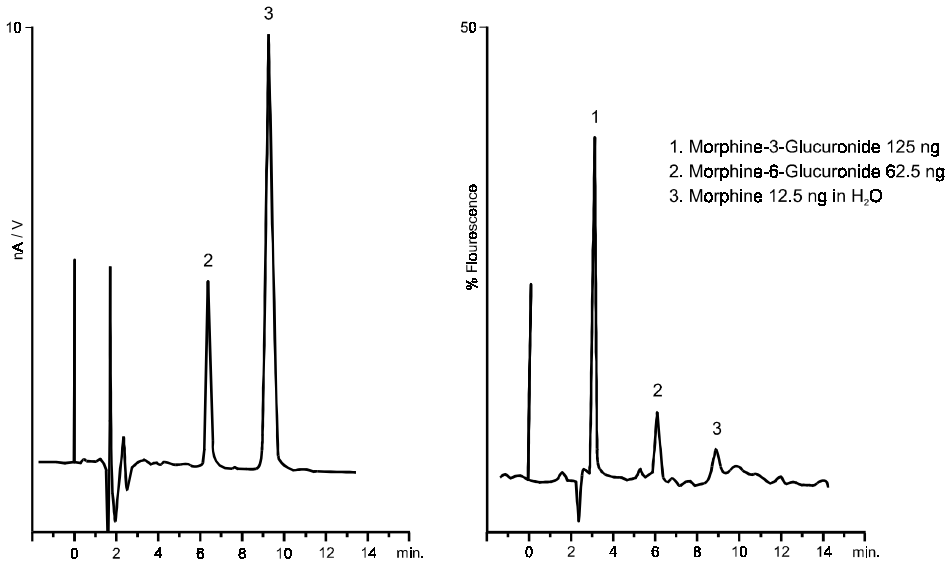
Separation of Morphine and Metabolites Extracted from Blood Plasma

Application Note

Forensic Toxicology

Robert Ricker

Detection and quantitation of morphine and its metabolites in blood is important for forensic applications.



A) electrochemical detect. (BAS Unijet) 720 mV

B) fluorescence detect. (Waters 470) exc. = 285 nm, em. = 352 nm

Courtesy of J. Visser, Center for Pharmacy, University of Groningen, The Netherlands

Conditions:
ZORBAX SB-C18 (3.5µm; 4.6 x 150 mm) (Agilent P/N: 863953-902)
Mobile Phase: (97 : 3) 70mM KH_2PO_4 + 1mM EDTA : ACN, pH 4.5
Injection volume 50µL, 1.5 mL/min

Highlights

- *Good resolution and peak shape for morphine and two metabolites using ZORBAX SB-C18 at moderate pH.*
- *Electrochemical detection sensitively detects morphine and the metabolite morpine-6-glucuronide; while fluorescence allows sensitive detection of morphine-3-glucuronide.*

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SAMPLE PREPARATION

1. *Blood plasma (0.5 mL) is mixed with 0.1 ml internal standard, 0.5 mL H_2O, 1 ml of 0.2M borate buffer pH 9, and 0.2 mL of 0.1M pentane-1-sulphonate. After each addition, the mix should be vortexed. The pentane-1-sulphonate solution must be prepared fresh weekly.*

2. *A solid phase C18 cartridge is activated with 3 x 1 mL MeOH followed by 3 x 1 mL H_2O. The mixture above is then added to the cartridge and flushed with 3 x 1 mL H_2O. Elution follows with 1 mL MeOH. This fraction is dried under nitrogen at 60° C, dissolved in 200µL of solvent, and vortexed. 50µL is injected onto the column.*

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Printed in the USA
April 25, 2002
5988-6381EN

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