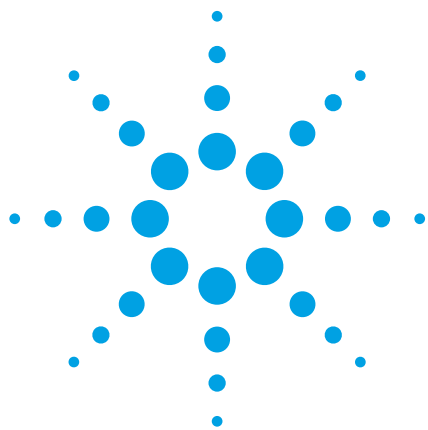




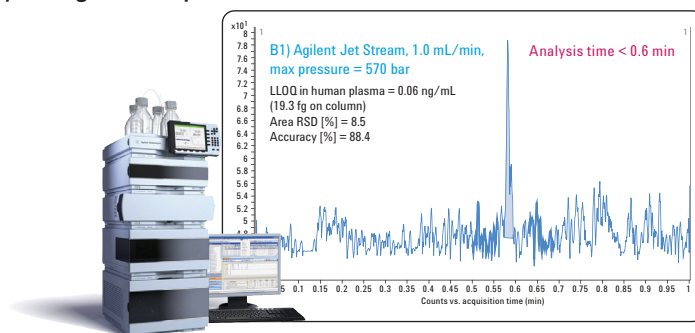
High-throughput bioanalytical method development using UHPLC/triple quadrupole mass spectrometry

Application Note

Drug Discovery, Drug Development

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Abstract

Several factors cause fast LC/MS/MS method development in the bioanalytical area to be an arduous task. In order to maintain sensitivity while speeding up analysis time, target analytes should not elute in the chromatographic region affected by ion suppression. The scan speed of the mass spectrometer must be fast enough to acquire an adequate number of data points to define the narrow peaks generated using sub-2 μm columns. At typical fast LC conditions, current HPLC systems (pressure limit ≤ 400 bar (5800 psi)) would yield back pressures close to, or greater than the threshold limit. In this application example, we utilized the Agilent 1290 Infinity LC system coupled to an Agilent 6460 Triple Quadrupole mass spectrometer comprising thermal gradient focusing ESI (Agilent Jet Stream technology, AJS) to streamline high-throughput bioanalytical method development using alprazolam spiked in human plasma (concentration range: 2 nM to 5000 nM, corresponding to 0.06 ng/mL to 1544 ng/mL). A 100- μL sample of spiked plasma was precipitated with three parts of ACN and centrifuged. A 200- μL amount of the supernatant was diluted with three parts of H_2O containing 0.1% formic acid (FA). Each sample was injected three times. AJS technology was compared to conventional orthogonal ESI using generic source values.

The Agilent 1290 Infinity LC Triple Quadrupole MS/MS system, which allows flow rates up to 2 mL/min, pressures up to 1200 bar, and dwell times as low as 1-2 ms achieved an analysis time of less than 0.5 min without sacrificing quantitative data quality. The greater column efficiency of the Agilent rapid resolution high definition columns (RRHD) resulted in narrow peaks, increased analyte peak height, excellent resolution from matrix components, and improved analyte response (sensitivity).



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Instrument Conditions

Agilent 1290 Infinity LC MS/MS system: Agilent 1290 Infinity LC System comprising binary pump with integrated degasser, high performance autosampler with thermostat and thermostatted column compartment, Agilent 6460A Triple Quadrupole LC/MS with AJS Technology or with conventional orthogonal ESI.

Conditions

Column: RRHD ZORBAX Eclipse Plus C18, 2.1 mm × 50 mm, 1.8 μm
Mobile phase: A= 0.1% FA in H₂O, B= 0.1% FA in ACN
Injection volume: 5 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 1.5 min

Method 2

Column temperature: 50 °C
Flow rate: 1.2 mL/min
Gradient: 0 min 25% B, 0.67 min 90% B, 0.68 min 25% B, stop time 1.37 min.
MS Scan type: MRM
Polarity: Positive

