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Validation of ion chromatographic methods for the trace analysis of ions in pharmacopoeial grades of water

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Abstract

Ion chromatography was investigated as an alternative technique to conventional pharmacopoeial methods for the determination of inorganic anions and cations in pharmacopoeial grades of water. These methods were validated by generating data on parameters such as specificity, linearity, precision, accuracy, sensitivity, ruggedness and stability of solution. The validation data demonstrate that ion chromatography is a viable alternative technique to current pharmacopoeial methods which enables automation of pharmacopoeial water analyses.

Keywords: Water analysis; Pharmaceutical analysis; Inorganic ions

1. Introduction

All water used in pharmaceutical processes must conform to appropriate specifications detailed in the relevant pharmacopoeias [1–5]. Pharmacopoeial methods for the analysis of inorganic anions and cations utilise classical 'wet' chemistry techniques that only provide qualitative and/or semi-quantitative information, and it is well established that the precision of the tests may not be as good as modern instrumental methods of analysis [6].

In recent years, ion chromatography (IC) has become a powerful alternative technique for the determination of low levels of inorganic anions and cations [7–10]. Use of IC enables automation of the analysis of large numbers of samples with permanent records of the analysis. Such quantitative data allow trend analysis of water systems, enabling alert and action limits to be set. This paper aims to show that IC is a suitable alternative technique for the analysis of pharmacopoeial grades of water by presenting typical method validation data.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade and all reagents, eluents and standard solutions were prepared using de-ionised water further purified with a Milli-Q system (Millipore) with a specific resistance of 18.2 MΩ cm, filtered through a 0.2 μm membrane filter. NaCl, $NaNO_3$ and Na_2SO_4 (Analar BDH, Merck, Dorset, UK) were used to prepare anion stock standard solutions at the specification limits (0.1 ppm, 0.2 ppm and 1.0 ppm, respectively). NH_4Cl, $MgCl_2$ and $CaCl_2$ (Aldrich, Gillingham, UK) were used to prepare cation stock standard solutions at the specification limits (0.2 ppm, 1.0 ppm and 2.0 ppm, respectively).

Sodium carbonate and sodium hydrogencarbonate (Dionex, Sunnyvale, CA, USA) were used for the preparation of the eluent for anion determinations and methanesulphonic acid (puriss grade, Fluka, Buchs, Switzerland) for cation determinations.

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2.2. Plasticware

All analysis were carried out using pre-washed polypropylene flasks and vials.

2.3. Instrumentation

A Dionex DX500 ion chromatograph with a Dionex GP4O gradient pump, Dionex ED4O electrochemical detector (in conductivity mode), a Thermo Separation Spectra SYSTEM AS 3500 autosampler, a Rheodyne injection valve and a 100 μl sample loop were used. All flow paths in the system were constructed from chemically inert, metal-free polydiether ketone (PEEK).

Anion separation was achieved with Dionex AS4A-SC analytical (4 mm I.D.) and AG4A-SC guard columns. Suppression was given by a Dionex Anion self-regenerating suppressor (ASRS-1, 4 mm). Cation separation was achieved with Dionex CS 12 analytical (4 mm I.D.) and CG12 guard columns. Suppression was given by a Dionex Cation self-regenerating suppressor (CSRS-1, 4 mm).

2.4. Anion-exchange method

Chloride, nitrate and sulphate anions were eluted using 1.8 m*M* $NaCO_3$:1.7 m*M* $NaHCO_3$ with a flow-rate of 2.0 ml/min. 100 μl injection volume was used with a run time of 10 min. The conductivity detector range was 30 μS.

2.5. Cation-exchange method

Ammonium, magnesium and calcium cations were eluted using 20 m*M* methanesulphonic acid with a flow-rate of 1.0 ml/min. 100 μl injection volume was used with a run time of 10 min. The conductivity detector range was 30 μS.

2.6. Analyses

Validation of the IC methods was performed as described in the European Pharmacopoeia purified water monograph. (The monograph in the British Pharmacopoeia is identical to the European Pharmacopoeia.) Prior to the IC validation exercises, the pharmacopoeial specification limit concentrations for chloride, sulphate, magnesium and calcium ions were determined using conventional methods described in the European Pharmacopoeia [4], as the monograph only contains pass/fail criteria for these tests. A series of solutions spiked with the ions of interest at varying concentrations were tested. The concentrations at which each ion failed the test were deemed to be the pharmacopoeial specification limits which were subsequently used for validating the ion chromatographic methods. The European Pharmacopoeia gives specification limit concentrations for nitrate and ammonium ions as less than 0.2 ppm for each of the two ions, respectively.

The following validation exercises were carried out on the IC methods, with responses for both peak area and height collected.

2.6.1. Specificity

A solution containing fluoride (5 ppm), bromide (5 ppm), nitrite (5 ppm), chlorate (5 ppm), phosphate (5 ppm) and sulphite ions (5 ppm) together with the anions in the pharmacopoeial monograph [chloride (5 ppm), nitrate (5 ppm) and sulphate (5 ppm)] were separated by the anion method to demonstrate appropriate specificity and lack of interference from potential problematical anions.

