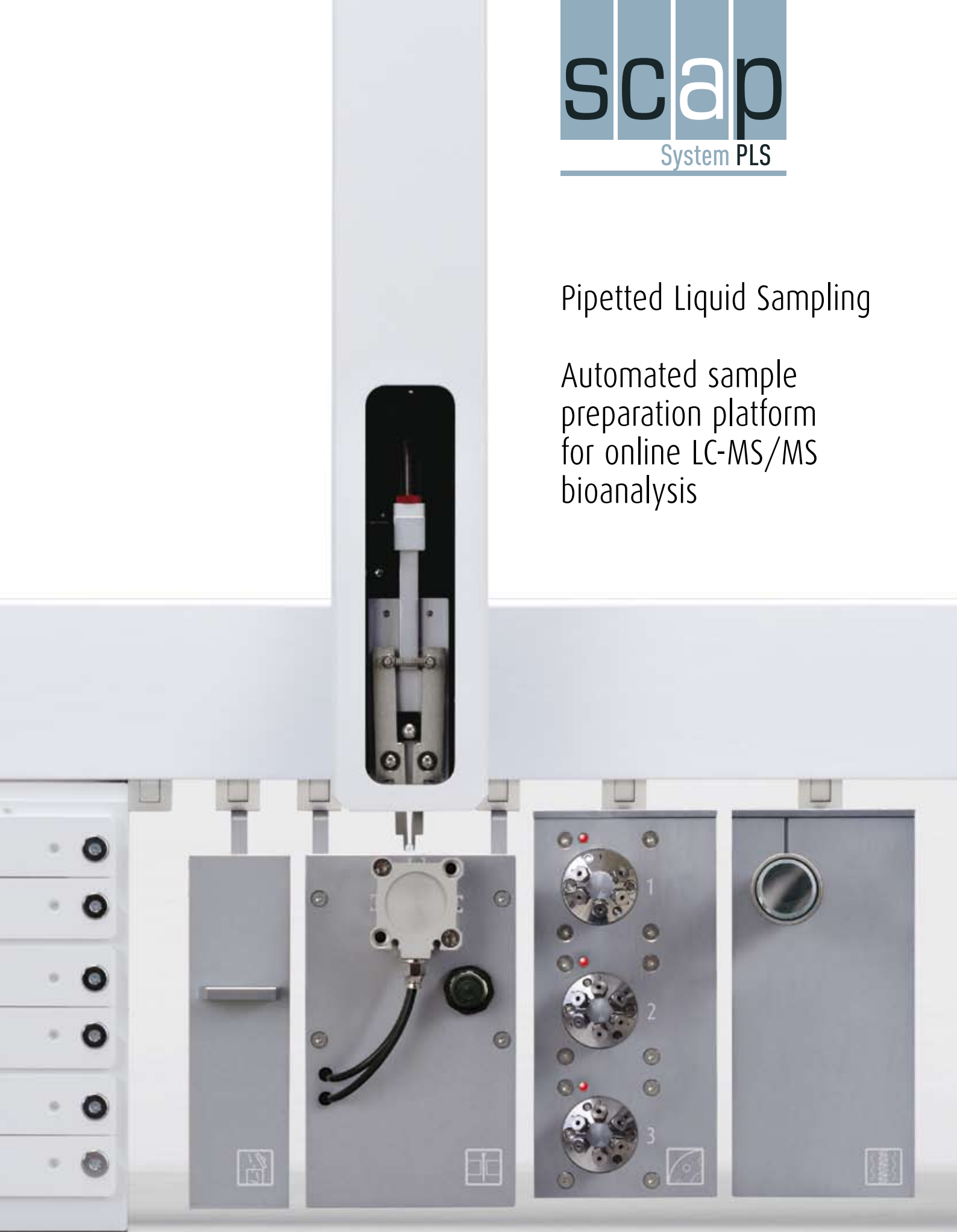


## Pipetted Liquid Sampling

Automated sample  
preparation platform  
for online LC-MS/MS  
bioanalysis



# Integrated Sample Processing



SCAP PLS system components mounted on a robotic platform

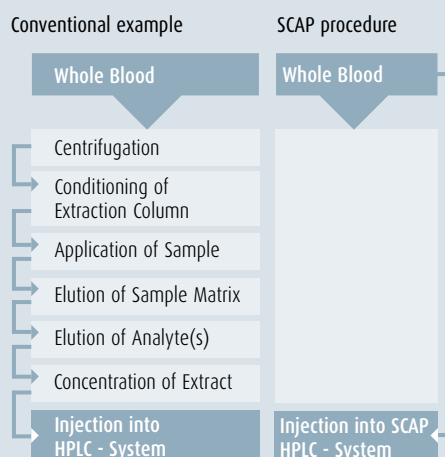
## Integrated Sample Processing

Both the healthcare and pharmaceutical industry do a tremendous number of analyses of substances in biological fluids, of which blood is one of the most common. In the healthcare industry, complete measurements are done to diagnose patients to determine the efficacy of administered drugs or to screen for early detection of diseases. In the pharmaceutical industry, measurements are done during the development process of new drugs (e.g. PK/TK studies, etc.).

