Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1080 (2005) 99–106
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development and application of a new on-line SPE system combined with LC–MS/MS detection for high throughput direct analysis of pharmaceutical compounds in plasma

Yazen Alnouti, Karthik Srinivasan, David Waddell, Honggang Bi, Olga Kavetskaia, Arkady I Gusev *

Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Eastern Point RD MS 4022, Groton, CT 06340, USA

Received 9 February 2005; received in revised form 4 April 2005; accepted 7 April 2005

Abstract

A technique using a fully automated on-line solid phase extraction (SPE) system (Symbiosis, Spark Holland) combined with liquid chromatography (LC)–mass spectrometry (MS/MS) has been investigated for fast bioanalytical method development, method validation and sample analysis using both conventional C_{18} and monolithic columns. Online SPE LC–MS/MS methods were developed in the automated mode for the quantification of model compounds (propranolol and diclofenac) directly in rat plasma. Accuracy and precision using online SPE LC–MS/MS with conventional C_{18} and monolithic columns were in the range of 88–111% and 0.5–14%, respectively. Total analysis cycle time of 4 min per sample was demonstrated using the C_{18} column. Monolithic column allowed for 2 min total cycle time without compromising the quality and validation criteria of the method. Direct plasma sample injection without on-line SPE resulted in poor accuracy and precision in the range of 41–108% and 3–81%. Furthermore, the increase in back pressure resulted in column damage after the injection of only 60 samples.

Keywords: LC–MS/MS; SPE; Plasma

1. Introduction

The inherent specificity of high-performance liquid chromatography and mass spectrometry (LC–MS) detection reduces reliance on comprehensive chromatographic separation and sample extraction to achieve reliable qualitative and quantitative analysis from complex biological matrices [1,2]. However, sample preparation is still required to remove proteins and non-volatile endogenous substances from the biological samples. The presence of such interferences might overload the LC system, contaminate the MS source and lead to suppression/enhancement effects of the MS signal. Furthermore, sample preparation in many cases has becomes the bottleneck step in method development and sample analysis [3]. Protein precipitation, liquid–liquid extraction (LLE) and solid phase extraction (SPE) are the traditional techniques employed to clean samples in biological matrices. In semi-automated operation, steps of solvent evaporation, reconstitution and transfer to the LC autosampler are still performed manually (off-line) [4]. Accordingly, intensive efforts were made to carry out sample extraction and clean up on-line with LC–MS/MS analysis. On-line extraction and clean up instrumentation and techniques are being continuously developed and has proven to be applicable for routine analytical assays [5,6].

On-column extraction has gained popularity as a high-throughput on-line extraction technique. This approach involves turbulent flow chromatography (TFC), restricted access media (RAM) and column switching. In these techniques, short (30–50 mm) columns packed with large (30–60 μm) particles are used under high flow rates

* Corresponding author. Tel.: +1 860 715 3009; fax: +1 860 715 3239.
E-mail address: arkady.gusev@pfizer.com (A.I. Gusev).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.04.056

(4–10 ml/min) to clean up biological samples [7] followed by sample elution into the mass spectrometer [8–14]. This approach resulted in inferior chromatographic performance because of the inherent large particle size of the column material [15,16]. This problem was circumvented by the use of a second analytical column i.e. the extraction column is used for sample extraction and clean up while the second analytical column is used to achieve the desired chromatographic separation [17–21]. Although this approach proved very effective in different applications, the usage life of the extraction column was limited to 100–250 injections when plasma samples were directly injected [8,9,11,12,21]. To increase extraction column lifetime and avoid column and system clogging, samples needed to be diluted or extracted prior to injection.

On-line SPE systems with single-use (i.e. disposable) cartridges provide an alternative technique to on—column extraction for direct analysis of biological samples. Sample dilution prior to injection is not required since the disposable cartridges are used once. The utility of on-line SPE extraction has been demonstrated for a variety of pharmaceutical, biological and environmental applications [22–26]. The system carries multiple cartridge trays and can run more than 1000 samples. Furthermore, the system provides automated features including automated and unattended method development.

The goal of this paper is to investigate the use of on-line SPE technique combined with LC–MS analysis for fast bioanalytical method development, method validation and sample analysis without compromising method and analysis validation criteria. We also explored the synergy of the on-line SPE with monolithic columns. Both on-line SPE and monolithic columns can utilize high flow rates without significant increases in the backpressure or degradation of chromatographic performance [27–34]. To provide comparison, LC–MS methods were developed to quantify model compounds, i.e. propranolol and diclofenac directly in rat plasma without any prior treatment using on-line SPE with conventional C_{18} column and then with monolithic column (Chromolith). Finally, we investigated direct plasma injection on the monolithic column without any sample pretreatment. Propranolol and diclofenac were chosen as model compounds because they represent typical small pharmaceutical molecules with different physicochemical properties. Diclofenac is an acidic compound and propranolol is a basic compound that ionize in the negative and positive ionization modes, respectively.

