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Rapid development of analytical method for antiepileptic drugs in plasma using UHPLC method scouting system coupled to LC/MS/MS

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Introduction

Method development for therapeutic drug monitoring (TDM) is indispensable for managing drug dosage based on the drug concentration in blood in order to conduct a rational and efficient drug therapy. Liquid chromatography coupled with tandem quadrupole mass spectrometry is increasingly used in TDM because it can perform selective and sensitive analysis by simple sample pretreatment. The UHPLC method scouting system coupled to tandem quadrupole mass spectrometer used in this study can dramatically shorten the total time for optimization of analytical conditions because this system can make enormous combinatorial analysis methods and run batch program automatically. In this study, we developed a high-speed and sensitive method for measurement of seventeen antiepileptics in plasma by UHPLC coupled with tandem quadrupole mass spectrometer.

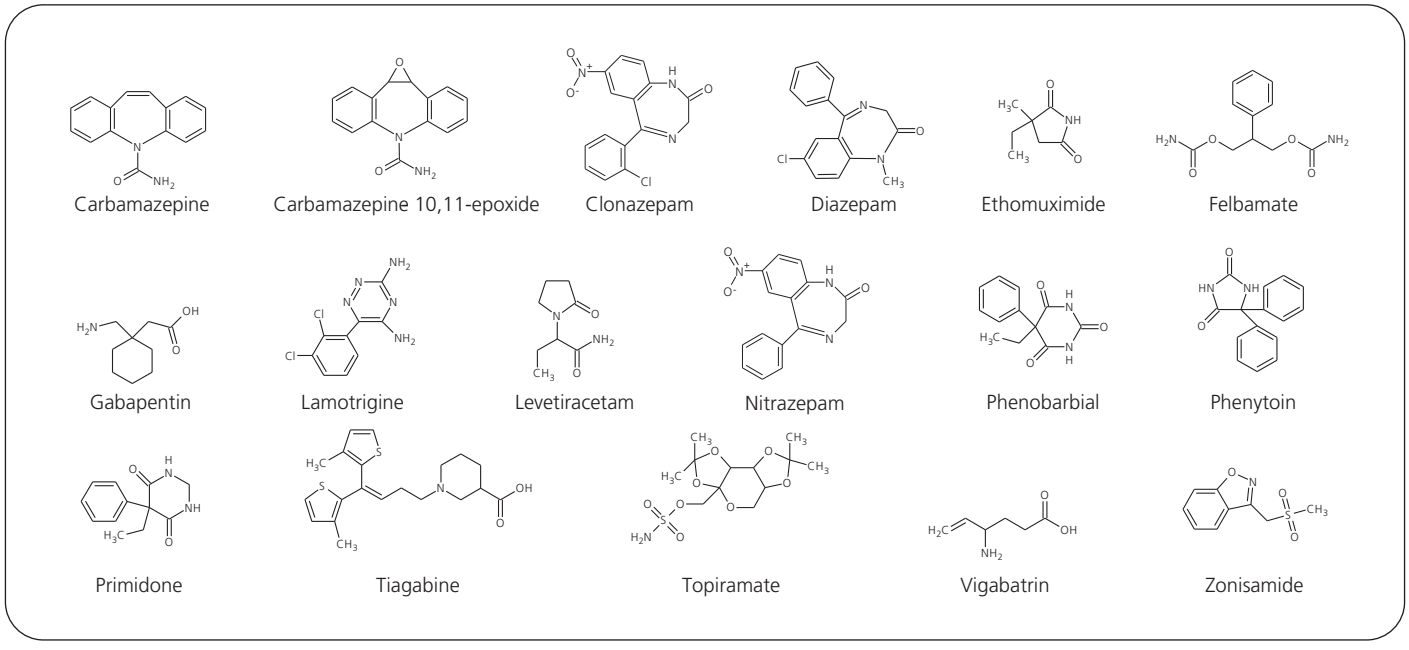

Figure 1 Antiepileptic drugs used in this assay

Experimental

Instruments

UHPLC based method scouting system (Nexera X2 Method Scouting System, Shimadzu Corporation, Japan) is configured by Nexera X2 UHPLC modules. For the detection, tandem quadrupole mass spectrometer (LCMS-8050, Shimadzu Corporation, Japan) was used. The system can be operated at a maximum pressure of 130 MPa, and it enables to automatically select up to 96 unique combinations of eight different mobile phases and six different columns. A dedicated software was newly developed to control the system (Method Scouting Solution, Shimadzu Corporation, Japan), which provides a graphical aid to configure the different type of columns and mobile phases. The software is integrated into the LC/MS/MS workstation (LabSolutions, Shimadzu Corporation, Japan) so that selected conditions are seamlessly translated into method files and registered to a batch queue, ready for analysis instantly.

