

DETERMINATION OF PENDIMETHALIN HERBICIDE IN WATER AND VEGETABLE SAMPLES BY MICROWAVE-ASSISTED SOLVENT EXTRACTION AND HPLC METHOD

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ABSTRACT

A method for High Performance Liquid Chromatography (HPLC) for the residue determination of pendimethalin herbicide in water and food samples was developed. The chromatographic analysis was carried out, on a C₁₈ packed capillary column with gradient elution, 20 µl injection volume and ultraviolet detector at 240 nm. Samples were spiked with amount between 5µg g⁻¹ and 15µg g⁻¹ of herbicide and were isolated from samples by applying microwave assisted extraction (MAE) acetone, ethanol and water as extraction solvent. Percent recoveries were improved by optimizing solvent types, solvent volume and time. Calibration curve range determined by HPLC was 0.2-40µgm L⁻¹. The interaction of different variables for maximum % recovery response was found to be in range from 91.22±0.01-99.32±0.01. Application of this procedure to the analysis of herbicide in water and vegetable samples showed the effectiveness of the methodology proposed.

Key words: MAE, pendimethalin, water, vegetable samples, HPLC.

INTRODUCTION

The intensive use of herbicides and other classes of chemicals products, in agricultural practice has resulted in serious impacts on the environment, causing an increase in the level of herbicide residues in natural water, soil, and foodstuffs. Herbicide pendimethalin *N*-(1-ethylpropyl)-2, 6- dinitro-3-4 xylidine is dinitroaniline group-containing compound used as selective herbicide to control annual grasses and broad-leaved weeds in cultivation of corn, potato, rice, wheat, onion, tomato, soya bean, cabbage, and pepper (Andressa *et al.* 2011; Lin *et al.* 2007). Pendimethalin is available in markets as the preparation of Stomp 330E or mixed with other herbicide. Pendimethalin degrades slowly in aerobic soil and rapidly in anaerobic soil conditions. Acting as a microtubule inhibitor, the dinitroaniline herbicide pendimethalin has been used in agricultural applications as a selective pre-emergence

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herbicide in various plant cultures. Field dissipation studies by (Kol, 2002) have revealed that pendimethalin is persistent, and its half life is 98 days at 30°C. Pendimethalin is classified as a non-leaching compound (Kaleem, 2006) Toxicological report of pendimethalin by shows that pendimethalin causes liver and kidney damages and number of mutagenic effects (Dimitro *et al.*, 2006).

The methods available in the literature for determination of pendimethalin are mostly based on gas chromatography (Engebretson *et al.*, 2001; Bruzzoniti *et al.*, 2006; Shen *et al.*, 2007; Arora *et al.*, 2008; Zhang *et al.*, 2008). The degradation products metabolism of pendimethalin was measured by (Jenson *et al.*, 2007; Ramakrishna *et al.*, 2008).

The purpose of the present study was to develop simple, rapid and sensitive method for the analysis of pendimethalin using HPLC system with UV/Visible detector.

MATERIALS AND METHODS

Instruments

The Perkin Elmer Series 200 CA, USA with HP series 1100 LC system was used. The system equipped with gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop and ultraviolet detector at 240 nm was used. Separation column Zorbax SB- C₈ (80 Å, 4.6 X 150 mm, 5mm particle size) with a Zorbax C₁₈ (4x4 mm) as a guard cartridge. Microwave extractions were performed with KEN ST/SS25 KENWOOD (China) microwave with temperature control system.

Reagents

Pendimethalin, N-(1-ethylpropyl)-2, 6-dinitro-3-4 xylidine 99% was purchased as standards from Dr. Ehrenstofer GmbH, Germany. HPLC-grade acetonitrile and ethanol were obtained from Rathburn, Walkerburn, UK. LC grade water was used by purifying distilled water with a Milli-Q water purification system (Millipore Co, USA). All other chemicals used were of analytical-reagent grade purity obtained from Merck (Darmstadt, Germany).

Standard pendimethalin solution (1000 µg mL⁻¹)

Stock solution of pendimethalin was prepared by dissolving 10 mg of pendimethalin in 10 mL of acetonitrile and diluted with acetonitrile. Working standards were prepared from this stock solution in range of 2-40 µg mL⁻¹ by dilution with acetonitrile.

