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Important parameters in liquid chromatography–continuous-flow fast atom bombardment mass spectrometry

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ABSTRACT

Continuous-flow fast atom bombardment (CF-FAB) is an attractive technique of interfacing reversed-phase high-performance liquid chromatography and mass spectrometry (LC–MS). Some of the important parameters affecting the performance of CF-FAB were studied. The wettability of the CF-FAB target and liquid film properties on the surface of the CF-FAB target play an important role in obtaining stable performance in LC–CF-FAB-MS. The wettability of the target can be improved by an acid treatment. The thermal conductivity of the CF-FAB target material together with the flow-rate and the mobile phase composition influence the liquid film properties of the target. The optimum supply rate of glycerol is observed to be 0.3 μl/min for LC–CF-FAB-MS under the studied conditions. Some bioanalytical examples of LC–CF-FAB-MS are shown.

INTRODUCTION

Continuous-flow fast atom bombardment (CF-FAB) is an important development in the rapidly growing research field of interfacing high-performance liquid chromatography and mass spectrometry (LC–MS). The reason for this is the improved performance of CF-FAB compared with conventional FAB and the possibility of using FAB as an ionization method in LC–MS. FAB is made available to on-line LC–MS applications by using either a frit-FAB interface, as described first by Ito *et al.*[1], or a direct introduction type of interface, as described by Caprioli *et al.*[2]. The

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TABLE I

SYSTEMS INVESTIGATED

Parameter	*System A*	*System B*	*System C*	*System D*	*FIA I*	*FIA II*
Pump	Pharmacia	Pharmacia	LKB	LKB	LDC/Milton Roy	LKB
Mobile phase[a]	TFA–Gly–ACN–H_2O	TFA–Gly–ACN–H_2O	TFA–Gly–ACN–H_2O	Gly–ACN–buffer	Gly–ACN–H_2O	TFA–Gly–MeOH
Composition (%, w/w)	0.26:10:30:59.74	0.25:10:30:59.75	0.25:9.9:29.8:60.05	10.2:41.4:48.4	10:30:60	0.28:10.1:89.62
LC flow-rate (ml/min)	1.0	1.0	1.2	1.0	5–7	500
Injector (μl)	20	20	20	20	0.5	20
Column	Techopak	Techopak	Techopak	PRP-1	–	–
Split capillary	19 cm × 200 μm I.D.	19 cm × 200 μm I.D.	44 cm × 150 μm I.D.	20 cm × 220 μm I.D.	–	40 cm × 200 μm I.D.
CF-FAB capillary	85 cm × 75 μm I.D.	85 cm × 75 μm I.D.	75 cm × 75 μm I.D.	80 cm × 75 μm I.D.	100 cm × 75 μm I.D.	80 cm × 75 μm I.D.
Splitting ratio	1:180	1:200	1:170	1:160	–	1:74
CF-FAB flow-rate (μl/min)	6	5	7	6	5–7	6
Mass spectrometer	MAT 8200	MAT 8200	MAT 90	MAT 90	MAT 90	MAT 90
Source temperature (°C)	40	40	60	75	40	40
Cold trap	No	No	Yes	Yes	No	Yes
Wick	No	No	Yes	Yes	Yes	Yes

[a] TFA = trifluoroacetic acid; Gly = glycerol; ACN = acetonitrile; MeOH = methanol; buffer = 0.1 *M* ammonium acetate (pH 8.3) and H_2O = water.

latter interface is usually referred to as the continuous-flow FAB or the dynamic FAB interface, and is the topic of this paper.

In CF-FAB, the maximum flow-rate is *ca.* 10 μl/min, leading to the necessity to split the eluent after conventional LC columns (3–4.6 mm I.D.). As a result, the concentration detection limits are increased by a factor of 50–100. Miniaturization of the LC system, *e.g.*, to 1 mm I.D. columns, reduces the splitting ratio but does not improve the concentration limits, because the injection volume in microbore systems is decreased correspondingly.

Despite the mentioned difficulties, impressive results have been reported with a broad variety of compounds[1–15]: bile acids[1,13], peptides[2–8], dansylated amino acids[3], antibiotics[4,14], oligosaccharides[9], drugs[10], glucosinolates[11], non-ionic detergents[12], polyethylene glycol oligomers[14] and herbicides[15]. In these applications, either flow-injection analysis (FIA)[1–3,5,6,8,9,13,14] or packed fused-silica capillary[1,4,9,12–15], microbore[3,4,6] or conventional bore[7,10,11] columns have been used.

