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ON-LINE TRACE ENRICHMENT FOR IMPROVED SENSITIVITY IN LIQUID CHROMATOGRAPHY WITH DIRECT LIQUID INTRODUCTION MASS SPECTROMETRIC DETECTION

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SUMMARY

Two model systems consisting of (1) phenylurea herbicides and (2) nitro aromatics were chosen to demonstrate the potential of on-line trace enrichment. The liquid chromatography–mass spectrometry system consists of a 2-mm-I.D. precolumn and analytical column combined with a direct liquid-inlet interface with a helium jet principle. The mass spectrometer has been operated in the positive and negative chemical-ionisation modes. For the investigated compounds, on-line trace enrichment can give enrichment factors of two to three orders of magnitude. For dinitroaromatics detection limits of 0.01 parts per billion were obtained, which corresponds to absolute detection limits of 1 pg. Band broadening was of the order of 1 sec (σ_t).

INTRODUCTION

In recent years, there has been a rapidly increasing interest in the use of mass spectrometry (MS) for detection in column liquid chromatography (LC). Considerable effort has been made to develop interfaces suitable for on-line LC–MS. Today, such interfaces include (see, *e.g.*, ref. 1) moving belt systems and a variety of direct liquid-introduction (DLI) interfaces, with and without splitting. In the latter case, the LC effluent is used as chemical-ionisation reagent gas. However, we have to realise that frequently the optimal separation medium is not necessarily also the optimal ionisation medium in the MS source; hence compromise will often have to be made.

In our group, we have designed[2] a DLI interface in which a jet of helium gas is used to aid nebulisation of the vaporizing LC effluent and sample into the MS source. This interface is coupled with narrow-bore LC–MS systems equipped with *ca.* 1-mm-I.D. analytical columns. Today, many workers use miniaturised chromatographic equipment, because the low mobile-phase flow-rates (10–100 μl/min) allow the introduction of the total LC effluent into the MS ion source. It is generally realised, however, that with miniaturised LC systems the injection volumes must also be reduced. They are in the order of 0.5–5 μl only. In such a situation, it seems attractive to improve sensitivity —in terms of concentration— via trace enrichment,

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i.e., by the on-line use of a short precolumn. The principles of such a procedure have been discussed extensively in, for example, ref. 3.

This communication presents two examples in the field of on-line trace enrichment for LC–MS, which illustrate the potential and general applicability —and also the limitations— of the technique.

EXPERIMENTAL

Materials

All the chemicals and solvents used were of analytical-grade quality. The six phenylurea herbicides (for their structures, see Table I) were gifts from Sandoz Ltd. (Basle, Switzerland) and the Food Inspection Service (Amsterdam, the Netherlands).

Methods

LC. The mobile phases for LC were delivered by two Gilson (Villiers-le-Bel, France) Model 302 pumps. A Kontron (Zürich, Switzerland) Model 812 pulse damper and a home-made membrane pulse damper were used in conjunction with the Gilson pumps. Samples were either directly introduced with a home-made micro injection valve with a 0.5-μl internal injection loop or a Valco (Houston, TX, U.S.A.) six-port injection valve with a 10-μl loop, or via the precolumn. The general set-up and operation of an LC system involving the use of a precolumn and the manual packing procedure of such columns have been described elsewhere[3,4]. In the present study, precolumns (5 × 2 mm I.D.) packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) or 5-μm Hypersil ODS (Shandon, Runcorn, U.K.) were used. The analytical stainless-steel column (200 × 2 mm I.D.), packed with 5-μm Hypersil ODS, was a gift from Hewlett-Packard (Waldbronn, F.R.G.).

MS. A Finnigan (Sunnyvale, CA, U.S.A.) Model 4021 quadrupole mass spectrometer, with Varian (Palo Alto, CA, U.S.A.) Model M4 and HS2 diffusion pumps for the vacuum system, was used. The Finnigan Model 2100 INCOS data system was employed for data acquisition and processing. The MS ion-source temperature was 300°C. Under the conditions used, the pressure in the ionisation chamber was *ca.* 0.3 Torr, and in the analyzer $6 \cdot 10^{-5}$ Torr. The electron multiplier was operated at *ca.* 1250 V with a dynode voltage of −3 kV when working in the positive- and negative-ion mode.