Extraction from samples

For the analysis of pendimethalin in water, 10 mL of water, passed through filtration screen and homogenized. Fortified samples were prepared by adding 5, 10 and 15 µg g⁻¹ of standard pendimethalin solution to water sample. After fortification 10 mL of acetone was

added and to get high extraction rate, microwave rapid heating were used on closed-vessel during a sample extraction procedure. The starting parameters setting in microwave system were 2.0 min at 350 W and 3.0 min at 500 W. Once the extraction program was completed the vessel were cooled down to room temperature before opening the vessel. After extraction the contents were centrifuged for 10 min and filtered. On equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 2 mL then 5mL of methanol was added to it centrifuged and passed through 0.45 μm pore. The extract was evaporated near to dryness and redissolved in 10 mL of acetonitrile before HPLC analysis.

Similarly for extraction from vegetable samples, fortified vegetable samples were prepared by adding 5, 10 and 15 $\mu\text{g g}^{-1}$ of standard pendimethalin solution to onion sample. After fortification the samples were kept at room temperature for 6.0 h to allow evaporation of the solvent. For microwave extraction vegetable samples (10g) were added to extraction vessels and 10 mL of acetone was added. In case of vegetable samples were kept in closed vessels and heated for 2-3min at optimized temperature in a microwave. This was followed by filtration and determination as above. Similarly for water samples the same optimized extraction procedure was applied to 10 mL of water samples.

Optimization of extraction procedure for determination of pendimethalin using reverse phase HPLC

After optimization of instrumental and physical parameters it was important to optimize extraction procedure for pendimethalin. For this purpose the effects of various experimental parameters of mechanical extraction and microwave extraction (MASE) were studied. Parameter optimized were solvent type, ratio of mixtures of solvents, MASE time, and temperature.

Pendimethalin is partially soluble in water and highly soluble in acetone ethanol and methanol. These solvents were used for extraction of pendimethalin from water and vegetables samples. For investigation of the effect of temperature for MASE on the extraction efficiency of, extractions pendimethalin were carried out at 25, 40, 60, 80 and 100 $^{\circ}\text{C}$, respectively. Similarly for the effect of extraction time, extraction of spiked samples was carried out at 60 $^{\circ}\text{C}$ for 3, 5, 7, 9 and 11 minutes, using acetone as the extraction solvent.

RESULTS AND DISCUSSION

HPLC method was developed for determination of pendimethalin using reverse phase Zorbax SB-C₈ column. Sample injection was 20 μL . The mobile phase consisted of acetonitrile and water. The gradient elution was carried out according to the

programme (5 min at 80% acetonitrile with 20% water and 12 min for 100% acetonitrile and 0% water). The flow rate was kept at 1 ml min⁻¹. The ultraviolet detector was adjusted at 240 nm for absorption measurement.

Preliminary experiments were performed to select the optimum operation conditions for the microwave-assisted solvent extraction of pendimethalin. The parameters like temperature, time and solvent type for extraction were optimized.

Optimization of Solvent for MASE

Selection of solvent for extraction is important because it affects the percent recoveries of herbicides. Pendimethalin is partially soluble in water and highly soluble in acetone, ethanol and methanol. These solvents were used for extraction of pendimethalin from soil and vegetables. The maximum extraction was achieved with acetone as extraction solvent (Fig. 1). The % recoveries were in the range of 70-78 in mechanical extraction and were improved to 92-97% by microwave assisted solvent extraction in different samples.

Optimization of temperature

The temperature of microwave irradiations control the energy supplied to the sample under analysis. To study the effect of extraction temperature on the extraction efficiency of pendimethalin, extractions were carried out at 25, 40, 60, 80 and 100 °C, respectively. The results of extraction recoveries are shown in Fig. 2. The recovery of pendimethalin increased as the temperature of extraction increased from 25°C to 60°C and the recovery decreased above 60 °C. This is probable due to degradation of pendimethalin at higher temperatures.

