High Performance Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of 2CC-NBOMe and 25I-NBOMe in Human Serum:

HPLC/MS/MS Method for 2CC-NBOMe and 25I-NBOMe in Serum

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Abstract

2CC-NBOMe (4-chloro-2,5-dimethoxyphenethyl-N-[(2-methoxyphenyl) methyl] ethanamine) and 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl] ethanamine) are of a class of N-benzyl phenethylamine derivatives whose synthesis was first reported in the scientific literature in 2011. Recent reports from "personal drug experience websites" and in the popular press indicate these drugs are the latest in a series of designer "Bath Salt" drugs of abuse. The presented high performance liquid chromatography triple quadrupole mass spectrometry (HPLC/MS/MS) method was developed for the detection and quantification of 2CC-NBOMe and 25I-NBOMe in serum of intoxicated emergency department patients. The assay applies 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl) ethanamine (25H-NBOMe) as the internal standard (ISTD). Samples were extracted using solid phase extraction (SPE) columns. The chromatographic separation was performed on a Luna 3µ C8 (2)100Å 100×2.0 mm, column. Detection was accomplished by multiple-reaction monitoring (MRM) via electrospray ionization (ESI) source operating in the positive ionization mode. The calibration curves were linear over the investigated concentration range, 30 to 2000 pg/mL, with a lower limit of detection (LOD) of 10 pg/mL for both 2CC-NBOMe and 25I-NBOMe. The method proved suitable for serum clinical toxicology testing. Two severely intoxicated emergency department patients were determined to have serum concentrations of 250 pg/mL and 2780 pg/mL of 25I-NBOMe using the presented method.

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Introduction

2CC-NBOMe (4-chloro-2,5-dimethoxyphenethyl-N-[(2-methoxyphenyl) methyl] ethanamine) and 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl] ethanamine) (figure 1) are of a class of N-benzyl phenethylamine derivatives whose synthesis was first reported in the scientific literature in 2011 (Ettrup et al., 2011). *In vitro* binding studies have demonstrated that these compounds are potent serotonin 2A (5-HT2A) receptor agonists (Nichols et al., 2008; Zuba et al., 2012; 2013). The 5-HT2A receptor has been closely linked to complex behaviors including working memory, cognitive processes and affective disorders such as schizophrenia. These receptors are believed to mediate the primary effects of hallucinogenic drugs (Braden et al., 2006). Little to no pharmacokinetic or pharmacological data concerning man or whole animals is presently available in the professional literature. Recent reports from "personal drug experience websites" and in the popular press indicate these drugs are the latest in a series of designer "Bath Salt" drugs of abuse. These drugs may serve as replacements for the previously popular beta-keto derivatives of amphetamine: methcathinone and methedrone, as well as the methylenedioxy ring derivatives similar to methylenedioxymethamphetamine (MDMA, "Ecstasy"), methylone and methylenedioxypyrovalerone (MDPV), which are now controlled substances in Europe and the United States (Gibbons, 2012). As with earlier "Bath Salts" both 2CC-NBOMe and 25I-NBOMe can be obtained easily over the internet. Blotter papers containing 25I-NBOMe appeared on the market in 2011 (Zuba et al., 2013). These compounds are sometimes sold under the name 25I, N-bomb, or Smiles.

Only a single case of a 25I-NBOMe overdose has been published to date. The patient displayed initial signs and symptoms consistent with sympathomimetic toxidrome (tachycardia, hypertension, mydriasis, agitation, and hypokalemia) plus hallucinations and bizarre behavior likely associated with serotonergic toxicity (Rose et al., 2013). Two abstracts of presentations have been published describing case series of 25I-NBOMe exposures of 4 and 10 patients, respectively (Kelly et al., 2012; Rose et al., 2012). The most common effects in these patients were tachycardia (13 of 14), agitation (10 of 14), and hypertension (8 of 14). Five of 14 patients experienced tonic/clonic seizures and one suffered a cerebral hemorrhage. However, toxicological analysis for 25I-NBOMe in patient specimens was performed in only one of the 14 reported cases (Rose et al., 2013).

