BENZODIAZEPINES in serum by LC/MS – Code LC80210

(Bromazepam, Lorazepam, Clonazepam, Flunitrazepam, Chlordemethyldiazepam, Nordiazepam, Clobazam, Diazepam, Midazolam, Nitrazepam, Oxazepam, Flurazepam, Triazolam, Norclobazam, Zopiclone, Trazodone, Demoxepam, Chlordiazepoxide)

INTRODUCTION

The main effects of benzodiazepines are hypnotic, anxiolytic, anticonvulsant, myorelaxant and amnesic.

There is a large range in potency and elimination rates between different benzodiazepines.

The action of GABA is allosterically modulated by benzodiazepines which interact with the GABA-A receptor complex. Flumazenil is a potent benzodiazepine antagonist that competitively blocks the central effects of benzodiazepines.

Long-term treatment with benzodiazepines induces drug-dependence.

It should be pointed out that the intensity the action of benzodiazepines also depends on the production of active metabolites. Benzodiazepines are composed of a benzene ring fused to a seven membered diazepine ring, a 5-aryl substituent and a 1,4-diazepine ring. Modifications in the structure of the rings give compounds with different properties. Due to their molecular hydrofobic structure, all benzodiazepines are completely absorbed orally and distributed. There is rapid uptake of benzodiazepines into the brain and in all perfused tissues. They bind to plasma proteins and are metabolised in the liver. Benzodiazepine metabolism involves modification and/or removal of substituents, producing desalkylated compounds at position 1 and hydroxylation at position 3, with formation of less hydrofobic compounds. These metabolites are conjugated with glucuronic acid and excreted by urine.

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CE

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.

TECHNICAL FEATURES

Principle of the Method:
Benzodiazepines are deproteinized with a suitable reagent containing the internal standard, centrifuged and directly injected into LC / MS.

Recovery :

	Cloq	Cm	Cs
Bromazepam	96,31	110,31	133,87
Lorazepam	109,19	116,58	136,87
Clonazepam	105,89	119,77	141,36
Flunitrazepam	107,25	119,08	143,84
Chlordemethyldiazepam	100,47	110,07	133,03
Nordiazepam	106,68	109,54	132,95
Clobazam	111,82	117,11	136,88
Diazepam	103,37	111,17	132,93
Midazolam	118,80	125,11	145,12
Nitrazepam	110,17	115,99	138,75
Oxazepam	100,44	108,31	128,13
Flurazepam	108,78	115,45	136,57
Triazolam	116,09	119,85	143,42
Norclobazam	110,91	117,98	136,45
Zopiclone	81,02	84,20	97,67
Trazodone	115,08	106,74	133,85
Demoxepam	88,77	95,41	115,81
Chlordiazepoxide	91,88	100,11	118,51

Sensitivity :

Bromazepam : 0,2 µg/L
Lorazepam : 0,2 µg/L

Clonazepam : 0,05 µg/L
Flunitrazepam : 0,05 µg/L
Chlordemethyldiazepam : 0,1 µg/L
Nordiazepam : 1,0 µg/L

