Application Note

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Analytical Determination of Drugs in Serum Using the Ultivo Triple Quadrupole LC/MS

Figure 1. Ultivo Integrated into LC Stack.

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Abstract

This Application Note demonstrates a sensitive and precise method for analyzing eight drugs in serum on the Agilent Ultivo triple quadrupole LC/MS system. Ultivo was designed to save laboratory space, while maintaining the performance required for high-throughput analyses. Drug discovery in the small molecule space is an important field, and requires robust and reliable detection solutions to suit the needs of the field. The Ultivo LC/TQ delivers this in the smallest triple quadrupole mass spectrometer footprint on the market, and surpasses limits of competitor instruments over three times its size. Excellent method precision was achieved on the Ultivo system, with relative standard deviations (RSD%) of <10 % at the lowest level of quantitation. Little to no sample prep is require for the Ultivo triple quadrupole to achieve sensitive and precise detection of drugs.

Introduction

Directed quantitation of small molecule drug targets is an essential step in drug discovery, regulatory generic filings, and drug development. These analyses are often performed in serum, adding complexity to the detection of these targets. Frequently, lab space is a critical factor in decisions involving instrumentation, and can result in delays in the business pipeline when it is a limiting factor.

These eight drugs represent a diverse set of small molecules that are commonly used, and are good probes for drug discovery, drug detection, and drug quantification studies.

This Application Note demonstrates the analytically sensitive and precise quantification of up to eight drug compounds in serum using the novel Ultivo triple quad LC/MS.

Experimental

Reagents and chemicals

All reagents used in this Application Note were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral system with a LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid and ammonium acetate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Chemical standards were purchased from Cerilliant.

Sample preparation

All drugs were obtained from Cerilliant. Serum sample preparation: 250 µL human serum (obtained from Golden West Biologicals) was crashed with 500 µL of acetonitrile, vortexed for one minute, and centrifuged for four minutes at 10,000 rpm. Then, 500 µL of supernatant were transferred and diluted with 500 µL of water. The 11-point calibration range for all drugs was from 0.001 to 100 ng/mL. Internal standard stock solution was added directly to each matrix standard and calibrator to create a consistent concentration of 25 ng/mL across all sample types injected.

Instrumentation

- Agilent 1290 Infinity II UHPLC
  - 1290 Infinity high speed pump (G7120A)
  - 1290 Infinity II multisampler with cooler (G7167B)
  - 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent Ultivo triple quadrupole LC/MS system
  - Agilent Jet Stream (AJS) electrospray ionization source (ESI)

