Agilent Automated Card Extraction Dried Blood Spot LC/MS System

Method Development Guide

This guide explains the steps typically used to develop an Agilent Automated Card Extraction Dried Blood Spot LC/MS (AACE DBS LC/MS) method.

An example test method that you can use for demonstration, training, or testing 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.*

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What is an AACE DBS LC/MS Method?

A complete AACE DBS LC/MS method defines the parameters required for automated analyte extraction and analysis of DBS samples. Because the AACE DBS LC/MS system is applicable to a wide range of analytes and matrices, development of a sensitive and robust method involves determining the correct choice of HPLC columns, solvents, and DBS extraction conditions. Method development is performed in two phases; one phase establishes the optimum parameters for the online extraction, and the other the analytical LC/MS detection parameters. Creation and changes to AACE DBS LC/MS methods are performed using the SCAP DBS software provided with the system.

For more information on using the Agilent Automated Card Extraction Dried Blood Spot LC/MS system, see the

- *Agilent Automated Card Extraction Dried Blood Spot LC/MS System QuickStart Guide*
- *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*

For more information on using and developing LC/MS analytical methods, see the documentation provided with the LC/MS.

Developing an AACE DBS LC/MS Method

This example is based on an AACE DBS LC/MS system with a binary high pressure gradient pump for the extraction, an isocratic pump for the dilution and a binary high pressure gradient pump for the chromatographic separation.

The AACE DBS LC/MS system functions as the master of the analytical setup and samples are analyzed according to the sample list created in the SCAP DBS software. The SCAP DBS method starts the extraction pump program, the dilution pump program and the analytical LC-MS/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 overlaps the analytical run and thereby shortens the cycle time (time duration from one injection until next injection).

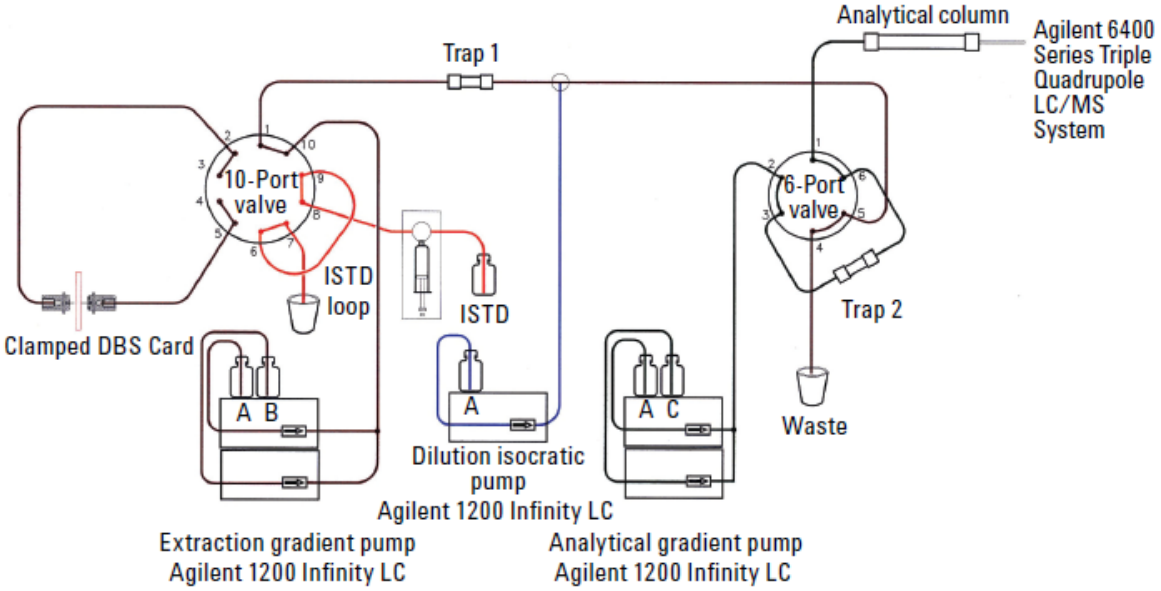

Figure 1 AACE DBS LC/MS system initial configuration

Selection of precolumns and solvents

For the extraction of the sample from the Auto DBS card and subsequent trapping of the analyte, determine and adapt suitable precolumns (trapping columns) and solvents. In order to allow for an optimized separation of matrix components, make sure the properties (selectivity) of the trapping columns are different.

Developing a method for extraction and analysis

For the determination of the extraction parameters, modify the AACE DBS LC/MS system accordingly.

