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Journal of Chromatography A, 970 (2002) 131–140

JOURNAL OF
CHROMATOGRAPHY A

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Developing strategies for isolation of minor impurities with mass spectrometry-directed fractionation

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Abstract

Efficient and automated purification of new chemical entities/potential drug substances and isolation of minor impurities are important aspects of early drug discovery and development strategies, especially when combinatorial synthesis is applied. LC–MS controlled preparative LC and automated fraction collection have been developed for this purpose. The success of such an approach is greatly determined by the quality of the software controlling the application, the coordination between software and hardware, and the reliability of the hardware. The performance of a commercially-available LC–MS controlled autopurification system was evaluated by fractionating four impurities of buspirone as a model compound, eluting closely to the major component under both acidic and basic mobile-phase conditions. A purification strategy for these four components is proposed.
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Keywords: Purification strategy; Mass spectrometry; Fractionation

1. Introduction

The development of combined liquid chromatography–mass spectrometry (LC–MS) has always been of great importance for the pharmaceutical industry, initially primarily for quantitative bioanalysis and for identification of drug metabolites [1]. With the advent of robust and reliable LC–MS technology in the early 1990s, the use of LC–MS was also promoted in other stages of drug development, e.g. drug discovery. The open-access approach [2–5], enabling synthetic chemists to perform an automated, rapid molecular-mass check on their synthetic products, was one of these developments. LC–MS also plays an important role in analytical strategies related to the application of combinatorial chemistry in drug discovery [6–9]. LC–MS was not only applied to ascertain the presence of the expected component in each particular well of a 96-well plate, but also to assess its purity [9,10]. Subsequently, LC–MS controlled purification and preparative-scale chromatography of combinatorial libraries was described [11–14]. LC–MS–MS controlled preparative LC for the purification of drug metabolites was also described [15,16]. The advantages of LC–MS controlled purification over LC–UV-based strategies are in the sensitivity and selectivity at which impurities can be detected, i.e. at a selected mass rather than at an absorbance wavelength, and in the ability to obtain immediate mass spectral information on the impurities.

In a LC–MS controlled purification strategy,

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PII: S0021-9673(02)00399-0

preparative LC is carried out. Unlike UV detection, MS is a destructive technique. Therefore, a post-column split must be applied to send a small fraction of the column effluent to the MS, which continuously monitors for impurities to elute. As soon as an impurity is detected, a fraction collection trigger is sent and the peak of interest is collected from the major fraction of the column effluent into a tube. The success of such a strategy is primarily determined by the "intelligence" of the software controlling the complete procedure and the coordination between software and hardware. A variety of (mostly multi-vendor) systems for MS controlled preparative LC are available on the market, in most cases based on Gilson sample managers (both injection and fraction collection) in combination with a mass spectrometer from one of the MS manufacturers. For reasons of robustness, a single-vendor solution would be preferred over a multi-vendor system. Recently, a new single-vendor LC–MS controlled preparative LC system was introduced, enabling rapid switching between analytical and preparative LC. This system enables either LC–MS or LC–UV (or both) controlled fraction collection from one and the same software platform. The performance of this system in LC–MS control mode was evaluated in this study.

One of the difficulties in impurity profiling [17] by LC–MS is related to the selectivity of the ionization techniques applied, i.e. either electrospray or atmospheric-pressure chemical ionization (APCI). The ionization, and thus the response in LC–MS analysis, is essentially limited by the ability to protonate or deprotonate the analytes of interest. Therefore, relatively nonpolar impurities, not prone to liquid-phase or gas-phase (de)protonation, will be "transparent" in LC–MS-based impurity profiling. The performance of electrospray and APCI is known to be influenced by the solvent composition as well. Therefore, impurity-profiling strategies should be carried out using different solvent conditions and different modes of ionization, e.g. both positive-ion and negative-ion mode. In addition, it is known that coeluting interferences may significantly influence the ionization yield due to matrix suppression effects, especially for electrospray. During preparative LC, such ion suppression effects might be caused by the major component. Non-linear behaviour in the concentration–response relation of the ionization may be another issue of importance, especially when quantitative statements on the amount of particular impurities in the sample are required.

