



BENZODIAZEPINES AND BARBITURATES IN URINE BY LC-MS/MS

REF LC80310

ALLOBARBITAL, ALPRAZOLAM, AMOBARBITAL, BARBITAL, BROMAZEPAM, BUTALBITAL, CATHINONE, CLOBAZAM, CLONAZEPAM, COCAETHYLENE, DIAZEPAM, HEXOBARBITAL, FENTANYL, FLUNITRAZEPAM, HYDROXY-ALPRAZOLAM, HYDROXY-BROMAZEPAM, LORAZEPAM, LORMETAZEPAM, LSD, Mescaline, NORDIAZEPAM, NORKETAMINE, OXAZEPAM, PENTOBARBITAL, SECButARBITAL, SECOBARBITAL, TEMAZEPAM, THIOPENTAL

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.



INSTRUCTION FOR USE

IVD

For *in vitro* diagnostic use

CE



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INTENDED USE

In vitro diagnostic medical device for the detection and quantitative analysis of ALLOBARBITAL, ALPRAZOLAM, AMOBARBITAL, BARBITAL, BROMAZEPAM, BUTALBITAL, CATHINONE, CLOBAZAM, CLONAZEPAM, COCAETHYLENE, DIAZEPAM, HEXOBARBITAL, FENTANYL, FLUNITRAZEPAM, HYDROXY-ALPRAZOLAM, HYDROXY-BROMAZEPAM, LORAZEPAM, LORMETAZEPAM, LSD, Mescaline, NORDIAZEPAM, NORKETAMINE, OXAZEPAM, PENTOBARBITAL, SECbutarbital, SECOBARBITAL, TEMAZEPAM, THiopental in urine as an aid to the diagnosis of benzodiazepine and barbiturate intoxication and for forensic use. Manual method for professional use.

KIT CONTENT FOR 100 TESTS

CONTENT	DESCRIPTION	PACKAGING	WARNINGS
Reagent A	Buffer Solution	1 x 4 ml	Store at $+2^{\circ}\text{C}$ 
Reagent B	Enzyme of Hydrolysis	1 x 3 ml	Store at $+2^{\circ}\text{C}$ 
Reagent C	Internal Standard Solution	1 x 2 ml	Store at -25°C 
Reagent D	Diluting Solution	1 x 40 ml	Store at $+2^{\circ}\text{C}$ 
Multiparametric Calibrator in urine		4 x 2 x 1 ml	(packed separately – Code LC80316 – see data sheet)
Reagent M1	Mobile Phase M1	2 x 500 ml	Store at $+2^{\circ}\text{C}$ 
Reagent M2	Mobile Phase M2	2 x 500 ml	Store at $+2^{\circ}\text{C}$ 

ACCESSORIES AND CONSUMABLES AVAILABLE SEPARATELY

CODE	DESCRIPTION	PACKAGING
LC80316	Calibrator for Benzodiazepines and Barbiturates in urine	4 x 2 x 1 ml
LC80319	Control for Benzodiazepines and Barbiturates in urine – Levels 1 and 2	2 x 5 x 1 ml
SK80310	Starter kit for Benzodiazepines and Barbiturates in urine	33 x 1 ml
LC80311	Mobile Phase M1	4 x 500 ml
LC80321	Mobile Phase M2	4 x 500 ml
LC80330	Deuterated Internal Standard mix Solution	1 x 2 ml
Z959757902	RRHD Eclipse Plus C18 Analytical Column (50 x 2,1 mm - 1,8 µm) for UHPLC	1 Pc
S29057U	Standard glass vials of 2 ml with screw caps	1 x 100 Pcs

INSTRUMENTS

DESCRIPTION	WARNINGS
LC-MS triple quadrupole (MRM Modality) Divert Valve at 6 ways ESI Interface (+) Binary Pump	Required
Operational Computer	Required
Autosampler	Required

MATRIX - COLLECTION AND STORAGE PROCEDURE

MATRIX	PROCEDURE
Urine	Collect 10 ml of urine. Stable for 3 months at  Centrifuge urinary samples before processing them.

REFERENCE VALUES

There are no shared cut-off values for confirmatory methods for urinary benzodiazepine and barbiturate dosage. Please, refer to the bibliography on page 19.