Optimization of time

For optimization of time of extraction spiked samples were extracted at 60 °C after 3, 5, 7, 9 and 11 min. using acetone as the extraction solvent. When the extraction time was increased from 3 min to 11 min the recovery of pendimethalin decreased (Fig. 3). This is due to degradation of pendimethalin caused by increasing irradiation time. Therefore, 3 minutes irradiation time was selected for MASE.

Construction of calibration plot

Calibration curve was obtained at concentration range 2.0-25 µg mL⁻¹ using HPLC with ultraviolet detector at 240 nm (Fig. 4). The linearity of the curve was verified by the correlation coefficient of 0.995. The limits of detection (LOD) and limits of quantification (LOQ) were calculated using the following formula.

$LOD = 3s/b$ and $LOQ = 10s/b$; where b is slope of the curve. The LOD and LOQ were found to be 0.059 µg mL⁻¹ and 0.17 µg mL⁻¹, respectively.

Application of the developed method

The control samples of, water and onion were spiked at three levels (5, 10 and 15 $\mu\text{g g}^{-1}$) of pendimethalin concentration and were analyzed in triplicate. The results are given in Table-2 and chromatograms are shown in Fig. 6 (a, b) and 7 (a, b). The method validation studies for spiked samples indicated that the MASE method gives good recoveries and precision for pendimethalin at three levels (5, 10, 15 $\mu\text{g g}^{-1}$).

The recoveries were found to be in the range of 90-100 % for soil samples and 97-100 % for onion samples and 92-99% for water samples. The developed microwave-assisted solvent extraction procedure using acetone as extracting solvent for extraction of pendimethalin from water and vegetable samples was found to be the best for water and vegetables samples. The results indicated that the MASE method is precise and efficient. MASE are now preferred over the traditional laborious techniques of blending, shaking or soxhlet extraction of analyte. Chromatogram of 10 $\mu\text{g mL}^{-1}$ of standard solution of pendimethalin is shown in Fig. 5. Chromatograms 5 and 15 $\mu\text{g g}^{-1}$ spiked water samples in Fig. 6 (a and b) chromatogram for 5,15 $\mu\text{g mL}^{-1}$ spiked onion samples are shown Fig. 7 (a and b).

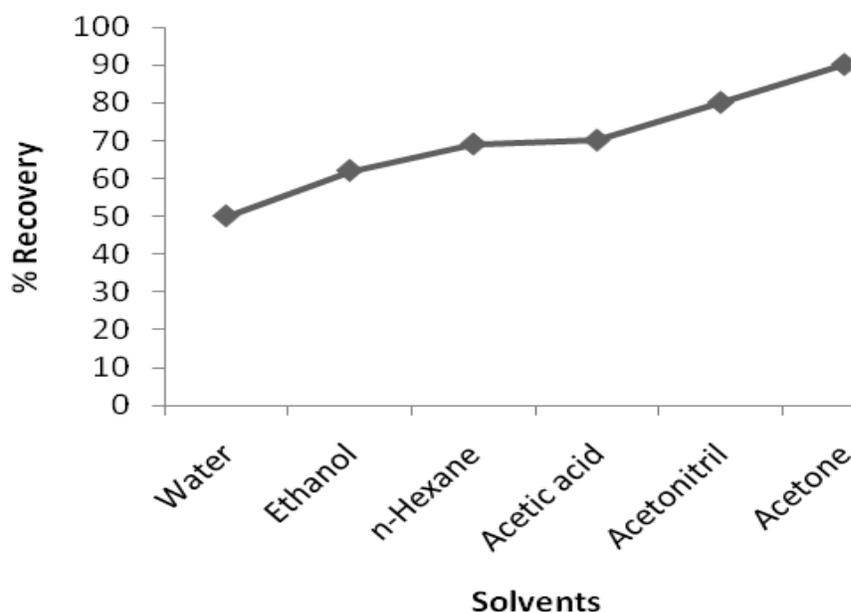


Figure 1. Investigation of suitable solvent for pendimethalin extraction.

