

59th TSRC Atlanta

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

**BREGÉON Bernard**



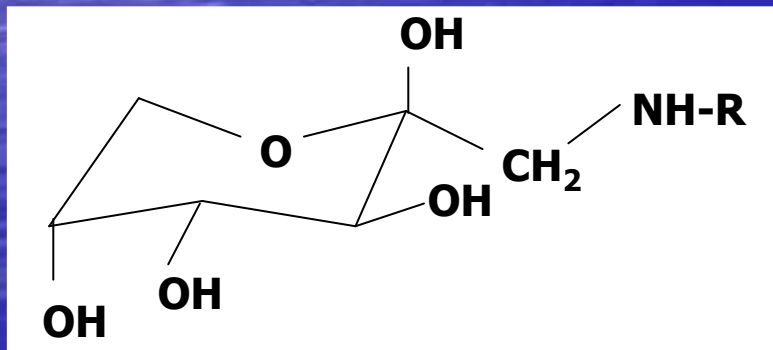
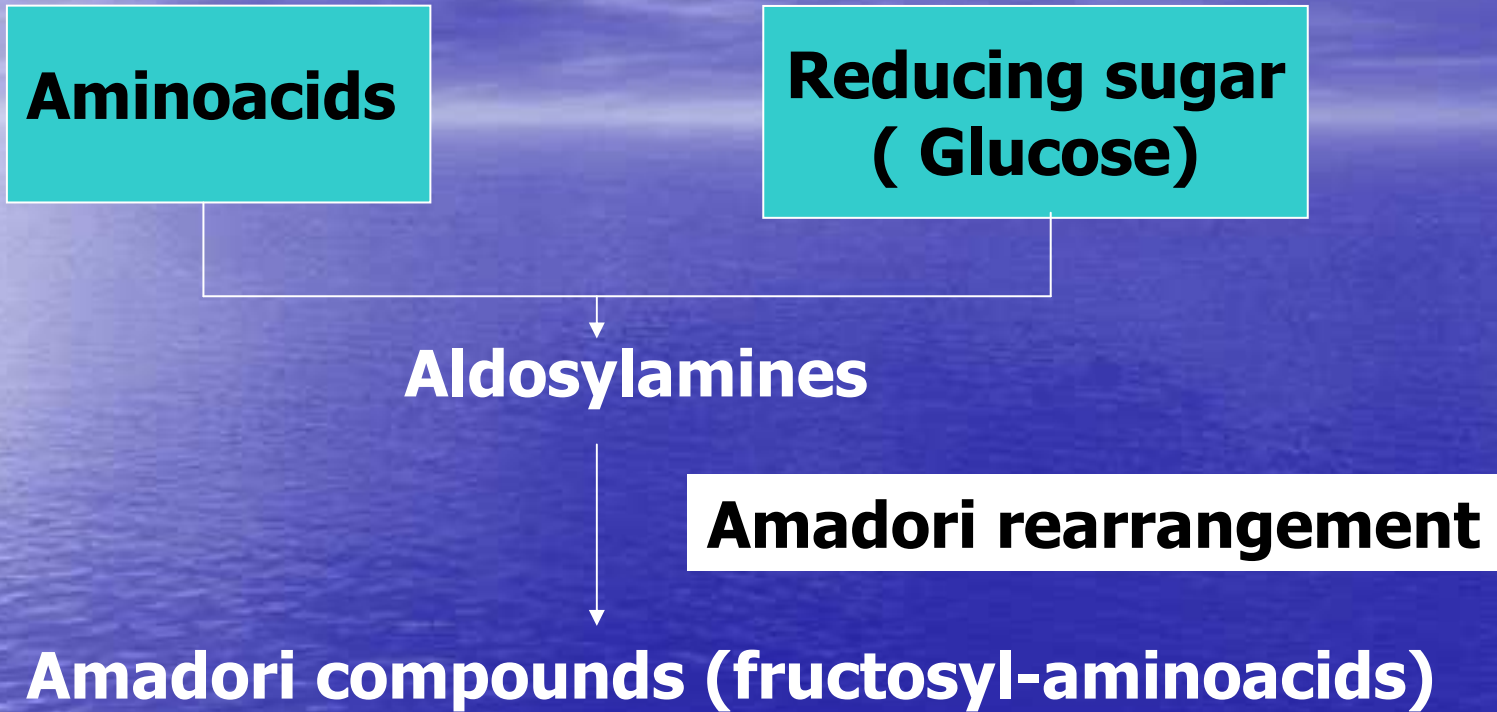
# Objective :

**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:



Coding of A. C.

