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Determination of protamine sulphate in drug formulations using high performance liquid chromatography

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Abstract

Protamine sulphate, which has the property of neutralising heparin, is determined in pharmaceutical formulations using spectrophotometric (BP 1995) or biochemical methods (USP XXIII 1995). Accuracy of these methods is not very high. We applied the HPLC technique for the assay of protamine sulphate in a gel formulation. The assay was carried out on a diol-type column with a mobile phase containing 8% acetonitrile in 0.15% trifluoroacetic acid at pH 2.5. The flow rate was 1.0 ml min^{-1}. The results obtained show that HPLC can be used for the determination of protamine sulphate in pharmaceutical preparations. The method is rapid and more accurate than those described until now.

Keywords: Protamine sulphate; Hydrophilic gel; High performance size exclusion chromatography

1. Introduction

For some years HPLC method has played an important part in separation of compounds in mixtures and has been a very powerful technique offering possibility of qualitative and quantitative determination of different drugs. Protamine sulphate is a purified mixture of simple protein principles obtained from the sperm or testes suitable species of fish, which has the property of neutralizing heparin [1]. It is used two pharmaceutical formulations: injection and gel [2]. Protamine sulphate injection is indicated in the treatment of heparin overdosage and protamin sulphate gel is used in stomatology in treatment of paradontopathy. Protamine sulphate is included in USP, British and European Pharmacopeias in monographs relating to the raw material and injection formulations. Unfortunately, the assay methods described are non-specific and time consuming as biological methods [1,3,4].

High performance size exclusion chromatography separates molecules according to their size in solution and has been an important tool for the analysis of polymers. Several chromatographic methods have been reported for identification of protamines derived from vertebrate sperm but procedures developed specifically for the determination active component in gel formulation have not been reported [5–7].

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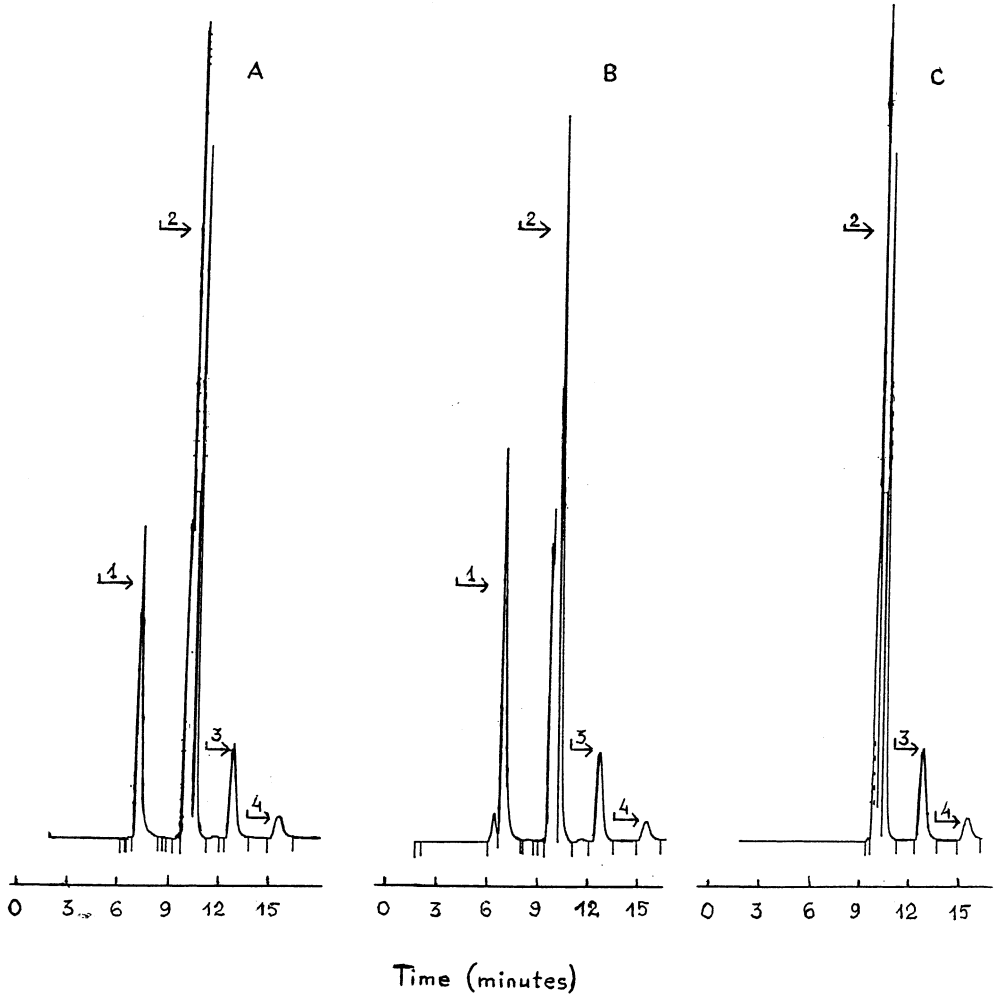

Fig. 1. Chromatograms of standard (A), sample (B), placebo (C). Peak 1, protamine sulphate; Peak 2, vehiculum; Peak 3, 4, preservatives.

