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Assay of potassium bitartrate using ion chromatography

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Keywords

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Goal

To develop an ion chromatography (IC) method to replace the titrimetric assay for potassium bitartrate in the United States Pharmacopeia (USP) Potassium Bitartrate monograph

Introduction

Potassium bitartrate, also known as potassium hydrogen tartrate, is a laxative for medical use and is commonly used together with sodium bicarbonate to produce carbon dioxide. The active ingredient is bitartrate (hydrogen tartrate). The USP has embarked on a global initiative to modernize many of the existing monographs across all compendia.[1] The current USP monograph for potassium bitartrate uses titration to determine the tartrate content.[2] In response to the modernization initiative, we propose a selective and sensitive IC method to replace the titrimetric assay for potassium bitartrate.

Our IC method offers a significant improvement to the existing assay in the USP monograph. It is a 5 min method that uses a Thermo Scientific™ Dionex™ IonPac™ AS20 ion-exchange column, an electrolytically generated KOH eluent, and suppressed conductivity detection on a Thermo Scientific™ Dionex™ Reagent-Free™ Ion Chromatography (RFIC™) system. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software allows the analyst to run analyses compliantly in an enterprise environment—from method creation to final reporting. Because the RFIC system requires only

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deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. This 5 min method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods.[3-5]

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC™ system* including:
  - – Eluent Generator
  - – Pump
  - – Degasser
  - – Conductivity Detector
  - – Column oven
  - – Detector-suppressor compartment
  - – Tablet
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074989)

* This method can be run on any Dionex RFIC system.

HPIC consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS™ 600 Anion Dynamically Regenerated Suppressor (2 mm, P/N 088667)
- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fitting Tubing Assembly Kit (P/N 088798)

Software

- Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System software

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Thermo Scientific™ Dionex™ Combined Seven Anion Standard I, 50 mL (P/N 056933)
- Potassium bitartrate USP reference standard (Sigma-Aldrich® P/N 1549840-3G)

Sample

- Potassium L-tartrate monobasic, (puriss., meets analytical specification of Ph.Eur., BP, FCC, 99.5–100.5%), (Sigma-Aldrich® P/N 25506-1KG)

IC conditions	
Columns:	Dionex IonPac AG20 Guard, 2 × 50 mm (P/N 063066) Dionex IonPac AS20 Analytical, 2 × 250 mm (P/N 063065)
Eluent source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600 trap column
Eluent:	20 mM KOH
Flow rate:	0.7 mL/min
Column temperature:	30 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm), recycle mode, use recommended voltage at constant voltage mode or 35 mA at constant current mode
System backpressure:	~3900 psi (100 psi = 0.6894 MPa)
Background conductance:	~0.2 µS/cm
Run time:	5 min

Preparation of solutions and reagents

Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.

USP potassium bitartrate stock solution, 1000 mg/L

Accurately weigh 100.0 mg of USP potassium bitartrate into a 125 mL polypropylene bottle and dissolve in 100 mL (100.00 g) of DI water. Mix thoroughly and store at 4 °C.

USP potassium bitartrate standard solution for assay, 20 mg/L

Mix 1.0 mL (1.0 g) of 1000 mg/L of USP potassium bitartrate stock solution and 49.0 mL (49.0 g) of DI water to make the standard solution for assay. Prepare fresh for each chromatography sequence.

Calibration standard for linearity and detection limit determinations

Mix 10.0 mL (10.0 g) of 1000 mg/L of USP potassium bitartrate stock solution and 90.0 mL (90.0 g) of DI water to make 100 mg/L USP potassium bitartrate solution. Further dilute the 100 mg/L standard solution to 0.2, 0.5, 1, 2, 5, 10, 15, 18, 20, and 25 mg/L with DI water. Dilute the 0.2 mg/L standard to 0.02 mg/L with DI water.

System suitability solution

Dilute the USP stock solution and common anions stock solutions with DI water to make the system suitability solution containing 20 mg/L of USP potassium bitartrate, 2 mg/L of fluoride, 10 mg/L of chloride, 10 mg/L of nitrite, 10 mg/L of bromide, 10 mg/L of nitrate, and 20 mg/L of phosphate. Mix thoroughly and store at 4 °C.

