

ANTIEPILEPTICS IN PLASMA BY LC-MS/MS

REF LC05015

10,11-DIHYDRO-10-HYDROXY-CARBAMAZEPINE, CARBAMAZEPINE, CARBAMAZEPINE EPOXIDE, DESMETHYLSUXIMIDE, ETHOSUXIMIDE, LACOSAMIDE, LAMOTRIGINE, LEVETIRACETAM, METHSUXIMIDE, OXCARBAZEPINE, PHELBAMATE, PHENOBARBITAL, PHENYTOIN, PERAMPANEL, PRIMIDONE, RUFINAMIDE, TOPIRAMATE, VALPROIC ACID, ZONISAMIDE

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.



INSTRUCTIONS FOR USE



For *in vitro* diagnostic use



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INTENDED USE

In vitro diagnostic medical device for the detection and the quantitative analysis of 10,11-Dihydro-10-Hydroxy-Carbamazepine, Carbamazepine, Carbamazepine Epoxide, Desmethysuximide, Ethosuximide, Lacosamide, Lamotrigine, Levetiracetam, Methsuximide, Oxcarbazepine, Phelbamate, Phenobarbital, Phenytoin, Perampanel, Primidone, Rufinamide, Topiramate, Valproic Acid and Zonisamide in plasma as monitoring of antiepileptic therapy. Manual method for professional use.

KIT CONTENT FOR 500 TESTS

CONTENT	DESCRIPTION	PACKAGING	WARNING
Reagent A	Deproteinization Solution + Internal Standards	1 x 250 ml	Store at  -15 °C -25 °C
Reagent B	Stabilization Solution	1 x 250 ml	Store at  +8 °C +2 °C
Reagent C	Buffer Solution	1 x 15 ml	Store at  +8 °C +2 °C
Multiparametric Calibrator in plasma		7 x 3 x 1 ml	(packed separately – Code LC04916 -see data sheet)
Reagent M1	Mobile Phase M1	4 x 500 ml	Store at  +8 °C +2 °C
Reagent M2	Mobile Phase M2	4 x 500 ml	Store at  +8 °C +2 °C

ACCESSORIES AND CONSUMABLES AVAILABLE SEPARATELY

CODE	DESCRIPTION	PACKAGING
LC04916	Calibrator for Antiepileptics in plasma	7 x 2 x 1 ml
LC04919	Control for Antiepileptics in plasma – Levels 1, 2 and 3	3 x 5 x 1 ml
SK05010	Starter kit for Antiepileptics in plasma	25 x 1 ml
LC05011	Mobile Phase M1	4 x 500 ml
LC05021	Mobile Phase M2	4 x 500 ml
LC05030	Deuterated Internal Standard Mix Solution in plasma	1 x 50 ml
Z699675902	Poroshell 120-EC C18 Analytical column (50 x 2,1 mm - 1,9 µm)	1 Pc
S51843550	Clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml	1 x 100 Pcs
S51820717	Caps for clear glass vials with reduced volume goblet form from 15 µl to 1,5 ml and for amber vials	1 x 100 Pcs
L-30-210	Eppendorf Test tubes by 1,5 ml with caps	1 x 1000 Pcs

INSTRUMENTS

DESCRIPTION	WARNING
LC-MS triple quadrupole instrument with Divert Valve – Work modality MRM – ESI Positive/negative	Required
Operational Computer	Required
Autosampler	Required

MATRIX - COLLECTION AND STORAGE PROCEDURE

MATRIX	PROCEDURE
Plasma	Take 3 ml of whole blood in a test tube without gel or with EDTA as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate the plasma and store at  -15 °C . Stable for 4 weeks at  +2 °C or  +8 °C 4 months at  -15 °C .