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Figure 2 Nexera Method Scoutuing System and LCMS-8050 triple quadrupole mass spectrometer

Calibration standards and QC samples

The main standard mixture was prepared in methanol from individual stock solutions. The calibration standards were prepared by diluting the standard mixture with methanol.
QC sample was prepared by adding 4 volume of acetonitrile to 1 volume of control plasma, thereby precipitating proteins, and subsequently adding the standard mixture to the supernatant to contain plasma concentration equivalents stated in Table 4. The QC samples were further diluted 100 times (10 µL sample added to 990µL methanol) before injection.
Next step of preparation procedure was divided into three groups by the intensity of each compound. For ethomuximide, phenobarbial and phenytoin, the supernatant was used for the LC/MS/MS analysis without further dilution. For zonisamide, 10 µL supernatant was further diluted with 990 µL methanol. For others, 100 µL supernatant was further diluted with 900 µL methanol. The diluted solutions were used for the LC/MS/MS analysis.

Result

MRM condition optimization

The MS condition optimization was performed by flow injection analysis (FIA) of ESI positive and negative ionization mode, and the compound dependent parameters such as CID and pre-bias voltage were adjusted using automatic MRM optimization function. The transition that gave highest intensity was used for quantification. The MRM transitions used in this assay are listed in Table 1.

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Table 1 Compounds, Ionization polarity and MRM transition

Compound	Retaintion (min)	Polarity	Precursor m/z	Product m/z
Carbamazepine	3.84	+	237.1	194.2
Carbamazepine-10,11-epoxide	3.24	+	253.1	180.15
Clonazepam	3.93	+	316.1	269.55
Diazepam	4.79	+	284.9	154.15
Ethomuximide	2.50	+	239.3	117.20
Felbamate	2.86	+	172.2	154.25
Gabapentin	2.27	+	256.2	211.05
Lamotrigine	2.96	+	171.2	126.15
Levetiracetam	2.32	+	281.9	236.20
Nitrazepam	3.90	+	219.2	162.15
Phenobarbial	3.06	+	376.2	111.15
Phenytoin	3.64	+	130.2	71.15
Primidone	2.83	+	213.1	132.10
Tiagabine	4.28	-	140.0	42.00
Topiramate	3.14	-	231.0	42.05
Vigabatrin	0.82	-	337.9	78.00
Zonisamide	2.58	-	143.1	143.10

UHPLC condition optimization

36 analytical conditions, comprising combinations of 9 mobile phase and 4 columns, were automatically investigated using Method Scouting System. Schematic representation of scouting system was shown in Figure 3. From the result of scouting, the combination of 10 mM ammonium acetate water and methanol for mobile phase and Inertsil-ODS4 for separation column were selected. Using this combination of mobile phase and column, the gradient condition was further optimized. The final analytical condition was shown in Table 2.

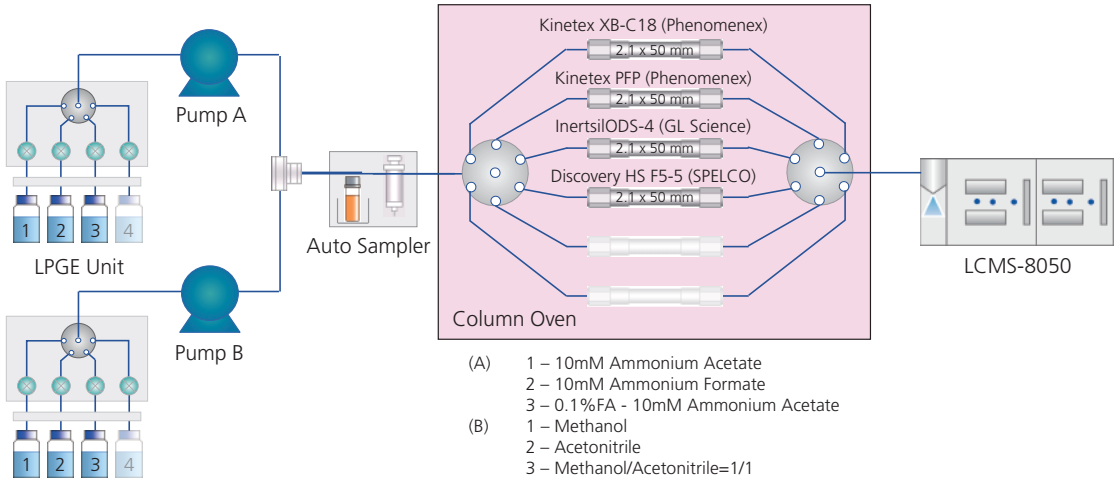

Figure. 3 Schematic representation and features of the Nexera Method Scouting System.

