Combining Chemometrics and Paper Spray Mass Spectrometry for Forensic Analysis and Food Authenticity Testing

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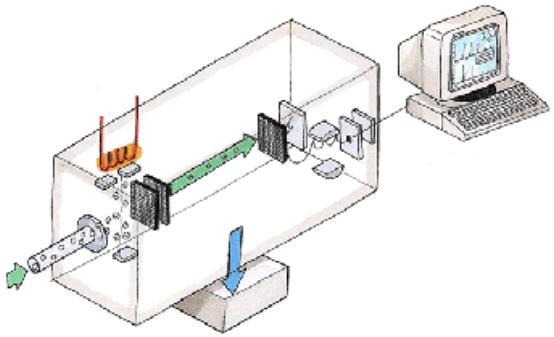
Ambient Ionization Mass Spectrometry

Modern MS is useful for characterizing complex matrices.

MS provides specific molecular information through the interpretation of the obtained fingerprints.

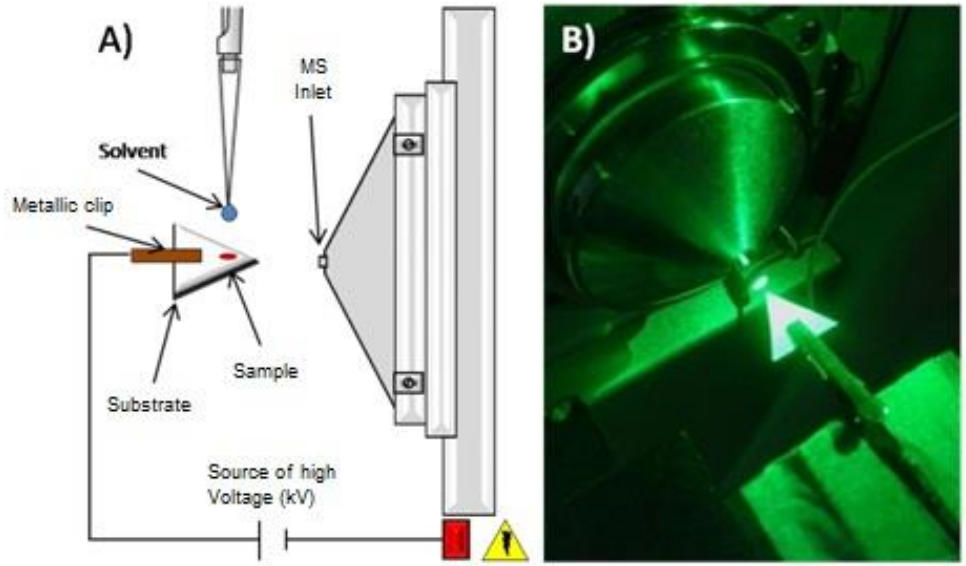


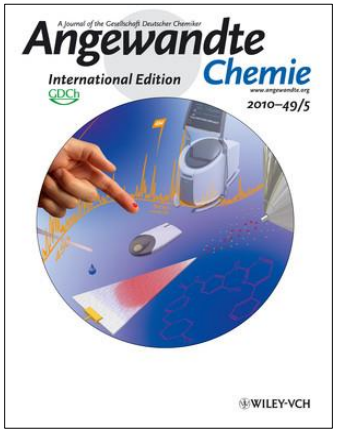
In a search of simplification of sample pretreatment, several ambient ionization MS techniques have been developed in the last years (EASI, DESI, DART, etc.).

Paper Spray Mass Spectrometry (PS-MS)

An ambient ionization technique, developed in 2010.

This is a simple and low cost technique that has been applied to complex matrices demanding a very small amount of solvents.





Mass Spectrometry

DOI: 10.1002/anie.200906314

Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry**

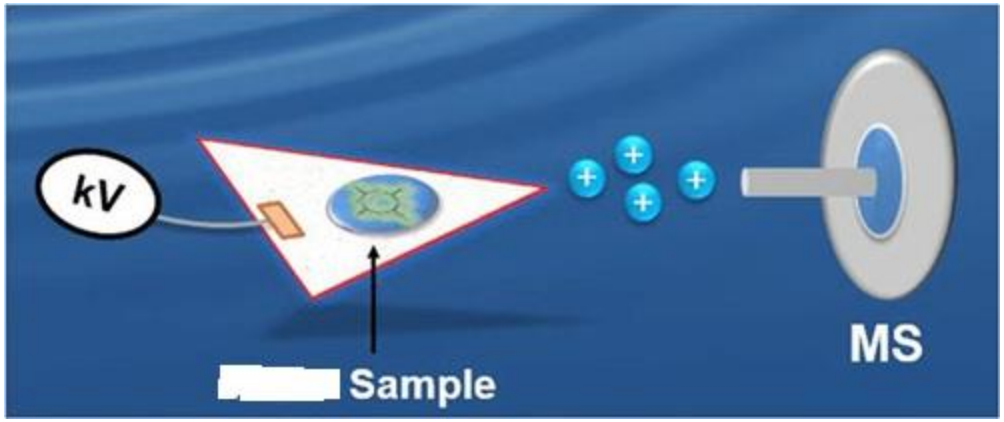
He Wang, Jiangjiang Liu, R. Graham Cooks, and Zheng Ouyang**

PURDUE UNIVERSITY

R.G. Cooks

Paper Spray Mass Spectrometry (PS-MS)

The porous substrate used can retain some interference compounds from complex matrices, minimizing matrix and ionic suppression effects, and thus improving the ionization efficiency.



S. Maher et al., *Scientific Reports* 6:35643 (2016).

OBJECTIVE

The combination of chemometrics and PS-MS to develop **screening methods** for **forensic analysis** and **food authenticity** testing.

1) Supervised Classification ⇒ **Forensic applications**
2) Multivariate Calibration
3) Data Fusion

Collaborators

Prof. Rodinei Augusti (UFMG)

Prof. E. Piccin

PhD Students

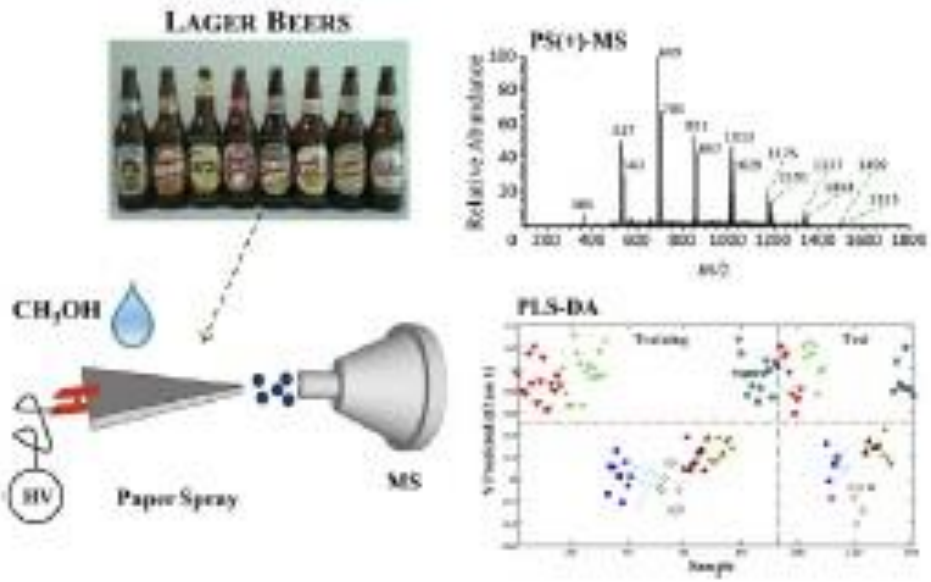
Hebert Pereira

Victória Amador

Janaína Teodoro

COUNTERFEIT BEER

PS-MS, PLS-DA and Variable Selection Applied to the Detection of Counterfeit Beer



COUNTERFEIT BEER

Beer counterfeiters switch labels and bottle caps of less expensive brands by those of the more expensive market leader brands.

BUDWEISER DOWNPLAYS COUNTERFEIT BEER OPERATION IN CHINA

1st June, 2017 by Natalie Wang

Budweiser has downplayed the scale of a counterfeit beer operation busted in China's southern Guangdong province, after a video showing an unhygienic beer canning line at an underground factory went viral.

"Throughout the world, Budweiser is brewed and packaged with great care and passion and according to the highest quality standards," the company says in ar statement sent to *dbHK*.

"The video that has been circulating on some social networks is from a small-sc counterfeit operator in China. We have been working with local authorities to shu down immediately".

The illegal operation was churning out 60,000 crates of counterfeit Budweiser canned beer a month, before it was busted by local authorities.

"Budweiser takes great care in every detail of its product and packaging. Cheap counterfeits have telltale signs that they are fakes such as imperfect seals, incor

ALL INTERNATIONAL BREWERS FACE SAME THREAT: HEINEKEN

Heineken 'absolutely on top' of fake beer threat after Vietnam gang bust

By Ben BOUCKLEY

25-Jun-2013 - Last updated on 04-May-2017 at 10:39 GMT

Brazilian beer market is dominated by 4 big groups producing American standard lager.

Only one group (68% of the market) produces the 3 most consumed brands (Brahma, Skol, Antárctica), which are target of counterfeit by cheaper brands (Cintra, Crystal, Glacial, Lokal, A Outra).

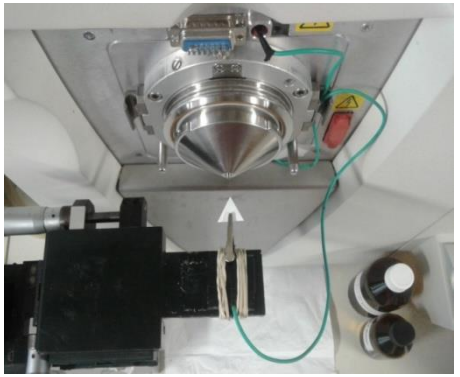
COUNTERFEIT BEER

141 samples of 8 brands and different batches.

