Journal of Chromatography, 577 (1992) 354–357
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6331

Short Communication

Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection

A. I. Bouquillon, D. Freeman and D. E. Moulin*

Pain Research Program, Departments of Clinical Neurological Sciences and Medicine (Clinical Pharmacology), University of Western Ontario, Victoria Hospital, 375 South Street, London, Ontario N6A 4G5 (Canada)

(First received October 8th, 1990; revised manuscript received February 10th, 1992)

ABSTRACT

High-performance liquid chromatography has become an important analytical tool for the quantitation of opioid drugs. Using solid-phase extraction and coulometric electrochemical detection, we have developed a chromatographic method for the simultaneous measurement of morphine and hydromorphone which is both sensitive and specific. Using 1 ml of plasma, intra-assay and inter-assay data show that the detection limit for accurate quantitation of these compounds is about 1.2 ng/ml (coefficient of variation 11.6%) for morphine and 2.5 ng/ml (coefficient of variation 10.5%) for hydromorphone. The method is simple and readily adaptable to most pharmacokinetic studies and toxic screens involving these drugs.

INTRODUCTION

High-performance liquid chromatography with coulometric electrochemical detection (HPLC–ED) has become an important analytical tool in quantitating compounds containing a phenolic hydoxyl group. These include catecholamines [1] and opiates [2] which have been measured in the picogram range.

Analytical techniques for measuring opiates include radioimmunoassay [3,4], HPLC with ultraviolet detection [5–7] and HPLC with amperometric detection [8,9]. However, radioimmunoassay may lack specificity and the latter two techniques lack the sensitivity that is often required in pharmacokinetic studies measuring low levels of opiates. We have developed a chromatographic method for the simultaneous determination of morphine and hydromorphone using coulometric ED which is both sensitive and specific. Coulometric ED is based on the principle that 100% of the analyte is oxidized at the detector while amperometric detection provides only 5–10% oxidation. Using naltrexone as the internal standard, we also modified an existing solid-phase method [10] for the extraction of morphine and hydromorphone from plasma to avoid a more elaborate and time-consuming liquid extraction technique [11,12]. This method, using smaller volumes of extraction buffer with improved re-

0378-4347/92/$05.00

covery from plasma, permitted the separation and quantitation of these three opiates in a single HPLC run of 20 min.

EXPERIMENTAL

Materials

Morphine sulphate was donated by Victoria Hospital Pharmacy (London, Canada) and hydromorphone hydrochloride (Dilaudid) was supplied by Knoll Pharmaceuticals (Whippany, NJ, USA). Naltrexone was obtained from Health and Welfare Canada. 1-Heptanesulphonic acid (disodium salt) was laboratory grade from BDH (Poole, UK). Disodium hydrogenphosphate was HPLC grade from Mallinckrodt (Paris, KY, USA). Ammonium sulphate, orthophosphoric acid and ammonium hydroxide were analytical grade from BDH (Toronto, Canada). Methanol was HPLC grade from Baker (Phillipsburg, NJ, USA). Triethylamine was HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). Water was purified by a Barnstead Sybron water purifier from Fisher Scientific (Ottawa, Canada). Aliquots (1 ml) of morphine and hydromorphone stock solution (1 mg/ml free base) in purified water were frozen at −20°C until ready for use. Stock solution of naltrexone (1 mg/ml free base) was stored at 4°C.

Chromatographic separation and quantitation

The chromatographic columns used were an Upchurch Scientific (Oak Harbor, WA, USA) C_{18} guard column and a Spherisorb reversed-phase (Phase Sep, Clwyd, UK) C_8, 5-μm analytical column (10 cm × 3.2 mm I.D.). The chromatographic system was composed of an ESA Model 5700 solvent delivery module (Bedford, MA, USA), a Rheodyne Model 7125 injector with a 20-μl injection loop and a Coulochem electrochemical detector (ESA). The Coulochem detector is composed of a solvent conditioning cell, a guard cell (detector 1) and an analytical cell (detector 2). These were set at +650, +250 and +600 mV, respectively. The column effluent was monitored at the analytical cell (detector 2) and the signal quantitated using peak heights on a Spectra-Physics SP4290 recorder (San Jose, CA, USA).

