

High-Throughput, Cost-Efficient LC-MS/MS Forensic Method for Measuring Buprenorphine and Norbuprenorphine in Urine

Xiaolei Xie, Joe DiBussolo, Marta Kozak; Thermo Fisher Scientific, San Jose, CA

Key Words

Buprenorphine, norbuprenorphine, urine, TSQ Quantiva, multi-channeling, Transcend, forensic toxicology

Goal

To develop a fast, cost-efficient method for measuring buprenorphine and norbuprenorphine in urine for forensic toxicology.

Introduction

Buprenorphine is an opioid used in the treatment of acute pain and as an alternative treatment of heroin addiction. However, it has quickly been abused by millions, leading to drug-related offenses and forcing forensic and testing labs to run drugs of abuse tests routinely to look for the presence of buprenorphine and its metabolite norbuprenorphine. Forensic toxicology labs are looking for cost-efficient analytical methods to run their tests on hundreds of samples to get rid of their case backlog. Hence, we developed a high-throughput screening and quantitation LC-MS/MS method in which urine samples are hydrolyzed by beta-glucuronidase, diluted, and analyzed by a short chromatographic method implemented on a four-channel LC-MS system for improved productivity.

Experimental

Sample Preparation

Urine samples were spiked with internal standards (buprenorphine- d_4 and norbuprenorphine- d_3), enzymatically hydrolyzed with beta-glucuronidase, and diluted 20-fold with water.

Chromatography

A 10 μ L aliquot of processed urine sample was injected onto a Thermo Scientific™ Accucore™ PFP, 2.6 μ m, 50 x 2.1 mm HPLC columns (P/N 17426-052130) at ambient temperature. A four-minute gradient-elution method was implemented on a Thermo Scientific™ Transcend™ II LX-4 multi-channel LC system. Mobile phase A was 10 mM ammonium acetate in water and mobile phase B was 10 mM ammonium acetate in methanol.

Mass Spectrometry

The detector used was a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI II) source. Two SRM transitions for each analyte and internal standard (Table 1) were measured and ion ratios were calculated for confirmation.

Table 1. List of SRM transitions (quantifying and qualifying ions in order) for each analyte and internal standard.

Analyte	Precursor m/z	Product m/z	Collision Energy (V)	RF lens (V)
Norbuprenorphine	414.3	187.1	40	89
	414.3	211.1	42	89
Norbuprenorphine-d_3	417.3	187.1	40	84
	417.3	211.1	41	84
Buprenorphine	468.3	414.3	35	118
	468.3	55.1	53	118
Buprenorphine-d_4	472.3	400.2	42	92
	472.3	59.1	55	92

Data Processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software version 3.2.

Method Performance Evaluation

Calibration standards in the range of 1–5000 ng/mL and quality control (QC) samples at concentrations of 2, 100, and 500 ng/mL were prepared in synthetic urine. Accuracy and precision were determined by analyzing five replicates of all three QC levels along with a calibration curve on three different days. Matrix effects were determined by comparing calculated concentrations of QCs prepared at nominal concentrations of 2, 10, and 50 ng/mL in donor urine (n=5) to QCs prepared in water.

Results and Discussion

Limits of quantitation were 2 ng/mL for both compounds, and calibration ranges were 2–1000 ng/mL with linear fit and 2–5000 ng/mL with quadratic fit for both analytes. Figure 1 shows representative calibration curves for buprenorphine, and Figure 2 shows representative calibration curves for norbuprenorphine. Figure 3 shows chromatograms for the lowest calibration standards for both compounds and their internal standards.