REFERENCE VALUES

Antiepileptics	Therapeutic Range
10,11-Dihydro-10-Hydroxycarbamazepine	3 - 40 µg/ml
Carbamazepine	6 – 12 mg/l
Carbamazepine Epoxide	2 - 4 mg/l
Desmethyloximide	10 - 40 mg/l
Ethosuximide	40 - 100 µg/ml
Lacosamide	1 - 10 mg/l
Lamotrigine	3 - 14 µg/ml
Levetiracetam	10 - 37 µg/ml
Methsuximide	10 - 40 mg/l
Oxcarbazepine	0 - 4 mg/l
Perampanel	0,1 - 1 µg/ml
Phelbamate	10 - 80 mg/l
Phenobarbital	10 - 40 mg/l
Phenytoin	10 - 20 mg/l
Primidone	5 - 12 mg/l
Rufinamide	1 - 50 mg/l
Topiramate	2 - 25 mg/l
Valproic Acid	40 - 100 mg/l
Zonisamide	10 - 38 µg/ml

ANALYTICAL PROCEDURE

STEP	DESCRIPTION																				
1	Dispense into Eppendorf tubes: <table><tr><td></td><td>Calibrator</td><td>Sample</td><td>Control</td></tr><tr><td>Calibrator</td><td>50 µl</td><td></td><td></td></tr><tr><td>Sample</td><td></td><td>50 µl</td><td></td></tr><tr><td>Control</td><td></td><td></td><td>50 µl</td></tr><tr><td>Reagent C</td><td>25 µl</td><td>25 µl</td><td>25 µl</td></tr></table> <u>Wait 3 minutes</u>		Calibrator	Sample	Control	Calibrator	50 µl			Sample		50 µl		Control			50 µl	Reagent C	25 µl	25 µl	25 µl
	Calibrator	Sample	Control																		
Calibrator	50 µl																				
Sample		50 µl																			
Control			50 µl																		
Reagent C	25 µl	25 µl	25 µl																		
2	Add 500 µl of Reagent A at each Eppendorf of STEP 1 <u>Warning: deproteinize directly on Vortex for at least 20 seconds</u>																				
3	Incubate at +2°C  +8°C for 15 minutes Centrifuge at 14.000 rpm for 15 minutes																				
4	Pipette 50 µl of supernatant into a glass vial. Add 450 µl of Reagent B (or an appropriate volume for the instrument sensitivity) <u>Vortex for 10 seconds</u>																				
INJECTION	Inject 1 – 10 µl of the solution into the LC system																				

GRADIENT

UHPLC SYSTEMS [Poroshell 120-EC C18 Analytical column (50 x 2,1 mm – 1,9 µm)]

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (µl/min)
0	100	0	500
0.53	100	0	500
0.54	87	13	500
1.80	87	13	500
1.81	79	21	500
2.80	79	21	500
3.30	50	50	500
3.80	50	50	500
3.90	20	80	500
4.20	20	80	500
4.30	5	95	500
4.80	5	95	500
4.90	100	0	500
6.00	100	0	500

PARAMETERS

Parameters set on triple quadrupole SCIEX 4500

	ANTIEPILEPTICS ESI POSITIVE	ANTIEPILEPTICS ESI NEGATIVE
CURTAIN GAS (CUR)	40	40
COLLISION GAS (CAD)	8	8
ION SPRAY VOLTAGE (IS)	5500 V	-4500 V
TEMPERATURE (TEM)	600°C	600°C
ION SOURCE GAS 1 (GS1)	40	30
ION SOURCE GAS 2 (GS2)	30	30

