

Determination of methamphetamine and amphetamine enantiomers in human urine by chiral stationary phase liquid chromatography-tandem mass spectrometry

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Abstract Methamphetamine (MA) is currently the most abused illicit drug in Korea and its major metabolite is amphetamine (AP). As MA exist as two enantiomers with the different pharmacological properties, it is necessary to determine their respective amounts in a sample. Thus a chiral stationary phase liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed for identification and quantification of *d*-MA, *l*-MA, *d*-AP, and *l*-AP in human urine. Urine sample (200 μ L) was diluted with pure water and purified using solid-phase extraction (SPE) cartridge. A 5- μ L aliquot of SPE treated sample solution was injected into LC-MS/MS system. Chiral separation was carried out on the Astec Chirobiotic V2 column with an isocratic elution for each enantiomer. Identification and quantification of enantiomeric MA and AP was performed using multiple reaction monitoring (MRM) detection mode. Linear regression with a $1/x^2$ as the weighting factor was applied to generate a calibration curve. The linear ranges were 25-1000 ng/mL for all compounds. The intra- and inter-day precisions were within 3.6 %, while the intra- and inter-day accuracies ranged from -5.4 % to 11.8 %. The limits of detection were 2.5 ng/mL (*d*-MA), 3.5 ng/mL (*l*-MA), 7.5 ng/mL (*d*-AP), and 7.5 ng/mL (*l*-AP). Method validation parameters such as selectivity, matrix effect, and stability were evaluated and met acceptance criteria. The applicability of the method was tested by the analysis of genuine forensic urine samples from drug abusers. *d*-MA is the most common compound found in urine and mainly used by abusers.

Key words: chiral separation, LC-MS/MS, *d,l*-methamphetamine, *d,l*-amphetamine, urine

1. Introduction

Methamphetamine (MA), a highly addictive synthetic drug, is the most commonly abused illicit drug in Korea.¹ The precursors for MA can be more easily obtained compared to those for other synthetic drugs.

For this reason, there have been cases of drug offenders with some knowledge of chemical synthesis, who actually synthesize MA using pharmaceutical compounds containing the related ingredients.^{2,3} According to the World Drug Report, there is an increasing trend in MA abuse not only in Korea but

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also in the other countries as well.⁴ The use of MA as an alternative drug is spreading quickly, since it is relatively less expensive and easier to purchase compared to other illicit drugs. MA abuse is becoming a serious social issue in Korea and abroad.^{5,6}

MA is a phenylethylamine-based compound that exists as two enantiomers, *d*- and *l*-MA. It has been reported that *d*-MA is usually used for abuse.⁷ Both *d*-MA and *l*-MA are known to have stimulant and hallucinogenic effects, but *l*-MA has a longer lasting stimulant effect and a shorter hallucinogenic effect than *d*-MA.^{8,9} While *d*- and *l*-MA is not used as a pharmaceutical in Korea, drugs containing *l*-MA are registered as over-the-counter drugs and used as nasal decongestants in some countries, including the United States.¹⁰ Selegiline, which can be regarded as a prodrug for MA or its major metabolite amphetamine (AP), is metabolized to produce *l*-MA and *l*-AP in humans.¹¹ There are cases in Korea where such precursors are being sold as pharmaceuticals. Therefore, the MA enantiomer taken can be verified through separation and analysis results on the MA and AP enantiomers.

Various analytical methods based on gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), capillary electrophoresis (CE), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) have been developed for the separation and analysis of MA and AP enantiomers in urine samples.¹²⁻¹⁸ GC-MS methods are the most widely used tools for drug analysis, which often includes time-consuming derivatization steps to enhance chromatographic performance of the compounds. In this technique, a chiral derivatizing agent *l*-*N*-trifluoroacetyl-1-prolyl chloride or *R*-(-)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride was used for the separation and quantification of the MA and AP enantiomers.^{12,13} A recent trend of gradually expanding the analytical methods from GC-MS to LC-MS has been observed in drug testing. This is because LC-MS is more useful for analyzing highly polar substances in biological samples than is GC-MS, and it has the advantage of a shorter pretreatment time since the derivatization

