Quality Control Botanicals

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Problems quality control botanicals

- Unknown active compound
- Contamination with pesticides
- Contamination with mycotoxins
- Adulterations with similar plants
- Large variation in level active compound(s)
- Toxic compounds

Approaches for quality control

- Analysis focused on active compound(s)
- Analysis focused on marker compound(s)
- Analysis focused on contaminations
- Fingerprinting

Applications metabolomics

- In functional genomics
- Safety assessment GMOs
- Phenotyping for breeders rights
- Dereplication: Identifying active compounds in plants
- Quality control: botanicals, wine, etc.
- In systems biology, e.g. studies of activities medicinal plants

Requirements analytical method

- High reproducibility
- Easy sample preparation
- Broad variety compounds in single analysis
- Selectivity
- Identification compounds

Fingerprinting by metabolomics

- TLC
- LC-MS
- GC-MS
- MS(-MS)
- NMR

Chromatographic methods

- Selectivity
- Sensitivity
- Identification
- Robotization
- Large dynamic range
- Peak capacity (ca. 200-1000)

- Differences in sensitivity compounds
- Reproducibility
- Calibration curve for each compound
- Elaborate sample preparation
- Volatility compounds

^{1}HNMR

- Very selective (physical data)
- Direct comparison amounts of compounds without internal standards
- Reproducibility
- Fast

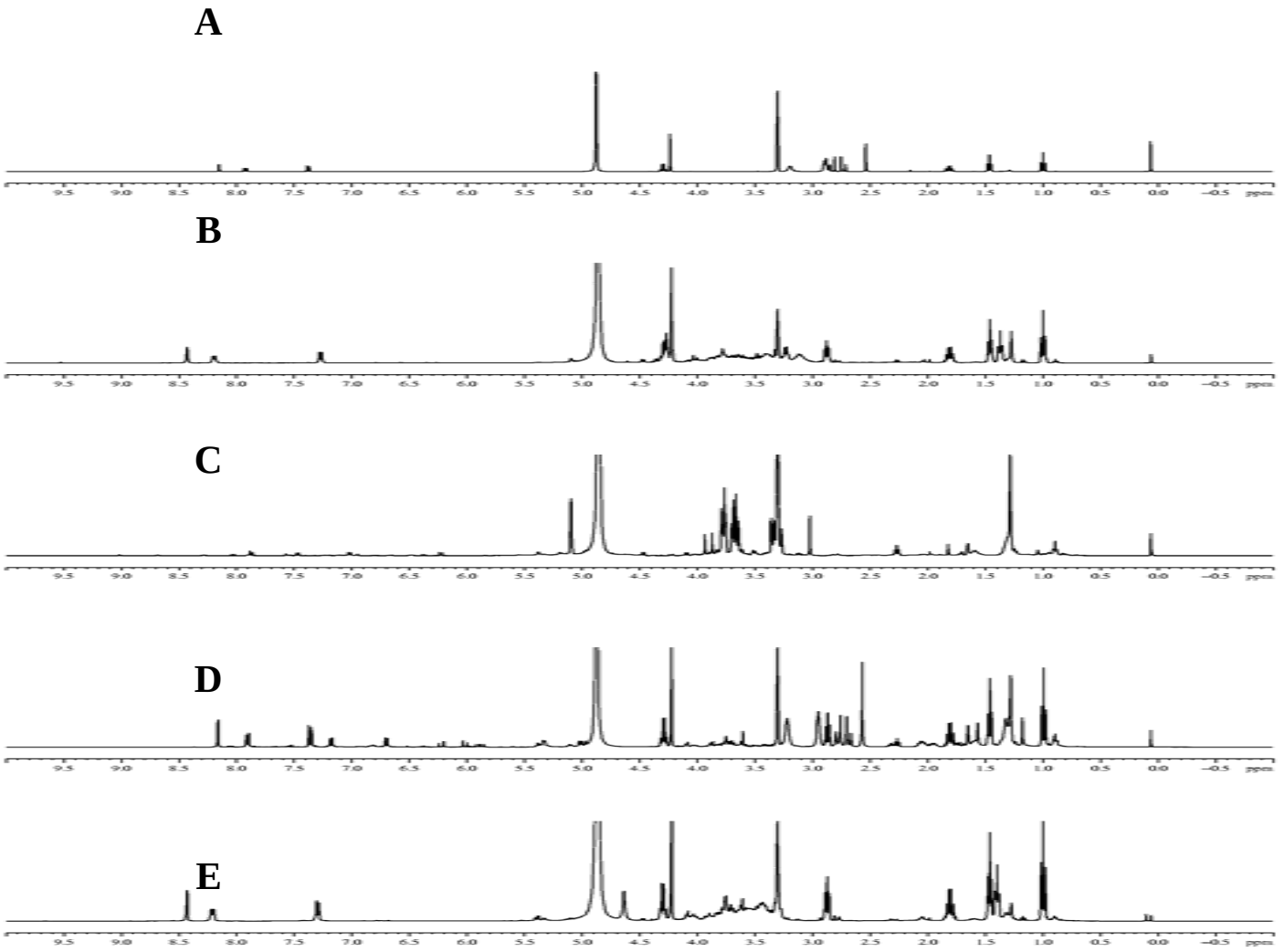
- Compounds not separated
- Polarity range
- Sensitivity
- Dynamic range (1:100)
- Peak capacity (ca. 50-100)

Methods	*Target Compounds*	*Sample preparation*	*Reproducibility*	*Resolution*	*Detector*
TLC	General	Simple	Low	Low	UV, MS, color reagents
GC	Nonpolar low MW	Elaborate derivatization	Medium	High	FID, TCD, NPD, MS
HPLC	Polar (chromophore)	Elaborate	Medium	Medium	UV, RI, MS, ELSD, fluorescence, NMR
CE	Ionic	Elaborate	Medium	High	UV, MS

Comparison metabolomic tools

	LC-MS	GC-MS	TLC	MS-MS	NMR
Sample prep	-	--	++	+	+++
Reproducible	--	+	-	-	+++
Absolute qnt	-	-	-	-	+++
Relative qnt	+	++	+	++	+++
Identity	++	++	+	++	++
Compound no	++	+++	+	+++	+
Sensitive	++/-	++/-	+	+++/-	-

Globalization: the best of two worlds?



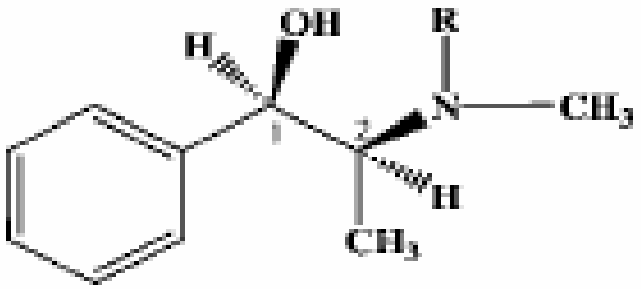
^{1}H-NMR Spectra (400 MHz, MeOD) of sildenafil (A), Libidoforte® (B),SWA 0404/058 tablet (C), SWA 0404/059 tablet (D), and SWA 0404/060 tablet (E) in the range of -1 – 10 ppm

Quality control medicinal plants

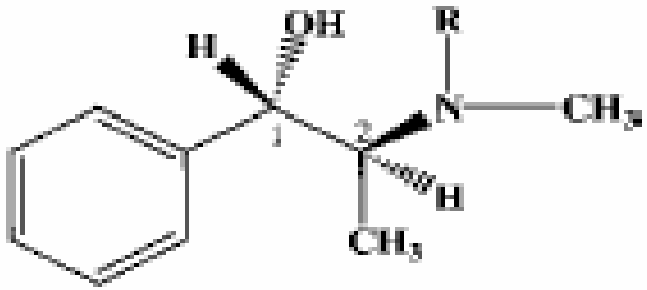
Metabolomic Profiling of Ephedra sold on markets in Taiwan (Ma Huang)

Quality control

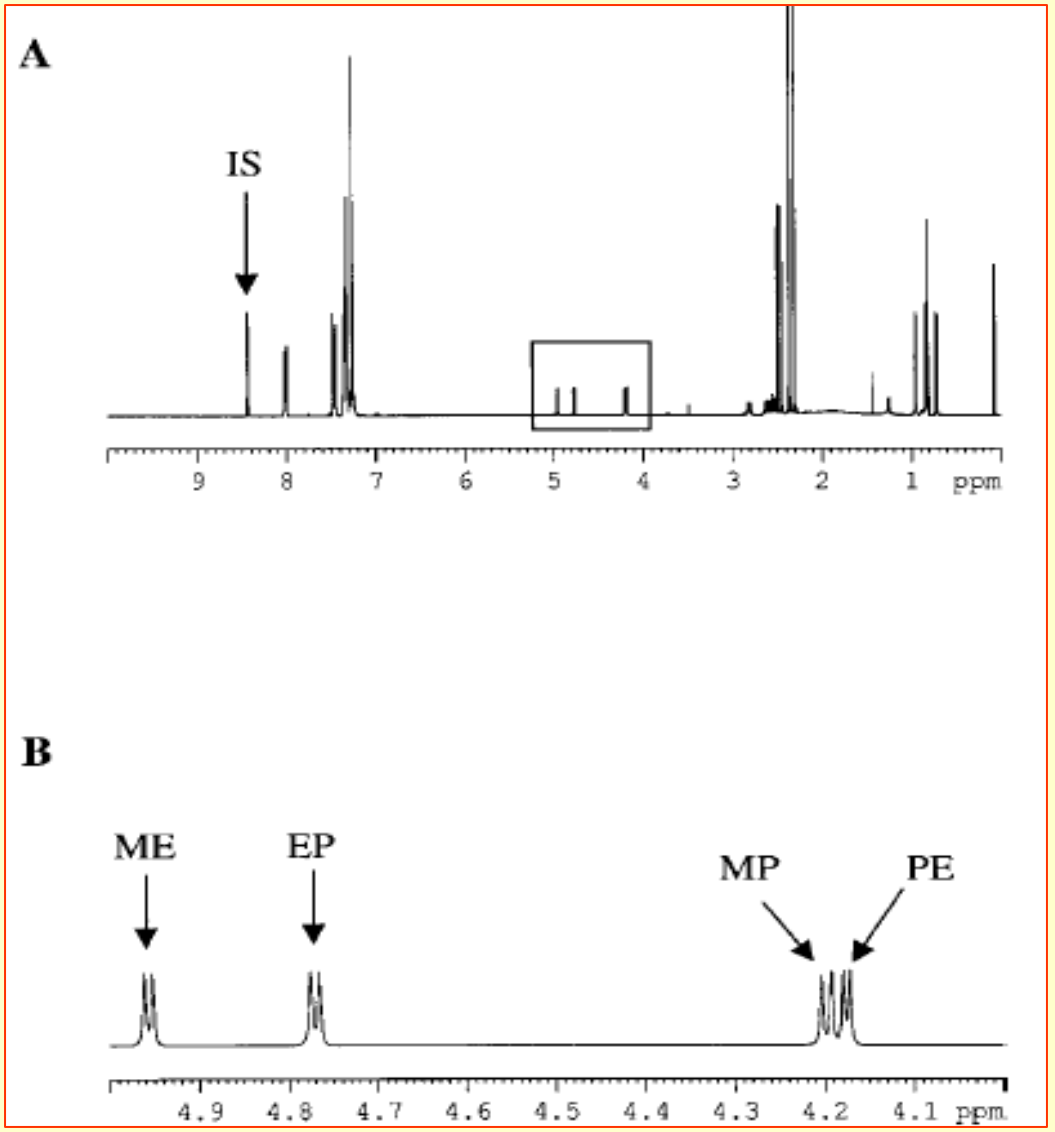
^{1}HNMR of ephedrine and analogues



R = H, Ephedrine
R = CH_3, Methylephedrine



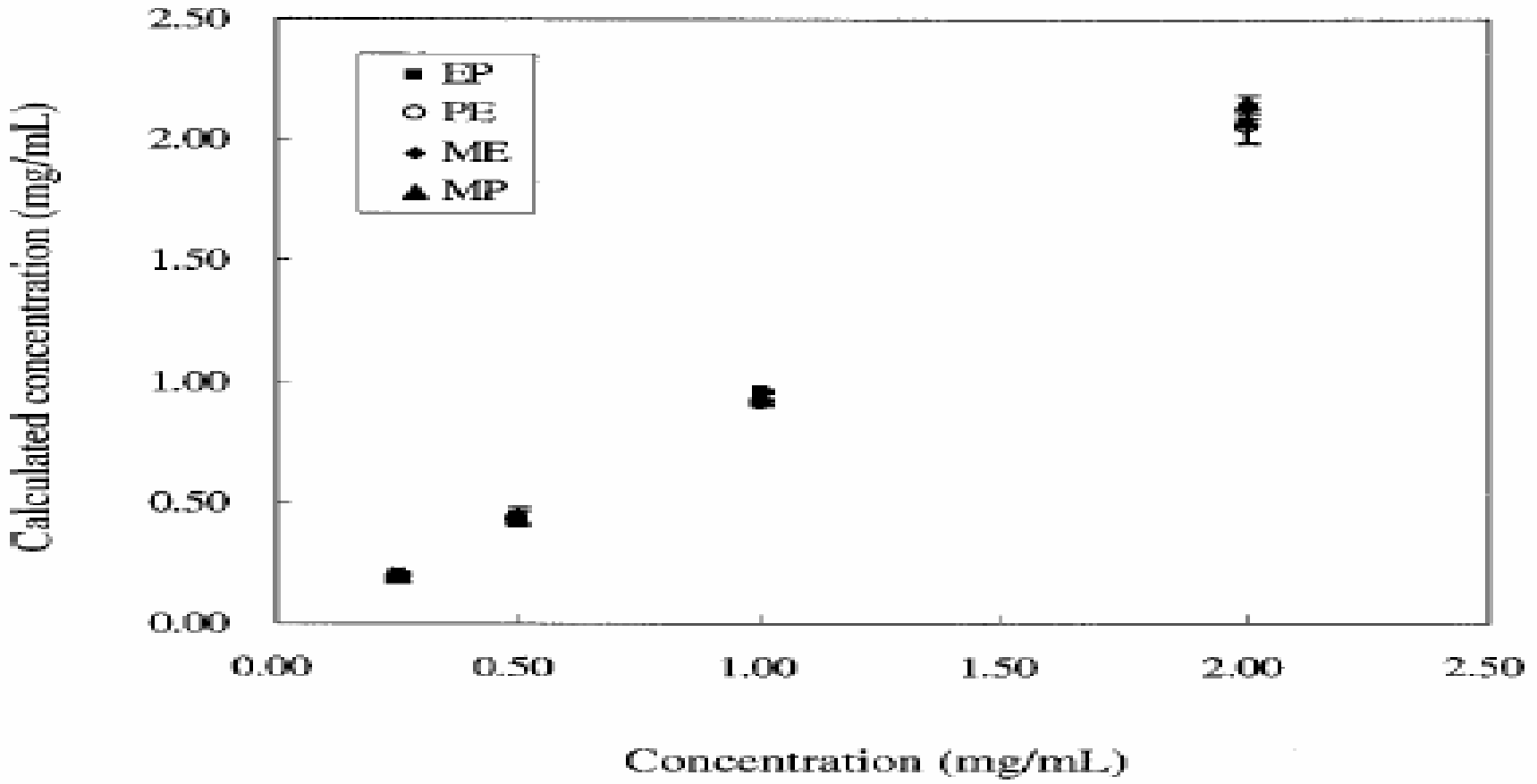
R = H, Pseudoephedrine
R = CH_3, Methylpseudoephedrine



