ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Organic Acid Workshop Rome

22th October 2021

1. **Different methods and quantitative analysis of organic acids in urine-** Clothilde Roux (SA QTOU)
2. **Slight increase of some organic acids, difficulty in the diagnosis-** Judit García (SA QLOU Barcelona)
3. **New/newly identifiable disorders-** Camilla Scott (SA QLOU Sheffield)

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Clothilde Roux

Lausanne QTOU

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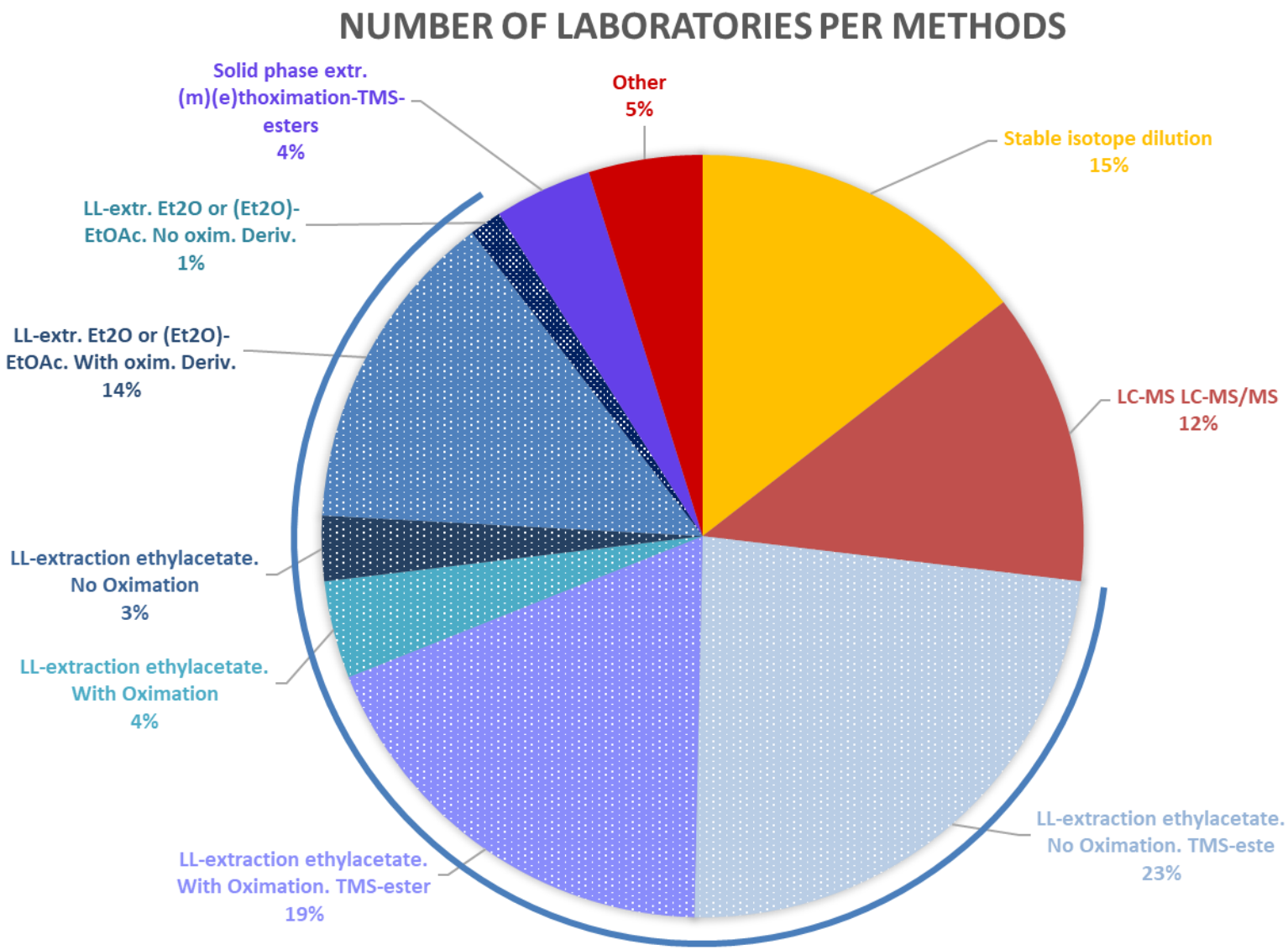
Organic acids workshop



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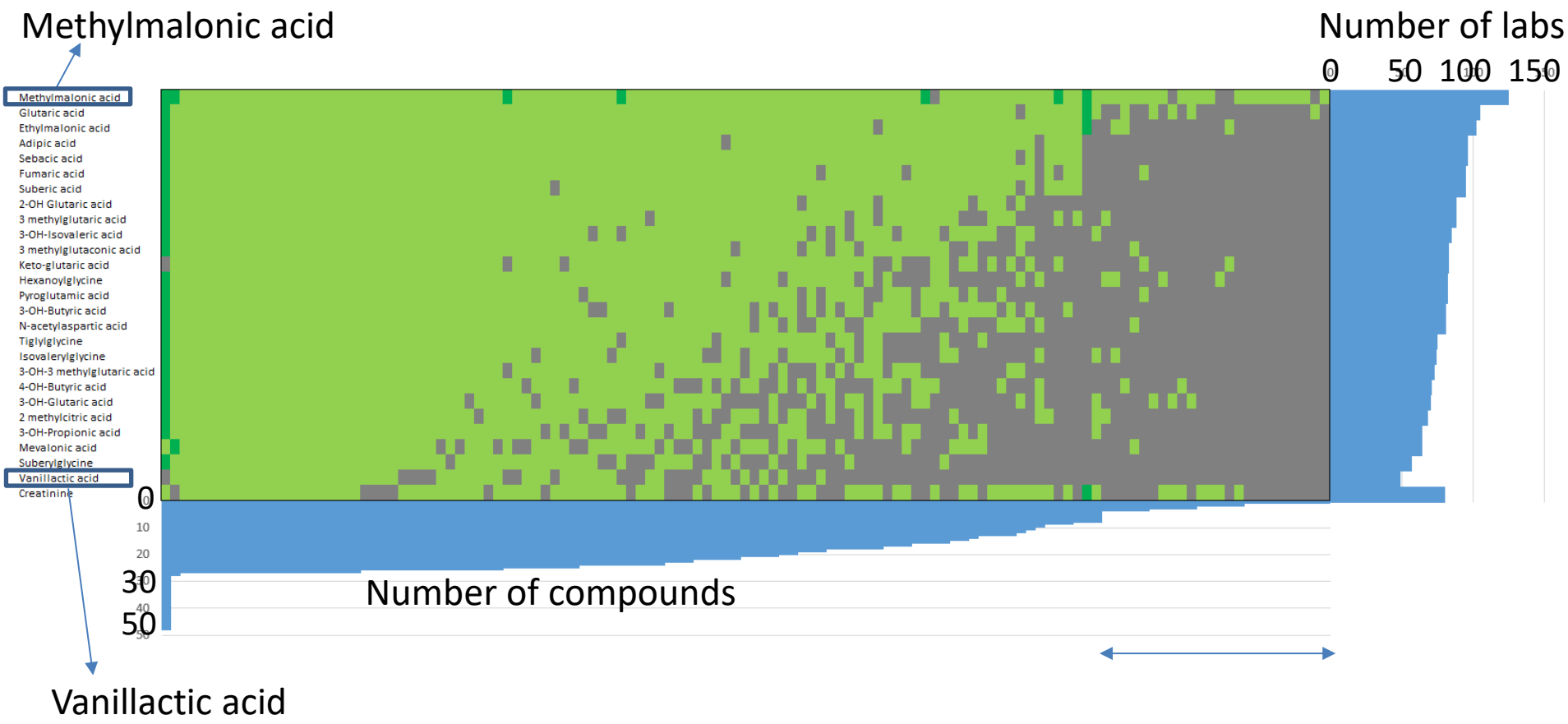
Organic acids workshop

Overview of laboratories participation



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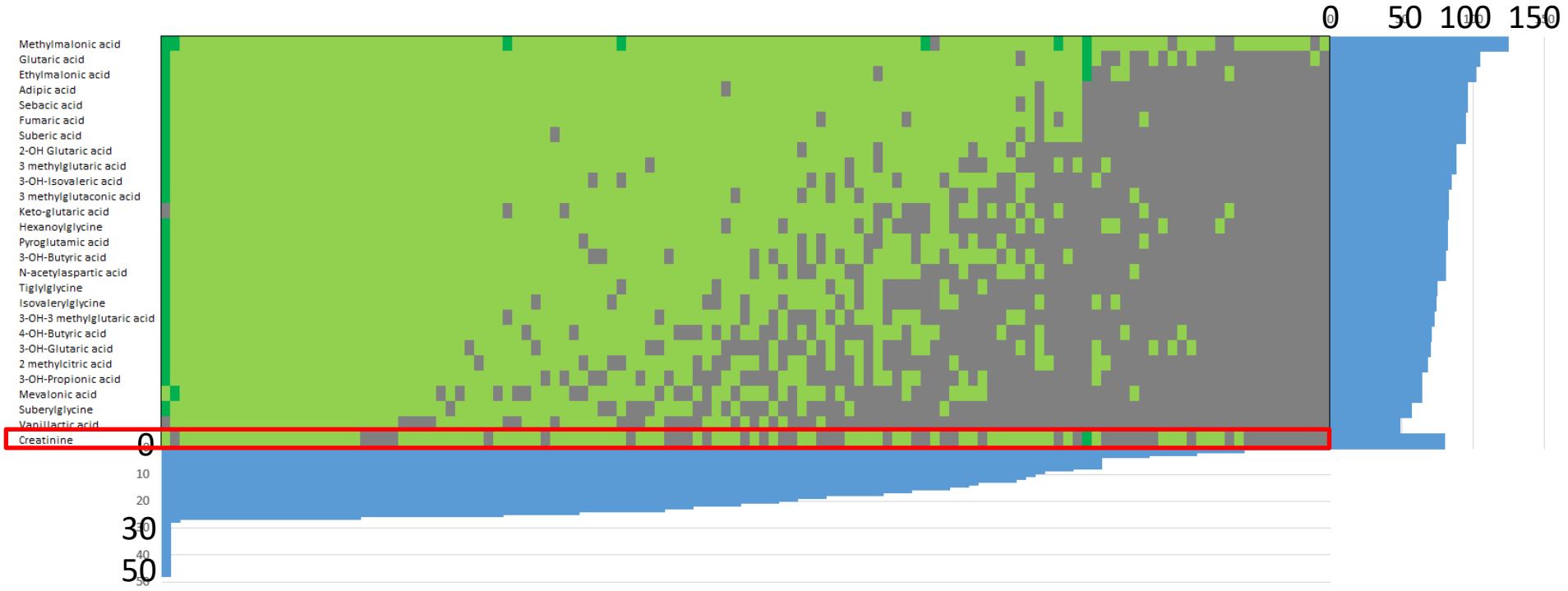
Organic acids workshop

Overview of laboratories participation



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Organic acids workshop

Overview of laboratories methods

Étiquettes de lignes	LL-extraction ethylacetate. No Oximation. TMS-este	LL-extraction ethylacetate. With Oximation. TMS-es	LL-ext. E2O or (E2O)-EtOAc. With oxim. TMS-este	LL-extraction ethylacetate. With Oximation	LL-ext. E2O or (E2O)-EtOAc. No oxim. TMS-esters	Solid phase extr. (m)ethoximation-TMS-esters	LC-MS - LC-MS/MS	Stable Isotope Dilution	LC-MS/MS Stable Isotope Dilution	LL-extraction ethylacetate. No Oximation	Other extraction. No oximation	Other extraction. With oximation. TMS	Oximation + TMS derivatisation	Urease , Derivatization of BSTFA	Other	Solid phase extr.	LL-ext. EtOAc. No oxim. TBDMS-esters.	LL-ext. EtOAc. With oxim. TBDMS-esters.	LL-ext. E2O or (E2O)-EtOAc. With oxim. Me-ester	Other extraction. With oximation	Total général
Methylmalonic acid	32	24	16	5	8	3	6	13	6	2	2	1	1	1	3	1	1				125
Glutaric acid	31	23	17	6	7	3	4	4	2	1	1	1	1	1	1	1	1				105
Ethylmalonic acid	31	21	18	6	5	5	3	3	1	2	1	1	1	1	1	1	1				102
Adipic acid	31	20	16	6	6	4	3	1	1	2	1	1	1	1		1	1				96
Sebacic acid	30	21	16	6	6	4	3		1	2	1	1	1	1	1	1	1				96
Fumaric acid	31	19	16	6	6	4	4	1		2	1	1	1	1	1		1				95
Suberic acid	30	20	16	6	6	4	3		1	2	1	1	1	1	1	1	1				95
2-OH Glutaric acid	28	18	16	5	4	3	3	2	1	2	1	1	1	1	1	1					88
3 methylglutaric acid	27	21	14	5	4	4	3	1	1	2	1	1	1		1	1	1				88
3-OH-Isovaleric acid	27	17	12	6	6	4	3	1	2	1	1	1	1	1	1	1					85
3 methylglutaconic acid	25	17	13	5	5	4	2	4	1	1	1	1	1	1	1	1					83
Keto-glutaric acid	21	20	15	6	4	3	4	1	1	1	1	1	1	1	1			1	1		83
Hexanoylglycine	26	18	11	5	3	4	4	5	1	1	1	1	1	1							82
Pyroglutamic acid	24	16	16	5	4	4	5			2	1	1	1	1	1	1					82
3-OH-Butyric acid	25	16	15	6	5	3	3			1	1	1	1	1	1	1	1				81
N-acetylaspartic acid	26	16	11	5	4	4	3	2	2	1	1	1	1	1	1	1	1				81
Creatinine	9	2	2	1	2	1	1		2		1				58					1	80
Tiglylglycine	25	16	10	5	4	4	3	2	1		1	1	1	1	1						75
Isovalerylglycine	25	13	9	5	5	4	3	4	2		1	1		1	1						74
3-OH-3 methylglutaric acid	21	16	12	4	4	4	3	1	1		1	1	1	1	1	1	1				73
4-OH-Butyric acid	22	16	13	3	4	4	1		1	2	1	1	1	1	1						71
3-OH-Glutaric acid	23	16	10	3	5	4	2	3	1		1	1	1								70
2 methylcitric acid	20	15	10	4	5	4	3	3	1		1	1		1							68
3-OH-Propionic acid	23	13	10	4	2	2	3		1		1	1	1	1	1	1					64
Mevalonic acid	18	12	8	4	4	3	1	7	2		1	1	1	1	1						64
Suberylglycine	20	14	7	3	1	3	3	3	1		1	1									57
Vanillactic acid	16	10	9	3	2	3	1		1			1	1	1	1						49
Total général	667	450	338	128	121	96	80	61	35	27	27	26	23	23	81	15	11	1	1	1	2212

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Organic acids workshop

Overview of laboratories methods

Étiquettes de lignes	LL-extraction ethylacetate. No Oximation. TMS-este	LL-extraction ethylacetate. With Oximation. TMS-es	LL-ext. E2O or (E2O)-EtOAc. With oxim. TMS-este	LL-extraction ethylacetate. With Oximation	LL-ext. E2O or (E2O)-EtOAc. No oxim. TMS-esters	Solid phase extr. (m)ethoximation-TMS-esters	LC-MS - LC-MS/MS	Stable Isotope Dilution	LC-MS/MS Stable Isotope Dilution	LL-extraction ethylacetate. No Oximation	Other extraction. No oximation	Other extraction. With oximation. TMS	Oximation + TMS derivatisation	Urease , Derivatization of BSTFA	Other	Solid phase extr.	LL-ext. EtOAc. No oxim. TBDMS-esters.	LL-ext. EtOAc. With oxim. TBDMS-esters.	LL-ext. E2O or (E2O)-EtOAc. With oxim. Me-ester	Other extraction. With oximation	Total général
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Keto-glutaric acid	21	20	15	6	4	3	4	1	1	1	1	1	1	1	1			1	1		83
Hexanoylglycine	26	18	11	5	3	4	4	5	1	1	1	1	1	1							82
Pyroglutamic acid	24	16	16	5	4	4	5			2	1	1	1	1	1	1					82
3-OH-Butyric acid	25	16	15	6	5	3	3			1	1	1	1	1	1	1	1				81
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2 methylcitric acid	20	15	10	4	5	4	3	3	1		1	1		1							68
3-OH-Propionic acid	23	13	10	4	2	2	3		1		1	1	1	1	1	1					64
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Suberylglycine	20	14	7	3	1	3	3	3	1		1	1									57
Vanillactic acid	16	10	9	3	2	3	1		1			1	1	1	1						49
Total général	667	450	338	128	121	96	80	61	35	27	27	26	23	23	81	15	11	1	1	1	2212

Organic acids workshop

ERNDIMQA - Annual report

ERNDIMQA - ANNUAL REPORT
Quant.org.acids 2020
ERN [redacted]

Methodset :

	Accuracy (mean)		Precision (CV% duplicates)		Linearity (r)		Recovery (%added analyte)		Data All Labs	
Analyte	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	Your Lab	All Labs	n	Interlab CV
2 methylcitric acid	25.2	26.2	8.6%	26.7%	0.997	0.981	77%	78%	68	74.8%
2-OH Glutaric acid	84.1	68.0	6.5%	13.0%	0.996	0.987	129%	102%	88	36.0%
3 methylglutaconic acid	83.4	92.3	3.9%	14.7%	0.999	0.992	95%	101%	83	35.5%
3 methylglutaric acid	47.0	48.6	7.7%	11.2%	0.998	0.992	100%	100%	88	24.6%
3-OH-3 methylglutaric acid	112	93.6	10.3%	25.7%	0.996	0.961	121%	96%	73	47.5%
3-OH-Butyric acid	143	167	3.3%	16.2%	0.993	0.991	75%	97%	81	47.5%
3-OH-Glutaric acid	11.1	9.44	17.1%	27.5%	0.934	0.881	123%	70%	70	85.2%
3-OH-Isovaleric acid	51.0	60.7	7.6%	31.2%	0.991	0.965	80%	95%	85	63.5%
3-OH-Propionic acid	144	144	3.0%	22.6%	0.998	0.990	99%	94%	64	88.0%
4-OH-Butyric acid	89.9	102	11.1%	20.1%	0.996	0.981	78%	90%	71	52.0%
Adipic acid	44.4	49.0	4.0%	13.8%	0.999	0.986	92%	100%	96	23.3%
Ethylmalonic acid	58.8	77.3	4.6%	15.2%	0.998	0.987	74%	101%	102	30.7%
Fumaric acid	56.4	57.0	15.3%	15.2%	0.996	0.995	91%	92%	95	37.5%
Glutaric acid	95.4	101	8.1%	10.0%	0.998	0.994	97%	103%	105	24.8%
Hexanoylglycine	6.12	7.11	17.3%	22.1%	0.992	0.980	60%	67%	82	49.8%
Isovalerylglycine	19.2	20.0	11.9%	22.9%	0.993	0.964	83%	81%	74	43.9%
Keto-glutaric acid	173	160	11.5%	20.0%	0.996	0.991	112%	99%	83	49.8%
Methylmalonic acid	53.8	52.9	23.1%	11.4%	0.993	0.998	106%	98%	125	28.9%
Mevalonic acid	14.5	15.0	19.1%	27.2%	0.991	0.976	75%	82%	64	52.9%
N-acetylaspartic acid	55.1	48.1	5.7%	27.8%	0.997	0.973	93%	78%	81	68.1%
Pyroglutamic acid	217	182	3.8%	19.0%	0.999	0.988	115%	86%	82	38.7%
Sebacic acid	46.8	65.8	3.7%	14.7%	0.998	0.992	74%	101%	96	40.6%
Suberic acid	30.2	35.0	4.4%	12.9%	0.998	0.989	90%	99%	95	28.1%
Suberylglycine	70.1	78.6	8.1%	38.8%	0.994	0.960	93%	102%	57	109%
Tiglylglycine	68.1	71.6	13.1%	16.1%	0.993	0.990	93%	94%	75	44.3%
Vanillactic acid	10.1	12.2	3.6%	17.8%	0.999	0.992	74%	89%	49	46.9%
Overall	69.6	71.0	9.1%	19.8%	0.994	0.980	92%	92%	82	48.9%

Organic acids workshop

ERNDIMQA - Annual report

Interlab CV% > 50% for:

- 2-Methylcitric acid
- 3-Hydroxyglutaric acid
- 3-Hydroxypropionic acid
- 3-Hydroxyisovaleric acid
- 4-Hydroxybutyric acid
- Mevalonic acid
- N-Acetylaspartic acid
- Suberylglycine

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3-OH-Propionic acid	144	144	3.0%	22.6%	0.998	0.990	99%	94%	64	88.0%
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Tiglylglycine	68.1	71.6	13.1%	16.1%	0.993	0.990	93%	94%	75	44.3%
Vanillactic acid	10.1	12.2	3.6%	17.8%	0.999	0.992	74%	89%	49	46.9%
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Organic acids workshop

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- Mevalonic acid
- N-Acetylaspartic acid
- Suberylglycine

3-Hydroxyglutaric acid

- glutaric aciduria type I (with glutaconic and glutaric acids)
- glutaric aciduria type I (low excretors)
- short chain 3-hydroxyacyl-coA deshydrogenase (SCHAD) (22-114 with normal glutarate)

Low excretors!

Glutaryl-coA Deshydrogenase deficiency (GCDH)

Classic form :	**Low excretors** :
hight excretion of Glutaric acid and 3-Hydroxyglutaric	normal or slight increased excretion of Glutaric acid and 3-Hydroxyglutaric slightly higher than controls
Mutations : A293T R402W	Mutations : A293T (heterozygosity only) R402W (heterozygosity only) V400M R227P
Glutarate > 3-Hydroxyglutarate	3-Hydroxyglutarate >Glutarate
Controls: median of 3 (2-10) for glutarate and 7 (2-14) for 3-Hydroxyglutarate	Controls: median of 3 (2-10) for glutarate and 7 (2-14) for 3-Hydroxyglutarate
Pathologicals values: Median of 2817 (204-8955) for glutaric acid and 420 (75-3573) for 3-Hydroxyglutaric acid	Pathologicals values: Median of 10 (2-84) for glutaric acid and 101 (16-571) for 3-Hydroxyglutaric acid

Busquets et al., Pediatric Research, 2000: 48: 315-322

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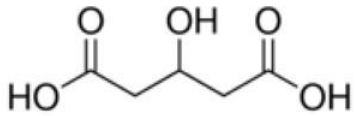
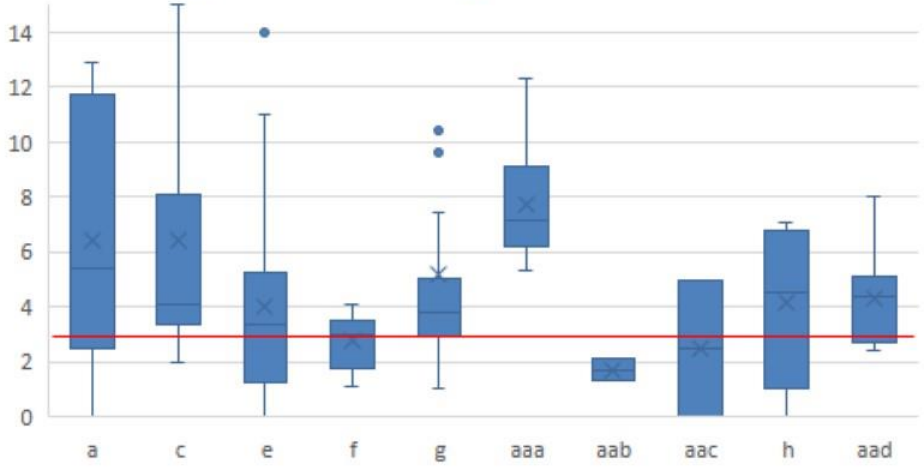
Organic acids workshop



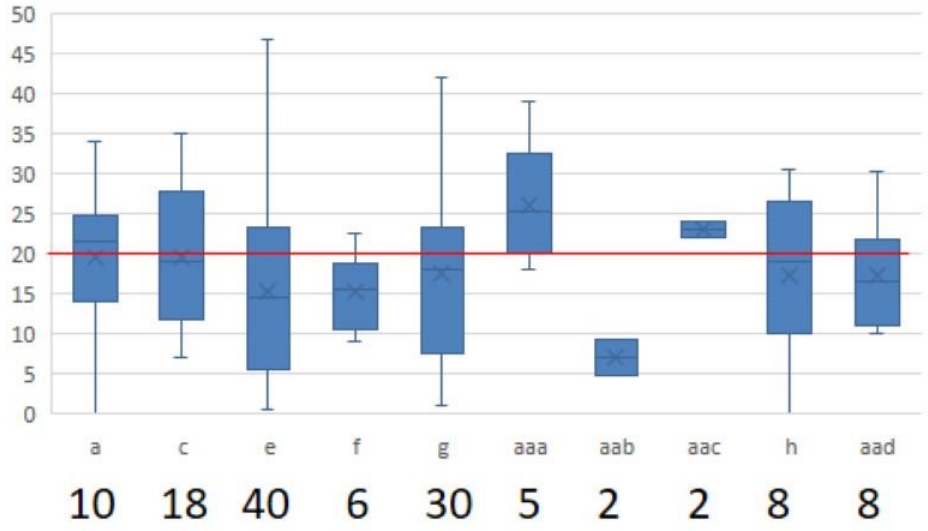
~ Controls value

~ Low excretors value

3-OH-glutarate 3.0 µmol/l - 2 scheme



3-OH-glutarate 20.0 µmol/l – 2 scheme



a	LL-extr. Et2O or (Et2O)-EtOAc. No oxim. TMS-esters
c	LL-extr. Et2O or (Et2O)-EtOAc. With oxim. TMS-este
e	LL-extraction ethylacetate. No Oximation. TMS-este
g	LL-extraction ethylacetate. With Oximation. TMS-es
d	LL-extraction ethylacetate. No Oximation
f	LL-extraction ethylacetate. With Oximation
h	Solid phase extr. (m)(e)thoximation-TMS-esters
aaa	LC-MS
aab	Other extraction. No oximation
aac	Urease+ Derivatization of BSTFA
aad	Stable Isotope Dilution

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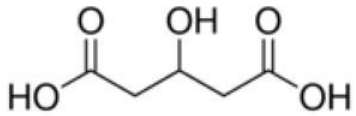

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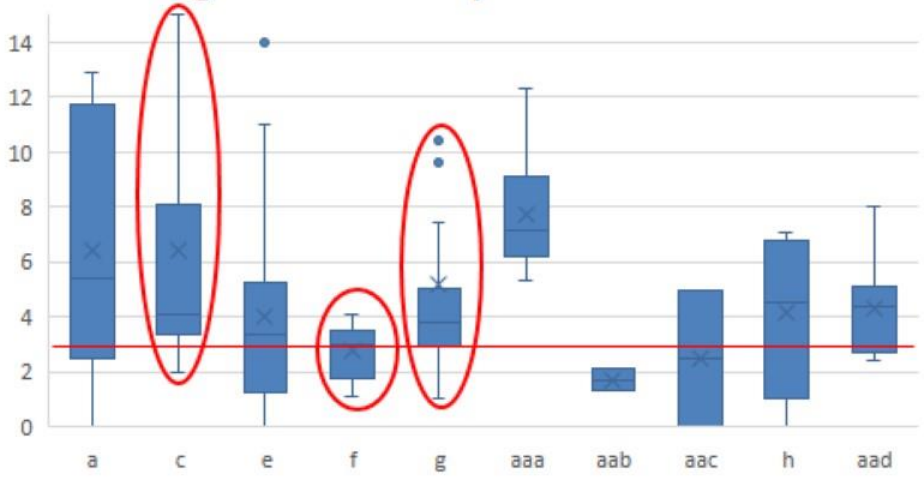
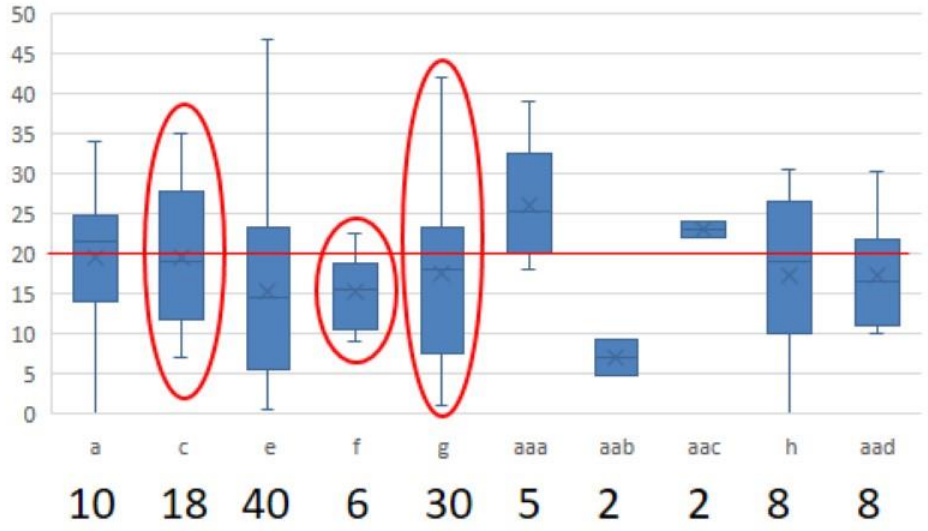


3-OH-glutarate 20.0 µmol/l – 2 scheme



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g	LL-extraction ethylacetate. With Oximation. TMS-es
d	LL-extraction ethylacetate. No Oximation
f	LL-extraction ethylacetate. With Oximation
h	Solid phase extr. (m)(e)thoximation-TMS-esters
aaa	LC-MS
aab	Other extraction. No oximation
aac	Urease+ Derivatization of BSTFA
aad	Stable Isotope Dilution

ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

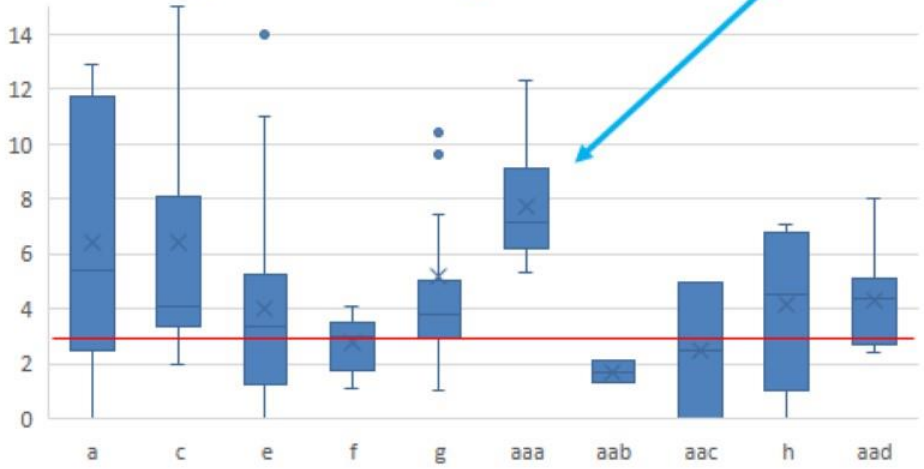
Organic acids workshop

LC-MS

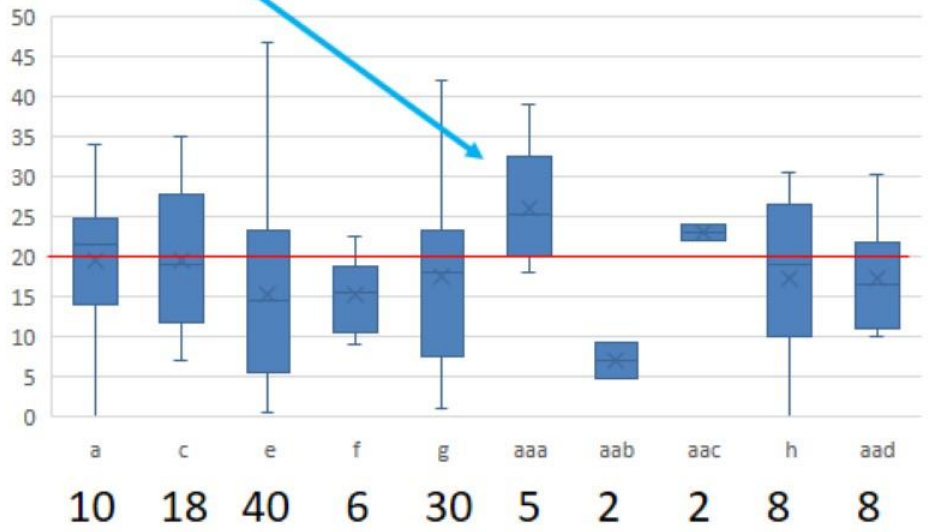
Controls value

Low excretors value

3-OH-glutarate 3.0 µmol/l - 2 scheme



3-OH-glutarate 20.0 µmol/l – 2 scheme



a	LL-extr. Et2O or (Et2O)-EtOAc. No oxim. TMS-esters
c	LL-extr. Et2O or (Et2O)-EtOAc. With oxim. TMS-este
e	LL-extraction ethylacetate. No Oximation. TMS-este
g	LL-extraction ethylacetate. With Oximation. TMS-es
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ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

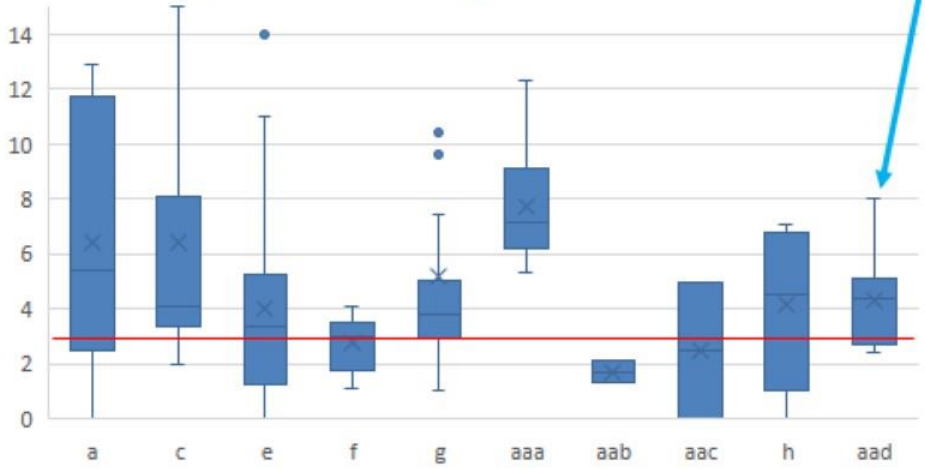
Organic acids workshop

SI dilution

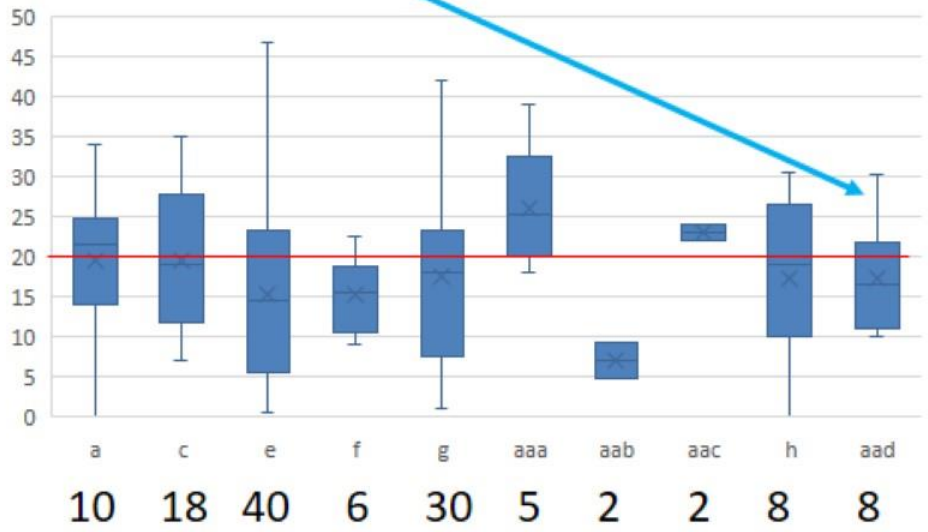
Controls value

Low excretors value

3-OH-glutarate 3.0 µmol/l - 2 scheme



3-OH-glutarate 20.0 µmol/l – 2 scheme



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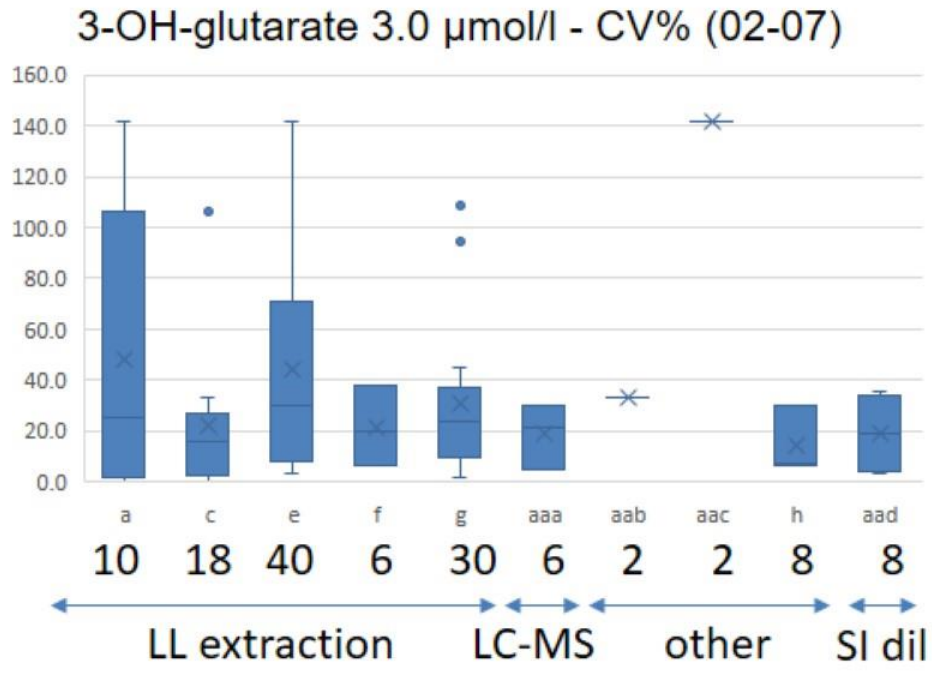
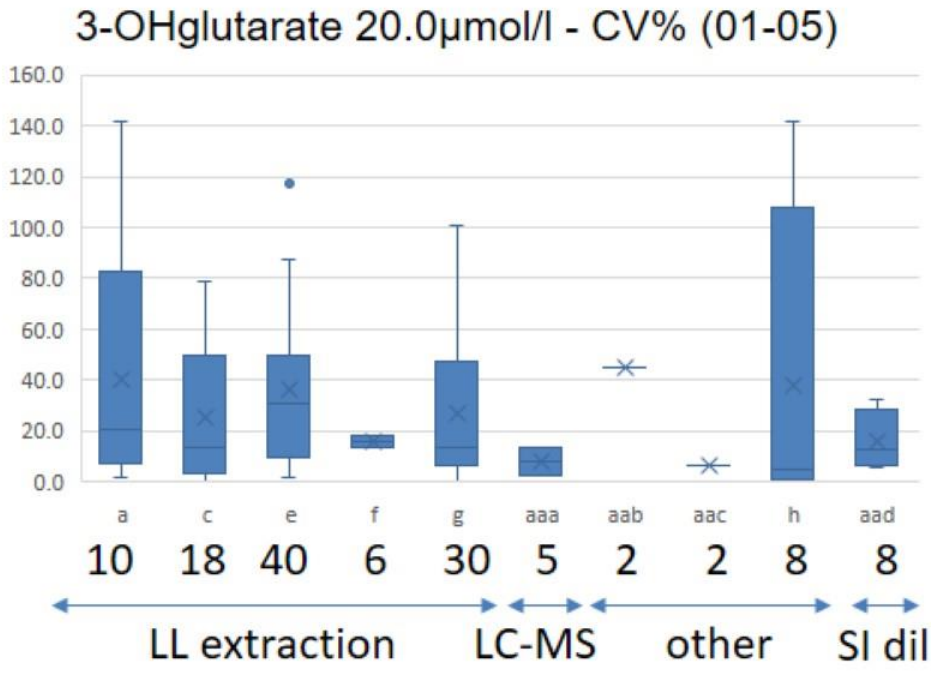
Organic acids workshop