2. Experimental

2.1. Chemicals and materials

Propranolol, diclofenac, ibuprofen and ketoconazole were purchased from Sigma (St.Louis, MO, USA). Fig. 1 shows the chemical structures of propranolol and diclofenac as well as their internal standards (IS), ketoconazole and ibuprofen, respectively. Rat plasma was obtained from Biochemed Pharmacologicals (Winchester, VA). HPLC grade methanol, acetonitrile and water were obtained from J.T.Baker (Philipsburg, NJ, USA). Ammonium acetate, ammonium formate, formic acid, acetic acid and ammonium hydroxide were purchased from J.T.Baker (Philipsburg, NJ, USA). C_{18} Luna column (2.1 mm × 50 mm, 5 μm) was purchased from Phenomenex (Torrance, CA, USA). The C_{18} Chromolith (2.1 mm × 50 mm) column was purchased from Merck KgaA (Darmstadt, Germany). The C_{18} HD cartridges (2 mm × 10 mm) were obtained from Spark Holland (Netherlands).

2.2. Chromatographic conditions

The new on-line SPE Symbiosis System (Spark Holland, Netherlands) is composed of two integrated units: the (Reliance) autosampler with a pair of binary LC pumps

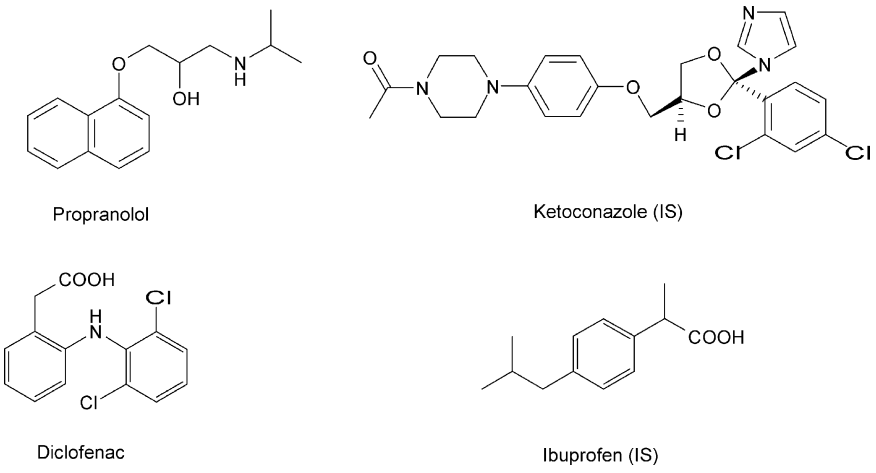

Fig. 1. Chemical structures of propranolol, ketoconazole (IS), diclofenac and ibuprofen (IS).

Table 1
HPLC gradient profiles for propranolol and diclofenac using the Luna and Chromolith columns

Luna column				Chromolith column[a]	
Propranolol		Diclofenac		Propranolol/diclofenac	
Time (min)	B%	Time (min)	B%	Time (min)	B%
0:00	0	0:00	0	0:00	10
1:30	0	1:30	0	0:45	10
2:30	70	2:30	70	1:15	80
3:00	70	2:45	70	1:40	80
3:05	95	2:50	95	1:45	10
3:25	95	3:25	95	2:00	10
3:30	0	3:30	0		
4:00	0	4:00	0		

[a] The HPLC gradient profile for propranolol and diclofenac analysis using the monolithic column was the same.

and the on-line SPE unit with a pair of high pressure solvent delivery pumps (HPDs). The entire system is operated by one software package (SparkLink). LC and extraction methods as well as run tables are created with the SparkLink software and submitted to the MS controlling software (Analyst 1.3). The traditionally packed [C_{18} Luna column (2.1 mm × 50 mm, 5 μm)] and the monolithic [C_{18} Chromolith (2.1 mm × 50 mm)] columns were used in this study. The injection volume for all experiments was 10 μl. Flow rates were set at 0.8 ml/min with the Luna column and at 3.5 ml/min with the monolithic column. In the later, the flow was split after the column directing 1.5 ml/min toward the MS instrument and 2 ml/min to waste. The LC pumps gradient profiles for the different methods are shown in Table 1. The mobile phase consisted of solvent A (5 mM ammonium acetate buffer, pH 7) and solvent B (acetonitrile).

2.3. Mass spectrometry conditions

Mass spectrometry detection was carried out in ESI mode using API 4000 (AB-Sciex, Concord, Ontario, Canada) triple quadrupole system. Table 2 shows the MS parameters used for the detection of propranolol/ketoconazole (IS) and diclofenac/ibuprofen (IS). Propranolol and ketoconazole produced signals in the positive ionization mode only, while diclofenac and ibuprofen produced signals in both ionization modes with much higher signal intensities in the negative ionization mode. Using the automatic MS tuning feature of the Analyst 1.3 software, the highest abundant product ion of each of the four compounds and the optimum MS parameters were automatically selected. Finally, all MS parameters were manually fine-tuned to obtain the highest MRM signals. No signal suppression/enhancement of the analyte signals due to their internal standards was observed. Furthermore, there was no evidence of cross talk between any of the analytes and their internal standards.

