Application Note: 529

Quantitative LC-MS Analysis of 14 Benzodiazepines in Urine Using TraceFinder 1.1 Software and High Resolution Accurate Mass

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Key Words

- TraceFinder Software
- Exactive
- Clinical Research

Introduction

Thermo Scientific TraceFinder 1.1 software is developed for quantitative analysis for clinical research laboratories. The software is designed for routine data acquisition, quantitation, qualitative screening and reporting on all Thermo Scientific liquid chromatography mass spectrometry (LC-MS) systems, including high resolution accurate mass (HRAM) instruments, with fully integrated support for the Thermo Scientific Transcend multiplexing system.

TraceFinder[™] 1.1 quantitative software simplifies routine analysis for the operator by executing a stepwise workflow from batch creation to reporting. For clinical research laboratories employing multiple types of LC-MS systems, TraceFinder 1.1 software eliminates the need to learn and maintain multiple software programs.

TraceFinder 1.1 software provides many easy approaches to execute workflow routines for operators and lab managers. The work presented here demonstrates the workflow used by lab managers during method development and includes processing method creation using the compound data store (CDS). The operator's workflow includes batch submission, real time monitoring, data review and report generation.

Goal

To demonstrate a new, easy-to-use workflow-driven quantitative method for 14 benzodiazepines in urine using the Thermo Scientific Exactive high performance benchtop mass spectrometer and TraceFinder 1.1 routine quantitative software.

Methods

Sample Preparation

Urine was spiked with internal standards and hydrolyzed with beta-glucuronidase. Acetonitrile was added to the hydrolyzed sample and the resulting mixture was centrifuged. Supernatant was further diluted and subjected to LC-MS analysis.

LC-MS/MS conditions

LC-MS analysis was performed on an ExactiveTM mass spectrometer with a heated electrospray ionization (HESI) source coupled with a TranscendTM TLX system used in

LX mode. Full scan mass spectrometry analysis was done with resolution of 100,000 (FWHM at m/z 200) with a mass isolation window of 3 ppm. Exact mass was used for compound identification. High performance liquid chromatography (HPLC) was carried out on a Thermo Scientific Hypersil GOLD PFP column (100 × 2.1 mm, 5 µm particle size) at room temperature.

The MS conditions were as follows:

Ionization	HESI-II
Polarity	Positive
Vaporizer temp (°C)	350
Capillary temp (°C)	350
Spray voltage (V)	3500
Sheath gas (AU)	40
Auxillary gas (AU)	10
Data acquisition mode	Full scan
AGC target	1.00E+06
Lock mass (m/z)	279.2591
Scan range (m/z)	135-600
Max injection time (ms)	100
Resolution	100,000

Software

Method development, data acquisition, data processing and report generation were all executed in TraceFinder 1.1 routine quantitation software.

Results and Discussion

Streamlined Workflow:

The entire workflow in TraceFinder 1.1 software is easy to set up and is summarized in Figure 1.



Figure 1. TraceFinder 1.1 workflow for quantitative analysis



Main Tabs in TraceFinder 1.1

Figure 2 shows the four main tabs: Configuration, Method Development, Data Review and Acquisition.



Figure 2. TraceFinder 1.1 welcome screen

Compound Data Store (CDS)

Figure 3 shows the CDS for this benzodiazepines application. Entries in this CDS are built based on the accurate masses. CDS can be later updated with retention times of analytes.

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Figure 3. Compound Data Store for benzodiazepines application

Master Method

The "Master Method" contains information on data acquisition (including instrument method), data processing, and analysis. In detail, it contains settings for 5 main categories: General (including method type, injection volume, instrument method, etc), Compound (acquisition list selected from CDS, detection, calibration, etc), Flags, Groups and Reports. Selected tabs in "General" and "Compound" are shown in Figure 4. To complete the

master method setup, settings in "Flags", and "Reports" can also be customized. TraceFinder software provides 50 predefined report templates.

Instrument Method

The instrument method is comprised of individual LC, autosampler and MS portions. The software allows for optimization of chromatography and customizable autosampler programming.

