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응용 자료

Simultaneous Quantitative Determination of Opioid Dependency Treatment Drugs in Human Urine Using UPLC-MS/MS

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For forensic toxicology use only.

Abstract

In this application note we develop and validate a single simple and rapid UPLC-MS/MS method for the simultaneous quantitative determination of the opioid dependency treatment drugs methadone, buprenorphine, and dihydrocodeine in human urine.

Introduction

- Across the developed countries of the world, 0.4–0.8% of adults develop a dependence on illicit opioids.[1]
- Common treatment methods include detoxification by supervised withdrawal and tapered doses of replacement drugs.[2]
- Buprenorphine, methadone and more recently, dihydrocodeine have been shown to be effective as replacement drugs for the treatment of opioid dependency.[3–5]
- Urine analysis of these compounds is essential to monitor abstinence and detect or confirm relapses.
- The associated overdose risk, potential for abuse and links with criminal activity has made the analysis of these compounds widespread in other areas of toxicology such as post-mortem and forensic.

Figure 1. Waters ACQUITY TQD System.

Experimental

Specimens

Validation was performed using human urine samples obtained from Concateno (London, UK) and Salford Royal NHS Foundation Trust Hospital (Manchester, UK). All samples were stored at -20 °C until analysis. Blank urine obtained from volunteers was used as the control material to prepare all the calibrators and quality controls (QC).

Standards

Standard reference material, deuterated analogues and drug metabolites were purchased from LGC Promochem (Teddington, UK). A mixed standard stock solution containing buprenorphine (BUP) and norbuprenorphine (NBUP) at 12.5 µg/mL and methadone (METH), 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine (EDDP) and dihydrocodeine (DHC) at 125 µg/mL was prepared in methanol. A mixed internal standard (IS) stock solution containing buprenorphine-D4 (BUP-D4) and norbuprenorphine-D3 (NBUP-D3) at 1.25 µg/mL and methadone-D9 (METH-D9), 2-ethylidene-1, 5-dimethyl-3, 3-

diphenylpyrrolidine-D3 (EDDP-D3) and dihydrocodeine- D6 (DHC-D6) at 2.5 µg/mL was prepared in methanol.

Sample Preparation

Enzymatic hydrolysis was performed on all samples, calibrators and QC's (250 µL) after the addition of the IS (10 µL). 1 M Sodium acetate, pH 5 (20 µL) and β-glucuronidase (10 µL – Helix pomatia, 100,000 units/mL, Sigma-Aldrich, Gillingham, UK) were added to the samples which were then heated at 56 °C for 1 hour. After hydrolysis, saturated disodium tetraborate buffer (250 µL) was added to all samples and a liquid/liquid extraction (LLE) using a mixture of dichloromethane, hexane, diethyl ether and isoamyl alcohol (30:20:50:0.5) was performed. The supernatant was taken to dryness using a sample concentrator block (50 °C) under nitrogen, before being reconstituted with a 50/50 mix of methanol and mobile phase A (250 µL).

LC Conditions

LC system:	Waters ACQUITY UPLC
Column:	ACQUITY UPLC HSS T3 column (2.1 x 100 mm, 1.8 µm)
Column temp:	30 °C
Flow rate:	300 L/min.
Mobile phase A:	5 mM Ammonium acetate containing 0.025 % formic acid in water
Mobile phase B:	Methanol
Gradient:	15-95% B over 5 min.
Injection volume:	10 L
Strong Wash Solvent:	Mobile phase B (800 µL)
Weak Wash Solvent:	Mobile phase A (2400 µL)

MS Conditions

MS system:	Waters TQ Detector
Ionization mode:	ESI Positive
Capillary voltage:	3 kV
Collision Gas Pressure:	4.5×10^{-3} mbar
Acquisition Mode:	Multiple reaction monitoring (MRM)
Data Processing:	MassLynx v4.1 with TargetLynx

Results and Discussion

The MRM conditions used for the measurement of all compounds of interest and their respective internal standards are summarised in Table 1.

Compound	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)
BUP	**468** 468	**55** 414	**60** 60	**50** 35
NBUP	**414** 414	**83** 101	**55** 55	**50** 45
METH	**310** 310	**265** 223	**30** 30	**15** 20
EDDP	**278** 278	**234** 186	**45** 45	**30** 30
DHC	**302** 302	**199** 128	**50** 50	**35** 35
BUP-D4	472	59	65	50
NBUP-D3	417	83	55	45
METH-D9	319	268	35	15
EDDP-D3	281	234	45	30
DHC-D6	308	202	50	35

Table 1. MRM conditions used for all compounds and their internal standards.

