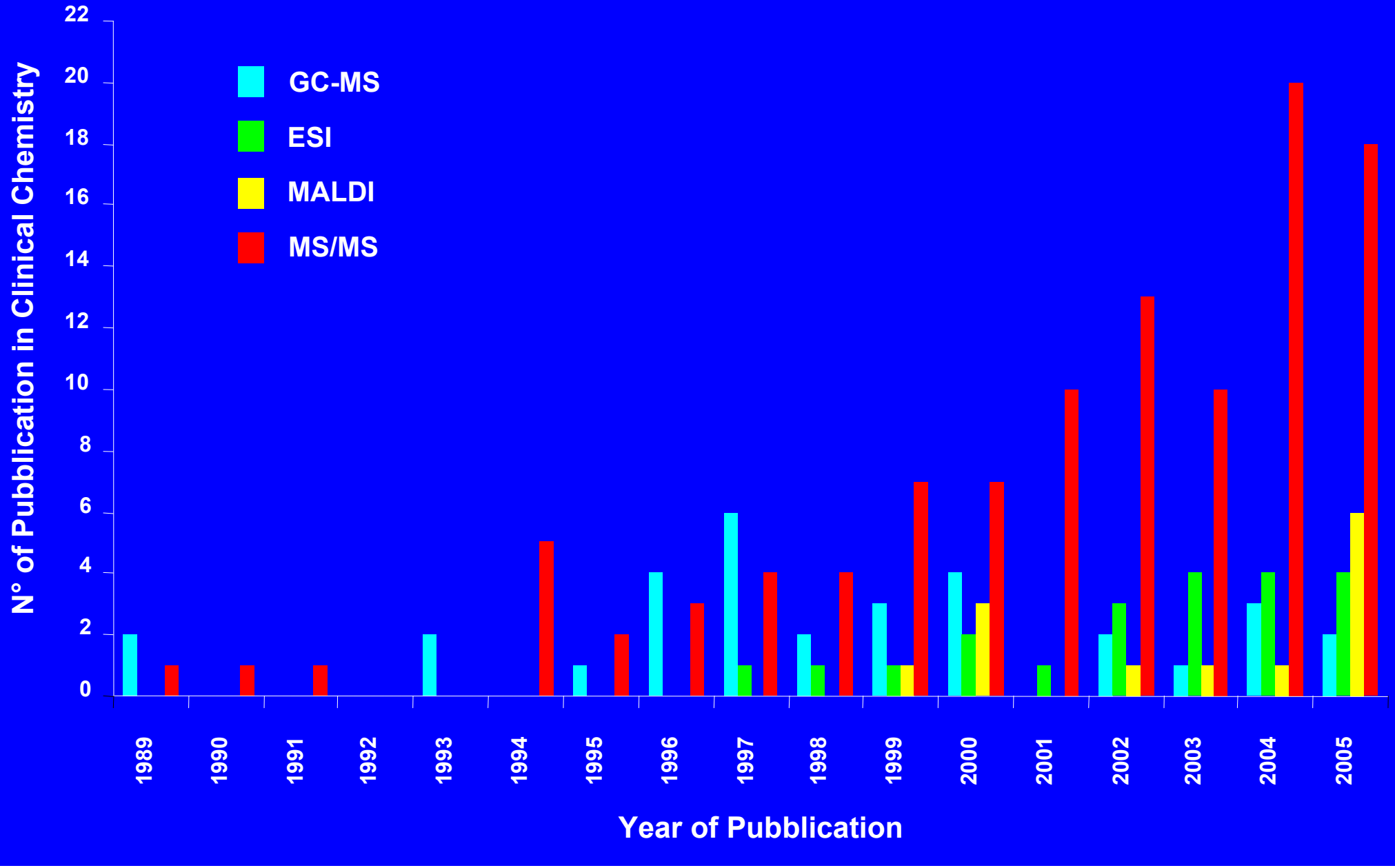
LA SPETTROMETRIA DI MASSA IN MS/MS OLTRE LO SCREENING

Giuseppe Giordano
giordano@pediatria.unipd.it

Dipartimento di Pediatria,
Universita' degli Studi di Padova

Pesaro 2006

Mass Spectrometry in Clinical Chemistry



Aminoacid

- **MSUD (allo)**
- **Argininosuccinic (ASA)**
- **Tyrosinemia type I (SUAC)**

Bile acid

- **3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-HSD)**
- **Peroxisomal 3-ketothiolase deficiency (pseudo-Zellweger syndrome)**

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography B, 831 (2006) 267–273
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Method for the quantification of underivatized amino acids on dry blood spots from newborn screening by HPLC–ESI–MS/MS

Mariella Zoppa, Lorena Gallo, Franco Zacchello, Giuseppe Giordano*

Department of Pediatrics, University of Padova, 35128 Padova, Italy

Received 1 September 2005; accepted 8 December 2005
Available online 4 January 2006

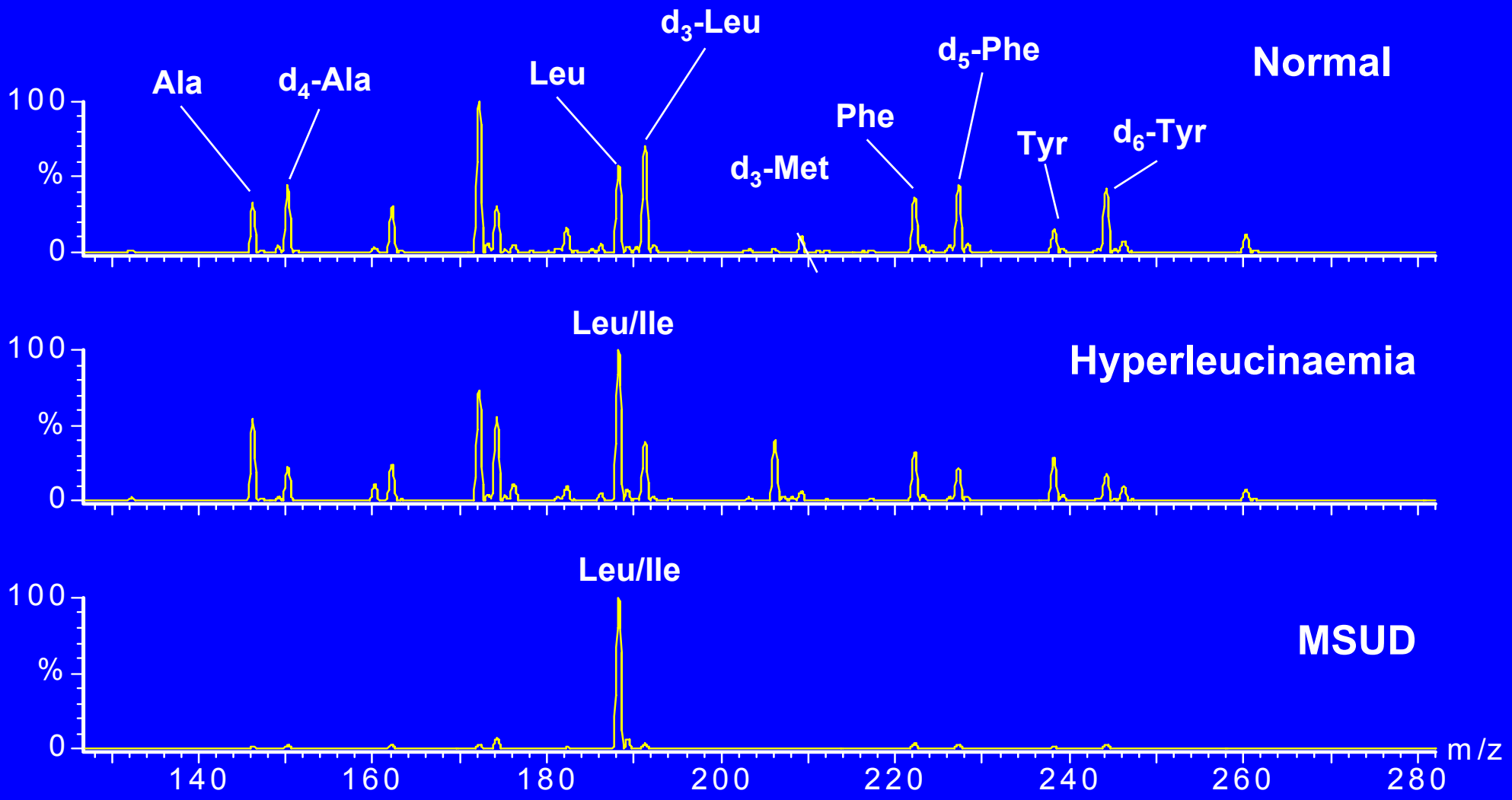
Abstract

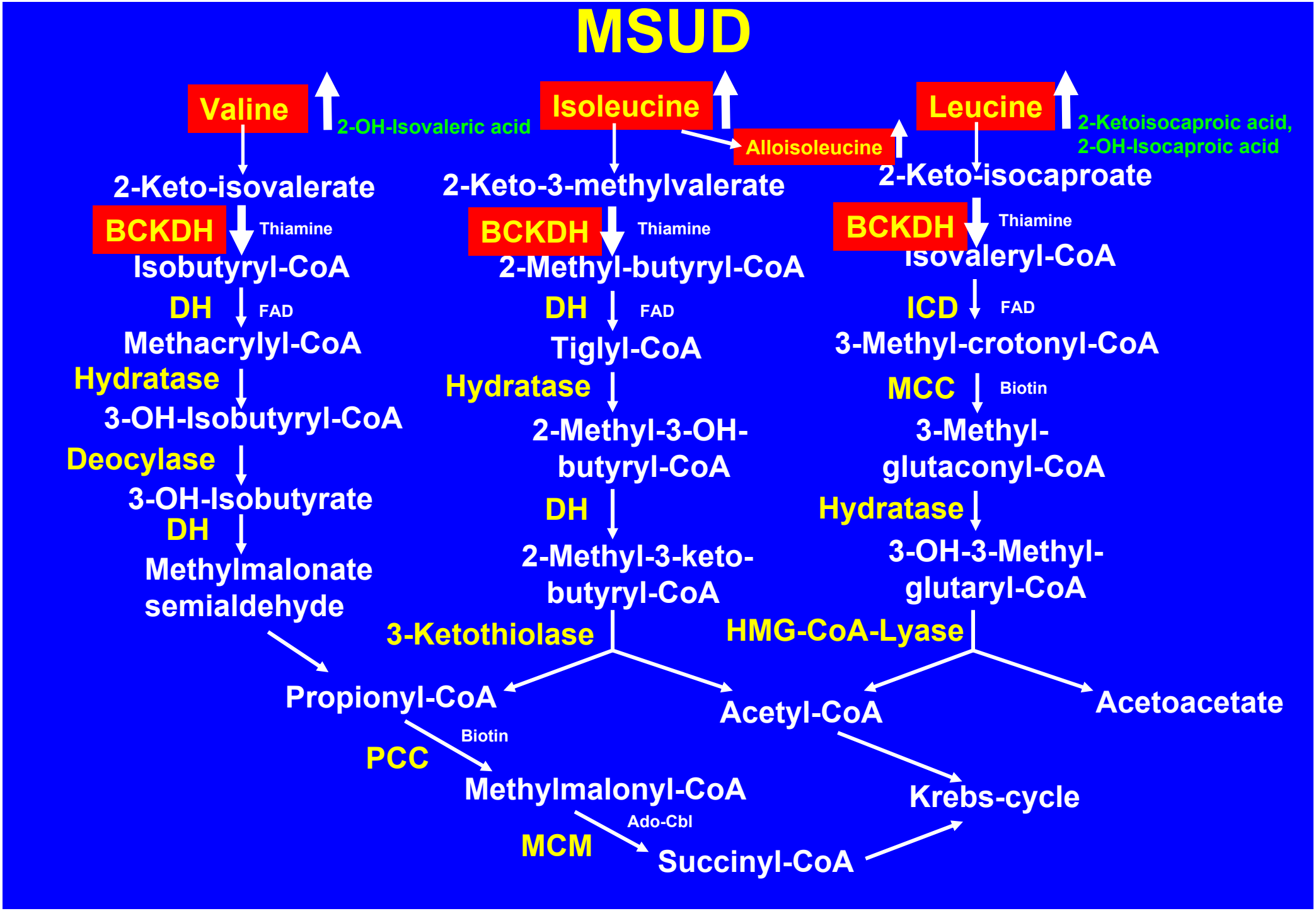
In our study we have developed an HPLC–ESI–MS/MS method for qualitative and quantitative analysis of underivatized amino acids on dry blood spots. The sensitive and specific instrumental performances permitted the chromatographic separation of 40 amino acids and their isomers within 10 min. The method has been set up for cases of suspected metabolic diseases revealed by newborn screening. What is new is that it is applied on the same blood spots used for newborn screening, instead of plasma, in order to avoid involvement of doctors, increased anxiety for parents, stress for patients for plasma collection, long time of waiting and further costs for analysis.

Keywords: Newborn screening; Blood spot; Underivatized amino acids; HPLC–ESI–MS/MS

L’ importanza diagnostica della leucina e suoi isomeri

Normal *vs* Hyperleucinaemia *vs* MSUD



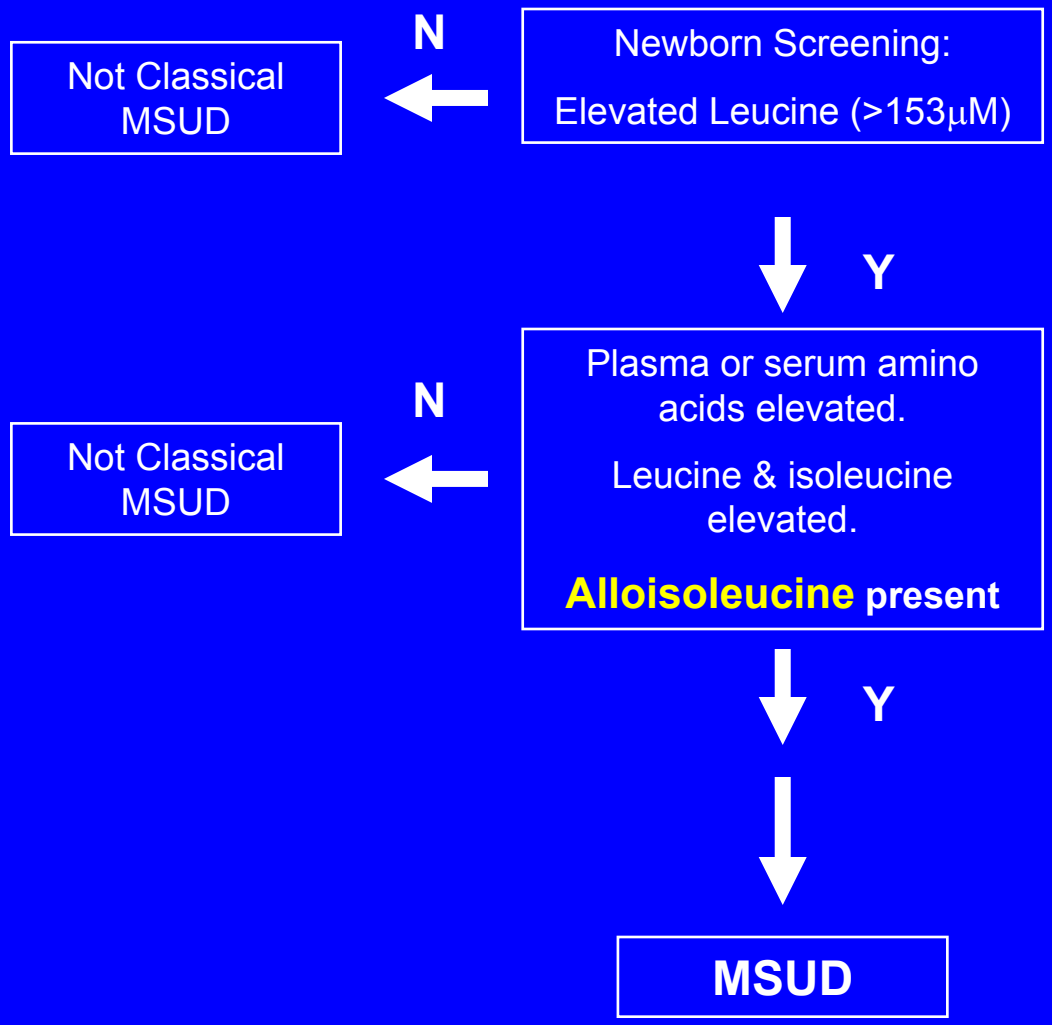

MSUD
Valine
2-OH-Isovaleric acid
Isoleucine
Alloisoleucine
Leucine
2-Ketoisocaproic acid,
2-OH-Isocaproic acid
2-Keto-isovalerate
2-Keto-3-methylvalerate
2-Keto-isocaproate
BCKDH
Thiamine
BCKDH
Thiamine
BCKDH
Thiamine
Isobutyryl-CoA
2-Methyl-butyryl-CoA
Isovaleryl-CoA
DH
FAD
DH
FAD
ICD
FAD
Methacrylyl-CoA
Tiglyl-CoA
3-Methyl-crotonyl-CoA
Hydratase
Hydratase
MCC
Biotin
3-OH-Isobutyryl-CoA
2-Methyl-3-OH-
butyryl-CoA
3-Methyl-
glutaconyl-CoA
Deocylase
DH
Hydratase
3-OH-Isobutyrate
2-Methyl-3-keto-
butyryl-CoA
3-OH-3-Methyl-
glutaryl-CoA
DH
Methylmalonate
semialdehyde
3-Ketothiolase
HMG-CoA-Lyase
Propionyl-CoA
Acetyl-CoA
Acetoacetate
Biotin
PCC
Methylmalonyl-CoA
Krebs-cycle
Ado-Cbl
MCM
Succinyl-CoA

Normal Reference Values for Inborn Errors of Leucine Metabolism

Age	Valine	Isoleucine	Leucine	Alloisoleucine
Premature	99-220	23-85	151-220	0
0-1 month	86-190	26-91	48-160	0
1-24 months	64-294	31-86	47-155	0
2-18yrs	74-321	22-107	49-201	0
Adult	119-336	30-108	72-201	0

Blau, Duran & Blaskovics, 1996

The presence of allo-isoleucine is a critical factor in the diagnosis of defects of leucine metabolism



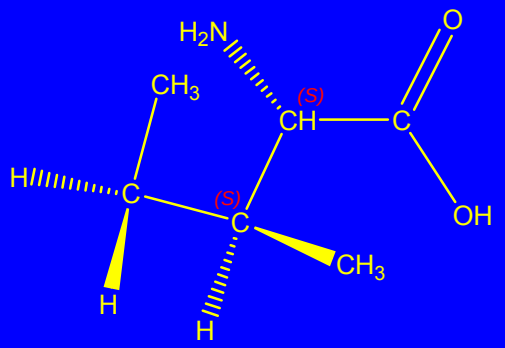
Leucine values in MSUD

Condition	Valine	Isoleucine	Leucine	Alloisoleucine
Classical	496-1846	199-1298	518-5091	72-310
Intermediate	Elevated (to 1000)	Elevated (to 1000)	400-2000	Present
Intermittent	Elevated (to 1000)	Elevated (to 1000)	50-4000	Present
Thiamine Responsive	Elevated (to 1000)	Elevated (to 1000)	50-5000	Present

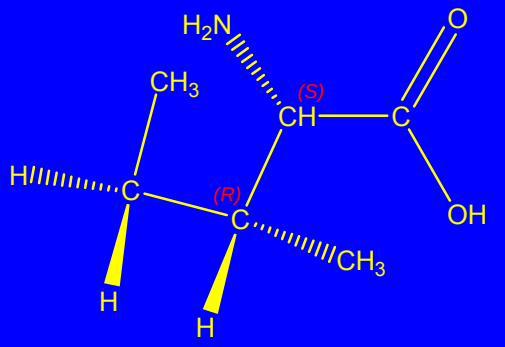
Blau, Duran & Blaskovics, 1996

ISOMERI

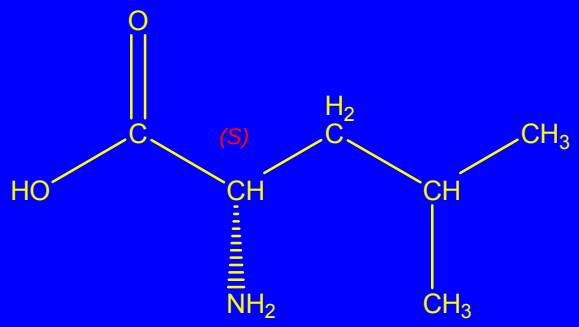
L-Isoleucine
(2S,3S)



L-Alloisoleucine
(2S,3R)

