Journal of Chromatography, 554 (1991) 125–140
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2360

On-line derivatization of eluted substances in dynamic high-performance liquid chromatography–mass spectrometry through the particle-beam interface

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ABSTRACT

Derivatization reagents were added through the particle beam high-performance liquid chromatograph–mass spectrometer interface in order to improve the detectability of polar compounds separated by high-performance liquid chromatography and detected by mass spectrometry by formation of the corresponding derivative. Mainly qualitative aspects are considered in this work. Dynamic derivatization in the gas phase of several substances containing carboxylic, sulphonic, hydroxy and other acidic or basic functional groups is described.

INTRODUCTION

Since the development of the first high-performance liquid chromatographic–mass spectrometric (HPLC–MS) interface based on the monodispersed areosol technique [1], a number of improvements have been achieved by modifying the geometry of the nebulizer and of the design of the momentum separator. Although the latest developments of the particle-beam HPLC–MS interface allow its use for a wide range of applications, *e.g.*, to compounds of higher polarity such as acids, bases and sulphonic acids, in principle derivatization may extend its applicability to a larger number of compounds by producing derivatives of higher volatility.

Another aspect of the applicability of the particle-beam HPLC–MS interface is the observation that high concentrations of water present in the mobile phase do not favour the detectability of the analytes. This seems to be related to the efficiency of both the nebulization and desolvation processes. This paper considers the possibility of dynamically derivatizing certain types of substances in the vapour or gas phase as they fly through the particle beam interface into the ion source of the mass spectrometer.

The objective of this work was the examination of the following aspects: to improve the volatility of some polar substances that are hardly brought into the vapour phase by the heat generated by the ion source; to provide additional qualitative information about the eluting compounds by examination of the spectra of the derivatized analytes; to improve the detectability of the sample by bringing the ions of

0021-9673/91/$03.50

interest into a higher mass range where the matrix does not interfere; to study the effect of transforming water as a mobile phase flowing through the nebulizer or at the outlet from the particle beam interface, in order to improve the operation of the particle-beam interface at high water concentrations of the mobile phase; and possibly to improve the detectability of a substance by the formation of a polyfluoro derivative and the use of chemical ionization and detection of negative ions.

A few common derivatization procedures were considered, as follows.

Silylation

This is one of the most widely used derivatization procedures in gas chromatography. Silyl derivatives are generally more volatile, less polar and more thermally stable than their parent compounds. Mass spectra obtained by electron impact and chemical ionization present intense ions in the higher mass range, often out of the region of the background.

Many compounds are completely derivatized as soon as they come into contact with the reagent. This is especially true for monofunctional molecules, where an active hydrogen is replaced with an alkylsilyl group, typically trimethylsilyl. Polyfunctional groups in terms of the number of active hydrogens or sterically hindered groups may react only partially. Silylation is a well known and common derivatization technique in the analysis of acids, alcohols, phenols, amines (primary and secondary), carbohydrates and some inorganic ions. The substances are derivatized in the liquid phase, and generally the reaction is complete rapidly under mild conditions. Less explored is derivatization into the vapour phase, as happens in an on-line configuration through the particle-beam HPLC–MS interface, as described.

Moreover, when the reagent is present in large excess, in the source of the mass spectrometer, additional ion–molecule reactions can occur in the gas phase inside the ion source.

Reagents used for this work are the well known trimethylchlorosilane (TMCS) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) [molecular weight (MW) 257; b.p. 146°C or 40°C at 12 mmHg]. The reaction generates product ions and by-products following the general reaction scheme

$$\underset{\text{(MW 257)}}{CF_3CON[Si(CH_3)_3]_2} + HOR \rightarrow \underset{\text{(if R = H, MW 90)}}{(CH_3)_3SiOR} + \underset{\text{(MW 185)}}{CF_3CONHSi(CH_3)_3}$$

Acylation

Fluorinated anhydrides are widely used acylating reagents for alcohols, phenols and amines and produce stable, highly volatile derivatives. The presence of fluorine makes them particularly interesting for electron-capture detection and negative-ion detection in mass spectrometry. Trifluoroacetic anhydride (TFAA) is the most volatile, and it can be easily brought into the particle beam interface in the vapour phase. N-Methylbistrifluoroactamide (MBTFA) does not produce acidic by-products and can be more conveniently used for derivatizing phenols and alcohols [2]. The pentafluoropropionic (PFPAA) derivatives could provide more fluorine atoms for electron capture by chemical ionization and negative-ion detection [3].

There are several acylating procedures in the liquid phase, which usually involve heating at 60–100°C for 15–60 min and eventually washing the organic phase

with water [4] or methods specially designed for the derivatization of sugars [5] or catecholamines [6].

Less well known are acylation reactions in the gas phase, induced by heat and ionization. "On-line" combination in the gas phase through the particle beam interface allows these types of chemical reactions to be studied under particular conditions of vacuum and plasma. Hydrolysis of the anhydrides and the formation of derivatives and adducts can be observed in the mass spectra.

In this work, only TFAA and PFPAA were tested as acylating reagents.

Use of water scavangers and modifiers

2,2-Dimethoxypropane (DMP) is used as a water scavanger for some alkylation reactions as for the formation of butylboronate derivatives. DMP converts water into organic compounds. Some tests have been done using the particle-beam interface by adding a certain amount of DMP in the gas phase, together with the helium flow in the nebulizer section, with the objective of transforming water-rich effluents chemically into a more rich organic phase, and verifying changes in the detectability of the eluted substances.

EXPERIMENTAL

Reagents

TMCS and BSTFA were used for the experiments in which silylation was involved. Acylation was performed with TFAA and PFPAA. DMP was used as a water scavenger. All these reagents were supplied by Supelco (Bellefonte, PA, USA).