2.4. Samples extraction

A method development tray containing 12 different types of SPE cartridges, which represent a wide spectrum of packing material polarities, was automatically screened for the appropriate cartridge to use. The C_{18} HD (2 mm × 10 mm) was the cartridge of choice because it yielded the highest recovery, retention and satisfactory peak shape for both propranolol and diclofenac. Table 3 lists the steps of the sample extraction process as performed by the Symbiosis system. In summary, a new cartridge is conditioned with an organic followed by aqueous solvents. Then, a transfer solvent transfers the sample from the autosampler loop injector onto the conditioned cartridge. The loaded cartridge is then flushed with a highly aqueous solvent to wash out salts and endogenous interferences present in the biological sample. Then the cartridge is physically moved with a robotic arm to a different position for cartridge elution. During elution a new cartridge is placed in the conditioning position and undergoes conditioning, loading and washing. Sample extraction was run at a faster rate when the Chromolith column was used, Table 3. Sample recovery of the analytes were determined by comparing the peak area of plasma samples vs. samples prepared in water.

2.5. Preparation of standards and calibration curves

Stock solutions of propranolol, ketoconazole, diclofenac and ibuprofen were prepared in acetonitrile at 1 mg/ml con-

Table 2
MS parameters for monitoring propranolol, ketoconazole (IS), diclofenac and ibuprofen (IS)

Ionization mode	Propranolol Positive	Ketoconazole Positive	Diclofenac Negative	Ibuprofen Negative
Curtain gas (CUR)	10 psi	10 psi	10 psi	10 psi
Collision gas (CAD)	5	5	5	5
Ion spray voltage (IS)	3000 V	3000 V	−3000 V	−3000 V
Temperature (TEM)	700 °C	700 °C	750 °C	750 °C
Ion source gas 1 (GS1)	85 psi	85 psi	80 psi	80 psi
Ion source gas 2 (GS2)	80 psi	80 psi	40 psi	40 psi
Declusterin potential (DP)	71 V	106 V	−45 V	−45 V
Entrance potential (EP)	10 V	10 V	−10 V	−10 V
Collision energy (CE)	27 eV	43 eV	−16 eV	−10 eV
Collision cell exit potential (CXP)	8 V	10 V	−15 V	−15 V
MRM	260/116 amu	531/489 amu	294/250 amu	205/161 amu

Table 3
The sample extraction process as performed by the on-line SPE system (Symbiosis) with Luna and Chromolith columns

	Solvent	Method	Flow rate (ml/min)	Volume (ml)	Duration (min)
Equilibration 1	Acetonitrile	Luna	5	1	0.3
		Chromolith	10	0.5	0.15
Equilibration 2	Ammonium acetate[a]	Luna	5	1	0.3
		Chromolith	10	0.5	0.15
Loading[b]	Ammonium acetate[a]	Luna	2	0.5	0.35
		Chromolith	5	0.5	0.2
Washing	Ammonium acetate[a]	Luna	5	1	0.3
		Chromolith	7.5	1	0.25
Elution	Acetonitrile	Luna	0.14	0.2	1.5
		Chromolith	0.5	0.25	0.6

[a] 5 mM ammonium acetate buffer (pH 7).
[b] In this step, the sample is transferred from the injector loop to the SPE cartridge.

centrations. By serial dilution with deionized water, standard solutions of concentrations: 20, 18, 16, 10, 6, 2, 0.2, 0.1, 0.06, 0.04 and 0.02 μg/ml were prepared from the stock solutions of propranolol and diclofenac. 10 μg/ml standard solutions were prepared from the ketoconazole (IS for propranolol) and ibuprofen (IS for diclofenac) stock solutions.

Blank rat plasma (1 mL) was spiked with 10 μg/ml of internal standard solution and the proper analyte standard solution, 50 μl each to prepare the calibration curve and the quality control (QC) points. Calibration curves were constructed of the following concentrations: 1, 2, 10, 50, 100, 300, 900 and 1000 ng/ml. Five QC points of the concentrations 1, 3, 500, 800 and 1000 were used to validate the calibration curves. The internal standard concentration, ketoconazole for the propranolol method and ibuprofen for the diclofenac method, was 500 ng/ml.

2.6. Method development

Method development, including SPE cartridge selection and extraction condition optimization, is an automatic process using the Symbiosis system. A method development tray with 12 different types of cartridges, which represent a wide spectrum of packing polarities, is automatically screened using a generic extraction method. This initial screening process identifies one type of cartridge with satisfactory recovery, retention and peak shape with no further optimization. Then, washing and elution conditions are optimized by varying solvent composition, duration or rate of solvent application to obtain desired chromatographic retention and elution.

