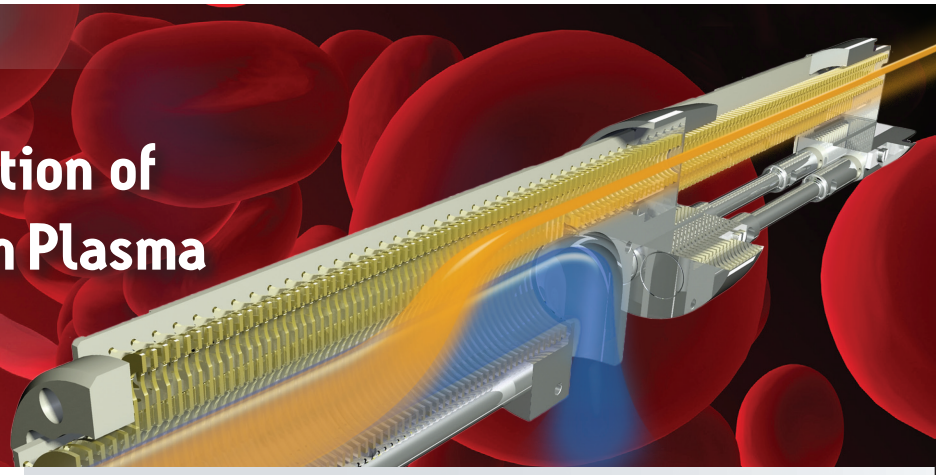
[TECHNOLOGY BRIEF]

Low Level Quantification of Pharmaceuticals from Plasma Using SYNAPT G2-S



GOAL

To assess the sensitivity gains of the SYNAPT® G2-S for bioanalytical studies, and to demonstrate the impact of the StepWave™ Ion Guide and QuanTof™ Detector combined within the SYNAPT G2-S for exact mass quantitation.

BACKGROUND

A crucial phase of the drug discovery process is the quantitative analysis of candidate pharmaceuticals and their metabolites in biological fluids. The information generated is used to determine key pharmacokinetic parameters, such as clearance, half-life, Tmax, and bioavailability. This type of quantitative analysis of low exposure compounds is typically performed using a nominal mass instrument, such as a triple quadrupole mass spectrometer. Historically, this has primarily been a consequence of the sensitivity and selectivity of multiple reaction monitoring (MRM) methodology. Although triple quadrupole instrumentation has taken the early lead in this application area, significant progress has been achieved with innovations in accurate mass spectrometry, and sensitivity and specificity gains are continually being made across diverse platforms. This progress can be seen with the introduction of the SYNAPT G2-S, which incorporates StepWave source geometry to improve sensitivity. With this novel source design, sensitivity has increased by as much as 30x for full scan exact mass analysis.

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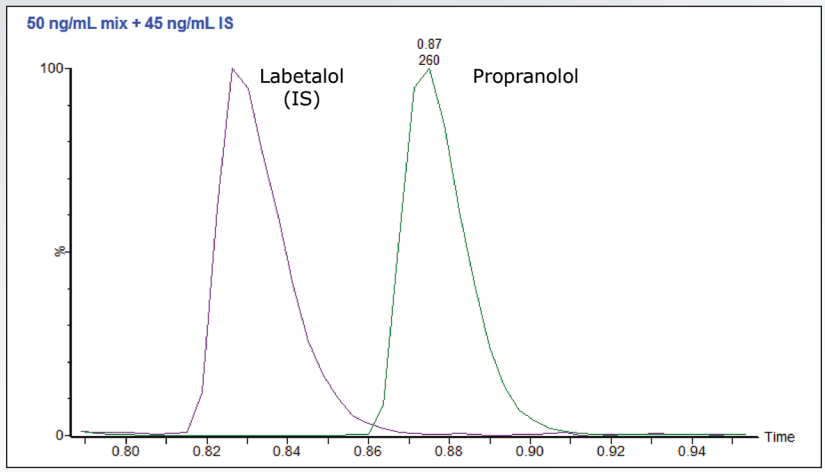

Figure 1. Extracted ion chromatogram of the analyte at 50 ng/mL and internal standard 45 ng/mL.

