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APPLICATION OF A COMBINED LIQUID CHROMATOGRAPHIC–MASS SPECTROMETRIC INSTRUMENT USING AN INTERFACE FOR DIRECT LIQUID INTRODUCTION

M. DEDIEU* and C. JUIN

Nermag SA, 49 Quai du Halage, 92500 Rueil-Malmaison (France)

and

P. J. ARPINO, J. P. BOUNINE and G. GUIOCHON

École Polytechnique, Laboratoire de Chimie-Physique, 91128 Palaiseau (France)

CONTENTS

1. INTRODUCTION

Coupling of high-performance liquid chromatography (HPLC) and mass spectrometry (MS) has been achieved using several different techniques[1]. Excellent results have been obtained by direct introduction of liquid (fractions of the HPLC eluent) into the ion source using a nebuliser that has a diaphragm only a few microns in diameter[2]. However, even this introduction technique has difficulties associated with it. For example, even with strong heating it is not easy to obtain complete or good evaporation of droplets in the ion source. To overcome this problem it is necessary to work with very small diaphragms and high pressures[33], which may cause jet instabilities.

Sensitivity is also affected: above 300 a.m.u. the sensitivity decreases rapidly and the total ion current (TIC) then often has the appearance of a ragged line from which it is difficult or even impossible to match elution peaks of the solute. Single ion research is then necessary, which requires a knowledge of the ions formed. A method for overcoming these problems is presented in this paper.

2. THEORETICAL

2.1. Simple method for calculation of average jet speed and droplet diameter at the outlet of diaphragms with a small aperture

Conditions for the nebulization of liquids through small apertures under vacuum has been studied previously[3]. For droplets of liquid effluents to be fully evaporated it is important that their speed is not too high. *i.e.*. the jet speed must be as low as possible without being so low as to cause collapse into drops and subsequent freezing of the jet[3].

First. it is easy to measure the optimal flow-rate of effluent into the ion source; an example of this is to make adjustments to give the correct parameters and flow into the mass spectrometer, then to remove the probe and weigh the collected effluent. Hence a weight *versus* time relationship is obtained. For example:

Methyl cyanide–water (8:2). $\varrho_{25 \cdot C}$ = 0.838 mg/μl

Weight collected, 323 mg; time. 40 min.

Flow-rate is 9.64 μl/min or 0.16 μl/sec. No cold trap.

With the use of a cold trap this value becomes 46 μl/min. These results are in agreement with those obtained by Arpino *et al.*[3].

Then. if R is the radius of the diaphragm hole and U is the optimal flow-rate of effluent. the average speed. $\bar{v}$. can be calculated using

$$\bar{v} = \frac{U}{\pi R^2}$$

The calculated speeds *versus* R are in agreement with the theoretical calculations of Arpino *et al.*[3] and Melera[4] (see Table I).

As the total path length available between the diaphragm and the walls of the ion source is 12 mm. the time allowed for evaporation. τ. is $0.6 \cdot 10^{-4}$ sec for $R = 0.5$ μm and $29.2 \cdot 10^{-4}$ sec for $R = 3.5$ μm. the latter being the limit of the diaphragm radius that can be used.

At this point it is necessary to determine the average radius. $\bar{r}$. of the droplets generated by such diaphragms (Fig. 1).

TABLE I

AVERAGE JET SPEED AND MAXIMUM EVAPORATION TIME ALLOWED *VERSUS* RADIUS R OF THE DIAPHRAGMS USED IN NORMAL CONDITIONS

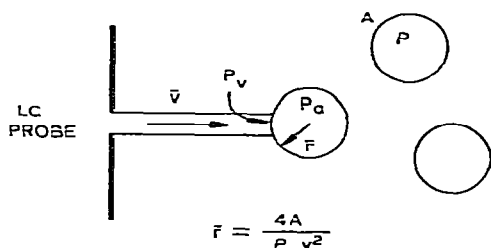| R (μm)	$\bar{v}$ (m/sec)	τ ($\times 10^{-4}$ sec)
0.5	203.7	0.6
1	50.9	2.3
1.5	22.6	5.3
2	12.7	10
2.5	8.1	14
3	5.6	21
3.5	4.1	29.2
4	3.2	Lowest practical speed
4.5	2.5	