Brewery	Brand	Brand Code	Batches	Bottles
Anheuser-Busch InBev	Antarctica*	A1	10	24
	Brahma*	A2	10	24
	Skol*	A3	10	24
Brasil Kirin	Cintra	B1	5	14
	Glacial	B2	5	14
Grupo Petrópolis	Crystal	C1	5	13
	Lokal	C2	5	14
Krill	A Outra	D1	5	14

* Brands with higher commercial price

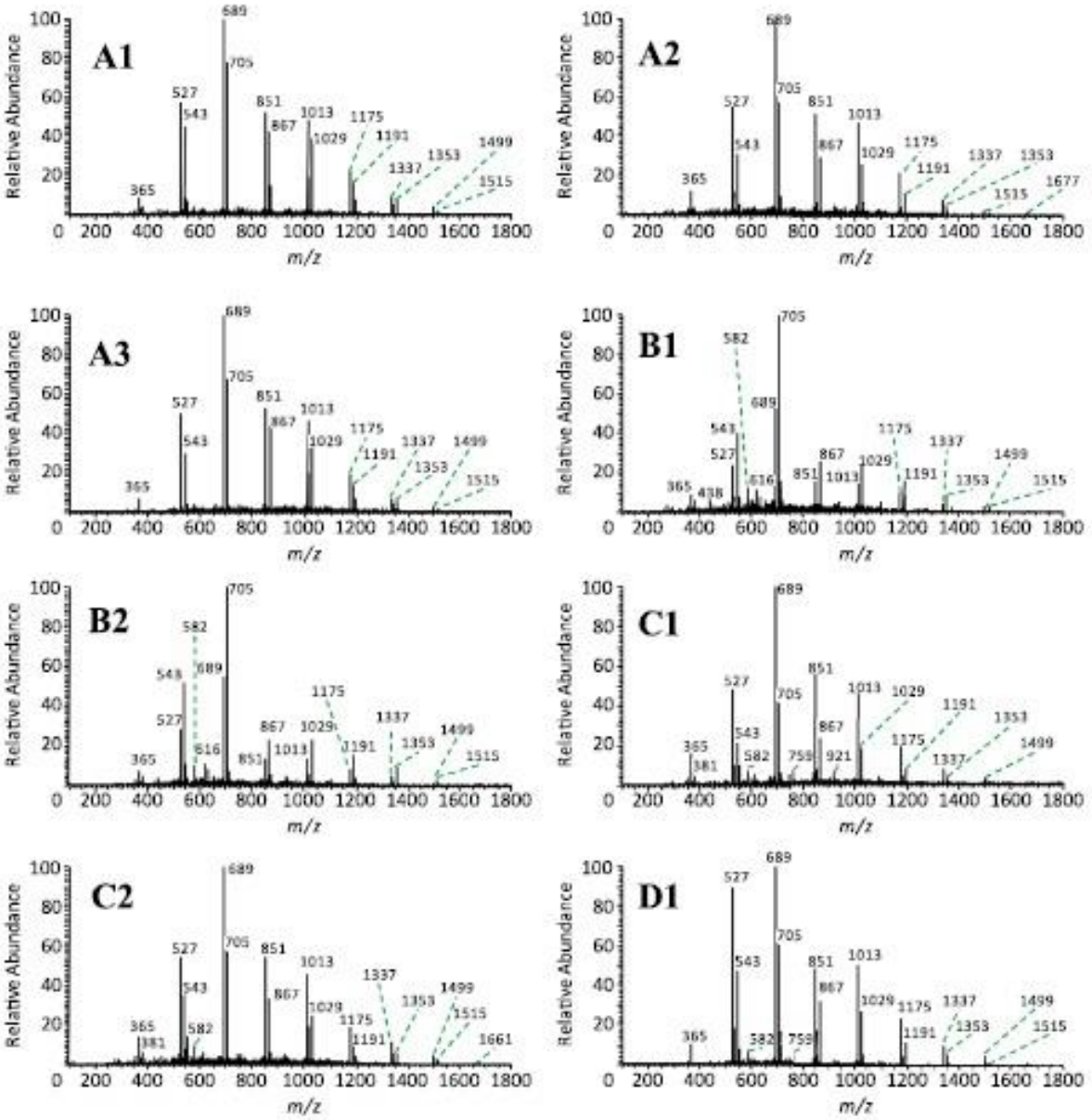
Positive Mode



Thermo LCQ FLEET – *Ion trap*

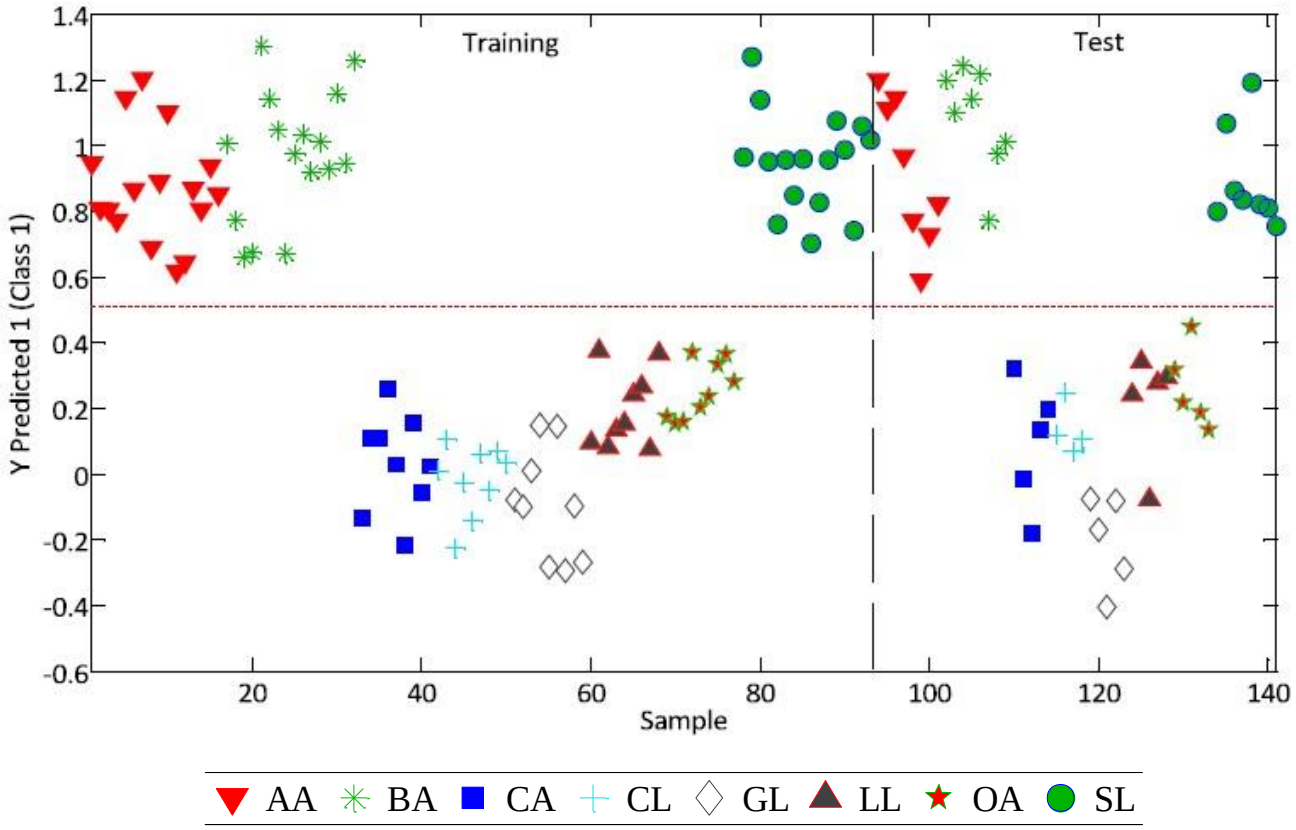
COUNTERFEIT BEER

Mass Spectra



COUNTERFEIT BEER

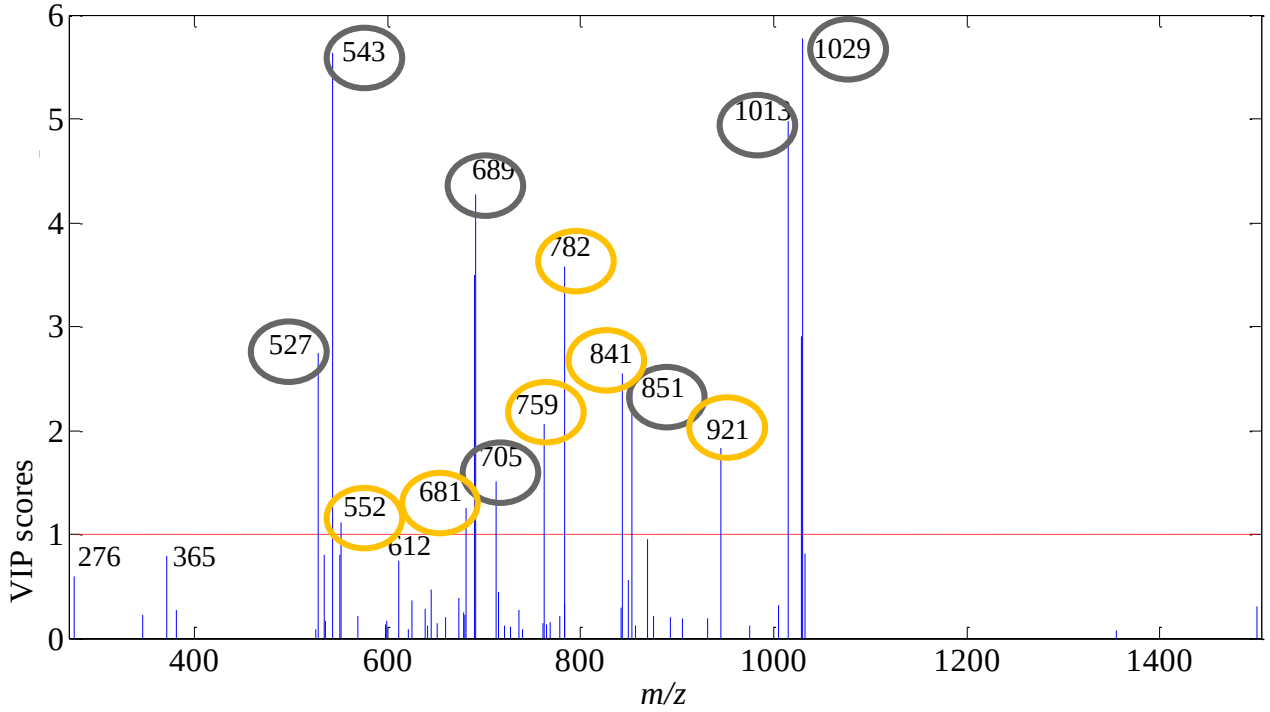
PLS-DA improved by OPS (*Ordered Predictors Selection*) variable selection: from 1701 (full spectra) to 60 *variables*



