

Analytical method, validation and degradation kinetics of Fluroxypyr-Meptyl in onion using gas chromatography-tandem mass spectrometry

S. MUKHERJEE,¹S. MUKHERJEE,¹A. KUNDU,²G. K. DAS AND¹A. BHATTACHARYYA

Dept. of Chemistry, Visva Bharati, Santiniketan-731235, West Bengal

¹Dept of Ag. Chemicals, BCKV, Mohanpur-741252, West Bengal

²Dept. of Ag. Chemistry and Soil Science, Calcutta University, Kolkata-700019

Received: 20-08-2014, Revised: 25-09-2014, Accepted: 30-09-2014

ABSTRACT

A simple, fast and inexpensive analytical method was developed and validated for screening of Fluroxypyr-Meptyl, a selective post-emergent systemic broadleaf herbicide in onion (plant leaf, bulb) and soil using GC-MS/MS. The method comprised of extraction with ethyl acetate using matrix solid phase dispersion method followed by column chromatographic and dispersive solid phase extraction cleanup and quantified in GC-MS/MS. The method was validated in concentration ranging from 0.02-0.1 $\mu\text{g g}^{-1}$. The recovery of Fluroxypyr-Meptyl in onion (all substrates) and soil was ranging from 88-98% at different levels of analytes with RSD (HorRat < 0.51) of 8-15%. The global uncertainty was calculated at 0.02 $\mu\text{g g}^{-1}$. In order to evaluate in safety use in India and degradation kinetics, a field study was conducted following the validated method.

Keywords: Degradation, fluroxypyr-Meptyl, GC-MS/MS, onion, validation, uncertainty,.

Onion (*Allium cepa* L.) is one of the important vegetable in India. The important weeds found in onion field are yellow sweetclover, white sweetclover, nutsedge, dodder etc. (UC IPM Guideline 2010). Residue levels of many pesticides in onion leaf and in bulb have also been reported (Otake *et al.*, 2011, Chakraborty *et al.*, 2005).

Fluroxypyr-Meptyl [1-methylheptyl((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetate] is a herbicide, belongs to the chemical family of Pyridinooxy acid. It is used for the control of broadleaf weeds in many kinds of crops, including wheat, barley, corn, rice, orchard, sugar cane, cereals etc. (Kirkland *et al.*, 2001). After predominantly foliar uptake, the ester is rapidly hydrolyzed to the parent acid Fluroxypyr in plant, which is the herbicidally active form, and translocated rapidly to other parts of the plants. It acts by inducing characteristic auxin-type responses *e.g.* leaf curling (Tomlin, 1994). It is classified by EPA as Toxicity Category II and as “not likely” human carcinogen (Aramend *et al.*, 2005).

The aim of this study was to optimize and validate an analytical method based on Matrix Solid Phase Dispersion (MSPD; QuEChERS approach) followed by glass column and dispersive SPE clean up. After validation of method, a field trial was conducted to determine the degradation kinetics of Fluroxypyr, in onion and soil matrices by GC-MS/MS.

MATERIALS AND METHODS

Pesticide reference standard of Fluroxypyr-Meptyl (purity > 99.9% m/m) and Fluroxypyr-Meptyl

Email: anjan_84@rediffmail.com

formulation (48% EC) were obtained from Dow Agro Sciences India Pvt. Ltd., Mumbai. HPLC grade organic solvent ethyl acetate (JT Baker, Phillipsburg, USA), hexane (Ranksolv), dichloromethane (RANKEM, India) and purified water (Millipore, Bedford, MA) were used. Analytical reagent grade acetic acid, anhydrous sodium sulfate, sodium chloride (Merck India Ltd., Mumbai, India), Primary secondary amine (PSA; 40 μm particle size), Bondesil C₁₈-ODS (Varian, Harbor City, CA), Graphitized carbon black; GCB (United Chemical Technology, Bellefonte, PA), activated acidic aluminium oxide (alumina; Sisco Research Laboratories Pvt. Ltd. India), 0.2 μm ultipor N₆₆ nylon 6,6 membrane filter (Pall Corporation) and Silica gel (60–120 mesh; Qualigens, Mumbai) were used for sample analysis. Anhydrous Na₂SO₄ was heated in a muffle furnace at 400–450 °C for 5 h before use and kept in desiccator.

