

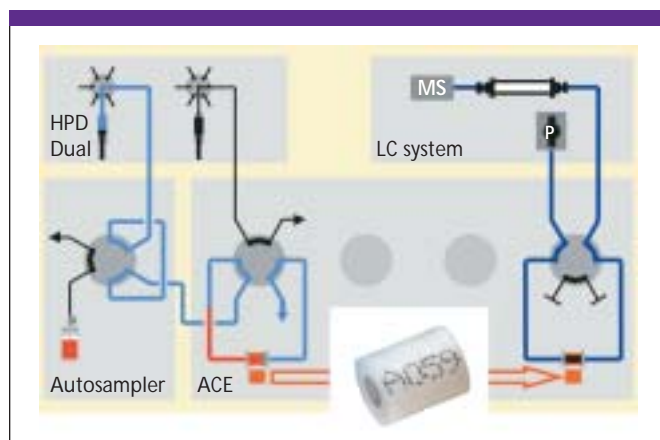


## Rapid Development of High-Speed On-Line Solid-Phase Extraction Methods for LC-MS in Bioanalysis

Dries Vrieling, Emile Koster, and Bert Ooms,  
Spark Holland B.V., Emmen, The Netherlands  
Anniek Schellen, Diosynth b.v., Oss, The Netherlands

**B**ioanalytical laboratories in the pharmaceutical industry are facing an increasing pressure to reduce turnaround time for preclinical and clinical studies in order to keep pace with the increasing rate at which new drug candidates are entering the development stage (1). For preclinical studies (a few hundred samples), reduction or elimination of method development time is crucial, whereas for clinical studies (thousands of samples), the cycle time of the assay is of vital importance.

Recent developments in on-line SPE were focused to meet those challenges. New instrumentation for on-line SPE-LC-MS has been designed to permit total SPE-LC-MS assay cycle times of less than 1 min. A generic protocol for SPE and LC has been developed to provide zero method development for new preclinical assays or a starting point for rapid development of high-throughput assays for clinical studies. Dedicated system configurations and strategies for rapid and smart method development have been developed with automated procedures to select the optimum of all SPE parameters, including the SPE temperature (Figure 1). Examples of high-throughput SPE-LC-MS, generic SPE-LC-MS method and smart automated method development are presented and discussed.



**Figure 1:** Parallel SPE and elution with Propekt•2™ with dual cartridge clamps. SPE solvents for solvation, equilibration, sample application and clean up are provided by the High-Pressure Dispenser (HPD) at programmable flow rates, independent of SPE cartridge back pressure. Raw sample is introduced by the autosampler. After SPE on the left clamp, the cartridge is transferred to the right clamp for elution into the LC-MS-MS system. During LC-MS-MS analysis, SPE of the next sample is carried out on a new cartridge in the left clamp. SPE cartridge exchange is programmable per analysis.

### Fast Generic SPE-LC Method For On-Line SPE-LC-MS-MS

**Generic SPE:**  
SPE cartridge: HySphere Resin GP (10 mm × 2 mm and 10 mm × 3 mm)

Solvation: 1.5 mL methanol (10.0 mL/min)

Equilibration: 1.5 mL water (10.0 mL/min)

Sample application and cleanup:

2.0 mL water (5.0 mL/min)

Cycle time: < 60 s

### Generic fast-gradient LC:

Column: 30 mm × 4 mm, 3- $\mu$ m particles C18

Flow rate: 2.0 mL/min

Gradient: 0.00–0.10 min 100% A, 0.10–1.40 min 100% B, 1.40–1.50 min 100% B, 1.50–2.00 min 100% A; A = 5% ACN, 0.1% HCOOH, 10 mM NH<sub>4</sub>AC, B = 95% ACN, 0.1% HCOOH, 10 mM NH<sub>4</sub>AC (Figure 2)

### Mass spectrometry:

Instrument: API 3000 with LINAC collision cell (Applied Biosystems)

