

Quantitative Analysis of Benzphetamine in Human Plasma using LC-MS/MS

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Abstract

Benzphetamine hydrochloride is a sympathomimetic amine with pharmacologic activity similar to the amphetamine class of compounds. Presented is data from the bioanalytical validation of a high performance liquid chromatography, tandem mass spectrometry (HPLC-MS/MS) method for the analysis of benzphetamine in human heparanized plasma. Sample preparation was accomplished using a liquid-liquid extraction with chromatographic separation using a C-8 column. The relationship between benzphetamine concentration to peak area ratio (drug/internal standard) was established over the range of 0.050 to 100 ng/mL. Routine use of this method with clinical samples has shown this method to be rugged, sensitive, specific, precise, and accurate.

Methods

Sample cleanup was accomplished by basifying a 300 μ L aliquot of human plasma followed by a liquid-liquid extraction of benzphetamine and the internal standard, methamphetamine-D₅. The organic phase was acidified and evaporated to dryness. Dried residue was reconstituted in a buffered aqueous : organic mobile phase. Sample aliquots were injected on a C-8 column and eluted under isocratic mobile phase composition. Samples were introduced using an ion spray source in the positive ion mode. The chemical structures of benzphetamine and methamphetamine-D₅ and typical product ion mass spectrum are presented in Figures 1 and 2 respectively. The tandem mass spectrometer was operated in a multiple reaction monitoring (MRM) scanning mode. Equipment used and relevant instrumental parameters are presented in Table 1.

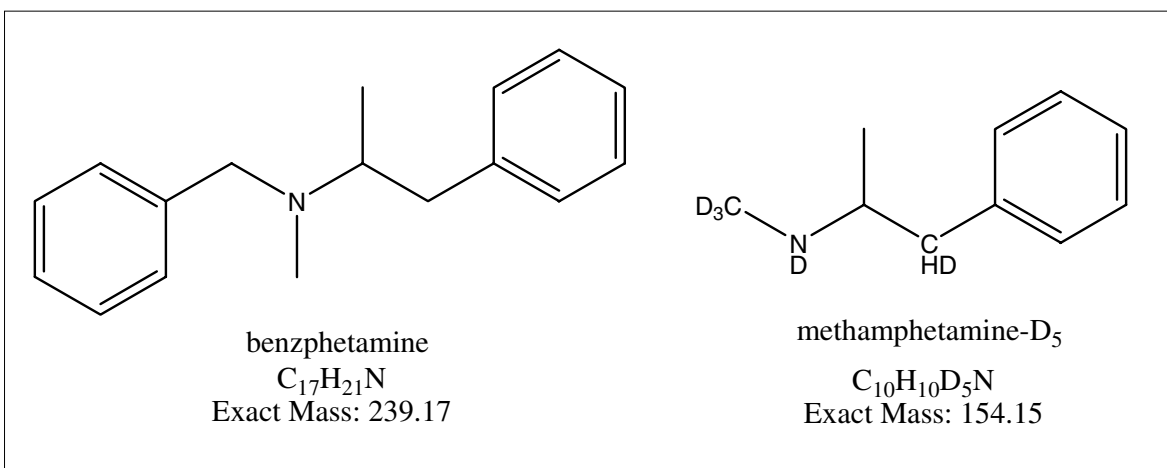


Figure 1. Chemical Structures of Benzphetamine and Methamphetamine-D₅

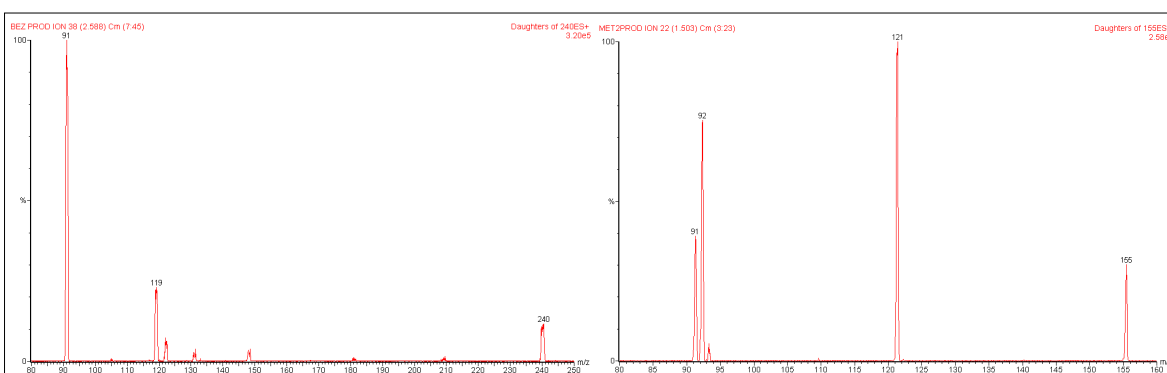


Figure 2. Representative Product Ion Spectra of Benzphetamine (left) and Methamphetamine-D₅ (right)

Table 1. Equipment Used and Typical Instrumental Parameters

Mass Spectrometer	Micromass Quattro LC
Ionization Mode	electrospray, positive ions, MRM mode
Solvent System	Methanol, Aqueous Ammonium Formate Buffer
Column	C-8
Source Block Temp	150 °C
Desolvation Temp	300 °C
Desolvation Gas Flow	700 - 900 L/hr
Capillary	0.5 KV
Cone	15 V
Collision Energy	Benzphetamine: 20 eV
MRM	m/z = Q1 240.1 Q3 91.1 benzphetamine m/z = Q1 155.1 Q3 120.0 IS
Dwell Time	400 ms
Run Time	5 min

During the validation of benzphetamine a quadratic regression model, weighted by 1/concentration squared yielded the best fit of the data over the range 0.050 to 100 ng/mL. A representative quadratic calibration curve weighted by 1/concentration squared is presented in Figure 3 with the respective area ratio response and concentration axes displayed in log-log format.