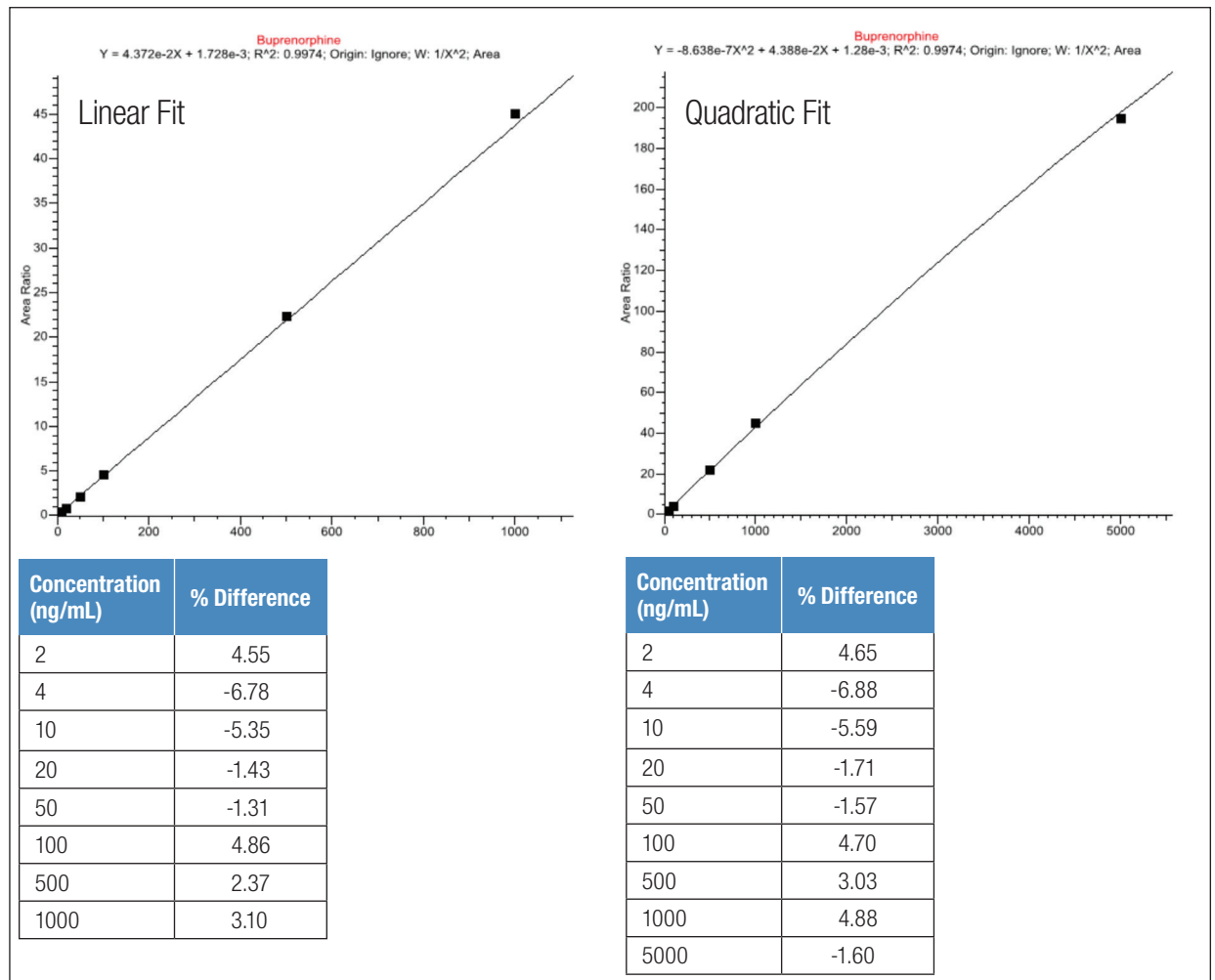


Figure 1. Buprenorphine representative calibration curve 2–1000 ng/mL with linear fit (left). Buprenorphine representative calibration curve 2–5000 ng/mL with quadratic fit (right).

