Journal of Chromatography, 474 (1989) 149–162
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 423

DEVELOPMENT OF OPTIMIZATION STRATEGIES IN THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

C. E. M. HEEREMANS, R. A. M. VAN DER HOEVEN, W. M. A. NIESSEN*, U. R. TJADEN and J. VAN DER GREEF

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, University of Leiden, P.O. Box 9502, 2300 RA Leiden (The Netherlands)

SUMMARY

In order to optimize the sensitivity and reproducibility of thermospray liquid chromatography–mass spectrometry in (bio)analytical applications, some of the experimental parameters that influence thermospray buffer ionization have been investigated systematically.

Attention was paid to the vaporizer temperature, which is especially important in the analysis of thermolabile compounds, the ammonium acetate concentration, the methanol content and the repeller potential. General optimization strategies for thermospray buffer ionization have been developed. The usefulness of extensive optimization is discussed for qualitative and quantitative analysis. In quantitative target compound analysis optimization on the analyte is necessary. In qualitative analysis, however, usually unknown compounds have to be analysed and no parent compound is available for optimization purposes.

INTRODUCTION

The thermospray interface, developed by Blakley and Vestal[1], is a valuable analytical tool, which has given a strong impetus to developments in liquid chromatography–mass spectrometry (LC–MS). In many laboratories thermospray LC–MS is now routinely used and many interesting applications have been described. In our laboratory thermospray LC–MS is applied in both qualitative and quantitative bioanalysis. In the development of bioanalytical methods, the optimization of selectivity, sensitivity and reproducibility is of utmost importance. This paper deals with the optimization of thermospray LC–MS in the buffer ionization mode. Many interdependent experimental parameters are important in this respect: the mobile phase composition (amount and type of buffer and organic modifier), the solvent flow-rate, the design and temperature of the vaporizer, the design and temperature of the ion source, and the geometry, position and potential of the repeller electrode. Other important parameters, which are difficult to adjust reproducibly, are the spray performance, the condition of the vaporizer, and the degree of contamination of the repeller electrode and the ion source. Although the thermospray interface has been

0021-9673/89/$03.50

commercially available for five years now, little attention has been paid to a systematic investigation of the influence and optimization of the various experimental parameters. Such systematic studies are hampered by the fact that there are large differences in the design and performance of the various commercially available thermospray interfaces, for instance with respect to the type of vaporizer and the temperature that has to be applied for stable spray conditions. The optimization of the temperature of the vaporizer, the type and concentration of the buffer salt, and the modifier content of the LC mobile phase has been studied by Voyksner and Haney[3] for triazine herbicides and organophosphorus pesticides. The effects of the vaporizer temperature have been investigated for some drugs by Lindberg and Paulson[4]. The influence of the potential applied to the repeller electrode, positioned either opposite to the sampling cone or slightly downstream, has been studied by Lindberg and Paulson[4] and by Bencsath and Field[5]. Various other geometries of the repeller electrode, *e.g.* a needle tip, are currently under investigation (*cf.* ref. 6). Considerable effort has been put into the improvement of the thermospray vaporizer performance, especially with respect to the reproducibility[7–12]. Both the vaporizer design[7–10] and the thermospray controller[11,12] are under investigation.

In the present paper the influence of various parameters on the eluent and analyte signals and mass spectra have been investigated systematically with a Finnigan MAT TSP interface. The results of the variation of the vaporizer temperature, the repeller potential, the ammonium acetate concentration and the methanol content of the mobile phase are reported here. From the results an optimization strategy for thermospray LC–MS studies is suggested.

EXPERIMENTAL

Thermospray mass spectrometry

The solvents were delivered by a Model 2150 high-pressure pump (LKB, Bromma, Sweden). The flow-rate was 1.2 ml/min. Thermospray MS was performed on a Finnigan MAT TSQ 70 triple quadrupole mass spectrometer equipped with a Finnigan MAT TSP interface (Finnigan MAT, San José, CA, USA). For all experiments the block temperature was kept at 200°C.

Repeller potential variation/vaporizer temperature variation

The mobile phase consisted of 50 m*M* ammonium acetate in water–methanol (80:20, v/v). For the systematic investigation of the influence of the repeller potential on the eluent background spectrum, an instrument control procedure was written, which automatically increases the repeller potential from 0 to 200 V in 10 V increments. After each potential adjustment, a stabilization time of 2 s is applied. At each repeller potential ten scans (m/z 10–100) are acquired. For investigation of the influence of the repeller potential on the analyte signal the repeller potential was increased by 10 V before every injection. These experiments were repeated at several vaporizer temperatures in order to get information about the influence of the vaporizer temperature on the eluent background, the analyte signals and mass spectra.