To validate the method and sample analysis for quantification of Fluroxypyr, two season experiment was carried out at Experimental Research Farm, BCKV, Mohanpur, cv. on onion Suksagar. The herbicide was applied in the field at the doses of 360 g a.i. h⁻¹ and 720 g a.i.h⁻¹ during December 2011 to March 2012 (*Rabi*) and December 2012 to March 2013 (*Rabi*). The average temperature range was 15.30 to 28.05 °C and 15.10 to 28.43 °C, with average relative humidity 48.75-91.75% and 50.25-90.75% and total rainfall were 59.20 mm and 7.30 mm in the two seasons respectively.

Preparation of standard solution

Stock solution of Fluroxypyr-Meptyl standard was prepared by accurately weighing 10 ± 0.01 mg with a digital balance (Sartorius, CP 225D, Germany) of the herbicide in volumetric flasks (certified "A" class) and dissolving it in 100 ml methanol. This stock solution was stored in a refrigerator at 4°C . A working standard of $1.0 \mu\text{g ml}^{-1}$ was prepared by diluting the intermediate stock standard solution. From freshly prepared working standard, the calibration standards within the range 0.01 – $0.50 \mu\text{g ml}^{-1}$ were prepared by serial dilution with ethyl acetate.

Onion samples and fortifications

Onion leaf, bulb and field soil samples were collected from the control plots of trial field. The collected samples were used in fortification experiments and as matrix blanks for matrix-matched calibration standards. The matrices used for residue study were the onion leaf, bulb and field soil. The recovery study was carried out by using the control

sample (all three matrices) fortified with Fluroxypyr-Meptyl at 20 (Limit of quantification), 50 and 100 ng g^{-1} concentration levels. The fortified sample was mixed well and left standing for 2 hrs. to distribute herbicide evenly and given time to interact with the matrix before extraction.

Selection of extracting solvent

Three organic solvents and solvent mixture, viz. ethyl acetate, acetonitrile and ethyl acetate + cyclohexane mixture (9:1; v/v) were taken in neutral, acidic (acetic acid to make the $\text{pH}^{\text{H}}4$) and basic (sodium hydroxide to make the $\text{pH}^{\text{H}}9$) medium to compare the extraction efficiency of fluroxypyr from onion and soil matrices by MSPD method using QuEChERS approach. The comparative data of the extracting solvents was presented in Fig. 1. Here it is clear that pure ethyl acetate in acidic medium (at $\text{pH}^{\text{H}}4$) is the ideal solvent to extract and estimate fluroxypyr from onion leaves, bulb and soil matrices.

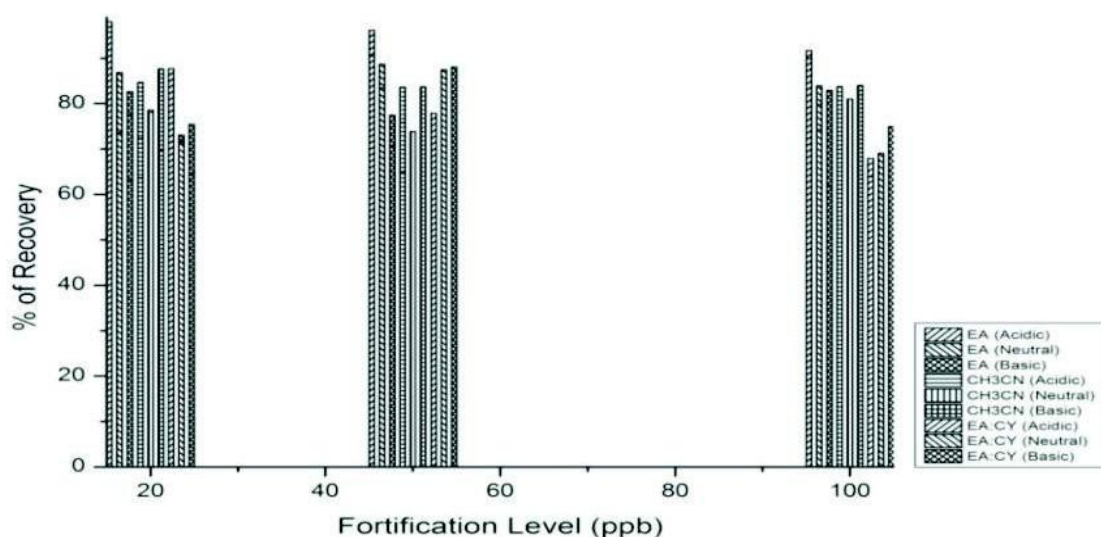


Fig. 1: Comparative Study between different extracting solvent considering different pH (Acidic, neutral and basic) using three different fortification levels