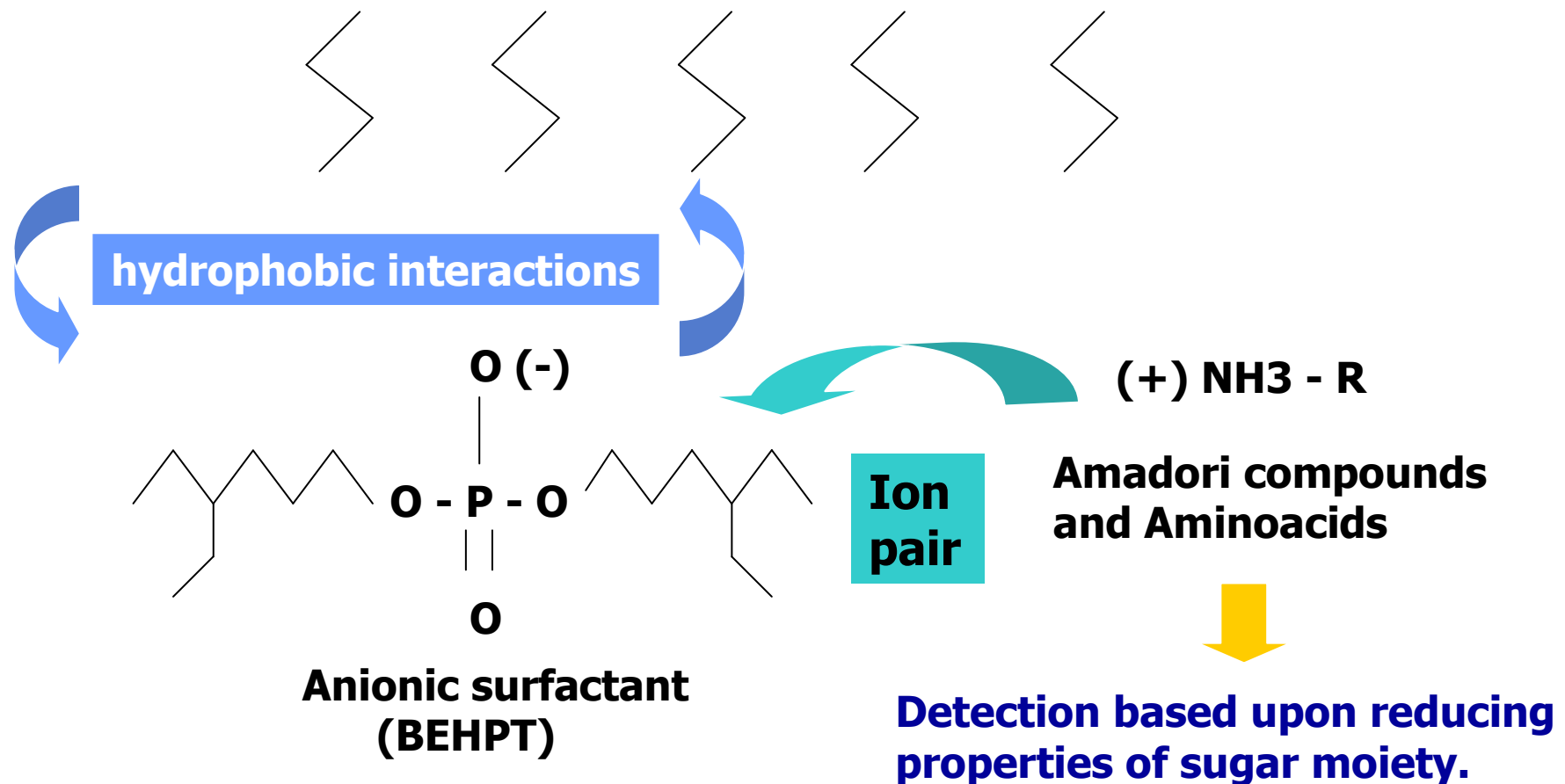
For Fructosyl-asparagine  
> FASN

# Analytical difficulties for Amadori compounds:

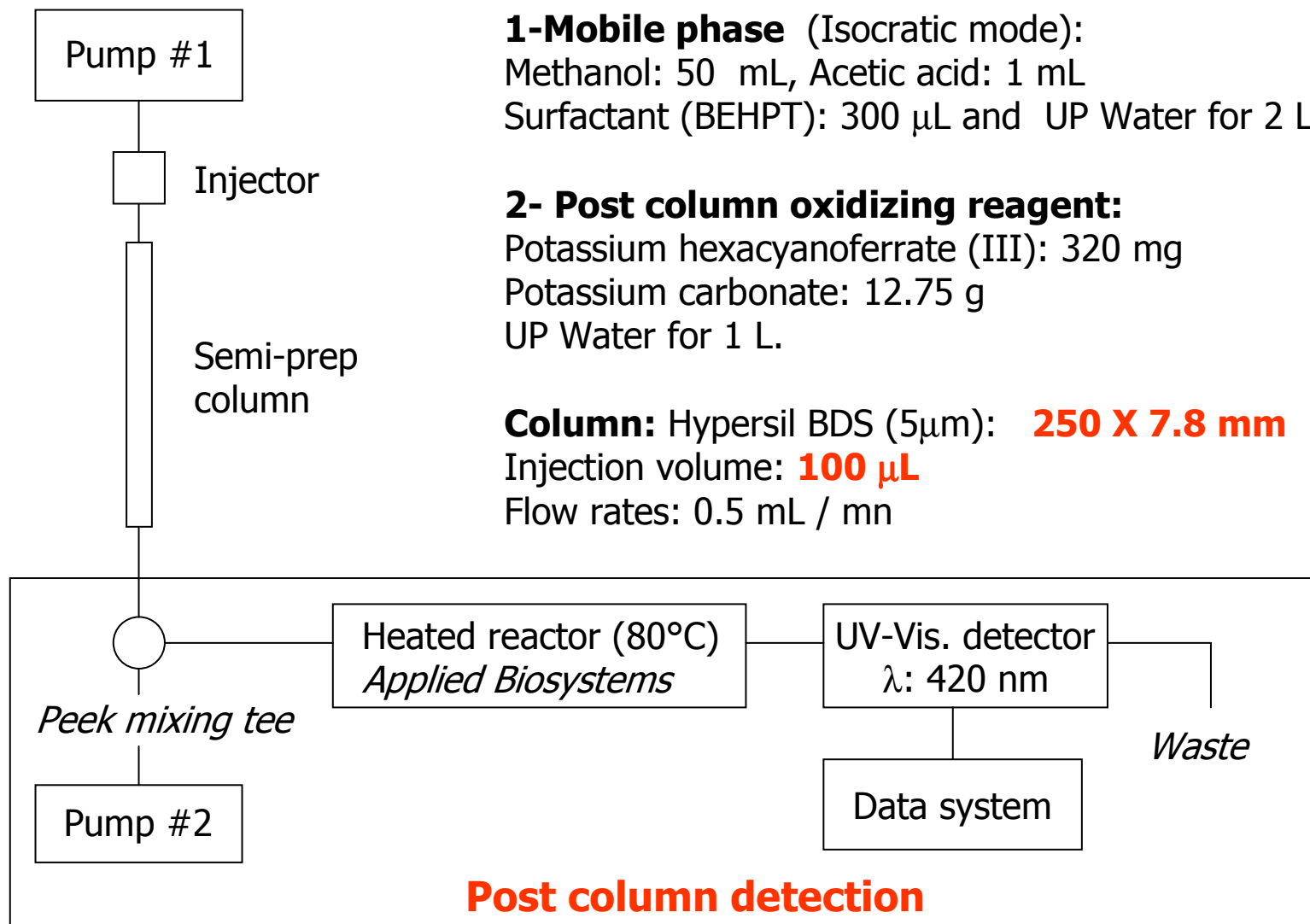
- Insufficient separation efficiency in analytical methods.
- Lack of commercial availability of Amadori products
- No efficient synthetic methods for their preparations (poor yields, hygroscopic properties of products...).

