Bull Environ Contam Toxicol (2011) 86:149–151
DOI 10.1007/s00128-011-0190-6

Gas Chromatographic Method for Residue Analysis of Metsulfuron Methyl from Soil

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Received: 13 September 2010 / Accepted: 7 January 2011 / Published online: 21 January 2011
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Abstract Gas Chromatographic method has been developed for the determination of metsulfuron methyl residues from soil. Due to the thermal unstability of this herbicide and its mono methyl derivative, it was derivatized to a dimethyl derivative using diazomethane. Structure of the derivatized product was confirmed by GC–MS. Time for methylation reaction was standardized and 24 h was found to be the optimum time in ethylacetate solvent. Using this method, recovery of metsulfuron methyl from soil was above 70%. Limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.1 μg mL^{-1} and 0.2 μg g^{-1} respectively.

Keywords Metsulfuron methyl · Herbicide · GC analysis · Dimethyl derivative

Modernization of agriculture has introduced very low dose and high potency molecules as plant protection chemicals. Therefore, analytical science is facing more and more challenges due to the changing paradigms in the field of pesticide chemistry. Metsulfuron methyl [Methyl-2-[[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]-carbonyl]-amino]-sulfonyl] benzoate] (I, Fig. 1), a sulfonylurea herbicide, discovered in the mid 70s, is a low application rate herbicide recently registered for use in India (Roberts and Bond 1984). Direct determination of metsulfuron methyl is not possible by gas chromatography (GC) due to its thermal unstability and extremely low volatility. Most of the methods reported for its determination are by High performance liquid chromatography (HPLC) (Lian et al. 1996; Sanyal et al. 2006; Paul et al. 2009). Bioassay has also been used for the detection of sulfonyl ureas (Paul et al. 2009). GC method can be used for ureas and sulfonyl ureas after derivatization of the parent compound. As there are two imino hydrogens in these compounds derivatization results in a mixture of two products mostly in unequal proportions. For estimation of a compound a single product after derivatization is desired. Keeping this in view chemical derivatization of metsulfuron methyl was standardized to over come the problem of thermal unstability and to standardize the time for obtaining a single dimethylated product for estimation by GC.

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Materials and Methods

Analytical grade metsulfuron methyl (99% purity) supplied by E.I. Du Pont, India Pvt. Ltd. had a melting point of 158°C. The solvents were laboratory grade and distilled prior use. Chemicals and reagents were analytical grade.

A Shimadzu gas liquid chromatograph instrument (model GC-17A) equipped with a ^{63}Ni electron capture detector (ECD) was used for this study. The column was an OV-1 megabore column (20 cm × 0.53 mm i.d.). The instrument had a microprocessor control data system that allowed automatic calculation of detector response in terms of peak area. The mobile phase was nitrogen gas maintained at a flow rate of 1 mL min^{-1}. The oven temperature was programmed at 150°C (1 min)… @ 10°C min^{-1}… 250°C (7 min) with a total run time of 20 min. Injector and detector temperatures were maintained at 280°C and 300°C, respectively. 3 μL of the sample solution was injected in split less mode with the help of a 10 μL GC syringe. Each treatment was repeated thrice and chromatograms were recorded on computer recorder.

