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NEW APPROACHES TO ON-LINE ION-PAIR EXTRACTION AND DERIVATIZATION FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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CONTENTS

1. INTRODUCTION

The use of ion-pairing techniques for the analysis of ionic compounds using conventional mass spectrometric ionization methods —*i.e.*, electron impact (EI) or chemical ionization (CI)— is a known but not frequently employed approach. It has been shown for example that the volatility of many organic compounds with highly polar or ionic functional groups can be enhanced significantly by combination of the solute(s) of interest with an appropriate reagent (counter-ion) and the subsequent liberation of a volatile derivative at the elevated temperatures of the inlet of the mass spectrometer[1]. A similar ion-pairing approach has been utilized for the EI analysis of ionic dyes[2] and biologically important quaternary amines such as various substituted cholines[3].

In view of the growing interest in combined high-performance liquid chromatography–mass spectrometry (HPLC–MS) (see, *e.g.*, ref. 4) and the effective use of ion-pairing for the HPLC analysis of ionic compounds, (see, *e.g.*, ref. 5), we recently examined the compatibility of ion-pairing techniques with HPLC–MS[6,7]. In these studies we demonstrated the feasibility of conducting on-line HPLC–MS in the ion-pairing reversed-phase mode by incorporating a continuous extraction interface in the HPLC–MS system[7]. It was further shown that ion-pair formation facilitated the

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extraction of ionic compounds into an organic phase which was readily adaptable to reversed-phase HPLC–MS coupling using a moving-belt transport system[8]. Useful mass spectra of ion-pairs were obtained with the mass spectrometer operated in either the EI or the CI mode.

As indicated in our earlier publications, in order to be compatible with MS analysis, it is essential that both components of the ion-pair be vaporized under normal MS operating conditions and that they provide spectra consistent with the structures of the unpaired materials. Moreover, it is necessary that spectral overlap of the two ion-pair constituents be kept at a minimum in order to permit retrieval of as much relevant information as possible from a given mass spectrum.

In our initial studies we examined ion pairs formed from *n*-alkylsulfate or *n*-alkylsulfonate counter-ions and primary and/or secondary amine solutes. Our selection of the *n*-alkylsulfate and sulfonate counter-ions was made on the basis of the frequent use of such species for extraction and HPLC separation. It was noted that many of the fundamental requirements for effective MS determination of various amine solutes were, indeed, met by using this type of an ion-pair system. Nevertheless, there are instances where the general applicability of such long chain counter-ions may be limited. This limitation arises from the well known fragmentation of the *n*-alkyl chain which gives rise to ion peaks every 14 mass units extending up to the mass of an ion of formula $C_nH_{2n}^+$. The occurrence of these peaks may be particularly troublesome when analyzing polar solutes of relatively low molecular weight and/or when structurally significant solute fragment ion peaks overlap with the alkyl chain fragments. While this problem may be partially circumvented via the use of CI as opposed to EI[6,7], this alternative may not be feasible or desirable, since CI may not provide structurally informative data. As a result, we have studied the use of different types of counter-ions for the analysis of ion-pairs by HPLC–MS using EI. Counter-ion salts were selected on the basis of the structural requirements for fragmentation in EI ionization MS as well as the compatibility of the counter-ions with reversed-phase HPLC. In contrast to HPLC with UV detection, it is shown in this paper that aromatic counter-ions are highly suited to HPLC–MS as their EI fragmentation is minimal.

In the ion-pair extraction approach cited above, charge neutralization on the moving belt is caused by proton transfer. In principle, it should also be possible to neutralize an anionic species by alkylation. In this paper we present this possibility in which methylation is accomplished using trimethylanilinium hydroxide as alkylating reagent. On the basis of the results, it is clear that one can selectively derivatize by the judicious choice of the counter-ion. The examples of this paper further support the contention that incorporation of chemical steps on-line between the column and the MS can significantly enhance the power of LC–MS.

2. EXPERIMENTAL

2.1. *Equipment*

A Varian Model 5000 liquid chromatograph (Varian Assoc., Palo Alto, CA, U.S.A.) equipped with a LDC Model 1205 UV detector (Laboratory Data Control, Riviera Beach, FL, U.S.A.) was used throughout. The injection valve consisted of a Valco air actuated CV6-UHPa-N60 injection valve (Valco Instruments, Houston, TX, U.S.A.). Chromatographic columns were packed in our laboratory with either 6.5 μm particle diameter Zorbax-CN (DuPont, Wilmington, DE, U.S.A.) or home-made C_{18} using 5 μm Hypersil silica gel (Shandon Southern Instruments, Sewickley,

PA, U.S.A.). The post-column continuous extractor employing liquid segmentation was similar to that previously described[8]. Special care was taken to maintain pressure for the delivery of the organic phase to the mixer and for the removal of the aqueous waste from the phase separator.

A Finnigan 4000 quadrupole mass spectrometer (Finnigan Instruments, Sunnyvale, CA, U.S.A.) interfaced to a Finnigan Incos 2300 data system and equipped with a moving-belt interface (Kapton) was used for the on-line LC–MS experiments. Deposition onto the belt of the mobile phase or the on-line extracting solvent was achieved by means of a hot spray nebulization technique adapted from the work of Smith and Johnson[9]. Off-line spectra were also obtained with a Nuclide 12-90-G magnetic spectrometer (Nuclide Corp., State College, PA, U.S.A.). The probe temperature was programmed from ambient temperature to 250 C (at approximately 100 C/min).

