Research article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Introduction of A Novel Binary Solvent System For The Determination of Pesticides By High-Performance Liquid Chromatography With Ultra Violet Detection

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ABSTRACT

The present research intends to confirm successful implication of propylene carbonate and methanol in the ratio of 60:40 (Solvent-X) as a mobile phase component in the analysis of carbendazim, isoproturon and pendimethalin by high pressure liquid chromatography (HPLC) with Ultra Violet (UV) detection. Chromatography was performed on (250 x 4.6 mm), 5-µm, Inertsil ODS C_{18} column with a flow rate of 1.0 ml/min for all the three pesticides with varying compositions of mobile phase. UV detection was carried out at 254 nm, 230 nm and 240 nm for Carbendazim, Isoproturon and Pendimethalin respectively. For each pesticide the method was validated two times initially with acetonitrile in the mobile phase and later by substituting it with Solvent-X maintaining all other chromatographic conditions unvaried to correlate the discrepancy in the figures of merit and other validation parameters. However it was ascertained that both the methods provide comparable results. Linearity was established between a concentration range of 25.0 to 75.0 µg/ml for Carbendazim, for Isoproturon it was 0.75 to 11.25 µg/ml and for Pendimethalin 0.30 to 4.50 µg/ml using internal standard method with a correlation coefficient of 0.9995, 0.9994 and 0.9994 with acetonitrile and 0.9998, 0.9984 and 0.9995 with Solvent-X.

Keywords: Acetonitrile; Carbendazim; Isoproturon; Pendimethalin; Propylene carbonate

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INTRODUCTION

While selecting an organic solvent to be used as mobile phase in liquid chromatography, several physical and chemical properties of the solvent should be considered. One useful approach to this is the Snyder Classification. This scheme classifies solvents on the basis of P' (polarity index) values, and also takes into account the possibility of specific effects. Each solvent is assigned three classification parameters: x_e(proton acceptor parameter), x_d(proton donor parameter), and x_n(strong dipole parameter). The classification then separates solvents into eight groups based on similarity of the x-parameters. Solvents belonging to the same group will show similar behavior[1]. Hence, in order to replace acetonitrile we choose another solvent from the same group as that of acetonitrile i.e. Group 6[2]in which propylene carbonate is also present hence a quick examination of its physical and chemical properties implies why propylene carbonate can be an attractive solvent substitute to acetonitrile.

Propylene carbonate (4-methyl-1, 3, dioxolan-2-one) is a five-membered alkylene carbonate manufactured most commonly by carbon dioxide insertion into the appropriate oxirane[3]. Besides, Propylene carbonate having properties similar to that of acetonitrile it also has few advantages over acetonitrile with respect to higher dielectric constant (64.4) and polarity Index value (6.1) as compared to acetonitrile which has a Dielectric constant of 36.6 and Polarity index value as 5.8[4,5]. Propylene carbonate has a Vapor pressure of 0.045 mm Hg at 25°C and flashpoint temperature at 135°C which makes it highly conducive and a safer solvent to handle in lab and also reduces the chances of accidental fire as compared to that of acetonitrile which has a Vapor pressure of 91.1 mm Hg at 25°C and flashpoint temperature 5.6°C[6,7]. Toxicity studies have found that propylene carbonate is less toxic than acetonitrile because LD50 value (on rats g/kg) of propylene carbonate is >5.0 whereas that of acetonitrile is in the range of 2.46-6.5[7,8]. The lower Log Po/w (-0.41) value of propylene carbonate determines a lower ability to bio-accumulate in nature hence making it an eco-friendly substitute to acetonitrile which has a Log Po/w value of -0.34[9].

Propylene carbonate which mainly has applications in cosmetics and personal care products, as an electrolyte in extraction of metals and many more[10], has till date not been used as a mobile phase component in HPLC. Hence to this aim experiments are performed in parallel with the conventional solvent that is using acetonitrile and with Solvent-X for the separation and estimation of Carbendazim (methyl benzimidazol-2-ylcarbamate), Isoproturon (3-(4-isopropylphenyl)-1, 1-dimethylurea) and Pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidene) using internal standard method.

