

Method Development and Validation for Simultaneous Estimation of Trace Level Ions in Purified Water by Ion Chromatography

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Abstract

Background: A simple, accurate, precise and reproducible Ion Chromatography simultaneous estimation method development and validation for estimation of trace level ions in purified water.

Method: A specific and sensitive isocratic IC method was developed and validated for analysis of trace level ions in purified water using thermo scientific AS23 (4×250 mm) with flow rate 1.0 ml/min and Temperature 30 °C. The isocratic mode was used for elution and the mobile phase was 30 mM KOH.

Result: The samples were analyzed using 70 µl injection volume. The developed method was validated as per ICH Q2R1 for linearity, specificity, accuracy, precision, limit of detection, limit of quantification and robustness. The linearity of the proposed method was investigated in the range of 0.1-2.0 ppm for Fluoride, Chloride, Nitrite, Bromide, Nitrate, and Sulfate ($R^2 = 0.9991, 0.9974, 0.9997, 0.9982, 0.9979, 0.9968$). The % accuracy was found to be in the range of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate was found to be in range of 98.05 – 99.79%, 98.17 – 101.04%, 99.62 – 101.06%, 98.96 – 102.04%, 99.80 – 101.04%, 99.59 – 102.31%. The RSD for precision and robustness was found less than 2.

Conclusion: A rapid and sensitive isocratic elution mode analytical method was developed and validated for the simultaneous analysis for Purified water. Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate been validated in accordance with ICH Q2 (R1) requirements.

Keywords: Purified water; Analytical Method validation; Trace level Anions; Ion Chromatography.

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INTRODUCTION

Water is a crucial component in the pharmaceutical industry and plays an essential role in drug production, quality control, and research and development. Water is used in various stages of pharmaceutical manufacturing, including as a raw material, a solvent, a reagent, and a cleaning agent. Overall, the use of water in the pharmaceutical industry is critical for ensuring



the safety, efficacy, and quality of pharmaceutical products. The quality of water used in pharmaceutical manufacturing must meet stringent requirements to avoid contamination and ensure that the products meet the required standards. Nitrate and Nitrite are naturally occurring ions that are part of nitrogen cycle. Excess concentration of nitrate causes disease. Methemoglobinemia oxygen transport depends on the maintenance of intra cellular haemoglobin in the reduced (Fe^{2+}) state. Chlorides may get into surface water from several sources including: rocks contain chlorides, agricultural run-off, waste water from industries, oil wellwastes, and effluent waste water from waste water treatment plants. Chlorides can corrode metals and affect the taste of food products. when chloride levels in water exceed the recommended levels, it can have several negative effects on health, including: Gastrointestinal problems, Dehydration, High blood pressure, Kidney problems.

Fluoride concentrations above 1.5 ppm in drinking water cause dental fluorosis and much higher concentration skeletal fluorosis. Sulfates occur naturally in numerous minerals and are used commercially, principally in the chemical industry. drinking water with high levels of sulfate can have some potential health effects, such as, Laxative effect, Gastrointestinal problems, Dehydration. Bromide is commonly found in nature along with sodium chloride, owing to their similar physical and chemical properties, but in smaller quantities. which have been associated with several negative health effects, including, increased cancer risk (bladder cancer, colon cancer, and rectal cancer), Reproductive problems, Nervous system effects, Kidney problem).

Experimental Part

Materials and instrumentation

A sample of purified water using Thermo Scientific IC.

Optimized chromatographic condition

Chromatographic separation was performed using isocratic elution mode with Dionex Ion Pac AS23 (4×250 mm) column and mobile phase used the composition of 30 Mm KOH. Injection volume was set to 70 μL . The column oven temperature was set to 30 °C. The flow rate was set to be 1.0 ml/min.

Preparation of Solutions

Selection of Diluents

Milli-Q water was used as diluent because it does not contain any contaminants, such as minerals,

ions, or organic compounds, that may interfere with the chemical or biological properties of the sample or reagent being diluted.

