The RP-HPLC method development and validation for simultaneous determination of oryzalin and ethofumesate pesticides in soil and water

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Received: July 1, 2021 • Accepted: August 1, 2021

ABSTRACT

A sensitive and reliable method for simultaneous determination of oryzalin and ethofumesate residues in pantnagar soil and water was validated. The compounds were extracted by LLE with dichloromethane from water, and acetonemethanol mixture from soil followed by SPE cleanup. Detection and quantification was done by RP-HPLC using mobile phase methanol:water (70:30, v/v) at 280 nm. The developed method showed satisfactory validation results with linearity (0.99), relative standard deviations (1.55 and 1.73%), and limit of quantification (0.002 µg g⁻¹ and 0.005 µg g⁻¹) for ethofumesate and oryzalin, respectively. Recoveries ranged for oryzalin and ethofumesate from 79.80–90.52, 75.58–86.04% (soil) and 83.50–95.92, 82.28–94.60% (water), respectively. The method could be used for routine high-throughput detection and determination of these compounds.

KEYWORDS

method validation, oryzalin, ethofumesate, soil & water, HPLC

INTRODUCTION

Injudicious use of pesticides pollutes environment, as they enter the biosphere through different modes after being discharged on soil surface or canopy. Only 1% of the sprayed pesticides are effective, while 99% of pesticides applied are released to non-target soils, water bodies and atmosphere, and finally absorbed by almost every organism leading to contamination of environment, residues in soil, water and food, damage to soil microorganisms including earthworms, negative effect on plant biodiversity, toxicity to human and animals, shifts in weed flora and appearance of resistance in pests species [1–4].

Oryzalin (4-(dipropylamino)-3,5-dinitrobenzenesulfonamide) (Fig. 1) is surface-applied, selective herbicide of dinitroaniline group that acts by inhibiting cell division in germinating weeds. Oryzalin exhibits moderate to very high persistence in soil with half-life of two or more months. Oryzalin had been classified as a Group C carcinogen, causing human cancer, causes mammary gland tumors in females and skin and thyroid tumors in both sexes. It causes accumulation of an iron-containing pigment in the kidneys, an increase in the weights of several organs, affects blood, bone marrow and liver in mammals. It also possesses a high risk to non-target plants, including threatened and endangered plants [5]. Technical grade oryzalin has been shown to be in toxicity category IV for acute oral toxicity and category III for acute dermal and inhalation toxicity. Oryzalin poses a potential risk to endangered aquatic species that occur in shallow regions of water and also non target plants [6].

Ethofumesate [(±) 2-ethoxy-3,3-dimethyl-2H-1-benzofuran-5-yl methanesulfonate] (Fig. 1) is selective systemic herbicide belonging to benzofuran group. Ethofumesate
mode of action is related to inhibition of mitosis plus reduced photosynthesis and respiration. Ethofumesate is toxic to fishes and zooplanktons, having moderate persistence in soil and is a potential ground water contaminant [7, 8].

Pesticide monitoring in soil and water is essential to know the status of pesticide in environment. Individually, an oryzalin and ethofumesate residue in different matrixes has been reported using [9–19]. As per literature search no study had been reported on simultaneous determination of these pesticides residues from soil and water. Viewing the above facts, a sensitive analytical HPLC method capable of estimating in micro quantities from soil and water is required. In developing countries most of the laboratories don’t have/use the facility of gas/liquid chromatography tandem mass spectrometry for routine analysis and rely on the LC analysis for determination. The objective of this study is to improve extraction procedure and develop simple, sensitive method which can be used conveniently for determination of ethofumesate and oryzalin from soil/water simultaneously and provide information to laboratories which analyses pesticide contamination in environment or are interested in it.

EXPERIMENTAL

Instruments, chemicals and glasswares

Agilent 1120 Compact LC technologies HPLC (Agilent, USA), Buchi rotatory evaporator (Buchii, Switzerland), VacElut (Analytical Chemical International, USA), Double beam UV-VIS spectrophotometer (Systronics, India). Analytical grade oryzalin and ethofumesate of 99.3 and 99.2% purity, respectively were obtained from M/s CO PCCPL (Punjab Chemicals and Crop Protection Limited), India. All the chemicals used were of analytical reagent (AR) or HPLC grade purchased from E-Merck or Hi Media. The glasswares used were of Pyrex (USA), Schott Duran (Germany), and Borosil (India).

Determination of $\lambda_{\text{max}}$

A stock solution of oryzalin and ethofumesate (100 µg mL$^{-1}$) was prepared by dissolving 1.0 mg of oryzalin and ethofumesate in 10 mL of methanol and stored at $-20^\circ$C. Working solutions of 0.0002–10.0 µg mL$^{-1}$ were prepared by serial dilution of the stock solution with methanol. Ten mg L$^{-1}$ solution of analytical ethofumesate and oryzalin were scanned in the range of 200–400 nm using methanol as reference on UV-VIS spectrophotometer. The instrument operating condition was: Bandwidth: 0.5 nm, Mode: Scan, Scan speed: Slow.