Bold transitions used as the quantifier ion.

Figure 2 shows the MRM chromatograms obtained from a 5 µL injection of a low level urine calibrator (50 ng/mL for METH, EDDP, and DHC, 5 ng/mL for BUP and NBUP). The quantifier/qualifier ion ratios for all compounds were monitored for all calibrators, QC's and samples and were found to be within ±20% of the target ion ratios.

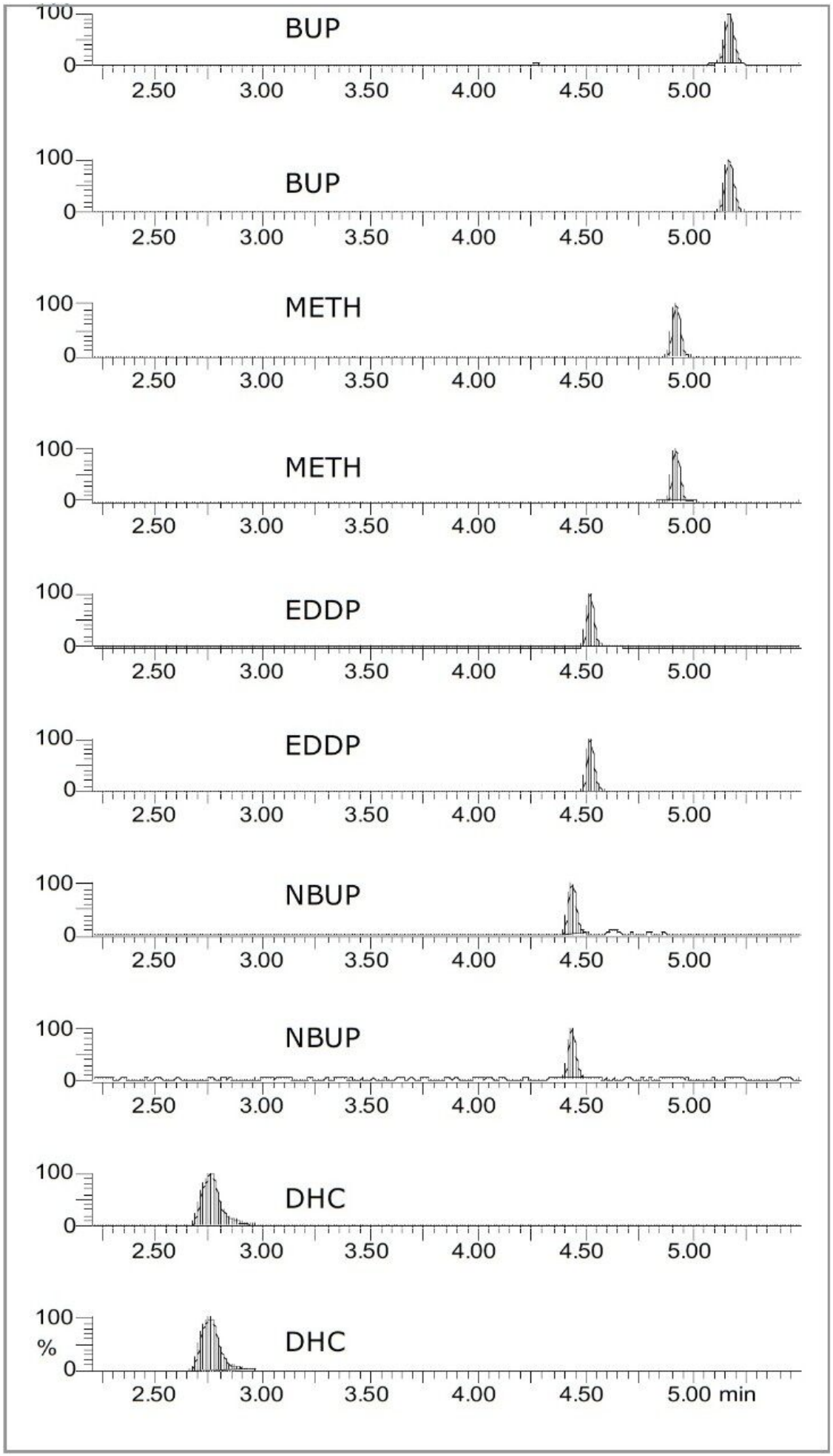

Figure 2. MRM chromatograms for qualifier and quantifier ions obtained from a low level calibra

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