Journal of Chromatography, 271 (1983) 51–60
Chromatographic Reviews
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,498

DETERMINATION OF PHENYLUREAS BY ON-LINE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The performance of an interface for direct introduction of the total effluent of a micro high-performance liquid chromatograph into a mass spectrometer is described. The mass spectrometer is operated under chemical ionization (CI) conditions were the solvent acts as CI reagent gas. The separation and identification of various phenylureas was optimized. It is demonstrated that this approach can be used as a prescreening of surface water for phenylureas.

INTRODUCTION

At present, gas chromatography–mass spectrometry (GC–MS) represents the method of choice for the identification of organic pollutants in water as it combines the high separation efficiency of capillary GC with the specificity and high sensitivity of MS. Unfortunately, only relatively volatile compounds are amenable to this technique, these comprise only 20–30% of the total organics in water. Thus considerable efforts have been made to separate and identify the less volatile compounds by liquid chromatography (LC). For these studies, on-line LC–MS is desirable for two reasons: (a) the commercially available LC detectors are neither very specific nor as sensitive as GC detectors; (b) it would allow a rapid, and ideally, an unequivocal identification of less volatile organics in water. To this end, a variety of LC–MS interfaces have been developed[1–7], among which the moving belt system[4] and the direct liquid introduction (DLI) system[5] have been used most frequently. With the latter interface the LC effluent is usually split prior to introduction into the mass spectrometer where the solvent acts as chemical ionization (CI) reagent gas. The coupling is facilitated if micro LC is used. In this case the total effluent can be introduced into the mass spectrometer as first demonstrated by Henion and Maylin[6]. Our approach is based on their concept. The application of this system to the analysis of polycyclic aromatic compounds, phenols and aliphatic acids has been reported previously[7,8].

0378-4355/83/$03.00

EXPERIMENTAL

Interface and apparatus

The interface is strikingly simple. Basically it consists of a stainless-steel capillary (30 cm × 0.1 mm I.D.) which acts as a flow restriction between the micro liquid chromatograph and the ion source of the mass spectrometer. Technical details of this interface have been published previously[7]. It can be introduced into the mass spectrometer via a sliding rod and connected rapidly to the chromatograph using PTFE tubing.

For this study a JASCO Familic-100 N micro high-performance liquid chromatograph was employed with acetonitrile–water solvents, flow-rates of 10 μl/min, pressure *ca.* 100 bar and a 20-cm reversed-phase RP-18 micro column. The sample was injected onto the column via a loop injector (capacity 0.3 μl). In a first step the separation was optimized using a UV detector (UVIDEC 100 II). The UV detector was then disconnected and the components identified mass spectrometrically. For this purpose the mass spectrometer (Finnigan 44 S) was operated in the CI mode (both positive and negative CI) where the LC solvent acted as reagent gas (source temperature 250°C, source pressure 150–500 μbar).

Performance of the interface

The interface has minimal dead volume. It can be operated reliably over extended periods of time. In particular, plugging of the capillary rarely occurs. Depending on the compound to be studied, a lower detection limit of 30 pg may be possible if the mass spectrometer is operated in the single ion monitoring mode. During operation the end of the interface capillary is heated by the hot ion source. This appears to be a prerequisite for proper performance of the interface, but precludes the analysis of thermally very labile compounds which at least in part can still be handled if a diaphragm is used as flow restrictor[9].

It is important to note that the ease of coupling of the chromatograph to the mass spectrometer is achieved at the expense of chromatographic resolution which is poorer with micro LC as compared with standard LC.

As the mass spectrometer is operated in the CI mode (see above) the mass spectra of thermally stable compounds exhibit mainly quasimolecular ions, *i.e.*, $[M + H]^+$ or $[M - H]^-$, but hardly any fragments. Thus the MS information is reduced to determination of the molecular weight. However, in general, the quasimolecular ion and the chromatographic retention time should allow identification of "targeted compounds".