We present a high performance liquid chromatography triple quadrupole mass spectrometry (HPLC/MS/MS) method for the identification and quantification of 2CC-NBOMe and 25I-NBOMe in human serum. The assay was developed in response to an outbreak of N-benzyl-phenethylamine derivative abuse and non-fatal overdose cases in our state during early 2012. The method is novel in that presently there are no published methods for the analysis of 2CC-NBOMe in biological specimens and our initial method involving liquid/liquid extraction of 25I-NBOMe from serum is only the procedure in the literature to date (Rose et al., 2013).

Materials and Methods

Reagents

The phenethylamine derivative primary reference materials for 25H-NBOMe, 2CC-NBOMe and 25I-NBOMe were purchased from Cayman Chemical Company (Ann Arbor, Michigan) as hydrochloride salts. Acetic acid, acetonitrile, ammonium acetate, dichloromethane, ethanol, formic acid, isopropanol, methanol and water were purchased from Fisher Scientific (Hanover Park, Illinois). Ammonia was purchased from Macron Chemicals (Charlotte, North Carolina). Hydrochloric acid and sodium phosphate dibasic were purchased from J.T. Baker (Phillipsburg, New Jersey). Sodium phosphate monohydrate was purchased from Sigma Aldrich (St. Louis, Missouri). All reagents were ACS grade or better. Medical grade nitrogen was purchased from National Welders Supply Company (Richmond, Virginia) and the Clean Screen ZSDUA020 sold phase extraction (SPE) columns were purchased from UCT (Horsham, PA). Liquichek™ controls were purchased from Bio-Rad Laboratories, Inc. (Hercules, California).

Stock solution preparation and dilutions

A series of working standard solutions of 1 and 10 ng/mL were prepared by appropriate dilution with ethanol of the stock standard solution of 2CC-NBOMe and 25I-NBOMe. An internal standard (ISTD) working solution of 10 ng/mL 25H-NBOMe was prepared by appropriately diluting the internal standard stock solution with ethanol. Working standards were stored at −20°C.

Preparation of calibrators and quality control specimens

In-house drug-free serum provided the matrix for all prepared calibrators, quality control (QC) and other study specimens. Drug-free out of date serum was obtained in-house from the Department of Transfusion Medicine. The drug-free serum specimens were analyzed by gas chromatography/mass spectrometry and found not to contain common drugs of abuse, phenethylamine derivatives of interest in this study or their metabolites. Appropriate volumes of the working solutions of 2CC-NBOMe and 25I-NBOMe were added to serum to obtain an eight-point calibration curve of 0, 30, 50, 100, 250, 500, 1000 and 2000 pg/mL of each analyte. Calibrators were prepared fresh in duplicate before analysis of each batch of samples. The following QC serums specimens for 25CC-NBOMe and 25I-NBOMe were prepared and analyzed with each batch of test specimens: limit of quantification quality control (LOQC), target concentration of 30 pg/mL; low control (LQC), target concentration of 75 pg/mL; medium control (MQC), target concentration of 750 pg/mL; and high control (HQC), target concentration of 1500 pg/mL. A drug free control (negative control) that contained neither 2CC-NBOMe nor 25I-NBOMe with ISTD added and a double negative control containing neither 2CC-NBOMe and 25I-NBOMe nor ISTD were also analyzed with each test batch. All QC samples were stored at -20°C until testing.