2.2. *Solvents and reagents*

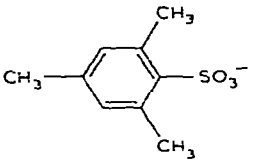
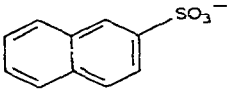
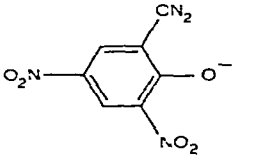
All solvents were either Baker Analyzed Reagent for HPLC (J. T. Baker, Phillipsburg, NJ, U.S.A.) or Mallinckrodt ChromAR (Mallinckrodt, Paris, KY, U.S.A.). The ion-pairing reagents were purchased from Eastman Kodak (Rochester, NY, U.S.A.) except for picric acid which was Baker Analyzed Reagent and trimethylbenzenesulfonic acid from Alfred Baker Library of Rare Chemicals (Milwaukee, WI, U.S.A.). In the latter case, we found that it was necessary to recrystallize from methylene chloride in order to minimize impurity peaks in the mass spectrum of this species. Trimethylanilinium hydroxide was purchased as a solution of 0.2 *M* in methanol from Supelco (Bellefonte, PA, U.S.A.). Solutes and buffer components were of analytical quality from various commercial sources and were used without further purification.

3. RESULTS AND DISCUSSION

Since the mass spectrum of an ion pair consists of the sum of the mass spectra of each of its constituents, it is obviously desirable to minimize the number of fragments arising from the counter-ion in order to obtain all the spectral information derived from the solute. In our initial studies on HPLC–MS of ion pairs, we selected counter-ions consisting of *n*-alkylsulfates and sulfonates, species well suited for reversed-phase HPLC analysis using a UV detector. In order to obtain a "clean" mass spectrum of the solute we could employ computer subtraction of the background produced from the counter-ion. While adequate, this approach is not entirely satisfactory and, as a result, we have now considered an alternative and more generally applicable approach. Accordingly, the counter-ions selected in this paper consisted of small aromatic compounds in order to take advantage of the well known phenomenon of the limited degree of fragmentation of such systems in EI-MS[10]. It is interesting to note that this approach contrasts with the requirements of the standard UV detector for HPLC where ion-pair chromatography is normally carried out with non-chromophoric counter-ions. It should also be pointed out that purification of the counter-ion salts by recrystallization was necessary in some cases (*e.g.*, 2,4,6-trimethylbenzenesulfonate to remove homologous impurities). Counter-ion purity would thus appear to be more critical for HPLC–MS than for the more conventional HPLC–UV detection because impurities can produce interfering spectral peaks in the MS detector. Such effects can often be averaged out in a UV detector.

TABLE I

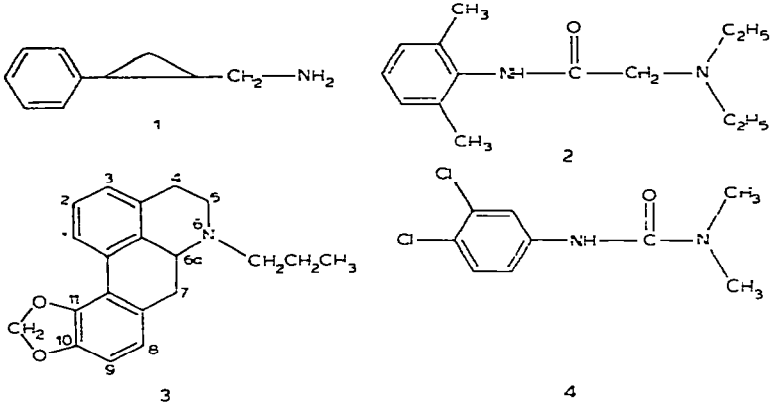
COUNTER-IONS AND MASS SPECTRAL CONTRIBUTIONS

Counter-ion	*Structure*	*m/z values of principal ions (EI)*
n-Decylsulfate	*n*-$C_{10}H_{21}OSO_3^-$	41, 55, 69, 70, 83, 84, 97, 112, 140
n-Octylsulfonate	*n*-C_8H_{17}-SO_3^-	41, 55, 56, 69, 70, 83, 84, 97, 112
2,4,6-Trimethylbenzenesulfonate		200, 118
2-Naphthalenesulfonate		144, 128, 115
Picrate		229, 199

The counter-ions examined in this study are listed in Table I. The *m/z* values of the principal fragment ions are presented along with those of the C_{10}-alkylsulfate and C_8-alkylsulfonate for comparison purposes. The picrate was selected because salts of this species are often useful derivatives of amines and heterocyclic bases and are readily adaptable for ion-pair chromatography and/or extraction[11]. Moreover, the mass spectrum of picric acid (*i.e.*, the neutral compound produced from the thermal decomposition of picrate salts) is dominated by three major ion peaks, at *m/z* 229 ($M^{-\cdot}$), *m/z* 199 [$(M - NO)^-$] and *m/z* 30 (NO^-) (ref. 12). Naphthalenesulfonate was selected because of the resistance of the naphthalene aromatic nucleus to electron impact induced fragmentation. Moreover, a related compound, 9,10-dimethoxy-2-anthracenesulfonate, was previously employed as a counter-ion by Brinkman *et al.*[13] for the analysis of amines, in which ion pairs were extracted on-line after the HPLC column into an organic solvent and detected by fluorescence. In our case, because of the MS detector, it was preferable to select instead a counter-ion with the non-substituted naphthalene nucleus in order to avoid additional peaks originating from the fragmentation of the methoxy group (*e.g.*, losses of CH_3 or CH_2O) (ref. 14). Contribution from the naphthalenesulfonate counter-ion to the mass spectra of ion-pairs was thus limited to major peaks at *m/z* 144 and 115 and a peak of lesser abundance at *m/z* 128. No molecular ion of significant intensity was observed. *m/z* 144 corresponds to an ion of hydroxynaphthalene formed via elimination of SO_2 from $M^{-\cdot}$, while *m/z* 115 is probably formed by further loss of HCO from the *m/z* 144 fragment ion to yield an ion of likely composition $C_9H_7^+$.