Step 1: Determine gradient pump program and timer variables

Instead of connecting the DBS clamp to the 10-port valve, a loop is implemented and the outlet of trapping column 1 (Trap 1) is directly connected to the MS instrument (diluting pump and analytical gradient pump OFF). (See Figure 2.)

In this configuration, determine the extraction gradient pump program to inject the analyte /internal standard (ISTD) via the ISTD loop and trap it on Trap 1. In addition, define and check the 4 TIMER variables of the SCAP DBS method:

Timer 1: DBS to Trap 1

Timer 2: Trap 1 Sample Wash

Timer 3: Trap 1 to Trap 2

Timer 4: Wash and Condition Trap 1

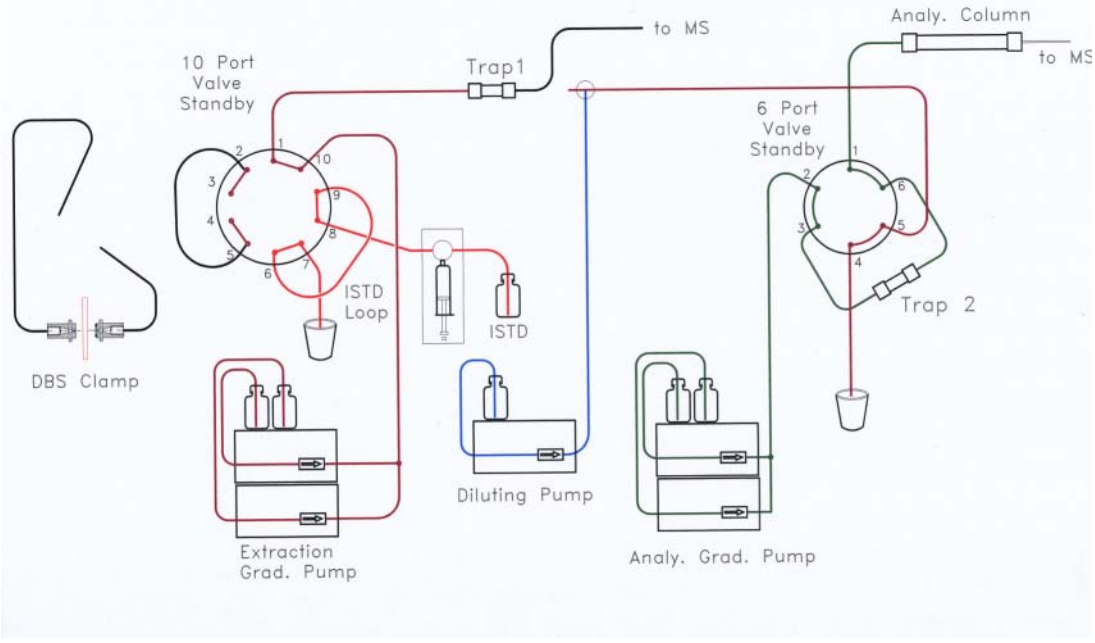

Figure 2 AACE DBS LC/MS method development configuration

Step 2: Load ISTD and DBS sample

Switch back to the initial configuration. In this initial configuration, the Auto DBS card is positioned and the ISTD loop is filled with ISTD. (See Figure 3.)

