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Determination of furosemide in rat plasma using HPLC and liquid scintillation

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Abstract: A rapid and convenient HPLC method for the determination of furosemide in plasma is described. The method uses a buffered mobile phase containing 22% (v/v) acetonitrile. The precision, detection limit and the correlation between the HPLC method and a liquid scintillation determination of furosemide are satisfactory. A pharmacokinetic study of furosemide in the rat is described.

Keywords: *Reversed phase HPLC; liquid scintillation; furosemide determination; rat plasma.*

Introduction

Furosemide is a commonly used diuretic. Knowledge of its concentration in plasma is important in the therapy of many diseases. Several types of assay are currently in use, including UV spectrometry [1], spectrofluorimetry [2], gas chromatography [3] and liquid scintillation [4, 5]. To improve the specificity, HPLC is most appropriate. Liquid chromatography methods have used a reversed-phase column with acetonitrile–buffer mixtures as an eluent. For the lower plasma concentrations, a poor recovery is found with a standard deviation greater than 10%. Fluorimetric detection does not offer a better precision [6–12]. Improved precision can be obtained by working in the optimal chromatographic conditions. Therefore, in this work, these conditions are established by calculation of some retention parameters on varying the pH, ionic strength and acetonitrile composition of the mobile phase. The new HPLC method is applied to the estimation of furosemide in rat plasma and to elaborate a pharmacokinetic study. A comparison with liquid scintillation is also carried out.

Experimental

Chemicals

Furosemide and ^{35}S furosemide were purchased from Hoescht (West Germany), sodium phenobarbital (internal standard) from Fluka Chemicals (Switzerland), acetonitrile (UV grade) from Eastman Kodak (USA), acetate salts and acetic acid from Merck

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(West Germany). Ultrapure water was obtained through a Milli Q apparatus from Millipore (USA).

Instrumentation

Separations were performed on a 30 × 0.4 cm Microbondapak C_{18} column along with a 2 × 0.4 cm C_{18} Corasil guard column (Waters, USA). The liquid chromatograph was a Waters 6000 A solvent delivery system with a dual UV model 440 Waters (USA) detector operating at 254 and 280 nm. Samples were injected with a U 6K loop valve. The flow rate was 2 ml min^{-1}. Several eluents were tried, made up from acetate buffers of different pH and ionic strength, containing 20, 22, 25, 30, 35, 40 and 50% (v/v) of acetonitrile. The ionic strengths were fixed to 0.01, 0.02, 0.04 and 0.1 M, the pH varying from 2.5 to 6.7.

The liquid scintillation instrument was a Packard model 3255 (USA). All plasma samples were counted using the 0.17 MeV channel. The ^{35}S furosemide used had a specific activity of 5.47 mg^{-1}.

Pharmacokinetic study

Twenty Wistar rats (10 for HPLC and 10 for liquid scintillation) with an average weight of 250 g were studied. For each rat, two cannulas were placed, one in the left jugular vein, the other in the carotid. Doses of 2.5 mg kg^{-1} of furosemide (for HPLC) or ^{35}S furosemide (for liquid scintillation) were injected into the jugular vein. Serial plasma collections (200 µl) were made after 5, 10, 20, 30, 60, 90, 120, 150, 180 and 210 min from the carotid and analysed.

Extraction

HPLC. Samples (200 µl) of plasma were collected, then 20 µl aliquots of an aqueous solution of 120 mg l^{-1} sodium phenobarbital were added. After filtration through a sample clarification kit (Millipore, USA), 20 µl aliquots were injected on to the chromatograph; sodium phenobarbital and furosemide concentrations were measured at 254 and 280 nm respectively.

Liquid scintillation. Plasma samples (50 µl) were transferred to a counting flask which contained 10 ml of Picofluor[15] (Packard, USA) and counted. Corrections were made for quench differences using an automatic standardization technique.

Results and Discussion

Determination of the optimal chromatographic conditions

To establish the optimal chromatographic conditions, the effects of pH, ionic strength and amount of acetonitrile in the eluent on three chromatographic parameters were investigated.

Capacity factor k'. The first parameter examined was the capacity factor of furosemide. Typical results are shown in Fig. 1. The following remarks can be made: (i) for a constant ionic strength and pH, log k' decreases linearly with the amount of acetonitrile; (ii) for a constant ionic strength, the slopes of the straight lines have an irregular variation with pH; (iii) for a constant pH, the slopes have maximum values at an ionic strength of 0.02 M.

