

Automated Dried Blood Spot Analysis for Anti-Doping Testing



Keywords

Anti-doping, competitive sports, cycling, WADA regulations, List of Prohibited Substances, performance-enhancing drugs

Introduction

In autumn 2019, the World Anti-Doping Agency (WADA) announced together with seven Anti-Doping Organizations (ADOs) the development and implementation of dried blood spot (DBS) testing for routine analysis in time for the 2022 Winter Olympic and Paralympic Games in Beijing. The implementation of DBS into anti-doping was announced as a game-changer, as the sampling intervals for blood can be increased, while the cost for sample shipment and storage can be significantly decreased.

Scope

To demonstrate the capabilities of fully automated DBS testing for anti-doping purposes, we developed a method for the simultaneous analysis of tramadol and its metabolite O-desmethyltramadol. The narcotic has been on the WADA monitoring program since 2012 and has been banned by the UCI for medical reasons across all cycling disciplines in 2019. The main focus of the project was on offering reliable high-throughput analysis combined with minimal turnaround time. Furthermore, with the integrated hematocrit (HCT) detection module, the CAMAG DBS-MS 500 HCT permits to go beyond the capabilities of manual DBS handling by normalizing the result to a target haematocrit value.

NOTE: The presented results are to be regarded as examples only!

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Recommended devices

- CAMAG DBS-MS 500 HCT
- Binary UHPLC pump to run a gradient
- Sensitive QqQ mass spectrometer (e.g. 8050 Shimadzu, 5500 Sciex, or higher)
- Column oven

Samples

Dried blood spot samples with a minimum volume of 10 μL (≥ 6 mm \varnothing) on DBS AutoCollect cards (85 x 53 mm). Volumetric sampling is not required.

Standards

The following standards can be obtained from Cerilliant (Round Rock, TX, USA):

- Cis-tramadol HCl (1 mg/mL, as free base)
- O-desmethyl-cis-tramadol (1 mg/mL, as free base)
- Cis-tramadol- ^{13}C , D_3 HCl (100 $\mu\text{g/mL}$, as free base)
- O-desmethyl-cis-tramadol- D_6 (100 $\mu\text{g/mL}$, as free base)

Analytical Process

First, the built-in image processing and reflectance analysis module documents and characterizes each DBS (size, area, roundness, and hematocrit). Then, 20 μL internal standard solution (25 ng/mL *cis*-tramadol- ^{13}C , D_3 , and 10 ng/mL O-desmethyl-*cis*-tramadol- D_6 in methanol) is sprayed in a homogenous layer using the spray module of the CAMAG DBS-MS 500 HCT. For the DBS elution, a water/methanol mixture (90/10, v/v) and a volume of 20 μL was chosen.

Analytical Column: Phenomenex Kinetex 2.6 μm biphenyl (100 \times 2.1 mm)

Mobile phase A: water + 0.1% formic acid

Mobile phase B: acetonitrile + 0.1% formic acid

Flow rate: 0.3 mL/min (0.5 mL/min from 2.5 min to 3.0 min)

Column temperature: 50°C

Analytical time: 3 min

Table 1: Parameters used for the selected reaction monitoring (SRM) of tramadol and O-desmethyltramadol

Analyte	Precursor [m/z]	Product [m/z]	Retention Time [min]
Tramadol SRM 1	264.1	58.25	2.15
Tramadol SRM 2	264.1	264.1	
Tramadol- ^{13}C , D_3	268.1	58.3	
O-desmethyltramadol SRM 1	250.2	58.15	1.87
O-desmethyltramadol SRM 2	250.2	250.2	
O-desmethyltramadol- D_6	256.2	64.15	

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Results

The method was successfully validated. Baseline separation and a nearly symmetric peak shape was achieved for the two analytes after the separation on the analytical column:

