NEW OBJECTIVE

Correlating Extended Dynamic Range with Flow-Rate in Static NanoESI-MS Analysis of Small-Molecules

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Introduction

Nanobore ESI-MS analysis has proven its functionality as a standard-free, semi-quantitative vehicle for parent-molecule and associated metabolite analysis[1].Prior investigations have revealed this equimolar response can occur over a limited flow-rate interval. Further research has affirmed the capacity of silicone-chip-based nanobore electrospray mass spectrometry to provide extended dynamic ranges in absolute quantitative analysis[2,3]. A similar linear-dynamic-range behavior for internal-standard (IS)-spiked solutions containing verapamil and testosterone analytes in a canine-plasma matrix using metallic-coated static nanospray emitters of various tip IDs was observed. Chemical structures for both analytes and their respective internal standards are illustrated in Figures 1 and 2.

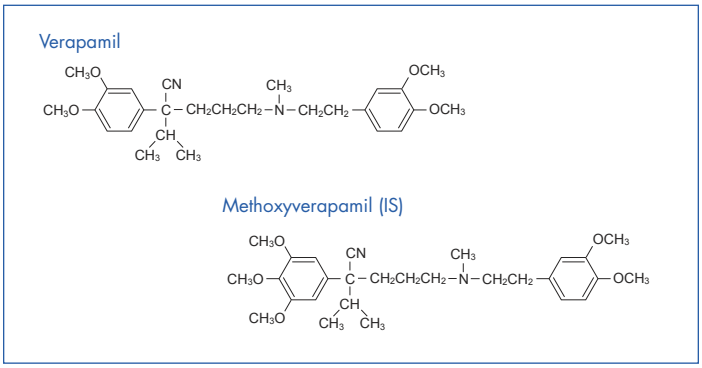

Figure 1 *The chemical structures of verapamil and methoxyverapamil*

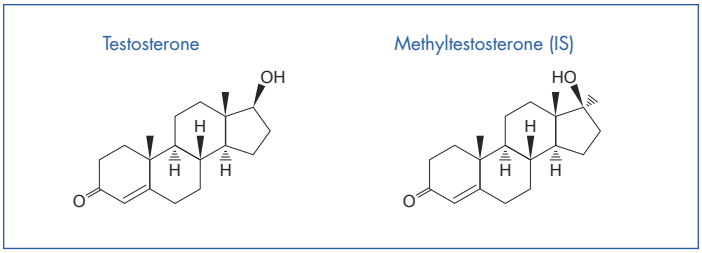

Figure 2 *The chemical structures of testosterone and methyltestosterone*

Methods & Materials

Instrumentation and Components

- Ion-trap mass spectrometer (LCQ Deca™, Thermo-Fisher), full-scan mode
- Custom-nanospray source (Digital PicoView® 150, New Objective) modified for offline analysis (Figure 3)
- Eppendorf Centrifuge 5415
- Micro-Centrifuge Tubes with Graduations (1.7 mL, VWR)
- A 0.5-10 µm Eppendorf® Single-Channel Research Pipette
- GlassTips™ (BG12-94-4-CE) and EconoTips™ (Econo12) offline nanospray emitters (New Objective)

Sample Preparation

- Centrifugation of 500 µL canine plasma with 1000 µL MTBE was followed by aspiration/expulsion of 250 µL organic layer into each of 10 clean 1.7 mL micro-centrifuge tubes; evaporation to dryness was allowed for expelled solvent[1].
- Through serial dilution in 50% aqueous methanol/0.1% formic acid, two suites of analytical standards were prepared in the aforementioned plasma-spiked micro-centrifuge tubes; one suite contained 0.01, 0.05, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0 µg/mL small-molecule analyte, and the other suite possessed identical concentrations in steroid analyte.
- Verapamil (Aldrich) was used as the small-molecule analyte, and testosterone (Alltech) was used as the steroid analyte.
- Both suites contained 5.0 µg/mL internal standard with chemical structure similar to respective analytes—Methoxyverapamil (Aldrich) was the IS employed in verapamil standards, and methyltestosterone (Alltech) was the IS for testosterone standards.
- Standard solutions for both analytes were loaded into distal ends of static nanoelectrospray emitters having 1µm, 2µm, and 4 µm tip inner diameters and analyzed via an ion-trap mass spectrometer in full-scan mode.

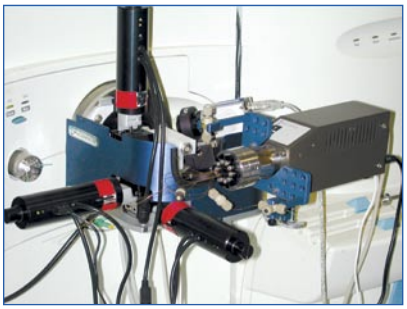

Figure 3 *Digital PicoView® nanospray source on LCQ Deca™ mass spectrometer*

Results

The analyte-to-internal-standard molecular-ion peak intensity ratio was computed upon analysis of each standard solution. Figures 4 and 5 display representative mass spectra of verapamil and testosterone, respectively. The molecular ions selected for quantitative analyses of each analyte are shown in Table 1.

