



LC-MS/MS Method Development for Buprenorphine, Norbuprenorphine, Propoxyphene and Norpropoxyphene Analysis in Human Urine

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LC-MS/MS is a rapidly adopted methodology in the analysis of pain management drugs in biological matrices. Conventional analysis uses immunoassay followed by confirmation testing using GC-MS or LC-MS. Immunoassay encounters problems with poor antibody specificity in combination with abundant cross-reacting. The essential strength of LC-MS/MS technology for clinical research includes specificity, wide range of applicability, flexibility, and information rich detection¹.

Buprenorphine is used for the treatment of chronic pain and also in treatment of heroin addiction as an alternative to methadone. Propoxyphene is structurally similar to the synthetic opiate methadone, and is usually prescribed for relief of mild to moderate pain. These drugs are prone to abuse and so there is a need for forensic and clinical laboratories to analyze for them. Buprenorphine, Propoxyphene and their metabolites are excreted in urine, almost exclusively as glucuronides.

The LC-MS/MS method for the analysis of 4 major pain management drug products in one method was desired. The assay consists of Buprenorphine, Norbuprenorphine, Propoxyphene and Norpropoxyphene.

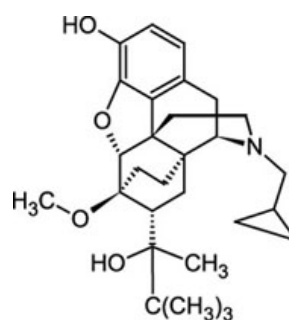
Methodology

The sample preparation is a simple "dilute and shoot" methodology using a 50 µL sample size. The method uses a 25µL injection volume with a 6.5 minute run time. The assay was run on an API 3200™ or 3200 QTRAP® LC/MS/MS system.

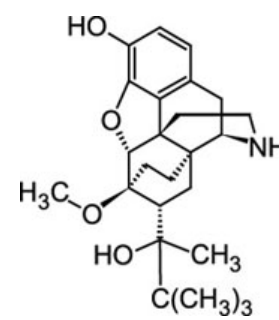


The assay incorporates a hydrolysis step to release the glucuronides to the free form for quantitation. The method uses a 4-point calibration curve covering the linear ranges for quantitation as follows:

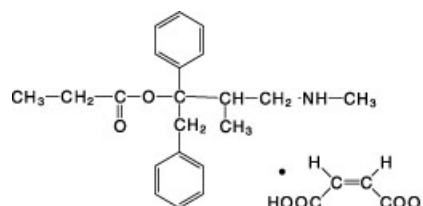
Analyte	Range (ng/mL)
Buprenorphine	40 - 1000
Norbuprenorphine	40 - 1000
Norpropoxyphene	60 - 1500
Propoxyphene	60 - 1500



