

## Evaluation of the Quattro Premier for the Analysis of Verapamil in Protein-Precipitated Plasma

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### Abstract

This application note demonstrates to evaluate the source robustness by injecting samples of verapamil spiked into human plasma.

## Benefits

The robustness and reproducibility for samples on biological matrices has been evaluated by analyzing verapamil in protein-precipitated plasma

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## Introduction

The Quattro Premier Tandem Quadrupole Mass Spectrometer has a newly developed ZSpray interface designed to meet the demands of high throughput analyses from biological matrices in the pharmaceutical industry. In this environment robustness and sensitivity are the driving forces for successful bioanalysis.

The following study has been developed to evaluate the source robustness by injecting samples of verapamil spiked into human plasma. The spiked plasma was protein-precipitated and the supernatant injected directly into the source. The study ran over a 24-hour period in positive ion electrospray ionization mode (ESI+ve).

## Overview

The newly designed ZSpray source for the Waters Micromass Quattro Premier incorporates the best features of the Quattro micro API but with a smaller ion block that reduces the volume of the source chamber. The new features are highlighted in Figure 1 and are explained in Tech Note [720000826EN](https://www.waters.com/nextgen/us/en/library/application-notes/2004/evaluation-of-the-quattro-premier-for-the-quantification-of-sulfadimethoxine-in-hanks-balanced-salt-solution.html) < <https://www.waters.com/nextgen/us/en/library/application-notes/2004/evaluation-of-the-quattro-premier-for-the-quantification-of-sulfadimethoxine-in-hanks-balanced-salt-solution.html>> .

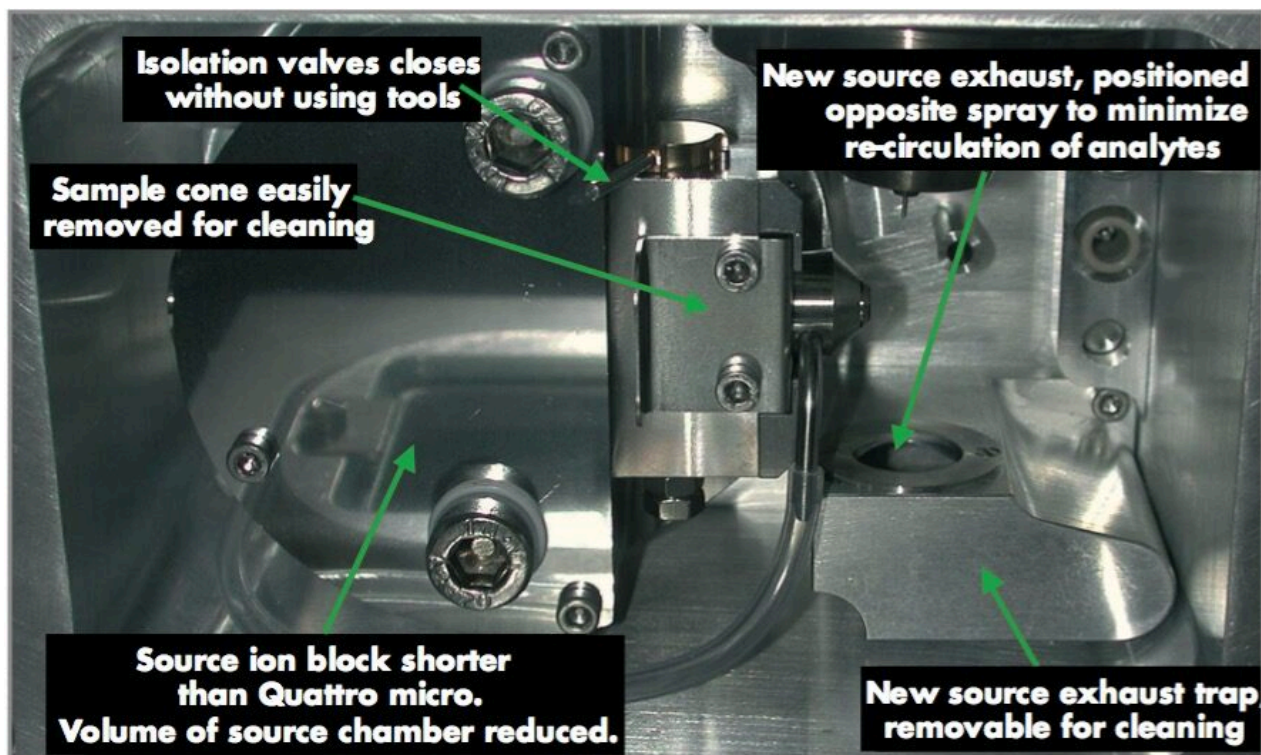


Figure 1. Features of the new ZSpray source of the Quattro Premier.

