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Determination of Opiates in Dried Blood Spots Using Novel Flow-Through Technology Coupled to LC/MS/MS

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Introduction

Dried blood spot (DBS) methodologies coupled to highly sensitive LC/MS/MS systems promises significant advantages in bioanalytics both in the toxicology and pharmaceutical industry. However, two major disadvantages that limit the acceptance of DBS technology are: (1) the laborious, time-consuming, error prone sample pre-treatment required, and (2) poor assay sensitivity due to reduced sample volume. An automated card extraction system virtually eliminates off-line sample preparation with direct online sample extraction of DBS cards without the need for hole punching while triple quadrupole mass spectrometry technology provides unparalleled sensitivity and superior quantitation for the low sample volumes typically encountered with DBS analyses.

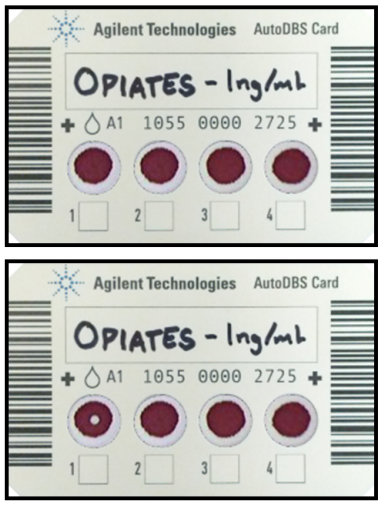

Figure 1. Images of cards before (A) and after (B) extraction

This presentation will describe our application of this integrated analysis system to the quantification of opiates in blood in the ng/mL concentration range. The use of dried blood spots (DBS) technology for clinical research and pharmacokinetics studies has advantages compared to conventional plasma sampling because it allows sampling of small blood volumes, easy sample shipping and storage, and removes many concerns related the handling of biohazardous materials. Compared with offline hole punching and manual extraction methods, the fully integrated Agilent Automated Card Extraction (AACE) LC/MS System enables automated flow-through analysis of DBS cards.

Experimental

Sample Preparation

Calibration standards were prepared by spiking the following compounds: codeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone , and 6-MAM at concentrations ranging from 1 to 1000 ng/mL in whole blood. These standards were spotted onto dried blood spot cards designed for flow-through analysis (Prolab, Switzerland) in triplicate and dried overnight before analysis. The respective deuterated compounds were used as internal standards.

Instrumentation

The AACE system (Figure 2) consists of the AACE instrument for automated flow-through analysis of DBS cards, two Agilent 1260 Infinity Binary LC pumps (one for sample extraction and cleaning and the other for analytical LC separation), an Agilent 1260 Infinity Isocratic LC pump for sample dilution, and an Agilent 6460 QQQ for analyte detection. A single software is used to control all components, and the entire process of DBS card extraction, sample trapping, elution, and LC/MS analysis. A build-in camera captures card images (Figure 1) and records the barcode, allowing multiple extractions on the same blood spot and ensuring unambiguous assignment of results.

Method Development

The separation of matrix components and washing of the trapping columns are key to development of a sensitive method. Method development takes place in several phases to establish optimal parameters for online extraction and LC/MS/MS analysis.

Solvent A	5mM NH4 Formate & 0.01% Formic acid in water
Solvent B	5mM NH4 Formate & 0.01% Formic acid in methanol
Dilution solvent	5mM NH4 Formate & 0.01% Formic acid in water
Trap 1 column	CC 8/4.6 mm, Nucleosil 100-5 C_6H_5ec, Ambient
Trap 2 column	CC 8/4 mm, Nucleosil 100-5 C18 HD, Ambient
Analytical column	75x2.1 mm, Agilent Poroshell EC-C18, 5 µm, 55 °C

