BNZ2

cobas®

ONLINE DAT Benzodiazepines II

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056927190	ONLINE DAT Benzodiazepines II (850 tests)	System-ID 2028 001	**cobas c** 303, **cobas c** 503
Materials required (but not provided):			
03304671190	Preciset DAT Plus I CAL 1-6 (1 x 5 mL)	Codes 20431-20436	
03304680190	Preciset DAT Plus II CAL 1-6 (1 x 5 mL)	Codes 20437-20442	
03304698190	C.f.a.s. DAT Qualitative Plus (6 x 5 mL)	Code 20698	
04590856190	C.f.a.s. DAT Qualitative Plus Clinical (3 x 5 mL)	Code 20699	
03312950190	Control Set DAT I (for 300 ng/mL assay) PreciPos DAT Set I (2 x 10 mL) PreciNeg DAT Set I (2 x 10 mL)	 Code 20123 Code 20124	
03312968190	Control Set DAT II (for 100 ng/mL assay) PreciPos DAT Set II (2 x 10 mL) PreciNeg DAT Set II (2 x 10 mL)	 Code 20125 Code 20126	
04500873190	Control Set DAT Clinical (for 100 ng/mL assay) PreciPos DAT Clinical (2 x 10 mL) PreciNeg DAT Clinical (2 x 10 mL)	 Code 20129 Code 20130	
03312976190	Control Set DAT III (for 200 ng/mL assay) PreciPos DAT Set III (2 x 10 mL) PreciNeg DAT Set III (2 x 10 mL)	 Code 20127 Code 20128	

English

For use in the USA only

System information

BZ1Q2: ACN 20280 (Urine): for qualitative assay, 100 ng/mL

BZ2Q2: ACN 20281 (Urine): for qualitative assay, 200 ng/mL

BZ3Q2: ACN 20282 (Urine): for qualitative assay, 300 ng/mL

BZ1S2: ACN 20284 (Urine): for semiquantitative assay, 100 ng/mL

BZ2S2: ACN 20285 (Urine): for semiquantitative assay, 200 ng/mL

BZ3S2: ACN 20286 (Urine): for semiquantitative assay, 300 ng/mL

BZQ1C: ACN 20283 (Urine): for qualitative assay, 100 ng/mL; using C.f.a.s. DAT Qualitative Plus Clinical

BZ3-QP: ACN 20288 (Urine): for qualitative assay, 300 ng/mL; using C.f.a.s. DAT Qualitative Plus

Intended use

Benzodiazepines II (BNZ2) is an in vitro diagnostic test for the qualitative and semiquantitative detection of benzodiazepines in human urine on **cobas c** systems at cutoff concentrations of 100 ng/mL, 200 ng/mL, and 300 ng/mL.

Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program. Semiquantitative assays are intended to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas chromatography/mass spectrometry (GC-MS), or Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS).

Benzodiazepines II provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC-MS) or Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method.[1,2] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

The benzodiazepines constitute a class of versatile and widely prescribed central nervous system (CNS) depressant drugs with medically useful anxiolytic, sedative, hypnotic, muscle relaxant, and anticonvulsant activities.[1,2,3,4,5] The absorption rates, distribution, metabolism, and elimination rates differ significantly among the benzodiazepine derivatives.

The quantitative differences in their potencies, pharmacodynamic spectra, and pharmacokinetic properties have led to various therapeutic applications. Clinical distinction of short-acting versus long-acting benzodiazepines have been observed in their efficacy, side effect, withdrawal, and dependence potential.[3,6,7] The extensive and efficacious therapeutic use of the benzodiazepines over the last several decades has inadvertently led to their misuse. Benzodiazepine overdoses are frequently associated with co-administration of drugs of other classes.[8,9] Acute or chronic alcohol ingestion and benzodiazepines co-administered may lead to various significant toxicological interactions. The net effect may be influenced by internal, external, and pharmacokinetic factors. Abuse patterns may involve relatively low benzodiazepine doses, as well as high-dose overuse; therefore, urinary drug/metabolite detection requires the proper selection of a cutoff that suits the requirements of the drug testing program.