Whole blood is a difficult, complex matrix to handle. In order to determine and quantify substances in blood, various intermediate preparation steps are required (e.g. Blood Spots, Dialysis, Liquid-Liquid Extraction, Precipitation, (Hemo)Lysis, Membrane Filtration, Anticoagulation). The major drawback of all these protocols is the time consuming, error prone and labour intensive nature of manual sample pretreatment. The SCAP PLS system from Prolab GmbH represents a unique approach for biofluid analysis and eliminates the drawbacks of current methods. It delivers a fully integrated workflow from sample collection until the final result without any manual sample preparation. A newly designed disposable SCAP PLS system sample cartridge is used for clean and safe blood collection.

The SCAP PLS cartridge is used for sample collection directly at the subject. Transferred to the SCAP PLS system it serves afterwards as a disposable sample loop prior to the following automated sample preparation steps.

## Comparison of conventional and SCAP PLS system sample preparation



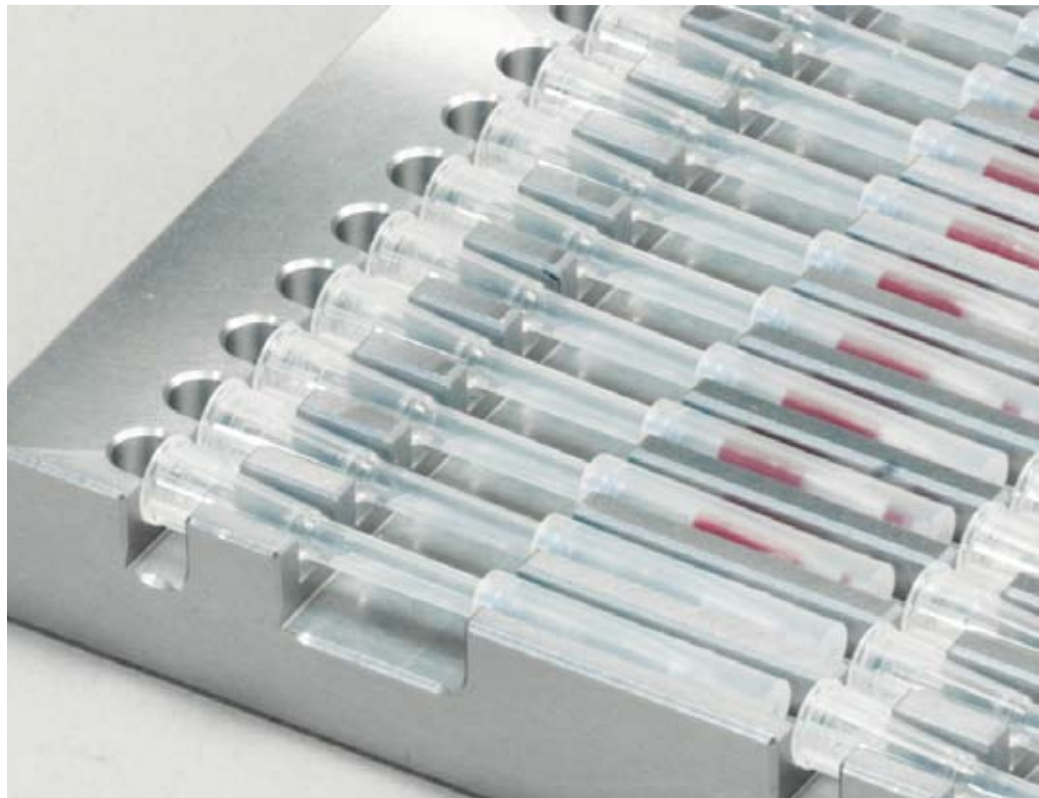
## Advantages

- Fully automated sample processing of biofluids including whole blood samples
- Integrated workflow from sample collection until the final analysis result
- One step sample manipulation, simplifies sample logistics, avoids sample mix-up
- Requires a minimum amount of sample for quantitative results (typically 5 µl)
- Minimized infection and contamination risk due to completely sealed and disposable sample containers
- No re-used syringe or sample loops. Disposable sample container, eliminates any carryover
- Simplifies method development, use of same generic method for different matrices
- Fully automated process saves time and labour costs versus traditional sample preparation
- Saves additional costs for SPE materials, solvents and other consumables

