CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Morphine In Serum

Purpose

Determination of morphine in plasma or serum samples

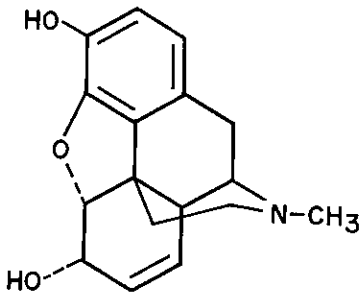

Figure 1. Structure of morphine.

Morphine (F1) is well known as a potent narcotic analgesic because of its effectiveness against all types of acute or severe pain. The mechanism of its action is not exactly known, but the drug is believed to bind to receptor sites for endogenous opioids (endorphins) in the limbic system of the brain. Morphine is used medicinally to reduce pain. Therapeutic doses average 70 ng/mL blood. Side effects associated with its use are increased intracranial pressure, respiratory depression and drug addiction.

Existing Methods

GCMS and GLC using flame ionization or electron capture detection, radioimmunoassay and enzyme immunoassay. These may require derivatization or have low sensitivity or selectivity. LC has been used (references 1-3) but detection is a problem. LCEC provides the desirable combination of high sensitivity, high selectivity, and a fast, effective sample work-up.

Conditions

System: BAS 400 Liquid Chromatograph
Detector: BAS LC-4B
Electrode: BAS glassy carbon
Potential: + 0.70 V vs Ag/AgCl

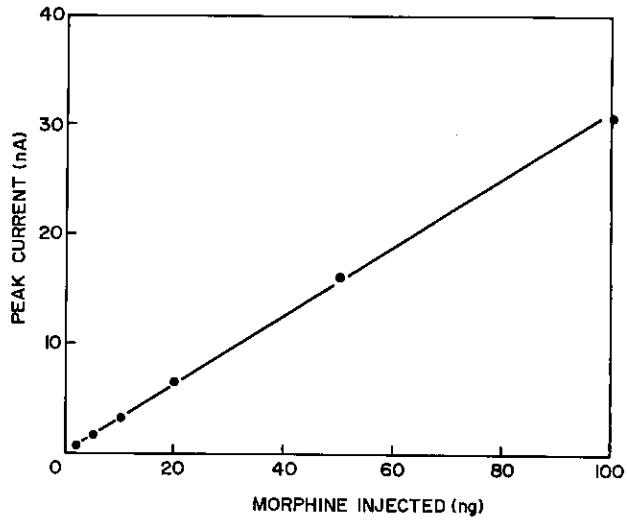

Figure 2. Calibration curve for morphine standards.

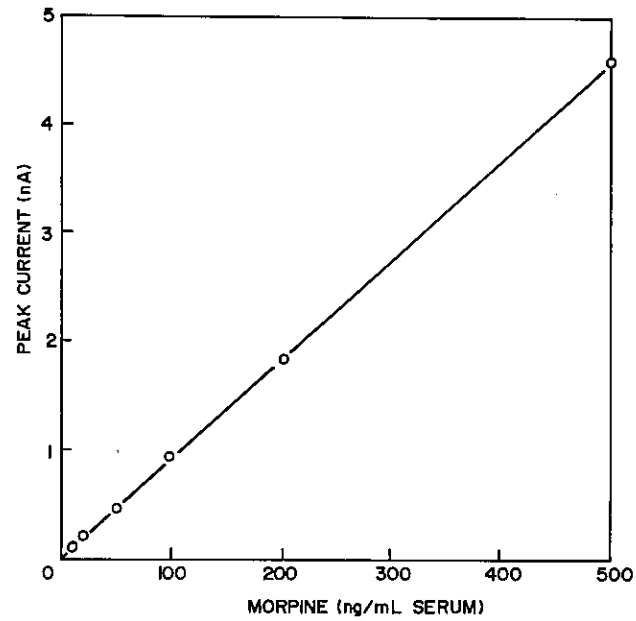

Figure 3. Calibration curve for spiked serum samples.

