Direct CNO-X Analysis of Solid Forms

X-ray fluorescence spectroscopy enables direct CNO-X elemental analysis of solids with minimal sample preparation and short analysis times.

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INTRODUCTION

The pharmaceutical industry is a fast-paced environment, requiring frequent sample testing throughout development and manufacturing to ensure product purity and quality. In a setting where speed is crucial, employing analytical methods that require minimal sample preparation while offering rapid analyses is highly advantageous. This is readily achieved through analysis of solids via X-ray fluorescence spectroscopy (XRF), which can provide the elemental composition of solids measured directly, with minimal to no sample preparation.

The primary elements of interest for a particular pharmaceutical application are carbon, nitrogen, and oxygen (CNO), all of which can be quantified easily with XRF instrumentation. CNO composition is critical to the determination of stoichiometry in the solid form selection processes – salt selection and polymorph, hydrate, solvate evaluations. Elements beyond CNO can be measured by XRF systems due to the broadly applicable mechanism underlying XRF measurement technology. This makes the technology useful in many different pharmaceutical applications, such as analysis of catalyst residues in APIs and intermediates, evaluation of catalysts for poison element impurities, and as a tool to analyze for blend uniformity of key excipients and certain APIs in formulations.

This webcast summary covers the basics of XRF as applied to solid analysis as well as experimental results from representative pharmaceutical samples.

PHARMA APPLICATIONS FOR X-RAY FLUORESCENCE SPECTROSCOPY

Although XRF has yet to be widely used as an analytical technique in the pharmaceutical manufacturing sector, there are several crucial areas where the approach is advantageous compared to more time-consuming techniques. XRF can reveal both

qualitative data and quantitative elemental sample content for evaluation of pharmaceuticals throughout the development and production processes. In pharmaceutical applications, these types of sample information are key in analyzing catalyst residues, detecting catalyst poisons, confirming proper salt formation and stoichiometry, and evaluating excipient blend uniformity.

Given its advantages, it is somewhat surprising XRF is not more widely used in pharmaceutical manufacturing. XRF offers features not found in other instrumentation systems common to production environments. The system's level of elemental specificity, coupled with the non-destructive nature of XRF analysis, allows for nearly complete sample recovery. As a non-destructive technique that permits direct measurement of both solids and liquids, the minimal sample preparation simply requires materials to be added to the instrument sampling vessel. Once samples are ready for analysis, XRF systems provide real-time results, fast enough for making quick decisions about production workflows, synthesis procedures, and other processes dependent upon quantitative or qualitative results. The high accuracy and reproducibility of data obtained from XRF measurements are readily suitable to pharmaceutical products, with systems capable of analyzing elemental species on a linear range as low as ppm levels to 100 mass% content, and even sub-ppm for certain elements.

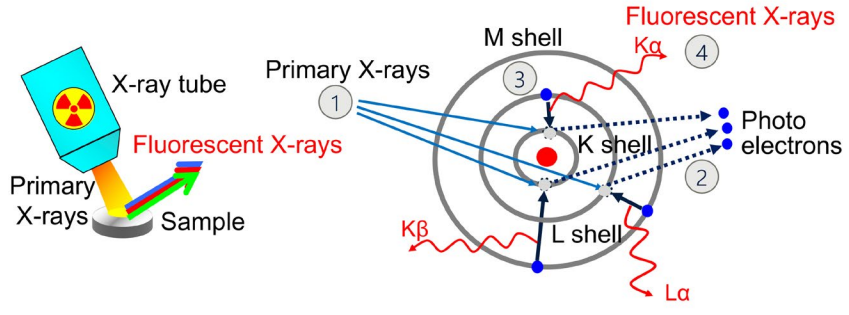
After initial calibration, XRF systems can maintain quantitative calibrations for years, owing to the instrumentation's stability. Correspondingly, the capabilities of XRF render the instrumentation suitable across the spectrum of potential applications, such as quality control, process control, exploration research, research and development, and material screening.