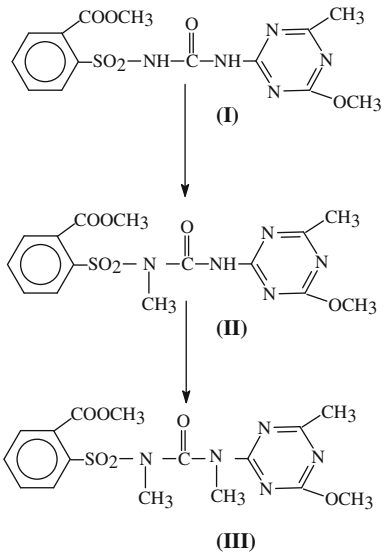

Fig. 1 Structure of mono and dimethyl derivatives of metsulfuron methyl

For the preparation of methyl derivative of metsulfuron methyl, diazomethane was prepared using nitrosomethylurea according to reported method (Arndt 1943) except a modification that diazomethane was collected as ethyl acetate (free from acetic acid) solution. For methylation, standard solution of metsulfuron methyl (100 μg mL^{-1}) was prepared in acid free, dry and distilled ethyl acetate. Working standard of 10 μg mL^{-1} was prepared from this by serial dilution in ethyl acetate. Twenty five graduated test tubes were taken and 1 mL of standard solution of metsulfuron methyl (10 μg mL^{-1}) was added to 24 test tubes. Ethyl acetate (1 mL) was added to one test tube to serve as blank. Freshly prepared diazomethane (as ethyl acetate solution, 1 mL) was added to each test tube and were tightly closed with the help of rubber stoppers. The test tubes with reaction mixture were kept at room temperature in a fume cupboard. The rubber stoppers of tubes were opened at an interval of 0.5, 1.0, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 h. Excess diazomethane was allowed to evaporate out, ethyl acetate was concentrated to minimum and hexane was added to makeup the final volume 5 mL. The samples were analyzed by GC-ECD. Complete methylated sample of 10 μg mL^{-1} was also serially diluted to 5, 2, 1, 0.5, 0.2, 0.1, 0.05 μg mL^{-1} for LOD calculation. The structures of mono and dimethyl derivatives of metsulfuron methyl were further confirmed by GC–MS.

For recovery studies from soil, two sets of 20 g soil were fortified with metsulfuron methyl at level of 0.1 and 0.2 μg g^{-1}. Each set was replicated thrice along with control. Soil samples were extracted with water, partitioned with dichloromethane and solvent evaporated to dryness (Paul et al. 2009). One set of the residues was analysed by HPLC using the reported method (Paul et.al. 2009), while to the second set of extracted residues, diazomethane ethyl acetate solution (0.5 mL) was added, stoppered (rubber) and kept for 24 h. After 24 h the stoppers were removed, the residues diluted with hexane and analysed by GC. In case of methylated samples the recoveries were calculated with respect to the peak area of methylated standard of known amount of metsulfuron methyl.

Result and Discussion

Due to thermal unstability and non-volatile nature, sulfonylureas are not detected by GC, although GC is considered as more sensitive technique than HPLC. There are two –NH– groups in metsulfuron methyl. Monomethyl derivatives of sulfonylureas are not very stable, thus for GC analysis, a dimethyl derivative was prepared. Two protons of sulfonylurea are quite acidic in nature and can be methylated by diazomethane. Etheral solution of diazomethane (Kulshrestha and Singh 1995) is commonly used for methylation. Metsulfuron methyl had poor solubility in ether. Therefore ethylacetate was choosen as the better solvent for methylation reaction. Solution of metsulfuron methyl was prepared in ethylacetate and diazomethane was also collected as solution in ethyl acetate.

For standardization of methylation time, reaction was monitored at different intervals by GC analysis. Parent herbicide does not respond to GC. Thus two peaks in GC at retention times 5.72 and 14.56 min were considered for mono and dimethyl derivatives of metsulfuron methyl (II and III, Fig. 1). As the reaction time increased relative area of the peak at 14.56 min. increased while the other peak at 5.72 min decreased (Fig. 2). The percentage of compound formed was arbitrarily calculated on the basis of peak area. The results of progress of methylation reaction are given in Table 1.

