TECHNICAL NOTE 73574

Quantification of 36 benzodiazepines in human plasma or serum by LC-HRAM(MS) for clinical research

Authors: Claudio De Nardi[1], Katharina Kern[2], Steffen Peters[2]

[1]Thermo Fisher Scientific GmbH, Dreieich, Germany
[2]RECIPE Chemicals + Instruments GmbH, Munich, Germany

Keywords: Benzodiazepines, offline sample preparation, plasma, serum, Orbitrap, high-resolution mass spectrometry

Application benefits

- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- High-resolution mass spectrometry for improved selectivity
- Robust, sensitive hardware enables increased confidence in data
- Simple offline sample preparation by protein precipitation
- 36 benzodiazepines in a single quantitative method

Goal

Implementation of an analytical method for the quantification of 36 benzodiazepines in human plasma or serum on a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer.

Introduction

Benzodiazepines (BZP) are a group of psychoactive drugs with a broad range of therapeutic effects. These controlled substances are some of the most frequently prescribed medications worldwide and are frequently prone to abuse by those without valid medical prescriptions.

An analytical method for clinical research for the quantification of 36 benzodiazepines in human plasma or serum is reported in this study. A comprehensive list of analytes, corresponding internal standards and concentration ranges covered by the method are reported in Table 1. Most reported LC-MS analyses of the above-mentioned benzodiazepines involve triple quadrupole mass spectrometers, traditionally used for targeted, sensitive

quantitation assays. However, in this report we present LC-MS data acquired using high-resolution accurate-mass (HRAM) mass spectrometry leveraging Orbitrap technology. This report demonstrates the capability of HRAM for routine quantitation analyses in addition to its use for performing in-depth qualitative investigations.

Table 1. Analytes, internal standards, and concentration ranges

Analyte	Internal standard	Conc. range (ng/mL)
3–Hydroxybromazepam	7–Aminoflunitrazepam–D_7	16.6–258
7–Aminoclonazepam	7–Aminoclonazepam–D_4	5.02–70.3
7–Aminoflunitrazepam	7–Aminoflunitrazepam–D_7	5.36–76.5
7–Aminonitrazepam	7–Aminoclonazepam–D_x	21.6–304
alpha–OH–Alprazolam	alpha–OH–Alprazolam–D_5	5.74–81.1
alpha–OH–Midazolam	alpha–OH–Midazolam–D_4	10.6–135
alpha–OH–Triazolam	alpha–OH–Triazolam–D_4	3.92–63.5
Alprazolam	Alprazolam–D_5	5.14–76.4
Bromazepam	alpha–OH–Alprazolam–D_5	21.3–319
Brotizolam	Temazepam–D_5	2.00–30.0
Chlordiazepoxide	Chlordiazepoxide–D_5	260–3512
Clobazam	Nordiazepam–D_5	27.7–460
Clonazepam	Clonazepam–D_4	4.94–72.3
Demoxepam	7–Aminoflunitrazepam–D_7	231–3524
Desalkylflurazepam	Zolpidem–D_6	17.1–274
Desmethylflunitrazepam	Oxazepam–D_5	4.78–84.3
Diazepam	Diazepam–D_5	128–1733
Estazolam	Estazolam–D_5	44.6–679
Flunitrazepam	Flunitrazepam–D_7	5.25–76.6
Flurazepam	Midazolam–D_4	10.4–155
Lorazepam	Lorazepam–D_4	19.2–276
Lormetazepam	Midazolam–D_4	1.97–27.2
Medazepam	Prazepam–D_5	75.3–1047
Midazolam	Midazolam–D_4	20.5–300
Nitrazepam	Nitrazepam–D_5	18.9–293
Norclobazam	Estazolam–D_5	240–4281
Nordiazepam	Nordiazepam–D_5	116–1653
Oxazepam	Oxazepam–D_5	117–1808
Prazepam	Prazepam–D_5	89.4–1259
Temazepam	Temazepam–D_5	115–1587
Tetrazepam	Prazepam–D_5	45.0–640
Trazodone	Midazolam–D_4	180–2396
Triazolam	Triazolam–D_4	3.46–43.8
Zaleplone	7–Aminoflunitrazepam–D_7	17.3–253
Zolpidem	Zolpidem–D_6	36.6–603
Zopiclone	Chlordiazepoxide–D_5	16.5–279

