

New Differential Pulse Polarographic Method for the Determination of Isoproturon Herbicide in Environmental Samples

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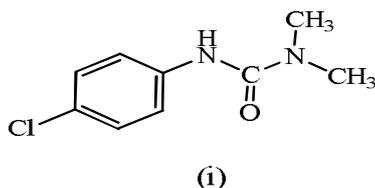
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ABSTRACT: The excessive use of isoproturon herbicide in agriculture can cause some serious health and environmental hazards. This necessitates the development of some simple methodologies for its determination in various environmental samples. A new differential pulse polarographic method based on the measurement of dimethyl amine (formed from hydrolysis of isoproturon with potassium *tert.* butoxide) as dimethyldithiocarbamate complex through reaction with carbon disulphide and copper(II) perchlorate yielding an analytically useful diffusion-controlled peak at -96 mV has been developed. The analysis has been accomplished by constructing calibration graph between peak current and concentration showing linear relationship in the range 0.21-4.13 $\mu\text{g mL}^{-1}$ of isoproturon solution. The method has been validated for the determination of this herbicide in its commercial formulation, water, soil and agricultural produces to monitor environmental pollution. The proposed method is accurate and precise as shown by the high recoveries (97.09-99.02%) with low relative standard deviation values (0.42-1.47 %). The method has also been validated to evaluate the leaching behaviour of this herbicide for assessing ground water contamination risk. The value of ground water ubiquity score (2.30-2.67) showed that it is a transition leacher herbicide and poses a potential risk towards environmental pollution.

Keywords: Differential pulse polarography; Environmental samples; GUS and Isoproturon.

INTRODUCTION: Isoproturon (i) belonging to the class of phenylureas is a selective and systemic herbicide used to control annual grasses and broadleaf weeds in cereals.¹⁻² The extensive use of this toxic chemical due to its direct exposure or through residues in food and drinking water³ poses serious health risks and environmental pollution. To ensure its proper and safe use, monitoring environment viz. soil, water and agriculture produce for its residues its determination is very important. Of no less importance is to study its adsorption in soil for the evaluation of its leaching potential to assess the risk of groundwater contamination.⁴⁻⁹ To accomplish above objectives its determination in commercial formulations, environmental samples and agricultural products is essential to obtain reliable residue and adsorption data.



The determination of this herbicide has been reported by HPLC¹⁰⁻¹⁵ and GC-MS¹⁶⁻¹⁹, capillary electrophore-

sis²⁰ and fluoroimmuno sensor methods.²¹⁻²² Despite the precision and accuracy of chromatographic and capillary electrophoresis methods, these are time-consuming, expensive, requiring high analytical skill thus limiting their wide applicability. Polarography is now finding much greater use in pesticide analysis. With the inexpensive and sensitive differential pulse polarography (DPP) technique the determinations can be done even at larger dilutions and with smaller volumes of solutions without any interference from inert carriers commonly present in their formulations. The above advantages of the technique have been applied in the present work for the DPP determination of isoproturon.

The microwave assisted hydrolysis of isoproturon with potassium *tert.* butoxide (PTB) to dimethyl amine and the derivatisation of the latter to copper dimethyldithiocarbamate complex through reaction with carbon disulphide and copper(II) perchlorate, yielding analytically useful diffusion-controlled peak at -96 mV in sodium perchlorate supporting electrolyte at dropping mercury electrode (DME) has been made the basis of the proposed method. The method has been applied to the analysis of isoproturon in its commercial formulation and recovery from fortified

grains, vegetables and water samples to monitor health hazards. The proposed method has also been validated to study the adsorption of this herbicide on five Indian soils of different soil characteristics to evaluate the risk of contamination of ground water due to this herbicide.

MATERIALS AND METHODS:

Apparatus and reagents: All Polarographic measurements were made with an Elico (India) Polarographic Analyser (model CL-362) equipped with DME as working electrode, saturated calomel electrode (SCE) as reference electrode and platinum as an auxiliary electrode. Domestic microwave oven, Samsung, India was used to carry out hydrolysis. Genei (TM) shaking incubator, Bangalore was used in soil adsorption study.