# Smart Sample Collection



Left: Assembled SCAP PLS system sample cartridge  
Right: Numbered SCAP PLS system tip and cap

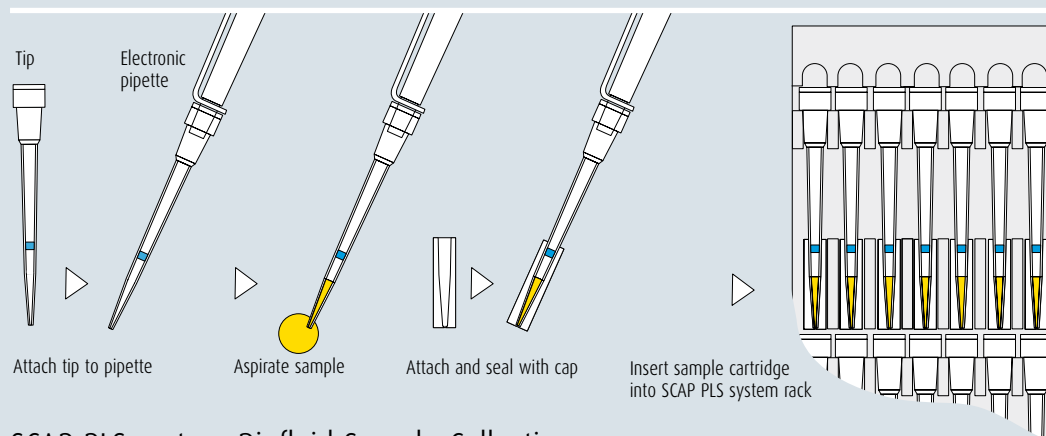


Loaded sample cartridges inserted in the SCAP PLS system rack

## Sample Collection Process

The SCAP PLS system sample collection process is as easy as any other standard pipetting task. The SCAP PLS system tip is attached to an electronic pipette and the desired sample volume is aspirated. The lower tip end is sealed with a SCAP PLS system cap. The completely sealed cartridges are then placed into the 54 position SCAP PLS system rack and are ready for immediate analysis. Alternatively the filled sample racks can be stored in a freezer for later analysis or may be shipped to an external location.

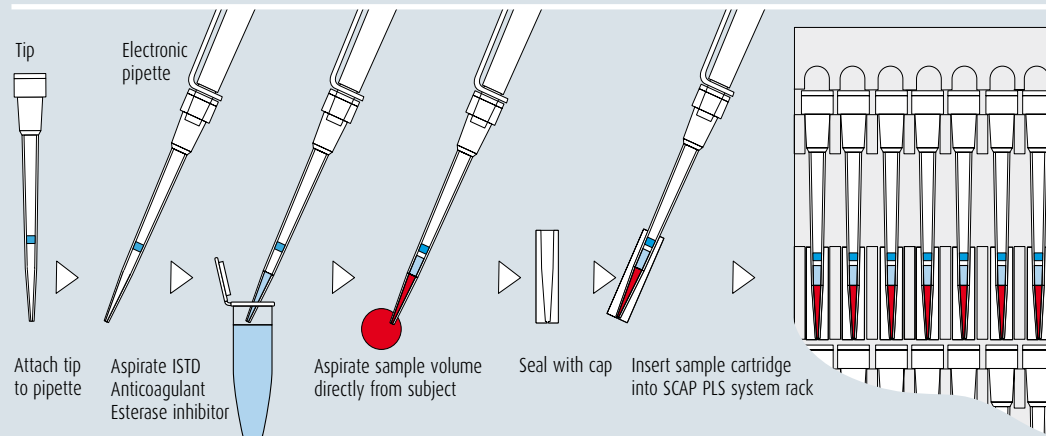
## SCAP PLS system Standard Sample Collection



## SCAP PLS system Biofluid Sample Collection

Sampling of supernatant (e.g. serum, plasma) or whole blood involves some additional pipetting steps. First an anticoagulant and if desired an esterase inhibitor are aspirated. Additionally the sample may be spiked with an appropriate internal standard. Finally the biofluid is aspirated and the cartridge is sealed.

In case of whole blood, samples are directly collected at the subject (e.g. finger, vein, heel, ear lobe) and are ready for immediate analysis without any further manual sample preparation steps.



