TXRF analysis of cell culture media and medical microsamples – saving time, money and lives

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Innovation with Integrity
Welcome

Speakers

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Berlin, Germany
Itinerary

Part I: Cell cultures
- Cell cultures – background
- Method development for element analysis of cell culture media
- TXRF analysis of high performance cell culture media
- Summary and conclusion

Part II: Biological and medical microsamples
- Protein analysis
- Bioassay analysis
- Summary and outlook
Part I: Cell cultures

Background
Cell cultures

Background

Application of cell cultures

- Biopharmaceuticals pharmaceutically active proteins and nucleic acids
- Clinical research: tissue cells for therapeutic purposes
- Study of elementary cellular processes without classical animal experiments
Cell culture media

- Natural environment of cells has to be imitated
- Standardized media, e.g. DMEM, 1 g/l or 4.5 g/l glucose
- Media contain amino acids for protein biosynthesis, vitamins and inorganic salts for metabolic processes, carbohydrates for energy production
- Nutrient (P, K, Ca, Zn, Fe) and trace elements (Se, Mn) nutrient sources, co-factors in enzymatic reactions
- Contaminations reduce yield of the biopharmaceuticals may cause necrosis of the cells
Cell culture media

Objective

Objective 1

• Development of a TXRF method for cell culture media
  1. Optimize sample layer on disc, standard DMEM media
  2. Determination of LOD / LOQ
  3. Reproducibility

Objective 2

• Measurement of nutrient elements in cell culture media

Objective 3

• Apply method to modern high performance media
  1. QC: identify contaminants
  2. Spike experiments for method detection limits
Cell culture media

Objective

Samples

- Commercially available DMEM, 1 g/l or 4.5 g/l glucose
- Mammalian media from two German suppliers
  Sugars conc. 8 g/l
  - Human Embryonic Kidney (HEK)
  - Chinese Hamster Ovary (CHO)
  - Baby Hamster Kidney (BHK)
  - Invertebrate (IKZ)
  - Feed media (20 g/l glucose)
Cell culture media
TXRF spectrometer

S4 T•STAR - **Unique benefits**

- Three excitation modes
to detect most elements of the PSE
- 60/100 mm² detectors improved sensitivity for lowest limits of detection
- New analytical capabilities angle scan for depth profiling and layer analysis
- Sample geometry flexibility measurement of discs, microscopy slides, wafers etc.
- Motorized beam path automatic beam adjustment and QC procedures
- Large sample capacity up to 90 sample discs, multi-user operation
- Most modern software instrument/measurement status display, statistical functions
Method development for element analysis of cell culture media
Cell culture media
Method development

1. Optimize sample layer on disc

Comparison of different modifier solutions (smoothing agents)

- DMEM media with 50 – 200 ppm P, S, K, Ca
- Addition of 10% modifier (1% Triton X-100, Ethanol, PVA (0.3 g/l))
- Dilutions down to 1 : 100
- Internal standard Sc

Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 µA
- W-L excitation, 50 kV, 1000 µA
- Measurement time 1000 s
Results

- Ethanol leads to satisfying recovery rates and standard deviations < 5% (< 10% at 1:100)
- Overlap of IS Sc with Ca, following experiments with V
- Mo excitation did not provide good data for P and S
Cell culture media
Method development

Results

- W-L excitation delivers reproducible results for P and S, if Ethanol was used.
2. Reproducibility

- Two preparations
  - 700 µl sample + 10 µl IS + 290 µl EtOH
  - 100 µl sample + 100 ml EtOH + 790 µl H₂O + 10 µl IS

- Better than 10%, typically < 3%

- Dilution recommended due to high amount of macro nutrient

- EtOH contained trace impurities (Fe) -> ultrapure or HPLC grade required
Cell culture media
Method development

3. LOQ

- EtOH treated media

<table>
<thead>
<tr>
<th></th>
<th>LOQ (mg/l)</th>
</tr>
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<tbody>
<tr>
<td>Magnesium*</td>
<td>122,7</td>
</tr>
<tr>
<td>Phosphor</td>
<td>4,165</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1,205</td>
</tr>
<tr>
<td>Potassium</td>
<td>0,200</td>
</tr>
<tr>
<td>Calcium</td>
<td>0,122</td>
</tr>
<tr>
<td>Iron</td>
<td>0,016</td>
</tr>
</tbody>
</table>

*) W-L excitation

- Sample preparation
  - 700 µl media
  - 290 µl EtOH
  - 10 µl V (IS)
TXRF analysis of high performance cell culture media
Modern high performance media
Quality control

Analysis of mammalian cell culture media

- Method developed for standard media
- 24 media in triplicate = 72 discs

Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 µA
- W-L excitation, 50 kV, 1000 µA
- W-Brems excitation, 50 kV, 1000 µA
- Measurement time 1000 s

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>290 µl</td>
</tr>
<tr>
<td>IS Vanadium</td>
<td>10 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>700 µl</td>
</tr>
<tr>
<td>Volume on disc</td>
<td>10 µl</td>
</tr>
<tr>
<td>Drying</td>
<td>30° C</td>
</tr>
</tbody>
</table>
Modern high performance media
Quality control

Results mammalian cell culture media

- Micro-nutrient and contamination test of different media batches
- Application of Mo and W-L excitation
- Typical concentration range 1 to 1000 ppb
- Distinct differences between cell lines

![Graph showing concentration of various elements for different cell lines and media types.]

