Application Note: 52100

Confirmatory Quantitation of Benzodiazepines in Human Blood and Urine using LC/MS with Online TurboFlow Technology

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Key Words

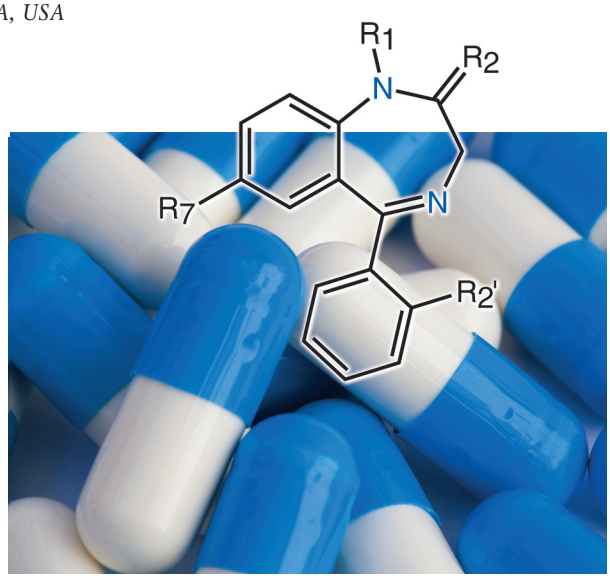
- MSQ Plus
- Transcend TLX
- TurboFlow Technology
- Benzodiazepine
- Drug Analysis
- Toxicology

Goal

Develop a simple, fast, robust and cost-effective LC/MS method for automated extraction and confirmatory quantitation of benzodiazepines in human blood and urine for clinical and forensic applications.

Introduction

Benzodiazepines are widely prescribed psychotropic drugs indicated for the management of anxiety, sleeping disorders, muscle spasms and seizures. Short-term therapeutic doses of these central nervous system depressants are generally safe and highly effective. High dosages over prolonged periods, however, can lead to tolerance, physical and psychological dependence, and severe withdrawal symptoms. These medications are subject to abuse, particularly by individuals with a history of drug and/or alcohol addiction, and are classified accordingly as Schedule IV controlled substances by the United States Drug Enforcement Agency (DEA). While benzodiazepine intoxication alone is rarely fatal, concurrent use with alcohol or other drugs can be life-threatening. Misuse of benzodiazepines, especially those with longer duration of action and faster onset, is on the rise. Between 2004 and 2008, the estimated number of emergency department visits involving the non-medical use of benzodiazepines increased by 89%, from 143,500 to 271,700; by individual benzodiazepines, the steepest increases in emergency visits over this period were related to the use of alprazolam (eg. Xanax®) (125%), clonazepam (eg. Klonopin®) (72%), diazepam (eg, Valium®) (70%), and lorazepam (eg. Ativan®)(107%).[1] Alprazolam, the most prescribed and the most frequently diverted and abused benzodiazepine, accounted for 104,800 of the 271,700 estimated benzodiazepine-related emergency visits in 2008, which is more than double the number associated with the second most abused benzodiazepine, clonazepam (48,400). Benzodiazepines encountered on the illicit market are either diverted legitimate pharmaceuticals or smuggled foreign products; there is no evidence of clandestine manufacture in the United States. In 2009, law enforcement agencies submitted 35,332 alprazolam, 9,530 clonazepam, 7,123 diazepam, 2,143 lorazepam and 375 temazepam exhibits for forensic analysis.[2] Flunitrazepam, which is not approved for use in the United States but is marketed legally in other parts of the world, is implicated in drug-facilitated sexual assault and is a Schedule IV controlled substance with Schedule 1 penalties. Methods that can rapidly and accurately identify, confirm and quantify multiple benzodiazepines in biological matrices in a single analysis are essential for many clinical and forensic toxicology applications.



Several techniques for the analysis of multiple benzodiazepine drugs and metabolites in human blood and urine samples have been developed. Immunoassay methods are generally effective for rapid screening but lack sufficient sensitivity and selectivity for accurate and robust confirmation and quantitation. HPLC with UV detection is not selective nor is it sensitive enough to detect therapeutic ranges of several low-dose benzodiazepines. GC/MS is highly sensitive and selective but requires chemical derivatization prior to the analysis of thermally labile and polar benzodiazepines and metabolites. LC/MS, in contrast, offers exceptional sensitivity and selectivity without the need for derivatization and may be coupled with on-line sample preparation techniques to increase throughput, augment efficiency and maximize reproducibility.

The Thermo Scientific Transcend TLX system coupled to the Thermo Scientific MSQ Plus Mass Detector enables rapid sample cleanup and LC/MS analysis on a single integrated benchtop platform. The Transcend™ system combines Thermo Scientific TurboFlow on-line extraction technology with fast LC to reduce sample preparation, minimize ion suppression, improve LC/MS sensitivity, reduce overall analysis times and maximize throughput. The high-sensitivity, fast-scanning MSQ™ Plus Mass Detector is ideally suited for accurate and reliable screening and quantitation of a diverse range of analytes in demanding high-throughput applications. Cost-effective and easy to operate, the automated TLX-MSQ platform provides a complete LC/MS solution for clinical and forensic laboratories. In this note, we demonstrate rapid and robust extraction, separation, identification and quantitation of ppb levels of benzodiazepines in human urine and blood samples using the TLX-MSQ LC/MS platform.

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Materials and Methods

Standards and Samples

All benzodiazepine standards (23) and the deuterated internal standards (10) are from Cerilliant (Round Rock, TX). Human plasma is from Sigma. Post-mortem samples were obtained from a proprietary source.

Sample Pre-treatment

Human Plasma

Human plasma was spiked with benzodiazepine standards at concentrations ranging from 5–1000 ng/mL for calibration. Quality control samples were prepared by spiking plasma with benzodiazepine standards at 200 ng/mL concentrations. All plasma samples were spiked with 100 ng/mL deuterated internal standards. Every plasma sample was mixed with acetonitrile (1:1) to precipitate proteins and centrifuged at 10,000 rpm for 5 minutes. 50 µL of the supernatant was loaded on the TurboFlow™ column for LC/MS analysis.

Human Urine

All human urine samples were centrifuged at 10,000 rpm for 5 minutes, and the resulting supernatant was used as the spiking blank. Urine was spiked with benzodiazepine standards at concentrations ranging from 5–1000 ng/mL for calibration. Quality control samples were prepared by spiking with benzodiazepine standards at 200 ng/mL concentrations. All urine samples were spiked with 100 ng/mL deuterated internal standards. 50 µL of the supernatant was loaded on the TurboFlow column for LC/MS analysis.

Human Urine Hydrolysis with β-glucuronidase

In this study, some urine samples were hydrolyzed with β-glucuronidase (pH 5) prior to analysis. A urine sample (0.5 mL) was hydrolyzed by spiking 75 µL of deuterated internal standards (1000 ng/mL alprazolam D5 and 1000 ng/mL diazepam D5) and adding 250 µL of β-glucuronidase solution (5000 units per sample). The sample was vortexed, incubated at 57 °C for 6 hours or overnight, cooled to room temperature and centrifuged at 10,000 rpm for 10 minutes. 50 µL of the supernatant was injected into the LC-MS system.

