Application of a Slotted Bandpass Ion Guide to Increase Robustness in Tandem Quadrupole LC-MS/MS Bioanalysis
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INTRODUCTION

Tandem quadrupole LC-MS/MS has become the technology of choice for quantitative bioanalysis [1], enabling drug quantification in biofluids in the low pg/mL range [2]. The selectivity, specificity and sensitivity of modern mass spectrometers has allowed sample preparation to be significantly simplified, with protein precipitation/removal being the preferred approach [3,4]. However, this results in an increased amount of matrix material entering the MS source. The high mass components of which can, if not eliminated, accumulate on the MS1 quadrupole, causing charging which reduces signal response and assay sensitivity. Restoring MS response often requires breaking the vacuum and cleaning of the quadrupole.

Here we describe a new resolving ion guide to mitigate MS1 quadrupole contamination. The new ion guide combines resolving DC and an axial field to create a bandpass filter (Fig. 1), protecting downstream ion optics from contamination by preventing unwanted high mass ion transmission into the MS1 quadrupole. Filtering out high *m/z* ions from biological matrices prevents quadrupole charging and the associated loss in sensitivity caused by the accumulation of these ions on the MS1 quadrupole rods.

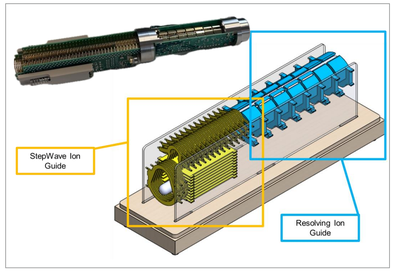

Figure 1. StepWave™ Resolving Ion Guide

STUDY DESIGN

Rat plasma samples (100 µL) were protein precipitated with cold acetonitrile (200 µL). Samples were vortex mixed, refrigerated (-20 °C) for 1 h, then centrifuged at 14,000 g for 5 mins. Supernatant layer was removed and diluted with water (1:1) containing, nefazodone, verapamil, amodiaquine, propafenone, chlorpromazine, dextromethorphan, nifedipine, phenacetin, gefitinib, O-desmethyl metabolite of gefitinib, ibuprofen, d6-gefitinib and ^{13}C3-ibuprofen to give a final concentration of 5 ng/mL.

Plasma extracts (2 µL) were analysed using an ACQUITY™ Premier UPLC™ System connected to a Xevo™ TQ Absolute XR Tandem Quadrupole Mass Spectrometer. Chromatographic separations were performed on a 2.1 x 50 mm ACQUITY Premier HSS T3, 1.7 µm column (60 °C) and eluted with a linear 5-95% B reversed – phase gradient over 1 minute at 600 µL/min, where mobile phase A = aqueous 0.1% formic acid and B = 95:5 acetonitrile / water containing 0.1% formic acid.

Analytes were monitored in MRM mode using ESI +ve / -ve switching using the following transitions: nefazodone (470.26 => 83.00), verapamil (455.31 => 165.09), amodiaquine (356.14 => 283.16), propafenone (342.19 => 116.0), chlorpromazine (319.08 => 85.99), dextromethorphan (272.19 => 171.08), nifedipine (347.11 => 315.19), phenacetin (180.04 => 109.96), gefitinib (447.18 => 128.1) gefitinib transition 2 (447.18 => 100.2) d6-gefitinib (453.16 => 134.2), ibuprofen (205.0 => 161.0).

Ethics Statement

This study is compliant with the corresponding projects APAFIS #32640-2021101419119467 v5. This project was reviewed by the Evotec Management and Ethical Committee (identified as CEPAL: CE 029) and compliant with national (UK) and EU regulations.

PLASMA ROBUSTNESS RESULTS

The plasma extracts were analyzed in batches of 1,000 samples with the sampling cone cleaned between each batch. A total of 10 batches were collected with no break in acquisition or cleaning of the ion guide or Q1 regions of the MS. The data below illustrates the reproducibility of the raw peak area responses for over 10,000 injections of protein precipitated plasma for nefazodone, dextromethorphan, nifedipine and propafenone (Fig. 2). The coefficients of variation (%CV) of the raw peak areas ranged from 13.1 – 20.4% for the analytes in the study and are listed in Table 1.