BASICS OF XRF: THEORY AND INSTRUMENTATION

The mechanism behind XRF spectroscopy is highly reproducible and yields signals with high specificity for each element. An incident beam of primary X-rays is generated by an X-ray tube, where the beam is aimed at the sample. The primary X-rays then strike the inner-shell electrons of the sample's constituent atoms, exciting the inner-shell electrons with enough energy to be ejected from the atom as photo electrons.

With inner-shell electrons kicked out, the higher energy outer-shell electrons will fall into the vacancy left by the ejected photo electrons achieving a more stable state. As the outer-shell electrons drop to the lower energy shells, characteristic fluorescent X-rays are emitted and are energetically equivalent to the energy difference between the inner and outer shells involved in the process, as illustrated in **FIGURE 1**.

FIGURE 1: Generation of X-ray Fluorescence



① Primary X-rays strike inner shell electrons.
② An inner shell electron is kicked out as a photo electron.
③ An outer shell electron transfers to fill the vacancy.
④ Fluorescent x-ray is emitted with equivalent energy difference.

XRF platforms suited to every level of analytical need are available commercially. The systems offered by Rigaku include both wavelength-dispersive and energy-dispersive models, with the wavelength-dispersive instrumentation featuring either single-channel simultaneous or multi-channel simultaneous detection capabilities. Highly flexible, sequential wavelength-dispersive XRF systems from Rigaku are available in both bench-top and floor-standing high-power formats. For high-power systems, such as the Rigaku ZSX Primus series and Simultix 15, the elemental measurement range can fully cover the elements from beryllium to uranium when appropriately equipped. The more compact bench-top XRF systems, like the Rigaku Supermini200, have power sufficient for detecting the elemental range beginning with oxygen and up to uranium.

Though XRF systems are still relatively unknown in the broader pharmaceutical manufacturing market, some Rigaku systems are currently used by pharmaceutical manufacturers, including the Primus IV, Primus IVi, Primus III+, SM200, and NEX DE platforms. The Primus IV and Primus IVi are free-standing systems that both feature 4-kW power, which is preferable for applications requiring detection of the lighter elements. A 3-kW system, the Primus III+, is also available as a free-standing instrument that retains similar capabilities to the IV and IVi, but with slightly lower wattage. For environments that may not need a high-power system and where a bench-top model would suffice, the Rigaku SM200 and NEX DE are economical XRF options. For this demonstration, the Primus IV was used.

XRF ANALYSIS AND SAMPLE PREPARATION

The method development process for quantitative XRF analysis follows the same general process as necessary for other types of instrumentation. Standard samples with known analyte concentrations are analyzed as a series across a concentration range. The standard samples can be prepared from essentially any organic species, which allows operators the flexibility to create methods according to their sample type needs rather than being constrained by instrumental requirements.

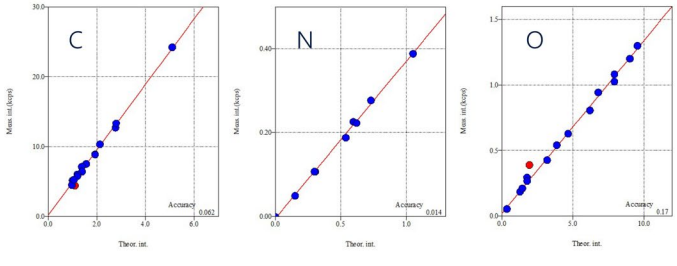
As the standard samples are measured, the XRF signal at each concentration will serve as a point within the calibration. Linear regression analysis of the XRF signals as a function of analyte concentration will then generate a calibration curve for the method being developed.

