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Determination of zinc oxide in sunscreen using ion chromatography with visible absorbance detection

Authors
Hua Yang and Jeffrey Rohrer
Thermo Fisher Scientific
Sunnyvale, CA

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Dionex IonPac CS5A Column, pharmaceutical, USP monograph, monograph modernization, drug substance, drug product, sample preparation, post column derivatization, transition metal, ICS-5000+, ICS-6000

Goal

To develop an ion chromatography method for the assay of zinc oxide in sunscreen

Introduction

Sunscreens are classified as over-the-counter (OTC) drug products in the USA. Zinc oxide is one of the active ingredients approved (up to 25%) by the U.S. Food and Drug Administration (FDA) for use in sunscreens.[1, 2] Zinc oxide is a powdered mineral that sits on the outermost layer of the skin scattering, reflecting, and absorbing ultraviolet radiation. Zinc oxide is unique among sunscreen ingredients in that it is a broad-spectrum blocker, protecting from both UVA and UVB radiation.

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 FDA approved drugs, including OTC drugs. The USP also embarked on a global initiative to modernize many of the existing monographs across all compendia. As part of this USP effort, an ion chromatography (IC) method has been proposed for addition to General Chapter <591>, Zinc Determination.[3] 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[4-6]).

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Thermo Scientific™ Dionex™ IonPac™ CS5A ion-exchange columns were designed for the separation of transition and lanthanide metals. The transition metal (e.g. Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, and Fe^{2+}) separation uses a pyridine-2,6-dicarboxylate (PDCA) eluent.[7,8] After separation, transition metals are detected using a post column reagent (4-(2-pyridylazo) resorcinol) (PAR)) and absorbance detection at 530 nm. The Dionex IonPac CS5A column (USP L100) is proposed for the determination of zinc.

This application demonstrates an IC method developed for the assay of zinc oxide in sunscreen based on the method in the proposed zinc oxide monograph. This application uses a Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system and the Dionex IonPac CS5A column. Method validation followed the guidelines[9-12] in USP General Chapter <1225>, Validation of Compendial Methods[9] and the USP General Chapter <621> Chromatography[12] (Figure 1).

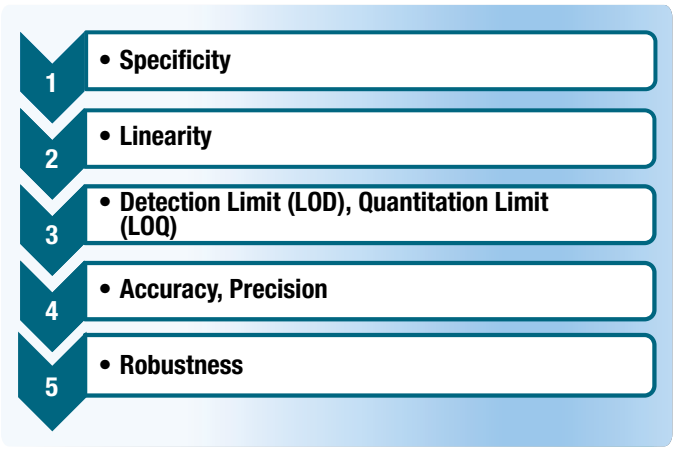

Figure 1. Validation steps

Experimental

Equipment

- A Dionex ICS-5000+ HPIC system* was used in this work. This system includes:
  - Dual Pump
  - Column Heater
  - Degasser
  - UV/Vis Absorbance Detector
- Thermo Scientific™ Dionex™ AS-AP Autosampler, with 250 µL syringe (P/N 074306), 1.2 mL buffer line assembly (P/N 074989), 2.5 µL injection loop
- Thermo Scientific™ Chromeleon™ 7.2.5 Chromatography Workstation

*This method can be run on any dual-pump Thermo Scientific™ Dionex™ ion chromatography system (Dionex ICS-3000, Dionex ICS-5000, or Dionex ICS-6000).

