



High SPEed™

On-line multidimensional SPE-SPE-LC of raw biofluids



Clinical Diagnostics

Therapeutic
Monitoring

Metabolite
Identification

Biomarker Discovery

SPE-SPE-LC



On-line SPE workstation for undisturbed LC-MS-MS Analysis



Direct injection of raw biofluids, e.g. plasma, serum, urine



Elimination of time-consuming and cost-intensive off-line operations



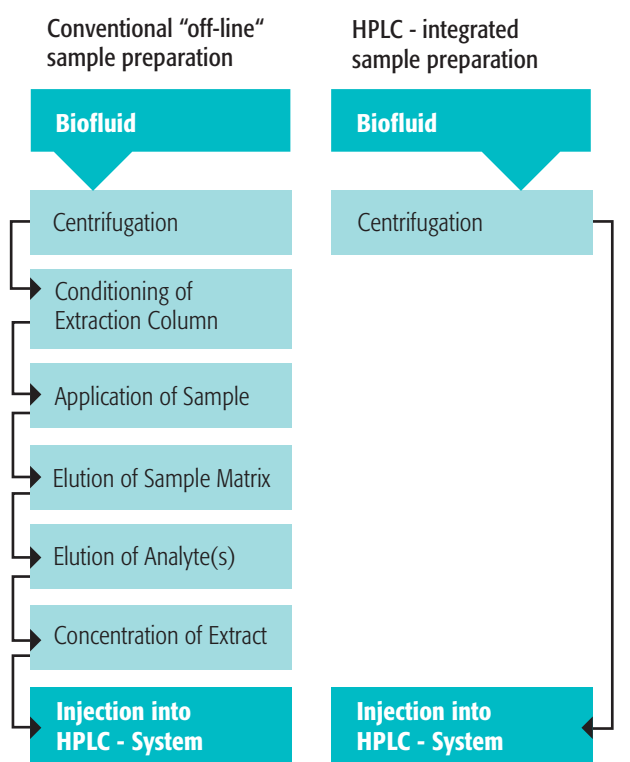
Dedicated SPE material for highly efficient matrix depletion



Unique in-line dilution for peak compression prior separation



Ready to go PAL System including SPE consumables



Comparison of conventional and integrated sample preparation

Conventional "off-line" sample cleanup

Over the last few years, cost-reduction and time to market have rapidly become highly important in every bioseparation research laboratory. In this context, automation of analytical procedures is undoubtedly one of the key parameters in method development and in its routine application. With regard to HPLC of complex biofluids, the main drawback of many protocols is the time consuming and labour-intensive nature of sample pretreatment. Currently, extraction of analytes by adsorption chromatography on bonded stationary phases (Solid-Phase Extraction, SPE) is the dominant clean-up technique. Many different types and sizes of pre-packed, disposable cartridges are commercially available. By proper selection of the bonded phase and the washing as well as the elution solvents, a large variety of compounds can be isolated from complex matrices to facilitate subsequent chromatographic separation and quantification. Conventional off-line SPE, however, is labour- and cost-intensive, error-prone and time consuming as it involves multi-step operations. Optimization of sample pre-treatment thus should be directed towards improved selectivity, high sample throughput and total automation with simultaneous cost-reduction and improvement of the overall analytical quality.

HPLC integrated "on-line" Solid Phase Extraction

Partially these requirements can be accomplished by integrating the extractive sample clean-up process into a HPLC-system (on-line SPE). For this purpose a SPE column is connected to a conventional analytical HPLC column via a six-port valve (column-switching, SPE-LC coupling). Such special SPE column packings, allowing the direct and multiple injection of untreated, complex samples (e.g. biofluids such as plasma, serum, saliva, urine) are called Restricted Access Materials (RAM). These porous silica or polymer based packings are characterized in that they possess a defined exclusion barrier and a non-adsorptive outer particle surface towards macromolecular matrix components (e.g. proteins). Low molecular compounds, i.e. target molecules, have free access to the binding centers and thus can be selectively extracted and enriched prior to their analytical separation.



