Analysis of 16 amino acids in tobacco by ion pair LC ESI MS MS without derivatization

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Introduction

- Amino acids (AA) are chemical markers of tobacco type, maturity, origin...
- Two drawbacks for their analysis : lack of chromophoric agents and polarity
  - Derivation before their detection by UV or fluorescence : This step is often time consuming and can lead to extra errors
  - No derivation by using ionic exchange or ion pair chromatography : problems of specificity
  - Mass spectrometry can overcome this problem

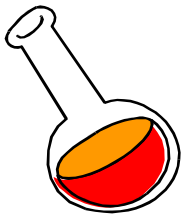
LC MS MS analysis of Amino Acids

- Work of the Organic and Analytic Chemistry Institute of Orléans University (ICOA)

  - Determination of 20 underivatized proteinic amino acids by ion pairing chromatography and pneumatically assisted electrospray mass spectrometry.
    - P Chaimbault, K. Petritis, C. Elfakir, M. Dreux.
    - *J. Chromatogr. A 855 (1999) 191 - 202.*

  - Parameter optimization for the analysis of underivatized protein amino acids by liquid chromatography and ionspray tandem mass spectrometry.
    - K. Petritis, P. Chaimbault, C. Elfakir, M. Dreux.
    - *J. Chromatogr. A 896 (2000) 253 - 263.*

Purpose



- Adapt the method to tobacco

Extraction → Separation Ion pair HPLC → Detection ESI MS MS

- Study its feasibility, performance
- Compare its results with our traditional method

- This method allows to analyze minor amino acids we don't currently analyze
- Study the interest of these new amino acids for the characterization of tobaccos

MS MS Conditions

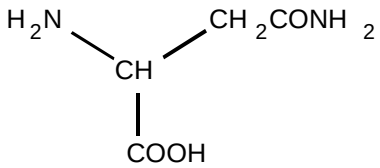
- Apparatus : Perkin Elmer Sciex API 300 triple quadrupole
- Ionization : electrospray, positive mode
- Curtain and collision gas : Nitrogen

- According to the publications, we verified
  - MS parameters for ionization
  - Fragmentation products for each AA
  - Best collision energy suitable for all the AA : 20 eV

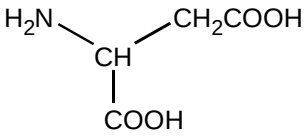
- Scan mode : Multiple Reaction Monitoring

Compounds	Q1/Q3
Alanine (ALA)	90/44
Arginine (ARG)	175/175
Asparagine (ASN)	133/87
Aspartic Acid (ASP)	134/88
Glutamic Acid (GLU)	148/130
Glutamine (GLN)	147/130
Lysine (LYS)	
Histidine (HIS)	156/110
Leucine (LEU)	132/86
Isoleucine (ILE)	
Methionine (MET)	150/104
Phenylalanine (PHE)	166/120
Proline (PRO)	116/70
Serine (SER)	106/60
Threonine (THR)	120/74
Tryptophane (TRP)	205/188
Tyrosine (TYR)	182/165
Valine (VAL)	118/72