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Zinc oxide USP reference standard (Sigma-Aldrich®, P/N 1724747-2G)
- Zinc oxide (Sigma-Aldrich, P/N 14439-100G)
- Hydrochloride acid 37% (Sigma-Aldrich, P/N 258148)
- Thermo Scientific™ Dionex™ MetPac PDCA Eluent Concentrate (5X) (P/N 046088)
- Thermo Scientific™ Dionex™ MetPac PAR Post Column Diluent (P/N 046094)
- Thermo Scientific™ Dionex™ PAR Reagent (4-(2-Pyridylazo) resorcinol monosodium salt, monohydrate) (P/N 039672)
- Samples: Sunscreens were purchased from a local store.

Conditions

Table 1. Chromatography conditions

Columns:	Dionex IonPac CS5A Analytical (2 × 250 mm) (P/N 052576) Dionex IonPac CG5A Guard (2 × 50 mm) (P/N 052836)
Eluent:	PDCA Eluent: 7.0 mM pyridine-2,6-dicarboxylic acid (also called dipicolinic acid), 66.0 M potassium hydroxide, 5.6 mM potassium sulfate, 74.0 mM formic acid in deionized water
Flow Rate:	0.3 mL/min
Injection Volume:	2.5 µL in Push-Full mode
Temperature:	30 ˚C
Postcolumn Reagent (PAR):	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

Preparations of solutions and reagents

Diluent 0.2% (w/v) hydrochloric acid

Prepare 0.2% (w/v) hydrochloric acid by diluting 5.4 g of 37% hydrochloric acid to 1.0 L by DI water.

Hydrochloric acid, 6N

Prepare ~6 N hydrochloric acid by 1 to 1 dilution of the 37% hydrochloric acid with DI water.

Standard stock solution, 1500 µg/mL zinc

Transfer an appropriate portion of USP Zinc Oxide USP reference standard to a suitable volumetric flask, add 6 N hydrochloric acid to about 10% of the total volume to dissolve it, and bring to the final volume by slowly adding DI water. Keep stock standard solutions at 4 ˚C.

Standard solutions

Dilute the 1500 µg/mL standard stock solution to 15.0 µg/mL (1 mL to 100 mL) and 30 µg/mL (2 mL to 100 mL) using diluent. The 15.0 µg/mL zinc standard solution is used for assay and 30 µg/mL is further diluted to make 11 levels of standards for calibration (Table 2).

Mobile phase

Prepare mobile phase (7.0 mM pyridine-2,6-dicarboxylic acid (also called dipicolinic acid), 66.0 M potassium hydroxide, 5.6 mM potassium sulfate, 74.0 mM formic acid in DI water) by diluting the Dionex MetPac PDCA Eluent Concentrate (5X) with DI water. Filter through a 0.2 µm filter.

Post column complexation reagent

To prepare the post column reagent solution, 0.5 mM PAR, accurately weigh 0.127 g of PAR (4-(2-pyridylazo) resorcinol) into 1000 mL of Dionex MetPac PAR Post Column Diluent (1.0 M 2-dimethylaminoethanol, 0.50 M ammonium hydroxide, and 0.30 M sodium bicarbonate), dissolve, and filter through a 0.2 µm filter. Cover the bottle with aluminum foil.

Table 2. Preparation of zinc standards for calibration

Level	1	2	3	4	5	6	7	8	9	10	11
Zinc (µg/mL)	0.1	1	2	5	10	12.5	15	17.5	20	25	30
30 µg/mL Zinc (mL)	0.02	0.2	0.4	1	2	2.5	3	3.5	4	5	6
Diluent (mL)	5.98	5.8	5.6	5	4	3.5	3	2.5	2	1	0
Total (mL)	6	6	6	6	6	6	6	6	6	6	6

Robustness study

Following the guidelines of USP General Chapter <621> Chromatography,[12] the robustness of this method was evaluated by examining the retention time (RT) and peak asymmetry after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature). A standard containing 15 mg/L zinc was injected and system suitability parameters were evaluated. The same procedure was applied to two column sets from two different lots. The following variations were tested:

- Flow rate of eluent/post column reagent at 0.3/0.15, 0.33/0.165, and 0.27/0.135 (mL/min)
- Column temperature at 30 °C, 27 °C, 33 °C
- Eluent concentrations at the target, 10% higher and 10% lower of the target concentration (by diluting the Dionex MetPac PDCA Eluent Concentrate (5×, 4.5×, and 5.5×) using DI water.