Nº LV = 4 **X** Variance = 75.8% **y** Variance = 85.8%

COUNTERFEIT BEER

15 diagnostic ions were detected from the most significant VIP scores (>1)



Aducts of Na^+ e K^+ of malto-oligossacharides

m/z not assigned

COUNTERFEIT BEER

- MS *fingerprints* allowed the discrimination between brand beers target of counterfeit and brands used as counterfeit.
- The method is very rapid, requiring a minimum of sample preparation and consuming a very small volume of solvent (some µL).

Analytica Chimica Acta 940 (2016) 104–112

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

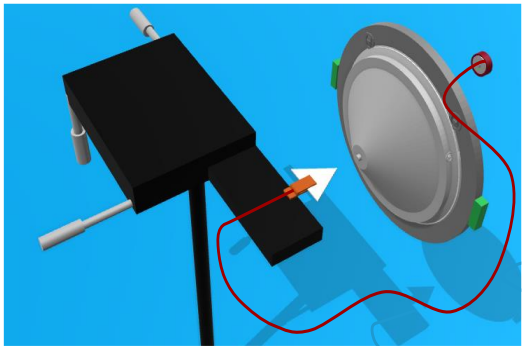
Paper spray mass spectrometry and PLS-DA improved by variable selection for the forensic discrimination of beers

Hebert Vinicius Pereira [a], Victória Silva Amador [a], Marcelo Martins Sena [a, b], Rodinei Augusti [a], Evandro Piccin [a, *]

[a] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Instituto Nacional de Ciência e Tecnologia em Bioanalítica, Campinas, SP, Brazil

COUNTERFEIT WHISKY

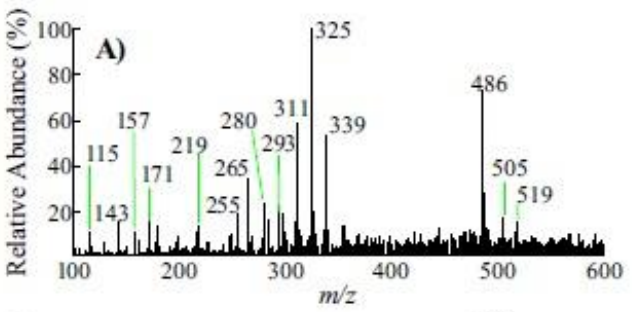
PS-MS and PLS-DA Applied to the Detection of Seized Samples of Counterfeit Whisky

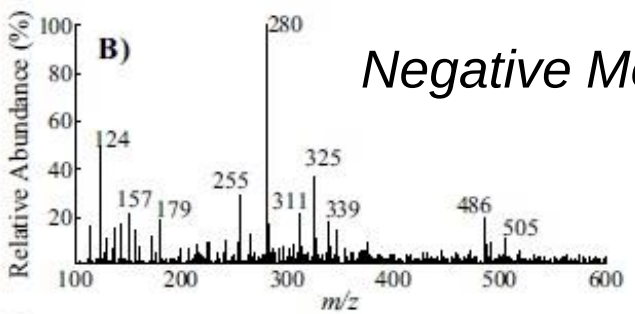


Seized samples obtained from Brazilian Federal Police

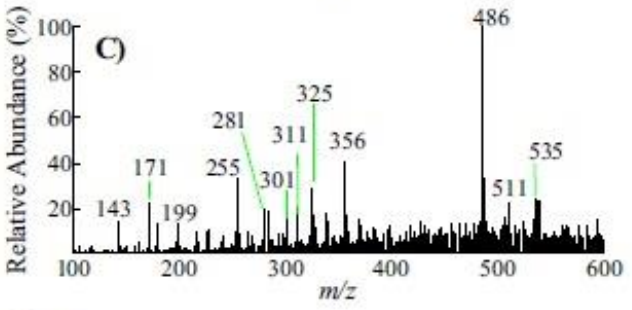
COUNTERFEIT WHISKY

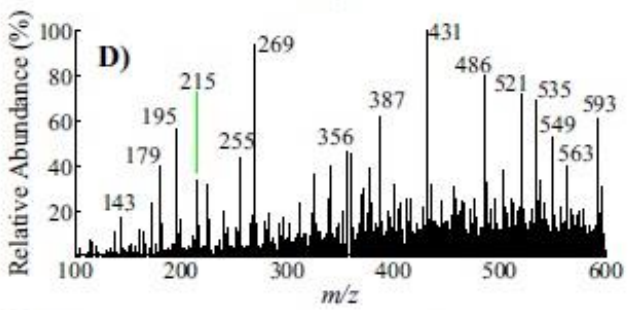
- 44 seized samples of 3 whisky brands
- 44 authentic samples of 3 whisky brands

