Detection of Morphine and Morphine-Glucuronide in Saliva and Urine with a Single, Rapid, LC Ion Trap Method Without Derivatization

Application Note

Drugs of Abuse Testing

Author

Jason S. Wood
Agilent Technologies, Inc.
5301 Stevens Creek Boulevard
Santa Clara, CA 95051
USA

Abstract

Rapid (5 min), dilute-and-shoot method for the determination of morphine and one of its major metabolites (morphine-glucuronide) in biological fluids (saliva and urine) using the Agilent 500 Ion Trap LC/MS. It does not require lengthy derivatization processes and can be used in a high-throughput screening environment.

Introduction

Drugs of abuse (DoA) testing is becoming more prevalent in many environments as well as for participants in sporting events. Add to the already existing markets of forensics and social justice, a steady increase in DoA testing is evident in several markets worldwide. Non-heroin opiates such as morphine, oxycodone, and hydrocodone are appearing increasingly in drug indicator data. A US Department of Justice report from the Bureau of Justice Statistics in 2004 showed that the top four drugs utilized by high school seniors (and the percentage of those reporting their use in the last 12 months) were: 1) Alcohol (70.6%), 2) Marijuana (34.3%), 3) Stimulants (10.0%) and 4) Non-heroin Opiates (9.5%) [2]. Moreover, the US Drug Enforcement Administration (USDEA) reports that since 1990, there has been about a 3-fold increase in morphine products in the United States [3].

This application note describes a rapid (5 min), « dilute-andshoot » method for the determination of morphine and one of its major metabolites (morphine-glucuronide) in biological fluids (saliva and urine). It has the advantage that it does not require lengthy derivatization processes (utilized in GC/MS) and, because of its speed, can be used in a high-throughput screen environment. It utilizes the advanced features of the Agilent 500 Ion Trap to divert salts and contaminating proteins away from the mass spec ion source and includes Agilent's OnTrak OraTube as a sampling mechanism for the saliva.

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Instrumentation

- The Agilent 500 Ion Trap LC/MS equipped with an ESI source
- Agilent ProStar 210 Solvent Delivery System (2)
- CTC Analytics HTS PAL AutoSampler

Materials and Reagents

All chemicals were reagent or HPLC grade from Sigma-Aldrich (St. Louis, MO) with the exception of the morphine and morphine-glucuronide (Cerilliant, Little Rock, TX). Drugs of abuse urine control were obtained from Utak Laboratories, Inc (Valencia, CA). OnTrak OraTube was obtained from the local Agilent sales office.

Sample Preparation

All stock solutions were prepared in 50:50 methanol:water at 1 mg/mL. All dilutions were prepared in 5% aqueous methanol + 0.1% acetic acid.

Agilent OnTrak OraTube (Cat# 6902051) was used to obtain a saliva sample and was used according to instructions.

Utak Laboratories drugs of abuse urine standard (Cat# 98815) was prepared, according to instructions, by adding 10 mL of HPLC grade water to the dried samples provided.

All saliva and urine samples were diluted 1:10 with 5% aqueous methanol containing 0.1% acetic acid.

Conditions

Mass spectrometry conditions

Ionization mode	ESI (positive)
Isolation window	5
API drying gas	37 psi at 350 °C
API nebulizing gas	45 psi
Data rate	0.45 Hz
Mulitplier offset	200
Needle	5000 V
Capillary	100 V
RF storage	(See 500 Ion Trap segment parameters)
Shield	600 V
Damping gas	0.8 mL/min
Excitation time	20 msec
Waveform	Resonant

HTS CombiPAL conditions

Injection mode	LC
Read bar code	Never
Required syringe	100 µL liquid
Pre-inj washes solvent 1	0
Pre-inj washes solvent 2	2
Pre-inj sample flushes	0
Sample vial penetration depth pct	90%
Plunger fill speed	5.0 µL/sec
Fill strokes	0
Viscosity delay	0.3 sec
Air volume below sample	0 µL
Injector	LC Vlv1
Pre-injection delay	0.5 sec
Plunger inject speed	5 µL/sec
Post-injection delay	0.5 sec
Post-inj washes solvent 1	0
Post-inj washes solvent 2	3
Post-inj valve washes solvent 1	0
Post-inj valve washes solvent 2	2
LC cycle time (prep ahead)	OFF

