Capel electrophoresis systems Solutions for pharmaceutical industry

- Highest separation efficiency
- Extraordinary short runtime
- Low consumption of reagents
- Multicomponent analysis in one run
- Simple sample pre-treatment
- Cost efficiency

Tasks facing the pharmaceutical industry:

- safety and quality control:
  - synthetic substances and raw materials,
  - active pharmaceutical ingredients,
  - excipients,
  - final pharmaceutical products;
- in-house and interoperable control of technological processes;
- development of new drugs and methods for their analysis;
- study of pharmakokinetics;
- evaluation of water quality for technological processes;
- detection of falsified drugs.

To solve these problems, modern, reliable and economical methods of analysis are needed.
Capillary electrophoresis (CE) as a separation technique, has gained a huge popularity in the last decades.

A various number of the electrophoresis methods are used to separate:

- charged particles and complexes,
- neutral molecules,
- hydrophobic, hydrophilic components,
- positional and optical isomers,
- proteins and oligonucleotides.

The widespread use of capillary electrophoresis happened due to many advantages of this method compared to other typically used analytical methods in this field:

- the highest, unique separation efficiency;
- fast analysis;
- extremely low volume of reagents and solvents;
- absence of expensive sorbent;
- low cost of instrument and single analysis;
- analysis simplicity.

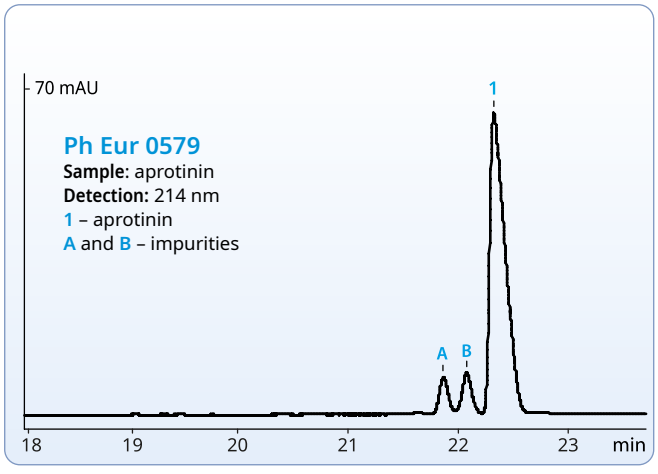
Pharmacopoeial articles

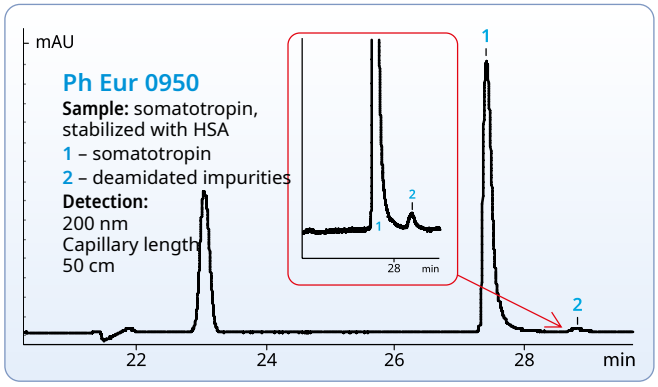
The CE method is included in all modern pharmacopoeias:
- Ph. Eur. 2.2.47;
- USP General Information Ch. <1053> Biotechnology-derived Articles – Capillary Electrophoresis;
- JP General Information 4. Capillary Electrophoresis;
- Brit. Ph. Vol. IV, Appendix III G. Capillary electrophoresis.

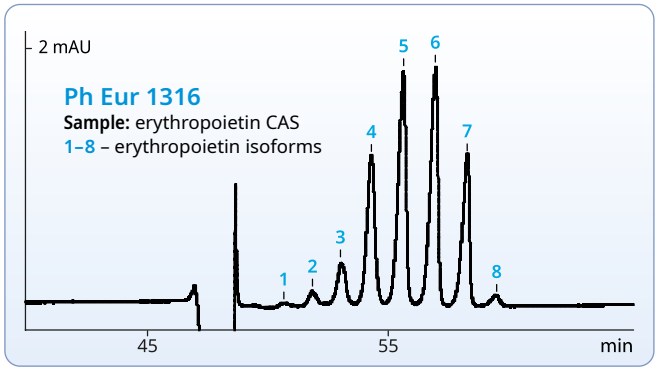
Moreover, it is implemented in pharmacopoeia articles for the following analyses:
- USP 129 – purity assessment of recombinant therapeutic monoclonal antibodies;
- Ph. Eur. 0579, 0580 – determination ofVimpurities in aprotinin;
- Ph. Eur. 0950, 0951, 0952, 2370 – impurities analysis in somatropin;
- Ph. Eur. 1316 – identification of recombinant erythropoietin;
- Ph. Eur. 1670 – determination of impurities in glutathione;
- Ph. Eur. 2335 – determination of enantiomeric purity of ropivacaine.