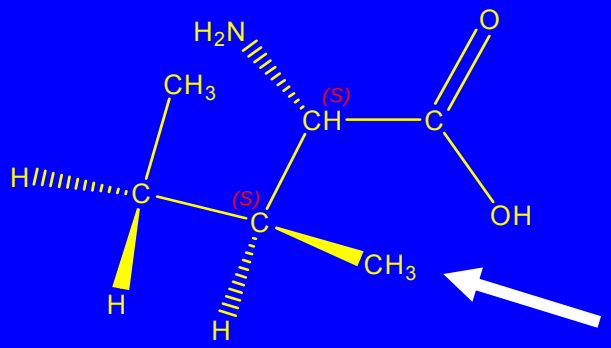


L-Leucine
(2S)

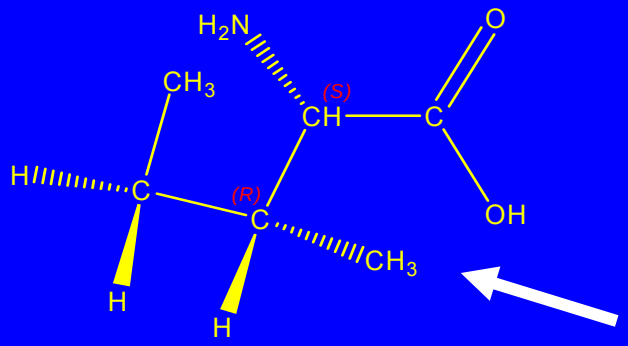


STEREO ISOMERI

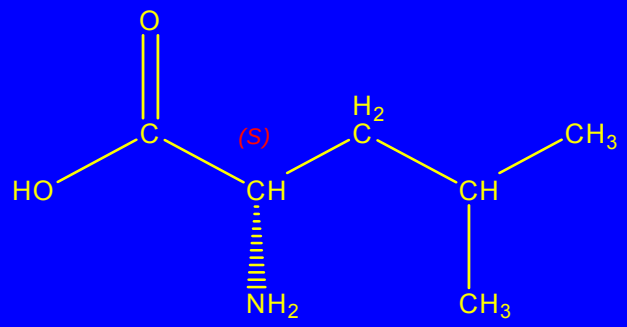
L-Isoleucine
(2S,3S)



L-Alloisoleucine
(2S,3R)

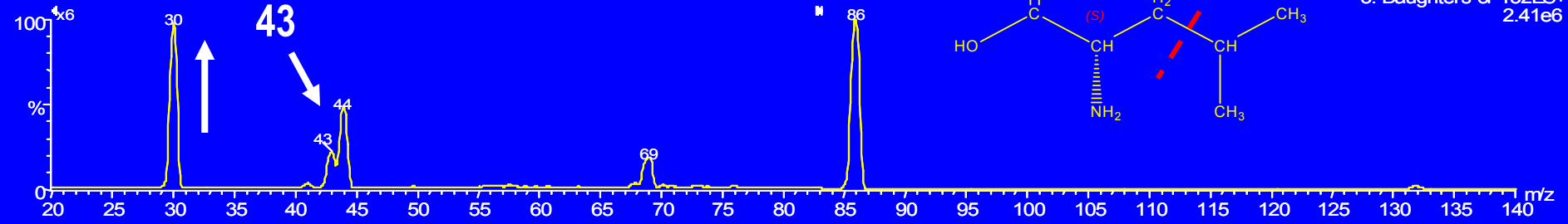


L-Leucine
(2S)



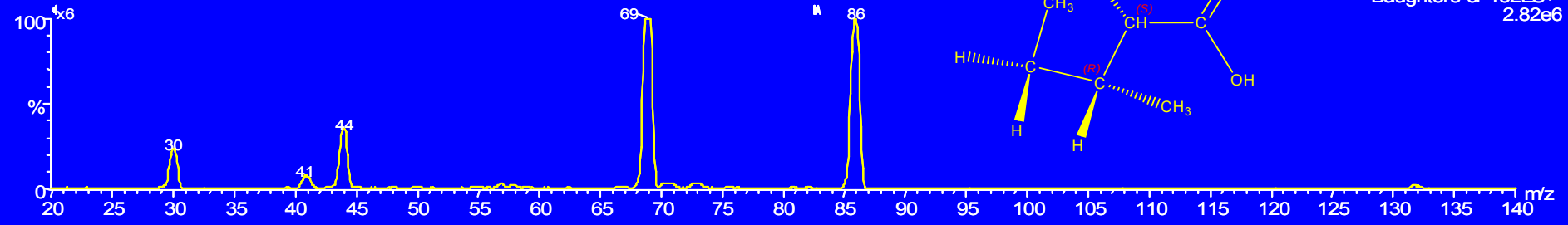
PATTERN DI FRAMMENTAZIONE PER GLI ISOMERI LEUCINA, ALLOISOLEUCINA E ISOLEUCINA

LEUCINA



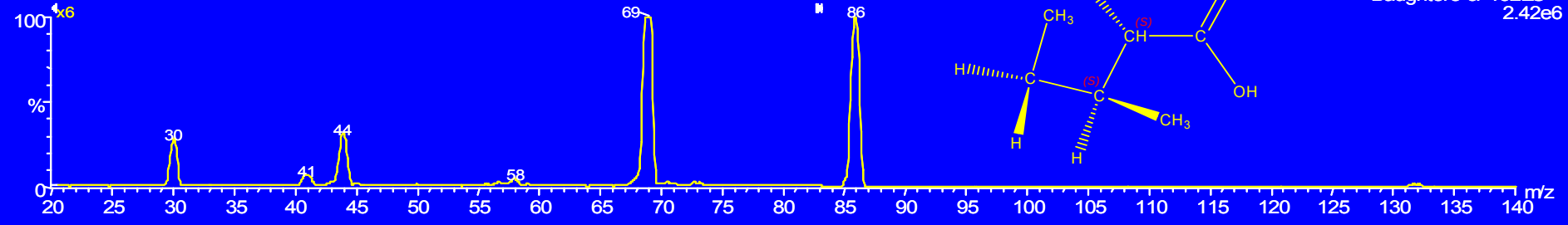
3: Daughters of 132ES+
2.41e6

ALLOISOLEUCINA



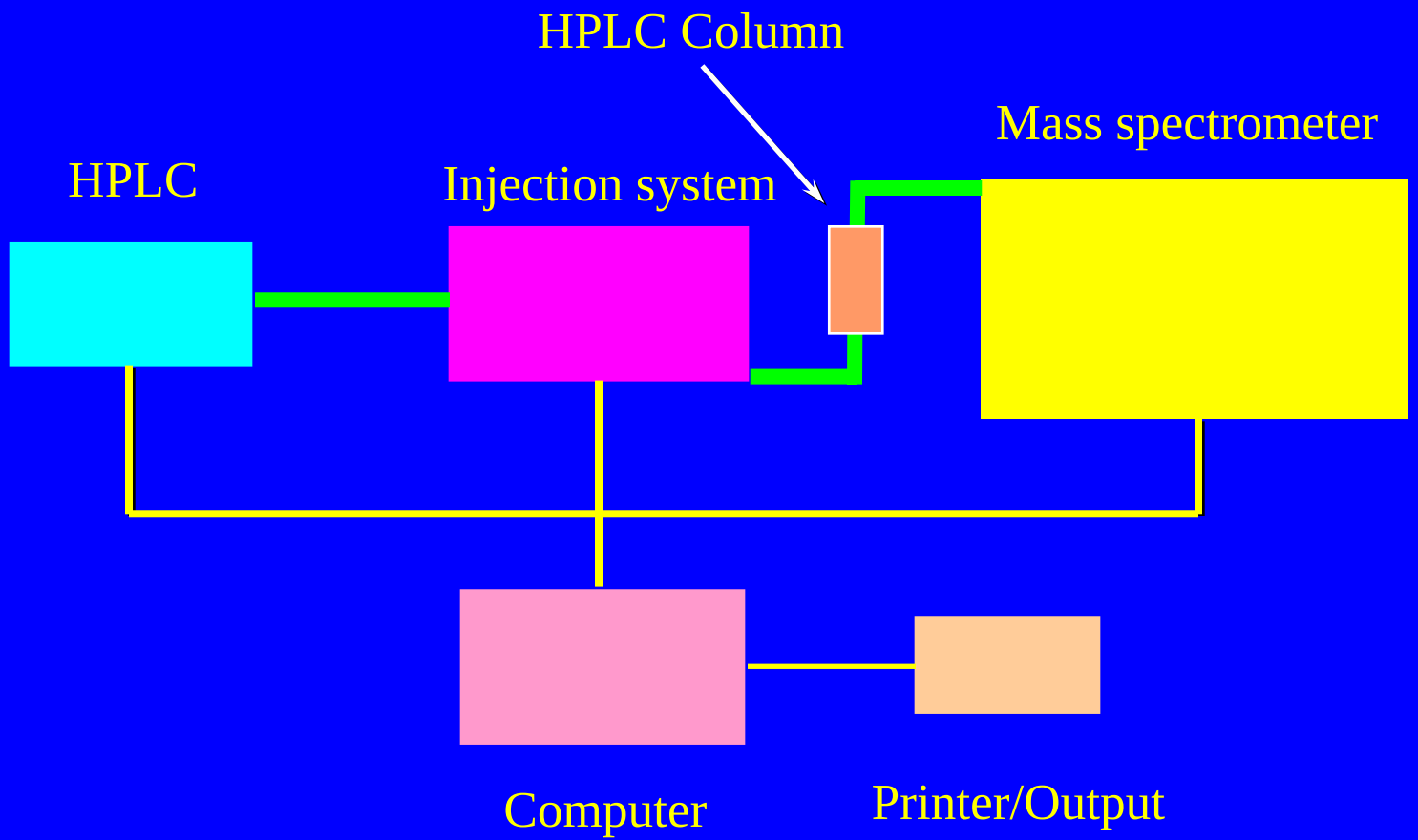
Daughters of 132ES+
2.82e6

ISOLEUCINA



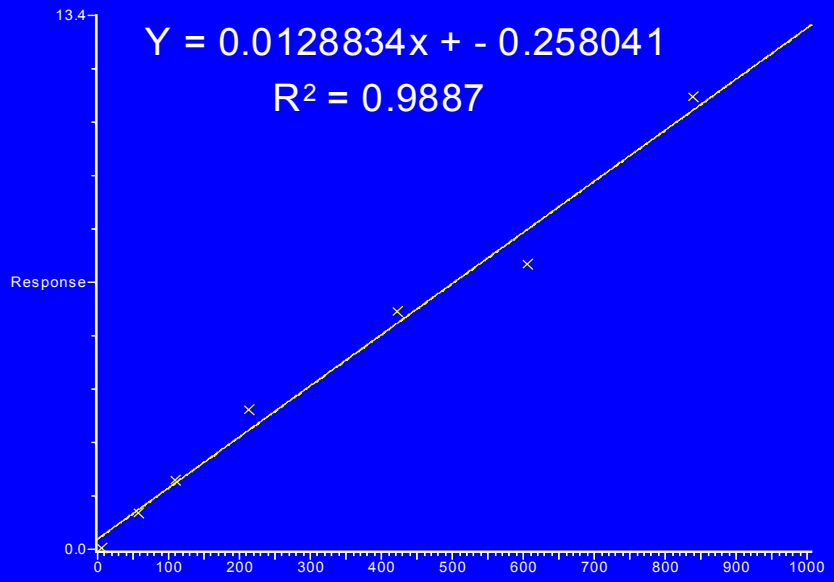
Daughters of 132ES+
2.42e6

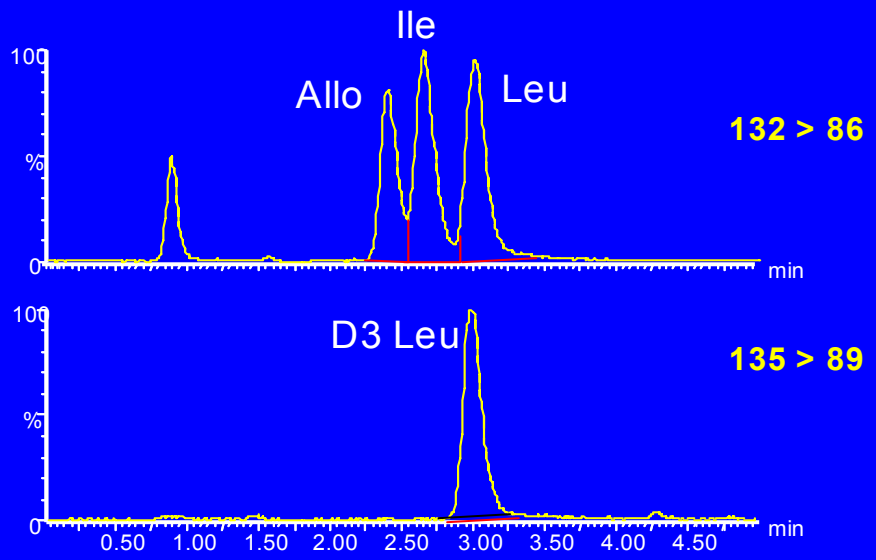
Schema di un sistema automatizzato d'analisi