Specimen extraction

To 1 mL aliquots of calibrators, QC specimens and patient serums was added 50 μL of ISTD consisting of 10 ng/mL (500 pg total) of 25H-NBOMe followed by the addition of 1 mL of

100 mM phosphate buffer (pH 6). The samples were then mixed for 5 minutes and centrifuged for 10 minutes at 3000 rpm. The Clean Screen ZSDUA020 sold phase SPE columns were conditioned with 3 mL of methanol followed by 3 mL of Di water and lastly 1 mL of 100 mM phosphate buffer (pH 6). The samples were added to the columns and aspirated under gravity. The columns were then washed with 3 mL of DI water followed by 1 mL 100mM acetic acid and finally 3 mL of methanol. Columns were dried under vacuum. 2CC-NBOMe, 25I-NBOMe and ISTD were then eluted with 3 mL of 78:20:2 dichloromethane/isopropanol/ammonia (v:v:v). One hundred microliters of 1% HCl in methanol (v:v) and 200 μL of DI water were added to the eluate. The samples were evaporated under nitrogen leaving approximately 200 μL of DI water. This solution was transferred to auto-sampler vials for analysis.

Instrumental Analysis

The HPLC/MS/MS analysis was performed with an Applied Biosystems 3200 Q trap with a turbo V source for TurbolonSpray attached to a Shimadzu SCL HPLC system controlled by Analyst 1.4.2 software. The chromatographic separation was performed on a Luna 3μ C8(2)100Å 100x2.0 mm, column (Phenomenex, Torrance, California). The mobile phase consisted of A: Water with 10 mM ammonium acetate and 0.1% formic acid and B: Acetonitrile. The following gradient was applied: 0.00-1.10 min, 20% B, a linear gradient to 33% B at 8.00 min, hold for 6.90 min, then return to 20% B at 8.00 min. The source temperature was set at 650°C and had a curtain gas flow rate of 30 mL/min. The ionspray voltage was 5000 V, with the ion source gases 1 and 2 at flow rates of 25 mL/min. The acquisition mode used was multiple reaction monitoring (MRM). The retention times were: 2CC-NBOMe, 4.4 min; 25I-NBOMe, 4.8 min; and 25H-NBOMe, 3.8 min. 2CC-NBOMe and 25I-NBOMe had a declustering potential of 38 eV and 25H-NBOMe had a declustering potential of 19 eV. The following transition ions (m/z) were monitored in MRM mode with their corresponding collection energies (eV) in parentheses: 2CC-NBOMe: 336>121 (26) and 336>91 (60); 25I-NBOMe: 428>121 (26) and 428>91 (50); and 25H-NBOMe: 302>121 (28) and 302>91 (70). The chromatographic separation is presented in figure 2. The total run time for the analytical method was 10 minutes.

Assay Performance

The evaluation of the assay was conducted over five separate days. The samples batches were analyzed as recommended for biomedical assay validation (Bioanalytical Method Validation., 2001) for linearity, LOQ, accuracy/bias, precision, selectivity, freeze and thaw stability, bench-top stability, long-term stability and post-preparative stability. Validation sample batches contained calibrators in duplicate, drug-free samples with internal standard added, drug-free samples without internal standard and replicates of the prepared LOQC, LQC, MQC and HQC samples.

Linearity, LOQ and LOD

An eight-point calibration of 0, 30, 50, 100, 250, 500, 1000 and 2000 pg/mL in duplicate of 2CC-NBOMe and 25I-NBOMe was prepared in drug-free serum. The calibration curve was constructed by a linear regression plot of the ratio of the peak area of the abundance quantification ion of 2CC-NBOMe and 25I-NBOMe to the peak area abundance

quantification ion of 25H-NBOMe ISTD, versus the calibrator concentrations. The lower limit of quantification (LOQ) of 30 pg/mL and the lower limit of detection (LOD) of 10 pg/mL for 2CC-NBOMe and 25I-NBOMe were administratively set. LOQC samples were used to verify the LOQ was within +20% of the 30 pg/mL target value and had a response at least five times greater than the signal to noise ratio of the response to drug-free serum. Samples prepared at the LOD of 10 pg/mL were analyzed with each batch to verify that there was a response at least three times greater than the signal to noise ratio of the response to drug-free serum.

