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Note

Determination of morphine, morphine-6-glucuronide and normorphine in plasma and urine with high-performance liquid chromatography and electrochemical detection

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In a previous paper we described a method for the simultaneous determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide and normorphine [1] This method includes sample purification with Sep-Pak C_{18} cartridges, ion-pair reversed-phase high-performance liquid chromatography (HPLC) and UV detection at 210 nm Plasma concentrations as low as 17 5 nmol/l (5 ng/ml) were determined The method was used for studies of morphine kinetics both in man and in animals [2—4] Methods using electrochemical detectors of the amperometric type are reported to detect concentrations of 3.5 nmol/l (1 ng/ml) in plasma [5—9]

Using a coulometric detector with two flow-through porous graphite electrodes, the sensitivity is further improved and morphine, morphine-6-glucuronide and normorphine concentrations as low as 1 nmol/l (0 29 ng/ml) can be determined in a 1-ml plasma sample.

EXPERIMENTAL

The experimental conditions were the same as previously described [1] except that a pulse-dampener (Tillqvist, Solna, Sweden) was installed before the injector, which was equipped with a 2-ml loop, and that a 5100 A Coulochem detector with a 5010 detector cell (ESA, Bedford, MA, U S.A) was coupled to the outlet of the UV detector The injection volume was 1 0 ml instead of 400 μl

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RESULTS AND DISCUSSION

The principal advantage of the coulometric detector, compared to an amperometric detector, is that all of the eluting component can be oxidized (or reduced) at a certain potential With two electrodes coupled in series, it is thus possible to pre-react the more easily oxidizable components at a low potential on the first electrode, and then detect the component of interest at a higher potential on the second electrode This results in a lower background current, and consequently a lower noise level, and a cleaner chromatogram.

Maximum morphine peak height was reached at 0.35 V potential on the

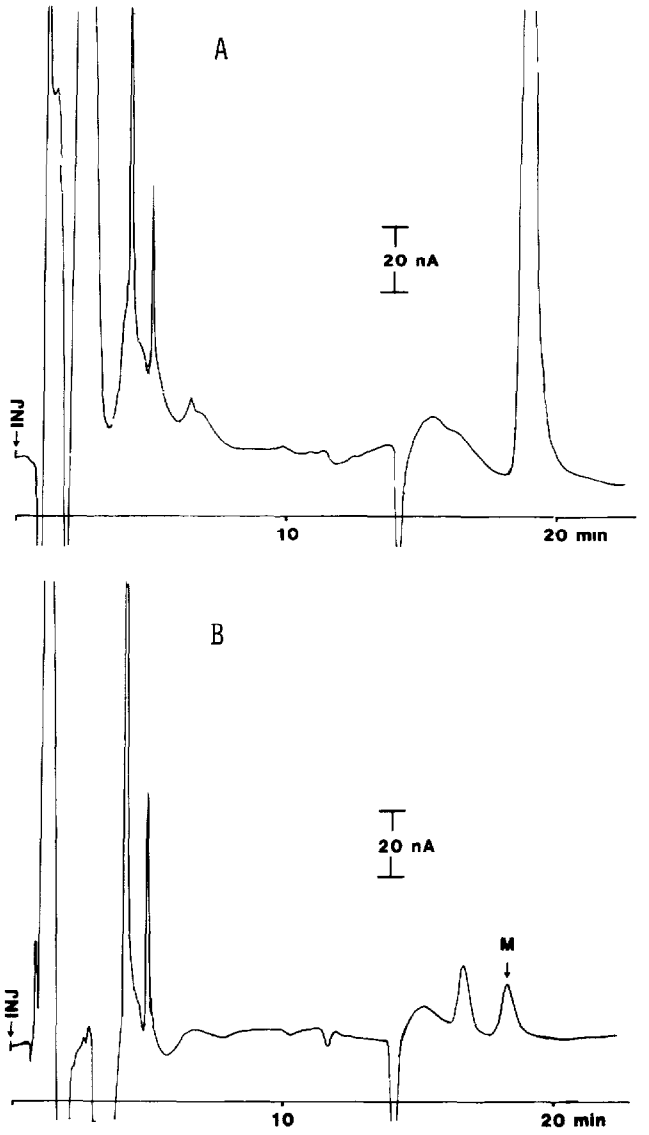

Fig 1

(Continued on p 176)