RESULTS AND DISCUSSION

Analysis of pure phenylureas

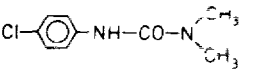
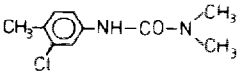
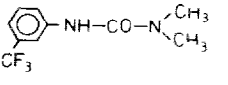
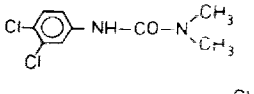
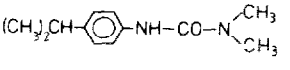
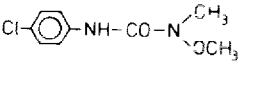
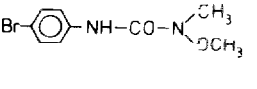
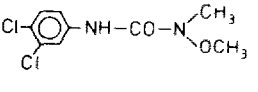
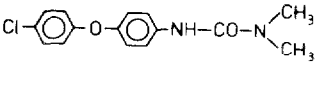
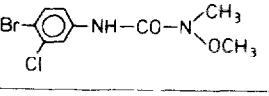
Phenylureas are widely used as herbicides and are released as such or as metabolites into the environment[10,11]. Gas–liquid chromatography (GLC) has been applied to the separation and identification of these compounds, but partial thermal decomposition during GLC analysis may cause erratic results[11]. Thus the application of LC to determination of phenylureas has been reported[12,13] and the potential of an off-line LC–MS coupling, in which several preseparation and prepurification steps

were necessary, has been explored[14]. We have used on-line LC–MS coupling to separate and identify phenylureas.

First, the separation of these compounds was optimized using a mixture of ten commercially available phenylureas, Table I. Fig. 1a shows the total ion chromatogram (mass range 140–300) obtained under positive chemical ionization (PCI) conditions at a concentration of 30 ng for each component injected onto the column. As a result of the limited chromatographic resolution, some components are not separated, *i.e.*, chlorotoluron and fluomethuron, chloroxuron and chlorbromuron. Note that a complete separation is possible with conventional LC[14]. The mass chromatograms of the protonated molecules reconstructed from the complete spectra are show in Fig. 1b and c. It is apparent that components having identical retention times can still be distinguished on the basis of their molecular weights. The complete mass spectra of these phenylureas are, as expected, dominated by the quasimolecular ions.

TABLE I

INVESTIGATED PHENYLUREAS

Common name		*Formula*	*Molecular weight*
(1)	Monuron		198
(2)	Chlorotoluron		212
(3)	Fluomethuron		232
(4)	Diuron		232
(5)	Isoproturon		206
(6)	Monolinuron		214
(7)	Metobromuron		258
(8)	Linuron		248
(9)	Chloroxuron		290
(10)	Chlorbromuron		292

