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Enantiomeric purity control of levocetirizine in pharmaceuticals using anionic cyclodextrin mediated capillary electrophoresis separation and fiber-based diode array detection

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The present work illustrates potential of anionic cyclodextrin (CD) mediated capillary electrophoresis (CE) separation technique coupled with on-capillary diode array detector (DAD) for highly reliable enantioselective determination of dextrocetirizine (DCET) besides its major active compound (levocetirizine, LCET) in pharmaceuticals (commercial tablets). DAD detection was utilized for the characterization of composition of each separated zone via match of corresponding tested (analyte in dosage form) and reference (standard analyte in water) UV-VIS spectrum (scanned in interval 200–800 nm) being expressed mathematically through Pearson's correlation coefficient (PCC). It was demonstrated, comparing the reference and real spectra of CET enantiomers, that the applied separation method was selective enough to produce pure (spectrally homogeneous) zones of interest without any interfering comigrating compound (PCCs values were equal or higher than 0.99). Successful validation of the proposed CE-DAD method suggests its routine use for the control of enantiomeric purity of pharmaceuticals.

1. Introduction

In pharmacotherapy the use of single-enantiomer forms can often lead to an improvement in the efficacy of the drug or the suppression of side effects related to the other enantiomer (Daniels and Jorgensen 1982; Innes and Nickersen 1970). Levocetirizine (LCET), 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl-methyl]piperazin-1-yl]ethoxy]acetic acid, is a third generation non-sedative antihistamine, developed from the second generation antihistamine cetirizine (CET) (Oppenheimer and Casale 2002). Chemically, LCET is the active enantiomer of CET and it is claimed to be more effective and with fewer side effects than the second generation drugs. It is obvious, from above mentioned facts, that the control of enantiomeric composition of pharmaceuticals containing LCET is important.

Among high performance separation techniques, capillary electrophoresis (CE) can be advantageously used for the analytical separations of ionic compounds because of its extremely high peak efficiency, versatility, simplicity, short analysis time, good compatibility with aqueous samples and low consumption of chiral selector (low cost of enantioselective analyses) as it has been demonstrated on many examples (Bonato 2003; Preinerstorfer et al. 2009; Ward and Hamburg 2004). In our previous work (Mikuš et al. 2005), we proposed an enantioselective CE method for the separation and determination of racemic CET based on migration and differentiation of zwitterionic (i.e. effective charge was near to zero) enantiomers with the aid of negatively charged sulphated-β-cyclodextrin (S-β-CD). In fact, this was the first case of the electrokinetic chromatography performed in hydrodynamically closed CE system. Thereafter, this method has been adapted (with some modifications) to the conventional hydrodynamically open CE system by several authors (Chou et al. 2008; Eeckhaut and Michotte 2006; Peng et al. 2006). Eeckhaut and Michotte (2006) demonstrated that heptakis(2,3-diacetyl-6-sulfato)-β-CD and acetonitrile as running buffer additives were suitable for enantiomeric purity testing of the drug substance. Limits of detection (LOD) and quantification (LOQ) were 1.6 and 5.0 μg/ml, respectively. However, due to peak overloading and the fact that the resolution between both enantiomers was not high, only samples of approximately 0.5 mg/ml of LCET could be injected. Therefore, the method allowed the detection of 0.3% m/m (1.6 μg/0.5 mg) and the precise and accurate quantification of approximately 1.0% m/m (5.0 μg/0.5 mg) enantiomeric impurity (DCET) in samples of LCET substance.

The aim of this work was to present the potential of electrokinetic chromatography in a hydrodynamically closed CE system for enantiopurity testing of zwitterionic CET. Here, using a capillary with a higher internal diameter (I.D.) should increase sample load capacity and eliminate overloading that can help to increase the detectable ratio of major and minor enantiomers in the injected sample. Moreover, diode array detection (DAD), on-line combined with CE in this work, should enable direct characterization of spectral purity (homogeneity) of separated zones.

Such spectral characterization should confirm or exclude mixed zones (spectrally inhomogeneous) that can be current when are large amounts of sample matrix injected.