Methanol content variation

The mobile phase consisted of 50 m*M* ammonium acetate in water–methanol

(v/v %) mixtures. The amount of methanol was increased from 0 to 80%, in 10% increments. The vaporizer temperature was decreased with increasing methanol content (*ca.* 5°C decrease for 10% methanol content increase). At every mobile phase composition the repeller potential was varied from 0 to 200 V.

Ammonium acetate concentration variation

The concentration of ammonium acetate in water–methanol (90:10, v/v) was increased from 10^{-5} to 10^{-2} *M*. For each experiment the buffer concentration was increased by an order of magnitude. The concentration of the analyte was *ca.* $2 \cdot 10^{-4}$ *M*. In this way spectra were obtained with ammonium acetate concentrations lower than, equal to, and higher than the analyte concentration. For every mobile phase composition the repeller potential was varied from 0 to 200 V. The vaporizer temperature was 115°C.

Samples

Standard solutions of analytes, dissolved in the mobile phase used in the described experiments, were injected (*ca.* 500 ng/20 μl) in the flow-injection analysis (FIA) mode, *i.e.* direct injection into the liquid stream without a chromatographic column. The analytes were obtained from various sources.

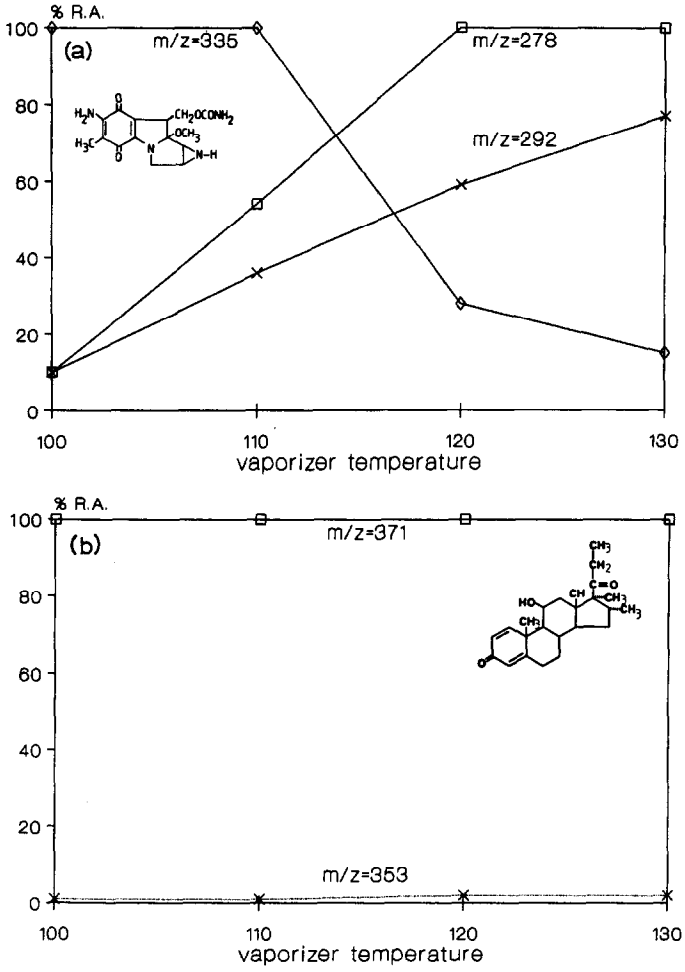
RESULTS AND DISCUSSION

Vaporizer

In the thermospray interface used, the vaporizer is a directly heated stainless-steel capillary. After installation of a new vaporizer a good spray is produced by squeezing the end of the capillary at ambient temperature and pressure. When a proper spray is established the thermospray interface can be made operational. The spray performance obviously is one of the parameters that cannot easily be reproduced. The use of apparently more reproducible systems with replaceable fused-silica capillaries or sapphire diaphragms has been suggested recently by Vestal[10].

The degree of vaporization of the eluent is an important experimental parameter, which depends on the composition of the mobile phase, the solvent flow-rate, and the vaporizer temperature. At a constant mobile phase composition and flow-rate, relatively stable signals can be obtained within a temperature range of *ca.* 30°C. Below this temperature range the spray is too wet and unstable signals are observed; above this range a dry spray is obtained and no signals are observed at all for the compounds studied. The vaporizer temperature is typically *ca.* 110°C for 1–1.5 ml/min of water–methanol mixtures, depending on the condition of the vaporizer (*e.g.* aperture of the capillary tip after squeezing, age and history of the capillary). The vaporizer temperature has to be adjusted for each combination of the mobile phase composition and the flow-rate in order to obtain stable background signals. Within the temperature range that provides relatively stable signals, optima in absolute intensities of the eluent and the analyte can be obtained. In some cases these optima coincide, as observed by others[3,4]. However, the gain in absolute intensities that can be achieved by optimization is very compound-dependent. In favourable cases a gain of an order of magnitude can be realized. The optimum vaporizer temperature for a particular analyte also depends on the condition of the vaporizer. Vaporizer temper-

ature optimization for a particular analyte may give different results on different days. This implies a limited usefulness of reporting "optimum" vaporizer temperatures. Apparently, the vaporizer temperature has a considerable influence on the mass spectra of thermolabile compounds. At high vaporizer temperatures these compounds will tend to decompose in the eluent stream[13]. The decomposition products can also