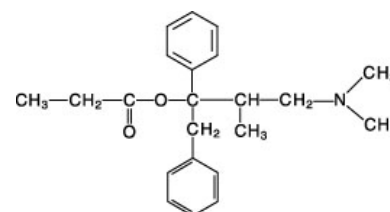
Buprenorphine



Norbuprenorphine



Norpropoxyphene



Propoxyphene

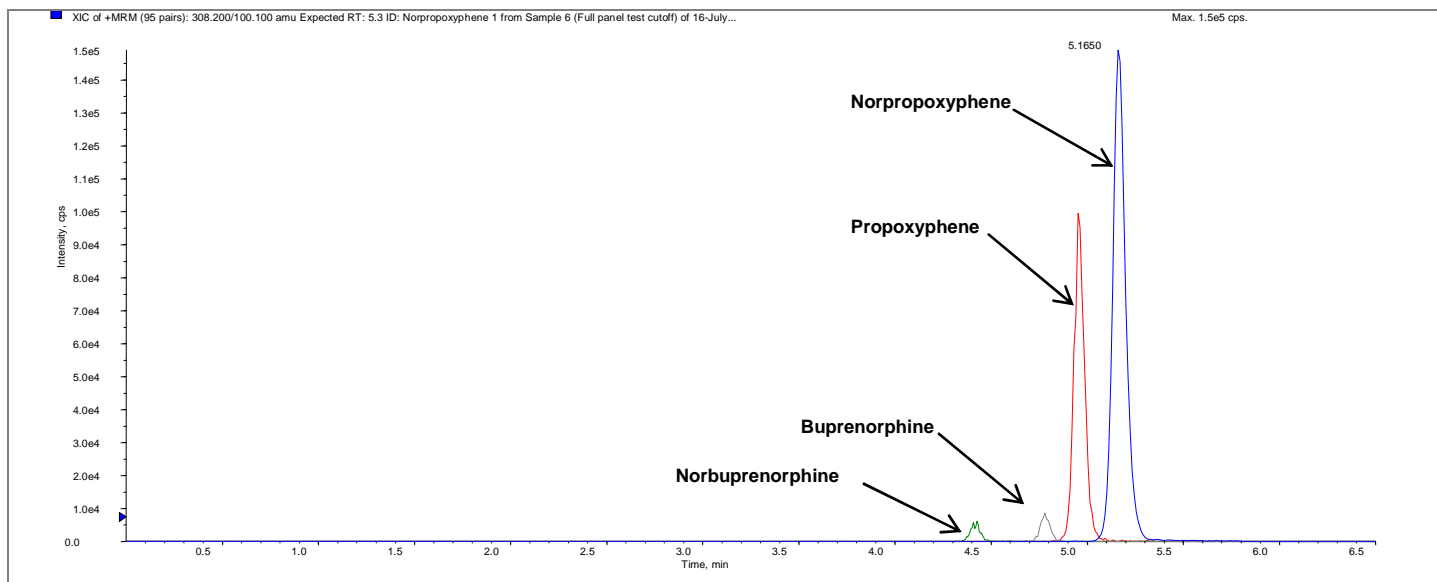


Figure 1: Representative chromatogram at the cutoff level.

Results

The analysis produced accurate and reproducible results across the 4-point calibration range for all analytes.

Table 1. Fifteen calibration standard replicates at the each calibration level produced accurate and reproducible statistics. Cutoff level at 100 ng/mL for Buprenorphine/Norbuprenorphine and 150 ng/mL for Propoxyphene/Norpropoxyphene.

Analyte	%CV at 40% Cutoff	Accuracy at 40% Cutoff (%)	%CV at Cutoff	Accuracy at Cutoff (%)	%CV at 3 x Cutoff	Accuracy at 3 x Cutoff (%)	%CV at 10 x Cutoff	Accuracy at 10 x Cutoff (%)
Buprenorphine	5.79	98.9	5.62	104	6.64	96.8	6.55	101
Norbuprenorphine	7.28	100	5.57	98.5	4.59	101	3.09	99.8
Norpropoxyphene	4.43	102	3.96	94.6	3.83	105	4.35	98.2
Propoxyphene	2.52	99.6	2.84	101	2.27	98.8	1.70	100

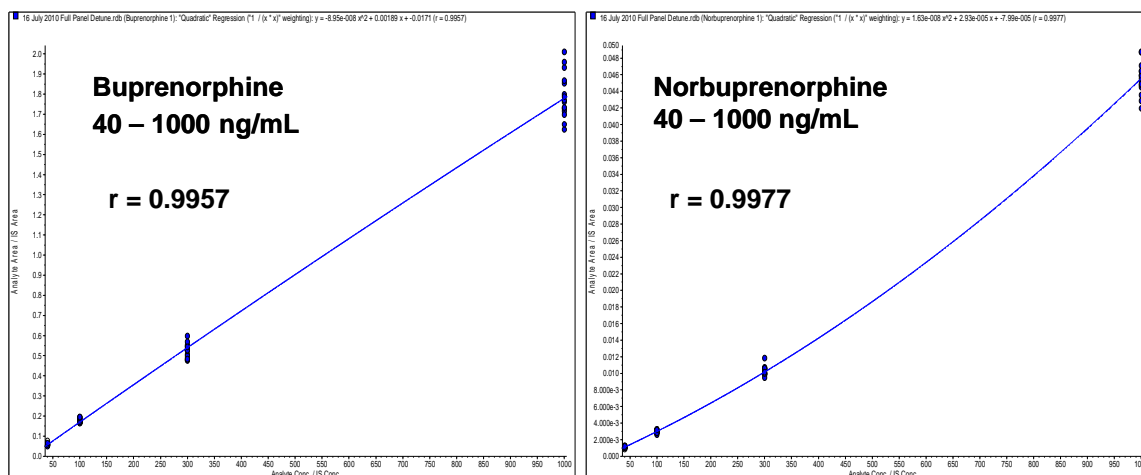


Figure 2a: Representative calibration curves.

