

**HPTLC and HPTLC/MS studies
on some**

Bioactive Natural Products

**International Symposium for
Thin-Layer Chromatography**

06-08 July 2011

BASEL, Switzerland



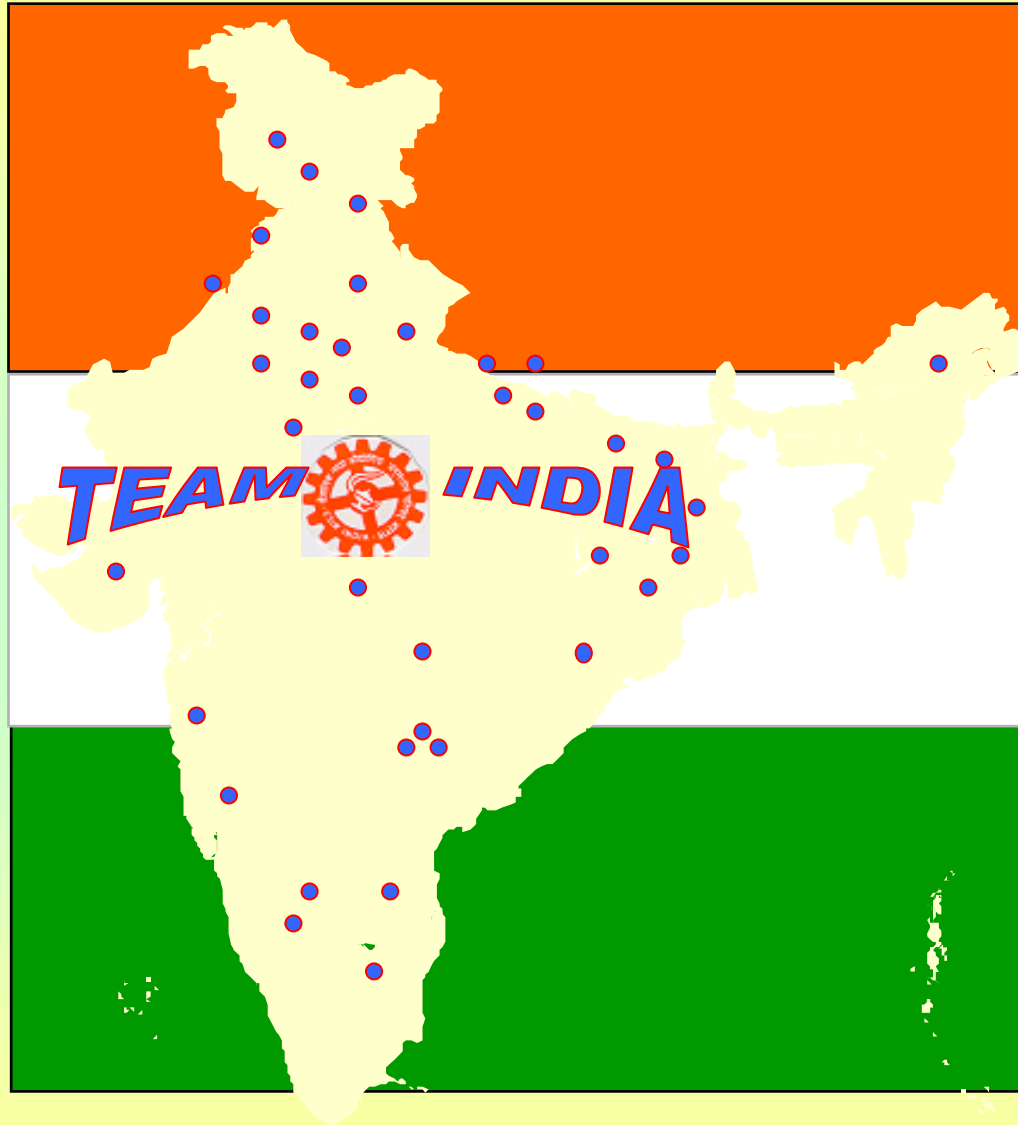
Dr. J. Madhusudana Rao

Director Grade Scientist & Head, Organic Chemistry Division-1

Indian Institute of Chemical Technology

Hyderabad-500607, India

Gateway to Technologies



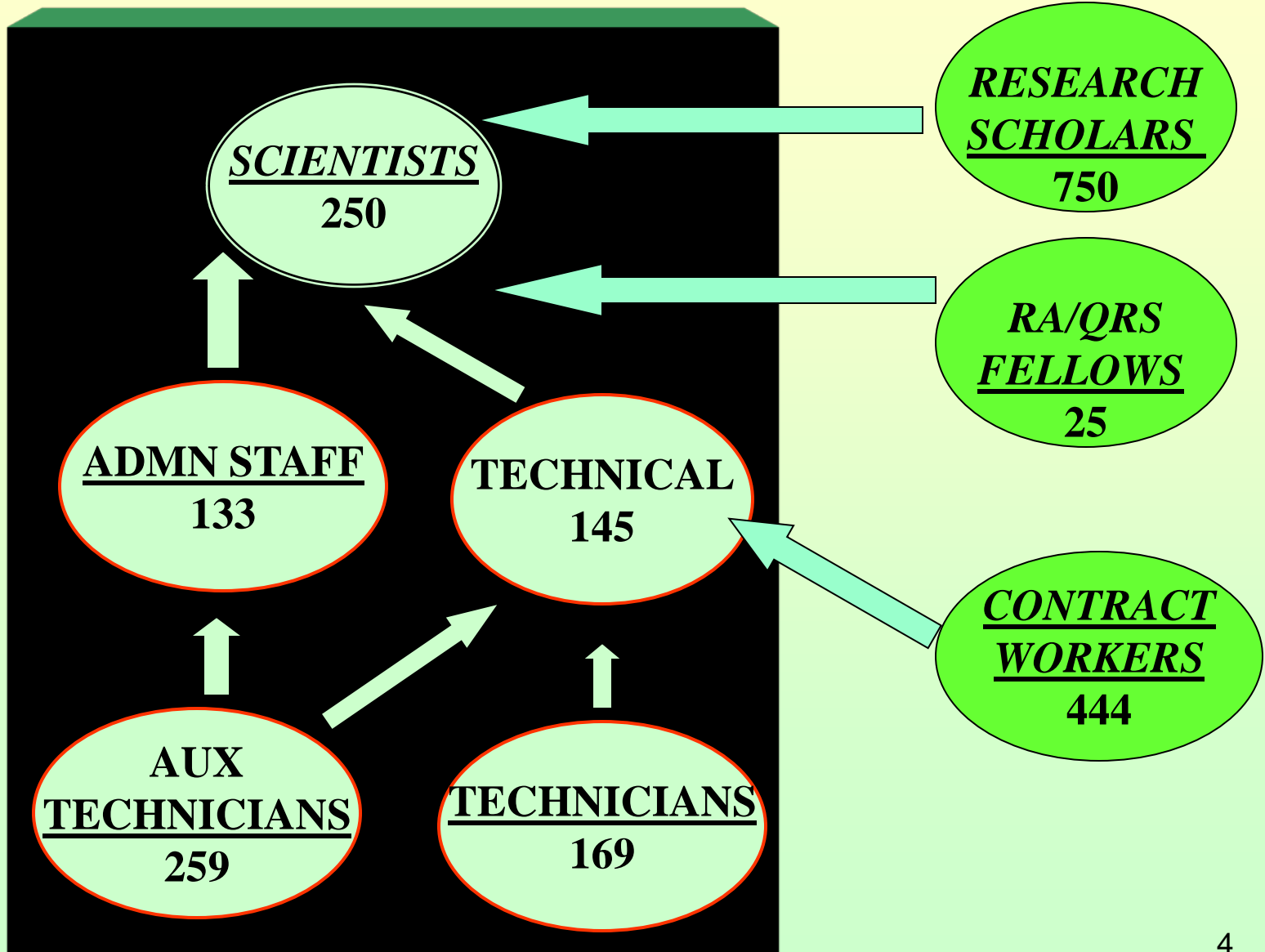
CONCEPT TO COMMERCIALIZATION

LAB SCALE – PILOT SCALE – DESIGNS FOR COMMERCIAL PLANT



**Sixty Five Years in Service of Chemical Industry
(1944 - 2009)**

HUMAN RESOURCE STRENGTHS OF IICT



ORGANIZATION



MANAGEMENT COUNCIL

DIRECTOR

RESEARCH COUNCIL

ADMINISTRATION
FINANCE
STORES AND PURCHASE

BUSINESS
HRD
RESEARCH
MANAGEMENT

NATURAL
PRODUCTS

AGRO
CHEMICALS

DRUGS &
INTERMEDIATES

PILOT PLANTS

COMPUTER
CENTRE

INORGANIC &
PHYSICAL
CHEMISTRY

LIPID SCIENCE &
TECHNOLOGY

COAL, GAS AND
ENERGY

GENERAL
ENGINEERING

BIO ENGINEERING
& ENVIRONMENTAL
CENTRE

FLURO
ORGANICS

MECHANICAL
DESIGN &
ENGINEERING

CHEMICAL
ENGINEERING
SCIENCES

INSTRUMENTATION

LIBRARY &
DOCUMENTATION
(IMA)

