



Application Note AN-U-076

Zinc oxide assay as per USP General Chapter <591>

USP monograph modernization utilizing ion chromatography as a valid analysis method

SUMMARY

Zinc is an essential trace element which is used in different pharmaceuticals and supplements [1]. In the form of zinc oxide, zinc is an integral part of skin care creams, pastes, and supplements [2,3]. In order to meet the stringent quality standards for pharmaceutical products, manufacturers and laboratories must employ validated methods as from the United States Pharmacopeia and National Formulary (USP-NF). In the course of their modernization activities, USP-NF also updated the zinc monograph and replaced the existing

identification procedure with titration by ion chromatographic analyses. Ion chromatography (IC) qualified as a methodical approach for the zinc assay in General Chapter <591>, Zinc Determination [4]. The analysis involves separation of zinc using, e.g., L91 column material (Metrosep A Supp 10) followed by post-column reaction using 4-(2-pyridylazo)resorcinol (PAR) and subsequent detection at 530 nm. IC was validated according to USP procedures as a highly specific and accurate method to ensure product safety and quality.

SAMPLE AND SAMPLE PREPARATION

All analyses are performed with a solution of ultrapure zinc oxide. No additional sample preparation is

required unless stated in the individual USP monographs.

EXPERIMENTAL

A sample stock solution is prepared by hydrolyzing 0.1868 g of ultrapure zinc oxide powder in 10 mL of 6 mol/L hydrochloric acid. This solution is made up to 100 mL in a volumetric flask with ultrapure water. The zinc concentration of this sample stock solution corresponds to 1500 µg/mL. To prepare the final sample solution, the sample stock solution is diluted 1:100 with 0.2% (w/v) HCl to obtain a final concentration of 15 µg/mL zinc.

The working standard solution is prepared from a

certified 1000 µg/mL zinc standard.

Samples and standard solutions were injected directly into the IC (**Figure 1**) using an 889 IC Sample Center – cool. Zinc is separated from all other cations using L91 column material (Metrosep A Supp 10 - 250/4.0 and Metrosep A Supp 10 Guard/4.0) applying the MetPac™ PDCA (pyridine-2,6-dicarboxylic acid) eluent (concentrate dilution 1:5) followed by post-column reaction (PCR) with MetPac™ PAR and subsequent detection at a wavelength of 530 nm.



Figure 1. Instrumental setup including a 930 Compact IC Flex with a 947 Professional UV/VIS Detector Vario, a 800 Dosino for PCR delivery and mixing, and an 889 IC Sample Center – cool. Cooling can prolong sample stability.

The calibration was performed using a single 15 $\mu\text{g/mL}$ zinc standard injected six times. The sample was analyzed in duplicate.

Table 1. Requirements for IC method as per USP General Chapter <591>.

Column with L91 packing	Metrosep A Supp 10 - 250/4.0
Eluent	MetPac™ PDCA concentrate (dilution 1:5)
Flow rate	1.2 mL/min
Temperature	30 °C
Injection volume	10 μL
PCR reagent	PAR (0.12 g MetPac™ PAR reagent in 1000 mL MetPac™ PAR diluent)
PCR flow rate	0.6 mL/min
Detection	Vis: 530 nm after PCR

RESULTS

The IC assay for zinc content was validated according to USP General Chapter <591>, Zinc Determination [4]. Ultrapure zinc oxide was analyzed for its zinc content. The accuracy of the zinc determination was calculated as 99% (Figure 2).

All acceptance criteria were fulfilled, e.g., asymmetry (tailing factor) for the zinc peak was <2, or the relative standard deviation of the standard solutions was <0.73% (n = 6, USP requirement no more than (NMT) 0.73) (Table 2).

Table 2. Required acceptance criteria according to General Chapter <591>.

Parameter	Actual	USP requirement	Status
% RSD	0.582	NMT 0.73	Pass
Tailing factor	1.465	NMT 2.0	Pass
Result standard	98.9%	+/- 2%*	Pass
Results sample	99.2%	+/- 2%*	Pass