Sample preparation

Four sunscreens samples with different formulations and zinc oxide contents were purchased from a local store (Table 3).

Table 3. Sunscreen samples

Sunscreen	SPF	Zinc Oxide (%)	Water Resistant (min)
1	15	3.0	na
2	45	8.0	80
3	50	21.6	80
4	50	5.0	40

Sample solutions

Sample preparation Method A

Weigh 50 to 200 mg of sunscreen into a tared container (vial or beaker) and record the weight. Add 6 N hydrochloric acid to dissolve zinc oxide (about 2 mL per 100 mg sample), rinse the container using diluent, carefully transfer into a suitable volumetric flask* (25 mL to 100 mL), and carefully dilute with DI water to the final volume (*or transfer into a clean and dry container, then determine the final volume by weight.) Filter through a 0.2 µm syringe filter before analysis. If the sample concentration is too high, add diluent to achieve a concentration of about 15.0 µg/mL.

Sample preparation 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.

Spiked sample solutions

Weigh 50 to 200 mg sunscreen into a 20 mL glass vial. Spike in zinc with either a 100 or 1500 µg/mL zinc standard stock solution. Thoroughly mix the zinc stock solution with sunscreen. Then follow the Method B procedure to prepare the spiked sample solution.

Results and discussion

Chromatograms

The zinc oxide content of a sunscreen product was determined using the Dionex ICS-5000⁺ HPIC system with a Dionex IonPac CS5A ion-exchange column. The determination of zinc is achieved by the formation of an anionic complex with a chromatographic eluent that contains pyridine-2,6-dicarboxylic acid (PDCA), separation of that complex, a subsequent post column complexation with 4-(2-pyridylazo)resorcinol (PAR), and detection of that complex by its absorbance at 530 nm. This IC method is selective for zinc as it is well separated from other transition metals (Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, and Fe^{2+}) with these conditions.[7,8]

Four purchased sunscreen samples were tested. Figure 2 shows the chromatogram of a standard prepared from the Zinc Oxide USP reference standard and chromatograms of the sunscreen samples. The retention time of the zinc complex is at about 7.9 min using the first of two columns used for this study, column A.

Columns:	Dionex IonPac CG5A, 2 × 50 mm and Dionex IonPac CS5A, 2 × 250 mm
Eluent*:	7.0 mM Pyridine-2,6-dicarboxylic acid (PCDA), 66.0 M Potassium hydroxide, 5.6 mM Potassium sulfate, 74.0 mM Formic acid in deionized water
Flow Rate:	0.3 mL/min
Inj. Volume:	2.5 µL
Column Temp.:	30 °C
Postcolumn Reg.:	0.5 mM 4-(2-pyridylazo)resorcinol (PAR) 1.0 M 2-Dimethylaminoethanol 0.50 M Ammonium hydroxide 0.30 M Sodium bicarbonate
Detection:	UV/VIS Absorbance at 530 nM
Peak	1. Zinc
Samples:	A. Standard (Zn^{2+} 15.0 µg/mL) B. Sunscreen 1 C. Sunscreen 2 D. Sunscreen 3 E. Sunscreen 4

