METABONOMICS

ULB/ERASME

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Jean-Marie Colet

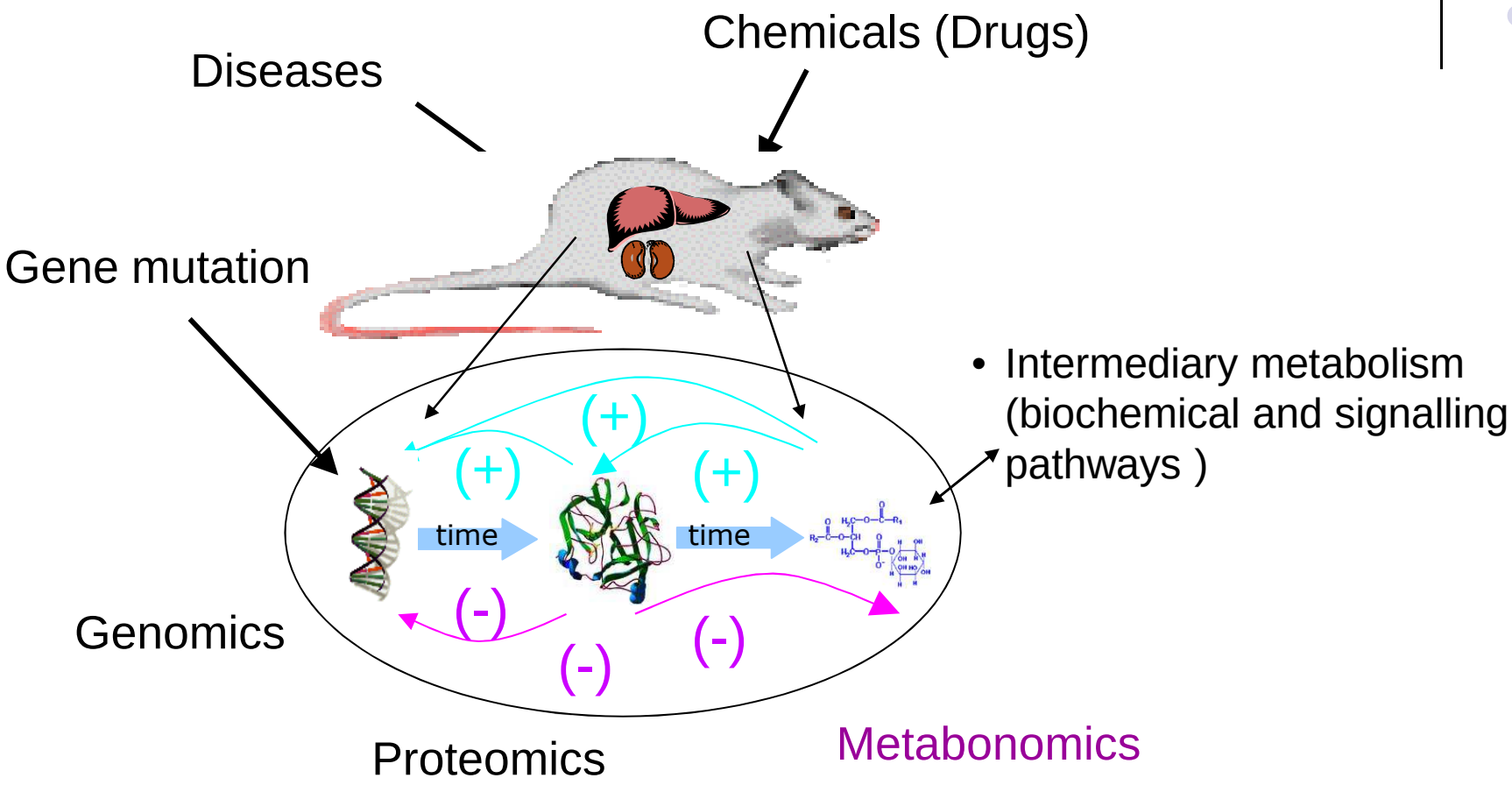
UMONS
Université de Mons

"Le Pentagone“
Aile 2A
Avenue du champ Mars, 6
7000 Mons

School of Medicine et Pharmacy

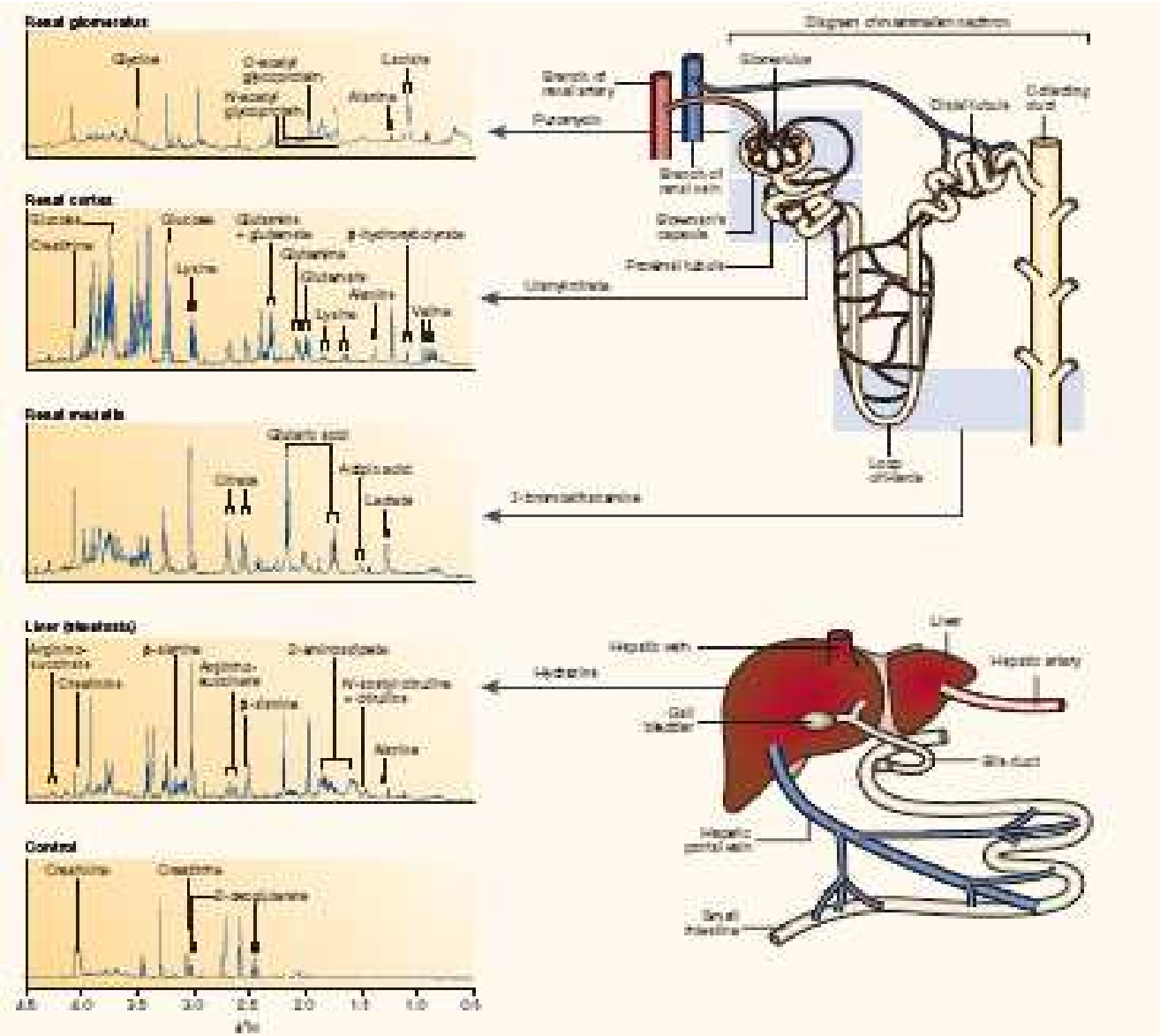
Department of Human Biology & Toxicology

http://portail.umons.ac.be/FR/universite/facultes/fmp/services/toxico/Pages/default.aspx



- Intermediary metabolism (biochemical and signalling pathways)

^{1}H-NMR spectrum of biofluid ~ « metabolic signature? »

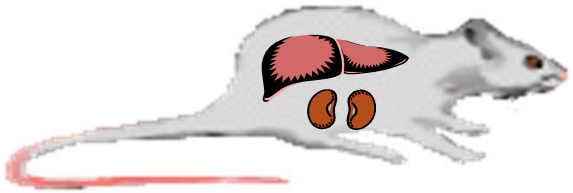


- Due to complexity of generated data , need multivariate data analysis

⇒ Spectroscopy (^{1}H-NMR, MS, …) + Multivariate data analysis

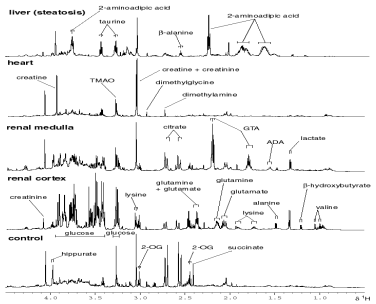
⇒ **METABONOMICS concept**

Nicholson JK Nat rev Drug Disc 1(2):153-161 (2002)