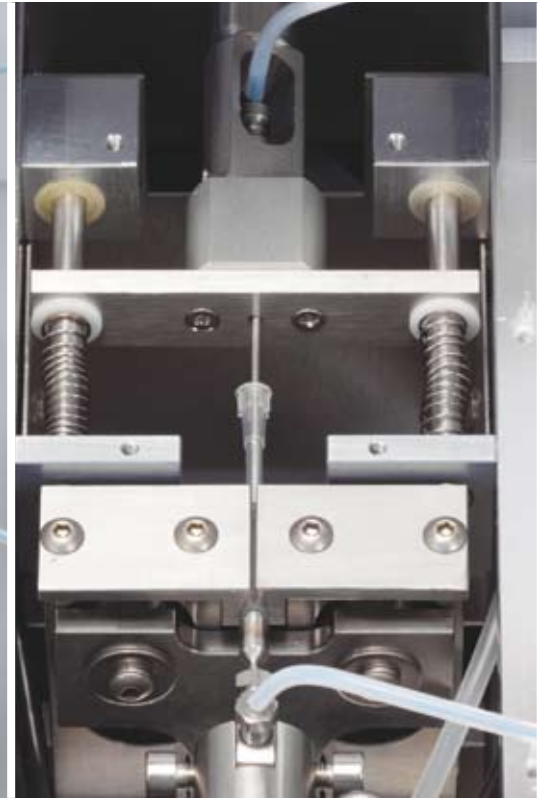
# Efficient LC-MS/MS Workflow



SCAP PLS system robotic gripper tool picks-up sample cartridge...



...and places it into the SCAP PLS system clamp module...



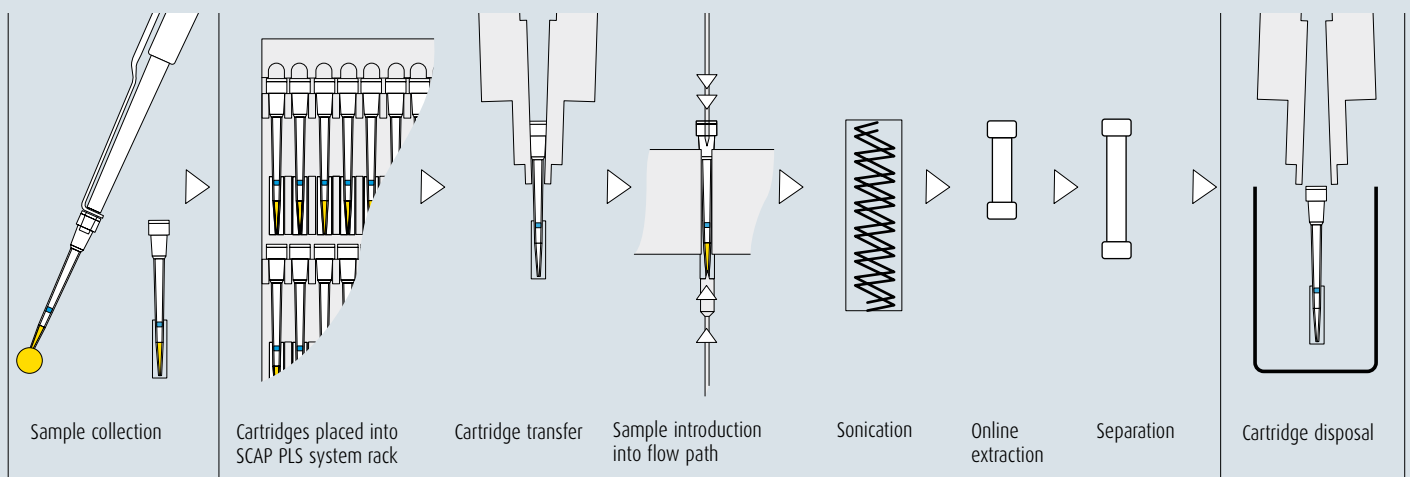
...where it is pierced at both ends and flushed into the analytical flow path.

## SCAP PLS system Sample Preparation Process

The SCAP PLS system samples directly from disposable cartridges without prior solid-phase extraction or any other sample desalting/pre-processing step. After placing the cartridges into the SCAP PLS system sample rack the samples may be stored in a freezer, shipped to another location or are directly analyzed. The cartridges are automatically picked-up by the autosampler gripper tool and are inserted into the clamp module. Both ends of the cartridge are pierced to eject the sample into the analytical flow path. The sample passes

first through a pulsed ultrasonic module, where the blood cell components are disintegrated and homogenized. This patented technology avoids any clogging of the fluidic path or the SPE pre-columns. Pre-columns may be used up to 1000 extractions before exchange. Subsequently the analyte reaches a restricted access material column (RAM) to remove selectively higher molecular components such as proteins. The trapped lower molecular compounds are eluted again and finally separated on the analytical LC column.

