IC: The all-rounder in pharmaceutical analysis

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Ion chromatography is a flexible technique with a wide variety of practical uses in the pharma sector – here we take a look at some pertinent trends and recent advances in its application.

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Testing in a regulated environment

High standards have to be met by the pharmaceutical industry when it comes to drug quality and safety. These standards are documented in pharmacopoeias as officially recognised pharmaceutical rules, and published as legal tools of customer protection by authorities such as governments and medical societies. The identification of a drug depends on sensitive, reliable instruments and methods – as does the determination of the drug's compliance with applicable regulations.

Ion chromatography (IC) is the method of choice to determine active ingredients, excipients, and traces of impurities, as well as metabolites in the form of organic and inorganic ions or polar substances, in a number of pharmaceuticals, pharmaceutical solutions, and even body fluids. It can determine the presence of several substances within a very short time in a single analysis – and even distinguish chemically similar analytes. The concentration of analytes can vary from ng/L up to the per cent range. The large selection of separation columns and elution systems available makes IC useful for almost any kind of analyte. Interfering effects caused by the sample matrix can easily be avoided by using the right sample preparation or choosing a suitable detection method. Inline sample preparation is a feature of many modern IC systems, as the focus of recent advances in IC has been mainly on ease of use. However, convenience is not the only advantage brought by the automation of the IC process: reducing human interference to a minimum also means reducing the chances of mistakes and contamination.

Depending on the requirements of analyte and matrix, there is a broad range of detection methods to choose from:

- Conductivity detection with and without suppression
- Electrochemical detection
- Spectrophotometric detection with and without post-column derivatisation (ultraviolet–visible spectrophotometry)
- Coupled detection methods such as IC–mass-spectrometry (MS) and IC–inductively-coupled-plasma-MS

Pharmaceutical samples come in many different forms which require different ion chromatographic approaches. What follows is an overview of frequent sample types with relevant example analyses.

Pharmaceutical solutions

The term 'pharmaceutical solutions' denotes isotonic, haemodialysis, or infusion solutions. They contain anions, cations, carbohydrates, and organic acids, the concentrations of which frequently differ from one another by several orders of magnitude. Within the context of production monitoring and final quality control, an analysis method is required that can determine these ingredients with a high degree of precision. In addition, the analysis should be quick and require minimal effort. With its intelligent analytical procedure and automatic inline sample preparation, IC fully accomplishes this task.

Two example analyses of haemodialysis solutions are shown in Figures 1 – 2. Patients suffering from renal failure require haemodialysis to compensate for the loss of the kidney's blood-cleansing function. During the process, the patient's blood exchanges solutes with a haemodialysis solution through a semi-permeable membrane. The exchanged solutes include, among others, waste products such as urea and phosphate, which diffuse out of the blood and into the dialysis solution along the concentration gradient. The composition of dialysis solutions is complex, because the removal of solutes from the blood changes its osmotic activity; therefore, it has to take place at a controlled rate, which is achieved by the right solute concentration. A strong change in osmotic activity can cause dialysis disequilibrium syndrome where, due to the low solute concentration in blood, solutes are washed out from other body compartments.

Figure 1 shows the simultaneous determination of citrate and acetate in diluted haemodialysis solution. In part A, an anion standard was measured; part B shows the sample determination. Citrate is added to haemodialysis solutions for its anticoagulant properties and acetate is added as a buffer substance. It is transferred to the patient's bloodstream during haemodialysis and stabilises the blood's pH value. This is necessary because the kidneys of dialysis patients are not capable of excreting acid components – therefore, patients are often acidotic.

Besides citrate and acetate, the chromatogram reveals the presence of a close to physiological concentration of chloride. By using physiological solute concentrations, the concentration gradient is reduced to a minimum, and a dynamic equilibrium is reached between blood and dialysis solution. The loss of certain solutes – including chloride – is thereby prevented. Figure 2 shows the determination of cations in haemodialysis concentrate after an automated inline dilution step. Like chloride, the cations are present in close to physiological concentrations to avoid their drainage from patients' blood by osmosis.

