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Short Communication

Determination of stabilizers in human serum albumin preparations

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ABSTRACT

A rapid isotachophoretic method was used for the simultaneous determination of sodium octanoate and N-acetyl-DL-tryptophan, which are used for the stabilization of human serum albumin (HSA) solution. The methods of external calibration and standard addition were used with conductimetric and spectrophotometric detection. The operational system applied is suitable also for the simultaneous determination of organic acids present in HSA solution without further treatment.

INTRODUCTION

Commercially prepared solutions of 20 or 5% human serum albumin (HSA) concentration are thermally treated in the course of their production in the presence of stabilizers [1], *e.g.*, a mixture of 20 or 5 mmol each of sodium octanoate and N-acetyl-DL-tryptophan. In order to test the quality of the final products, various analytical methods have been utilized [2–10], but all of them require treatment of the samples prior to the analysis. In this paper, a rapid isotachophoretic method without treatment such as extraction or protein precipitation is suggested. The operational system described by Everaerts *et al.* [11] was modified and used for the simultaneous determination of organic acids in the HSA solution.

EXPERIMENTAL

Samples of albumin were from Imuna (Šarišské Michal'any, Slovak Republic). Sodium octanoate and N-acetyl-DL-tryptophan were supplied by Merck (Darmstadt, Germany), the reagents for the preparation of the operational electrolyte system by Serva (Heidelberg, Germany) and acetate and citrate by Lachema (Brno, Czech Republic).

A CS isotachophoretic analyser (VVZ PJT, Spišská Nová Ves, Slovak Republic) was used. The separation unit was assembled in a coupled-column configuration [12–14] with a 40 × 0.85

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mm I.D. preseparation column coupled to a 150 × 0.30 mm I.D. analytical column with conductimetric and spectrophotometric (254 nm) detection. The detector signals were automatically recorded and evaluated with a computer using ITP software from KasComp (Bratislava, Slovak Republic). The operational electrolyte system was as follows: the leading electrolyte consisted of 10 mmol/l each histidine and histidine monochloride, with 0.1% hydroxylethylcellulose (HEC) solution added to suppress electroendoosmosis, and the terminating electrolyte contained 5 mmol/l 2-(N-morpholino)ethanesulphonic acid (MES) and 9.6% ethanol. The pH was adjusted to 6.0 by adding tris(hydroxymethyl)aminomethane (Tris).

Samples were prepared by diluting the proteins to a concentration of 0.2% with water and ethanol and analysed without further treatment such as extraction or protein precipitation. A 30-μl aliquot of the sample was analysed. External calibration and standard addition were used. The calibration solutions contained 0.2% of protein (human albumin), 4.8% (v/v) of ethanol, 0.40–0.05 mmol of sodium octanoate and 0.05–0.40 mmol of N-acetyl-DL-tryptophan. The standard addition solution contained 0.40 mmol of sodium octanoate, 0.40 mmol of N-acetyl-DL-tryptophan, 0.70 mmol of citrate, 0.80 mmol of acetate and 4.8% (v/v) of ethanol. The sample solution was injected into the terminating electrolyte near the boundary between the leading and terminating electrolytes. The driving current in the detection capillary (I_2) was 50 μA.

RESULTS

The signals were evaluated from the conductimetric detector of the detection capillary in the case of sodium octanoate and from both the conductimetric and UV detectors of the detection capillary in the case of N-acetyl-DL-tryptophane.

Fig. 1 shows the isotachopherogram of 20% HSA diluted 100-fold. The zones of the components determined are perfectly separated, which enables not only sodium octanoate (zone 3) and acetyltryptophan (zone 4) to be deter-

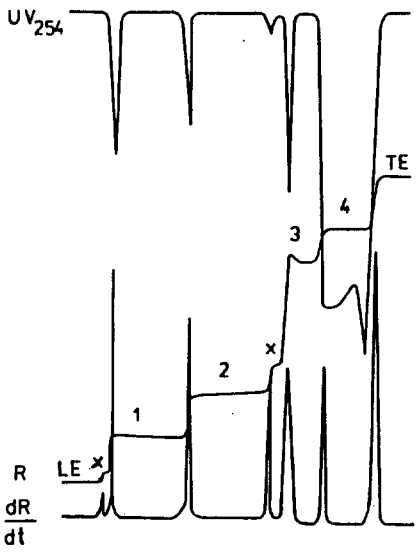

Fig. 1. Isotachopherogram of 20% HSA diluted 100-fold. 1 = citrate; 2 = acetate; 3 = sodium octanoate; 4 = N-acetyl-DL-tryptophan; × = unidentified impurities from electrolyte system. R = response of the conductimetric detector; dR/dt = derivative of the conductimetric detector response with time; A = absorbance of the UV detector at 254 nm; $I_1 = 250$ μA; $I_2 = 50$ μA; amount of sample = 30 μl.