Based on the general reasoning of the need for a stable aromatic system to minimize fragmentation, a third counter-ion was also examined, namely 2,4,6-trimethylbenzenesulfonate. Spectral contributions from this counter-ion were limited pri-

marily to a peak at *m/z* 200 (the molecular ion of the corresponding sulfonic acid) and a fragment peak at *m/z* 118, formed by elimination of H_2SO_3 from *m/z* 200. Two additional counter-ions (benzenesulfonate and toluenesulfonate) were also explored briefly, but gave unsatisfactory results due to the poor extraction efficiency of their ion-pairs.



Several amine solutes were examined in this study. The results outlined for the biologically significant amines α-methylparnate (1), lidocaine (2), 10,11-methylenedioxy-N-*n*-propylnorapomorphine (3) and the pesticide diuron (4) were representative of those obtained with the compounds investigated. Mass spectra both on-line and off-line of these compounds with different counter-ions are shown in Figs. 1–6. For off-line MS ion pairs of the various solutes and counter-ions were collected by extraction into methylene chloride from an aqueous solution (80% water, phosphate, pH 2.5, 0.1 *M* and 20% methanol) containing the solute of interest and a 4–5-fold excess of the counter-ion. These are conditions essentially identical to those employed for on-line work using the post column extraction interface.

The EI mass spectra of α-methylparnate and its corresponding ion pairs with C_{10}-alkylsulfate, picrate and naphthalenesulfonate are compared in Fig. 1. The spectrum of the parent compound shows structurally relevant peaks at *m/z* 147, *m/z* 130, *m/z* 119, *m/z* 91 and *m/z* 77. These correspond to $M^{+\cdot}$, $[M - NH_3]^{+\cdot}$, $[M - CH_2NH_2]^+$, $C_7H_7^+$ and $C_6H_5^+$, respectively, a pattern typical of compounds of this type[15,16]. Fortuitously, in the mass spectrum of the α-methylparnate/C_{10}-alkylsulfate ion pair (Fig. 1b) the hydrocarbon fragment ions (*m/z* 140, 112, 97, 83, 70) do not interfere with the major solute fragments. Nevertheless, the potential for confusion of solute and counter-ion peaks is apparent, especially when a solute reference spectrum is not available.

Besides computer subtraction, one possible approach to overcoming such potential confusion is demonstrated in the spectrum of the 2-naphthalenesulfonate ion pair (Fig. 1d). Three major peaks are contributed by the counter-ion at *m/z* 144, 128 and 115. (Note *e.g.*, the relative increase in the intensity of the peak at *m/z* 115 in the cluster *m/z* 115–*m/z* 119 due to contribution from the naphthalenesulfonate constituent). Other than that, a "clean" spectrum of the solute is evident. The flexibility

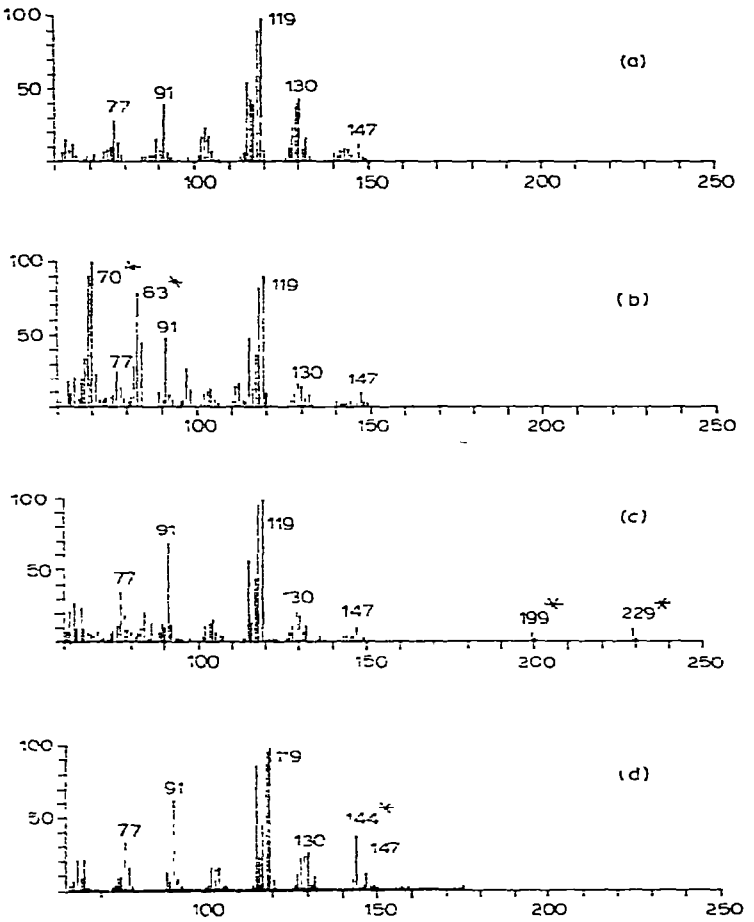


Fig. 1. (a) EI mass spectrum of α-methylparnate (I); (b) EI mass spectrum of α-methylparnate/C_{10}-alkylsulfate ion pair; (c) EI mass spectrum of α-methylparnate/picrate ion pair; (d) EI mass spectrum of α-methylparnate naphthalenesulfonate ion pair. All spectra recorded off-line with Finnigan 4000 mass spectrometer.

provided by the proper selection of the counter-ion species is further illustrated in Fig. 1c which shows the mass spectrum of the α-methylparnate/picrate ion-pair. Contribution from the counter-ion is limited to the peaks at *m/z* 199 and 299, which are entirely removed from the spectral region associated with the solute, thus providing a definitive spectrum of α-methylparnate.