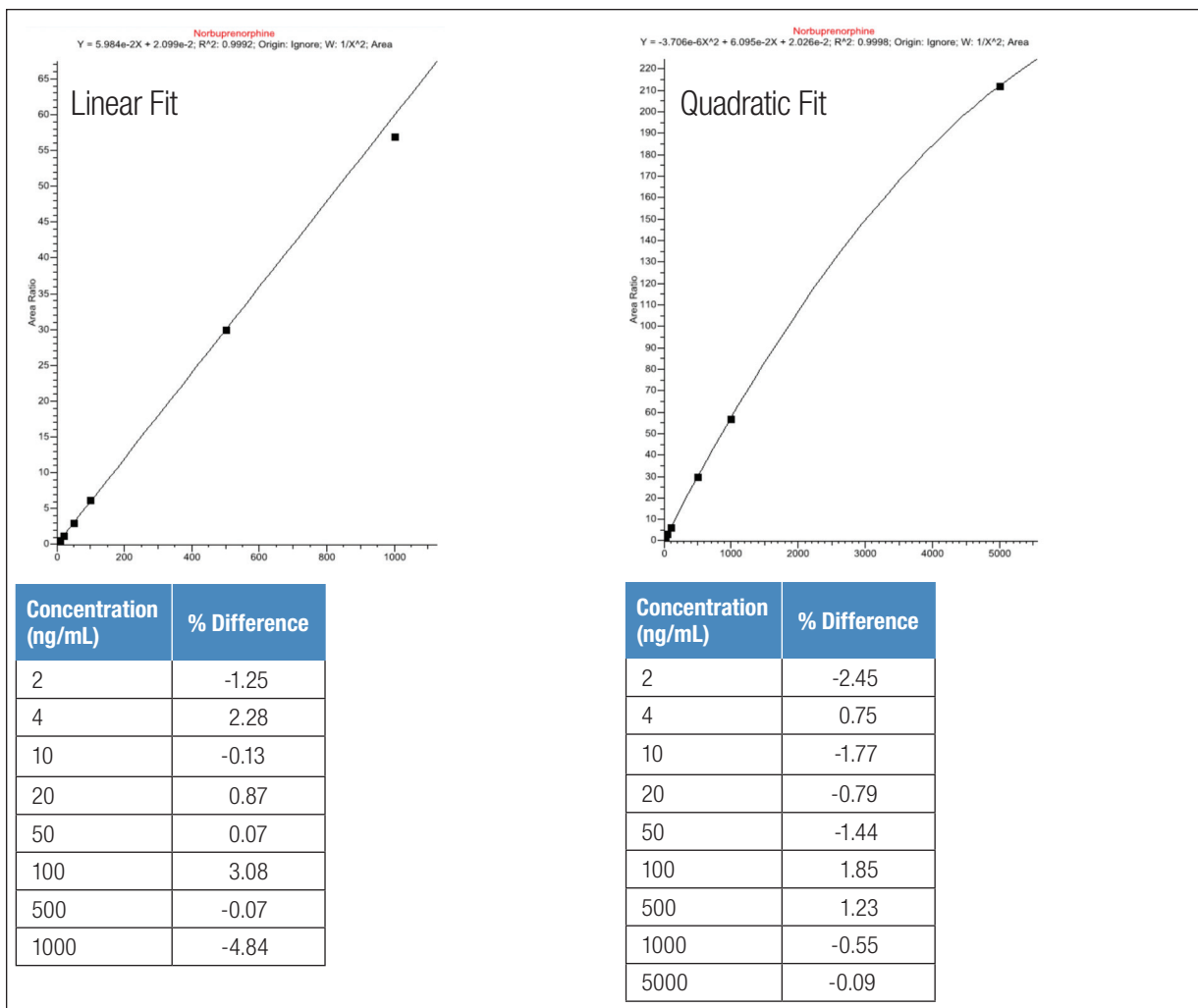


Figure 2. Norbuprenorphine representative calibration curve 2–1000 ng/mL with linear fit (left). Norbuprenorphine representative calibration curve 2–5000 ng/mL with quadratic fit (right).