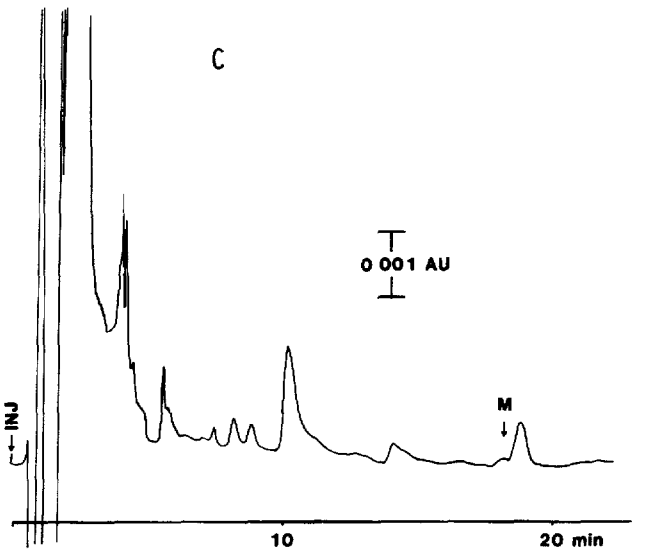

Fig 1 Chromatogram of blank plasma spiked with 10 nmol/l morphine (M) (A) First electrode (+ 0 22 V), (B) second electrode (+ 0 30 V), (C) UV detector at 210 nm

second electrode. This was taken as 100% oxidation. The lower oxidation potential (ca. 0.3 V difference) on the coulometric detector compared to amperometric detectors [5—9] is, according to the manufacturer, due to differences in reference electrode material and arrangement.

The first electrode was set at a potential of +0.22 V. At this potential, ca. 1.6% of the morphine was oxidized and the background current was ca. 310 nA. The second electrode potential was set at +0.30 V. At this potential, ca 95% of the morphine was oxidized, with a background current of ca. 60 nA

Fig 1 shows chromatograms of blank plasma spiked with 10 nmol/l morphine. A component eluting near morphine, also visible in UV, is pre-oxidized at the first electrode, and thus does not interfere with the morphine peak.

A standard curve was obtained by analysis of plasma spiked with 10, 20, 30, 40 and 50 nmol/l morphine The peak areas (peak height × peak width at half height) in mm^2 were determined at 200 nA f.s. recorder deflection. The standard curve was linear ($y = 8.44x - 4.11$; $r = 0.9991$). The lower limit of detection was 1 nmol/l. The coefficient of variation for morphine in plasma was 5.9% at 5 nmol/l ($n = 6$).

Fig 2 shows chromatograms of plasma from a morphine-treated cirrhosis patient. The signal-to-noise ratio is about seven times higher on the electrochemical detector than on the UV detector. Morphine-3-glucuronide is not detected by the electrochemical detector as it has no oxidizable phenolic hydroxyl group

Fig. 3 shows a chromatogram of urine from a morphine-treated cancer patient. Normorphine can only be detected in plasma and urine from patients receiving high doses of morphine.

Since morphine-6-glucuronide is not available as a reference substance, a standard curve for morphine-3-glucuronide was used for the UV detection