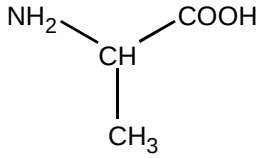
Amino acids



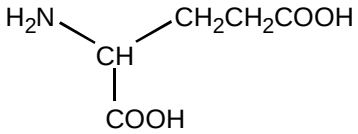
Asparagine (ASN)
133 / 87



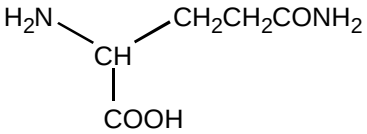
Aspartic acid (ASP)
134 / 88



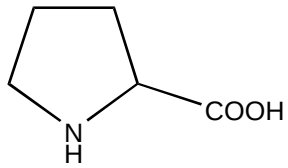
Alanine (ALA)
90 / 44



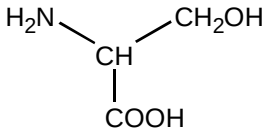
Glutamic Ac. (GLU)
148 / 130



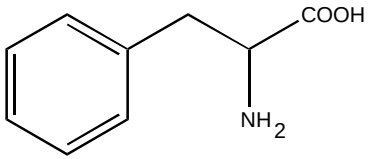
Glutamine (GLN)
147 / 130



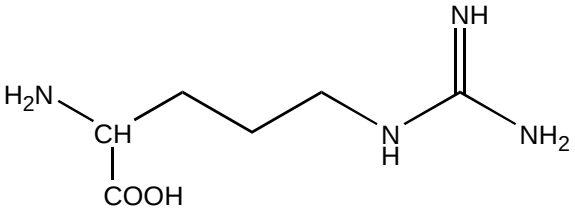
Proline (PRO)
116 / 70



Serine (SER)
106 / 60



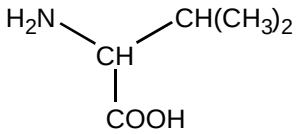
Phenylalanine (PHE)
166 / 120



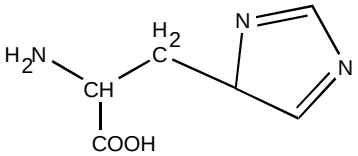
Arginine (ARG)
175 / 175

TSRC 2003 - Norfolk

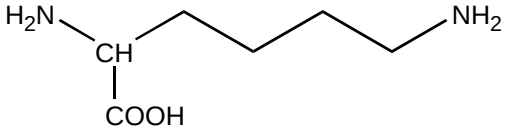
Amino acids (2)