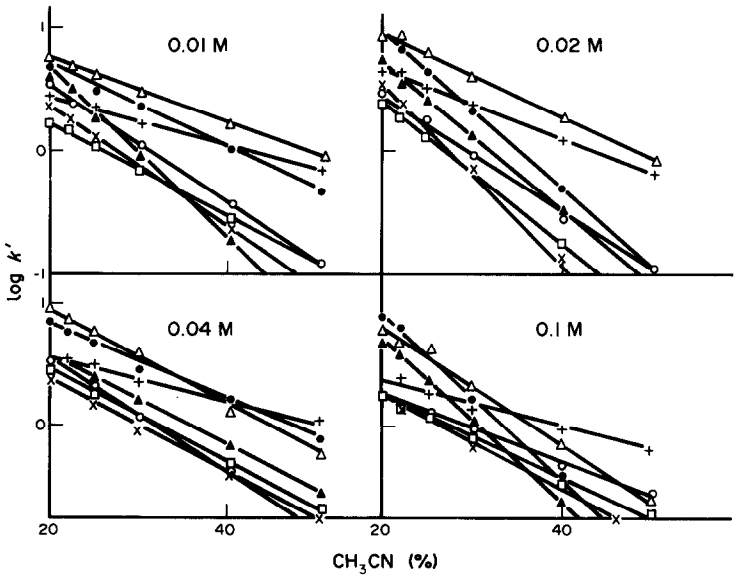

Figure 1
Retention (log k') of furosemide, in acetate buffer eluents of various concentrations at pH 3.2 (△); 2.5 (●); 4 (+); 5.8 (○); 4.5 (▲); 5.3 (□); 6.7 (×) determined as a function of acetonitrile concentration in the eluent.

Number of theoretical plates. The second parameter studied was the number of theoretical plates, N, calculated for each mobile phase. Figure 2 shows the variation of N with acetonitrile composition, taking account of pH and ionic strength. This figure suggests the following conclusions: (i) when the pH and ionic strength vary, N has a maximum value at 22% (v/v) of acetonitrile; (ii) the maxima obtained for the four concentrations of buffer used show that the greatest corresponds to the ionic strength of 0.02 M; (iii) for a constant ionic strength, the maximum value of N occurs at pH 5.3, with 22% v/v acetonitrile. The optimal eluent is thus an aqueous acetate buffer, 0.02 M, pH = 5.3, with 22% of acetonitrile.

Asymmetry factor. To confirm these conditions, the asymmetry factor of the furosemide peak was calculated for an ionic strength of 0.02 M. This parameter estimates the peak tailing which affects quantitation, especially in the detection limit range. In Fig. 3, the asymmetry factor AF is plotted against the amount of acetonitrile in the eluent at different pHs. The minimum value of AF is given by the same mobile phase which gives the maximum value of N. In these conditions, the retention times of furosemide and the internal standard were respectively 5 and 7 min and the resolution between the two peaks is 5.5.

Calibration and quantitation

The calibration curve for furosemide in plasma has the following regression values: slope, 0.182; intercept, 0.025; correlation coefficient, 0.995. Comparison with a similar calibration curve prepared in the absence of plasma (slope, 0.190; intercept, 0.026; correlation coefficient, 0.993) indicates a recovery of 96%. For the lower concentration range (0.01–1 mg l^{-1}; a satisfactory precision is obtained, with a relative standard deviation of 5–6%. The lowest detectable concentration, defined as twice the signal-to-

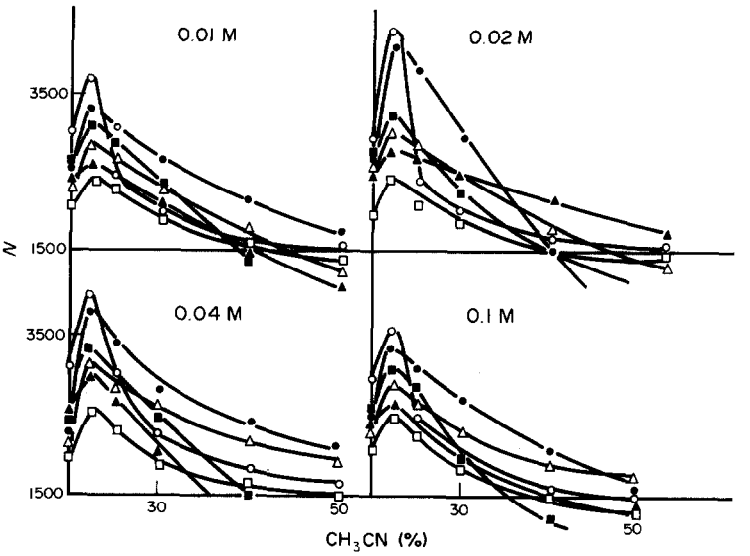

Figure 2
Number of theoretical plates in acetate buffer eluents of various concentrations at pH 5.3 (○); 4.5 (●); 6.7 (■); 5.8 (△); 3.2 (▲); 4 (□).