Clobazam : 0,4 µg/L

Diazepam : 1,0 µg/L

Midazolam : 0,2 µg/L

Nitrazepam : 0,1 µg/L

Oxazepam: 1,0 µg/L

Flurazepam: 0,05 µg/L

	Triazolam:	0,04 µg/L
	Norclobazam:	1,0 µg/L
	Zopiclone:	0,05 µg/L
	Trazodone:	1,5 µg/L
	Demoxepam:	1,5 µg/L
	Chlordiazepoxide:	0,4 µg/L
Minimum concentration analyzable (LLOQ) :	***Bromazepam :***	0,6 µg/L
	Lorazepam :	0,6 µg/L
	Clonazepam :	0,2 µg/L
	Flunitrazepam :	0,2 µg/L
	Chlordemethyldiazepam :	0,4 µg/L
	Nordiazepam :	5,0 µg/L
	Clobazam :	1,2 µg/L
	Diazepam :	4,0 µg/L
	Midazolam :	0,6 µg/L
	Nitrazepam :	0,4 µg/L
	Oxazepam:	5,0 µg/L
	Flurazepam:	0,2 µg/L
	Triazolam:	0,1 µg/L
	Norclobazam:	10,0 µg/L
	Zopiclone:	0,2 µg/L
	Trazodone:	5,0 µg/L
	Demoxepam:	6,0 µg/L
	Chlordiazepoxide:	1,2 µg/L
Linearity :	***Bromazepam :***	0,6 - 1440 µg/L
	Lorazepam :	0,6 - 1440 µg/L
	Clonazepam :	0,2 - 480 µg/L
	Flunitrazepam :	0,2 - 480 µg/L
	Clordemethyldiazepam :	0,4 - 960 µg/L
	Nordiazepam :	5,0 - 12000 µg/L
	Clobazam :	1,2 - 2880 µg/L
	Diazepam :	4,0 - 9600 µg/L
	Midazolam :	0,6 - 1440 µg/L
	Nitrazepam :	0,4 - 960 µg/L
	Oxazepam:	5,0 - 6000 µg/L
	Flurazepam:	0,2 - 480 µg/L
	Triazolam:	0,1 - 240 µg/L
	Norclobazam:	10,0 - 9000 µg/L
	Zopiclone:	0,2 - 480 µg/L
	Trazodone:	5,0 - 8400 µg/L
	Demoxepam:	6,0 - 14400 µg/L
	Chlordiazepoxide:	1,2 - 7200 µg/L

Therapeutic Range in serum :

Bromazepam :	50 – 200 µg/L
Lorazepam :	10 – 15 µg/L
	20 – 250 µg/L*
Clonazepam :	4 – 80 µg/L
Flunitrazepam :	5 – 15 µg/L
Chlordemethyldiazepam :	10 – 100 µg/L
Nordiazepam :	20 – 800 µg/L
Clobazam :	30 – 300 µg/L
Diazepam :	200 – 2500 µg/L
Midazolam :	6 – 15 µg/L
	40 – 250 µg/L*
Nitrazepam :	30 – 100 µg/L
Oxazepam:	200 – 1500 µg/L
Flurazepam*:	20 - 100 µg/L
Triazolam:	2 - 20 µg/L
Norclobazam:	300 – 3000 µg/L
Zopiclone:	10 - 50 µg/L
Trazodone:	700 - 1000 µg/L
Demoxepam*:	500 - 740 µg/L
Chlordiazepoxide:	400 - 3000 µg/L

Ref. AGNP Conseus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011
*Ref. Schulz et al.: Therapeutic and toxic blood concentration of nearly 1000 drugs and other xenobiotics. Critical care 2012

Accuracy intra serie (relative error %) Bromazepam:

Ci	Cs
18 µg/L	144 µg/L
2,36%	2,0%

Accuracy inter serie (relative error %) Bromazepam:

Ci	Cs
18 µg/L	144 µg/L
3,64%	4,0%

Reproducibility intra serie (coefficient of variation %) Bromazepam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
3,98%	2,55%	1,44%

Reproducibility inter serie (coefficient of variation %) Bromazepam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
5,07%	3,22%	2,38%

Coefficient of correlation R^2 + Dev Std 0,9966 ± 0,0010 Bromazepam

Accuracy intra serie (relative error %) Lorazepam:

Ci	Cs
18 µg/L	144 µg/L
3,31%	2,58%

Accuracy inter serie (relative error %) Lorazepam:

Ci	Cs
18 µg/L	144 µg/L
4,5%	2,95%

Reproducibility intra serie (coefficient of variation %) Lorazepam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
3,29%	0,98%	1,32%

Reproducibility inter serie (coefficient of variation %) Lorazepam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
5,02%	2,18%	1,63%

Coefficient of correlation R^2 + Dev Std 0,9966 $\pm$ 0,0010 Lorazepam

Accuracy intra serie (relative error %) Clonazepam:

Ci	Cs
6 µg/L	48 µg/L
2,99%	3,61%

Accuracy inter serie (relative error %) Clonazepam:

Ci	Cs
6 µg/L	48 µg/L
3,44%	4,25%

Reproducibility intra serie (coefficient of variation %) Clonazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
6,11%	2,05%	1,86%

Reproducibility inter serie (coefficient of variation %) Clonazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
7,01%	2,8%	2,04%

Coefficient of correlation R^2 + Dev Std 0,9982 $\pm$ 0,0003 Clonazepam

Accuracy intra serie (relative error %) Flunitrazepam:

Ci	Cs
6 µg/L	48 µg/L
1,75%	1,82%

Accuracy inter serie (relative error %) Flunitrazepam:

Ci	Cs
6 µg/L	48 µg/L
2,46%	2,07%

Reproducibility intra serie (coefficient of variation %) Flunitrazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
5,41%	1,96%	1,73%

Reproducibility inter serie (coefficient of variation %) Flunitrazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
7,23%	3,05%	2,38%

Coefficient of correlation R^2 + Dev Std 0,9982 ± 0,0005 Flunitrazepam

Accuracy intra serie (relative error %) Chlordemethyldiazepam:

Ci	Cs
12 µg/L	96 µg/L
1,67%	4,53%

Accuracy inter serie (relative error %) Chlordemethyldiazepam:

Ci	Cs
12 µg/L	96 µg/L
3,63%	6,25%

Reproducibility intra serie (coefficient of variation %) Chlordemethyldiazepam:

Cl	Cm	Cs
0,4 µg/L	48 µg/L	240 µg/L
1,00%	1,31%	0,40%

Reproducibility inter serie (coefficient of variation %) Chlordemethyldiazepam:

Cl	Cm	Cs
0,4 µg/L	48 µg/L	240 µg/L
2,82%	2,11%	0,87%

Coefficient of correlation R^2 + Dev Std 0,9966 ± 0,0010 Chlordemethyldiazepam

Accuracy intra serie (relative error %) Nordiazepam:

Ci	Cs
150 µg/L	1200 µg/L
3,74%	4,21%

Accuracy inter serie (relative error %) Nordiazepam:

Ci	Cs
150 µg/L	1200 µg/L
4,35%	4,01%

Reproducibility intra serie (coefficient of variation %) Nordiazepam:

Cl	Cm	Cs
5 µg/L	600 µg/L	3000 µg/L
4,90%	1,10%	0,67%

Reproducibility inter serie (coefficient of variation %) Nordiazepam:

Cl	Cm	Cs
5 µg/L	600 µg/L	3000 µg/L
5,29%	1,64%	1,78%

Coefficient of correlation R^2 + Dev Std 0,9984 ± 0,0005 Nordiazepam

Accuracy intra serie (relative error %) Clobazam:

Ci	Cs
36 µg/L	288 µg/L
1,70%	4,83%

Accuracy inter serie (relative error %) Clobazam:

Ci	Cs
36 µg/L	288 µg/L
3,49%	5,19%

Reproducibility intra serie (coefficient of variation %) Clobazam:

Cl	Cm	Cs
1,2 µg/L	144 µg/L	720 µg/L
3,41%	0,88%	0,42%

Reproducibility inter serie (coefficient of variation %) Clobazam:

Cl	Cm	Cs
1,2 µg/L	144 µg/L	720 µg/L
5,64%	3,60%	1,46%

Coefficient of correlation R^2 + Dev Std 0,9976 ± 0,0011 Clobazam

Accuracy intra serie (relative error %) Diazepam:

Ci	Cs
120 µg/L	960 µg/L
4,10%	2,73%

Accuracy inter serie (relative error %) Diazepam:

Ci	Cs
120 µg/L	960 µg/L
4,43%	4,10%

Reproducibility intra serie (coefficient of variation %) Diazepam:

Cl	Cm	Cs
4 µg/L	480 µg/L	2400 µg/L
4,97%	1,11%	1,04%

Reproducibility inter serie (coefficient of variation %) Diazepam:

Cl	Cm	Cs
4 µg/L	480 µg/L	2400 µg/L
4,93%	1,97%	1,05%

Coefficient of correlation R^2 + Dev Std 0,9986 ± 0,0002 Diazepam

Accuracy intra serie (relative error %) Midazolam:

Ci	Cs
18 µg/L	144 µg/L
4,02%	3,60%

Accuracy inter serie (relative error %) Midazolam:

Ci	Cs
18 µg/L	144 µg/L
5,13%	4,00%

Reproducibility intra serie (coefficient of variation %) Midazolam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
2,20%	0,58%	1,08%

Reproducibility inter serie (coefficient of variation %) Midazolam:

Cl	Cm	Cs
0,6 µg/L	72 µg/L	360 µg/L
4,30%	1,27%	1,74%

Coefficient of correlation R^2 + Dev Std 0,9987 ± 0,0002 Midazolam

Accuracy intra serie (relative error %) Nitrazepam:

Ci	Cs
12 µg/L	96 µg/L
1,73%	0,65%

Accuracy inter serie (relative error %) Nitrazepam:

Ci	Cs
12 µg/L	96 µg/L
2,65%	1,94%

Reproducibility intra serie (coefficient of variation %) Nitrazepam:

Cl	Cm	Cs
0,4 µg/L	48 µg/L	240 µg/L
3,40%	1,70%	0,55%

Reproducibility inter serie (coefficient of variation %) Nitrazepam:

Cl	Cm	Cs
0,4 µg/L	48 µg/L	240 µg/L
4,19%	2,30%	1,25%

Coefficient of correlation R^2 + Dev Std 0,9985 ± 0,0003 Nitrazepam

Accuracy intra serie (relative error %) Oxazepam:

Ci	Cs
150 µg/L	1200 µg/L
4,53%	2,35%

Accuracy inter serie (relative error %) Oxazepam:

Ci	Cs
150 µg/L	1200 µg/L
5,30%	2,75%

Reproducibility intra serie (coefficient of variation %) Oxazepam:

Cl	Cm	Cs
5 µg/L	600 µg/L	3000 µg/L
6,22%	0,65%	1,12%

Reproducibility inter serie (coefficient of variation %) Oxazepam:

Cl	Cm	Cs
5 µg/L	600 µg/L	3000 µg/L
6,98%	1,57%	1,36%

Coefficient of correlation R^2 + Dev Std 0,9970 ± 0,0009 Oxazepam

Accuracy intra serie (relative error %) Flurazepam:

Ci	Cs
6 µg/L	48 µg/L
3,48%	2,48%

Accuracy inter serie (relative error %) Flurazepam:

Ci	Cs
6 µg/L	48 µg/L
5,46%	3,48%

Reproducibility intra serie (coefficient of variation %) Flurazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
6,00%	0,62%	1,12%

Reproducibility inter serie (coefficient of variation %) Flurazepam:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
6,79%	2,34%	1,66%

Coefficient of correlation R^2 + Dev Std 0,9988 ± 0,0003 Flurazepam

Accuracy intra serie (relative error %) Triazolam:

Ci	Cs
3 µg/L	24 µg/L
2,39%	2,26%

Accuracy inter serie (relative error %) Triazolam:

Ci	Cs
3 µg/L	24 µg/L
4,30%	3,59%

Reproducibility intra serie (coefficient of variation %) Triazolam:

Cl	Cm	Cs
0,1 µg/L	12 µg/L	60 µg/L
7,11%	0,91%	0,91%

Reproducibility inter serie (coefficient of variation %) Triazolam:

Cl	Cm	Cs
0,1 µg/L	12 µg/L	60 µg/L
6,85%	1,20%	1,22%

Coefficient of correlation R^2 + Dev Std 0,9984 ± 0,0003 Triazolam

Accuracy intra serie (relative error %) Norclobazam:

Ci	Cs
300 µg/L	2400 µg/L
4,80%	1,57%

Accuracy inter serie (relative error %) Norclobazam:

Ci	Cs
300 µg/L	2400 µg/L
7,64%	2,02%

Reproducibility intra serie (coefficient of variation %) Norclobazam:

Cl	Cm	Cs
10 µg/L	1200 µg/L	6000 µg/L
2,41%	0,30%	0,27%

Reproducibility inter serie (coefficient of variation %) Norclobazam:

Cl	Cm	Cs
10 µg/L	1200 µg/L	6000 µg/L
4,84%	2,89%	1,44%

Coefficient of correlation R^2 + Dev Std 0,9995 ± 0,00004 Norclobazam

Accuracy intra serie (relative error %) Zopiclone:

Ci	Cs
6 µg/L	48 µg/L
2,84%	3,90%

Accuracy inter serie (relative error %) Zopiclone:

Ci	Cs
6 µg/L	48 µg/L
3,70%	4,29%

Reproducibility intra serie (coefficient of variation %) Zopiclone:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
6,21%	0,71%	0,50%

Reproducibility inter serie (coefficient of variation %) Zopiclone:

Cl	Cm	Cs
0,2 µg/L	24 µg/L	120 µg/L
6,64%	1,25%	1,03%

Coefficient of correlation R^2 + Dev Std 0,9982 ± 0,0001 Zopiclone

Accuracy intra serie (relative error %) Trazodone:

Ci	Cs
180 µg/L	1200 µg/L
7,72%	1,39%

Accuracy inter serie (relative error %) Trazodone:

Ci	Cs
180 µg/L	1200 µg/L
9,23%	4,43%

Reproducibility intra serie (coefficient of variation %) Trazodone:

Cl	Cm	Cs
5 µg/L	600 µg/L	2800 µg/L
4,66%	1,81%	0,34%

Reproducibility inter serie (coefficient of variation %) Trazodone:

Cl	Cm	Cs
5 µg/L	600 µg/L	2800 µg/L
5,53%	3,53%	1,18%

Coefficient of correlation R^2 + Dev Std 0,9980 ± 0,0009 Trazodone

Accuracy intra serie (relative error %) Demoxepam:

Ci	Cs
180 µg/L	1440 µg/L
2,73%	3,87%

Accuracy inter serie (relative error %) Demoxepam:

Ci	Cs
180 µg/L	1440 µg/L
4,21%	5,33%

Reproducibility intra serie (coefficient of variation %) Demoxepam:

Cl	Cm	Cs
6 µg/L	720 µg/L	3600 µg/L
1,06%	1,07%	1,00%

Reproducibility inter serie (coefficient of variation %) Demoxepam:

Cl	Cm	Cs
6 µg/L	720 µg/L	3600 µg/L
6,73%	2,62%	2,06%

Coefficient of correlation R^2 + Dev Std 0,9970 ± 0,0009 Demoxepam

Accuracy intra serie (relative error %) Chlordiazepoxide:

Ci	Cs
180 µg/L	1440 µg/L
3,59%	1,92%

Accuracy inter serie (relative error %) Chlordiazepoxide:

Ci	Cs
180 µg/L	1440 µg/L
6,89%	1,95%

Reproducibility intra serie (coefficient of variation %) Chlordiazepoxide:

Cl	Cm	Cs
1,2 µg/L	720 µg/L	3600 µg/L
4,54%	1,20%	1,32%

Reproducibility inter serie (coefficient of variation %) Chlordiazepoxide:

Cl	Cm	Cs
1,2 µg/L	720 µg/L	3600 µg/L
6,23%	2,56%	1,52%

Coefficient of correlation R^2 + Dev Std 0,9994 ± 0,0002 Chlordiazepoxide

Components of the kit : All the components are ready-to-use. Stability: 3 years at 2-8 °C.

Reagent A – Deproteinization Solution + Internal Standards, **1 x 33 ml** **Store at -20°C**

Serum Calibrator – Level 0, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 1, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 2, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 3, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 4, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 5, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**
Serum Calibrator – Level 6, 1 x 1 ml	**Code LC80016 (pace separately – see data sheet)**

Reagent M1 – Mobile Phase M1, **2 x 500 ml**

Reagent M2 – Mobile Phase M2, **1 x 500 ml**

Minimum Instrumental equipment required:

LC/MS System with triple quadrupole
MRM Work Mode, positive ESI
Chromatograms Recorder

Optional Equipment:

Autosampler.
Operational Computer

Whole Blood Collection Procedure:

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 serum and store at – 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE

STEP 1 : Dispense in an eppendorf:

	Calibrator	Sample	Controls
Calibrator	100 µl		
Sample		100 µl	
Controls			100 µl
Reagent A – Deproteinization Sol. + Internal Standards	300 µl	300 µl	300 µl

Vortex for 20 seconds

STEP 2 : Centrifuge at 14.000 rpm for 5 minutes

STEP 3 : Take the surnatant

N.B.: at this step, the sample is stable 2 days at 2-8 °C and 1 month at – 20 °C

INJECTION :

- Inject 1 - 10 µl of the eluate into the chromatographic system.

BENZODIAZEPINES - Warnings

COLUMN CONDITIONING

Install a new analytical column RRHD Eclipse Plus C18 (50 x 2,1 mm - 1,8 um) thermostated at 50°C. Disconnect the detector and flux a solution of H_2O : Mobile Phase M2 (10 : 90 v/v) set flow at 600 µl / minute. **Don't recycle the washing solutions**. Condition further on the column with a solution of Mobile Phase M1 : Mobile Phase M2 (10 : 90 v/v) set flow at 600 µl / minute for 10 minutes. Then, flux Mobile Phase M1 : Mobile Phase M2 (90 : 10 v/v). Then do 3 injections of mobile phase before analytical session.