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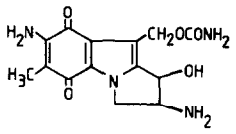

Fig. 1. Relative intensities of the protonated molecule and some fragments as a function of the vaporizer temperature (°C) for mitomycin C (MW = 334) (a) and rimexolone (MW = 370) (b). Repeller potential, 20 V; eluent, 50 m*M* ammonium acetate in water–methanol (80:20, v/v). (c) Structure of 2,7-diamino-1-hydroxy-mitosene.

be ionized in the ion source, resulting in a spectrum of both the analyte and its decomposition products. Real fragment peaks may also be observed, when such a process is thermodynamically favourable or when collisionally induced dissociation takes place in the source[14].

Fig. 1 shows the effect of the vaporizer temperature on the mass spectra of mitomycin C (MMC) and (11β,16α,17β)-11-hydroxy-16,17-dimethyl-17-(1-oxopropyl)androsta-1,4-dien-3-one (rimexolone), a thermolabile and a thermally stable compound, respectively. With increasing vaporizer temperature the relative abundance of the protonated molecule of the anti-cancer drug MMC (MW = 334) decreases compared with the fragment peaks at *m/z* 292 and 278. The peak at *m/z* 292 results from the loss of HNCO from the protonated molecule, which might take place both by thermal decomposition of the neutral and by fragmentation of the protonated molecule. The peak at *m/z* 278 is probably due to a loss of HNCO from the protonated molecule of a hydrolysis product of MMC, 2,7-diamino-1-hydroxymitosene (MW = 320, structure in Fig. 1c)[15], which is formed by cleavage of the methoxy group, resulting in an unsaturated bond and opening of the aziridine ring by addition of water. In this study, 2,7-diamino-1-hydroxymitosene obviously is a thermal hydrolysis product of MMC formed in the eluent stream, which after loss of HNCO, either by thermal decomposition of the neutral or fragmentation of the protonated species, results in a peak at *m/z* 278. In the thermospray mass spectrum of 2,7-diamino-1-hydroxymitosene, a peak at *m/z* 278, resulting from the loss of HNCO from the protonated molecule, is observed as well[6]. In the thermospray mass spectrum of MMC, a peak at *m/z* 321, due to the protonated molecule of 2,7-diamino-1-hydroxymitosene, is observed at low abundance. In the chemical ionization (CI) and desorption chemical ionization (DCI) spectra of MMC, a peak at *m/z* 292 is observed[17], whereas the peaks at *m/z* 321 and *m/z* 278 are absent. For a thermally stable compound, such as rimexolone (Fig. 1b), no influence of the vaporizer temperature is observed on the relative abundances of the protonated molecule at *m/z* 371 and the major fragment at *m/z* 353, resulting from a loss of water.

Solvent composition

Ammonium acetate concentration. The presence of a volatile buffer (50–100 m*M*), *e.g.* ammonium acetate, in the mobile phase is essential for thermospray buffer ionization[1]. The influence of the ammonium acetate concentration has been studied by Voyksner and Haney[3]. They report a strong dependence of the relative analyte signal in a concentration range between 10^{-3} and $8 \cdot 10^{-2}$ *M*, and a plateau is reached at higher concentrations. In the present experiments the buffer concentration was varied between 10^{-5} and 10^{-2} *M* in order to investigate the influence of the ammonium acetate concentration on the signal of 10^{-4} *M* of analyte. The results obtained are shown in Fig. 2 for caffeine. When the ammonium acetate concentration is ten times lower than the analyte concentration, the intensity of the protonated molecule of caffeine is very weak. Sodium and potassium cationized molecules are observed as the most intense signals. When the ammonium acetate concentration is equal to the analyte concentration, the protonated molecule becomes the most abundant peak, whereas with a buffer concentration ten times higher than the analyte concentration a large increase (three orders of magnitude) in the absolute intensity of the protonated molecule is observed. The absolute intensity of the ammoniated molecule increases by

