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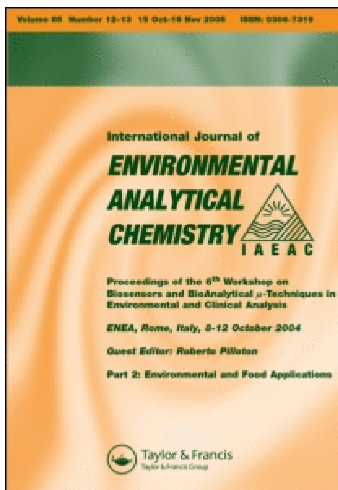

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Trace Analysis of 2,4,5,TP and other Acidic Herbicides in Wheat Using Multicolumn-HPLC†

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In continuation of our work dealing with multicolumn HPLC (MC-HPLC) techniques and their applicabilities for tracing a few compounds out of complex multicomponent matrices a residue analysis of the herbicides 2,4,5T and MCPA (phenoxyacids) in wheat is described.

A simple plant extract with aqueous basic buffer is loaded in quantities of several 100 μl onto a strong anion exchanger (column 1, C1) performing extraction of the acidic compounds, while the neutral and cationic substances are eluted thus attaining on-column trace enrichment. Via mobile phase selection (pH change) elution from C1 is possible, the fraction (zone-cut) containing the compounds of interest is transferred onto C2 (reversed phase, RP 2 and RP 18) on which peak compression is performed followed by (step)gradient elution. Detection limits in the lower ppb range are routinely obtained. A MC-HPLC chromatographic setup separation of eleven acidic herbicides in a formulation is also shown.

KEY WORDS: Multicolumn HPLC, Herbicide, MCPA, 2,4,5T, Trace Analysis.

INTRODUCTION

In a previous paper[1] we reported on the residue analysis of the herbicide Pyridate using multicolumn HPLC (MC-HPLC) as analytical technique. This contribution deals with some advantages

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of column switching techniques demonstrated by trace analysis of water-soluble samples, in particular of acidic herbicides in plant matrices.

MC-HPLC offers ideal features as a separation method in residue analysis and other fields where one has to detect (qualitatively and quantitatively) a few compounds out of a complex multicomponent mixture. The general concept of MC-HPLC performed in an on-line mode is the coupling of LC-systems (columns) with different selectivity thus creating multidimensionality based on chromatographic parameters resulting in a significant increase of the overall selectivity and in relation to, its total peak capacity.[2-5]

By coupling columns of the same separation mechanism only an increased plate number N can be performed which is under practical aspects mostly not sufficient to resolve the questionable peaks from the matrix within a k′ range of about 20 to keep the peak width and sensitivity, respectively, acceptable for trace analysis. Enhanced selectivity in the overall chromatographic system should cause consequently that the time consuming off-line sample clean-up steps may be evaded and can be transferred to an on-line multi-column system, respectively.

From this point of view we developed MC-HPLC setups in consideration of some general aspects in modern trace analysis, which are: (a) selectivity (trace identification), (b) sensitivity, often performed by trace enrichment, (c) quantification, (d) reproducibility, (e) overall short analysis time.

The less selective and sensitive the detection principles of the compounds to be quantitated are, the more input the chromatographic selectivity (separation power) gets, if one takes into account the hundreds of possible by-products which may interfere in trace analysis of complex samples in the ppb range.

Enhanced selectivity in the on-line coupled chromatographic system should include possibilities to reduce some time consuming off-line sample cleanup steps like extractions, concentrations etc.; these manipulations should rather be performed on-line and in an automatic way. Considering the above mentioned requirements this paper describes residue analysis of phenoxy acids (MCPA and 2,4,5T) in wheat corn applying MC-HPLC. By combining an anion exchange column with reversed phase columns, sample enrichment and sample clean-up steps are on-line integratable into the overall

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analysis scheme. Besides this also a MC-HPLC setup is performed to characterize chromatographically eleven acidic herbicides.