CHO | Chinese Hamster Ovary
HEK | Human Embryonic Kidney
IZK | Invertebrate cell cultures
Feed | Feed media
Limit of detection

- LOD down to single digit ppb
- W-L significantly improves the detection of light elements (factor 2 - 4)
- Recommended measurement time for routine analysis = 300 s
Modern high performance media
Spike experiments

Preparation of spike media

- 1 ml of one high performance medium
- Two spike elements per sample concentrations see table
- Measurement in triplicate
  15 samples = 45 discs

Measurement parameter S4 T-STAR

- Mo excitation, 50 kV, 1000 μA
- W-L excitation, 50 kV, 1000 μA
- W-Brems excitation, 50 kV, 1000 μA
- Measurement time 1000 s
Modern high performance media
Spike experiments

W-Brems spectra
  • Cd spike (4 ppm)

Mo spectra
  • Cr spike (200 ppb)
Modern high performance media
Spike experiments

Measurements with Mo excitation

- Very low concentrations of Ni, Se
- Values close to ICP

<table>
<thead>
<tr>
<th></th>
<th>Ni</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP</td>
<td>2.1 µg/l</td>
<td>5.2 µg/l</td>
</tr>
<tr>
<td>TXRF spike</td>
<td>7.5 µg/l</td>
<td>3.6 µg/l</td>
</tr>
<tr>
<td>TXRF direct</td>
<td>5.3 µg/l</td>
<td>3.4 µg/l</td>
</tr>
</tbody>
</table>

ICP 2.1 µg/l
TXRF spike 7.5 µg/l
TXRF direct 5.3 µg/l

ICP 5.2 µg/l
TXRF spike 3.6 µg/l
TXRF direct 3.4 µg/l

Ni

\[ y = 1.0256x + 0.0077 \]
\[ R^2 = 0.9982 \]

Se

\[ y = 1.0371x + 0.0037 \]
\[ R^2 = 0.9977 \]
Modern high performance media
Spike experiments

Measurements with Mo excitation

<table>
<thead>
<tr>
<th></th>
<th>ICP</th>
<th>TXRF spike</th>
<th>TXRF direct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiked Pb</td>
<td>n.a.</td>
<td>17 µg/l</td>
<td>7.6 µg/l</td>
</tr>
<tr>
<td>Spiked Mn</td>
<td>2.6 µg/l</td>
<td>4.4 µg/l</td>
<td>15.8 µg/l</td>
</tr>
</tbody>
</table>

\[ y = 1.0411x + 0.0177 \]
\[ R^2 = 0.9883 \]

\[ y = 1.0037x + 0.0044 \]
\[ R^2 = 0.9978 \]
Modern high performance media
Spike experiments

- ICP values for Cr questionable
- Cd quantification not successful

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP</td>
<td>1.9 µg/l</td>
</tr>
<tr>
<td>TXRF spike</td>
<td>24.7 µg/l</td>
</tr>
<tr>
<td>TXRF direct</td>
<td>14.7 µg/l</td>
</tr>
</tbody>
</table>

**ICP**
- Spiked values: < 0.2 µg/l
- TXRF spike: < 45 µg/l
- TXRF direct: < 77.3 µg/l

**Cr**
- Measured concentration vs. Spiked Cr concentration
  - $y = 1.0908x + 0.0269$
  - $R^2 = 0.9919$

**Cd**
- Measured concentration vs. Spiked Cd concentration
  - $y = 1.1423x - 0.0658$
  - $R^2 = 0.9967$
Summary and conclusion

• A rapid method for TXRF measurements of cell culture media was developed

• EtOH has to be used as smoothing agent for optimal layer formation on quartz sample discs

• For contamination control detection limits in the one digit ppb range can be achieved

• The measurement of nutrient elements after a 1:10 dilution provides a reproducibility < 10%
Part II: Biological and medical microsamples
Protein analysis

Introduction

Metal ions and enzymes

• Metal ions are important for the biological function of enzymes
• Various modes of metal-protein interaction: metal-, ligand-, enzyme-bridge complexes
• Metals serve as electron donors or acceptors, Lewis acids or structural regulators


C. Arnaud: CEN, January 7, 2008 Volume 86, Number 1, p. 8
Examples of metal ions in enzymes

<table>
<thead>
<tr>
<th>Role</th>
<th>Metals</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen transport and storage</td>
<td>Fe, Cu</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>Electron transport</td>
<td>Fe, Cu</td>
<td>Cytochromes</td>
</tr>
<tr>
<td>Nitrogen fixation</td>
<td>Fe, Mo, V</td>
<td>Nitrogenase</td>
</tr>
<tr>
<td>Oxygen atom transfer</td>
<td>Mo, W</td>
<td>Oxidases, Reductases</td>
</tr>
<tr>
<td>Alkyl group transfer</td>
<td>Co</td>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Zn, Cu, Mn</td>
<td>Hydroxylases, Peptidases</td>
</tr>
<tr>
<td>Storage and transport</td>
<td>Fe, Cu, Zn</td>
<td>Ferritin, Metallothioneins</td>
</tr>
</tbody>
</table>
Commonly used analytical techniques