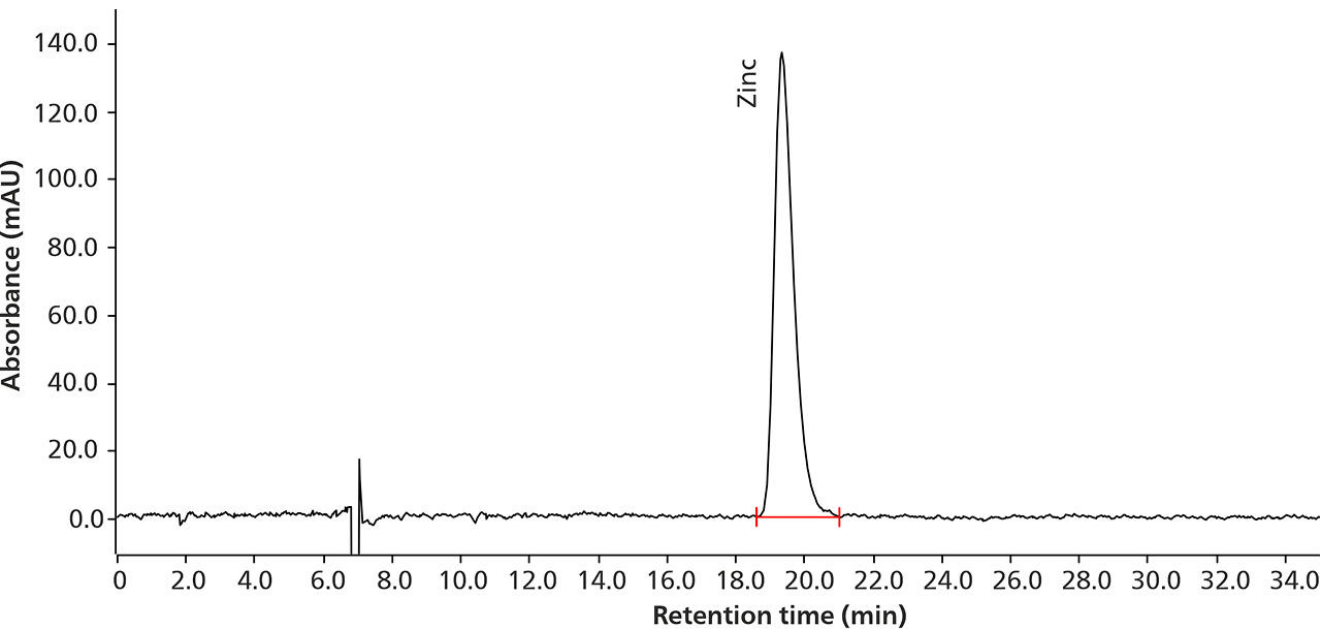


Figure 2. Chromatogram of zinc in a zinc oxide sample containing 14.865 µg/mL Zn (99.1% recovery of the nominal concentration).

CONCLUSION

Ion chromatography qualifies for the determination of zinc as per USP General Chapter <591>. Using the column material L91, zinc can be reliably determined in pharmaceuticals and other samples using IC with post-column reaction and UV detection. The high degree of automation possibilities for IC

systems from Metrohm (e.g., autosampler, Metrohm intelligent Partial Loop Injection Technique, Inline Dilution, etc.) and the traceability of all steps during analysis make it a user-friendly, efficient, and valuable analytical technique for pharmaceutical quality control processes.

REFERENCES

1. Royal Society of Chemistry. *Zinc*. The Royal Society of Chemistry's interactive periodic table. <https://www.rsc.org/periodic-table/element/30/zinc>.
2. Juch, R. D.; Rufli, T.; Surber, C. Pastes: What Do They Contain? How Do They Work? *Dermatology* **1994**, 189 (4), 373–377. <https://doi.org/10.1159/000246882>.
3. Maier, T.; Korting, H. C. Sunscreens - Which and What For? *Skin Pharmacol Physiol* **2005**, 18 (6), 253–262. <https://doi.org/10.1159/000087606>.
4. U. S. Pharmacopeia/National Formulary. <591> Zinc Determination. In *General Chapter*, USP/NF, Rockville, MD, USA. https://doi.usp.org/USPNF/USPNF_M99350_05_01.html.

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CONFIGURATION



930 Compact IC Flex Oven/ChS/PP/Deg

The 930 Compact IC Flex Oven/ChS/PP/Deg is the intelligent Compact IC instrument with **column oven**, **chemical suppression** and a **peristaltic pump** for suppressor regeneration and built-in **degasser**. The instrument can be used with any separation and detection methods.

Typical areas of application:

- Anion determinations with chemical suppression and conductivity detection
- Organic acids with ion-exclusion chromatography and inverse suppression



889 IC Sample Center – cool

The 889 IC Sample Center – cool is the appropriate automation solution when you have only a very small amount of sample. In comparison with the 889 IC Sample Center, it possesses in addition a cooling function and is thus the ideal sample changer for biochemically relevant or thermally unstable samples.



947 Professional UV/VIS Detector Vario SW

As an intelligent, single-wavelength detector, the 947 Professional UV/VIS Detector Vario SW permits a secure and reliable quantification of substances active in the ultraviolet or visible range. One wavelength can be selected.



Metrosep A Supp 10 - 250/4.0

The Metrosep A Supp 10 - 250/4.0 separation column is based on a high-capacity polystyrene-divinylbenzene copolymer with a particle size of only 4.6 μm . The longest column of the A Supp 10 product range offers the greatest selectivity and flexibility. Utilization of the MSM-HC is particularly recommended with longer chromatogram duration. Changes in temperature, flow and composition of the eluent also enable a wide variety of separations of anions on this separation column.

The Metrosep A Supp 10 - 250/4.0 has a very high capacity. It is suitable for samples with high ionic strength, for complex separation tasks and for analyzing samples in which great differences in concentration between the individual components are present.



Metrosep A Supp 10 Guard/4.0

The Metrosep A Supp 10 Guard/4.0 reliably protects the Metrosep A Supp 10 analytical separation columns against contamination. Thanks to the "On Column Guard System", the guard column is very easy to handle. The guard column screws easily and directly onto the analytical column. No tools are required.