A Rapid iMethod™ Test for Benzodiazepines in Urine

iMethod™ Test for Benzodiazepines V.1.1 for Cliquid® Software

The following information outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the quantitation of Benzodiazepines in urine when using an AB SCIEX 3200 QTRAP® or API 3200™ LC/MS/MS System. This method has also been optimized for use on the 4000 QTRAP® or API 4000™ LC/MS/MS System.

The method included is for the routine analysis of Benzodiazepines in urine. Calibration is performed by injecting stock solutions with concentration ranges from 20-2000 ng/ml for a total run time of 8.3 minutes per sample. The method uses deuterated internal standards for Aminoflunitrazepam, Aminoclonazepam, Diazepam, Nordiazepam, Oxazepam, and Hydroxyalprazolam.

The sample preparation is based upon dilution with acetate buffer, hydrolysis, further dilution, and then centrifugation followed by injection onto a Phenomenex Synergi, 4µ Hydro-RP 50 x 2 mm HPLC column, included with the method, connected to the Turbo V™ ion source of the mass spectrometer.

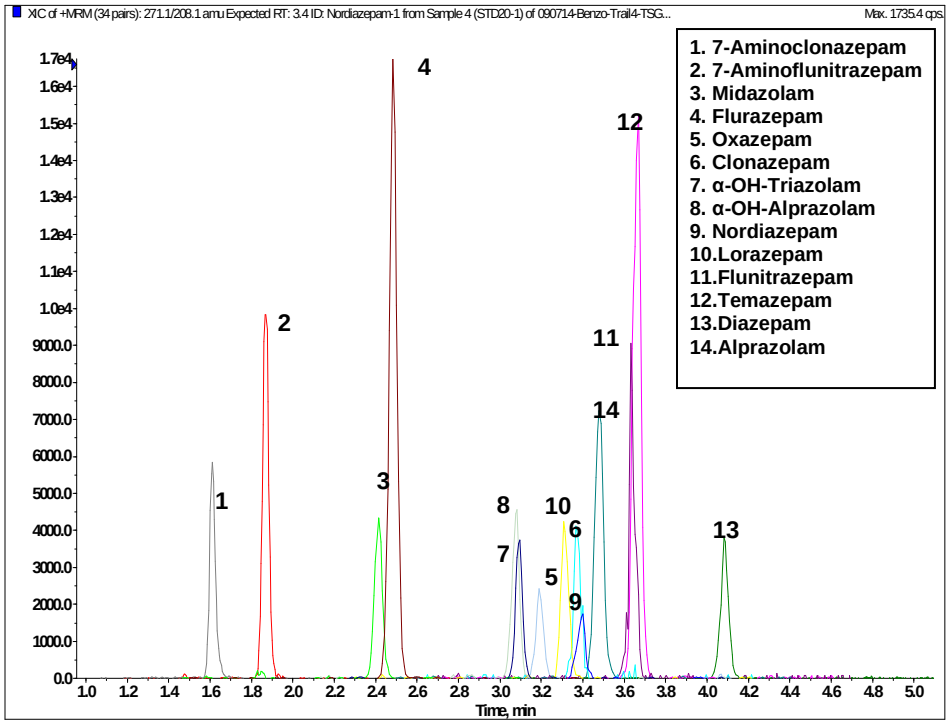


Figure 1. Representative chromatogram of spiked urine at 20 ng/mL performed on a 3200 QTRAP® LC/MS/MS system with a Shimadzu LC system in a 8.3-minute run

Quality control samples included 3 replicates at 100 ng/mL. %CV and S/N values for the target analytes were obtained using 6 replicates at 200 ng/mL. Recoveries ranged from 80 to 110%. The estimated detection limits for each analyte are more than sufficient to allow the analytical method to be used as either a screening or confirmation technique. The use of labeled internal standards for the target analytes further improves quantitation and allows for consistent cutoff levels in urine samples.

