Application Note AN-D-003

Quality control of dialysis concentrates

Comprehensive analysis of anions, acetate, and cations by IC

Hemodialysis is a medical treatment applied to sustain life when renal functions decline and the kidneys' endogenic detoxification abilities fail [1,2]. Dialysis fluids (hemodialysis solutions), composed of electrolytes, buffers, and carbohydrates (glucose) identical to the blood, are a central element of this treatment [1,3–5]. The diffusion gradient between the blood and the dialysis fluid allows removal of metabolic waste and normalization of electrolyte content [1,2]. Dialysis fluids are prepared by adding concentrates that contain electrolytes, carbohydrates, and buffers to water. These require the highest standards for manufacturing and on-site preparation, specified by e.g., the European Pharmacopeia, ISO 11663, ISO 23500, or ISO 13958 (for hemodialysis concentrates) [1,2,4].

Atomic absorption spectroscopy (AAS) is often used for quality control purposes but is restricted to cationic (metallic) components and a limited number of concurrently determined analytes. Ion chromatography (IC) is an automated, fast, and sensitive solution to accurately quantify cationic and anionic components including acetate simultaneously. This comprehensive approach makes IC an economic alternative to traditional analytical techniques for the quality control of pharmaceutical solutions like hemodialysis concentrates. Ease-of-use, accuracy, and the high throughput capabilities of IC increase productivity and comply with the demands of modern routine and research laboratories.

SAMPLE AND SAMPLE PREPARATION

Dialysis fluids must closely mimic blood plasma composition to remove toxic components from blood by diffusion. These fluids are typically composed of water, electrolytes that provide cations and anions (e.g., sodium, potassium, calcium, chloride), buffers (e.g., acetate or carbonate), and carbohydrates (e.g., glucose) [**1**,**3–5**]. In this application example, cations, anions, and acetate content were analyzed in two hemodialysis concentrates (**Table 1**). Optimal results were obtained with a dilution in the range of 1:500 to 1:750 using ultrapure water (UPW).

The dialysis concentrates were provided by MTN Neubrandenburg GmbH a Nipro company, an established producer of high-quality hemodialysis products. Both were acid concentrates (A-concentrates) for bicarbonate dialysis with different compositions (**Table 1**). The production of such concentrates is subject to strong standardized quality criteria as e.g., ISO 13958, ISO 11663, and ANSI/AAMI RD 61:2000 [**1**]. Strict standards also apply to the other components necessary for the preparation of the final dialysis fluid, including water and the basic concentrates (B-concentrates) [**1**,**3–5**].

EXPERIMENTAL

Anions and cations were analyzed with a dual channel IC setup (**Figure 1**) using conductivity detection (sequentially suppressed for anions). A UV/VIS detector (947 Professional UV/VIS detector Vario) can be used as well to exclude nitrite, nitrate, and bromide contaminations in the concentrates.

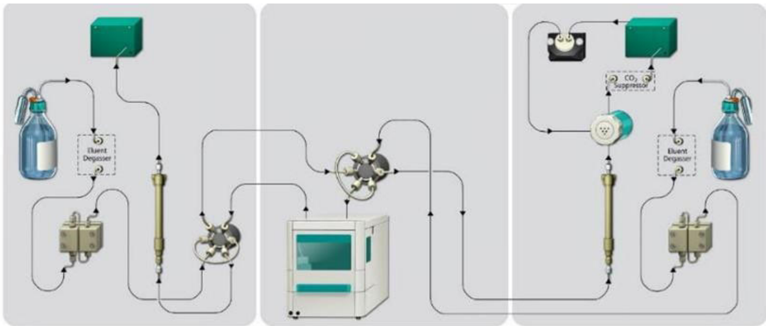

Figure 1. Flow path for a dual channel Metrohm IC system. Injection was performed using the 889 Sample Center – cool (middle). Non-suppressed conductivity was used for cation detection, while anions were detected with suppressed conductivity as well as UV (205 nm).

These impurities can be determined with high precision and sensitivity even in presence of elevated chloride concentrations (Table 1). Method performance tests with nitrate- and nitrite-spiked concentrates yielded recoveries of 90–110%.

Table 1. Composition (average and range) of two tested hemodialysis acid concentrates (A-concentrates) according to the manufacturer.