Selection of Mobile Phase

Mobile phase selection involved selection of solvent. Proper selection of the IC method depends upon the nature of the sample, its molecular weight and solubility. The mobile phase was selected on the basis of good separation, peak purity, Tailing factor, Number of theoretical plates etc. Various mobile phases were tried in different compositions at different to achieve sharp peak of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate.

Preparation of Standard stock solution

Accurately transfer 2.0 mL each Fluoride Standard Solution (1000 ppm), Nitrite Standard Solution (1000 ppm), Chloride Standard Solution (1000 ppm), Sulfate Standard Solution (1000 ppm), nitrate Standard Solution (1000 ppm), Bromide Standard Solution (1000 ppm) in to 20 mL of volumetric flask and dilute up to volume with diluent and mix well.

Preparation of standard stock solution 2(10 ppm):

Accurately transfer 5.0 mL of stock solution 1 in to 50 mL volumetric flask and dilute up to volume with diluent and mix well.

Preparation of standard solution (1 ppm):

Accurately transfer 5.0 mL of standard stock 2 in to 50 mL of volumetric flask and dilute up to volume with diluent and mixed well.

Preparation of Mobile phase:

Accurately transfer 30.0 ml of potassium hydroxide solution (1000 mM) in to 1000 ml volumetric flask and dilute up to volume with diluent and mix well

METHOD VALIDATION

Analytical validation parameters for this proposed method were determined according to ICH Q2 (R1) guideline.³⁴

Linearity

By plotting calibration curve of peak area v/s concentration, Linearity has been determined over 7 different concentrations.

Accuracy

Accuracy performed by drug-to-drug spiking at three levels at 50%, 100% and 200%. For each level three sets were prepared and percentage recovery

was calculated.

Precision

Replicates of Assay concentration for Repeatability and 3 Concentration of 3 Replicates of intermediate precision studies were carried out. The result was recorded as Relative Standard Deviation (RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were found by the equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times \sigma / S \text{ and } \text{LOQ} = 10 \times \sigma / S$$

Specificity

The percentage interference was calculated after injecting a blank (mobile phase), placebo, standard.

Robustness

Robustness was performed by deliberate change in flow rate (± 1.0 ml/min) and column oven temperature ($30 \pm 1^\circ\text{C}$). Robustness was calculated in terms of RSD.

RESULTS AND DISCUSSION

Preliminary method development

Preliminary studies were performed using various combinations of mobile phase. From preliminary studies, it was found that 30 mM KOH composition and flow rate of 1.0 mL/min with 30°C column oven temperature shows good separation with acceptable suitability parameter. In optimized chromatographic condition Retention time of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate in Fig. 1.

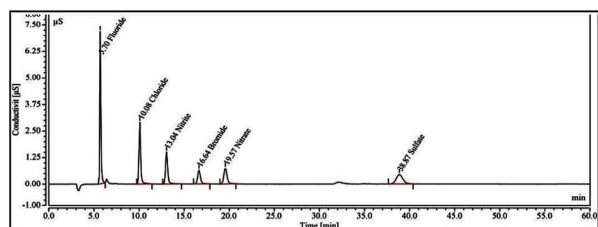


Fig. 1. Optimized Chromatogram

System suitability parameter

After calculating the system suitability parameters, it was discovered that all parameters fall within the permissible range. It mentioned in Table 1. Acceptance criteria for tailing factor is less than 2, for resolution greater than 2 and for theoretical plate it is not less than.

