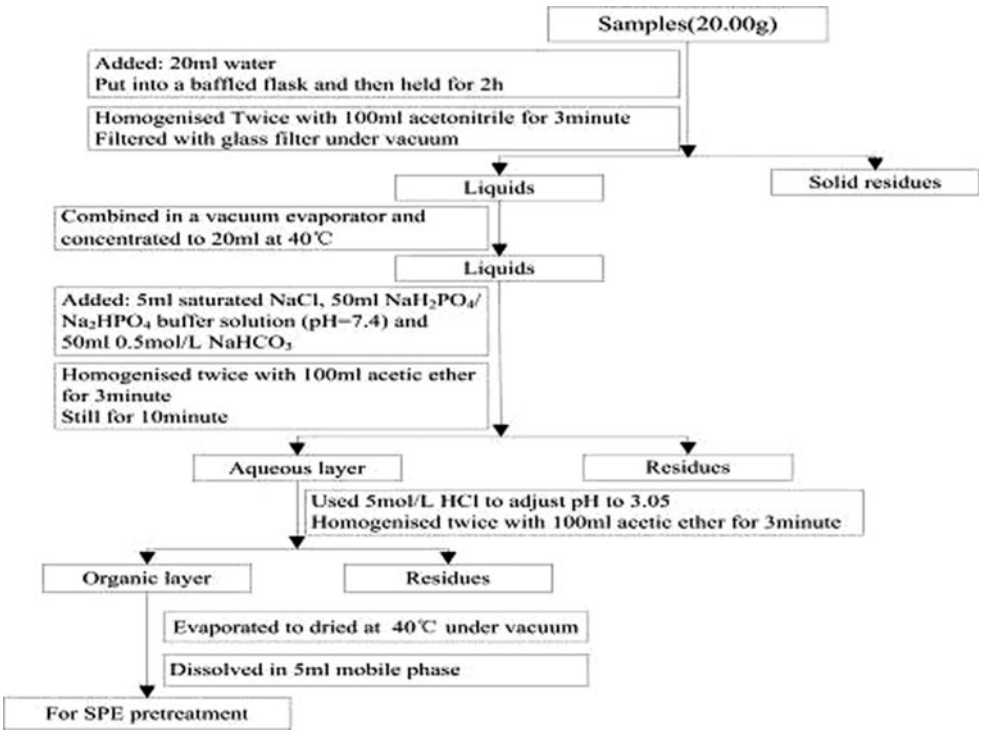

Fig. 2 The solvent extraction scheme of bispyribac-sodium

0.06, 0.03 mg/kg) in triplicate with all calibration curves showing excellent linear regression ($y = 43.36x + 0.468$, where y is the peak area, x is the bispyribac-sodium concentration, $R^2 = 0.9999$, at least) within the plotted range.

Table 1 shows the average recoveries of bispyribac-sodium from different rice samples. The rice samples were fortified at 0.10, 0.50 and 1.00 mg/kg before extraction by adding an appropriate volume of standard solution. The recoveries and relative standard deviation (RSD) obtained were in the acceptable range of 83.98–98.51% and 0.56–6.36%, respectively. Figure 3 shows the chromatograms of the bispyribac-sodium standard and spiked samples with different spiked levels. The limit of detection and limit of quantification (LOQ) were defined at a signal-to-noise ratio of 3 and 10 under the stated chromatographic conditions. The LOQ of bispyribac-sodium was found to be 0.01 mg/kg, which meets the MARLs in most countries and regions.

Bispyribac-sodium is a faintly acid compound and is expected to exist in an ionic state over a wide pH range. As such, a complex set of equilibria can govern its physiochemical behavior and affect matrix extraction efficiencies and chromatographic separations. In this work, the pesticide was extracted from the samples with ethyl acetate owing to the iso-electric point nature of the bispyribac-sodium, extracted by organic-aqueous liquid–liquid partition at a pH of 3.05 and combined and purified with SPE columns. The liquid–liquid partition between the organic solvents and alkali-based solution was found to be a reasonable alternative for removing fats and other products from the aqueous matrix. These partitions followed by SPE analyte isolation-elution, eliminated most interfering peaks and allowed for good recoveries at low fortification levels (Fig. 3).

