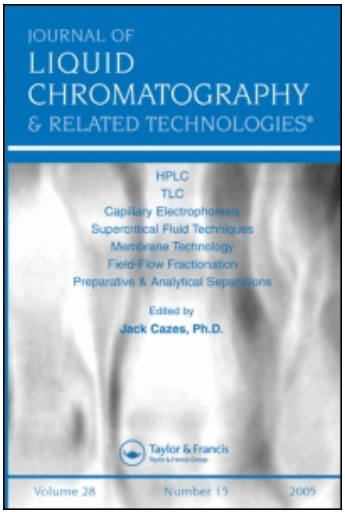

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR MEPTAZINOL AND METABOLITE IN PLASMA AND URINE USING A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE

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ABSTRACT

An HPLC method for quantitating meptazinol, a synthetic narcotic analgesic, and its glucuronide in plasma and urine is presented. The method involves extraction from plasma with methylene chloride under alkaline conditions (pH = 9.7). Urine samples are injected without further preparation following addition of internal standard. The conjugate metabolite of meptazinol was hydrolyzed with ß-glucuronidase. Samples are analyzed using an Ultrasphere Si column with an aqueous mobile phase of 65% acetonitrile and 5 mM dibasic ammonium phosphate at pH = 7.0. The flow rate is 1.0 ml/min. Fluorescent detector settings were 275 nm and 315 nm for excitation and emission wavelengths, respectively. Detection limits were 2 and 5 ng/ml in plasma and 50 and 70 ng/ml in urine for meptazinol and the hydrolyzed glucuronide, respectively. The coefficients of variation for interday and intraday precision were less than 10%. This method has been used in pharmacokinetic studies of i.v. and i.m. formulations involving 1360 plasma and 640 urine samples.

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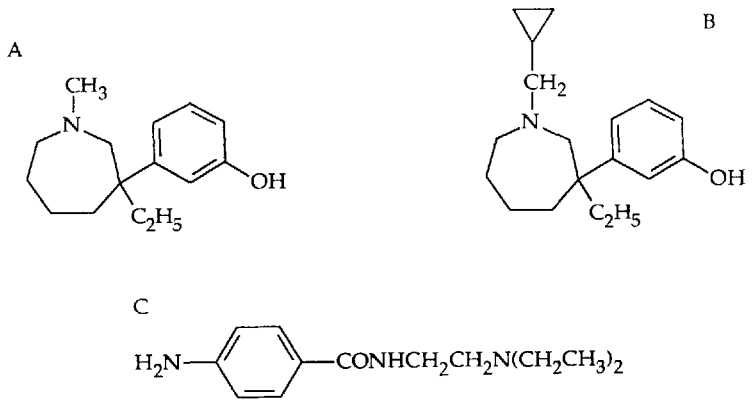

Figure 1: Structures of (A) Meptazinol Base, (B) Plasma Internal Standard Base, and (C) Urine Internal Standard Base (Procainamide).

INTRODUCTION

Meptazinol [3-(3-ethylhexahydro-1-methyl-1H-azepin-3-yl)phenol] (Figure 1) is a new synthetic narcotic analgesic and has been used for control of moderate and severe pain (1). In pharmacokinetic studies, assays for this drug have used gas-liquid chromatography with flame ionization detection (2), high-performance liquid chromatography (HPLC) with fluorescence detection (3) and HPLC with electrochemical detection (4,5). In this paper, an HPLC method with fluorescence detection for quantitation of this drug in human plasma and urine is presented in which an increasing popular technique, separation on a silica gel column run with an aqueous mobile phase (6), is used. This technique has been successfully used in this laboratory to assay plasma and urine for several amines (7,8,9). The advantages of this technique, such as chromatography with a clean base line and resultant high signal to noise ratio at low concentrations, absence of interfering late peaks, good peak symmetry, and consistent results are demonstrated again in this paper.

