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應用手冊

Evaluation of the Quattro *micro* for the Quantification of Diazepam in Human Plasma

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The robustness of the Quattro *micro* Tandem Quadrupole System has been evaluated with diazepam in protein-precipitated human plasma.

Introduction

In recent years triple quadrupole mass spectrometers have become the instruments of choice for quantitative analysis of drug-related compounds in the pharmaceutical industry. The sensitivity of atmospheric pressure ionization (API) coupled with the specificity and speed of analysis that can be achieved with triple quadrupole mass spectrometers operating in multiple reaction monitoring (MRM) mode make LC-MS/MS the method of choice for quantitative applications.

The Quattro *micro* is a benchtop tandem quadrupole incorporating a Z SPRAY atmospheric pressure ionization source with a vertically mounted probe. The instrument has been designed as a compact, high performance instrument for optimized high throughput quantification. Experiments have been designed to evaluate the robustness of this interface for the analysis of samples derived from in vivo drug testing.

The robustness of the system has been evaluated with Diazepam in protein-precipitated human plasma.

Experimental

The proteins from a plasma sample were precipitated by the addition of an equal volume of acetonitrile. The plasma sample was then centrifuged for 5 minutes and the supernatant decanted. Diazepam was spiked into the supernatant at a concentration of 200 pg/µL and 350 repeat injections of the sample were performed. The analytical conditions are described below.

LC Conditions

LC system:	Waters Alliance

Column:	Waters Symmetry C_{18}, 3.5 µm, 100 x 2.1 mm
Flow rate:	0.2 mL/min
Injection volume:	10 µL
Gradient:	Isocratic at 50:50 A = 0.1% Formic acid in water B = 0.1% Formic acid in acetonitrile

MS Conditions

Ion mode:	ESP+
Cone voltage:	40 V
Collision energy:	25 eV
Detection mode:	MRM (284.9 > 154.7)
Dwell:	0.5 seconds
Collision gas:	Argon

Results and Discussion

Figure 1 shows the plot of peak area against injection number for the integrated MRM chromatograms from the analysis of diazepam in protein-precipitated plasma. The average response for the detected peak was 54187 with a relative standard deviation of 2.1%. The quantitative analysis of the samples in biological matrices in pre-clinical and clinical studies requires both high throughput and good analytical accuracy and precision. No discernible reduction in response was observed over the analysis period despite all of the injected matrix being passed into the source. The RSD of 2.1% demonstrates excellent reproducibility over the 35-hour analysis period

which is easily within the requirements of precision for quantitative bioanalysis.