FRAGMENTATION OF ANALYTES AND DEUTERATED INTERNAL STANDARDS

ANALYTES	RT (min)	FRAGMENTATION		DEUTERATED I.S. USED FOR CALIBRATION	RT (min)	FRAGMENTATION	
		PARENT ION (Q1)	FRAGMENT ION (Q3)			PARENT ION (Q1)	FRAGMENT ION (Q3)
10,11-Dihydro-10-Hydroxycarbamazepine (+)	2.90	255.2	237.2 – 194.1	Lacosamide-D3	2.55	254.0	108.0
Carbamazepine (+)	3.70	237.2	194.2	Lacosamide-D3	2.55	254.0	108.0
Carbamazepine Epoxide (+)	3.45	253.0	210.0 – 180.0	Lacosamide-D3	2.55	254.0	108.0
Desmethysuximide (+)	3.14	188.0	143.0	Desmethysuximide-D5	3.14	193.0	148.0
Ethosuximide (-)	2.02	140.1	140.1	Ethosuximide-D3	2.02	143.1	143.1
Lacosamide (+)	2.55	251.2	91.2 – 108.2	Lacosamide-D3	2.55	254.0	108.0
Lamotrigine (+)	2.66	256.1	211.1 – 159.1	Lacosamide-D3	2.55	254.0	108.0
Levetiracetam (+)	1.70	171.1	126.1	Levetiracetam-D6	1.70	177.1	132.1
Methsuximide (+)	3.69	204.1	126.2 – 79.2	Desmethysuximide-D5	3.14	193.0	148.0
Oxcarbazepine (+)	3.55	253.2	236.2 – 180.2	Lacosamide-D3	2.55	254.0	108.0
Perampanel (+)	4.71	350.1	219.0	Lacosamide-D3	2.55	254.0	108.0
Phelbamate (+)	2.63	239.3	178.2	Phelbamate-D4	2.63	243.2	182.1
Phenobarbital (-)	3.03	231.0	188.1	Zonisamide-D4	2.48	215.0	123.0
Phenytoin (+)	3.70	253.1	104.3 – 182.3	Lacosamide-D3	2.55	254.0	108.0
Primidone (+)	2.44	219.2	162.2 – 91.2	Lacosamide-D3	2.55	254.0	108.0
Rufinamide (+)	2.69	239.3	127.2	Lacosamide-D3	2.55	254.0	108.0
Topiramate (+)	3.52	357.1	264.1	Lacosamide-D3	2.55	254.0	108.0
Valproic Acid (-)	3.86	143.0	143.0	Zonisamide-D4	2.48	215.0	123.0
Zonisamide (-)	2.48	211.0	119.0 – 147.0	Zonisamide-D4	2.48	215.0	123.0

TECHNICAL FEATURES - PART I

ABBREVIATION	MEANING
C LLOQ	Concentration at low limit of quantification
C1	Low concentration
C2	Medium concentration
C3	High concentration
Max C	The highest concentration

ANALYTES	CONCENTRATIONS USED TO CALCULATE REPRODUCIBILITY AND ACCURACY (µg/ml)				
	C LLOQ	C1	C2	C3	MAX C
10,11-Dihydro-10-Hydroxycarbamazepine	1,8	7,7	19	31,6	70,4
Carbamazepine	0,6	2,1	5,0	8,0	18,6
Carbamazepine Epoxide	0,3	1,2	3,2	5,0	12,4
Desmethysuximide	1,8	5,9	17,4	27,7	63,6
Ethosuximide	3,8	15,8	36,9	59,5	131,7
Lacosamide	0,5	2,0	5,0	7,4	18,3
Lamotrigine	0,8	3,1	7,6	13,6	29,8
Levetiracetam	1,7	7,5	19,3	29,3	72,6
Methsuximide	1,7	6,0	16,2	25,2	61,1
Oxcarbazepine	0,2	1,1	2,8	4,5	11,3
Perampanel	0,05	0,2	0,5	0,9	2,0
Phelbamate	3,2	12,6	30,7	57,9	120,1
Phenobarbital	2,2	7,7	20,5	31,8	68,7
Phenytoin	0,7	4,6	12,0	19,0	42,2
Primidone	0,9	3,7	8,7	14,4	31,1
Rufinamide	1,4	4,6	10,6	15,0	36,1
Topiramate	0,8	4,1	8,9	15,8	36,2
Valproic Acid	3,3	16,8	36,6	61,7	139,8
Zonisamide	1,5	5,9	13,9	23,5	56,5