EXPERIMENTAL

Materials

Meptazinol hydrochloride and the internal standard [(*m*-(1-cyclopropylmethyl-3-ethylhexahydro-1H-azepin-3-yl)phenol) fumarate] for the plasma assay were provided by Wyeth Laboratories Inc., (Philadelphia, PA), lot numbers 1/E/11593 and 185-AW-155, respectively. The internal standard for the urine assay, procainamide hydrochloride, was purchaced from Pfaltz & Baur Inc. (Flushing, NY). ß-glucuronidase was purchased from Sigma Chemical Co. (St. Louis, MO), lot number 123F-3833. All other chemicals were analytical reagent grade. Solvents were HPLC grade.

Chromatographic Conditions

An HPLC Model 110 solvent delivery system (Beckman Instrument, Berkeley, CA) equipped with an automatic sample processor WISP 710B (Waters Associates, Milford, MA) and an Altex ultrasphere Si column (4.6 x 25 cm, 5 µm particle size, Beckman Instruments, Berkeley, CA) were employed. A Shimadzu RF 530 fluorescence detector (Shimadzu Scientific Instruments Inc., Columbia, MD) and an HP integrator Model 3392 (Hewlett-Packard, Santa Clara, CA) were used. The detector operating wavelengths were: excitation at 275 nm and emission at 315 nm. The extraction was carried out in a Glas Col rotator (Glas-Col Apparatus Co., Terre Haute, IN).

Mobile phase was prepared by dissolving 2.6 g of dibasic ammonium phosphate in 1400 ml of nanopure water and 2600 ml of acetonitrile, pH was adjusted to 7.0 with 75% phosphoric acid. The solvent was filtered and degassed before use. The flow rate was 1.0 ml/min. All separations were performed at room temperature.

Sample Preparation

Plasma (0.5 ml) to be assayed for free drug is combined with 100 µl of internal standard solution (0.32 µg/ml of plasma internal standard

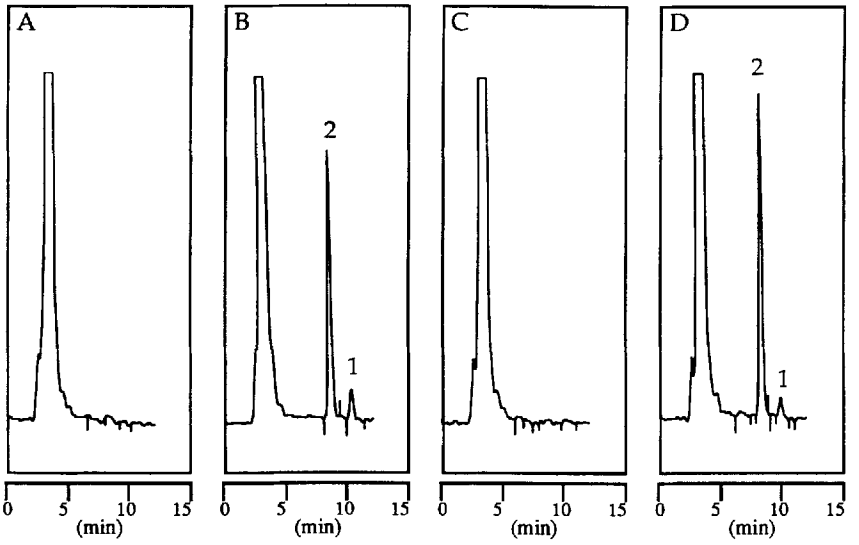

Figure 2: Plasma chromatograms: A (blank) and B (spiked to 8 ng/ml with meptazinol) before hydrolysis. C (blank) and D (spiked to 10 ng/ml with meptazinol) following hydrolysis. (1: Meptazinol, 2: Internal Standard).

as fumarate in water) and 100 µl of a 1 M sodium bicarbonate solution (at pH 9.7). The mixture is extracted (rotate 10 min) with methylene chloride (5 ml). After centrifugation, the aqueous layer is discarded. The organic layer is transferred and evaporated to dryness under a nitrogen stream. The residue is dissolved in 250 µl of 60% acetonitrile and 15 - 35 µl is injected onto the column.

