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Liquid Chromatographic Separation and Quantification of Imidacloprid in Different Modes of Formulations

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Abstract: The aim of the present study was to develop a sensitive chromatographic method by using HPLC with UV detector for quantitative determination of imidacloprid (insecticide) in multiple modes of products available in market. For the separation of analyte, C-18 beckman column (5µm x 0.46cm x 15cm) was used as stationary phase, whereas acetonitrile and water (70:30 v/v) was suitably used as mobile phase. For the optimization of method, different associated parameters were also investigated those may influence the efficiency of separation. Pump was adjusted to flow at 1mL/min. throughout the analysis was performed at ambient temperature and 270nm wavelength. Linearity of the chromatographic results was checked between the range of 0.5 to 300µg/mL and R^2 was found to be 0.999. LoD and LoQ of the method were found to be 4 and 12µg/mL respectively and the reliability of the method was found to be good; up to the concentration level of 300µg/mL. Method validation tests ensured the proposed method with good precision and accuracy. Overall the results show that the proposed method can be successfully implemented for the quantitative determination of different modes of formulated products containing imidacloprid as active ingredient.

Key words: Imidacloprid · HPLC · Formulation · Pesticides

INTRODUCTION

Pesticides are used to control pests and most commonly used in agriculture to control weeds, herbs, insect infestation and diseases to protect the crop [1]. These pesticides are applied on crops using formulated products of respective pesticide [2]. Formers use these different formulated products depending upon the selection of application mode or types of pest control. Since, the pesticides' formulations contain specific percentage of labeled active ingredient. Therefore, correctness of these labeled percentage to meet the recommended doses of active ingredient are significantly important in order to ensure the control use of these pesticides. Therefore, deviation in active ingredient from the claimed percentage in pesticide formulation may impose adverse effect. The use of pesticide formulations with excessive active ingredient may produce the agricultural crop exceeding to maximum residual limit (MRL) whereas using formulated product with active ingredient below the claimed percentage, may not be sufficient enough to protect the crop from targeted pest. In this context, ensuring the claimed percentage of active ingredient in the products is highly important. In classical agricultural practices, several formulations with different active ingredients are available in market. Imidacloprid (IMI) is one of the important insecticides which are available in market with variety of formulations named Gaucho, Merit, Admire, Confidor, Macho and Winner. It is now considered to be the most widely used insecticide globally [3-5]. Although, IMI has been in use for a relatively short period of time compared to other pesticides but these days, frequent use of this insecticide has been observed in more than 140 agricultural corps and somewhat applications of IMI has been reported for pets and household pests [5]. Since, it is widely applied insecticide on crops therefore, the control use of IMI formulation is also very important. For this purpose it is

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necessary to ensure that the labeled percentage of IMI in particular formulation must be present within the range of recommended percentage. For this purpose several methods have been developed for the determination of numerous active ingredients including IMI in their respective formulations [6]. For the quantification of active ingredient of IMI in formulated products, different chromatographic techniques are commonly practiced these include HPLC-DAD [7, 8], LC-MS and GC-FID [9, 10]. Some alternative techniques, such as Enzyme-linked immune sorbent assay, [11, 12], flourimetry [13], Thermal Lense Spectrophotometric detection, pulse polarography [14], fourier transform infrared spectroscopy [15] and voltammetry [16] have also been employed to analyze different imidacloprid formulations. But the above mentioned analytical methods for the determination of IMI demand relatively high running cost. These analytical procedures are however not economically favored. Therefore, the study was conducted to develop a new chromatographic method based on HPLC-UV for quantitative determination of imidacloprid formulation products that should be simple, quick and inexpensive with same precision and accuracy.

MATERIALS AND METHODS

All chemicals and reagents (methanol and acetonitrile) those were utilized are of HPLC grade. De-ionized water was used throughout the experimental work. The reference standard of IMI and different formulation samples i.e. Imidacloprid 25%WP, Imidacloprid 70%WG, Imidacloprid 200SL (W/V) and 95% Technical imidacloprid were obtained from the Department of Plant Protection (DPP), Karachi, Pakistan. Some of the formulations were also purchased from the local market.

Instrumentation: The chromatographic determinations were serviced by Shimadzu with UV-visible detector, binary solvent delivering pumps and 20µL loop attached with a rheodyne. C-18 Beckman column (5µm x 4.6mm x 15cm) and a C-18 discovery column (5µm x 4.6mm x 25cm) were used as stationary phase placing an ambient temperature. Column selection was made considering the identical specification for ruggedness study. Data integration was finalized by using Shimadzu SPD-10AV UV-detector with communication bus module and all the data were collected and evaluated on chromatography station for window version 1.7 DLL, Build 160502, S/N: 11-8199 software (version 2).