2.7. Method validation

Intra-day and inter-day accuracy and precision as calculated from five QC points were used to validate the calibration curves. Intra-day precision was calculated as percent relative standard deviation (% RSD) of the analyte to internal standard peak areas obtained from replicates ($n = 5$) of each QC point. Accuracy was calculated as the % bias of the calculated concentration relative to the nominal concentration of each QC point. The process was repeated over three days using freshly prepared standards. Results from the three days were pooled to calculate inter-day accuracy and precision ($n = 15$).

3. Results

The primary objective of this work was to investigate, develop and utilize the on-line SPE system (Symbiosis) for fast bioanalytical method development, method validation and direct sample analysis (no sample pretreatment) without compromising method and analysis validation criteria. Additionally, we aimed to investigate the merits of combing monolithic columns with the on-line SPE system for higher throughput analysis. Therefore, our approach involved method development for the analysis of the model compounds using (i) direct plasma injections with on-line SPE/conventional C_{18} column, (ii) direct plasma injections with on-line SPE/monolithic column, and (iii) direct plasma injection (no SPE)/monolithic column

3.1. On-line SPE/conventional C_{18} column

Method development for SPE extraction was performed automatically within 4 h as described in Section 2. The C_{18} HD cartridge was selected based on a ca.100% recovery for both propranolol and diclofenac. Extraction and clean up cycles were completed in ca.1.3 min. Analytes and IS eluted from the SPE cartridge at 1.2–1.5 min before being transferred to the Luna C_{18} analytical column. Propranolol, ketoconazole, diclofenac and ibuprofen eluted from analytical column at 2.7, 3.0, 2.7 and 2.7 min, respectively. Fig. 2 shows representative LC–MS/MS chromatograms for propranolol and diclofenac obtained in rat plasma. All compounds had a symmetrical peak shape with <0.2 min peak width.

Table 4 shows the method validation accuracy and precision results for both compounds in rat plasma. Accuracy and

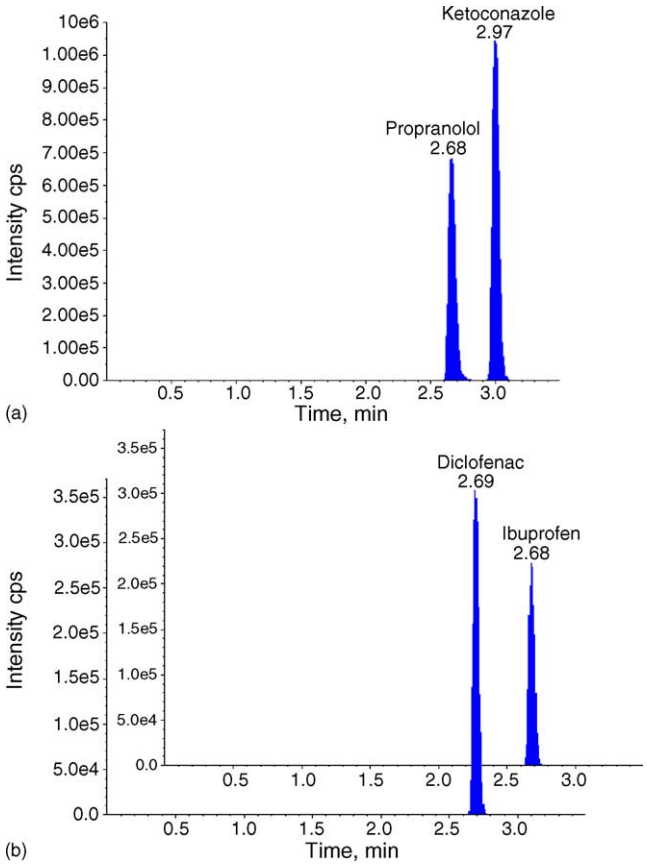

Fig. 2. Representative chromatograms of (a) propranolol/ketoconazole and (b) diclofenac/ibuprofen in plasma obtained with the on-line SPE and Luna C18 column combination.

precision were in the range of 90–110% and 0.5–14%, respectively. All calibration curves had r^2 value in excess of 0.99. The total analysis cycle time per sample was 4 min. Sixty two plasma samples for each validation day were run over 3 days. No noticeable increase in the system back-pressure or changes in system performance were observed after a total of 186 samples.