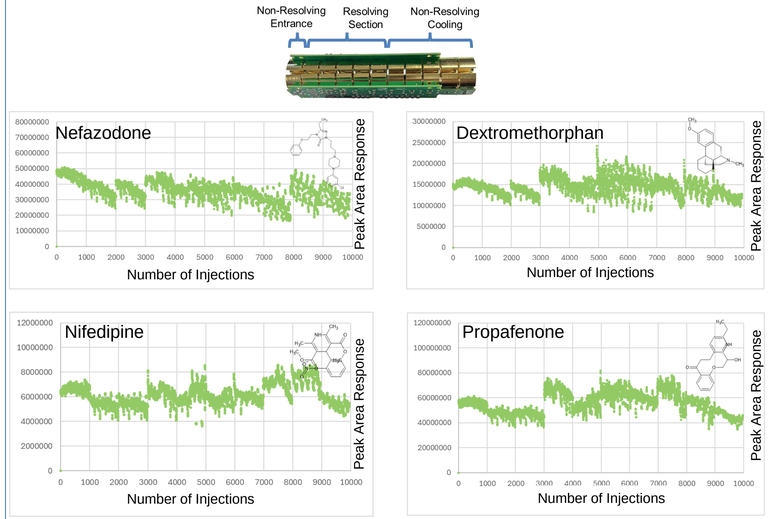

Figure 2. Variation in peak area responses for nefazodone, dextromethorphan, nifedipine, and propafenone

Analyte	Peak Area %CV
Nefazodone	20.4
Verapamil	17.9
Amodiaquine	16.9
Propafenone	14.9
Chlorpromazine	18.6
Dextromethorphan	13.7
Nifedipine	13.1
Phenacitin	14.8
Ibuprofen	13.9

Table 1. Coefficient of variation for analytes over the course of a 10,000-injection study of protein precipitated rat plasma

GEFITINIB & METABOLITES

The ACQUITY Premier – Xevo TQ Absolute XR LC-MS/MS system was used to quantify gefitinib and its major metabolites in rat plasma. The variation in peak area ratio of gefitinib and O-desmethyl metabolite to d6-gefitinib internal standard over the course of the 10,000-injection study are shown below (Fig. 3).

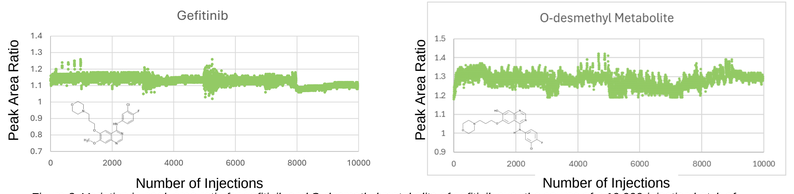

Figure 3. Variation in peak area ratio for gefitinib and O-desmethyl metabolite of gefitinib over the course of a 10,000-injection batch of protein precipitated rat plasma.

RAT GEFITINIB STUDY

The UHPLC-MS/MS system was employed for the analysis of rat plasma and urine from the subcutaneous administration of gefitinib to male Wistar rats at 10 mg/kg. Blood samples were collected 0-24 h post dose via tail bleed, urine samples were collected 0-1, 1-3, 3-8 and 8-24 h post dose. The plasma samples were prepared by protein precipitation with acetonitrile containing d6-gefitinib as the internal standard. The derived calibration and pharmacokinetic data are given below (Fig. 4).

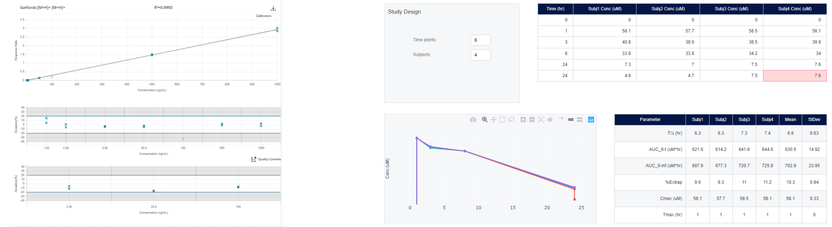

Figure 4. Assay calibration data and pharmacokinetics for gefitinib following the subcutaneous administration at 10 mg/kg to male Wistar rats.

CONCLUSION

- **Wide bandpass slotted ion guide for maximum robustness in high throughput bioanalysis studies.**
- **Long term instrument stability for large batch analysis and maximum uptime demonstrated by more than 10,000 LC-MS/MS analyses of protein precipitated plasma with no loss in signal response.**
- **Simple user maintenance, simple source and cone cleaning with no need to "break instrument vacuum".**

References

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