High Sensitivity Quantitative Analysis of Buspirone in Rat Plasma Using the High Resolution Accurate Mass Agilent 6550 iFunnel Q-TOF LC/MS System

Application Note

Authors

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Abstract

This application note demonstrates the utility of the Agilent 6550 iFunnel Q-TOF LC/MS for the high sensitivity quantitative analysis of buspirone in rat plasma with low pg/mL lower limit of quantitation (LLOQ) and greater than three orders of linear dynamic range. A simple protein precipitation sample preparation method was used to extract buspirone and the bioanalytical performance with regard to the sensitivity, linearity, assay precision, accuracy, and robustness is demonstrated. The simultaneous acquisition of targeted and nontargeted data provides additional information which can be used for metabolite identification to increase laboratory productivity and workflow efficiency.

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Introduction

Bioanalytical information is essential for the evaluation of pharmacokinetic parameters, safety assessment, and the interpretation of efficacy and toxicological observations in drug discovery and development. Triple quadrupole LC/MS instruments using multiple-reaction-monitoring (MRM) have been the workhorses for the targeted quantitation of small molecule drugs and their metabolites in plasma and other biological matrices. However, as this platform is optimized for high-sensitivity targeted quantitation, there is a lack of information for nontargeted components and no possibility of retrospective data mining in order to search for compounds such as metabolites and endogenous interferences. With recent advances in high resolution accurate mass (HRAM) quadrupole time-of-flight (Q-TOF) mass spectrometers, there is a growing interest in the utility of HRAM LC/MS for bioanalysis.[1-2] This technique offers a number of advantages: it allows for rapid method development with no MRM optimization required; it provides quantitation for compounds of interest, and allows re-interrogation of data to obtain additional information, such as drug related metabolites, biomarkers, and endogenous components. It provides insights for troubleshooting potential assay interference, without reanalyzing samples.

This application note evaluates the utility of the 6550 iFunnel Q-TOF LC/MS System for the quantitation of buspirone in rat plasma. A simple protein precipitation sample preparation method was used to extract buspirone and the internal standard from rat plasma samples. The bioanalytical performance with regard to the sensitivity, linearity, assay precision, accuracy, and robustness is demonstrated.

Experimental

Sample preparation

Plasma calibration standards (12.5 pg/mL – 50 ng/mL) and QCs (100 pg/mL and 25 ng/mL) were prepared by spiking appropriate amounts of buspirone in blank rat plasma. Each plasma calibration standard (100 µL) and QC (100 µL) was extracted using acetonitrile containing 50 pg/mL of verapamil (internal standard). The supernatant was then dried with N_2 and reconstituted with 100 µL of 10 % acetonitrile/water.

LC/MS conditions

Instruments used for the analysis included an Agilent 1290 Infinity LC System, comprised of a binary pump with an integrated degasser, a high-performance autosampler with a thermostat, and a thermostatted column compartment, and a 6550 iFunnel Q-TOF LC/MS System with a Dual Agilent Jet Stream source. Data acquisition was performed using an Agilent MassHunter Workstation (version B.04.01). Agilent MassHunter Quantitative Analysis software (version B.05.00) was used for data processing and generation of buspirone calibration curve. Table 1 shows the experimental conditions.

Table 1. LC/MS conditions.

LC conditions		
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)	
Mobile phase	A = 0.1 % formic acid in water, B = 0.1 % formic acid in acetonitrile	
Injection volume	10 µL	
Column temperature	50 °C	
Flow rate	0.4 mL/min	
Gradient	Time (min)	% B
	0.5	10
	2.5	50
	2.8	95
	3.5	95
	3.8	10
Post time	1 minute	
MS conditions		
Ion mode	Positive	
Drying gas	250 °C at 12 L/min	
Sheath gas	400 °C at 12 L/min	
Nebulizer	35 psi	
Capillary voltage	3,500 V	
Nozzle voltage	300 V	
Instrument mode	Extended dynamic range	
Mass range	100 – 1,000 *m/z*	
Acquisition rate	MS mode (3 Hz), targeted MS/MS mode (3 Hz)	
Collision energy	32 V (buspirone), 28 V (Verapamil)	
Reference ions	118.086255 and 922.009798	

Results and Discussion

Sensitivity and selectivity

The 6550 iFunnel Q-TOF LC/MS System, incorporating breakthrough iFunnel technology, provides a new level of sensitivity for the quantitation of buspirone in rat plasma. Figure 1 shows extracted ion chromatograms (EICs) of 12.5 pg/mL buspirone in rat plasma and that for the blank plasma. A ± 10 ppm mass extraction window was used at *m/z* of 386.2551 (A) and at the targeted MS/MS of 386.2551/122.0713 (B) respectively. Excellent signal-to-noise ratios (S/N > 71) were achieved for buspirone, with no detectable interference from the blank rat plasma.