#	Type	Std. Conc	RT	Area	Response	Conc.	%Dev
1	Blank	45	0.83	6777.259	6777.259	46.142	2.5
2	Standard	45	0.83	6976.384	6976.384	47.498	5.6
3	Standard	45	0.83	6781.436	6781.436	46.107	2.6
4	Standard	45	0.83	6601.790	6601.790	44.947	-0.1
5	Standard	45	0.83	6468.758	6468.758	44.042	-2.1
6	Standard	45	0.83	6710.381	6710.381	45.687	1.5
7	Standard	45	0.83	6606.152	6606.152	44.977	-0.1
8	Standard	45	0.83	6611.368	6611.368	45.013	0.0
9	Standard	45	0.83	6505.438	6505.438	44.291	-1.6
10	Standard	45	0.83	6520.000	6520.000	44.390	-1.4
11	Standard	45	0.83	6313.585	6313.585	42.985	-4.5

Table 1. Reproducibility of injection for internal standard giving an %RSD of 2.77%.

In addition, QuanTof Technology, which is already well-established on the G2 Platform, provides a wide usable dynamic range and allows the collection of both qualitative and quantitative data in the same analysis with no compromise in sensitivity or data quality. This brings together traditionally separate analyses into a single platform by removing redundancy in analysis time, instrument use, sample preparation, and many other aspects that are often duplicated due to a multiple instrument workflow approach. In this technical brief we will explore the quantitative performance of the UPLC®/SYNAPT G2-S System.

THE SOLUTION

Propranolol was the substrate chosen for this analysis with labetalol serving as an internal standard. A serial dilution was performed and spiked into plasma at concentrations ranging from 500 ng/mL to 0.01 ng/mL. For each sample in the standard curve protein precipitation was performed by the addition of three volumes of acetonitrile containing 60 ng/mL IS; bringing the final concentrations to 125 ng/mL to 0.0025 ng/mL for the standard curve and 45 ng/mL for IS. The acceptable tolerance criteria for quantitation was set at < 20% concentration deviation on the lowest standards and < 15% for the remainder of the curve. An injection of 3 µL was then performed and standard quantification curves were built using the QuanLynx™ Application Manager. An ACQUITY UPLC® HSS T3 C_{18} Column (2.1 x 50 mm, 1.8 µM) was used and the temperature maintained at 40 °C. The mobile phase consisted of A: water + 0.1% formic acid (v/v) and B: acetonitrile + 0.1% formic acid (v/v) with an initial hold at 95% A for 15 sec, and a subsequent linear gradient to 100% B at 1.45 min.

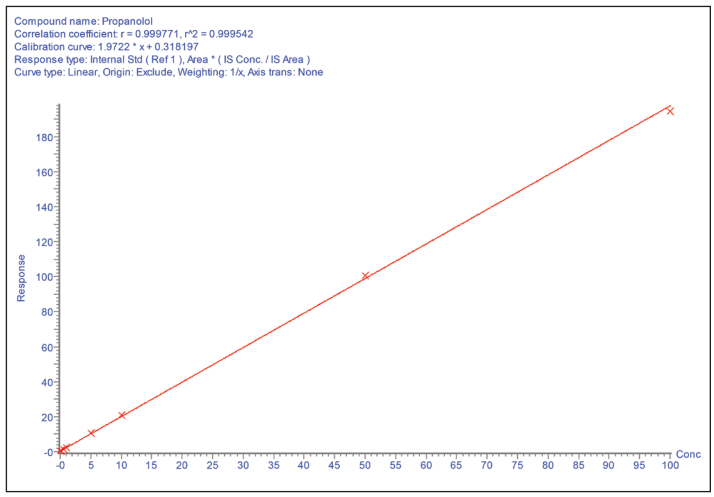

Figure 2. Standard curve showing linearity from 7.5 fg to 75.0 pg on column.

Figure 1 shows an extracted mass chromatogram of the analyte and internal standard from the 50 ng/mL sample. This shows both compounds are baseline resolved with peak widths of 2.4 sec at 5%. The standard curve for propranolol is shown in Figure 2, covering a linear quantitative dynamic range from 0.01 ng/mL (7.5 fg on column) to 100 ng/mL with R^2 0.9995.

In order to demonstrate reproducibility of the injections, Table 1 lists the internal standard response across all samples; an RSD of 2.77% was observed over 12 injections.

SUMMARY

In this technology brief, we have shown the applicability of exact instrumentation to routine low level quantitation, in time frames that are consistent with current methods. We have previously shown that comprehensive metabolite identification is accessible using the same methods and samples.[1] The combination of these two analyses on the same platform, in the same analytical run allows consistent, quantitative and efficient analysis using a comprehensive metabolite identification solution that can be applied throughout the drug discovery process.

Reference

1. Comprehensive Sub-2 Minute Metabolite Identification and Characterization Using Xevo G2 QTof and MS^E, Waters technology brief 720003837en.

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