process is not required.¹⁹⁻²¹

In the present study, a chiral stationary phase LC-MS/MS technique was developed for the separation and analysis of *d*-MA, *l*-MA, *d*-AP, and *l*-AP. The chiral stationary phase used to separate and analyze the enantiomers could be divided into five categories.²² It has been reported that cellulose and amylose-based chiral stationary phases are the most frequently used.²³ When the separation capability for two enantiomer peaks was measured and compared between the vancomycin-based chiral stationary phase analytical method used by Ward et al. and the method used in the present study, their chiral resolution values were 1.62 and 1.83, respectively. It shows that the present method had superior separation capability.¹⁸ In this study, the solid-phase extraction method was applied to obtain a purified extract, with the elimination of matrix effects in urine samples. Moreover, the usefulness of this method was tested by employing it to determine the concentration and detection frequency of MA and AP enantiomers in 93 real-case urine samples of MA abusers.

2. Experimental

2.1. Reagents and analytical standards

Standards *d*-MA, *l*-MA, *d,l*-MA, *d*-AP, *l*-AP, and *d,l*-AP and internal standards *d,l*-MA-*d*₅ and *d,l*-AP-*d*₅ were all purchased from Cerilliant (Austin, TX, USA). Fig. 1 shows the chemical structures of the

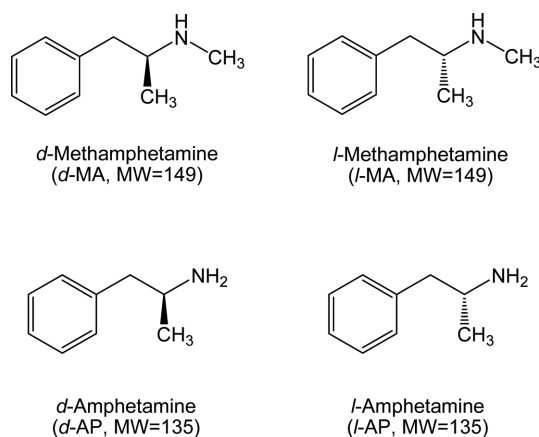


Fig. 1. Chemical structures of the analytes.

analytes. Acetic acid (HPLC grade) and ammonia (puriss pa plus, $\geq 25\%$ in water), added to the mobile phase, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (HPLC grade), used as the organic component for the mobile phase, was purchased from J.T. Baker/Avantor (Center Valley, PA, USA), and distilled water (LiChrosolv grade) was purchased from Merck (Darmstadt, Germany). All other reagents were ACS grade or higher.

Standards *d*-MA, *l*-MA, *d,l*-MA, *d*-AP, *l*-AP, and *d,l*-AP, diluted in methanol to a final concentration of 1 $\mu\text{g/mL}$. Internal standards *d,l*-MA- d_5 and *d,l*-AP- d_5 , diluted in methanol to a final concentration of 0.5 $\mu\text{g/mL}$. The prepared standard solutions were stored at $-20\text{ }^\circ\text{C}$ until use.

2.2. Urine samples

Urine samples from non-drug users were used as the blank for the construction of the calibration curve and preparation of QC samples. To construct the calibration curve, analytes *d*-MA, *l*-MA, *d*-AP, and *l*-AP were added to the blank to prepare calibration samples with concentrations of 25, 50, 125, 250, 500, and 1000 ng/mL . The QC samples were prepared at concentrations of 25, 75, 250, and 750 ng/mL corresponding to lower limit of quantitation (LLOQ), low, medium, and high concentrations within the calibration range.

The samples were obtained from 93 actual drug abusers, whose urine samples were collected by the district prosecutor's office and police stations in the Yeongnam region, was tested positive for MA. The samples were stored at $4\text{ }^\circ\text{C}$ for 20 days, while urine samples that subsequently required additional analysis were kept separately in a freezer at $-20\text{ }^\circ\text{C}$ for reanalysis if required.

2.3. Instrumentation

The HPLC equipment used in the present study was an Agilent 1260 Infinity HPLC system (Santa Clara, CA, USA), which included a vacuum degasser, binary pump, autosampler, and column oven. For separation of the enantiomers, ChiroSil RCA(+) (4.6 mm \times 150 mm,

5 μm), ChiroSil SCA(-) (4.6 mm \times 150 mm, 5 μm), and Supelco Astec Chirobiotic V2 (2.1 mm \times 150 mm, 5 μm) were used. For the mobile phase, a mixed solvent containing 0.02% ammonium hydroxide, 0.1% acetic acid, 10% distilled water, and 90% methanol was used. The flow rate of the mobile phase was set to 150 $\mu\text{L/min}$, and isocratic elution was performed for 20 min.