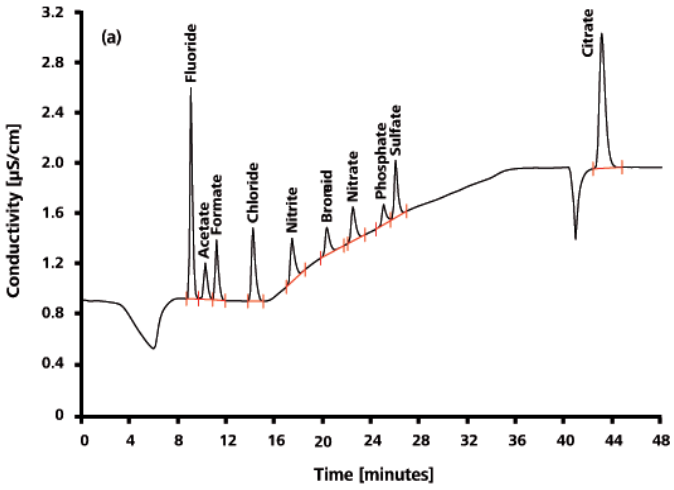

Figure 1A. IC measurement on a Metrosep A Supp 7 - 250/4.0 using Na_2CO_3 gradient elution, followed by sequential suppression and conductivity detection. Anion standard including acetate and citrate.

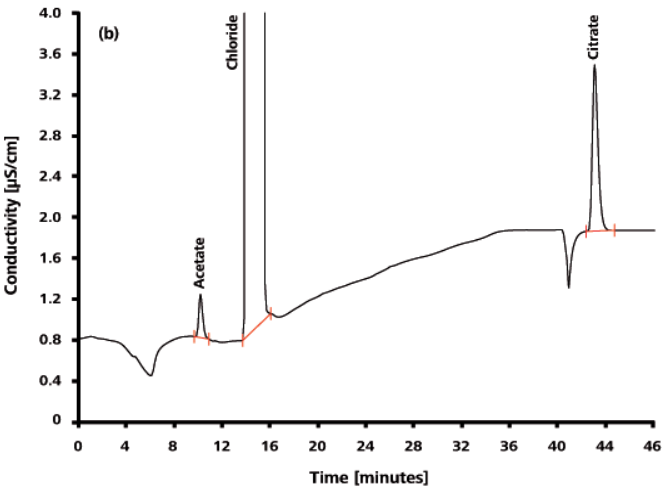

Figure 1B. IC measurement on a Metrosep A Supp 7 - 250/4.0 using Na_2CO_3 gradient elution, followed by sequential suppression and conductivity detection. Acetate and citrate in haemodialysis solution.

Active pharmaceutical ingredients

Active pharmaceutical ingredients (APIs) in medicines such as gentamicin, neomycin, cefadroxil, or bethanechol chloride can be determined by IC in accordance with the regulations of the US Pharmacopeia and the European Pharmacopoeia. The requirements regarding precision, separation, and recovery of the analytes are described in detail in the pharmacopoeias. Figure 3 depicts the ion chromatogram of an analysis of gentamicin, an antibiotic belonging to the group of aminoglycosides. Aminoglycosides are bactericidal antibiotics that block bacterial protein biosynthesis by binding to ribosomes, thereby causing mistakes in the translation from messenger ribonucleic acid to DNA. Gentamicin consists of several closely related compounds, namely gentamicin C_1, gentamicin C_{1a}, and gentamicin C_2, C_{2a}, and C_{2b}. In spite of their structural similarity, IC achieves a good separation of the different gentamicin components.

