

Determination of Pesticides by Solid Phase Extraction Based on Central Composite Design

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Pesticides used frequently in farmlands and homes around the world, which easily passes to agricultural products. Therefore, optimum analyses of these widely used compounds are urgently needed. In this study, our main aim is to determine the effects of sorbent, solvent desorption, flow rate, sample volume and methanol desorption on the extraction efficiency for parathion-methyl, azinfos-ethyl and isoproton according to the central composite design. Each factor and their combinations were studied and the optimum conditions for an solid phase extraction were determined in which 32 mL solvent with a 2.28 mL min⁻¹ flow rate can extract 59.45 mL sample in a short time. This method was used due to its limited data requirement and the frequency of its use in recent studies.

Key Words: Central composite design, Significant values, Solid phase extraction, Pesticides.

INTRODUCTION

Pesticides are used to kill pests, microorganism, unwanted insects, worms, *etc.* and have been in use since the ancient Romans who used hot sulfur to kill insects and controlled the growth of weeds with the use of salt. Due to connectivity of the natural resources, these chemicals can easily pass from one medium to another. There has been a great attempt to determine the concentration of these products in water¹⁻³. Since these products are found in homogenized mode in the environment, there is a urgent need in developing sensitive methods for their determination. Colorimetric^{4,6}, chromatographic⁷⁻⁹ and electrochemical methods^{10,11} have been proposed in determining these compounds. However, there are some short comings that necessitates a fresh look at these products and methods. To ensure the required sensitivity is met, the way in which the experiments are performed is an important one. In this work, we have tried to devise a plan to increase sensitivity in determining parathion-methyl, azinfos-ethyl and isoproton through the use of statistical model, central composite design (CCD).

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EXPERIMENTAL

All pesticides and insecticides were obtained from Aldrich. Methanol, ethyl acetate, sulfuric acid and sodium sulfate were from Merck. The sorbent was ODS, with 40-60 μ mesh from Macherey-Nagel Company. Water used was HPLC grade, produced by Millipore A10 water purifier. GC was model 6890 N from Agilent Technologies with FID detector with HP5, (30 \times 0.5 mm) column and H₂, N₂ gases were used. Solid phase extraction (SPE) was prepared with a HPLC pump Kontron 420 model. pH meter was CG 824 from schott and all measurements were done on A&D analytical balance model D003.

Cartridge preparation: In a clean empty syringe a fritted glass is fitted on the bottom and proper amount of the packing dissolved in THF and 2-propanol (1:1), was pumped through the cartridge with the HPLC pump using methanol as a carrier liquid. In short intervals, syringe is tapped to ensure the uniformity of the packing materials. A second fritted glass is then fitted to the top of the syringe to block the packing material.

Conditioning the cartridge: 5.0 mL volume of methanol passed through the sorbent for 5 min with the flow rate of 1.0 mL min⁻¹, then 5.0 mL of pure water with a pH of 3.0, Prepared by addition of the right amount of 0.01 M sulfuric acid, passed through the sorbent with the flow rate of 1.0 mL min⁻¹. It was made certain that the conditioning did not last long and the elution volume was not excessive.

Adsorption steps: Predetermined samples were passed through the column and then the column was washed with 5.0 mL pure water with a flow rate of 0.2 mL min⁻¹. After which, the column was dried with 0.2 mL min⁻¹ nitrogen gas flow for 10 min, the extracts were not collected at this time.

Desorption step: Desorption of the samples was done according to the GC retention time for the pure samples based on the followings: isoproton 12.4 min, parathion-methyl 13.2 min and azinfos-ethyl 22.1 min. A nitrogen gas flow was set up in such a way that nitrogen tank was connected to a pipette by rubber tubing. The pipette was inserted in the solution vial and the flow rate was controlled to 0.2 mL min⁻¹. The evaporation step stopped when the solution volume reached 0.5 mL. Care was taken not to dry out the vials completely and the nitrogen flow rate was never more than 0.2 mL min⁻¹. The extracts were kept in cool place and used within two days.