The SCAP PLS system is delivered with a ready to go control software, which triggers all necessary auxiliary hardware and valve switching operations. It can be interfaced to any leading mass spectrometer and data acquisition system.

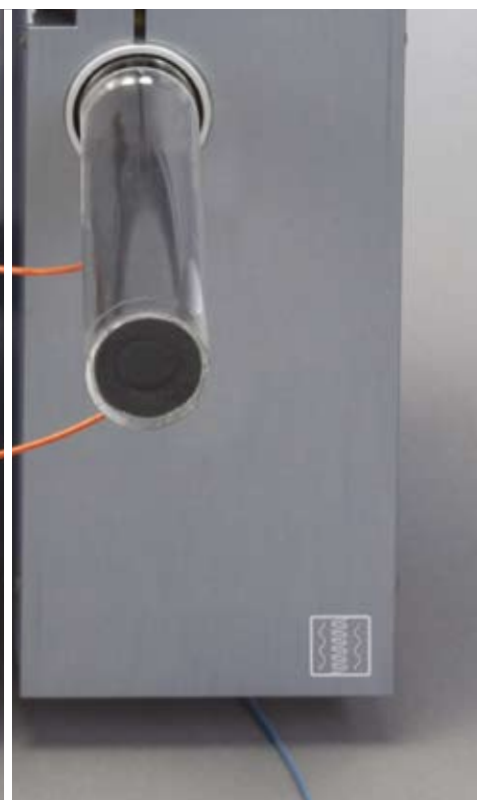




# Minimized Sample Volume - Maximized Return



Valve module



SCAP PLS system ultrasonic module



SCAP PLS system waste box

## Clean Sample Introduction

In contrast to current automated HPLC sample introduction technology the SCAP PLS system works with an exchangeable sample loop. Each sample is prepared and processed in a disposable SCAP PLS system cartridge which is discarded after sample injection. No glass syringes, injection ports and time consuming wash procedures are involved. This ensures fast and virtually carry-over free operation. For convenient sample handling, the SCAP PLS system cartridges are delivered in 54 position tip boxes. To load the tips a conventional pipette is used. Prepared cartridges are completely sealed and may be stored or directly analyzed.



Cartridge preparation with electronic pipette

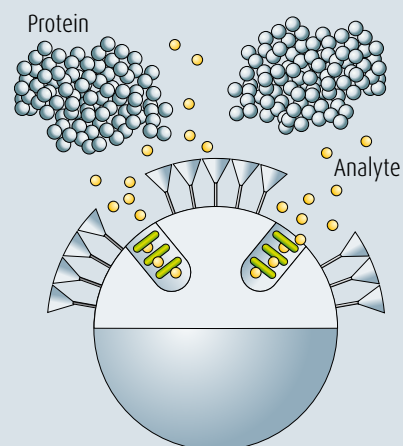
## SCAP PLS system Valve module

The valve module consists of up to 3 valve drives and connects the clamp module containing the disposable sample cartridge to the analytical separation column. Depending on the application the drives are equipped with up to 3pcs. 6-port valves for regular HPLC or UPLC operation. Only one valve is used if the sample is injected without prior online sample preparation. The two valve configuration enables a regular pre-column solid phase extraction step.

Three valves are recommended if a dual stage cleaning procedure on a RAM and an additional SPE pre-column is performed. All necessary valve switching operations are controlled by pre-stored timetable events and may be optimized for the individual analytical method. Optionally the valve module can be equipped with high pressure valves for UPLC operation up to 20'000 psi.

## RAM Column

RAM columns allow the direct extraction and enrichment of hydrophobic, low molecular analytes from untreated samples such as haemolysed blood, plasma, serum, milk, fermentation broth, supernatants of cell cultures as well as food homogenates. It allows fully automated preparation of the sample prior to the analytes being separated in the analytical column. This allows the untreated biofluid to be directly injected without negative effects on either the column or the results achieved.

