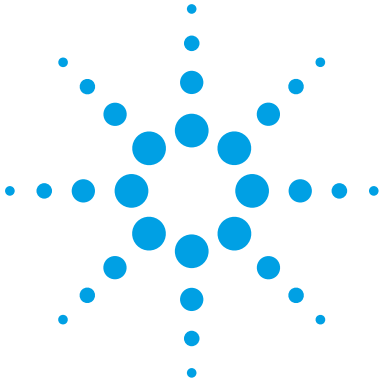

Agilent Automated Card Extraction Dried Blood Spot LC/MS System

Quick Start Guide

This Quick Start Guide provides an overview of how to use the Agilent Automated Card Extraction Dried Blood Spot LC/MS (AACE DBS LC/MS) system step-by-step instructions on how to start the program and run an extraction.

Agilent Technologies

What is the AACE DBS LC/MS System?

The Agilent Automated Card Extraction Dried Blood Spot (AACE DBS LC/MS) system is a fully integrated instrument for the automated analysis of Dried Blood Spots (DBS) and other Dried Matrix Spots (DMS). The system consists of the AACE instrument which enables automated flow-through analysis of Agilent Auto DBS cards, together with software to control the online analyte extraction, trapping, and LC/MS analysis. The AACE instrument is a CTC-based PAL autosampler platform integrated with SCAP DBS software (Prolab Instruments GmbH) and the Agilent 1200 Series Infinity HPLC to provide analytical LC separation. Quantitative analysis is performed using any of the Agilent 6400 Triple Quadrupole instruments (6400 Series LC/MS). Data processing and reporting is performed using Agilent MassHunter software.

Figure 1 Agilent Automated Card Extraction Dried Blood Spot LC/MS (AACE DBS LC/MS) system

System Overview

The AACE DBS LC/MS system consists of a high pressure binary gradient pump for extraction, an isocratic pump for dilution and another high pressure binary gradient pump for chromatographic separation.

The AACE DBS instrument functions as the master controller of the analytical setup and samples are analyzed according to the DBS Sample List.

- Sample information imported/entered into single location
- Camera – captures card image and records barcode
- Integrated software control of extraction, dilution, and analytical processes

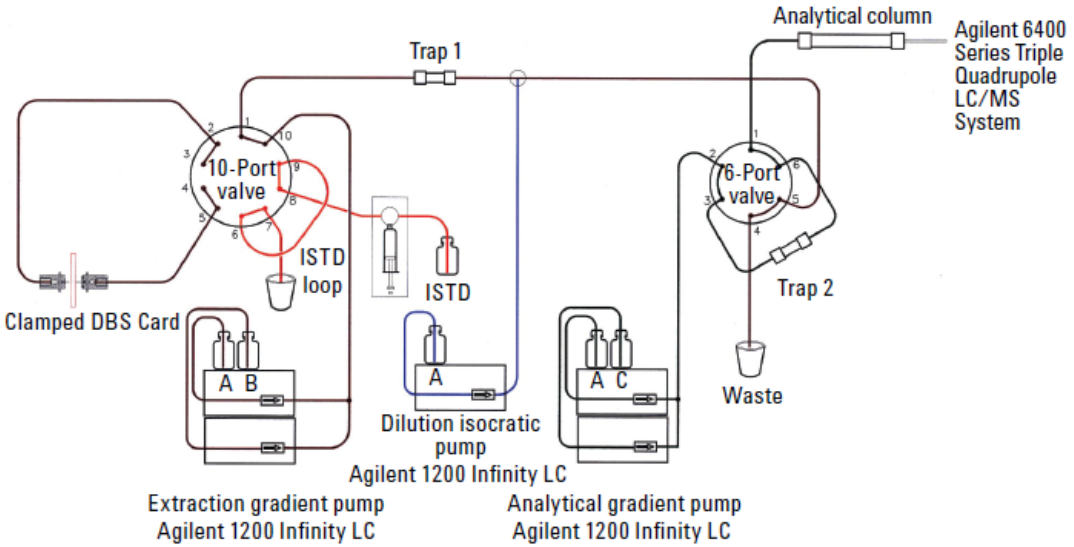

Figure 2 AACE DBS LC/MS system configuration

Operational Overview

The SCAP method starts the extraction pump program, the dilution pump program and the 6400 Series LC/MS system. The start for the next DBS extraction is performed from the extraction pump program while the analytical gradient is still running. Thus, the extraction step overlaps the analytical run, which shortens the cycle time (duration from one injection until next injection).