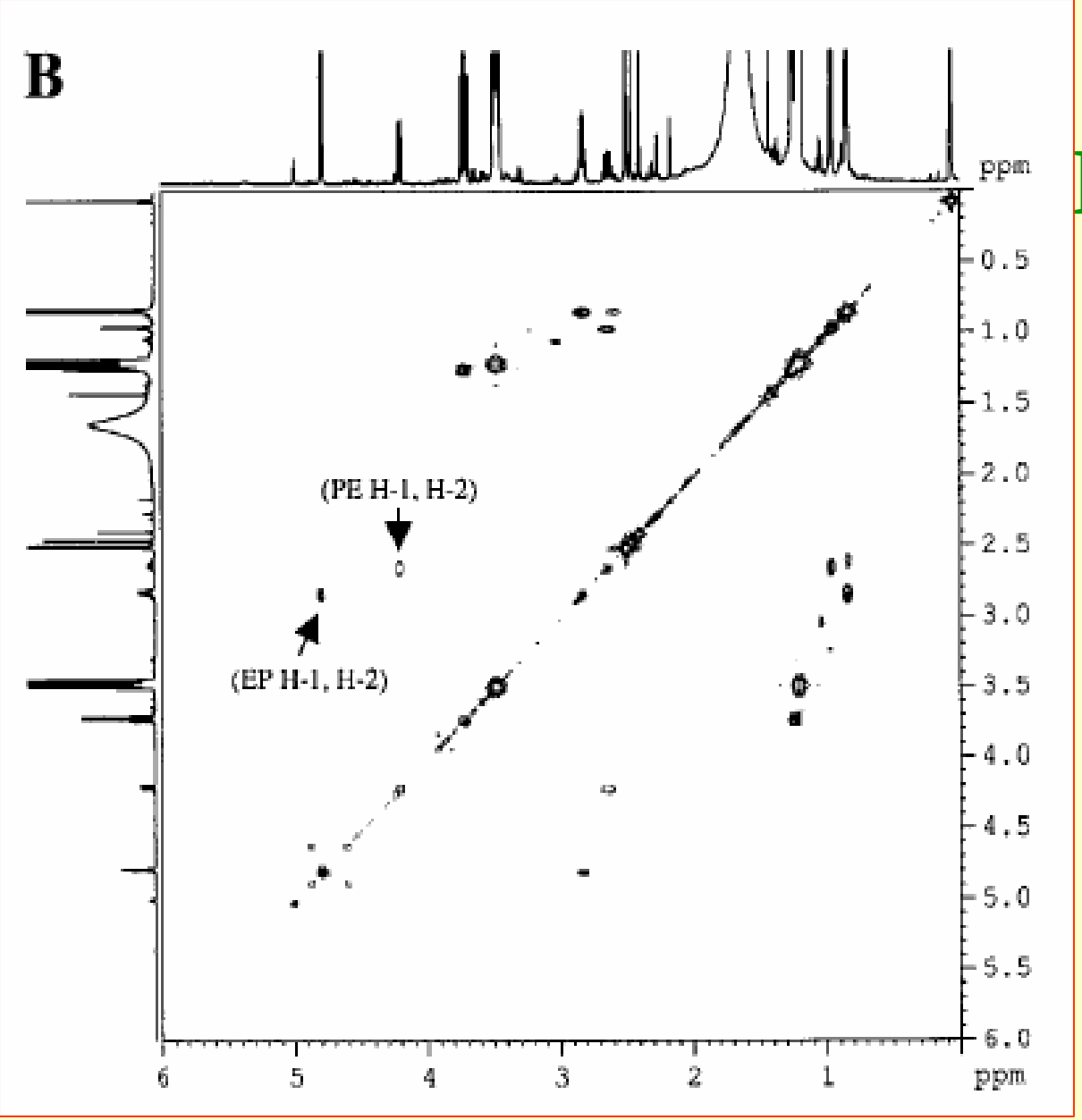
^{1}HNMR analysis *Ephedra* samples

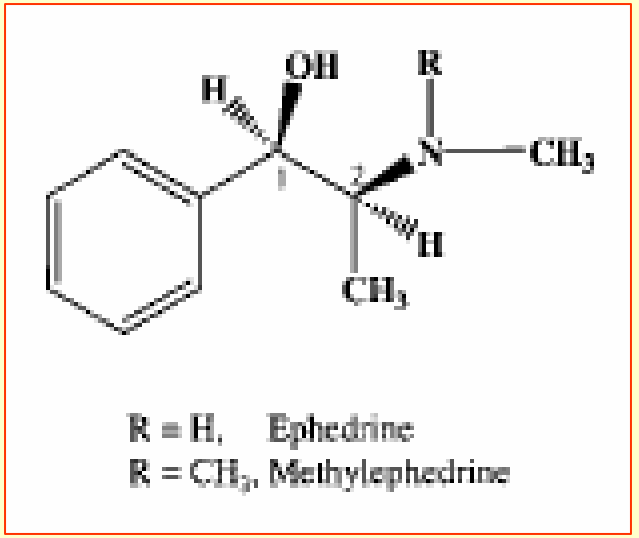
- Extraction with 0.5 M H2SO4, basification with KOH, extraction with Et2O (2x)
- Dry
- Dissolve in CDCl3
- 400 MHz Bruker NMR
- Recovery alkaloids 88.3-92.9% (± 2%)
- Internal standard anthracene

Calibration curves ephedrine and analogues



^{1}H- ^{1}H COSY NMR *Ephedra sinica*

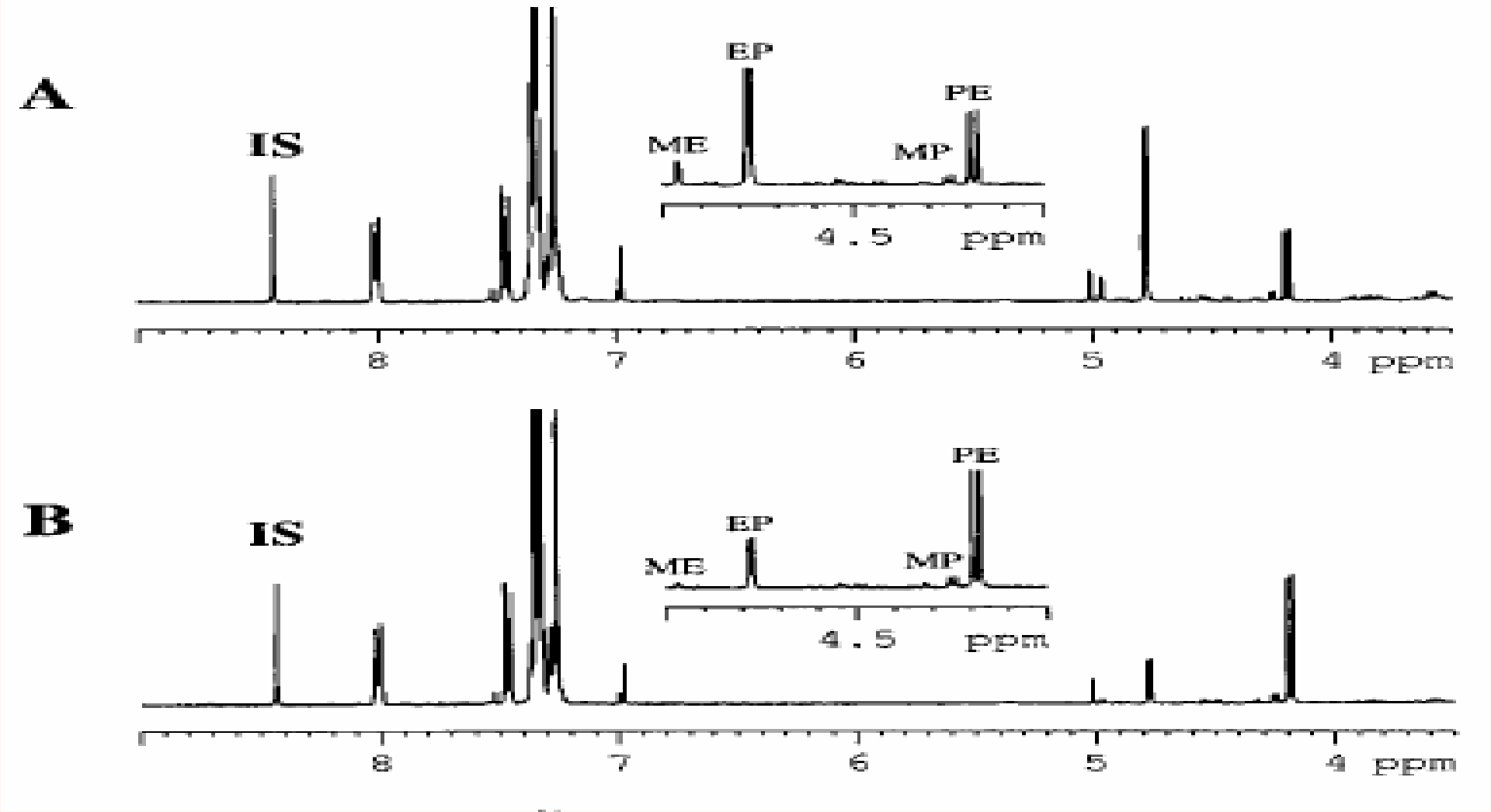




EP=ephedrine, PE=pseudoephedrine

Ephedra sinica (A), *E. intermedia* (B)

ME=methyl ephedrine, EP=ephedrine,
MP=methylpseudoephedrine, PE=pseudoephedrine



Quantitative analysis *Ephedra* samples

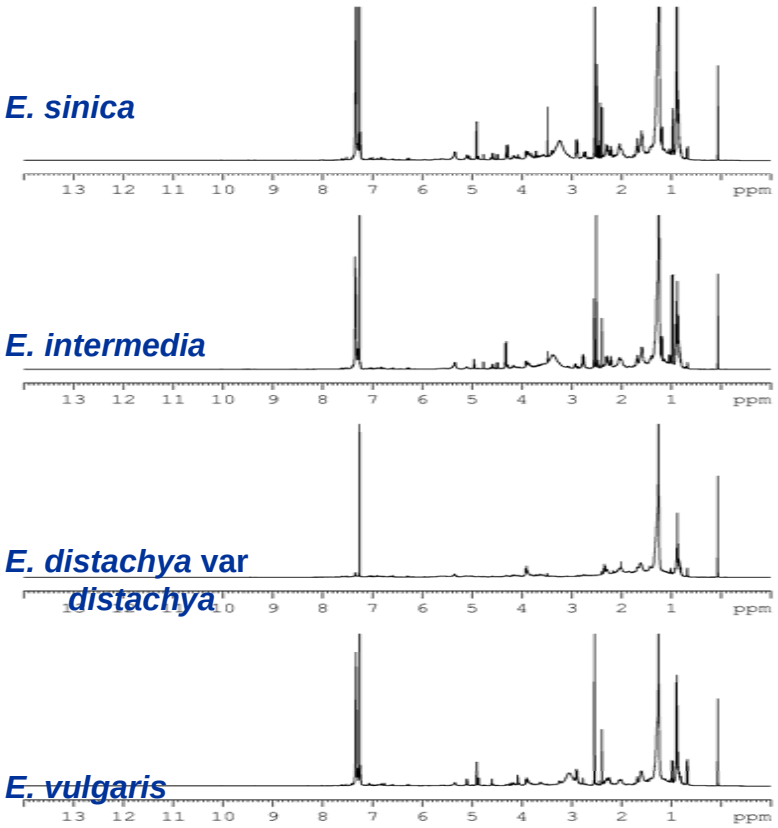
Table 1. The Content of Ephedrine Alkaloids of Nine Different *Ephedra* Plants Including *Ephedra sinica* and *Ephedra intermedia*

Sample	Ephedrine (EP)	Pseudoephedrine (PE)	Methylephedrine (ME)	Methylpseudoephedrine (MP)	Total alkaloids[a)] (%)
E. sinica	8.98±1.23	3.92±0.44	1.30±0.18	0.75±0.10	1.49
E. intermedia	2.15±0.31	6.29±0.87	0.22±0.04	0.76±0.14	0.94
1	2.61±0.15	9.44±0.85	0.38±0.03	1.48±0.07	1.39
2	1.57±0.29	6.99±1.01	0.20±0.04	1.07±0.22	0.98
3	2.84±0.14	8.22±0.46	0.39±0.02	1.07±0.07	1.25
4	1.87±0.18	8.70±0.99	0.26±0.08	1.27±0.27	1.21
5	1.59±0.09	8.63±0.55	0.21±0.03	1.25±0.15	1.17
6	9.12±0.45	5.09±0.17	1.10±0.03	0.91±0.02	1.62
7	1.69±0.11	9.06±0.53	0.17±0.04	1.49±0.09	1.24
8	1.64±0.16	8.73±0.95	0.23±0.04	1.06±0.02	1.17
9	2.23±0.04	9.88±0.23	0.32±0.02	1.43±0.14	1.39