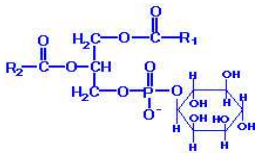


Metabolic cages on refrigerated & automated racks

Plasma &t urine : clinical chemistry parameters



Chemometric analysis

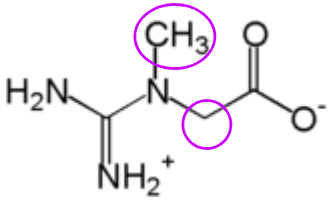


Biomarkers ID

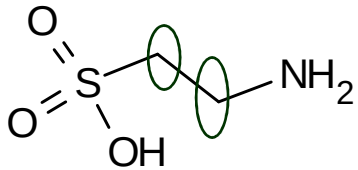
Pattern recognition

Spectroscopy:
NMR, MS, IR, …

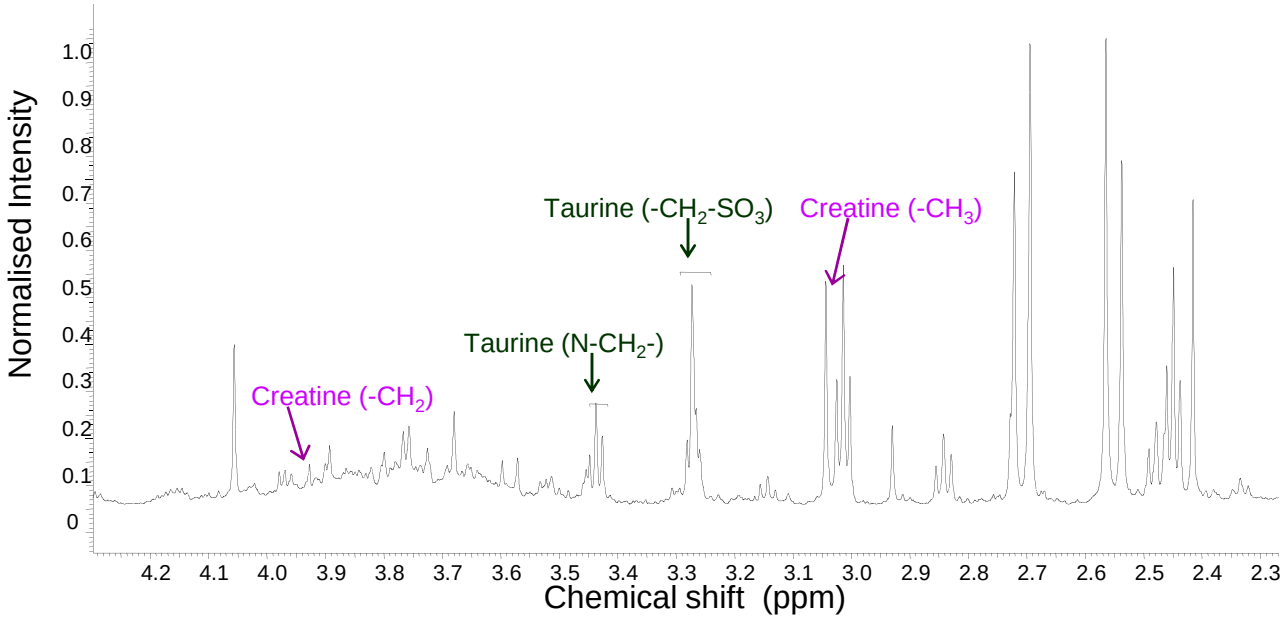
Chemical shift (δ) & multiplicity



Creatine



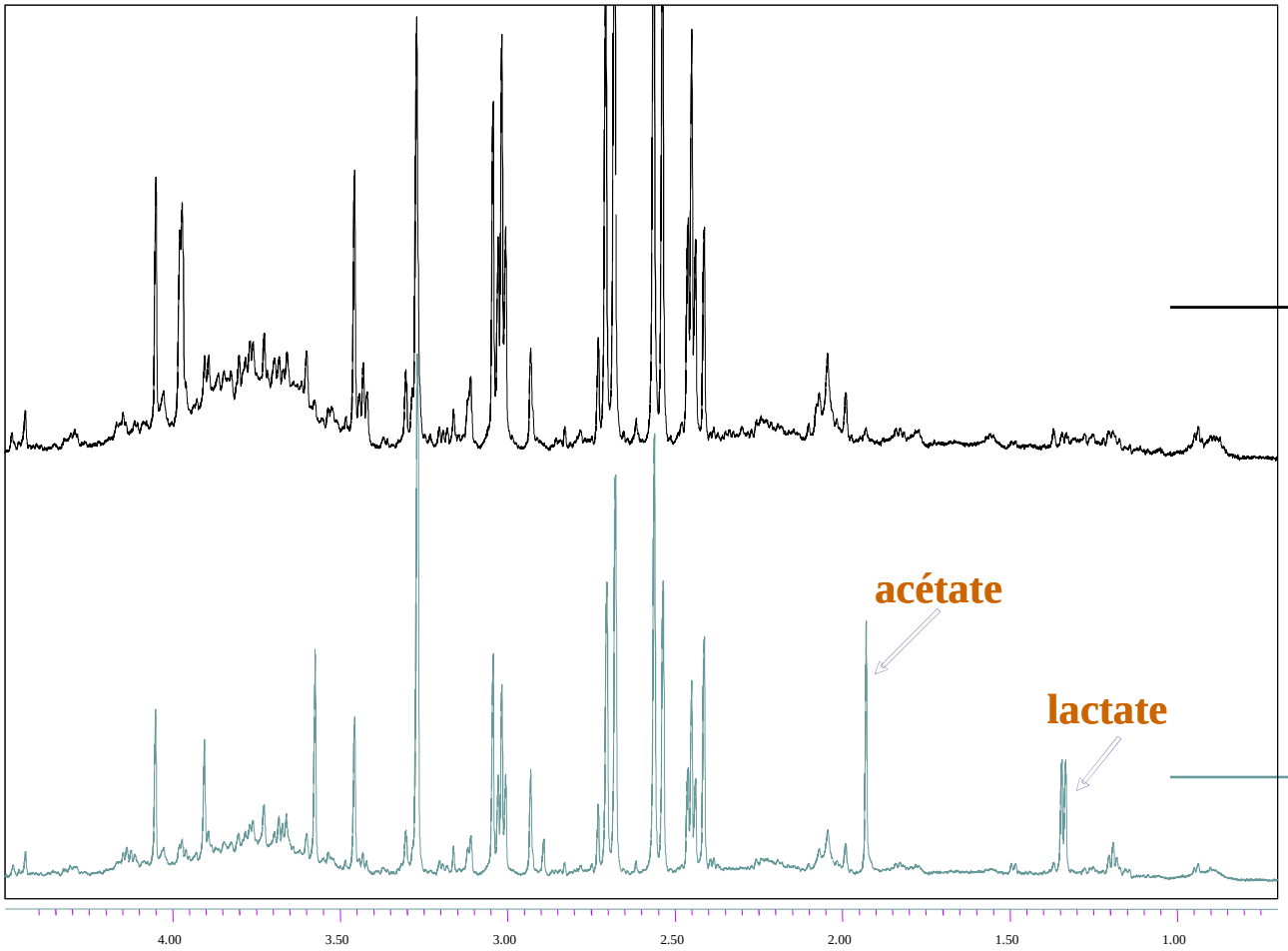
Taurine



- Animals placed in metabolic cages for urine collection
- Acclimatation (at least 2 days) to minimize stress
- Water and food ad libitum or controlled if necessary

Avoid bacterial contamination !

Wrong data interpretation due to bacterial co,ntamination…



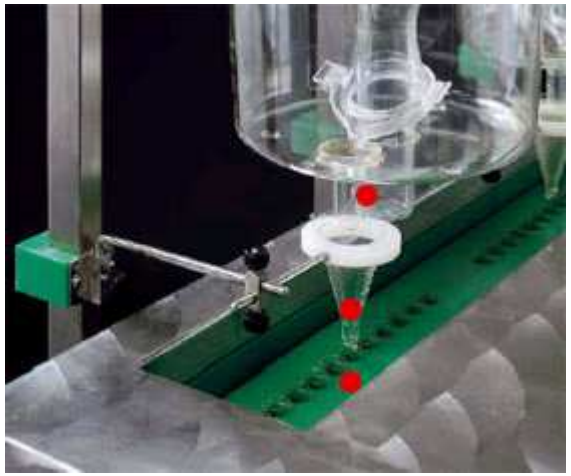
Urine collected at 4°C + NaN_3

Bacterial contamination

- After drug administration, urine fractions collected over first 0-8h period to remove spectral contamination from exogenous molecules (parent compound + potential DRC's)

- Next, urine collection over time

- In healthy Sprague-Dawley rat, daily urine volume ~ 15 ml



What biofluids?

Mixing of various substances

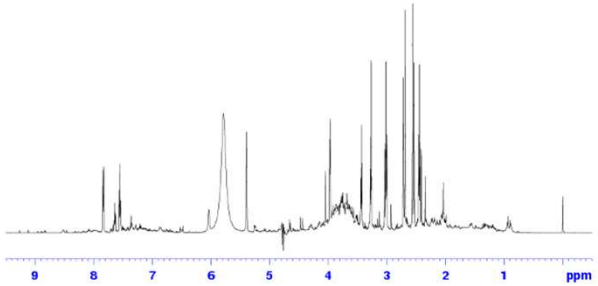
(Metabolites, proteins, sugars, lipids, salts)

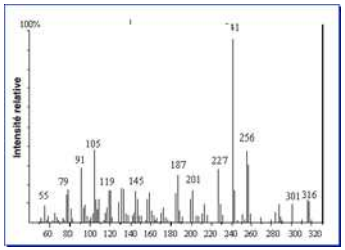
+

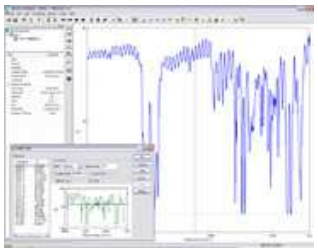
H_2O

- Urine
- Blood (Plasma / Sérum)
- CSF
- Bile
- Saliva
- ...

