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Modernization of USP Methods Using Ion Chromatography (IC) for Active Pharmaceutical Ingredients (API)

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ABSTRACT

Purpose: Modernization of USP methods using ion chromatography (IC) technology

Methods: Three IC methods used in two USP monographs were validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods[1]:

1st and 2nd: An isocratic method to assay sodium thiosulfate and a gradient method to determine the ionic impurities in sodium thiosulfate using IC with a Thermo Scientific™ Dionex™ IonPac™ AS12A column and suppressed conductivity detection.

3rd: An assay of zinc oxide using IC with a Thermo Scientific™ Dionex™ IonPac™ CS5A column and visible absorbance detection. Using pyridine-2,6-dicarboxylate (PDCA) eluent and 4-(2-pyridylazo) resorcinol) (PAR) as a post-column reagent, zinc is separated from transition metals and quantified using absorbance detection at 530 nm.

Results: All IC methods had good performance (linear, sensitive, accurate, precise, and robust) showing the value of IC to USP modernization.

INTRODUCTION

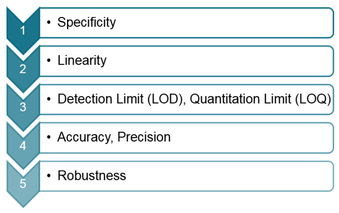
The United States Pharmacopeia (USP) is engaged in the ongoing challenge of obtaining the information needed to create and sustain up-to-date quality drug product monographs for U.S. Food and Drug Administration (FDA)-approved drugs, including over-the-counter (OTC) drugs. The USP also embarked on a global initiative to modernize many of the existing monographs across all compendia. As part of the USP modernization effort, ion chromatography (IC) methods have been proposed to replace many existing titration-based methods in monographs.

Sodium thiosulfate is an active pharmaceutical ingredient (API) approved by the United States Food and Drug Administration (FDA). Dosing sequentially with sodium nitrite, Sodium Thiosulfate Injection solution is used for the treatment of acute cyanide poisoning that is judged to be life-threatening[2]. Sodium thiosulfate is being tested as an extravasation antidote for cancer treatment to lessen the side effects of cisplatin (a chemotherapy agent)[3] in the Sodium Thiosulfate and Sodium Thiosulfate Injection revision monographs. An IC method has been proposed to replace existing titration-based assays for sodium thiosulfate, and another IC method has also been proposed for determining chloride, sulfate, and sulfite impurities in sodium thiosulfate; and sulfate and sulfite impurities in Sodium Thiosulfate Injection.[4,5]

Sunscreens are classified as OTC drug products in the U.S. Zinc oxide is one of the active ingredients approved (up to 25%) by the U.S. FDA) for use in sunscreens. Zinc oxide is unique among sunscreen ingredients in that it is a broad-spectrum blocker, protecting from both UVA and UVB radiation. An IC method has been proposed for addition to General Chapter <591>, Zinc Determination.[6] This IC method will replace existing titration-based assays in the zinc-containing drug product monographs (such as Zinc Oxide, Zinc Oxide Neutral, and Zinc Sulfate Ophthalmic Solution[7-9]). Here we demonstrates an IC method developed for the assay of zinc oxide in sunscreen based on the method in the proposed zinc oxide monograph.

This poster demonstrates the validation of these methods followed the guidelines in USP General Chapter <1225>, Validation of Compendial Methods (Figure 1).

Figure 1. Method validation steps.



MATERIALS AND METHODS

All samples and standards (use USP Reference Standard) were prepared in polymeric containers using deionized (DI) water (18 MΩ-cm resistance or better).

A Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system with dual pump was used for the tests.

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.2 SR4 was used for data collection and data analysis.

Sodium Thiosulfate

Sample Preparations

Sodium thiosulfate assay

Sample and standards: 100 mg/L sodium thiosulfate salt in DI water.