Absolute recovery and ion suppression

The percent recovery was determined by first extracting and preparing residues of drug free serum. These residues were then reconstituted with water to prepare test samples containing the target concentrations of the 750 pg/mL of 2CC-NBOMe and 25I-NBOMe and 500pg/mL of the ISTD. The addition of the drugs to the residue of extracted serum mitigated any matrix effects on recovery studies. The absolute recovery of the assay was determined by comparing the absolute area of the extracted aliquots of 750 pg/mL 2CC-NBOMe and 25I-NBOMe and 500 pg/mL ISTD compared to the absolute peak area of reconstituted matrix with unextracted 750 pg/mL 2CC-NBOMe and 25I-NBOMe and 500 pg/mL ISTD, multiplied by 100. The ion suppression of the assay was determined by comparing the absolute area of the reconstituted matrix with unextracted 750 pg/mL 2CC-NBOMe and 25I-NBOMe and 500 pg/mL ISTD added compared to the absolute peak area of unextracted 750 pg/mL 2CC-NBOMe and 25I-NBOMe and 500 pg/mL ISTD samples, multiplied by 100 and then minus 100.

Accuracy/Bias and Precision

Accuracy/bias and precision of the method were determined from analysis of five different batches of the prepared QC samples. The percent accuracy/bias of the method was calculated as the ratio of the mean 2CC-NBOMe and 25I-NBOMe concentration of five aliquots of each QC sample analyzed in the same batch of samples, to the target concentration of the QC samples times 100. The criteria for acceptable assay mean accuracy/bias were quantified 2CC-NBOMe and 25I-NBOMe results within +15% of the target value of the prepared QC samples. The intra-day precision of the method was determined by quantified results of replicate analysis of three aliquots of the four different prepared QC samples. The inter-day precision was determined from quantified results of the three intra-day aliquots and triplicate analysis of the four prepared controls on five different days.

Stability

Stability experiments in serum were performed at three QC concentrations, the LQC, MQC and HQC with three replicas at each concentration. The stability in serum was assessed during storage and after three freeze-thaw cycles at -20°C with 24 hr passing in-between two cycles. The bench-top stability at room temperature was assessed in serum for 48 hr to cover the processing time of the samples including transportation. Long term storage was assessed by keeping the QC frozen at -20°C for one month before analysis. These QC samples were then run against freshly prepared calibrators. The post-preparative stability or the auto-

sampler stability was assessed by re-injection reproducibility after storage of the samples in the auto-sampler for 72 h at room temperature. Under these conditions 2CC-NBOMe and 25I-NBOMe were considered to be stable in serum as their concentrations were within 20% of the target values for the QC samples tested.

Results

Each calibrator concentration for each of five calibration curves, of the duplicate curves, was determined to be within + 15% of the expected value except the LOQ which was determined to be within 20%. The linear regression correlation coefficients (r^2) for the calibration curves of 2CC-NBOMe and 25I-NBOMe in the five batches yielded a mean r^2 of 0.996 + 0.003 and 0.996 + 0.002, respectively with a range of 0.992 – 0.999. LOQ and LOD for 2CC-NBOMe and 25I-NBOMe were administratively set at 30 pg/mL and 10 pg/mL, respectively. The LOD had a response greater than 10 times the signal to noise ratio of the response to drug-free serum. 10 pg/mL samples were analyzed in triplicate and were found to a have at least five times the signal to noise ratio of the drug-free serum.

The absolute recovery of the assay for 2CC-NBOMe and 25I-NBOMe at the 750 pg/mL concentration (n=3) was 103% and 97%, respectively. The absolute recovery for the 25H-NBOMe, ISTD, at 500 pg/mL (n=3) was 86%. The ion suppression for 2CC-NBOMe and 25I-NBOMe at the 750 pg/mL (n=3) was 8% and 7%, respectively. The ion suppression for 25H-NBOMe, ISTD, at 500 pg/mL (n=3) was 3% (table 1).