It should be noted that contributions from the methylene chloride extraction solvent cannot be always entirely subtracted out. This is illustrated in Fig. 2 which shows the peaks at *m/z* 84, 86, and 88 arising from $CH_2Cl_2^{+\cdot}$ and its various chlorine isotope combinations. Moreover, it should also be pointed out that, consistent with some of our previous observations[6], some minor quantitative variations in the spectrum of the solute are apparent when recorded from an ion pair, compared to that obtained from the free base (reference compound). Note, for example, the small variations in the ion clusters around *m/z* 131, 119, and 104 (Figs. 2a–c).

Fig. 2 shows the mass spectra of lidocaine along with those of lidocaine/C_{10}-alkylsulfate and lidocaine/picrate ion pairs. The molecular ion peak of lidocaine is

well defined at m/z 234 in all three spectra. Interference from the CH_2Cl_2 solvent peaks, however, masks the base peak of lidocaine at m/z 86 which arises from cleavage of the amide bond beta to the aromatic system to give an ion with structure a (Fig. 2a, c). Again, the value of the picrate over the C_{10}-alkylsulfate counter-ion is illustrated in the comparison of spectra 2b and 2c. Excluding some confusion arising from the solvent contributions at m/z 84 and 86, the picrate spectrum (Fig. 2c) is considerably simpler.

$$O=C=\overset{+}{N}-(CH_3)_2$$

a

The spectra of lidocaine and its ion-pairs which were recorded by introduction of the sample into the MS via the moving-belt interface exhibited some variations from those obtained via the direct insertion probe. Most notable is the abnormally high relative intensity of the peak at m/z 217 in spectra obtained with the moving belt, while in the probe spectra the dominant peak in that region of the spectrum occurs at m/z 219, $[M - CH_3]^+$. It is not clear whether such discrepancies are due to catalytic

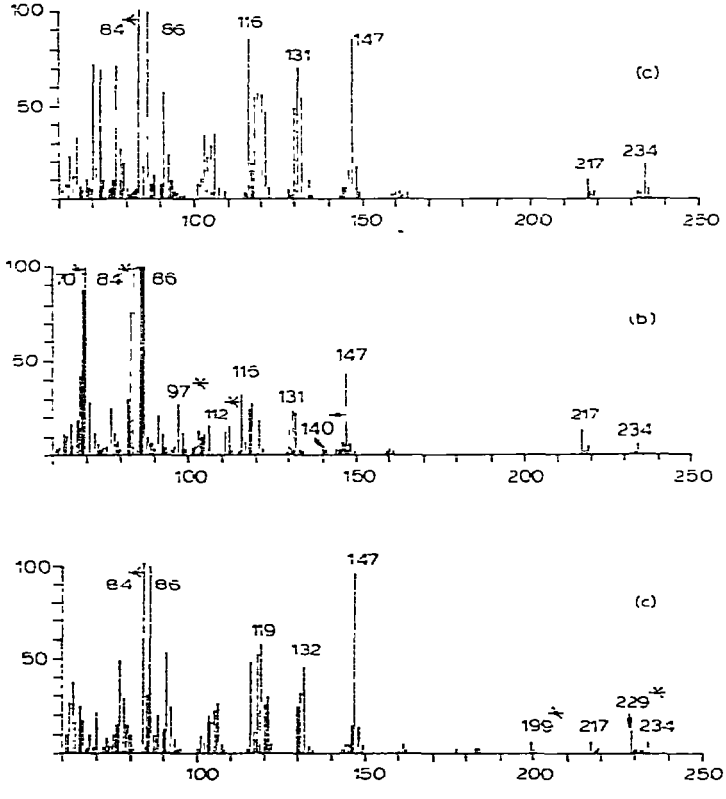


Fig. 2. (a) EI mass spectrum of lidocaine (2); (b) EI mass spectrum of lidocaine C_{10}-alkylsulfate ion pair; (c) EI mass spectrum of lidocaine-picrate ion pair. All spectra were obtained with Finnigan 4000 mass spectrometer on-line with a Varian 5000 HPLC (for typical chromatograms see ref. 7).

thermal decomposition effects associated with the belt material or not. Additional work to clarify such phenomena is currently under consideration.

Perhaps a somewhat different situation is illustrated in Fig. 3 which shows the mass spectra of 10,11-methylenedioxy-N-*n*-propylnorapomorphine (Fig. 3a) and those of its ion-pairs with C_{10}-alkylsulfate, 2,4,6-trimethylbenzenesulfonate and 2-naphthalenesulfonate. Typical of the mass spectral behavior of alkaloids of this type[17,18] the peaks of structural significance occur in the high mass region of its spectrum. These are exemplified by an intense molecular ion peak at *m/z* 307, the peak at *m/z* 306 arising from the loss of the 6a-tertiary hydrogen and the $[M - 29]^+$ ion at *m/z* 278 arising from cleavage of the *n*-propyl C–C bond beta to the nitrogen and elimination of a $C_2H_5^{\cdot}$ radical. Further loss of 29 a.m.u. from *m/z* 278, a characteristic fragmentation of N-*n*-propylnoraporphines[18], is responsible for the ion of *m/z* 249. Additional fragmentations associated with the methylenedioxy function and resulting in loss of 30 a.m.u. (CH_2O group) from *m/z* 306 and *m/z* 249 are responsible for the peaks at *m/z* 276 and 219 respectively.

