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MASS SPECTROMETRY OF BIOGENIC CATECHOLAMINE ION PAIRS BY DIRECT LIQUID INTRODUCTION

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SUMMARY

The mass spectrometry of ion pairs of dopamine, noradrenaline and 3,4-dihydroxyphenylacetic acid with *n*-octylsulphonate has been investigated by direct liquid introduction in an acidic buffer currently used for their high-performance liquid chromatographic separation. In order to follow the fate of the ion pairs, they were first studied by desorption/chemical ionization as dichloromethane extracts or in the buffer.

The results show that characteristic spectra of the solutes can be obtained by direct liquid introduction provided that the interface between the chromatographic system and the mass spectrometer permits the desolvation of the ion pairs from the cluster of solvent molecules

INTRODUCTION

Biogenic catecholamines dopamine (DA) and noradrenaline (NA) and one of their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), are currently determined in biological samples by reversed-phase high-performance liquid chromatography (HPLC) and electrochemical detection as ion pairs[1]. However, the specificity of this method is not absolute. Extra peaks can interfere with those of the compounds under analysis, arising either from endogenous substances when analysing samples of different biological origin or from exogenous substances (or their metabolites) when applying pharmacological treatments, for example There are several ways of confirming the identity and purity of an HPLC peak.

We were interested in investigating the possibility of using direct liquid introduction (DLI) into a mass spectrometer, taking advantage of the Nermag R10–10C instrument facility[2], to solve this type of problem. Such small molecules might not represent the best example of the application of coupling of liquid chromatography (LC) and mass spectrometry (MS) They have low molecular weights and other solutions could be envisaged However, from the chromatographic point of view, this system works routinely in our laboratory and one of our aims was to become familiar with LC–MS coupling. Moreover, we believe that in many circumstances LC–MS coupling will be useful only if one can couple the separation system one is currently using with other types of detectors to a mass spectrometer without needing to adapt

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it. In our case, this results in two major difficulties: first, to introduce a mobile phase buffered at low pH values (*ca.* 3) and containing dissolved salts, and second to be able to ionize ion pairs with the production of spectra that are characteristic of the solute.

After a first negative attempt, we undertook a systematic study of the ionization of ion pairs when introduced directly in the ion source, taking advantage of a recently developed interface. Our results are compared to those obtained by Vouros and co-workers[3,4], who used a moving belt interface and a continuous dichloromethane extraction device[5].

EXPERIMENTAL

Chemicals

DA and NA hydrochloride salts were purchased from Fluka (Switzerland), DOPAC from Sigma (U.S.A.) and sodium *n*-octylsulphonate from Eastman-Kodak (U.S.A.), and were used as received. Water was doubly distilled. Methanol (Merck, G.F.R.) and other solutes were of analytical-reagent grade and were used without further purification.

Chromatographic conditions

The liquid chromatographic system consisted of a Waters (U.S.A.) 6000 pump, an Altex 210 (U.S.A.) injector equipped with a 100-μl loop and a TL-5 glassy carbon electrochemical detector (ElCD) (BAS, U.S A.) operated at a potential of 0.9 V *versus* a silver–silverchloride reference electrode. The column (25 × 4.6 mm I.D.) was packed with 7-μm RP-18 material (Knauer, G.F.R.). For the DLI or LC–MS experiments, the mobile phase consisted of either methanol-water (1.4) or buffer (2 vols. of 0.02 *M* citric acid and 1 vol. of 0.02 *M* Na_2HPO_4)–methanol (80.20), made 2.5 m*M* with sodium *n*-octylsulphonate and 0.05 m*M* with Na_2EDTA. The pH was then adjusted to 3.2 with phosphoric acid. The flow-rate was 1 ml/min and the splitting ratio to the mass spectrometer was 1% to achieve an optimal flow-rate of 10 μl/min in the ion source.

Mass spectrometry

A Nermag R10–10C system monitored by a PDP 11/23 computer was used. The running conditions of the mass spectrometer and the interface between the chromatographic system are described below.

RESULTS

The three compounds (solutes) were first introduced into the ion source by DLI in the methanol–water mobile phase in order to obtain their chemical ionization-positive mode (CI^+) spectra, which are shown in Fig. 1. They exhibit a base peak corresponding to the $(M + H)^+$ ion (except for NA) and at least one important fragment corresponding to the basic structure. In addition, the catechol acid DOPAC produced an ion corresponding to the addition of one molecule of methanol to the pseudo-molecular ion.