Acetonitrile, methanol and water, all of HPLC grade, were supplied by Merck, Darmstadt, Germany. Water was quartz distilled twice before use.

Addition of derivatization reagents through the PB interface

The addition of derivatization reagents into the particle beam interface was performed as follows (see Fig. 1): through a tee-piece before the nebulizer (modifier acetonitrile) at position (a); together with the helium flow at position (b) (modifier DMP); and at the inlet of the gas for chemical ionization at position (d), together or without the methane reactant gas. For some preliminary tests not reported here the reagent was placed in the bulb that is normally used for the calibrant (perfluorotributylamine) of the mass spectrometer when this is used in the HPLC–MS mode. The

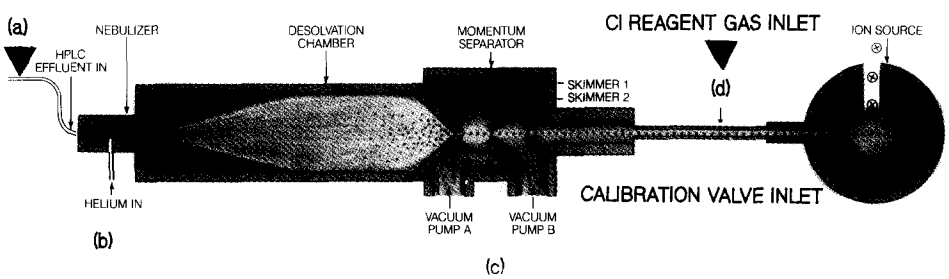

Fig. 1. Schematic representation of the particle beam HPLC–MS interface and locations (a–d) where derivatization reagents can be added.

point of addition is at position (d). The advantage is that these is no need for modification of the standard hardware, but the control of the amount of derivatization reagent flowing into the source is more critical.

The series of experiments performed are summarized in Table I.

TABLE I
DERIVATIZATION REAGENTS USED AND LOCATION OF ADDITION THROUGH THE PARTICLE BEAM INTERFACE

Derivatization method	Derivatization reagent	Location (see Fig. 1)	Remarks
Silylation	TMCS, BSTFA	(d)	Used for steroids, sugars, carboxylates, sulphonates, polyfunctional compounds. Chemical ionization has been used
Acylation	TFAA, PFPAA	(d)	Used for steroids. Chemical ionization has been used
Water scavenger	DMP	(b)	Experiments on nebulization process. Electron impact has been used
Mobile phase modifier	Acetonitrile	(a)	Increase percentage of organic solvent in the mobile phase for better detectability

On-line silylation and acylation

At the outlet of the particle-beam interface [position (d) in Fig. 1], a mixture of methane and silylating or acylating reagent was added. The total pressure into the ion source was 130 Pa with a ratio of methane to reagent of about 1:1. These are the conditions under which most of the actual work has been done and the majority of the results have been obtained. A necessary modification to the standard hardware is the addition of a tee-piece and a bulb containing the derivatization reagent with a flow regulator, on the line that is normally used for the chemical ionization reactant gas. This minor modification can be retained even for the usual HPLC–MS work, as the reactant can be isolated from the flow line by shutting off the flow regulator.

The analyte elutes from the HPLC column into the particle-beam interface and at position (d) (Fig. 1) comes into contact with the derivatization reagent. Note that, at this point, most of the mobile phase has been already removed by the nomentum separator.

The amount of analytes injected using this instrumental configuration ranged from 100 to 1000 ng. Separation through the HPLC column was performed for the analysis of steroids and, in some instances, for diuretics. For the other components flow injection was used.

Addition of a water scavanger at the nebulizer

In order to acquire a better knowledge of the nebulization process and consider the reasons why the presence of high concentrations of water in the mobile phase can decrease the performance of the transport process of substances through the interface, the helium flow that sustains the nebulization was doped with DMP as a water scavenger.

Modification is required to the standard hardware, *i.e.*, a Hewlett-Packard (Palo Alto, CA, USA) solvent-delivery system, part number 05985-60238, which is part of the kit of the previous direct liquid introduction HPLC–MS interface, is installed and connected to the line of the helium flow. The helium bubbles into the liquid DMP, or other modifier, and flows into the nebulizer. The helium flow-rate is about 2 l/min and the DMP flow-rate, vaporized into the helium, corresponds to about 2 ml/min of liquid unter these conditions.

The experiment consisted in injecting repeatedly 2 μl of a 50 ng/μl solution of benzidine in acetonitrile, bypassing the analytical column, and gradually changing the composition of the mobile phase from 100% acetonitrile to 100% water without readjusting the position of the fused-silica tubing of the nebulizer. Moreover, the helium flow for the nebulizer was bubbled through a bomb containing about 20 ml of water scavenger. During this test, the presence of DMP was monitored, in addition to the benzidine signal. In particular, the relative intensity of the signal of the molecular ion of benzidine at m/z 184 indicates how well this substance is transported through the interface by changing the composition of the mobile phase.

The experiment lasted about 20 min before all the water scavenger had been consumed. Hence it can be calculated that the flow of DMP into the nebulizer area was ca. 1 ml/min. The signal of benzidine was monitored under electron impact conditions, scanning from 45 to 220 u at 2 scans/s, and plotting the extracted ion chromatogram for the molecular ion at m/z 184.

HPLC–MS combination

An HP 1090L liquid chromatograph (Hewlett-Packard) equipped with a dual pumping system, variable-volume injector and diode-array detector was used for all tests. An additional HP 1050 pump (Hewlett-Packard) was occassionally used to add modifiers at the end of the HPLC column.

Model 59980B particle-beam interfaces (LC–PB-MS) from Hewlett-Packard were used. The experiments were conducted at a desolvation temperature of typically 50°C and a helium head pressure of 300 kPa.

