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Experiences with monolithic LC phases in quantitative bioanalysis

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Abstract

The applicability of monolithic liquid chromatographic (LC) phases in the field of quantitative bioanalysis has been evaluated. Two existing methods with fluorescence detection (the determination of bexarotene in plasma and the determination of dextromethorphan plus metabolites in urine) were successfully transferred from a conventional reversed-phase column to a 10 cm × 4.6 mm i.d. monolith. By simply increasing the mobile phase flow-rate, run times were about 3-fold reduced, while the chromatographic resolution of the analytes remained unaffected. In both cases, a very good correlation was found between the results of clinical samples obtained with the original method and those obtained with the adapted method. Two methods with tandem mass spectrometric detection were set up. For one of these methods (nifedipine in plasma), the separation of the analyte from interfering matrix components did not need a high plate number; the resolution found on a 10-cm monolith at 6 ml/min and that on a 3-cm conventional column at 2 ml/min were comparable and achieved in the same period of time. As the validation results on both column types were similar and considering the limited compatibility of mass spectrometric detection with high solvent flow rates, the conventional column was concluded to be the best choice for this application. For the determination of estradiol in plasma, however, there was so much interfering material that needed to be separated from the analyte, that the best results were obtained with three 10-cm monolithic columns coupled in series and because of the possibility to apply a relatively high flow-rate, a reasonable run time was still achieved.

Keywords: Monolithic LC phases; Bioanalysis; Bexarotene; Dextromethorphan; Nifedipine; Estradiol

1. Introduction

For the past two to three decades, liquid chromatographic (LC) methods have dominated the field of quantitative bioanalysis. Although much progress has been made with regard to the efficiency and speed of these methods, during the last years an optimum seems to have been found. For most bioanalytical applications, LC columns packed with 3–5 μm spherical particles are used, which typically yield 100 000 theoretical plates per meter. Very roughly, these columns produce 1000 plates per min in actual practice. For LC with

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conventional detection, columns 10–25 cm in length (10 000–25 000 plates) are generally used which give run times of about 10–25 min. For methods with–more selective–tandem mass spectrometric (MS/MS) detection, 2000–5000 theoretical plates normally suffice; typical column lengths are, therefore, 2–5 cm and analysis times 2–5 min. Up to now, a further improvement of separation efficiency per unit time, by using either smaller particles or higher mobile phase velocities, has been limited by the accompanying increase in system back-pressure, which cannot be handled by current analytical instrumentation.

In the late 1990s, it was demonstrated that this limitation can be overcome by using monolithic silica, because of its bimodal pore structure [1–3]. Briefly, a monolithic LC column consists of a single rod of silica-based material, which contains relatively large through-pores (typically 2 μm) next to smaller mesopores (12 nm). While the large pores are responsible for a low flow resistance and thus allow the application of high eluent flow-rates at a limited back-pressure, the small pores simultaneously ensure sufficient surface area for uncompromised separation efficiency. Since then it has been reported that monolithic silica can be efficiently applied to the separation of various compounds [4,5]. For 100 × 4.6 mm i.d. columns, flow-rates as high as 9 ml/min can be applied at acceptable back-pressures, resulting in run times of just a few minutes for multi-analyte assays. In addition, the application of flow-gradients was described as an alternative for the more usual gradients with organic modifiers [6,7].

So far, published applications of monolithic columns in the bioanalytical field have been relatively scarce. The use for combinatorial chemistry [6,7] and drug metabolite identification [8,9] has been described and it has been demonstrated that they can also be applied for quantitative purposes, mainly with MS/MS detection and often resulting in an impressive sample throughput [10–13]. It is the aim of this paper to present an overview of our experiences with monolithic columns for quantitative bioanalysis and evaluate their general usefulness in this field. In order to cover as broad an area as possible, their use for LC with conventional and with MS/MS detection is reported. Transfer of existing methods—the determination of bexarotene in human plasma [14] and the determination of dextromethorphan and metabolites in human urine [15]—as well as the development of new methods—the determination of nifedipine and of estradiol in human plasma—are described and discussed. The applicability of the columns is illustrated by presenting (cross-) validation results.