CV %age





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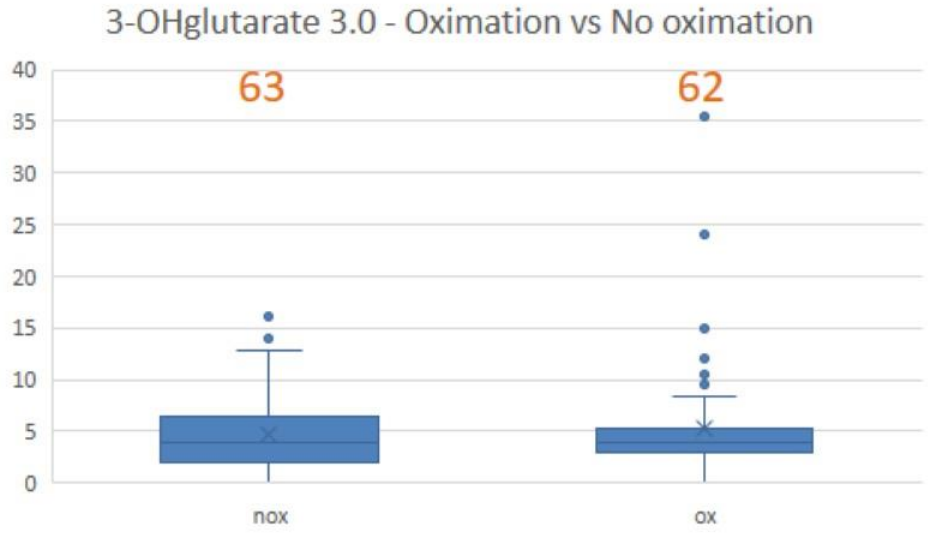
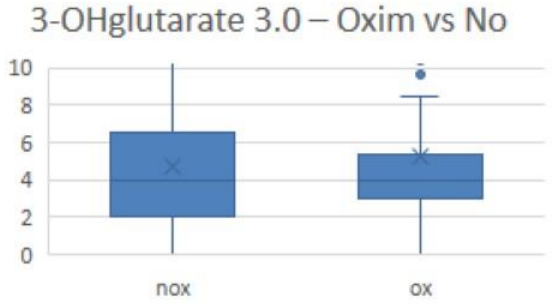
Organic acids workshop

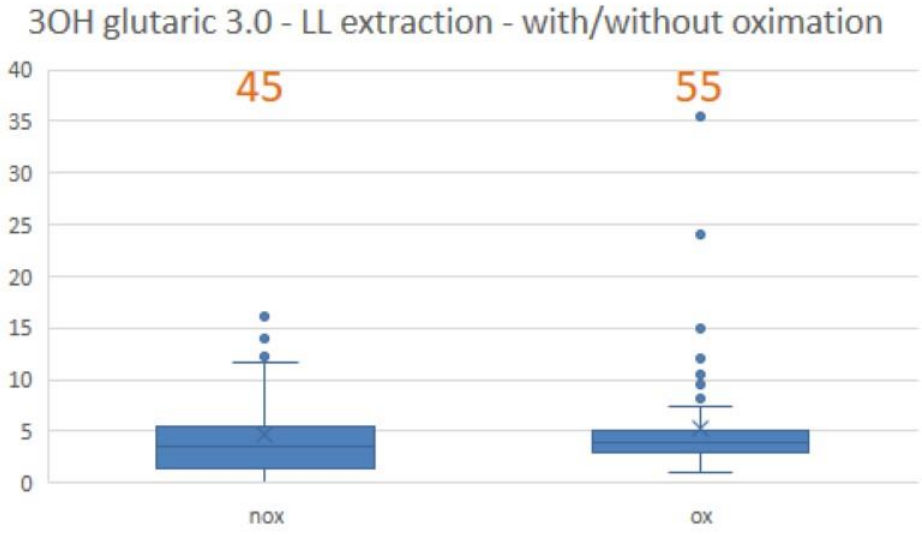
Oximation :

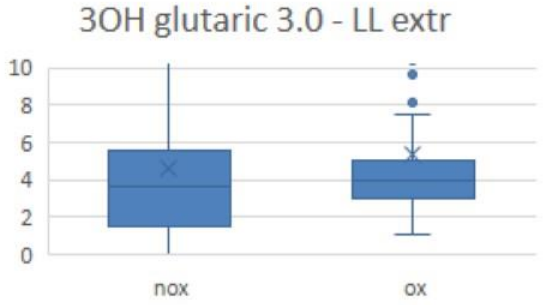
With or without oximation for all methods





With or without oximation for LL extraction with ethylacetate or diethyl-ether





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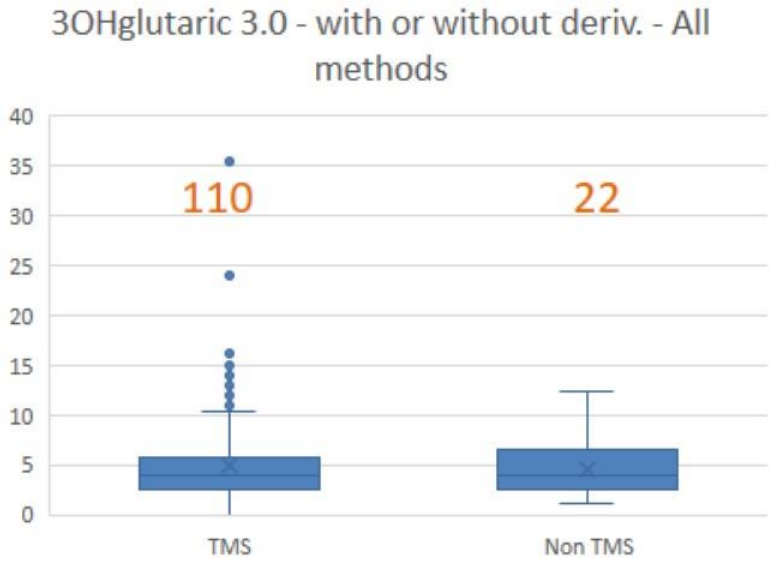
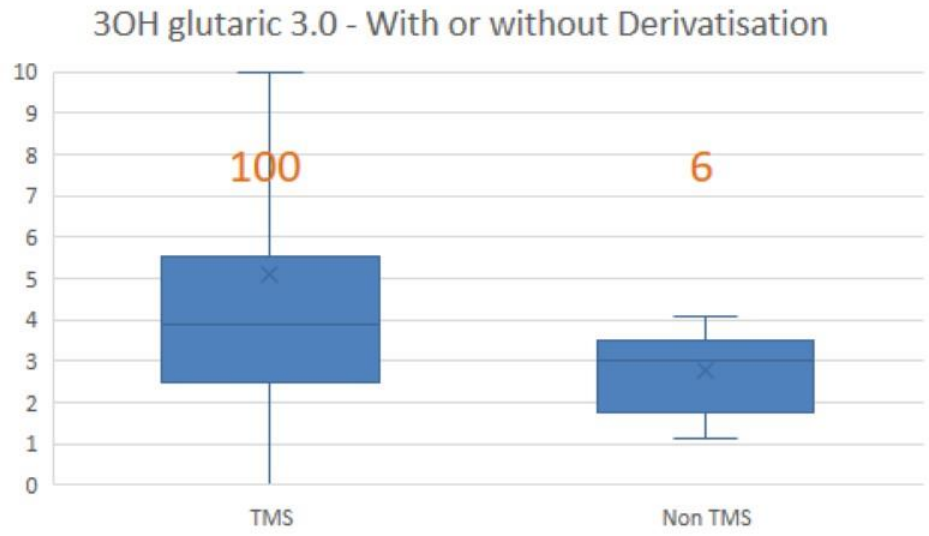
Organic acids workshop

Silylation :

With or without TMS for all methods



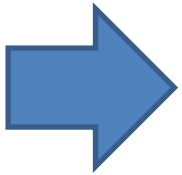
With or without TMS for LL extraction with ethylacetate or diethyl-ether



Organic acids workshop

Interlab CV% > 50% for:

- 2-Methylcitric acid
- 3-Hydroxyglutaric acid
- 3-Hydroxypropionic acid
- 3-Hydroxyisovaleric acid
- 4-Hydroxybutyric acid
- Mevalonic acid
- N-Acetylaspartic acid
- Suberylglycine



Mevalonic acid

Hyper IgD Syndrome !

Mevalonic aciduria

Classic form

Mevalonate kinase deficiency:

- Autosomal recessive disorder
- Mutation of the MVK gene
- Varying degrees of clinical severity

- Autoinflammatory syndrome
- Gastrointestinal complaints
- Recurrent episodes of fever
- Lymphadenopathy
- Arthralgia
- Myalgia
- Dysmorphic features
- Severe failure to thrive
- Developmental delay
- Hepatosplenomegaly
- Hypotonia

- Massive quantities of MVA in urine

Mild form

Hyper IgD Syndrome (HIDS):

- Autosomal recessive disorder
- Mutation in MVK gene (different from those reporting for MKD

- Autoinflammatory syndrome
- Gastrointestinal complaints
- Recurrent episodes of fever
- Lymphadenopathy
- Arthralgia
- Myalgia
- Absence of dysmorphic features

- **During the recurrent crisis**:
- Elevated level of Ig D
- Slight increase (median : 11 mmol/mol of CRT) excretion of MVA in urine
- Increased inflammatory markers

Jeyaratnam et al., JIMD Reports, 2016; 27: 33-38

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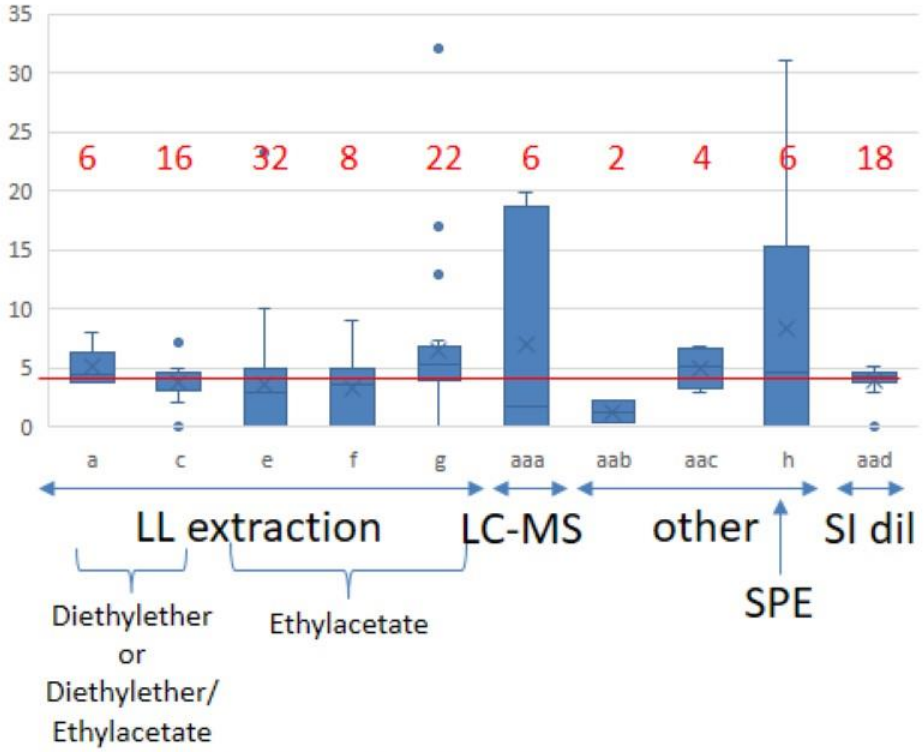
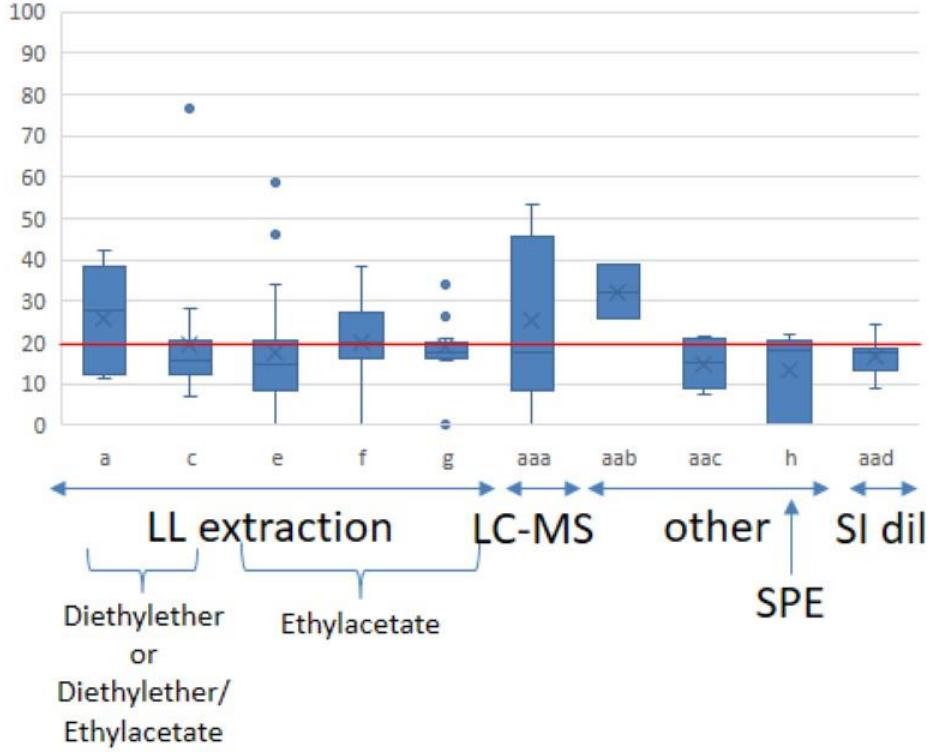
Organic acids workshop

Mevalonic acid 5.0 µmol/l - 2 Surveys (02 & 07)



Mevalonic acid 20 µmol/l - 2 Survey (04 & 06)



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g	LL-extraction ethylacetate. With Oximation. TMS-es
d	LL-extraction ethylacetate. No Oximation
f	LL-extraction ethylacetate. With Oximation
h	Solid phase extr. (m)(e)thoximation-TMS-esters
aaa	LC-MS
aab	Other extraction. No oximation
aac	Urease+ Derivatization of BSTFA
aad	Stable Isotope Dilution

Mevalonic extraction 0.152µmol (2nd point of our calibration curve)

Column: HP-5 MS, 60 m × 0.25 mm ID, 0.25 µm df
without oximation and TMS ester derivatization

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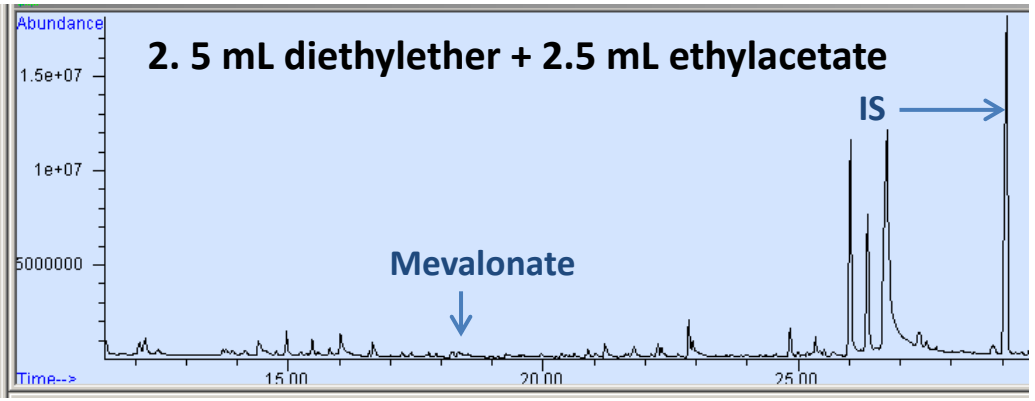
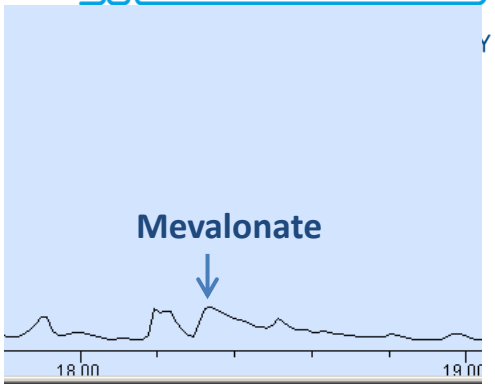
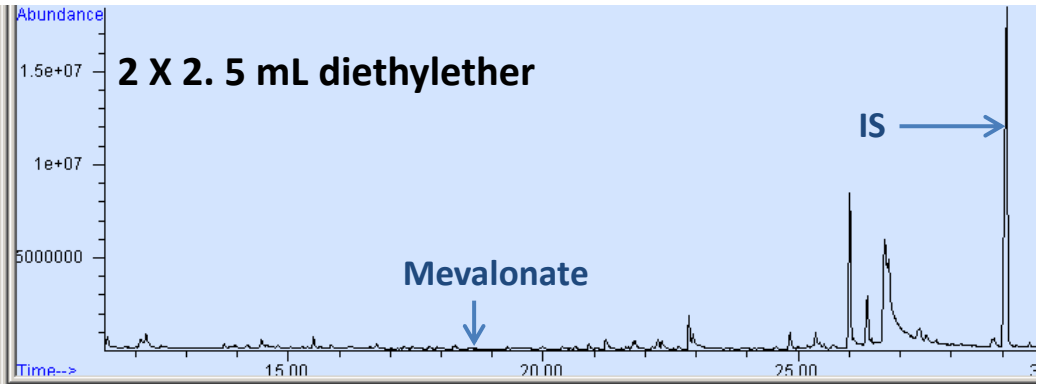
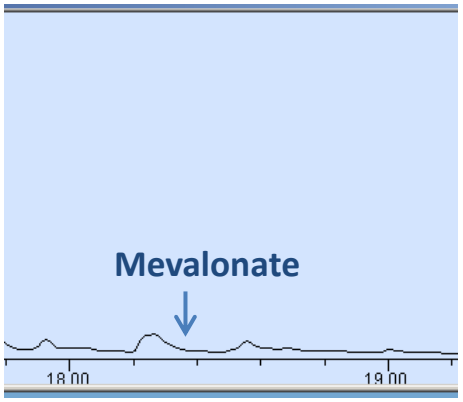
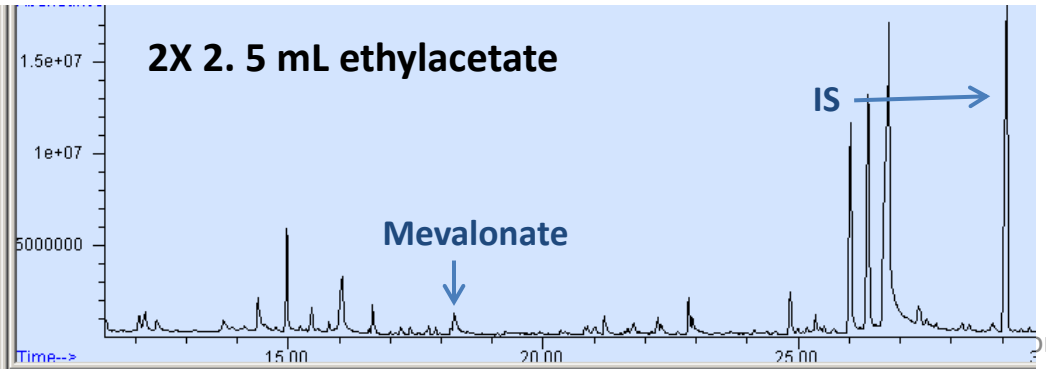
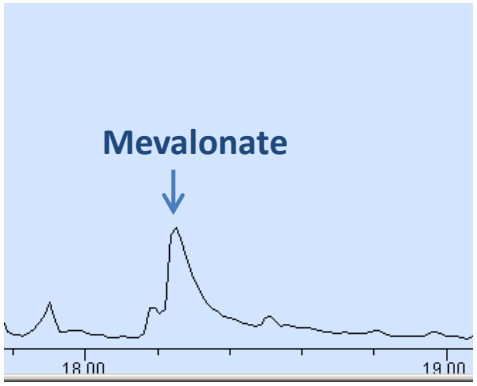






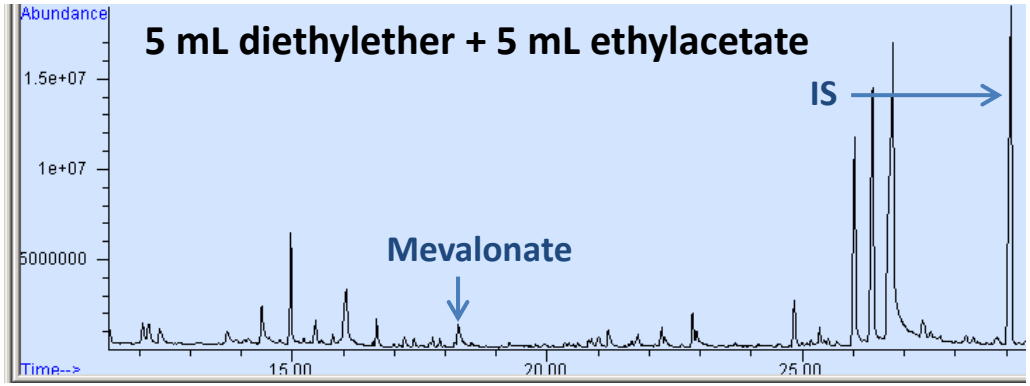


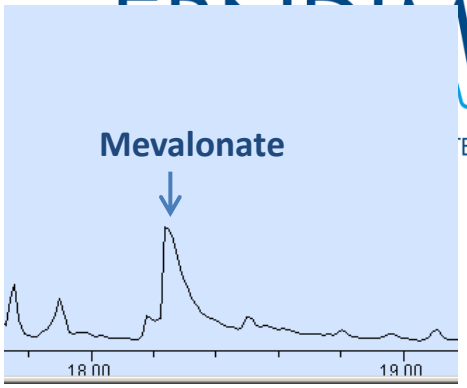


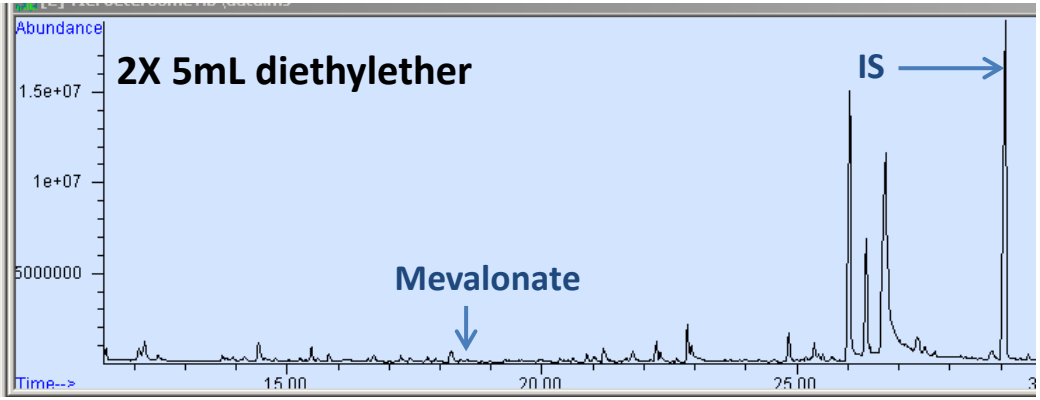


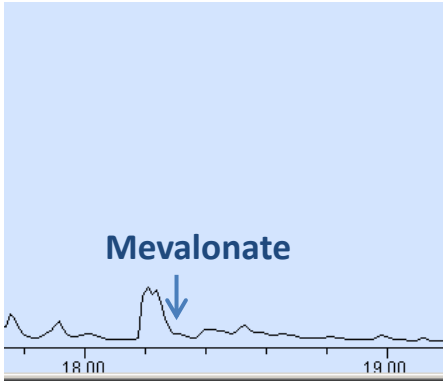
Mevalonic extraction 0.152µmol (2nd point of our calibration curve)

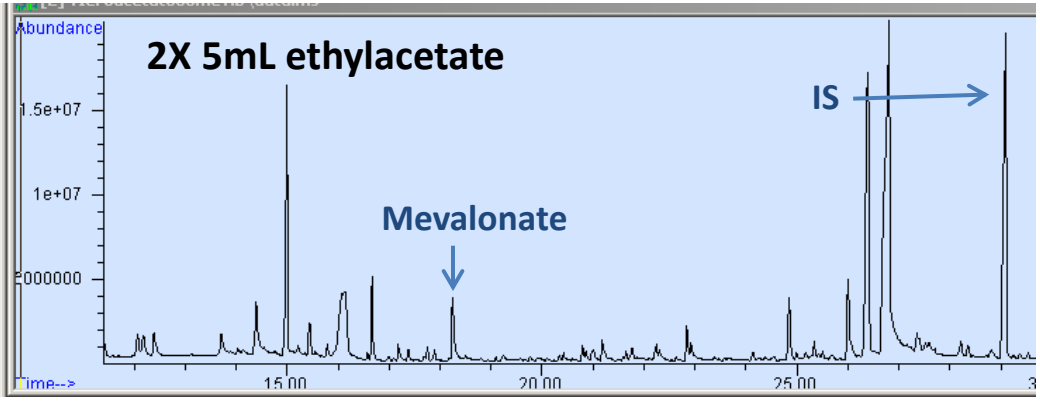
Column: HP-5 MS, 60 m × 0.25 mm ID, 0.25 µm df

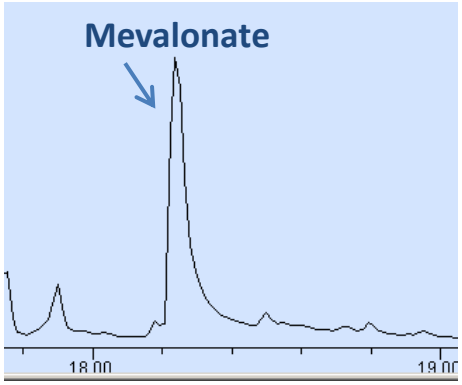












Mevalonic extraction 0.152µmol (2nd point of our calibration curve)

Column: HP-5 MS, 60 m × 0.25 mm ID, 0.25 µm df
without oximation and TMS ester derivatization

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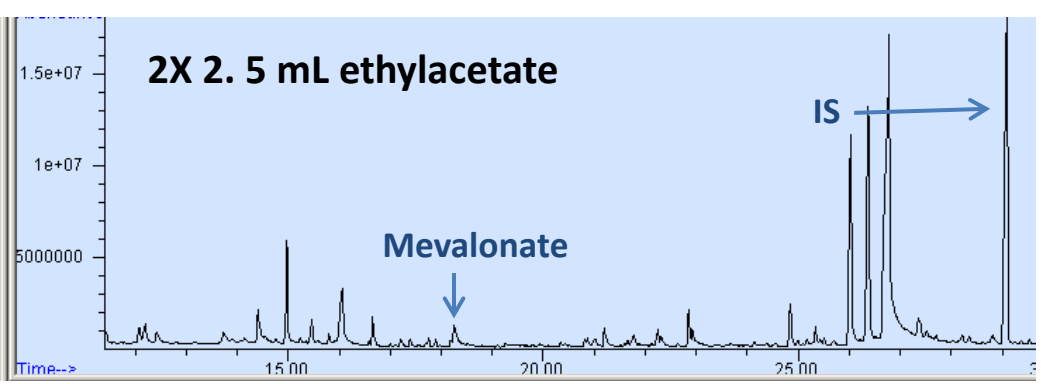

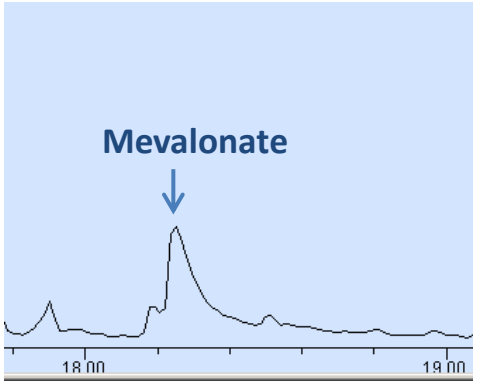

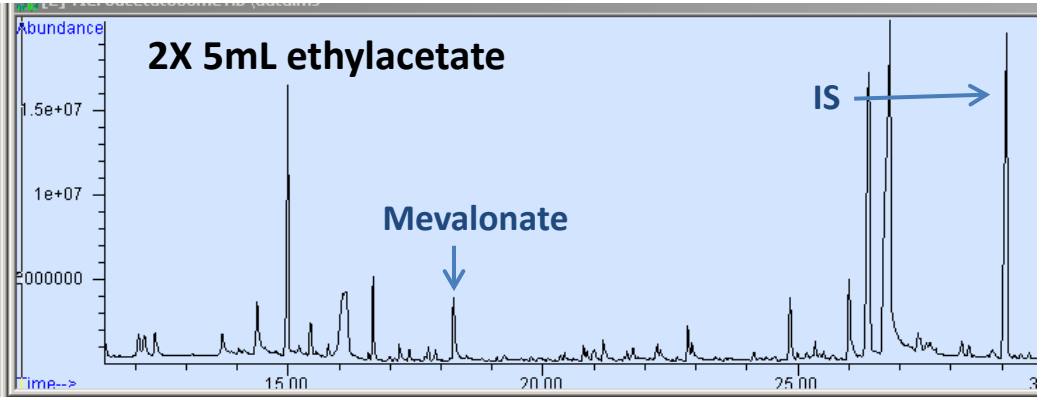

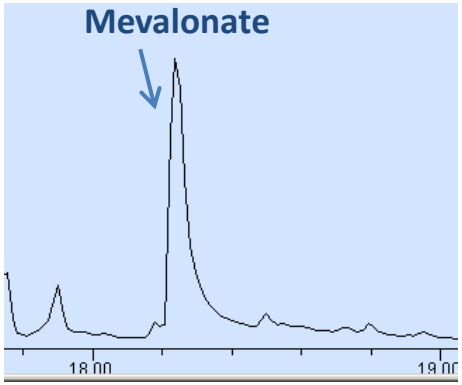

The best

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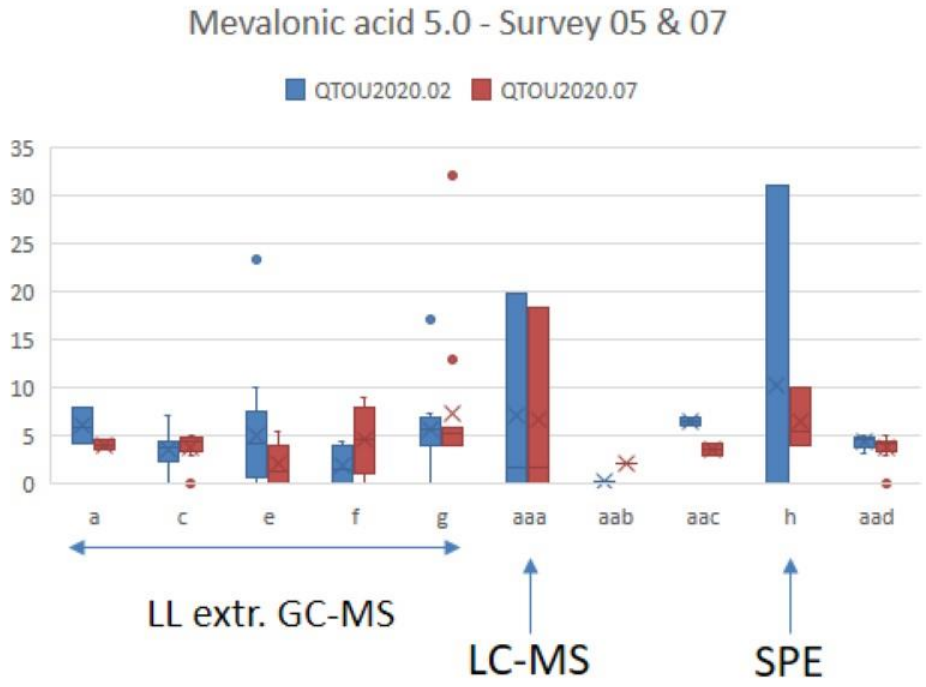
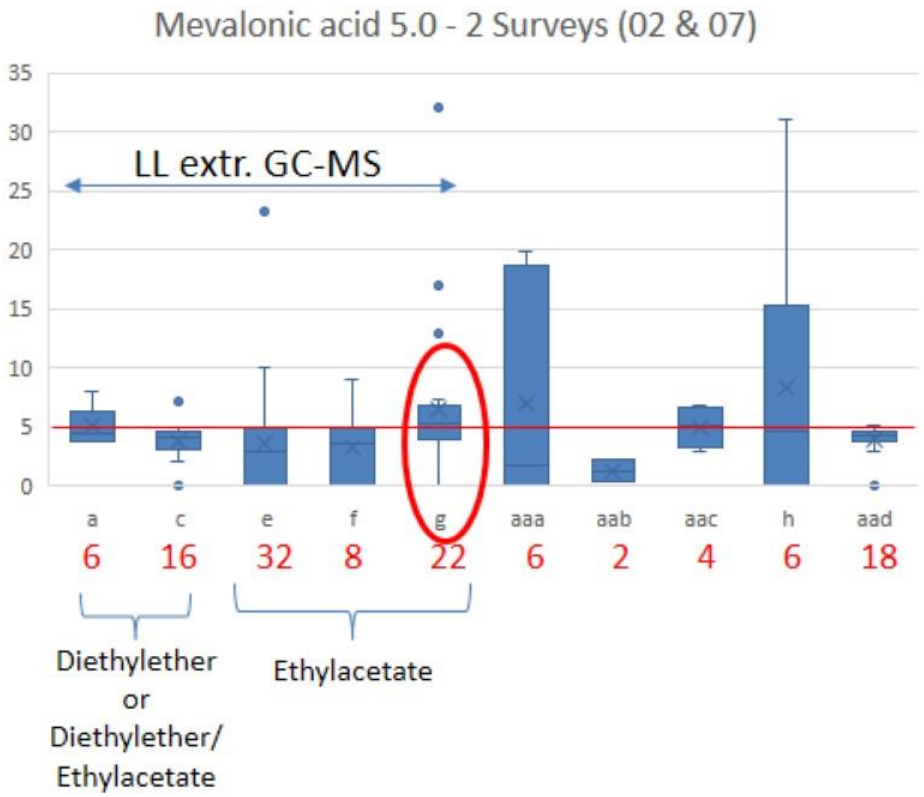
Organic acids workshop





