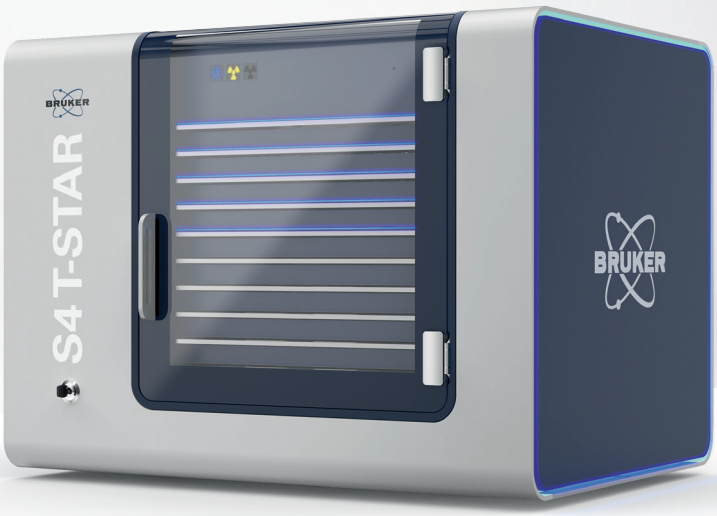

TXRF

Biopharmaceutical production: Rapid nutrient and contaminant analysis of cell culture media by TXRF spectroscopy

Application Note # XRF 468

Introduction

Cell cultures offer a wide range of applications in research and industry. In particular mammalian cells are used in most industrial cell culture systems.

- Biopharmaceuticals (pharmaceutically active proteins and nucleic acids) are produced by mammalian cell culture systems.
- In research, tissue cells can be cultured for therapeutic purposes.
- Elementary cellular processes can be studied on cells without having to carry out classical animal experiments.

Total Reflection **X**-**R**ay **F**luorescence (TXRF) spectroscopy is a well established method for the analysis of biological and environmental samples [1]. However, a systematic investigation of cell culture media has not been published yet.

This lab report describes different preparation methods for cell culture media (CCM) and the rapid and accurate TXRF element analysis of nutrients and contaminants.

Figure 1
Cell cultures in research

Cell culture media

In order to ensure the function of all physiological processes of the cell culture also ex vivo, the natural environment of the cells has to be imitated in defined media. Standardized media, such as Dulbecco's Modified Eagle's Medium (DMEM), are used for cell cultures.

Cell culture media (CCM) contain amino acids for protein biosynthesis, vitamins and inorganic salts for metabolic processes and carbohydrates such as glucose (typically 1 g/l or 4.5 g/l) for energy production. Nutrient elements (P, Zn, Cu, Fe) as well as trace elements (Se, Mn) serve as nutrient sources for the cells or are required to maintain the membrane potential and the osmotic pressure. Some ions also serve as co-factors in enzymatic reactions.

In order to achieve reproducible results with a cell line, the composition of the medium must always be kept constant and free from contamination. Nutrients and trace elements are fed via highly concentrated feed solutions. Small fluctuations in the added components and minimal contaminations may reduce the yield of the biopharmaceutical product and, in the extreme case, leads to necrosis of the cells.

Objective

The aim of the present study was to develop a preparation method for the rapid and accurate element TXRF analysis of cell culture media (CCM) in order to control nutrient elements and to identify undesirable contaminants.

Sample preparation

DMEM

The measurement of cell culture media with high sugar concentrations requires the development of a dedicated preparation method. Commercially available DMEM (1 g/l or 4.5 g/l glucose) was used to optimize the preparation and to determine reproducibility and detection limits for trace elements.

To optimize the preparation of sample layers on quartz carrier discs different smoothing agents (1% Triton X-100, ultrapure ethanol, or 0.3% polyvinylalcohol PVA) were added to the DMEM. Four different sample series with cell culture media dilutions from 1:5 up to 1:100 of the media were prepared and tested.

Procedure 1: In a micro reaction tube 1 ml of a mixture with the following components were prepared:

- DMEM: 10 to 200 µl
- Smoothing agent: 100 µl
- Internal standard Sc: 10 µl
- Filling up with water to 1 ml (resulting in dilution factors from 1:5 to 1:100)
- After careful homogenization 10 µl of each sample were prepared on a quartz disc.
- The samples were dried on a heating plate at 30°C.