Although the applicability of the CF-FAB method has been studied intensively by various groups, little attention has been paid to a systematic study of the key parameters determining the performance of CF-FAB, both from a mass spectrometric point of view and as an LC–MS interface. In this work, some of the important parameters of CF-FAB were investigated, such as the target material and conditions enhancing the wettability and liquid film properties of the target. Some illustrative examples of CF-FAB applied in LC–MS are given.

EXPERIMENTAL

Liquid chromatography

The six different experimental set-ups that have been used are summarized in Table I and are referred to in the text as systems A–D and FIA I and II. In systems A–D conventional-bore LC columns in combination with a splitter were used. FIA was performed using either a 0.5-μl injector (FIA I) and a low flow-rate or a 20-μl injector (FIA II) and a high flow-rate in combination with a splitter.

The LC system consisted of either a Pharmacia (Uppsala, Sweden) P-3500 pump, an LKB (Bromma, Sweden) 2150 pump or an LDC/Milton Roy (Riviera Beach, FL, U.S.A.) MicroMetric metering pump and a Rheodyne (Berkeley, CA, U.S.A.) Model 7125 (20-μl) or a Model 7410 (0.5-μl) injection valve.

For the determination of dextromethorphan in plasma (systems A–C), a Techopak reversed-phase C_{18} column (15 cm × 3.9 mm I.D., packed with 10-μm material) (HPLC Technology, Macclesfield, U.K.) was used. A laboratory-packed PRP-1 (Hamilton, Reno, NV, U.S.A.) column (10 cm × 3.9 mm I.D.) with 10-μm particles was used for the determination of erythromycin 2'-ethylsuccinate in plasma (system D). The temperature of the column and the stainless-steel capillaries (systems A–D) was kept at 56°C in order to reduce the viscosity of the mobile phase.

The eluent from the LC column (systems A–D) and from FIA II was directed to a laboratory-made splitting device described in detail elsewhere[10]. FIA I and the splitter were connected to a Finnigan MAT (Bremen, F.R.G.) prototype CF-FAB probe by means of a 75-μm I.D. fused-silica capillary, the so-called CF-FAB capillary.

Mass spectrometry

The CF-FAB probe was fitted to either a Finnigan MAT 8200 or a Finnigan MAT 90 double-focusing mass spectrometer, operating at 3 and 5 kV, respectively. Both instruments were equipped with a FAB gun (Ion Tech, Teddington, U.K.) using xenon and producing a beam of neutral atoms with 5–8 kV energy. The FAB spectra were recorded in the positive ion mode by scanning from 20 to 1000 with a scan speed of 1 s per decade.

In the MAT 90 instrument an exchangeable ion volume with a wick was applied. The exchangeable ion volume reduces contamination problems. The wick was prepared from compressed paper and positioned at the bottom of the ion volume. Additional vacuum pumping at the ion source housing was obtained with a liquid nitrogen trap. In the MAT 8200 instrument there was no possibility of using an exchangeable ion volume, a wick or a cold trap.

Acid treatment of the target

Four different metal targets were used at the CF-FAB probe, *viz.*, stainless-steel, copper, nickel and silver. The stainless-steel target was pretreated with concentrated hydrochloric acid for 30 min. The target was washed in an ultrasonic bath with both water and methanol for 15 min and subsequently air dried. The nickel and silver targets were pretreated with concentrated nitric acid for 15 min and washed as described above. The copper target was quickly washed with 30% hydrochloric acid and subsequently rinsed with water.

Reagents

Dextromethorphan hydrobromide, erythromycin, erythromycin 2′-ethylsuccinate and erythromycin d_5-2′-ethylsuccinate were obtained from the Research Center of Orion Pharmaceutica (Espoo, Finland). Vindesine was supplied by Dr. D. E. M. M. Vendrig (Department of Pharmaceutical Analysis, Faculty of Pharmacy, University of Utrecht, The Netherlands).

Trifluoroacetic acid, hydrochloric acid, nitric acid, methanol, hexane, ammonium acetate, ammonia, iodomethane and diethyl ether were supplied by E. Merck (Darmstadt, F.R.G.). Glycerol (98% pure) was purchased from Lamers & Pleuger ('s-Hertogenbosch, The Netherlands), sodium carbonate and potassium carbonate (AnalaR) from BDH (Poole, U.K.), triethylamine by Pierce (Rockford, IL, U.S.A.) and acetonitrile (ChromAR) from Promochem (Wesel, F.R.G.). The pH of the ammonium acetate buffer was adjusted with concentrated ammonia solution. Water was distilled before use.

