Fast and Selective Quantification of Benzodiazepines in Urine using LDTD-TripleTOF 5600 System

Gregory Blachon[1], Serge Auger[1] , Sarah Demers[1], Pierre Picard[1] & Michael Jarvis[2]
[1]Phytronix Technologies, Quebec, Canada, [2]AB Sciex, Concord, ON, Canada

Keywords: Benzodiazepines, Urine, Liquid-Liquid Extraction, LDTD

Introduction

Benzodiazepines and their metabolites in urine are drugs frequently analyzed in Toxicology labs. These analyses are generally performed on LC-MS/MS systems, requiring run-times as long as 15 minutes.

Laser Diode Thermal Desorption technology allows a direct vaporization-ionization of the sample into gas phase, providing fast analysis without the use of any solvent. Using such a shotgun approach for 14 molecules (benzodiazepines) with a high potential of cross-talk interference due to similar structure and molecular weight is a challenge. High resolution accurate mass AB SCIEX TripleTOF® 5600 is used to gain specificity over triple quadrupole systems.

The high throughput LDTD™ ion source coupled with a high resolution accurate mass AB SCIEX TripleTOF® 5600 System constitutes a Fast and Specific system for the quantification of benzodiazepines.

LDTD-MS/MS System

Figure 1: LDTD interfaced to AB SCIEX TripleTOF®5600 System

Sample preparation

Extraction procedure

The following drugs and metabolites (7-aminoclonazepam, 7-aminoflunitrazepam, chlordiazepoxide, clonazepam, diazepam, estazolam, lorazepam, nordiazepam, OH-alprazolam, OH-ethylfluorazepam, OH-midazolam, OH-triazolam, oxazepam and temazepam) were spiked in urine to obtain a standard curve from 1 ng/mL to 1000 ng/mL.

Preparation of an IS mixture in aqueous solution:

- 0.2 N NaOH
- 100 ng/mL of each deuterated IS (d5-Temazepam, d5-Oxazepam, d7-7aminoflunitrazepam, and d4-OH-triazolam)

A liquid-liquid extraction is performed as follows:

- 25 µL of urine sample
- 75 µL of IS mixture
- Vortex
- 200 µL of Ethyl Acetate
- Vortex
- Centrifugate for 2 minutes at 16000 rpm
- Dispense 6 µL of upper layer on Lazwell plate and let it dry at room temperature

LDTD-MS/MS Parameters

LDTD

Gas Flow:	3 L/min	
Laser pattern:	Time (s)	Power (%)
	0	0
	2	0
	5	55
	7	55
	7.1	0
	8	0

The analysis was performed using the Product Ion mode on a TripleTOF® 5600 System. The inlet parameters for the source are: Ion Source Gases (GS1/GS2) at 0, Curtain Gas (CUR) at 10, Temperature (TEM) at 0 and Nebulizer Current (NC) at 3.0.

All product ions use 100 as Declustering Potential (DP), an accumulation time of 11 ms and a scan window from 100 to 350 amu in positive mode. **Table 1** summarizes the specific transitions for the compounds

Compound	Q1	TOF (± 10 ppm)	CE
7-AminoClonazepam	286.1	121.0747	38
7-AminoFlunitrazepam	284.1	135.0907	35
Chlordiazepoxide	300.1	227.0492	30
Clonazepam	316	270.0552	30
Diazepam	285.1	222.1154	33
Estazolam	295.1	205.0752	45
Lorazepam	321	275.0139	30
Nordiazepam	271.1	208.0985	38
OH-Alprazolam	325.1	297.0648	32
OH-EthylFluorazepam	333.1	211.0785	44
OH-Midazolam	342.1	203.0361	35
OH-Triazolam	359	331.0114	35
Oxazepam	287.1	241.0523	30
Temazepam	301.1	255.0685	30
Temazepam D5	306.1	260.0996	30
Oxazepam D5	292.1	246.0839	30
7-AminoFlunitrazepam D7	291.1	138.1098	35
OH-Triazolam D4	363	335.0400	35

Table 1 Analysis of Benzodiazepines on TripleTOF® 5600 System

Results and Discussion

Using the parameters in the previous table, standard curves are generated for every compound between 1 and 1000 ng/mL as shown in **Figure 2** for clonazepam.

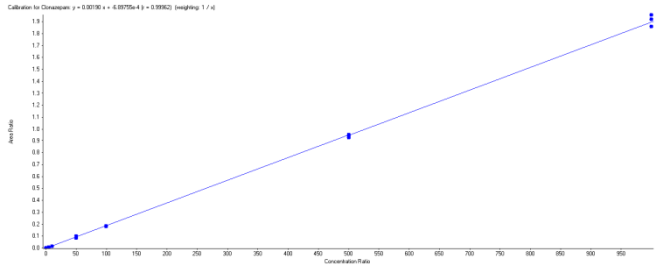

Figure 2 Clonazepam calibration curve.

The 14 benzodiazepines show R^2 > 0.999 and LOD < 5 ng/mL. 62 real urine samples have been tested with this method to correlate with LC-MS/MS method. **Figure 3** shows a correlation >0.99 between results for temazepam using both methods.

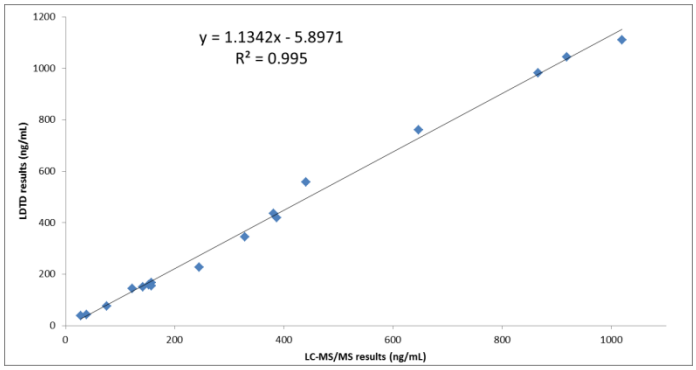

Figure 3 Correlation between Temazepam concentrations in real urine samples obtained with LDTD-MS/MS and LC-MS/MS

Conclusion

With the High-Resolution versatility of the TripleTOF® 5600 System and the high-throughput of the LDTD™ ion source, we achieved an ultra-fast benzodiazepine quantification method running 1 sample **every 7 seconds**. Sample preparation consists in a liquid-liquid extraction, with the organic phase directly spotted onto LazWell plates.