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Note

Gas chromatographic determination of furosemide in plasma using an extractive alkylation technique and an electron capture detector

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Furosemide (Fig. 1) is a widely prescribed diuretic drug, and methods for the determination of plasma levels have been described[1,2]. These methods are based on the spectroscopic determination of either an organic plasma extract directly or a degradation product of furosemide (2-amino-4-chloro-5-sulphamidobenzoic acid), which also has been reported to be a metabolite[2]. This degradation product can also be formed from another metabolite of furosemide[3], a conjugate, probably together with glucuronic acid. Higher specificity is obtained by employing the present method, in which the intact furosemide molecule is converted into its trimethyl derivative (Fig. 1) by extractive alkylation; this is analogous to a procedure described for chlorthalidone[4]. The derivative is then analysed on a gas chromatograph, using the triethyl analogue as internal standard.

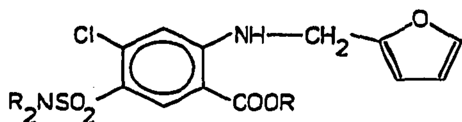

Fig. 1. Structure of furosemide (R = H) and its trimethyl derivative (R = CH_3).

EXPERIMENTAL

Instrumental

Analyses were performed on a Packard-Becker 409 gas chromatograph, equipped with a ^{63}Ni electron capture detector. The samples were chromatographed on a 3% JXR column, operated at 245°. The injector and detector temperatures were 260° and 275°, respectively. The JXR stationary phase (Supelco, Bellefonte, Pa., U.S.A.) was coated on 100–120-mesh Gas-Chrom Q and packed in a 1.8 m × 2 mm silanized glass column. The derived furosemide was identified on an LKB 9000 gas chromatograph–mass spectrometer. The radioactivity was measured on a Packard Tri-Carb 2405 instrument.

Internal standard

The internal standard was prepared by the same extractive alkylation tech-

nique as that used for the preparation of the trimethyl derivative of furosemide. In this procedure, exchange of methyl iodide for ethyl iodide yielded the triethyl derivative; this was purified by preparative TLC (silicic acid plates developed in dichloromethane–*n*-heptane, 2:1). Extraction of the silicic acid with dichloromethane, followed by evaporation of the solvent, yielded triethylfurosemide in a crystalline form.

Method

A 1-ml volume of plasma was diluted with 2 ml of water and acidified with 0.15 ml of 4 *M* hydrochloric acid. The mixture was extracted twice with 5 ml of diethyl ether in a screw-capped tube. After centrifugation, the ether phases were combined and transferred to another screw-capped tube. The ether solution was evaporated to dryness with a stream of nitrogen, then 2 ml of 0.2 *M* sodium hydroxide solution, 50 μl of 0.1 *M* tetrahexylammonium hydrogen sulphate solution and 5.0 ml of 0.5 *M* methyl iodide in dichloromethane were added to the residue. The tube was shaken for 20 min at 50° and, after centrifugation, 4.0 ml of the organic phase were transferred to another tube and evaporated to dryness with a stream of nitrogen at room temperature. To the residue was added 0.2 ml of *n*-hexane, containing the internal standard. The tube was placed in an ultrasonic bath for 5 min and, after centrifugation, 5 μl were injected on to the gas chromatograph.

RESULTS

Extraction

Extraction of furosemide from plasma, according to the procedure described above, yielded 98% of the substance in the organic phase after one extraction. This was studied by adding 0.5 μg/ml of ^{35}S-labelled furosemide to a plasma sample before extraction. The degradation product of furosemide, 2-amino-4-chloro-5-sulphamidobenzoic acid, was also extracted under the conditions described.

Extractive alkylation

Furosemide could not be gas chromatographed without derivatization, which was accomplished by exchanging the acidic protons of the carboxylic and sulphamidic groups for methyl groups. Furosemide was extracted into dichloromethane containing methyl iodide from an alkaline solution as an ion pair with tetrahexylammonium ion. The trimethyl derivative was obtained in the organic phase. The alkylation was completed in about 10 min when carried out at 50°. By using combined gas chromatography–mass spectrometry (GC–MS), it was shown that amounts of furosemide up to 80 μg could be quantitatively trimethylated when treated according to the conditions described above. The efficiency of the extractions was also demonstrated by using radioactive furosemide.

Gas chromatography

The GC determinations were carried out on a 3% JXR column, which gave appreciable separation between the trimethyl and triethyl derivatives of furosemide. Three or four injections of an extracted and alkylated plasma sample were often necessary in order to obtain maximum sensitivity and good peak symmetry. This operation probably served as a deactivation of the column. It was necessary to wait

for 15 min under the given conditions before making the next injection because of substances eluting after the trimethyl and triethyl derivatives of furosemide. Typical gas chromatograms are shown in Fig. 2.

According to the method described, dichloromethane was evaporated after alkylation. The dry residue contained trimethylfurosemide and tetrahexylammonium iodide. When the residue was treated with a solvent in which the tetrahexylammonium iodide is soluble, one of the methyl groups of trimethylfurosemide was exchanged for a hexyl group after injection on the column, as shown by combined GC–MS. Because of the low solubility of the tetrahexylammonium iodide in *n*-hexane, this solvent was used for the injection of the samples. The sensitivity in minimal detectable quantity (MDQ)[5] units for trimethylfurosemide was $7 \cdot 10^{-15}$ mole/sec.

Calibration graph

Fig. 3 shows the calibration graph, prepared by adding different amounts of furosemide to plasma samples and analysing them according to the above method. The ratio of the areas of the peaks of trimethylfurosemide and the added standard was plotted against the concentration of furosemide. The graph is linear in the range investigated (0.3–5.4 μg/ml) and passes through the origin. Plasma samples containing 0.9 and 3.6 μg/ml of furosemide were used to determine the relative standard deviation, which was found to be 6.3 and 3.4%, respectively. The method is suited for determinations down to 0.1 μg/ml plasma.

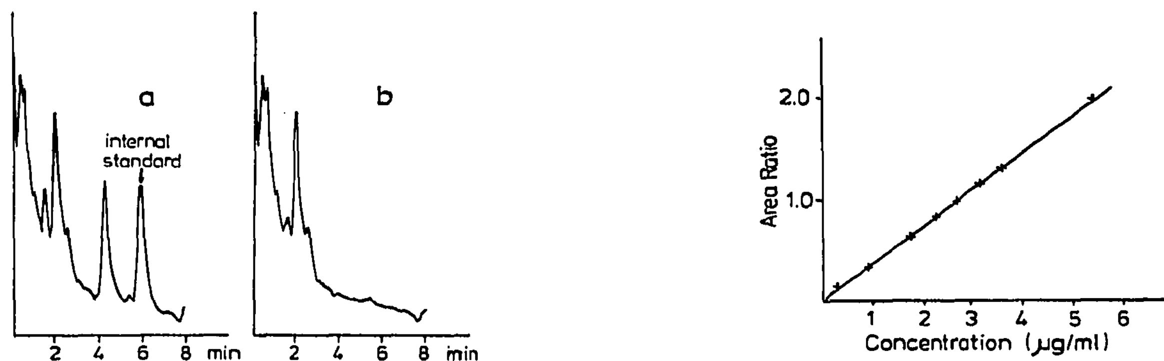

Fig. 2. (a) Gas chromatogram of a plasma extract analysed according to the method described. The plasma contained 2.6 μg/ml of furosemide. (b) Gas chromatogram of a blank plasma extract treated according to the conditions in the method described.

Fig. 3. Furosemide/internal standard calibration graph.

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