

Application Data Sheet

No.47

LC-MS

Liquid Chromatograph Mass Spectrometer

High-Speed Quantitative Analysis of Anti-epileptic Drugs Using Triple Quadrupole LC/MS/MS

This data sheet illustrates high-speed quantitative analysis of 16 anti-epileptic drugs (AEDs) using the Shimadzu UFMS Triple Quadrupole Mass Spectrometer, LCMS-8050.

Without having any time-consuming sample pretreatment, the LCMS-8050 yielded excellent quantitative results for all 16 AEDs in plasma with a 1 μ L injection volume, all in only 7 minutes.

■ Compound Panel

Table 1: Compounds, Ionization polarity and MRM transition

Compounds	Polarity	Precursor m/z	Product m/z
Levetiracetam	+	171.10	126.15
Zonisamide	+	212.90	132.00
Felbamate	+	239.10	117.10
Primidone	+	219.00	162.15
Lamotrigine	+	255.70	211.05
Carbamazepine-10,11-epoxide	+	252.70	180.05
Carbamazepine	+	236.60	194.10
Clonazepam	+	316.10	270.05
Nitrazepam	+	281.90	236.10
Tiagabine	+	376.00	111.15
Diazepam	+	284.70	154.05
Ethosuximide	-	140.20	140.20
Phenobarbital	-	231.30	42.05
Topiramate	-	337.95	78.00
Valproic acid	-	143.20	143.10
Phenytoin	-	251.10	102.05

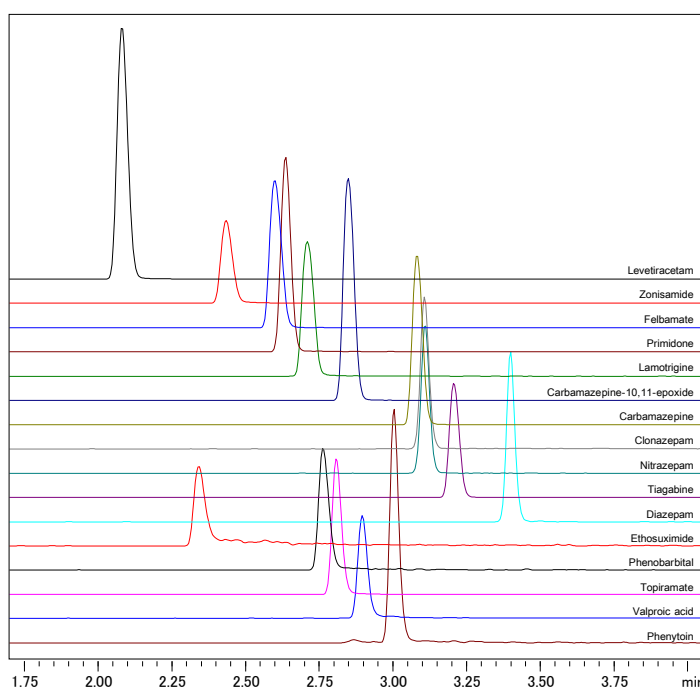


Figure 1: Representative chromatogram of 16 AEDs

Table 2: Analytical Conditions

Column	: Inertsil ODS-4 (50 mmL. X 2.1 mmI.D., 2.0 μ m)
Mobile Phases	: A 10mM Ammonium acetate : B Methanol
Gradient	: 3% (0.0-0.5min) \rightarrow 90%(3.0-5.0min) \rightarrow 3(5.01-7.0min)
Flow Rate	: 0.4 mL/min
Column Temperature	: 40 $^{\circ}$ C
Injection Volume	: 1 μ L
Probe Voltage	: +4.5 kV (ESI-positive mode) / -3.5 kV (ESI-negative mode)
DL Temperature	: 150 $^{\circ}$ C
Block Heater Temperature	: 200 $^{\circ}$ C
Interface Temperature	: 400 $^{\circ}$ C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min

■ Analysis of QC samples

Table 3 illustrates linearity of 16 AEDs and accuracy of 16 QC samples at three concentration levels. QC samples were prepared by spiking each AED on protein precipitated plasma. For this data sheet, we measured plasma samples with a 100 times dilution by methanol without making tedious sample pretreatment. Excellent linearity and accuracy for all 16 AEDs were obtained, even at a 1 uL injection volume.