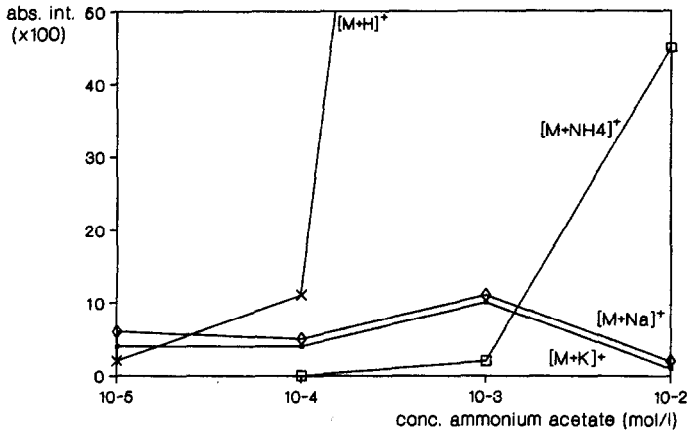

Fig. 2. Response of the protonated, ammoniated and sodium and potassium cationized molecules of caffeine ($2 \cdot 10^{-4}$ *M*) as a function of the ammonium acetate concentration. Repeller potential, 50 V; eluent, ammonium acetate in water–methanol (90:10, v/v).

two orders of magnitude in the buffer range studied, whereas the intensities of the sodium and potassium cationized molecules remain constant.

Methanol content. Because in bioanalysis gradient high-performance liquid chromatographic (HPLC) runs often have to be performed with the organic modifier content of the mobile phase changing over a wide range, *e.g.* from 0 to above 50%, the influence of the methanol content on the analyte intensities and mass spectra has been investigated. With increasing methanol content the vaporizer temperature has to be lowered to obtain similar spray conditions, because of the higher volatility of the mobile phase with increasing methanol content. When no methanol is present in the mobile phase the highest sensitivity is obtained, which is in accordance with the results, reported by others (*cf.* ref. 3). For the test compounds (adenosine, caffeine and coniferic aldehyde) a slight decrease is observed with increasing methanol content from 0 to 40%. When the methanol content exceeds 40%, the sensitivity drops significantly. External ionization sources, filament or discharge, are needed at these modifier contents.

Repeller potential

Eluent. In the thermospray source design used in these experiments, a flat circular tip repeller electrode is placed opposite to the sampling cone. Significant influence of the repeller potential on the intensity and the appearance of the mass spectrum of the eluent cluster ions has been reported[4]. The repeller potential influences both the total ion current and the abundance of the various species. The ions observed depend on the mobile phase. In Tables I and II compositions of the various ions observed with two different eluents are proposed. Fig. 3 shows the influence of the repeller potential variation on the total ion current (*m/z* 10–100) and on the intensities of some of the more abundant ions for 50 m*M* ammonium acetate in water. Similar plots for 50 m*M* ammonium acetate in water–methanol (20:80, v/v) are given in Fig. 4. The plots of the total ion current and the intensities of the various ions as a function of the repeller potential differ from the profiles reported by Lindberg and

TABLE I

COMPOSITION OF THE VARIOUS POSITIVE IONS OBSERVED IN THERMOSPRAY BUFFER IONIZATION WITH 50 m*M* AMMONIUM ACETATE IN WATER

Asterisk indicates present in the given repeller potential region.

m/z	*Composition*	*Repeller potential < 100 V*	*Repeller potential > 100 V*
15	$[CH_3]^+$		*
18	$[NH_4]^+$	*	
19	$[H_3O]^+$		*
33	$[H_2O + CH_3]^+$		*
35	$[2NH_3 + H]^+$	*	
36	$[NH_3 + H_2O + H]^+$	*	
37	$[2H_2O + H]^+$		*
43	$[CH_3CO]^+$		*
51	$[2H_2O + CH_3]^+$		*
53	$[2NH_3 + H_2O + H]^+$	*	
54	$[NH_3 + 2H_2O + H]^+$	*	
55	$[3H_2O + H]^+$		*
61	$[H_2O + CH_3CO]^+$		*
73	$[4H_2O + H]^+$		*
77	$[2NH_3 + CH_3CO]^+$	*	
78	$[NH_3 + H_2O + CH_3CO]^+$	*	
79	$[2H_2O + CH_3CO]^+$		*
95	$[2NH_3 + H_2O + CH_3CO]^+$	*	
96	$[NH_3 + 2H_2O + CH_3CO]^+$	*	
97	$[3H_2O + CH_3CO]^+$		*

Paulson[4]. The potentials at which the highest intensities for the various ions are observed are lower in our experiments. These differences cannot be attributed to the difference in solvent composition (43% methanol in ref. 4 and 20% methanol in this study).