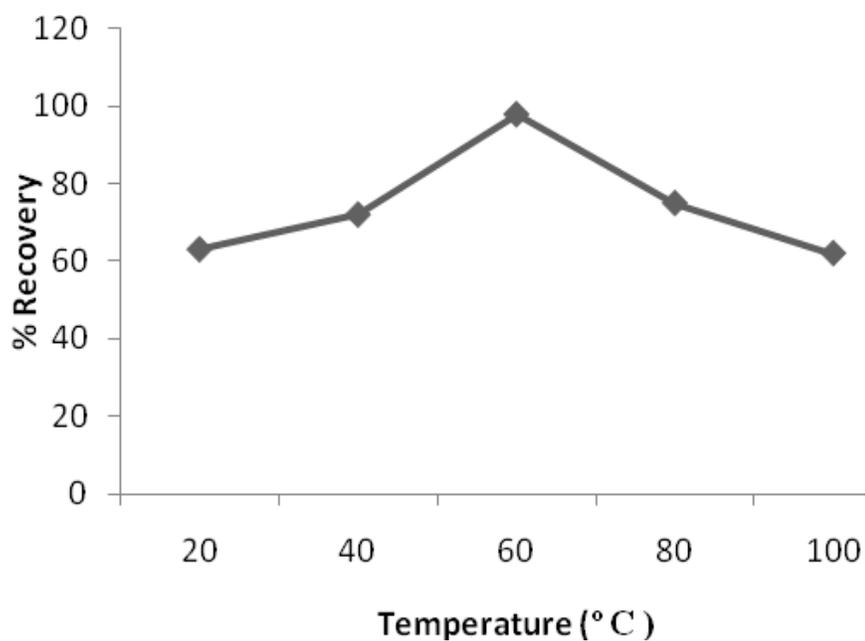


Figure 2. Optimization of temperature using MAE for maximum % recovery of pendimethalin.

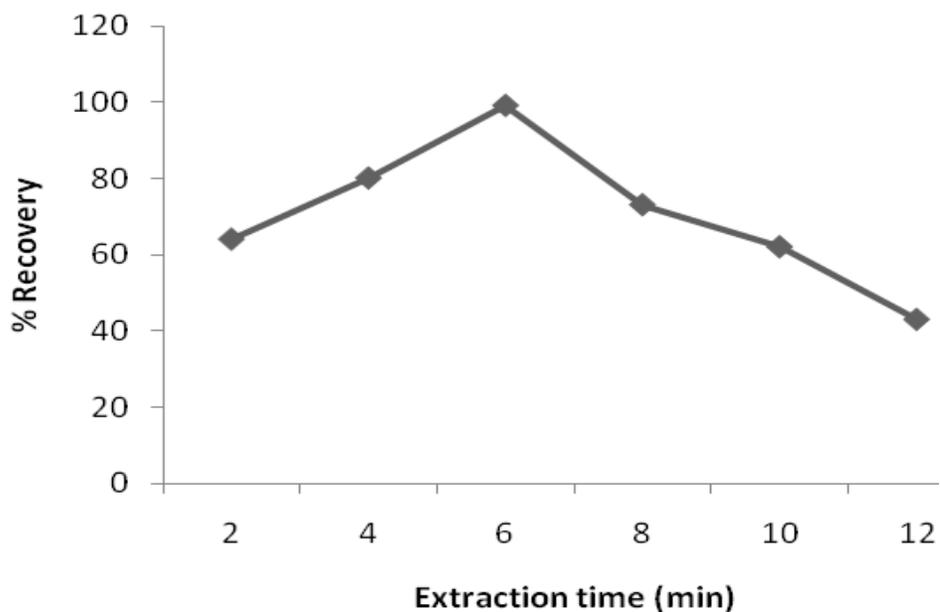


Figure 3. Optimization of extraction time for MAE.