Matrix solid phase dispersion (MSPD) method

Fifty gram (50 g) onion leaf and bulb samples were blended separately using Polytron homogenizer (Polytron, PT-MR-3100, Kinematica AG, Switzerland). In order to estimate Fluroxypyr-Meptyl residue, the onion bulb and leaf (10 g) was taken in a 50 ml fluorinated ethylene propylene (FEP) centrifuge tube (Nalgene, Rochester, NY) separately. Then 10 ml of ethyl acetate, 10 ml of 5% aqueous NaCl solution was added to it and acetic acid was used to maintain pH value 4. Then it was subjected to vortex (Spinix) for 1

min. and followed by shaking for 1 hour in a mechanical shaker (ZHWHY-200D; Zhicheng, China). After that adding 5 g of activated Na_2SO_4 , the sample was again vortex for 3 min. Then the sample was centrifuged with refrigerated centrifuge (Model Avanti J-30I; Beckman coulter, USA) with the rotor heads JA-30.50 T1 for 10 min at 5,000 rpm and afterwards 6 ml supernatant liquid was taken out and placed in a glass tube. Finally the sample was evaporated to dryness under nitrogen evaporator (Turbo Vap LV instrument from Caliper Life Science;

Degradation kinetics of fluroxypyr meptyl in onion

Hopkinton, MA, USA) and dissolved in 2 ml of Dichloromethane.

Column chromatographic clean up

Acidic alumina (2 g) was packed in a glass column using hexane. The 2 ml extract was loaded over the alumina column. Then the impurities were eluted with 10 ml of hexane. Then the sample was eluted 30 ml of dichloromethane and the collected eluent was evaporated to dryness using a rotary vacuum evaporator (EYELA) at 40°C. The dried mass was dissolved in 6 ml of ethyl acetate.

Dispersive SPE clean up

After column chromatographic clean up the 6 ml aliquot was taken, afterwards 75 mg PSA, 75 mg GCB

(for plant leaf samples only) and 450 mg of Na₂SO₄ was added to it and vortex for 2 min and the sample was again centrifuged for 5 min at 5,000 rpm. Then 4 ml supernatant liquid was collected from it. The extract thus obtained was filtered through a 0.2 μm nylon membrane filter.

Field soil extraction

Field soil sample (10 g) was taken in a 50 ml centrifuge tube. Ethyl acetate (10 ml), 5% aqueous NaCl solution (10 ml) was added to it and acetic acid was used to maintain the pH value at 4. Then it was subjected to vortex for 2 min. and after that adding 5 g of activated Na₂SO₄, the sample was again vortex for 3 min. Then the sample was centrifuged for 10 min at 5,000 rpm and then 6 ml supernatant liquid was taken

Table 1: Recovery^a, RSD^b, HorRat and Matrix Effect (ME %) of Fluroxypyr-Meptyl from onion matrices and soil

	Substrate	Fortified level (ng g ⁻¹)	Recovery ^a (%)	RSD ^b (%)	HorRat ^c	ME ^d (%)
Level of fortification	Onion leaf (ng g ⁻¹)	20	97.62	8.413	0.294	-04.46
		50	96.15	9.535		
		100	91.68	8.505		
	Onion bulb (ng g ⁻¹)	20	98.02	11.610	0.406	-01.98
		50	89.32	14.864		
		100	88.89	13.239		
	Field soil (ng ml ⁻¹)	20	95.74	14.642	0.513	-04.26
		50	90.43	13.899		
		100	89.89	10.335		
LOQ (Limit of quantification)		Onion leaves	Onion bulb	Soil		
(ng g ⁻¹)		20	20	20		

Recovery^a (%) is the average percentage value of five replications (n=5) of each matrix, RSD^b (%) is the relative standard deviation of five replications (n=5) of each matrix, HorRat^c at 20 ng g⁻¹ for all matrices, ME^d (%) (Matrix Effect) pertains to matrix-induced signal suppression ("-" sign) or enhancement at 20 ng g⁻¹.

Table 2: Overview of the Mass Spectrometric residue monitoring parameters of Fluroxypyr-Meptyl

Qamu	Q ₁ (amu)	Dwell	CE (V)	Quad temp (°C)	Q ₂ (amu)	Dwell	CE (V)	Quad temp (°C)	Scan type	Ion source
254.0	208.9>181.10	10	15.0	150	254.0>181.1	10	10.0	150	MRM	EI

Q: protonated parent ion; Q1: quantifier ion; Q2: second transition and CE: collision energy.

