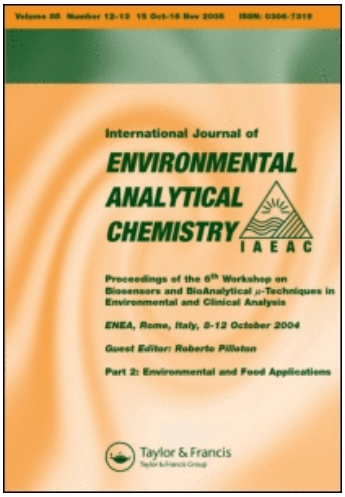

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HPLC—Residue Analysis of the Herbicide Pyridate in Cereals†

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For many reasons residue analysis of plant protecting chemicals is becoming of increasing importance. The demand of modern trace analysis techniques is to detect significantly and sensitively values down to levels in the ppb range or even less. Aggravating circumstances are the complex multicomponent sample matrices out of whom the residue analysis has to be performed; theoretically thousands of compounds could be present and interferences with substances of interest are quite likely. One way to get around these difficulties is to employ multidimensional HPLC (MD-HPLC). A method using this invaluable analytical tool tracing Pyridate and its main metabolites in various plant extracts is presented.

Based on a brief discussion about requirements of modern trace analysis HPLC in connection with column switching we designed an automatizable setup combining a weak anionexchanger (dimethylamine phase) with a reversed phase system handling relatively large aqueous sample volumes. Depending on specifically described sample pretreatment schemes detection limits down to 30 ppb are routinely obtained. At this level acceptable on-line UV-spectra can be obtained via inserting a spectrophotometric detector in a loop of a 6-port valve. The usefulness of the method described has been demonstrated by analysing several hundred samples.

KEY WORDS: Multidimensional HPLC, column switching technique, trace analysis, Pyridate, sample cleanup.

INTRODUCTION

High performance liquid chromatography (HPLC) is becoming a widely used technique in the trace analysis of pesticides and various

†Parts of this paper have been presented at the 12th Annual Symposium on the Analytical Chemistry of Pollutants, Amsterdam, April 14–16, 1982.

‡Dedicated to Prof. Dr. E. Schauenstein on the occasion of his 65th birthday.

other plant protecting compounds. It is not the purpose of this article to review HPLC on this subject, but there it is our intention to suggest multicolumn HPLC (MC-HPLC) as a powerful tool in residue analysis demonstrated on a new herbicide, Pyridate and its main metabolites (see Figure 1).

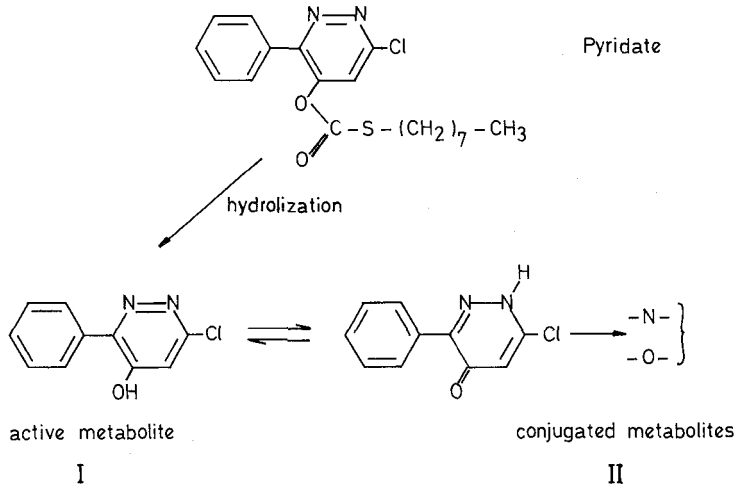

FIGURE 1 Formula of Pyridate and its main metabolites I and II.