2. Experimental

2.1. Chemicals

The analytical reference standards and internal standards used in the various assay methods were obtained as follows. Bexarotene, its two metabolites and LG100130 (internal standard) were provided by Ligand Pharmaceuticals (San Diego, CA, USA). Dextromethorphan hydrobromide monohydrate, 3-hydroxymorphinan hydrochloride and 3-methoxymorphinan hydrobromide were supplied by Roche (Basel, Switzerland). Dextrorphan tartrate, nifedipine and estradiol were obtained from Sigma (St. Louis, MO, USA). Levallorphan tartrate and nitrendipine were purchased from Promochem (Wesel, Germany) and d4-estradiol from CDN Isotopes (Pointe-Claire, Canada).

Acetonitrite, methanol, acetic acid, hydrochloric acid, *n*-pentane, *n*-hexane, ethyl acetate, isoamylalcohol, triethylamine (TEA), ammonium formate, ammonium acetate, ammonium hydroxyde and sodium carbonate were obtained from Merck (Darmstadt, Germany). Phosphoric acid and potassium dihydrogen phosphate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Standard solutions, calibration and quality control samples

Methanolic stock solutions for bexarotene and LG100130 were prepared at 500 and 50.0 μg/ml, respectively. For dextromethorphan, its metabolites and levallorphan, separate stock solutions

were prepared in 0.1% aqueous TEA at 500 μg/ml of the free base for levallorphan and 1000 μg/ml of the free base for the other compounds. For nifedipine, nitrendipine, estradiol and d4-estradiol 1000 μg/ml methanolic stock solutions were prepared.

Calibration samples were prepared from these stock solutions at the following levels: for bexarotene at 3.00, 10.0, 25.0, 100, 400, 800, 1200 and 1500 ng/ml in human plasma; for dextromethorphan and dextrorphan at 10.0, 25.0, 75.0, 250, 1000, 2500, 5000 and 10 000 ng/ml and 25.0, 50.0, 100, 250, 750, 2500, 5000 and 10 000 ng/ml in human urine, respectively; for nifedipine at 0.500, 1.00, 2.50, 5.00, 10.0, 50.0, 100 and 200 ng/ml in human plasma; for estradiol at 5.00, 20.0, 50.0, 100, 200, 500, 1000 and 2000 pg/ml in human plasma.

Quality control samples were prepared from separately prepared stock solutions at the following levels: at 10.0, 400 and 1200 ng/ml; for dextromethorphan and dextrorphan at 25.0 and 50.0, 250 and 250 and 5000 and 5000 ng/ml; for nifedipine at 1.50, 50.0 and 160 ng/ml. For estradiol no quality control samples were prepared.

2.3. Equipment

For the methods for bexarotene and dextromethorphan, the following equipment was used: a Model 717 autosampler (Waters, Milford, MA, USA), a Julabo (Seelbach, Germany) water bath, a Waters Model M510 pump and a Waters Model 474 fluorescence detector. For the methods for nifedipine and estradiol, the following equipment was used: a HTC PAL (CTC Analytics, Zwingen, Switzerland) autosampler, a CTO-10 (Shimadzu, Kyoto, Japan) column oven, an LC-10 (Shimadzu) pump and an API 4000 (MDS Sciex, Concord, Canada) tandem mass spectrometer.

2.4. Sample preparation

For bexarotene, sample preparation was as described elsewhere [14]. Briefly, after addition of 1250 μl acetonitrile and 1000 μl 0.5 M hydrochloric acid to 1000-μl human plasma, samples were extracted with 5 ml *n*-pentane/isoamylalcohol (98:2, v/v). After evaporation of the extraction solvent, the residue was reconstituted in 500 μl mobile phase, of which 50 μl was injected.

The sample preparation procedure for dextromethorphan and metabolites has also been reported earlier [15]. A 500-μl aliquot of human urine was subjected to hydrolytic deglucuronidation by incubation with 150 μl 10 M hydrochloric acid at 100 °C for 90 min. Subsequently, the sample was alkalinised with 1000 μl saturated sodium carbonate solution and extracted with 6 ml *n*-hexane/ethyl acetate (50:50, v/v). Back-extraction with 150 μl 3% acetic acid was performed and 30 μl of the aqueous phase was injected.

