

Complementary Role of GC/MS in Clinical Laboratory and Beyond

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Alternate Title

GC/MS is not dead yet !!

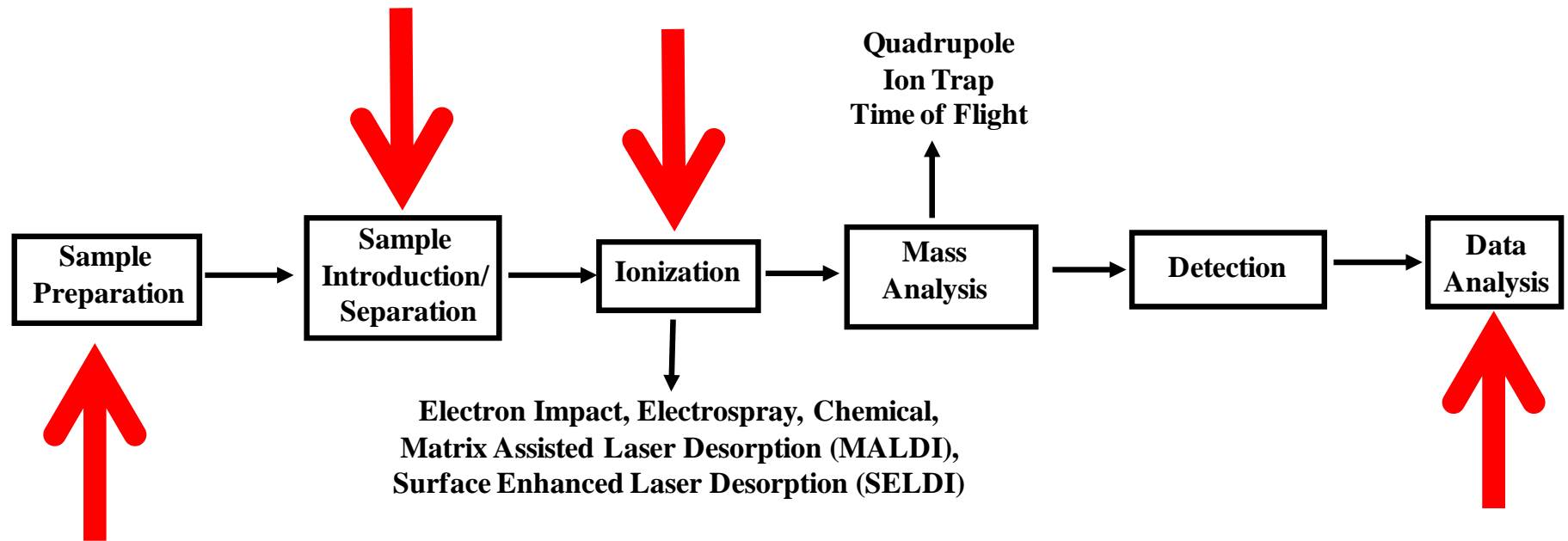
Conflict of Interest

NONE

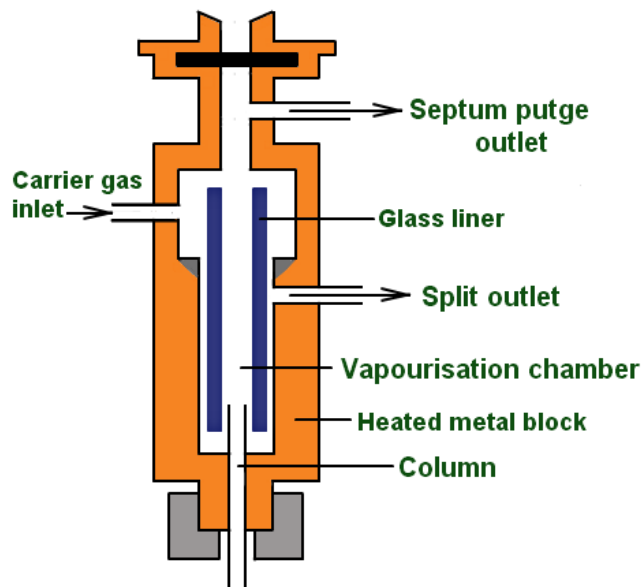
Objectives

- **Describe functioning of GC/MS, and compare GC/MS and LC/MS**
- **Describe clinical applications of GC/MS**
- **Describe role of GC/MS in emerging clinical needs such as metabolomics and biomarker discovery**

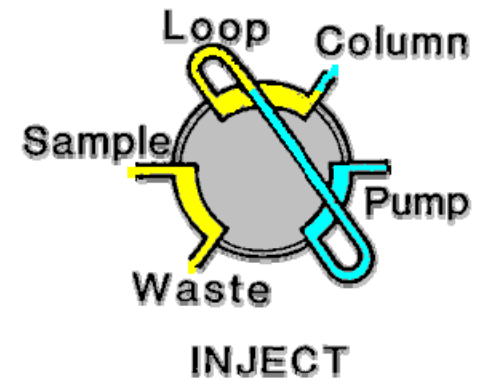
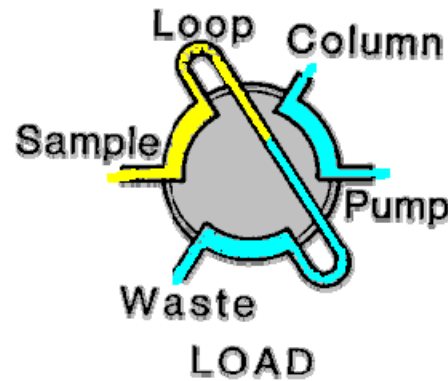
Mass Spectrometry Analysis