The critical performance test of the commercially available system was done using buspirone, an anxiolytic drug, as a model compound. The study was not aimed at a comprehensive impurity profiling, but primarily directed at isolation of the minor impurities eluting closely to the major component. These compounds, present at approximately 0.1% level or less, were thought to be most challenging in terms of a performance test. The preparative LC is performed in positive-ion electrospray mode and under both acidic and basic conditions, which is a unique feature of the new-generation preparative columns used. Acidic and basic eluent conditions resulted in different chromatographic behaviour of the impurities, both in terms of peak shape, chromatographic resolution, and retention order. From the results obtained, a final purification strategy is proposed.

2. Experimental

2.1. Chemicals and materials

All reagents used were of analytical grade. Methanol, acetonitrile, and formic acid were obtained from J.T. Baker (Deventer, The Netherlands), trifluoroacetic acid (TFA) from Fluka (Buchs, Switzerland), and ammonium hydroxide and ammonium bicarbonate from Sigma (St Louis, MO, USA). High-purity water was produced using a Millipore Milli-Q system (Bedford, MA, USA).

Buspirone ($C_{21}H_{31}N_5O_2$, M_r 385.2 Da, delivered as HCl salt, Lot 91H0802) was purchased from Sigma. It was dissolved at concentrations of 1, 20 or 200 mg/ml in water.

XTerra MS C_{18} 5-μm columns were purchased from Waters (Milford, MA, USA). In analytical chromatography 50 mm×4.6 or 3.0 mm I.D. columns were applied, while in preparative chromatography, 50 mm×19 mm I.D. columns were applied. Separate columns were used for acidic and basic mobile phases.

2.2. Instrumentation

A Waters LC–MS controlled preparative LC system was used throughout this study. The system consists of a 2767 one-bed injection-collection Sample Manager, a 2525 binary high-pressure LC Pump, a Column/Fluidic Organizer (CFO), a 2996 Photodiode Array detector (PDA), a ZQ single-quadrupole mass spectrometer (MS), equipped with a Z-spray electrospray interface, and a WFCII as waste collector. In addition, a Reagent Manager was used for make-up liquid delivery. The CFO contains a 1:1000 Acurate splitter, manufactured by LC Packings (Amsterdam, The Netherlands). The complete system was controlled by MassLynx software version 3.5. A fluidic schematic of the system is shown in Fig. 1.

The electrospray source was running at 3.5 kV capillary voltage, 120 and 250 °C source and desolvation temperature, respectively, and 350 and 50 l/h desolvation and cone gas, respectively. The cone voltage was 30 V in most applications, while cone voltages of 50 and 80 V were applied in some cases to induce fragmentation. Full-scan acquisition between m/z 150 and 600 was performed at a scan speed of 1 s/scan with a 0.1-s inter-scan delay (three 0.3 s/scan channels for cone-voltage fragmentation studies). Positive-ion detection was applied, while some runs in negative-ion mode using the same parameters were performed as well. The solvent delivered to the electrospray interface was split in a 1:5 ratio, delivering ca. 200 μl/min to the interface (100 μl/min when the 3.0 mm I.D. column was used). The on-line PDA UV detector was monitoring between 210 and 400 nm with a scan speed of 1 s/spectrum. UV spectra are only used for compound characterisation but not used as collection trigger.

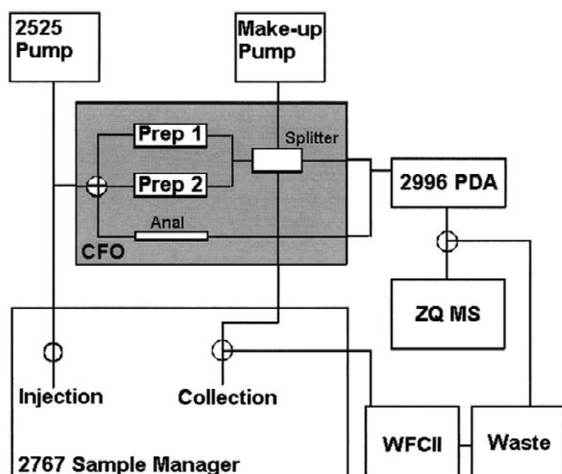

Fig. 1. Schematic diagram of the MS controlled preparative LC system.

