An Executive Summary

Are You Ready?
What USP Monograph Modernization Means for Your Lab

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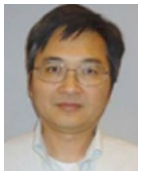

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Industry standards and analytical techniques that can improve selectivity and sensitivity.

Introduction

USP is a scientific nonprofit organization that sets standards for the identity, strength, quality, and purity of medicines, dietary supplements, and food ingredients manufactured, distributed, and consumed worldwide.

Pharmaceutical laboratories rely on monographs from the United States Pharmacopeia and National Formulary (USP–NF) to test drug substances and drug products. USP reviews existing monographs to evaluate their suitability with regard to advances in technology, safety, and to ensure they are up to date. The organization has embarked on a global initiative to modernize monographs across all of its compendia to include more selective and sensitive analytical methodologies.

The primary emphases of USP's monograph modernization efforts are on identification, assay, impurity analysis, and other tests. Revisions may include adding or updating identification tests, replacing outdated equipment, eliminating hazardous solvents, replacing non-specific tests, deleting non-value adding procedures, replacing organoleptic tests, updating Reference Standards, adding or combining impurity tests, and incorporating reagent tagging. Other monograph information may be updated as well such as chemical structures and names or packaging and storage conditions.

The modernized versions are designed to reflect "state-of-the-industry" practices that are suitable for their intended use and employ readily available, appropriate Reference Standards. All components should be clear, complete, and correct, with unnecessary tests removed. The updated monographs and general chapters must be added in a timely manner, while omitting those that are considered obsolete (see **Figure 1**).

Monograph Modernization Using Ion Chromatography

As part of the USP monograph modernization process, specific identification tests and assay procedures for zinc determination are repressed with the most specific and accurate method—ion chromatography (IC)—to ensure product quality and safety.

The analytical method for zinc determination has evolved from using wet chemistry methods to utilization of modern, state-of-the-art technologies such as IC systems.

When selecting an analytical method, the dynamic range and detection limit are both equally important for the assay procedure

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and the impurity test. The wide dynamic range and lower detection limit allow computation of both the active substance and stress impurity in a single injection or a single analytical run, which translates into cost savings because it reduces sample preparation and analysis time.

Inductively coupled plasma optical emission spectrometry (ICP-OES) and ion chromatography were both considered for modernizing the zinc family monographs (see **Figure 2**), but there were several reasons for choosing ion chromatography. First, ion chromatography provides better precision in measurements when compared with ICP-OES. ICP-OES also has numerous steps involved for measurement, each of which can contribute to variation. Another reason to choose ion chromatography for this method is because USP monograph users are very familiar with the technique from the high performance liquid chromatography (HPLC) assay procedure and impurity testing in terms of instrument parameter settings and system suitability requirements.

In one demonstration of the specificity of the ion chromatography method in General Chapter <591>, a standard solution mixture of 36 elements with 5.0 µg/mL each was analyzed. Zinc was well separated from its neighboring peaks (nickel and cobalt). Aluminum and other metals were not detectable by the method because either they do not react with the compressing agent in the mobile phase or they are not forming chromophore after post-column derivatization.

Magnesium monographs that currently specify manual titration using EDTA and visual indication methods are being updated with IC methods. Similarly, the fluoride family of monographs that uses ion selective methods for assays and wet chemistry methods for identification are being replaced with selective and sensitive IC methods for identification and assay. Updating the monographs is an ongoing process and based on input from the FDA. USP prioritizes its monograph modernization efforts.

Figure 1: Monograph modernization prioritization scheme.