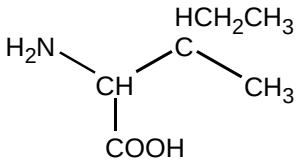
Valine (VAL)
118 / 72



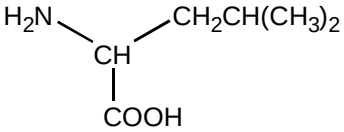
Histidine (HIS)
156 / 110



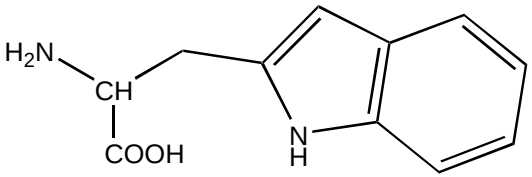
Lysine (LYS)
147 / 130



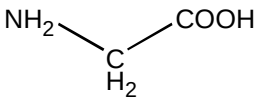
Isoleucine (ILE)
132 / 86



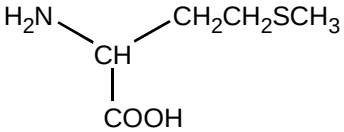
Leucine (LEU)
132 / 86



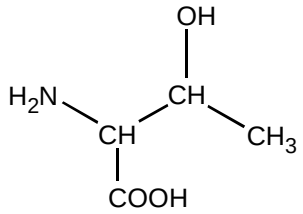
Tryptophane (TRP)
205 / 188



Glycine (GLY)
76 / 30



Methionine (MET)
150 / 104

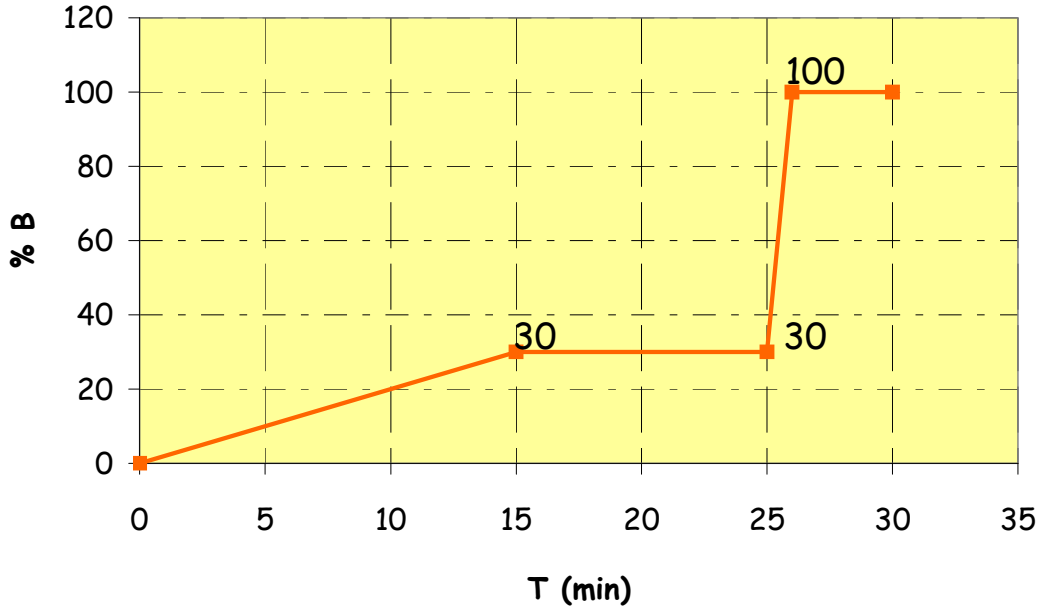


Threonine (THR)
120 / 74

LC Conditions

- Column : C18 silica. Lichrocart (Purospher) 125 x 2 mm, 5 µm
- Mobile phase :
  - A = Heptafluorobutyric acid (HFBA) 0.1 % / ACN 97.5 / 2.5
  - B = ACN
- Flow rate : 0.2 mL/min, split 1/10 before ion source