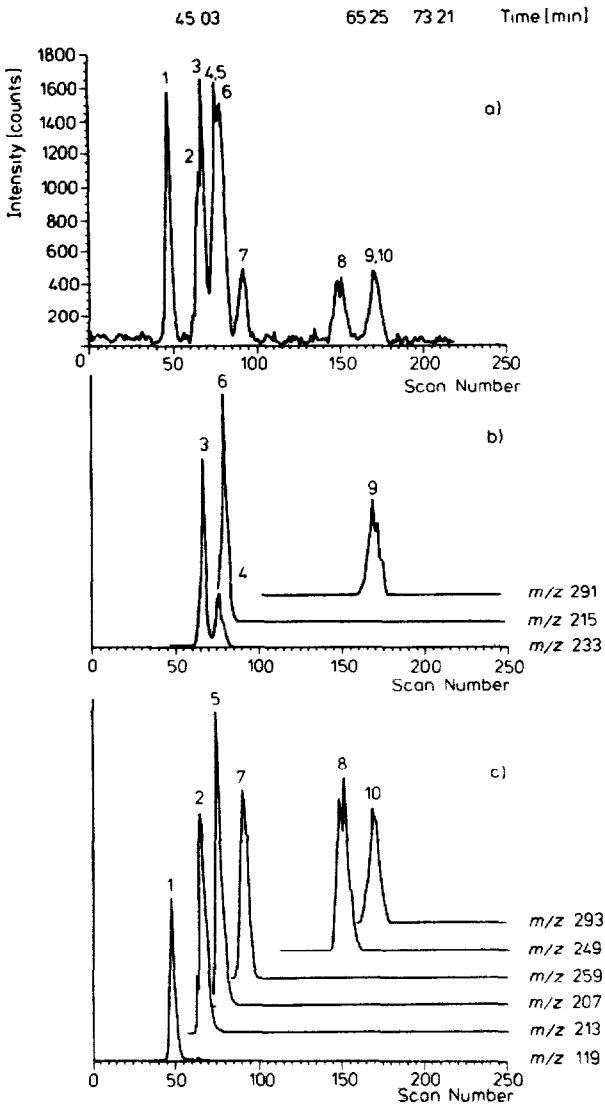


Fig. 1. LC–MS analysis of a mixture of ten phenylureas, 30 ng per compound injected onto the column (*m/z* values of protonated molecules in parentheses): 1 = monuron (199), 2 = chlorotoluron (213); 3 = fluomethuron (233), 4 = diuron (233), 5 = isoproturon (207), 6 = monolinuron (215), 7 = methobromuron (259), 8 = linuron (249), 9 = chloroxuron (291); 10 = chlorbromuron (293). Experimental conditions: acetonitrile–water (40:60), 19-cm RP-18 column, 5-μm particles; flow-rate 10 μl/min, LC pressure 105 bar, CI pressure 230 μbar, source temperature 290°C. a, Total ion chromatogram; b, c, reconstructed mass chromatograms of the protonated molecules.

This is demonstrated in Fig. 2 for linuron and in Fig. 3 for metobromuron. The mass spectrum does not only give information on the molecular weight. In addition, the cluster of isotope peaks reveals the presence of two chlorine atoms in the case of linuron and a bromine atom in the case of metobromuron, while structure specific fragments are missing. Fig. 4 shows the mass chromatogram of monuron (protonated

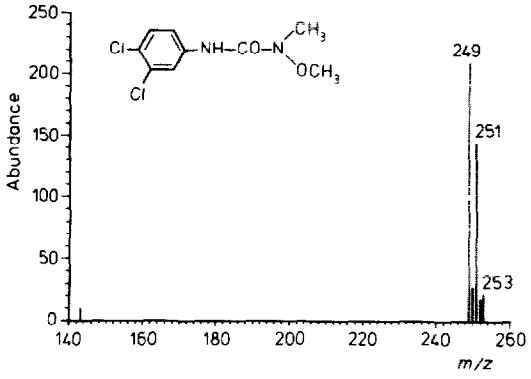


Fig. 2. Mass spectrum corresponding to peak 8 in Fig 1 (linuron)

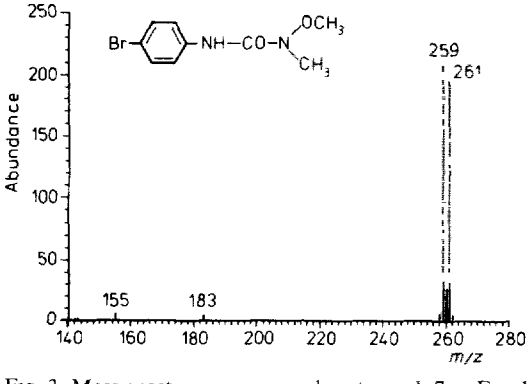


Fig 3 Mass spectrum corresponding to peak 7 in Fig 1 (metobromuron)

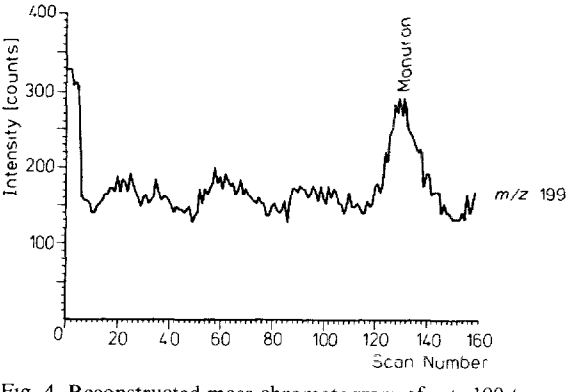


Fig. 4 Reconstructed mass chromatogram of *m/z* 199 (monuron); 30 pg injected onto the column (conditions as in Fig. 1)

molecule) obtained with 30 pg injected onto the column, demonstrating that at present about 30 pg represents the lower detection limit of the system.