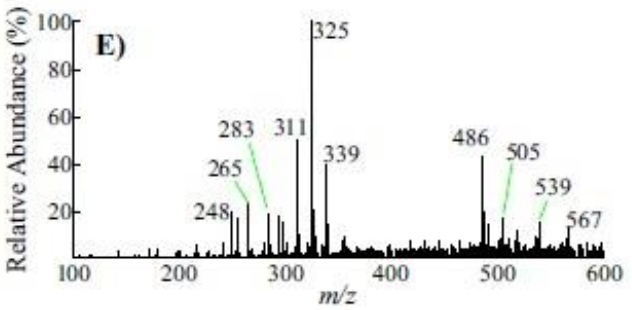


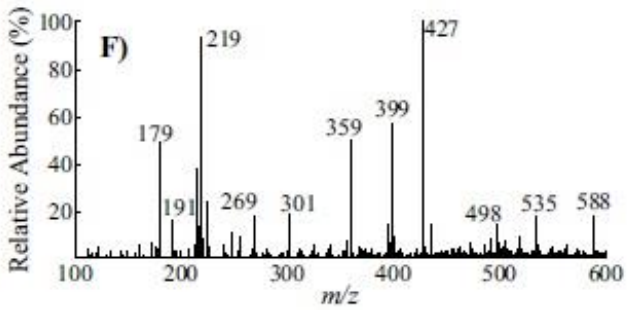


Negative Mode





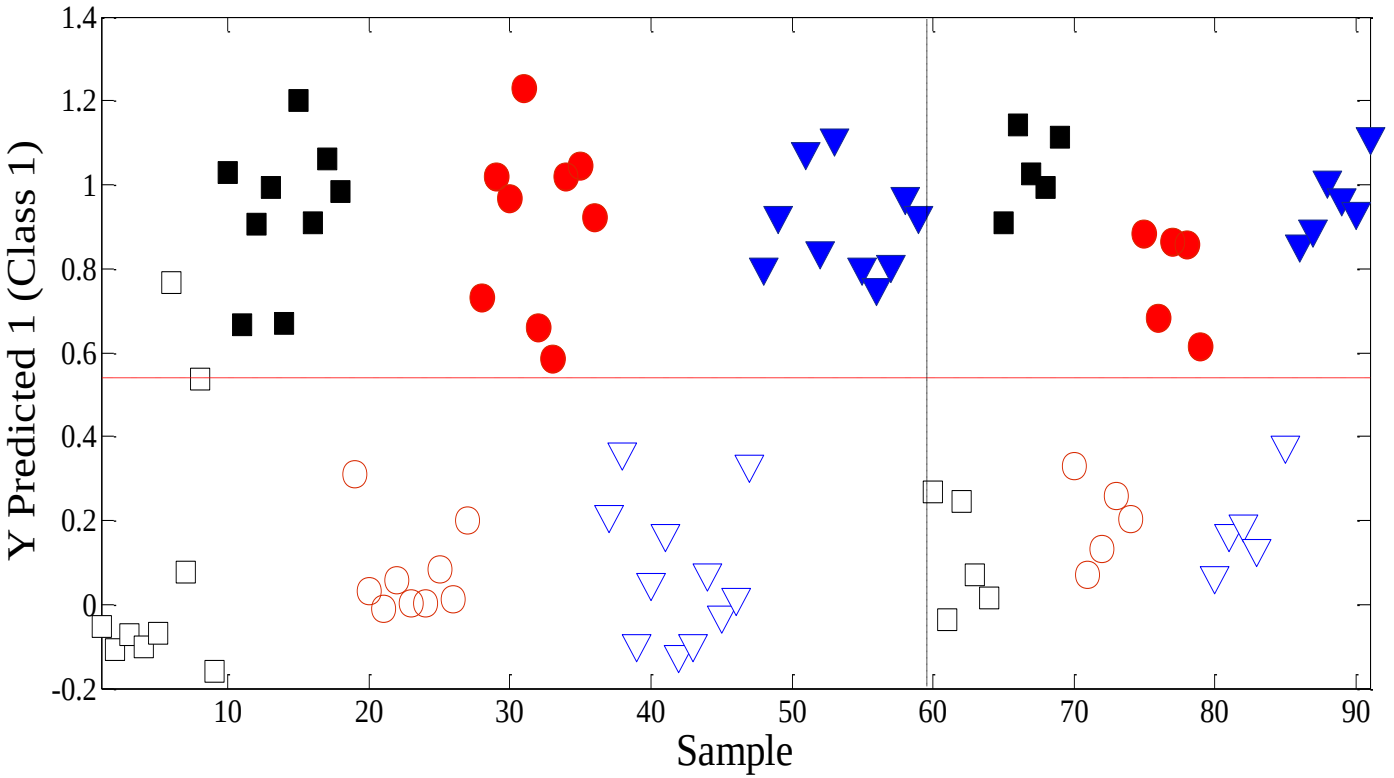




AUTHENTIC **COUNTERFEIT**

COUNTERFEIT WHISKY

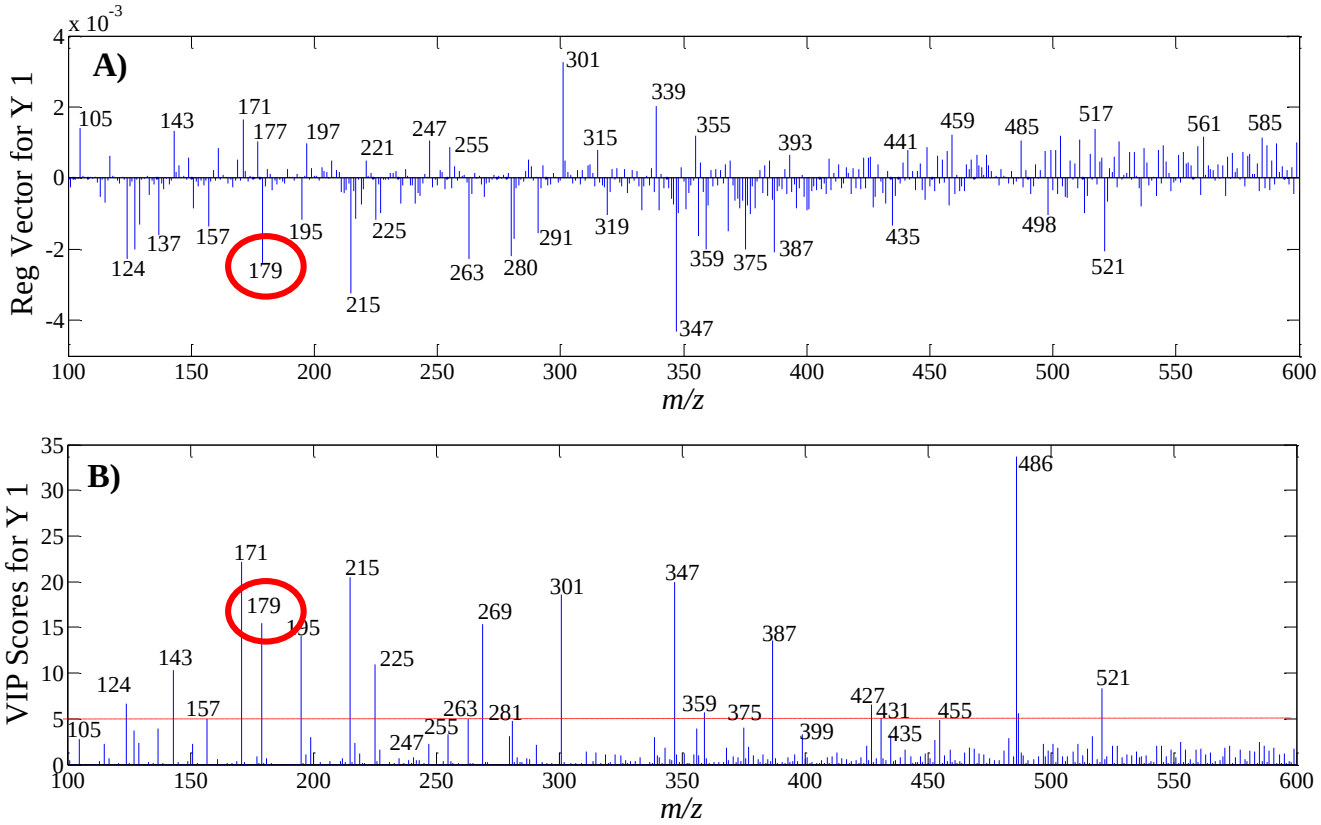
PLS-DA Model Predictions

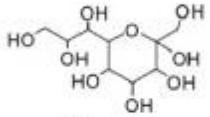
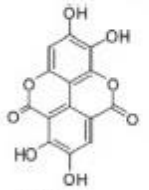
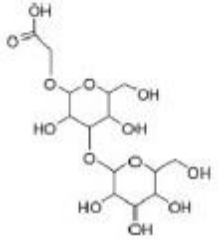


Only one False Positive in the Training Set

COUNTERFEIT WHISKY

Spectral Interpretation : (A) Regression Vector (B) VIP scores



m/z [M–H]⁻	Proposed structure
143	
171	
199	
269	
301	
399	

Ion of *m/z* 179: deprotonated form of a monosaccharide (marker of counterfeit whisky)

COUNTERFEIT WHISKY

Food Chemistry 237 (2017) 1058–1064

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Paper spray mass spectrometry and chemometric tools for a fast and reliable identification of counterfeit blended Scottish whiskies

Janaína Aparecida Reis Teodoro [a], Hebert Vinicius Pereira [a], Marcelo Martins Sena [a], Evandro Piccin [a], Jorge Jardim Zacca [b], Rodinei Augusti [a,*]

[a] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Polícia Federal, Ministério da Justiça, Instituto Nacional de Criminalística, 70390-145 Brasília, DF, Brazil

COUNTERFEIT PERFUME

Discriminant (PLS-DA) *versus* One-Class Modeling (SIMCA) Applied to the Detection of Seized Samples of Counterfeit Perfumes

Seized samples obtained from Civil Police of the State of São Paulo (Brazil)

COUNTERFEIT PERFUME

60 Samples (31 counterfeit + 29 authentic) of 10 brands from “O Boticário”

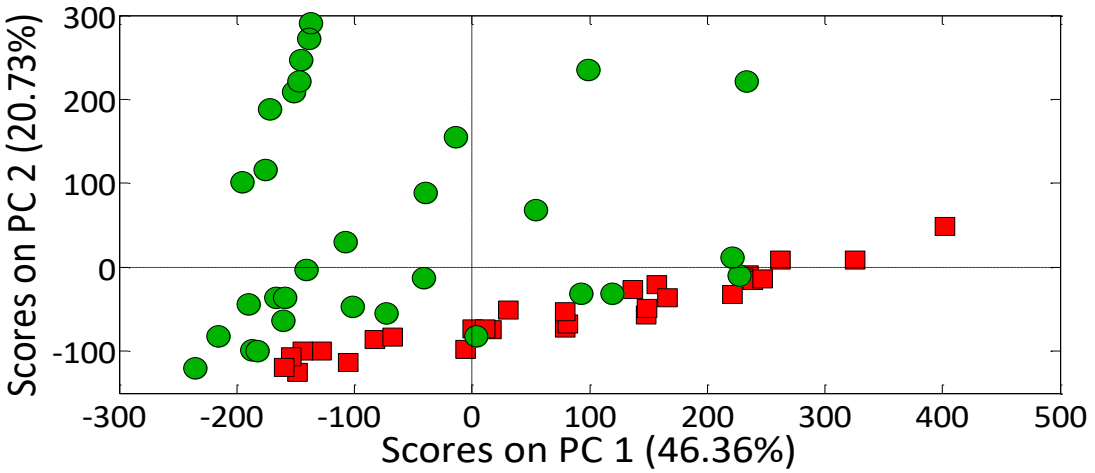
Table 1 - Description of the perfume samples analysed by PS-MS.

Brand	Brand codes	Authentic samples	Counterfeit samples
Arbo	AB	3	2
Egeo dolce	ED	3	4
Egeo Woman	EW	3	1
Egeo Man	EM	3	3
Floratta in blue	FB	3	3
Floratta in rose	FR	2	2
Malbec	MB	3	8
Portinari	PT	3	3
Quasar azul	QA	3	4
Quasar vermelho	QV	3	1
TOTAL		29	31

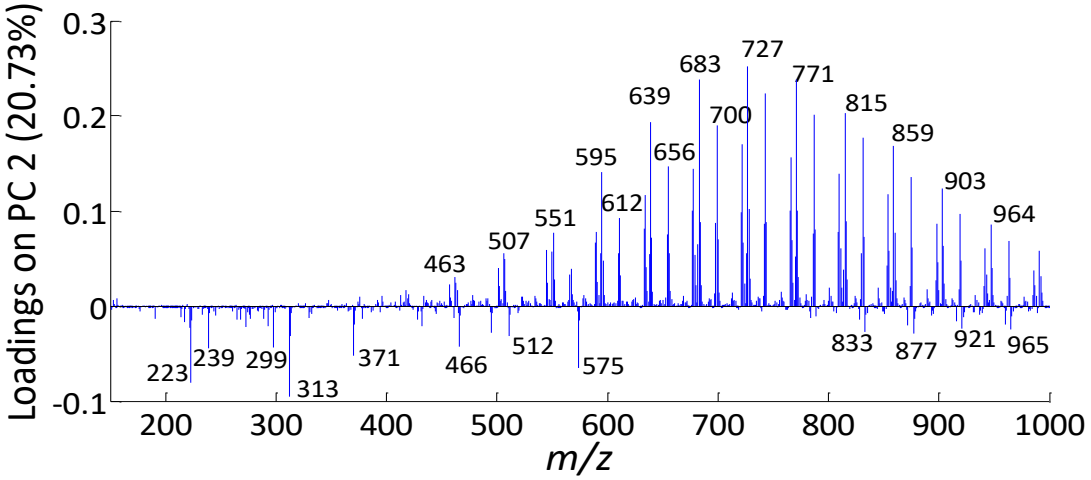
COUNTERFEIT PERFUME

PCA MODEL

A)

