New Approaches in the Analysis of Amadori Compounds

T. Davidek, I. Blank, K. Kraehenhuehl, J. Hau and S. Devaud

Nestlé Research Centre
Lausanne, Switzerland
Structure of the presentation

- **Introduction**
  - Analysis of Amadori compounds by HPLC

- **Analysis of Amadori compounds using HPAEC-ECD**
  - Separation of glucose-based Amadori compounds
  - Simultaneous analysis of Amadori compounds, precursors and degr. products

- **Separation of Amadori compounds on CS-17 column**
  - coupling to ECD
  - coupling to MSD

- **Analysis of Amadori compounds by CE-MS**
Analysis of Amadori compounds by HPLC

- HPLC (refractometer, UV-VIS)
  - 😊 Rapid (no derivatisation prior to analysis)
  - 😞 Generally low separation efficiency and sensitivity/selectivity

An efficient HPLC method was developed by Eichner and co-workers (1989, 1990)

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DEAE-Si
Post-column derivatisation with TTC
Analysis of Amadori compounds using HPAEC

Column: Carbopac PA-1 (strong anion exchange column)
Detection: Electrochemical detector (PAD)
Eluents: Gradient of NaOH and CH₃COONa in water
Equipment: Dionex DX 500 system
Sample: Glucose + 8 amino acids 4h 70°C in MeOH

Separation of 8 glucose derived Amadori compounds

Time (min)

PAD Response (μC)

0.0 20.0 40.0 60.0

0.00 0.100 0.200 0.300 0.400 0.500

Glucose
Fru-Pro
Fru-Ala
Fru-Val
Fru-Gly
Fru-Leu & Ile
Fru-Met
Fru-Phe
Simultaneous analysis of Amadori compounds, precursors and degradation products

Chromatogram of Fru-Gly heated 1h at 120°C (pH 8)

(A) Electrochemical detection
(B) UV (λ=285nm)
(C) UV (λ=350nm)

(Davidek et al. 2003)
Analysis of Amadori compounds using HPAEC

- **Advantages**
  - ☻ Rapid - no derivatisation and no or limited clean-up
  - ☻ High separation efficiency
  - ☻ High selectivity and sensitivity
  - ☻ Simultaneous detection of parent compounds and degradation products
  - ☻ Particularly suitable for analyses of model systems (monitoring of known compounds)

- **Limitations**
  - ☹ No unequivocal identification
  - ☹ Eluents not compatible with MS detection
  - ☹ Not suitable for analysis of pentose based Amadori compounds
Analysis of xylose-based Amadori compounds using column CS-17

Chromatograms of reaction mixtures containing xylose and amino acid

<table>
<thead>
<tr>
<th>Reaction Mixture</th>
<th>Conditions</th>
<th>Chromatogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyl / Ala+Ile</td>
<td>70°C, 4h, MeOH</td>
<td><img src="Xyl-Ala.png" alt="Chromatogram" /></td>
</tr>
<tr>
<td>Xyl / Gly</td>
<td>80°C, 30min, Phosphate pH 6</td>
<td><img src="Xyl-Gly.png" alt="Chromatogram" /></td>
</tr>
</tbody>
</table>

Formation of Xyl-Gly

![Formation of Xyl-Gly](Formation.png)
Analysis of Amadori compounds using column CS-17 (HPCEC)

Chromatograms of Fru-Gly and Xyl-Gly

Co-elution of glucose-based and xylose based Amadori compounds

Lower separation efficiency as compared with CarboPac PA-1 column

Chromatograms of Reaction mixtures containing 8 amino acids and glucose or xylose
Analysis of Amadori compounds using HPCEC-MS/MS

**HPCEC:**
- an integrated Agilent-1100 system (Agilent, Palo Alto, CA).
- a IonPac CS-17 ion exchange column
- Flow rate 0.25 mL/min

**MS conditions:**
- a Q-Trap tandem mass spectrometer (AB/MDS Sciex, Concord, Canada).
- positive electrospray (EPI) mode
- enhanced scan, enhanced product ion or MRM (SRM) program
- Turboionspray temperature 450°C
- Gas speed 1: 35 L/h; gas speed 2: 60 L/h
- DP (declustering potential) : 40
- CE = - 20 eV; LIT: 20ms
Fragmentation of Amadori compounds

