GC/MS Analysis for Morphine and Other Opiates in Urine

GC/MS

Varian Application Note

Number 59

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Key Words: *Opiates, Urine, Drugs, Saturn*

Introduction

In regulated forensic urine drug testing (FUDT) morphine and codeine are the only opiate target analytes[1]. However, as a Clinical Toxicology Laboratory offering 24 hr/day services, we require a confirmation method which is robust and yields unambiguous chromatographic separations of all common opiate drugs. Derivatization by acetylation or TMS yields acceptable chromatography for FUDT analytes[2,3], but will not permit routine separation and MS identification of all the common opiates. Hydromorphone, hydrocodone and oxycodone upon heating during anhydride or TMS derivatization convert to their tautomeric enol form; thus, yielding a chromatographic doublet (one for the hydroxyl derivative and the other for the parent keto form of the underivatized drug). These compounds are difficult to resolve from similar structured opiates. Several different approaches have been applied to this problem with varying success including: TMS derivatization at room temperature[4], TMS-iodide derivatization[4,5] and reduction with sodium borohydride[6]. We have found a recently presented method involving the conversion of keto-opiates to their oxime derivatives during glucuronide hydrolysis with subsequent TMS derivatization to meet all our requirements for opiate GC/MS analysis[7]. Thus using a single extraction and single injection in GC/MS the following opiates are resolved and identified: codeine, morphine, hydromorphone, 6-monoacetyl-morphine, hydrocodone, and oxycodone.

Procedure

The sample preparation for analyzing opiates in urine involves alkaline extraction and derivatization. Add 2 mL of urine sample to a disposable test tube containing 1000 ng of Morphine d-3 and 1000 ng of hydromorphone d-3. Next add 500 µL of 2M acetate buffer pH 5.0 with 3.6 mg of β-glucuronidase (Patella vulgata, type L-II, powder). Cap the vial and vortex briefly. Place in a heater block for 2 hours at 55°C.

Cool the tube and add 200 µL of 10% Hydroxamine HCl (0.1 gm/mL in water). Return to the heating block for an additional 30 minutes. Cool and add 1 mL of saturated carbonate/ bicarbonate buffer (1:1, N:N, pH 9.5) and 3 mL of chloroform:2-propanol (9:1). Cap the vial and rotate mix for 5 minutes at 20 rpm. Centrifuge at 2500 rpm for 5 minutes. Aspirate the top aqueous layer, transfer the organic layer to a clean test tube and evaporate to dryness in a 40°C water bath under a constant stream of air. Derivatize the residue by adding 50 µL of BSTFA (N,O-bis(trimethylsilyl)-trifluoroacetamide) + 10% TMCS (Trimethylchlorosilane) to the tube, capping and heating for 30 minutes at 70°C. This sample is ready for GC/MS analysis.

Results

In order to positively identify opiates in real samples the spectral quality must remain consistent over a wide range of concentrations. Additionally, sensitivity is important since the spectral quality at the detection limit is often poor due to the matrix interference. The greater the signal-to-noise, the better the quality of the spectrum is at low concentrations. Figures 1 and 2 show the chromatographic results of a GC/MS analysis of a typical calibrator prepared at 300 ng/mL of each analyte in drug free urine.

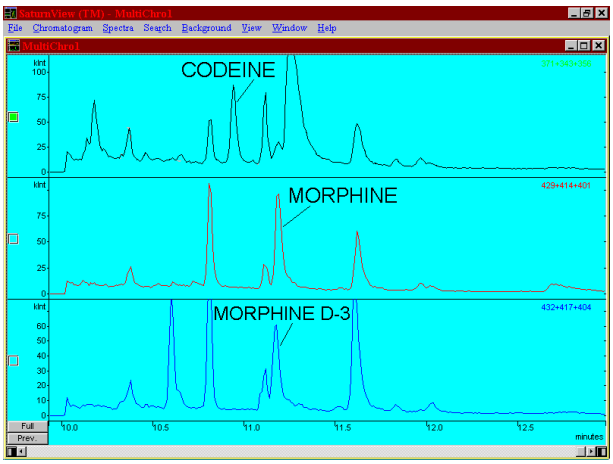

Figure 1: 300 ng/mL calibrator added to drug free urine.

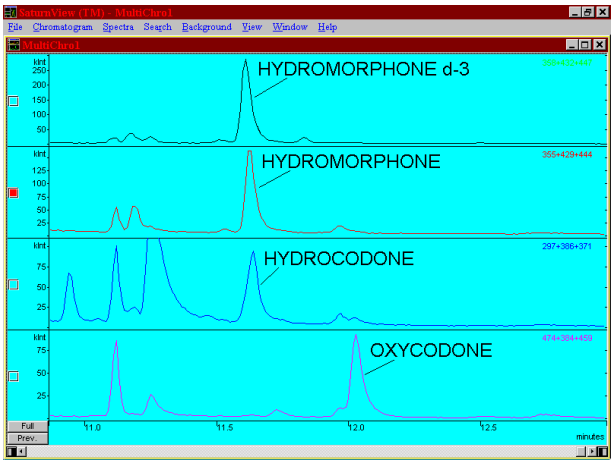

Figure 2: 300 ng/mL calibrator added to drug free urine.