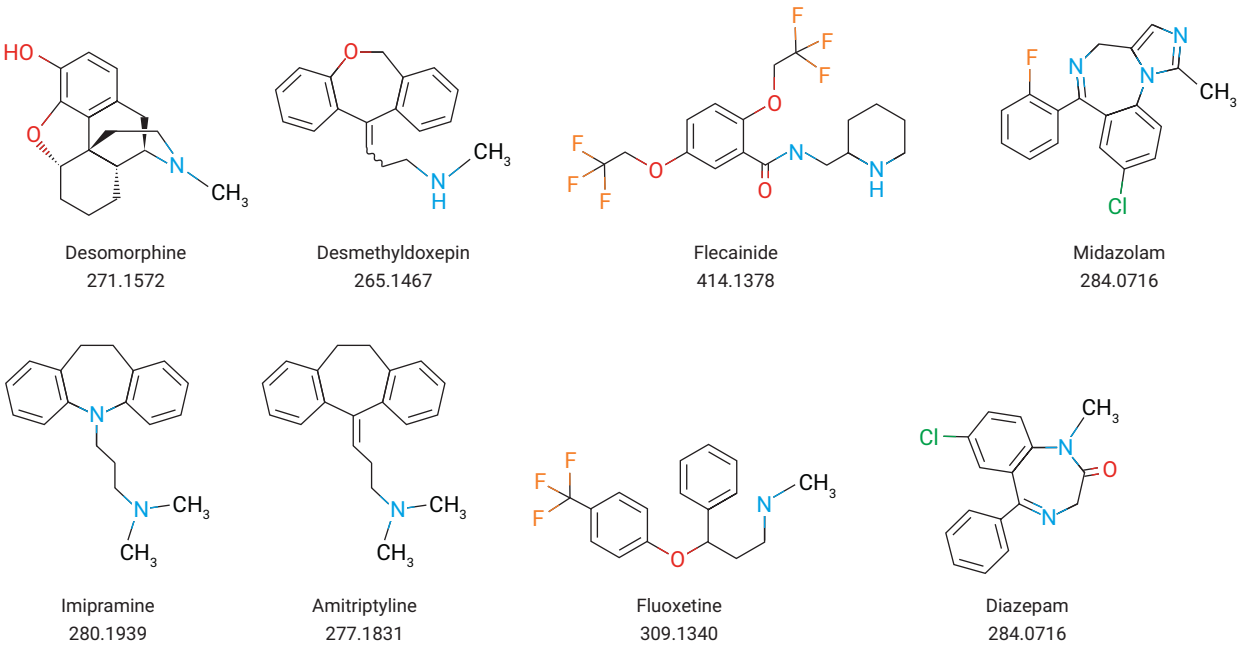

Figure 2. Structure and monoisotopic mass (g/mol) for all drugs studied.

Method

Table 1 summarizes the 1290 Infinity II UHPLC conditions. Table 2 summarizes theUltivo triple quadrupole parameters and Agilent Jet Stream ESI source parameters. Analysis was carried out with positive ionization and dynamic multiple reaction monitoring (dMRM). Data were evaluated using the Agilent MassHunter Quantitative Analysis Software B.09 with the Quant-My-Way feature.

Table 1. 1290 Infinity II UHPLC parameters.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C8, 2.1 × 100 mm, 1.8 µm (p/n 959758-906)
Column temperature	40 °C
Injection volume	2 µL
Mobile phase	A) Water + 0.1 % formic acid B) Acetonitrile + 0.1 % formic acid
Flow rate	0.6 mL/min
Gradient	Time %B 0 5 0.5 35 2 40 3 50 3.5 75 3.51 95 4 95 Post run time: 1.0 minutes Method cycle time: 5 minutes

Table 2. Ultivo triple quadrupole and AJS source parameters.

Parameter	Value
Drying gas temperature	200 °C
Drying gas flow	11 L/min
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Capillary voltage	2,750 V(+)
Nozzle voltage	0 V(+)
Cycle time	350 ms

Table 3. Transitions for drug detection in dMRM mode.

Compound	ISTD	Precursor ion (*m/z*)	Product ion (*m/z*)	Retention time (min)	Retention window (min)	Fragmentor (V)	CAV (V)	Collision energy (V)	Polarity
Flecainide	No	415.2	398.1	1.43	1	163	9	16	+
Flecainide	No	415.2	301	1.43	1	163	9	28	+
Midazolam	No	326.1	291.2	1.31	1	168	9	20	+
Midazolam	No	326.1	249.1	1.31	1	168	9	36	+
Fluoxetine	No	310.1	148.1	1.94	1	89	9	0	+
Fluoxetine	No	310.1	44.2	1.94	1	89	9	0	+
Diazepam	No	285.1	193.1	3.14	1	151	9	28	+
Diazepam	No	285.1	154	3.14	1	151	9	20	+
Amitriptyline D3	Yes	281.2	105	1.74	1	124	9	16	+
Amitriptyline D3	Yes	281.2	91	1.74	1	124	9	24	+
Imipramine	No	281.2	86	1.64	1	109	9	8	+
Imipramine	No	281.2	58.1	1.64	1	109	9	36	+
Amitriptyline	No	278.2	105	1.74	1	124	9	16	+
Amitriptyline	No	278.2	91	1.74	1	124	9	24	+
Desomorphine D3	Yes	275.2	167.1	0.94	1	153	9	36	+
Desomorphine D3	Yes	275.2	152.1	0.94	1	153	9	56	+
Desomorphine	No	272.2	167.1	0.94	1	163	9	32	+
Desomorphine	No	272.2	152.1	0.94	1	163	9	56	+
Desmethyldoxepin	No	266.2	235.1	1.37	1	114	9	4	+
Desmethyldoxepin	No	266.2	107.1	1.37	1	114	9	12	+

Results and discussion

Quant-My-Way is a set of tools designed to assist in showing only the capabilities of interest to create a streamlined version of MassHunter Quantitative Analysis software for each specific assay. The new ribbon can be customized with just the actions needed, and can hide as much or as little as the assay requires. In Figure 3, the drug quantitative analysis has been processed using a preconfigured user interface, and the peaks at 5 ppt of each compound can be seen. The top ions depicted are simplified from the original MassHunter Quantitative Analysis interface.

