On the Direct Characterization and Quantification of Active Ingredients in Commercial Solid Drugs using PIXE, PIGE and ToF-SIMS techniques

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  - Sample Preparation
  - PIXE/PIGE/ToF-SIMS set up
- Results
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    - Celecoxib, anti-inflammatory (Celebrex®, Celex®, pfizer®)
    - Atorvastatin, antihyperlipidymic (Lipitor®, Lipinorm®, Strovas®)
    - Clidinium bromide, anticholinergic
    - trifluoperazine, antipsychotic

- Molecular analysis (ToF-SIMS)
  - Fluphenazine dihydrochloride®
  - Fludinium®

- Conclusion & Overview
Introduction

- Drug

  - Active Ingredient (therapeutic molecule)
  - Excipients (inactive - major role in drug formulation)
    - Carrier system (to ensure the delivery of the A.I to the site of action),
    - Control the time of residence of the A.I, physical stability, drug dissolution,
    - antioxidant, coating material, binder (tablets), desintegrants,…

- The quantification of active ingredients in drugs is a crucial step in the drug quality control process

- Classical and universal wet chemical analytical techniques: LC/MS/MS, UV, Voltametry, etc…
If the A.I contains one or more heteroatom F, S, Cl, Br,...

Elemental IBA (PIXE, PIGE) can be explored

Advantages of IBA in Drug Quality Control (Rapidity, Precision)

✓ Analysis of drugs under solid form: simple sample preparation procedure
✓ Analysis time is few minutes/sample
✓ Simultaneous determination of different active ingredients
✓ Results can be done with good precision(< 5%)

# Ability of the TT-PIXE and TT-PIGE for the quantification of heteroatom containing active ingredient in drugs

# Potential of the TOF- SIMS for heteroatom free active ingredients quantification
Experimental

- **Sample category**: capsule, tablet, coated tablet

- **Sample preparation for elemental analysis (PIXE-PIGE)**

  Decapsulating
  
  Decoating → Milling → Pellet with external binder (0.1-0.2g) → Carbon coating → Sample/Al sample holder
Experimental

- PIXE, PIGE

Light elements

\[ \gamma \text{ ray} \quad Z > 10 \]

\[ \chi \text{ ray} \]

- Characteristic \(\gamma\)-rays
- External standard is needed

Matrix correction

Relative quantification: If composition sample ~ composition std

NEC 5-SDH 1.7 MV LAEC-Tandem accelerator
Proton 3MeV

Si(Li): 170 eV at 5.9 keV
HPGe: 1.9 keV at 1.33 MeV

- Absolute Analysis (std less)
- Relative analysis: external std
Experimental

- **ToF-SIMS technique**
  - Ar$^{3+}$ 9MeV delivered by the 4MV VDG accelerator of the IPNL
  - ToF detection with high transmission
  - Ion/Ion technique (static mode ~1000-5000 ions/s over ~300 um beam diameter)
  - Negative and positive ion mode
  - Samples (thin film, pellet, ..)

Surface analysis (few Å)

Emission of charged characteristic secondary species

Molecular

$[M+H]^+$

Chemical (low mass region)

Structure (fragments)
Results: Elemental analysis

CELECOXIB (F, S)

- Celebrex-pfizer origin A 200mg (A.I ~ 70% - 75%)
- Celebrex-pfizer origin B 200mg
- Celex-alpha 100 mg
- Celexocib Standard (A.I = 100%)

Celecoxib, Celebrex, Celex (PIGE)  Celecoxib (PIXE)
Results: Elemental quantification

CELECOXIB (F, S)

Organic matrix: Method validation!!
- Stability under beam irradiation
- Matrix composition Std and Drugs: Matrix correction?

