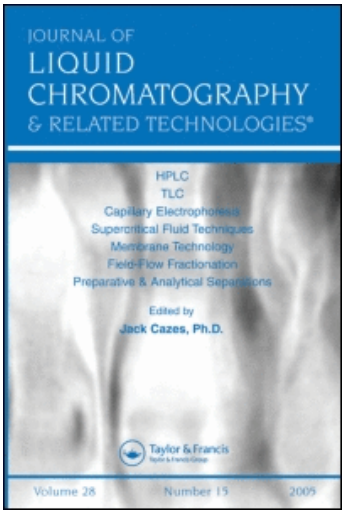

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF HYDROMORPHONE HYDROCHLORIDE INJECTION

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ABSTRACT

A stability indicating high performance liquid chromatographic (HPLC) method for determining hydromorphone hydrochloride in dosage forms is described. The drug was chromatographed on a C_{18} reverse phase column, using a mobile phase consisting of sodium lauryl sulfate, acetic acid, acetonitrile and water, and detected at 280 nm. Linearity of detector response to the concentration was confirmed. The procedure showed excellent reproducibility and gave quantitative recoveries of the drug from spiked placebos. Photodegraded samples of the dosage form, were assayed by the HPLC procedure and the current USP spectrophotometric procedure. Comparison of the results showed that the USP procedure is only partially stability indicating.

INTRODUCTION

Opiate analgesics comprise a large group of drugs which control pain by causing a depressant effect on the central nervous system. Morphine and codeine are the oldest known and still the most widely prescribed members in this family of drugs. In humans, the major detoxification pathway for morphine and structurally related drugs is conjugation with glucuronic acid. The glucuronides accumulate in the kidney and are excreted through urine (1 - 3). In the case of patients with impaired or compromised liver and/or kidney functions, potential for accumulation of these drugs and their metabolites in the body and subsequent onset of their toxic effects are very real. Use of more potent analgesics, which in smaller amounts can provide equieffective pain relief and at the same time reduce the potential for accumulation is the viable alternative in such situations.

Hydromorphone, which is a semisynthetic alkaloid derived from morphine is reported to be 6 to 7 times more potent than morphine in its analgesic action. Currently dosage forms of this drug are available as injectables in vials, ampuls and syringes at 1, 2, 3 and 4 mg/mL. Patient Controlled Analgesia (PCA) devices that were introduced recently have brought into vogue a new dimension of capability in the management of acute pain. These devices can help maintain therapeutic levels of the analgesic in the blood, through programable intermittent intravenous infusions of small quantities of the drug. For use in PCA devices, it is desirable to have the drug formulated at lower concentrations, for example 0.2 mg/mL.

The procedure described in the United States Pharmacopeia for the determination of hydromorphone hydrochloride in dosage

forms is based on the nitroso color reaction of phenols (4). Other alkaloids such as morphine, oxymorphone and thebaine are known to undergo similar color reactions (5 - 7). The presence of either a free or a masked phenolic group appears to be the only structural requirement for a positive response to this reaction. Hence, the procedure can not be expected to be specific for hydromorphone. A literature search for analytical methodology for opiate alkaloids revealed a number of procedures based on GLC, HPLC and RIA for morphine, codeine, thebaine, papaverine, etc (8 - 12). An HPLC method using ion-pair chromatography conditions and electrochemical detection has been recently reported for determination of hydromorphone in plasma (13). However, a selective and stability indicating assay method for hydromorphone hydrochloride in parenteral dosage forms has not been published. This manuscript describes the development of a simple, rapid,accurate, precise and stability indicating HPLC assay for hydromorphone hydrochloride in parenteral dosage forms.

MATERIALS AND METHODS

Apparatus and Chromatography Conditions

a) The liquid chromatograph included a Model 8700 Solvent Delivery System (Spectra Physics, San Jose, CA), Model 710B autosampler (Waters Associates, Milford, MA), and a Spectroflow model 773 variable wavelength UV detector (Kratos, Ramsey, NJ).

b) Integrator cum Recorder - Chromatopac CR3 A Integrator with FDD1A Dual Floppy Disk Drive and a monochrome monitor (Shimadzu, Kyoto, Japan).

c) Columns - Nucleosil C_{18}, 5 micron, 15 cm x 4.6 mm i.d. (Alltech/Applied Science, Deerfield, Il).

Note: A uBondapak C_{18}, 10 micron, 30 cm x 4 mm i.d.column (Waters, Milford, MA) was also found to be suitable for the analysis. However all validation data presented here were generated using the Nucleosil column.

d) Chromatography Conditions: Detector Wavelength, 280 nm; Injection Volume, 100 microliters; Detector Range and Integrator Attenuation Settings appropriate to keep peaks on scale; and Mobile Phase flow rate, 1.5 mL per minute.