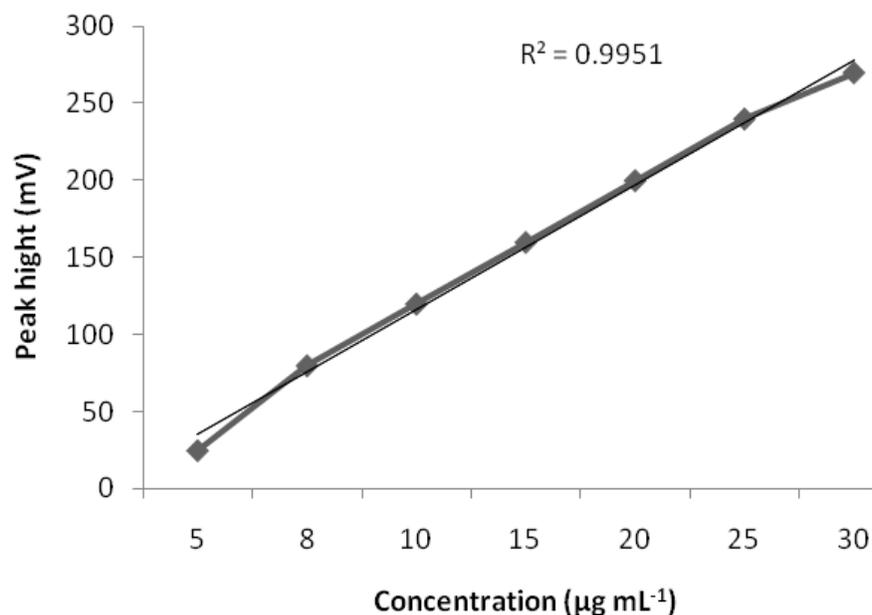


Figure 4. Calibration plot for pendimethalin determination using HPLC.

Table-1. Percent recovery of herbicide from fortified sample.

S.N	Spiked Sample	Added ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)		% Recovery		Average % Recovery	
			ME	MAE	ME	MAE	ME	MAE
01	Vegetables	5	4	5.2	80 \pm 0.38	104 \pm 0.76	83.3 \pm 5.7	98 \pm 2.3
		10	9	9.75	90 \pm 0.41	97.5 \pm 1.15		
		15	12	15.10	80 \pm 0.21	100.6 \pm 1.5		
02	Water	5	4.5	5.12.5	90 \pm 0.37	102.0 \pm 0.7	87.3 \pm 8.9	99.6 \pm 4.7
		10	9.5	9.25	98 \pm 0.27	92.5 \pm 0.43		
		15	14	14.75	97 \pm 0.15	98.3 \pm 0.15		

ME (Mechanical Extraction)

MAE (Microwave Assisted Extraction)

Table-2. Residue level of herbicide found in real sample.

S.N	Samples	Residue $\mu\text{g g}^{-1}$
1.	Water	1.97 \pm 1.24
2.	Onion	1.31 \pm 2.18
3.	Cabbage	1.41 \pm 1.26

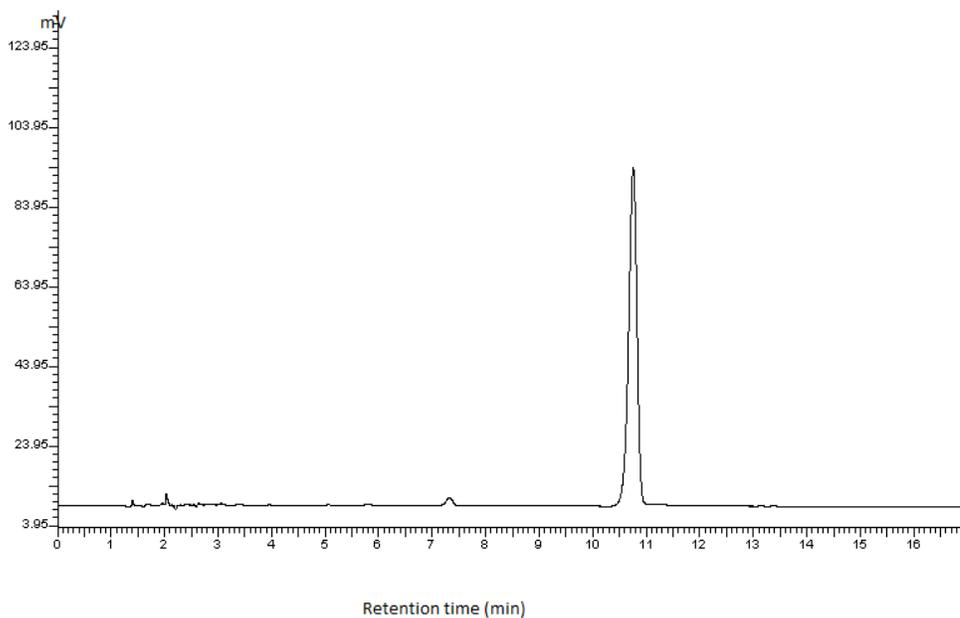


Figure 5. Chromatogram of standard pendimethalin ($10\mu\text{g mL}^{-1}$) using reverse phase HPLC.