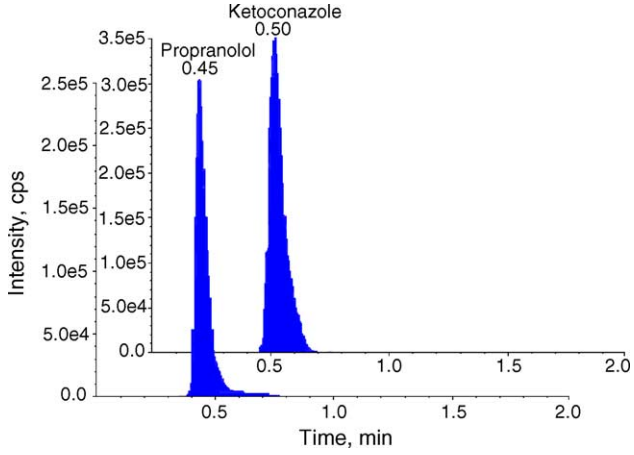

Fig. 3. Representative chromatograms of propranolol/ketoconazole elution profile from the cartridge at 500 μl/min acetonitrile without the presence of the analytical column.

3.2. On-line SPE/monolithic column

A combination of high flow on-line SPE extraction with high flow monolithic columns would allow for faster analysis without compromising chromatographic performance and method validation criteria. Fig. 3 shows chromatograms of propranolol/ketoconazole elution profile obtained from the C_{18} HD SPE cartridge (SPE cartridge directly connected to MS without the presence of the analytical column). Higher elution flow (i.e. 500 μl/min) allowed for faster extraction and clean up cycles. Elution from the SPE cartridge was completed in ca. 0.8 min compared to 1.3 min obtained with conventional C_{18} column.

Fig. 4 shows representative LC–MS/MS chromatograms for propranolol and diclofenac with on-line SPE and using

Table 4
Accuracy and precision of method validation for propranolol and diclofenac in rat plasma using on-line SPE and conventional C_{18} column

QC (ng/ml)	Day 1		Day 2		Day 3		Inter-day[a]	
	Accuracy[b]	Precision[c]	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
Propranolol								
1	99.8	3.8	110	4.2	94.1	6.2	101.3	8.2
3	98.1	2.2	105	5.4	96	3.3	99.7	5.4
500	101.8	0.8	100	2.3	97	1.6	99.5	2.7
800	94.3	1.5	91.7	1.9	90	0.5	91.9	2.4
1000	93.1	0.8	91.9	1.7	92.4	1.7	92.5	1.5
Diclofenac								
1	93.4	4.8	102.9	13.9	113.1	12.5	101.9	13.74
3	93.9	1.6	107.8	4.8	89.0	2.7	96.2	9.5
500	99.2	1.43	100.5	0.54	97.4	1.14	99.0	1.73
800	101.8	1.56	95.04	1.34	93.4	2.21	96.8	4.19
800	101.8	1.56	95.04	1.34	93.4	2.21	96.8	4.19
1000	103.8	1.94	92.7	0.92	88.4	0.83	95.0	6.8

[a] Inter-day: all data from the three validation days are pooled ($n = 15$).
[b] Accuracy: [measured/theoretical] % ($n = 5$).
[c] Precision: % RSD ($n = 5$).

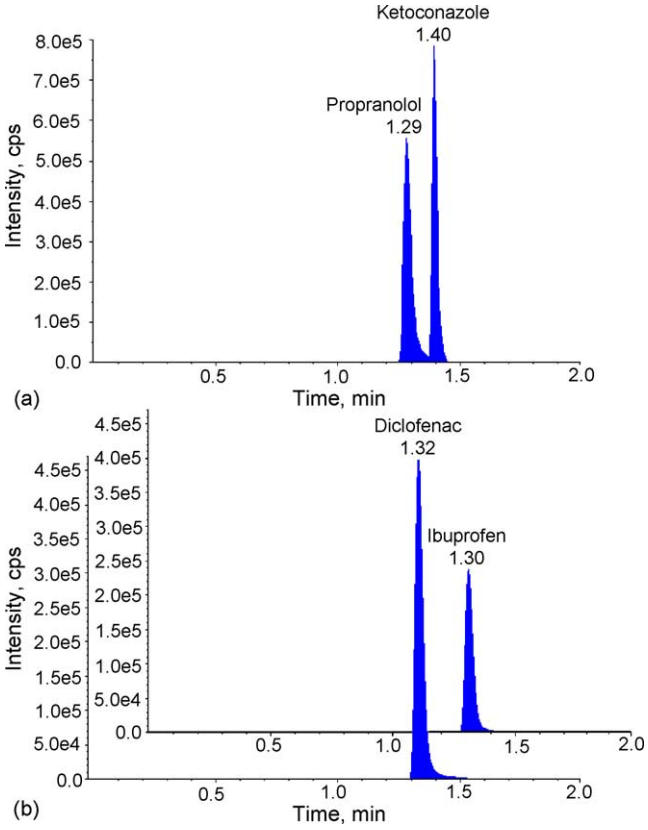

Fig. 4. Representative chromatograms of (a) propranolol/ketoconazole and (b) diclofenac/ibuprofen in plasma obtained with the on-line SPE and the Chromolith monolithic column combination.