*Prepared from the Dionex MetPac PDCA Eluent Concentrate

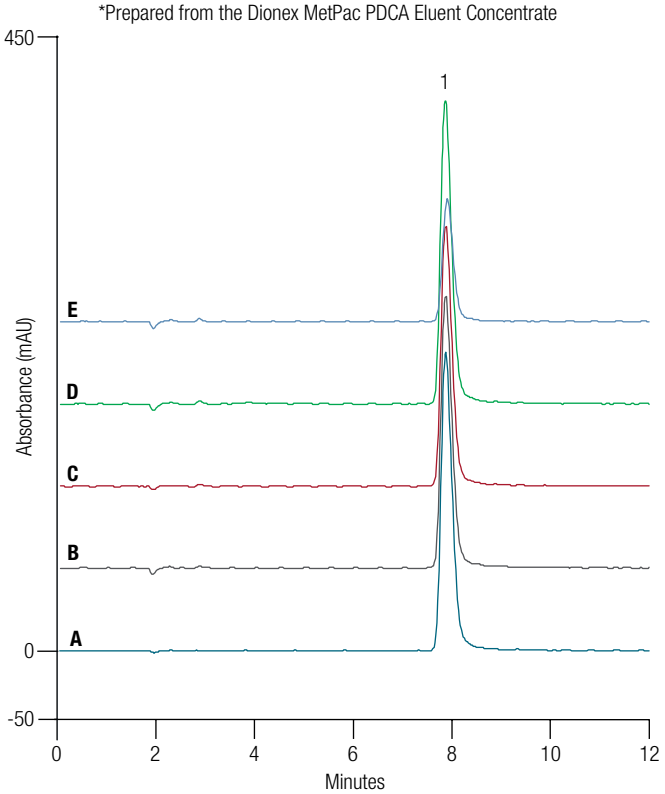

Figure 2. Chromatograms of a zinc oxide standard and sunscreen samples

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

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay. In this study, zinc was calibrated at 11 concentration levels ranging from 0.1 to 30 µg/mL. The results yield a linear relationship (Figure 3) of peak area to concentration with a coefficient of determination (r^2) of 0.9995 (Table 4).

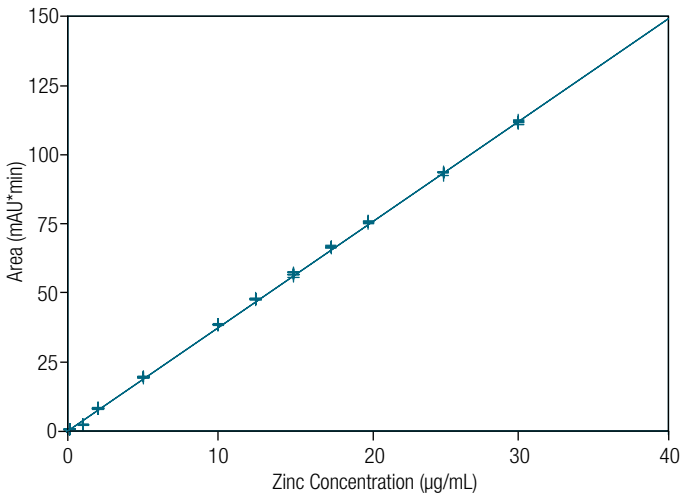

Figure 3. Calibration plot for zinc illustrating linearity

As a linear relationship has been established, the zinc concentration is calculated using a single zinc standard at about 15 µg/mL as follows:

$$cu = cs \times \frac{ru}{rs}$$

- *ru* = Peak area from the sample solution
- *rs* = Peak area from the standard solution
- *cs* = Concentration of zinc prepared from USP Zinc Oxide RS in the standard solution (µg/mL)
- *cu* = Concentration of zinc in the sample solution (µg/mL)

Table 4. Calibration linearity, LODs, and LOQs of zinc

Standard(s) (µg/mL)	Calibration Type	r^2	Response 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		

To determine the LODs and LOQs, 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 0.2 µg/mL Zn^{2+}. The LODs and LOQs were then determined by multiplying the signal-to-noise ratio 3x and 10x, respectively (Table 4). The IC method is sensitive with a LOD of 0.1 µg/mL (Figure 4) and a LOQ of 0.3 µg/mL.

Sample analysis, accuracy, and precision

The test results for the four sunscreen samples are listed in Table 5 and the results were compared to the labeled zinc oxide contents to assess method accuracy.

The sample solutions were first prepared following preparation Method A, which is similar to the method in the proposed USP zinc oxide monograph. However, the results were inaccurate for some sunscreen formulations. For non-water-resistant sunscreen 1 and lightly (40 min) water-resistant sunscreen 4, the test results are in agreement with the labeled value (99–100%). While for sunscreens 2 and 3, which are each 80 min water-resistant sunscreens, the measured zinc oxide contents were significantly less than the labeled value.