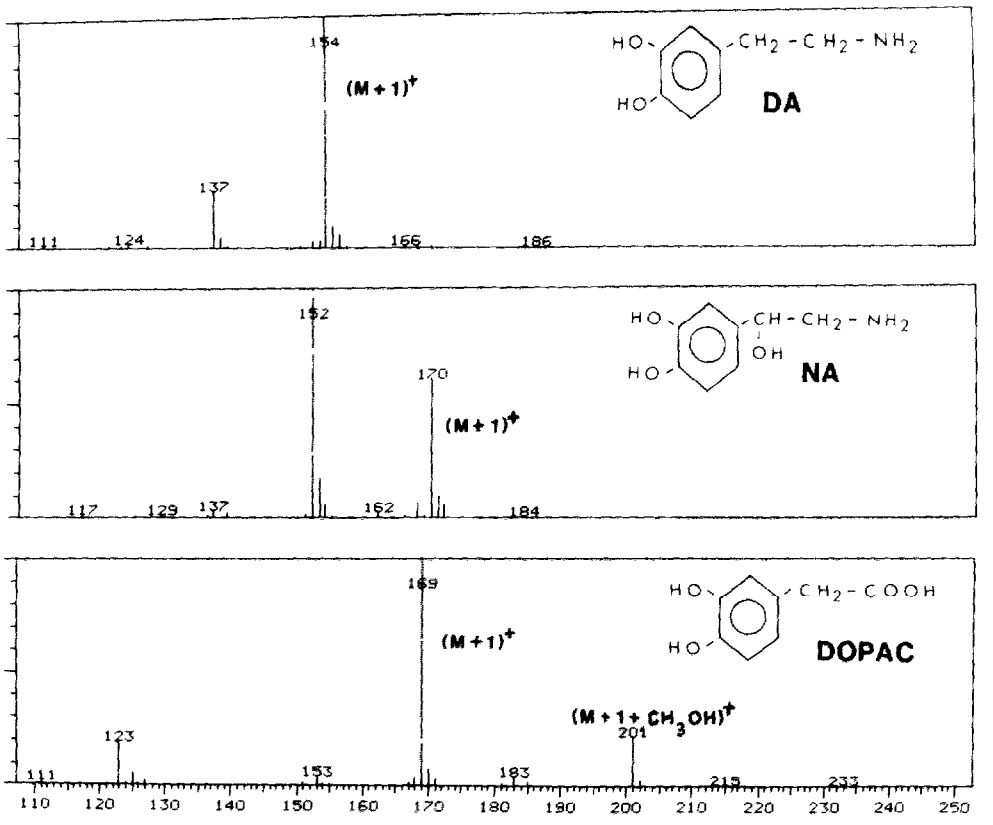

Fig 1 Mass spectra of DA, NA and DOPAC by DLI (CI^+) in methanol–water (1·4) of the pure compounds

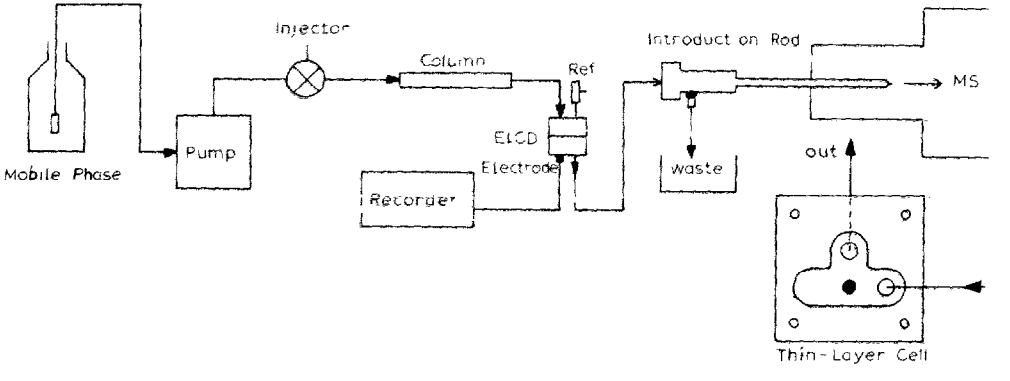

Fig. 2 Schematic diagram of the HPLC–ElCD–MS system The modification of the thin-layer is shown on the lower right.

