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An Efficient Analytical Method for Analysis of Spirotetramat and its Metabolite Spirotetramat-Enol by HPLC

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Abstract Spirotetramat is a new compound which belongs to the chemical class of ketoenols. As per the available literature analysis of spirotetramat and its metabolites spirotetramat-enol is carried out by high pressure liquid chromatograph with mass spectrometry (HPLC-MS/MS). In this study we have standardized a method where analysis of both the compounds is carried out by HPLC. The extraction and cleanup of spirotetramat and its metabolites spirotetramat-enol was carried out by QuEChERS method. The cleaned up residues were estimated by HPLC equipped with a photo diode array detector at a wavelength of 250 nm. The mobile phase used was acetonitrile: water at a proportion of 40:60. The limit of quantification (LOQ) of the method was 0.05 mg kg^{-1} for both spirotetramat and its metabolite spirotetramat-enol. The recoveries of both the compounds at the LOQ level were in the range of 72.72%–86.76% from mango and 74.82% to 86.92% from cabbage.

Keywords Acetonitrile · High pressure liquid chromatography (HPLC) · QuEChERS method · Spirotetramat · Spirotetramat-enol

Spirotetramat (*cis*-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro[4.5]dec-3-en-2-one) belongs to the chemical class of ketoenols and acts as a systemic insecticide for the control of a broad spectrum of sucking insects. It is used in a wide range of crops against aphids, whiteflies, scales and mealy bugs etc. Spirotetramat exhibits an excellent systemic and translaminar efficacy. This compound is mobile within the phloem of the plants and can control hidden pests. When applied as a foliar spray it can even protect the roots from aphid attack and newly-grown leaves that develop after the spray application are also protected. These outstanding properties described as a two-way systemicity are unique among recently developed insecticides (Anonymous 2008). Spirotetramat is considered to be a safer insecticide compared to traditional ones against non-target organisms. Spirotetramat once penetrated into the plant is hydrolyzed to its enol form. Spirotetramat-enol (*cis*-3-(2,5-dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4,5]dec-3-en-2-one) is a major metabolite in plants. Due to its physicochemical properties the primary metabolite spirotetramat-enol fulfills the requirements for a phloem-systemic insecticide. Spirotetramat gives effective control of pests of pepper, chilli and cotton etc. (Kay and Herron 2011; Kumar and Kuttalam 2009; Kumar et al. 2009). It is in the process of being registered in India for use against pests of fruits and vegetable crops. The analytical method available to our knowledge for analysis of spirotetramat and its metabolites in plant material requires usage of HPLC-MS/MS (Schoning 2008). Due to the high cost of the equipment it may not be possible for all laboratories to have HPLC-MS/MS, especially in the developing nations. Therefore we have standardized this method for analysis of spirotetramat and its metabolite spirotetramat-enol in mango and cabbage by HPLC.

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Materials and Methods

Reference standards of spirotetramat (99.4% purity) and metabolite spirotetramat-enol (99.4% purity) were obtained

from M/S Bayer Crop Science Limited (Mumbai, India). The chemical structure of spirotetramat and spirotetramat-enol are given in Fig. 1. Primary secondary amine (PSA) sorbent was procured from Varian India Private Ltd. All reagents and chemicals used were of analytical and gradient HPLC grade and purchased locally.

Standard stock solutions of spirotetramat (1,000 μg mL^{-1}) and its metabolite spirotetramat-enol (1,000 μg mL^{-1}) were prepared with gradient HPLC grade acetonitrile. Further dilutions were made with gradient HPLC grade acetonitrile to obtain the working standards. Pesticide free mango and cabbage were locally grown at the Experimental Farm of Indian Institute of Horticultural Research, Bangalore, India. Mango whole fruit, peel and pulp were cut into small pieces and homogenized in a Robot Coupe Homogenizer (Germany). Similarly cabbage samples were cut into small pieces and homogenized. Mango and cabbage samples (10 g) in 5 replicates were fortified with spirotetramat and spirotetramat-enol at 0.05, 0.1, 0.5 and 1.0 mg kg^{-1}. Mango and cabbage samples without insecticides served as controls.