The mobile phase was prepared by weighing the solvents. It was filtered and degassed ultrasonically before use. The mobile phase was prepared daily in order to avoid bacterial growth.

Sample preparation

Stock solutions containing 15 mg of dextromethorphan hydrobromide were prepared in 10 ml of water. Plasma samples containing 1.8 μg/ml of dextromethorphan were prepared by adding the appropriate amount in 50 μl of water to 2.0 ml of blank human plasma. The sample pretreatment procedure, consisting of a liquid–liquid extraction step, has been described in detail elsewhere[10].

Stock solutions of erythromycin 2′-ethylsuccinate (1.18 mg/ml) and erythromycin d_5-2′-ethylsuccinate (1.30 mg/ml) were prepared in acetonitrile. Duplicate plasma samples containing 0.25, 0.49, 0.98, 2.5, 4.9 and 9.8 μg/ml of erythromycin 2′-ethylsuccinate were prepared by adding the appropriate amount of erythromycin 2′-ethylsuccinate in 50 μl of acetonitrile to 2.0 ml of blank human plasma; 13 μg of d_5-erythromycin 2′-ethylsuccinate in 50 μl of acetonitrile was added to the samples as an internal standard. After rapid mixing, 200 μl of saturated sodium carbonate solution and 5.0 ml of diethyl ether were added. The tubes were gently shaken on a horizontal shaker for 15 min. After centrifugation for 5 min (1000 *g*), 4.0 ml of the organic layer were separated and evaporated to dryness in a gentle stream of helium at 30°C. The residue was dissolved in 50 μl of acetonitrile. An aliquot of 20 μl was injected onto the HPLC column (system D).

Methylation of dextromethorphan

Methylation of dextromethorphan was performed according to Kidwell *et al.*[16]. To 950 μl of water 10 mg of potassium carbonate and 2.5 μg of the dextromethorphan in 50 μl of water were added. After mixing for 30 s, 250 μl of methanol and 50 μl of iodomethane were added. The mixture was heated at 60°C for 5 min until the solution was homogeneous and 20 μl of the sample were injected onto the FIA II system.

RESULTS AND DISCUSSION

CF-FAB target

In LC–CF-FAB-MS the sample from the LC column or from the flow-injection system is introduced to the CF-FAB target via a fused-silica capillary. The sample in a glycerol matrix is bombarded at the target with a beam of 5–8-kV xenon atoms to produce ions which are mass analysed. A schematic diagram of the CF-FAB target is given in Fig. 1. The positioning of the fused-silica capillary at the target tip is an important aspect in producing a homogeneous film of the mobile phase on the target. If the tip of the capillary protrudes too much, droplets of the mobile phase are formed on the surface of the target and unstable vaporization of the mobile phase takes place. When the tip of the capillary protrudes *ca.* 0.5 mm out of the surface of the target, it is possible to obtain a good liquid film. With the help of an adjustment screw in the CF-FAB probe the positioning of the tip is simplified. Compressed paper at the

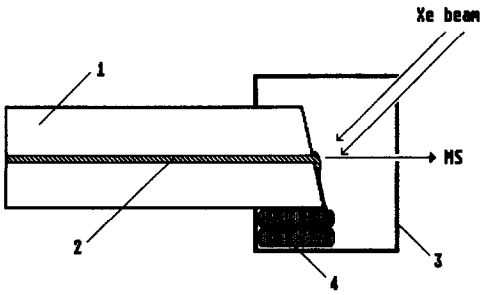

Fig. 1. Schematic diagram of the CF-FAB target with an exchangeable ion volume. 1 = CF-FAB target; 2 = CF-FAB capillary; 3 = exchangeable ion volume; 4 = wick.

bottom of the ion volume, the so-called wick, is used to collect the excess of mobile phase from CF-FAB target to prevent droplet formation at the CF-FAB target and to maintain a liquid film of the mobile phase on the target.

The wettability of the target is a key factor for the stable performance of the LC–CF-FAB-MS system. Treatment of the target surface with a concentrated acid roughens the surface of the target and thereby enchances the wettability. The roughness is maintained longer when an acid is also included in the mobile phase, *e.g.*, 0.2% trifluoroacetic acid (TFA). The wick in combination with the correctly adjusted capillary tip and the acid-treated target improve the liquid film properties, resulting in improved stability of the chemical background ions.

