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Note

Determination of vephylline in blood plasma by high-performance liquid chromatography

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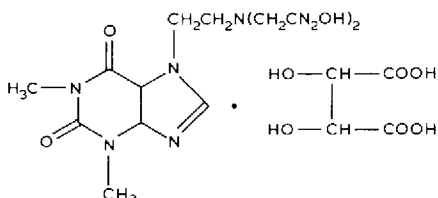
Vephylline, 7-[2-bis(2-hydroxyethyl)aminoethyl]theophylline tartrate, is a theophylline derivative with the following formula:



It was synthesized in 1979 by Gagauzov and co-workers [1].

Although it is similar to theophylline, vephylline has some essential advantageous differences. Pharmacological investigations showed that this compound is effective in the relief of bronchospasm and it has a lower toxicity than theophylline [2]. These properties of vephylline provoked particular interest, and the drug was the subject of profound investigations.

The structural similarity of vephylline and theophylline leads to the assumption that the latter may appear as a major metabolite. Correspondingly, in the present paper, a high-performance liquid chromatographic (HPLC) method for the analysis of vephylline and theophylline is proposed. The procedure is developed with regard to pharmacokinetic and biopharmaceutical studies of the substance and its formulations.

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EXPERIMENTAL

Instrumentation

Chromatography was carried out on a Perkin-Elmer Series 1/1 liquid chromatograph using a fixed-wavelength (254 nm) detector (Perkin-Elmer LC-15). Detection was effected at 0.008 a.u.f.s. A Rheodyne 7010 with an injection loop of 100 μl was used.

The potentiometric investigations were performed by means of a Radiometer PHM 52 digital pH meter with a Radiometer G 202B glass electrode and a Radiometer K 401 saturated calomel electrode.

Drugs and reagents

Vephylline was obtained as a pure compound from the Department of Pharmaceutical Chemistry, Faculty of Pharmacy (Sofia, Bulgaria). Phenobarbital (an internal standard) and theophylline were of pharmacopoeial purity. Methanol, chloroform and isopropanol were used after redestillation.

HPLC conditions

A 250 × 4 mm I.D. column (Hibar-LiChrosorb RP-8, 5 μm; Merck, Darmstadt, F.R.G.) was used.

Since in the molecule of vephylline a tertiary amino group is present, this drug has pronounced acid–base properties, in contrast to theophylline. Hence, by varying the pH value of the mobile phase, proper conditions for the separation of these substances could be achieved. Horváth and co-workers [3, 4] were the first to show such dependence for ionogenic substances in water as a mobile phase. In this laboratory, similar relations were derived for non-aqueous mobile phases [5]. According to Pashankov et al. [5], the graphical dependence of k' versus $[SH_2^+]/K_a$ values* gives relatively complete information concerning the chromatographic behaviour of the substances investigated and allows suitable conditions to be selected for their separation. The graphical presentation of this dependence is a sigmoidal curve, restricted from below with a k'_1 value (the capacity factor of the charged form of the substance) and with a k'_0 value from above (capacity factor of the uncharged form), the inflection point being $pH^* = pK_a^*$ (see footnote and Fig. 2).

The acid—base constant (pK_a^* value) of vephylline was determined previously in a mixture of methanol—water (30:70). For this purpose, a straightforward potentiometric titration method was used in which no preliminary calibration of the cell was needed [6]. In Fig. 1, a Gran plot of this titration is presented and the pK_a^* value of vephylline was determined (5.976 ± 0.003; t_0 = 22°C, μ = 0.1). As expected, theophylline showed an absence of acid–base properties in this medium (methanol–water, 30:70).

The capacity factors k'_0 and k'_1 were determined from two chromatograms.

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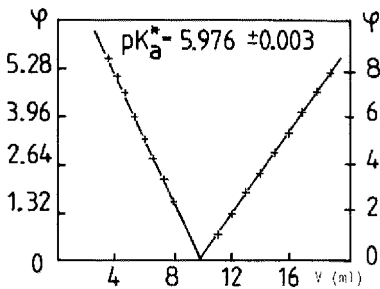
*In the present treatment, the concentration of hydrogen ions and not their activity are meant, hence the pH value in the non-aqueous medium is denoted with an asterisk and defined as $pH^* = pc_H^*$ (see ref. 5). For the same reason, the pK_a value determined in the non-aqueous medium is denoted with an asterisk. In fact, pK_a^* is a concentration equilibrium constant in the medium in question.



