[TECHNOLOGY BRIEF]

Quantitative Analysis of Alprazolam in Rat Plasma Using ACQUITY UPLC Coupled with Xevo G2 QTof

GOAL

To demonstrate the quantitative analysis of alprazolam in rat plasma with four orders of magnitude linearity using ACQUITY UPLC® coupled with Xevo™ G2 QTof.

BACKGROUND

In drug discovery and metabolism studies there is a requirement to carry out both metabolite identification and quantitation experiments. These are typically achieved using separate workflows on tandem quadrupole instruments operated in the multiple reaction monitoring (MRM) mode for quantitative studies and full scan, exact mass instruments such as quadrupole time of flight (QTof) for metabolite identification. The goal of many laboratories is to streamline their studies into a single workflow that encompasses both quantitative and qualitative experiments. The QuanTof technology incorporated in the Xevo G2 QTof allows sensitive, exact mass quantitative and qualitative experiments to be carried out simultaneously.

Alprazolam is a small drug molecule that is primarily used to treat moderate to severe anxiety disorders and panic attacks, and is used as an adjunctive treatment for anxiety associated with moderate depression. In this technical brief, we present a study using ACQUITY UPLC coupled with Xevo G2 QTof to quantify alprazolam in rat plasma.

Xevo G2 QTof has the ability to acquire both quantitative and qualitative data on the same instrument, increasing laboratory productivity significantly.

Figure 1. Xevo G2 QTof System.

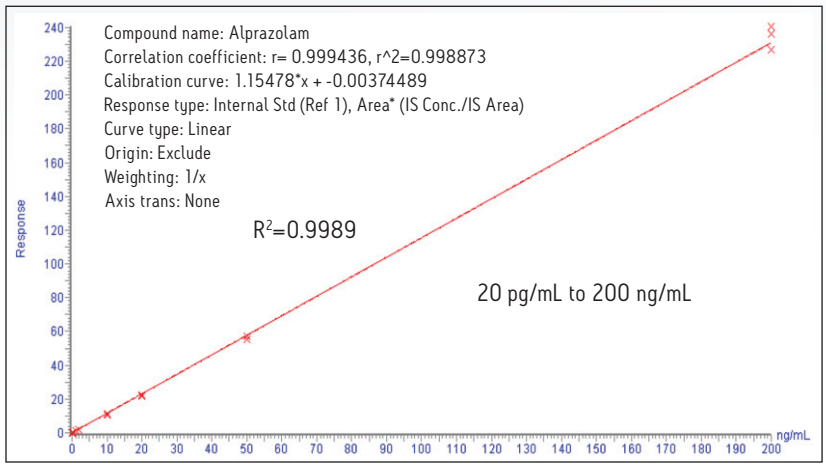

Figure 2 Alprazolam quantitation curve from concentration of 20 pg/mL to 200 ng/mL prepared in blank plasma, with four orders of magnitude linearity observed.

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THE SOLUTION

A quantitation curve for alprazolam was prepared in blank rat plasma at concentration levels from 20 pg/mL to 200 ng/mL. Alprazolam D5 was used as the internal standard. 10 µL of the sample was injected (with triplicate injections) for analysis. Experiments were performed using a Waters® ACQUITY UPLC System coupled with Xevo G2 QTof. An ACQUITY UPLC BEH C_{18}, 2.1 X 50 mm 1.7 µm Column was used with the temperature maintained at 45 °C. The optimized mobile phases were A: water, 1% NH_4OH and B: methanol 1% NH_4OH. A linear gradient of 5% B to 95% B in 1.5 min was used at a flow rate of 600 µL/min, with a total runtime of 2.5 min (including column re-equilibration time). Xevo G2 QTof was operated in the positive ion mode.

Figure 2 shows the alprazolam quantitation curve from concentration of 20 pg/mL to 200 ng/mL prepared in blank plasma, with four orders of magnitude linearity observed. Xevo G2 QTof provides excellent quantitative accuracy and sensitivity, with a high level of specificity by using accurate mass chromatograms with narrow mass windows. Figure 3 shows the extracted mass chromatogram (XIC) for the lowest concentration level of 20 pg/mL alprazolam, within a 20 mDa window at *m/z* 309.0907.

This demonstrates the ability of Xevo G2 QTof to perform sensitive, quantitative experiments over four orders of linear dynamic range. The low end and high end QC samples are at the concentration level of 50 pg/mL and 100 ng/mL respectively. The precision (% CV) for them are 1.08 and 0.99. And the accuracies (% Dev) are -6.6, -5.5, -7.5; and 5.3, 3.9, 5.9 (with triplicate injections).

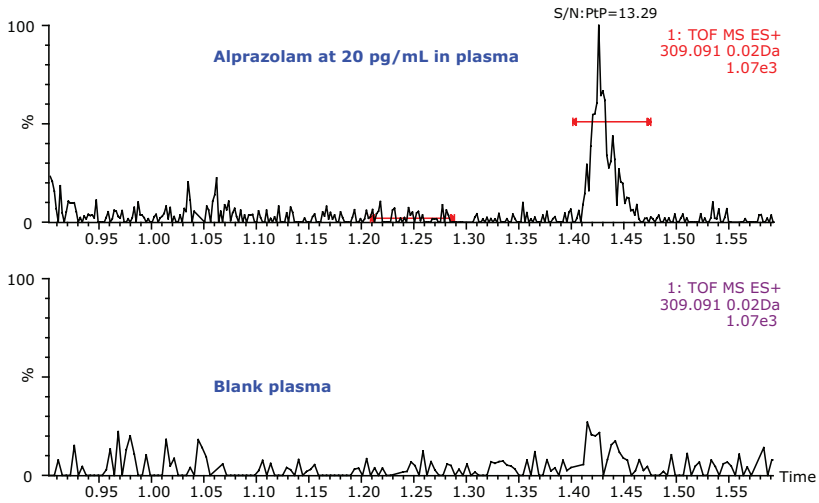

Figure 3. Alprazolam (top, at 20 pg/mL) and blank plasma (bottom) extracted ion chromatograms (XIC).

SUMMARY

This technical brief demonstrates the ability to perform quantitative analysis of alprazolam in rat plasma by using ACQUITY UPLC coupled with Xevo G2 QTof. Four orders of magnitude linearity were observed from concentration levels of 20 pg/mL to 200 ng/mL for aprazolam. The ability of Xevo G2 QTof to acquire both quantitative and qualitative data on the same instrument increases laboratory productivity significantly.

Reference

1. Fast and Sensitive *In Vitro* Metabolism Study of Rate and Routes of Clearance for Ritonavir using UPLC coupled with the Xevo QTof MS System, Castro-Perez, J., Yu, K., Shockcor, J., Shion, H., Marsden-Edwards, E., Goshawk, J., Yamada, Y., and Oshikata, M. Waters 2009 application note 720003025en.

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