Plasma or serum samples were processed by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with heated electrospray ionization (H-ESI II). Detection was performed by full scan coupled to data-dependent fragmentation (fullMS-ddMS^2) using 21 deuterated internal standards. The full scan experiment was used for quantification, and fragmentation for confirmation. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass® Add-On Set for Benzodiazepines from RECIPE® Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, lower limit of quantification (LLOQ), carryover, accuracy, trueness of measurements, and intra- and inter-assay precision for each analyte.

Experimental

Target analytes

The concentration ranges covered by the calibrators (MS6013 batch #1069) used are reported in Table 1.

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS6082 batch #1267), as well as 20 deuterated internal standards for the quantification. Samples of 50 µL of plasma or serum were protein-precipitated using 100 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged. 100 µL of supernatant were transferred to a clean plate or vial and diluted with 100 µL of a dilution solution provided with the kit (MS9022).

Liquid chromatography

A Vanquish Duo UHPLC system, a dual-channel instrument configured for both LC-only and online SPE applications (Figure 1), was used for chromatographic separation. The LC-only channel was used in this case, utilizing mobile phases and an analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 6.8 minutes.

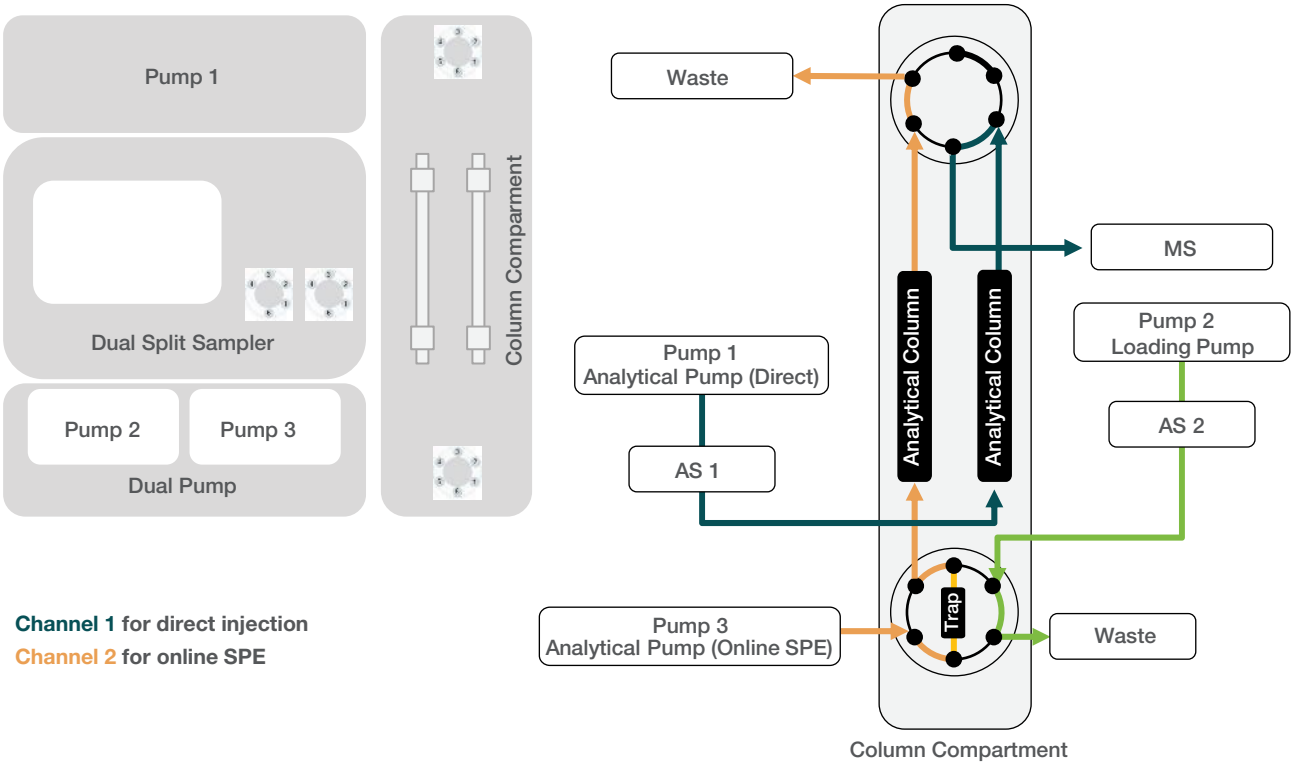

Figure 1. Schematic representation of the Vanquish Duo UHPLC system setup

Table 2. Liquid chromatography method description

Gradient profile			
Time (min)	**Flow rate (mL/min)**	**A (%)**	**B (%)**
0.00	0.6	90	10
0.21	0.6	90	10
0.30	0.6	76	24
3.00	0.6	74	26
6.20	0.6	40	60
6.21	0.6	15	85
6.40	0.6	15	85
6.41	0.6	90	10
6.80	0.6	90	10
Other parameters			
Injection volume		15 µL	
Column temperature		40 °C	

Table 3. MS settings

Parameter	Value
Source type	Heated electrospray ionization (H-ESI II)
Vaporizer temperature	450 °C
Capillary temperature	275 °C
Spray voltage (positive mode)	3000 V
Sheath gas	58 AU
Sweep gas	0 AU
Auxiliary gas	16 AU
S–Lens RF level	60
Data acquisition mode	FullMS–ddMS^2
FullMS resolution @ *m/z* 200	70,000
