

Quality Control of Chondroitin Sulphate used in Dietary Supplements

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Abstract

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Chondroitin sulphate (CS), a complex polysaccharide isolated from animal cartilage, is used as an ingredient in dietary supplements to support joint health and/or to treat osteoarthritis. Several low-quality CS raw materials were identified in marketed products of dietary supplements. For the evaluation of the quality of CS, we present here a capillary isotachophoretic method (cITP). As a leading electrolyte for the anionic analysis of CS, a mixture of 5 mM HCl + 10 mM glycine + 0.01% of 2-hydroxyethylcellulose of pH 2.8 was used. A solution of 10 mM citric acid served as the terminating electrolyte. On a series of CS raw material samples we proved that the cITP is a suitable method for this purpose.

Keywords: capillary isotachopheresis; electrophoresis

Chondroitin sulphates (CS) are naturally occurring glycosaminoglycans (GAG) having repeating disaccharide units formed by glucuronic acid and *N*-acetyl-galactosamine residues linked by β bonds. The significant feature of the molecular structure of CS is the presence of a sulphate ester group in the *N*-acetyl-galactosamine and/or glucuronic acid residues, which makes the CS molecule a highly negatively charged ion. Depending on the position of the sulphate group in CS, several forms of CS are distinguished; the most common forms are chondroitin sulphate A also known as chondroitin 4-sulphate (sulphate group is located on C4 of *N*-acetyl-galactosamine) (Figure 1A), chondroitin sulphate B also known as dermatan sulphate, and chondroitin sulphate C also known as chondroitin 6-sulphate (sulphate group is located on C6 of *N*-acetyl-galactosamine) (Figure 1B). Molecular weight of CS varies from 9000 to 93 000 g/mol (S1M *et al.* 2005). CS is isolated mainly from bovine, porcine, or shark cartilages. Fish derived CS is currently referred to as the best quality raw material on the market, considering the degree of purity and sulphonation pattern. The identity and quality of CS is the major issue related to its therapeutic activity. Some dietary

supplements contain less than the labelled amount of CS, in some cases as little as 10%. Due to the poor CS quality of some nutraceuticals, there is a need

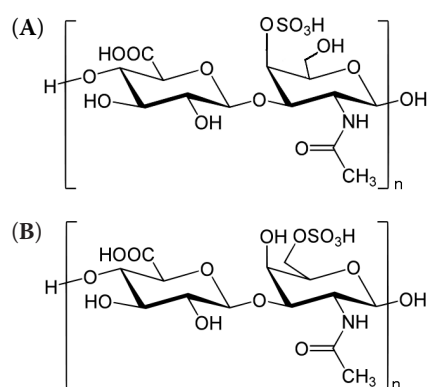


Figure 1. Structural formulas chondroitin sulphates: (A) chondroitin sulphate A (CS-A) is the alternative name for chondroitin 4-sulphate, i.e., chondroitin sulphate which is sulphated on the C4 position of the *N*-acetyl-galactosamine; (B) chondroitin sulphate C (CS-C) is the alternative name for chondroitin 6-sulphate, which is sulphated on the C6 position of the *N*-acetyl-galactosamine
MW of the disaccharide unit of CS-A, CS-B and CS-C is 443.34 g/mol, $n = 20\text{--}200$

for stricter regulations regarding the quality of CS as well as specific and accurate analytical procedures to confirm the purity and label claims for both the raw bulk materials and finished products (VOLPI 2009).

A number of methods for the determination of CS quality of pharmaceutical and/or nutraceutical preparations have been reported involving size-exclusion chromatography (CHOI *et al.* 2003), ion-pair HPLC (JIN *et al.* 2009), and CE (MALAVAKI *et al.* 2008). For the determination of chondroitin disaccharides highly selective methods have also been published based on enzymic digestion of CS using chondroitinase followed by HPLC (GRØNDHAL *et al.* 2011) or CE (OKAMOTA *et al.* 2004; MALAVAKI *et al.* 2008).

We present a capillary isotachopheresis (cITP) method for the evaluation of CS quality. This method was firstly used for the determination of CS in cosmetic products (YAMAMOTO *et al.* 1982). The authors used acidic electrolyte system (pH 3.2) for the analysis of CS after extraction of interferences with cetylpyridinium chloride. The recovery of CS was 92.4% and the method was applicable for the qualitative and quantitative analysis of CS in commercial cosmetic products. Recently, we reported on the application of cITP methods for the determination

of CS and glucosamine quantities in dietary supplements (VÁCLAVÍKOVÁ & KVASNIČKA 2013). In this study we present the modified cITP method for the evaluation of quality of CS raw materials used for dietary supplements production.

