Methamphetamine and Amphetamine Drug Screening in Urine via Solid Phase Extraction

Application Note CL0213

Keywords

SPE (Solid Phase Extraction), Drug Screening, mAMP (Methamphetamine), AMP (Amphetamine), Urine Analysis

Introduction

Routine drug screening is often a hiring requirement for a new employee. Urine is the most common biological matrix sampled for testing of mAMP (Methamphetamine) and AMP (Amphetamine) concentrations, with a confirmation test indicating concentrations of ≥500 µg/L of mAMP and 200 µg/L of AMP. Urine testing is the preferred biological fluids because mAMP and AMP is detectable from one to seven days, depending on use.[2] This application note focuses on comparing LC-MS/MS results from drug screening method for detecting mAMP and AMP, along with additional common drugs detectable in urine, using four different SPE cartridge manufacturers to identify the cartridge producing consistent recoveries at the lowest detection level, as well as consistent recoveries at 50x the lowest detection level with associated ion suppression values.

Materials & Methods

Materials

- Automated Solid Phase Extraction:
  - ASPEC™ HLB 3 mL/60 mg
  - Gilson Part Number: 54350562

Sample Preparation

- 10 mL of urine was treated with 100 µL of TFA

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Solid Phase Extraction Steps

1. **Condition 1:** 3 mL of MeOH @ 8 mL/min
2. **Condition 2:** 3 mL of H_2O@ 8 mL/min
3. **Load:** 1 mL of prepared urine sample@ 2.5 mL/min
4. **Wash 1:** 3 mL (5/95) MOH/H_2O, 2% NH_4OH (v/v)@ 6 mL/min
5. **Wash 2:** 3 mL (20/80) MeOH/H_2O, 2% NH_4OH (v/v))@ 6 mL/min
6. **Wash 3:** 1 mL (80/20) MeOH/H_2O (v/v) @ 6 mL/min
7. **Elution Fraction 1:** 3 mL MeOH @ 3.0 mL/min
8. **Elution Fraction 2:** 3 mL 2% formic acid in MeOH @ 3.0 mL/min

Sample Reconsititution:

- Sample fractions were evaporated at 40°C for 20 minutes with nitrogen
- Evaporated fractions were reconstituted with 3 mL of the mobile phase solution

Chromatographic Conditions

- **Mobile Phase:** 0.800 mL/min
  - **A:** 1 mM ammonium formate in (70/30) MeOH/H_2O
  - **B:** 0.1% NaOH (v/v)
- **Column:** 4.6 x 50 mm C18, 5 µm @ 23°C
- **Detector:** Sciex API 3000
  - **Turbo Ion Spray Heater Gas Flow:** 8,000 cc/min
  - **Turbo Ion Spray Heater Temperature:** 350°C, ESI^+, MRM SCAN
- **Injection Volume:** 5 µL

Results

Analysis results from LC-MS/MS at the lower level of detection (5 ng/mL) yielded acceptable recovery results for the ASPEC HLB, competitor A, and competitor C cartridges, based on the recovery consistency for all compounds (Figure 1A). Competitor B recoveries resulted in significantly lower recovery for Amphetamine compared to the other three cartridge manufacturers at 5 ng/mL. Other recoveries at the same concentration were slightly lower for the remaining compounds.

Analysis results from LC-MS/MS at 50x the lower level of detection (250 ng/mL) yielded acceptable recovery results for the ASPEC HLB, competitor A, and competitor C cartridges, based on the overall recovery consistency (Figure 1B). The ASPEC HLB cartridge recoveries were consistently the highest across all compounds, with acceptable ion suppression values measured at 250 ng/mL (Table 1).

Figure 1: Recovery Results at 5 ng/mL (A) and Recovery Results at 250 ng/mL (B)

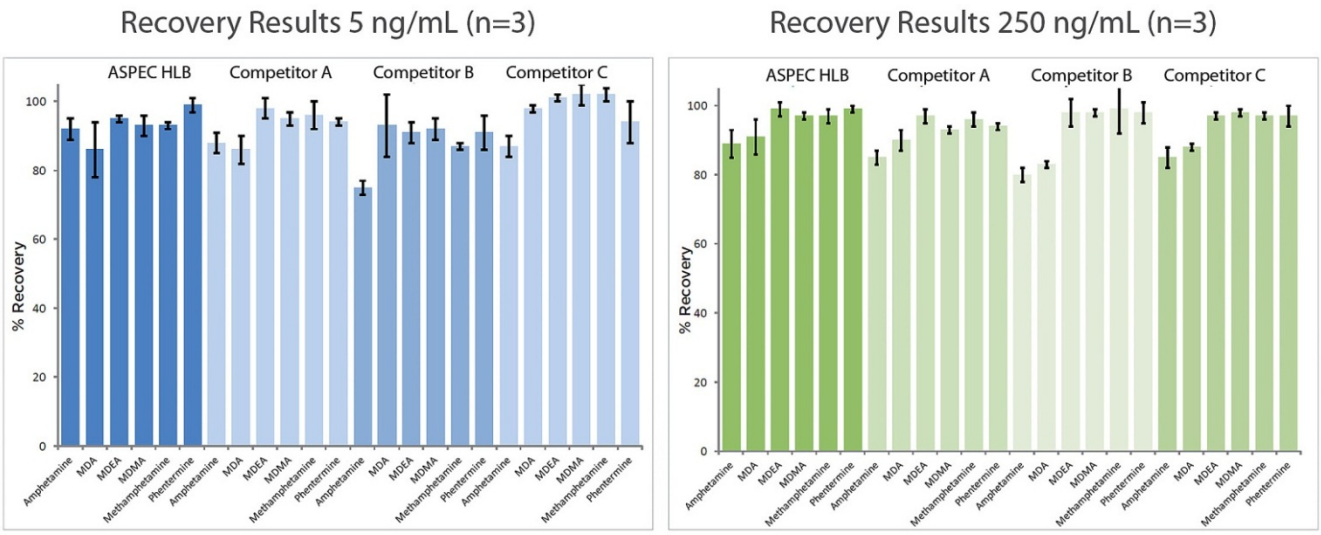

Table 1: Ion Suppression Results Expressed as a Percentage at 250 ng/mL

ION SUPPRESSION MEASURED AT 250 NG/ML (N=3)				
Compounds	ASPEC HLB (%)	Competitor A (%)	Competitor B (%)	Competitor C (%)
Amphetamine	-9	-4	-4	7
MDA	-7	-5	-5	3
MDEA	-6	-5	-4	-2
MDMA	-12	-11	-12	-10
Methamphetamine	-7	-3	-4	7
Phentermine	11	15	15	21

Conclusion

The LC-MS/MS analysis provided data at the lower level of detection and at higher levels (50x) to determine the acceptability of cartridge manufacturer based on recoveries and ion suppression values. Recovery results from the SPE method were acceptable for compound selectivity, which is a direct result of obtaining a clean SPE extract to inject. Ion suppression results were acceptable, confirming the clean extract was obtained prior to LC-MS/MS analysis.

References

1. Clinical Chemistry (2002). Duration of Detectable Methamphetamine and Amphetamine Excretion in Urine after Controlled Oral Administration of Methamphetamine to Humans. http://www.clinchem.org/content/48/10/1703.long
2. National Highway Traffic Safety Administration. Drugs and Human Performance Fact Sheets: Methamphetamine (and Amphetamine). http://www.nhtsa.gov/people/injury/research/job185drugs/methamphetamine.htm