# CAMAG
# DBS-MS 500

## FULLY AUTOMATED DRIED BLOOD SPOT EXTRACTION SYSTEM

## DRIED BLOOD SPOT APPLICATION IN DOPING CONTROL


- High throughput analysis of up to 500 DBS cards per run
- Integrated optical card recognition and barcode reading module
- Automated internal standard application module
- Unique extraction module with wash station to eliminate carry-over
- Online coupling to analysis system (LC-MS, MS or Sample Collector)
- Full control through Chronos software

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# FULLY AUTOMATED LCMS DETECTION OF COTININE AND NICOTINE FROM DRIED BLOOD SPOTS IN DOPING CONTROL

Athletic doping carries high medical risks and is a violation of the spirit of sport. To avoid doping, a fast and cost-effective analysis method feasible for a large population is needed. Dried blood spots (DBS) analysis offers such a workflow and is gaining interest as more and more analysis methods are published. To ease the DBS workflow and to facilitate high throughput applications, the whole process has been automated by the DBS-MS 500 [1].

Prohibited substances are surveilled as part of the Monitoring Program of the World Anti-Doping Agency (WADA) [2]. Among others, nicotine is listed as a stimulant influencing various bodily functions correlated with physical performance, such as dose-dependent increase in heart rate, blood pressure, muscle blood flow, intensified release of blood sugar and catecholamines. Additionally, mental effects such as reduction of stress and increased cognitive function have been reported. Nicotine is mostly administered by smokeless tobacco, like snus, chewing tobacco or snuff [3].

LCMS methods for small molecules can generally easily be transferred to a DBS interface. Using DBS offers the advantage of improved sample handling and fully automated sample pre-workup. Here, a method for the LCMS analysis of nicotine and its metabolite cotinine [4] was transferred to the automated DBS-MS 500 platform and further optimized.

Cotinine and Nicotine both need a relatively high water fraction for the extraction, which needs to be compatible with the chromatographic system. This was accomplished by using a polymer-based column (ODP2 HP) from Shodex. The method only takes a few minutes and achieves a limit of detection below 1 ng/ml for both nicotine and cotinine.

Table 1, MRM setting

| Compound Name | Precursor Ion | Product Ion | Dwell | Fragmentor | Collision Energy | Cell Accelerator Voltage |
|---|---|---|---|---|---|---|
| Cotinine | 177 | 98.1 | 50 | 130 | 28 | 2 |
| Cotinine qualifier | 177 | 80.1 | 50 | 130 | 28 | 2 |
| D4-Nicotine | 167 | 134.1 | 50 | 110 | 25 | 2 |
| D4 Nicotine qualifier | 167 | 121.2 | 50 | 110 | 25 | 2 |
| Nicotine | 163 | 130.1 | 50 | 110 | 25 | 2 |
| Nicotine qualifier | 163 | 117.1 | 50 | 110 | 25 | 2 |

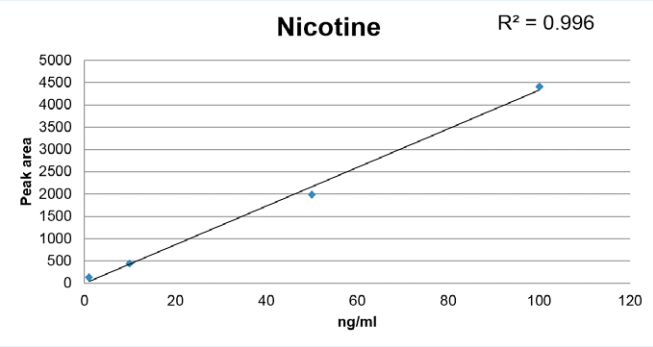


Figure 1, Calibration of nicotine from the spiked DBS sample

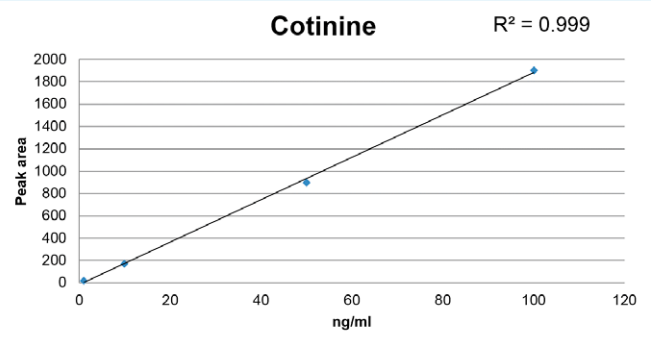


Figure 2, Calibration of cotinine from the spiked DBS sample


[1] "CAMAG DBS" 2017. [Online]. Available: http://www.camag.com/en/dbs/what_is_dried_blood_spot_sampling.cfm. [Accessed: 29-Aug-2017].
[2] "World Anti-Doping Agency, The 2015 Monitoring Program." [Online]. Available: https://www.wada-ama.org/en/content/what-is-prohibited. [Accessed: 20-Feb-2018].
[3] F. Chague *et al.*, Smokeless tobacco, sport and the heart, Arch. Cardiovasc. Dis. 108, 75–83, 2015
[4] I. A. Abdallaha *et al.*, "A fully validated LC–MS/MS method for simultaneous determination of nicotine and its metabolite cotinine in human serum and its application to a pharmacokinetic study after using nicotine transdermal delivery systems with standard heat application in adult," J. Chromatogr. B, vol. 1020, pp. 67–77, 2016


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