HPLC Conditions and method Optimization: The chromatographic parameters were established by checking multiple compositions of mobile phase, flow rate and wave-length of detector. Different ratios of solvents, acetonitrile or methanol were checked with water (50:50 to 100:0 at interval of 10). These were practicedfor the optimization of mobile phase in term of obtaining good separation of analyte with best resolution.In order to obtain the shortest retention time (without losing the optimized chromatographic response of analyte), flow rates of mobile phase were varied between 0.5mL/min to 1.0mL/min at interval of 0.1mL/min. Throughout the analysis isocratic mode of mobile phase flow was employed. Each mobile phase solvent was filtered through 0.45µm filter membrane using vacuum pump filter system followed by degassing of mobile phase by ultrasonic bath. The separation was accomplished by using C-18 column at ambient temperature. According to Guzsvany *et al*. [3], different wavelengths in UV range were tested between 200 and 300nm at internal of 10nm to determine λ_{max} and optimum chromatographic responses with the minimization of interferences of inert materials that was found in some formulated products. For performing the robustness test, the optimized wavelength and flow rates were deliberately altered. Results obtained by alteration in each parameter were accordingly compared.

Standard Solutions Preparation: Stock solution of IMI was prepared by dissolving accurately weighed 0.01g of standard in 25mL diluent (a mixture of ACN and water 70:30 – v/v). The stock solution was further used for the preparation of working standard solution upto 0.5µg/mL.

Calibration Standards Preparation: For standard calibration curve, nine (9) working standard solutions between the range from 0.5 to 300 µg /mL were prepared. These working standards were also subjected for evaluating inter-day and intra-day precision in chromatographic responses.

Sample Preparation: Specific quantities of different modes of formulated products were individually transferred in 25mL each. Each of the sample contained 0.01g active ingredient (i.e. imidacloprid). The samples were initially dissolved in diluent – a homogenous mixture of acetonitrile: water – 70:30. These samples were then sonicated to allow complete solubilization of active contents and volume were increased up to the mark with diluent. The samples were thereafter filtered through 0.45micron Millipore filter membrane before injecting to HPLC.

Method Validation: For the proposed chromatographic method, the method has been validated by ensuring the ICH guidelines for system suitability test, specificity, linearity, range, precision and robustness.

System Suitability Test: This test was performed by injecting ten consecutive injections of 50µg/mL of each formulationwithoptimized conditions. On each day of validation, system suitability test was performed and was found to be in acceptable criteria.

Specificity: All modes of products were individually tested for investigation of interfering effects of inert materials with the peak of IMI. Effects of inert materials were however not found conflicting to the chromatographic responses.

Linearity: Calibration curve was designed between the peak area and height and the concentration of analyte with nine concentration levels of IMI and linearity of the newly developed method was obtained. The calibration curve was further utilized for obtaining regression characteristics including slope, correlation coefficient and standard deviation.

Limit of Detection and Quantification: Lowest limit of detection (LoD) and limit of quantification (LoQ) for the proposed method were determined by the given formula. Data obtained from linearity was employed for these quantifications.

$LoD = 3.3\ \sigma/S$
$LoQ = 10\ \sigma/S$

σ = the standard deviation of the response
S = the slope of the calibration curve

Precision: Precision of the method was confirmed by checking the responses on inter-day and intra-day analysis. For evaluating intra-day precision, four different formulation types were used. Similarly, inter-day precision was assessed using the solutions of similar products. The results were obtained for three consecutive days. The%RSD determined by using the following formula:

%RSD = (SD/Mean x100)

SD=Standard Deviation of analyte (specific conditions)
Mean=Average of analyte (specific conditions)

Accuracy: Different modes of formulated products of IMI were used for their assay employing the proposed method. All the samples were also simultaneously analyzed in two other laboratories. This inter-laboratory comparison was performed for the accuracy check of the proposed method.

Robustness and Ruggedness: Ruggedness of the newly developed method was investigated using two separate columns of same specification. Between the analyses on two different columns, all other parameters were kept constant including wave-length of detector, flow rate and mobile phase composition. On the other hand, robustness was also performed by deliberate alteration in detector wave-length 270±2, mobile phase (ACN: Water – 80:20) ratio ±2 and flow rate 1.0±1mLmin^{-1}.

RESULTS AND DISCUSSION

Method Development and Optimization: Analytical methods for the quantitative determination of IMI are already available but the existing methods demand sophisticated chromatographic instruments i.e. GC, GC-MS, GC-FID or LC-MS etc. This attempt was therefore made to develop an analytical method for consistent quantitative determination of IMI in multiple modes of formulations. This is cost effective with optimized chromatographic conditions using HPLC with Ultraviolet detector. The optimized parameters were checked and developed method was also validated.