A Sciex QTrap 4500 triple-quadrupole mass spectrometer (AB SCIEX, Foster city, CA, USA) was connected to a liquid chromatograph and equipped with an electrospray ionization as the interface. Electrospray ionization was carried out in the positive mode, and the amount of gas supplied was set to nebulize gas 50, curtain gas 20, and turbo ion spray heater gas 50. The turbo-gas temperature was set to $600\text{ }^\circ\text{C}$, and the ionization voltage was set to 5500 V. The multiple reaction monitoring (MRM) method was used for quantitative analysis, and nitrogen gas was used as the collision gas for the fragmentation of the precursor ions.

2.4. Sample preparation

The urine sample (200 μL), distilled water (2 mL), and 0.5 $\mu\text{g/mL}$ internal standard containing *d,l*-AP- d_5 and *d,l*-MA- d_5 (50 μL) were placed in a test tube (12 \times 100 mm) and mixed. Prior to sample loading, an Oasis HLB (Waters, Milford, MA, USA) cartridge (60 mg, 3 cc) loaded on the automatic solid phase extractor was activated by sequentially running 3 mL of methanol and 3 mL of distilled water on the cartridge. After running the sample on the activated cartridge at a flow rate of 7 mL/min, 2 mL of distilled water was run at a flow rate of 15 mL/min for washing, followed by drying for 2 min with nitrogen gas. The analytes were extracted with 3 mL of methanol as the eluent at a flow rate of 3 mL/min.

2.5. Validation of the analytical method

To validate the analytical method, the selectivity, limit of detection (LOD), LLOQ, linearity, accuracy and precision, dilution integrity, matrix effect, recovery, process efficiency, and stability were assessed.^{24,25}

The selectivity was compared and evaluated by

analyzing six different urine samples, testing the influence of interfering substances on the retention time of the analyte and internal standard, based on the chromatographic peaks.

For LOD, the standard deviation (SD) of the signal (S) in the analysis of six samples with the same concentration and the noise (N), obtained from six blank samples, was used to check for the concentration that gave an S/N ratio ≥ 3 . The concentration suitable for the analytical objectives was selected as the LLOQ, with the S/N ratio ≥ 10 , precision (% CV) $< 20\%$, and accuracy (% bias) $\pm 20\%$.

For the quantitation range of the calibration curve, the linearity of the calibration curve constructed, using 25–1000 ng/mL of *d*-MA, *l*-MA, *d*-AP, and *l*-AP, was assessed by calculating the coefficient of determination (r^2) and weighting factor ($1/x^2$) was applied to generate a calibration curve.

To verify the repeatability of the analysis results, the intra- and inter-day precision and accuracy were measured. To test the precision (closeness of the measured values obtained from repeated analysis using several aliquots of a homogenous sample) and accuracy (the difference between the actual and measured values), LLOQ and QC samples with three different concentrations (low, medium, and high) were prepared, and five samples per concentration were measured. The accuracy of the mean measured value was set to within 15% (bias) of the actual measured values, and the precision was set such that the coefficient of variance (CV) does not exceed 15%. As an exception, the samples with concentration corresponding to the LLOQ were managed to within 20%.

For dilution integrity, the blank sample was sequentially added to the medium (250 ng/mL) and high (750 ng/mL) QC samples to prepare 10- and 20-fold diluted samples. The diluted samples were prepared by dividing them into six aliquots, which were analyzed after the pretreatment process.

The matrix effect, recovery, and process efficiency were determined using samples prepared at five aliquots each from sets A, B, and C according to Matuszewski *et al.*²⁶ Set A was prepared by adding

the analyte and internal standard to the mobile phase; set B was prepared by adding the analyte and internal standard to the eluent after extracting the blank sample; and set C was prepared by extraction after adding the analyte and internal standard to the blank sample. The ratios of the peak areas obtained by analyzing the aliquots of each set were calculated to assess the matrix effect ($ME = B/A \times 100$), recovery ($RE = C/B \times 100$), and process efficiency ($PE = C/A \times 100$).