MATERIAL AND METHODS

Chemicals and samples. The European Pharmacopeia reference standard of chondroitin sulphate (CS-CRS, manufacturer Bioiberica, cat. No. Y0000280, batch 2.0) was purchased from Sigma and utilised as a standard in these analyses. Chondroitin sulphate A (CS-A) from bovine trachea, sodium salt (cat. No. C9819-5G, lot 038K1276), chondroitin sulphate C (CS-C) from shark cartilage, sodium salt (cat. No. C4384-5G, lot 42K1434), and chondroitin sulphate Shark (CS-Shark) from shark cartilage, sodium salt (cat. No. C4384-1G, lot 1412853) were purchased from Sigma-Aldrich (Prague, Czech Republic). Sodium salts of unsaturated CS disaccharides (Δ Di-0S: α - Δ UA-[1 \rightarrow 3]-GalNAc (cat. No. C3920-5MG, lot 125K1392); Δ Di-4S: α - Δ UA-[1 \rightarrow 3]-GalNAc-4S (cat. No. C4045-5MG, lot 124K1170), Δ Di-6S: α - Δ UA-[1 \rightarrow 3]-

Table 1. List of analysed chondroitin sulphate standards and samples with their description and chondroitin sulphates (CS) content claimed on label

Sample	Content of CS (% w/w)	Description
CS-CRS	99.6 ^a	European Pharmacopeia standard, from bovine trachea
CS-A	~ 90	from bovine trachea
CS-C	~ 85	from shark cartilage
CS-Shark	–	from shark cartilage
CS-1	> 95 ^b	from bovine trachea
CS-2	> 90	unknown origin
CS-3	97.5	unknown origin
CS-4	> 90	from shark cartilage
CS-5	~ 90	from pork cartilage
CS-6	~ 90	unknown origin
CS-7	> 90	from shark cartilage
CS-8	> 90	from pork cartilage
CS-9	94.6	granulated, unknown origin
CS-10	> 90	from pork cartilage
CS-11	~ 40	pork cartilage hydrolysate
CS-12	~ 90	unknown origin
CS-13	> 90	unknown origin
CS-14	> 90	unknown origin

^acontent of CS hydrate; ^bin dry matter

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GalNAc-6S (cat. No. C4170-5MG, lot 124K1169), chondroitinase ABC, chondroitin ABC lyase (cat. No. C2905-2UN, lot. 095K4083, 0.85 U/mg solid). All chemicals used for the electrolyte preparation, i.e., hydrochloric acid volumetric standard (1 N solution in water), phosphoric acid (85% w/v), Trizma base (tris-(hydroxymethyl)-aminomethane, TRIS), glycine (GLY), and 2-hydroxyethylcellulose (HEC) were obtained from Sigma-Aldrich (Prague, Czech Republic). Citric acid, sodium nitrate, sodium phosphate dibasic, and methanol were obtained from Lach-Ner, Ltd. (Neratovice, Czech Republic). All chemicals were of analytical grade. Deionised water of Milli-Q quality was used for the electrolyte, standard, and sample preparations. The samples of various chondroitin sulphates were obtained from several manufacturers (Table 1).

Capillary isotachopheresis (cITP). Isotachopheretic analyses of CS were carried out using the of column coupling electrophoretic analyser EA 101 with contact conductivity detectors and a UV detector operating at 254 nm (Villa-Labeco, Spišská Nová Ves, Slovakia). The separation compartment of EA 101 analyser consisted of pre-separation FEP capillary (90 mm × 0.8 mm ID) coupled with the analytical FEP capillary (90 mm × 0.3 mm ID). The analyser was controlled with the help of PC software package ITPPro32 (KasComp Ltd., Bratislava, Slovakia) supplied with the analyser. The instrument operates with hydrodynamically closed separation system, i.e., electro-osmotic flow is suppressed. For the analysis of CS, an electrolyte system consisting of 5 mM HCl + 10 mM GLY + 0.01 % HEC (pH 2.8) was used as the leading electrolyte and 10 mM citric acid as the terminating one. The driving current applied to the pre-separation capillary was 250 μ A and to the analytical capillary 50 μ A which was decreased to 25 μ A during the detection. The samples were injected by the sample valve with an internal fixed volume of 30 μ l or by Hamilton 10- μ l micro syringe. Each analysis required 15 minutes.

Capillary zone electrophoresis (CZE). For the analysis of unsaturated CS disaccharides, the electrophoretic analyser ^{3D}CE was used (Hewlett-Packard, Palo Alto, USA). The analysis was performed in a fused silica capillary having a total length of 43.5 cm (35 cm effective length, 50 μ m ID, 60°C), and the constant voltage applied to the capillary was –20 kV (reversed polarity setting, i.e., positive electrode was at the outlet vial). The optimised background electrolyte (BGE) consisted of 25 mM-H₃PO₄ + 21 mM-Tris,

pH 3.0. The samples were injected by pressure (25 mbar for 25 s), the separated analytes being detected at 232 nm. One analysis took 10 minutes. The instrument operated with the hydrodynamically open separation system (open tubular capillary), i.e., electro-osmotic flow was not suppressed. The analyser was controlled with the help of PC software package HP ^{3D}CE ChemStation, Rev. A.10.02 (Hewlett-Packard, Palo Alto, USA) supplied with it.

