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Simultaneous Quantitative Determination of Nine Hallucinogenic NBOMe Derivatives in Human Plasma Using Liquid Chromatography Tandem Mass Spectrometry

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Abstract : We developed a bioanalytical method for simultaneous determination of nine NBOMe derivatives (25H-NBOMe, 25B-NBOMe, 25E-NBOMe, 25N-NBOMe, 25C-NBOH, 25I-NBOH, 25B-NBF, 25C-NBF, and 25I-NBF) in human plasma using liquid chromatography tandem mass spectrometry (LC-MS/MS). Human plasma samples were pre-treated using solid-phase extraction. Separation was achieved on a C18 column under gradient elution using a mobile phase containing 0.1% formic acid in acetonitrile and 0.1% formic acid in water at a flow rate of 0.3 mL/min. Mass detection was performed in the positive ion mode using multiple reaction monitoring. The calibration range was 1-100 ng/mL for all quantitative analytes, with a correlation coefficient greater than 0.99. The intra- and inter-day precision and accuracy varied from 0.85 to 6.92% and from 90.19 to 108.69%, respectively. The recovery ranged from 86.36 to 118.52%, and the matrix effects ranged from 27.09 to 99.72%. The stability was acceptable in various conditions. The LC-MS/MS method was validated for linearity, accuracy, precision, matrix effects, recovery and stability in accordance with the FDA guidance. The proposed method is suitable for reliable and robust routine screening and analysis of nine NBOMe derivatives in forensic field.

Keywords : New psychoactive substances, simultaneous determination, LC-MS/MS, solid-phase extraction, human plasma

Introduction

In the last decade, new psychoactive substances (NPS) that are synthetic alternatives to traditional illicit drugs have emerged and rapidly supplied the illicit drug market[1,2] and the number of NPS is estimated at approximately 500 according to the United Nations Office on Drugs and Crime (UNODC).[3] Since NPS are easier to obtain though internet or and fall outside of drug legislation, the explosive increase in the use of NPS result in serious problem such as the physical and mental health of the human population[4,5] and increase in the number of crimes. Because of the diversity and rapid development of NPS, there is resulted in analytical challenge regarding detection and quantification. Accordingly, it is evident that the development of analytical method in biological matrices is required to provide new information.

NBOMes are N-(2-methoxy)benzyl derivatives of the 2C hallucinogenic drugs, which acts as a potent agonist for the 5-HT2A receptor.[6,7] The most common side effects and adverse reactions were agitation, tachycardia, hypertension, fear, paranoia and nausea.[6] So, NBOMes are classified as NPS and have become popular as drug of abuse. So, many countries considered that these compounds was controlled substance. Johnson et al.,[8] Poklis et al.,[9] and Cunha et al.[10] reported the LC/MS/MS methods for NBOMe derivatives. But no currently published studies have reported the simultaneous analytical method of nine hallucinogenic NBOMe derivatives (25H-NBOMe, 25B-NBOMe, 25E-NBOMe, 25N-NBOMe, 25C-NBOH, 25I-NBOH, 25B-NBF, 25C-NBF, and 25I-NBF). Especially, 25N-NBOMe, 25C-NBOH, 25I-NBOH, 25B-NBF, and 25C-NBF have not been studied about analytical method using LC-MS/MS in biological material.

We developed a fast, specific, and sensitive simultaneous quantification method of nine NBOMe derivatives (25H-

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NBOMe, 25B-NBOMe, 25E-NBOMe, 25N-NBOMe, 25C-NBOH, 25I-NBOH, 25B-NBF, 25C-NBF, and 25I-NBF; Figure 1) in human plasma using LC-MS/MS. The method will apply to rapidly screen nine NBOMe derivatives of abuse in biological specimens.

Experimental

Chemicals and materials

25H-NBOMe (98.10%), 25B-NBOMe (97.16%), 25E-NBOMe (97.58%), 25N-NBOMe (98.28%), 25C-NBOH (98.55%), 25I-NBOH (98.41%), 25B-NBF (97.05%), 25C-NBF (97.59%), and 25I-NBF (97.77%) were obtained from the National Institute of Food and Drug Safety Evaluation Ministry of Food and Drug Safety (Cheongju, Korea) (see Table 1. for IUPAC names and Figure 1. for structures). Diphenhydramine was used as an internal standard (IS). Trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO), and formic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade organic

Table 1. Common name, IUPAC name, and MRM conditions of NBOMe derivatives and internal standard.

