Application Note
Clinical Research

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Analytical Development of a Four-Stream Multiplexed LC/MS Method for the Simultaneous Determination of SDMA and ADMA

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Abstract

A profound technical challenge to laboratories today is achieving the highest productivity while meeting the demands for precise, accurate, and cost-effective test results. The Agilent StreamSelect LC/MS System can help deliver all this, by seamlessly multiplexing up to four concurrent HPLC separations with one triple quadrupole mass spectrometer. The system can accommodate a single method mirrored across all HPLC systems, or can have an unique method assigned to each HPLC. With MassHunter-based software-driven scheduling and acquisition, a four-fold increase in throughput can be realized without any loss of analytical fidelity. This Application Note demonstrates the development and confirmation of an analytical method for asymmetric and symmetric dimethyl arginine (ADMA and SDMA respectively).

Introduction

ADMA and SDMA are two modified amino acids that are emerging biomarkers. A method for the simultaneous determination of ADMA and SDMA by LC/MS/MS was developed and rigorously confirmed on a four-stream Agilent StreamSelect LC/MS system. The data demonstrate excellent and equivalent analytical performance across all four streams, and show that the system can meet strict analytical performance targets.

Experimental

Stock solutions of ADMA and SDMA were prepared at 1 mg/mL in water. Calibrators for ADMA and SDMA were prepared in charcoal-stripped human serum at 10, 20, 50, 100, 250, and 500 ng/mL by appropriate dilution of stock solutions, and stored frozen. A 50-µL volume of human serum or plasma was mixed with 150 µL of methanol containing 60 ng/mL of heavy-labeled ADMA to precipitate proteins and provide an internal standard. The precipitate was removed by centrifugation, and the supernatant transferred to a clean vial, and subsequently diluted with 200 µL of 0.5 % heptafluorobutyric acid in water prior to injection.

Instrumentation

An Agilent 6490 triple quadrupole LC/MS system with Agilent StreamSelect software was configured with an HTC-PAL autosampler with four injection ports, four Agilent 1260 Infinity binary pumps, and a 9-port/8-position stream selection valve.

Instrument conditions

LC conditions	
Analytical column	Phenomenex Kinetix 2.6 µM 2.1 × 50 mm, alternatively, an Agilent Poroshell
Column temperature	Ambient
Injection volume	3 µL
Mobile phase	A) H_2O + 0.005 % HFBA B) Methanol + 0.005 % HFBA
Flow rate	0.4 mL minutes
Stop time	5 minutes
Isocratic separation	5 % mobile phase B for 2.5 minutes with a wash at 95 %B for 2.5 minutes
MS/MS conditions	
Instrument	Agilent 6490 triple quadrupole LC/MS
Ion mode	Positive
Drying gas temperature	250 °C
Gas flow	14 L/min
Nebulizer	20 psi
Sheath gas temperature	300 °C
Shealth gas flow	12
Capillary voltage	3,000
EMV	+100 V

MRM transitions

Analyte	Precursor	Product	Fragmentor	Dwell	Collision energy
ADMA-d6-quant	209.2	69.9	380	100	27
ADMA-d6-qual	209.2	52	380	100	22
SDMA-quant	203.2	172.1	380	100	10
SDMA-qual	203.2	69.9	380	100	10
ADMA-quant	203.2	69.9	380	100	27
ADMA-qual	203.2	46	380	100	22

Results and discussion

Imprecision studies were carried out running eight replicate injections from each of five unique batches over a span of 14 days. In the absence of reference material, bias was determined against assigned values established for synthetic and sample pools from calibrators and reagents prepared separately from the ones used in this study. Each individual stream was evaluated separately. For both analytes, the mean bias was below 4.0 % for a high QC pool, and below 2.5 % for a low QC pool. Interbatch imprecision was below 3.0 % CV, and intrabatch imprecision was below 2.4 % CV, as shown in Table 1.

Table 1. Assay performance.

	ADMA	SDMA
Intra-assay imprecision	<1.7%	<2.4%
Inter-assay imprecision	<3.0%	<2.8%
Mean Bias at 30ng/mL	0.7%	2.5%
Mean Bias at 90 ng/mL	1.2%	3.9%

Calibration stability

Calibration samples were run with each batch. Linear regression with 1/x weighting was used to generate calibration curves. To illustrate stability, calibration statistics for slope, intercept, R^2 , and maximal back-calculated deviation at day 1 and day 14 are found along with mean internal standard intensity in Table 2. The individual HPLC streams generate calibration curves that are essentially indistinguishable from each other.

Table 2. ADMA calibration statistics.

	Slope	
Stream	Day 1	Day 14
1	1.0000	0.9996
2	1.0000	1.0010
3	1.0020	1.0010
4	0.9997	0.9950
	Intercept	
1	0.0180	-0.0184
2	-0.0158	0.0093
3	-0.0493	-0.0207
4	-0.0312	-0.0160
	R^2	
1	0.9997	0.9999
2	0.9998	1.0000
3	0.9998	0.9998
4	0.9997	0.9999
	Maximum % backcalculated deviation	
1	3.0	-4.0
2	2.0	2.0
3	-3.0	-4.0
4	-3.0	3.0
	Mean IS area for calibrator	
1	33433	38601
2	40489	43467
3	34397	40618
4	48449	37296

Stream-by-stream performance

Figures 1A and 1B show the individual data points for each observation for sample pool with eight observations per day per stream carried out over 14 days, and emphasizes the quality of the analytical data generated in multistream mode. The data revealed no dependence on injection order and very good performance, with only 12 of 320 analyses having a bias of more than 5 %.