The accuracy/bias of the assay for 2CC-NBOMe and 25I-NBOMe over the linear range of the assay varied from a low of 92% at a concentration of 1500 pg/mL exhibited by 2CC-NBOMe to 110% at a concentration of 30 pg/mL exhibited by 25I-NBOMe (table 2). The inter-day precision at the four different QC concentrations ranged between 97% to 103% for 2CC-NBOMe and 99 to 110% 25I-NBOMe of their target values. The intraday precision of the 2CC-NBOMe QC specimens ranged between 90% and 104%, while the intra-day precision for 25I-NBOMe QC specimens ranged between 93% and 100% of their target values (table 3).

The selectivity of the assay was determined using six different lots of drug-free serum. Each individual lot was analyzed with and without internal standard. No peaks were detected that co-eluted with the targeted 2CC-NBOMe and 25I-NBOMe or with the internal standard. This ensured that endogenous serum components did not interfere with the assay. No interferences were observed from compounds in the following commercially available controls; Liquichek™ Immunoassay Plus Control, level 3; Liquichek™ Therapeutic Drug Monitoring, level 3; and Liquichek™ Urine Toxicology Control, Level C3 (table 4).

Sample carryover was evaluated in each of the five validation batches using two different procedures. Immediately following the injection of the 2000 pg/mL 2CC-NBOMe and 25I-NBOMe calibrator, an extract of a drug-free serum was injected. The rejection criterion for carryover was set at the detection of 2CC-NBOMe and 25I-NBOMe at a concentration less than 20% of the 10 pg/mL LOD. Neither 2CC-NBOMe nor 25I-NBOMe carried over to into the injected aliquot drug-free serum. As an additional measure to evaluate possible

carryover, an injection of the extracted HQC (1500 pg/mL) sample was immediately followed by injection of the LQC (75 pg/mL) sample. This procedure was routinely applied each time HQC and LQC samples were analyzed. The rejection criterion for carryover was set at a concentration with a bias of less than 20% of the target value of the LQC. Lack of carryover was confirmed as none of the 2CC-NBOMe and 25I-NBOMe LQC samples demonstrated a significant quantified bias.

Under the stability tested conditions for the three freeze thaw cycles, room temperature for 48 hours and frozen for one month stability 2CC-NBOMe and 25I-NBOMe were determined to be stable the for all LQC, MQC and HQC samples. Stability of the extracted 2CC-NBOMe, 25I-NBOMe and the ISTD, 25H-NBOMe were also considered stable as the concentrations of the re-injected QC samples were within + 20% of their target concentrations (table 5).

Patient Serums

Emergency room serum samples collected from two intoxicated patients displaying symptoms as previously described by Rose et al. (2013) after the development of the presented method were determined to contain 250 pg/mL and 2,780 pg/mL 25I-NBOMe. During hospitalization, both patients admitted using 25I-NBOMe.

Discussion

The presented HPLC/MS/MS method demonstrated acceptable reliability and reproducibility for the detection and quantification of 2CC-NBOMe and 25I-NBOMe in serum specimens. During the development of this assay, we observed a consistent loss of both 2CC-NBOMe and 25I-NBOMe was observed if we the SPE elution solvent was evaporated to dryness. The addition of methanolic HCl and water to the elution solvent combined with limited evaporation of the solvent resulted in acceptable recoveries. Accuracy as well as intra-day and inter-day precisions were determined not to exceed CVs of <15% over the dynamic range of the assay. The variance in these parameters may be reduced with the use of deuterated internal standards. Tables 1, 2 and 3 present data from 202 different tests. The quantified results of these tests were within the acceptable performance of + 20% of their targeted values with application of only 25H-NBOMe as a single non-deuterated internal standard. This demonstrates the robustness of the assay. When the assay was developed, no deuterated internal standards were commercially available for 2CC-NBOMe or 25I-NBOMe. 25H-NBOMe was used as the ISTD because of it's structurally similarity to 2CC-NBOMe and 25I-NBOMe, and it had not been reported as an abused designer drug. The assay was free of significant interference from matrix effects and free from significant analyte carryover. The 30 pg/mL LOQ for 2CC-NBOMe and 25I-NBOMe is well below concentrations detected in serum from intoxicated patients to date.