$$\bar{r} = \frac{4A}{\rho\ v^2}$$

Fig. 1. Simple calculation of the average radius, $\bar{r}$, of the droplets generated by the jet stream of LC effluent P_v = Dynamic pressure inside the jet of velocity $\bar{v}$; P_a = excess pressure created by the internal forces of the solvent droplet; A = surface tension of the liquid; ρ = density of the liquid.

If

Δp_v = dynamic pressure generated by the jet on the cohesion path of the liquid;

Δp_a = excess pressure inside a droplet of radius $\bar{r}$
A = surface tension of the liquid.

The conditions for the formation of a droplet require that $\Delta p_v = \Delta p_a$. Now,

$$\Delta p_v = \tfrac{1}{2}\ \rho v^2$$

where ρ is the density of the liquid,

$$\Delta p_a = \frac{2A}{\bar{r}}$$

$$\bar{r} = \frac{4A}{\rho v^2}$$

The results are given in Table II.

Melera[4] has shown that for a water droplet with a radius of 5 μm, bearing no charge, at 200°C, and at 1 Torr pressure, this radius is still 1 μm after $2.4 \cdot 10^{-3}$ sec. With methanol at 300°C a 5 μm radius decreases to 1 μm after $6.5 \cdot 10^{-4}$ sec. This means that it is impossible to use pure water if the ion source is heated to 200°C and with diaphragms larger than 2 μm. With smaller diaphragms the viscosity causes the jet stream to produce drops.

Solutions to increase the flight time of such droplets have been developed by Melera[5] using a desolvation chamber.

2.2. *Speed of the gaseous flow resulting from the evaporation of the liquid effluent under optimal conditions in a vacuum (Fig. 2)*
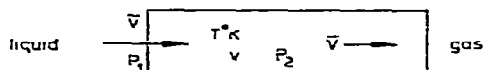

Fig. 2.

TABLE II

RADIUS $\bar{r}$ OF THE DROPLETS *VERSUS* RADIUS r OF THE DIAPHRAGMS USED FOR SOME COMMON SOLVENTS

Constant flow-rate $= 10 \, \mu l/min$.

Solvent	$R \, (\mu m)$	$2 \, \bar{r} \, (\mu m)$
Acetonitrile	1	0.11
$(A = 27 \cdot 10^{-3} \, N/m)$	1.5	0.5
	2	1.7
	2.5	4.2
	3	8.8
	3.5	16.5
Methanol		
$(A = 22.6 \cdot 10^{-3} \, N \cdot m)$	1	0.1
	1.5	0.5
	2	1.4
	2.5	3.5
	3	7.4
	3.5	13.8
Water		
$(A = 72 \cdot 10^{-3} \, N/m)$	1	0.22
	1.5	1.13
	2	3.6
	2.5	8.7
	3	18.5
	3.5	34.2

The speed, $\bar{V}$, of the gaseous flow generated at $T°K$ and P_2 atm may be calculated from

$$\sqrt{2 \, vg \, \overline{(P_1 - P_2) + \bar{r}}}$$

where v is the gaseous volume per unit time (P_2, T). Some results are shown in Table III.

The high gas flow-rate observed may be used to evaporate and nebulize solvent droplets through a convenient device.