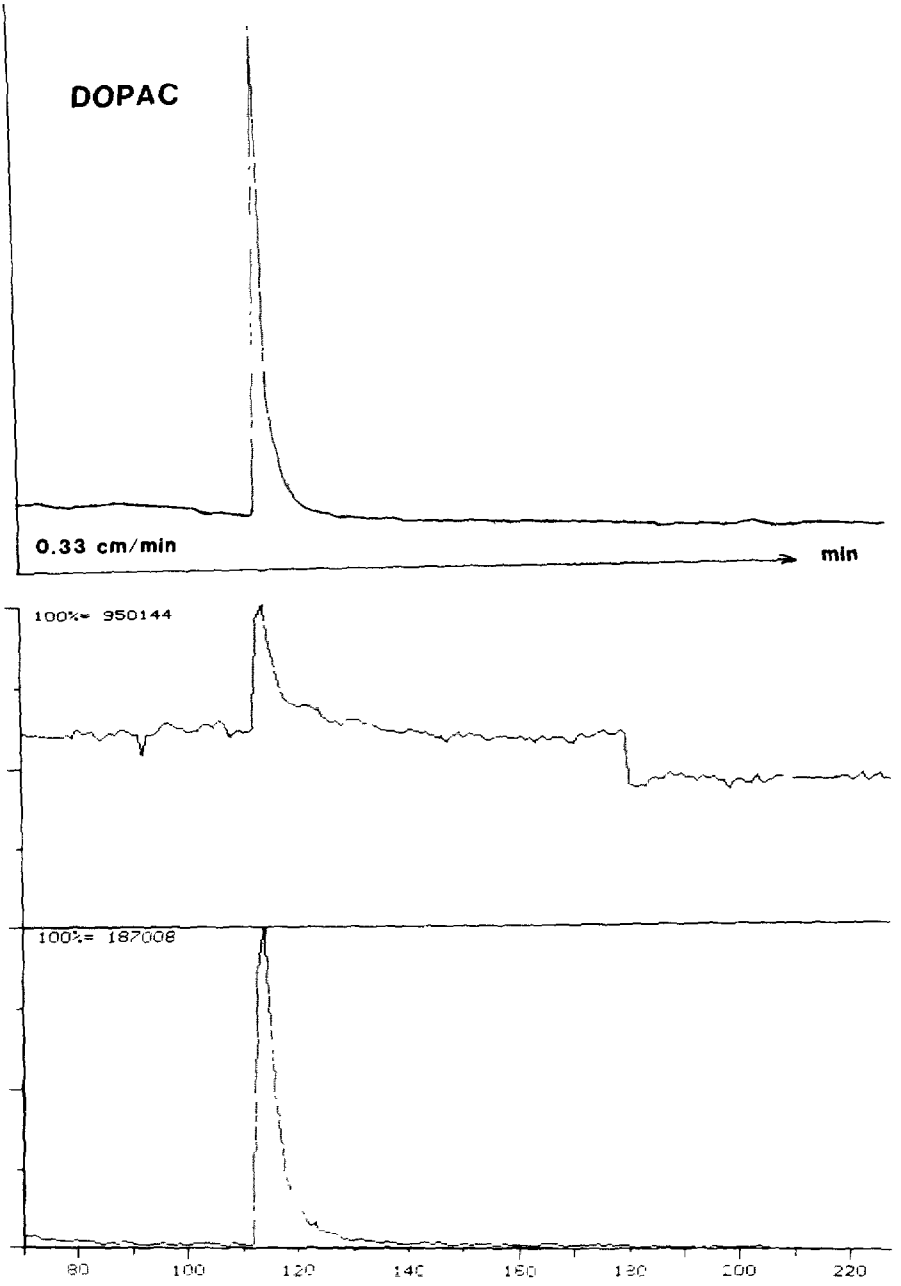

Fig 3 ElCD recording (top), total ion current (middle) and *m z* 169 ion current (bottom) traces fo DOPAC *n*-octylsulphonate ion pair DOPAC was injected onto the RP-18 column in a methanol solutior The buffer (pH 3 2) mobile phase contained *n*-octylsulphonate (2 5 m*M*) (for details, see text) Cl^{+}

LC–MS of the ion pairs

We then directly attempted to couple our chromatographic system to the mass spectrometer, keeping the ElCD in series. For this purpose, we had to modify the thin-layer cell of the ElCD: in order not to lose resolution, the thin plastic film forming the cell was enlarged to expose the small dead volume exit from the detector (initially the mobile phase left the detector through the large-volume reference electrode assembly), as shown in Fig. 2. The only substance that produced ions in this configuration was DOPAC (Fig. 3) In fact, this compound is an acidic metabolite of deaminated dopamine and probably does not form an ion pair.