Sample preparation

Accurately weigh 100.0 mg potassium bitartrate into a 125 mL polypropylene bottle and dissolve in 100 mL (100.00 g) of DI water to make a 1000 mg/L sample stock solution. Further mix 1.0 mL (1.0 g) of 1000 mg/L of sample stock solution and 49.0 mL (49.0 g) of DI water to make a 20 mg/L sample solution for the assay of potassium bitartrate.

Recovery test sample solution

To make 10 mg/L potassium bitartrate sample solution spiked with 2, 5, 8, 10, and 12 mg/L of USP potassium bitartrate in triplicate, mix 20 mg/L sample solution, 100 mg/L USP potassium bitartrate, and DI water to the corresponding concentrations (Table 1).

Robustness study

Following the guidelines in USP General Chapter <1225>, Validation of Compendial Methods[5] and USP General Chapter <621> Chromatography[6], the robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and resolution of the analyte after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature). The same procedure was applied to two column sets from two different lots. The following variations were tested:

- Flow rate at 0.7 mL/min, 0.63 mL/min, and 0.77 mL/min
- Column temperature at 30 ˚C, 27 ˚C, and 33 ˚C. In order to apply temperatures below 30 °C, the minimum temperature of the Dionex Integrion oven, a Dionex ICS-6000 system was used for this test.
- Eluent concentrations at 20 mM KOH, 18 mM KOH, and 22 mM KOH

Table 1. Preparation of spiked samples for assay recovery test (n=3)

Potassium bitartrate spiked (mg/L)	2	5	8	10	12
20 mg/L Sample solution (mL)	5	5	5	5	5
100 mg/L USP solution (mL)	0.2	0.5	0.8	1	1.2
DI water (g)	4.8	4.5	4.2	4	3.8

Results and discussion

Separation

The separation of bitartrate (separated and detected as tartrate) was achieved within 5 min using a Dionex IonPac AS20 column set with 20 mM KOH. Figure 1, chromatogram A, shows the separation of seven common anions and potassium bitartrate USP reference standard using 20 mM KOH eluent. Tartrate was well resolved from common anions that could be in the sample. Figure 1, chromatogram B, shows a 20 mg/L potassium bitratrate sample.

Column:	Dionex IonPac AG20 Guard, 2 × 50 mm Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent:	20 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge with CR-ATC 600
Temperature:	30 °C
Flow Rate:	0.7 mL/min
Inj. Volume:	2.5 µL
Detection:	Dionex ADRS 600 suppressor, 2 mm, recycle mode. Use recommended voltage.
Peaks:	1. Fluoride 2. Chloride 3. Nitrite 4. Bromide 5. Carbonate 6. Nitrate/Phosphate 7. Tartrate

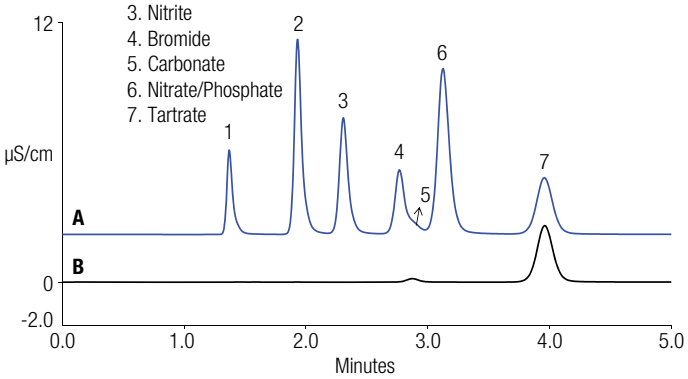

Figure 1. Separation of (A) seven common anions and potassium bitartrate USP reference standard and (B) a 20 mg/L potassium bitartrate sample.

Calibration, limit of detection (LOD), and limit of quantitation (LOQ)

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay.[3-5] For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration. In this study, bitartrate was calibrated at 10 concentration levels ranging from 0.2 to 25 mg/L. The results yielded a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.9999 (Figure 2).

