Sensitive & Robust Accurate Mass Analysis of Small Molecules Using a Dual-Spray Thermal Gradient Focusing Electrospray Ionization Source

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Introduction

It has been demonstrated that TOF instruments can reliably yield low part-per-million (ppm) mass errors through the use of real time reference mass correction. Although reference mass ions can be produced by "teeing in" suitable reference compounds to the main analyte flow, this approach often suffers from ionization suppression or solvent gradient effects. Each of these factors can affect either the analyte or reference mass ion response. Loss of analyte ion response will affect limits of detection and quantitation (LOD/LOQ) and overall linearity, while loss of reference mass ion response typically leads to increased mass errors.

In this study, we utilize a high resolution Q-TOF instrument equipped with a novel dual-spray thermal gradient focusing electrospray ionization (ESI) interface (Dual Agilent Jet Stream) in which a pneumatically nebulized reference mass solution is sprayed into the thermal confinement region of electrosprayed droplets. The resulting reference mass ions are largely unaffected by LC gradient conditions and are highly tolerant of matrix effects. Furthermore, the reference spray did not affect the analyte response for the compounds studied.

Experimental

Agilent 1290 Infinity UHPLC:

An Agilent 1290 Infinity UHPLC system comprising a binary pump, thermostatted autosampler and column compartment was used to develop high matrix and wide gradient narrow-bore analytical methods to test the robustness of the new source design.

Agilent 6540 Ultra High Definition (UHD) Q-TOF:

An Agilent 6540 Q-TOF was used to evaluate the performance of a novel dual-spray thermal gradient focusing ESI interface. The instrument was operated in Extended Dynamic Range mode for the gradient study and High Resolution mode for the matrix study.

Dual-Spray Agilent Jet Stream Source:

An Agilent Jet Stream interface was modified to add a thermally shielded reference nebulizer in addition to the analytical nebulizer with active cooling. (Figure 1). A 1200 series Isocratic pump and 1:100 splitter was used to provide variable flow control of the reference mass solution for the reference sprayer. A precision sapphire splitter was used to provide a 1:3 split of the analytical sprayer gas supply to control gas pressure for the reference sprayer.

Experimental

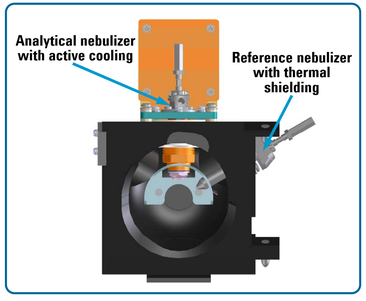


Figure 1: Dual Agilent Jet Stream source provides enhanced sensitivity and robust reference mass ionization.

Gradient Study:

Alprazolam standards from 5 to 5000 pg/µL concentrations were separated at 0.75 mL/min on a 2.1 x 50 mm C18 column using a rapid gradient from 5-95% acetonitrile with 0.1% formic acid (FA). A 5ppm extraction window was used to generate quantitative data. Reference masses at 121.050873 and 922.009798. were used to provide internal mass correction throughout the gradient study.

Sample Preparation:

A commercially available alprazolam standard (Cerilliant) was used to prepare neat calibration standards from 5 to 5000 pg/µL in water/acetonitrile. Reference ion standards (Agilent) were prepared in 95% acetonitrile at 2µM for the purine standard and 1µM for the HP-0921 standard.

UHPLC Conditions:

Mobile Phase	A: 0.1% FA in Water	
	B: 0.1% FA in ACN	
Injection Volume	1 µL + needle wash, 10 sec.	
Column	Eclipse Plus, 1.8 µM, 2.1 x 50 mm	
Column Temp.	50 °C	
Binary Flow	0.75 mL/min	
Binary Gradient	0.0 min	5% B
	0.25	5% B
	1.75	95% B
	2.17	5% B
	3	5% B
Stop Time	3.0 min	
Post Time	Off	

Results and Discussion

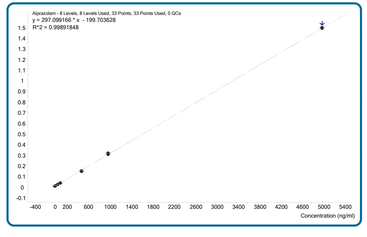
Q-TOF Conditions:

Ionization mode	Positive ion
	Dual-Spray AJS
Operational mode	Extended dynamic range
Drying gas temp.	300 °C
Drying gas flow	7 L/min
Sheath gas temp.	350 °C
Sheath gas flow	10 L/min
Nebulizer pressure	40 psi
Nozzle voltage	0 V
Capillary voltage	3250 V
Fragmentor	130 V
Skimmer	47 V

Mass Range	m/z 50 - 1000
Acquisition rate	4 spectra/sec
Reference Mass Flow	20 µL/min

Quantitative Results from Gradient Study:

The quantitation results for Alprazolam with the rapid gradient method yielded an excellent fit to a linear calibration curve across three orders of magnitude with a correlation coefficient (R^2) of 0.99937.



Area %RSD for the lowest level (5 pg on-column) was 7.3% with five replicates. Calculated accuracies were within 15% across the range of sample concentrations, demonstrating that quantitation is not compromised by the second sprayer or the use of reference mass ions.