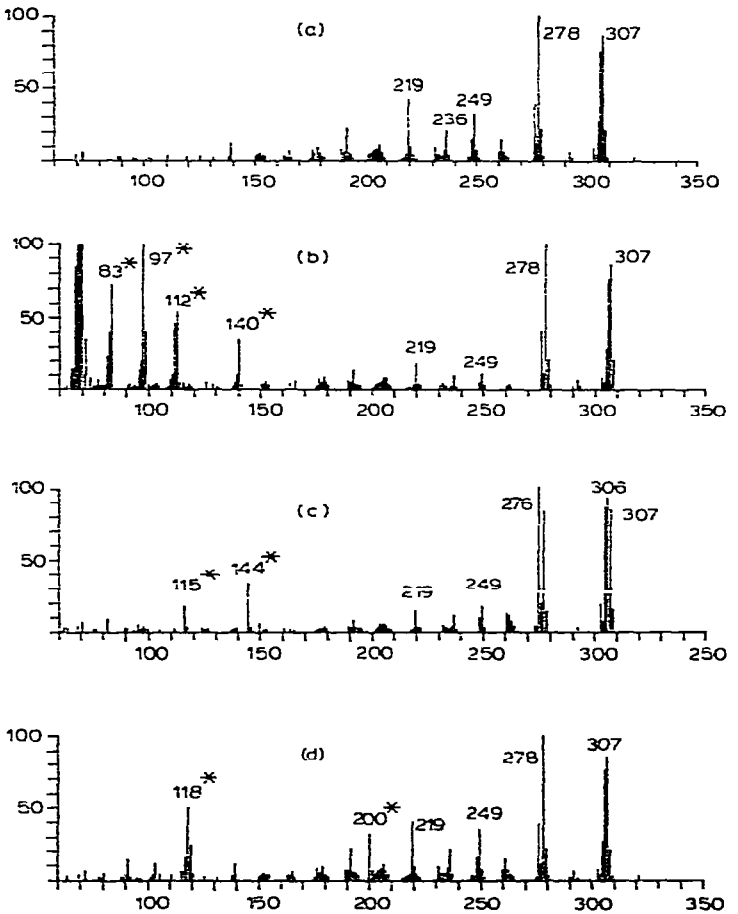


Fig. 3. (a) EI mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine (3); (b) EI mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine/C_{10}-alkylsulfate ion pair; (c) EI mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine/2-naphthalenesulfonate ion pair; (d) EI mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine/2,4,6-trimethylbenzenesulfonate ion pair. All spectra recorded off-line with Nuclide 12-90-G mass spectrometer.

In view of the fact that the most significant fragment ions in the spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine occur above the *m/z* 200 region, no interferences from the C_{10}-alkylsulfate counter-ion are possible (Fig. 4b). Nevertheless, the repeating pattern of hydrocarbon fragment peaks still presents an unnecessarily rich spectrum which may distract one's attention from the spectrum of primary interest, namely, that of the solute. It is for this reason that the naphthalenesulfonate counter-ion which contributes only three major peaks at *m/z* 115, 128 and 144 is advantageous (Fig. 3c). A similar advantage is realized with the picrate counter-ion (spectrum not shown here) as well as with the 2,4,6-trimethylbenzenesulfonate (Fig. 4d) which exhibits major peaks only at *m/z* 200 and *m/z* 118.

For general comparison purposes the methane-CI spectra of the methylenedioxy compound, 3, and its ion-pairs with C_{10}-alkylsulfate and picrate are shown in Figure 4. As expected, the spectra are greatly simplified, the solute spectrum being dominated by the peaks at *m/z* 308 and *m/z* 306, $[(M + H)^+$ and $(M + H - 2H)^-]$ respectively. A single major peak is contributed by the C_{10}-alkylsulfate (*m/z* 141) and two peaks by the picrate (*m/z* 230 and 200).