Abbreviation or Common Name	IUPAC name	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (eV)	Collision Energy (eV)
25H-NBOMe	2-(2,5-Dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine	302.1	121	110	17
25B-NBOMe	2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine	379.9	121	105	21
25E-NBOMe	2-(4-ethyl-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine	330.1	121	110	17
25N-NBOMe	2-(2,5-Dimethoxy-4-nitrophenyl)-N-(2-methoxybenzyl)ethanamine	347.1	121	85	13
25C-NBOH	2-({[2-(4-chloro-2,5-dimethoxyphenyl)ethyl]amino}methyl)phenol	322	199	75	17
25I-NBOH	2-((2-(4-Iodo-2,5-dimethoxyphenyl)ethylamino)methyl)phenol	414	107	125	33
25B-NBF	2-(4-bromo-2,5-dimetoxifenyl)-N-[(2-fluorophenyl)methyl]ethan-1-amine	367.9	242.9	110	17
25C-NBF	2-(4-chloro-2,5-dimetoxifenyl)-N-[(2-fluorophenyl)methyl]ethan-1-amine	324	199	105	13
25I-NBF	2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-fluorophenyl)methyl]ethan-1-amine	415.9	290.9	130	17
Diphenhydramine (IS)	2-(diphenylmethoxy)-N,N-dimethylethanamine	256.1	167	90	10

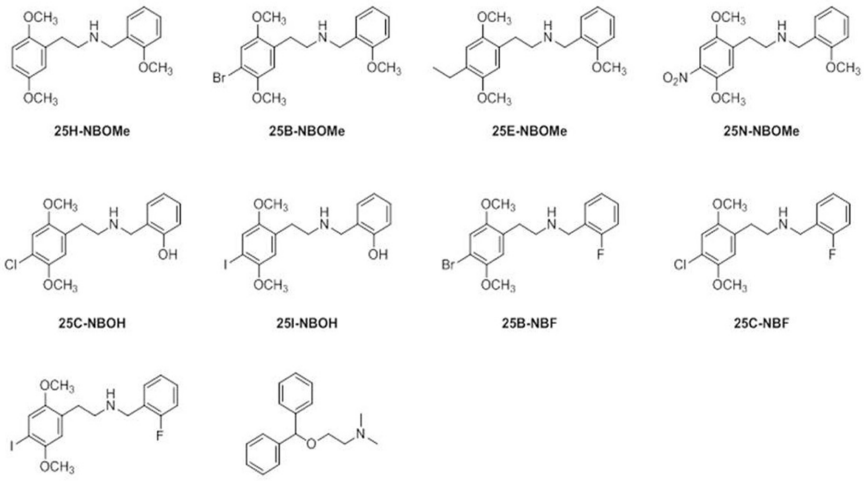

Figure 1. Chemical structures of NBOMe derivatives and diphenhydramine as an internal standard.

solvents (acetonitrile and methanol) were purchased from Burdick & Jackson Inc. (Muskegon, MI, USA). Distilled water was produced using a Millipore water purification system (Milli-Q system, Millipore, Bedford, MA, USA). Human plasma (with K2EDTA as anticoagulant) was purchased from Innovative research Inc. (Novi, MI, USA).

High-performance liquid chromatography and mass spectrometry conditions

The described analytical method was developed using an Agilent Infinity 1260 HPLC system (Agilent, Palo Alto, CA, USA) equipped with an Agilent 6460 triple-quadrupole mass spectrometer. The analytes were separated using a Poroshell 120 EC-C18 column (2.1 × 100 mm, 2.7 μm, Agilent) maintained at 35°C. The flow rate of 0.3 mL/min was used for sample analysis. The mobile phase consisted of 0.1% formic acid in distilled water (eluent A) and 0.1% formic acid in acetonitrile (eluent B). The elution was performed using the

following gradients: 0–0.5 min: A:B = 85:15 (v/v); 0.5–8.0 min: A:B 75:25(v/v); 8.0–15.0 min: A:B = 70:30 (v/v); an 15.0–20.0 min: A:B 15:85 (v/v). The flow was maintained at an 85% of eluent B (v/v) for a further 1 min (20.0–21.0 min) before returning to the initial conditions. The total run time was 25 min. Mass spectrometry was performed in the positive electrospray ionization mode. The source temperature was set to 350°C. All analyses were performed in the multiple reaction monitoring (MRM) mode in order to monitor specific transitions. The MRM conditions are presented in Table 1.