Procedure 2: In a micro reaction tube 1 ml of a mixture with the following components were prepared:

- DMEM or HP-CCM: 700 µl
- Ethanol: 290 µl
- Internal standard V (1 g/l): 10 µl
- After careful homogenization 10 µl were transfered to a quartz disc.
- The samples were dried on a heating plate at 30°C.

Pb	Mn	Se	Ni	Cr	Cd
5	20	5	10	15	300
10	50	10	20	30	500
20	100	20	50	50	1000
50	200	50	100	100	2000
100	500	100	200	200	4000

Table 1
Element concentrations of the prepared solutions for spike experiments in µg/l.

HP-CCM

Modern high performance cell culture media (HP-CCM) are used for mammalian cells of the following types:

- Human Embryonic Kidney (HEK)
- Chinese Hamster Ovary (CHO)
- Baby Hamster Kidney (BHK)
- Invertebrate (IKZ)

In high performance cell culture media the amount of glucose is significantly higher, e.g. 8 g/l or 20 g/l in feed media, respectively.

The HP-CCM samples were prepared according procedure 2.

Spiked HP-CCM

For an accurate and reliable quantification of trace metals in the low ppb range spiked samples were prepared. Three micro reaction tubes with 1 ml of a HP-CCM were each spiked with five concentrations of different elements given in Table 1.

Measurements

All measurements were performed for 1000 s with a S4 T-STAR® spectrometer at the conditions shown in Table 2. The spiked samples were measured with Mo-K (Mn, Se, Pb, Ni) , W-L (Cr), and W-Brems (Cd) excitation.

Parameter	Specification
X-ray tube 1	Mo target, 50 kV, 1000 µA
X-ray tube 2	W target, 50 kV, 1000 µA
Excitations	Mo-K 17.5 keV; W-L 8.4 keV
Detector	XFlash SDD, 60 mm^2, energy resolution < 145 eV
Measurement time	1000 s

Table 2
Measurement parameters of the S4 T-STAR®

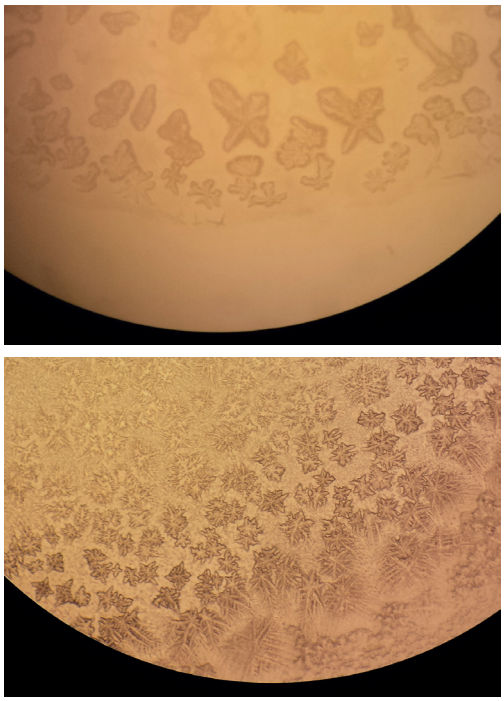

Figure 2
10-fold magnified images of DMEM on quartz sample carriers after dilution with water (top) and ethanol (bottom).

