Quantification of alprazolam in dried blood spots using the Agilent 1290 Infinity LC System and Agilent 6460 Triple Quadrupole LC/MS System

Application Note

Drug Metabolism and Pharmacokinetic (DMPK)/Bioanalysis

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Abstract

A method to quantitatively determine alprazolam in dried blood spots (DBS) was developed using ultra high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC/MS/MS).

Fifteen microliters of whole rat blood were spotted onto FTA Elute MicroCards. From the center of the DBS, cores of 6 mm ID were punched using a Harris Uni-Core puncher. Extraction was done by ultrasonicating the cores for 15 min using 100 µL of a mixture of acetonitrile/water (60:40 v/v) containing 2% formic acid. Chromatographic separation was performed on an Agilent ZORBAX Eclipse Plus RRHD C18 column (50 mm × 2.0 mm, 1.8 µm) with a fast gradient using water/acetonitrile, at flow rates of 1.0 mL/min or 1.8 mL/min. This led to run times of only 1.0 min and 0.64 min respectively. Column back pressures reached approximately 1,000 bar when working at a flow rate of 1.8 mL/min. Three microliters of each sample were injected three times.

The triple quadrupole mass spectrometer was operated in the positive ion mode and multiple reaction monitoring (MRM) was used for drug quantitation. At 1 mL/min the method was validated over a range of 0.15–1500 ng/mL in rat blood. Accuracies ranged from 84.6% to 107.2% and area precision from 2.4% to 8.6%. At the 1.8 mL/min flow rate, concentrations ranged from 0.30 to 1500 ng/mL, accuracies were between 84.6% and 116.0 % and area precisions between 2.4% and 15.3%. The Agilent 6460 Triple Quadrupole LC/MS system allows for flow rates up to 2 mL/min, at pressures up to 1200 bar and dwell times as low as 1-2 ms. This made it possible to achieve an analysis time of less than 0.64 min without sacrificing quantitative data quality.

The greater column efficiency of the Agilent Rapid Resolution High Definition (RRHD) columns resulted in narrow peaks, increased analyte peak height, excellent resolution from matrix components and, therefore, improved analyte response (sensitivity).

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Instrument conditions

LC/MS/MS system: Agilent 1290 Infinity LC System comprised of a binary pump with integrated degasser, high performance autosampler with thermostat and thermostatted column compartment, and an Agilent 6460A Triple Quadrupole LC/MS system with Agilent Jet Stream Technology.

Conditions	
Column:	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm, 1.8 µm
Mobile phase:	A= 0.1% FA in H_2O, B= 0.1% FA in ACN
Injection volume:	3 µL
Method 1:	
Column temperature:	50 °C
Flow rate:	1.0 mL/min
Gradient:	0 min 25% B, 0.8 min 90% B, 0.81 min 25% B, stop time 1.0 min, post time 0.5 min. Scan type: MRM
Method 2:	
Column temperature:	50 °C
Flow rate:	1.8 mL/min
Gradient:	0 min 25%B, 0.44 min 90%B, 0.45 min 25%B, stop time 0.64 min.
MS Scan type:	MRM
Polarity:	Pos.
Parameters	
Drying gas temperature:	350 °C
Drying gas flow:	10 L/min
Sheath gas:	400 °C and 12 L/min
Nebulizer:	35 psig, 60 psig
Nozzle:	0 V
Capillary:	3500 V
Collision cell acceleration voltage:	-2 V
Transition:	309.2→281.1
Fragmentor:	145 V
CE:	24 V
Dwell time:	50 ms

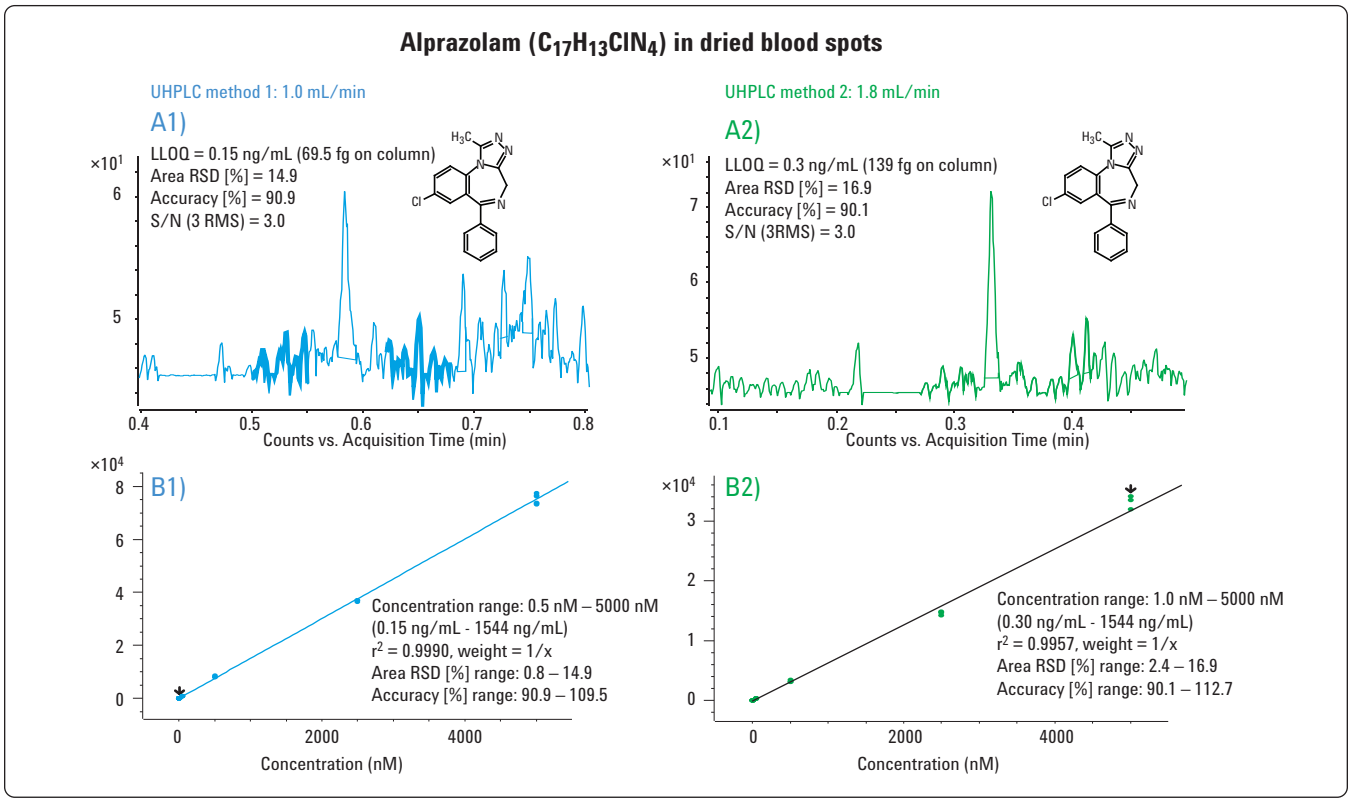

Figure 1
A1) MRM chromatogram at the LLOQ (0.15 ng/mL, 69.5 fg on column) using a flow rate = 1.0 mL/min. B1) Calibration curve, Area RSD [%] and Accuracy obtained at 1.0 mL/min over the range 0.15 ng/mL to 1544 ng/mL. A2) MRM chromatogram at the LLOQ (0.3 ng/mL, 139 fg on column) using a flow rate = 1.8 mL/min. B2) Calibration curve, Area RSD [%] and Accuracy obtained at 1.8 mL/min over the range 0.3 ng/mL to 1544 ng/mL.

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