MATERIALS AND METHOD

REAGENTS AND CHEMICALS

Working standards of Carbendazim (technical grade 98.5% purity) and Pendimethalin (technical grade 99.0% purity) was procured from Organic Phosphorus Pvt. Ltd., India. Isoproturon (technical grade with purity of 99.4%) and Metamitron (technical grade purity98.0%) was procured from Gharda Chemicals Pvt. Ltd. India.

Isoproturon (technical grade purity 99.4%) was used as internal standard for carbendazim and pendimethalin analysis whereas Metamitron (technical grade purity98.0%) was used as internal standard for Isoproturon analysis.

The formulation of carbendazim, Bevastin 50% WP was procured from market. The formulation, Isoproturon 75% WP was procured from Gharda Chemicals Pvt, Ltd. and the formulation of Pendimethalin 30% EC manufactured by Rallis India Ltd. was procured from the company.

HPLC grade acetonitrile and methanol were obtained from Merck Limited, Mumbai. HPLC grade propylene carbonateofSigma Aldrich was imported from Germany. Double distilled water was used for solutionpreparations throughout the project. Mobile phase was always filtered through 0.45 membranefilter paper and degassed before use.

STANDARD PREPARATION

Standard stock solution of carbendazim, Isoproturon and pendimethalin was prepared by dissolving 10 mg of working standard in 5 mL of acetonitrile and Solvent-X each separately, followed by sonication for about 15 minutes and diluted to 10 mL with the respective solvents. Further dilutions were made in mobile phase.

The stock solution of internal standard was prepared by dissolving 10 mg of Isoproturon (99.4% purity) and Metamitron (98.0% purity) respectively in 5 mL of acetonitrile followed by sonication for about 15 minutes and diluted to 10 mL with acetonitrile. Similar procedure was followed for the preparation of internal standard stock solution using Solvent-X. Further dilutions were made in mobile phase.

SAMPLE PREPARATION

Bevastin 50%, a wettable powder (WP) formulation of carbendazim, Isoproturon 75% WP and Pendimethalin 30% EC was accurately weighed equivalent to 1 mg and transferred to 10 ml volumetric flask containing 5 ml of acetonitrile for Method A and 5ml of Solvent-X for Method B and sonnicated

for 15 minutes and then diluted to 10 ml with acetonitrile and Solvent-X individually. This solution was filtered through Whatman no. 1 filter paper to obtain clear solution before injection.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

The LC system consisted of a JASCO HPLC-900 series equipped with PU-980 intelligent pump, AS-950 intelligent auto sampler (1-100 l) and UV-975 intelligent UV-Vis detector with 8 l flow cell. Chromatograms and data were recorded by means of Borwin Chromatographic software version 1.5. Compounds were separated on an Inertsil ODS C18 (250 x 4.6 mm, 5) column. In the method A, the mobile phase consisted of acetonitrile: water, 60:40 (v/v) for carbendazim, acetonitrile: water 60:40 (v/v) for isoproturon and acetonitrile: water 90:10 (v/v) for pendimethalin. The flow rate was 1 ml/min. 10 l of sample was injected and the detection wavelength was 254 nm, 230 nm and 240nm for carbendazim, isoproturon and pendimethalin respectively.

The retention time of carbendazim and internal standard Isoproturon was 3.61 min and 6.83 min respectively. Also the retention time of isoproturon with internal standard metamitron (3.84 min) was 9.30 min. Similarly internal standard isoproturon eluted ahead at 3.47 min followed by pendimethalin at 6.01 min. For method B, the mobile phase consisted of Solvent-X: water with the same compositions. All other chromatographic conditions were kept unchanged. The retention time of carbendazim and internal standard Isoproturon was 4.15 min and 8.54 min respectively. Also the retention time of isoproturon with internal standard metamitron (3.61 min) was 5.61 min. Similarly internal standard isoproturon eluted ahead at 3.40 min followed by pendimethalin at 6.22 min. The typical HPLC chromatogram obtained in method A for carbendazim is shown in Fig.1A and the chromatogram obtained by Method B is shown in Fig. 1B. Similarly for Isoproturon and pendimethalin chromatograms obtained by Method A are shown in Fig. 2A and Fig. 3A and for Method B is shown in Fig. 3A and Fig. 3B respectively.