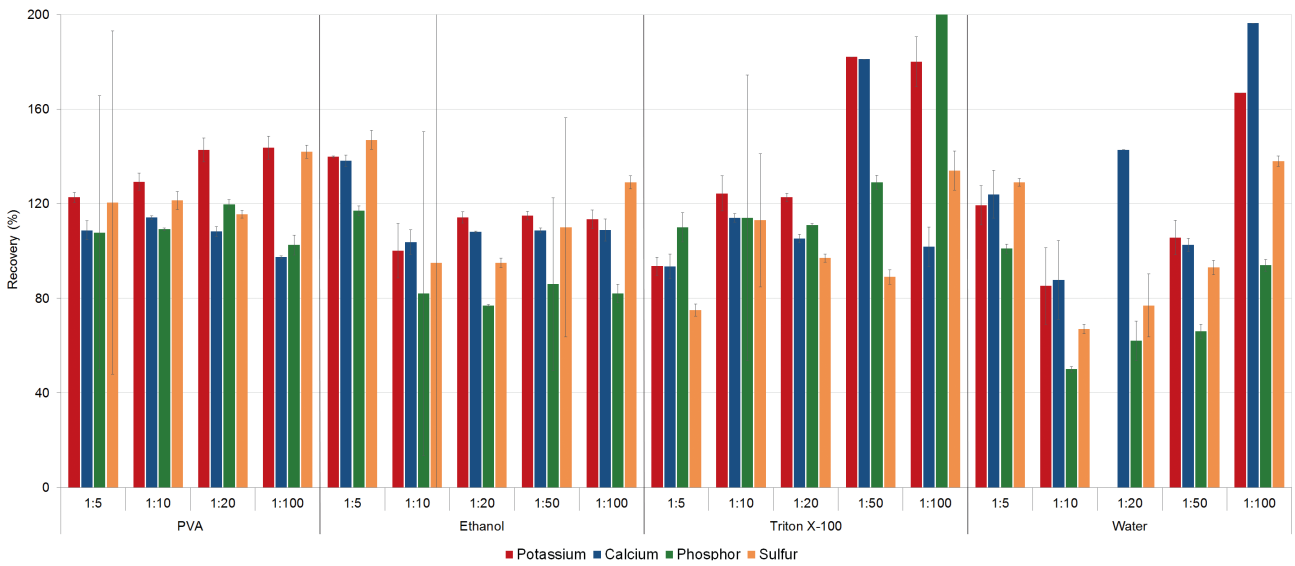

Figure 3
Recovery of the nutrient elements K and Ca (measured with Mo-K excitation) and S and P (measured with W-L excitation) in DMEM with different smoothing agents and dilution factors.

Results for DMEM

Selection of the smoothing agent

Cell culture media contain high amounts of sugars and other nutrients. During the drying process of such a medium on a TXRF carrier unwanted crystallization may occur. The formation of a homogenous flat sample layer can be achieved by addition of a smoothing agent like Triton X-100, ethanol or polyvinylalcohol. In a first experiment the effect of different smoothing agents was compared.

After dilution with water and drying of the DMEM on quartz sample carriers the formation of large crystals was observed in an optical microscope (Figure 2, top). The addition of ethanol as smoothing agent leads to smaller crystals and a more homogeneous layer (Figure 2, bottom).

The TXRF analysis of CCM at different dilutions from 1:5 to 1:100 does not provide satisfying recovery rates in case of water (Figure 3). The use of 1% Triton X-100 or polyvinylalcohol as smoothing agents is well established for sample preparation and leads to better recovery rates. Comparing all smoothing agents and all dilutions, the DMEM sample prepared with 1:10 Ethanol delivers the most accurate results with standard deviations typically better than 5% (< 10% at 1:100).

While the Mo-K excitation works well for the quantification of Ca and P, only the W-L excitation in combination with the smoothing agent Ethanol shows reproducible results for the light elements P and S.

During these experiments scandium was selected as internal standard element (see procedure 1). Due to the overlap of Sc with Ca, the following LOQ and reproducibilty experiments were performed with DMEM samples prepared according to procedure 2 with vanadium as internal standard.

Reproducibility

The reproducibility for the measurement of nutrient elements was proven with the two different methods described in the Sample preparation section:

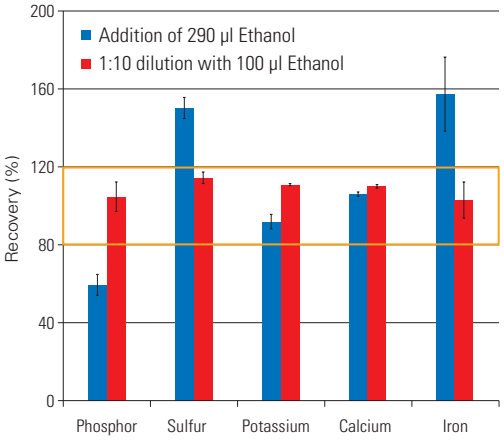

Figure 4
Reproducibility of the measurement of nutrient elements in DMEM. The acceptable recovery limit of ± 20 % is defined with the yellow frame.

