



Acetylsalicylic acid (ASA)

Pipette

Quartz discs

S2 PICOFOX

Result

Lab Report XRF 92

S2 PICOFOX TRACE ELEMENT ANALYSIS APPLIED TO AUTHENTICITY AND PURITY CONTROL OF PHARMACEUTICAL SAMPLES

Introduction

The characterization of brand drugs and their generics is an essential task in pharmaceutical analysis. Several analytical techniques are available for the determination of grain size distribution, morphology and phase distribution. To date, the determination of chemical drug composition has not turned out satisfactorily due to sample preparation requirements. Additionally, the elemental composition of raw materials and end products must be analyzed regularly to comply with public and internal regulations.

The applicability of common X-ray fluorescence (XRF) techniques for these analytical tasks is limited due to the high demand on sensitivity, the oftentimes small sample amount available and the lack of suitable calibration standards. The use of other methods for trace element analysis like atomic adsorption spectroscopy (AAS) or inductively-coupled plasma optical emission spectroscopy (ICP-OES) is often restricted by sample quantity, the necessity for sample digestion and matrix-related difficulties.

With total reflection X-ray fluorescence spectroscopy (TXRF) sample quantities of less than a milligram can be

analyzed for their trace element distribution. As this method is based on internal standardization, no standards for external calibration are needed. Furthermore, the S2 PICOFOX TXRF spectrometer does not require any consumables, gases or cooling media.

The topic of this report is the application of TXRF spectroscopy for authenticity analysis of pharmaceutical samples. Different commercially available acetylsalicylic acid based drugs were tested by qualitative and quantitative TXRF analysis.

For this purpose the following drugs were analyzed:

- Aspirin 500 mg, Bayer AG, Germany
- Aspirin +C (fizzy tablet), Bayer AG, Germany
- ASA 100 mg, Hexal AG, Germany
- ASA, Ratiopharm GmbH, Germany
- "Aspirin", no name, U.S.A.

To confirm the suitability of the S2 PICOFOX for purity control a typical raw material, sodium chloride (NaCl), was spiked with different trace amounts of arsenic to determine the detection limits of this contaminant.

Measurement parameters

The spectrometer is equipped with a low-power micro focus Mo tube (37 W) and a 30 mm² XFlash® Silicon Drift Detector. The samples were measured at a high voltage setting of 50 kV, 750 µA for 1000 s.

Sample preparation

All samples were ground manually in an agate mortar to obtain a grain size of < 75 µm.

A qualitative fingerprint analysis was done by weighing sample powder amounts of about 60 mg and placing the sample material into plastic vessels. For slurry preparation 2.5 ml of an aqueous 1% Triton X100 solution were added. Triton X100 is a common detergent for adjusting the viscosity of solutions. Applied for slurry preparation in TXRF analysis, it enhances the quality of the specimens.

After thorough homogenization 10 µl of each slurry were placed on quartz glass sample carriers, dried in a desiccator and subsequently measured.

Quantification of TXRF measurement data is done by means of internal standardization. For this purpose, slurries were prepared in the same way as for the qualitative analysis. As an internal standard approximately 30 µl of a 100 mg/l Se-solution (Merck) were added.

After homogenization 10 µl of the slurries were pipetted onto quartz glass sample carriers, dried in a desiccator and measured at the same conditions as the qualitative samples.

For contamination control about 60 mg of NaCl (p.A., As < 0.4 mg/kg) were dissolved in 1 ml ultrapure water and spiked with As solution to reach final concentrations in the range from 0 to 4 mg/kg.

Figure 1: Typical TXRF spectrum of three different ASA drugs: Aspirin Bayer (green), Aspirin Hexal (blue) and „no name“ ASA (red).