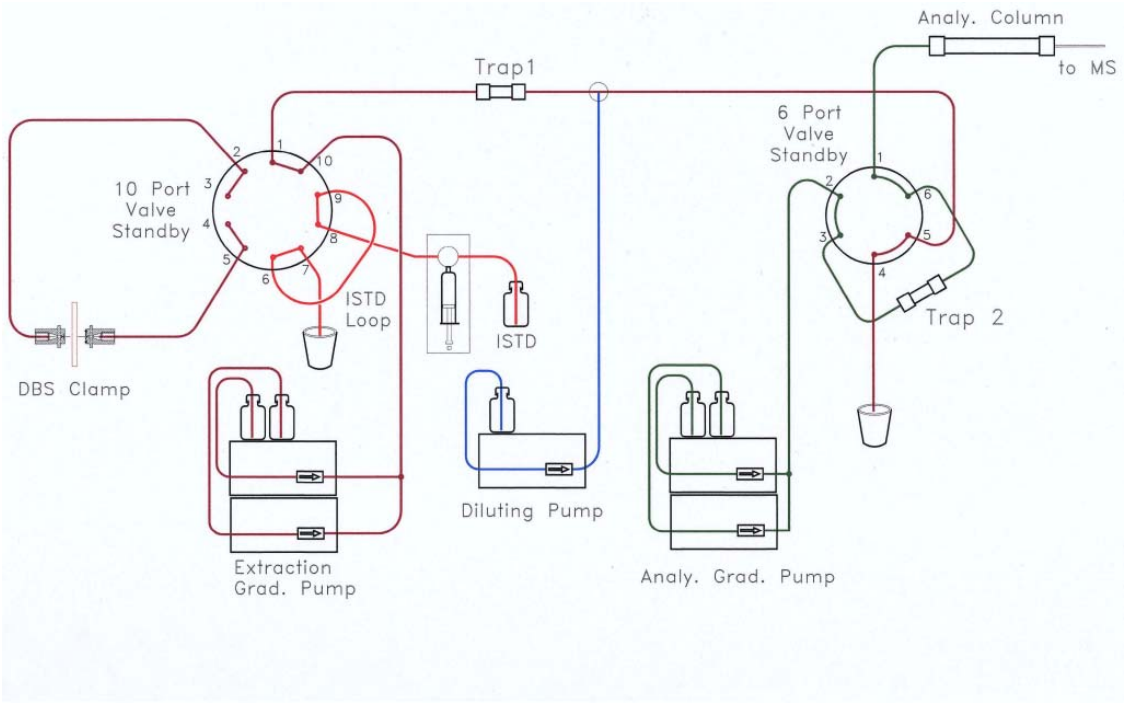

Figure 3 AACE DBS LC/MS system initial configuration

Step 3: Determine Timer 1: DBS extraction to Trap 1

In the next configuration, the ISTD and the extract of the dried blood spot are loaded onto Trap 1 (aqueous extraction solvent A). This step determines the timing for DBS extraction to Trap 1. (See Figure 4.)

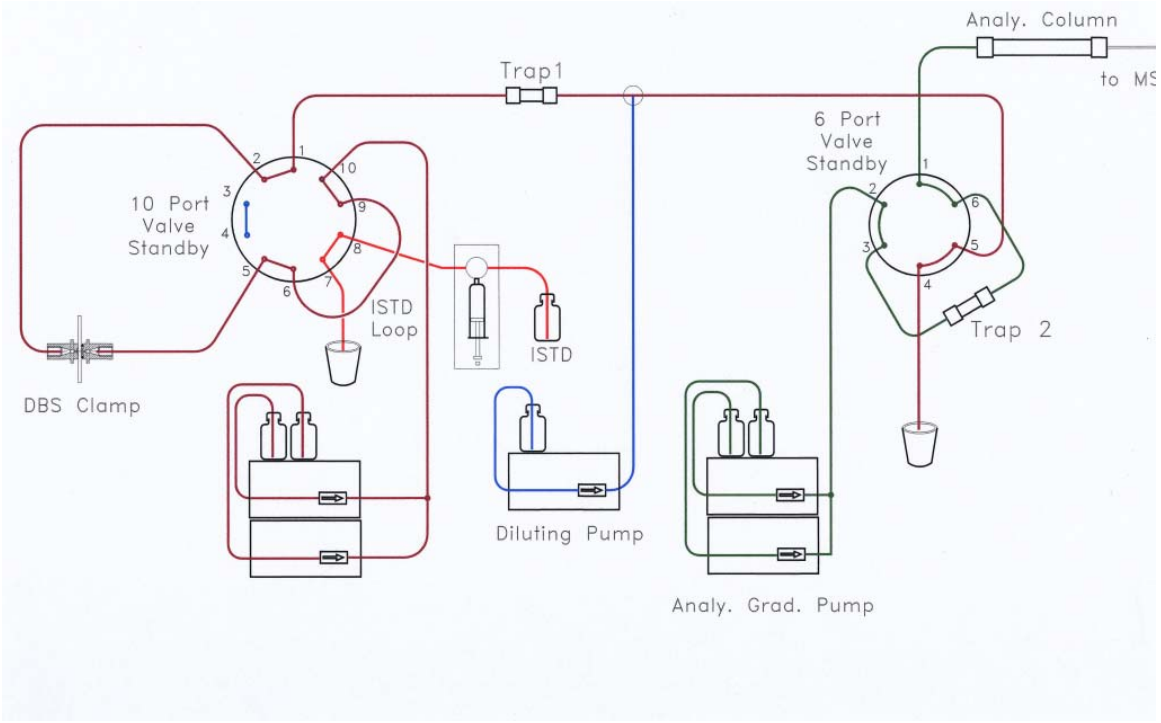

Figure 4 DBS extraction to Trap 1 (Timer 1)

Step 4: Determine Timer 2: Trap 1 Sample Wash

After expiration of Timer 1, the 10-port valve switches back and the washing phase of the sample on Trap 1 starts. During the Trap 1 Sample Wash (Timer 2) phase, the diluting pump is activated. (See Figure 5.)

