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Pesticide residue analysis in crops by LC-MS/MS method

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Abstract: An LC-MS-MS assay is described for fluazifop residue analysis in crops. The residues are extracted with acidified organic solvent, the esters and conjugates are hydrolysed with 6.01 M hydrochloric acid, then the extracts are cleaned-up by solid phase extraction using C_2(EC) and Si cartridges in tandem. Quantitative analysis is performed by gradient liquid chromatography coupled to triple quad- rupole mass spectrometer using atmospheric pressure chemical ionization. All fluazifop-P-butyl, free fluazifop- P and any conjugates are quantified as fluazifop-P. The limit of quantification is 0.012-0.054 mg/kg depending on crop matrices. The clean-up method is also suitable for LC-UV analysis with a compromise in higher limit of quantification 0.052-0.22 mg/kg.

Keyword: LC-MS-MS, Pesticide, LC-UV, fluazifop

INTRODUCTION

Fluazifop-P-butyl is a systemic, post-emergence herbicide that selectively controls both annual and perennial grass weeds in non-graminaceous crops. It is rapidly hydrolysed by esterases and the main metabolite is its acid derivative fluazifop. Conjugates may also contribute to the overall residues, so methods have to capture all the parent and major metabolites. Most of the published methods for the determination of fluazifop residues have used either gas-liquid chromatography (GLC) or liquid chromatography (HPLC). GLC procedures require extensive clean-up and derivatisation, e.g. pentafluorobenzylation [1, 2], al- kylation using diazomethane [3, 6] or various chlorofor- mates [7]. For GLC detection, electron capture [1, 5, 7], mass selective [2-4, 7] or nitrogen selective detectors [6] have been used. HPLC methods [8-11] are also labour intensive and lack selectivity. Both UV [8-10] and am- perometric [11] detection have been used with HPLC.

LC-MS-MS determination of acidic herbicides [12] has been previously published for water using negative ion electrospray ionisation (ESI). In this paper a robust, highly specific and sensitive LC-MS-MS method is described for crops using positive ion atmospheric pressure chemical ionisation (APcI).

To facilitate the speed of procedure and minimise possible contamination, disposable labware and solid phase extraction cartridges were used. The method is also suitable for rapid screening of residues using ultaviolet detection with a compromise in sensitivity.

EXPERIMENTAL

CHEMICALS AND REAGENTS

a) Solvents and reagents: acetone, acetonitrile, dichloromethane, ethyl acetate, methanol, hydrochloric acid, glass distilled/HPLC grade solvents and analytical grade reagents.

b) Standard solutions: fluazifop-P (R)-2-[4-(5-trifluoromethyl-2-pyridyloxy) phenoxy] propionic acid (IUPAC) analytical standard > 98% (Zeneca Agrochemicals) dissolved in acetonitrile.

c) Solid phase extraction cartridges (standard polypropylene sy-ringe format): C_2 (EC) - end capped 500 mg adsorbent in 10 mL XL cartridge (IST International Sorbent Technology/Jones Chromatography, UK). Si - high activity silica (500 mg adsorbent in 3 mL cartridge) declared moisture content 7-8% (Varian).

APPARATUS

1. Ultra Turrax homogeniser - for homogenisation at 10000--15000 rpm.
2. Thermostatic heating block - at 60 °C for hydrolysis.
3. Solid phase extraction manifold.
4. HPLC column - Kromasil KR-100 C_{18} 5 |am, 100 A, 50 mm x 3.2 mm for LC-MS-MS determination or 150 mm x 3.2 mm for LC-UV determination.
5. LC-MS-MS system. - Hewlett Packard 1050 Quaternary HPLC system coupled to a Perkin Elmer Sciex API *III* triple quadrupole mass spectrometer.