Preparation of standard and quality control (QC) samples

The standard stock solutions of NBOMe derivatives were prepared by dissolving the compounds in DMSO at concentration of 10 mg/mL for each analyte and diluted with methanol to give concentration of 100 μg/mL of NBOMe derivatives, respectively. Working standards solutions were prepared as mixtures of nine NBOMe derivatives at concentration of 1000 ng/mL as high standard concentration and sequentially diluted in methanol at the concentrations of 10, 30, 200, 500, and 800 ng/mL. The diphenhydramine (as IS) was prepared to final concentration of 25 ng/mL using methanol. Calibration standards and Quality control (QC) samples were prepared in blank human plasma by the addition corresponding working solutions. The QC samples were prepared at four concentration levels to final concentrations of 1 ng/mL (lower limit of quantification, LLOQ), 3 ng/mL (low quality control, LQC), 50 ng/mL (medium quality control, MQC), 80 ng/mL (high quality control, HQC). Stock and working solution were stored at -80°C until analysis.

Sample preparation

The procedure for extracting the target analytes from human plasma employed a Waters Sep-pak C18 SPE cartridge (1 mL, 50 mg; Waters, USA). Initially, 200 μL of plasma spiked with 25 ng/mL diphenhydramine (as IS) was diluted using 1000 μL of 0.1 M HCl. The Sep-pak C18 SPE cartridge was conditioned with 2 mL of 100% acetonitrile, followed by 2 mL of 0.1% TFA in 50% acetonitrile/distilled water, and finally, with 2 mL of 0.1% TFA in distilled water. For the SPE, the spiked, diluted plasma sample was loaded into the conditioned SPE cartridge and aspirated under gravity, and subsequently the cartridge was washed with 2 mL of 0.1% TFA in distilled water. For the preparation of the sample for injection into the HPLC, the analytes were eluted with 1 mL of 0.1% TFA in 50% acetonitrile/distilled water. The eluted liquid was evaporated under vacuum and re-dissolved in 200 μL of 50% methanol/distilled water.

Validation procedure

The analytical method was validated in accordance with the FDA guidance for Bioanalytical Method Validation.[11]

The selectivity, linearity, accuracy, and precision of the described method for simultaneous determination of NBOMe derivatives were validated. The selectivity of the method was evaluated by comparing blank plasma samples and plasma sample spiked with the NBOMe derivatives at concentrations of 1 ng/mL as the LLOQ to ensure that no interfering peaks were present. The calibration curves for the NBOMe derivatives ranged from 1 to 100 ng/mL. The regression curve was calculated using a weighted (1/x) linear regression model. Precision and accuracy of the method were estimated using replicate samples (n = 5).

Intra- and inter-day accuracy and precision were evaluated at the LLOQ of 1 ng/mL and three different concentrations of 3, 50, and 80 ng/mL in a single day and over 5 days, respectively. LQC, MQC, and HQC must be between 85% and 115% and the LLOQ range should be 80-120% for good accuracy. Also, LQC, MQC, and HQC must be within 15% and the LLOQ within 20% for precision. The accuracy was calculated as the percentage of the deviation between the theoretical and calculated concentration. The precision was expressed as the relative standard deviation (RSD).[12]

The matrix effect and recovery were estimated by analyzing three sets of samples at three concentrations (3, 50, and 80 ng/mL). The recovery was determined by comparing the peak area of each analyte when spiked into plasma before extraction (set 1) with analytes spiked after extraction (set 2). To calculate the matrix effect, peak areas obtained for set 2 were compared with those obtained for the analytes in the mobile phase (set 3).[13]

To evaluate the stability of NBOMe derivatives in human plasma, short-term and long-term stability tests were performed. The short-term stability was evaluated under variable conditions, including repeated freeze–thaw cycles, bench top (1 day at room temperature), and post-preparative stability (1 day at 4°C). Long-term stability involved maintaining the samples at -80°C for 30 days.

Results and Discussion

Mass spectra and chromatography

The product ion mass spectra of nine NBOMe derivatives are shown in Figure 2. The mass spectra and the selected MRM mode resulted in high sensitivity and selectivity of acquisition data. Table 1 summarizes the MRM transitions and the detailed mass spectrometry conditions used for the quantification of nine NBOMe derivatives. As shown in Figure 3, nine NBOMe derivatives have been well separated under reversed phase conditions. The use of poroshell 120 EC-C18 column showed excellent resolution, peak shape and reproducibility for nine NBOMe derivatives as well as the IS in the plasma matrix.

