ThP19 - 396

Selective High-Throughput method for simultaneous analysis of 14 Benzodiazepines in urine by LDTD-MS/MS

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OVERVIEW

Purpose
- Simultaneous quantitation of 14 benzodiazepines in high-throughput using LDTD-MS/MS

Method
- Liquid-Liquid extraction of Benzodiazepines spiked in Human Urine
- Standard curve is a cocktail of 14 benzodiazepines mixed together at concentrations between 1 and 1000 ng/mL
- Evaluation of possible cross-talk between similar molecules

Results
- Excellent linearity over the calibration range ($R^2 > 0.99$) for all benzodiazepines
- Accuracy ranging from 85 and 111.6% using area ratio value
- Precision ranging from 1.7 and 10 % using area ratio value
- Optimization of the conditions limiting the potential interference (1)
- **All samples are analysed with a run time of 9.6 seconds using LDTD-MS/MS**

INTRODUCTION

Benzodiazepine metabolites in urine are drugs frequently analyzed in Toxicology labs. High-throughput is a definite requirement for these laboratories due to the quantity of samples. Analyzing in a shotgun approach 14 molecules (Benzodiazepines) with a high potential of cross-talk interference due to similar structure and molecular weight is a challenge. Major chlorine atom isotopes may interfere with adjacent masses. Specific MS/MS transitions are used to increase the selectivity.

LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 well plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation with strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This innovative process allows for high-throughput capabilities of 9 seconds sample-to-sample analysis time, without carry over.

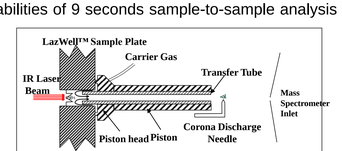

Figure 1 Schematic of the LDTD Ionization Source.

METHOD

Sample preparation
- 25 µL of Urine spiked sample
- 75 µL of NaOH 0.2N containing 100 ng/mL IS
- Vortex
- 200 µL Ethyl-Acetate
- Vortex
- Centrifuge at 16000RPM
- Dispense 6 µL into LazWell plate

LDTD Parameters
- Laser Power pattern :
  - ➢ Increase laser power to 55 % in 3.0 s
  - ➢ Stay at 55% for 2 s
  - ➢Decrease laser power to 0 %
- Carrier gas flow : 3 L/min (Air)

MS Parameters
- APCI (+)
- Dwell Time 11 ms

Instrumentation
- LDTD model S-960, Phytronix Technologies
- AB Sciex TripleTOF System

- LDTD model T-960, Phytronix Technologies
- Thermo Vantage TripleQuad

Table 1

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

* Same transitions are used with the TripleQuad system, without High-Resolution

Method Validation
- Individual Standards at 1000 ng/ml are analyzed while monitoring all transitions to identify potential interferences
- Only OH-Alprazolam contribute to Estazolam transition under heat effect at a level varying between 0,3 to 8% upon maximum power used (Table 2).
- Laser Pattern with 20% as maximum is used to limit eventual interferences

Table 2

Maximum laser power (%)	Area response Estazolam	Area response Estazolam (OH-Alpraz QC)	% interference
60	2539194	203461	8,0
45	2601699	157102	6,0
30	1288211	34603	2,7
20	968214	8348	0,86
15	697052	2132	0,31

- Pooled calibration curve in urine constructed by spiking urine sample at concentrations from 1 to 1000 ng/ml
- OH-Alprazolam concentration is lowered 10 times (0.1 to 100 ng/ml) into the pooled curve to avoid contribution to Estazolam signal.