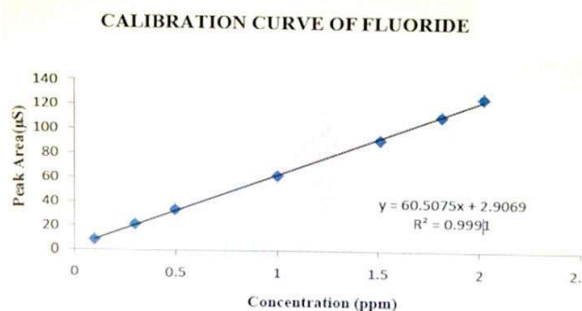
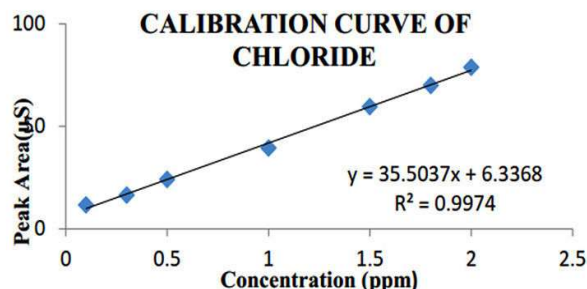
Table 1: System Suitability Parameter of Optimized Condition

Chromatographic Parameter	Optimize Condition
Flow rate	1.0 ml/min
Mobile Phase composition	30 Mm KOH
Column	Dionex Ion Pac AS23 (4×250 mm)
Injection volume	70 μl
Temperature	30°C

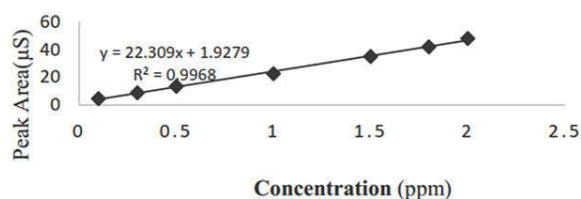
Method Validation

Linearity

Linear responses were found for Fluoride, Chloride, Nitrite, Bromide, Nitrate, and Sulfate in the concentration range of 0.1-2.0 ppm. Fig. 5 calibration curves for Fluoride, Chloride, Nitrite, Bromide, Nitrate, and Sulfate respectively.



