Automated Extraction of 6-Monoacetylmorphine from Urine using VERSA 1100 SPE prior to GC-MS

The detection of 6-Monoacetylmorphine (6-MAM) in urine is considered a reliable marker of heroin use, making it a critical analyte for forensic toxicology laboratories. The manual extraction of 6-MAM from urine samples can be a time-consuming and labor-intensive process. In this application note, we describe an automated extraction method for 6-MAM from urine using VERSA 1100 solid phase extraction (SPE) workstation.

Introduction

6-MAM is a metabolite of heroin that is excreted in urine after heroin use. As a metabolite specific to heroin, 6-MAM is reliable indicator of recent heroin consumption, aiding in drug abuse investigations. A typical workflow for 6-MAM analysis involves SPE and Gas Chromatography-Mass Spectrometry (GC-MS). Manual SPE of 6-MAM from urine samples is laborious, time-consuming, and prone to variability due to human error. Consequently, there is a need for automated SPE technique that can enhance efficiency and reproducibility in forensic toxicology laboratories. By implementing the VERSA 1100 automated SPE system, forensic toxicology laboratories can benefit from improved workflow efficiency, reduced hands-on time, and enhanced reproducibility in the analysis of 6-MAM in urine samples.

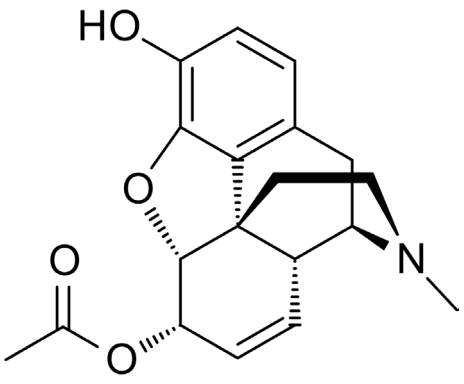

FIGURE 2. 6-MAM structure

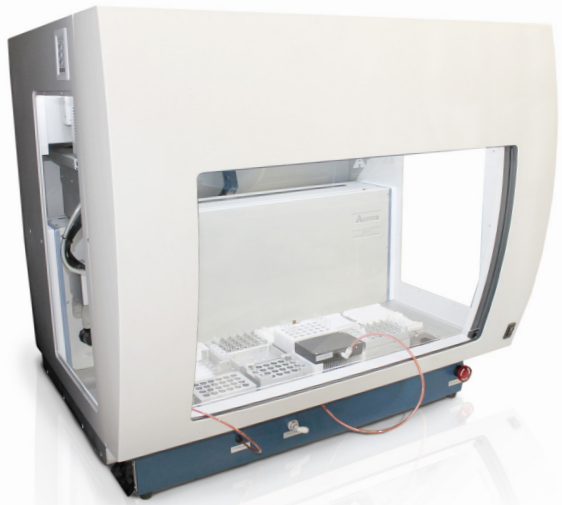

FIGURE 1. VERSA 1100 SPE

VERSA 1100 SPE FEATURES

- 4 or 8 channel pipetting head with single channel functionality offers enhanced versatility in liquid handling
- 5 to 8 channel ReagentDrop provides dispensing of bulk reagents without disposable pipette tips
- SPE decks compatible with 1, 3, and 6 mL cartridges as well as 96-well SPE cartridge plates
- Gripper transports modules for on-deck pressure application (positive or negative) and nitrogen drying
- Shaker-heater (RT to 90°C) for efficient sample pre-processing and derivatization
- UV HEPA filtered enclosure to minimize risk of sample contamination
- Open system allows for incorporation of third-party accessories and consumables
- Liquid-liquid extraction functionality available

