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Short communication

Capillary electrophoresis with indirect UV detection for the determination of stabilizers and citrates present in human albumin solutions

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ABSTRACT

Sodium caprylate and N-acetyltryptophan are the most frequently used stabilizers that protect the albumin from aggregation or heat induced denaturation. In turn citrates – excipients remaining after fractionation process – can be treated as by-product favoring leaching aluminum out of glass containers whilst albumin solution is stored. With ionic nature these substances have all the markings of a subject for capillary electrophoresis analysis. Thus CE methods were proposed as new approach for quality control of human albumin solution in terms of determination of stabilizers and citrates residue.

Human albumin solutions both 5% and 20% from various manufacturers were tested. Indirect detection mode was set to provide sufficient detectability of analytes lacking of chromophores. As being anions analytes were separated with reversed electroosmotic flow. As a result of method optimization two background electrolytes based on p-hydroxybenzoic acid and 2,6-pyridinedicarboxylic acid were selected for stabilizers and citrates separation, respectively. The optimized methods were successfully validated. For citrates that require quantification below 100 μM the method demonstrated the precision less than 4% and the limit of detection at 4 μM. In order to check the new methods accuracy and applicability the samples were additionally tested with selected reference methods. The proposed methods allow reliable quantification of stabilizers and citrates in human albumin solution that was confirmed by method validation as well as result comparison with reference methods. The CE methods are considered to be suitable for quality control yet simplifying and reducing cost of analysis.

1. Introduction

Stabilizers, like preservatives, are a considerable group of compounds which are used in pharmaceutical products as excipients. Their variety covers substances of different chemistry including amino acids, polyalcohols, carboxylic acid salts, carbohydrates and certain proteins [1–4]. Such substances are added to a pharmaceutical formulations to prevent the active substance from losing its biological activity. Most commonly they are added to pharmaceutical products containing peptides or proteins, including human albumin solutions.

Due to its specific structure the human albumin molecule shows a distinct tendency to form polymers and aggregates. In particular, heating, concentration, dilution or lyophilization are important factors affecting aggregate formation [5–9]. Sodium caprylate and N-acetyl-DL-tryptophan are the most frequently used albumin stabilizers [1,10]. These compounds bind specifically to the albumin molecule preventing aggregation or heat induced denaturation.

In the literature several methods have been described for the determination of the stabilizers mentioned above. Acetyltryptophan present in human albumin preparations has been determined using reversed phase HPLC methods [11,12], and a spectrophotometric method after albumin precipitation has been employed [13]. Gas chromatography is frequently used to determine sodium caprylate [12–14]. Whilst an enzymatic method, making use of the activity of acyl-coenzyme A synthetase towards free fatty acids has also been described [15]. However, to the authors best knowledge, no publication has offered a method allowing simultaneous determination of sodium caprylate and acetyltryptophan in albumin preparations. The ionic nature of the mentioned compounds suggests that capillary electrophoresis can be used for the simultaneous determination of both stabilizers in albumin solutions.

The capabilities and advantages of capillary electrophoresis can be also applied for determination of citrate ions in the human albumin medicinal products. Sodium citrate and citric acid used as anti-coagulants during blood collection are substantially removed in the subsequent fractionation process to produce the albumin solution but a considerable amount of citrate ions remain in the product. Thus in the case of fast administration of high volumes of albumin solution some adverse effects as: hypocalcaemia, hypomagnesaemia as well as disorders in the acid/base equilibrium can

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result [16–18]. Recently, the necessity to control the citrate ion content in the final pharmaceutical product has been often stressed since citrates can form complexes with inorganic cations, including aluminum [19–21].

This paper simplified quality control method for the simultaneous determination of stabilizers (sodium caprylate and acetyltryptophan) as well as citrate ions in human albumin preparations using capillary electrophoresis.