Following ingestion, the benzodiazepines of the 1,4-substituted class (including the triazolobenzodiazepine derivatives) are absorbed, metabolized, and excreted in the urine at different rates as a variety of structurally related metabolites. Metabolite diversity reflects the different physiochemical properties and metabolic pathways of the individual drugs. Overall metabolic similarities include removal of substituents from the β ring of the 1,4-substituted benzodiazepines, α-hydroxylation of the triazolobenzodiazepines, demethylation, hydroxylation of the three-position carbon of the β ring, and conjugation of hydroxylated metabolites followed by urinary excretion predominantly as glucuronides.[1,2,3,4,5] The enzymatic hydrolysis of glucuronidated benzodiazepines can increase their cross-reactivities to benzodiazepine immunoassays.[10,11,12,13,14]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[11,15] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates that are photometrically detected by turbidity measurements. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

The presence of β-glucuronidase enzyme enhances the Benzodiazepines II assay cross-reactivity to some of the glucuronidated metabolites. Enzymatic

cleavage makes the benzodiazepine part of the glucuronides more accessible for the antibody.

Reagents - working solutions

R1 Benzodiazepines antibody (sheep polyclonal); buffer; β-glucuronidase enzyme; bovine serum albumin (BSA); 0.09 % sodium azide

R2 Conjugated benzodiazepine derivative microparticles; buffer; 0.09 % sodium azide

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	26 weeks

Do not freeze.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[16]

Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs*.[17]

Centrifuge highly turbid specimens before testing.

See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC-MS or LC-MS/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine

	Semiquantitative	Qualitative
Reporting time	10 min	10 min
Wavelength (sub/main)	– /546 nm	– /546 nm
Reagent pipetting		Diluent (H_2O)
R1	63 µL	–
R2	28 µL	–
Sample volumes	*Sample*	*Sample dilution*
100 and 200 ng/mL cutoffs		*Sample* *Diluent (NaCl)*
Normal	3.2 µL	– –
Decreased	3.2 µL	– –
Increased	3.2 µL	– –
300 ng/mL cutoff		
Normal	1.4 µL	– –
Decreased	1.4 µL	– –
Increased	1.4 µL	– –

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Semiquantitative applications

Calibrators	*100 and 200 ng/mL cutoff assays* S1-6: Preciset DAT Plus II, CAL 1-6 0, 50, 100, 200, 400, 1000 ng/mL *300 ng/mL cutoff assay* S1-6: Preciset DAT Plus I, CAL 1-6 0, 150, 300, 600, 1000, 3000 ng/mL
Calibration mode	Non-linear
Calibration frequency	Full calibration - after reagent lot change - every 13 weeks on-board - as required following quality control procedures

Qualitative applications

Calibrators	*100 ng/mL cutoff assay* S1: Preciset DAT Plus II, CAL 3, *(Test BZ1Q2)*, 100 ng/mL S1: C.f.a.s. DAT Qualitative Plus Clinical, *(Test BZQ1C)*, 100 ng/mL *200 ng/mL cutoff assay* S1: Preciset DAT Plus II, CAL 4, 200 ng/mL *300 ng/mL cutoff assay* S1: Preciset DAT Plus I, CAL 3, *(Test BZ3Q2)*, 300 ng/mL S1: C.f.a.s. DAT Qualitative Plus, *(Test BZ3-QP)*, 300 ng/mL
Cutoff Calibrator	A value of "0" is encoded in the e-barcode in order to ensure flagging of positive samples with >Test and negative absorbance values for negative samples.

Calibration K factor	The K factor of -1000 is predefined in the application settings.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 13 weeks on-board - as required following quality control procedures

The drug concentrations of the calibrators have been verified by GC-MS.

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against a primary reference method (GC-MS).

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Drug concentrations of Control Set DAT I, II, III, and Clinical have been verified by GC-MS.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Results

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between preliminary positive and negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Preliminary positive samples are flagged with >Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

For the semiquantitative applications, **cobas c** systems automatically calculate the drug or metabolite concentration of each sample in the unit ng/mL. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

Preliminary positive results should be confirmed by another method.

The semiquantitation of preliminary positive results should only be used by laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC-MS or LC-MS/MS. It also permits the laboratory to establish quality control procedures and assess control performance.