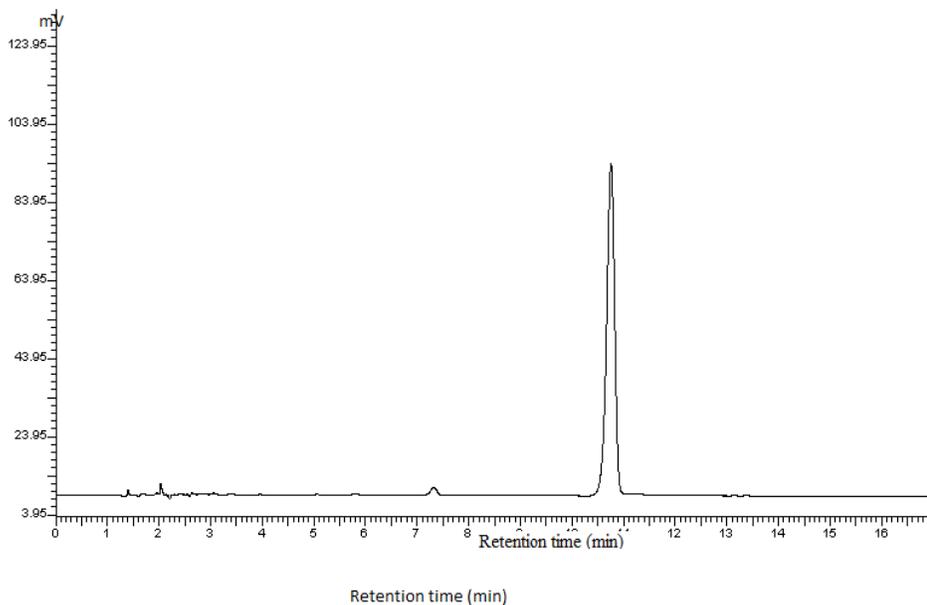


Figure 6 (a). Chromatogram for $5\mu\text{g mL}^{-1}$ fortified water samples using reverse phase HPLC.

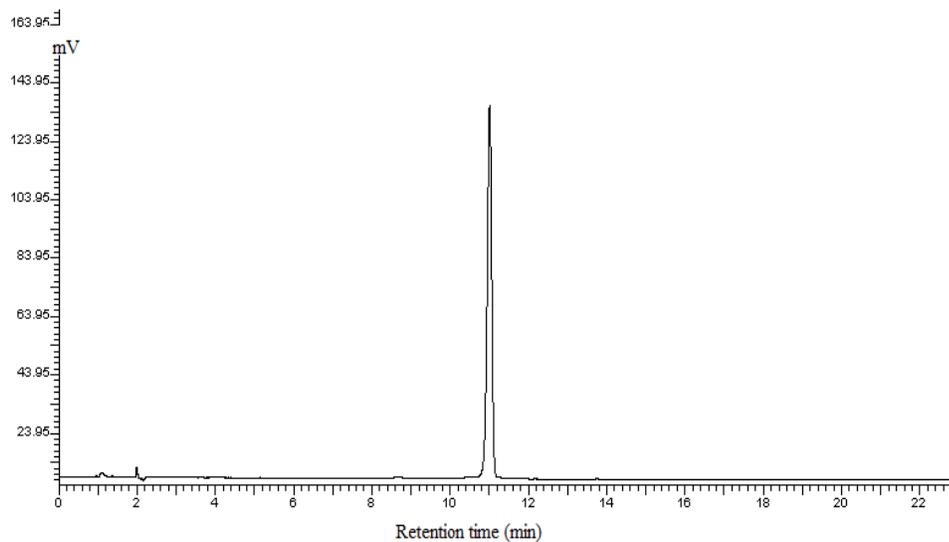


Figure 6 (b). Chromatogram for 10 $\mu\text{g mL}^{-1}$ fortified water samples using reverse phase HPLC.