SAMPLE PREPARATION, EXTRACTION AND HYDROLYSIS

Samples were chopped and homogenized using an internationally accepted method [12]. For crops with a low water content, (dry crops e.g. seeds/oilseeds) a sub-sample (10 g) was soaked overnight (or for a minimum of 2 h) in 1 M hydrochloric acid (50 mL minus water content of 10 g sample). Acetonitrile (50 mL) was added and then sample was extracted using an Ultra Turrax homogeniser at > 10000 rpm for 5 min. Crops with a high water content were extracted by homogenising a sub-sample (20 g) directly with freshly prepared acetonitrile: hydrochloric acid mixture 98: 2 v/v (100 mL minus the average water content of 20 g sample) for 5 min at > 10000 rpm. With all crop types, the homogenate was separated from the extract by centrifugation at 3500 rpm for 2 min. An aliquot of the supernatant equivalent to 0.5 g sample (2.5 mL) was transferred into a 7 mL vial. The organic solvent was removed by evaporation with a stream of dry air at < 60 °C until an aqueous solution remained. An extra aliquot was taken from an untreated control sample extract in order to prepare a standard in the presence of crop matrix for LC-MS-MS determination. The volume of the extract was measured using a pipette and an equal volume of hydrochloric acid (12 M) was added to set the overall acid concentration to approximately 6 M. The vial was tightly sealed and hydrolysed at 60 ±2 °C for 1 h using a thermostatted heating block. After hydrolysis, the hydrolysate was diluted to six times its original volume using ultra-pure water. This dilution was taken as a preventative measure to minimise acid hydrolysis of the modified silica phase in the clean-up below.

CLEAN-UP

A C_2 (EC) solid-phase extraction column was conditioned with methanol (2.5 mL) followed by water (2.5 mL). The diluted hydrolysate was applied onto the column at a speed of 3-5 mL/min. The column was washed with ultra-pure water (3 x 1 mL) then dried for 3-5 min by drawing air through the cartridge. Any remaining water droplets inside the reservoir were wiped off with a clean piece of tissue paper. The column was washed with hexane (2 x 1 mL) then dried as above for 15 min. (It was found vital that no residual water was transferred to the silica clean up to ensure robust elution characteristics). While the C_2 (EC) cartridges were drying, the silica cartridges were conditioned with dichloro- methane (2.5 mL). A 2-3 mm layer of dichloromethane was left on the top of the Si adsorbent by stopping the flow of solvent before the C_2(EC) cartridge was coupled to the Si cartridge, in tandem, using a suitable adapter. The sample was quantitatively eluted from the C_2 (EC) column directly onto the silica with a 95: 5 (v/v) mixture of dichloromethane and acetone (2 x 1.5 mL). The upper C_2 column was detached and the Si column was washed with di- chloromethane (2 x 1 mL), followed by ethyl acetate (2 x 1.5 mL). The washes were discarded. The analyte was eluted from the Si cartridge with methanol (2 x 1 mL) into an HPLC vial. The sample was evaporated to dryness with a stream of dry air at < 60 °C and reconstituted in HPLC mobile phase (1.0 mL of 10:90 v/v acetonitrile: 0.4% v/v HCOOH in water) ready for HPLC analysis. A short ultra-sonication was used to assist re-dissolution of the residuum. If precipitation or floating particulates were present prior to HPLC analysis, the sample was centrifuged at 3500 rpm for 1 min and the clear supernatant transferred into a new vial.

PREPARATION OF CALIBRATION STANDARDS

Due to matrix related response enhancement observed with LC-MS-MS analysis, matrix matched standards were prepared in HPLC mobile phase containing the appropriate crop matrix at the relevant sample concentration. Matrix matched standard were prepared by adding the appropriate amount of fluazifop-P standard to the extra aliquot of the control matrix at the methanol eluate stage of the silica clean-up. The eluate is evaporated to dryness and redissolved in 1.0 mL of HPLC mobile phase in the same fashion as used with the sample.

LC-UV detection is invariable of sample matrix, therefore calibration standards were prepared in HPLC mobile phase corresponding to the initial composition of the gradient.

With both detection strategies, quantification was based on comparison of peak areas of a sample with that of the relevant standard.
Instrumental analysis

LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

Gradient HPLC was found satisfactory for APcl/LC-MS-MS. Mobile phase flow rate was 1 mL/min, column temperature 40 °C, injection volume 50 pL. Mobile phase A was water: acetonitrile: formic acid (95: 5 : 0.4 v/v); mobile phase B was acetonitrile: water: formic acid (95 : 5 : 0.4 v/v). The initial composition was 20% B held from 0-0.75 min, followed by a linear gradient to 80% B from 0.75-2 min. This is held at 80% B from 2-3 min, rising to 100% B from 3-3.2 min. 100% B is held from 3.2-5 min, then the system is re-equilibrate at initial conditions (20% B) from 5.2-7 min.