Impurities in pharmaceuticals

Apart from API analysis, it is also possible to determine impurities in pharmaceutical products by IC. Even small concentrations of an impurity can cause significant side-effects. For example, in the synthesis of the antihypertensive irbesartan, azide can be detected in traces as an impurity in the product. Azide is strongly toxic to humans and its concentration in irbsartan is therefore subject to rigorous controls. The US Pharmacopeia recommends ion chromatographic azide determination after direct injection according to USP 621. In this method, a transfer solution consisting of the IC eluent and suitable organic solvent is used to remove the API from the analytical column. However, this procedure is tedious, time-consuming and cannot be automated.

Azide determination is more selective, more sensitive, and, above all, quicker with the use of inline matrix elimination, where the interfering pharmaceutical matrix is separated from the target analyte in the course of sample preparation. The ion chromatogram shows the analysis of an irbesartan sample spiked with different concentrations of azide (see Figure 4).

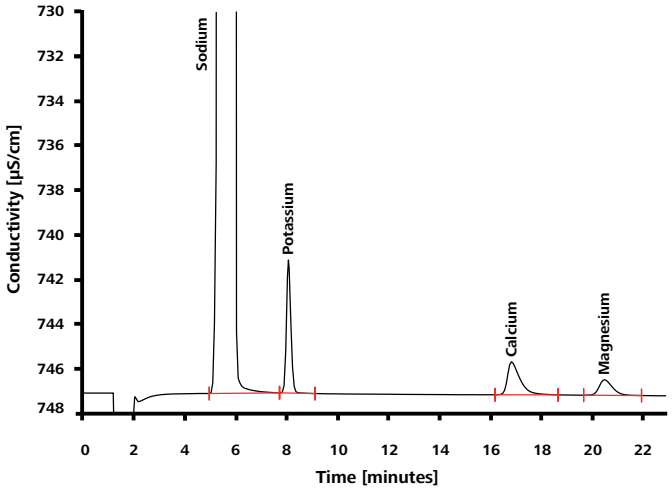

Figure 2. Cations in diluted haemodialysis concentrate using the Metrosep C 4 - 150/4.0 column and non-suppressed conductivity detection.

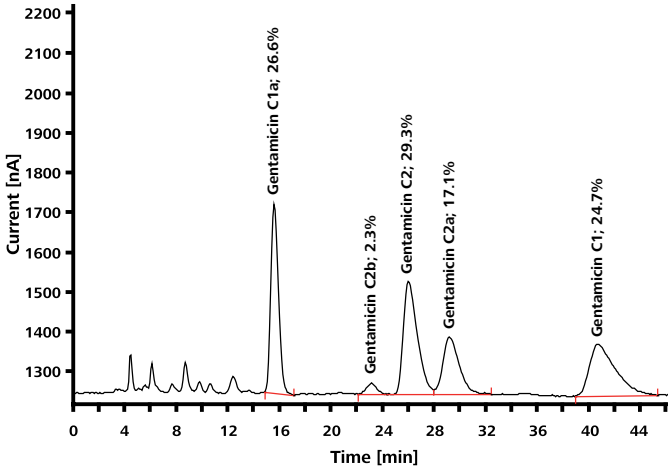

Figure 3. IC determination of the antibiotic gentamicin by pulsed amperometric detection; column: Polymer Laboratories RP-S; eluent: 60g/L Na_2SO_4, 1.75g/L sodium octanesulfonate, 1.34g/L NaH_2PO_4, 8mL/L THF (pH = 3, H_3PO_4); post-column addition: 300mmol/L NaOH.

High sensity thanks to matrix elimination

The signal is recorded by a conductivity detector following sequential suppression. Table 1 lists the average recovery values of azide that were achieved over three measurements, as well as the mean conductivity measured by the detector and the relative standard deviation. The determination of azide in irbesartan with preceding matrix elimination fulfils all requirements of the regulatory authorities, which concern the selectivity of the method, its limits of detection and quantitation, precision, linearity, accuracy, and robustness. Thus, it can be used as a quicker and more sensitive alternative to the proposed determination according to USP 621.