It's NOT possible to do analysis at recycling phase.

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux a solution of H_2O : Mobile Phase M2 (10 : 90 v/v) set flow at 600 µl / minute for 15 minutes and discharge. Store the column in a solution of H_2O : Mobile Phase M2 (10 : 90 v/v).

WASH AUTOSAMPLER SYRINGE

Wash with H_2O:ACN:IPA 33:33:33 + 0.1% Formic Ac.

PARAMETERS ESI+ (optimized on triple quadrupole Sciex 4500)

Curtain Gas (CUR): 35
Collision Gas (CAD): 10
Ion Spry Voltage: (IS): 5500 V
Temperature (TEM): 600°C
Ion Source Gas 1 (GS1): 60
Ion Source Gas 2 (GS2): 50

Ionization	Analyte	Internal Standard
Positive	Nordiazepam	*Nordiazepam-d5*
Positive	Nitrazepam	*Nitrazepam-d5*
Positive	Diazepam	*Diazepam-d5*
Positive	Demoxepam	*Demoxepam-d5*
Positive	Oxazepam	*Oxazepam-d5*
Positive	Norclobazam	*Norclobazam-d5*
Positive	Chlordiazepoxide	*Chlordiazepoxide-d5*
Positive	Clobazam	*Norclobazam-d5*
Positive	Chlordemethyldiazepam	*Diazepam-d5*
Positive	Flunitrazepam	*Flunitrazepam-d7*
Positive	Bromazepam	*Bromazepam-d4*
Positive	Clonazepam	*Clonazepam-d4*
Positive	Lorazepam	*Lorazepam-d4*
Positive	Midazolam	*Midazolam-d4*
Positive	Triazolam	*Triazolam-d4*
Positive	Trazodone	*Midazolam-d4*
Positive	Flurazepam	*Norclobazam-d5*
Positive	Zopiclone	*Zopiclone-d8*

FLOW SET

GRADIENT			
Time (min)	**% M1 (PUMP A)**	**% M2 (PUMP B)**	**Flow (µl/min)**
0	90	10	600
0.30	90	10	600
0.31	76	24	600
2.00	60	40	600
5.00	30	70	600
5.70	15	85	600
5.71	15	85	600
6.70	90	10	600
7.50	90	10	600

ANALYTE	**Q1**	**Q3**
NORDIAZEPAM	271.1	165.0
NITRAZEPAM	282.1	236.1
DIAZEPAM	285.0	193.1
OXAZEPAM	287.1	269.0
CLOBAZAM	301.0	259.1
CHLORDEMETHYLDIAZEPAM	305.0	140.0
BROMAZEPAM	316.0	182.1
CLONAZEPAM	316.0	270.0
MIDAZOLAM	326.1	291.0
TRIAZOLAM	343.0	308.0
TRAZODONE	372.1	176.1
FLURAZEPAM	388.4	288.1
FLUNITRAZEPAM	316.0	268.1
ZOPICLONE	389.0	245.0
CHLORDIAZEPOXIDE	300.1	227.1
NORCLOBAZAM	287.1	245.1
DEMOXEPAM	287.0	180.0
LORAZEPAM	321.0	275.0
NORDIAZEPAM-D5	276.1	140.1
NITRAZEPAM-D5	287.1	185.1
DIAZEPAM-D5	290.1	198.1
OXAZEPAM-D5	292.1	246.1

BROMAZEPAM-D4	320.0	186.0
CLONAZEPAM-D4	320.1	274.1
MIDAZOLAM-D4	330.1	295.1
TRIAZOLAM-D4	347.0	312.0
FLUNITRAZEPAM-D7	321.0	275.0
ZOPICLONE-D8	397.0	245.0
CHLORDIAZEPOXIDE-D5	305.1	286.1
NORCLOBAZAM-D5	292.2	250.0
LORAZEPAM-D4	327.0	281.0
DEMOXEPAM-D5	292.1	180.1