Variations in parameters as described in section 2.2, resulted peak asymmetry, low resolution and/or merging of IMI peak with co-extractives of inert materials of formulation samples. Peak symmetry with good separation was obtained at mobile phase ratio 70:30, flow rate 1ml/min (Table 1) and wave-length 270nm (considered to be the λ_{max}) Figure 1.

Method Validation: The developed method allows the analyte to elute within 3.5min but the runtime was programmed for 7min to completely elute UV-sensitive co-extractives of formulation and to reproduce smooth baseline. Validation parameters throughout used for this method. To validate this analytical method, specificity, linearity, accuracy, precision, robustness and ruggedness were checked.

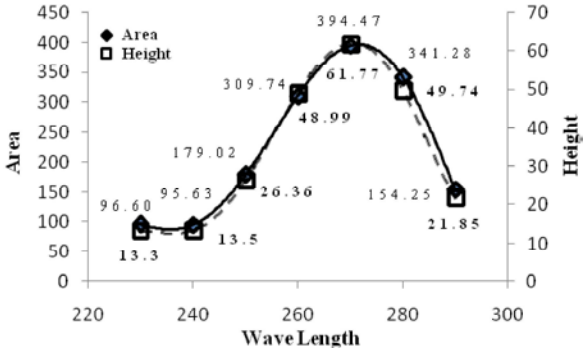

Fig. 1: Chromatographic response of imidaclopird at different wave-lengths

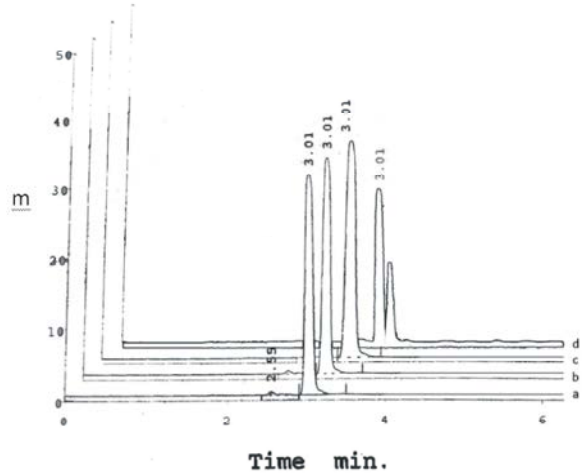

Fig. 2: Peaks of multiple modes of imidacloprid formulations; a) 25% WP, b) 70% WG, c) 95% Tech, d) 20% SL

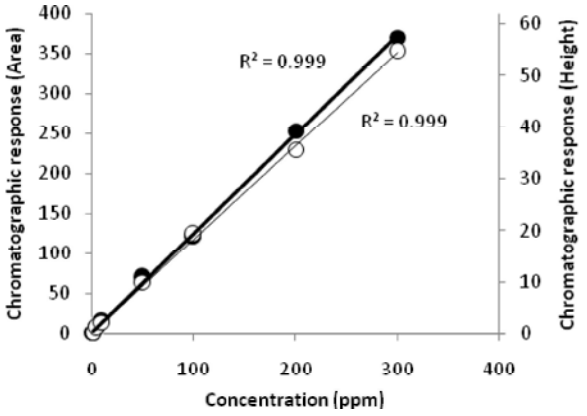

Fig. 3: Calibration curve of IMI with both chromatographic responses (Area and Height)

Specificity: Samples of multiple modes of pesticide formulations were analyzed to evaluate the possible interferences of inert materials in the samples. The results showed good separation between the peak of analyte and inert material in any mode of formulated product (Figure 2).

Table 1: System Suitability test at optimized conditions

S. No.	Formulation type	$W_{1/2}$	T	K'	N
1	25% WP	0.080	1.444	0.99	2933
2	70%WG	0.083	1.667	0.99	2914
3	20% SL	0.084	1.300	1.04	2876
4	95% Technical	0.087	1.345	0.97	3072

$W_{1/2}$=Half Width, N = no of theoretical plates
T= Tailing factor, K' = Capacity Factor

Table 2: Intra-day anInter-day precision

		Intra-day Analysis		Inter-day Results	
S.No.	Formulation Type	Results	%RSD	Results	%RSD
1	25% WP	24.73	1.54	24.89	2.48
2	70% WG	70.55	0.67	70.50	0.84
3	20% SL	19.58	2.33	19.78	2.01
4	95% Tech	94.90	0.61	94.61	0.91

System Suitability Test: The replicate results of all the formulation types were found to be close to each other representing%RSD <2. Number of theoretical plates and tailing factor were considered for system suitability test (Table 1).