A-concentrate	#293	#570
Sodium (mol/L)	3.61 (3.51–3.70)	4.64 (4.52–4.75)
Potassium (mmol/L)	70.00 (66.50–73.50)	90.00 (85.50–94.50)
Magnesium (mmol/L)	17.50 (16.63–18.37)	22.50 (21.38–23.62)
Calcium (mmol/L)	52.50 (49.88–55.12)	56.25 (53.44–59.06)
Chloride (mol/L)	3.82 (3.62–4.01)	4.88 (4.64–5.13)
Acetic acid (mol/L)	0.11 (0.10–0.11)	0.14 (0.13–0.14)
Glucose (g/L)	35.00 (33.25–36.75)	45.00 (42.75–47.75)

The complete system (**Figure 1**) was controlled by Waters EmpowerTM 3 software. A refrigerated autosampler (889 IC Sample Center – cool) was used to extend the stability of the highly diluted samples.

Anions were separated using the Metrosep A Supp 19 - 150/4.0 column (standard eluent and flow rate, **Figure 2 A**, **C**). This high-capacity IC column exhibits excellent separation capabilities, even for highly loaded matrices.

The unique properties of the Metrosep A Supp 19 column allow adequate separation and quantification of acetate even in presence of high concentrations of chloride. In addition to acetate (0.4–20 mg/L) and chloride (6–300 mg/L), the system calibration included fluoride (0.02–1 mg/L), nitrite, and bromide (0.04–2 mg/L), as well as nitrate, phosphate, and sulfate (0.2–10 mg/L).

Cations were separated using a Metrosep C 6 -

150/4.0 column (standard eluent, flow rate: 1.3 mL/min, **Figure 2 B**). Cation calibration was performed for sodium (4–200 mg/L), ammonium (0.02–1 mg/L), and potassium, calcium, and magnesium (0.2–10 mg/L). The special column chemistry of the Metrosep C 6 guarantees optimal peak resolutions and enables quantification of low concentrations of analytes (e.g., ammonium) that elute close to more highly concentrated components (e.g., sodium).

Anions and cations were analyzed simultaneously from the same sample in less than 25 minutes (**Figure 2**). The robustness of both separation columns permits high flow rates, speeding up the overall run time.

RESULTS

A summary of the results, including the recoveries calculated compared to the manufacturer values, is shown in **Table 2**. Relative standard deviations (RSDs) of less than 1% for anions and cations for repeated sample measurements reveal adequate repeatability of the method. The recoveries calculated according to the manufacturer data fell between 91–106% for all analytes (**Tables 1 and 2**).

The major components of the tested A-concentrates are sodium and chloride, corresponding to the main fractions in blood plasma, with 136–145 mEq/L and 98–106 mEq/L, respectively [2]. However, this also shows that these concentrates are highly saline solutions — analytically challenging and often requiring matrix elimination steps for accurate analyte determination. When present in high concentrations, both sodium and chloride can overlap nearby peaks (e.g., acetate, nitrite, or ammonium) making their quantification impossible or overloading the column, resulting in peak broadening and substantial retention time shifts.

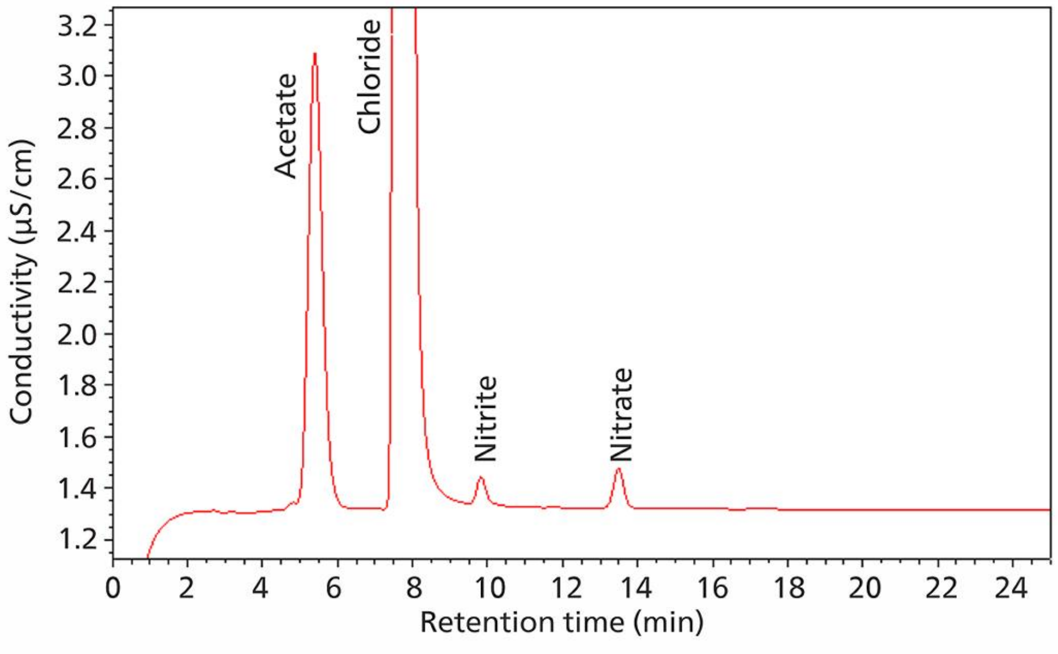

Figure 2 A.