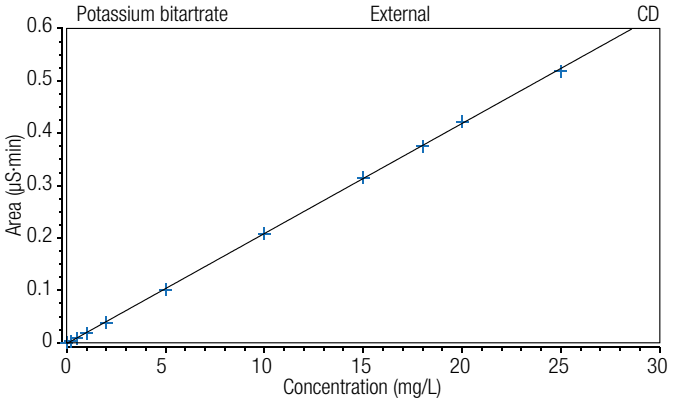

Figure 2. Linearity of potassium bitartrate response to concentration (0.2–25 mg/L)

To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peaks of interest. The signal was determined from the average peak height of seven injections of 0.02 mg/L USP potassium bitartrate solution. The LOD and LOQ were then determined by multiplying the signal-to-noise ratio 3× and 10×, respectively. The LOD and LOQ of potassium bitartrate were 9.13, and 30.4 µg/L, respectively (Table 2).

Table 2. Calibration, LOD, and LOQ of potassium bitartrate

Analyte	Calibration range (mg/L)	r^2	LOD (µg/L)	LOQ (µg/L)
Potassium bitartrate	0.2–25	0.9999	9.13	30.4

Sample analysis

The USP monograph requires that potassium bitartrate contains not less than 99.0% and not more than 101.0% potassium bitartrate calculated on the dried basis.[2] In this study, the USP potassium bitartrate reference standard was used to prepare 20 mg/L standard solution of potassium bitartrate. Sigma-Aldrich potassium bitartrate was used to prepare 20 mg/L sample solution.

Two quantitative methods were compared and evaluated to calculate the percentage of potassium bitartrate in the sample solution (Table 3).

Potassium bitartrate percentage method A: Single Standard Point (20 mg/L)

Calculate the percentage of potassium bitartrate ($C_4H_5KO_6$) in the portion of potassium bitartrate taken:

$$\text{Result} = \left(\frac{r_u}{r_s}\right) \times \left(\frac{Cs}{Cu}\right) \times 100$$

r_u = peak response from the sample solution

r_s = peak response from the 20 mg/L standard solution

Cs = 20 mg/L USP potassium bitartrate standard solution

Cu = nominal concentration of potassium bitartrate in sample solution (mg/L)

Potassium bitartrate percentage method B: USP Potassium Bitartrate Calibration Standard Curve

Prepare potassium bitartrate sample solution at certain concentration. Calculate the true concentration of sample solution using the calibration curve. Calculate the percentage of potassium bitartrate in the sample.

$$\text{Result} = \frac{\textit{Calculated concentration of sample solution}}{\textit{Nominal concentration of sample solution}} \times 100$$

As shown in Table 3, the potassium bitartrate percentage calculated from the Method A (Single Standard Point) gives a similar result to the standard curve calibration method (Method B).

Table 3. Potassium bitartrate percentage in sample using two quantitative methods

	Method A (%)	Method B (%)
Average	99.2	99.5
RSD (n=5)	0.22	0.22

Sample accuracy and precision

Method accuracy was validated by spiked recovery of USP Potassium Bitartrate Reference Standard in potassium bitartrate samples over five concentration levels, with three replicates of each concentration. Table 4 summarizes recovery results of potassium bitartrate. Potassium bitartrate recovery ranges from 97.1% to 102% using the two quantitative methods.