The mobile phase consisted of methanol–50 m*M* disodium hydrogenphosphate (1.5:8.5, v/v) with 3 m*M* 1-heptanesulphonic acid adjusted to pH 3.5 with orthophosphoric acid. The mobile phase was filtered with a 0.45-μm filter (Millipore, Bedford, MA, USA) and degassed by vacuum and sonication. It was pumped at room temperature at a flow-rate of 0.8 ml/min.

Maximal response is desired for all compounds of interest and so a hydrodynamic voltammogram was generated for each of the three opiates. This was done by increasing the potential at detector 2 in increments of 50 mV from +500 to +1000 mV. The potential setting of +600 mV was finally selected as it was at the beginning of the plateau in the voltammogram for morphine which has the lowest oxidation potential of the three opioids in accordance with published data [2]. Higher settings were found to reduce the signal-to-noise ratio.

Standard curves for morphine and hydromorphone were generated from standards made up in plasma. This was processed and quantitated by internal standardization using naltrexone. Typical retention times for morphine, hydromorphone and naltrexone were 5.2, 8.0 and 16.4 min, respectively.

Extraction

Sample purification was carried out using a method based on that described by Svensson [10]. Several modifications were made to this extraction technique including the use of smaller buffer volumes and a single extraction step. Frozen (−20°C) plasma samples were thawed and centrifuged at 50 g for 2 min at room temperature to remove any proteinaceous material. Extraction cartridges (1-ml C_{18} columns, Baxter Healthcare, Muskegon, MI, USA) were installed on a vacuum manifold (Baker SPE) and conditioned with 3 ml of methanol followed by 3 ml of water. A 1-ml plasma aliquot buffered with 2 ml of 500 m*M* ammonium sulphate (pH 9.3) and 30 μl of internal standard (naltrexone, 1 ng/μl) was then applied to the column and washed with 3 ml of 5

m*M* ammonium sulphate (pH 9.3) followed by 3 × 1 ml of water. Vacuum was applied to withdraw residual water from the columns. The retained morphine, hydromorphone and naltrexone were eluted with 1 ml of methanol containing 0.5% triethylamine. The eluent was then dried under nitrogen at room temperature (Canox, London, Canada) and reconstituted with 100 μl of mobile phase. A 20-μl aliquot of the sample was chromatographed. The percentage recovery was determined by comparing extracted *versus* unextracted samples in the absence of the internal standard.

RESULTS

The recoveries of opiates from plasma extracted with Baxter C_{18} columns were similar for both hydromorphone (88%) and morphine (86%) and highest for naltrexone (94%). These findings demonstrate a strong correlation with recoveries from liquid extractions [2,8,11].

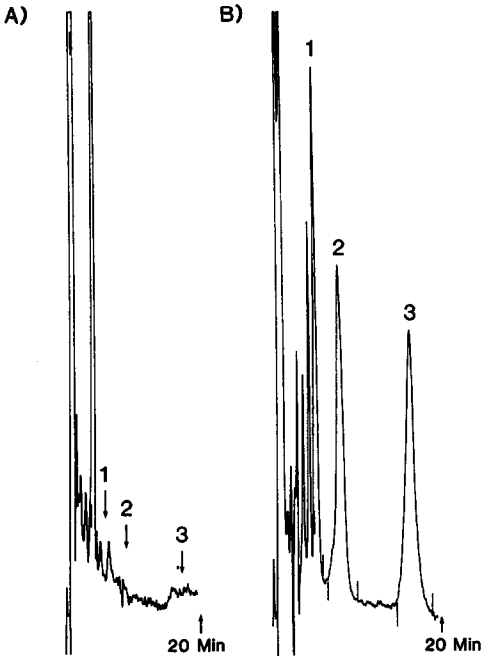

Fig. 1. (A) Chromatogram of blank plasma; (B) chromatogram of extracted plasma containing (1) morphine (20 ng/ml), (2) hydromorphone (80 ng/ml) and (3) naltrexone (internal standard) (90 ng/ml).