GC vs LC Injection Ports



The split / splitless injector



GC vs LC Columns



Examples

- **Broad Spectrum Drug screening**
- **Metabolic Screening**
 - **Organic acids screening**
- **Other**
 - **Metabolomics**
 - **Environmental testing**

Drug Screening

- **Clinical testing for patient management**
- **Workplace drug testing**
- **Pain clinic/management drug testing**
- **Post-mortem drug testing**
- **Doping control**

General Process of Drug Screening

- **Immunoassays**
- **Immunoassays followed by mass spectrometry**
- **Mass spectrometry only**

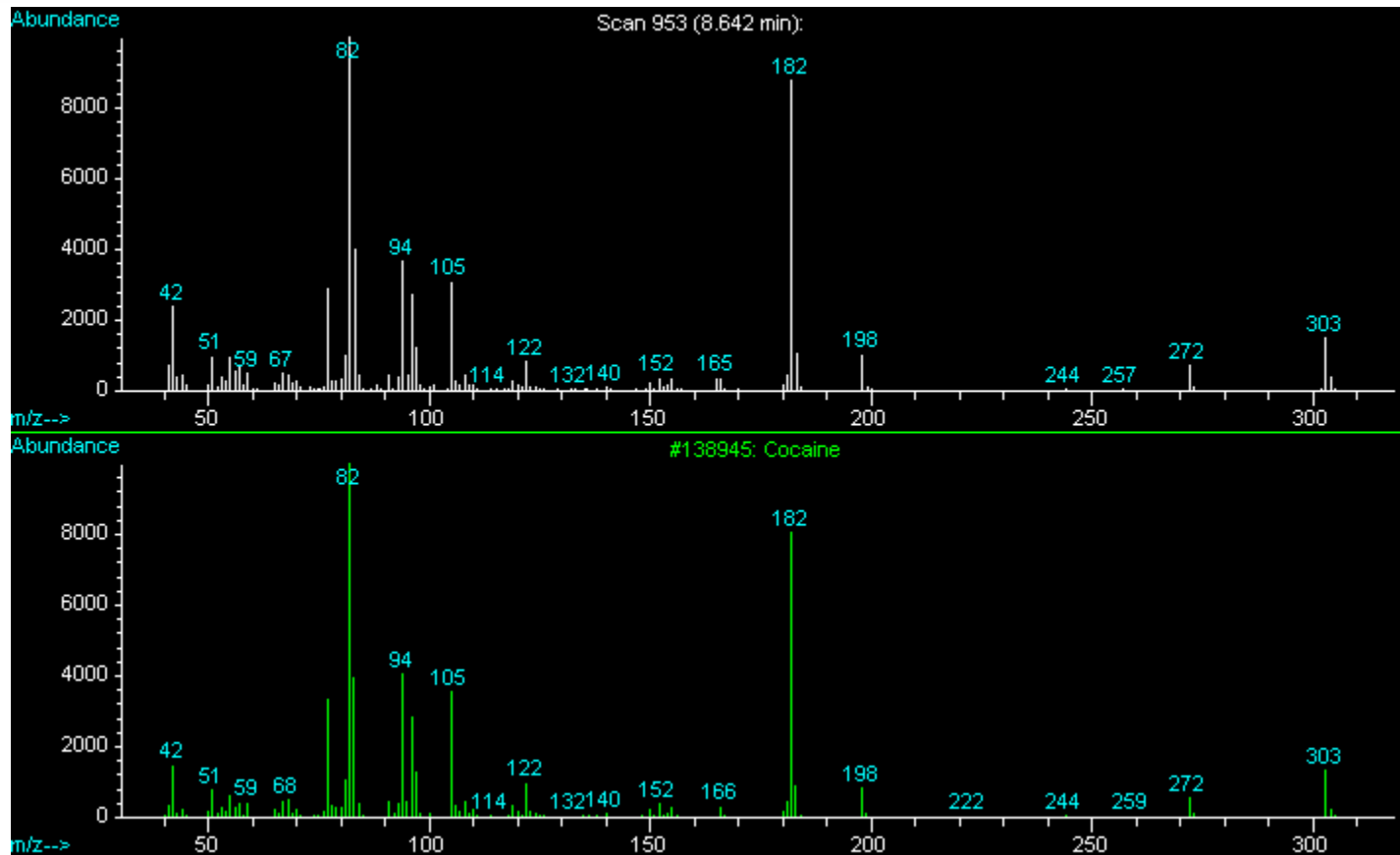
Drug Screening - MS

- GC/MS
- GC/MS/MS
- GC/TOF-MS
- LC/MS
- LC/MS/MS
- LC/TOF-MS

Advantages of GC/MS

- **Probably the best technique for volatiles, non-polar analytes**
- **Highly robust reproducible mass spectra (EI)**
 - Universal fragmentation conditions
- **Libraries:**
 - Transferable among instruments (universal)
 - Commercially available mass spectral libraries
- **Automated identification by mass spectra through deconvolution**