To measure the stability of the analytes in urine, repeated measurements were carried out on QC samples ($n = 5$) prepared with concentrations of 75 and 750 ng/mL for *d*-MA, *l*-MA, *d*-AP, and *l*-AP. For short-term stability, samples placed on the bench-top were assessed for 12 and 24 h, which corresponded to the conditions for applying the pretreatment and analyzing the samples. For long-term stability, the samples were compared with one another after cold storage at 4 °C within 20 days, which corresponded to the conditions for sample storage. The stability, which was similar to HPLC autosampler storage, was assessed by comparing the analysis results obtained from samples reinjected after they were stored at 20 °C for 12 h. Five replicates corresponding to each concentration of low and high QC samples were analyzed, and the results were compared against each concentration of QC samples that were analyzed initially. The analytes in urine were determined to be stable if the accuracy was within 85%–115% and the precision did not exceed 15%.

3. Results and Discussion

3.1. Sample preparation

SPE was applied to reduce the matrix effect in the samples during LC-MS/MS analysis. The SPE method is not only useful for eliminating interfering substances in the extract, but also offers the advantage of being able to obtain clean extracts.

Comparison of the chromatographic baselines of urine samples subjected to the dilute-and-shoot and SPE methods revealed that the baseline was lower in

the latter case. This suggested that SPE is more effective in minimizing matrix interference.

3.2. Optimization of HPLC conditions

To optimize the retention time and shape of the chromatographic peaks, the separation capability for the analytes was tested using different organic solvents. The separation was enhanced when methanol were used as the mobile phase. Moreover, isocratic elution and gradient elution with varying compositions of the mobile phases were applied. Stable results could be obtained under isocratic elution conditions, with a constant composition under the applied pressure.

Three different columns were selected for the separation and analysis of the enantiomers ChiroSil RCA(+) (4.6 mm × 150 mm, 5 μm), ChiroSil SCA(−) (4.6 mm × 150 mm, 5 μm), and Supelco Astec Chirobiotic V2 (2.1 mm × 150 mm, 5 μm) and their separation capability was compared. ChiroSil RCA (+) and SCA (−) gave similar results under the mobile phase conditions of 0.02 % ammonium hydroxide, 0.1 % acetic acid, 10 % distilled water, and 90 % methanol. Among the three columns, Chirobiotic V2

showed superior separation capability for *d*- and *l*-enantiomers. However, the present study did not employ separation columns filled with various types of chiral stationary phases. The use of such columns is limited due to cost implications, with chiral stationary-phase columns being more expensive than reverse-phase columns.

3.3. MS/MS analysis

The MS/MS parameters were optimized to achieve maximum analyte sensitivity. The retention time of the analytes was specified, and specific MRM ion pairs were selected for use in the analysis. The MS parameters for the analytes used in quantitative analysis are listed in *Table 1*, while representative LC-MS/MS chromatograms for *d*-MA, *l*-MA, *d*-AP, and *l*-AP are presented in *Fig. 2*.

3.4. Validation

To verify the validity of the chiral stationary phase LC-MS/MS technique, the selectivity, LOD, LLOQ, linearity, accuracy and precision, dilution integrity, matrix effect, recovery, process efficiency, and stability

Table 1. Retention times, MRM transitions and compound dependent parameters for LC-MS/MS analysis of the analytes and internal standards

Compound	Retention time (min)	MRM transitions		DP (V)	EP (V)	CE (V)	CXP (V)
		Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)				
<i>d</i> -AP	9.2	136.0	<u>91.0</u>	1	10	15	8
		136.0	119.1	1	10	8	6
		136.0	65.0	1	10	35	6
<i>l</i> -AP	10.3	136.0	<u>91.0</u>	1	10	15	8
		136.0	119.1	1	10	8	6
		136.0	65.0	1	10	35	6
<i>d</i> -MA	10.5	150.1	<u>91.0</u>	51	10	15	8
		150.1	119.1	51	10	10	8
		150.1	65	51	10	35	6
<i>l</i> -MA	11.6	150.1	<u>91.0</u>	51	10	15	8
		150.1	119.1	51	10	10	8
		150.1	65	51	10	35	6

DP: declustering potential.

EP: entrance potential.

CE: collision energy.

CXP: collision cell exit potential.

Product ion underlined was used for quantification.

