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Determination of Pesticide Residues in Water using On-line SPE-HPLC Coupling System

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Abstract : The on-line SPE-HPLC coupling system was developed for the efficient separation and determination of trace pesticides, such as phenoxyacetic acids and esters, and triazines in aqueous solutions. By using the developed SPE-HPLC on-line system, the band broadening usually observed in single precolumn switching mode was greatly reduced, consequently, the quantitative determination of trace pesticides could be achieved. Besides, since most of the analytes preconcentrated by SPE column could be injected directly into HPLC system, the limit of detection can be improved down to ppt level.

Keyword : solid phase extraction, triazines, phenoxyacetic acids / phenoxyacetic esters, preconcentration, on-line SPE-HPLC

1. Introduction

Determination of trace amounts of pesticides in aqueous samples requires a preconcentration step before LC analysis. Preconcentration is mainly carried out by means of liquid-liquid extraction (LLE) [1-3], but the drawbacks are obvious. LLE has some disadvantages : it is laborious and time-consuming, emulsion may be formed, large volumes of organic solvents are used, and sample losses can hardly be avoided[4]. The use of off-line or on-line solid-phase extraction as a preconcentration technique is therefore to be preferred. This approach allows easy automation at relatively low costs, with high sample throughput and good repeatability[5]. In the on-line system, the analytes are trapped on a small precolumn packed with a suitable sorbent. However, the low flexibility in setting the desorption conditions in the on-line system is the problems that have to be coped with when one tries to obtain a narrow elution profile from the precolumn, and to get the optimum separation in the analytical column[6]. The composition of the mobile phase used for the on-line SPE-HPLC system has to be primarily chosen in accordance with the requirements for a good separation in the analytical column. However, it becomes more critical, when a sorbent having stronger interactions with these analytes than a conventional C_{18} material should be used in the precolumn because of low breakthrough volume. If the precolumn containing more hydrophobic material is connected to a C_{18} analytical column, the danger of additional band broadening is obvious[7]. Therefore, the aim of this study was to solve the quantitative problems encountered in the determination of trace-level polar pesticides, such as phenoxyacetic acids, its esters, and triazines which are in common use, determinations by using the on-line SPE-HPLC coupling system.

2. Experimental

2.1. Apparatus

The chromatographic system consists of a Shimadzu LC-10AD pump unit, an SPD-10A spectrophotometric detector, a Rheodyne 7125 sample injector with a 100 μL loop and home-made water bath column oven. A Shodex C18-5B column (250×4.6mm i.d., 5 μm spherical octadecyl silica) was used. The pH measurements were carried out with a Fisher Model 15 pH meter. A three-way inlet valve (Hamilton), a ten-port valve (Valco C10W) and a six-port stream selection valve (Upchurch V240) were used for column switching. On-line sample loading was performed with MasterFlex Microprocessor pump. The precolumn(20×2mm i.d.) was filled manually with packing materials.

2.2. Reagents

All pesticides were purchased from Polyscience Inc. The individual stock solutions of all pesticides were prepared in aqueous acetonitrile mixtures. The solid sorbents, XAD-2, XAD-4, XAD-7 and XAD-8 are macroreticular resins purchased from Rohm and Haas and obtained by grinding and sieving (150-200mesh). The resin was purified by first shacking in 1M HCl (in 30% MeOH). The resins were then placed in a Soxhlet extractor and sequentially extracted for 24h each with methanol and acetonitrile. HPLC grade acetonitrile, methanol, tetrahydrofuran were purchased from Burdick & Jackson Laboratories, Inc. Water was purified by using a Milli-Q filtration system. (Millipore, Bedford, MA, U.S.A.). All other chemicals used were of analytical reagent grade.

2.3 Sample preconcentration

The preconcentration has to be carried out by on-line system, that is, use of a small precolumn and a larger analytical column connected in series. The desired solutes in aqueous sample solution are concentrated by precolumn containing sorbent. After rapid and complete elution with the mobile phase which well suited to separate the solutes from the analytical column, this on-line system has been proved as more advantageous with respect to simplicity and high reproducibility in comparison with off-line preconcentration system. The steps of the preconcentration procedure are schematically represented in *Fig. 1* and summarized in *Table 1*. The percentage recoveries of pesticides were calculated directly as proportions of standard and sample peak area.