Table 3: Overview of the GC details of Fluroxypyr-Meptyl

Oven Temperature		Rate ($^{\circ}\text{C min}^{-1}$)		Temp. ($^{\circ}\text{C}$)	Hold (min)	Total (min.)	
		Initial		120	1	1	
		25		260	4	10.6	
Source Temp.	Aux Heater	Column Flow	Stop Time	Post Run	Injector Temp.	Injection Mode	Injection Volume
230 $^{\circ}\text{C}$	280 $^{\circ}\text{C}$	1.0 ml min^{-1}	10.6 min.	290 $^{\circ}\text{C}$ (for 3 min.)	260 $^{\circ}\text{C}$	Splitless	1 μL
Carrier Gas				He			
Collision Gas (N_2)				Flow: 1.5 ml min^{-1}			
Quench gas (He)				Flow: 2.25 ml min^{-1}			
LOD (Limit of detection)				10 ng g^{-1}			
Retention Time				9.228 \pm 0.1 min.			
Column Temperature				25$^{\circ}\text{C} \pm 0.8^{\circ}\text{C}$			

in 10 ml centrifuge tube. Then d-SPE clean up procedure was followed as mentioned in d-SPE clean up section. The samples were finally ready for GC-MS/MS analysis.

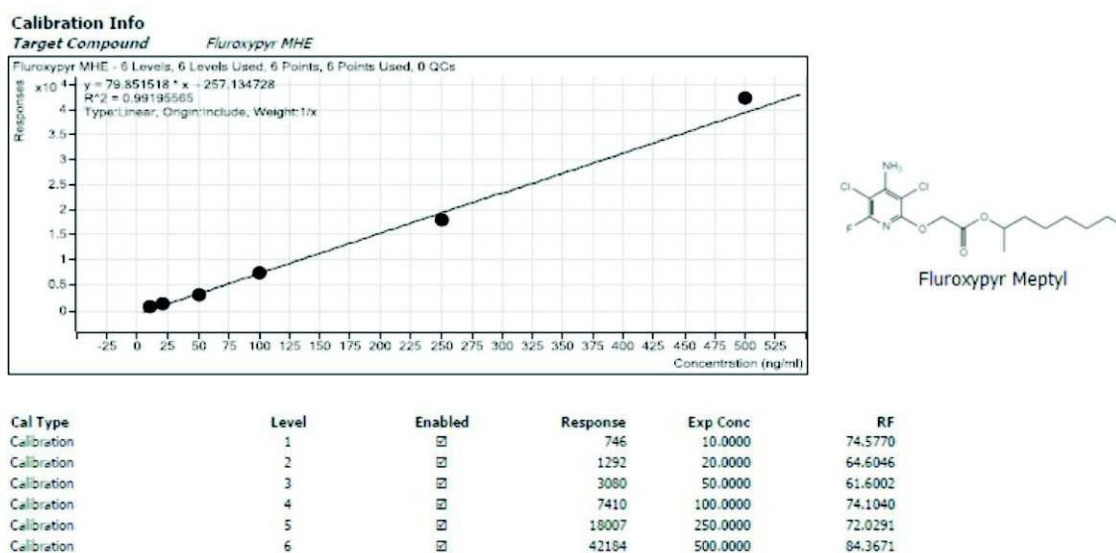
GC-MS/MS analysis

The residue analyses were performed by an Agilent Technologies GC 7890A coupled with QQQ 7000 mass spectrometer using electron impact in the positive ion (EI+) mode with Mass Hunter software. The gas chromatographic separation was performed via autosampler on a HP-5 (30m \times 250 $\mu\text{m} \times$ 0.25 μm) column (Agilent Technologies, USA). Table 2 indicates the mass spectrometric parameters and table 3 indicates the GC conditions of the entire analysis. All

the graphs, chemical structures were designed using OriginPro 8.5 and ChemDraw Ultra 6.0 softwares.

Preparation of matrix-matched calibration standards

For calibration in GC-MS/MS, six concentration levels of Fluroxypyr-Meptyl (10, 20, 50, 100, 250 and 500 ng g^{-1}) were prepared. The standard calibration curve is presented in Fig. 2. Qualifier as well as quantifier ion ratio, mass transition chromatograms and recovery chromatogram of Fluroxypyr-Meptyl in LOQ level at 20 ng g^{-1} are presented in Fig. 3 and Fig. 4 respectively. To evaluate the matrix effect, matrix-matched calibration curve was prepared by using the extracts of blank matrices.