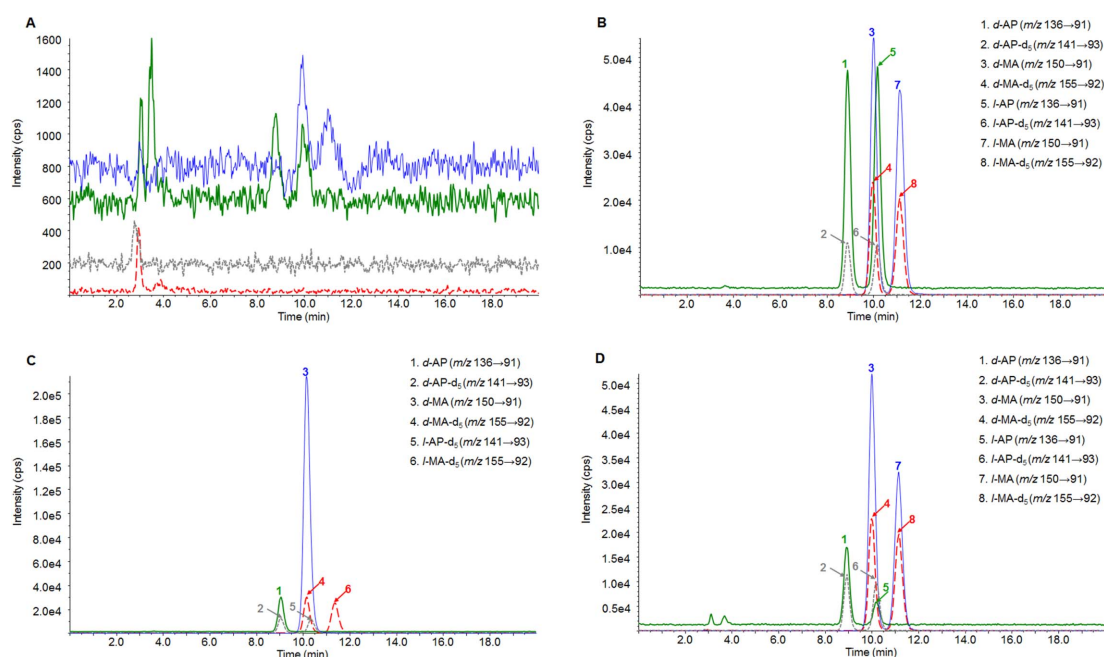


Fig. 2. Representative MRM chromatograms of (A) blank urine, (B) spiked urine containing 250 ng/mL of *d*-MA, *l*-MA, *d*-AP, and *l*-AP, (C) *d*-MA positive urine and (D) *d,l*-MA positive urine samples.

Table 2. Method calibration

Compound	Concentration range (ng/mL)	Weighting factor	Slope	y-Intercept	Linearity (r^2)	LOD (ng/mL)	LLOQ (ng/mL)
<i>d</i> -AP	25-1000	$1/x^2$	0.0087 ± 0.0002	-0.0144	0.9992	7.5	25
<i>l</i> -AP	25-1000	$1/x^2$	0.0087 ± 0.0001	-0.0193	0.9992	7.5	25
<i>d</i> -MA	25-1000	$1/x^2$	0.0040 ± 0.0001	0.0028	0.9994	2.5	25
<i>l</i> -MA	25-1000	$1/x^2$	0.0040 ± 0.0001	-0.0093	0.9992	3.5	25

Linearity is described by the coefficient of determination for the calibration curve.

Limit of detection (LOD) was based on the concentration corresponding to a signal plus 3 standard deviations above from the mean of ten replicates of blank urine.

Lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curve with precision (% CV) less than 20 % and accuracy (% bias) within ± 20 %.

were assessed. For the selectivity assessment, urine samples from non-drug users ($n = 6$) were analyzed, and the results showed that constituents that may influence the analysis of *d*-MA, *l*-MA, *d*-AP, and *l*-AP could not be identified.

The coefficient of determination (r^2) of the calibration curve with a weight coefficient of $1/x^2$ for the analytes was ≥ 0.999 , indicating acceptable linearity along the calibration range. The LOD and LLOQ were identified to be 2.5–7.5 ng/mL and 25 ng/mL, respectively (Table 2).