Liquid chromatography condition

The HPLC condition used during the experiments was ODS II C-18 column (250 × 4.6 mm i.d.) 5 µm particle size, mobile phase methanol:water (70:30, v/v), UV detection 280 nm, flow rate 1 mL min$^{-1}$. The sample injection volume was 5 µL. The room temperature was 26 ± 1°C.

Soil and water samples

Soil samples was silty clay loam of 0–15 cm depth was collected from five randomly selected spots with the help of tube auger from Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, India. Water sample was collected from the borewell. The soil was air-dried, powdered, passed through 2 mm sieve and stored in polythene bags. Soil and water were analyzed for their physico-chemical properties by standard analytical procedure. All experiments were laid in completely randomized block design (CRD) fashion and treatments were replicated thrice. Data were subjected to analysis to determine standard deviation among the replicates.

Method validation

The method was validated by evaluating analytical curves and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (%RSD), recovery, precision (repeatability and intermediate precision), and specificity [20–23].
Analytical curve and linearity

The linearity of the instrument and the method was evaluated by analytical curves with the concentration levels of 0.002, 0.01, 0.1, 0.5, 1.0, 5.0 and 10.0 μg mL⁻¹ in pure methanol of standard ethofumesate and oryzalin and their peak areas were calculated. Five μL of each concentration was injected in HPLC in triplicate and average detector response in terms of area under the peak was used for preparation of calibration curve. A plot of peak area against concentrations and its respective standard deviation (SD) and coefficient of correlation was also calculated.

Limit of detection and quantification

Limit of detection (LOD) is the lowest amount of compound that can be detected with signal-to-noise ratio of 3:1 and limit of quantification (LOQ) is the lowest amount of compound which can be quantified by signal-to-noise ratio 10:1 with adequate precision and specificity. The LOD and LOQ of ethofumesate and oryzalin were calculated. The values were also checked experimentally.

Precision

Precision specifies random errors. Precision in terms of repeatability was obtained by carrying out the extraction and the analysis of fortified samples. Each spiked level, was extracted in three replicates and injected three times (n = 9). The intermediate precision was estimated in the same way as the repeatability, but on different days.

Specificity

Specificity of the assay was demonstrated by obtaining chromatograms for blank and observing the lack of interfering peaks at the retention time for the compounds. Specificity was performed to compare the standard ethofumesate and oryzalin in soil and water extracts. It was calculated by comparing the retention time of the peak, peak start, peak apex, and peak end of the standard and extracts. The spectral scan of both the standards and extracts were compared.

Accuracy

Accuracy is the concordance between true value of analyte in the sample and the value measured by the analytical process. Percent relative standard deviation was calculated to check the accuracy of the result. Results were expressed in percent relative standard deviation (% RSD).

Recovery

Recovery is measured as response of a processed spiked matrix standard with response of a pure standard which has not been subjected to sample pre treatment. For recovery five different concentrations of ethofumesate 0.005, 0.05, 0.10, 0.25, and 0.5 μg mL⁻¹ (n = 5) and oryzalin at 0.01, 0.5, 1.0, 2.5, and 5.0 μg mL⁻¹ (n = 5) was performed. Processed soil (250 gm) and ground water (250 mL) was taken in a glass tray/glass bottle. The soil/water was spiked with standard concentration (5 mL) of ethofumesate and oryzalin to get desired concentrations. Samples were extracted as standardized below. Peak areas of standard added to samples were calculated and average percent recovery was estimated.

Extraction procedure

Surface soil (0–20 cm depth) was shade dried, powdered and sieved through 2 mm sieve. Acetone: methanol (75 mL, 5:1 v/v ratio) solvent mixture was added to fortified soil sample (20 gm) and samples were shaken on an orbital shaker for 30 min and the process was repeated three times. The extract was filtered, pooled and transferred into 500 mL separatory funnel and 100 mL of 5% sodium chloride was added. The solution was washed twice with 25 mL n-hexane. The hexane washing were discarded and the aqueous phase was partitioned thrice with 50 mL dichloromethane. Organic layer was passed through anhydrous sodium sulphate and the solvent was removed by rotary vaccum evaporation at 40 ± 1°C.

Water samples were taken from borewell. Fortified/spiked water sample (50 mL) were partitioned with 100 mL (50 + 30 + 20) dichloromethane. The organic layers were collected, pooled, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure at 40 ± 1°C. The residues were dissolved in 1 mL HPLC methanol (100%). The samples were filtered through 0.22 μm PTFE disc filter prior to HPLC analysis. No cleanup was required.

RESULTS

Soil and water were analysed for their physico-chemical properties as per standard procedure showed that texture was clay loam having alkaline pH and rich in organic carbon content. Water was slightly alkaline in nature, electrical conductivity (0.90 mS m⁻¹), high in total dissolve solids, and hardness (Table 1). The UV absorption spectrum revealed that absorption maximum (λ_max) of ethofumesate was 221.2 and 277.6 while maximum absorbance of oryzalin was at 238.5 and 280.0 nm. The detector was kept at 280 nm for HPLC conditions for analysis of mixture of both herbicides in soil and water with good resolution (Fig. 2).

