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Effective Monitoring for Enantiomeric Forms of Methamphetamine and Related Compounds by LC/MS

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Introduction

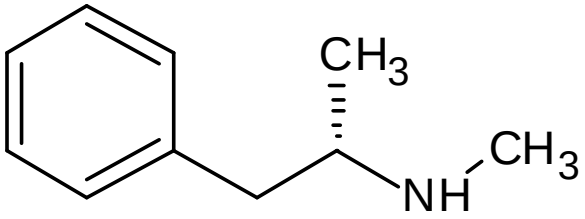
- Methamphetamine is a controlled substance (Class A in Europe and Schedule II in the USA). The R-isomer (levo) is used legally in several over the counter medicines (e.g., Vicks® Vapoinhaler) and so, can alter the true level arising from drug abuse.
- The **S-isomer (dextro)** of methamphetamine is the illicit simulant often abused for recreational purposes.
- Analysis is further complicated by the fact that R-methamphetamine is also a metabolite of certain therapeutic drugs such as selegiline, a compound used in the treatment of Parkinson's disease, depression and dementia.
- Immunoassay does not differentiate between the legal and illicit versions and therefore will report a positive finding if either are detected in the sample above cutoff concentrations. The same holds true for reversed-phase LC-MS techniques that are commonly used in toxicology for drugs of abuse quantitation.

Introduction (contd.)

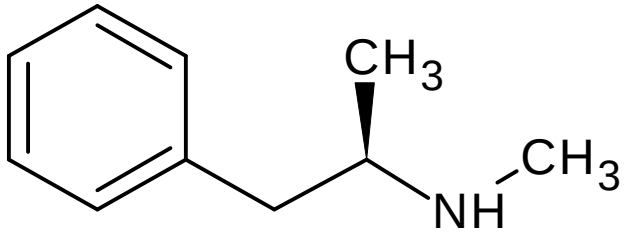
- The presented work describes the chromatographic screening procedure used on urine samples for methamphetamine. Sample recoveries and detection limits for the method are provided.
- The same method was also used to determine enantiomers of amphetamine in urine.

Experimental

Figure 1. Compounds Used in this Study



S(+) Methamphetamine (dextro)



R(-) Methamphetamine (levo)

LC Conditions Experiments-Optimum Mobile Phase

Initially, a study was undertaken to investigate the chromatographic results of different buffer additives for the chiral analysis of (+/-)-methamphetamine using a CHIROBIOTIC® V2 column.

In this experiment, two different mobile phases were evaluated based on previously reported work (1).