Cocaine – GC/MS Spectrum



Other Advantages of GC/MS

- **In general, higher analyte resolution, better columns**
- **Instruments are more stable and easier to operate**
- **No liquid phases**
- **Lower cost**
 - Instrument
 - Operating
- **No ion suppression**
 - Better quantification when labeled internal standards are unavailable

GC/MS – Limitations

- Analyte must be volatile or made volatile by chemical derivatization
- Analyte or its derivative should be thermally stable
- Sample preparation is generally longer
- Larger sample volume
- Lack of direct sample analysis
- Molecular ion is often lost when electron impact (EI) ionization is used

LC/MS/MS in Drug Screening

- In the last ~10 years LC/MS/MS have taken important role in drug screening
- Pushed by need for detection of drugs not amenable to GC
- Data Acquisition
 - Selected reaction monitoring (SRM)
 - Full spectrum mode

Strengths of LC/MS/MS

- **Compatibility with larger number analytes**
- **Easier sample preparation**
- **Smaller sample volume**
- **Generally derivatization not required**
- **Direct sample analysis**
- **Shorter sample analysis time**
- **Molecular ion - easier identification of unknowns**

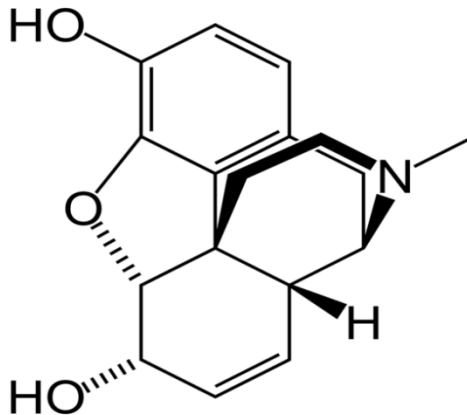
Limitations of LC/MS/MS

- **Limited/lack of spectral libraries**
- **Less reproducible fragmentation pattern**
- **Mass spectra across different instruments are variable**
- **Ion suppression**

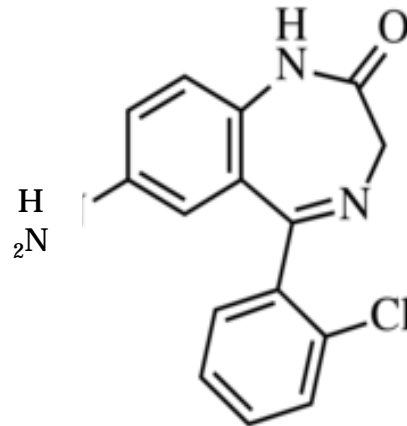
LC- High Resolution MS

- **Orbitrap and TOF/MS**
- **Identification based on accurate mass and isotopic pattern**
- **Mass resolution of 1-5 ppm can be achieved**
- **Presumptive identification can be made**
- **Confirmation by reference standard**
- **TOF-MS/MS**

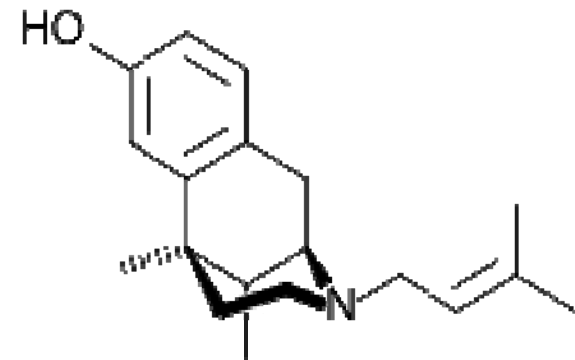
High Resolution MS



Morphine: $C_{17}H_{19}NO_3$
MW 285.3377



7-aminoclonazepam: $C_{15}H_{12}ClNO_3$
MW 285.7283



Pentazocine : $C_{19}H_{27}NO$
MW 285.2093



Clinical History

A drug screen and quantitation was ordered by a physician of a young man with a history of narcolepsy and drug abuse.

