

Analysis of Buprenorphine and Norbuprenorphine in Urine by Ion-Exchange HPLC-MS/MS

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Introduction

- Buprenorphine (*Buprenex*[®], *Subutex*[®], *Temgesic*[®]) is licensed in the UK for treating moderate to severe pain and for opiate withdrawal. It is being used increasingly instead of methadone because it has lower abuse potential and is relatively safe in overdose¹.
- There is a need to monitor adherence in patients undergoing buprenorphine treatment for opiate withdrawal.
- Buprenorphine is primarily metabolised by *N*-dealkylation to norbuprenorphine, which is pharmacologically active. Both drug and metabolite are excreted in urine as D-glucuronides². Current immunoassays employ a positive 'cut-off' for buprenorphine in urine of 5 µg/L, but are subject to interferences from certain opioids³.
- We have developed a sensitive method for the analysis of both buprenorphine and norbuprenorphine in urine based on enzymatic hydrolysis of the glucuronide conjugates followed by strong cation-exchange liquid chromatography with MS/MS detection.
- Buprenorphine, norbuprenorphine, and their respective deuterated internal standards fragment poorly in LC-MS/MS. Optimum sensitivity was gained by monitoring surviving MH⁺ ions in Q3. Selectivity was ensured by ramping collision energy voltages to produce qualifier ion fragments for buprenorphine and norbuprenorphine at m/z 396.3 and 101.2, respectively.

Experimental

Sample Preparation:

- Urine samples, calibration standards (2-50 µg/L for both analytes) or internal QC samples (3.0 mL), to which 100 µL aqueous internal standard (1 mg/L each buprenorphine-D₄ and norbuprenorphine-D₃, LGC Promochem) had been added, were hydrolysed overnight (37 °C) with 500 µL β-glucuronidase solution (*Helix aspersa*, Sigma; 134785 units/100 mL, pH 5.0).
- SPE columns (*Phenomenex* Strata Screen C) were preconditioned with (i) methanol and (ii) phosphate buffer (pH 6.8). Hydrolysed samples were applied and the columns washed (1 mL volumes) with (i) acetate buffer (pH 4.0), (ii) deionised water, and (iii) methanol. Analytes were eluted with 2 mL dichloromethane/2-propanol (80+20) with 2 % (v/v) ammonia solution (25% w/v). The extract was evaporated to dryness under compressed air (50 °C) and reconstituted in 100 µL LC eluent.