DLI interface. The home-made interface has been described in detail in an earlier paper[2]. It consists of a *ca.* 35-cm-long outer stainless-steel jacket which fits into the solid sample probe of the MS instrument. In our experiments, the LC effluent enters the MS source through a fused silica capillary (0.16 mm O.D. × 50 μm I.D.). This capillary is coaxial with a stainless-steel capillary ($^1/_{16}$ in. O.D. × 0.3 mm I.D.), and helium (at an inlet pressure of 1–2 bar) flows in between both capillaries and into the MS source. For routine work, fused-silica capillary diameters of 80–100 μm are recommended to avoid clogging problems.

RESULTS AND DISCUSSION

The general set-up of the on-line trace enrichment with LC–MS is shown in Fig. 1. An easy comparison of signal intensity (peak height or peak area) recorded

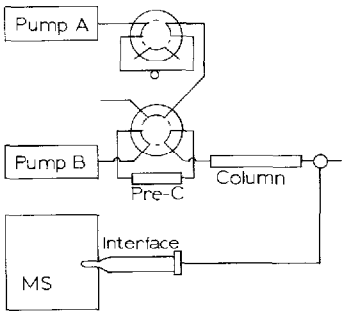


Fig. 1. Schematic of the LC–MS system including the precolumn used for trace enrichment. Pump A delivers water or 10^{-3} *M* formic acid (for loading the precolumn), pump B delivers the eluent (for desorption and LC separation).

after loop injection, *versus* on-line trace enrichment, is possible. Fig. 1 presents the configuration for desorption of the preconcentrated analytes from the precolumn with subsequent separation on the analytical column and MS detection. For both experiments described in this paper the following conditions were used:

(1) The aqueous solutions containing the model compounds were loaded on to the precolumn at a flow-rate of 500 μl/min.

(2) Acetonitrile–water (70:30), at a flow-rate of 200 μl/min, was the mobile phase used for desorption and LC separation.

At present, 2 to 11-mm-long precolumns with an inner diameter of 2 to 4.6 mm are used for various research projects carried out in our laboratory[3]. Recently, a precolumn having an inner diameter of 1 mm has been designed[5] and shown to perform satisfactorily in the trace enrichment of chlorophenols and the cytostaticum etoposide. Unfortunately, use of this precolumn is not yet as easy as that of the larger bore 2- to 4.6-mm-I.D. precolumns. Therefore, for the present work, a 2-mm-I.D. precolumn and analytical column were selected and, inevitably, a splitter had to be inserted between the outlet of the analytical column and the DLI interface; 10% of the LC mobile phase was directed to the MS source.

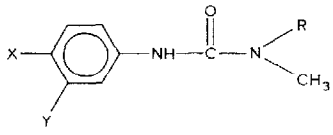
Phenylurea herbicides

For a first investigation of the trace-enrichment procedure, a rather simple test mixture was selected, consisting of six phenylurea herbicides (see Table I). From their known behaviour in reversed-phase LC[6,7] it was expected that they can be preconcentrated on a 5-mm-long precolumn packed with LiChrosorb RP-18 from at least 10 ml of sample solution without breakthrough except for the early eluting fenuron. Typical results are shown in Fig. 2. The same amount of each of the analytes was dissolved in either 0.5 μl, and analysed via direct loop injection, or in 10 ml, and analysed via on-line trace enrichment. Comparison of peak areas shows that the difference between trace enrichment and loop injection is less than 10% for all phenylureas with the exception of fenuron. The latter compound is almost completely absent from the trace-enrichment chromatogram, since most of it has been lost due to breakthrough during the sample-loading step.