Experimental design

One of the most common second order experimental designs is central composite design (CCD). This is a planner factorial design with an extra point in the center in such a way that $2f = Na$ where f is the number of factorials. Therefore, CCD needs $2^f + 2f + 1$ experiment based on complete factorials and in comparison to the 3 dimensional factorials, it requires fewer experiments to be performed. The mathematical model for a CCD with four variables is:

$$Y = \beta_0 + \sum \beta_j \chi_j + \beta_{jj} \chi_j^2 + \beta_{jk} \chi_j \chi_k$$

where Y is response, χ_j, χ_k are variables, $\beta_0, \beta_j, \beta_{ij}, \beta_{jk}$ are coefficient of regression, constant, linear, square and interaction, respectively. Our main purpose is to determine the parameters that affected the extractions efficiency and present study focused on the statistical method, CCD. Calibration curve and equations for each compound were determined using standard solution injection in GC to be as follow: isoproton $Y = 459.6X - 38.9$ and for parathion-methyl $Y = 323.9X - 117.7$ and for azinfos-ethyl is $Y = 279.12X - 206.7$ with the regression of 0.9998, 0.9999 and 0.9978, respectively. Many parameters affect the outcome of any extraction. However, we chose those that have the most influence on the efficiency of an extraction for a CCD analysis (Table-1). The stat graphics @ plus computer program was used separately for each compound.

TABLE-1
PARAMETERS HAVING MOST AFFECT ON THE EXTRACTION

Symbol	A	B	C	D	E
Names of parameters	Sorbent	Methanol desorption solution	Sample flow rate	Volume sample	V desorption solution
Unit	mg	%	mL min ⁻¹	mL	mL

RESULTS AND DISCUSSION

In this study, we have tried to look at different parameters that may influence efficiency of an extraction. Certainly, the amount of the sorbent, desorption solution strength, sample volume, flow rate and volume of the desorption solution have vital importance in any successful extraction. Therefore, this need prompted us to look further into each factor. Each one of these factors alone could alter the course of any extraction, therefore, not only did we have to look at them individually but also as interacting species. Table-2 shows the estimated affect for each parameter and their affects were also studied using variance to determine significant or insignificant values. As it can be seen from the tables, there is some merit in each factors involvement in the extraction. In all cases, sample volume proved to have negative response due to increase in retention time as the sample increases. Sample volume showed a negative response except in parathion-methyl and that could be due to its spatial orientation in relation to the sorbent. Variation of the sorbent weight, factor A, had little affect on the extraction, since the sample is in minute amount and too large of the sorbent would not have any tangible effect on the optimization. The two factors 'B' and 'C' had regular chromatographic affects on the extraction efficiency with 'B' being 42.82 % as optimum solution and 2.28 mL min⁻¹ proved to be the best flow rate. Different flow rates and per cent volumes caused leading and tailings in the GC chromatograms. It appears that the factor 'E', desorption volume, has the most affected (Tables 2, 4 and 6). The interacting parameters CE, CD, BD, AE, BE and AD have significant values and the factors CE and CD have relatively large

TABLE-2
EFFECT OF EACH PARAMETER AND THEIR INTERACTION ON AZINFOS-ETHYL

Effect	Estimate	Standard error	V.I.F
Average	21.390500	2.42073	–
A: Sorbent	-2.008330	2.27207	1.00000
B: methanol Desorp.	-1.191670	2.27207	1.00000
C: Flow rate	2.558330	2.27207	1.00000
D: sample volume	-2.808330	2.27207	1.00000
E: solvent V Desorp.	11.591700	2.27207	1.00000
AA	0.466554	2.09641	1.04359
AB	4.787500	2.78271	1.00000
AC	-104875	2.78271	1.00000
AD	7.187500	2.78271	1.00000
AE	10.462500	2.78271	1.00000
BB	-0.183446	2.09641	1.04359
BC	-1.862500	2.78271	1.00000
BD	10.912500	2.78271	1.00000
BE	9.487500	2.78271	1.00000
CC	1.416550	2.09641	1.04359
CD	-13.612500	2.78271	1.00000
CE	-15.237500	2.78271	1.00000
DD	0.291554	2.09641	1.04359
DE	-0.712500	2.78271	1.00000
EE	-0.483446	2.09641	1.04359

