SHIMADZU

MP-173

Probe ESI Screening and Quantification for Drugs in Whole Blood Combined with Quadrupole Time-of-Flight Mass Spectrometer

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1. Introduction

LC-QTOF-MS based drug screening analysis is becoming a gold standard in forensic toxicology, because it allows for the simultaneous determination of drugs in a single run. One of the major point of concern is the substantial ineffectiveness of current toxicological sample pretreatment and screening methods to identify the compounds in biological samples. The recent increase in the usage of drugs highlighted the need for fast and reliable identification of compounds with the simple sample preparation.

This work describes qualitative analysis and targeted quantitative analysis using DPiMS QT which is a unique probe ESI ionization technique. Additionally, DPiMS QT installed newly designed quadrupole time-of-flight (Q-TOF) mass spectrometer (Fig. 1) which achieves rapid polarity switching and prominent mass stability. This combination enables to conduct direct analysis and minimize the time from sample preparation to analysis.

Fig. 1 Appearance of Shimadzu Q-TOF and DPiMS QT

2. Sample Preparation and Analysis conditions

In this study, amoxapine, clozapine, nortriptyline, pentobarbital, quetiapine, risperidone and salicylic acid were selected as the test compounds which were spiked to a human whole blood as the concentration of 1 µg/mL. 10 µL of the whole blood was mixed with 90 µL of water, and the solution was diluted with 100 µL of isopropyl alcohol which consisted 1 µg/mL diazepam-d5 and 10 µg/mL phenobarbital-d5. Then, the mixtures were centrifuged, and 10 µL of the supernatant was dripped on the sample plate for probe ESI.

iDIA was used for qualitative analysis. This is a notable identification technique by a combination of the DPiMS QT and upgraded shimadzu Q-TOF. This acquisition obtained MS/MS spectra which set the precursor m/z window to a minimum. In this study, MS/MS spectra were acquired comprehensively with a precursor *m/z* window as 3 Da. Both positive and negative MS/MS spectra were acquired in a single run. Table 1 shows the parameters for qualitative analysis.

7 drugs spiked to whole blood and prepared 9 calibration samples set as 10, 25, 50, 75, 100, 250, 500, 750, 1000 ng/mL. Quantitative test was performed using TOF-MS analysis. Diazepam-d5 was used as internal standard for positive ions and phenobarbital-d5 was for negative ions. Table 2 shows the settings of quantitative analysis.

Table. 1 Analytical settings for qualification

System	: DPiMS QT and upgraded shimadzu Q-TOF		
Polarity	: Negative/Positive	Precursors of MS/MS	: 100-500 *m/z* window 3 Da
DL temp	: 250 ˚C	MS/MS	: 20-500
Heat block temp	: 50 ˚C	Collision energy ramp	: 10-50
Interface Voltage	: 3.5 kV (Positive) -2.5 kV (Negative)	Measurement Time TOF-MS	: Negative: 0.00-1.40 min Positive:1.50-2.90 min
TOF-MS	: *m/z* 100-500	Measurement Time MS/MS	: 0.2 min/group 7 groups for each polarity

Table2. Analytical settings for quantification

System	: DPiMS QT and upgraded shimadzu Q-TOF		
Polarity	: Positive/ Negative	Interface Voltage	: 3.5 kV (Positive) -2.5 kV (Negative)
DL temp	: 250 ˚C	TOF-MS	: *m/z* 100-500
Heat block temp	: 50 ˚C	Measurement Time TOF-MS	: Positive:0.00-0.50 min Negative: 0.60-1.10 min

3. Results

Qualification Analysis

Table 3 shows the mass errors for 7 drugs at the concentration of 1 µg/mL spiked in whole blood. The results indicate the good mass stability for both positive and negative mode. Fig. 2 shows the MS/MS spectra of 7 drugs in human whole blood (1 µg/mL) using *iDIA* (upper) and reference MS/MS spectra acquired by LC-ESI-Q-TOF-MS (lower). The fragment patterns from DPiMS QT was equivalent to the patterns by conventional LC-ESI-Q-TOF-MS. High mass accuracy and excellent MS/MS spectral patterns improved reliable qualification result for positive and negative ions within 2.9 min.

