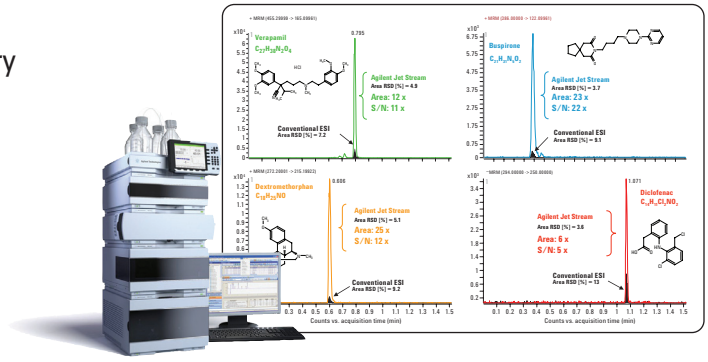
Extended ionization capability of thermal gradient focusing ESI in high-throughput in-vitro ADME assays

Application Note

Drug Discovery



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Abstract

Several factors cause fast LC/MS/MS method development in the drug discovery area to be an arduous task. Combination of ESI/APCI sources offers broad response with multiple ionization modes, but optimization can be difficult and some sources limit flow rates to 1 mL/min, while others compromise chromatographic performance. The scan speed of the mass spectrometer needs to be fast enough to acquire an adequate number of data points across the narrow peaks generated using sub-2 µm columns. At typical fast LC conditions, current HPLC systems (pressure limit ~ 400 bar) would yield back pressures greater than the threshold limit. In this application example, we utilized the Agilent 1290 Infinity UHPLC system coupled with 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 in-vitro metabolic stability samples. Incubations of the substrates buspirone, verapamil, dextromethorphan or diclofenac were carried out separately. An aliquot was taken at increasing time points from each incubate and then pooled together for analysis. 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, dwell times as low as 1-2 ms, and polarity switching time of 30 ms, achieved an analysis time of less than 1.1 min without sacrificing quantitative data quality. Due to the high data acquisition rate provided by the Agilent 6460A Triple Quadrupole mass spectrometer, compounds ionizing in positive and negative modes were analyzed in a single run. An adequate number of data points (>10) could be collected across the extremely narrow peaks (Average full width half maximum (FWHM) < 1.3 sec) generated by the Agilent 1290 Infinity LC system. AJS showed enhanced area response and signal-to-noise in comparison to conventional orthogonal ESI.

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Instrument Conditions

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

Conditions	
Column:	RRHD ZORBAX SB-C18, 2.1 mm × 50 mm, 1.8 µm
Mobile phase:	A= 0.1% FA in H_2O, B= 0.1% FA in ACN
Injection volume:	1 µL
Method:	
Column temperature:	25 °C
Flow rate:	1.0 mL/min
Gradient:	25% to 80% B in 1 min, 1.25 min 80% B, 1.26 min 25% B, stop 1.8 min
Scan type:	MRM
Polarity:	Pos/Neg

Parameters	
Drying gas temperature:	350 °C (ESI / ESI + AJS)
Drying gas flow:	10 L/min (ESI + AJS), 13 L/min (ESI)
Sheath gas:	400 °C and 12 L/min (ESI + AJS)
Nebulizer:	35 psig (ESI + AJS), 60 psig (ESI)
Nozzle:	0 (+) 1500 V (–) (ESI + AJS)
Capillary:	3500 V (±) (ESI / ESI + AJS)
Dwell time:	5 ms

Chromatograms

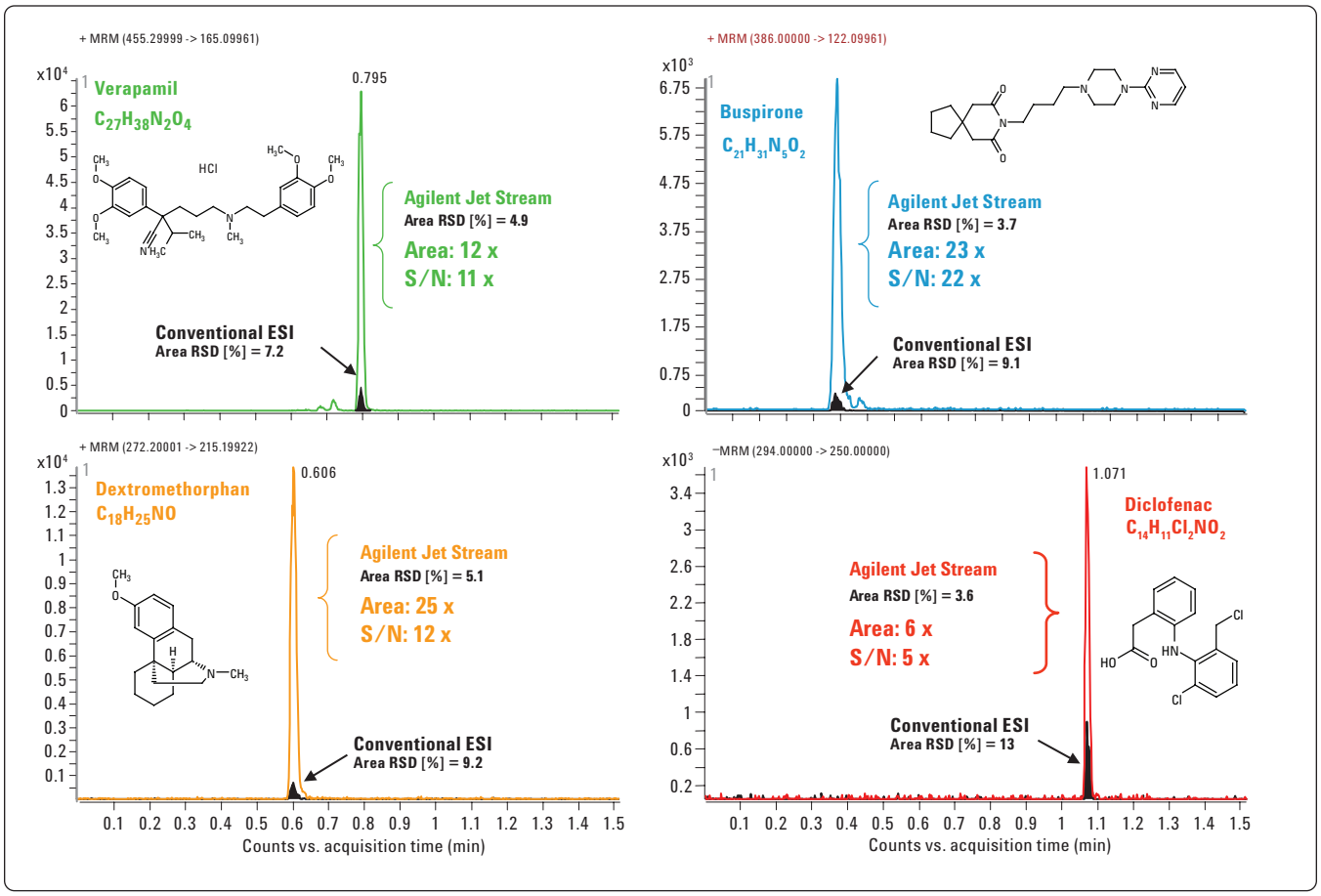

Figure 1.
Overlaid MRM chromatograms obtained using AJS in comparison to conventional orthogonal ESI for the metabolic stability substrates after 35 minutes of incubation with rat liver S9 fraction.

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