Linearity: Linearity was determined by constructing calibration curve with nine different concentrations ranging between 0.5 and 300µgmL^{-1}. The calibration curve showed correlation coefficient greater than 0.999 using both chromatographic responses (area and height) against respective concentrations (Figure 3).

Limit of Detection and Quantification: Using the linearity data and applying the formula given in section 2.6.4, LoD of the proposed method was found to be 4µg/mL and LoQ of the method is 12µg/mL.

Precision: The proposed method was also investigated for intra-day and inter-day precision. Reproducibility for different modes of formulations was checked to represent the intraday precision. Inter-day precision was determined for period of three days in all formulations of pesticide. Variation in results were reported as%RSD that was found to be <2.5 in all analysis. This reflects the high precision of the proposed method (Table 2).

Accuracy: Accuracy for the proposed method was checked by inter-laboratory comparison test of multiple modes of imidacloprid formulation samples. This test was

Table 3: Inter Laboratory Comparison Test for multiple pesticide formulations

	Lab 1		Lab 2		Lab 3	
Formulation Type	Result	%RSD	Result	%RSD	Result	%RSD
25% WP	24.82	1.126	24.38	1.056	25.11	1.587
70%WG	68.87	0.843	71.3	1.058	69.18	0.706
20% SL	19.91	1.069	20.17	1.358	19.83	0.974
95% Technical	94.77	0.480	95.87	0.785	95.74	1.121

Lab 1: Food Quality and Safety Research Institute, Pakistan Agriculture Research Council
Lab 2: Department of Plant Protection, Karachi, PakistanLab 3: Bayer CropScience (PVT) Ltd

Table 4: Robustness and Ruggedness Test

		25% WP		70% WG		20% SL		95% Technical	
Parameters	Variables	N	T	N	T	N	T	N	T
Flow rate (mL/min)	0.6	3217	1.590	3068	1.731	3054	1.564	3117	1.638
	0.7	2933	1.444	2914	1.667	2876	1.300	3072	1.345
	0.8	3105	1.773	2976	1.849	2972	1.843	3155	1.932
Mobile Phase (ACN: Water)	68:32	2926	1.765	2836	1.439	2916	1.453	2876	1.778
	70:30	2962	1.219	2944	1.348	2861	1.443	2945	1.54
	72:28	2855	1.334	2876	1.453	2804	1.583	2852	1.63
Wave Length	268	2880	1.532	2855	1.653	2895	1.443	2904	1.874
	270	3027	1.654	2974	1.342	2986	1.217	2875	1.576
	272	2873	1.438	2911	1.804	2875	1.359	2816	1.612
Column	Beckman	2983	1.334	3174	1.639	2972	1.219	3012	1.384
	Discovery	2763	1.378	2876	1.834	2684	1.447	2946	1.564

N = Theoretical Plate
T = Tailing factor

simultaneously conducted among three laboratories. Analytical results of each sample obtained by using the proposed method were found to be close to the results obtained from other laboratories. Table 3 depicts all the results obtained and ensured the suitability of proposed method for quantitative determination.

Robustness and Ruggedness: To check the flexibility and authenticity of the developed method, deliberate variations were made in some parameters of developed method for the determination of IMI. Ratio of mobile phase was altered by ±2, flow by ±0.1mL/min and wavelength was varied by ±1nm and their effect was observed. Two different columns were also checked for ruggedness check of the method. Table 4 shows that change of stationary phase or small change in the mobile phase combination, flow rate and in wavelength imparted negligible effect on analytical results of the developed method. Overall the developed method was found to be robust and rugged.

CONCLUSIONS

This study was conducted to develop an improved HPLC-UV based identical analytical method that can suitably be used for quantitative determination of imidcaloprid in multiple modes of formulated products. The performance of method was successfully assessed by studying different validation parameters. Optimization of this method was found with C-18 Beckman column (5µm x 0.46cm x 15cm) as stationary phase whereas, mobile phase i.e. acetonitrile and water (70:30) was used. For the optimization of method, different associated parameters were also investigated those may influence the efficiency of separation. Pumps were program to flow @ 1mL/min and UV-detector was adjusted at 270nm wavelength. Linearity of the chromatographic results was checked between the range of 0.5 to 300µg/mL and R^2 was found to be 0.999. LOD and LoQ of the method were found to be 4µg/mL and 12µg/mL respectively and concentration level up to 300µg/mL was the maximum quantifiable level of this insecticide. Method validation tests ensured the proposed method with good precision and accuracy. Overall the results show that the proposed method may be successfully implemented for the quantitative determination of IMI as active ingredient in different available formulation mode of products.

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