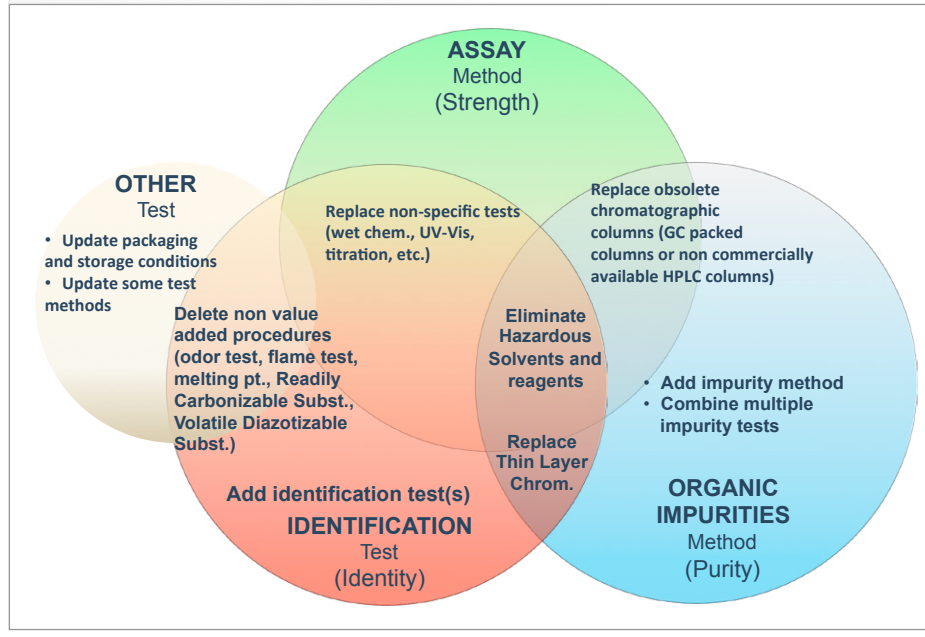
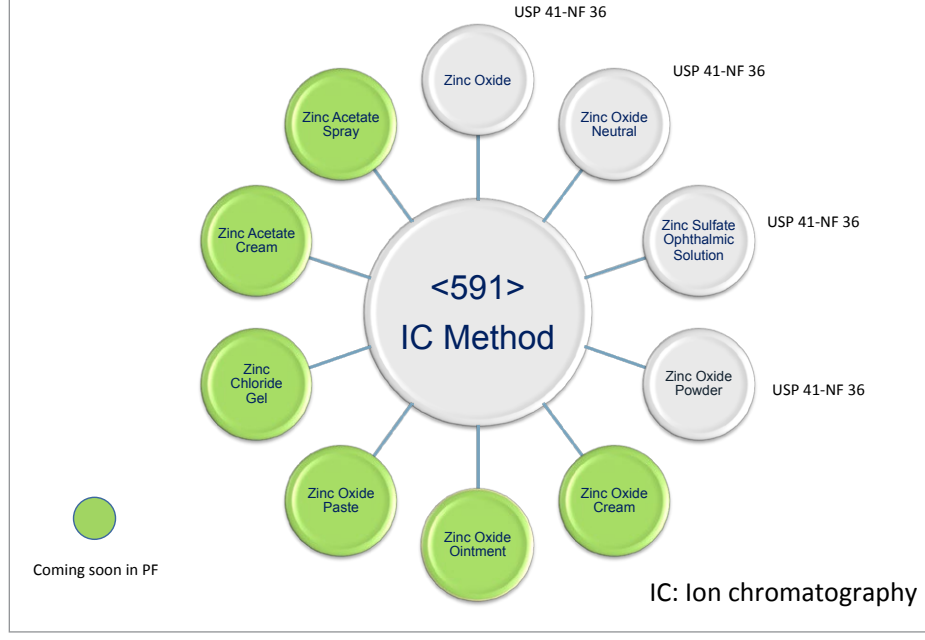


Figure 2: USP zinc family monographys modernization.



Drug Analysis Methodologies

Several analytical technologies can be used to evaluate drug substances and drug products. Some common analytical techniques are summarized and shown in **Figure 3**, which specifies their typical roles and contributions to

monographs. The broad applicability of ion chromatography makes it ideal for many drug assessments because it is the only test in the group that is amenable to the analysis of organics, inorganics, anions, cations, and metals and can be used for identification, assay, and impurity evaluation.

IC instruments can be interfaced with four different detectors: conductivity, UV-Vis, electrochemical, and mass spectrometry. Conductivity detectors are commonly used for anions, cations, aliphatic amines, and organic acids in conjunction with ion exchange, ion exclusion, and ion pair separation modes. Two types of conductivity detection are typically employed. Suppressed conductivity detection is the recommended methodology for anionic species, whereas non-suppressed conductivity detection offers a wide linear dynamic range for cationic species quantification such as lithium, magnesium, potassium, or calcium.

IC with conductivity detection is applicable for many of the USP General Chapters and individual monographs for identification and assays. The analysis can also be used for extractable and leachable ionic species detection as well as pharmaceutical-grade water. The fluoride, lithium, sodium, potassium, and magnesium families are all undergoing USP monograph modernization using IC with conductivity detection. USP General Chapters that incorporate IC-conductivity analysis are <191>, <345>, <476>, <1086>, <1230>, <1231>, <1663>, and <1664>. Utilization of suppressed conductivity for fluoride determination shown in **Figure 4** achieved excellent specificity in the analysis of anti-cavity mouthwash.