2. Experimental

2.1. Materials

N-acetyl-DL-tryptophan reference standard (AcTrp), sodium caprylate reference standard were purchased from Sigma–Aldrich (Poznań, Poland) and trisodium citrate monohydrate reference standard was purchased from JT Baker (Łódź, Poland)). All reagents used for buffers preparation, a capillary conditioning and sample pretreatment were of analytical-reagent grade. The materials used: tris(hydroxymethyl)-aminomethan (Tris), p-hydroxybenzoic acid (PHBA), o-phtalic acid (Phtal) were all from Merck (Darmstadt, Germany); 1,2,4,5-benzenetetracarboxylic acid (BTCA), 1,2,4-benzenetricarboxylic acid (BtriCA), 2,3-naphtalenedicarboxylic acid (NDA), 2-(N-morpholine)-ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES), N-[tris(hydroxymethyl)-methyl]glycine (TRICINE), sorbic acid (2,4-hexadienoic acid, Sorb) were all supplied by Sigma–Aldrich (Poznań, Poland); bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methan (BisTris), 2,6-pyridine-dicarboxylic acid (PDC), polybrene (hexadimetrine bromide, HDB) were from Fluka (Sigma–Aldrich, Poznań, Poland); sodium hydroxide (NaOH) from Applichem (Darmstadt, Germany) and isopropanol from Lab-Scan Ltd. (Dublin, Ireland).

Human albumin solutions for parenteral infusion 5% and 20% obtained from various manufacturers were used as test samples.

Two enzymatic kits: NEFA C enzymatic kit for free fatty acid determination (cat. no. 999-75406, Wako Chemicals GmbH, Germany) and citric acid (UV method; for the determination of citric acid in foodstuffs and other materials; Enzymatic Bioanalysis/Food Analysis) (cat. no. 10 139 076 035, Boehringer Mannheim/R-Biopharm, Germany) were used for caprylate and citrate reference assays, respectively.

Aqueous solutions of aromatic carboxylic acids (PHBA, Sorb, Phtal, NDA, PDC, BtriCA and BTCA) were prepared in a concentration of 50 mM each adding 0.2 M NaOH for complete solubilization, where appropriate. Buffering reagents (MES, HEPES, TRICINE, BisTris and Tris) were dissolved in water to obtain stock solutions with a concentration of 200 mM each. Background electrolytes were prepared by mixing suitable amount of selected aromatic carboxylic acid solution and buffer stock solution to reach the desired concentration. The pH of electrolyte was adjusted with 0.2 M NaOH when necessary.

Acetyltryptophan and caprylate reference solutions were prepared at a concentration of 8 mM and citrate reference solution was prepared at a concentration of 2 mM. Reference standard substances were dissolved in water with addition of the lowest volume of 0.2 M NaOH needed for complete solubility.

Highly purified deionized water was prepared using EASY Pure RF deionizer (Barnstead-Thermolyne, Dubuque, IA, USA). All buffers and solutions applied to a capillary were filtered through GDX MF (micro-fiber) syringe filter 0.45 μm (Whatman, Maidstone, UK).

2.2. Equipment

Capillary electrophoresis equipment BioFocus 2000 (BioRad, Hercules, CA, USA) with UV detector was used during the study. Separations were carried out in 50 cm (45.4 cm to detector), 50 μm ID fused silica capillaries (Beckman Coulter, Fullerton, CA, USA) thermostated at a temperature of 20 °C with voltage 440 V/cm (negative polarity). Various injection modes and detection wavelengths were applied depending on analyte assayed and chromophore component used in BGE.

A new capillary has been flushed with isopropanol, and then with 0.2 M NaOH solution, deionized water for 100 s at 100 psi each. Before use the capillary has been conditioned with 0.2 M NaOH solution, deionized water, 1 mg/ml HDB solution and background electrolyte for 100 s each. Between runs reconditioning included flushing with 0.1 M NaOH solution, 1 mg/ml HDB solution and background electrolyte for 50 s each.

Spectra analysis and enzymatic assays of caprylates and citrates were done with UV-VIS spectrophotometer U-2000 (Hitachi, Japan) in 1 cm quartz cuvette. Shimadzu 10A VP HPLC isocratic equipment (Shimadzu, Tokyo, Japan) with UV detector set at 280 nm was used for acetyltryptophan determination.

2.3. CE procedures

2.3.1. Stabilizers determination

Electrophoresis was performed with a background electrolyte containing 5 mM PHBA, 20 mM Tris at pH 8.5. Samples were injected hydrodynamically applying 5 psi pressure for 1 s and peaks were recorded at 254 nm. Albumin samples were diluted with water up to final analyte concentration 0.16 mM each in a solution injected into the capillary. For quantification a standard addition method was used in order to reduce the matrix influence on a peak area. Standard addition at three levels was applied covering 80–120% of amount of each of stabilizers in the sample The corrected peak area was taken for calculation and each result was obtained in triplicate.