Detection limits

All eight drugs are well separated with baseline resolution for each peak within a four-minute window (Table 4). The limit of quantification (LOQ) is lower than 5 ppt for each drug in serum using the miniature Ultivo triple quad LC/MS, rivaling detection limits of much larger top-of-the-line systems.

Table 4. LOQs for drugs in this study.

Compound	LOQ	
	Ultivo (fg on column)	Ultivo (pg/mL)
Desomorphine	17.3	1.73
Desmethyldoxepin	27.8	2.78
Flecainide	11.7	1.17
Midazolam	40.0	4.0
Imipramine	6.57	0.66
Amitriptyline	14.2	1.42
Fluoxetine	15.6	1.56
Diazepam	18.4	1.84

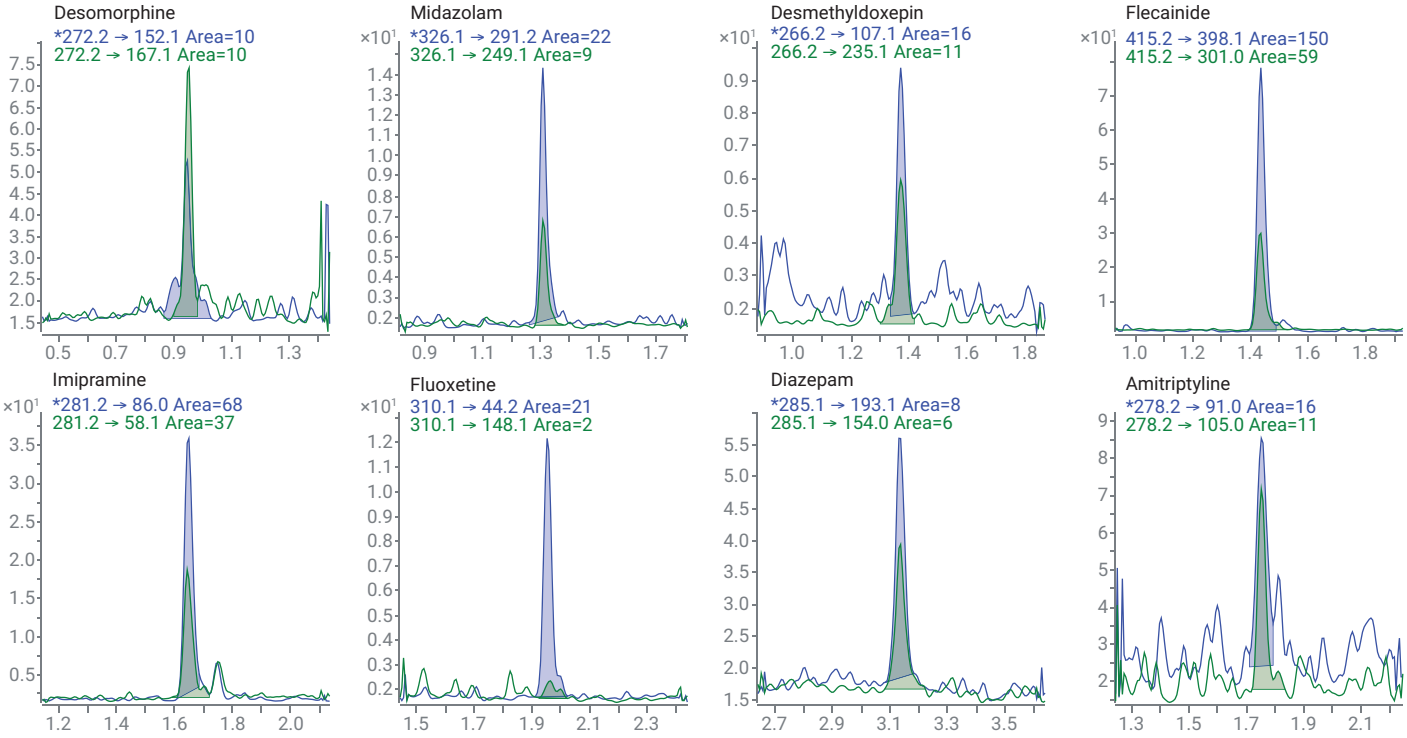

Figure 3. Excellent sensitivity demonstrated for all eight drugs at 5 ppt in serum.