Materials

All reagents were analytical grade. Nanopure water and HPLC grade acetonitrile were used in the mobile phase preparation. Hydromorphone Hydrochloride USP Reference Standard and Morphine Sulfate USP Reference Standard were used in the standard and the system suitability solution preparations (See below). Dosage forms and addition samples in placebo were prepared using a lot of hydromorphone hydrochloride purchased from Mallinckrodt, Inc. (St. Louis, MO). Prior to use in these studies, this material was tested to ensure that it met all the specifications for Hydromorphone Hydrochloride, USP.

Mobile Phase

Twenty milliliters of glacial acetic acid, 10 g of sodium lauryl sulfate and 800 mL of acetonitrile were mixed and stirred with sufficient water to make up a volume of 2000 mL. The mixture was filtered through 0.4 um polycarbonate filter membranes (Millipore Corporation, Milford, MA), using house vacuum, changing the membrane each time after about 300 mL of

the mixture had gone through. The pooled solution was filtered once more, this time using a single membrane for the whole volume.

Note: The membranes tended to get plugged frequently when this mobile phase was filtered.

Standard Solution

A stock solution containing 1 mg/mL of Hydromorphone Hydrochloride USP Reference Standard in water was diluted 4 mL to 100 mL to make a standard solution which was 0.04 mg/mL in drug concentration.

Sample Preparation

A suitable volume of the sample (e.g. 5 mL) was quantitatively diluted to an appropriate volume (e.g. 25 mL) to give a sample solution which contained approximately 0.04 mg/mL of hydromorphone hydrochloride.

System Suitability Solution

The system suitability solution contained 0.03 mg/mL of morphine sulfate and 0.04 mg/mL of hydromorphone hydrochloride in water.

Chromatography

Resolution

New columns were conditioned over-night by passing the mobile phase at a flow rate of 0.5 mL/minute. With all system

components in place, the column was equilibrated at a mobile phase flow rate of 1.5 mL/minute for 30 minutes or until a steady base line was obtained and until two successive 100 uL injections of the standard solution gave peak responses within 1.5% of each other. Subsequent 100 uL injection of the system suitability solution gave two well resolved peaks. The resolution factor, was equal to or greater than 2.5. The tailing factor for hydromorphone hydrochloride was less than 1.5. Upon completion of the analysis the column was washed with about 100 mL of water and stored. Validation data presented in here were generated using a pair of dedicated columns.

Procedure

Equal volumes (100 uL) of the standard and the sample preparations were injected into the chromatograph and the respective peak area responses (A_s and A_u) were obtained from the integrator. Average A_s and A_u were obtained from duplicate injections of the standard and sample solutions, respectively.

Calculation

The concentration of hydromorphone hydrochloride in the sample was calculated by the following equation:

$$\frac{Au}{As} \times \text{Standard Concentration (mg/mL)} \times \text{Dilution Factor}$$

$$= \text{mg/mL Hydromorphone Hydrochloride in sample}$$

where: A_U = Average peak area response for sample solution

A_S = Average peak area response for standard solution

Validation of the Assay Method

Linearity

Five solutions of hydromorphone reference standard ranging in concentrations from 0.01 mg/mL to 0.08 mg/mL were prepared. Averaged hydromorphone peak response data from duplicate injections of each, were plotted against the respective concentration.

Reproducibility and Recovery

Reproducibility of the method was established by replicate analyses of the same two lots of the dosage form over a period of four days, by two analysts. Recovery was determined by addition of 89 to 111% of label claim of hydromorphone hydrochloride to a placebo(excipient mixture). The placebo was a citric acid/sodium citrate buffer (0.5 mg/mL of total citrate, pH 4.5), containing sodium chloride (8.75 mg/mL) and disodium edetate (0.5 mg/mL).

Photodegradation studies

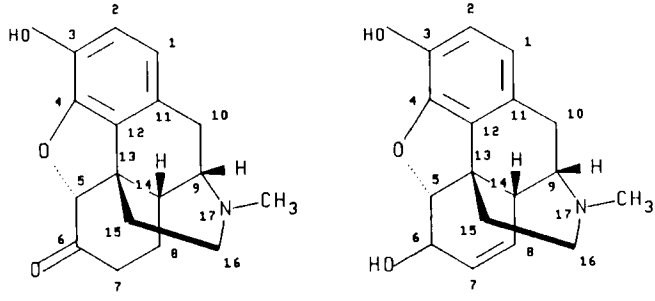
The stability indicating nature of the method was evaluated using solutions that were subjected to degradation by light. One lot of the dosage form was prepared, in 50 mL glass ampuls. A sufficient number of the ampuls were kept in a temperature controlled (25 ± 3° C) light box (Approximately 5' x 4' x 3'). Illumination (1000 foot candles) was provided by 16 fluorescent lamps (Each 40 W, 48", Philips). Samples were assayed by the procedure after seven days and again after 49 days of irradiation.