EXPERIMENTAL

Chemicals

The analytical standards Pichloram, Suffix (as acid), Barnon (as acid), MCPP, MCPA, 2,4,5T, 2,4,5TP, 2,4D and 2,4DP (international abbreviations) were from Chrompack (Netherland) and a national laboratory for pesticide analysis (Graz, Austria), and were of highest grade of purity for commercially available materials. The analytical standard Pyridate (carbonithiocic acid-0-(6-chloro-3-phenyl-4-pyridazinyl)-S-n-octyl-ester) and its main metabolite CL9673 (3-phenyl-4-hydroxy-6-chloro-pyridazin) were obtained from Chemie Linz AG (Austria). The solvents and reagents for sample pretreatment and chromatography, methanol, acetic acid, ammonia, potassium-dihydrogen-phosphat and phosphoric acid were all of p.a. grade and obtained from Merck (West Germany). The water was deionized and doubledestilled in our laboratory.

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Chromatographic components

The columns (stationary phases) used were: Anion exchange-silica of 10 μm (Lichrosorb, 60 × 4.6 mm I.D., Kontron MPLC-cartridge), reversed phase RP 18 (Lichrosphere ODS 5 μm, 100 × 4.6 mm I.D.) and RP 2 (Lichrosorb 10 μm, 100 × 4.6 mm I.D.) (Kontron MPLC-cartridges).

The equipment to set up the multicolumn HPLC system consisted of: Two high pressure pumps Model 410; spectrophotometric detector 720 LC; valve switching unit Tracer 670; programmer Model 200; recorder 21 (all units Kontron, Switzerland); loop injector Model 7210 (Rheodyne, U.S.A.) with 20 μl and 850 μl loop volumes.

The time programmes of the valve switchings (activating the different columns and mobile phases) are schematized in the block diagrams (Figure 3 and Figure 6) belonging to the chromatogram Figure 2 and Figure 5).

The quantification was persued by peak height measurements and external standards at a detection-wavelength of 230 nm.

Sample pretreatment

Equipment: Omni mixer (Sorvall, U.S.A.); cooling apparatus −20°C (Holzwath, West Germany); rotary evaporator (Büchi, Switzerland); centrifuge, Labofuge 1 (Heraeus, West Germany); filtration funnels and filters.

Procedure for trace analysis of MCPA and 2,4,5T in wheat

Fifty gramms wheat were doted with various amounts of MCPA and 2,4,5T (between 20 and 200 ppb), extracted twice with 100 ml of a mixture of methanol and 0.05 m aqueous ammoniumacetate (70/30; pH = 7.0) in a high speed blender (omnimixer) for 10 minutes at a temperature of around 10°C. The combined pulpy extractions were filtered prior to its evaporation at 50°C. The residue was taken up in 10 ml mobile phase M 1 (see Figure 3), centrifuged and 850 μl of this solution was injected onto the multicolumn HPLC system. For the qualitative analysis of the herbicide mixtures (formulations), solutions of the various standards in methanol served as samples.

Chromatography

As pointed out in the introduction, we set up column switching systems (see Figure 1 and 4) which allowed to work dominantly in a multidimensional LC mode performed by combining an anion exchanger and reversed phase (RP 2 and for RP 18) systems involving various step gradients (mobile phase changes) generated by switching low pressure solvent selection valves. Thus multidimensional chromatographic selectivity was created via mobile- and stationary phase switching.

Working with two pumps, A and B, (see Figure 1 and 4) guarantees that the separation system I can be run simultaneously at the time the final analysis is performed on column II. The simultaneous principle (in slightly different mode often boxcar principle called) considered, results in an overall acceptable analysis time.