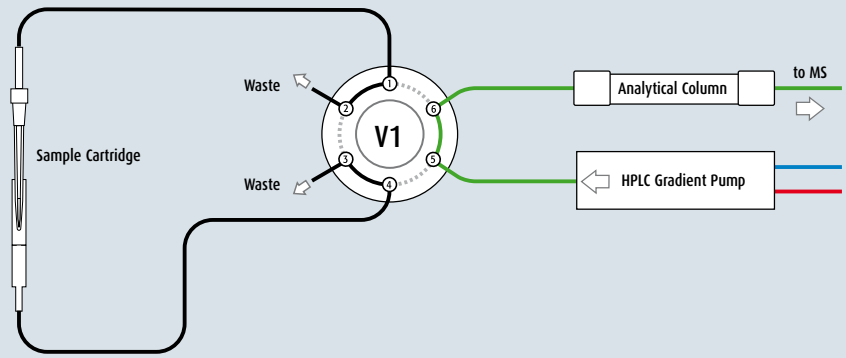


Restricted Access Material principle

# Column Switching Options

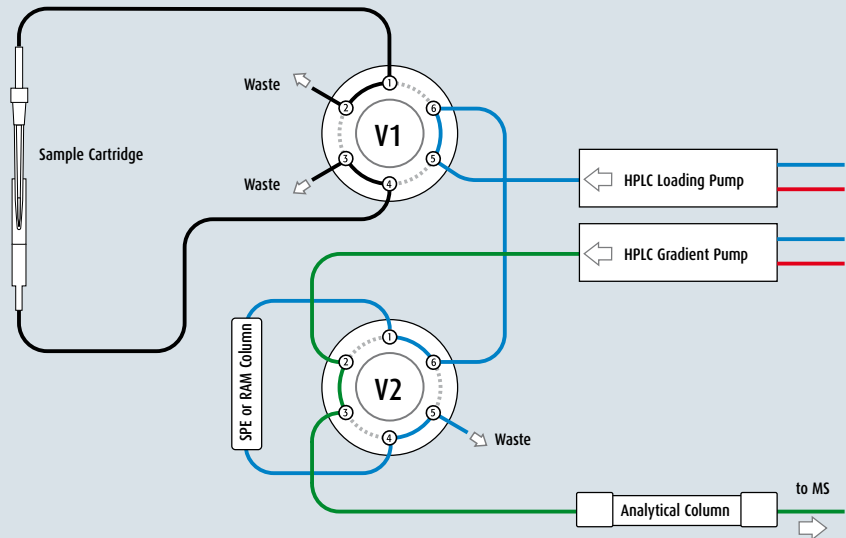
## Single Valve Option

The single valve configuration shows how a sample is loaded directly onto an analytical column without prior sample preparation steps. Thereby the SCAP PLS system cartridge acts as a disposable sample loop and is automatically exchanged after each injection. This setup is limited to a system pressure of 2'500 psi (170 bar)



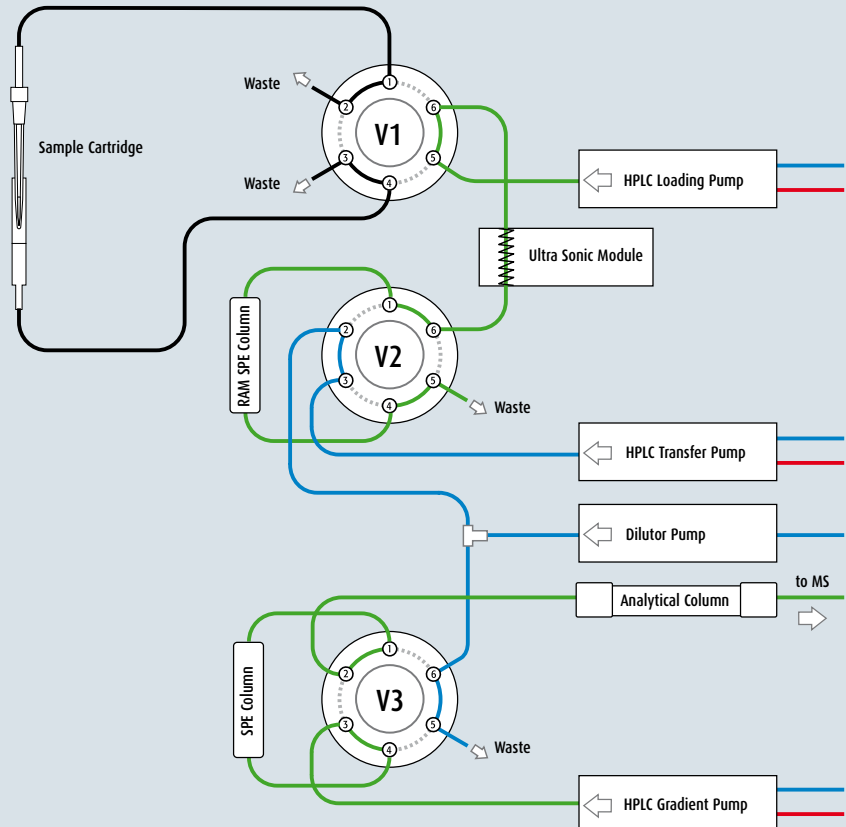
## Dual Valve Option

The dual valve configuration is used if prior to the separation on an analytical column a classical SPE cleaning step is required (e.g. serum, plasma samples). For higher throughput the SPE column is regenerated during the analytical run. Each sample is introduced into a disposable SCAP PLS system cartridge which is automatically exchanged before each run. No syringes, dilutors, injection ports or time consuming wash procedures are involved. This ensures fast, contamination free operation, with virtually no carry-over. This setup can be operated either for regular HPLC or fast UPLC applications up to 20'000 psi.