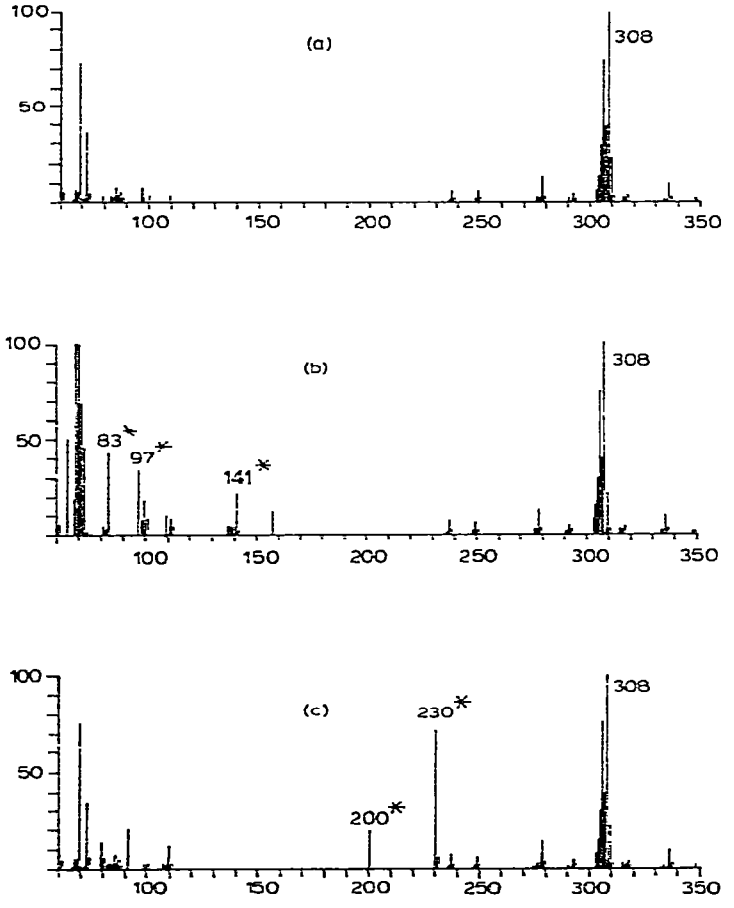


Fig. 4. (a) CI (CH_4) mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine; (b) CI (CH_4) mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine/C_{10}-alkylsulfate ion pair; (c) CI (CH_4) mass spectrum of 10,11-methylenedioxy-N-*n*-propylnorapomorphine/picrate ion pair. Spectra recorded off-line with Finnigan 4000 mass spectrometer.

As a final example the on-line LC-mass spectrum of the pesticide diuron and several ion pairs was considered. Figure 5a shows the EI spectrum of the free base. In addition to the weak molecular ion peak (*m/z* 232), the spectrum shows intense peaks at *m/z* 187, $[M - HN(CH_3)_2]^{+\cdot}$, *m/z* 159 (loss of CO from *m/z* 187) and *m/z* 124 (loss of Cl from *m/z* 159), along with the corresponding isotope clusters. These characteristics are retained, as expected, in the spectra of the C_{10}-alkylsulfate and picrate ion pairs (Figs. 5b and 5c, respectively). Some variations due to previously noted interference from the CH_2Cl_2 solvent are more evident in the picrate spectrum (Fig. 5c). The overall picture was simplified considerably when the on-line spectra were recorded in the CI (CH_4) mode (Fig. 6), at the expense, of course, of some structural detail. The spectrum of the diuron solute now exhibits primarily only the peaks at *m/z* 233, $(M + H)^+$, and *m/z* 188, $[M - NH(CH_3)_2]^-$, while the alkylsulfate, naphthalenesulfonate and picrate counter-ions show their previously noted peaks (see CI spectra of the methylenedioxy compound, Fig. 4). Adduct ions from the CI reagent gas may be noted at *m/z* values above 233 corresponding to $(M + 29)^+$ and $(M + 41)$.

In the examples discussed thus far, vaporization of the solute from the ion pair into the MS was accomplished by neutralization of the counter-ion salt via the effec-

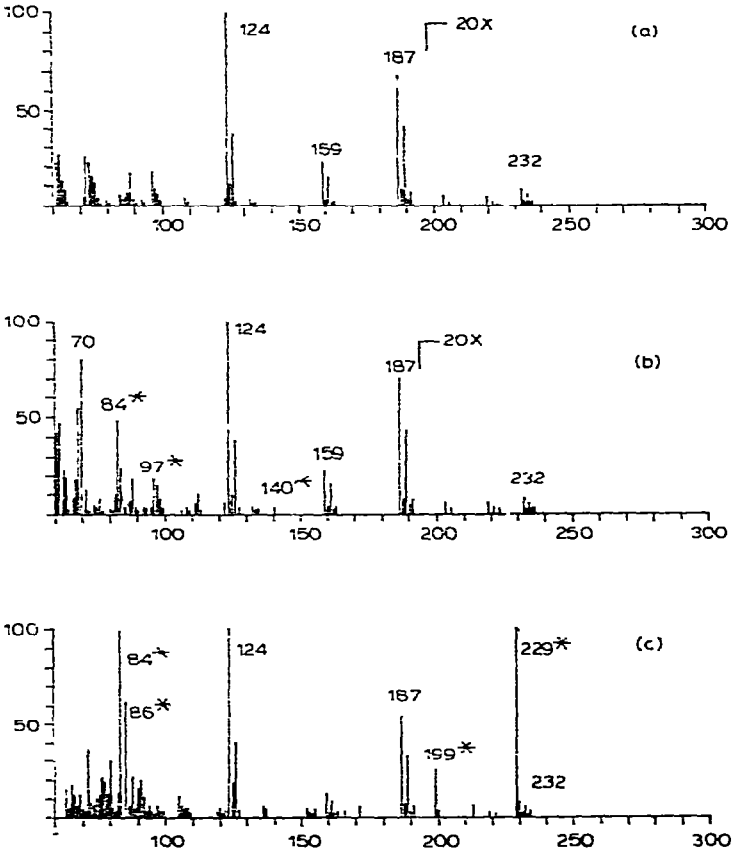
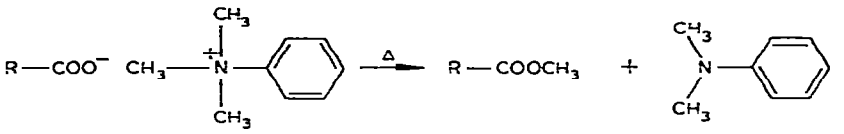


Fig. 5. (a) EI mass spectrum of diuron (4); (b) EI mass spectrum of diuron/C_{10}-alkylsulfate ion pair; (c) EI mass spectrum of diuron/picrate ion pair. Spectra recorded with Finnigan 4000 mass spectrometer interfaced with Varian 5000 liquid chromatograph.

tive transfer of a proton in a thermally induced mechanism. In principle it should be possible to accomplish the same process by transferring an alkyl group instead of proton. To demonstrate this we selected an example from a well known methylation reaction in the injection port of a gas chromatograph, based on the use of trimethylanilinium hydroxide (TMAH) as reagent for the esterification of carboxylic acids[19,20]. The reaction process is outlined in Scheme 1.