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Table.2 UHPLC analytical conditions

Column	: Inertsil ODS-4 (50 mmL. x 2.1mmi.d., 2um)
Mobile phase	: A) 10mM Ammonium Acetate
	B) Methanol
Binary gradient	: B conc. 3% (0.65 min) → 40% (1.00 min) → 85% (5.00 min)
	→ 100% (5.01-8.00 min) → 3% (8.01-10.00 min)
Flow Rate	: 0.4 mL/min
Injection vol.	: 1 µL
Column Temp.	: 40 deg. C

Precision, accuracy and linearity of AEDs

Figure 4 shows MRM chromatograms of the 17 AEDs. It took only 10 minutes per one UHPLC/MS/MS analysis, including column rinsing.

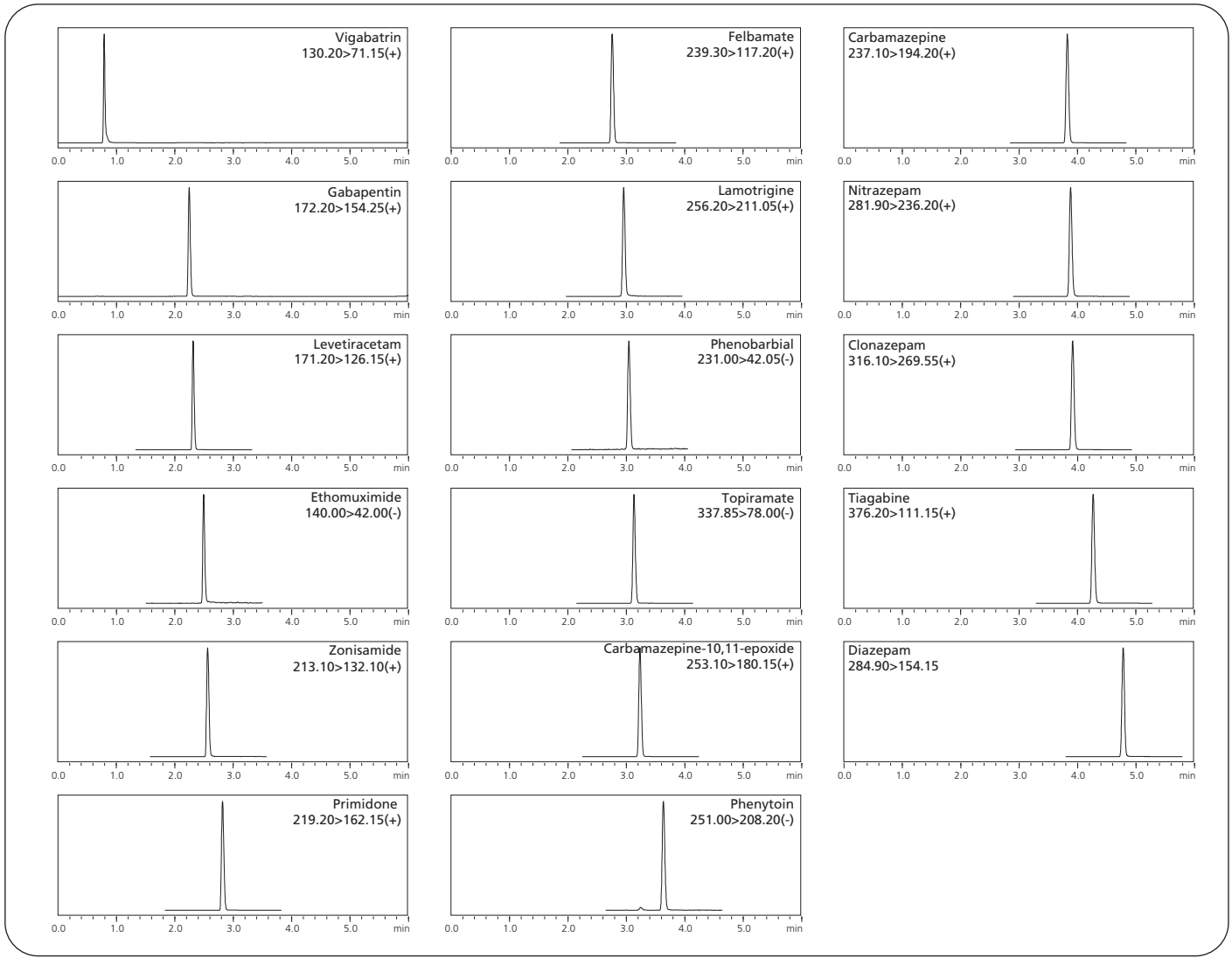

Figure. 4 Chromatogram of 17 AEDs calibration standards

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Table 3 illustrates linearity of 17 AEDs and Table 4 illustrates accuracy and precision of the QC samples at three concentration levels. Determination coefficient (r^2) of all calibration curves was larger than 0.995, and the precision and accuracy were within +/- 15%. Excellent linearity, accuracy and precision for all 17 AEDs were obtained at only 1 µL injection volume.

Table.3 Linearity of 17 AEDs QC sample

Compound	Linarity (ng/mL)			r^2
Carbamazepine	0.25	-	50	0.999
Carbamazepine-10,11-epoxide	0.25	-	50	0.998
Clonazepam	0.005	-	2.5	0.998
Diazepam	0.01	-	5	0.999
Ethomuximide	25	-	2500	0.998
Felbamate	0.5	-	100	0.998
Gabapentin	2	-	50	0.999
Lamotrigine	0.25	-	50	0.999
Levetiracetam	0.5	-	100	0.999
Nitrazepam	0.005	-	1	0.999
Phenobarbial	5	-	500	0.996
Phenytoin	5	-	500	0.998
Primidone	0.25	-	10	0.996
Tiagabine	0.25	-	50	0.998
Topiramate	0.5	-	100	0.998
Vigabatrin	0.5	-	50	0.998