TurboFlow Extraction and LC/MS Analysis

Instrumentation

On-line sample extraction and LC/MS analysis were performed on a Thermo Scientific Transcend TLX system coupled to a Thermo Scientific MSQ Plus Mass Detector. Transcend TLX system include one Open Accela Autosampler, two Accela 600 pumps and one VIM box for valve control.

Software Platform

Xcalibur 2.1 with Foundation 1.0
Open Accela AS driver (LC devices 2.4)
Ariax 1.2 driver
MSQ 2.0 SP 2

Chromatographic Conditions

The Transcend TLX system is optimized to use turbulent flow chromatography to separate analytes from biological fluids prior to MS analysis. A large particle TurboFlow extraction column operating under a high flow rate removes high molecular weight matrix components and retains smaller target analytes by diffusion and column chemistry. The analytes are subsequently eluted via column switching to an LC column for further separation. The chromatographic focusing effect provided by the LC column improves peak shape, which helps to optimize sensitivity and quantitation. A schematic of the Transcend TLX dual-column configuration is shown in Figure 1.

LC Parameters

TurboFlow Columns:	Thermo Scientific Cyclone MCX-2 (0.5 × 50 mm)
Analytical Columns:	Thermo Scientific Hypersil GOLD PFP (150 × 3 mm, 3 µm)
Mobile Phase:	**Loading Solvent** A: Water: Acetonitrile (1:1) + 10 mM Ammonium Formate + 0.1% Formic Acid B: Water: Acetonitrile (1:1) + 100 mM Ammonium Formate + 0.1% Formic Acid C: Acetonitrile:Isopropanol: Acetone (7:2:1) D: Water
	Eluting Solvent A: Water + 10 mM Ammonium Formate + 0.1% Formic Acid B: Acetonitrile + 0.1% Formic Acid C: Methanol + 0.1% Formic Acid D: Methanol

Loading Gradient:

Time	A %	B %	C %	D %	Flow Rate (mL/mm)	Tee	Loop	Div
1.00	–	–	–	100.0	2.0	–	Out	Waste
3.00	–	–	–	100.0	0.2	T	In	Waste
4.50	–	100.0	–	–	0.5	–	In	Waste
6.50	–	100.0	–	–	0.5	–	In	Waste
8.50	–	–	–	100.0	0.5	–	In	Det
16.50	–	–	100.0	–	0.5	–	In	Det
17.50	100.0	–	–	–	2.5	–	In	Det
18.50	–	100.0	–	–	2.5	–	In	Det
20.00	100.0	–	–	–	2.0	–	Out	Waste
20.17	–	–	–	100.0	1.0	–	Out	Waste

Eluting Gradient:

Time	A %	B %	C %	D %	Flow Rate (mL/mm)
1.00	90.0	1.0	4.0	5.0	0.8
3.00	90.0	1.0	4.0	5.0	1.2
4.50	75.0	2.5	10.0	12.5	0.8
6.50	60.0	4.0	16.0	20.0	0.8
8.50	44.0	5.6	22.4	28.0	0.8
16.50	25.0	7.5	30.0	37.5	0.8
17.50	5.0	9.5	38.0	47.5	1.0
18.50	5.0	9.5	38.0	47.5	1.0
20.00	90.0	1.0	4.0	5.0	1.0
20.17	90.0	1.0	4.0	5.0	1.0

MS Conditions

Ionization:	Positive Electrospray
Probe Temperature:	500 °C
Cone Voltage:	80/100/120 V
Scan Mode:	Full scan 200-400 *m/z* or selected ion monitoring (SIM)
ESI Voltage:	3.0 kV
Scan Time:	0.5 s