MC-HPLC, as seen from a survey of recent literature,[1,2] offers ideal features in residue analysis and other fields where one has to detect (qualitatively and/or quantitatively) a few compounds in a complex multicomponent mixture. The general concept of MC-HPLC and for several advantages performed in an on-line mode, is the coupling of n ($n=2$ or more LC separation mechanisms) LC columns to increase significantly the overall chromatographic selectivity. Thus the general term MC-HPLC becomes more limited and one deals ideally with multidimensional HPLC (MD-HPLC) systems. Both, the stationary phases and the mobile phases are to be selected independently. To attain this flexibility a simple general LC setup is necessary and is schematized in Figure 2. It shows a configuration which uses basically two pumps with two low pressure solvent selection valves and at least two high pressure valves to

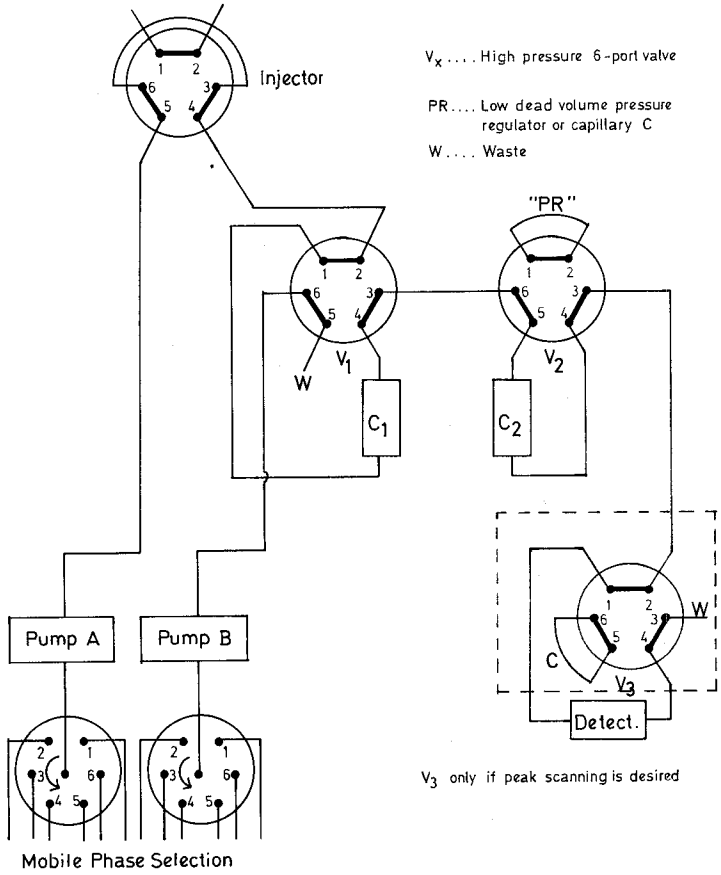

FIGURE 2 Column switching setup. Column C_1 Nucleosil dimethylamine 10 μm; Column C_2 RP, ODS 5 μm Spherisorb; V_1, V_2, V_3 high pressure 6-port valves; SSV low pressure solvent selection valve.

achieve time programmable zone cutting and on-line transfer of their effluent fractions from one LC separation system to the other. This automatic column switching configuration capable to perform MD-HPLC is in part similar to the work of Erni *et al.*[3,4] however, some new aspects have to be considered. As can be seen from the literature[5] various other column switching setups have also demonstrated their potential as MC-HPLC techniques for increasing

the selectivity in trace analysis in complex matrices (biological materials etc.).

In the following the possibilities of a two column system schematized in Figure 2 will be briefly discussed with regard to:

a) sample clean-up;
b) trace enrichment;
c) trace identification and quantification;
d) reproducibility;
e) (overall short analysis time).

a) Sample clean-up

Off-line sample clean-up is a well known procedure and dominantly performed by selective (pH controlled) multiple extractions. This time consuming technique with the risk of low recovery values (for several reasons) can mostly be avoided by chromatographic extraction techniques, whereby only the effluent fractions in which the compound(s) of interest is eluted are collected and transfered from one LC separation column to the other. The effluent cuts/fractions are termed in respect of their zones in a chromatogram and should be named as indicated in Figure 3.

