

Novel Walk Away Automation for DBS Sample Extraction from 4-Spot Cards to LC-MS

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Overview:

The Dry Blood Spot (DBS) technique has a long history in various areas of whole blood sample collection, preservation, transportation and laboratory analysis. It has been applied where very little blood sample is available for many different reasons. As its popularity increased during the last few years in new areas such as pre-clinical drug discovery research, the call for automation in the laboratory also increased. (Fig. 1 and 2)



Figure 1 Drawing a small blood value



Used



Unused

Figure 2 Typical DBS filter cards

Introduction:

Automation no longer has just the value of labor cost savings. It is clearly recognized for error reduction and more consistent results. Manual sample preparation has always raised questions of consistency. Technology often simplifies the process and manual intervention. In this case several steps of the sample prep process are eliminated and escape a complex automation task. These are punching out filter paper samples from the original carrier as well as the elution process in a solvent vial. Eliminating the elution process from the filter paper as an intermediary step avoids unnecessary dilution, inconsistency, and contamination normally possible during a manual handling process.

Method:

The automated method on the SCAP platform (Fig. 3) applies a direct elution process from the DBS card to the LC or LC/MS system. An automatic internal standard addition during the elution process solidifies the consistency of results. The Robotic Platform is a well proven XYZ system (LEAP PAL) that is used in many configurations in research settings, production and quality assurance situations. The workflow is simple and can be easily observed on the SCAP PAL. A gripper for card handling, card racks, a clamp-elution (Fig. 4) station and the necessary valving system are the key components of the system. There is no syringe needed for sample injections and there is no injection valve. The sample plus the added internal standard are coming off the filter paper card to a pre-column by means of an LC loading pump. The gradient pump will elude the trapped sample analytes off the pre-column onto a normal LC or UHPLC system with UV or MS detector (see Fig.5). The clamp device can have different diameter dyes, allowing small to large diameter elution spots on the card. This has proven to be a useful variable to eliminate possible dilution steps when concentrations of analytes are relatively high.