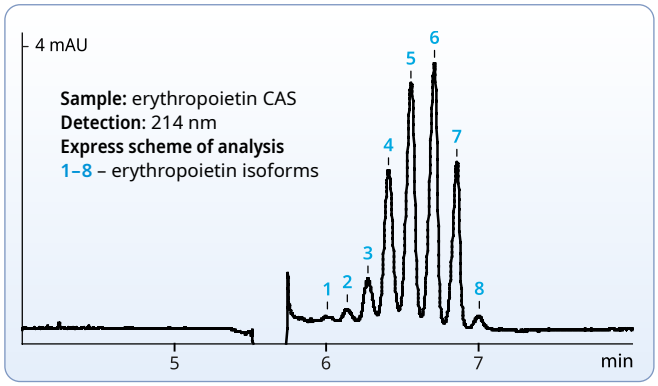
The use of CE systems in pharmacy is not limited only to these pharmaceutical articles. According to experts, about half of all CE instruments produced in the world are used in the pharmaceutical sector for a wide range of tasks - from assessing the quality of finished products to clinical trials.

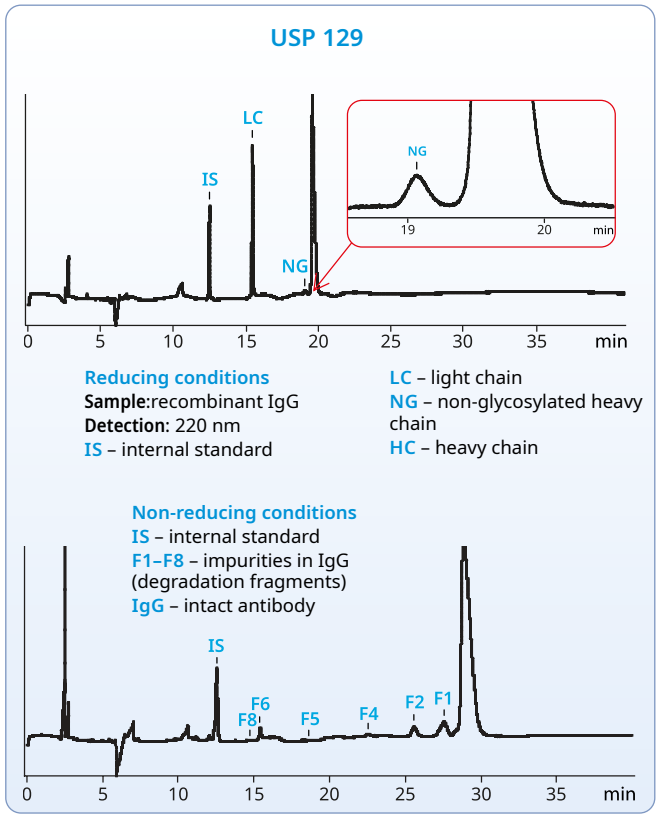
Lumex Instruments is one of the world's leading manufacturers of CE systems. We adapted the most of pharmacopoeia articles for the use with our Capel systems series. This was performed together with our customers all over the world. Some examples are:











Biomolecule analysis

A. Capillary gel electrophoresis

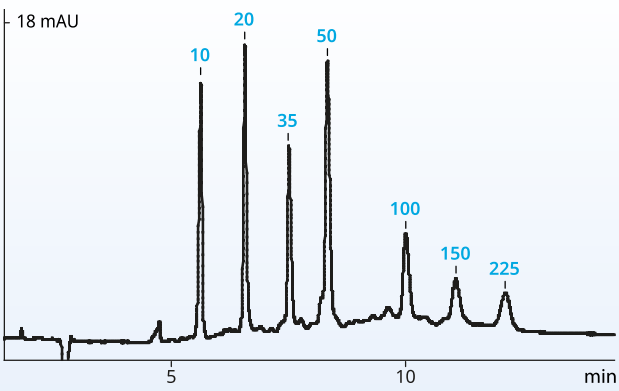
Antibodies and other **recombinant proteins** are important pharmaceutical and biotechnological products. Determining their **purity, stability, and heterogeneity** is a very important task, since post-translational modifications, as well as protein degradation processes after biosynthesis, can radically change their biological activity.