In general and especially in trace analysis the elution strength of the solvent (mixture) of the effluent cuts should be weak for the following stationary phase to perform on the top of this column a so

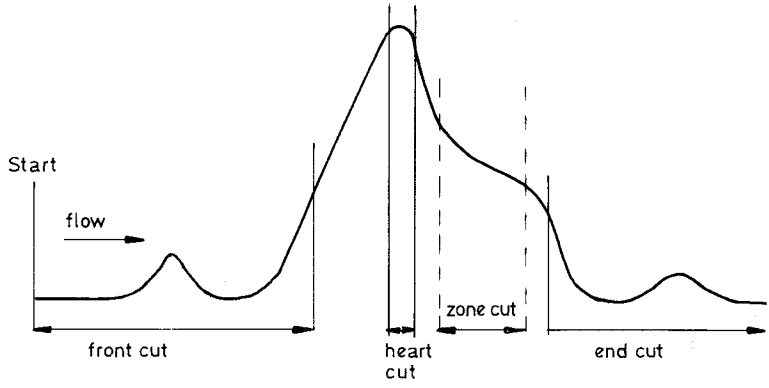

FIGURE 3 Effluent cut techniques, nomenclature.

called on-column concentration effect. Large sample volumes (cut volumes) are injectable without significant band broadening as long as the k' values are around 100 or higher. Detailed information on this subject were recently reported in the literature.[6, 7, 8]

b) Trace enrichment

In chromatography trace enrichment is feasible by on-column concentration effects as mentioned above. Large sample (effluent cut) volumes (up to several 100 ml) carrying the trace compound(s), will be passed through a column; the solvent has to have a very weak elution strength for the traces on the particular stationary phase to achieve on-column concentration with recovery values up to 100%.

c) Trace identification

Interferences, especially in complex matrices, are more likely to disturb the determination of lower concentrations of the trace compound. In the 1 ppm range e.g. one has to take into account empirically ca 1000 components in the matrix.[9] To some extent MD-HPLC is a way of getting around this problem via increased chromatographic selectivity and identification, respectively. The more different the coupled separation systems, the higher the significance of peak identity will be, simultaneously the risk of misinterpretation qualitatively and quantitatively decreases. Additional significant information in term of peak identity could also be gained using selective detection principles (UV-scan, fluorescence, mass spectroscopy etc.).

d) Reproducibility and overall short analysis time

With modern programmable valve switching devices the requirement of reproducibility is mostly fulfilled. The chromatographic reproducibility depends on the column and its irreversible coatings by matrix components. This effect can be significantly reduced by washing the columns independently after each run. Column switching techniques should be used to reach this goal with an overall reduction in analysis time. This can be achieved by running the (two) columns (for on-column concentration, preseparation,

reconditioning, final separation) independently and simultaneously in a so-called "box car" or better "simultaneous" principle.

In consideration of the basic requirements of MD-HPLC approaching trace analysis we applied this technique to quantify the herbicide Pyridate and its main metabolite CL 9673, free and in conjugated form, in the ppb range in maize and other cereals of different plant physiological growth stages.

A residue analysis method of Pyridate is already published by J. F. K. Huber and coworkers[10] applying multidimensional LC, but in a quite different mode as it is presented in this paper.

EXPERIMENTAL AND RESULTS

Chemicals

The analytical standards Pyridate (carbonicthionic acid-0-(6-chloro-3-phenyl-4-pyridazinyl)-S-n.octyl ester) and the metabolite I (3-phenyl-4-hydroxy-6-chloro-pyridazin) were obtained from Chemie Linz AG (Austria). The solvents for the sample pretreatment (see Figure 4) and chromatography, acetone, dichloromethane, acetic acid, ammonia, sulfuric acid, methanol and n-octylamine were all p.a. grade obtained from Merck (West Germany). The water was deionized and doubledestilled in our laboratory.