Human plasma samples assayed for nifedipine were pretreated by protein precipitation. To 50-μl aliquots of plasma, 50 μl of methanolic calibration solution (for calibration samples) or 50 μl of methanol (other samples) and 200 μl of methanol, containing the internal standard nitrendipine at 10.0 ng/ml, were added. After mixing and centrifugation at 15 000 × *g* for 5 min, the supernatant was mixed with 200 μl of a 10 mM ammonium formate solution. A 10-μl aliquot of this mixture was injected.

Sample preparation for estradiol was as follows. A 1500-μl aliquot of human plasma was mixed with 75 μl of methanolic calibration solution, 500 μl of water and 50 μl of methanolic internal standard solution (10.0 ng/ml d4-estradiol). This mixture was transferred to a Waters Oasis HLB solid-phase extraction cartridge, which had been conditioned with 1000 μl of methanol and 1000 μl of water. After washing the cartridge with 500 μl of methanol/2% aqueous acetic acid (40:60, v/v) and 500 μl of methanol/2% aqueous ammonium hydroxyde (40:60, v/v), elution was performed with 400 μl of acetonitrile/methanol (70:30, v/v). The eluate was evaporated under nitrogen at 40 °C, the residue dissolved in 75 μl of a mixture of acetonitrile and 0.2 mM ammonium hydroxyde solution (20:80, v/v) and 50 μl was injected.

2.5. Chromatographic and detection conditions

For all applications, separation was performed on a Chromolith Performance RP18e (100 × 4.6

mm i.d.) monolithic column (Merck). For bexarotene, the mobile phase was a mixture of acetonitrile and a 10 mM ammonium acetate buffer (pH 3.0) (80:20, v/v) and the column temperature was 35 °C. The mobile phase flow rate was varied between 1.0 and 4.0 ml/min. Fluorescence detection was performed at an excitation wavelength of 260 nm and an emission wavelength of 430 nm.

The method for dextromethorphan and metabolites used a mobile phase of methanol, 50 mM potassium phosphate buffer (pH 3.0) and TEA (68:32:0.1, v/v). The column was kept at 25 °C and the mobile phase flow-rate was varied between 1.0 and 4.0 ml/min. Fluorescence detection was performed at an excitation wavelength of 230 nm and an emission wavelength of 310 nm.

For nifedipine, the mobile phase was a mixture of methanol and 10 mM ammonium formate solution (70:30, v/v), pumped at a flow rate of 1.0 ml/min (completely transferred to the detector) or 6.0 ml/min (1.5 ml/min directed into the detector and 4.5 ml/min to waste). The column temperature was 40 °C. The MS/MS detector was operated in negative ion mode using an ionspray interface at 550 °C. The mass transition of the m/z 345 to the m/z 122 ion was monitored for nifedipine, versus the mass transition of the m/z 359 to the m/z 122 ion for nitrendipine.

In the estradiol method, three columns were coupled in series. The column temperature was 40 °C and gradient elution was performed with a mobile phase of acetonitrile and 0.2 mM ammonium hydroxyde solution, with the acetonitrile content linearly increasing from 20 to 90% over 12 min. The flow rate was 2.5 ml/min, of which half was transferred into the detector. Detection took place in positive ion mode using an ionspray interface at 700 °C. The m/z 271 to the m/z 145 mass transition was monitored for estradiol, the m/z 275 to the m/z 147 mass transition for d4-estradiol.

2.6. Validation

The feasibility of method transfer from a conventional to a monolithic column was investigated by analysing a set of clinical samples along with calibration and quality control samples, both with the original and the new method. The results obtained by the new method were plotted against the results from the original method and regression analysis was performed. For bexarotene, 14 plasma samples with concentrations varying between 25 and 900 ng/ml were analysed; the mobile phase flow rate through the monolithic column was 4.0 ml/min in this case. For dextromethorphan, 14 urine samples (concentrations 10–900 ng/ml for dextromethorphan and 200–10 000 ng/ml for dextrorphan) were analysed using the original method and on a monolithic column, with a mobile phase flow rate of 2.0 ml/min from 0 to 3 min and 4.0 ml/min from 3 to 8 min.

A limited (one-run) validation was performed for the method for nifedipine, at a flow rate of 6.0 ml/min. Validation samples at five concentration levels (0.500, 1.50, 50.0, 160 and 200 ng/ml) were analysed in 6-fold together with a calibration curve in duplicate. Bias and precision were calculated by means of analysis of variance. Because of its explorative nature, no validation was carried out for the method for estradiol.