A solution containing lithium (0.1 ppm), sodium (0.4 ppm) and potassium (0.4 ppm) together with the ions in the pharmacopoeial monograph [ammonium (0.8 ppm), magnesium (0.4 ppm) and calcium (2 ppm)] were separated by the cation method to show that all the components of interest were resolved.

2.6.2. Linearity

Five standards (in duplicate) containing all components of interest for each method were prepared covering a concentration range of 25–150% of the pharmacopoeial specification limit.

2.6.3. Precision

(1) Method Repeatability: Milli-Q water spiked with the ions of interest at the pharmacopoeial specification limit concentrations were analyzed six times on the same day, using the same equipment in the same laboratory, by the same analyst.

(2) Method Reproducibility: The method repeatability sample above was analyzed in duplicate on six different days by different analysts. As only

one IC and set of columns for each analysis was available it was not possible to perform the analyses on different instruments, different columns and in different locations.

2.6.4. Accuracy

Three solutions were prepared accurately at approximately 75%, 100% and 125% of normal standard concentrations by spiking purified water with the ions of interest. A recovery exercise was then performed. This was performed in duplicate.

2.6.5. Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) were determined by performing a linearity exercise covering a range of concentrations close to the limit of detection. All solutions (including Milli-Q water as a blank solution) were injected six times and a mean response was taken. An RS/1 computer program (LOD) was utilised to plot concentration versus detector response and calculate the LOD and LOQ using the following formulae:

$$\text{Limit of detection} = \frac{3Sx/y}{b}$$

$$\text{Limit of quantification} = \frac{10Sx/y}{b}$$

where b = slope of best fit regression line and Sx/y = estimate of residual standard deviation. Further details of this calculation are available on request from the author.

2.6.6. Ruggedness

Flow-rate and concentration of the eluent were varied by ±10% of the values quoted in the method. The effects on retention times were noted. The ion chromatograph was located in a temperature-controlled laboratory. The separation columns, suppressors and detector cell were housed in an insulated Dionex LC2O Chromatography enclosure; therefore, effects of changes in room temperature were deemed to be insignificant.

2.6.7. Stability in container

The stability of a solution of the ions of interest (stored in polypropylene flasks at ambient temperature) were evaluated by assessing whether there was a change in assay over a period of days. Results from the method reproducibility exercise were used.

3. Results and discussion

In order to assess whether a method is valid for a particular use, the validation data generated using that method must be compared to pre-determined acceptance criteria. In the case of the anion and cation methods described earlier, the only acceptance criteria set were that the methods should be equal to or more sensitive and precise than the associated 'classical' method; see Table 1.

3.1. Anion method validation

Summaries of results for the anion validation exercises are tabulated in Tables 2 and 3. The method was found to be specific for chloride, nitrite, bromide, chlorate, nitrate, phosphate, sulphite and sulphate ions. No interference was noted among the component ions and the other ions investigated, with the exception of chlorate and nitrate ions, which were only partially resolved. Altering flow-rate and concentration of the eluent by ±10% of the values quoted in the method gave only slight shifts in retention times and relative retention remained the same. Therefore, the method was found to be robust with regard to mobile-phase composition and flow-rate.

Of the three anions investigated, the chloride ion was the most difficult to analyze. The chloride ion is poorly retained on the column, with a retention time of approximately 2.5 min, which is close to a negative peak caused by water.

Table 1
Specification limits for classical methods

Ion	Specification limit (ppm)
Chloride	0.1
Nitrate[a]	0.2
Sulphate	1.0
Ammonium[a]	0.2
Magnesium	1.0
Calcium	2.0

[a] Taken from the European Pharmacopoeia.

Table 2
Summary of anion validation results by area response

	Chloride	Nitrate	Sulphate
Specification limit	0.1 ppm	0.2 ppm	1.0 ppm
Limit of detection	0.02 ppm	0.01 ppm	0.04 ppm
Limit of quantification	0.05 ppm	0.02 ppm	0.14 ppm
Linearity (5 data points)	$y=0.605x=1.65$	$y=0.620x=1.01$	$y=4.303x=11.94$
Correlation coefficient	0.9973	0.9996	0.9996
p value	0.370	0.357	0.186
Method repeatability			
Mean ($n=6$)	0.10 ppm	0.19 ppm	1.00 pm
% R.S.D.	5.0%	3.3%	0.7%
Method reproducibility			
Mean ($n=6$)	0.12 ppm	0.19 ppm	1.05 ppm
% R.S.D.	12.4%	7.8%	4.0%
Accuracy			
% Recovery ($n=6$)	105.1%	104.5%	105.3%
% R.S.D.	14.7%	3.7%	4.0%
Stability in container at ambient temperature	>7 days	>7 days	>7 days

Reproducible integration of the peak proved difficult, since the response was small. This was overcome by integrating the chloride peak manually each time using the Beckman chromatography management system.

