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Forensic toxicology quantitation of 30 benzodiazepines in whole blood using a high-resolution, accurate-mass (HRAM) mass spectrometer

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Keywords

Benzodiazepines, whole blood, liquid chromatography, high resolution, accurate mass, mass spectrometry, Orbitrap, Q Exactive Focus

Goal

Development and analytical evaluation of a method for the analysis of benzodiazepines in whole blood with a high-resolution, accurate-mass instrument.

Application benefits

- Large panel of 30 benzodiazepines in one run
- Short method based on full scan acquisition
- Minimal sample preparation

Introduction

The analysis of drugs in forensic toxicology laboratories is often handled in two stages. An initial step involves screening for a large panel of drugs. Positive detection of the analytes of interest is subsequently followed with quantitation of the drugs found. For the quantitation step, a large number of samples are tested because calibrators and quality controls must be used for each analyte to achieve the highest accuracy of a method. It is therefore of interest to the analyst to have an efficient and simple method to ensure accurate results in a short time.

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Benzodiazepines quantitation is often necessary in forensic toxicology. The main challenge of this determination is the large number of compounds to consider, including some benzodiazepines that are extensively metabolized. Therefore, quantitation is based on the presence of metabolites of the parent drug.

A method based on the use of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ benchtop high-resolution, accurate-mass (HRAM) mass spectrometer was developed for the analysis of 30 benzodiazepines in whole blood. Straightforward sample preparation based on protein precipitation followed by evaporation and reconstitution was performed before analysis.

Experimental

Target analytes

A panel of 30 benzodiazepines and metabolites was analyzed. The list of the target compounds is presented in Table 1.

Table 1. Mass spectrometric properties of the tested target compounds and internal standards.

Compound Name	Chemical Formula	Adduct	Parent *m/z*	Fragment *m/z*	Retention Time (min)
1-Hydroxymidazolam	$C_{18}H_{13}ClFN_3O$	M+H	342.0803	N/A	4.72
3-Hydroxybromazepam	$C_{14}H_{10}BrN_3O_2$	M+H	332.0029	N/A	4.34
7-Aminoclonazepam	$C_{15}H_{12}ClN_3O$	M+H	286.0741	N/A	3.49
7-Aminoflunitrazepam	$C_{16}H_{14}FN_3O$	M+H	284.1193	N/A	4.03
Alpha-hydroxyalprazolam	$C_{17}H_{13}ClN_4O$	M+H	325.0850	N/A	5.41
Alprazolam	$C_{17}H_{13}ClN_4$	M+H	309.0901	N/A	5.7
Bromazepam	$C_{14}H_{10}BrN_3O$	M+H	316.0080	N/A	4.81
Bromazepam D4	$C_{14}H_6BrN_3OD_4$	M+H	320.0331	N/A	4.81
Chlordiazepoxide	$C_{16}H_{14}ClN_3O$	M+H	300.0898	N/A	4.32
Clobazam	$C_{16}H_{13}ClN_2O_2$	M+H	301.0738	259.0626	5.82
Clonazepam	$C_{15}H_{10}ClN_3O_3$	M+H	316.0483	N/A	5.48
Clotiazepam	$C_{16}H_{15}ClN_2OS$	M+H	319.0666	N/A	6.23
Desalkylflurazepam	$C_{15}H_{10}N_2OClF$	M+H	289.0538	N/A	5.68
Diazepam	$C_{16}H_{13}ClN_2O$	M+H	285.0789	N/A	6.1
Estazolam	$C_{16}H_{11}ClN_4$	M+H	295.0745	N/A	5.48
Flunitrazepam	$C_{16}H_{12}FN_3O_3$	M+H	314.0935	N/A	5.65
Flurazepam	$C_{21}H_{23}ClFN_3O$	M+H	388.1586	N/A	4.78
Loprazolam	$C_{23}H_{21}ClN_6O_3$	M+H	465.1436	N/A	4.65
Lorazepam	$C_{15}H_{10}C_{l2}N_2O_2$	M+H	321.0192	N/A	5.99
Lormetazepam	$C_{16}H_{12}C_{l2}N_2O_2$	M+H	335.0348	N/A	5.93
Medazepam	$C_{16}H_{15}ClN_2$	M+H	271.0996	N/A	4.7
Midazolam	$C_{18}H_{13}ClFN_3$	M+H	326.0854	N/A	4.65
Nitrazepam	$C_{15}H_{11}N_3O_3$	M+H	282.0873	N/A	5.3
Norclobazam	$C_{15}H_{11}ClN_2O_2$	M+H	287.0581	245.0475	5.46
Nordiazepam	$C_{15}H_{11}ClN_2O$	M+H	271.0632	N/A	5.69
Oxazepam	$C_{15}H_{11}ClN_2O_2$	M+H	287.0581	241.0531	5.46
Prazepam	$C_{19}H_{17}ClN_2O$	M+H	325.1102	N/A	6.77
Temazepam	$C_{16}H_{13}ClN_2O_2$	M+H	301.0738	255.0677	5.81
Tetrazepam	$C_{16}H_{17}ClN_2O$	M+H	289.1102	N/A	5.96
Zolpidem	$C_{19}H_{21}N_3O$	M+H	308.1757	N/A	4.03
Zolpidem D6	$C_{19}H_{15}N_3OD_6$	M+H	314.2134	N/A	4.03
Zopiclone	$C_{17}H_{17}ClN_6O_3$	M+H	389.1123	N/A	3.25