Sodium thiosulfate ionic impurity method: Sample

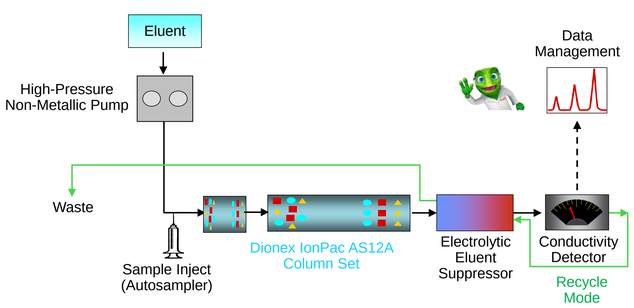
Sample: 2000.0 mg/L sodium thiosulfate salt in diluent (2.0 g/L of D-mannitol in water).

Standards: 0.04–10 mg/L of sodium chloride, sodium sulfite and sodium sulfite in diluent.

Table 1. Chromatography conditions for sodium thiosulfate test methods

IC methods	1-Sodium thiosulfate assay	2-Sodium thiosulfate ionic impurity method
Columns:	Dionex IonPac AS12A 4mm Analytical, 4 x 250 mm (P/N 046034) Dionex IonPac AG12A 4mm Guard, 4 x 50 mm (P/N 079801)	
Eluent:	Solution A: 2.7 mM Na_2CO_3/0.3 mM $NaHCO_3$ Solution B: 13.5 mM Na_2CO_3/1.5 mM $NaHCO_3$	
	Solution B, Isocratic	Solution A (-5–5 min), A to B(14–16 min), B (16–21 min), B to A (21–23 min), A (23–30 min)
Flow Rate:	1.5 mL/min	
Inj. Volume	25 µL in Push-Full mode	
Temperature:	30 ˚C	
Detection:	Suppressed conductivity, Thermo Scientific™Dionex™ AERS™ 500 (4mm) Anion Electrolytically Regenerated Suppressor, recycle mode, 106 mA current	
Backpressure:	~2500 psi	
Background :	~26 µS	~13 to 26 µS
Noise:	<5 nS/min	
Run Time	10 min	35 min (includes 5 min equilibrium time)

Figure 2. Illustration of an IC system with suppressed conductivity detection for the sodium thiosulfate tests.



Zinc Oxide

Sample Preparations

Four sunscreens samples with different formulations and zinc oxide contents were purchased from a local store.

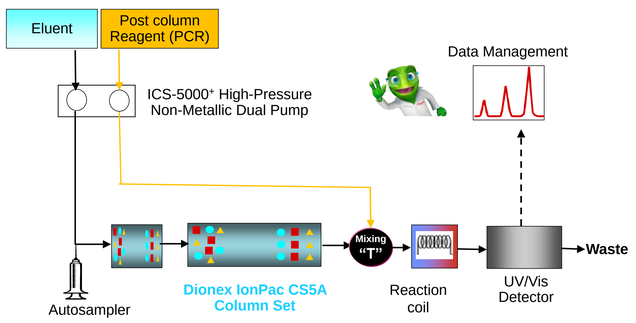
Method A (sample preparation method described in General Chapter <591>, Zinc Determination.[6]): Weigh 50 to 200 mg of sunscreen. Add 6 N hydrochloric acid to dissolve zinc oxide (about 2 mL per 100 mg sample), carefully transfer into a volumetric flask, and dilute with DI water to final volume. Filter through 0.2-µm syringe filter before analysis. If the sample concentration is too high, add diluent to achieve a concentration of about 15.0 mg/mL.

Method B: For water-resistant sunscreen, it is necessary to add acetonitrile to disperse the sunscreen before adding 6 N hydrochloric acid. Add 1 mL acetonitrile per 100 mg water-resistant sunscreen. Other steps are the same as Method A.