Figure 2 shows the selectivity of buspirone in rat plasma as a function of the mass extraction window. It is evident that the narrow mass extraction window allows discrimination against other compounds present in the plasma sample. As the mass extraction window is reduced from ± 500 ppm to ± 10 ppm, the plasma endogenous components are eliminated. The best selectivity is achieved using a mass extraction window of ± 10 ppm.

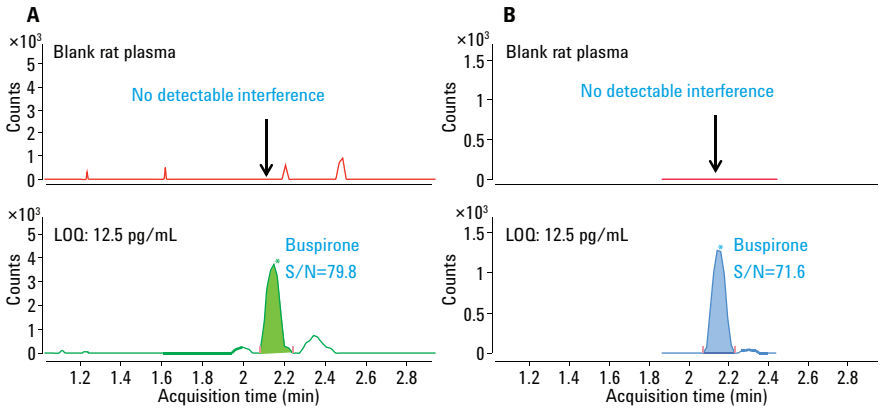

Figure 1. EICs of buspirone at lower limit of quantitation (12.5 pg/mL) and blank rat plasma, MS (A) and MS/MS (B).

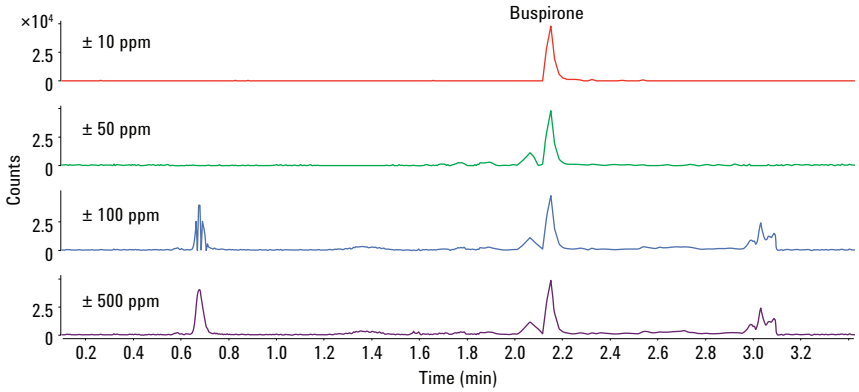

Figure 2. Selectivity of buspirone (250 pg/mL) in rat plasma sample as a function of mass extraction window.

Linearity, accuracy, precision, and robustness

Figure 3 shows a calibration curve of buspirone in rat plasma. A linear regression model was used with 1/x weighting. Excellent linearity over the concentration range of 12.5 pg/mL to 50.0 ng/mL was achieved with an R^2 of 0.998. The assay precision and accuracy were also evaluated for seven calibration standards, and low and high QCs. As shown in Table 2 the assay accuracies for the low (100 pg/mL) and high (25 ng/mL) QCs are 103 and 99.7, and the precision measurements (% CV) are 8.49 and 7.61 respectively, based on the triplicate results. Figure 4 demonstrates the robustness of the HRAM Q-TOF LC/MS method for buspirone quantitation in rat plasma. The peak area ratios of the analyte/IS are consistent over 100 consecutive analyses of 5 ng/mL buspirone in rat plasma extract. These results demonstrate the ability of 6550 iFunnel Q-TOF LC/MS System to perform sensitive, rugged, accurate, and precise quantitation of buspirone in rat plasma with regard to the bioanalytical acceptance criteria.[3]