ANALYTES	ACCURACY INTRA SERIE (RELATIVE ERROR %)		ACCURACY INTER SERIE (RELATIVE ERROR %)	
	C1 (%)	C3 (%)	C1 (%)	C3 (%)
10,11-Dihydro-10-Hydroxycarbamazepine	1,37	1,84	7,66	2,58
Carbamazepine	9,68	2,59	12,12	6,08
Carbamazepine Epoxide	4,12	1,64	9,83	2,05
Desmethyloximide	2,90	1,64	9,13	3,20
Ethosuximide	0,42	3,18	3,89	3,27
Lacosamide	3,50	5,51	5,51	5,57
Lamotrigine	2,35	2,15	3,53	3,02
Levetiracetam	2,83	5,23	6,82	6,43
Methsuximide	5,75	2,95	10,51	7,51
Oxcarbazepine	1,37	4,56	3,91	4,91
Perampanel	2,36	1,41	3,63	4,68
Phelbamate	4,82	3,97	5,65	4,97
Phenobarbital	7,24	2,57	10,35	2,77
Phenytoin	1,88	3,70	4,98	4,36
Primidone	2,85	2,32	4,08	2,82
Rufinamide	7,19	2,72	7,50	4,03
Topiramate	1,75	3,13	5,39	6,23
Valproic Acid	3,42	2,37	3,77	2,44
Zonisamide	2,49	1,79	3,90	4,13

TECHNICAL FEATURES - PART II

ANALYTES	REPRODUCIBILITY INTRA SERIE (COEFFICIENT OF VARIATION %)			REPRODUCIBILITY INTER SERIE (COEFFICIENT OF VARIATION %)		
	C LLOQ %	C2 %	Max C %	C LLOQ %	C2 %	Max C %
10,11-Dihydro-10-Hydroxycarbamazepine	6,21	2,29	3,59	8,19	4,12	3,79
Carbamazepine	9,92	4,96	5,00	11,38	5,72	7,76
Carbamazepine Epoxide	3,77	2,74	1,54	6,47	6,01	2,93
Desmethysuximide	4,19	4,77	2,62	8,46	5,52	3,86
Ethosuximide	5,78	4,87	3,63	6,40	7,00	6,73
Lacosamide	5,43	3,94	4,94	9,58	4,79	6,95
Lamotrigine	3,02	3,02	3,48	6,99	5,18	4,67
Levetiracetam	1,04	3,56	3,70	5,26	5,69	4,59
Methsuximide	6,56	6,67	3,41	13,42	8,76	8,10
Oxcarbazepine	5,20	4,88	4,29	5,24	6,53	5,15
Perampanel	4,28	3,20	5,14	4,76	6,07	6,16
Phelbamate	4,81	6,07	2,61	7,01	8,77	4,44
Phenobarbital	6,81	4,36	2,07	7,29	4,90	3,58
Phenytoin	8,05	3,30	4,46	11,85	8,79	8,06
Primidone	4,67	3,60	1,90	8,50	8,88	4,50
Rufinamide	5,29	3,71	1,50	6,34	11,37	3,64
Topiramate	5,63	4,76	3,08	9,88	8,45	5,26
Valproic Acid	4,35	2,80	1,96	6,57	9,39	11,98
Zonisamide	5,73	1,66	3,28	5,80	7,85	3,77

TECHNICAL FEATURES - PART III

ANALYTES	LLOD (µg/ml)	LINEARITY µg/ml	COEFFICIENT OF CORRELATION R ² + STD DEV
10,11-Dihydro-10-Hydroxycarbamazepine	0,6	1,8 - 100	0,9972 ± 0,0031
Carbamazepine	0,2	0,6 - 30	0,9941 ± 0,0044
Carbamazepine Epoxide	0,1	0,3 - 100	0,9974 ± 0,0023
Desmethyloximide	0,6	1,8 - 100	0,9977 ± 0,0025
Ethosuximide	1,3	3,8 - 150	0,9982 ± 0,0004
Lacosamide	0,2	0,5 - 30	0,9970 ± 0,0025
Lamotrigine	0,3	0,8 - 30	0,9977 ± 0,0010
Levetiracetam	0,5	1,7 - 100	0,9984 ± 0,0003
Methsuximide	0,5	1,7 - 100	0,9944 ± 0,0036
Oxcarbazepine	0,6	0,2 - 100	0,9984 ± 0,0010
Perampanel	0,02	0,05 - 5	0,9986 ± 0,0002
Phelbamate	1,1	3,2 - 150	0,9978 ± 0,0007
Phenobarbital	0,7	2,2 - 100	0,9973 ± 0,0017
Phenytoin	0,2	0,7 - 50	0,9971 ± 0,0010
Primidone	0,3	0,9 - 30	0,9976 ± 0,0019
Rufinamide	0,5	1,4 - 100	0,9979 ± 0,0012
Topiramate	0,3	0,8 - 100	0,9975 ± 0,0014
Valproic Acid	1,1	3,3 - 200	0,9968 ± 0,0022
Zonisamide	0,5	1,5 - 100	0,9982 ± 0,0003