**Fig. 2: Calibration curve of analytical standard (10-500 ng g^{-1}) of FluroxypyrMeptyl (solvent only)**

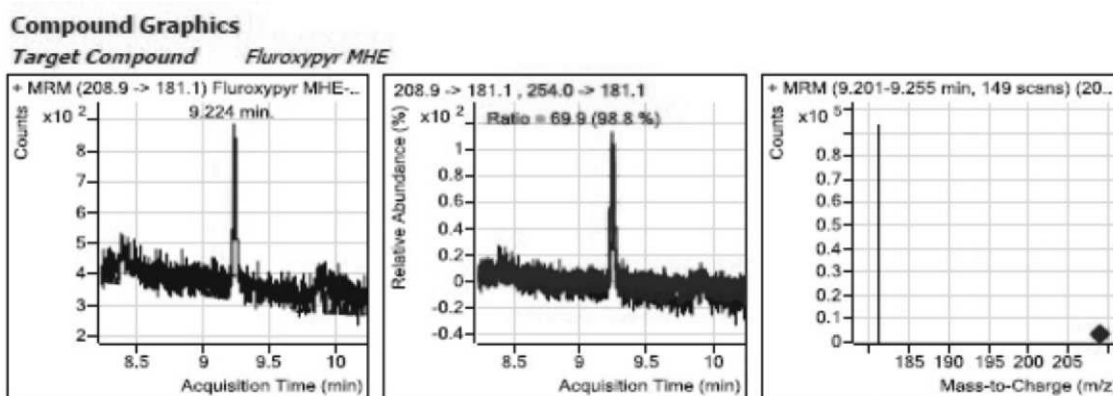


Fig. 3: Chromatograms of Fluroxypyr-Meptyl standard (conc. 20 ng g⁻¹) with qualifier, quantifier ion transition and ion ratio

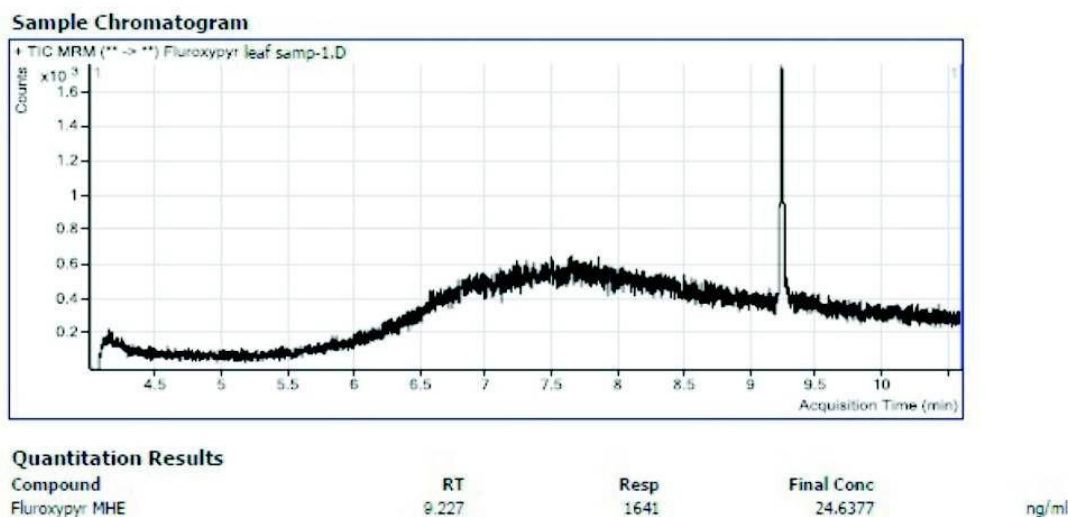


Fig. 4: Recovery chromatogram of Fluroxypyr-Meptyl at 20 ng g⁻¹ in GC-MS/MS

Method validation

The analytical method was validated as per the single laboratory validation (SLV) approach (SANCO guideline, 2009). The performance of the method was evaluated considering different validation parameters that include the precision, accuracy-recovery experiments, matrix effect and measurement of uncertainty.

The calibration curves for the compound in pure solvent and matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at six calibration levels ranging between 10 and 500 ng g⁻¹.