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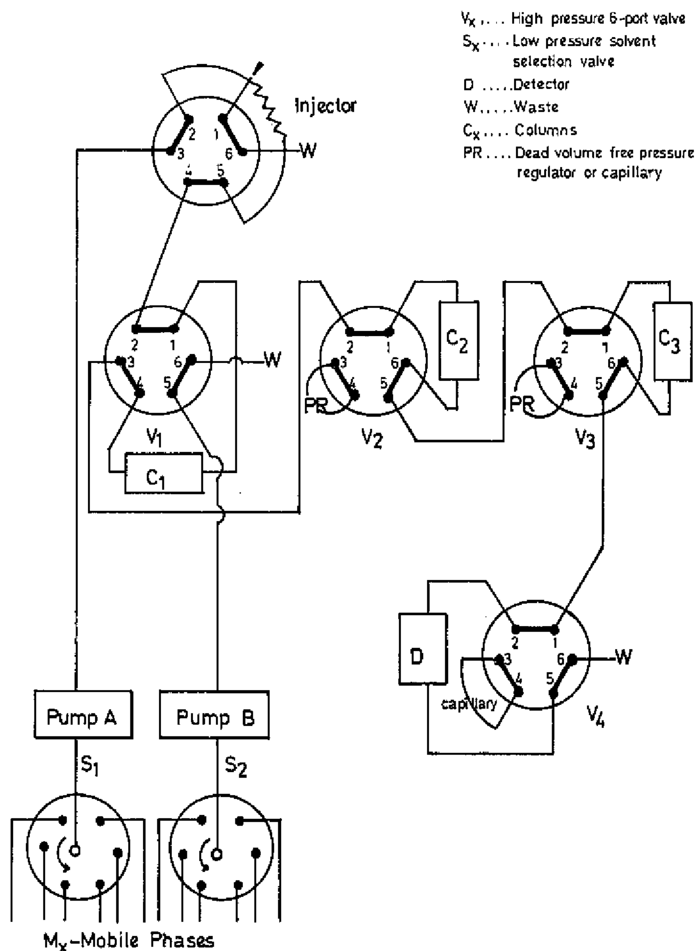

FIGURE 1 MC-HPLC valve switching configuration used for trace analysis of MCPA and 2,4,5T in wheat.

Description of the MC-HPLC setups

(a) *Analysis of plant extracts:* The valve switching configuration shown in (Figure 1) is designed that column C1 will be sample loaded in the foreflush mode, followed by several washing steps (see block diagram Figure 3). Since C1 is an anion exchanger it retains

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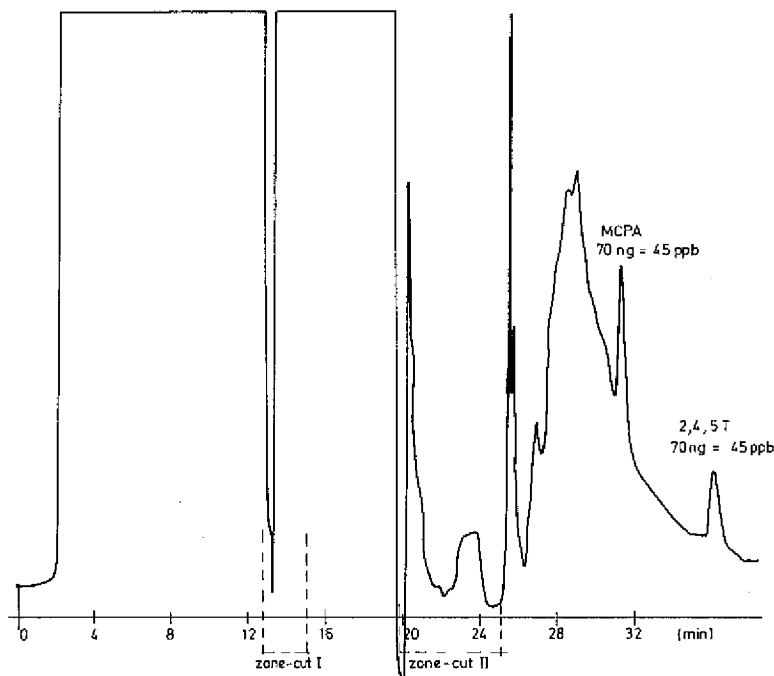

FIGURE 2 MC-HPLC chromatogram based on the column switching setup according Figure 1.