Buffer additives in 95:5, methanol:water

- 0.1% acetic acid, 0.02% ammonium hydroxide
- 0.05% ammonium trifluoroacetate

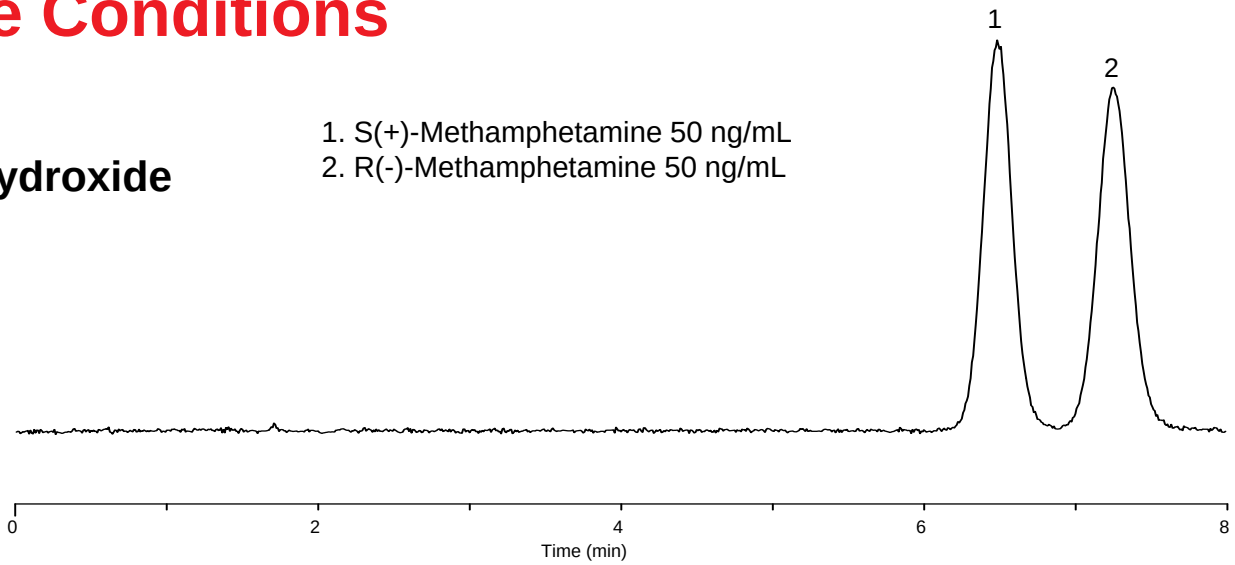
Results and Discussion

- Figure 2 shows two LC conditions for the separation. Both conditions provide baseline resolution of the enantiomers.
- The acetic acid/ammonium hydroxide mobile phase provides more retention and more resolution (resolution = 2.0) of the compounds as shown in Figure 2. Ammonium trifluoroacetate (ATFA) provides less retention and resolution (resolution = 1.7).
- ATFA is also less amenable to MS as it tends to precipitate over time, leading to a drift in retention time.
- There is an improved response as indicated by the greater signal to noise ratio, (50 vs 25) for the hydroxide vs ATFA, respectively).
- Therefore, the acetic acid/ammonium hydroxide mobile phase was used for the remainder of the study.
- The final optimized method conditions are presented in Figure 3.

Figure 2. Chromatograms to Determine Optimum Mobile Phase Conditions

0.10% acetic acid
0.02% ammonium hydroxide
resolution = 2.0
s/n = 50

1. S(+)-Methamphetamine 50 ng/mL
2. R(-)-Methamphetamine 50 ng/mL



0.05% ammonium trifluoroacetate
resolution = 1.7
s/n = 25

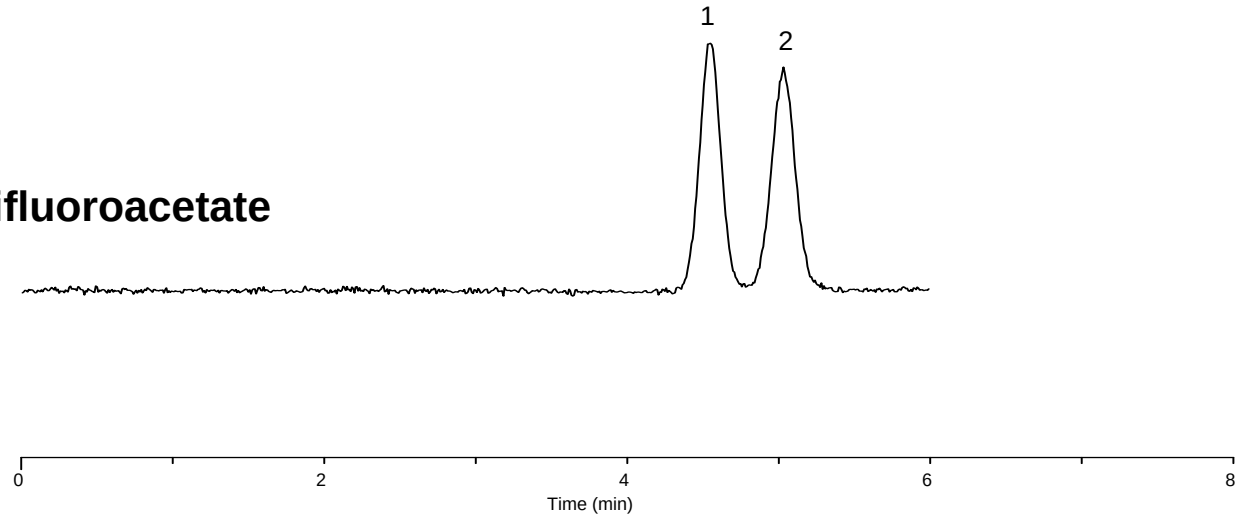


Figure 3. LC Chromatogram Using Optimum Conditions

column:	CHIROBIOTIC V2, 15 cm x 4.6 mm, 5 µm particle size (15023AST)
mobile phase:	95:5:0.1:0.02, methanol:water:acetic acid:ammonium hydroxide
flow rate:	1.0 mL/min
pressure:	1220 psi, 84 bar
temp.:	20 ºC
det.:	MS,SIR m/z 150.1
	injection: 2 µL
sample:	1. S(+)-Methamphetamine, 50 ng/mL
	2. R(-)-Methamphetamine, 50 ng/mL
instrument:	Waters Acquity QDa