CALIBRATION CURVE OF SULFATE



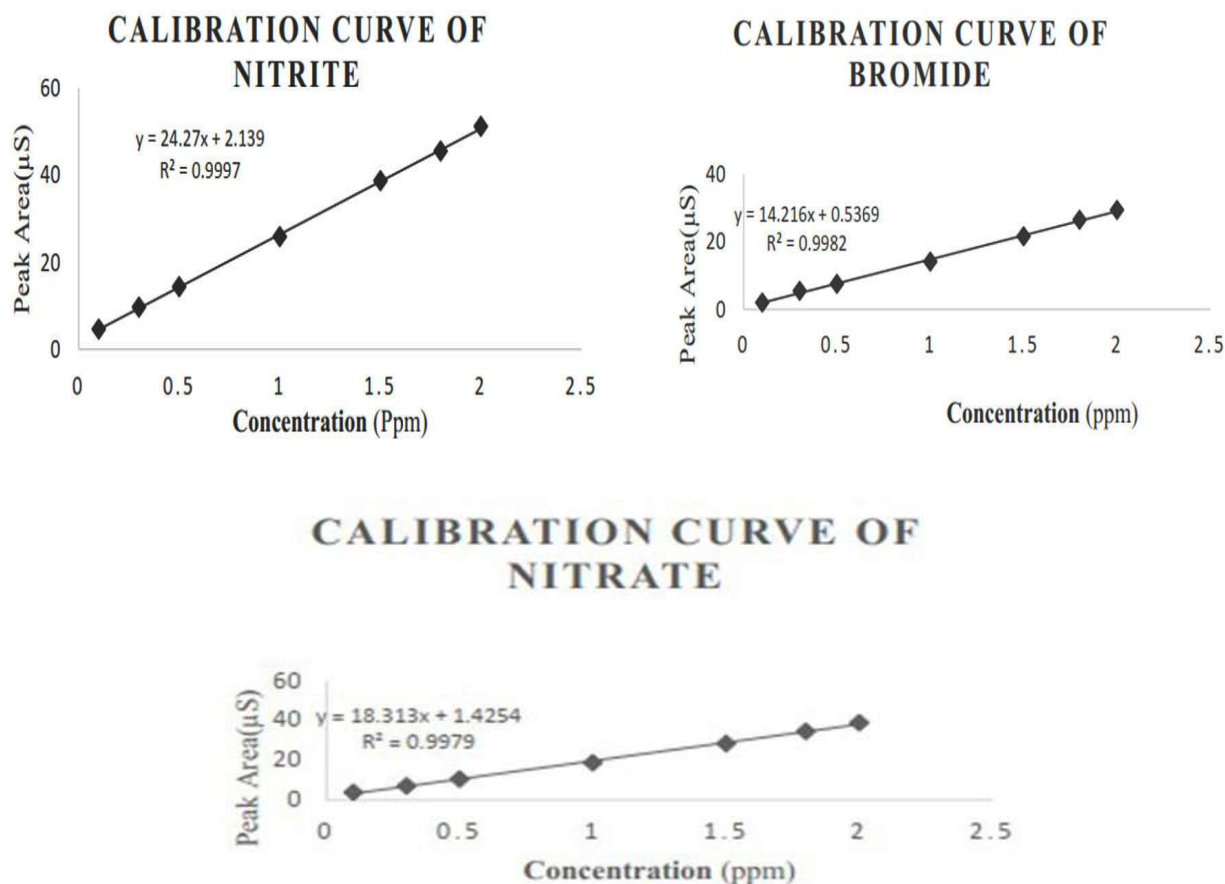


Fig. 2: Calibration curve

Specificity: It is proved that there is no any interference of excipient with the peak of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate by

comparing the chromatogram of blank, mobile phase, and standard solution.

Table 2. Accuracy data

Level	Conc.(ppm)	Bromide		Nitrate		Sulfate	
		Area (μS)	%Recovery	Area (μS)	%Recovery	Area(μS)	%Recovery
50%	0.5 ppm	10.5	100.05	241.29	100.72	192.36	100.23
		10.41	98.96	241.37	101.44	192.28	99.59
		10.46	99.58	241.2	99.8	192.53	101.54
		16.41	100.23	249.74	102.79	201.72	102.13
100%	1.0 ppm	16.27	99.17	249.68	102.45	201.38	100.61
		16.56	101.25	249.56	101.8	201.25	99.98
		31.74	99.87	269.95	101.003	227.88	101.55
200%	2.0 ppm	31.75	99.9	269.79	100.58	227.42	100.6
		31.77	99.98	269.89	100.84	227.29	100.33

Accuracy: Percentage recovery for Silodosin was in the range of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate by IC was found to be in the

range of 98.05 – 99.79%, 98.17 – 101.04%, 99.62 – 101.06%, 98.96 – 102.04%, 99.80 – 101.04% and 99.59 – 102.31% data are shown in Table 3.

Level	Conc. (ppm)	Fluoride		Chloride		Nitrite	
		Area (μS)	% Recovery	Area (μS)	% Recovery	Area (μS)	% Recovery
50%	0.5 ppm	35.77	98.48	2708.85	99.95	14.25	99.62
		35.81	98.61	2708.95	100.41	14.36	100.38
		35.63	98.05	2708.46	98.17	14.34	100.21
		62.84	98.75	2725.46	100.001	25.34	99.9
100%	1.0 ppm	62.94	98.9	2725.86	101.04	25.64	101.06
		63.18	99.31	2725.34	99.69	25.58	100.85
		127.35	99.15	2773.59	100.01	53.35	100.44
200%	2.0 ppm	127.55	99.31	2773.89	100.36	53.55	100.81
		128.16	99.79	2773.54	99.95	53.16	100.07

Precision

Replicates of Assay concentration for Repeatability and 3 Concentration of 3 Replicates

of intermediate precision studies were carried out. The method is precise as RSD and was found to be less than 2. Results are shown in Table 3