Table 3: Linearity of 16 AEDs and accuracy of QC samples

Compounds	Linearity (µg/mL)	r ²	QC samples concentration (ug/mL)			Accuracy (%)		
			Low	Medium	High	Low	Medium	High
Levetiracetam	0.25 - 25	0.995	2	4	20	86	87	92
Zonisamide	0.5 - 10	0.996	0.8	4	8	125	91	98
Felbamate	0.5 - 10	0.995	0.8	4	8	135	95	101
Primidone	0.25 - 25	0.995	2	4	20	88	88	89
Lamotrigine	0.25 - 25	0.997	2	4	20	97	97	96
Carbamazepine-10,11-epoxide	0.5 - 25	0.997	2	4	20	97	95	97
Carbamazepine	0.025 - 25	0.997	0.4	4	20	113	93	109
Clonazepam	0.005 - 0.5	0.996	0.04	0.08	0.4	106	100	109
Nitrazepam	0.005 - 0.5	0.998	0.04	0.08	0.4	96	98	99
Tiagabine	0.025 - 25	0.999	2	4	20	100	102	96
Diazepam	0.05 - 1	0.996	0.08	0.2	0.8	101	87	88
Ethosuximide	5 - 500	0.996	20	40	400	113	115	92
Phenobarbital	2.5 - 50	0.998	10	20	40	86	90	94
Topiramate	0.5 - 100	0.996	0.8	4	40	103	109	87
Valproic acid	2 - 200	0.996	8	16	160	109	110	92
Phenytoin	0.5 - 100	0.996	8	40	80	117	90	91

■ Intuitive Data Processing by LabSolutions Quant Browser

Quant Browser makes intuitive data reprocessing with multiple data files possible. Figure 2 illustrates MRM chromatograms of the standard and QC sample. Reviewing multiple results in a single panel saves time during data processing.

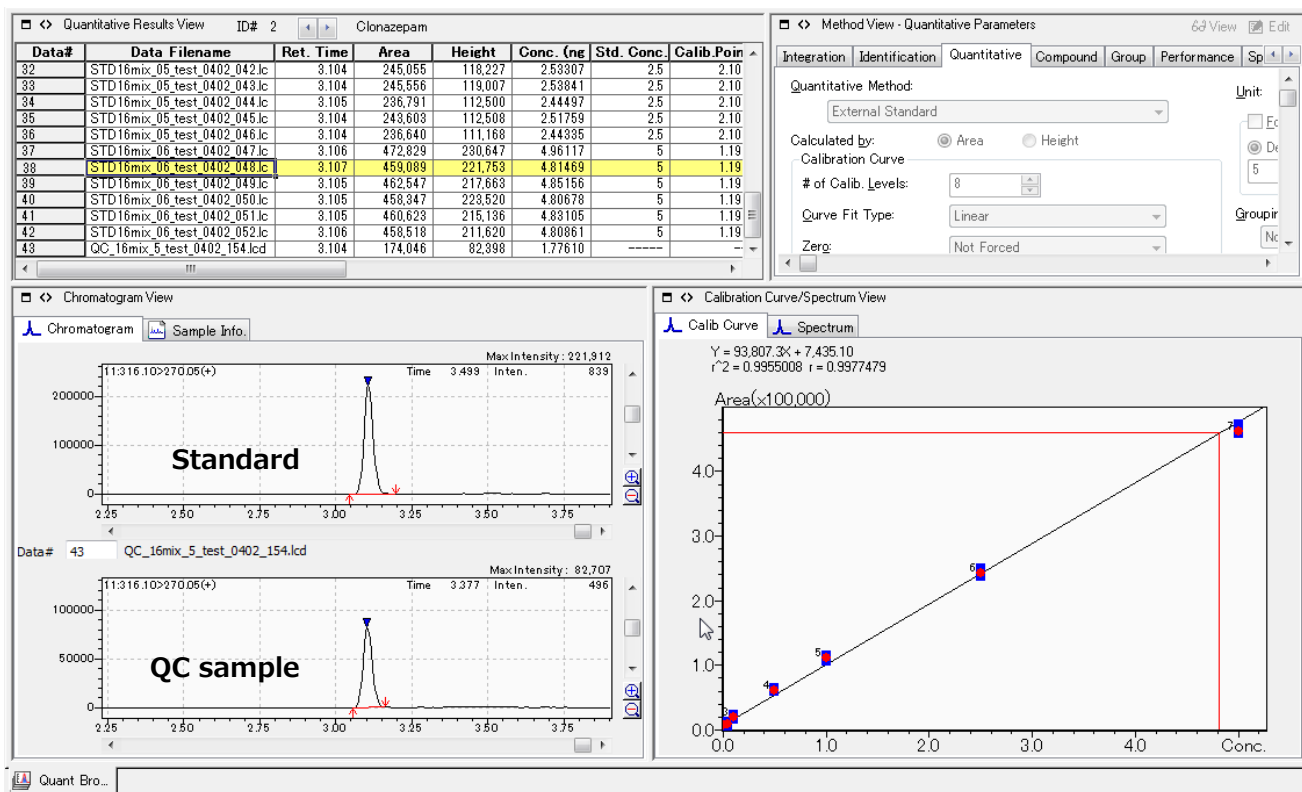


Figure 2: Data Review by Quant Browser

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