2. Investigations, results and discussion

2.1. Separation selectivity and spectral characterization of zones

The basic CE separation conditions were optimized in our previous work (Mikuš et al. 2005) and adapted to a CE-DAD combination in the present paper. The main purpose was to investigate the separation selectivity of the proposed method, especially, in the situation of (i) high concentration ratio of LCET : DCET (>99 : 1), and (ii) high concentration of water soluble matrix (dosage form) constituents, where a risk of a peaks overlapping can increase.

Optimized separating conditions, resulting in a sufficient enantioseparation of CET in a wide scale of concentration ratios of CET enantiomers, reasonable separation efficiency and analysis time, consisted of: 25 mmol/l MES adjusted to pH 5.2 with EACA in presence of 0.1% (w/v) HEC, and 5 mg/ml S-β-CD. An electropherogram from the enantioseparation of racemic CET standard is shown in Fig. 1a and the corresponding UV-VIS spectra (serving as reference) are shown in Fig.1b.

Following experiments were devoted to the analyses of model samples containing LCET : DCET at a high concentration ratio. Thanks to the larger I.D. (320 μm) of the separation capillary, it was possible to load an increased amount of the sample (up to 1000 μg of LCET) without overloading of capillary, and hence, monitor lower concentrations of the accompanied enantiomeric impurity (DCET) in samples. Our experiments revealed an amount of ∼0.7% of DCET impurity in the LCET reference substance and the capability of the proposed CE-DAD method to separate and determine DCET besides LCET at enantiomeric ratio 0.7 : 99.3 (the determinable ratio was limited by the purity of LCET reference substance). In the situation, where an analyte is present at a very low concentration level (∼LOQ), the spectral processing (section 3.3.) can be very useful to obtain the relevant spectral response. This is obvious comparing the PCC values of raw (0.9846) and processed (0.9934) spectra of DCET present at the concentration of 7 μg/ml (besides 1000 μg/ml of LCET in model sample) where the processing provided the relevant spectral response toward DCET. In this work, spectral testing of the vale between two peaks of very different high/area was proposed as a very useful tool for the indication of completeness of separation of enantiomers present at a high concentration ratio in the sample. It was found that the PCC value of the processed spectrum of valley between LCET and DCET peaks (enantiomeric ratio 99.3 : 0.7) was 0.9467, i.e. the peaks were fully resolved as no match was found between the spectra of valley and tested enantiomers.

2.2. Validation of the method

The full resolution (R) of CET enantiomers in a wide scale of their concentration ratios and short analysis time are indicating the effective enantioseparation process suitable for the fast monitoring of the trace enantiomeric impurity. The optimized CE method was validated (see section 3.2.) and the resulting data are given in Tables 1 and 2. These data clearly indicate that parameters of the proposed method, i.e. sensitivity, linearity, precision, accuracy/recovery and robustness, are favorable for its routine use in pharmaceutical analysis. This is obvious also from the good separation efficiency given by (i) the number of theoretical plates (N) as well as (ii) the height equivalent to one theoretical plate (H). In this method, LOD and LOQ of CET enantiomers were improved, in comparison with our previous method (Mikuš et al. 2005), replacing the FEP (fluorinated ethylene-propylene copolymer) capillary by fused silica one.

2.3. Pharmaceutical application

The approved CE method was applied in pharmaceutical analysis to determine the enantiomeric ratio of LCET and DCET in commercial pharmaceuticals. The representative electropherogram, shown in Fig. 2a, illustrates detailed CE profile of the sample prepared from tablets Xyzal (similar results were obtained also for other batches of this pharmaceutical).