FullMS scan range	110 – 400 *m/z*
ddMS^2 resolution @ *m/z* 200	17,500
ddMS^2 isolation window	2.0 *m/z*
Stepped normalized collision energy (NCE)	15, 25, 35

Mass spectrometry

Analytes and internal standards were detected by FullMS-ddMS^2 mode on a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with heated electrospray ionization (H-ESI II) operated in positive ion mode. A summary of the MS conditions is reported in Table 3.

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, LLOQ, carryover, accuracy, and intra- and inter-assay precision for all the analytes.

To determine the LLOQ, a four-step serial dilution of the lowest calibrator using blank matrix was performed; a full set of calibrators (three levels), diluted calibrators (four levels), and controls (two levels) were extracted in replicates of five (n=5), injected in a single batch, and all used for the linear interpolation. The LLOQ was set as the lowest level that could be determined with a CV < 20% across the entire batch of samples.

Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected immediately after it.

Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations at two different levels using the quality control samples provided by RECIPE, prepared and analyzed in replicates of five on three different days.

Trueness of measurement was also evaluated as percentage bias using certified external quality controls (GTFCh BZF1-2019 A and B, Instand 876, 877 and 878 3-2019 Probes 31 and 32) prepared and analyzed in replicates of five on a single day.

Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

A linear response (quadratic for trazodone) with 1/x weighting was obtained for all the analytes not only in the calibration range covered by the calibrators, but also down to a LLOQ reported in Table 4. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators (±15% for the lowest calibrator) in all the runs. Representative chromatograms for the LLOQ for midazolam, prazepam, and the corresponding internal standards are depicted in Figure 2. Representative calibration curves for the same analytes in the concentration range covered by the kit (three calibrators) are shown in Figure 3.

No significant carryover was registered, with no peak detected in the blank sample injected immediately after the highest calibrator.