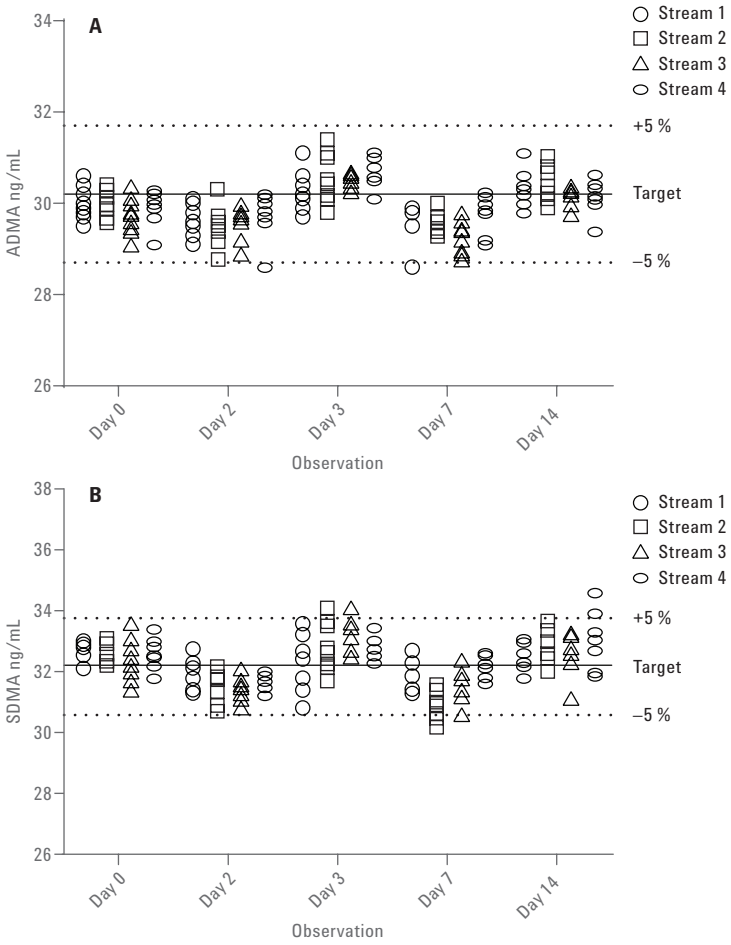

Figure 1. Low QC pool precision and accuracy for ADMA (A) and SDMA (B).

The suitablity for the four-stream system to be fully multiplexed and thought of as one system with no distinction between streams can conveniently be evaluated using total error, which incorporates estimates of both bias and imprecision. To ensure that each stream performed acceptably across a wide dynamic range, we examined the total error for each stream as a function of concentration. Figure 2 shows that the total error for each of four streams remains below the threshold for ADMA and SDMA: between 7.5 and 400 ng/mL for ADMA, and 5.0–400 ng/mL for SDMA. This performance indicates that full multiplex operation where calibrators, unknowns, and QC specimens are distributed across all four streams is acceptable, providing the highest throughput.

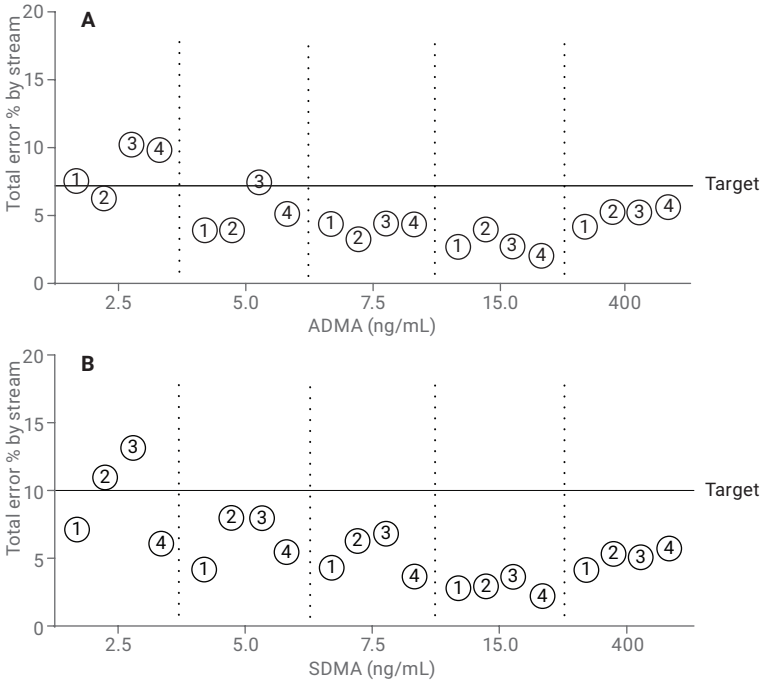

Figure 2. Total error by stream for ADMA (A) and SDMA (B).

Conclusions

Deploying analytical methods that retain optimal performance as throughput increases is a challenging task. The StreamSelect LC/MS System provides an effective multiplexing platform. We have developed a four-stream method that meets the stringent analytical performance criteria for the simultaneous analysis of SDMA and ADMA. The data support the view that the four-stream HPLC system can be considered as one system, allowing distribution of calibrators, QC samples, and unknowns across all available streams for highest throughput.

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