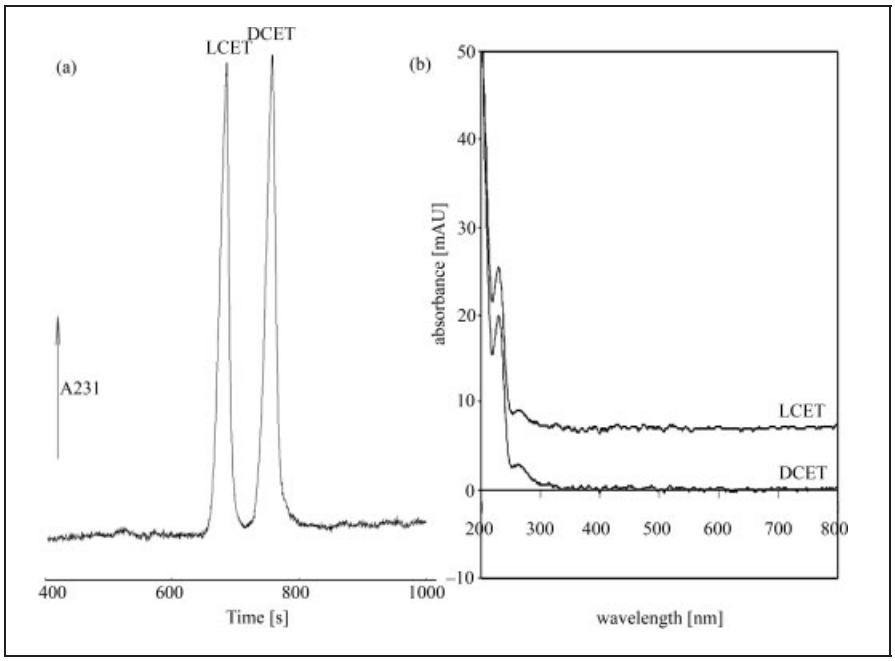

Fig. 1:
Enantioselective analysis of racemic CET in model sample. Electropherogram (a) and corresponding processed UV-VIS spectra of CET enantiomers (b). For the processing of DAD spectra, scanned in interval of wavelengths of 200–800 nm, see section 3.3. The separating conditions as in section 2.1. The driving current was stabilized at 150 μA and detection wavelength for recording of the electropherogram was set at 231 nm. The concentration of CET in the sample was 50 μg/ml. For the model sample preparation and other working conditions see sections 3.5.1. and 3.1., respectively

Table 1: Performance parameters of CE-DAD method and parameters of calibration lines of cetirizine enantiomers[a]

Parameter	LCET	DCET
t_m [s]	681.258	752.127
s_{tm} [s]	9.029	11.752
a [mAU]	31.895	34.451
s_a [mAU]	4.883	5.497
b [mAU · μg^{-1} · ml]	9.51035	9.41117
s_b [mAU · μg^{-1} · ml]	0.1648	0.1983
RSS	141.60	223.27
r^2	0.99832	0.99808
LOD [$\mu g \cdot ml^{-1}$]	1.7	1.9
LOQ [$\mu g \cdot ml^{-1}$]	5.1	5.8
N	13700	14200
H [μm]	10.9	10.6
Robustness (ΔR) [%]	< 3.4	
Enantioresolution, R	3.29	

[a] Separating conditions as in section 2.1. and other working conditions as in Experimental section. The concentration of racemic CET in model samples was 50 μg/ml unless otherwise stated. The parameters of calibration lines were calculated from corrected peak areas

The migration positions of CET enantiomers in electropherograms were confirmed by spiking of real samples with the standards while the purities of zones of CET enantiomers in particular experiments (reflecting the achieved separation selectivity) were confirmed through their processed spectra (see section 3.3.). It is shown in Fig. 2b that the spectra of CET enantiomers obtained in real pharmaceutical samples (tablets Xyzal) were comparable to the reference spectra of CET enantiomers (similar results were obtained also for other batches of this pharmaceutical). This observation was also supported mathematically via corresponding PCCs. The PCC values equal or higher than 0.99 (see Table 3) confirmed spectral identity of CET enantiomers present in pharmaceutical samples with the reference CET spectra. This reflected producing of the pure (non-mixed, i.e. spectrally homogeneous) zones of CET enantiomers in pharmaceutical matrices (i.e. no detection interferences from the sample solvent, impurities and dosage form excipients occurred in CET spectra in the interval of wavelengths of 200–800 nm) and, hence, the sufficient separation selectivity of the proposed method.