Repeatability Data

Sr. No	Conc. (ppm)	Area (μS)					
		Fluoride	Chloride	Nitrite	Bromide	Nitrate	Sulfate
1	1	62.15	38.56	25.87	13.85	18.5	22.52
2	1	61.45	38.86	25.39	13.89	18.41	22.22
3	1	61.14	39.48	25.57	13.93	18.41	22.23
4	1	60.16	38.77	24.84	14.02	18.51	21.91
5	1	61.57	38.18	25.26	13.85	18.68	20.96
6	1	60.59	38.36	25.26	14.04	18.73	22.06
Mean		61.18	38.7	25.37	13.93	18.54	22.15
SD		0.71	0.45	0.34	0.08	0.13	0.22
%RSD		1.17	1.17	1.35	0.59	0.73	1.00

Intraday precision

Precision		Fluoride	Chloride	Nitrite	Bromide	Nitrate	Sulfate
50%	Mean Area (μS)	33.80	24.16±	14.48±	7.86±	10.82±	13.81±
	± SD	±0.04	0.05	0.20	0.15	0.17	0.17
	%RSD	0.13	0.21	1.41	1.98	1.60	1.26
100%	Mean Area (μS)	61.28±	39.30±	25.64±	13.89±	19.11±	23.68±
	± SD	0.15	0.97	0.23	0.03	1.57	0.87
	%RSD	0.25	0.97	0.92	0.27	1.57	0.872

table cont.....

200%	Mean Area (μ S) $\pm$ SD	124.70 $\pm$ 1.49	78.72 $\pm$ 0.21	51.47 $\pm$ 0.31	29.49 $\pm$ 0.23	38.66 $\pm$ 0.59	47.68 $\pm$ 0.30
	%RSD	1.20	0.21	0.60	0.810	0.597	0.629

Interday precision

Precision		Fluoride	Chloride	Nitrite	Bromide	Nitrate	Sulfate
50%	Mean Area (μ S) $\pm$ SD	33.19 $\pm$ 0.48	23.29 $\pm$ 0.16	14.58 $\pm$ 0.21	7.35 $\pm$ 0.13	10.79 $\pm$ 0.09	13.86 $\pm$ 0.08
	%RSD	1.46	0.69	1.50	1.81	0.83	0.61
100%	Mean Area (μ S) $\pm$ SD	61.39 $\pm$ 0.37	38.44 $\pm$ 0.29	25.92 $\pm$ 0.07	14.71 $\pm$ 0.12	19.30 $\pm$ 0.17	23.81 $\pm$ 0.13
	%RSD	0.60	0.77	0.27	0.87	0.917	0.566
200%	Mean Area(μ S) $\pm$ SD	124.70 $\pm$ 1.49	78.73 $\pm$ 0.09	51.71 $\pm$ 0.27	29.50 $\pm$ 0.21	38.56 $\pm$ 0.27	47.58 $\pm$ 0.17
	%RSD	1.20	0.12	0.53	0.71	0.71	0.36

LOD and LOQ

LOD and LOQ of Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate were determined using average of slope and standard deviation of intercepts. LOD was found to be 0.02, 0.02, 0.03, 0.04, 0.02, 0.02 ppm for Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate respectively. LOQ was found to be 0.05, 0.06, 0.09, 0.07, 0.05, 0.05 ppm for Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate respectively.

Robustness

The method was found to be robust when different factors such as flow rate and Temperature were deliberately changed. The relative standard deviation of peak area was less than 2 percent when the parameters were deliberately changed.

CONCLUSION

The current work developed and validated an Ion Chromatography technique for determining Ions in Purified Water. Fluoride, Chloride, Nitrite, Bromide, Nitrate and Sulfate been validated in accordance with ICH Q2 (R1) requirements. As a recovery study, the accuracy of Fluoride, Chloride,

Nitrite, Bromide, Nitrate and Sulfate by IC was found to be in the range of 98.05 – 99.79%, 98.17 – 101.04%, 99.62 – 101.06%, 98.96 – 102.04%, 99.80 – 101.04% and 99.59 – 102.31% percent, respectively, and precision was found to be less than 2% in terms of RSD, and all other parameters were found to be within the specified limit as per ICH guidelines.

Conflict of Interest

The author shows no conflict of interest

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