Chromatographic materials

The stationary phases used were dimethylamine-silica of 10 μm (Nucleosil, Macherey, Nagl, West Germany) and reversed phase, Spherisorb ODS 5 μm (Phase Separation, Great Britain). The RP 18 column (250 × 4.6 mm I.D.) was packed by the company, the dimethylamine column (200 × 4.6 mm I.D.) was slurry packed (MeOH-CCl_4/10–90) in our laboratory.

APPARATUS

Sample pretreatment

Omni mixer (Sorvall, U.S.A.); shaking machine (Bühler, West

Germany); cooling apparatus −20°C (Holzwath, West Germany); rotary evaporator (Büchi, Switzerland); centrifuge, Labofuge 1 (Heraeus, West Germany); filtration funnels and filters.

MD-HPLC equipment and conditions

Two pumps Model 410: spectrophotometric detector equipped with λ-scan option Model 720 LC; valve switching unit, prototype of the Model Tracer 670; programmer Model 200; recorder 21; all units (Kontron, Switzerland); loop injector with 850 μl loop volume, Model 7210 (Rheodyne, U.S.A.). The detection wavelength was set to 280 nm. Peaks with the appropriate retention time of the metabolite I were routinely scanned between 200 and 380 nm down to an absorption maximum of 3.10^{-3} A.U. at the peak maximum (280 nm). The chromatograms and UV spectra were registered with the potentiometric recorder, the quantification was persued by peak height measurements and external standards.

Samples and sample pretreatment

The plant materials to be analyzed for Pyridate, metabolites I and II were green plants of different growth stages, straw and grain of maize, wheat, barley and oat, as well as rape seeds and poppy seeds. Except for the seeds the plant materials were kept deep frozen between the harvest and the sample pretreatment. According to the work-up procedure schematized in Figure 4 we developed a sample pretreatment which allows a preseparation of the three compounds of interest. It is based on selective, pH controlled, extractions between organic and aqueous phases combined with hydrolization steps of Pyridate and the metabolite II to get metabolite I. The resulting aqueous extracts containing the metabolite I are analysed by MD-HPLC, whereby Pyridate and the metabolite II are determined as metabolite I. By this procedure only one LC separation system has to be controlled. Pyridate hydrolizes very rapidly under basic, acidic and physiological conditions, a fact which was used evaluating the herbicide Pyridate knowing that the hydrolizate (metabolite I) has the intrinsic plant physiological activity. Pyridate is only a carrier form.

Based on that it seemed rather logically for routine work to design

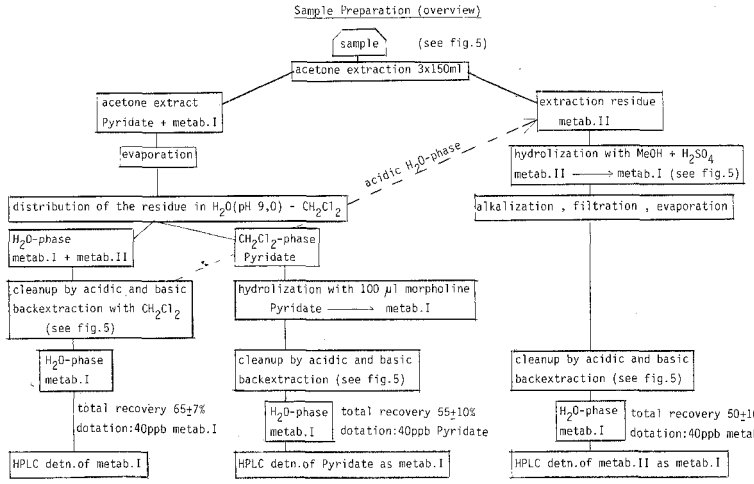

FIGURE 4 Sample pretreatment scheme for individual determination of Pyridate and its metabolites I and II.