Method validation

Selectivity was assessed by analyzing the chromatograms of blank plasma with IS, and blank plasma spiked with mixed standards and IS. Typical MRM chromatograms obtained for blank plasma, LLOQ (1 ng/mL) samples, and samples at concentration of 100 ng/mL are shown in

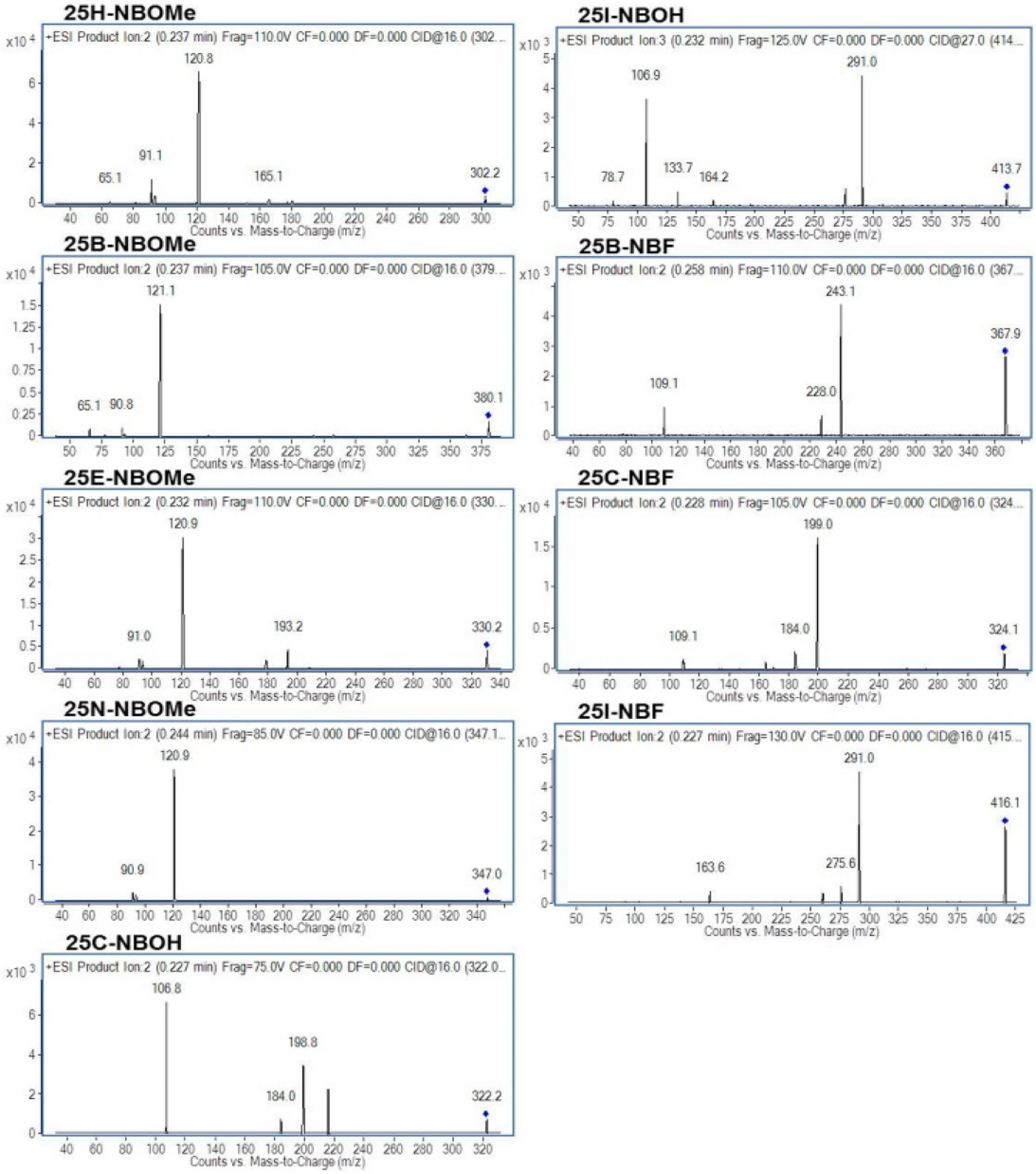

Figure 2. The product-ion scan spectra of NBOMe derivatives.