Size-exclusion chromatography (SEC). SEC analyses of CS were performed on Dionex Summit HPLC instrument consisting of the pump P680, column oven STH585, refractometric detector RI 101 3000, and autosampler ASI 100T (Dionex, Santa Clara, USA) controlled by Chromeleon 6.40 software package. Chromatographic separation of CS was carried out on three columns PLaquagel-OH Mixed H in series (3 × 300 × 8 mm, 8 μ m; Varian, Palo Alto, USA). The column temperature was kept at 30°C. The mobile phase consisted of 200 mM sodium nitrate + 10 mM sodium phosphate dibasic, pH 8.2. An isocratic elution was applied at the flow rate of 1 ml/minutes. Amount of 50 μ l of the standard or sample solution were injected onto the column and the eluate was monitored by refractive index detector. One analysis lasted 30 minutes.

Standard and sample preparation. All the electrolytes, mobile phases, standards, and samples solutions were filtered through 0.45 μ m membrane filter prior to analysis.

Stock solution of CS standard (10 mg/ml) was prepared by dissolving 50 mg of CS-CRS in 5 ml of demineralised water. The obtained solution was divided after filtration into 0.5 ml portions and stored in Eppendorf vials at –20°C. For the cITP analysis, the calibration solutions of CS (0.1 and 1 mg/ml) and the relationship between the injected amounts of CS (0.2–4 μ g) on the step length of CS were evaluated. Linear regression was applied for the calculation of the intercept, slope, and correlation coefficient of CS calibration curve. The samples of CS for isotachopheretic analysis were dissolved in demineralised water in the concentration of 1 mg/ml (50 mg/50 ml).

For CZE analyses, stock solutions of unsaturated disaccharides (Δ Di-0S, Δ Di-4S, and Δ Di-6S) were prepared in the concentration of 2 mg/ml in demineralised water. After filtration the solutions were divided into 0.1 ml portions and stored in Eppendorf vials at –20°C. The response factor of each disaccharide (i.e., concentration divided by the peak area corrected on the migration time) was evaluated

and used for the quantitation of disaccharides in the samples.

As we did not have standards of chondroitins with different molecular masses we used the standards of dextrans (molecular weight (MW) 10 000, 20 000, 40 000, 70 000, and 150 000 g/mol) for the calibration of SEC analysis, prepared in the concentration of 5 mg/ml of the mobile phase. The relationship between the retention time of the respective dextran and log MW was evaluated.

Digestion of CS by chondroitinase ABC was applied to determine the composition and content of disaccharides. We followed the published procedure (VOLPI 2007) with the following modifications: into a 0.5-ml Eppendorf vial, 160 µl of buffer (15 mM HCl + 25 mM Tris, pH 7.5), 20 µl of CS solution (1 mg/ml) and 20 µl of chondroitinase ABC solution (1 mU/µl) were injected. The samples were digested at 37°C for 2 h, this time being sufficient. A prolonged time period did not increase the content of carbohydrate fragments. After inactivation of the enzyme by im-

mersion of the vial into boiling water bath for 5 min, the mixture was analysed by CZE.

RESULTS AND DISCUSSION

Isotachopheresis as one of the basic electrophoretic techniques is almost exclusively carried out in a capillary using a free solution of appropriately chosen electrolytes. Either anions or cations can be separated in a single separation run in the given electrolyte system.

Disaccharide unit of CS contains one carboxylic group ($pK_a \sim 3.3$) (TOMMERAAS & WAHLUND 2009; WANG *et al.* 1991) and one (or more) *O*-sulphate group ($pK_a \sim -1$). CS is a strong poly-acid negatively charged even at low pH (< 3). This property allows performing electrophoretic separation in acidic electrolytes. For the evaluation of CS quality we applied capillary isotachopheresis with a leading electrolyte of pH 2.8. The analysis with the use of a leading electrolyte of

Table 2. Results of capillary isotachopheresis (cITP) and size-exclusion chromatography (SEC) analyses of chondroitin sulphate samples

Sample	RSH	Degree of sulphatation	CS	Sodium sulphate	UVC	M_w	M_n	Dispersity
		(–)		(g/100 g)		(Da)		(–)
CS-A	0.267	0.96	82.0	1.50	1.5	30 700	22 100	1.39
CS-C	0.220	1.16	74.6	3.93	0.7	60 800	35 000	1.74
CS-Shark	0.220	1.16	89.3	0.86	1.3	83 200	36 500	2.28
CS-1	0.275	0.93	86.0	0.69	0.4	49 300	33 000	1.49
CS-2	0.262	0.98	75.0	1.61	4.0	36 600	25 900	1.41
CS-3	0.272	0.94	81.0	1.23	3.6	36 200	24 600	1.47
CS-4	0.286	0.90	8.7	0.53	0.1	14 200	8 700	1.64
CS-5	0.261	0.98	74.4	2.05	3.0	26 300	20 000	1.31
CS-6	0.273	0.94	82.4	4.75	11.0	40 200	23 200	1.74
CS-7	0.225	1.14	75.6	1.06	2.3	80 800	44 600	1.81
CS-8	0.287	0.89	8.9	0.49	0.2	14 900	8 700	1.70
CS-9	0.256	1.00	71.6	0.97	4.4	34 400	19 000	1.51
CS-10	0.284	0.90	75.7	1.02	0.9	40 600	26 900	1.51
CS-11	0.294	0.87	37.2	1.21	0.8	30 300	23 000	1.32
CS-12	0.280	0.91	76.8	1.58	1.8	44 000	29 300	1.50
CS-13	0.245	1.05	2.4	33.96	0.3	25 900	22 600	1.14
CS-14	0.266	0.96	85.8	2.25	1.1	39 300	29 000	1.36