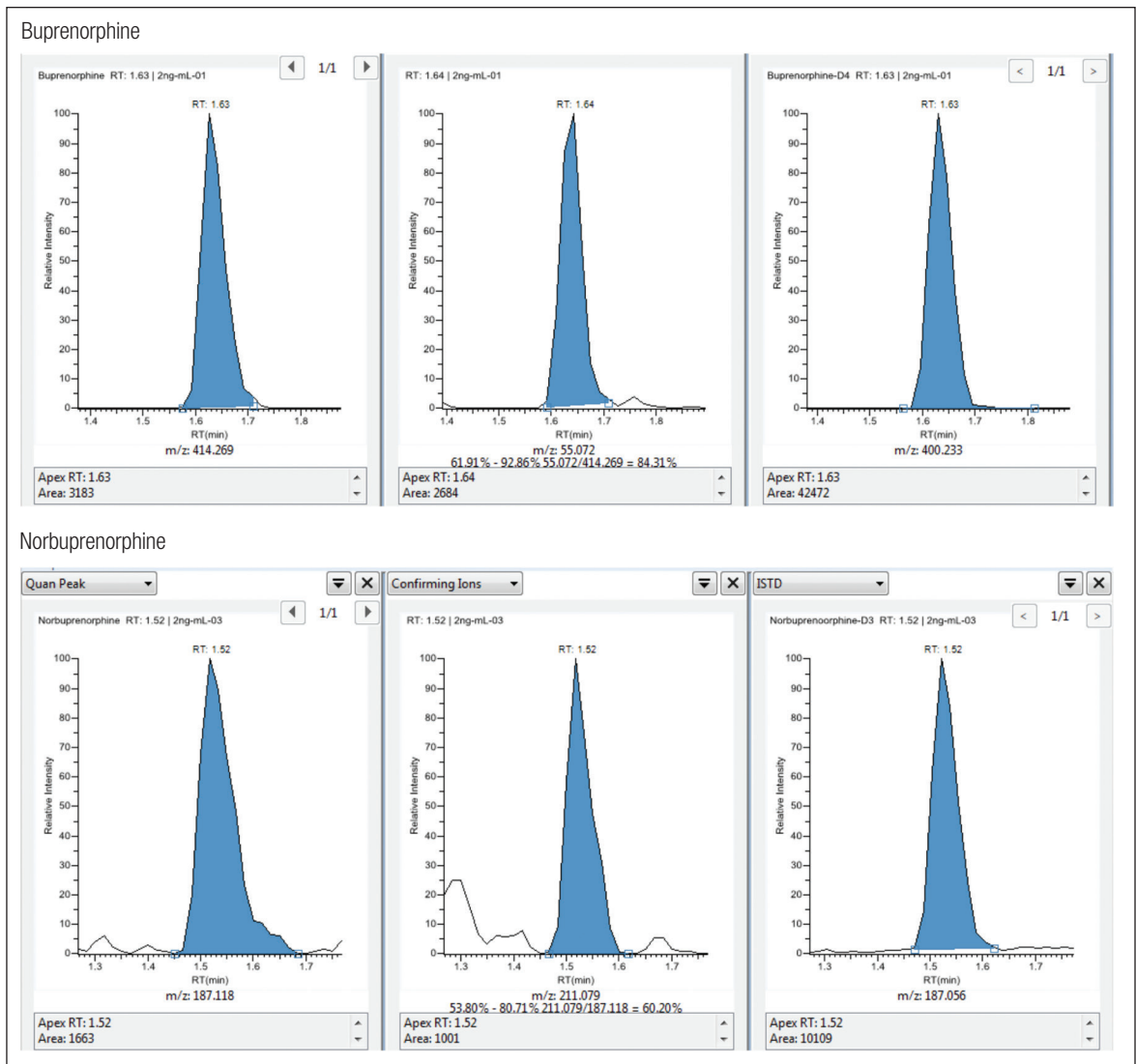


Figure 3. Buprenorphine chromatograms of the lowest calibration standard (2 ng/mL) - quantifying and confirming ions and corresponding internal standard (Buprenorphine- d_4) (top). Norbuprenorphine chromatograms of the lowest calibration standard (2 ng/mL) - quantifying and confirming ions and corresponding internal standard (Norbuprenorphine- d_3) (bottom).

As shown in Table 2, intra-assay precision was better than 8.1% RSD for buprenorphine and better than 12.4% RSD for norbuprenorphine. Inter-assay precision was better than 7.5% RSD for buprenorphine and 13.1% RSD for norbuprenorphine. Insignificant matrix effects were observed in the method. Average %Recoveries in spiked urine samples compared to a sample prepared in water were within $\pm 20\%$ for norbuprenorphine and within $\pm 10\%$ for buprenorphine.

Table 2. Precision.

Analyte	%RSD					
	Intra-assay			Inter-assay		
	QC1	QC2	QC3	QC1	QC2	QC3
Norbuprenorphine	<12.4	<5.1	<4.0	13.1	3.7	4.4
Buprenorphine	<8.1	<2.6	<1.5	7.5	1.9	1.0

Figure 4 presents chromatograms of a donor sample spiked with buprenorphine and norbuprenorphine at a concentration of 2 ng/mL. This method was successfully implemented on a Transcend LX-4 multi-channeling LC system in a production laboratory. Figure 5 shows how multi-channeling the method across four channels achieved a throughput of 60 injections per hour.