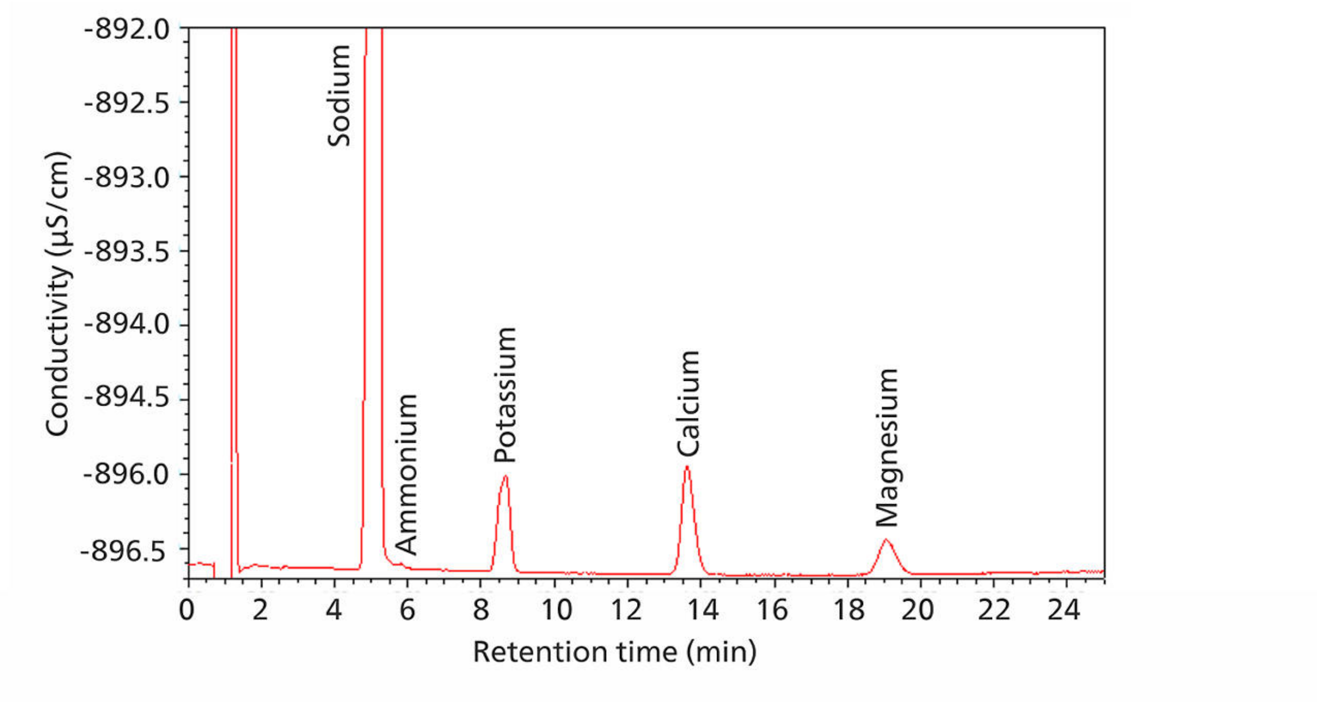

Figure 2 B.