Plasma (0.2 ml) to be assayed for total drug is hydrolyzed by incubation with 400 µl of ß-glucuronidase solution (300 units/ml in 0.05 M sodium acetate, pH = 4.5) at 37°C overnight. Following addition of 100 µl of internal standard solution (0.09 µg/ml of plasma internal standard as fumarate in water) and 600 µl of 1 M sodium bicarbonate solution (at pH = 9.7), the mixture is extracted and processed as described above.

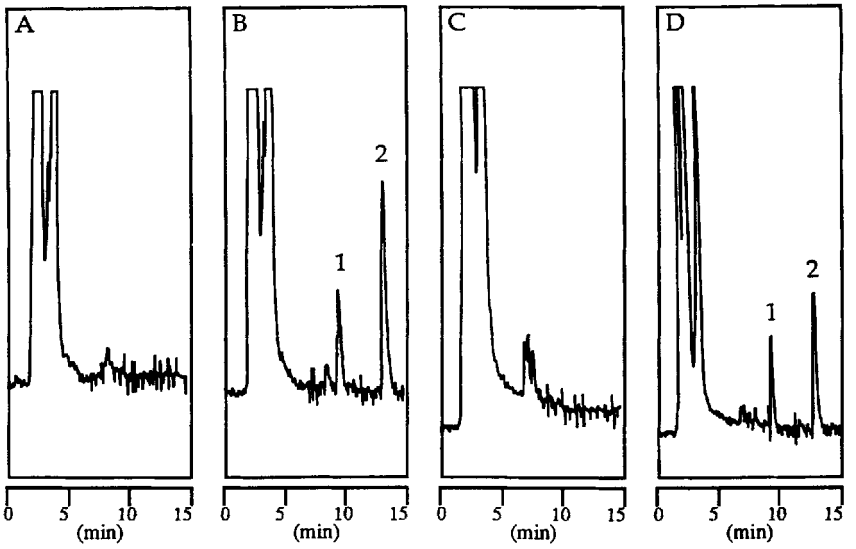

Figure 3: Urine chromatograms: A (blank) and B (spiked to 0.75 µg/ml with meptazinol) before hydrolysis. C (blank) and D (spiked to 10 µg/ml with meptazinol) following hydrolysis. (1: Meptazinol, 2: Internal Standard).

Urine (0.2 ml) to be assayed for free drug is mixed with 50 µl of internal standard solution (12.2 µg/ml of procainamide as hydrochloride in water), and 2 - 20 µl is injected onto the column.

Urine (0.2 ml) to be assayed for total drug is hydrolyzed by incubation with 400 µl of ß-glucuronidase solution (2500 units/ml in 0.05 M sodium acetate , pH 4.5) at 37°C overnight. Then 100 µl of internal standard solution (36.6 µg/ml of procainamide as hydrochloride in water) is added and 2 - 20 µl is injected onto the column.

Plasma standard curve samples were prepared from drug free human plasma at concentrations ranging from 2 ng/ml to 400 ng/ml for the free drug assay and from 5 ng/ml to 1200 ng/ml for the total drug assay.

TABLE 1A: Precision of Plasma Assay for Meptazinol

Spiked Concentration (ng/ml)	Mean (n = 6)	Standard Deviation	Coefficient of Variation (percent)
	Intraday		
400	398	4.72	1.19
200	200	1.75	0.88
40.0	40.2	0.46	1.14
3.00	3.03	0.34	11.2
	Interday		
400	415	8.49	2.05
200	206	4.32	2.10
40.0	42.0	0.96	2.31
3.00	3.05	0.18	5.90

TABLE 1B: Precision of Urine Assay for Meptazinol

Spiked Concentration (μg/ml)	Mean (n = 6)	Standard Deviation	Coefficient of Variation (percent)
	Intraday		
10.0	10.6	0.25	2.36
3.00	3.12	0.08	2.56
0.750	0.756	0.01	1.32
0.100	0.096	0.007	7.29
	Interday		
10.0	10.0	0.26	2.60
3.00	2.94	0.12	4.08
0.750	0.764	0.02	2.56
0.100	0.102	0.01	9.80

Urine standard curve samples were prepared from drug free human urine at concentrations ranging from 0.05 μg/ml to 20 μg/ml for the free drug assay and from 1 μg/ml to 400 μg/ml for the total drug assay.