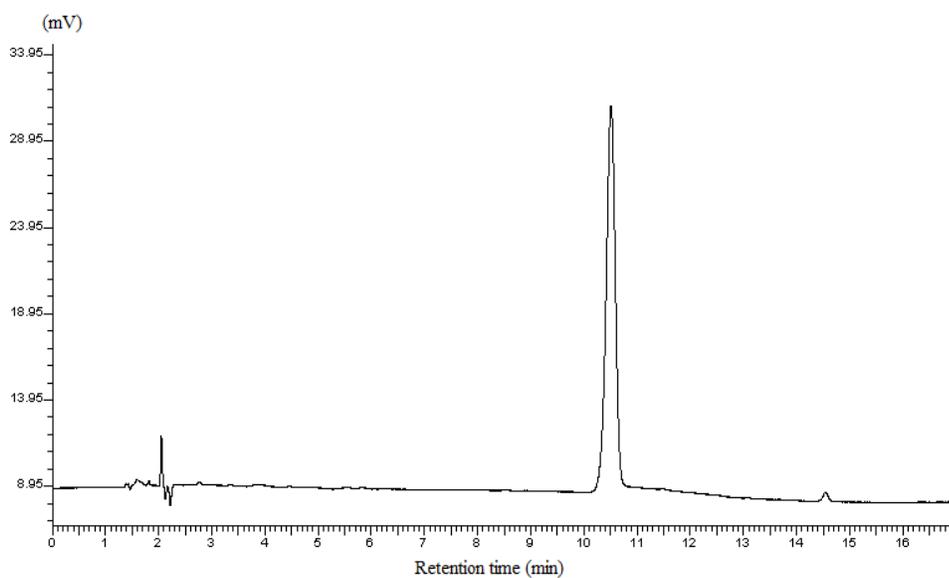


Figure 7 (a). Chromatogram for 5 $\mu\text{g mL}^{-1}$ fortified onion samples using reverse phase HPLC.

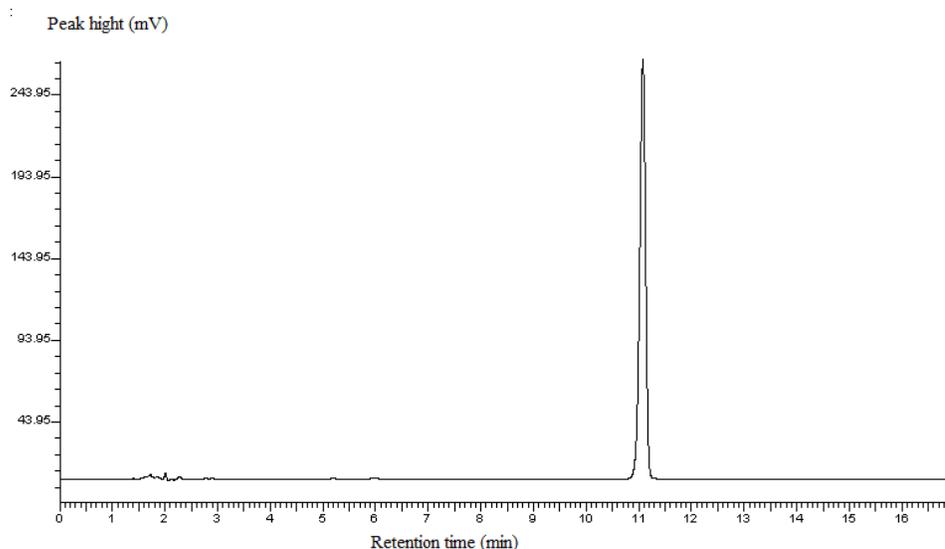


Figure 7 (b). Chromatogram for 15 $\mu\text{g mL}^{-1}$ fortified onion samples using reverse phase HPLC.

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