3. Results and discussion

3.1. Bexarotene

The original method for the determination of the anti-cancer agent bexarotene [14] is based on a rather straightforward chromatographic separation using a 150×4.6, mm i.d. octadecylsilica (ODS) column. Because of the hydrophobic nature of the analyte, the mobile phase consists of 80% acetonitrile and 20% ammonium acetate buffer, which at a flow rate of 1.0 ml/min and a temperature of 35 °C gives rise to a typical backpressure of 70 bar. Under these conditions, bexarotene elutes around 8.5 min and the internal standard LG100130 around 10.5 min; the total analytical run time is 12 min. In plasma samples taken from subjects dosed with bexarotene, two additional peaks show up in the early part of the chromatogram, which are due to metabolites.

The method could be easily transferred to a monolithic LC phase, with the 100×4.6 mm i.d. monolith used, a comparable separation was

obtained, when the same column temperature and mobile phase composition and flow rate were applied (see Fig. 1); the somewhat shorter run times are obviously caused by the reduced column length. The effect of increasing the mobile phase flow rate was investigated on run time, system back-pressure and the chromatographic resolution between the two metabolites. Table 1 summarises the results. An almost 4-fold reduction in run time could be achieved by simply increasing the flow rate from 1.0 to 4.0 ml/min, while the pressure drop still did not exceed 70 bar. Up to 2.0 ml/min, increasing the flow rate had no effect on the resolution between the metabolites; a further increase in flow rate was accompanied by a decrease in separation efficiency.

The bexarotene concentrations in 14 plasma samples were determined using the monolithic column at 4.0 ml/min and these were plotted against the corresponding concentrations obtained with the conventional method. Table 2 shows that there is an excellent correlation. The major advantage is the reduction in run time: for the 34 injections (study samples plus calibration and quality control samples) it took 6 h 50 min with the conventional method, but only 1 h 25 min using the monolithic phase. In other words, the sample throughout increased from 5 to 24 per hour.

3.2. Dextromethorphan

The quantification of dextromethorphan and its three metabolites in urine is of importance in the field of phenotyping for the cytochrome P450 isoenzymes CYP2D6 and CYP3A4. In the original method [15], an ODS column (150 × 4.6 mm i.d.) and isocratic elution using 24% acetonitrile and 76% phosphate buffer (pH 3.0) are applied; 0.1% TEA is added to prevent peak tailing. The analytical run time is 21 min and the back-pressure (at 1.0 ml/min and 30 °C) typically 70 bar.

It appeared that this method could not be directly transferred to a monolithic column. With acetonitrile as the organic modifier a very poor separation of the analytes was obtained, indicating an entirely different selectivity of the monolithic column is this case. Separation of the four analytes and the internal standard in a similar run time of 21 min could be achieved by using 32% methanol