2.3.2. Citrates determination

Citrates were determined using electrophoretic system based on 2,6-pyridinedicarboxylic acid (PDC). Background electrolyte containing 5 mM PDC 20 mM Tris, pH 8.6 required detection at 272 nm. Albumin samples were used undiluted. Electrokinetic injection 15 kV (negative polarity) for 10 s was applied in order to increase analyte signal and to avoid high amount of sample matrix to be introduced into the capillary. Since electrokinetic injection depends on some of sample related parameters (i.e. pH, ionic strength) attempts were made to avoid significant variations between the composition of the reference and test solution. Therefore the quantification was performed with a standard addition method assuring that the addition of reference solution was not greater than 20% of total sample volume. In case of citrate level higher than 0.5 mM samples were diluted appropriately. Standard addition at three levels was applied covering 80–120% of amount of citrate in the sample. The corrected peak area was taken for calculation and each result was obtained in triplicate.

2.4. Reference methods

The following methods are selected by a manufacturer for stabilizers and citrates determination in an albumin solution, hence they were used as reference methods for evaluation of CE method accuracy.

2.4.1. N-acetyltryptophan determination by HPLC-SEC

Size exclusion TSK gel G3000 SWXL 300 mm × 7.8 mm column (TosoH Corporation, Japan) was selected for the test. Small molecule of acetyltryptophan demonstrates the highest retention comparing to the other constituents present in the pharmaceutical product. As the stabilizer peak was detected at 280 nm, caprylates did not interfere with the detection. The mobile phase contained 27.4 mM

$Na_2HPO_4 \cdot 12\ H_2O$; 12.6 mM $NaH_2PO_4 \cdot 2\ H_2O$; 200 mM NaCl and 0.005% NaN_3 and pH was not adjusted. The mobile phase was pumped with 0.6 ml/min flow (run time 35 min) and the column was thermostated at 25 °C.

Acetyltryptophan reference solution was diluted with mobile phase to obtain a standard curve covering the range of 0.2–0.6 mM. The samples were diluted with a mobile phase up to final concentration of stabilizer of 0.4 mM. Salicylic acid at a concentration of 0.2 mM was used as internal standard.

2.4.2. Enzymatic determination of caprylic acid with VIS detection

Chemical principle of the assay makes use of free fatty acid (FA) conversion into the thiol esters of coenzyme A (CoA) known as acyl-CoA after treating with acyl-CoA synthetase in the presence of adenosine triphosphate (ATP), magnesium cations and CoA. Next the acyl-CoA is oxidized by added acyl-CoA oxidase to produce hydrogen peroxide which in the presence of peroxidase allows the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple colored product with absorption maximum at 550 nm.

The tests were done according to the manufacturer instruction with exact 10 min incubation time at 37 °C. 1 mM FA solution (from the kit) was used as a reference standard. Albumin solutions were diluted with water up to final caprylate concentration of 1 mM.

2.4.3. Enzymatic determination of citrates using UV detection

The method is based on enzymatic conversion of citrate finally to oxaloacetate and pyruvate that are reduced to L-maleate and L-lactate, respectively, by reduced nicotinamide-adenine dinucleotide (NADH). The amount of oxidized NADH is stoichiometric to the amount of citrate and is determined by recording absorbance at 340 nm. The test was performed at 25 °C, following the manufacturer procedure, taking care to assure the exact 5 min reaction time and adjusting the amount of sample (up to 2 ml) to reach optimum absorbance difference between the sample and the blank. Undiluted samples were analyzed.

3. Results and discussion

3.1. CE method development

The acidic properties of all studied compounds, the lack of chromophore in sodium caprylate and sodium citrate molecules, as well as literature data on analysis of low molecular weight carboxylic acids [22–26], suggested the application of zone electrophoresis with reversed electroosmotic flow and indirect detection.

Indirect detection was accomplished by use of background electrolyte containing aromatic carboxylic acids (PHBA, Sorb, Phtal, NDA, PDC, BtriCA and BTCA) as they show significant absorbance in UV range. The detection wavelength was optimized by taking into account the maxima of UV-spectra for carboxylic acids and acetyltryptophan. Based on spectroscopic data the most favorable detection was found in the range of 240–254 nm, corresponding to the minimum absorbance of acetyltryptophan.