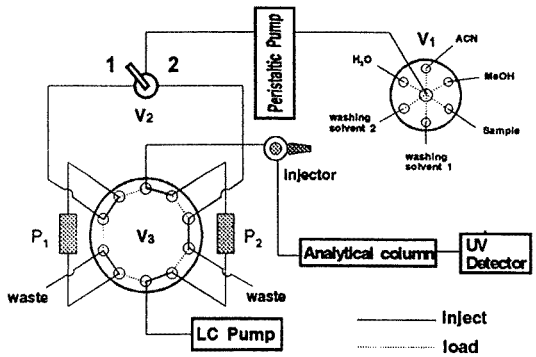

Fig. 1. The schematic diagram of an on-line SPE-HPLC coupling system.
P_1 , P_2 : Precolumn ; V_1 , V_2 , V_3 : Valve

Table 1. Summary of the Procedure of Precolumn of Preconcentration (Fig. 1)

Step	Operation	Valve position		
		V1	V2	V3
1	Washing the precolumn 1 (20mm×2mm i.d., dry-packed) with 10mL of ACN. (2.5mL/min)	ACN	1	inject
2	Conditioning them with 10mL of MeOH and then 10mL of LC-grade water. (2.5mL/min)	MeOH H_2O	1	inject
3	Preconcentration of sample (5mL/min)	Sample	1	inject
4	Wash step with 2.5mL of water (2.5mL/min)	H_2O	1	inject
5	Desorption of precolumn 1 on-line to the analytical column & repeat the 1,2,3,4 step		2	load
6	Desorption of precolumn 2 on-line to the analytical column		2	inject

ACN : acetonitrile, MeOH : methanol

3. Results and Discussion

3.1 Adsorption

Five triazines were examined for their adsorption characters on precolumn containing different sorbents, activated carbon, Amberite XADs, and C_{18}. As shown in *Table 2*, XAD-2 and XAD-4 were found to be the most effective sorbents for triazines as showing high

Table 2. Recovery of Triazines as a Function of the Sample Volume, or Each Sorbent

compound	%, Recovery											
	Activated carbon	XAD-2				XAD-4				XAD-7	XAD-8	C_{18}
	100mL	100mL	250mL	500mL	1000mL	100mL	250mL	500mL	1000mL	100mL	100mL	100mL
simazine	64	100	100	97	94	100	91	86	82	79	65	51
prometon	91	99	100	100	103	98	97	100	101	87	76	66
atrazine	101	100	105	100	98	100	91	89	86	96	77	93
propazine	100	100	101	101	95	98	90	88	87	93	90	90
prometryme	84	101	101	95	90	99	92	94	86	95	87	73
average	88	100	101	99	96	99	92	91	89	90	79	75

Amount of compounds spiked : 4×10^{-9}mol
Elution with 5mL 0.008M H_3PO_4 in ACN. results are the average of 3 individual results

recovery(> 95%) with different sample volumes. In general, the polar triazines such as simazine and prometon (pk_a range : 3- 4) showed lower recoveries. The adsorption behavior of phenoxyacetic acids and its esters was similar to that of triazines. For the efficient adsorption, it is necessary to adjust the pH of the sample solution using trifluoroacetic acid : TFA. Since the number of interfering matrix components in the chromatogram is increased with decreasing pH, the optimum pH adjusted was fixed at 3.5(*Fig. 2*). When phosphoric acid was used instead of TFA, the more interfering components were found.

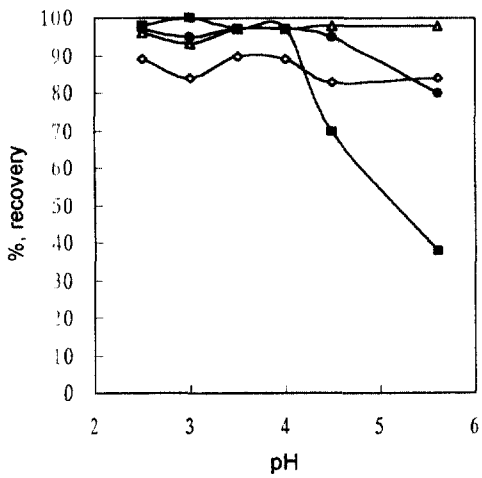

Fig. 2. Recovery dependence on sample pH
■ 2,4,5 D ; △ 2,4 D ; ◇ 2,4 DB ; ● dactal