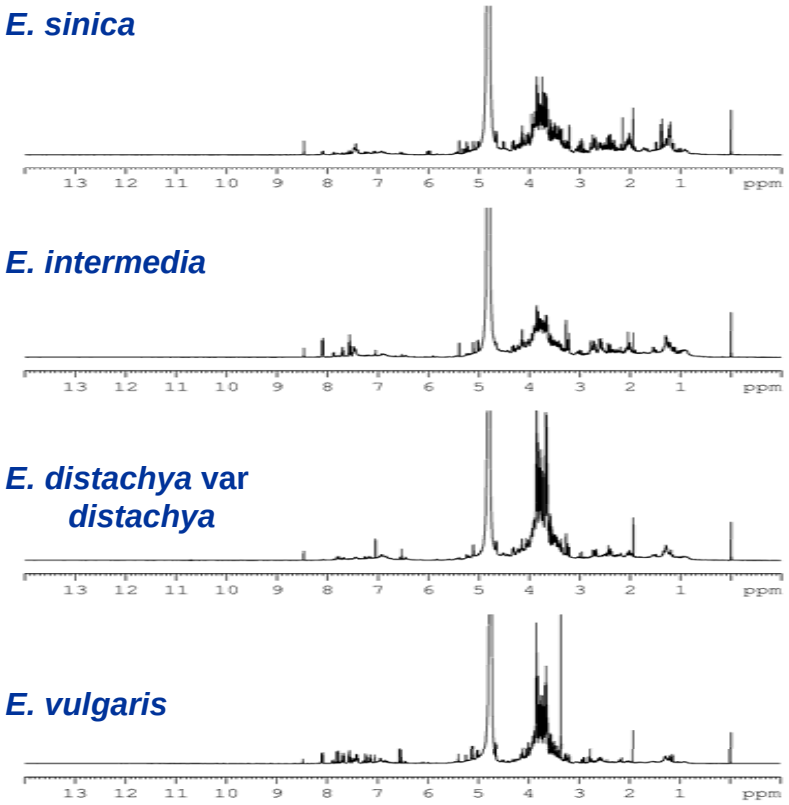
The amounts were calculated from the peak area of H-1 compared to that of 200 μg of anthracene (mg/g dry weight±S.D.). All experiments were done in triplicate. *a*) Sum of four alkaloids (EP, PE, ME, MP) analyzed in this study was expressed in percentage of 1 g of dry weight.

Metabolic profiling of Ephedra using ^{1}H-NMR and multivariate analysis

Overview of all metabolites, possible to differentiate all species

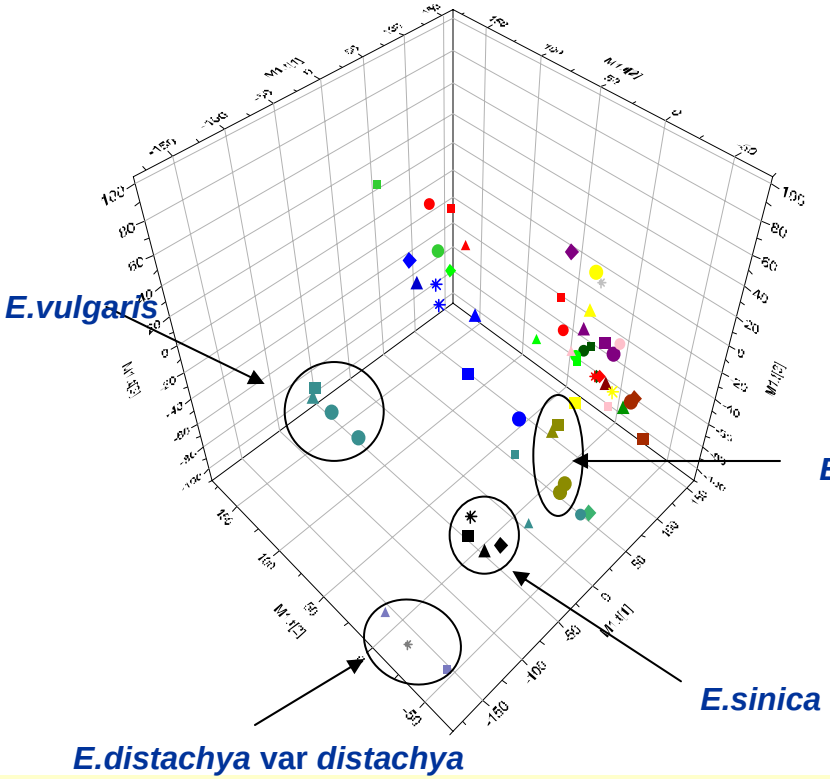


Chloroform fraction

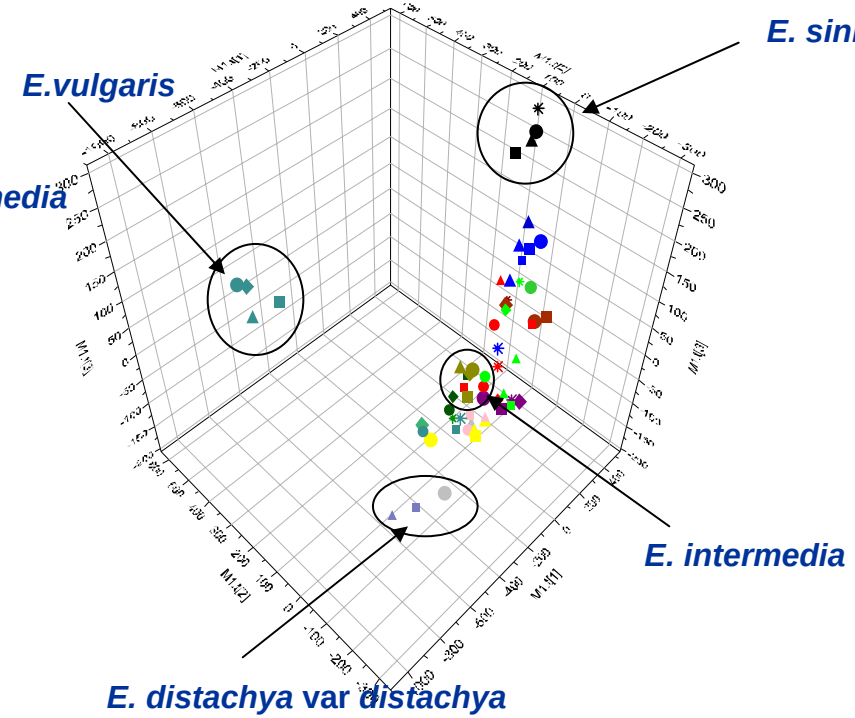


Water fraction

PCA plots of *Ephedra* samples



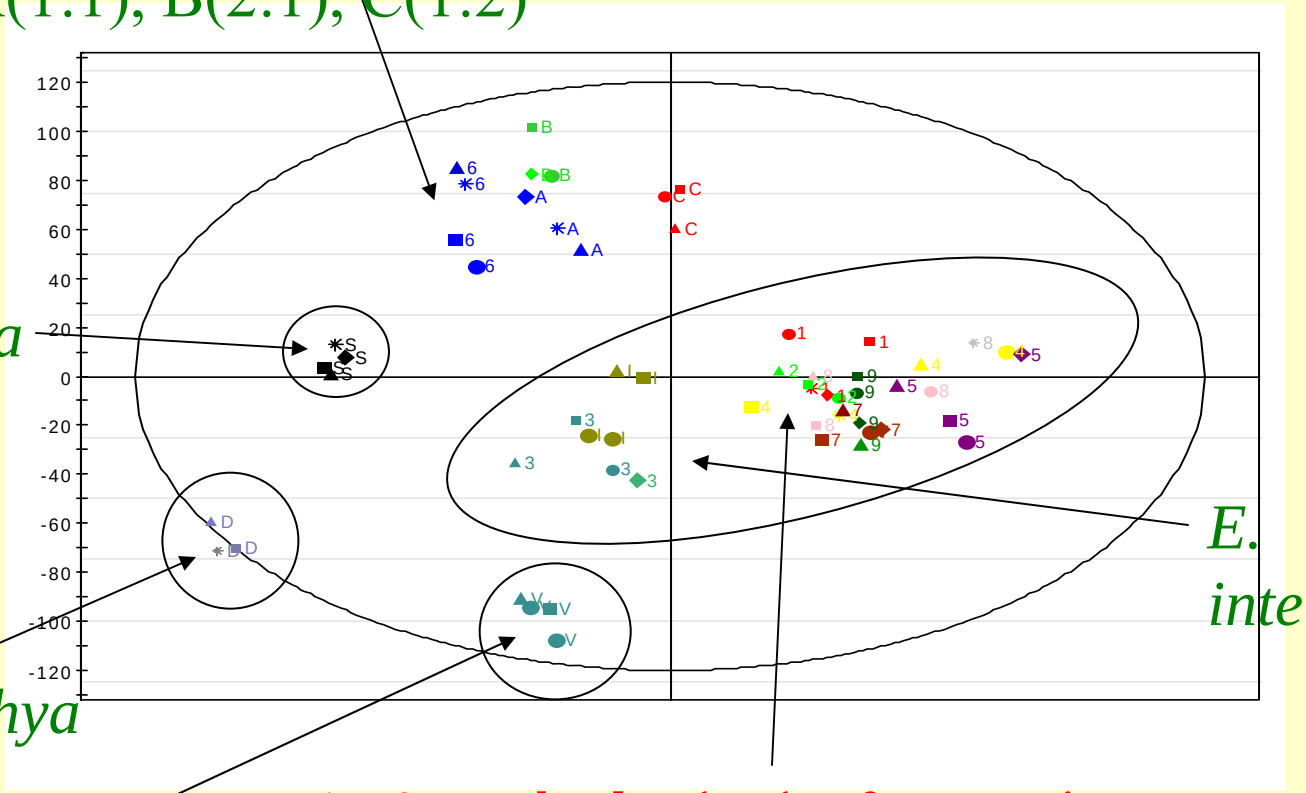
water fraction



Chloroform fraction

PCA plot of water fraction (PC1 vs PC3)

Mixture of *Ephedra sinica* & *E. intermedia*:
A(1:1), B(2:1), C(1:2)



E. sinica

E. intermedia

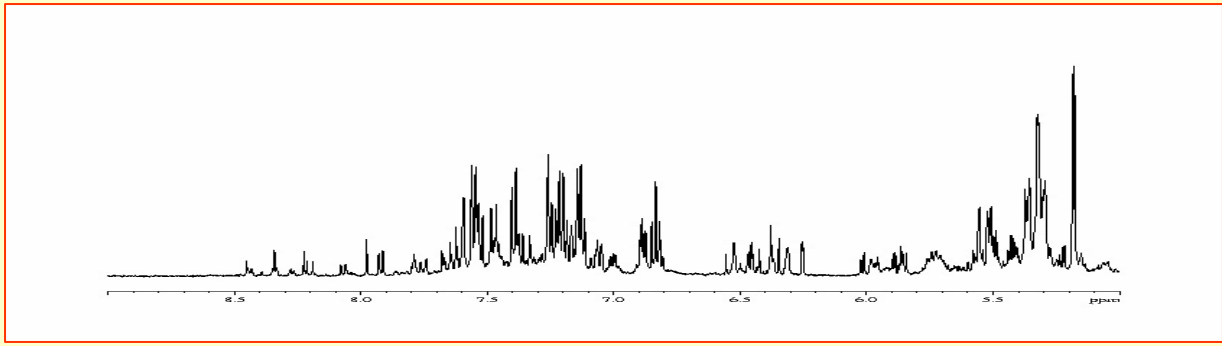
E. distachya

E. vulgaris

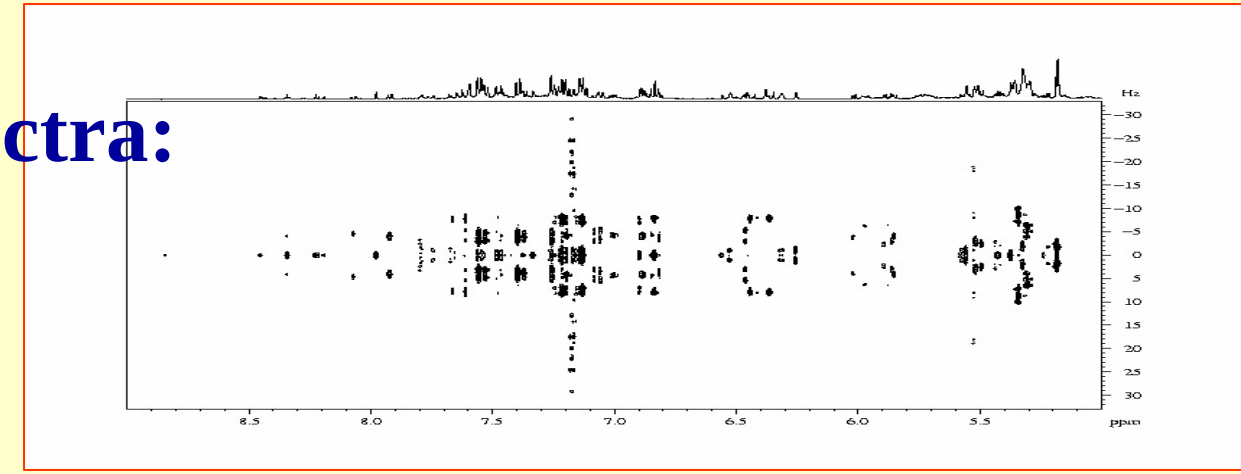
1~6 : *Ephedra* herbs from Taiwanese market