A closer look at the various cluster ions reveals some interesting features. Clusters containing NH_3 are observed only at repeller potentials below 100 V for both mobile phases. The NH_4^+ ion (*m/z* 18) is most intense at *ca.* 100 V. Clusters containing H_2O and no NH_3 are observed only at repeller potentials above 100 V. For the mobile phase containing 20% methanol, intense methanol clusters are observed over the whole range of repeller potentials. Protonated methanol clusters ($[CH_3OH_2]^+$, $[2CH_3OH + H]^+$ and $[3CH_3OH + H]^+$) are observed at high potential. Methanol clusters containing NH_3 are observed at low potential, whereas methanol clusters containing H_2O are observed at high potential. A nice illustration of the changeover in cluster composition from NH_3-containing clusters to H_2O-containing clusters with increasing repeller potential is given in Fig. 5, where the intensities of *m/z* 77 $[2NH_3 + CH_3CO]^+$, *m/z* 78 $[NH_3 + H_2O + CH_3CO]^+$ and *m/z* 79 $[2H_2O + CH_3CO]^+$) are plotted as a function of the repeller potential. The NH_3-containing clusters at *m/z* 77 and 78 have a maximum intensity at low repeller potential, whereas the H_2O-containing cluster at *m/z* 79 is observed at high repeller potential. Similar effects have been observed for other clusters, such as *m/z* 35, 36 and 37, *m/z* 53, 54 and 55, and *m/z* 95, 96 and 97. Another interesting feature, also illustrated in Fig. 5, is observed for the

TABLE II

COMPOSITION OF THE VARIOUS POSITIVE IONS OBSERVED IN THERMOSPRAY BUFFER IONIZATION WITH 50 m*M* AMMONIUM ACETATE IN WATER–METHANOL (80:20, v/v)

Asterisk indicates present in the given repeller potential region.

m/z	*Composition*	*Repeller potential < 100 V*	*Repeller potential > 100 V*
15	$[CH_3]^+$		*
18	$[NH_4]^+$	*	
19	$[H_3O]^+$		*
31	$[CH_2{=}OH]^+$		*
33	$[CH_3OH + H]^+$		*
35	$[2NH_3 + H]^+$	*	
36	$[NH_3 + H_2O + H]^+$	*	
43	$[CH_3CO]^+$		*
47	$[CH_3{-}O{-}CH_3 + H]^+$		*
50	$[CH_3OH + NH_3 + H]^+$	*	
51	$[CH_3OH + H_2O + H]^+$		*
54	$[NH_3 + 2H_2O + H]^+$	*	
60	$[NH_3 + CH_3CO]^+$	*	
61	$[H_2O + CH_3CO]^+$		*
65	$[2CH_3OH + H]^+$		*
68	$[CH_3OH + NH_3 + H_2O + H]^+$	*	
75	$[CH_3OH + CH_3CO]^+$		*
77	$[2NH_3 + CH_3CO]^+$	*	
78	$[NH_3 + H_2O + CH_3CO]^+$	*	
79	$[2H_2O + CH_3CO]^+$ $[3CH_3OH - H_2O + H]^+$		*
82	$[2CH_3OH + NH_3 + H]^+$	*	
83	$[2CH_3OH + H_2O + H]^+$		*
96	$[NH_3 + 2H_2O + CH_3CO]^+$	*	
97	$[3H_2O + CH_3CO]^+$ $[3CH_3OH + H]^+$		*

clusters at *m/z* 43 $[CH_3CO]^+$, 61 $[H_2O + CH_3CO]^+$ and 79 $[2H_2O + CH_3CO]+$. It appears that the larger clusters fragment at higher repeller potentials. The cluster at *m/z* 79, containing two water molecules, consecutively loses the two water molecules with increasing repeller potential, resulting in clusters at *m/z* 61 and 43. More detailed discussions on these probably collisionally induced effects will be reported elsewhere[18].

The above-described instrument control procedure can also be used as a diagnostic tool to ascertain a good instrument performance. When no response of the total ion current of the solvent background is observed as a result of the increasing repeller potential, either the vaporizer spray performance has to be checked, or the repeller electrode and the ion source are seriously contaminated. Cleaning of the repeller electrode and the ion source has to be performed about every two weeks, which is obviously dependent on the nature of the samples.

The analyte. The influence of the repeller potential on the analyte signal has been investigated by means of repetitive injection at various repeller potentials between 0 and 200 V. With repeller potentials between 0 and 100 V not much influence