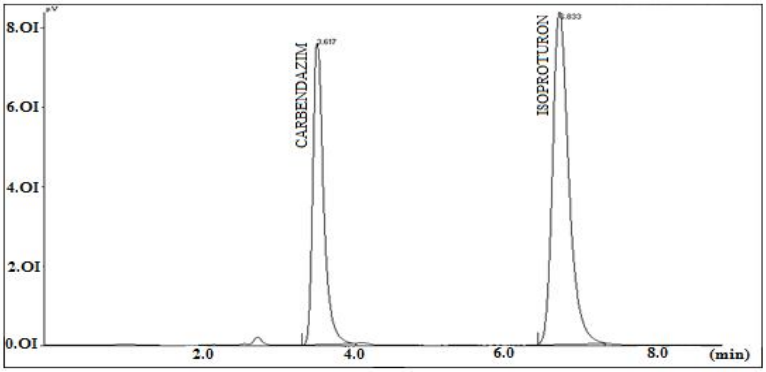

Figure 1A: Typical HPLC chromatogram obtained for carbendazim and isoproturon in a standard preparation by method A.

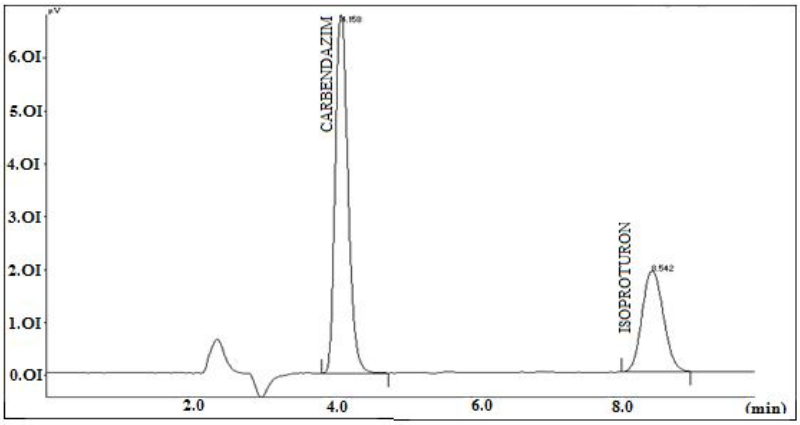

Figure 1B: Typical HPLC chromatogram obtained for carbendazim and isoproturon in a standard preparation by method B.

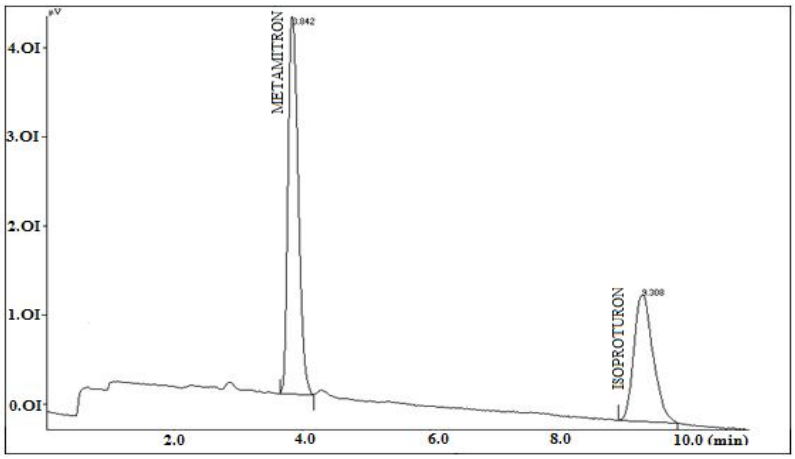

Figure 2A: Typical HPLC chromatogram obtained for metamitron and isoproturon in a standard preparation by method A.

