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High-performance liquid chromatography of deacetylmetipranolol in plasma*

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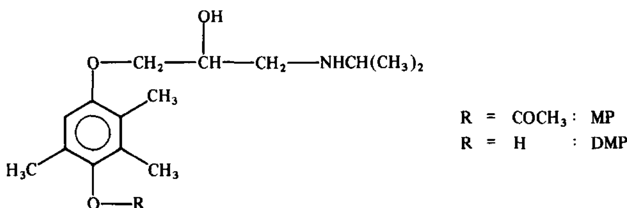
Abstract: A sensitive high-performance liquid chromatographic method with electrochemical detection is developed for the determination of deacetylmetipranolol (DMP), an active metabolite of beta-sympatholytic drug metipranolol (MP). DMP is extracted from the plasma after basifying with dichloromethane. After evaporation, the residue is reconstituted in the mobile phase, injected onto the reversed-phase ODS column and chromatographed at 50°C. The sensitivity of the method is 2 ng of DMP in 1 ml plasma. Pindolol, another beta-blocking agent is used as internal standard. The method is used for a pharmacokinetic investigation of MP in healthy volunteers and in the patients with liver cirrhosis.

Keywords: *High-performance liquid chromatography; beta-blocking agents; metipranolol; deacetylmetipranolol; electrochemical detection.*

Introduction

The beta-blocking agent metipranolol (MP) is very rapidly metabolized *in vivo* to yield deacetylmetipranolol (DMP) which has a non-selective beta-receptor blocking activity [1]. MP is produced and sold as medicament, e.g. by Boehringer–Mannheim, FRG (Disorat Tablets) and by Spofa, Czechoslovakia (Trimepranol Tablets).

The determination of DMP in plasma is possible by gas chromagraphy (GC) [2] or gas chromatography–mass spectrometry (GC–MS) [3] methods and by thin-layer chromatography (TLC) with densitometric evaluation [4]. The disadvantage of the GC and GC–MS methods is the necessity of derivatization of the sample. The TLC method is not sensitive enough for pharmacokinetic studies.



Scheme 1

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Consequently, a high-performance liquid chromatographic (HPLC) method has been developed which is suitable for the determination of plasma levels of DMP.

Experimental

Drugs and formulated drugs

The materials used in the investigation were as follows: metipranolol (Slovakofarma, Hlohovec, Czechoslovakia); deacetylmetipranolol hydrochloride, chromatographically pure (Research Institute for Pharmacy and Biochemistry, Praha, Czechoslovakia); pindolol (INFA, Milan, Italy); propranolol hydrochloride, metoprolol tartrate, timolol maleate, practolol hydrochloride (Research Institute for Pharmacy and Biochemistry, Praha, Czechoslovakia); carazolol–suacron (Boehringer, Mannheim, FRG); trimepranol tablets — 40 mg each (Slovakofarma, Hlohovec, Czechoslovakia).

Reagents

All were of analytical grade and were obtained from different suppliers. Distilled water was HPLC grade.

Apparatus

The chromatographic system consisted of a model LC-6A high-performance liquid chromatograph, integrator Chromatopac C-R 3 A, UV-vis detector SPD-6AV, electrochemical detector L-ECD-6A and spectrofluorimetric detector RF-530, all components from Shimadzu (Japan). Manual injection using a Rheodyne Model 7125 valve fitted with a 20-μl loop was employed.

An amperometric detector comprising three electrodes, namely a working glassy carbon electrode, a reference Ag/AgCl electrode and an auxiliary steel capillary electrode, with a cell volume of 3.7 μl was used.

The analytical stainless steel column Zorbax ODS 250 × 4.6 mm, i.d., containing 5 μm particles was used with a guard-column packed with Zorbax ODS (20 μm particles). The mobile phase consisted of methanol–0.05 M phosphoric acid (40:60, v/v) containing 10 mg ethylenediamine tetraacetic acid disodium salt (Na_2EDTA) per litre and adjusted to pH 3.0 with sodium hydroxide. The mobile phase was degassed under a vacuum with sonication before use and the system operated at a flow rate of 0.8 ml min^{-1}. The operating potential of the working electrode was +0.9 V with reference to the Ag/AgCl electrode.

