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Note

High-performance liquid chromatographic determination of phosphocreatinine and creatinine in pharmaceutical preparations

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Phosphocreatinine is used in some pharmaceutical preparations since it is an important phosphagen in transphosphorylation reactions[1,2]. It readily undergoes hydrolysis to creatinine, which is not active.

This paper describes a simultaneous determination of phosphocreatinine and creatinine in pharmaceutical preparations. None of the methods in the literature were very satisfactory for our purposes[4–6]. Those that use titration in anhydrous solution with perchloric acid or nitrogen determination also detect the creatinine derived from hydrolysis. Those that use the determination of inorganic phosphorus[4] do not distinguish the original phosphocreatinine from that hydrolysed to creatinine plus phosphate. The determination of creatinine in phosphocreatinine may be carried out by thin-layer chromatography (TLC)[6]; this appears to be a long and approximate method because of the error introduced by comparing the intensity of the creatinine spot in the sample with a standard amount of creatinine. The Jaffe alkaline picrate method for creatinine quantitation[7–9] is not applicable in the presence of phosphocreatinine: our studies showed that values were too high because of the hydrolysis of the phosphocreatinine that occurs during the reaction.

It, therefore, seemed necessary to us to work out a method for the simultaneous determination of phosphocreatinine and creatinine, the latter being always present as an impurity in pharmaceutical preparations containing phosphocreatinine.

EXPERIMENTAL

Apparatus

A high-pressure liquid chromatograph, Hewlett-Packard 1084 A, was equipped with two independent pumps, a column containing LiChrosorb-NH_2 (amino phase chemically bonded to 10 μm silica gel) (Brownlee Labs, Santa Clara, Calif., U.S.A.), a variable wavelength spectrophotometer (Hewlett-Packard 1030B) to monitor the column effluent, and a liquid chromatograph terminal (Hewlett-Packard 79850 A) to programme the chromatography conditions. The detector wavelength was set at 234 nm.

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Operating conditions

For creatinine, the operating conditions were adjusted to give a mobile phase flow-rate of 2.0 ml/min (resulting operating pressure 23–26 atm). The column oven temperature was set at 33°. The mobile phase consisted of acetonitrile (LiChrosolv; Merck, Darmstadt, G.F.R.), double distilled water (90:10). Creatinine can also be determined by means of the mobile phase described below.

For creatinine and phosphocreatinine, the operating conditions were adjusted to give a mobile phase flow-rate of 2.5 ml/min (resulting operating pressure 38–40 atm). The column oven temperature was set at 55°. The mobile phase consisted of acetonitrile–phosphate buffer pH 8 (60:40) prepared fresh daily.

Reagents

LiChrosolv was filtered through a 0.2 μm membrane filter (Sartorius type SM 11607) and double-distilled water was filtered through another 0.2 μm membrane filter (Sartorius type SM 11307). Phosphate buffer (pH 8) was prepared by dissolving 50.0 ml of potassium dihydrogen phosphate (0.2 *M*) with 46.8 ml of sodium hydroxide (0.2 *M*) to 200 ml with double-distilled water. This solution was filtered through the same membrane filter as the water.

Column purge

In the determination of creatinine, to preserve column efficiency daily after analyses, it was advantageous to remove the phosphocreatinine from the column by washing it with phosphate buffer (pH 8) for 10 min at 55° and then to flush the whole apparatus with double-distilled water for 10 min (flow-rate 2.5 ml/min).

Preparation of standards

Standard solution of creatinine. Approximately 100 mg of creatinine (Carlo Erba, Milan, Italy), accurately weighed, was transferred to a 100-ml volumetric flask and brought to volume with double-distilled water (final concentration 1 mg/ml). This solution was diluted to a final concentration of 500, 200, 100, 50, 25 or 10 μg/ml and assayed.

Adenine internal standard solution. Approximately 40 mg of adenine (Merck), accurately weighed, was transferred to a 100-ml volumetric flask and brought to volume with double-distilled water, gently warming for dissolving if necessary (final concentration 400 μg/ml). Aliquots of this solution were mixed with aliquots of concentrated creatinine solution to get the following concentration ratios of creatinine to adenine internal standard (expressed in μg/ml): 50:200, 100:200, 400:200, 250:100.