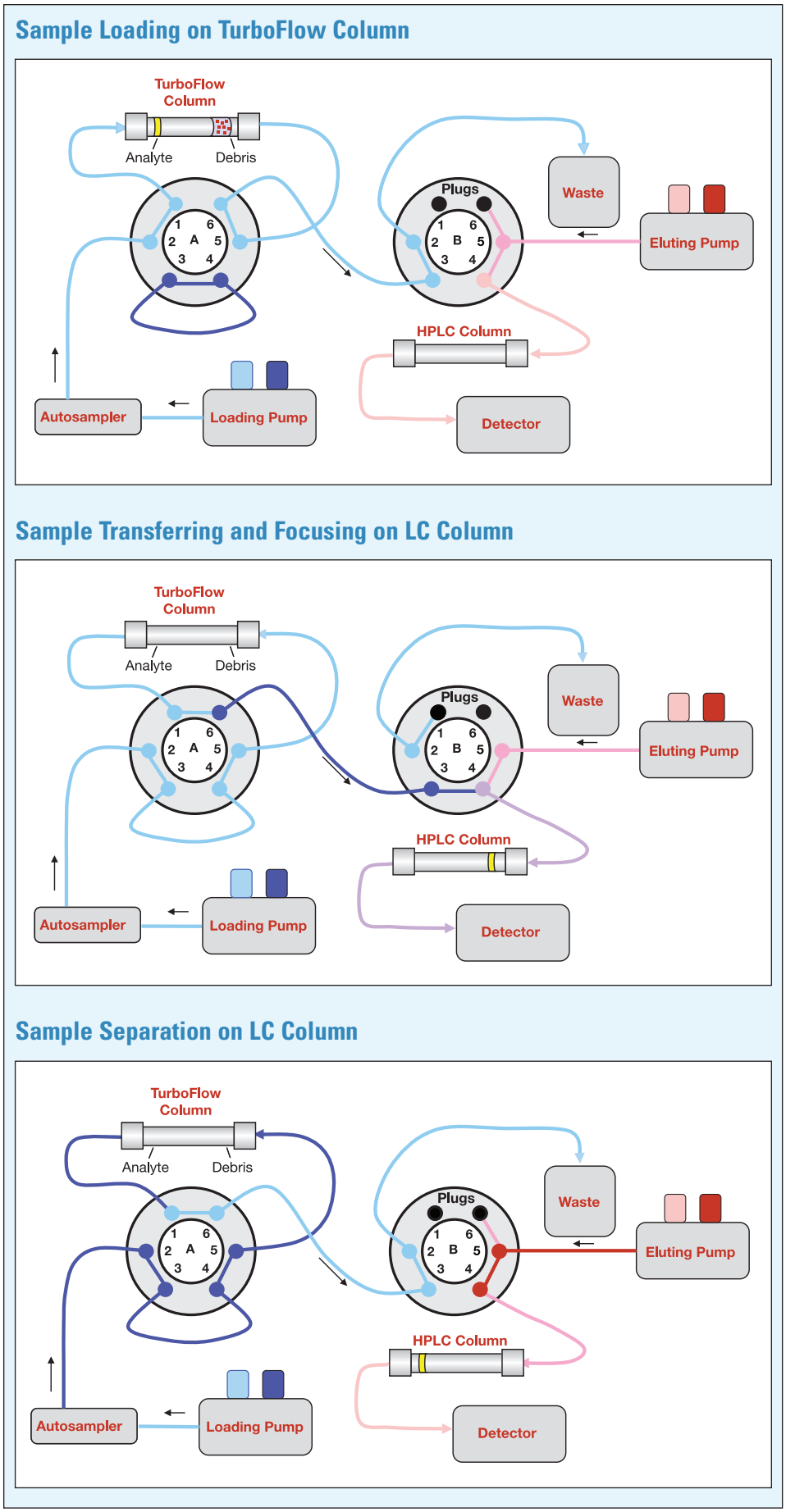

Figure 1: Transcend TLX dual-column configuration

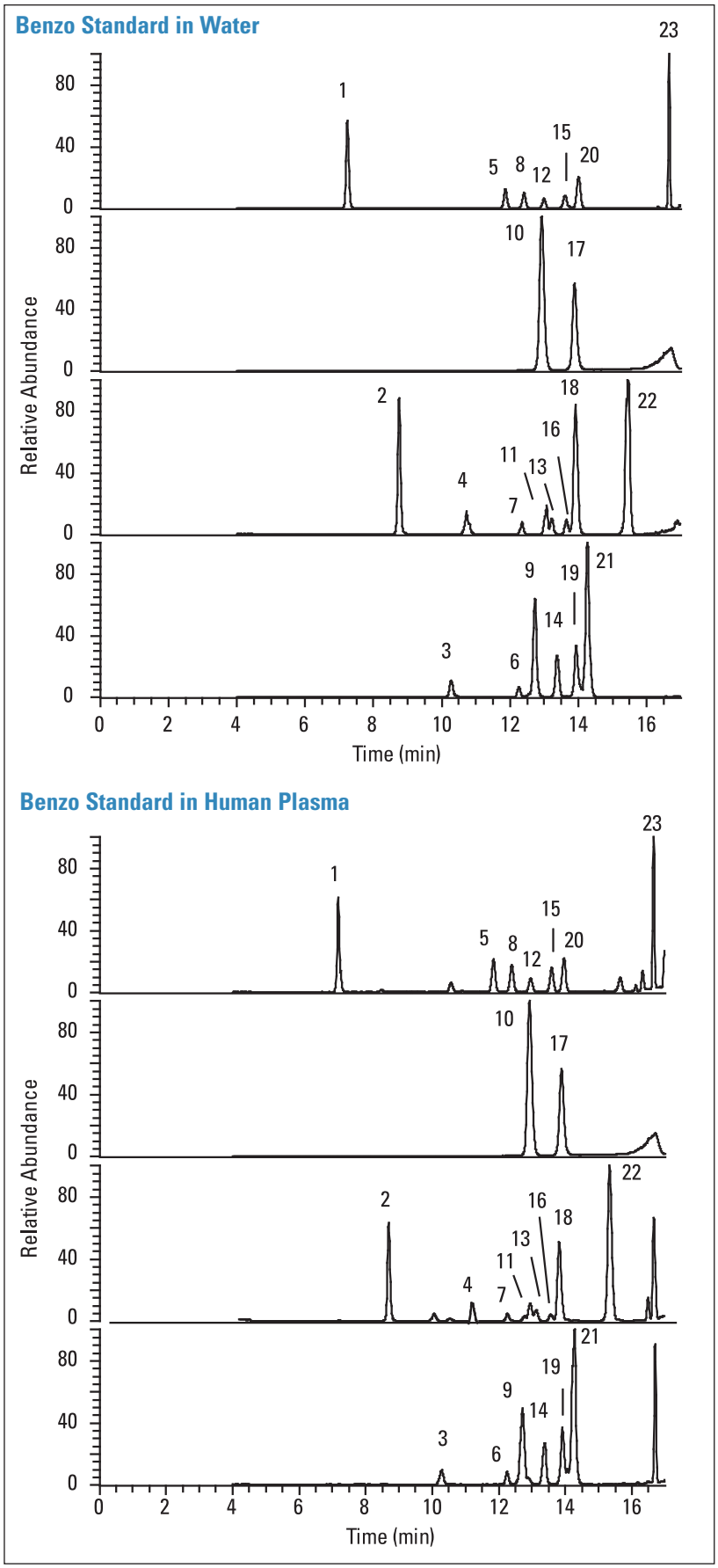

Figure 2: Separation and detection of 23 benzodiazepine standards at 100 ppb concentrations in (a) water and (b) human plasma using the Transcend TLX system coupled to the single quadrupole MSQ Plus mass Detector. Note: This method has 2 min less time for TurboFlow column washing step compared with the stated LC/MS method in Chromatographic conditions.

Results and Discussion

On-line Sample Preparation and LC Separation

The Transcend TLX system drastically minimizes the tedious sample preparation steps required for LC/MS analysis of biological fluids, enabling direct injection of plasma and urine samples with minimal pre-treatment. In this study, plasma samples required only a simple protein precipitation step, while urine samples were either directly injected or hydrolyzed prior to injection.

Figure 2 shows chromatograms of 23 benzodiazepine standards spiked in water and in human plasma. On-line sample preparation, separation and detection were achieved in less than 20 minutes. Matrix interferences were virtually eliminated by on-line extraction and cleanup using TurboFlow technology. Good peak shapes were obtained, ensuring maximum detection sensitivity and robust quantitation.

Peak Number	Compound Name
1	7-Aminoclonazepam
2	7-Aminoflunitrazepam
3	Bromazepam
4	Chlordiazepoxide
5	Alphahydroxytriazolam
6	Lorazepam
7	Oxazepam
8	Alphahydroxyalprazolam
9	Estazolam
10	Midazolam
11	Nitrazepam
12	Clobazam
13	Clonazepam
14	Desalkylflurazepam
15	Temazepam
16	Lormetazepam
17	Flurazepam
18	Triazolam
19	Nordiazepam
20	Flunitrazepam
21	Alprazolam
22	Diazepam
23	Prazepam

Table 1: Peak names of the 23 benzodiazepine in Figure 2

Identification and Confirmation

The identification of the benzodiazepine compounds in sample matrices can be realized by the retention times of LC separations and confirmed by their MS spectra. The MS spectra with rich fragmentation information of the MSQ Plus Mass Detector can be collected with in-source collision induced dissociation (CID) via accelerating the ions by increasing the cone voltage. The MS spectra library for the benzodiazepines were created using three cone voltages at 80, 100 and 120 v. The representative fragmentation MS spectra was shown in Figure 3 for flunitrazepam and 7-amino-flunitrozepam. With the current HPLC/MS method, target benzodiazepine compounds can be identified and confirmed by matching their mass spectra against the MS spectra library. The Thermo Scientific Xcalibur software will display the searching result with a list of compounds ranked by their matching scores. The implementation of the MS spectra library in compound identification provided more convenient and confirmative results in analyzing the real world samples.

Quantitation and Confirmation

Selected ion monitoring (SIM) assays were developed for quantitation and confirmation. Two ions, the primary *m/z* ion and a secondary confirmation ion, were monitored for all target benzodiazepines. Confirmation ions were created through in-source collision induced dissociation (CID) to provide verification of the presence of a suspected benzodiazepine in a sample. Table 2 lists the quantitative results with the primary and confirmative ions for the targeted benzodiazepines in plasma, urine and hydrolyzed urine matrices. Extracted ion chromatograms of representative benzodiazepines spiked at 200 ppb in urine and in plasma matrices are shown in Figure 4. Quantitative results based on confirmation ions were within 5% of those based on molecular ions in plasma, urine and hydrolyzed urine matrices. The method is highly reproducible, with RSDs of three replicate injections ranging from 0.8–6.9% (Table 2) and quantitation accuracy of less than 10% for all benzodiazepines in plasma, urine and hydrolyzed urine matrices.