Table 4. Analytes and corresponding LLOQ

Analyte	LLOQ (ng/mL)
3–Hydroxybromazepam	8.30
7–Aminoclonazepam	0.251
7–Aminoflunitrazepam	0.268
7–Aminonitrazepam	1.08
alpha–OH–Alprazolam	2.87
alpha–OH–Midazolam	0.53
alpha–OH–Triazolam	1.96
Alprazolam	0.514
Bromazepam	10.7
Brotizolam	2.00
Chlordiazepoxide	13.0
Clobazam	13.9
Clonazepam	2.47
Demoxepam	11.6
Desalkylflurazepam	0.855
Desmethylflunitrazepam	2.39
Diazepam	6.40
Estazolam	2.23
Flunitrazepam	0.525
Flurazepam	0.52
Lorazepam	1.92
Lormetazepam	0.985
Medazepam	3.77
Midazolam	1.03
Nitrazepam	0.945
Norclobazam	120
Nordiazepam	5.80
Oxazepam	5.85
Prazepam	4.47
Temazepam	5.75
Tetrazepam	2.25
Trazodone	9.00
Triazolam	1.73
Zaleplone	0.865
Zolpidem	18.3
Zopiclone	1.65

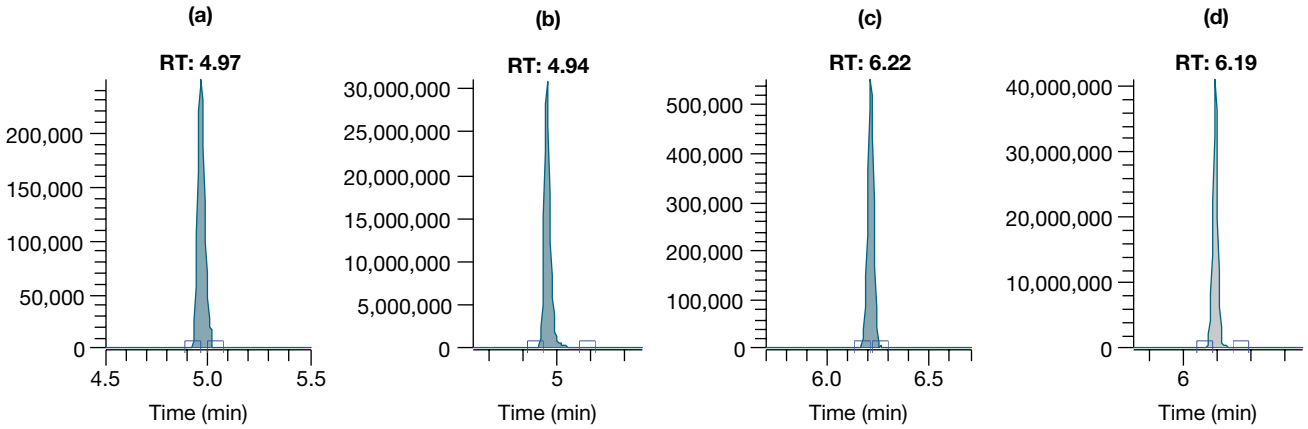

Figure 2. Representative chromatograms of the LLOQ for (a) midazolam, (b) midazolam-D_4, (c) prazepam, and (d) prazepam-D_5