Alternatively the mass spectrometer can be operated under negative chemical ionization (NCI) conditions. If acetonitrile–water is used as solvent, the dominant $C_2H_2N^-$ anion abstracts a proton from the sample molecules thus giving rise to the formation of $[M - H]^-$ quasimolecular ions. The NCI mass chromatogram of the mixture of ten phenylureas at a concentration level of 30 ng per component is shown in Fig. 5; the $[M - H]^-$ ions were monitored.

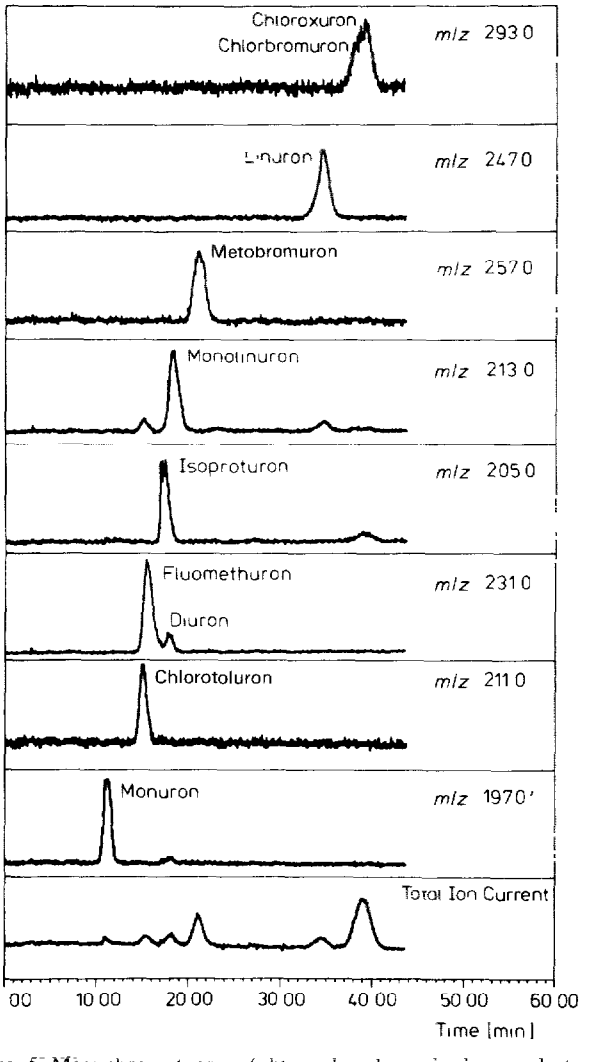


Fig. 5. Mass chromatogram (obtained in the multiple ion selection mode under negative chemical ionization conditions) of a mixture of ten phenylureas, 30 ng per compound injected onto the column. The $[M - H]^-$ ions were monitored (conditions as in Fig. 1).

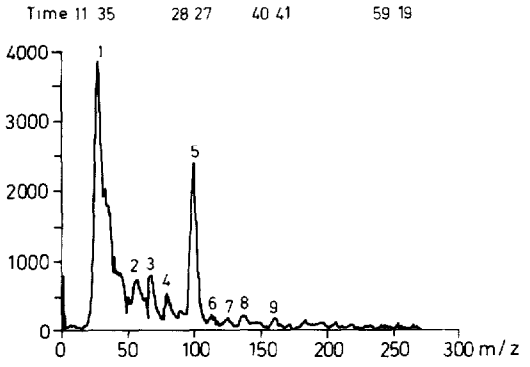


Fig 6 Total ion chromatogram of a Rhine water extract (m/z 140–300) spiked with the ten phenylureas listed in Fig 1 at the concentration level of 6 ng Conditions as in Fig 1

Identification of phenylureas in surface water

While separation of a mixture of pure phenylureas and their MS identification is readily achievable, the analysis is expected to be much more complicated if these compounds are to be identified from complex matrices such as water samples. To test the potential of our method for the analysis of organic pollutants in water we spiked a water extract from the river Rhine with the ten phenylureas listed in Table I such that the injected sample contained 6 ng of each herbicide. For the analysis only 1 % (0.3 μl) of the total water extract (30 μl) was injected. Thus the 6 ng of herbicide injected onto the column correspond to 600 ng in the original sample. Fig. 6 shows the total ion chromatogram (m/z 140–300) of the water extract. MS analysis demonstrates that the two major peaks 1 and 5 do not correspond to any phenylurea. Although the retention times of some of the smaller peaks discernible in the total ion chromatogram correspond to those of the phenylureas, an unambiguous identification based on the total ion chromatogram alone is not possible.

