Complementary Role of GC/MS in Clinical Laboratory and Beyond

Uttam Garg, PhD, DABCC, FABFT, FACB

Professor, University of Missouri School of Medicine

Division Chief, Laboratory Medicine

Director, Clinical Chemistry and Toxicology Labs

Children's Mercy Hospital

Kansas City, MO

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Alternate Title

GC/MS is not dead yet !!

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Conflict of Interest

NONE

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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**

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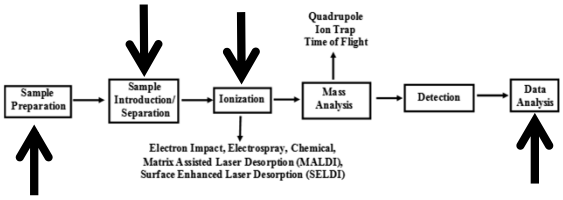
Mass Spectrometry Analysis



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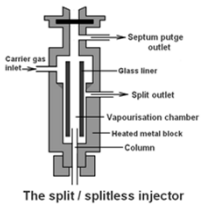
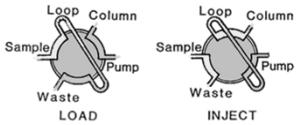
GC vs LC Injection Ports



The split / splitless injector



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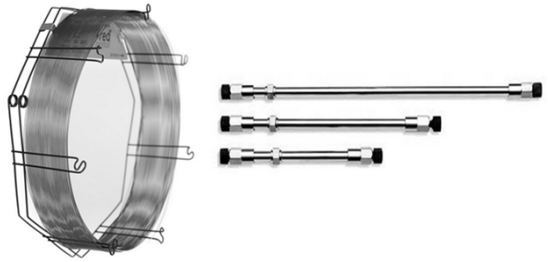
GC vs LC Columns



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Examples

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

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Drug Screening

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

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General Process of Drug Screening

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

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Drug Screening - MS

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

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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**

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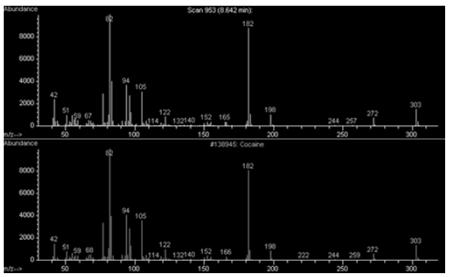
Cocaine – GC/MS Spectrum



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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

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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

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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

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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

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Limitations of LC/MS/MS

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

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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**

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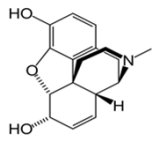
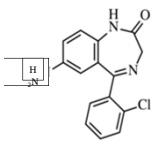
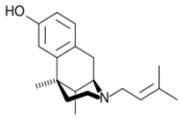
High Resolution MS



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

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AHB Wu and J Colby, Clin App Mass Spec: Methods and Protocols (2015, in Press)

COLLEGE of AMERICAN PATHOLOGISTS

T-A SURVEYS 2015 | **Toxicology** Participant Summary

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**

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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**

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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**

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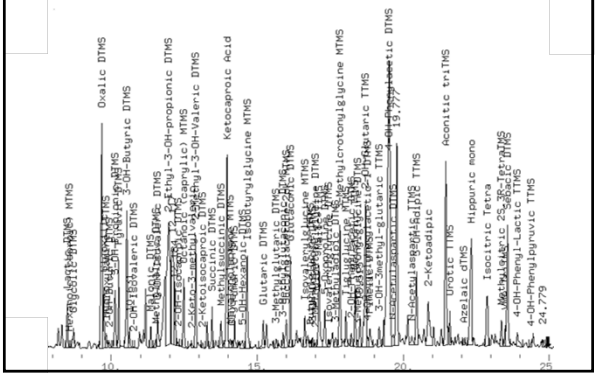
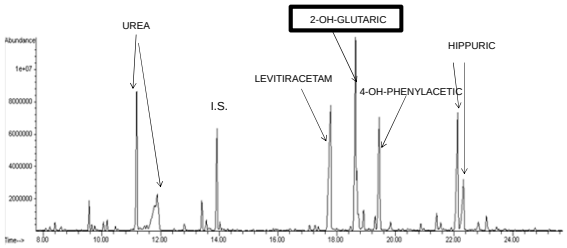
Urine Organic Acid Profile



2-Hydroxyglutaric Aciduria



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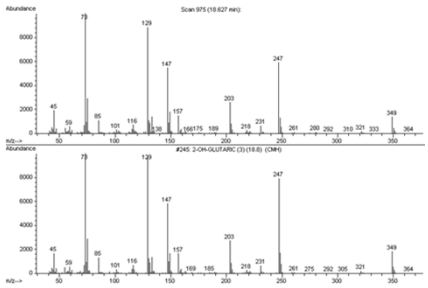
2-Hydroxyglutaric acid



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Children's Mercy Hospital and Clinics - Laboratory Services

Urine Organic Acid Quantitation (mmoles / mole Creatinine)

COMPOUND	VALUE	U Limit	COMPOUND	VALUE	6M 0-2Yr
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-Ketohexavaleric	0	10	3-OH-Glutaric	12	78
4-OH-Butyric	0	8	Phenyllactic	1	3
Benzoic	23	24	Pimelic	1	6
2-Ethyl-3-OH-Propionic	3	22	3-OH-3-Methylglutaric	3	24
3-OH-Valeric	0	1	4-OH-cyclohexylacetic	1	3
3-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	3-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	Homogentisic	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
2-Methylglutaconic	3	17	N-acetyltyrosine	0	2
Glutaconic	0	0	Suberylglycine	0	0
Decanoic	0	2	---END OF REPORTING---	0	0

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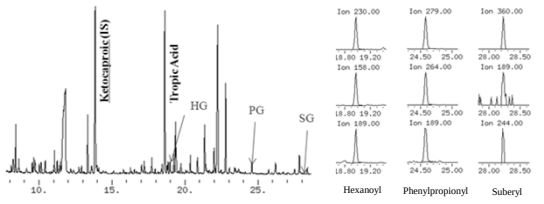
Urine Organic Acid Profile: MCAD Patient



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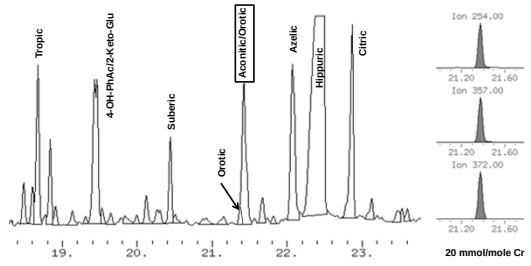
Organic Acid Profile: OTC Patient



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CAP/ACMG 2015 Biochemical Genetics

Participant Summary

Urine Organic Acid Analysis

Method used for organic acid analysis:

Method	Participants No.	(81) %
Gas chromatography/mass spectrometry	79	97.6
Gas chromatography	1	1.2
Gas chromatography/MSMS	1	1.2

Method used for organic acid extraction:

Extraction Method	Participants No.	(78) %
Ethylacetate extraction	38	48.7
Ethylacetate and ether	29	37.1
Urease	3	3.8

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

CHROMATOGRAPHY TODAY February / March 2014

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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.

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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

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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**

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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)	✓✓✓✓	✓✓

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Take Home Message

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