# Mechanism of separation and detection principle:

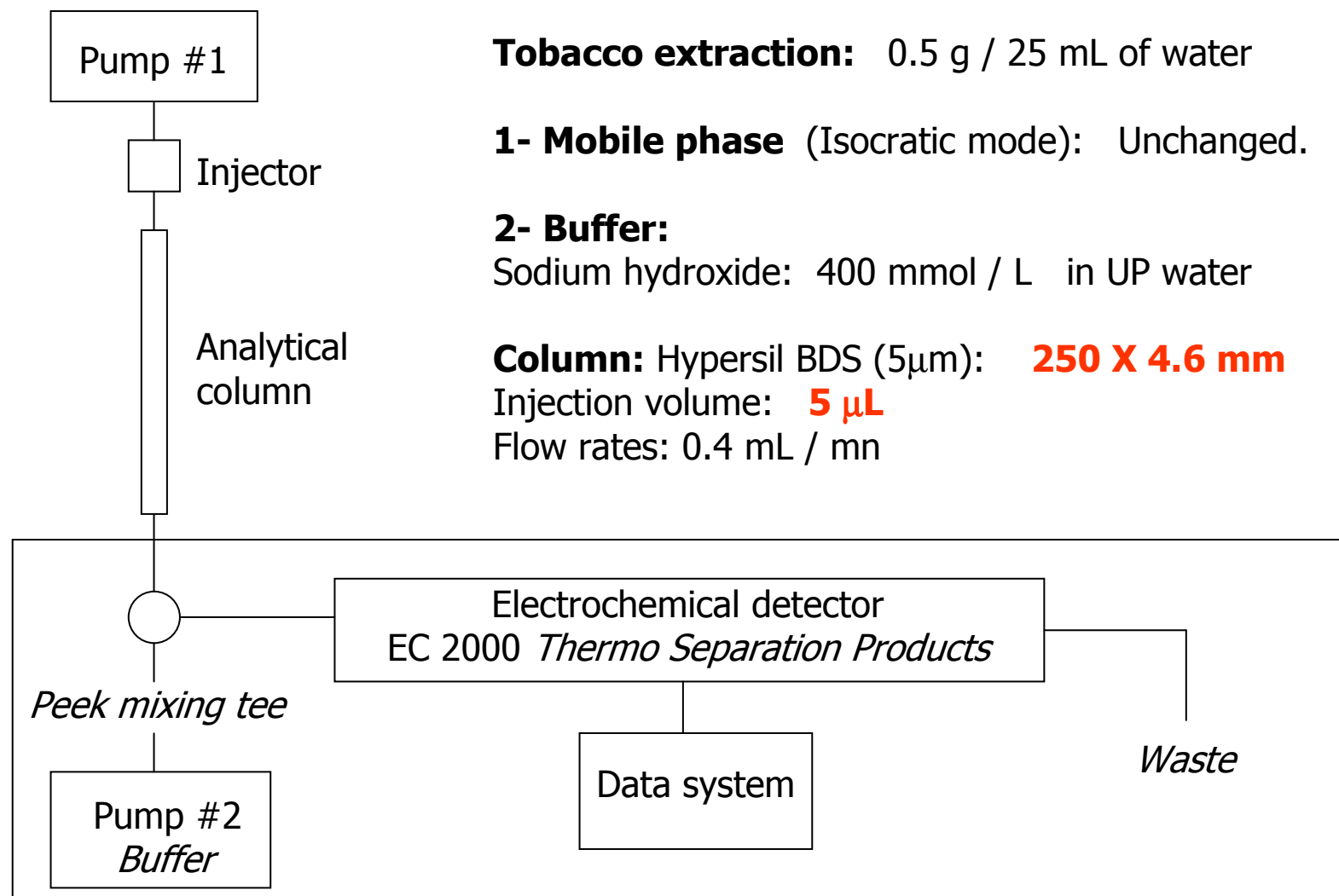
**Stationary phase: Hypersil BDS (C18)**



