Direct Plasma Injection On-Line SPE-LC-MS/MS for the Quantitative Analysis of Drugs in Human Plasma Samples

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ON-LINE SPE-LC FOR DRUG ANALYSIS

Bioanalytical laboratories are under continuous pressure to analyze the fast-growing number of drug candidates that enter clinical studies. Instrumental developments in LC-MS technology during the last decade have enabled significantly accelerated analysis of biological samples. However, sample preparation, such as liquid-liquid extraction or off-line solid-phase extraction (SPE), is the most time-consuming step and presents a bottleneck to increased sample throughput. By utilizing an automated SPE process, it is possible to remove this bottleneck and significantly improve throughput.

Advantages of on-line SPE-LC for the quantitative analysis of drug assays include:

- Automated sample preparation for reduced analysis time and cost
- Less experimental variability
- Less contact with biological or pharmaceutical samples
- Small sample quantities

This poster presents an on-line SPE-LC-MS/MS method for the quantitative analysis of fexofenadine, an antihistamine drug, in plasma. Fexofenadine hydrochloride (brand names include Allegra and Telfast) is used for treatment of hay fever and other allergy symptoms. Its structure is depicted in Figure 1. Fexofenadine is 60–70% bound to plasma proteins. After a 60 mg oral dose the Cmax is about 209 ng/mL in plasma, within in 1–3 hours.

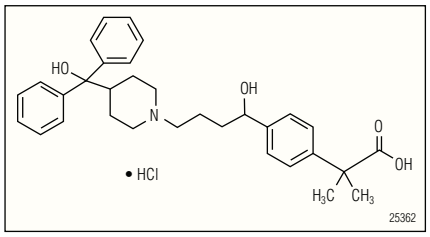


Figure 1. Molecular structure of fexofenadine, used for hay fever treatment.

This LC-SPE-MS/MS method has been validated for accuracy, precision, selectivity, sensitivity, linearity, and stability.

SYSTEM CONFIGURATION FOR ON-LINE SPE-LC-MS/MS

A Dionex UltiMate® 3000 ×2 Dual-Gradient HPLC system was used. This comprises two integrated gradient pumps, which were used for sample loading and cleanup and separation on the analytical column. Chromeleon® Chromatography Software 6.80 was used to control the system.

The setup for on-line SPE-LC is shown in Figure 2. The workflow is as follows:

1. Sample cleanup step on the SPE column separates analytes from matrix. The interfering matrix components are flushed to waste.

2. Analytes are transferred to the analytical column, separated, and detected.

3. The SPE column and analytical column are equilibrated.

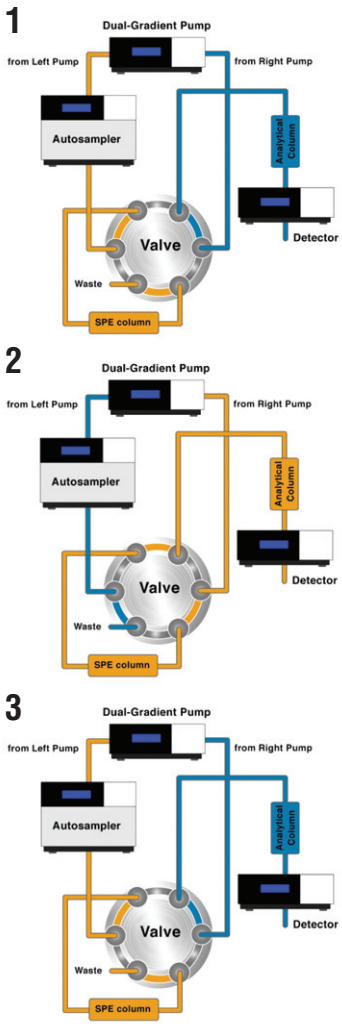


Figure 2. Column setup for on-line SPE-LC.

The SPE column packing features a hydrophilic surface and hydrophobic pores that are size restricted. This unique packing creates a restricted access for large plasma proteins and allows the injection of plasma samples for the extraction of small drug molecules.

Columns and Solvents

- SPE Column: Bio Trap 500 C18, 20 × 4.0 mm
- Extraction Mobile Phase: 96% water/4% isopropyl alcohol/ 0.2% formic acid
- Extraction Phase Flow Rate: 2.0 mL/min
- Analytical Column: Acclaim® C8, 5 µm, 50 × 4.6 mm (40 °C)
- Mobile Phase: 0% 10 mM ammonium acetate/buffer/ 60% acetonitrile; pH 7.0
- Flow Rate: 1.5 mL/min

MS/MS detection was performed in positive ion mode with a triple quadrupole MS (Applied Biosystems, API 2000) used in the SRM mode. Selected reaction monitoring was performed for fexofenadine HCl (*m/z* 502.3 and 262.2) and cetirizine HCl (m/z 389.2 and 201.1).