Table 3 shows the precision and accuracy of the analytical method. The intra- and inter-day precisions were within 3.6 % and 2.2 %, respectively. The intra- and inter-day accuracies were -5.4 % to 9.6 % and -4.7 % to 11.8 %, respectively. Both precision and accuracy showed favorable results with deviation within 15 % and CV within the range of -15 % to 15 %.

Based on the results of the dilution integrity experiment, the upper concentration limit of the analytes could be expanded up to 20000 ng/mL. When the

Table 3. Intra- and inter-day precision and accuracy

Compound	Nominal concentration (ng/mL)	Intra-day (n = 5)		Inter-day (n = 15)	
		Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
<i>d</i> -AP	25	2.1	5.0	0.9	3.9
	75	1.8	0.8	0.9	0.9
	250	0.7	-4.5	1.4	-3.4
	750	1.0	-5.4	0.6	-4.7
<i>l</i> -AP	25	3.4	-0.4	0.5	0.2
	75	1.3	-0.5	0.5	-0.2
	250	1.2	-5.0	1.5	-4.3
	750	0.9	-4.1	0.7	-4.2
<i>d</i> -MA	25	3.6	9.6	1.9	11.8
	75	1.7	2.2	2.2	3.9
	250	0.9	-1.9	1.3	-0.9
	750	1.8	-2.9	1.5	-1.4
<i>l</i> -MA	25	1.0	2.2	1.3	2.9
	75	1.6	1.7	1.2	3.0
	250	1.8	-1.1	1.4	-0.3
	750	1.3	-3.7	1.9	-1.7

Precision is expressed as the coefficient of variance (CV) of the peak area ratios of analyte/internal standard.

Accuracy is calculated as [(mean calculated concentration - nominal concentration)/nominal concentration] × 100.

Table 4. Dilution integrity

Compound	Concentration before dilution (ng/mL)	Dilution factors	Concentration after dilution (ng/mL)	Dilution integrity (n = 6)		
				Determined concentration (ng/mL)	Precision (% CV)	Accuracy (% bias)
<i>d</i> -AP	250	10	25.0	23.4	4.0	-6.6
		20	37.5	37.9	1.4	1.1
	750	10	75.0	76.1	3.4	1.5
<i>l</i> -AP	250	10	25.0	23.1	3.3	-7.6
		20	37.5	36.9	2.3	-1.6
	750	10	75.0	74.5	3.2	-0.6
<i>d</i> -MA	250	10	25.0	22.1	4.0	-11.7
		20	37.5	35.8	4.5	-4.6
	750	10	75.0	74.8	3.5	-0.2
<i>l</i> -MA	250	10	25.0	23.1	2.2	-7.7
		20	37.5	36.6	1.3	-2.4
	750	10	75.0	73.8	3.1	-1.6

QC samples with medium and high concentrations were diluted 10- and 20-fold, the results showed a precision of 1.3 %–4.5 % and accuracy of -11.7 % to 1.5 % (Table 4).

The matrix effect, recovery, and process efficiency of the analytes were measured. The results showed that the matrix effect in *d*-MA, *l*-MA, *d*-AP, and *l*-AP was 98.7 %–109.3 %, 100.0 %–105.6 %, 92.8 %–

Table 5. Matrix effect, recovery and process efficiency

Compound	Concentration (ng/mL)	Matrix effect (% mean, n = 5)	Recovery (% mean, n = 5)	Process efficiency (% mean, n = 5)
<i>d</i> -AP	25	92.8	113.5	105.3
	75	105.4	108.3	114.1
	250	100.7	104.7	105.4
	750	95.0	108.7	103.2
<i>l</i> -AP	25	95.2	114.6	109.1
	75	106.9	107.1	114.5
	250	101.4	103.8	105.3
	750	95.8	108.3	103.8
<i>d</i> -MA	25	98.7	110.8	109.3
	75	109.3	104.8	114.6
	250	102.2	103.1	105.3
	750	100.2	107.8	108.0
<i>l</i> -MA	25	100.0	114.6	114.6
	75	105.6	108.6	114.8
	250	100.2	104.6	104.8
	750	101.3	108.0	109.4

105.4 %, and 95.2 %–106.9 %, respectively. These results indicate that matrix effect remained consistent over the range of concentrations tested. The results for the recovery and process efficiency of each analyte are described in detail in Table 5.