ANALYTICAL PROCEDURE

STEP	DESCRIPTION																												
1	Sample preparation In vials of 2 ml dispense in sequence: <table><tr><td></td><td>Calibrator (µl)</td><td>Sample (µl)</td><td>Controls (µl)</td></tr><tr><td>Calibrator</td><td>100</td><td></td><td></td></tr><tr><td>Sample</td><td></td><td>100</td><td></td></tr><tr><td>Controls</td><td></td><td></td><td>100</td></tr><tr><td>Reagent A</td><td>40</td><td>40</td><td>40</td></tr><tr><td>Reagent B</td><td>30</td><td>30</td><td>30</td></tr><tr><td>Reagent C</td><td>20</td><td>20</td><td>20</td></tr></table> Close the vials with caps. Vortex per 5 sec.		Calibrator (µl)	Sample (µl)	Controls (µl)	Calibrator	100			Sample		100		Controls			100	Reagent A	40	40	40	Reagent B	30	30	30	Reagent C	20	20	20
	Calibrator (µl)	Sample (µl)	Controls (µl)																										
Calibrator	100																												
Sample		100																											
Controls			100																										
Reagent A	40	40	40																										
Reagent B	30	30	30																										
Reagent C	20	20	20																										
2	Incubate for 1 hour at 55°C. Cool down at room temperature.																												
3	Add 400 µl of <u>Reagent D</u> Vortex for 5 sec.																												
INJECTION	Inject 20 µl of the solution into the LC System.																												

GRADIENT

TIME(min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.0	95	5	400
0.5	95	5	400
8.0	25	75	400
8.1	0	100	400
10.0	0	100	400
10.1	95	5	400
12.0	95	5	400

PARAMETERS

Parameters set on triple quadrupole

	BENZODIAZEPINES AND BARBITURATES ESI POSITIVE (SCIEX 4500)	BENZODIAZEPINES AND BARBITURATES ESI NEGATIVE (SCIEX 4500)
CURTAIN GAS	25	25
COLLISION GAS	10	10
ION SPRAY VOLTAGE	4.500 V	-4.500 V
TEMPERATURE	450°C	450°C
ION SOURCE GAS 1	55	55
ION SOURCE GAS 1	55	55

FRAGMENTATION OF ANALYTES AND DEUTERATED INTERNAL STANDARDS (IS)

ANALYTES	RT	FRAGMENTATION		DEUTERATED IS USED FOR CALIBRATION	RT	FRAGMENTS	
		PARENT ION (Q1)	FRAGMENT ION (Q3)			PARENT ION (Q1)	FRAGMENT ION (Q3)
Allobarbitol (-)	4.1	207,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Alprazolam	6.6	309,0	281,0	Lorazepam-D4	6.6	327,1	281,0
Amobarbital (-)	5.9	225,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Barbital (-)	2.9	183,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Bromazepam	5.7	316,1	209,0	Bromazepam-D4	5.7	320,1	186,0
Butalbital (-)	5.3	223,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Cathinone	1.5	150,0	105,0	Bromazepam-D4	5.7	320,1	186,0
Clobazam	6.4	301,1	259,1	Oxazepam-D5	6.5	292,1	246,1
Clonazepam	6.1	316,7	270,0	Clonazepam-D4	6.1	320,1	274,1
Cocaethylene	4.6	318,1	196,1	Bromazepam-D4	5.7	320,1	186,0
Diazepam	7.2	285,1	154,0	Diazepam-D5	7.2	290,1	198,1
Fentanyl	5.2	337,0	188,2	Bromazepam-D4	5.7	320,1	186,0
Flunitrazepam	6.3	314,1	239,1	Clonazepam-D4	6.1	320,1	274,1
Hexobarbital (-)	5.6	235,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Hydroxy- Alprazolam	6.6	325,1	297,1	Lorazepam-D4	6.6	327,1	281,0
Hydroxy - Bromazepam	5.0	332,1	303,0	Bromazepam-D4	5.7	320,1	186,0
Lorazepam	6.6	321,0	275,0	Lorazepam-D4	6.6	327,1	281,0
Lormetazepam	6.9	335,0	289,0	Lorazepam-D4	6.6	327,1	281,0
LSD	4.7	324,1	223,0	Bromazepam-D4	5.7	320,1	186,0
Mescaline	2.8	212,1	165,0	Bromazepam-D4	5.7	320,1	186,0
Nordiazepam	6.9	271,1	140,0	Diazepam-D5	7.2	290,1	198,1
Norketamine	3.5	225,1	126,0	Bromazepam-D4	5.7	320,1	186,0
Oxazepam	6.5	287,1	104,0	Oxazepam-D5	6.5	292,1	246,1
Pentobarbital (-)	5.9	225,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Secbutarbitol (-)	5.7	211,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Secobarbital (-)	6.3	237,0	42,0	Bromazepam-D4	5.7	320,1	186,0
Temazepam	6.7	301,1	255,1	Oxazepam-D5	6.5	292,1	246,1
Thiopental (-)	6.5	241,0	58,0	Bromazepam-D4	5.7	320,1	186,0