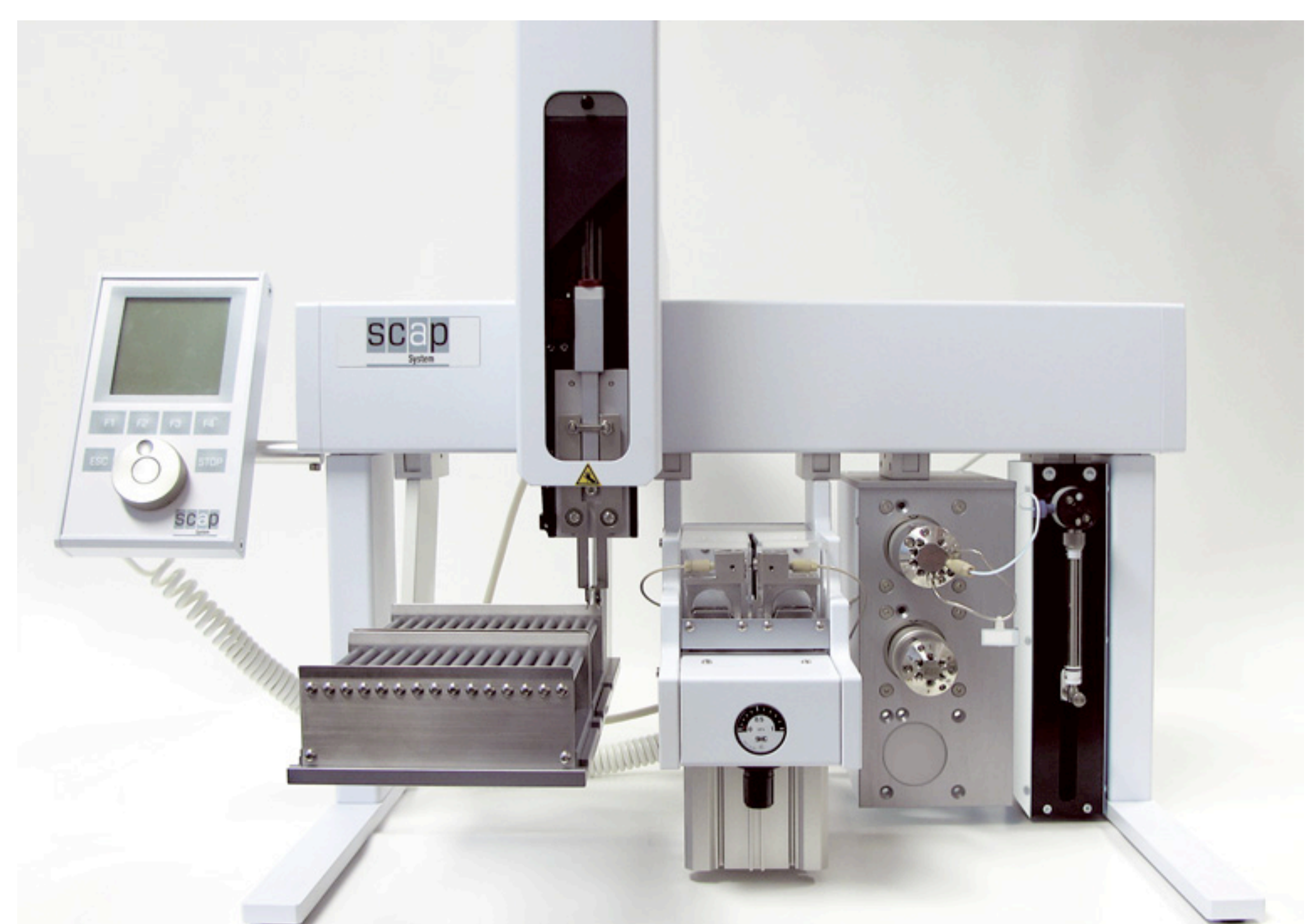


Figure 3 SCAP DBS PAL

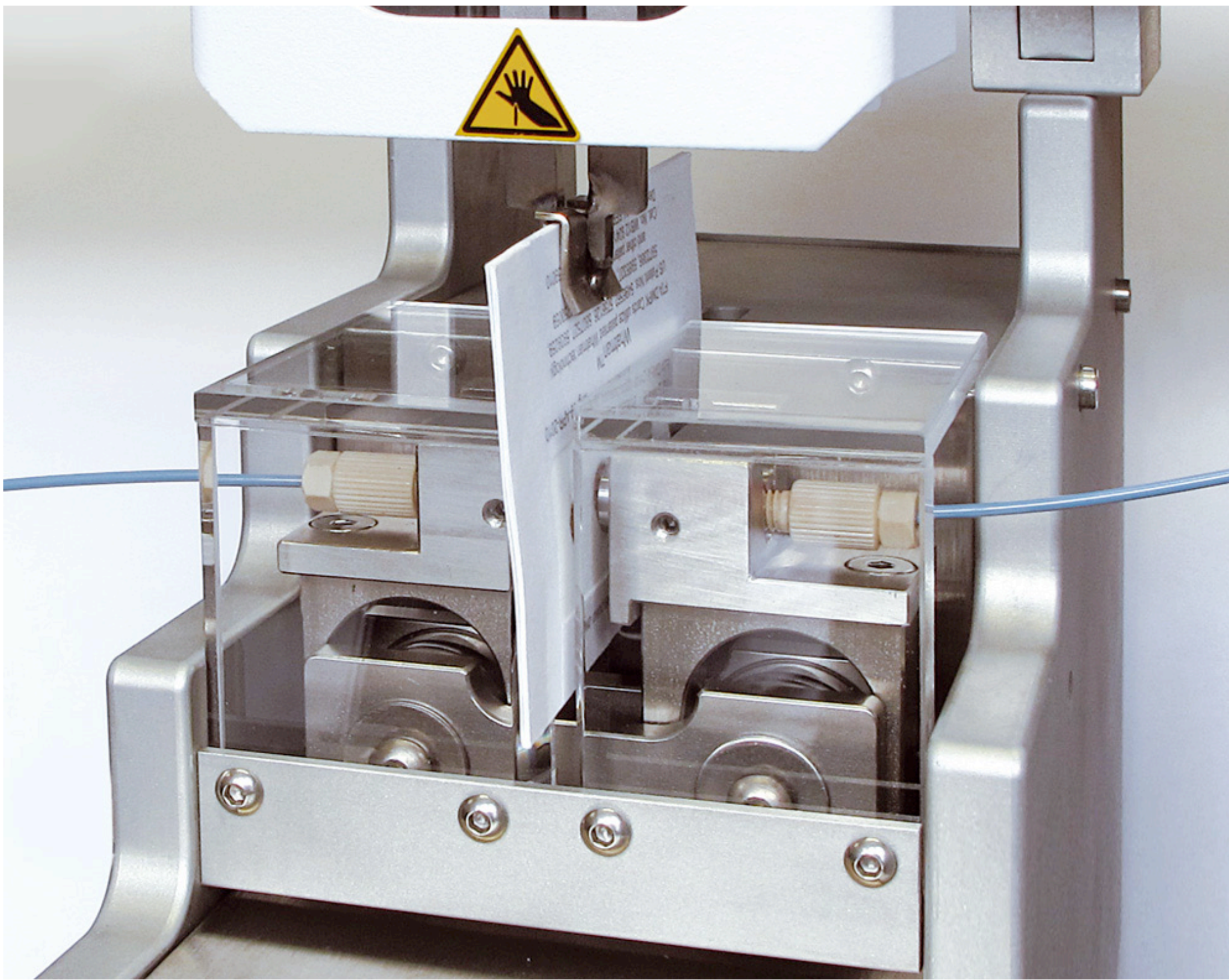


Figure 4 DBS Card Clamp for LC

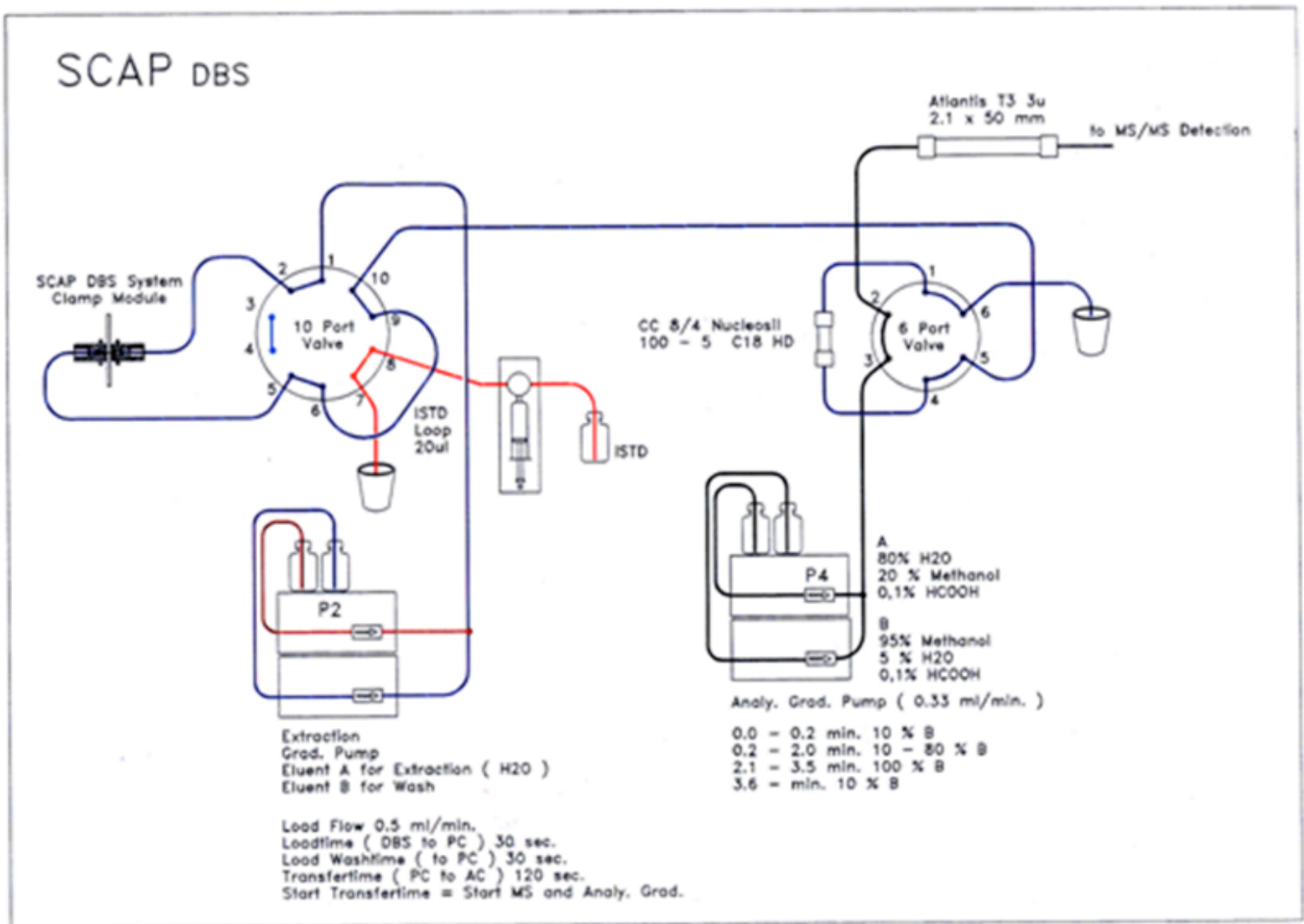


Figure 5 SCAP DBS Flow Path

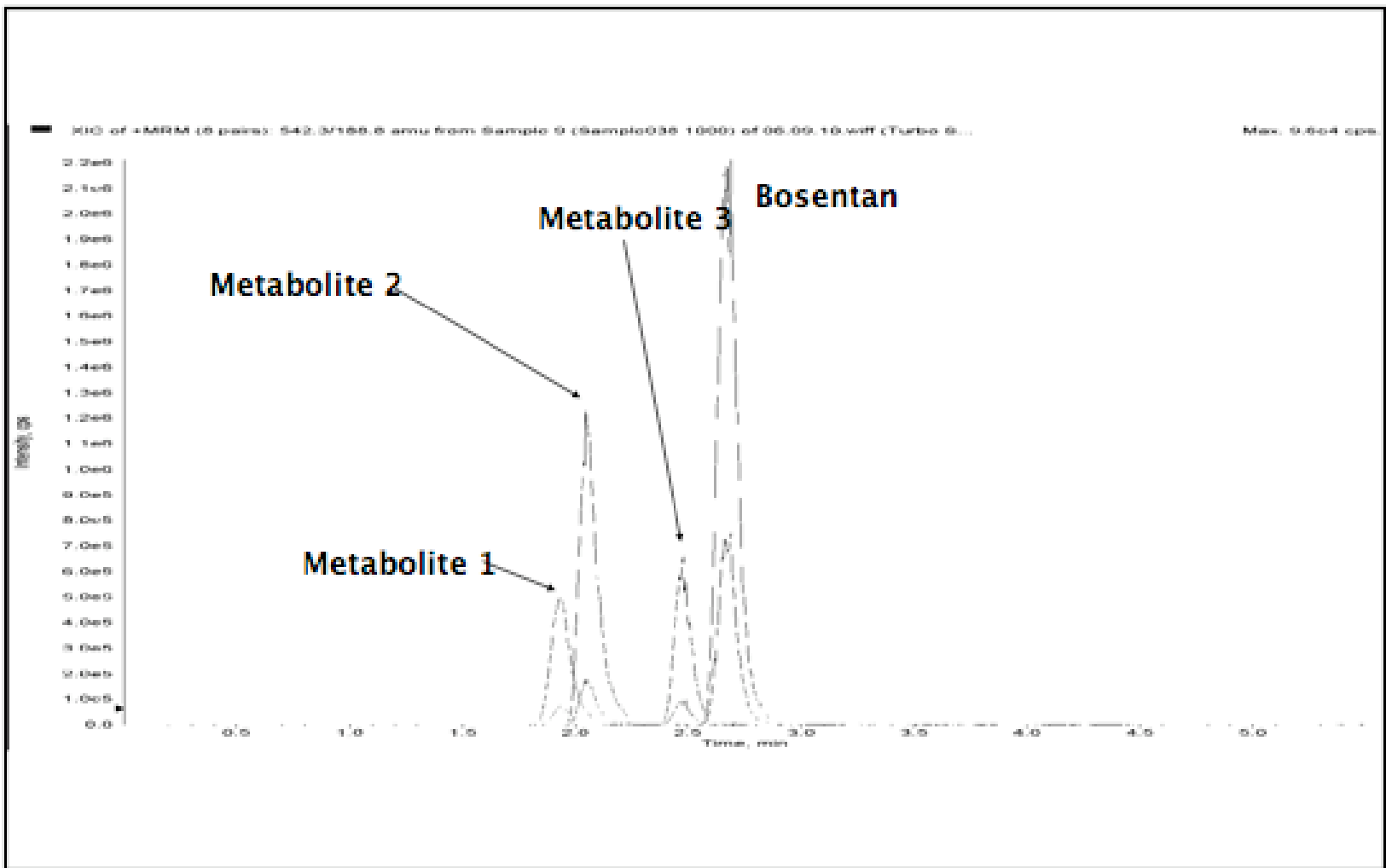


Figure 6 LC-MS /MS Chromatograms of Analytes and ISTDs

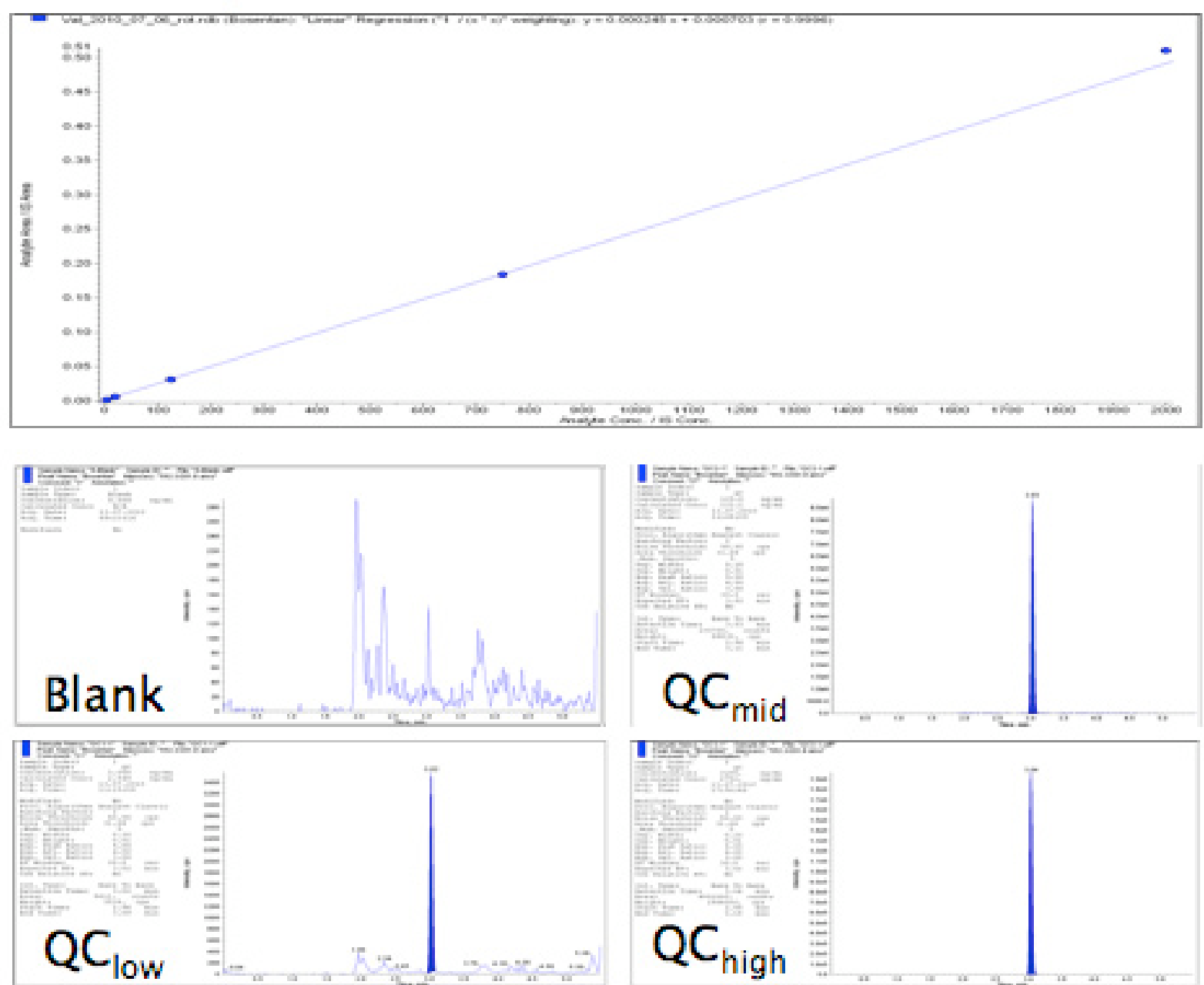


Figure 7 Linearity - Calibration Curve of Bosentan

Results:

Good linearity is shown in Figure 7 . Since trapping/ clean-up is fairly common when handling biological samples end-users will optimize many of the SPE and analytical separation themselves. Since these optimizations depend on the analytes to be identified and