Quantitation alkaloids *Catharanthus roseus*

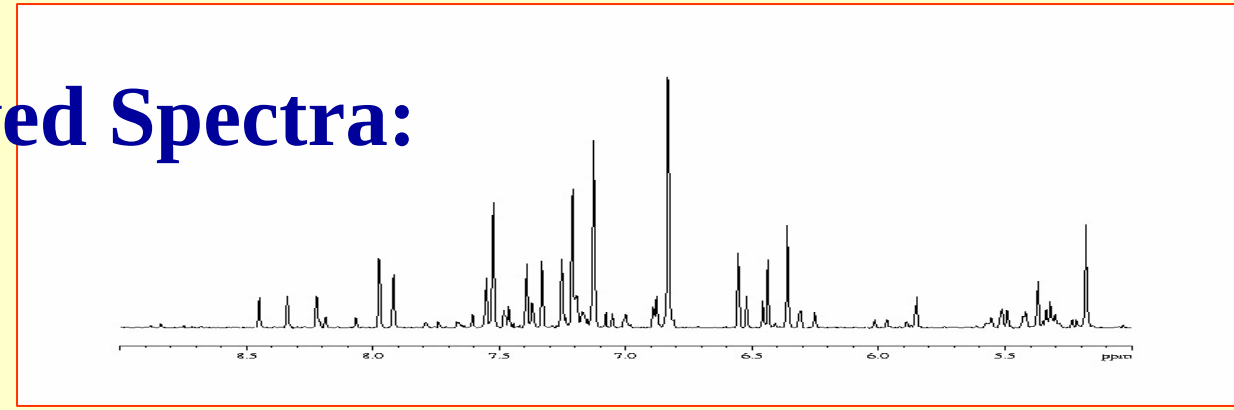
^{1}H-NMR Spectrum:
Complex signals



2D-J-resolved Spectra:
High resolution

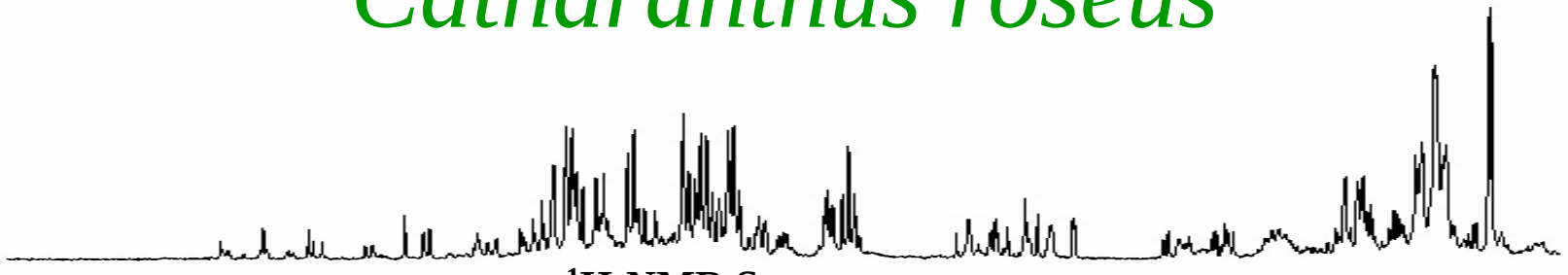


Projected-J-resolved Spectra:
^{1}H decoupled

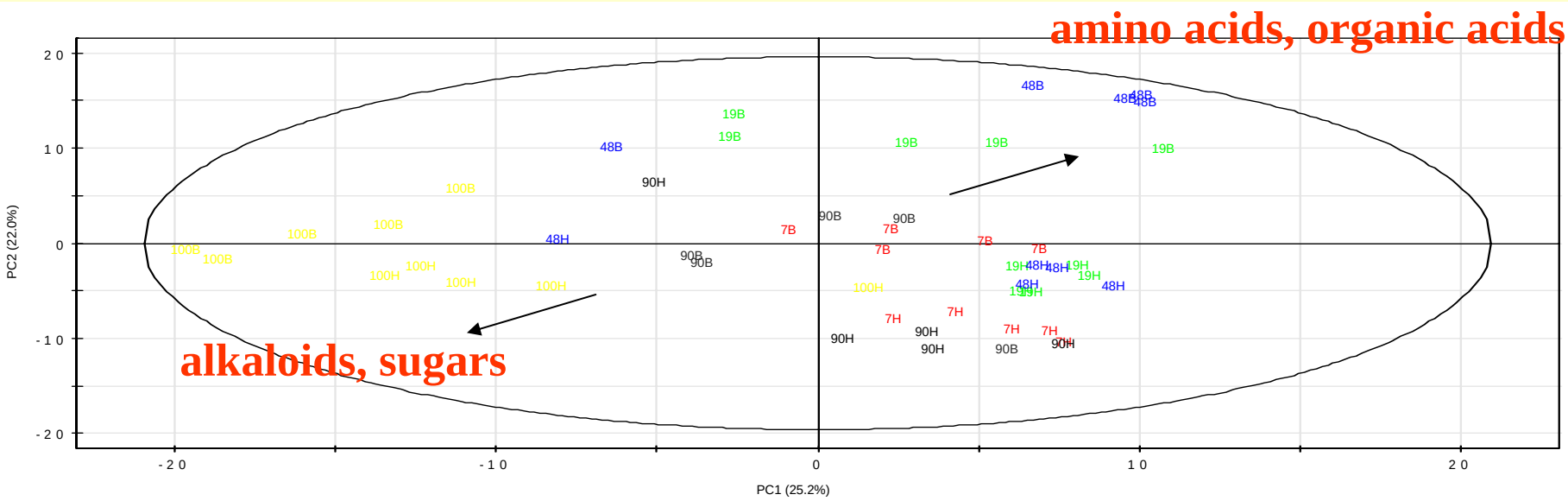


Differentiation 5 Varieties

Catharanthus roseus



^{1}H-NMR Spectrum

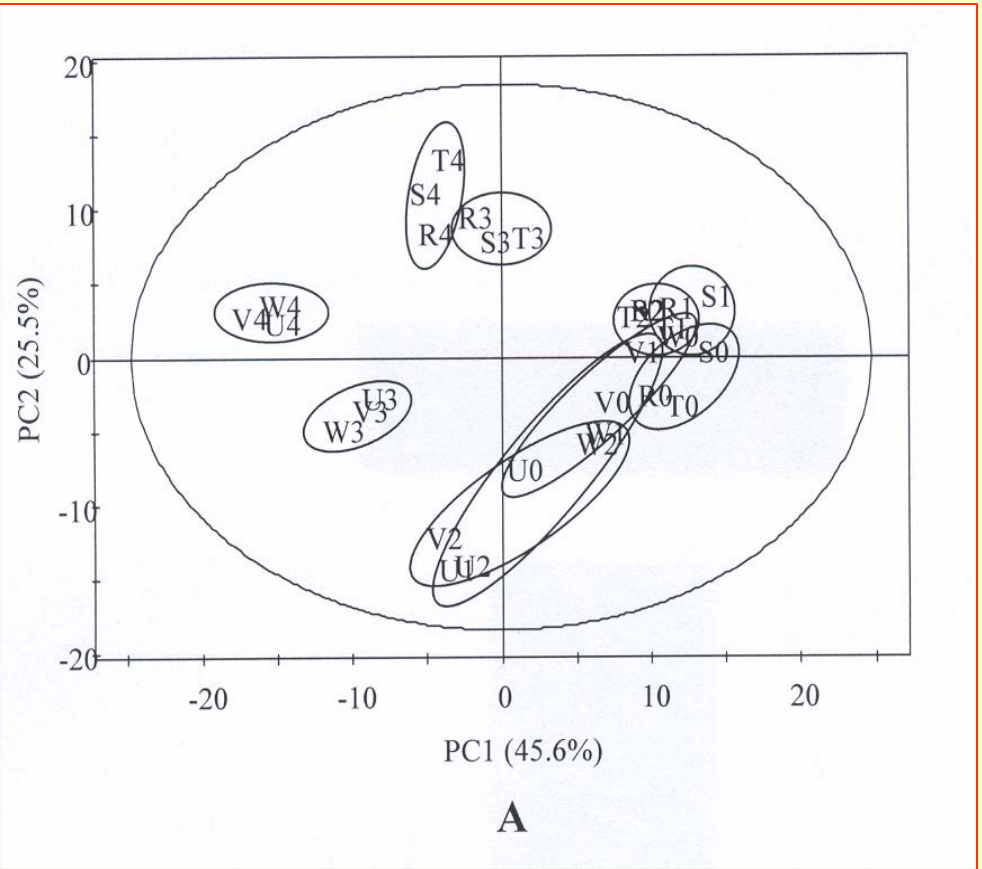


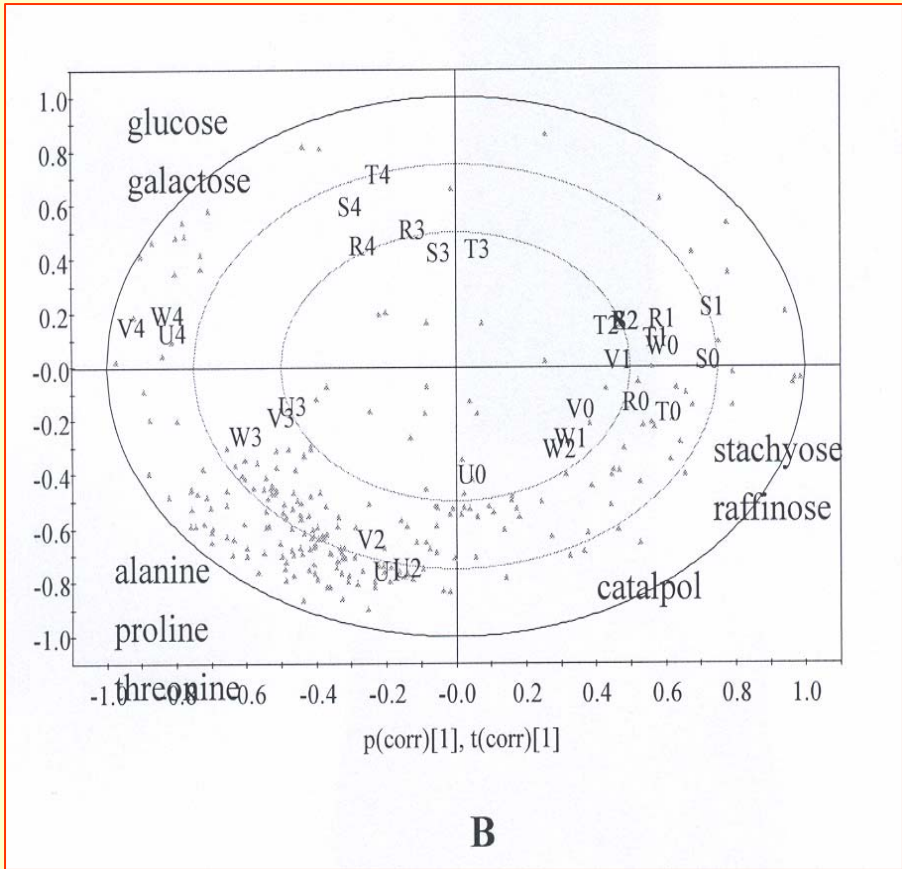
Principal Components Analysis for ^{1}H-NMR Spectrum

Rehmania glutinosa roots

R, S and T grown at 22^0 C U,V and W grown at 22^0 C

0 = fresh, 1 = dried, 2 = processed,
3 = processed 4x, 4 = processed 9x





Traditional Processing *Rehmania glutinosa* roots: drugs with different pharmacological use

- **Rinse 15 min with rice wine**
- **Steam 60 min**
- **Bake at 55^0 C**
- **Repeat 5-9 times**

Rehmania glutinosa roots
2D J-resolved NMR

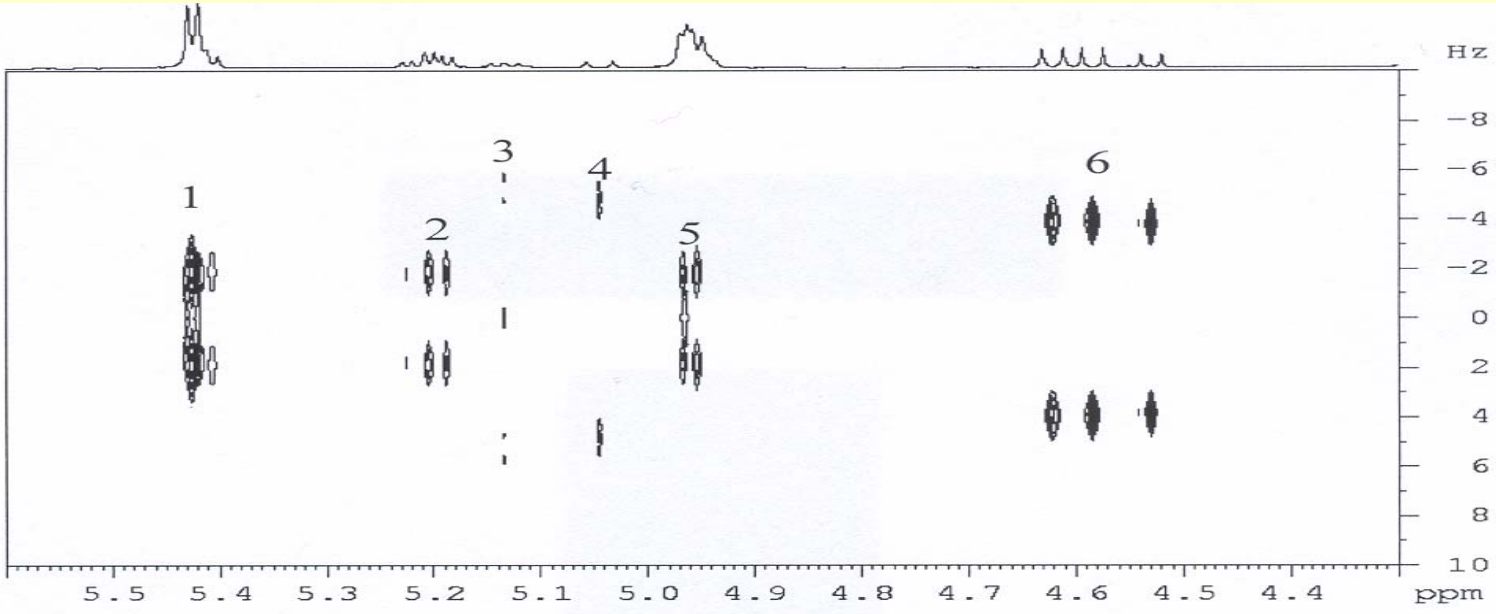
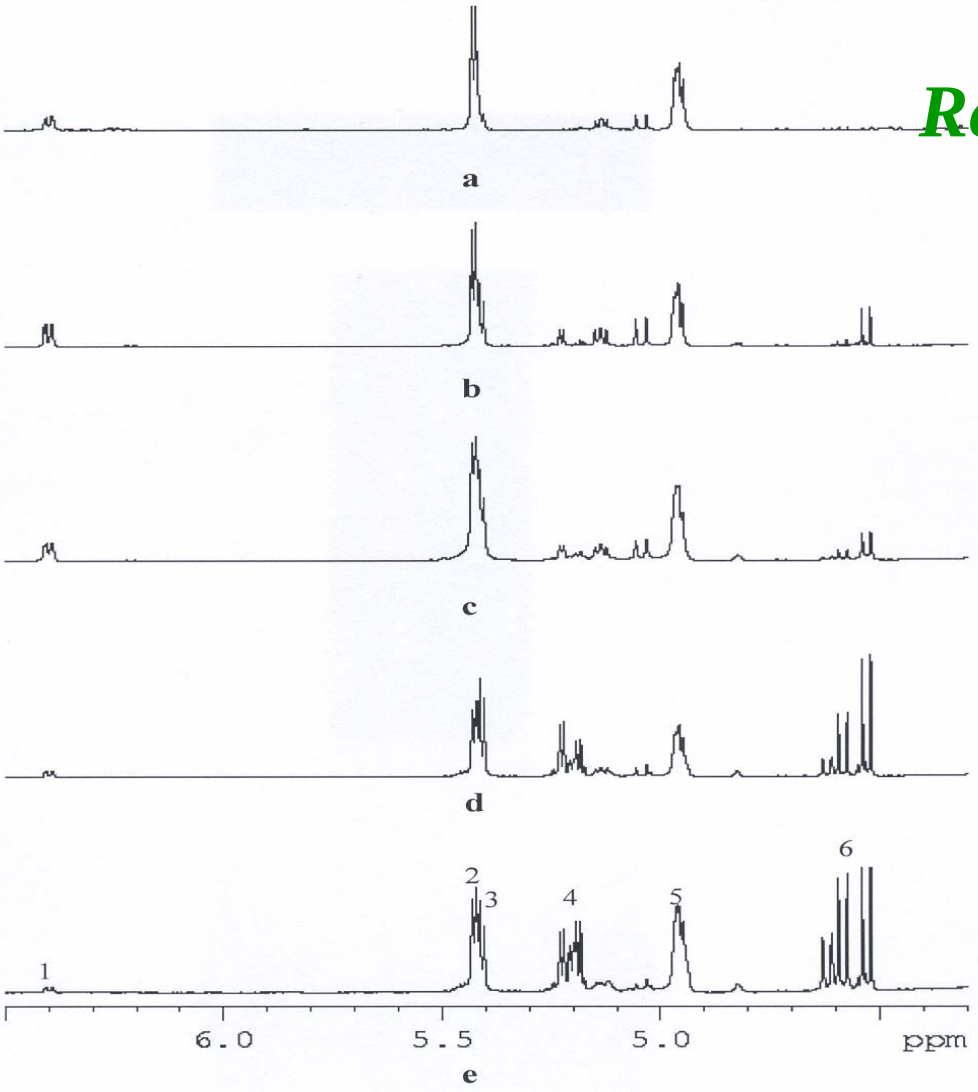