Classical single SPE column system

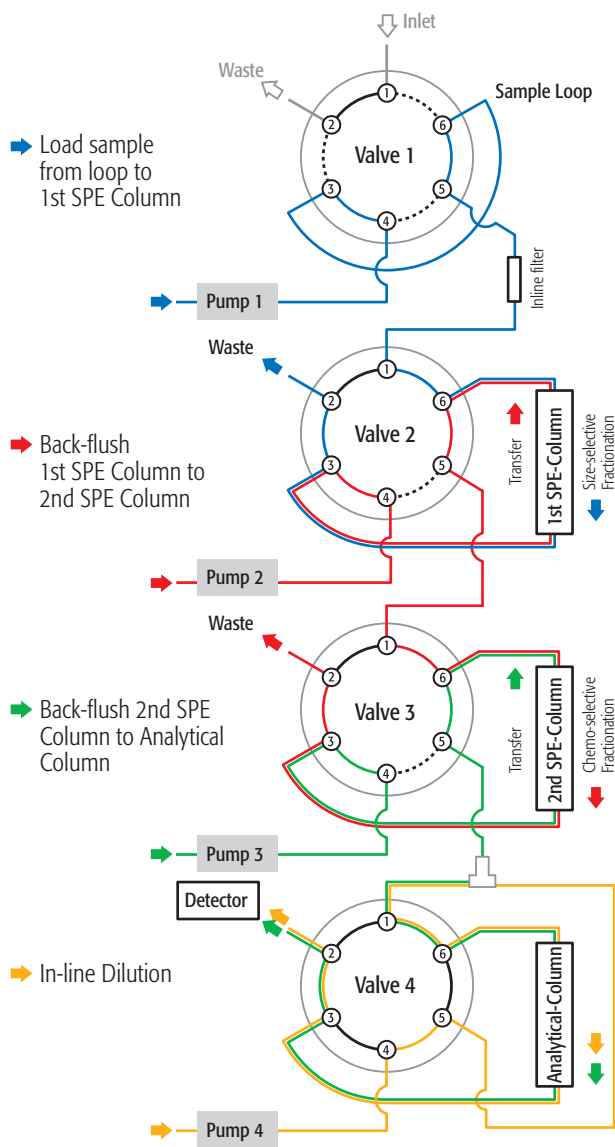
High SPEed™ Multidimensional SPE-SPE-LC System

The combination of two different SPE columns with an analytical LC column allows to set-up very efficient, multidimensional (MD) separation systems for highly selective on-line separation of complex mixtures.

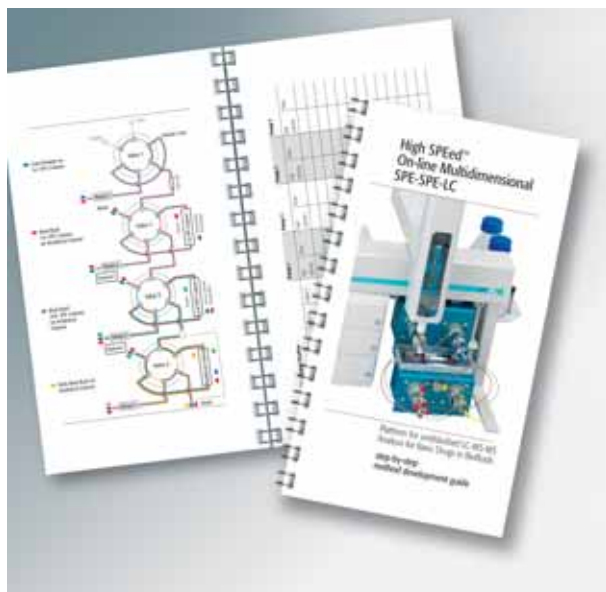
The step-by-step method development guide provided with the High SPEed™ option and the example described, is the combination of a SPE column having SEC and RP properties with a second SPE column exhibiting IEC (Ion Exchange Chromatography) and RP properties. The second SPE column is additionally coupled to a RP analytical LC column. It has been shown that such a MD-SPE platform is highly efficient with regard to the depletion of low and high molecular weight components, which otherwise would disturb the electrospray ionization process in LC-MS-MS analysis. This MD-SPE procedure is generic with regard to basic drugs (e.g. antidepressants) present in plasma or urine.



