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Note

Determination of heptaminol in pharmaceutical preparations by high-performance liquid chromatography

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6-Amino-2-methyl-2-heptanol hydrochloride (heptaminol hydrochloride) is a sympatomimetic drug with adrenergic action, widely used in therapeutics. Two methods have been described for its determination in complex mixtures: the first is based on measuring the fluorescence of its dansylated product after separation of the latter by thin-layer chromatography[1], and the second method relies on a gas chromatographic system which permits the identification of heptaminol in urine after extraction and its quantification by use of an internal standard[2].

This paper describes a simple and rapid procedure for the determination of heptaminol using high-performance liquid chromatography (HPLC) under isocratic elution conditions. This compound has no UV–visible spectral properties. Also, derivatization is necessary if one wishes to use a classical spectroscopic method of detection.

As Roth first reported[3] *o*-phthaldialdehyde (OPA) in the presence of 2-mercaptoethanol in alkaline medium forms highly fluorescent adducts with primary amines (λ_{ex} = 330 nm; λ_{em} = 455 nm). This derivatization has been widely used for the detection of amino acids in chromatographic column effluents[4]. However, the condensation with OPA may also be performed before chromatographic analysis[5–7].

In the procedure described we adopted pre-column OPA derivatization because of its easier application and lower cost, as post-column derivatization requires special reaction chambers and a second pump as part of the chromatographic system. The relatively large amounts of heptaminol in pharmaceutical preparations allow UV detection of the OPA-heptaminol adduct with sufficient sensitivity.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. Solvents were distilled twice before use.

o-Phthaldialdehyde (OPA) was purchased from Fluka (Buchs, Switzerland) and heptaminol hydrochloride from Finorga (Courbevoie, France). The purity of the latter was measured by non-aqueous acidimetric titration and was at least 99%. Pharmaceutical preparations (tablets, packets, oral solutions) were commercially available products.

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HPLC instrumentation

The liquid chromatographic system consisted of an isocratic solvent delivery pump (Model 8500; Varian, Palo Alto, CA, U.S.A.), an injection valve provided with a 20-μl sample loop (Model 7125; Rheodyne, Berkeley, CA, U.S.A.), a reversed-phase column (250 × 4 mm I.D.) prepacked with LiChrosorb RP-18 (particle size 7 μm) (Hi bar EC 250-4; E. Merck, Darmstadt, G.F.R.) and a variable-wavelength detector (Variscan; Varian) operated at 330 nm. A pre-column (30 × 4 mm I.D.) packed with LiChrosorb RP-18 (particle size 40 μm) was used in-line during all chromatographic runs.

The mobile phase consisted of aqueous 12.5 m*M* disodium hydrogen orthophosphate buffer (pH 7.2)–methanol (30:70), which was filtered through a 0.6-μm microfilter (type BD; Millipore, Bedford, MA, U.S.A.). A flow-rate of 70 ml/h was used with an operating pressure of 2500 p.s.i.

Chromatographic recordings and all calculations were performed on an integrator (Model ICR-1; Intersmat, Courtry, France).

Assay procedure

A standard solution of heptaminol hydrochloride was prepared in methanol (0.1%, w/v; $5.5 \cdot 10^{-4}$ *M*).

Solid preparations were ground in a mortar to a fine powder. An aliquot of the resulting powder was weighed and mechanically stirred in methanol for 30 min. The volume of the solution was adjusted with methanol in order to obtain the same heptaminol concentration as that in the standard solution.

If an internal standard was used, it was added to the assay solutions at a molarity equal to that of heptaminol.

These solutions were passed through a 0.45-μm microfilter (type BD; Millipore).

Borate buffer (pH 9.5) was prepared by dissolving orthoboric acid in water (3.3%, w/v) and adjusting the pH to the correct value with 10 *M* sodium hydroxide solution.

The derivatization solution was prepared by dissolving 0.1 g of OPA in methanol–borate buffer–2-mercaptoethanol (9:1:0.1). This reagent is stable for 1 day at room temperature when protected from light. Samples of 1 ml of standard or assay solution were made alkaline by adding 1 ml of borate buffer.

Derivatization was performed by adding 0.75 ml of reagent and 2 ml of methanol, followed by vigorous mixing. The mixture was allowed to react at room temperature for 5 min and an aliquot was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The optimum mobile phase was established by selecting mixtures of solvents used for the separation of OPA–amino acid adducts in gradient runs as described in previous papers[5–7].

A pH of 7.2 was found to be the optimum both for stability and partition of OPA–amine adducts on reversed-phase columns. Methanol was chosen as the organic modifier of the mobile phase. Its optimum proportion, established by several experiments at different levels, was 70% (v/v) (Fig. 1). Under these conditions, the retention time of the OPA–heptaminol adduct is about 9 min.