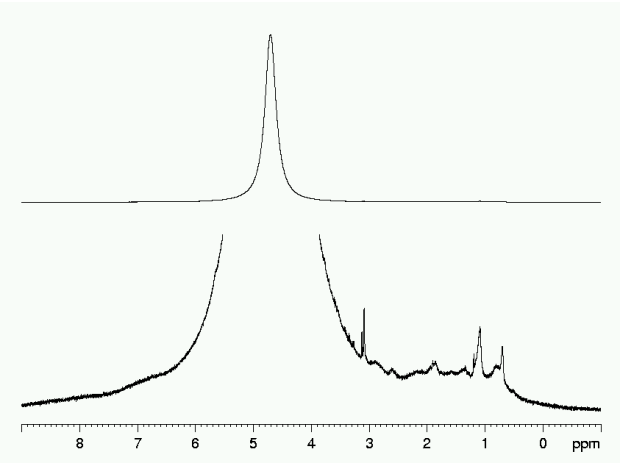
Spectroscopic techniques





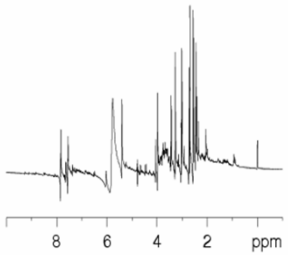


Importance de l'acquisition des spectres

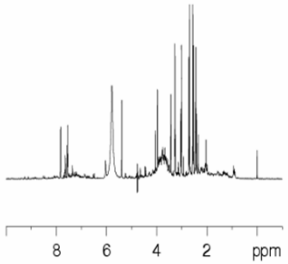


Serum
+ 10% D_2O

… et du traitement des spectres

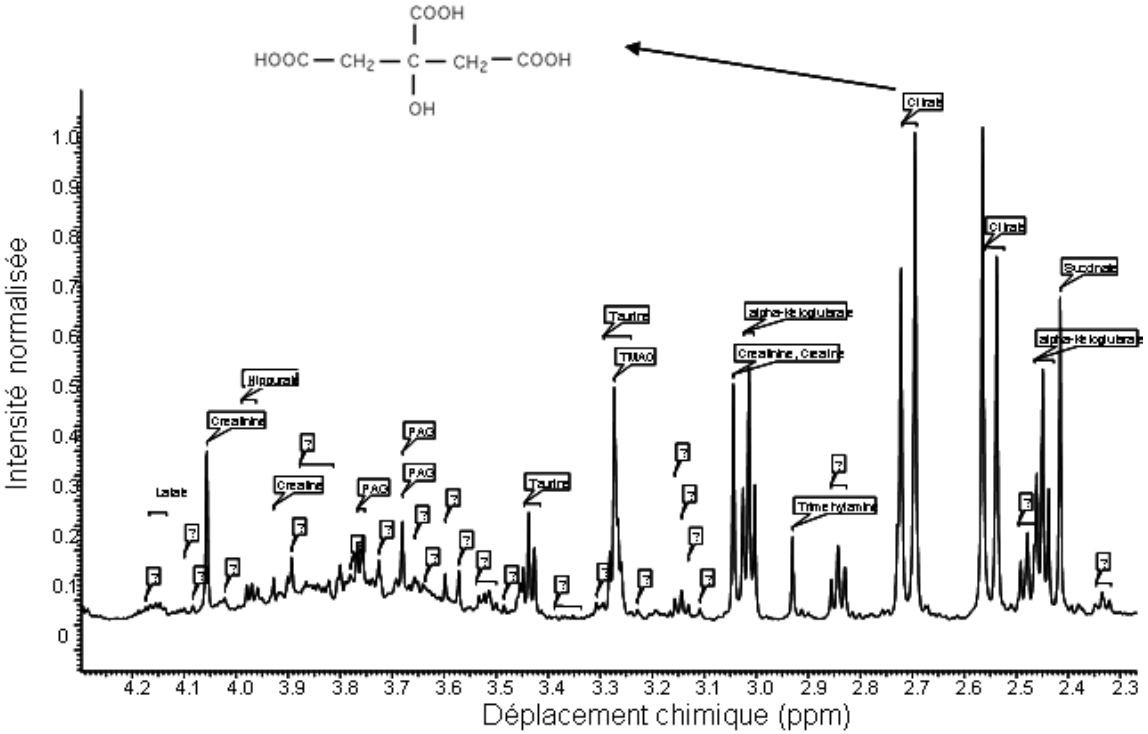


Correction de phase



Correction de la ligne de base

In NMR peak identification remains weak



… multivariate data set

Multivariate data analysis

Two basic approaches :

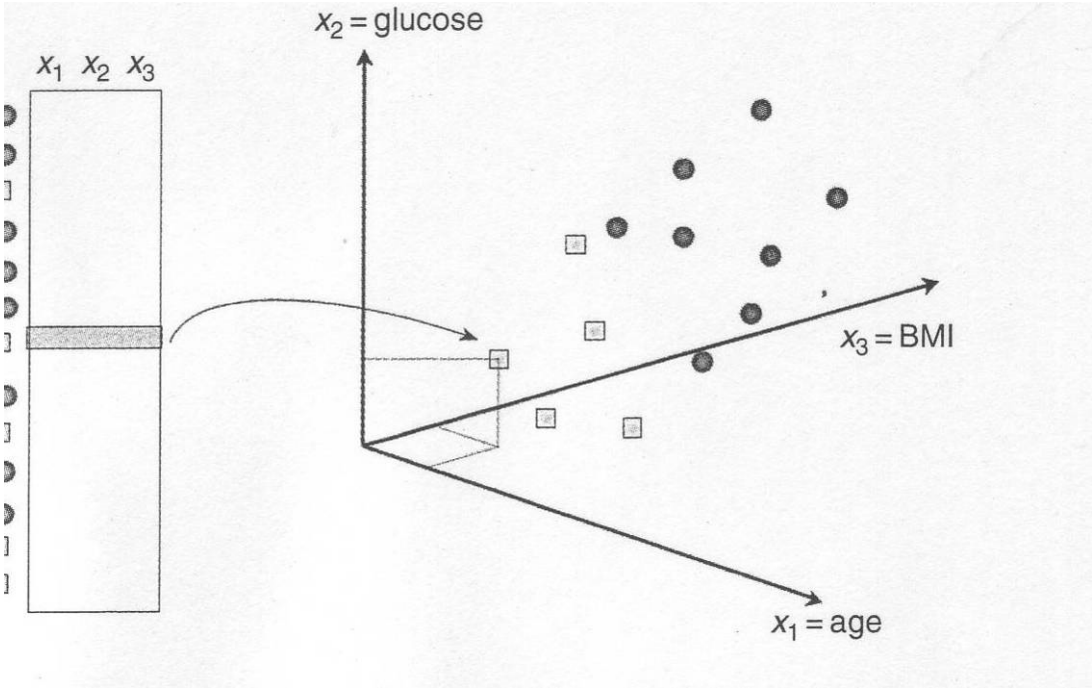
Non supervised

- No information on individual sample provided to the system
- PCA (principal component analysis)
- …

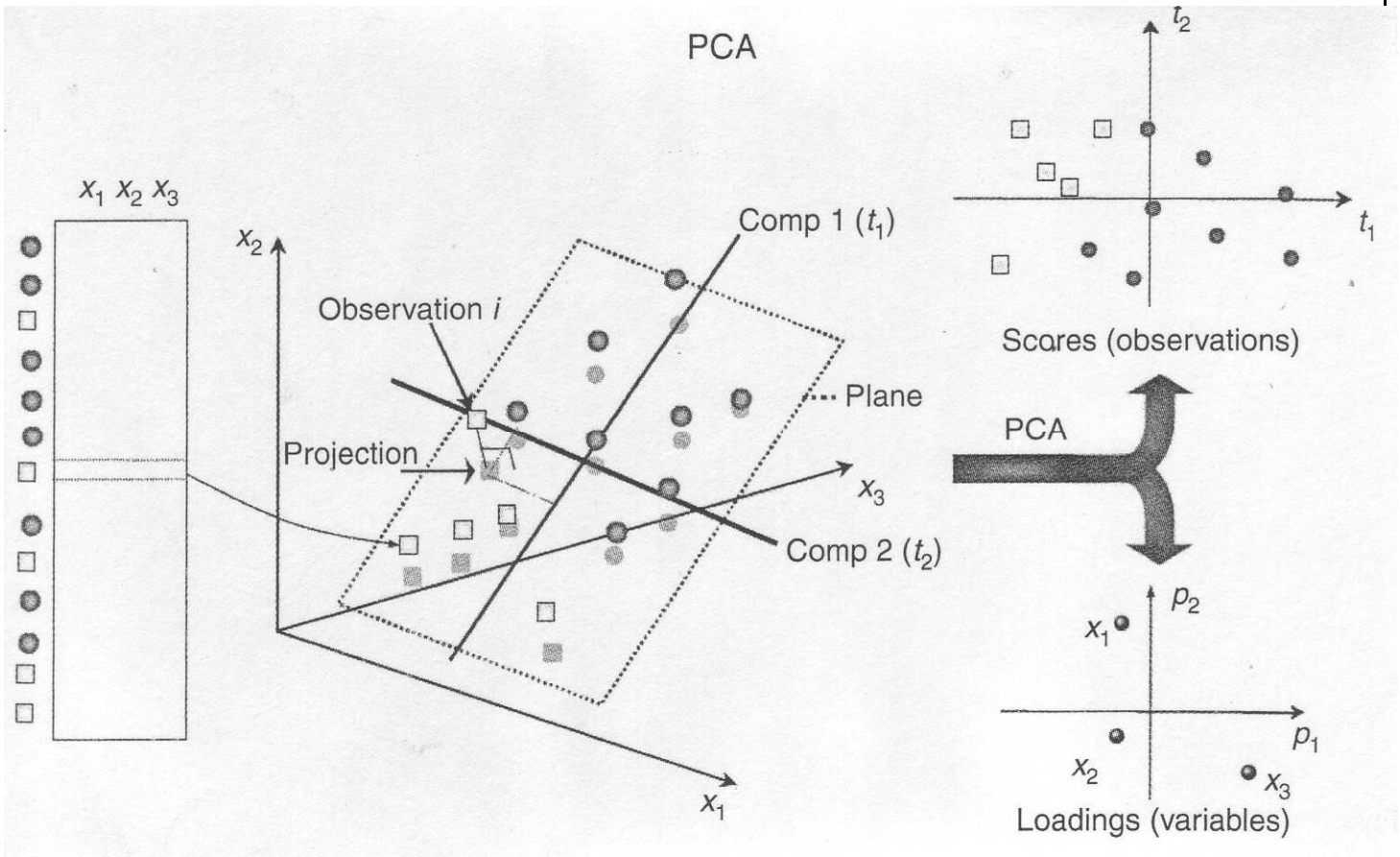
Supervised

- At least one information provided to the system (for instance classification)
- PLS-DA
- …