Columns:	Dionex IonPac CG5A, 2 × 50 mm and Dionex IonPac CS5A, 2 × 250 mm
Eluent*:	7.0 mM Pyridine-2,6-dicarboxylic acid (PCDA), 66.0 M Potassium hydroxide, 5.6 mM Potassium sulfate, 74.0 mM Formic acid in deionized water
Flow Rate:	0.3 mL/min
Inj. Volume:	2.5 µL
Column Temp.:	30 °C
Postcolumn Reg.:	0.5 mM 4-(2-pyridylazo)resorcinol (PAR) 1.0 M 2-Dimethylaminoethanol 0.50 M Ammonium hydroxide 0.30 M Sodium bicarbonate
Detection:	UV/VIS Absorbance at 530 nM
Sample:	A. Standard (Zn^{2+} 0.1 µg/mL)

*Prepared from the Dionex MetPac PDCA Eluent Concentrate

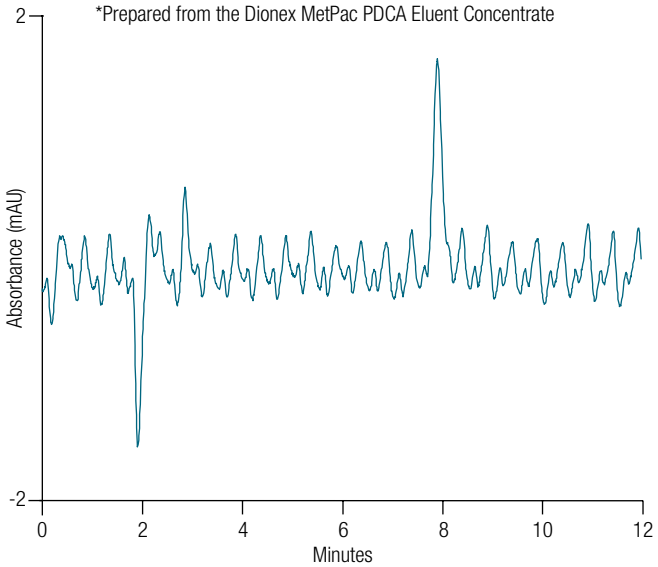

Figure 4. Chromatogram of a zinc oxide standard at LOD level

Table 5. Determination of zinc oxide in sunscreens

Sample	Preparation Method	Zinc Oxide in Sunscreen (%)			Accuracy Measured/Label
		Label	Measured	RSD	
Sunscreen 1	A	3.0	3.0	0.3 (n=18)	100%
	B	3.0	3.0	0.8 (n=6)	101%
Sunscreen 1*	A	3.0	3.0	0.2 (n=3)	99%
	B	3.0	3.0	1.1 (n=3)	96%
Sunscreen 2	A	8.0	0.19	-	2%
	B	8.0	8.1	1.0 (n=9)	101%
Sunscreen 2*	B	8.0	7.9	0.7 (n=3)	99%
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%

Two Columns were used. The rows marked with * are the results from the second column.

A better sample preparation method (Method B) was developed for all sunscreens. It was observed that water-resistant sunscreen is not miscible with aqueous 6 N hydrochloric acid (HCl) and this prevents the release of zinc oxide. To release zinc oxide from these samples an organic solvent addition is necessary. Different organic solvents were tested, and acetonitrile gave the best result. Sample preparation Method B was used to prepare all four sunscreen samples. The results show good agreement with the labeled values (96–101%).

Method accuracy was also validated by spiked recovery of Zn^{2+} standard solutions into non-water-resistant Sunscreen 1, and water-resistant Sunscreen 2. The results are listed in Table 6. Although sample preparation Method B was used here, it is observed that when a large volume (>0.5 mL) of aqueous Zn^{2+} standard solution spiking was used there was a low recovery from water-resistant sunscreen. This is likely due to the water diluting the acetonitrile and thus interfering with its ability to release zinc oxide from water-resistant sunscreen. When a small volume of the spike standard solution is used, the Zn^{2+} recovery is good (92% to 105%) for the water-resistant sunscreen. The Zn^{2+} recovery ranges from 94% to 102% for the non-water-resistant sunscreen, and spiking volume has no impact on the Zn^{2+} recovery.