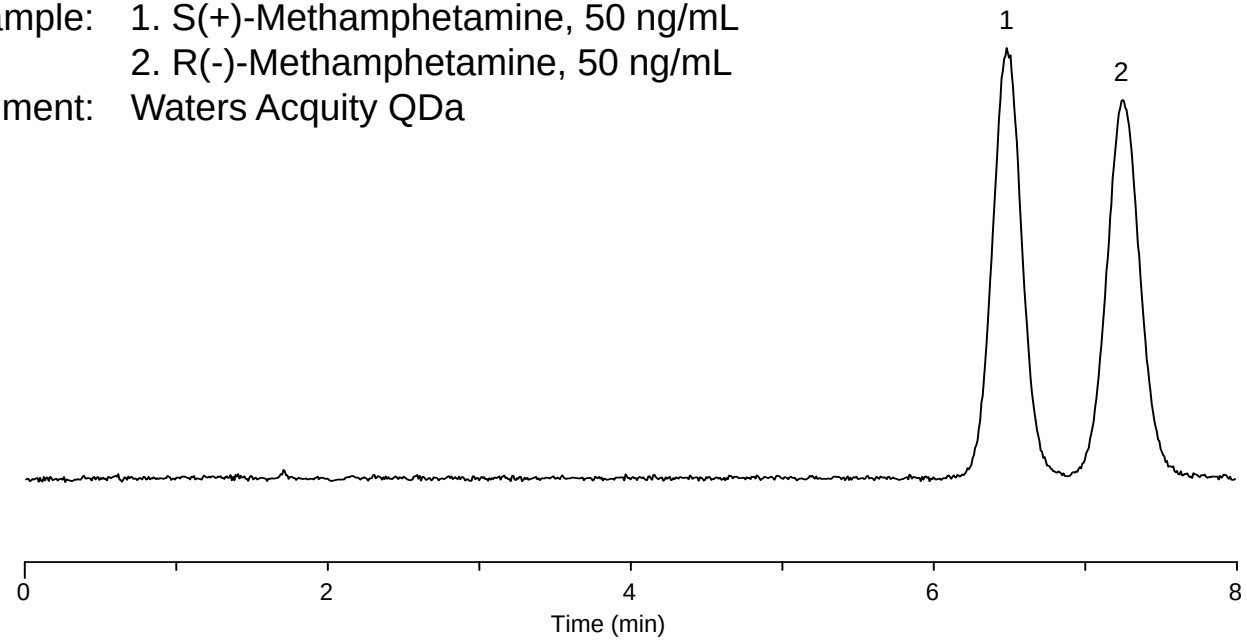
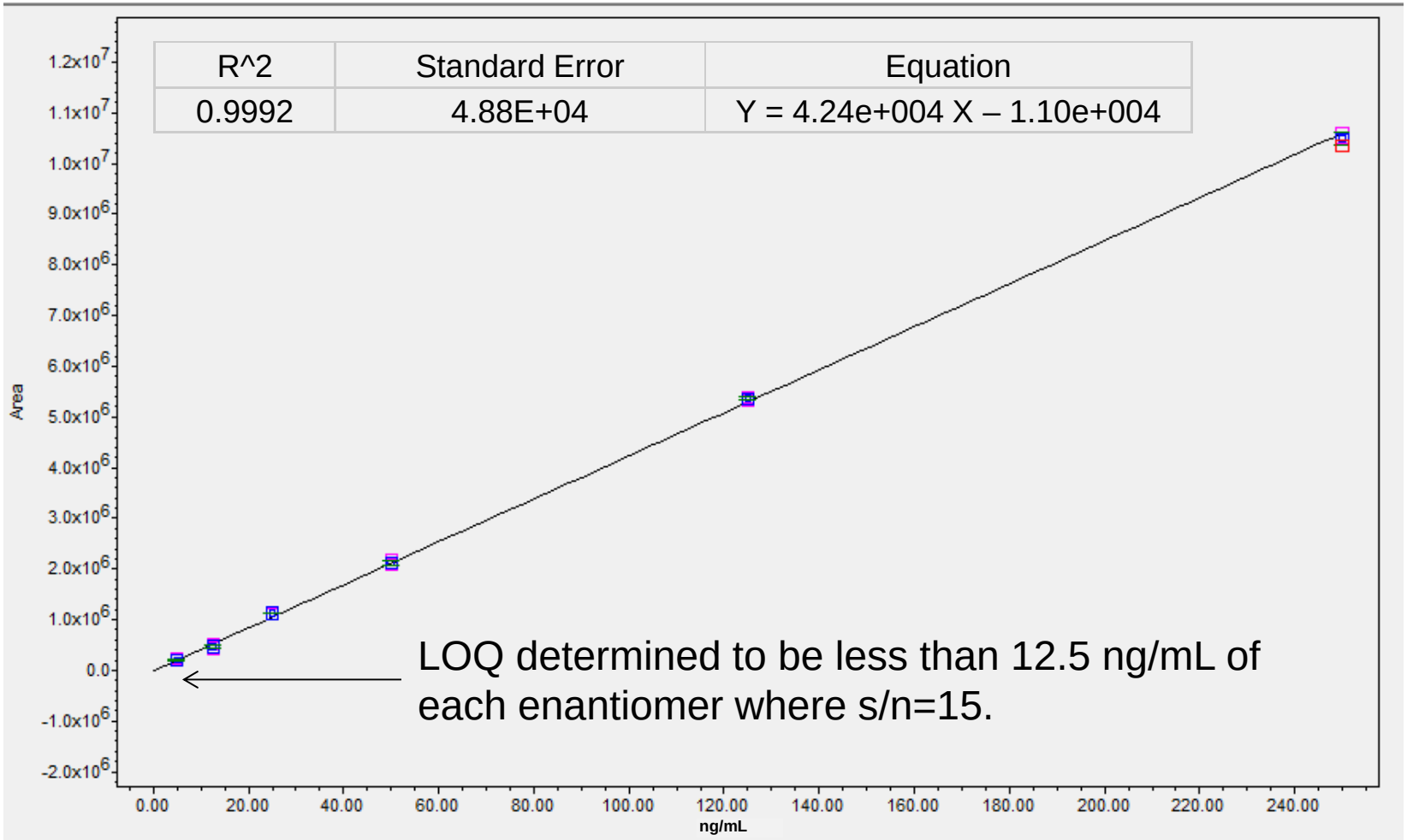