2. Experimental

2.1. Apparatus and chromatografic conditions

The HPLC system consisted of a controller (SCL-10A Shimadzu), two pumps (LC-10AS Shimadzu) connected to an autosampler with 50 μl volume loop (SIL-10A Shimadzu) and a variable wavelength UVspectrophotometric detector (SPD-10A Shimadzu). For the constant chromatographic column temperature control a column oven was incorporated into the system (CT0-10AC Shimadzu). Chromatograms were recorded with C-R6A Chromatopac integrator (Shimadzu).

A 300×7.8 mm i.d. stainless-steel column packed with 10 μm silica diol column (Waters) was used with a mobile phase containing 8% acetonitrile (Lab-Scan Ireland) in 0.15% trifluoroacetic acid (BDH) v/v, adjusted to pH 2.5. It was filtered, degassed and pumped at a flow rate of 1 ml min^{-1}. The column temperature was maintained at 25°C and the detection wavelength was 214 nm. The injection volume was 50 μl.

Table 1
Repeatability

Sample number	Concentration (%)
1	1.909
2	1.907
3	1.907
4	1.895
5	1.883
6	1.896
7	1.888
8	1.892
9	1.925
10	1.911

2.2. Preparation of stock standard solution

Protamine sulphate (supplied by WHO/NIBSC-UK) was dissolved in water to produce solution containing 1 mg ml.$^{-1}$ Working standards for preparation of a standard curve were prepared containing 15, 20, 30, 40, 60, 80, and 100 μg ml^{-1}. These solutions were prepared just before use.

The composition of drug 'protaminum sulfuricum' gel (supplied by Unia, Warsaw, Poland) was:

Active agent	Protamine sulphate-2.0% (w/w)
Preservatives	Methyl-4-hydroxybenzoate
	Propyl-4-hydroxybenzoate
Vehiculum	Hydroxyethyl cellulose
	Glycerol
	Ethanol
	Purified water

2.3. Preparation of samples

A quantity of a gel (0.125 g) equivalent to 2.5 mg of protamine sulphate was placed in a 25 ml volumetric flask, 20 ml of water were added, the flask was shaken vigorously for 3 min to dissolve this gel and made up to volume with water. After stirring 5 ml of this solution was transferred to each of two 25 ml volumetric flasks. The first flask was filled up to volume with water. The chromatogram was recorded. For recovery determination to the second volumetric flask 5 ml of working standard solution of protamine sulphate (concentration = 100 μg ml^{-1}) was added and the procedure followed as described above. Ten repeated determinations were made.

3. Results and discussion

Under the described chromatographic conditions the absolute retention times were found to be 7.2 min for protamine sulphate, 14.9 min for methyl-4-hydroxybenzoate and 18.8 min for propyl 4-hydroxybenzoate (Fig. 1). The preservatives did not interfere with the found of protamine sulphate.

3.1. Validation

The method was tested for linearity, repeability, intermediate precision and recovery. The linearity of chromatographic response for the protamine sulphate was determined over the range 15–100 μg ml^{-1} ($R = 0.9926$, $n = 7$, RSD = 114.27, slope = 8951 and intercept = 0).

Repeatability data of ten independent determi-

Table 2
Intermediate precision

Determination	Concentration (%) day 1	Concentration [%] day 2	Concentration [%] day 3
1	1.907	1.938	1.881
2	1.907	1.916	1.932
3	1.902	1.943	1.902

nations of active ingredient were made on the same sample of drug. The results are presented in Table 1. RSD value was 0.67%.

Intermediate precision data for three series of determinations of protamine sulphate response were made on different days. The results are presented in Table 2. As these data shown precision (RSD values) of the determination were 1.05%.

The recovery was 99.9% with RSD of 1.15 ($n = 10$).The limit of quantitation for the method has been determined as 1 μg ml^{-1} and the limit of detection was determined as 15 μg ml^{-1}.

Protamine sulphate was stable during the analytical procedure and method can be used as a stability-indicating assay.

4. Conclusion

This method was very simple because the sample preparation consisted only in the dissolution of a portion of the gel in water. It has been shown that this method is suitable for the routine qualitative and quantitative control of protamine sulphate in the hydrophilic gels.

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