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Note

High-performance liquid chromatographic analysis of methadone in sustained release formulations

JENG-WU HSIEH, JOSEPH K. H. MA, JOHN P. O'DONNELL and NICOLAS H. CHOULIS*
School of Pharmacy, West Virginia University, Medical Center, Morgantown, W.Va. 26506 (U.S.A.)
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Dole and Nyswander[1] emphasized the importance of methadone in the treatment of heroin addiction. Recently, Choulis and co-workers[2–5] have succeeded in the preparation of sustained release methadone formulations, to be used as substitute to methadone, in the maintenance programs.

The increased need for accurate determination of this compound in the various pharmaceutical preparations and in *in vivo* studies, has led to the development of a number of techniques. The use of UV spectrophotometry was recommended by Wallace *et al.*[6] while McGonigle[7] utilized a fluorometric method. Thin-layer chromatographic (TLC) separation of methadone was carried out by Choulis[8] while Beckett and co-workers[9] detected methadone and its metabolites using gas–liquid chromatography (GLC). Choulis and Papadopoulos[10] measured methadone in sustained release tablets by the same method.

High-performance liquid chromatography is a valuable separation technique due to its superior resolving power and the rapidity with which analysis can be performed. Several types of columns with different functionalities are available and many have already been used in the analysis of drugs of abuse[11,12]. In more recent studies, Beasley and Ziegler[13] described the use of high-performance liquid chromatography (HPLC) for the analysis of methadone hydrochloride in the dosage form of a flavored oral solution. The absorbance was monitored continuously at 280 or 254 nm using a flow-through, UV, double-beam photometer. The authors claim that drug recovery from a syrup was better than 99.8%. However, this method delt with milligram quantities.

In the present investigation the quantitative determination of methadone extracted from sustained release tablets is examined with a ion-pair HPLC technique. Also, the sensitivity of the method for the detection of microgram quantities of methadone is investigated for possible *in vivo* application.

EXPERIMENTAL

Equipment

A high-performance liquid chromatograph (Waters Assoc., Milford, Mass.,

* To whom correspondence should be addressed.

U.S.A.) equipped with a Model 6000A pump, a U6K liquid chromatography injector and a UV detector (Monochromator GM 770) connected to a spectroflow monitor operating at 230 nm was used. The output of the detector was displayed on a recorder (Omniscribe Model A 5213-15, Houston Instruments, Austin, Texas, U.S.A.) having a full-scale range of 10 mV.

HPLC separation of methadone was carried out using a reversed-phase μBondapak C_{18} column (Waters Assoc.).

Materials

Methadone hydrochloride (kindly supplied by Eli Lilly and Co., Indianapolis, Ind., U.S.A.) was used without further purification. Sustained release methadone tablets were prepared according to the methods outlined elsewhere[4]. HPLC grade methanol (Fisher Scientific, Pittsburgh, Pa., U.S.A.), 1-pentanesulfonic acid sodium salt (Eastman-Kodak, Rochester, N.Y., U.S.A.), anthracene (Aldrich, Milwaukee, Wisc., U.S.A.) and glacial acetic acid, all chemically pure, were used.

Mobile phase

To 0.961 gram of the sodium salt of 1-pentanesulfonic acid was added 250 ml double-distilled water. The solution was filtered through a 0.45-μm Millipore filter and degassed. A 750-ml portion of HPLC-grade methanol was added to the water solution and the mixture was adjusted to pH 3.5 with glacial acetic acid.

Chromatographic conditions

The column temperature was ambient. The electrometer was set at 0.01 a.u.f.s. with a recorded chart speed of 2 in. per 10 min. The volume of the samples introduced into the column was 10 μl. The solvent (mobile phase) flow-rate was controlled at 1.0 ml/min.

Internal standard solution

A stock solution of 0.1 mg/ml anthracene in methanol, which was used as an internal standard, was made and transferred into the HPLC samples using a microsyringe. In all samples the final concentration of the internal standard was 0.2 μg/ml.

Preparation of standard and assay methadone solutions

Standard. Solutions containing varying amounts of methadone (5–50 μg/ml) and 0.2 μg/ml of anthracene were injected into the HPLC system with a microsyringe. The results were then used as a standard curve for the assay of sustained release methadone tablets.

Assay. A number of sustained release methadone tablets (containing 10 mg methadone per 40 mg tablet; other ingredients are cellulose acetate hydrogen phthalate, carbopol-934 and magnesium stearate) were weighed and pulverized in a mortar. Quantities of powder containing 5, 10, 15 and 20 mg of tablet powder, or 1.25, 2.50, 3.75 and 5 mg methadone, respectively, were dispensed into 50-ml volumetric flasks, using the mobile phase as solvent. The preparations were shaken well to insure extraction of methadone from the powder. A 1-ml portion of each of the above solutions was then filtered and diluted 5-fold with the mobile phase solvent. After spiking with 0.2 μg/ml of anthracene, 10 μl of the solutions were injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

The results are depicted in Figs. 1 and 2. Fig. 1a represents a typical chromatogram of methadone hydrochloride using a mobile phase of methanol–water (75:25), while Fig. 1b illustrates the response of the same solution when an ion-pair agent, namely the sodium salt of 1-pentanesulfonic acid is present in the mobile phase. It is seen that the ion-pair agent increases the absorption and the resolution of the methadone peak as a result of ion-pair formation between the ammonium ion of the methadone and the sulfonic group of the sodium salt of 1-pentanesulfonic acid. The ion-pair formation proved to be valuable in reversed-phase chromatography[14]