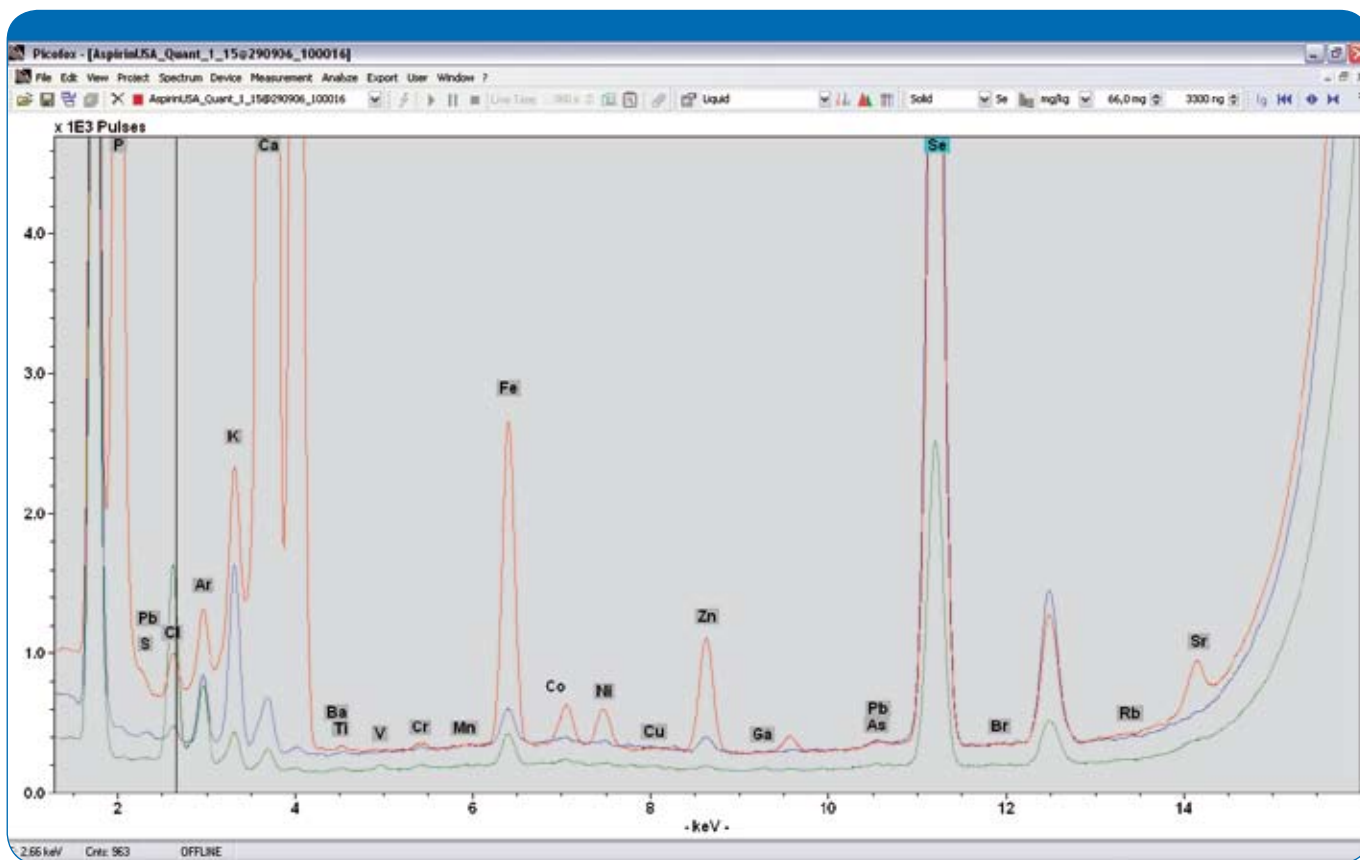
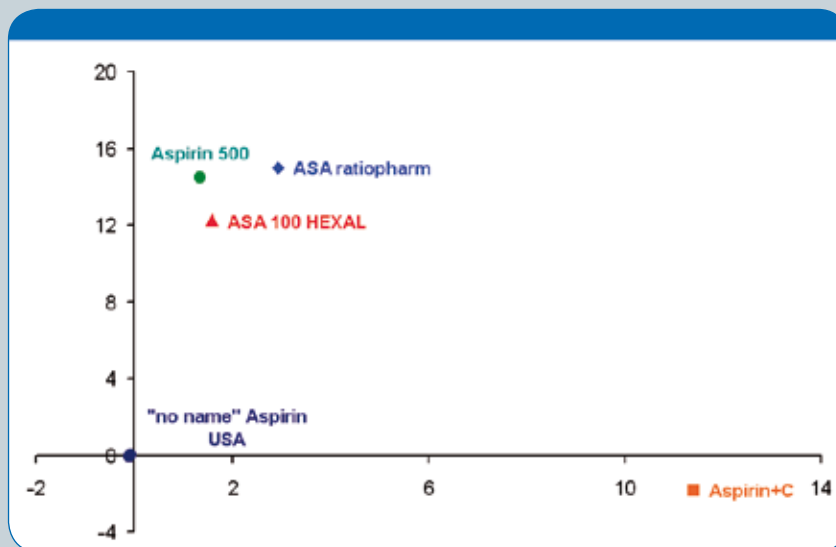


Table 1: Example of different element concentrations of Aspirin drugs.