Calibration standards and QC samples

Calibration solutions were prepared by spiking whole blood (from Etablissment Français du Sang) with a mixture of standards in aqueous solution. Eight calibration levels were used from 2 ng/mL up to 1000 ng/mL. The same process was used to prepare three quality controls, with a different set of standard solutions used.

Sample preparation

Samples were prepared by mixing 200 µL of each sample with 610 µL of an internal standard solution at 42 ng/mL in acetonitrile. The internal standards used for this method were bromazepam-D4 and zolpidem-D6. After vortex mixing and centrifugation at 10,000 × g for 10 min, 600 µL of supernatant were evaporated to dryness with a nitrogen stream and reconstituted in 100 µL of a mixture of mobile phase solvents 30% A / 70% B, described below, for their further injection.

Liquid chromatography

A chromatographic method of 10 minutes was used for the analysis of the benzodiazepines using a Thermo Scientific™ UltiMate™ 3000RS system consisting of an HPG pump, a column oven, and an autosampler. The separation was performed on a Thermo Scientific™ Accucore™ phenyl-hexyl 100 × 2.1 mm, 2.6 µm column at 40 °C. Mobile phases consisted of 2 mM ammonium formate with 0.1% of formic acid in water for phase A, and 2 mM ammonium formate with 0.1% of formic acid in methanol/acetonitrile 50/50 V/V for phase B. The mobile phase was set to 10% B for 1 minute, then increased linearly to 85% B in 6 minutes where it remained for 1 minute, and was increased to 99% B in 30 seconds and maintained 30 seconds in this condition. Equilibration to 10% B lasted 1 minute. Flow rate was set to 0.5 mL/min.

Mass spectrometry

Compounds were detected on a Q Exactive Focus benchtop quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Ion Max source and an HESI-II probe. Data were acquired with two different scan types in parallel: Full Scan and Parallel-Reaction Monitoring (PRM). Full Scan was performed for a mass range from *m/z* 110 to *m/z* 610 at a resolution of 70,000 (FWHM) at *m/z* 200. PRM mode was applied for only 4 compounds extracted in pairs with similar *m/z* ratio, norclobazam, oxazepam, clobazam, and temazepam, and only between 4.5 and 6 minutes. In this mode, a single precursor ion was selected in the quadrupole with an isolation width of 3.0 *m/z* and fragmented in the HCD cell using optimized compound-specific collision energy. The resulting MS/MS product ion spectrum was detected in the Thermo Scientific™ Orbitrap™ analyzer at a resolution of 17,500 (FWHM) at *m/z* 200.

Method evaluation

Linearity was evaluated by collecting calibration curve data (n=6). Calibration samples were run on each day of analysis and calibration curves were obtained by plotting the ratio between the peak area of the substance / peak area of deuterated IS against the concentrations of the calibration standards. The calibration was performed in quadratic mode with a 1/X weighting.