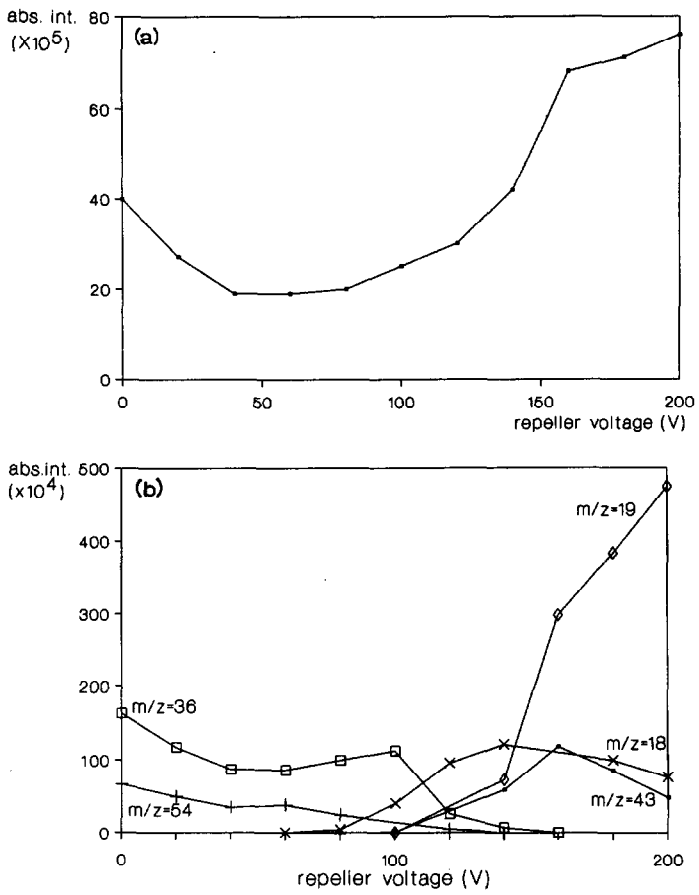

Fig. 3. Influence of the repeller potential on the total ion current (a) and on the intensities of the more abundant solvent cluster ions (*m/z* 18, 19, 36, 43, 54) (b) for 50 m*M* ammonium acetate in water. Vaporizer temperature, 120°C.

on the analyte signal is observed. Flat optima, which are to some extent compound-dependent, are found in plots of intensity *versus* repeller potential (*cf.* for instance the signal obtained for the protonated molecule of adenosine at *m/z* 268 with various repeller potentials given in Fig. 6). Above 100 V the signal drops significantly, and it decreases further with increasing potential. This repeller potential range, in which the analyte signals reach an optimum in absolute intensity, coincides with the repeller potential range in which the NH_3-containing solvent clusters have their optimum intensity. Apparently, proton transfer from the ammoniated clusters is an important contribution in the mechanism of thermospray buffer ionization. This is also in agreement with the results obtained in the studies on the influence of the ammonium acetate concentration on the analyte signals, which are described above. The potential at which the signal drops is shifted to higher repeller potential when the repeller electrode becomes contaminated. This may be attributed to changes in the effective potential. The various compounds that were investigated in these experiments, adenosine, MMC and rimexolone, reach their optimum response at similar repeller po-

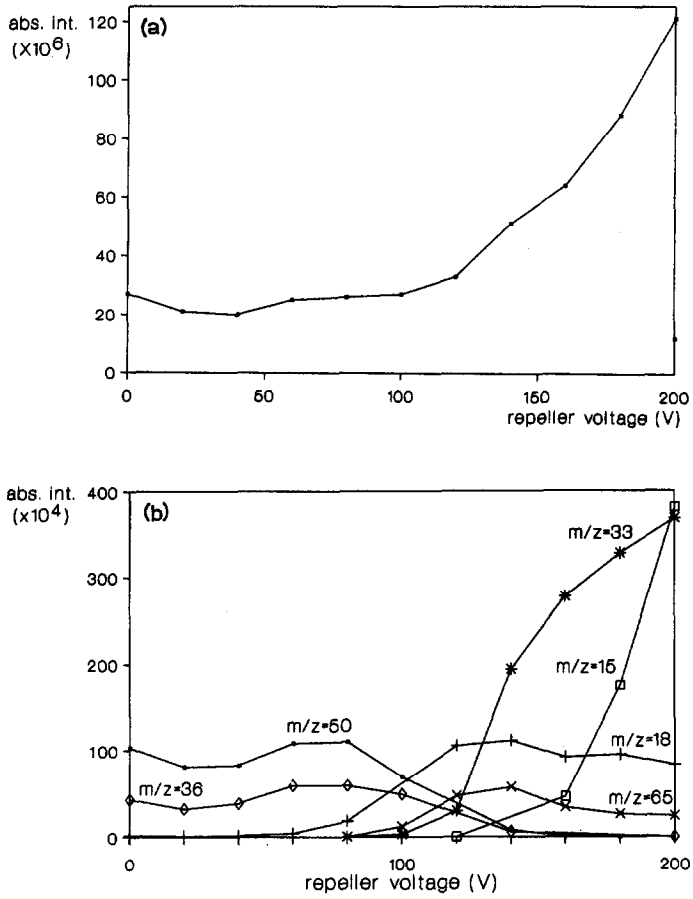

Fig. 4. Influence of the repeller potential on the total ion current (a) and on the intensities of the more abundant solvent cluster ions (*m/z* 15, 18, 33, 36, 50, 65) (b) for 50 m*M* ammonium acetate in water–methanol (20:80, v/v). Vaporizer temperature, 110°C.

tentials. This is in accordance with the results reported by Lindberg and Paulson[4]. However, the optimum repeller potentials observed in the present study are significantly lower than those reported elsewhere[4]. Thus, while the trends observed are similar, the actual potentials differ considerably between these two studies.

