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Increasing sample throughput in pharmacological studies by using dual-column liquid chromatography with tandem mass spectrometry

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Abstract

A robust novel technology of parallel chromatography combined with tandem mass spectrometry was successfully applied to a biological matrix extract for analyte detection. The presented study shows how only by using an additional isocratic pump, a second column and a 10-port valve the throughput is twice of that of a conventional single column system with the same sensitivity. Analytes and matrix were separated and eluting peaks of the first column were detected while the second column was equilibrated. The system was tested and used for the determination of several drugs, metabolites and endogenous compounds (i.e., propiverine, talinolol, scopolamine and leukotrienes).

Keywords: Pharmaceutical analysis; Column switching; Propiverine; Talinolol; Scopolamine; Leucotrienes

1. Introduction

To achieve high-quality analytical data for samples originally in biological fluids, good chromatographic retention of the analyte is preferred to minimise signal suppression and other matrix effects. Thus, at the beginning of the separation quite a large portion of the chromatographic cycle time usually does not contain any useful information. Furthermore gradient elution mode in chromatography is widely used for the screening and a gradient needs to be recycled for each sample. As a result, at the end of the separation another large portion of the cycle time does not contain any useful information either. Column switching can accelerate the analytical method [1–8].

A robust novel technology of parallel chromatography using two columns was successfully applied to a biological matrix extract for analyte detection. Eluting peaks of a column were detected while the chromatography of the analyte from the next injection was performed on the other column. Quantitative liquid chromatography–tandem mass spectrometry (LC–MS–MS) data were acquired using parallel chromatography. The system was tested and used for the measurement of the anticholinergic bladder spasmolytic drug propiverine and its main metabolite [9], the β-adrenoceptor antagonist talinolol [10], the anticholinergic akaloid scopolamine [11] and the postulated endogenous compound 16-carboxy-tetranor-leukotriene E3 (LTE3) [12,13] derived from arachidonic acid.

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2. Experimental

2.1. Chemicals

Propiverine hydrochloride and its N-oxide were kindly provided by Apogepha Dresden (Dresden, Germany). Talinolol was a product of Arzneimittelwerk Dresden (Dresden, Germany). Scopolamine hydrobromide DAB 10 was provided by Caesar u. Kretz (Hilden, Germany). 16-Carboxy-tetranorleukotriene E3 was obtained from Cayman (Ann Arbor, MI, USA). Acetonitrile LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography), formic acid (analytical-reagent grade) and ammonium acetate (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany). Pure water (18 MΩ) was obtained using an ion-exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. LC–MS–MS analysis – apparatus and chromatographic conditions

The used LC–MS–MS system was an API 3000 (PE Sciex, Concord, Canada) equipped with a turbo ion spray interface. Full scan mass spectra were acquired by continual infusion of standard solutions (concentration 100 ng/ml with 10 μl/min). The product ion mass spectra were obtained by choosing the molecular ions as the precursor ions, scanning Q2 from m/z 100–450 and optimised with the help of the program Autotune. For the measurements of the analytes the multiple reaction monitoring (MRM) mode was used. The capillary voltages were 5300 V for propiverine and its N-oxide, 5500 V for talinolol, 5800 V for scopolamine (all positive ionisation) and −4500 V for LTE3 (negative ionisation). The temperature in the turbo ion spray source was 400°C at a gas flow of 8 ml/min (N_2).

A 10-port valve, the use of an isocratic pump and a binary pump allow a simultaneous use of two C_{18} endcapped, 55 mm×2 mm, 5 μm Purospher STAR (Merck) columns. The HPLC equipment consisted of an isocratic pump (LC6 Shimadzu, Kyoto, Japan), a binary pump system, an autosampler (both series 200, Perkin-Elmer, Norwalk, CT, USA) and a precolumn C_{18}, 4×2 mm (SecurityGuard, Phenomenex, Torrance, CA, USA) (Fig. 1).