Comparison with USP Assay Procedure

For evaluating how well the HPLC procedure compares with the USP monograph procedure for the assay of hydromorphone, appropriate amounts of the drug solutions were lyophillized and reconstituted at 1 mg/mL strength and subjected to the USP assay. Typical lots of the dosage form, solutions used in the recovery studies, and light degraded solutions were evaluated by the USP procedure.

RESULTS AND DISCUSSION

Hydromorphone is a semisynthetic alkaloid. It is a valence isomer of morphine. Hydromorphone is conveniently prepared by hydrogenation of the 7,8 - double bond of morphine and subsequent oxidation of the hydroxy group (Figure 1). Since morphine could be expected as one potential trace contaminant in hydromorphone, acceptable resolution between morphine and



HYDROMORPHONE MORPHINE

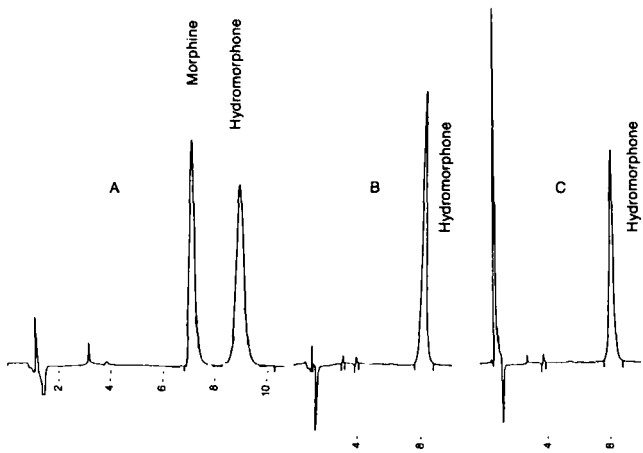
FIGURE 1

STRUCTURES OF MORPHINE AND HYDROMORPHONE

hydromorphone was set as a condition for system suitability in the present method. The chromatographic conditions that were developed, does indeed result in good separation of the two drugs (Figure 2). In fact identical HPLC conditions could be used for quantitation of morphine in parenteral dosage forms.

A plot of peak area response versus concentration of hydromorphone is linear with a correlation coefficient of 1.0000 (Figure 3). Reproducibility data generated using two lots of the dosage form showed a relative standard deviation of ± 0.5% (Table 1).

Addition of the drug into a mixture of excipients gave a mean recovery of 100.1% (RSD ± 0.5%). (Table 2). The excipient



A. System Suitability Preparation B. A Typical Hydromorphone Sample Solution
C. Light Degraded Sample

FIGURE 2

Chromatograms of a System Suitability Preparation, Typical Hydromorphone Hydrochloride Sample Solution and a Light Degraded Sample Solution

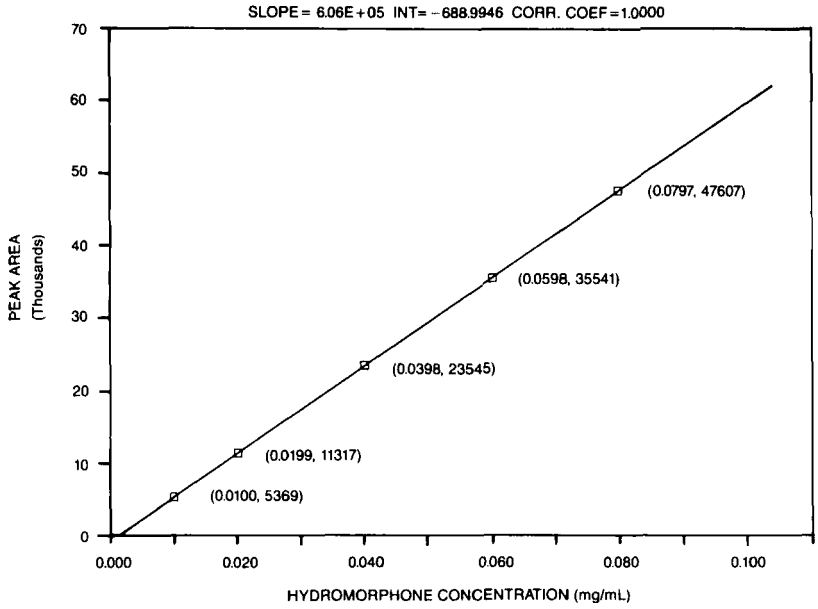


FIGURE 3

Plot of Detector Response (Peak Area) versus Hydromorphone Hydrochloride Concentration (mg/mL)