Injection precision was evaluated by injecting seven replicates of 20 mg/L potassium bitartrate sample solution and expressed as the RSDs of RT and peak area. The intra-day variability was examined within one day in five independently prepared 20 mg/L potassium bitartrate samples, three injections each sample. The inter-day precision was carried out for three successive days using the sample solutions prepared for the intra-day variability, three injections each sample. The RT RSDs were <0.1% and the peak area RSDs ranged from 0.17% to 0.72% (Table 5). The assay exhibited good short-term precision.

Table 4. Recovery of potassium bitartrate (n=3)

Added (mg/L)	Peak area RSD	Method A		Method B	
		Total found (mg/L)	Recovery (%)	Total found (mg/L)	Recovery (%)
0	0.22	9.94	-	9.97	-
2	0.83	12.0	102	12.0	102
5	0.71	14.9	99.7	15.0	99.9
8	0.79	17.7	97.1	17.8	97.3
10	0.94	19.7	97.7	19.8	98.0
12	0.18	21.8	98.4	21.8	98.8

Table 5. Assay precision

Injection precision		Intra-day precision		Inter-day precision	
RT RSD	Peak area RSD	RT RSD	Peak area RSD	RT RSD	Peak area RSD
0.0	0.2	0-0.04	0.17–0.72	0.067	0.47

Robustness

Assay robustness was evaluated by measuring the influence of small variations (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature) on the RT, peak asymmetry, and resolution of the analyte on two column sets from two different lots. The peak asymmetry was evaluated following the USP formula. The resolution was determined relative to the previous peak in a chromatogram using the USP formula.

A standard mix (20 mg/L of USP potassium bitartrate, 2 mg/L of fluoride, 10 mg/L of chloride, 10 mg/L of nitrite, 10 mg/L of bromide, 10 mg/L of nitrate, and 20 mg/L of phosphate) was injected three times at each chromatographic condition. Table 6 summarizes the results. These results indicate the method was robust to both changes in chromatography conditions and column lots.

Conclusion

This study developed and validated an IC-based assay of potassium bitartrate to modernize the USP Potassium Bitartrate monograph. This method uses a high-performance anion-exchange column to separate tartrate in samples with an electrolytically generated potassium hydroxide eluent, and suppressed conductivity detection on an IC system supported by regulatory compliant chromatography workstation software. This 5 min method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods. Compared to the time-consuming assay in the USP Potassium Bitartrate monograph, this IC-based assay executed with an RFIC system offers a simple, accurate, and robust measurement of the analyte instead of titration with sodium hydroxide that the current USP technique uses and without manual mixing of hazardous reagents. Therefore, this method is an alternative to replace the existing potassium bitartrate assay in the USP's Potassium Bitartrate monograph.

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Table 6. Robustness of the IC-based assay for potassium bitartrate (n=3)

Parameter		Column 1						Column 2					
		RT (min)	Diff. (%)	Asym.	Diff. (%)	Resol.	Diff. (%)	RT (min)	Diff. (%)	Asym.	Diff. (%)	Resol.	Diff. (%)
Flow rate (mL/min)	0.70	3.96	-	1.00	-	3.93	-	3.72	-	1.04	-	4.03	-
	0.63	4.38	10.7	1.00	0.00	4.02	2.29	4.10	10.4	1.04	0.00	4.09	1.49
	0.77	3.63	-8.34	1.00	0.00	3.88	-1.27	3.39	-8.67	1.02	-1.92	3.98	-1.24
Eluent conc. (mM)	20	3.96	-	1.00	-	3.93		3.72	-	1.04	-	4.03	-
	18	4.69	18.4	0.98	-2.00	4.20	6.87	4.37	17.5	1.03	-0.96	4.29	6.45
	22	3.45	-12.9	0.99	-1.00	2.67	-32.1	3.24	-12.9	1.03	-0.96	2.70	-33.0
Column Temp. (°C)	30	3.87	-	1.10	-	3.62	-	3.72	-	1.04	-	4.03	-
	27	3.67	-5.16	1.08	-1.82	2.33	-35.6	3.52	-5.30	1.03	-0.96	3.61	-10.4
	33	4.09	5.53	1.08	-1.82	3.10	-14.4	3.91	5.33	1.04	0.00	4.75	17.9

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