2.3. Analytical chromatography

Samples were analysed on a 50 mm×4.6 mm I.D. column under either acidic, using a mobile-phase gradient of water and acetonitrile, both containing 0.05% TFA (pH* 2.4), or basic conditions, using a mobile-phase gradient of 10 mM aqueous ammonium bicarbonate, set to pH 10 using ammonium hydroxide, and acetonitrile. A 10-min linear gradient from 5 to 60% acetonitrile (acidic) or 10 to 60% acetonitrile (basic) was applied. Both gradient programs featured 1 min washing at 95% acetonitrile and 3-min column equilibration prior to the next injection. The total run time was 15 min. Flow-rates were 1.2 ml/min under both conditions.

In the system repeatability tests, five repetitive injections of 20 μl of a 20 mg/ml buspirone solution were carried out under both acidic and basic conditions. To evaluate carry-over effects and repeatability of injection, alternating 20-μl injections of a blank (water) and a 1.0 mg/ml buspirone solution were carried out under acidic conditions. The total number of injections is 11 (six blanks and five samples). These repeatability tests were evaluated using mass chromatograms of the components involved.

2.4. Preparative purification

For preparative LC, the same mobile phases were applied as in analytical LC. However, the gradient programs were slightly different. Sample injection (250 μl, except when specified otherwise) was performed at 4 ml/min for 1 min. Next, the flow-rate was increased to 20 ml/min, taking about 0.2 min, and kept at 20 ml/min for 0.5 min, before the gradient program was started (5–60% acetonitrile under acidic and 10–60% acetonitrile under basic conditions). After washing at 95% acetonitrile for 2 min and column equilibration at starting conditions

for 2 min, the flow-rate was reduced to 4 ml/min prior to the next injection. The total run time was 15 min. A make-up flow-rate consisting of 85% methanol in 0.1% aqueous formic acid at a flow-rate of 1 ml/min was delivered at the splitter in the CFO.

In order to evaluate the fraction collection repeatability, five 250-μl injections of a 200 mg/ml buspirone solution were performed under both acidic and basic conditions. For proper fraction collection, the signal slope at the leading and tailing edge of the chromatographic peak have been specified, typically 60 and 40–60%, respectively, as well as a minimum intensity threshold, depending on the signal of the peak to be collected. The delay between the peak detection by the MS and the arrival of the compound at the collection valve was 25 s for the set-up used. No peaks were collected during the first 90 s after injection in order to avoid eventual collection triggering by the injection peak. Parts of the chromatogram not collected were directed to the WFCII waste collector.

2.5. Purity check of collected fractions

Collected fractions were analysed directly from collection tubes without any additional liquid handling. For each fraction, duplicate injections were carried out on a 50 mm×3.0 mm I.D. column under acidic and/or basic conditions. The same mobile phases and gradient programs were applied as in analytical LC. The flow-rate was set at 0.5 ml/min. The choice of a 3.0-mm I.D. column rather than a 4.6-mm I.D. column is based on the need to improve the sensitivity, as the collected fractions were expected to have very low concentrations. No sample concentration, e.g. by solvent evaporation, was performed.

For a proper purity check of collected fractions by the same system directly after a preparative purification, it was found that an appropriate injector wash procedure was of utmost importance. The 2767 injector-collector has one single injection needle, but two dedicated injection ports for injecting either the highly concentrated solutions onto a preparative column or the diluted collected fractions onto an analytical column. A 4× overfill of the 20-μl loop as well as two wash cycles were applied with methanol as wash solvent. Prior to each series of analytical runs, additional needle washing was enforced and a blank injection with water was run.

Relative peak areas in mass chromatograms were used to evaluate peak purity of collected fractions relative to the major component, thus assuming comparable response factors for the impurities and the major component.

3. Results and discussion

3.1. Preliminary analytical studies

In order to evaluate the LC–MS controlled fraction-collection system, buspirone was selected as a model compound. Initial chromatographic conditions (with both acidic and basic mobile phases) were selected in order to have the buspirone eluting in the centre of the gradient, i.e. around 6 min. This enables both more polar and less polar impurities to be separated.