Several experiments were repeated and reconfirmed using two different mass spectrometers: an HP 5988A and an HP 5989A (Hewlett-Packard). The ion source was maintained at 250°C and the quadrupole housing at 100°C.

Chemical ionization (CI) was used for all tests except those for which a modifier (acetonitrile or DMP) was added at the nebulizer (see Table I), when the electron impact mode was used. For the CI experiments methane was used both as reagent gas for chemical ionization and as a "carrier" of the derivatizing reagents. The total pressure into the ion source was 130 Pa.

Chromatographic conditions

For the separation of steroids, a stainless-steel column (100 × 2.1 mm I.D.) from Hewlett-Packard packed with 5-μm Hypersil ODS was used with a gradient of water–methanol from 40:60 to 1:99 (v/v) in 30 min at a flow-rate of 0.4 ml/min.

For the analysis of diuretics, the mobile phase was water–acetonitrile–formic acid (55:45:0.5, v/v/v). Flow-injection analysis bypassing the column was occasionally performed. Chromatographic analysis was done with a 25 × 2.1 mm I.D. stainless-steel column from Hewlett-Packard packed with 5-μm Hypersil ODS. The flow-rate was 0.4 ml/min.

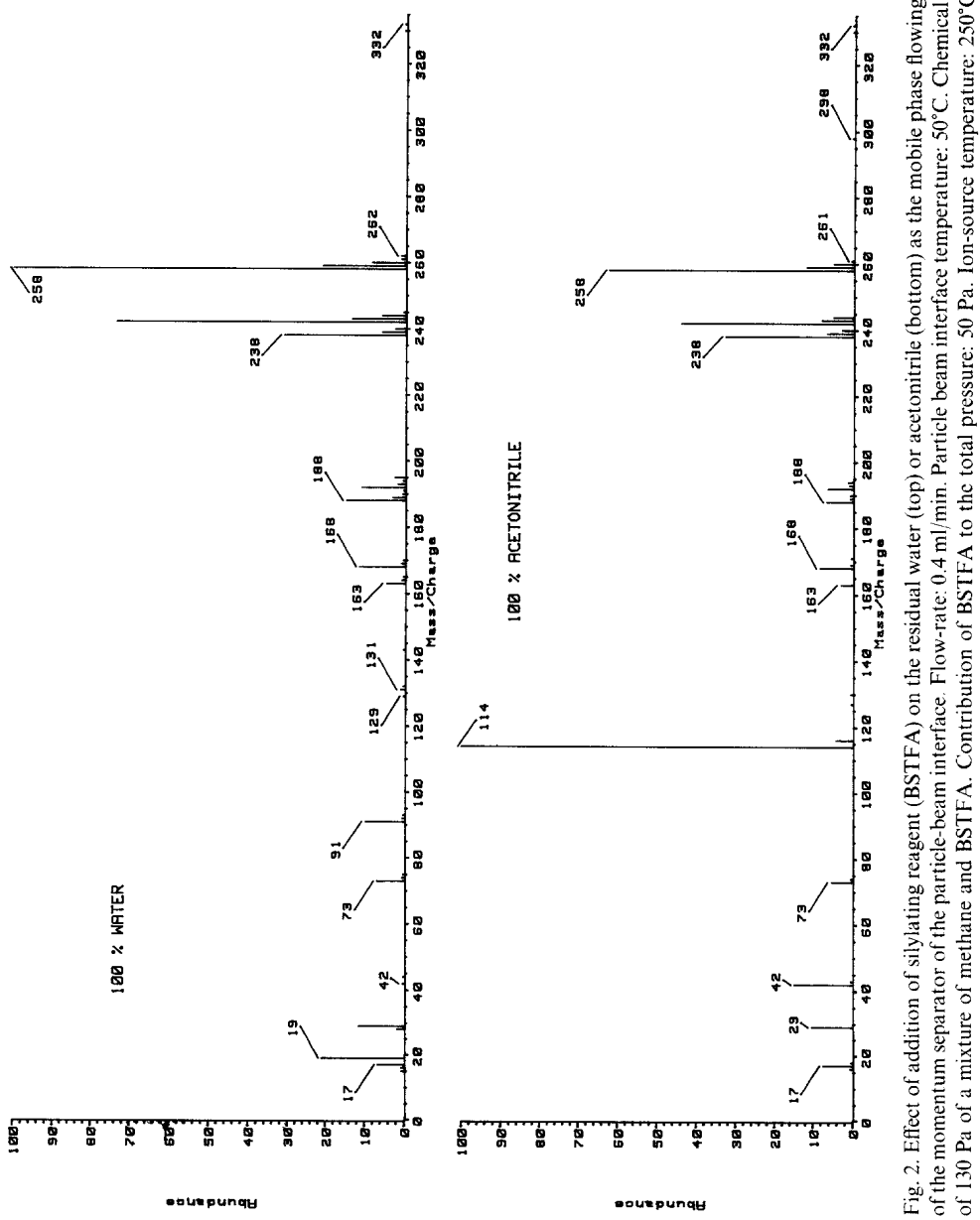

Fig. 2. Effect of addition of silylating reagent (BSTFA) on the residual water (top) or acetonitrile (bottom) as the mobile phase flowing from the HPLC column out of the momentum separator of the particle-beam interface. Flow-rate: 0.4 ml/min. Particle beam interface temperature: 50°C. Chemical ionization at a total pressure of 130 Pa of a mixture of methane and BSTFA. Contribution of BSTFA to the total pressure: 50 Pa. Ion-source temperature: 250°C.

Flow injection bypassing the column was used for the other substances analysed, using methanol as mobile phase at a flow-rate of 0.4 ml/min.