A number of counterions was taken into consideration for BGE optimization. As zwitterionic compounds, MES, HEPES, TRICINE generated in most cases a system peak co-migrating with acetyltryptophan, thus only monovalent cationic buffers (BisTris and Tris) were found suitable for the separation. Additionally, using these buffers a wide pH range can be covered.

During the study TTAB, CTAB and hexadimetrine bromide (HDB) were used as EOF modifiers. However, the presence of these compounds in the background electrolyte can result in the appearance of additional system peaks, and also hinder formation of chloride peak, due to interference with bromides from the modifier [22].

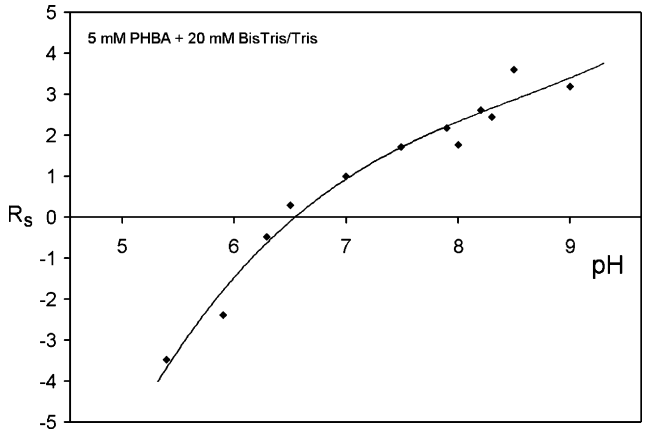

Fig. 1. The effect of pH of BGE (5 mM PHBA with 20 mM BisTris or Tris depending on the pH range) on resolution between caprylate and AcTrp. Negative R_s values represent reversed migration order of analytes.

Among all the modifiers tested only HDB can form on silica a dynamic coating which is stable enough to make its presence in BGE not necessary [22]. Therefore, before each separation the capillary has been reconditioned with HDB solution (1 mg/ml), and subsequently flushed with the separation buffer free from modifier. This procedure for pretreatment of the capillary between runs assured stable reversed EOF and good repeatability of solute migration times.

During the first stage, the work was focused on developing an electrophoretic system allowing effective separation and determination of all three compounds. The goal was to select a BGE composition for what the mobility was comparable to those of analytes being separated so as to achieve good peak symmetry and high efficiency of the system. A model mixture containing 0.16 mM AcTrp 0.16 mM caprylate 0.05 mM citrate was used with hydrodynamic injection. The separation efficiency as well as resolution and peak symmetry was checked for a number of electrolytes using all the above-mentioned aromatic carboxylic acids. Buffers used contained both 5 mM of acid and 10 mM BisTris, pH 6.0, with detection carried out at 254 nm.

The best separation for acetyltryptophan and caprylate was obtained in the buffers containing mono- or dicarboxylic acids. However, using Phtal or PDC, negative peaks of caprylate and citrate but positive peak of AcTrp were recorded.

In the BGEs containing NDA or Sorb considerable baseline fluctuations were observed, hindering integration and decreasing peak area repeatability. Only the PHBA containing electrolyte yielded a stable baseline and short analysis time. Moreover for all analytes negative peaks were recorded. However, regardless of the buffer used the peak corresponding to citrate was asymmetric. Thus this tailing peak, considering rather low content of this analyte in the sample, adversely affected the method sensitivity.

The influence of the electrolyte pH on separation of analytes was then checked. The electrophoresis was performed in buffers of different pH within the range from 5.5 to 9.0, containing both 5 mM of PHBA and BisTris (pH 5.5–7.3) or Tris (pH 7.0–9.0), respectively, depending on pH.

Fig. 1 presents resolution (R_S) obtained between caprylate and AcTrp depending on pH of the BGE (5 mM of PHBA and 20 mM of BisTris or Tris, respectively, depending on pH range). For pH 6.3–6.5 no separation of AcTrp and caprylate was observed, since both analytes showed the same electrophoretic mobility. In buffers of pH below 6.3, AcTrp migrated faster than caprylate (depicted in the diagram as a negative value of R_S), but with pH above 6.5 the migration order was reversed. The system efficiency increased for all peaks with increasing pH of the BGE. The best separation of two analytes