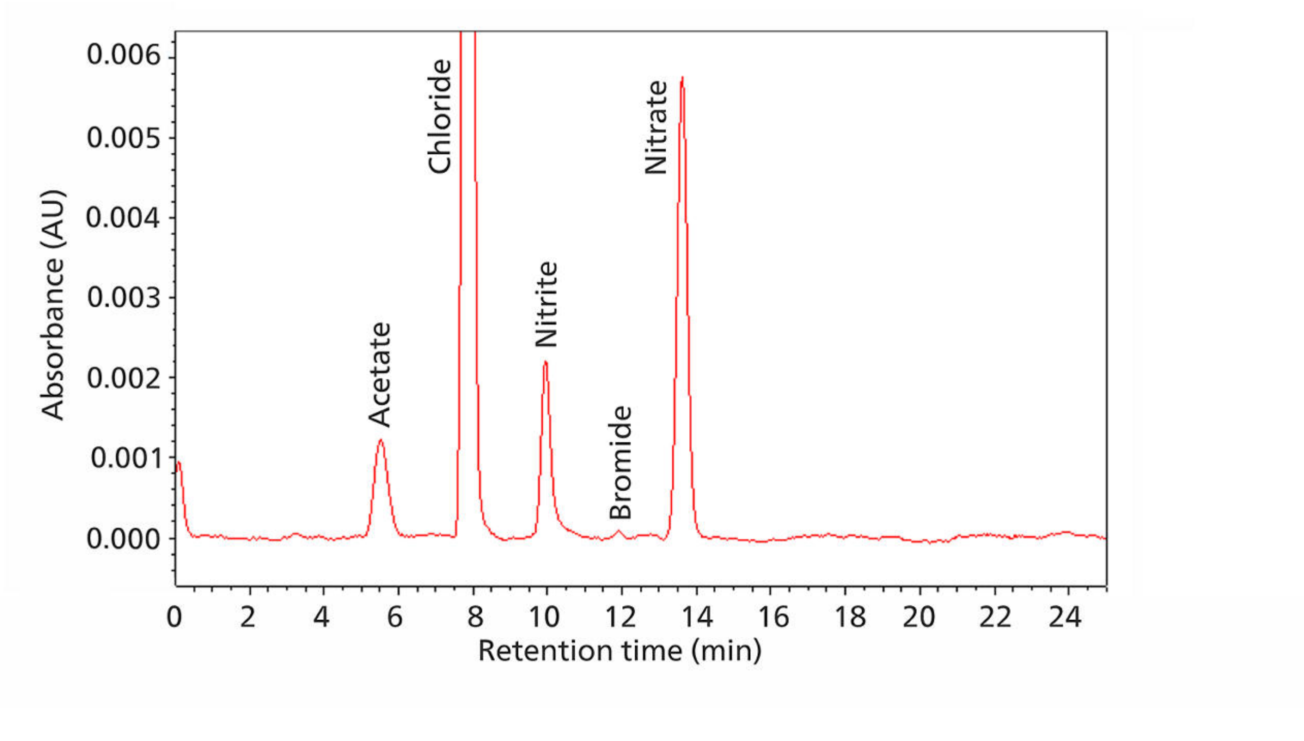

Figure 2 C. Chromatograms showing conductivity (A, B) and UV (C) signals for IC analysis of anions (including acetate) and cations in the hemodialysis concentrate sample #293. All samples were diluted by a factor of 750 with UPW. Injection volume was 20 µL.

For the A-concentrates, the accurate determination of all components (acetate, chloride, sodium, potassium, calcium, and magnesium, Table 1) is indispensable and requires appropriate peak separation combined with sharp and symmetric peaks. The use of the Metrosep A Supp 19 and Metrosep C 6 columns prevents the aforementioned problems – the high column capacities prevent matrix overload and guarantee excellent peak separation.

Table 2. Data for the major components in the hemodialysis A-concentrate samples #293 and #570 from MTN Neubrandenburg GmbH a Nipro company. The data show average values and RSDs for two separately prepared and analyzed samples (dilution 1:500) as well as the recoveries based on the manufacturer data.

	#293 $Conc_{AVG}$ ± SD (RSD (%))	Recovery (%)	#570 $Conc_{AVG}$ ± SD (RSD (%))	Recovery (%)
Sodium (mol/L)	3.70 ± 0.04 (1.0)	103	4.90 ± 0.03 (0.6)	106
Potassium (mmol/L)	66.21 ± 0.52 (0.8)	95	86.75 ± 0.42 (0.5)	96
Magnesium (mmol/L)	15.95 ± 0.11 (0.7)	91	21.47 ± 0.08 (0.4)	96
Calcium (mmol/L)	50.36 ± 0.56 (1.1)	96	55.18 ± 0.19 (0.3)	98
Chloride (mol/L)	3.84 ± 0.01 (0.2)	103	4.97 ± 0.01 (0.1)	104
Acetic acid (mol/L)	0.11 ± <0.01 (<0.1)	102	0.14 ± <0.01 (0.2)	102

Acetate (8 g/L) can be determined directly beside high chloride concentrations (180 g/L) on the Metrosep A Supp 19 column. No additional steps are required, such as matrix elimination or using different dilution factors. Cations can be determined in parallel from the same sample (**Figure 1**, cation channel) as the Metrosep C 6 column is also ideal for high matrix samples.

