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Short communication

High-performance liquid chromatographic determination of pholedrine in human serum using ion-pair extraction and amperometric detection

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Abstract

The method for the quantitation of pholedrine in human serum involves extraction of the sample with benzene utilizing an ion-pairing reagent, bis(2-ethylhexyl)phosphoric acid, and re-extraction into diluted phosphoric acid. Analysis is carried out on a ODS reversed-phase column with heptanesulfonate as the ion-pairing reagent. The procedure allows quantitation at the lower nanogram level and is useful for pharmacokinetic investigations. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Pholedrine

1. Introduction

Pholedrine, *p*-(2-methylaminopropyl)phenol (Fig. 1), an indirectly acting sympathomimetic amine, undergoes considerable first-pass metabolism passing through the gut wall, forming the sulfate conjugate (pholedrine-*O*-sulfate). Only about 10% of the total serum pholedrine exists in the free (unchanged) form [1]. Therefore, therapeutical dosage of pholedrine results in maximum serum levels of less than 100 ng/ml.

In the context of quantification of pholedrine for pharmacokinetics in human serum only one method has been published by Petzsche and Fürst [2]. Pholedrine, isolated by permeation via a matrix stabilized lipid membrane, is fluorimetrically determined after oxidation with Fremy's salt to the *o*-quinone, reaction with ethylendiamine and oxidation to the fluorescent quinoxaline. The procedure is very tedious, time-consuming and the limit of quantitation (LOQ) does not fulfill the requirements for pharmacokinetics. A radioimmunoassay procedure, developed in our laboratory [3], prerequisites the isolation of free and conjugated pholedrine.

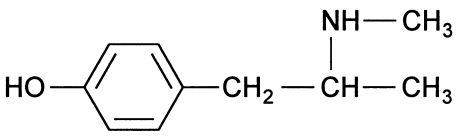

Fig. 1. Structure of pholedrine.

Pholedrine, the *p*-hydroxymethamphetamine (*p*-OHMA), is the main metabolite of methamphetamine. Against the background of drug abuse there are a variety of methods in literature for analysis of *p*-OHMA (see Ref. [4]), especially in urine, but few methods are published for the determination in serum [4–6]. With respect to require-

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ments for pharmacokinitic studies the gas chromatography–nitrogen–phosphorus detection (GC–NPD) assay of Cheung et al. [4] seems to be useful but the preliminary steps in sample preparation – extraction with ethylacetate, back-extraction in 0.1 *M* HCl, second extraction with ethyl acetate, derivatization after evaporation – are laborious.

Liquid chromatographic methods for the analysis of the substance at the lower nanogram level were performed by fluorescence [7] and chemiluminescence [8,9] detection after derivatization, by liquid chromatography–mass spectrometry (LC–MS) [6,10] and by electrochemical detection [11,12].

Pholedrine is an ampholyte with ionization constants of 9.5 and 10.9. The molecule is ionized at all pH values for aqueous solutions, hence sufficient extraction by ion-suppression is not possible. Therefore, sample pretreatment is the critical step of the analytical procedure and a general problem in the bioanalysis of ampholytes of the hydroxyphenylalkylamine and hydroxyphenylaminoalkanole type. The diversity of methods used in this context is representative of the problem. For instance, deproteinization with solvents [13], solid-phase extraction on ion-exchange [14,15], reversed-phase [11,12] or silica [16] cartridges, liquid–liquid ion-pair extraction with tetraphenylborate [17] or bis(2-ethylhexyl)phosphate (HDEHP) [18] are reported, furthermore combined techniques and clean-up with immunochromatography.

It was the objective of this investigation to select conditions for an effective isolation of free pholedrine from human serum followed through an LC procedure which allows quantitation of free pholedrine for pharmacokinetic studies.

2. Experimental

2.1. Reagents

Pholedrine sulfate was a gift from Isis Pharma (Zwickau, Germany). [^{3}H]Pholedrine was prepared according to Ref. [19]. HDEHP (pract.) was from Serva (Heidelberg, Germany). Heptanesulfonic acid, sodium salt (puriss. analytical-reagent grade; ion-pair grade) was from Fluka (Buchs, Switzerland). All other reagents were analytical reagent grade, from commercial sources, and were used without further purification.