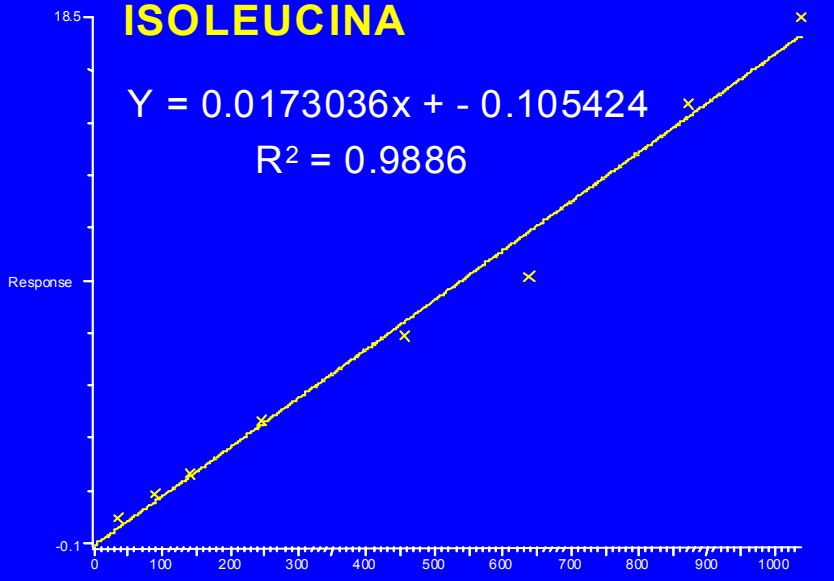
CURVE DI CALIBRAZIONE SU SPOT DI SANGUE PER AMINOACIDI NON BUTILATI

ALLOISOLEUCINA

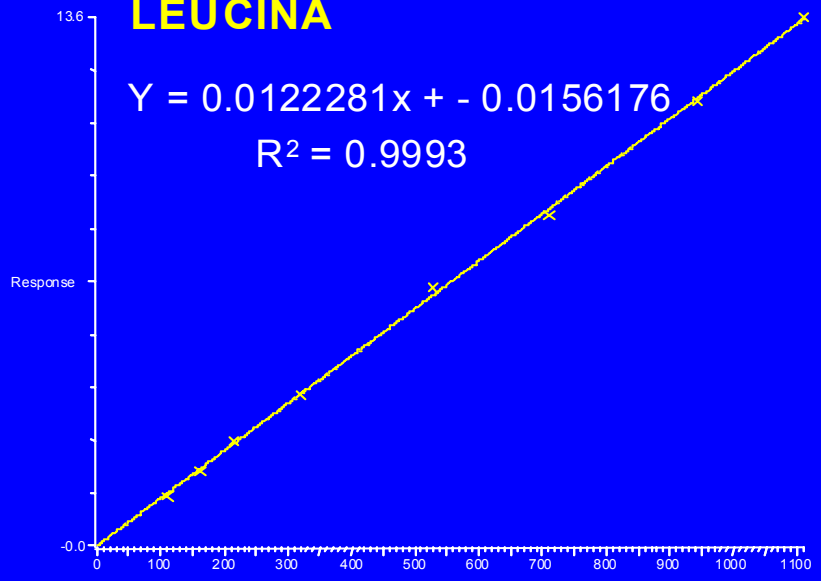




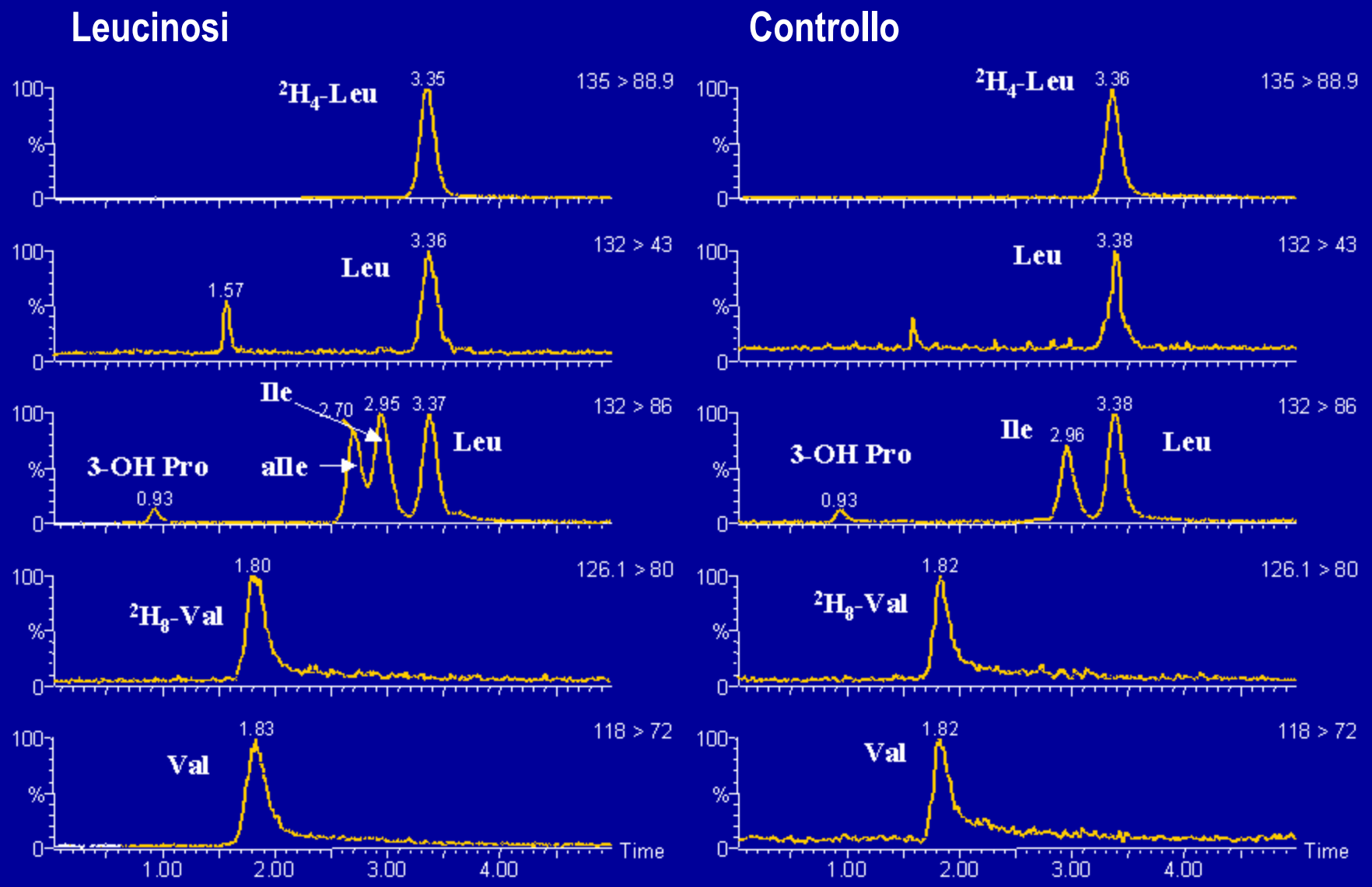
ISOLEUCINA



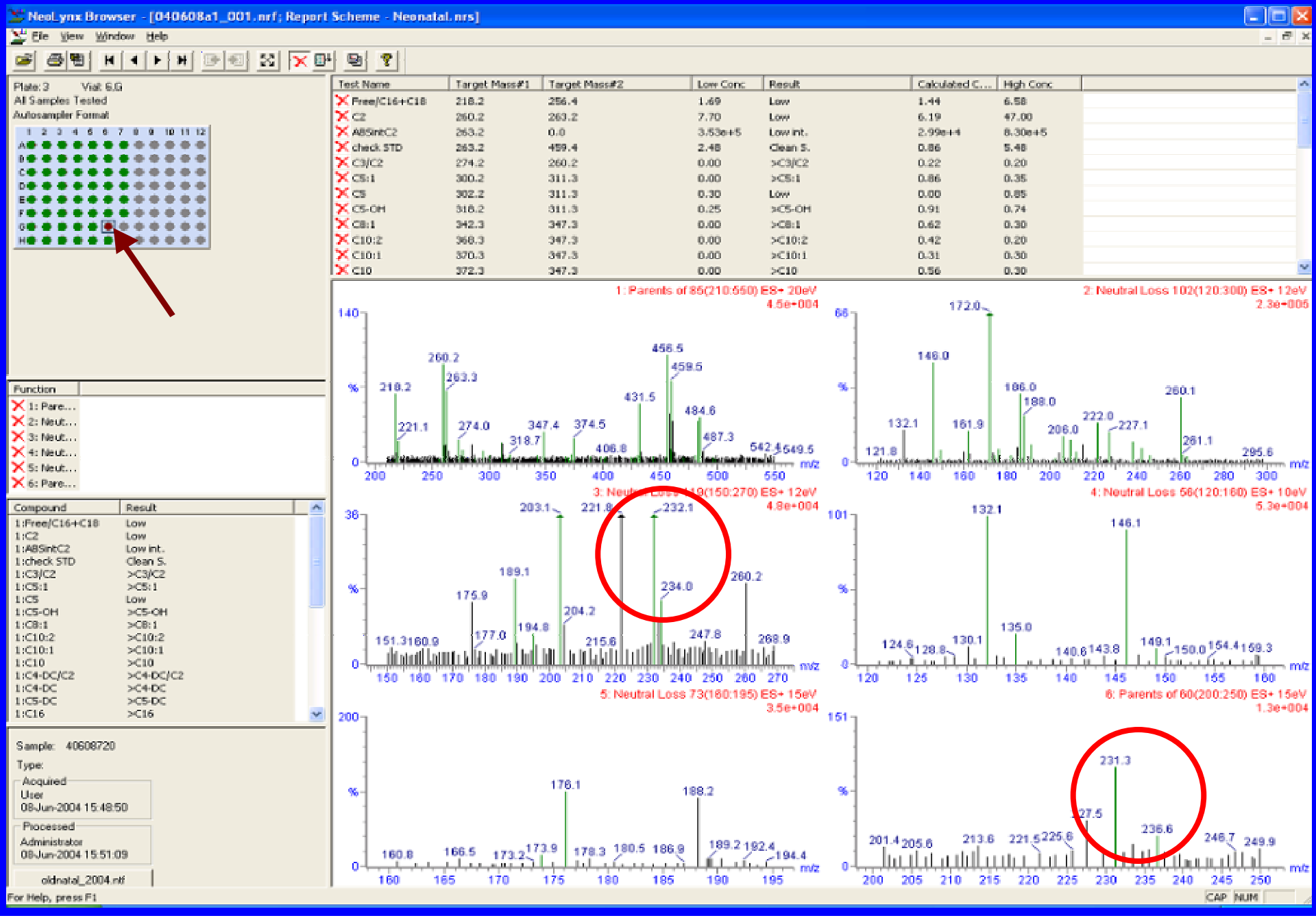
LEUCINA



Spot in 150 µL Methanol with int. Std and Inj. 5 µL

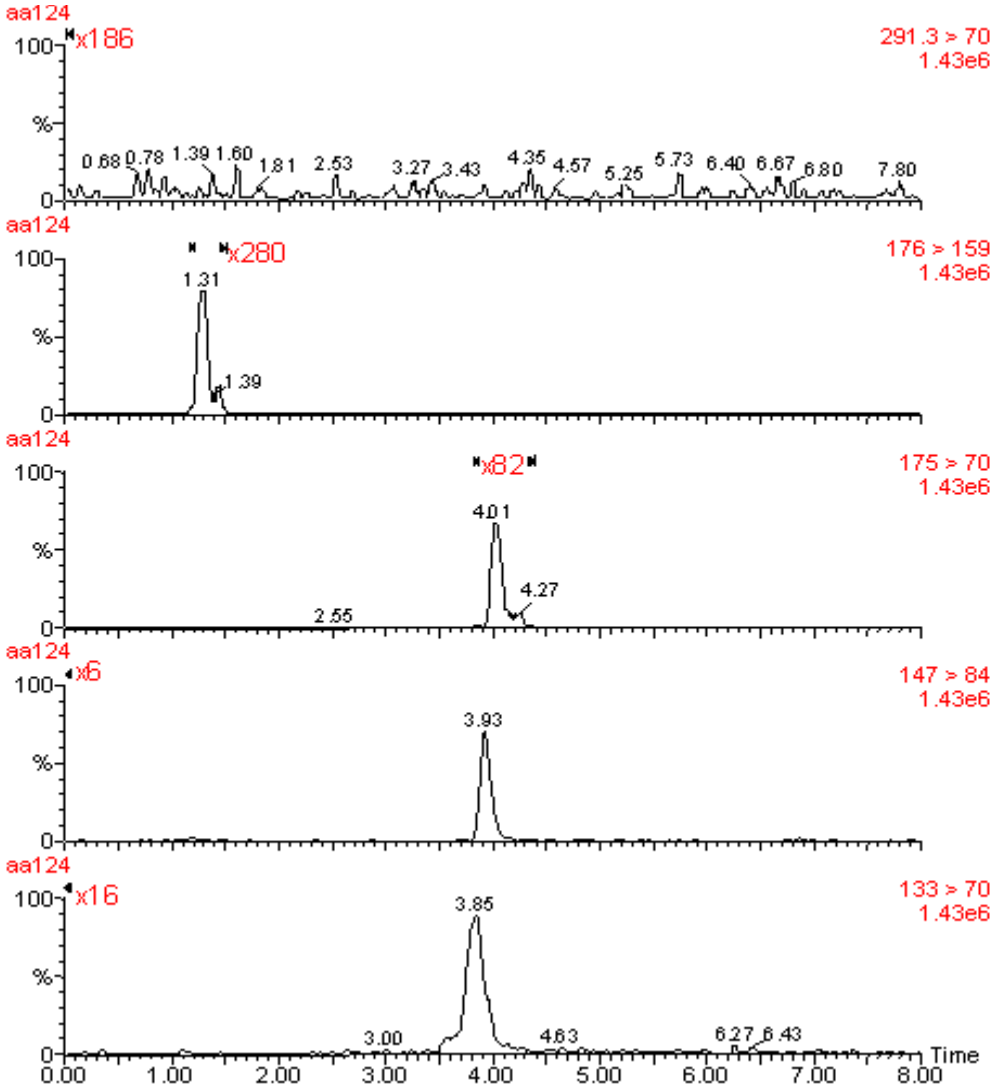


Prima identificazione del campione affetto (ASA)

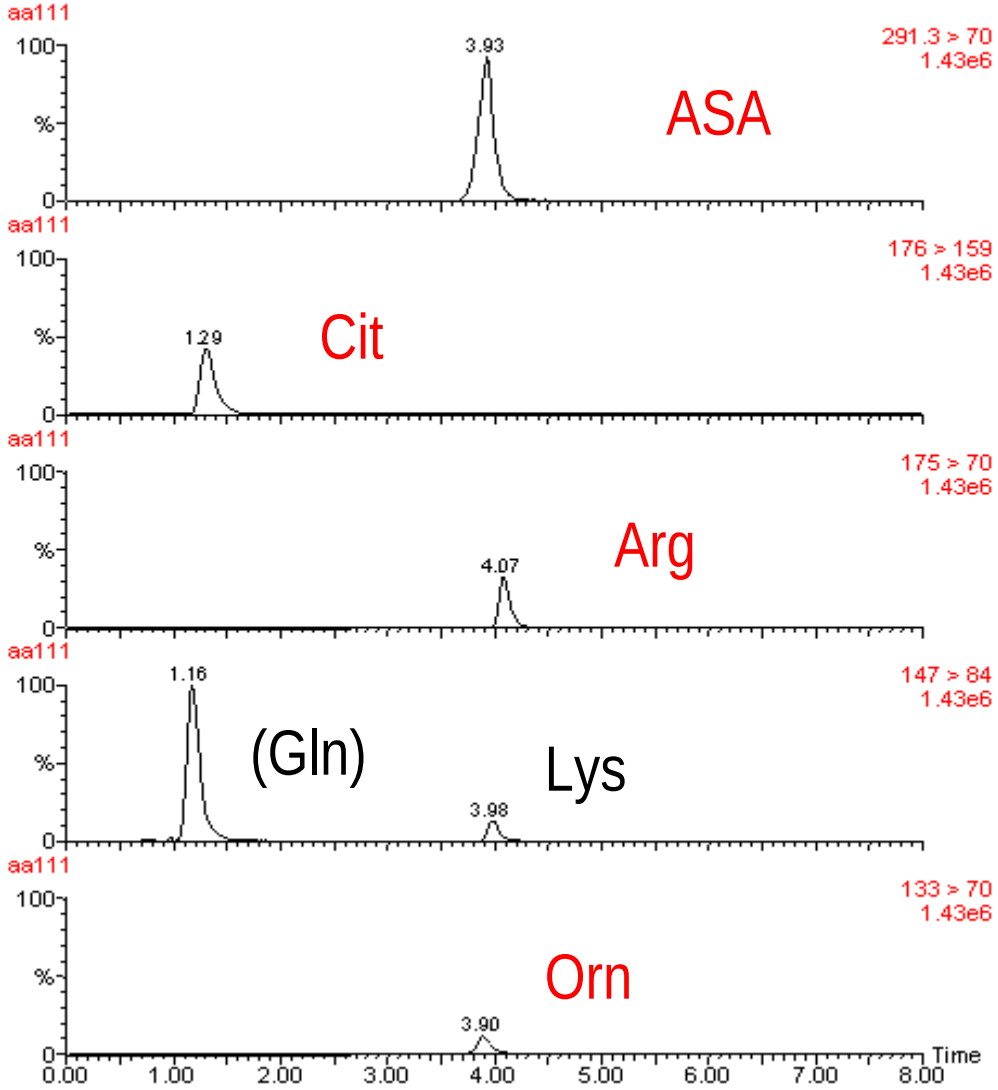


Confronto di un campione affetto (ASA) con un controllo

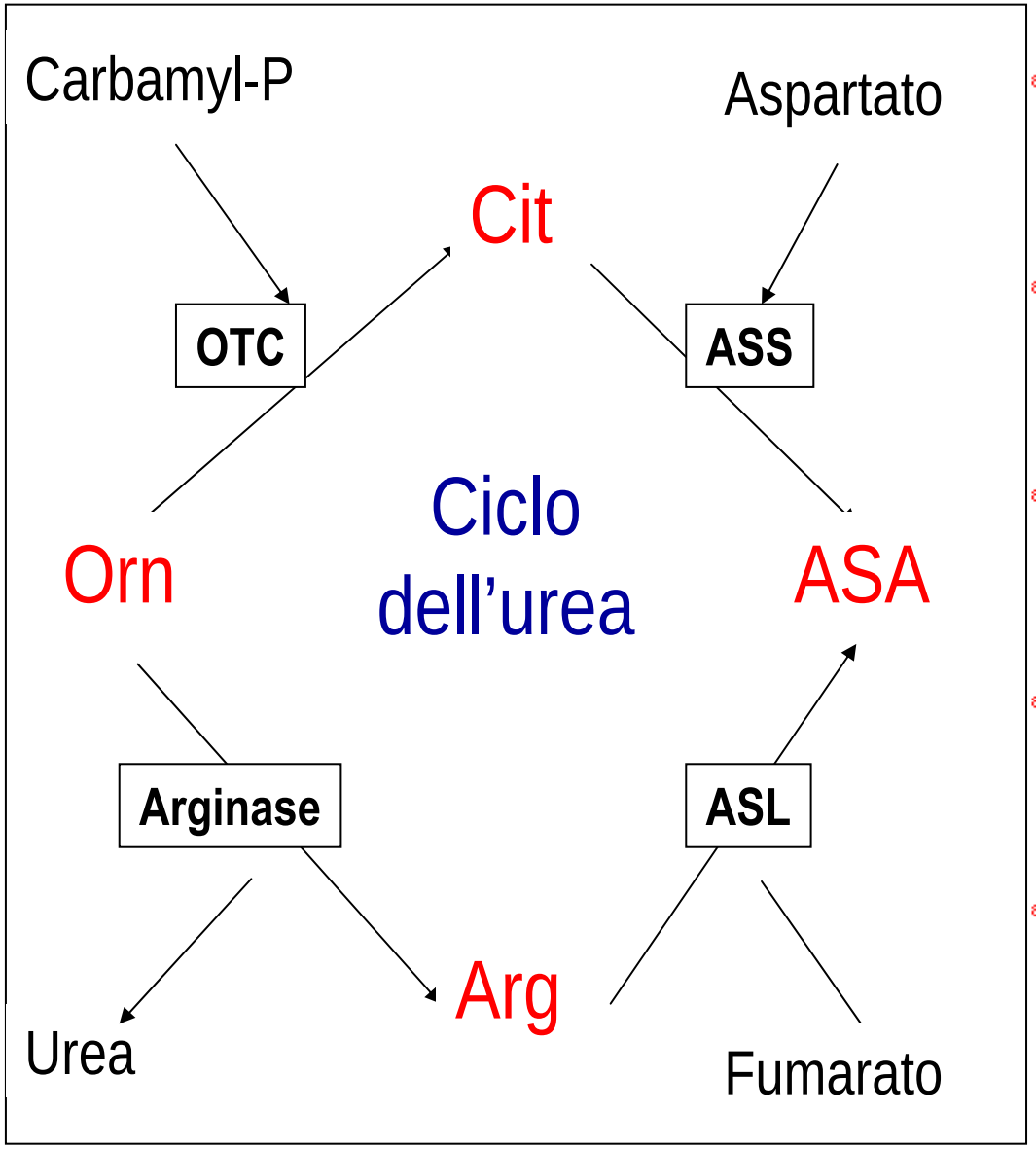
Spot controllo



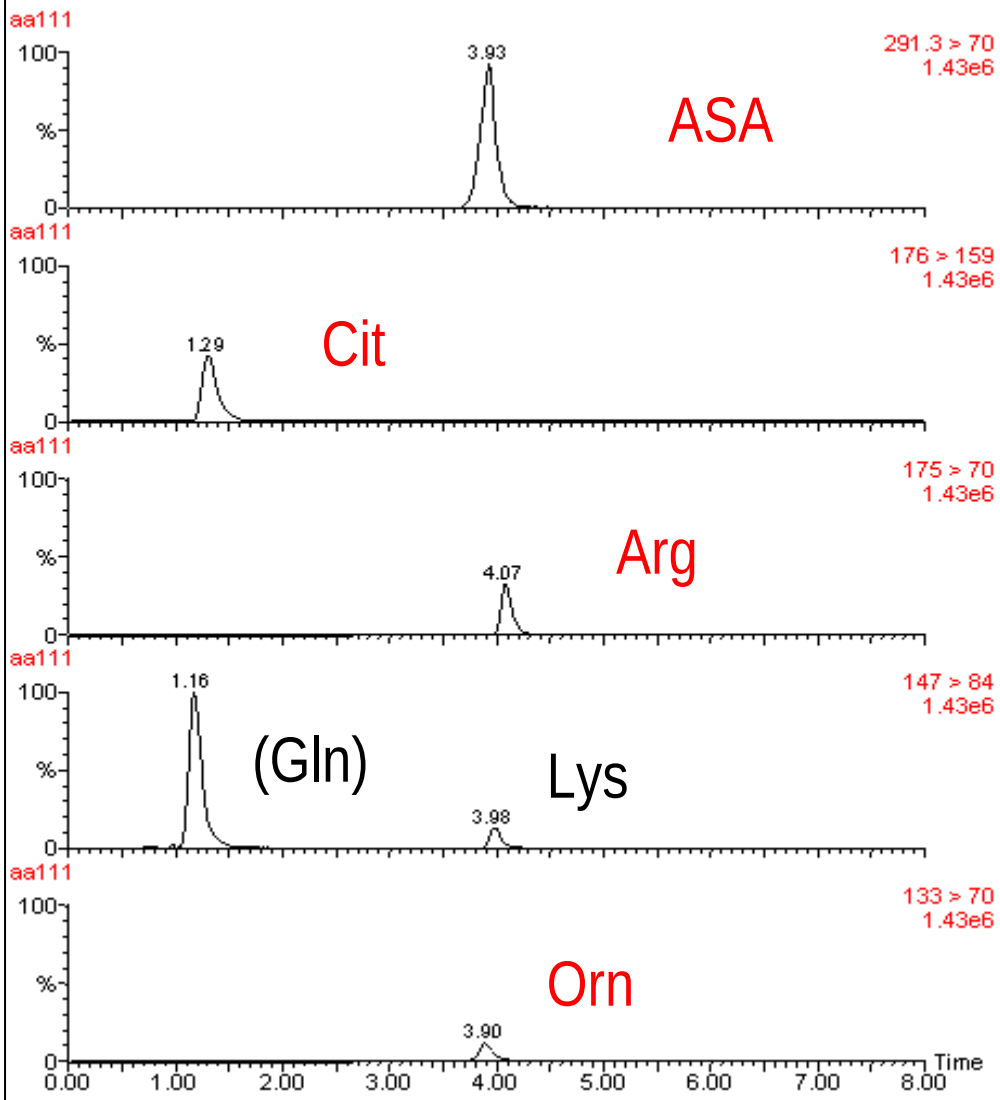
Spot campione ASA



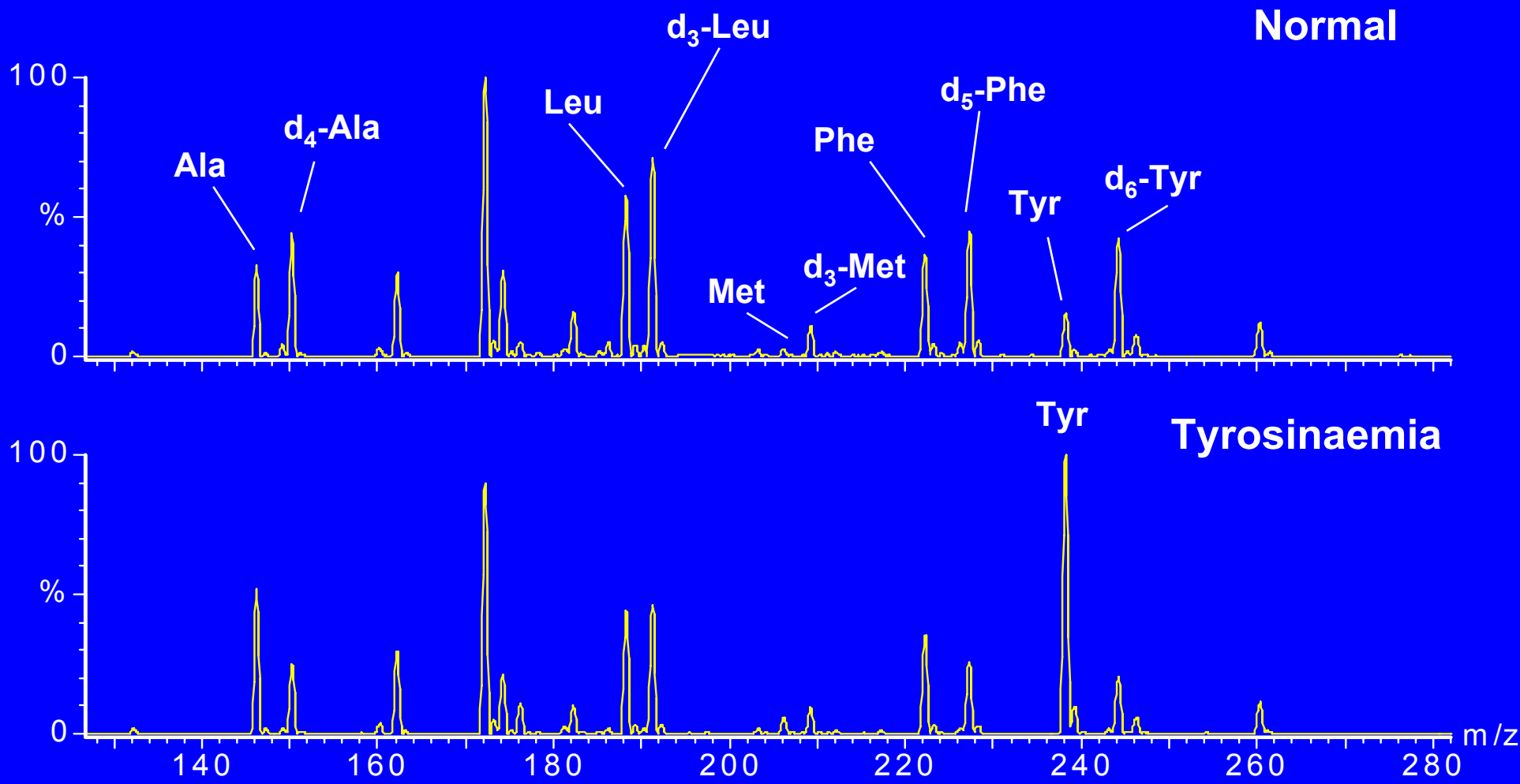
Confronto di un campione affetto (ASA) con un controllo