TECHNICAL FEATURES - PART I

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

ANALYTES	CONCENTRATIONS USED TO CALCULATE REPRODUCIBILITY AND ACCURACY (ng/ml)				
	C LLOQ	C1	C2	C3	MAX C
Allobarbital	100	200	500	1.000	2.000
Alprazolam	1	100	200	500	1.000
Amobarbital	10	100	200	500	1.000
Barbital	10	100	200	500	1.000
Bromazepam	1	100	200	500	1.000
Butalbital	100	200	500	1.000	2.000
Cathinone	1	100	200	500	1.000
Clobazam	1	100	200	500	1.000
Clonazepam	1	100	200	500	1.000
Cocaethylene	1	100	200	500	1.000
Diazepam	1	100	200	500	1.000
Fentanyl	1	100	200	500	1.000
Flunitrazepam	10	100	200	500	1.000
Hexobarbital	10	100	200	500	1.000
Hydroxy-Alprazolam	1	100	200	500	1.000
Hydroxy-Bromazepam	1	100	200	500	1.000
Lorazepam	1	100	200	500	1.000
Lormetazepam	1	100	200	500	1.000
LSD	1	100	200	500	1.000
Mescaline	1	100	200	500	1.000
Nordiazepam	1	100	200	500	1.000
Norketamine	1	100	200	500	1.000
Oxazepam	1	100	200	500	1.000
Pentobarbital	10	100	200	500	1.000
Secbutarbitol	10	100	200	500	1.000
Secobarbital	10	100	200	500	1.000
Temazepam	1	100	200	500	1.000
Thiopental	10	100	200	500	1.000

ANALYTES	ACCURACY INTRA SERIE (RELATIVE ERROR %)		ACCURACY INTER SERIE (RELATIVE ERROR %)	
	C1 (%)	C3 (%)	C1 (%)	C3 (%)
Allobarbital	9,53	4,82	12,53	8,43
Alprazolam	3,78	1,89	10,15	5,67
Amobarbital	6,13	7,63	7,83	8,35
Barbital	7,14	3,70	8,65	6,14
Bromazepam	5,86	4,05	10,60	8,21
Butalbital	4,80	1,56	9,54	2,07
Cathinone	1,63	1,26	4,81	5,12
Clobazam	5,75	3,37	11,62	5,93
Clonazepam	5,28	5,58	9,08	8,46
Cocaethylene	4,92	4,15	12,56	8,23
Diazepam	7,95	4,47	10,84	7,89
Fentanyl	3,55	3,84	6,60	4,86
Flunitrazepam	9,75	0,94	10,20	4,20
Hexobarbital	4,38	6,30	8,51	7,10
Hydroxy-Alprazolam	9,97	3,66	9,97	6,19
Hydroxy-Bromazepam	5,79	4,65	7,02	6,98
Lorazepam	5,35	4,69	8,27	7,45
Lormetazepam	6,60	1,78	7,88	6,27
LSD	7,79	6,28	11,85	6,55
Mescaline	10,61	1,14	11,70	4,60
Nordiazepam	4,34	5,21	5,52	6,45
Norketamine	4,61	2,51	6,86	5,51
Oxazepam	6,49	8,85	5,95	11,33
Pentobarbital	10,98	3,12	13,00	7,60
Secbutarbital	3,23	3,79	7,53	4,61
Secobarbital	3,32	6,70	6,60	8,72
Temazepam	2,96	6,72	8,95	6,82
Thiopental	7,30	6,30	8,26	6,88

