Analytical Method Development for Imazapyr and Imazapic Herbicides using High Performance Liquid Chromatography

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***Abstract*—**The mixture of imazapyr and imazapic herbicides was recently introduced to kill the weedy rice and increase the rice yield in agricultural areas around the world. Recent studies have proven these herbicides as a potential risk to non-target organisms and eliciting the risks to humans as well. Applications of imazapyr have been discontinued in Norway and France due to its persistency in soils. Its residues were also detected in groundwater in Sweden after 8 years application to the related crops. However, previous studies only manage to detect a single compound of the herbicide. Therefore, a simple and rapid method was developed for the quantitative simultaneous determination of imazapyr and imazapic herbicides residues in water by using Agilent 1200 HPLC equipped with UV detector. This method allows the separation of these two analytes in a sample in 6 minutes. Reverse phase HPLC was performed using an Agilent Zorbax SB-C18 (4.6 x 250mm, 5µm) column in the gradient mode where mobile phase A consisted of Acetonitrile (100%) and mobile phase B consisted of water acidified with 10% acetic acid (pH adjusted to 2.8). The best peak separation was identified at the ratio of 35:65 v/v. The highest peak area for imazapyr and imazapic was detected at 251nm, a flow rate of 1.0 ml/min and injection volume, 17µL. In the method development step, the herbicides was dissolved in water individually before mixing and quantified by injecting the sample into HPLC system without undergoing any clean-up process. The retention time for imazapyr and imazapic in this system was 3.62 and 4.66 min, respectively. The lowest detection for imazapyr was 10 ppt while imazapic was 1ppb. The developed method was very simple, precise and can be used for routine monitoring of the herbicide in surface water especially for the irrigation water in a rice field to reduce the costing of weeding management.

***Keywords*—**herbicide, HPLC, imazapic, imazapyr, water.

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I. Introduction

IMIDAZOLINONE herbicides are widely used for weed control in many crops such as paddy rice, corn and wheat that functions by inhibiting the action of plant enzyme acetohydroxyacid synthase (AHAS), which causes the weed to stop growth and dies [1], [2]. AHAS is a key enzyme in biosynthesis of branched amino acid in plants [2] but absent in animals and humans. Imidazolinone herbicides are assumed to be environmentally safe because of their low application rate and low toxicity to mammals [3]. In view of this, the herbicide formulation containing imazapyr [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid] and imazapic [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid] was introduced in Malaysia to control the weedy rice in paddy fields [4], [5].

However, there are several environmental concerns regarding imidazolinone herbicides. Imazapyr had been prohibited in Norway [6] and France [7] due to its persistency in soil. In addition, imazapyr residues were detected in groundwater in Sweden after 8 years application to the related crops [8]. Up to now, no analytical method for the simultaneous determination of these compounds in the environment has been reported. Therefore, this paper presents a simple and sensitive HPLC procedure for analysis of imazapyr and imazapic in water where the analyses were performed without clean-up of the water samples and can be used for routine monitoring of herbicide in surface water.

II. Experimental

A. Chemicals, reagents and apparatus

Standards of imazapyr and imazapic with 99.5% and 99.9% of purity respectively were purchased from Sigma-Aldrich. Acetic acid and acetonitrile with HPLC grade was purchased from Merck (Malaysia). All solutions were prepared with ultra pure deionized water (Milli-Q water, Millipore). 0.2µm Whatman membrane filter were used for HPLC analysis.

B. Preparation of stock solutions

Standard stock solutions of the two herbicides, imazapyr and imazapic, were made individually in ACN and diluted

into several concentration range from 50 mgL^{-1} to 10 ngL^{-1} before mixed and stored below 4°C.

C. Water fortification

Tap water sample were spiked with the mixed standard of imazapyr and imazapic to obtain aqueous solution with range 5 mg/L to 0.05 mg/L in concentration and then directly injected into HPLC for quantification without undergoing any treatment.

D. Reverse Phase HPLC Analysis

An Agilent 1200 HPLC equipped with UV detector was used to quantify simultaneously imazapyr and imazapic in the spiked samples. Chromatographic separation was performed using an Agilent Zorbax SB-C18 (4.6 x 250mm x 5µm) column in the gradient mode where mobile phase A consisted of Acetonitrile (100%) and mobile phase B consisted of water acidified with 10% acetic acid (pH adjusted to 2.8). The mobile phase, wavelength, flow rate and injection volume were varied to obtain the best separation with highest peak area for imazapyr and imazapic. The optimization of the HPLC was conducted prior to analyzing of the spiked sample.

III. Result And Discussion

A. Determination of HPLC Condition

After varying the mobile phase, wavelength, flow rate and injection volume (Table I), the best separation peak for these two herbicides was identified at the ratio of 35 (mobile phase A):65 (mobile phase B) v/v. The highest peak area for imazapyr and imazapic was detected at 251nm with flow rate, 1.0 ml/min and injection volume, 17µL. The retention time for imazapyr and imazapic in this system was 3.62 and 4.66 min, respectively. The peaks position from the mixed standard was compared to the individual peak point for imazapyr and imazapic with the same wavelength, flow rate and injection volume. For this HPLC system, the lowest detection for imazapyr was 10 ngL^{-1} while imazapic was 1 μgL^{-1}.

TABLE I
SUMMARY OF SIMULTANEOUS DETERMINATION OF IMAZAPYR AND IMAZAPIC[a]

Mobile Phase (A:B, v/v)	Peak Area (mAU), Retention Time (min)	
	Imazapyr	Imazapic
- 40:60[b]	1286.33, 3.32	1655.04, 4.05
- 35:65[c]	1286.62, 3.62	1638.33, 4.66
- 30:70[b]	1279.50, 4.08	1631.50, 4.65

[a]The flow rate, 1.0 mL/min; wavelength, 251nm; injection volume, 17µL
[b]Peak for both analytes showed good separation, however not sharp
[c]Peak for both analytes showed good separation and sharp

B. Evaluation of spiked water samples

Imazapyr and imazapic in water was quantified by injecting the samples into HPLC without undergoing any treatment. The recovery data (>85.00%) obtained from three determinations for each concentration examined are shown in Table II.

TABLE II
RECOVERY OF IMAZAPYR AND IMAZAPIC FROM SPIKED WATER SAMPLES

Spiking Level (mg/L)	Imazapyr		Imazapic	
	Recovery (%)	SD (%)	Recovery (%)	SD (%)
5.00	98.68 (99.68,98.42,97.94)	0.90	98.69 (99.92,97.98,98.18)	1.07
0.50	94.13 (91.60,97.40,93.40)	2.97	92.33 (87.60,91.80,97.60)	5.02
0.05	92.67 (86.00,98.00,94.00)	6.11	92.00 (92.00,96.00,88.00)	4.00

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