Initial results generated for the chloride ion validation were thought not to be a true reflection of the method. Method repeatability (0.07 ppm/20.53% R.S.D. by area), method reproducibility (0.10 ppm/41.89% R.S.D. by area) and accuracy (79.18% of claim, 16.05% R.S.D. by area) all gave poor precision. Investigations demonstrated that there was a significant carry over from the Milli-Q water system. This was eliminated by extensively flushing the pipework prior to initiation of analysis. This technique was incorporated into sample and standard preparations for all chloride validation experiments. Results for nitrate and sulphate ions previously generated were deemed to be acceptable; therefore, they were not repeated, since they represented a

Table 3
Summation of anion validation results by height response

	Chloride	Nitrate	Sulphate
Specification limit	0.1 ppm	0.2 ppm	1.0 ppm
Limit of detection	0.02 ppm	0.004 ppm	0.04 ppm
Limit of quantification	0.05 ppm	0.01 ppm	0.12 ppm
Linearity (5 data points)	$y=0.175x+0.52$	$y=0.095x+0.84$	$y=0.297x+0.57$
Correlation coefficient	0.9966	0.9891	0.9997
p value	0.534	0.354	0.301
Method repeatability			
Mean ($n=6$)	0.10 ppm	0.19 ppm	1.00 ppm
% R.S.D.	4.0%	3.3%	0.7%
Method reproducibility			
Mean ($n=6$)	0.12 ppm	0.19 ppm	1.05 ppm
% R.S.D.	13.9%	7.7%	4.1%
Accuracy			
% Recovery ($n=6$)	112.0%	105.6%	105.4%
% R.S.D.	13.8%	1.6%	3.6%
Stability in container at ambient temperature	>7 days	>7 days	>7 days

Table 4
Summary of cation validation results by area response

	Ammonium	Magnesium	Calcium
Specification limit	0.2 ppm	1.0 ppm	2.0 ppm
Limit of detection	0.01 ppm	0.06 ppm	0.10 ppm
Limit of quantification	0.03 ppm	0.20 ppm	0.35 ppm
Linearity (5 data points)	$y=1.951x+37.717$	$y=16.010x+(-112.074)$	$y=20.101x+(-226876)$
Correlation coefficient	0.9986	0.9995	0.9993
p value	9.4×10^{-6}	5×10^{-4}	1.0×10^{-5}
Method repeatability			
Mean ($n=6$)	0.21 ppm	1.10 ppm	2.36 ppm
% R.S.D.	2.0%	2.2%	2.0%
Method reproducibility			
Mean ($n=6$)	0.19 ppm	1.09 ppm	2.36 ppm
% R.S.D.	7.1%	0.9%	2.0%
Accuracy			
% Recovery ($n=6$)	98.6%	98.9%	98.3%
% R.S.D.	3.4%	1.9%	4.8%
Stability in container at ambient temperature	>7 days	>7 days	>7 days

worst-case scenario. All three anions investigated were shown to be linear and passed through the origin with 95% confidence.

3.2. Cation method validation

Summaries of results for the cation validation exercises are given in Table 4 Table 5. The methodology was shown to be specific for lithium, sodium, ammonium, potassium, magnesium and calcium ions. No interference was noted among the component ions and the other ions investigated. It was also shown to be robust with regard to mobile-phase composition and flow-rate.

Linearity plots of all three cations investigated did not pass through the origin with a 95% confidence interval. The small positive intercept was attributed to residual ions present in the water or plasticware, but is not considered to be an issue since it is less than the LOD for the method.

Table 5
Summary of cation validation results by height response

	Ammonium	Magnesium	Calcium
Specification	0.2 ppm	1.0 ppm	2.0 ppm
Limit of detection	0.01 ppm	0.08 ppm	0.10 ppm
Limit of quantification	0.02 ppm	0.25 ppm	0.35 ppm
Linearity (5 data points)	$y=0.082x+2.840$	$y=0.698x+(-3.878)$	$y=0.748x+(-6.023)$
Correlation coefficient	0.9970	0.9997	0.9993
p value	1.8×10^{-6}	5×10^{-4}	10×10^{-5}
Method repeatability			
Mean ($n=6$)	0.21 ppm	1.10 ppm	2.29 ppm
% R.S.D.	2.7%	1.7%	0.4%
Method reproducibility			
Mean ($n=6$)	0.19 ppm	1.08 ppm	2.33 ppm
% R.S.D.	5.4%	1.7%	2.6%
Accuracy			
% Recovery ($n=6$)	97.9%	98.5%	98.4%
% R.S.D.	5.4%	1.8%	4.0%
Stability in container at ambient temperature	>7 days	>7 days	>7 days

4. Conclusions

Both the anion and cation ion chromatographic methods are more sensitive and more precise than the classical methods found in the European and United States Pharmacopoeias. The IC methods provide suitable alternatives to pharmacopoeial methods for both qualitative and quantitative analysis by peak area or height response.

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