Conclusion

A method for the determination of 2CC-NBOMe and 25I-NBOMe in serum was developed. The assay used a simple SPE extraction procedure prior to chromatographic analysis. The assay is particularly suited for analysis of serum samples form emergency room patients.

Further, the assay may be easily adapted for the analysis of 2CC-NBOMe and 25I-NBOMe in research specimens.

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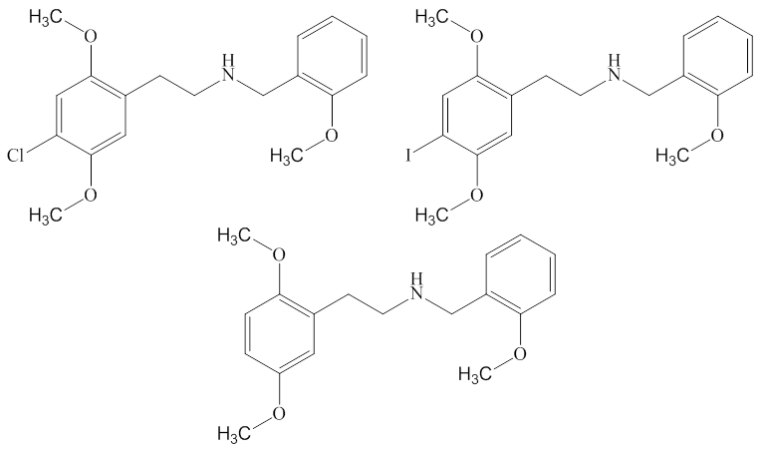

Fig 1.
Chemical Structure of 2CC-NBOMe, 25I-NBOMe and 25H-NBOMe

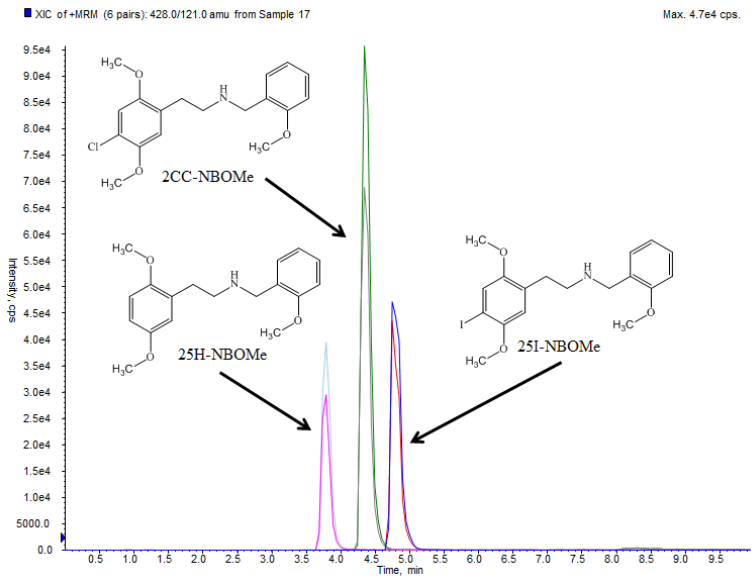

Fig 2.
Chromatographic separation of 2CC-NBOMe, 25I-NBOMe and 25H-NBOMe

Table 1

Recovery and Ion Suppression

Recovery & Suppression (n=3)		
Designer Drug	**Conc. (750 pg/mL)**	**% Mean +/− % SD**
2CC-NBOMe	Recovery (%)	100 +/− 3
	Suppression (%)	8+/−14
25I-NBOMe	Recovery (%)	97+/−10
	Suppression (%)	7+/−14
ISTD	Conc. (500 pg/mL)	% Mean +/− % SD
25H-NBOMe	Recovery (%)	86+/−14
	Suppression (%)	3+/−14