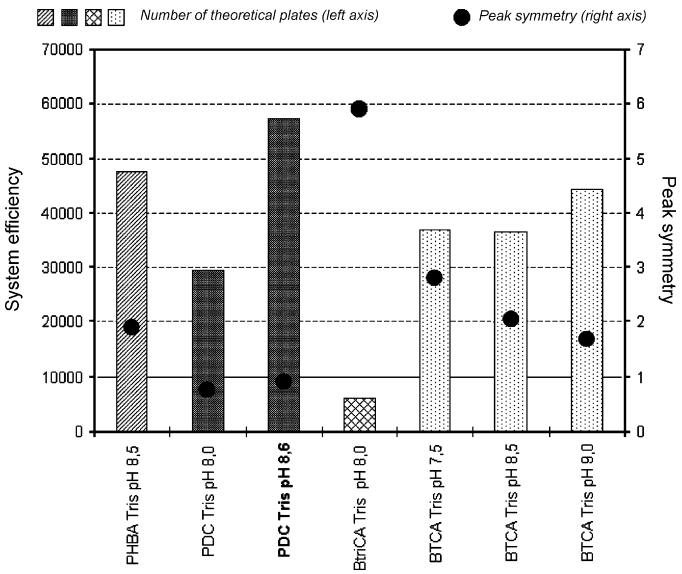

Fig. 2. The effect of BGE composition (5 mM selected carboxylic acid + 20 mM Tris) and pH on system efficiency (left axis) and peak symmetry (right axis) calculated for citrate peak. The highest number of theoretical plates and best peak symmetry close to 1 were achieved with BGE 5 mM PDC 20 mM Tris pH 8,6. Sample: model mixture 0.16 mM AcTrp 0.16 mM caprylate 0.05 mM citrate. Injection: hydrodynamic 5 psi 1 s.

was achieved in the buffer containing 5 mM of PHBA, 20 mM Tris of pH 8.5 (Fig. 1).

Unfortunately, the above described electrophoresis conditions were not suitable for citrates due to peak shape (symmetry factor A_S 1.73) that, in case of quantification at a level below 0.1 mM (0.03 mg/ml) in albumin solution did not assure sufficient method sensitivity.

The results of previous experiments were reviewed to select the system providing the optimal conditions for the determination of citrate ions at low level concentration. Fig. 2 shows comparison of system efficiency and citrate peak symmetry obtained for buffers containing different chromophores (mono-, di-, tri- and tetracarboxylic acids) and pH. The best parameters for the citrate peaks were obtained using a buffer containing 5 mM of PDC 20 mM and Tris at pH 8.6.

As a result of the method optimization, the following buffer composition was chosen for determination of stabilizers in human albumin preparations: 5 mM PHBA, 20 mM Tris at pH 8.5. However for determination of traces of citrate the buffer containing 5 mM PDC, 20 mM Tris of pH 8.6 was chosen.

3.2. Method validation

The above-proposed methods for determination of the stabilizers and traces of citrate in human albumin preparations have been validated for linearity range, detection and quantification limits, precision and intermediate precision. The results are presented in Table 1.

Good correlation was found between normalized peak area and concentration within the range from 0.025 to 2.0 mM for both caprylate and AcTrp. However, for concentrations higher than 1.7 mM a small deviation from linearity was observed (peak area was smaller than expected). It is typical for methods using indirect detection that the linearity range is rather narrow compared to the methods applying direct detection. In the case of citrate ions, given their low concentration in the samples, the linearity was tested in limited range, i.e. from 0.01 to 0.20 mM.

Limit of detection (LOD) for analytes was the concentration at which the signal to noise ratio was equal 3, whereas for the limit of quantification (LOQ) the signal to noise ratio was equal 10.

Validation of the methods was completed by checking precision and intermediate precision. The precision of the method is expressed as relative standard deviation (R.S.D.) of six consecutive assays performed the same day. The intermediate precision is calculated as R.S.D. of six assays performed on successive days. The higher R.S.D. values obtained for AcTrp reflect the fact that it migrates as the last peak and the peak shows higher asymmetry than that of caprylate. The R.S.D. values for precision and intermediate precision are below 2% and 4%, respectively, for stabilizers. The precision and intermediate precision for the citrates determination, assessed for the concentration range within 2 × LOQ–3 × LOQ, were found below 5%. Based on the validation results it can be considered that both methods are appropriate for the intended purpose.