Both the verapamil and testosterone analyses displayed acceptable linearity over a concentration range spanning four orders of magnitude. Verapamil analyses using 1 µm, 2 µm, and 4 µm tip IDs yielded linear dynamic ranges with sensitivities and R^2 values of 0.9994, 0.9991, and 0.9995, respectively. Testosterone analyses employing 1 µm, 2 µm, and 4 µm tip IDs displayed similar sensitivities and R^2 values of 0.9985. 0.9970, and 0.9998, respectively. Table 2 summarizes the slopes and R^2 values obtained from linear regressions for each analyte and tip ID.

Figures 6 and 7 illustrate tip-dependent regression analyses for verapamil and testosterone respectively (analyte-to-internal-standard intensity ratio), plotted as a function of analyte concentration in µg/mL.

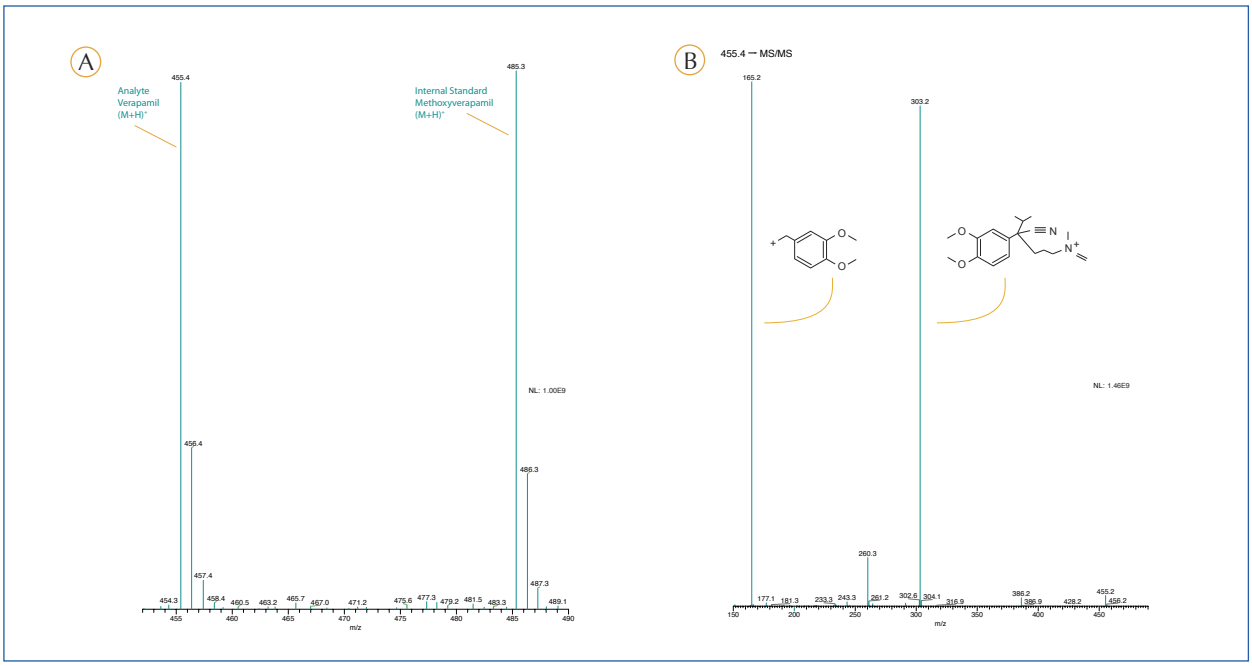

Figure 4 *A) Mass spectrum of verapamil with methoxyverapamil (IS); B) MS/MS of verapamil (M+H)+*

Compound	Molecular-Ion Peak (m/z)
Verapamil (analyte)	455.3
Methoxyverapamil (IS)	485.1
Testosterone (analyte)	289.2
Methyltestosterone (IS)	303.2

Table 1 *Summary of analytes and corresponding molecular ions*

Analyte	Tip ID (µm)	R^2	Regression Slope
Verapamil	1	0.9994	0.1739
	2	0.9991	0.1671
	4	0.9995	0.1741
Testosterone	1	0.9985	0.1139
	2	0.9970	0.1150
	4	0.9998	0.1052