It can be seen that with rather non-polar compounds, on-line trace enrichment

TABLE I

NAME, STRUCTURE AND SELECTED *m/z* OF SIX PHENYLUREA HERBICIDES



Name	*Code*	*Substituents*			*m/z*
		X	*Y*	*R*	
Fenuron	Fe	H	H	CH_3	165
Monuron	Mo	Cl	H	CH_3	199
Diuron	Di	Cl	Cl	CH_3	233
Metobromuron	Mb	Br	H	OCH_3	259
Linuron	Li	Cl	Cl	OCH_3	249
Chlorbromuron	Cb	Br	Cl	OCH_3	293

can easily cause a considerable improvement in sensitivity (from 200 ppm to 10 ppb, in this example), which greatly enhances the potential of LC–MS. However, two remarks should be made. Firstly, the 20000-fold increase in sensitivity that can be calculated from the present example is somewhat exaggerated, since a 0.5-μl injection volume is rather small for a 2-mm-I.D. LC system. Secondly, comparison of the chromatograms in Fig. 2 clearly shows that on-line trace enrichment causes some

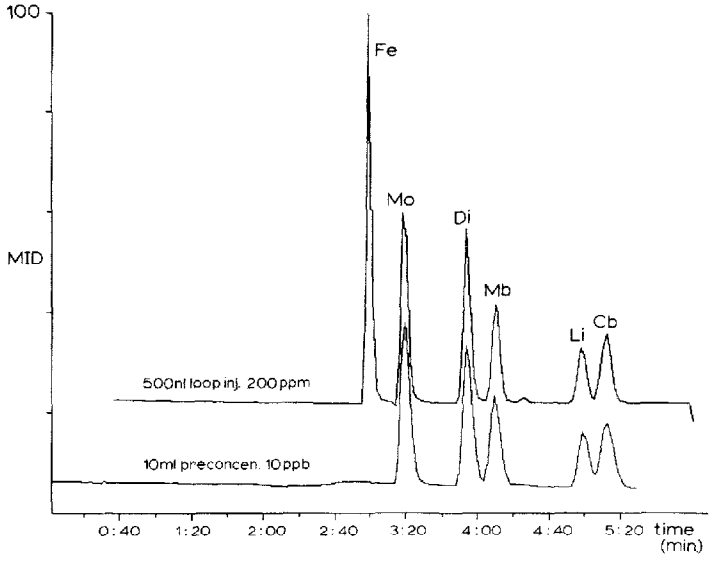


Fig. 2. Multiple-ion detection (MID) mass chromatograms of positive ions for a mixture of six phenylurea herbicides injected via a 0.5-μl loop and after 10-ml trace enrichment. Conditions: column (200 × 2 mm I.D.) packed with 5-μm Hypersil ODS; eluent, acetonitrile–water (70:30); flow-rate, 200 μl/min. Scanned ions: 165, 199, 233, 249, 259, 293; scan time, 1.5 sec.

TABLE II

NAME, CAPACITY RATIO*, k', AND SELECTED m/z OF SIX NITROAROMATICS

Name	*Peak no.*	k'	m/z
4-Nitrophenol	1	0.38	139
1,3-Dinitrobenzene	2	0.63	168, 138
2-Nitrophenol	3	0.67	139
2-Nitrotoluene	4	1.03	137
3-Nitrotoluene	5	1.13	137
1.5-Dinitronaphthalene	6	1.16	218

* LC separation: 5 μm-Hypersil ODS/acetonitrile–10^{-3} *M* formic acid (70:30).

additional band broadening, which is, of course, a drawback with closely eluting peaks. Both aspects are further evaluated with the help of the second example below.

Nitroaromatics

The second test mixture was composed of four mononitro- and two dinitroaromatics, which can be expected to give high sensitivity in LC–MS with negative chemical ionisation[8]. Analysis of a mixture of these compounds is distinctly more difficult than that of the six phenylureas, since they all elute rather rapidly (k' range, 0.4–1.2; see Table II) under the LC conditions selected, which will result in a poor resolution and early breakthrough. In addition, the presence of two nitrophenols and two nitrotoluenes requires a LC separation prior to MS detection. Results obtained with 0.5- and 10-μl loop injections are shown in Fig. 3. It is evident that working with the larger injection volume causes a noticeable loss in resolution in the case of the early eluting 1,3-dinitrobenzene and 2-nitrophenol. Typical results of trace-en-