2.3. *Description of a high-speed direct liquid introduction (DLI) interface*

First, it is evident that if we want to transform pressure into speed, we must take into consideration the ratio P_2/P_1. If the gaseous effluents are taken at relatively low pressures and high temperatures then they assume a behaviour close to that of perfect gases, *i.e.*, $\gamma = C_p/C_v = 1.3$ for superheated steam and 1.4 for perfect gases.

$$\frac{P_2}{P_1} = \left(\frac{2}{\gamma + 1}\right)\left(\frac{\gamma}{\gamma - 1}\right)$$

For superheated steam $P_2/P_1 = 0.54$ and for perfect gases $P_2/P_1 = 0.52$.
Here $P_2/P_1 \simeq 10^{-6} \ll 0.54$, so the device used must have a convergent–divergent shape and have the proper dimensions (Fig. 3).

TABLE III

GAS STREAM SPEED AND MAXIMAL RADIUS R_m OF THE DROPLETS GENERATED *VERSUS* INTERFACE TEMPERATURE

Solvent	Parameter	Temperature (°C)					
		100	150	200	250	300	350
Acetonitrile	$\bar{V}$ (m/sec)	153	163	172	181	189	197
	$\bar{V}_0$ (m/sec)	313	334	353	371	388	405
	$\bar{R}_m$ (μm)	0.6	0.53	0.47	0.43	0.39	0.36
Water	$\bar{V}$ (m/sec)	260	277	292	307	322	336
	$\bar{V}_0$ (m/sec)	473	504	533	560	586	611
	$\bar{R}_m$ (μm)	1.61	1.42	1.27	1.15	1.05	0.96

The maximum speed in the transition zone is known to be the sonic velocity. $\bar{V}_0$ (T,P) according to

$$\bar{V}_0 = \sqrt{\gamma gnRT} \ (\gamma = 1.3)$$

where n = number of moles/kg and R = thermodynamic gas constant.

In this zone, droplets of large size may be split into smaller droplets and hence be readily evaporated by the stream of heated vapour from the pre-evaporation chamber before undergoing final acceleration. Because of the high velocity conditions it is assumed that the wall effects will be minimal and can be ignored. It is also noted that in the case of $\bar{V}_0$, the smaller the molecular mass of the liquid effluent, the higher will be the relative speed. This means that the best heat fluid to use is water, which ensures the best calorific transportation and the highest speed possible with LC–MS effluents.

Verification of this hypothesis was carried out using pure water in the system.

Droplets injected near the transition zone undergo a change of speed of $\bar{V}_0 - \bar{r}$ m/sec. If ϱ_g is the density of the gas, the average radius of the droplets is given by $\bar{r} = 4A/\varrho_g \ (\bar{V}_0 - \bar{V})^2$ (Table III).
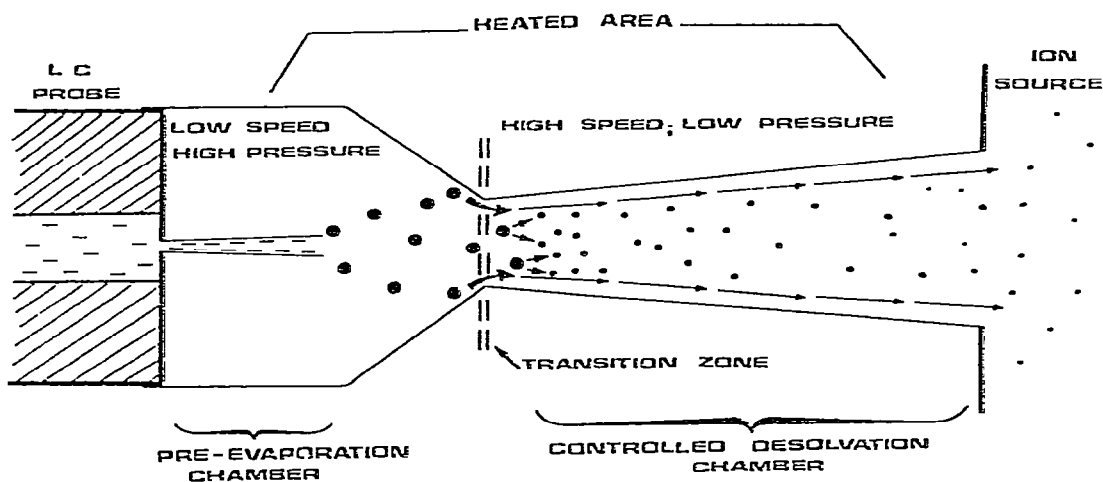

Fig. 3. High-speed direct liquid introduction device. The maximal velocity in the transition zone is sonic velocity (T, p).