Polyacrylamide gel electrophoresis (SDS-PAGE) is currently widely used to separate proteins with different molecular weights.

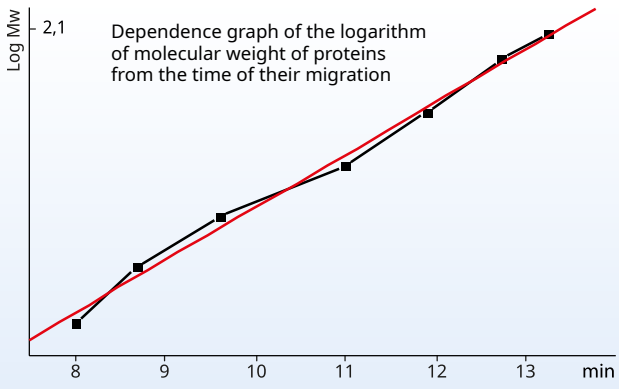
An important alternative to it is capillary gel electrophoresis (CGE). It uses a capillary filled with buffer with polymer additives. Before analysis, the protein sample is denatured by heating in the presence of sodium dodecyl sulfate (SDS) under reducing (with a reducing agent) or non-reducing conditions. After rapid cooling, the sample is injected into the capillary, high voltage is applied, and the proteins begin to move through the polymeric buffer solution. Same as in gel electrophoresis, **separation is achieved through differences of molecular weights (the so-called sieving effect)**.

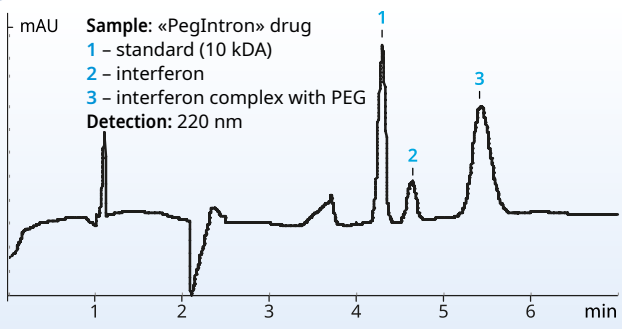
There is a direct dependence between the logarithm of the molecular weight of a protein and its migration time. This allows to directly determine the molecular weight of unknown proteins by their electromigration time.

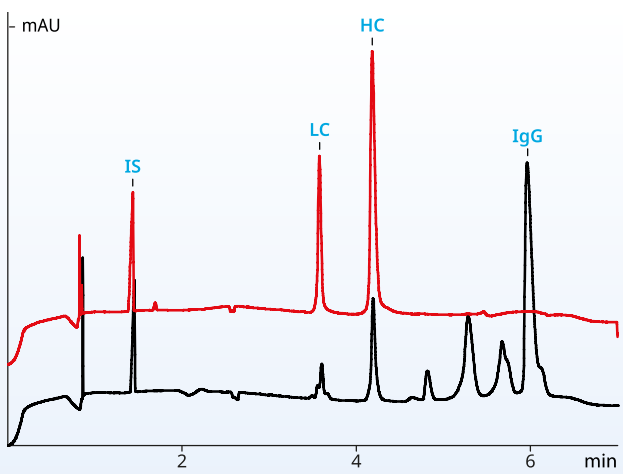
Using the CGE method, it is possible to **separate proteins that differ in mass just by 4% and higher.**



Sample: mixture of polypeptides with a known molecular weight
Detection: 220 nm







Sample: recombinant immunoglobulin IgG in reducing (red) and non-reducing (**black**) conditions
IS – internal standard
LC – light chain (25 kDa)
HC – heavy chain (55 kDa)
IgG – antibody (146 kDa)
Other peaks – antibody degradation products
Detection: 220 nm