Table3. compound list and its mass errors from 1 µg/mL in whole blood

#	Compounds	Polarity	Formula	Theoretical *m/z*	Measured *m/z*	Mass error (ppm)
1	Amoxapine	+	C17H16ClN3O	314.1055	314.1050	-1.6
2	Clozapine	+	C18H19ClN4	327.1371	327.1364	-2.1
3	Nortriptyline	+	C19H21N	264.1747	264.1743	-1.5
4	Pentobarbital	-	C11H18N2O3	225.1245	225.1246	0.4
5	Quetiapine	+	C21H25N3O2S	384.1740	384.1732	-2.1
6	Risperidone	+	C23H27FN4O2	411.2191	411.2181	-2.4
7	Salicylic acid	-	C7H6O3	137.0244	137.0242	-1.5

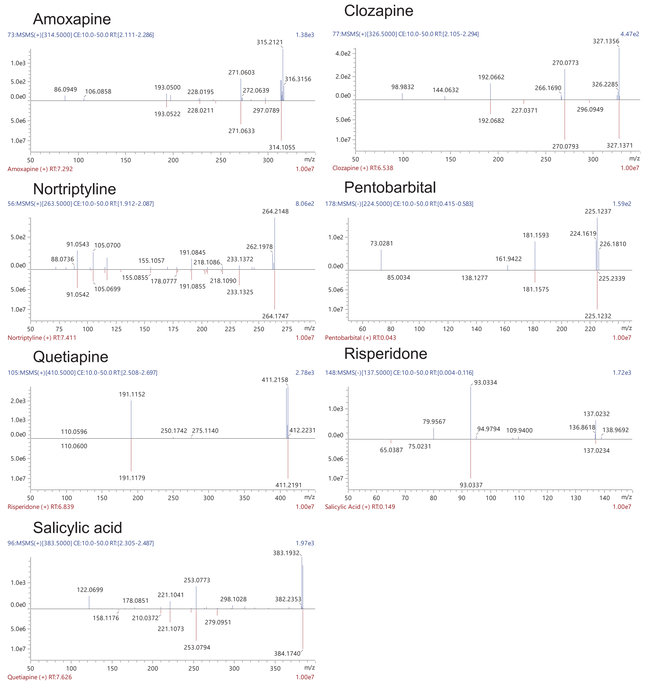

Fig2. MS/MS spectra of 7 drugs in human whole blood (1 µg/mL) using *iDIA* (upper) and MS/MS spectra acquired by LC-ESI-Q-TOF-MS (lower)

Quantification Analysis

Fig.3 illustrates the calibration linearities and calibration range for 7 drugs. Good linearities were obtained in the set calibration range. Quantification analysis was performed within 1.1 min including both positive and negative measurement. The combination of DPiMS QT and upgraded shimadzu Q-TOF provide the time savings compared with conventional LC based analysis and quantitative result immediately.

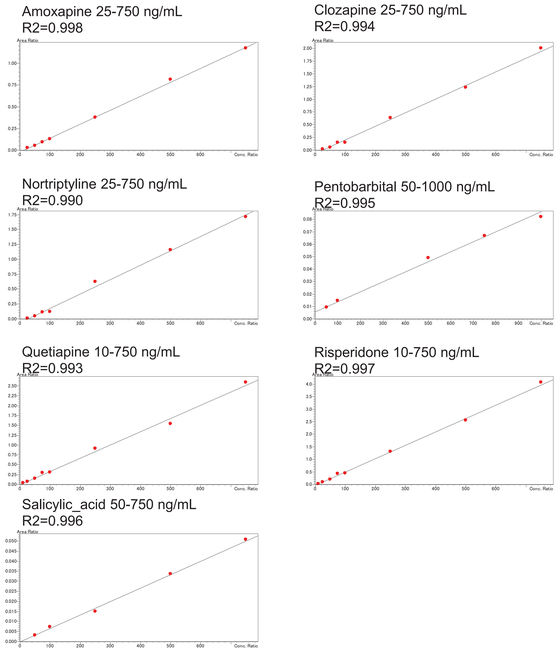

Fig3. Calibration curves for 7 drugs in whole blood

5. Conclusion

The *iDIA* measurement comprehensively acquiring the MS/MS spectra of all positive and negative ionized components in a sample was developed.

Qualification analysis provided high mass accuracy and excellent MS/MS spectral patterns which was equivalent to conventional LC-ESI-Q-TOFMS.

Good linearities were achieved for each calibration range.