Results and Discussion

Chromatographic conditions for the determination of DMP in model solutions

First the relationship between the signal given by the amperometric detector and the set polarizing voltage was studied systematically. Electrochemical detector oxidation voltage for maximum sensitivity for DMP was chosen by sequential injection of known amounts of standard DMP (20 μl of the solution 250 ng ml^{-1}) at different analytical cell potential settings (0.4–1.3 V) and was found to be at an optimum value at +0.9 V (see Fig. 1).

The sensitivity and linearity of the EC detector response were evaluated by means of seven injections of increasing concentration of DMP (10–100 ng ml^{-1}). The linearity in this concentration range was found to be given by the expression:

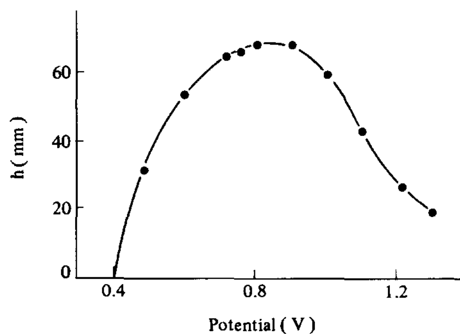

Figure 1
Dependence of the electrochemical response on the applied potential (versus Ag/AgCl) for injections of 5 ng DMP.

$$y = 1.27x + 1.35, (r = 0.999)$$

The precision (reproducibility) of repeated injections of DMP solution containing 100 ng ml^{-1} evaluated from the peak heights gave RSD = 1.7% for $n = 8$.

Internal standard
Several beta-blocking agents, viz. carazolol, metipranolol, metoprolol, pindolol, praktolol, propranolol, and timolol were tested. From these pindolol was found to be the best internal standard.

The comparison of EC, UV and fluorimetric detectors
A comparison of different detection methods was performed under the above chromatographic conditions and the detection limit for DMP was taken as the criterion of performance. The results are summarized in Table 1.

As expected UV detection was the least sensitive. Measurement at 220 nm improved by 10-fold the detection limit compared with 283 nm as analytical wavelength but the noise level increased and with plasma samples measurements were practically impossible owing to the UV absorption of endogenous substance.

The greater sensitivity of the fluorimetric detection compared UV detection was confirmed. However difficulties were experienced with this form of detection in the form of high noise levels presumably caused by the presence of endogenous substances and also by traces impurities in the chemicals and solvents used.

The best sensitivity was obtained with the amperometric detector, which was able to detect 10-fold smaller amounts than the fluorimetric detector and 200-fold smaller quantities than UV detector.

Table 1
The sensitivity of detectors

Detector	Detection limit (ng ml^{-1})
Spectrophotometric	
λ 283 nm	2000
λ 220 nm	200
Fluorimetric	
λ_{ex} 283/λ_{em} 320 nm	10
Electrochemical	1

Application to plasma samples

The therapeutic levels of DMP in 1 ml of plasma are of the order of a few nanograms, and as a consequence, assay by direct injection of plasma onto the LC column is not feasible.

Extraction and determination of DMP

The following extraction step was developed on a knowledge of distribution coefficient for DMP between water and organic solvents [5]. For the analysis of DMP in plasma at concentrations above 5 ng ml^{-1} the following procedure was developed. A 0.5 ml vol. of plasma (from volunteer or patient) was transferred into a screw-cap separatory tube to which was added 10 ng of pindolol, 1 ml of borate buffer (pH 9.0) and 5.0 ml of redistilled dichloromethane. After shaking for 10 min the extraction mixture was centrifuged at 3500 rpm for another 10 min. The organic layer was removed and evaporated under a gentle stream of nitrogen. The residue was reconstituted by vortexing for 30 s in 0.25 ml of the mobile phase, and a 20 μl vol. was injected onto the chromatographic column which was operated at 50°C. Typical chromatograms with EC detection are shown in Fig. 2.