REFERENCE CHROMATOGRAMS

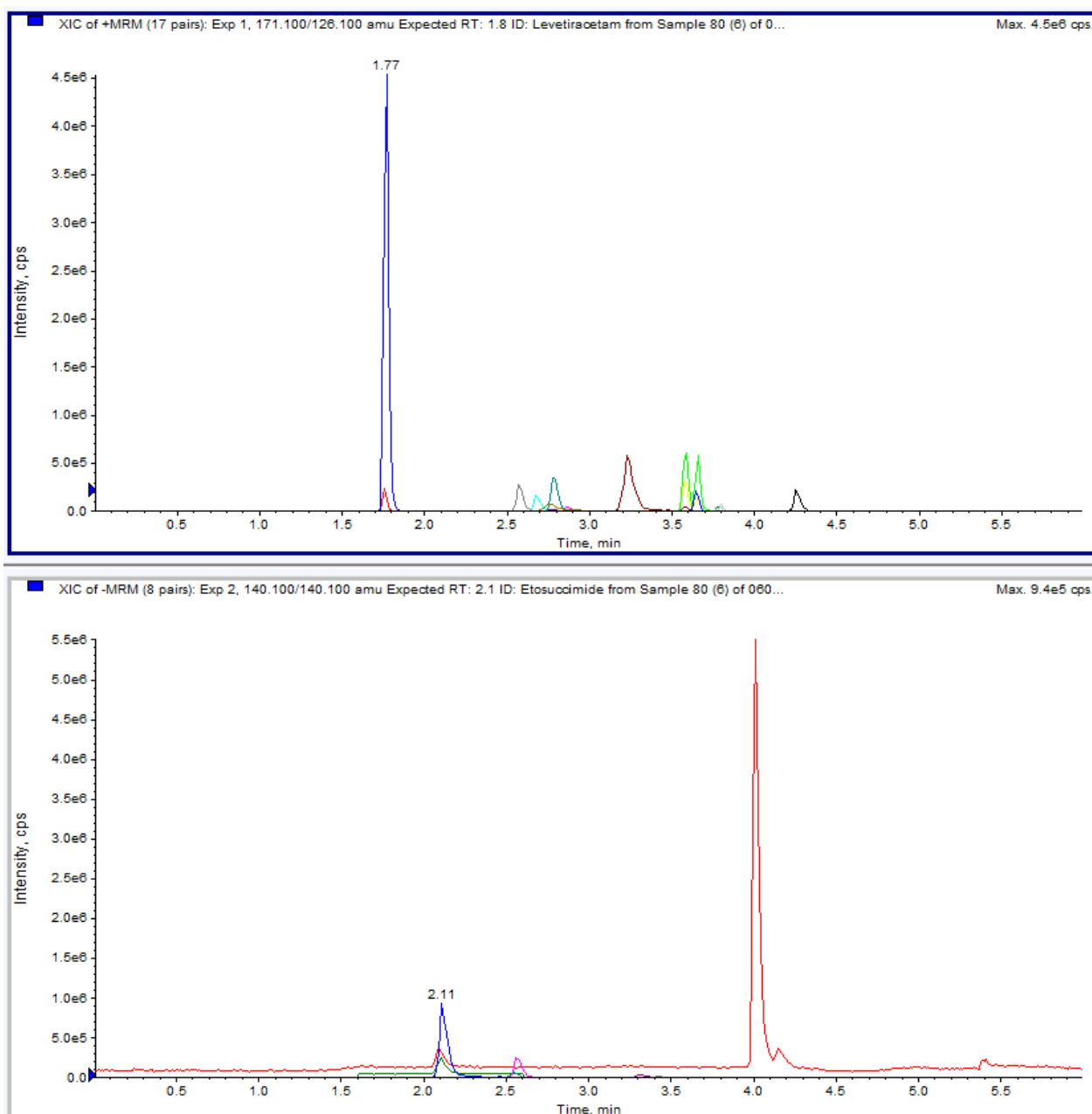


Fig. 1 Calibrator level 6 (at the top positive transitions, at the bottom negative transitions)