RESULTS

Derivatization with TMCS and BSTFA: effects on the mobile phase

In order to evaluate the effectiveness of derivatization, two parameters were changed initially: solvent composition, from 100% water to 100% acetonitrile; and the amount of derivatization reagent flowing into the mass spectrometer from position (d).

The spectra obtained by using a relatively small amount of BSTFA and water as mobile phase show that under chemical ionization conditions m/z 19 is still the base peak (protonated water), while m/z 91 appears at low intensity as protonated monohydroxytrimethylsilane. An ion at m/z 258 appears as protonated BSTFA.

In a similar manner, for acetonitrile as mobile phase, m/z 42 can be observed (protonated acetonitrile) as the base peak, with the presence of an ion at m/z 114 representing a charged species constituted by a trimethylsilyl group on the acetonitrile molecule.

By adding a relatively larger amount of BSTFA, the reaction can be brought almost to completion as shown in Fig. 2. The ratio between the abundances of the m/z 91 and m/z 19 ions changes in favour of the m/z 91 ion and anion of m/z 163 (two trimethylsilyl groups on the water molecule) is present. A similar behaviour is observed for the ions at m/z 114 and 42 when acetonitrile is used as the mobile phase. An abundant peak of protonated BSTFA at m/z 258 is present, of course. Derivatization with TMCS is also effective: no ion at m/z 19 is found with water flow as the mobile phase, m/z 91 and m/z 163 ions being present instead.

In Table II the relative abundances of some significant ions are related to the absolute pressure of BSTFA in the ion source as measured with a Pirani gauge, without taking into account any eventual response factor. It should be pointed out that the total pressure in the ion source was maintained the same in the different experiments by compensating with addition of more or less methane. It is not known at which point of the HPLC–MS interface the reaction with water actually takes place. Formation of adduct can be observed as the derivatization reagent also contributes to the chemical ionization process.

It should be pointed out that a similar behaviour has been reported [7] by using chemical ionization conditions with mixtures of methane and tetramethylsilane. The use of silylating reagents instead of tetramethylsilane shows an additional reactivity on the active sites of the molecules.

Derivatization of different analytes

The derivatization of analytes was performed by using an excess of derivatization reagent, in order possibly to bring the reaction to completion. A trial-and-error procedure with inspection of the resulting spectrum of the analyte generally allows a convenient pressure of the reagent to be established.

Using BSTFA pressures between 50 and 90 Pa, no important qualitative changes to the mass spectrum of the derivatized analyte can be observed. A practical advantage of this situation is that the response also is relatively constant.

TABLE II

RELATIVE ABUNDANCES OF SOME IONS IN THE MASS SPECTRUM OF THE MOBILE PHASE (WATER FLOWING AT 0.4 ml/min) IN RELATION TO THE AMOUNT OF BSTFA ADDED TO THE PARTICLE-BEAM INTERFACE

The total pressure in the ion source was constant at 130 Pa in all experiments, by compensation with methane.

Absolute BSTFA pressure (Pa)	Protonated water (*m/z* 19)	*m/z* 91	*m/z* 163	Protonated BSTFA (*m/z* 258)
13	100	16	7	20
45	20	17	19	100
70	–	35	45	100

TABLE III

MOST ABUNDANT IONS IN THE MASS SPECTRUM OF SOME POLYFUNCTIONAL SUBSTANCES WITH ON-LINE SILYLATION

Relative abundances of the most significant ions expressed in % are given and the corresponding mass units are reported in parentheses.

Substance	Functional groups[a]	MW	$[M+1]^+$	1 × Silyl	2 × Silyl	3 × Silyl	Remarks
Bumetanide	Sulphonamide, sec. amine, carboxylic acid	364	Abs.	18% (437)	100% (509)	10% (581)	
Probenecid (*m/z* 504)	Carboxylic acid, sulphonamide	285	8% (286)	89% (358)	100% (430)	8% (504)	Adducts
Furosemide	Aromatic amine, carboxylic acid, sulphonamide	330	Abs.	13% (403)	100% (475)	8% (547)	
Ethacrynic acid	Carboxylic acid, methylene, keto	303	Abs.	35% (375)	100% (447)	4% (519)	Adducts
Piretanide	Carboxylic acid, sulphonamide	362	Abs.	Abs.	72% (507)	Abs.	Base peak *m/z* 385
Testosterone	Hydroxy	288	Abs.	100% (361)			Adducts present
Androsterone	Hydroxy	290	Abs.	100% (363)			Adducts present
Cortisol	3 × Hydroxy	362	Abs.	100% (435)	4% (507)	Abs.	
Cortisone[b]	2 × Hydroxy	360	Abs.	100% (433)	8% (505)		
Glucose	5 × Hydroxy	180	Abs.	12% (253)	11% (325)	9% (397)	4 × Silyl present; base peak *m/z* 235
17-α-Hydroxy-progesterone	Hydroxy	330	Abs.	100% (403)			
Acid Red 88[c]	Hydroxy, sulphonate	378[d]	Abs.	–[e]			Base peak *m/z* 205

[a] Only functions containing active hydrogens are mentioned.
[b] See Figs. 3 and 4.
[c] No signal was observed on injecting this compound without on-line derivatization.
[d] Calculated as acid.
[e] Other ions present at *m/z* 481 (5%), 374 (12%), 368 (17%), 302 (32%), 132 (22%). All mentioned fragments show the effect of the presence of silicon in the isotopic pattern.

Qualitative similar spectra can be observed for different concentrations of an analyte. The consequence is that, for example, a linear calibration graph could constructed for testosterone injected in amounts between 20 and 1000 ng.