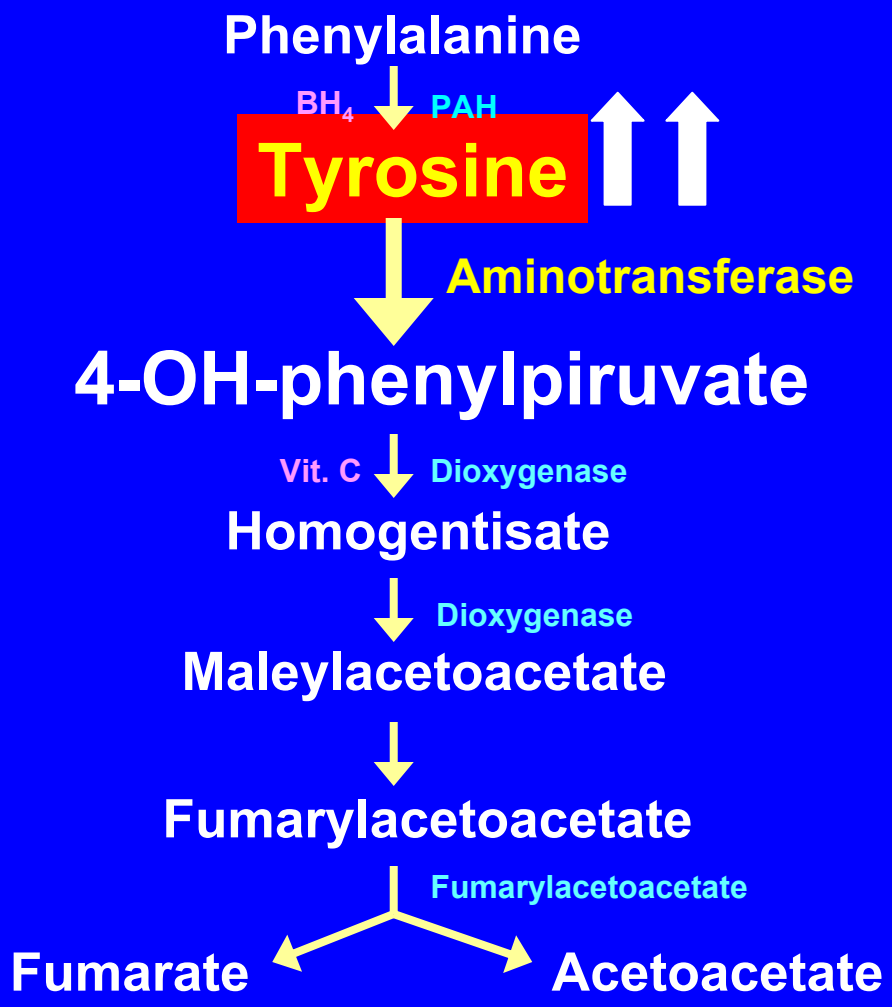
Spot campione ASA



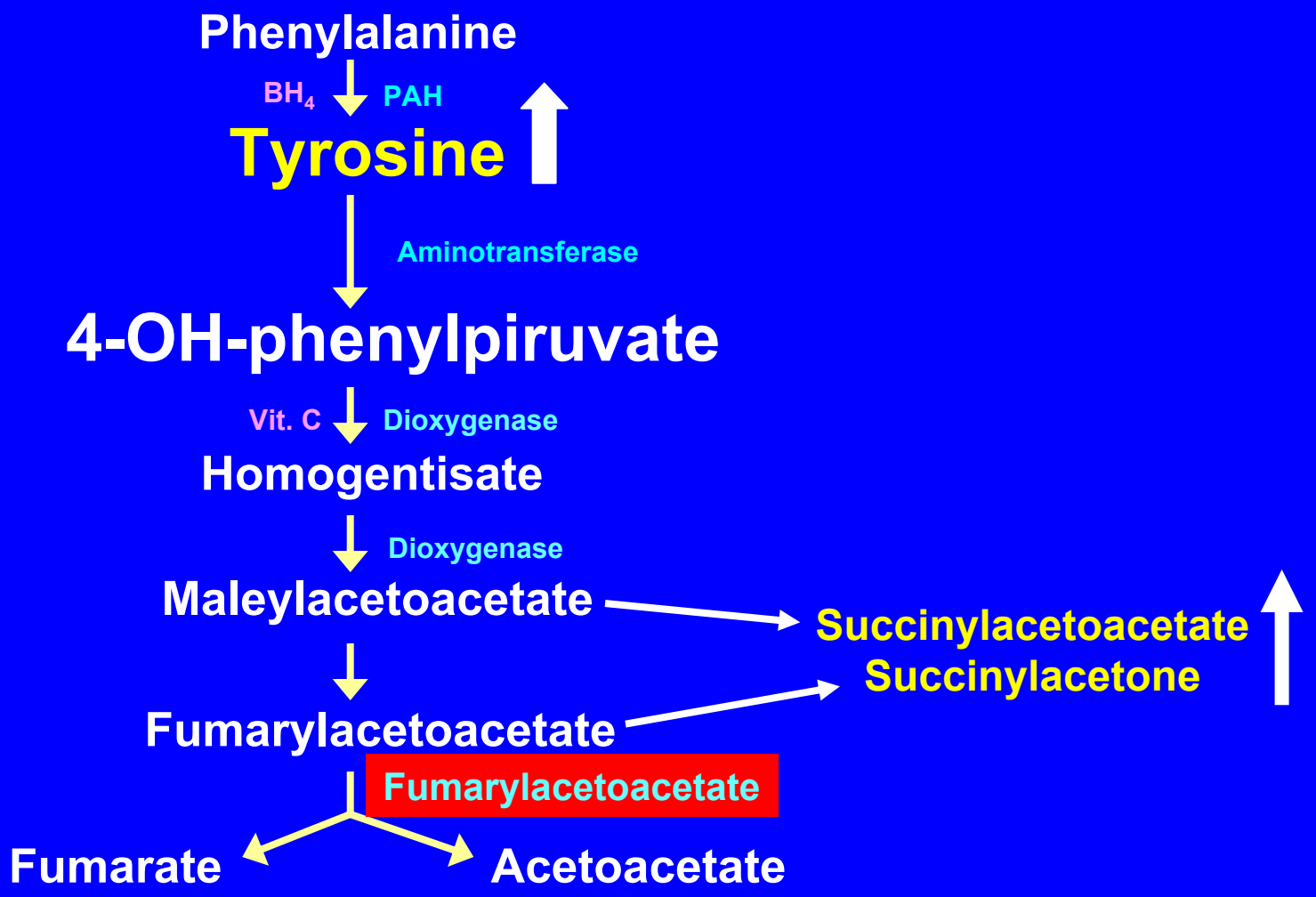
Normal *vs* Tyrosinaemia



TYROSINAEMIA II



TYROSINAEMIA I



Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Genetics and Metabolism xxx (2006) xxx–xxx
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I

Mark J. Magera [a], Nishantha D. Gunawardena [a], Si Houn Hahn [a], Silvia Tortorelli [a], Grant A. Mitchell [b], Stephen I. Goodman [c], Piero Rinaldo [a], Dietrich Matern [a,*]

[a] *Biochemical Genetics Laboratory, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[b] *Department of Pediatrics, Hôpital St. Justine, Montreal, Canada H3T 1C5 PQ*
[c] *Biochemical Genetics Laboratory, University of Colorado Health Science Center at Fitzsimons, Aurora, CO 80045, USA*

Received 18 October 2005; received in revised form 13 December 2005; accepted 14 December 2005

Abstract

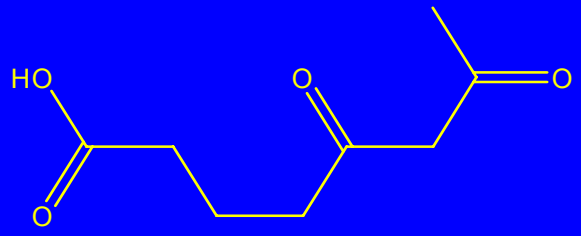
Background: Tyrosinemia type I (TYR 1) is a severe disorder causing early death if left untreated. While tyrosine can be determined in dried blood spots (DBS), it is not a specific marker for TYR 1 and most often associated with benign transient tyrosinemia of the newborn. Succinylacetone (SUAC) is a specific marker for TYR 1 but not detectable by routine newborn screening. We developed a new assay that determines SUAC in DBS by liquid-chromatography tandem mass spectrometry (LC–MS/MS).

Methods: Whole blood is eluted from a 3/16-in. DBS by an aqueous solution containing deuterium labeled SUAC as internal standard (IS). SUAC and IS are oximated, then extracted, butylated, and analyzed by LC–MS/MS. Quantitation is from SUAC spiked calibrator DBS over the range 0–200 μM using selected reaction monitoring of transitions *m/z* 212 to 156 and *m/z* 214 to 140 for SUAC and IS, respectively. Analysis time is 5 min. To assess the effectiveness of a two-tier screening approach for TYR 1 we applied this assay to our newborn screening program over the last 15 months.

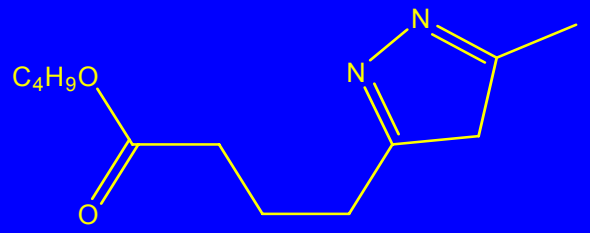
Results: The intra-assay precision was determined for three different levels of SUAC (5, 20, and 50 μmol/L) and the CV calculated to be 4.7, 2.6, and 3.1%, respectively ($n = 5$). Inter-assay precision CVs were 12.7, 8.2, and 7.8%, respectively on the same samples. SUAC levels in DBS from 10 confirmed TYR 1 cases not treated with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) were clearly abnormal (16–150 μmol/L; mean: 61 μmol/L; controls: <5 μmol/L). Over a 15-month period, SUAC was determined in newborn screening samples with elevated tyrosine concentrations when applying different cut off values until it was settled at 150 μmol/L. No case of TYR 1 was detected in 124,780 newborns tested.

Conclusion: We have developed a new LC–MS/MS based method for the determination of SUAC in DBS. This assay has the potential to significantly reduce the number of false positive results in newborn screening for TYR 1 and can also be used for the laboratory follow up of patients treated for TYR 1.

ANALYSIS OF SUCCINYLACETONE FROM DRIED BLOOD SPOTS

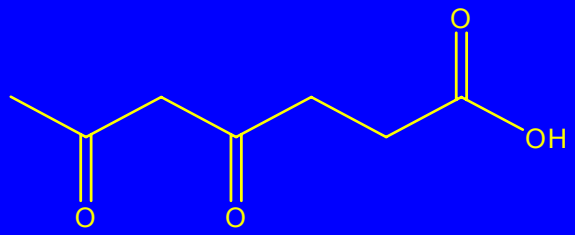


5-7-dioxooctanoic acid

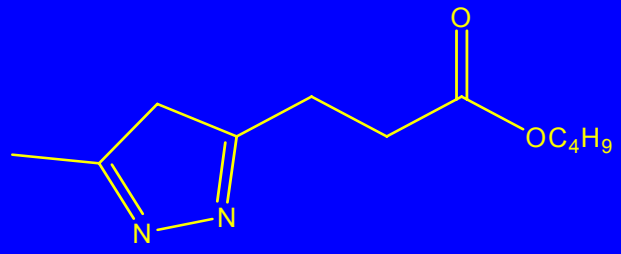


$C_{12}H_{20}N_2O_2$
Exact Mass: 224.1

oximated extracted and butylated



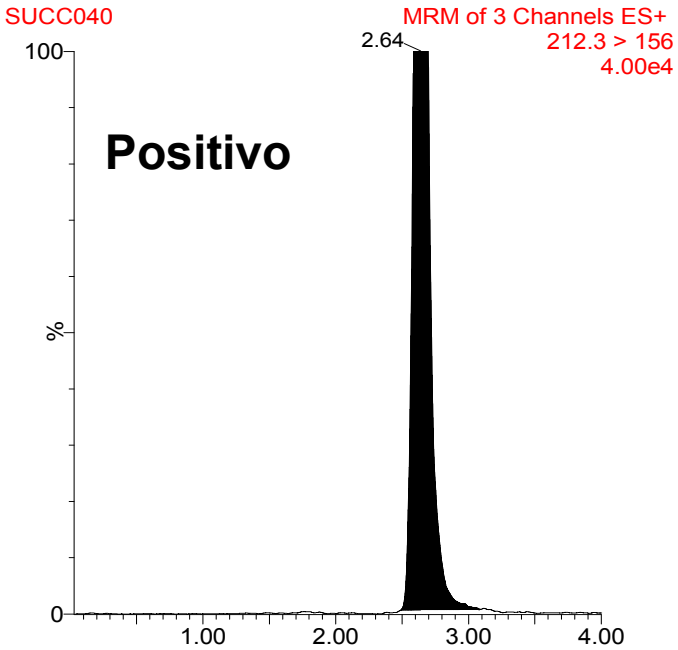
4-6-Dioxoheptanoic acid Succinylacetone

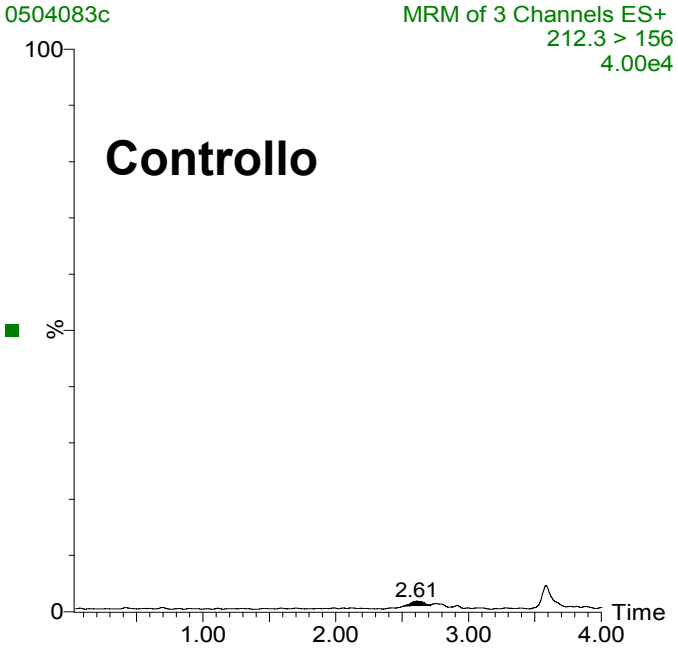


$C_{11}H_{18}N_2O_2$
Exact Mass: 210.1

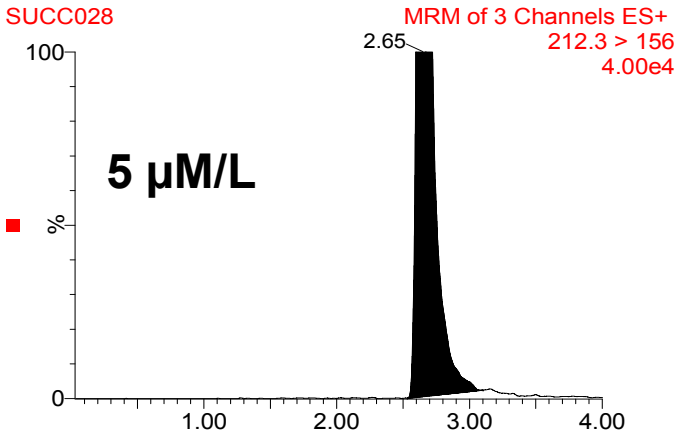
Clinical Biochemistry 37 (2004) 1010– 1015

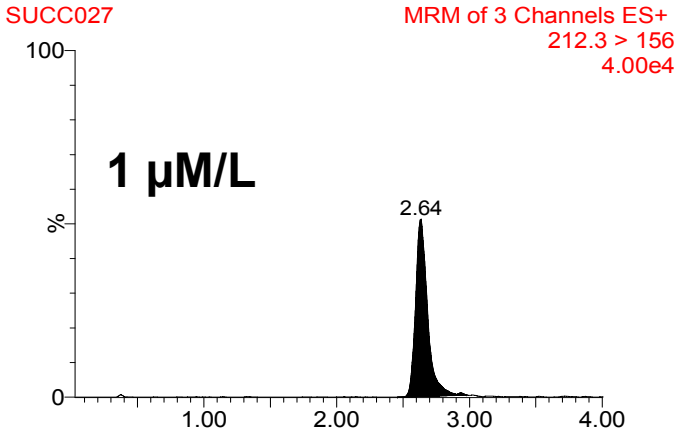
Urine SUAC

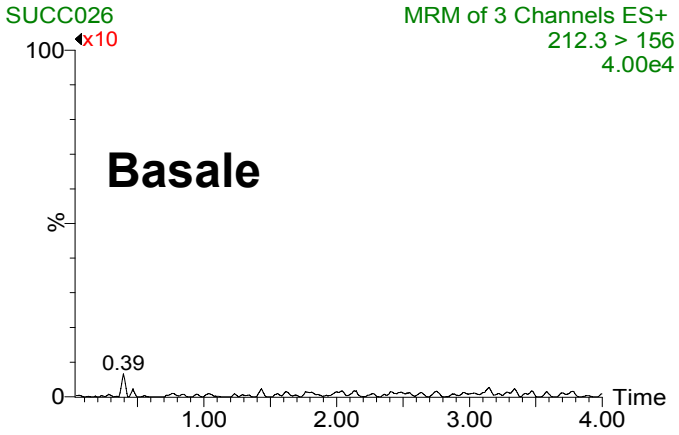




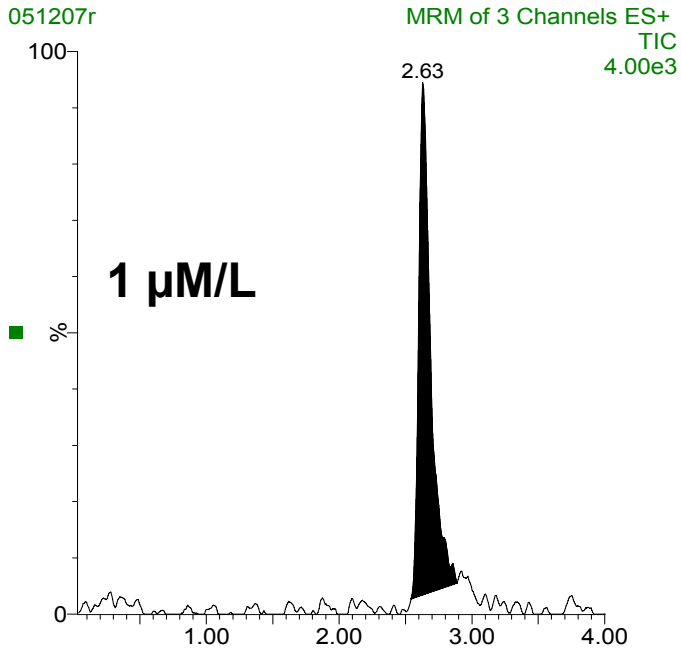
Spot + aggiunta SUAC

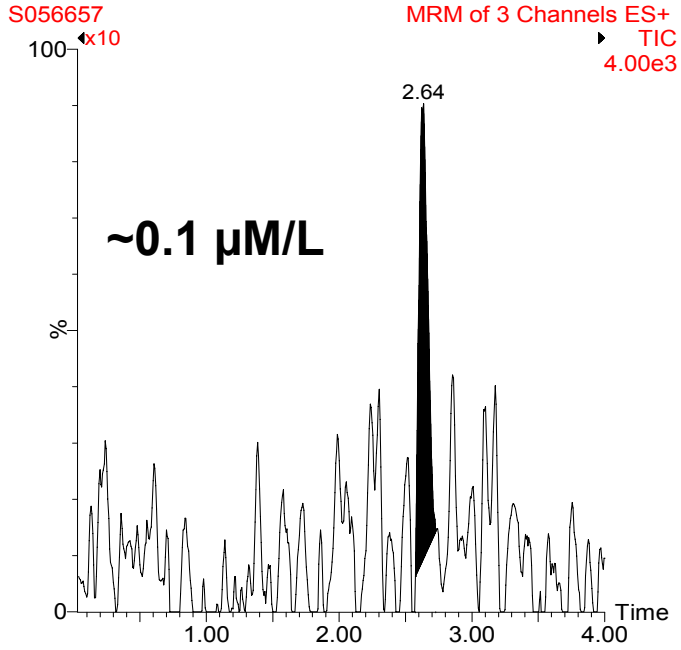






Spot





Aminoacid

- **MSUD (allo)**
- **Tyrosinemia type I (SUAC)**
- **(ASA)**

Bile acid

- **3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-HSD)**
- **Peroxisomal 3-ketothiolase deficiency (pseudo-Zellweger syndrome)**

