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Determination of opiates and other basic drugs by high-performance liquid chromatography with electrochemical detection*

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Abstract: A procedure is described for the extraction and determination of morphine (M), hydromorphone (HM), codeine (C) and metoclopramide (MCP) present in human plasma. The drugs are separated by reversed-phase liquid chromatography and detected amperometrically at a glassy carbon electrode. The method provides high sensitivity and selectivity and has been used successfully in bioavailability studies.

Keywords: *Morphine; codeine; hydromorphone; metoclopramide; opiates; reversed-phase LC and electrochemical detection.*

Introduction

Single dose oral administration of morphine (M), codeine (C), hydromorphone (HM), and other basic drugs such as metoclopramide (MCP) produce low plasma concentrations (0–50 ng ml^{-1}) due to their respective large volumes of distribution and/or a large first pass metabolism. Consequently, the detection and quantitation of such low plasma levels require assays which are both highly selective and sensitive. Most procedures in current use for the detection of opiates in biological materials employ either gas–liquid chromatography, which requires derivatization [1], or radioimmunoassay [2, 3] where, unless combined with high-performance liquid chromatography (HPLC), cross-reactivity between structurally similar opiate compounds is a problem. Methods which employ HPLC with UV detection may not provide the sensitivity necessary for the detection of opiates. Coulometry is a relatively new analytical technique [4] which is becoming increasingly popular due to its high sensitivity. An HPLC–electrochemical detection system has been developed which enables the accurate and precise determination of concentrations as low as 1 ng ml^{-1} for M and HM, and 5 ng ml^{-1} for C and MCP levels in plasma. The method has been applied successfully to bioavailability studies of M, C and MCP in the authors' laboratory.

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Experimental

Instrumentation

The HPLC system consisted of a Constametric II HPLC pump (Milton Roy, Florida, USA), a Model 7125 valve injector (Rheodyne Berkeley, California, USA) equipped with a 20 μl loop and a 250 × 4.6 mm column packed with 5 μm Spherisorb cyanopropyl (Hichrom, UK). The detector was a Model 5100A coulometer purchased from ESA instruments equipped with an analytical cell comprised of two glassy carbon electrodes. A low pulse damper (LP-21 SSI) was used at all times to minimize background noise caused by flow fluctuations. Chromatograms were recorded by means of a Model 3390A electronic integrator (Hewlett Packard, Montreal, Canada).

Oxidation potentials

The screening electrode was set at +0.27 V, a potential sufficient to eliminate endogenous contaminants without oxidizing any of the drugs. The working electrode was operated at +0.45 V for the detection of M, HM and their internal standards and at +0.77 V for the analysis of C and M together, MCP and their respective internal standards.

Extraction of drugs from plasma

Plasma aliquots of 0.5 ml were pipetted into 3 ml silanized glass tubes together with internal standard (12.5, 25 or 60 ng nalorphine HCl for M and HM, and 30 ng N-ethyl morphine for C and MCP) and 0.2 ml borate buffer (pH 8.8 for M, C and HM, and pH 9.5 for MCP). The tubes were vortexed and their contents poured onto Clin–Elut (CE 1001) celite extraction columns fixed on a Vac–Elut system. After waiting 3 min for the sample to adsorb onto the column packing, chloroform–isopropanol (95:5, v/v, 7 ml) was added and allowed to elute by gravity into 150 mm silanized glass tubes after which a slight vacuum was applied to maximize recovery. The extracts were evaporated to dryness under a stream of nitrogen gas at 40°C. The residues were reconstituted in 0.3 ml of the mobile phase. For M and HM, the aqueous phase was extracted with 1 ml of chloroform, the organic phase was removed and discarded and the aqueous phase was then washed with 1 ml of pure hexane. For C and MCP the aqueous phase was extracted with 1 ml of hexane and then with 1 ml of 15% chloroform in hexane. These washing procedures removed endogenous impurities without affecting drug concentrations. Volumes of 10 μl of the aqueous phase were injected into the HPLC system. The mobile phase flow rate was maintained at 1.5 ml min^{-1}.