The second commonly used detector for IC is UV-Vis, which detects various ionic species that absorb light in those regions of the spectrum. With this technique, iodide or sulfide species can be directly determined after ion exchange (IEX) separation. Non-chromophoric ions such as zinc or other transition metals are often detected after post-column reaction (PCR) with a suitable complexing agent. The combination of an ionic exchange separation followed by the post-column reaction offers optimal selectivity and sensitivity.

IC-IEX-UV-vis is applicable for various USP General Chapters including the identification of extractable and leachable ionic species as well as pharmaceutical-grade water analysis. An example of this

Figure 3: Selective and sensitive drug and drug products analysis.

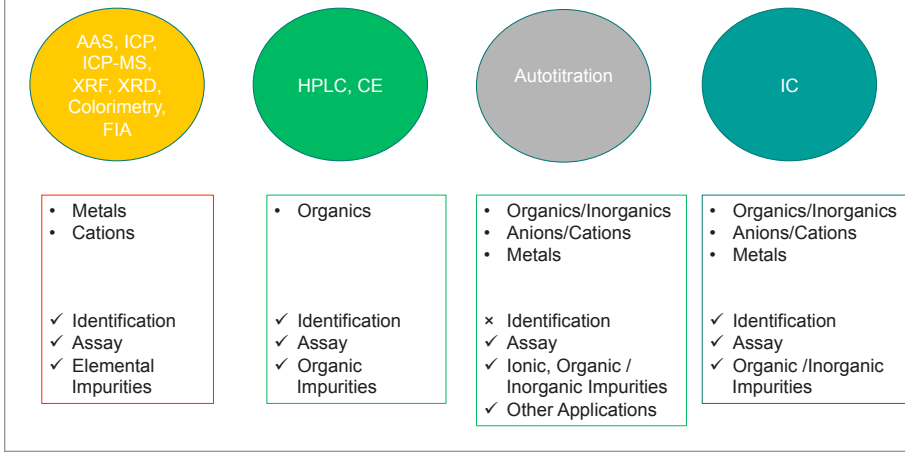
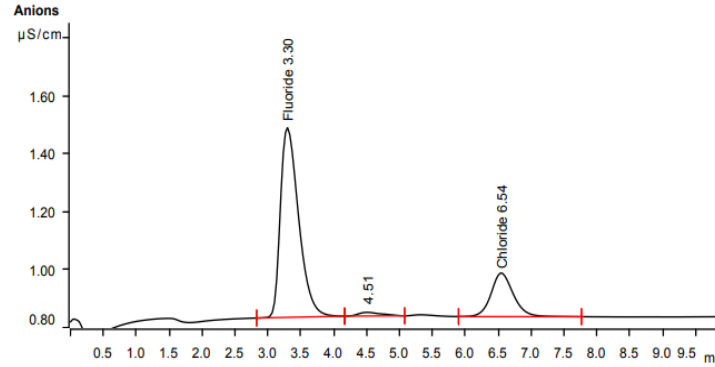


Figure 4: Fluoride family monograph modernization – Anti-cavity fluoride mouthwash sample.

Column (L91)	Metrosep A Supp 16 -100/4.0/ A Supp 16 Guard/4.0
Eluent	150 mg/L Na_2CO_3/1.0 mmol/L NaOH
Eluent flow	1.0 mL/min
Column temperature	30 °C
Sample volume	20.0 µL
Detection	Sequentially Suppressed Conductivity



Retention time	Component name	Area	Height	Resolution	Asymmetry	Concentration
min		(µS/cm) x min	µS/cm			ppm
3.297	Fluoride	0.2103	0.654	2.166	1.533	1.150
4.513		0.0052	0.013	3.310	1.450	invalid
6.543	Chloride	0.0589	0.150	invalid	1.141	1.500

Source: Metrohm Application Note, AN-S120.

mode of detection, widely used for USP General Chapter <591> for the zinc family monograph modernization, is shown in **Figure 5**'s zinc oxide assay. In addition to Chapter <591>, Chapters <191>, <1230>, <1231>, <1663>, and <1664> also involve this technology.

Ion chromatography combined with electrochemical detection is more appropriate for electrochemically active inorganic and organic species. It is particularly pertinent for USP Chapter <212> for oligosaccharides, as well as USP Chapter <476>, which involves organic impurities in drug substances and drug products. Additional Chapters employing electrochemical detection are <191> and <1086>.