The analytical standard of isoproturon (99.7%), Ghar-da Chemicals Mumbai was used. A commercial formulation containing 75% isoproturon WP (PRIMI-LON-75) was procured from the local market. Acetonitrile (Merck, AR) was used as recieved. Potassium *tert.* butoxide (Loba Chem., AR),(1 mol L⁻¹solution in *tert*-butanol) was used for hydrolysing the herbicide. Sodium perchlotate (Merck, Germany, 0.1 mol L⁻¹ solution in acetonitrile) was used as supporting electrolyte. Triton-X 100 (Merck), 0.002% in acetonitrile was used as suppressor.

Preparation of calibration graph: Aliquots (0.1 to 2.0 mL) of the stock solution (0.0002 mol L⁻¹) of isoproturon in acetonitrile were taken in 20 mL measuring flasks and volume made to 2 mL with acetonitrile. Each solution was mixed with 0.7mL of 0.02mol L⁻¹ solution of PTB followed by 0.3mL solution of water in acetonitrile (0.02 mol L⁻¹) and kept in microwave oven for 60 sec in each case. Each solution was mixed with one drop of carbon disulphide (~100 µL), 1.0 mL of copper(II) perchlorate (0.002 mol L⁻¹ in acetonitrile)and transferred into polarographic cell, mixed with Triton-X-100 (1 mL, 0.002% in acetonitrile) and final volume was made to 20 mL with sodium perchlorate (0.1 mol L⁻¹in acetonitrile). Nitrogen gas was bubbled through each solution for 5 min. and polarograms were recorded with the instrumental parameters listed in Table 1. A typical polarogram was shown in Figure 1. Calibration curve was plotted between the concentration of herbicide and the peak current (Figure 2) and the various calibration characteristics are given in Table 2.

Formulation analysis: A single large sample of the herbicide formulation viz. Primilon-75 containing 75% isoproturon (W.P.) formulation was weighed, shaken with acetonitrile and filtered. The residue was

washed 2-3 times with 5mL of acetonitrile. The filtrate and washing were diluted to a known volume (25 mL) with same solvent. Suitable aliquots of the extracts of each formulation were taken for analysis and processed in same manner as described for pure compounds. The assay results are given in Table 3.

Table 1: Optimized instrumental parameters.

Parameter	Set up	Parameter	Set up
Initial applied voltage	200 mV (vs. SCE)	Sensitivity	1 µA/V
C.C. Compensation	100 %	Acquisition	Fast
I.R. Compensation	0	Pulse amplitude	50mV
Drop time	1.0 sec	Scan rate	6mV/sec
Height of Hg column	90 cm	Temperature	25 ± 1°C

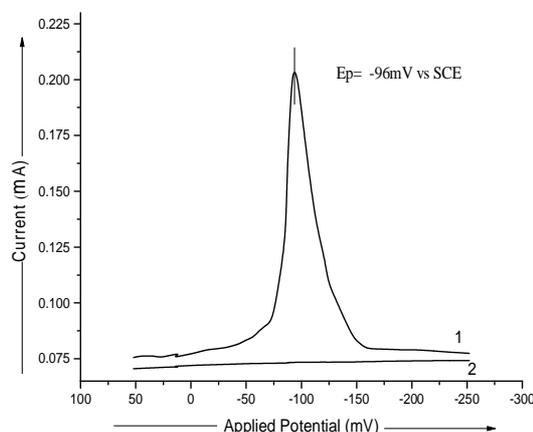


Figure 1: A typical differential pulsepolarogram[1. Cu(II)-dimethyldithiocarbamate reaction 2. Supporting electrolyte at DME].

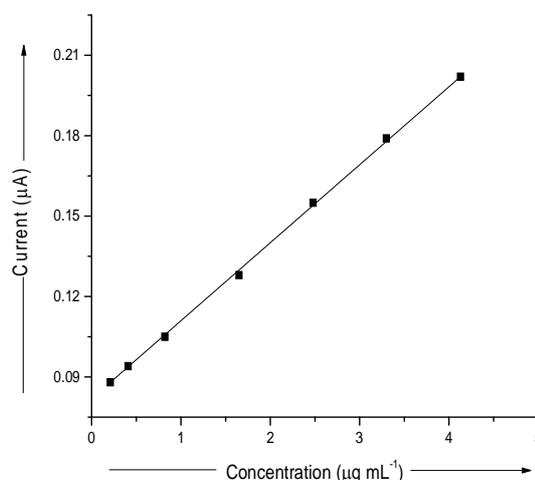


Figure 2: Relationship between peak current and concentration (calibration graph) for isoproturon.