Table 1. Solvents and columns

Automated Card Extraction System

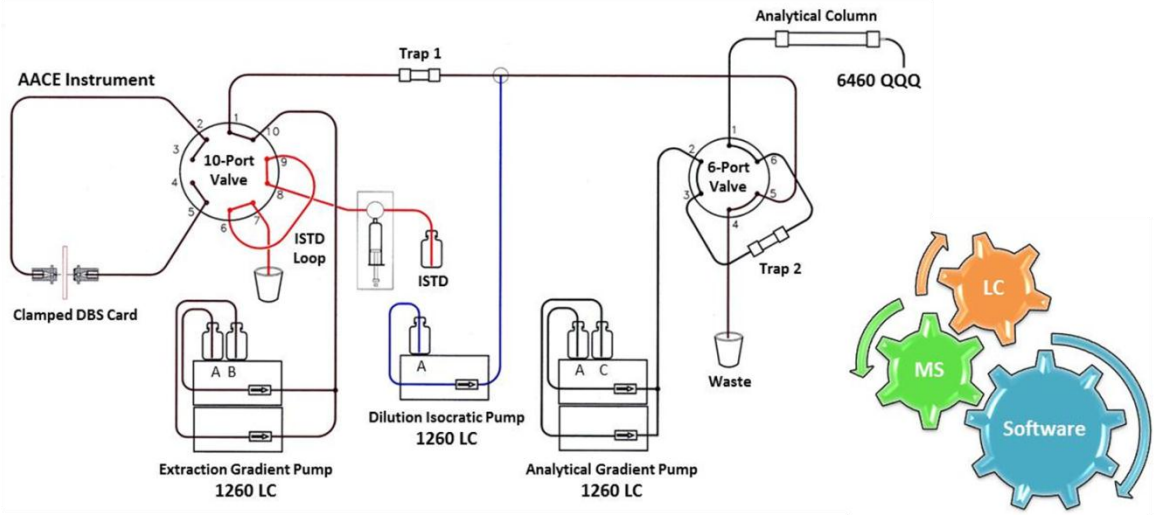

Figure 2. Agilent Automated Card Extraction (AACE) LC/MS System configuration

Gas temperature and flow	250 °C at 12 L/min
Sheath gas temp and flow	350 °C at 11 L/min
Nebulizer pressure	50 psi
Capillary voltage	2000 V (+)

Table 2. MS source conditions

Name	Prec Ion	Prod Ion	Frag (V)	CE (V)
6-MAM	328.2	211.1	166	24
Codeine	300.2	215.1	140	21
Hydrocodone	300.2	199.1	160	29
Hydromorphone	289.2	185.1	160	29
Oxycodone	316.2	298.2	166	13
Oxymorphone	302.1	284.2	140	17
Morphine	286.2	201.1	160	25

Table 3. MRM transitions

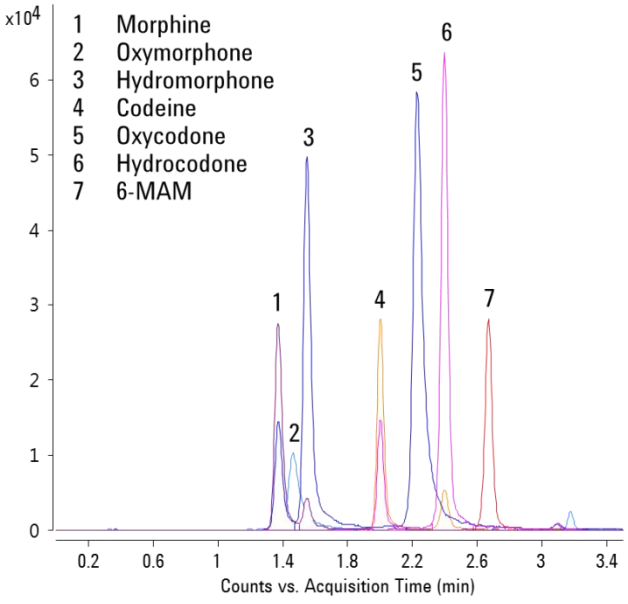

Figure 3. MRM chromatograms for codeine, hydrocodone, hydromorphone, oxycodone, morphine and 6-MAM

