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ANALYTICAL METHOD DEVELOPMENT FOR IMAZAPIC HERBICIDE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(Pembangunan Kaedah Analitikal Bagi Racun Rumpai Imazapic Menggunakan Kromatografi Cecair Prestasi Tinggi)

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Abstract

A reliable method using High Performance Liquid Chromatography (HPLC) was developed for the optimization of imazapic herbicide extraction and clean-up method from water. For this purpose, an Agilent HPLC 1200 Series fitted with a UV detector was used. The column used was a Zorbax StableBond C_{18} (4.6mm x 250mm I.D., 5µm particle size). For the extraction and clean-up procedure, a solid-phase extraction (SPE) method was applied. Two types of cartridges were chosen namely styrene-divinylbenzene polymer bond elut PPL (PPL) and octadecyl/ silica bond elut C_{18} (C_{18}), for comparison of the recovery performance. Good resolution of imazapic was optimum at λ=252nm wavelength. The mobile phase was acetonitril: ultra-pure water (pH 3.0), in the ratio of 45:55. The flow rate of the mobile phase and injection volume were 1.2 mL/min and 20 µL, respectively. Efficacy of extraction method used was determined through recovery test conducted using water samples spiked with imazapic standard. The recovery was 112% and 73% for C_{18} cartridge and 10.35% and 10.14% for PPL at concentration of 2 and 10 mg L^{-1}, respectively. In conclusion, C_{18} cartridge was chosen as the best cartridge for the extraction, clean-up method and determination of imazapic in water.

Keywords: bond elut C_{18} (C_{18}), bond elut PPL (PPL), High Performance Liquid Chromatography (HPLC), imazapic, solid - phase extraction (SPE)

Abstrak

Satu kaedah boleh dipercayai menggunakan Kromatografi Cecair Prestasi Tinggi (KCPT) telah dibangunkan untuk mengoptimumkan pengekstrakan dan prosedur pembersihan racun rumpai imazapic dari sampel air. Untuk tujuan ini, sebuah Agilent KCPT 1200 Siri dilengkapi dengan pengesan UV telah digunakan. Turus yang digunakan adalah Zorbax StableBond C18 (4.6mm x 250mm ID, saiz zarah 5µm). Untuk pengekstrakan dan prosedur pembersihan, kaedah pengekstrakan fasa pepejal (SPE) telah digunakan. Dua jenis kartrij telah dipilih iaitu stirena-divinylbenzene bond elut polimer PPL (PPL) dan oktadesil / silika bond elut C_{18} (C_{18}) bagi membandingkan prestasi pemulihan mereka. Resolusi imazapic yang baik adalah optimum pada ketika panjang gelombang λ=252nm. Fasa bergerak adalah acetonitril: air ultra-tulen (pH 3.0), dalam nisbah 45:55. Kadar aliran dan jumlah suntikan ialah 1.2 mL/min dan 20 µL, masing-masing. Keberkesanan kaedah pengekstrakan yang digunakan ditentukan melalui ujian pemulihan yang dijalankan menggunakan sampel air yang dicampurkan dengan standard imazapic. Keputusan bagi kadar pemulihan adalah 112% dan 73% untuk kartrij C_{18} dan 10.35% dan 10.14% untuk PPL pada kepekatan 2 dan 10 mg L^{-1}, masing-masing. Kesimpulannya, kartrij C_{18} dipilih sebagai kartrij yang terbaik untuk pengekstrakan, kaedah pembersihan dan penentuan imazapic dalam air.

Kata kunci: Bond elut C_{18} (C_{18}), Bond elut PPL (PPL), Imazapic, Kromatografi Cecair prestasi Tinggi (KCPT), Pengekstrakan fasa pepejal (SPE).

Introduction

For decades, the pattern of pesticide usage in agriculture industry has steadily increased both globally and simultaneously with the increase of population growth in order to meet the demands of food production [1 - 4]. Almost 30% global pesticide usage is in the developing country [3] with Malaysia alone consuming more than 200000 tons pesticide annually comprising of more than 50000 tons of active compounds [5]. Pesticide is a well-known artificially synthesized substance used in agriculture practices as an effective way to increase the quantity and quality of food production by controlling pests, plague and weeds, all of which can be harmful to crops and reduces productivity [2,3,6]. There are different classes of pesticides, depending on its purpose or target organism, and this includes herbicide, insecticide, fungicide, nematicide, and bactericide [1,6]. Herbicide is preferred by the majority of the agricultural community due to its advantages in high-yield crops as it helps the farmers to remove crop competing weeds without the need of farm labourers [7]. One such herbicide is Imazapic, a compound that belongs to the imidazolinone family [2 - 4, 8 - 12]. Imazapic is a well-known herbicide used by farmers to kill weedy rice in paddy fields [11] and was introduced in Malaysia around the year 2012 [13]. However, few studies have been conducted for the optimization of the extraction and analysis of this compound from the environmental samples, especially from water [4,3,11,14]. Therefore, this research aims to optimize an extraction and clean-up method for imazapic herbicide from water.