Stainless-steel[1,9–11,13], copper[2,10], gold[3,4,6] and silver[12] have been used as target and frit materials in CF-FAB. Takeuchi *et al.*[14] compared the performances of stainless-steel, silver, glass, paper and PTFE as frit materials. They concluded that the highest intensities for the analyte are observed when stainless-steel and silver frits are used, and that this is due to the good wettability of these materials by glycerol. Nickel, silver, copper and stainless-steel have been tested as target materials in our laboratory. The performance of the target material was evaluated with respect to the stability of the baseline by monitoring the intensities of protonated glycerol (*m/z* 93) and protonated acetonitrile (*m/z* 42). A typical example of a background signal is given in Fig. 2 (FIA I, with an acid-treated nickel target). When nickel, silver or copper are used as target materials, good stability of the background ions is achieved after a few minutes. The latter takes up to 15 min with a stainless-steel target. The observed good liquid film properties are probably due to the good thermal conductivity of these target materials resulting in enhanced evaporation of the mobile phase. A disadvantage of the nickel target is that the time-consuming roughening had to be done every 2 h to maintain the wettability of the target and consequently the stability of the baseline. The silver and copper targets are difficult to use in practice because of the strong clustering of copper and silver ions (no TFA can be used for the latter material). The best long-term stability was obtained with the acid-treated stainless-steel target. Although it takes more time to achieve stability of the baseline with a stainless-steel target, its performance can be maintained for the whole working day. The roughness of the stainless-steel target has to be renewed daily. So far stainless-steel is preferred as the most useful target material, but there is still a need for inert target materials, with good thermal conductivity and wettability.

The analytical performances of the copper and stainless-steel targets were compared by studying the within-run precision of the LC–CF-FAB-MS method for the determination of dextromethorphan in plasma. The plasma samples were spiked with 1.8 μg/ml of dextromethorphan, corresponding to 5–7 ng introduced into the mass spectrometer after splitting. The protonated molecule of dextromethorphan at *m/z* 272 and the protonated glycerol cluster at *m/z* 277 were recorded in the selected ion monitoring mode. In Table II the relative standard deviations for 5–6 measurements of the peak areas are summarized. By using the acid-treated copper target (system A) a better precision is obtained than by using the untreated stainless-steel target (system B). However, the performance of the stainless-steel target is improved when the wick and the cold trap are used after acid treatment of the target (system C).

TABLE II

WITHIN-RUN PRECISION OF THE PEAK AREAS FOR DETERMINATION OF DEXTROMETHORPHAN IN HUMAN PLASMA BY MEANS OF LC–CF-FAB-MS WITH A COPPER AND A STAINLESS-STEEL TARGET

Plasma spiked with 1.8 μg/ml of dextromethorphan.

Target	*n*	*Relative standard deviation (%)*	*Conditions*
Copper	5	13	No wick No cold trap Acid-treated target System A
Stainless-steel	5	24	No wick No cold trap Non-treated target System B
	6	15	Wick Cold trap Acid-treated target System C

Mobile phase

In CF-FAB glycerol is generally used as the ionization matrix, because of its good solubility properties and its low vapour pressure. In CF-FAB usually 5–20% of glycerol is added to the mobile phase of the LC system in either the pre-column[1–6,8–15] or post-column mode[7]. For the frit-FAB approach, Takeuchi *et al.*[14] reported that the optimum supply rate of glycerol in the mobile phase is 0.1 μl/min, which means 10% (v/v) of glycerol in the mobile phase at a flow-rate of 1 μl/min and 5% (v/v) at 2 μl/min. In the frit approach, glycerol and solute are left on the surface of the frit, while the other solvents immediately evaporate from the surface of the frit[1,9,13,14]. In CF-FAB, the mobile phase is constantly directed to the CF-FAB target and the surface layer is renewed at the rate of the mobile phase flow. When conventional FAB, frit-FAB and CF-FAB are compared, the disturbing background and chemical noise derived from the glycerol are greatly reduced in the latter two instances and the most in the continuous-flow approach.