3.1.1. Acidic conditions

Under acidic conditions, upon injection of 20 μl of a 20 mg/ml solution, only two impurities are detected in the total-ion chromatogram (TIC), i.e. at 4.1 min (most likely $[M+H]^+$ at *m/z* 455.3) and 6.8 min (most likely $[M+H]^+$ at *m/z* 458.3), next to the major component, detected at 5.4 min ($[M+H]^+$ at *m/z* 386.3). A typical chromatogram is shown in Fig. 2a and related information is summarised in Table 1.

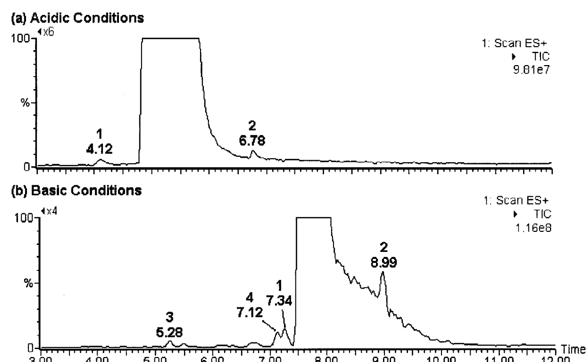

Fig. 2. Total-ion chromatograms of buspirone (20 mg/ml) analysed under (a) acidic and (b) basic conditions. Peaks indicated are: 1, *m/z* 455; 2, *m/z* 458; 3, *m/z* 402; and 4, *m/z* 384. The major peak is due to buspirone (*m/z* 386).

Table 1
Summary of analytical LC data, obtained under acidic and basic conditions

Peak Nr.	Tret (min)	m/z	Peak area	Area%
Acidic conditions				
1	4.12	455.3	3×10^4	0.1
Buspirone	5.40	386.3	6×10^7	99.7
2	6.78	458.3	1.5×10^5	0.2
Basic conditions				
3	5.28	402.3	9×10^4	0.2
4	7.12	384.3	1×10^5	0.2
1	7.34	455.3	2×10^5	0.4
Buspirone	7.81	386.3	4×10^7	97.2
2	9.05	458.3	9×10^5	2

Relative standard deviations (RSD) of the retention time were found to vary between 0.18 and 0.49% (mean 0.33%, $n=5$). The carry-over was found to be 0.26%. The RSD of the peak area was found to be 2%.

3.1.2. Basic conditions

The column used in this study allows the use of both acidic and basic mobile phases without deterioration of its performance [18]. Under basic conditions, with the same amount injected, a larger number of impurities are detected in the total-ion chromatogram. A typical chromatogram is shown in Fig. 2b and related information is summarised in Table 1.

In addition to the impurities already found under acidic conditions, two new impurities have been detected. Impurities at m/z 402.3 and 384.3 have been found to elute, respectively at 5.28 and 7.12 min.

A search for these two impurities under acidic conditions showed that they could be detected in mass chromatograms at 4.14 and 6.12 min, respectively. The impurity with m/z 402.3 co-eluted with m/z 455.3, while the second one (m/z 384.3) was in the peak tailing of the major peak (m/z 386.3).

The sample was also analysed in negative-ion mode (under basic conditions), but no peaks were detected in the TIC. No further experiments in negative-ion mode were carried out.

Relative standard deviations (RSD) of the retention time were found to vary between 0.16 and 0.37% (mean 0.23%, $n=5$). The somewhat lower RSD under basic conditions reflects the generally better peak shape observed under these conditions.

3.1.3. Mass spectra of the impurities collected

The mass spectra of buspirone and some of its impurities were evaluated using three cone voltages (30, 50 and 80 V). Generally, protonated molecules are observed for all compounds without significant adduct ions. For most compounds, a high cone voltage is needed to induce significant fragmentation.

The spectrum of buspirone at a cone voltage of 80 V is shown in Fig. 3, together with a tentative identification of most of the major fragments observed. The mass spectra for all compounds are summarized in Table 2. It may be concluded that buspirone and its impurities show quite different fragmentation patterns with little common fragments or common neutral losses. MS–MS-based strategies as well as NMR spectroscopy will be needed to elucidate the identity of these impurities. No attempts were made to identify the impurities found.