Table 2: Recovery, accuracy and precision of the CE method for cetirizine in dosage form matrices obtained from quality control (QC) samples[a]

QC sample	added concentration [mg/l]		found concentration, mean[b] [mg/l]		Recovery (%)		accuracy[b] RE (%)		precision[b] RSD (%)	
	L	D	L	D	L	D	L	D	L	D
Low	10	10	9.55	9.69	95.50	96.90	−4.50	−3.10	1.92	1.31
Medium	50	50	48.59	48.98	97.18	97.96	−2.82	−2.04	0.74	0.86
High	100	100	97.03	96.64	97.03	96.64	−2.97	−3.36	0.84	0.99

[a] Separating conditions as in section 2.1. and other working conditions as in Experimental section. [b] The mean of concentration, RE and RSD values were obtained from six consecutive experiments. The water extracts of pharmaceutical tablets Xyzal were diluted to a 50 μg/ml concentration of LCET (plus spiked amounts of CET enantiomers)

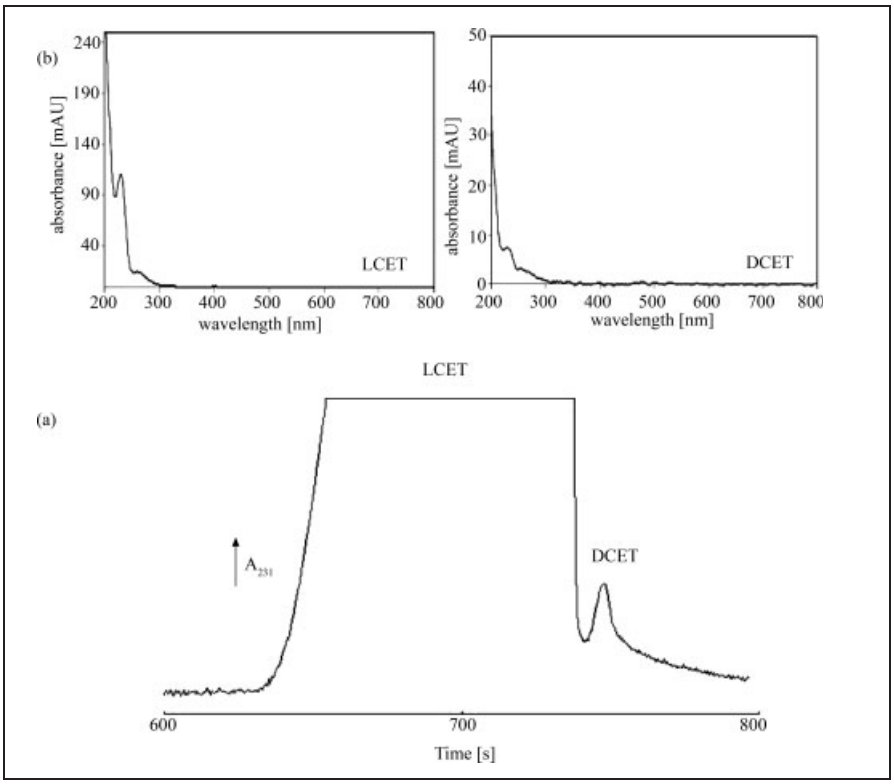

Fig. 2:
Enantiopurity testing of LCET in commercial pharmaceutical preparation (tablets Xyzal). (a) Detailed electropherogram from the separation and determination of LCET present at the concentration of 1000 μg/ml besides of the trace (6.8 μg/ml) enantiomeric impurity (DCET). (b) Corresponding processed UV-VIS spectra of CET enantiomers. The separating conditions as in section 2.1. The driving current was stabilized at 150 μA and detection wavelength for recording of the electropherogram was set at 231 nm. For the pharmaceutical sample preparation and other working conditions see sections 3.5.2. and 3.1., respectively

Table 3: Enantiopurity testing of LCET in pharmaceuticals[a]

Parameter	I	II	III	IV
Determined content of LCET ± SD (%)[b]	96.5 ± 1.6	98.1 ± 0.9	97.3 ± 1.1	96.2 ± 1.1
Percentual amount of DCET ± SD (%)[c]	0.68 ± 0.04	0.83 ± 0.06	0.89 ± 0.05	0.76 ± 0.05
PCC: LCET, DCET[d]	0.99935, 0.98991	0.99991, 0.99326	0.99966, 0.99087	0.99963, 0.98745

[a] Separating conditions as in section 2.1., sample preparation procedures and other working conditions as in Experimental section.
[b] Average content of LCET in the tested pharmaceuticals (four batches of tablets Xyzal, I-IV) was calculated from six consecutive CE runs. Declared content of LCET in each pharmaceutical was 5 mg per 1 tablet. Concentration of LCET in the injected samples was ~50 μg/ml.
[c] Average percentual amount of DCET in the tested pharmaceuticals (four batches of tablets Xyzal, I-IV) was calculated from six consecutive CE runs. Concentration of LCET in the injected samples was ~1000 μg/ml.
[d] For the spectral characterization of CET peaks see section 3.3. For the analyzed samples see paragraph (c) in this table.