strongly acidic compounds (herbicides and others) at a pH around 7. Consequently one gets effective on-column concentration effects injecting the sample dissolved in mobile phase M1 (pH = 7.0) (even large volumes as 850 μl or higher). The lipophilic mobile phase M2 serves as cleanup eluent for lipophilic and cationic by-products. Elution of weak anionic compounds of the plant extract was performed with the weak acidic mobile phase M3. By changing the eluent to strong acidic conditions (M4) elution of the herbicides is attainable; via backflush elution and zone-cutting only a relatively small fraction is on-line transferred to the following column C2 (RP 2) via pump B and the aqueous mobile phase M4 (pH = 2.0). Under these acidic conditions the phenoxy acids are non ionized and the transferred fractions are compressed on the top of the following moderate lipophilic RP 2 column. By mobile phase changing from M4 to M5 elution from C2 is performed via step gradient. However,

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Time (min): 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 (Start)

Valve	Function activated	Time (min) 0 → 44
J	Injector	Start; zone-cut I; zone-cut II
V 1 SSV 2	Column C 1	PB M 1; PB - M 2; PB - M 3; PA - M 4; PB - M 7; PB - M 1; off or sample precleaning of next injection with PB-M1-M2-M3
V 2 SSV1	Column C 2	off; PA-M4; PA - M 5; off; PA-M4
V 3 SSV1	Column C 3	off; PA - M5; PA - M 6; PA-M5; off
V 4	λ - scan	off; on; off; on; off
	Flow PA ml	0; 2; 2
	Flow PB ml	2; 9; 0; 4; 2

Column	Description	Mobile phase	Composition	Mobile phase	Composition
C 1	Anion Exchanger 10 µm,60x4,6 mm	M 1	0,05m NH_4Ac (pH7,0)-MeOH/60-40		
C 2	RP 2 10 µm,100x4,6 mm I.D.	M 2	H_2O-MeOH / 10-90	M 5	0,2m KH_2PO_4(pH2,0)-MeOH/65-35
C 3	RP 18 5 µm,100x4,6 mm I.D.	M 3	0,45m H_2O+AcOH-MeOH / 50-50	M 6	0,2m KH_2PO_4(pH2,0)-MeOH/55-45
		M 4	0,2m KH_2PO_4 (pH 2,0)	M 7	0,2m NH_4Ac (pH 9,0)

FIGURE 3 Time function table of the chromatographic parameters used in the MC-HPLC system described in Figure 1 and Figure 2.

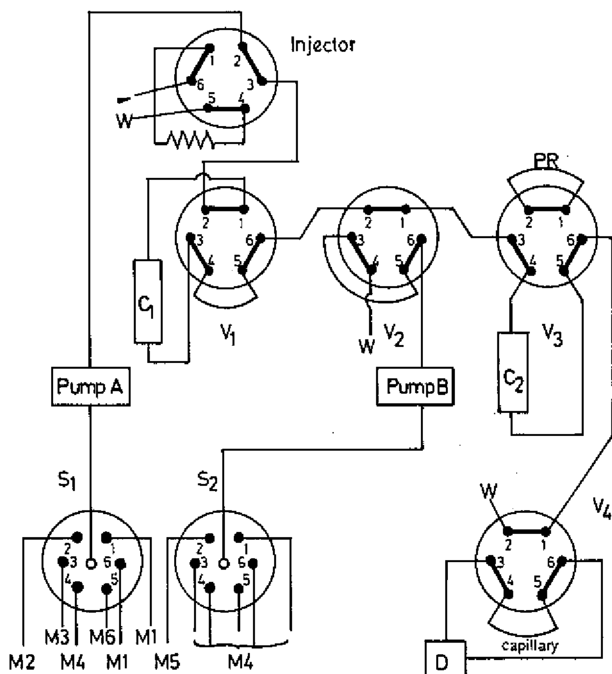

FIGURE 4 A typical valve switching configuration for MC-HPLC analysis in a multidimensional mode. Abbreviations see Figure 1.