Results and Discussion

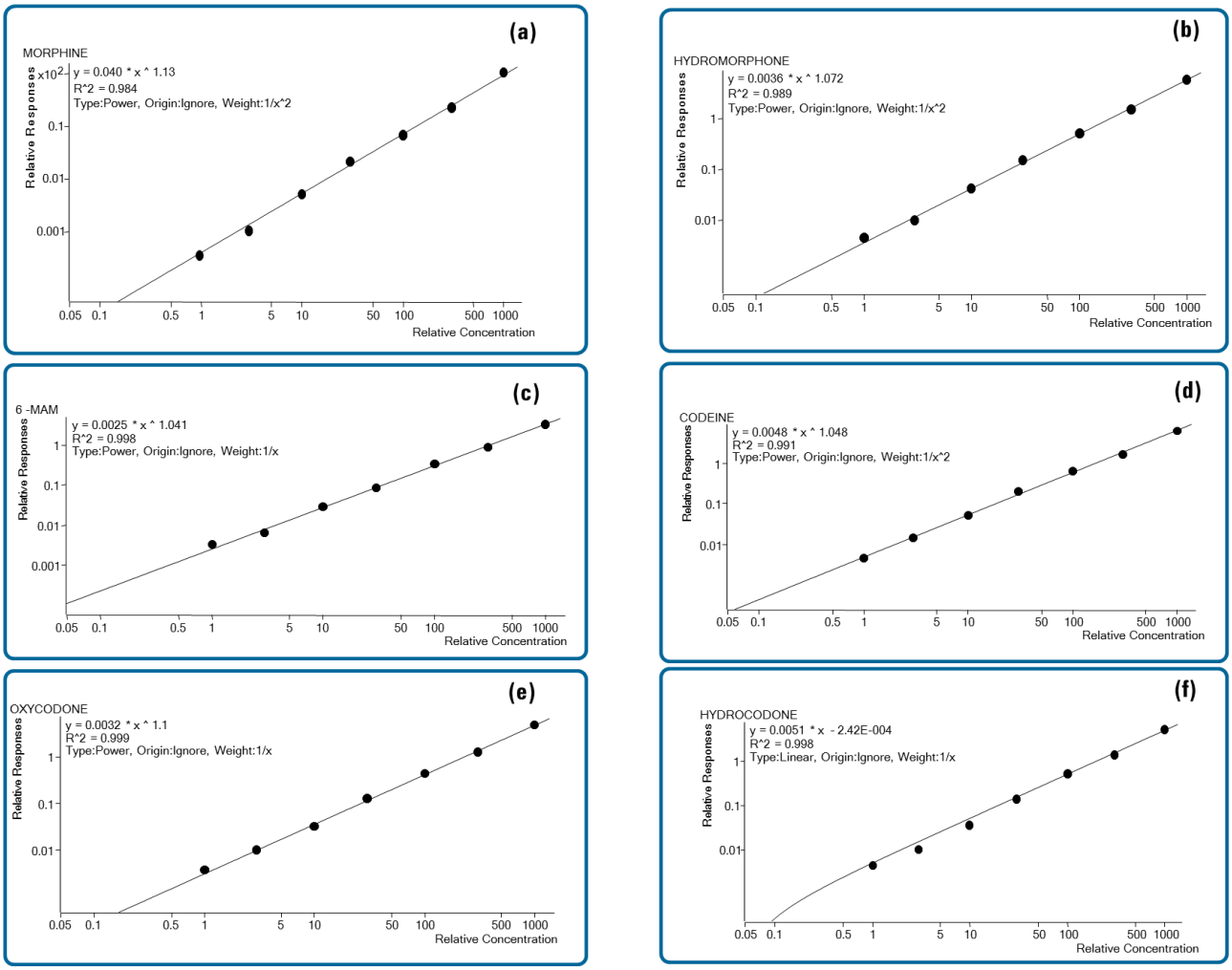

Figure 4. Calibration curves for morphine (a), hydromorphone (b), 6-MAM (c), codeine (d), oxycodone (e), and hydrocodone (f) from 1-1000 ng/mL

Results and Discussion

- Agilent Automated Card Extraction (AACE) LC/MS System offers automated flow-through DBS analysis with fully integrated software control, efficient method development, and data processing and reporting.
- This system was used for the DBS analysis of codeine, hydrocodone, hydromorphone, morphine oxycodone, oxymorphone, and 6-MAM in whole blood delivering excellent sensitivity with an LOQ of 1 ng/mL in less than 3.5 minutes.
- Calibration curves of the seven compounds in whole blood show linearity over 3 orders of magnitude (Figure 4). An order of magnitude can be readily achieved with further chromatographic optimization in trapping efficiencies for early opiate eluters.
- The AACE LC/MS system greatly reduces analysis time and manual experimental errors. Excellent sensitivity, linearity, dynamic range, precision, accuracy, and reproducibility, and the quantitative performance capabilities of the online extraction method were demonstrated. This DBS analysis approach using the AACE LC/MS system can be readily applied to clinical research, forensic toxicology and pharmaceutical, research studies.