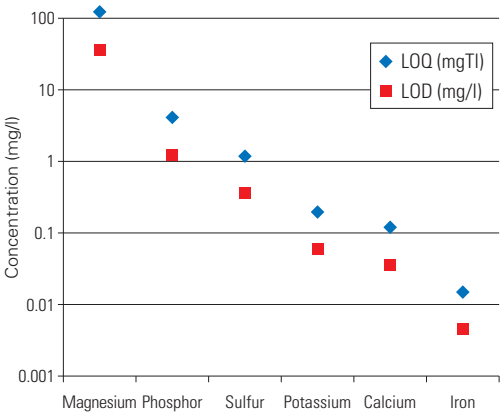

Figure 5
Limits of detection (LOD) and quantification (LOQ) for nutrient elements in cell culture media.

Element	LOQ
Magnesium*	122.700
Phosphor	4.165
Sulfur	1.205
Potassium	0.200
Calcium	0.122
Iron	0.016

Table 3
Limit of quantification (LOQ) of nutrient elements in DMEM in mg/l.
* measured with W-L excitation

Procedure 1: dilution factor 1:10, 100 µl sample + 100 µl ethanol + 790 µl H2O + 10 µl internal standard Sc

Procedure 2: no dilution, 700 µl sample + 290 µl ethanol + 10 µl internal standard V.

Figure 4 demonstrates the improved reproducibility after dilution of the high glucose media. Typically, standard deviations were better than 3% for nutrients and better than 10% for traces at low ppb concentrations.

Limit of quantification

Since ethanol has proven to be the most suitable smoothing agent providing the best results for the quantification of nutrient elements, the preparation procedure 2 was also applied for the DMEM samples used for the determination of the limits of detection (LOD) and quantification (LOQ). The results are shown in Figure 5 and given in Table 3.

Although the matrix content of the media induces a higher background, a low LOQ value of 16 µg/l for a trace metal like iron could be achieved. For the measurement of light elements the W-L excitation was tested the first time. While the Mo excitation did not provide reliable data for Mg, the use of W-L excitation leads to a LOQ of about 123 mg/l.

Results for high performance media (HP-CCM)

High performance media samples for the growth of mammalian cell cultures were prepared according to procedure 2 and measured with the S4 T-STAR® using Mo-K excitation at 17.5 keV and W-L excitation at 8.4 keV. Between two and eight samples of each cell culture type (HEK – Human Embryonic Kidney, CHO – Chinese Hamster Ovary, BHK – Baby Hamster Kidney, and IKZ - Invertebrate) were analyzed.

Various micro nutrients and contaminants were detected in all media in concentrations from 1 ppb to about 10 ppm as displayed in Figure 6. While the trace element concentrations within one cell line were almost identical (data not shown), significant variations between the cell lines were observed. Therefore, all media are clearly distinguishable by the amount of trace elements.

Limits of detection

Figure 7 shows the detection limits for several nutrient and trace elements for two different high performance cell culture media (CHO and feed) and the two applied excitation modes. HP-CCM contain high amounts of sugars, in case of the CHO medium about 8 g/l.

Nonetheless, the ethanol preparation method (procedure 2) leads to limits of detection down to 1.5 ppb for CHO and Mo-K excitation. Even feed media with 20 g/l glucose could be analyzed efficiently with TXRF down to 7 ppb.

While the common X-ray tube with Mo target is most suitable to detect the elements in the range from Mn to Y, W-L radiation efficiently excites light elements. Using W-L excitation the detection limits for the element range from Mg to Cr can be improved by a factor of 2 or more as shown in Figure 7.

Due to the sensitivity of the S4 T-STAR® TXRF spectrometer, for all possible contaminants measurement times (here: 1000 s) can be significantly reduced to increase the sample throughput.

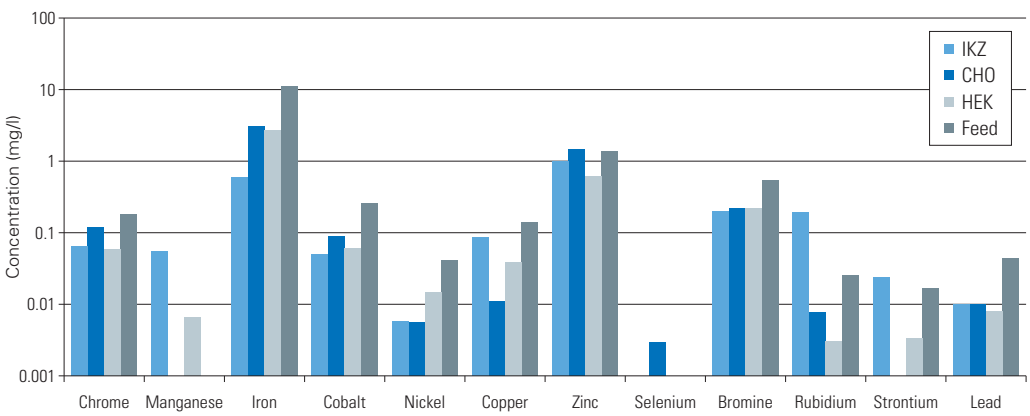

Figure 6
Concentration of micronutrients and contaminants in different types of high performance cell culture media.