When the thermospray interface is used in discharge-on mode, high repeller potentials can induce significant fragmentation of the analytes[14]. Using thermospray buffer ionization these effects are usually not observed. However, for some compounds, differences in the mass spectra are observed when the repeller potential is varied. For instance for heptabarbital (Fig. 7) at low repeller potential an intense ammoniated molecule (*m/z* 268) is observed as well as a peak at *m/z* 285, which might be due to $[M + 2NH_3 + H]^+$; no fragmentation occurs. At high repeller potential the protonated molecule (*m/z* 251) becomes the base peak and an intense fragment (*m/z* 157) corresponding to the loss of the heptenyl-group is observed. Similar effects were observed by others for some other compounds (*cf.* ref. 4).

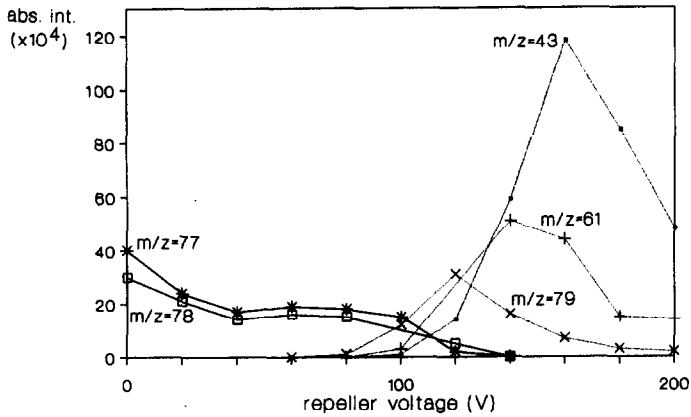

Fig. 5. Response of some solvent cluster ions (*m/z* 43, 61, 77, 78, 79) as a function of the repeller potential for 50 m*M* ammonium acetate in water. Vaporizer temperature, 120°C.

Optimization strategies

As a result of the described systematic studies, some general guidelines for optimization of thermospray buffer ionization have been developed. A clean ion source and repeller electrode are always necessary for the best results. When ammonium acetate concentrations between 50 and 100 m*M* are used, the buffer concentration generally will be higher than the analyte concentration and the highest sensitivity will be obtained. Gradient runs, with the methanol content of the mobile phase ranging from 0 to 40% for example, can easily be performed in combination with the thermospray buffer ionization mode. Above a methanol content of 40% additional sources of ionization are needed. These guidelines can be applied in both qualitative and quantitative analysis with thermospray LC–MS. In general, the usefulness of exten-

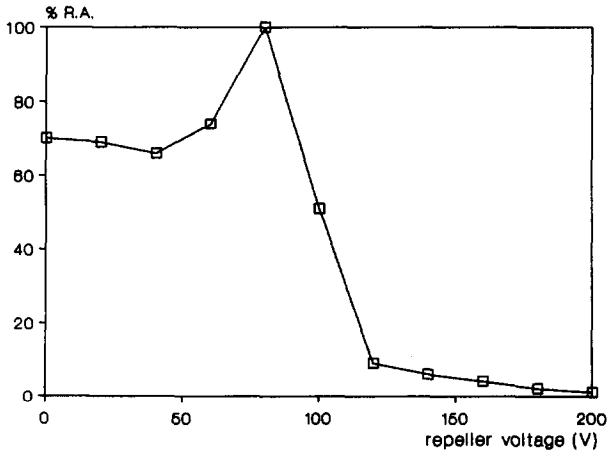

Fig. 6. Relative abundances of the protonated molecule of adenosine (MW = 267) as a function of the repeller potential obtained in the FIA mode. Vaporizer temperature, 120°C; eluent, 50 m*M* ammonium acetate in water–methanol (80:20, v/v).