As an example of XRF system calibration linearity, a set of 15 organic small molecules were analyzed according to carbon, nitrogen, and oxygen (CNO) mass%. The signals were normalized to the respective CNO compositions (ignoring the H component) and used to construct calibration curves for each of the three elements, as shown in **FIGURE 2**. Since

FIGURE 2: CNO - X Analysis with Small Samples

Representative Standards Used

Element Standard	C	N mass%	O
L-Tryptophan	68.765	14.581	16.654
Folic Acid	54.046	23.220	22.734
Arginine	45.015	34.997	19.987
EDTA	38.982	9.092	51.926
Cellulose	47.393	0	52.607
Lysine	54.563	21.210	24.227
Malic Acid	37.523	0	62.477
Glutamine	44.137	20.589	35.275
Sorbitol	42.881	0	57.119
Glycine	34.304	20.002	45.693
Aspirin	62.814	0	37.186
Vitamin C	42.881	0	57.119
Citric Acid	39.154	0	60.846
Kapton	70.985	7.526	21.490
Cholesterol	95.299	0	4.702



Data is normalized w/o H

hydrogen atoms are too light to be measured by XRF and are essentially transparent to X-ray beams, the normalization process is necessary to obtain correct stoichiometries. The calibration analytes in this example included amino acids, weak acids, vitamins, polymers, carbohydrates, drugs, and cholesterol, but together yielded linear calibration curves and thereby demonstrate the suitability of XRF across molecular classes.

The sample preparation process for Rigaku XRF systems has been minimized to a simple procedure requiring only the sample, a disposable and compressible boric acid shell sample holder, and a simple hydraulic press, as shown in **FIGURE 3**. Two stainless steel dies, known as a pancake press, sandwich the consumable boric acid shell made with a sample well that accepts a small volume of powdered analyte. The powdered analyte and shell are compressed by force in a standard manual or automatic press, compacting the sample enough that the material is flat and contained. Preparing samples with compression eliminates the need for binding additives, allowing for analysis without possible binder interference and the recovery of samples free from undesirable additives.

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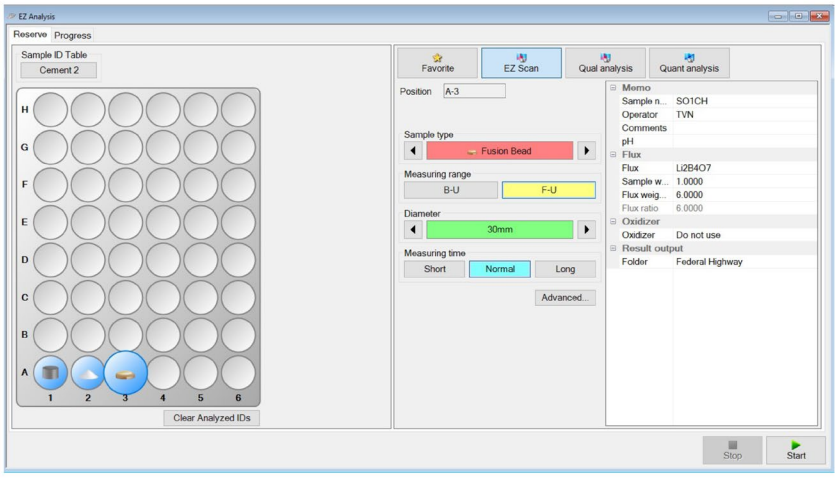
Although the most commonly used boric acid shells have 13 mm wells and use sample sizes of 50-200 mg, smaller well sizes are available to accommodate sample masses below 50 mg. Compression to a 5-mm diameter is suitable for 10-50 mg of powder even for CNO analysis. The instrument diaphragm diameter can be used as small as 500 µm, but this is unsuitable for analysis of light elements such as carbon, nitrogen, and oxygen which produce weaker X-rays.

Once ready for analysis, operators can set up the sample queue with Rigaku's streamlined software interface (see **FIGURE 4**). Samples are loaded into a grid array, with analytical parameters specifically designated for each individual sample. Different methods can be used for each sample rather than requiring use of a single method for each batch. Some of the pertinent parameters used in the

FIGURE 3: How Do I Prep the Samples?