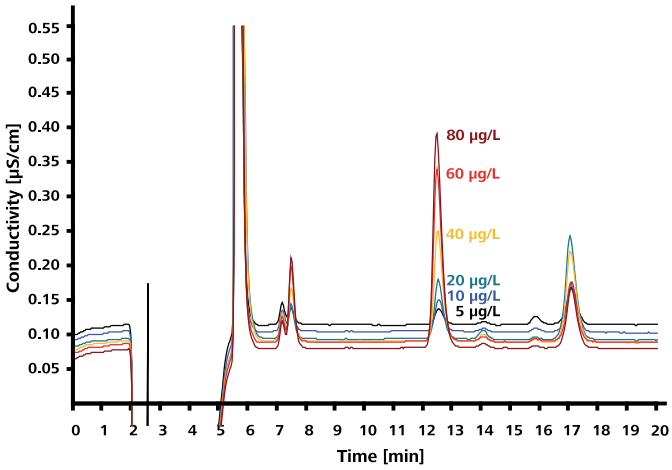

Figure 4. Irbesartan sample spiked with 5-80µg/L azide; column: Metrosep A Supp 10 - 250/4.0; eluent: 5mmol/L Na_2CO_3, 5mmol/L $NaHCO_3$; inline matrix elimination with 70:30(v/v) methanol/water.

Radio IC

Radio IC aims to determine the radiochemical purity of radiopharmaceuticals. These are radioactive substances that are used for medical purposes, mainly in diagnostics, but also in the treatment and prevention of certain diseases. [^{18}F]fluorodeoxyglucose and [^{18}F]fluorocholine are two prominent examples of radiotracers that are used in diagnostics by positron emission tomography (PET). They are labelled with the radionuclide [^{18}F]fluorine. During the radioactive decay of this unstable isotope, a proton in the nucleus of [18F]fluorine changes to a neutron. This is accompanied by the emission of a neutrino and a positron. The latter combines with an electron in the surrounding tissue, resulting in annihilation of both particles and emission of two photons (gamma rays) in opposite directions, each with an energy of 0.511 MeV. From the data acquired through coincidence detection of the photon pair, the location of its emission in the patient's body is calculated. This location coincides closely with the location of the original radiotracer molecule, and thus reveals information on its activity.

The purity of radiotracers is of crucial importance. The highly energetic gamma rays emitted during the combination of a positron with an electron are harmful to the human body. By using a pure radiotracer, thereby avoiding the injection of free [^{18}F]fluorine or other radioactive contaminants, the amount of radioactive substance administered to the patient can be kept to a minimum.

The quality control of radiotracers is established by radio IC in the short time between their synthesis and the recording of the three-dimensional PET scan. The separation step in radio IC is the same as in regular IC – apart from the fact that it happens behind lead doors. What really sets radio IC apart from conventional IC is the detection step, in which a radioactivity detector is added to the setup. The radioactivity chromatogram reveals the presence – or, ideally, the absence – of radioactive contaminants.

Table 1. Precision and recovery of azide

Peak area			
	Mean value (µS/cm)	Relative standard devitation (%)	Recovery (%)
5 µg/L spike	± 5.00	1.96	101.71
30 µg/L spike	± 0.30	0.14	103.38