Such an approved method was successfully used for the highly reliable determination of enantiomeric purity of LCET in commercial pharmaceuticals. The results indicated an amount of less than 0.9% of the enantiomeric impurity (i.e. DCET) in commercial pharmaceuticals tested. The content of LCET, obtained by the proposed method, was in a good agreement with this one declared by the manufacturer (see Table 3).

Summarizing, the proposed separation method was approved for its effective use in enantiopurity testing of zwitterionic (electroneutral) CET enantiomers. The use of a capillary with a higher (320 μm) internal diameter (in comparison with 25–75 μm I.D. capillaries used in conventional CE systems) increased sample load capacity and eliminated overloading that helped to increase the detectable ratio of major and minor enantiomers in the injected sample. An added value of this method, in comparison with our previous enantioselective method for CET (Mikuš et al. 2005), was enhanced sensitivity and enhanced reliability thanks to the on-line spectral information in purity (i.e. spectral homogeneity) of separated zones of analytes. Spectral testing of the vale between two peaks of very different high/area was proposed as a useful tool for the indication of completeness of separation of enantiomers present at a high concentration ratio in the sample. It can be concluded that the proposed CE-DAD method is useful for routine pharmaceutical applications.

3. Experimental

3.1. Instrumentation

A capillary electrophoresis analyzer EA-101 (Villa-Labeco, Spišská Nová Ves, Slovakia), assembled in the single column configuration of the separation unit, was used in this work for performing the CE runs. The samples were injected by a 200 nl internal sample loop of the injection valve of the analyzer. The CE column was provided with a 320 I.D. fused silica capillary tube of a 160 mm total length.

A multiwavelength photometric absorbance diode array detector Smartline PDA Detector 2800 (Knauer, Germany) was connected to an on-column photometric detection cell, mounted on the CE column, via optical fibers. The detector operated under the following conditions: (1) scanned wavelength range 200–800 nm; (2) integration time 6 ms; (3) scan interval 0.2 s; (4) number of accumulations 1.

Prior to the use, the capillary was not treated by any rinsing procedures to suppress an electroosmotic flow (EOF). A dynamic coating of the capillary wall by means of hydroxyethylcellulose (HEC 30 000; Serva, Heidelberg, Germany) in background electrolyte solution served for this purpose. The separating electrolyte in the capillary was replaced by a fresh one between each run. CE analyses were carried out in the anionic regime of the separation (i.e. anodic movement of S-β-CD as well as complex forms of the analytes). The experiments were performed in constant current mode at laboratory temperature. The driving current applied was 150 μA.

3.2. Data evaluation and performance parameters

The absorption maximum wavelength of CET (231 nm) was used for the evaluation of analytical parameters, as given in Tables 1 and 2. The racemic CET standard (see section 3.5.1.) was used for validation experiments and performance parameters were evaluated according to the ICH guideline (ICH Guideline 2005). Peak areas of CET enantiomers were corrected for their migration times to compensate for their differential detector residence times (Huang et al. 1989). Parameters of calibration lines for CET enantiomers were calculated by using QCExpert ver.2.5 statistical software (Trilobyte, Prague, Czech Republic).

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as the ratio of standard deviation of y-intercept of regression line (s_a) and the slope of the regression line (b) multiplied by factor 3.3 (LOD) or 10 (LOQ). Linearity, tested in the concentration range given in section 3.5.1., was expressed via determination coefficient (r^2).

Precision was expressed via relative standard deviation of intercept (s_a) and slope (s_b) of calibration lines, and migration times of CET enantiomers (s_{tm}) as well as via residual sum of squares (RSS). Each concentration point was calculated as average from six consecutive CE runs.

Recovery was evaluated by spiking of dosage form and water samples with CET at three different concentration levels, given in section 3.5.1. (each point was measured six times), and comparing the peak areas of CET enantiomers obtained in the different matrices. Accuracy (expressed via relative error, RE) was evaluated through the recovery test.