Mobile phases

For the analysis of M and HM, the mobile phase consisted of 10% acetonitrile in 0.015 M phosphoric acid adjusted to pH 4.4. The mobile phases for MCP and the simultaneous assay of C and M were prepared in a similar manner, except that they contained 20% and 15% acetonitrile, respectively. Water was distilled and filtered through an organic cartridge. The mobile phases were degassed prior to use and recirculated in all instances.

Reagents

Morphine sulphate, HM, C base, N-ethylmorphine and nalorphine hydrochloride were a generous gift from the Bureau of Dangerous Drugs, (Ottawa, Canada).

Metoclopramide USP was obtained from Purdue Frederic Inc. (Toronto, Ontario, Canada). Acetonitrile, phosphoric acid and methanol of HPLC grade were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA).

Standard curves

Stock solutions of all drugs were prepared at a concentration of 0.2 mg % in methanol and kept refrigerated. Plasma standards were prepared by adding appropriate amounts of the stock solutions to plasma blanks. Plasma standards were extracted as described. Standard curves were obtained by plotting drug to internal standard peak height ratios vs drug concentration.

Results

The voltammograms illustrated in Fig. 1 indicate the relative response of the analytical cell for the different opiates run with their respective mobile phases. Morphine, HM and their internal standard N presented similar curves. Codeine and its internal standard NEM showed overlapping voltammograms and required higher oxidation potentials. The response of the cell to MCP was comparable to that for C (data not shown). Thus, when analysing M or HM alone, a working potential of +0.45 V was selected. Codeine and MCP were analysed at +0.77 V. The data in Fig. 1 indicate, however, that in pharmacokinetic studies, C and its metabolite M, can be studied simultaneously by setting the working electrode at +0.77 V.

As shown in Fig. 2 the mobile phase in conjunction with a 5 μm cyanopropyl column in the reversed-phase mode resulted in excellent separation of M, N, C and NEM when standard mixtures were injected. The resolution of these drugs from spiked plasma samples also was achieved without the presence of interfering endogenous substances. Figures 3 and 4 show plasma blanks with internal standards and plasma extracts from patients entered in clinical trials for M and C, respectively. Figure 5 illustrates the chromatography of a blank plasma extract with internal standard and an extract from a patient receiving MCP. The separation of HM from M, C and their internal standards has been achieved in the authors' laboratory. However, no chromatograms for HM are presented here.

Data supporting the validity of the analytical methods reported for M, C and MCP are presented in Tables 1, 2 and 3. Results are expressed as mean values ±SD for each drug