a more simple sample pretreatment so as to detect only the metabolite I as a sum-total of hydrolized Pyridate. Metabolite I and hydrolyzed metabolites II, are probably also physiologically active compounds. To hydrolize the conjugated metabolites II to metabolite I, the solid residue of the acetone extraction combined with the aqueous acidic phase of the first extraction has to be suspended in 100 ml Methanol-2n-H_2SO_3 and refluxed for 20 min. After alkalization with ammonia to pH 9.0, filtration and evaporation to dryness of the methanolic filtrate, the residue was treated like the fraction carrying the metabolite I as seen in Figure 4. Under these hydrolization conditions the metabolite I is chemically stable, because the solid residue buffers the H_2SO_4 to a large extent. Hydrolization without the solid residues as performed in the simplyfied sample pretreatment (see Figure 5) leads with 2n H_2SO_4 to a significant degradation. With 0.2 n methanolic H_2SO_4 the recovery is higher than 97%. The total recoveries of metabolite I for the different extraction procedures are shown in Figure 4 and Figure 5. The values are rather low (between 50 and 70%), however, they are reproducible and high enough to reach overall detection limits of

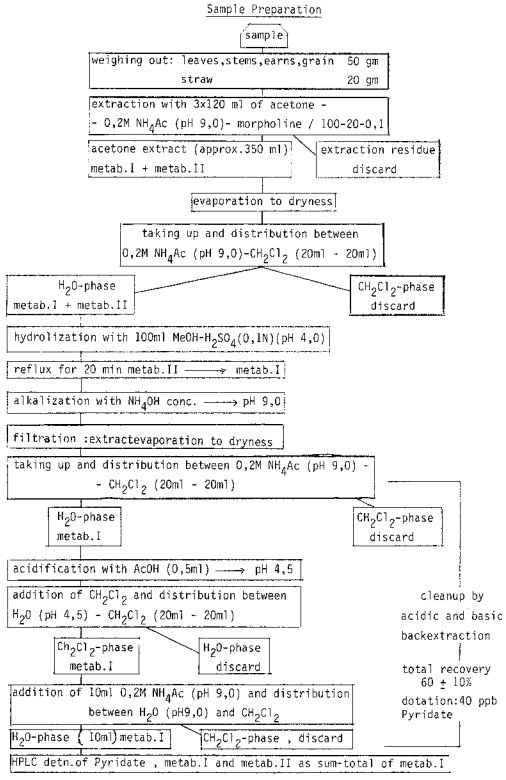

FIGURE 5 Simplified sample pretreatment scheme determining the total amount of Pyridate and its metabolites I and II as metabolite I.

metabolite I (sample pretreatment + HPLC analysis) in the low ppb range.

Chromatography

As pointed out in the introduction, we set up a column switching system (see Figure 2), which allowed to work primarily in a multidimensional LC mode performed by combining a weak anion exchanger (dimethylamine phase) and a reversed phase (RP 18) involving various step gradients (mobile phase changes) generated by switching low pressure solvent selection valves. Working with two

pumps (A and B) guarantees that the separation system I can be run simultaneously at the time the final analysis is performed on column II. The simultaneous principle considered results in an overall acceptable analysis time. As one can see in the chromatogram (Figure 6), the matrix peaks are sufficiently separated from the metabolite I peak, also its "stop flow scan" UV spectrum measured in an 8 μl flow cell is depictured. The absolute amount of 75 ng corresponds to ca.30 ppb according to the sample pretreatment scheme (Figure 4) (50 g plant material ending with 10 ml endextract) and an injection volume of about 850 μl. With this column switching setup the run to run reproducibility (retention time and peak height for quantification) was sufficient ($s = \pm 5\%$ and $n = 6$ at an absolute peak concentration of 100 ng). Nevertheless, we run routinely after six matrix samples a standard sample to check the system and especially the time window of the appropriate zone cut to be transferred from column I to column II. We found some degradation of column I (Nucleosil, dimethylamine phase) not knowing if it was only physically or also chemically caused. However, by refilling this void in the column approximately every 30th injection we could use the column I for at least 400 matrix injections before replacing it. Column II lasted longer than 1000 injections before an intolerable loss (more than 30% of the initial conditions) on efficiency occurred. To get to this relatively high life time we found it is of great importance to reduce rapid changes of pressure drops on the columns, a factor which one can hardly get around entirely in