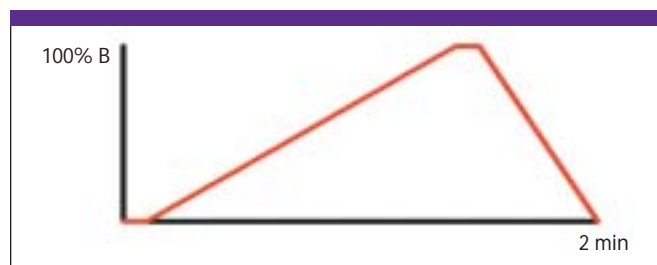
Flow: Split to 400  $\mu$ L/min

Ionization mode: Turbo-ion spray; positive-ion mode

Scan mode: Selective reaction monitoring

### Fast Generic High-Speed SPE-LC-MS-MS — Results

The generic protocol was tested for the 11 highly different compounds shown in Figure 3. Table I shows the results of generic SPE-LC-MS-MS analysis of 100- $\mu$ L spiked porcine serum. Precision and accuracy were better than 15% RSD (typical acceptance criteria for preclinical studies) for all compounds. Figure 4 shows a typical example of a seven-in-one analysis of serum spiked at the 1-ng/mL level.



**Figure 2:** The LC gradient profile.

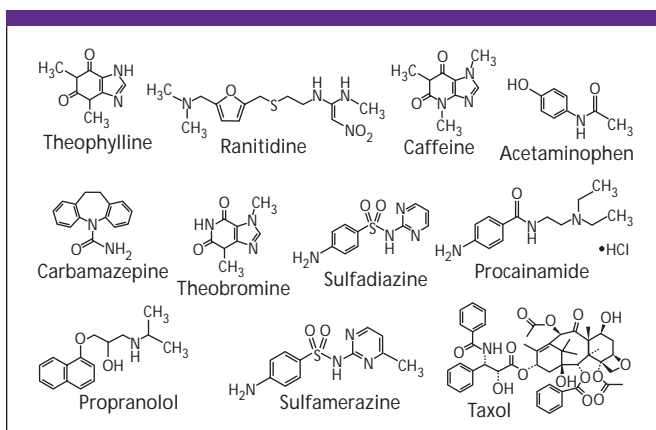
**Table I: Results of generic SPE-LC-MS-MS\***

Compound	SRM ( <i>m/z</i> )	Recovery† (%)	Linearity (ng/mL)	Correlation Coefficient	Carryover‡ (%)	LLO (ng/mL)
Sulfadiazine	251.4 → 156.2	99	1–1000	0.9982	<0.05	1.0
Sulfamerazine	264.8 → 156.2	103	1–1000	0.9998	<0.05	1.0
Taxol	854.5 → 285.9	97	5–500	0.9953	<0.05	1.0
Propranolol	259.8 → 183.0	100	2–1000	0.9995	0.08	1.0
Carbamazepine	237.6 → 193.8	99	2–1000	0.9997	<0.1	0.2
Procainamide	236.4 → 163.4	98	2–1000	0.9995	0.07	0.2
Caffeine	195.2 → 138.2	99	1–10000	0.9999	0.05	0.2
Ranitidine	315.2 → 176.0	98	2–5000	0.9993	0.07	0.25
Theophylline	181.2 → 124.0	99	2–10000	0.9995	0.02	0.25
Acetaminophen	152.2 → 110.2	100	5–1000	0.9992	<0.1	2.0
Theobromine	—	101	—	—	—	—

\* Twenty  $\mu$ L phosphoric acid per 1 mL of sample was added to release the strongly protein-bound sulfas, and a 3-mm SPE cartridge was used for the polar acetaminophen and theobromine. MS detection of theobromine could not be accomplished within the limited time we had access to this MS system. Compounds with identical colors were determined in a single run (seven-in-one experiments).

† Recovery was determined using diode-array detection.

‡ Standard autosampler wash procedure; no optimization.