Table 2 The chromatography conditions of Zinc Oxide assay

IC method	Zinc oxide assay
Columns:	Dionex IonPac CS5A Analytical (2x250mm) (PN 052576)
	Dionex IonPac CG5A Guard (2x50mm) (PN 052836)
Eluent:	PDCA Eluent: 7.0mM Pyridine-2,6-dicarboxylic acid (also called dipicolinic acid) , 66.0 M Potassium hydroxide, 5.6mM Potassium sulfate, 74.0mM Formic acid in Deionized water
Flow Rate:	0.3 mL/min
Injection Volume	2.5 µL in Push-Full mode
Temperature:	30 ˚C
Post column Reagent (PCR):	0.5 mM 4-(2-Pyridylazo) resorcinol (PAR) monosodium salt, 1.0 M 2-Dimethylaminoethanol, 0.50 M Ammonium hydroxide, and 0.30 M Sodium bicarbonate
PCR flow rate:	0.15 mL/min
Detection:	Vis (530 nm)
System Backpressure:	<3000 psi
Run Time	12 min

Figure 3. Illustration of a IC system with UV/Vis detection for zinc oxide assay.

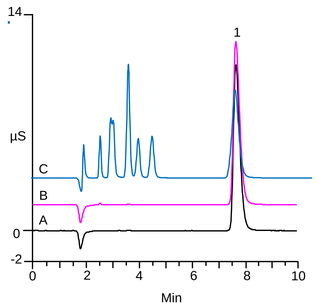


RESULTS

Sodium Thiosulfate

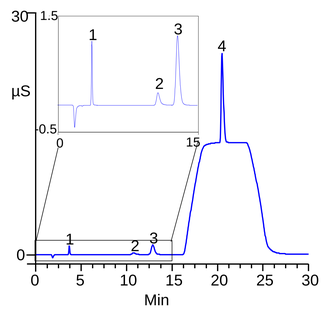
Separations

Figure 4. Chromatograms of sodium thiosulfate and separation from anions.



A: 100 mg/L of Sodium Thiosulfate USP reference
B: 100 mg/L of Sodium Thiosulfate sample
C: 50 mg/L of Sodium Thiosulfate USP reference in DI-water spiked with anions.(4 to 30 mg/L of Fluoride, Chloride, Nitrite, Bromide, Nitrate, Phosphate, and Sulfate)

Figure 5. Chromatogram of chloride, sulfite, and sulfate in sodium thiosulfate.



Peaks:		mg/L
1	Chloride	2.0
2	Sulfite	5.0
3	Sulfate	10.0
4	Thiosulfate	100.0

Calibration, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

Table 3. Calibration range, limit of detection (LOD), and limit of quantitation (LOQ) of the IC methods.

IC Method	Analyte	Calibration Standards (mg/L)	(r^2)	LOD (µg/mL)	LOQ (µg/mL)
Method-1 (Assay)	Sodium thiosulfate	0.2-200	0.999	0.05	0.17
Method-2 (Impurities)	Chloride	0.04–2	1	0.004	0.01
	Sulfite	0.2–10	0.9995–0.9998	0.09	0.3
	Sulfate	0.2–10	1	0.02	0.08

Accuracy and Precision

Table 4. Accuracy and precision of the IC methods.

IC Method	Analyte	Accuracy		Precision (RSD, %)		
		Spike (µg/mL)	Recovery (%)	Injection precision**	Intraday range**	Interday
Method-1 (Assay)	Sodium thiosulfate	100 in DI water	98-101	0.04-0.3	0.2- 0.6	0.8 (4 days)
		10 in 100 mg/L sample	103			
		25 in 100 mg/L sample	105			
Method-2 (Impurities)	Chloride	1*	99-100		0.2-0.8	4.1 (5 days)
	Sulfite	5*	90-96		1.0-3.3	1.9 (5 days)
	Sulfate	5*	108		0.04-0.9	1.9 (5 days)

* Impurities were spiked into 2000mg/L sodium thiosulfate sample
** n =3 injections/sample, n=3 or > 3 samples for each day.

Robustness – See Thermo Scientific Application Note: Assay of sodium thiosulfate and ionic impurities in sodium thiosulfate using ion chromatography.[11]

Zinc Oxide in Sunscreen

Figure 6. Chromatograms of zinc oxide standard and sunscreen samples.