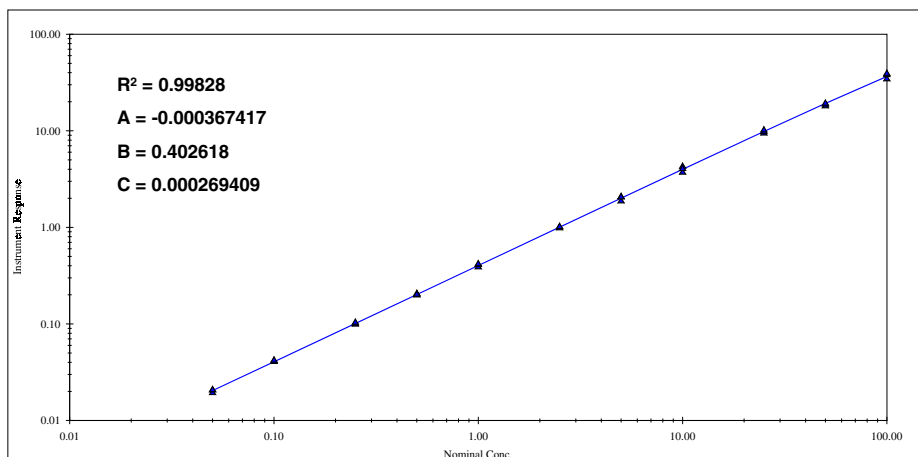


Figure 3. Representative Quadratic, Weighted (Conc.)² Calibration Curve

The accuracy of the assay was defined as the difference between the back-calculated and nominal values of the quality control samples divided by nominal values and expressed as a percentage. The intra-day accuracy for the quality control samples over the three day validation ranged from 5.00 to 13.50% for benzphetamine. Inter-day accuracy for quality control samples is presented in Table 5.

The precision of the assay was expressed as the percent coefficient of variation of the quality control samples, extracted in groups of six replicates at benzphetamine concentrations of 0.050, 0.100, 4.00, and 80.0 ng/mL. Intra-day precision for the quality control samples ranged from 1.85 to 7.83%. Inter-day precision of the quality control samples is also presented in Table 5.

Table 5. Inter-day Accuracy of Quality Control Samples

Run Date	Curve Number	Low (0.0500 ng/mL)	Mid (0.100 ng/mL)	Mid (4.00 ng/mL)	High (80.0 ng/mL)
20-Oct-2003	1	0.0517	0.100	4.42	92.0
		0.0578	0.0987	4.56	77.8
		0.0526	0.110	4.09	89.4
		0.0565	0.104	4.26	89.2
		0.0561	0.109	4.10	92.3
		0.0576	0.109	4.34	81.9
21-Oct-2003	2	0.0527	0.114	4.66	83.2
		0.0547	0.114	4.48	83.3
		0.0552	0.112	4.45	77.9
		0.0539	0.109	4.83	90.1
		0.0555	0.113	4.48	83.0
		0.0543	0.106	4.36	86.9
22-Oct-2003	3	0.0501	0.105	3.74	84.4
		0.0538	0.114	4.30	87.4
		0.0527	0.110	4.66	87.4
		0.0596	0.110	4.64	98.4
		0.0615	0.116	4.49	93.3
		0.0574	0.119	4.49	90.7
Mean		0.0552	0.110	4.41	87.1
S.D.		0.00288	0.00533	0.253	5.43
%CV		5.22	4.85	5.74	6.23
%Theoretical		110.40	110.00	110.25	108.88
%Bias		10.40	10.00	10.25	8.88
n		18	18	18	18
Overall % CV		5.51			

The stability of benzphetamine in spiked human plasma after 24 hours at ambient temperature was determined. Triplicate samples spiked at concentrations of 0.100 and 80.0 ng/mL of benzphetamine were kept at ambient temperature for 24 hours before extraction. The mean concentrations of the stability samples were compared to the theoretical concentrations. Summary results are found in Table 7. The stability of the extracted samples in the autosampler was evaluated by reinjecting three quality control samples (0.100 and 80.0 ng/mL) and comparing the mean, back-calculated concentrations to the theoretical concentrations of benzphetamine after 47 hours at ambient temperature. The results for the extracted samples on the autosampler are also presented in Table 7.

Table 7. Ambient Temperature (24 h) and Autosampler (47 h) Stability of Benzphetamine

Benzphetamine Stabilities	Ambient Temperature Stability (24 h)		Autosampler Stability (47 h)	
	Theoretical Concentration (ng/mL)		Theoretical Concentration (ng/mL)	
	0.100	80.0	0.100	80.0
Mean Measured Concentration	0.102	75.5	0.104	76.5
Precision (% CV)	3.31	2.82	3.91	4.64
Recovery (%)	102	94	104	96
Number of Samples	3	3	3	3

Long term and frozen storage stability was demonstrated by the analysis of quality control samples at concentrations of 0.200 and 50.0 ng/mL benzphetamine stored at -20 °C and quantitated against a freshly prepared, never frozen, standard line and quality controls. Frozen storage stability was demonstrated for at least 417 days with all test samples quantitating within 15% of the theoretical concentration.

Conclusions

The analytical method described is suitable for the analysis of benzphetamine in human plasma. The method is rugged, sensitive, specific, precise and accurate. The calibration curve extends from a limit of quantification of 0.050 ng/mL to 100 ng/mL using a 0.300 mL aliquot of human plasma.

This assay has been used to analyze several thousand clinical samples with comparable analytical performance and ruggedness as demonstrated in the method validation.

Acknowledgements

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