Recovery was evaluated by comparing the signal obtained for six different donor whole blood samples spiked before sample treatment with 100 ng/mL of the compounds against the signal obtained for the same samples spiked with the same concentration of the analytes after sample treatment. The matrix effect was evaluated by comparing the mobile phase solvents at 30% A / 70% B spiked with 100 ng/mL of the compounds to these six donor whole blood matrices but spiked after sample treatment.

Intra- and inter-assay accuracy and precision were obtained for the analysis of three quality controls to the LOQ, in the middle range (80 ng/mL), and in the upper concentration range (800 ng/mL). For intra-assay results, precision was obtained in terms of percentage coefficient of variation (%CV) and accuracy as the bias of the result expressed in percentage for the three levels with six replicates prepared and analyzed in one batch. Inter-assay precision and accuracy were obtained in the same way but for three quality controls prepared and analyzed on six different days.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. For most of the compounds, the parent mass was used for the quantitation, except in the case of norclobazam, oxazepam, clobazam, and temazepam for which the most abundant fragment was used for quantitation. The resulting chromatograms were extracted and reconstructed with a mass accuracy of 5 ppm.

Results and discussion

The calibration curves were obtained by internal calibration for concentrations going from the LOQ up to a concentration of 1000 ng/mL. The LOQ was defined as the lowest concentration in the studied concentration range (2 ng/mL to 1000 ng/mL) for which intra- and inter-day precision and accuracy were less than 20%. Table 2 presents these results as well as the internal standards used for each compound. Representative chromatograms at the LOQ and reconstructed with a mass accuracy of 5 ppm are reported in Figure 1.

Table 2. Results obtained for LOQ, recovery, and matrix effects.

Compound Name	Internal Standard	Type	Weighting	R^2	LOQ (ng/mL)	Recovery	Matrix Effects
1-Hydroxymidazolam	Bromazepam D4	QUAD	1/X	0.9919	2	73%	94%
3-Hydroxybromazepam	Bromazepam D4	QUAD	1/X	0.9943	2	77%	102%
7-Aminoclonazepam	Bromazepam D4	QUAD	1/X	0.9938	5	56%	86%
7-Aminoflunitrazepam	Bromazepam D4	QUAD	1/X	0.9963	5	55%	77%
Alpha-hydroxyalprazolam	Bromazepam D4	QUAD	1/X	0.9951	2	77%	97%
Alprazolam	Bromazepam D4	QUAD	1/X	0.9922	2	63%	79%
Bromazepam	Bromazepam D4	QUAD	1/X	0.9947	2	65%	107%
Chlordiazepoxide	Bromazepam D4	QUAD	1/X	0.9931	5	56%	75%
Clobazam	Bromazepam D4	QUAD	1/X	0.9942	2	59%	99%
Clonazepam	Bromazepam D4	QUAD	1/X	0.9938	2	81%	86%
Clotiazepam	Bromazepam D4	QUAD	1/X	0.9947	2	111%	114%
Desalkylflurazepam	Bromazepam D4	QUAD	1/X	0.9938	2	63%	96%
Diazepam	Bromazepam D4	QUAD	1/X	0.994	2	107%	106%
Estazolam	Bromazepam D4	QUAD	1/X	0.9939	2	51%	83%
Flunitrazepam	Bromazepam D4	QUAD	1/X	0.9924	2	57%	83%
Flurazepam	Bromazepam D4	QUAD	1/X	0.9938	2	92%	74%
Loprazolam	Bromazepam D4	QUAD	1/X	0.9922	2	69%	76%
Lorazepam	Bromazepam D4	QUAD	1/X	0.9956	2	90%	98%
Lormetazepam	Bromazepam D4	QUAD	1/X	0.9945	2	63%	104%
Medazepam	Bromazepam D4	QUAD	1/X	0.9943	2	55%	78%
Midazolam	Bromazepam D4	QUAD	1/X	0.9944	2	71%	75%
Nitrazepam	Bromazepam D4	QUAD	1/X	0.9931	2	61%	88%
Norclobazam	Bromazepam D4	QUAD	1/X	0.9955	2	96%	91%
Nordiazepam	Bromazepam D4	QUAD	1/X	0.9941	2	54%	94%
Oxazepam	Bromazepam D4	QUAD	1/X	0.9961	2	61%	90%
Prazepam	Bromazepam D4	QUAD	1/X	0.9959	5	46%	81%
Temazepam	Bromazepam D4	QUAD	1/X	0.9947	2	66%	100%
Tetrazepam	Bromazepam D4	QUAD	1/X	0.9937	2	65%	75%
Zolpidem	Zolpidem D6	QUAD	1/X	0.9949	2	58%	88%
Zopiclone	Zolpidem D6	QUAD	1/X	0.9925	2	68%	78%