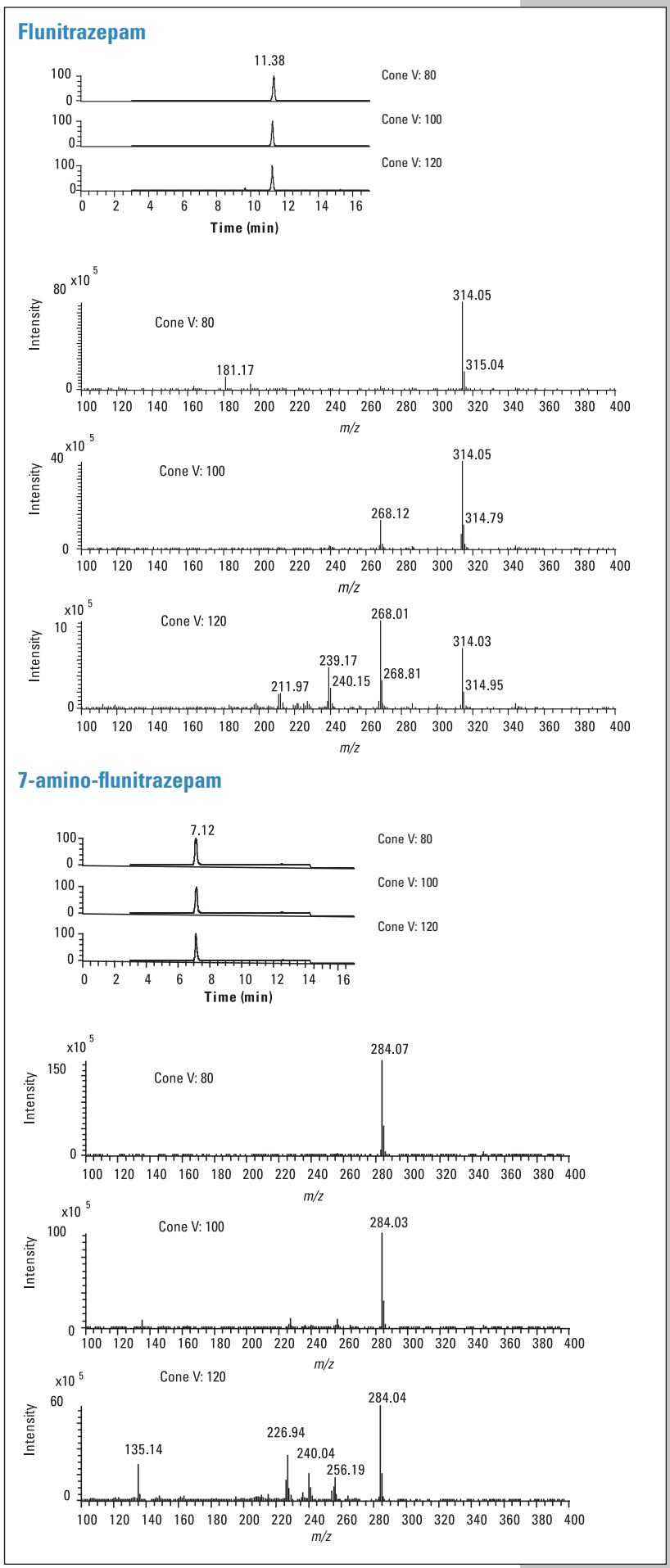

Figure 3: The MS spectra of flunitrazepam and 7-amino-flunitrazepam at 80, 100 and 120 v cone voltage

Compound Name	Quantitation Ion	Internal Standard	Quantitation Results in Human Plasma at 200 ppb	RSD% (n=3)	Quantitation Results in Urine at 200 ppb	RSD% (n=3)	Quantitation Results in Urine Hydrolysis at 200 ppb	RSD% (n=3)
7-Aminoclonazepam	286.10	7-Aminoclonazepam-D4	194.6	1.6	199.7	3.9	209.1	1.2
7-Aminoclonazepam	250.10	7-Aminoclonazepam-D4	195.2	1.3	197.8	3.8	206.4	3.3
7-Aminoflunitrazepam	284.08	7-Aminoflunitrazepam-D7	213.6	1.1	216.9	1.2	208.7	1.6
7-Aminoflunitrazepam	256.10	7-Aminoflunitrazepam-D7	215.4	1.5	210.4	4.2	207.1	2.0
Bromazepam	315.96	Estazolam-D5	184.6	5.1	186.7	3.1	217.8	1.8
Bromazepam	287.84	Estazolam-D5	183.0	1.7	187.4	4.1	N.A.	N.A.
Chlordiazepoxide	300.08	Estazolam-D5	206.6	2.3	191.0	0.5	237.0	6.9
Chlordiazepoxide	282.08	Estazolam-D5	207.0	1.6	195.0	0.4	215.3	5.9
Alphahydroxytriazolam	358.90	Estazolam-D5	191.1	5.3	190.4	3.5	200.5	2.8
Alphahydroxytriazolam	330.87	Estazolam-D5	189.6	2.2	188.6	2.9	205.8	4.4
Lorazepam	321.00	Estazolam-D5	193.3	6.5	203.4	5.2	189.4	4.9
Lorazepam	274.96	Estazolam-D5	190.2	5.0	206.9	4.4	N.A.	N.A.
Oxazepam	287.00	Oxazepam-D5	185.4	3.9	196.8	5.6	202.7	4.4
Oxazepam	241.05	Oxazepam-D5	190.6	2.8	191.6	5.7	181.1	4.9
Alphahydroxyalprazolam	325.06	Alphahydroxyalprazolam-D5	190.9	4.4	198.3	6.5	197.1	3.2
Alphahydroxyalprazolam	297.00	Alphahydroxyalprazolam-D5	196.2	4.8	203.6	3.3	196.5	3.5
Estazolam	295.04	Estazolam-D5	205.3	5.4	187.5	3.2	197.1	2.7
Estazolam	267.01	Estazolam-D5	198.3	1.2	197.9	4.6	207.7	2.8
Midazolam	326.08	Alprazolam-D5	205.6	0.9	205.8	3.4	200.8	2.5
Midazolam	291.00	Alprazolam-D5	211.0	1.9	198.9	4.4	199.2	3.0
Nitrazepam	282.4	Alprazolam-D5	194.2	5.9	196.8	4.1	201.2	1.5
Nitrazepam	236.00	Alprazolam-D5	195.8	4.5	201.4	3.8	218.0	4.8
Clobazam	301.08	Alprazolam-D5	188.5	3.4	190.3	4.2	210.7	2.1
Clobazam	259.00	Alprazolam-D5	212.5	1.1	197.6	5.7	N.A.	N.A
Clonazepam	315.93	Alprazolam-D5	188.3	4.1	208.5	3.6	180.2	6.7
Clonazepam	269.98	Alprazolam-D5	192.8	3.9	203.2	2.7	209.7	4.5
Desalkylflurazepam	261.23	Alprazolam-D5	208.4	5.1	102.1	1.8	216.6	4.3
Desalkylflurazepam	226.13	Alprazolam-D5	195.2	2.6	104.2	2.8	213.4	1.3
Temazepam	301.06	Alprazolam-D5	190.2	3.2	215.4	3.9	N.A.	N.A
Temazepam	255.09	Alprazolam-D5	183.5	0.4	213.3	2.5	206.5	4.7
Lormetazepam	262.01	Alprazolam-D5	192.9	5.1	203.2	1.2	195.8	3.3
Lormetazepam	243.04	Alprazolam-D5	185.8	2.6	194.7	3.5	213.5	2.5
Flurazepam	388.01	Alprazolam-D5	197.5	5.0	N.A.	N.A	196.2	2.4
Flurazepam	314.91	Alprazolam-D5	198.3	6.0	183.3	2.1	198.2	0.8
Triazolam	342.91	Alprazolam-D5	207.3	4.7	183.5	1.2	219.6	0.3
Triazolam	308.01	Alprazolam-D5	208.2	2	196.4	4.7	217.9	4.5
Nordiazepam	271.05	Nordiazepam-D5	194.3	2.9	207.9	5.4	197.5	4.2
Nordiazepam	243.00	Nordiazepam-D5	187.7	1.7	201.2	4.3	215.5	1.4
Flunitrazepam	314.07	Flunitrazepam-D7	203.1	2.9	180.6	3.0	200.8	6.7
Flunitrazepam	268.05	Flunitrazepam-D7	202.3	3.6	192.8	5.5	210.6	5.8
Alprazolam	309.10	Alprazolam-D5	194.2	1.5	196.3	4.9	210.5	2.9
Alprazolam	281.00	Alprazolam-D5	200.3	4.0	202.9	4.8	201.5	1.5
Diazepam	285.10	Diazepam-D5	193.4	4.4	201.3	4.8	204.8	1.6
Diazepam	257.20	Diazepam-D5	193.1	1.6	201.1	3.2	197.5	3.4
Prazepam	325.09	Prazepam-D5	202.9	2.1	186.9	1.8	199.3	0.8
Prazepam	271.10	Prazepam-D5	201.6	3.0	193.1	1.3	196.0	2.1