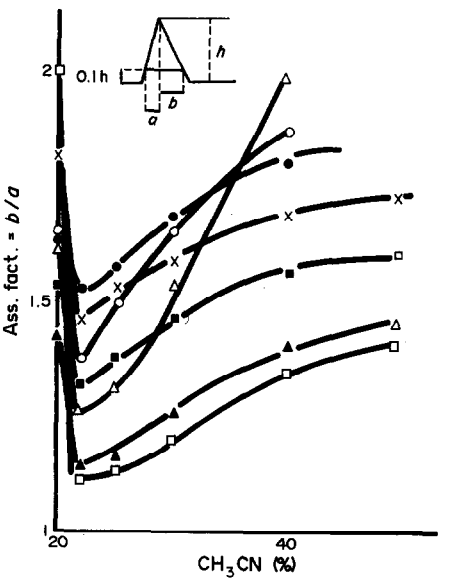

Figure 3
Asymmetry factor of furosemide peak in acetate buffer eluents 0.02 M at pH 4 (●); 4.5 (○); 3.2 (△); 2.5 (▲); 5.3 (□); 5.8 (×); 6.7 (■).

noise ratio, is 0.01 mg l^{-1}. Figure 4 shows a representative chromatogram of a plasma extract at 254 and 280 nm.

Using liquid scintillation, serial plasma samples with known concentrations of ^{35}S furosemide were counted and the results compared with the HPLC results. A good correlation was found (Fig. 5). It is important to notice that in earlier work [4, 13] it was shown that the levels of metabolites in plasma is insignificant compared with furosemide concentrations. Therefore, the total radioactivity measured is proportional to the furosemide concentration in plasma.

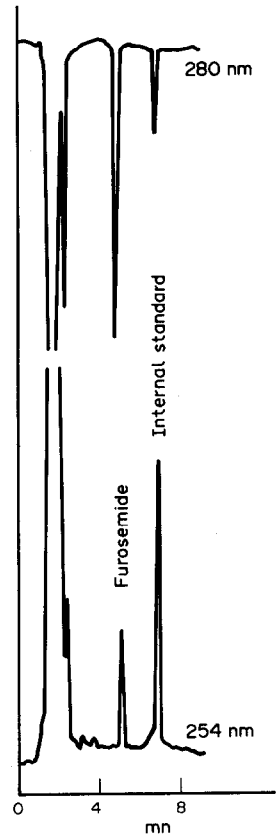

Figure 4
Representative chromatogram of a plasma extract at 254 and 280 nm. Mobile phase — acetate buffer 0.02 M, pH = 5.3: acetonitrile 78:22 v/v.

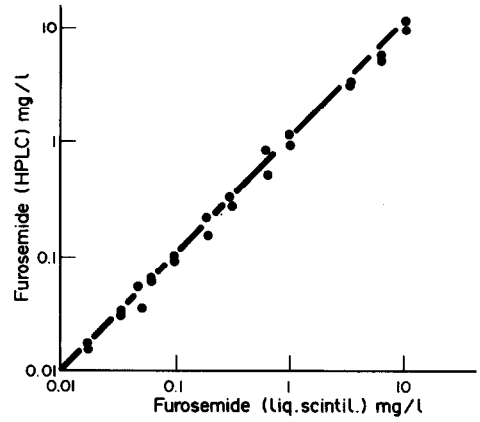

Figure 5
Correlation of plasma HPLC furosemide concentrations with plasma liquid scintillation furosemide concentrations.

Pharmacokinetic results

To prove its applicability, the HPLC method described was tested on rat plasma samples after intravenous administration of furosemide in a pharmacokinetic study. The results were again compared with those obtained by liquid scintillation. The log (average plasma concentration) versus time curve (Fig. 6) fitted a two compartment model with an elimination half life ($t_{1/2}$) of 0.45 h.

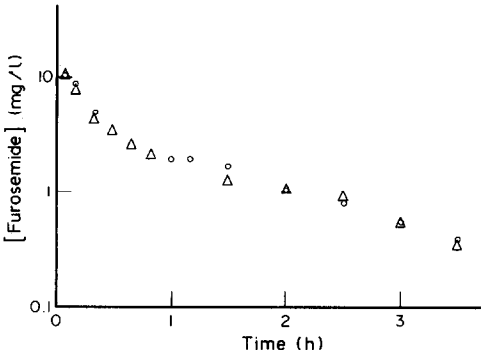

Figure 6
Log (plasma concentration) versus time curve after administration of 2.5 mg kg^{-1} of furosemide: HPLC (○), liquid scintillation (△).

Conclusion

The HPLC method described in this paper provides a simple and reliable procedure for the determination of furosemide in plasma. A good precision is found for the lower concentrations. A satisfactory correlation is obtained using HPLC and liquid scintillation. The present method is appropriate for use both in pharmacokinetic studies and in therapeutic drug monitoring in routine hospital service laboratories.

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