



# Quantitative Analysis of Opiates in Urine Using Accurate Mass LC/MSD TOF

## Application

## Forensics

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### Abstract

**Urine samples were quantitatively analyzed at the 6 ng/mL level using liquid chromatography/mass selective detector time-of-flight. The advantage of accurate mass measurement to enhance selectivity is presented. The instrumental detection limit is 2 pg on-column with a signal/noise ratio of 5:1.**

### Introduction

Until now quantitative time-of-flight (TOF) has not been broadly applicable. This application demonstrates that the Agilent liquid chromatography/mass selective detector time-of-flight (LC/MSD TOF) can routinely quantify compounds at low levels in matrices important to the forensic

scientist. Both direct injection of urine and solid phase extraction (SPE) are performed to demonstrate the robustness, sensitivity, and selectivity of the LC/MSD TOF.

### Experimental

#### Sample preparation

Direct injection samples were spiked at the specified concentrations with no further handling. Accubond II Evidex SPE Cartridges (part number 188-2946) were used as per extraction protocol for opiates (see step-by-step instructions that comes with cartridges). Five milliliters of either blank or spiked urine was treated with 0.5 mL concentrated HCl, 0.75 mL 10 N NaOH, and then adjusted to pH 6.5-7.5 with 2.5 mL 0.5 M phosphoric acid. The heating step was not included because acid hydrolysis of glucuronides were not expected. After conditioning, this solution was loaded onto the cartridge, rinsed, and then eluted with the prescribed solution of methylene chloride/isopropanol/ammonium hydroxide. The eluant was taken to dryness with nitrogen (no heat) and then reconstituted in 0.5 mL 40:60 water:acetonitrile.



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## Instrument

Agilent 1100 Series LC/MSD TOF with Agilent 1100 binary pump and well plate autosampler

**Table 1** Experimental Conditions

### LC Conditions

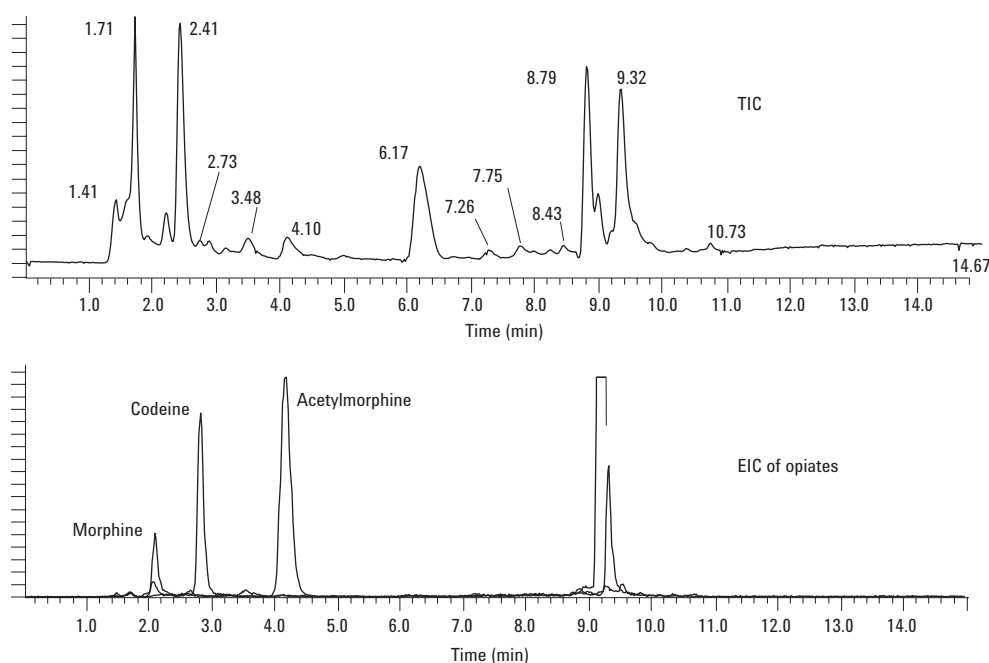
Column	ZORBAX XDB-C18, 2.1 mm × 50 mm, 3.5 μm P/N 971700-902
Mobile Phases	A: Acetonitrile with 0.1 % formic acid B: Water with 0.1 % formic acid
Gradient	35% to 95% A in 5 min, then to 100% in 6 min
Flow rate:	0.35 mL/min