- Gradient elution



Necessity to add a washing step with pure ACN between two runs

Equilibrating time 30 min

Sample preparation

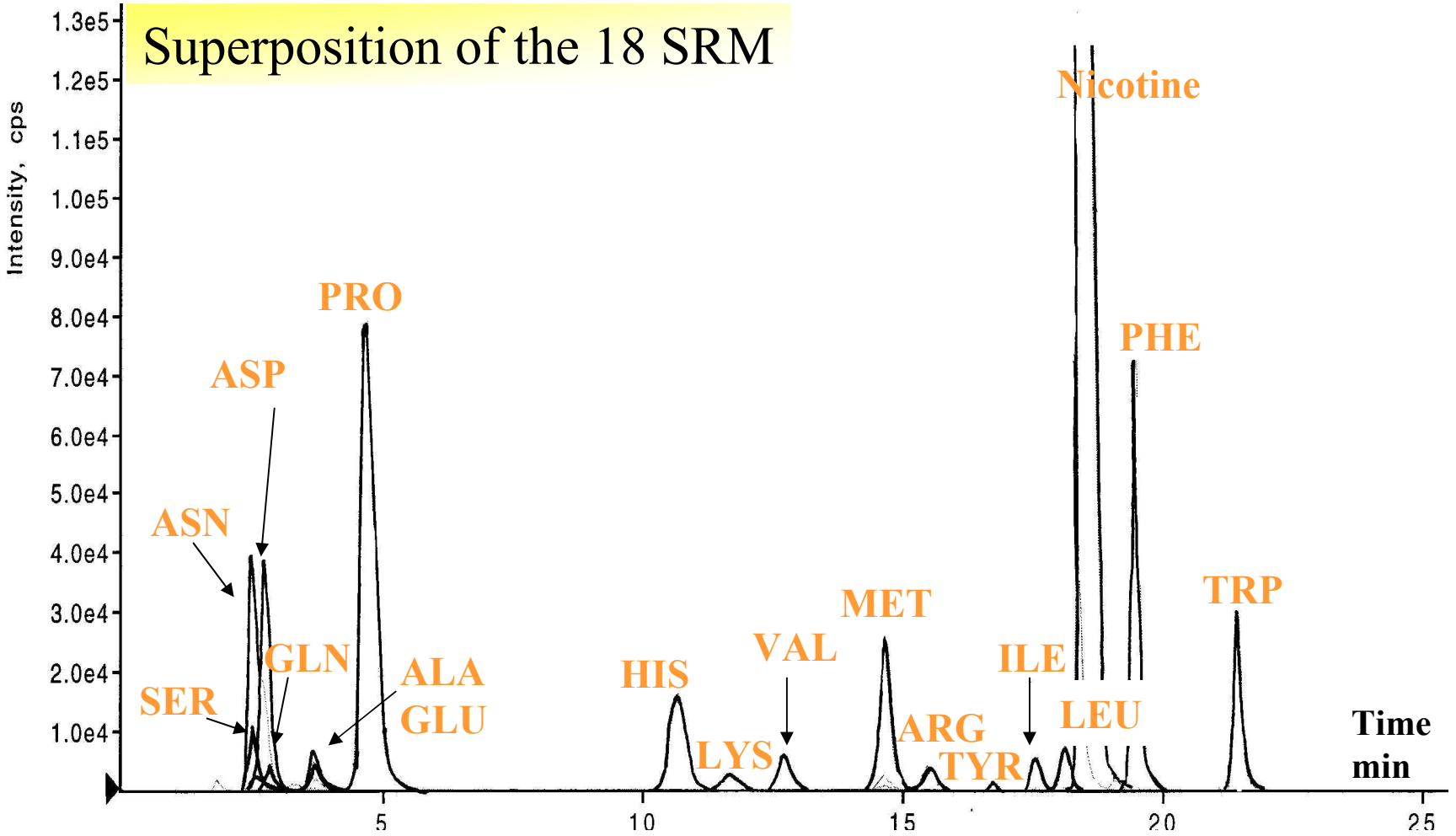
Standards

- Necessity to add HFBA in the sample to improve the formation of ion pair of HIS, ARG, LYS at the injection
- Internal Standard : choice of AA not detected in a series of tobaccos : methionine & threonine

Tobacco

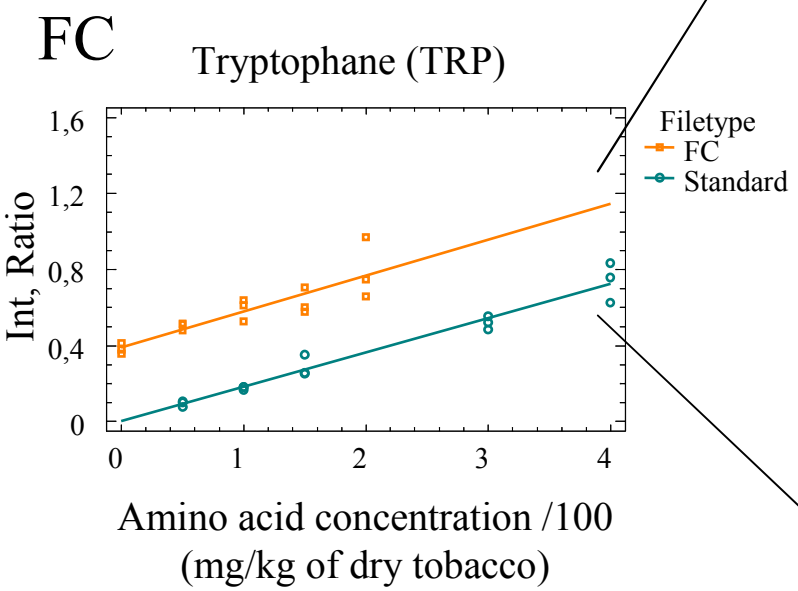
- Extraction
  - 1 g of tobacco powder in 50 mL of water
- Dilution of the extract by 2
- Addition of HFBA : 0.3 % of HFBA are necessary to obtain symmetric peaks for HIS, ARG, LYS.
- Methionine chosen as Internal Standard. Threonine was rejected due to matrix effect on tobacco.

Analysis of a Burley tobacco



- Specificity of MS MS allows to decrease the separation

Matrix Effect



Tobacco
(Burley, Flue-Cured, Oriental)

5 spikes
3 repetitions

Blank

5 concentrations
3 repetitions

2

Linearity of the response

3

Comparison with standard

4

Recovery calculation

1

Linearity of standards

Recoveries

AA	Burley Recovery (%)	Flue Cured Recovery (%)	Oriental Recovery (%)
ASN	98	106	109
SER	87	102	104
ASP	103	103	105
GLU	99	84	93
GLN	98	103	103
ALA	94	102	106
PRO	97	102	107
HIS	108	110	103
LYS	103	98	98
VAL	81	81	82
ARG	102	95	94
ILE	89	103	100
LEU	86	100	97
PHE	100	109	102
TRP	105	103	102
TYR	86	82	95