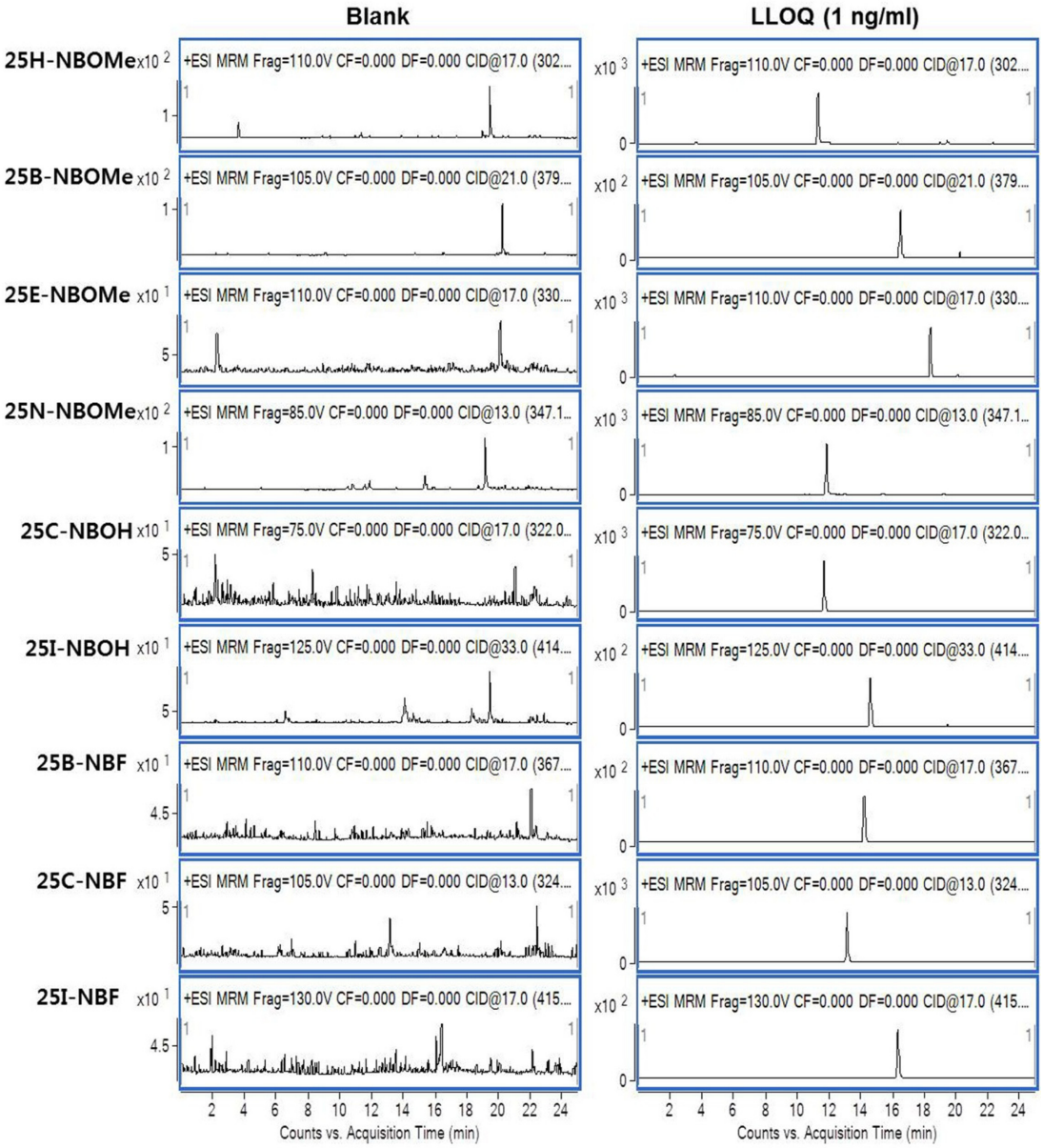

Figure 3. Representative chromatograms obtained in the multiple reaction monitoring mode for blank human plasma and human plasma spiked with NBOMe derivatives at 1 ng/mL (LLOQ).

Figure 3. The results showed that there were no interfering peaks directly overlapping with those arising from the analytes or IS.