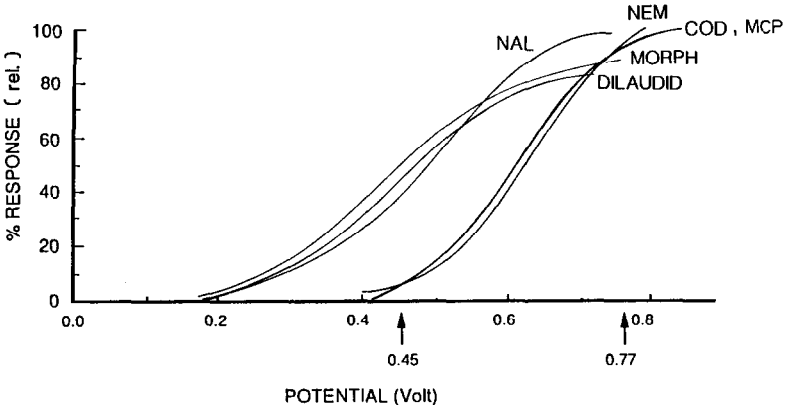

Figure 1

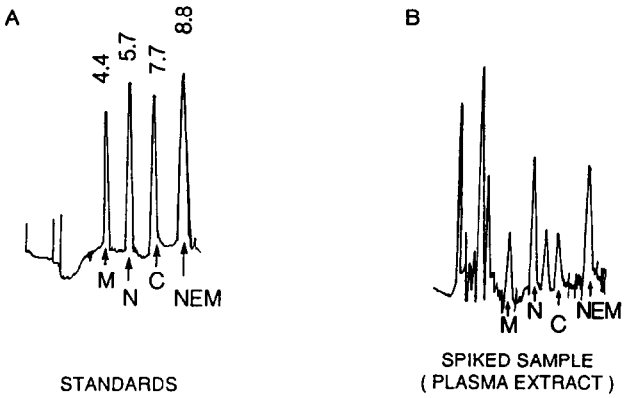

Figure 2

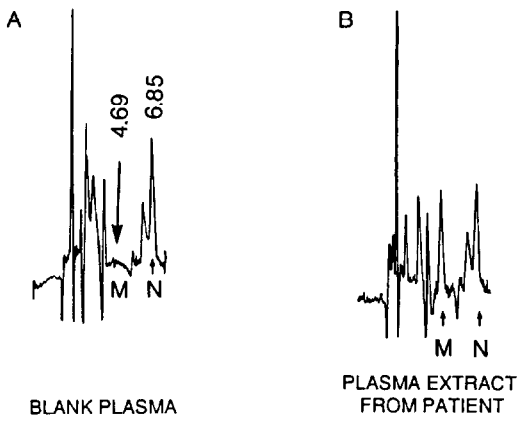

Figure 3

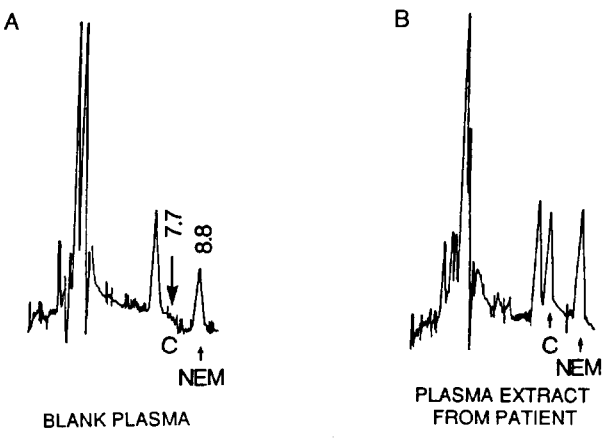

Figure 4

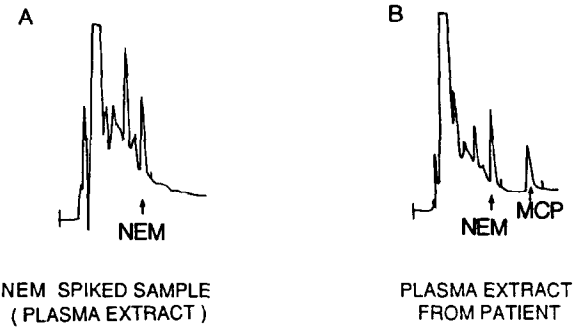

Figure 5

Table 1
Coulometric assay morphine — principal characteristics

Concentration (ng ml^{-1})	Mean peak* height ratio	±SD	RSD (%)	Relative error (spiked samples) (%)	Percentage recovery Morphine	Nalorphine
0	0		0			
1	0.080	0.007	8.71		82.4	
2	0.157	0.014	8.91			
5	0.333	0.025	7.45	8.42		
10	0.683	0.051	6.35		83.4	86.1 (12.5 ng)
15	1.063	0.068	6.42			
20	1.428	0.078	5.49	7.36		
25	1.750	0.094	5.35			87.1
30	1.958	0.095	4.84		83.0	(25 ng)
50	3.312	0.128	3.85	4.25		86.5
100	6.594	0.267	4.05	3.85	85.4	(60 ng)
Mean values			5.62	6.00	83.6	86.6

Equations of the standard curves: $y = 0.0657x + 0.0363$; $y = 0.0354x + 0.0712$; $y = 0.0115x - 0.05$.
Quantity of nalorphine (I.S.) used: 12.5 ng (present data); 25 ng, 50 ng (performed simultaneously with C).
*Mean values of three triplicate analyses performed on different days.