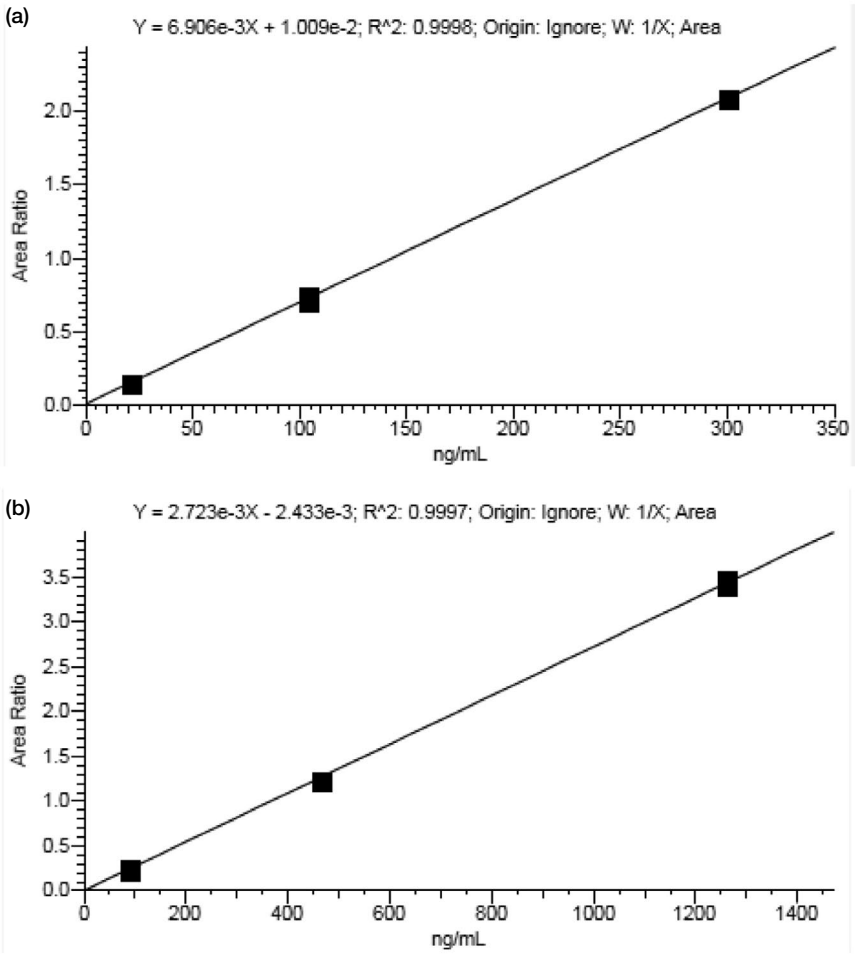

Figure 3. Representative calibration curves for (a) midazolam and (b) prazepam

The data presented in this report demonstrate the outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -13.2% and 8.6% (Table 5).

Table 5. Analytical accuracy results for controls MS6082 batch #1267

Analyte	Control 1			Control 2		
	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)
3-Hydroxybromazepam	42.4	45.1	6.4	144	156	8.6
7-Aminoclonazepam	14.3	14.5	1.6	49	47.8	-1.4
7-Aminoflunitrazepam	15.0	15.7	4.7	50	50.8	2.7
7-Aminonitrazepam	63.5	65.2	2.7	211	207	-1.8
alpha-OH-Alprazolam	16.2	16.3	0.7	54.1	54.7	1.1
alpha-OH-Midazolam	55.0	53.7	-2.3	177	174	-1.5
alpha-OH-Triazolam	15.7	15.3	-2.2	51.9	52.6	1.3
Alprazolam	16.9	15.5	-8.2	57.2	52.3	-8.5
Bromazepam	93.3	88.1	-5.6	305	306	0.4
Brotizolam	4.56	4.12	-9.6	15.4	14.0	-8.9
Chlordiazepoxide	631	635	0.6	2053	1998	-2.7
Clobazam	89.5	87.8	-1.9	292	289	-0.9
Clonazepam	8.13	7.90	-2.8	61.3	58.9	-4.0
Demoxepam	646	657	1.7	2189	2255	3.0
Desalkylflurazepam	29.9	29.5	-1.4	101	103	1.7
Desmethylflunitrazepam	14.6	15.0	2.7	50.9	52.1	2.3
Diazepam	290	297	2.6	939	955	1.7
Estazolam	127	134	5.5	425	444	4.4
Flunitrazepam	16.2	15.2	-6.2	54.2	51.1	-5.6
Flurazepam	62.3	59.4	-4.7	199	193	-3.1
Lorazepam	65.6	56.9	-13.2	203	186	-8.3
Lormetazepam	5.65	5.38	-4.8	18.3	18.2	-0.5
Medazepam	274	288	5.2	838	879	4.9
Midazolam	30.2	30.0	-0.6	78.9	81.1	2.8
Nitrazepam	45.3	41.3	-8.9	148	140	-5.6
Norclobazam	771	799	3.6	2733	2709	-0.9
Nordiazepam	244	222	-9.2	782	719	-8.0
Oxazepam	360	355	-1.3	1205	1197	-0.6
Prazepam	271	259	-4.6	866	842	-2.7
Temazepam	202	185	-8.4	562	501	-10.8
Tetrazepam	126	131	4.2	418	427	2.2
Trazodone	509	496	-2.5	1664	1509	-9.3
Triazolam	7.55	7.96	5.5	24.1	25.0	3.8
Zaleplone	24.7	25.6	3.8	83.2	88.0	5.8
Zolpidem	128	124	-3.1	426	409	-4.0
Zopiclone	18.9	18.3	-3.0	66.2	66.1	-0.2