Extraction and clean up of the samples were carried out as per QuEChERS method (Anastassiades et al. 2003). The fortified mango and cabbage samples (10 g) were placed in 50 mL Teflon tubes. To the tube 10 mL of gradient HPLC grade acetonitrile was added and shaken vigorously for 1 min. To the tube containing the fortified samples 4 g anhydrous magnesium sulphate and 1 g sodium chloride was added and spinned for 2 min. The tubes were centrifuged at 10,000 rpm for 10 min. An aliquot (4 mL) of the upper acetonitrile extract was placed in a centrifuge tube containing 50 mg primary secondary amine (PSA) sorbent and 150 mg anhydrous magnesium sulphate. The tubes were shaken vigorously for 1 min and centrifuged for 10 min at 10,000 rpm. About 2 mL of the supernatant acetonitrile phase was taken in a vial and injected into HPLC after passing through Millipore 0.45 μm filters. The control mango and cabbage samples were processed in the similar manner. The residues of spirotetramat and metabolite, spirotetramat-enol were estimated by HPLC (Shimadzu LC-2010 CHT) fitted with an auto sampler and equipped with a photo diode array (PDA) detector, at a wavelength of 250 nm. The column used was Phenomenex C18, Luna, 250 × 4.6 mm i.d. Acetonitrile:water at a proportion of 40:60 was used as the mobile phase at a flow rate of 1 mL min^{-1}. The injection volume taken was 20 μL.

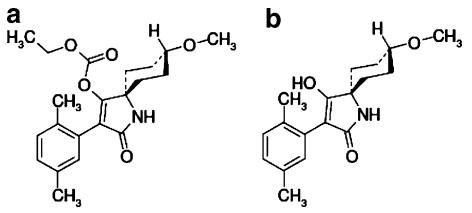

Fig. 1 Chemical structure of **a** spirotetramat and **b** spirotetramat-enol

Results and Discussion

Analysis of spirotetramat and its metabolite was carried out using HPLC with a PDA detector as described above. The constituents of the mobile phase and its proportion were found to be the most important factor. When examined with the mobile phase with 0.04% formic acid in water as described by Schoning (2008) for HPLC-MS/MS analysis) the peaks were not well resolved. Next we examined with acetonitrile : water as the mobile phase without formic acid at different proportions. The proportion of acetonitrile:water were, 15:85, 20:80, 30:70, 35:65 and 40:60. Only with acetonitrile:water at a proportion of 40:60 the peaks could be detected. With other mobile phase proportion both spirotetramat and its metabolite were not detected. With all other solvent combinations tried with or without formic acid, this mobile phase proportion gave the best peak resolution. Using a PDA detector wavelength for detection of both spirotetramat and its metabolite with minimum matrix interference and maximum resolution was achieved at 250 nm. With these operating parameters, spirotetramat-enol and spirotetramat were eluted at the retention time of 7.7 and 8.9 min, respectively (Fig. 2).

The only other method available to our knowledge for extraction of spirotetramat and its metabolite involved use of acetonitrile: water at a proportion of 4:1 (v:v) containing 0.22 mL L^{-1} formic acid (Schoning 2008). In this method only 10 g sample was taken and after extraction the final volume was made up to 200 mL. The dilution factor is too high and HPLC (with a PDA detector) is not sensitive to analyze at such a low level. In the present method extraction was carried out with only 10 mL acetonitrile without any added water or formic acid, reducing the use of solvents drastically. Extraction with only acetonitrile also reduced formation of emulsions which otherwise happened by using the solvent mixture as described above.

Cleanup procedure using QuEChERS method was very quick, easy and reduced the usage of any additional organic solvent. This method was equally efficient for both the matrices (mango and cabbage). This clean up procedure did not give rise to interfering peaks during HPLC analysis and can be easily adopted for other matrices. The limit of detection (LOD) of spirotetramat and its metabolite, spirotetramat-enol was 0.016 ppm. The calibration curve was linear in the range 0.1–2 ppm. The limit of quantification (LOQ) of the method was 0.05 mg kg^{-1} for spirotetramat and metabolite, spirotetramat-enol. This LOQ of spirotetramat and metabolite

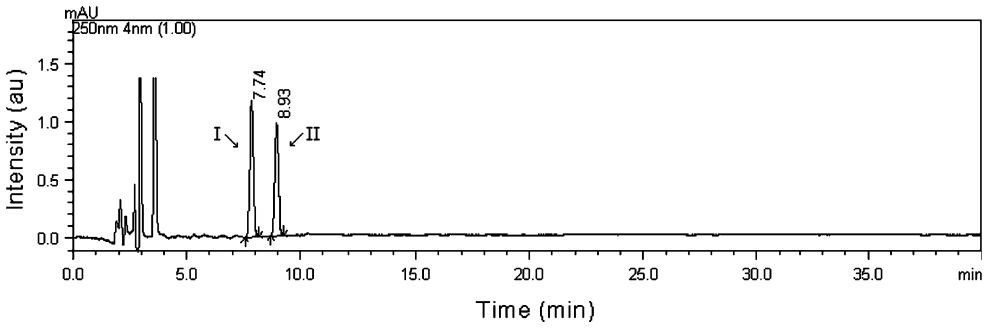

Fig. 2 Chromatogram of 1 ppm reference standards; metabolite, spirotetramat-enol (I) and spirotetramat (II)

was below the maximum residue limit (MRL) of all the commodities (Anonymous 2007).