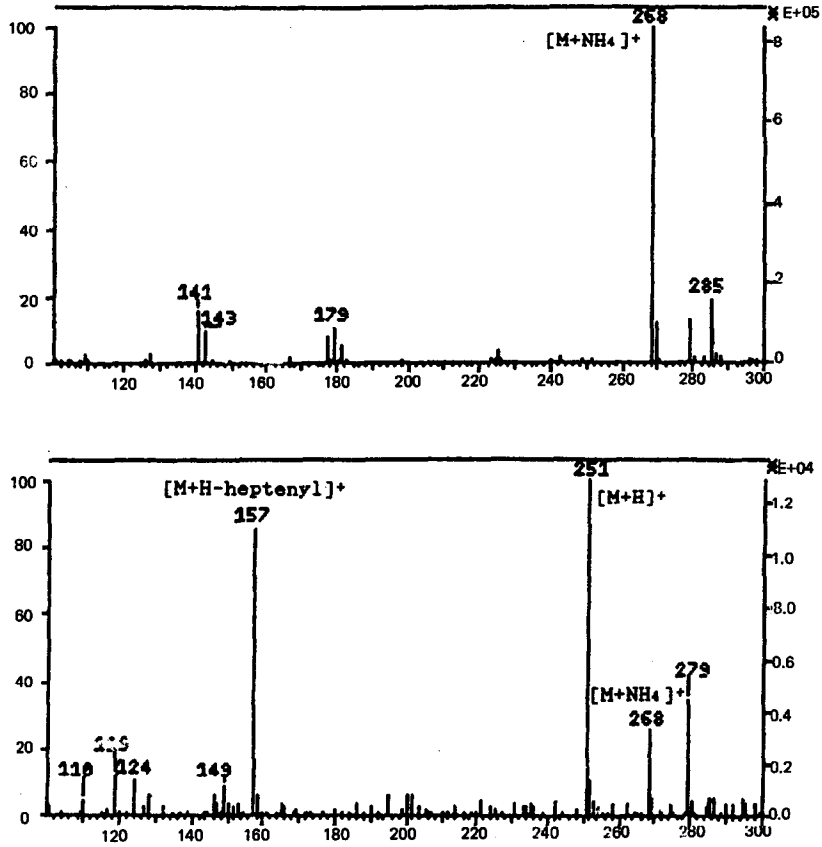

Fig. 7. Two thermospray mass spectra of heptabarbital (MW = 250) obtained at a repeller potential of 50 V (a) and 160 V (b). Vaporizer temperature, 120°C; eluent, 50 m*M* ammonium acetate in water–methanol (60:40, v/v).

sive optimization strategies for thermospray buffer ionization can be questioned. Many applications of thermospray LC–MS are concerned with qualitative analysis, *i.e.* confirmation or identification of (unknown) compounds. In some cases optimization on one of the constituents of the sample mixtures to be analysed can be useful, but in other cases the various components can show widely differing thermospray behaviour. An example of the former case is the analysis of desulphoglucosinolates, in which for example the readily available sinigrin can be used for optimization of the vaporizer temperature and the repeller potential. Other desulphoglucosinolates show similar behaviour under thermospray buffer ionization conditions, especially with respect to their thermolability[19]. An example of the latter case is the thermospray LC–MS analysis of metabolites and degradation products of mitomycin C, where the parent compound is highly sensitive to the vaporizer temperature, because of its thermolability, whereas the other products are hardly influenced at all by the vaporizer temperature[16]. In many applications, however, parent compounds are not available for optimization purposes.

The chromatographic conditions are another aspect of importance in optimiza-

tion of the analysis. Quite often the mobile phase used for the HPLC separation is not compatible with on-line thermospray LC–MS, especially because of the use of non-volatile buffers in the mobile phase. In some favourable cases the non-volatile buffers can be replaced by volatile ones without influence on the separation. In other cases the buffers are merely present for reasons of reproducibility of the retention times and can be replaced easily by volatile ones for qualitative analysis. In many applications with mixtures, correspondence of the peaks obtained with UV or fluorescence detection and the peaks observed with MS detection is obligatory. Changes in the mobile phase are not attractive in such cases. Flow-rate incompatibility, which is also encountered in practice, can be easily overcome, either by using higher flow-rates through the analytical column, which is sometimes acceptable because of the high selectivity of the mass spectrometer, or by post-column addition of buffer[20]. In general optimization for maximum intensity with direct FIA injections of (complex) mixtures can be considered as well. This approach may give some indication of the influence of the vaporizer temperature and repeller potential for some major compounds in the mixture. The highly analyte-dependent response has probably to be accepted as a fact in the analysis of complex mixtures.

The optimization strategies are more useful and applicable in quantitative analysis, especially in target compound analysis. In order to obtain the highest sensitivity and the lowest detection limits the thermospray MS system has to be optimized for each compound or group of compounds along the lines drawn in the previous sections of this paper. This optimization has to be repeated before every experimental series, because of the unpredictable influence of contamination and vaporizer condition, or even better, isotopically labelled internal standards should be used.

For some compounds the pH of the mobile phase might be an important parameter as well, because it appears that it is not only the gas-phase ion–molecule reactions that are of importance in thermospray buffer ionization but the solvent chemistry as well[21–23]. Some systematic studies on the influence of the pH of the mobile phase and the pK_a values of the analytes on the analyte signals have been described[21,24,25], but the results have been ambiguous. Systematic pH variations of the mobile phase are currently under investigation in our laboratory, and the results will be reported in due course.

CONCLUSIONS

Various experimental parameters influencing thermospray buffer ionization have been investigated systematically and as a result optimization strategies are suggested. Extensive optimization is not always very useful and in many cases, *e.g.* when dealing with unknown compounds, optimization for a certain analyte is not possible at all. For these cases some general guidelines for optimization are given.

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