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 %
Allobarbital	7,88	7,85	7,56	11,38	10,41	10,56
Alprazolam	1,54	1,57	2,18	10,32	9,81	7,35
Amobarbital	1,83	5,67	3,20	10,42	21,49	22,23
Barbital	9,52	2,88	5,54	14,46	12,61	15,07
Bromazepam	5,31	6,53	3,43	7,49	9,22	9,87
Butalbital	5,60	8,15	8,72	12,57	14,53	10,49
Cathinone	1,89	7,25	4,90	8,61	32,46	20,93
Clobazam	7,10	3,87	3,30	8,24	14,18	5,51
Clonazepam	3,69	2,32	3,54	10,53	5,03	4,76
Cocaethylene	7,11	5,17	1,80	7,31	9,28	5,26
Diazepam	6,59	5,11	2,52	6,96	5,61	5,81
Fentanyl	6,34	5,47	2,66	13,95	8,55	9,83
Flunitrazepam	2,99	4,05	0,63	3,07	7,32	3,98
Hexobarbital	4,25	6,70	7,23	14,36	7,50	8,39
Hydroxy-Alprazolam	1,17	1,75	2,17	1,13	12,06	8,74
Hydroxy-Bromazepam	0,68	3,31	2,17	6,95	4,97	5,64
Lorazepam	5,01	5,43	3,08	12,28	8,71	3,76
Lormetazepam	4,18	5,33	2,41	11,78	6,55	6,44
LSD	3,01	5,95	2,17	8,84	7,00	4,75
Mescaline	1,47	7,82	1,32	12,07	8,75	19,93
Nordiazepam	6,14	4,36	6,12	10,31	8,84	6,28
Norketamine	7,65	5,54	2,33	12,03	7,75	16,14
Oxazepam	1,97	4,55	3,15	11,11	10,38	6,81
Pentobarbital	1,71	8,51	7,34	9,43	10,38	10,32
Secbutarbital	5,59	4,59	6,52	12,31	1262	12,04
Secobarbital	4,87	5,39	6,47	13,45	13,40	12,76
Temazepam	3,24	3,81	4,31	9,96	11,95	6,74
Thiopental	14,17	7,55	6,95	15,62	23,91	18,48

TECHNICAL FEATURES - PART III

ANALYTES	LLOD (ng/ml)	LINEARITY ng/ml	COEFFICIENT OF CORRELATION R2 + DEV STD
Allobarbital	33,3	100 – 2.000	0,9907 ± 0,0008
Alprazolam	0,3	1 – 2.000	0,9916 ± 0,0012
Amobarbital	3,3	10 – 2.000	0,9956 ± 0,0006
Barbital	3,3	10 – 2.000	0,9923 ± 0,0039
Bromazepam	0,3	1 – 2.000	0,9949 ± 0,0031
Butalbital	33,3	100 – 2.000	0,9936 ± 0,0021
Cathinone	0,3	1 – 2.000	0,9952 ± 0,0029
Clobazam	0,3	1 – 2.000	0,9922 ± 0,0015
Clonazepam	0,3	1 – 2.000	0,9937 ± 0,0028
Cocaethylene	0,3	1 – 2.000	0,9916 ± 0,0018
Diazepam	0,3	1 – 2.000	0,9941 ± 0,0032
Fentanyl	0,3	1 – 2.000	0,9922 ± 0,0033
Flunitrazepam	3,3	10 – 2.000	0,9952 ± 0,0027
Hexobarbital	3,3	10 – 2.000	0,9935 ± 0,0018
Hydroxy-Alprazolam	0,3	1 – 2.000	0,9928 ± 0,0019
Hydroxy- Bromazepam	0,3	1 – 2.000	0,9955 ± 0,0031
Lorazepam	0,3	1 – 2.000	0,9939 ± 0,0013
Lormetazepam	0,3	1 – 2.000	0,9906 ± 0,0005
LSD	0,3	1 – 1.000	0,9922 ± 0,0025
Mescaline	0,3	1 – 2.000	0,9946 ± 0,0045
Nordiazepam	0,3	1 – 2.000	0,9952 ± 0,0021
Norketamine	0,3	1 – 2.000	0,9943 ± 0,0039
Oxazepam	0,3	1 – 2.000	0,9910 ± 0,0016
Pentobarbital	3,3	10 – 2.000	0,9904 ± 0,0075
Secbutarbital	3,3	10 – 2.000	0,9931 ± 0,0017
Secobarbital	3,3	10 – 2.000	0,9933 ± 0,0033
Temazepam	0,3	1 – 2.000	0,9942 ± 0,0016
Thiopental	3,3	10 – 2.000	0,9930 ± 0,0020