Gli acidi biliari

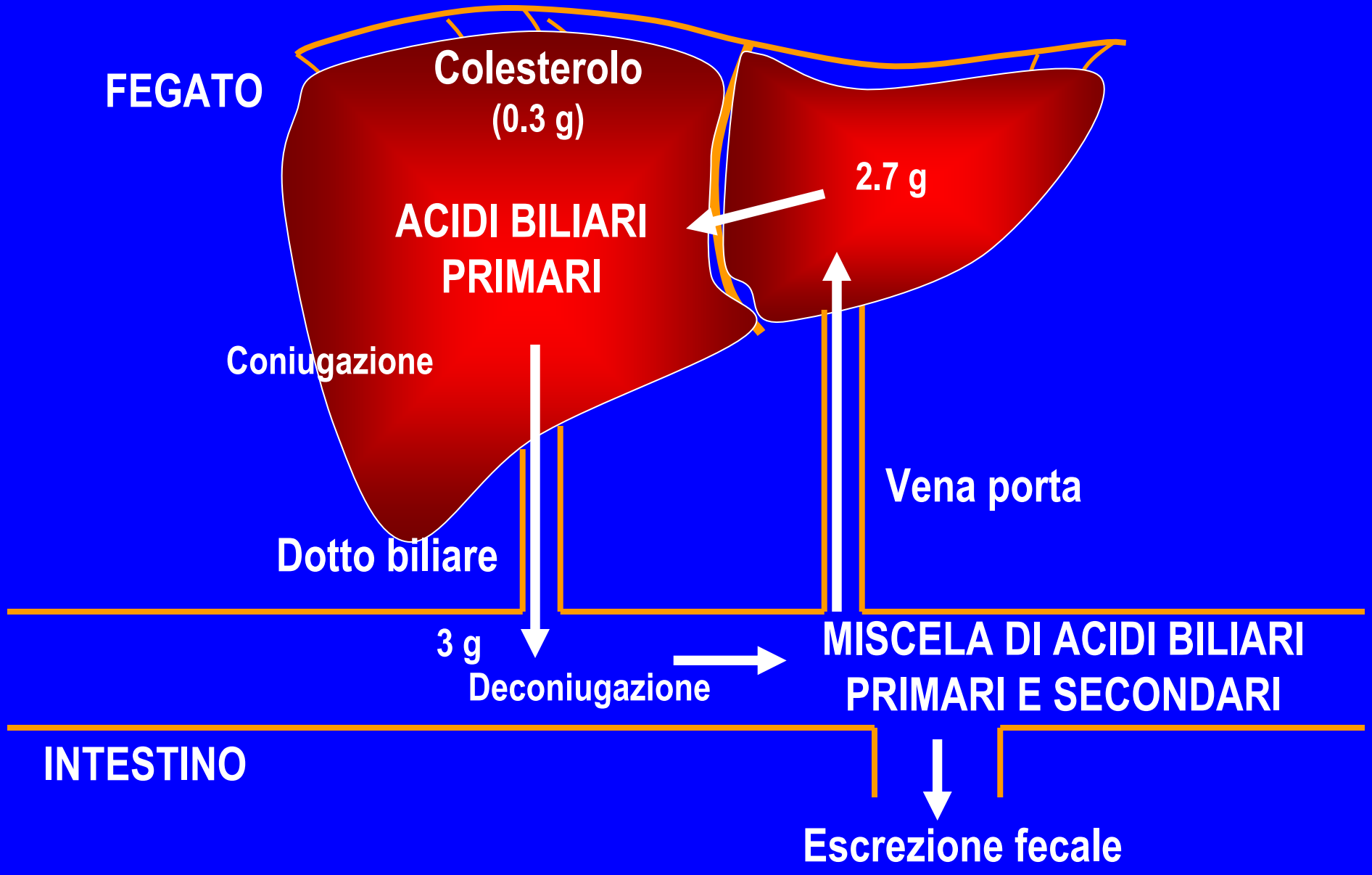
Gli acidi biliari vengono prodotti dal catabolismo del colesterolo

Nel fegato vengono sintetizzati gli acidi biliari primari (colico e chenodeossicolico). Dopo coniugazione con glicina e taurina vengono secreti nelle vie biliari attraverso le quali passano nell'intestino dove svolgono la loro funzione prodigestiva di emulsionamento dei lipidi

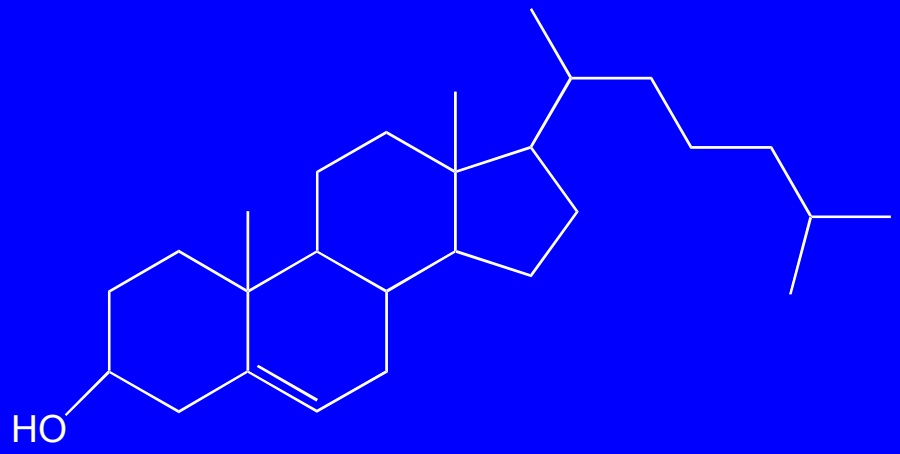
Nel lume intestinale parte degli acidi primari viene trasformata in acidi secondari (deossicolico e litocolico)

Acidi primari e secondari sono infine riassorbiti a livello intestinale dopo deconiugazione ritornano al fegato per mezzo dei vasi portali

Circolo enteroepatico degli acidi biliari



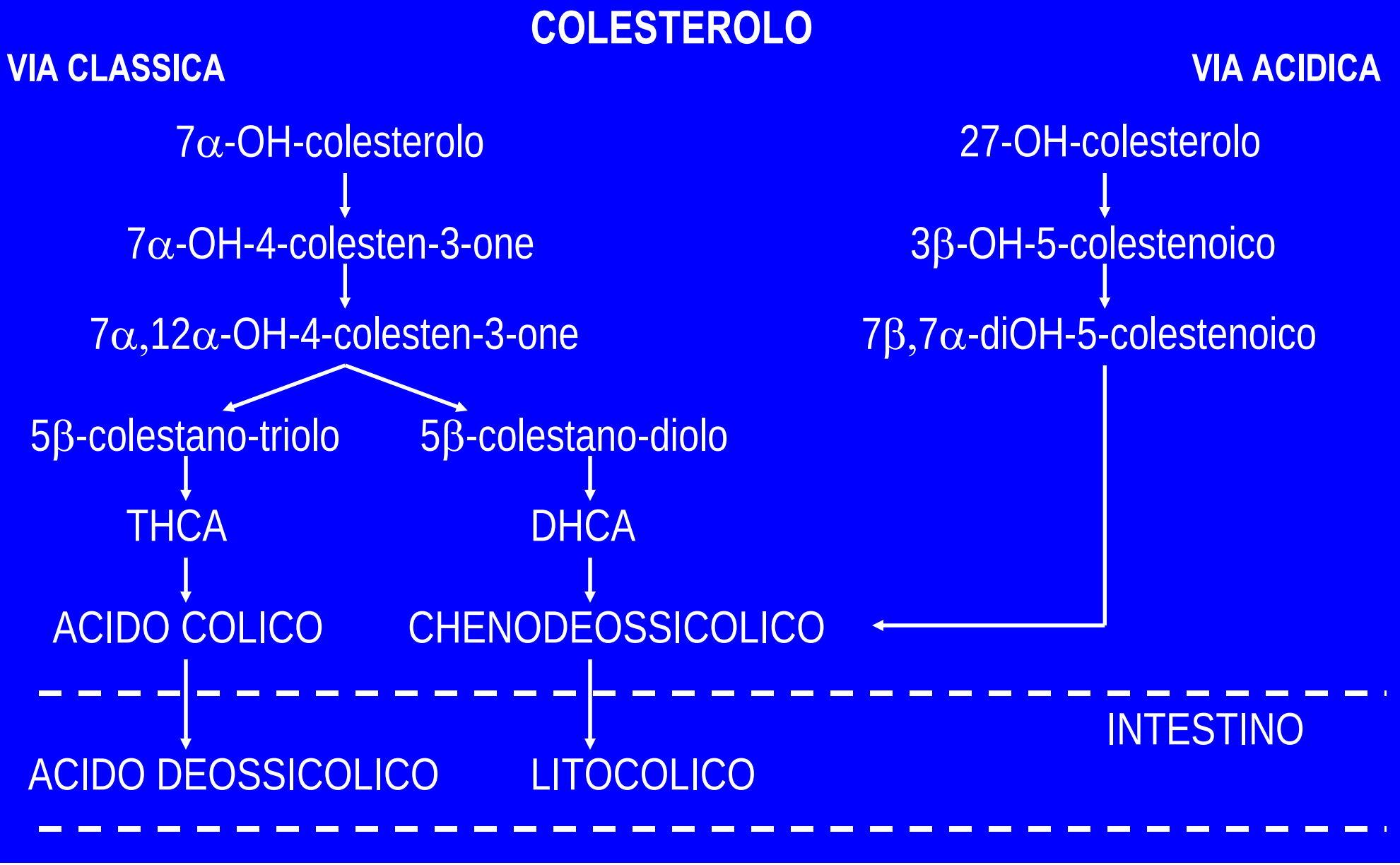
Il colesterolo e gli acidi biliari



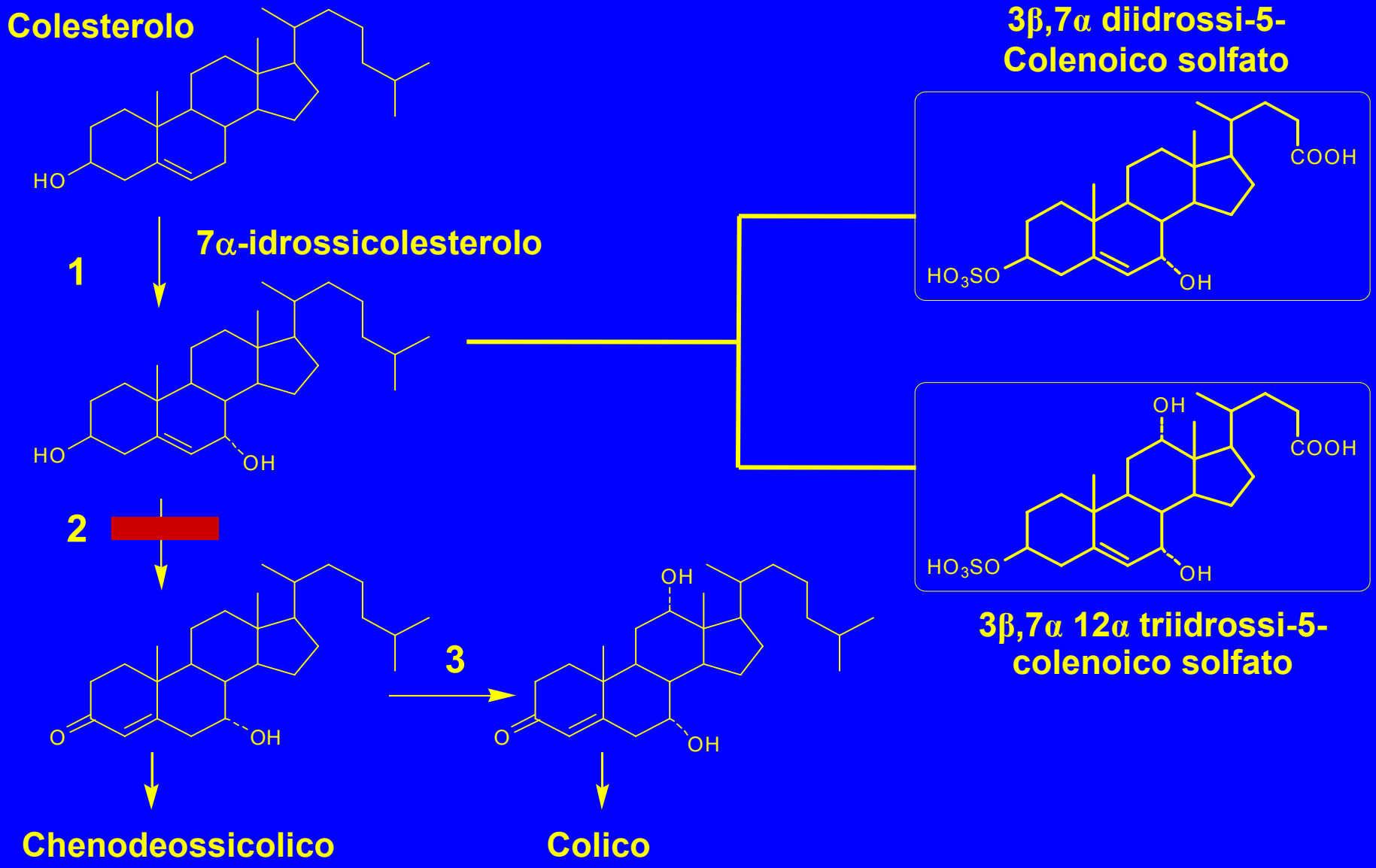
Colesterolo

Acido biliare

Sintesi degli acidi biliari dal colesterolo



Biochimica del difetto di HSD



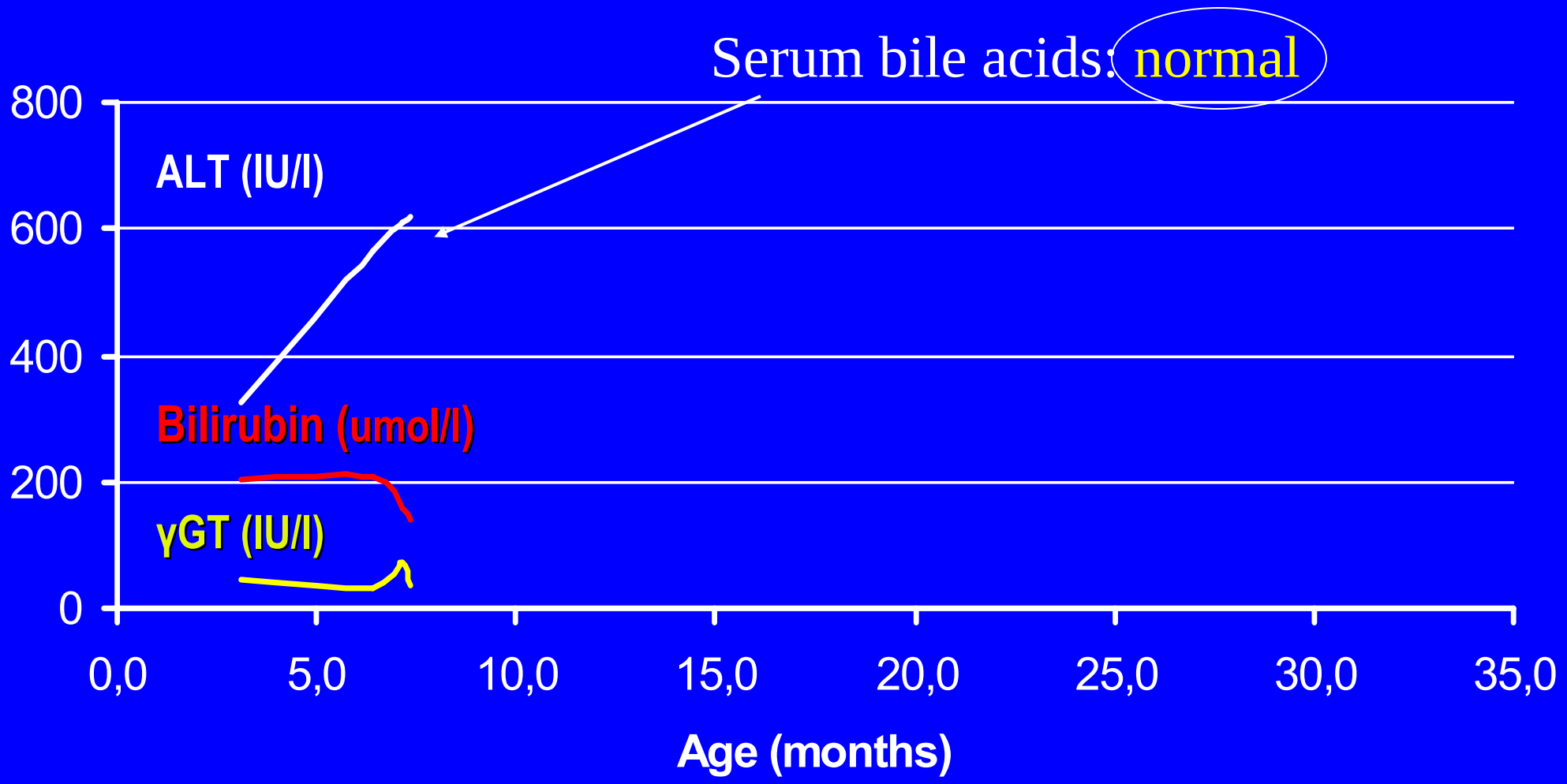
Background

3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-HSD) deficiency is a rare inborn error of bile acid metabolism

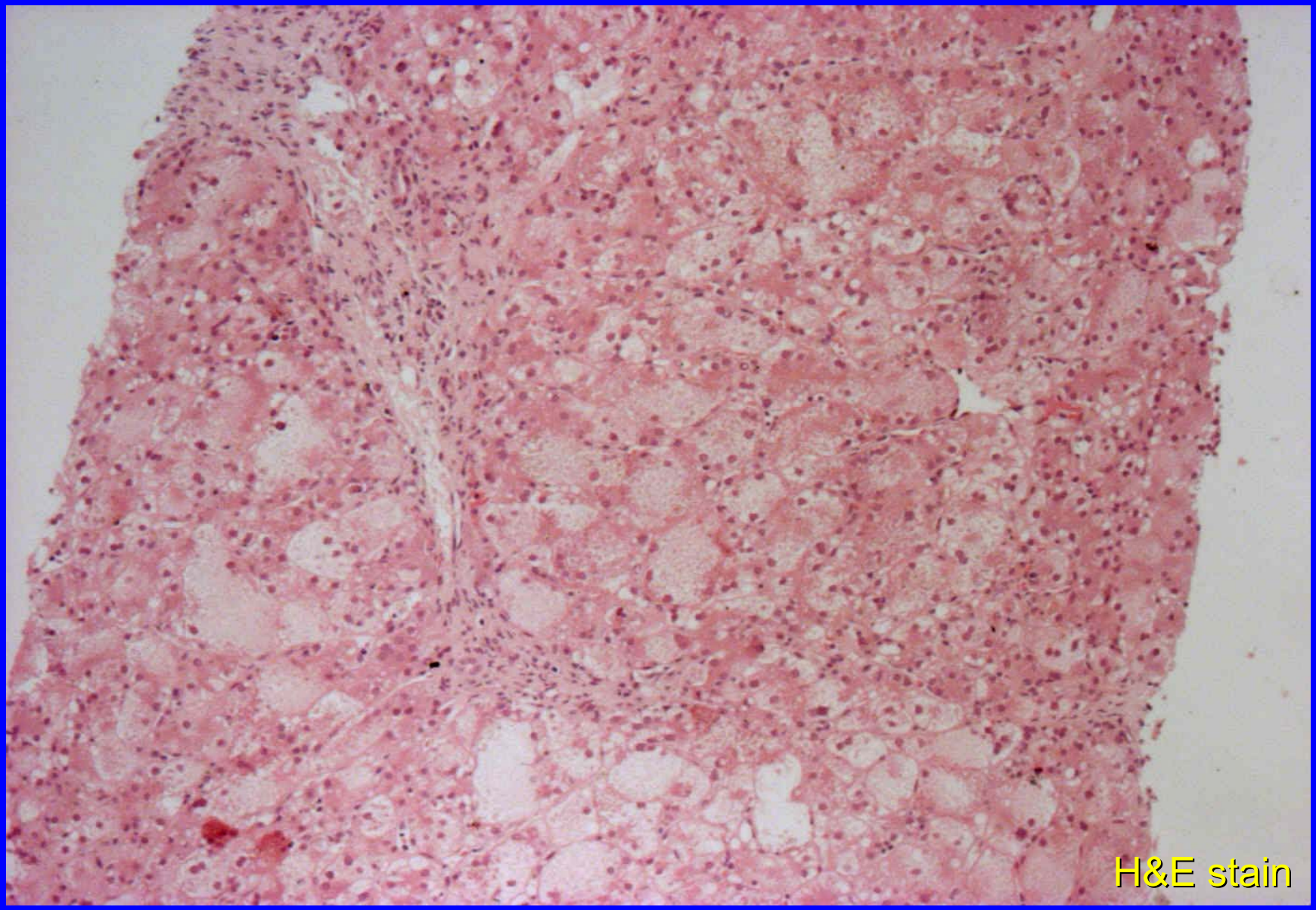
The metabolic derangements of the disorder involve