Figure 3. Two dimensional J-resolved NMR spectra of processed *Rehmannia glutinosa* roots in the range of δ4.3 –δ5.6. 1: H-1 of raffinose, stachynose and sucrose; 2: H-1 of α–glucose and α–galactose; 3: H-4 of catalpol; 4: H-1' of catalpol; 5: H-1 of internal α–glucose and α-galactose moiety of raffinose and stachynose; 6: H-1 of β-glucose and β-galactose.

Rehmania glutinosa roots

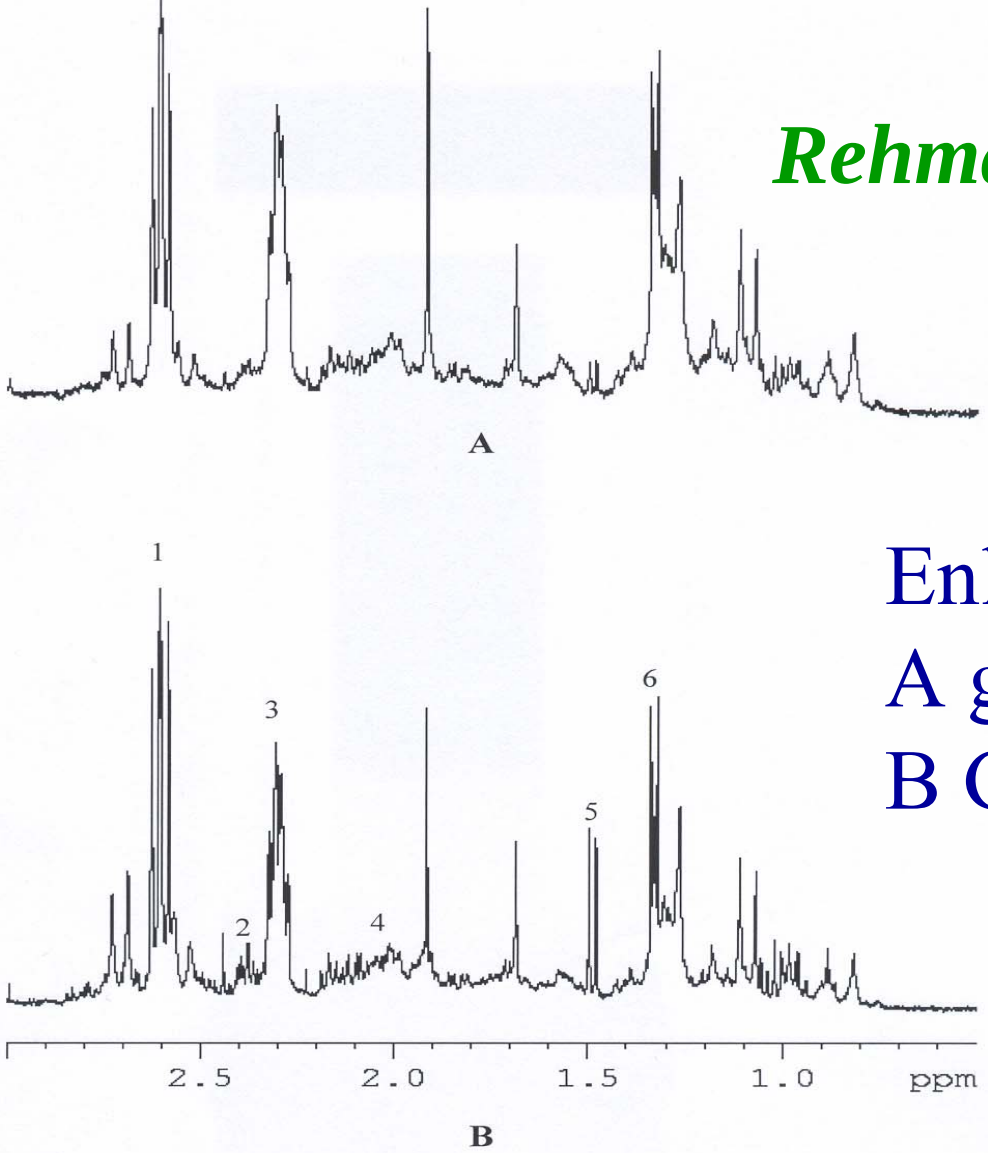


Enlarged NMR
Anomeric sugar protons

-a fresh
-b dried
-c one process cycle
-d five process cycles
-e nine process cycles

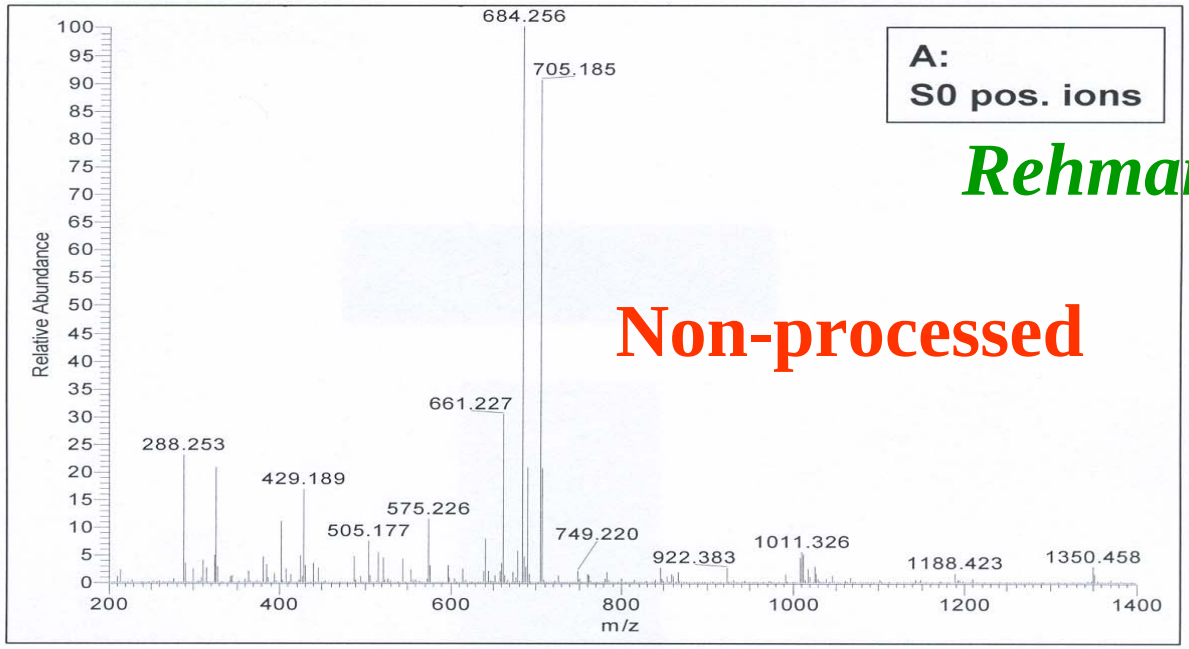
Figure 4. ^{1}H-NMR spectra of processed *Rehmannia glutinosa* roots in the range of δ 4.3 – δ 6.5. a: fresh root; b: dried root; c: one cycle processing (rinsed with Chinese rice wine, steamed and baked); d: five cycles processing; e: nine cycles processing. 1: H-3 of catalpol; 2: H-1 of raffinose and stachynose; 3: H-1 of sucrose; 4: H-1 of α-glucose and α -galactose; 5: H-1 of internal α–glucose and α-galactose moiety of raffinose and stachynose; 6: H-1 of β-glucose and β-galactose.

Rehmania glutinosa roots



Enlarged NMR
A grown at 22^0 C
B Grown at 25^0 C

Figure 5. ^{1}H-NMR spectra of processed *Rehmannia glutinosa* roots in the range of δ 0.5 – δ 3.0. A: processed root grown in 22°C; B: processed root grown in 25°C. 1: H-9 of catalpol; 2: H-3 of proline; 3: H-5 of catalpol; 4: H-3' of proline; 5: H-3 of alanine; 6: H-5 of threonine.



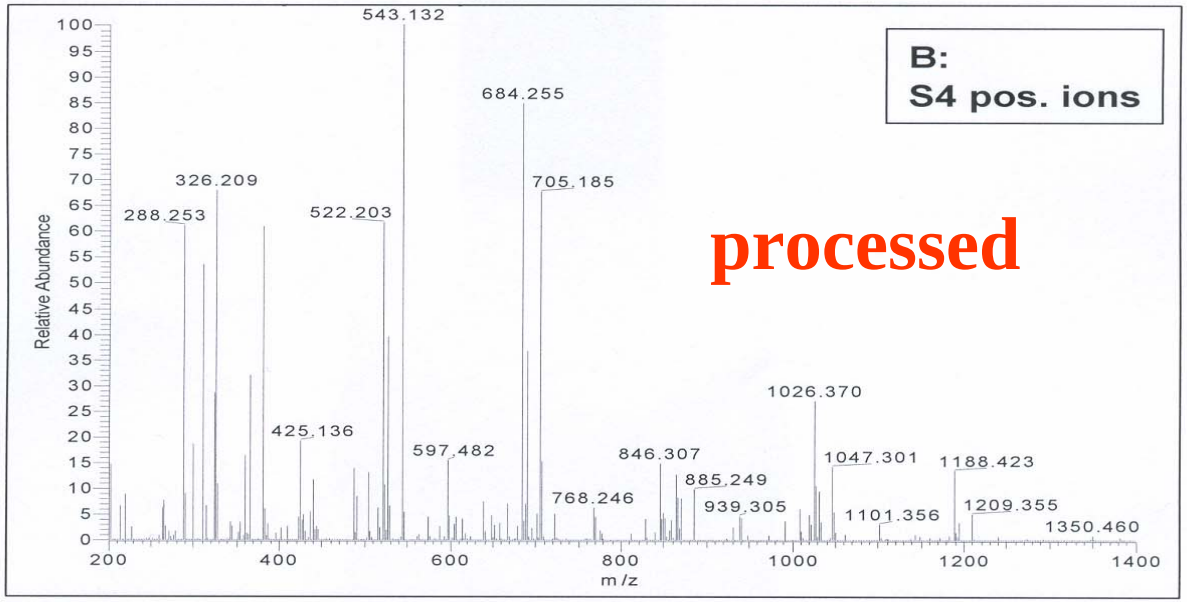


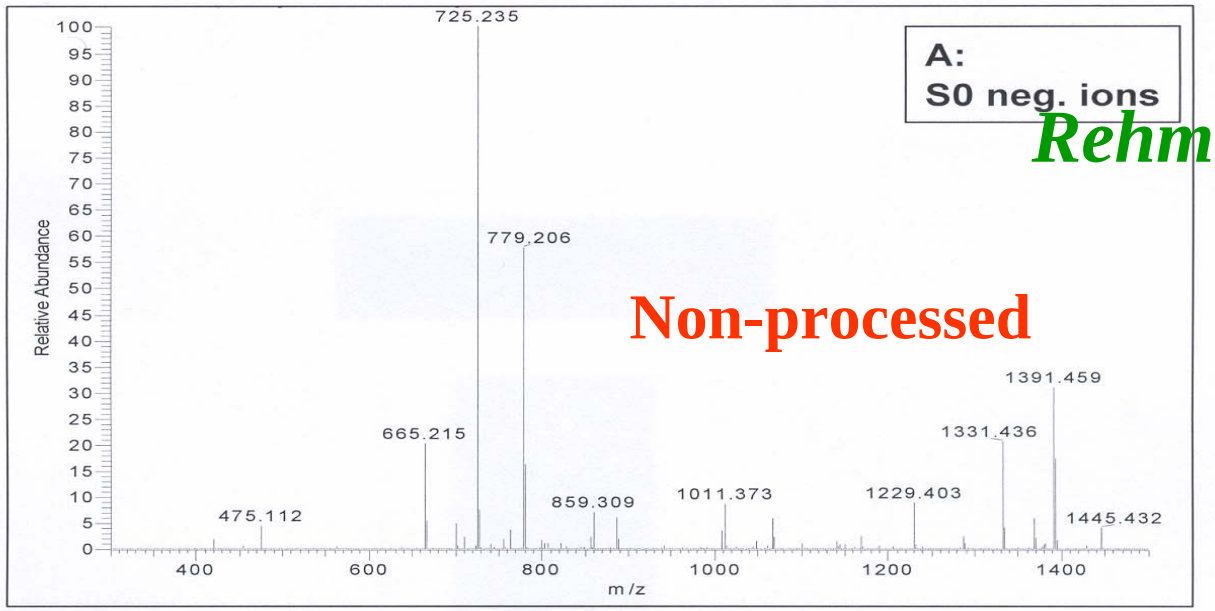
Figure 6. The positive ion FT-MS spectrum of the non-processed root of *Rehmannia glutinosa* (S0, A) and processed by rinsed with Chinese rice wine, steamed and baked for nine cycles (S4, B).