RESULTS:

Table 3: LDTD – AB Sciex TripleTOF™ 5600 System

Compound	IS	LOD (ng/mL)	100 ng/mL QC (n=3)	
			Accuracy %	Precision %
7-AminoClonazepam	Temazepam – D5	5	86	15
7-AminoFlunitrazepam	7-AminoFlunitrazepam – D7	1	89	4.9
Chlordiazepoxide	Temazepam – D5	1	103	1.4
Clonazepam	Temazepam – D5	1	104	7.4
Diazepam	Temazepam – D5	5	103	4.3
Estazolam	Temazepam – D5	1	111	5.5
Lorazepam	Oxazepam – D5	1	88	10
Nordiazepam	7-AminoFlunitrazepam – D7	1	99	15
OH-Alprazolam	Oxazepam – D5	10	96	2.9
OH-EthylFluorazepam	Temazepam – D5	1	110	6.0
OH-Midazolam	Oxazepam – D5	5	96	7.8
OH-Triazolam	OH-Triazolam – D4	5	102	5.0
Oxazepam	Oxazepam – D5	1	93	12
Temazepam	Temazepam – D5	1	97	2.3

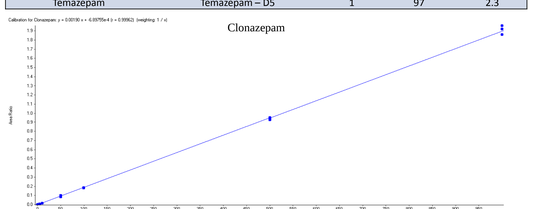

Figure 2

Table 4: LDTD – Thermo Scientific TSQ

Compound	IS	LOD (ng/mL)	100 ng/mL QC (n=3)	
			Accuracy %	Precision %
7-AminoClonazepam	Temazepam – D5	5	96	10
7-AminoFlunitrazepam	7-AminoFlunitrazepam – D7	5	96	2.9
Chlordiazepoxide	Temazepam – D5	1	94	2.9
Clonazepam	Temazepam – D5	1	100	6.3
Diazepam	Temazepam – D5	5	93	8.9
Estazolam	Temazepam – D5	1	96.7	4.3
Lorazepam	Oxazepam – D5	1	93.5	1.7
Nordiazepam	7-AminoFlunitrazepam – D7	5	98.1	4.3
OH-Alprazolam	Oxazepam – D5	5	107	13
OH-EthylFluorazepam	Temazepam – D5	1	98.3	3.9
OH-Midazolam	Oxazepam – D5	5	110	8.3
OH-Triazolam	OH-Triazolam – D4	5	98.0	5.2
Oxazepam	Oxazepam – D5	5	89.3	5.9
Temazepam	Temazepam – D5	1	100	2.1

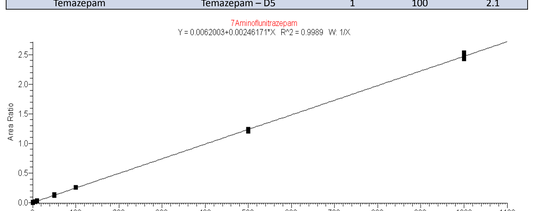

Figure 3

High-Resolution Performance
- Uses of AB Sciex TripleTOF™ 5600 System provide higher-selectivity giving better S/N ratio and lower blank response
- MS/MS speed capability enable to achieve 18 complete products ion scan in 250 msec giving at least 10 points per desorption peak
- The MS/MS mode of the QqToF system allows to use a quantitation ion and as many confirmation ions as needed
- Figure 4 present the product spectra of a compound used in calibration curve and highlight the chosen fragments
- Equivalent method with 2 confirmation ions in triple quadrupole require 54 transitions monitored at the same time. For a 3 seconds wide peak, this overcome the instrument capability. The experiment should be divided in 3 desorptions of the same sample

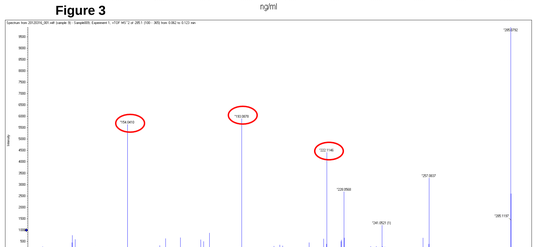

Figure 4

CONCLUSIONS

- LDTD Ion Source enables High-Throughput simultaneous analysis of 14 benzodiazepines in urine extract in **9.6 seconds sample-to-sample**
- Only one limited potential interference. Care must be taken if 1% of the concentration of OH-Alprazolam is higher than the cut-off value of Estazolam
- Good linearity, accuracy, specificity and precision for the quantification method of benzodiazepines using LDTD-MS/MS or LDTD-TripleTOF