### MS Conditions

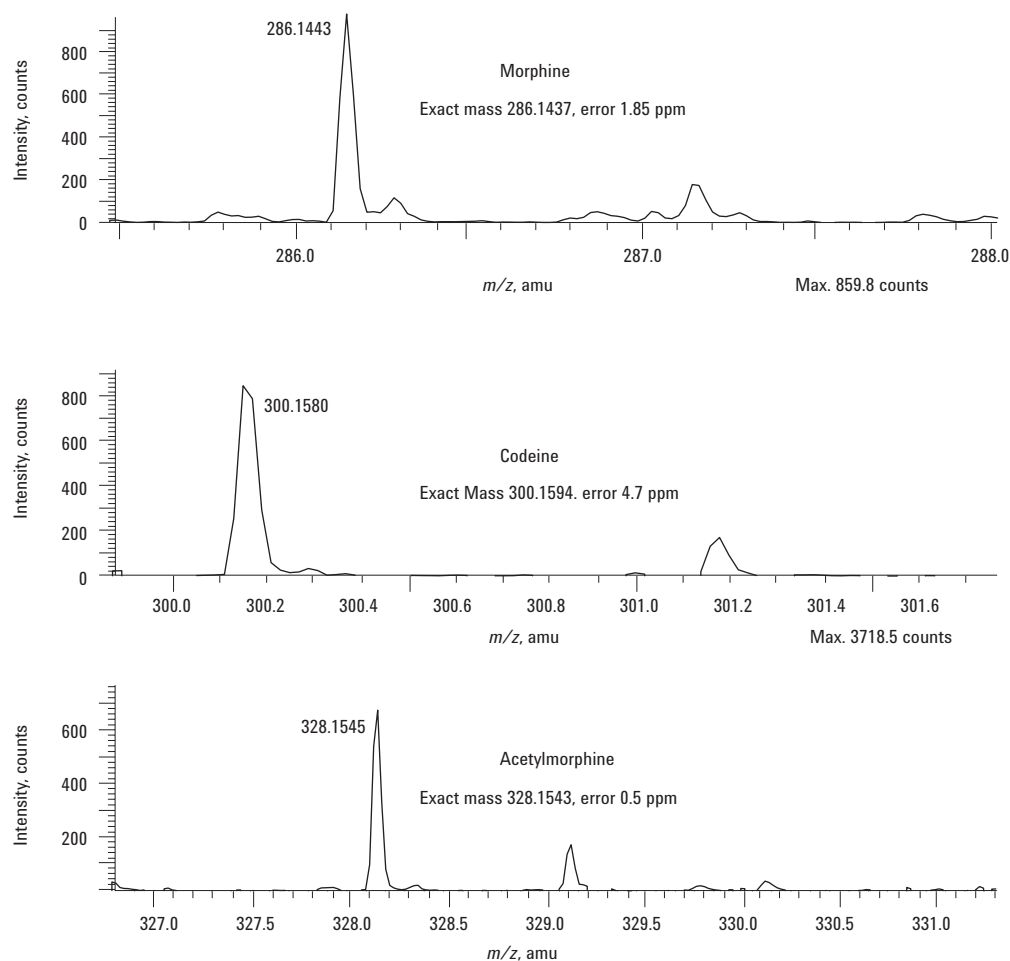
Standard autotune conditions with calibrant delivery system providing constant low flow of ~2 μM purine and HP-921 calibrant to dual ESI for continuous auto-calibration

## Results

Shown in Figure 1 (upper panel) is the total ion chromatogram (TIC) and in the lower panel, overlaid extracted ion chromatograms (EICs) for morphine, codeine, and acetylmorphine, in a direct injection of urine at 300 ng/mL. The EIC has a mass window of 20 ppm (~ ±0.002 u). Accurate mass spectra for these opiates is given in Figure 2. Table 1 shows the quantitative results obtained with direct injection. Table 2 shows the results obtained with the solid phase extraction (SPE).



**Figure 1.** The upper panel shows the TIC of a direct urine injection spiked with 300 ng/mL of each opiate. The lower panel shows the EIC of each compound.



**Figure 2. Mass spectra of M+H ions for opiates showing both mass resolution and mass accuracy at 2 pg on-column.**

**Table 2. Quantitative results (in ng/mL) of spikes at 1000 ng/mL and 300 ng/mL obtained by LC/MSD TOF direct injection of urine.**

Urine direct injection (Spike 1000 ng/mL)				Urine direct injection (Spike 300 ng/mL)			
	Morphine	Codeine	Acetylmorphine		Morphine	Codeine	Acetylmorphine
	241	446	715		66.7	93.8	176
	222	402	653		78.5	94.8	203
	238	426	683		73.7	93	199
	195	338	687		73.7	96.5	201
	200	351	588		76.6	94.3	185
Mean	219.2	392.6	665.2	Mean	73.8	94.5	192.8
SD	21.2	46.8	48.4	SD	4.5	1.3	11.8
RSD (%)	9.7	11.9	7.3	RSD (%)	6.1	1.4	6.1

These are typical concentration and cut-off range of immunoassay. Note that difference between spiked value and measured concentration represents degree of ion suppression at source.

**Table 3. Quantitative results of spikes at 6 ng/mL and 60 ng/mL obtained by LC/MSD TOF with Accubond Evidex SPE sample preparation.**

Accubond Evidex 5 mL Urine (Spike 6 ng/mL) Expected Conc. 60 pg/μL				Accubond Evidex 5 mL Urine (Spike 60 ng/mL) Expected Conc. 600 pg/μL			
	Morphine	Codeine	Acetylmorphine		Morphine	Codeine	Acetylmorphine
	6.97	8.62	3.74		508	499	182
	8.56	9.57	4.21		567	543	193
	10	8.41	4.03		525	504	183
	9.24	8.5	3.81		521	502	191
	7.07	8.15	3.48		595	532	193
	9.46	8.99	3.5		591	532	192
	7.66	8.91	3.79		582	540	196
Mean	8.4	8.7	3.8	Mean	555.6	521.7	190.0
SD	1.2	0.5	0.3	SD	36.6	19.2	5.4
RSD (%)	14.4	5.3	6.9	RSD (%)	6.6	3.7	2.8

Difference in spiked value and measured concentration represents both recovery of SPE method and ion suppression (if any).

## Conclusions

The data shown demonstrates the ability of LC/MSD TOF to confirm - with accurate mass measurement, and quantify- with selective narrow mass window.

- Direct injection of urine shows the robustness of the LC/MSD TOF.
- Typical clean-up (SPE) shows excellent sensitivity.
- High-mass resolution and accuracy (of every spectrum) provides the selectivity for reduction of chemical noise for quantitation and confirmation.

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