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3 4.62 7-Aminoflunitrazepam 4 5.33 a-Hydroxytriazolam 5 5.39 Lorazon	5.000 5.000 5.000 5.000	5.000 11 5.000 10 5.000 10	10.000 3 10.000 3 10.000 3	25.000 25.000 25.000	50.000 50.000 50.000	125.000	250.000	500.000	1000.000	4	5.33 a-Hyd 5.39 Loraz	xytriazolam pam	15.000	300.000		
3 4.52 7.Aminofluminazepam 4 5.33 6-Hydroxythazolam 5 5.39 Lonazepam 6 5.42 A-Hydroxythazolam	5.000 5.000 5.000 5.000 5.000	5.000 11 5.000 10 5.000 10 5.000 10	10.000 : 10.000 : 10.000 : 10.000 :	25.000 25.000 25.000 25.000	50.000 50.000 50.000 50.000	125.000 125.000 125.000 125.000	250.000 250.000 250.000	500.000 500.000 500.000	1000.000	4 5 6	4.62 7-Ame 5.33 a-Hyd 5.39 Loraz 5.42 a-Hyd	xytniazolam xytniazolam xwaitnazolam	15.000	300.000 300.000 300.000 300.000		
3 4.62 7-Aminoflumitracepam 4 5.33 a-Hydroxyfuacolam 5 5.99 Loracepam 6 5.42 a-Hydroxyfurocolam 7 5.42 o-Hydroxyfurocolam	5.000 5.000 5.000 5.000 5.000 5.000	5.000 11 5.000 11 5.000 10 5.000 10 5.000 10	10.000 3 10.000 3 10.000 3 10.000 3	25.000 25.000 25.000 25.000 25.000	50.000 50.000 50.000 50.000 50.000	125.000 125.000 125.000 125.000	250.000 250.000 250.000 250.000	500.000 500.000 500.000 500.000	1000.000 1000.000 1000.000	3 4 5 6 7	4.62 7.4mi 5.33 a-Hyd 5.39 Loraz 5.42 a-Hyd 5.42 Oxaze	xytriazolam xytriazolam xytriazolam xytalprazolam	15.000 15.000 15.000 15.000	300.000 300.000 300.000 300.000 300.000		
3 4.82 7 Aminofur/tazegam 4 5.33 a-Hydrosythacolam 5 5.39 Loxazgam 6 5.42 a-Hydrosythacolam 7 5.42 Oxazgam 6 5.51 2.41/drosythythuzegoam	5.000 5.000 5.000 5.000 5.000 5.000 5.000 5.000	5.000 11 5.000 11 5.000 10 5.000 10 5.000 10 5.000 10	10.000 3 10.000 3 10.000 3 10.000 3 10.000 3 10.000 3	25.000 25.000 25.000 25.000 25.000 25.000	\$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000	125.000 125.000 125.000 125.000 125.000 125.000	250.000 250.000 250.000 250.000 250.000	500.000 500.000 500.000 500.000 500.000	1000.000 1000.000 1000.000 1000.000	3 4 5 6 7 8	4.62 7-Ami 5.33 a-Hyd 5.39 Loraz 5.42 a-Hyd 5.42 Oxaze 5.42 Oxaze 5.51 2-Hyd	xythiszolam xythiszolam am xysigrazolam am xyethytifurazegam	15.000 15.000 15.000 15.000 15.000	300.000 300.000 300.000 300.000 300.000		
3 4.62 7 Aminofluminacepam 4 5.03 A Hydroxybicacelom 5 5.73 Lorazepam 6 5.42 A Hydroxybicacelom 7 5.42 O Hydroxybicacelom 7 5.42 O Hydroxybicacelom 9 5.52 D Angeogram 9 5.52 D Angeogram	5.000 5.000 5.000 5.000 5.000 5.000 5.000 5.000	5.000 11 5.000 11 5.000 10 5.000 10 5.000 10 5.000 10 5.000 10	10.000 3 10.000 3 10.000 3 10.000 3 10.000 3 10.000 3 10.000 3	25.000 25.000 25.000 25.000 25.000 25.000 25.000	50.000 50.000 50.000 50.000 50.000 50.000 50.000	125.000 125.000 125.000 125.000 125.000 125.000 125.000	250.000 250.000 250.000 250.000 250.000 250.000	500.000 500.000 500.000 500.000 500.000 500.000	1000.000 1000.000 1000.000 1000.000 1000.000 1000.000	3 4 5 6 7 8 9	4.62 7.4mm 5.33 a-Hyd 5.39 Loraz 5.42 a-Hyd 5.42 Oxaze 5.51 2.Hyd 5.57 Desal	xytriazolam xytriazolam xyvalprazolam am xyvethyfflurazepam xthurazepam	15.000 15.000 15.000 15.000 15.000 15.000 15.000	300.000 300.000 300.000 300.000 300.000 300.000		