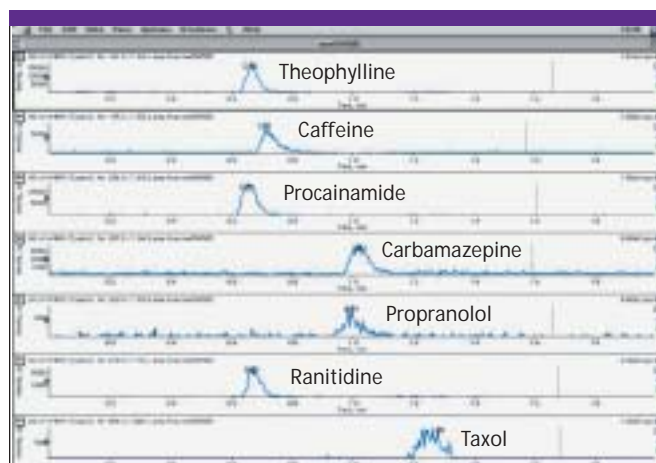


**Figure 3:** Fast generic SPE-LC-MS-MS — test compounds. The compounds selected for testing of the generic protocol. The compounds varied from polar (e.g. theophylline) to nonpolar (e.g. taxol), from acidic (e.g. sulfadiazine) to basic (e.g. procainamide), and from linear (e.g. ranitidine) to cyclic (e.g. carbamazepine).

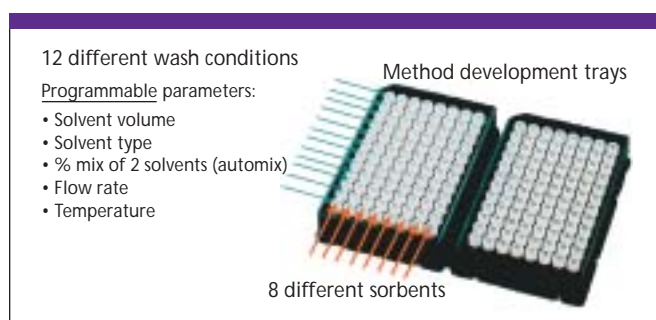
**2-min cycle time:** With dual-cartridge clamps, a new sample can be extracted on one clamp while analyzing the previous one on the other clamp. Hence, the overall assay cycle time equals the longest of the two parallel processes, which is the gradient LC-MS-MS run: 2 min.

### Rapid Method Development for High-Throughput Clinical Assays

For further optimization in terms of speed (fast LC-MS-MS run) and analytical quality, the data from the generic method can be taken as a starting point. SPE performance for different sorbents as a function of cleanup variables can be determined automatically using the concept described below and special method development cartridge trays (Figure 5).



**Figure 4:** Seven-in-one analysis of spiked porcine serum at 1 ng/mL for each compound. (Courtesy: TNO Pharma, Zeist, The Netherlands.)