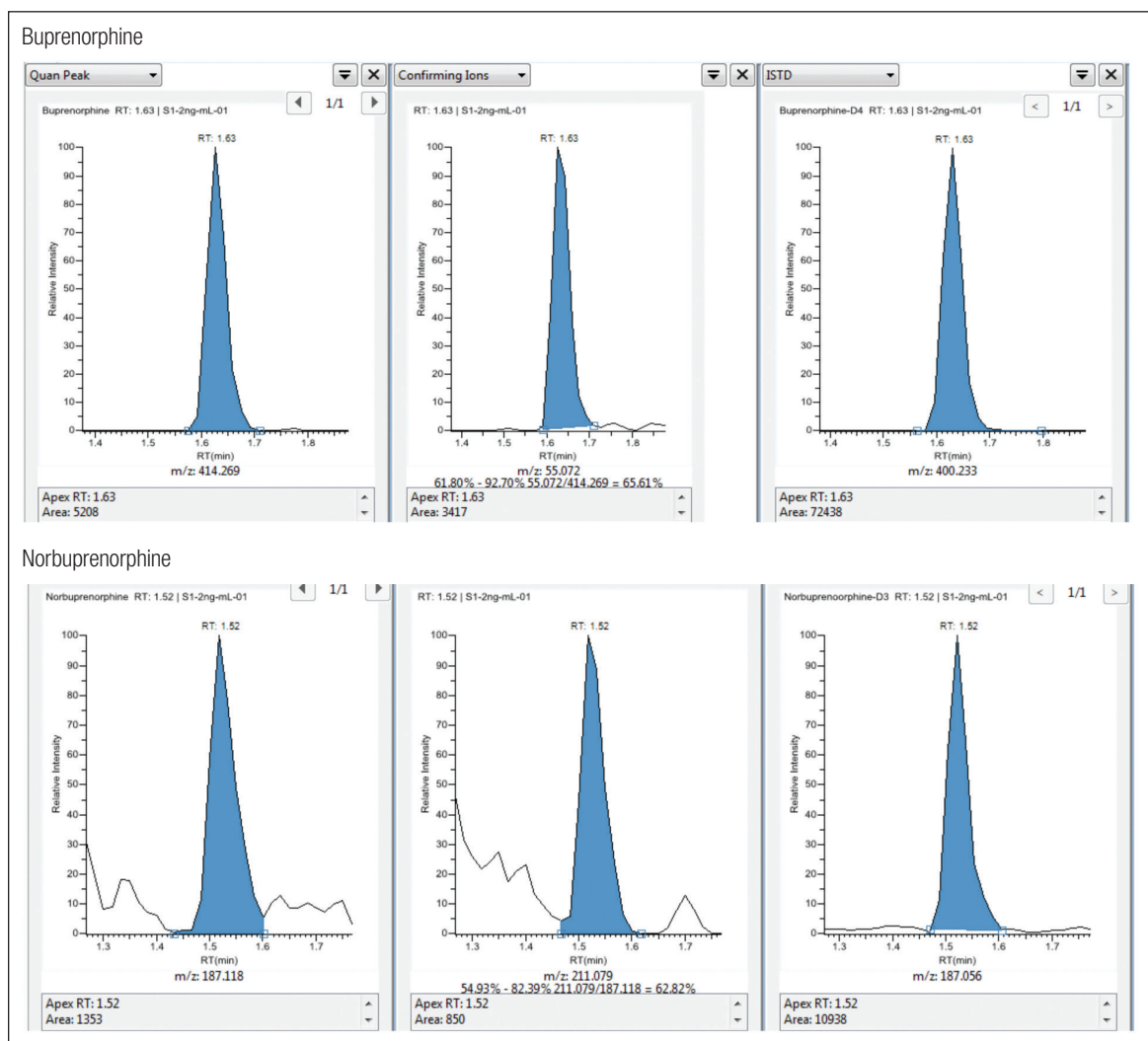


Figure 4. Chromatograms of buprenorphine in donor urine sample spiked to concentration of 2 ng/mL - quantifying ion, confirming ion, and internal standard. No significant matrix effects were observed (top). Chromatograms of norbuprenorphine in donor urine sample spiked to concentration of 2 ng/mL - quantifying ion, confirming ion, and internal standard. No significant matrix effects were observed (bottom).