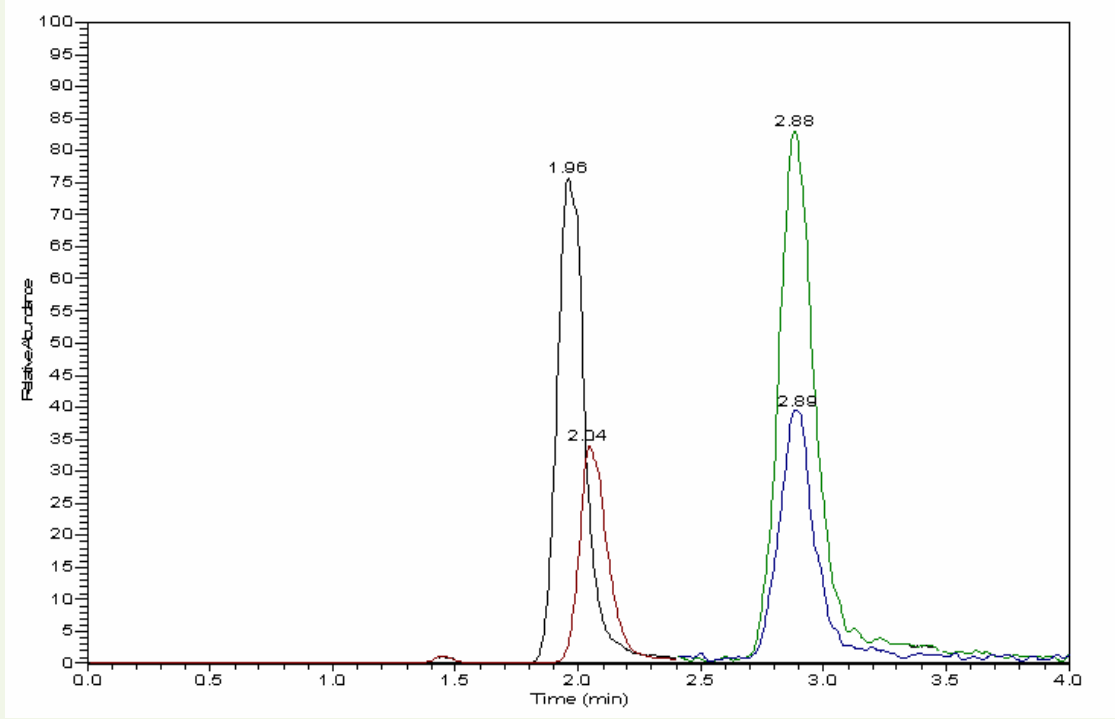
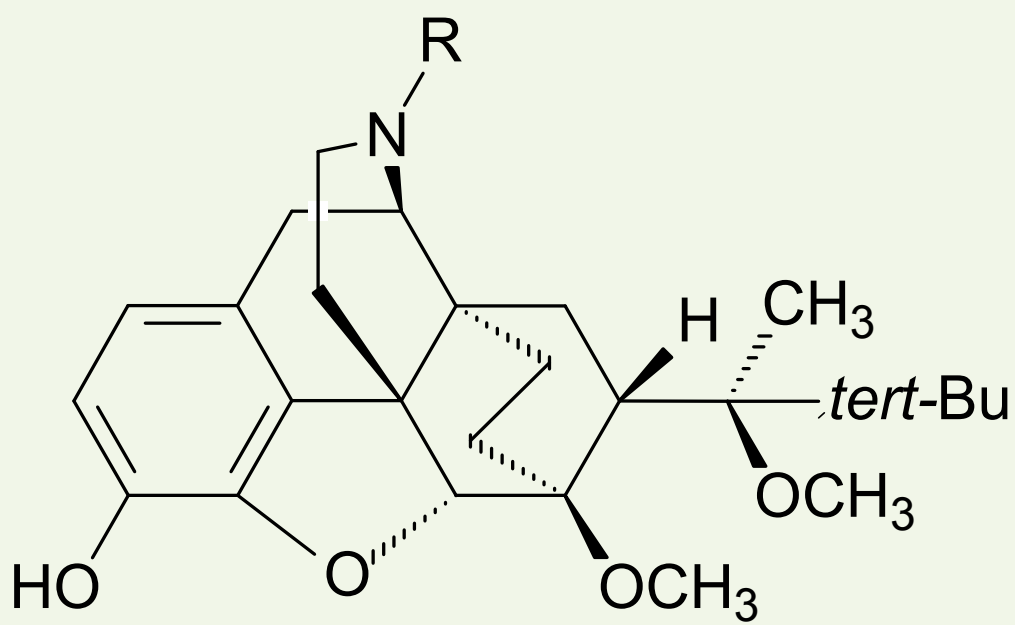
Liquid chromatography-Mass spectrometry

- 100 x 2.1 mm Waters Spherisorb S5SCX with 40 mmol/L methanolic ammonium acetate (pH* 6.0) as eluent (flow-rate 0.5 mL/min). Injection volume: 20 µL.
- Thermo TSQ Quantum Access MS/MS in positive atmospheric pressure chemical ionisation (APCI) mode (discharge current 5 µA, vapourizer temperature 350 °C, sheath gas 60 psi, capillary temperature 225 °C).

