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Sensitive, robust quantitative analysis of a mixture of drug candidates in plasma using a TSQ Altis triple quadrupole mass spectrometer

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Goal

To develop a sensitive, robust, reproducible LC-MS/MS assay for determination and quantitation of a mixture of compounds of pharmaceutical interest in human plasma and plasma from preclinical animal species.

Introduction

Small molecules represent a significant proportion of drug discovery and development in the search for new chemical entities, in addition to the extensive work involved in the regulatory filings of generics. Targeted quantitation assays are a critical part of an optimal workflow, which is required to successfully develop a small molecule drug. These targeted quantitation analyses must be done in biological matrices, which can often create analytical challenges. In this study, we report the development of a sensitive, robust, reliable, and reproducible LC-MS/MS assay for multiple drug standards in rat plasma.

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Experimental

Sample preparation

Crashed plasma stock solutions were prepared using an acetonitrile (ACN) crash at a ratio of 3:1, ACN to plasma. The resulting solution was centrifuged at 10,000 rpm for 10 minutes. The supernatant was removed and added to an equivalent volume of water to make the final crashed plasma stock solution. Stock solutions of each standard compound at 1 mg/mL were diluted in a pooled mix in the crashed plasma stock to concentration ranges of 1 pg/mL to 25,000 pg/mL and 10 pg/mL to 100,000 pg/mL. Isotopically labeled internal standards were added to each calibration level to produce a final internal standard concentration of 0.5 ng/mL. All reagents were obtained from Cerilliant Corporation, Round Rock, Texas, at 1 mg/mL in methanol.

Liquid chromatography

Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Horizon HPLC system. The column used was a Thermo Scientific™ Hypersil GOLD™ aQ C18 Polar Endcapped LC column (100 × 2.1 mm, 1.9 µm particle size). Mobile phases A and B consisted of 10 mM ammonium formate in Fisher Chemical™ Optima™ grade water and 0.1% formic acid in Fisher Chemical™ Optima™ grade acetonitrile, respectively. The column temperature was 50 °C. The total run time was 3.5 minutes (Table 1).

Table 1. Chromatography gradient for analysis.

Time (min)	Flow Rate (mL/min)	%A	%B
0	0.6	95	5
0.4	0.6	95	5
0.5	0.6	65	35
1.5	0.6	64	36
1.6	0.6	55	45
2.2	0.6	53	47
2.3	0.6	5	95
2.95	0.6	5	95
2.995	0.6	95	5
3.5	0.6	95	5

Mass spectrometry

Mass spectrometry analysis was carried out on a Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer equipped with the Thermo Scientific™ OptaMax™ NG source housing. Tables 2 and 3 show mass spectrometer source and SRM parameters used in the experimental setup.

Table 2. Mass spectrometer set-up.

Parameter	Setting
Run Time	3.5 min
Ion Source	HESI
Spray Voltage	3500 V
Sheath Gas	40 Arb
Auxiliary Gas	15 Arb
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	350 °C
Vaporizer Temperature	325 °C
Experiment Type	SRM
Cycle Time	0.3 s
Chromatography Peak Width	6 s
Collision Gas Pressure	1.5 mTorr
Q1 Resolution	0.7 FWHM
Q3 Resolution	0.7 FWHM

Data analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software.

Table 3. SRM properties for experimental set-up.

Compound Name	Start Time (min)	End Time (min)	Polarity	Precursor *m/z*	Product *m/z*	Collision Energy (V)	RF Lens
Desomorphine	0.760	1.060	Positive	272.062	215.054	26	69
Desmethyldoxepin	1.230	1.530	Positive	266.062	107.000	23	56
Flecainide	1.310	1.610	Positive	415.050	398.054	24	84
Midazolam	1.410	1.710	Positive	326.012	291.054	28	87
Imipramine	1.660	1.960	Positive	281.462	86.054	17	48
Amitriptyline	1.800	2.100	Positive	278.075	233.111	18	53
Fluoxetine	1.890	2.190	Positive	310.362	43.889	11	39
Diazepam	2.230	2.530	Positive	285.012	193.071	33	78

Results and discussion

Table 4 shows the lower limits of quantitation (LLOQ) obtained with the TSQ Altis MS for each of the drug candidates, which were significantly lower than those obtained from previous generation MS systems. In addition, significantly lower %CV values for the IS also implies increased robustness and reproducibility for the TSQ Altis MS. The representative chromatogram of QC 2 at 300 pg/mL is show in Figure 1. Further details on linearity and reproducibility of the QCs are shown in Table 5.

Table 4. Limits of quantitation for the drug candidates in plasma and %CV (n=3) for the internal standards.

Compound	LOQ (pg/mL)	IS %CV
Desomorphine	5	3.5
Desmethyldoxepin	2.5	3.5
Flecainide	1	3.5
Midazolam	2.5	4.4
Imipramine	2.5	4.4
Amitriptyline	2.5	4.4
Fluoxetine	5	5.1
Diazepam	2.5	3.4

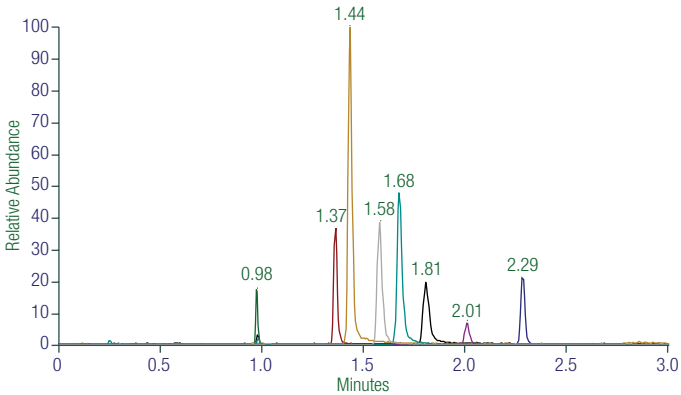

Figure 1. Representative chromatogram of QC 2 – 300 pg/mL.

Table 5. Linearity and reproducibility data for four QC points per calibration curve.

Compound	QC 1 %CV 30 pg/mL	QC 2 %CV 300 pg/mL	QC 3 %CV 3000 pg/mL	QC 4 %CV 15,000 pg/mL	R^2 Linear Fit
Desomorphine	7.93	4.72	3.47	1.15	0.9945
Desmethyldoxepin	5.28	1.55	0.67	1.01	0.9904
Flecainide	4.20	4.88	2.46	2.97	0.9924
Midazolam	2.96	1.52	1.71	2.77	0.9917
Imipramine	2.50	1.26	0.38	1.24	0.9913
Amitriptyline	7.04	3.16	0.68	0.83	0.9908
Fluoxetine	3.15	2.80	2.03	2.87	0.9901
Diazepam	5.77	3.15	0.53	2.69	0.9927

Conclusion

The method referenced in this application note shows excellent linearity and reproducibility over the dynamic range of the assay. This method demonstrates that the TSQ Altis MS provides the sensitivity and reproducibility required in the analysis of pharmaceutical compounds.

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