# Initial method:

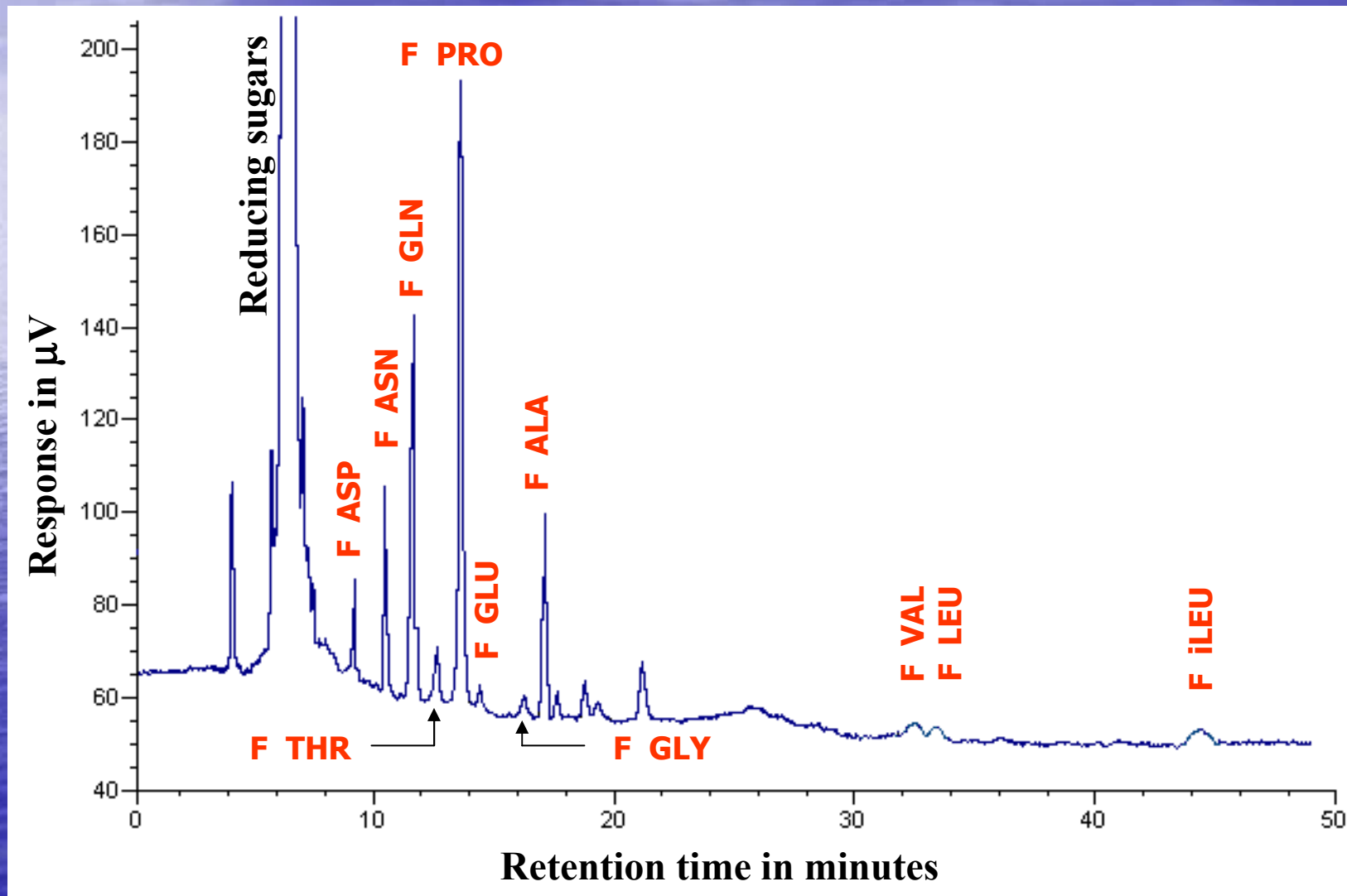


# New approach:

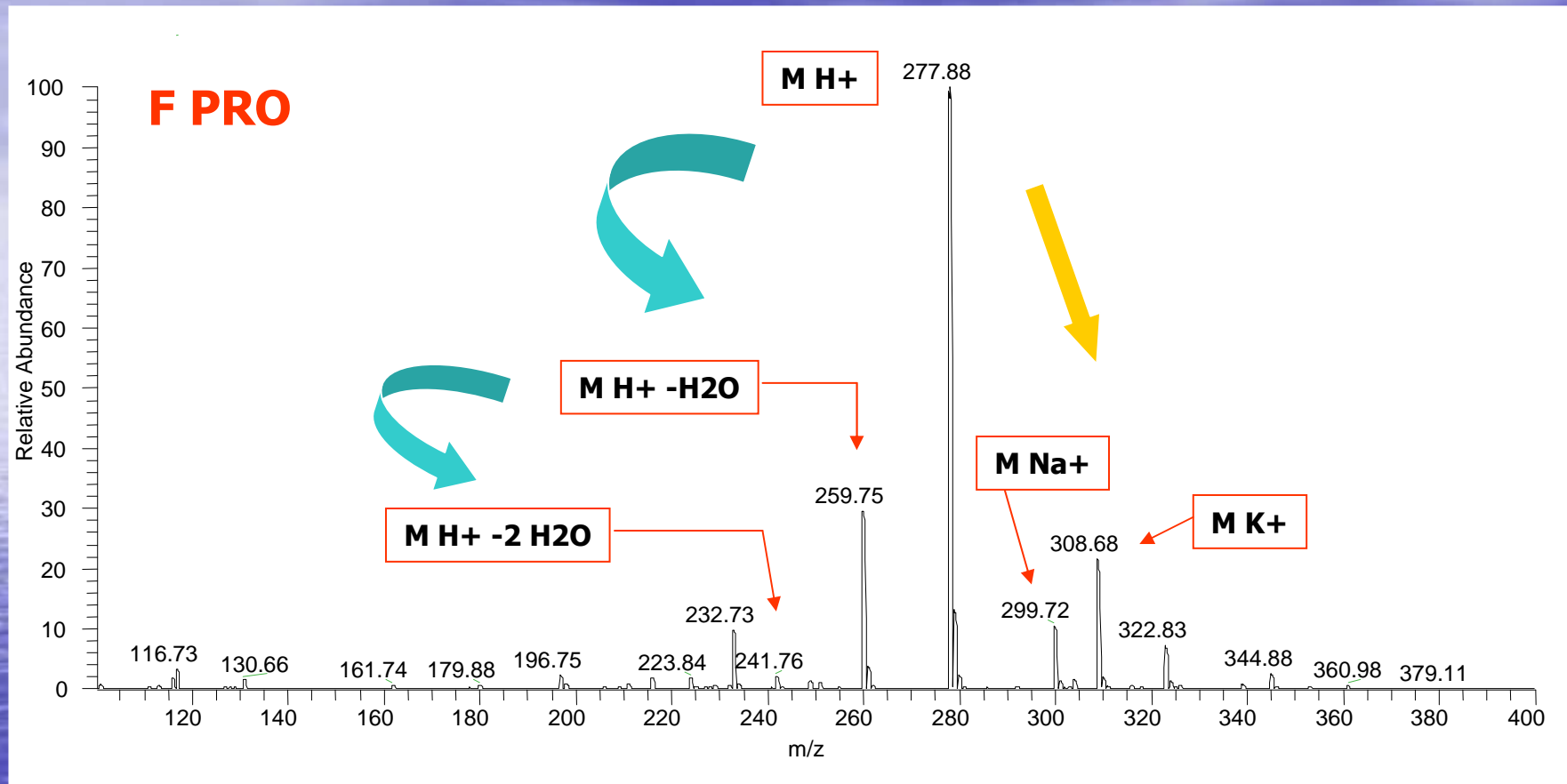




# ECD Chromatogram (Oriental tob.):



# 