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RAPID QUANTIFICATION OF CODEINE BY DESORPTION/IONIZATION ON SILICON (DIOS) TIME-OF-FLIGHT MASS SPECTROMETRY

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INTRODUCTION

Quantitative analysis of small molecules using laser desorption methods has long been an appealing concept due to high throughput capabilities of laser desorption time-of-flight mass spectrometers combined with the inherent mass selectivity. Both matrix-assisted laser/desorption ionization (MALDI)[1-3] and desorption/ionization on silicon mass spectrometry (DIOS)[4-7] have a number of applications showing small molecule quantification. In most cases, the key to successful quantification is the presence of an internal standard and appropriate sample preparation.[8]

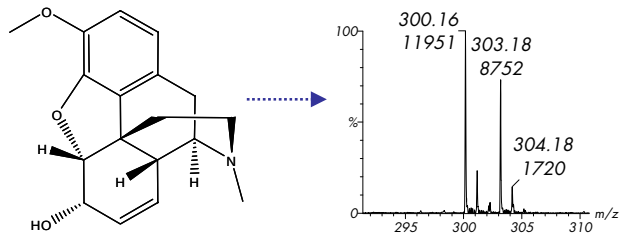
Key Advantages of DIOS-TOF MS for Small Molecule Quantification:

1. Rapid sample cycle times are generally less than 1 minute
2. DIOS is a matrix-free method, so the analyte signal is not obscured by chemical noise from the matrix
3. Method development is minimal and simple providing for quick results
4. The technique provides both qualitative and quantitative information

GOALS

1. Perform relative quantification of codeine by DIOS-TOF MS and LC/MS
2. Compare the sample cycle time, precision and accuracy of quantification by DIOS-TOF MS and LC/MS
3. Quantify codeine in a pain medication elixir

Codeine (m/z 300)



DIOS-TOF MS with the Waters Micromass® MassPREP™ DIOS-target™ Plate adds a new dimension to the capabilities of the Waters Micromass® MALDI micro MX™ Time-of-Flight Mass Spectrometer.

RESULTS

DIOS-TOF MS Codeine Quantification: *Codeine was quantified relative to a deuterated internal standard. The standard curve is based on the correlation between the mass spectral intensity ratio (codeine/codine-_d3) and the concentration of codeine (Figure 1A). Examples of the DIOS-TOF MS spectra are shown in Figure 1B with mono-isotopic peaks for codeine (m/z 300) and the internal standard codeine_d3 (m/z 303).*

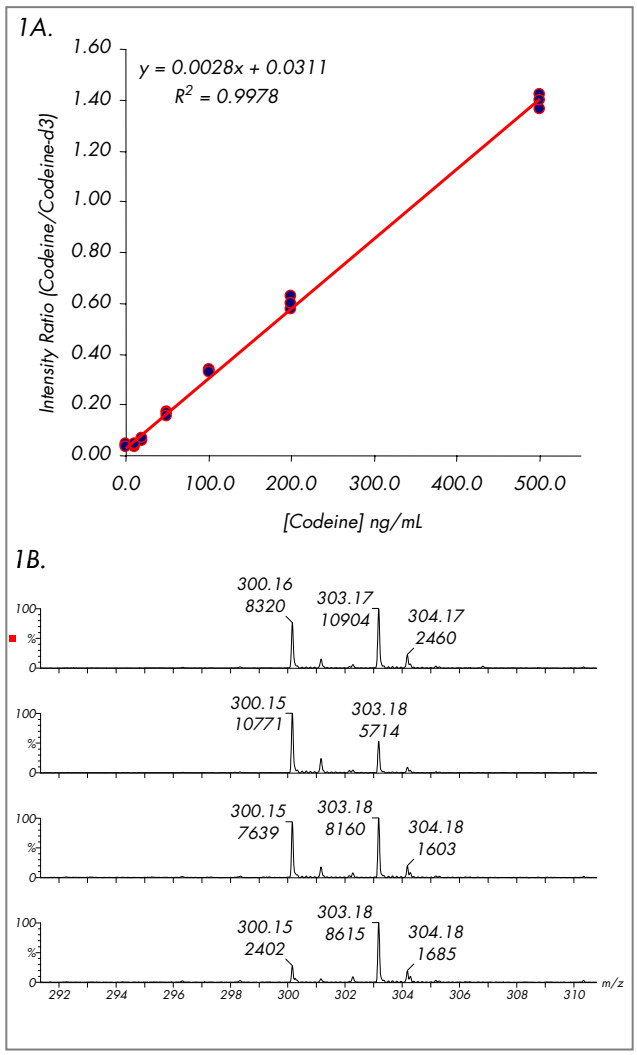


Figure 1A. The standard calibration curve for quantification of codeine derived from DIOS-TOF MS peak intensity ratios. Figure 1B. Representative DIOS-TOF MS mass spectra of quality control (QC) and pain medication elixir samples.