The samples were prepared by adding 50 µL of internal standard (cetirizine dihydrochloride) to 1000 µL of spiked plasma, vortexing for 30 s, and centrifuging the plasma at 10,000 rpm for 8 min. The supernatant was transferred to the autosampler vial. The typical injection volume for analysis was 20 µL.

SOFTWARE WIZARD FOR ON-LINE SPE-LC

Chromeleon Chromatography Software features a dedicated wizard for on-line SPE-LC, which greatly simplifies automated sample preparation for rapid creation of methods. An example of the on-line SPE-LC wizard for setting the valve switching times is shown in Figure 3.

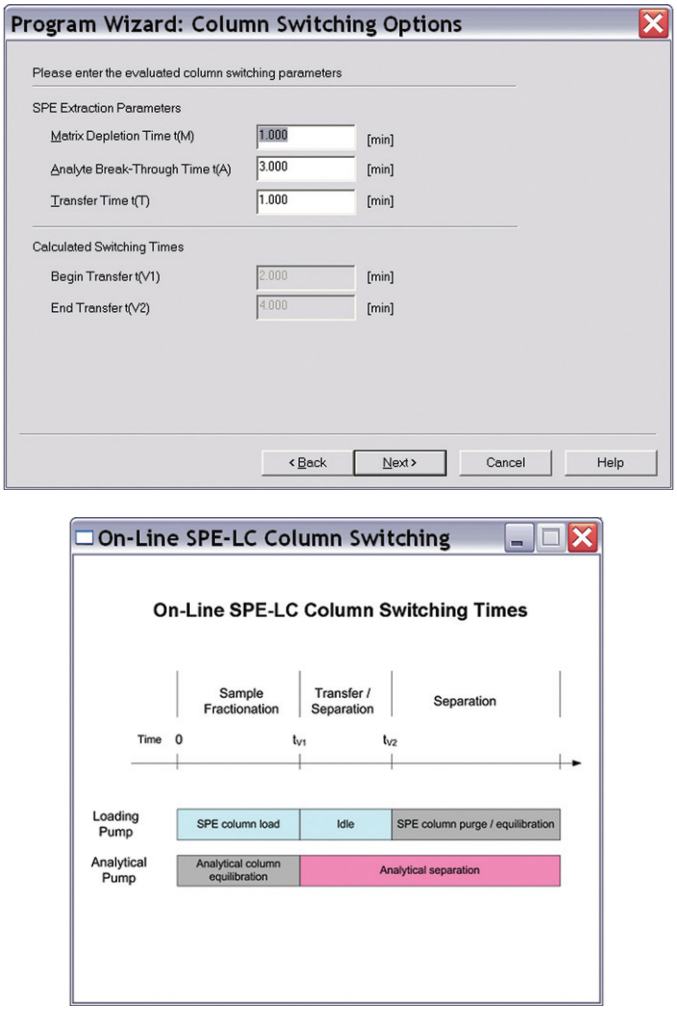


Figure 3. Chromeleon wizard for on-line SPE-LC method development.

The valve switching times for the extraction of fexofenadine are listed in Table 1.

Table 1. On-Line SPE-LC Method

Time (Min)	Flow Rate (mL/min)	Valve Position	Remark
0	2.0	1-2	Wash and extraction
2.0	2.0	6-1	Elution and separation
3.2	2.0	1-2	Equilibration
5.0	2.0	1-2	

SELECTED REACTION MONITORING DETECTION

Figure 4 shows the Selected Reaction Monitoring (SRM) chromatogram of fexofenadine. The total analysis time including on-line SPE is 5 minutes. The elution of fexofenadine is approximately 2.9 minutes at the end of the separation period. The internal standard cetirizine and fexofenadine have the same retention time and are selectively detected by applying the SRM transitions.