Stability of the matrix under ion irradiation was checked
3 Mev p+ 0.1 nA-2 nA -10 nA different acquisition time
Accumulated charge during the analysis 0.5-5µC
Dose and dose effect assessment

→ Count rates of F and S per µC were practically stable

Matrix similarity of the Std and the analysed drugs was confirmed by RBS

Quantification by relative calculation/external standard without matrix correction

<table>
<thead>
<tr>
<th>% of Celecoxib in analyzed drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>S</td>
</tr>
</tbody>
</table>
Results: Elemental analysis

TIEMONIUM METHYLSULFATE (S)

- Timozin 50 mg (A.I. ~ 16%)
- TIE Standard (A.I. = 100%)

Labeled 50 mg TIE

PIXE relative quantification:
External Standard
60 mg ± 1.2

Matrix correction (PIXE absolute quantification)
48.7 mg ± 1.1

ATORVASTATIN (F)

- Storvas 10 mg
- Lipinorm 10 mg (A.I. ~ 6.5%)
- Lipitor 10 mg
Results: Elemental analysis

**CTORVASTATIN (F)**

Elemental composition of the Atorvastatin standard (100 % atorvastatin) is significantly different from the composition of the analyzed drug samples (RBS measurements).

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>C&lt;sub&gt;m&lt;/sub&gt;</th>
<th>H&lt;sub&gt;t&lt;/sub&gt;</th>
<th>H&lt;sub&gt;m&lt;/sub&gt;</th>
<th>F&lt;sub&gt;t&lt;/sub&gt;</th>
<th>F&lt;sub&gt;m&lt;/sub&gt;</th>
<th>N&lt;sub&gt;t&lt;/sub&gt;</th>
<th>N&lt;sub&gt;m&lt;/sub&gt;</th>
<th>O&lt;sub&gt;t&lt;/sub&gt;</th>
<th>O&lt;sub&gt;m&lt;/sub&gt;</th>
<th>Ca&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Ca&lt;sub&gt;m&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin std.</td>
<td>68.61</td>
<td>69.42</td>
<td>5.93</td>
<td>6.00</td>
<td>3.29</td>
<td>3.29</td>
<td>4.85</td>
<td>4.78</td>
<td>13.85</td>
<td>12.82</td>
<td>3.47</td>
<td>3.69</td>
</tr>
<tr>
<td>Lipitor®</td>
<td>40.3</td>
<td>6.76</td>
<td>0.25</td>
<td>4.16</td>
<td>35.6</td>
<td>12.9</td>
<td></td>
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</tr>
</tbody>
</table>
Results: Elemental analysis

**ATORVASTATIN (F)**

\[
\frac{Y_{\text{Sample}}}{Y_{\text{Ref}}} = \frac{C_{\text{Sample}}}{C_{\text{Ref}}} \times \frac{S_{\text{Ref}}(E_{1/2})}{S_{\text{Sample}}(E_{1/2})}
\]

**Atorvastatin Active Ingridient (mg)**

<table>
<thead>
<tr>
<th>Storvas® 10 mg</th>
<th>Lipitor® 10 mg</th>
<th>Lipinorm® 10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured relatively/std</td>
<td>measured with matrix correction</td>
<td>UV</td>
</tr>
<tr>
<td>12.5±0.3</td>
<td>11.2±0.3</td>
<td>10.9±0.4</td>
</tr>
</tbody>
</table>

PIGE RESULTS ARE IN A GOOD AGREEMENT WITH UV MEASUREMENTS
Results: Elemental analysis

Fludinium (Br, S, Cl, F)

Binary drug

Clidinium bromide

Fludinium® spectra

Clidinium bromide

Trifluoperazine

Fludinium® spectra

PIXE

PIGE

$^{19}\text{F}(P, P')^{19}\text{F}$
Results: Elemental analysis

**FLUDINUM (Br, S, Cl, F)**

**Analysed Samples:**
- Standards with different % of the two A.I. and excipients.
- Fludinium commercial drug with different compositions

**Stability under irradiation:**
- Samples are **stable** under analysis conditions (3MeV, 0.2 nA, 0.1µC, 15 min acquisition)
- Loss of Cl under ion irradiation even under condition of analysis
- Cl is not related to the A.I by covalent band it is thermo fragile.