Table 2: Quantitation parameters and data for benzodiazepines

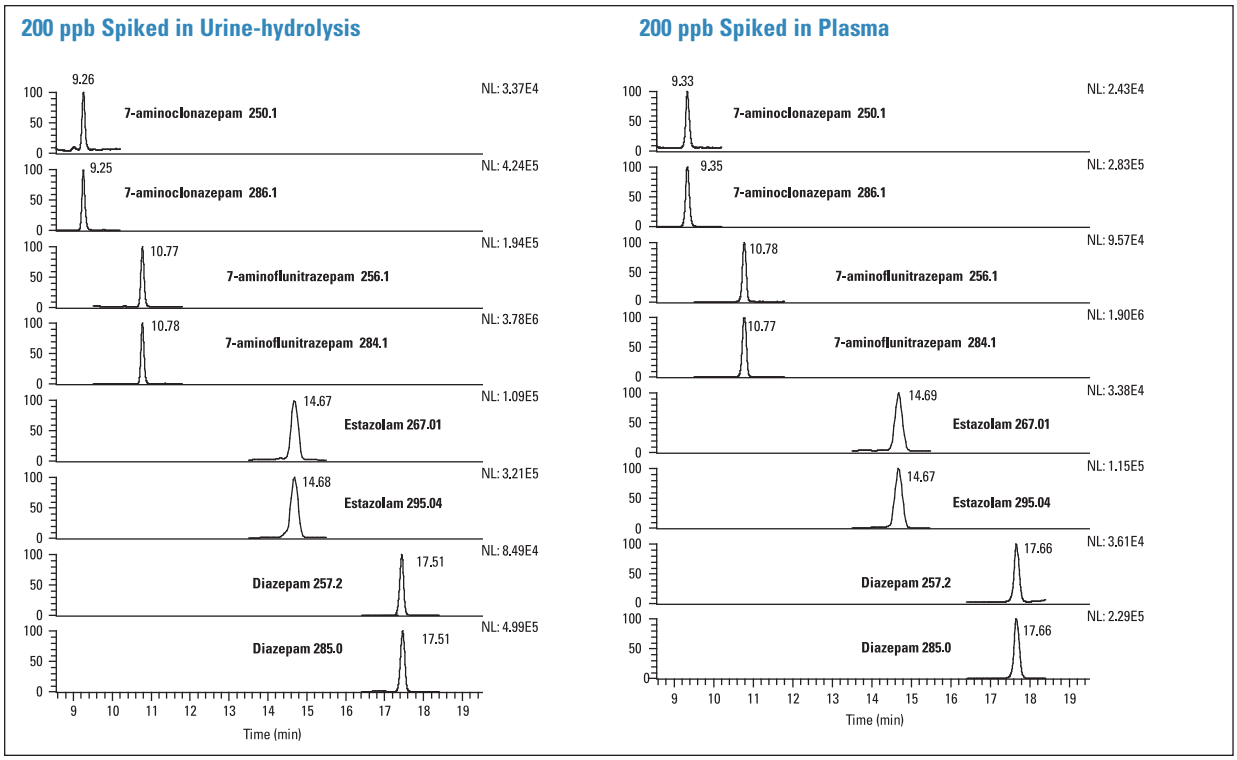

Figure 4. Extracted ion chromatograms of representative benzodiazepines in human plasma and urine matrices. The primary *m/z* ion and a confirmation ion were monitored for each benzodiazepine.

Linearity and Sensitivity

Excellent linearity in detector response was observed over the range of 5–1000 ng/mL (ppb) for benzodiazepine standards in human plasma, urine and hydrolyzed urine matrices, with correlation coefficients greater than 0.999 for most analytes (Table 3). Representative calibration curves are shown in Figure 5a–5c. Most compounds were detectable at levels as low as 5 ppb in plasma and urine matrices (Figure 6), which is below the lower limit of typical therapeutic concentrations of most benzodiazepines. The sensitivity of this LC/single quadrupole MS method is comparable to those that utilize triple quadrupole mass spectrometers.