- **Atomic Absorption Spectrometry**
  - Ca, Co, Cu, Fe, Mg, Mo, Ni, Se, Zn

- **Flame/Inductively Coupled Emission Spectrometry**
  - for most metals occurring in proteins

- **Neutron activation analysis**
  - limited availability
  - most reliable technique for a number of elements
  - very sensitive for some elements
  - some critical interferes, e.g. Na

- **Inductively Coupled Mass Spectrometry**
  - detection of ppb levels of >40 elements in one minute
Protein analysis

Introduction

Analytical issues

- High matrix (polypeptides, buffers, salts)
- Sample viscosity, turbidity
- Removal of buffer, salts by dialysis or gel filtration total destruction of organic matter
- Significant sample amount needed microanalysis impossible
- Methods are expensive and laborious

Mounicou et al. (2004), Analyst, (2), 116-123
Protein analysis
TXRF spectroscopy

Samples
Certified reference materials
- “BCR 273 – Singe cell protein”
- “BCR 274 – Single cell protein”
500 mg powderous sample were suspended in 25 ml pure water
Sample preparation

First approach:
mixing of microsamples in **vials**

- 100 µl sample + internal standard
  + 10 µl Sc (10 mg/l)
  + 10 µl Ga (10 mg/l)

- Preparation of
  - 10 µl
  - 5 µl
  - 3 µl
  - 1 µl
  - 0.5 µl
Protein analysis
TXRF spectroscopy

Sample preparation

Second approach: mixing of microsamples on discs

- 5 µl sample + 5 µl Sc/Ga solution (20 mg/l)
- 3 µl sample + 3 µl Sc/Ga solution (20 mg/l)
- 1 µl sample + 1 µl Sc/Ga solution (20 mg/l)
Protein analysis
TXRF spectroscopy

S4 T•STAR
• Mo tube, 50 kV/1000 μA
• W-tube, 50 kV/1000 μA
• 60 mm² XFlash SDD
• 90 position sample changer
• Mo-K excitation, 1000 s
• W-L excitation, 1000 s
• W-Brems, 1000 s
Protein analysis
TXRF spectroscopy

Results mixing in vials

![Bar chart showing concentration (mg/kg) for various elements (P, K, Ca, Mn, Fe, Cu, Zn, Se) in different volumes (10 μl, 5 μl, 3 μl, 1 μl, 0.5 μl) and a reference sample.](image)
Protein analysis
TXRF spectroscopy

Results mixing in vials

Accuracy for the light elements P (S and Cl) fluctuates with deposited sample amount → effect of sample height and size
Results mixing in vials

Other major and trace elements (K, Ca, Mn, Fe, Cu, Zn and Se) are not affected.
protein analysis
TXRF spectroscopy

Results mixing in vials
• The detection limits are directly related to the deposited amount
Protein analysis
TXRF spectroscopy

Results mixing on discs

![Bar chart showing concentration (mg/kg) for different elements (P, K, Ca, Mn, Fe, Cu, Zn, Se) with varying volumes (5 + 5 µl, 3 + 3 µl, 1 + 1 µl, Reference).]
Protein analysis
TXRF spectroscopy

Results mixing on discs

Limited accuracy for the light elements P (S, Cl)

Effect of sample height and size
Protein analysis
TXRF spectroscopy

Results mixing on discs
Other major and trace elements (K, Ca, Mn, Fe, Cu, Zn and Se) are not affected
Protein analysis
TXRF spectroscopy

Results mixing on discs
• The detection limits are directly related to the deposited amount
Bioassay analysis
Introduction

Saving lifes...?
Not directly theirs...
Bioassay analysis
Introduction

Saving lifes...?
But theirs...
Bioassay analysis
TXRF spectroscopy

Sample preparation

Sampling and preparing non-lethal amounts of blood (urine, saliva ...)

20 µl sample
+ 10 µl Ga (4 mg/l)

Preparation of
• 10 µl (duplicate)
Bioassay analysis
TXRF spectroscopy

Results
- Seronorm human serum CRM, S2 PICOFOX
Results

- Serum samples from lab mice (publication in preparation)
The analysis of minute protein or serum samples by means of TXRF is possible

- Mixing of internal standard and sample in a vial is recommended
- Determination of detection limits for trace elements requires careful consideration of the sample amount

Outlook

- Intensive analysis of bioassays is ongoing
- Measurements with W-Brems excitation (S4 T•STAR) to detect elements like Mo, I
- Method development for light elements with optimized calibration factors
Q & A

Any Questions?

Please **type in** the questions you may have for our speakers in the **Questions Box** and click **Submit**
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Thank you for your attention!

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