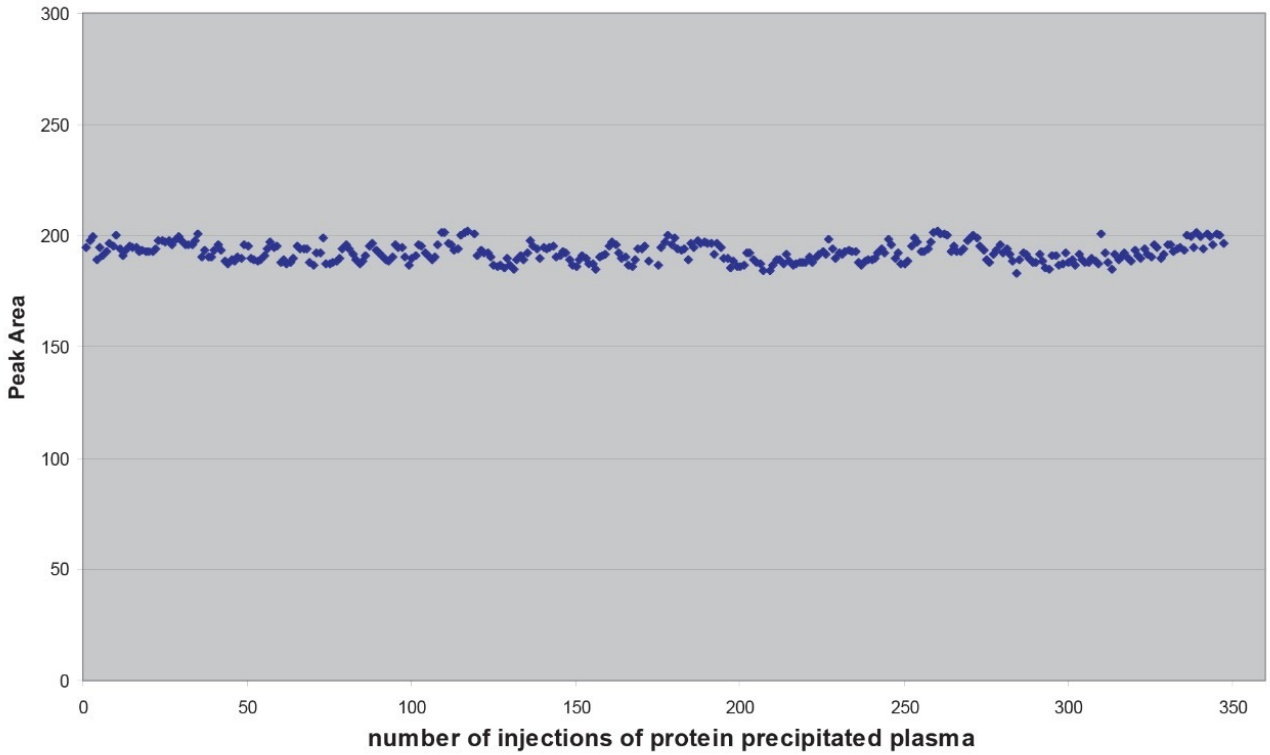

Figure 1. A plot of peak area for diazepam against number of injections.

A calibration series of diazepam in protein-precipitated plasma was prepared over the concentration range of 0.5 pg/µL to 5 ng/µL. These were injected in duplicate and peaks are plotted against concentration. A plot of peak area against concentration shows good linearity over the range 0.5 pg/µL to 5 ng/µL. The calibration line was plotted using a linear fit with 1/x weighting and resuted in a coefficient of determination of 0.9984 (Fig. 2.)

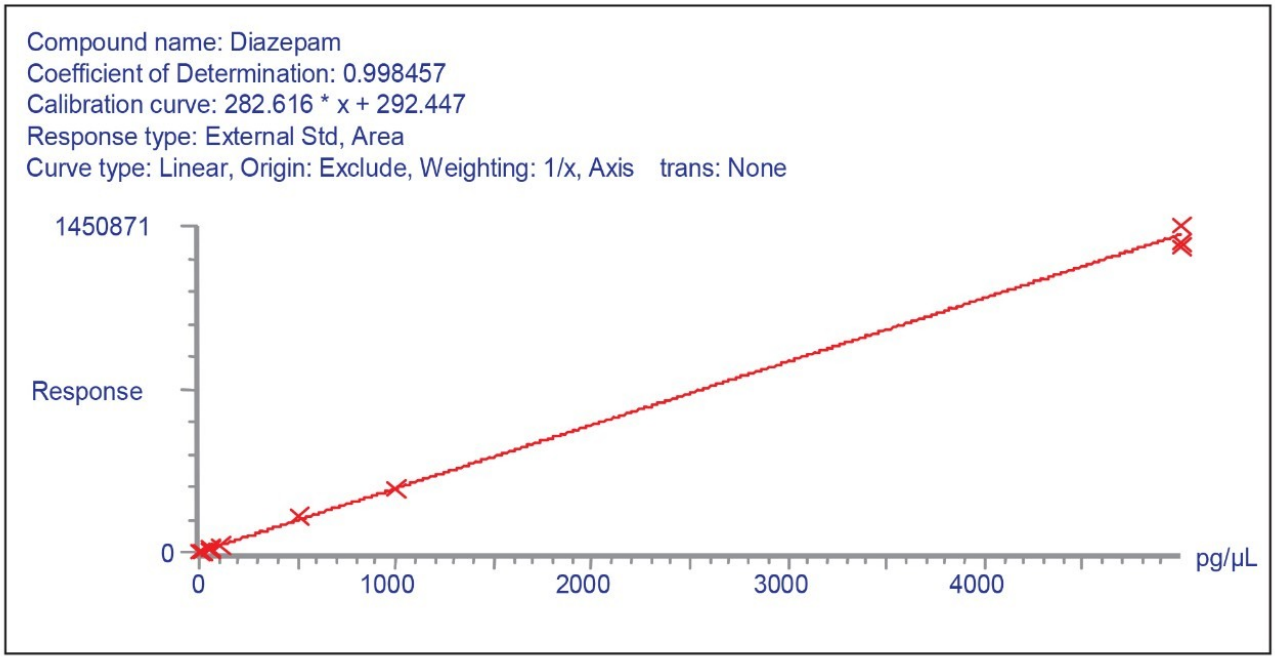

Figure 2. A calibration line for diazepam in protein precipitated plasma.

Conclusion

The Quattro *micro* tandem quadrupole system has been developed for quantitative LC-MS/MS. The instrument has been evaluated by analyzing a diazepam in protein-precipitated human plasma over a period of 35 hours (350 injections) in order to show its robustness and tolerance to protein-precipitated plasma and the linear increase in response with concentration.

The results showed that with over 350 injections of the Diazepam the sensitivity was maintained with a relative standard deviation of 2.1%. The calibration line resulted in a linear plot over the range 0.5 pg/µL to 5 ng/µL (Figure 2) with a correlation coefficient of 0.9984 demonstrating that the Quattro micro shows good linearity over 4 orders of magnitude.

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