Drug	No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
Amphetamine, ng/mL							
GC-Mass Spectrometry	31	532.94	100.38	18.8	540.0	154.0	715.0
LC-MS/MS	21	561.54	126.34	22.5	557.0	300.0	920.0
All Method Mean	59	544.35	108.40	19.9	556.0	154.0	920.0
Codeine, ng/mL							
GC-Mass Spectrometry	34	333.42	38.33	11.5	339.8	265.0	451.6
LC-MS/MS	31	345.75	48.48	14.0	343.0	260.0	530.0
All Method Mean	71	342.45	46.20	13.5	340.5	260.0	530.0
Morphine, ng/mL							
GC-Mass Spectrometry	34	82.77	15.81	19.1	87.1	41.1	105.5
LC-MS/MS	31	91.48	17.00	18.6	91.0	65.1	155.0
All Method Mean	69	87.51	18.19	20.8	88.0	41.1	155.0

Metabolic Testing

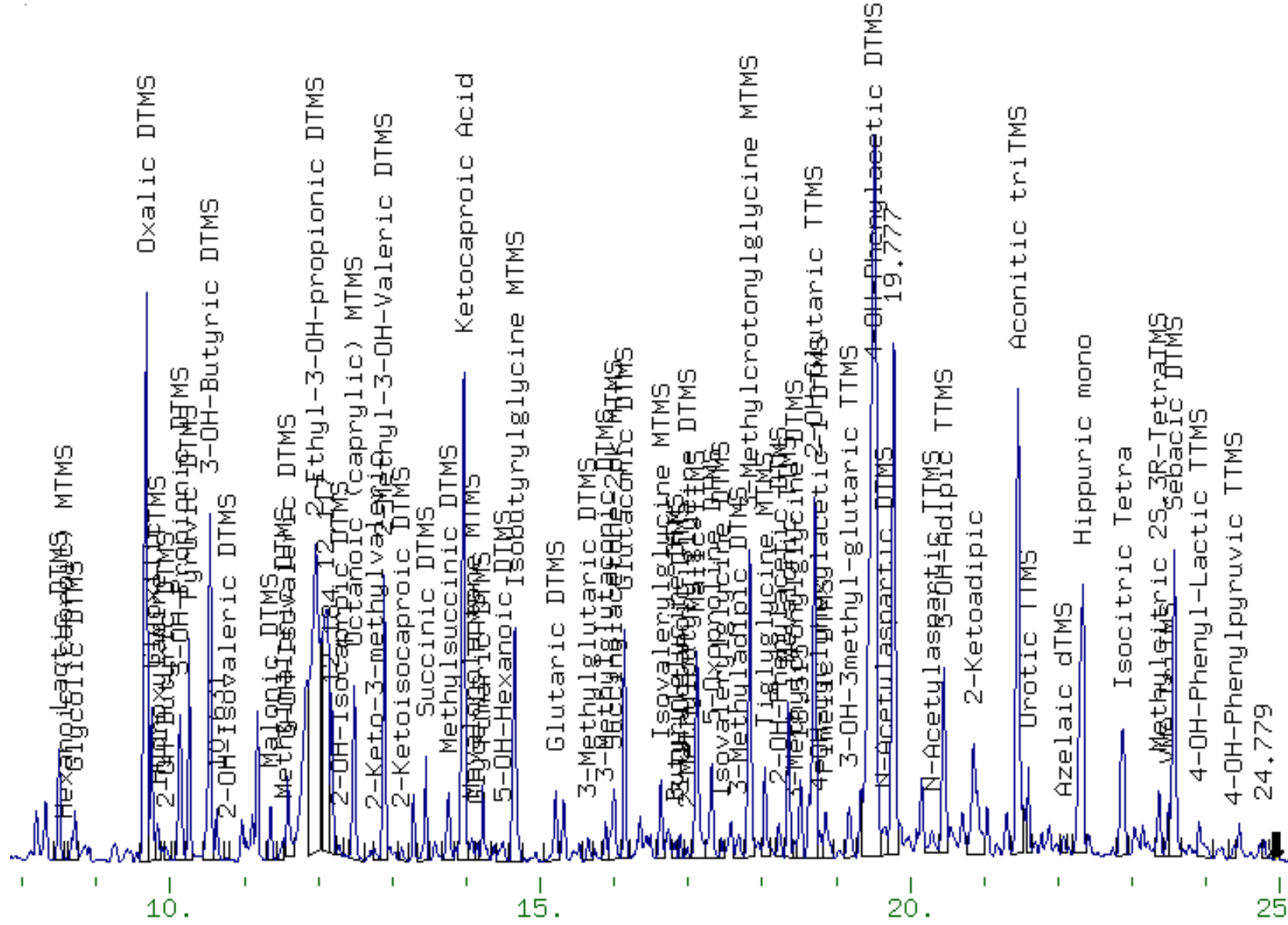
Organic Acidurias by GC/MS

- Organic acidurias are characterized by the excretion of non-amino organic acids in urine due to defects in metabolism of carbohydrate, fatty acids and nucleic acids
- Symptoms include vomiting, poor feeding, neurologic symptoms such as seizures and abnormal tone, and lethargy leading to coma
- GC/MS is widely used in the diagnosis of organic acidurias
- There are >100 organic acids of clinical importance