3.2. Preparative purification

3.2.1. Fraction collection procedure

In order to appreciate the fraction collection procedure, it is described here in more detail. The CFO allows a rapid software-controlled switch-over between analytical and preparative LC, as is demonstrated in the schematic diagram in Fig. 1. The CFO contains one 10-port and one six-port switching

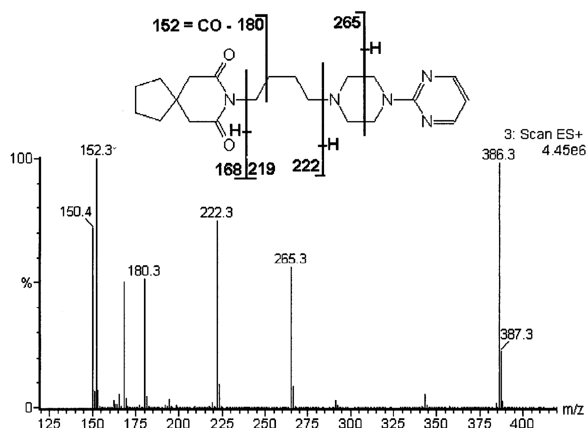

Fig. 3. Positive-ion mass spectrum and proposed fragmentation pattern of buspirone at a cone voltage of 80 V (acidic conditions).

Table 2
Positive-ion mass spectra of buspirone and collected impurities acquired at cone voltages of 30 and 80 V

Compound	Cone at 30 V	Cone at 80 V
Buspirone *m/z* 386	386	386, 343, 265, 222, 180, 168, 152, 150
m/z 402	402	402, 281, 238, 220, 196, 178, 150
m/z 400	400	400, 279, 236, 194, 182, 166
m/z 384	384	384, 162
m/z 455	455	455, 416, 252, 219, 150
m/z 458	458	458, 219, 222, 180, 168, 152, 150

valve: the first one to select either analytical or preparative operation and the second one to select between two preparative columns.

To collect as much of the target compounds as possible, one would prefer loading as much sample to the preparative column as possible. In this study, typically 250 μl of a 200 mg/ml aqueous buspirone solution was loaded onto the 19-mm I.D. column, run at 20 ml/min. This corresponds to a sample load of 0.18 mg/mm^2, which is about seven times higher than in analytical LC. As illustrated by Fig. 1, the column effluent is directed to a 1:1000 splitter, which delivers 19.98 ml/min for fraction collection, while 20 μl/min is delivered towards the MS. However, for MS detection, this column effluent is far too concentrated. Therefore, after splitting the 20 μl/min is diluted with 1 ml/min of make-up liquid. This also increases the linear velocity and reduces the residence time in the connecting tubes. This actually avoids the fact that the trigger from the MS to start the fraction collection comes too late, i.e. when the fraction to be collected has already been sent to waste. The 1.02 ml/min goes via the PDA to the 1:5 splitter in front of the electrospray interface, delivering typically ca. 200 μl/min of a 50-times diluted solution for MS detection. It must be added here that LC–MS controlled preparative LC was described already much earlier, using a similar approach of splitting and make-up liquid by Hsu et al. [19]. However, manual fraction collection was performed in this case.

The software continuously monitors the MS data and searches for a response at the *m/z* specified by the user in the sample list (up to 10 *m/z* values can be selected for one run). As soon as a peak with a specified *m/z* is detected and fulfils collection criteria like minimum intensity threshold and/or leading edge slope, a trigger is sent to the fraction collector. After the specified split-collector delay, the fraction is collected into the next empty tube in the series. When the end of the peak is detected, a second trigger is sent to the fraction collector in order to stop the collection. The collector needle moves to the next empty tube and waits for the next collection trigger. On the chromatogram, the collected fraction of the chromatogram is indicated by a coloured band and the trigger *m/z* and the tube in which the fraction was collected, are specified. Typical examples of such chromatograms are shown in Fig. 4. In this way, one can easily keep track of all the fractions.