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.

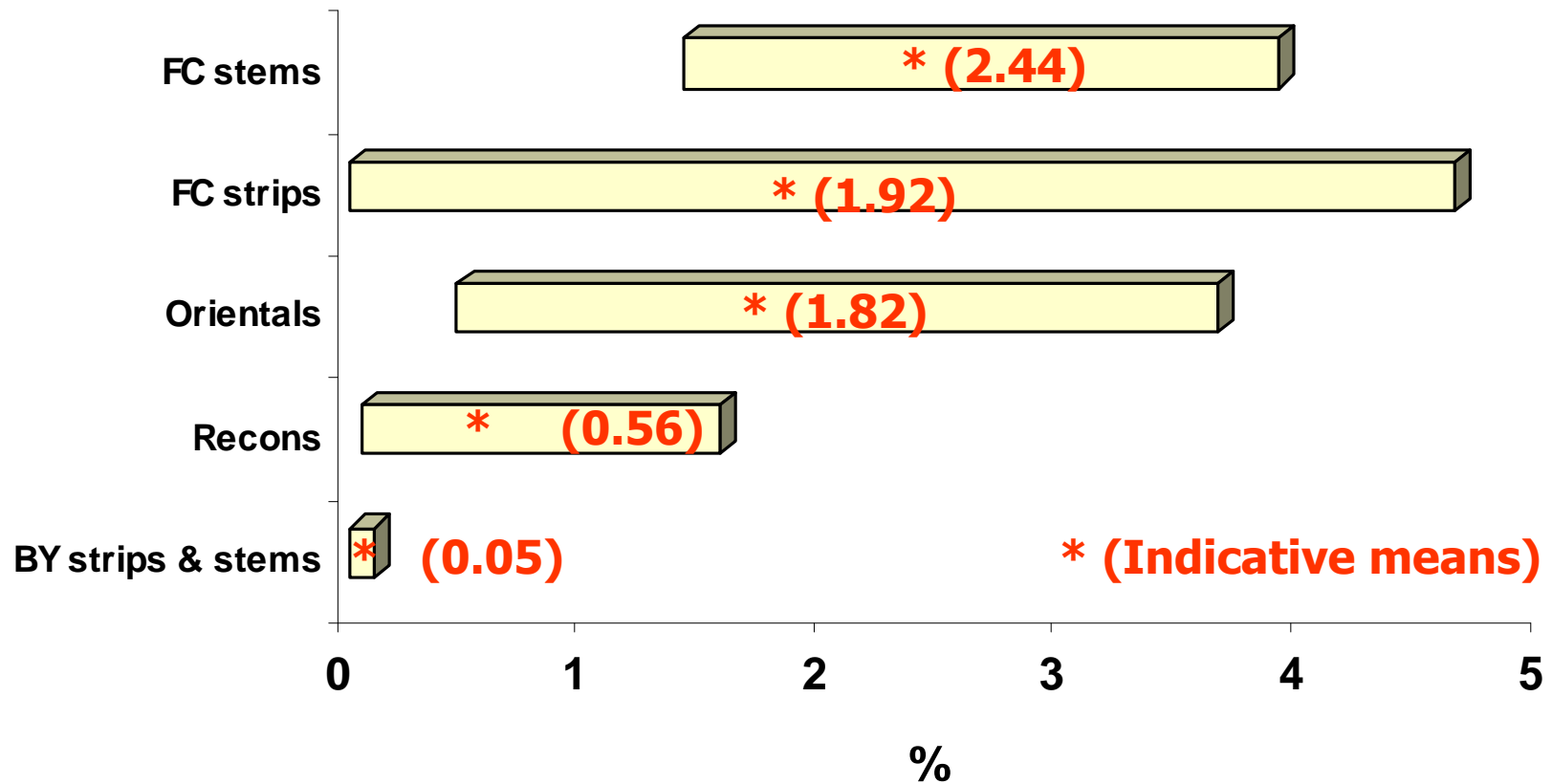
The minor Amadori compounds quantities are estimated by peak area percentage of F ASN amount.