Atmospheric pressure chemical ionisation operating in the positive ion mode was found to be the most satisfactory ionisation technique. With the Perkin Elmer API *III* mass spectrometer, the system was optimised using a 5.5 kV corona discharge voltage, a 40 V orifice voltage, 20 V collision energy and a collision gas thickness of 280 (using 90 : 10 argon: nitrogen as the collision gas). The APcI probe temperature was set at 480 °C. Protonated molecular ions generated in the ion source (m/z 328) were selected and subjected to further fragmentation by collisional activation. The most abundant ion, (m/z 282, identified as carboxyl loss), in the re re-sulting daughter ion spectrum was then monitored and used for quantitative analysis. The mass spectrometer scanning method was set up

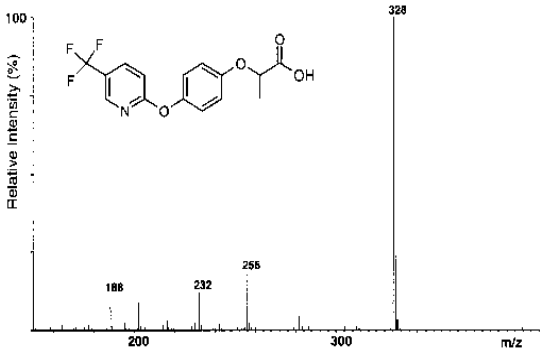

Fig:1 Parent ion[M+H]+ mass spectrum of fluazifop-P

with a dwell time of 250 ms and a pause time of 0.02 ms leading to a 0.5 s scan speed. Parent and daughter ion spectra are shown in Figs. 1 and 2.

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LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

Ultraviolet detection was also used when higher residues (generally > 0.1 mg/kg) were expected. Mobile phase flow rate on a longer column was 1 mL/min, column temperature 40 °C, injection volume 200 pL and detector wavelength 270 nm. Mobile phase A was water containing 0.4% formic acid, mobile phase B was acetonitrile. A linear gradient was employed from 10% to 90% B within 0-15 min, then a second step from 90% to 95% B within 15-20 min, then re-equilibrated at initial 10% B from 20-25 min.

RESULT AND DISCUSSION

For fast LC-MS-MS analysis, isocratic elution was initially attempted but occasionally false results were obtained. It was found that late eluting matrix components from previous sample injections could interfere with the APcI ionisation processes, consequently affecting the detector response and the quantification. This effect was overcome with gradient elution, where high percentages of organic solvent were used to remove all matrix components from the system prior to subsequent injections. This procedure was necessary to ensure robust operation and quantification.

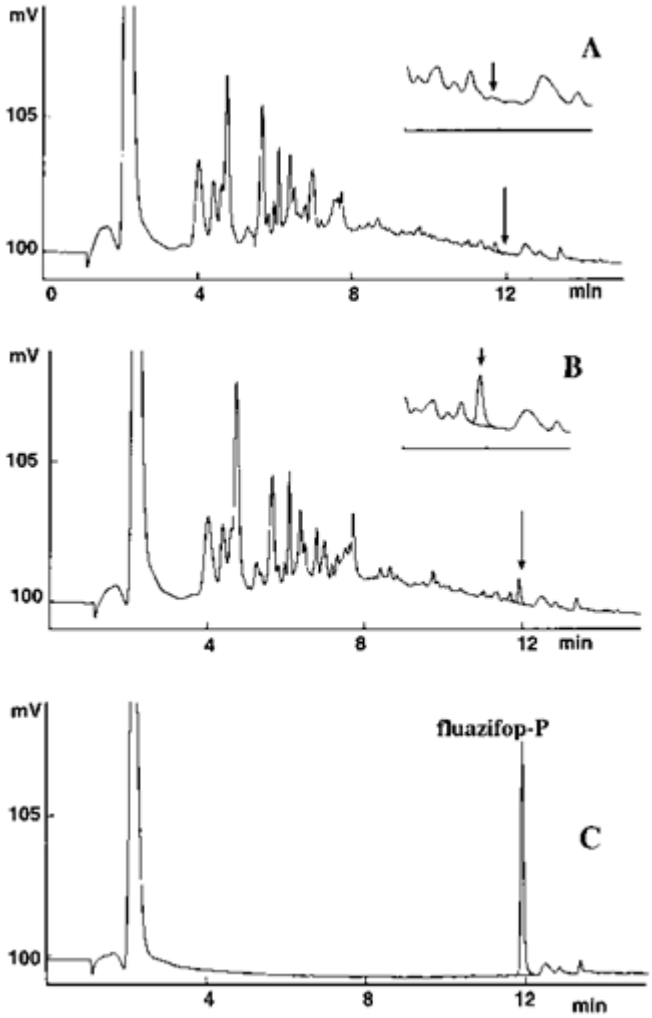

Fig. 4 LC-UV chromatograms at 270 nm of untreated cabbage A, fortified cabbage at 0.1 mg/kg B and fluazifop-P standard (0.5 ^g/ mL) C