ASA brand	Cr	Zn	Pb
Aspirin 500	n.d.	0.583	0.079
Aspirin + C	1.097	0.668	0.839
ASA 100 Hexal	n.d.	0.73	0.163
ASA ratiopharm	n.d.	25.31	0.405
ASA USA	0.826	7.659	n.d.
All values in mg/kg			

Figure 2: Correspondence analysis plot of the element composition of five different Aspirin drugs.



Results - ASA authenticity test

Fig. 1 shows the spectra of the different ASA samples. P, Ni, Cu and Sr are present in the „no name“ Aspirin and V, Cr, As and Se are present in the Hexal sample. Elements like Cl, K, Ca, Fe, Zn and Pb show differences above one order of magnitude across all five drugs. For some representative elements (Cr, Zn, Pb) the differences in concentrations are shown in Table 1.

The difference of the ASA drugs can be compared after a data transformation of the specific element contents. This correspondence analysis performed by a so-called Biplot analysis gives a visual interpretation of the chemical composition (Fig. 2). The size of each data point represents the error of the TXRF analysis. Obviously, all drugs can be differentiated reliably.

The reproducibility of the measurements was proven by a tenfold preparation and analysis of the sample „Aspirin, no name, U.S.A.“ Table 2 shows the average values of the most important elements. The standard deviation exceeds an acceptable range only in case of concentrations close to the detection limits.

Table 2: Reproducibility of TXRF measurements of drugs, sample „Aspirin, no name, USA“, average of 10 measurements

Element	Average	Std. dev. (mg/kg)	Std. dev (%)	LLD (mg/kg)
P	1175	66	3.7	5.8
Cl	25	5.8	23.5	2.3
Ca	5712	77	1.3	1.9
Fe	39.2	3.9	9.9	0.21
Zn	0.77	0.25	32.7	0.11
Sr	1.5	0.14	9.3	0.09
Pb	0.15	0.18	117.7	0.10

Results - sodium chloride purity

The measured NaCl concentrations show a very good correlation with the spiked As concentrations ($R^2 = 0.9972$, Fig. 3). Furthermore, the measurements indicate the presence of about 0.2 mg/kg arsenic in the unspiked reagent, which fits well to the specified value of < 0.4 mg/kg.

The detection limits for all measurements are shown in Table 3. For all tested solutions the detection limits for As are significantly below 0.1 mg/kg, which is a factor of 4 below the specified value.

Conclusions

Differences in element composition - especially for P, S, Cl, Ca, Fe, Zn, Sr, Pb - of five different aspirin drugs were accurately determined by the S2 PICOFOX TXRF spectrometer.

The results of the drug authenticity test were visualized by a correspondence analysis plot. This quantitative fingerprint analysis delivered valuable information which, in combination with other methods like XRD, can lead to an unambiguous identification of brand products.

In addition, the S2 PICOFOX has been proven for the purity control of raw, solid chemicals, which are commonly used during production of pharmaceuticals. The As contamination of NaCl was accurately measured by achieving detection limits down to 70 ± 14 ppb.

The specific benefits of TXRF for the analysis of pharmaceuticals in comparison to other analytical methods are:

- The required sample amount is extremely low (below mg).
- The sample preparation is fast and simple enough for routine application.
- Instrument calibration and the use of calibration standards is not required.
- All detectable elements are analyzed simultaneously.

Figure 3: Correlation of NaCl purity measurements with spiked As contaminations

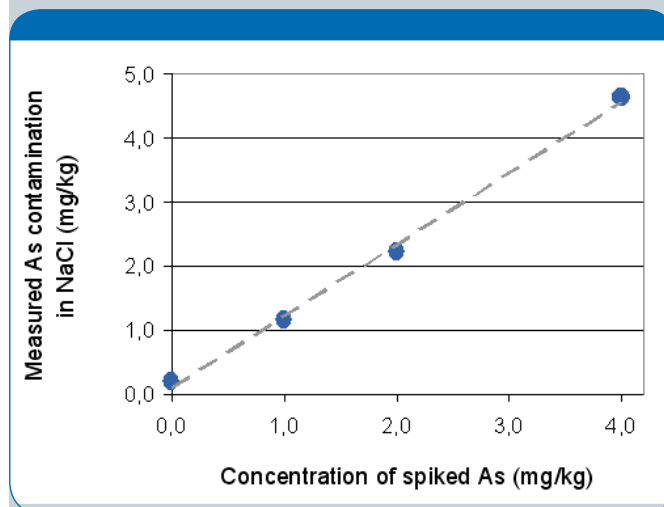


Table 3: Detection limits of As in NaCl

Spiked As cont. (mg/kg)	Detection limit (mg/kg)
0	0.089
1	0.067
2	0.066
4	0.054
Average	0.069

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