Calibration curve

Calibration samples containing 0, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 50.0, 75.0 and 100.0 ng of DMP, and 10.0 ng of pindolol in 1 ml of blank plasma were analysed as described above.

For the concentration investigated, a linear calibration was found which may be defined by the expression:

$$y = 0.0606x + 0.0012, (r = 0.997)$$

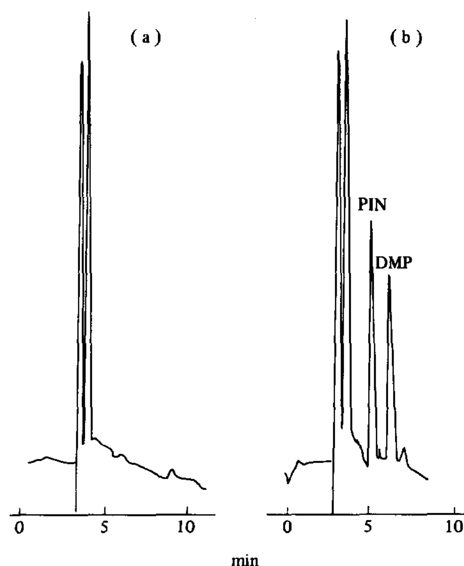

Figure 2
Representative chromatograms for the DMP determination. (a) Blank human plasma, (b) 0.5 ml human plasma spiked with 10 ng of pindolol (PIN, retention time = 5.2 min) and 10 ng of DMP (retention time = 6.3 min).

Precision of the method

The precision was evaluated using the results obtained by repeated analysis of samples containing a known amount of DMP in blank plasma (see Table 2).

A comparison of added and found amounts of DMP in spiked plasma samples (Table 2), found by the analytical procedure, indicate a percentage recovery of 88%.

Consistent with previous knowledge, no other metabolites were found in the samples investigated therefore confirming the specificity of the present method for DMP.

Application of the method

The method has been employed to determine the pharmacokinetics of DMP in plasma of healthy volunteers and patients with liver cirrhosis following a single oral dose of 40 mg MP. Blood samples were taken in K_2EDTA-containing tubes at 0, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after drug administration. They were immediately centrifuged and the plasma stored frozen at −18°C until required for analysis. The plasma DMP concentrations observed for the two healthy subjects and the two patients are illustrated in Fig. 3.

It is intended to use the method in further clinical pharmacokinetic studies in the Institute's laboratories. Also, it may be applied to the pharmacokinetics or therapeutic drug level monitoring of pindolol.

Table 2
Precision of the method

DMP (ng) Added to 1.0 ml of blank plasma	Found in 1.0 ml of spiked plasma ±SD	RSD (%) (n = 5)
5.0	4.1 ± 0.28	6.9
10.0	9.2 ± 0.53	5.7
25.0	21.5 ± 1.37	6.4
50.0	45.5 ± 2.18	4.8

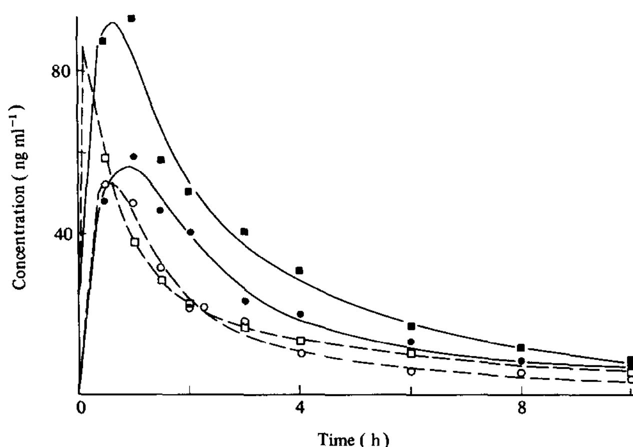

Figure 3
Plasma DMP concentration–time curve obtained from two healthy volunteers (●—●; ○---○) and two patients with liver cirrhosis (■—■; □---□) after a 40 mg oral dose of MP (Trimepranol tablets).

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