The limit of detection (LOD) was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained from blank sample,

whereas the limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10 irrespective of the onion leaves, bulb and soil matrices by using matrix-matched standards.

Precision

In the single-laboratory validation (SLV) studies precision was determined from the reproducibility (R) and repeatability (r). The experiment was carried out separately for a standard concentration of 20 ng g⁻¹ in onion leaf, bulb and soil, which is previously determined as LOQ and presented in Table 1. The Horwitz ratio (HorRat) pertaining to within-laboratory precision, which indicates the acceptability of a method with respect to precision (Horwitz *et al.*, 1980; AOAC Off. Method, 2012) was calculated for Fluroxypyr-Meptyl in the following way:

$$\text{HorRat}(r) = \frac{\text{RSD}(r)}{\text{PRSD}(R)}$$

Where RSD is the relative standard deviation and PRSD is the predicted RSD = $2C^{-0.15}$ and where C is the concentration expressed as a mass fraction ($20 \text{ ng g}^{-1} = 20 \times 10^{-9}$).

Accuracy-recovery experiments

Onion obtained from an untreated plot (which did not receive any treatment of the test herbicide

i.e. Fluroxypyr-Meptyl) was used as blanks. The recovery experiments were carried out on fresh untreated onion leaves, bulb and soil by fortifying the sample in five replicates with Fluroxypyr-Meptyl at three concentration levels *i.e.* 20, 50 and 100 ng g^{-1} in onion leaves, bulb and soil. The results are presented in table 1.

Matrix effect

The percentage of matrix effect (ME %) was assessed by employing matrix matched standards. The

Table 4: Overview of the GC details of Fluroxypyr-Meptyl

Oven Temperature		Rate ($^{\circ}\text{C min}^{-1}$)		Temp. ($^{\circ}\text{C}$)	Hold (Min)	Total (min.)	
		Initial		120	1	1	
		25		260	4	10.6	
Source Temp.	Aux Heater	Column Flow	Stop Time	Post Run	Injector Temp.	Injection Mode	Injection Volume
230 $^{\circ}\text{C}$	280 $^{\circ}\text{C}$	1.0 ml Min $^{-1}$	10.6 Min.	290 $^{\circ}\text{C}$ (for 3 min.)	260 $^{\circ}\text{C}$	Splitless	1 μL
Carrier Gas				He			
Collision Gas (N ₂)				Flow: 1.5 ml min $^{-1}$			
Quench gas (He)				Flow: 2.25 ml min $^{-1}$			
LOD (Limit of detection)				10 ng g $^{-1}$			
Retention Time				9.228 \pm 0.1 Min.			
Column Temperature				25 $^{\circ}\text{C} \pm 0.8^{\circ}\text{C}$			

Table 5: Individual, combined, standard and expanded uncertainties for Fluroxypyr-Meptyl in different matrices

Substrate	Uncertainties									
	U ₁	U ₂	U ₃	U ₄	U ₅	U ₆	U ₇	U _c	U _s	U
Onion leaf	0.000280	0.0376	1.64e $^{-06}$	0.000577	0.0131	0.0013	0.0132	0.0420	0.40	0.80
Onion bulb	0.000323	0.0519	2.45e $^{-06}$	0.000577	0.0134	0.0013	0.0132	0.0552	0.52	1.05
Field soil	0.000195	0.0655	1.48e $^{-06}$	0.000577	0.0054	0.0013	0.0132	0.0670	0.57	1.13

Note: Uncertainty associated with U₁: Calibration curve; U₂: Recovery; U₃: Precision; U₄: Standard; U₅: Weighing; U₆: Volumetric flask; U₇: Pipette; Each contributing uncertainties (U₁– U₇) were reported as relative uncertainty U_c: combined uncertainty; U_s: standard uncertainty was calculated multiplying the combined uncertainty with the result of recovery experiment; U: Expanded or global uncertainty was twice the value of the standard uncertainty. A coverage factor of 2 was decided at a confidence level of 95% to evaluate the expanded uncertainty at 20 ng g $^{-1}$ of fortification.