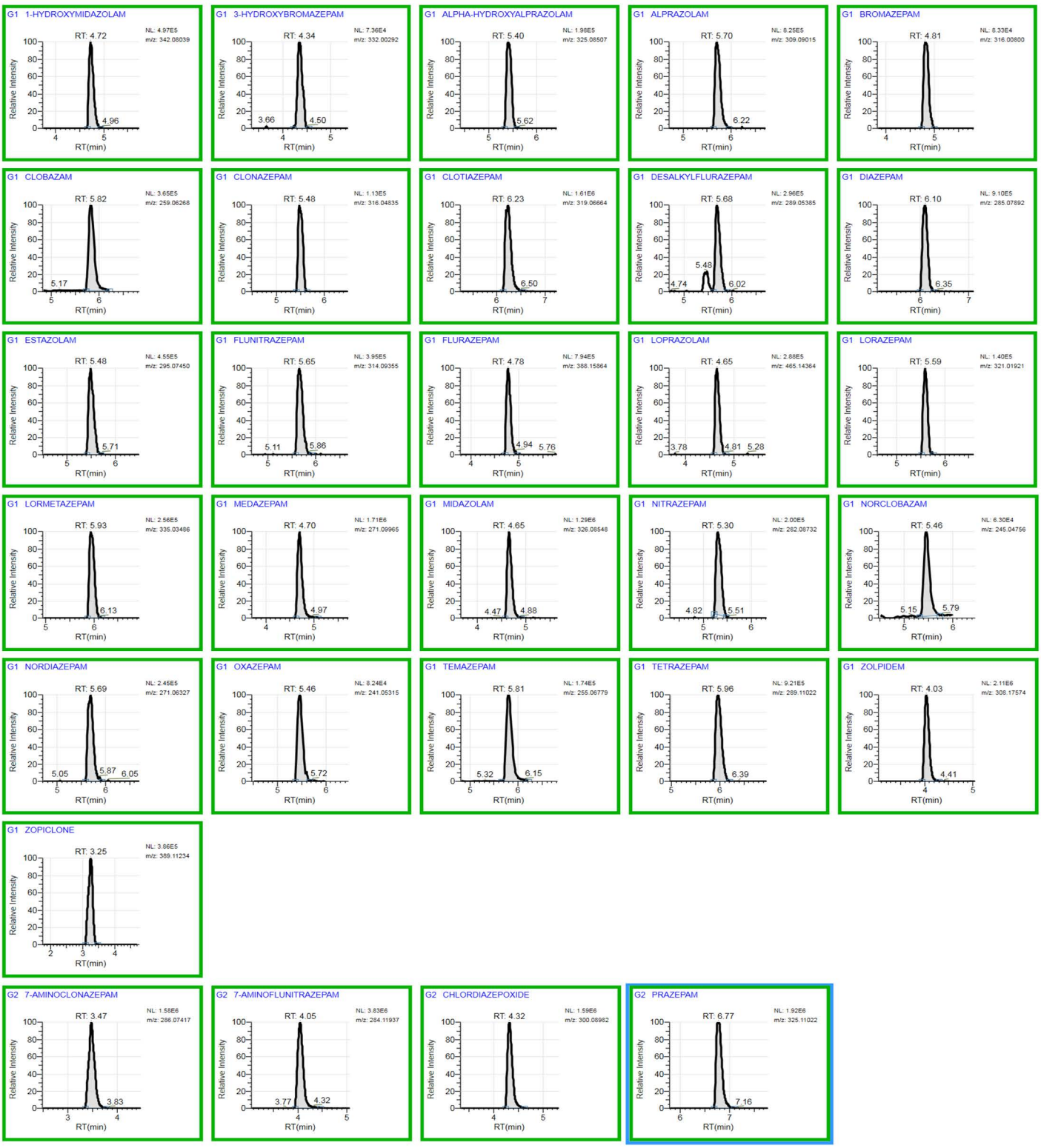

Figure 1. Representative chromatograms at the LOQ for the analyzed benzodiazepines.