For most of the monofunctional substances (one active hydrogen atom) analysed here, the reaction is complete and no original $[M+1]^+$ is observed. Polyfunctional substances may be derivatized at all possible positions, but in some instances the reaction is not complete (see Table III).

In the chromatogram obtained under dynamic conditions where a mixture of testosterone, epitestosterone, andreosterone and etiocholanolone (3-α-hydroxy-5-β-androstan-17-one) was separated by reversed-phase HPLC (see Fig. 3) and silylated on-line with BSTFA, the spectra of the mono-functional components show a complete reaction, while cortisol and cortisone (Fig. 4) are not totally silylated at all possible active positions.

However, for the qualitative analysis of some polyfunctional substances such as diuretics [8], the mass spectra show that the silylation reaction is complete in some instances. Adducts produced by ion–molecule reactions under chemical ionization conditions may be present in the spectra (see Table III). For the analysis of furosemide, "one-line" derivatization with BSTFA increases the positive-ion detection in the chemical ionization mode by a factor of about 20, although the most sensitive method is still the detection of negative ions and chemical ionization with methane or ammonia. A similar behaviour can be found for diuretics of analogous molecular structure. For the electron impact response taken as a reference, the measured response ratios using different detection methods were as reported in Table IV.

The advantage of derivatization, in this instance, is that the measurement is made at higher mass values, hence an improvement in the signal-to-noise and repeatability (see R.S.D. values in Table IV) is observed.

On-line acylation with TFAA and PFTFA

These anhydrides were at the end of the particle beam interface at position (d) (see Fig. 1) together or without the reagent gas. Nitrogen, argon or methane were tested as additional "carriers" for these anhydrides, although the volatility of the anhydrides was sufficient to transfer them into the inlet line of the mass spectrometer. Methane was found to give the best performance.

Under chemical ionization conditions and with detection of the negative ions, the molecular ion of pentafluoropropionic anhydride is observed and the base peak is constituted by the corresponding acyl group at m/z 147.

As with silyl derivatization, water flowing from the HPLC column into the interface reacts with the acylating reagent to produce hydrolysis products.

The addition of TFAA "on-line" for the analysis of polyfunctional compounds produces a partial derivatization; this behaviour was observed on injecting cortisol or cortisone.

As shown in Fig. 5, the derivatization of cortisone "off-line", following the usual procedure, produces a spectrum that, under chemical ionization conditions and with flow injection of the mixture containing the reactant, corresponds to the reaction of three trifluoroacyl groups (indicated here as TFA). The ion at m/z 648 derives from MW (360) − 3 H (3) + 3TFA (97). An additional adduct of the trifluoroacetic acid anion is observed at m/z 761.