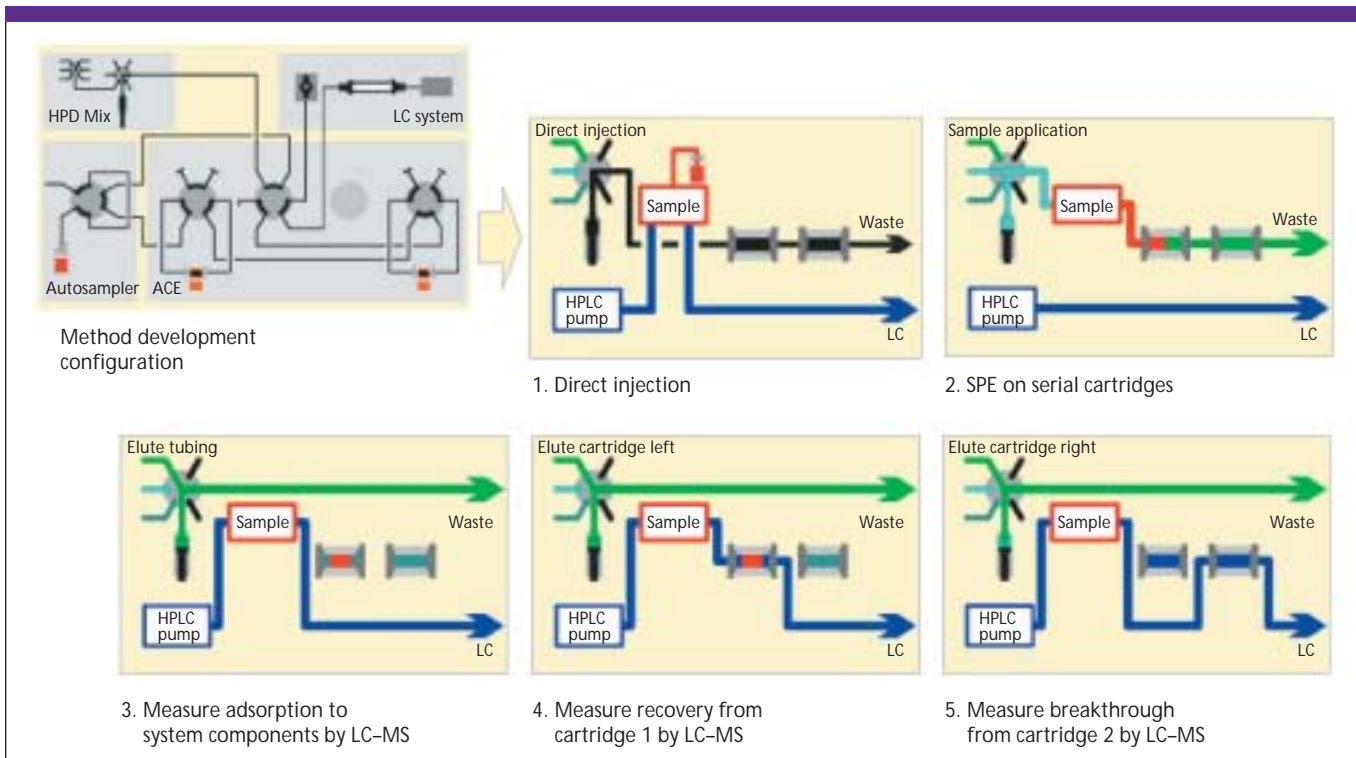
**Figure 5:** Method development trays.

### Intelligent Automated Method Development — Concept

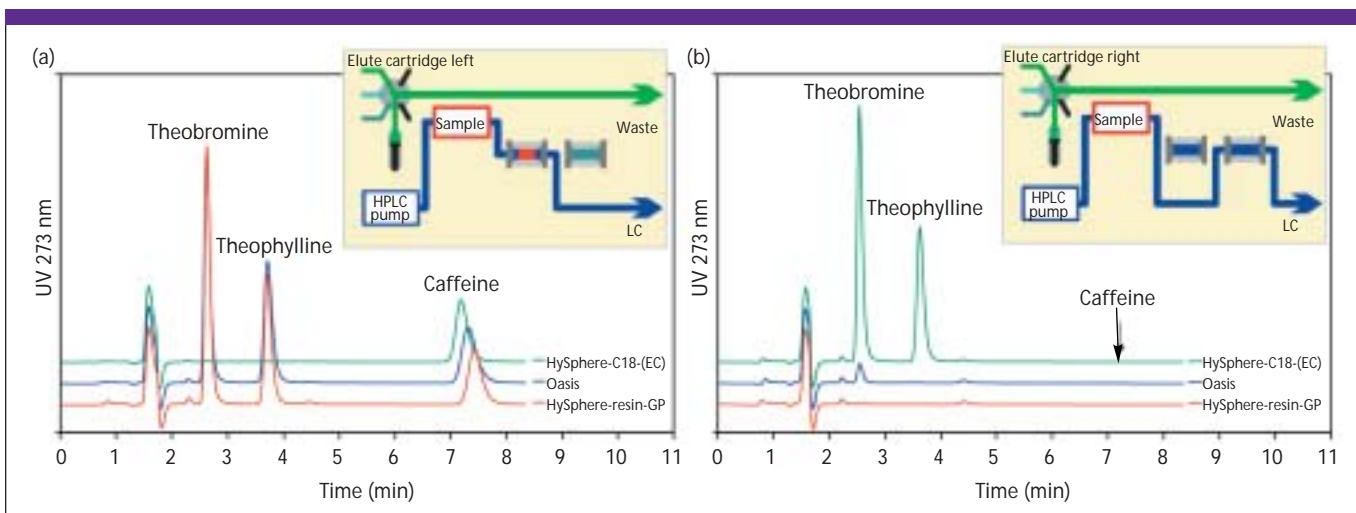
The configuration and procedure as illustrated in Figure 6 is used for intelligent method development to measure percent recovery, percent breakthrough, and percent adsorption to system components for every combination of sorbent and cleanup parameters.