The global recovery and the matrix effects observed for each analyte based on the average for six different whole blood matrices from donor samples are also documented in Table 2. The donor samples as well as the matrix used for calibration curves preparation were tested before and the absence of all of the studied compounds was confirmed. The reported averaged matrix effect is based on the comparison of the signal obtained from a real sample spiked after preparation as compared to a standard at the same concentration. Observed matrix effects are between 74% and 114% and are still in the acceptance limit of <15% CV for the variability of matrix effects between matrix batches. The matrix effects can be reduced if isotopically labelled internal standards are used for each compound. Most of the compounds have approximately 70% recovery, and this recovery is consistent within the different matrix sources (n=6). This recovery is in agreement with the usual concentrations to be assessed in whole blood samples.

The results are correct for the precision and accuracy assays within the concentration ranges tested in this work. Intra-assay and inter-assay accuracy in %bias and precision in %CV were found below 20% for the low concentration (2 ng/mL or 5 ng/mL depending on the LOQ) quality control, and below 15% for the other quality controls. The complete results of this evaluation are found in Table 3.

Table 3A. Intra- and inter-assay results.

Molecule	Intra-assay results				Inter-assay results			LOQ
	Theoretical Concentration (ng/mL)	Average Concentration (ng/mL)	%Bias	%CV	Average Concentration (ng/mL)	%Bias	%CV	
1-Hydroxymidazolam	2	2.2	12.0	3.6	2.1	5.0	10.7	LOQ
	5	4.7	-5.8	1.7	5.2	4.5	10.3	
	80	87.1	8.9	2.6	88.8	10.9	11.1	
	800	856.0	7.0	1.8	825.6	3.2	11.4	
3-Hydroxybromazepam	2	1.9	-2.8	5.0	1.9	-6.8	11.3	LOQ
	5	4.5	-9.5	6.1	5.2	3.4	8.9	
	80	80.7	0.9	2.1	80.6	0.7	9.7	
	800	825.9	3.2	1.3	794.9	-0.6	5.6	
7-Aminoclonazepam	2	2.1	6.8	6.0	1.8	-10.6	21.5	
	5	5.1	1.4	7.3	4.8	-4.5	11.9	LOQ
	80	84.2	5.2	4.7	80.5	0.6	11.3	
	800	792.6	-0.9	2.8	773.6	-3.3	8.6	
7-Aminoflunitrazepam	2	1.7	-17.4	8.0	1.6	-21.7	12.6	
	5	4.8	-4.0	7.7	4.7	-5.5	11.1	LOQ
	80	86.5	8.1	4.7	78.3	-2.1	11.1	
	800	752.0	-6.0	3.8	713.6	-10.8	7.4	
Alpha-hydroxyalprazolam	2	2.1	7.3	2.7	2.1	6.9	10.4	LOQ
	5	4.3	-13.1	3.6	5.0	-0.1	16.1	
	80	79.4	-0.7	0.8	80.4	0.4	15.4	
	800	835.4	4.4	1.6	802.0	0.3	6.7	
Alprazolam	2	1.9	-5.7	4.6	2.1	3.4	5.5	LOQ
	5	4.4	-11.3	2.5	4.5	-9.4	8.0	
	80	82.7	3.4	0.9	71.1	-11.1	12.7	
	800	806.6	0.8	1.9	772.2	-3.5	6.8	
Bromazepam	2	2.3	13.1	16.2	2.0	1.9	12.0	LOQ
	5	5.0	0.9	5.7	4.8	-4.2	6.8	
	80	77.4	-3.3	1.8	79.5	-0.6	7.1	
	800	820.4	2.6	1.5	824.7	3.1	4.1	

Table 3B. Intra- and inter-assay results.