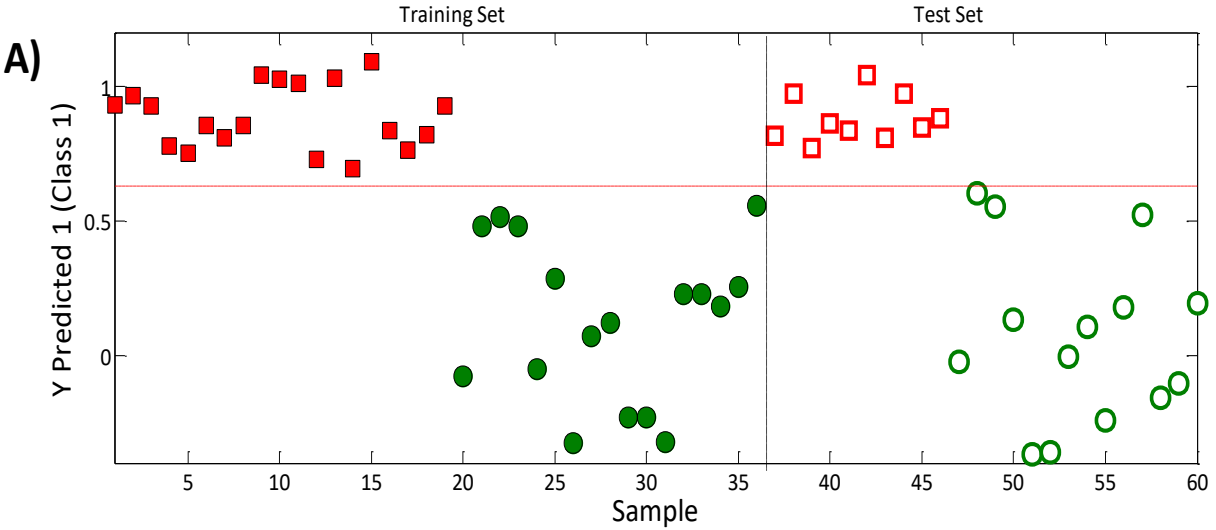


B)

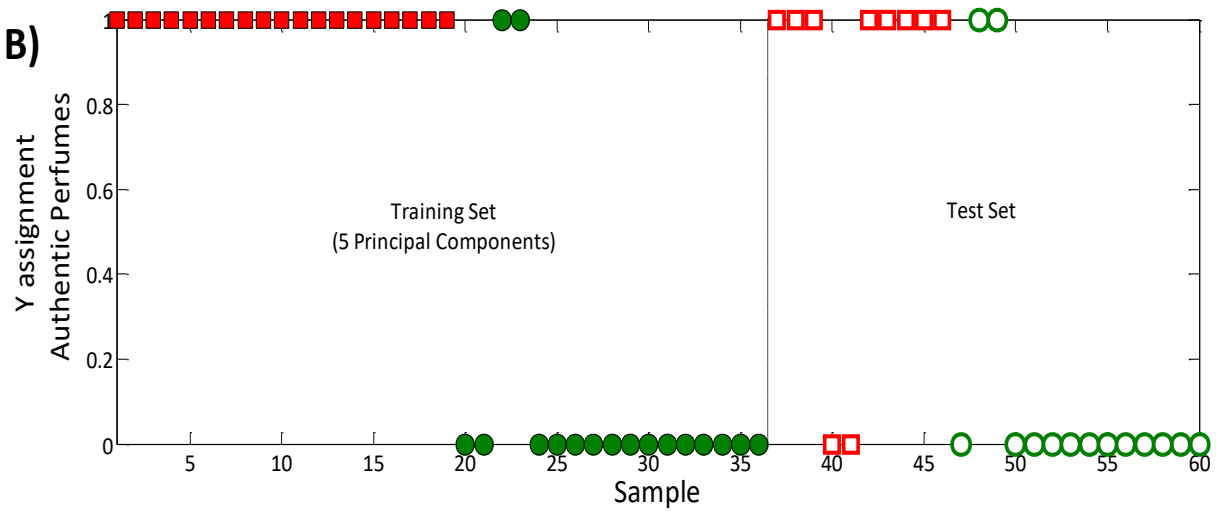


COUNTERFEIT PERFUME

Comparison between PLS-DA and *one-class* SIMCA



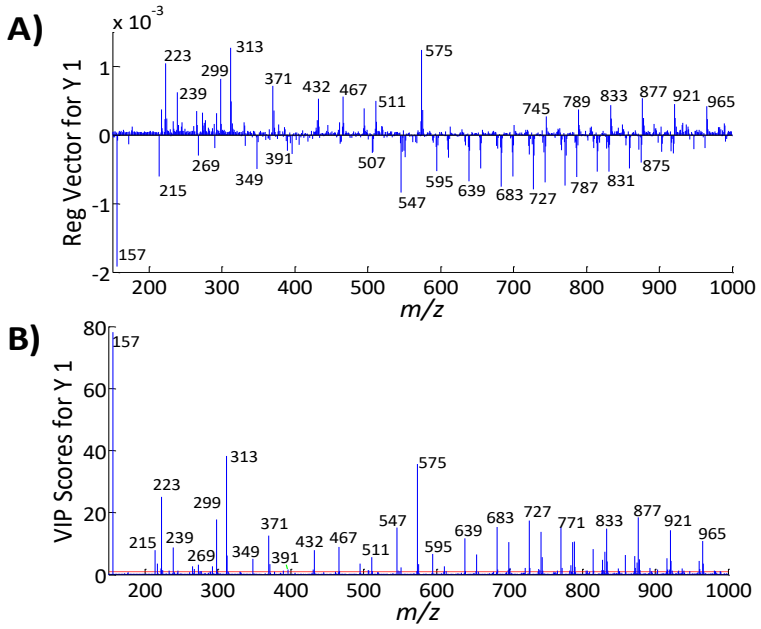
PLS-DA



SIMCA

COUNTERFEIT PERFUME

Spectral Interpretation : (A) Regression Vector (B) VIP scores



Diagnostic ions for authentic and counterfeit perfumes were proposed based on this spectral interpretation.

Some of the diagnostic ions for counterfeit perfumes were suggested to be compounds of allergenic potential.

Table 2 - Proposed structures for some diagnostic ions detected by PS(+)-MS and highlighted in the informative vectors of the PLS-DA model.

m/z	Ion Species	Suggested attributions	Perfumery application
157	$[M+H]^+$	Citronellol[11,45]	Fragrance
215	$[M+Na]^+$	Damascene[11]	Fragrance
	$[M+Na]^+$	Dimehtyl benzyl carbonyl acetate (DMBCA)[11]	Fragrance
269	$[M+Na]^+$	Benzopheone-2[48,49]	UV radiation filter
	$[M+H]^+$	2-tert-butyl-4,6-dinitro-5 methylanisole (Musk ambrette)[46]	Fragrance
	$[M+Na]^+$	Methyl cedryl Ketone (Acetycedrene)[11]	Fragrance precursor
313	$[M+Na]^+$	Ethylhexyl methoxycinnamate[50]	UV radiation filter
371	$[M+H]^+$	Decamethylcyclopentasiloxane[47]	Solvent
391	$[M+H]^+$	Bis (2-ethylhexyl)phthalate (DEHP)[9,46]	Solvent/ fixative
	$[M+H]^+$	Di-n-octylphthalate (DNOP)[46]	Solvent/ fixative

COUNTERFEIT PERFUME

Analytical Methods

ARTICLE

Received 00th January 2017,

Forensic discrimination
perfumes using paper spra
supervised classification

J. A. R. Teodoro,[a] H. V. Pereira,[a] D. N, Co

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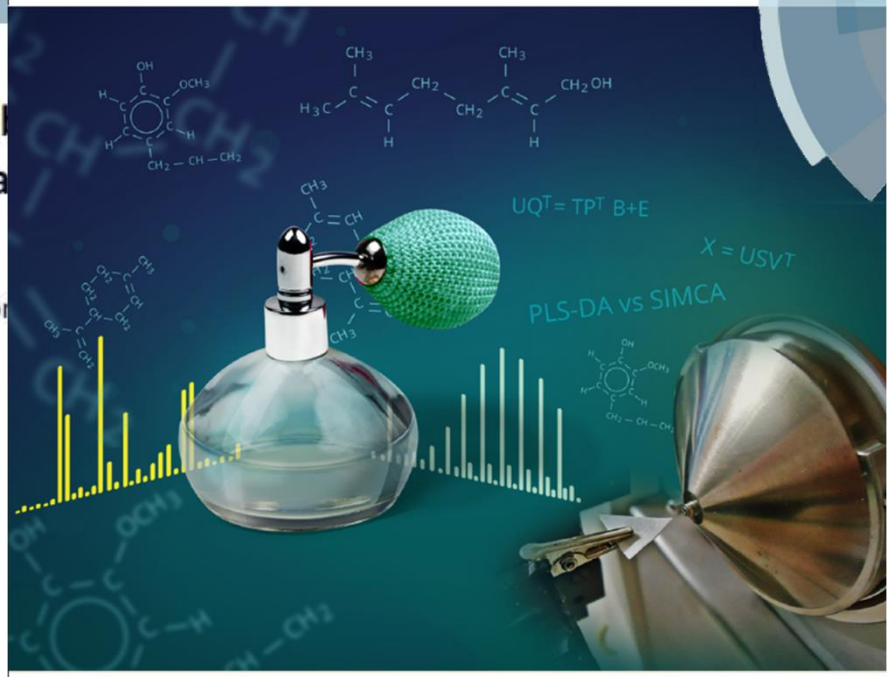
Analytical Methods

Accepted Manuscript



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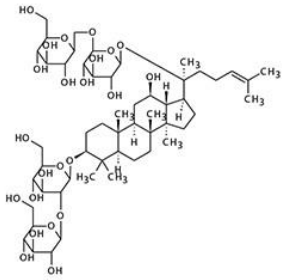
rsc.li/methods

GINSENG AUTHENTICATION

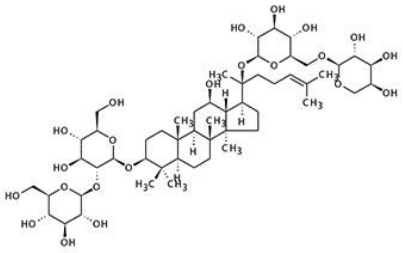
A Multivariate Calibration Model for Quantifying Ginseng Adulteration using PS-MS

Ginseng is the root of plants in the genus *Panax*, typically characterized by the presence of ***ginsenosides***, that has been used in traditional medicine for its health effects.