In conclusion, when sample preparation Method B was used, this IC method is an accurate and precise method for zinc oxide determination in sunscreen.

Robustness

Method robustness was evaluated by measuring the influence of small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature) on the retention time (RT), peak asymmetry, and peak area RSDs on two column sets from two different lots. A standard containing 15 µg/L of Zn^{2+} was injected three times at each chromatographic condition. Table 7 summarizes the results for both columns. Although the RT of Zn^{2+} changes, the peak asymmetry of Zn^{2+} does not change for all conditions. Peak area RSD ranged from 0.15% to 0.71%, which passed the suitability test in the zinc oxide monograph (Peak area RSD < 0.73%). These parameter changes did not have impact on Zn^{2+} concentration determination. These results indicate the method is robust.

Table 6. Recovery data for Zn^{2+} spiked in sunscreen samples

Sunscreen	Sample Weight (mg)	Total V (mL)	Zn^{2+} SD Con. (µg/mL)	Zn^{2+} SD added (mL)	Recovery (%)
1	133.8	50	100	1	94
1	102.09	50	1500	0.1	99
1*	73	50	1500	0.2	101
1*	81.9	50	1500	0.2	102
2	105.98	50	100	1	-2379
2	79.43	50	1500	0.1	92
2	92.6	50	1500	0.5	-324
2*	53.5	50	1500	0.2	92
2*	60.7	50	1500	0.2	105

*Column B

Table 7A. Column A robustness of the IC-based method for zinc determination (injected sample: 15 µg/L of Zn^{2+}; average of three injections)

Parameter		Peak area (mAU*min)			Ret. Time (min)		Asym.	
		Average	RSD	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	0.33/0.165	46.0	0.25	9	7.18	-9	1.34	1
	0.3/0.15	50.5	0.47		7.90		1.33	
	0.27/0.135	56.2	0.27	11	8.76	11	1.34	1
Column Temp (˚C)	27	52.5	0.28	-4	8.03	2	1.33	0
	30	50.5	0.47		7.90		1.33	
	33	49.3	0.29	-2	7.76	-2	1.35	2
Eluent (Dilution of MetPac PDCA Eluent Concentrate (5×))	5.5	51.1	0.71	-1	8.81	12	1.34	1
	5	50.5	0.47		7.90		1.33	
	4.5	50.9	0.15	1	7.23	-8	1.34	0

Table 7B. Column B robustness of the IC-based method for zinc determination (injected sample: 15 µg/L of Zn^{2+}; average of three injections)

Parameter		Peak area (mAU*min)			Ret. Time (min)		Asym.	
		Average	RSD	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	0.33/0.165	45.6	0.50	10	7.97	-9	1.24	-1
	0.3/0.15	50.8	0.56		8.81		1.25	
	0.27/0.135	56.0	0.60	10	9.77	11	1.25	0
Column Temp (˚C)	27	52.5	0.60	-3	9.87	12	1.24	-1
	30	50.8	0.56		8.81		1.25	
	33	49.7	0.69	-2	8.04	-9	1.25	0
Eluent (Dilution of MetPac PDCA Eluent Concentrate (5×))	5.5	50.4	0.37	1	8.97	2	1.24	-1
	5	50.8	0.56		8.81		1.25	
	4.5	50.2	0.45	-1	8.65	-2	1.26	0

Conclusion

This study demonstrated an IC method developed for the assay of zinc oxide in sunscreen based on the proposed USP Zinc Oxide monograph. This work demonstrated that adding acetonitrile to the sunscreen before adding 6 N hydrochloric acid is important for the preparation of water-resistant sunscreen samples. The IC method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods. The method is linear, accurate, precise, and robust for the determination of zinc oxide in sunscreen lotions.

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