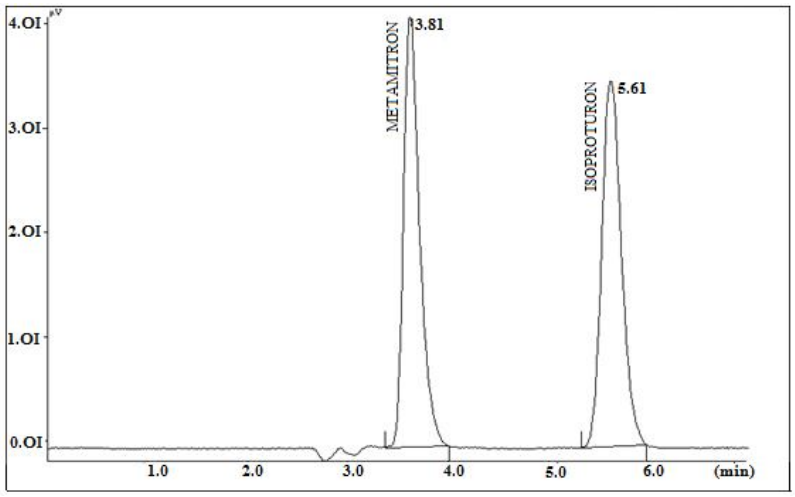

Figure 2B: Typical HPLC chromatogram obtained for metamitron and isoproturon in a standard preparation by method B.

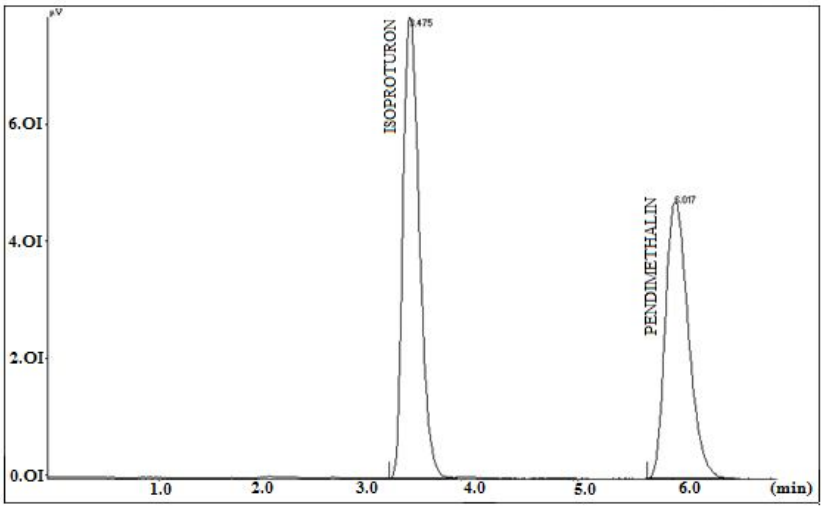

Figure 3A: Typical HPLC chromatogram obtained for isoproturon and pendimethalin in a standard preparation by method A.

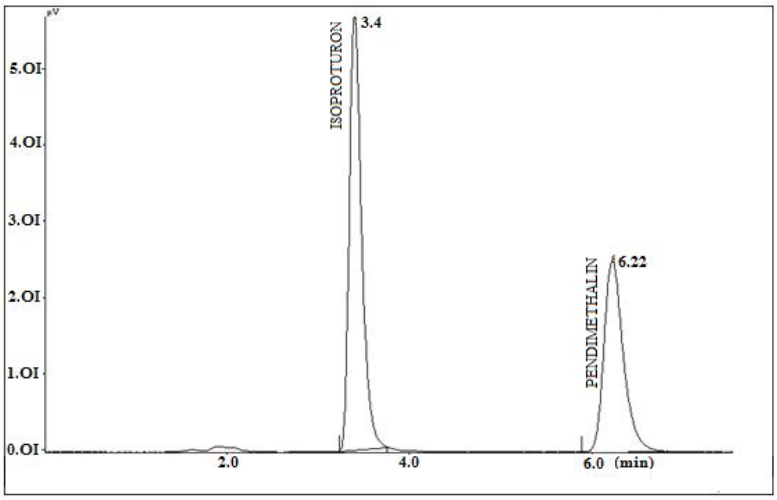

Figure 3B Typical HPLC chromatogram obtained for isoproturon and pendimethalin in a standard preparation by method B.