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the combined two separation systems (chromatographic selectives) of C1 and C2 were not satisfactory to separate the remaining matrix peaks from MCPA and 2,4,5T to be traced sufficiently. To get around this, we connected to the system above an additional reverse phase column with increased lipophilicity (RP 18 > RP 2) to attain again on-column concentration effects when transferring a zone cut from C2 to C3 with mobile phase M5. The final analysis was performed on C3 with M6 in a step gradient mode and is shown on the chromatogram Figure 2). Simultaneously C1 (anion exchanger) was washed with M7 and re-equilibrated with M1 to guarantee initial conditions for the next multicolumn analysis. As indicated on block diagram Figure 3), in case of peaks with the appropriate retention times of 2,4,5T and MCPA, we made on-line UV spectra of this fractions by switching the valve V4 to work in a "stop flow

mode" via a bypass valve switching setup. For this the baseline had to be stored in a previous run. The detection limit for acceptable UV-spectra was around 10 miliabsorption units at 230 nm.

(b) *Analysis of synthetic mixtures of acidic herbicides:* Based on the general concept combining ion exchange columns with reversed phase systems (pH step gradients and variation of their elution strength on RP columns) we set up a relatively simple valve switching configuration shown in Figure 4). Via the valve V3 the two separate chromatographic systems (pump A—mobile phase selection S1–C1) and (pump B–S2–C2) are linkable or dislinkable from each other. Thus, simultaneously with the final analysis on C2 the ion exchanger C1 can be regenerated and brought into equilibrium for the following analysis.

As can be seen on the chromatogram (Figure 5) the most herbicides are separated from each other, indicating that the

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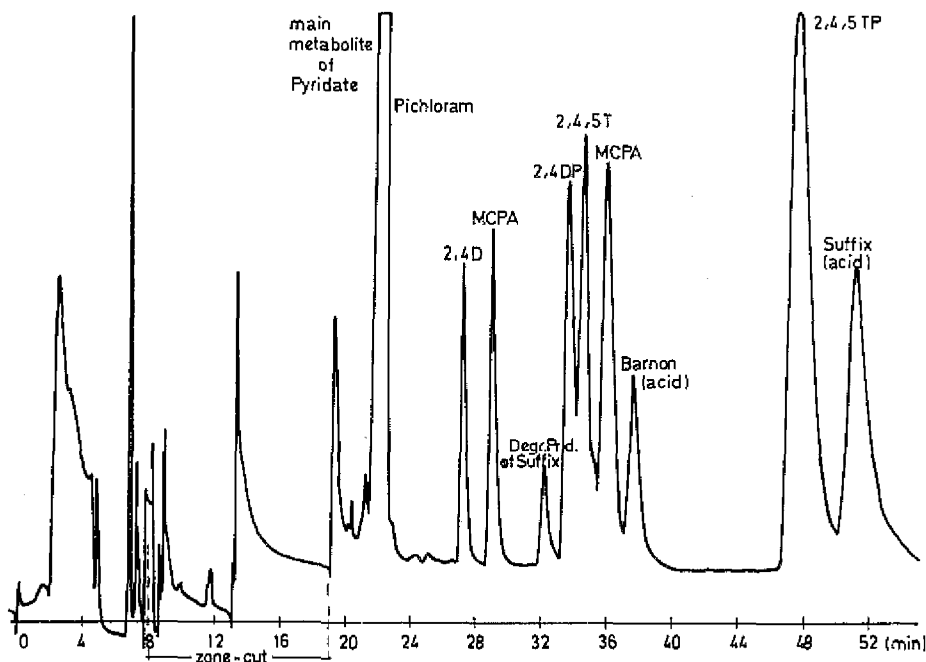

FIGURE 5 MC-HPLC chromatogram of acidic herbicides to the column switching setup Figure 4.