Concentration (pg/µL)	5	25	50	250	500	2500	5000
% Accuracy	112.0	87.8	100.4	91.6	104.0	99.9	100.1
Reproducibility (%RSD, n = 5)	7.3	4.5	3.3	1.9	1.4	1.6	1.8
Signal/Noise	12.4	53.3	145.1	560.2	871.1	1178.0	1165.1

Matrix Study:

A red wine sample was spiked with reserpine at 150 pg/µL and separated at 0.6 mL/min on a 2.1 x 150 mm C18 column using a 40 minute gradient from 1-99% acetonitrile with 0.1% formic acid. Empirical formula confirmation was applied to 30 replicate runs spanning 24 hours of continuous operation to calculate the mass accuracy of the reserpine standard. Reference masses at 121.050873 and 922.009798. were used to provide internal mass correction throughout the gradient study.

Sample Preparation:

A red wine sample was spiked with reserpine at 150 pg/µL and vortexed for 5 seconds. No filtration or centrifugation was applied to the sample prior to analysis. Reference ion standards (Agilent) were prepared in 95% acetonitrile at 2µM for the purine standard and 1µM for the HP-0921 standard.

UHPLC Conditions:

Mobile Phase	A: 0.1% FA in Water	
	B: 0.1% FA in ACN	
Injection Volume	1 µL + needle wash, 10 sec.	
Column	Eclipse Plus, 1.8 µM, 2.1 x 150 mm	
Column Temp.	60 °C	
Binary Flow	0.6 mL/min	
Binary Gradient	0.0 min	1% B
	40 min	99% B
Stop Time	40 min	
Post Time	5.0 min	

Q-TOF Conditions:

Ionization mode	Positive ion
	Dual-Spray AJS
Drying gas temp.	325 °C
Drying gas flow	10 L/min
Sheath gas temp.	350 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Nozzle voltage	0 V
Capillary voltage	3250 V
Fragmentor	125 V
Skimmer	47 V

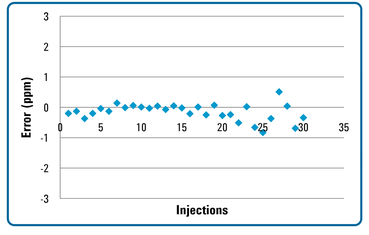
MS Acquisition: Reserpine in Wine

Mass Range	m/z 50 - 1000
Acquisition rate	1 spectra/sec
Reference Mass Flow	20 µL/min

Results and Discussion

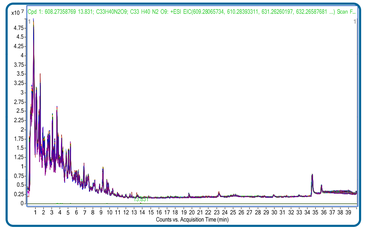
Mass Accuracy Results from Matrix Study:

A scatter plot of the mass errors from the formula confirmation results exhibited mass errors of less than 1-ppm. Given the 24 hour duration of the study, these results would be impressive even without the presence of a complex, heavy matrix. This illustrates the stability of the UHD Q-TOF system and the effectiveness of the real-time mass correction.



Robustness & Reproducibility of Dual-Spray AJS Source:

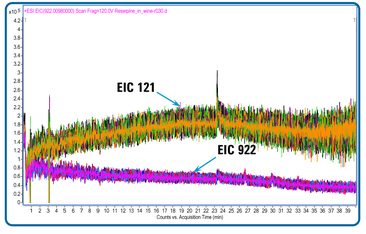
An overlay of the Total Ion Chromatogram (TIC) results for each of the 30 runs shows the remarkable consistency of both the 1290 Infinity UHPLC system and the Dual Agilent Jet Stream source.



The Extracted Ion Chromatogram (EIC) trace of the reserpine masses indicates the relative scale of the sample peak at 13.831 minutes. Elution times for the reserpine peak varied by less than 0.05 minutes and the %RSD for the peak areas was 1.66%.

Stability of Reference Mass Ion Response:

An overlay of the EIC traces from each of the 30 runs for the 121.0509 and 922.0098 reference masses demonstrates the stability of the reference mass ion signal across a wide gradient with a complex matrix. Given the low flow of reference mass solution (20 µL/min), it is likely that the reference ions are desolvated prior to encountering the thermal confinement region of the analyte spray. This would largely eliminate solvent and matrix effects and substantiates the utility of the dual sprayer approach for reference ion generation.



Conclusions

- The Gradient study demonstrated that quantitative results could be obtained across a 5-95% acetonitrile gradient using a Dual-Spray Agilent Jet Stream (AJS) source with external reference mass correction.
- The Matrix study demonstrated that the reference mass ion response of the Dual-Spray AJS source was largely unaffected by LC gradient conditions or matrix effects.
- The Matrix study demonstrated the remarkable consistency of the Dual-Spray AJS source analyte response and the chromatographic performance of the 1290 Infinity UHPLC system.
- When a Dual-Spray AJS source is used in conjunction with an Agilent Ultra High Definition (UHD) Q-TOF system, it is possible to obtain sub-ppm mass accuracies throughout a 24 hour study.