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.

Detection limit (LOD):	0.01 %
Quantification limit (LOQ):	0.02 %

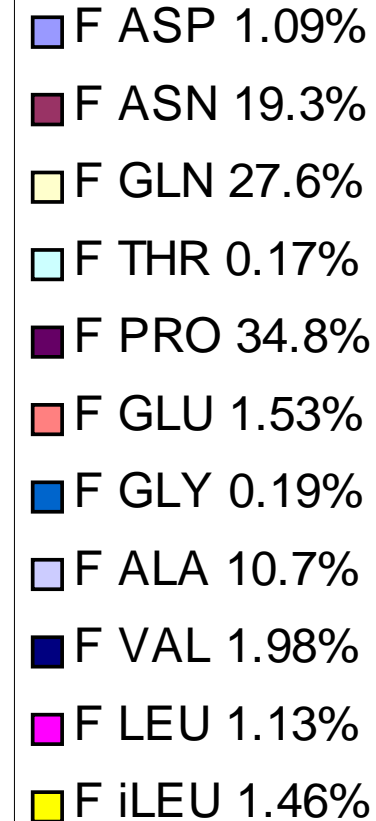
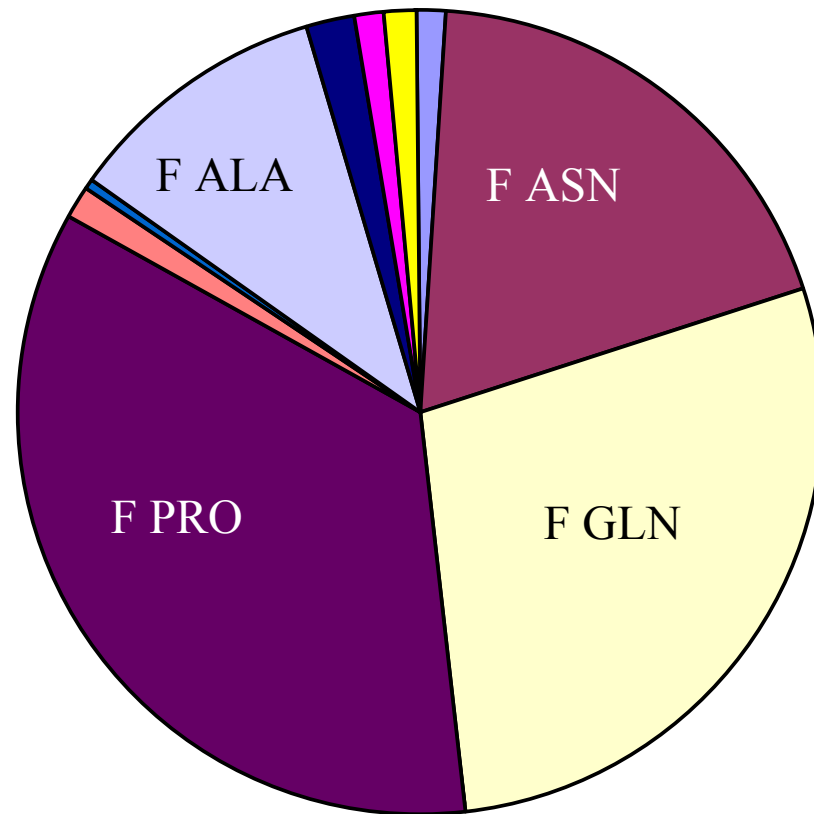
# Tobacco applications:

## Total Amadori compounds per blend components:



1500 Representative lots

# Balance of Amadori compounds amounts in a Flue Cured lot (FRA):

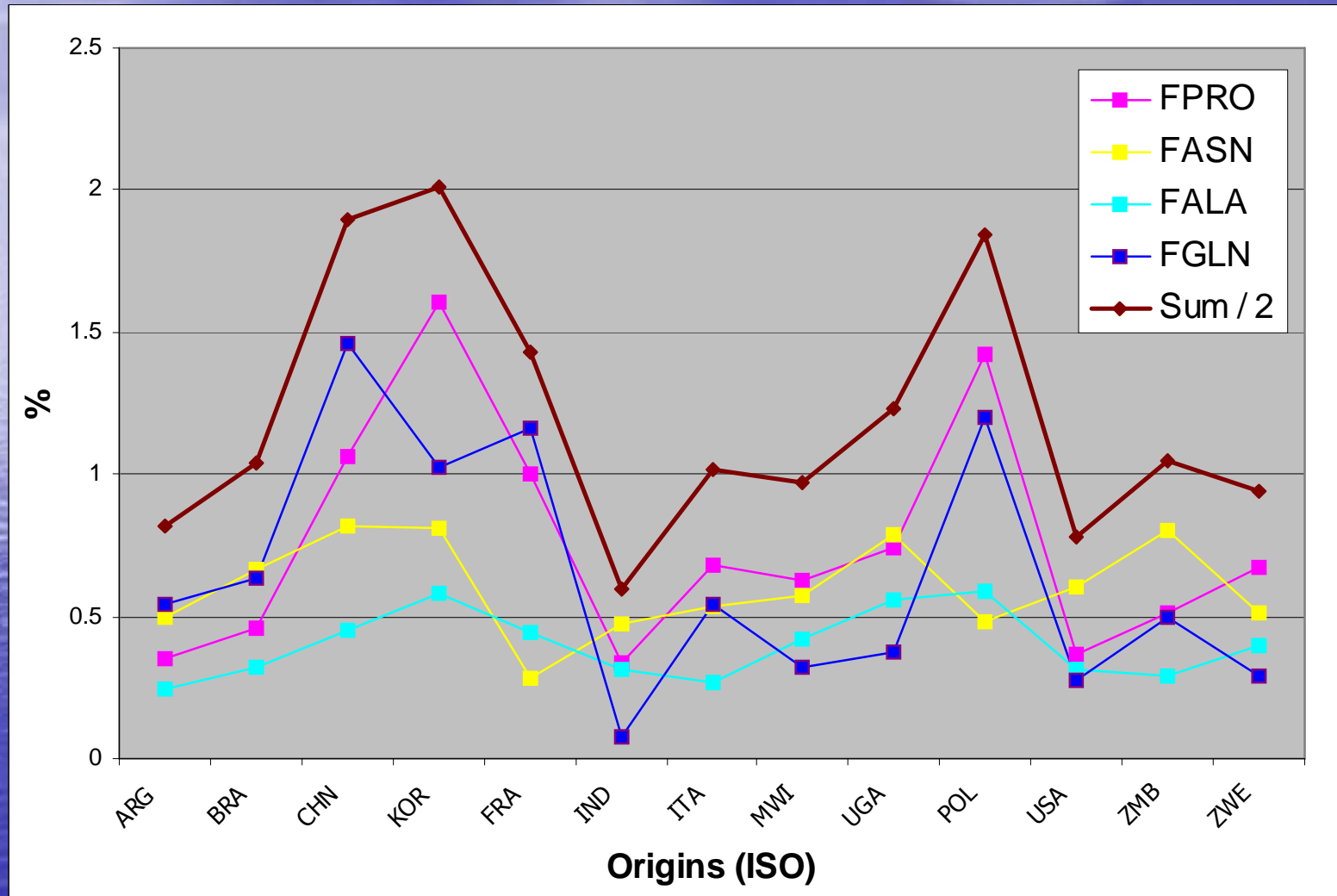


Expressed in % of total amount

>>

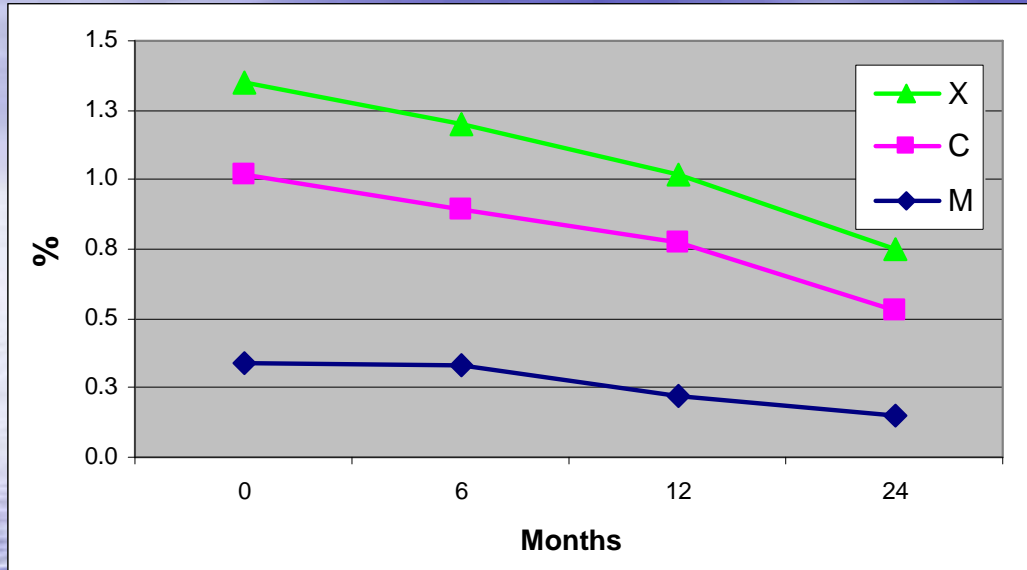
4 Major = 92%

# Incidence of origins (F.C. Medians):



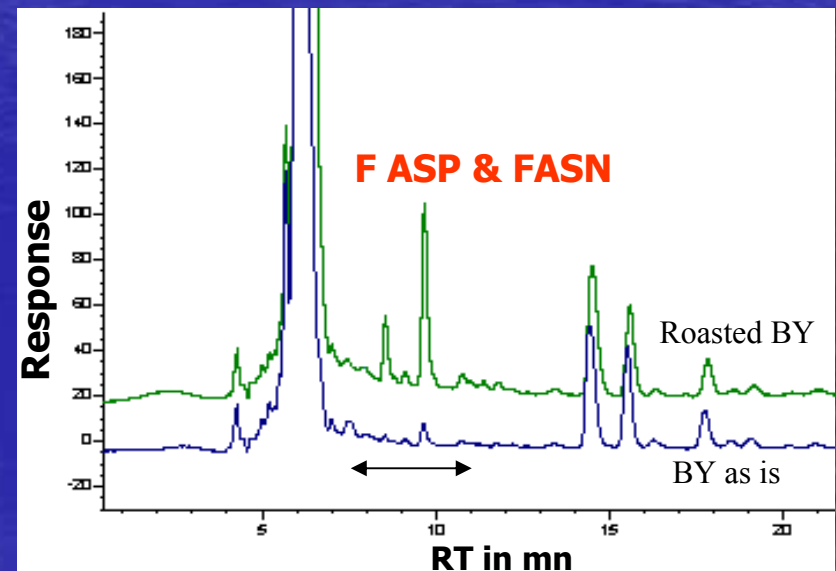