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC80016	Serum Calibrator for Benzodiazepines	7 x 2 x 1 ml
LC80019	Controllo sierico per Benzodiazepine – Livelli 1, 2 e 3	3 x 5 x 1 ml
SK80210	Starter kit Benzodiazepines	32 x 1 ml
LC80211	Mobile Phase M1	4 x 500 ml
LC80221	Mobile Phase M2	4 x 500 ml
LC80230	Deuterated Internal Standard Mix Solution in serum	1 x 33 ml
Z959757902	RRHD Eclipse Plus C18 (50 x 2,1 mm – 1,8 µm) Analytical Column	1 Pc
Z51843550	Glass vial with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
Z51820717	Caps for Glass vial with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
L-30-210	Eppendorf Tubes by 1,5 ml with caps	1 x 1000 Pcs

BENZODIAZEPINES IN SERUM

(Reference Chromatograms/Spectra)

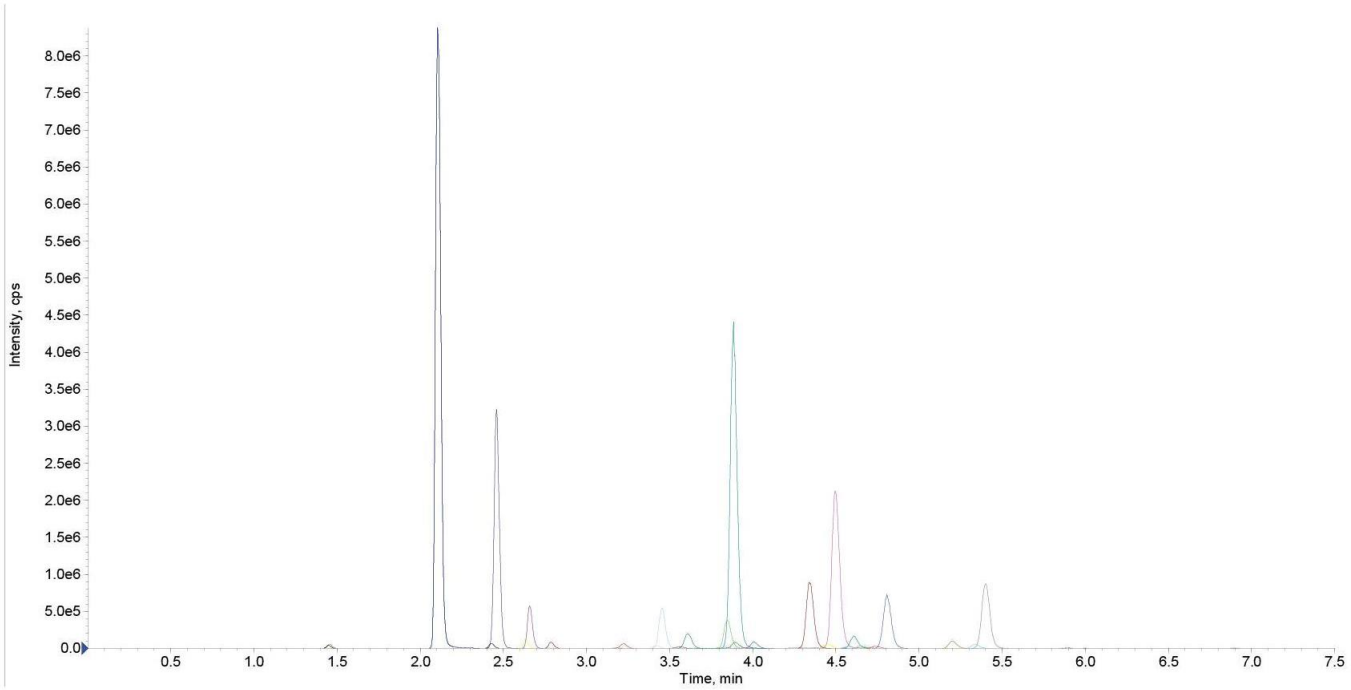

Fig. 1 :	**Benzodiazepines in serum**

N°	Active Principle Name	Commercial Name			
1	Chlordemethyldiazepam	1	EN		
2	Chlordiazepoxide	1	Endequil	8	Psicoterina
		2	Equibral	9	Reliberan
		3	Labican	10	Seren-vita
		4	Librium	11	Smail
		5	Lixin	12	Ansiacal
		6	Philcorium	13	Benzodiapin
		7	Psicofar		
3	Diazepam	1	Aliseum	16	Apozepam
		2	Ansioli	17	AspaserineB_6Tranq
		3	Avex	18	Atensine
		4	Eridan	19	Benzopin
		5	Noan	20	Betapam
		6	Qetinil	21	Calmaven
		7	Quievita	22	Calmigen
		8	Tranquirit	23	Complutine
		9	Valitran	24	Diaceplex
		10	Valium	25	Dialar
		11	Vatran	26	Diaquel
		12	Alupran	27	Diazemuls
		13	Aneurol	28	Ansium
		14	Antenex	29	Anxicalm
		15	Vivol		
4	Flunitrazepam	1	Rohypnol	6	Roipnol
		2	Rohipnol	7	Darkene
		3	Hypnodorm	8	Hypnor
		4	Narcozep	9	Noriel
		5	Valsera		
5	Lorazepam	1	Temesta	13	Lorans
		2	Tran-Qil	14	Tranqipam
		3	Somagerol	15	Novolorazem
		4	Pro-Dorm	16	Duralozam
		5	Orfidal	17	Idalprem
		6	Donix	18	Tolid
		7	Securit	19	Sedizepam
		8	Tavor	20	Almazine
		9	Somnium	21	Ativan
		10	Tèmesta	22	Quait
		11	Punktyl	23	Lorax