Table 2: Calibration characteristics for isoproturon.

Characteristic	Corresponding value
Linearity range, ($\mu\text{g mL}^{-1}$)	0.21-4.13
Slope	0.0291
Intercept	0.0818
Correlation coefficient (r^2)	0.998
Limit of detection (LOD), ($\mu\text{g mL}^{-1}$)	0.07
Limit of quantification(LOQ), ($\mu\text{g mL}^{-1}$)	0.21

Table 3: Assay results of commercial formulation of isoproturon containing 75% active ingredient[†].

Active ingredient added (μg)	Active Ingredient*	
	Found (μg)	Recovery (%)
0.52	0.51±0.008	98.07±1.47
1.03	1.00±0.011	97.09±1.04
1.55	1.52±0.017	98.06±1.09
2.06	2.04±0.018	99.02±0.90
2.58	2.53±0.011	98.06±0.42

*Values are mean of five determinations with standard deviation (\pm)

[†]Maker's specifications established by an independent method²³

Determination of isoproturon in grains (wheat and barley): A known weight (5g) of grains (wheat and barley) was mixed with various amounts of herbicide formulation. The samples were well mixed and extracted with 2-3 installments of 5mL of chloroform. The combined extracts were shaken for 5 min. and filtered. The solvent from the filtrate was removed by heating at 40°C in a water bath. The residue was transferred into a 10mL measuring flask with 2 mL of acetonitrile and processed for analysis as described above. Results are summarized in Tables 4.

Table 4: Recovery of Isoproturon from fortified grains samples.

Sample (μg)	Active ingredient added	Recovery of active ingredient* (%)	
		Found (μg)(%)	Recovery (μg)(%)
Wheat	1.03	0.90±0.007	87.73±0.72
	1.65	1.49±0.014	90.30±0.88
	2.27	2.01±0.028	88.54±1.23
	2.89	2.64±0.033	91.35±1.16
Barley	1.03	0.96±0.014	93.20±1.37
	1.65	1.53±0.015	92.73±0.92
	2.27	2.15±0.028	94.71±1.22
	2.89	2.60±0.027	89.96±0.95

*Values are mean of five determinations with standard deviation (\pm)

Determination of isoproturon in vegetables (cauliflower and pea) and water samples: Suitable aliquots of the standard solution of isoproturon were added to 25 g portions of vegetables and were mixed thoroughly. These samples were blended with 25 mL chloroform and filtered through coarse filter paper and the filtrate was transferred into 250 mL separatory funnel. Sodium chloride (5 g) was added, the funnel was shaken for one minute and two phases were allowed to separate for 15 min. The lower aqueous phase and any emulsion were discarded. Anhydrous sodium sulphate (4 g) was added to separatory funnel, and the funnel was shaken for 30 sec. The dried extract was filtered through coarse filter paper and evaporated to dryness. The residue was dissolved in 2 mL of acetonitrile and was processed for analysis as described above. Assay results are listed in Table 5.

In case of water samples, various amounts of herbicide solution were added to 25 mL water samples and were mixed thoroughly. The samples were extracted with 2 installments of 5 mL chloroform and the extract processed for analysis in the same manner as described above. The results are given in Table 5.

Table 5: Recovery of isoproturon from vegetables and water samples.