To study the optimum supply rate of glycerol for CF-FAB, the within-run precision in flow injection (FIA II) of dextromethorphan as a test compound was investigated by adding different amounts of glycerol to the mobile phase. A sample containing 40 ng of dextromethorphan in water was injected, which corresponds to 500 pg introduced into the mass spectrometer. In Table III the relative standard deviations of the peak areas are summarized. The content of trifluoroacetic acid was constant during the measurements and only the ratio of glycerol and methanol was changed. When 1–2.3% (w/w) of glycerol is used in the mobile phase, a moderate precision is achieved for these injections of dextromethorphan, but after some time the baseline becomes very unstable and it is impossible to obtain reproducible results. This

TABLE III

INFLUENCE OF SUPPLY RATE OF GLYCEROL ON WITHIN-RUN PRECISION OF FLOW INJECTION OF DEXTROMETHORPHAN

Injected amount 40 ng (500 pg in the mass spectrometer after splitting). For conditions, see text. FIA II with an acid-treated stainless-steel target.

Amount of glycerol in mobile phase		*CF-FAB flow-rate (μl/min)*	*Supply rate of glycerol (μl/min)*	*Relative standard derivation (%)*	*n*
% (w/w)	*% (v/v)*[a]				
1.0	0.6	5.6	0.03	26	8
2.3	1.5	5.3	0.08	27	4
5.0	3.2	5.5	0.18	19	6
6.9	4.4	6.1	0.27	9	9
10.1	6.6	6.5	0.43	19	8

[a] Made up as % (w/w), recalculated in % (v/v).

is probably due to a lack of glycerol on the target, resulting in an unstable liquid film on the target.

The best overall performance under thes experimental conditions (FIA II) was obtained with 7% (w/w) of glycerol in the mobile phase, indicating that the optimum supply rate of glycerol is 0.3 μl/min for CF-FAB, which is three times higher than that in the frit approach. From this result it can be calculated that for 1% (v/v) of glycerol in the mobile phase, the optimum flow-rate is *ca.* 30 μl/min, for 5% (v/v) 6 μl/min and for 10% (v/v) 3 μl/min. However, it must be pointed out that the stability of the system depends not only on the supply rate of glycerol, but also on the applied mobile phase composition, the evaporation rate of that mobile phase and the pumping capacity of the mass spectrometer. A mobile phase containing only trifluoroacetic acid, methanol and glycerol as used in this experiment (FIA II) is especially suitable for the desorption step in phase-system switching (PSS) experiments reducing the flow-rate[10] and for general FIA procedures. The key parameters are the fast baseline stabilization due to the good liquid film properties of this mobile phase, the fast evaporation rate of methanol and the absence of water.

The influence of the flow-rate on the baseline stability of CF-FAB is illustrated in Fig. 2. With 5 μl/min irregular acetonitrile evaporation within the CF-FAB capillary and droplet formation from glycerol on the target took place, resulting in a very unstable baseline. At 7 μl/min the evaporation of acetonitrile took place from a stable liquid film on the target surface. As a result, a very stable baseline with low glycerol (cluster) intensity can be obtained under the latter conditions.

The ion-source temperature affects the evaporation rate of the components of the mobile phase. When the flow-rate or the water content of the mobile phase is changed, the ion-source temperature has to be optimized. Generally, an ion-source temperature of 40–45°C is used for non-aqueous mobile phases and up to 80°C for mobile phases containing high percentages of water in order to prevent freezing of the solvents on the target.

In CF-FAB, typically reversed-phase mobile phases, *e.g.*, methanol, acetonitrile, water and mixtures thereof, are used. In a solvent mixture containing 10% of glycerol,