The present bio-analytical method provided lower limit of quantitation and good range of linearity. The weighted regression (1/x) was used for the quantification of all analytes. The calibration curves for nine NBOMe derivatives ranged from 1 to 100 ng/mL, and displayed a correlation coefficient of > 0.99. The back-calculated concentration at all the concentrations tested showed accuracy within 15% of the nominal concentration and precision that does not exceed 15% of the CV(data not shown).

The intra- and inter-day reproducibility of all analytes is summarized in Table 2. The intra-day accuracy ranged from 90.19 to 108.69%, with RSD values ranging from 0.85 to 6.25% and the inter-day accuracy ranged from 98.35 to 106.60%, with RSD values ranging from 1.65 to 6.92%, thus confirming the excellent accuracy of the developed method. In this context, the present LC-MS/MS method developed for the simultaneous assessment of nine NBOMe derivatives was demonstrated to meet the accepted limits for accuracy and precision experiments.[11]

Table 2. Inter-and inter- day assay for 9 compounds in human plasma (n=5).

Compound	Nominal concentration (ng/mL)	Intra-day			Inter-day		
		Found Concentration (ng/mL)	Accuracy[a] (%)	Precision[b] (%)	Found Concentration (ng/mL)	Accuracy[a] (%)	Precision[b] (%)
25H-NBOMe	1	0.99 ± 0.02	98.52	2.41	1.07 ± 0.03	107.09	2.48
	3	3.07 ± 0.05	102.22	1.78	3.01 ± 0.09	100.35	2.91
	50	49.34 ± 0.55	98.68	1.11	46.77 ± 1.77	93.54	3.79
	80	82.09 ± 1.68	102.61	2.05	79.11 ± 3.83	98.89	4.84
25B-NBOMe	1	0.95 ± 0.06	95.47	6.21	1.05 ± 0.05	105.06	4.63
	3	3.07 ± 0.09	102.43	2.95	2.98 ± 0.15	99.45	5.19
	50	48.22 ± 0.92	96.43	1.90	46.91 ± 1.89	93.82	4.03
	80	79.97 ± 1.30	99.96	1.62	77.53 ± 4.85	96.92	6.26
25E-NBOMe	1	0.92 ± 0.03	92.47	2.97	1.03 ± 0.04	102.69	4.03
	3	3.03 ± 0.10	100.99	3.24	2.97 ± 0.19	98.86	6.49
	50	48.73 ± 1.46	97.45	3.00	46.50 ± 1.54	92.99	3.32
	80	79.76 ± 1.91	99.70	2.39	76.58 ± 5.30	95.72	6.92
25N-NBOMe	1	0.95 ± 0.02	94.64	1.82	1.04 ± 0.03	104.42	2.42
	3	3.11 ± 0.05	103.71	1.69	3.05 ± 0.10	101.56	3.12
	50	50.26 ± 0.50	100.52	1.00	47.41 ± 1.89	94.81	3.98
	80	82.48 ± 1.59	103.10	1.92	78.76 ± 3.86	98.45	4.90
25C-NBOH	1	0.95 ± 0.04	95.23	3.74	1.03 ± 0.02	102.95	1.65
	3	3.10 ± 0.05	103.22	1.63	3.06 ± 0.10	101.90	3.14
	50	50.00 ± 0.65	100.01	1.30	48.04 ± 2.28	96.08	4.74
	80	81.71 ± 1.67	102.13	2.04	79.10 ± 4.04	98.88	5.11
25I-NBOH	1	0.95 ± 0.04	95.17	4.47	1.02 ± 0.02	102.36	2.06
	3	3.17 ± 0.07	105.83	2.28	3.00 ± 0.13	100.12	4.24
	50	50.19 ± 0.88	100.38	1.75	47.76 ± 2.20	95.51	4.61
	80	83.73 ± 0.71	104.66	0.85	78.68 ± 4.01	98.35	5.10
25B-NBF	1	0.90 ± 0.03	90.19	3.32	1.04 ± 0.04	103.66	3.80
	3	3.26 ± 0.08	108.69	2.52	2.98 ± 0.10	99.24	3.39
	50	49.90 ± 1.51	99.80	3.03	47.56 ± 2.38	95.12	5.01
	80	83.69 ± 1.33	104.61	1.59	78.22 ± 4.45	97.77	5.69
25C-NBF	1	0.94 ± 0.04	93.52	4.57	1.05 ± 0.04	105.07	4.00
	3	3.19 ± 0.07	106.24	2.31	3.00 ± 0.08	100.16	2.65
	50	49.86 ± 0.75	99.71	1.51	47.24 ± 2.19	94.48	4.64
	80	83.72 ± 0.99	104.65	1.18	79.00 ± 3.97	98.75	5.02
25I-NBF	1	0.95 ± 0.06	95.09	6.25	1.07 ± 0.07	106.60	6.39
	3	3.11 ± 0.09	103.81	3.04	3.03 ± 0.12	101.15	3.89
	50	49.50 ± 1.26	99.00	2.55	47.38 ± 2.02	94.76	4.27
	80	81.52 ± 1.29	101.90	1.58	77.83 ± 4.87	97.29	6.26