RSH – Relative Step Height; degree of sulphatation – number of sulphate groups per disaccharide unit, it is calculated as the ratio of RSH_{CRS}/RSH_i multiplied by 0.97 (= degree of sulphatation of CS-CRS estimated from the content of unsaturated disaccharides in CS-CRS, $RSH_{CRS} = 0.264$); CS – content of CS based on the calibration by CRS standard; UVC – content of UV (254 nm) absorbing compounds expressed as CS; M_w – weight-average molar mass; M_n – number-average molar mass; dispersity – ratio of M_w/M_n

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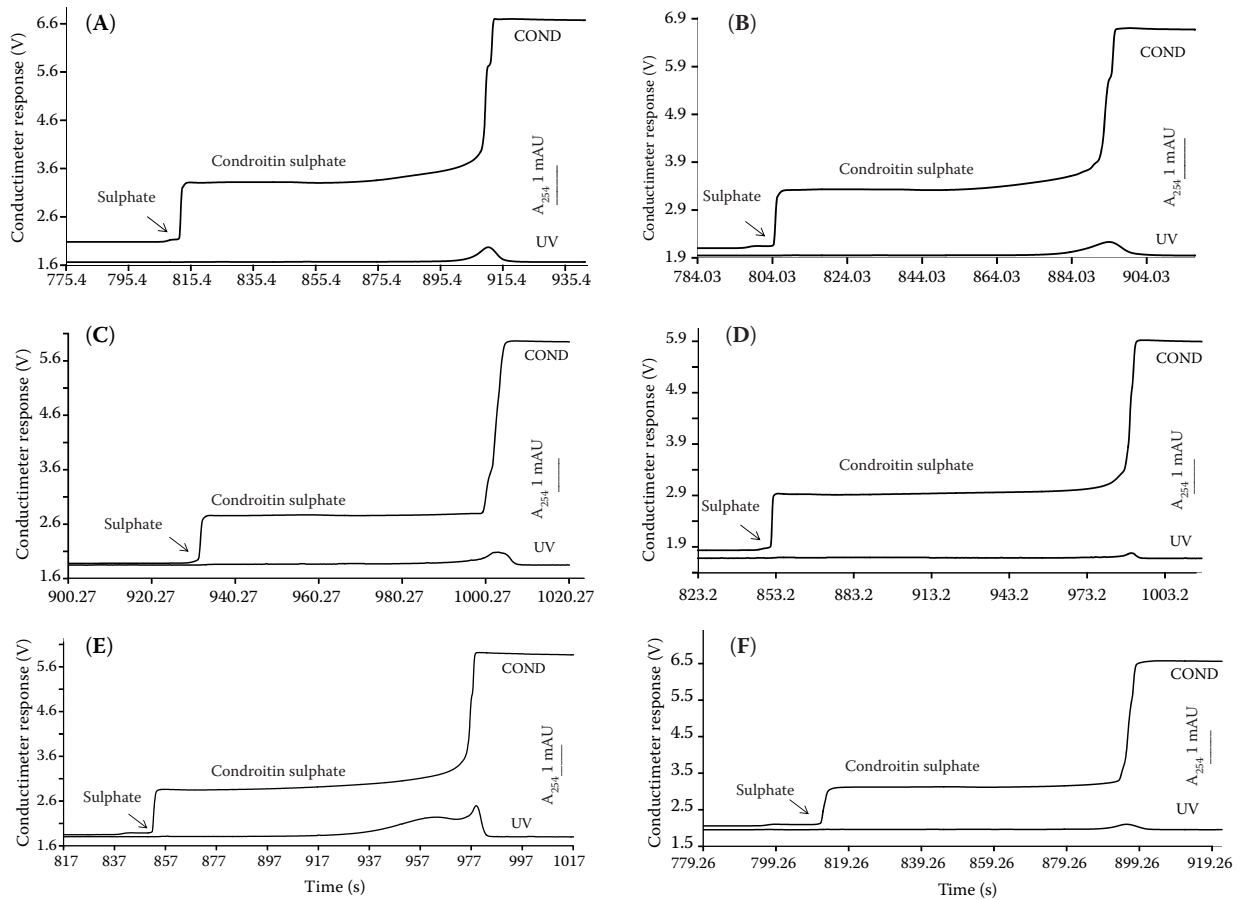