### Automated Method Development

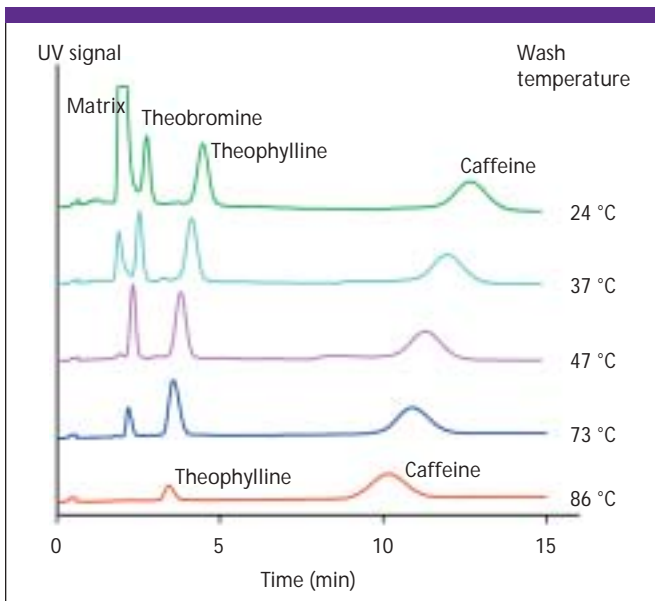
**Example — Testing of sorbent capacity for on-line SPE of xanthines:** SPE-LC-UV traces from cartridge 1 (Figure 7a) and cartridge 2 (Figure 7b) reveal that HySphere GP has 100% recovery for all xanthines (no breakthrough) and that C18 could be used for selective determination of caffeine.



**Figure 6:** Method development configuration. 1. Direct injection, 2. SPE on serial cartridges, 3. Measure adsorption to system components by LC-MS, 4. Measure recovery from cartridge 1 by LC-MS, 5. Measure breakthrough from cartridge 2 by LC-MS.



**Figure 7:** SPE-LC-UV elution profiles from (a) cartridge 1 to measure the recovery and (b) cartridge 2 (strong hydrophobic sorbent) to measure breakthrough from cartridge 1.



**Figure 8:** Chromatograms of xanthines in human plasma after on-line SPE-LC using a Hysphere resin GP cartridge and a 0.5 mL wash with water at different temperatures.

#### Temperature Assisted On-Line SPE (TASPE)

Figure 8 is an example of optimization of wash temperature for on-line SPE-LC as alternative to modifier addition or larger wash volume. Elevated wash temperature enables faster removal of early eluting matrix compounds. Temperatures greater than 47 °C lead to breakthrough of the polar xanthines.

#### Conclusions

- Parallel on-line SPE permits SPE-LC-MS-MS cycle times to 1 min or less (Figure 9).
- The generic SPE-LC-MS protocol had a success rate of 10 out of 11 model preclinical assays.



**Figure 9:** Prospekt•2™ in high-throughput configuration.

- Application of the intelligent SPE method development concept enables fast automated testing of all SPE variables. The concept enables discrimination between analyte breakthrough, adsorption losses, and ionization suppression as possible causes of reduced analyte signal.
- Temperature is a valuable parameter to optimize SPE performance.

#### Acknowledgment

We would like to thank Dick van de Lagemaat, William van Dongen, and Elwin Verheij from TNO Pharma (Zeist, The Netherlands) for giving us access to the API 3000 mass spectrometer and for giving their valuable technical assistance.

#### Reference

- (1) Mike S. Lee and Edward H. Kerns, *Mass Spec. Rev.* **18**, 187-279 (1999).

**Spark Holland B.V.**

P.O. Box 388, 7800 AJ Emmen, The Netherlands  
tel. +31 (591) 631700, fax +31 (591) 630035  
www.spark-holland.nl