An unequivocal detection of the "targeted" compounds is, however, possible, if the information from the mass spectra is used. Fig. 7 shows the mass spectrum of peak 2. The quasimolecular ions of chlorotoluron and fluomethuron with the correct isotopic pattern can readily be detected. These results demontrate that even in com-

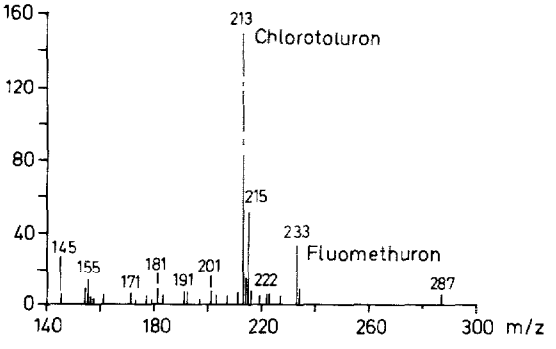


Fig 7 Mass spectrum of peak 2 in Fig 6. Quasimolecular ions of chlorotoluron (m/z 213 and 215) and fluomethuron (m/z 233)

plex matrices phenylureas can be detected by this method at the lower nanogram level.

In a second step a freeze-dried sample (corresponding to 5 l water) from the river Main was screened for the presence of phenylureas. The sample was extracted with chloroform, concentrated to dryness and dissolved in 30 μl acetonitrile. 0.3 μl from this extract were injected onto the column In order to obtain optimum sensitivity the mass spectrometer was repeatedly scanned first over a mass range from m/z 195 to 240 then from m/z 240 to 300. (The molecular weights of all phenylureas should fall within these mass ranges.) The total ion chromatograms for these two mass ranges are shown in Fig 8a and b. From the mass spectra, the mass chromatograms of the protonated molecules and those of the corresponding isotope peaks (if the suspected phenylurea contains halogen atoms) were reconstructed as shown in Fig. 9 for m/z 207, 249 and 251. Comparison with the test mixture run on the same day

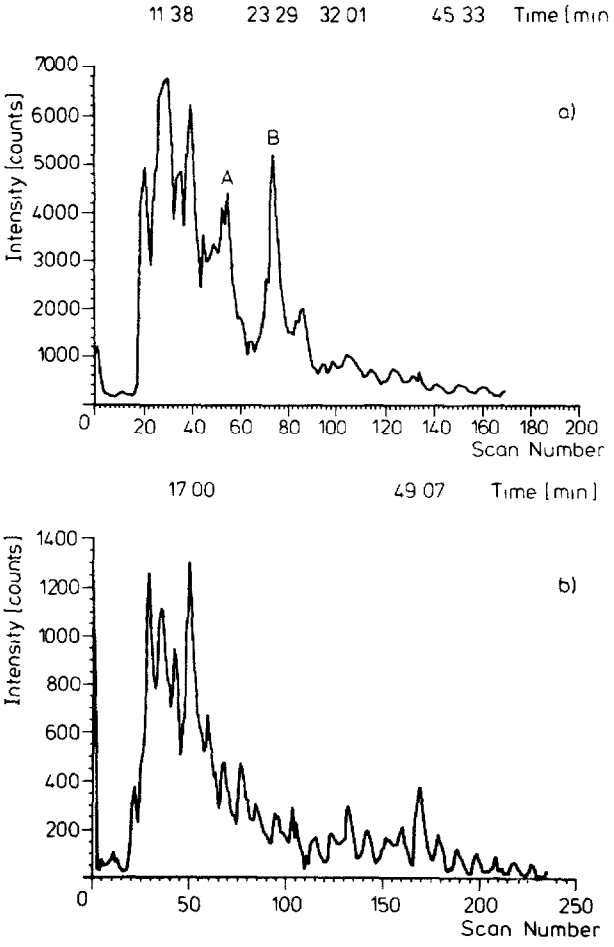


Fig 8 Total ion chromatogram of a water extract from the river Main (conditions as in Fig. 1) a, mass range from m/z 195 to 240 (peak B = isoproturon, see Fig. 9a); b, mass range from m/z 240 to 300 (conditions as in Fig 1)