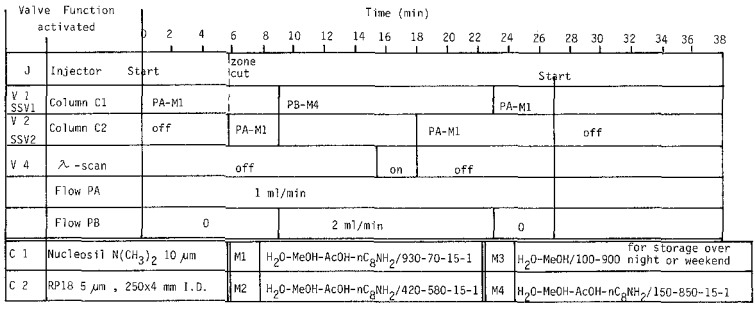

FIGURE 6(a) Time table and block diagram of the functions changed in the setup of FIGURE 2 during a chromatographic run shown in FIGURE 6.

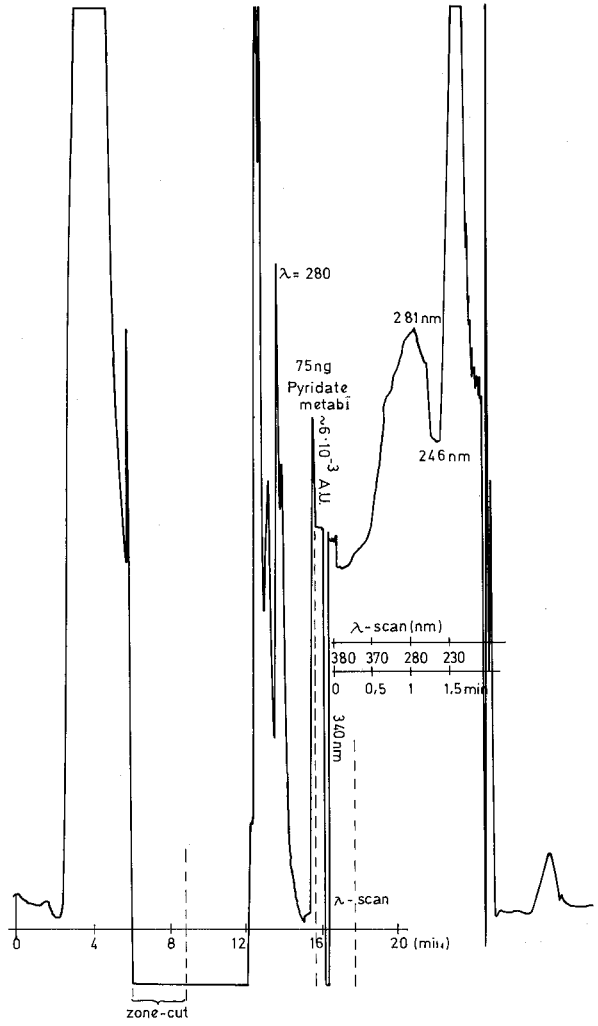

FIGURE 6 Typical chromatogram of a plant extract sample containing metabolite I identified by UV scanning.

multicolumn liquid chromatography and effluent zone cutting techniques, respectively. One way is the logical insert of adjustable low deadvolume pressure regulators "PR" (see Figure 7 and Figure 2) in the effluent streams bypassing the columns. The flow is directed by high pressure 6-port valves. Adjustability is necessary to adapt the "PR" units according to the particular pressure drops of the columns in use. Low dead volume is desirable to avoid significant peakbroadening effects. We used successfully this "PR" unit with different column switching configurations. In all cases the total column life time was significantly increased.