Multivariate Calibration Models with PS-MS are very rare.



Ginsenoside RB_1



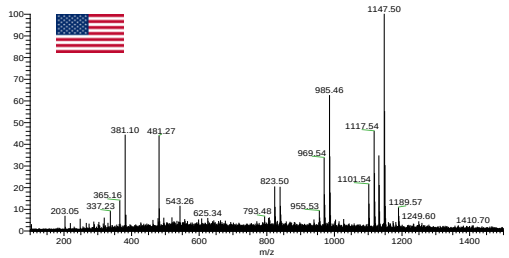
Ginsenoside RB_2

GINSENG AUTHENTICATION

AMERICAN GINSENG

Panax quinquefolius

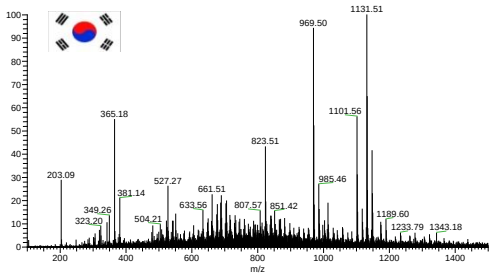
- Strengthens the body.
- Aphrodisiac.
- Gives energy.
- Tones the stomach.



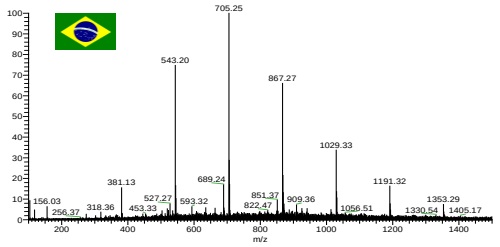
KOREAN GINSENG

Panax ginseng)

- Stimulates memory.
- Fight anemia.
- Decreases tiredness.
- Improves blood circulation.



BRAZILIAN GINSENG (***Pfaffia paniculata***) doesn't have the ginsenosides responsible for therapeutic effect. The phytochemical characteristics are similar to korean ginseng.



GINSENG AUTHENTICATION

CBS NEWS NEWS SHOWS VIDEO CBSN MORE

Herbal supplements filled with fake ingredients, investigators find

41 Comments / Share / Tweet / Stumble / Email

Last Updated Feb 3, 2015 9:44 PM EST

Numerous store brand supplements aren't what their labels claim to be, according to an ongoing investigation that subjected popular herbal supplements to DNA testing.

The investigation, led by New York Attorney General Eric Schneiderman, focused on a variety of herbal supplements from four major retailers: GNC, Target, Walmart and Walgreen Co. Lab tests determined that only 21 percent of the products actually had DNA from the the plants advertised on the labels.

"This investigation makes one thing abundantly clear: The old adage 'buyer beware' may be especially true for consumers of herbal supplements," Schneiderman said. His office issued cease and desist letters to the retailers on Monday telling them to stop sales of the products.

The investigation found supplements, including echinacea, ginseng, St. John's wort, garlic, ginkgo biloba and saw palmetto, were contaminated with substances including rice, beans, pine, citrus, asparagus, primrose, wheat, houseplant and wild carrot. In many cases, unlisted contaminants were the only plant material

Dried Ginseng roots are used as medicinal herbal supplements, having relative high market value.

Commonly, Ginseng frauds occur by substitution (total or partial) of American Ginseng by Korean Ginseng.

GINSENG AUTHENTICATION

Journal of Pharmaceutical and Biomedical Analysis 97 (2014) 129–140

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Discrimination of leaves of *Panax ginseng* and *P. quinquefolius* by ultra high performance liquid chromatography quadrupole/time-of-flight mass spectrometry based metabolomics approach

Qian Mao[a,1], Min Bai[a,b,1], Jin-Di Xu[a], Ming Kong[a], Lin-Yin Zhu[a], He Zhu[a], Qiang Wang[b,*], Song-Lin Li[a,*]

J Ginseng Res 40 (2016) 395–399

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

Discrimination of Korean ginseng (*Panax ginseng* Meyer) cultivar Chunpoong and American ginseng (*Panax quinquefolius*) using the *auxin repressed protein* gene

Jong-Hak Kim[2,☆], Min-Kyeoung Kim[1,☆], Hongtao Wang[2], Hee-Nyeong Lee[2], Chi-Gyu Jin[2], Woo-Saeng Kwon[2], Deok-Chun Yang[2,*]

Several methods has been proposed to detected this kind of fraud, most of them based on expensive or laborious analytical techniques.

Analytica Chimica Acta 753 (2012) 73–81

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Rapid differentiation of *Panax ginseng* and *Panax quinquefolius* by matrix-assisted laser desorption/ionization mass spectrometry

Ying-Han Lai[a,b,1], Pui-Kin So[a,b,1], Samual Chun-Lap Lo[a,b], Eddy Wing Yin Ng[c], Terence Chuen Wai Poon[c], Zhong-Ping Yao[a,b,*]

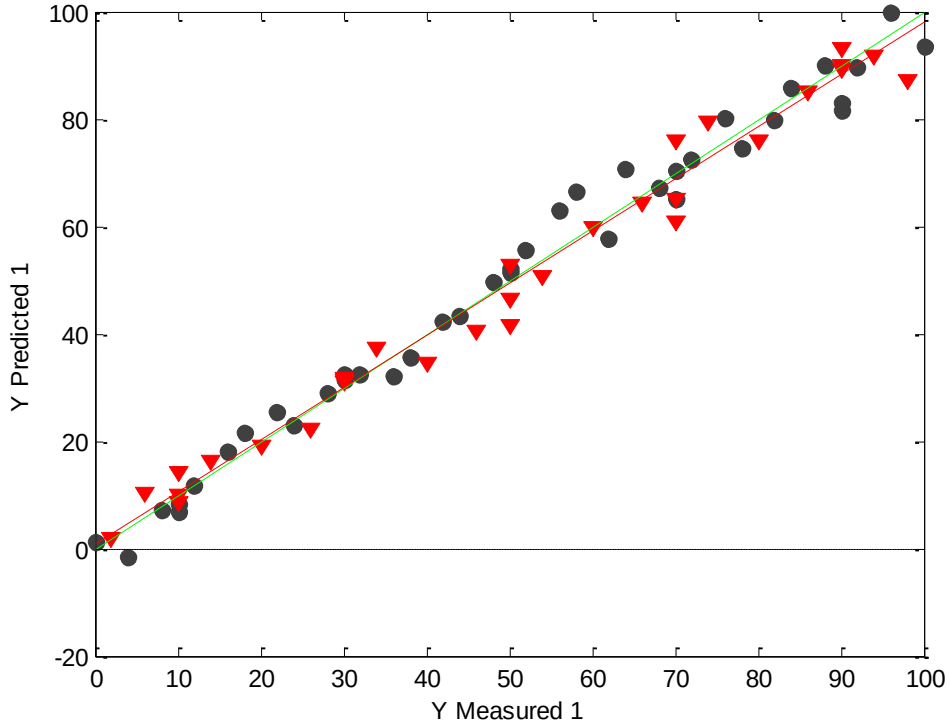
GINSENG AUTHENTICATION

American Ginsengs were adulterated with Korean Ginsengs in the range of 0-100 %

#	% m/m teórica	Volume de amostra Total (uL)	Volume extrato Ginseng Coreano (uL) Reg Ginseng	Volume extrato Ginseng Americano (uL) American Ginseg Triple Leaf
1	0	1000	0	1000
2	2	1000	20	980
3	4	1000	40	960
4	6	1000	60	940
5	8	1000	80	920
6	10	1000	100	900
7	10	1000	100	900
8	10	1000	100	900
9	10	1000	100	900
10	10	1000	100	900
11	12	1000	120	880
12	14	1000	140	860
13	16	1000	160	840
14	18	1000	180	820
15	20	1000	200	800
16	22	1000	220	780
17	24	1000	240	760
18	26	1000	260	740
19	28	1000	280	720
20	30	1000	300	700
21	30	1000	300	700
22	30	1000	300	700
23	30	1000	300	700
24	30	1000	300	700
25	32	1000	320	680
26	34	1000	340	660
27	36	1000	360	640
28	38	1000	380	620

GINSENG AUTHENTICATION

PLS MODEL



$n_{LV} = 5$

RMSEC = 3.7%

RMSEP = 4.2%

R = 0.990

Spectral interpretation: variables assigned to Na and K adducts of oligosaccharides and protonated ginsenosides were important in this model (VIPscores).