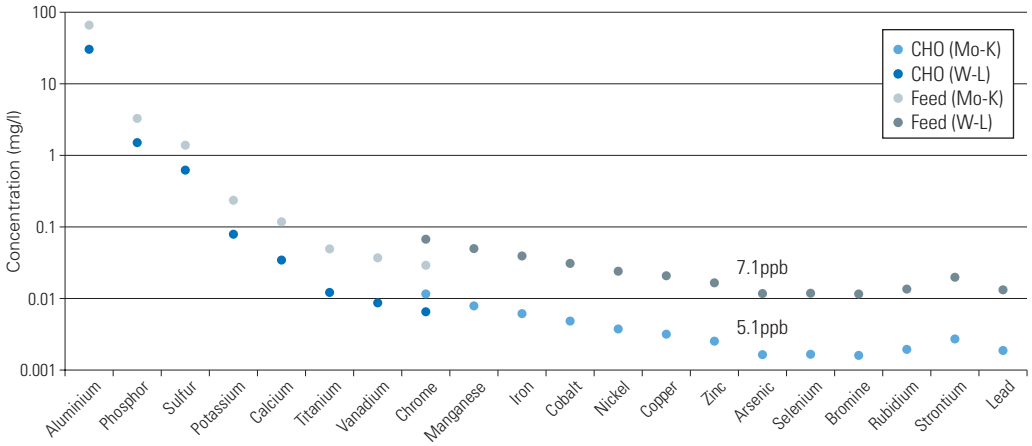

Figure 7
Detection limits of micro-nutrients and contaminants in CHO and feed of high performance cell culture media.

Results for spike experiments

To detect trace metals in the low ppb range with high accuracy, spiked HP-CCM samples were prepared and measured with the S4 T-STAR® (see Table 1). For this series of experiements the element Scandium was chosen as internal standard.

A typical spectrum of a HP-CCM spiked with Cr and measured with the W-L excitation is shown in Figure 8. In addition to Cr (spike concentration 100 µg/l) traces of Mn, Fe, Cu, and Ni were detected.

Element	TXRF (µg/l)	TXRF Spike (µg/l)	ICP-MS (µg/l)
Cr	24.7	14.7	1.9
Mn	15.8	4.4	2.6
Ni	5.3	7.7	2.1
Pb	7.6	17.0	–
Se	3.4	3.6	5.2

Table 4
Comparison of TXRF measurements in triplicate of a HP-CCM with TXRF results of the spike experiment and ICP-MS data.