- Severe cholestasis from lack of primary bile acids
- Chronic liver injury from toxic metabolites

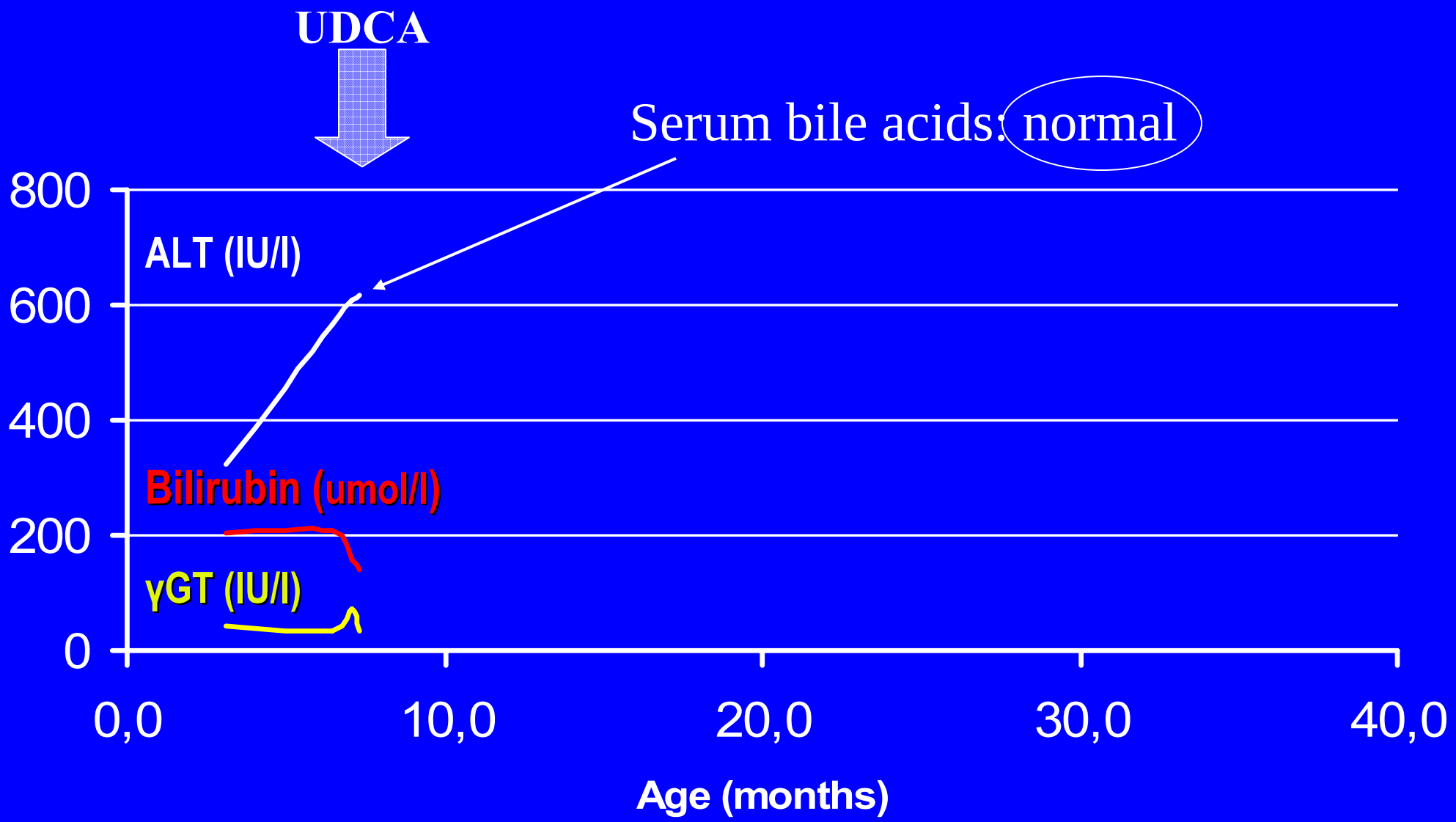
Liver function tests at presentation



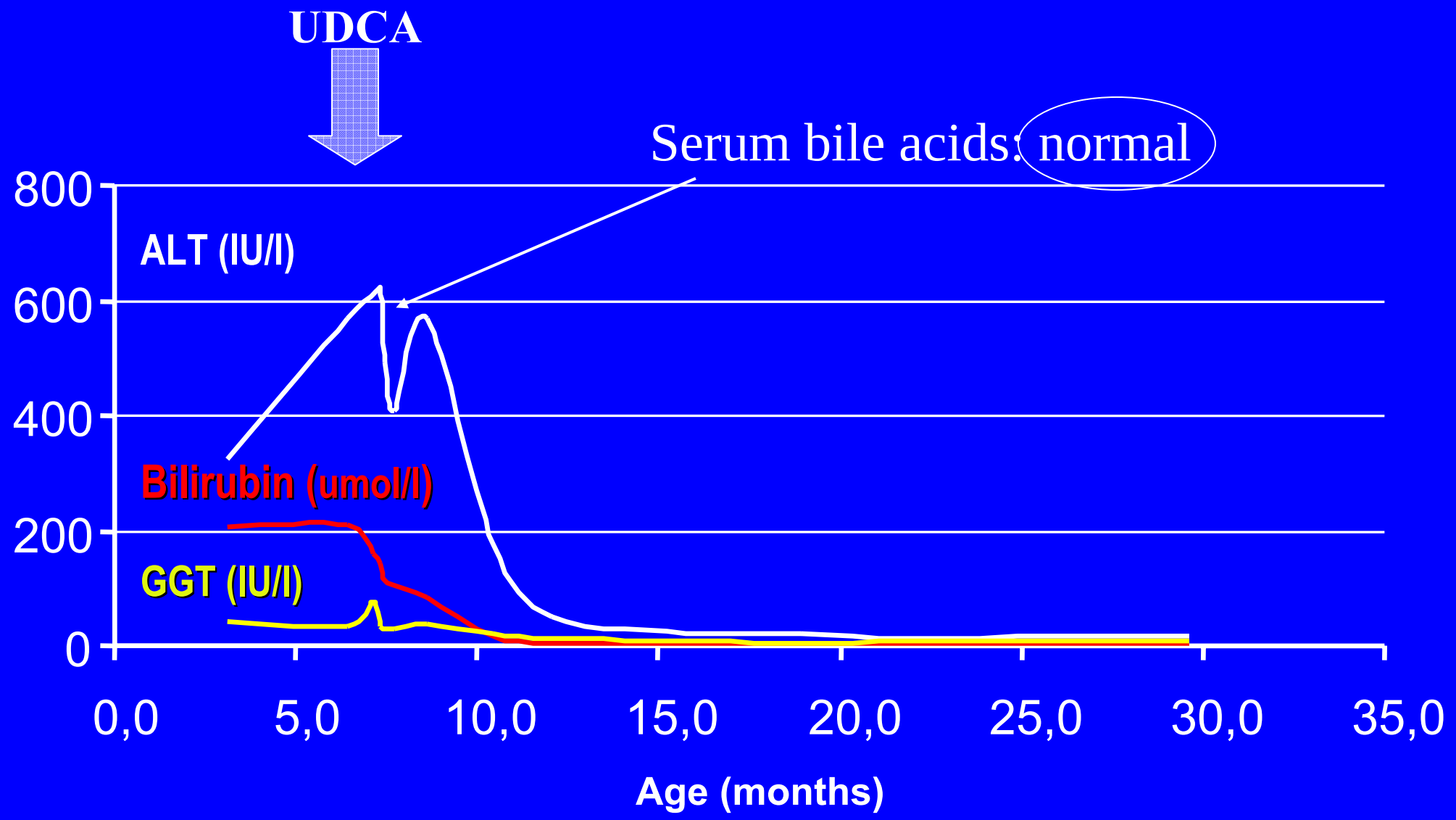
Liver biopsy at presentation



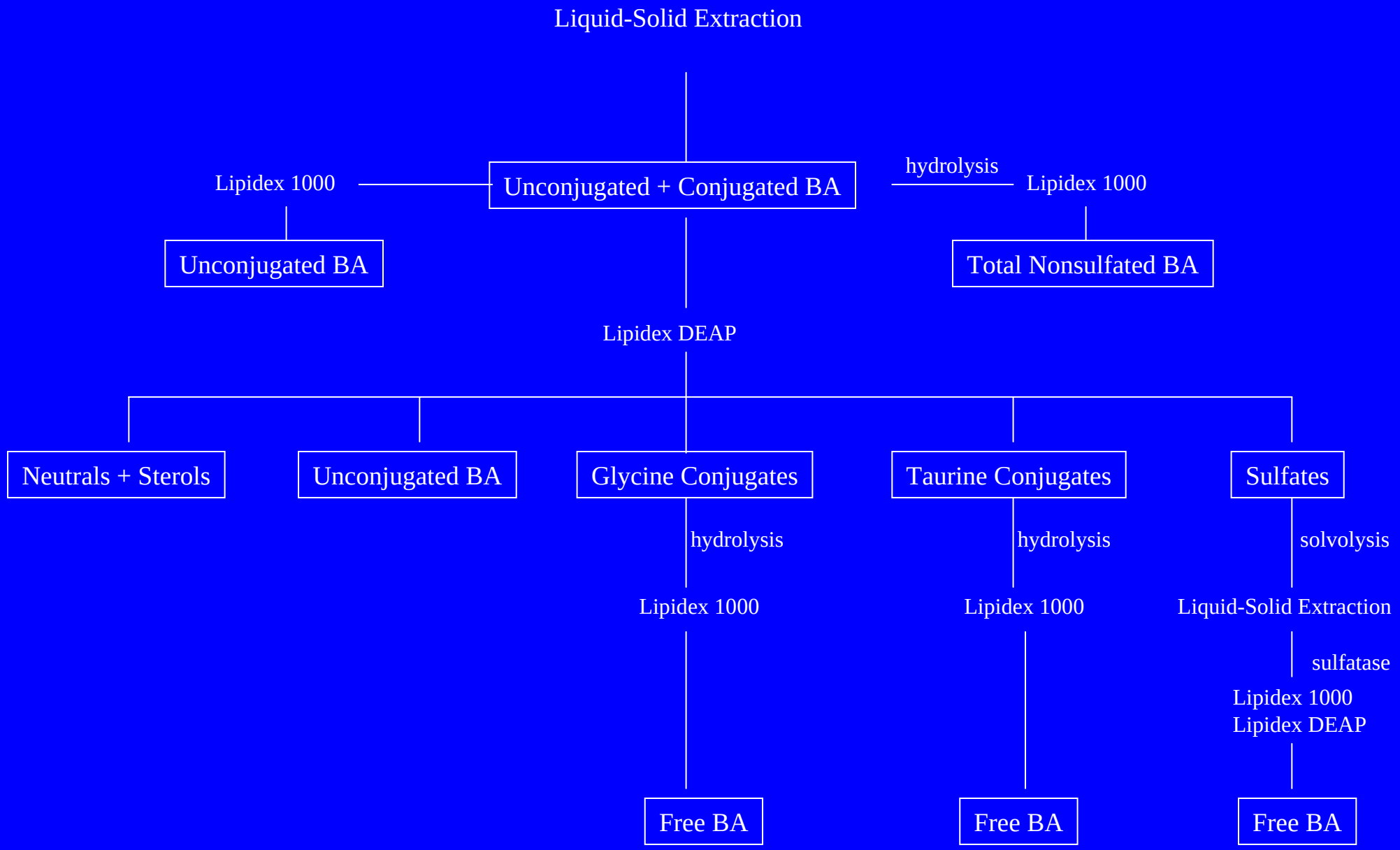
Liver function tests at presentation



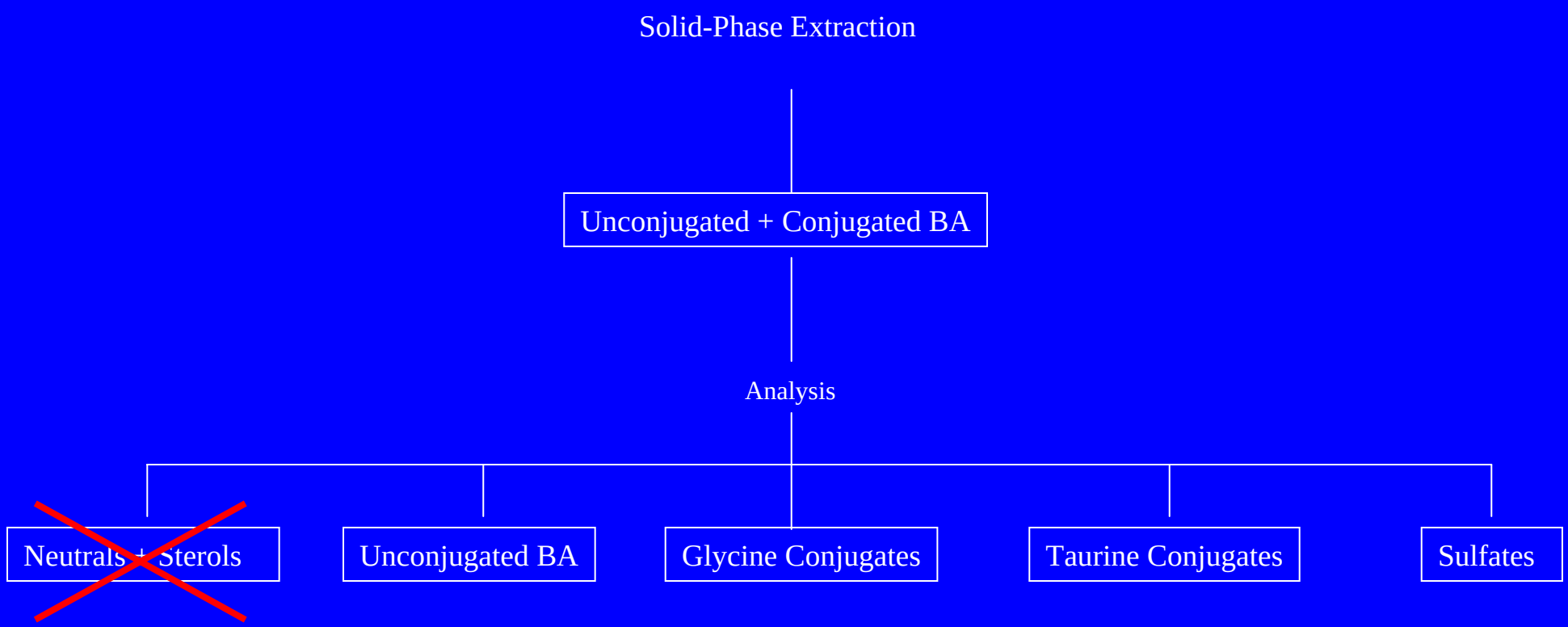
Liver function tests at presentation



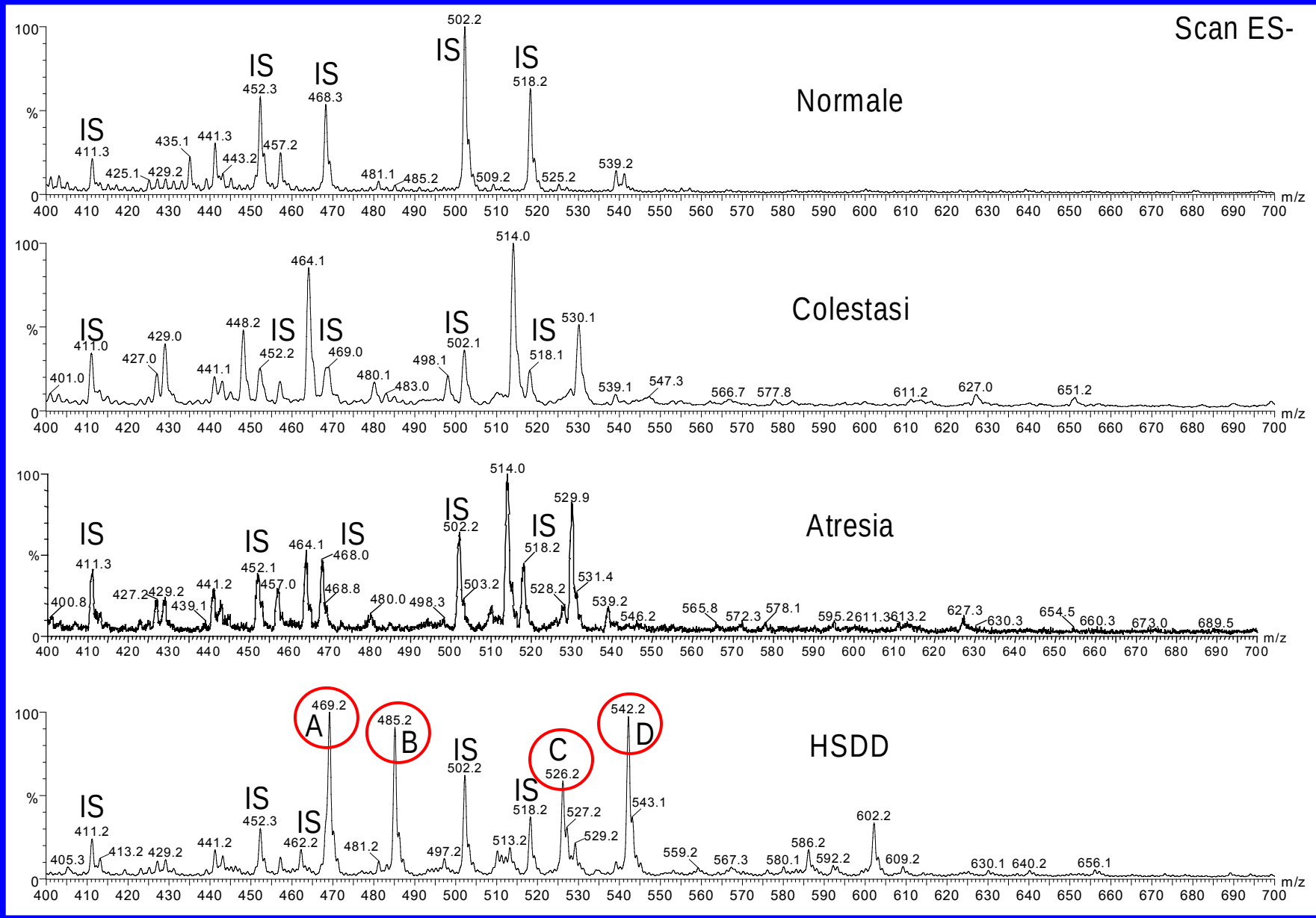
Schema dell'analisi degli acidi biliari in GC-MS