Table 5. Accuracy and RSD (%) for each drug studied in serum.

Level	Desomorphine		Midazolam		Desmethyldoxepin		Flecainide		Imipramine		Amitriptyline		Fluoxetine		Diazepam	
ng/mL	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD
0.005	101.0	9.31	102.9	44.66	87.6	14.97	85.6	6.33	83.0	3.55	78.3	7.65	100.1	8.39	129.1	9.91
0.01	105.1	6.96	156.1	10.78	103.9	8.44	96.1	11.29	99.0	5.90	97.6	4.53	110.4	8.46	147.3	6.19
0.05	94.4	3.66	91.6	2.69	99.5	6.12	88.0	4.17	96.7	2.52	101.9	3.03	101.1	2.21	101.5	4.59
0.1	96.7	3.26	93.1	3.55	99.9	3.70	93.4	3.63	98.5	1.11	98.1	4.41	99.2	2.43	103.3	2.92
0.5	97.1	1.90	99.0	1.49	102.7	2.06	96.2	2.08	101.0	1.34	108.2	1.47	103.1	1.98	106.6	2.79
1	96.8	1.49	97.5	2.28	99.5	2.25	93.8	1.99	96.6	0.95	96.0	1.69	100.4	1.47	103.0	1.23
5	97.2	0.79	97.3	1.85	101.6	1.86	94.7	0.86	98.6	1.33	98.3	1.07	100.0	1.54	102.8	1.40
10	96.6	1.18	95.3	0.82	99.3	0.65	93.4	1.03	96.7	1.20	96.7	0.95	97.6	0.85	100.1	0.68
50	102.2	0.83	100.2	1.22	102.2	0.44	99.8	0.94	101.3	1.63	101.6	1.03	102.0	1.59	102.5	1.25
100	99.5	1.57	100.5	1.28	98.9	1.41	101.1	1.27	99.8	0.58	99.6	1.01	99.2	0.57	98.5	0.92

Method sensitivity

Figure 4 demonstrates the excellent signal response of the eight drugs analyzed in this method, at 100 ppt. Separation and sensitive detection of all eight drug standards was achieved, even at sub ppb levels on this small LC/TQ.

Figure 3 shows extracted ions of all eight drugs at 5 ppt. LOQs were defined as having four out of seven replicate injections with accuracy of 80–120 %, and a signal-to-noise ratio greater than 10.