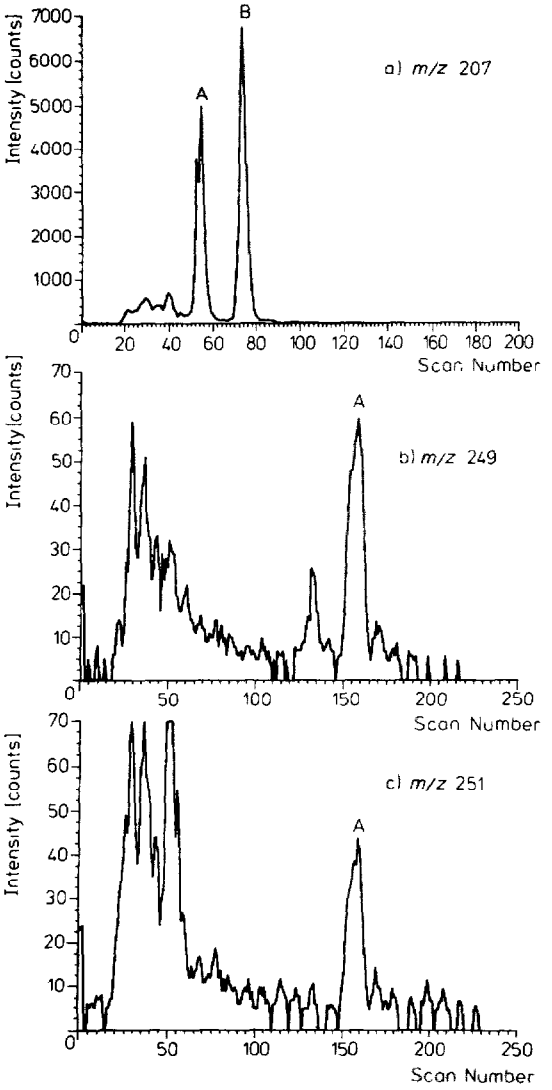


Fig 9. Reconstructed mass chromatogram of a. m/z 207 (peak B isoproturon, peak maximum at scan number 76 in test mixture), b. m/z 249, c. m/z 251 (peak A linuron peak maximum at scan number 161 in test mixture).

allowed tentative assignment of peak B in Fig. 9a as isoproturon, while peak A in Fig. 9b is probably due to linuron. The latter assignment does not only rest on the correct retention time and mass of the protonated molecule, but also on the mass and intensity of the isotope peak at m/z 251 (Fig. 9c) which is due to the two chlorine atoms. (The observed intensity ratio m/z 251.249 = 1.42 is close to the theoretical ratio of 1.54.) Furthermore it is very likely that the sample contained also minor amounts of monolinuron and chlorotoluron as supported by the correct retention time and isotope pattern. Owing to the large number of organic compounds in surface water, the

interference from other compounds of the same mass and retention time cannot be excluded. Thus it is desirable to confirm the proposed constituents by other methods, *e.g.*, high-resolution measurement.

CONCLUSIONS

The described method of on-line LC–MS allows the rapid prescreening of surface water samples for "targeted" compound classes provided that these compounds are thermally stable. However, as a result of the micro columns employed, the chromatographic resolution is poorer than with standard LC equipment. The identification of the compounds rests on the determination of the retention time and the molecular weight, and on the interpretation of the isotope pattern if halogen atoms are present in the sample molecules. If complex matrices such as water samples are analyzed this information may not be sufficient for an unequivocal identification of a given compound. Thus the results should be confirmed by methods such as high-resolution measurements or tandem MS[15]. One disadvantage is that the sample volume injected onto the column is only 0.3 μl, as it is difficult to concentrate a water extract to this size. Thus although targeted compounds at a concentration level below 10 ng injected onto the column can be identified from complex matrices, this detection limit can in practice only be realized if the above mentioned sampling method is improved.

ACKNOWLEDGEMENT

Financial support from the "Umweltbundesamt Berlin" is kindly acknowledged.

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