+ESI-MS/MS Spectrum of [M+H]^+ ion of Fru-Gly

+ESI-MS/MS Spectrum of [M+H]^+ of Xyl-Gly
Analysis of Amadori compounds in sugar-AA mixture by HPCEC-MS/MS

**Glucose derived Amadori compounds**

- Fru-Pro
- Fru-Ala
- Fru-Gly
- Fru-Val
- Fru-Leu/Ile
- Fru-Met
- Fru-Phe

**Xylose derived Amadori compounds**

- Xyl-Pro
- Xyl-Ala
- Xyl-Gly
- Xyl-Val
- Xyl-Leu/Ile
- Xyl-Met
- Xyl-Phe

SRM mode ([M+H]⁺ → [M+H-H₂O]⁺)
Analysis of Amadori compounds in dried tomatoes by HPCEC-MS/MS

SRM mode ([M+H]⁺ → [M+H-H₂O]⁺)

- Fru-Glu
- Fru-Ala
- Fru-Gly
- Fru-Val
- Fru-Leu/Ile
- Fru-Met
- Fru-Phe

Electrochemical detection
Analysis of Amadori compounds in dried tomatoes by HPCEC-MS/MS

SRM mode ([M+H]+ → [M+H-H₂O]+)

- Fru-Glu
- Fru-Ala
- Fru-Gly
- Fru-Val
- Fru-Leu/Ile
- Fru-Met
- Fru-Phe

MS/MS Spectrum of [M+H]+ ion of Fru-Glu in dried tomatoes

MS/MS Spectrum of reference compound (Fru-Glu)
Separation of Amadori compounds using CE-MS

**CE:**
- **Equipment:** Hewlett-Packard HP³D CE System equipped with the manufacturer's CE-MS interface kit
- **Capillary:** uncoated fused silica capillary (132 cm, with 50 μm i.d., 375 μm o.d.)
- **Run buffer:** 20 mmol/L ammonium acetate in water (pH 6.9)
- **Conditions:** 30kV; 10 mbar pressurised capillary

**CH₃COONH₄**

**CE**

**MS/MS**

**MS:**
- **Equipment:** Finnigan TSQ 700 triple quadrupole mass spectrometer scan mode
- **Sheath flow** 2 μl/min (20% run buffer / 80% 2-propanol)
- **No sheath or auxiliary gas used**
- **Collision gas** - Argon at 4 hPa (3.0 mTorr)
Detection of Amadori compounds using CE-MS

Extracted trace [M+H]^+

Sample: Glucose + 6 amino acids
4h 70°C in MeOH

MS/MS spectrum of [M+H]^+ ion of Fru-Pro
Conclusions

- **HPAEC-ECD (CarboPac PA-1)**
  - ☺ for the analyses of hexose-based Amadori compounds in model systems
  - ☺ Very useful for kinetics studies
  - ☹ No unequivocal identification
  - ☹ Not suitable for analysis of pentose-based Amadori compounds

- **HPCEC-ECD (CS-17)**
  - ☺ for the analyses of pentose-based Amadori compounds in model systems
  - ☺ High sensitivity
  - ☹ Low separation efficiency
  - ☹ No unequivocal identification

- **HPCEC-MS (CS-17)**
  - ☺ Universal method for the analyses of pentose and hexose-based Amadori compounds
  - ☺ Sensitive (0.2-0.4 ppm in SRM mode) and highly selective
  - ☺ Very useful for analysis of food samples
  - ☹ Expensive (parent sugars not retained)

- **CE-MS**
  - ☺ Alternative method to HPIC-MS
Back–up slides
Methods for the analysis of Amadori compounds

- Gas Chromatography
  - ☺ High separation efficiency and sensitivity
  - ☺ Sugar and amino acid may be analysed in the same run
  - ☹ Time consuming - need derivatisation and clean up

Separation of 11 Amadori compounds on OV-101 in the form of trimethylsilyl-oxim derivatives

Eichner and co-workers (1990)
High Performance Anion Exchange Chromatography

Huyghues-Despointes and Yaylayan (1994) Detection of Amadori compounds (Fru-Pro, Fru-Trp) using PAD

Ge and Lee (1996) Separation of Phe, Fru-Phe and Glu on Carbopac PA-1