Quantitative Metabolomics Facility at C-CAMP

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Introductions

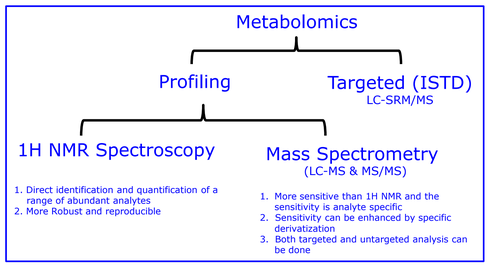
Metabolomics:

Metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind" or "Identification and quantification of the compounds (<1500 Da) in the metabolome".

Untargeted and Targeted Metabolomics:

Untargeted metabolomics is commonly used to profile the entire metabolome in an organ, tissue, cell, or biological fluid (e.g., urine, plasma, saliva, or culture medium). Mainly used to discover and identify differential metabolites.

Targeted metabolomics is mainly used to elucidate the association between known metabolic pathways and modifications/perturbations that arise as a result of drug intervention, disease, or gene modification.

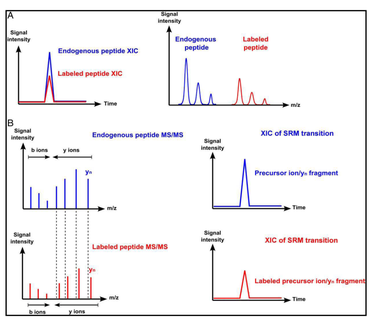


Absolute Quantification:

By using specific internal standards (ISTDs) trying to quantify the molecule of interest with the help of advanced LC-MS system. Quantification of the known metabolites by using its stable isotope analog (^{13}C, ^{15}N, ^{3}D) by generating standard curve (Ratio of L/H versus Concentration).

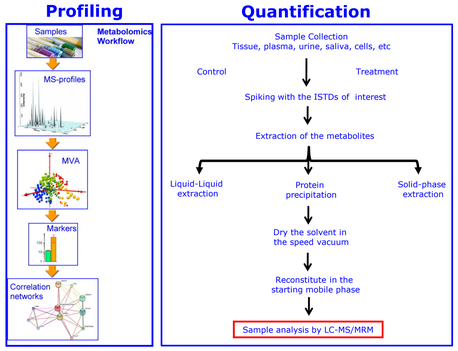
Stable Isotope Dilution Method:

Normally involves the use of a stable isotope-labeled internal standard, which is spiked into a sample at a known concentration. The response ratio between the analyte and the labeled compound obtained by LC-MRM-MS can then be interpolated onto a standard curve to calculate the absolute amount of the analyte in the unknown sample.



Using stable isotope analogue one can quantify molecules using Full scan, MS/MS scan and SRM scan.

Metabolomics Work Flow



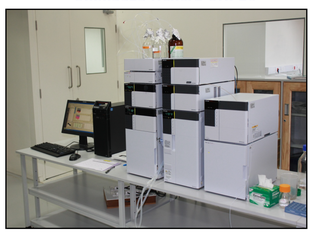
Facilities Offered

- **Full scan analysis of known/purified compounds (Solid/ Liquid)**
- **Product ion scan (MS/MS) analysis of known/purified compounds (Solid/Liquid)**
- **Analysis of known compounds in biological matrix**
- **Method development for specific metabolites to know the absolute quantification (STDs and ISTDs to be obtained from the companies)**
  - Limit of detection (LOD) and quantitation (LOQ)
  - Precision, Accuracy and Stability (Freez-Thaw)
  - Standard curve
  - Quality control samples (lower, middle, upper)
  - Intra and inter-day validation

Instruments

TSQ Vantage-Agilent 1290 UHPLC (LC-MS)

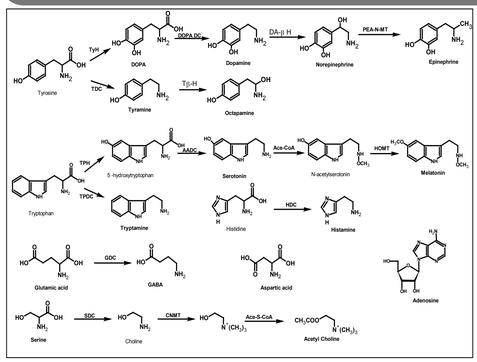
Shimadzu Nexera UHPLC



Methods developed

- **Quantification of sixteen neurotransmitters from Planarian extract**
- **Quantification of neurotransmitters from biological fluids (Tissue, Sera and Urine)**
- **Quantification of Olanzapine (Psychotic drug) and its metabolites from sera**
- **Quantification of bio-pesticides (azadirachtin, nimbin and salanin) from plant extract (Leaf and Seed)**
- **Quantification of amino acids form biological fluids**

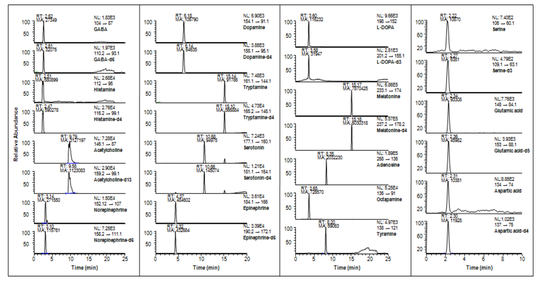
Quantification of Neurotransmitters and method to quantify Olanzapine from sera



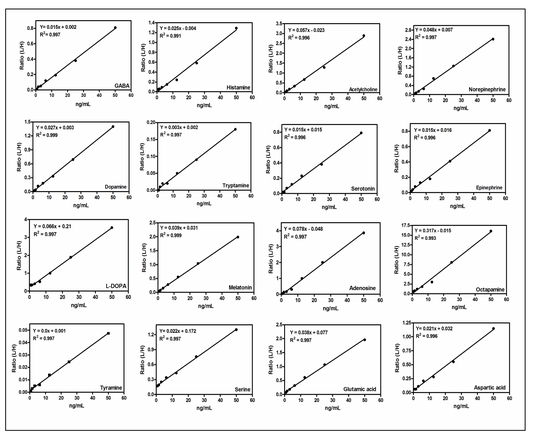
Biosynthetic pathways for the formation of neurotransmitters in biological systems.