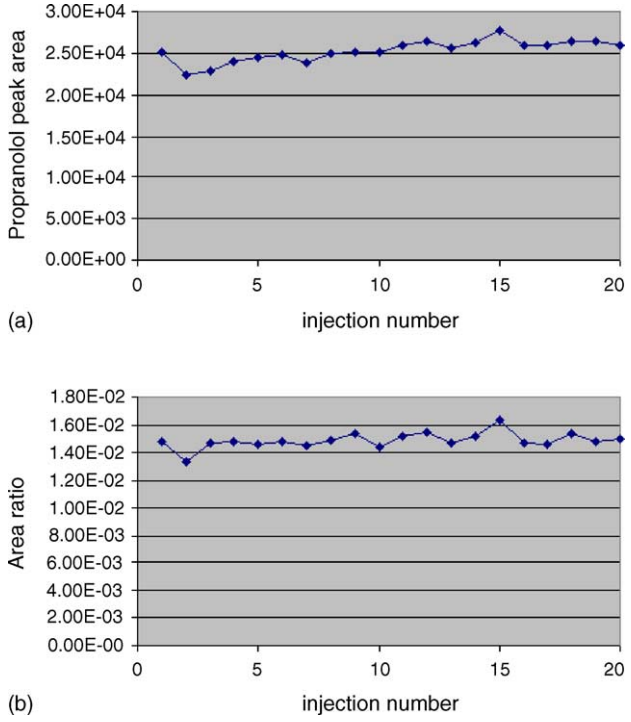

Fig. 5. (a) Signal intensities for propranolol peak at 5 ng/ml and (b) peak area ratio of propranolol/ketoconazole (IS) vs. injection number for multiple injections onto the same SPE cartridge.

the Chromolith column obtained in rat plasma. Propranolol, ketoconazole, diclofenac and ibuprofen eluted at 1.3, 1.4, 1.3 and 1.3 min, respectively. All compounds had narrow peaks with peak width of 0.1–0.15 min.

Table 5 shows the accuracy and precision results of methods validation for both compounds obtained in rat plasma. The accuracy and precision ranged from 88 to 111% and 2 to 14%, respectively. An r^2 value greater than 0.99 was obtained for all calibration curves. Accuracy and precision results obtained with on-line SPE and using the Chromolith column (see Table 5) are comparable to the results obtained with conventional C_{18} column (see Table 4). However, the run cycle time was reduced to 2 min compared to 4 min obtained with conventional C_{18} column.

We also explored the option of reusing one SPE cartridge for multiple injections. Fig. 5 shows peak areas and ana-

Table 5
Accuracy and precision of method validation for propranolol and diclofenac in rat plasma using on-line SPE/monolithic column

QC (ng/ml)	Day 1		Day 2		Day 3		Inter-day[a]	
	Accuracy[b]	Precision[c]	Accuracy	Precision	Accuracy	Precision	Accuracy	Precision
Propranolol								
1	87.68	7.4	105.2	12.6	111.2	12.8	101.3	13.74
3	96.82	4.3	101.9	5.4	97.58	6.1	98.83	4.9
500	104.4	2.7	111.2	2.3	109.8	2.5	108.5	3.83
800	97.24	3.6	106.3	2.4	100.8	2.0	101.5	4.6
1000	95.85	3.7	104.5	3.2	99.33	5.9	99.89	5.5
Diclofenac								
1	89.98	2.72	103.8	9.1	98.13	8.35	97.3	9
3	97.35	4.19	98.87	4.1	96.1	9.01	97.44	5.95
500	98.62	2.8	93.98	2.03	98.2	2.45	96.93	3.19
800	103.2	2.32	94.97	1.82	100.9	2.44	99.68	4.09
1000	103.9	4.08	101.7	2.35	103.5	3.34	103.02	3.28

[a] Inter-day: All data from the 3 validation days are pooled ($n = 15$).
[b] Accuracy: [measured/theoretical] % ($n = 5$).
[c] Precision: % RSD ($n = 5$).