To analyze the potential impurities nitrite, bromide, and nitrate, higher sensitivity can be achieved using a UV/VIS detector at a wavelength of 205 nm.

CONCLUSION

Dialysis concentrates used for hemodialysis treatments are highly saline solutions, requiring matrix-tolerant, accurate, and sensitive quality control analytics. By using a dual channel IC system, anions and cations can be determined accurately and simultaneously from the same sample. In less than 25 minutes, major concentrate components of acetate, chloride, sodium, potassium, calcium, and magnesium, along with impurities (e.g., nitrite, nitrate, or ammonium) can be quantified. Although analyzing high salinity matrices is often challenging, the high capacity separation columns Metrosep A Supp 19 and Metrosep C 6 reduce the common risks for column overload and inaccurate peak identification and quantification. Simultaneous analysis of anionic and cationic components and impurities enables a comprehensive examination of all analytes from a single sample, presenting IC as an accurate, sensitive, efficient, high-throughput analytical technique for the quality control of pharmaceutical solutions such as hemodialysis concentrates.

Metrohm IC systems can be fully controlled (including the intelligent and automated features) by different software: MagIC Net (Metrohm), Empower™ 3 (Waters), or OpenLab CDS (Agilent). These options provide a flexible solution for many analytical laboratories.

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CONFIGURATION

Metrosep A Supp 19 - 150/4.0

出色的分性能和高容量 - Metrosep A Supp 19 品系列因此在色柱品品中脱而出。其特点在于最佳峰称性和性,以及高的、机械和化学定性,其因此在更高流速和力下其固和定。

150 mm 器型号被是子色的准色柱,因其可靠解决了大多数用,并且用途非常广泛。Metrosep A Supp 19 - 150/4.0 分柱容量高,因此特用于具有要求苛基的用。Metrosep A Supp 19 - 150/4.0 具有出色的分性能,因此用域非常广泛,例如包括以下用:

- 定各水中的准子(子、子、硝酸根、子、硝酸根、酸根和硫酸根);
- 定品基中的准子和有机酸,例如:境或食品品;
- 定炉水中的准子和有机酸,保障厂安全行;
- 定物品中的准子。

Metrosep A Supp 19 Guard/4.0

Metrosep A Supp 19 Guard/4.0 Metrosep A Supp 19 品系列的子分柱行保,使其不受品或洗淋液染,并从而着延其使用寿命。Metrosep A Supp 19 品系列的保柱和分柱材 PEEK,并用相同材料填充。保了色分效率不会受到任何不利影。

«On Column Guard System» 的作用是,便于直接并且方便地将保柱到子分柱上。Metrosep A Supp 19 Guard/4.0 价格惠且操作,因此是保分柱的理想。

Metrosep C 6 - 150/4.0

Le matériau haute capacité de la C 6 fait de la colonne de séparation Metrosep C 6 - 150/4,0 la solution optimale pour la séparation des cations standard à des concentrations très différentes avec des temps de rétention raisonnables. Les eaux potables présentant de faibles teneurs en ammonium peuvent etre déterminées à l'aide de cette colonne.

Metrosep C 6 Guard/4.0

Metrosep C 6 Guard/4.0 包含 C-6 柱材料,用于防微粒和防染。用它可著延分析用分柱的使用寿命。Metrosep C 6 Guard/4.0 按照 «On Column Guard System» 来行,可几乎无死点容地直接安装到相分柱上。

940 Professional IC Vario TWO/SeS/PP

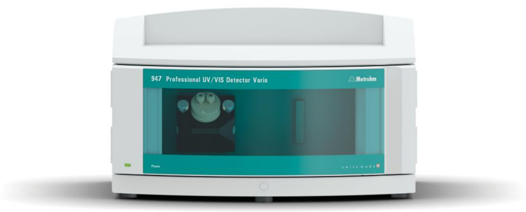
940 Professional IC Vario TWO/SeS/PP 是智能型**双通道** IC 子色器,有**序列抑制**(通道)和一个**蠕**用于抑制器再生。器可使用各分和方法。

典型的用范:

- 并行定子和子的准器
- 子和子的痕量分析
- 子和子的在控

889 IC Sample Center – cool

889 IC Sample Center – cool 自化解决方案也用于品量少的情况。与 889 IC Sample Center 相比,它具有冷却功能,因此是用于生物化学相域或定性差的品的最佳自器。



947 Professional UV/VIS Detector Vario MW

智能多波器,947 Professional UV/VIS Detector Vario MW,可紫外光或可光区域内的活性物行安全可靠的定量操作。最多可自由八波。通二管列行。