TABLE-3
VARIANCE STUDY OF EACH PARAMETER ON
THE AZINFOS-ETHYL EXTRACTION

Source	Some of squares	DF	Mean square	F-Ratio	P-Value
A: sorbent	24.200400	1	24.200400	0.78	0.3975
B: methanol Desorp.	8.520420	1	8.520420	0.28	0.6114
C: Flow rate	39.270400	1	39.270400	1.27	0.2865
D: Sample volume	47.320400	1	47.320400	1.53	0.2447
E: Solvent V Desorp.	806.200000	1	806.200000	26.03	0.0005
AA	1.534070	1	1.534070	0.05	0.8284
AB	91.680600	1	91.680600	2.96	0.1161
AC	8.850630	1	8.850630	0.29	0.6046
AD	206.641000	1	206.641000	6.67	0.0273
AE	437.856000	1	437.856000	14.14	0.0037
BB	0.237169	1	0.237169	0.01	0.9320
BC	13.875600	1	13.875600	0.45	0.5185
BD	476.331000	1	476.331000	15.38	0.0029
BE	360.051000	1	360.051000	11.62	0.0067
CC	14.141900	1	14.141900	0.46	0.5146
CD	741.201000	1	741.201000	23.93	0.0006
CE	928.726000	1	928.726000	29.98	0.0003
DD	0.599074	1	0.599074	0.02	0.8922
DE	2.030620	1	2.030620	0.07	0.8031
EE	1.647170	1	1.647170	0.05	0.8223
Total error	309.739000	10	30.973900	–	–
Total (corr.)	4521.640000	30	–	–	–

TABLE-4
EFFECT OF EACH PARAMETER AND THEIR
INTERACTIONS ON PARATHION-METHYL

Effect	Estimate	Standard error	V.I.F
Average	22.23410	1.12420	
A: Sorbent	1.09340	1.08976	1.00000
B: methanol Desorp.	1.12990	1.11300	1.00000
C: Flow rate	-0.06775	1.13876	1.00000
D: sample volume	0.97712	1.12899	1.00000
E: solvent V. Desorp.	16.76540	1.12888	1.00000
AA	1.02561	1.02543	1.01100
AB	-2.16500	1.42110	1.00000
AC	0.89900	1.14011	1.00000
AD	-2.99100	1.28999	1.00000
AE	-0.29900	1.40987	1.00000
BB	-0.00987	1.00989	1.03990
BC	0.69900	1.40110	1.00000
BD	-0.14900	1.40110	1.00000
BE	-0.69900	1.40110	1.00000
CC	1.59900	1.49900	1.03760
CD	-0.79700	1.40880	1.00000
CE	0.23000	1.40860	1.00000
DD	-0.29900	1.04980	1.04359
DE	-1.58000	1.39330	1.00000
EE	0.29000	1.04880	1.03996

Standard errors are all based on total errors with 10 d.f.