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3 4.52 7.4mindluritaxpam 4 5.53 a-tydooptacken 5 5.93 Losespen 6 5.42 o-tydooptackom 7 5.42 o-tydooptackom 9 5.57 Decatyptin 9 5.57 Decatyptin 9 5.57 Decatyptin 10 6.58 Temacapam 10 5.57 Decatydtacepon	5.000 5.000 5.000 5.000 5.000 5.000 5.000 5.000 5.000 5.000	5000 11 5000 11 5000 10 5000 10 5000 10 5000 10 5000 10 5000 10 5000 10	10.000 3 10.	25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000	\$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000	125.000 125.000 125.000 125.000 125.000 125.000 125.000 125.000 125.000	250.000 250.000 250.000 250.000 250.000 250.000 250.000 250.000	500.000 500.000 500.000 500.000 500.000 500.000 500.000 500.000	1000.000 1000.000 1000.000 1000.000 1000.000 1000.000 1000.000	3 4 5 6 7 8 8 9 10 11	4.62 7.4mm 5.33 a-Hyd 5.39 Loraza 5.42 a-Hyd 5.42 Oxaze 5.51 2.Hyd 5.57 Desal 5.63 Tema 5.63 Tema	wydriazolam wydriazolam am wyedrydfurazolam wyedrydfurazopam dfurazopam ppam opam	15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000	300.000 300.000 300.000 300.000 300.000 300.000 300.000 300.000		
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3 4.52 74mindhurhasepan 4 5.33 64tydoxylacadom 5 5.33 Lexempen 6 5.23 Lexempen 7 5.42 Oksopyan 9 5.57 Decalylitacatom 9 5.57 Decalylitacatom 10 5.51 24tydoxylylitacatom 11 5.70 Neodoppan 12 5.72 Ajkacdam 12 5.57 Decalylitacatom	5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000 5,000	5.000 111 5.000 111 5.000 111 5.000 110 5.000 110 5.000 110 5.000 110 5.000 110 5.000 110	10.000 : 1 10.000	25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000 25.000	\$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000 \$0.000	125.000 125.000 125.000 125.000 125.000 125.000 125.000 125.000 125.000 125.000	250.000 250.000 250.000 250.000 250.000 250.000 250.000 250.000 250.000	500.000 500.000 500.000 500.000 500.000 500.000 500.000 500.000 500.000 500.000	1000.000 1000.000 1000.000 1000.000 1000.000 1000.000 1000.000 1000.000 1000.000	3 4 5 6 7 8 9 10 11 11 12 13	4.62 74mm 5.33 a-Hyd 5.39 Loraz 6.42 a-Hyd 5.42 Oxaz 5.51 2-Hyd 5.53 Tema 5.63 Tema 5.57 Alpras 5.51 Diase	wytharum azepam wytharolam aam aam aam wyethyfflurazepam yflurazepam yflurazepam apam lam am	15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000 15.000	300.000 300.000 300.000 300.000 300.000 300.000 300.000 300.000 300.000		