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Workflow chart

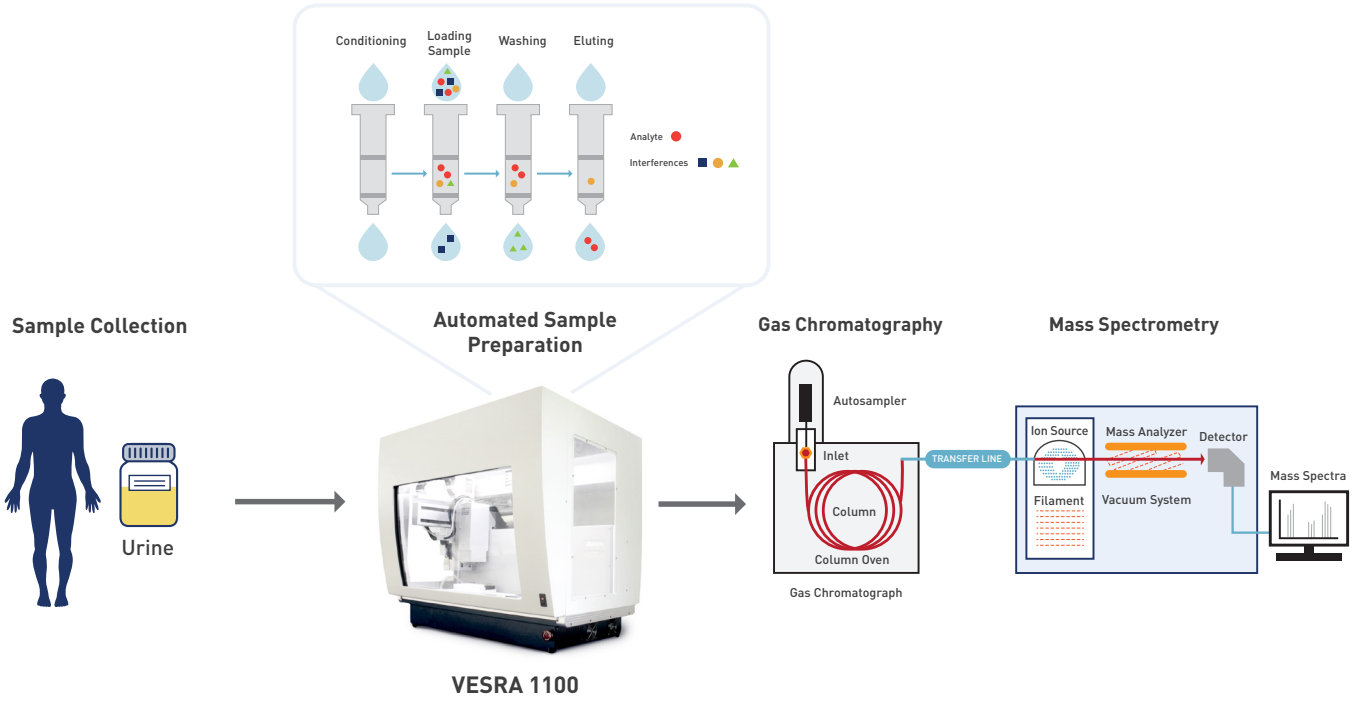

FIGURE 3. Workflow of 6-monoacetylmorphine analysis using VERSA 1100 SPE and GC-MS

Experimental

We used VERSA 1100 SPE equipped with a 96-well SPE module for the automated extraction of 6-MAM from urine samples. The SPE module used Agilent Bond Elut Certify cartridges with a 30 mg sorbent bed. The following protocol was used for the extraction:

1. Transfer 4 mL urine into a tube and add 2 mL of 0.1M pH 6.0 potassium phosphate buffer. Shake for 2 minutes.
2. Condition columns by sequential addition of 2 mL of ethanol, 2 mL of deionized water, and 2mL of the 0.1M pH 6.0 potassium phosphate buffer. Allow complete aspiration of each washing before adding the next and use minimal vacuum during washing.
3. Apply samples to columns without vacuum and the increase vacuum to a minimal rate to provide slow passage of samples through the column.
4. Rinse columns with 2 mL of 0.1 M pH 4.5 potassium acetate buffer.
5. Rinse columns with 2 mL of methanol.
6. Use vacuum to dry columns for 3 minutes.
7. Add 2 mL of freshly prepared elution solution (ethyl acetate, isopropyl alcohol, NH_4OH (18:12:4 v/v)) to each column and then increase the vacuum for an aspiration rate of 1 mL/minute.
8. Evaporate extracts to dryness with nitrogen at 40°C.
9. Add 50 uL toluene to dissolve the dried sample.
10. Transfer to a vial containing 100 uL insert.