Standard solution of phosphocreatinine. Phosphocreatinine disodium 5-hydrate salt from Fabbrica Italiana Sintetici (Vicenza, Italy) had the following characteristics: water (K. Fischer), 27%; free creatinine, less than 0.5% determined by TLC, then exactly determined by our method by HPLC to be 0.42%. An accurately weighed sample of *ca.* 100 mg of the salt was transferred to a 100-ml volumetric flask and brought to volume with double-distilled water (final concentration of 1 mg/ml). This solution was diluted to a final concentration of 500, 200, 100 or 50 μg/ml and assayed.

Thiamine monophosphate ester phosphoric acid salt internal standard solution. Approximately 50 mg of thiamine ester internal standard (Merck), accurately weighed, was transferred to a 100-ml volumetric flask and brought to volume with double-

distilled water (final concentration of 500 μg/ml). Aliquots of this solution were mixed with aliquots of concentrated phosphocreatinine solution to get the following concentration ratios of phosphocreatinine to thiamine ester internal standard (expressed in μg/ml): 100:200, 500:500, 500:200 and 500:100.

Preparation of samples

The contents of capsules and lyophilized ampoules were dissolved in water and diluted. Internal standard solutions were added in the concentrations detailed above. Before assays all these solutions were filtered through a membrane filter (Sartorius type SM 11307).

RESULTS AND DISCUSSION

Six determinations of each standard solution were carried out. The retention times are given in Table I. Figs. 1 and 2 show typical chromatograms.

TABLE I

RETENTION TIMES (MIN) OF STANDARD SOLUTIONS

Mobile phase	*Acetonitrile–water (90:10)*	*Acetonitrile–buffer pH 8 (60:40)*
Creatinine	5.0	1.6
Phosphocreatinine	Retained in column	3.5
Adenine	4.2	—
Thiamine monophosphate ester	—	4.7

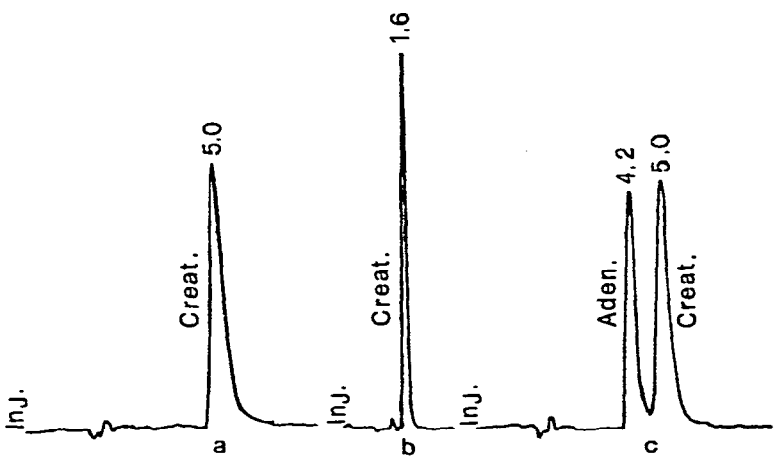

Fig. 1. Chromatograms of (a) creatinine with acetonitrile–water (90:10); (b) creatinine with acetonitrile–phosphate buffer, pH 8 (60:40); (c) creatinine + adenine internal standard with acetonitrile–water (90:10).

Figs. 3 and 4 show that in the range examined excellent linearity ($P < 0.001$) was found between the concentrations and the peak areas.