REFERENCE CHROMATOGRAMS

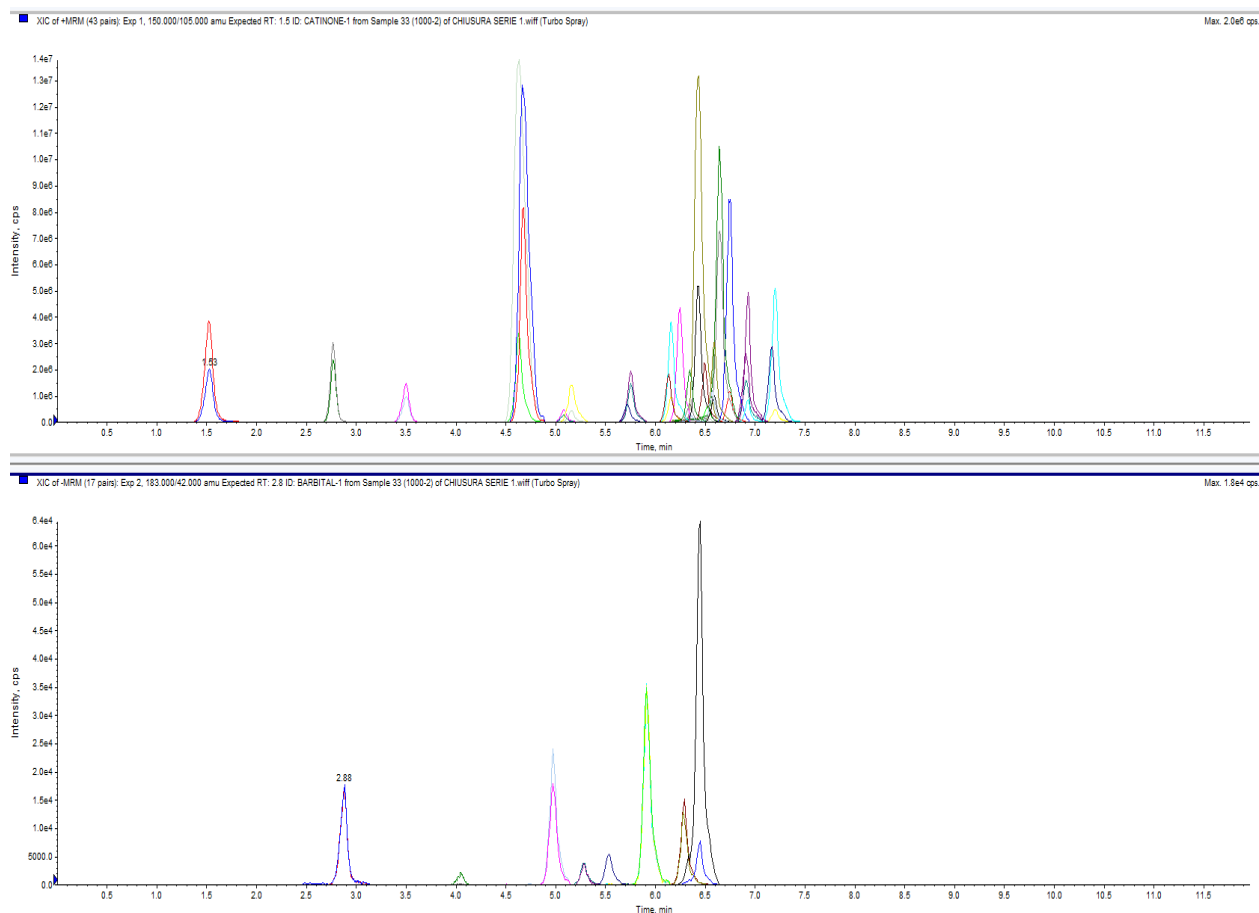
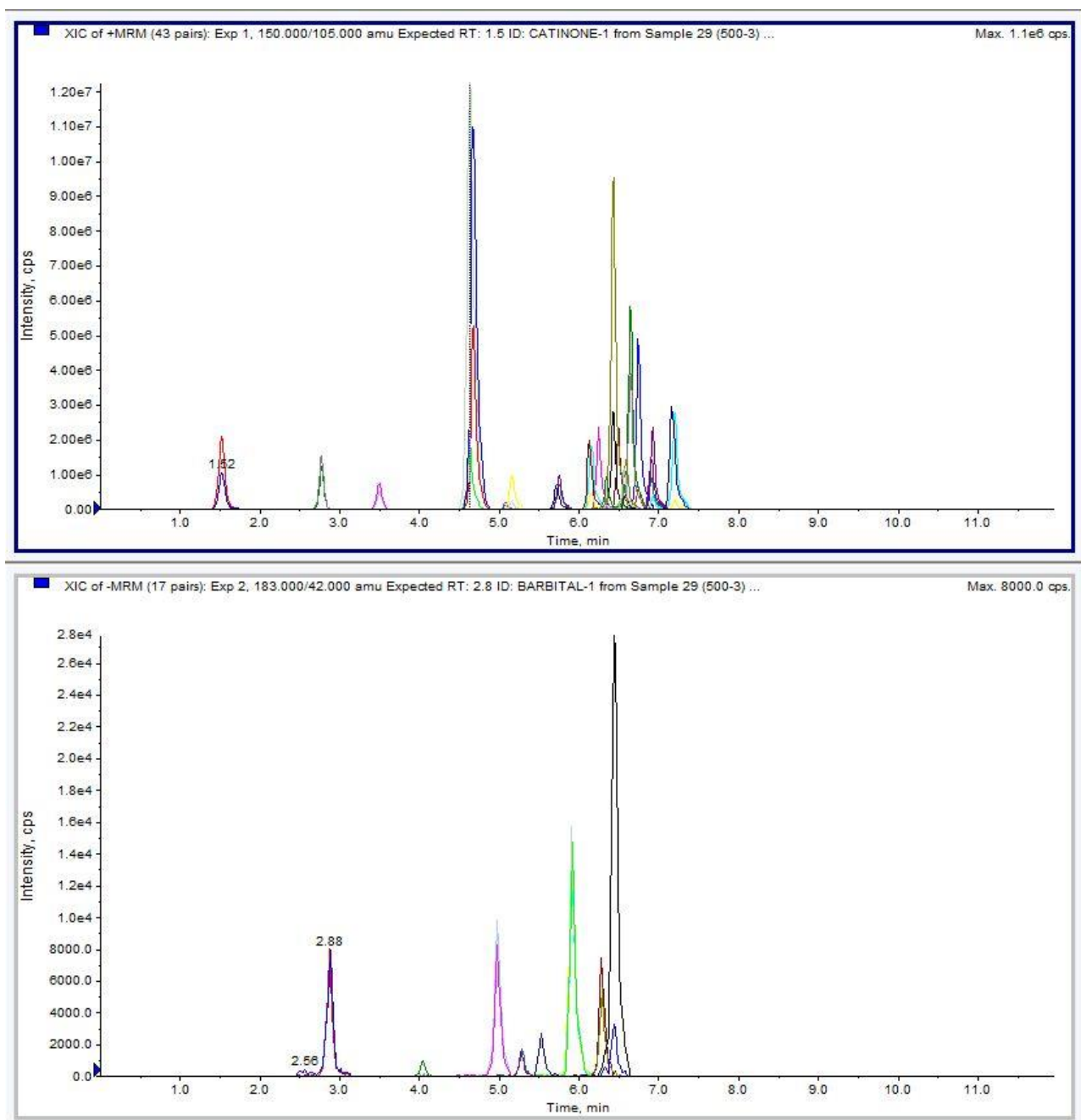