Figure 2. Isotachopherograms of chondroitin sulphate; trace from the conductivity (red) and the UV (254 nm) detector (blue) of the analytical capillary, respectively: (A) CS CRS standard (3 µg injected); (B) CS-A (3 µg injected); (C) CS-Shark (3 µg injected); (D) CS-1 (4 µg injected); (E) CS-5 (4 µg injected); (F) isotachopherogram of CS-B (3 µg injected)

COND – indication of record from conductivity detector

pH 3.2 (VÁCLAVÍKOVÁ & KVASNIČKA 2013) gave a horizontally smooth step of CS which is favourable for the step length evaluation, i.e. for the quantitative analysis. CS, however, can contain “impurities” such as partially desulphated or unsaturated chains (fragments). Most of these molecules are less acidic than CS and therefore the shift of the pH of the leading electrolyte to 2.8 enables their separation from CS. Isotachopherograms of various CS are depicted in Figure 2. In the dependence on the step height and shape we can deduce the size and homogeneity of charge-to-mass ratio of CS, respectively. The lower the RSH, the higher the charge-to-mass density of CS. From the RSH data summarised in Table 2 we calculated the degree of sulphatation of CS samples of various animal origins. For fish CS, this value is higher than 1. For bovine, porcine, and chicken CS is this degree of sulphatation 0.9–1.0, so it is necessary to know the ratio of CS-4/CS-6. The obtained values are

in good agreement with the published results (VOLPI 2007). This means that the origin of CS can be deduced from RSH value. Only with sample CS-4, denoted as shark CS, the calculated degree of sulphatation degree was lower than 1. The explanation of this is that the sample was of low purity (desulphated CS).

It is known that in free solution the electrophoretic mobility of a polymer is independent when the total charge on the molecule is proportional to its length (GROSSMAN & COLBURN 1992). The horizontally smooth step of CS indicated that the sample contained polysaccharide chains with homogeneous charge-to-mass ratio (Figures 2C, D, and F), while the ascending step indicated heterogeneity in the charge-to-mass ratio (Figures 2A, B, and E). In such a case the sample contained polysaccharides with different electrophoretic mobilities in the given conditions. The results of the NMR determination of pK of carboxylic group in heparin suggested that

the molecular size affects pK (WANG *et al.* 1991). For example, the pK of unsaturated disaccharide was 3.35 while for unsaturated hexasaccharide it was 2.24. Analogy should hold for CS, the shorter are the CS chains the less acidic carboxylic groups are present and thus the electrophoretic mobility is lower. CS can contain partially desulphated chains of lower mobility than is that of CS. As a result, the higher RSH of such analytes makes the CS step ascendant.

These less mobile compounds were UV absorbing, as shown in Figure 2. We suppose that these com-

pounds could be unsaturated oligomers originated from CS. To prove, it we digested CS by chondroitinase ABC. The results of the experiment are depicted in Figure 3. It is clear that chondroitinase ABC gradually released from chondroitin sulphate the UV absorbing fragments of mobility lower than was that of CS (ascending step; Figure 3A) and unsaturated disaccharides (Figures 3B and C).

As already mentioned, the uniformly charged polymers of different molecular masses cannot be separated in free-solution electrophoresis. The application of soluble linear polymers (entangled polymers) into the electrolyte as a sieving media enables such a separation (GROSSMAN & SOANE 1991). The influence of the addition HEC (MW 1 300 000 g/mol) into the leading electrolyte on CS separation is shown in Figure 4. The increase of RSH of CS (decrease of electrophoretic mobility) was proportional to the molecular mass of CS. The plot of relative RSH vs. logarithm of MW was almost linear (Figure 4C). Using this equation, the molecular mass of CS can be estimated. From the step shape the dispersity of the CS molecular mass can be deduced. CS-CRS standard (dispersity 1.3) gave an almost horizontally smooth step, while CS-Shark (dispersity 2.28) gave several steps (Figures 4A and B) indicating a mixture of polysaccharide chains with different molecular mass.

It is worth saying that under the given conditions heparin (highly sulphated GAG) and hyaluronan (non-sulphated GAG) did not disturb the analysis of CS. Heparin and hyaluronan had, respectively, a higher and a lower mobility than CS (data not shown).

For quantitative analysis of CS (purity), we used an external standard method. The linear response between the analysed amount of CS and the step length was verified (Figure 5). As a standard, we bought CRS substance. The absolute amount of chondroitin sulphate in CRS standard was calculated as the amount of unsaturated disaccharides (Δ Di-0S, Δ Di-4S, and Δ Di-6S) released by chondroitinase ABC divided by a factor of 1.036 (ratio of MW of disaccharide and MW of disaccharide unit in CS). Capillary zone electrophoresis (CZE) was used for disaccharides determination. To avoid splitting of the peaks of disaccharides due to the presence of α - and β -anomers, which complicates their quantitation, we ran the analyses at elevated temperature, i.e. 60°C. The chromatogram of analysis of disaccharide standards at 60°C is shown in Figure 6A. From the electropherograms of CS-CRS and CS-Shark (Figures 6B and C), it is