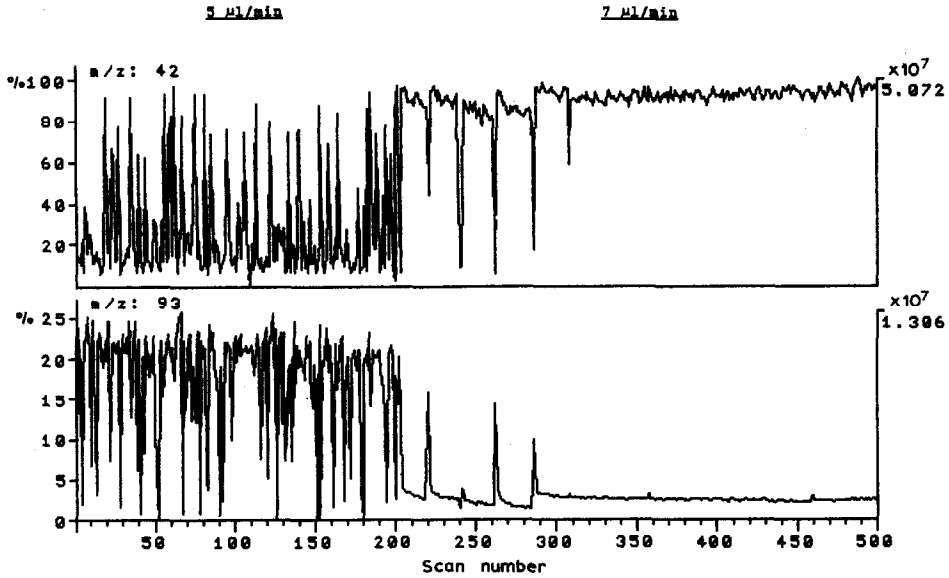

Fig. 2. Influence of flow-rate on the baseline stability of the LC–CF-FAB-MS system. Reconstructed mass chromatograms at *m/z* 42 for protonated acetonitrile (top) and at *m/z* 93 for protonated glycerol (bottom). FIA I (see Table I) with an acid-treated nickel target.

only up to 45% of acetonitrile can be mixed. Higher acetonitrile percentages result in an emulsion. With methanol–water–glycerol mixtures these problems are absent: these solvents can be mixed in any required composition. The composition of the solvent mixture influences the stability and the time required to obtain stable conditions after installing the probe. It is more difficult to achieve a stable performance with high water contents, because of the increased amount of vapour. The cold trap improves the pumping capacity of the mass spectrometer and, by that means, the overall performance and stability of the system.

One of the difficulties in LC–MS interfacing is the limitation to the buffers that can be used in the mobile phase. The applied buffers have to be volatile. Trifluoroacetic acid has been used with excellent results to adjust the pH of the mobile phase, to improve and maintain the wettability of the CF-FAB target and also to enhance the sensitivity for particular analytes. In order to study the possibilities of using the volatile ammonium acetate buffer in the mobile phase, a method for the determination of erythromycin 2'-ethylsuccinate in human plasma using LC–CF-FAB-MS has been developed.

Application: erythromycin 2'-ethylsuccinate in plasma

Erythromycin is a macrolide antibiotic. To improve the pharmacokinetic and pharmaceutical properties of erythromycin, several 2'-esters have been synthesized. Because these esters are antibacterially inactive, it is important that an analytical method is capable of separating erythromycin and its esters. Only a few LC methods have been published for the determination of erythromycin and erythromycin

esters[17,18] in biological fluids. The determinations are based on coulometric[17] and UV detection[18]. Erythromycin 2'-ethylsuccinate has also been determined in human plasma by FAB-MS[19]. A method for the determination of erythromycin 2'-ethylsuccinate in plasma using LC–CF-FAB-MS has been developed. The separation of erythromycin and erythromycin 2'-ethylsuccinate is achieved by applying a polymer-based stationary phase and a mobile phase that contains ammonium acetate, glycerol and acetonitrile. A good separation of erythromycin and its 2'-ethylsuccinate ester is obtained. Erythromycin d_5-2'-ethylsuccinate is used as an internal standard for the determination of erythromycin 2'-ethylsuccinate.

Selected ion monitoring chromatograms of the protonated molecule of erythromycin 2'-ethylsuccinate and internal standard at *m/z* 862 and 867 are given in Fig. 3. The plasma samples were spiked with 9.8 μg/ml of erythromycin 2'-ethylsuccinate (corresponding to 40 ng introduced into the mass spectrometer) and 6.5 μg/ml of erythromycin d_5-2'-ethylsuccinate (26 ng introduced). The protonated molecule of erythromycin at *m/z* 734 was also monitored. The blank plasma is free from an interfering background at the *m/z* values monitored. The linearity (ratio of peak area of analyte and internal standard *vs.* concentration) of the method was checked in the range 0.25–9.8 μg/ml of erythromycin 2'-ethylsuccinate in plasma. The calibration graph is linear over this range, corresponding to 1–39 ng of erythromycin 2'-ethylsuccinate introduced into the mass spectrometer. The determination limit with a signal-to-noise ratio of 10:1 is *ca.* 0.25 μg/ml in the selected ion monitoring mode. The recovery of the extraction method was calculated to be 81% at the 5 μg/ml level by comparing the peak areas obtained from extracted spiked plasma samples and from extracted blank samples, to which erythromycin 2'-ethylsuccinate was added after extraction.

