

Nota applicativa

Codeine and its Glucuronide Metabolite in Human Urine by LC/UV

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

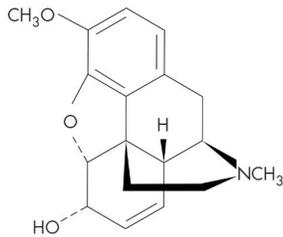
Abstract

This application brief highlights on the analysis of codeine and its glucuronide metabolite in human urine.

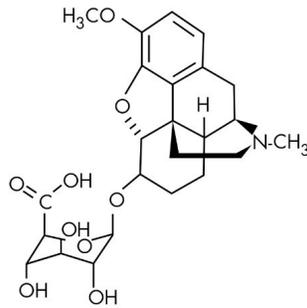
Introduction

The compounds analyzed in this study are:

1. Ranitidine (I.S.)
 2. Codeine-6-Glucuronide (C-6-G)
 3. Codeine
-



CODEINE



CODEINE-6-GLUCURONIDE
(C-6-G)

Compound	% Recovery 0.12 µg/mL	% RSD 0.6 µg/mL
Codeine (n=3)	88.5 (2.7)	99.5 (1.2)
Codeine-6-Glucuronide (n=3)	99.3 (5.4)	98.7 (0.4)
Codeine Interday (n=6)	102.5 (3.5)	105.4 (4.0)
Codeine Interperson (n=9)	91.6 (7.1)	104.1 (5.4)
C-6-G Interday (n=6)	116.1 (9.4)	111.5 (3.3)
C-6-G Interperson (n=9)	102.4 (7.7)	107.4 (7.3)

Experimental

HPLC Conditions

Column:

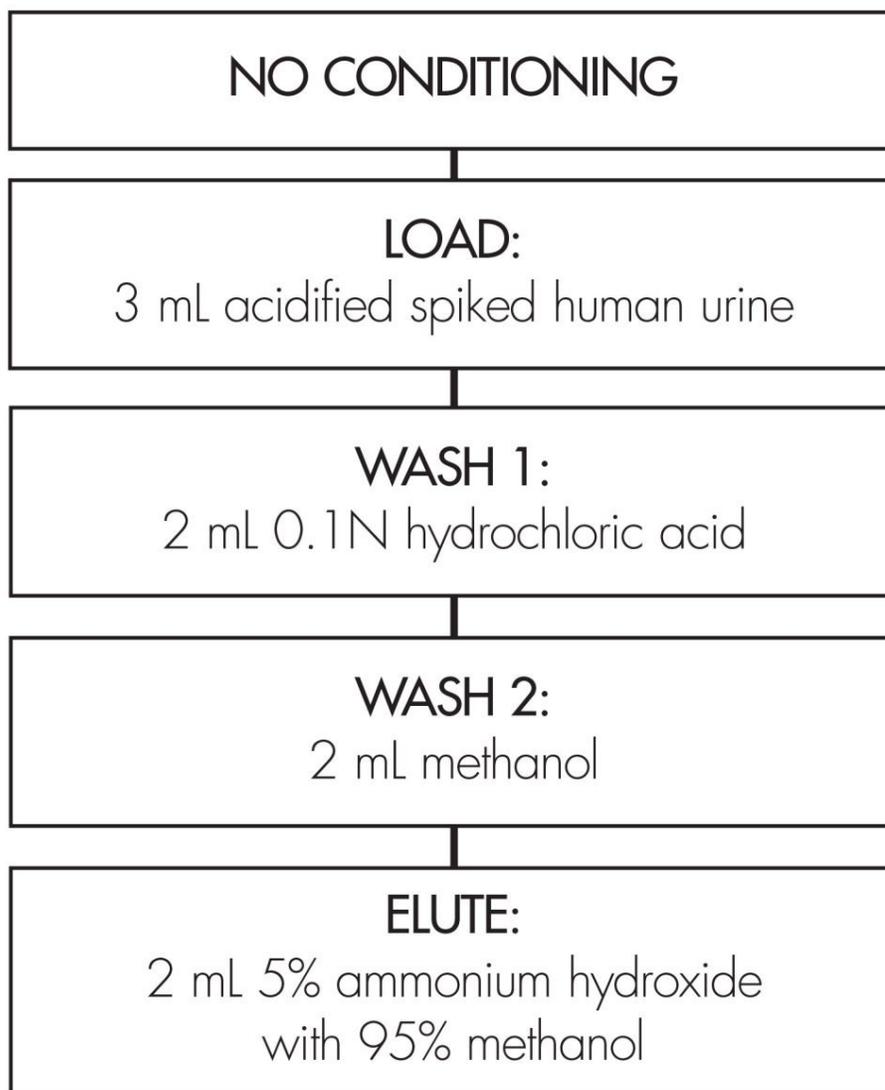
Symmetry C₁₈, 2.1 x 150 mm, 3.5 µm

Guard column:	Symmetry C ₁₈ , 2.1 x 10 mm, 3.5 μm
Part numbers:	Column - 186000174, Guard - 186000169
Mobile phase:	Methanol/Acetonitrile/0.05% TFA in water 5:5:90
Flow rate:	0.3 mL/min
Injection volume:	80 μL urine extract
Temperature:	30° C
Detection:	UV @ 220 nm (0.04 AUFS)

OASIS[®] MCX EXTRACTION METHOD

Oasis[®] MCX Extraction Cartridge, 3 cc/60 mg

Part Number 186000254



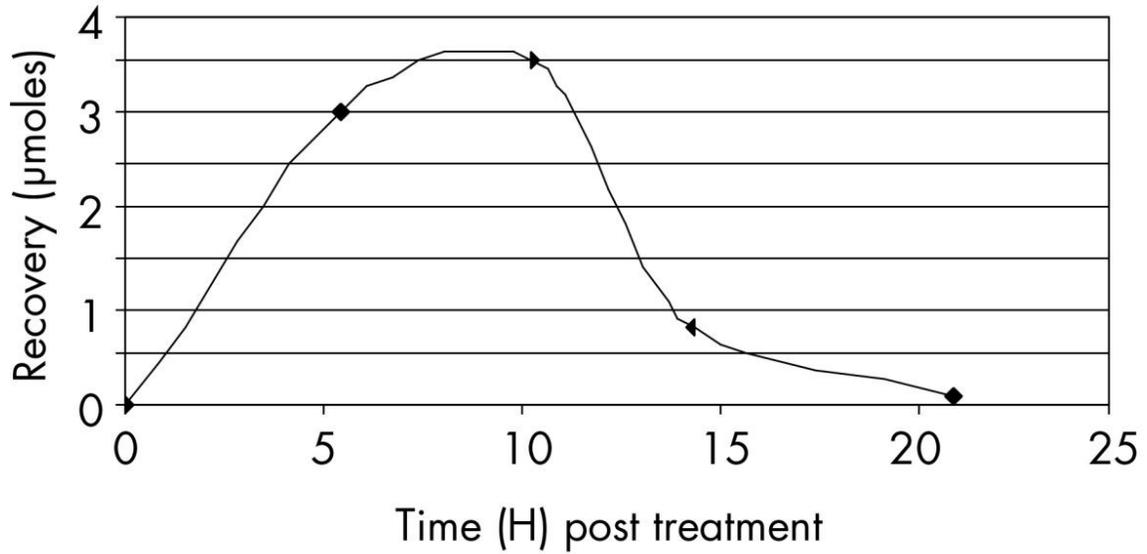
After elution, the extract is diluted 1:3 with water for HPLC analysis