We initially chose Supelco LC-Florisil, ProElut PXA and Oasis MAX 6 cc for use after the liquid–liquid partitioning procedures. For the Supelco LC-Florisil, the complex ionic behavior of bispyribac-sodium made it difficult to adjust to an appropriate pH for consistent and quantitative extraction although it resulted in a good recovery. ProElut PXA is a strong anion-exchanger and RP-hybrid cartilage. Quaternary ammonium groups were bonded to the adsorbent to attract the targets. Oasis MAX extraction products contain the dimethyl butylamine group, which extracts acidic compounds with anion-exchange groups. ProElut PXA and Oasis MAX 6 cc are both suited for the extraction of bispyribac-sodium, where the results from the Oasis MAX 6 cc were slightly better than that of ProElut PXA (Fig. 4). The loading, washing, and elution steps were examined carefully. The optimal loading condition was 2 mL aqueous solution of sample. The washing step carried out with basic or acidic water methanol mixtures alone resulted in a decrease in analyte recovery, while other kinds of washing solutions (water alone, different kinds of organic solvent) did not result in the satisfactory purification of the samples. The best results were obtained when washing the cartridge with 3 mL 0.05 M sodium acetate

Table 1 Average recoveries of bispyribac-sodium from different rice examples

Samples	Fortified level (mg/kg)	Average recovery (%)	RSD (%)
Jing'an County	1.00	95.02	1.45
	0.50	85.83	0.56
	0.10	84.78	5.84
Xiaogan County	1.00	97.20	2.24
	0.50	94.17	6.36
	0.10	81.30	2.63
Yongfeng County	1.00	98.51	4.21
	0.50	85.34	5.92
	0.10	83.98	3.37

solution. In the elution step, water or basic methanol/water mixtures did not give satisfactory results for analyte recovery. On the contrary, 3 mL 2% formic acid/methanol solution resulted in good extraction yields, and this was chosen as the elution solution. The eluate was then dried under vacuum and re-dissolved in 1 mL acetonitrile/water (65:35 v/v) for HPLC analysis.

Confirmation tests by HPLC-mass spectrometry (HPLC–MS) were used to determine whether peaks were detected at the retention times of the analyte. Bispyribac-sodium was identified by its retention time and the specific molecule ion peak $[M+H]^+$ was found at m/z 431.0 and 453.1 in the HPLC–MS spectrum according to the proposed conditions (Fig. 5).

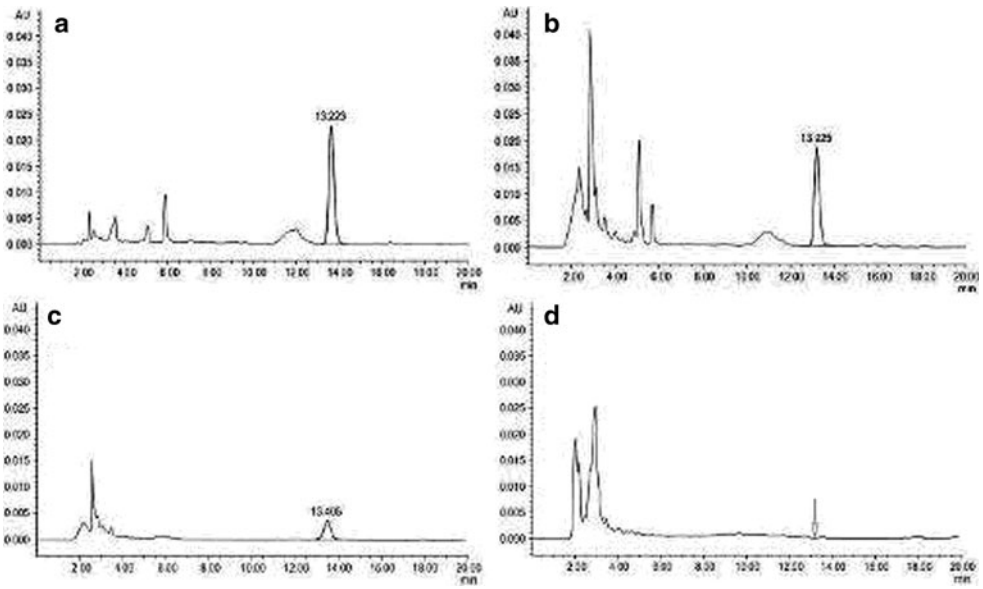

Fig. 3 Chromatogram of **a** bispyribac-sodium standard (0.50 mg/kg); **b** spiked rice sample at 0.50 mg/kg; **c** spiked rice sample at 0.10 mg/kg; **d** control rice sample