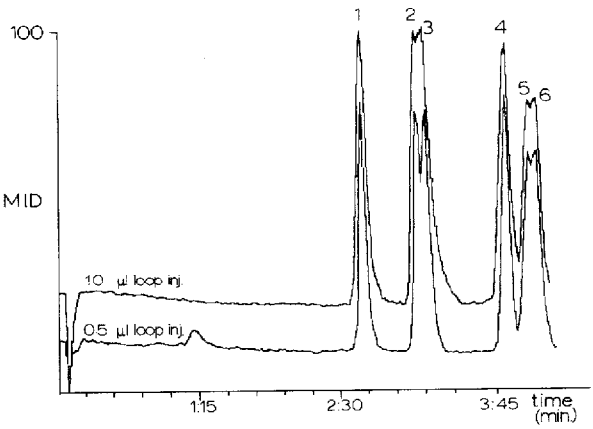


Fig. 3. MID mass chromatograms of negative ions for a mixture of six nitroaromatics injected on the column via a 0.5-μl and a 10-μl loop. Conditions: column (200 × 2 mm I.D.) packed with 5-μm Hypersil ODS; eluent, acetonitrile–10^{-3} *M* formic acid (70:30), flow-rate 200 μl/min; sample concentration: dinitroaromatics, 2 μg/ml; mononitroaromatics 20 μg/ml (*i.e.*, 2 and 20 ppm, respectively), for the 0.5-μl loop injection; 20 times diluted for the 10-μl loop injection. Scanned ions: 137, 138, 139, 168, 218; scan time, 1.5 sec.

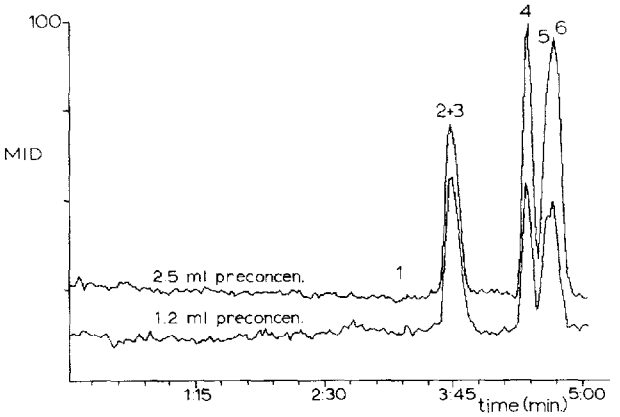


Fig. 4. MID mass chromatograms of negative ions for a mixture of six nitroaromatics injected after 1.2- and 2.5-ml trace enrichment, respectively. Conditions and scanned ions: see Fig. 3. Sample concentration: 0.1 ppb for the dinitroaromatics and 1.0 ppb for the mononitroaromatics.

richment experiments (from an aqueous solution containing 10^{-3} *M* formic acid on a precolumn packed with Hypersil ODS) are presented in Fig. 4. The slightly retained 4-nitrophenol is seen to be completely lost even after trace enrichment from only 1.2 ml of solution. The next two nitroaromatics have a recovery of over 90% in the 1.2-ml trace-enrichment experiment, but breakthrough and loss of analyte occurs in going from sampling volumes of 1.2 to 2.5 ml. With the three remaining compounds —2-nitrotoluene, 3-nitrotoluene and 1,5-dinitronaphthalene— recovery still is quantitative after loading with 2.5 ml of test solution. In other words, trace enrichment does not present any problems with analytes showing sufficient retention on the stationary phase used in the precolumn. With compounds of relatively high polarity, such as 4-nitrophenol, breakthrough occurs readily and a more hydrophobic material such as the styrene–divinylbenzene copolymer PRP_1 (Hamilton, Reno, NV, U.S.A.) should be selected[4].

Comparison of the chromatograms in Figs. 3 and 4 indicates that the additional band broadening caused by on-line trace enrichment is rather similar to that found in the case of the 10-μl loop injections. Or, in other words, even for the rather polar analytes a 100-fold improvement in sensitivity (10 μl *vs.* 1.2 ml) can be obtained, without loss in resolution. In the trace-enrichment experiments discussed here, the concentration of the mononitro compounds was 1 ppb, and that of the dinitro compounds 0.1 ppb. Detection limits obviously are in the 0.1–0.01 ppb range, which, taking into account the 1:10 split ratio, corresponds to absolute detection limits of 1–10 pg. This offers good possibilities for the sensitive detection of nitro-substituted polynuclear aromatics, which can be expected to display breakthrough volumes of at least 10 ml.