Recovery of spirotetramat and spirotetramat-enol from mango whole fruit, peel and pulp are given in Table 1. Since mangoes are often consumed as whole fruit and without peel, all three matrices were taken separately. Recovery of spirotetramat at the lowest fortification level of 0.05 mg kg^{-1} was in the range of 72.72%–74.36% from all the three matrices described above. At the fortification level of 0.1 mg kg^{-1} the recovery of spirotetramat was in the range of 76.23%–79.62% (Table 1). At the next higher fortification level of (0.5 mg kg^{-1}) it increased to 82.09%–87.23%. By further increasing the fortification level to 1.0 mg kg^{-1} the recovery percent increased tremendously to 89.66%–93.51%. There was no significant difference in the recovery percent of both spirotetramat from the three matrices. Recovery of spirotetramat-enol by the above method was much higher compared to the parent compound. At the lowest fortification level the recovery was about 86% (Table 1). By increasing the fortification level recovery percent increased and at the highest fortification level of 1.0 mg kg^{-1} the percent recovery was in the range of 103.18–116.58%. Similar trend was observed in case of cabbage as well (Table 2). The recovery of spirotetramat from cabbage increased from 74.82% to 91.38% by increasing the fortification level from 0.05 to 1.0 mg kg^{-1}. Recovery of the metabolite from cabbage was much better, being 86.92% at the lowest fortification level itself. When the fortification level was increased to 1.0 mg kg^{-1} the recovery percent increased to 109.28%. The MRL value of spirotetramat for both mango and cabbage is not available. But MRL value of spirotetramat for all major crops is more than 0.1 mg kg^{-1} and it is 2.1 mg kg^{-1} for cherry preserve (Anonymous 2007). Since the method LOQ is less than 0.1 mg kg^{-1} this method can be used for analysis of spirotetramat and its metabolite in mango and cabbage. The chromatogram of mango peel, pulp, whole fruit and cabbage fortified with spirotetramat and its metabolite are given in Figs. 3, 4, 5 and 6.

Table 2 Recovery of spirotetramat and its metabolite spirotetramat-enol from cabbage

Fortified concentration (mg kg^{-1})	Mean recovery (%) ± SD[a]	
	Spirotetramat	Spirotetramat-enol
0.05	74.82 ± 4.69	86.92 ± 5.29
0.10	74.06 ± 5.84	91.38 ± 3.74
0.50	81.60 ± 6.37	105.26 ± 8.15
1.00	91.38 ± 7.05	109.28 ± 4.38

[a] Average of five replicate analyses ± standard deviation

The analytical method standardized for analysis of spirotetramat and its major metabolite, spirotetramat-enol is simple and easily reproducible. The recovery of both the compounds from mango to cabbage was well within the accepted range when fortified at the LOQ level and higher than LOQ level. The method was repeated 5 times and

Table 1 Recovery of spirotetramat and its metabolite spirotetramat-enol from mango

Fortified concentration (mg kg^{-1})	Mean recovery (%) ± SD[a]					
	Mango whole fruit		Mango peel		Mango pulp	
	Spirotetramat	Spirotetramat-enol	Spirotetramat	Spirotetramat-enol	Spirotetramat	Spirotetramat-enol
0.05	72.72 ± 5.64	84.66 ± 7.24	74.36 ± 4.36	86.76 ± 3.07	73.20 ± 6.82	86.11 ± 6.05
0.10	78.53 ± 7.20	89.34 ± 6.58	76.23 ± 5.48	89.12 ± 6.24	79.62 ± 3.29	92.22 ± 1.67
0.50	82.09 ± 4.33	94.42 ± 5.57	85.47 ± 6.07	98.02 ± 4.68	87.23 ± 4.75	101.06 ± 8.56
1.00	89.66 ± 5.74	103.18 ± 8.52	92.03 ± 7.64	112.47 ± 6.55	93.51 ± 7.31	116.58 ± 3.20