LC conditions

Column	Agilent Pursuit C18 3 mm 100 × 2 mm		
Solvent A	0.1% acetic acid in water		
Solvent B	0.1% acetic acid in methanol		
Flow Rate	0.2 mL/min		
Injection volume	10 µL		
LC program	Time	%A	%B
	0:00	95	5
	0:30	95	5
	1:30	95	5
	2:00	57	43
	3:45	5	95
	4:30	5	95
	4:45	95	5
	5:00	95	5

500 Ion Trap segment parameters

#	Name	Time (min)	Precursor	Ex stor	Ex amp	Prod ion start	Prod ion end	RF load
1	Divert	0–1	NA	NA	NA	NA	NA	NA
2	Mor	1.01–2	286.1	94.5	2	95	296	85
3	MGluc	2.01–5	462.2	155.7	1	200	350	60

Discussion

The structures of morphine and one of its metabolites are shown in Figure 1. Glucuronidation (catalyzed by uridineglucuronic transferase or UGTs) is a Phase II metabolism reaction and involves the addition of glucuronic acid from uridinediphosphoglucuronide to the xenobiotic being metabolized. In general, morphine metabolism is undertaken by UGT2B7.

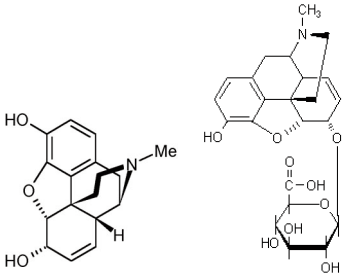

Figure 1. Structures of morphine and morphine-3-glucuronide.

Typically, simple onsite kits such as the Agilent OnTrak TesTcard perform preliminary screening for morphine usage, either on a random basis or as an incident-driven event. However, to eliminate false positives a second, more specific chemical method, such as GC/MS, is used as a confirmatory test. However, GC/MS methods require the use of solid-phase extraction and derivatization with pentafluoropropionic anhydride/pentafluoropropanol.

Figures 2 and 3 are the calibration curves for morphine and morphine-glucuronide, respectively. Curves were determined to be linear in the range of 20 to 1000 pg (six points, triplicate runs) for morphine and 40 to 1000 pg (five points, triplicate runs) for morphine-glucuronide. Limits of detection and quantification are 10 and 20 pg (respectively) for morphine and 40 and 60 pg (respectively) for morphine-glucuronide. These limits are well within the limits required by the National Institute of Drug Abuse (NIDA) for opiates in saliva and urine which are 40 ng/mL and 300 ng/mL, respectively.

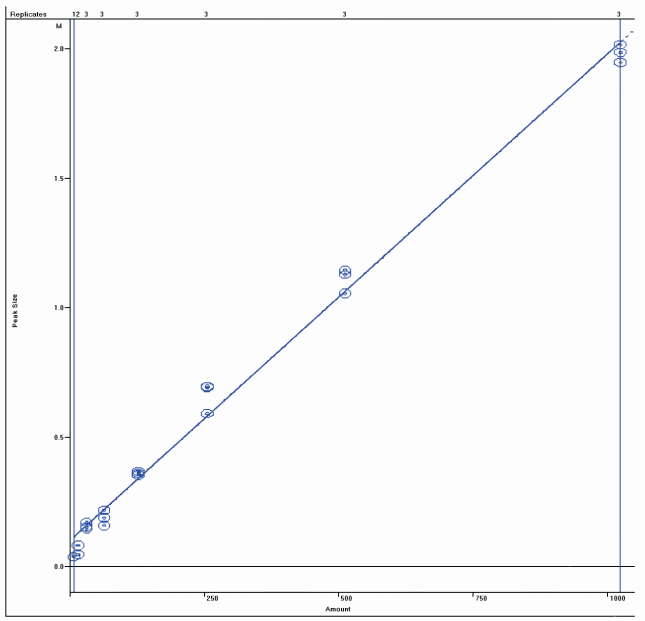

Figure 2. Morphine calibration curve (triplicate runs).