CHEMICAL &
INSTRUMENTAL
ANALYSIS

ORGANIC
COATINGS &
POLYMERS

BIOLOGY

QUALITY
MANAGEMENT

CHEMICAL
BIOLOGY

PHARMACOLOGY



Natural Products Division (Organic Division-1)

1. Natural Products
2. Synthetic Chemistry
3. Molecular Modeling
4. Pheromones
5. Chemical Biology



NATURAL PRODUCTS

1. NEW BIOACTIVE MOLECULES FOR DRUGS AND PESTICIDAL APPLICATIONS
2. LEAD OPTIMIZATION USING COMBINATORIAL NATURAL PRODUCTS AND ANALOGUE BASED APPROACH
3. HERBAL DRUGS AND THEIR STANDARDIZATION



HERBAL INDUSTRY

Global Herbal drug sales: \$100.0 Billion

Developed Countries Share: 50%

Indian Share: Low



Traditional Systems of Medicine

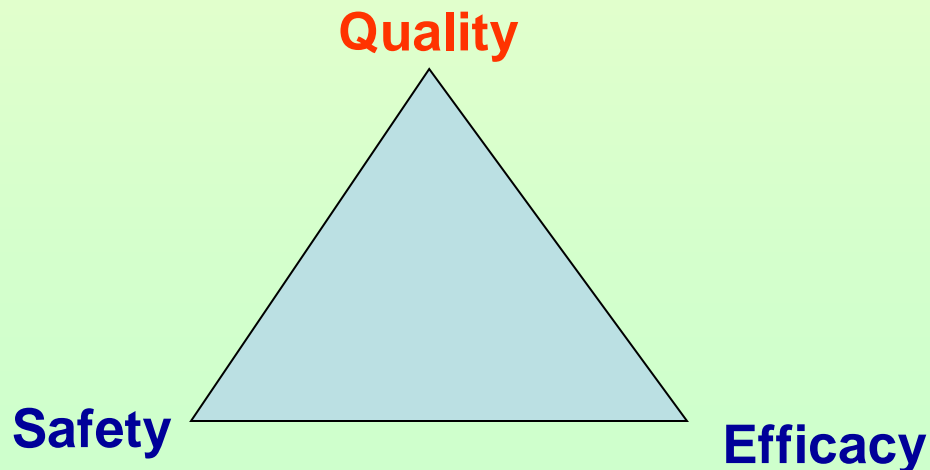
- **Ayurveda**
- **Sidha**
- **Unani**
- **Folklore Medicine.**



The Three Major Issues with Formulations are

- 1. Quality**
- 2. Safety**
- 3. Efficacy**

The Issues Safety and efficacy are directly dependent on Quality





Herbal Drugs & Standardization

Projects completed:

1. Hamdard's Herbal Formulation-**HRF**
2. Preparation and Standardization by HPLC and HPTLC of Amla extract-**M/S Arjuna Natural Extracts Ltd., Kerala**
3. Comparison of Chemical fingerprints of *Solanum melongena* and BT *Solanum melongena*-**M/S Mahyco-Mosanto Ltd., Mumbai**
4. Process and Product Standardization of Classical Ayurvedic Drugs with Special reference to Rheumatism
DST/ICT/AVS Project
5. Quality Assurance and Validation of some Ayurvedic Formulations for Life Style related and Gynaecological disorders :**DST/ICT/IMPCL, Mohan**
6. Isolation of Licoricidine and Licorisoflavone-A from Lico Rice Extract-**M/S Colgate Palmolive**



The Various Methods Known for Standardization include

- 1. Marker compound based standardization**
- 2. Chemical Fingerprinting.**
- 3. Based on Active ingredients.**