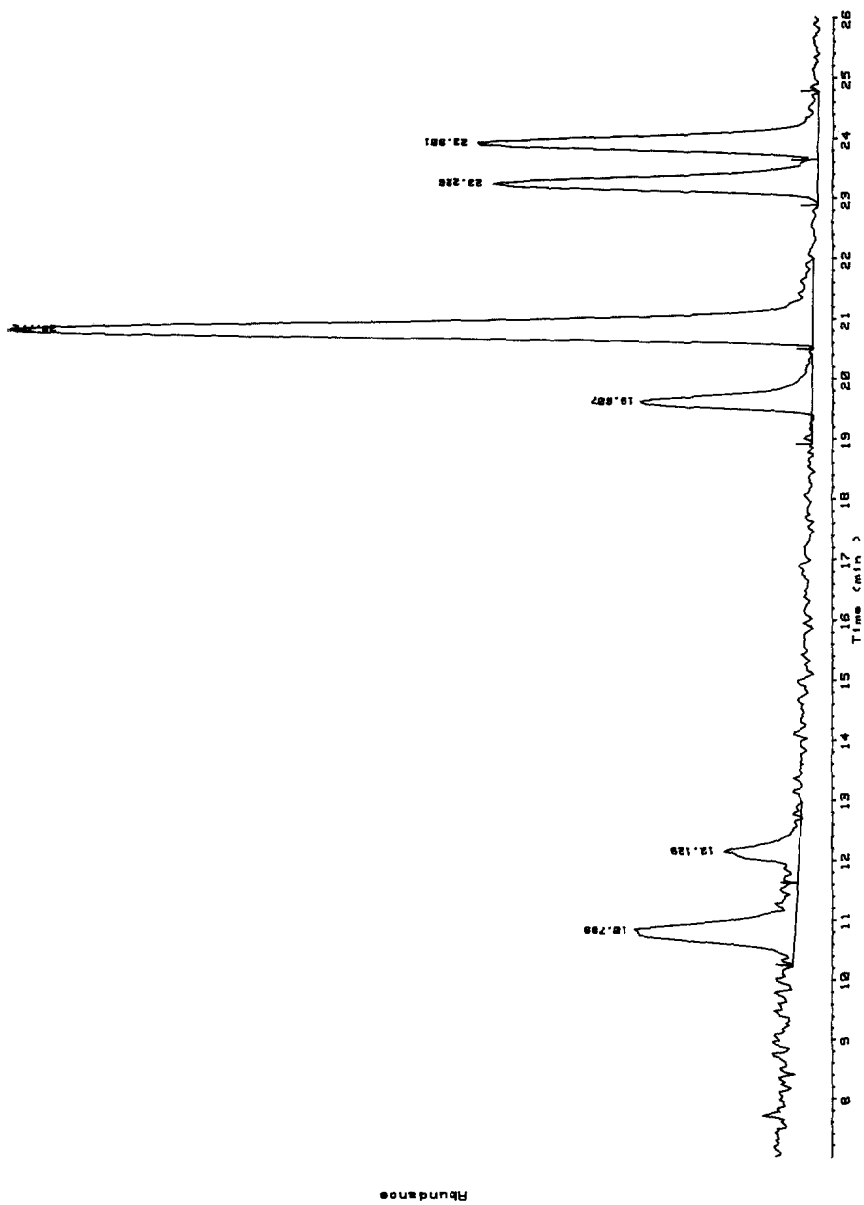

Fig. 3. Chromatogram of a mixture of hydro cortisone, cortisone, testosterone, epitestosterone, androsterone and epiandrosterone by on-line derivatization with BSTFA. *Amount injected: 200 ng for all components except epitestosterone (600 ng).* Column: *100 × 2.1* mm I.D. stainless steel (Hewlett-Packard) packed with 5-μm Hypersil ODS. Mobile phase: gradient of water–methanol from 40:60 to 1:99 (v/v) in 30 min. flow-rate: 0.4 ml/min. Desolvation chamber temperature: 50°C. Chemical ionization conditions and detection of positive ions. Source temperature: 250°C. Total pressure into the ion source: 130 Pa. Reactant gas: methane–BSTFA (*ca.* 2:1).

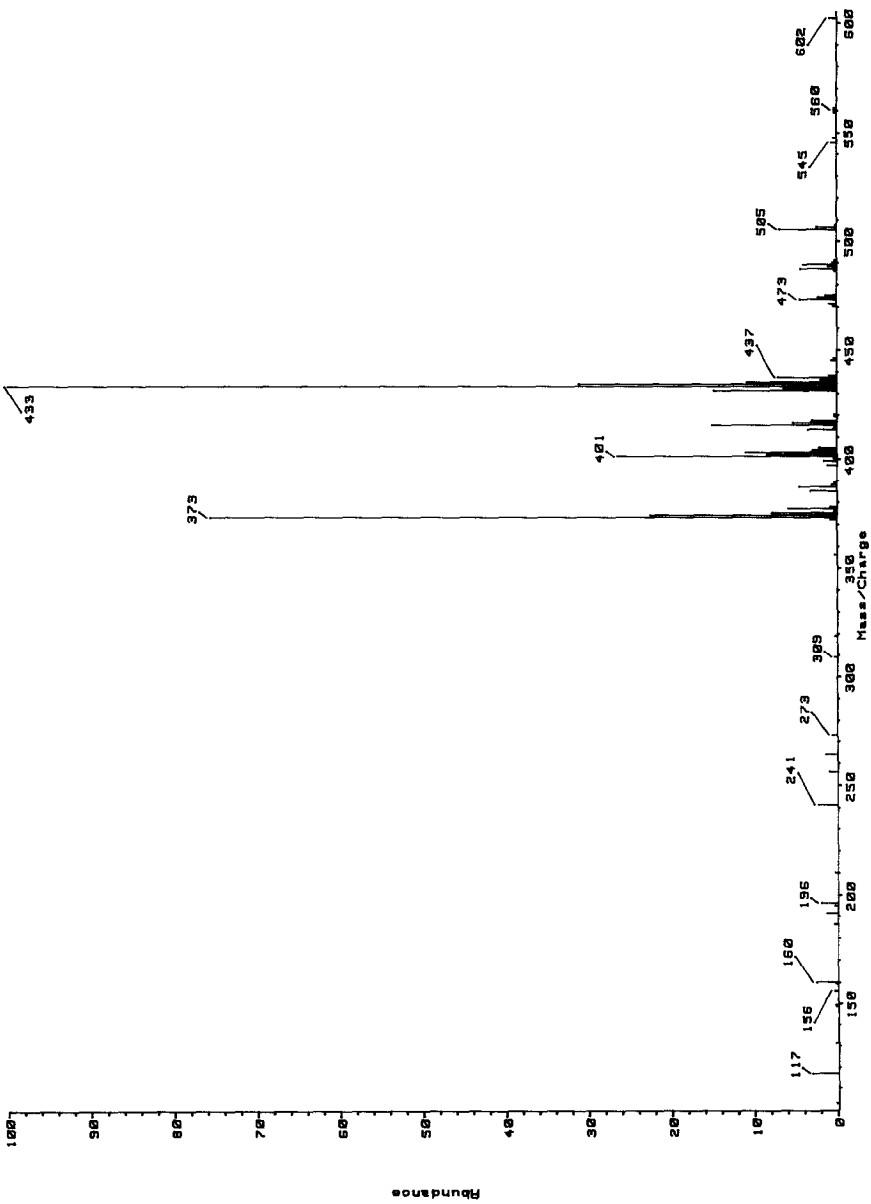

Fig. 4. Mass spectrum of cortisone by on-line derivatization with BSTFA. Conditions as in Fig. 3.