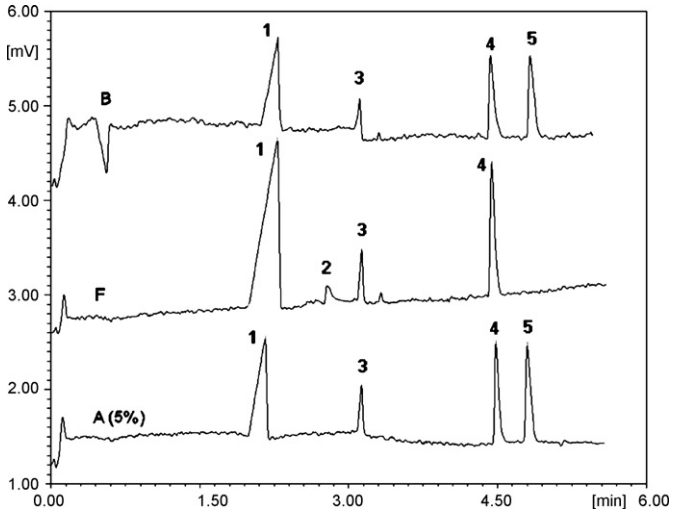

Fig. 3. The electrophoregrams of stabilizes in human albumin solution. Peak identification: (1) chlorides; (2) citrates; (3) system peak; (4) caprylates; (5) acetyltryptophan. Letters represent a manufacturer of each albumin preparation (see Table 2). Separation conditions: 5 mM PHBA with 20 mM Tris pH 8.5; 50 cm (45.4 cm to detector), 50 μm ID fused silica capillary; 20 °C; voltage 440 V/cm (negative polarity); det. 254 nm.

Table 1
Results of method validation (precision and intermediate precision: $N = 6$).

Compound tested	Linearity range (mM)	LOD (μM)	LOQ (μM)	Precision R.S.D. (%)	Intermediate precision R.S.D. (%)
Sodium caprylate	0.025–2.0 $r^2 = 0.9955$	10	25	0.98%	2.95%
N-acetyltryptophan	0.025–2.0 $r^2 = 0.9974$	10	25	1.30%	3.19%
Citrates	0.01–0.20 $r^2 = 0.9938$	4	10	3.64%	4.55%

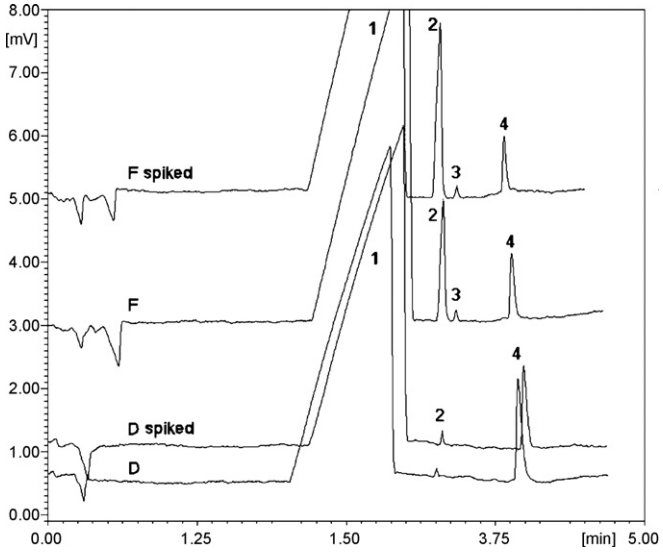

Fig. 4. The electrophoregrams of citrates in human albumin solution. Peak identification: (1) chlorides; (2) citrates; (3) unknown; (4) system peak. Letters represent a manufacturer of each albumin preparation (see Table 2). "spiked"—indicates sample fortified with citrate reference standard. Separation conditions: 5 mM PDC with 20 mM Tris pH 8.6; 50 cm (45.4 cm to detector), 50 μm ID fused silica capillary; 20 °C; voltage 440 V/cm (negative polarity); det. 272 nm; electrokinetic injection 15 kV for 10 s.

3.3. Method accuracy and real sample tests

The developed CE methods were applied for determination of stabilizers and trace of citrate in pharmaceutical products containing 5% or 20% of human albumin, originating from different local or foreign manufacturers. Most of the preparations contain both stabilizers at approximately of 0.08 mmol per 1 g of albumin. However, some of albumin solutions are stabilized with sodium caprylate only, but using a concentration twice higher.

All determinations were done using the standard addition method with calibration curve. Since the matrices were contained in the same amount in each sample set, the chloride peak was used as the internal standard to compensate for any injection error. Representative electroforegrams of the samples tested are shown in Figs. 3 and 4.