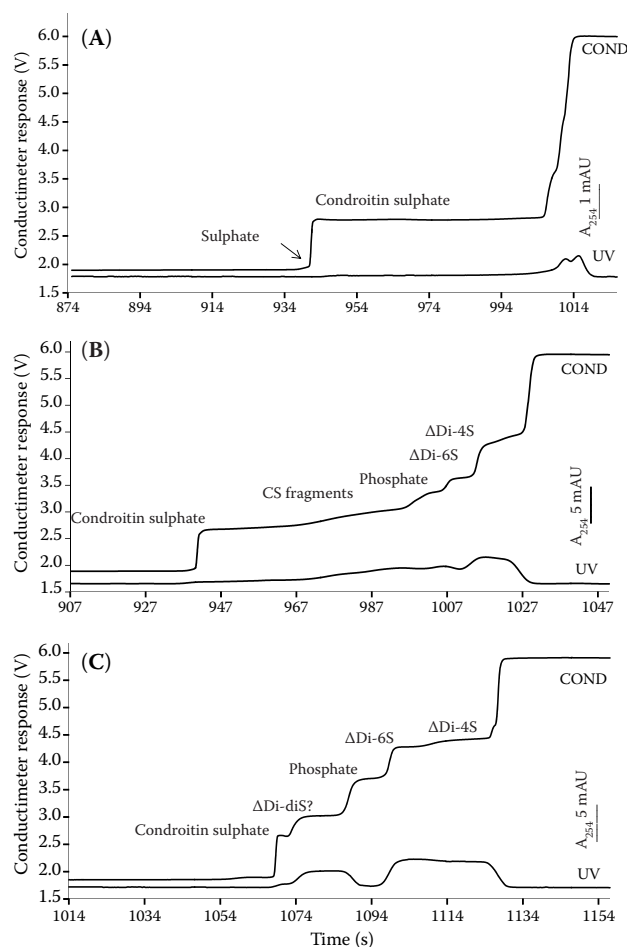


Figure 3. Hydrolysis of CS-Shark by chondroitinase ABC; trace from the conductivity (red) and the UV (254 nm) detector (blue) of the analytical capillary, respectively: (A) isotachopherogram of CS-Shark – partially hydrolysed (250 mg of CS + 10 mU of chondroitinase ABC in 0.5 ml of hydrolysis buffer, 1 min of hydrolysis, 5 ml injected); (B) isotachopherogram of CS-Shark – partially hydrolysed (the same conditions as A, 20 min of hydrolysis, 5 ml injected); (C) isotachopherogram of CS-Shark – almost totally hydrolysed (20 mg of CS + 20 mU of chondroitinase ABC in 0.2 ml of hydrolysis buffer, 60 min of hydrolysis, 10 ml injected); the phosphate comes from the chondroitinase ABC

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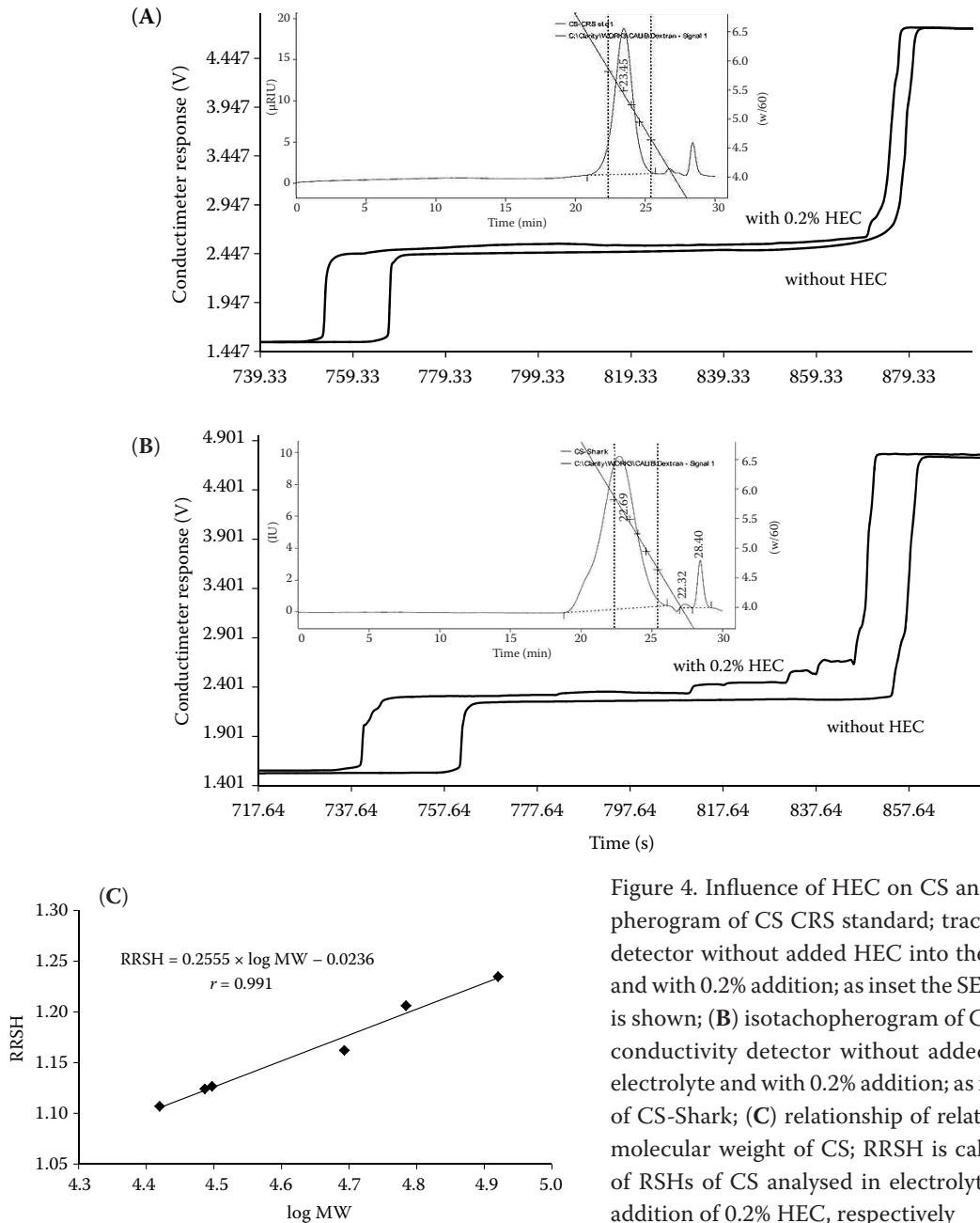