		12	Control	24	Laubeel
6	N-Demethyldiazepam	1	Madar		
7	Clobazam	1	Clarmyl	5	Sederlona
		2	Clopax	6	Urbadan
		3	Frisium	7	Urbanol
		4	Noiafren	8	Urbanyl

	Active Principle Name	*Commercial Name*			
8	***Nitrazepam***	1	Somnite	15	Ipersed
		2	Pelson	16	Mitidin
		3	Remnos	17	Mogadon
		4	Paxadorm	18	Surem
		5	Eatan-N	19	Mitidin
		6	Imeson	20	Dormo-Purem
		7	Insoma	21	Serenade
		8	Dormigen	22	Somnipar
		9	Nitrados	23	Ormodon
		10	Mogadan	24	Alodorm
		11	Somnibel-N	25	Apodorm
		12	Unisomnia	26	Arem
		13	Pelsonfilina	27	Persopir
		14	Novanox	28	Tri
9	***Flurazepam***	1	Staurodorm New		
10	***Flurazepam Dihydrochloride***	1	Dalmadorm	5	Midorm
		2	Dalmane	6	Novoflupam
		3	Dormodor	7	Remdue
		4	Irdal		
11	***Flurazepam Monohydrochloride***	1	Dalmadorm	5	Paxane
		2	Dalmane	6	Somnol
		3	Felison	7	Valdorm
		4	Flunox		
12	***Clonazepam***	1	Iktorivil	3	Rivotril
		2	Klonopin		
13	***Delorazepam***	1	Briantum	2	EN
14	***Bromazepam***	1	Bromazanil	8	Lexil
		2	Brozam	9	Lexomil
		3	Compendium	10	Lexotan
		4	Durazanil	11	Lexotanil
		5	Gityl	12	Neo-OPT
		6	Lectopam	13	Normoc
		7	Lexatin		
15	***Lormetazepam***	1	Ergocalm	4	Loretam
		2	Lembrol	5	Minias
		3	Loramet	6	Noctamid
16	***Brotizolam***	1	Lendormin	2	Lendormine
17	***Midazolam***	1	Dormicum	2	Hypnovel
18	***Midazolam Hydrochloride***	1	Dormicum	3	Versed
		2	Hypnovel		

	Active Principle Name	***Commercial Name***			
19	***Midazolam Maleate***	1	Dormicum		
20	***Aprazolam***	1	Tafil	4	Xanax
		2	Trankimazin	5	Xanor
		3	Valeans		
21	***Oxazepam***	1	Adumbram	6	Sedotin
		2	Isodin	7	Serpax
		3	Limbial	8	Serax
		4	Quen	9	Serenid
		5	Quilibrex		
22	***Prazepam***	1	Prazene	5	Mono-demetrin
		2	Centrax	6	Reapam
		3	Demetrin	7	Trepaidam
		4	Lysanxia		
23	***Triazolam***	1	Halcion	4	Novodorm
		2	Apo-Diazo	5	Songar
		3	Dumozolam		
24	***Temazepam***	1	Restoril	3	Levanxol
		2	Euhypnos		