Materials and Methods

Materials and Reagents

Standard of imazapic (99% purity) was purchased from Sigma-Aldrich (M) Sdn Bhd (Selangor, Malaysia). HPLC grade solvents including 2-propanol, methanol (MeOH), acetonitrile (ACN), and dichloromethane (DCM) were purchased from MERCK (Damstadt, Germany). Acetic Acid (Glacial) was also purchased from the same supplier. All solutions were prepared with ultra-pure water using a Purite-Select BIO 160 water system. For the solid-phase extraction (SPE) method, a 12-port-SPE manifold was purchased from Supelco, Sigma-Aldrich (M) Sdn Bhd (Selangor, Malaysia). A range of 500mg per 6mL SPE cartridges including Agilent Bond Elut-C_{18} (C_{18}) and Agilent Bond Elut PPL (PPL) were purchased from Agilent Technologies Sales (Malaysia) Sdn Bhd (Selangor, Malaysia). The sorbent structure for both C_{18} and PPL used for the SPE method has a based structure of octadecyl/ silica bonded and styrene-divinylbenzene polymeric respectively.

Stock and Working Standard Preparation

Standard stock solution of imazapic was prepared in ACN at a concentration of 50 mg L^{-1} by diluting 2.5 mg of imazapic stock powder with ACN in 50 mL volumetric flask. The working standard solutions of 10, 2, 1, and 0.5 mgL^{-1} was prepared from the stock solution.

Extraction and Solid-Phase Clean-Up

For recovery test, two different concentrations of imazapic, 2 and 10 mg L^{-1} were prepared. The samples were prepared by spiking the purified water samples with 2 mL and 10 mL of 50 mg L^{-1} stock solution in 50 mL volumetric flask to produce 2 and 10 mg L^{-1} water samples, respectively. Before analysis, samples preparation procedure was carried out, and this comprise of sample extraction, concentration, and isolation of analytes. This sample preparation has a great influence on the reliability and the accuracy of the analysis. SPE was chosen as a method for extraction because it is a recognised method of extraction which can reduce sample handling, labor and solvent consumption [2]. Prior to sample application, the SPE column was conditioned by passing consecutively two times 3 mL DCM, two times 3 mL MeOH, and three times 2 mL purified water acidified (pH 3.0) with acetic acid 1:1 (v/v). After adjusting the pH to 3.0 by adding acetic acid, the samples were well mixed and passed through the SPE tubes at 10 mL min^{-}. The tubes were then eluted with two times of 3 mL DCM before being dried and 4 mL of 2-propanol was then added and allowed to dry until it reaches 1 mL before being injected into HPLC-UV for analytes separation with the injection volume of 20 µL [15].

High Performance Liquid Chromatography Analysis

The analysis was carried out using Agilent HPLC-UV (Agilent, Wilmington, DE, USA) system that is equipped with a vacuum degasser, a quaternary pump, an autosampler, a thermostatic column compartment, and a variable

wavelength detector that was used for the reversed phase analysis. The collected data was processed using a LC workstation with Chemstation software. All of the solvents and solutions used in the mobile phase were previously filtered and degassed by ultrasonic application. The chromatography separation was done using column Zorbax StableBond (SB-C18) column (4.6mm x 250mm I.D., 5µm particle size) with the variation of its wavelength, mobile phase ratio, pH of the mobile phase, and flow rate while the temperature of the column was kept constant at 30°C for the optimization of the separation method of analytes. The method was validated using the following criteria: calibration, limit of detection (LOD), limit of quantification (LOQ), repeatability and the recovery percentage. As for the validation of method, samples were analysed using optimal condition of the HPLC column.

Results and Discussion

Optimization of HPLC Condition: Determination of the Optimum Wavelength by HPLC_UV

In order to optimize the HPLC parameters, the conditions were monitored by varying the detector wavelength: 250, 251, 252, 253, 254, 255, and 256 nm. It was observed that the maximum absorbance of the analyte were at the detector wavelength (λ_{max}) of 252 nm for imazapic (Figure 1).