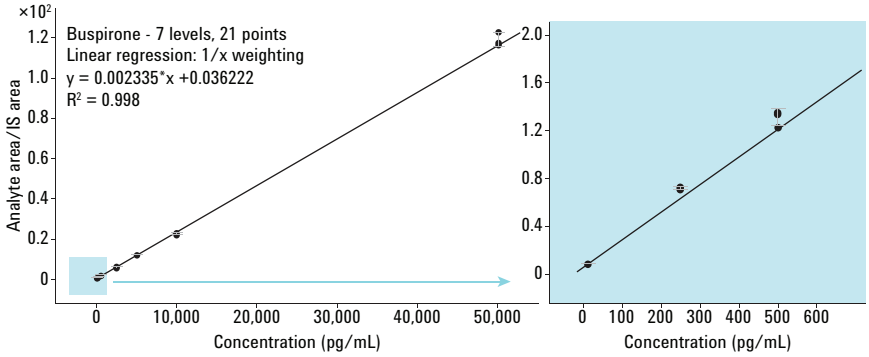

Figure 3. Buspirone calibration curve from 12.5 pg/mL to 50 ng/mL in rat plasma, with a zoom in view of buspirone plasma standards from concentrations of 12.5 – 500 pg/mL.

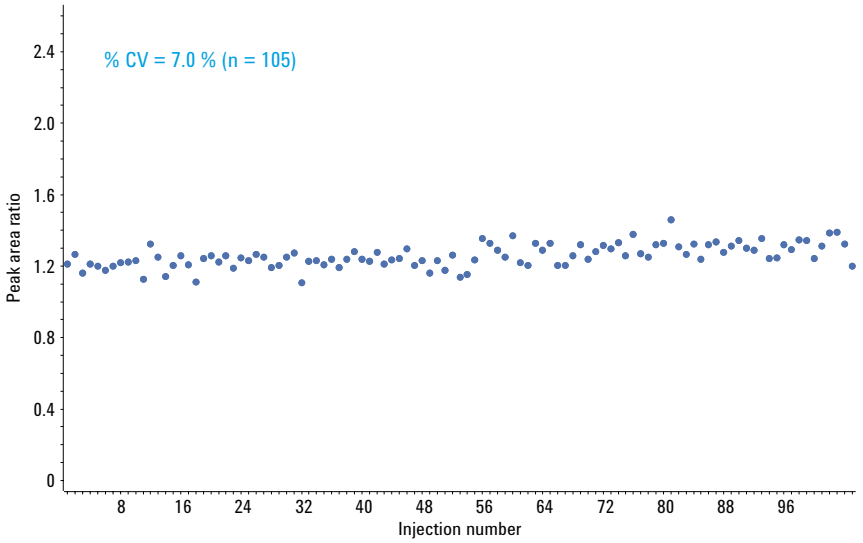

Figure 4. Reproducibility over 100 consecutive analyses of buspirone (5 ng/mL) in rat plasma extract.

Table 2. Assay accuracy and precision.

	Calibration standards							QCs	
Concentration (pg/mL)	12.5	250	500	2,500	5,000	10,000	50,000	100	25,000
Mean concentration (pg/mL) (n=3)	11.9	284	540	2,238	4,865	9,412	50,912	103	24,922
% Accuracy (n=3)	95.6	114	108	89.5	97.3	94.1	102	103	99.7
Standard deviation (n=3)	4.31	2.14	6.03	6.34	0.458	2.60	2.95	8.73	7.59
% CV (n=3)	4.51	1.88	5.59	7.08	0.471	2.76	2.90	8.49	7.61

Post acquisition data mining

One of the advantages of full scan HRAM Q-TOF MS is the ability to reprocess the data for additional information (data mining). Unlike targeted triple quadrupole multiple reaction monitoring (MRM) methods, the Q-TOF can simultaneously detect ions over a wide mass range and this data can be retrieved and interrogated at any time without re-analyzing samples. This is illustrated in Figure 5 and Figure 6, in which EICs from endogenous phospholipids and major oxidation metabolites of buspirone are shown.