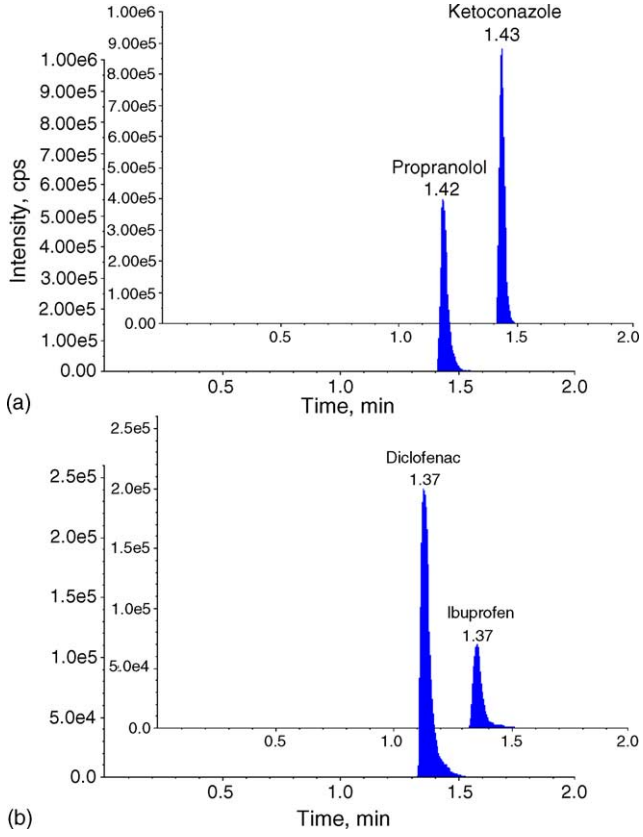

Fig. 6. Representative chromatograms of (a) propranolol/ketoconazole and (b) diclofenac/ibuprofen in plasma with direct injection (no SPE) onto the Chromolith monolithic column.

lyte/IS peak area ratio for 20 consecutive injections of plasma onto the same cartridge. The variation in the analyte peak area and the cartridge back-pressure was less than 5%. No significant signal suppression or changes in recovery were observed.

3.3. Direct plasma injection/monolithic column

The same chromatographic conditions from the on-line SPE/monolithic column experiment were applied for direct injection of plasma samples onto the monolithic column without on-line SPE clean up. Fig. 6 shows representative chromatograms for propranolol and diclofenac. Peak shape and chromatographic separation is comparable with results obtained using the on-line SPE/monolithic column combination (see Fig. 3). However, omitting the on-line extraction step resulted in a poor accuracy and precision results for both compounds. Accuracy in the range of 41–108% and precision in the range of 3–81% were obtained and calibration curve r^2 value was as low as 0.6. Therefore, direct plasma injection (without SPE) yielded unacceptable results, as can be seen from the poor precision and accuracy data. Furthermore, a gradual increase in backpressure and shifts in elution times were encountered after only 60 plasma injections.

4. Discussion

The new on-line SPE Symbiosis System (Spark Holland, Netherlands) is a fully integrated system controlled with one software (SparkLink). The automation feature offered by the Symbiosis system allowed for automated and unattended method development and was used for developing all assays in this report. The refrigerated autosampler can accommodate 24 96-well blocks. The SPE extraction unit can adapt 12×96-cartridges plates, allowing the fully automated and unattended analysis of 1152 samples assuming the single use of each cartridge.

A total run cycle time of 4 min per sample was easily achieved for direct plasma analysis with the on-line SPE system using a conventional C_{18} column. When a monolithic column was used in combination with the on-line SPE system, the cycle time was decreased to 2 min per sample without increasing carryover or matrix signal suppression. A 2 min cycle time with the accuracy and precision levels achieved in this study is applicable for routine sample analysis. Therefore, it was possible to achieve the same precision and accuracy criteria using monolithic columns as conventional columns with half the cycle time.

Direct plasma injection onto the monolithic column without sample pretreatment yielded poor accuracy, precision and robustness. This is probably due to lack of plasma dilution, which is usually required for analysis using on-column extraction columns to avoid column clogging. It is important to note that capabilities to inject plasma sample without any dilution or manipulation will facilitate integration of the system with automated sample collection and processing systems.

On-line SPE is based on the sequential extraction of individual samples whereas off-line 96 well plate format processes samples in parallel. The minimum on-line SPE extraction time achieved was ca.45 s per sample (see Fig. 3). Assuming that on-line SPE sample preparation adds 45 s per sample, an entire 96 well plate of samples would require ca. 70 min. By comparison, off-line extraction may require more than 70 min to extract, evaporate and reconstitute 96 samples. However, if a large number of 96 well plates need to be analyzed, off-line extraction may prove to be more time efficient.

5. Conclusions

The Spark Holland Symbiosis on-line SPE system was proved useful in developing high throughput methods for direct plasma analysis with no sample pretreatment. Method development requires only 4 h and was carried out in a fully automated mode. The combination of on-line SPE with monolithic columns allowed for the development of high throughput methods with 2 min total analysis time without compromising the method validation criteria. 4 min cycle time per sample was required to achieve the same preci-

sion and accuracy criteria using a conventional C_{18} column. Such a fast assay would potentially allow for daily analysis of plasma 720 samples per instrument without compromising quality and validation criteria. On-line SPE is more time efficient than semi-automated off-line SPE provided that the number of samples to be extracted and analyzed is less than a few 96 well plates. Furthermore, the cost of analysis can be reduced by reusing the same cartridge up to 20 times without a significant change in the analyte recovery or the cartridge back pressure.