Excellent results were obtained also from the evaluation of trueness of measurement, with a percentage bias between -8.7% and 15.2% (Table 6 and Table 7).

Table 6. Analytical accuracy results for controls GTFCh BZF1-2019 and Instand 876 3-2019

	GTFCh BZF1-2019						Instand 876 3-2019					
	Probe A			Probe B			Probe 31			Probe 32		
Analyte	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)
7-Aminoflunitrazepam	N/A	N/A	N/A	17.6	19.3	8.9	N/A	N/A	N/A	N/A	N/A	N/A
Alprazolam	46.0	47.3	2.8	N/A	N/A	N/A	32.1	32.8	2.0	10.7	10.9	1.4
Bromazepam	146	165	11.3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chlordiazepoxide	N/A	N/A	N/A	N/A	N/A	N/A	2248	2417	7.0	634	704	9.9
Clonazepam	59.2	63.5	6.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Desalkylflurazepam	N/A	N/A	N/A	N/A	N/A	N/A	68.4	77.3	11.5	38.6	36.8	-5.0
Desmethylflunitrazepam	N/A	N/A	N/A	20.5	21.1	2.8	N/A	N/A	N/A	N/A	N/A	N/A
Diazepam	368	396	7.1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Flunitrazepam	N/A	N/A	N/A	14.8	15.1	2.2	N/A	N/A	N/A	N/A	N/A	N/A
Lorazepam	N/A	N/A	N/A	119	119	0.1	N/A	N/A	N/A	N/A	N/A	N/A
Lormetazepam	N/A	N/A	N/A	15.5	15.7	1.3	N/A	N/A	N/A	N/A	N/A	N/A
Midazolam	N/A	N/A	N/A	91.5	94.7	3.3	N/A	N/A	N/A	N/A	N/A	N/A
Nitrazepam	109	105	-3.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nordiazepam	416	413	-0.7	N/A	N/A	N/A	236	225	-5.1	321	318	-0.9
Oxazepam	N/A	N/A	N/A	292	289	-0.9	639	667	4.2	1259	1343	6.2
Trazodone	N/A	N/A	N/A	N/A	N/A	N/A	661	657	-0.6	1379	1275	-8.2
Zolpidem	108	109	0.6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Zopiclone	N/A	N/A	N/A	59.1	69.7	15.2	N/A	N/A	N/A	N/A	N/A	N/A

Table 7. Analytical accuracy results for controls Instand 877 and 878 3-2019

	Instand 877 3-2019						Instand 878 3-2019					
	Probe 31			Probe 32			Probe 31			Probe 32		
Analyte	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)
Bromazepam	N/A	N/A	N/A	N/A	N/A	N/A	167	186	10.3	106	112	5.0
Clobazam	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	85.4	83.2	-2.6
Clonazepam	91.3	98.3	7.1	13.2	12.7	-3.6	N/A	N/A	N/A	N/A	N/A	N/A
Flunitrazepam	43.7	44.8	2.5	30.4	31.1	2.1	N/A	N/A	N/A	N/A	N/A	N/A
Flurazepam	317	320	0.8	286	274	-4.4	N/A	N/A	N/A	N/A	N/A	N/A
Lorazepam	N/A	N/A	N/A	N/A	N/A	N/A	21.4	22.1	3.2	12.5	13.4	6.5
Nitrazepam	94.6	88.3	-7.2	151	139	-8.7	N/A	N/A	N/A	N/A	N/A	N/A
Temazepam	613	591	-3.7	104	103	-0.9	N/A	N/A	N/A	N/A	N/A	N/A

The %CV for intra-assay precision was always below 9.1%. The maximum %CV for inter-assay precision was 8.5%. Results for intra- and inter-assay precision are reported in Table 8 and Table 9, respectively.