quantified some more generalized indications have been looked at. A typical flow diagram is shown in Figure 5. The key steps and proposed starting point selections are:
Elution (DBS) solvent provided by gradient pump1 :500ul/min H2O:Me 60:40; 2.1um x10mm RP polar C18 pre-column; gradient pump 1 also uses high org. for cleaning pre-column.
Elution (from pre-column): gradient pump 2: 400ul/min H2O: Org 60:40 plus 1.0ml/min H2O infusion before analytical column
For adjusting concentration levels for analytical column different dyes can be chosen for the elution clamp; standard is 3.2 mm, range 1.5mm to 5.0mm.

Figure 6 shows results for analytes and an ISTD (Bosentan).

The typical cycle time for getting the samples off the card onto the pre column is 45 seconds. Cleaning of the pre-column and its re-equilibration takes 30 seconds. This trap and clean-up cycle and the card handling fits easily into a typical analytical cycle for LC-MS.

Conclusions:

This SCAP PAL makes the DBS process in the laboratory a hands off process. The cycle time is short and the analysis time is more likely the throughput limiting factor. Even though field experience is still limited, the deployment of the PAL platform into this emerging application area provides proven reliable, simple automation for higher consistency and less sample mix-up and contamination.
The Dry Blood Spot (DBS) technique of eluting the blood sample from filter paper cards still lacks simple, reliable and inexpensive automation. Recently, Prolab Instruments, GmbH introduced a system (SCAP DBS System) built on the popular PAL XYZ robotic platform. LEAP Technologies has teamed up with the system developer Prolab to offer the SCAP DBS system in the U.S. and Canada.

There are approx.100methods/applications known for these sample carrier DBS cards. In many such areas automation would be a tremendous benefit. It would not only be labor saving, but also decrease handling, which means less errors and more consistency in getting the samples introduced into a separation and then the analytical system. The presentation goes through the work flow of the automation approach step by step. The experience with hardware and software so far will be reviewed, including experience with the built-in internal standard addition. The SCAP DBS has patent pending technology applied and is simple to use. The approach of eluting the dry blood sample off the card directly to a multi-column system by-passing all manual and cumbersome treatment steps of the sample cards. First results from needed cycle times and throughput will be presented. Beta test results with analytical data compared to the traditional manual method will also be shown.