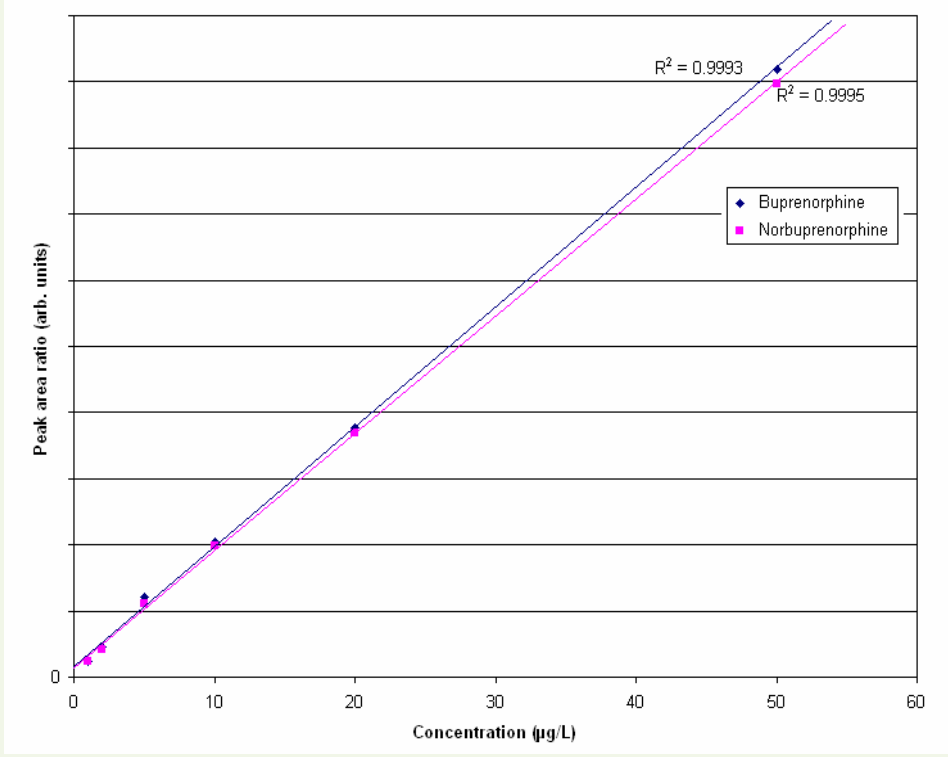
MRM transitions and settings for the analytes of interest

Analyte	Q1 (m/z)	Q3 (m/z)	Tube Lens Voltage (V)	Collision Energy (V)	Collision (Q2) Pressure (mTorr)
Buprenorphine	468.3	468.3	140	20	20
"	468.3	414.4	140	33	33
"	468.3	396.3	140	38	38
Buprenorphine-D ₄	472.3	472.3	140	20	20
Norbuprenorphine	414.2	414.2	130	20	20
"	414.2	396.1	130	5	5
"	414.2	224.3	130	56	56
Norbuprenorphine-D ₃	417.2	417.2	140	20	20

Results



Chromatogram showing an extracted 20 µg/L calibration standard



Typical buprenorphine and norbuprenorphine calibration curves

- Peak area ratios analyte:internal standard gave linear calibration over the range 1–50 µg/L ($r^2 > 0.99$) for both buprenorphine and norbuprenorphine.
- Ion suppression was investigated by repeated blank matrix injections, with post-column infusion of the four analytes, and was insignificant at the retention times corresponding to the peaks of interest.

Conclusions

- Using SPE for sample preparation, SCX chromatography at specific pH* for separation, and MS/MS with qualifier ions for detection, very high specificity for both analytes was achieved (limit of detection 0.2 µg/L for buprenorphine and 0.5 µg/L for norbuprenorphine).
- This methodology allows for the simultaneous analysis of buprenorphine and norbuprenorphine, which is useful in (i) confirming results obtained using CEDIA[®] (Microgenics; limit of detection 5 µg/L) and (ii) in identifying urine samples to which buprenorphine has been added.

References

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- Pavlic M, Libiseller K, Grubweiser P, Rabl W. Cross-reactivity of the CEDIA buprenorphine assay with opiates: an Austrian phenomenon? *Int J Legal Med* 2005; 119: 378-81