FIGURE 4: Simple Sample Queue Setup



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sample queue interface include specifying the sample type, measurement range, sample diameter, and measurement time, according to each sample position and can be pre-set.

EXAMPLE APPLICATION OF XRF CNO-X ANALYSIS TO PHARMACEUTICALS

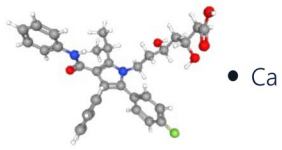
The CNO calibration obtained from the set of 15 standards was used as the analytical method for characterizing two common small molecules: lactose and aspirin. Although lactose was not included as a standard within the calibration curve, XRF analysis of a lactose sample demonstrated great stoichiometric accuracy. The theoretical CNO composition of lactose was 45.02% carbon, 0% nitrogen, and 54.98% oxygen, which was matched closely by the experimental results of 45.52% carbon, 0.2% nitrogen, and 54.28% oxygen.

The XRF experimental results for aspirin were similarly close to theory, where the theoretical composition was 62.81% carbon, 0% nitrogen, and 37.19% oxygen, and experimental values were 62.95% carbon, 0.16% nitrogen, and 36.88% oxygen. One point of note is that the lactose and aspirin samples were not analyzed under conditions controlling for the presence or absence of hydrates, which can impact stoichiometric measurements.

For CNO-X measurements wherein the X includes elements beyond CNO, XRF systems can be configured to detect these heavier elements using a built-in method library. The library methods are appropriate for obtaining semi-quantitative results that can be used in concert with CNO results gathered using the in-house system calibration. Inclusion of the heavier elements along with CNO is especially valuable in pharmaceutical analysis of salt species, such as the drug atorvastatin, which is produced as a calcium salt.

The theoretical elemental composition of the atorvastatin calcium salt, shown on the left in **FIGURE 5**, including

FIGURE 5: CNO-X Results



Atorvastatin

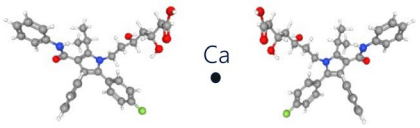
33	2	5	1	1
C	N	O	Ca	F
mass%	mass%	mass%	mass%	mass%
70.3	5.0	14.2	7.1	3.4

Calcium Salt

CNO-X Analysis

Semi-Quant "X" Ca and F

C	N	O	Ca	F
mass%	mass%	mass%	mass%	mass%
72.1	5.5	15.9	3.7	3



66	4	10	1	2
C	N	O	Ca	F
mass%	mass%	mass%	mass%	mass%
72.9	5.2	14.7	3.7	3.5

Hemi-Calcium Salt

determination of calcium and fluorine in addition to CNO, did not appear to match well with the semi-quantitative composition obtained for the sample shown in the middle of **FIGURE 5**. However, closer scrutiny of the two sets of values shows the experimental calcium mass% is roughly half that of the theoretical quantity, which is indicative that atorvastatin may be forming a hemi-calcium salt. Calculation of the theoretical hemi-calcium composition, shown on the right in **FIGURE 5**, was then found to match well with the experimental stoichiometry. With just a few minutes of XRF analysis, the salt composition was determined without requiring in-depth assessment of the sample's physical properties to identify the species — leaving the sample recoverable for other uses.

CONCLUSIONS

X-ray fluorescence (XRF) spectroscopy is an appealing instrumental approach for pharmaceutical manufacturing due to the speed, simplicity, accuracy, and precision of analyses it offers. Research and production workflows also can benefit from the streamlined sample preparation and analysis that allow data collection in essentially real time with nearly full sample recovery from the non-destructive technique. Moreover, the suitability of XRF for a broad array of small molecules, as well as small sample masses, is ideal for the pharmaceutical production environment.