Active ingredient added(μg)	Recovery of active ingredient* (%)					
	Cauliflower		Pea		Water	
	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
1.03	0.88±0.012	85.43±1.22	0.84±0.009	81.55±0.97	0.99±0.008	96.12±0.81
1.65	1.43±0.016	86.67±0.98	1.41±0.022	85.45±1.37	1.57±0.012	95.15±0.73
2.27	1.87±0.020	82.37±0.89	1.89±0.021	83.26±0.93	2.20±0.029	96.92±1.28
2.89	2.36±0.029	81.66±1.00	2.42±0.038	83.74±1.36	2.75±0.038	95.15±1.31

*Values are mean of five determinations with standard deviation (\pm)

Evaluation of leaching potential: Triplicate soil samples (2g) of each soil with different soil characteristics (Table 6) were taken in 50mL conical flasks and were equilibrated with 3 mL acetonitrile solution of isopro-

turon in the concentration range from 18.65-93.24 $\mu\text{g mL}^{-1}$ and 7 mL distilled water. The contents of each flask were stirred to bring effective mixing by shaking mechanically at 140 rpm for 24 hr equilibration time

at 25±1°C. After 24 hr the suspensions were centrifuged and equilibrium concentration (C_e) was determined in the supernatant in the same manner as described above for pure compound. Freundlich adsorption model (equation 1) was used to evaluate various adsorption parameters.

$$\log X = \log K_f + n_f \log C_e \quad (1)$$

Where; X is the amount of insecticide adsorbed (mg Kg⁻¹) on the adsorbent; K_f and n_f are adsorption coefficients which are related to the adsorption capacity and adsorption intensity respectively. The various adsorption parameters viz. K_f, n_f, K_{OC} were calculated from the plot of log X versus log C_e from Freundlich's adsorption equation by the reported method²⁴ and are presented in Figure 3 and Table 7.

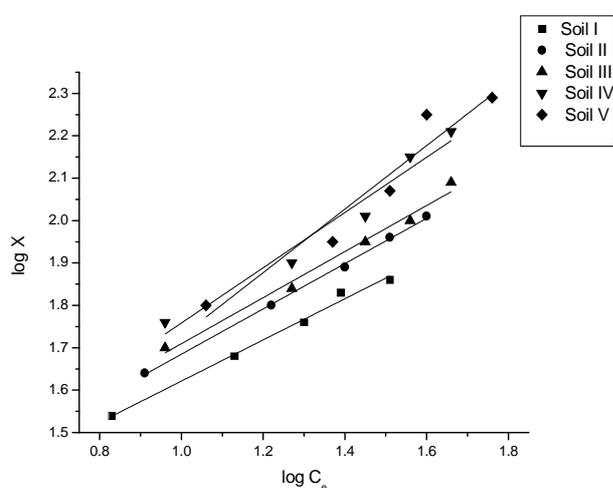


Figure 3: Plots of log X versus log C_e for isoproturon on five soils.

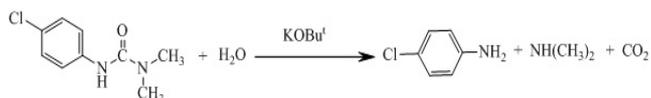
Table 6: Characteristics of five soils used in the adsorption study of isoproturon.

Soil Sample	pH	Clay (%)	Organic Carbon (%)	Cation Exchange Capacity (meq/100 g)
I	6.90	10.2	0.7	12.8
II	7.40	5.0	0.8	12.7
III	7.50	26.3	0.9	13.1
IV	7.62	18.2	1.0	12.9
V	6.50	20.0	1.5	11.5

Table 7: Adsorption parameters for isoproturon on five soils at 25±1°C.

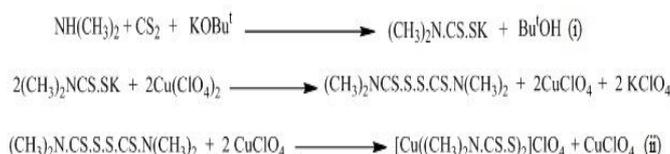
Soil Sample	K _f	n _f	K _d	K _{oc}	GU S	ΔG° (KJmol ⁻¹)
I	0.82	0.53	0.56	80.64	2.30	-1.38
II	0.42	0.99	0.54	68.45	2.38	-1.47
III	0.50	0.82	0.53	58.94	2.45	-1.54
IV	0.39	1.06	0.55	55.64	2.48	-1.42
V	0.37	1.13	0.56	37.68	2.67	-1.39

RESULTS AND DISCUSSION: The proposed method involves the microwave assisted hydrolysis of herbicide to dimethyl amine and the derivatisation of the latter with carbon disulphide and copper (II) perchlorate to a copper dithiocarbamate derivative. Microwave assisted hydrolysis is advantageous than conventional heating in terms of higher reaction rates and greater selectivity and reproducibility and reduces the problems associated with analyte losses and atmospheric contamination.²⁵ PTB in *tert.* butanol has been used for selective hydrolysis of amide function present in isoproturon.