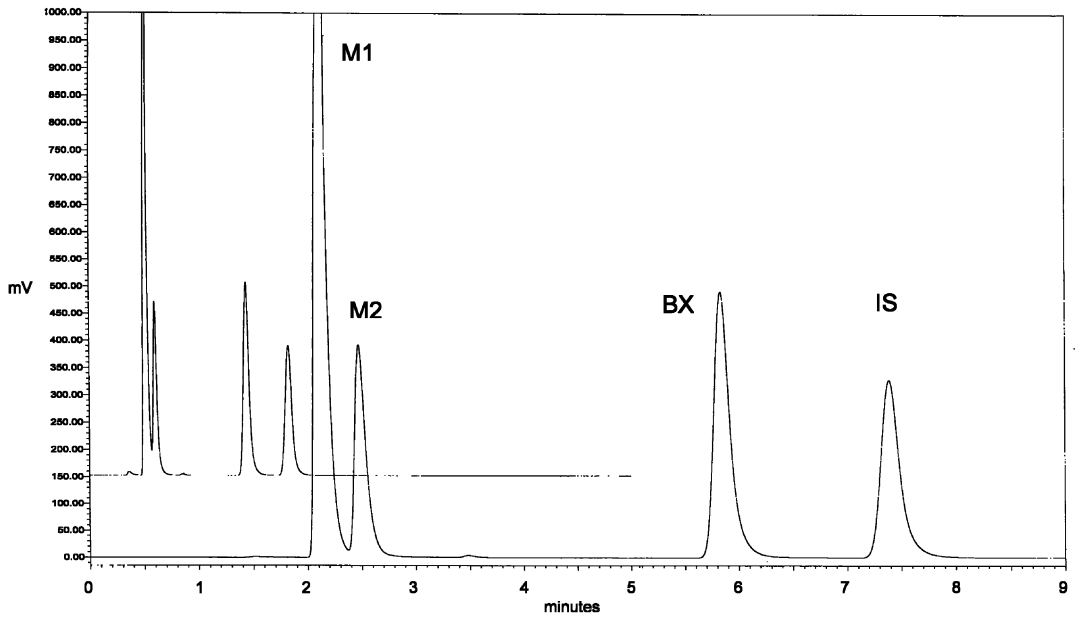

Fig. 1. LC-fluorescence chromatograms of a standard solution containing two bexarotene metabolites (M1 and M2), bexarotene (BX) and internal standard (IS) on a 10 cm monolithic column; upper trace: 4.0 ml/min, lower trace: 1.0 ml/min.

Table 1
Analytical characteristics of the method for bexarotene on a 100 × 4.6 mm i.d. monolithic column

Mobile phase flow rate (ml/min)	Back-pressure (bar)	Run time (min)	R_s (M1 and M2)
1.0	15	8.5	1.6
2.0	30	4.2	1.6
3.0	50	2.8	1.4
4.0	70	2.2	1.2

and 68% phosphate buffer at a column temperature of 25 °C and a flow rate of 1.0 ml/min (see Fig. 2). Also on this column the addition of 0.1% TEA was necessary to reduce peak tailing, which illustrates that secondary interaction with free silanol groups also takes place in the monolithic column used.

Table 3 shows the effect of increasing the flow rate on run time, system back-pressure and the chromatographic resolution between the two most polar metabolites hydroxymorphinan and dextrorphan. As for bexarotene, increasing the flow rate from 1.0 to 4.0 ml/min causes a 4-fold reduction in run time, but the back-pressure at 4.0 ml/min is much higher, obviously due to the higher water content of the mobile phase and the lower column temperature. Again, the separation efficiency decreases above 2.0 ml/min, as can be concluded from the decreasing resolution between hydroxymorphinan and dextrorphan at 4.0 ml/min. In order to decrease the analytical run time and at the same time maintain an optimum resolution between the metabolites, a flow gradient was applied. After elution of hydroxymorphinan and dextrorphan (3 min) the flow rate was increased from 2.0 to 4.0 ml/min, thus reducing the run time to 8 min (Fig. 2).

Using this flow gradient, the concentrations of dextromethorphan and DXT (the two analytes determining the CYP2D6 phenotype) were determined in 14 urine samples and compared with the results obtained with the original method. Again, the correlation was very good (Table 2). The reduction of the total run time for 34 injections is from 11 h 55 min to 5 h 10 min, which corresponds to an increase in sample throughput from less than 3 per h for the original method to 7.5 for the method using the monolithic column.

3.3. Nifedipine

For methods with mass spectrometric detection, it is important that the analytes are chromatographically separated from compounds interfering with their ionisation. Because of the high selectivity of the detection, these interfering compounds generally do not show up in the chromatograms, but they can be visualised. By post-column addition of a standard solution of the analyte to the column effluent and subsequently into the ion source of the mass spectrometer, an elevation of the baseline is brought about and any compound present in the sample, which interferes with the ionisation process, is seen as a decrease or increase in the baseline [16].

The separation between the cardiovascular drug nifedipine and interfering plasma components is shown in Fig. 3. A distinct decrease in the baseline

Table 2
Results of regression analysis of concentrations in study samples obtained on a monolithic column vs. those obtained on a standard ODS column

Compound	Slope of regression line	Correlation coefficient	Mean relative difference (%)
Bexarotene	1.010	1.0000	0.8
Dextromethorphan	0.980	0.9992	4.8
Dextrorphan	1.031	0.9973	3.8