TABLE-5
VARIANCE STUDY OF EACH PARAMETER AND THEIR
INTERACTION ON PARATHION-METHYL EXTRACTION

Source	Some of squares	DF	Mean square	F-Ratio	P-Value
A: sorbent	6.98045	1	6.98045	0.99	0.3432
B: methanol Desorp.	8.158887	1	8.158887	1.05	0.3299
C: Flow rate	0.030055	1	0.030055	0.00	0.9550
D: Sample volume	5.03924	1	5.03924	0.65	0.4397
E: Solvent V Desorp.	1600.19	1	1600.19	218.39	0.0000
AA	7.59999	1	7.59999	0.98	0.3464
AB	17.9988	1	17.9988	2.43	0.1500
AC	2.99878	1	2.99878	0.49	0.5006
AD	40.8997	1	40.8976	5.26	0.0447
AE	0.49876	1	0.49876	0.07	0.7935
BB	0.0008999	1	0.0008999	0.00	0.9915
BC	2.3987	1	2.3987	0.31	0.5907
BD	0.0899	1	0.0899	0.01	0.9165
BE	2.3999	1	2.3999	0.31	0.5907
CC	17.9999	1	17.9999	2.43	0.1501
CD	2.60000	1	2.60000	0.33	0.5790
CE	0.2009	1	0.2009	0.03	0.8751
DD	0.8999	1	0.8999	0.12	0.7380
DE	11.0023	1	11.0023	1.40	0.2643
EE	0.60214	1	0.60214	0.08	0.7893
Total error	77.7667	10	7.7.7667	–	–
Total (corr.)	1908.34	30	–	–	–

negative for azinfos-ethyl, while they have small negative or positive values for parathion-methyl and isoproton. As it can be seen from the tables, the factors BC, CD and CE, in all three cases, show little or no significant values. The significant value for these parameters is shown in Fig. 1. Data found show the estimated effect of each parameter and their interaction on parathion-methyl, Table-4 and to determine significance or insignificance of the values found, the variance analyses were performed which is shown in Table-5, As it can be seen from table, similar to azinfos-ethyl, the factor E, desorption volume, has the most positive effect for the parathion-methyl. However, contrary to the previous substance, none of the interacting factors have significant values. Normal paper diagram confirms the valued factors in which four significant values can be seen in Fig. 2. However, the factors AB, AD and CC don't have a measurable effect, where E shows a great value. The result of present study on isoproton is shown in the Tables 6 and 7 (Fig. 3). Based on these tables and figures, the most effective factor is E and there are four significant values with E being the most noticeable one. Having performed the experiments on the three substances in a CCD design separately, the data found are recorded and the optimum conditions for the extractions were determined to be according to the Table-8.