Table 8. Intra–assay precision results for control MS6082 batch #1267

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
3-Hydroxybromazepam	45.1	2.6	45.8	2.2	44.5	2.9	154	2.1	155	2.6	159	2.0
7-Aminoclonazepam	14.5	5.1	14.1	7.3	15.0	5.2	47.8	3.1	45.8	3.2	49.8	5.3
7-Aminoflunitrazepam	15.2	2.8	16.0	2.3	15.8	2.5	48.5	2.4	52.1	4.2	51.9	3.5
7-Aminonitrazepam	65.7	3.1	63.4	8.5	66.6	4.3	215	3.1	192	3.4	215	3.5
alpha-OH-Alprazolam	16.1	2.6	16.2	4.3	16.6	3.5	52.6	3.0	54.5	2.6	57.1	3.1
alpha-OH-Midazolam	55.1	4.2	52.2	2.6	54.2	3.0	179	2.1	173	0.6	171	1.4
alpha-OH-Triazolam	15.0	4.4	15.1	4.4	15.9	4.3	49.8	1.9	53.7	3.4	54.3	3.6
Alprazolam	15.9	2.7	15.4	2.0	15.4	3.5	53.8	2.7	51.7	2.6	51.4	1.8
Bromazepam	87.2	2.7	89.3	1.9	87.6	3.6	299	4.3	309	4.4	311	6.5
Brotizolam	4.11	4.6	4.01	4.4	4.25	4.3	13.6	1.6	14.6	6.0	13.8	2.7
Chlordiazepoxide	641	4.3	623	4.9	641	3.8	1979	1.7	1984	1.0	2029	2.2
Clobazam	90.5	4.7	84.2	7.5	89.2	4.4	295	4.1	277	3.6	297	2.2
Clonazepam	7.93	7.0	8.25	8.1	7.53	9.1	59.0	2.2	58.6	3.9	58.9	4.3
Demoxepam	660	2.0	669	6.7	643	3.6	2137	3.5	2366	3.2	2261	2.1
Desalkylflurazepam	29.9	3.6	29.6	4.3	29.1	2.9	100	5.0	105	3.8	103	2.9
Desmethylflunitrazepam	15.8	6.4	15.0	4.8	14.3	3.9	53.2	2.6	53.7	2.1	49.4	2.4
Diazepam	300	2.4	297	2.2	297	1.9	963	1.8	950	2.3	951	2.2
Estazolam	136	1.6	131	2.4	136	2.7	436	1.5	441	2.3	454	3.4
Flunitrazepam	15.4	3.2	15.3	2.7	14.9	3.7	50.5	3.8	51.7	3.0	51.2	3.7
Flurazepam	59.7	4.0	56.2	4.9	62.3	6.7	196	3.9	187	2.5	196	1.9
Lorazepam	56.5	0.9	57.2	2.1	57.0	1.5	181	1.7	190	2.5	187	2.3
Lormetazepam	5.65	1.6	5.30	4.7	5.23	5.1	17.8	3.1	18.0	4.4	18.9	5.4
Medazepam	286	4.7	274	7.7	304	1.6	883	1.3	857	3.2	897	3.7
Midazolam	29.8	5.2	29.5	4.7	30.7	5.9	81.9	2.4	80.6	3.7	80.8	2.2
Nitrazepam	41.2	4.2	41.6	4.4	40.9	4.8	142	5.2	145	1.6	133	1.8
Norclobazam	790	6.1	812	2.8	793	2.8	2811	5.4	2770	4.2	2546	4.4
Nordiazepam	220	6.2	227	4.7	218	3.0	715	2.4	719	1.8	724	2.2
Oxazepam	355	2.6	355	3.2	356	3.8	1212	5.3	1194	2.6	1186	2.3
Prazepam	264	1.3	258	2.1	255	2.0	837	1.8	858	1.5	832	1.5
Temazepam	181	0.4	183	2.5	190	3.0	497	1.4	500	2.9	507	3.3
Tetrazepam	131	2.2	130	2.4	134	3.4	420	2.2	430	3.4	432	1.4
Trazodone	496	3.2	483	1.6	509	5.3	1490	2.1	1490	3.5	1548	4.5
Triazolam	7.90	2.0	8.15	3.9	7.83	5.4	25.4	3.0	25.0	2.3	24.6	2.6
Zaleplone	24.5	5.0	26.9	3.8	25.3	3.0	84.9	5.8	86.7	3.6	92.4	2.3
Zolpidem	125	2.8	124	5.1	123	2.2	408	2.3	411	3.3	409	2.2
Zopiclone	19.2	1.3	17.4	7.2	18.6	2.4	67.4	3.4	64.6	3.7	66.3	2.9