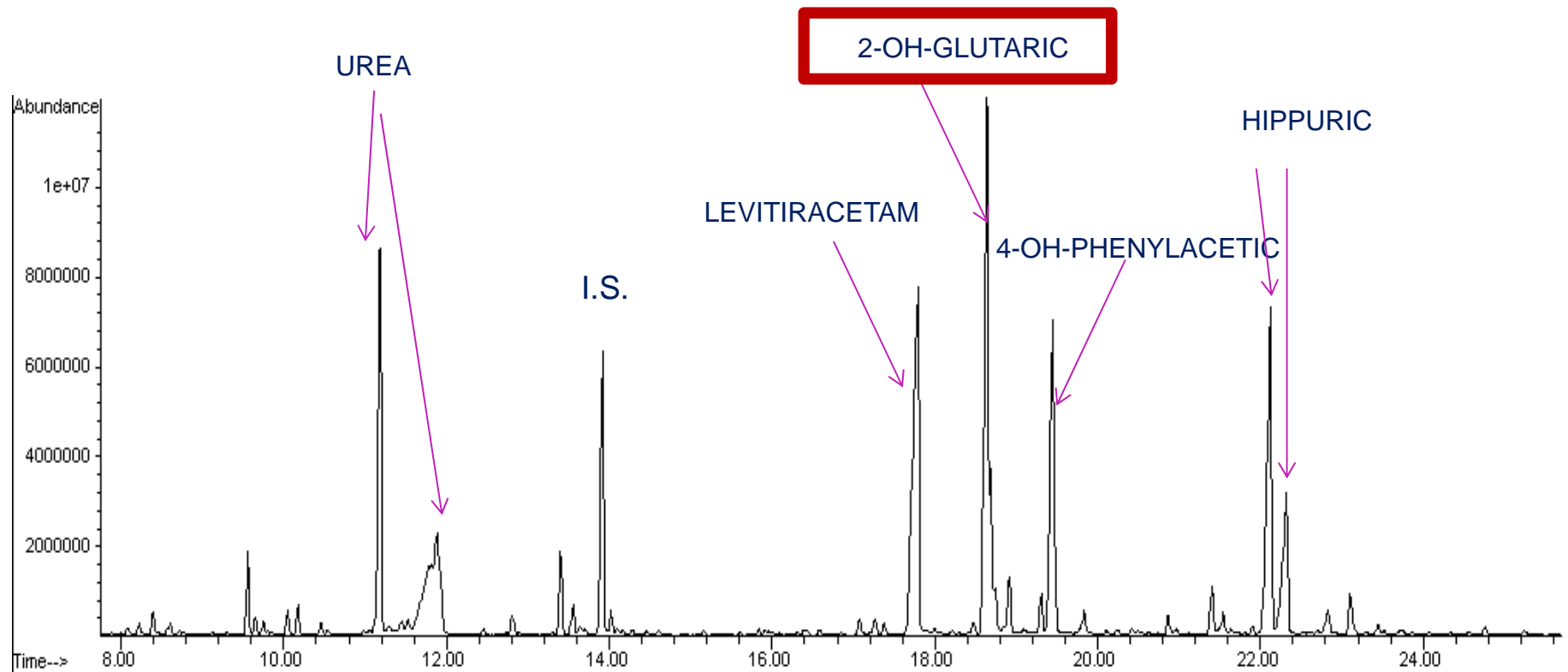
Organic Acids Analysis

- **Extraction of organic acids from acidified urine**
- **Derivatization – commonly using TMS**
- **GC/MS analysis**
- **Data collection – Total ion**
- **Identification by full spectra/ ion extraction**
- **Many organic acids are not available**
- **Quantification**