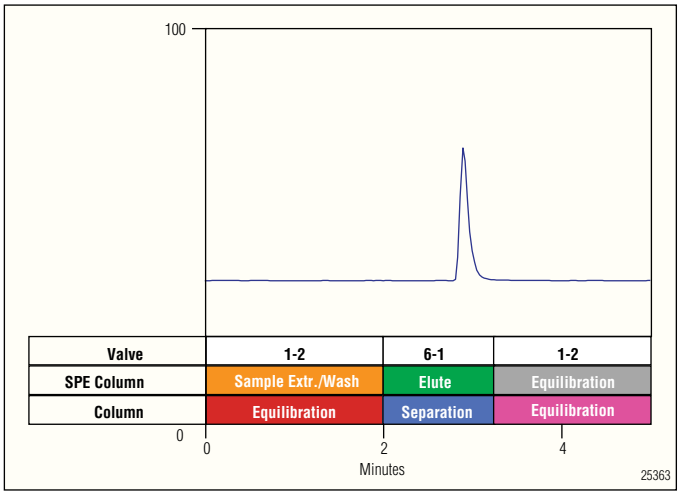
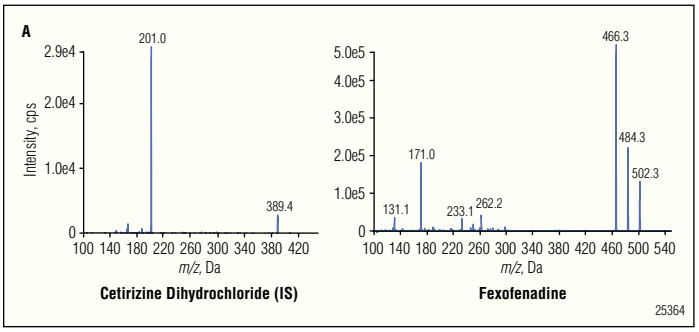


Figure 4. On-line SPE-LC-MS of fexofenadine in human plasma.

The SRM transition applied for MS detection determines the sensitivity, stability and selectivity of the method and should be chosen carefully. The product-ion spectra of fexofenadine and the internal standard cetirizine are shown in Figure 5A. The applied SRM transitions for fexofendaine and cetirizine resulted in a selective method as can be seen in Figure 5B. No certirizine or fexofendaine was detected in the blank plasma. When adding the internal standard (certirizine) a peak is detected with *m/z* transition 389>201 in the zero plasma.



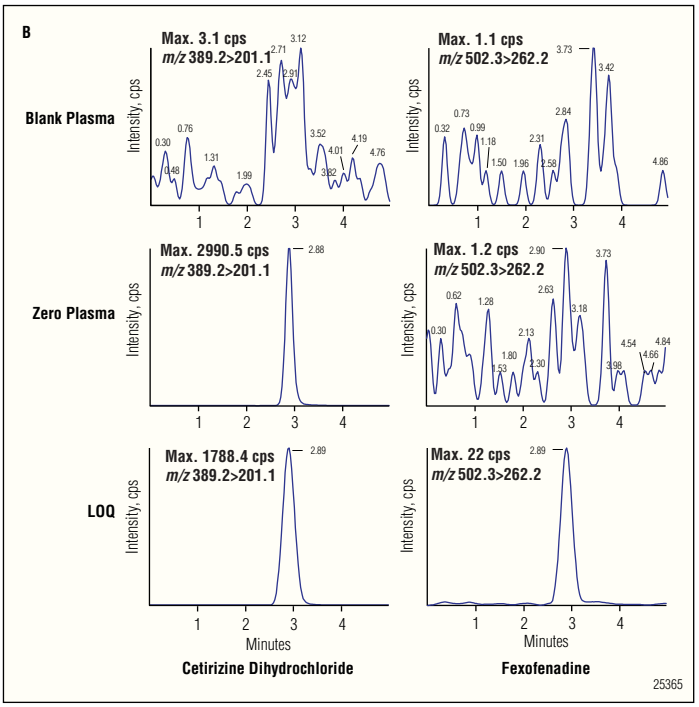


Figure 5. A) Product ion spectra of cetirizine dihydrochloride (IS) (left) and fexofenadine (right). B) Selectivity of the method.

VALIDATION OF THE SPE-LC-MS/MS METHOD

To validate the method, calibration curves were prepared on 3 days. All calibration standards met the criterion of a deviation smaller than 15% from nominal concentration, as can be seen in Table 2. The accuracies of the LOQ samples containing 10 and 30 ng/ml fexofenadine were 88.77% and 96.84%, respectively.