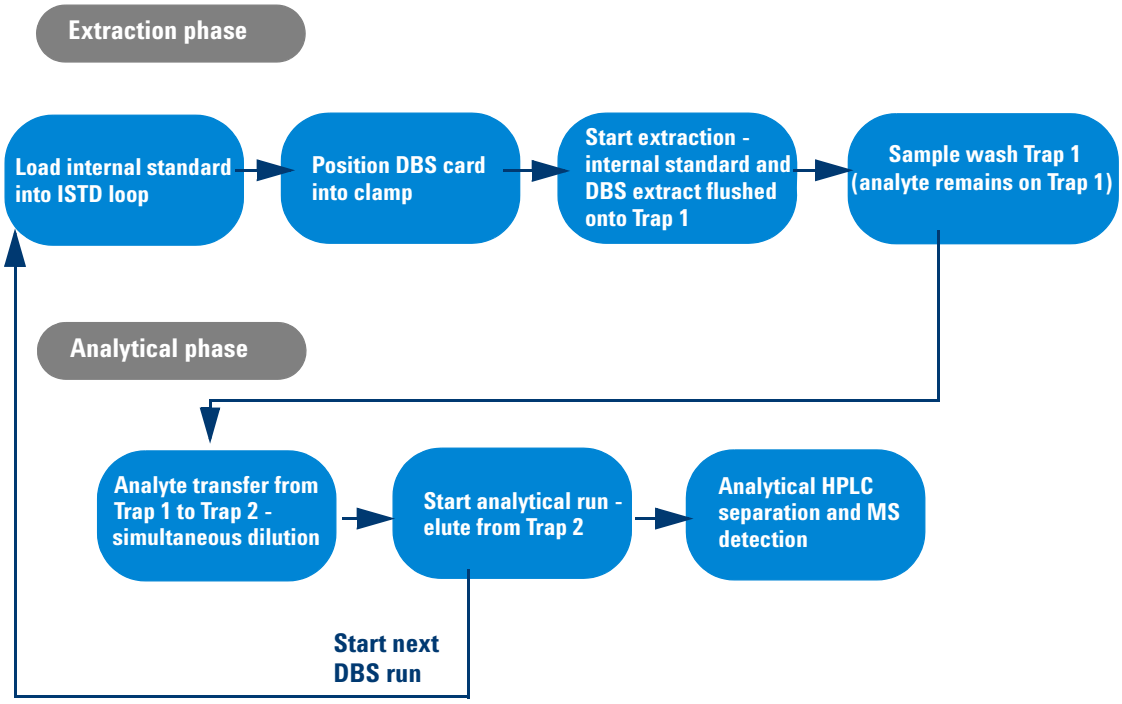

Figure 3 Operational overview

Method Development

In order to successfully analyze target compounds in blood or other matrices which are spotted onto an Agilent Auto DBS card, it is necessary to develop the optimal analytical conditions for their extraction, analytical LC and MS analysis. For more information, see the *Agilent Automated Card Extraction Dried Blood Spot LC/MS System Method Development Guide*.

Method development takes place in two phases:

Exraction Method Development – Establishes the optimum parameters for the online extraction

Analytical Method Development – Establishes optimum parameters and gradients for the HPLC separation and MS detection.

Extraction Method

When the analysis starts, the internal standard is first loaded into an injection loop. The valve switches, and the loop containing the internal standard(s) and the clamped Auto DBS card is connected with the flow path. An organic solvent is introduced in order to extract analytes from the card onto a trapping column along with the internal standard(s). As the analytes elute off the first trapping column, a dilution pump adds aqueous mobile phase to allow them to be retained on the second trapping column. The second trapping column is then switched into the analytical flow path.

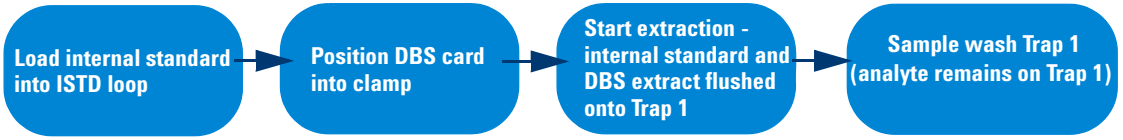

Figure 4 Sample extraction phase

Analytical Method

During the analytical HPLC separation and MS Detection, the second trapping column is connected with the analytical flow path. The analytes are then separated on the analytical column and detected by the 6400 Series LC/MS instrument. The optimal MS parameters (such as fragmentor voltage and collision energies) are established for each multiple reaction monitoring (MRM) transition.