Rehmania glutinosa roots FT-MS

Non-processed

processed

Rehmania glutinosa roots FT-MS



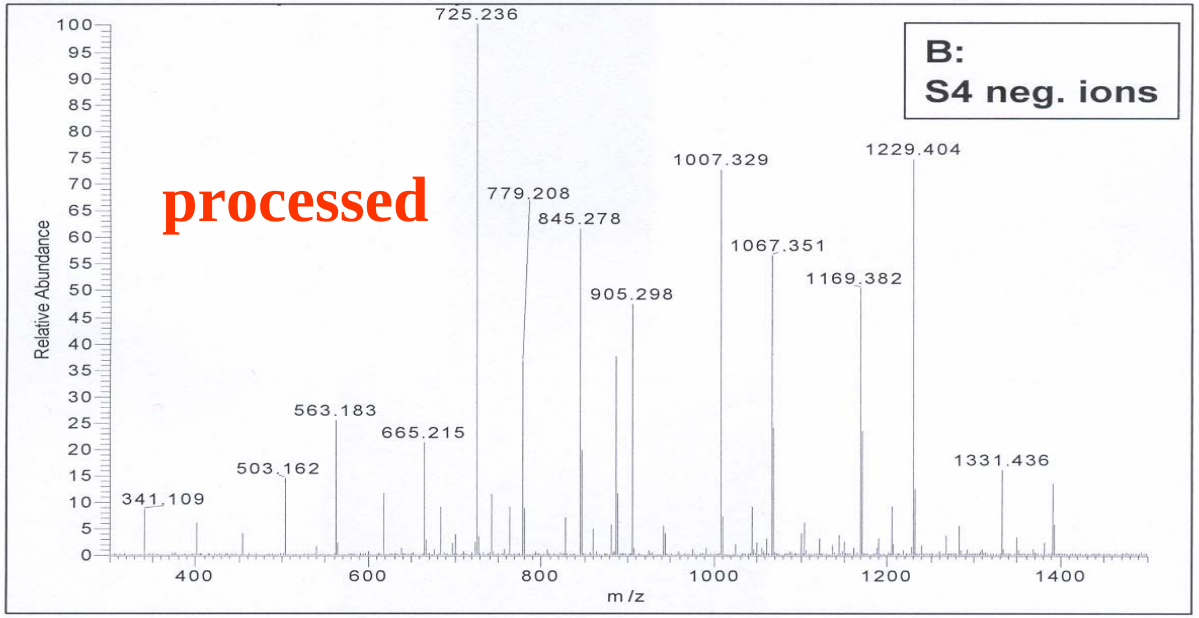
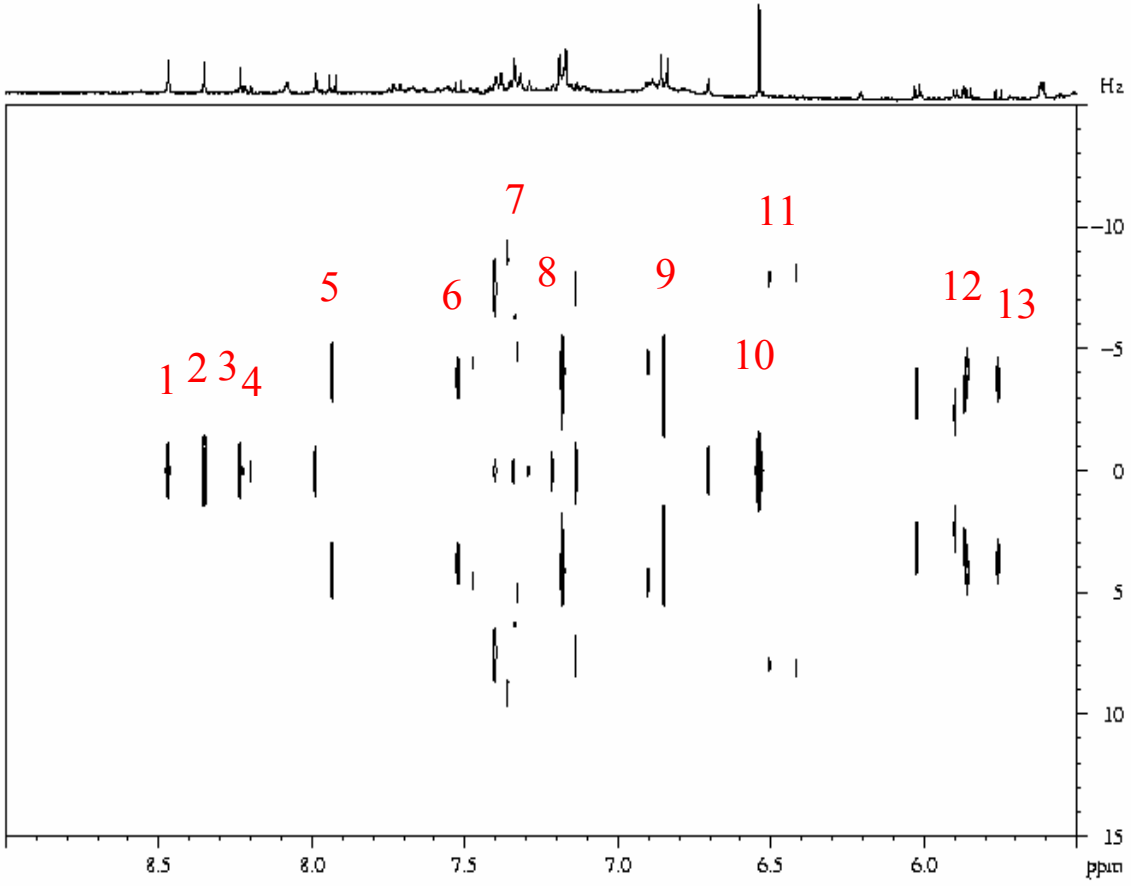


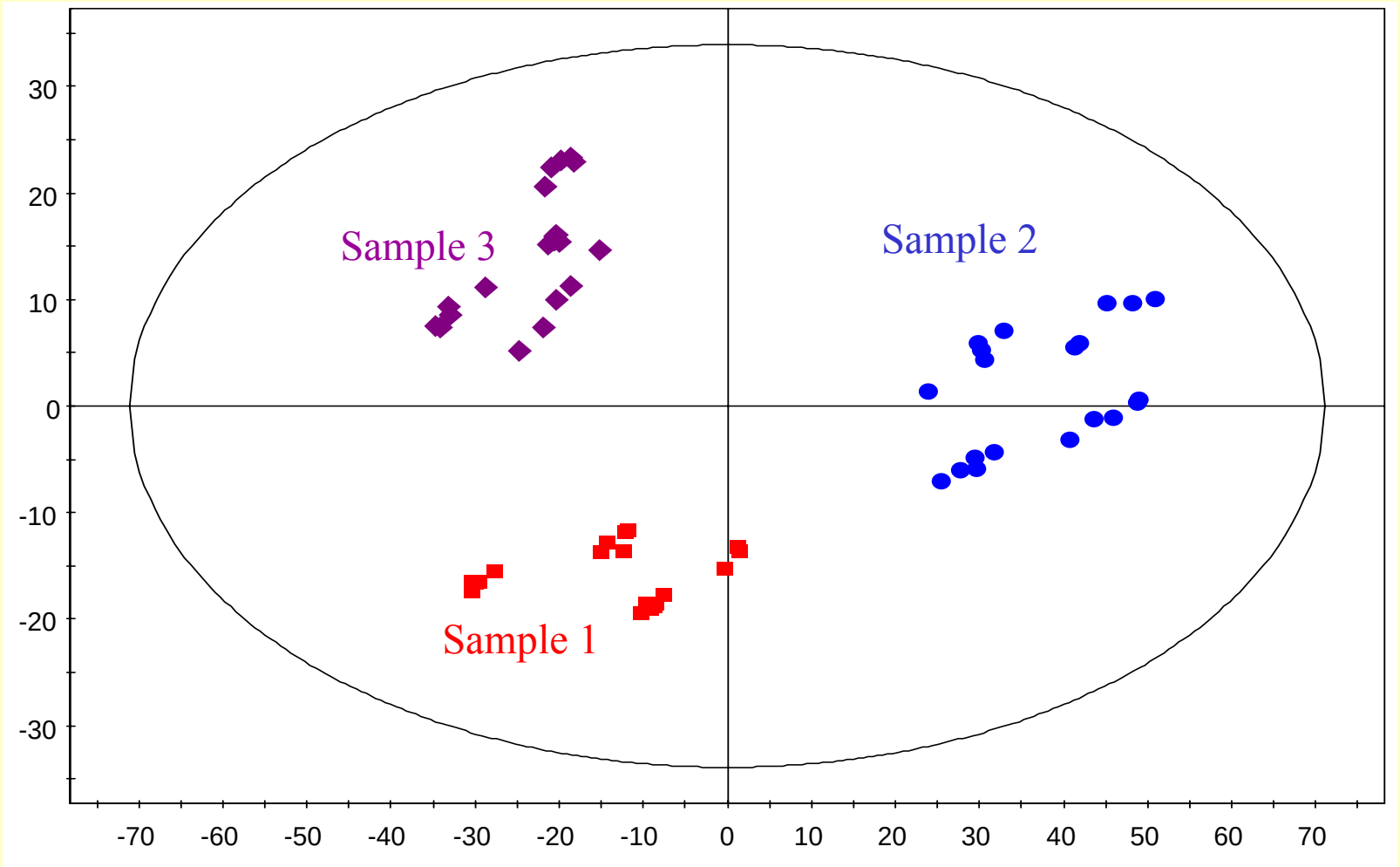
Figure 7. The negative ion FT-MS spectrum of the non-processed root of *Rehmannia glutinosa* (S0, A) and processed by rinsed with Chinese rice wine, steamed and baked for nine cycles (S4, B).

2D J-resolved spectrum Ginseng

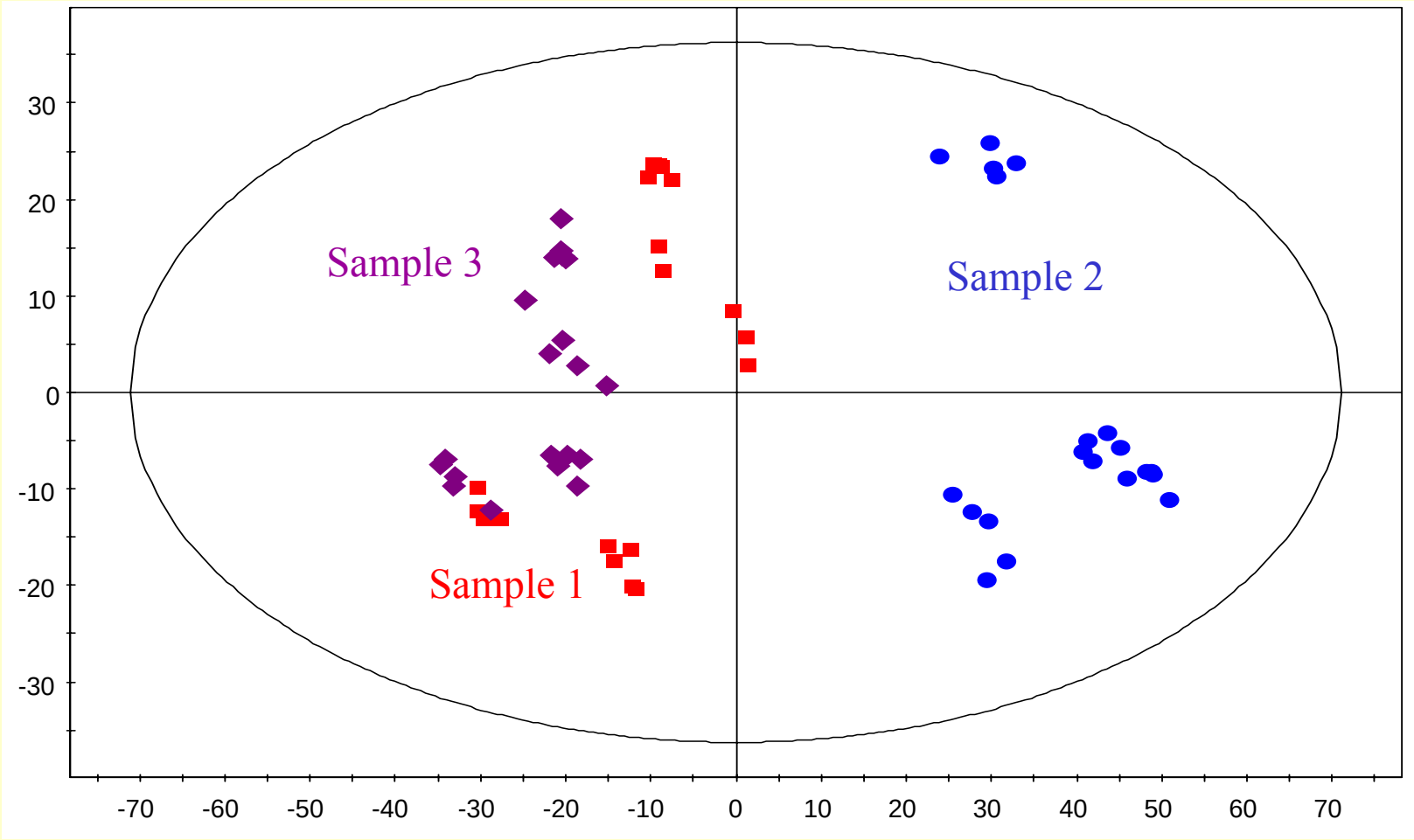


1; H-2 adenosine monophosphate, **2**; H-2 adenosine, **3**; H-7 adenosine monophosphate, **4**; H-7 adenosine, **5**; H-6 uracil, **6**; H-6 cytosine, **7**; aromatic protons phenyl alanine, **8**; H-5 and H-9 of tyrosine, **9**; H-6 and H-8 tyrosine, **10**; H-2 fumaric acid, **11**; H-8 phenylpropanoids, **12**; H-5 uracil, **13**; H-5 cytosine