The results indicate that droplets generated by diaphragm holes of radius from 2 to 3.5 μm must split into smaller droplets even when water is used. This process offers the possibility of using fairly large diaphragm holes, which give very stable jet streams of liquid effluents.

Finally, we obtain a mist of fine droplets in a hot gas stream which may or may not undergo complete evaporation at the interface temperature. Subsequent introduction into the ion source, which may remain at a lower temperature, gives better conditions for ionization.

3. EXPERIMENTAL AND RESULTS

A Waters 6000 A liquid chromatograph was used with LiChrosorb (Merck, Darmstadt, G.F.R.) (250 × 4.6 and 10 × 4.6 mm I.D.) and Zorbax ODS (DuPont, Wilmington, DE, U.S.A.) (250 × 4.6 mm I.D.) columns.

Mass spectrometry was performed on a Nermag R 10-10 C apparatus.

The solvents were analytical-reagent grade methyl-cyanide and methanol from Merck and Carlo Erba (Milan, Italy).

Water was first deionized and distilled on 800°C quartz heaters under a stream of filtered air. Complete apyrogenic water was thus obtained. Subsequent ultrasonic treatment for degassing just before use was sufficient.

3.1. Is filtration of the solutions necessary?

It was concluded that filtration is necessary because it prevents clogging of the diaphragms, and in the reverse phase filtering to 0.2 μm has a great effect on the background noise and the background noise observed is no higher than that with a normal phase. This result indicates that this noise in the reverse phase may come from fine particles of the phase itself.

For this purpose solvents are filtered, just before use, on a 0.5-μm PTFE Millipore membrane and stored in clean bottles. Finally, 0.2-μm PTFE Millipore
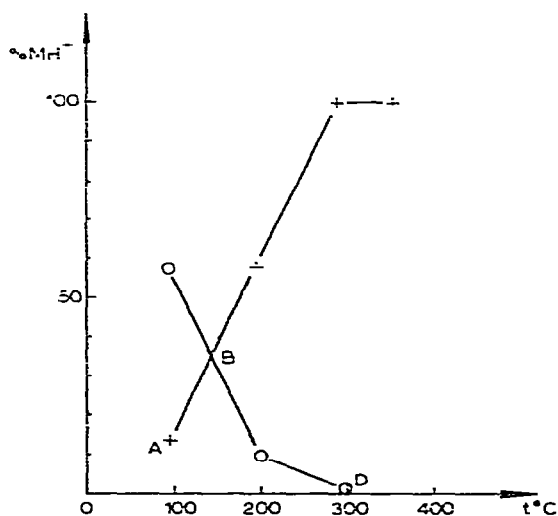