3.2.2. Fraction collection of buspirone impurities under acidic conditions

When selecting the impurities to be isolated, it was decided to focus on the most difficult peaks, i.e. eluting just in front or after the major component,

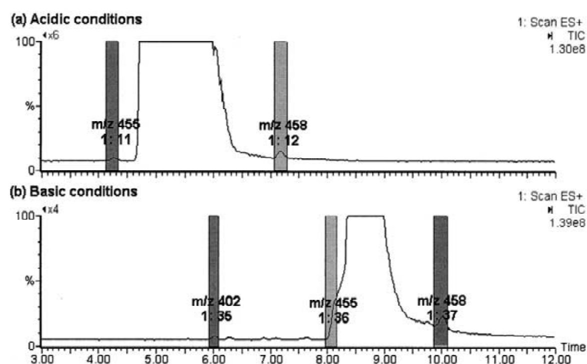

Fig. 4. Total-ion chromatograms of the preparative LC of buspirone under (a) acidic and (b) basic conditions. The fractions collected are indicated as a grey band, with target *m/z* and the tube in which the fraction was collected (rack no. 1: tube no.).

because this would be the most challenging performance test.

Preparative LC under acidic conditions was performed first with two target masses: m/z 455 eluting just in front of the major component, and m/z 458 in the tail of the major component.

After preliminary preparative runs, which allowed the determination of the optimum collection parameters (intensity threshold and start/end slope conditions), a series of five preparative LC runs using 250-μl injections of a 200 mg/ml buspirone solution were performed, enabling evaluation of the between-run repeatability of the width of the fraction collected. One of the chromatograms obtained is shown in Fig. 4a, with the collected fractions indicated. The collection time is ca. 12 s, corresponding to ca. 4-ml fractions collected. The RSD of the fraction width is within 4.5%. The RSD of retention times were better than 0.13% under these conditions.

3.2.3. Fraction collection of buspirone impurities under basic conditions

Next, a series of preparative LC runs were carried out under basic conditions with four target masses: m/z 402, 384, 455 and 458. Five preparative LC runs using 250-μl injections of a 200 mg/ml buspirone solution were performed. The collection time is between 10 and 14 s, corresponding to 3- to 5-ml fractions collected. The RSD of the fraction width is within 5.3%. The RSD of retention times were better than 0.10% under these conditions.

Although in each preparative run, four target masses were used, only three fractions were obtained. Two impurities, i.e. with target masses m/z 384 and 455, eluting just in front of the major component, were not resolved under preparative conditions (Fig. 4b), although they were separated with a chromatographic resolution of ca. 0.8 under analytical LC conditions (cf. Fig. 2b). Moreover, a closer observation of the data acquired with the 250-μl injection of the 200 mg/ml solution showed that the peak of the major component is having a 40-s long plateau at about 10% of its final height before it starts the steep rise to its maximum height (Fig. 5a). The co-eluting peaks of the target masses m/z 384 and 455 actually elute in this fronting plateau. This loss of chromatographic resolution is due to the overloading of the preparative column.

These findings initiated an additional series of experiments to estimate the optimum loadability of the preparative column without compromising the chromatographic resolution. The results are summarized in Fig. 5. Good and repeatable results in terms of chromatographic resolution with respect to the major component were achieved at 140-μl injection, corresponding to 99 $\mu g/mm^2$ (Fig. 5c,d). With a 175-μl injection (123 $\mu g/mm^2$), good resolution was achieved in the first injection, but it was lost in the second one (Fig. 5e,f). While providing a better separation, this almost twofold reduction in sample loading would require a larger number of injections in order to collect the same amount of the impurity. This means that the total time required for the preparative runs would be twice as long.

3.3. Fraction purity check

After the preparative runs, the purity of different fractions was checked by the same system. For these purity checks, samples were injected to the analytical LC directly from the tubes where the fractions were collected.