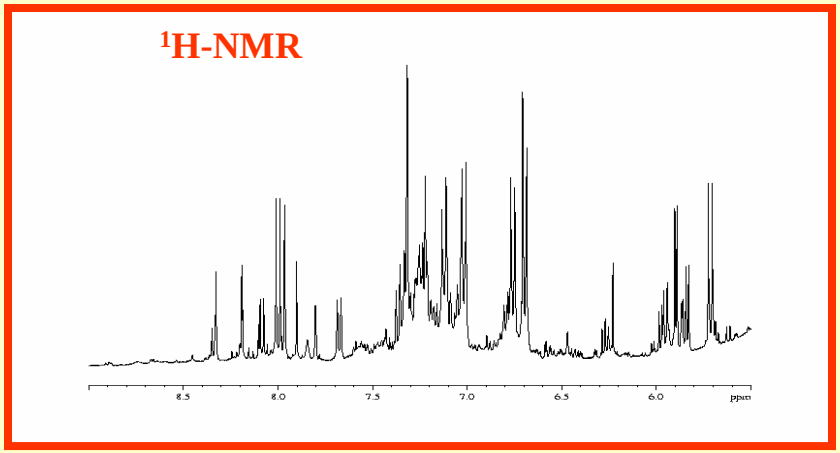
PCA of NMR spectra different brands of Ginseng

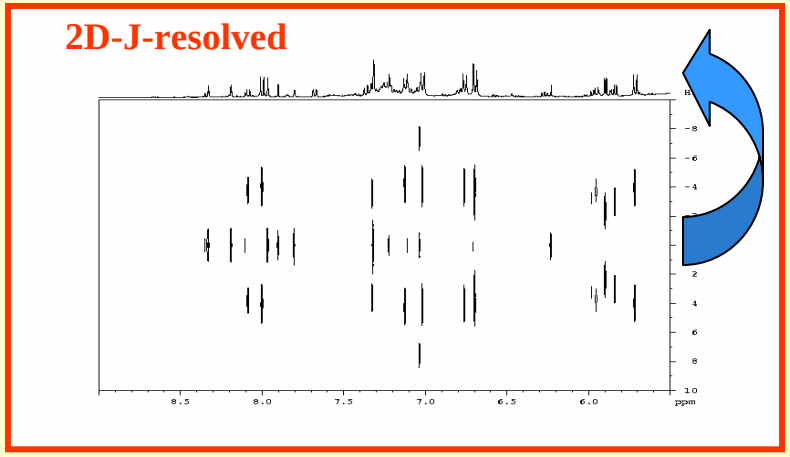


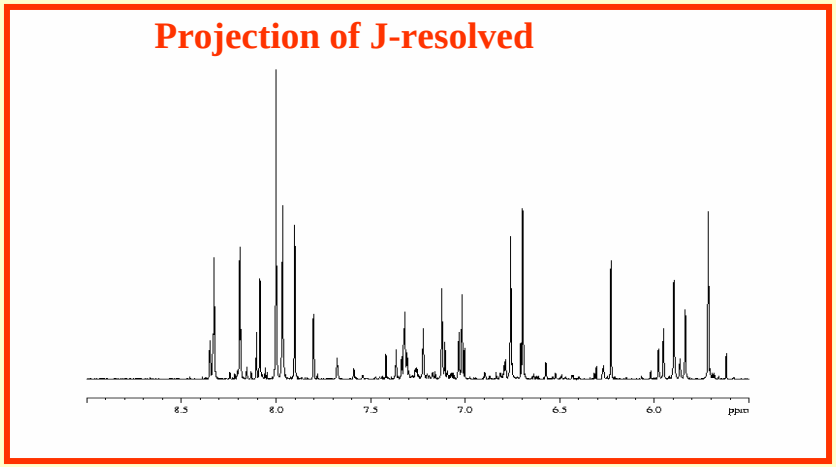
PCA of NMR different brands of Ginseng



Ginseng
Projected 2D J-resolved spectra

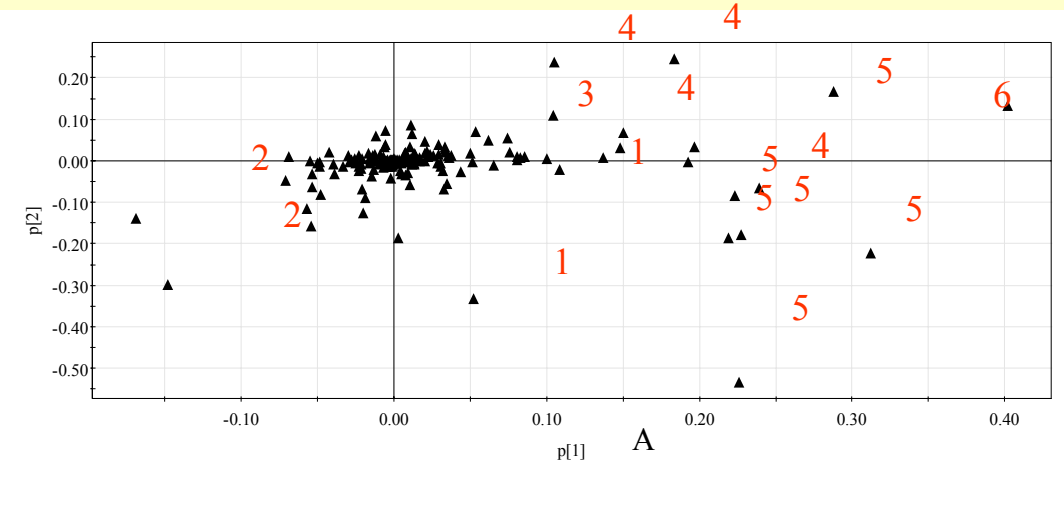


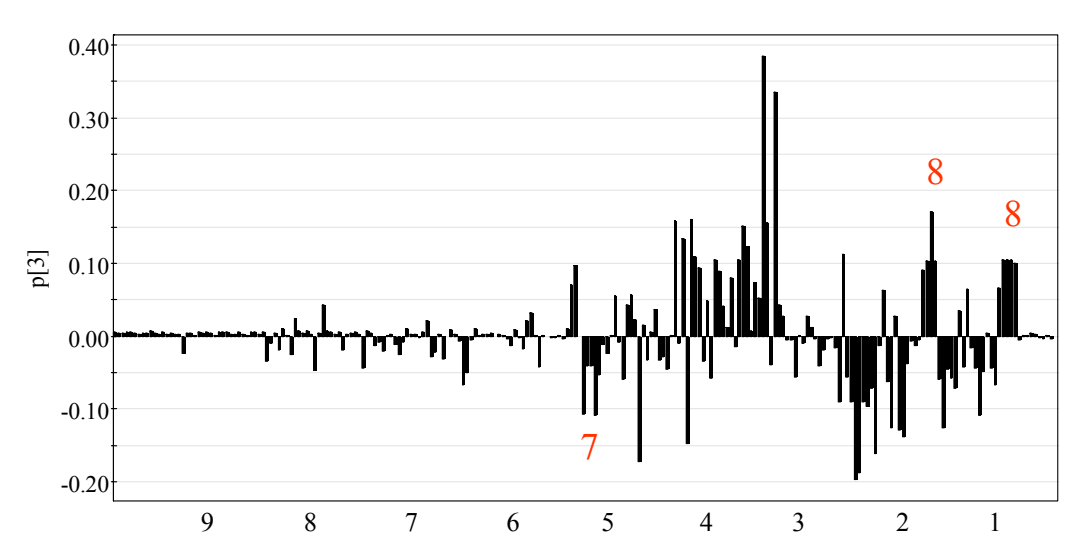




- **Less complex spectra**
- **Decoupled spectra like ^{13}C**
- **Higher resolution**
- **Better separation in PCA**

Ginseng loading plot: find differentiating compounds



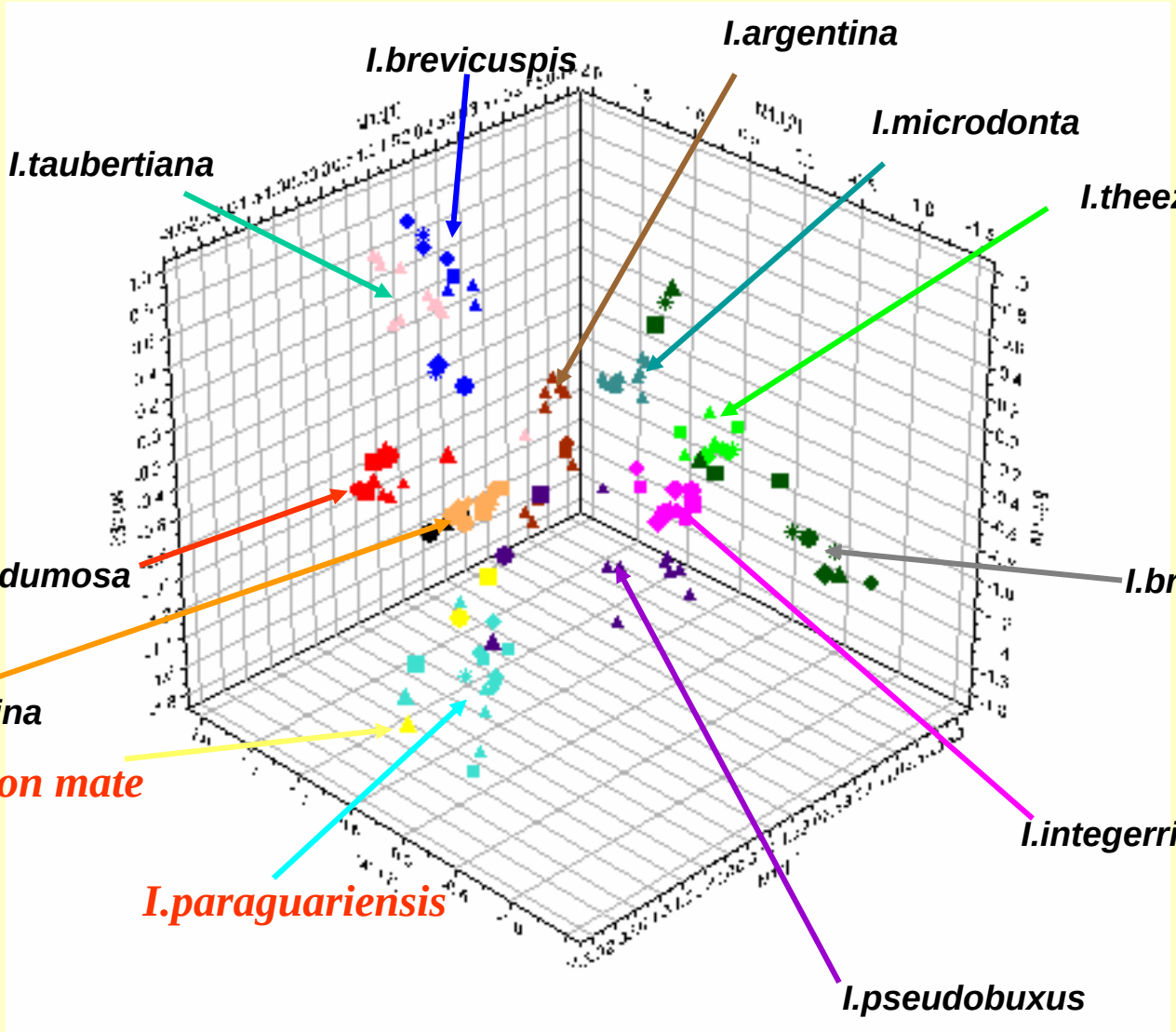


1: alanine
2: arginine
3: fumaric acid
4: inositol
5: sucrose
6: acetate
7: anomeric protons sugars
8: methyl protons saponins