NOTE: If a result of Calc.? or Samp.? alarm is obtained, review the Reaction Monitor data for the sample and compare with the Reaction Monitor data for the highest calibrator. The most likely cause is a high concentration of the analyte in the sample, in which case the absorbance value for the sample will be less than that of the highest calibrator. Make an appropriate dilution of the sample using the 0 ng/mL calibrator and rerun the sample. A normal drug-free urine may be substituted for the 0 ng/mL calibrator if the urine and procedure have been validated by the laboratory. To ensure that the sample was not over-diluted, the diluted result, prior to multiplying by the dilution factor, must be at least half the analyte cutoff value. If the diluted result falls below half the analyte cutoff value, repeat the sample with a smaller dilution. A dilution that produces a result closest to the analyte cutoff is the most accurate estimation. To estimate the preliminary positive sample's concentration, multiply the result by the appropriate dilution factor. Dilutions should only be used to interpret results of Calc.? or Samp.? alarms, or when estimating concentration in preparation for GC-MS or LC-MS/MS.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

Limitations - interference

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A preliminary positive result with this assay indicates the presence of benzodiazepines and/or their metabolites in urine. It does not reflect the degree of intoxication.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Expected values

Qualitative assay

Results of this assay distinguish preliminary positive (≥ 100 ng/mL, ≥ 200 ng/mL, or ≥ 300 ng/mL depending on the cutoff) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Semiquantitative assay

Results of this assay yield only approximate cumulative concentrations of the drug and its metabolites (see "Analytical specificity" section).

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogeneous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Semiquantitative precision - 100 ng/mL

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	54.1	1.19	2.2
Urine - 25 %	78.4	2.04	2.6
DAT2N	79.1	1.67	2.1
Cutoff urine	98.9	1.30	1.3
Urine + 25 %	126	1.55	1.2
DAT2P	127	1.16	0.9
Urine + 50 %	148	1.19	0.8
Intermediate precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	54.1	1.47	2.7
Urine - 25 %	78.4	2.56	3.3
DAT2N	79.1	2.56	3.2
Cutoff urine	98.9	2.05	2.1
Urine + 25 %	126	2.35	1.9
DAT2P	127	3.10	2.4
Urine + 50 %	148	2.22	1.5

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ONLINE DAT Benzodiazepines II

Qualitative precision - 100 ng/mL

Cutoff (100)	Number tested	Correct results	Confidence level
Urine - 50 %	84	84	> 95 % negative reading
Urine - 25 %	84	84	> 95 % negative reading
DAT2N	84	84	> 95 % negative reading
Cutoff urine	84	n.a.*	n.a.*
Urine + 25 %	84	84	> 95 % positive reading
DAT2P	84	84	> 95 % positive reading
Urine + 50 %	84	84	> 95 % positive reading

*n.a. = not applicable

Semiquantitative precision - 200 ng/mL

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	99.3	1.28	1.3
Urine - 25 %	148	1.33	0.9
DAT3N	152	1.31	0.9
Cutoff urine	207	1.74	0.8
Urine + 25 %	251	7.32	2.9
DAT3P	252	1.65	0.7
Urine + 50 %	302	2.34	0.8
Intermediate precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	99.3	1.88	1.9
Urine - 25 %	148	2.16	1.5
DAT3N	152	3.73	2.4
Cutoff urine	207	2.74	1.3
Urine + 25 %	251	8.65	3.4
DAT3P	252	5.72	2.3
Urine + 50 %	302	4.55	1.5

Qualitative precision - 200 ng/mL

Cutoff (200)	Number tested	Correct results	Confidence level
Urine - 50 %	84	84	> 95 % negative reading
Urine - 25 %	84	84	> 95 % negative reading
DAT3N	84	84	> 95 % negative reading
Cutoff urine	84	n.a.*	n.a.*
Urine + 25 %	84	84	> 95 % positive reading
DAT3P	84	84	> 95 % positive reading
Urine + 50 %	84	84	> 95 % positive reading