Conclusions

This application note demonstrates the use of the Agilent 6550 iFunnel Q-TOF LC/MS System to effectively quantify small molecule drugs in a biological matrix. High-sensitivity analysis is significantly enhanced by using the 6550 Q-TOF LC/MS System with iFunnel technology to allow quantitation of buspirone in rat plasma with an LLOQ at 12.5 pg/mL and an assay linearity greater than 3 orders of magnitude. The bioanalytical performance is excellent in terms of the assay selectivity, linearity, precision and accuracy, and robustness. This approach of using high resolution accurate mass is especially beneficial in drug discovery bioanalysis, as it allows the use of generic LC/MS method development and eliminates time-consuming MRM optimization for each analyte. Furthermore, the simultaneous acquisition of targeted and nontargeted data provides additional information which can be used for metabolite identification. Thus, it has potential to increase laboratory productivity and workflow efficiency.

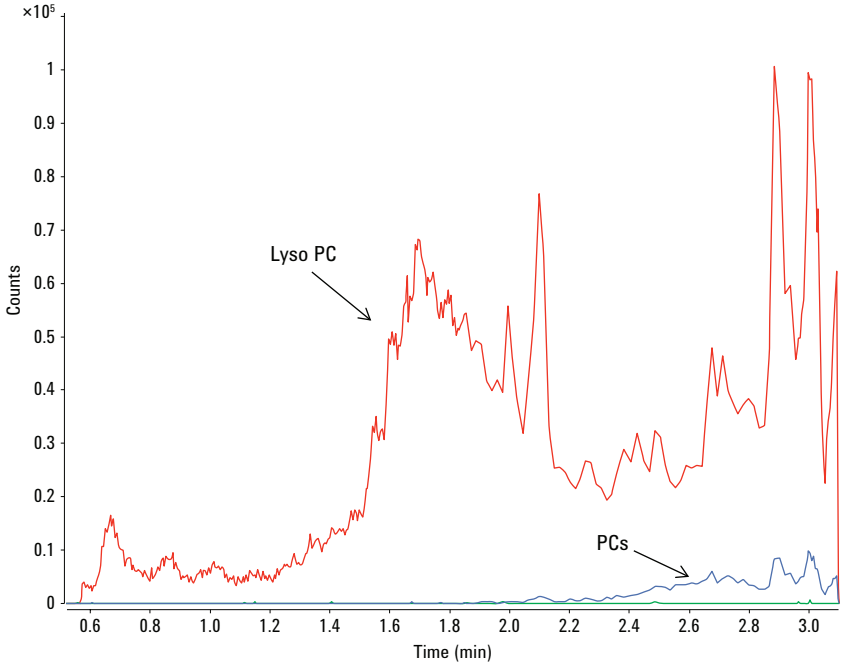

Figure 5. EICs of selected phospholipids in blank rat plasma. The red trace is lysophosphatidylcholine (lyso PC) (exact mass of $[M+H]^+$ = 496.3398), the blue trace is phosphatidylcholine (PC) C16:0/C18:2 (exact mass of $[M+H]^+$ = 760.5851), and the green trace is phosphatidylcholine (PC) C16:0/C18:1 (exact mass of $[M+H]^+$ = 758.5094).

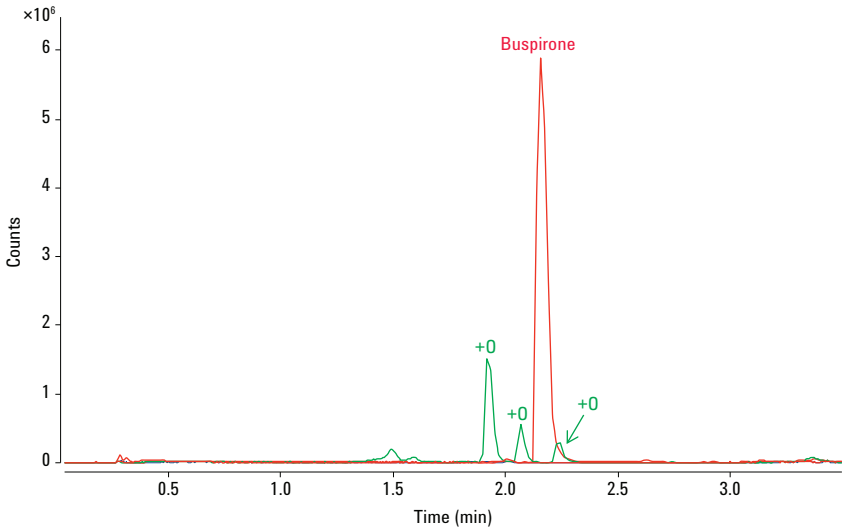

Figure 6. EICs of buspirone and major oxidation metabolites from a rat plasma spiked with an *in vitro* buspirone HLM incubation sample.

References

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