- Linearity of standards and spiked tobaccos always validated
- Some matrix effects but acceptable

Repeatability & Limit of quantification

- Analysis of a Burley tobacco during 6 weeks over 4 months

AA	CV %
ALA	7
ARG	15
ASN	13
ASP	10
GLN	18
GLU	13
HIS	15
ILE	9
LEU	14
LYS	17
PHE	15
PRO	13
SER	11
TRP	13
TYR	20
VAL	14

Necessity to calibrate every 3 days

Deuterated Internal Standards should improve the repeatability

Limits of quantification compatible with the analysis of tobaccos

- Estimation of LOQ, comparison with the mean value observed after analysis of 106 various tobaccos

LOQ mg/Kg	Mean
6	411
3	149
13	5424
3	5443
3	713
25	561
4	271
10	40
2	49
3	192
0.3	464
2	3572
4	275
0.5	276
4	71
2	82

Comparison with our current method

New Method

Extraction

↓

Ion pair HPLC

↓

ESI MS MS

↓

Asn,Asp, Glu, Gln, Ser, Pro

+

Ala, Arg, His, Tyr, Val, Leu, Ile, Trp, Phe, Lys

Analysis of tobaccos

28 Burleys
38 Flue Cured
13 Oriental

Current Method

Extraction

↓

Derivation

↓

Reverse phase HPLC

↓

UV or Fluorimetry

↓

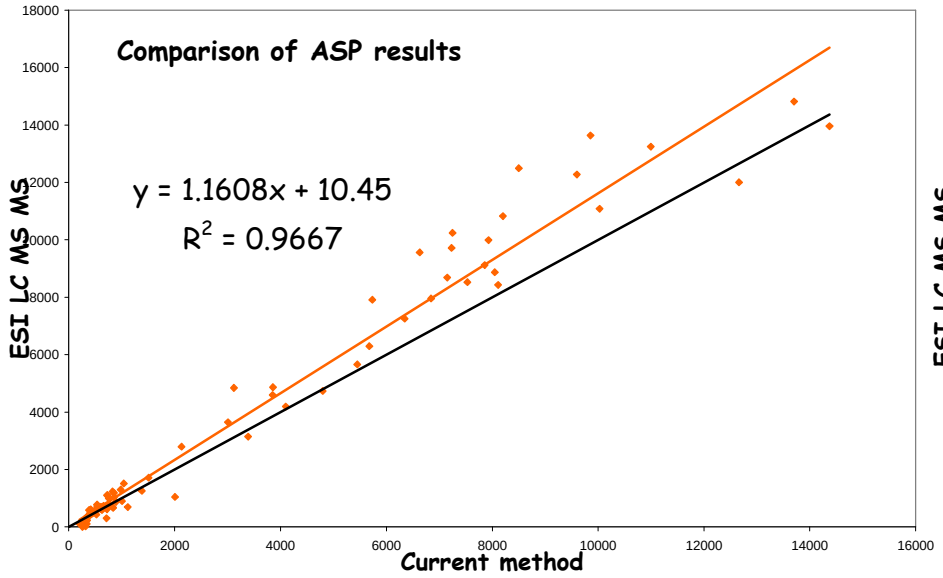
Asn,Asp, Glu, Gln, Ser, Pro

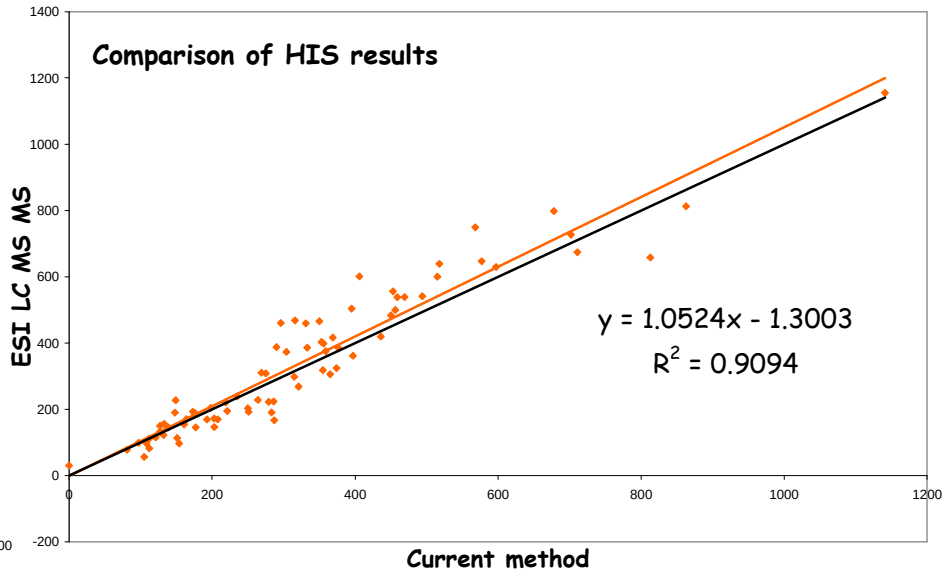
+

Ala, Arg, His

Comparison with our current method

AA	R2 adjusted				Slope
	Global	By	FC	O	Global
ASN	91.6	91	81	99	1.03
SER	77	84	51	96	1.13
ASP	96.6	84	84	99	1.16
GLN	80	80	67	75	1.02
GLU	79	95	9	93	0.94
PRO	91	95	77	64.6	0.88
ARG	95	92	58	96	1.16
HIS	90.9	94	78	94	1.05
ALA	16.6	62	3	32	0.3





Comparison with our current method

- Similar results
- Shorter analysis time
  - no derivation
  - faster separation
- Lower limits of detection for amino acids : at least a factor 10
- Possibility of analyzing new amino acids
- Reproducibility should be improved