n = 3 measurements

Table 2. Selection of IC applications in the pharma industry

Pharmaceutical or excipient	Analyte
Acamprosate calcium	Acetate
Acifluorfen, sodium	Acetate
Adrenaline	Adrenaline
Amisulpride	Dimethyl and diethyl sulfate
Anticoagulation solution	Phosphate, citrate
Arsenic trioxide	Arsenate, arsenite
Atovaquone	Acetate
Atorvastatin calcium salt	Cyanide, tetrabutylammonium
Sulfobutylether-ß-cyclodextrin	ß-cyclodextrin
Bethanechol chloride	Bethanechol, sodium, calcium, decomposition product (HPTA)
Bromide salt	Chloride
Busulfan	Methanesulfonic acid
Calcium gluconate	Oxalate
Calcium salt	Borate
Camphorsulfonic acid	Camphorsulfonic acid
Carbamazepine	Chloride, bromide
Carbidopa	EDTA, hydrazine, sodium disulfite
Cefadroxil	Cefadroxil
Cefdinir	Iron, EDTA
Cefepime hydrochloride	N-methyl-pyrrolidinium
Ceftazidime sodium	Sodium
Clopidogrel besylate	Anions, carbonate, cations
Colesevelam	Quaternary alkylamines
Copovidone EP	Acetate, formate
Dasatinib	Ethylenediamine
Dextromethorphan HBr	Formic acid
(2,3-Dichlorophenyl) oxoacetonitrile	Cyanide, tetrabutylammonium
Diclofenac sodium	Sodium, potassium
Dicyclopropylmethylamine	Dicyclopropylmethylamine
Doxazosin, methanesulfonic acid	Bromide
Drospirenone	Propargyl alcohol
Enoxaparin sodium	Sulfate
Esomeprazole magnesium	Tartrate
Febuxostat	Hydroxylamine
Felodipine	Silicate, sodium
Fenofibrate	Sodium lauryl sulfate (SLS)
Ferumoxide (contrast enhancer)	Citrate
Fluorouracil (also fluoruracil)	Fluoride
Gabapentin	Chloride
Gadopentetate dimeglumine	Gadolinium
Gentamicin sulfate (see page 17)	Gentamicin

Pharmaceutical or excipient	Analyte
Glycine carbonate, sodium salt	Carbonate
Glimepiride	Trans-4-methylcyclohexylamine
Guaifenesin	Epichlorhydrine
Heparin sodium	Glucosamine and galactosamine
Ibandronic acid sodium	Ibandronate, phosphite, phosphate
Indinavir sulfate	Ethyl sulfate
Indomethacin sodium	2-ethylhexane acid
Irbesartan	Cyanide, azide
Ibuprofen	Ibuprofen, valerophenone
Lamotrigine	Cyanide
Lanthanum carbonate	Nitrate
Levetiracetam	Tetrabutylammonium
Levofloxacin	Fluoride
Linezolid	Morpholine
Losartan potassium	Azide
Meropenem	EDTA, dimethylamine
Metformin hydrochloride	Dimethylamine
(Mono)sulfiram (temosol)	Cyanide
Montelukast sodium	Methanesulfonic acid, acetate
Multivitamin tablets	Cations, Vitamin C
Mycophenolate mofetil	Morpholine
Nebivolol hydrochloride	Monomethylamine
Neomycin sulfate	Neomycin
Oxaliplatin	Chloride
Pioglitazone hydrochloride	Piperidine
Piperacillin	Chloride
Piperazine	Piperazine, N-methylpiperazine
RA-Thermoseal toothpaste	Potassium, zinc
Ribitol	Ribitol (adonitol)
S-Adenosyl methionine	Sulfate
Sevelamer	Binding capacity of phosphate
Suxamethonium chloride	Choline chloride
Tadalafil	Methanolic methylamine
Terbinafine hydrochloride	Monomethylamine, tetrabutylammonium
Topiramate	Carbohydrates, sulfate and sulfamate
Triclosan	Potassium
Timolol maleate	Chlorite
Varenicline tartrate salt	Trifluormethanesulfonic acid
Voriconazole	Camphorsulfonic acid
Zingisol	Potassium and zinc
Zoledronic acid	Phosphite, phosphate

Detection method: conductivity detection with suppression; direct conductivity detection; conductivity detection with and without suppression; amperometric detection; spectrophotometric detection

Summary and conclusion

Today, IC covers a diverse field of applications in the pharma industry. A selection of ca. 40 applications is listed in Table 2. The technique has become an extremely versatile method due to the large number of different columns, eluent and gradient options, sample preparation techniques, and automation possibilities that are available to the user.

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