Molecule	Intra-assay results				Inter-assay results			LOQ
	Theoretical Concentration (ng/mL)	Average Concentration (ng/mL)	%Bias	%CV	Average Concentration (ng/mL)	%Bias	%CV	
Chlordiazepoxide	2	1.9	-7.1	8.0	1.5	-25.0	19.6	
	5	4.7	-6.9	7.3	4.8	-3.9	13.8	LOQ
	80	82.8	3.6	4.3	84.2	5.2	7.1	
	800	797.5	-0.3	3.4	786.2	-1.7	9.2	
Clobazam	2	2.0	0.5	2.9	1.9	-3.4	7.0	LOQ
	5	4.8	-4.2	3.0	5.3	5.8	11.5	
	80	86.7	8.4	1.4	82.0	2.5	15.1	
	800	717.7	-10.3	2.6	787.1	-1.6	10.2	
Clonazepam	2	2.1	6.5	4.6	2.1	3.5	9.4	LOQ
	5	4.5	-9.4	2.6	4.9	-2.0	13.5	
	80	81.9	2.4	0.9	79.0	-1.2	10.5	
	800	833.7	4.2	1.5	787.8	-1.5	6.1	
Clotiazepam	2	1.9	-4.7	2.6	2.1	6.8	20.4	LOQ
	5	4.6	-8.5	3.6	4.8	-4.1	8.0	
	80	81.5	1.9	0.8	75.8	-5.2	5.3	
	800	782.1	-2.2	4.1	808.6	1.1	5.3	
Desalkylflurazepam	2	2.1	7.3	9.6	2.1	6.7	8.1	LOQ
	5	4.8	-4.7	3.3	5.0	0.3	11.0	
	80	83.3	4.1	1.0	79.0	-1.2	9.6	
	800	771.9	-3.5	9.1	791.9	-1.0	13.5	
Diazepam	2	1.9	-3.1	6.7	2.0	0.1	8.3	LOQ
	5	4.4	-12.7	2.8	4.8	-3.2	9.9	
	80	82.4	3.1	1.0	81.9	2.4	7.1	
	800	797.6	-0.3	1.3	802.3	0.3	4.6	
Estazolam	2	2.0	2.4	4.6	2.3	13.1	4.4	LOQ
	5	4.4	-12.2	1.4	5.0	0.3	7.5	
	80	80.7	0.9	0.6	83.1	3.9	6.1	
	800	815.0	1.9	1.8	851.6	6.4	5.0	
Flunitrazepam	2	1.9	-4.4	3.1	2.0	-1.8	8.3	LOQ
	5	4.4	-11.3	2.1	4.7	-5.0	5.4	
	80	83.5	4.4	0.7	76.9	-3.9	6.2	
	800	817.4	2.2	1.5	821.4	2.7	5.2	
Flurazepam	2	1.7	-14.5	1.7	2.0	1.9	12.9	LOQ
	5	4.7	-6.4	2.4	5.2	3.5	10.5	
	80	78.7	-1.6	0.7	78.2	-2.2	13.4	
	800	793.9	-0.8	1.8	830.3	3.8	7.8	
Loprazolam	2	2.0	1.3	4.2	2.1	3.1	12.5	LOQ
	5	4.4	-12.7	3.2	4.7	-6.0	7.4	
	80	76.9	-3.9	1.2	76.8	-4.1	11.1	
	800	759.5	-5.1	2.2	761.8	-4.8	9.7	
Lorazepam	2	2.0	2.3	3.9	2.2	8.3	10.8	LOQ
	5	4.5	-10.5	2.4	4.8	-4.9	7.6	
	80	80.2	0.3	0.9	76.0	-4.9	5.6	
	800	819.1	2.4	2.1	803.3	0.4	2.1	

Table 3C. Intra- and inter-assay results.