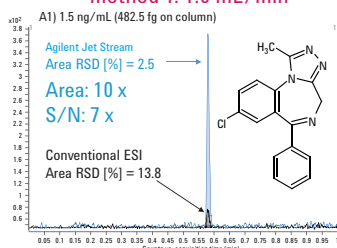
Parameters

Drying gas temperature: 350 °C (ESI and ESI + AJS)
Drying gas flow: 10 L/min (ESI + AJS), 13 L/min (ESI)
Sheath gas temperature: 400 °C (ESI + AJS)
Sheath gas flow: 12 L/min (ESI + AJS)
Nebulizer pressure: 35 psig (ESI + AJS), 60 psig (ESI)
Nozzle: 0 V (ESI + AJS)
Capillary: 3500 V (ESI and ESI + AJS)
Transition: 309.2→281.1
Fragmentor: 145 V, CE: 24 V
Dwell time: 50 ms

Chromatograms

Alprazolam (C₁₇H₁₃ClN₄) spiked in human plasma, RRHD ZORBAX Eclipse Plus C18

Agilent 1290 Infinity LC
method 1: 1.0 mL/min



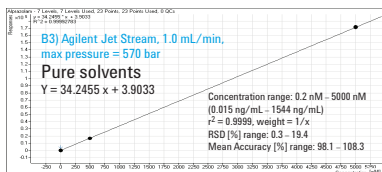
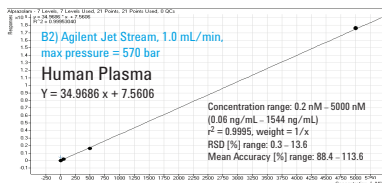
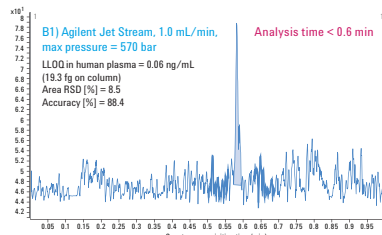
A1) Overlaid MRM chromatograms obtained using AJS in comparison to conventional orthogonal ESI for 1.5 ng/mL alprazolam spiked in human plasma.

B1) MRM chromatogram at the LLOQ for alprazolam spiked in human plasma using AJS and flow rate = 1 mL/min (LLOQ = 0.06 ng/mL, 19.3 fg on column).

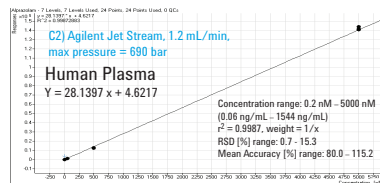
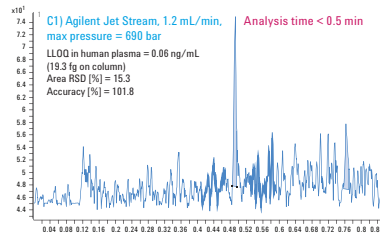
B2) Calibration curve obtained using AJS. 4.5 orders of linear dynamic range in human plasma was demonstrated.

B3) Calibration curve obtained at flow rate 1.0 mL/min for alprazolam spiked pure solvent. The amount on column for these samples corresponds to the amount on column obtained for the spiked and treated human plasma samples. The comparison between the slopes of the calibration curves obtained in spiked human plasma (slope = 34.97) and in pure solutions (slope = 34.25) shows that the slopes are practically the same, suggesting the absence of any significant matrix effect on quantification.

Agilent 1290 Infinity LC
method 1: 1.0 mL/min



Agilent 1290 Infinity LC
method 2: 1.2 mL/min



C1) MRM chromatogram at the LLOQ for alprazolam spiked in human plasma using AJS and flow rate = 1.2 mL/min (LLOQ = 0.06 ng/mL, 19.3 fg on column). Increasing the flow rate resulted in a run time < 0.5 min without sacrificing quantitative data quality.

C2) Calibration curve obtained using AJS at flow rate = 1.2 mL/min. 4.5 orders of linear dynamic range, good accuracy and precision were demonstrated in human plasma at 1.2 mL/min.

Figure 1.

A1) Overlaid MRM chromatograms obtained using AJS in comparison to conventional orthogonal ESI. B1) and C1) MRM chromatograms at the LLOQ (0.06 ng/mL, 19.3 fg on column) using AJS and flow rates = 1.0 and 1.2 mL/min, respectively. B2) and C2): Calibration curves obtained using AJS at 1.0 mL/min and 1.2 mL/min, respectively. B3) Calibration curve of alprazolam in pure solvents obtained using AJS at 1.0 mL/min shows practically the same slope in comparison to human plasma indicating the absence of any significant matrix effect.

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