The precision for the lower LOQ sample containing 10 ng/ml fexofenadine was 11.21% with a signal that was approximately 10 times greater than the blank response. The precision and sensitivity for the lower LOQ sample met the acceptance criteria of <20% and 5 times the blank response, respectively. Therefore, the concentration of 10 ng/ml can be considered as lower limit of quantification.

Table 2. Calibration Data for Fexofenadine

Actual Conc. (ng/mL)	Observed Conc. (ng/mL) Day-1	Day-2	Day-3	Mean	S.D.	% R.S.D (<15%)	Mean % Accuracy (80–120%)
9.78	9.11	8.41	8.80	8.77	0.351	4.002	89.67
29.34	28.6	28.61	28.15	28.45	0.263	0.924	96.97
48.90	49.54	49.34	53.33	50.74	2.248	4.430	103.76
146.71	162.08	166.35	162.88	163.77	2.270	1.386	111.63
244.51	259.55	268.04	251.24	259.61	8.400	3.236	106.18
391.22	416.03	396.85	409.92	407.60	9.798	2.404	104.19
586.82	585.68	613.74	626.43	608.62	20.853	3.426	103.71
782.43	828.07	839.98	838.75	835.60	6.550	0.784	106.80
1173.65	1171.68	1136.60	1152.89	1153.72	17.555	1.522	98.30
1760.47	1693.9	1685.55	1662.05	1680.50	16.515	0.983	95.46
r	0.9992	0.9986	0.9985	0.9988			
Slope	0.001	0.00094	0.00094	0.00096			
Y-intercept	0.00233	0.00283	0.00115	0.00210			

The calibration curve on day 3 was obtained with a new SPE column. No differences were observed between the new SPE column and one that had been used for 800 prior plasma injections. Figure 6 shows the comparison of the SRM signals for calibration standard on the old and new SPE columns.

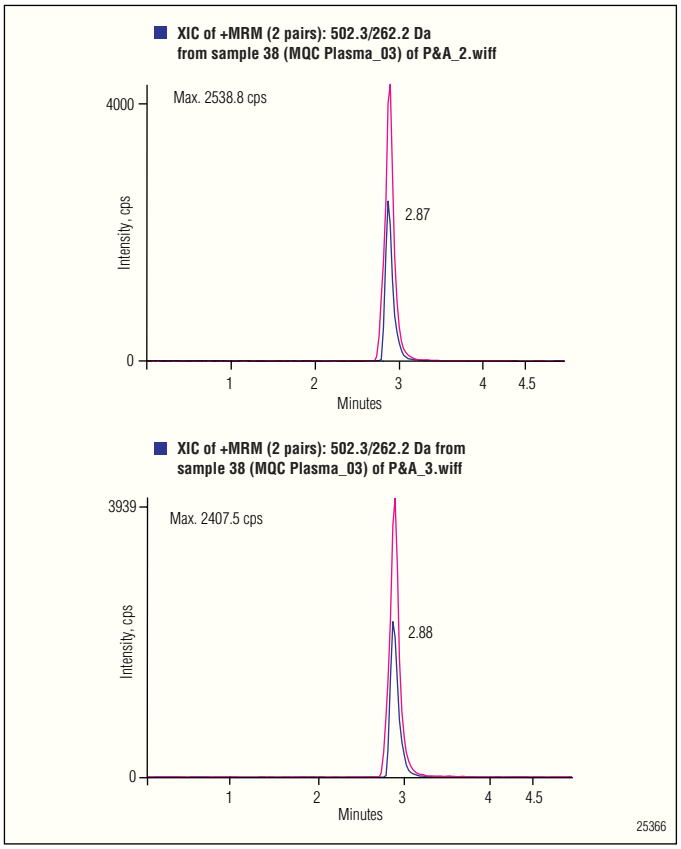


Figure 6. SRM signals for the MQC fexofenadine sample (490 ng/ml) obtained on an old SPE column (top) and new SPE column.

CONCLUSIONS

This presentation shows a solution for on-line SPE-LC-MS/MS quantification of fexofenadine in plasma using the UltiMate 3000 x2 Dual-Gradient HPLC system. The same system has meanwhile been successfully used for the quantification of carbamazepine, losartan and lamotrigin in plasma as well.

The Bio Trap SPE column allows direct injection of plasma and proved to be stable for extraction of at least 800 plasma injections of 20 µL each. The method met criteria for validation parameters, including accuracy, precision, and linearity according ICH guidelines.[1]

REFERENCE

1. Bioanalytical Method Validation. Food and Drug Administration Center for Drug Evaluation and Research (CDER). May 2001.

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