Table 6: Half life of Fluroxypyr-Meptyl in two season field study in Onion leaves and field soil from days vs. log (residue \times 1000) data

Substrate	Season	T ₁ (360 g a.i. h $^{-1}$)			T ₂ (720 g a.i. h $^{-1}$)		
		T _{1/2} (Days)	Regression equation	R ²	T _{1/2} (Days)	Regression equation	R ²
Onion Leaf	1	1.350	Y= -0.223x + 3.178	0.998	1.375	Y= -0.219x + 3.538	0.997
	2	1.260	Y= -0.239x + 3.489	0.964	1.584	Y= -0.190x + 3.787	0.884
Field Soil	1	1.870	Y= -0.161x + 3.336	0.998	1.930	Y= -0.156x + 3.591	0.993
	2	1.831	Y= -0.166x + 3.345	0.986	1.893	Y= -0.159x + 3.632	0.990

slope of the calibration graph based on the matrix-matched standards of onion leaves, bulb and soil was compared with the slope of the pure solvent-based calibration graph. A higher slope of the matrix calibration indicates matrix-induced signal enhancement (positive value), whereas, a lower slope represents signal suppression (negative value). The results were presented in Table 1.

Measurement uncertainty

Global uncertainty was determined for Fluroxypyr-Meptyl at the level of 20 ng g⁻¹ as per the statistical procedure of the EURACHEM/CITAC Guide CG 4 (2000) in the same way as reported by Kanrar *et al.* (2010). Seven individual sources of uncertainty were taken into account. The combined uncertainty (U_C) was calculated as

$$U_C = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 + U_5^2 + U_6^2 + U_7^2)}$$

and was reported as expanded uncertainty, which is twice the value of the combine uncertainty. The uncertainty values for Fluroxypyr-Meptyl are presented as relative uncertainties in table 4.

RESULTS AND DISCUSSION

MSPD method of extraction using QuEChERS approach

In case of Acetonitrile extraction, the recovery of Fluroxypyr-Meptyl was only within 63.70-87.65% in neutral, acidic and basic medium for all matrices. With and ethyl acetate + cyclohexane (9:1; v/v) mixture the recovery was in between 35.04-88.06% (for all matrices), as the recovery percentage was gradually decreases with the increasing percentage of cyclohexane. But for pure ethyl acetate the recovery was in between 41.82-88.70% (for basic and neutral medium) and 89.89-97.62% (for acidic medium) when quantified with matrix-matched calibrations. Precision in terms of HorRat (single laboratory) at 20 ng g⁻¹ level was less than 0.51 and RSD is less than 20% for Fluroxypyr-Meptyl (Table 1), indicating satisfactory repeatability and ruggedness of the methodology. A relatively less recovery of Fluroxypyr-Meptyl was found irrespective of neutral and basic medium.

Measurement of uncertainty

If we consider the measurement of uncertainty, there was a satisfactory laboratory precision [determined from the reproducibility (R) and repeatability (r)] result and analysts' confidence level (*i.e.* 95%), precision in terms of HorRat (values are in

between 0.294-0.513) and percentage of RSD (8.413-14.864%). The analytical method was validated as per the SLV approach. Here seven parameters of uncertainty were considered and calculated the combined uncertainty (0.0420, 0.0552 and 0.0670); standard uncertainty (0.40, 0.52, and 0.57) and lastly the expanded or global uncertainty (0.80, 1.05 and 1.13) for onion leaf, bulb and field soil respectively.

From the entire study it clearly revealed that at acidic medium pure ethyl acetate gave higher recovery percentage than other solvent or solvent mixtures used for extraction of onion and soil matrices. Although, pure ethyl acetate is economically cheaper, environment friendly, less toxic than other extracting solvent *viz.* dichloromethane, acetonitrile. That is why it is very much appropriate in selecting solvent ethyl acetate for extraction of a matrix like onion leaf, bulb and field soil samples.

ANALYSIS OF REAL SAMPLE

After validation of the proposed method, we perform a field trial of Fluroxypyr-Meptyl on onion in two seasons as mentioned in field trial details. Fluroxypyr-Meptyl was applied after 20 days of the transplantation, following recommended package of practices. The T_{1/2} of Fluroxypyr-Meptyl was determined using Hoskins formula (1961) and the values were presented in table 5 and figure 5. In onion leaves Fluroxypyr-Meptyl residues were found below detection limit (BDL) at 10 days but in the field soil it was not detected at 15 days from the date of application. On the time of harvest there is no residue of Fluroxypyr-Meptyl in the plant, bulb and soil samples, irrespective of doses and seasons.