## Triple Valve Option

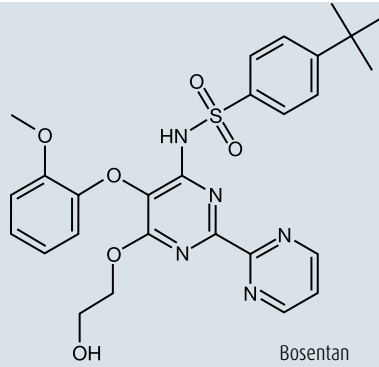
The triple valve configuration enables a dual stage cleaning step including additional sample sonication at the SCAP PLS system ultrasonic module. This setup is recommended for whole blood samples. First the sample passes the ultrasonic module where the blood cell components are disintegrated and homogenized. The sample reaches the RAM and SPE column where all unwanted matrix is discharged. Afterwards the trapped analytes are desorbed again to the analytical column with strong organic solvents. If the amount of organic modifier used for backflash is too high, an optional enrichment step may be included by adding water via a T-piece. While separation and detection take place, the pre-columns are re-equilibrated with the initial eluent composition to be ready for the next sample injection.



# Example Application

## Description

A new method for the detection of Bosentan and three metabolites is presented. Bosentan and its three metabolites were spiked in whole blood and analyzed by the SCAP PLS system. Mass spectrometric detection was performed on an MDS Sciex API 4000™ operating in positive electrospray ionization mode.

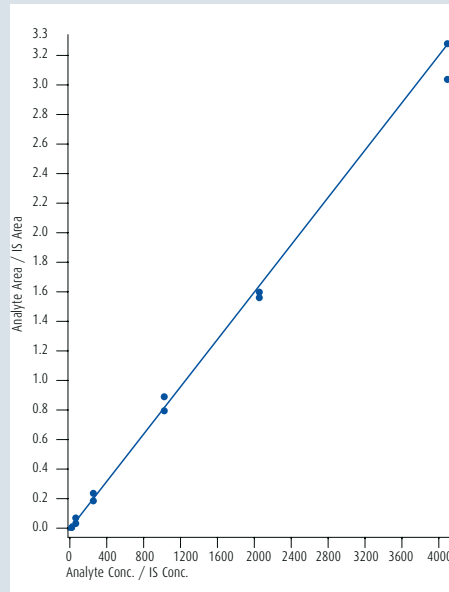


## Method

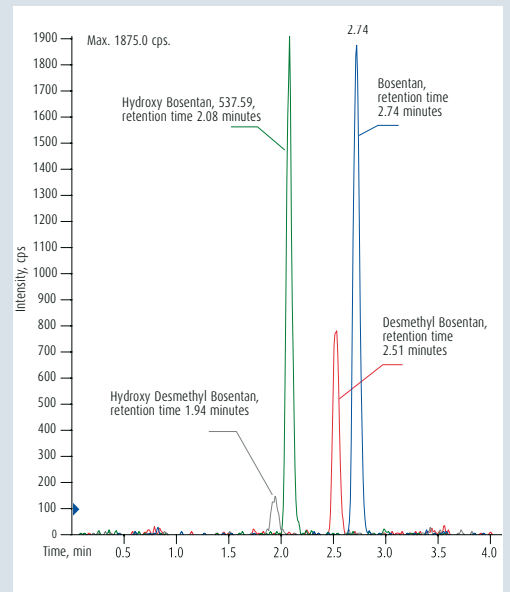
8 levels of calibration and 3 levels of quality control samples were spiked in whole blood with a linear range from 1.00 ng/mL to 4096 ng/mL for Bosentan and 2.00 to 512 ng/mL for the three metabolites.

For measurement of calibration and quality control samples, 5  $\mu$ L of each level were aspirated into the sample cartridge together with 1  $\mu$ L of internal standard solution (containing the ISTD's for all 4 compounds).

The mass spectrometer was operated in positive electrospray ionization mode with dwell times of 50 msec for each MRM transition and an HPLC flow rate of 300  $\mu$ L/min on the analytical column. The whole blood samples were cleaned on a Merck RAM column, pre-concentrated on a C18 trapping column and separated on a C6-Phenyl HPLC column.