	Neurotransmitter	Parent Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	S-lens voltage	Retention Time (min)
1	GABA	104.08	87.05	7	58	2.82
2	GABA-d6	110.0	93.111	7	58	2.82
3	Histamine	112.08	95.02	14	43	3.91
4	Histamine-d4	116.0	99.102	14	43	3.91
5	Acetylcholine	146.118	87.07	13	46	6.35
6	Acetyl choline-d3	149.35	91.122	13	46	6.35
7	Norepinephrine	170.0 (170.1114)	107.05	17	56	9.14
8	Norepinephrine-d6	176.2 (176.2)	111.115	17	56	9.14
9	Dopamine	154.0 (0)	91.05	23	60	8.38
10	Dopamine-d4	158.14	95.1	23	60	8.38
11	Serotonin	177.1	160.104	11	40	10.648
12	Serotonin-d4	181.14	164.155	11	40	10.648
13	Epinephrine	184.13	166.08	7	43	4.32
14	Epinephrine-d6	190.25	172.188	7	43	4.32
15	L-DOPA	198.08	152.083	13	51	5.8
16	L-DOPA-d3	200.2	155.136	13	51	5.8
17	Melatonin	233.1 (0)	174.08	14	57	13.57
18	Melatonin-d4	237.2	178.232	14	57	13.57
19	Tryptamine	161.1	144.126	13	56	13.14
20	Tryptamine-d4	165.3	148.155	13	56	13.14
21	Adenosine	268.08	136.062	16	64	8.35
22	Tyramine	138.08	121.077	10	44	8.3
23	Octopamine	154.16 (154.0)	91.079	17	64	3.88
24	Serine	106.08	60.1	10	40	3.22
25	Serine-d3	109.1	63.1	10	40	3.22
26	Glutamic acid	148.08	84.1	14	42	3.34
27	Glutamic acid-d5	153.08	88.1	14	42	3.34
28	Aspartic acid	134.06	74	13	43	3.35
29	Aspartic acid-d3	137.06	75	13	43	3.35

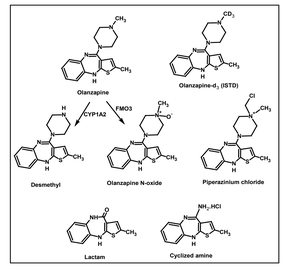
SRM Table for NTs analyzed



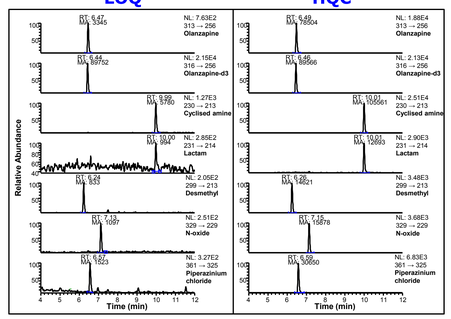
UHPLC-MS/SRM chromatogram of all sixteen NTs and thirteen ISTDs in HQC level. NL-Normalized Level



Standard curves and regression line analysis of sixteen NTs.



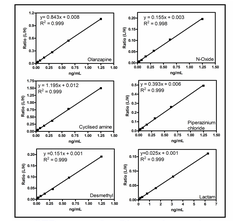
Structure of Olanzapine, Olanzapine-d_3 (ISTD), two metabolites and three of its impurity standards.



LC-MS/SRM chromatogram of Olanzapine and its impurity metabolites (LOQ and HQC)

Compound	Parent ion (m/z)	Product ion (m/z)	Collision Energy (CE)	S-lens voltage
Olanzapine	313.4	256.07	23	105
Olanzapine-d3	316.4	256.07	23	105
Cyclized amine	230.06	213.05	19	88
Lactam	231.2	214.03	20	106
Desmethyl	299.4	213.02	26	107
Olanzapine N-oxide	329.4	229.05	17	92
Piperazinium chloride	362.9	325.12	11	81

MRM Table for Olanzapine and its Impurities



STD curves for Olanzapine and its impurity metabolites.

Bangalore Bio-cluster users

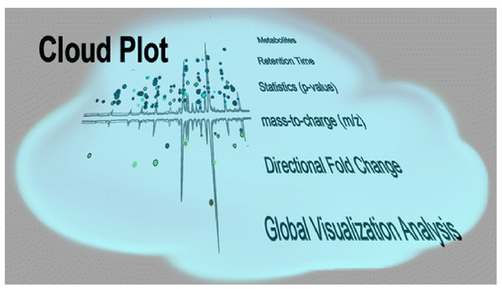
Dr. Panicker from Neurobiology lab
Dr. Dasaradhi from inStem
Dr. Yashoda from inStem
Dr. Malali Gowda from C-CAMP

Outside users

Dr. Prakash Halan from BITS Pilani, Goa
Dr. M.G.Sridhar from JIPMER
Dr. Jayanth Haldar from JNCSAR

Future expansions

Planning to setup the facility to do the profiling of small molecules from the biological system and using XCMS software to do the global visualization of data analysis.



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