Zonisamide	0.5	-	20	0.996

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Table.4 Accuracy and precision of 17 AEDs QC sample

Compound	Plasma concentration equivalents (μg/mL)			Precision (%)			Accuracy (%)		
	Low	Middle	High	Low	Middle	High	Low	Middle	High
Carbamazepine	1.8	18	71	2.2	0.9	0.9	106.1	103.9	95.8
Carbamazepine-10,11-epoxide	1.8	18	71	2.4	1.9	1.3	104.2	105.0	98.2
Clonazepam	0.04	0.9	1.8	3.3	0.7	0.5	106.7	102.1	90.1
Diazepam	0.1	0.7	2.9	3.2	1.7	1.4	105.8	106.6	100.6
Ethomuximide	18	446	714	7.8	1.5	1.4	104.3	99.9	97.0
Felbamate	3.6	89	179	1.7	0.4	0.8	97.1	106.3	91.7
Gabapentin	18	36	143	1.3	0.7	0.7	85.8	98.8	89.5
Lamotrigine	1.8	45	71	10.5	1.2	1.7	107.7	98.4	99.2
Levetiracetam	3.6	89	179	2.1	0.5	1.1	99.5	104.9	90.4
Nitrazepam	0.04	0.4	1.4	3.3	1.4	1.5	105.0	105.2	97.9
Phenobarbial	3.6	71	143	3.5	6.2	1.6	100.9	108.4	95.8
Phenytoin	3.6	89	143	7.8	1.9	1.2	103.2	100.1	96.2
Primidone	1.8	18	45	3.2	0.7	0.7	99.5	112.6	97.1
Tiagabine	1.8	18	71	1.8	1.8	1.0	107.6	105.7	97.5
Topiramate	3.6	36	143	12.5	1.5	1.2	105.4	101.6	96.1
Vigabatrin	8.9	18	89	1.4	1.1	2.1	105.9	101.6	88.8
Zonisamide	36	89	179	3.3	1.3	1.6	111.7	100.4	95.2

Conclusions

- We could select the most suitable combination of mobile phase and column from 36 analytical condition without time-consuming investigation.
- We have measured plasma sample as it is after 100-10,000 times dilution by methanol without making tedious sample pretreatment. Excellent linearity, precision and accuracy for all 17 AEDs were obtained at only 1 uL injection volume.

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