Projection methods …



Projection methods…



Principal component analysis (PCA)

Yearly consumption (kg/year) of various nutriments in Europe

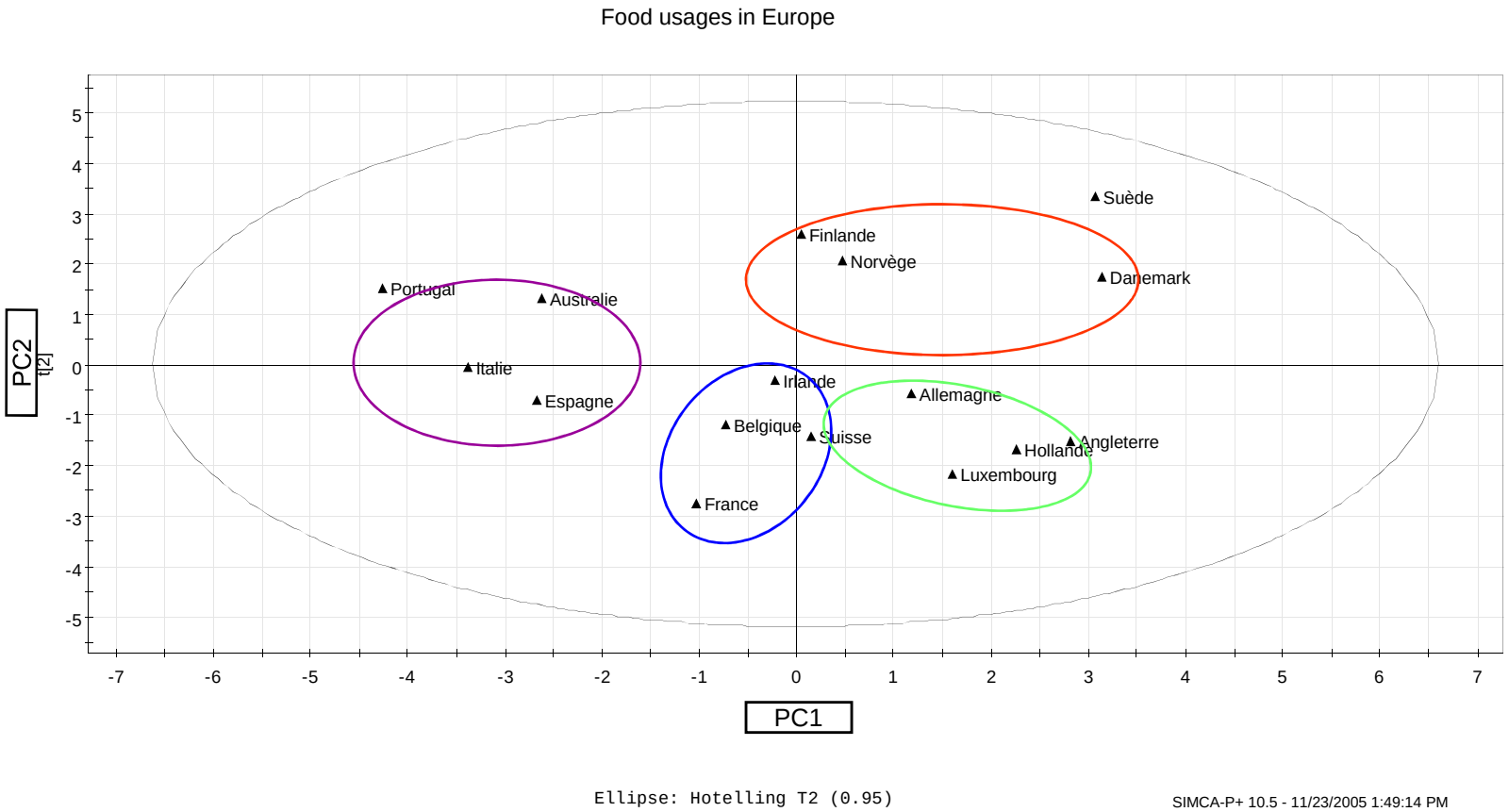
observations

Initial variables

Pays	Café moulu	Café instantané	thé	Édulcorant	biscuit	poisson	légume	pomme	orange	fruit	jambon	ail	beurre	margarine	huile	yoghurt	crackers
Allemagne	90	49	88	19	57	27	21	81	75	44	71	22	91	85	74	30	26
Italie	82	10	60	2	55	4	2	67	71	9	46	80	66	24	94	5	18
France	88	42	63	4	76	11	5	87	84	40	45	88	94	47	36	57	3
Hollande	96	62	98	32	62	14	14	83	89	61	81	15	31	97	13	53	15
Belgique	94	38	48	11	74	13	12	76	76	42	57	29	84	80	83	20	5
Luxembourg	97	61	86	28	79	26	23	85	94	83	20	91	94	94	84	31	24
Angleterre	27	86	99	22	91	20	24	76	68	89	91	11	95	94	57	11	28
Portugal	72	26	77	2	22	20	3	22	51	8	16	89	65	78	92	6	9
Australie	55	31	61	15	29	15	11	49	42	14	41	51	51	72	28	13	11
Suisse	73	72	85	25	31	19	15	79	70	46	61	64	82	48	61	48	30
Suède	97	13	93	31		54	45	56	78	53	75	9	68	32	48	2	93
Danemark	96	17	92	35	66	51	42	81	72	50	64	11	92	91	30	11	34
Norvège	92	17	83	13	62	30	15	61	72	34	51	11	63	94	28	2	62
Finlande	98	12	84	20	64	18	12	50	57	22	37	15	96	94	17		64
Espagne	70	40	40		62	23	7	59	77	30	38	86	44	51	91	16	13
Irlande	30	52	99	11	80	5	3	57	52	46	89	5	97	25	31	3	9

Eriksson *et al.* Multi- and Megavariate Data Analysis. Principles and Applications. Umetrics Academy, 2001.

Principal component analysis (PCA)



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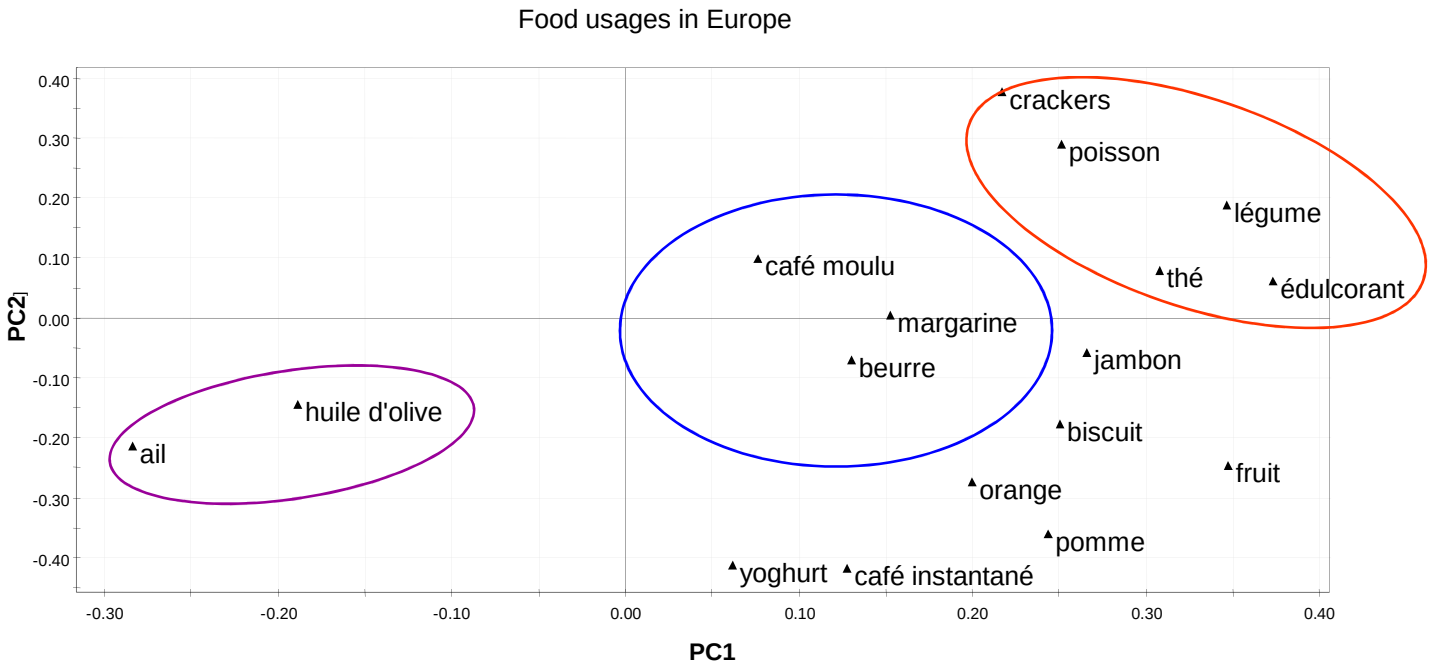