Table 2 *Summary of regression data by analyte and tip ID*

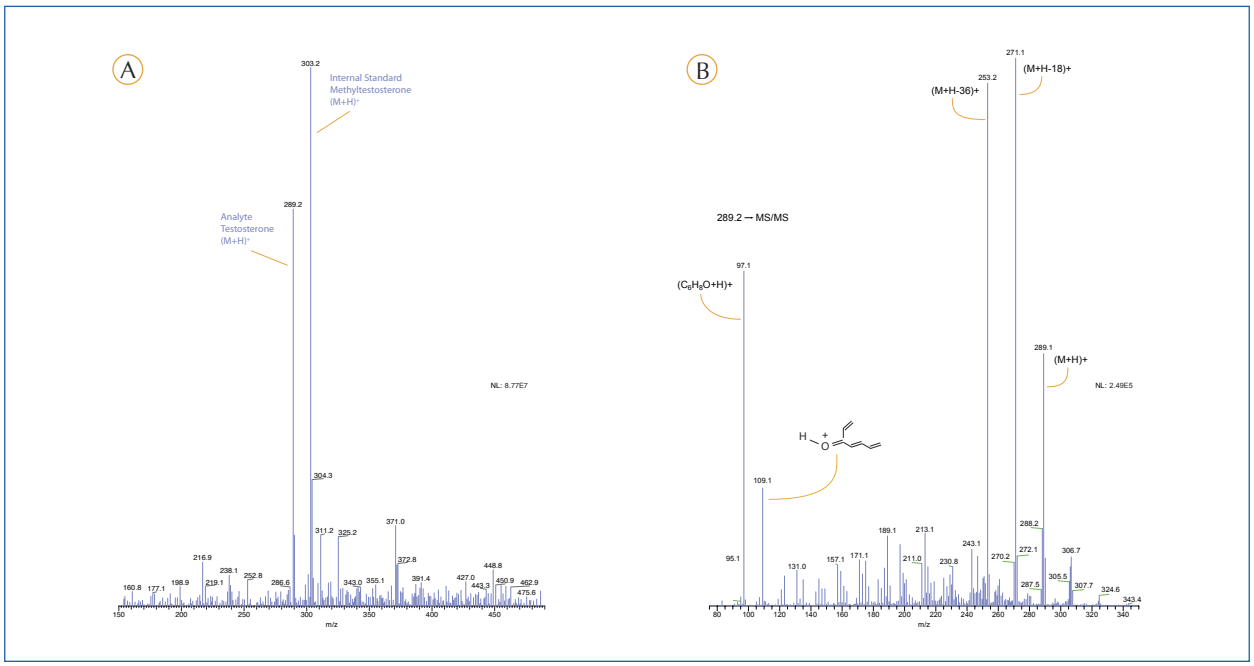

Figure 5 *A) Mass spectrum of testosterone with methyltestosterone (IS); B) MS/MS of testosterone $(M+H)^+$*

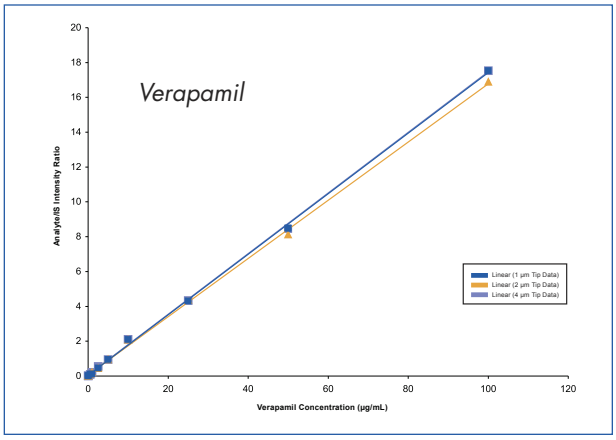

Figure 6 *Linear dynamic range behavior of verapamil using three tip IDs*

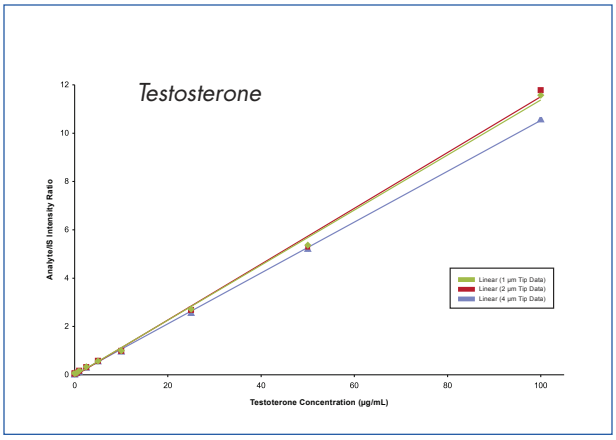

Figure 7 *Linear dynamic range behavior of testosterone using three tip IDs*

Conclusions

- A four-order-of-magnitude linear dynamic range was observed for verapamil and testosterone with traditional static-emitter-based nanoelectrospray
- Linearity was observed at relatively high concentrations, up to 100 µg/mL
- Similar sensitivities were observed for various tip IDs in plasma-spiked verapamil and testosterone samples, with $R^2 \geq 0.9970$ for each analyte and tip ID.
- Performance limitations in the current investigation originated from technological boundaries of ion-trap mass spectrometry
- Future work will involve the use of triple-quadrupole mass spectrometry for enhanced sensitivity of analyteson biological matricies

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Originally published June 2007, at the American Society of Mass Spectrometry Meeting in Indianapolis, Indiana, U.S.A.