2.2. Sample extraction

One ml of plasma, 500 μl of phosphate buffer (0.5 *M*, pH 5.0) and 5 ml of HDEHP in benzene (0.5 *M*) were mixed by shaking mechanically for 10 min and then centrifuged. A 4-ml volume of the organic phase was re-extracted with 600 μl of phosphoric acid (0.05 *M*) as above. A 500-μl volume of the aqueous phase was evaporated under nitrogen and the residue was reconstituted with 100 μl of mobile phase.

2.3. Chromatography

High-performance liquid chromatography (HPLC) was performed at ambient temperature on a Hewlett-Packard 1084B liquid chromatograph.

The amperometric detector was self-constructed and consisted of a thin-layer cell with a 10 mm^2 glassy carbon working electrode and, in the opposite position, a glassy carbon auxiliary electrode, both separated by a 100-μm spacer. The Ag/AgCl (3 *M* KCl) reference electrode was positioned in the outlet of the cell, the electronic unit was constructed according to Ref. [20]. The mobile phase consisted of 2.5 m*M* heptanesulfonic acid and 0.1 *M* acetic acid, adjusted to pH 5.5 with ammonia, containing 0.2% (v/v) diethylamine and 15% (v/v) acetonitrile.

The other conditions were as follows: column, LiChrosorb RP-18, 10 μm (250×4.6 mm I.D.); flow-rate, 1 ml/min; applied potential, 1050 mV; injection volume, 70 μl.

For the calculation of results the external standard method was used regarding the efficiency of extraction. The calibration curves were obtained by plotting the peak-height against the concentration of pholedrine dissolved in the mobile phase.

3. Results and discussion

3.1. Extraction and re-extraction

The use of bis(2-ethylhexyl)phosphoric acid as an ion-pairing reagent and benzene as the solvent for

the extraction of catecholamines has been reported [18]. An estimation of the extraction efficiency of pholedrine was made using a spike of [^{3}H]pholedrine. After ion-pair extraction the organic phase was evaporated and the residue was counted in scintillation cocktail. Independent of the concentration within the controlled range of 1 to 100 ng/ml the extraction efficiency was determined to be 83% and was identical from aqueous solutions and from serum, respectively. The re-extraction efficiency, using 0.05 *M* phosphoric acid, was quantitative. With chloroform or ethylacetate as extractants instead of benzene pholedrine was totally extracted, but in HPLC large extraneous peaks were obtained. The same results gave the clean-up procedures for pholedrine with reversed-phase cartridges and by membrane permeation [2].

3.2. Chromatography and detection

Pholedrine is amphiprotic in nature. More than 80% of the neutral form exists as a zwitterion. Therefore, pholedrine interacts with residual silanol groups of reversed phases. From the reversed-phase material used in this study pholedrine was eluted neither with methanol nor acetonitrile and neither with methanol–phosphate buffers nor acetonitrile–phosphate buffers within the pH range of 4.5 to 7.5. This phenomenon was also observed in solid-phase extraction of pholedrine on a Bond-Elut C_{18} cartridge whereas the LC procedure was performed with an acetonitrile–phosphate buffer on a highly deactivated reversed-phase column [11,12].

Ion-pair reversed-phase LC has been the most commonly used approach for the determination of serum hydroxyphenylalkylamines, hydroxyphenylaminoalkanols and catecholamines. Heptanesulfonic acid as the ion-pairing reagent was useful for the determination of pholedrine. A typical chromatogram is shown in Fig. 2. To select the proper detection potential, a hydrodynamic voltagram was registered (Fig. 3).

3.3. Validation

An overall estimate of the precision of the serum assay at a concentration of 25 ng/ml gave a relative standard deviation (RSD) of 6.2% (n=10). The limit of detection was 0.5 ng/ml (195 pg on column) and the LOQ was 2.5 ng/ml (950 pg on column) at a signal-to-noise ratio of 2.