Validation method

Limit of detection (LOD) and limit of quantification (LOQ). The instrument detector gave response for 0.0002 ppm of ethofumesate and oryzalin solution when 5 μL of pure sample (extraction without matrix/solutions before extraction) were injected so, the limit of detection (LOD) for ethofumesate and oryzalin was 0.001 μg g⁻¹. A good quantifiable peak was observed when 5 μL of 0.0004 ppm of ethofumesate and 0.001 ppm of oryzalin were injected so, limit of quantification (LOQ) for ethofumesate and oryzalin (extraction of compound with matrix) were established to be 0.002 μg g⁻¹ or mL⁻¹ and 0.005 μg g or mL⁻¹, respectively (Fig. 3).
Calibration curve/linearity. Calibration curve of ethofumesate and oryzalin were linear and value of determination coefficient \( R^2 \) for each was 0.999 that represented that the calibration curves were satisfactory and concentration of herbicides dependent on detector response.

Accuracy/relative standard deviation (RSD). Percent relative standard deviation (% RSD) was calculated to check the accuracy of the result. The %RSD value is based on the spread of results compared to the average. The accuracy of the method was found to be good. Accuracy of the method was deemed acceptable if the value of percent RSD value is lower than 2. In our study the value percent RSD of ethofumesate and oryzalin were found to be 1.55 and 1.73%, respectively. Low percent (<2.5%) means the small spread of results indicating the good accuracy of calibration curve.

Specificity and retention time. Under the optimised condition as written above, the compounds were well separated and retention time of ethofumesate and oryzalin were found to be 5.4 and 6.6 min. The chromatogram showing peak of ethofumesate and oryzalin is shown in Fig. 4. Since there was no interfering matrix peak showing good specificity of the method.

Recovery study. The recoveries at 0.005–0.50 ppm concentration for ethofumesate and oryzalin in soil sample varied from 75.58–86.04 and 79.80–90.52%, respectively while, in water it were 82.28–94.60 and 83.50–95.92%, respectively (Table 2). The intermediate precision ranged from 72 to 91% with RSD 2.3658%–2.9154%.

### DISCUSSION

The simultaneous detection of ethofumesate and oryzalin by the HPLC method can be performed as there is noteworthy difference in their retention time and proper peak separation. The mobile phase methanol: water (70:30, v/v) was adequate for proper peak separation and accurate analysis. The wave length selected (280 nm) permits good detection without loss in sensitivity of both herbicides. All the values were in the acceptable range for detection and trace residue analysis which is usually between 70 and 120%, RSD 20% maximum and LOQ according to MRL (maximum residue limit) of the compound [22]. Scientists had reported earlier,

### Table 1. Physico-chemical properties of soil and water

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<thead>
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<th>Soil</th>
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<td>pH</td>
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<td>Organic carbon (%)</td>
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<tr>
<td>Water Holding Capacity (%)</td>
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<td>Electrical conductivity at 19°C (dS m(^{-1}))</td>
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<td>Temporary hardness (ppm)</td>
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**Fig. 2.** UV-Vis absorption spectrum of ethofumesate (a) and oryzalin (b)

**Fig. 3.** Limit of quantification of ethofumesate and oryzalin

**Fig. 4.** Chromatogram showing ethofumesate and oryzalin separation by HPLC
single method of analysis for both herbicides and the detection limit of oryzalin residues from soil/water by analytical methods ranged from 0.001 to 0.05 μg/g and recovery ranged from 87 to 91% for soil and 70–94.7% for water while, for ethofumesate the detection limit were 0.0001–0.0005 μg/g by different chromatographic methods, and percent recovery ranged from 78 to 94% for soil and 90–96% for water, respectively [11, 12, 14, 15, 16].

### CONCLUSION

The method developed and validated was simple, efficient and sensitive. The method is equally versatile as that of single method of analysis of these pesticides and can detect both pesticides simultaneously without compromise in recovery and sensitivity. The recovery, linearity, calibration, accuracy and precision shows that method is rapid, accurate and precise for the determination of oryzalin and ethofumesate residues simultaneously in soil and water samples with less or no interference of matrix. The total optimized method is therefore useful for both qualitative and quantitative analysis in routine assays by agrochemicals industry and institutes within acceptable limits.

*Application of the proposed method:* The method developed is rapid, accurate and sensitive with high-throughput trace level detection of both active ingredients simultaneously, in soil and water for the purpose of routine detection and determination of pesticide residue analysis by agrochemical and research laboratories.

Conflict of interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGMENTS

The authors are thankful to PCCPL (M/S Punjab Chemicals & Crop Protection Ltd., India) for providing analytical grade and formulation of metamitron for research purpose. Authors are also thankful for G.B. Pant University of Agriculture & Technology authorities for providing necessary facilities for doing thesis research work.

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