Finally, the reconstructed ion current of the selected ions as well as the single-ion mass chromatograms for the 1.2-ml trace-enrichment experiments are shown in Fig. 5. Two aspects are worth mentioning:

(1) The increase in sensitivity caused by utilising single-ion monitoring even allows the detection of 4-nitrophenol.

(2) The combined use of LC and MS easily permits the identification of all six test solutes. With MS alone the two mononitrophenols and the two mononitrotoluenes cannot be distinguished, but they can easily be separated chromatographically. On the other hand, the most difficult pairs to separate chromatographically (1,3-dinitrobenzene and 2-nitrophenol, and 3-nitrotoluene and 1,5-dinitronaphthalene) are easily distinguished by the mass spectrometer.

CONCLUSIONS

The use of on-line trace enrichment for narrow-bore LC–MS easily allows a 100 to 1000-fold improvement in sensitivity (in terms of concentration units) for non-polar and even relatively polar analytes. Compared with a 0.5-μl loop injection, additional band broadening is of the order of σ_t = 1 sec (calculated for 2-nitrotoluene; see Figs. 3 and 4), which is quite acceptable. Under favourable conditions, *viz.*, negative chemical ionisation for suitable test compounds and selected ion monitoring, detection limits of 0.01 ppb can readily be obtained.

On the other hand, we have to be aware of the fact that relatively polar compounds will breakthrough early, which results in low trace-enrichment factors.

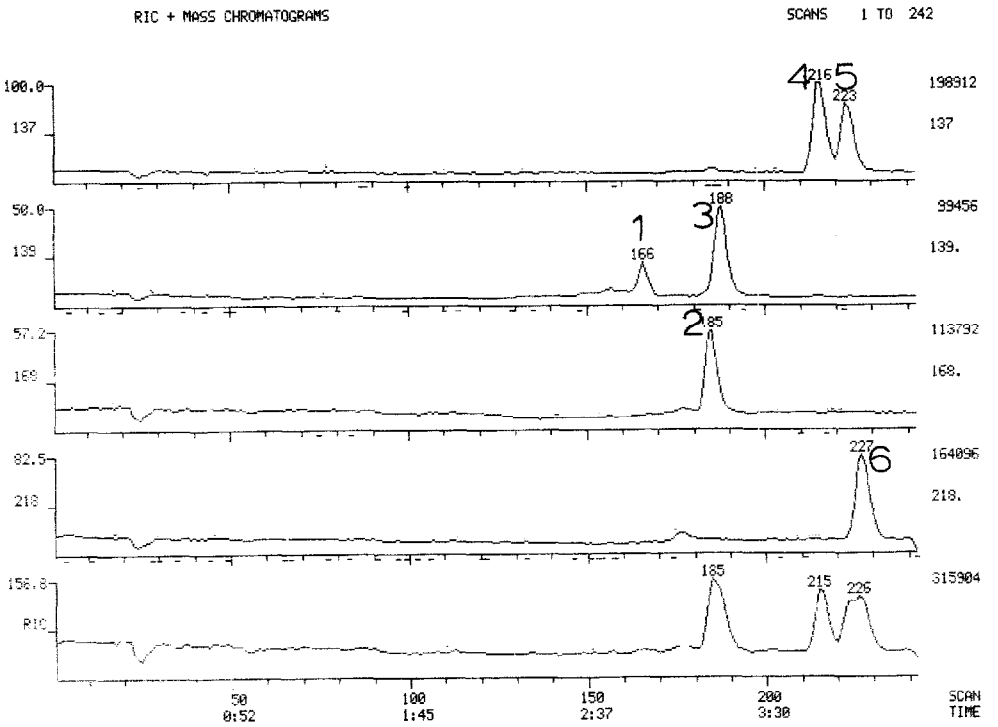


Fig. 5. Multiple- and single-ion monitoring of negative ions for a mixture of six nitroaromatics injected after 1.2-ml trace enrichment. Conditions: see Fig. 3. Sample concentration: see Fig. 4.

Current research is directed at the application of the on-line trace-enrichment/LC–MS principle to real samples and to further exploring the use of chemical phenomena in LC–MS interfacing.

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