[a] Average of five replicate analyses ± standard deviation

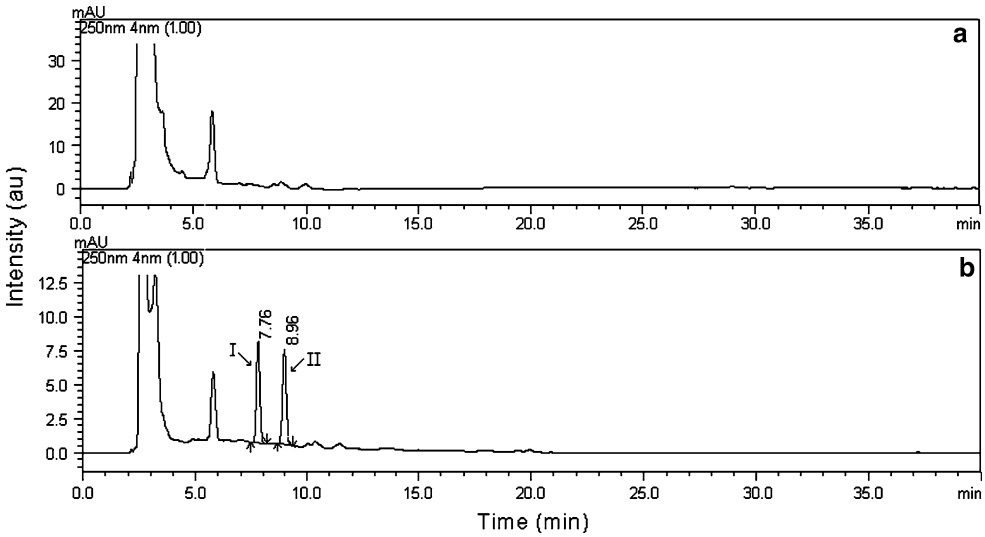

Fig. 3 Chromatogram of mango peel: **a** untreated control, **b** fortified with spirotetramat-enol (I) and spirotetramat (II)

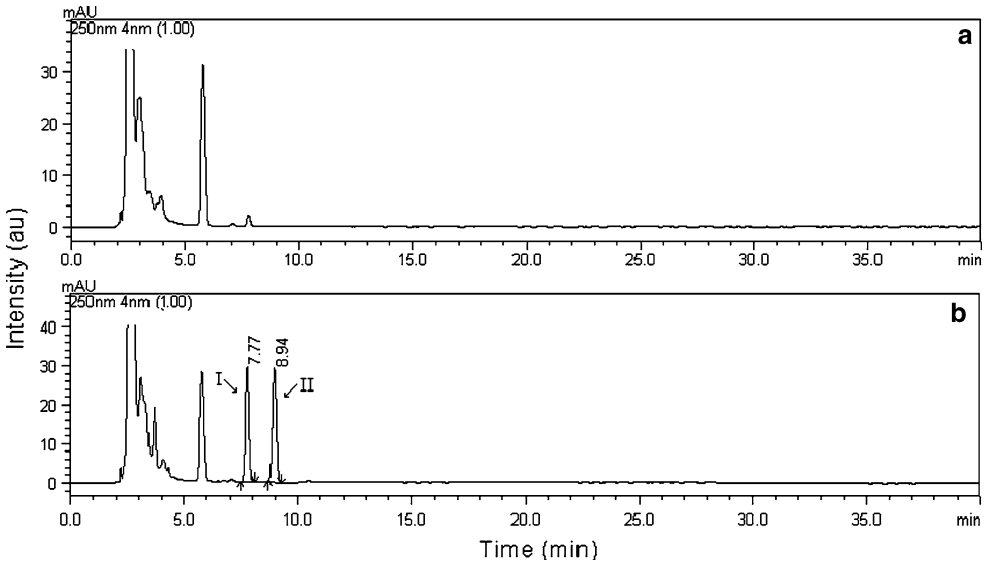

Fig. 4 Chromatogram of mango pulp: **a** untreated control, **b** fortified with spirotetramat-enol (I) and spirotetramat (II)