RESULTS AND DISCUSSION

The influences of column type, mobile phase composition, flow rate etc. were systematicallyinvestigated to ensure accurate analysis of the pesticides. The final chromatographic conditions revealed to provide best separation with the mobile phase composed of acetonitrile: water::60:40 for carbendazim and Isoproturon whereas acetonitrile:water::90:10 for pendimethalin. Wavelength was selected by scanning the reference standards over the wide range of the wavelength 200-400 nm. Once the method was developed, Solvent-X was substituted for acetonitrile (Method B),

to check the chromatographic performance. It was observed that Solvent-X was a weaker eluent as compared to ACN, though it showed good resolution and peak shape.

METHOD VALIDATION

Both the above chromatographic methods developed were validated for the followingparameters, the results of which are listed in Table 2:

SYSTEM SUITABILITY TEST (SST)

It was assessed by injecting 6 replicates of the standard preparation of 100% strength. The percentage relative standard deviation (% RSD) of the peak area responses of each pesticide was calculated and they were found to be < 2% for both the methods. The values for resolution, peak symmetry, theoretical plates etc. are listed in Table 1.

Table 1: Chromatographic figures of merit for carbendazim, Isoproturon and pendimethalin usingacetonitrile/Solvent-X in mobile phase

Figures of Merit	Carbendazim		Isoproturon		Pendimethalin	
	Method A	Method B	Method A	Method B	Method A	Method B
Capacity factor (k')	1.78	1.77	1.77	2.53	3.02	1.38
Selectivity (α)	3.17	4.87	2.96	3.37	8.16	3.86
Resolution (R_s)	3.57	5.48	3.78	4.75	5.45	3.73
Peak Symmetry (A_s)	1.25	1.2	1.25	1.0	1.2	1.2
Theoretical plate no.(N)	1804	2325	2919	1888	2160	2321
HETP (h)	0.0138	0.0107	0.0085	0.0132	0.0115	0.0107

LINEARITY

It was evaluated by analysis of the standard solutions of carbendazim, Isoproturon and pendimethalin at different concentrations. The linearity was studied in the range of 25.0 to 75.0 µg/ml for Carbendazim, 0.75 to 11.25 µg/ml for Isoproturon and 0.30 to 4.50 µg/ml for Pendimethalin using internal standard method. The concentration and the peak area response of each pesticide were subjected to regression analysis for calculating the calibration equations and correlation coefficients. The regression data obtained for all the three pesticides by both the methods have been listed in Table 2. The results show that in both the methods, within these concentration ranges, there was excellent correlation between peak area ratio and concentration of each pesticide.

PRECISION

Precision study was assessed by injection repeatability and sample repeatability. Injection repeatability was confirmed by performing replicate injection of the standard solution and calculating the % RSD of the peak area responses for the content. The sample repeatability was studied by analyzing the same sample for six times and calculating the % assay value and % RSD (Table 2).

LIMITS OF QUANTITATION (LOQ)

Limits of Quantification (LOQ) were calculated and confirmed by injecting 6 replicates of the standard preparation of lowest strength that gave reproducible and precise responses. The LOQ for carbendazim, Isoproturon and pendimethalin by both the methods have been listed in Table 2.

ACCURACY

The accuracy of the method was determined by measuring the recovery of the pesticide by themethod of standard additions. To the 100 % strength solution, known amount of each pesticide corresponding to its 80, 100 and 120 % were added. Each set of additions was repeated 6times. The results listed in Table 2 indicate that the method enables highly accuratedetermination.

ROBUSTNESS

Robustness of the method was determined by deliberately varying certain parameters like flow rate (ml/min), wavelength, proportion of acetonitrile (ml) and Solvent-X (ml) in themobile phase. Each parameter was studied at 3 levels. Oneparameter at a time was changed to estimate the effect. Percentage recovery was calculatedin each case and was found to be within the acceptance limit of 85 %-115 %.