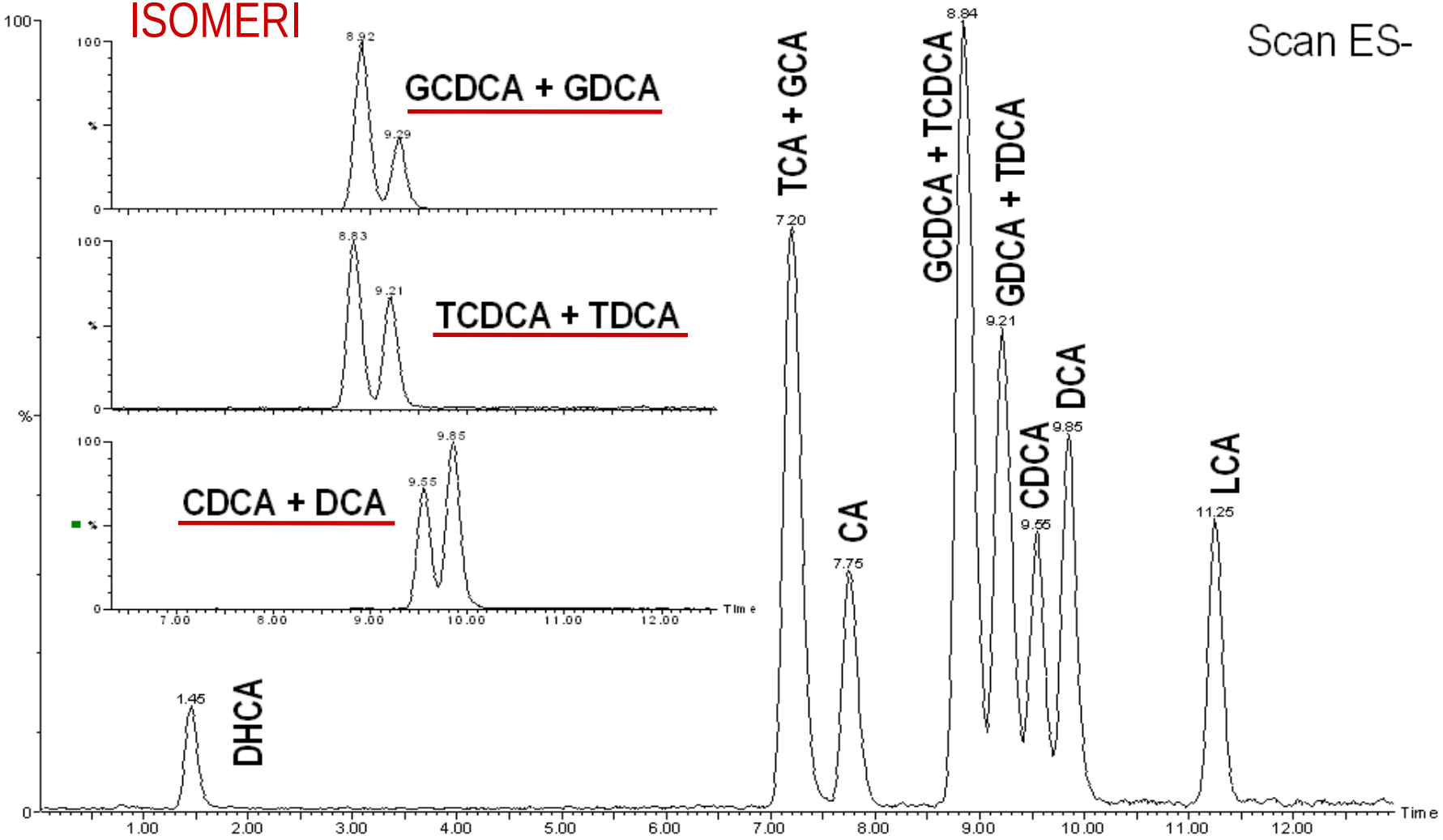
Schema dell'analisi acidi biliari in FIA dopo una SPE extraction



Profilo degli acidi biliari in ES-MS

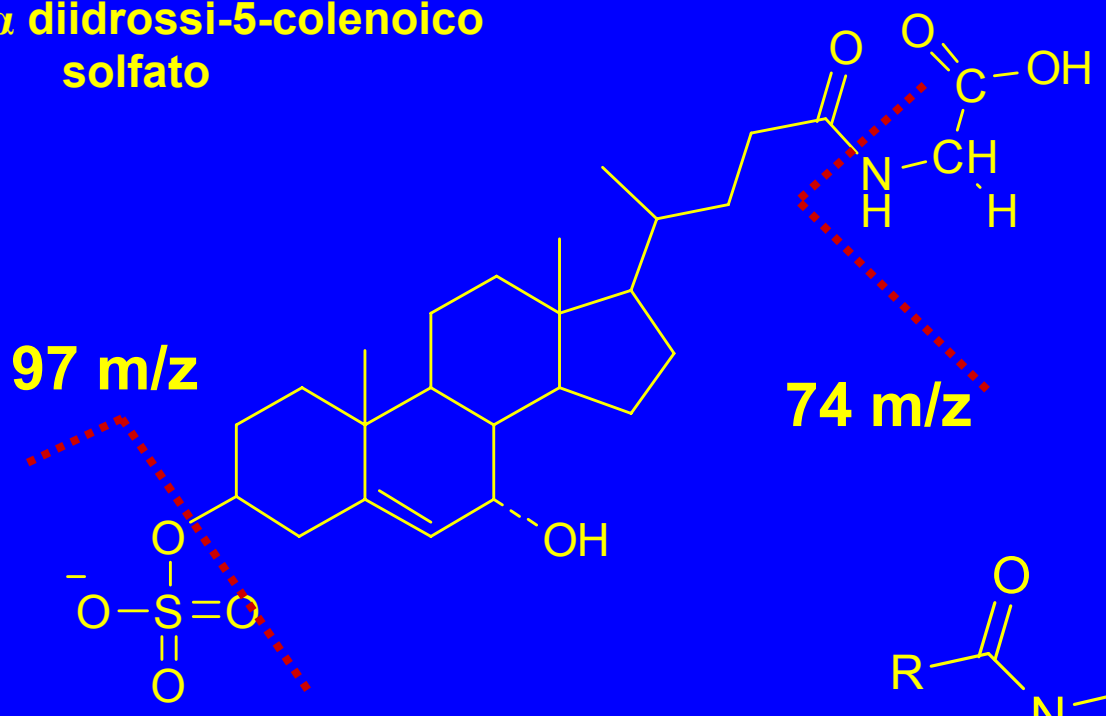


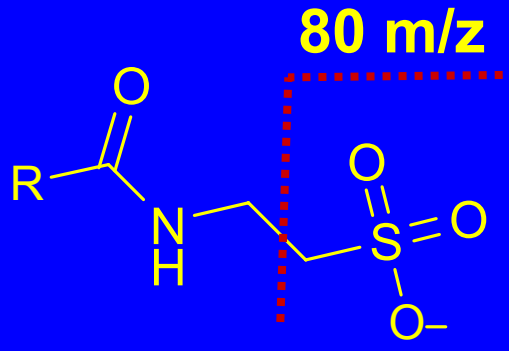
Separazione cromatografica degli acidi biliari in scansione



IONI FRAMMENTO

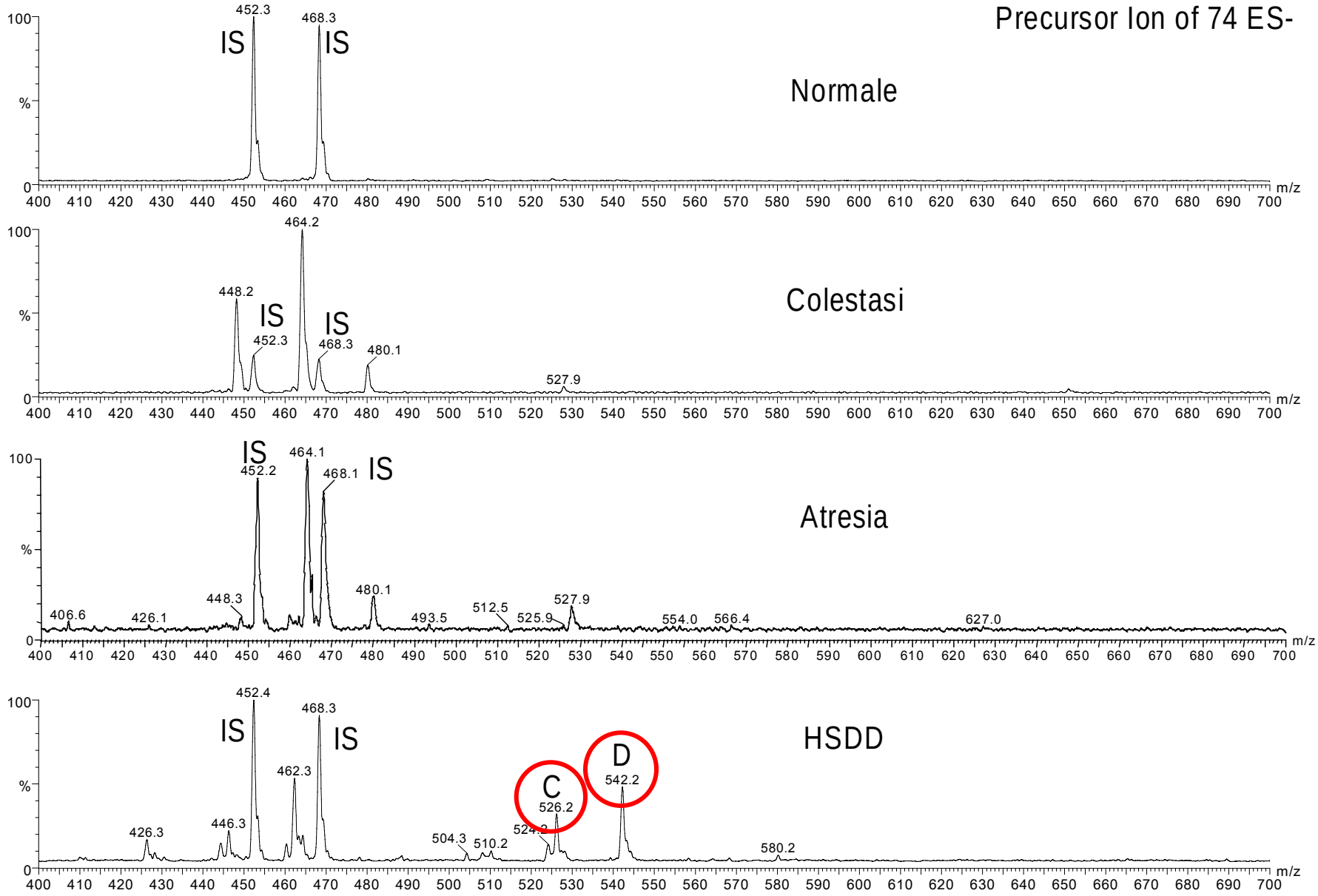
Glico 3β,7α diidrossi-5-colenoico solfato



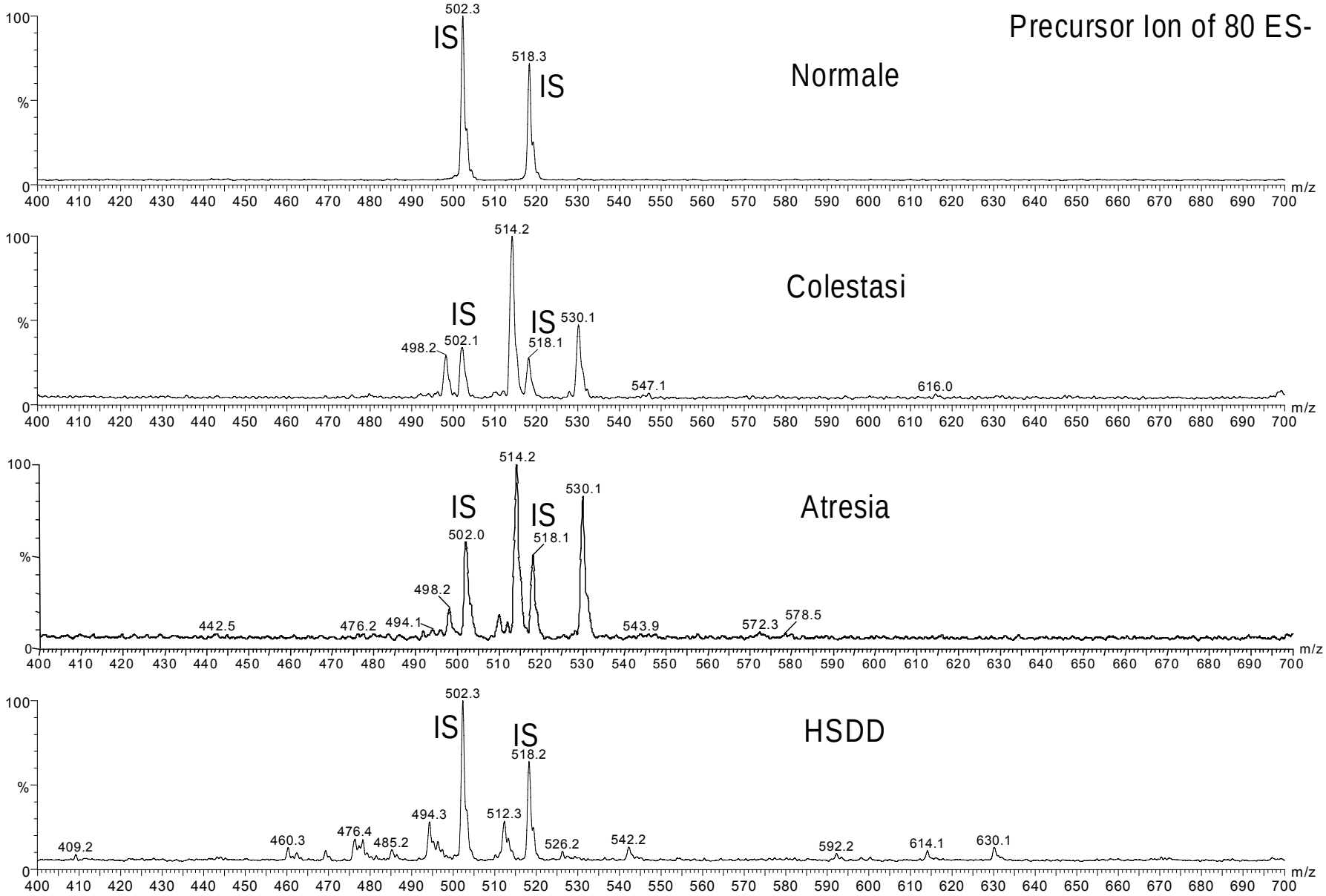


Tauro 3β,7α diidrossi-5-colenoico

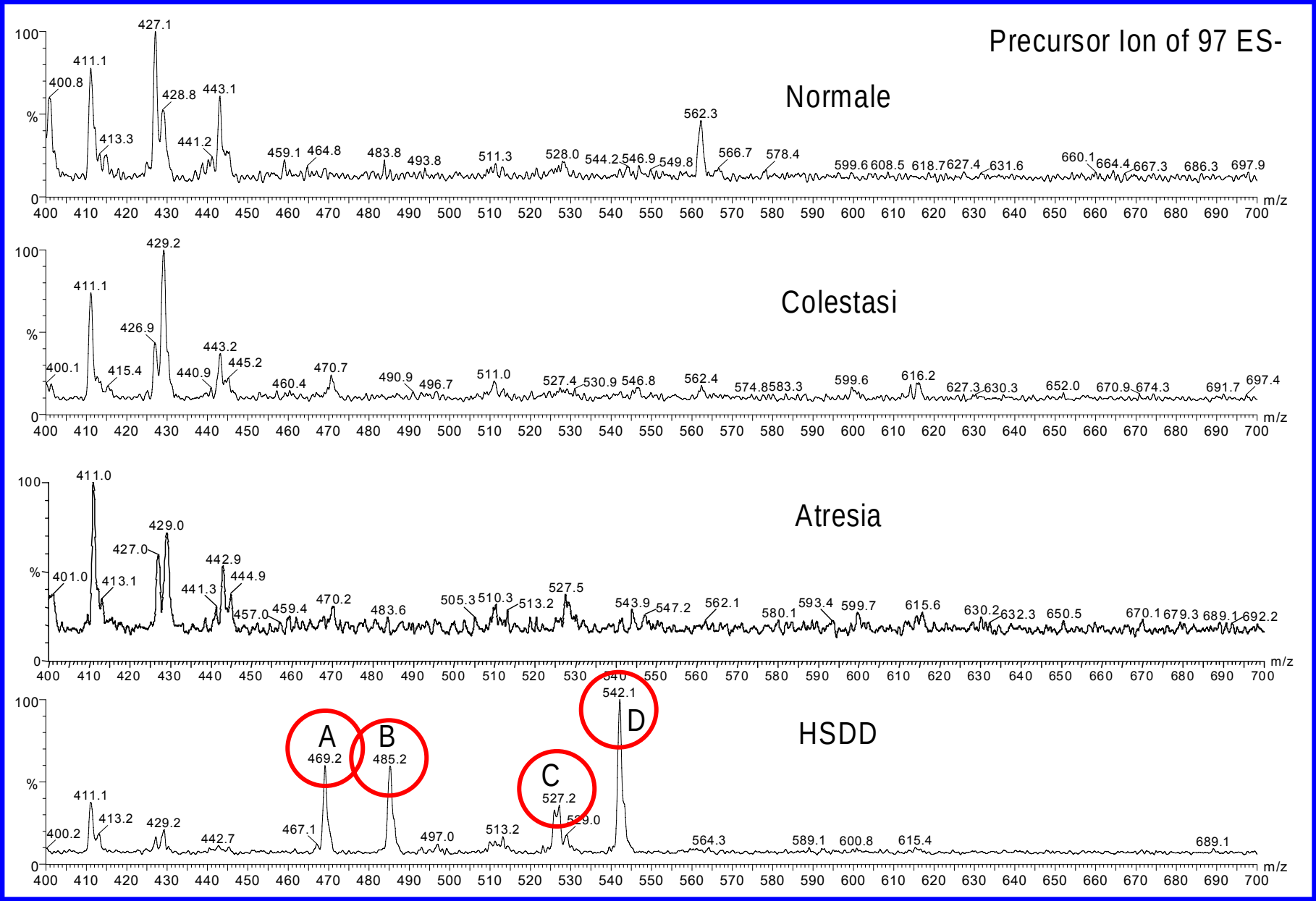
Profilo degli acidi biliari coniugati con glicina in ES-MS/MS



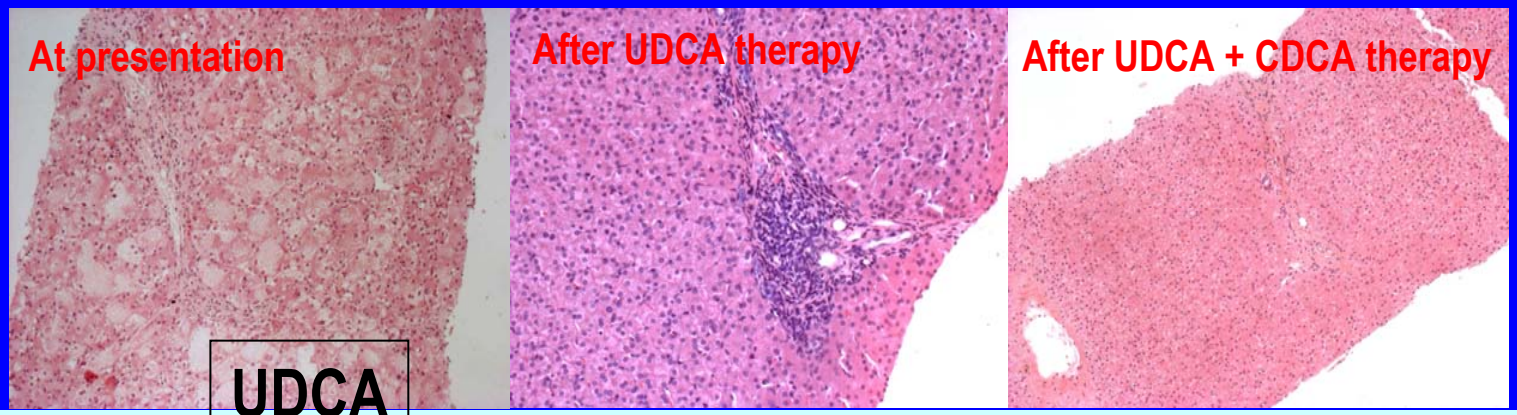
Profilo degli acidi biliari coniugati con taurina in ES-MS/MS