Ion pairs by desorption/chemical ionization-positive mode (D/CI$^+$) in dichloromethane extracts and buffer

In order to follow more closely the fate of the ion pairs, we followed an approach parallel to that of Kirby *et al.*[3], who first extracted the ion pairs by with dichloromethane and studied them by direct EI-MS and LC–MS using a moving-belt interface

In our case, as expected, the dichloromethane extract of pure *n*-octylsulphonate (counter ion) or solutes did not produce ions. However, when the dichloromethane extracts of the ion pairs were introduced, we obtained spectra corresponding to the sum of the spectra of both components (Fig 4).

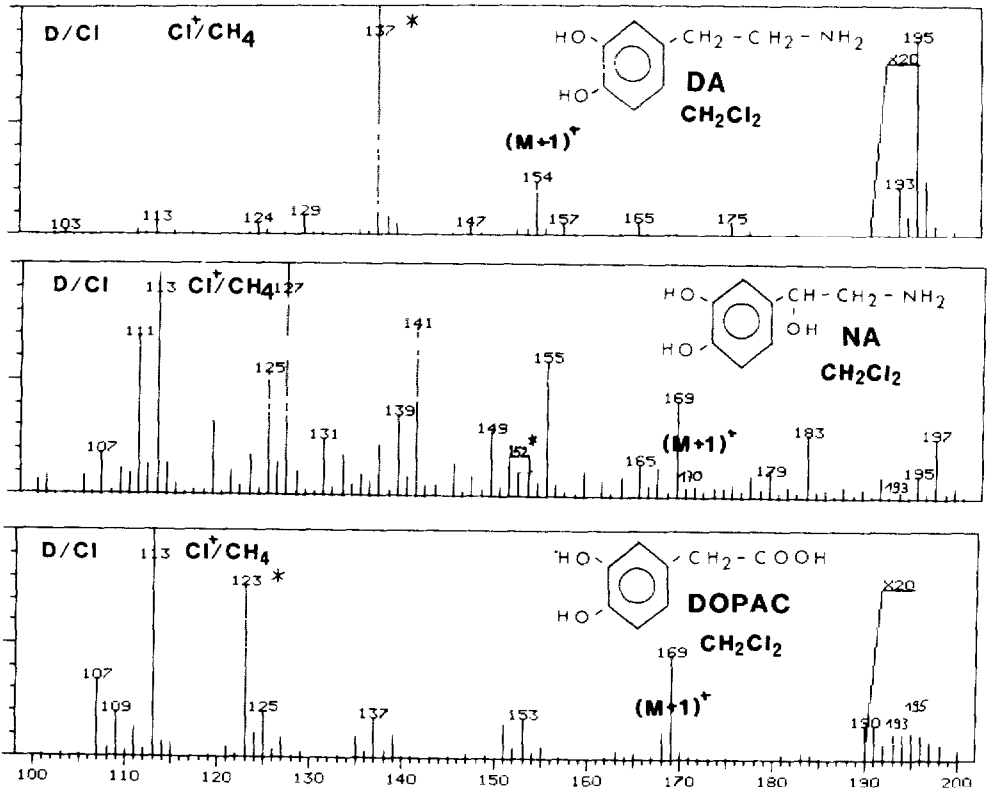

Fig 4 Mass spectra of DA, NA and DOPAC ion pairs by D/CI$^+$ (methane) in dichloromethane extracts

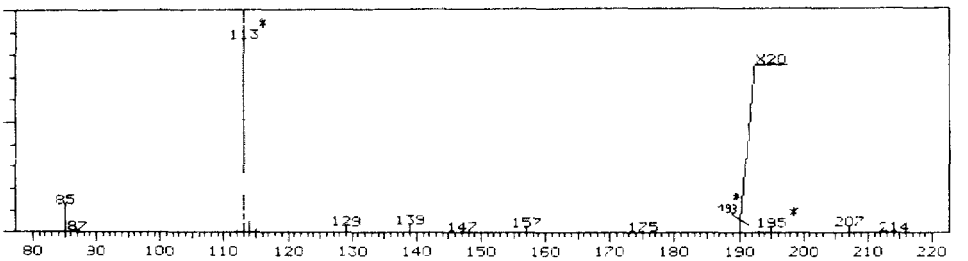

Fig 5 Mass spectrum of sodium *n*-octylsulphonate by D/CI$^+$ (methane) in buffer (pH 3 2) * Characteristics fragment ions

When the buffer solution was deposited on the desorption/chemical ionization (D/CI) filament without any solute, a spectrum of the counter ion was obtained (Fig. 5) in which the base-peak was at *m/z* 113 and two major fragments at *m/z* 195 and 193.