Figure 4. Master Method creation process (selected tabs)

Batch

After creation of the master method, a new sample batch can be created for data acquisition. Creating a batch involves assigning a project, linking to the master method, building a run-sequence and submitting. Figure 5 shows an exemplary batch view containing six calibrators and two levels of "Check Standards" (or QCs, n=5).

I- 118 -14	•											👖 Re	al time status 🛛 😥 D
Batch V	iew - E	3enzo14Mix*											
Local Met	hod: Ber	izo14Mix		~	Upd	ate Ir	nstrument:	Thermo	Scientific	c Instrument	User:		
	Status	Filename	Sample type	Sample	e level	Sample ID	e Sam nam	nple C	omment	Vial posi	ion	Injection volume	Conv Factor
▶ 1	•	Cal1	Cal Std	Cal1		1				CStk1-01	:01	50.0	1.000
2	•	Cal2	Cal Std	Cal2		1				CStk1-01	:2	50.0	1.000
3	•	Cal4	Cal Std	Cal4		1				CStk1-01	:3	50.0	1.000
4	•	Cal6	Cal Std	Cal6		1				CStk1-01	:4	50.0	1.000
5	•	Cal7	Cal Std	Cal7		1				CStk1-01	:5	50.0	1.000
6	•	Cal8	Cal Std	Cal8		1				CStk1-01	:6	50.0	1.000
7	•	QA001	Chk Std	QC1		1				CStk1-01	:7	50.0	1.000
8	•	QA002	Chk Std	QC1		1				CStk1-01	:8	50.0	1.000
9	•	QA003	Chk Std	QC1		1				CStk1-01	:9	50.0	1.000
10	•	QA004	Chk Std	QC1		1				CStk1-01	:10	50.0	1.000
11	•	QA005	Chk Std	QC1		1				CStk1-01	:11	50.0	1.000
12	•	QB001	Chk Std	QC2		1				CStk1-01	:12	50.0	1.000
13	•	QB002	Chk Std	QC2		1				CStk1-01	:13	50.0	1.000
14	•	QB003	Chk Std	QC2		1				CStk1-01	:14	50.0	1.000
15	•	QB004	Chk Std	QC2		1				CStk1-01	:15	50.0	1.000
16	•	QB005	Chk Std	QC2		1				CStk1-01	:16	50.0	1.000
								~ ~					2
Automated	d Batch	Reports								Compoun	d Active	Status	
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	Repo	rt Name		Туре	Print	Create	Create XMI	Create		▶ 1	7-Amine	oclonazepam	
1	Blank	Benort		Standard						2	7-Amino	clonazepam-D4	
2	Check	Standard Benort		Standard						3	7-Amino	onitrrazepam	
	Chron	atogram Benort		Standard					- 11	4	7-Amino	oflunitrazepam	
1 1	Comp	ound Calibration Bar	port	Standard					-11	5	7-Amino	oflunitrazepam-D7	
	Comp	ound Calibration Per	oort - Alternate	Standard						6	a-Hydro	xytriazolam	
	Confin	mation Report	Jon - Mileinidie	Standard						7	a-Hydro	xytriazolam-D4	
- 0	Confin	mation Report 2		Standard					- 1	8	Lorazep	bam	×
· · ·	- Confin	mauori mepori 2	deed Deered	Standard					- 1	9	Lorazep	bam-D4	
8	High L	vensity Internal Star	idard Heport	Standard					-	10	a-Hydro	xyalprazolam	

Figure 5. Acquisition Batch view

Data Acquisition and Real Time Status

After batch submission, data will be acquired and real time chromatograms can be shown in customizable ways (Figure 6). Status of acquisition (pressure profile, event log), device status, and sample queue can all be monitored in Real Time Status. TraceFinder software allows for multiple batches submission prioritization.



Figure 6. Real Time Status view

Data Review

Data Review (Figure 7) allows for flagging for any items that require attention (retention time drift, limit of quantitation, ion ratio discrepancy, etc.).