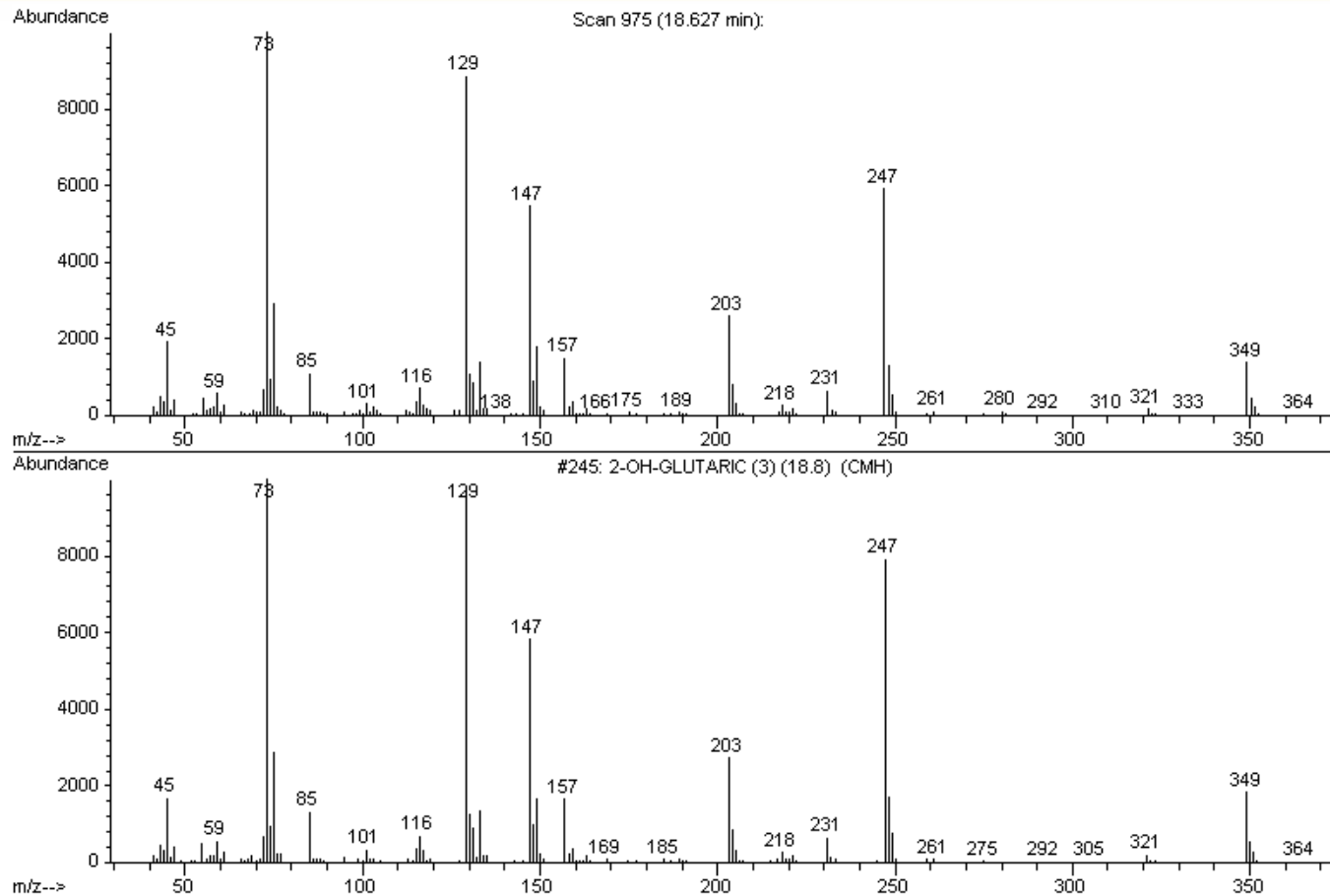
Urine Organic Acid Profile



2-Hydroxyglutaric Aciduria



2-Hydroxyglutaric acid

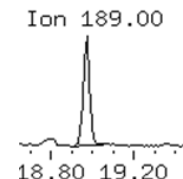
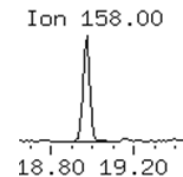
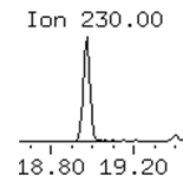
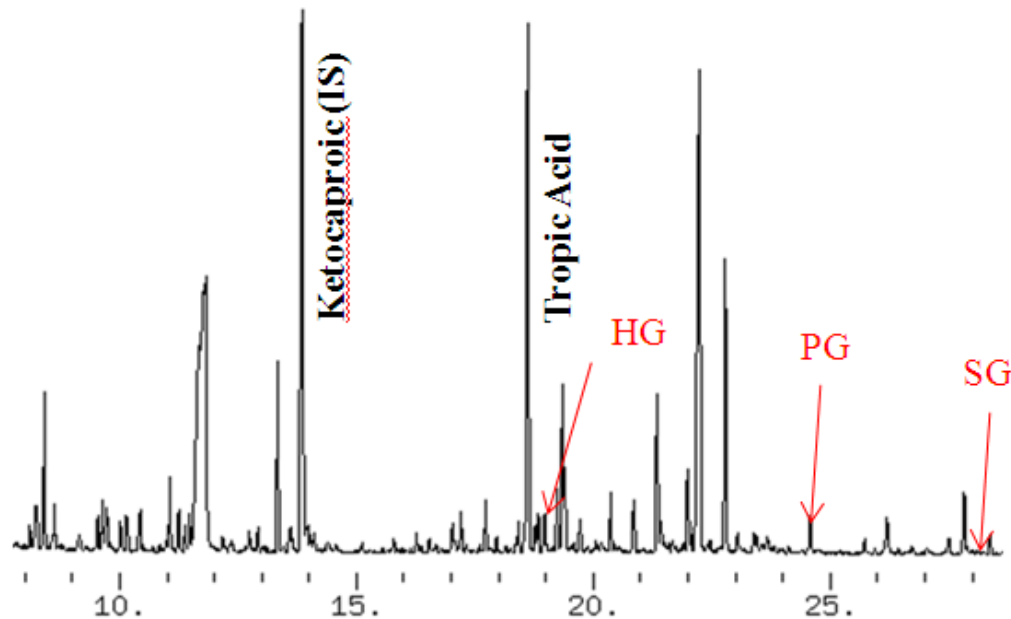


Children's Mercy Hospital and Clinics - Laboratory Services

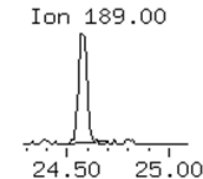
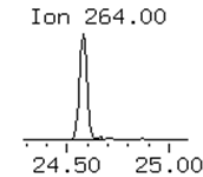
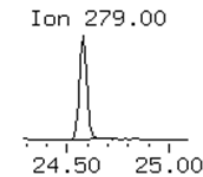
Urine Organic Acid Quantitation (mmoles / mole Creatinine)

