# Robustness of an Agilent 6470 Triple Quadrupole Mass Spectrometer for Analysis of Pharmaceuticals in Plasma Matrices

## Application Note


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### Introduction

Pharmaceutical drug discovery requires a host of *in vitro* and *in vivo* assays to screen potential drug candidates for further development. *In vivo* identification and quantification of metabolites are specifically challenging due to the presence of matrix components (plasma, urine, and so forth), and require the use of sensitive and robust analytical techniques for high-throughput screening of drug candidates. The most common analytical approach is to use triple quadrupole mass spectrometers in multiple reaction monitoring (MRM) mode. However, the analytical performance of these systems can degrade over time as nonvolatile sample matrix components accumulate in the ion source and ion transfer optics. As a result, frequent cleaning may be required to maintain optimal performance. Agilent 6470 Triple Quadrupole Mass Spectrometry (MS) systems feature several key design innovations for sensitive, precise, and robust quantification of target analytes in heavy sample matrices over a wide linear dynamic range. These design innovations include:

- Optimized ion optics and prefilter geometries, which increase ion transmission and minimize contamination
- A curved and tapered hexapole collision cell, which enables high efficiency MS/MS fragmentation and transmission of ions
- An ion detector with a high-energy conversion dynode and low noise characteristics, which enables efficient positive and negative ion detection across a wide *m/z* range

This Application Note demonstrates the advantages of using an Agilent 6470 Triple Quadrupole Mass Spectrometer coupled to an Agilent RapidFire 365 High-throughput MS (HTMS) System for the rapid and robust analysis of pharmaceuticals in plasma.

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## Robustness Evaluation of an Agilent 6470 Triple Quadrupole Mass Spectrometer System

Analytical robustness of a 6470 Triple Quadrupole Mass Spectrometer coupled with an RapidFire 365 HTMS System was evaluated using a mixture of alprazolam and clozapine, and their corresponding internal standards (ISs) (alprazolam-D5 and clozapine-D4, respectively) spiked in precipitated porcine plasma at a concentration of 5 ng/mL (each). Pig plasma was precipitated using a 1:3 volume ratio of acetonitrile followed by centrifugation at 13,500 rpm for 5 minutes. The supernatant was then diluted with water (1:10) prior to spiking with the analytes and ISs. The spiked plasma sample was transferred to 96-well microtiter plates and sealed using an Agilent PlateLoc Thermal Microplate Sealer prior to RapidFire/MS analysis.

The RapidFire 365 HTMS parameters were optimized to yield the following RapidFire method conditions:

**Wash solvent 1** (Buffer A, 1.50 mL/min flow rate) water + 2 mM ammonium acetate + 0.1 % formic acid

**Wash solvent 2** (Buffer B, 1.25 mL/min flow rate) 20 % acetonitrile (in water) + 10 mM ammonium acetate + 0.1 formic acid

An elution solvent (Buffer C, 75 % ethyl acetate in methanol) at a flow rate of 1.25 mL/min was used to elute analytes from Agilent type A (C4) cartridges. Analysis of the small molecule pharmaceuticals in plasma was performed at a rate of approximately 15 seconds per sample, over five consecutive days (2,000 injections/day). Optimized 6470 Triple Quadrupole ion source and MS parameters used in this study are summarized in Tables 1 and 2.

Table 1. Agilent Jet Stream source parameters.

| Parameter | Value |
|---|---|
| Drying gas temperature | 325 °C |
| Drying gas flow | 7 L/min |
| Nebulizer pressure | 50 psi |
| Sheath gas temperature | 300 °C |
| Sheath gas flow | 12 L/min |
| Capillary voltage | 4,000 V |
| Nozzle voltage | 500 V |

Table 2. Agilent 6470 Triple Quadrupole MRM parameters.

| Compound | Q1 | Q3 | CE |
|---|---|---|---|
| Alprazolam | 309.1 | 281.1 | 20 |
| Clozapine | 327.1 | 270.1 | 24 |
| Alprazolam-D5 (IS) | 314.1 | 286.1 | 20 |
| Clozapine-D4 (IS) | 331.1 | 272.2 | 24 |

Figure 1 shows the peak area ratios for alprazolam (A) and clozapine (B) from injection of 50 picograms of each analyte as a function of RapidFire injection number (a total of 10,000 injections).

Stable peak area ratios were obtained over the 10,000 injections, indicating robust analytical performance of the 6470 Triple Quadrupole MS system.

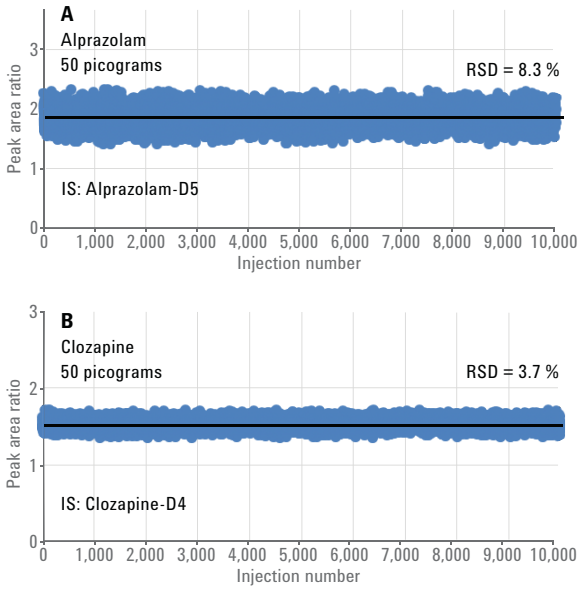


Figure 1. Peak area ratios for alprazolam (A) and clozapine (B) over 10,000 injections.

Figure 2 shows the overlaid MRM chromatograms for alprazolam (A) and clozapine (B) at the beginning and end of the 10,000 sample injections. Figure 2 shows that peak shape and area (height) for alprazolam and clozapine remain unchanged after 10,000 injections.

## Conclusion

The innovative ion transfer optics design of the Agilent 6470 Triple Quadrupole Mass Spectrometer minimizes the adverse effects of sample accumulation to provide a robust analytical platform for the ultra high throughput analysis of target drug candidates in precipitated plasma samples.

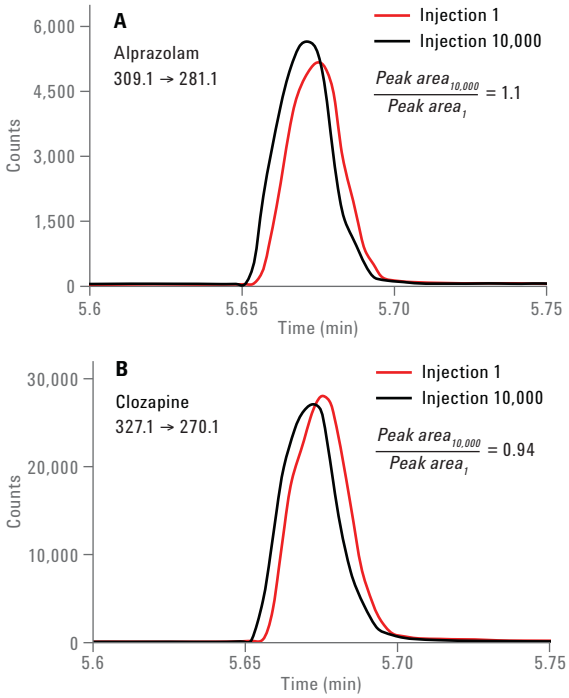


Figure 2. Overlaid MRM chromatograms for alprazolam (A) and clozapine (B) at the beginning and end of the 10,000 injections.

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