Common applications of electrochemical detection include cyanide, sulfide, amino acids, sugar, and sugar alcohol analysis. An example of adrenaline quantification in an EpiPen® using Direct Current (DC) measurement technology with a glassy carbon electrode is displayed in **Figure 6**.

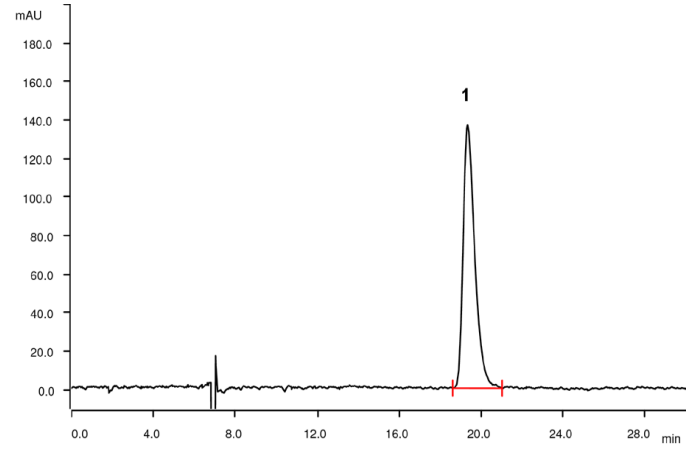
A mass spectrometer may also be hyphenated with an ion chromatograph. This combination is highly selective as well as sensitive. It is particularly advantageous for the analysis of complex pharmaceutical formulations.

Titration

In some cases, titration is the only technology that is suitable for pharmaceutical analysis. One very common use is for the determination of the residual acidity or basicity of a raw material, drug substance, or drug product for which there are no alternative analytical techniques available.

Figure 5: Zinc Oxide assay.

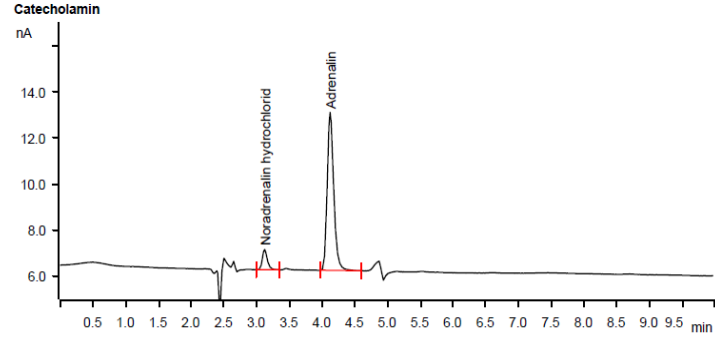
Column (L91)	Metrosep A Supp 10 - 250/4.0
Eluent	7.0 mmol/L dipicolinic acid 66.0 mmol/L potassium hydroxide 5.6 mmol/L potassium sulfate 74 mmol/L formic acid (pH 4.2)
Eluent flow	1.2 mL/min
PCR	0.5 mM 4-(2-pyridylazo) resorcinol monosodium salt, 1.0 M 2-methylaminoethanol, 0.50 M ammonium hydroxide, and 0.3 M sodium bicarbonate in water
PCR Flow	0.6 mL/min
Column temperature	30 °C
Sample volume	10.0 µL
Detector	UV-VIS/530nm



	Cation	Concentration [mg/L]	Recovery [%]
1	Zinc	14.83	98.9

Figure 6: Active ingredients: Antibiotics using ampherometric detection.

Sample	1:25000 diluted Adrenalin solution from an "EpiPen" injector
Eluent	38 mmol/L NH_4SO_4 + 3 mL/L conc. acetic acid
Eluent flow	1.0 mL/min
Column	C18 – (250 x 4.0mm)
Column temperature	35 °C
Sample volume	20 µL
Detector	Ampherometric – DC Mode
Working Potential	+800 mV
Working Electrode	GC