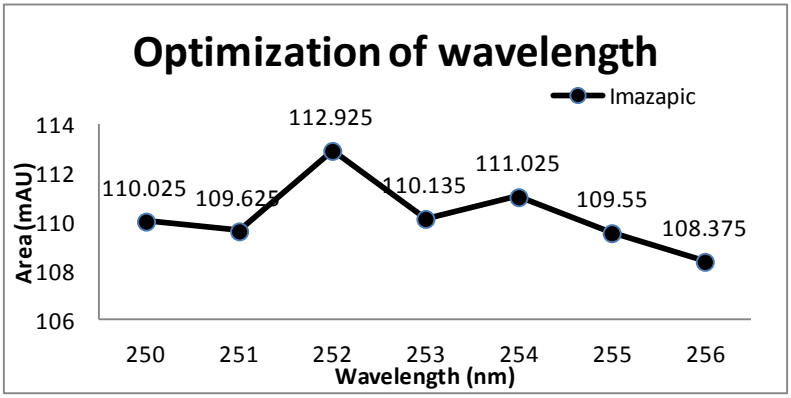

Figure 1. Maximum absorption area (mAU) for the wavelength range from 250nm to 256nm

Effect of the pH of Mobile Phase

The result showed that the peak response was higher at lower pH especially at a pH lower than pH 3 (Figure 2). This could be due to the fact that imazapic is an imidazolinone compound which has a tendency to be a pH dependent compound since pK_a value (dissociation constant) for imidazolinone group – with the exception of imazamethabenz-methyl of its carboxylic acid group – is in the range of 3.0 to 3.5. Therefore, as the pH of the mobile phase descended below 3 or 3.5, the water solubility will eventually decrease while increasing the water partition coefficient [8]. This explains the better peak of imazapic compound when the pH was set at pH 3.0 (Figure 2). As the pH of the mobile phase change, it will also help to increase the efficiency of the column since it will alter both the ionization of the analyte and the residual silanols apart from minimizing secondary interactions between analytes and the silica surface that is the usual cause of poor peak shape [16].

Effect of Mobile Phase Composition

In HPLC-UV analysis that uses reverse phase method, mobile phase composition plays an important part in the separation of the compound. Acetonitrile and water was chosen as the mobile phase in the analysis because they were reported to be the best initial choice for mobile phase in method development [16]. The mixture of mobile phase A (acetonitrile) and phase B (water: acetic acid; pH3.0) with the ratio (45A:55B; v/v) was found to be at optimum for better sharp peak of the compound with more stable baseline (Figure 3). Thus mobile phase ratio (45A : 55B; v/v) was finally chosen.

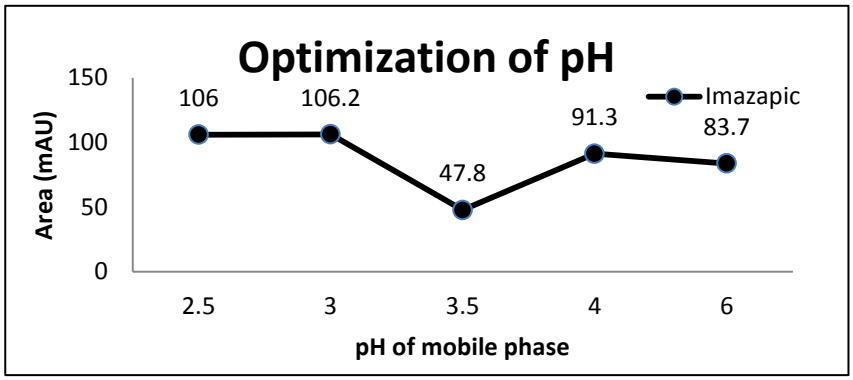

Figure 2. Maximum absorption area (mAU) using five different pH of the mobile phase from 2.5, 3.0, 3.5, 4.0 and 6.0.

Effect of the Flow Rate

Flow rate has an im portant role in influencing the retention time, peak area, but little on the separation [17]. A flow rate of 1.2 mL min^{-1} was chosen to be the optimum setting for the HPLC analysis due to its satisfactory area size and retention time which falls at 3.12 minutes for complete elution of the compound from the column especially when the setting was used to run samples (Figure 3).

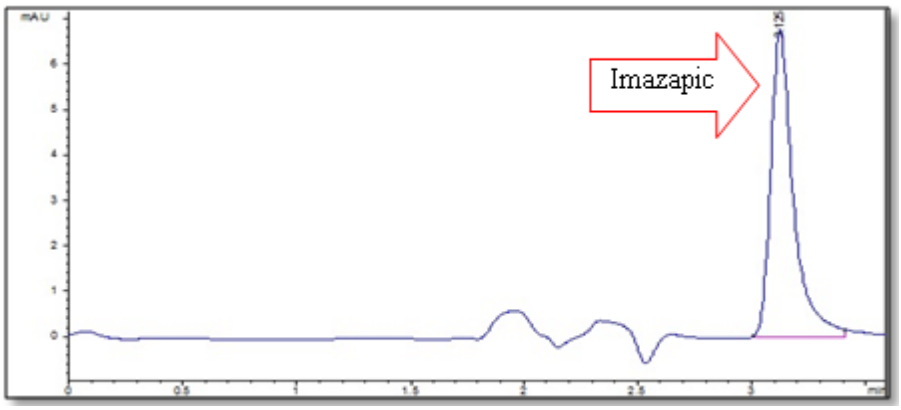

Figure 3. Chromatogram of imazapic herbicide obtained during analysis.