Moreover, precision evaluation assay, according to Saunders and Fleming[3], shows that in this range the precision limit mean is 1.2%. The method is simple and

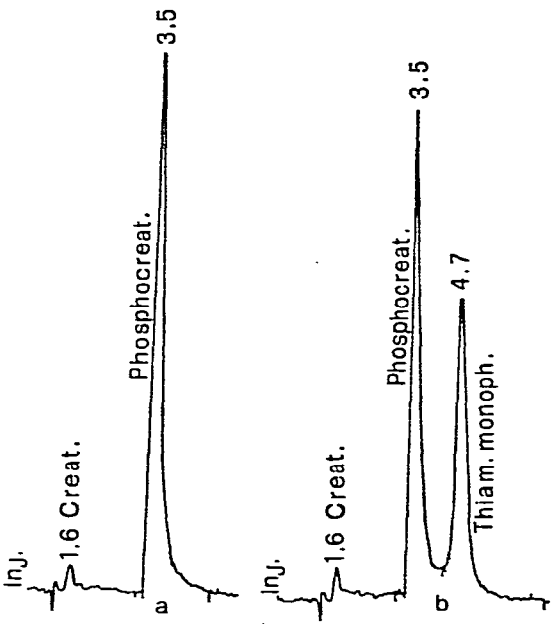

Fig. 2. Chromatograms of (a) phosphocreatinine with acetonitrile–phosphate buffer, pH 8 (60:40); (b) Phosphocreatinine + thiamine monophosphate ester internal standard with acetonitrile–phosphate buffer, pH 8, (60:40).

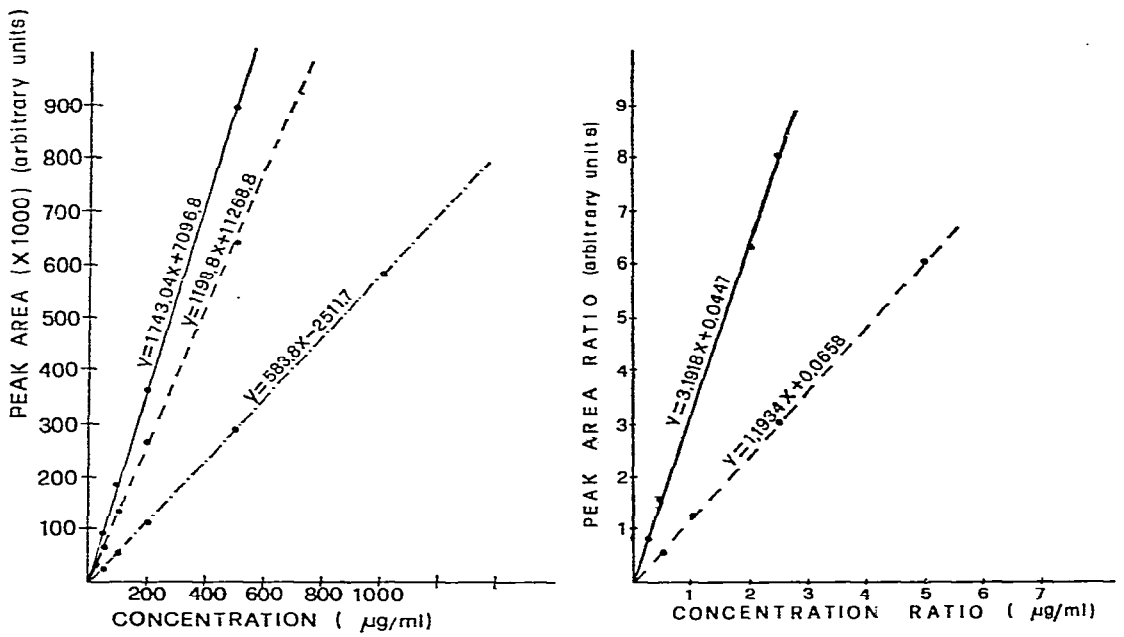

Fig. 3. Calibration curve: creatinine and phosphocreatinine concentrations against peak area. Each point is the mean of six determinations. ———, Creatinine; — — —, creatinine (buffer); -·-·-·-, phosphocreatinine

Fig. 4. Calibration curve: concentration ratio of creatinine and phosphocreatinine to internal standard against peak area ratio of creatinine and phosphocreatinine to internal standard. Each point is the mean of six determinations. ———, Creatinine + internal standard; — — —, phosphocreatinine + internal standard.

rapid and this is important for quality control and stability testing of pharmaceutical preparations. Therefore we think that the specificity, ease and rapidity will make the method important for the analysis of phosphocreatinine and its main degradation product, creatinine.

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