Finally, the buffer solution containing the solutes was deposited on the D/CI filament and spectra containing characteristic ions from both the solutes and the counter ion were observed (Fig. 6)

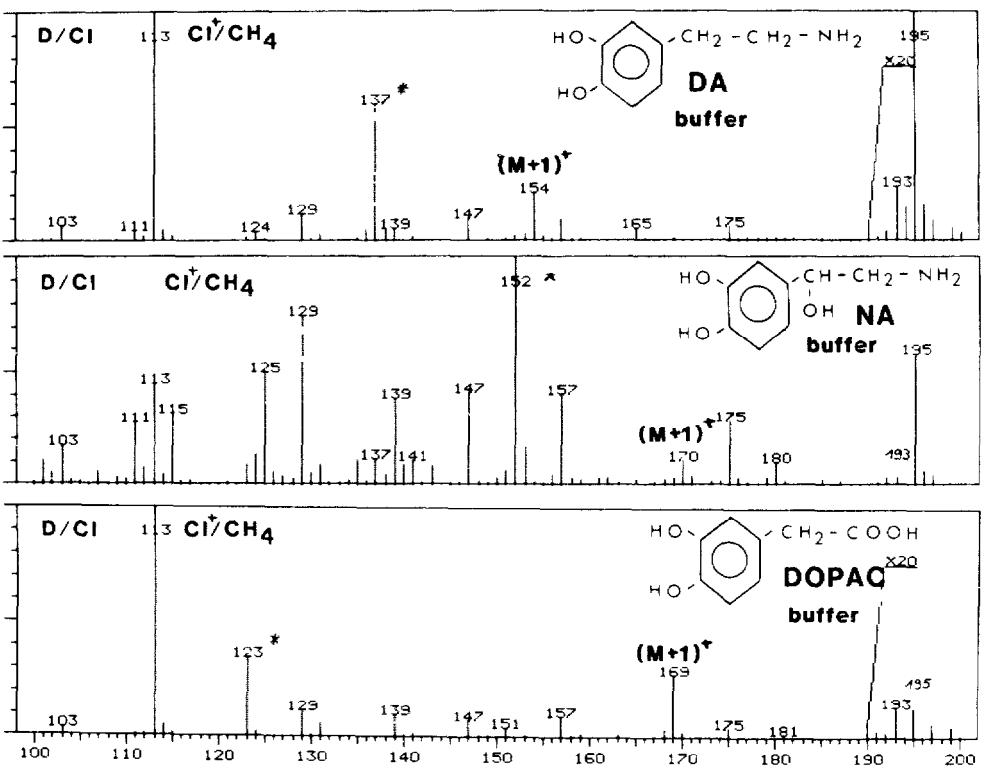

Fig. 6 Mass spectra of DA, NA and DOPAC ion pairs by D/CI$^+$ (methane) in buffer * Characteristic fragment ions of the solutes.

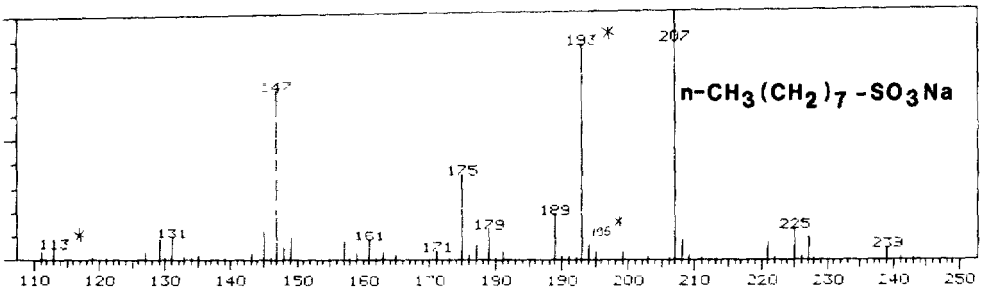

Fig 7 Mass spectrum of sodium *n*-octylsulphonate by DLI (CI^+) in methanol–water (1 4) mobile phase with desolvation chamber (operated at 250°C)

Ion pairs by DLI in methanol–water and buffer mobile phases

In order to facilitate the ionization of the ion pairs, we then tentatively used the desolvation chamber described by Dedieu *et al.*[6]. An exploratory series of experiments showed that the optimal temperature of the desolvation chamber depends on the compound under study. Therefore, we chose a compromise temperature of 250°C for all the subsequent experiments.