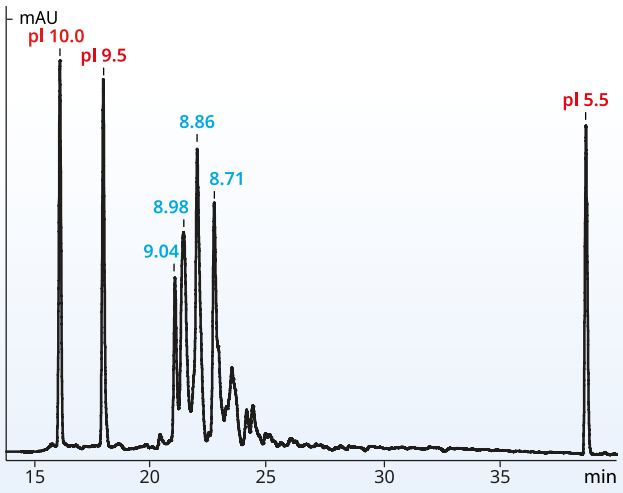
B. Capillary isoelectric focusing

The method of capillary isoelectric focusing (CIEF) **is used to separate proteins with different isoelectric points** (separation of isoforms with different charges). In this CE mode even proteins with the same or very similar molecular weight can be resolved.

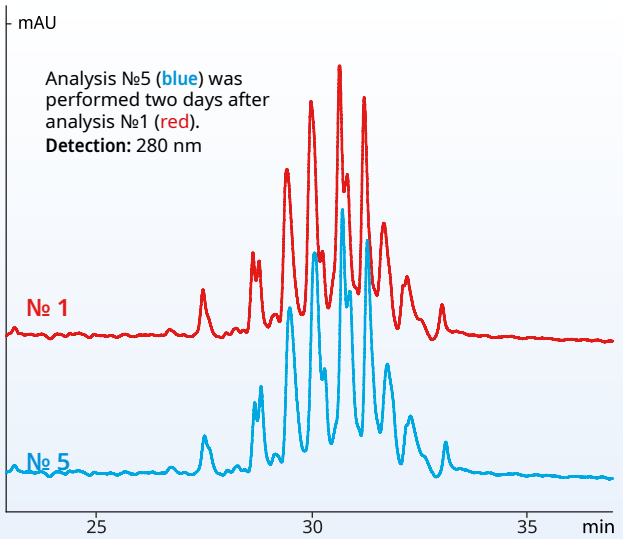
In a two-stage version of this method, the capillary is filled with a mixture of buffer solution, ampholytes, and the sample. On the first stage (focusing), high voltage is applied, resulting in a pH gradient in the capillary and the protein molecules, in accordance

with their isoelectric points, are focused into narrow zones, in which they lose their charge and electrophoretic mobility. On the second stage (mobilization), the outlet vial is replaced with the mobilizing solution vial and the high voltage is applied again, after which the focused zones begin to move towards the detector.

There is a direct proportional dependence between isoelectric points of the proteins and their apparent migration time, which permits to calculate pI's of the unknown proteins using the so-called pI markers, added to the sample mixture. Proteins with isoelectric points **differing by as little as 0.04 pI units** can be separated using this method.



Sample: recombinant IgG
Separated 4 protein isoforms with slightly different pI values.
Markers were added to determine pI of protein isoforms.
Detection: 280 nm



Sample: protein compound
Several differently charged isoforms were separated, showing the heterogeneity of the protein compound (it was shown that the compound is homogeneous). A series of analyses were carried out over several days to assess reproducibility.

Physico-chemical studies

Such physicochemical characteristics of molecules as dissociation constants, complexation constants, binding constants (for proteins), distribution coefficients are the most important indicators that must be taken into account when developing new pharmacologically active compounds. They largely determine the bioavailability of substances, their transport in the blood system, and membrane permeability. Given the large volume of newly synthesized biologically active compounds, it is the CE method that is indispensable for determining these parameters.

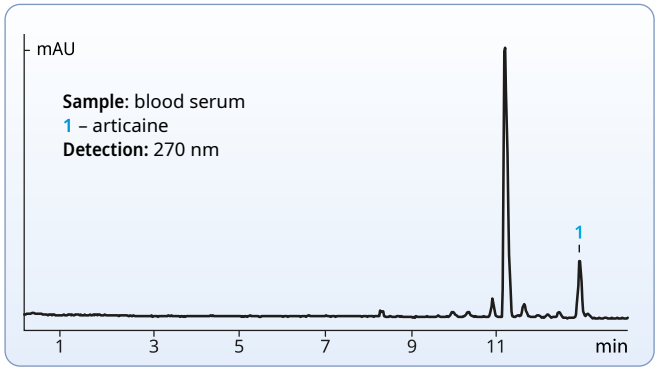
Ultra-low and ultra-high pH values are unfavorable conditions for HPLC analysis, but they do not interfere with the **logP determination of acids and bases** in their uncharged form by CE. For compounds with known logP values (markers), you can build a diagram logP by migration time, and then use it to calculate the logP value for the chemical compound.