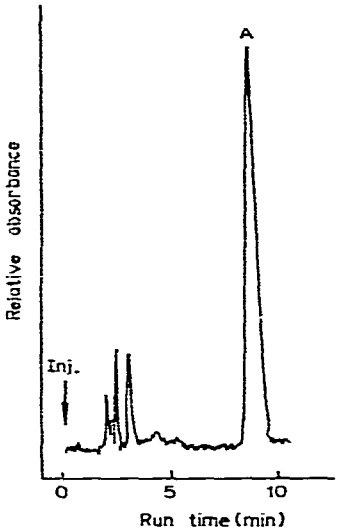

Fig. 1. Chromatogram of heptaminol–OPA adduct (A). Column, LiChrosorb RP-18 (7 μm); eluent, aqueous 12.5 m*M* Na_2HPO_4 buffer (pH 7.2)–methanol (30:70); flow-rate, 70 ml/h; UV detection, λ = 330 nm (0–0.1 a.u.f.s.).

The complete recovery of heptaminol from pharmaceutical preparations was achieved with use of methanol or water as solvent. Nevertheless, methanol is preferred because it performs a sample clean-up step when sugars are the vehicles. The proportions of the different reagents in the derivatization reaction were studied in order to achieve the maximum signal in the chromatographic system. The optimum molar proportions of heptaminol, OPA and 2-mercaptoethanol were established as 1:100:5000.

The derivatization reaction occurs quickly at room temperature. The substitued isoindole product formed is detected with a UV absorption system at a wavelength which is used for excitation in a fluorimetric system (λ_{ex} = 330 nm). The detection limit achieved is low enough for measurement of the OPA–heptaminol adduct in pharmaceutical preparations. The usual concentrations of this drug are in the range 1–5%.

The stability of the OPA–heptaminol adduct was tested by chromatographing the same sample after various times. The signals obtained showed no significant variation 5 min, 1 h and 4 h after derivatization.

No interferences from other compounds contained in the pharmaceutical preparations (water-soluble vitamins, amino acids, preservatives, vehicles, etc.) were observed.

The linearity of the response was studied for heptaminol concentrations in the range 0–10^{-3} *M* (Fig. 2). The usual measurements were carried out by injecting into the HPLC system an amount equivalent to $1.1 \cdot 10^{-8}$ mol of heptaminol.

The accuracy of the method, including the derivatization and chromatographic steps, was calculated for fifteen replicate determinations; the coefficient of variation was 1.38%. Consequently, assays of heptaminol in pharmaceutical preparations may be realized with a precision of ±5%, as is generally required in industrial control

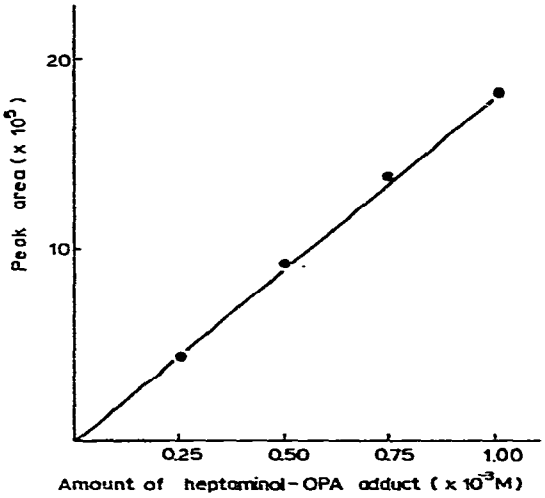

Fig. 2. Calibration graph for heptaminol–OPA adduct in the HPLC system; correlation between peak area and amount of heptaminol in the sample.

laboratories. An internal standard may be used to increase the precision. For this purpose, two compounds were tested: ethanolamine and lysine hydrochlorides. Both were found to be suitable and had retention times of about 3.7 and 4.8 min, respectively (Fig. 3).

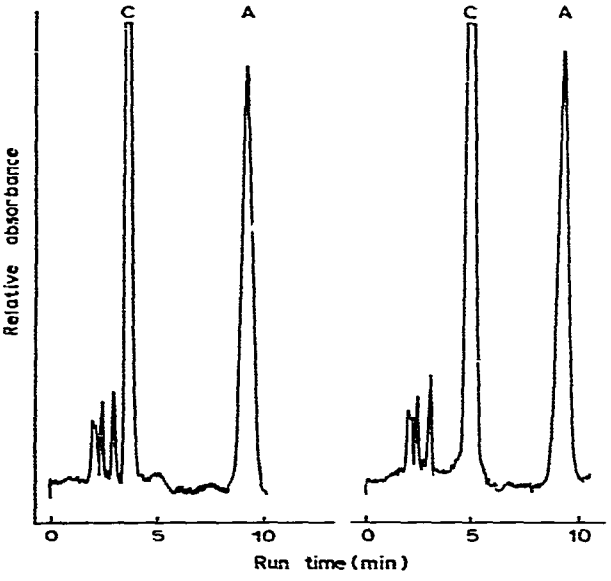

Fig. 3. Chromatograms of OPA adducts of (A) heptaminol, (B) ethanolamine and (C) lysine. Lysine and ethanolamine were used as internal standards.

CONCLUSION

The proposed method offers many advantages over previous described procedures. The derivatization of heptaminol with OPA is easier than dansylation[1]. The reagents are stable in water and the reaction quickly occurs at room temperature and requires no heating. No extraction procedure is necessary, in contrast to gas chromatography[2]. The sensitivity of the method may be greatly increased by using fluorimetric detection, which would allow monitoring of the drug in biological fluids.

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