Acknowledgments

The authors would like to thank Spark Holland, Inc. and in particular Rob Caspien, for providing the Symbiosis Pharma system for evaluation. Special thanks go to Steven Eendhuizen, Dirk Hiemstra and Emile Koster of Spark Holland and John Lawrence of Pfizer for their technical assistances.

References

[1] M. Jemal, Biomed. Chromatogr. 14 (2000) 422.
[2] R.T. Gallagher, M.P. Balgoh, P. Davey, M.R. Jackson, I. Sinclair, L.J. Southern, Anal. Chem. 75 (2003) 973.
[3] R.S. Plumb, G.J. Dear, D.N. Mallett, D.M. Higton, S. Pleasance, R.A. Biddlecombe, Xenobiotica 8–9 (2001) 599.
[4] M.C. Hennion, J. Chromatogr. A 856 (1999) 3.
[5] J. Kling, Scientist 11 (1997) 16.
[6] M. Swartz, M. Jones, P. Fowler, M. Andrews, LC GC North Am. (2002) 49.
[7] M.J. Berna, B.L. Ackermann, A.T. Murphy, Chem. Acta 1 (2004) 1.
[8] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, Rapid Commun. Mass Spectrom. 11 (1997) 1953.
[9] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, J. Chromatogr. A 828 (1998) 199.
[10] J. Ayrton, R.A. Clare, G.J. Dear, D.N. Mallett, R.S. Plumb, Rapid Commun. Mass Spectrom. 13 (1999) 1657.
[11] M. Jemal, Y. Qing, D.B. Whigan, Rapid Commun. Mass Spectrom. 12 (1998) 1389.
[12] Y. Hsieh, M.S. Bryant, G. Gruela, J.M. Brisson, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 14 (2000) 1384.
[13] Y. Hsieh, M.S. Bryant, G. Gruela, J.M. Brisson, N. Kwokei, W.A. Korfmacher, J. Chromatogr. B 767 (2002) 253.
[14] A. Haque, J.T. Stewart, Biomed. Chromatogr. 13 (1999) 51.
[15] M. Jemal, Z. Ouyang, Rapid Commun. Mass Spectrom. 14 (2000) 1757.
[16] R. Plumb, G. Dear, D. Mallett, J. Ayrton, Rapid Commun. Mass Spectrom. 15 (2001) 986.
[17] S. Souverian, M. Mottaz, S. Cherkaouri, J.L. Veuthey, Anal. Bioanal. Chem. 377 (2003) 880.
[18] L. Ynddal, S.T. Hansen, J. Chromatogr. A 1020 (2003) 59.
[19] R.P. Grant, C. Cameron, S. Mackenzie-McMurter, Rapid Commun. Mass Spectrom. 16 (2002) 1785.
[20] W. Zeng, A.Q. Wang, A.L. Fisher, D.G. Musson, Rapid Commun. Mass Spectrom. 17 (2003) 2475.
[21] S. Hsieh, T. Tobien, K. Koch, J. Dunn, Rapid Commun. Mass Spectrom. 18 (2004) 285.
[22] R.D. Estrela, M.C. Salvadori, G. Suarez-Kurtz, Rapid Commun. Mass Spectrom. 18 (2004) 1147.
[23] A. Pastoris, L. Cerutti, R. Sacco, L. Devecchi, A. Sbaffi, J. Chromatogr. B 664 (1995) 287.
[24] C. Opper, W. Wesemann, G. Barka, H. Kerkadijk, G. Haak, LC GC 12 (1994) 684.
[25] A. Schellen, B. Ooms, D. van de Lagematt, R. Vreeken, W.D. van Dongen, J. Chromatogr. B 788 (2003) 251.
[26] R.S.L. Raices, M.C. Salvadori, R.D.F. Esterla, F.R.D. Neto, G. Suarez-Kurtz, Rapid Commun. Mass Spectrom. 17 (2003) 1611.
[27] Y. Hsieh, M. Chintala, H. Mei, J. Agans, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 15 (2001) 2481.
[28] N. Tanaka, H. Nagayama, H. Kobayashi, T. Ikegami, K. Hosoya, J. High Resolut. Chromatogr. 23 (2000) 111.
[29] K. Nakanishi, H. Shikata, N. Ishizuka, N. Koheiya, N. Soga, J. High Resolut. Chromatogr. 23 (2000) 106.
[30] Y. Hsieh, G. Wang, Y. Wang, S. Chackalamannil, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 16 (2002) 944.
[31] G. Dear, R. Plumb, D. Mallett, Rapid Commun. Mass Spectrom. 15 (2001) 152.
[32] N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hiaro, N. Tanaka, Colloid Surf. A 187–188 (2001) 273.
[33] J.T. Wu, H. Zeng, Y. Deng, S.E. Unger, Rapid Commun. Mass Spectrom. 15 (2001) 1113.
[34] Y. Hsieh, G. Wang, Y. Wang, S. Chackalamannil, W.A. Korfmacher, Anal. Chem. 75 (2003) 1812.