Column: BAS 3 µm Phase II ODS (100 x 3.2 mm) (PN MF-6213)
Mobile Phase: 95% (v:v) 0.2 M sodium perchlorate, 5 mM sodium citrate, pH 5.0; 5% acetonitrile.

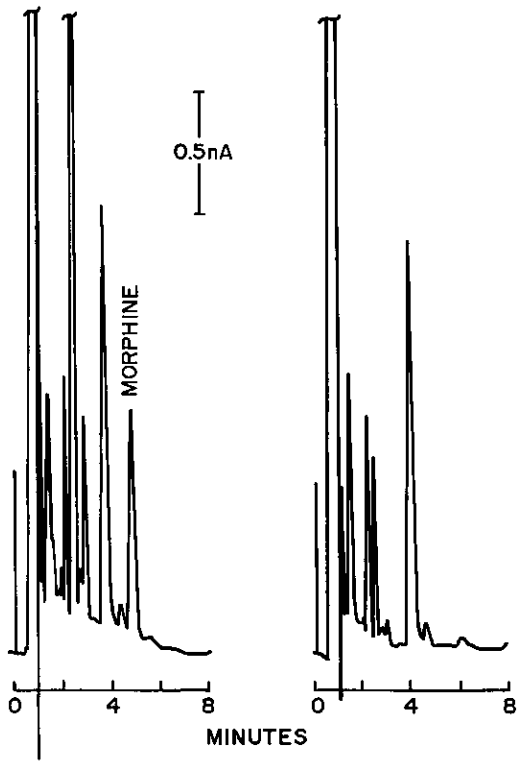

Figure 4. Typical chromatograms for spiked (left, 100 ng/mL serum) and unspiked serum.

Flow Rate: 0.9 mL/min.
Detection Limit: 100 pg injected standard (S/N = 4); 5 ng/mL serum
Linear Range: 100 pg - 500 ng injected standards; 5 - 500 ng/mL serum

Sample Preparation

1. Prepare phosphate buffer: 0.1 M Na_2HPO_4, pH 8.9. Keep refrigerated and replace weekly.

2. Prepare organic extraction solvent: 900 parts chloroform, 100 parts isopropanol.

3. In a 15 mL nalgene centrifuge tube add 125 µL phosphate buffer and 200 µL plasma or serum. Vortex.

4. Add 5 mL chloroform/isopropanol solvent. Cap tubes and vortex 120 seconds.

5. Centrifuge 5 minutes at 1000 x g.

6. Remove the white aqueous layer with a disposable pipet. It is important to remove it all since it contains interfering peaks.

7. Transfer 4 mL of the organic layer to a clean nalgene tube and evaporate to dryness under a stream of nitrogen at 40°C or in a vacuum evaporator.

8. Reconstitute the sample in 200 µL mobile phase. Filter the sample through a 0.45 µm membrane (PN MF-5655) in a microfiltration tube (PN MF-5500) by centrifugation at 1600 x g. Inject a 50 µL volume into the chromatograph.

Notes

A calibration curve for injected morphine standards (morphine sulfate) is presented in F2. A calibration curve for serum samples spiked with morphine is presented in F3. F4 shows typical chromatograms of spiked and unspiked serum.Recovery of morphine from spiked serum was 89%.

Nalgene tubes are used for this procedure because small concentrations of morphine (< 10 ng) are known to be readily adsorbed to active sites on unsilanized glassware [3]. Although the extraction efficiency of morphine from plasma was very good, the addition of an internal standard may be of benefit: suggested compounds are nalorphine, normorphine, or dihydromorphine.

The determination of morphine presented in this report can be duplicated with the BAS 200 Problem Solver.

References

1. M.W. White, J. Chromatogr. 178 (1979) 229-240.

2. J.E. Wallace, S.C. Harris and M.W. Peek, Anal. Chem. 52 (1980) 1328-1330.

3. W. Sadee and G.C.M. Beelen, "Drug Level Monitoring," John Wiley & Sons, New York, 1980, p. 344.