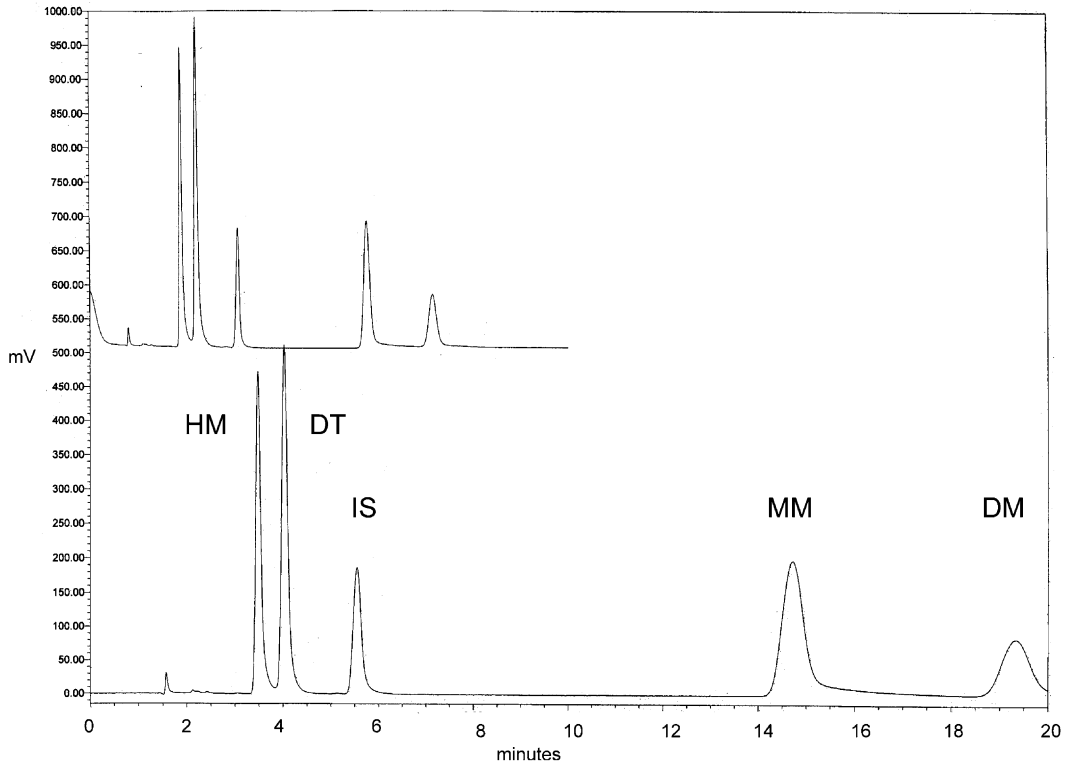

Fig. 2. LC-fluorescence chromatograms of a standard solution containing hydroxymorphinan (HM), dextrorphan (DT), internal standard (IS), methoxymorphinan (MM) and dextromethorphan (DM) on a 10 cm monolithic column; upper trace: 2.0 ml/min from 0 to 3 min and 4.0 ml/min from 3 to 10 min, lower trace 1.0 ml/min.

at the column dead time clearly shows the elution of endogenous plasma compounds, which suppress the ionisation of nifedipine. On a monolithic column, increasing the mobile phase flow rate from 1.0 to 6.0 ml/min causes a 5-fold reduction of the run time to just 0.4 min (Table 4), while the separation between the analyte and suppressing matrix components, although somewhat less favourable at 6.0 ml/min, is still sufficient. Although the feasibility of using monolithic columns with MS detection was thus demonstrated, the major practical problem of this approach lies in the limited compatibility of MS detection with high eluent flow rates. Traditional MS detectors can only handle up to typically 0.5 ml/min and for the newer types, of which one was used for this study, flow rates introduced into the detector above 2.0 ml/min are not recommended, which means that a

Table 3
Analytical characteristics of the method for dextromethorphan on a 100 × 4.6 mm i.d. monolithic column

Mobile phase flow rate (ml/min)	Back-pressure (bar)	Run time (min)	R_s (HM and DT)
1.0	45	21.0	2.6
2.0	90	10.5	2.6
4.0	160	5.5	2.1
2.0 (0–3 min)	90 (0–3 min)	8.0	2.6
4.0 (3–8 min)	180 (3–8 min)		