Table 2

Accuracy/Bias

Accuracy/Bias (n=5)		Mean Conc. +/− SD	Average
Designer Drug	**Control**	**(pg/mL)**	**(%)**
2CC-NBOMe	LOQ (30 pg/mL)	30 +/− 3	100
	LQC (75 pg/mL)	72 +/−5	96
	MQC (750 pg/mL)	718 +/− 25	96
	HQC (1500 pg/mL)	1375 +/− 107	92
25I-NBOMe	LOQ (30 pg/mL)	34 +/− 2	111
	LQC (75 pg/mL)	74 +/− 6	98
	MQC (750 pg/mL)	755 +/− 95	101
	HQC (1500 pg/mL)	1481 +/− 200	99

Table 3

Precision

Precision		**Intra Day (n=3)**		
Designer Drug	**Control**	**Mean Conc. (pg/mL)**	**CV**	**(%) Accuracy/Bias**
2CC-NBOMe	LOQ (30 pg/mL)	27	9	90
	LQC (75 pg/mL)	72	11	96
	MQC (750 pg/mL)	733	1	98
	HQC (1500 pg/mL)	1563	9	104
25I-NBOMe	LOQ (30 pg/mL)	30	15	100
	LQC (75 pg/mL)	71	6	95
	MQC (750 pg/mL)	694	7	93
	HQC (1500 pg/mL)	1473	12	98
		Inter Day, 5 days (n=15)		
Designer Drug	Control	Mean Conc. (pg/mL)	CV	(%) Accuracy/Bias
2CC-NBOMe	LOQ (30 pg/mL)	31	12	103
	LQC (75 pg/mL)	75	11	100
	MQC (750 pg/mL)	712	10	95
	HQC (1500 pg/mL)	1460	12	97
25I-NBOMe	LOQ (30 pg/mL)	33	9	110
	LQC (75 pg/mL)	76	12	101
	MQC (750 pg/mL)	742	10	99
	HQC (1500 pg/mL)	1536	11	102

Table 4

Compounds found in the commercially available Liquichek™ controls;