The within-run precision of the method was studied by analysing spiked plasma samples containing 5 μg/ml of erythromycin 2'-ethylsuccinate. The relative standard

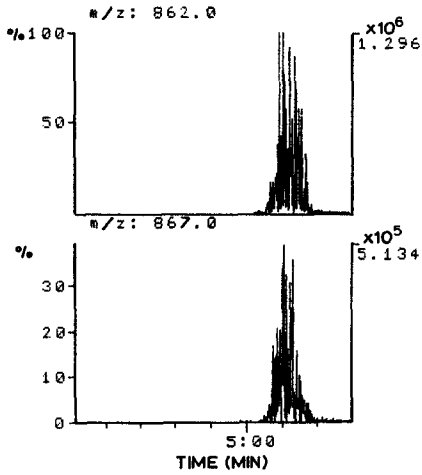

Fig. 3. Selected ion monitoring chromatograms of protonated erythromycin 2'-ethylsuccinate (upper trace, *m/z* 862) and protonated erythromycin d_5-2'-ethylsuccinate (lower trace, *m/z* 867). Plasma was spiked with 9.8 μg/ml of erythromycin 2'-ethylsuccinate and 6.5 μg/ml of erythromycin d_5-2'-ethylsuccinate. System D (see Table I) with an acid-treated stainless-steel target.

deviation of the peak areas is 19% for five measurements. Because there is a high buffer concentration [48% (w/w) of 0.1 *M* ammonium acetate] in the mobile phase, only a moderate precision of the method is achieved. However, when a dilute ammonium acetate buffer is used, the method can be applied to the determination of erythromycin 2'-ethylsuccinate. In spite of the mandatory splitting, the sensitivity of the method is sufficient for typical concentration levels of erythromycin 2'-ethylsuccinate in human plasma[19]. It can be concluded that ammonium acetate can be used in the mobile phase with LC–CF-FAB-MS while sufficient selectivity of the LC system is achieved.

Sample solution

In LC, the solvent of the sample solution usually influences both the sensitivity of the method and the chromatographic peak shape. In CF-FAB in the FIA mode, the solvent plug injected is expected to affect the ionization as a result of its influence on the liquid film at the target surface. The latter effect is demonstrated in Fig. 4, in which the reconstructed total ion current chromatogram (RIC, *m/z* 20–1000) and the reconstructed mass chromatograms of the protonated acetonitrile (*m/z* 42) and protonated dextromethorphan (*m/z* 272) are given. When the peak from a replicate injection (without splitting) of a sample containing 11 ng of dextromethorphan in aqueous solution elutes to the surface of the target, the liquid film of the target is disturbed for a few seconds and a decrease in the intensity of the protonated acetonitrile is observed.

In FIA, when small flow-rates (5–7 μl/min) are used, a 0.5- or 1.0-μl injector is usually applied without a splitter. However, the use of such an injector is very critical and readily causes extra peak broadening. The peak broadening of dextromethorphan in Fig. 4 resulted from the use of a 0.5-μl injector. Better peak shapes have been obtained using a 20-μl injector in combination with a high flow-rate and a splitting