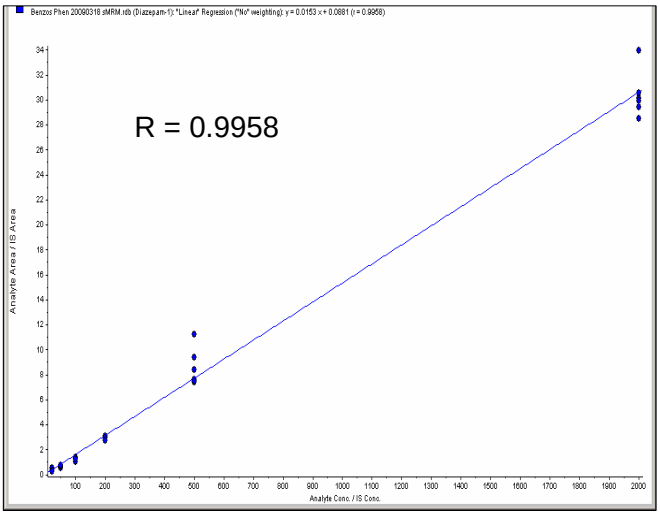
Table 1. Representative Signal-to-Noise Ratios of Spiked Urine Sample at 20 ng/mL

Analyte (used Internal Standard)	S/N	%CV	Estimated limit of detection (ng/mL)	Estimated limit of quantitation (ng/mL)
7-Aminoclonazepam (7-Aminoclonazepam-D4)	128.0	5.76	0.5	1.6
7-Aminoflunitrazepam (7-Aminoflunitrazepam-D7)	359.5	7.17	0.2	0.6
Alprazolam (Oxazepam-D5)	83.4	5.86	0.7	2.4
Clonazepam (Oxazepam-D5)	52.9	10.86	1.1	3.8
Diazepam (Diazepam-D5)	70.9	3.76	0.8	2.8
Flunitrazepam (Oxazepam-D5)	85.1	10.60	0.7	2.4
Flurazepam (Oxazepam-D5)	190.7	7.19	0.3	1.0
Lorazepam (Oxazepam-D5)	143.6	6.75	0.4	1.4
Midazolam (Oxazepam-D5)	96.9	14.58	0.6	2.1
Nordiazepam (Nordiazepam-D5)	31.1	14.86	2.0	6.4
Oxazepam (Oxazepam-D5)	21.5	9.80	2.8	9.3
Temazepam (Oxazepam-D5)	16.5	21.10	3.6	12.0
α-OH Alprazolam (α-OH Alprazolam-D5)	80.3	8.98	0.7	2.5
α-OH Triazolam (Oxazepam-D5)	18.2	9.31	3.3	11.0

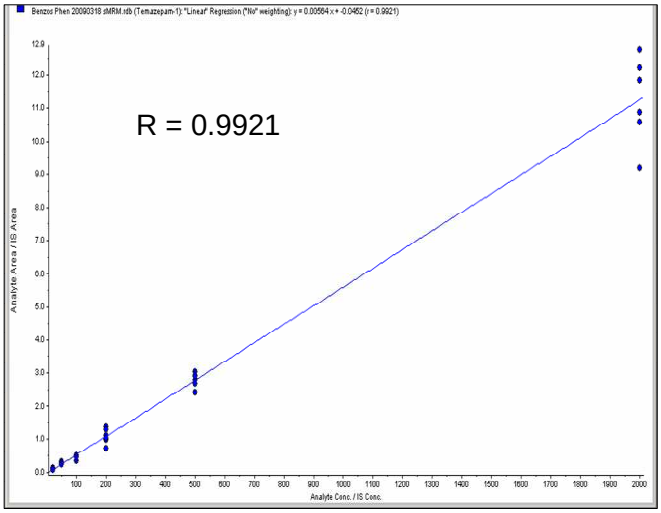
Calibration Curves

The following calibration curves, using the spiked calibration standards, are provided as examples, showing the range and linearity expected for the assay. Representative calibration curves are shown for four of the compounds included in the method. Standards at 20, 50, 100, 200, 500, and 2000 ng/mL were used to calibrate the analysis. Calibration curves for all other analytes were similar in linearity, precision, and accuracy. The data shown below was acquired on a 3200 QTRAP® LC/MS/MS system.