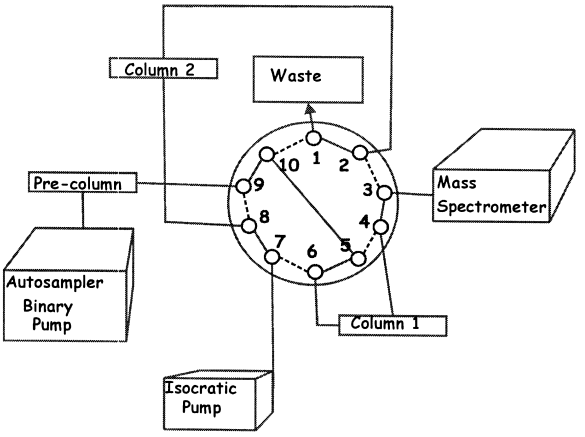

Fig. 1. Schematic flow diagram of the employed column-switching system.

Mobile phase gradients were applied with solvent A (5:95:0.2, v/v) and solvent B (95:5:0.2, v/v) of a mixture of acetonitrile–ammonium acetate in water (2 mmol/l)–formic acid. A typical time program which was used for the drug propiverine and its N-oxide is shown:

Time (min)	0	0.2	1.2	2.7	3.2	4.2
A (%)	100	100	0	0	100	100

The flow-rate was 0.4 ml/min. Within this time (4.2 min) analytes and matrix were separated, eluting peaks of the first column were detected and quantitative LC–MS–MS data were acquired while the second column was equilibrated with solvent A for the next injection with the additional isocratic pump. After 4.2 min the 10-port valve was switched and the next sample were injected onto the second column. All subsequent analyses were performed alternating during the whole procedure. The mobile phase gradients and the column switching procedure for the other compounds were similar.

Peak areas and the calibration curve were obtained using the program TurboQuan (PE Sciex).

2.3. Sample preparation

Propiverine and its N-oxide, talinolol, scopolamine

in serum samples and the LTE3 in urine samples were extracted and cleaned up by using an automated solid-phase extraction method in an ASPEC XL sample processor (Gilson) [9,11,12]. All liquids and air were pressed through the cartridges. This is in contrast to the most other tools which draw the liquids through the cartridges. Following cartridges were used: Oasis (Waters) for scopolamine and talinolol, Nexus (Varian) for propiverine and its N-oxide and a combination of Discovery (Supelco) and Nexus for LTE3.

3. Results

3.1. Mass spectrometry

The mass spectra of propiverine, propiverine-*N*-oxide, talinolol and scopolamine revealed base peaks at m/z 368, 384, 364 and 304, respectively, corresponding to the molecular ion $(M+H)^+$; and LTE3 revealed a base peak at m/z 414 corresponding to the molecular ion $(M-H)^-$. The product ion mass spectra were obtained by choosing the molecular ions as the precursor ions. The fragment ions observed at m/z 105, 116, 183 and 308 for propiverine, at m/z 183 and 225 for propiverine-*N*-oxide, at m/z 209, 226 and 308 for talinolol, at m/z 138 and 156 for scopolamine and at m/z 211, 265, 309, 327 and 396 for LTE3 (Table 1). Normally fragment ions with the highest intensity were chosen but in cases of a complex matrix and incomplete chromatographic separation a high specificity of the transition is likewise important for the selection of the best quantification method.

3.2. Chromatography

To achieve high-quality analytical data for samples originally in biological fluids, good chromatographic retention of the analyte is preferred to minimise signal suppression and other matrix effects. A mixture of acetonitrile–ammonium acetate in water (2 mmol/l)–formic acid gave a high sensitivity of the analytes: propiverine and its N-oxide, talinolol, scopolamine and LTE3. Using an acetonitrile gradient, small peaks were observed. To exclude interferences from the biological matrix chromatograms of two transitions between the molecular ions and the product ions were controlled separately for all analytes. Negligible interferences and a low background noise were found for propiverine (Fig. 2) and its N-oxide (Fig. 3), talinolol and scopolamine. The most intensive transition of LTE3 between m/z 414 and m/z 327 showed often interferences in urine samples in the concentration range