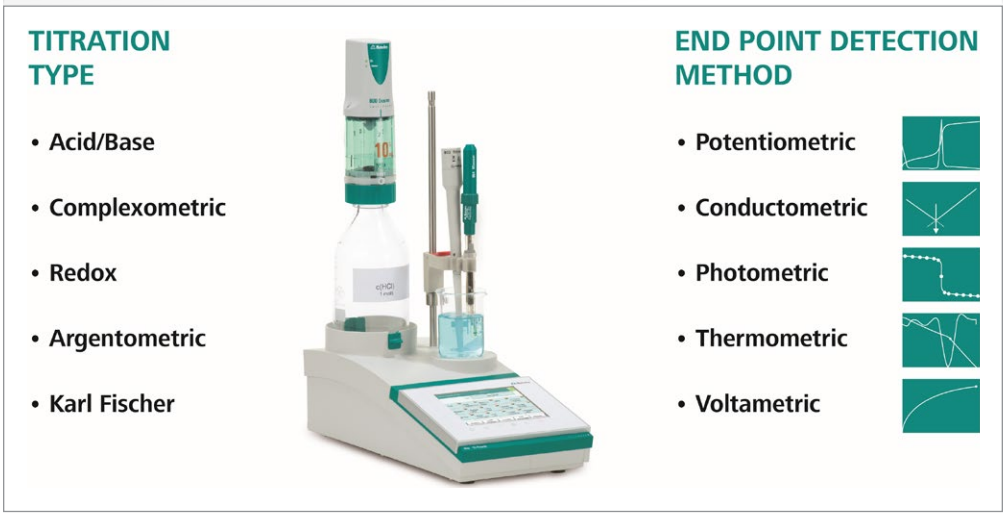
Peak number	Component name	Height	Area	Concentration	Retention time
		nA	(nA) x min	µg/L	min
1	Noradrenalin hydrochlorid	0.875	0.076	92124.421	3.12
2	Adrenalin	6.870	0.789	1053233.932	4.12

Source: Metrohm White Paper, WP-019.

Conventional titrations, chosen according to the substance being analyzed, are acid/base, complexometric, redox, argentometric, and Karl Fischer.

USP General Chapter <541> elaborates on the various types of titration and how they are connected to several hundred individual monographs. The majority of the procedures refer to manual titration, which has many shortcomings. Although manual titration is fast and easy to implement, it has signifiant limitations due to its user dependency. The system is controlled entirely by the operator, including the addition of titrant and evaluation of the end point. The data is handled manually as well. All of these steps have the potential to introduce error to the results.

Figure 7: Autotitration for drug and drug products analysis.



In contrast, autotitration eliminates the possibility of error, as the operation of the system is well-controlled with no need for manual intervention. It automatically detects the end points and reports the values, which makes method development, validation, and transfer much more straightforward compared to manual titration. In addition, the built-in intelligence offers a fully traceable result by recording the serial numbers of the electrode and dispensing devices. Thus, data integrity is far superior to manual titration.

Various autotitration end point detection methods are available for different types of analytes. Based on the chemistry involved, potentiometric, conductometric, photometric, thermometric, or voltammetric detection methodology may be chosen to maximize the specificity or sensitivity (**Figure 7**). USP modernization for titration of pharmaceuticals is focused on moving from manual titration to autotitration in order to ensure data integrity and eliminate manual intervention.

As previously mentioned, there are times when titration is the only technique that can successfully provide critical answers. In these cases, modernization means moving from manual titration to autotitration and other possible detection methods to improve selectivity. For example, the potassium carbonate and bicarbonate assay is not amenable to IC methods due to species conversion under IC separation conditions. As a result, ion chromatography is incapable of speciation and can only measure total carbonate. However, potentiometric titration can easily differentiate the carbonate and bicarbonate end points, thereby allowing a simple calculation of the bicarbonate impurities in the sample. Thus, autotitration can be a simpler and faster solution to do a selective and sensitive analysis of this formulation.

Another example in which the sample matrix poses a challenge are the citrate and the sulfuric citric acid assays. USP General Chapter <345> includes the assay of citrate and citric acid. However, this procedure is only relevant for measuring the total citrate because everything is converted into total citrate under IC conditions. Alternatively, autotitration offers a simple, faster way to speciate and quantify the available citric acid concentration in the formulation.

Conclusion

Techniques for the identification, assay, and impurities analysis of pharmaceuticals are chosen based upon the desired information, as well as the type of sample and its matrix. Potential complications to consider include species conversion, degradation, and sample handling that could result in erroneous data. With its widespread applicability, ion chromatography offers selective and sensitive analysis with four detector configurations to cover a large variety of USP Chapters and monographs.

Nevertheless, there are some instances in which IC conditions limit the success of speciation for assays and impurity analyses. In many of those cases, titration is recommended. Autotitration is particularly suitable for those applications because it offers data integrity while minimizing human intervention. USP monograph modernization includes updating IC and titration methods to reflect advances in both of these essential technologies.