Fig. 4. Curve response at 170 (+) and 240 u (O) and % MH⁺ of N-nitrosodiphenylamine *versus* interface temperature. A, Temperature too low; B, proper conditions; D, decomposition.
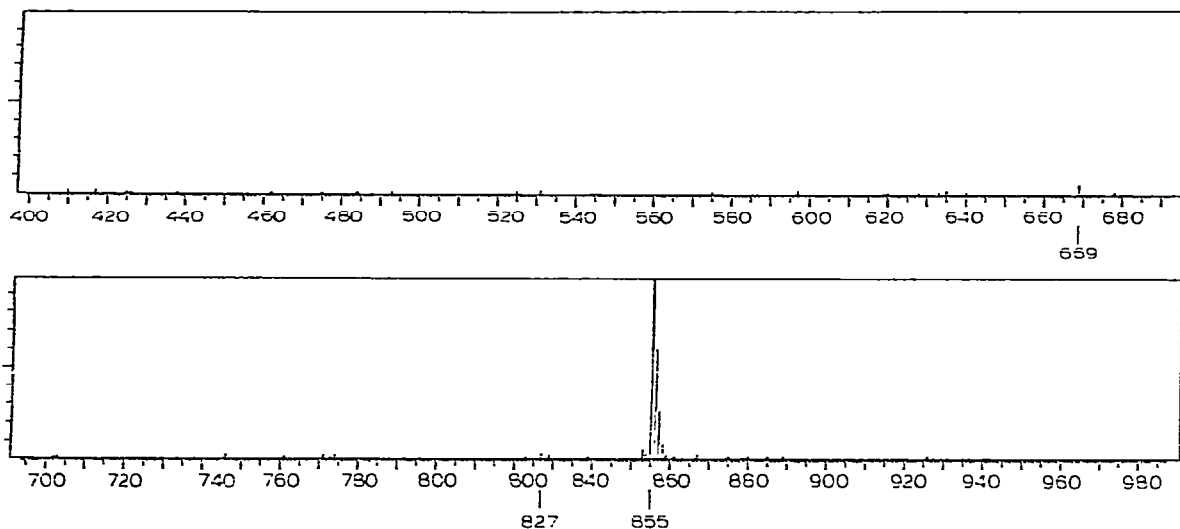
Fig. 5. Mass spectrum of labile sulphone dyestuff $C_{52}H_{-6}O_6N_2S$ (M = 856) using heptane–dichloromethane (80:20). Interface temperature: 80°C.

membranes are placed at the inlet and the outlet of the column.

With these pre-filtered solutions there is no clogging of the diaphragms. It is concluded that clogging is due mainly to the presence of fine particles rather than dissolution of silica in the water of the LC solvent[6].

3.2. Influence of the interface temperature on spectral patterns

For this study we used a labile substance, N-nitrosodiphenylamine ($C_{12}H_{10}N_2O$) of molecular weight 198. The solvent was acetonitrile–water (80:20). The ions observed were MH^-, 170 and 240 (addition of methyl cyanide).

3.3. Curve response of 170.240 a.m.u. and $\%$ MH^- versus interface temperature (see Fig. 4)

At A (Fig. 4) the temperature interface is too low; at B proper conditions are realized; at D the molecule of solute has lost its cluster of solvent and complete decomposition occurs. D was named the "cut-off" point of the curve.
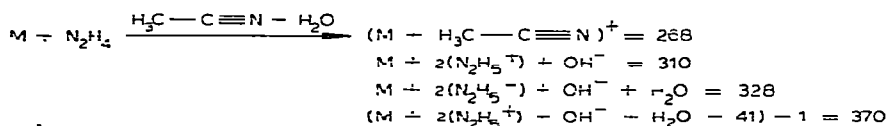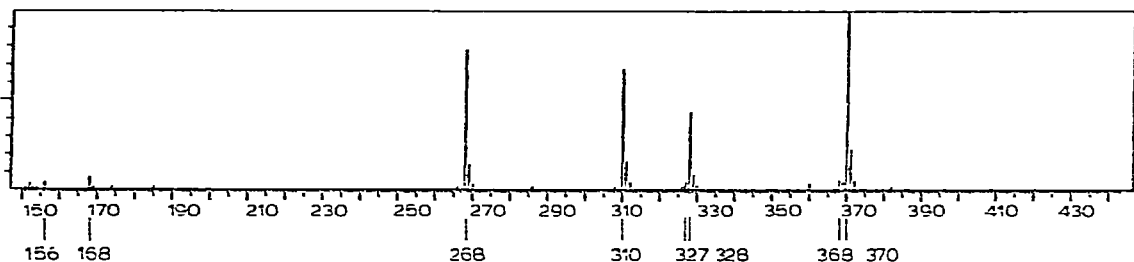