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e	LL-extraction ethylacetate. No Oximation. TMS-este
g	LL-extraction ethylacetate. With Oximation. TMS-es
d	LL-extraction ethylacetate. No Oximation
f	LL-extraction ethylacetate. With Oximation
h	Solid phase extr. (m)(e)thoximation-TMS-esters
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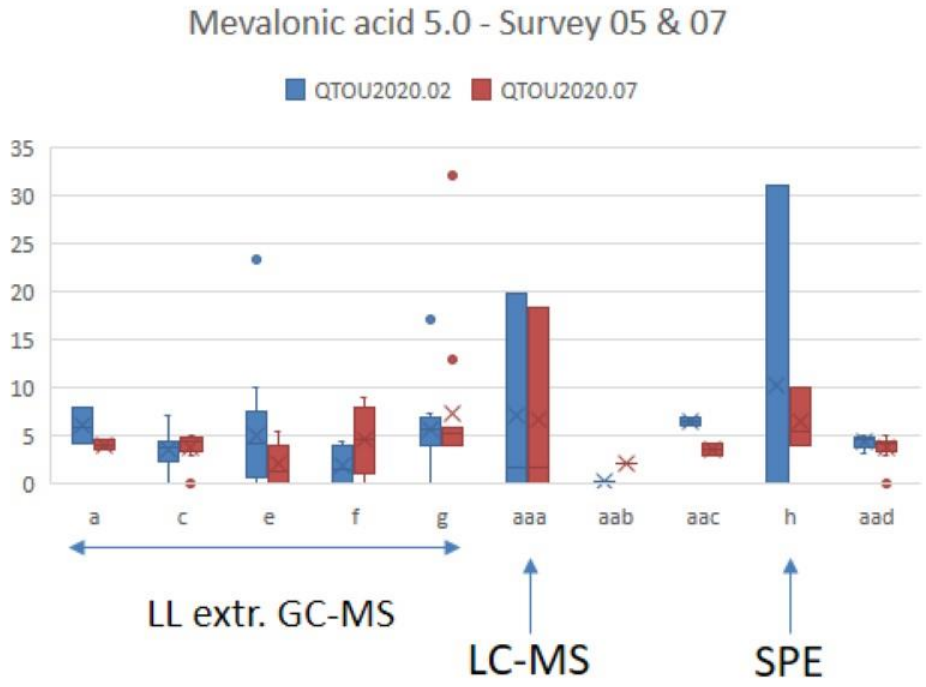
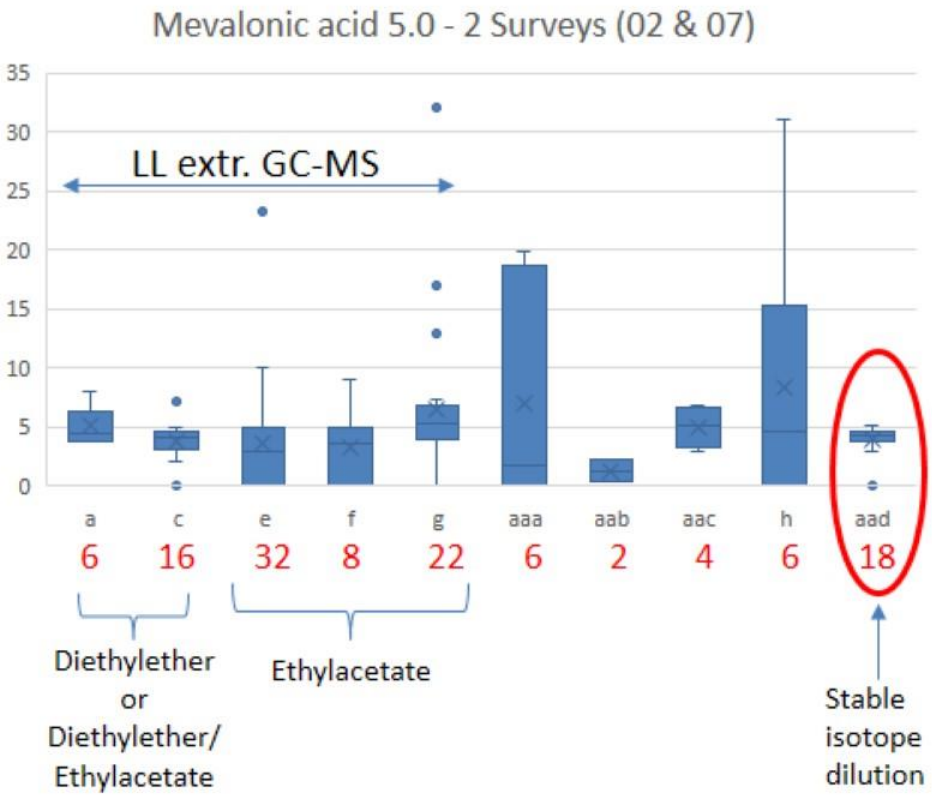
Organic acids workshop





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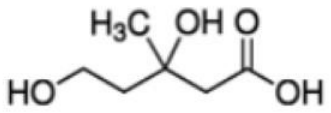
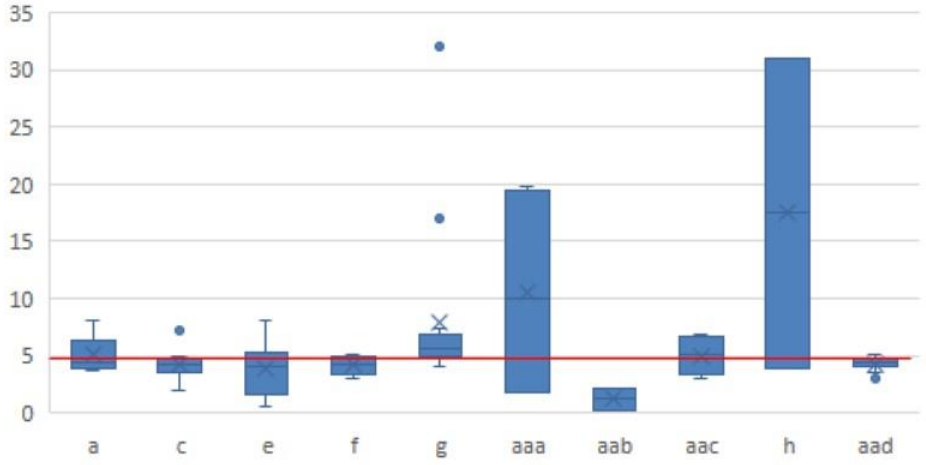
Organic acids workshop

Only 60 labs / 129 measure mevalonic acid:



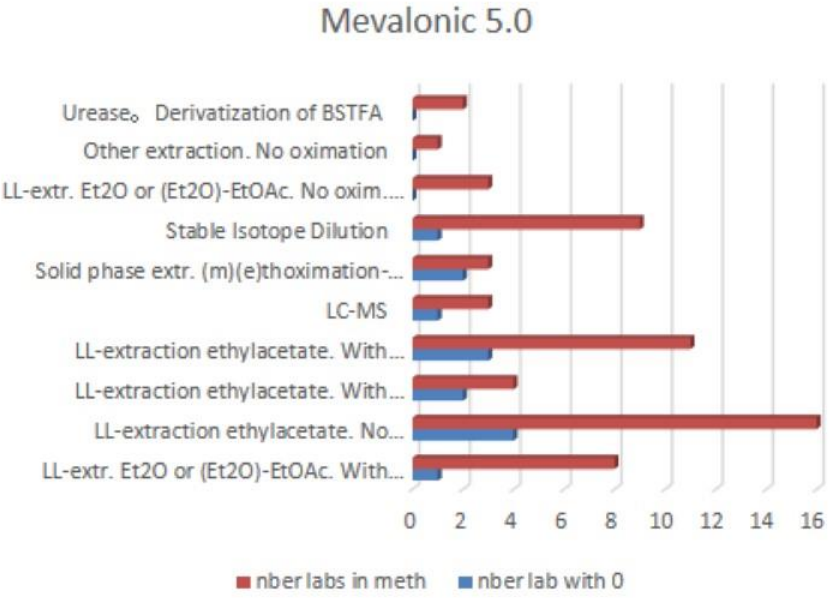
Mevalonic acid 5.0 - 2 surveys 02-07



6 14 20 4 16 4 2 4 2 16

Results of 44 labs (only the one without 0)

14 labs give 0 instead of 5.0 µmol/l for at least one survey on two :



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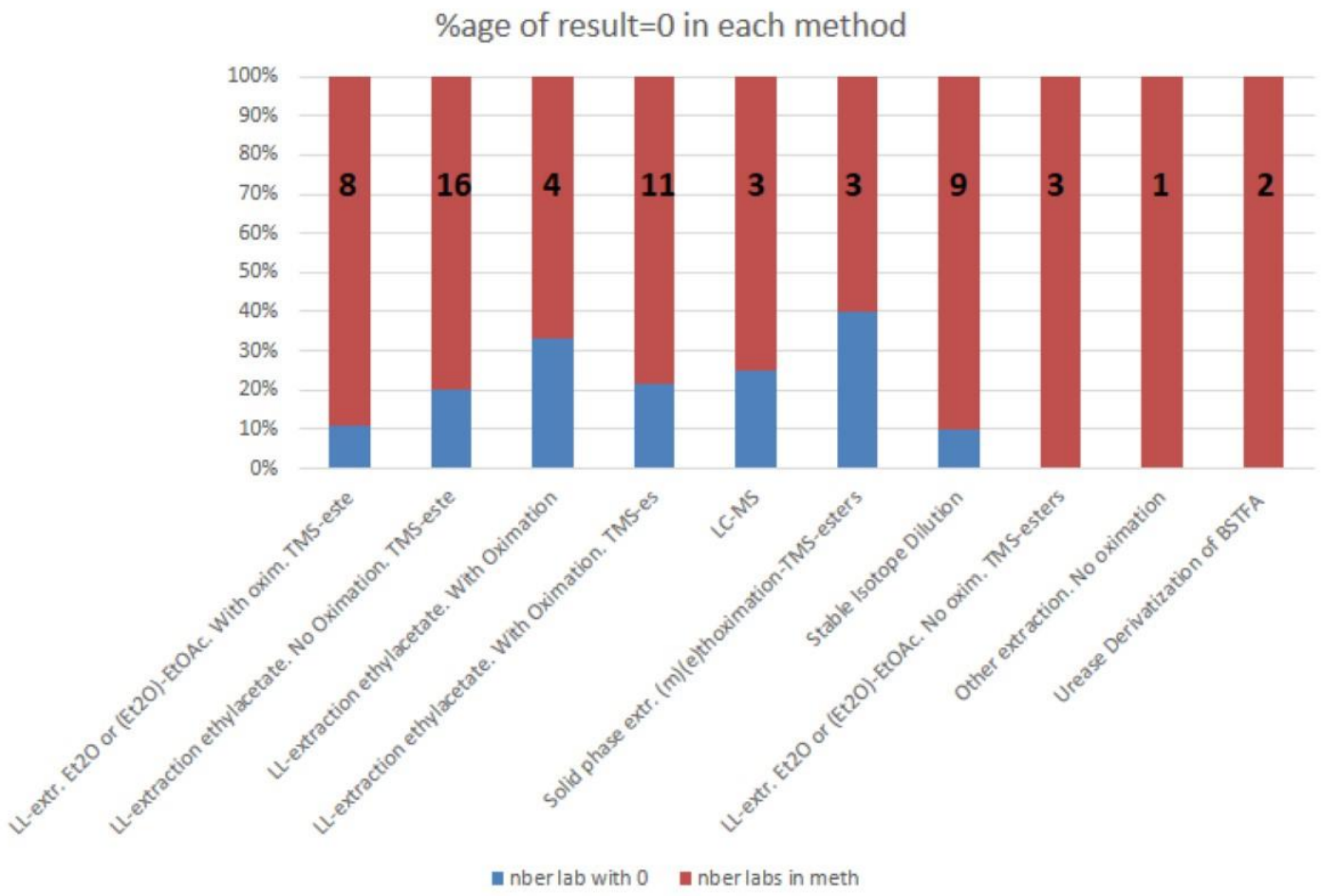
Organic acids workshop



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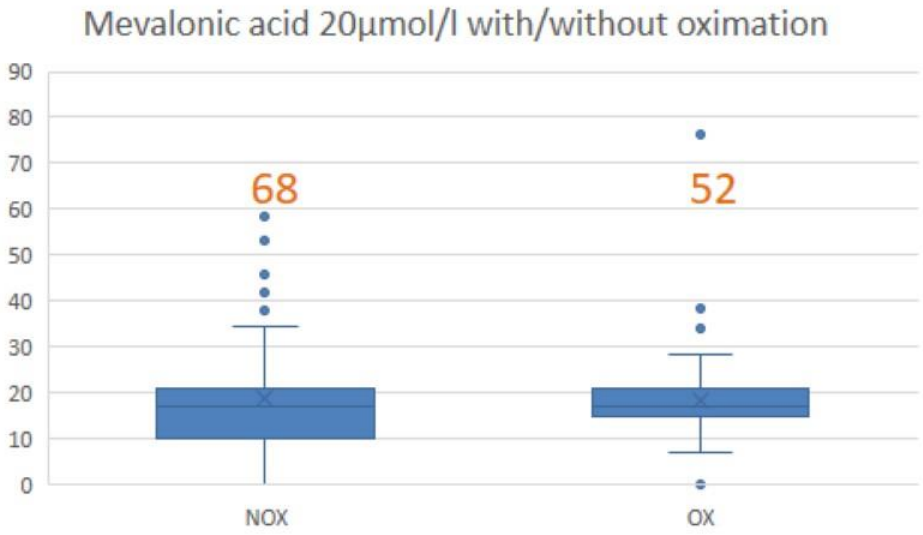
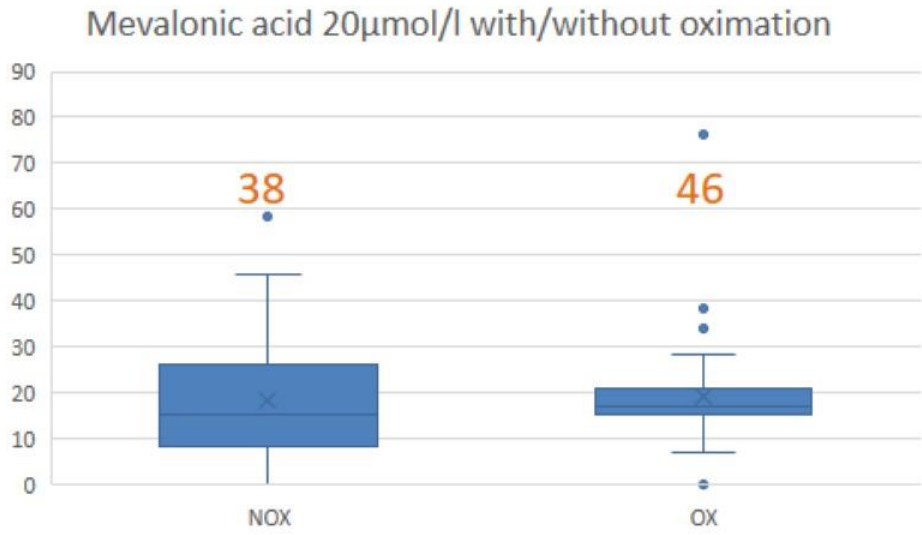
Organic acids workshop

Oximation :

With or without oximation for all methods



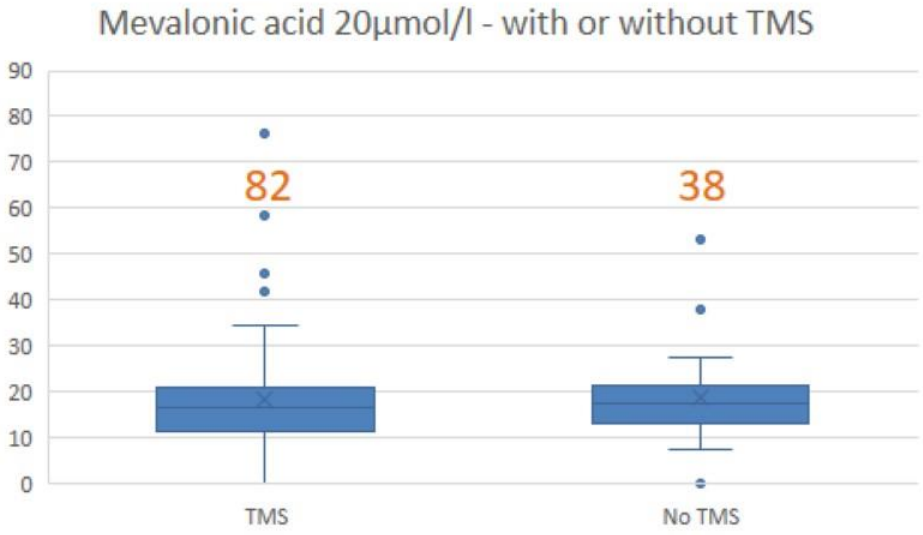
With or without oximation for LL extraction with ethylacetate or diethyl-ether



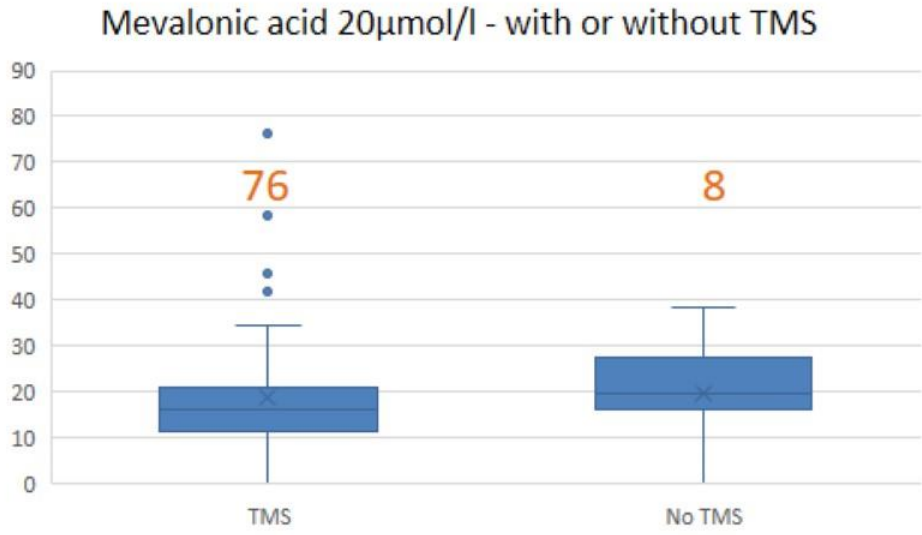
Organic acids workshop

Silylation :

With or without TMS for all methods



With or without TMS for LL extraction with ethylacetate or diethyl-ether



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Summary

- Organic acid profile include several compounds type (organic acids, acylglycine..)
- Large variety of very different methods
- The number of parameters quantitatively measured by the lab is really variable
- The traditional historical methods are the most used but some compounds have huge interlab CVs.
- We can’t extract a better method over others. There are good performers for each type of method
- LC methods are not significantly better but too small number of labs to be statistically relevant
- Stable isotopic dilution (GC and LC methods) have better performance of precision and exactitude
- Oximation and silylation seems to improve also the precision

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Recommendation

- Each method is a trade-off : No ideal method !
- Use the quantitative surveys to access your own analytical limits
- Determine and document your method specifications (reference interval, LLOQ, ..)
- Communicate openly to clinicians your blind spots
- Determine together the list of diseases you will and you won’t able to diagnose

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Organic Acid Workshop Rome

22th October 2021

Judit García Villoria
SA Barcelona QLOU

2. Slight increase of some organic acids, difficulty in the diagnosis

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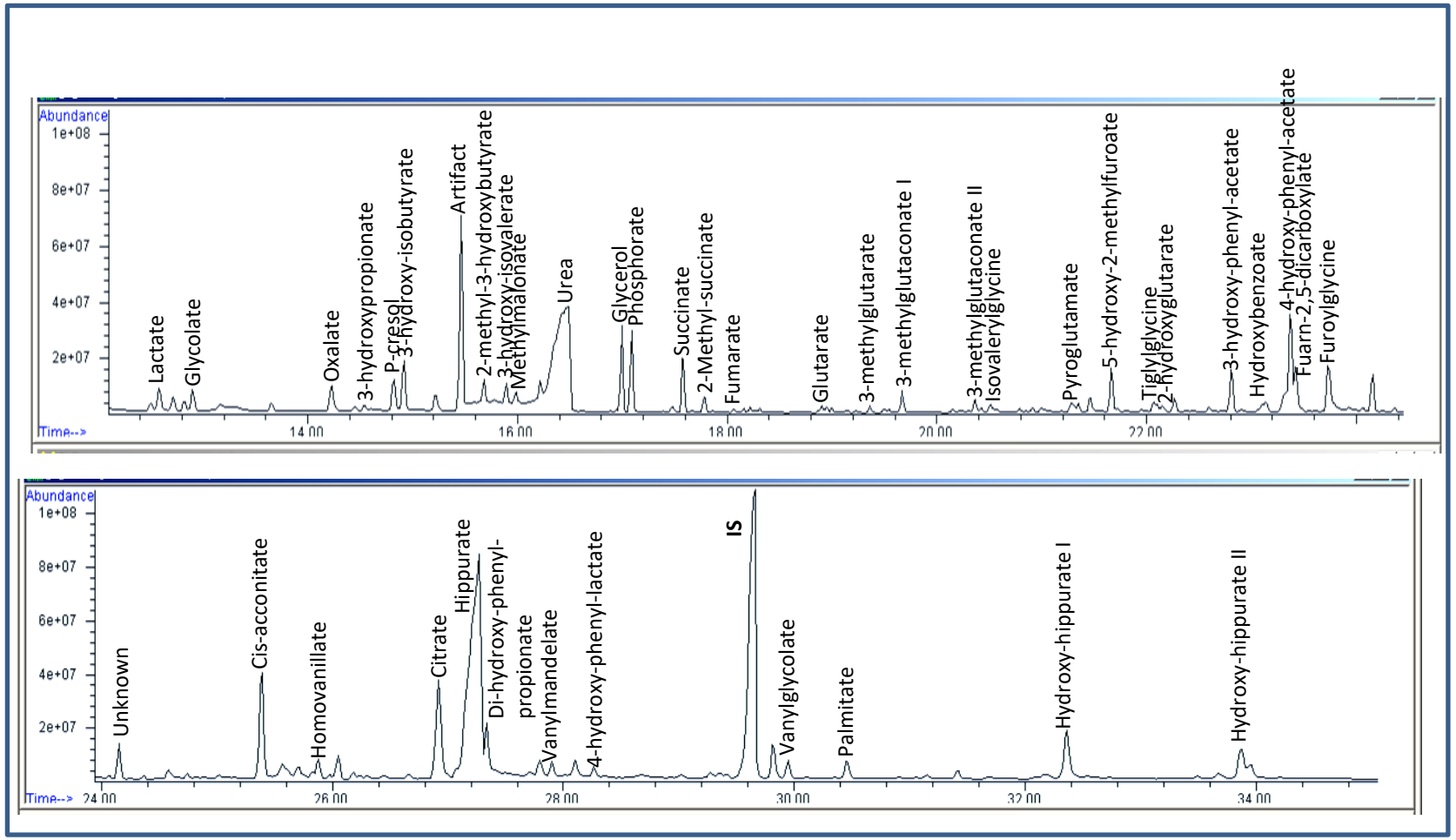
Organic acids workshop

Chromatogram obtained with the analysis of organic acids extracted with ethylacetate without oximation and with TMS derivatization (BSTFA). The organic acids were analyzed by GC-MS using the 60 m x 0.25 mm ID HP-5MS capillary column.



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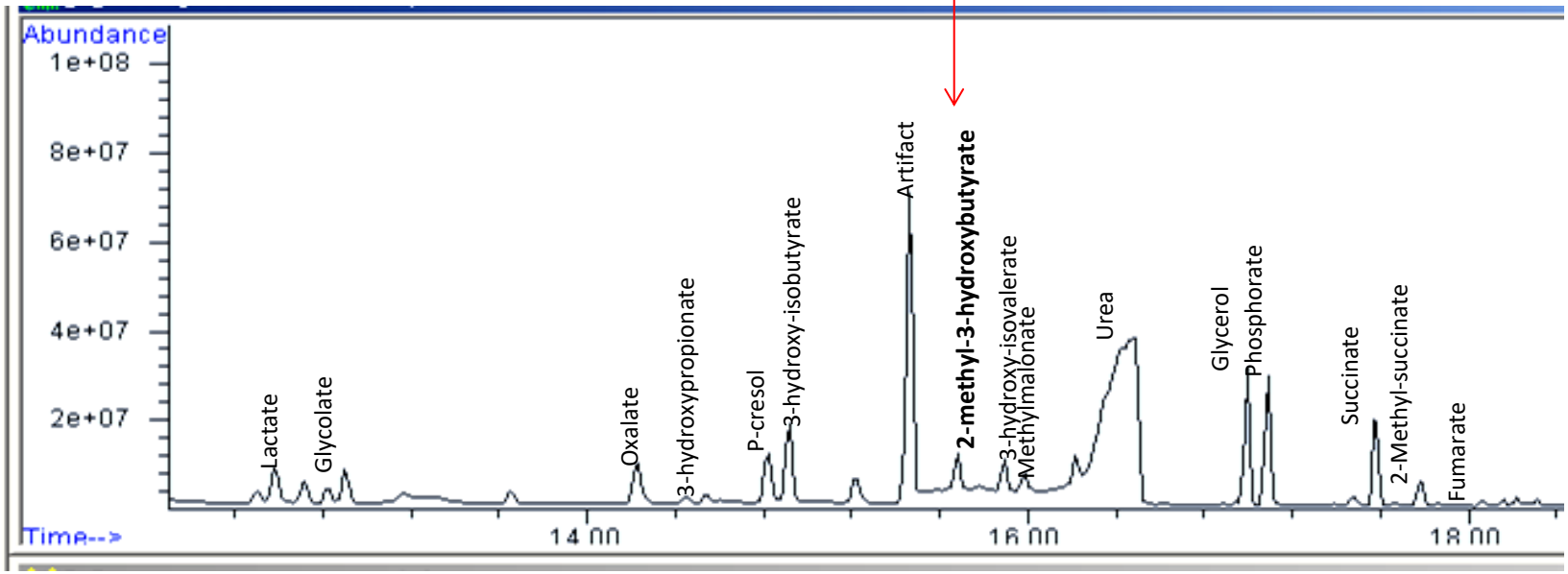
2-methyl-3-hydroxybutyric acid

2-methyl-3-hydroxybutyric acid?
Yes: search tiglylglycine and 2-methylacetoactic acid



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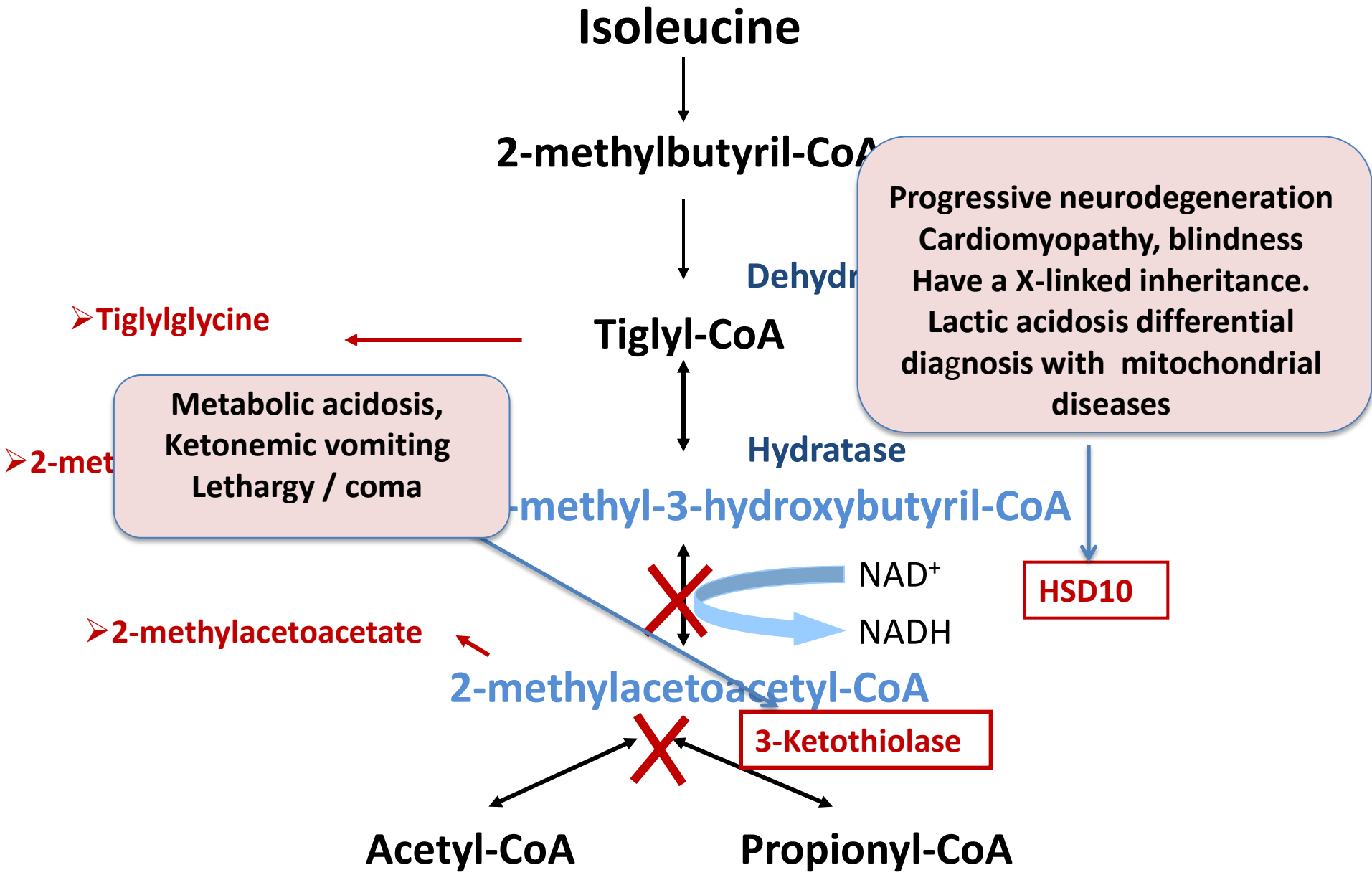
2-methyl-3-hydroxybutyric acid



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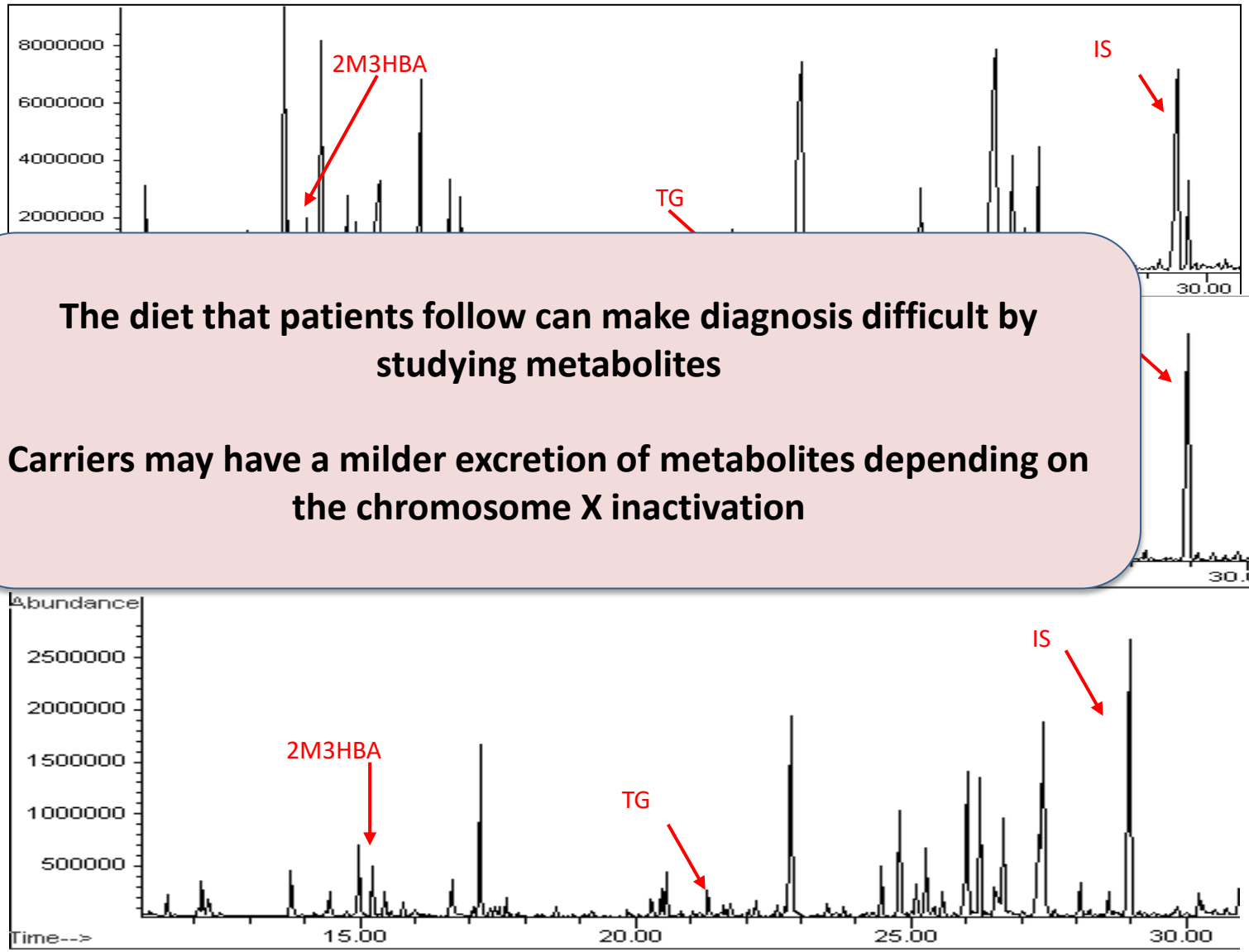
2-methyl-3-hydroxybutyric acid

2-methyl-3-hydroxybutyril-CoA dehydrogenase or HSD10 deficiency

Control

Patient 1

Patient 2 (Hipoproteic diet/famale)



The diet that patients follow can make diagnosis difficult by studying metabolites

Carriers may have a milder excretion of metabolites depending on the chromosome X inactivation

2M3HBA: 2-methyl-3-hydroxybutyrate; TG: tiglylglycine; IS: internal standard

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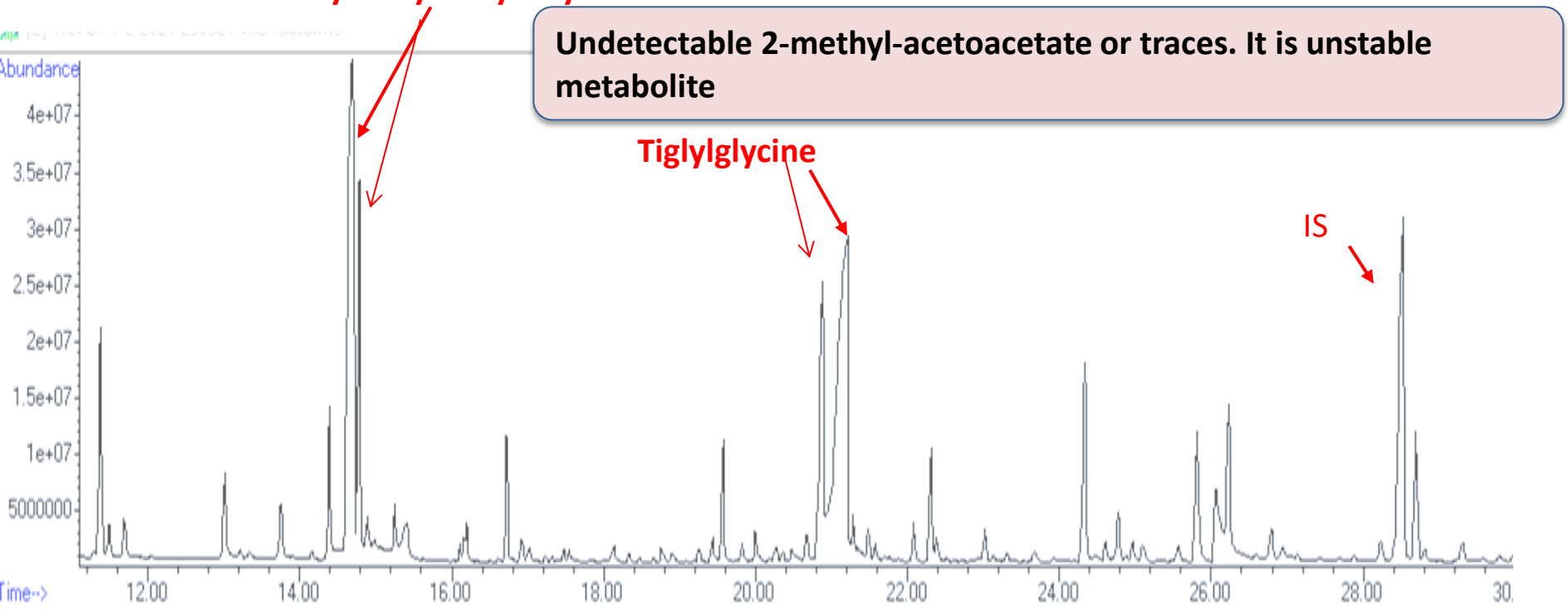
2-methyl-3-hydroxybutyric acid

Beta-ketothiolase deficiency

first week of life, vomiting, tachypnea, **metabolic acidosis with ketonuria**, but no hypoglycemia and no hyperammonemia.

DPT: Overall proficiency 92%



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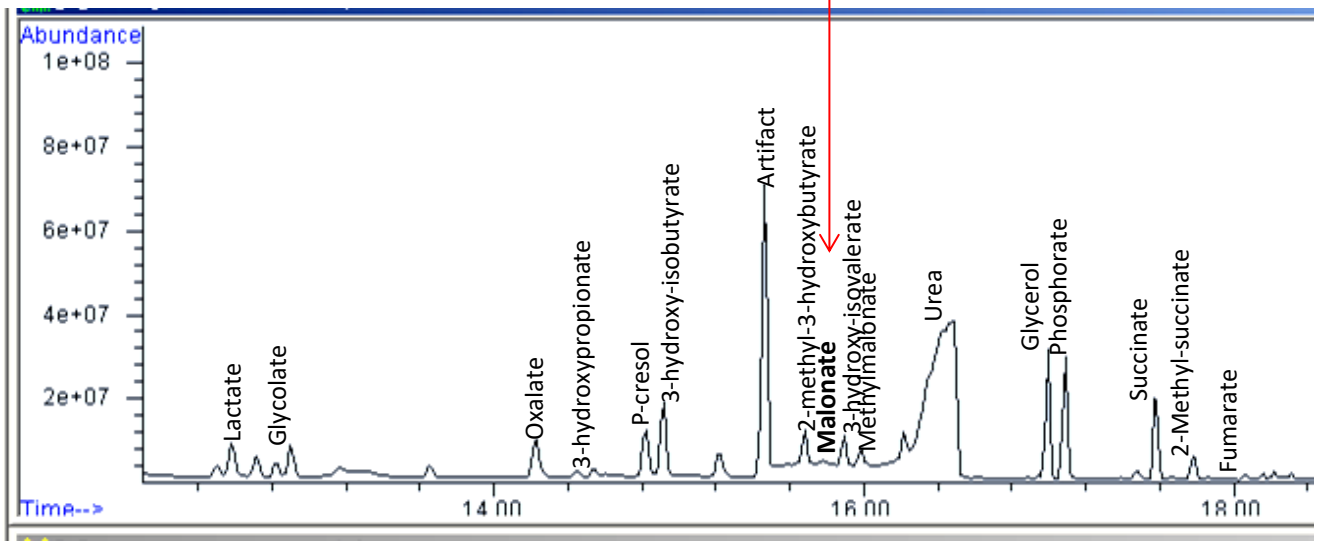
Malonic acid

Malonate?