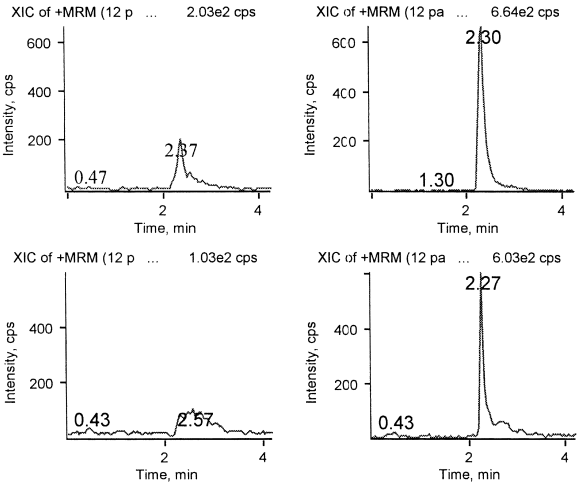

Fig. 2. MRM chromatograms of propiverine extracted from serum. MRM was performed by monitoring the transitions between m/z 365 and m/z 105 and between m/z 365 and m/z 308; (left) blank serum, (right) blank serum spiked with 780 pg/ml propiverine (lowest point of the standard curve).

Table 1
Mass spectrometry; used ions

Substance	Molecular mass	Precursor ion	Product ions	Used transition
Propiverine	367	368	105, 116, 183 and 308	368→105
Propiverine-*N*-oxide	383	384	183 and 225	384→183
Talinolol	363	364	209, 226 and 308	364→308
Scopolamine	303	304	138 and 156	304→138 and 304→156
Leucotriene E3	415	414	211, 265, 309, 327 and 396	414→211

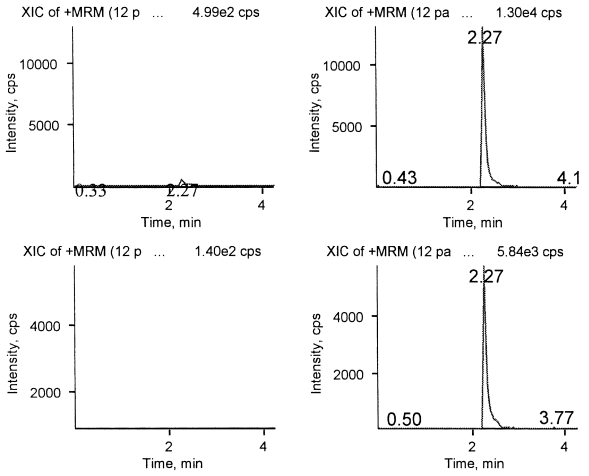

Fig. 3. MRM chromatograms of propiverine-*N*-oxide extracted from serum. MRM was performed by monitoring the transitions between *m*/*z* 384 and *m*/*z* 183 and between *m*/*z* 384 and *m*/*z* 225; (left) blank serum, (right) blank serum spiked with 3.12 ng/ml propiverine-*N*-oxide (lowest point of the standard curve).

lower than 5 ng/ml. The interferences of the urine matrix with the transition between *m*/*z* 414 and *m*/*z* 211 of LTE3 was about 10-times lower.

The retention times of all described compounds were between 2.5 and 4 min. The overall chromatographic cycle time without column switching was 10 min because the gradient needs to be recycled for each sample. A robust novel technology of parallel chromatography using two equal columns was applied to biological matrix extract for analyte detection. Analytes and matrix were separated and eluting peaks of the first column were detected while the second column after the gradient were equilibrated for the next injection. After 4.2 min the 10-port valve was switched and the next sample was injected.

Quality control samples or standards were injected twice to control the precision and show the property of the columns. The samples had to give the same results irrespective of the used column. If the difference was more than 5% the columns were changed.