High SPEed™ "on-line" SPE-SPE-LC Workstation



High SPEed™ Flow schematic system overview (including "In-line Dilution" option)



Method development guide

Method Development Overview

- **Step 1 Analyte Separation**
Despite of being the last step in an on-line procedure this step should be evaluated at first.
- **Step 2 Determination of Switching Time of Valve 2**
Elution Profile of Matrix and Analytes
- **Step 3 Determination of Switching Time of Valve 3**
Depletion of low and high molecular weight components which otherwise would disturb the electro spray ionization process in LC-MS/MS analysis.
- **Step 4 Evaluation of Analyte(s) Recovery**
The recovery of the analyte(s) gives a good indication of the efficiency of the two clean-up steps.

Advanced Method

- **In-Line Dilution**
If the amount of organic modifier used to back-flush the second SPE column is too high the analyte(s) can not be focused at the top of the analytical column. An enrichment is possible by adding water from Pump 4 via a T-piece at the entrance of Valve 4.
- **Post Column Infusion**
To monitor any suppression or enhancement of the ionization of the analyte by the matrix constituents is a constant flow of a diluted analyte added to the sample stream.
- **Early Back-flush of Analytical Column**
An early back-flush would allow increasing the throughput if the later eluting part is of no interest.

On-line SPE-SPE-LC

High SPEed™ represents a dedicated, easy to use automated platform for undisturbed LC-MS-MS of basic drugs in biofluids. The "on-line SPE-SPE" clean-up procedure for basic analytes present in raw biofluids such as urine and plasma eliminates all matrix effects influencing the ionization process which is one of the major problems in bioanalytical LC-MS-MS analysis. Eliminating matrix effects ensures a high quality identification and quantification of the target analyte compounds and allows to speed up the chromatographic process. Typical cycle times are 5 to 10 minutes. High SPEed™ is a unique and flexible workstation for the routine analysis in clinical and other bioanalytical labs. The concept is based on the work of Prof. Dr. Dr. Boos and his co-workers*. A step-by-step method development guide is provided to establish the valve switching times and recoveries of the individual classes of basic drugs.

* Prof. Dr. Dr. Boos, Institute of Clinical Chemistry, University Hospital, Grosshadern Munich, Germany, and co-workers.

PAL LC Versions General Specifications

System Type

XYZ robot with syringe only concept, no tubing in sample path

Local User Interface

Control panel with 4 function keys, graphical LCD display, unique scroll knob for teach functions

Remote Control

Cycle Composer control software Windows 2000 / XP
Third party instrument drivers for all major LC/LC-MS Systems

Maintenance

Accessibility to all maintenance parts from front
Preventative maintenance kits and IQ/OQ documents available

Electrical Control

up to 2x RS232
up to 3x TTL Input
up to 2x Opto Coupler Input
up to 2x Relay Output

Power Requirements

100-240V, 120W, 50/60Hz

Electrical Safety Standards

CAN/CSA C22.2 No. 61010-1 / ANSI/UL 61010-1 / EN 61010-1

Environment

4°C - 40°C constant temperature, < 80% humidity (non condensing)

Weight

~ 10kg (without accessories)

Specifications are subject to change without notice

PAL High SPEed™ System Configuration

Basic Instrument Platforms

PAL HTX9 or PAL HTS9 or PAL HTC9

Valve Switching Module

PAL 4VlvDrv-C2V6, 4-Valve Module incl. 4pcs. 6-port Cheminert Valves

Sample Storage

PAL StkCooler, Peltier cooled 6DW Stack 4°C to 40°C
for 6 DW or 6 MT Plates or 324 2ml vials

Injection Syringes

PAL SyrLCX100µl, 100µl Syringe Kit incl. SYRX G100-22S-3
(optional 25µl and 50µl available)

WashStation

PAL WashActive Station or PAL WashFast Station

Software

PAL CycCompCD, Cycle Composer incl. High SPEed™ startup macro's

Documentation

Step by Step High SPEed™ Application Development Guide

SPE Columns

1st SPE column Lichrospher® ADS RP RAM Selectivity Kit
2nd SPE column Oasis® WCX online column 30µm 2.1 x 20mm
(The High SPEed™ System is compatible with a wide variety of different column materials and column dimensions)

In-line Filter

Recipe In-line filter FK 7400

PAL LC Sample Injection Systems

perfect sample loader for leading Mass Spectrometers



Distributed by:

CTC Analytics has dedicated the last 20 years to the continued development and high reliability of advanced sample injection technology. To learn more about the unique PAL Series of LC/LC-MS sample handling systems or any of our GC/GC-MS sample injection systems contact your CTC Analytics distributor.

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