Cl will not considered for quantification

S, F: quantification of A.I.2
Br: quantification of A.I.1
Results: Elemental analysis

FLUDINIUM (Br, S, Cl, F)

VALIDITY OF THE ANALYSIS

- S direct quantification in drugs/standard is highly affected by matrix composition of the analyzed drugs. Matrix correction is needed (A.I.2)
- Br can be quantified directly in drugs/standard (A.I 1)
- F can be quantified directly in drugs/standard (A.I 2)

Stopping power

Matrix composition

Absorption Coefficient

$K_\alpha (S) = 2.3$ keV: highly affected by variation of matrix absorption coefficient

$K_\alpha (Br) = 11.9$ keV

$\gamma (F) = 197$ KeV

Practically not affected
### Results: Elemental analysis

#### FLUDINUM (Br, S, Cl, F)

<table>
<thead>
<tr>
<th>Sample labeled composition</th>
<th>S (matrix correction) PIXE-Gupix</th>
<th>F (direct analysis) PIGE</th>
<th>Br (direct analysis) PIXE</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mg A.I.1, 50mg A.I.2</td>
<td>3.33% S, 5.9% F, 9.25% Br</td>
<td>3.37%±0.16</td>
<td>5.7% ±0.2</td>
</tr>
<tr>
<td>40mg A.I.1, 40mg A.I.2, 20 mg Excipient</td>
<td>2.67% S, 4.7% F, 7.4% Br</td>
<td>2.60%±0.12</td>
<td>4.61% ±0.22</td>
</tr>
<tr>
<td>25mg A.I.1, 25mg A.I.2, 50mg Excipient</td>
<td>1.67% S, 2.97% F, 4.6% Br</td>
<td>1.62%±0.03</td>
<td>2.73% ±0.13</td>
</tr>
<tr>
<td>12.2mg A.I.1, 4.9mg A.I.2, 82.9mg Excipient</td>
<td>0.32% S, 0.58% F, 2.25% Br</td>
<td>0.31%±0.01</td>
<td>0.57% ±0.03</td>
</tr>
<tr>
<td>7.9mg A.I.1, 2.6mg A.I.2, 89.5mg Excipient</td>
<td>0.175% S, 0.31% F, 1.46% Br</td>
<td>0.18%±0.01</td>
<td>0.35% ±0.02</td>
</tr>
<tr>
<td>0.58mg A.I.1, 1.45mg A.I.2, 97.2mg Excipient</td>
<td>388ppm S, 691ppm F, 2692ppm Br</td>
<td>434ppm±43</td>
<td>705ppm±35</td>
</tr>
</tbody>
</table>
Results: Molecular analysis (ToF-SIMS)

**FIRST APPROACH RESULTS AND DISCUSSION**

- Larger number of A.I. are **heteroatom free**.

- Molecular characterization in stead of element analysis

- Surface analysis - Semi quantitative

- The secondary Ion Emission is highly dependent on the matrix (composition, **texture**, inter and intra molecular interaction, surface structure)
Results: ToF-SIMS

FLUHENAZINE DIHYDROCHLORIDE

Excipients are trade secret

Positive emission
Fluphenazine
Finger print

Molecular fragments
[Flu+H]

High emission of Excipient

Negative emission
Results: ToF-SIMS

FLUHENAZINE DIHYDROCHLORIDE

Any semi-quantititation needs that the thickness of the sample > secondary ion escape depth

In positive emission semi-quantification can be extracted

Negative emission
Results: ToF-SIMS

**FLUDINIUM (BINARY DRUG)**

2.5 mg Clidinium Br, 1 mg Trifluoperazine

Semi-quantification in a prepared mixture as thin films (spin coating)??

Composition checked by RBS

Surface segregation!!?

ESCA

Fludinium® positive emission

Positive emission is rich in molecular and chemical structure

Composition checked by RBS

Surface segregation!!?

ESCA
Conclusion and Overview

- PIXE and PIGE techniques are appropriate for rapid and accurate quantification of A.I. containing heteroatoms like S, F, Cl, Br.
- Methode validation: - Ensure the stability of the sample under ion irradiation, - Elemental composition of the standard and the analyzed drugs. – Quantification was validated with or without matrix correction.
- TOF-SIMS can be promising technique for A.I. heteroatom free characterization

- Effort to be done in the sample preparation techniques to ensure analytical reproducibility and trueness (ToF-SIMS)
- A.I. determination in liquid form (syrup) to be analyzed as thin film
- Elemental and molecular techniques for analysis of solid drug with several A.I. (>3A.I. with low concentration ppm range)
- TOF-SIMS Exploration of the use of internal standards for direct quantification (d-labeled A.I. or chemically similar molecule(s) to A.I.)
Thank you for your attention