## Experimental

A quality control (QC) solution of verapamil in human plasma was prepared at a concentration of 10 pg/mL. The QC's were then protein-precipitated by adding acetonitrile (1:1), the resultant mixture was centrifuged (ca 3000 rpm, 10 mins) and the supernatant taken for analysis by LC-MS/MS, 200 repeat injections were performed.

## HPLC Conditions

LC system:	Waters 2695
Column:	Waters Atlantis, C <sub>18</sub> 3.5 $\mu$ m, 2.1 x 100 mm
Flow rate:	0.2 mL/min
Gradient:	Isocratic at 55% water: 45% acetonitrile 0.1%

Formic acid

## MS Conditions

Mass spectrometer:	Waters Micromass Quattro Premier
Ion mode:	ESI +ve
Capillary voltage:	3.2 V
Cone voltage:	40 V
Collision energy:	27 eV
Detection mode:	MRM (455.40 > 165.10)
Dwell:	0.5 seconds
Collision gas:	Argon ( $3.6 \times 10^{-3}$ mbar)

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## Results and Discussion

The MRM chromatograms for the verapamil analysis were integrated automatically using Waters proprietary ApexTrack peak integration algorithm and the resulting peak areas were plotted against injection number to show the reproducibility of response over the analysis period. A relative standard deviation of 3.0% was observed across all 200 analyses (see Figure 2).

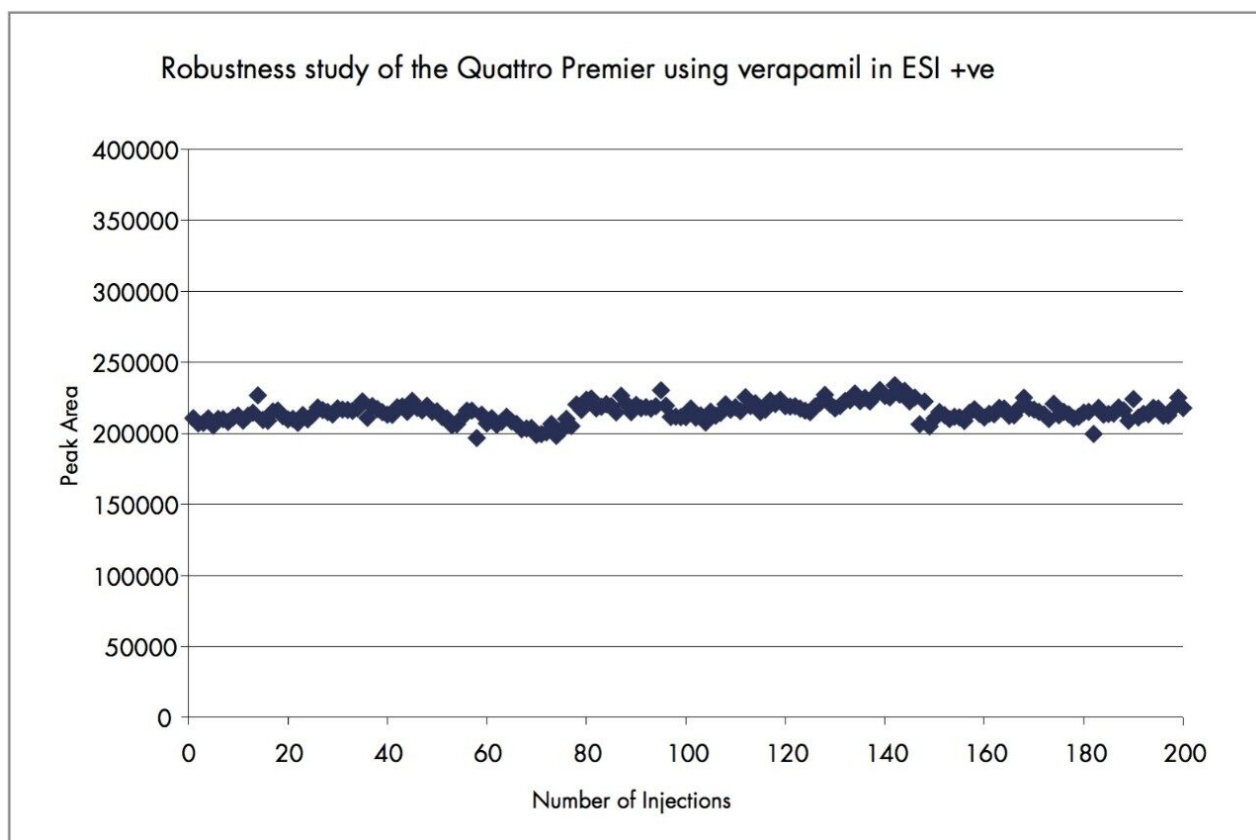


Figure 2. A plot of peak area for verapamil against a number of injections.