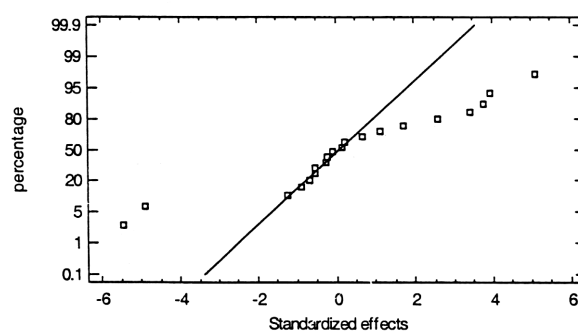


Fig. 1. Normal paper graph for the extraction of azinfos-ethyl

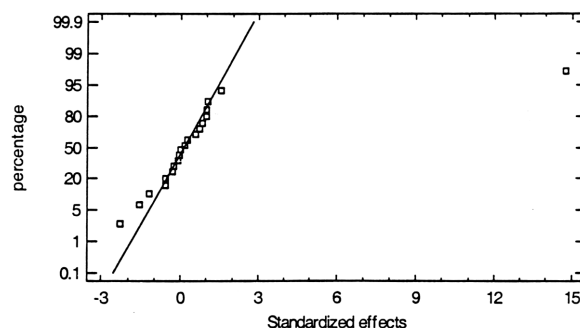


Fig. 2. Normal paper graph for the extraction of parathion-methyl

TABLE-6
EFFECT OF EACH PARAMETER AND THEIR INTERACTION ON ISOPROTON

Effect	Estimate	Standard error	V.I.F
Average	22.037800	2.86870	–
A: Sorbent	1.816670	2.69254	1.00000
B: methanol Desorp.	3.283330	2.69254	1.00000
C: Flow rate	1.900000	2.69254	1.00000
D: sample volume	-0.416667	2.69254	1.00000
E: solvent V Desorp.	17.316700	2.69254	1.00000
AA	-4.091220	2.48437	1.04359
AB	0.400000	3.29767	1.00000
AC	-3.250000	3.29767	1.00000
AD	-0.900000	3.29767	1.00000
AE	0.800000	3.29767	1.00000
BB	-3.141220	2.48437	1.04359
BC	1.150000	3.29767	1.00000
BD	-1.500000	3.29767	1.00000
BE	-1.450000	3.29767	1.00000
CC	-0.416216	2.48437	1.04359
CD	3.300000	3.29767	1.00000
CE	-1.300000	3.29767	1.00000
DD	-0.841216	2.48437	1.04359
DE	0.250000	3.29767	1.00000
EE	0.908784	2.48437	1.04359

Standard errors are based on total error with 10 d.f

TABLE-7
VARIANCE STUDY OF EACH PARAMETER AND THEIR INTERACTION ON ISOPROTON

Source	Some of squares	DF	Mean square	F-Ratio	P-Value
A: Sorbent	19.8017	1	19.80170	0.46	0.5152
B: Methanol Desorp.	64.6817	1	64.68170	1.49	0.2507
C: Flow rate	21.66	1	21.66000	0.50	0.4965
D: Sample volume	1.04167	1	1.04167	0.02	0.8801
E: Solvent V Desorp.	1799.2	1	1799.20000	41.36	0.0001
AA	117.963	1	117.96300	2.71	0.1306
AB	0.64	1	0.64000	0.01	0.9059
AC	42.25	1	42.25000	0.97	0.3476
AD	3.24	1	3.24000	0.07	0.7905
AE	2.56	1	2.56000	0.06	0.8132
BB	69.5405	1	69.54050	1.60	0.2348
BC	5.29	1	5.29000	0.12	0.7345
BD	9.0	1	9.00000	0.21	0.6589
BE	8.41	1	8.41000	0.19	0.6695
CC	1.2209	1	1.22090	0.03	0.8703
CD	43.56	1	43.56000	1.00	0.3406
CE	6.76	1	6.76000	0.16	0.7016
DD	4.98721	1	4.98721	0.11	0.7419
DE	0.25	1	0.25000	0.01	0.9411
EE	5.82054	1	5.82054	0.13	0.7221
Total error	434.986	10	43.4986	–	–
Total (corr.)	2652.75	30	–	–	–

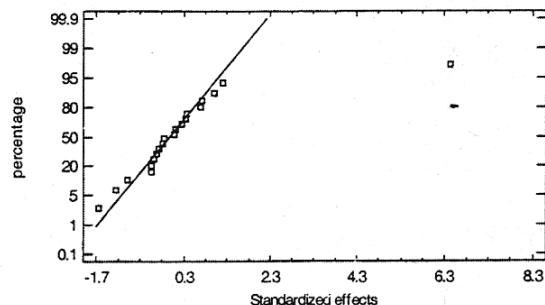


Fig. 3. Normal paper graph for the extraction of isoproton

TABLE-8
OPTIMUM CONDITIONS FOR THE EXTRACTIONS

Factors	Low level	High level	Optimum amount
Adsorption (mg)	100	500	465.49
Methanol (%)	0	100	42.82
Flow rate (mL min ⁻¹)	2	10	2.28
Sample V (mL)	200	1000	59.45
Desorption (Volume)	10	35	32.23

Conclusion

An overview of the work done on the compounds shows acceptable results. Desorption volume shows a salient affect on all compounds, whereas, the sample volume showed a negative response except in parathion-methyl. Adsorbent weight had no significant importance on the results while different interactions among tested factors had some significant values in each extraction but negligible compare to desorption volume.

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