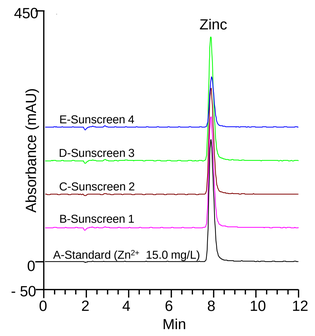


Table 5. Sunscreens from different manufacturers.

Sunscreen	SPF	Zinc Oxide (%)	Water resistance (min.)
Sunscreen 1	15	3.0	na
Sunscreen 2	45	8.0	80
Sunscreen 3	50	21.6	80
Sunscreen 4	50	5	40

Table 6. Calibration linearity, LOD, and LOQ of the IC method for zinc.

Standard(s) (µg/mL)	Calibration Type	r^2	Respond Factor (mAU*min/(µg/mL))	LOD (µg/mL)	LOQ (µg/mL)
15	One level, through origin	n. a	3.78	0.1	0.3
0.1-30	Linear, through origin	0.9995	3.76		

Table 7. Determination of zinc oxide in the four sunscreens.

Sample	Preparation Method	Zinc Oxide in the Sunscreen			Accuracy
		Label (%)	Measured	RSD (%)	Measured/label
Sunscreen 1	A	3.0	3.0	0.3% (n=18)	100 %
	B	3.0	3.0	0.8%(n=6)	101%
Sunscreen 2	A	8.0	0.19	-	2%
	B	8.0	8.1	1.0% (n=9)	101%
Sunscreen 3	A	21.6	2.7	1.4% (n=3)	13%
	B	21.6	20.7	0.3% (n=3)	96%
Sunscreen 4	A	5	5.0	0.3% (n=3)	99%
	B	5	5.0	0.7% (n=3)	99%

Robustness and more data – See Thermo Scientific Application Note: Determination of zinc oxide in sunscreen using ion chromatography with visible absorbance detection.[12]

The isocratic method to assay for sodium thiosulfate and an gradient method to determine the ionic impurities in sodium thiosulfate were validated. It is found that the assay method, a 10 min isocratic method, is linear (r^2=0.999) over the established analytical range of 0.2–200 mg/L. The method is sensitive (LOQ at 0.17µg/mL), accurate (intraday and inter-day accuracy 99–105%), precise (precision <0.6%), and specific for sodium thiosulfate determination. The sodium thiosulfate ionic impurities method, a 35 min gradient method, is linear over the established analytical range for impurities (chloride: 0.04–2.00 mg/L r^2=1.00, sulfite: 0.1-5.0 mg/L, r^2=0.9998 and sulfate: 0.5-10.0 mg/L r^2=1.00). We found the method was sensitive (LOQ of chloride =0.01, sulfite =0.2 and sulfate = 0.05 µg/mL), and accurate (recovery 90–108%).

Following the method described in the USP General Chapter <591>, zinc oxide in sunscreens was determined using IC with visible absorbance detection. Table 7 shows that adding acetonitrile to the sunscreen before adding 6 N hydrochloric acid is important for the preparation of water-resistant sunscreen samples. The method is linear ranging from 0.1 to 30 µg/mL (r^2=0.9995), accurate (96-101% of label), precise (RSD=0.3-1.4%).

Both methods demonstrated robustness in response to deliberate changes of method parameters[11,12]. Overall, both methods had good performance showing the value of IC to USP modernization.

CONCLUSIONS

Modernization of USP Methods Using IC for APIs was demonstrated by validation of three IC methods used in two USP monographs following the USP guidelines.

- Two IC methods using IC with conductivity detection are used to assay sodium thiosulfate and to determine the ionic impurities in sodium thiosulfate. It was found that both IC methods are specific, sensitive, linear, accurate, precise and robust.
- An IC method using IC with post-column derivatization and visible absorbance detection is used to determine zinc oxide in sunscreen. It was found that, with modification of sample preparation, the method is also specific, sensitive, linear, accurate, precise and robust.

As all IC methods had good performance, these experiments demonstrate that IC is both valuable and important to the USP modernization effort.

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