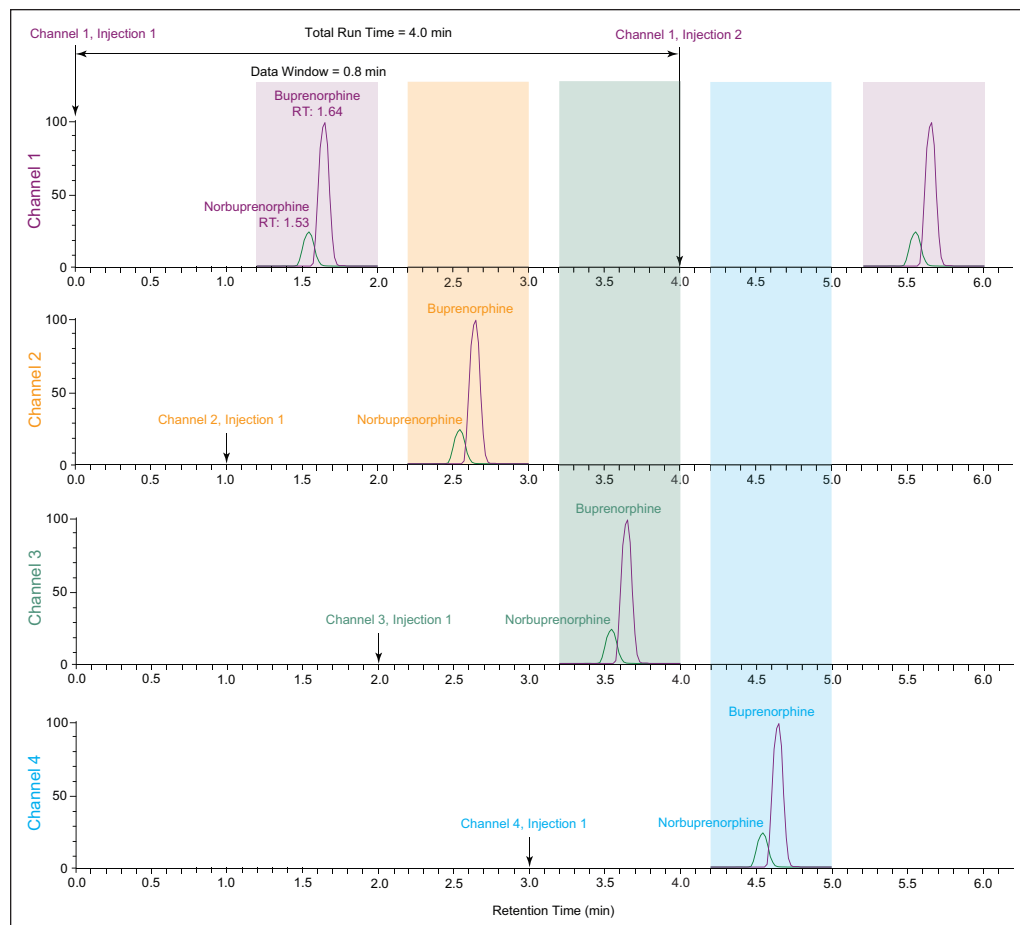


Figure 5. Method multi-channeling scheme on Transcend II LX-4 system.

Conclusion

A high-throughput, cost-efficient, and sensitive forensic method for measuring buprenorphine and norbuprenorphine in urine has been developed. Method performance met forensic toxicology research lab requirements. Implementation on a four-channel LC system allowed one sample result to be generated every minute resulting in a throughput of 60 samples per hour, which is quite comparable to running an immunoassay method.

For forensic toxicology use only.

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Denmark +45 70 23 62 60

Europe-Other +43 1 333 50 34 0

Finland +358 9 3291 0200

France +33 1 60 92 48 00

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India +91 22 6742 9494

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Russia/CIS +43 1 333 50 34 0

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