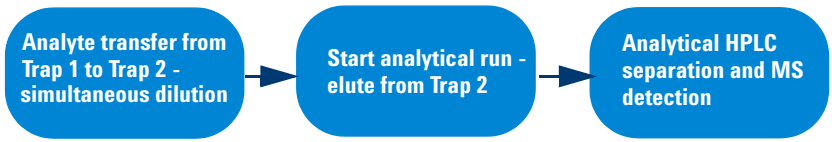

Figure 5 Analytical method phase

Data Processing and Reporting

Once the analysis is complete, a report can be generated. The report includes the MRM chromatograms, the quantitation results, and photographic images (“before” and “after”) for each DBS sample. This provides an easy way to review each analysis and inspect for any anomalies related to spot integrity or sampling. Sample reports are generated by the MassHunter software. For information on how to use the MassHunter software, see the user guides provided with the MassHunter software.

Using the AACE DBS LC/MS System

For a typical method, the steps to use the AACE DBS LC/MS system are outlined in this section. Steps that you do are shown in **black**. Steps that are automatically done by the AACE DBS LC/MS system are shown in blue.

A complete example of a real method is described in the Agilent Application Note 5991-0295EN, *Automated Online Card Extraction LC/MS System for the Determination of Clozapine and its Metabolites in Rat Blood.*

Using the AACE DBS LC/MS System

Steps	Detailed Instructions
1 Collect blood samples.	After the sample is collected, drop a small volume of the sample matrix onto the Auto DBS card, and allow it to dry. The dried card is easily stored and transported under normal ambient temperature conditions.
2 Load cards into sample tray.	Insert the Auto DBS cards in the sample tray. The sample tray is specially designed to hold up to 40 DBS cards.
3 Place tray in reader.	Place the DBS trays in the automated card extraction instrument. Depending on the model, the instrument can hold up to 4 or 8 trays at a time, for a maximum of 320 cards.
4 Set up the run.	Using the SCAP software, create a sample worklist. A worklist provides information about the samples, including which of the four spots on the card to analyze, and the position of sampling within each spot. You can manually enter the sample information or import the sample list electronically from an existing file. For details on how to set up a worklist, see the Agilent Automated Card Extraction Dried Blood Spot LC/MS System SCAP DBS Software User Guide.
5 Start the run.	To start a worklist run, click **Start**.

Using the AACE DBS LC/MS System (continued)

Steps	Detailed Instructions
6 Sample is extracted.	**a** When the worklist run starts, the gripper picks up the first Auto DBS card and the system camera takes a photograph of it. The image is used for accurate positioning of the card in the clamping mechanism to ensure optimal analyte extraction. The exact positioning also allows multiple sampling of each spot. **b** The gripper places the card in the clamping unit for analyte extraction. **c** The internal standard is loaded into a loop. **d** The 10-port valve switches, and the loop containing the internal standards and the Auto DBS cards are connected in the flow path. **e** An organic solvent is introduced to extract the analytes off the card onto the first trapping column along with the internal standard.
7 Sample is analyzed.	**a** As the analytes elute off the first trapping column, the dilution pump adds aqueous mobile phase to facilitate analyte retention on the second trapping column. **b** The second trapping column is switched into the analytical flow path. **c** First trapping column is washed, and extraction of second sample begins. **d** The analytes are separated on the analytical column and are detected by the mass spectrometer. **e** While the analysis continues, the card is photographed and then returned to the tray. The image shows that the extraction was completed, and provides assurance that the sample was taken correctly.
8 Sample reports generated by MassHunter software.	The MassHunter software generates reports for the sample. Sample summary reports include before and after photographic images. This provides an easy way to review each analysis and reveals any problems in sampling integrity or chain of custody. For more information on using the MassHunter software, see the *Agilent Mass Hunter Software Quick Start Guide*.

Getting Help

AACE DBS User documentation

See the following documents for more information on the AACE DBS LC/MS system and how to use it:

Agilent Automated Card Extraction Dried Blood Spot LC/MS System Quick Start Guide (this document)

Agilent Automated Card Extraction Dried Blood Spot LC/MS System SCAP DBS Software User Guide

Agilent Automated Card Extraction Dried Blood Spot LC/MS System Technical Reference Guide

Agilent Automated Card Extraction Dried Blood Spot LC/MS System Method Development Guide

Agilent 6400 Series Triple Quad LC/MS System Quick Start Guide

Agilent MassHunter Workstation Software – Data Acquisition for 6400 Series Triple Quadrupole LC/MS Familiarization Guide

Agilent website

To find contact information for your country, click the following link, select your country, and then click **Go.**

http://www.chem.agilent.com/en-us/ContactUS/Pages/ContactUs.aspx

www.agilent.com

In this book

This book contains brief instructions to help you get started with the Agilent Automated Card Extraction Dried Blood Spot LC/MS System.

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