Scheme 1.

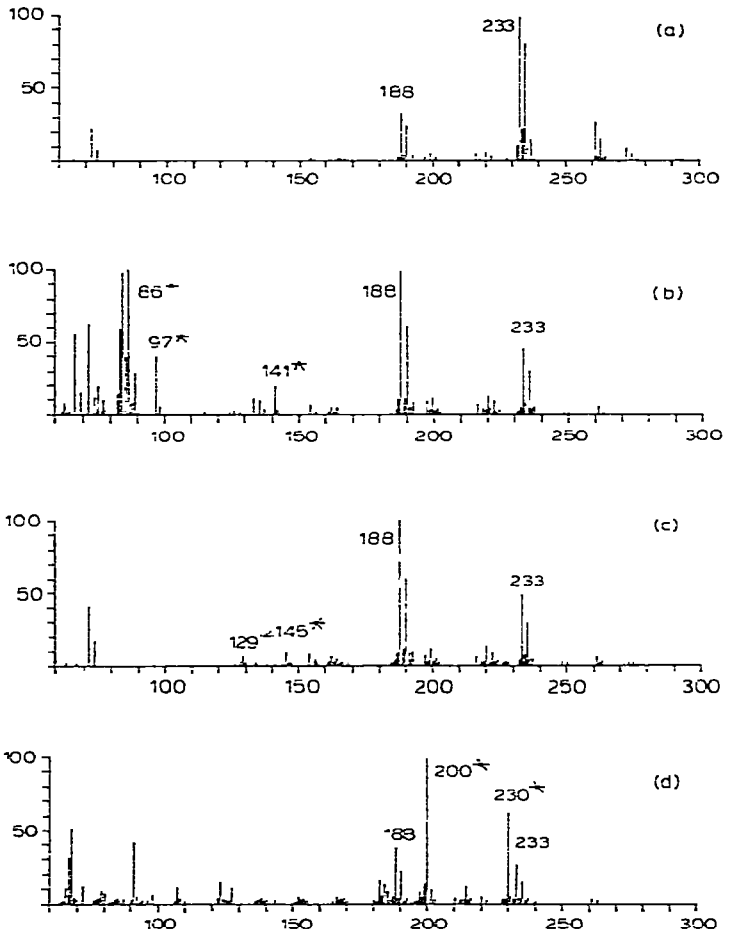


Fig. 6. (a) CI (CH_4) mass spectrum of diuron; (b) CI (CH_4) mass spectrum of diuron/C_{10}-alkylsulfate ion pair; (c) CI (CH_4) mass spectrum of diuron/naphthalenesulfonate ion pair; (d) CI (CH_4) mass spectrum of diuron/picrate ion pair. Spectra recorded with Finnigan 4000 mass spectrometer interfaced with Varian 5000 liquid chromatograph.

In our case, the flash vaporizer of the moving-belt interface serves the same function as the injection port of the gas chromatograph. The applicability of this methylation concept for HPLC–MS was tested with several long-chain fatty acids. The solutes were chromatographed as the free acids and the TMAH counter-ion was added after the HPLC column via a low dead-volume mixing tee. The mass spectrum of the methyl stearate formed from stearic acid is shown in Fig. 7 (chromatogram in the inset). Essentially complete conversion of the acid to the methyl ester is indicated in the spectrum. (Note, *e.g.*, the absence of any peak at *m/z* 284 from the free acid.) The spectrum thus obtained for the methyl ester derivative of the fatty acid solute is essentially identical to library reference spectra of methyl stearate. Contributions from the TMAH counter-ion are reflected in the peaks at *m/z* 121 $[\varnothing\text{-}N(CH_3)_2]^{+\cdot}$, *m/z* 120 $[121 - H]^+$, *m/z* 105 $[(\varnothing)(CH_3)N = CH_2]^+$ and $[105 - H]^-$. It should be pointed out that for complete esterification a 5–10-fold excess TMAH reagent is required. For solvents containing phosphate buffers —as was the case in our example— consumption of the reagent due to methylation of the phosphate has to be taken into consideration in estimating the reagent excess. In the example described here the TMAH reagent was added after the column. Clearly, TMAH can also be used directly as a counter-ion for reversed-phase HPLC in the ion-pair mode as well as for purposes of facilitating the solute extraction into a CH_2Cl_2 layer using the segmented flow interface. These aspects as well as other variations and applications of this alkylation process are currently under study.