The optimum time required for complete hydrolysis of isoproturon to dimethylamine in a microwave was optimized by varying the reaction time from 10-90sec. and measuring corresponding peak current intensity. It has been observed that maximum current intensity was obtained corresponding to 60sec. hydrolysis time. The effect of various concentrations and volume of reagents on the peak current was also studied and was found that 0.7 mL of 0.02 mol L⁻¹ solution of PTB followed by 0.3 mL solution of water in acetonitrile (0.02 mol L⁻¹) hydrolysed the herbicide completely.

The reaction of liberated dimethyl amine with carbon disulphide in the presence of PTB to form alkali dithiocarbamate and of the latter with copper (II) perchlorate in acetonitrile to form copper (III) dimethyl dithiocarbamate complex (ii) showing one electron reduction at DME at -96 mV in DPP and linear relationship between concentration and peak current formed the basis of proposed method.



The formation of copper (III) dithiocarbamate complexes of type (ii) have thoroughly been reported in literature²⁶. The other hydrolytic product and excess of carbon disulphide do not interfere in the method. Under the optimised experimental conditions and instrumental parameters the calibration curve was found to be linear in the range 0.21- 4.13 µg mL⁻¹ of the herbicide concentration. The linearity was also indicated by the high values of correlation coefficient (r² = 0.998) obtained (Table 2). The proposed DPP methods have been applied to the determination of isoproturon in commercial formulation, grains, vegetables and spiked water samples. The high recoveries in the ranges 97.09-99.02%, 87.73-94.71%, 81.55-

86.67% and 95.15-96.92% respectively of the nominal content of isoproturon with low RSD in the range 0.42-1.47 %, 0.72-1.37 % , 0.89-1.37% and 0.73-1.31 % shows good accuracy and precision of the method (Tables 3-5).

The method has also been applied to study the adsorption on five soils of different characteristics for the purpose of the evaluation of its leaching potential and consequently risk of contamination of water bodies. The adsorption isotherms have been evaluated by Freundlich's adsorption equation using batch equilibrium technique. The leaching potential of isoproturon in terms of Groundwater Ubiquity Score (GUS) index was determined by using experimentally observed K_{OC} value for each soil sample and literature reported half-life of isoproturon²⁷.

$$GUS = \log(t_{1/2}) [4 - \log(K_{oc})]$$

GUS value is used to study the leaching behaviour of pesticides, classifying them as lecher ($GUS > 2.8$); non-lecher ($GUS < 1.8$) and transition ($2.8 > GUS > 1.8$). The GUS score for isoproturon has been observed between 2.30-2.67 (Table 7), which is above 1.8 thus classifies it as a transition lecher pesticide. Thus possess potential to contaminate ground water. The toxicity due to this herbicide can be reduced by adjusting the application dose according to soil properties. The soils with higher organic carbon content increase its adsorption and consequently reduce its leaching losses.

CONCLUSION: With a view to ensure proper and safe use of isoproturon by monitoring environment for its residues in agriculture produce, water and soil a DPP method has been developed. The method is advantageous in that analysis can be done at low concentration ($LOQ, 0.21 \mu g mL^{-1}$) without any interference from inert carriers commonly present in its formulations. The simplicity, rapidity of the procedure and well-established stoichiometry and mechanism of electrode reaction are some other attributes of the proposed method. The high recoveries of isoproturon in formulation and residue analysis with low relative standard deviation values show good accuracy and precision of the method. For the assessment of the leaching risk and subsequent contamination of ground and surface water, the method has also been validated for its adsorption study on soils to calculate GUS index. The values in the range 2.30 to 2.67 classify classifies it as a transition lecher pesticide having potential to contaminate ground water. From adsorption studies it is concluded that by adjusting the application dose according to soil properties and their amendments with manure and compost which are rich in organic carbon content can reduce its leaching.

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