When injected in methanol–water as the mobile phase, the counter ion exhibited a spectrum containing fragments at *m/z* 195, 193 and 113 but, in contrast to the spectrum obtained by D/CI, the base peak was at *m/z* 193 (Fig. 7). When the ion

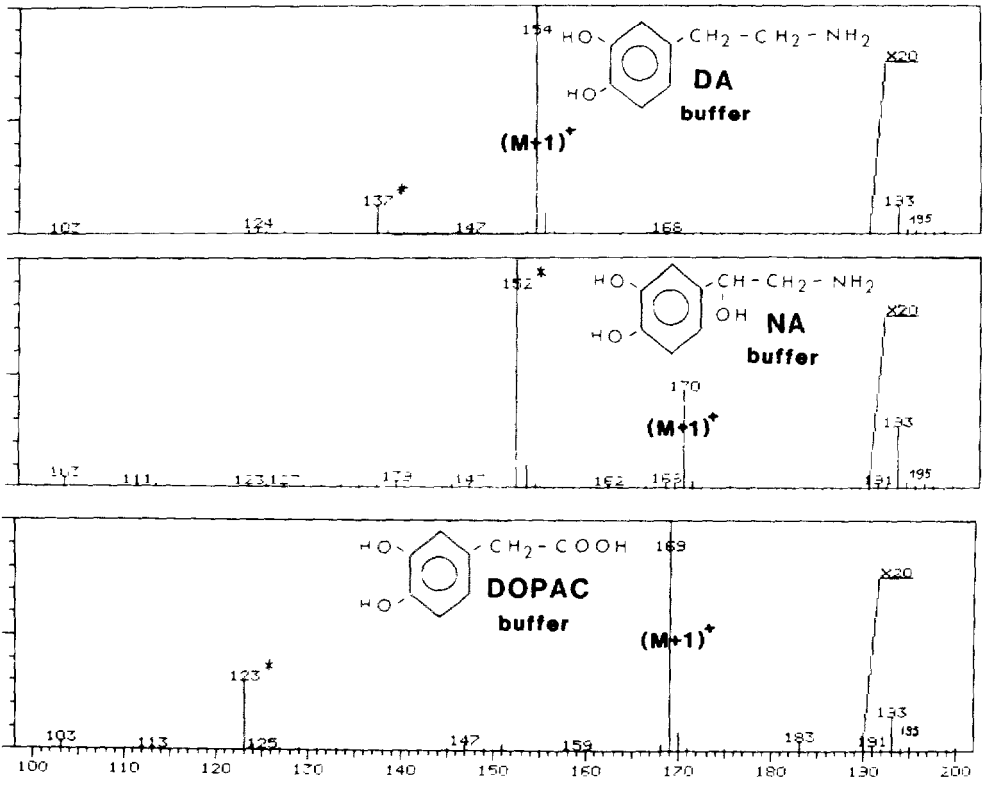

Fig 8 Mass spectra of DA, NA and DOPAC ion pairs by DLI (CI^-) in methanol–water (1 4) mobile phase with desolvation chamber (250°C)