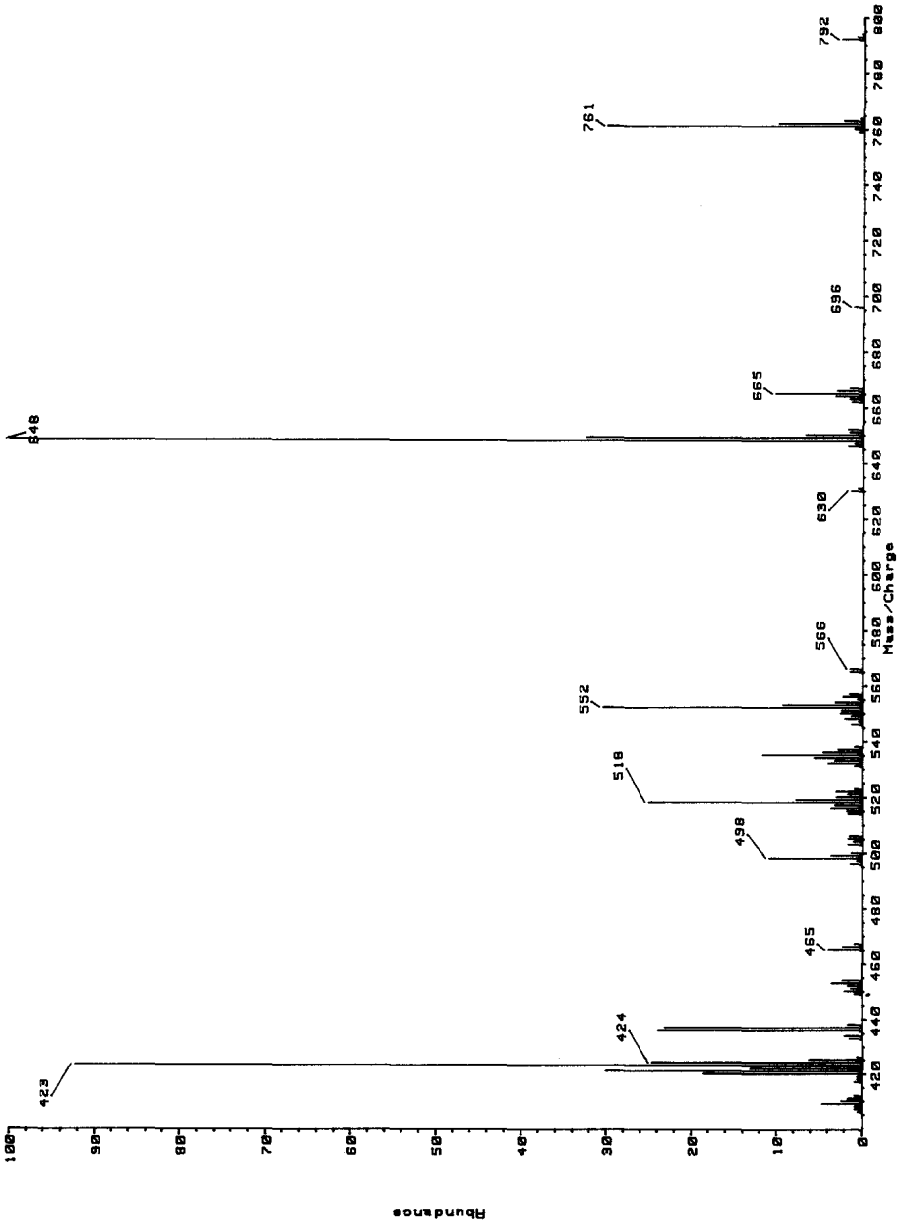

Fig. 5. Mass spectrum of cortisone after reaction with TFAA at 80°C for 1 h and injected bypassing the analytical column. Particle beam interface temperature: 50°C. Chemical ionization conditions with a pressure into the ion source of 130 Pa. Reagent gas: methane. Detection of negative ions. Mobile phase: acetonitrile at a flow-rate of 0.4 ml/min.

TABLE IV

RELATIVE RESPONSE OF FUROSEMIDE USING DIFFERENT DETECTION TECHNIQUES

Detection	R.S.D. (%)[a]	Response ratio[b]
Electron impact	3.5	1 (reference)
Negative ions (CI)	7.6	36.7
Positive ions (CI)	27.4	0.01[c]
Positive CI, on-line derivatization	3.2	0.2

[a] Relative standard derivation (n = 12).
[b] These data are based on absolute response (total ion current) under similar operating conditions.
[c] Methane was used as a chemical ionization reagent.

The "on-line" addition of TFAA (Fig. 6) in the gas phase to cortisone produces mainly the derivatization of one trifluoroacyl group with an addition of one TFAA anion (360 − 1 + 97 + 113) at m/z 569, and a smaller amount of the product of two trifluoroacyl substitutions with a resulting ion at m/z 665.

A similar behaviour was observed on using PFPAA as reactant, but the reaction seems to be less effective as only one group is reacted with the molecule. Ions are found at m/z 669 and 523. No traces of ions are found for the underivatized cortisone. It should be pointed out that the background signal of the derivatization reagent is relatively high with negative-ion detection and under chemical ionization conditions.

Effect of the addition of a water scavenger at the nebulizer

It is known that high water concentrations do not favour the transmission of substances through the particle-beam interface. The detectability of 100 ng of benzidine on changing mobile pahse composition from acetonitrile to pure water, without nebulizer readjustement, drops to about 15% (Table V). Note that if the nebulizer position is readjusted, the response for benzidine with water is about one third of the response obtained with acetonitrile as the mobile phase.

Addition of DMP to the helium used for the nebulization changes this behaviour to some extent by keeping the response less sensitive to changes in mobile phase composition. However, is should be pointed out that the absolute response of the benzidine signal is low.

Postcolumn addition of acetonitrile

It could be verified that the addition of acetonitrile at the outlet of the HPLC column [position (a)] to a water-rich mobile phase (95–100%) improves the detectability by an average factor of *ca.* 2–3, depending of the flow conditions (data not reported).

