HPLC application note

Morphine Determination by RP-LCEC

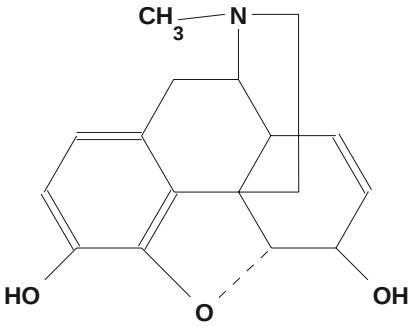
Abstract

A method is described for the determination of morphine by reversed phase LCEC (Liquid Chromatography with Electrochemical Detection). Sensitivity of the method is in the sub-ppm range and the retention time of morphine is approximately 7 minutes.

'White was the first to report a HPLC method... because of its sensitivity and specificity, this technique has become the method-of-choice for the determination of morphine in biological fluids...'

Morphine was first isolated in 1803 and since then a number of related compounds have been synthesised in an attempt to overcome the main drawbacks of the parent drug *i.e.*, tolerance and dependence on heroin and the 3,6-diacetate of morphine. In the body, heroin undergoes rapid enzymatic deacylation to 6-acetylmorphine which is further converted to morphine, presumably in the liver.

Immunological assays, specifically RIA, EIA and fluorescence polarisation immunoassays, have all been used for the analysis of morphine with varying degrees of success. TLC is simple and inexpensive but lacks sensitivity and specificity. GC methods require careful and time consuming sample preparation including derivatisation in order to achieve the same sensitivity as RIA.[1]



HPLC is the preferred method for morphine analysis due to its inherent features of specificity, reliability, sensitivity and reduced sample preparation requirements. White was the first to report a HPLC method for morphine use using electrochemical oxidation.[2] Because of its sensitivity and specificity, this technique has become the method-of-choice for the determination of morphine in biological fluids.[1]

Keywords:
Morphine, Electrochemical, LCEC, Pharmaceutical

Serum, plasma and blood preparation techniques for morphine analysis are well reviewed in reference 1.

Conditions

Column:	Sperhisorb S5 ODS2, 250 x 4.6 mm ID
Mobile Phase:	0.2 M Sodium Perchlorate, 0.005 M Sodium Citrate, 3 mM Triethylamine, (adjusted to pH 4.9 with conc.HCl)/ Acetonitrile (90:10) (Helium Sparging)
Flow Rate:	1.5 ml/min
Temperature:	30°C
Detection:	
Working Electrode:	Glassy Carbon
Reference Electrode:	Ag/AgC1(3 M KCl)
Auxiliary Electrode:	Cell Body
Applied Potential:	700 mV
Morphine Standard:	800 pg in column/ 150 mM (20 µl injection vol)

References

1. F.Tagliaro et.al.,'HPLC Determination of Morphine in Biological Samples: An overview of Separation Methods and Detection Techniques', *J.Chromatogr.*, 448, (1989), 215.

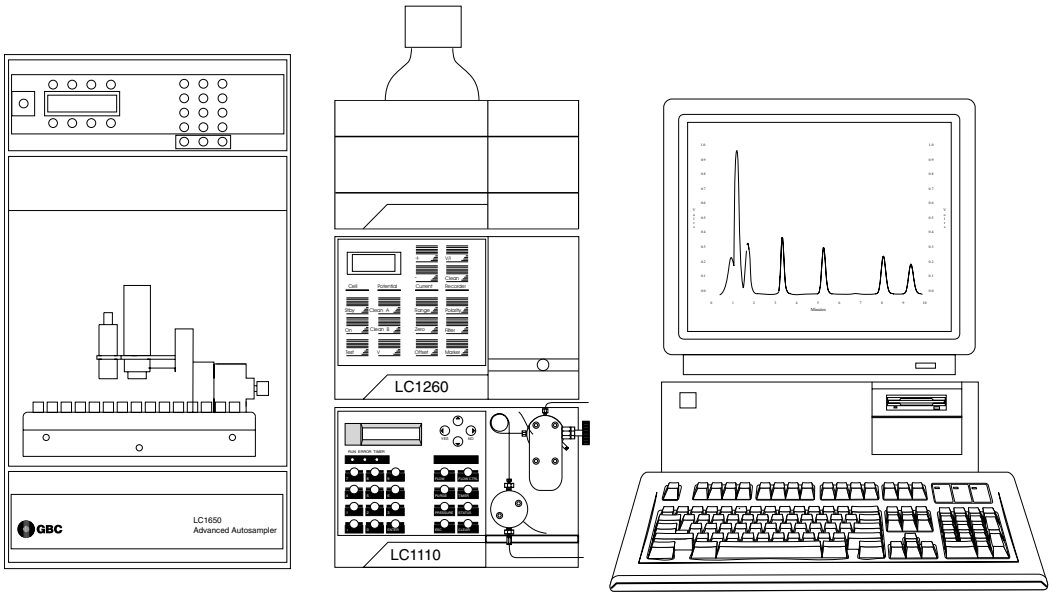
2. M.W.White, *J.Chromatogr.*, 178, (1979), 229.

GBC

P10
01-0381-00

GBC HPLC Instrumentation

LC1110 Dual Piston HPLC Pump
LC1260 Eelctrochemical Detector
LC1650 Advanced Autosampler
WinChrom Chromatography Data Management System
LC1440 System Organiser
GBC Column Heater



GBC

P10
01-0381-00

GBC Scientific Equipment Pty Ltd
A.C.N. 005 472 686
12 Monterey Road, Dandenong, Victoria, 3175, Australia
Phone: (03) 9213 3666 Fax: (03) 9213 3677

GBC publication number 01-0381-00
September, 1995