$$M \div N_2H_4 \xrightarrow{\quad H_3C \longrightarrow C \equiv N \div H_2O \quad} (M \; - \; H_3C \longrightarrow C \equiv N \,)^+ = 268$$

$$M \div 2(N_2H_5^+) \div OH^- = 310$$

$$M \div 2(N_2H_5^-) \div OH^- \; + \; H_2O = 328$$

$$(M \div 2(N_2H_5^+) - OH^- - H_2O - 41) - 1 = 370$$

Fig. 6. Mass spectrum of trinitrine (positive ion monitoring). Solvent: acetonitrile–water (70:30) + 0.05 % hydrazine hydrate. Successive additions of methyl cyanide, hydrazine hydrate and water can be seen.
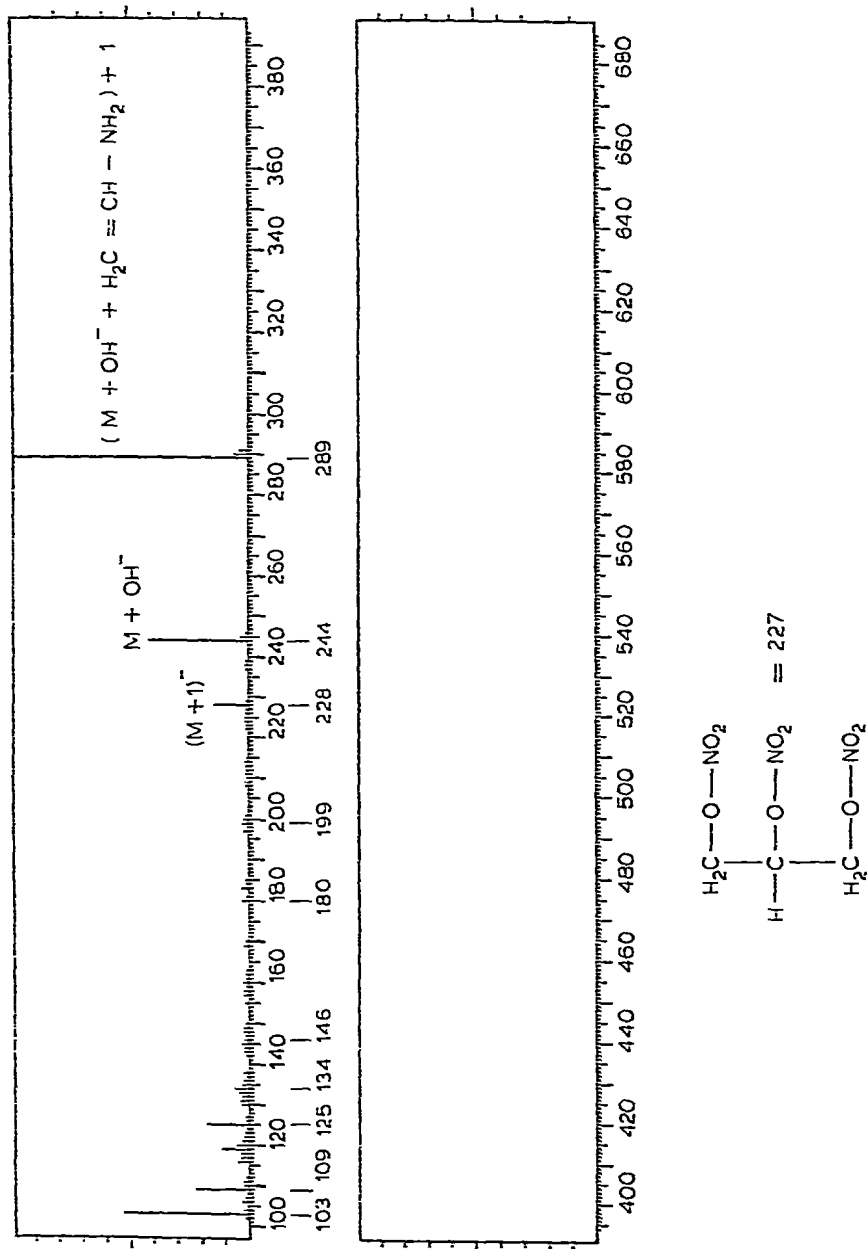
$(M + OH^- + H_2C = CH - NH_2) + 1$