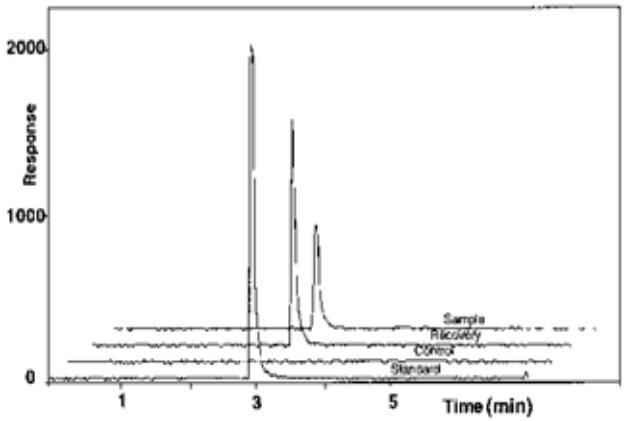

Fig. 3 LC-MS-MS chromatogram of fluazifop-P standard (0.1 ^g/ mL), untreated control sunflower seed, fortified control (recovery at 0.1 mg/kg - 105% recovery) and a field treated sample of sunflower seed (residue 0.065 mg/kg). Crop to solvent ratio - 0.5 g/mL

It was found that for a variety of samples, the presence of matrix could lead to signal enhancement (ranging from 0 to 35% dependent on matrix) when performing LC-MS- MS analysis. This led to inaccurate quantification of fluazifop concentration in some samples. No correlation between matrix type and enhancement was found, hence for consistency and accurate quantification, matrix matched standards were used in all LC-MS-MS analyses. (This effect was not seen when LC-UV detection was used.) For method validation untreated control and fortified crop samples in the range of 0.01-2 mg/kg were analysed by both detection techniques. Similar recoveries were obtained for both detection regimes and summaries of the resultant data are shown in Table 1. Examples of representative chromatograms for LC-MS-MS and LC-UV are given in Figs. 3 and 4, respectively. The LC-MS-MS chromatograms clearly demonstrate a better sensitivity and selectivity compared to that by LC-UV.

Conclusions

A sensitive and specific LC-MS-MS method using atmospheric pressure chemical ionisation has been developed to quantify fluazifop residues in crops. Disposable labware and adsorbents were used for convenience and to minimise contamination. The method has been validated for a variety of crop types covering most FAO/WHO Codex classification of crop categories [13]. The clean-up method is also suitable for monitoring purposes using less sophisticated instrumentation such as LC-UV.

TABLE .1: RECOVERIES AND LOQS BY LC-MS-MS AND LC-UV DETECTION

Crop	Average Recover (%)	RSD (%)	*n*	Range (%)	LOQ (mg/kg)	Average recovery (%)	RSD (%)	*n*	Range (%)	LOQ (mg/kg)
Potatoes	92	10	14	73-107	0.01	99	7.9	14	83-108	0.05
Spinach	99	7.4	14	90-121	0.05	96	6.2	14	79-101	0.05
Cabbage	96	8.3	20	84-119	0.01	101	5.2	20	95-116	0.01
Peas (mange tout)	93	4.5	16	86- 99	0.05	94	6.7	16	83-103	0.05
Tomato	84	6.7	14	74- 95	0.05	84	6.2	14	75- 91	0.05
Orange	96	7.1	14	83-110	0.01	90	11	10	69-103	0.05
Apples	92	7.6	14	70-101	0.01	91	13	10	72-103	0.05
Soya beans	92	8.8	14	80-104	0.05	107	6.4	6	100-116	0.2
All crops	93	8.5	120	70-121	0.01-0.05	95	9.8	104	69-116	0.01-0.2

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".