Samples to test plasma recovery were prepared as described above, except the internal standard was added after the organic layer was evaporated and redissolved in the 60% acetonitrile solution (250 μl). The solvent samples were prepared by spiking standard solution into the same volume of the 60% acetonitrile solution and adding the same volume of internal standard solution as in plasma.

RESULTS

Under the described chromatographic conditions, meptazinol and internal standard form well separated, symmetric peaks with no interference by endogenous substances, either in plasma or in urine samples, before or after hydrolysis (Figures 2 and 3).

The linearity of standard curves (the concentration of drug versus the peak height ratio of meptazinol to internal standard) was excellent, in both the plasma and the urine assays, and before or after hydrolysis. Coefficients of determination (r^2) were better than 0.990 for all standard curves (n = 24 for plasma and n = 11 for urine) used in one study. The detection limits of the method were 2 ng/ml for 0.5 ml plasma samples and 0.05 μg/ml for 0.2 ml urine samples.

The precision of the method at four concentrations selected to demonstrate precision throughtout the range of the standard curve, was estimated by analysis of replicate (n = 6) spiked plasma samples, or spiked urine samples. Coefficients of variation (CVs) ranged from 0.88 to 11.2%, as shown in Tables 1A-B.

The extraction recovery of the plasma assay was assessed at four different concentrations by comparing the assayed peak height ratio of meptazinol in solvent to the assayed peak height ratio of meptazinol in plasma when both set of samples were spiked with equivalent amounts of meptazinol. Mean (n = 3) plasma recovery for the four concentrations ranged from 91.8% to 94.5% and averaged 93.5%.