*n.a. = not applicable

Semiquantitative precision - 300 ng/mL

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	156	2.85	1.8
Urine - 25 %	224	2.97	1.3
DAT1N	234	3.02	1.3
Cutoff urine	305	4.65	1.5
DAT1P	372	3.26	0.9
Urine + 25 %	378	4.43	1.2
Urine + 50 %	456	2.65	0.6
Intermediate precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
Urine - 50 %	156	4.05	2.6
Urine - 25 %	224	4.87	2.2
DAT1N	234	5.14	2.2
Cutoff urine	305	4.82	1.6
DAT1P	372	7.90	2.1
Urine + 25 %	378	5.51	1.5
Urine + 50 %	456	6.90	1.5

Qualitative precision - 300 ng/mL

Cutoff (300)	Number tested	Correct results	Confidence level
Urine - 50 %	84	84	> 95 % negative reading
Urine - 25 %	84	84	> 95 % negative reading
DAT1N	84	84	> 95 % negative reading
Cutoff urine	84	n.a.*	n.a.*
DAT1P	84	84	> 95 % positive reading
Urine + 25 %	84	84	> 95 % positive reading
Urine + 50 %	84	84	> 95 % positive reading

*n.a. = not applicable

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Accuracy

54 urine samples for the 100 ng/mL cutoff, 68 urine samples for the 200 ng/mL cutoff, and 57 urine samples for the 300 ng/mL cutoff, that screened negative in a drug test panel, were evaluated with the Benzodiazepines II assay on a **cobas c** 503 analyzer and a **cobas c** 501 analyzer. 100 % of these normal urines were negative relative to all cutoffs on the **cobas c** 503 analyzer.

60 urine samples for the 100 ng/mL cutoff, 66 urine samples for the 200 ng/mL cutoff, and 57 urine samples for the 300 ng/mL cutoff, that screened preliminary positive for benzodiazepines relative to the corresponding cutoff on a **cobas c** 501 analyzer and subsequently confirmed by LC-MS/MS, were evaluated with the Benzodiazepines II assay on a **cobas c** 503 analyzer. For the 100 ng/mL cutoff, 98.3 % of these samples were positive on the **cobas c** 503 analyzer. For the 200 ng/mL cutoff, 98.5 % of these samples were positive on the **cobas c** 503 analyzer. For the 300 ng/mL cutoff, 100 % of these samples were positive on both the **cobas c** 503 analyzer and the **cobas c** 501 analyzer.

Benzodiazepines II correlation (cutoff = 100 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	59	0
	-	1	54

Benzodiazepines II correlation (cutoff = 200 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	65	0
	-	1	68

Benzodiazepines II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 503 analyzer	+	57	0
	-	0	57

Additional clinical samples were evaluated with this assay on a **cobas c** 303 analyzer and on a **cobas c** 501 analyzer. 54 urine samples for the 100 ng/mL cutoff, 68 urine samples for the 200 ng/mL cutoff, and 57 urine samples for the 300 ng/mL cutoff, that screened negative in a drug test panel, were evaluated with the Benzodiazepines II assay on a **cobas c** 303 analyzer and a **cobas c** 501 analyzer. 100 % of these normal urines were negative relative to the 100 ng/mL cutoff on both the **cobas c** 303 analyzer and the **cobas c** 501 analyzer. 98.5 % of these normal urines were negative relative to the 200 ng/mL cutoff on the **cobas c** 303 analyzer. 98.2 % of these normal urines were negative relative to the 300 ng/mL cutoff on the **cobas c** 303 analyzer.

63 urine samples for the 100 ng/mL cutoff, 66 urine samples for the 200 ng/mL cutoff, and 57 urine samples for the 300 ng/mL cutoff, that screened preliminary positive for benzodiazepines relative to the corresponding cutoff on a **cobas c** 501 analyzer and subsequently confirmed by LC-MS/MS, were evaluated with the Benzodiazepines II assay on a **cobas c** 303 analyzer. For the 100 ng/mL cutoff, 93.7 % of these samples were positive on the **cobas c** 303 analyzer. For the 200 ng/mL cutoff, 95.5 % of these samples were positive on the **cobas c** 303 analyzer. For the 300 ng/mL cutoff, 100 % of these samples were positive on both the **cobas c** 303 analyzer and the **cobas c** 501 analyzer.