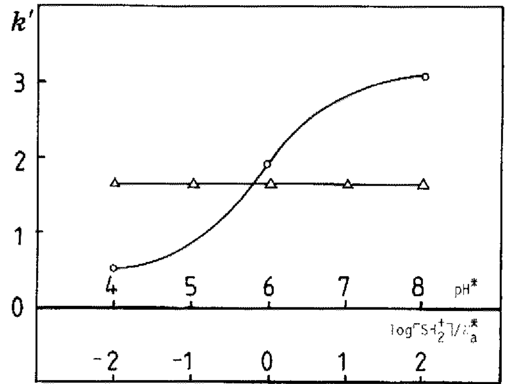


Fig. 1. Gran plot of a potentiometric titration of vephylline base with 0.0091 M hydrochloride acid in methanol—water (30:70). $\varphi = V \cdot 10^{E/59.16}$ before the equivalence point; $\varphi = (V_0 + V) \cdot 10^4 \cdot 10^{-E/59.16}$ after the equivalence point.

Fig. 2. Plot of k' versus pH* of vephylline (○) and theophylline (△) in methanol—water (30:70).

Vephylline was chromatographed at pH* 4.0 and pH* 8.0 with a mobile phase of methanol—water (30:70), from which two capacity factors were obtained; k'_1 = 0.43 and k'_0 = 3.16. As expected, the capacity factor of theophylline was not affected by pH* changes (k' = 1.67).

The normalized curve $k' = f[\log([SH_2^+]/K_a^*)]$ superimposed over the three experimental points k'_1, k'_0 and pK_a^* = pH* is drawn with a full line in Fig. 2. For comparison, the capacity factor of theophylline is also presented. It follows from this figure that rather good separation can be achieved at pH* $\leqslant$ 5 and pH* $\geqslant$ 7. At pH* $\leqslant$ 5, however, vephylline has a very low capacity factor (and a very short retention time). It will be separated excellently from theophylline but not from the plasma components. Much better conditions will be achieved at pH* $\geqslant$ 7 where not only are the two substances well separated but they are well separated from the plasma components as well as from the internal standard (see Fig. 4).

On the basis of these investigations, a mobile phase of methanol— water (30:70) with a pH* of 7.0 (adjusted with 0.001 M phosphate buffer) and a flow-rate of 1.0 ml/min was chosen. It was prepared by mixing 300 ml of methanol and 10.00 ml of phosphate buffer (containing $6.2 \cdot 10^{-2}$ M NaH_2PO_4 and $3.8 \cdot 10^{-2}$ M Na_2HPO_4) and making up to 1000 ml with distilled water. The mobile phase was used after degassing. All chromatographic investigations were performed at room temperature.

Sample preparations

To 0.2 ml of plasma, a 1.0-ml mixture of isopropanol—chloroform (1:1) containing 4.8 μg/ml phenobarbital (as internal standard) was added. After shaking for 1 min on a Vortex mixer, the mixture was centrifuged at ca. 1400 g for 5 min. A 0.5-ml aliquot of the clear organic solvent was evaporated at 65—70°C. The residue was dissolved in 0.2 ml of mobile phase and 100-μl aliquots were injected into the chromatograph.

Preparation of calibration curve

Standard solutions containing 1—5 μg/ml vephylline, 0.2—1 μg/ml theo-

phylline and 2.4 μg/ml phenobarbital were prepared in the mobile phase. These standards were chromatographed and analysed in duplicate.

Statistics

The calibration curve data were analysed by a least-squares method by means of an HP-85 microcomputer. The curves obtained were linear ($r = 0.997$ for vephylline; $r = 0.999$ for theophylline) and followed the equations: $y = -0.022 + 0.579x$ (vephylline) and $y = -0.009 + 0.305x$ (theophylline).

In order to determine the precision of the method, a five-fold analysis of three different concentrations was performed. The relative standard deviation found was ± 3%. The recovery of the method proposed was checked by means of in vitro experiments in which a known amount of the drug has undergone the whole procedure. The quantity found from six experiments showed a recovery of 96.7 ± 3.6%.

The limit of detection was found to be 0.5 μg/ml vephylline in plasma. It was determined by extrapolating a plot of peak height versus standard concentration to where the signal equalled three times the noise. The noise was determined from the standard deviation of several measurements of the response of blank plasma measured at the retention time of the drug.