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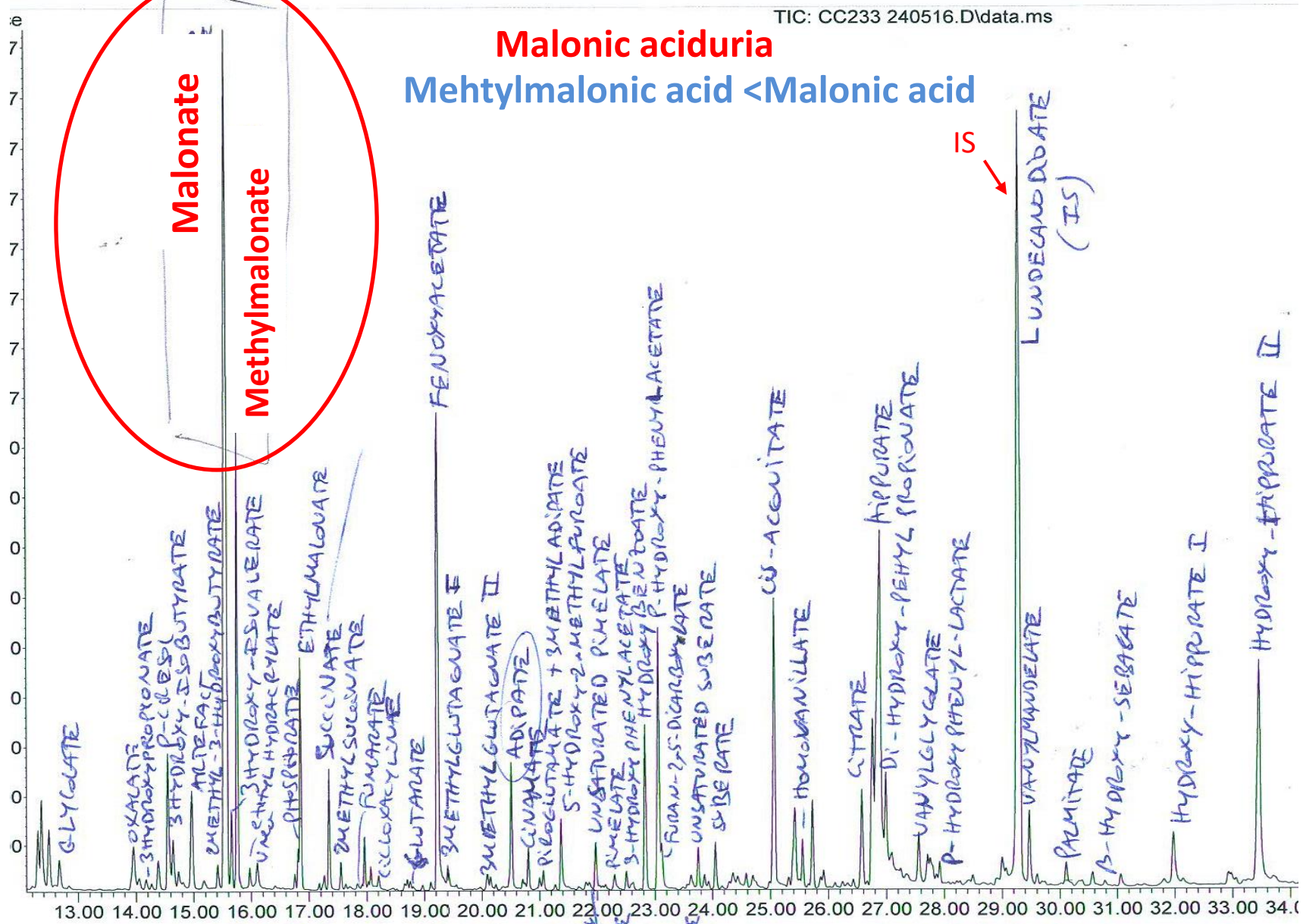
Malonic acid



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Malonic acid

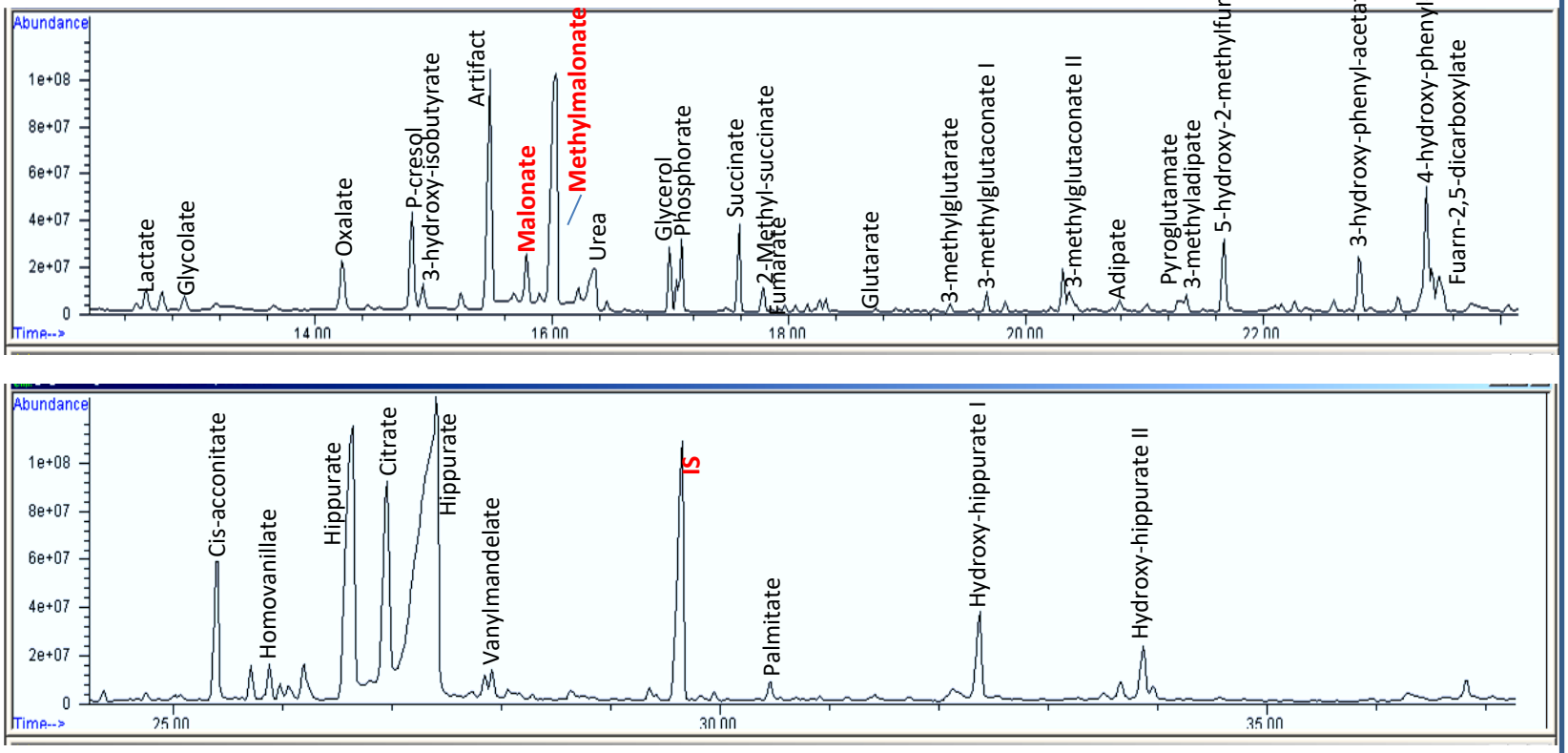
Combined malonic and methylmalonic aciduria (CMAMMA) , due to mutations in *ACSF3*

Mehtylmalonic acid >Malonic acid

DPT and QLOU: Overall proficiency 67% and 71% respectively

TIC QLOU-EB-2019-G

Methylmalonic acid: 172 mmol/mol creatinine



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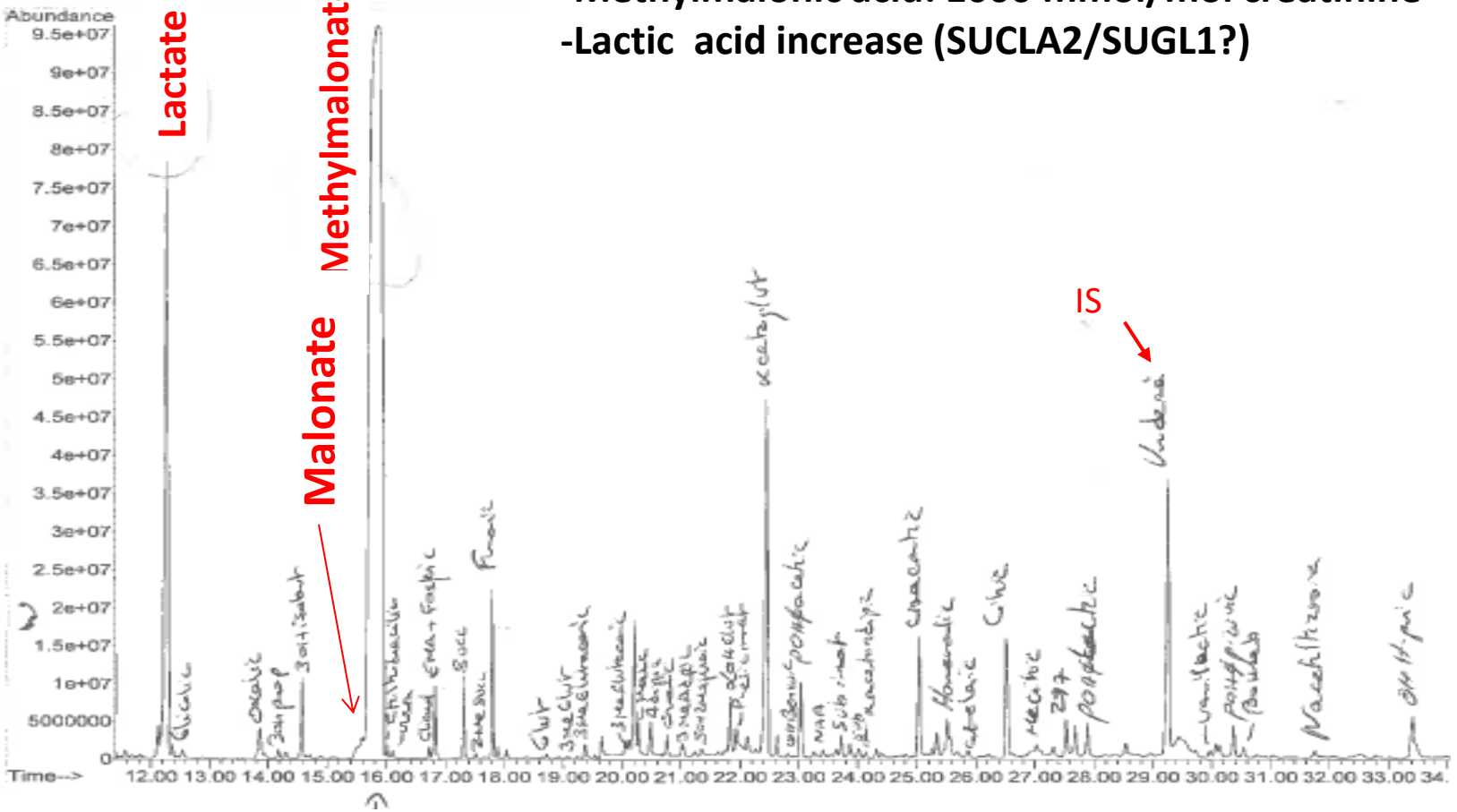
Malonic acid

Combined malonic and methylmalonic aciduria (CMAMMA) , due to mutations in *ACSF3*

-Newborn screening: increased C3 in DBS
-Methylmalonic acid: 1000 mmol/mol creatinine
-Lactic acid increase (SUCLA2/SUGL1?)

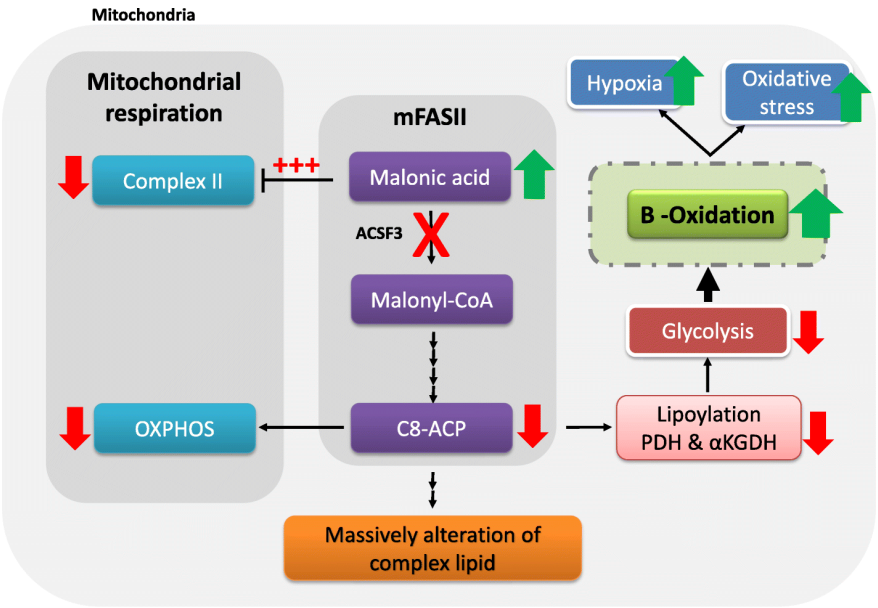


Combined malonic and methylmalonic aciduria (CMAMMA)

The clinical significance of combined malonic and methylmalonic aciduria due to ACSF3 deficiency is controversial. Levtova et.al. J Inherit Metab Dis. 2019 Jan;42(1):107-116

benign condition

-coma
-ketoacidosis
-hypoglycemia
-failure to thrive
-elevated transaminases
-microcephaly
-dystonia
-axial hypotonia
-developmental delay
-Seizures
-memory problems
-psychiatric disease
-cognitive decline



Brain metabolism and neurological symptoms in combined malonic and methylmalonic aciduria. Sara Tucci. *Orphanet Journal of Rare Diseases* **volume 15**, Article number: 27 (2020)

Older patients may present with neurological manifestations such as seizures, memory problems, psychiatric problems and/ or cognitive decline. In **fibroblasts from CMAMMA patients we have recently demonstrated a dysregulation of energy metabolism**

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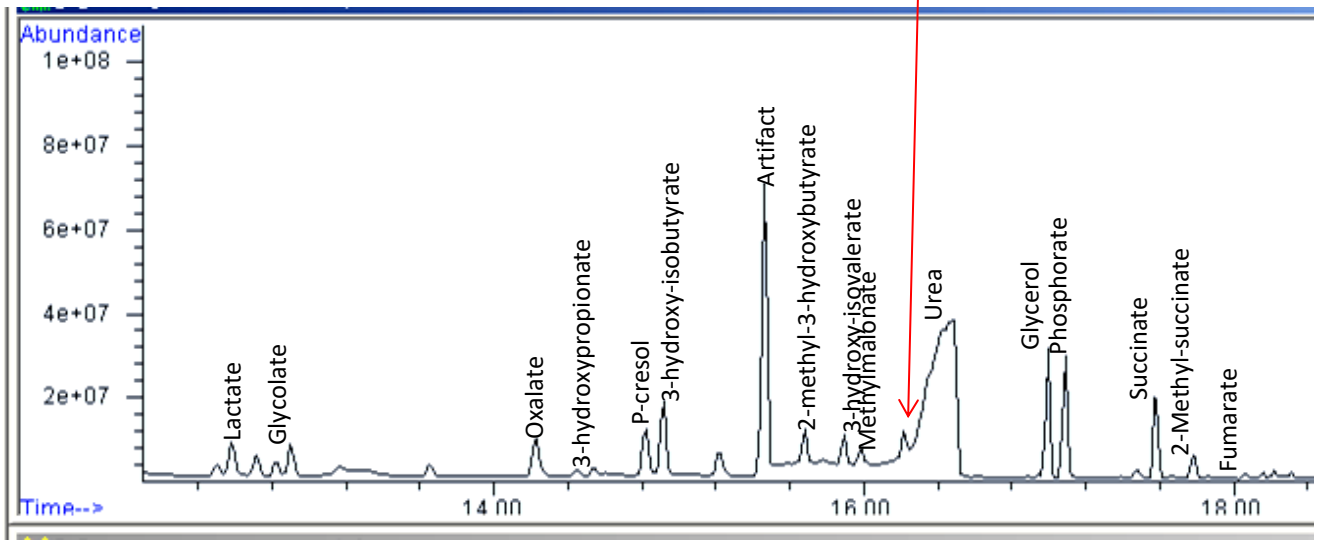
4-hydroxybutyric acid

4-hydroxybutyric acid?

Yes: search 4,5-di-hydroxyhexanoic acid

Drug vs genetic disorder



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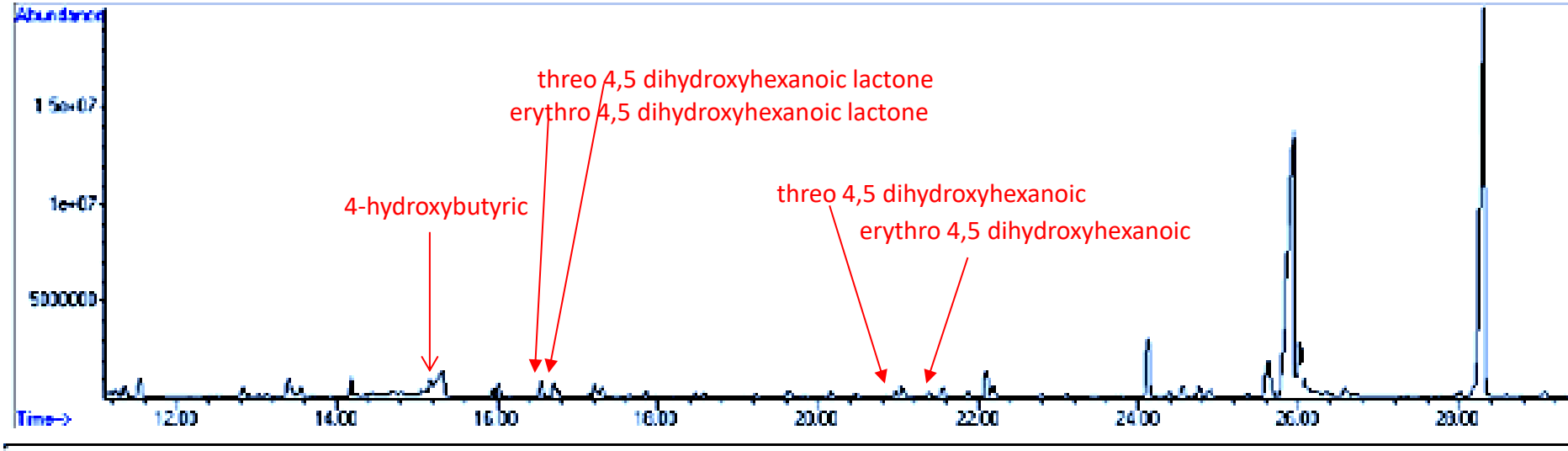
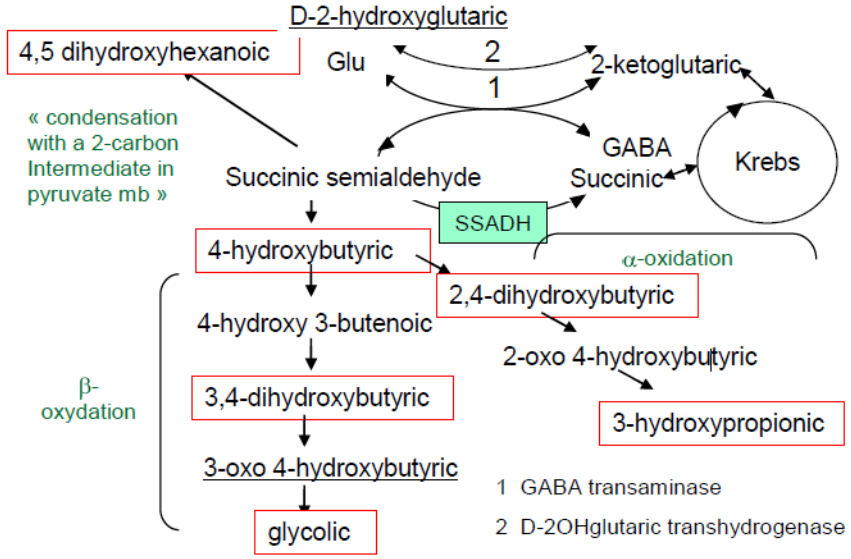
4-hydroxybutyric acid

4-hydroxybutyric aciduria (succinate semialdehyde dehydrogenase deficiency)





DPT and QLOU HD : Overall proficiency 81 and 93%

Patients with non-specific psychomotor and mental retardation

Other described symptoms are: language delay, hypotonia, motor delay, seizures, behavior problems, ataxia, hyperreflexia, white matter abnormalities…

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Uracil

Pyrimidine metabolism deficiency

Yes, search :

-thymine: MNGIE, Dihydropyrimidine dehydrogenase

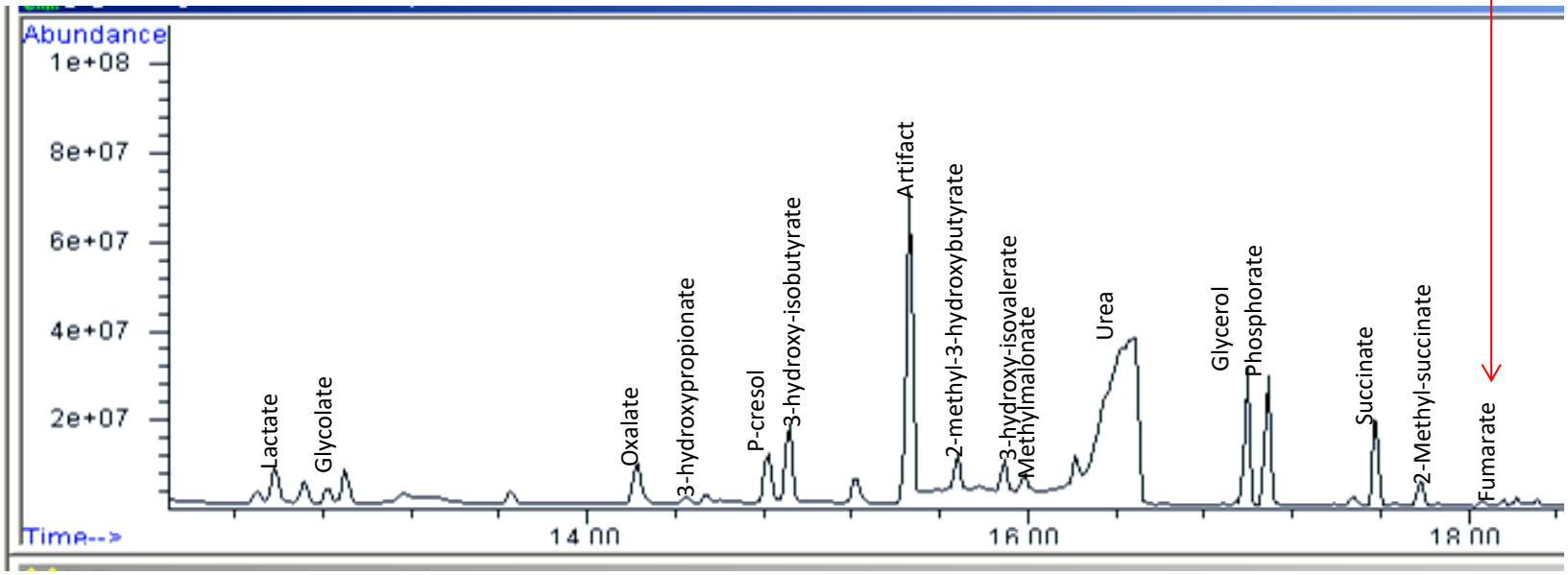
-dihydro-thymine, di-hydrouracil: beta-ureido propionase deficiency, Dihydropyrimidinase deficiency

-Orotic acid: OCT deficiency /HHH syndrome

-Citrulline cyclic metabolite: citrulinemia deficiency

Ura cycle disorder

Uracil?



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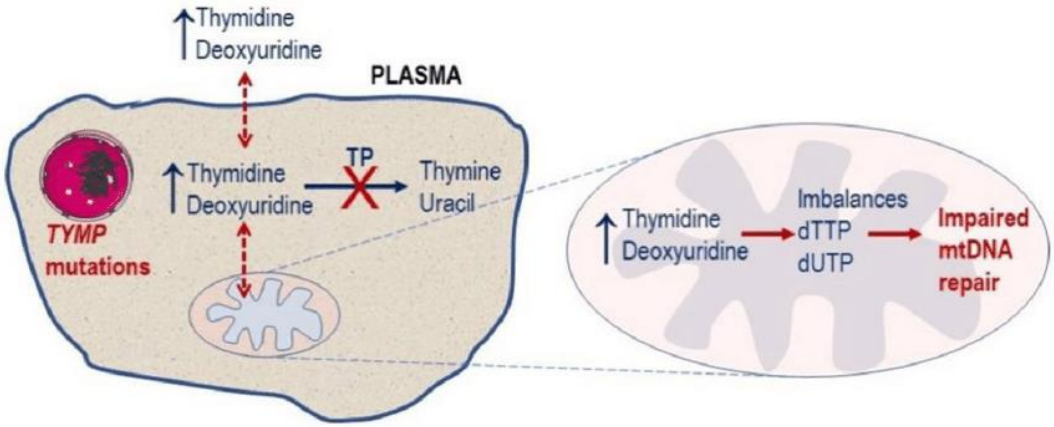
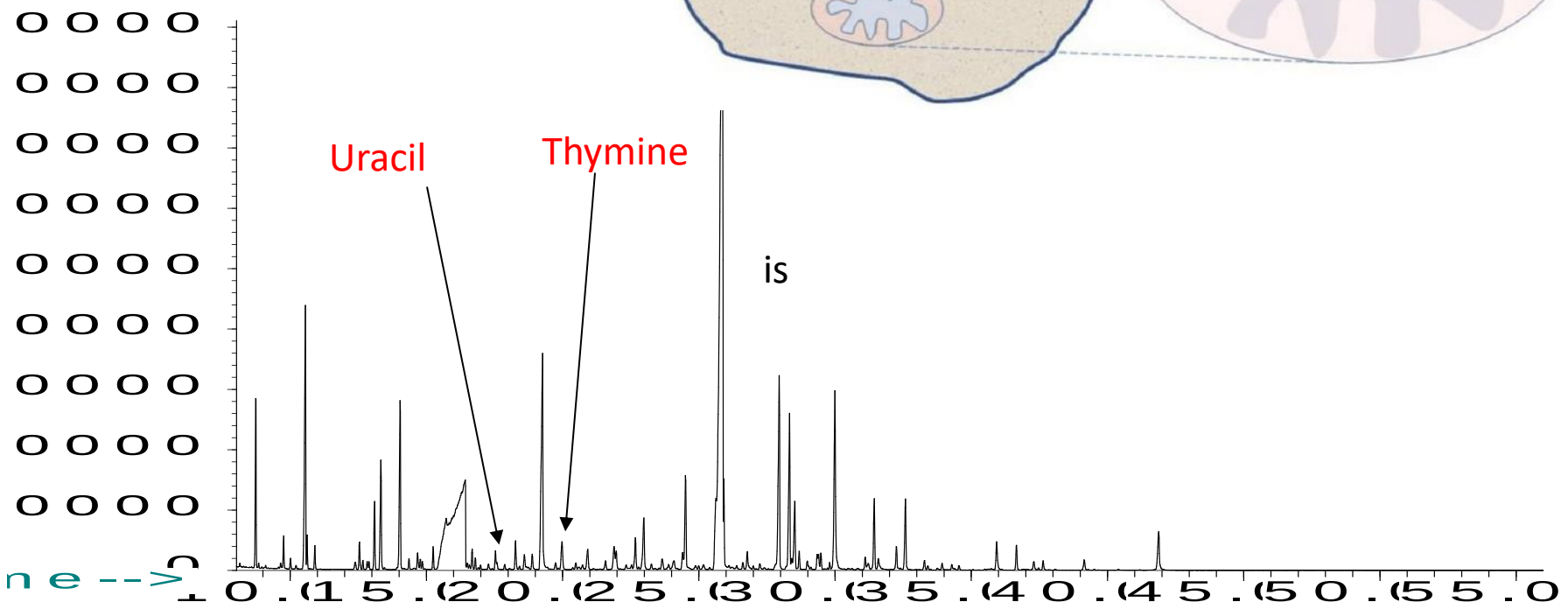
Uracil

MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOPATHY SYNDROME **MNGIE**

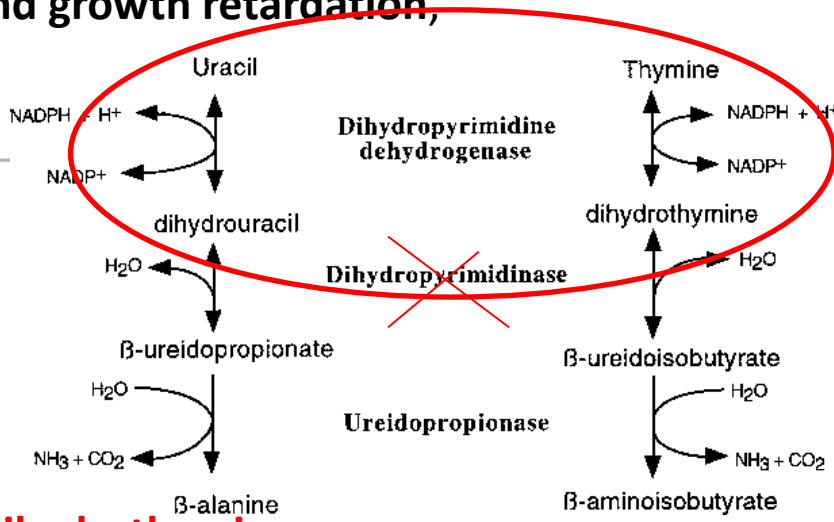
-impaired gastrointestinal motility
-peripheral neuropathy
-chronic progressive **external ophthalmoplegia**
-leukoencephalopathy

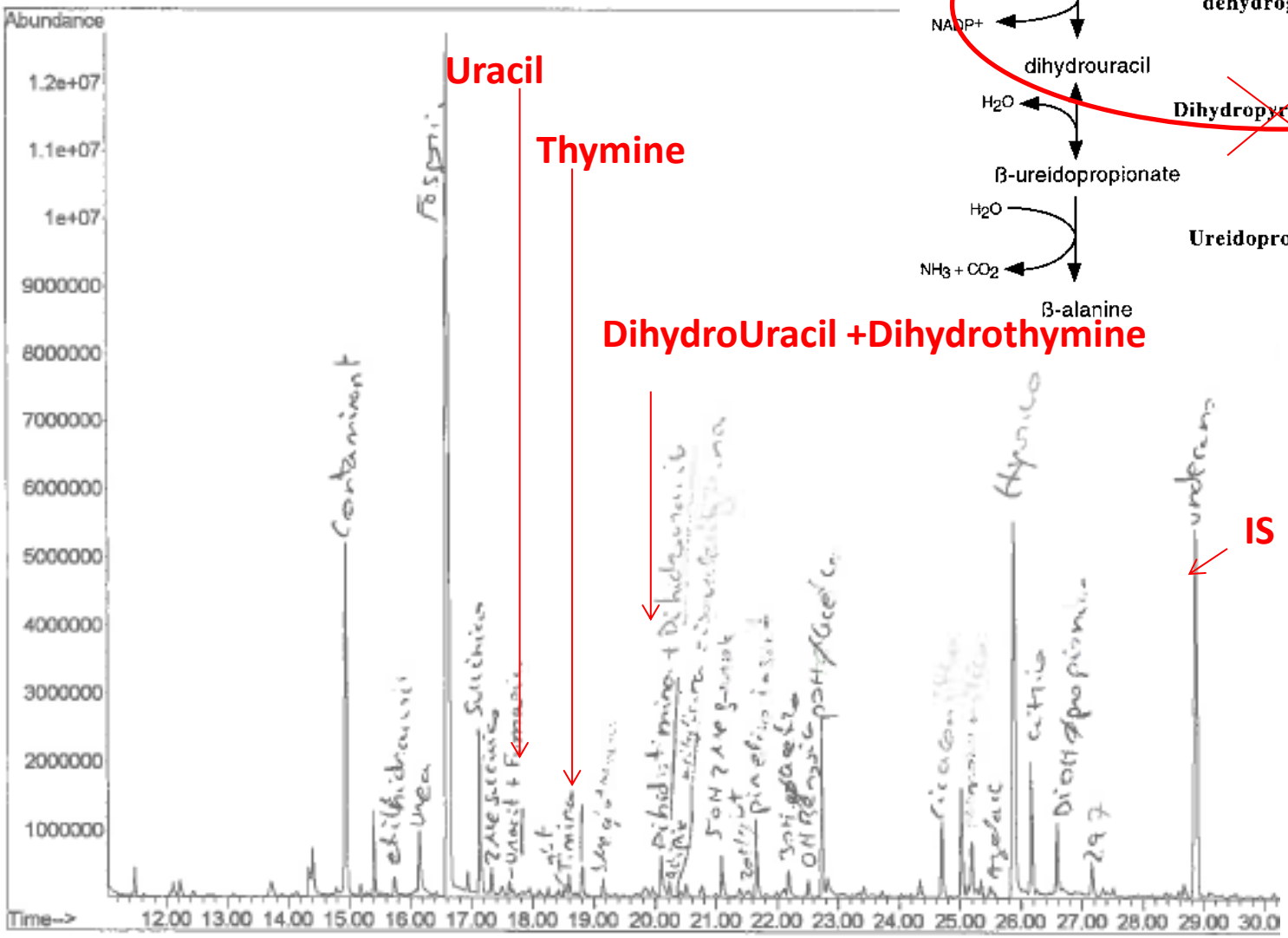




Uracil

Dihydropyrimidinase deficiency, seizures , mental and growth retardation, dismorfia. Our patient cardiomyopathy (2 years)





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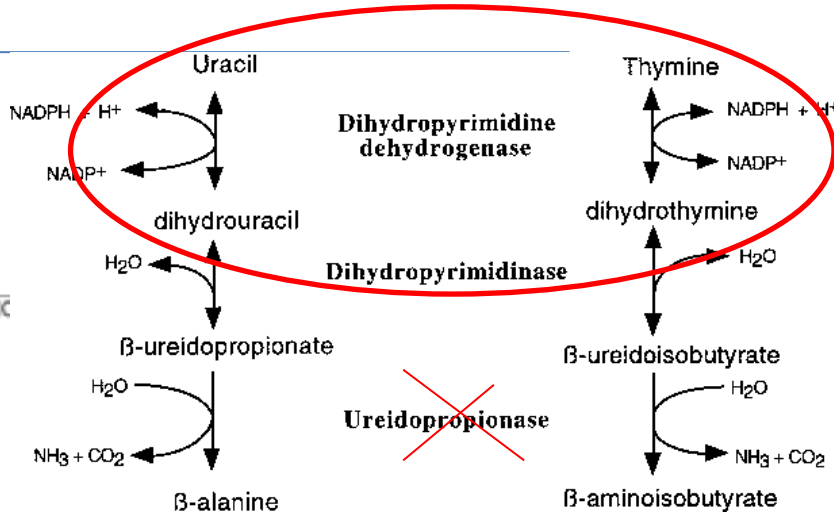
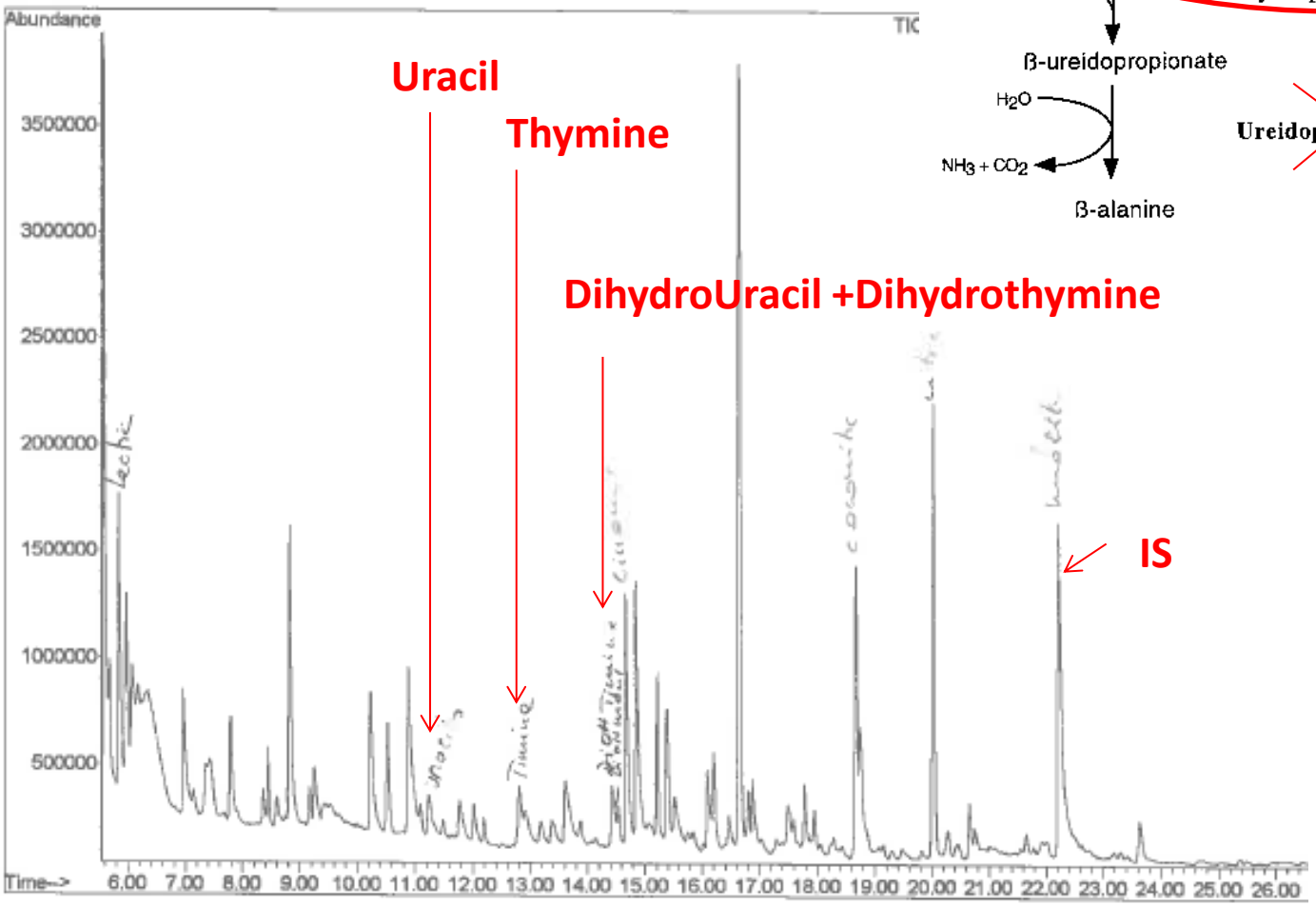
Uracil