CONCLUSIONS

On-line derivatization through the particle-beam HPLC–MS interface can be performed in different ways, with a relatively wide selection of derivatization reagents. Criteria for the selection of the derivatization reagent are based on both

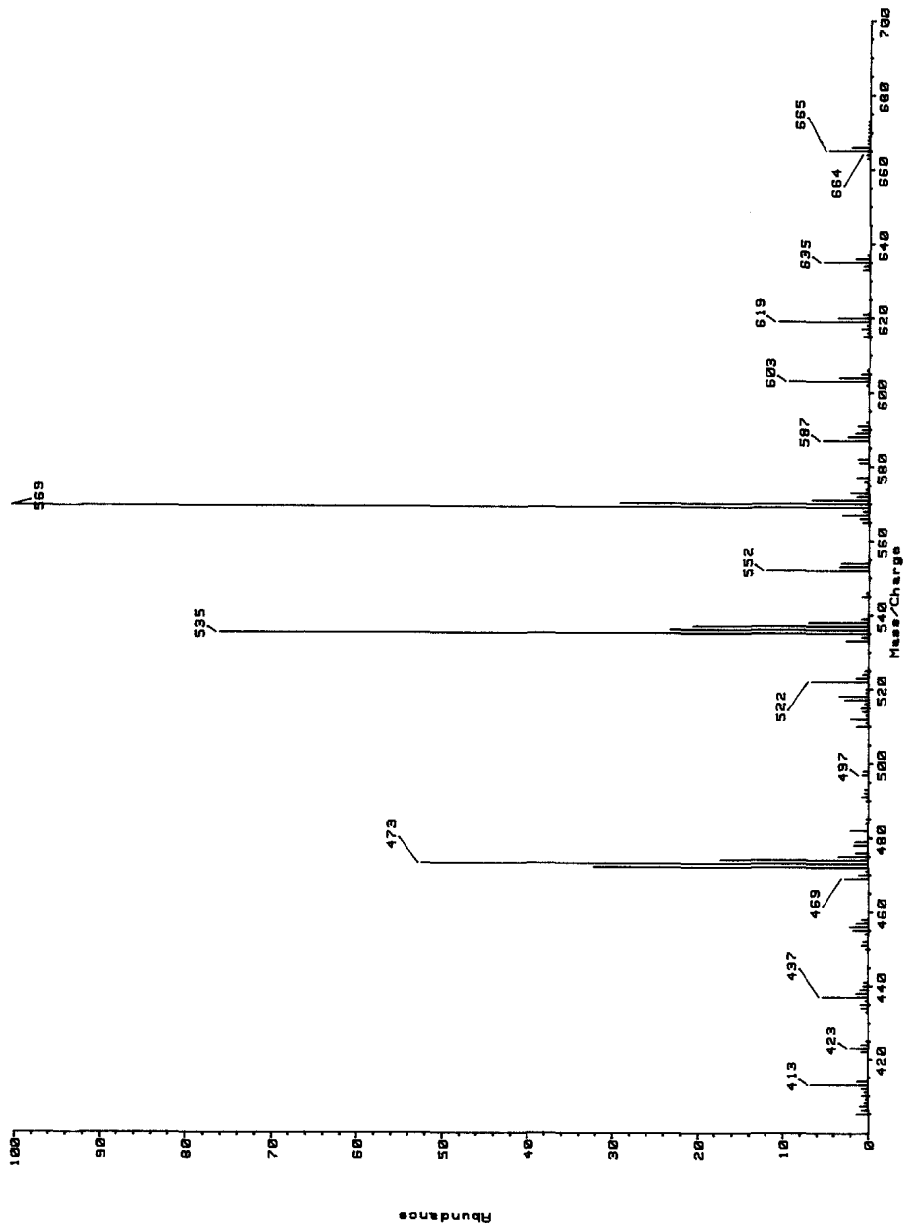

Fig. 6. Mass spectrum of cortisone injected bypassing the analytical column. Mobile phase: acetonitrile at a flow-rate of 0.4 ml/min. Particle-beam interface temperature: 50°C. Chemical ionization conditions. Reactant gas: TFAA. On-line derivatization conditions.

TABLE V

EFFECT OF THE ADDITION OF DMP TO HELIUM USED FOR NEBULIZATION ON THE RESPONSE FOR 100 ng OF INJECTED BENZIDINE

All data obtained without readjustment of the nebulizer position.

Conditions	Mobile phase composition (% of water in acetonitrile)					
	0	25	50	75	90	100
Without addition of DMP	100	88	73	41	20	15
With addition of DMP	18	9	8	8	8	7

volatility and functionality. Volatility is important to be able to bring the reagent into the mass spectrometer in the gas phase. Functionality depends on its activity and rate of reaction with the analyte.

Successful silylations and acylations of several substances have been obtained, and the results have been evaluated on the basis of the resulting mass spectra. On-line silylation reactions are complete for compounds containing one active functional group. For polyfunctional compounds the reaction may take place only for a limited number of functional groups or with all of them to a limited extent.

Some substances with relatively high polarity and low volatility have been derivatized using the described procedure.

Some aspects of the improvement of detectability of some of the substances without or with on-line derivatization, *e.g.*, Acid Red 88, furosemide, maltose and cortisol, are reported. These molecules contain, among others, sulphonate, carboxylic or hydroxy functions or their combinations. The detectability of furosemide with positive-ion detection and under chemical ionization conditions improved by a factor of 20. Reasons for the higher detectability are related to both a higher volatility and mass spectral behaviour of the derivative.

In order to obtain additional qualitative information about the nature of the analyte, the spectra of the derivatives are particularly useful and complementary to other mass spectral data obtained without derivatization.

Typical solvents used for reversed-phase HPLC separations have been used (water, acetonitrile, methanol). Derivatization of the residual solvent at the outlet of the momentum separator has been observed, and this happens even with water as the mobile phase.

Simple modifications to standard instruments were made in order to introduce the reagent into the particle-beam interface. As a general remark, BSTFA can be used more conveniently than TMCS for reliable long-term work, with a limited effect on the contamination of the ion source.

Other experiments involving the addition of a water scavenger to the nebulizer can only reconfirm that the water concentration is definitely an important parameter for the efficiency of both the nebulization process and the desolvation. No other conclusions have been drawn so far from those experiments. Therefore, these data

can be considered as a partial contribution to studies on the nebulization process, which can affect the performance of a particle-beam HPLC–MS interface.

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