Robustness test examined the effect that deliberate variations in operational parameters had on the analysis results, in our case, the enantioresolution (R).

3.3. Processing and comparing DAD spectra

The spectral data were acquired and processed by a EuroChrom program (version 3.05, Knauer). The raw spectra of CET enantiomers were processed in order to provide relevant spectral information (free of various noise effects).

The background correction, i.e. subtraction of background spectrum from the raw spectrum of the analyte (Strašík et al. 2003), was carried out to minimize the impact of the electrolyte system on the CET spectrum. Such a corrected spectrum was further smoothed by the procedure of Savitzky-Golay (1964), implemented in EuroChrom software, with a 5-point window.

Homogeneity of spectra of CET enantiomers in real samples was expressed via Pearson's correlation coefficients (PCCs) (Miller and Miller 1993). The value of PCC higher than 0.99 is assumed to provide an acceptable certainty in a confirmation of the identity of the analyte (Strašík et al. 2003), i.e. a match of the tested (CET in dosage form) and reference (CET in water) spectrum.

3.4. Chemicals and samples

The electrolyte solutions were prepared from chemicals obtained from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland) in water demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, U.S.A.). All chemicals used were of analytical grade. The solutions of electrolytes were filtered before the use through disposable membrane filters of a 0.45 μm pore size (Millipore, Molsheim, France).

Carrier electrolyte was prepared titrating morpholinoethanesulfonic acid (MES) (Merck) in the presence of HEC by ϵ-aminocaproic acid (EACA) (Merck) to reach a required pH. An appropriate amount of sulfated sodium salt of β-cyclodextrin (S-β-CD) (Fluka) was then dissolved in this solution in order to obtain the final separating buffer for the CE.

Cetirizine dihydrochloride (CET) was obtained as a racemic reference substance from Zentiva (Prague, Czech Republic). Reference substance of levocetirizine dihydrochloride (LCET) was obtained from UCB Farchim S.A. (Bulle, Switzerland). Pharmaceutical tablets, Xyzal®, produced by UCB Farchim S.A., were obtained from a local drug store. The declared content of LCET in one tablet was 5 mg.

3.5. Procedures for sample and standard solution preparations

3.5.1. Standard solutions

The stock solutions of racemic CET as well as LCET reference substances were prepared by dissolving 50 mg of the powder in 10 ml of demineralized water and they were stored at −8 °C in the freezer. Working solutions were made by appropriate dilutions of the stock solutions with demineralized water or by spiking CET or LCET from the stock solution into the dosage form.

The concentration levels of CET enantiomers (racemate) in the injected model calibration solutions (prepared in demineralized water) were in the

range of 5–150 μg/ml (5, 10, 20, 50, 75, 100, 150), and each calibration point was measured six times.

For the determination of the trace (at LOQ concentration level) enantiomeric impurity (DCET) besides the major constituent (LCET), the model samples were prepared containing 5 μg/ml of DCET (as 10 μg/ml of racemic CET standard) along with 500–2000 μg/ml (500, 1000, 1500, 2000) of LCET. The purity of the LCET reference substance was also tested preparing model samples containing 500–2000 μg/ml of LCET alone.

For the recovery experiments, CET was spiked from its stock solution into real matrices (dosage form). The samples with three concentration levels (10, 50 and 100 μg/ml) of the enantiomers of CET standard in Xyzal matrix (including 50 μg/ml of LCET) were prepared.

For the robustness test, the deliberate variations in operational parameters were as follows: the concentration of complexing agent (4–6 mg/ml) and carrier anion (23–27 mmol/l), pH (5.1–5.3).

3.5.2. Sample preparation

Ten pharmaceutical tablets were accurately weighed, powdered in a mortar and a portion of the powder equivalent to 50 mg CET was weighed accurately into a volumetric flask and suspended in 10 ml of demineralized water. After 2 h of mechanical shaking, the mixture was ultrasonicated for 10 min and then centrifuged (8000 rpm) for 10 min. The resulting solution was appropriately diluted with demineralized water prior to the analysis and filtered (a 0.45 μm pore size (Millipore)) before the injection into the CE equipment.

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