Beta-ureidopropionase deficiency, seizures ,
Mental retardation, hypotonia.
Our patient seizures and hypertonia (1 year)



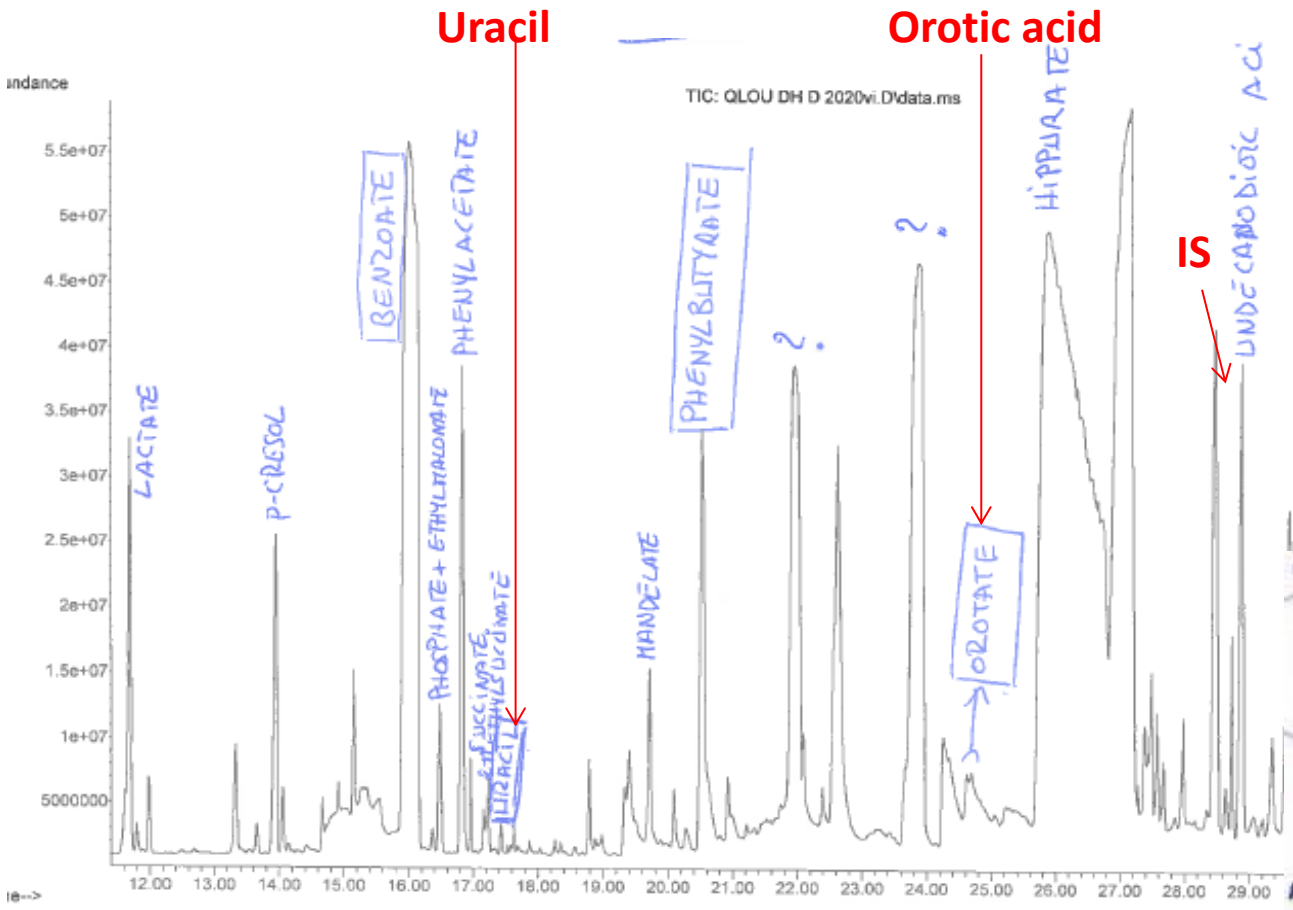


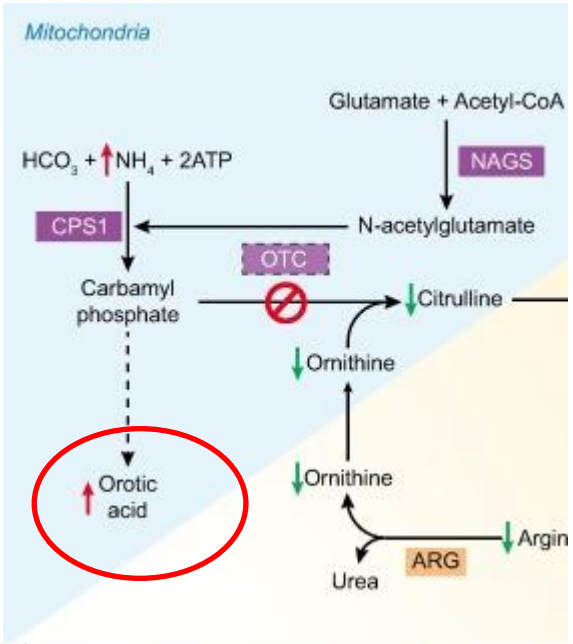
Uracil

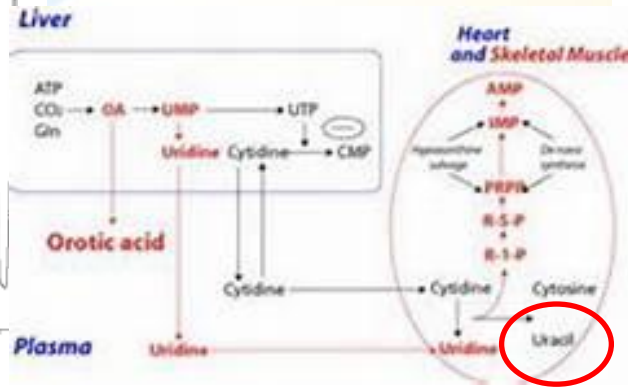
Ornithine transcarbamylase (OTC) deficiency, Watch out for the females, X-linked disorder

Female with development delay, diagnosed at 6 years old when she presented **hyperammonemia** after an episode of infection. At present she is under treatment

DPT: Overall proficiency 80% and QLOU 2020 overall proficiency 68% (no orotic acid educational sample)







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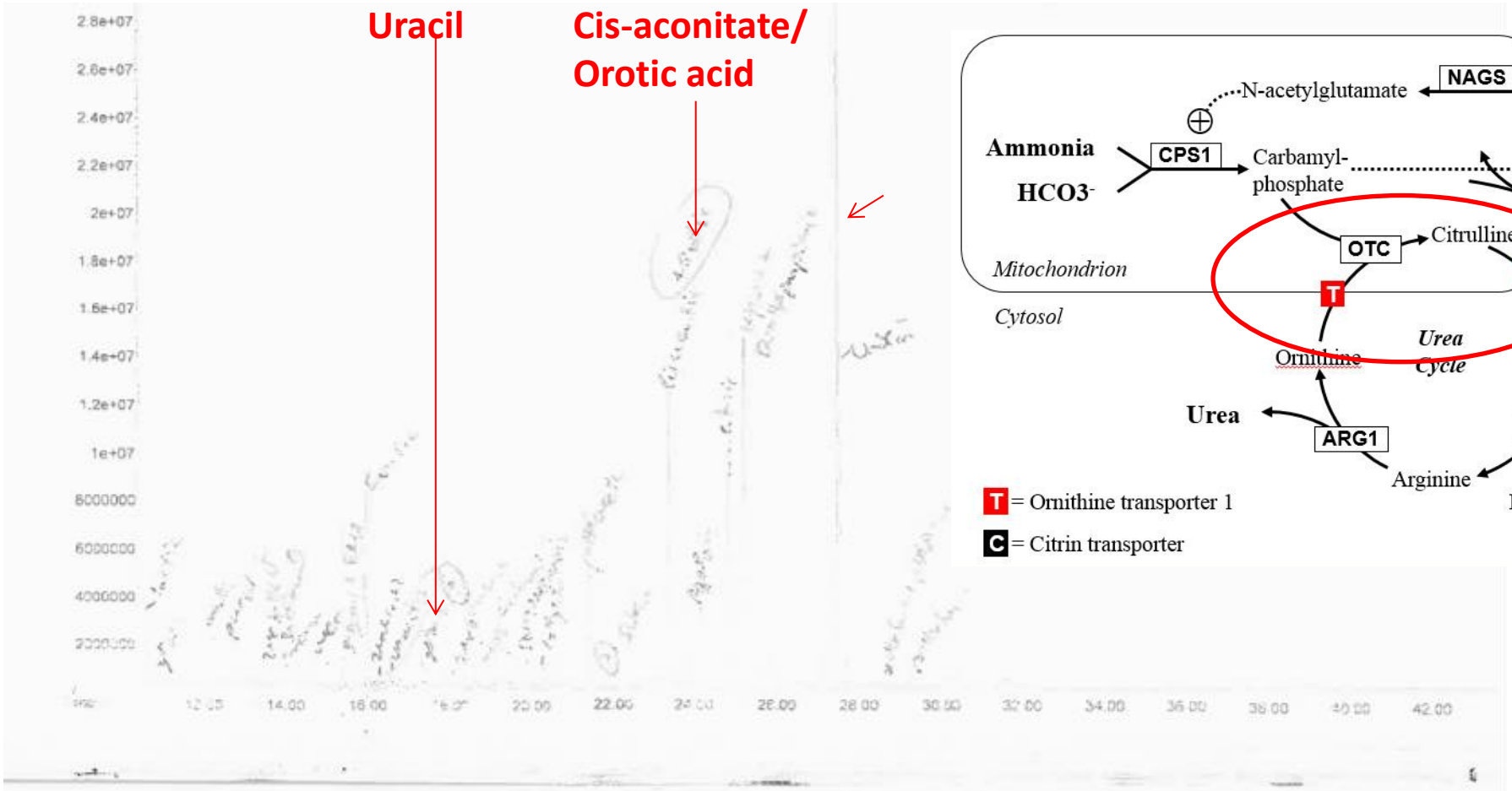
Uracil

Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome

DPT: Overall proficiency 80% (some labs OTC diagnostic)

Hypotonia at 6 months of age, later developmental delay, failure to thrive, elevated transaminases and **hyperammonemia.**



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Uracil

Depends on solvent OA extracion the cycle derivate could be lower

Citrullinemia

DPT: Overall proficiency 95%

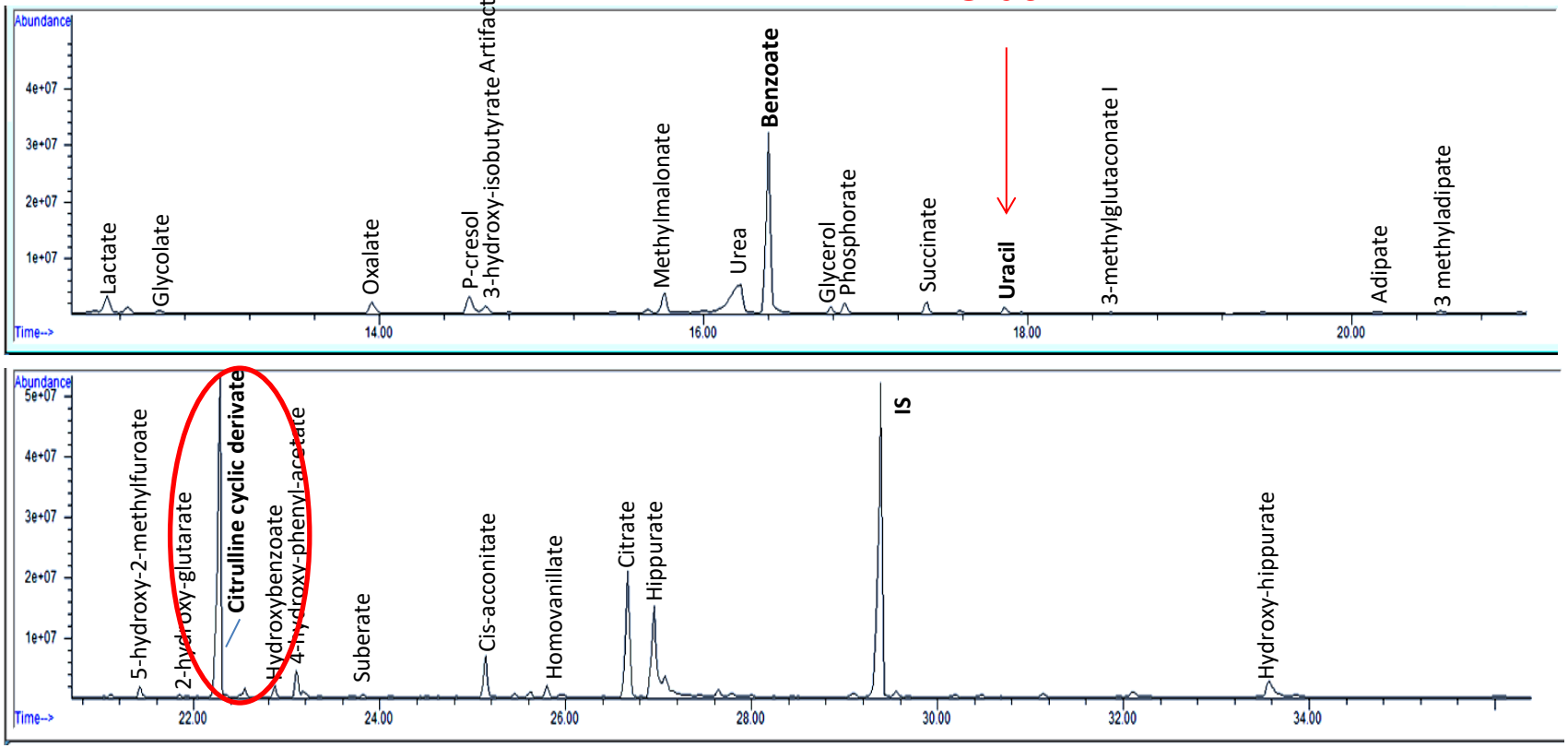
She presented at 3 days of life with hyporeactivity, grunting, which rapidly worsened.

Ammonaemia was >700 µmol/L.

QLOU: Overall proficiency 64%

Male under treatment that presents epilepsy and language delay from 4 years of age

Uracil



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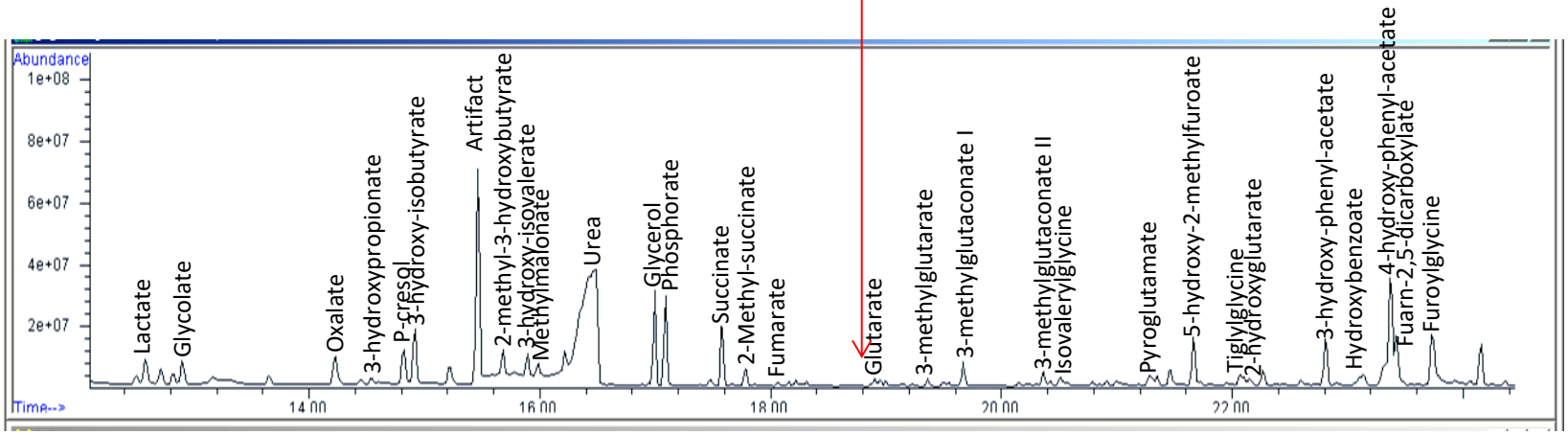
2-methyl-2,3-dihydroxybutyric acid

2-methyl-2,3-dihydroxybutyric acid?



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2-methyl-2,3-dihydroxybutyric acid

Mitochondrial short-chain enoyl-CoA hydratase deficiency (ECHS1D)

ECHS1 mutations in **Leigh disease**: a new inborn error of metabolism affecting valine metabolism. Peters, H. et al Brain 137: 2903-2908, 2014

3-Hydroxyisobutyryl-CoA hydrolase deficency (HIBCHD)

HIBCH mutations can cause **Leigh-like disease** with combined deficiency of multiple mitochondrial respiratory chain enzymes and pyruvate dehydrogenase. Ferdinandusse, S. et al. Orphanet J. Rare Dis. 8: 188, 2013.

ECHS1D and **HIBCHD**
are autosomal recessive inborn errors of metabolism characterized by severely delayed psychomotor development, **neurodegeneration, increased lactic** acid, and **brain lesions in the basal ganglia**

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2-methyl-2,3-dihydroxybutyric acid

ECHS1 and HIBCH common laboratory abnormalities

- Increased serum lactate
- Increased CSF lactate
- Secondarily decreased activity of the pyruvate dehydrogenase complex (PDC)
- Increased urinary S-(2-carboxypropyl)cysteine
- Increased **2-methyl-2,3-dihydroxybutyrate**

HIBCH specific lab. abnormalities

Increased 3-hydroxy-isobutyryl-carnitine (U)

Increased 3 hydroxyisovaleric acid excretion has been reported

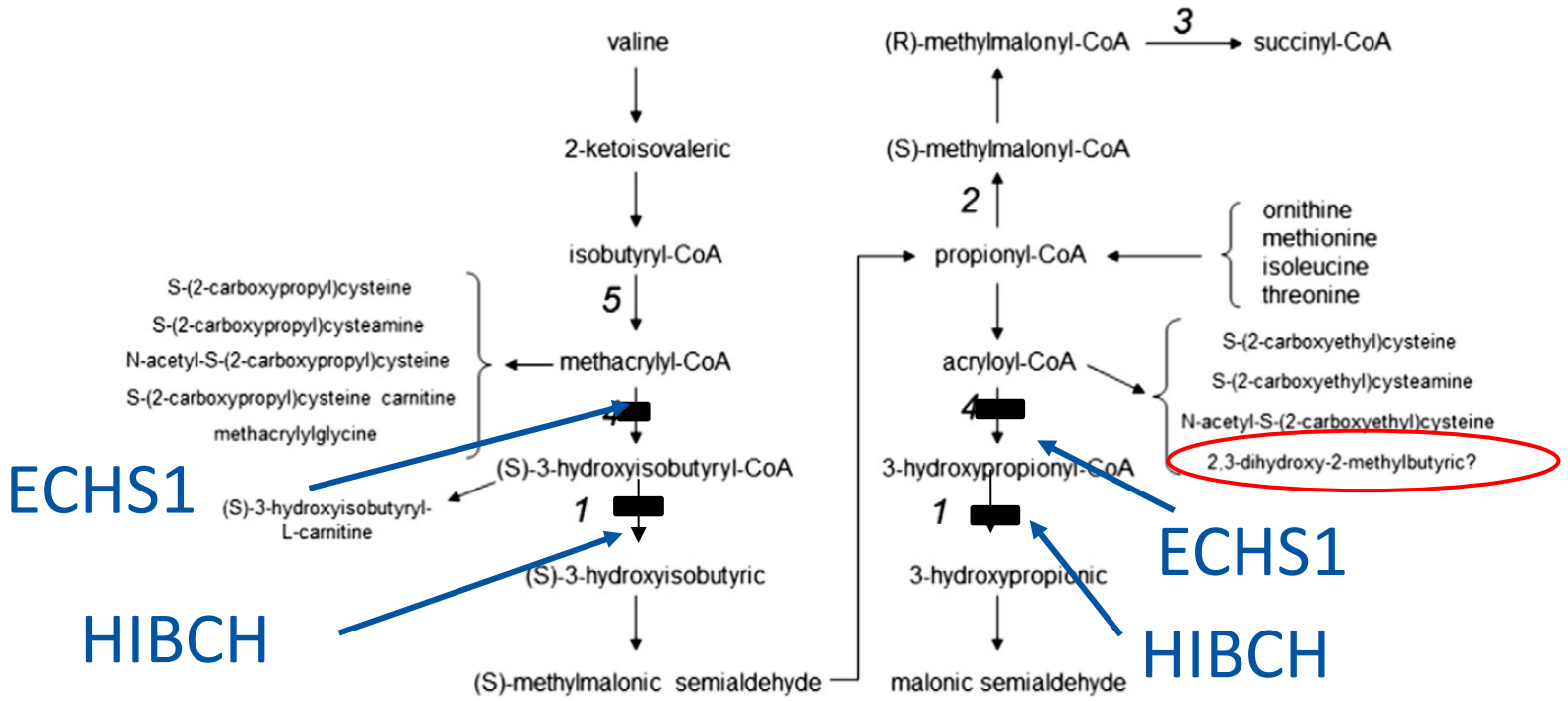

Fig. 1. Valine catabolic pathway showing the formation of metabolites in HIBCH deficiency, modified from [4]. Enzymes are numbered: 1 = 3-hydroxyisobutyryl-CoA hydrolase; 2 = propionyl-CoA carboxylase; 3 = (R)-methylmalonyl-CoA mutase; 4 = short-chain enoyl-CoA hydratase (*ECHS1* gene); 5 = isobutyryl-CoA dehydrogenase. Acryloyl-CoA as the possible metabolic origin of 2,3-dihydroxy-2-methylbutyric was indirectly inferred (see text).

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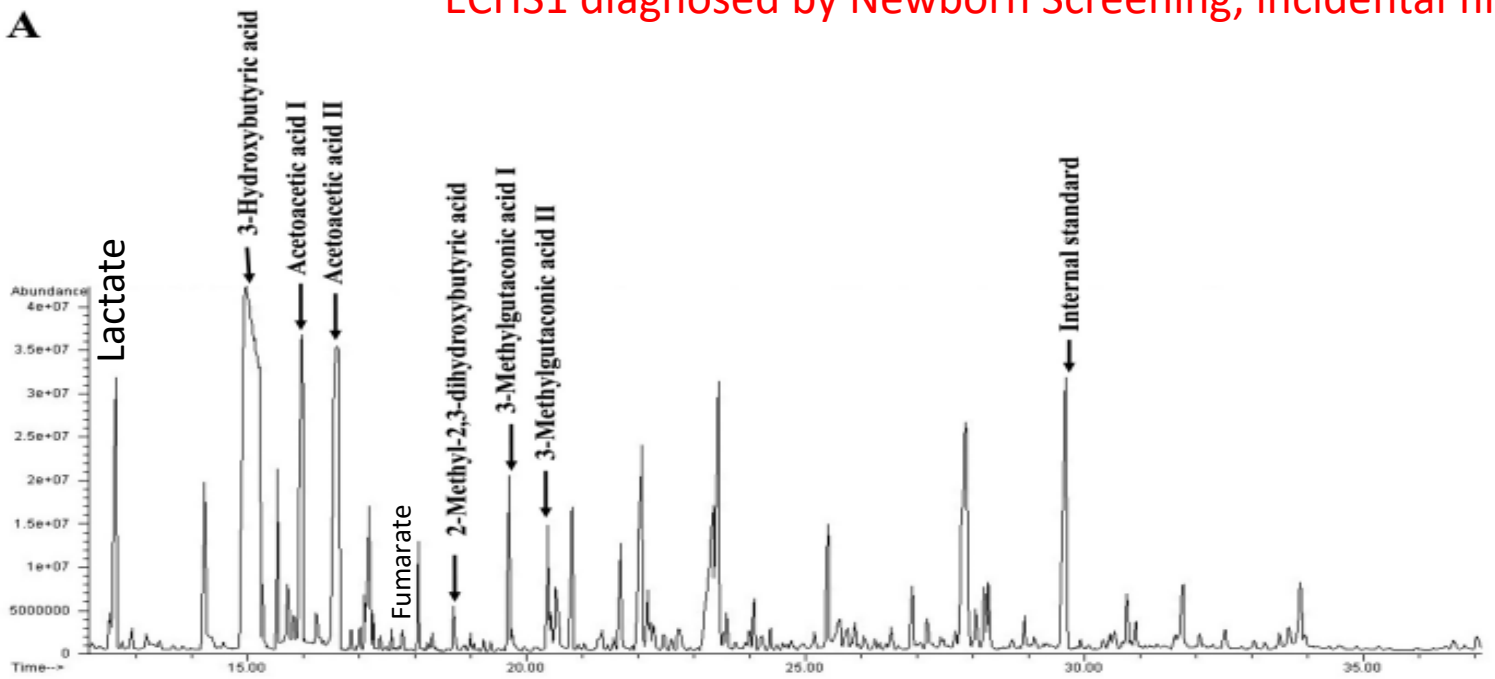
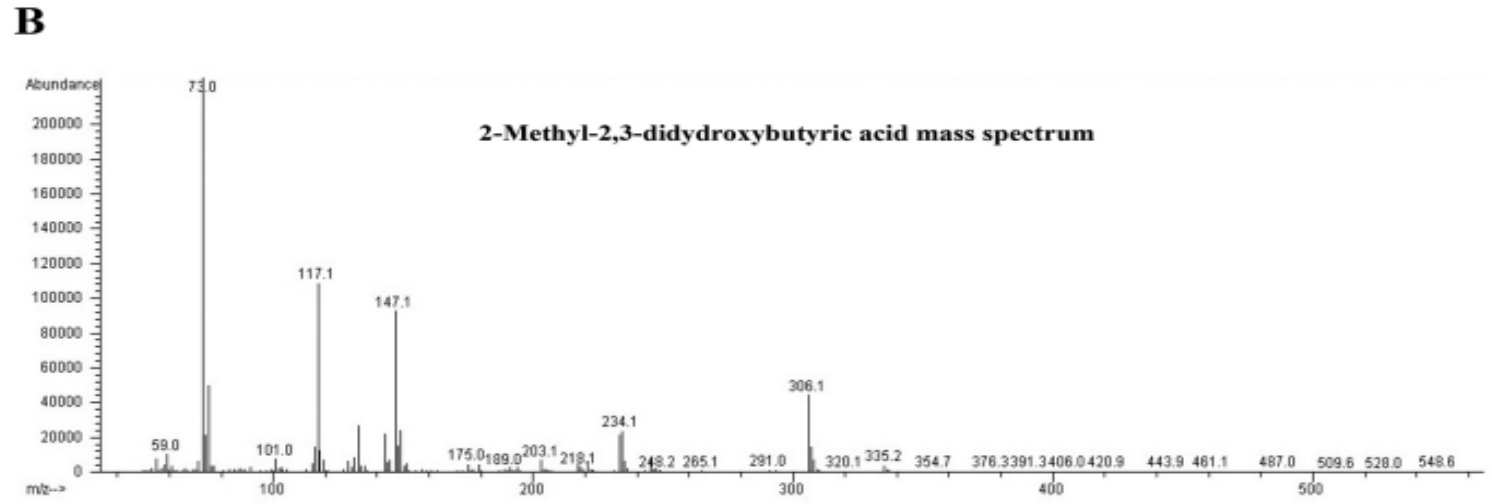
2-methyl-2,3-dihydroxybutyric acid

S. Pajares, et al. Molecular Genetics and Metabolism Reports 22 (2020) 100553

ECHS1 diagnosed by Newborn Screening, incidental finding





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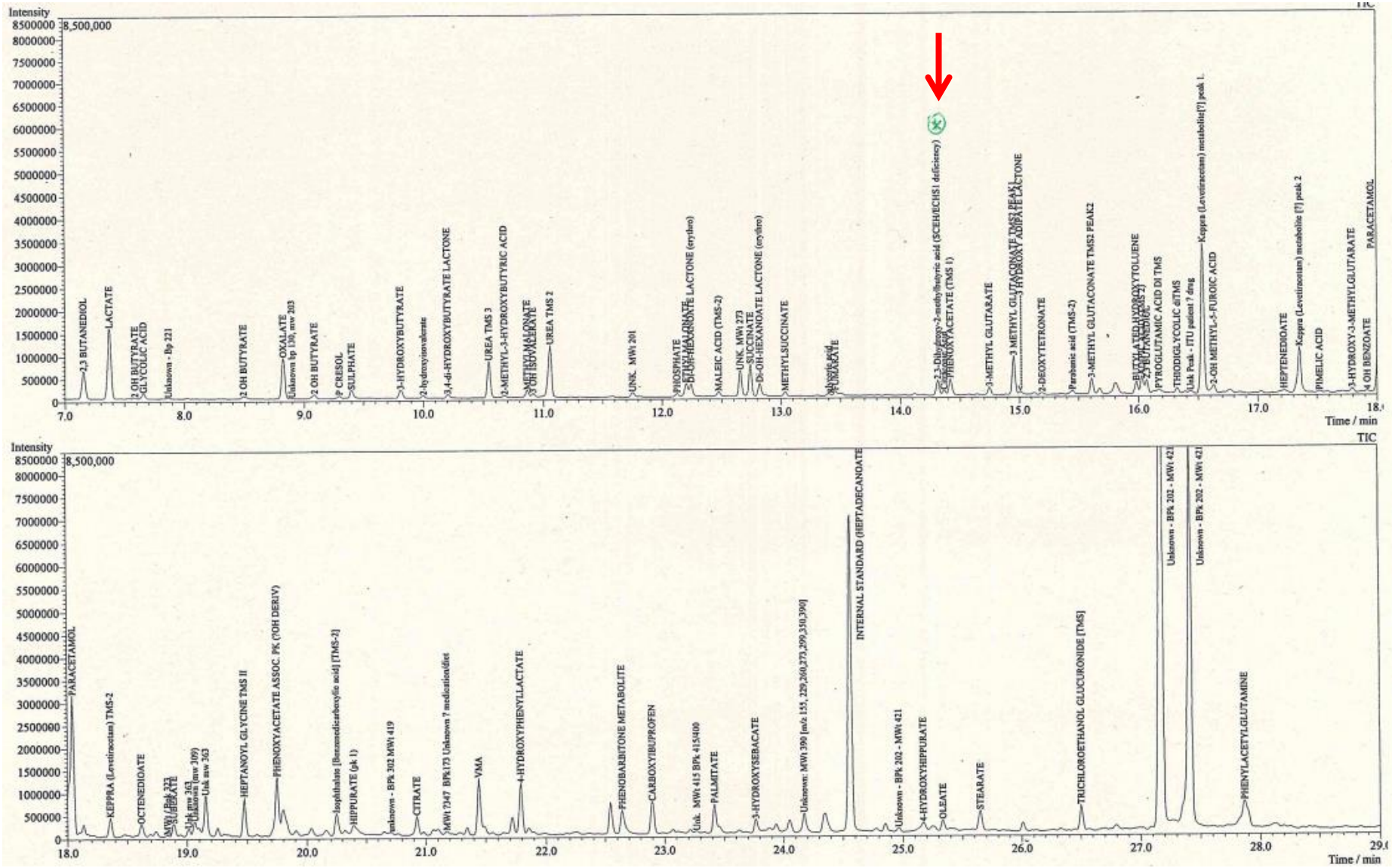
2-methyl-2,3-dihydroxybutyric acid

HIBCH



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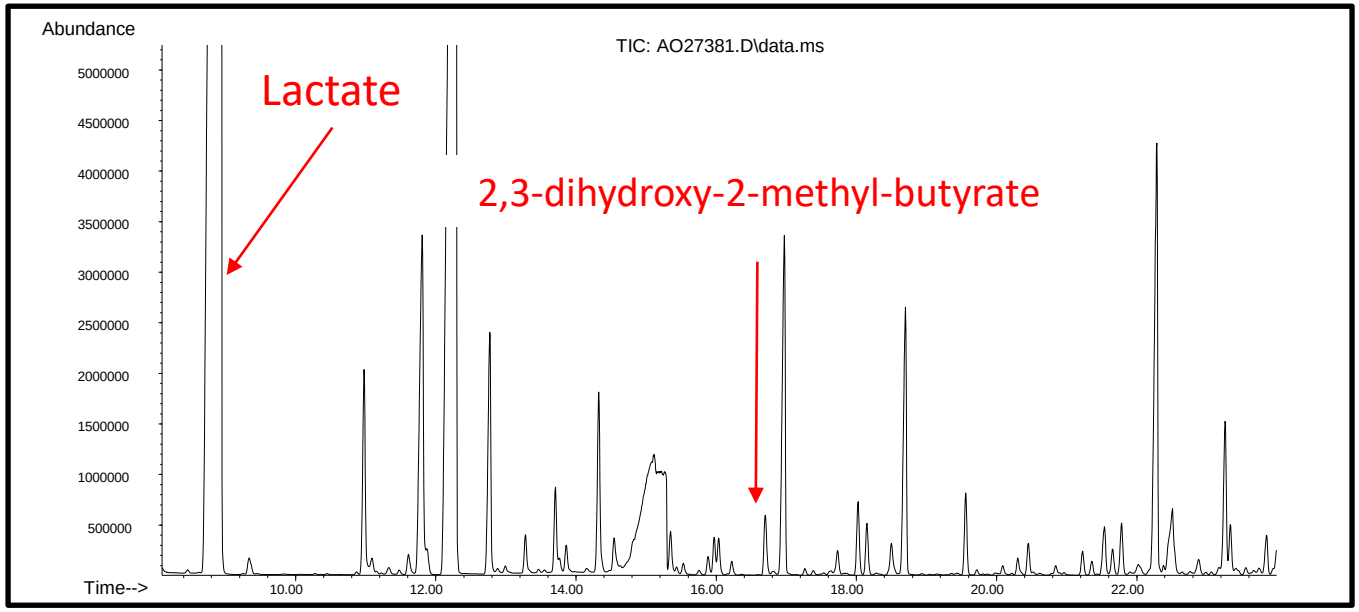
2-methyl-2,3-dihydroxybutyric acid



-Kearns-Sayre s
yndrome (KSS)
-Pearson
marrow-pancreas
syndrome (PMS)

Clin Chim Acta
. 2018 Jun;481:156-160.
doi: 10.1016/j.cca.2018.03.002. Epub 2018 Mar 10.