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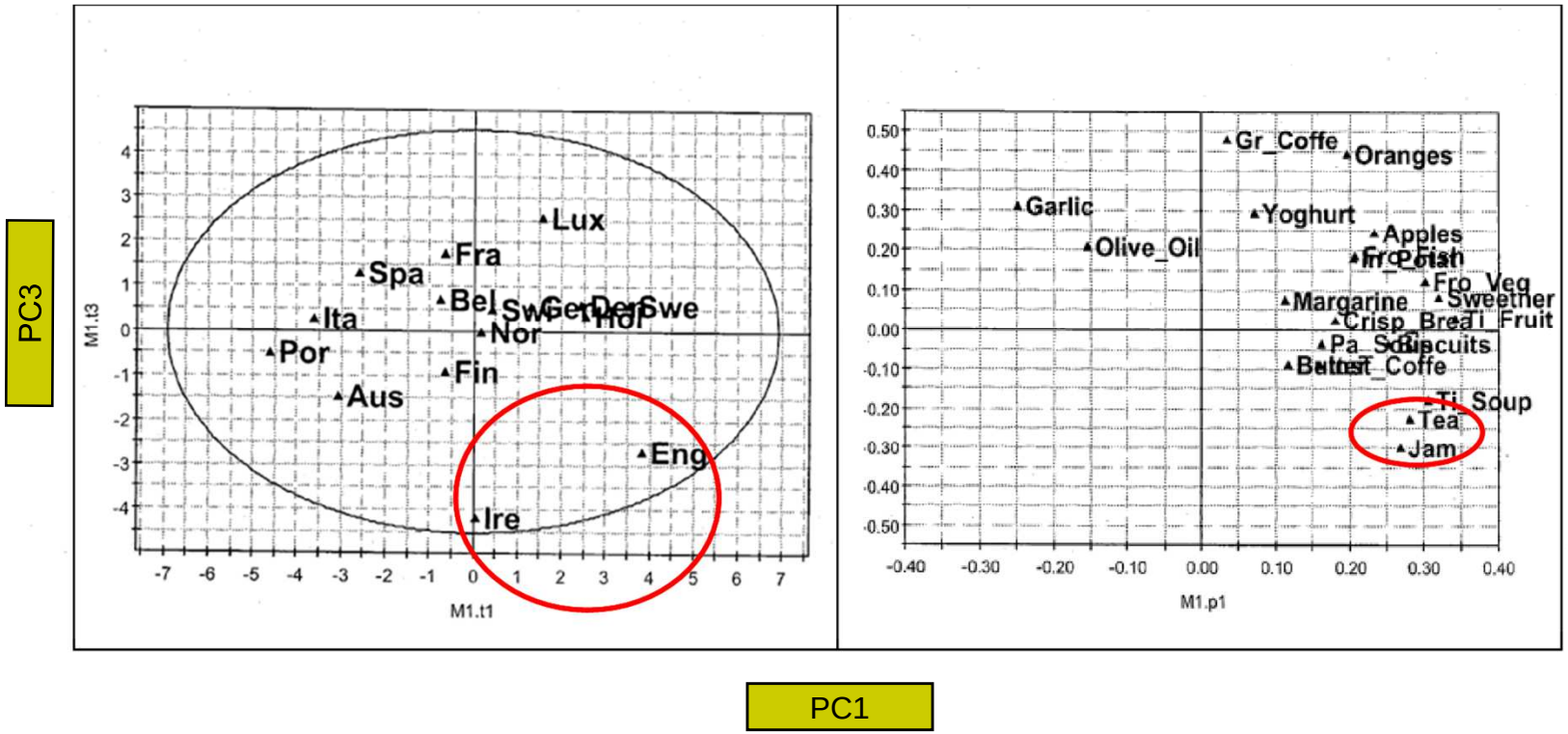
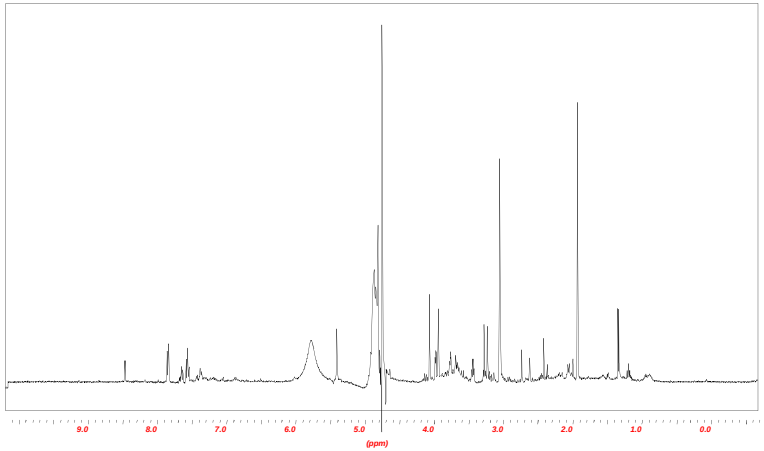
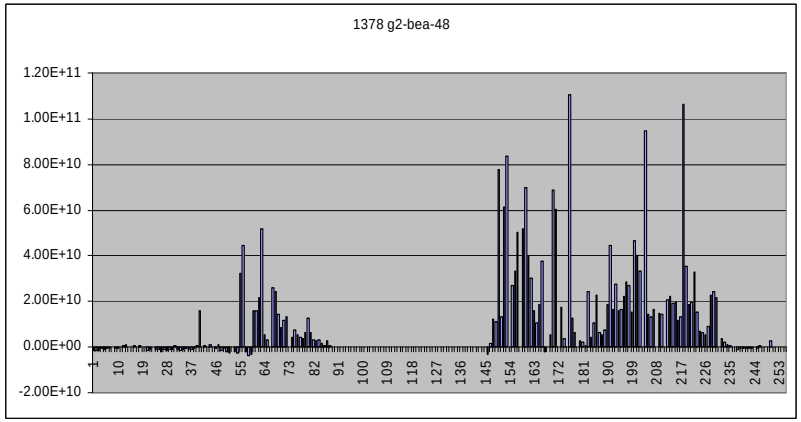


PC1

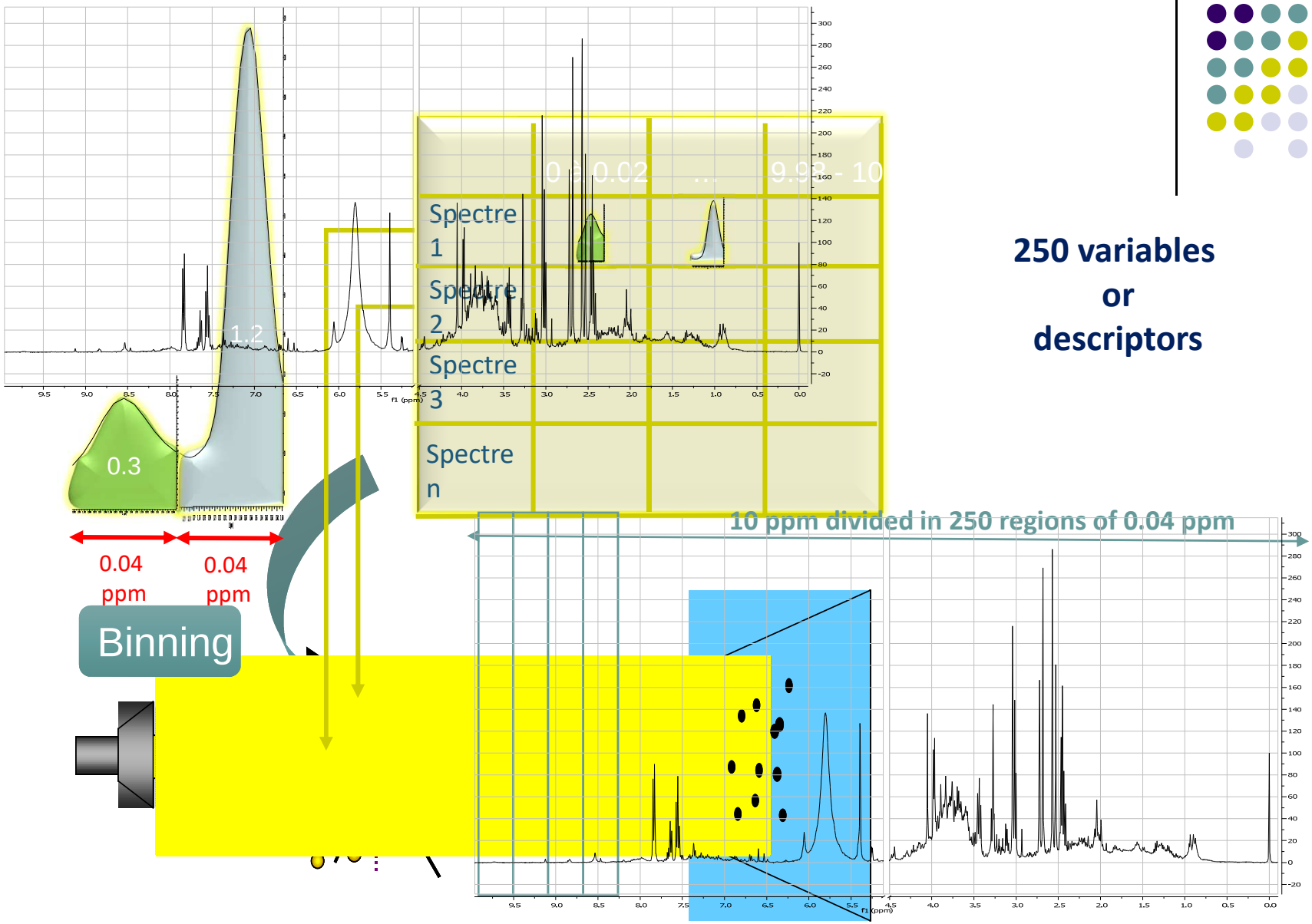
Eriksson *et al.* Multi- and Megavariate Data Analysis. Principles and Applications. Umetrics Academy, 2001.