Application to the characterization of tobaccos

Extraction → Separation Ion pair HPLC → Detection ESI MS MS

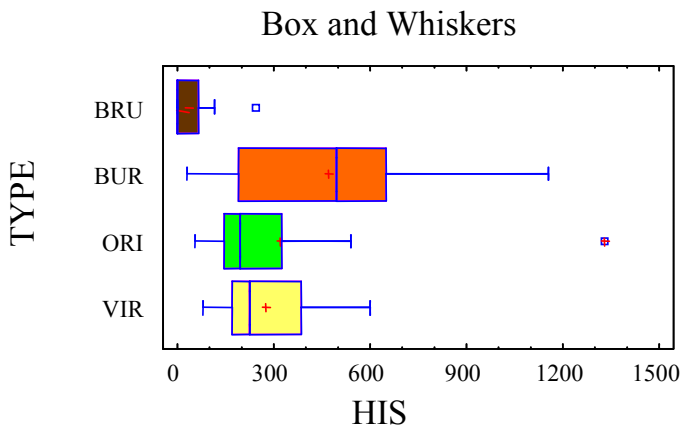
New AA compared to our current method

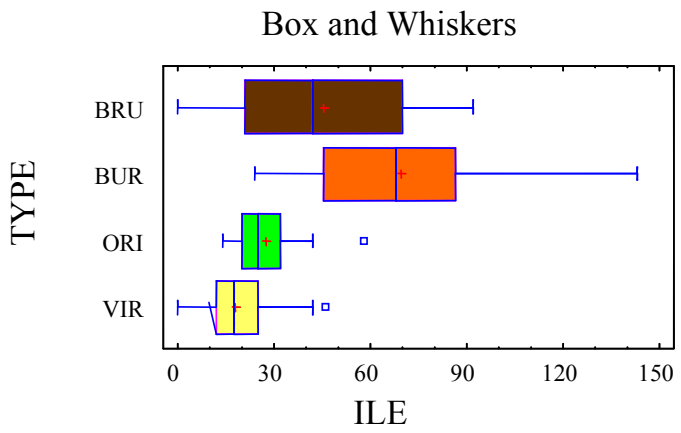
Analysis of tobaccos

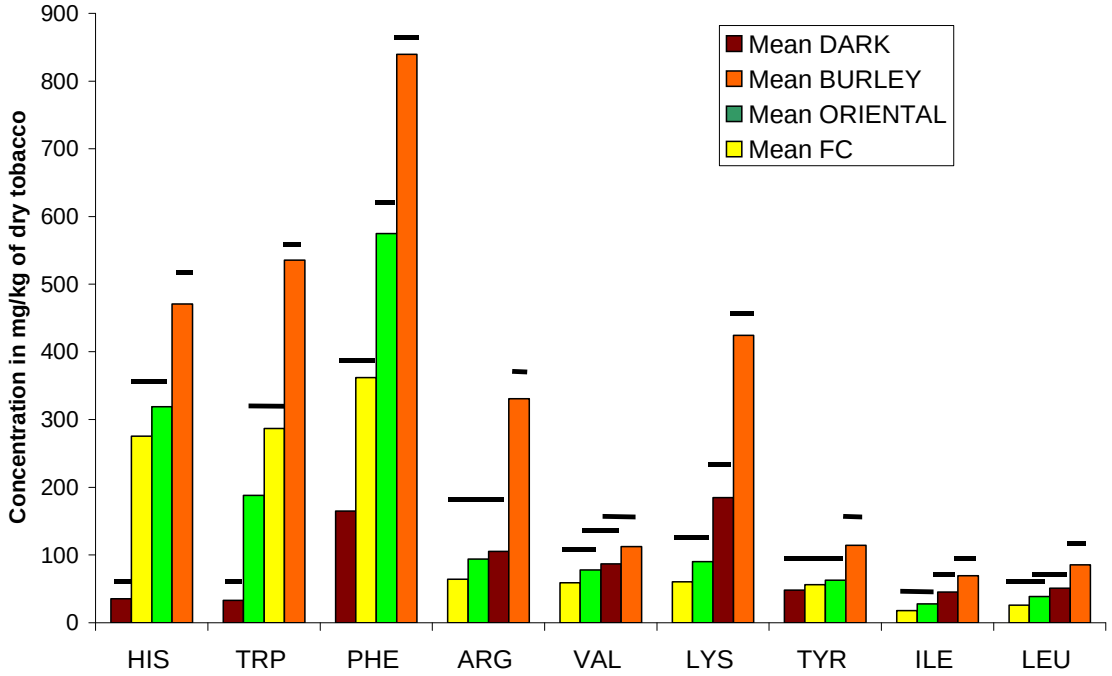
28 Burleys
27 Dark
38 Flue Cured
13 Oriental

Do these new AA improve the characterization of tobaccos ?