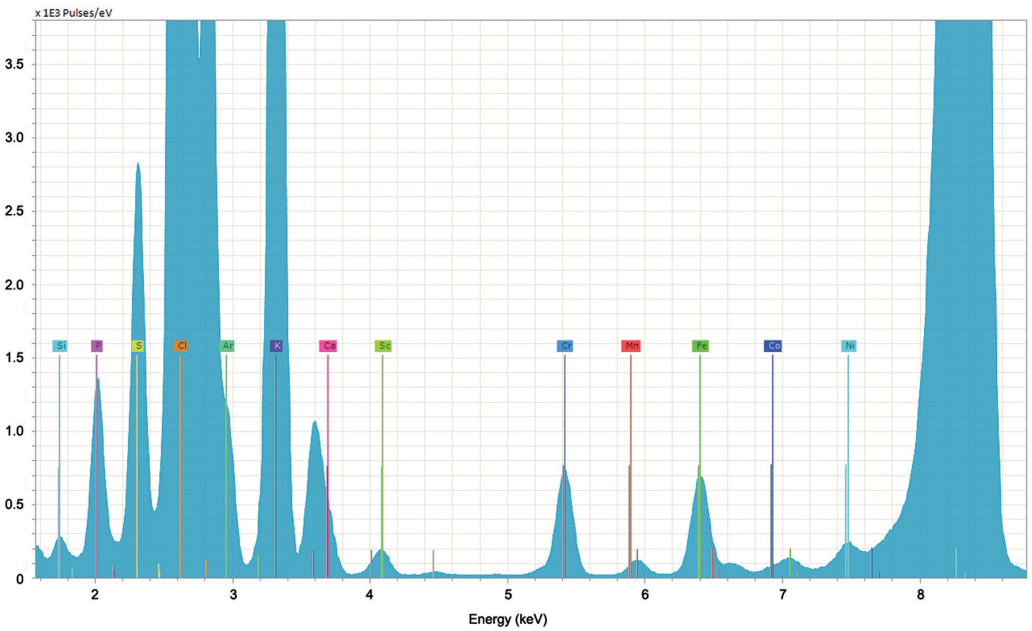

Figure 8
Typical spectrum of a HP-CCM spiked with 100 µg/l Cr and measured with W-L excitation.

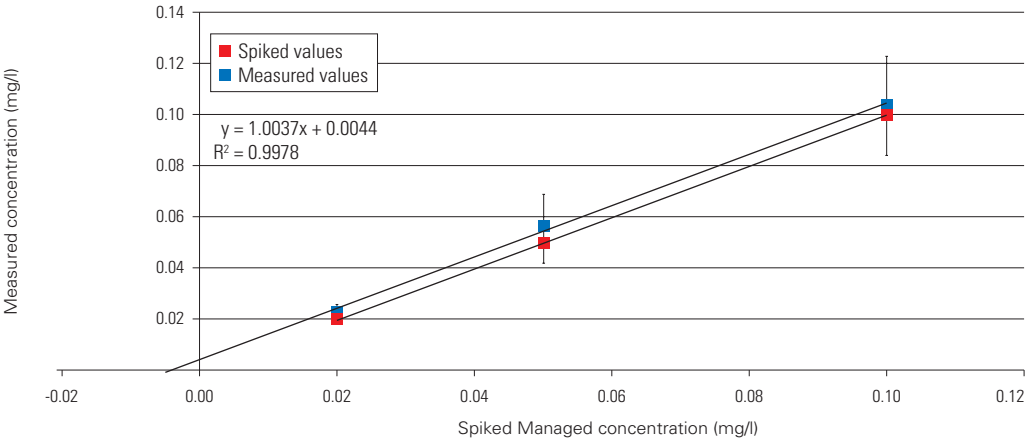

Figure 9
Calculation of the Mn concentration in HP-CCM by comparison of measured values (blue) and spiked concentrations (red).

The comparison of measured values with spiked concentrations for the element Mn is demonstrated in Figure 9. The original Mn concentration in the medium can be calculated by the intercept of the y-axis.

The concentrations of all elements quantified by the spike experiment are summarized in Table 4. For Ni and Se the results of both TXRF measurements are similar and at the same order of magnitude as the ICP values.

In case of Mn the spike experiment is much closer to the ICP data. The ICP result for Cr is much lower than the value of both TXRF measurements and is therefore highly doubtful.

Conclusion

Cell culture media are of great importance during the production of biopharmaceutical products. Up today the optimal supply with micro-nutrients and the impact of contaminants is not fully understood.

The systematic investigation of nutrients and contaminants in cell culture media by TXRF is described in this lab report for the very first time.

- A rapid sample preparation method for TXRF measurements of cell culture media with high sugar concentrations of up to 20 g/l was developed.
- High purity ethanol was used as smoothing agent for optimal sample layer formation on quartz sample carriers.
- The measurement of nutrient elements after a 1:10 dilution provides a reproducibility < 10%.
- For the control of contaminants, detection limits in the one digit ppb range can be achieved.

All measurements were performed with different excitation methods of the advanced TXRF spectrometer S4 T-STAR®. While Mo excitation is suitable for most metals, a W-L excitation improves the detection of light elements up to Cr by a factor of two or more.

In addition to the outstanding analytical performance the S4 T-STAR® is a plug and play system with low operation costs dedicated for research, routine or QC laboratories.

Literature

[1] ISO 18507 "Surface chemical analysis — Use of Total Reflection X-ray Fluorescence spectroscopy in biological and environmental analysis", First edition, 2015-07-15.

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