Fig. 1 Multiparametric Calibrator at 1000 ng/mL.

At the top positive MRM transitions; at the bottom negative MRM transitions.

**Fig 2.**

Multiparametric Control at 500 ng/mL

At the top positive MRM transitions, at the bottom negative MRM transitions

GENERAL WARNINGS

1. STORAGE AND SHELF LIFE

All the components are ready to use. They are stable for 3 years at  + 8 °C except the

Reagent C (Internal Standard Solution) that must be stored at  - 25 °C .

After opening the shelf life of our internal standards is 6 months if stored at  - 25 °C .

2. NEW COLUMN TREATMENT

Install the new column RRHD C18 (50 x 2,1 mm - 1,8 µm) thermostated at 40°C. Disconnect the detector and flux 30 ml of a solution of H₂O:Methanol (20:80 v / v) and set flow at 300 µl / minute. Don't recycle the washing solution.

3. COLUMN CONDITIONING

Conditionate the new analytical column RRHD C18 (50 x 2,1 mm - 1,8 µm) with Mobile Phases M2: M1 (95: 5). Set flow at 400 µl / minute for 15 minutes and throw the first 10 ml. Thermostate at 40°C. It's not possible to make analysis at recycling phase. If room temperature is >20°C store

the Mobile Phase at  + 2 °C from an analytical session to another.

4. COLUMN CLEANING

Disconnect the detector. Wash the column with a solution of H₂O: Methanol (20:80 v / v) for 30 minutes and throw it. Store the column in a solution of Mobile Phases M2:M1 (95:5).

5. AUTOSAMPLER SYRINGE WASHING

Wash the syringe with Methanol 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: 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

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2. Langman Lj *et al.* Clinical toxicology In: Rifai N, Horwath AR, Wittwer CT, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Elsevier; 2018:832-887

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