Compound Name	Quantitation Ion	Linear Range Human Plasma (ng/mL)	Correlation Coefficients (R2)	Linear Range Urine (ng/mL) (ng/mL)	Correlation Coefficients (R2)	Linear Range Urine Hydrolysis (ng/mL)	Correlation Coefficients (R2)
Midazolam	326.08	5–1000	0.9999	5–1000	0.9996	5–1000	0.9996
Midazolam	291.00	5–1000	0.9999	5–1000	0.9999	5–1000	0.9999
Clonazepam	315.93	10–1000	0.998	10–1000	0.998	50–1000	0.998
Clonazepam	269.98	10–1000	0.992	10–1000	0.997	50–1000	0.9998
Flurazepam	388.01	5–1000	0.9999	5–1000	0.999	5–1000	0.999
Flurazepam	314.91	5–1000	0.9996	5–1000	1.000	5–1000	0.999
Triazolam	342.91	5–1000	1.000	5–1000	0.998	5–1000	0.999
Triazolam	308.01	5–1000	0.9999	5–1000	0.9998	5–1000	0.9996
Nordiazepam	271.05	5–1000	0.999	5–1000	0.997	10–1000	0.999
Nordiazepam	243.00	50–1000	0.997	5–1000	0.993	50–1000	0.997
Flunitrazepam	314.07	5–1000	0.9998	5–1000	0.9998	5–1000	0.999
Flunitrazepam	268.05	5–1000	0.9999	5–1000	1.000	5–1000	0.999
Alprazolam	309.10	5–1000	0.997	5–1000	0.996	5–1000	0.999
Alprazolam	281.00	5–1000	0.993	5–1000	0.994	5–1000	0.999
Diazepam	285.10	5–1000	0.995	5–1000	0.996	5–1000	0.9998
Diazepam	257.20	5–1000	0.996	5–1000	0.996	5–1000	0.999
Prazepam	325.09	5–1000	0.9999	5–1000	0.999	5–1000	0.999
Prazepam	271.10	5–1000	0.9997	5–1000	0.9995	5–1000	0.999

Table 3: Method linearity for representative benzodiazepines in human plasma and urine matrices

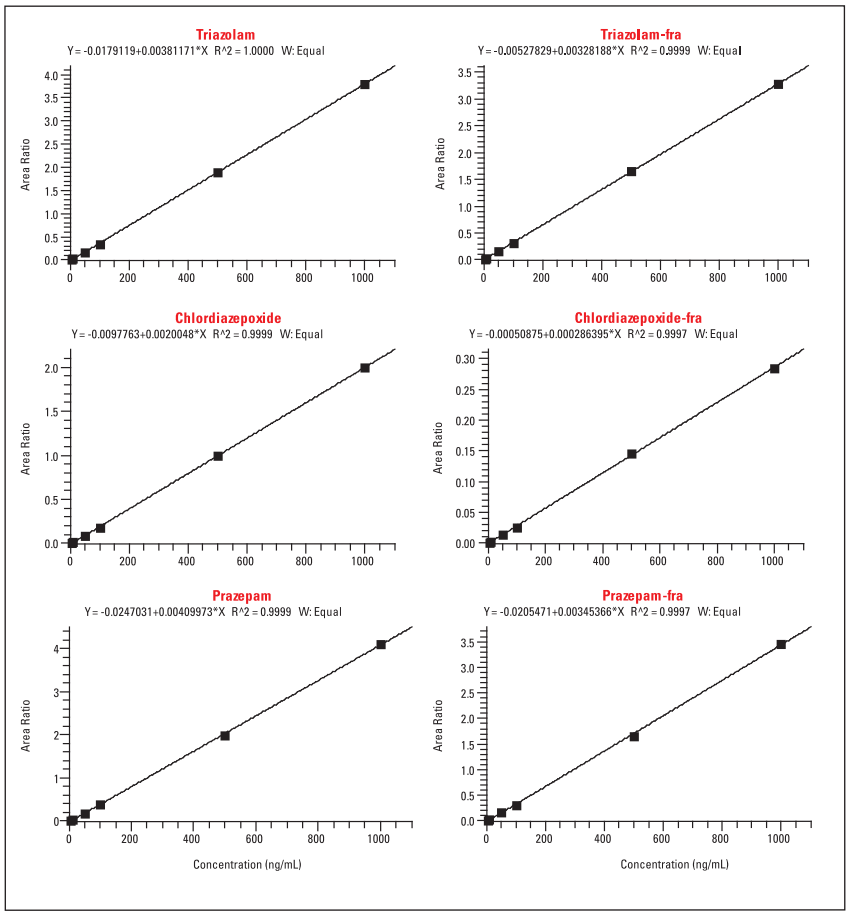

Figure 5a: Representative calibration curves of benzodiazepine standards in plasma matrix

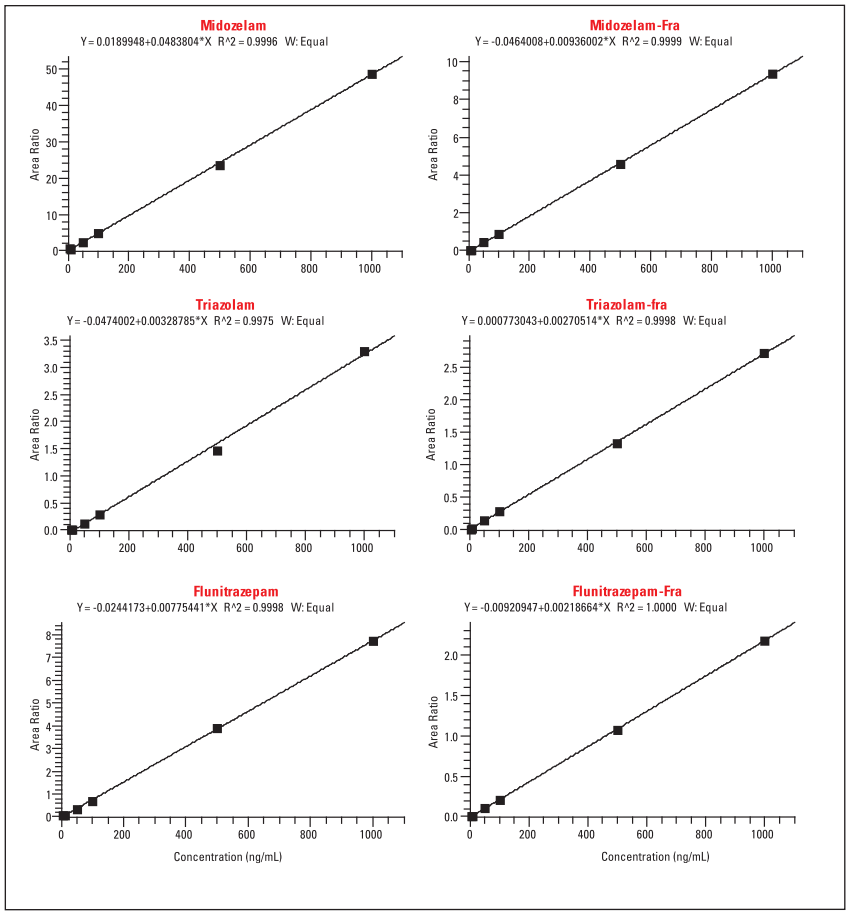

Figure 5b: Representative calibration curves of benzodiazepine standards in urine matrix

