Development and Validation of a LC/QToF Mass Spectrometry Method to Quantify Buprenorphine and its Metabolite in Urine

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Abstract

Quantitation of buprenorphine (BUP) and its metabolite, norbuprenorphine (NorBUP), in urine by Waters Acquity UPLC/Xevo G2 QToF mass spectrometry was developed and validated by this lab. All analytical parameters: intra- and inter-assay precision, LOD/LOQ, linearity, accuracy/recovery, stability, dilution, interference, and ion-suppression were investigated and met the established acceptance criteria. The method was compared to the reference lab's LCMSMS method. The correlations for BUP/NorBUP were y=0.9272x+0.9239, R^2=0.9875 and y = 1.003x -4.9, R^2 =0.9889. We demonstrated that this LC accurate mass time-of-flight mass spectrometry method is accurate, stable, and robust; suitable for routine urine drug of abuse confirmation testing.

Introduction

Buprenorphine was first approved by the FDA in 2002 to be used as an alternative medication to treat opioid addiction. More recently, buprenorphine has also been shown to be effective in the treatment of moderate to severe chronic pain. As like most opioids to control chronic pain, the potential for buprenorphine abuse is high. To support providers in our clinic system to manage pain patients, we developed and validated this quantitative method for buprenorphine (Bup) and its metabolite, norbuprenorphine (NorBup) in urine on our newly acquired Waters Acquity UPLC/Xevo G2 QToF mass spectrometer.

Methods

Sample preparation: Deuterated buprenorphine-D4 (Bup-D4) and norbuprenorphine-D3 (NorBup-D3) were added to 2 mL of urine sample, calibrators, and controls followed by beta-glucuronidase (5000 U/mL) enzymatic hydrolysis at 65ºC for 30 mins. Drugs were extracted by solid phase extraction utilizing UCT Clean Screen DAU columns to purify and concentrate the analytes.
LCQToF analysis: Waters Acquity UPLC/Xevo G2 QToF mass spectrometry was used for the identification and quantitation analysis.
LC condition: A 3 min gradient at flow rate of 0.5 mL/min starts at 75% mobile phase A (20 mM Ammonium formate, pH = 3) and 25% mobile phase B(100% LCMS grade methanol) to 100 % B and ends at the initial solvent ratio.
QToF parameters: ES + mode, capillary:2.3 KV, sampling cone: 62 V, extraction cone:4.2 V. source temp:150ºC, cone gas:20L/h, desolvation gas:800 L/h, mass range: 100 – 1200 Da. sample flow control:20.0 µL/min, lockspray flow control: 10.0 µL/min.

Table 1: Ion monitored

Analyte	Quantitating Ion	Qualifying Ion
Buprenorphine	468.31	396.21
Buprenorphine-D4	472.34	400.24
Norbuprenorphine	414.26	340.19
Norbuprenorphine-D3	417.28	399.27

Results

Precisions: Intra-assay precision was performed on three different samples at low, mid and high levels for Bup and NorBup 10 times. Inter-assay precision was performed on all three levels in 10 days. The mean CVs are reported in Table 2.

Table 2. Precision

	Intra-assay %CV	Inter-assay %CV
	N= 30	N= 30
Buprenorphine	2.17	6.23
Norbuprenorphine	3.0	7.57

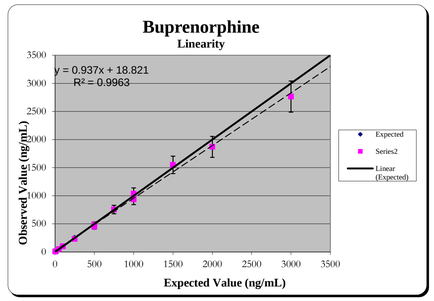
Performance characteristics Table 3: Limit of Detection (LOD), Limit of Quantitation (LOQ), and upper limit of linearity (ULOL)

Table 3. Analytical Characteristics

	LOD (ng/mL)	LOQ (ng/mL)	ULOL (ng/mL)
Buprenorphine	1.0	2.0	3000
Norbuprenorphine	3.0	5.0	2000

Linearity: Linearity was evaluated between 5 – 3500 ng/mL in duplicates.

Figure 1. Linearity



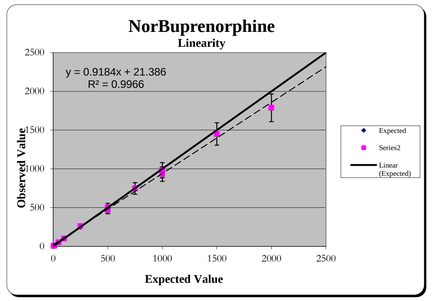
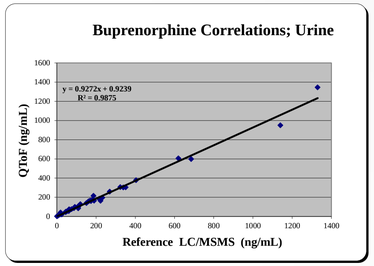
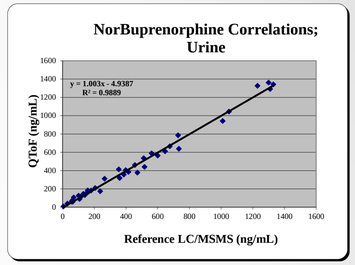


Figure 2. Patient Correlation Study: Split sample comparison performed for 40 patients





- Recovery study: 4 pools of serum, three of them were spiked with Bup/NorBup at 20, 100, 500 ng/mL each. Each pool was analyzed 4 times; the averaged recoveries were 104.1% for Bup and 98.3% for NorBup.
- Matrix effect (Ion suppression):Matuszewski1 protocol was used to assess the matrix effect and total process efficiency. Ion suppression was observed in Bup and NorBup at 47 and 34%. Total process efficiency for Bup and NorBup was at 80 and 85%. Quantitation was not affected from matrix effect by using the deuterated internal standard as shown by the ratio of analyte over internal standard from the solvent, post extraction addition and the normal extraction procedures.
- Carryover study: No carryover observed for both analytes up to ULOL.
- Dilution study: Four samples containing 4 different concentrations of Bup and NorBup were diluted with water, saline, and phosphate buffer at x2, x5, x10 and x20 dilutions. The % differences of all four levels were below 10% to their target levels.
- Interference study: interferences of the following classes of drugs were evaluated; amphetamine, antidepressants, barbiturates, benzodiazepines, opioids (6-MAM, hydrocodone, hydromorphone, codeine, morphine, oxycodone, oxymorphone), narcotics (ketamine, methadone EDDP, tramadol, fentanyl, meperidine, norpropoxyphene), cannabinoids, cocaine, benzyleconine and PCP. None showed appreciable interference.

Conclusion

We demonstrated that this method is robust and suitable for monitoring patients prescribed with buprenorphine in clinical settings.

Reference:
Matuszewski, B.K; Constanzer, M.L.; Chavez-Eng, C.M. Anal. Che,. 2003, 75, 3019-3030.