LC/MS Codeine Quantification: *The LC/MS method was operated in SIR (selected ion recording) mode and all data was analyzed using QuanLynx™ Application Manager for MassLynx™ 4.0 Software. The standard curve extends from 10.0 to 2000.0 ng/mL of codeine and the linear regression R^2 value of 0.9997 (Figure 2A), a significant improvement over the DIOS-TOF MS method calculations. However, the method development time and sample cycle times for the LC-based approach were markedly longer than those for the DIOS method. The DIOS method provides a significant advantage of increased sample throughput.*

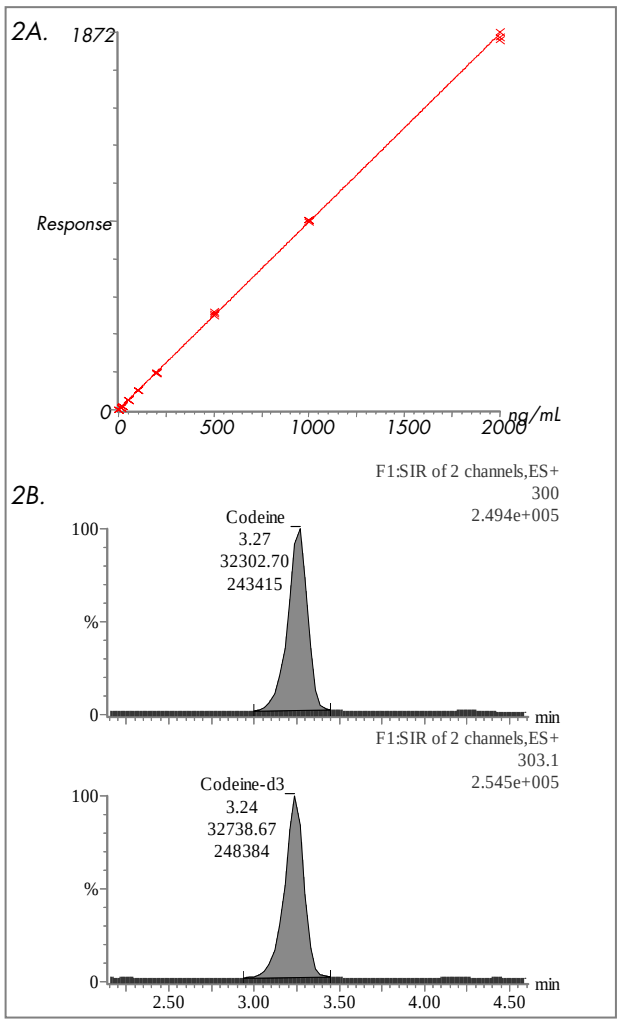


Figure 2A. The standard calibration curve for quantification of codeine from LC/MS SIR peak area ratios. Figure 2B. Peak integration of examples of codeine (m/z 300) and codeine_d3 (m/z 303).

Comparison of DIOS-TOF MS and LC/MS Codeine Quantification: *The data in Table 1 indicate that for the DIOS-TOF MS method, from 15.0 to 800.0 ng/mL, the average % error and % RSD are 8.6 and 10.0 respectively while the sample cycle time is 66 seconds. The LC/MS approach, from 10.0 to 2000.0 ng/mL provided an average % error of 2.3%, % RSD of 1.1% and sample cycle time of 20 minutes.*

		A. DIOS-TOF MS: MS Intensity Ratio			**B. LC/MS: Peak Area Ratio**		
Sample	Actual [Codeine] ng/mL	Average [Codeine] ng/mL	% Error	% RSD	Average [Codeine] ng/mL	% Error	% RSD
Elixir	240.0	270.8	12.8	0.9	239.6	-0.2	0.8
QC 15	15.0	15.3	1.7	43.2	15.7	4.4	0.4
QC 90	90.0	91.8	2.0	3.9	89.5	-0.6	0.9
QC 300	300.0	328.6	9.5	1.5	317.6	5.9	2.4
QC 800	800.0	662.5	-17.2	0.6	803.6	0.5	0.8
Root Mean Squared			8.6	10.0		2.3	1.1
Linear Dynamic Range		15.0 to 800.0 ng/mL			10.0 to 2000.0 ng/mL		
Cycle Time		**66 seconds**			**20 minutes**		

Table 1. Summary of quantification results for codeine using (A.) DIOS-TOF MS intensity ratios and (B.) LC/MS selected ion recording (SIR) peak area ratios.

CONCLUSIONS

- *DIOS-TOF MS provides for quantification of codeine over roughly 2 orders of magnitude dynamic range with 8.6% error and 10.0% RSD.*

- *LC/MS analysis of the same samples run by DIOS-TOF MS provides for at least 2.5 orders of magnitude dynamic range with 2.3% error and 1.1% RSD.*

- *The sample cycle time for DIOS-TOF MS (66 seconds) is significantly faster than that of LC/MS (20 minutes). This advantage, combined with simple and rapid method development makes DIOS-TOF MS an attractive alternative technique for high throughput semi-quantitative analysis.*

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Further reading for complete experimental details:

Wall, D, Finch J, Cohen C. Letter to the Editor: Quantification of codeine by desorption/ionization on silicon (DIOS) time-of-flight mass spectrometry and comparisons to liquid chromatography mass spectrometry. Rapid Commun. Mass Spectrom. 2004; in press.

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