3.3. Quantification

The calibration graphs were generated from MRM of increasing amounts of drug standard in blank serum samples. A quadratic calibration graph was constructed using least-squares regression of quantities versus peak area ratio. Using a sample volume of 200 μl a good response over the range of 780 pg/ml to 200 ng/ml serum was demonstrated for propiverine. The correlation coefficient of regression lines was 0.9992 or higher. The precision and

Table 2
Precision and accuracy of the analytical method for propiverine from six independent sets of spiked serum samples

	Concentration of propiverine (ng/ml)									*r*
	Added									
	0.78	1.56	3.12	6.25	12.5	25	50	100	200	
	Found									
(1) SC	0.91	1.27	3.45	5.58	13.2	23.9	48.8	104.0	198	0.9994
(2) SC	0.90	1.33	3.16	5.70	12.8	25.6	53.0	95.4	201	0.9993
(3) SC	0.70	1.68	2.74	7.04	13.1	25.2	47.6	101.0	200	0.9996
(4) SC	0.72	1.71	3.13	6.31	12.4	23.1	53.9	97.5	201	0.9993
(5) SC	0.67	1.58	3.25	6.76	11.8	26.2	52.3	95.1	202	0.9995
(6) SC	0.76	1.44	2.84	7.07	14.0	22.9	53.1	96.0	201	0.9992
Mean	0.78	1.50	3.10	6.41	12.9	24.5	51.5	98.2	201	
SD (%)	0.105	0.183	0.263	0.66	0.75	1.38	2.60	3.6	1.4	
RSD (%)	13.4	12.2	8.5	10.2	5.8	5.6	5.0	3.7	0.7	
Accuracy (%)	99.8	96.3	99.2	102.6	103.1	97.9	102.9	98.2	100.3	

SC=Standard curve; SD=standard deviation; RSD=relative standard deviation; *r*=coefficient of correlation.

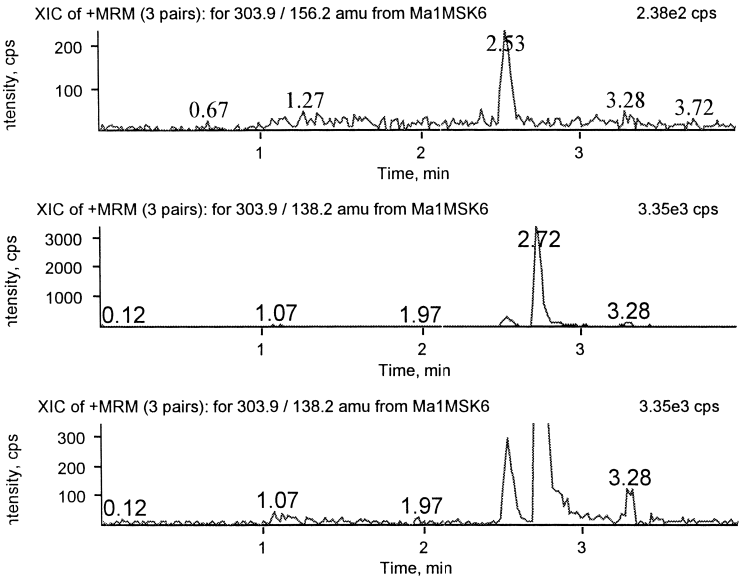

Fig. 4. TIC and MRM chromatograms of scopolamine extracted from blank serum spiked with 100 pg/ml scopolamine. (a) Transition between m/z 304 and m/z 156, (b) transition between m/z 304 and m/z 138, normalised to endogenous base peak, (c) transition between m/z 304 and m/z 138, normalised to scopolamine peak.

accuracy of the method was assessed by the determination of nine concentrations in six independent series of spiked serum samples as shown in Table 2. The accuracy for added propiverine ranged from 96.3 to 103.1%. The relative standard deviation (RSD) ranged from 0.7 to 13.4%. The lower limit of quantification, i.e., a relative standard deviation $<$20% for six repeated measurements, is 780 pg/ml. Typical chromatograms obtained from extracted serum samples are illustrated in Fig. 4. A recovery of 80% from the serum matrix was found irrespective of the concentration. Day-to-day precision data were obtained over a period of 5 working days by taking aliquots of serum with 7, 85 and 180 ng/ml propiverine, respectively, and processing them daily. Adequate relative standard deviations were found: 7.3% (lowest concentration), 10.4% (medium concentration) and 6.4% (highest concentration). The main metabolite propiverine-*N*-oxide was measured simultaneously with similar results.