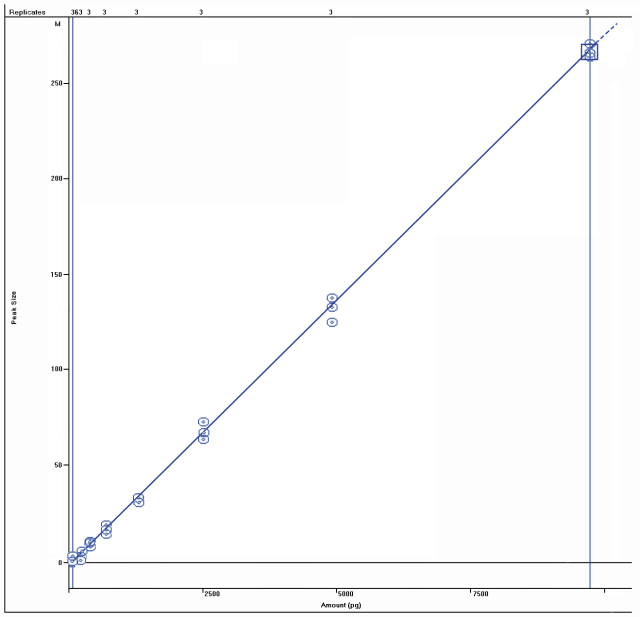

Figure 3. Morphine-glucuronide calibration curve (triplicate runs).

Figure 4 shows the separation of morphine that was added to a sample of saliva taken with the OraTube sampler. Panel 1 shows the separation of morphine (arrow marked B) from the salts and matrix interfering peaks (arrow A). These peaks are also removed when compared to a saliva sample further spiked with morphine (for confirmation of its identity) but run with the 6-port valve of the Agilent 500 Ion Trap LC/MS plumbed as a diverter valve. Figure 5 shows the configuration used to make the valve into a diverter instead of an injection valve.

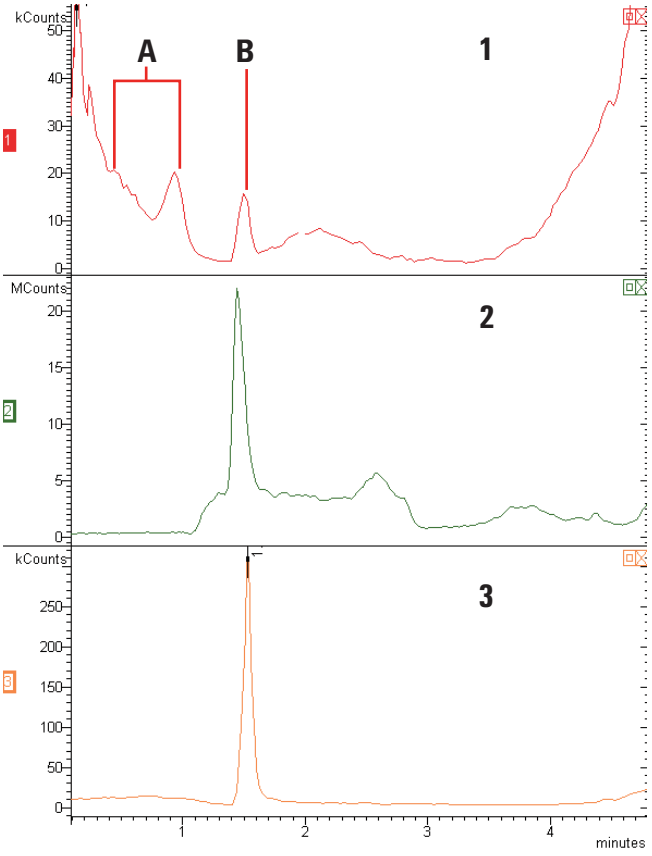

Figure 4. Detection of morphine in saliva. 1) morphine in saliva (10 ppb), no diverter valve, 2) spiked (1 ng) sample from above with diverter valve plumbed in, 3) morphine standard. 1A shows matrix interference that is diverted away from the source by use of the diverter valve (Panel 2).