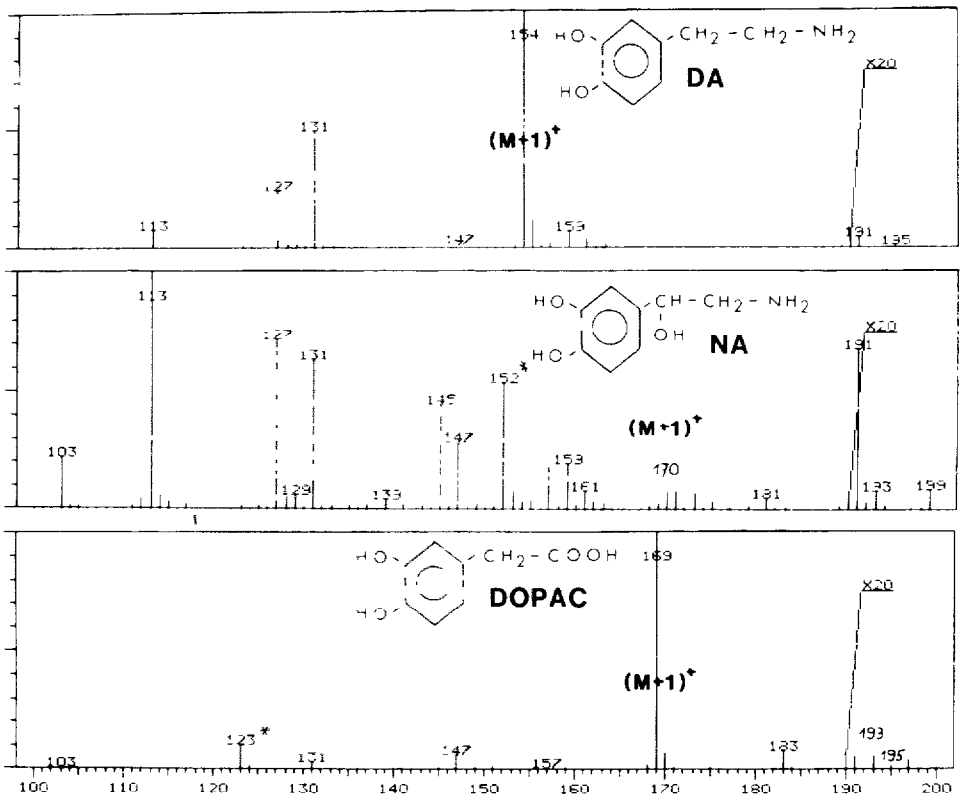

Fig 9 Mass spectra of DA, NA and DOPAC by DLI (CI^+) in buffer (pH 3 2) mobile phase containing *n*-octylsulphonate (2.5 m*M*) with desolvation chamber (250°C)

pairs were injected (they were formed by dissolving the solutes in the buffer solution), characteristic fragments from both the counter ion and the solutes were observed (Fig. 8).

We then used the buffer solution containing the counter ion (concentration 2.5 m*M*) as the mobile phase and injected the solutes dissolved in methanol. The same ions as above were observed but those originating from the fragmentation of the solutes were weaker (Fig. 9).

DISCUSSION

These experiments show that the ionization of ion pairs introduced by DLI is possible. In fact, similar results have also been obtained with the biogenic indole derivatives 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid. However, the interface system has to be more sophisticated than the straightforward introduction of the liquid effluent into the ion source. This is probably due to the difficulty of removing the cluster of solvent molecules surrounding the ion pairs. In our case, the desolvation has been achieved by using a heated interface, leading at the same time to an acceleration of the molecules at a lower pressure[6]. As the effect is more pronounced with effluents of low molecular weight, the situation of an aqueous buffer could be favourable. Moreover, this type of interface permits the ion source to be run at lower temperatures (*ca.* 125°C) which also favours better ionization.

We shall now discuss some problems encountered in this work, and also those which might be foreseen in future work.

Buffer the introduction of this type of mobile phase into the ion source first causes problems due to its acidic pH. All the tubing must be made from stainless steel containing no nickel or from platinum. Further, in our instrument, the diaphragms are normally made from pure nickel, the only metal that allows the manufacture of 2–3-μm holes. Such diaphragms are attacked by the buffer solution and permit work for only a few minutes To protect the nickel, they must be gold plated (*ca.* 1 μm thick). Second, the dissolved salts introduced into the ion source contaminate it very quickly It appeared that the contamination can cause changes in the relative intensities of the fragment ions It is therefore of prime importance to keep the source very clean The dichloromethane extraction device of Vouros and coworkers could overcome this drawback. However, the conditions of the extraction should be very carefully optimized as it appeared to us that the extraction yield could lower the sensitivity considerably.

Sensitivity all our experiments were conducted by introducing large amounts of compounds (hundreds of nanograms) in the source. Such large amounts are never found in practice, as indicated in the Introduction Several solutions now exist for increasing the splitting ratio before the introduction into the source. One can either use a microbore column, although at present there is not a wide choice on the market and a danger of overloading the column with the injection volume or the amount of solute exists, or one can use a small-bore column (*ca.* 2 mm I.D.) combined with a larger diaphragm hole and therefore increase the splitting ratio to 1:10 or even 1 5.