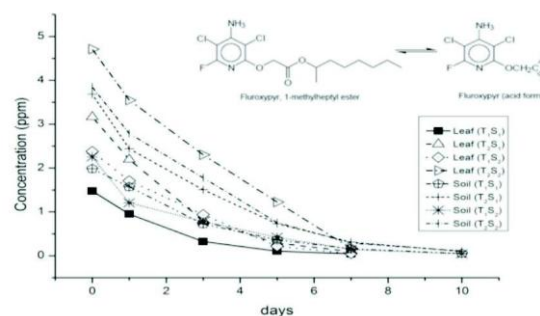


Fig. 5: Degradation kinetic study of Fluroxypyr-Meptyl in onion leaf and field samples of two Seasons

S₁: Season 1; S₂: Season 2; T₁: Single dose (360 a.i. ha⁻¹); T₂: Double dose (720 a.i. ha⁻¹)

The proposed simple method of extraction with ethyl acetate, cleanup by acidic alumina glass column, d-SPE with PSA + GCB + Na₂SO₄ and quantified by GC-MS/MS using MRM provided high cleanup efficiency and low matrix effects thus enabling adaptation of this sensitive and selective method for routine residue analysis of Fluroxypyr-Meptyl on onion and soil matrices with satisfactory recovery (88–98%). The method offers low cost of analysis, short time as well as satisfactory uncertainty parameters.

ACKNOWLEDGMENT

We are thankful to Indofil Industries Limited, Mumbai for financial assistance and we are grateful to Dept. of Ag. Chemicals, BCKV, Mohanpur, WB, India, for all instrumental facilities. We are also thankful to Dept of Chemistry, Visva-Bharati, Santiniketan, West Bengal.

REFERENCES

- Aramendra, M.A. Marinas, A. Marinas, J.M. Moreno, J.M. and Urbano, F.J. 2005. Photocatalytic degradation of herbicide fluroxypyr in aqueous suspension of TiO₂. *Catalysis Today*, **101**: 187–93.
- Chakraborty, A., Ghosh, M., Banerjee, H., Bhattacharyya, A. and Roy, A. 2005. Persistence behavior of Halexypop-A new Herbicide in/on onion. *J. Crop Weed*, **1**:41-44.
- Galdón, B.R. Rodríguez, C.T. Rodríguez, E.M.R. and Romero C.D. 2009. Fructans and major compounds in onion cultivars (*Allium cepa*). *J. Food Comp. Analysis*, **22**: 25-32.
- Guidelines for Standard Method Performance Requirements, 2012. *AOAC Official Methods of Analysis*. Appendix F: 13-14.
- Horwitz, W., and Albert R. 2006. The Horwitz Ratio (HorRat): A Useful Index of Method Performance with Respect to Precision. *J. AOAC Int.*, **89**: 1095-09
- Hoskins, W.M. 1961. Mathematical treatment of the rate of loss of pesticide residues. *FAO Pl. Prot Bull.*, **9**: 163-168.
- Kanrar, B. Mondal, S. and Bhattacharyya, A. 2010. Validation and Uncertainty Analysis of a Multiresidue Method for 67 Pesticides in Made Tea, Tea Infusion, and Spent Leaves Using Ethyl Acetate Extraction and Gas Chromatography/Mass Spectrometry. *J. AOAC Int.*, **93**: 411-24.
- Kirkland, K.J. Johnson, E.N. and Stevenson, F.C. 2001. Control of wild oat (*Avena fatua*) in wheat with MKH 6562. *Weed Technol*, **15**: 48-55.
- Method validation and quality control procedures for pesticide residues analysis in food and feed. Document no. SANCO/10684/2009, SANCO, 2009. 6th EU AQC, [http:// ec. europa.eu/ food/ plant/protection/ resources/ qualcontrol_en.pdf](http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf).
- Otake, T. Yarita, T. Aoyagi, Y. Kuroda, Y. and Numata M. 2011. Development of Green Onion and Cabbage Certified Reference Materials for Quantification of Organophosphorus and Pyrethroid Pesticides. *J. Agric. Food Chem.*, **59**: 8568–74.
- Quantifying Uncertainty in Analytical Measurement, 2000. EURACHEM/CITAC Guide CG 4, *EURACHEM/CITAC*, 2nd ed., [http://www. measurementuncertainty.org.html](http://www.measurementuncertainty.org.html).
- Tomlin, C.D.S. 1994. The Pesticide Manual - World Compendium. 10th ed. Surrey, *The British Crop Protection Council*, UK: 506.
- UC IPM Pest management guideline: Onion/Grape, 2010. *University of California Agriculture and Natural Resources*. publication: **3453**.