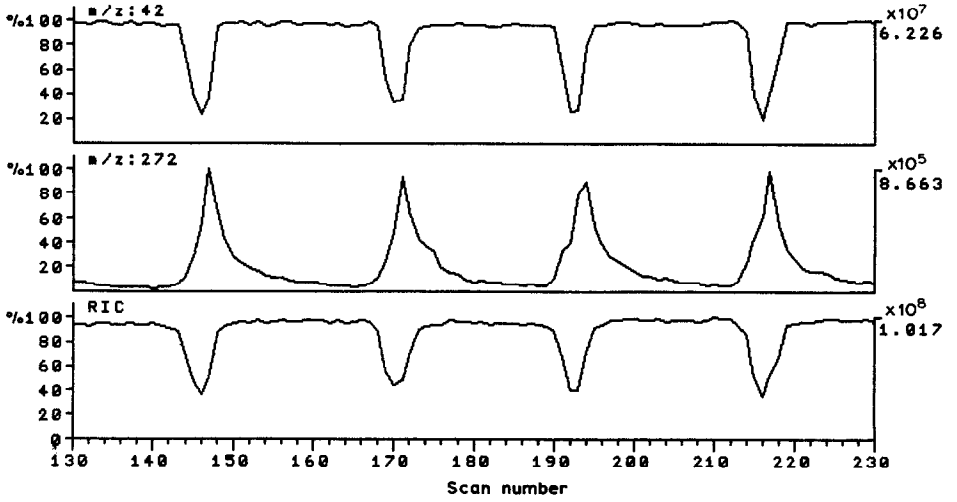

Fig. 4. Influence of the replicate injection of 11 ng of dextromethorphan in water on the baseline stability of the LC–CF-FAB-MS system. Reconstructed total ion current chromatogram (RIC, *m/z* 20–1000) and reconstructed mass chromatograms of protonated acetonitrile (*m/z* 42) and protonated dextromethorphan (*m/z* 272). FIA I (see Table I) with an acid-treated nickel target.

device. When a column between the pump and the injector is used, additional pulse damping is provided. This is shown in Fig. 5 for the flow injection of 960 ng of vindesine, a vinca alkaloid, corresponding to 13 ng of vindesine introduced into the mass spectrometer after splitting. In order to show the baseline stability, the reconstructed selected ion monitoring chromatogram obtained by adding traces of the protonated vindesine and the glycerol background at *m/z* 754 and 737 is given. Under these experimental conditions good peak shapes and stable performance are achieved.

Preformed ions of drugs in complex mixtures have been shown to enhance sensitivity in secondary ion mass spectrometry[16,20]. These methods include derivatization, *e.g.*, with Girard reagents or methylation. A fixed charge is introduced into the molecule before ionization and preformed ions are desorbed from the surface of the conventional FAB probe. To study the effect of preionization in CF-FAB, preprotonation and methylation were compared. A sample containing 40 ng of dextromethorphan (500 pg in the mass spectrometer after splitting, FIA II) was injected as an acidic aqueous solution of pH 2, well below the pK_a of dextromethorphan, which is 8.3. These results were compared with those obtained for 40 ng of methylated dextromethorphan. Single ion monitoring chromatograms of the protonated molecule of dextromethorphan, the protonated glycerol cluster $[3M + H]^+$ and methylated dextromethorphan at *m/z* 272, 277 and 286, respectively, were recorded. When dextromethorphan is methylated, a fixed charge is introduced into the molecule. The derivatization procedure is apparently not completed, because after methylation underivatized dextromethorphan was found. The protonated and methylated dextromethorphan showed similar responses. As a conclusion there does not seem to be any significant difference in sensitivity between a fixed charge or preprotonation for this compound in LC–CF-FAB-MS.

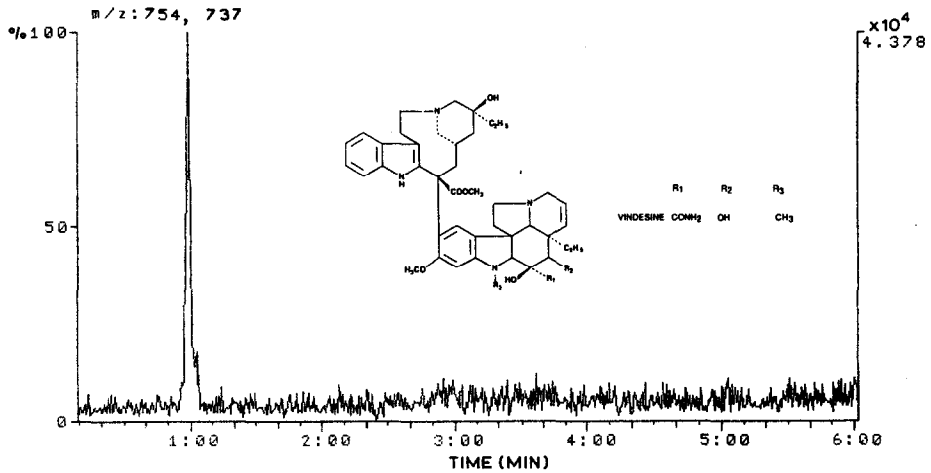

Fig. 5. Reconstructed selected ion monitoring chromatogram obtained by adding the signals corresponding to protonated vindesine (*m/z* 754) and the protonated glycerol cluster (*m/z* 737). The amount injected was 960 ng of vindesine (13 ng in the mass spectrometer). FIA II (see Table I) with an acid-treated stainless-steel target.

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