Use of the diverter valve feature is also important for the samples containing urine. Urine contains a large amount of salt that could potentially damage the electrospray needle if injected directly into the MS. To demonstrate this, an identical sample was analyzed by both MS and UV detectors. Figure 6 shows the detection of morphine-glucuronide in urine as detected by the Agilent 500 Ion Trap LC/MS with the diverter valve, the expanded region on top shows the same sample as detected by UV/Vis at 214 nm.

The bottoming out of the chromatogram (at approximately 0.6 min) is the sample void caused (usually) by a high salt concentration eluting from the LC column.

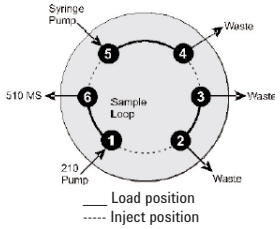

Figure 5. Plumbing to make the 500 Ion Trap front panel valve a diverter valve.

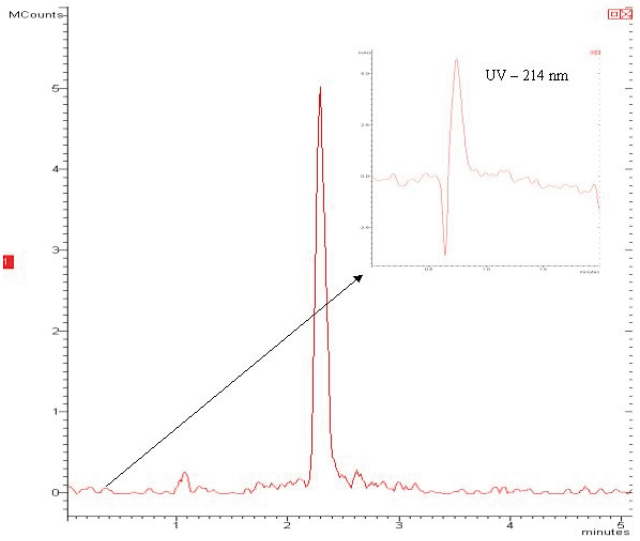

Figure 6. Overlay of morphine-glucuronide detection with both the 500 Ion Trap and a UV/Vis detector (expanded region, above).

Conclusion

This application note demonstrates the picogram detection, without derivatization, of morphine and its metabolite morphine-glucuronide in saliva and urine. The limit of quantitation for these analytes is well within the limits set by NIDA and SAMHSA for the detection of opiates in saliva and urine. More importantly, this method does not require lengthy derivatisations with pentafluoroproprionic anhydride as needed in a GC/MS method. All compounds were found to be linear in the calibration range tested and all had very good correlation coefficients (average r^2= 0.989).

This application demonstrates two unique features of the Agilent 500 Ion Trap LC/MS, the use of the built-in 6-port injection valve and the syringe pump. Together they can be used as a diverter valve to avoid potential damage to the electrospray interface by diverting salts away from the source and applying a make-up volume of solvent.

Finally, it should be noted that this method does separate both of the major metabolites of morphine, morphine- 3-glucuronide and morphine-6-glucuronide, if required. Therefore it was found that most, if not all, of the Utak urine standard was found to be morphine-3-glucuronide, consistent with the information provided in their catalog.

References

1. NIDA InfoFacts – Sept. 2004 (www.drugabuse.gov)
2. US Dept. Of Justice – Bureau of Justice Statistics (www.ojp.usdoj.gov/bjs/dcf/du.html)
3. DEA Briefs and Background, Drugs and Drug Abuse (www.usdoj.gov/dea/concern/morphine.html)

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