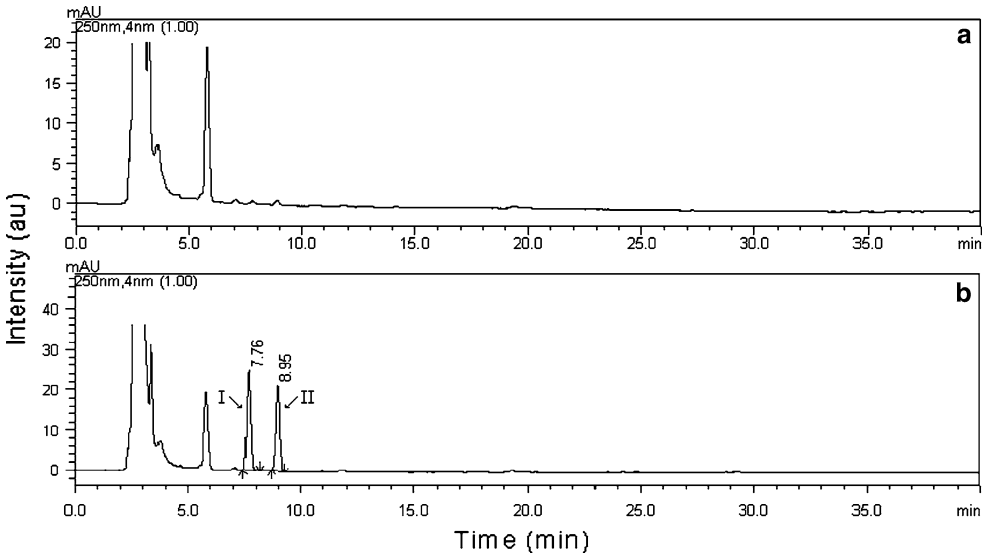

Fig. 5 Chromatogram of mango whole fruit: **a** untreated control, **b** fortified with spirotetramat-enol (I) and spirotetramat (II)

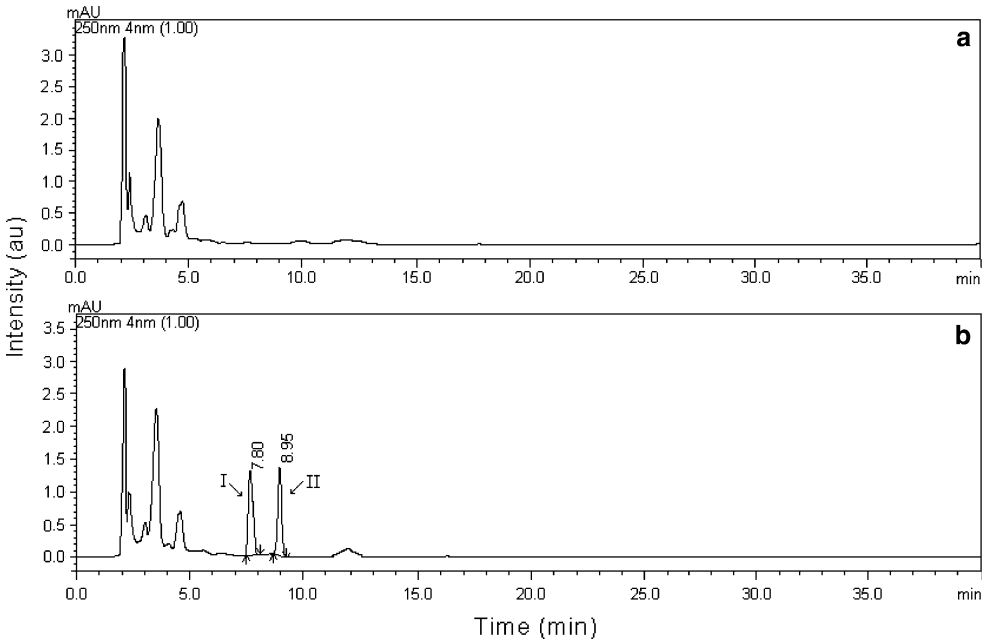

Fig. 6 Chromatogram of cabbage: **a** untreated control, **b** fortified with spirotetramat-enol (I) and spirotetramat (II)

every time the similar results were obtained. The method can be easily adopted for analysis of spirotetramat and its major metabolite, spirotetramat-enol in other matrices.

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References

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J AOAC Int 86: 412–431

Anonymous (2007) Spirotetramat-US Environmental Protection Agency, USA, 234:224–226

Anonymous (2008) Biological profile of spirotetramat (Movento®)—a new two-way systemic (ambimobile) insecticide against sucking pest species. http://www.bayercropscience.com/bcsweb/cropprotection.nsf/id/EN_8thArticle022008. Accessed 21 Jan 2011

Kay IR, Herron GA (2011) Evaluation of existing and new insecticides including spirotetramat and pyridalyl to control *Frankliniella occidentalis*. Aust J Entomol 49:175–181

Kumar BV, Kuttalam S (2009) Toxicity of spirotetramat 150 OD—a new insecticide molecule against natural enemies of chillies. J Plant Protec Environ 6:1–5

Kumar BV, Kuttalam S, Chandrasekaran S (2009) Efficacy of new insecticide spirotetramat against cotton whitefly. Pestic Res J 21:45–48

Schoning R (2008) Residue analytical method for the determination of residues of spirotetramat and its metabolites in and on plant material by HPLC-MS/MS. Bayer Crop Sci J 61:417–454