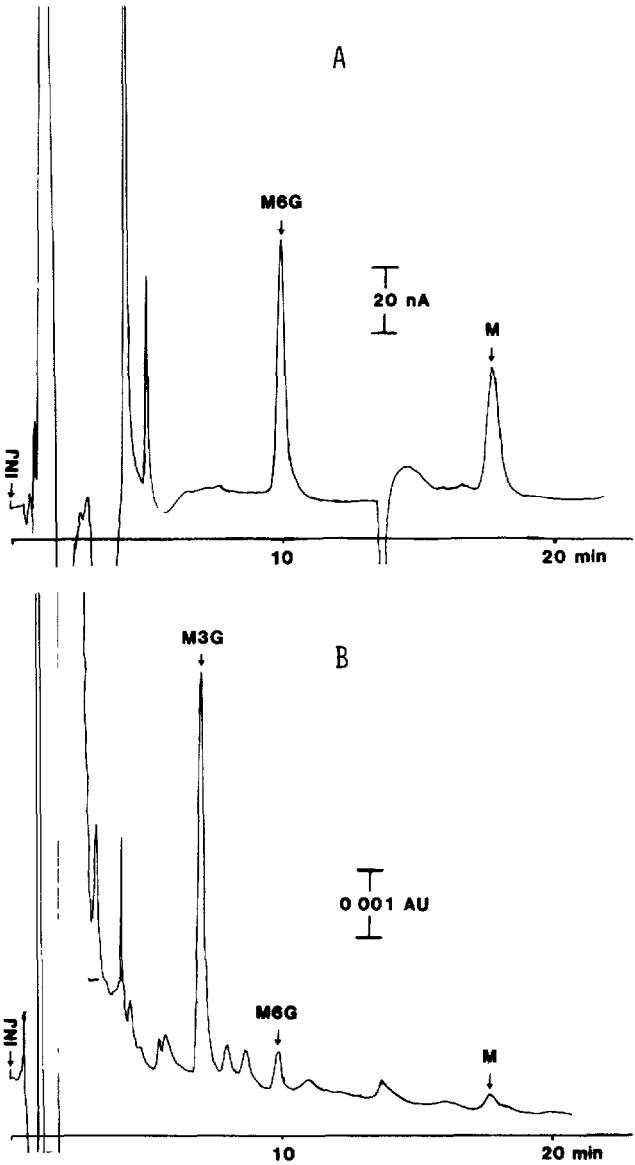

Fig 2 Chromatogram of plasma from a morphine-treated cirrhosis patient (A) Second electrode (+ 0 30 V), (B) UV detector at 210 nm Peaks M3G = morphine-3-glucuronide, M6G = morphine-6-glucuronide, M = morphine

[1]. Morphine, morphine-6-glucuronide and normorphine are oxidized at the same potential, and should, according to the theory of coulometric measurements, give the same molar signal in the coulometric detector. Therefore morphine-6-glucuronide can be quantitated using the standard curve for morphine. The two methods of quantitation of the 6-glucuronide give the same result.

Due to the extreme flow-sensitivity of the electrochemical detector, a pulse-

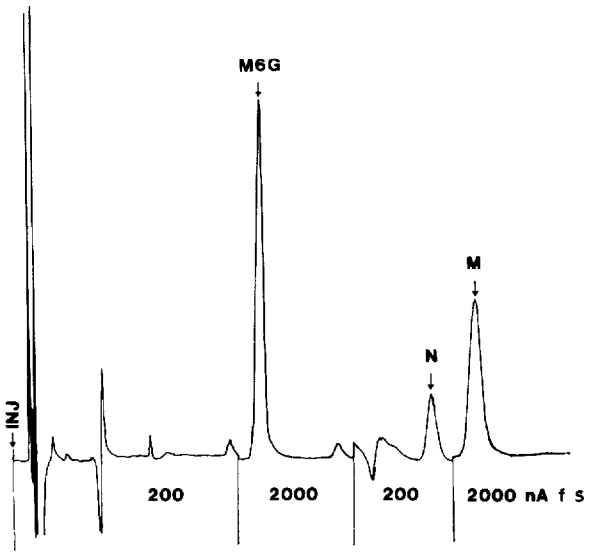

Fig 3 Chromatogram of urine from a morphine-treated cancer patient Second electrode (+ 0 30 V) Peaks M6G = morphine-6-glucuronide, N = normorphine, M = morphine Note the different sensitivities in nA f s , 40 μl injected

dampener placed after the pump is recommended In order to avoid adsorption of morphine, plastic should be used throughout the whole sampling and purification procedure, instead of glass.

This method has been used for several hundred samples, including kinetic studies of morphine in cancer patients, cirrhosis patients and patients with cardiac infarction No interfering peaks from concomitant drug therapy have been observed.

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