The Agilent Automated Card Extraction LC/MS System

Technical Overview

Introduction

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

Figure 1. The Agilent Automated Card Extraction LC/MS System.

The AACE LC/MS System uses a high pressure binary gradient pump for extraction, an isocratic pump for dilution, and another high pressure binary gradient pump for chromatographic separation. A single software package controls the analytical setup and sample analysis.

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Key Features

- Sample information is imported or entered into single a location.
- A camera captures the card image and records the barcode.
- Integrated software control of extraction, dilution, and analytical processes.

The AACE method starts the extraction pump program, the dilution pump program, and the 6400 Series Triple Quadrupole LC/MS System. The restart 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 and shortens the overall cycle time (defined as the duration from one injection until next injection). The AACC instrument configuration is illustrated in Figure 3.

In order to successfully analyze target compounds in blood or other matrices which have been spotted onto DBS cards, it is necessary to develop optimal analytical conditions for extraction, analytical LC, separation, and MS analysis.

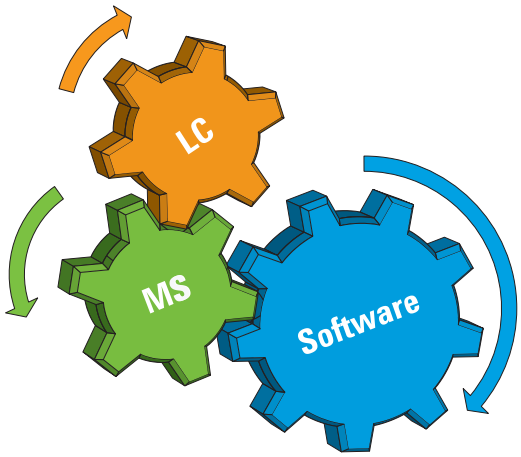

Figure 2. Integrated software controls the LC pumps and the Agilent 6400 Series Triple Quadrupole LC/MS System.

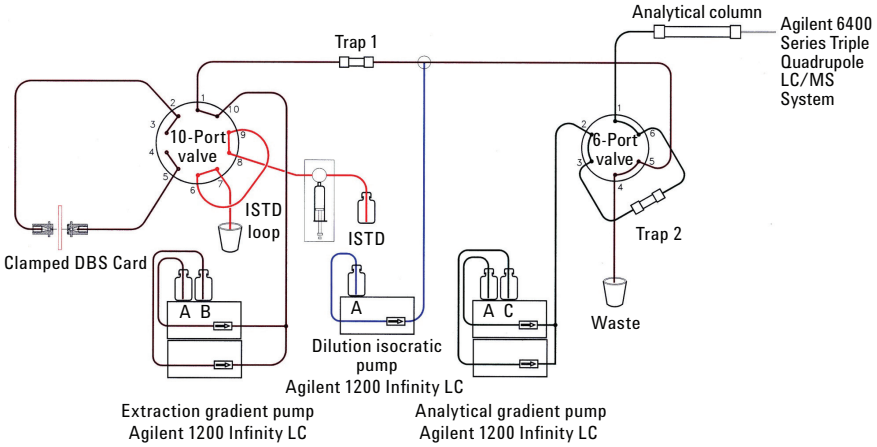

Figure 3. The Agilent Automated Card Extraction system configuration.

Method Development

The AACE System is designed for automated online analyte extraction and analysis of DBS samples. Depending on the choice of HPLC columns, solvents, and DBS extraction conditions, methodologies may be applicable to the analysis of a wide range of analytes in a variety of matrices. The separation of matrix components and the washing of the trapping columns are key to the development of a sensitive and robust method. Method development takes place in two phases to establish the optimal parameters for online extraction and analytical LC/MS detection.

Extraction

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 DBS card is connected with the flow path. An organic solvent is introduced in order to extract analytes from the card onto the first trapping column along with the internal standard(s). As the analytes elute off the first trap, a dilution pump adds the 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).

Analysis

During the analytical method development process, the second trapping column is connected to the analytical flow path. The analytes are separated on the analytical column and detected by the 6400 Series Triple Quadrupole LC/MS System instrument. The optimal MS parameters (such as fragmentor voltage and collision energies) are established for each multiple reaction monitoring (MRM) transition, as shown in Figure 5.

Figure 4. Extraction method development workflow.

Figure 5. Analytical method development workflow.

Data Processing and Reporting

Once the analysis is complete, a report can be generated that includes the MRM chromatograms, the quantitation results, and the before and after photographic images for each DBS sample. This provides an easy way to review each analysis and inspect for any anomalies related to spot integrity or sampling. The data reporting and review workflow is shown in Figure 6.

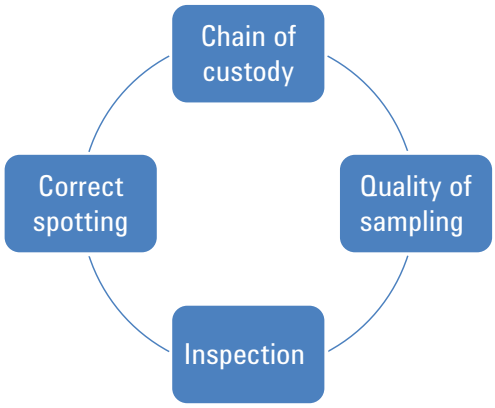

Figure 6. Data reporting and review workflow.

Summary

The AACE LC/MS system is a fully automated solution for analyzing dried blood spots or other dried matrices used in pharmaceutical bioanalytical measurements and clinical research. Excellent sensitivity, linearity, dynamic range, accuracy, and precision can be achieved using the system. In contrast to conventional hole punching and offline analyte extraction, the AACE system uses online analyte extraction and direct flow-through analysis of DBS cards which greatly reduces analysis time and manual sample preparation errors. Integrated system set up and control simplifies the analytical workflow and facilitates data processing and reporting.

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