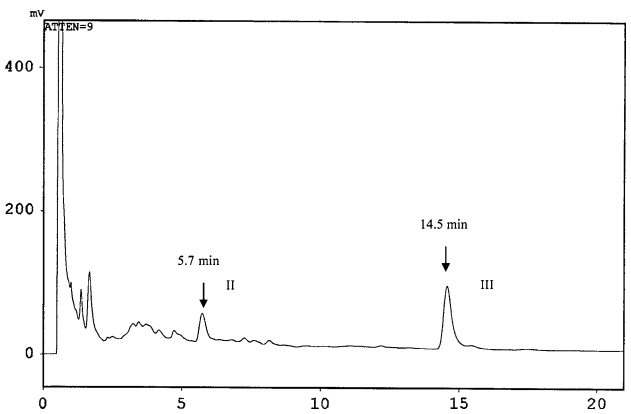

Fig. 2 GC chromatogram of the methylation reaction after 6 h

Table 1 Progress of metsulfuron methyl methylation reaction

Time (hours)	Monomethyl derivative (%)	Dimethyl derivative (%)
0	–	–
0.5	70	30
1.0	50	50
2.0	40	60
4.0	35	65
6.0	30	70
12.0	20	80
24.0	0	100
48.0	0	100

The percentage has been arbitrarily given on the basis of peak area

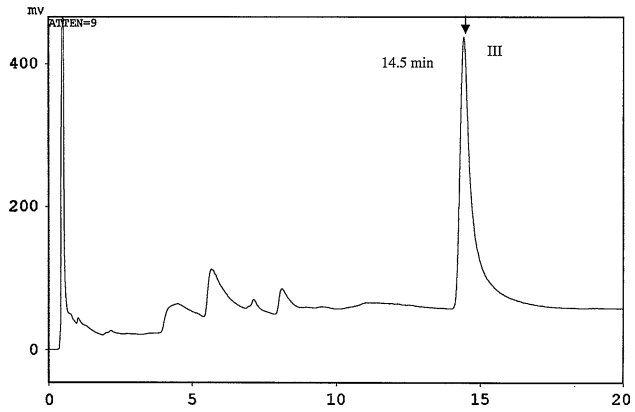

Fig. 3 GC chromatogram of the methylation reaction after 24 h

Nearly 70% of mono methyl and 30% of dimethyl derivative was formed just in 30 min. After 1 h reaction time, both the compounds were in equal proportions and finally after 24 h the peak at 5.72 min disappeared indicating the completion of the methylation reaction (Fig. 3). The reaction mixture was kept for 48 h also but there was no change in the peak area of dimethyl derivative. The probable structures of mono (II) and dimethyl (III) derivatives of metsulfuron methyl were proposed considering the more acidic nature of $–SO_2NH–CO–$ proton in comparison to –CO–NH– in the parent herbicide metsulfuron methyl (Fig. 1). The structure was further confirmed by GC–MS which showed M^+ at 395 and 409 for mono (II) and dimethyl (III) derivatives of metsulfuron methyl respectively. Reaction time 24 h was considered as the optimum methylation time for metsulfuron methyl with diazomethane.

Using the above methylation method and analysis by GC, recovery of metsulfuron methyl from fortified soil samples at 0.1–0.2 μg g^{-1} concentration was between 50.8–60.5 and 72–78% respectively (Table 2). The results

Table 2 Recovery of metsulfuron methyl from soil

S. no.	Fortification level (μg g^{-1})	Amount recovered (μg g^{-1})	Recovery (%)
1.	0.1	0.0605	60.5
2.	0.1	0.0514	51.4
3.	0.1	0.0508	50.8
4.	0.2	0.144	72.0
5.	0.2	0.1488	74.4
6.	0.2	0.156	78.0

were comparable to that of HPLC analysis. LOD and LOQ of the method were 0.1 μg mL^{-1} and 0.2 μg g^{-1} respectively.

Conclusions

Metsulfuron methyl can be analysed by GC after methylation by diazomethane. The technique is sensitive and can be used for micro-estimations. Using ethylacetate as the reaction medium for methylation is the best choice for complete methylation, as solubility of metsulfuron methyl in ether, a commonly used solvent for methylation by diazomethane, is very poor. More over any traces of ethyl acetate don't pose any problem in GC analysis. The optimum reaction time was found to be 24 h for complete methylation. As HPLC may not be accessible to many residue laboratories, the method can be utilized for residue analysis of metsulfuron from soil.

Acknowledgments Rupak Paul gratefully acknowledges the financial assistance provided by ICAR, New Delhi.

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