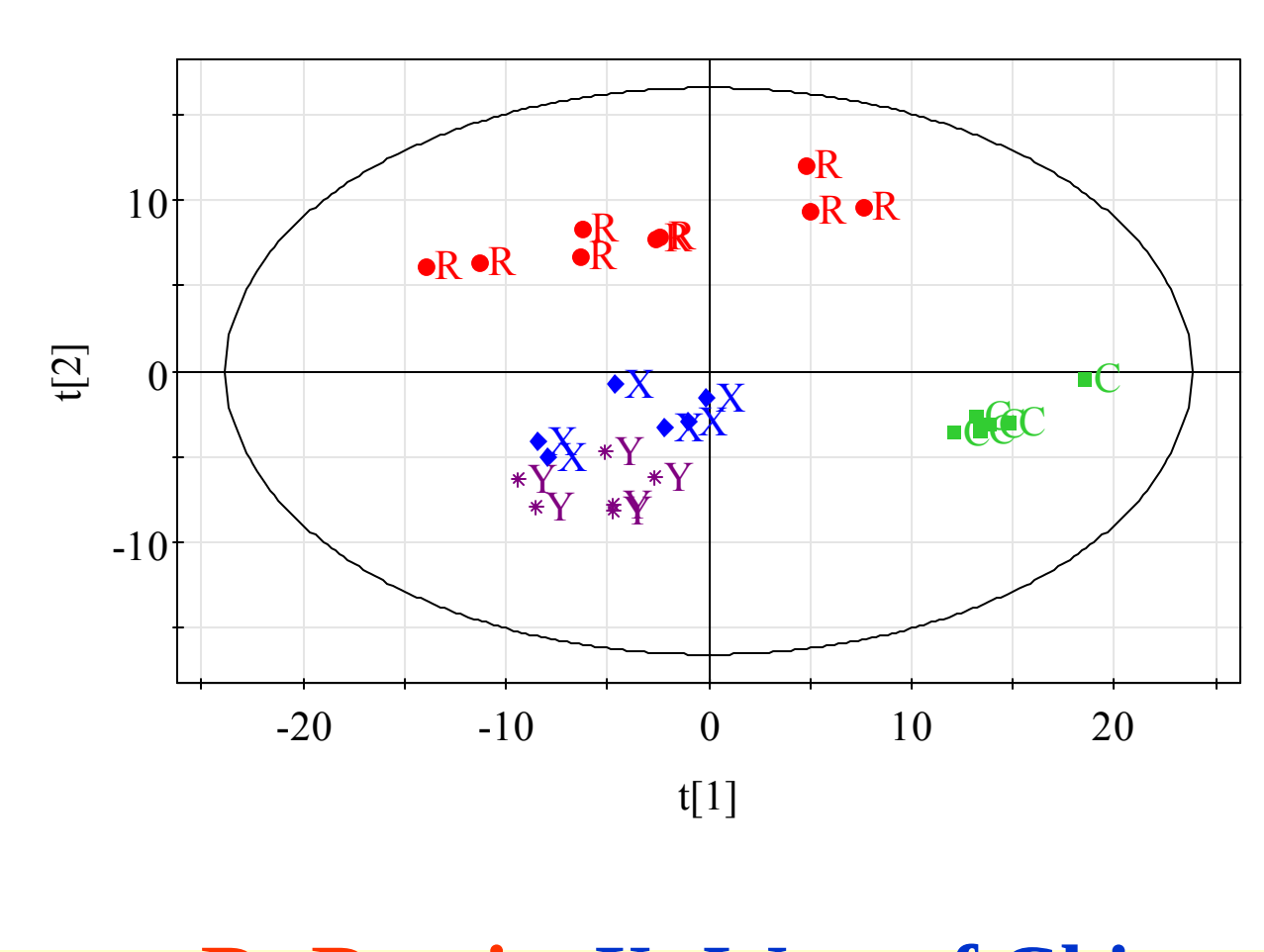
Local congeneric substitutes for *Ilex paraguariensis*

- ***Ilex brevicuspis***
- ***Ilex dumosa var.dumosa***
- ***Ilex dumosa var.guaranina***
- ***Ilex theezans***
- ***Ilex integerrima***
- ***Ilex brasiliensis***
- ***Ilex pseudobuxus***
- ***Ilex argentina***
- ***Ilex microdonta***
- ***Ilex taubertiana***

PCA analysis NMR spectra Ilex species



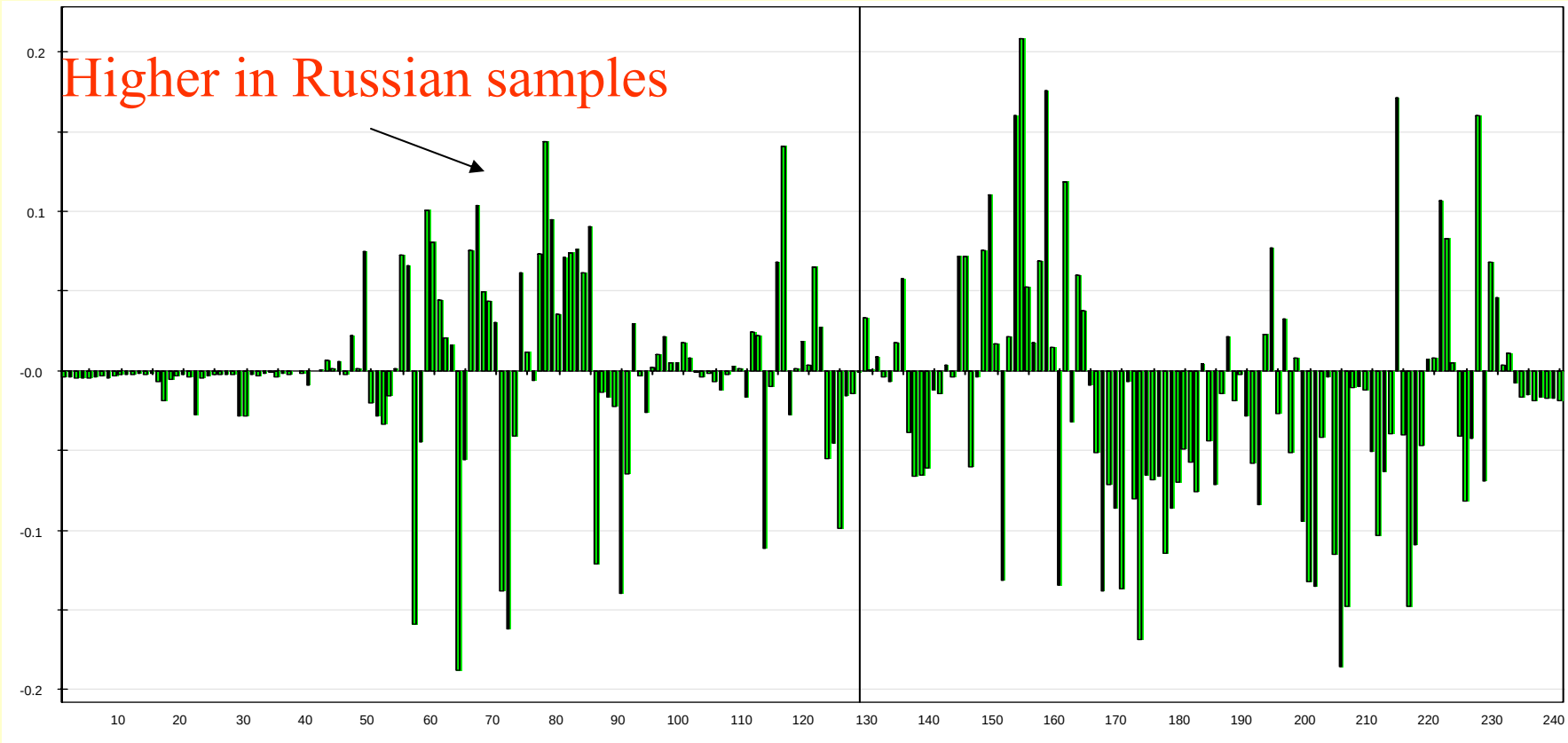
PCA NMR of Licorice samples



R: Russia, X: West of China,

Y: East of China, C: Cultivated

Flavonoids levels different in licorice samples

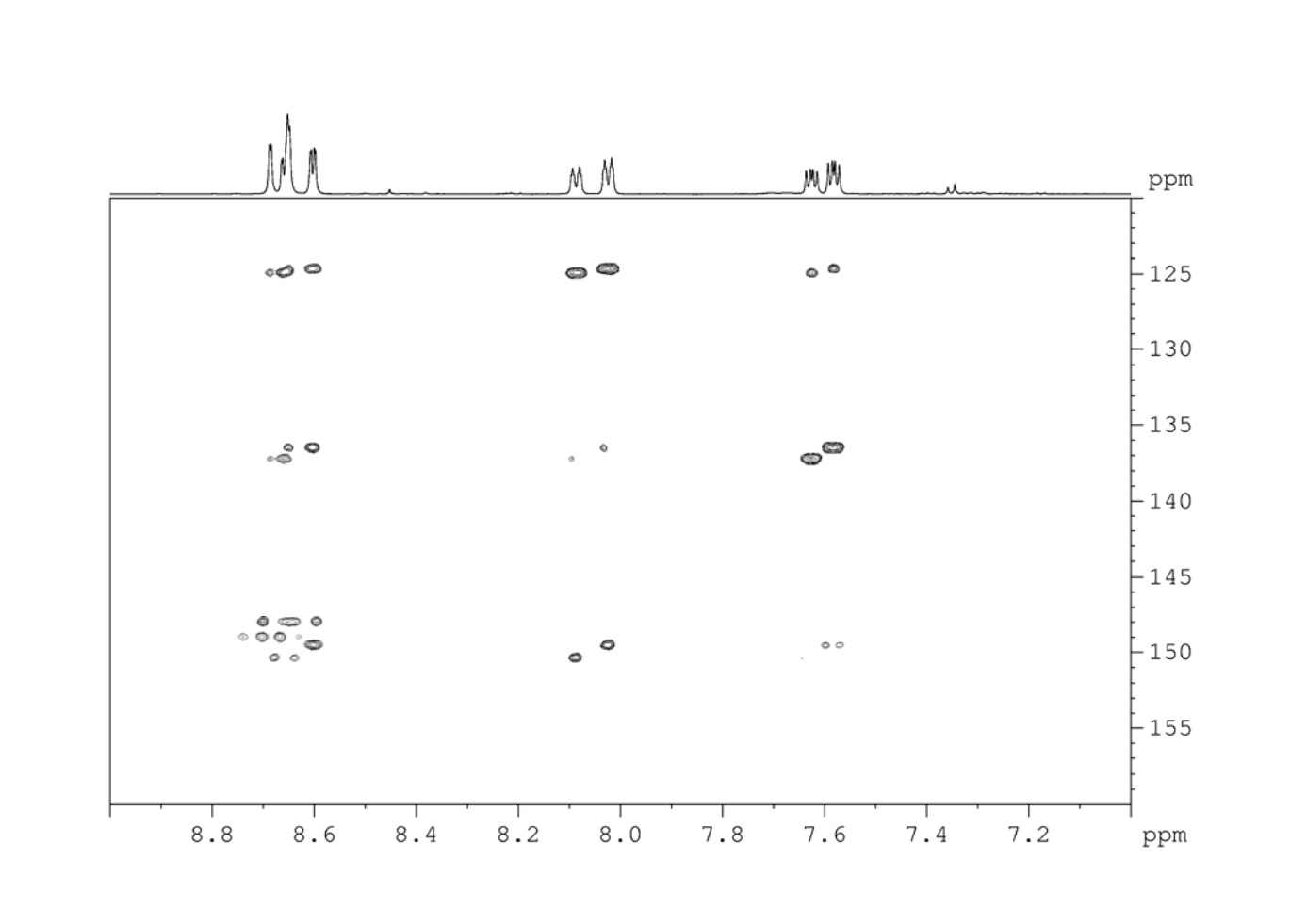


NMR as analytical method

- Very fast method
- Simple extraction
- No need for calibration curves and references
- Different compounds in single analysis
- High reproducibility
- Low costs per sample
- Suitable for public database

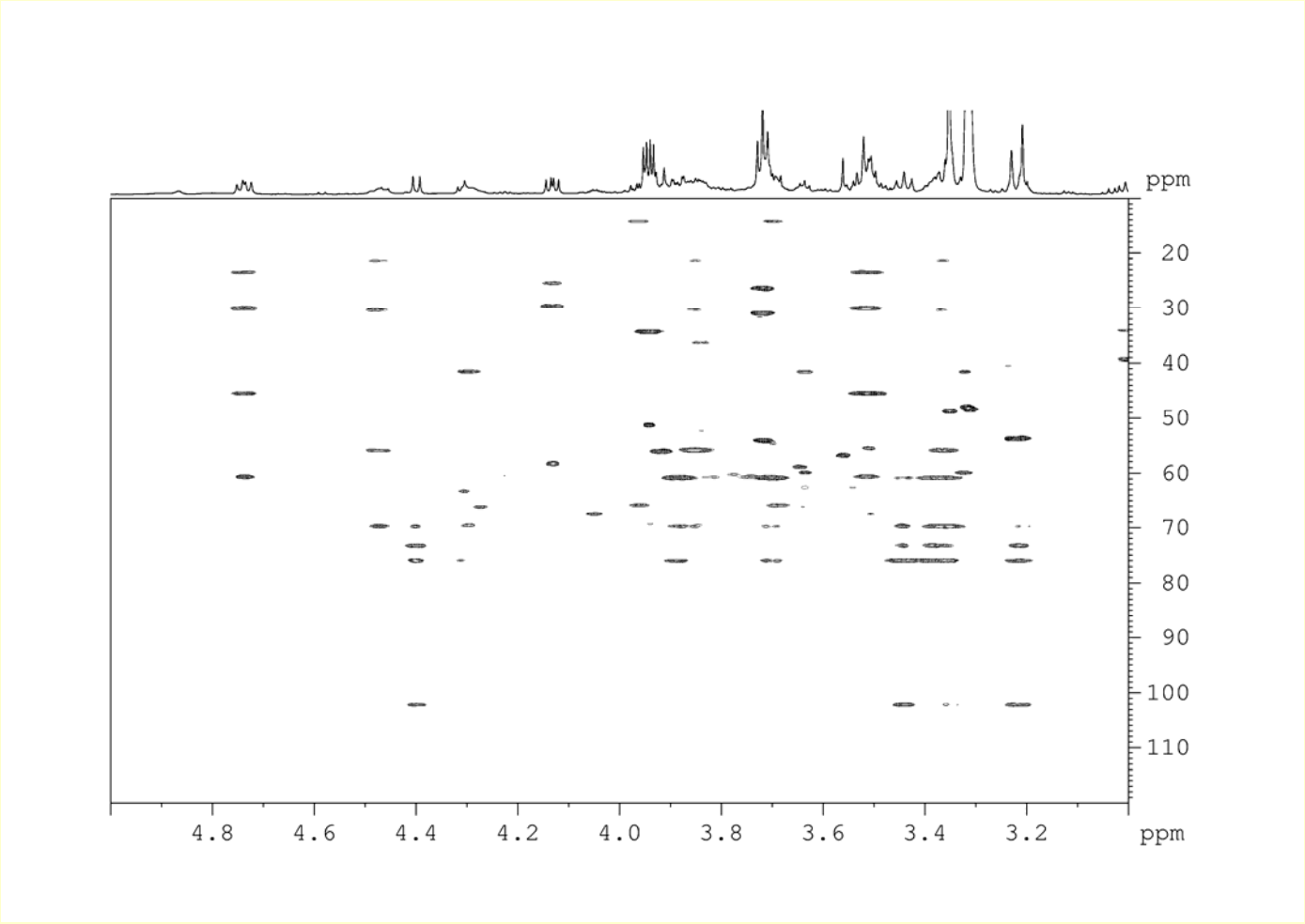
- High inititial investment
- Sensitivity (10-100µg)

Application of HSQC-TOCSY



Nicotine analogues in mixture

Application of HSQC-TOCSY



Sugars and amino acid

Application of HSQC-TOCSY