Quantitative Determination of Sodium-Octanoate in Human Serum Albumin Preparations

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Introduction

For many years commercially prepared human serum albumin (HSA) solutions for transfusion purposes has had to be heated for 10 h at 60°C in the presence of stabilizers to inactivate transmissible infectious agents, e.g. hepatitis or human immune deficiency viruses (HIV). As thermal stabilizers, mainly sodium octanoate (sodium caprylate) and/or sodium acetyltryptophanate are used to avoid heat-denaturation of albumin [1–3]. For quality control of the final HSA product, in order to determine the sodium octanoate content, we use a simple and fast reversed-phase high-performance liquid chromatography method, which we want to describe in this short report.

Material and Methods

Octanoic acid and decanoic acid were purchased from Merck (Darmstadt, FRG).

Sample preparation: To 2 ml of the final 5 % or 20 % HSA solution an internal standard of 8 or 32 µmol/l sodium decanoate from a stock solution was added and mixed well. 200 µl of this protein solution were added to 800 µl methanol and mixed with a vortex. The precipitate was centrifuged, and the supernatant was removed and filtered through a 0.45 µm Millex® HV filter (Millipore, Bedford, USA). Reversed-phase high-performance liquid chromatography of the extracts was done with a 4.6 × 250 mm Nucleosil®-C18 (5 µm) column (Machery-Nagel, Düren, FRG) with 0.1% trifluoroacetic acid (TFA) in methanol/water (80:20) as mobile phase at a flow rate of 0.8 ml/min. 20 µl was injected. Detection was done by ultraviolet (UV) absorption at 214 nm. Each sample extract was injected at least twice and the peak areas were averaged.

Results

For reproduceable results we have established an internal standard reversed-phase HPLC method with sodium decanoate as standard substance. This standard, converted in the free acid form by an acid eluent, is well resolved from the octanoic acid and other small impurity peaks in the chromatogram, shown in figure 1.

For determination of the relative response at 214 nm and the relative recovery of octanoic and decanoic acid extracted from human serum albumin preparations, equal molar amounts

CO
CD
λmL
Λ

Area
63317 63474
Cone
49.938 50.062
Name
Octanoic acid Decanoic acid
PKNO Time
1 6.817
2 12.167
Total
126790 100

Fig. 1. Reversed-phase HPLC chromatogram of a methanolic extract from a 5 % HSA preparation with 8 mmol/l octanoate (retention time of octanoic acid: 6.817 min) and 8 mmol/l decanoate (retention time of decanoic acid: 12.167 min) with relative peak areas of about 1:1. PKNO = peak number.

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(8 or 32 mmol/l) of sodium octanoate and decanoate were added to pure albumin solutions (5% or 20%) without any additional stabilizer. After mixing, the stabilizer and internal standard were extracted and analyzed as described above. Figure 1 shows the chromatogram of this determination and the relative peak areas of octanoic and decanoic acid. As shown in figure 1, no difference in 214 nm absorption and recovery could be detected.

In a second experiment an albumin solution with the stabilizer (octanoate) was heated for 10 h at 60 °C before adding an equal amount of internal standard (decanoate) and extraction. But this pasteurization step did not influence the recovery of one component.

With a response/recovery factor of 1.0, there is a simple equation for the calculation of the sodium octanoate content in human serum albumin preparations:

peak area

octanoic acid
mmol/l octanoate = $\frac{\text{peak area octanoic acid}}{\text{peak area decanoic acid}} \times \text{mmol/l decanoate added}$

peak area

decanoic acid

Discussion

The described method for quantitative determination of sodium octanoate in human serum albumin preparations is a simple and fast method. The extraction procedure of 1–20 samples took no longer than 10 min. The chromatographic run on a reversed-phase high-performance column and automatic registration and calculation by an integrator required 15 min for one sample. With an autoinjector up to 60 samples could be analyzed overnight.

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