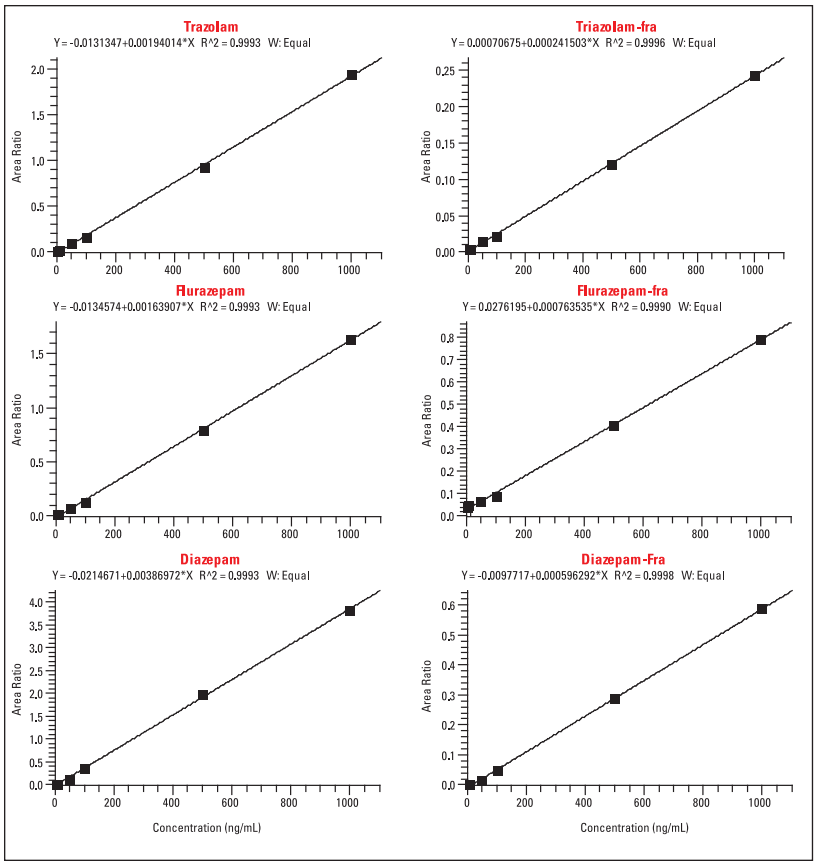

Figure 5c: Representative calibration curves of benzodiazepine standards in urine hydrolysis matrix

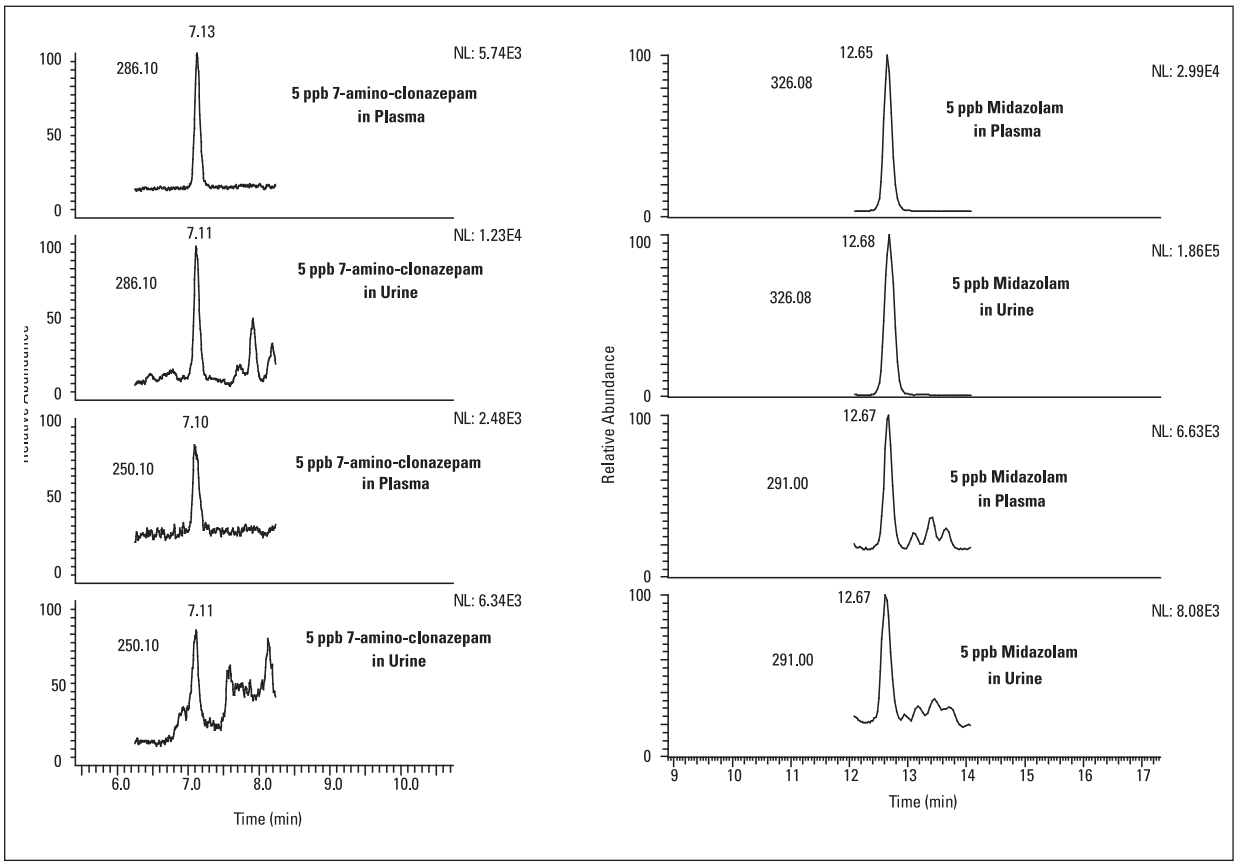

Figure 6: Extracted ion chromatograms of representative benzodiazepines at 5 ppb in human plasma and urine matrices

Quantitative Analysis of Benzodiazepines in Post-Mortem Samples

To evaluate the applicability of this quantitative LC/MS method to complex real-world samples, post-mortem blood and urine specimens were analyzed. Several benzodiazepines were detected at concentrations ranging between 22.8 ppb – 1193.2 ppb. Table 4 summarizes the benzodiazepines that were identified and quantified in each sample. The ion chromatograms of benzodiazepines in three blood samples and in one urine sample are shown in Figure 7. Alprazolam, the most frequently abused benzodiazepine, was detected in two blood samples. 7-aminoclonazepam, a metabolite of the commonly abused benzodiazepine clonazepam, was detected in one urine sample. Diazepam, another highly abused benzodiazepine, and its active metabolite nordiazepam were simultaneously detected in one blood specimen and in one urine specimen; this same urine sample also contained detectable levels of temazepam and oxazepam, a metabolite of diazepam and temazepam. Midazolam was detected in one blood sample. A discrepancy quantitative result was observed with urine sample #1, where the primary ion quantitative results are much lower than that of the confirmative ion for diazepam. It was noticed that the extracted chromatogram with the primary ion (285.1) showed a shoulder peak (Figure 7), indicating an interferences from matrices occurred at this particular mass. The quantitative results of the confirmative ion (257.2) in this case would be more reliable. For the rest benzodiazepine compounds in post-modem samples, the quantitative results using the confirmation ions were in excellent agreement with the quantitative results obtained using primary *m/z* ions.