COFFEE AUTHENTICATION

Data Fusion of Spectra from Different Techniques in the Development of PLS Models to Quantify and Characterize Coffee Blends

Camila Assis
PhD Student

Prof. Leandro Oliveira
UFMG

ARABICA *versus* ROBUSTA

Two more important cultivated species: Arabica (***Coffea arabica***) (56%) and Robusta (***Coffea canephora***) (44%). Arabica coffees present **20-25%** higher market prices

100% of Arabica coffees are **target of fraud by adulteration** with Robusta

Coffee is a high complex chemical matrix

Components	Coffee Arabica[a]	Coffee Robusta[a]
Caffeine	1.2	2.2
Trigonelline	1.0	0.7
Ashes	4.2	4.4
Acids:		
Chlorogenic	6.5	10.0
Aliphatic	1.0	1.0
Quinic	0.0	0.4
Sugars:		
Sucrose	8.0	4.0
Reducing Sugars	0.1	0.4
Polysaccharides	44.0	48.0
Lignin	3.0	3.0
Pectin	2.0	2.0
Proteins	11.0	11.0
Free Amino Acids	0.5	0.5
Lipids	16.0	10.0

[a]In g $100g^{-1}$ in dry basis

COFFEE AUTHENTICATION

Data Fusion models at low and mid levels

VARIABLE SELECTION

- Genetic Algorithm (GA)
- **Ordered Predictors Selection (OPS)**

MATERIALS & METHODS

Raw beans were obtained from different producers of Arabica (30) and Robusta (10)

Samples were **toasted** at three levels: light/185^{o}C, medium/195^{o}C, and dark/205^{o}C (N=40 for each level)

Toasted samples were **ground** and **sieved** (40 mesh)

Blends (10 g) were prepared in the range of **0-33%** (steps 1%) of Robusta

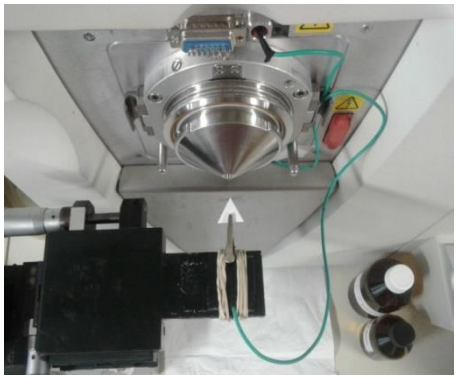
MATERIALS & METHODS

ATR-FTIR

IRAffinity-1S Shimadzu with an ATR accessory (ZnSe)

Range: 4000 a 800 cm^{-1}

PS-MS



Thermo LCQ FLEET – *Ion trap*
Range: *m/z* 100-500

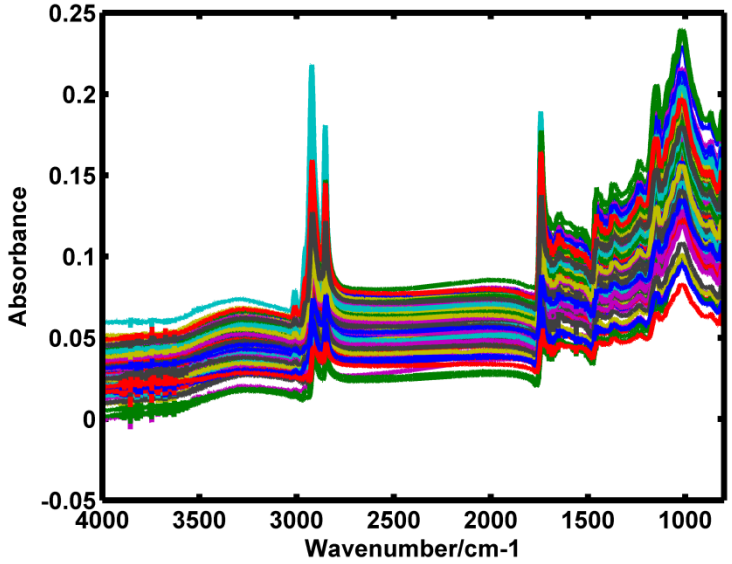
Positive Ion Mode

Samples: powder mixtures

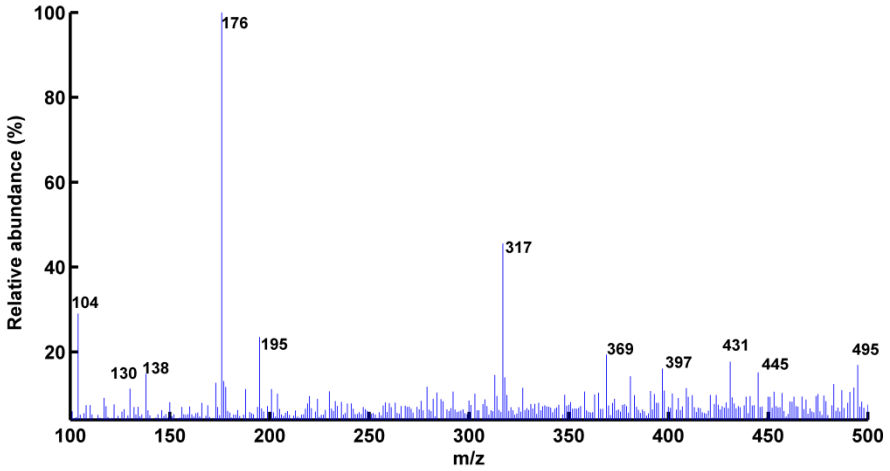
Samples: hot water extracts

SPECTRA

ATR-FTIR



PS-MS



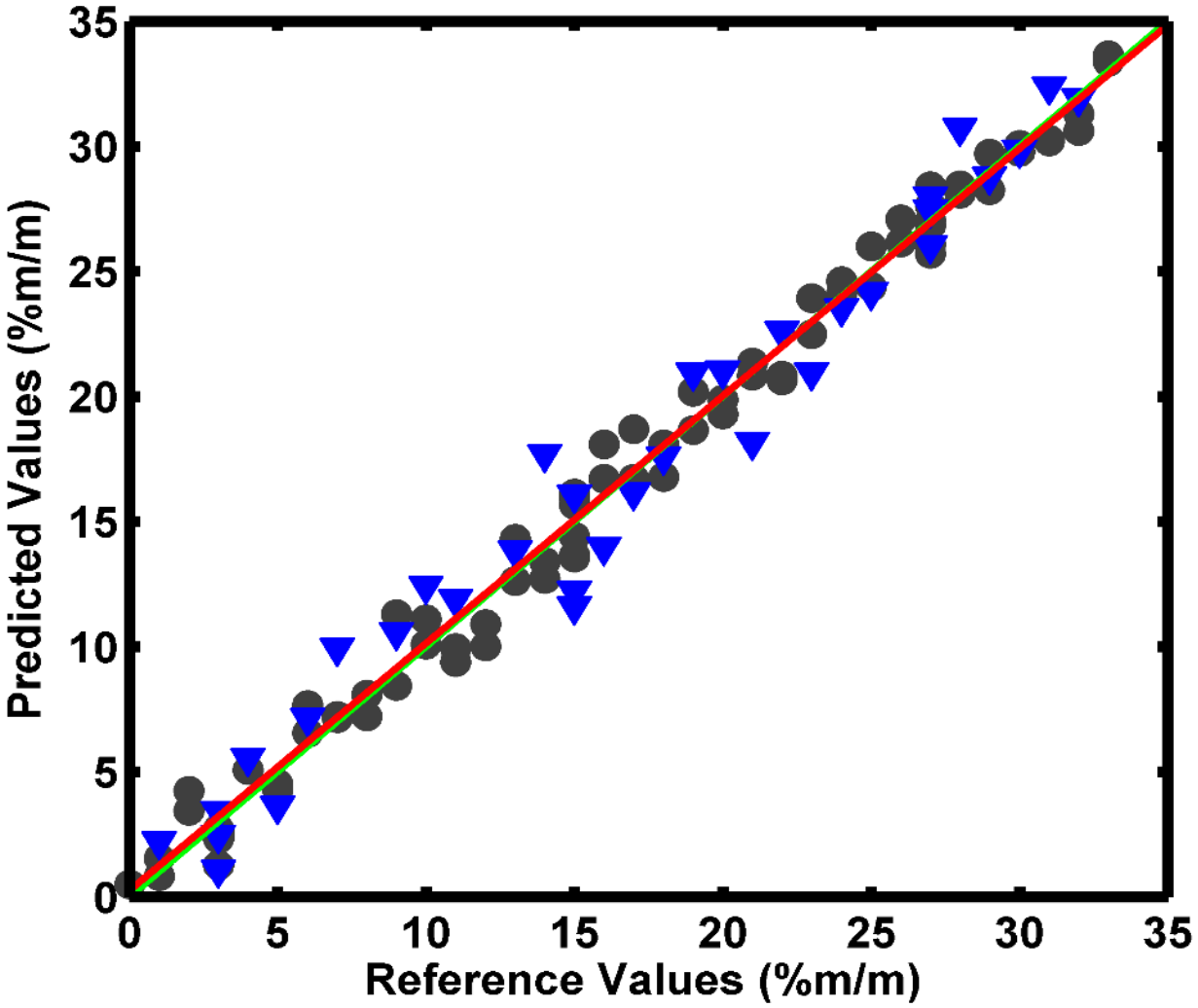
<u>Preprocessing</u>: MSC and mean centering (FTIR); mean centering (MS); autoscaling (Data Fusion)

