DETERMINATION OF AMADORI COMPOUNDS IN TOBACCO BY ION PAIR CHROMATOGRAPHY COUPLED TO ELECTROCHEMICAL DETECTOR – THE LASTEST IMPROVEMENTS.

BREGEON Bernard

Altadis
Objective:

Improve our analytical method for the determination of Amadori compounds in tobaccos.
Structure:

#1 - Introduction
#2 - Description of the initial method.
#3 - Optimized version.
#4 - Identification
#5 - Quantification and limits
#6 - Tobacco applications
#7 - Conclusions
Mechanism of the initial step of Maillard reaction:

1. **Aminoacids**
2. **Reducing sugar (Glucose)**

   **Aldosylamines**

   **Amadori rearrangement**

   **Amadori compounds (fructosyl-aminoacids)**

   **Coding of A. C.**

   For Fructosyl-asparagine

   > F ASN
Analytical difficulties for Amadori compounds:

- Insufficient separation efficiency in analytical methods.
- Lack of commercial availability of Amadori products.
- No efficient synthetic methods for their preparations (poor yields, hygroscopic properties of products...).
Mechanism of separation and detection principle:

Stationary phase: Hypersil BDS (C18)

- Hydrophobic interactions

- Ion pair

- Anionic surfactant (BEHPT)

- (+) NH3 - R

- Amadori compounds and Aminoacids

- Detection based upon reducing properties of sugar moiety.
Initial method:

1- **Mobile phase** (Isocratic mode):
Methanol: 50 mL, Acetic acid: 1 mL
Surfactant (BEHPT): 300 µL and UP Water for 2 L.

2- **Post column oxidizing reagent:**
Potassium hexacyanoferrate (III): 320 mg
Potassium carbonate: 12.75 g
UP Water for 1 L.

**Column:** Hypersil BDS (5µm): **250 X 7.8 mm**
Injection volume: **100 µL**
Flow rates: 0.5 mL / mn

**Heated reactor (80°C) Applied Biosystems**

**UV-Vis. detector**
λ: 420 nm

**Data system**

**Post column detection**
New approach:

Tobacco extraction: 0.5 g / 25 mL of water

1- Mobile phase (Isocratic mode): Unchanged.

2- Buffer:
Sodium hydroxide: 400 mmol / L in UP water

Column: Hypersil BDS (5µm): 250 X 4.6 mm
Injection volume: 5 µL
Flow rates: 0.4 mL / mn

Electrochemical detector
EC 2000 Thermo Separation Products

Data system

Waste
ECD Chromatogram (Oriental tob.):
LC/MS spectrum of F PRO:

**LC/MS Conditions:** (Unchanged conditions of LC separation)

**Apparatus:** QUANTUM ULTRA (Thermo Separation Products)

**Mode:** ESI (+)  
**Scan:** 100-600 amu.
Quantification:

The major Amadori compounds are quantified in tobaccos on the basis of calibration curves by comparing the peak areas to those of standard solutions containing known amounts of pure compounds (In-house preparations). Good linearity in the range of concentration of tobacco lots.

The minor Amadori compounds quantities are estimated by peak area percentage of F ASN amount.

Practically one referent tobacco (stored in a cool place) previously calibrated is used for the routine quantification and a second tobacco is used for controlling.

<table>
<thead>
<tr>
<th>Detection limit (LOD)</th>
<th>0.01 %</th>
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<tbody>
<tr>
<td>Quantification limit (LOQ)</td>
<td>0.02 %</td>
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Tobacco applications:

Total Amadori compounds per blend components:

- FC stems: * (2.44)
- FC strips: * (1.92)
- Orientals: * (1.82)
- Recons: * (0.56)
- BY strips & stems: * (0.05)

1500 Representative lots

* (Indicative means)
Balance of Amadori compounds amounts in a Flue Cured lot (FRA):

Expressed in % of total amount

F ASP 1.09%
F ASN 19.3%
F GLN 27.6%
F THR 0.17%
F PRO 34.8%
F GLU 1.53%
F GLY 0.19%
F ALA 10.7%
F VAL 1.98%
F LEU 1.13%
F iLEU 1.46%

Expressed in % of total amount >> 4 Major = 92%
Incidence of origins (F.C. Medians):

350 Representative lots of Flue cured (strips)
Some examples of process incidence:

- Detection of A. C. in roasted BY (Cased with reducing sugars).
- Ageing effect on F GLN (F. C. Origin: Brazil)
Post-harvesting treatment effect:

Comparative ECD chromatograms of green and correspondent as is. (Flue Cured / Origin: FRA).
Conclusions:

Increased sensitivity (X 20) with ECD detection.

Clear separation of Amadori compounds in tobacco:

> 11 A. C. quantifiable and fully separated.
> Simple, robust and reliable approach.

Tobacco results confirm or reveal:

> Quantitative importance of 4 Amadori compounds.
> Incidence of production origins.
> Some process effects: Ageing and roasting.