As mentioned above, comparable experiments have been performed by Kirby *et al.* using a moving-belt interface[3]. Some differences were observed in the spectra of the counter ion. Whereas they observed only a single ion when using *n*-octylsulphonate, corresponding to the protonated sulphonic acid of the salt, we observed a fragmentation pattern corresponding to that observed by Kirby *et al.* for the *n*-octylsulphate salt. However, in our case, it must be noted that the intensity of the fragments depended on the introduction conditions in the D/CI experiments, the base peak corresponded to the protonated alkene ion (m/z 113) but in the DLI experiments the base peak corresponded to an ion at m/z 193. As far as the spectra of the ion pairs are concerned, no essential differences were observed from those of Kirby *et al.* It must also be pointed out that the differences observed between the results of Kirby *et al.* and our D/CI results could have been caused by the different reagent gases used (isobutane by Kirby *et al.* and methane by us), and second between our D/CI and DLI experiments because in the latter no reagent gas was used We tentatively add methane and ammonia in the DLI experiments, but we observed no effects on the fragmentation pattern It can be said that DLI does not necessitate an extraction procedure with all its drawbacks and ensures that all the compounds separated by the HPLC column are introduced into the ion source

Fig. 10 shows a comparison of the spectra obtained in the different modes for DA as a representative example of the other compounds studied. In all modes, characteristic ions from both the counter ion and the solute are present, the $(M + H)^+$ ion of the solute being present in every instance. However, it is obvious that concerning the solute, the relative intensities of the $(M + H)^+$ ion and the fragments depend on the mode of introduction. As expected, DLI appears to produce a softer ionization than D/CI. If this could lead to a loss of specificity, it might increase the sensitivity of detection.

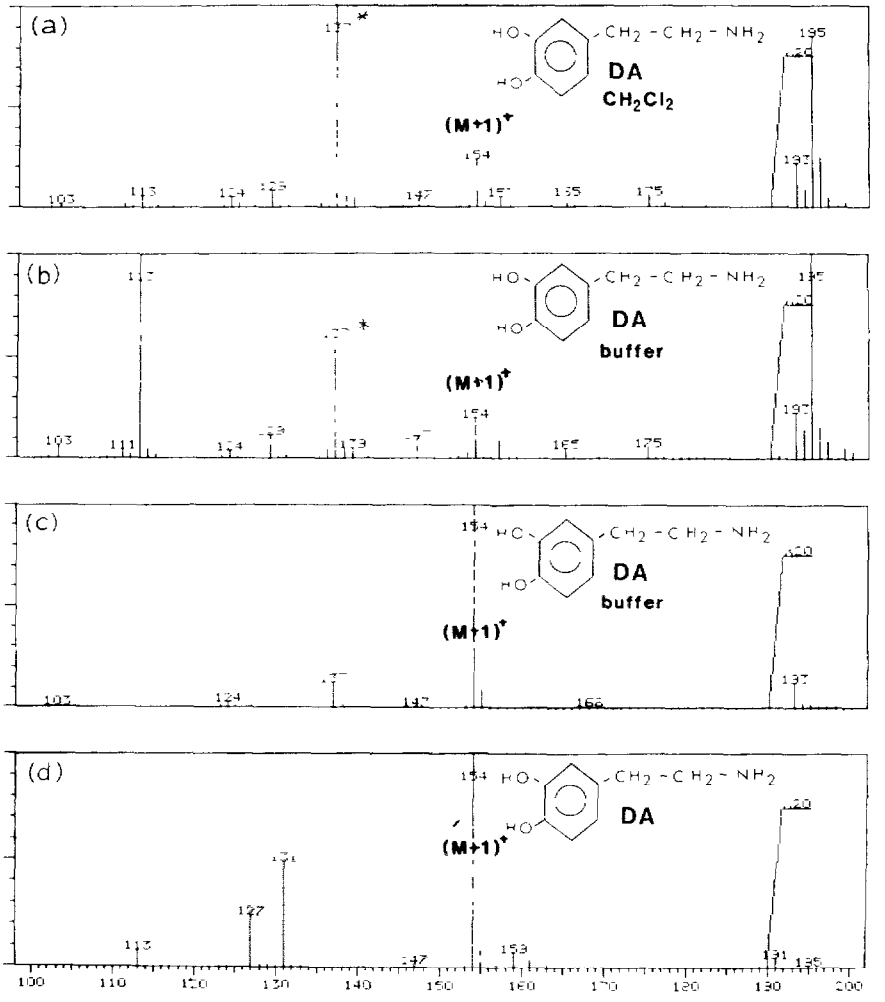

Fig 10 Mass spectra of DA in different modes showing the effect on the relative intensities of the fragment ion of DA and counter ion. a and b, D.CI$^+$ (methane), c, DLI (CI$^+$), methanol water (1 4); d, CI$^+$, buffer pH 3 2

In conclusion, we have shown that spectra of ion pairs can be obtained when injected by DLI, provided that the interface allows the desolvation of the ion pairs from the cluster of solvent molecules

Studies are under way to achieve the sensitivity required for biological work and to analyse other types of compounds necessitating the use of MS.

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