Table 2
Coulometric assay codeine — method of validation

Concentration (ng ml^{-1})	Mean peak* height ratio	±SD	RSD (%)	Relative error* (spiked samples) (%)	Percentage recovery Codeine	N-Ethylmorphine
0	0	0	0			
10	0.12	0.01	8.3		86.5	
20	0.22	0.02	9.1	8.8		
40	0.41	0.03	7.3		87.0	
80	0.86	0.06	7.4	5.4	86.2	
120	1.47	0.12	8.9			85.7
160	2.02	0.14	6.9	4.9	86.2	
Mean values			6.84	6.4	86.5	85.7

Equation of the curve: $y = 0.0125x - 0.03$; $r^2 = 0.9910$.
*Mean values of three triplicate analyses performed on different days.

Table 3
Coulometric assay metoclopramide — method of validation

Concentration (ng ml^{-1})	Mean peak* height ratio	±SD	RSD (%)	Relative error† (spiked samples) (%)	Percentage recovery Metoclopramide	N-Ethylmorphine
12.5	0.20	0.02	14.0	9.12	94.5	
25.0	0.34	0.02	7.8			
50.0	0.64	0.05	8.4			
60.0						98.5
90.0				6.12		
100	1.37	0.014	1.0		95.3	
150	2.19	0.12	5.5			
170				5.30	95.5	
200	3.00	0.25	8.3			
Mean values			7.5	6.85	95.1	98.5

Equation of the curve: $y = 0.0149x - 0.0384$; $r^2 = 0.9980$.
*Mean values of three triplicate analyses performed on different days.
†Mean values of triplicate analysis.

concentration. Relative standard deviations (RSD) and estimates of the relative errors are indicated for low, medium and high concentrations of analytes. The concentration of the internal standard used in the assays depended on the drug concentrations to be analysed and was adjusted to prevent peak height ratios greater than four. The recoveries of M, C and MCP from plasma also are included to show that they did not vary, irrespective of their plasma concentrations, indicating a reliable extraction procedure.

The analysis of HM is at a preliminary stage. When treated identically to M, results of triplicate assays of concentrations from 0 to 100 ng ml^{-1} plasma indicate a mean recovery of 85.3%, a linear analytical response curve ($y = 0.0269x - 0.02$, $R^2 = 0.999$) and a within-run RSD of 6.8%.

Discussion

The extraction of M, C, HM and MCP from plasma on solid-phase celite columns is shown to be reliable and efficient. The procedure provides clean extracts with very good drug recovery, provided that the eluates are collected in silanized glass tubes, otherwise significant and erratic drug losses occur due to adsorption after evaporation. Furthermore, the procedure is attractive since it involves a single step rather than several manipulations typical of liquid–liquid extraction.

The cyanopropyl column in the reversed-phase mode combined with an electrochemical detector provided the selectivity, specificity and sensitivity required for low nanogram concentrations of the drugs and their internal standards.

Recirculation of the mobile phases is cost efficient and it reduces the time necessary for the stabilization of the coulometer to approx. 1 h.

With the ESA Model 5100A coulometer it is not necessary to use a Faraday cage to overcome the electrostatic interferences around the electrodes. Furthermore, it offers a sensitivity such that the usual setting does not exceed 5% of the detectors maximal capacity even when determining concentrations in the low nanogram range.

For trouble free analysis it is important that the water to be used is glass distilled and filtered through an organic cartridge, to avoid the development of problems which completely impede drug analysis. Organic materials accumulate on the column and are slowly leached, thereby producing extraneous peaks and coating the electrodes of the analytical cell.

Pre-column inline filters must be replaced frequently to keep the pressure within the system at a minimum to avoid large demands on the HPLC pump. Pre-cell filters also must be installed to prevent the entry of any particles that could permanently clog the cell. The entire system should be rinsed with glass distilled water and methanol at the end of each working day, otherwise impurities accumulate at the surface of the electrodes thus forcing the analyst to clean the system with a sequence of water, DMSO, methanol, nitric acid (2–6 M) and water rinsing, a procedure which is long and tedious and that decreases the lifespan of the cell.

The high sensitivity obtained throughout the lifespan of the cell (*ca* 2000 analyses), the reproducibility of peak height ratios from one cell to another and the possibility of inverting a cell without losing reproducibility are indicative of a highly reliable analytical system.

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