The stability of the analytes in urine changed by < 9 % (short-term stability) upon storage of the samples for 24 h at room temperature and by < 13 %

(long-term stability) after storage for 20 days at 4 °C. The stability of processed samples in autosampler was ≤ 5 %. The results confirmed the sample stability during typical experimental conditions.

3.5. Quantitative analysis results and detection frequency

To prove method applicability to real-case urine

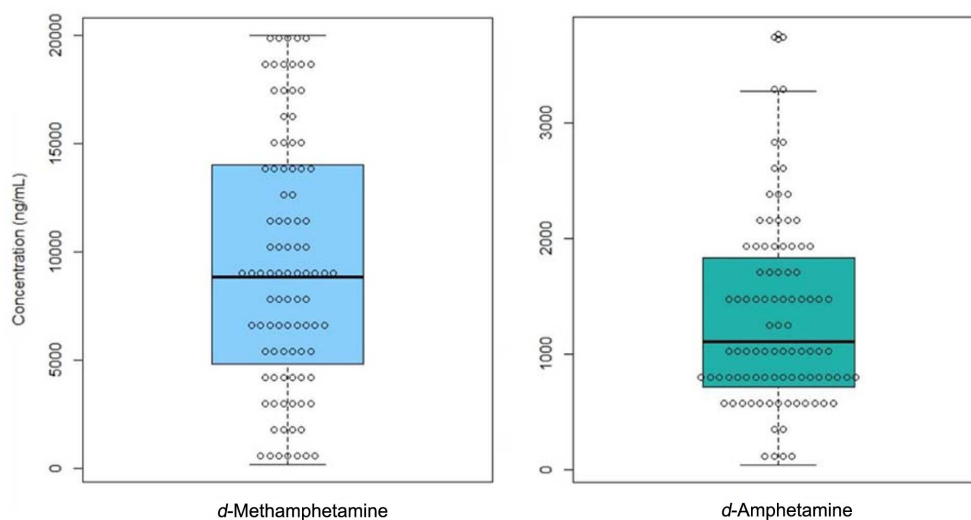


Fig. 3. Boxplot of the quantitative results for *d*-MA and *d*-AP in forensic urine samples (n = 93) obtained from drug abusers.

samples (n = 93), the method was used to analyze the urine samples, which tested positive for MA with an immunochemical analyzer (Cobas C311, Roche, Hitachi). As mentioned in the Introduction section, MA exists as two enantiomers (*d*- and *l*-MA), with *d*-MA known to be abused. However, when pharmaceuticals that are prodrugs of MA or AP, such as selegiline, are digested, *l*-MA and *l*-AP may be detected in urine. However, it is necessary to identify the MA enantiomer that was actually detected in urine samples tested positive for MA. Fig. 3 shows the distribution of the concentrations measured for *d*-MA and *d*-AP in urine. Figs. 2(C) and (D) show representative LC-MS/MS chromatograms of MA positive urine samples. Similar to the study by Li *et al.*, *d*-MA abuse was significantly higher in Korea. However, *d,l*-MA and *d,l*-AP were detected in urine samples from three subjects, which was suspected to be the result of abuse of the racemic form *d,l*-MA. The results of the enantiomer analysis performed on the confiscated MA confirmed the presence of the racemic form of MA. The results showed no cases with *l*-MA and *l*-AP, suggesting that the introduction of pharmaceuticals containing *l*-MA into Korea or the ingestion of pharmaceuticals that are precursors of *l*-MA or *l*-AP is uncommon. Based on this study, it was concluded that the analytical method developed is suitable for analyzing *d*-MA, *l*-MA, *d*-AP, and *l*-AP in urine and can clearly differentiate between the enantiomers of MA that are abused.

4. Conclusions

A chiral stationary phase LC-MS/MS method for the determination of *d*-MA, *l*-MA, *d*-AP, and *l*-AP in urine was developed. Quantitative reliability was assured, and matrix effects were not detected. The validity of this analytical method was verified by using it to analyze real-case urine samples. Interference from the chemical background noise was effectively eliminated by applying solid-phase extraction. Applying this analytical method to analyze urine samples obtained from 93 drug abusers, it was possible to perform

quantitative analysis on *d*-MA, *l*-MA, *d*-AP, and *l*-AP enantiomers with excellent separation capability without interference from other substances.

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