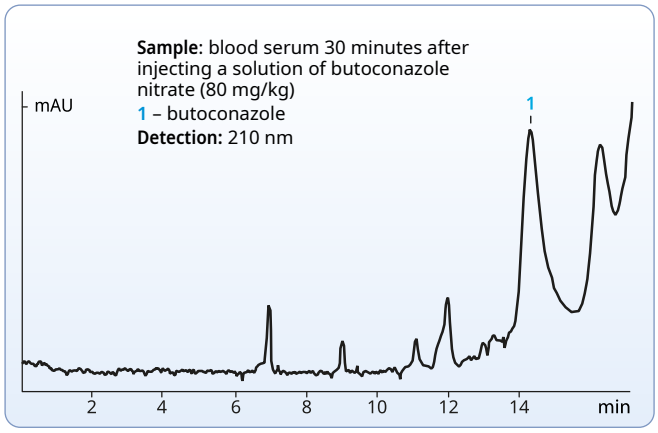
The second most important application of the CE method in physicochemical studies is the **determination of the dissociation constant pKa.** These values can be easily calculated from the migration times of the compound at different pH values.

When determining pKa by the CE method, the **researcher needs neither large portions of the compound** (this is important for screening), **nor the determination of the exact initial concentration**. All this is a huge advantage of the CE method over the titrimetric method. Since micro impurities and the test compound have different electrophoretic mobility, the impurities **do not interfere with the determination of pKa by the CE method**. The pKa values obtained by potentiometric titrations or spectrophotometrically can be strongly distorted.

Pharmacokinetics

The CE method has found a wide use for the analysis of not only the initial biologically active compounds, but also their metabolites in clinical studies.





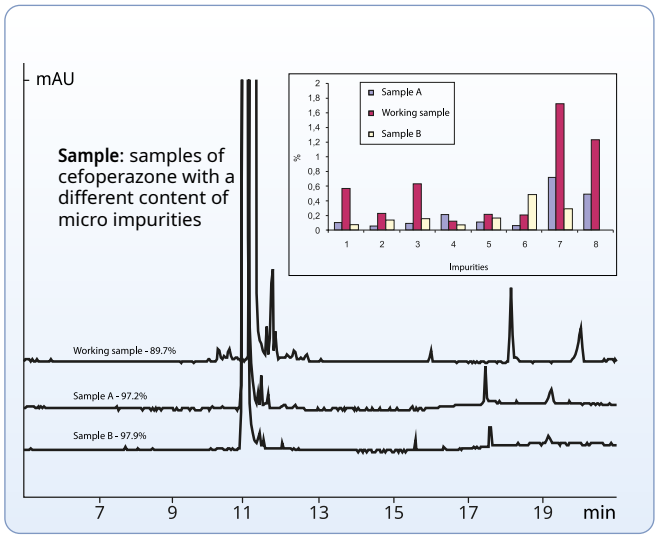
Determination of low molecular weight compounds

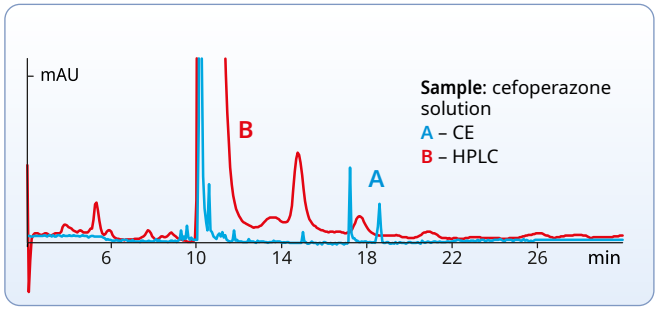
Analgesics and antibiotics (penicillin, cephalosporin, β-lactam), barbiturates and tricyclic antidepressants, purines and flavonoids, amino acids and vitamins – all these compounds can be determined by CE method either in the final pharmaceutical products or in raw materials.