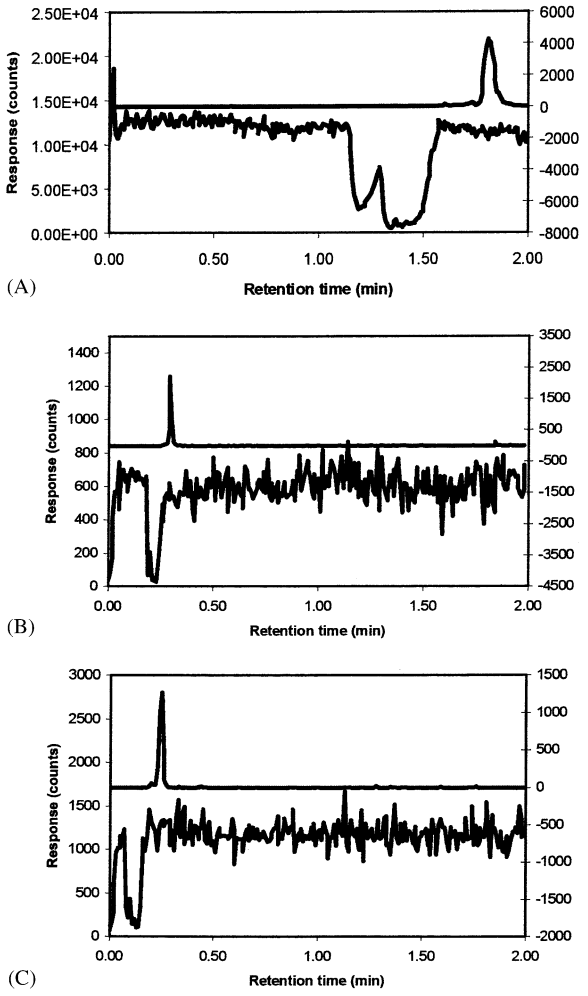

Fig. 3. LC-MS/MS chromatograms of a plasma extract containing 0.5 ng/ml nifedipine (upper traces, right-hand y-axes) and a blank plasma extract with post-column infusion of nifedipine (lower traces, left-hand y-axes) on (A) a 10 cm monolithic column at 1.0 ml/min, (B) idem at 6.0 ml/min and (C) a 3 cm ODS column at 2.0 ml/min.

major part of the eluent needs to be split off prior to detection. For this reason, an investigation was made to see if the same run time could be achieved at a lower flow rate. As is shown in Fig. 3, the application of a short (3 cm) ODS column at 2.0 ml/min gives an essentially similar elution profile for nifedipine and matrix components. In other words, for this particular application, the higher number of theoretical plates of the 10 cm monolith is not necessary to obtain an adequate separation between analyte and suppression matrix components.

A one-run validation of both methods showed comparable results for precision (CV between 1.7 and 9.3% for the conventional column and between 1.6 and 9.4% for the monolith) and accuracy (bias between −0.7 and +7.1% for the conventional column and between +2.0 and +11.4% for the monolith).

The total run time was 20 min for 50 samples in both cases, but whereas the mobile phase consumption on the conventional column was only 40 ml, it was 120 ml on the monolithic column, which illustrates that the use of a short conventional column is to be preferred over a 10 cm monolithic column.

3.4. Estradiol

The determination of the steroid hormone estradiol in plasma with LC-MS/MS is complicated by the very low concentrations of this compound and the presence of much higher levels of a large number of structurally very similar steroids in human plasma. The use of a monolithic column in the normal way, i.e. using isocratic elution at a relatively high modifier concentration (70% acetonitrile) and flow rate (2.5 ml/min), resulted in a very short chromatographic run

Table 4
Analytical characteristics of the method for nifedipine

Column	Mobile phase flow rate (ml/min)	Flow into MS (ml/min)	Back-pressure (bar)	Run time (min)
100 × 4.6 mm i.d. monolith	1.0	1.0	40	2.0
100 × 4.6 mm i.d. monolith	6.0	1.5	180	0.4
30 × 4.6 mm i.d. ODS	2.0	1.0	120	0.4