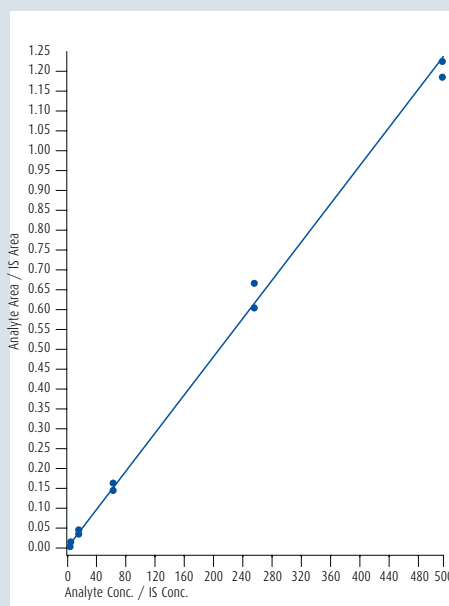
Calibration curve for Bosentan ( $r = 0.9979$ )



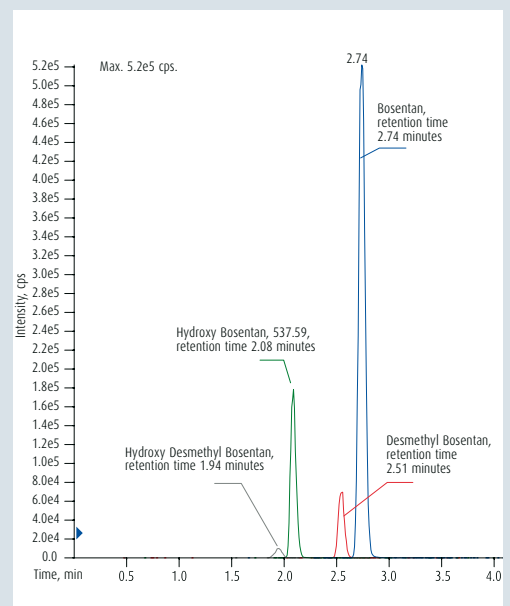
LLOQ for Bosentan and metabolites

## Data

Validation runs containing 2 calibration curves and 6 replicates of the 3 levels of quality control were analyzed. Typical calibration curves for Bosentan and one of the metabolites are presented beneath. The lower limit of quantification for all 4 compounds and a picture for a typical chromatogram are shown (traces for ISTD's are not shown).



Calibration curve for one Bosentan metabolite ( $r = 0.9977$ )



Typical chromatogram

# System Configuration

## Basic SCAP PLS system consisting of:

SCAP PLS system Clamp module  
 SCAP PLS system Gripper  
 Valve Drive module  
 (excl. valve drives and valves)  
 SCAP PLS system Ultrasonic module  
 SCAP PLS system Waste Box  
 SCAP PLS system Starter Kit



Electronic pipette



SCAP PLS system gripper



SCAP PLS system tips and caps

## SCAP PLS system Accessories

SCAP PLS system Rack  
 Set of 6pcs. SCAP PLS system Racks  
 Electronic Pipette



SCAP PLS system Ultrasonic module



Valve module



SCAP PLS system waste box

## SCAP PLS system Consumables

SCAP PLS system Tip Box, 54 Tips and Caps, numbered  
 SCAP PLS system Tip Refill, 110 Tips and Caps not numbered, bulk



SCAP PLS system tip box numbered



SCAP PLS system rack



SCAP PLS system clamp module

## PAL Configuration:

The following CTC Analytics PAL components are required to operate a SCAP System (Available either through CTC Analytics or Prolab GmbH)



PAL HTS9 or PAL HTS9-xt  
 PAL CycCompCD  
 PAL 1VlvDrv\*  
 PAL 2VlvDrv\*  
 PAL 3VlvDrv\*  
 C2V-1006D-CTC-K 6-port\*  
 C72VX-1696D-CTC-K\*  
 PAL StkCooler12MT  
 PAL MTHolder

PAL80 instrument 80cm X-axis  
 Cycle Composer Software  
 PAL 1-Valve Drive Module  
 PAL 2-Valve Drive Module  
 PAL 3-Valve Drive Module  
 VICI Cheminert Injection Valve 0.25mm bore, 5000psi  
 6-port VICI Cheminert Injection Valve, 0.25mm bore, 15'000psi  
 Peltier cooled Stack for 6pcs. SCAP Racks  
 Trayholder for 1pc. SCAP Rack

\*Choose one depending on your application

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 Instruments GmbH

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