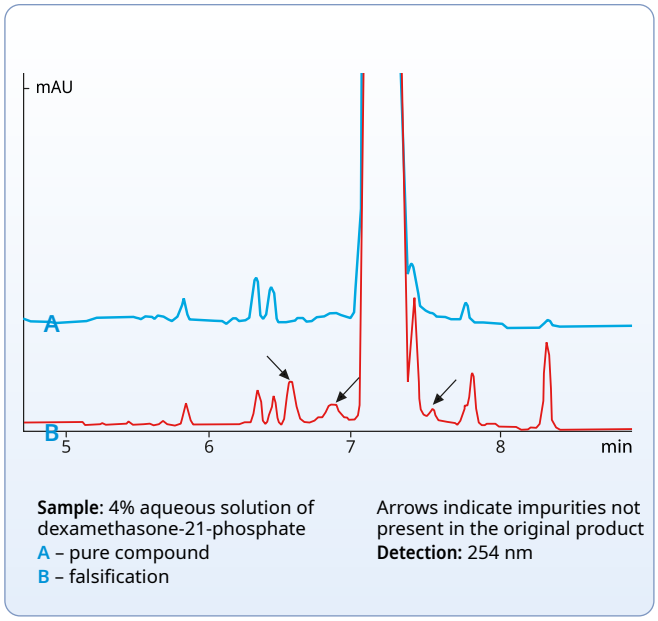
Capillary electrophoresis becomes attractive for the **analysis of the main active substance**, as well as **for determination of micro impurities** and **decomposition products**.

The method does not include a derivatization stage and simplifies the selection of separation conditions for compounds similar in structure and physicochemical properties. The new generations of capillary electrophoresis systems allow online concentration, which increases the determination sensitivity by several times.

Combination of **CE and HPLC is the only possible way to obtain reliable results on the chemical composition of the test sample**. Moreover, it can solve such an important problem as the detection of falsifications.







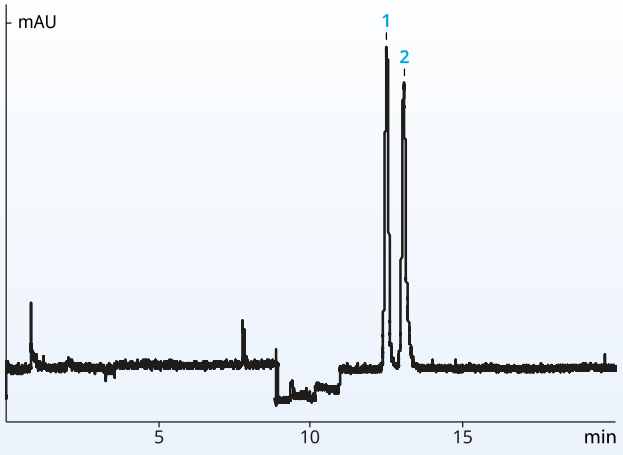
Separation of isomers

Many substances are used in the manufacture of medicines are racemate (a mixture of two optical isomers - **enantiomers**), but often only one of them shows biological activity. HPLC is the most commonly used method for determining enantiomeric purity, but its use has serious limitations: the high cost of specialized chromatographic columns, the difficulty in predicting the separation efficiency for different types of compounds,

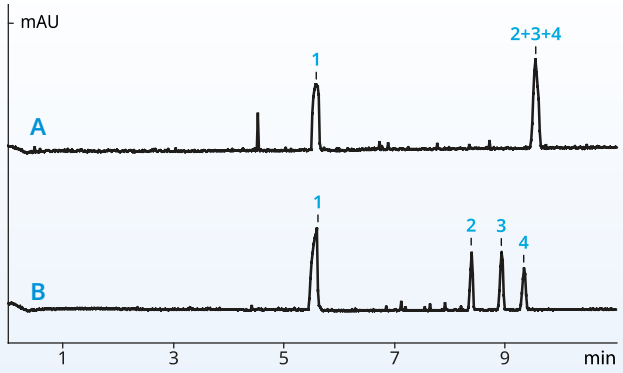
and the difficulty in assessing the degree of modification of the sorbents used. A convenient alternative to this is the CE method. The determination of enantiomeric purity is the most common task for capillary electrophoresis in the practice of pharmaceutical research laboratories.

Significant advantages of the CE method are:
- **ease of selecting additives (chiral selectors) and optimization of conditions for the separation of enantiomers,**
- **cost efficiency,**
- **fast analysis.**

Modified β- and γ-cyclodextrins, as well as macrocyclic antibiotics (vancomycin), most of which are commercially available, are currently the most commonly used additives.