The urinary organic acids profile in single large-scale mitochondrial DNA deletion disorders

Michela Semeraro [1], Sara Boenzi [2], Rosalba Carrozzo [3], Daria Diodato [3], Diego Martinelli [2], Giorgia Olivieri [2], Giacomo Antonetti [2], Elisa Sacchetti [2], Giulio Catesini [2], Cristiano Rizzo [2], Carlo Dionisi-Vici [2]

Also associated in methylmalonic acidurias and oder disorders of valine metabolism

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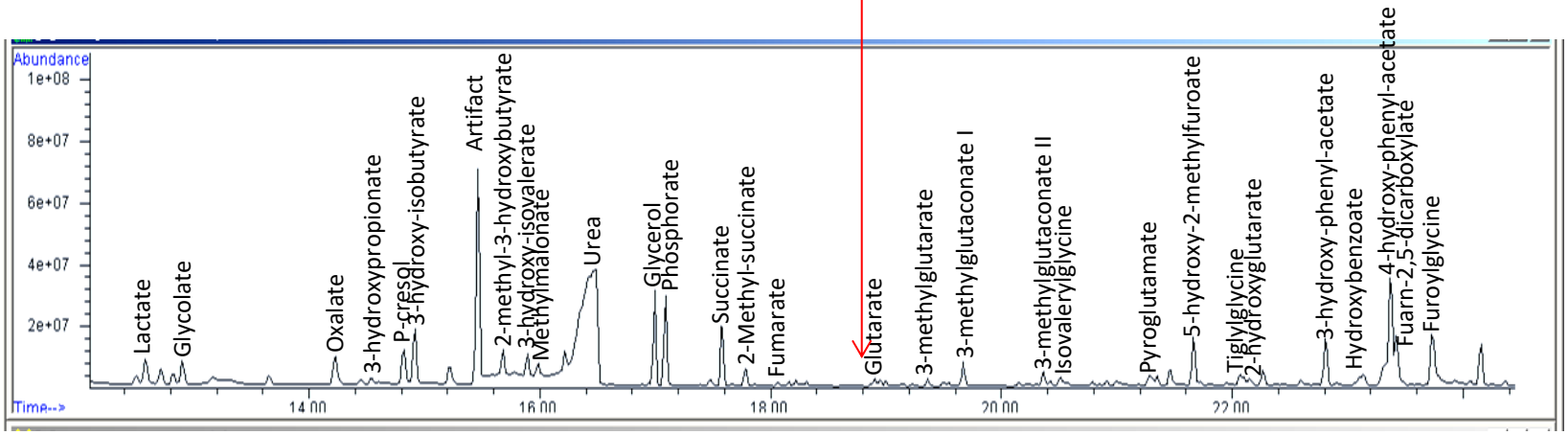
Isobutyrylglycine

Isobutyrylglycine?



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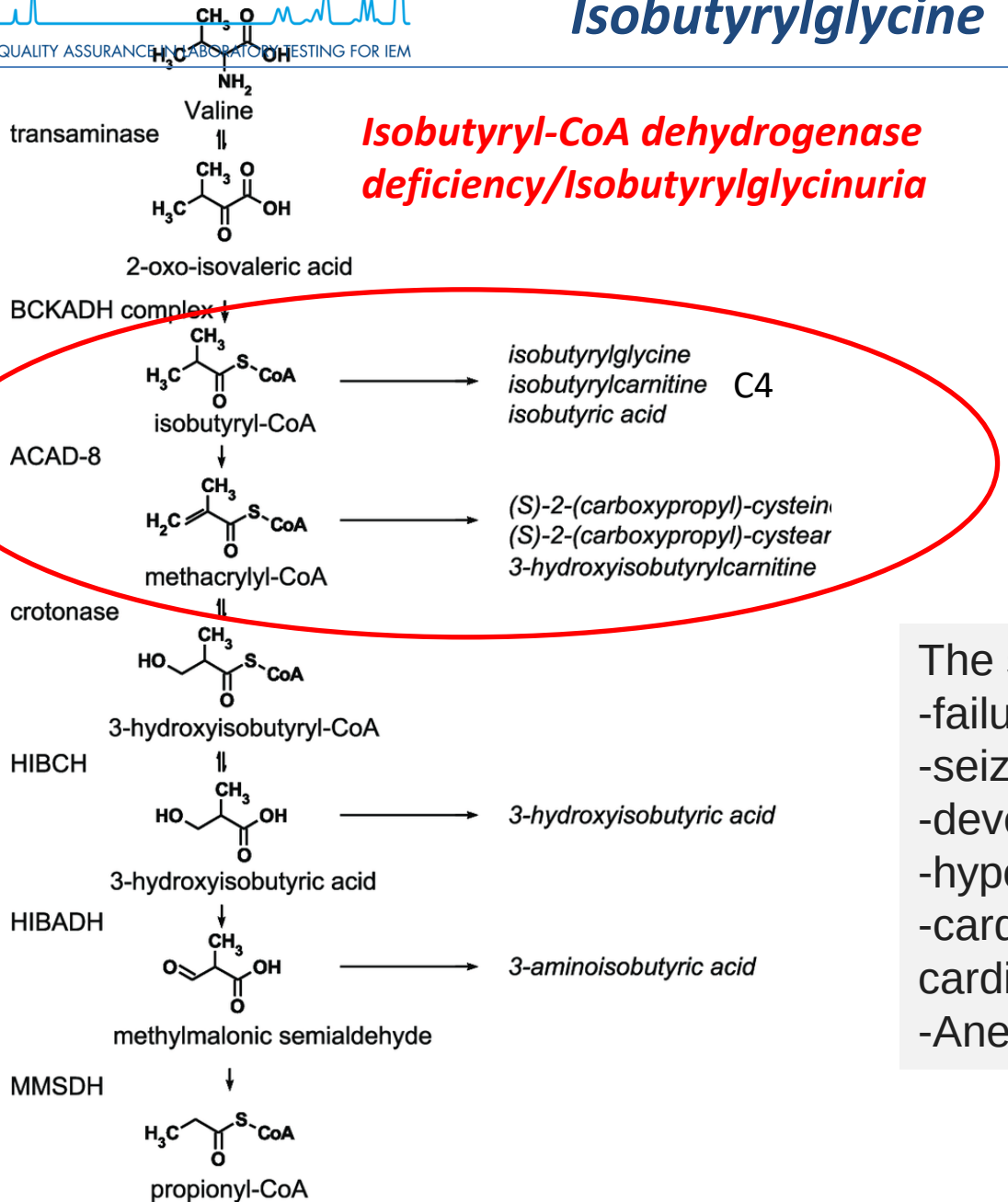
Isobutyrylglycine

Isobutyryl-CoA dehydrogenase deficiency/Isobutyrylglycinuria



The symptoms often include:
-failure to thrive
-seizures
-developmental delay
-hypotonia
-cardiomegaly or cardiomyopathy
-Anemia

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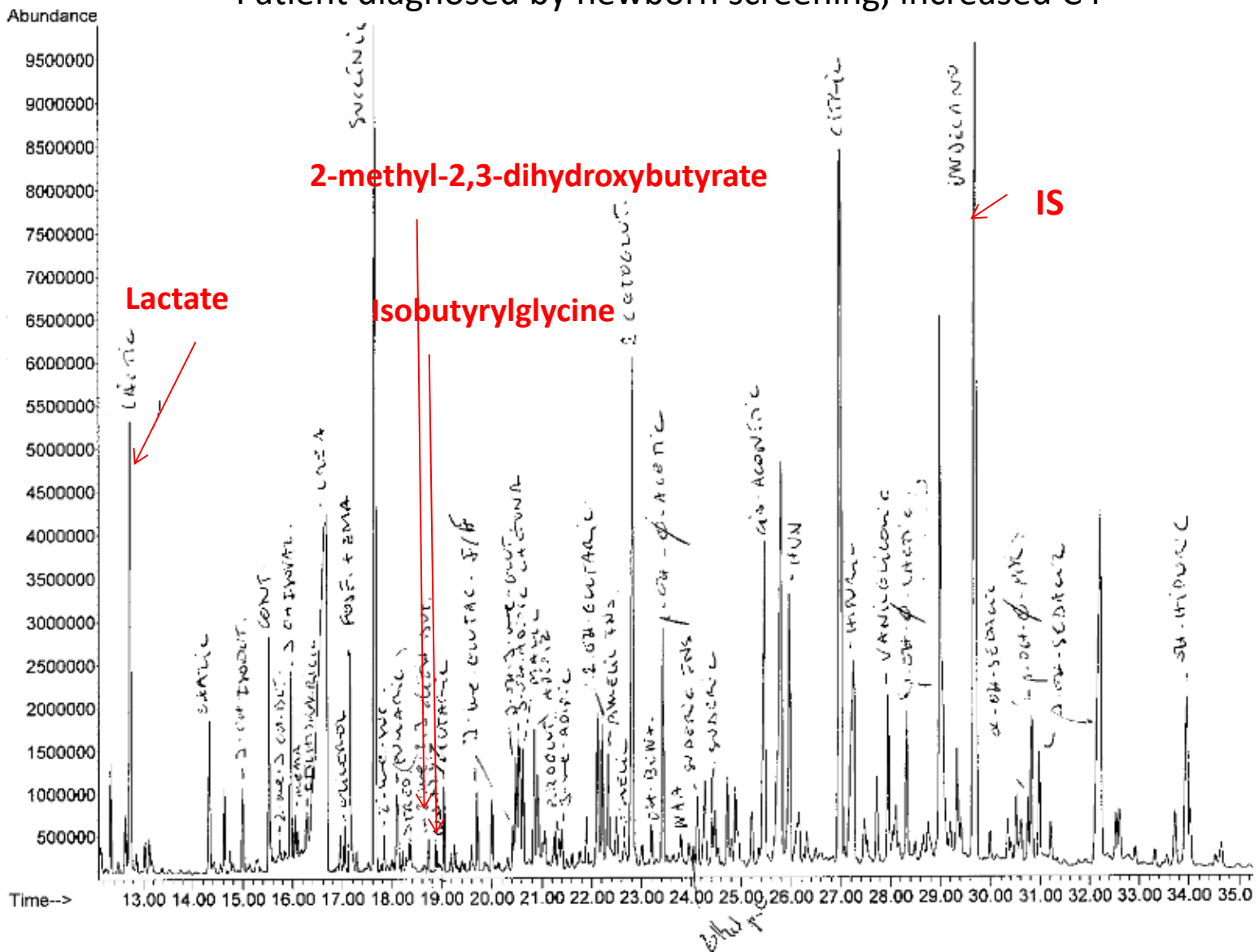
Isobutyrylglycine

Patient diagnosed by newborn screening, increased C4



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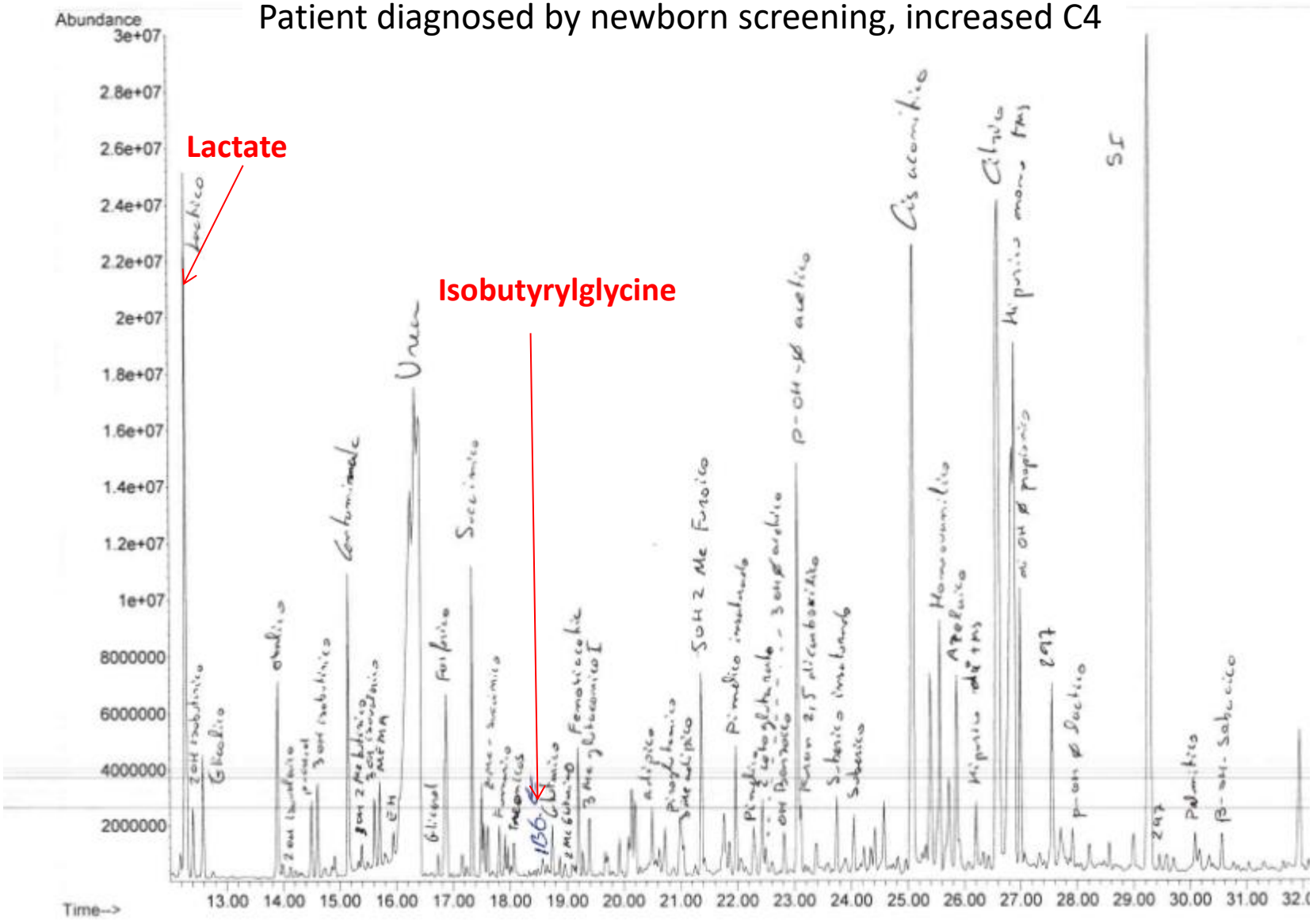
Isobutyrylglycine

Patient diagnosed by newborn screening, increased C4



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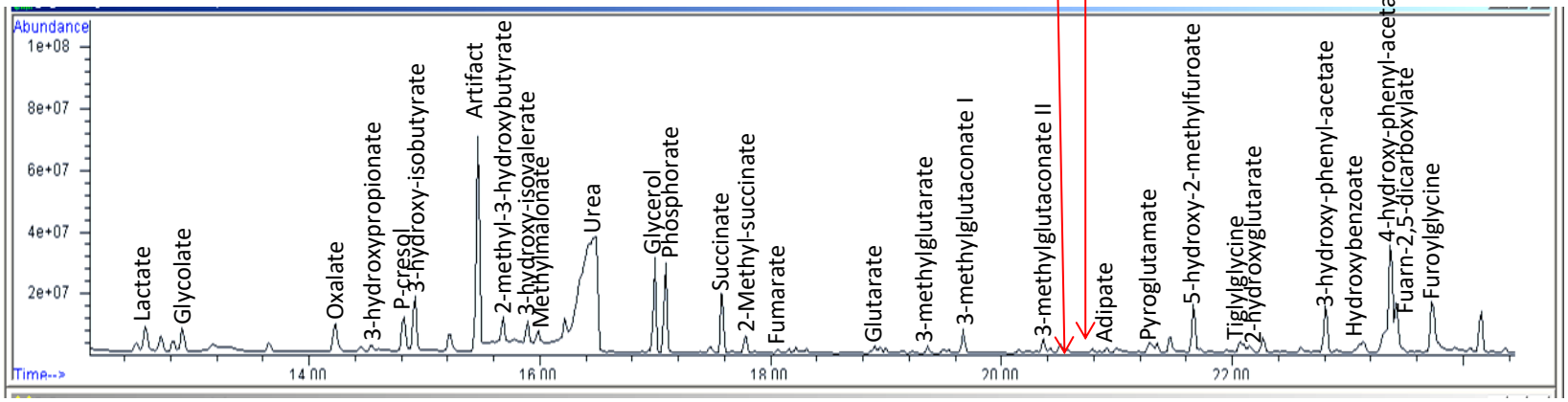
2-methylbutyrylglycine

2-methylbutyrylglycine mono o di-TMS?



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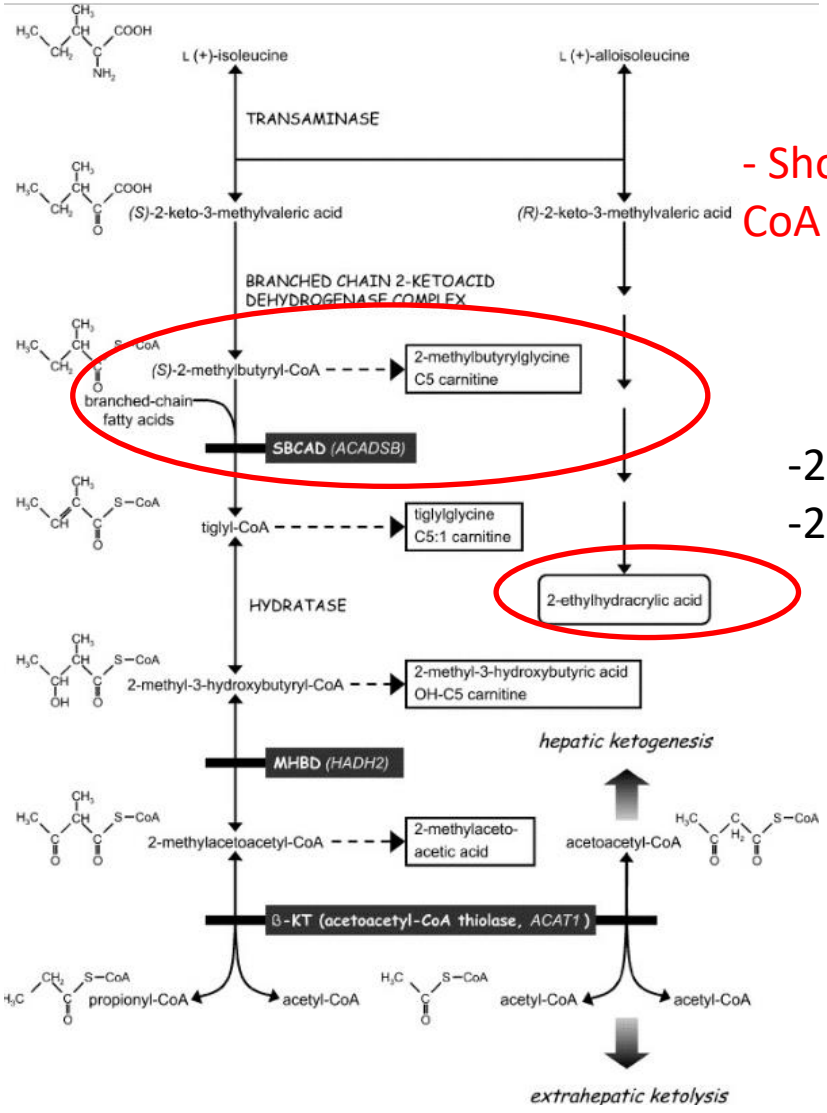
2-methylbutyrylglycine



- Short/branched chain acyl-CoA dehydrogenase/2-methylbutyrylglycinuria

-2-methylbutyrylglycine
-2-methylbutyrylcarnitine (C5)

Korman SH. Inborn errors of isoleucine degradation: a review. Mol Genet Metab. 2006 Dec;89(4):289-99

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2-methylbutyrylglycine

short/branched chain acyl-CoA dehydrogenase

Gibson, K. Met al **2-Methylbutyryl-coenzyme A dehydrogenase deficiency: a new inborn error of L-isoleucine metabolism.** Pediat. Res. 47: 830-833, 2000.

The symptoms often include:
-poor feeding
-lack of energy (lethargy)
-vomiting
-irritability
-difficulty breathing
-seizures
-coma

Additional problems can include:
-poor growth
-vision impairment
-learning disabilities
-muscle weakness,
-delays in motor skills such as standing and walking.

It is unclear why some people with SBCAD deficiency develop health problems and others do not

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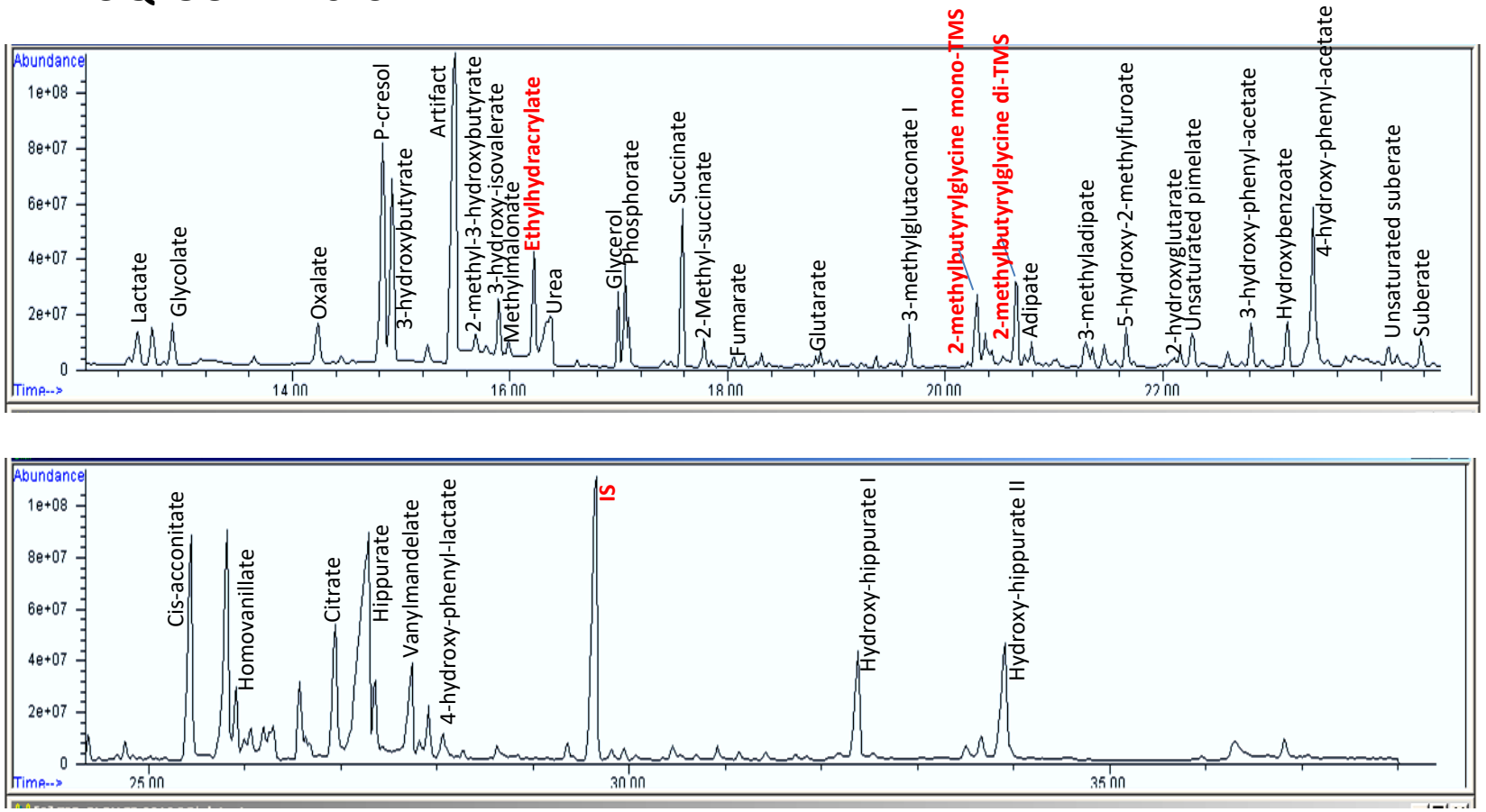
2-methylbutyrylglycine

QLOU: Overall proficiency 87%

This male infant was referred for delayed motor milestones and hypotonia at age 1 y. Currently, at age 6 y, his intellectual development is normal. He is receiving specific treatment.

TIC QLOU-EB-2019-I



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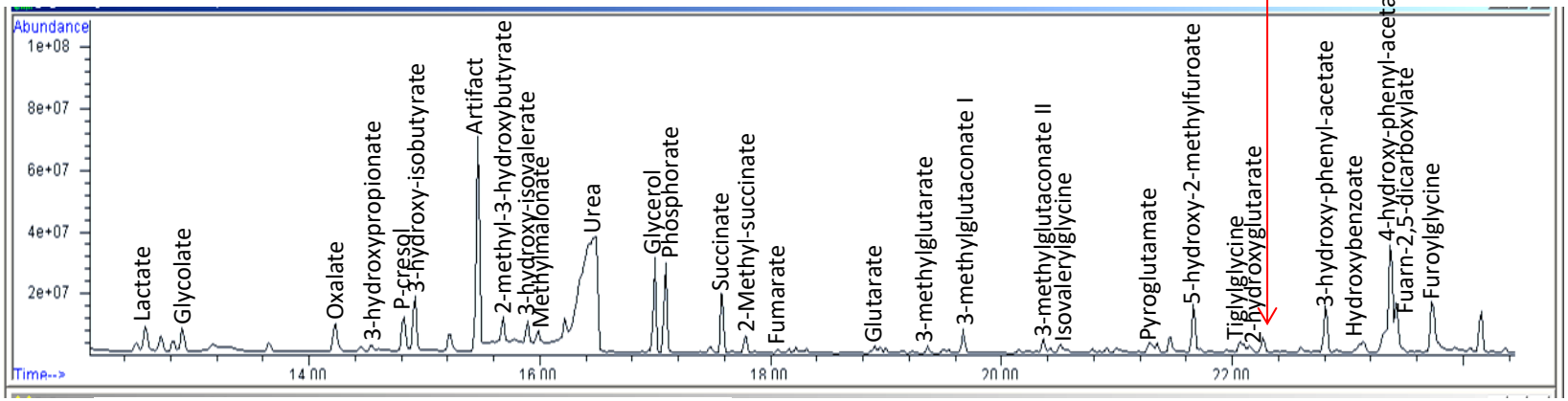
3-hydroxy-glutaric acid

3-hydroxy-glutaric acid?



Lysine, Hydrohylysine, tryptofan

2 - Oxoadipic acid

⇧Glutarylcarnitine ← Glutaril - CoA → Glutaric acid

GCDH

Glutaconyl - CoA

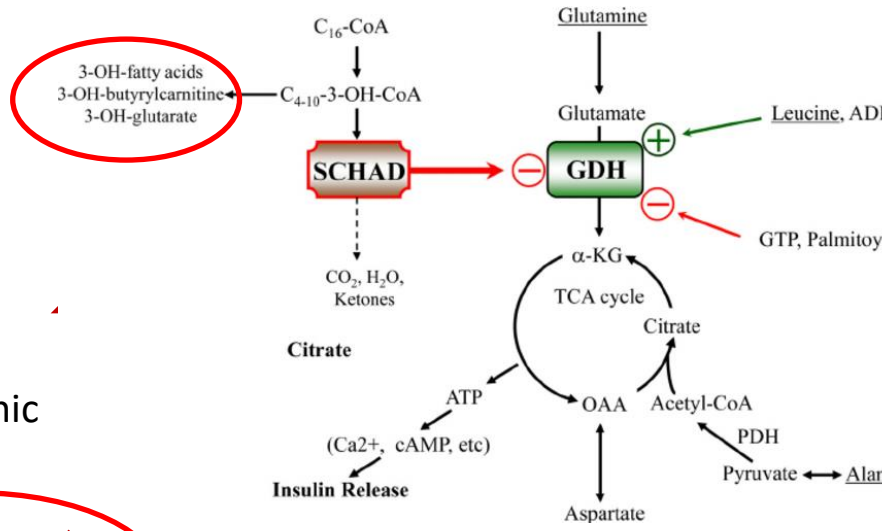
GA1 → Glutaconic acid

Crotonyl - CoA

3 – Hydroxybutyryl - CoA

Acetoacetyl - CoA

3- Hydroxy – ⇧ glutaric acid



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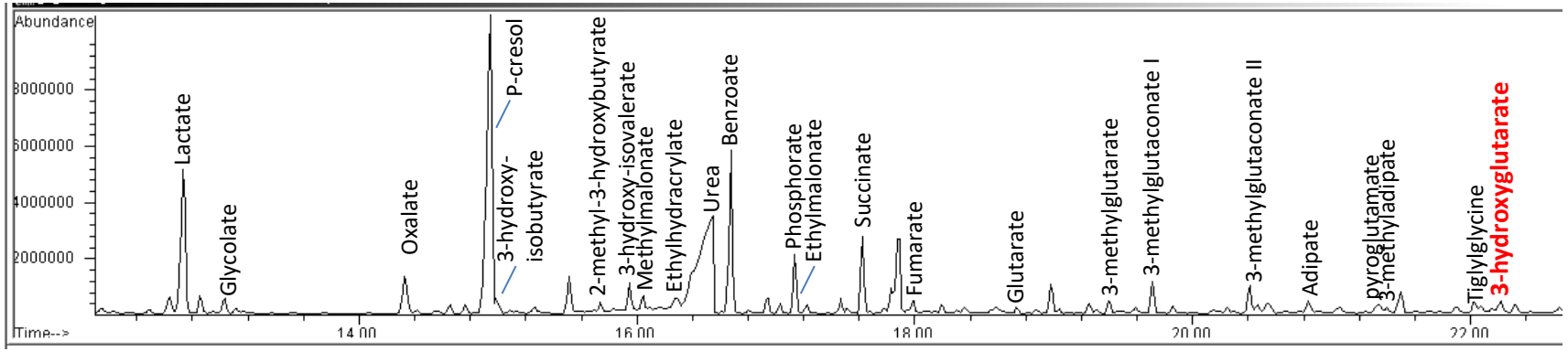
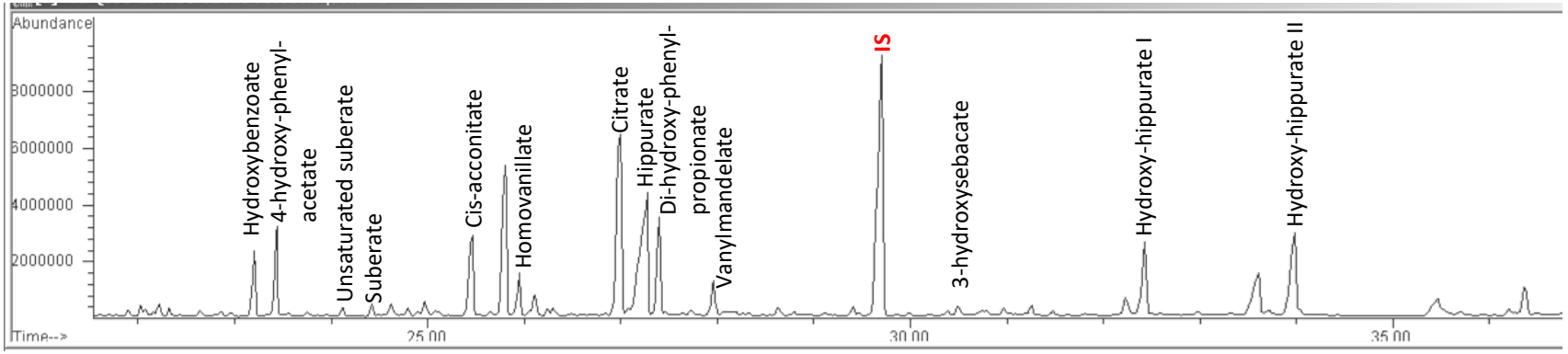
3-hydroxy-glutaric acid

Glutaric aciduria type I low excretor, only slight increase of 3-hydroxyglutaric acid. The sample was collected at 29 years of age, under carnitine treatment. Currently, the patient presents **spastic-dystonic tetraparesia** with severe disability and wheelchair dependency.

QLOU: overall proficiency 47%





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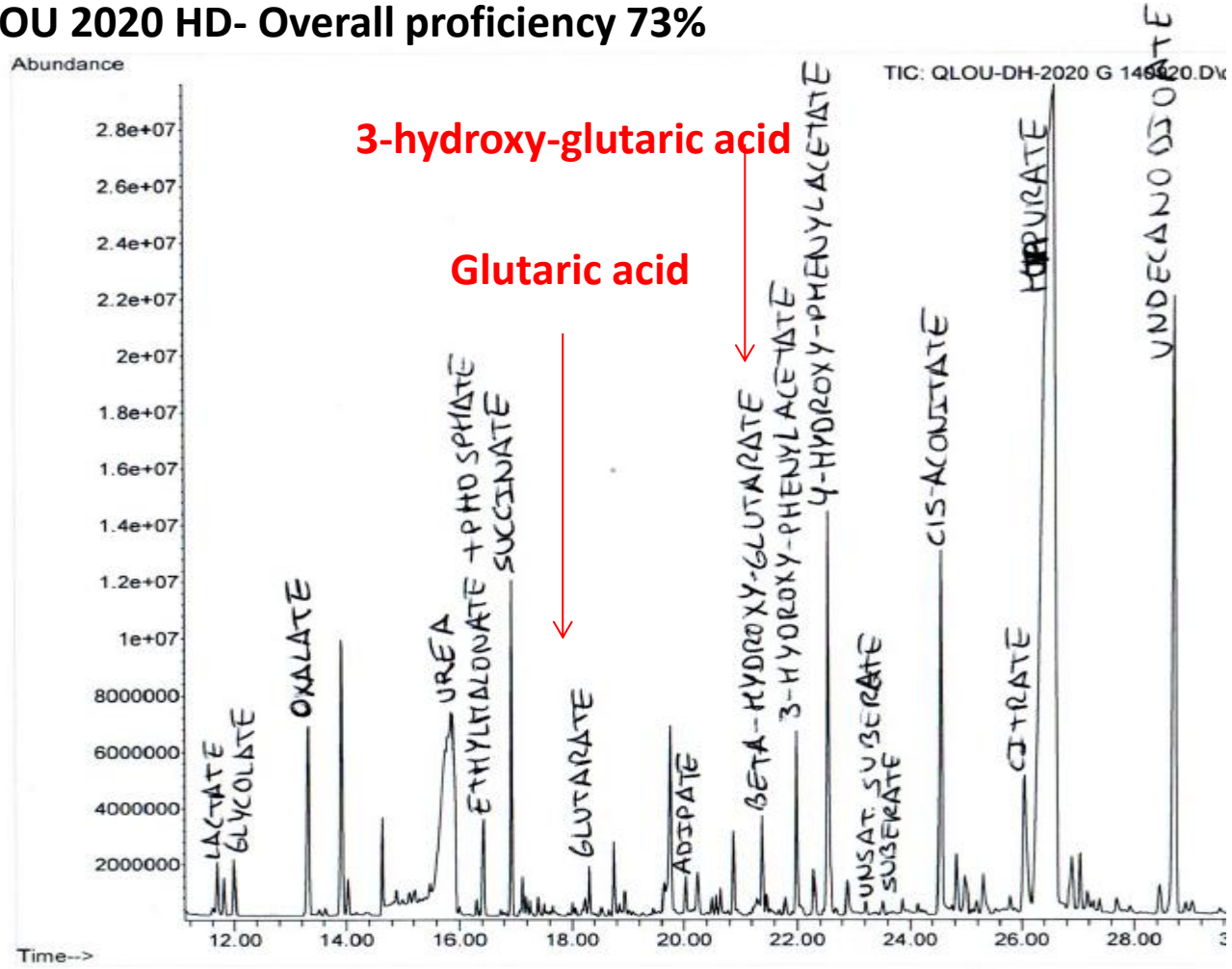
3-hydroxy-glutaric acid

Glutaric aciduria type I low excretor, 2-year-old girl with ataxia and tremor. This girl was hospitalized at 19 months of age with fever and vomiting, reduced consciousness and a generalized seizure

QLOU 2020 HD- Overall proficiency 73%



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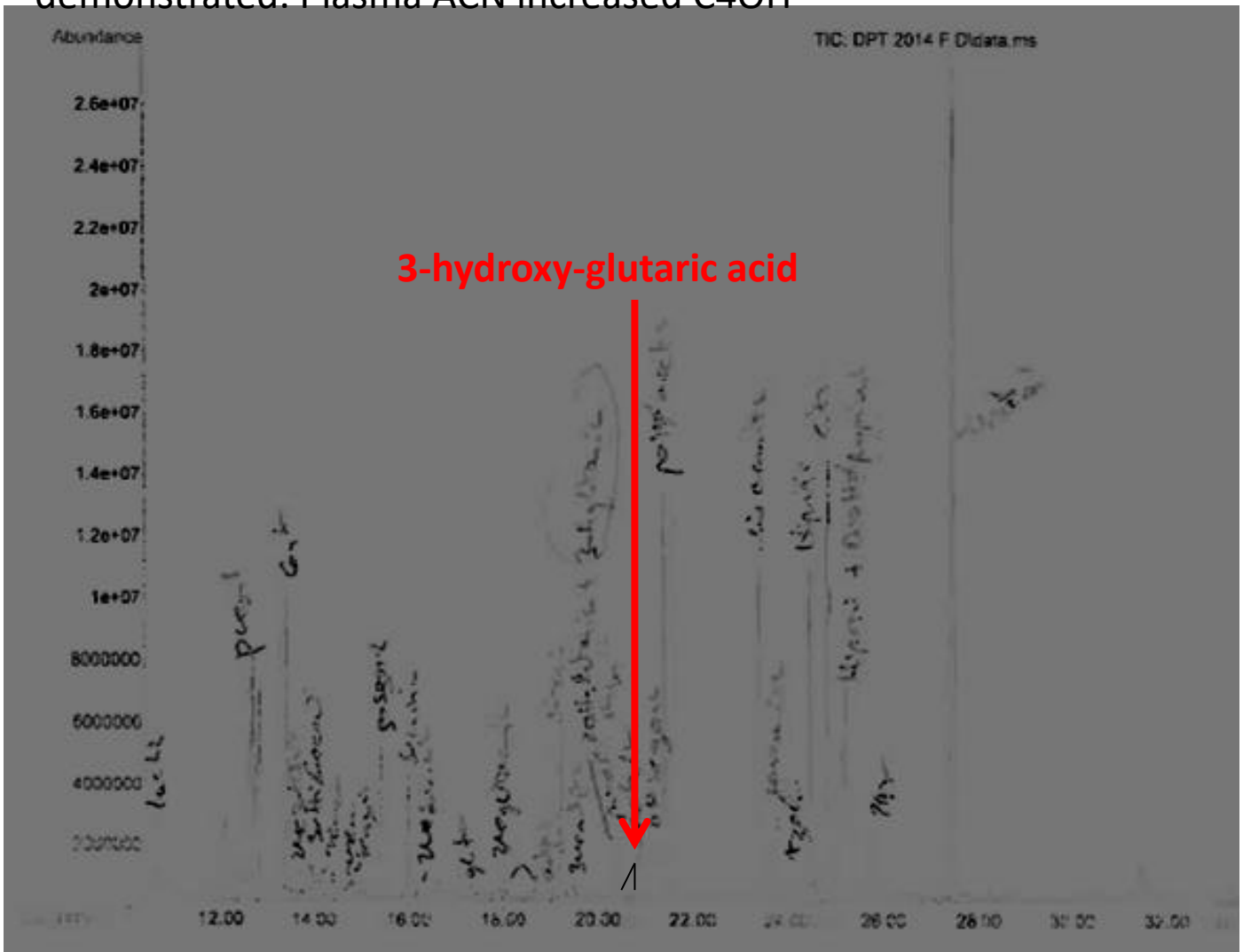
3-hydroxy-glutaric acid