Benzodiazepines II correlation (cutoff = 100 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 303 analyzer	+	59	0
	-	4	54

Benzodiazepines II correlation (cutoff = 200 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 303 analyzer	+	63	1
	-	3	67

Benzodiazepines II correlation (cutoff = 300 ng/mL)			
		cobas c 501 analyzer	
		+	-
cobas c 303 analyzer	+	57	1
	-	0	56

Analytical specificity

The specificity of the Benzodiazepines II assay for various benzodiazepines and benzodiazepine metabolites was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 100 ng/mL, 200 ng/mL, and 300 ng/mL cutoff. The following typical data were obtained on a **cobas c** 501 analyzer.

Compound[a)]	**ng/mL Equivalent to 100 ng/mL cutoff**	**Approx. % Cross-reactivity**
Bromazepam	76	132
3-Hydroxybromazepam	153	66
Demoxepam	76	131
Deschloretizolam	81	124
Estazolam	88	114
Oxazepam	89	113
Oxazepam glucuronide	135	74
Diazepam	90	111
Nordiazepam	101	99
Alprazolam	92	109
4-Hydroxyalprazolam	63	160
α-Hydroxyalprazolam	85	118
α-Hydroxyalprazolam glucuronide	190	53
Clobazam	95	106
Temazepam	94	106
Temazepam glucuronide	160	63
Nitrazepam	96	105
7-Aminonitrazepam	73	137
7-Acetamidonitrazepam	21421	0.5
Diclazepam	99	101
Nimetazepam	99	101
7-Aminonimetazepam	87	115
Clonazepam	103	97
7-Aminoclonazepam	124	80
Pyrazolam	103	97
Triazolam	103	97
α-Hydroxytriazolam	96	105
4-Hydroxytriazolam	98	102
Flubromazolam	105	95
Lorazepam	105	95
Lorazepam glucuronide	178	56
Midazolam	106	95
α-Hydroxymidazolam	103	97
α-Hydroxymidazolam glucuronide	179	56
Lormetazepam	107	94
Chlordiazepoxide	109	92
Desmethylchlordiazepoxide	108	93
Delorazepam	109	92
Clonazolam	110	91
Pinazepam	110	91
Flunitrazepam	113	88
Desmethylflunitrazepam	100	100
7-Aminoflunitrazepam	109	91
3-Hydroxyflunitrazepam	118	85
Tetrazepam	116	86
Etizolam	118	85
Meclonazepam	123	82
Phenazepam	124	81
Prazepam	124	80
Bentazepam	128	78
Nifoxipam	129	78
Flubromazepam	132	76
3-Hydroxyflubromazepam	130	77
Halazepam	132	76

Brotiazolam	138	73
Medazepam	138	72
Desmethylmedazepam	168	59
Flurazepam	161	62
Desalkylflurazepam	97	103
Hydroxyethylflurazepam	103	97
Didesethylflurazepam	116	86
Clorazepate	189	53

a) Indented compounds are metabolites of the preceding drug.

Compound[b)]	ng/mL Equivalent to 200 ng/mL cutoff	Approx. % Cross-reactivity
Bromazepam	154	130
3-Hydroxybromazepam	371	54
Deschloretizolam	159	126
Estazolam	168	119
Alprazolam	174	115
4-Hydroxyalprazolam	119	169
α-Hydroxyalprazolam	168	119
α-Hydroxyalprazolam glucuronide	399	50
Diazepam	175	115
Nordiazepam	198	101
Demoxepam	182	110
Clobazam	185	108
Nitrazepam	188	106
7-Aminonitrazepam	177	113
7-Acetamidonitrazepam	42005	0.5
Oxazepam	193	104
Oxazepam glucuronide	282	71
Temazepam	193	103
Temazepam glucuronide	318	63
Diclazepam	202	99
Nimetazepam	201	99
7-Aminonimetazepam	213	94
Pyrazolam	203	99
Flubromazolam	203	98
Midazolam	206	97
α-Hydroxymidazolam	201	100
α-Hydroxymidazolam glucuronide	372	54
Clonazepam	208	96
7-Aminoclonazepam	283	71
Lorazepam	208	96
Lorazepam glucuronide	360	56
Triazolam	207	96
α-Hydroxytriazolam	198	101
4-Hydroxytriazolam	203	99
Flunitrazepam	212	94
Desmethylflunitrazepam	196	102
7-Aminoflunitrazepam	219	91
3-Hydroxyflunitrazepam	259	77
Lormetazepam	214	94
Pinazepam	213	94
Delorazepam	217	92
Clonazolam	226	89
Tetrazepam	233	86
Etizolam	236	85
Prazepam	240	83
Phenazepam	252	79
Brotiazolam	265	76
Flubromazepam	266	75
3-Hydroxyflubromazepam	256	78
Halazepam	269	74
Medazepam	279	72
Desmethylmedazepam	399	50
Chlordiazepoxide	282	71
Desmethylchlordiazepoxide	304	66
Bentazepam	288	69
Meclonazepam	306	65
Flurazepam	312	64
Desalkylflurazepam	194	103
Hydroxyethylflurazepam	206	97
Didesethylflurazepam	231	87
Nifoxipam	310	64
Clorazepate	396	50