The results of the extraction recovery and matrix effect were investigated at three concentration levels, namely at 3, 50, and 80 ng/mL, and the results are summarized in Table 3. For all analytes evaluated, the recovery ranged from 86.36 to 118.52%, and the matrix effect ranged from 27.09 to 99.72%. The % recovery of the nine NBOMe derivatives was found to be more than 86.36%. The extent of recovery is considered acceptable in bioanalytical method development.[14,15] The matrix effect was found to be range from 80.09 to 99.72% excluding 25E-NBOMe. It

should also be noted that no significant ion suppression or enhancement was observed for the NBOMe derivatives except for 25E-NBOMe under the present experimental conditions. The mean matrix effect at 25E-NBOMe concentrations of 3, 50, and 80 ng/mL were 29.70, 27.09, and 28.86%, respectively. In a reverse column, the phospholipid in biological matrices may elute as organic solvent concentrations gradually increases. This may cause the ion suppression.[16,17] Thus, 25E-NBOMe might be co-eluted with phospholipids and it could result in significant matrix effects on 25E-NBOMe. These results suggest that there is some matrix effects, however, the signal intensity at the LLOQ level is satisfactory.

The stability of the biological matrices is very important to evaluate the effectiveness of analytical components under specific conditions and durations. The stability of all analytes was investigated at three QC levels (3, 50, and 80 ng/mL) under various conditions. As demonstrated in Table 4, the nine NBOMe derivatives showed good stability even at -80°C for an extended period of 30 days and post-preparative stability (1 day at 4°C). In addition, no detrimental impact was observed on the outcome of the

Table 3. Recovery (RE) and matrix effect (ME) (n = 3).

Compound	LQC (3 ng/mL)		MQC (50 ng/mL)		HQC (80 ng/mL)	
	RE[a] (%)	ME[b] (%)	RE (%)	ME (%)	RE (%)	ME (%)
25H-NBOMe	114.25	99.72	100.47	98.28	88.39	98.69
25B-NBOMe	113.00	83.73	99.33	80.46	86.36	80.09
25E-NBOMe	118.52	29.70	106.97	27.09	92.94	28.86
25N-NBOMe	113.44	94.45	98.41	91.35	88.57	93.36
25C-NBOH	113.95	95.86	99.21	94.04	88.15	94.47
25I-NBOH	111.03	92.68	97.39	93.12	86.82	94.04
25B-NBF	115.51	90.96	99.92	88.80	89.22	91.42
25C-NBF	112.24	94.18	98.82	93.74	86.93	95.05
25I-NBF	112.50	88.73	100.14	87.10	88.28	88.19

Table 4. Stability of 9 compounds in human plasma (n = 3).