Following lower limits of quantification were obtained for the other substances: talinolol 200 pg/ml, scopolamine 50 pg/ml and LTE3 500 pg/ml.

4. Discussion

LC–MS–MS is a powerful technique for highly specific and quantitative measurement of very low levels of analytes in biological matrices.

Because of the high specificity of the MS–MS method a complete chromatographic separation of analytes and matrix is not necessary. But in many cases, especially to measure extremely low levels of analytes in serum or urine, good chromatographic separation of the analyte is preferred to minimise signal suppression and other matrix effects. For instance in blank serum you often find the typical transition of scopolamine m/z 304$\rightarrow m/z$ 138. But the other typical transition m/z 304$\rightarrow m/z$ 156 is missing and the retention time of this substance is different to scopolamine. Using only one transition and measurement without sufficient chromatographic separation can lead to false results (Fig. 4).

To achieve small and high peaks a steep gradient from 5 to 95% acetonitrile was advantageous. For the peak shape and the reproducibility it is important to recycle the gradient for each sample completely.

Therefore a long chromatographic cycle time of about 10 min was found for the analytes, but a large portion of this time does not contain any useful information. The presented study shows how only by using an additional isocratic pump, a second column and a 10-port valve the throughput is twice of that of a conventional single column system. The parallel chromatography method for the determination of propiverine and its N-oxide were completely validated. The methods for talinolol, scopolamine and leucotriene E3 were adapted from hitherto existing single column methods [10,11].

The capacity of one ASPEC XL sample processor for the described automated solid-phase extraction methods was 6–10 samples per hour. Two systems were used for the increased sample throughput.

5. Conclusion

LC–MS–MS is the most sensitive method for quantitating propiverine, talinolol and scopolamine in serum and for LTE3 in urine samples. Furthermore the assays requires an automatic off-line sample preparation. The parallel chromatography configuration allowed the throughput to be twice of that of a conventional single column system. These methods are suitable for pharmacological studies.

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References

[1] S.H. Wong, J. Pharm. Biomed. Anal. 7 (1989) 1011.
[2] P. Campins-Falco, R. Herraez-Hernandez, A. Sevillano-Cabeza, J. Chromatogr. 619 (1993) 177.
[3] J. Zagrobelny, C. Chavez, M. Constanzer, B.K. Matuszewski, J. Pharm. Biomed. Anal. 13 (1995) 1215.
[4] R. Oertel, K. Richter, T. Gramatté, W. Kirch, J. Chromatogr. A 797 (1998) 203.
[5] A. Marchese, C. McHugh, J. Kehler, H. Bi, J. Mass Spectrom. 33 (1998) 1071.
[6] J. Zweigenbaum, K. Heinig, S. Steinborner, T. Wachs, J. Henion, Anal. Chem. 71 (1999) 2294.
[7] Z. Wang, C.E. Hop, K.H. Leung, J. Pang, J. Mass Spectrom. 35 (2000) 71.
[8] Y.Q. Xia, D.B. Whigan, M.L. Powell, M. Jemal, Rapid. Commun. Mass Spectrom. 14 (2000) 105.
[9] K. Richter, S. Scheithauer, D. Thümmler, J. Chromatogr. B 708 (1998) 325.
[10] R. Oertel, K. Richter, B. Trausch, A. Berndt, T. Gramatté, W. Kirch, J. Chromatogr. B 660 (1994) 353.
[11] R. Oertel, K. Richter, U. Ebert, W. Kirch, J. Chromatogr. B 750 (2001) 121.
[12] M. Mizugaki, T. Hishinuma, N. Suzuki, J. Chromatogr. B 729 (1999) 279.
[13] J.H. Yuan, J. Birkmeier, D.C. Yang, J.D. Hribar, N. Liu, R. Bible, E. Hajdu, M. Rock, G. Schoenhard, Drug Metab. Dispos. 24 (1996) 1124.