Liquichek™ Immunoassay Plus Control			
Acetaminophen	Digoxin	Insulin	SHBG
AFP	Disopyramide	Iron	Somatomedin-C
Aldosterone	Estradiol	Iron (TIBC)	T3 (Free)
17-Alpha-Hydroxyprogesterone	Estriol (Free)	LH	T3 (Total)
Amikacin	Estriol (Total)	Lidocaine	T3 Uptake/T-Uptake
Amiodarone	Estrogens (Total)	Lithium	T4 (Free)
Amitriptyline	Ethosuximide	NAPA	T4 (Total)
Androstenedione	Ferritin	Netilmicin	TBG
Angiotensin I	Flecainide	Nortriptyline	Testosterone
Anti-TG Ab	Folate	PAP	Testosterone (Free)
Anti-TPO Ab	Fructosamine	Phenobarbital	Theophylline
Caffeine	FSH	Phenytoin	Thyroglobulin
Carbamazepine	Gentamicin	Phenytoin (Free)	Tobramycin
Carbamazepine (Free)	hCG	Primidone	Tricyclic Antidepressants
CEA	hCG-Beta Subunit	Procainamide	(TCA) Screen
Chloramphenicol	hGH	Progesterone	TSH
CK-MB Isoenzyme	25-Hydroxy Vitamin D	Prolactin	Valproic Acid
Cortisol	Ibuprofen	Propranolol	Valproic Acid (Free)
Cyclosporine	Imipramine	PSA	Vancomycin
11-Deoxycortisol*	IgA	PSA (Free)	Vitamin B12
Desipramine	IgE	PTH-MM	
DHEA	IgG	Quinidine	
DHEA Sulfate	IgM	Salicylate	
Liquichek™ Therapeutic Drug Monitoring			
Acetaminophen	Digoxin	Nortriptyline	T4 (Free)
Amikacin	Disopyramide	Phenobarbital	T4 (Total)
Amitriptyline	Estriol (Total)	Phenytoin	Theophylline
Caffeine	Ethosuximide	Phenytoin (Free)	Tobramycin
Carbamazepine	Flecainide	Primidone	Tricyclic Antidepressant (TCA)
Carbamazepine (Free)	Gentamicin	Procainamide	Screen
Chloramphenicol	Haloperidol	Propranolol	TSH
Clonazepam	Imipramine	Quinidine	Valproic Acid
Cortisol	Lidocaine	Salicylate	Valproic Acid (Free)
Cyclosporine	Lithium	T3 (Free)	Vancomycin
Desipramine	Methotrexate	T3 (Total)	
Diazepam	NAPA	T3 Uptake/T-Uptake	
Liquichek™ Urine Toxicology Control			
d-Amphetamine	Pentobarbital	Benzoylecgonine	Codeine

d-Methamphetamine	Phenobarbital	Ethanol	Morphine-3-β-d-Glucuronide
MDMA	Secobarbital	Lysergic Acid Diethylamide (LSD)	Phencyclidine (PCP)
MDA	α-Hydroxyalprazolam	Methadone	Propoxyphene
MDEA	Nordiazepam	Methadone Metabolite	Norpropoxyphene
Amobarbital	Oxazepam	Methaqualone	
Butalbital	THC-COOH	6-Monoacetylmorphine	

Table 5

Stability

Freeze/Thaw				
Designer Drug	**Control**	**Mean Conc. (pg/mL)**	**CV**	**(%) Accuracy/Bias**
2CC-NBOMe	LQC (75 pg/mL)	84	5	112
	MQC (750 pg/mL)	803	3	107
	HQC (1500 pg/mL)	1684	3	112
25I-NBOMe	LQC (75 pg/mL)	77	13	103
	MQC (750 pg/mL)	842	1	112
	HQC (1500 pg/mL)	1690	2	113
BenchTop				
Designer Drug	Control	Mean Conc. (pg/mL)	CV	(%) Accuracy/Bias
2CC-NBOMe	LQC (75 pg/mL)	64	1	86
	MQC (750 pg/mL)	859	5	114
	HQC (1500 pg/mL)	1673	4	112
25I-NBOMe	LQC (75 pg/mL)	66	4	88
	MQC (750 pg/mL)	809	3	108
	HQC (1500 pg/mL)	1706	1	114
1 Month				
Designer Drug	Control	Mean Conc. (pg/mL)	CV	(%) Accuracy/Bias
2CC-NBOMe	LQC (75 pg/mL)	84	5	112
	MQC (750 pg/mL)	803	3	107
	HQC (1500 pg/mL)	1684	9	112
25I-NBOMe	LQC (75 pg/mL)	34	5	113
	MQC (750 pg/mL)	802	2	107
	HQC (1500 pg/mL)	1626	4	108
Post Prep at 48 hours				
Designer Drug	Control	Mean Conc. (pg/mL)	CV	(%) Accuracy/Bias
2CC-NBOMe	LQC (75 pg/mL)	74	16	99
	MQC (750 pg/mL)	768	19	102
	HQC (1500 pg/mL)	1397	19	93
25I-NBOMe	LQC (75 pg/mL)	73	18	97
	MQC (750 pg/mL)	769	16	103
	HQC (1500 pg/mL)	1333	14	89