TABLE 1

Reproducibility of Hydromorphone Hydrochloride

Day	Lot A mg/mL	Lot B mg/mL
1	0.199	0.200
2	0.199	0.200
3	0.198	0.200
4	0.201	0.199
Mean	0.199	0.200
Standard Deviation	± 0.001	± 0.0005
Relative Standard Deviation	± 0.5%	± 0.25%

TABLE 2

Recovery of Hydromorphone Hydrochloride added in Placebo

% Level of Addition	Hydromorphone Hydrochloride (mg/mL) Added	Recovered	% Recovery
89	0.177	0.177	100.0
	0.177	0.177	100.0
	0.177	0.176	99.4
	0.177	0.176	99.4
100	0.199	0.200	100.5
	0.199	0.198	99.5
	0.199	0.200	100.5
	0.199	0.199	100.0
111	0.221	0.222	100.5
	0.221	0.223	100.9
	0.221	0.221	100.0
	0.221	0.221	100.0
		Mean Recovery	100.1%
		Standard Deviation	± 0.5
		Relative Standard Deviation	± 0.5%

mixture consisted of a solution of sodium chloride (8.75 mg/mL), in a citric acid/sodium citrate buffer (equivalent 0.5 mg/mL of total citrate, pH 3.5 - 5.5), also containing disodium edetate (0.5 mg/mL). The levels of addition were 89% to 111% of the label claim for the dosage form (0.2 mg/mL).

Comparison of the assay results from the current procedure and the USP procedure revealed the superior stability indicating nature of the former. Light protected samples of the dosage form gave practically identical results by both procedures (Table 3). Practically identical assay results were also obtained when solutions containing known amounts of the drug in the excipient admixture were subjected to both the procedures (Table 3). In both these situations, the ratios of the respective assay results by the two procedures were practically close to unity.

Table 3

Comparison of HPLC and USP Procedures for the Assay of Hydromorphone Hydrochloride in Non-light Degraded Samples.

Lot # or Hydromorphone Theory (mg/mL)	Hydromorphone Hydrochloride (mg/mL) HPLC	USP	HPLC/USP
Dosage forms			
Lot 1	0.199	0.201	0.990
Lot 2	0.201	0.200	1.005
Lot 3	0.198	0.198	1.000
Lot 4	0.200	0.198	1.010
Lot 5	0.204	0.201	1.015
Lot 6	0.196	0.199	0.985
Addition in Placebo Samples			
0.177	0.176	0.179	0.983
0.177	0.177	0.178	0.994
0.177	0.177	0.179	0.989
0.199	0.200	0.197	1.015
0.199	0.200	0.200	1.000
0.199	0.198	0.199	0.995
0.221	0.222	0.221	1.005
0.221	0.221	0.221	1.000
0.221	0.221	0.221	1.000
		Mean	± 1.000
		Standard Deviation	± 0.010
		Relative Standard Deviation	± 1.0%

However for samples degraded by light, the USP procedure consistently gave higher assay results (Table 4). The ratio of assay results by HPLC to that by the USP procedure seem to depart farther from unity, with the extent of degradation.

Reisch and coworkers have investigated the effect of energy-rich radiation on analgesics such as morphine, hydromorphone and oxycodone (14, 15). These authors have

TABLE 4

Comparison of HPLC and USP Procedures for the Assay of Hydromorphone Hydrochloride in Light Degraded Samples.

Sample Number	Exposure Days *	Hydromorphone Hydrochloride (mg/mL) HPLC	USP	Ratio HPLC/USP
1	7	0.189	0.194	0.974
2	7	0.192	0.194	0.990
3	7	0.189	0.193	0.979
4	49	0.150	0.164	0.915
5	49	0.155	0.173	0.896

* The samples were exposed to 1000 foot-candles of illumination for the duration indicated

reported the formation of at least four degradation products when solutions of hydromorphone is subjected to gamma ray irradiation, and at least two degradation products when the drug is exposed to UV irradiation. These authors have further shown that the photoproducts from UV irradiation of hydromorphone are phenolic in nature. We feel that the photodegradation products of hydromorphone under our illumination conditions are also phenolic in nature. We also think that these photoproducts undergo the same nitroso color reaction as the parent drug and gets quantitated in the USP procedure along with the undegraded drug. We did not make any efforts to isolate and identify the photodegradation products. The HPLC profile of the photodegraded dosage form (Figure 2) did not show peaks due to the degradation products.

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