PCA on ^{1}H-NMR data ?



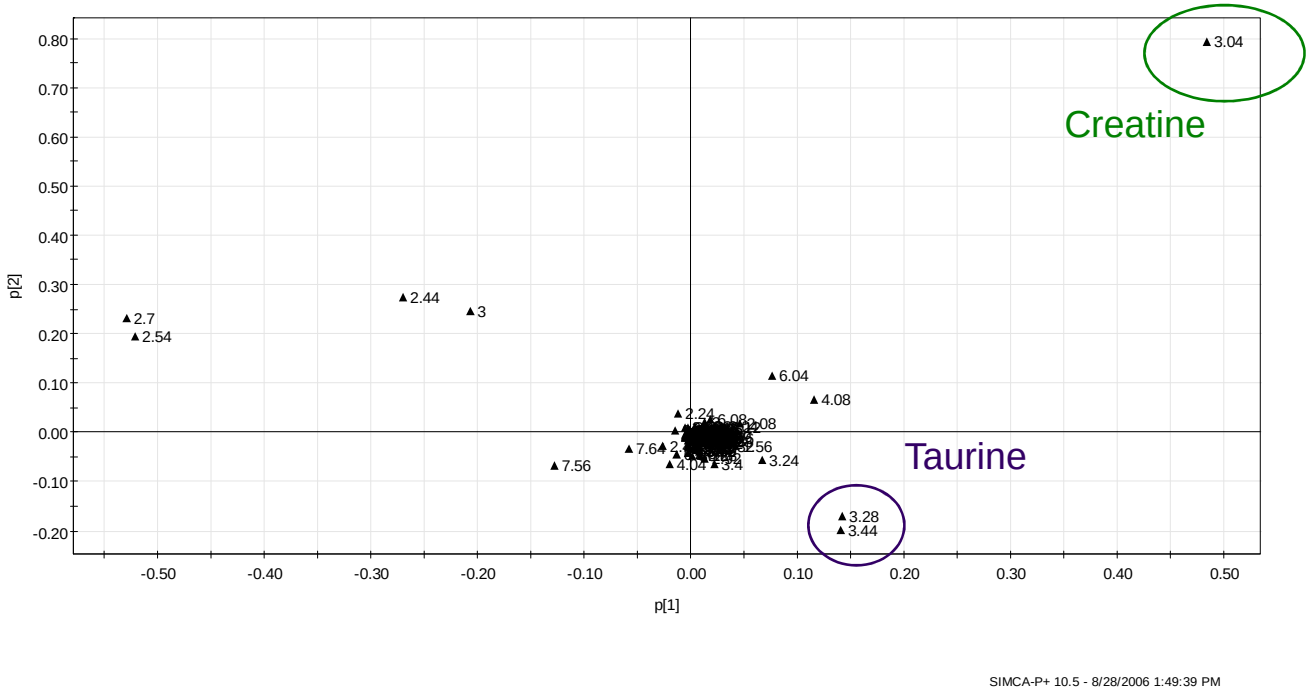


“Binning” procedure



250 variables or descriptors

Principal component analysis (PCA)



<u>Loadings plot</u> : what variables responsible for separation or gathering of observations

Two-entries Excel file

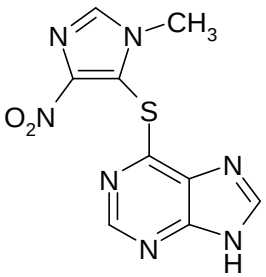
Median chemical shift of the descriptor 9.86 to 9.90 ppm

AUC of descriptor

	9.96	9.92	9.88	9.84	9.8	--	0.4	0.36	0.32	0.28	0.24
L20r01h-016	0.000983	0.0012	-0.00207	0.00186	0.00156	--	0.0076	0.00836	0.00545	0.00793	0.00748
L20r02h-016	0.000161	-0.0021	-0.00026	-0.00063	0.000468	--	0.00684	0.00484	0.00512	0.0104	0.00614
L20r03h-016	0.000428	-0.00026	0.0013	0.00138	0.0026	--	0.0125	0.0102	0.0072	0.0127	0.0111
L20r04h-016	-0.00148	-0.00016	0.000369	0.0016	0.00225	--	0.0117	0.0128	0.00979	0.0169	0.0131
L20r05h-016	-0.00036	-0.00026	0.00152	0.00103	0.00234	--	0.00642	0.00601	0.00581	0.00943	0.00666
L20r06h-016	-0.00061	-0.0006	-0.00058	0.00159	0.00479	--	0.00393	0.00468	0.00291	0.00667	0.00423
L20r07h-016	0.00214	0.00113	-0.00035	0.00188	0.00013	--	0.0104	0.00843	0.0065	0.0141	0.0114
L20r08h-016	0.000229	0.00145	-0.00075	0.000965	0.00115	--	0.00845	0.00668	0.00684	0.00786	0.00444
L20r09h-016	0.000514	0.000777	0.002	0.00194	0.00191	--	0.00659	0.00587	0.00633	0.0101	0.00842
L20r10h-016	0.000283	0.000981	0.00263	0.00134	0.00127	--	0.00415	0.00775	0.00331	0.00958	0.00826
--	--	--	--	--	--	--	--	--	--	--	--
L20r07h+168	-0.00319	-0.00076	0.000934	0.000282	0.00143	--	0.0136	0.0112	0.0105	0.0158	0.0128
L20r08h+168	-0.0013	-0.00024	-0.00041	0.00209	0.00142	--	0.00984	0.0074	0.00839	0.0108	0.00743
L20r09h+168	-0.00049	-0.00045	0.00201	0.000371	0.00199	--	0.0156	0.013	0.0138	0.0168	0.0168
L20r10h+168	-0.00025	0.000264	0.00029	0.00156	0.00154	--	0.0108	0.00908	0.00903	0.0113	0.0116
L20r16h+168	0.00016	0.000599	0.00181	0.00136	0.00153	--	0.00733	0.00573	0.00517	0.00865	0.00589
L20r17h+168	0.00121	0.00016	0.00136	-0.0009	0.000824	--	0.00889	0.00751	0.00737	0.0104	0.00948
L20r18h+168	0.000594	0.00148	0.000818	0.00105	0.000883	--	0.0121	0.0127	0.00947	0.0131	0.0141
L20r19h+168	0.0028	0.000391	-0.00018	0.000905	0.00325	--	0.0104	0.00985	0.00952	0.013	0.0117
L20r20h+168	-0.00058	-0.00076	0.00176	0.00163	0.00126	--	0.00643	0.00641	0.00398	0.0093	0.00709

Each line = ^{1}H-NMR spectrum of urine sample

How to apply MBX in preclinical toxicology …



Azathioprine

Azathioprine
Immunosuppresion drug
But …
Liver toxicity (vascular damages)

Sprague-Dawley rats receiving azathioprine in food for 3 to 4 weeks ⇒ liver necrosis with mitochondrial and endoplasmic proliferation

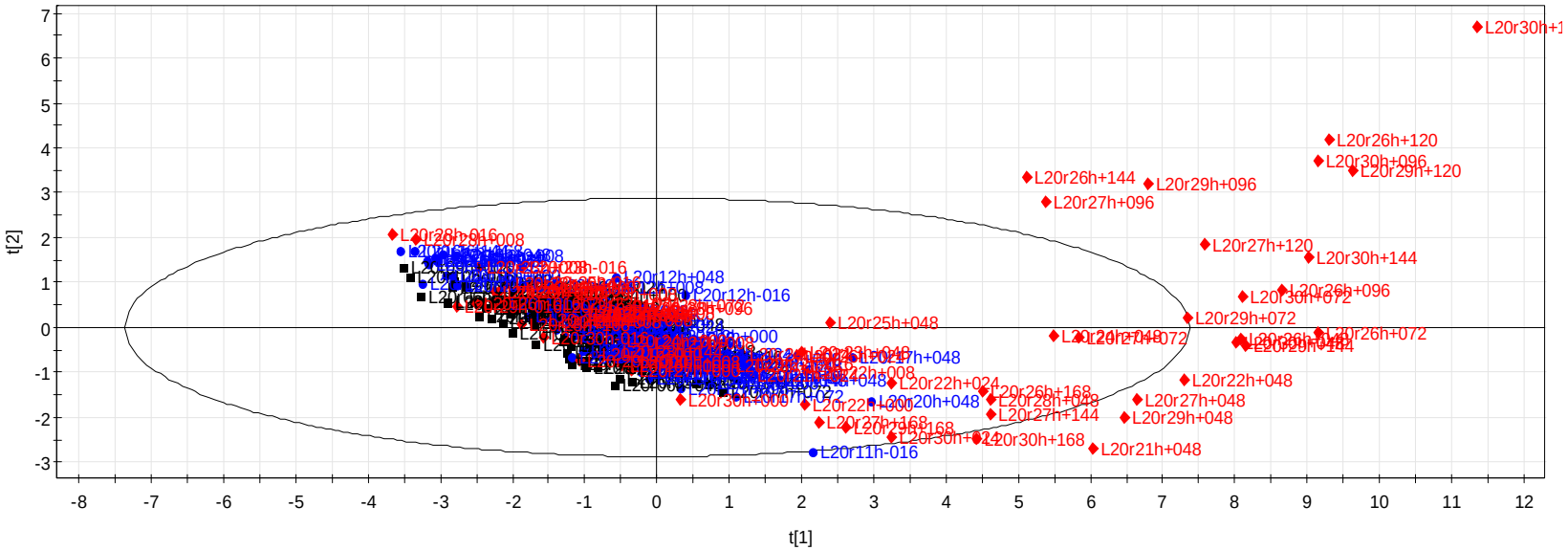
LD_{50} in rat = 400 mg/kg p.o. et 300 mg/kg i.p.