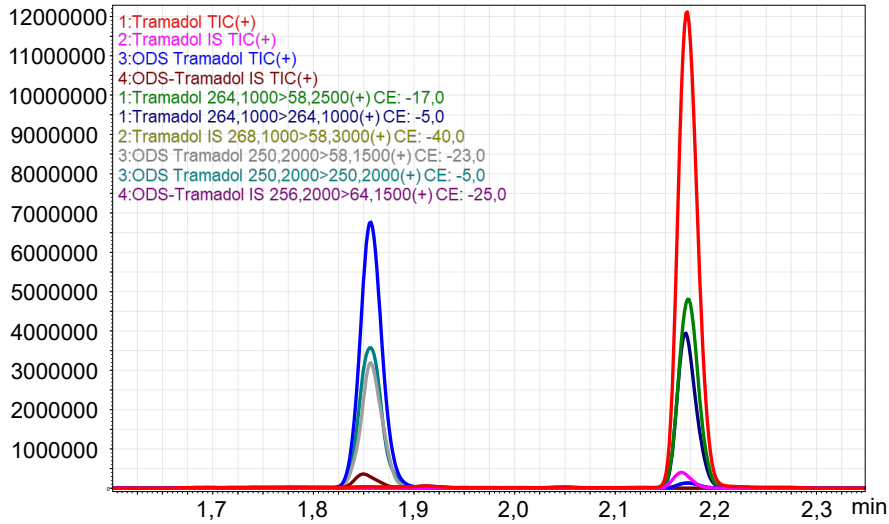


Figure 1: Separation of Tramadol (2.15 min) and O-desmethyltramadol (1.87 min) on the analytical column. The figure represents a blank blood sample sprayed with internal standard and spiked with 200 ng/mL of tramadol and 80 ng/mL of O-desmethyltramadol

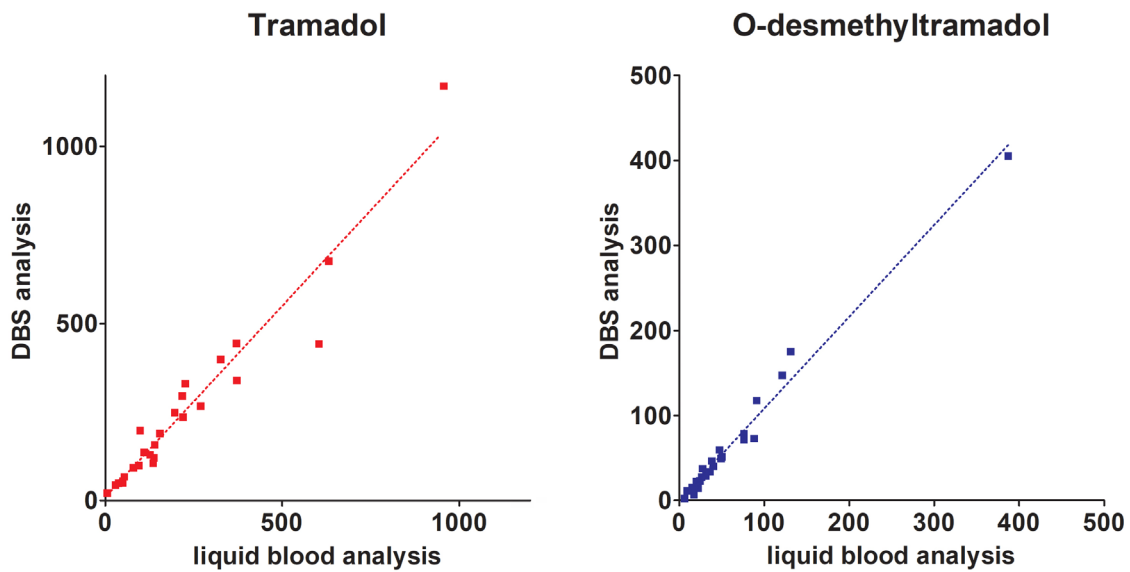


Figure 2: Cross-validation of 26 authentic samples using either liquid blood analysis or the fully automated DBS analysis with the CAMAG-DBS-MS 500 HCT

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Literature

Luginbühl M, Angelova S, Gaugler S, Längin A, Weinmann W. Automated high-throughput analysis of tramadol and O-desmethyltramadol in dried blood spots. *Drug Testing and Analysis*. 2020. doi:10.1002/dta.2819

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