Figure 4 shows a curve for S(+)-Methamphetamine standard demonstrating a linear response in concentration, with a range of 5-250 ng/mL, (R^2=0.9992). The limit of quantitation (LOQ) was determined to be less than 12.5 ng/mL, using a simple single quadrapole mass spectrometer.

Figure 4. Calibration Curve and Determination of LOQ, S (+)-Methamphetamine Standard, Range= 5-250 ng/mL



Sample cleanup was initially done with a liquid/liquid extraction of a urine sample. The results yielded poor recovery (~66 %) of the (+)methamphetamine.

Based on prior experience, a generic sample preparation method was then used and is detailed below.

Generic Extraction Conditions

matrix:	1 mL of synthetic urine spiked with 50 ng/mL of each enantiomer, acidified to pH 3-4 with formic acid
SPE well plate:	Supel™ Select SCX SPE 96-well Plate, 30 mg/well (575664-U)
conditioning:	1 mL 1% formic acid in acetonitrile, then 1 mL water
sample addition:	1.0 mL urine sample
washing:	2 mL water, then 1 mL 25% methanol
elution:	1 mL 10% ammonium hydroxide in acetonitrile
eluate post-treatment:	evaporate to dryness under nitrogen at 40 ºC, reconstitute in 1 mL mobile phase

Figure 5 shows an overlay of a urine blank and a 25 ng/mL spiked sample extraction on the same y scale indicating a good sample cleanup procedure.