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Function activated	Time (min) 0	4	8	12	16	20	24	28	32	36	40	44	48
Inject / Zone-cut (ZC)			backflushed ZC										
C 1	PA-M1, PA-M2	PA-M3	PA-M4			PA-M6		PA-M1					
C 2			PA-M4			PB-M5				PB-M4			
Pump A (PA) Flow	2 ml/min												
Pump B (PB) Flow				2 ml/min						4ml/min			
Valve V 2 position	a		b			a							
Valve V 3 position	a					b							
Valve V 4 position	b		a										
Selector valve S 1 position	1, 2	3	4			5		6					
Selector valve S 2 position				2						1			
Detector	UVIKON 720 LC, 230 nm, 0,1 AUFS												

Columns		Mobile Phases			
C 1	Anion exchange (10 μm), 60x4,6 mm	M 1	0,05M NH_4Ac (pH=9) : MeOH (60:40)	M 4	0,2M KH_2PO_4 (pH=2,0)
C 2	RP 18 (5 μm), 100x4,6 mm	M 2	H_2O : MeOH (10:90)	M 5	0,2M KH_2PO_4 : MeOH (56:44)
		M 3	H_2O	M 6	0,2M NH_4Ac (pH=9)

FIGURE 6 Time function table of the chromatographic parameters used in the MC-HPLC system described in Figure 4 and Figure 5.

described column switching setup could be used only with slight modification of the mobile phases for many of the acidic herbicides *on the market. The block diagram corresponding to the* chromatogram (Figure 5), is shown on (Figure 6) containing all informations concerning the chromatographic parameters.

RESULTS AND DISCUSSION

With respect to the requirements of trace analysis in complex multicomponent matrices the MC–HPLC setups described above show some general possibilities tracing ionizable compounds out of complex multi-component extracts, and which are: (a) The sample clean-up is transferred to an on-line chromatographic technique involving (b) trace enrichment to increase the overall sensitivity.

Unfortunately, also some by-products (also ionizable) will be enriched by loading the first column with a large sample volume, while neutral or oppositly charged components are only weak retained. Using a nonlipophilic ion exchanger, (e.g. based on silica gel backbone) in our case anion exchanger, but it is generally also true for a cation exchanger, and ideal and selective group separation system is given for aqueous samples containing ionic compounds.

With proper pH and/or ionic strength changes elution from ion exchangers can be performed selectively, based on the pk_a values of the particular compounds, resulting aqueous eluents containing the ionizable compounds in nonionic lipophilic form, ideal to be on-column concentrated on e.g. reversed phases (RP18). Transferring only the appropriate effluent zone cut containing the compounds of interest from system 1 to system 2 advantageous effects are combined, the sample cleanpup by effluent cutting, the zone or peak(fraction)-compression on column 2 to avoid untolerable peakbroadening because of inferior chromatographic starting conditions[6-9] (sample volume is too large), and finally the on-line combination of two quite different chromatographically selective separation systems, thus creating truely multidimensionality.

Summarizing the practical parameters of the application described, the overall recoveries (inclusive plant extraction) for MCPA and 2,4,5T trace analysis using the MC–HPLC system with three columns was 80% each at levels between 20 and 200 ppb with a

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standard deviation of ±7% tested on doted wheat samples. The detection limits reach the low ppb range.

The examples described represent typical two-dimensional column switching systems, whereby sample clean-up trace enrichment and preseparations are carried out by ion exchange processes, while the final analyses are performed via adsorption phenomena based on lipophilic interactions on reversed phase columns.

As a conclusion, some important effects in MC–HPLC we have learned from our experience and the study of literature, especially in the field of automated drug analysis[10–15]. If the sample solvent is aqueous it is very convenient and potentially interesting to couple on-line the following chromatographic systems and columns, respectively: size exclusion-ion exchanger-polar and non-polar bonden phases, which are quite different in their selectivity, whereby the column sequencing has dominantly to be determined by the fact to create on the top of each column so-called on-column concentration effects inclusive sample (trace) enrichment prior to their (step)-gradient elution by mobile phase changes.

Considering the latter as a necessity also the coupling of several non aqueous systems is convenient,[16–18] but sometimes less handy applicable.

However, the high and efficient separation power of multidimensional MC–HPLC, combined with the benefits of minimal sample pretreatment and cleanup procedures, respectively, has to be paid by increased requirements on chromatographic hardware and know-how. But the results you can get justify the effort for now and the future.

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