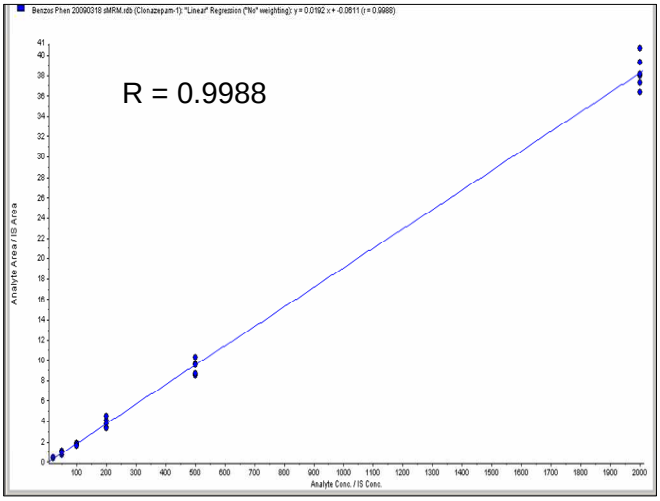
Diazepam



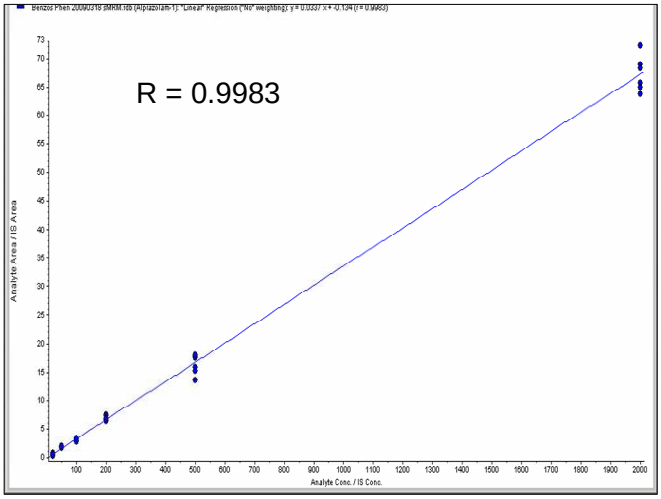
Temazepam



Clonazepam



Alprazolam



Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

System Requirements

To run this method as provided, the following equipment is required:

- An AB SCIEX 3200 or 4000 series LC/MS/MS System (3200 QTRAP®, API 3200™, API 4000™ or 4000 QTRAP® System)
- A Shimazdu Prominence 20A HPLC system with reservoir tray and bottles, CBM-20A system controller, 100 µl mixer, 2 isocratic LC-20AD pumps, 3 channel degasser , SIL-20AC autosampler and column oven or an Agilent 1100/1200 HPLC system with binary pump (no static mixer), well plate autosampler and thermostated column oven
- Synthetic urine blank (www.cerilliant.com)
- Benzodiazepine standards and deuterated internal standards (www.cerilliant.com)
- β-Glucuronidase (www.sigmaaldrich.com)
- HPLC grade water, acetonitrile, methanol and formic acid (www.sigmaaldrich.com)
- 1.5 ml Eppendorf tubes
- Phenomenex Synergi 4µ Hydro-RP 50 x 2 mm HPLC column
- A centrifuge able to accommodate Eppendorf tubes and run at 14,000 rpm
- Pipettes and standard laboratory glassware

Please note that the Phenomenex Synergi HPLC column is included with this iMethod™ Test. Also, that this method can also be run on other HPLC systems, given that they are supported for use by Cliquid® Software and the retention times are updated to reflect the configuration used.

Ordering Information

Product Name	Part Number
iMethod™ Test for Benzodiazepines V.1.1 for Cliquid® Software	1034374

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements.

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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