3.3.1. Analysis of collected fractions under acidic conditions

The purity of the two fractions collected in preparative LC under acidic conditions, i.e. the fractions with target masses m/z 455 and 458, was checked in duplicate, using the 3-mm I.D. column and the acidic mobile phase. The results are summarised in Table 3. From the data, it can be concluded that fraction 1 was obtained as a mixture: next to target mass m/z 455, a component with m/z 402 was collected. The fraction had a relatively good purity (84%) with respect to the major component, buspirone. Concerning fraction 2, the purity of the component with target mass m/z 458 is quite acceptable (91%).

3.3.2. Analysis of collected fractions under basic conditions

As the purity check of fraction 1 under acidic conditions did not allow an accurate purity assessment of the component at m/z 402 with respect to the co-eluting target mass m/z 455, this fraction has

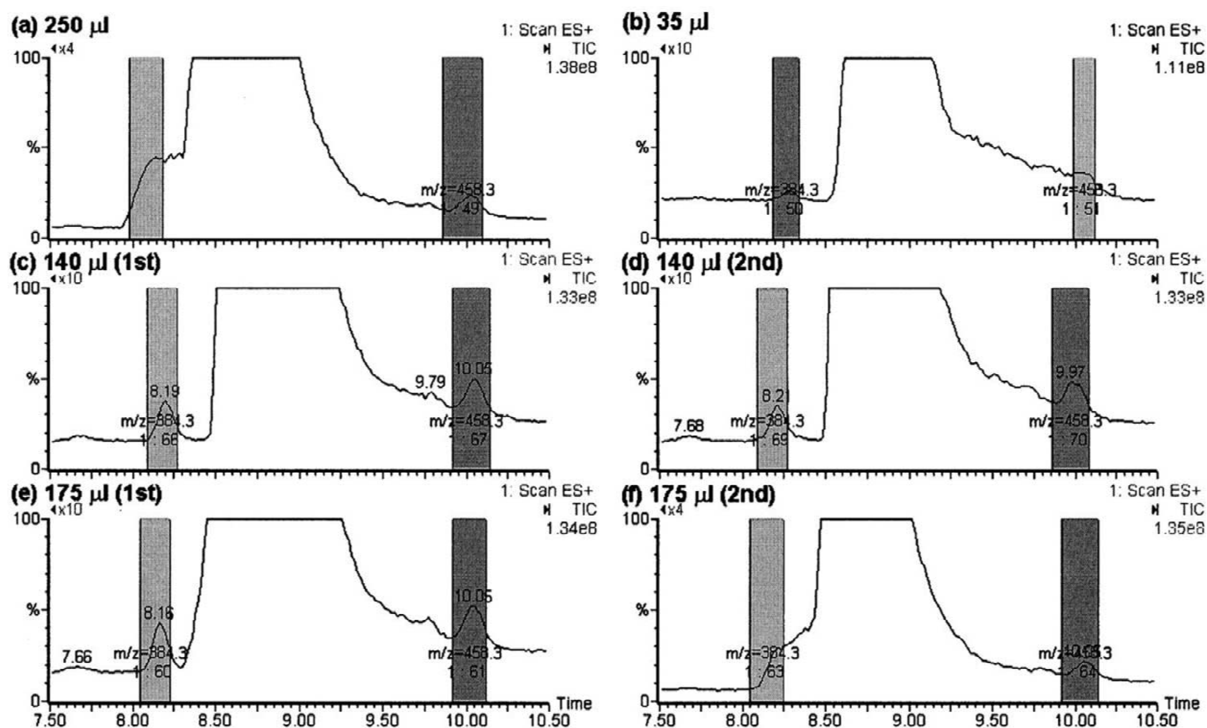

Fig. 5. Influence of the sample load to the preparative column on the chromatographic resolution between the m/z 384 and 455 impurity peak and the major component (m/z 386); (a) 250-µl injection, corresponding to a sample load of 176 µg/mm^2, (b) 35 µl (25 µg/mm^2), (c) first injection of 140 µl (99 µg/mm^2), (d) second injection of 140 µl (99 µg/mm^2), (e) first injection of 175 µl (123 µg/mm^2), (f) second injection of 175 µl (123 µg/mm^2). Basic conditions.