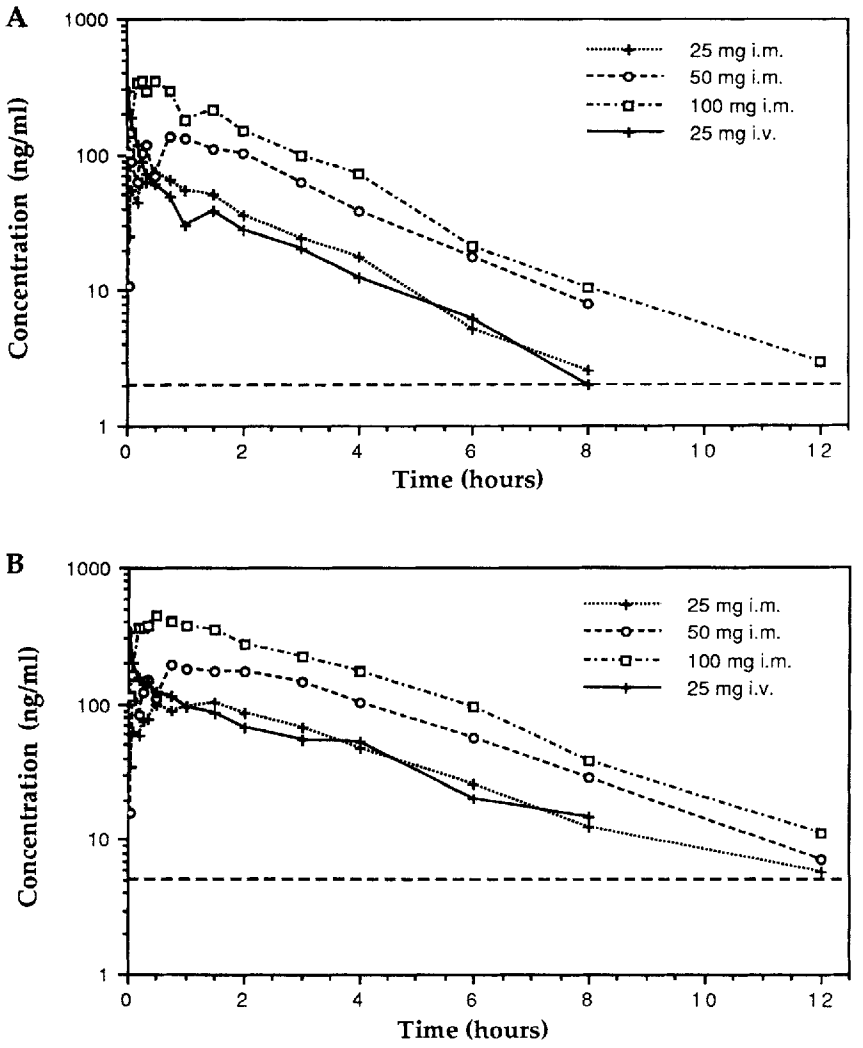

Figure 4: Plasma concentration of meptazinol versus time in one volunteer taking 25, 50, or 100 mg intramuscular or 25 mg intravenous doses of meptazinol HCl. (A): Free meptazinol concentration/time profile. (B): Total meptazinol (following hydrolysis) concentration/time profile.

DISCUSSION

In this method, an increasingly popular chromatographic technique, use of a bare (unbonded) silica gel column run with an aqueous mobile phase (6), is used. This technique is very useful in quantitating basic amine drugs in biological samples (especially in plasma). In this chromatographic system, the non-polar neutral compounds and unprotonated bases in biological samples, which are extracted into organic solvent when plasma samples are cleaned by solvent extraction, are not retained on the silica column and are eluted with the solvent front. Consequently, the chromatogram shows a very clean base line at the retention times of cationized amines (Figures 2 and 3). This invaluable phenomenon results mainly from the retention mechanism of this system, which is mediated primarily via cation exchange interactions with surface silanols, so that only positively charged species (e.g. protonized amines) are retained (6). Comparatively, in a traditional reverse phase system (alkyl-bonded silica as stationary phase), those neutral compounds and unprotonated bases are retained by the column and appear as late peaks in chromatograms, because the retention mechanism is mediated primarily via lipophilic partition. Consequently, these compounds interfere with and distort the peaks of interest, making the detection of drugs at low concentrations less accurate and less consistent. These disadvantages are avoided in the unbonded silica gel/aqueous mobile phase method. Therefore, a detection limit of 2 ng/ml for 0.5 ml plasma samples was obtained for the study described in this paper, which is nearly comparable to the more tedious electrochemical detection method(4). The advantages are also demonstrated in the assay for the conjugated form. Plasma or urine samples after hydrolysis also give clean chromatograms (Figures 2 and 3). Thus, this method is suitable for measuring the concentration of free meptazinol and total meptazinol after hydrolysis in plasma and urine. Thousands of samples in a pharmacokinetic study of this drug have been assayed. A typical drug concentration versus time profile of one volunteer taking 25, 50, and 100 mg intramuscularly and 25 mg intravenously of meptazinol

TABLE 2: Accumulated Urinary Excretion Amount of Meptazinol Over 12 Hours for One Volunteer Taking a 25 mg Intramuscular Meptazinol Dose

Time (h)	Accumulated Amount (μg)	
	Free Drug	Total Drug
0	0	0
1	228	1476
2	320	3008
4	436	5226
6	472	7109
8	489	8115
12	524	9515

hydrochloride is shown in Figure 4. The accumulated urinary excretion amount after a 25 mg i.m. dose is shown in Table 2. The major meptazinol congugate metabolites account for 35.3 ± 9.1% and meptazinol accounts for 3.42 ± 1.68% of the 25 mg intramuscular dose (n = 20) after 24 hours.

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