Chromatograms representing a typical separation of the opiates after extraction of blank plasma and patient plasma are shown in Fig. 1A and B. The blank plasma chromatogram verifies that there are no interfering peaks present. Baseline resolution was obtained between morphine, hydromorphone and naltrexone.

The standard curve generated for morphine over a concentration interval between 1.6 and 130 ng/ml was linear with a coefficient of correlation of 0.9976 and an intercept of 0.005. The regression was linear for the hydromorphone plasma standard curve with a coefficient of correlation of 0.9751 and an intercept of 0.292 over a concentration range between 1.2 and 30 ng/ml.

Table I shows the analytical precision of our method using intra-assay and inter-assay data. Judging from these data, an acceptable detection limit for accurate quantitation of these compounds is 1.2 ng/ml (coefficient of variation, C.V. = 11.6%) for morphine and 2.5 ng/ml (C.V. = 10.5%) for hydromorphone using 1 ml of plasma.

DISCUSSION

This is the first description of a method for the simultaneous solid-phase extraction of morphine and hydromorphone. Solid-phase extraction is less labour-intensive than the liquid extraction techniques employed by other authors [2,11,12] and can be carried out in volumes of plasma ranging from a few microlitres to 1 ml. This may be an advantage in the analysis of pediatric samples where plasma volume may be restricted. In addition, solid-phase extraction of plasma or serum provides high recoveries of the opioids.

This chromatographic method (HPLC–ED) is both sensitive and specific for the simultaneous measurement of morphine and hydromorphone in plasma. This is an advantage for clinical pharmacokinetic studies involving these drugs in cancer patients with chronic pain. Such studies require a fixed protocol for the administration of the opiate analgesic under investigation and the availability of a second narcotic analgesic to manage breakthrough pain. We suggest that the

TABLE I

ANALYTICAL PRECISION AND ACCURACY

Compound	Actual concentration (ng/ml)	*n*	Measured concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
Intra-assay				
Morphine	1.2	5	1.5 ± 0.17	11.6
	2.4	4	2.6 ± 0.31	11.8
	6.0	6	6.8 ± 0.35	5.1
Hydromorphone	1.2	8	0.9 ± 0.16	17.3
	2.4	10	2.6 ± 0.28	10.5
	6.0	12	6.5 ± 0.22	2.6
Inter-assay				
Morphine	1.6	7	1.7 ± 0.15	8.8
	2.4	7	2.5 ± 0.26	10.4
	6.0	9	5.8 ± 0.52	8.9
Hydromorphone	1.2	6	1.6 ± 0.38	24.0
	2.4	6	2.1 ± 0.22	10.5
	4.8	5	5.0 ± 0.45	9.0
	6.0	5	6.1 ± 0.55	9.0

lower limit of accurate quantitation based on the use of 1 ml of plasma and a C.V. of 10% is about 1.2 ng/ml for morphine and 2.5 ng/ml for hydromorphone. In adult patients where plasma samples larger than 1 ml are feasible, this value can probably be lowered to 1 ng/ml or less.

In summary, our method of simultaneous extraction and chromatographic analysis of morphine and hydromorphone is simple and readily adaptable to most pharmacokinetic studies and toxic screens involving these drugs.

ACKNOWLEDGEMENTS

This project was supported by a grant from Health and Welfare Canada (6606-3512-52). Dr. Moulin was a Clinical Trials Scholar of the National Cancer Institute of Canada.

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