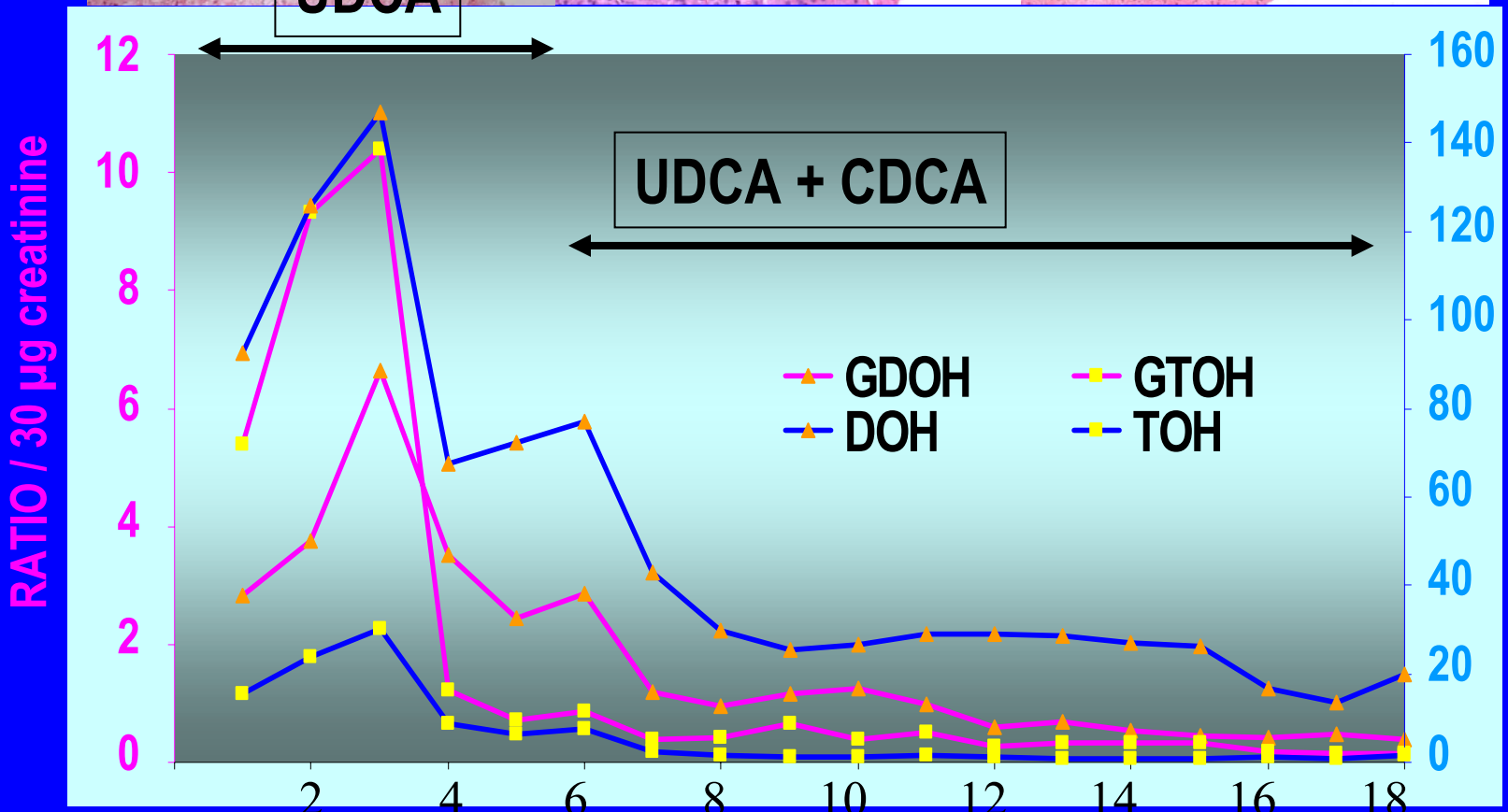


Profilo degli acidi biliari coniugati con solfato in ES-MS/MS

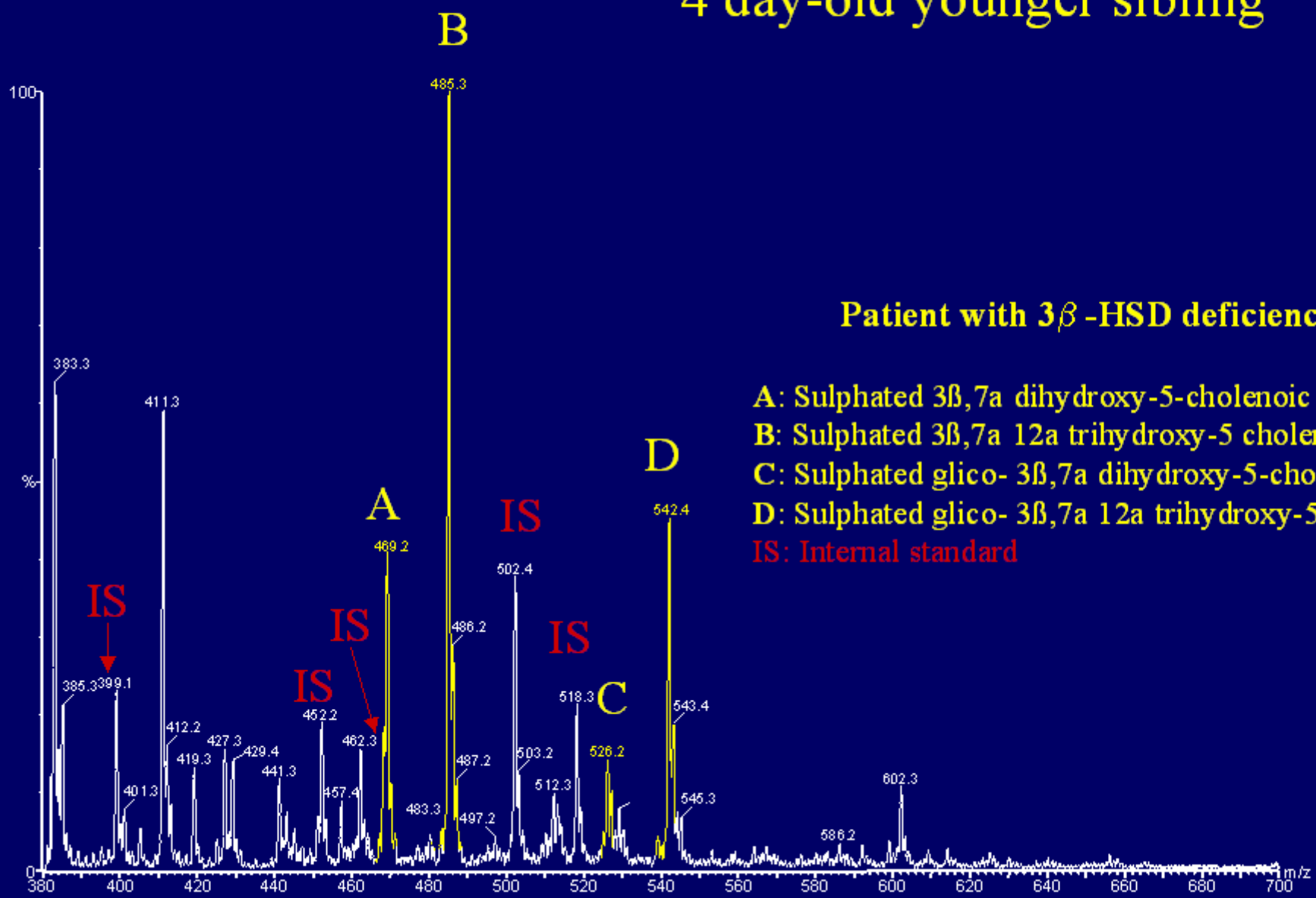


Liver biopsy of the first patient





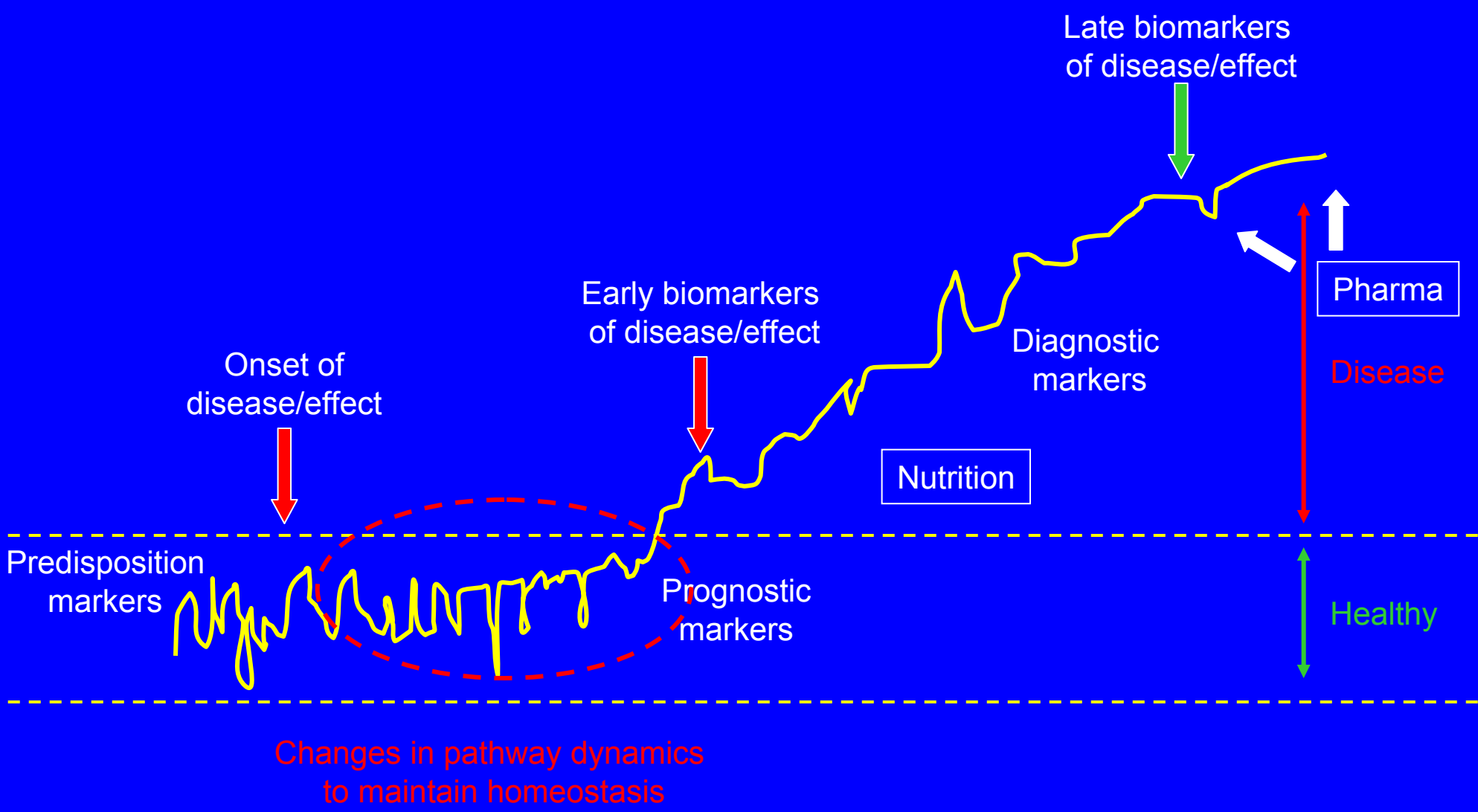
4 day-old younger sibling



Patient with 3β-HSD deficiency

A: Sulphated 3ß,7a dihydroxy-5-cholenoic
B: Sulphated 3ß,7a 12a trihydroxy-5 cholenoic
C: Sulphated glico- 3ß,7a dihydroxy-5-cholenoic
D: Sulphated glico- 3ß,7a 12a trihydroxy-5 cholenoic
IS: Internal standard

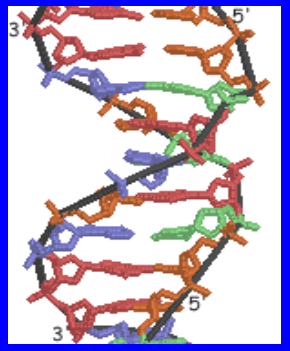
Scheme of development of disease from healthy



Van der Greef J. et. Kluver Academic Publishers; 2003: 170-198

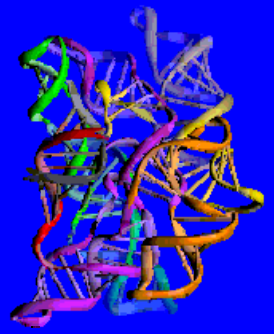
Biochemical Context of Genomics and Proteomics

DNA



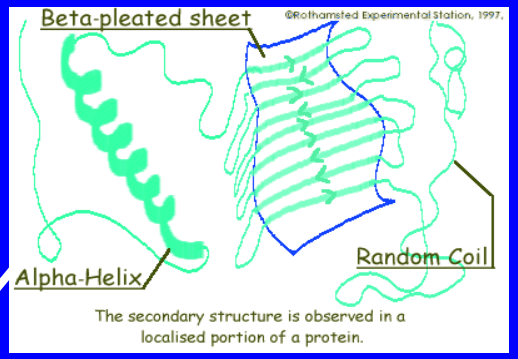
Genomics – 25,000 Gene

RNA



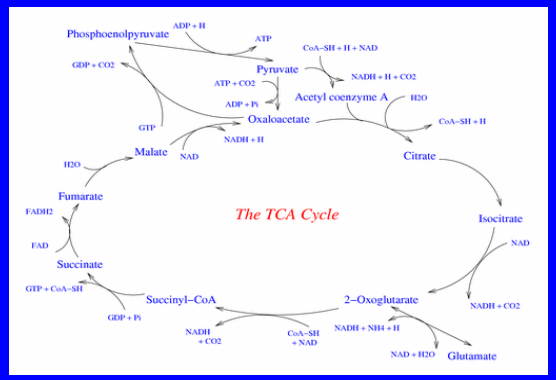
Transcriptomics – 100,000 Transcripts

Protein



Proteomics – 1,000,000 Proteins

Biochemicals (Metabolites)



Metabolomics – 2,400 Compounds

Metabolomics

METABOLOMICS

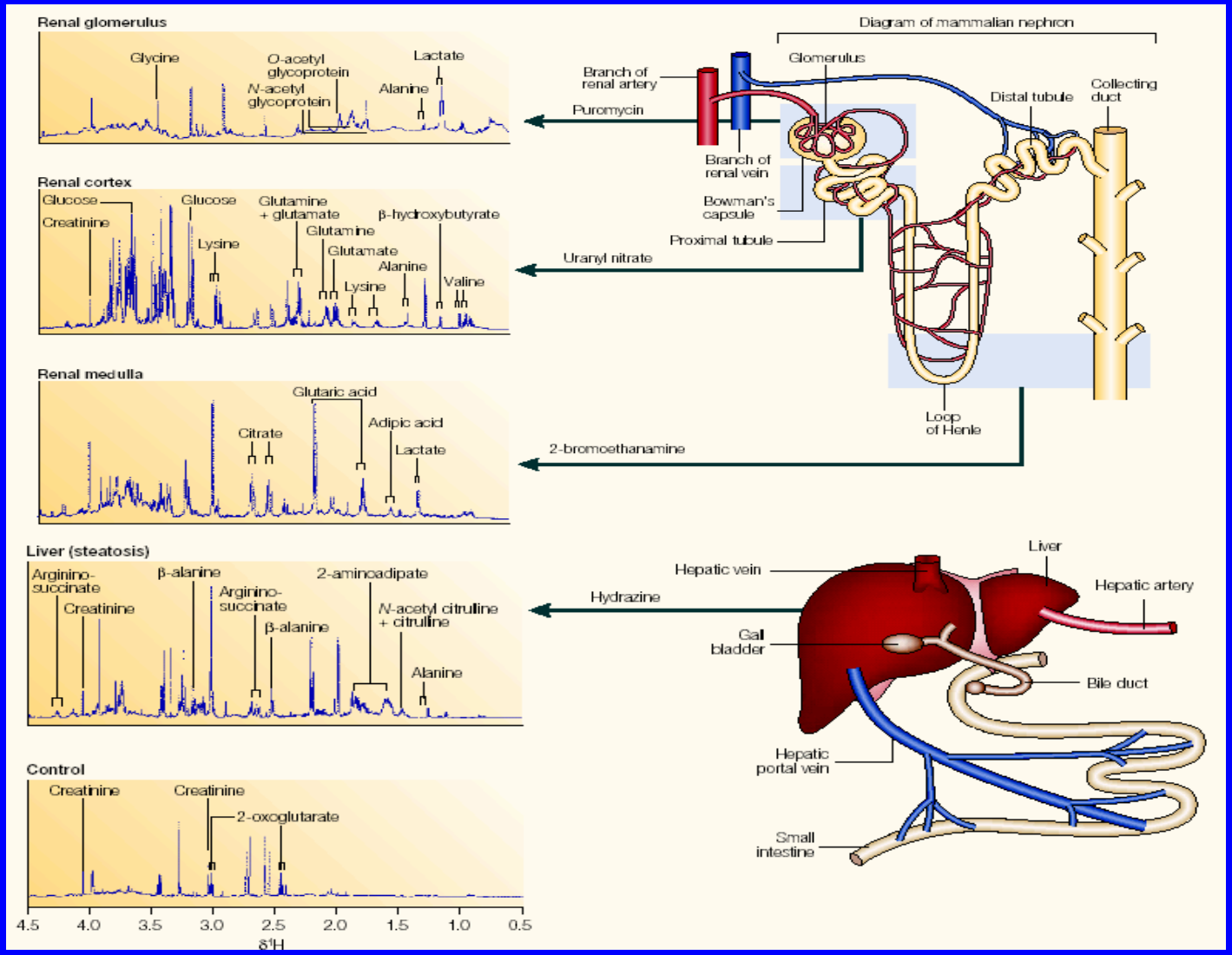
THE MEASUREMENT OF METABOLITE CONCENTRATIONS AND FLUXES AND SECRETION IN CELLS AND TISSUES IN WHICH THERE IS A DIRECT CONNECTION BETWEEN THE GENETIC ACTIVITY (GENE EXPRESSION), PROTEIN ACTIVITY (PROTEOME) AND THE METABOLIC ACTIVITY ITSELF.

METABONOMICS

THE QUANTITATIVE MEASUREMENT OF THE MULTIVARIATE METABOLIC RESPONSES OF MULTICELLULAR SYSTEMS TO PATHOPHYSIOLOGICAL STIMULI OR GENETIC MODIFICATION. AN APPROACH TO UNDERSTANDING GLOBAL METABOLIC REGULATION OF ORGANISM AND ITS COMMENSAL AND SYMBIOTIC PARTNERS.

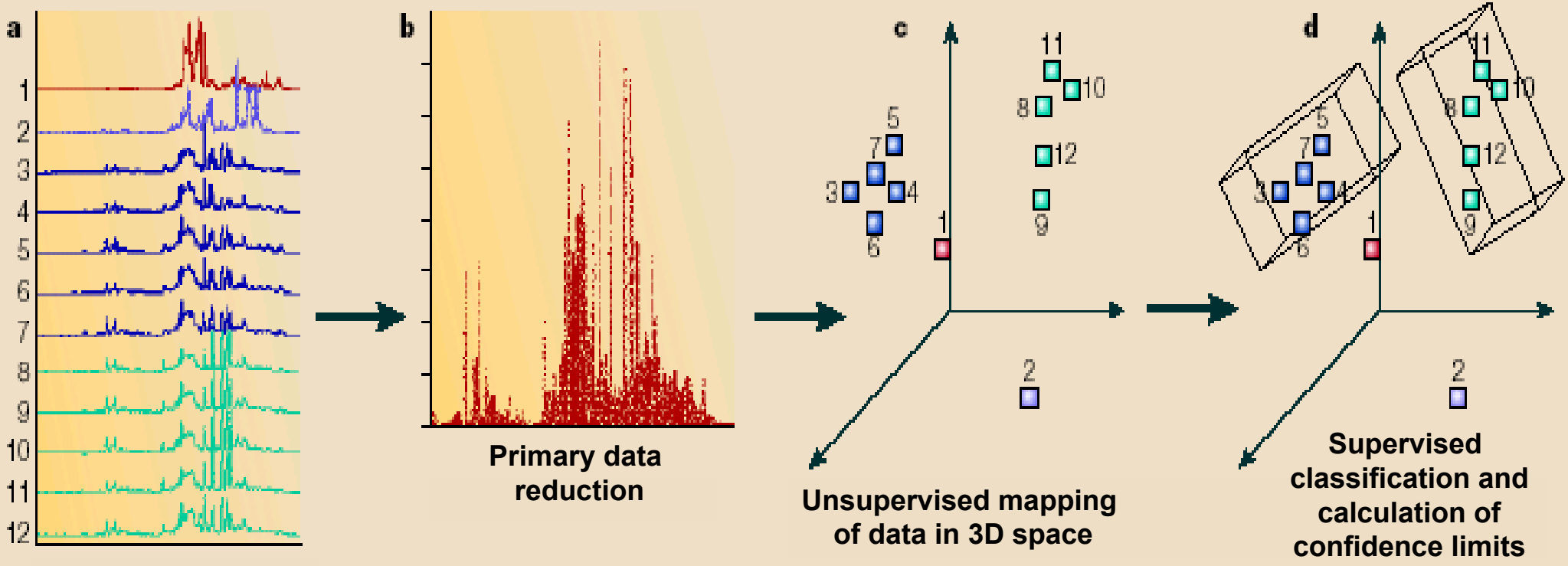
Ref.: Nicholson J. K. et al., 2002, *Nature Reviews* 1: 153-161.

METABONOMIC DETECTION OF LIVER AND KIDNEY TOXICITY



Ref.: Nicholson J. K. et al., 2002, *Nature Reviews* 1: 153-161.

IMPORTANT TECHNIQUES AND PROCEDURES IN METABONOMICS



Ref.: Nicholson J. K. et al., 2002, *Nature Reviews* 1: 153-161.

Conclusions

- LC-MS/MS has great potential for a clinical laboratory and as analytical engine for metabonomics (complementary to NMR)

- Chromatography is often necessary for these kind of experiments due to the large number of endogenous components present in biological fluids

- Data interpretation is an important part of the process and this maybe a “real bottleneck”

- Biomarkers may be identified by Exact Mass and MS/MS analysis together with NMR

- Rapid simple transfer of methodology from discovery to high throughput analysis using LC-MS/MS