$M + OH^-$

$(M + 1)^-$

380 360 340 320 300 289 280 260 244 240 228 220 200 199 180 180 160 146 140 134 125 120 109 103 100

680 660 640 620 600 580 560 540 520 500 480 460 440 420 400

$$\begin{array}{c} H_2C\text{---}O\text{---}NO_2 \\ | \\ H\text{---}C\text{---}O\text{---}NO_2 \quad = 227 \\ | \\ H_2C\text{---}O\text{---}NO_2 \end{array}$$

Fig. 7. Mass spectrum of trinitrine (negative ion monitoring). Solvent: acetonitrile–water (70:30) + 0.1 % allylamine.

The same phenomenon can be seen with cortisol. It is now possible to observe the $MH^+ = 363$ a.m.u. ion. The optimal temperature is near 300°C but at 350°C MH^- completely disappears. This indicates that molecules of solvent must be kept around the solute, even in small amounts, to prevent decomposition.

Another similar example arises with a labile sulphone dyestuff in the volatile LC solvent heptane–dichloromethane (80:20) (Fig. 5). If the temperature adjustment of the interface is higher than 80°C the MS response falls to zero.

Hence the system offers better control of the solvation or desolvation of the solute simply by temperature adjustment. In this way it is now possible to add some reactants to the liquid phase solution, such as hydrazine hydrate, allylamine, ammonia and nitromethane, which react at a suitable temperature with the solute to large changes in the spectral patterns. For example, the effects of hydrazine and allylamine on glycerol trinitrate (trinitrine) are shown in Figs. 6 and 7.

4. CONCLUSION

The direct liquid introduction devices described here offer the possibility of working with larger diaphragms than previously and of working with pure water, and they offer the facility of breaking clusters such as acetonitrile–water and the possibility of starting the acquisition at 60 a.m.u. Also, they give a very good response with a labile substance such as chloramphenicol (Fig. 8) or trinitrophenol (Fig. 9). Finally, the sensitivity gets much higher at high mass ranges and the ability to study high-molecular-weight substances such as porphyrins offers no difficulties.
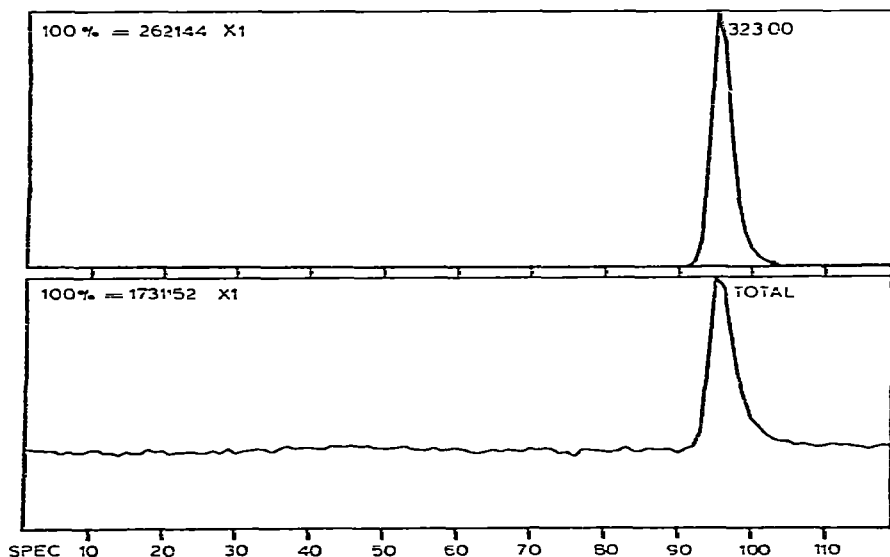