Figure 4. Influence of HEC on CS analysis: (A) isotachopherogram of CS CRS standard; trace from conductivity detector without added HEC into the leading electrolyte and with 0.2% addition; as inset the SEC record of CS-CRS is shown; (B) isotachopherogram of CS-Shark; trace from conductivity detector without added HEC into leading electrolyte and with 0.2% addition; as inset the SEC record of CS-Shark; (C) relationship of relative RSH (RRSH) on molecular weight of CS; RRSR is calculated as the ratio of RSHs of CS analysed in electrolyte with and without addition of 0.2% HEC, respectively

clear that all disaccharides were well separated within 10 minutes. Since we had no standards of Δ Di-diS, we quantified these disaccharides on the basis of average response factor (peak area corrected on migration time divided by concentration) of Δ Di-4S and Δ Di-6S. Under the term “charge density”, usually used in the literature (VOLPI 2007, 2009; VOLPI & MACCARI 2008), the number of sulphate groups per disaccharide unit of CS is intended. In this context is this term somewhat misleading. Generally, the term “charge density” means the number of charges per unit (molecule, monomer or area). At a low pH (< 2.5),

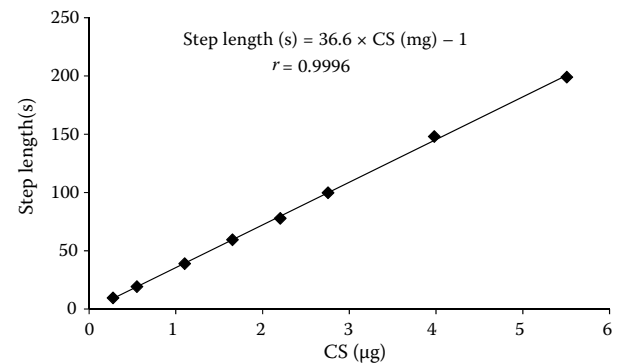


Figure 5. Calibration curve for CS – relationship of step length in seconds on injected amount of CS in μ g