Compound	LQC (3 ng/mL)			MQC (50 ng/mL)			HQC (80 ng/mL)		
	Mean	Accuracy[a] (%)	RSD[b] (%)	Mean	Accuracy[a] (%)	RSD[b] (%)	Mean	Accuracy[a] (%)	RSD[b] (%)
Freeze–thaw (-80°C, 3 cycle)									
25H-NBOMe	2.88	96.10	4.75	52.91	105.81	1.35	90.43	113.04	2.77
25B-NBOMe	2.80	93.24	4.86	50.65	101.31	1.37	88.58	110.72	3.89
25E-NBOMe	2.87	95.65	7.97	50.45	100.89	2.56	87.40	109.26	3.22
25N-NBOMe	2.84	94.66	6.19	52.46	104.93	0.74	89.65	112.06	3.79
25C-NBOH	2.85	95.01	4.94	54.31	108.62	0.62	88.71	110.89	3.56
25I-NBOH	2.84	94.77	6.12	52.25	104.51	0.91	88.59	110.74	3.67
25B-NBF	2.90	96.64	5.08	51.47	102.94	1.19	89.29	111.62	3.95
25C-NBF	2.91	96.89	5.19	52.30	104.60	0.24	88.96	111.20	3.49
25I-NBF	2.92	97.30	5.51	51.81	103.61	1.19	88.20	110.25	4.17
Bench (room temperature, 1 day)									
25H-NBOMe	2.75	91.55	1.59	46.61	93.22	0.59	76.40	95.50	1.20
25B-NBOMe	2.85	95.00	2.61	47.62	95.24	0.54	77.44	96.80	1.47
25E-NBOMe	2.93	97.68	5.34	50.07	100.15	0.99	79.45	99.31	3.45
25N-NBOMe	2.77	92.25	3.28	47.12	94.25	0.91	75.77	94.71	1.26
25C-NBOH	2.81	93.65	3.63	47.07	94.15	0.71	75.81	94.76	1.50
25I-NBOH	2.85	94.91	2.24	47.69	95.37	1.59	77.24	96.56	1.57
25B-NBF	2.82	93.90	3.96	47.57	95.14	1.72	77.25	96.56	1.78
25C-NBF	2.84	94.78	3.19	47.53	95.05	0.97	76.89	96.12	0.41
25I-NBF	2.87	95.71	1.86	47.35	94.70	0.74	76.64	95.80	1.02

Table 4. Continued.

Compound	LQC (3 ng/mL)			MQC (50 ng/mL)			HQC (80 ng/mL)		
	Mean	Accuracy[a] (%)	RSD[b] (%)	Mean	Accuracy[a] (%)	RSD[b] (%)	Mean	Accuracy[a] (%)	RSD[b] (%)
Post-preparative stability (4°C, 1 day)									
25H-NBOMe	2.96	98.52	4.38	48.12	96.24	2.09	77.11	96.39	3.97
25B-NBOMe	3.00	100.06	4.09	48.72	97.45	0.57	77.38	96.72	4.32
25E-NBOMe	2.88	95.96	2.13	48.01	96.02	4.42	76.67	95.84	0.77
25N-NBOMe	3.01	100.45	3.99	49.18	98.36	2.11	77.18	96.48	4.23
25C-NBOH	3.00	99.94	3.70	49.46	98.92	1.69	77.61	97.01	4.14
25I-NBOH	3.07	102.26	7.19	48.45	96.89	0.79	76.76	95.95	4.19
25B-NBF	2.94	97.84	3.85	48.03	96.06	0.46	76.84	96.05	2.63
25C-NBF	2.99	99.55	4.96	48.01	96.02	0.97	76.71	95.89	0.77
25I-NBF	3.00	100.01	4.46	48.95	97.91	1.23	77.31	96.63	4.40
Freezer (-80°C, 30 days)									
25H-NBOMe	2.66	88.58	0.72	45.92	91.83	5.78	73.57	91.96	2.17
25B-NBOMe	2.70	90.12	6.05	47.25	94.50	3.77	75.99	94.99	2.49
25E-NBOMe	2.91	97.05	4.52	52.24	104.49	5.45	81.33	101.66	3.66
25N-NBOMe	2.68	89.41	0.66	46.81	93.63	5.91	74.13	92.67	2.33
25C-NBOH	2.66	88.8	1.06	46.37	92.74	6.09	72.64	90.80	2.56
25I-NBOH	2.62	87.28	0.76	45.87	91.74	4.42	72.00	90.00	1.39
25B-NBF	2.71	90.39	2.12	44.90	89.80	2.49	70.43	88.04	2.10
25C-NBF	2.60	86.51	3.56	46.09	92.18	4.64	73.07	91.34	3.29
25I-NBF	2.68	89.23	3.58	46.04	92.07	3.69	73.58	91.97	1.34

analysis when the samples were subjected to three freeze thaw cycles and maintained at room temperature for 1 day. The remaining nine NBOMe derivatives exhibited good stability at room temperature as well as over three freeze and thaw cycles.

Conclusions

An LC-MS/MS method was developed and validated for the simultaneous determination and quantitation of nine NBOMe derivatives in human plasma. The developed method is useful for reliable and robust routine screening and analysis of nine NBOMe derivatives in forensic field.

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