Karl Fischer Titration of Pharmaceutical Products with AQUASTAR®

Pyridine-free Reagents

Introduction

Water content of both active pharmaceutical ingredients and excipients directly impacts the potency, efficacy, stability, and licensed shelf life of the final medicinal products of which they are a part. This makes accurate measurement of water content critical to the pharmaceutical development and production processes.

Since its invention by Dr. Karl Fischer in 1935, Karl Fischer (KF) analysis has progressed from an esoteric laboratory procedure to a widely accepted instrumental method routinely used in the pharmaceutical industry. The method is specified in the leading Pharmacopoeias, including the USP, and the Ph. Eur. It is estimated that nearly 500,000 KF determinations are performed daily around the world.

Many advances in titrator instrumentation have further improved the accuracy and reproducibility of KF analyses, and enabled true automation by use of such devices as sample changers, ovens, and homogenizers. Yet, despite the development of a variety of state-of-the-art instrumentation, the key to successful KF analysis remains the fundamental understanding of sample and KF reagent chemistries.

Challenges Posed by Pharmaceutical Samples

A number of pharmaceutical samples present certain challenges to conventional KF methods and reagents. These can be categorized into three general classes:

Reactivity Issues

Certain compounds will undergo interfering side reactions either with methanol or iodine. For example, the side reactions of aldehydes and ketones with methanol that produce acetals and ketals, also generate water. On the other hand, penicillins often contain various hydrolysis products which are oxidized by iodine. The following table lists a number of compound types known to interfere with KF by reacting with iodine.

Antimony oxide	Ascorbic acid	Arsenites
Arsenates	Boric acid	Tetraborates
Calcium oxide	Calcium peroxide	Carbonates
Copper (I) salts	Disulfites	Iron (III) salts
Hydrazine	Hydrazine derivatives	Hydroxides
Hydrogen carbonates	Lead (II) oxide	Magnesium oxide
Mercaptans/thiols	Mercury oxide	Nitrites
Selenites	Silanols	Silver oxide
Sulfites	Tellurites	Thiosulfates
Tin (II) salts	Zinc oxide	Zinc peroxide

Solubility Issues

Some pharmaceuticals have limited solubility in methanol. For example, atropine sulfate and calcium folinate are only partially soluble in methanol, as are many creams, ointments, and syrups.

Issues Related to pH

KF titration is pH-dependent reaction. The optimum reaction rate is observed in the pH range of 5.5 to 8. Below that range, titration proceeds very slowly, while above that range, the reaction rate increases due to

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interfering side reactions, leading to sluggish endpoints and erroneously high results. A number of pharmaceutical samples can change the pH of the KF solvent to either the acidic or basic extremes, and require buffering.

Strategies for Successful KF of Pharmaceutical Samples

Reactivity issues may be resolved by using specialty reagents, performing titrations at low temperatures, or by employing auxiliary equipment. For example, aldehydes and ketones may be titrated by using special methanol-free coulometric or volumetric reagents. Certain slow-occurring side reactions may be kinetically "frozen out" by performing titrations using a special jacked titration cell connected to a water bath circulating ice water, brine, or dry ice/methanol mix. Water content of compounds that undergo side reactions with KF reagents that cannot be suppressed using alternate solvents or thermal techniques may be determined by using a KF Oven coupled with a KF titrator. Here, moisture is released from the sample in the KF Oven, at temperatures between 100-300°C, and is then carried into the titration cell using a dry inert carrier gas, such as nitrogen.

Similarly, solubility issues may be overcome by using different specialty reagents and titration aides, performing titrations at elevated temperatures, or by employing auxiliary equipment. Co-solvents such as hexanol, decanol, chloroform, and xylene are frequently used to increase the solvent capacity of KF reagents in the titration cell. Alternatively, specially pre-formulated KF reagents incorporating one or several of these solvents are commercially available. Certain compounds will not dissolve in any co-solvents suitable for KF, yet will release all of their water content in the presence of formamide. Either in conjunction with use of formamide, or as a stand-alone technique, titration at elevated temperature using a jacked titration vessel can also be used for samples which are difficult to dissolve or extract moisture from at room temperature. Moisture content of substances that release their water only at very high temperatures and do not allow for effective extraction using formamide can be determined by using a KF Oven coupled with a KF titrator, as described above.

Finally, samples that create a highly acidic or basic environment for KF must be buffered to enable accurate water quantification. In the case of acidic samples, weak bases, such as imidazole, have proven to the most effective titration aides while in the case of basic samples, buffering using a weak acid, such as salicylic acid, is typically recommended.

The tables below provide examples of reagents and techniques used to analyze several pharmaceutical samples by volumetric and coulometric KF, respectively.

Sample	Titrant	Solvent System	Temp.	Sample Size
Acetyl salicylic acid	CombiTitrant 5	CombiMethanol	R/T	0.50 g
Atropine sulfate	CombiTitrant 5	CombiMethanol	50°C	0.50 g
Calcium folinate	CombiTitrant 5	CombiMethanol + Salicylic Acid	50°C	0.25 g
Erythromycin	CombiTitrant 2	CombiMethanol + Imidazole	R/T	0.25 g
Ointment, anti-itch	CombiTitrant 2	CombiMethanol + Chloroform + Formamide	R/T	0.10 g
Penicillin-G-potassium	CombiTitrant 5	CombiMethanol + Salicylic Acid	R/T	1.00 g
Sunblock cream	CombiTitrant 5	CombiSolvent Fats	R/T	0.05 g

Sample	Cell Type	Anolyte	Catholyte	Sample Size
Benzoin	Fritted	Coulomat AK	Coulomat CK	0.50 g
Benzylpenicillin procaine	Fritted	Coulomat A	Coulomat C	0.05 g
Eucalyptus oil	Fritless	CombiCoulomat Fritless	N/A	0.25 g
Monobutylamine	Fritless	CombiCoulomat Fritless + Salicylic Acid	N/A	0.25 g
p-Phenetidine	Fritted cell	Coulomat A	Coulomat C	0.20 g
PEG 1000	Fritted cell	Coulomat A	Coulomat C	0.50 g
Vitamin B2	Fritted cell	Coulomat A	Coulomat C	0.02 g

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