Analytica Chimica Acta, 108 (1979) 401—403

Short Communication

GAS CHROMATOGRAPHIC DETERMINATION OF XANTHINOL IN PLASMA

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(Received 19th December 1978)

Summary. The determination of xanthinol in plasma is described. After extraction of the drug, together with the internal standard (papaverine hydrochloride), the extract is evaporated to dryness and the drug is derivatized with acetic anhydride for chromatography. The method is linear for 2—100 $\mu g\ ml^{-1}$; the coefficient of variation is 3% and the recovery 80%. The resulting stable solution allows large numbers of samples to be processed with an automatic injector.

Xanthinol nicotinate (Complamin), used in the treatment of peripheral vascular disorders, has been claimed to be of value in the treatment of angina of effort, of cerebral and other arteriosclerotic conditions, [1, 2] and of placental malfunctions [3]. Xanthinol is metabolized to a small extent (7—8%) and is largely excreted unchanged in urine [4]. The absorption of the drug from slow-release and plain tablets has been studied [5] by measuring plasma levels of nicotinic acid, but this is not the active species. A method for the determination of xanthinol in plasma does not seem to have been described; without such a method it is impossible to establish therapeutic blood levels or to evaluate the bio-availability from different formulations. This communication describes a gas chromatographic method for this purpose. The drug is extracted from the plasma together with the internal standard; after derivatization with acetic anhydride, it is injected into the gas chromatograph.

Experimental

Reagents. The reagents used were chloroform, isopropanol, methanol and acetic anhydride (Merck, Darmstadt), xanthinol nicotinate (Nogépha, Amsterdam) and papaverine hydrochloride (Brocacef, Maarssen). All solvents were of reagent grade.

Gas chromatography. A Packard-Becker Model 419 gas chromatograph, equipped with flame ionization detectors was used. The glass column (1.5 m × 2 mm i.d.) was packed with 10% SE 30 on Chromosorb WHP, 80—100 mesh (Chrompack, Middelburg). The operating conditions were: injection port temperature, 270°C; column temperature, 245°C; detector temperature, 270°C; carrier gas (nitrogen) flow, 20 ml min^{-1}; hydrogen flow 30 ml min^{-1}; air flow 300 ml min^{-1}. Peak areas were measured with the chromatography Data Analyser System IV B (Spectra Physics, Schijndel).

Assay procedure. To 1 ml of plasma in a centrifuge tube was added 100 μl of 0.1 M NaOH and 4 ml of chloroform—isopropanol (95 + 5), containing papaverine hydrochloride (2.5 μg ml^{-1}) as the internal standard. After shaking for 30 s and centrifugation for 10 min at 3000 rpm, the organic phase was transferred to another tube by means of a Pasteur pipette. The extract was evaporated to dryness under nitrogen in a metal heating block at 60°C after which 100 μl of acetic anhydride was added to the residue. After heating for 10 min at 80°C, the excess of reagent was evaporated under nitrogen and the residue was dissolved in 50 μl of methanol; 1 μl of this solution was injected into the gas chromatograph. Standard curves were obtained by spiking blank plasma with xanthinol nicotinate and subjecting these samples to the above procedure.

Recovery of xanthinol from plasma. First xanthinol nicotinate was dissolved in methanol to a concentration of 0.5 mg ml^{-1}, and 100 μl of this solution was added to 4 ml of chloroform—isopropanol containing the internal standard. This solution was then evaporated and derivatized as described for the assay procedure. Secondly, the solution of xanthinol nicotinate in methanol (100 μl) was added to 1 ml of plasma to which was then added 100 μl of 0.1 M NaOH. This solution was extracted with 4 ml of chloroform—isopropanol without internal standard. After separating the phases, 4 ml of chloroform—isopropanol containing the internal standard was added to the organic phase; this solution was then evaporated and derivatized as described.

Results and discussion

Injection of underivatized xanthinol on various columns resulted in strongly tailing peaks; losses as high as 500 ng per injection, probably through irreversible adsorption on the column support material, were noted. Derivatization of the two hydroxyl groups in xanthinol with acetic anhydride resulted in symmetrical peaks and no significant losses occurred. Acetic anhydride has the advantage that the reaction is complete within minutes and the excess of reagent can be easily removed by evaporation. Under the described chromatographic conditions, xanthinol and papaverine gave well-resolved symmetrical peaks (Fig. 1) with retention times of 4.5 and 5.5 min, respectively. Chromatograms of blank plasma samples showed no peaks at the retention times of xanthinol or the internal standard, although a peak with a retention time of 11 min occurred. When a column packed with 3% OV17 was used, the peaks of xanthinol and papaverine were further apart but the retention time of the plasma peak was very close to that of the xanthinol peak.

The calibration curves were linear over the range studied (2—100 μg ml^{-1}) with a small negative intercept of 0.3—0.5 μg ml^{-1}. The coefficient of variation, determined at 10 μg ml^{-1} and at 50 μg ml^{-1} by analyzing six plasma samples at each concentration, was calculated to be 3%. The recovery of xanthinol was 80% with a coefficient of variation of 5% (n = 6).

After injection into the gas chromatograph the solutions were occasionally

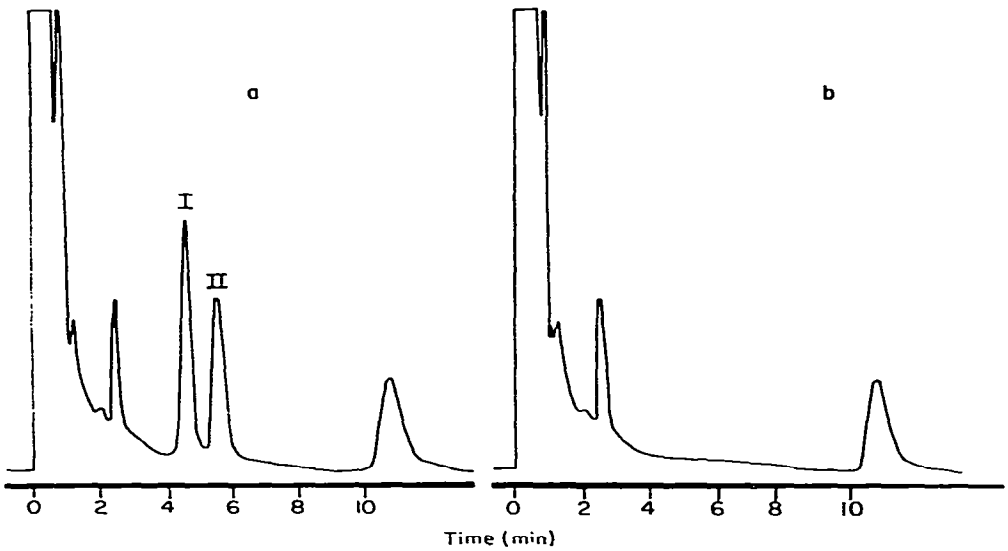

Fig. 1. Chromatograms of plasma samples; for conditions, see Experimental. (a) Blank plasma, spiked with xanthinol: (I) xanthinol; (II) papaverine. (b) Plasma blank.

stored in the dark at room temperature for 1 or 2 days; the peak area ratios did not change on storage. The stability of this solution should therefore allow the processing of a large number of samples with an automatic injector.

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