ASSAY

The validated HPLC methods were used for the estimation of carbendazim, Isoproturon and pendimethalin in their marketed formulations. In the assay experiment sixsamples were weighed separately and analyzed. The mean assay results, expressed as apercentage of the label claim, are listed in Table 2. The results indicate that the amount ofeach pesticide in the tablets is within the requirements of 90-110% of the label claim by both themethods.

SOLUTION STABILITY

The stability of analytical solutions of both the methods was assessed by comparing the arearesponse for standard preparation at different time intervals, (viz. 4 hrs, 8 days, 16 days, 21days and 30 days) with the freshly prepared standard solution. The % assay was compared.The results found within 98.0-102 % indicate that carbendazim, Isoproturon and pendimethalin can beconsidered stable under the conditions investigated.

CONCLUSION

Thework described in this paper has shown that the Methods A and Bfor the three pesticides carbendazim, Isoproturon and pendimethalin are precise, accurate,linear, and robust. From the above experimental work, it is also proved that, HPLC methods that use acetonitrile canbe replicated using exactly the same amount of Solvent-Xinstead to give reasonablygood results without any change in other chromatographic conditions.

However, as compared to the column pressure observed when acetonitrile based mobilephase is used, a slight increase of up to 60 kg/cm^2 was observed when Solvent-X based mobilephase was used. This may be attributed to the higher viscosity of propylene carbonate which forms 60% ofSolvent-X.

Thus, this work isexpected to be of interest to scientists who are working in the field of developing cleaner andgreener chemical technologies to create a better environment in the coming times.

Table 2: Summary of the validation study

Parameter		Carbendazim		Isoproturon		Pendimethalin	
		Method A	Method B	Method A	Method B	Method A	Method B
Linearity	Slope	0.0229	0.0225	0.1099	0.0911	0.3711	0.3594
	Intercept	-0.0494	0.0198	0.0139	0.0037	0.0101	0.0007
	Correlation	0.9995	0.9998	0.9994	0.9984	0.9994	0.9995
SST (%R.S.D)		1.61	0.01	1.38	0.34	1.96	1.76
Precision (%R.S.D)		0.92	0.50	0.045	0.045	1.42	1.91
LOQ (µg/ml)		0.18	0.18	0.61	0.82	0.0165	0.0165

Results from determination of the accuracy of the method										
Analyte	**Original Amount (µg/ml)**	**Amount added (µg/ml)**			**% Accuracy**					
					Method A			**Method B**		
		80%	**100%**	**120%**	**80%**	**100%**	**120%**	**80%**	**100%**	**120%**
Carbendazim	50	40	50	60	103.74	101.54	91.88	88.3	100.1	113.4
Isoproturon	7.5	6.0	7.5	9.0	88.38	92.04	96.03	89.69	95.31	96.09
Pendimethalin	3.0	2.4	3.0	3.6	101.87	101.45	101.01	99.89	110.67	94.74

Results from determination of Robustness of the method (% Accuracy)						
Parameter	**Carbendazim**		**Isoproturon**		**Pendimethalin**	
	Method A	**Method B**	**Method A**	**Method B**	**Method A**	**Method B**
Flow rate- 0.8mL/min	85.45	96.53	105.51	01.54	99.86	104.22
Flow rate-1.0mL/min	89.61	100.25	105.51	03.63	101.88	96.88
Flow rate-1.2mL/min	88.57	88.52	105.07	02.13	101.66	101.10
UV: - 2 nm	89.62	87.67	105.85	02.07	98.93	114.07
UV: ± 0 nm	89.61	100.25	105.51	03.63	101.88	96.88
UV: + 2 nm	89.20	88.52	105.54	04.46	101.26	110.60
Mobile Phase: – 2 ml	89.33	89.99	107.03	8.85	98.26	111.07
Mobile Phase: ± 0ml	89.61	100.25	105.51	03.63	101.88	96.88
Mobile Phase: + 2 ml	90.70	87.34	106.43	03.30	108.64	105.39

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