Figures 3-6 we see the spectrum of the morphine calibrator, and three samples containing morphine at widely varying concentrations. Note the similarity of the spectra in all four analysis. By scanning a range of 200 ions more spectral information can be obtained and a more valid identification can be obtained than obtained by single ion or 3 ion monitoring.

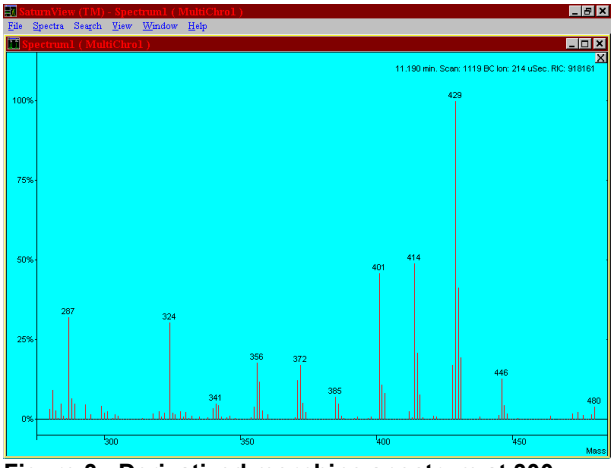

Figure 3: Derivatized morphine spectrum at 300 ng/mL added to drug free urine.

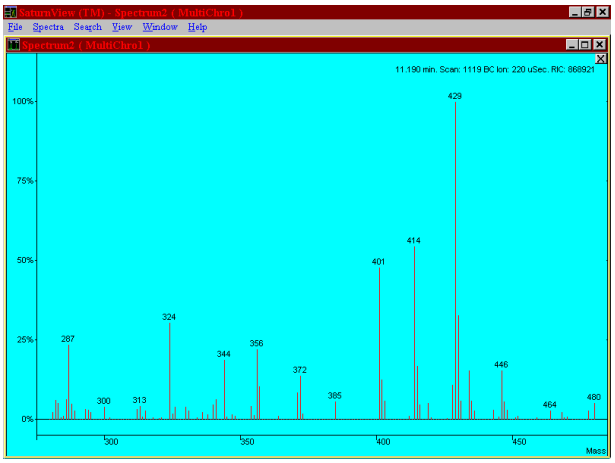

Figure 4: Derivatized morphine spectrum in urine sample at 150 ng/mL.

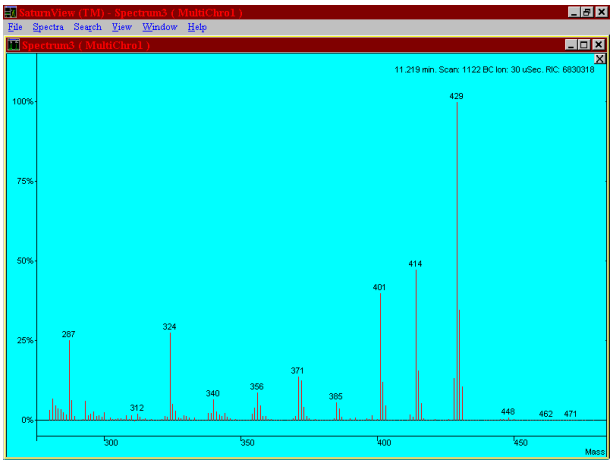

Figure 5: Derivatized morphine spectrum in urine sample at 1150 ng/mL.

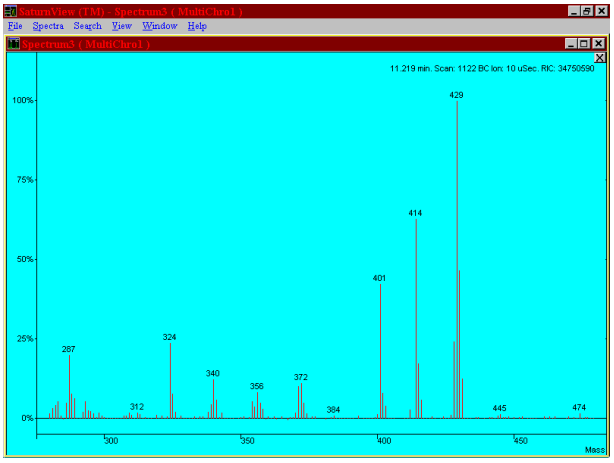

Figure 6: Derivatized morphine spectrum in urine sample at 4100 ng/mL

Instrumental

Gas Chromatograph
70°C and hold .1 minute, then heat at 25°C/min. to 280°C, and then 2°C/min. to 290°C.
Injection: 170°C, splitless for 0.7 minutes
Column: DB-5ms (J&W) 30m x 0.25 mm x 0.25µm
Transfer line: 280°C

Mass Spectrometer

Mass Range	280-480
Sec/scan	0.6
Filament	100 µ amps
Background mass	249
AGC Target	10000
Threshold	0 count
Ion trap temperature	170°C

Conclusion

The analysis of opiates in urine is a routine application in our laboratory using the above described procedure. A single point calibration is used to determine the concentration of various opiates in the samples. The data quality is reliable and accurate for both quantitative and qualitative analysis.

References

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