1 Image: Constraint of the sector of the secto	CalStd I2 CalStd I4 CalStd I6 CalStd I7 CalStd	CStk1-01:01 CStk1-01:2 CStk1-01:3 CStk1-01:3	5.34 5.35 5.34	Method Method	6116 12637	5.4901 9.9474	5.000 10.000	159884 124449		9.80	N/A	N/A	7-Aminoclonazepam-D4
2 • • C 3 • • C 4 • • C 5 • • C 6 • C C 7 • • • Q	I2 CalStd I4 CalStd I6 CalStd I7 CalStd	CStk1-01:2 CStk1-01:3 CStk1-01:4	5.35 5.34	Method	12637	9.9474	10.000	124449					Z Aminoflumitrazonam DZ.
3 • C 4 • C 5 • C 6 • C 7 • A	I4 CalStd I6 CalStd I7 CalStd	CStk1-01:3 CStk1-01:4	5.34	Ad a block of				124440		-0.53	N/A	N/A	- Availinioidinidazepaino7
4 O C 5 O C 6 O C 7 O A Q	l6 CalStd I7 CalStd	CStk1-01:4		Method	73596	45.9933	50.000	119990		-8.01	N/A	N/A	a-Hydroxyaiprazolam-Do
5 O C 6 O C 7 O A Q	I7 CalStd		5.36	Method	433035	236.4220	250.000	130543		-5.43	N/A	N/A	🍋 Alprazolam-D5
6 💊 C 7 💊 🥼 Q		CStk1-01:5	5.36	Method	1047	524.5294	500.000	141381		4.91	N/A	N/A	Desalkylflurazepam-D4
7 🕒 🔔 Q	l8 CalStd	CStk1-01:6	5.37	Method	1819	992.6177	1000.000	129451		-0.74	N/A	N/A	Diazapam-D5
	001 ChkStd	CStk1-01:7	5.36	Method	26294	14.3502	15.000	160274		-4.33	7.53	9.58	Midazolam-D4
8 🕒 Q	002 ChkStd	CStk1-01:8	5.36	Method	24801	13.4152	15.000	164482		-10.57	7.53	9.58	Nordiazepam-D5
9 🙆 🔥 0	003 ChkStd	CStk1-01:9	5.36	Method	21115	12.5994	15.000	151695		-16.00	7.53	9.58	📃 🎴 Oxazepam-D5
10 O 🧍 O	004 ChkStd	CStk1-01:10	5.36	Method	23553	13.4035	15.000	156381		-10.64	7.53	9.58	Temazepam-D5
11 🙆 🔔 🛛	005 ChkStd	CStk1-01:11	5.36	Method	21011	11.7254	15.000	165722		-21.83	7.53	9.58	7-Aminoclonazepam
12	1001 CbkStd	CStk1-01-12	5.36	Method	611744	293.8776	300.000	148015		-2 04	2.38	2 41	7 Aminoflunitrazepam
13	002 CbkStd	CStk1-01:13	5.36	Method	601486	299,4603	300.000	142795		-0.18	2.38	2.41	7-Aminonitrrazepam
14	1002 Christel	CS#1.01:14	5.36	Method	598636	284 8504	300.000	149480		-5.05	2.38	2.41	a-Hydroxyalprazolam
	1003 Chrstd	CSik1-01-15	5.37	Method	600854	297 5668	300.000	143561		.0.81	2.30	2.41	Alprazolam
	1004 Chicked	CSW1 01:10	5.36	Method	589322	303 4743	300.000	138039		1.10	2.00	2.41	Desalkylflurazepam
	Chksta	CSIKT-01.16	0.00	mound	OCOLL	000.4140	000.000	100000		1.10	2.30	2.41	Diazepam
													Lorazepam

an 🛛 🗌 Quan Pea			Calibrat	tion curve		Spectra	T	QED Spectra		Confirming	g lons		lon overlay
									Lorazepam				
ie	. 5.54 Gail					Y =	= 1.42e-2X -	3.97e-2; R^2	2: 0.9986; 0	Origin: Igr	nore; W:	1/X; Area	1
	RT: 5.34 AA: 6116-41		16-										
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50-			~ -							-			

Figure 7. Data Review view for lorazepam, one of the 14 benzodiazepines

Reporting

Figures 8 and 9 are two examples (compound calibration and check standard/quality control) of the Report View.



Figure 8. Compound Calibration Report for lorazepam

: View - Intra1	_			_	_	_
ew Only 🔵 Generate Only						
report: Check Standard Report	t 🔽 Sampl	e file: QA002	*			
} H ∢ → H 1	12 6	M 🔍 +				
itandard Report						
Lab Name: DefaultLabora Instrument: ThermoScient User: USSJO-TRCF Batch: Intra1	atory tific Instrument NDR2tthermo	Checks	itandard Report Method: Intra1_Ber Benzo14w Cali File: Intra1.calx	izo14Mix Iix		Page 1 of 2
Vial Pos Sample CStk1-01:8 1	ID File Name QA002	2 <u>Level</u> QC1	<u>Sample Name</u>	File Date 3/30/2011 1:59:40 I	<u>Com</u> ™	iment
Compound	Curve Type Dail	ly RF Mean RF Min F	FRF%D MaxRF%D	QC Amt Calc Amt	Amt%D	Max Amt %D Flag
7-Aminoclonazepam	L	0.0	00	15.0000 13.5286	-9.81	20.00 Pass
7-Aminonitrrazepam	L	0.0	00	15.0000 13.4464	-10.36	20.00 Pass
7-Aminoflunitrazepam	L	0.01	00	15.0000 13.4085	-10.61	20.00 Pass
a-Hydroxytriazolam	L	0.01	00	15.0000 14.0475	-6.35	20.00 Pass
Lorazepam	L	0.0	00	15.0000 13.4152	-10.57	20.00 Pass
a-Hydroxyalprazolam	L	0.0	00	15.0000 13.4446	-10.37	20.00 Pass
Oxazepam	L	0.0	00	15.0000 12.8524	-14.32	20.00 Pass
2-Hydroxyethylflurazepam	L	0.0	00	15.0000 13.6104	-9.26	20.00 Pass
Desalkylflurazepam	L	0.0	00	15.0000 13.7256	-8.50	20.00 Pass
Temazepam	L	0.0	00	15.0000 13.2345	-11.77	20.00 Pass
Nordiazepam	L	0.0	00	15.0000 14.4350	-3.77	20.00 Pass
Alprazolam	L	0.01	00	15.0000 14.2256	-5.16	20.00 Pass
					De UKAN	