PLS RESULTS

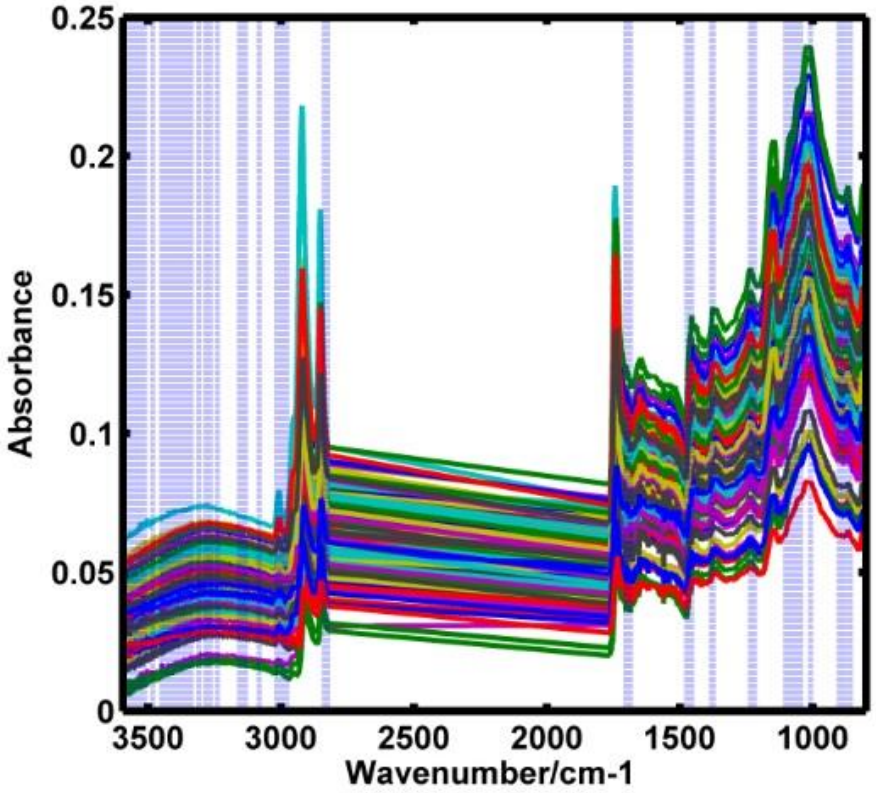
	Low Level			Mid Level		
	Full Spectra	OPS	GA	Full Spectra	OPS	GA
nVars	2202	230	193	2202	320	233
nLV	5	6	5	5	6	5
RMSEC (%)	2.7	1.0	2.1	1.9	1.5	1.7
Rc	0.96	0.99	0.98	0.97	0.99	0.98
RMSEP (%)	3.2	1.7	2.5	4.3	2.3	1.9
Rp	0.94	0.98	0.97	0.87	0.97	0.98

The best Data Fusion model (OPS/low level) was slightly better than a model built only with FTIR spectra, but much better than a model built only with PS-MS spectra

PLS RESULTS



SELECTED VARIABLES



IR: 111 variables

MS: 119 variables

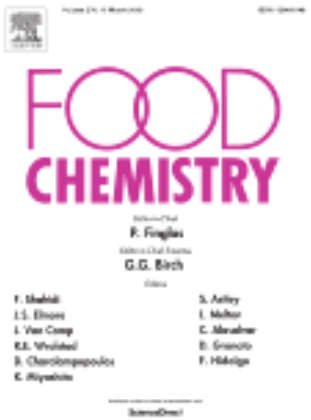
Many of the selected MS variables were assigned based on the literature and to specific coffee components, such as **trigonelline**, **caffeine**, **chlorogenic acids**, **sugars**, **quinic acid**, etc.

COFFEE AUTHENTICATION

40

*Manuscript

Click here to view linked References



1 **Combining mid infrared spectroscopy and paper spray mass spectrometry in a**

2 **data fusion model to predict the composition of coffee blends**

3

4 Camila Assis[a], Hebert Vinicius Pereira[a], Victoria Silva Amador[a], Rodinei Augusti[a],

5 Leandro Soares de Oliveira[b], Marcelo Martins de Sena[a,c,*]

6

More Data Fusion

ATR-FTIR and PS-MS were merged with NIRS and TXRF, and optimized by variable selection

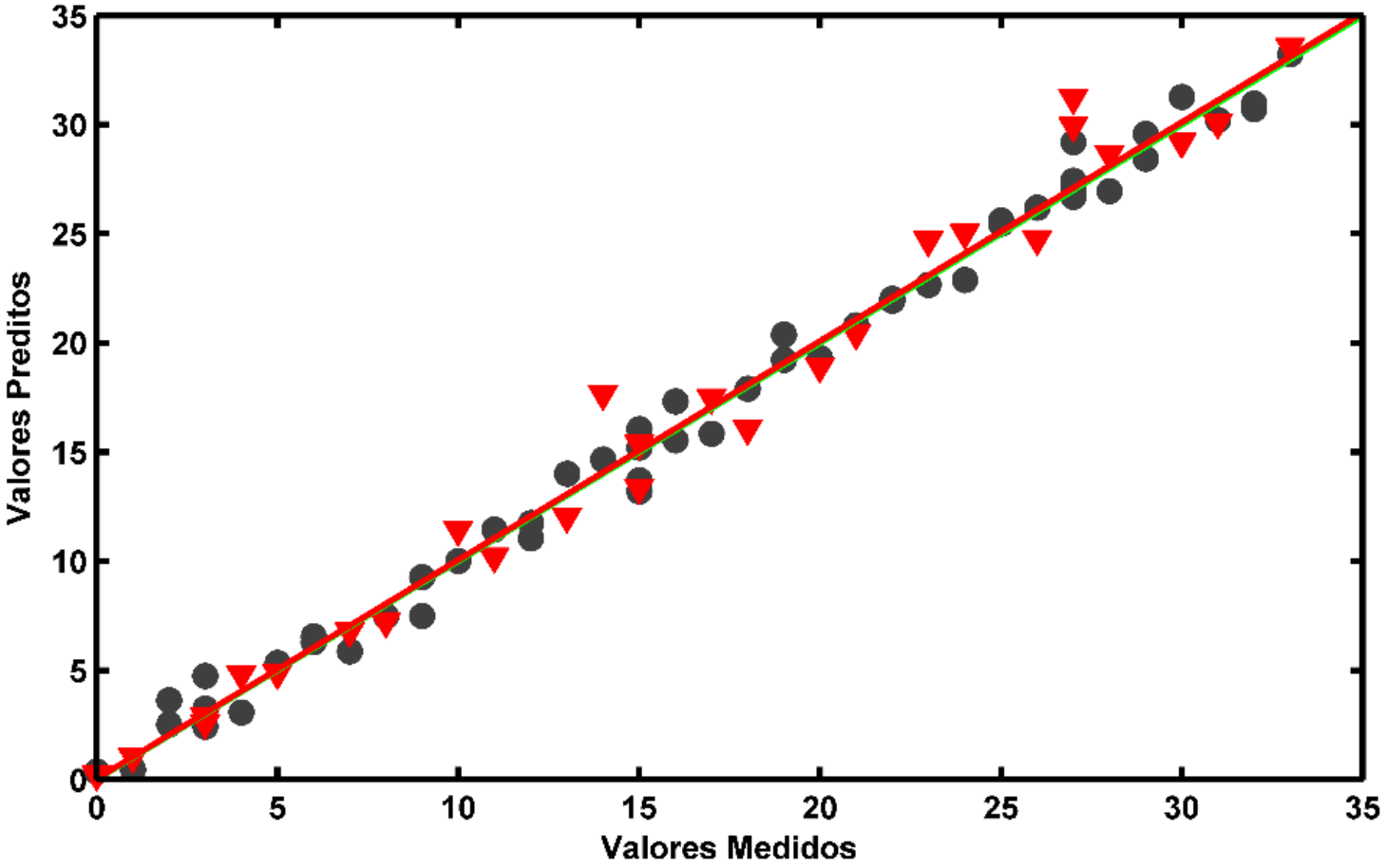
NIRS

Range: 900 a 2000 nm

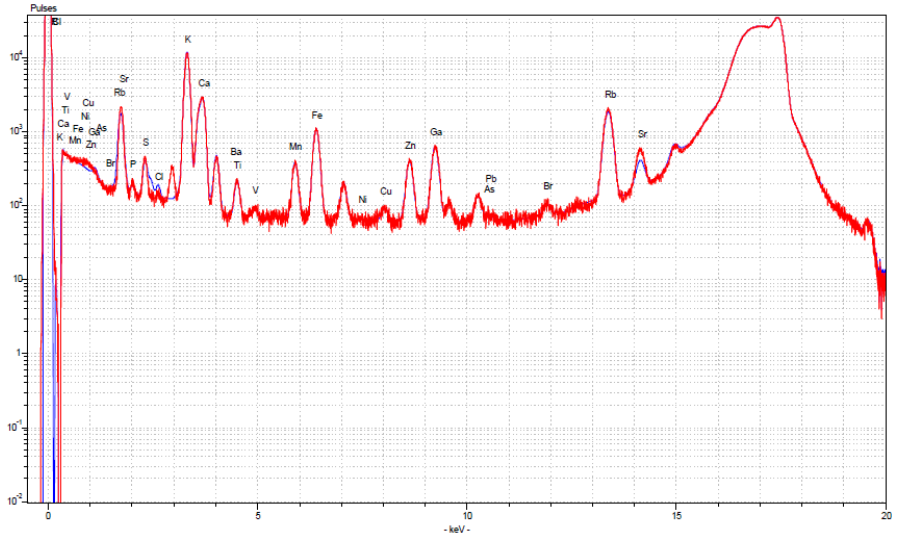
TXRF

14 detected elements : P, S, Cl, K, Ca, Ti, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr

More Data Fusion



TXRF Selected Elements



4 out of 14 elements/varibles selected by OPS:

K, Mn, Fe, Br

CONCLUSIONS & PERSPECTIVES

- ✓ **Multivariate methods based on PS-MS are simple, rapid and require a minimum sample pretreatment.**
- ✓ **Spectral interpretation of the models through selected variables and informative vectors provide specific and relevant information to characterize food/forensic matrices (*more objective than vibrational techniques*).**
- ✓ **Data Fusion models allow to find out correlations between molecular and atomic composition, which can be related to food/sample origin, variety, processing, etc.**
- ✓ **All the models showed good precision, at least at the repeatability level, and in some cases also at the intermediate precision level.**

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6th Bioanalytical School
28-30 November 2018
28 a 30 de Novembro de 2018
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6ª Conferência Internacional de Bioanalítica

Departamento de Química UFMG

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