Note the small shoulder on peak 1 in the spiked extraction sample. This peak was not observed in the standards but is detected in the urine blanks.

Removal of this small interference is likely possible through improved extraction procedures or possibly by MS/MS for detection.

As shown in Table 1, suitable recoveries for both enantiomers were observed at the 25 ng/mL level; 82% and 72% for S and R, respectively.

Figure 5. Overlay Urine Blank and Spiked Sample, same scale, (25 ng/mL)

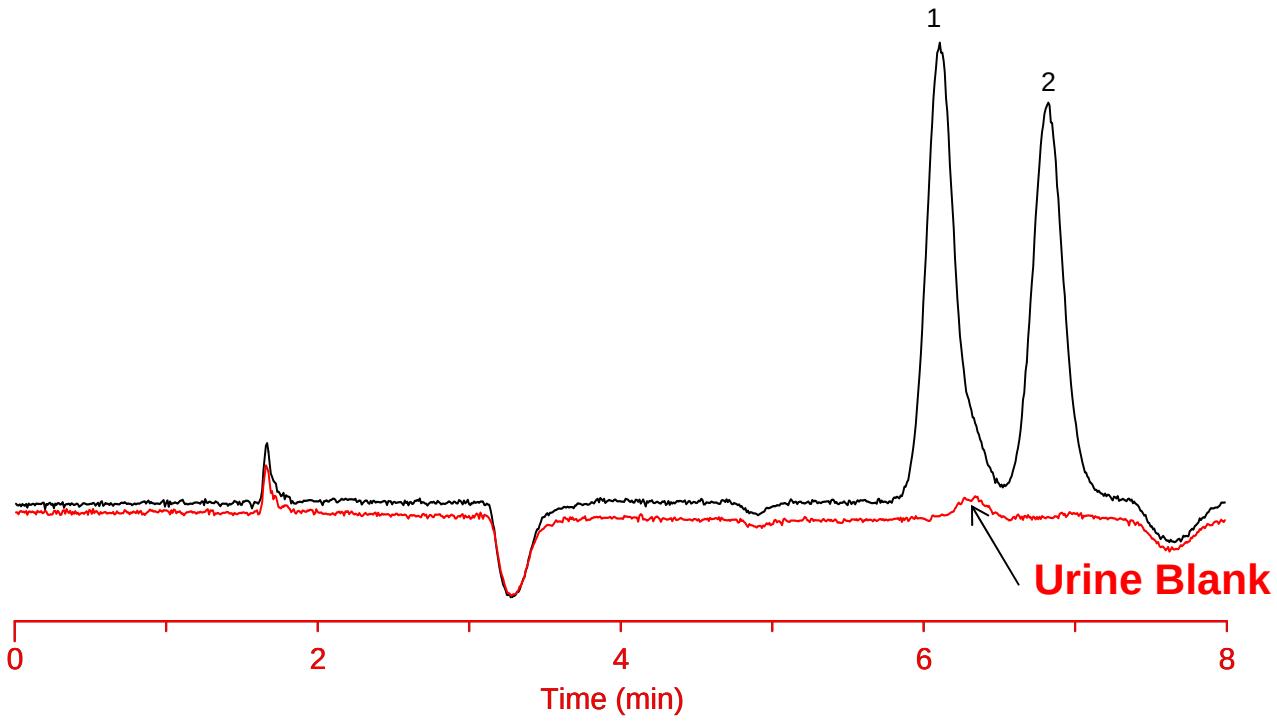


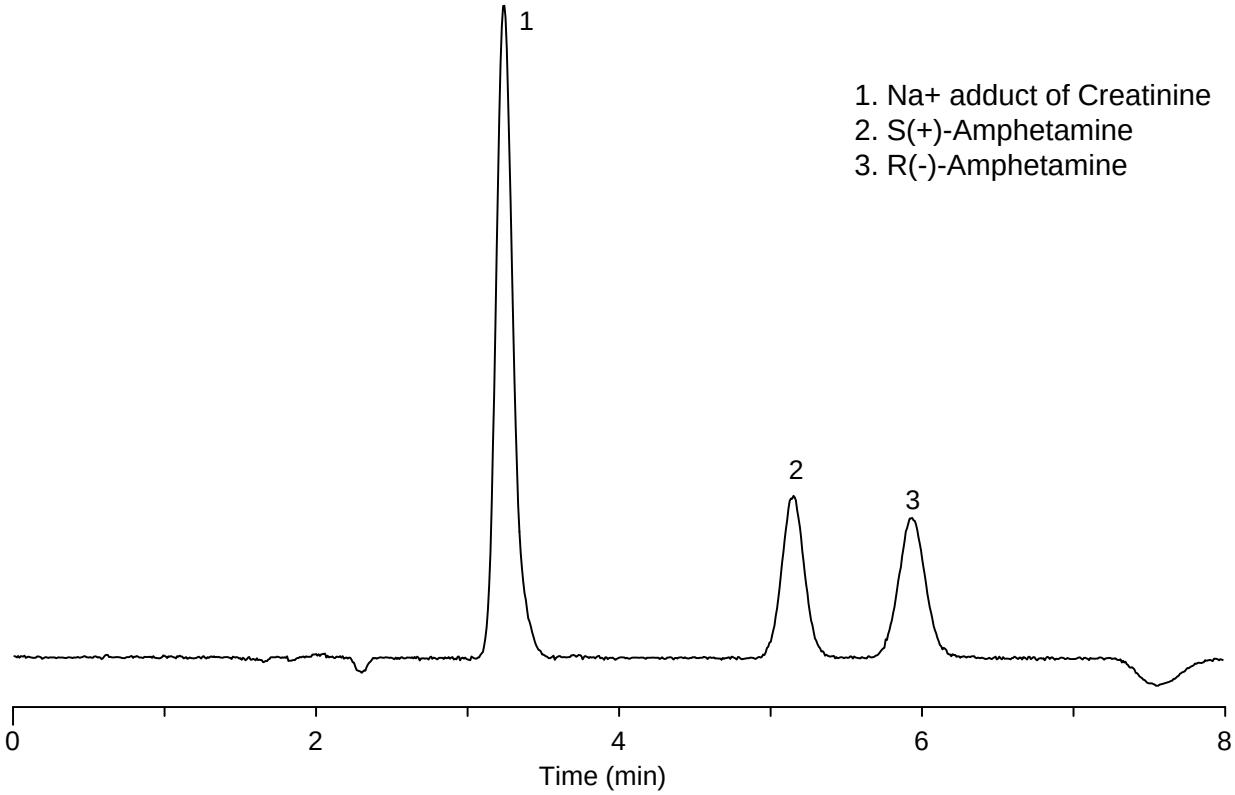
Table 1. Recovery (+/-)-Methamphetamine Using Supel Select SCX

	Spike (ng/mL)	Average Recovery n=3	% Recovery
S-(+)-Methamphetamine	25	20.7	82.8%
R-(-)-Methamphetamine	25	18.2	72.7%

In applying the same chromatographic conditions and extraction procedures to a related compound, a separation of the enantiomeric forms of amphetamine is shown in Figure 6.

Baseline resolution of the two enantiomers is observed without any interfering peaks. However, a large peak is detected in the chromatogram that has been identified as the sodium adduct of creatinine (peak 1) (m/z136). An additional washing step in the extraction procedure using ammonium phosphate, pH 8, resulted in significant removal of the creatinine response (data not shown).

Figure 6 . Enantiomeric Forms of Amphetamine Extracted from Urine (50 ng/mL)



Conclusions

A method for the determination of enantiomeric forms of methamphetamine and amphetamine from urine was developed. The choice of acetic acid and ammonium hydroxide as additives provided improved LOQ and suitable resolution. Sample preparation was shown to be optimal using SCX SPE material, although an additional wash step would be desirable to remove a low level interferent. Good linearity and recovery were demonstrated for methamphetamine after urine extraction.

The method is shown to be suitable for the differentiation of illicit and legal presence of methamphetamine in urine.

Reference

1. http://www.sigmaaldrich.com/technical-documents/articles/analytical/bioanalytical/chiral-lcms-methamphetamine.html – Reporter 31.2, Pages 24-25, 2013.

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