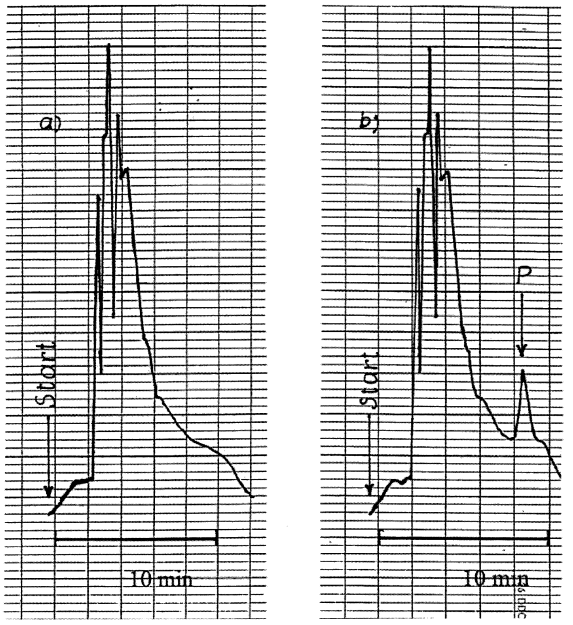

Fig. 2. Chromatograms of (a) blank serum and (b) serum taken 60 min after an oral dose of pholedrine (40 mg; aqueous solution), corresponding 60.7 ng/ml. P=pholedrine.

To control the linearity across the relevant range of 5 to 100 ng/ml spiked serum samples were

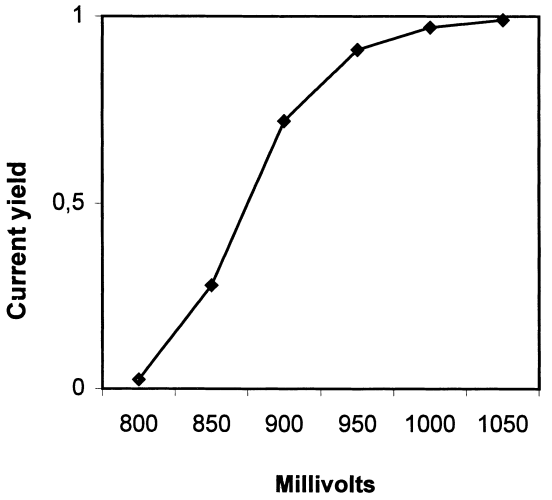

Fig. 3. Hydrodynamic voltagram of pholedrine.

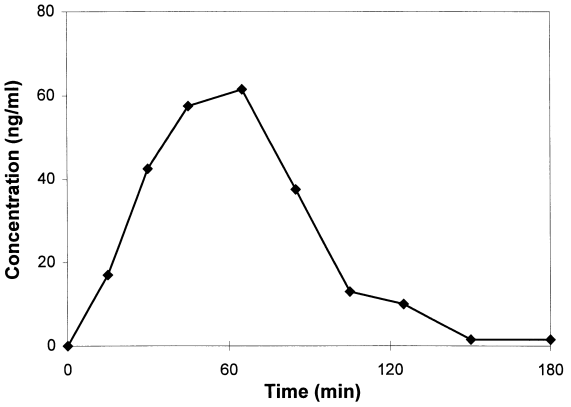

Fig. 4. Change in serum level after oral administration of pholedrine in a volunteer. Dose: 40 mg (aqueous solution).

analyzed. The coefficient of correlation was estimated to be 0.9984.

3.4. Application of the method

The present method was applied to a pharmacokinetic study of free pholedrine in humans after oral administration. A typical concentration–time profile after an oral single dose of 40 mg pholedrine sulfate is shown in Fig. 4.

The profile of the curve is different from the results published by Petzsche et al. [1]. The maximum plasma concentration of 60.7 ng/ml was attained 60 min after administration. After 3 h the concentration of free pholedrine was less than a 2% portion of the maximum level.

Acknowledgements

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