3.2 Band broadening by precolumn

On-line preconcentration of polar compounds, such as triazines, phenoxyacetic acids & esters, give several problems. Firstly, strong sorbents that adsorb polar compounds cause band broadening during precolumn elution process. Secondly, the band-broadening lowers the reproducibility of on-line precolumn elution compared to direct injection. Recently, Henninon, and et al[8] demonstrated that band-broadening for polar compounds could be reduced by gradient elution. Gradient elution, however, is not recommended for quantitative analysis since it causes raising the baseline. The difference in the results between on-line precolumn and direct elution is likely due to that organic solvents used for the stock solution which make solutes be broadened during LC separation process. In order to solve the problem, the following experiments were recommended. Firstly, by eluting the mobil phase which has the same composition of solvents for the direct elution, the band-broadening could be disappeared for up to 100μL injection that corresponds to void volume of precolumn. Secondly, as shown in *Fig. 1*, by using two separate precolumns, the stable baseline could be obtained without any disturbance due to single precolumn switching mode. Thirdly, by using back-flushing method could be employed. The chromatograms for direct and on-line precolumn elution of triazines, phenoxyacetic acids and its esters by using on-line preconcentration system were shown in *Fig.3 and 4*, respectively. Compared to the results in *Fig.3 and 4*, there is no band-broadening and the peak shapes of

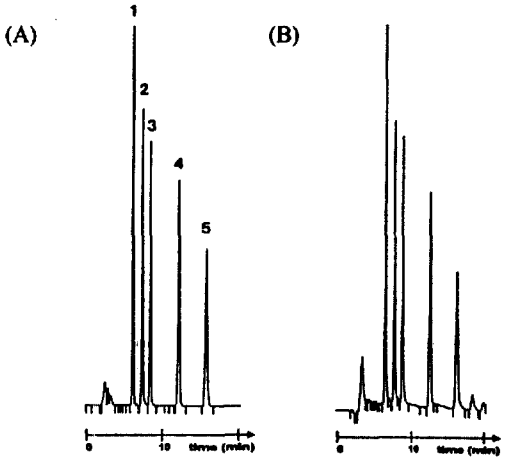

Fig. 3. Efficiency of the on-line coupling of a XAD-2 precolumn with a C_{18} analytical column for triazines (A) direct 100 μL loop injection ; (B) on-line preconcentration of 100mL of LC-grade water spiked with 10^{-8} M of each compound. Solute : 1=simazine ; 2=prometon ; 3=atrazine ; 4=propazine ; 5=prometryne. Mobile phase: 0.05M NaH_2PO_4 : Acetonitrile = 50 / 50 (pH 4.2),
Flowrate: 1mL/min, Detection: 220nm, A.U.F.S.: 1.28

on-line precolumn elution are almost the same as those of direct elution.

3.3 Validation

In order to verify no band-broadening, capacity factors and CV (coefficient of variance) for both direct and on-line elution were listed in *Table 3*. As shown in *Table 3*, the k' values of direct elution are almost the same as those of on-line elution, which proves that there is no band-broadening and the same is proved for the CV showing the excellent reproducibility of both methods. In addition, the recoveries of all pesticides are more than 90%. The limit of detection has been found to be below ppt.

3.4 Calibration by on-line preconcentration

It has been also proved from the chromatogram of on-line injection that there is no band-broadening. However, in order to confirm the possibility of determination at lower