MBX prediction of azathioprine liver toxicity after one single dose ?

Three groups of 10 rats :

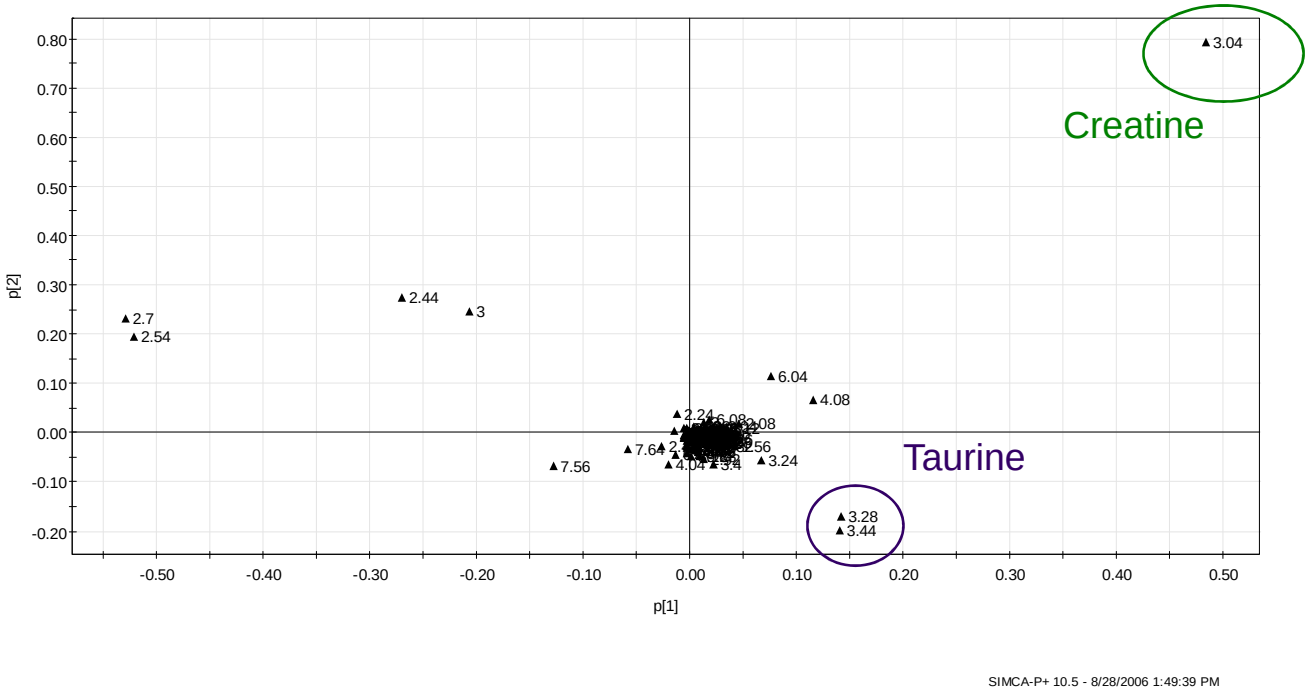
- Control group : vehicle
- Low dose group : 30 mg/kg azathioprine (pharmacological dose)
- High dose group : 300 mg/kg azathioprine (toxic in chronic exposure)

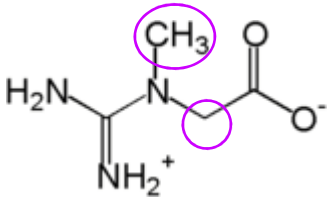
	9.96	9.92	9.88	9.84	9.8	--	0.4	0.36	0.32	0.28	0.24
L20r01h-016	0.000983	0.0012	-0.00207	0.00186	0.00156	--	0.0076	0.00836	0.00545	0.00793	0.00748
L20r02h-016	0.000161	-0.0021	-0.00026	-0.00063	0.000468	--	0.00684	0.00434	0.00512	0.0104	0.00614
L20r03h-016	0.000428	-0.00026	0.0013	0.00138	0.0026	--	0.0125	0.0102	0.0072	0.0127	0.0111
L20r04h-016	-0.00148	-0.00016	0.000369	0.0016	0.00225	--	0.0117	0.0128	0.00979	0.0169	0.0131
L20r05h-016	-0.00036	-0.00026	0.00152	0.00103	0.00234	--	0.00642	0.00601	0.00581	0.00943	0.00666
L20r06h-016	-0.00061	-0.0006	-0.00058	0.00159	0.00479	--	0.00393	0.00468	0.00291	0.00667	0.00423
L20r07h-016	0.00214	0.00113	-0.00035	0.00188	0.00013	--	0.0104	0.00843	0.0065	0.0141	0.0114
L20r08h-016	0.000229	0.00145	-0.00075	0.000965	0.00115	--	0.00845	0.00668	0.00684	0.00786	0.00444
L20r09h-016	0.000514	0.000777	0.002	0.00194	0.00191	--	0.00659	0.00587	0.00633	0.0101	0.00842
L20r10h-016	0.000283	0.000981	0.00263	0.00134	0.00127	--	0.00415	0.00775	0.00331	0.00958	0.00826
--	--	--	--	--	--	--	--	--	--	--	--
L20r07h+168	-0.00319	-0.00076	0.000934	0.000282	0.00143	--	0.0136	0.0112	0.0105	0.0158	0.0128
L20r08h+168	-0.0013	-0.00024	-0.00041	0.00209	0.00142	--	0.00984	0.0074	0.00839	0.0108	0.00743
L20r09h+168	-0.00049	-0.00045	0.00201	0.000371	0.00199	--	0.0156	0.013	0.0138	0.0168	0.0168
L20r10h+168	-0.00025	0.000264	0.00029	0.00156	0.00154	--	0.0108	0.00908	0.00903	0.0113	0.0116
L20r16h+168	0.00016	0.000599	0.00181	0.00136	0.00153	--	0.00733	0.00573	0.00517	0.00865	0.00589
L20r17h+168	0.00121	0.00016	0.00136	-0.0009	0.000824	--	0.00889	0.00751	0.00737	0.0104	0.00948
L20r18h+168	0.000594	0.00148	0.000818	0.00105	0.000883	--	0.0121	0.0127	0.00947	0.0131	0.0141
L20r19h+168	0.0028	0.000391	-0.00018	0.000905	0.00325	--	0.0104	0.00985	0.00952	0.013	0.0117
L20r20h+168	-0.00058	-0.00076	0.00176	0.00163	0.00126	--	0.00643	0.00641	0.00398	0.0093	0.00709
L20r26h+168	0.00134	7.73E-05	-0.00169	0.001381	0.005036	--	0.002316	0.003084	0.00461	0.001641	0.001101
L20r27h+168	-0.0023	-0.00115	4.49E-05	-0.0016	0.001532	--	0.002798	0.005161	0.002165	0.003737	0.003431
L20r28h+168	0.000189	0.000726	0.00234	0.00177	0.001878	--	0.005821	0.004346	0.003796	0.008211	0.00474
L20r29h+168	0.000576	-0.00195	7.77E-05	0.001938	0.001534	--	0.001797	0.002271	0.001989	0.002695	0.003977
L20r30h+168	0.009122	0.003687	0.004284	-0.00219	0.002493	--	0.006661	0.002273	0.004179	0.009415	0.001236



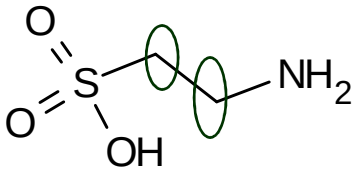
Scores plot (PCA: PC1 vs PC2):

- group 1 = control rats
- group 2 = low dose (30 mg/kg)
- group 3 = high dose (300 mg/kg)