COMPOUND	VALUE	U Limit	COMPOUND	VALUE	6Mo o-2Y r	
Lactic	36	141	Butyrylglycine	0		0
Hexanoic	0	2	3-OH-Octanoic	0		2
Glycolic	68	154	2-Methylbutyrylglycine	0		1
2-OH-Butyric	3	9	Malic	8		49
3-OH-Propionic	12	34	Adipic	5		53
Pyruvic	11	76	5-Oxoproline	64		207
3-OH-Butyric	1	42	Isovalerylglycine	0		5
3-OH-Isobutyric	22	83	3-Methyladipic	0		6
2-OH-Isovaleric	0	3	Tiglylglycine	0		2
Malonic	7	15	2-OH-Phenylacetic	3		11
2-Methyl-3-OH-Butyric	2	20	Mevalonic	0		0
Methylmalonic	2	11	3-methylcrotonylglycine	0		1
3-OH-Isovaleric	6	40	2-OH-Glutaric	3014	H	89
2-Ketoisovaleric	0	10	3-OH-Glutaric	12		10
4-OH-Butyric	0	8	Phenyllactic	1		3
Benzoic	23	24	Pimelic	1		8
2-Ethyl-3-OH-Propionic	3	22	3-OH-3-Methylglutaric	3		24
3-OH-Valeric	0	1	4-OH-cyclohexylacetic	1		3
2-OH-Isocaproic	0	1	4-OH-Phenylacetic	102		134
2-OH-3-Methylvaleric	0	1	2-Ketoglutaric	63		471
Octanoic	0	3	Phenylpyruvic	0		8
Acetoacetic	5	26	N-acetylaspartic	8		47
4-OH-Isovaleric	0	0	Hexonylglycine	0		3
2-Keto-3-Methylvaleric	0	10	2-OH-Adipic	1		3
2-Methyl-3-OH-Valeric	0	0	3-OH-Adipic	1		19
Ethylmalonic	3	23	Suberic	1		26
Phenylacetic	1	4	2-Ketoadipic	1		17
2-Ketoisocaproic	1	19	Orotic	1		7
Succinic	64	171	Aconitic	27		177
Methylsuccinic	2	10	Azelaic	1		37
2-Methyl-Acetoacetic	0	0	Hippuric	366		867
Mevalonolactone	0	0	Isocitric	26		149
Glyceric	34	131	Homogenetic	0		1
Fumaric	1	16	Citric	120		994
5-OH-Hexanoic	2	15	Methylcitric	1		7
Glutaric	3	20	Sebacic	0		10
Isobutyrylglycine	0	1	4-OH-PhenylLactic	0		15
3-Methylglutaric	1	3	4-OH-Phenylpyruvic	0		22
Propionylglycine	0	0	Phenylpropionylglycine	0		0
Succinylacetone	0	0	3-OH-Decanedioic	1		85
3-Methylglutaconic	3	17	N-acetyltyrosine	0		2
Glutaconic	0	0	Suberylglycine	0		0
Decanoic	0	2	---END OF REPORTING---	0		0

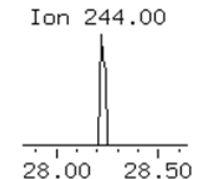
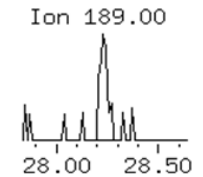
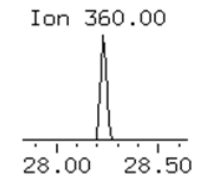
Urine Organic Acid Profile: MCAD Patient