Compound Name	Quantitation Ion	Internal Standard	Quan Blood #1 (ppb)	Quan Blood #2 (ppb)	Quan Blood #3 (ppb)	Quan Urine #1 (ppb)	Quan Urine #2 (ppb)
7-Aminoclonazepam	286.10	Alprazolam-D5	N.D.	N.D.	N.D.	N.D.	471.7
7-Aminoclonazepam	250.10	Alprazolam-D5	N.D.	N.D.	N.D.	N.D.	433.0
Oxazepam	287.0	Alprazolam-D5	N.D.	N.D.	N.D.	1158.9	N.D.
Oxazepam	241.05	Alprazolam-D5	N.D.	N.D.	N.D.	1193.2	N.D.
Midazolam	326.08	Estazolam-D5	32.1	N.D.	N.D.	N.D.	N.D.
Midazolam	291.00	Estazolam-D5	29.4	N.D.	N.D.	N.D.	N.D.
Temazepam	255.09	Alprazolam-D5	N.D.	N.D.	N.D.	464.4	N.D.
Nordiazepam	271.05	Nordiazepam-D5	N.D.	N.D.	26.4	167.2	N.D.
Nordiazepam	243.00	Nordiazepam-D5	N.D.	N.D.	22.8	168.9	N.D.
Alprazolam	309.10	Alprazolam-D5	N.D.	28.3	87.6	N.D.	N.D.
Alprazolam	281.00	Alprazolam-D5	N.D.	26.5	86.2	N.D.	N.D.
Diazepam	285.10	Diazepam-D5	N.D.	N.D.	75.6	38.5	N.D.
Diazepam	257.20	Diazepam-D5	N.D.	N.D.	71.7	69.5	N.D.

Table 4: Benzodiazepine concentrations in human post-mortem blood and urine specimens.
The post-mortem urine samples were treated with β-glucuronidase hydrolysis prior to analyses.

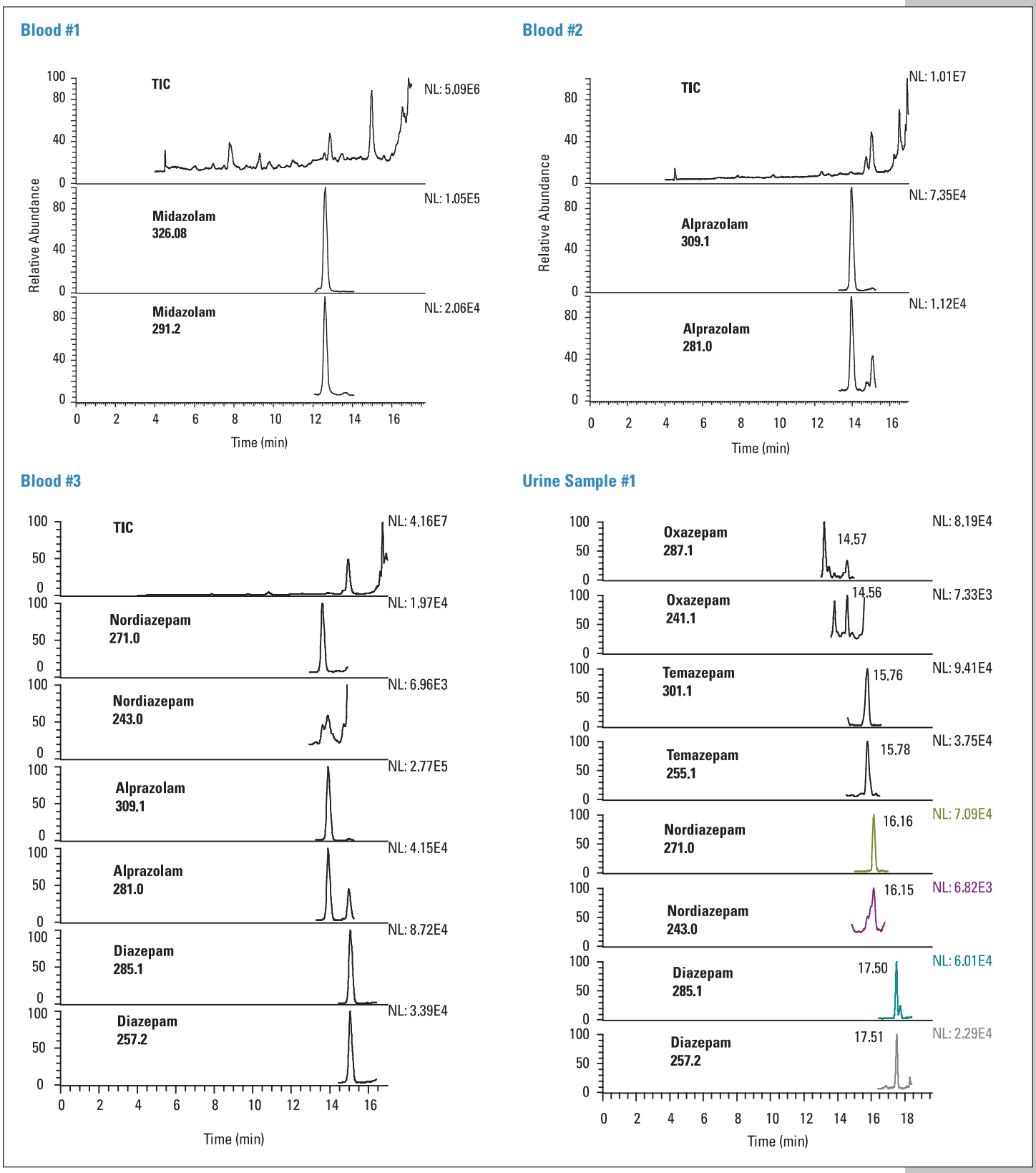

Figure 7: Chromatograms of benzodiazepines in human post-mortem blood and urine samples

Conclusion

A simple, rapid and robust quantitative LC/MS assay for benzodiazepines in biological fluids was developed. Online extraction, separation, detection and quantitation of 23 benzodiazepines in human plasma and urine were achieved in 20 minutes using the Transcend TLX system coupled to the MSQ Plus Single Quadrupole Mass Detector. The linear dynamic range obtained was 5–1000 ng/mL in matrix. Consistent ppb level quantitative results with primary ion and confirmative ion in blood and urine matrices with peak area RSD better than 5% were obtained. The quantitative results with confirmative ion on three post-mortem blood samples and two post-mortem urine samples were in excellent agreement with primary ion quantitative results.

TurboFlow technology drastically reduced sample preparation, eliminated matrix effects and significantly improved sensitivity to allow robust confirmatory quantitation of low ppb levels of benzodiazepines with single quadrupole mass spectrometry. Convenient and cost-effective, the automated benchtop TLX-MSQ LC/MS platform is ideally suited for routine use in clinical and forensic toxicology applications.

References

1. Centers for Disease Control and Prevention. Emergency Department Visits Involving Nonmedical Use of Selected Prescription Drugs–United States, 2004-2008. Morbidity and Mortality Weekly Report, June 18, 2010, 59(23); 705–709
2. United States Department of Justice, Drug Enforcement Administration, Office of Diversion Control. http://www.deadiversion.usdoj.gov/drugs_concern/benzo_1.htm

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