Results and Discussion

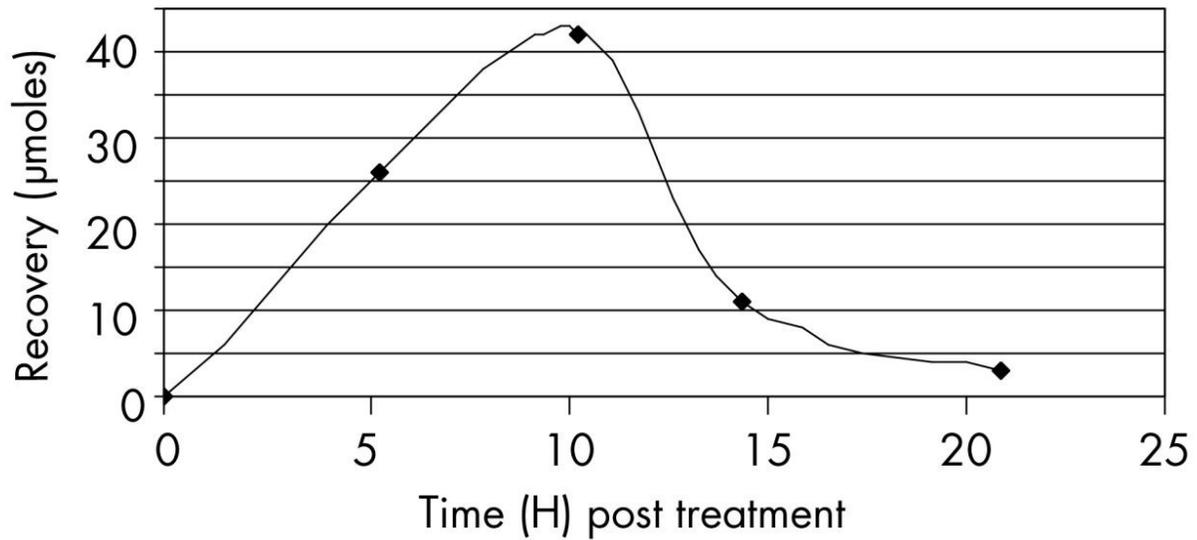
Clearance Study from Human Urine

Timepoint	Recovery % RSD (n=5)	
	Codeine (μmoles)	Codeine-6-Glucuronide (μmoles)
5.4 hours	3.0 (2.4)	26.1 (1.9)
10.3 hours	3.5 (2.9)	42.3 (2.9)
14.3 hours	0.8 (1.2)	11.6 (1.6)
20.9 hours	0.1 (15.3)	3.0 (1.6)

Codeine clearance



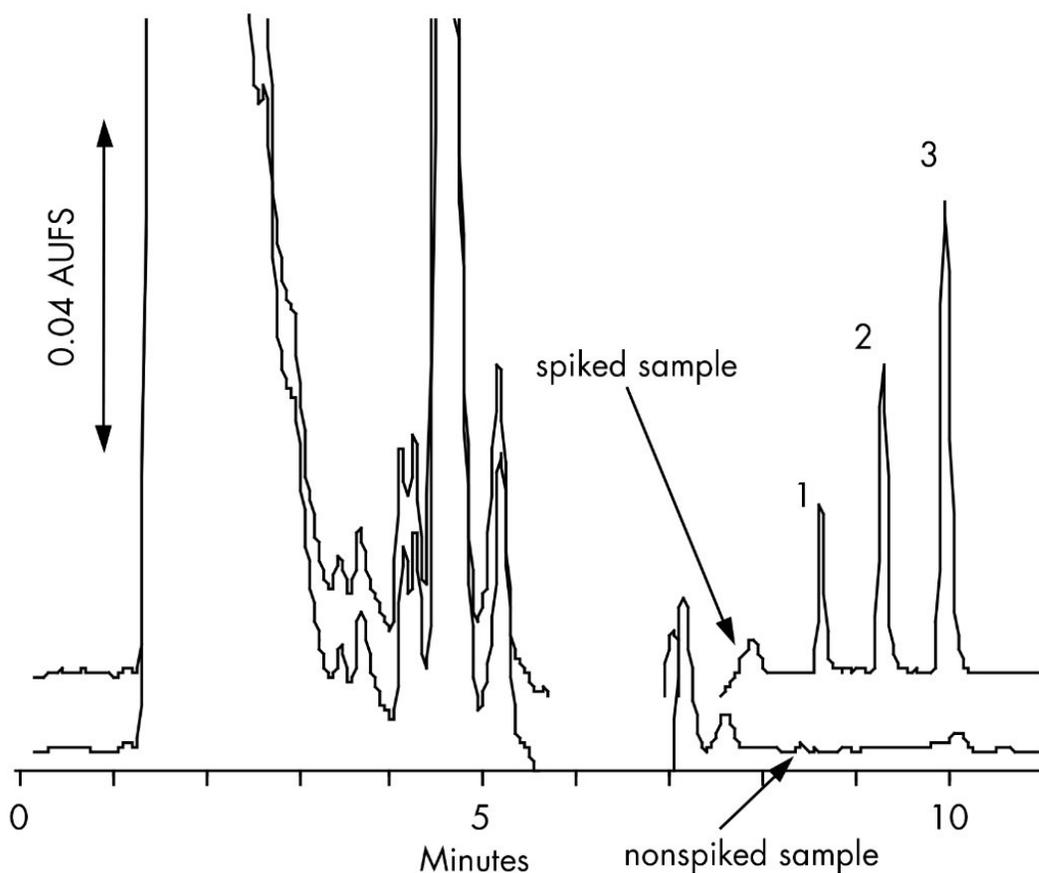
Codeine-6-Glucuronide clearance



Test subject took a therapeutic 60 mg oral dose of Codeine and subsequently had urine monitored over a 21 hour

timeframe. Peaks of interest were confirmed by LC-MS, at the 10.3 hour timepoint, to be Codeine-6-Glucuronide and Codeine

CHROMATOGRAM OF URINE EXTRACTS: A) BLANK B) SPIKED SAMPLE



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