350 Representative lots of Flue cured (strips)

# Some examples of process incidence:

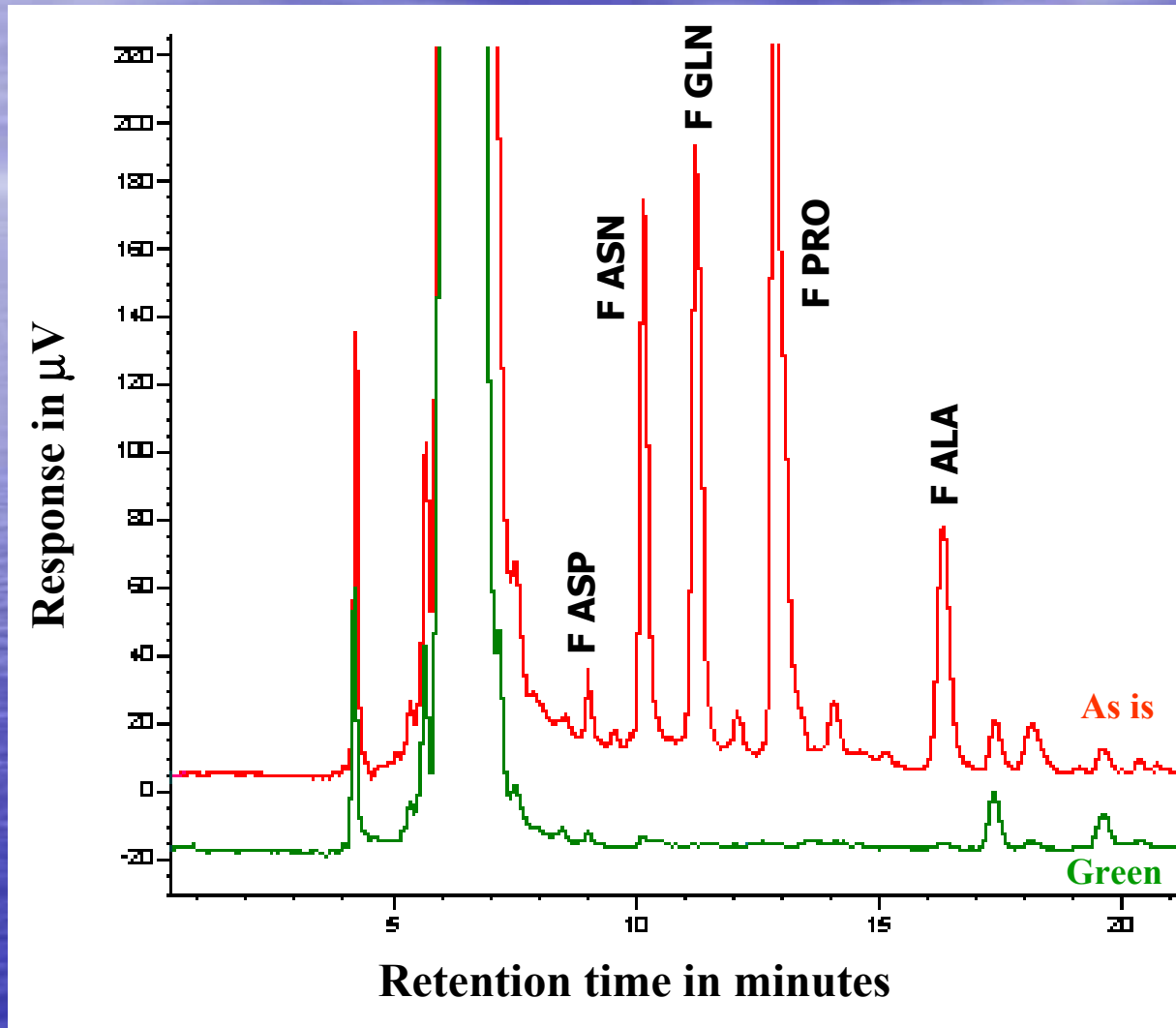


- Ageing effect on F GLN (F. C. Origin: Brazil)

- Detection of A. C. in roasted BY (Cased with reducing sugars).



# 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.**