Method Performance

Sample preparation for urine analysis of benzodiazepines was previously done with solid phase extraction (SPE). Here we tested a simple urine dilution strategy. The absolute recovery of deuterated benzodiazepine internal standards was tested with several lots of human urine. It was determined that the absolute recoveries of the internal standards ranged from 83.0% to 100.5% at 100 ng/mL from all lots of urine tested (data not shown). This method was linear from 5 to 1000 ng/mL for all 14 benzodiazepines with an accuracy of 85.4%-106.0%. Inter- (n=15) and intra-batch (n=5) coefficients of variation (CV) at two different concentration levels ranged from 0.5% to 11.7%. The method has a lower limit of quantitation (LLOQ) of 5 ng/mL for all 14 benzodiazepines tested. The method performance is summarized in Table 1. Figure 10 shows the extracted ion chromatograms (XICs) with 3 ppm mass isolation window of all 14 benzodiazepines at their LLOQ (5 ng/mL).

Table 1. Method performance for 14 benzodiazepines in urine

		QC level 1	l: 15 ng/mL	QC level 2	: 300 ng/mL		
Name	m/z	% Precision	% Accuracy	% Precision	% Accuracy	Linear Range (ng/mL)	LLOQ (ng/mL)
7-Aminonitrazepam	252.1131	2.9	88.7	2.9	106.0	5 - 1000	5
Nordiazepam	271.0633	5.7	89.6	2.9	100.9	5 - 1000	5
7-Aminoflunitrazepam	284.1194	3.4	91.2	4.0	100.9	5 - 1000	5
Diazepam	285.0789	8.8	96.0	2.6	99.7	5 - 1000	5
7-Aminoclonazepam	286.0742	2.0	89.1	2.1	99.4	5 - 1000	5
Oxazepam	287.0582	5.0	85.6	3.5	98.4	5 - 1000	5
Desalkylflurazepam	289.0539	5.5	88.5	2.9	98.6	5 - 1000	5
Temazepam	301.0738	3.4	89.1	2.7	97.6	5 - 1000	5
Alprazolam	309.0902	3.1	90.0	3.2	101.5	5 - 1000	5
Lorazepam	321.0192	7.6	85.4	3.4	95.3	5 - 1000	5
lpha-Hydroxyalprazolam	325.0851	3.0	87.0	1.8	97.3	5 - 1000	5
Midazolam	326.0855	3.6	91.3	2.6	101.2	5 - 1000	5
2-Hydroxyethylflurazepam	333.0801	3.7	89.0	2.5	99.7	5 - 1000	5
lpha-Hydroxytriazolam	359.0461	5.9	86.9	2.8	97.5	5 - 1000	5



Figure 10. Extracted ion chromatograms of 14 benzodiazepines in urine at their LLOQ (5 ng/mL, mass isolation window=3 ppm)

Conclusion

We have developed a fast and sensitive LC-MS method for 14 benzodiazepines in urine using a benchtop Exactive mass spectrometer with TraceFinder 1.1 software. TraceFinder 1.1 software is easy to use and effective in performing quick routine quantitative analysis of benzodiazepines in urine. The software enables easy method development, batch creation, submission and real time monitoring for clinical research laboratories. The data review functionality was very useful in quick review and verification of the calibration accuracy and linearity. The report templates make selecting and generating reports with all the necessary information easy and quick.

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