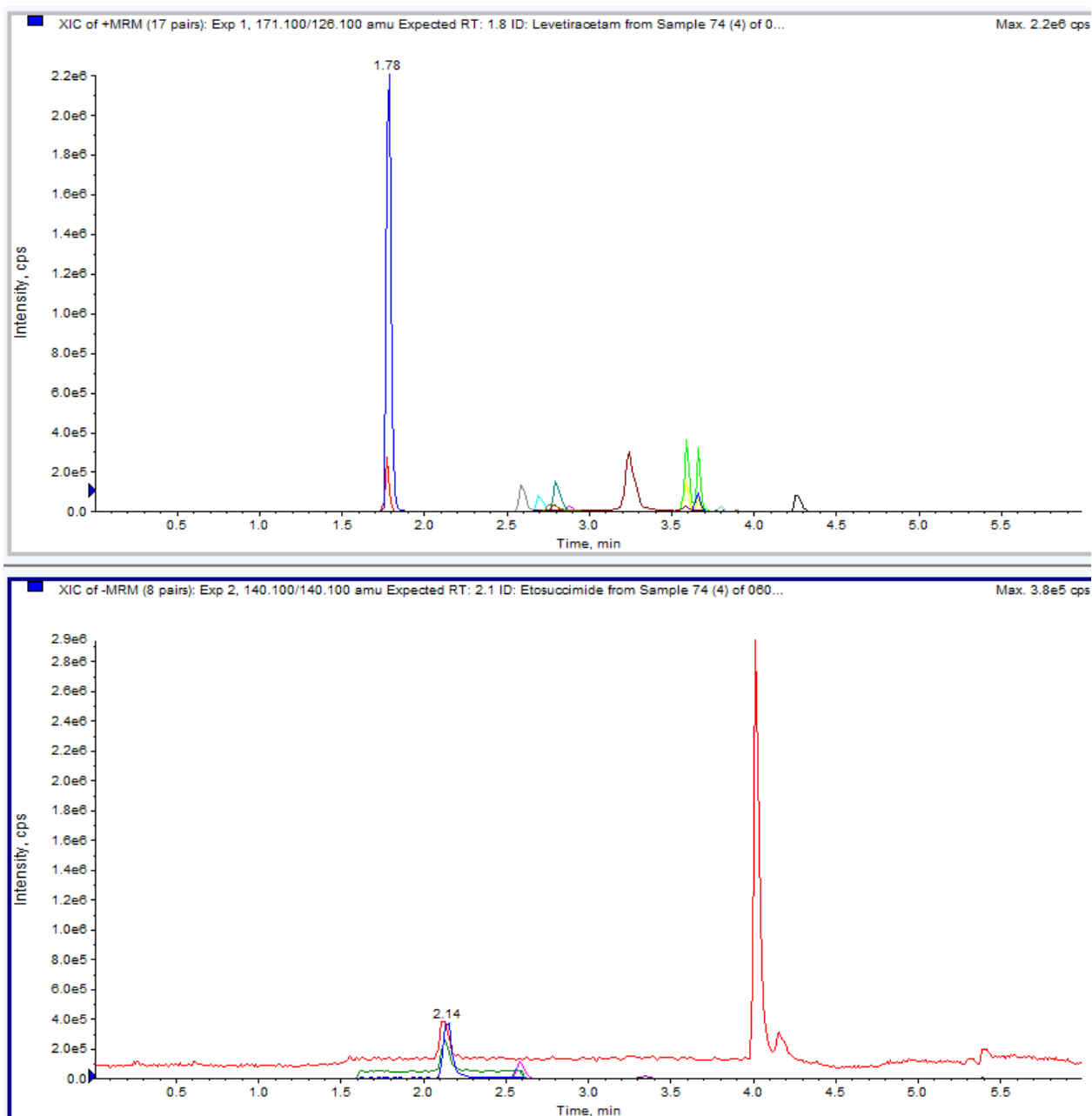


Fig 2 Control level 2 (at the top positive transitions, at the bottom negative transitions)