Molecule	Intra-assay results				Inter-assay results			LOQ
	Theoretical Concentration (ng/mL)	Average Concentration (ng/mL)	%Bias	%CV	Average Concentration (ng/mL)	%Bias	%CV	
Lormetazepam	2	2.2	9.4	3.3	2.1	5.2	8.3	LOQ
	5	4.5	-11.0	2.9	4.7	-6.2	8.7	
	80	79.9	-0.1	0.8	76.0	-4.9	7.6	
	800	789.4	-1.3	3.0	800.0	0.0	3.1	
Medazepam	2	2.1	3.4	1.5	2.1	5.5	12.3	LOQ
	5	4.6	-7.1	3.4	5.0	0.1	11.8	
	80	83.6	4.4	1.7	77.6	-3.1	6.2	
	800	788.3	-1.5	4.4	786.3	-1.7	9.3	
Midazolam	2	2.0	-1.3	3.9	2.1	4.8	11.9	LOQ
	5	4.7	-5.7	3.3	5.3	5.1	5.8	
	80	79.9	-0.1	3.9	89.1	11.4	6.1	
	800	898.0	12.2	4.3	868.1	8.5	9.6	
Nitrazepam	2	2.1	3.8	7.1	2.0	2.2	12.1	LOQ
	5	4.3	-13.2	2.0	4.7	-6.5	7.8	
	80	81.9	2.3	1.1	76.5	-4.4	7.4	
	800	816.8	2.1	0.8	805.0	0.6	2.5	
Norclobazam	2	2.2	10.5	2.9	2.1	2.9	13.3	LOQ
	5	4.8	-3.6	2.6	4.8	-3.2	8.1	
	80	83.6	4.5	1.7	79.4	-0.7	5.2	
	800	880.1	10.0	2.2	815.9	2.0	7.1	
Nordiazepam	2	2.0	1.2	6.8	2.1	7.4	9.7	LOQ
	5	4.4	-11.1	3.7	5.0	0.2	10.3	
	80	81.2	1.5	1.0	82.9	3.6	6.7	
	800	802.7	0.3	1.8	841.7	5.2	8.0	
Oxazepam	2	2.1	6.3	1.9	2.0	-0.2	11.1	LOQ
	5	4.6	-7.6	1.9	5.0	-0.9	7.2	
	80	82.9	3.7	1.0	82.6	3.3	5.2	
	800	835.6	4.5	3.0	834.6	4.3	9.5	
Prazepam	2	2.0	0.9	2.5	2.4	21.7	7.6	
	5	4.5	-9.3	2.8	4.9	-1.4	7.2	LOQ
	80	77.9	-2.6	1.8	77.8	-2.7	5.4	
	800	814.7	1.8	2.3	858.0	7.2	5.4	
Temazepam	2	2.1	6.0	2.1	2.1	2.6	8.2	LOQ
	5	4.7	-5.6	2.2	4.9	-2.8	7.0	
	80	83.9	4.9	1.0	81.7	2.2	9.4	
	800	846.8	5.9	2.0	852.1	6.5	7.3	
Tetrazepam	2	2.0	1.9	7.2	2.0	0.8	17.2	LOQ
	5	4.4	-12.3	8.3	4.5	-11.0	8.2	
	80	78.7	-1.6	2.6	73.2	-8.5	4.0	
	800	792.4	-1.0	2.5	757.8	-5.3	3.7	
Zolpidem	2	2.1	3.1	1.6	2.1	3.7	10.7	LOQ
	5	4.5	-9.8	1.6	4.8	-4.1	7.1	
	80	83.0	3.7	0.8	79.5	-0.7	7.1	
	800	823.6	2.9	1.2	792.0	-1.0	3.9	
Zopiclone	2	1.9	-6.3	5.3	2.2	9.3	8.2	LOQ
	5	4.6	-7.0	4.9	4.9	-2.2	7.3	
	80	78.5	-1.8	1.4	79.5	-0.7	8.7	
	800	798.4	-0.2	1.0	832.2	4.0	3.1	

Conclusions

An HPLC method coupled to HRAM Orbitrap detection was implemented for the quantitation of 30 benzodiazepines in whole blood. Sample preparation is simple, efficient, and economical with the use of only two internal standards for the whole benzodiazepines panel. Good accuracy and precision were obtained for the studied range of concentrations that is convenient for the forensic toxicology analysis of benzodiazepines. The results obtained herein show that the Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer, extensively used in forensic screening, can equally be used in quantitative analysis. Furthermore, the mobile phases and the column used for this project are the same that we have used before for screening purposes (refer to technical note TN65008[1]). This allows the fast and easy switching between screening and further quantitation of benzodiazepines in whole blood.

Reference

1. Thermo Scientific Technical Note TN650008, Targeted forensic screening and semi-quantitation of drugs using high-resolution, accurate-mass detection and online sample preparation, 2017 [online] http://tools.thermofisher.com/content/sfs/brochures/TN-65008-LC-MS-Forensic-Drug-Screening-Plasma-TN65008-EN.pdf

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