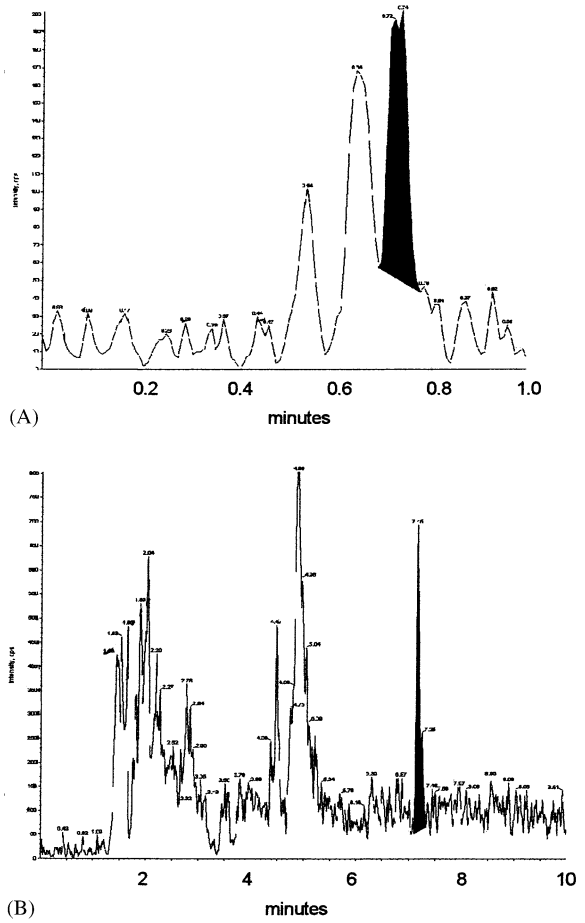

Fig. 4. LC-MS/MS chromatograms of a plasma extract containing 20 pg/ml estradiol on (A) one 10-cm monolithic column with isocratic elution and (B) three 10-cm monolithic columns with gradient elution.

time, but also to severe interference of matrix constituents in the chromatogram (Fig. 4A). Moreover, the peak responses of the analyte in plasma were approximately 10-fold lower than those in standard solutions of the same concentration, which clearly indicates the presence of co-eluting material with a suppressing effect on the ionisation of estradiol.

Clearly, for this application there was need for a better chromatographic separation of the analyte from the matrix and it was tested if better results could be obtained when several monolithic columns were coupled in series. Fig. 4B shows a chromatogram of a plasma sample analysed on three monolithic columns and using an acetonitrile gradient from 20 to 90% in 12 min. In this case, the estradiol peak was well separated from interfering peaks in the chromatogram and the reduction in peak response by switching from a test solution to a plasma extract was now less than 2-fold. Even though a 30-cm column was used, a flow rate of 2.5 ml/min could be applied at a back-pressure not exceeding 200 bar, resulting in a run time of 12 min. Increasing the number of columns to four or five extended the run time but did not improve the response of the analyte, which seems to indicate that it is difficult to completely separate the trace amounts of estradiol from the excess of its structural analogues that cause ionisation suppression.

4. Conclusion

Monolithic LC columns are a useful means of increasing the separation efficiency per unit time of chromatographic methods, which can very simply be achieved by increasing the mobile phase flow rate. In the field of quantitative bioanalysis, they can be successfully applied in combination with both conventional and tandem mass spectrometric detection. In conventional LC, the increased efficiency per unit time can routinely be applied to reduce chromatographic run times at least 3-fold, while maintaining the resolution between analyte peaks. Although the applicability may obviously vary from one method to the other, the results shown here support the idea that most chromatographic assays on standard reversed-phase columns can be rather easily transferred to commercially available monolithic LC phases. A useful feature is the possibility of applying flow gradients as an alternative for modifier gradients, which has the advantage that no equilibration time is necessary. The fact that the consumption of mobile phase per unit time is much higher than for conventional methods is compensated for by the much shorter run times of each analysis, which means that the total solvent consumption per analysis is comparable with that of conventional LC methods.

For LC-MS/MS methods, the main benefit of monolithic columns lies in their ability to improve the separation efficiency without extending the run times. As the incomplete separation of an analyte from matrix components often leads to difficulties with regard to the reproducibility of quantitation because of interference in the ionisation process, monoliths can be very helpful to tackle this often rather neglected problem. Still, the examples presented in this paper show that the extent to which ionisation suppression occurs depends very much on the application. If the effect is negligible, the application of conventional columns at conventional flow rates is to be preferred, because of the limited compatibility of MS/MS detection with high eluent flow rates. In this respect, the availability of narrow-bore monolithic columns, which could produce a similar effect at lower flow rates, would be of enormous significance for the applicability in the field of quantitative bioanalysis with LC-MS/MS.

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