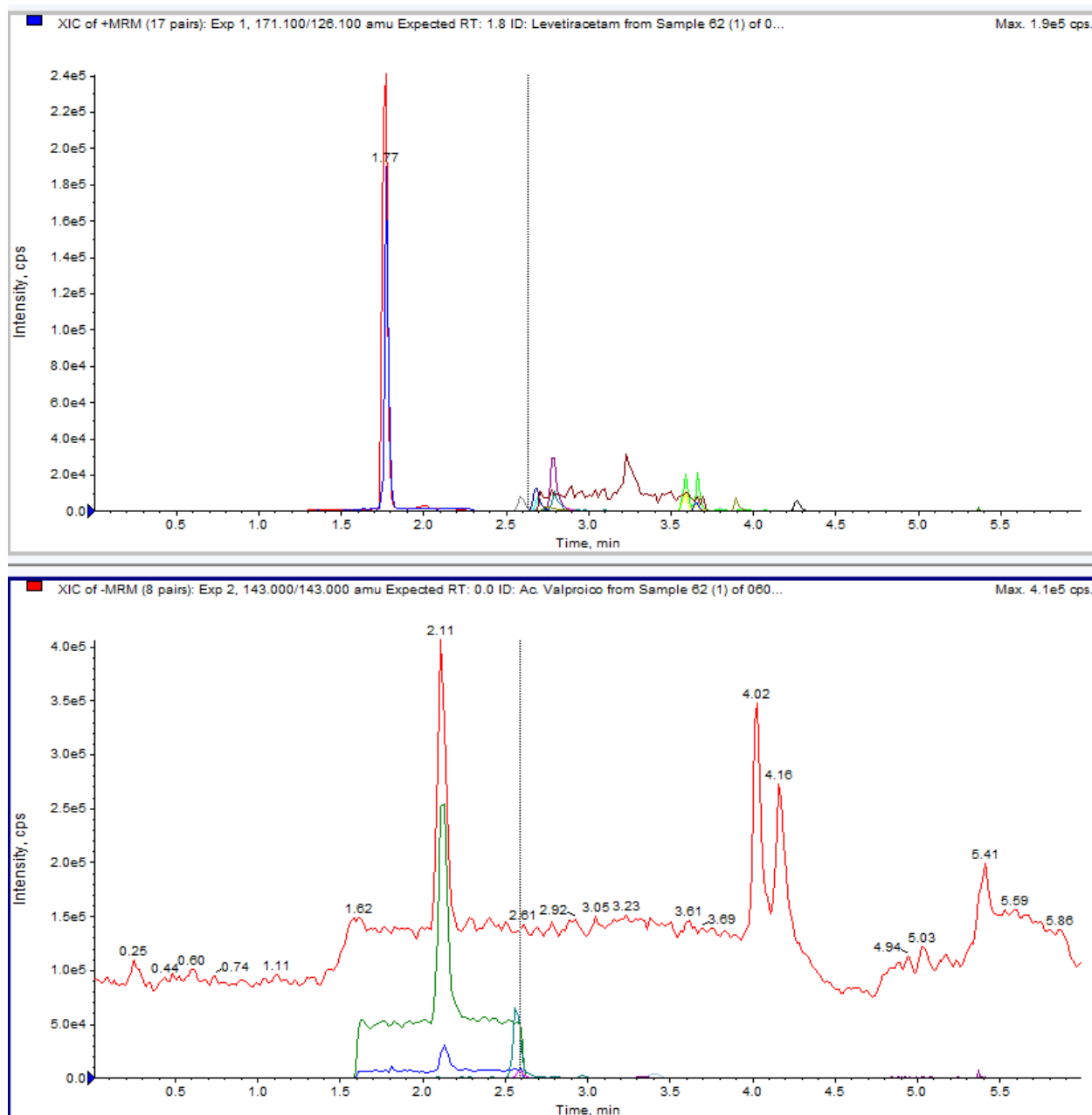
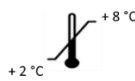
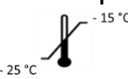
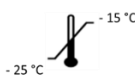