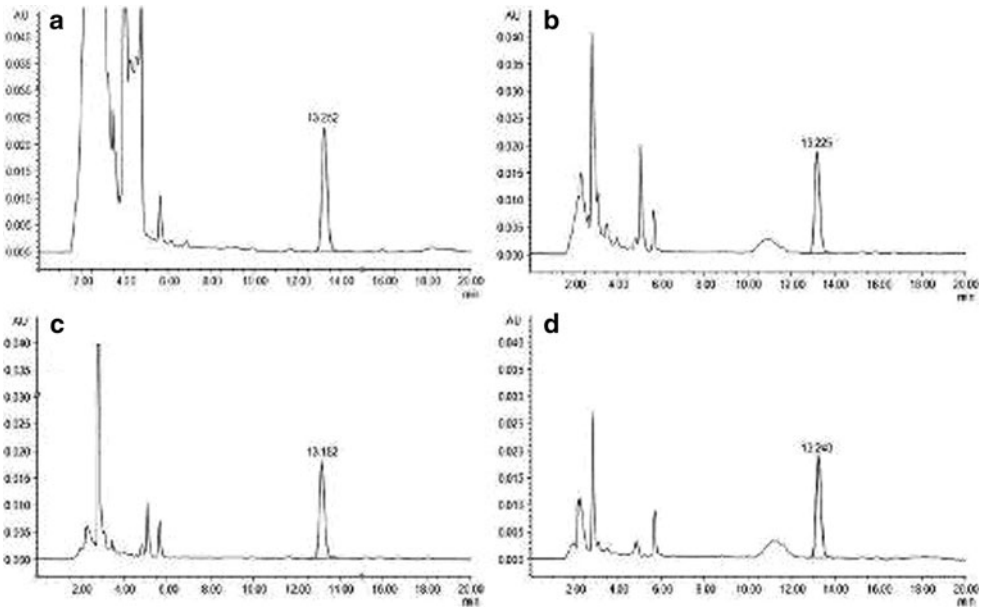

Fig. 4 HPLC chromatogram of **a** samples without purification; **b** samples purified by Oasis; **c** samples purified by ProElut PXA; **d** samples purified by Supelco LC-Florisil

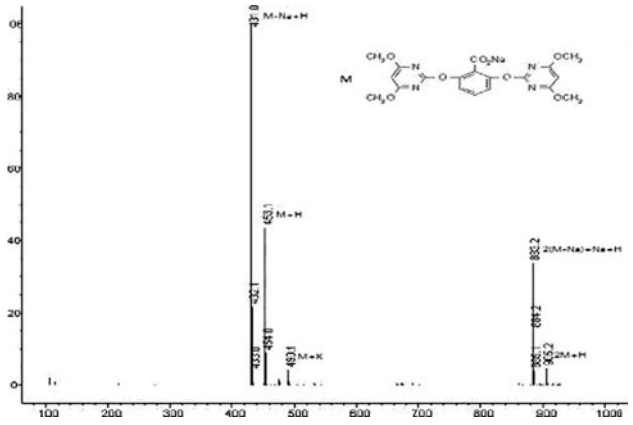

Fig. 5 LC-MS spectrum of bispyribac-sodium

The developed method was applied to the analysis of ten different rice samples collected from the field in Yongfeng Country, Jiangxi Province. No bispyribac-sodium residues (0.01 mg/kg) were detected in the rice at harvest time with a holding period of 5 months after treatment with the pesticide. The MRL of bispyribac-sodium set by the US and EU government for rice is 0.02 mg/kg and no MRL has been set in Chinese legislation or by the Food and Agriculture Organization. Based on the American and European MRL, applying bispyribac-sodium to rice in agriculture would be considered to be safe.

The proposed HPLC method offers clear advantages such as lower detection limits and faster analysis times as compared with the report of the Japanese Positive List System. When compared with other methods found in the literature, this method has the advantage of high precision and accuracy, good selectivity, and feasibility. Another favorable feature is the reduction of sample processing time. It is indeed reassuring to note that results from the application of the method indicate that the levels of bispyribac-sodium in rice are below the Japanese and other countries' legal limits especially as bispyribac-sodium is helpful in rice planting. To date, this compound has not been found in other crops.

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