Validation Method

The validation steps for imazapic using HPLC-UV was performed under the optimized condition of 252 nm as maximum wavelength, flow rate of 1.2 mL min^{-1}, and mixture of mobile phase A (acetonitrile) and phase B (water: acetic acid; pH3.0) for mobile phase elution with the ratio (45A:55B; v/v) and the duration of analysis of 3.12 min. Repeatability of the retention time and peak areas were estimated by injecting replicates of 1 mg L^{-1} and 2 mg L^{-1} (n=4) of imazapic standard solutions. The results obtained showed a good relative standard deviaton percentage (RSD %) of the retention time at 0.27% which is below 20% [2].

Linearity, Limit of Detection (LOD), Limit of Quantification (LOQ) and Recovery.

Based on the standard calibration curve obtained from the concentration of imazapic plotted against the mean peak of the imazapic compound eluted from the HPLC column, the standard calibration curve of imazapic was linear

from 0.5 to 10 mg L^{-1} with coefficient of determination (R^2) above 0.99 (Figure 4). The equation of the calibration curve is shown in Table 1.

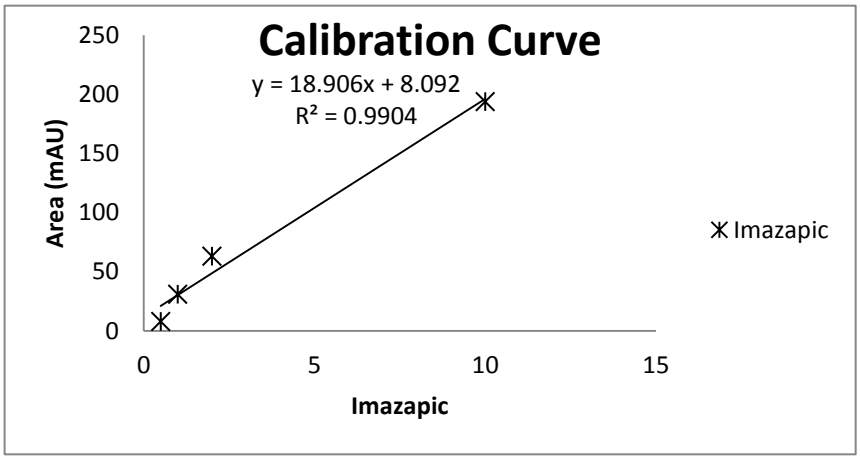

Figure 4. Calibration curve of imazapic at concentration level of 0.5 to 10 mg L^{-1}

Table 1. Validation of analytical method for imazapic by HPLC-UV

Compound	Retention time, R_T (minutes)	Calibration equation	R^2	RSD (%)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Number of standards (*n*)
Imazapic	3.12	y= 18.906x + 8.092	0.9904	0.08	0.412	1.248	4

For this analysis, limit of detection (LOD) and limit of quantification (LOQ) was estimated at 0.412 mg L^{-1} and 1.248 mg L^{-1} respectively (Table 1). The calculation for both LOD and LOQ was estimated based on the calculation from the standard deviation of the response (δ) and slope (S) of the regression line as shown in Equation (1) and (2) [18].

$$LOD = 3.3 \times \delta/S \quad (1)$$

$$LOQ = 10 \times \delta/S \quad (2)$$

The recovery percentage for solvent extraction using C_{18} cartridge for concentration of 2 and 10 mg L^{-1} was 112% and 73%, respectively. However, for solvent using PPL cartridge, the recovery for 2 and 10 mg L^{-1} was 10.35% and 10.14%, respectively. Based on this, extraction using C_{18} was found to be more consistent and reliable. This is because its recovery performance was in the range of 70% to 120% (Table 2) [2].

Table 2. Recovery of imazapic using SPE cartridge

SPE cartridge	Imazapic concentration in water sample (mgL^{-1})	Recoveries (%)
C_{18}	2	112
	10	73
PPL	2	10.35
	10	10.14

Conclusion

These findings suggest that in general, the analysis of imazapic using HPLC-UV can be successfully carried out through optimum parameters as follows; wavelength at 252 nm, 1.2 mL min^{-1} for the flow rate, mobile phase composition mixture of phase A (ACN) and phase B (distilled water; acetic acid, v/v) as mobile phase with elution ratio (45A : 55B) during the analysis time of (3.12 minutes), and also with the pH of phase B to be adjusted to pH 3.0 using acetic acid. As for the recovery test, C_{18} cartridge was chosen as the best cartridge for the extraction and clean-up method for imazapic compound for its consistency and reliability in the recovery performance with 112% and 73% at concentration of 2 and 10 mg L^{-1}, respectively.

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