RESULTS AND DISCUSSIONS

The method proposed was used for pharmacokinetic investigations of vephylline. The substance was applied intravenously and orally in thirty rabbits, five volunteers and fifteen patients. The drug was administered in the following doses: for rabbits, solutions and tablets containing 50 mg of vephylline; for humans, tablets containing 200 mg of vephylline.

Fig. 3 shows typical chromatograms for: (a) blank rabbit plasma and (b) rabbit plasma 15 min after intravenous administration. From the chromatograms, quantitative data are obtained by peak-height analysis according to the equation

$$C_x = \frac{C_{st} h_x}{F h_{st}} Z$$

where C_x is the concentration of vephylline (μg/ml); C_{st} is the concentration of internal standard (μg/ml); h_x and h_{st} are the peak heights of vephylline and internal standard, respectively; F is the factor of determination; and Z is the dilution coefficient.

In Fig. 4, a chromatogram of human plasma extract is presented. The sample is taken 360 min after oral administration of a tablet of vephylline. Besides the plasma components, vephylline and internal standard, a peak of theophylline can be seen also. The concentrations determined are 11.6 μg/ml vephylline and 4.5 μg/ml theophylline. The method proposed for simultaneous determination of vephylline and its metabolite theophylline could be very useful with regard to proper dosage and treatment. It is well known that theophylline has a narrow therapeutic range [7]. Its presence as a major metabolite must be taken into account in order to prevent some undesirable side-effects.

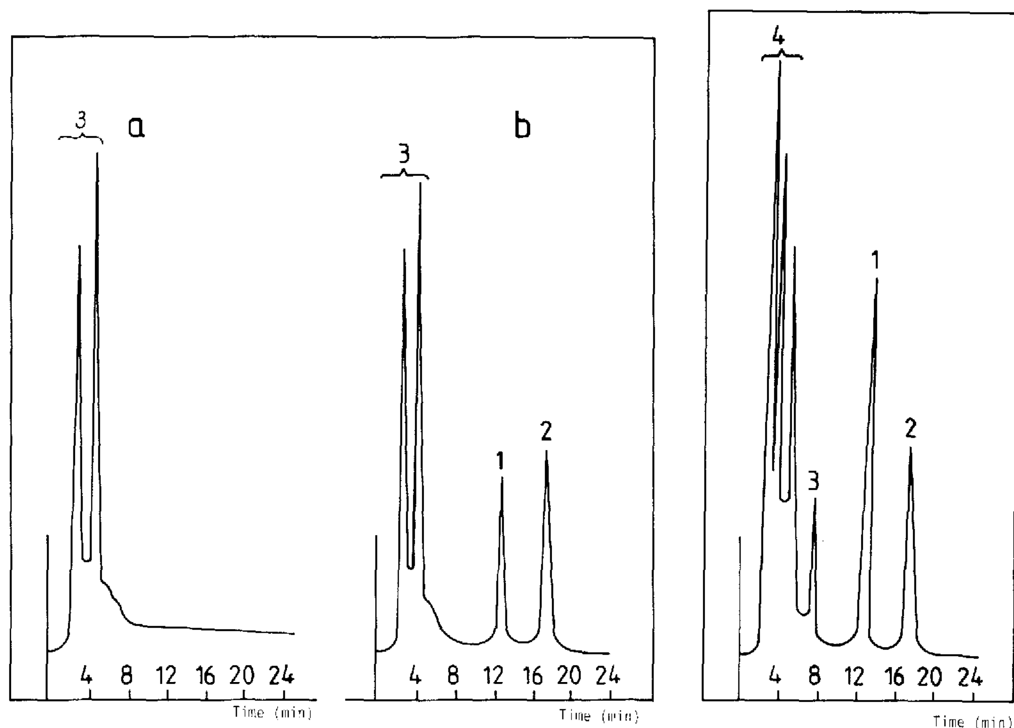


Fig. 3. Chromatograms of rabbit plasma extracts. (a) Vephylline-free plasma without internal standard; peaks 3 are plasma components; (b) plasma containing vephylline after intravenous administration; peaks: 1 = vephylline (3.2 μg/ml); 2 = phenobarbital, (internal standard 2.4 μg/ml); 3 = plasma components.

Fig. 4. Chromatogram of patient plasma extract 360 min after oral administration of a tablet of vephylline. Mobile phase is methanol—water (30:70) with a pH* of 7.0. Peaks: 1 = vephylline (11.6 μg/ml); 2 = phenobarbital, (internal standard 2.4 μg/ml); 3 = theophylline metabolite (4.5 μg/ml); 4 = plasma components.

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