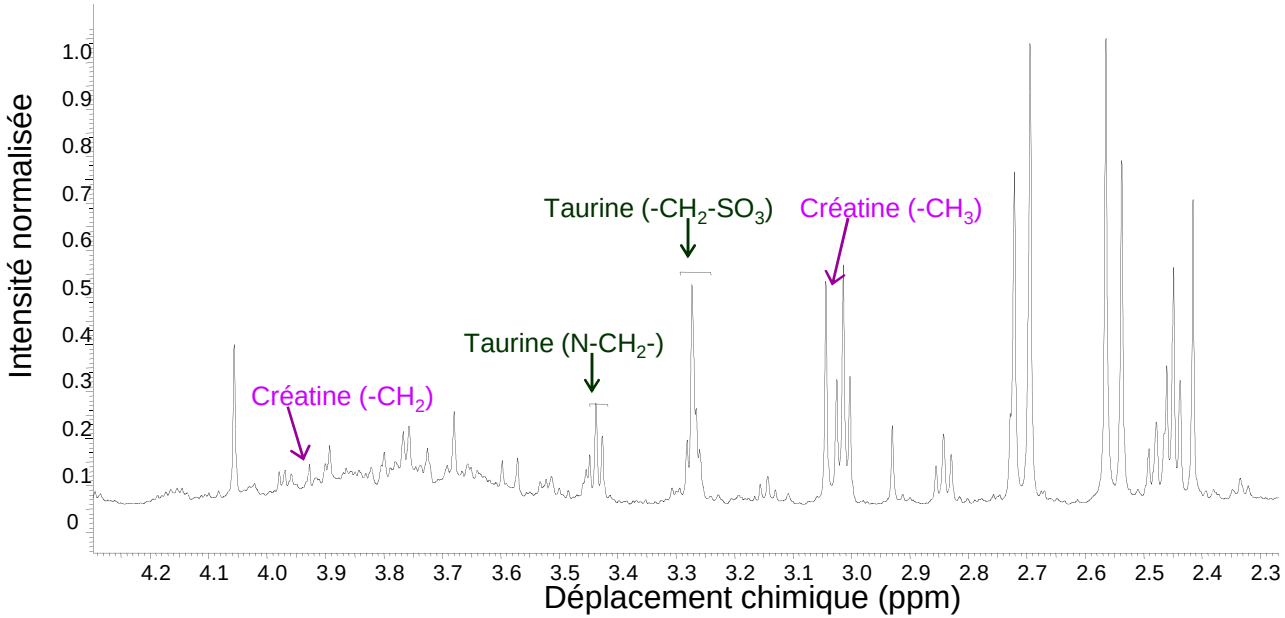

Creatine
Taurine
3.04
2.44
3
2.7
2.54
6.04
4.08
7.56
3.24
3.28
3.44
p[1]
p[2]
SIMCA-P+ 10.5 - 8/28/2006 1:49:39 PM



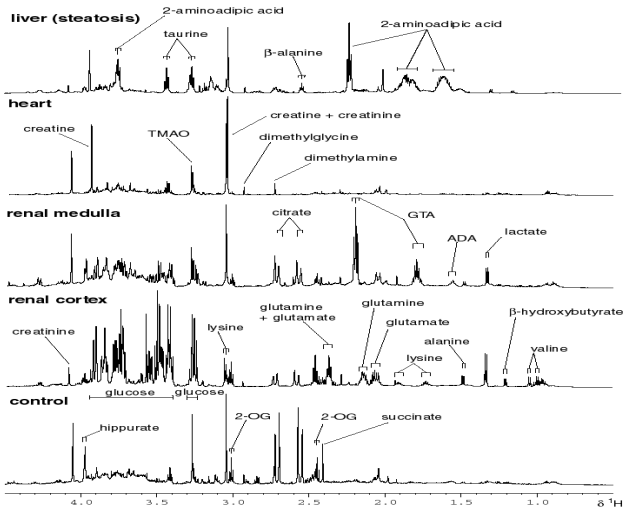
Créatine

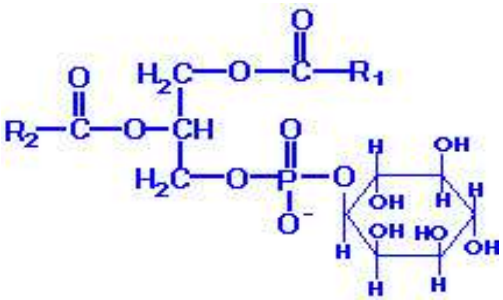


Taurine



Biomarkers Identification

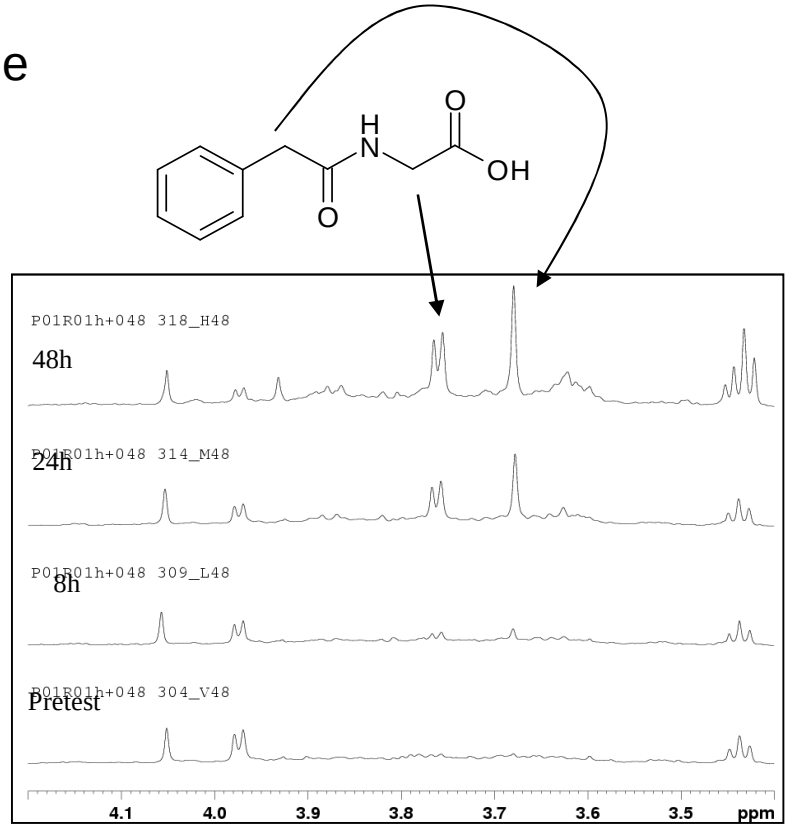
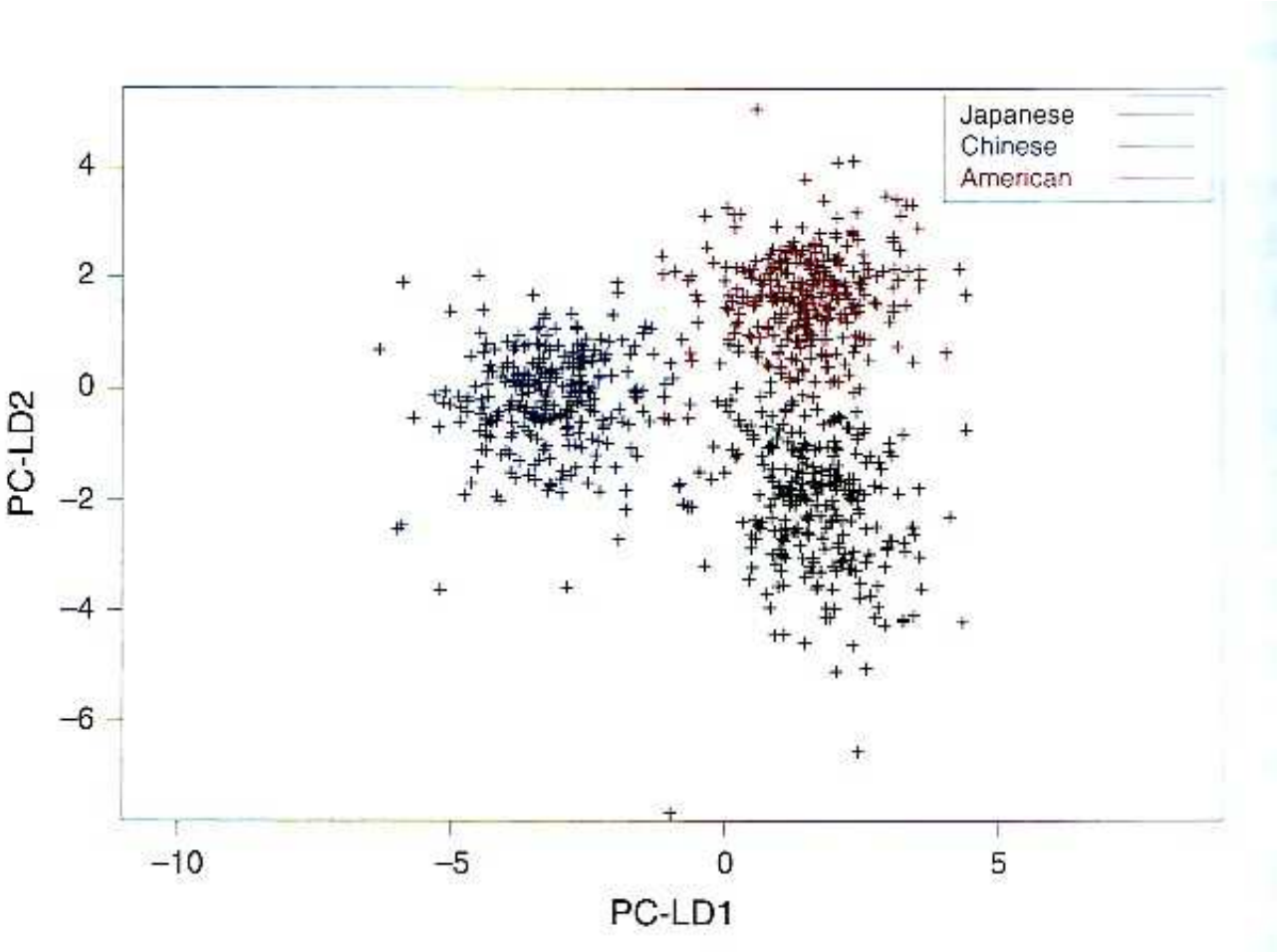




Phospholipidosis

Biomarker of drug toxicity

Phenylacetylglycine



Metabonomics in humans?