b) Indented compounds are metabolites of the preceding drug.

Compound[c)]	ng/mL Equivalent to 300 ng/mL cutoff	Approx. % Cross-reactivity
Bromazepam	229	131
3-Hydroxybromazepam	504	59
Demoxepam	256	117
Deschloretizolam	257	117
Estazolam	270	111
Diazepam	273	110
Nordiazepam	305	98
Temazepam	272	110
Temazepam glucuronide	496	61
Alprazolam	275	109
4-Hydroxyalprazolam	192	156
α-Hydroxyalprazolam	275	109
α-Hydroxyalprazolam glucuronide	567	53
Oxazepam	275	109
Oxazepam glucuronide	442	68
Clobazam	277	108
Nitrazepam	294	102
7-Aminonitrazepam	230	131
7-Acetamidonitrazepam	93148	0.3
Nimetazepam	309	97

7-Aminonimetazepam	283	106
Pyrazolam	311	96
Clonazepam	317	95
7-Aminoclonazepam	390	77
Diclazepam	317	95
Triazolam	315	95
α-Hydroxytriazolam	312	96
4-Hydroxytriazolam	319	94
Lorazepam	323	93
Lorazepam glucuronide	519	58
Flubromazolam	327	92
Lormetazepam	328	92
Pinazepam	327	92
Flunitrazepam	329	91
Desmethylflunitrazepam	301	100
7-Aminoflunitrazepam	343	87
3-Hydroxyflunitrazepam	399	75
Delorazepam	334	90
Midazolam	332	90
α-Hydroxymidazolam	326	92
α-Hydroxymidazolam glucuronide	569	53
Clonazolam	338	89
Etizolam	362	83
Prazepam	363	83
Tetrazepam	360	83
Chlordiazepoxide	374	80
Desmethylchlordiazepoxide	370	81
Phenazepam	382	78
Bentazepam	393	76
Medazepam	394	76
Desmethylmedazepam	549	55
Brotiazolam	407	74
Halazepam	406	74
Meclonazepam	409	73
Nifoxipam	412	73
Flubromazepam	435	69
3-Hydroxyflubromazepam	401	75
Flurazepam	487	62
Desalkylflurazepam	307	98
Hydroxyethylflurazepam	324	92
Didesethylflurazepam	352	85
Clorazepate	601	50

c) Indented compounds are metabolites of the preceding drug.

Many benzodiazepines appear in the urine largely as the glucuronidated conjugate. Glucuronidated metabolites may have more or less cross-reactivity than the parent compound. The presence of β-glucuronidase enzyme enhances the Benzodiazepines II assay cross-reactivity to some of the glucuronidated metabolites.

Drug interference

The following compounds were added at the concentrations listed below to pooled human urine containing benzodiazepine at the level of the negative control (75 ng/mL, 150 ng/mL or 225 ng/mL) and at the level of the positive control (125 ng/mL, 250 ng/mL or 375 ng/mL) corresponding to the given cutoff (100 ng/mL, 200 ng/mL or 300 ng/mL respectively). For each compound, the control level samples recovered properly with the Benzodiazepines II assay. The tested compounds do not cause any cross-over at the given concentration. The following results were obtained with the Benzodiazepines II assay on the **cobas c** 501 analyzer.