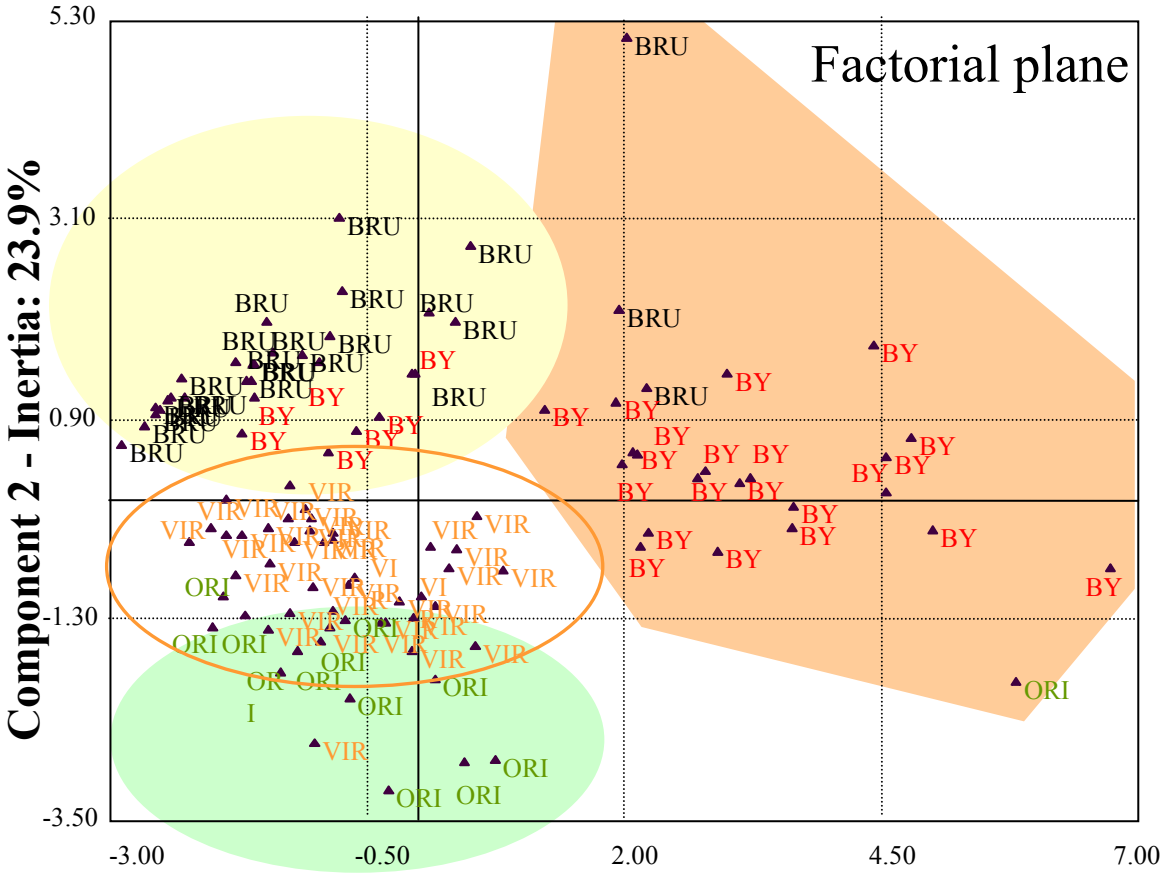
Differences between tobacco types

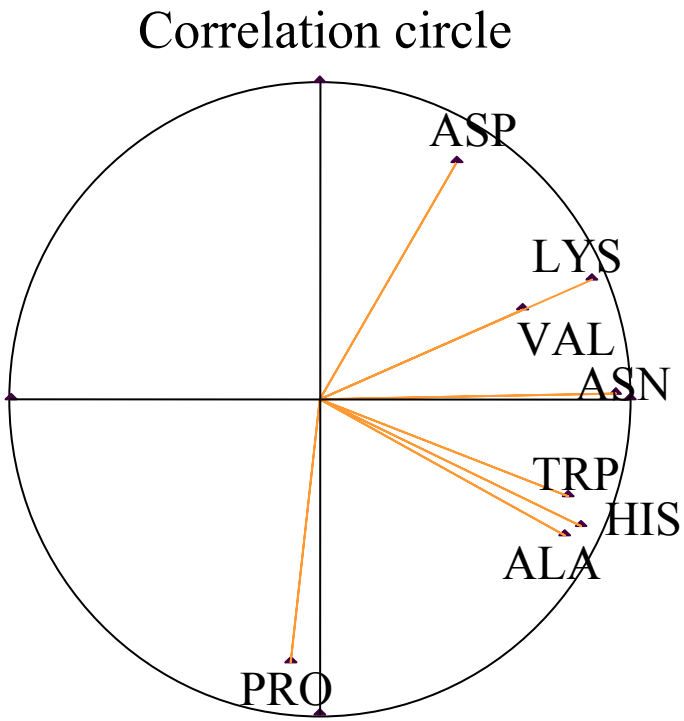






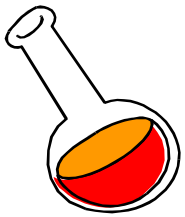
Selection of the best amino acids





- Ala, Trp, His, Val, Lys bring some information
- AA separate well Burley tobaccos

Conclusion



- Amino acids can be analyzed by ESI LC MS MS without derivation in tobaccos
- Results are comparable to our current method
- The major amino acids can be analyzed within 6 minutes
- Some new amino acids can be better detected

- A lot of minor amino acids are Burley markers
- Ala, Trp, His, Val, Lys help discriminating tobacco types
- Study the interest of the minor amino acids for the characterization of tobaccos from the same tobacco type (Burley)