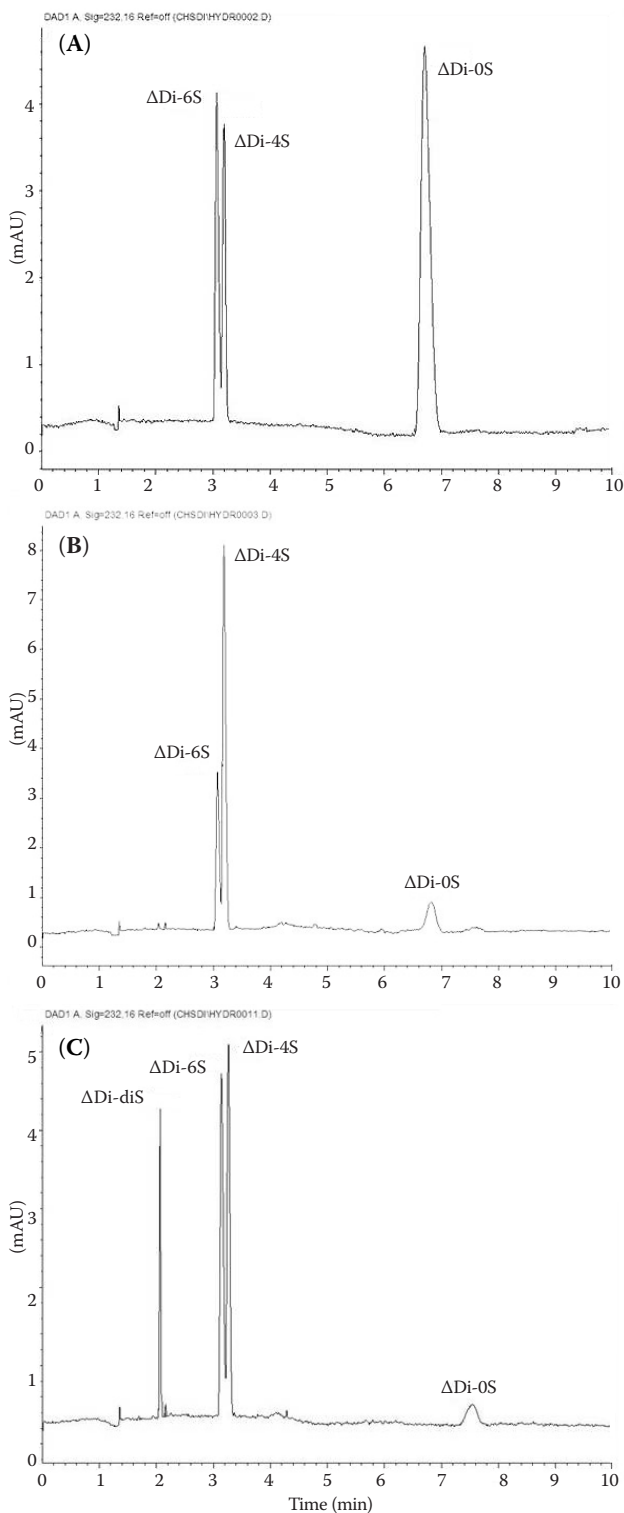


Figure 6. Electropherograms of chondroitin sulphate; trace from UV (232 nm) detector: (A) unsaturated disaccharide standards Δ Di-0S, Δ Di-4S, and Δ Di-6S (each 25 μ g/ml, capillary thermostated at 60°C); (B) CS-CRS after hydrolysis (100 μ g/ml, capillary thermostated at 60°C); (C) CS-Shark after hydrolysis (100 μ g/ml, capillary thermostated at 60°C)

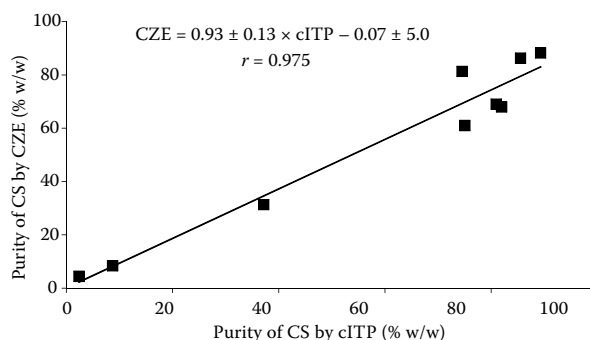


Figure 7. Comparison of capillary isotachopheresis (cITP) and capillary zone electrophoresis (CZE) results

carboxyl groups of CS are not dissociated and the charge density corresponds to the number of sulphate groups. However, at pH > 4 the carboxyl groups of CS are fully dissociated and thus the charge density is increased, but the number of sulphate groups is still the same. That is why we used the degree of sulphatation instead of charge density. The degree of sulphatation of CRS standard was calculated as the relative amount of non-sulphated disaccharide Δ Di-0S subtracted from 1.

By the isotachopheretic analysis of CS, free sulphate content in CS could be determined (Figure 2). This value could be considered as a measure of the of chondroitin sulphate degradation during isolation and/or purification. The content of unsaturated disaccharides, degree of sulphatation, and moisture content of CRS standard correspond to those published by VOLPI (2007). The higher MW of the standard used in this study was the only difference. The explanation could be in using different methods for the MW estimation.

On a series of selected CS samples it was proved that cITP (direct analysis of chondroitin sulphate content) gave comparable results with CZE (indirect analysis; content of CS was calculated as a sum of unsaturated disaccharides after chondroitinase ABC treatment). The relationship between these methods is depicted in Figure 7.

Generally, the obtained qualitative and quantitative data of various chondroitin sulphate raw materials obtained by cITP (Table 2) are in a good agreement with the literature (VOLPI 2007; VOLPI & MACCARI 2008). The only drawback of cITP is that it does not differentiate dermatan sulphate (chondroitin sulphate B, Figure 2F) from CS. For the detection of CS-B, the use of the specific enzyme, chondroitinase B, is necessary.

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CONCLUSIONS

We present the cITP method for the determination of the quality of chondroitin sulphate raw materials used for dietary supplements. The obtained results document the suitability of this method for the quality evaluation of CS raw material.

The presented cITP method enables:

- (1) estimation of CS origin,
- (2) estimation of the degree of CS sulphatation and its homogeneity,
- (3) estimation of the molecular mass of CS and dispersity of MW,
- (4) determination of CS purity,
- (5) detection of CS degradation products (UV absorbing compounds, free sulphate).

The cITP method can be easily used as an alternative to HPLC or CZE methods for the evaluation of chondroitin sulphate quality.

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