With the extraction gradient pump program, increase the organic amount %B until the analyte/ISTD elute from Trap 1. Using the AACE DBS LC/MS Method Development Configuration, the times for the Timer 1, 2, 3 and 4 are determined.

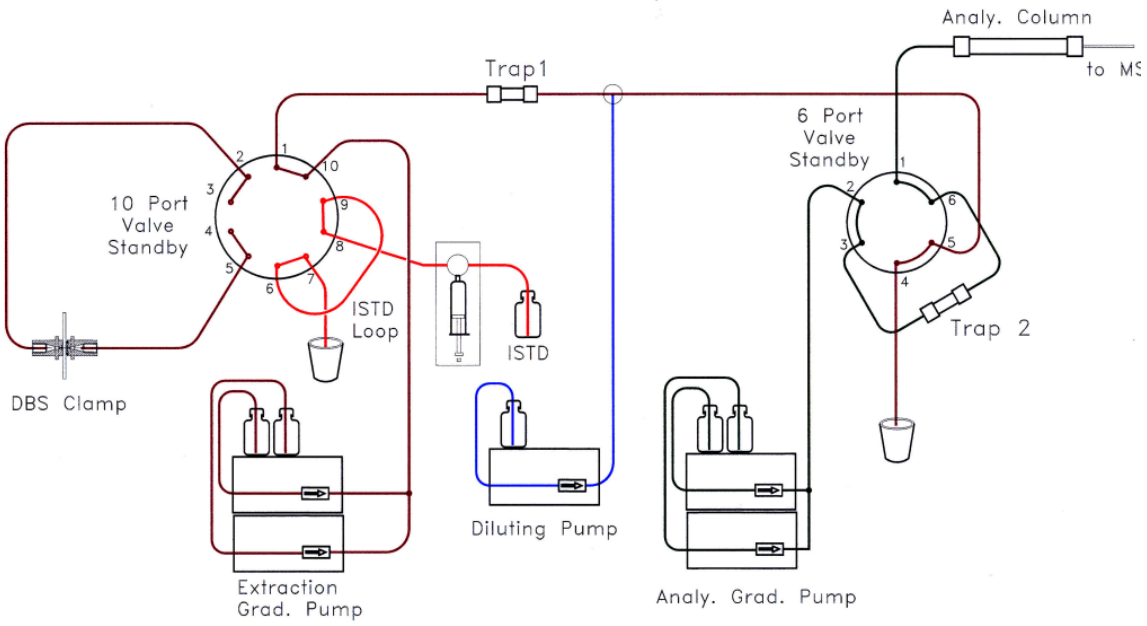

Figure 5 Trap 1 sample wash (Timer 2)

Step 5: Determine Timer 3: Transfer Trap 1 to Trap 2

In this configuration, the outlet of Trap 1 is connected to Trap 2. During Timer 3 phase the analyte/ISTD elutes from Trap 1. Using the diluting pump, the eluent is diluted with water in order to enable the analyte binding on Trap 2. (See Figure 6.)