Fig. 8. TIC and 323 a.m.u. specific ion monitoring on chloramphenicol. Solvent: acetonitrile–water (40:60). Interface temperature: 150°C.
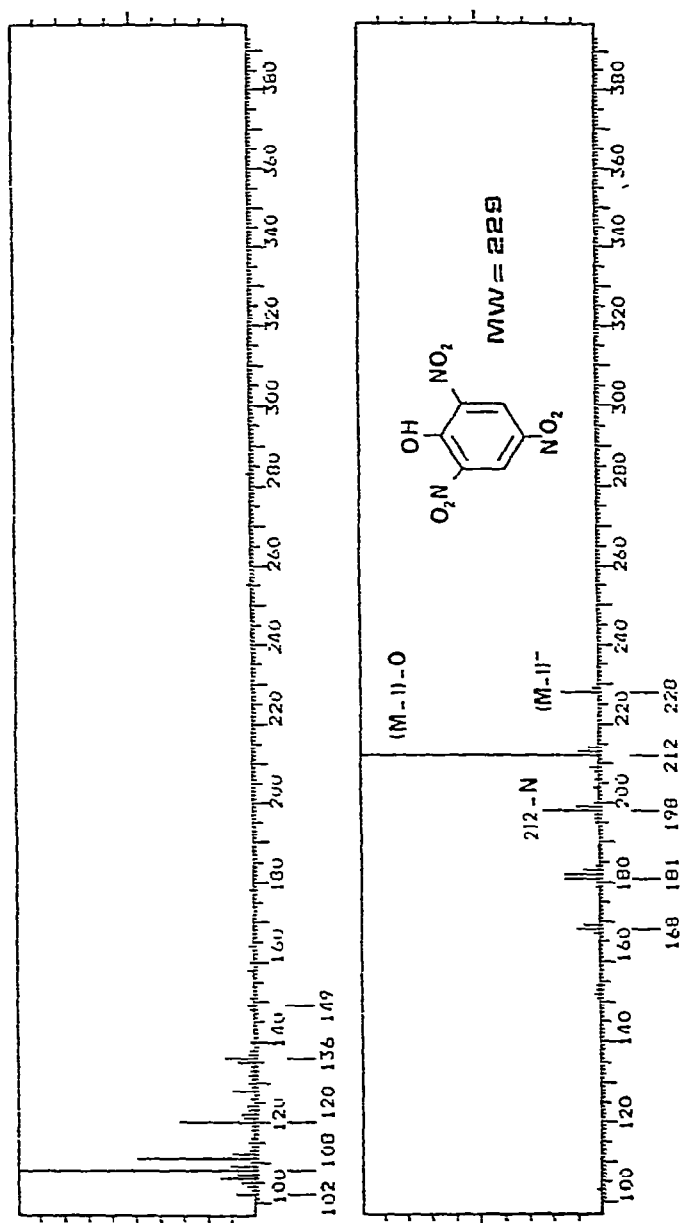
Fig. 9. Mass spectrum of trinitrophenol with positive (above) and negative ion monitoring (below). Solvent: water.

5. SUMMARY

A liquid chromatographic (LC)–mass spectrometric interface based on a new concept allows better introduction of LC effluents into the source and has a higher sensitivity than the standard system. This system operates in two steps: evaporation of a part of the solvent from LC stream and droplets of large diameter and the use of the gaseous mass obtained to achieve nebulization of the droplets. Finally evaporation and desolvation of the LC effluents are carried out. There is the possibility of breaking up molecular associations and of introducing compounds into the solvents such as hydrazine and nitromethane, which are used as reactant gases, leading to different solvent effects and remarkable changes of mass spectral patterns and ultimately the use of pure water as solvent. Spectra of labile compounds of high molecular weight are shown.

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