Fig. 3 Real sample (Levetiracetam 2.1 ug/mL), (at the top positive transitions, at the bottom negative transitions)

GENERAL WARNINGS

1. STORAGE AND SHELF LIFE

All the components are ready-to-use. They are stable for 3 years at  except from Reagent A (Deproteinization + Internal Standards Solution) that must be stored at . After opening the shelf life of our internal standards is 6 months if stored at .

2. NEW COLUMN TREATMENT

Install the new Poroshell 120-EC C18 Analytical column (50 x 2,1 mm – 1,9 µm) thermostated at 50°C. Disconnect the detector and flux 30 ml of a solution of Mobile Phase M1:Mobile Phase M2 (20 : 80 v/v) and set flow at 500 µl/minute. Don't recycle the washing solutions.

3. COLUMN CONDITIONING

Conditionate the new Poroshell 120-EC C18 Analytical column (50 x 2,1 mm – 1,9 µm) with Mobile Phases M1:M2 (90 : 10 v/v), set flow at 500 µl / minute and discharge the first 10 ml. It is not possible to make analysis at recycling phase. If room temperature is > 20°C store the Mobile Phase

at  from an analytical session to an another.

4. COLUMN CLEANING

Disconnect the detector. Flux a solution made of Mobile Phase M1:Mobile Phase M2 (30: 70 v/v) set flow at 500 µl / minute for 10 min and discharge. The column must be stored in a solution of Mobile Phases M1:M2 (30 : 70 v/v).

5. AUTOSAMPLER SYRINGE WASHING

Wash the syringe with Methanol at 75% before use.

ABBREVIATIONS

ABBREVIATIONS	MEANING
e.g. 5 x 1 x 1 ml	N° of levels x N° of vials x volume
e.g packaging of the starter kit 27 x 1 ml	N° of vials x volume Note: each vial contains one molecule (analyte or labelled IS)
IS	Internal Standard
pc/pcs	Piece/pieces
RT	Retention Time
STD DEV	Standard Deviation

BIBLIOGRAPHY

1. Faten Farouk *et al.* Simultaneous UPLC-MS/MS Determination of Antiepileptic Agents for Dose Adjustment Biomedical Chromatography 31(7) 2017
2. Tiedong Guo *et al.* Determination of levetiracetam in human plasma/serum/saliva by liquid chromatography-electrospray tandem mass spectrometry Clinica Chimica Acta 375 (2007) 115–118
3. Kwon-Bok Kima *et al.* Simple and accurate quantitative analysis of ten antiepileptic drugs in human plasma by liquid chromatography/tandem mass spectrometry Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 771– 777
4. Lei Yin *et al.* Simultaneous determination of ten antiepileptic drugs in human plasma by liquid chromatography and tandem mass spectrometry with positive/negative ion-switching electrospray ionization and its application in therapeutic drug monitoring J. Sep. Sci. 2016, 39, 964–972

REFERENCE STANDARDS

Quality Assurance System compliant with regulations:

UNI CEI EN ISO 13485:2016,

UNI EN ISO 9001:2015

Any modification made to the Medical Device unless authorized by Eureka S.r.l. - Lab Division makes this declaration invalid.

We declare that the devices comply with the following **Technical Standards**:

UNI CEI EN ISO 14971:2012

UNI CEI EN ISO 15223-1:2017

UNI EN ISO 18113-2:2012

We declare that the device is sold in a non-sterile package.

IVD SYMBOLS

NUMBER	SYMBOL	DESCRIPTION
1		Manufacturer
2		Product Model Number / Designation or Part Number
3		Caution, consult accompanying documents
4		Product Lot Number
5		Use By (Expiry)
6		Consult Operating Instructions
7		Date of manufacture
8		Temperature limitation
9		For <i>in vitro</i> diagnostic use
10		European Union conformity
11		Packaging