Sample: solution of carbamazepine derivatives
1 and 2 –enantiomers of trans-10,11-dihydroxycarbamazepine
Chiral selector: octakis-(2,3-dihydroxy)-6-sulfato-γ-cyclodextrin
Detection: 214 nm



Sample: simulated solution of positional isomers of nitrobenzoic acid
A: borate buffer
B: borate buffer with added β-cyclodextrin

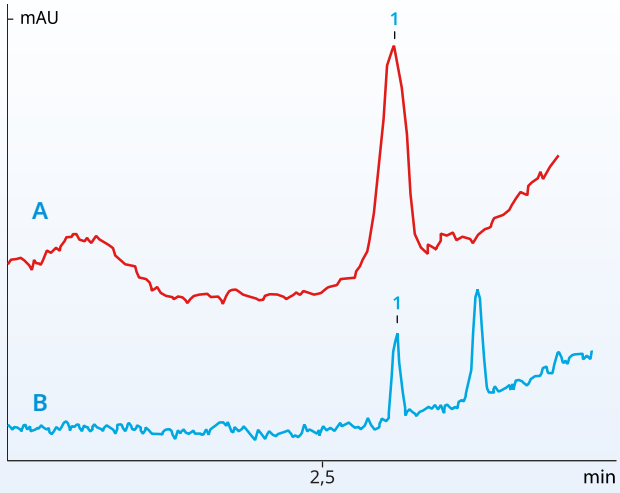
1 – electroosmosis flow
2 – *4-nitrobenzoic acid*
3 – 4-nitrobenzoic acid
4 – 2- nitrobenzoic acid
Detection: 254 nm

Determination of ions

A. Determination of the ionic composition of water

On all stages of pharmaceutical production, obtaining accurate information about the ionic composition of water is an important analytical task. Using the Capel systems, you can get **complete information about the concentrations of inorganic and organic anions** (fluorides, chlorides, bromides, iodides, nitrites, nitrates, phosphates, sulfates, and acetates) and **inorganic cations** (ammonium, potassium, sodium, lithium, magnesium, calcium, strontium and barium) in various types of water in just a couple of minutes. The detection limits of most of these ions do not exceed mg/l, and these values can be reduced by an order of magnitude by using on-line preconcentration methods.

Lumex Instruments has developed two proprietary analytical protocols for the determination of inorganic ions in water samples. In addition, such recognized protocols as EPA 6500 and ASTM D6508-10 can be also realized on Capel CE systems.



Sample: acetate ion solution (0,1 mg/l)
A – online concentration
B – standard sample input

1 – acetate ion
Achieved concentration by 10 times.
Detection: 254 nm, indirect detection

B. Determination of counterions

The majority of molecules of medicinal compounds are salts containing counterions, which ensure the stability of the substance, its solubility, transport, etc. Currently such analytical methods as titrimetry, IC or atomic emission spectrometry are being used for counterions determination, but CE is becoming more and more popular for these purposes.

Lumex Instruments has been producing innovative instruments that meet the requirements of SP XIV for instrumental methods of analysis for more than thirty years:

- infrared spectroscopy (general monograph 1.2.1.1.0002.15) – **Infrared Fourier Spectrometer «InfraLUM FT-08»** with a wide range of attachments and specialized spectra libraries;
- Capillary electrophoresis (general monograph.1.2.1.0022.15) – **capillary electrophoresis systems «Capel»**;
- atomic absorption spectrometry (general monograph.1.2.1.1.0005.15) – **atomic absorption spectrometer «MGA-1000»** and mercury analyzer «RA-915M» with attachments for liquid and solid samples
- fluorimetry (general monograph.1.2.1.1.0006.15) and photometry (general monograph.1.2.1.1.0003.15) – **spectrofluorimeter «Fluorat-02-Panorama»** and **fluorimeters series «Fluorat-02»**.

The fluorimetry method determines the aluminum content in the following objects:

- medicine;
- water for injections;
- purified water;
- potassium chloride;
- sodium chloride;
- citric acid.

Certified Lumex Instruments' products are successfully used for the analysis of plant raw materials, as well as biologically active additives.

The software used in the Capel CE systems fully meets the requirements for working in accordance with standards GMP/GLP, ISO and CFR 21 p.11.

The quality management system is certified for compliance with the requirements of the international standard ISO 9001:2015.

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