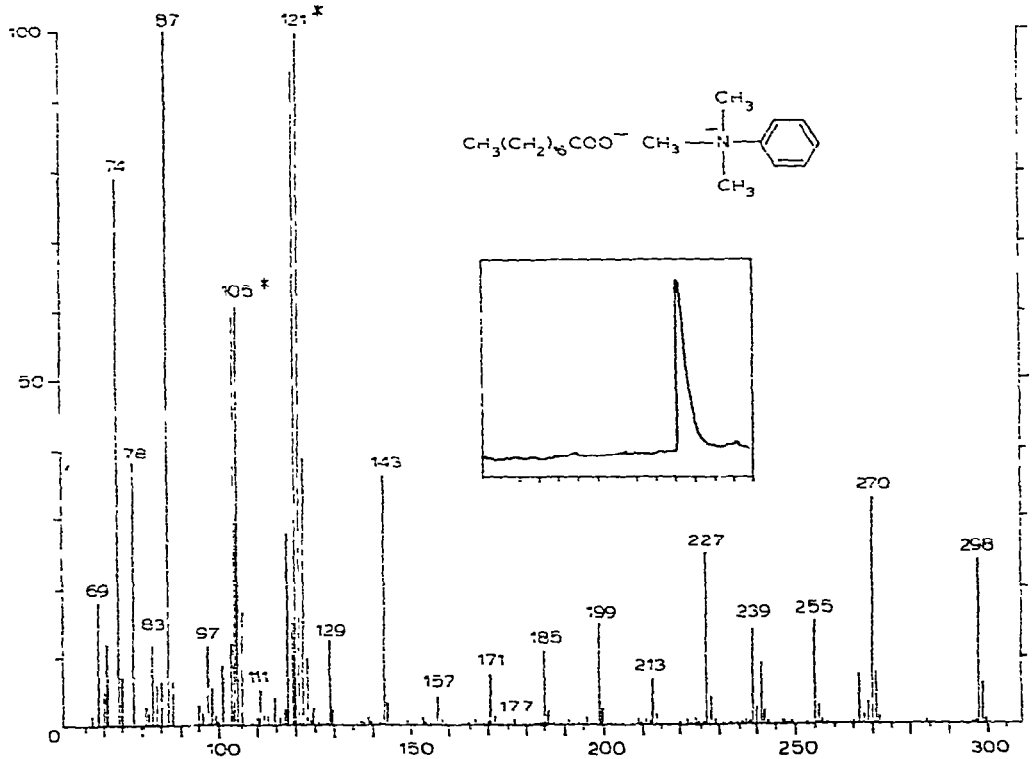


Fig. 7. EI mass spectrum of methylstearate obtained from the vaporization of stearic acid/trimethylanilinium hydroxide ion pair. Spectrum recorded with Finnigan 4000 mass spectrometer interfaced with Varian 5000 liquid chromatograph. Ion-pairing reagent added post-column. Chromatographic profile (molecular ion current) shown in inset.

4. CONCLUSIONS

The data presented above further demonstrate the utility and flexibility of ion-pairing techniques for carrying out combined HPLC–MS. Counter-ions can be selected according to the requirements imposed by the MS fragmentation of the solute and the specific needs of the analysis. For MS purpose these requirements are best met via the use of counter-ions with an aromatic structure which is most resistant to EI fragmentation. If the chromatography is conducted with the free solute, the ion pairing can be accomplished by post-column addition of a solution containing the most desirable counter-ion. Obviously, the counter-ion can be changed according to the requirements dictated by the MS detector. If, on the other hand, HPLC is being conducted in the ion-pair mode, a second counter-ion, more suitable for MS, can be added after the column to replace the original counter-ion, given the proper extraction constants. Alternatively, a polar bonded phase can be used in the reversed-phase ion-pair mode for direct introduction of the effluent into the on-line extractor[7].

Of the counter-ions examined in this study, the naphthalesulfonate and the picrate exhibit adequate hydrophobicity to provide for good extraction efficiency of the amine solutes examined. In addition, these counter-ions exhibit little fragmentation thus minimizing the probabilities for spectral overlap with different solutes. The obvious route to take for further improvement of the extraction characteristics of the counter-ion would be increasing its hydrophobicity. For example, in the case of the naphthalenesulfonate salts this can be readily accomplished via the use of a polymethyl-substituted naphthalene nucleus. The presence of several methyl groups should provide a system of improved solubility in organic solvents, without jeopardizing the MS requirements; it is expected that the peaks of m/z 144, 128 and 115 (see Table I) would be merely shifted by the appropriate mass units depending on the number of methyl groups present. Thus, if a variety of methylated naphthalenesulfonate counter-ions were available in sufficient purity, they could each be used selectively according to the analytical problem on hand. Of course, the same criteria can be applied to other systems which produce limited EI fragmentation, *e.g.*, anthracene.

Finally, the example of the TMAH demonstrates further the flexibility provided by the ion-pair derivatization of HPLC–MS. The transfer of a methyl group instead of hydrogen illustrated here opens the possibility for consideration of a variety of extractive alkylation techniques in conjunction with HPLC–MS[17]. It should be possible with judicious selection of pre- or post-column chemistry to increase the compatibility of HPLC with MS for more effective on-line coupling.

The incorporation of on-line chemical procedure between LC and MS thus provides practical approaches to the solution of a variety of analytical problems. Moreover, such approaches permit the optimization of LC separation procedures relatively independent of the specific mass spectrometer and interface. We are continuing our examination of various chemical procedures for such on-line LC–MS coupling.

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6. SUMMARY

In a continuation of earlier work, the use of ion-pairing reagents to increase the compatibility of high-performance liquid chromatography (LC) coupled with mass spectrometry (MS) is demonstrated. Counter-ions were selected for examination on the basis of the structural requirements for mass spectrometric fragmentation and the on-line extractability of their ion pairs with different solutes into organic solvents. The use of aromatic (*e.g.*, naphthalenesulfonate, picrate) instead of aliphatic (*e.g.*, *n*-decylsulfate) counter-ions shows a significant simplification of the resulting electron impact mass spectra of the solutes. Thus, structural information on the solutes can be easily obtained, especially when relevant peaks occur at lower *m/z* values. A second example of on-line chemical transformation for LC–MS is shown in the use of trimethylanilinium hydroxide for selective methylation. These results along with previous work, demonstrate practical approaches to enhance the on-line LC–MS method using the moving-belt interface.

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