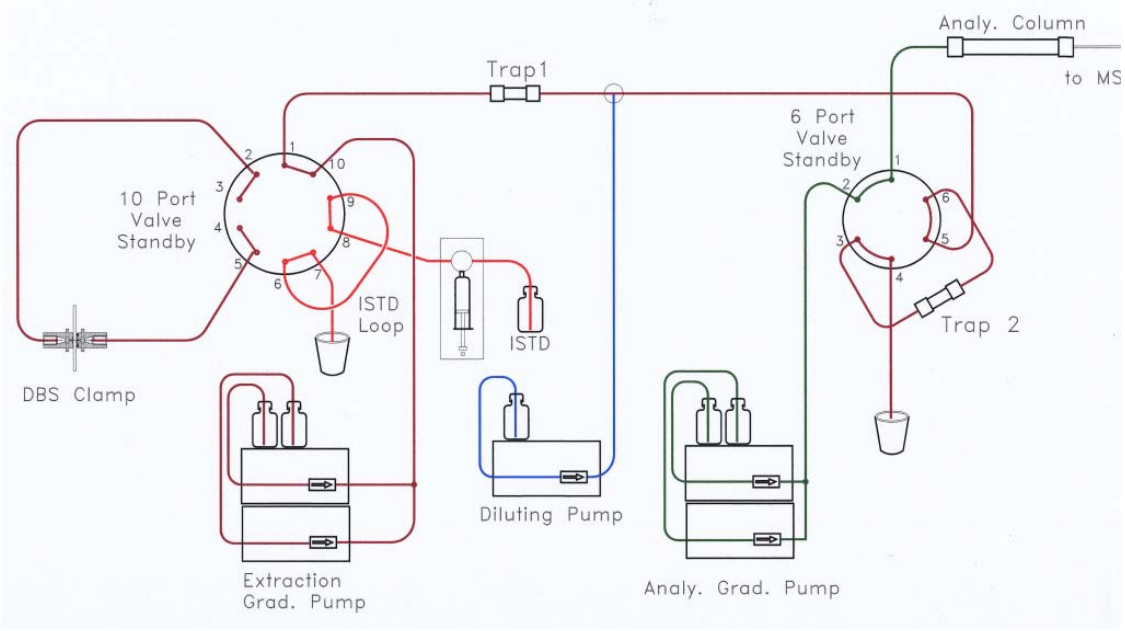

Figure 6 Transfer Trap 1 to Trap 2 (Timer 3)

Step 6: Determine Timer 4: Start analytical run and wash/condition Trap 1 (Timer 4)

As soon as the transfer from Trap 1 to Trap 2 (Timer 3) finishes, Trap 2 is switched into the analytical flow. This starts the analytical run. (See Figure 7.)

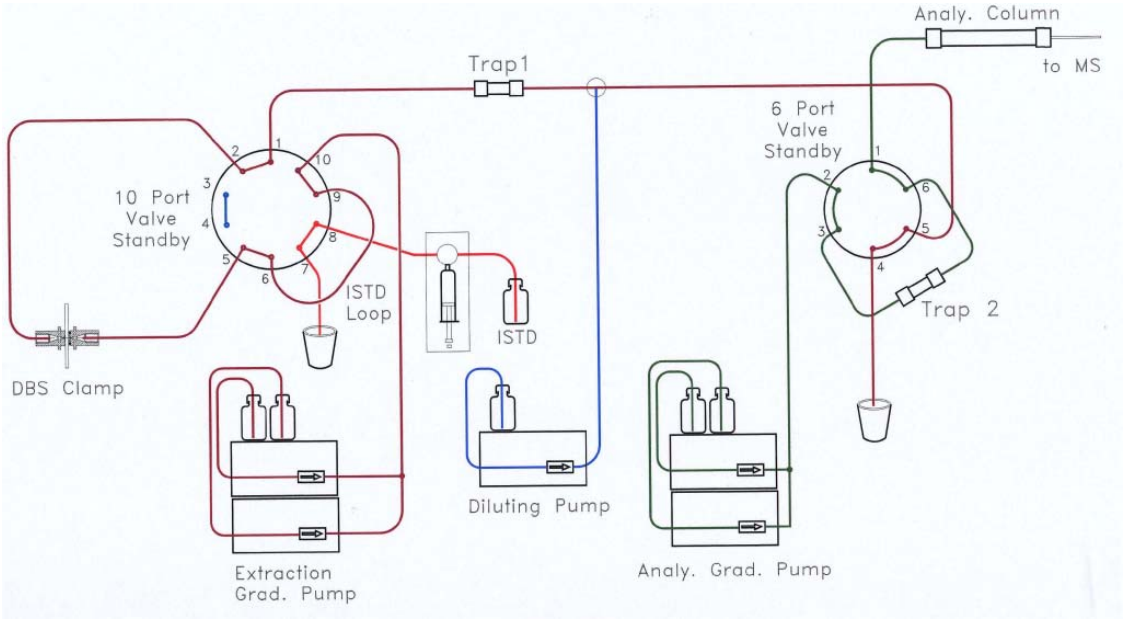

Figure 7 Start analytical run and wash/condition Trap 1 (Timer 4)

In this configuration the chromatographic separation and the MS data acquisition are carried out. In parallel, the Clamp line and Trap 1 are washed and reconditioned by use of the extraction pump system.

As soon as the initial conditions are reached by the extraction pump system, the AACE DBS LC/MS system is switched to the initial configuration (see Figure 3 on page 6). At this stage, the next DBS sample is loaded and a new extraction phase is started (see Figure 4 and Figure 5).

NOTE During Timer 1 and Timer 2 phase, the analytical run and the reconditioning of the analytical column and Trap 2 must finish.

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In this book

This book contains instructions on how to develop an AACE DBS LC/MS method for extraction and analysis of DBS samples. It also includes an example method that you can use for training or system testing.

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