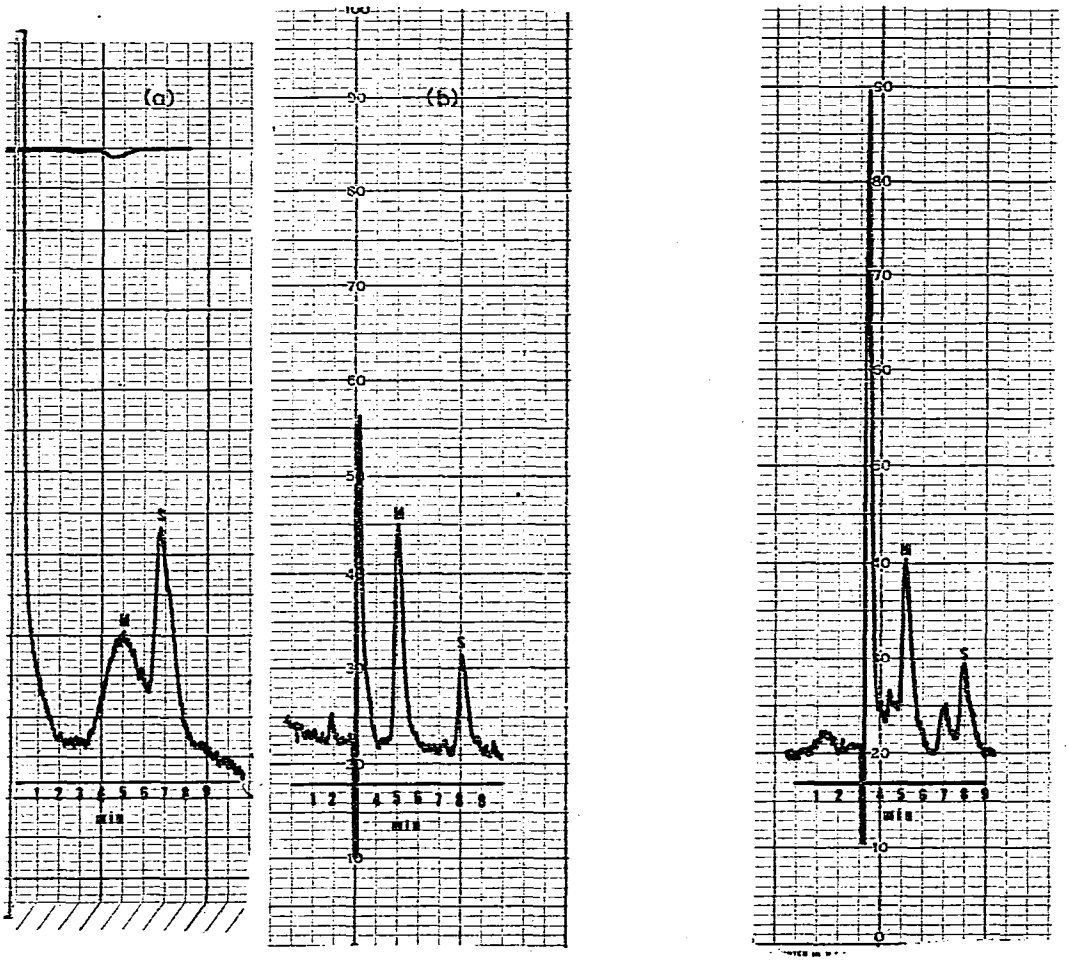

Fig. 1. (a) Typical chromatogram of methadone hydrochloride in a methanol–water (75:25) solution. (b) Chromatogram of methadone hydrochloride in the presence of an ion-pair agent (sodium salt of 1-pentanesulfonic acid). M = Methadone; S = internal standard.

Fig. 2. Chromatogram of methadone hydrochloride obtained from the material isolated from crushed tablets. M = Methadone; S = internal standard.

which is primarily used for separation of neutral species. An ion-pair acts like a neutral molecule which may be successfully retained by the column to achieve a higher resolution.

Fig. 2 shows the chromatogram of methadone obtained from the material isolated from crushed tablets. No interference to the methadone peak from the vehicle materials was detected.

Prior to the experiment, a calibration curve was obtained (Fig. 3) in which the concentration of the internal standard was maintained at a constant level while that of the methadone hydrochloride was varied according to the numbers shown.

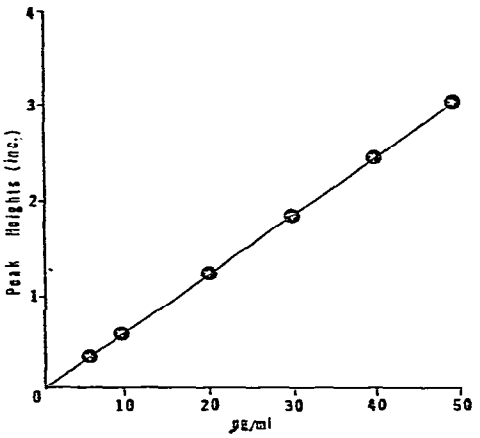

Fig. 3. Calibration curve of methadone hydrochloride.

Table I indicates the recoveries at the various concentration levels of the methadone obtained from the assayed sustained release tablets. A 93.25 ± 5% recovery of the active compound was detected, rendering the method as a sensitive one for the determination of methadone from pharmaceutical preparations using microgram quantities. Furthermore, the high sensitivity and the low quantities (μg) of drug detected utilizing HPLC indicates that this method may be successfully used for the *in vivo* determination of methadone and, therefore, facilitate in the drug abuse detection systems employed widely today.

TABLE I

RECOVERY DATA OF METHADONE FROM SUSTAINED RELEASE TABLETS

Weight of sample (mg)	*Methadone in sample (mg)*	*Methadone recovered (mg)*	*Recovery (±5%)*
5	1.1	1.020	93
10	2.2	2.050	93
15	3.3	3.100	94
20	4.4	4.090	93

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