Table 3
Purity check on the fraction collected after preparative LC

Fraction no.	Target m/z	Purity of target (Area%)	Other m/z (Area%)
Acidic conditions			
1	455.3	70.1	402 (14.2) 386 (15.7)
2	458.3	91.1	386 (8.9)
Basic conditions (250-µl injection)			
3	402.3	>99	
4	455.3	4.0	384 (8.6) 386 (87.4)
5	458.3	81.0	386 (19.0)
Basic conditions (140-µl injection)			
4	455.3	23.1	384 (63.0) 386 (13.9)

been analysed under basic conditions as well. In this case, the impurity with m/z 402 is well separated (retention time 5.39 min) from the component with target mass m/z 455 (retention time 7.74 min). The relative purities are 40 and 60% for the components with masses m/z 402 and 455, respectively.

The three fractions obtained in preparative LC under basic conditions, containing the components with target masses m/z 402 (fraction 3, cf. Table 1), 384 and 455 (fraction ‘‘1 and 4’’, cf. Table 1 and below), and 458 (fraction 2, cf. Table 1), respectively, were analysed in duplicate. Actually, two different fractions ‘‘1 and 4’’, obtained from 250- and 140-µl injections (cf. Fig. 5) were analysed separately. The results are summarised in Table 3.

From the results with 250-µl injections, it can be concluded that fraction 3 is >99% pure containing the component with target mass m/z 402, fraction ‘‘1 and 4’’ contains mainly buspirone (m/z 386, 87%) with minor amounts of the components with target

masses m/z 384 (9%) and m/z 455 (4%), and fraction 2 contains the impurity with target mass m/z 458 (81%) with a 19% contamination by buspirone (m/z 386).

In the results with 140-μl injections, which was determined as optimum column loading, no significant improvement of purity was observed for fractions 2 and 3. However, as expected, the buspirone contamination in fraction "1 and 4" was significantly reduced (14%), while the purity of the components with target masses m/z 384 and 455 increased to 63 and 23%, respectively.

3.4. Optimised purification strategy for the isolation of buspirone impurities

From the above analytical results, one may suggest a strategy for the isolation of the four main impurities. First, preparative LC should be performed under acidic conditions with three target masses of m/z 455, 384 and 458 in order to obtain enrichment of these impurities. In each preparative run, three fractions of ca. 4 ml each will be collected, with the first containing a mixture of the component with target masses m/z 402 and 455, the second containing the component with target mass m/z 384 significantly contaminated with buspirone (m/z 386), and the third one containing the component with target mass m/z 458 with a 9% contamination of buspirone. Assuming ca. 1 mg of each impurities should become available in the end and assuming the impurities are present in the buspirone at about 0.1% level, one would need ca. 20 injections of 250 μl of the 200 mg/ml solution, taking 5 h in total (each preparative run lasts 15 min). At the end of those 20 injections, the corresponding fractions can be pooled, resulting in three fractions of ca. 80 ml each, which have to be evaporated to dryness and re-dissolved in a small amount of solvent for further purification. Secondly, the three pooled fractions can be subjected to three preparative LC runs under basic conditions. The first pooled fraction would provide two pure fractions containing the components with target masses m/z 402 and 455, respectively (the retention time difference is 2.1 min under basic conditions). The second run would isolate the component with target mass m/z 384 from the buspirone contamination (retention time difference 0.7 min). The third run would separate the component with target mass m/z 458 from buspirone (retention time difference = 1.1 min). It is worthwhile pointing out that each pooled fraction only needs one injection, thus taking only 45 min, because they contain only a few milligrams of enriched impurities.

4. Conclusions

The potential and power of LC–MS controlled preparative fractionation to isolate minor drug impurities has been evaluated, focusing on some impurities eluting closely to the major component buspirone, which was selected as a model compound. The study shows that with a combination of appropriate software and fully software-controlled hardware, automated fractionation can be a simple and straightforward procedure. However, strategies for optimum fraction collection in a particular application may require some developmental time, especially when impurities eluting close to the major component have to be isolated. The ability of the columns used, enabling analytical and preparative chromatography on the same packing material and under both acidic and basic conditions, was of great help in this study, significantly reducing the developmental time in the isolation procedure. In addition, the current instrumental set-up enables a rapid switch-over between analytical and preparative runs.

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