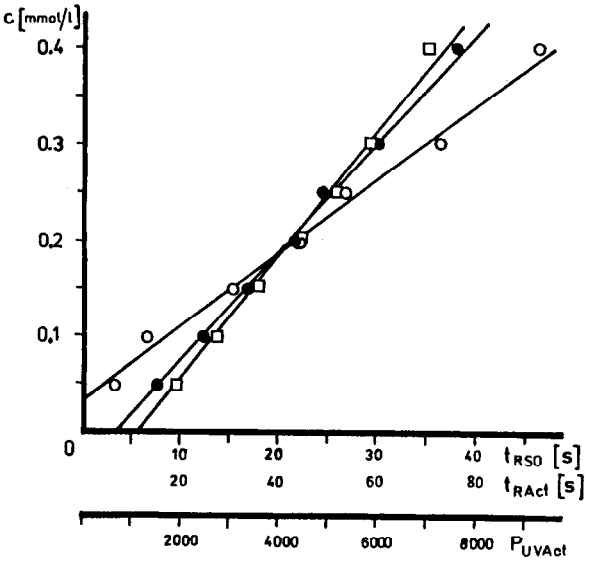

Fig. 2. Calibration graphs for (○) sodium octanoate and (●) N-acetyl-DL-tryptophan, both sets of values from the conductimetric detector, and (□) N-acetyl-DL-tryptophan from the UV detector at 254 nm. The horizontal axis represents zone length in seconds for conductimetric detection and the area of the peaks calculated electronically for UV detection.

mined, but also the citrate and acetate contents (zones 1 and 2, respectively).

For quantitative analysis the calibration graphs covered the range 0.05–0.40 mmol/l, obtained by analysing a series of standard solutions. The calibration graphs (see Fig. 2) were linear in this range and can be expressed by the following equations:

$$c_{RSO} = 0.0077t + 0.034 \quad r = 0.9960$$

$$c_{RAct} = 0.0058t - 0.042 \quad r = 0.9989$$

$$c_{UVAct} = 0.000065P - 0.82 \quad r = 0.9964$$

where c_{RSO} is the sodium octanoate concentration (mmol/l), c_{RAct} and c_{UVAct} are those for N-acetyl-DL-tryptophan obtained from conductimetric and spectrophotometric detection respectively (mmol/l), t(s) is the zone length, P is the peak area, and r is the correlation coefficient.

The results for the stabilizer content in the samples of 20% and 5% HSA are summarized in Table I. As can be seen, external calibration gives sufficiently accurate results (the declared values are 20 mmol). In spite of this, it is necessary to use the standard addition method when citrate and acetate in HSA are to be determined. These compounds are usually present in HSA solutions. Analysis is carried out in the presence of proteins, *i.e.*, without their removal prior to the isotachophoretic determination.

The reproducibility of the zone measurements with sodium octanoate is less than 1 s and with N-acetyl-DL-tryptophan less than 2 s. UV detection is more suitable for N-acetyl-DL-tryptophan determination.

CONCLUSIONS

The method described for the determination of sodium octanoate and N-acetyl-DL-tryptophan in HSA is rapid and simple. The time for one measurement of a multi-component mixture is *ca.* 22 min. It is possible, in addition to the above-mentioned components, to determine simultaneously also citrate and acetate ions. By the standard addition method, twenty samples of HSA were analysed isotachophoretically with a relative error of 1–15%.

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TABLE I

STABILIZER CONTENT OF 20% AND 5% HSA

Leading electrolyte: 10 mmol histidine–histidine monochloride (pH 5.95). NAct = N-acetyl-DL-tryptophan; $NAct^+$ = values obtained with UV detection.

Stabilizer	Stabilizer content (mmol/l)			
	20% HSA		5% HSA	
	External calibration	Standard addition	External calibration	Standard addition
Sodium octanoate	19.8	18.2	4.9	4.2
NAct	19.0	22.3	4.9	5.2
$NAct^+$	20.8	20.3	4.9	4.9

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