3-hydroxyacyl-coA dehydrogenase (SCHAD) deficiency, **DPT overall proficiency 85%**, 6 months seizures, hepatomegaly and hypoglycemia. At 14 months the **hyperinsulinism** was demonstrated. Plasma ACN increased C4OH



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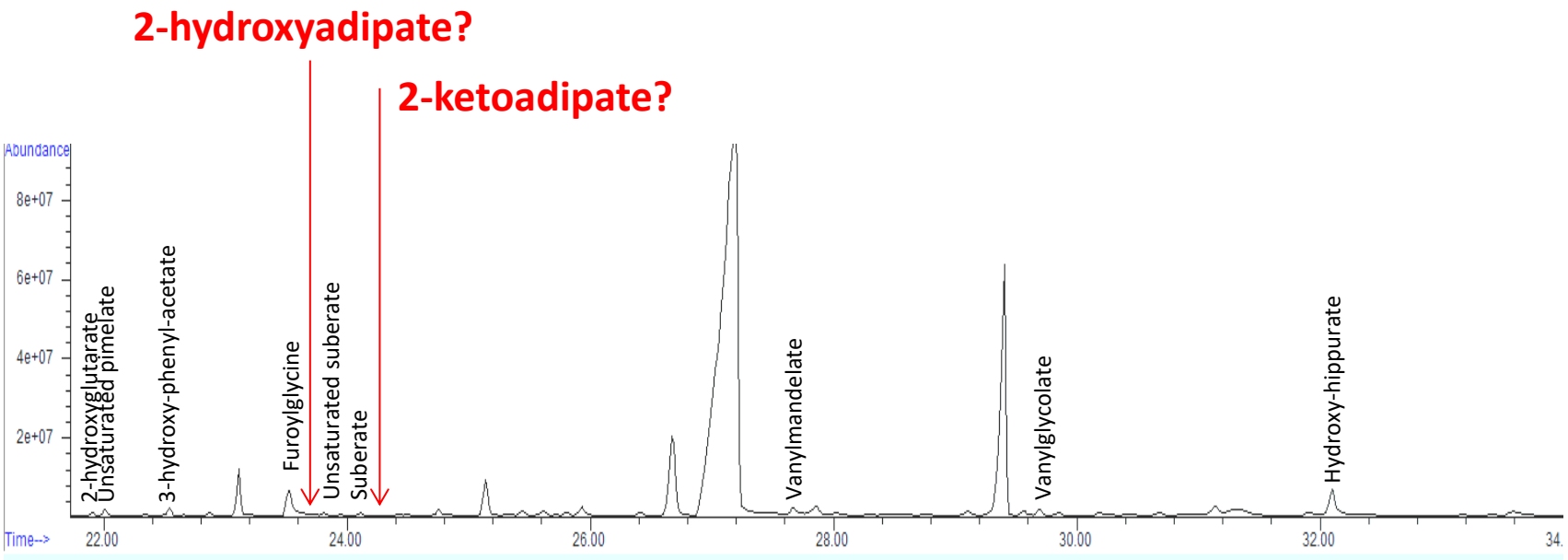
2-hydroxyadipic acid/2-ketoadipic acid

Yes: **increase of gluatarte and ketoglutarate?** → Deficiency of lipoic acid



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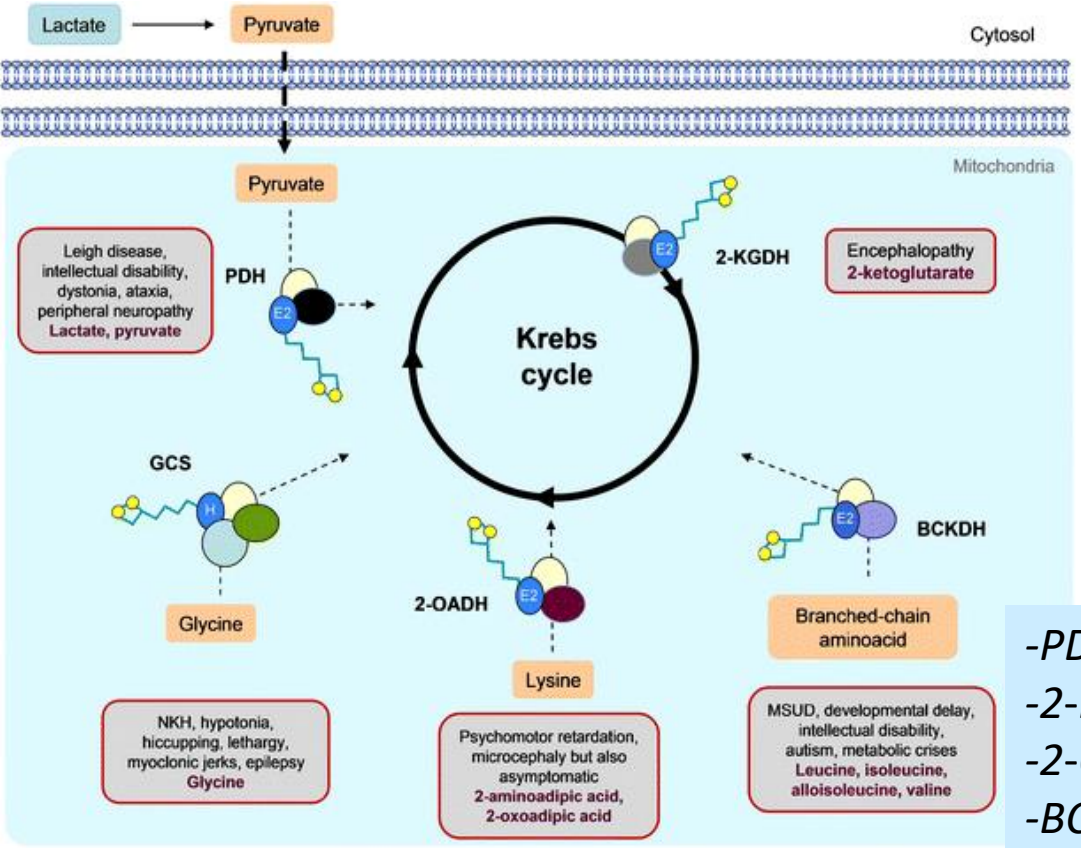
2-hydroxyadipic acid/2-ketoadipic acid

Lipoic acid Synthesis Defects



Lipoic-acid-dependent pathways. Lipoic acid is a cofactor of five enzymatic complexes that play a central role in the mitochondrial energy metabolism by connecting glycolysis with Krebs cycle and regulating the catabolism of branched-chain amino acids, lysine, and glycine.

-*PDH* pyruvate dehydrogenase
-*2-KGDH* 2-ketoglutarate dehydrogenase
-*2-OADH* 2-oxoadipate dehydrogenase
-*BCKDH* branched-chain ketoacid dehydrogenase,
-*GCS* glycine cleavage system

J Inherit Metab Dis (2016) 39:781–793

Differential diagnosis of lipoic acid synthesis defects

Frederic Tort[1] · Xènia Ferrer-Cortes[1] · Antonia Ribes[1]

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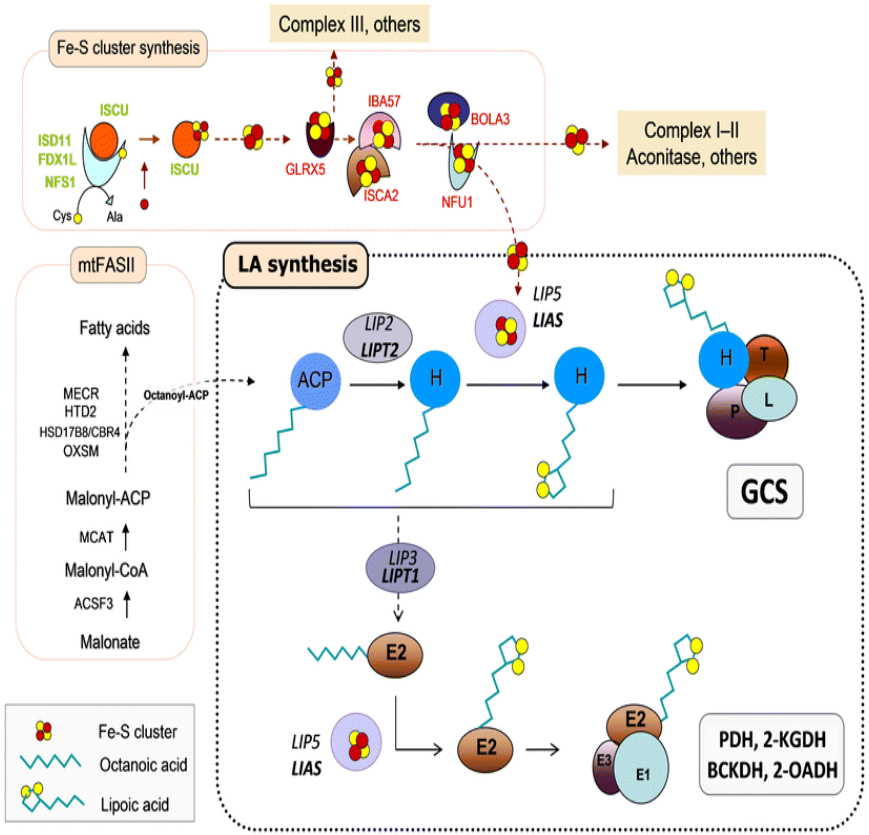
2-hydroxyadipic acid/2-ketoadipic acid

Lipoic acid biosynthesis.



Lipoic acid biosynthesis is a multistep process that involves mitochondrial **fatty acid synthesis** (mtFASII) and **iron–sulphur (Fe-S) cluster biogenesis.** According to knowledge gleaned from investigating yeast, acyl carrier protein (ACP)-conjugated octanoic acid derived from mtFASII is transferred to the H-protein of the glycine cleavage system (GCS) via action of lipoyl(octanoyl) transferase 2 (LIP2) (LIPT2 in humans). Octanoylated H-protein is the substrate for insertion of two sulfur atoms at C-6 and C-8 positions by LIP5 (LIAS in humans). The LIP3 protein (LIPT1 in humans) is responsible for octanoylation and subsequent lipoylation of E2 subunits of the 2-oxoacid dehydrogenase complexes (PDH, 2-KGDH, BCKDH, and 2-OADH). Fe-S cluster biogenesis and mtFASII pathways are summarized. Highlighted in red, Fe-S cluster proteins known to be associated with lipoic acid biosynthesis defects in human

Differential diagnosis of lipoic acid synthesis defects

Frederic Tort[1] · Xènia Ferrer-Cortes[1] · Antonia Ribes[1]

J Inherit Metab Dis (2016) 39:781–793

Table 2 Most common clinical and biochemical features of patients with lipoic acid deficiency

	LIPT2	*LIAS*	*LIPT1*	*NFU1*	*BOLA3*	*IBA57*	*ISCA2*	*GLRX5*
Metabolic pathway	LA synthesis	LA synthesis	LA transfer	Fe-S	Fe-S	Fe-S	Fe-S	Fe-S
Number of reported patients	1	3	4	43	6	3	7	3
Age at onset	8 y	neonatal	0–15 m	0–15 m	0–4 m	0–6 m	3–7 m	2–7 y
Clinical phenotype								
Psychomotor retardation	nr	+	+	+	+	+	+	+/–
Epilepsy	nr	+	–	–	+	nr	nr	+/–
Leukoencephalopathy	+	+	+	+	+	+	+	+
Cardiomyopathy	nr	+/–	+/–	+/–	+	nr	nr	–
Hypotonia	+	+	+	+	+	+	nr	–
Pulmonary hypertension	nr	–	+	+	nr	nr	nr	–
Biochemical phenotype								
Metabolites								
Lactate	–	+	+	+	+	+	+	+/–
Glycine	+	+	–	+	+	+	+	+
Altered organic acids	nr	2-KG	2-KG	2-KG 2-AA	2-KG	nr	nr	
Enzymatic activities								
PDH deficiency	+	+	+	+	+	+	–/ nr	+
Low substrate oxidation rate	+	+	+	+	nr	nr	nr	nr
Respiratory chain deficiency	–	+/–	–	I, II	I, II	I, II	–/ I	–
GCS	nr	+	–	+	+	nr	nr	+

PDH pyruvate dehydrogenase, *GCS* glycine cleavage system, *2-KG* 2-ketoglutarate, *2-AA* 2-aminoadipate, *m* month, *y* year, *nr* not reported

Differential diagnosis of lipoic acid synthesis defects

Frederic Tort[1] · **Xènia Ferrer-Cortes**[1] · **Antonia Ribes**[1]

J Inherit Metab Dis (2016) 39:781–793

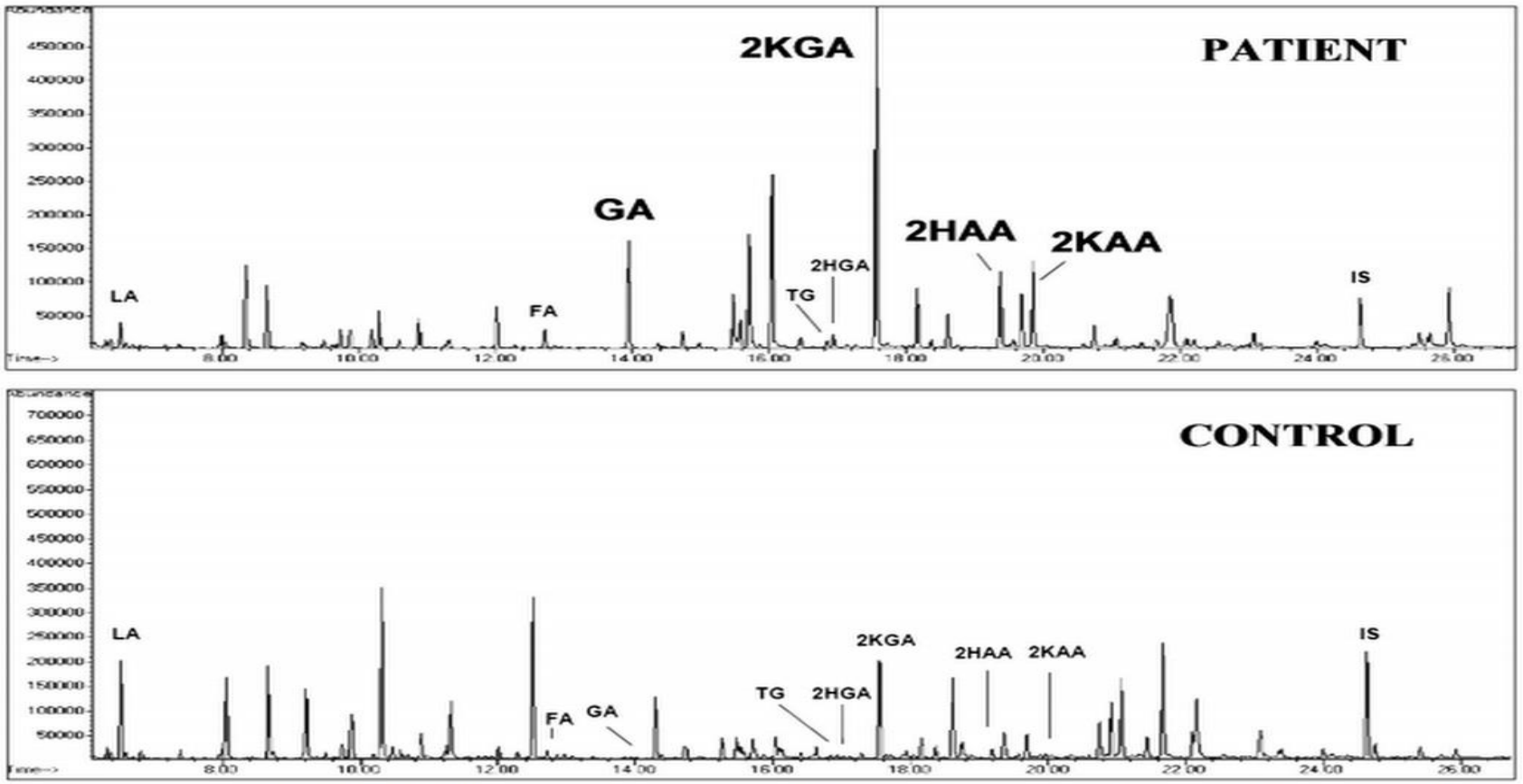


Fig. 3 Urine organic acid profile of a NFU1 patient analyzed by gas chromatography–mass spectrometry. Characteristic peaks are indicated in *bold*: 2-ketoglutaric acid (2KGA), 2-hydroxyadipic acid (2HAA), 2-ketoadipic acid (2KAA). The excretion of glutaric acid (GA) was probably due to spontaneous decarboxylation of 2-ketoadipic acid. The following metabolites were not increased in all cases: lactic acid (LA), fumaric acid (FA), 2-hydroxyglutaric acid (2HGA), and tyglylglycine (TG). *IS* internal standard (undecanoic acid). Modified from Navarro-Sastre et al. 2011

Differential diagnosis of lipoic acid synthesis defects

Frederic Tort[1] · Xènia Ferrer-Cortes[1] · Antonia Ribes[1]

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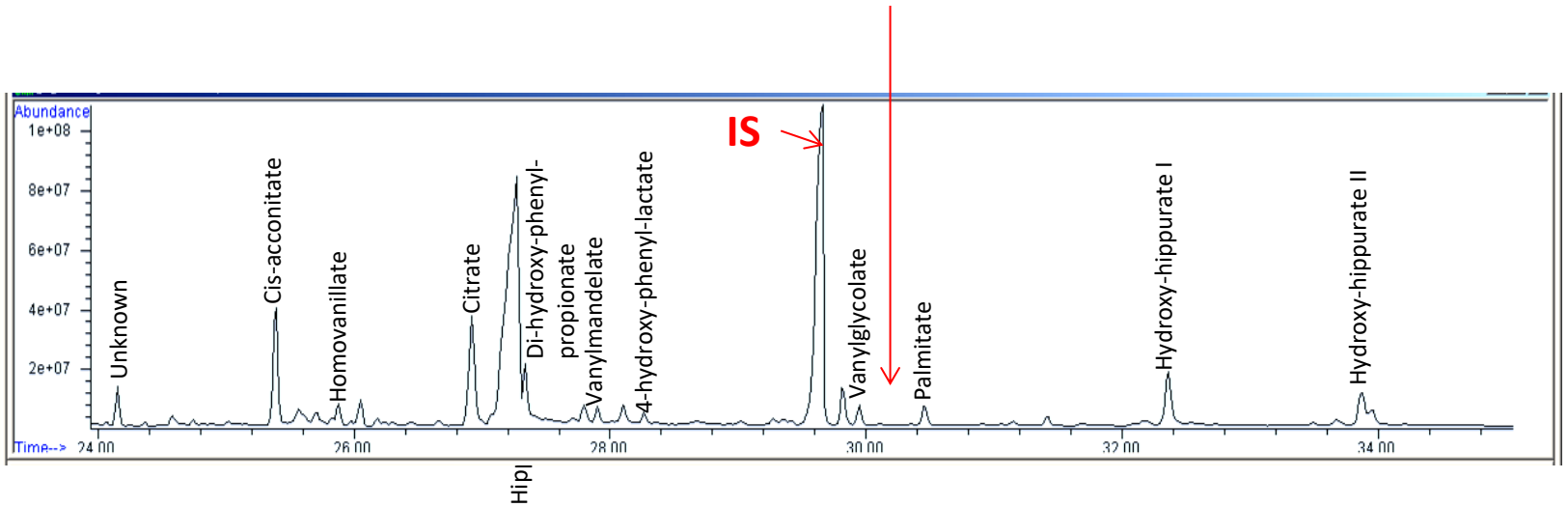
Vanillactic acid

Vanillactate?



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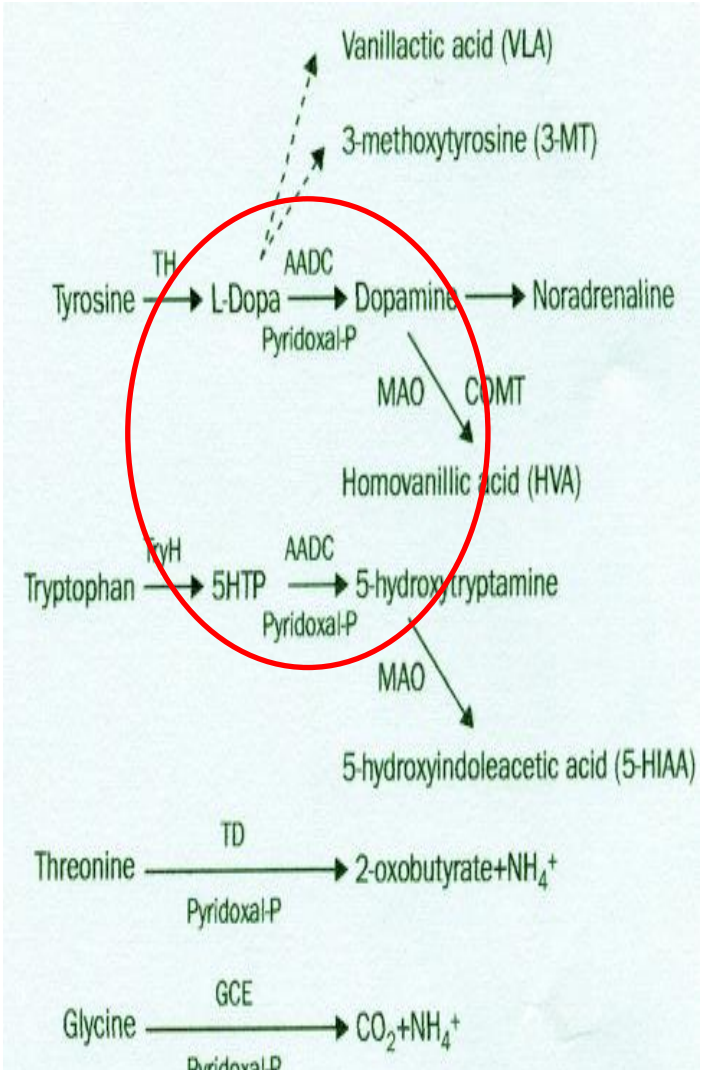
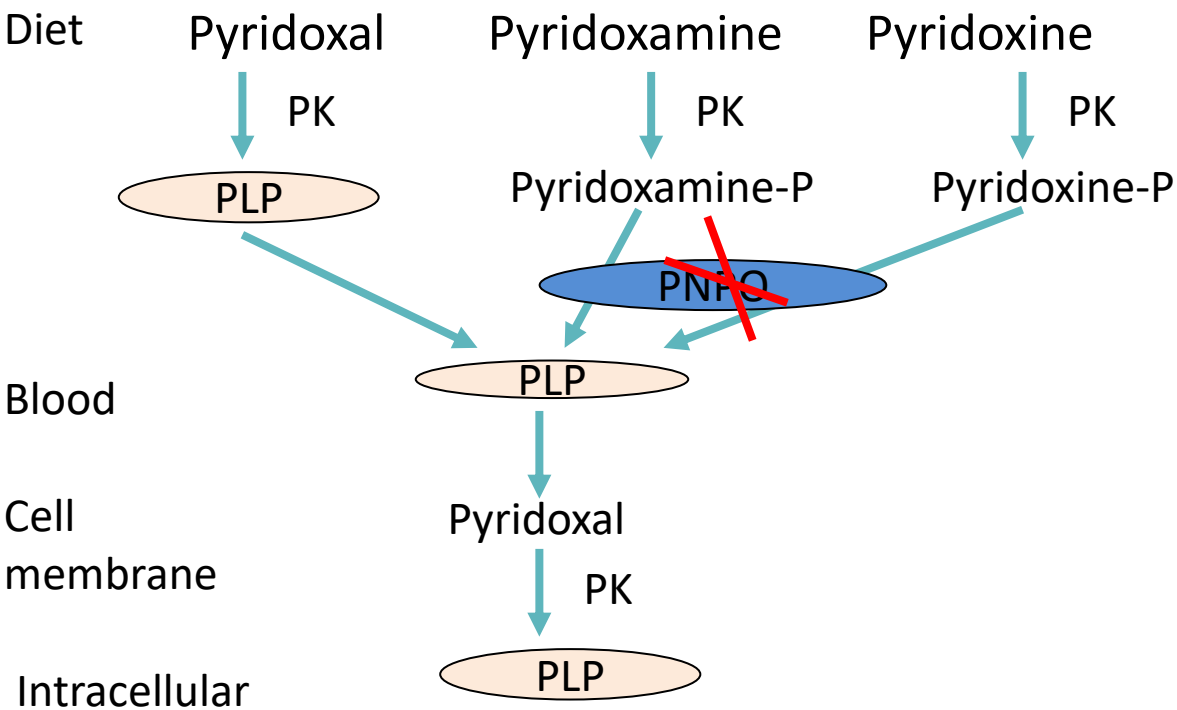
Vanillactic acid

Increase of vanillactate-difernetial diagnosis :

-Aromatic aminoacid decarboxylase (AADC) deficiency

-Pyridox(am)ine 5'-phospfate oxidase (PNPO) deficiency





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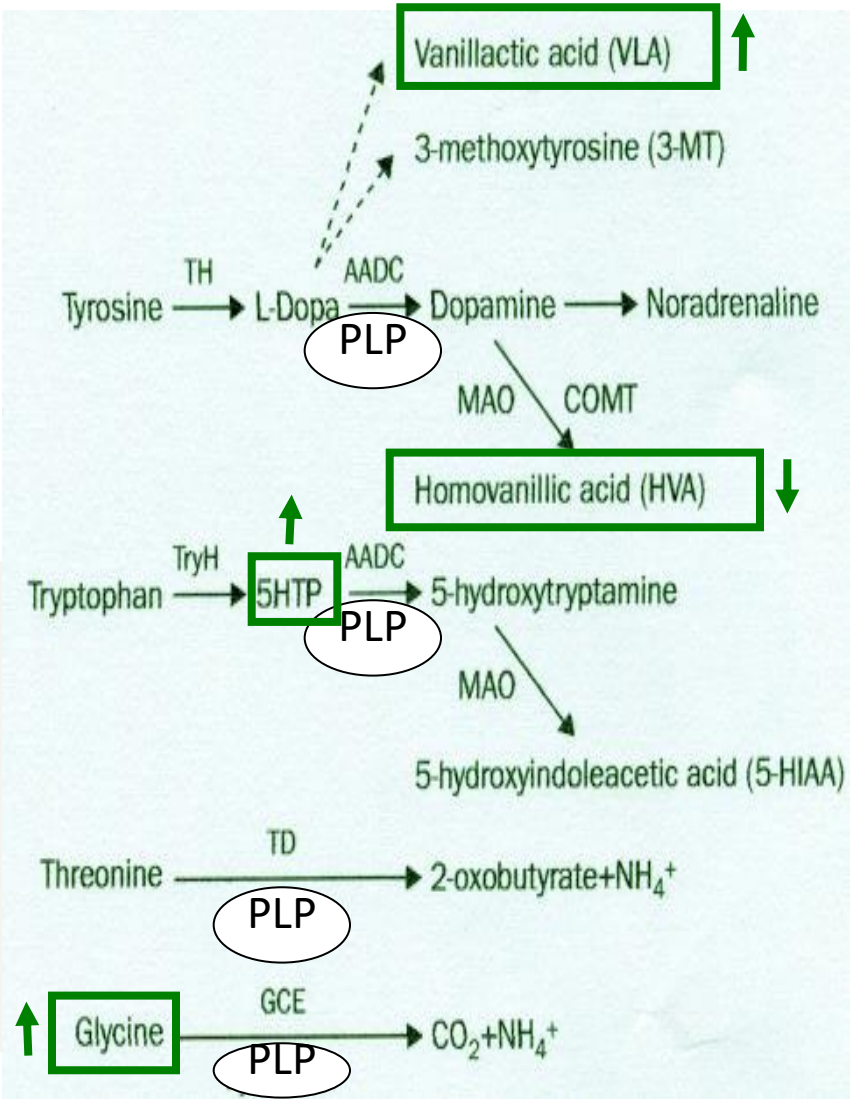
Vanillactic acid

Increase of vanillactate-difernetial diagnosis :

-Pyridox(am)ine 5'-phospfate oxidase (PNPO) deficiency

Pyridoxal Phosphate (PLP) cofactor:

- **Aromatic aminoacids**
- **Tryptophan synthase**
- **Threonine synthase**
- **Threonine dehydratase**
- **Glycine metabolism**
- **Glutamate metabolism**
- **Cysteine synthase**
- **Cystathionine beta-synthase**
- **Cysteine synthase**
- **Pyridoxal phosphate-dependent deaminase**
- **D-serine ammonia-lyase**
- **Threonine dehydratase**



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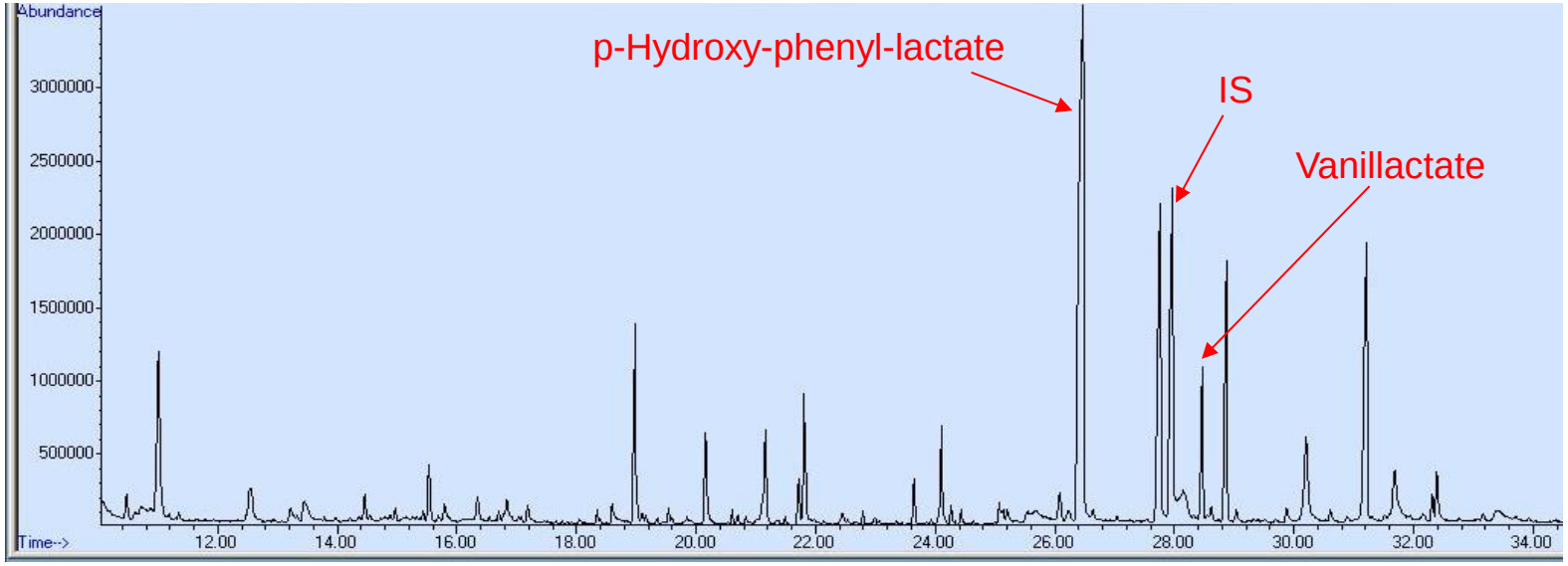
Vanillactic acid

-Patient that from birth he showed a faltering cry, continuous hypersalivation with orobuccal rhythmic movements, myoclonus and marked hyperexcitability. with hypochromic anemia, leukopenia, thrombocytopenia and coagulopathy that requiered tranfusion of hemoderivates. The boy developed renal dysfunction and hepatomegaly. **Sizures where present intra-utero and at birth without response to antiepileptic drugs**



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Summary

Try to perform extract ion or SIM detection for these small compounds:

- **2-methyl-3-hydroxibutirate/2-methylacetoacetate**
- **Malonate**
- **4-hydroxybutyrate**
- **Uracil/Thimine/di-Hydrouracil/di-hydrothymine/orotic acid/citrulline cyle derivate**
- **2-methyl-2,3-dihydroxybutyrate**
- **Isobutyrylglycine**
- **2-methylbutyrylglycine**
- **3-hydroxyglutarate**
- **2-Hydroxyadiate/2-ketoadipate**
- **Vanillactate**

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Organic Acid Workshop

22 October 2021

Camilla Scott
SA Sheffield QLOU

- (New/newly identifiable) Disorders that can be identified by clues in the qualitative organic acid profile:
  - Tango 2
  - HMG CoA Synthase deficiency
  - Aromatic L-amino acid decarboxylase(AADC) deficiency
  - Amino Acylase 1 deficiency
  - PEPCK deficiency

TANGO2

- Transport and Golgi Organization protein 2 deficiency.
- Identified in 2019.
- Only 29 cases described.

Tango 2

- TANGO-2 related metabolic encephalopathy
- Presenting features:
  - Cardiomyopathy
  - Metabolic crisis (hypoglycaemia, elevated lactate, mild hyperammonaemia)
  - Developmental delay
  - Regression
  - Seizures
  - Ataxia/muscle weakness
  - Intermittent episodes of Rhabdomyolysis

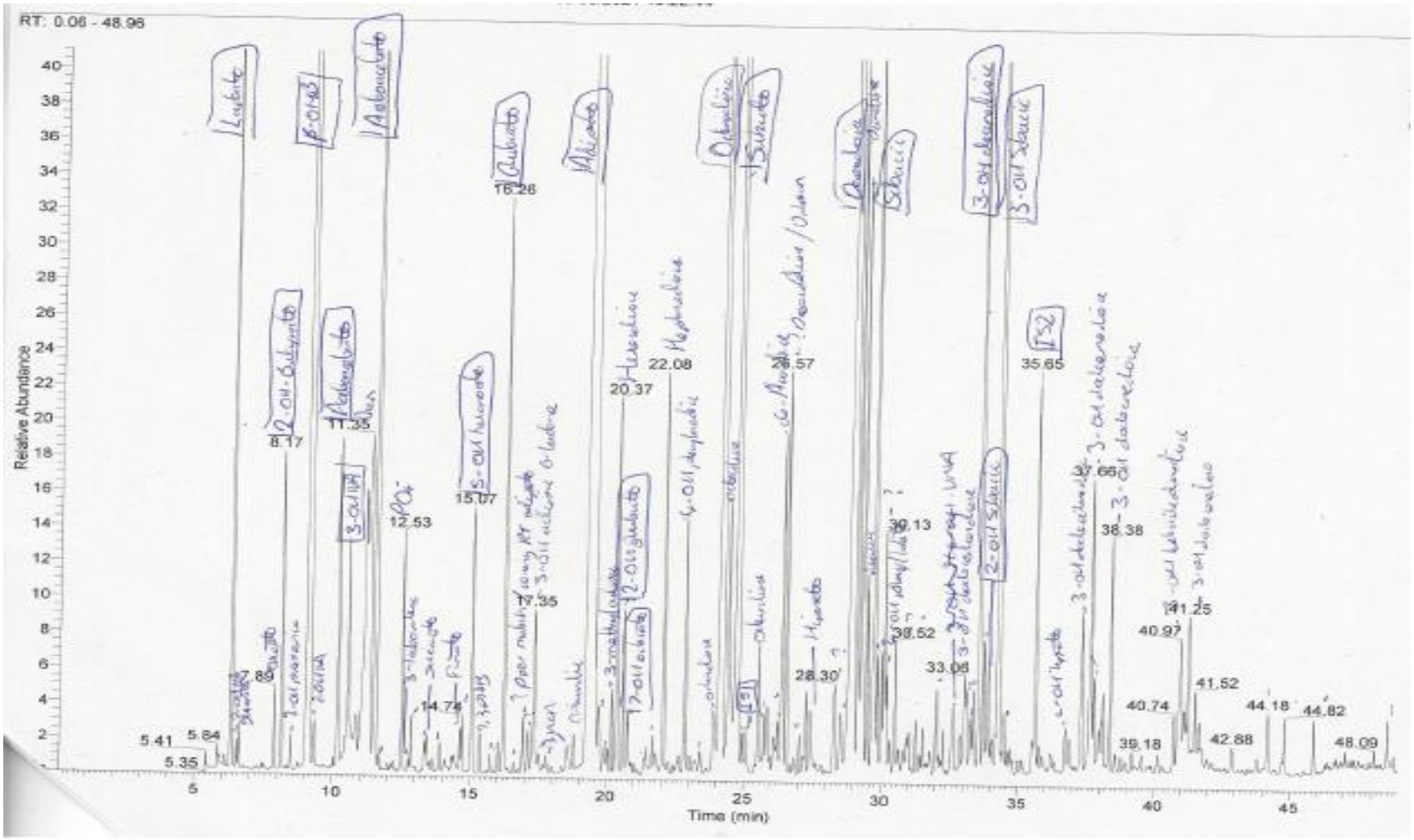
Diagnosis

- Bilateral variants in *TANGO2* on molecular testing.
- Organic acids can show marked ketoacidosis and lactic acidosis with a dicarboxylic aciduria.
- Acylcarnitine's may show elevated C14:1.