We also had some problems with column I caused by backflush elution. The column became disorientated probably mainly due to the fact of its tendency of degradation; the shrinked packing gets

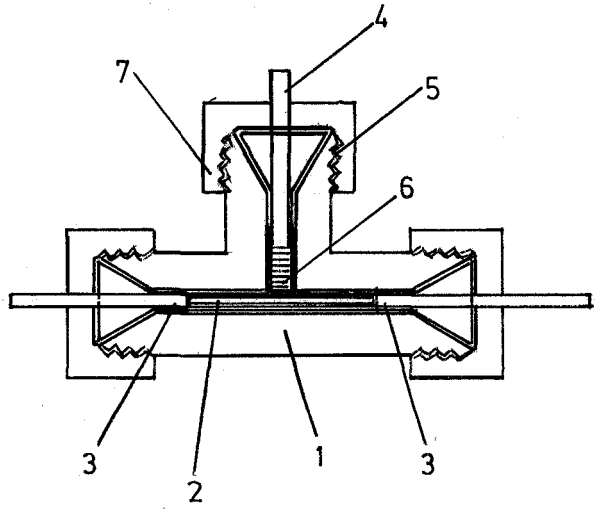

FIGURE 7 Homemade back pressure regulator "PR", adjustable and with low dead volume.

1) Union Tee for capillaries 1/16″ (drilled out to 1/16″ I.D.)
2) Teflon capillary 1/16″, 0.3 mm I.D.
3) Stainless steel capillaries 1/16″, 0.25 mm I.D. (steel ferrules prefixed)
4) Stainless steel plug 1/16″ (steel ferrule prefixed)
5) Ferrule wrapped around with teflon tape acting as seal
6) Teflon plug
7) Nut; capillary 2 gets squeezed by turning the nut.

pushed through the column like a plug. With the more stable reversed phase columns these phenomena occur much less frequently. Chromatographically, backflushing should be advantageous since the overall retention times and analysis time, (elution of the peaks of interest and the washing cycles) are tending to decrease.

CONCLUSION

The multidimensional HPLC setup described above in combination with the extractive sample precleaning steps according to the sample pretreatment scheme allows the performance of routine analysis of Pyridate and its main metabolites in plant biological materials and other sources. Significant detection limits down to 30 ppb can be obtained routinely. Several hundred different samples were analysed by this technique to demonstrate its workability.

In conclusion, combining the power of MD-HPLC results in an exponential increase of the total chromatographic selectivity and peak capacity. In addition a selective detection system offers a great potential in trace analysis of various complex multicomponent samples.

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References

1. R. E. Majors, *J. Chromatogr. Sci.* **18,** 571 (1980).
2. R. L. Miller, K. Ogan and A. F. Polle, International Laboratory, Sept. 1981, p. 60.
3. F. Erni and R. W. Frei, *J. Chromatogr.* **149,** 561 (1978).
4. F. Erni, H. P. Keller, C. Morin and M. Schmitt, *J. Chromatogr.* **204,** 65 (1981).
5. W. Lindner, H. P. Keller and F. Erni, *Chromatographia* in preparation (1983).
6. C. E. Werkhoven-Goewie, Thesis, Free University of Amsterdam (1983).
7. C. E. Werkhoven-Goewie, U. A. Brinkman and R. W. Frei, *Anal. Chem.* **53,** 2072 (1981).

8. P. E. Schoemakers, P. I. Billet and L. deGalan, *J. Chromatogr.* **185,** 179 (1979).
9. H. Hulpke and U. Werthmann, *Chromatographia* **13/7,** 395 (1980).
10. J. F. K. Huber, J. Fogy and C. Fioresi, *Chromatographia* **13/7,** 408 (1980).
11. J. F. K. Huber and R. Hirtz, Oral Presentation at the VI Int. Symposium on Column Liqu. Chromatogr., Philadelphia, June 1982.