Hexanoyl

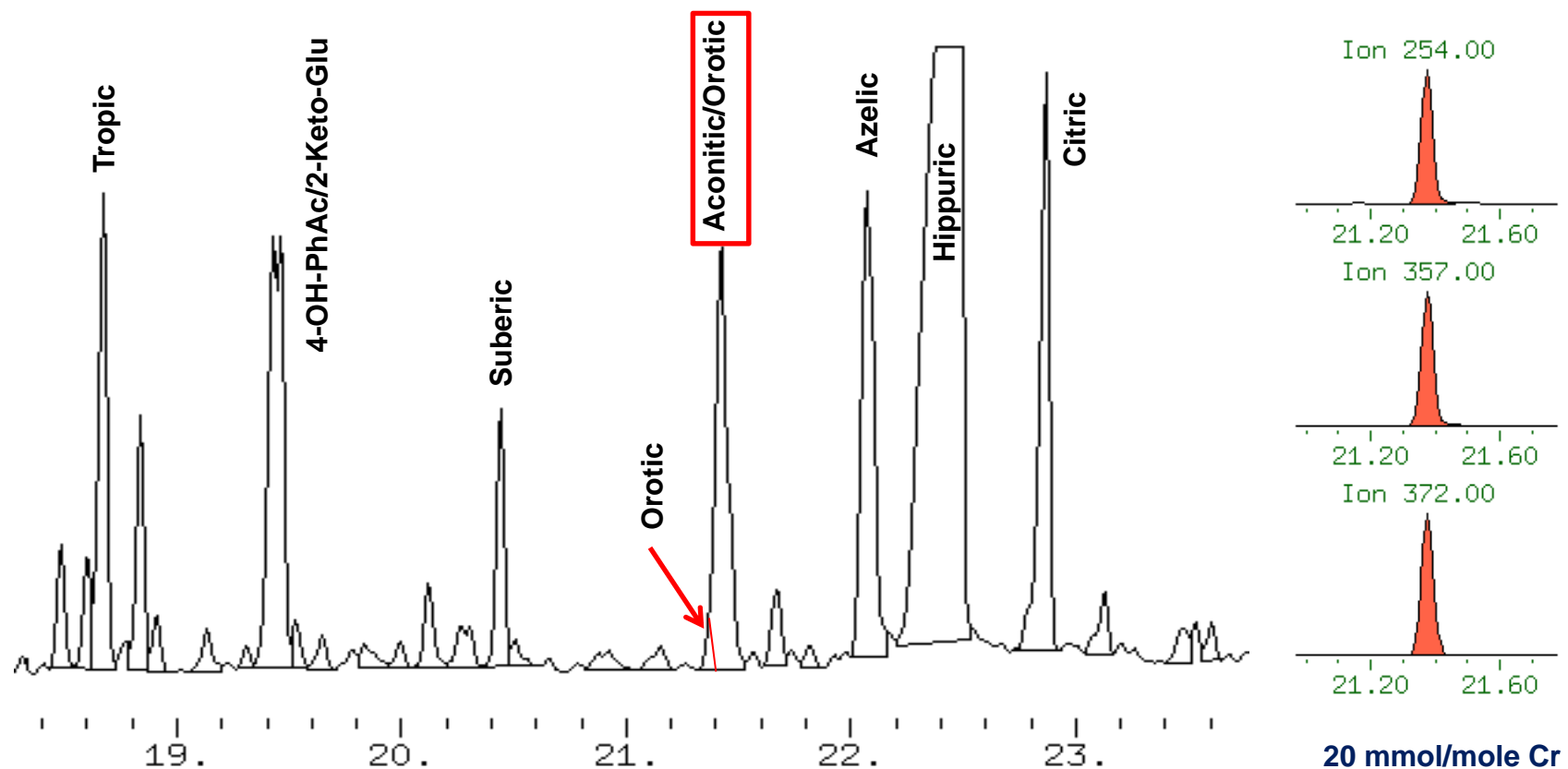


Phenylpropionyl



Suberyl

Organic Acid Profile: OTC Patient



CAP/ACMG 2015

Biochemical Genetics

Participant Summary

Urine Organic Acid Analysis

Method used for organic acid analysis:

Method

Gas chromatography/mass spectrometry
 Gas chromatography
 Gas chromatography/MSMS

Participants	(81)
No.	%

79	97.6
1	1.2
1	1.2

Method used for organic acid extraction:

Extraction Method

Ethylacetate extraction
 Ethylacetate and ether
 Urease

Participants	(78)
No.	%

38	48.7
29	37.1
3	3.8

Metabolomics

An Introduction into the Role of Gas Chromatography - Mass Spectrometry (GC-MS) in Metabolomic Analysis

Stephen Childs, Department of Pharmacy Health & Well-being, Faculty of Applied Sciences, University of Sunderland, Sciences Complex, Wharnclyffe Street, Sunderland, SR1 3SD, UK.

Dr. Lee Williams, Department of Pharmacy Health & Well-Being, Faculty of Applied Sciences, University of Sunderland, Sciences Complex, Wharnclyffe Street, Sunderland, SR1 3SD, UK.

CHROMATOGRAPHY
 TODAY

February / March 2014

Metabolomics

Gas Chromatography in Metabolomics Study

Yunping Qiu and Deborah Reed

Advances in Gas Chromatography © 2014

> 1000 metabolites have been analyzed, directly or through derivatization

GC-MS is one of the most efficient, sensitive, and reliable tools for metabolomics studies. GC-MS produces reproducible molecular fragmentation patterns making it an integral tool for metabolite identification.

Steroid Hormones

Journal of Steroid Biochemistry and Molecular Biology

Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 496–504

Review

Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS)[☆]

Nils Krone*, Beverly A. Hughes, Gareth G. Lavery, Paul M. Stewart, Wiebke Arlt, Cedric H.L. Shackleton

Centre for Endocrinology, Diabetes and Metabolism, School for Clinical and Experimental Medicine, University of Birmingham, United Kingdom

Environmental Biomonitoring

- **Hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are easier analyzed using GC-MS than LC-MS.**
- **Lack of easily ionizable groups**
- **Need stronger ionizing conditions such as EI**

GC vs LC Mass Spec

	GC/MS(n)	LC/MS(n)
Analyte range (Menu)	✓✓	✓✓✓✓
Commercial Libraries	✓✓✓✓	✓✓
Full ion Spectra	✓✓✓✓	✓✓
Molecular ion	✓	✓✓✓✓
Drug Screening	✓✓✓	✓✓✓✓
Ion suppression	None	✓✓ (Labeled IS)
Sample Preparation	✓✓	✓✓✓✓
Speed of analysis	✓✓	✓✓✓✓
Cost (Initial /Operation)	✓✓✓✓	✓✓