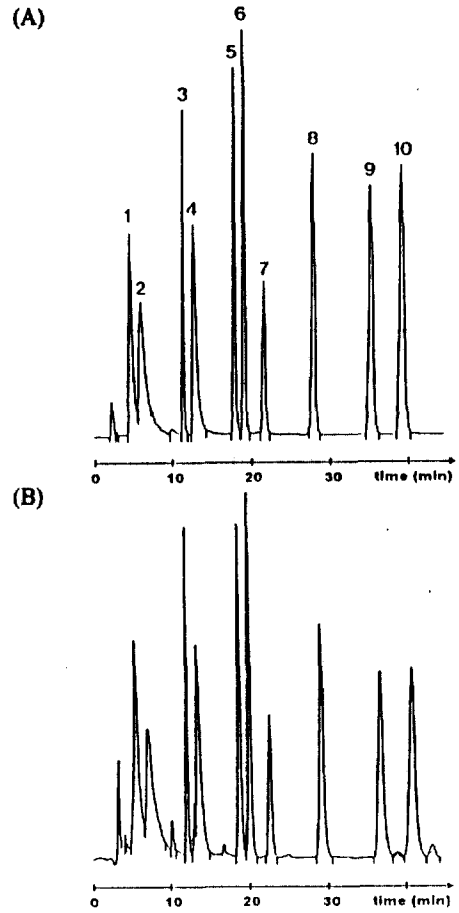

Fig. 4. Efficiency of the on-line coupling of a XAD-4 precolumn with a C_{18} analytical column for pesticides (A) direct 100 μL loop injection : (B) on-line preconcentration of 100mL of LC-grade water spiked with 2×10^{-8} M of each compound. Solute : 1=2,4,5 T ; 2=2,4 D ; 3=propanil ; 4=2,4 DB ; 5=2,4 DME ; 6=metolachlor ; 7=alachlor ; 8=2,4,5 TME ; 9=2,4 DBME ; 10=dacthal. Mobile phase: 0.05M NaH_2PO_4 / Acetonitrile = 50 / 50 (pH 4.3),
Flowrate: 1mL/min, Detection: 220nm, A.U.F.S.: 1.28

level, calibration curve for on-line preconcentration has been prepared as shown in *Fig.5*. The correlation coefficient(R) of larger than 0.99 have been found for all pesticides showing the good linearity. Besides, the peak areas of the procedural blanks were almost zero in all cases.

Table 3. Comparison between Direct Elution and On-line Elution for Pesticides

Solute	Direct Elution		On-line Elution		recovery[c], %	limit of detection[d] (ppt)
	k[a]	%, CV[b]	k[a]	%, CV[b]		
simazine	0.94	0.28	0.97	0.78	98	0.021
prometon	1.33	0.61	1.34	0.55	96	0.054
atrazine	1.67	0.39	1.67	0.23	100	0.054
propazine	2.86	0.86	2.81	0.86	97	0.053
prometryne	3.99	0.80	3.90	0.98	98	0.063
2,4,5T	1.17	0.92	1.18	1.70	97	0.51
2,4D	1.87	4.08	1.79	6.41	97	0.55
propanil	3.74	0.20	3.71	4.44	99	0.59
2,4DB	4.28	1.43	4.23	3.20	90	0.37
2,4 DME	6.42	0.79	6.31	2.17	98	0.38
metoalachor	6.94	0.58	6.80	0.71	98	0.40
alachlor	7.98	1.11	7.81	1.04	97	0.84
2,4,5 TME	10.56	2.35	10.32	2.88	101	0.38
2,4 DBME	13.60	1.54	13.26	1.42	96	0.34
dacthal	15.21	1.21	14.83	5.07	89	0.37
average		1.14		2.16	97	

[a] $(t_R-t_0)/t_0$

[b] standard deviation/mean × 100 (n=5)

[c] concentration of solute spiked 4×10^{-10} mol/100mL

[d] signal/noise=3

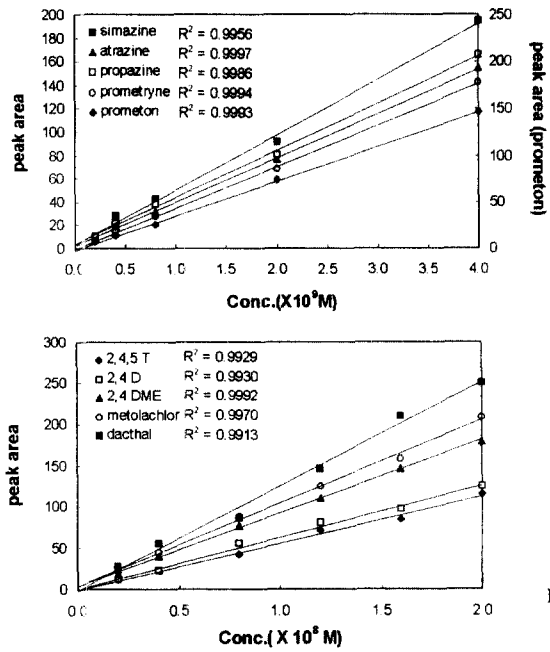

Fig. 5. Calibration curves for pesticides by on-line SPE-HPLC coupling system
Sample volume : 100mL

Conclusion

Among the different sorbents examined with regard to their functions for adsorbing and desorbing pesticides in water, XAD-2 and XAD-4 have been proved to be well suited for on-line SPE-HPLC system. On-line SPE-HPLC coupling system developed in this work allows the automatic, rapid and reliable quantification of pesticides at ppt level.

Acknowledgments

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