Table 9. Inter–assay precision results for control MS6082 batch #1267

Analyte	Control 1		Control 2	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
3-Hydroxybromazepam	45.1	2.7	156	2.6
7-Aminoclonazepam	14.5	6.3	47.8	5.2
7-Aminoflunitrazepam	15.7	3.1	50.8	4.7
7-Aminonitrazepam	65.2	5.8	207	6.3
alpha-OH-Alprazolam	16.3	3.5	54.7	4.4
alpha-OH-Midazolam	53.7	3.8	174	2.4
alpha-OH-Triazolam	15.3	4.8	52.6	4.9
Alprazolam	15.5	3.0	52.3	3.1
Bromazepam	88.1	2.8	306	5.1
Brotizolam	4.12	4.8	14.0	4.9
Chlordiazepoxide	635	4.2	1998	2.0
Clobazam	87.8	6.2	289	4.4
Clonazepam	7.90	8.5	58.9	3.4
Demoxepam	657	4.7	2255	5.1
Desalkylflurazepam	29.5	3.5	103	4.2
Desmethylflunitrazepam	15.0	6.2	52.1	4.4
Diazepam	297	2.0	955	2.1
Estazolam	134	2.7	444	3.0
Flunitrazepam	15.2	3.2	51.1	3.4
Flurazepam	59.4	6.8	193	3.6
Lorazepam	56.9	1.6	186	2.9
Lormetazepam	5.38	5.2	18.2	4.9
Medazepam	288	6.6	879	3.3
Midazolam	30.0	5.2	81.1	2.8
Nitrazepam	41.3	4.2	140	4.9
Norclobazam	799	3.9	2709	6.2
Nordiazepam	222	4.7	719	2.1
Oxazepam	355	3.0	1197	3.5
Prazepam	259	2.2	842	2.0
Temazepam	185	3.1	501	2.6
Tetrazepam	131	2.9	427	2.6
Trazodone	496	4.2	1509	3.8
Triazolam	7.96	4.2	25.0	2.8
Zaleplone	25.6	5.4	88.0	5.3
Zolpidem	124	3.4	409	2.5
Zopiclone	18.3	5.9	66.1	3.6

Conclusions

An LC-HRAM-based method (a Vanquish Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap MS) is reported here, demonstrating the power of Orbitrap technology in performing accurate qualitative analyses and routine quantitation with high efficiency. This method for clinical research was developed and implemented for the quantification of 36 benzodiazepines in human plasma or serum. The ClinMass TDM Platform with the ClinMass Add-On Set for Benzodiazepines from RECIPE was used. The method incorporates a quick and simple offline protein precipitation step with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

Find out more at **thermofisher.com/clinicalresearch**