Clinical Forensic Applications note

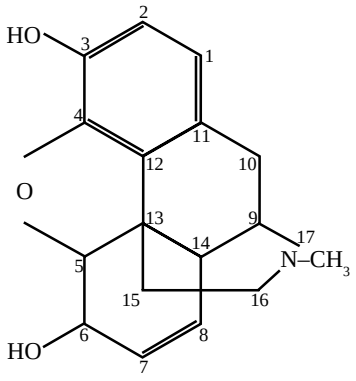
cat.# 59576

Opiate Analysis

Opiates or opioids are terms that classify a group of compounds with morphine-like actions. Their pharmacological properties include analgesia or pain relief, drowsiness and respiratory depression. **Figure 1** shows the structure for morphine. Substitutions at the 3, 6, and 17 positions produce compounds with varying degrees of potency and pharmacological activity. The National Institute for Drug Abuse

Figure 1

Morphine Structure



(NIDA) has targeted opiates as a class to be monitored in urine for detection of drug abuse. Testing guidelines have been established with a limit of detection of 0.3µg/ml for morphine. Screening of opiates is commonly done by using enzyme immunoassays. Enzyme immunoassays have the ability to cross react with a number of structurally similar opiates including codeine, hydromorphone, hydrocodone, levorphanol, and oxycodone. In order to differentiate between all of the possible substances being detected by enzyme immunoassay, confirmational analysis by GC/MS should be performed.

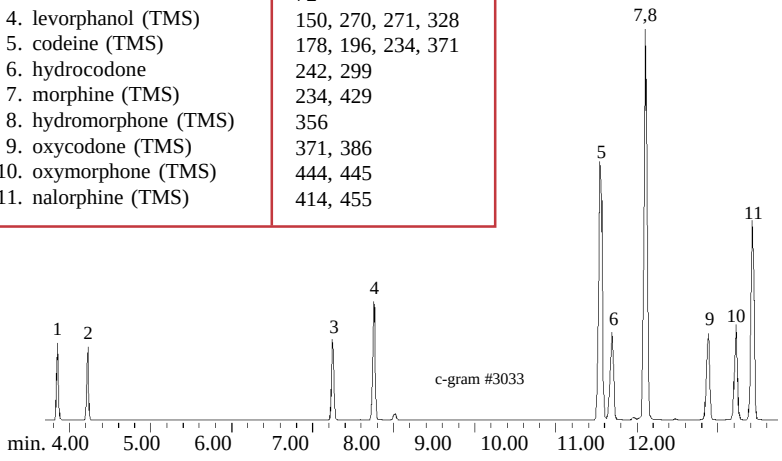
Chromatographic performance of the opiates is significantly affected by small changes in their chemical structure. The presence of hydroxyl groups at the 3 and 6 positions produce compounds that are more polar and reactive. Compounds with reactive hydroxyl groups in their chemical structure can suffer from adsorption and peak tailing, leading to diminished response in chromatographic systems that contain active sites. Sample preparation of sensitive compounds, like opiates, should take place in silanized glassware and samples should be stored in deactivated sample vials. Derivatization of reactive hydroxyl groups can improve chromatographic performance and detection limits and prevent sample loss on glassware and sample vials. Both trimethylsilyl and fluoroacyl derivatives of the opiates yield end products that are less polar and/or more volatile than the underivatized compound.

For this analysis, trimethylsilyl derivatives were prepared using BSTFA with 1% TMCS. Derivatizing the reactive hydroxyl group with a less polar trimethylsilyl group eliminates the tailing peaks commonly seen with compounds like morphine. **Figure 2** shows the analysis of a selection of opiates on an Rtx®-5 column. Compounds that have been derivatized prior to analysis are designated as TMS in the peak list. The TMS derivatized

Compounds	Ions Monitored
1. meperidine	71, 246
2. alphaprodine	172, 187
3. methadone	72
4. levorphanol (TMS)	150, 270, 271, 328
5. codeine (TMS)	178, 196, 234, 371
6. hydrocodone	242, 299
7. morphine (TMS)	234, 429
8. hydromorphone (TMS)	356
9. oxycodone (TMS)	371, 386
10. oxymorphone (TMS)	444, 445
11. nalorphine (TMS)	414, 455

Figure 2

Opiates analysis on an Rtx®-5 column.



30m, 0.25mm ID, 0.25µm Rtx®-5 (cat.# 10223). 2.0µl split injection of opiates.
Oven temp.: 200°C to 325°C @ 7°C/min.; **Inj./det. temp.:** 250°C/300°C;
Carrier gas: helium; **Linear velocity:** 30cm/sec. set @ 200°C;
Split ratio: 50:1 **Ionization:** EI **Mode:** SIM

opiates chromatograph well on a low polarity (Rtx®-5) column with good resolution and peak shape.

Sensitivity and specificity in confirming the presence of opiates in different samples can be enhanced by selectively choosing certain ions to monitor. Identification based upon the presence of distinctive, high mass ions is preferred, especially when analyzing derivatized compounds. Trimethylsilyl derivatives will add 72 amu for every hydroxyl group derivatized.

Effective protocols for opiate analysis include extensive sample preparation and optimized instrument parameters. Derivative formation and the use of deactivated glassware, sample vials, and inlet liners will ensure maximum recoveries and response. Optimized detector parameters using selected ions for detection will aid in the identification of different compounds.

Product Listing:
Rtx®-5
30m, 0.25mm ID, 0.25µm (cat.# 10223)

	Description	Concentration	cat.#
Pharmaceutical standards All standards are diluted in methanol. Packaged 1ml per ampul.	codeine	1000µg/ml	34000
	hydrocodone	1000µg/ml	34002
	hydromorphone	1000µg/ml	34063
	levorphanol	1000µg/ml	34003
	meperidine	1000µg/ml	34004
	methadone	1000µg/ml	34005
	morphine	1000µg/ml	34006
	oxycodone	1000µg/ml	34007
	oxymorphone	1000µg/ml	34065

For a complete list of Restek's Quantitative Drug Standards, please request a copy of our General Catalog.