	Concentration (ng/mL)		
Compound	**Cutoff 100 ng/mL**	**Cutoff 200 ng/mL**	**Cutoff 300 ng/mL**
Acetaminophen	3000000	3000000	3000000
Acetylsalicylic acid	100000	100000	100000
Amitriptyline	100000	100000	100000
Amobarbital	100000	100000	100000
d-Amphetamine	100000	100000	100000
l-Amphetamine	100000	100000	100000
Ampicillin	100000	100000	100000
Aspartame	100000	100000	100000
Atropine	100000	100000	100000
Benzocaine	100000	100000	100000
Benzoylecgonine	100000	100000	100000
Benzphetamine	100000	100000	100000
Buspirone	100000	100000	100000
Butabarbital	100000	100000	100000
Ca-dobesilate	1000000	1000000	1000000
Caffeine	100000	100000	100000
Calcium hypochlorite	100000	100000	100000
Cannabidiol	100000	100000	100000
Captopril	100000	100000	100000
Cefoxitin	2000000	4000000	6000000
Chloroquine	100000	100000	100000
Chlorpheniramine	40000	100000	100000
Chlorpromazine	100000	100000	100000
Cocaine	100000	100000	100000
Codeine	100000	100000	100000
Desipramine	100000	100000	100000
Dextromethorphan	100000	100000	100000
Dextropropoxyphene (*d*-Propoxyphene)	100000	100000	100000
Digoxin	100000	100000	100000
Diphenhydramine	40000	100000	100000
Doxepin	100000	100000	100000
Ecgonine	100000	100000	100000
Ecgonine methyl ester	100000	100000	100000
EDDP (2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	25000	50000	75000
EMDP (2-Ethyl-5-methyl-3,3-diphenylpyrroline)	25000	40000	80000
Enalapril	100000	100000	100000
d-Ephedrine	100000	100000	100000
l-Ephedrine	100000	100000	100000
Epinephrine	100000	100000	100000

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	Concentration (ng/mL)		
Compound	**Cutoff 100 ng/mL**	**Cutoff 200 ng/mL**	**Cutoff 300 ng/mL**
Erythromycin	100000	100000	100000
Estriol	100000	100000	100000
Fenoprofen	40000	100000	100000
Flumazenil	100000	100000	100000
Furosemide	100000	100000	100000
Gentamicin sulfate	400000	400000	400000
Gentisic acid	100000	100000	100000
Glutethimide	100000	100000	100000
Guaiacol glycerol ether	100000	100000	100000
Hydrochlorothiazide	100000	100000	100000
Hydroxyindole acetic acid	100000	100000	100000
Hydroxyindole carboxylic acid	100000	100000	100000
Ibuprofen	4000000	4000000	4000000
Imipramine	100000	100000	100000
Isoproterenol	100000	100000	100000
Ketamine	100000	100000	100000
Levodopa	1000000	1000000	1000000
Lidocaine	100000	100000	100000
LSD	100000	100000	100000
Melanin	100000	100000	100000
Meperidine (Pethidin)	100000	100000	100000
Methadone	40000	80000	100000
d-Methamphetamine	100000	100000	100000
l-Methamphetamine	100000	100000	100000
Methaqualone	100000	100000	100000
Methyldopa	2000000	2000000	2000000
Methylenedioxyamphetamine (MDA)	100000	100000	100000
Methylenedioxymethamphetamine (MDMA)	100000	100000	100000
Methylphenidate	100000	100000	100000
Morphine	100000	100000	100000
N-acetyl cysteine	10000	10000	10000
Naloxone	100000	100000	100000
Naltrexone	100000	100000	100000
Naproxen	100000	100000	100000
Niacinamide	100000	100000	100000
Nicotine	100000	100000	100000
Norethindrone	100000	100000	100000
l-Norpseudoephedrine	100000	100000	100000
Ofloxacin	900000	900000	900000
Omeprazole	100000	100000	100000
Oxaprozin[d)]	100	200	300
Penicillin G	100000	100000	100000
Pentazocine	100000	100000	100000
Pentobarbital	100000	100000	100000
Phenazopyridine	300000	300000	300000