In case of caprylate and acetyltryptophan determinations, the final concentration of analytes in the injected samples was equivalent to 1:100 dilution of the preparations under investigation. Despite this high sample dilution, the amount of protein still present in a solution interacts with capillary wall and affects subsequent separation. For this reason, before each run the capillary was additionally flushed with 0.1 M of NaOH to assure removal of albumin. The application of this procedure resulted in good migration time repeatability for chlorides, caprylates and AcTrp (R.S.D. 1.95%, 2.13% and 2.31%; $N = 12$, respectively).

In turn, the quantification of citrate, because of its low content, required injection of the undiluted sample solution. However, apart from adverse effect on the separation caused by the presence of protein, as mentioned above, the high ionic strength (120–140 mM sodium) of undiluted albumin solution also adversely affected the separation leading to an antistacking effect and improper peak shapes. Significant improvement was obtained after applying the technique of selective electrokinetic injection, which exclusively allowed anions to be introduced into the capillary. This also resulted in increase of sensitivity for citrate.

To evaluate the accuracy of method, the results for stabilizers and citrates determined for a number of samples by means of the CE method were compared with the results obtained with the reference methods. The results are presented in Table 2.

Table 2
The results of stabilizers and citrates determination with new CE methods in albumin samples from various manufacturers in relation to results obtained with reference methods ($N = 3$).

Manufacturer	Albumin solution (%)	Sodium caprylate				Acetyltryptophan				Citrates			
		Specification (mM)	Reference method (mM)	CE method		Specification (mM)	Reference method (mM)	CE method		Specification (mM)	Reference method (mM)	CE method	
				Result (mM)	R.S.D.%			Result (mM)	R.S.D.%			Result (mM)	R.S.D.%
A	5	≤4.2	3.44	3.26	1.41	≤4.2	3.61	3.89	1.62	–	0.055	0.061	3.95
A	20	≤16.8	13.51	13.64	2.57	≤16.8	14.20	13.96	2.49	–	0.070	0.067	3.10
B	20	12.8–19.2	18.16	17.08	2.93	12.8–19.2	15.63	16.26	2.41	≤0.1	0.041	0.042	5.28
C	20	12.8–19.2	16.40	16.72	1.82	12.8–19.2	15.20	15.18	1.65	≤0.15	0.009	0.010	9.08
C	20	12.8–19.2	16.40	16.28	1.07	12.8–19.2	14.37	13.99	2.24	≤0.15	0.028	0.032	4.74
D	20	13–19	14.04	14.74	0.93	14.5–17.5	14.97	14.52	1.56	≤0.08	0.032	0.037	6.55
E	20	14.4–17.6	17.20	16.85	1.97	14.4–17.6	16.00	15.75	1.49	–	0.049	0.045	3.85
F[a]	5	8[b]	9.28	9.65	2.06	–	–	–	–	–	1.46	1.44	1.86

[a] Only sodium caprylate is present as stabilizer.
[b] No specification, the value represents label claim.

The CE results were found to be concordant with the reference methods and showed similar R.S.D. values when comparing the intermediate precision data. In case of stabilizers the difference between values obtained using CE and reference methods, in general, did not exceed 5%. In case of citrates, the difference in results between the CE and reference method, expressed as percentage, was higher, but it was related to lower absolute quantities of the analyte. In order to additionally confirm concurrence of the results, a paired *t*-test was applied indicating that the difference between these methods is statistically insignificant ($p = 0.686$).

Citrate content is not always specified for albumin preparations. Nevertheless, in the majority of cases the citrate ions content was found to be below 0.1 mM. Only in the albumin preparation supplied by manufacturer F the citrate content exceeded 1 mM. This is probably a result of differences in the manufacturing process.

4. Conclusions

The proposed methods allow fast separation, identification and determination of stabilizing substances (N-acetyltryptophan and sodium caprylate) as well as citrate ion residue present in human albumin preparations. Validation of the methods confirm their applicability in pharmaceutical analysis. Comparable results have been obtained to reference methods demonstrating the accuracy of the proposed methods. Thus advantages of the developed methods make them attractive for albumin solution testing. The use of two electrophoretic systems, instead of three reference methods, brings in simplification of the overall analysis, resulting in time and a cost reduction in quality control of human albumin containing preparations.

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