The plot of peak area against concentration showed good linearity over the range 0.5–5000 pg/ $\mu$ L. The calibration line was plotted using a linear fit with 1/x weighting and gave a correlation coefficient of 0.9978, with all calibration points resulting in <8% deviation (see Figure 3)

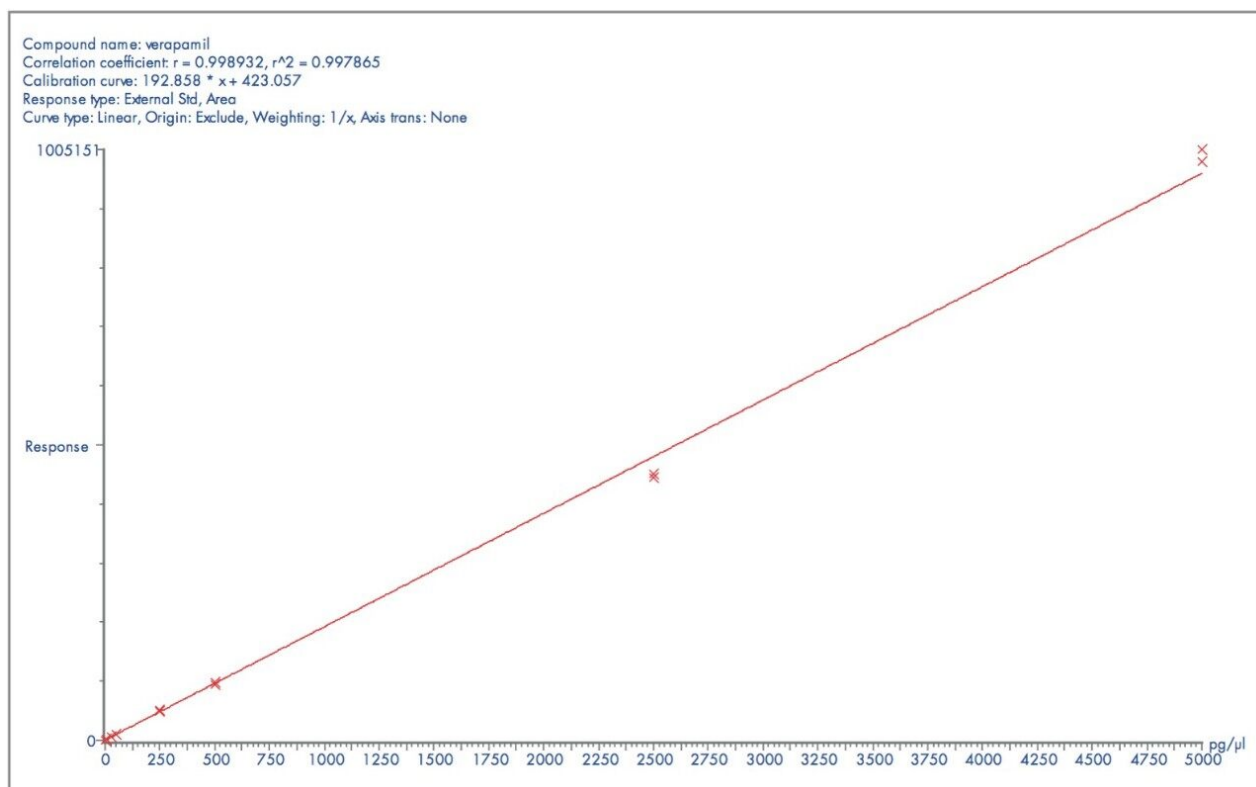


Figure 3. A calibration line for verapamil in protein-precipitated plasma.

## Conclusion

The new Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer has been developed for quantitative LC-MS/MS. The robustness and reproducibility for samples on biological matrices has been evaluated by analyzing verapamil in protein-precipitated plasma.

The results also show that the Quattro Premier sensitivity was maintained with a relative standard deviation of 3.0% during 200 injections. This demonstrates the robustness of the modified ZSpray source for analyses from complex matrices. The calibration line resulted in a linear plot over 4 orders of magnitude (0.5 to 5000 pg/μL) with a correlation coefficient of 0.9978.