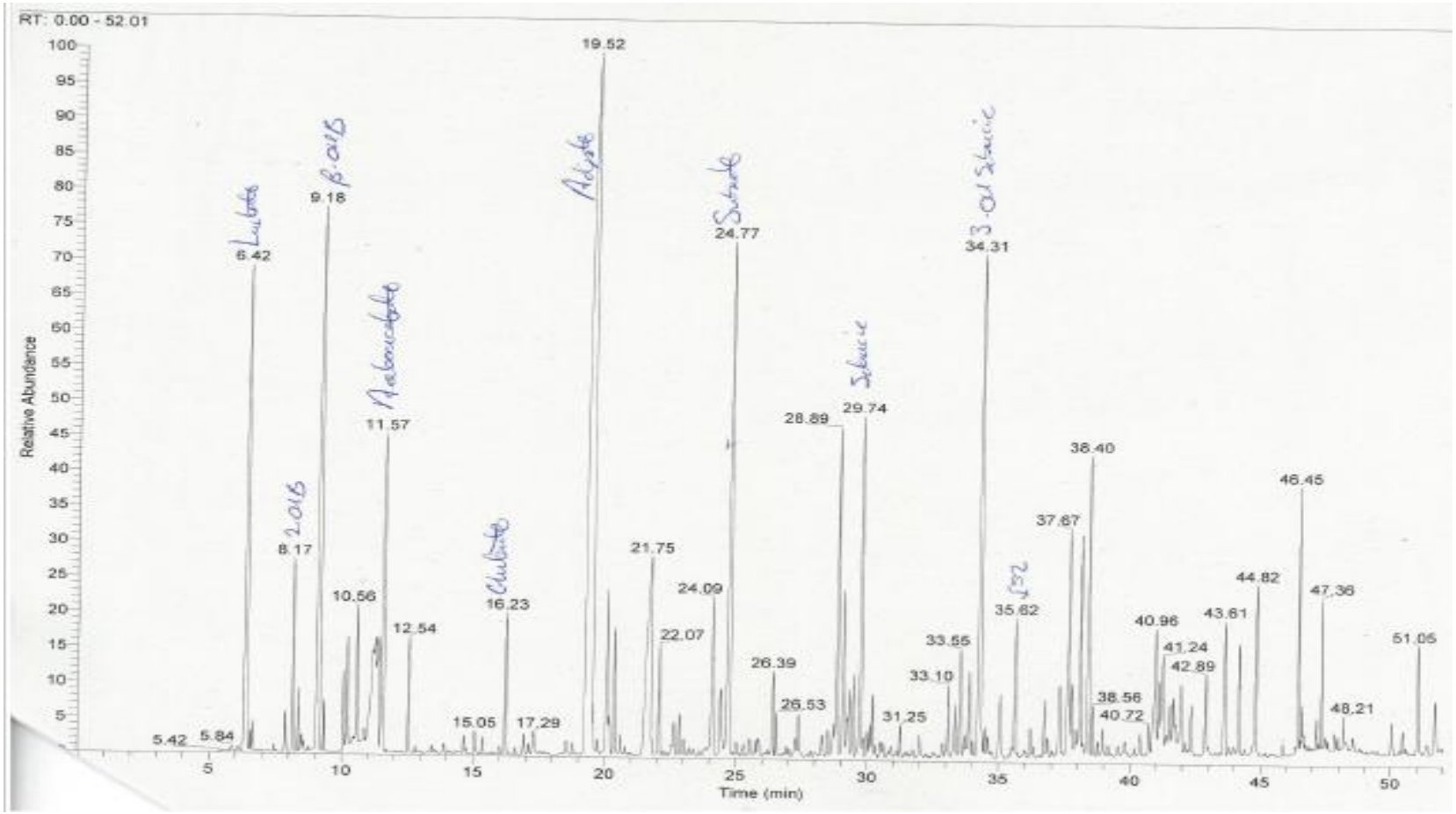
Clues to differentiate TANGO2 vs VLCADD

- Presenting features can be similar to VLCADD with rhabdomyolysis but also a more acute metabolic decompensation.
- Plasma acylcarnitine's can mimic VLCADD with an increased C14:1.
- Urine organic acids will demonstrate a patient in metabolic crises with ketosis DCA & lactic aciduria.
- One third of patients will also have hypothyroidism.

OA Chromatogram for TANGO2 patient



OA Chromatogram for TANGO 2 patient



Acylcarnitine profile TANGO2 in a recent case Bristol/Sheffield

- DBS consistent with VLCADD
- Plasma no significant abnormality
- Organic acids mixed picture of ketosis, lactate and dicarboxylic aciduria
- However clinical symptoms of the patient were more of a metabolic crises and the patient was subsequently confirmed to have TANGO2

Mitochondrial 3-hydroxy-3methylglutaryl-CoA synthase deficiency

- Autosomal recessive disorder of ketone body synthesis.
- Very rare (around 20 patients described)
- Associated with hypoketotic, hypoglycaemia, hepatomegaly and encephalopathy following a period of fasting.
- However its important to note that it is not always associated with hypoglycaemia and there are cases described of moderate ketosis.

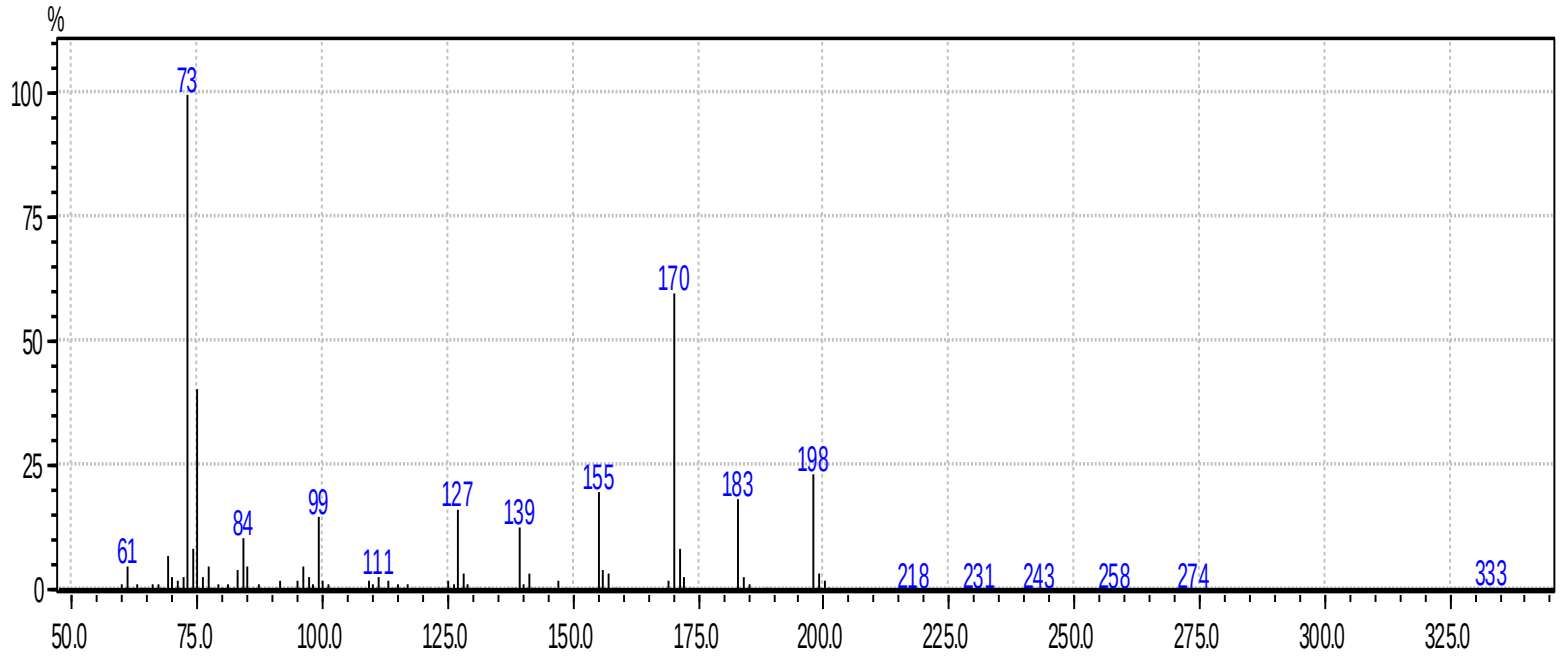
- Key biochemical & clinical clues:
  - Encephalopathy
  - Hepatomegaly
  - Severe metabolic acidosis
  - Mild hyperammonemia
  - Hypertriglycerideamia

  Overlap with many other IMD conditions and are relatively non specific

Urine organic acids

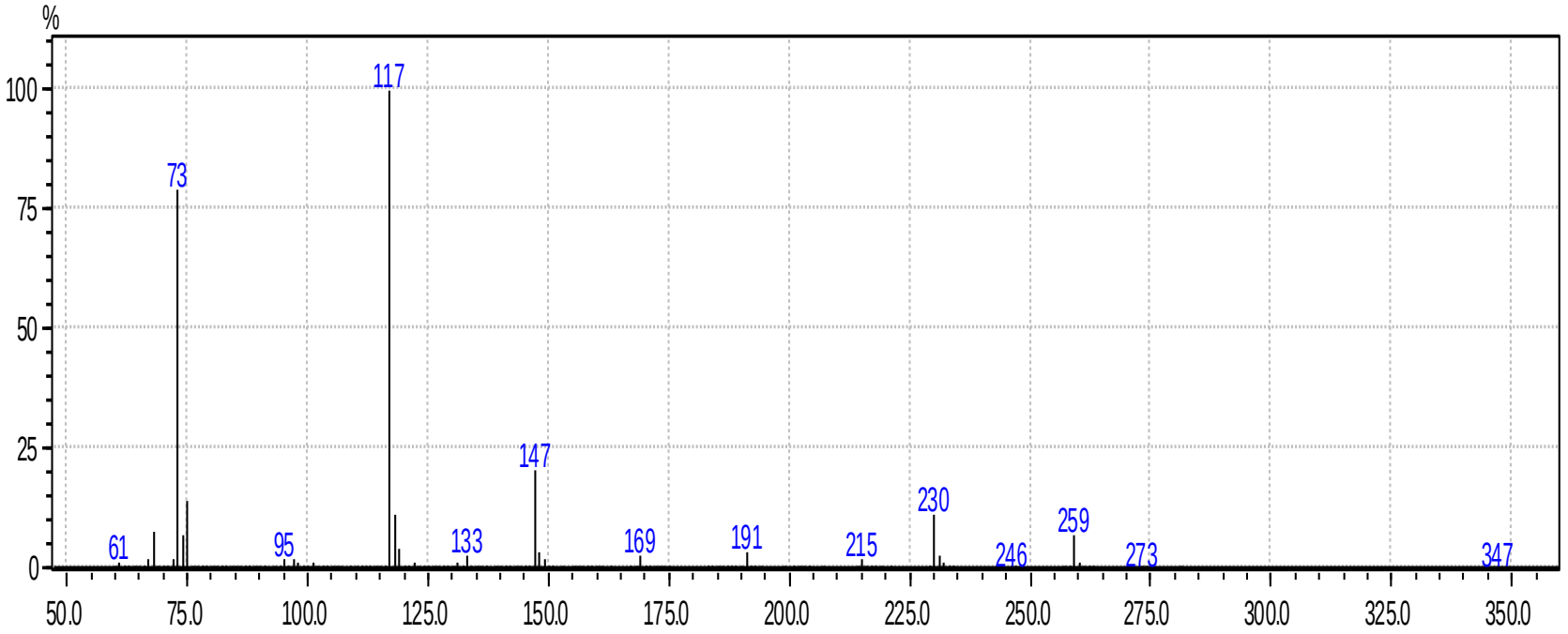
- 3-hydroxydicarboxylic aciduria
- 4-hydroxy-6-methyl-2-pyrone (4HMP) novel organic acid that appears during episodes of decompensation
- Trans-5-OH-hex-2-enoic
- (Note – this metabolite may be present in episodes of severe ketosis)
- Marked increased **glutarate**
- Marked increased adipate
- Mild increased 3 hydroxy glutarate

4 hydroxy-6-methyl-2-pyrone ion profile



RT 15 mins

Trans-5-OH-hex-2-enoic ion profile



RT 14.2 mins

Confirm by genetic analysis of *HMGCS2*

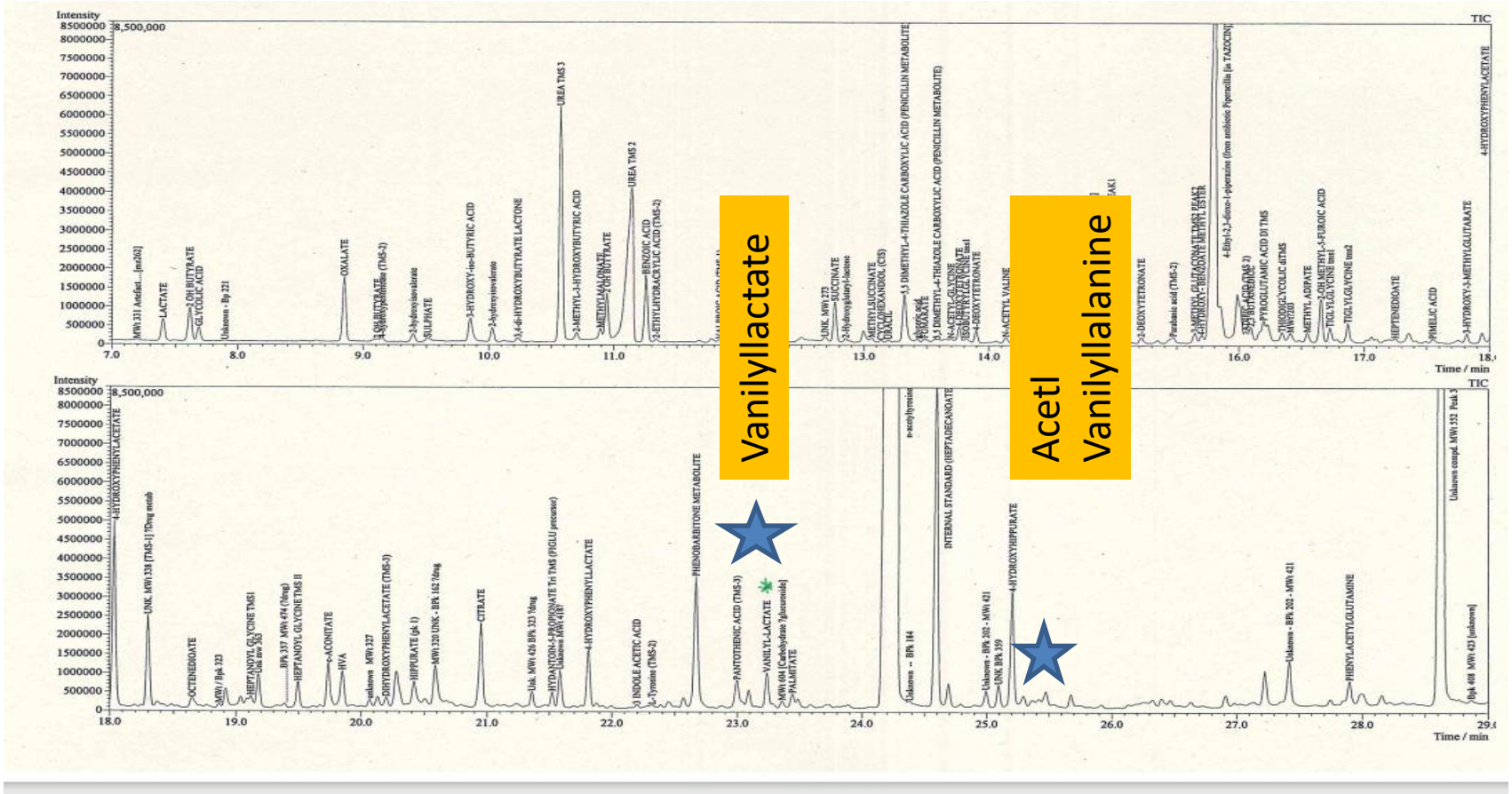
Aromatic L-amino acid decarboxylase deficiency (AADC)

- Not a new disorder.
- Decreased activity of aromatic-l-amino acid decarboxylase which is an enzyme involved in the synthesis of dopamine and serotonin.
- Clinically presenting with hypotonia, decreased muscle tone and movement disorder.
- Diagnosis is by CSF analysis/genetics.
- Urine organic acids can give a clue to the diagnosis.

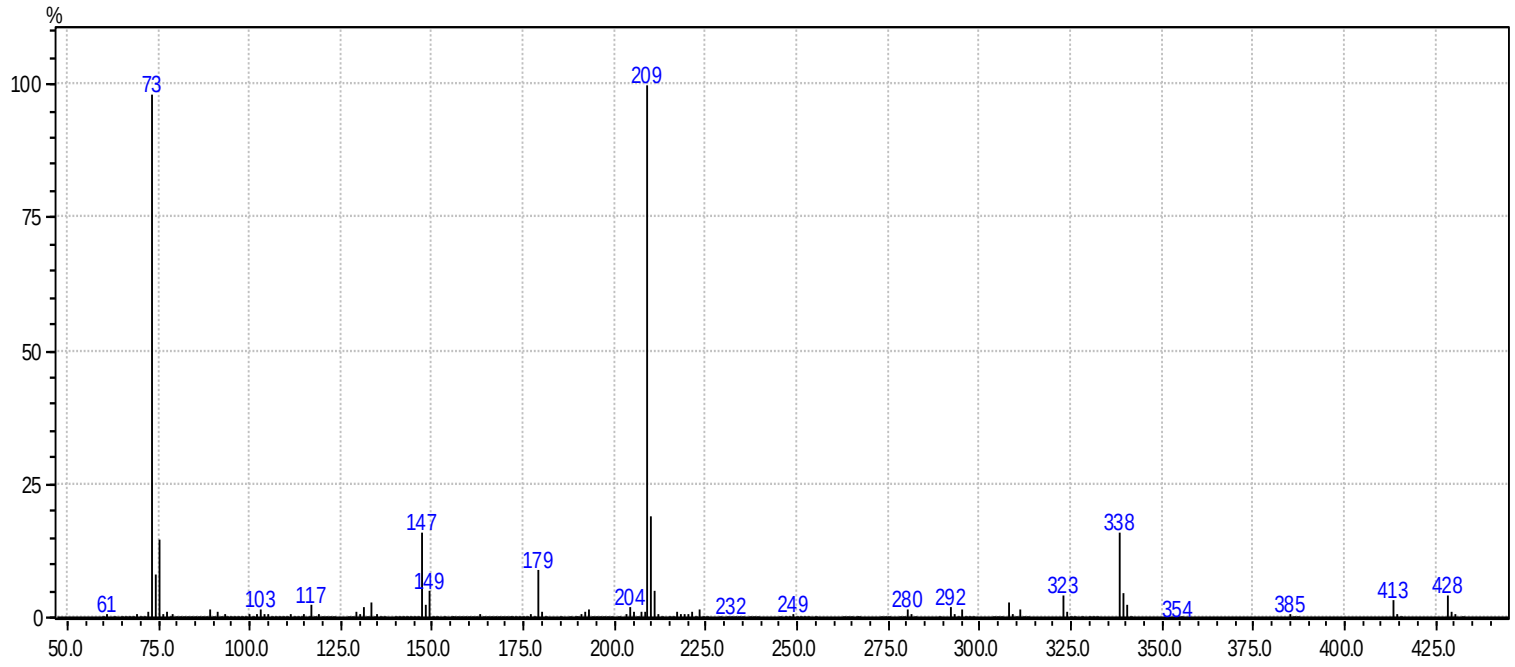
Urine organic analysis for AADC

- Presence of small of moderate peaks of vanillactic acid.
- N-acetyl-vanilylalanine is also present.
- Note the elevation is often mild.
- The VLA/VMA ratio by semi quantitative measurement has been described in the literature.

AADC

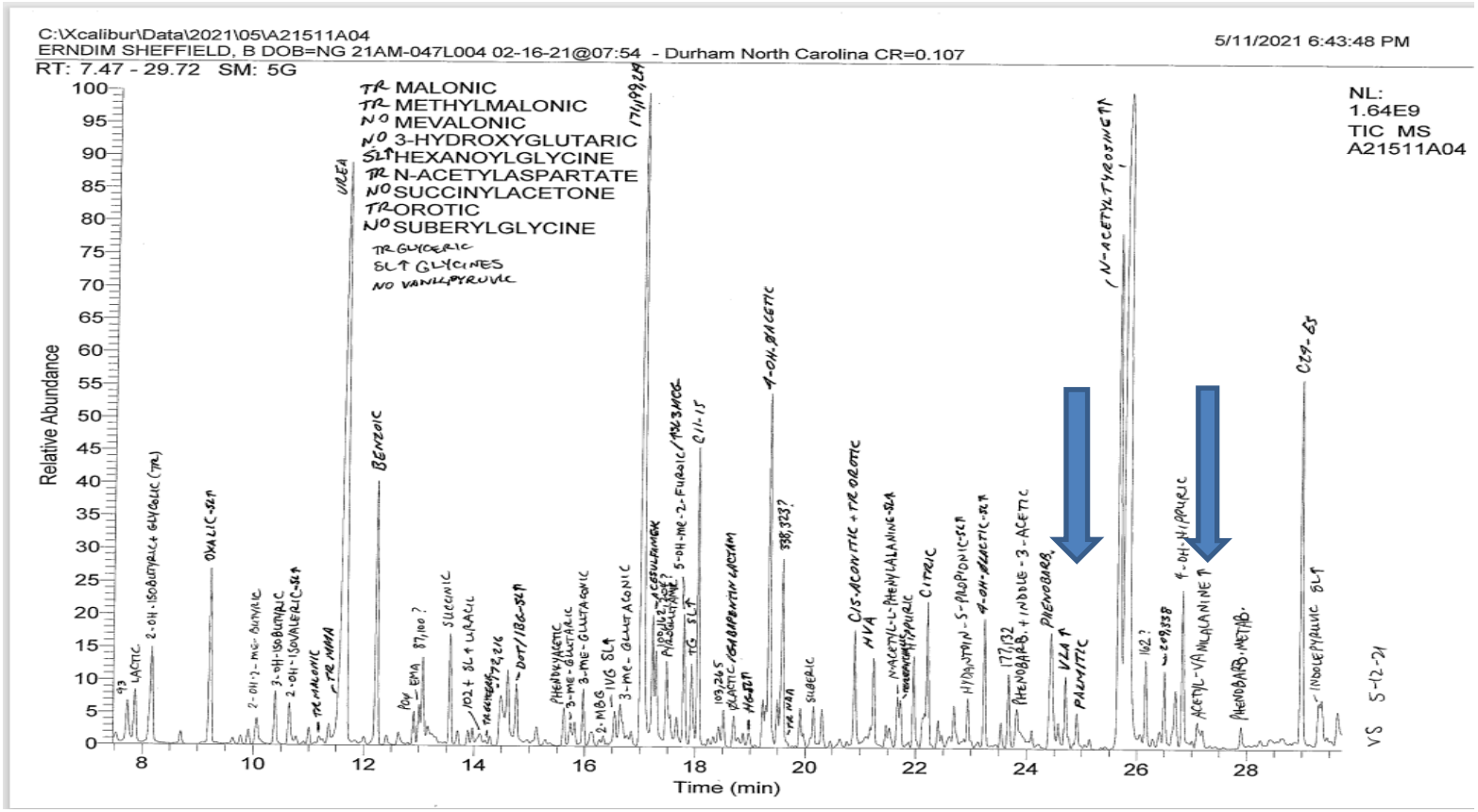

Vanilyllactate
Acetl Vanilyllalanine
Intensity
TIC
Time / min

Vanilyllactate ion profile

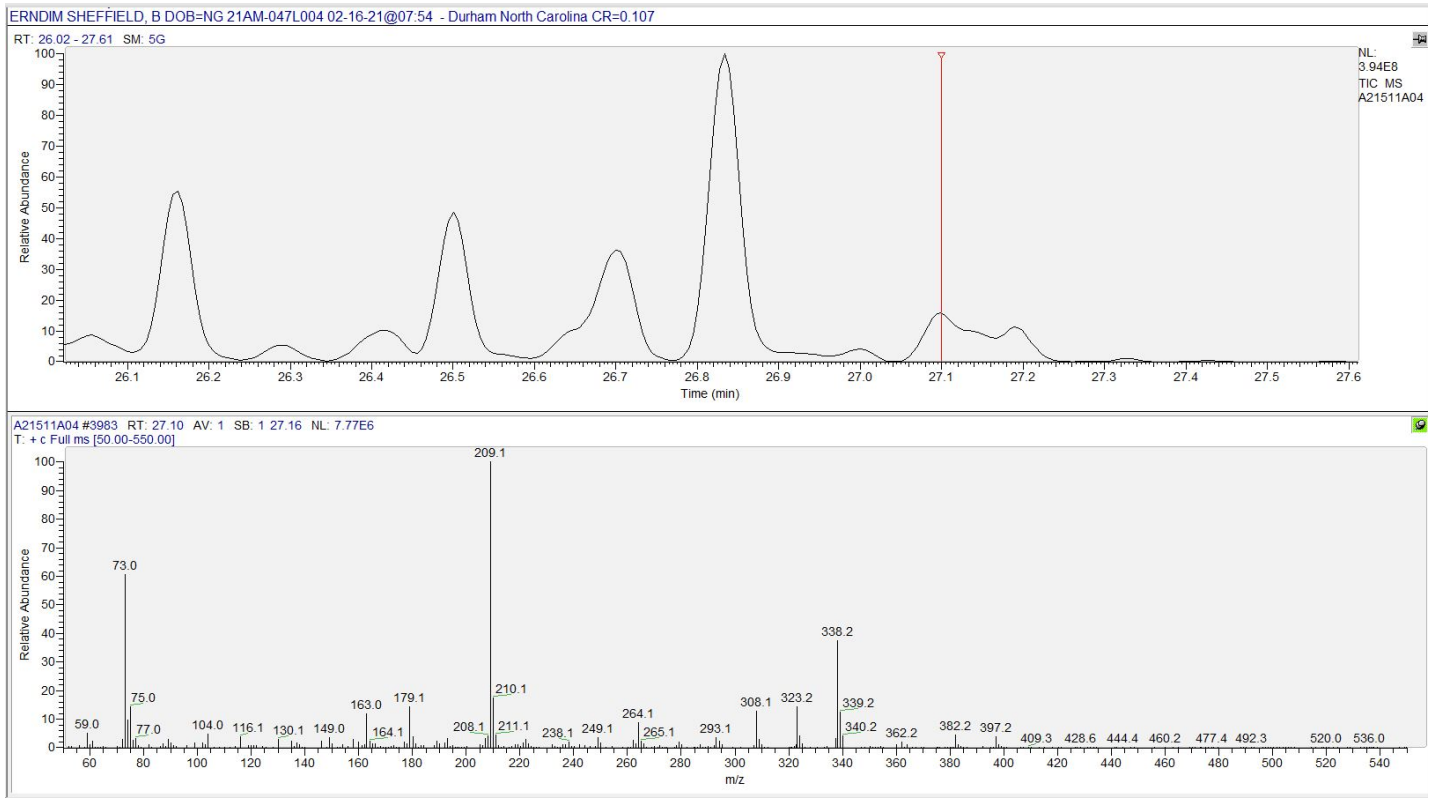


RT 23.5 mins

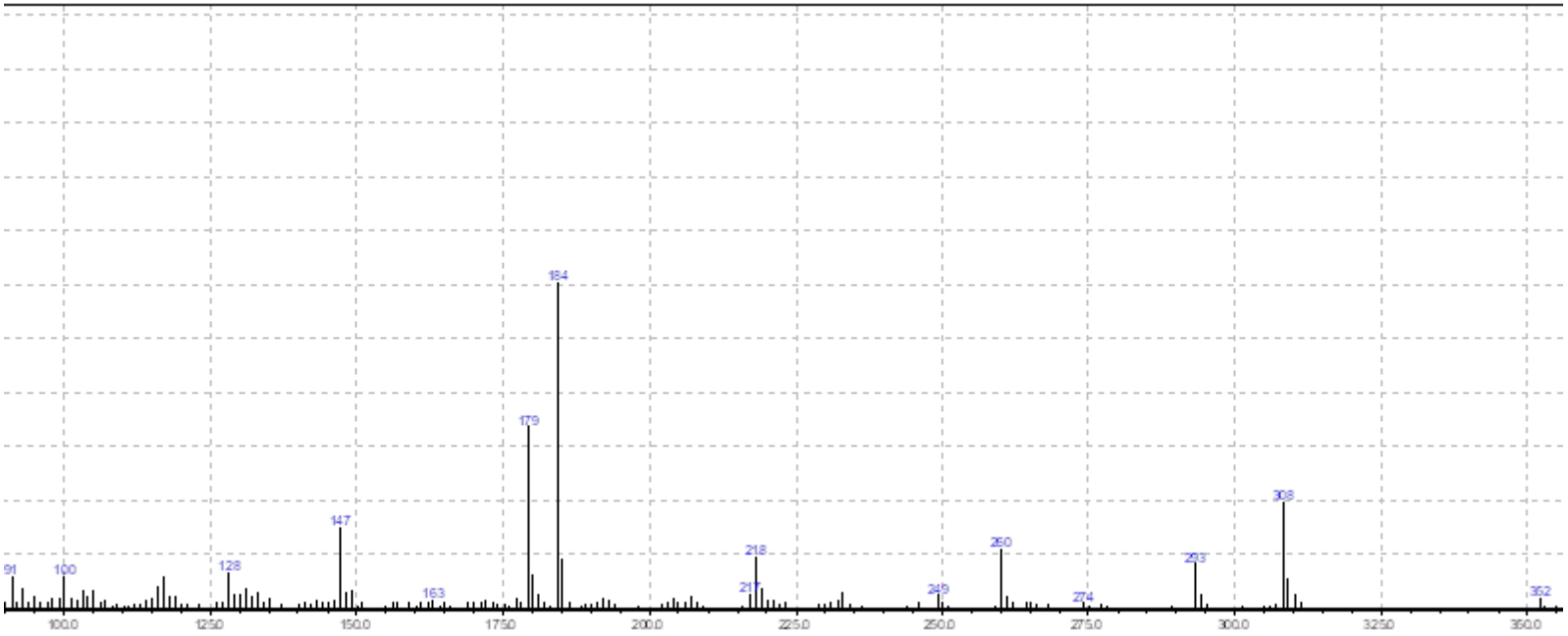
ERNDIM – participant profile



N-Acetyl-vanilalanine



N-Acetyl-vanilalanine

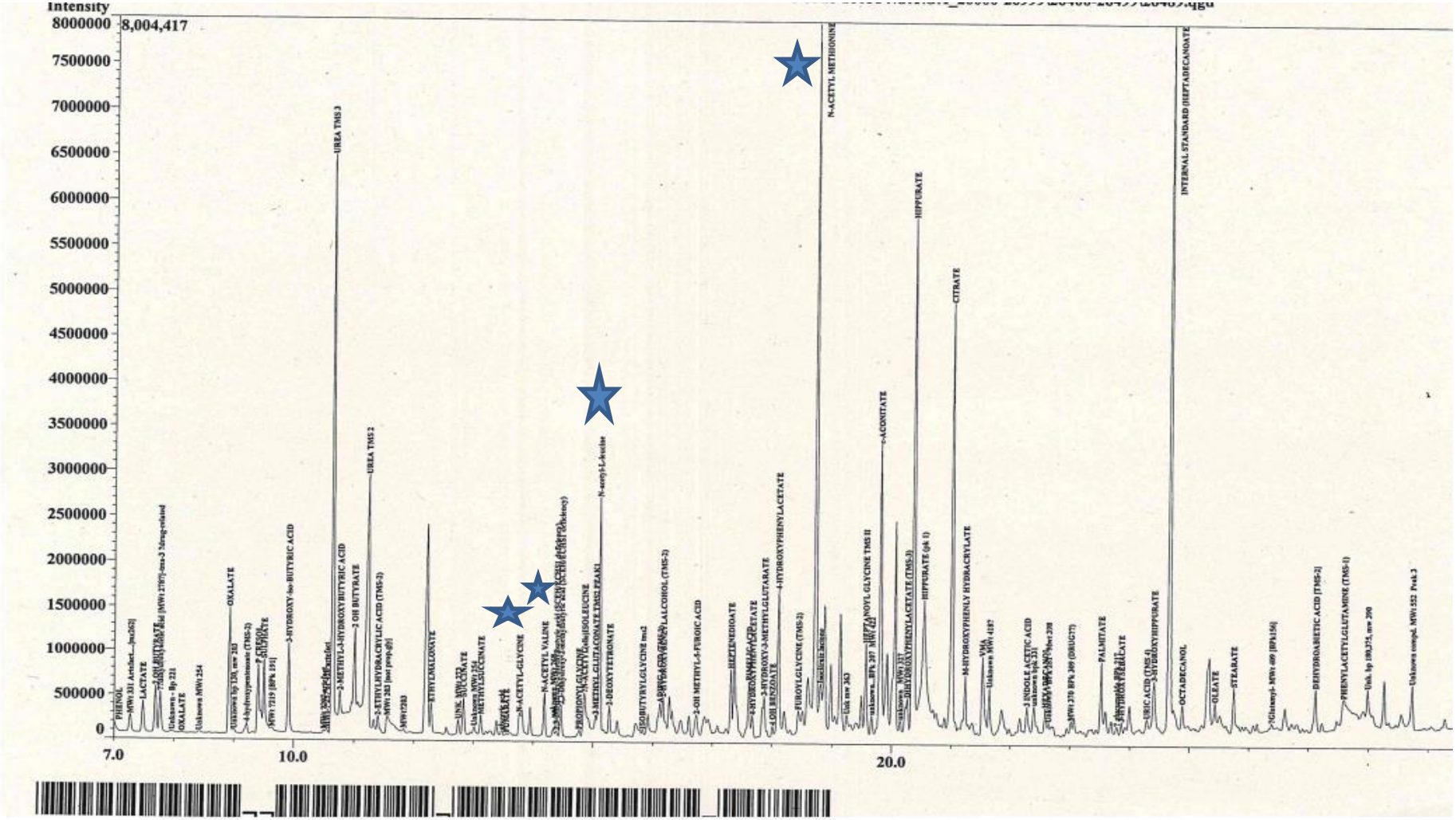


RT 25.5 mins

Aminoacylase 1

- Disorder of amino acid metabolism.
- A zinc-binding enzyme which hydrolyses the *N*-acetyl amino acids into free amino acids and acetic acid.
- Clinically diverse typically presenting with delayed development, psychomotor delay, hypotonia and seizures although there are cases of asymptomatic individuals.
- *ACY1* gene.
- Urine organic acids demonstrate increased *N*-acetyl amino acids and derivatives.

Chromatogram



PEPCK deficiency

- Disorder of carbohydrate metabolism.
- Deficiency of the enzyme phosphoenolpyruvate carboxykinase.
- Clinical features include hypoglycaemia, hepatomegaly and failure to thrive.
- Urine organic acids demonstrate hypoketotic response (although ketones may be present), Lactic aciduria, fumarate, succinate, 2 ketoglutarate and glutarate.
- Confirm with molecular analysis of *PCK1* gene.

Qualitative vs Quantitative measurement of organic acids.

- Pros

Qualitative	Quantitative
Can be taken in the context of the other metabolites	Values against a reference range
Typical well defined patterns	Auto pathways for out of the reference range can be set up
Many analytes in one profile	Useful for monitoring known patients response to treatment
One extraction	Can sometimes distinguish between carriers and affected
Faster turn around time for general screening	Age related ranges can be used to screen out immature profiles etc

cons

Qualitative	Quantitative
Subtle conditions can be missed e.g. mevalolactone, mild	Minor increases can result in over investigation
Reliant on libraries being up to date for peak annotation	More time consuming
Reliant on trained staff for pattern recognition	Risk of missing patterns
Subjectivity when it comes to immature profiles etc.	More instrument time required
Risk of miss identified peaks	More expensive if more stable isotopes are used.

Thank you for your attention

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ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

Question

Serum 2-Hydroxyglutarate and glutaric lactone determination in IDH-Mutated Acute Myeloid Leukemia

- 2-OHglutaric acid in IDH mutated patient in Glioma
- Poor recovery for extraction of organic acids in plasma
- Separation of L- and D-OHglutaric acid to calculate a ratio

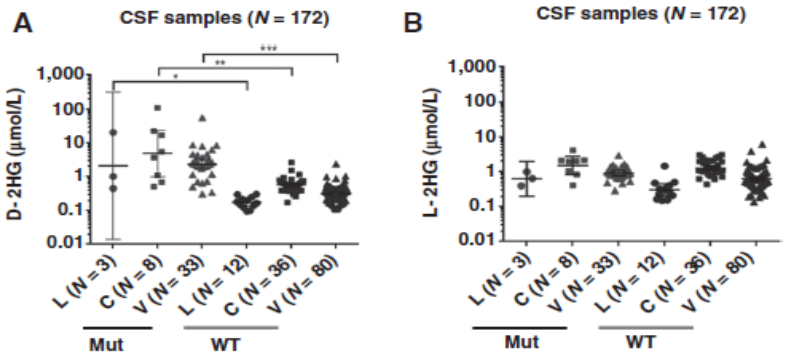

Figure 3.
The levels of D-2HG detected in CSF from lumbar (L), cisternal (C), or ventricular (V) draws were significantly different between WT and mutant IDH patient groups. **A,** Student *t* test comparisons show significant differences in the levels of D-2HG found in the CSF of patients with WT or Mut IDH gliomas from lumbar, $P = 0.0283$, cisternal, $P = 0.0022$, and ventricular, $P = 0.0002$, draws. **B,** There are no significant differences in L-2HG levels between WT and mutant groups. Geometric means with 95% CI are indicated. The number (*N*) of samples is indicated in the parentheses.

- Dedicated quantitative method which separate 3- and 2-OH glutaric acid and enantiomers D- and L-2OHglutaric acid.

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