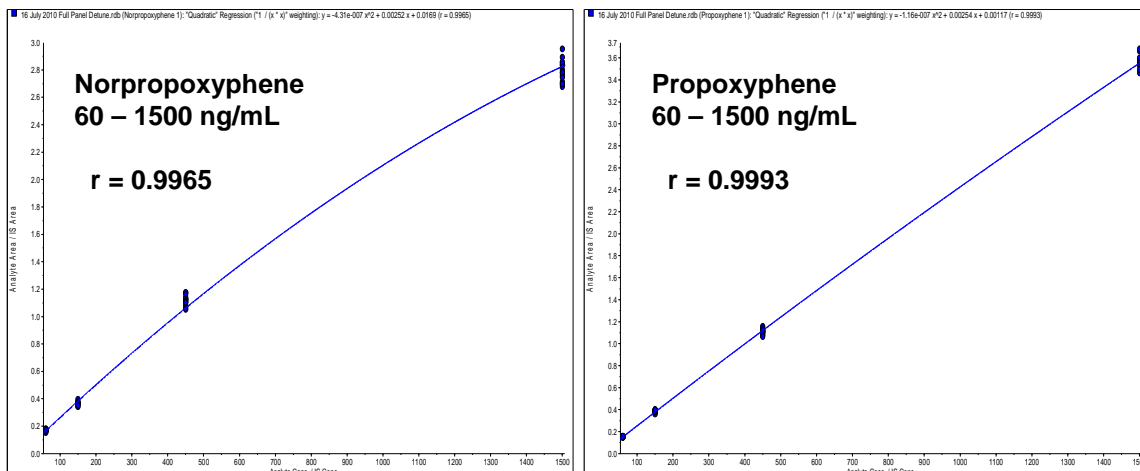


Figure 2b: Representative calibration curves.

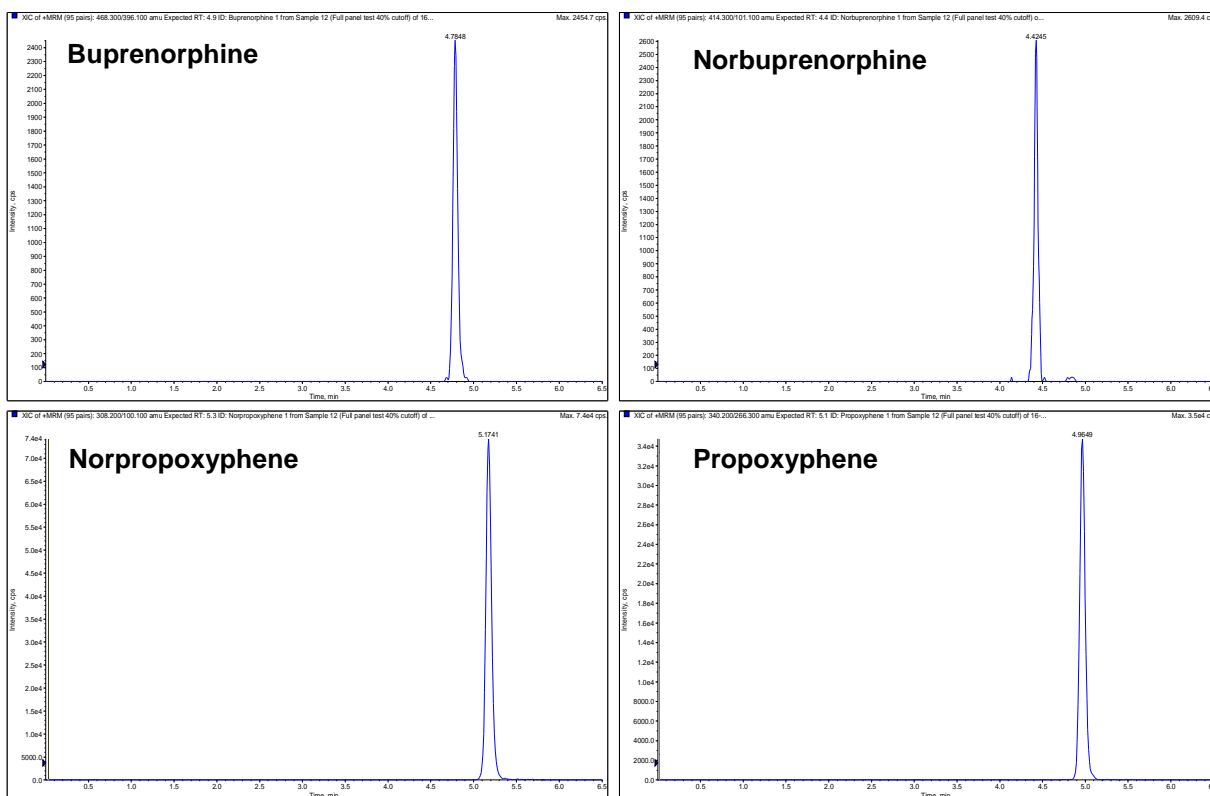


Figure 3: Representative Chromatograms at the 40% Cut off level.

The assay produced good linearity across the full range for all analytes. Limits of quantitation showed good sensitivity and peak resolution.

Conclusions

A method for the quantitation of 4 pain management drugs representing different treatment regimes with corresponding clinical relevant concentration ranges in human urine has been successfully developed utilizing ESI-LC/MS/MS.

A robust method for the quantitation of pain management drugs has been developed to allow for economical fast throughput analysis of human urine samples.

References

1. Vogeser M, Seger C, A decade of HPLC-MS/MS in the routine clinical laboratory—Goals for future development, J. Clinical Biochemistry 41 (2008) 649-662

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