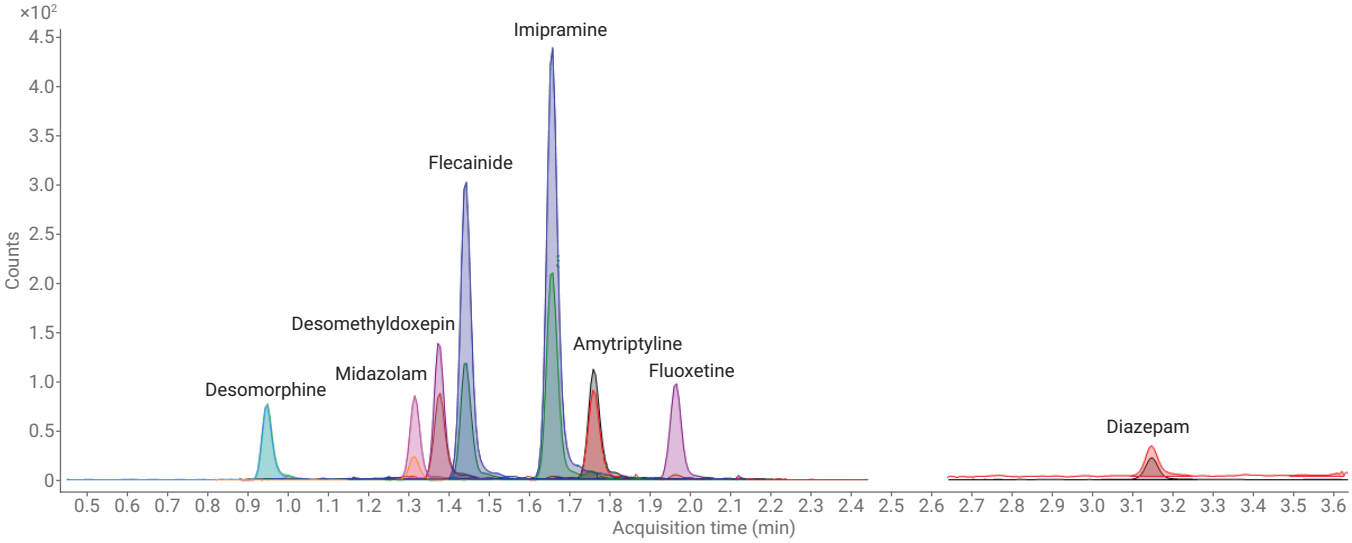

Figure 4. Composite MRM chromatogram of drugs spiked into a serum sample that was extracted.

Method precision and linearity

Excellent precision was achieved for all compounds studied. Figure 5 shows the calibration curves of all eight drugs at all levels. Outstanding linearity was demonstrated for all compounds. Every compound had R^2 values ≥0.99.

Conclusions

Ultivo is an exceptionally innovative mass spectrometer that can minimize laboratory workspace needs, as well as reduce maintenance challenges, creating a productive work environment for high-throughput laboratories. Ultivo is a small but powerful tool enabling the accurate and sensitive detection of drugs in human serum at part per trillion levels. MassHunter software provides an easy-to-use, all-inclusive tool for acquiring and reporting LC/MS data.

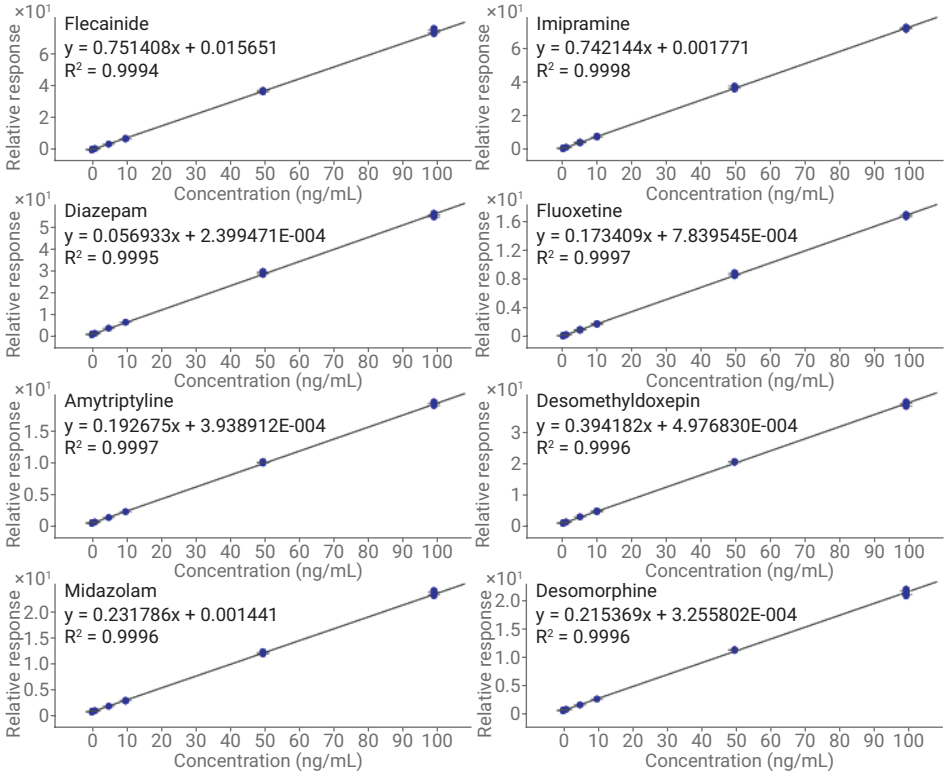

Figure 5. Calibration curves for all drugs studied.

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