	Concentration (ng/mL)		
Compound	**Cutoff 100 ng/mL**	**Cutoff 200 ng/mL**	**Cutoff 300 ng/mL**
Phencyclidine	100000	100000	100000
Phenobarbital	100000	100000	100000
Phenothiazine	100000	100000	100000
Phenylbutazone	100000	100000	100000
Phenylpropanolamine	100000	100000	100000
Phenytoin	100000	100000	100000
Procaine	100000	100000	100000
Promethazine	100000	100000	100000
d-Pseudoephedrine	100000	100000	100000
Quetiapine	5000	10000	20000
Quinidine	100000	100000	100000
Quinine	100000	100000	100000
Salicyluric acid	6000000	6000000	6000000
Secobarbital	100000	100000	100000
Sulindac	100000	100000	100000
Tetracycline	300000	300000	300000
Δ^9 THC-9-carboxylic acid	100000	100000	100000
Tetrahydrozoline	100000	100000	100000
Thioridazine	100000	100000	100000
Tolmetin	100000	100000	100000
Trifluoperazine	100000	100000	100000
Trimipramine	100000	100000	100000
Tyramine	100000	100000	100000
Verapamil	100000	100000	100000
Zaleplone	100000	100000	100000
Zolpidem	50000	100000	100000
Zopiclone	100000	100000	100000

d) In a drug-free matrix, the approximate quantity of oxaprozin that is equivalent in assay reactivity to a 100 ng/mL, 200 ng/mL, and 300 ng/mL cutoff was determined to be 790 ng/mL, 3091 ng/mL, and 3049 ng/mL respectively. This equals a cross reactivity of 13 % (at cutoff 100 ng/mL), 6 % (at cutoff 200 ng/mL), and 10 % (at cutoff 300 ng/mL).

When oxaprozin was added to pooled human urine containing benzodiazepine at the level of the negative control (75 ng/mL, 150 ng/mL or 225 ng/mL), positive results were observed at > 100 ng/mL, > 200 ng/mL, and > 300 ng/mL for the 100 ng/mL cutoff, 200 ng/mL cutoff, and 300 ng/mL cutoff, respectively. Patient samples containing benzodiazepines in the presence of oxaprozin may yield falsely elevated results. Results should always be assessed in conjunction with the patient's medical history, clinical examinations, and other clinicopathological findings.

Interfering substances were added to pooled human urine containing benzodiazepine at the level of the negative control (75 ng/mL, 150 ng/mL or 225 ng/mL) and at the level of the positive control (125 ng/mL, 250 ng/mL or 375 ng/mL) corresponding to the given cutoff (100 ng/mL, 200 ng/mL or 300 ng/mL, respectively) at the concentrations listed below. All samples recovered properly in the presence of the interfering substance in both semiquantitative and qualitative modes. The interfering substances do not cause any cross-over at the given concentration. The following results were obtained with the Benzodiazepines II assay on the **cobas c** 501 analyzer.

Compound	**Compound concentration**
Acetone	1000 mg/dL
Ascorbic acid	1500 mg/dL

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Compound	Compound concentration
Calcium (as calcium chloride)	133 mg/dL
Citrate (as potassium citrate monohydrate)	357 mg/dL
Creatinine	1000 mg/dL
Ethanol	1000 mg/dL
Glucose	7000 mg/dL
Hemoglobin	750 mg/dL
Human Albumin	250 mg/dL
Human Immunoglobulin (IgG)	110 mg/dL
Magnesium (as magnesium chloride)	238 mg/dL
Oxalate (as sodium oxalate)	20 mg/dL
Phosphate (as sodium dihydrogen phosphate dihydrate)	2028 mg/dL
Sodium chloride	5844 mg/dL
Urea	18000 mg/dL
Uric acid	100 mg/dL
Urobilinogen	15 mg/dL

Urine samples within a pH range from 4.0 to 9.0 and samples with specific gravities ranging from 1.001 to 1.034 containing benzodiazepine at the level of the negative control (75 ng/mL, 150 ng/mL or 225 ng/mL) and at the level of the positive control (125 ng/mL, 250 ng/mL or 375 ng/mL) corresponding respectively to the given cutoff (100 ng/mL, 200 ng/mL or 300 ng/mL) recovered properly in both semiquantitative and qualitative modes.

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