Chemical Standardisation

Most Common Ayurvedic Formulations

1.Choornam

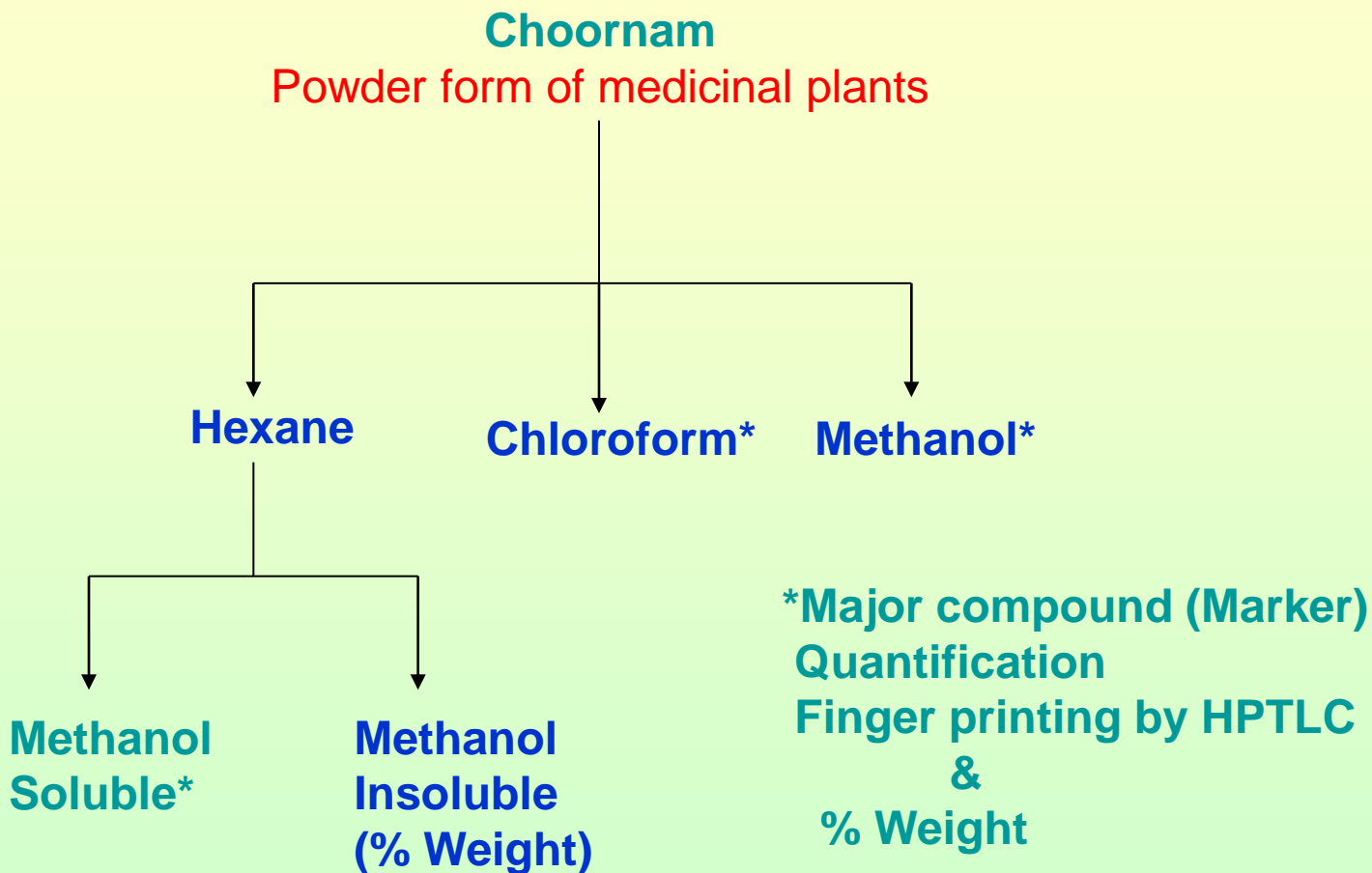
2.Kwatham

3.Ghritam

4. Electuary



Chemical Standardisation

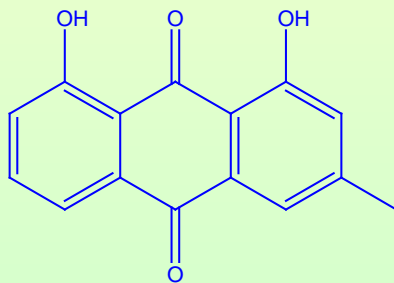




Marker Compound Based Standardisation:

To standardize the herb based on a particular herb with respect to a Marker compound irrespective of whether the compound is responsible for the said physiological effect.

Eg., Standardisation of *Rheum emodi* with respect to Chrysophanol, the major Compound.



Chrysophanol

Ref: *J Planar Chromatography-Modern TLC*, 2002, 15, 128.

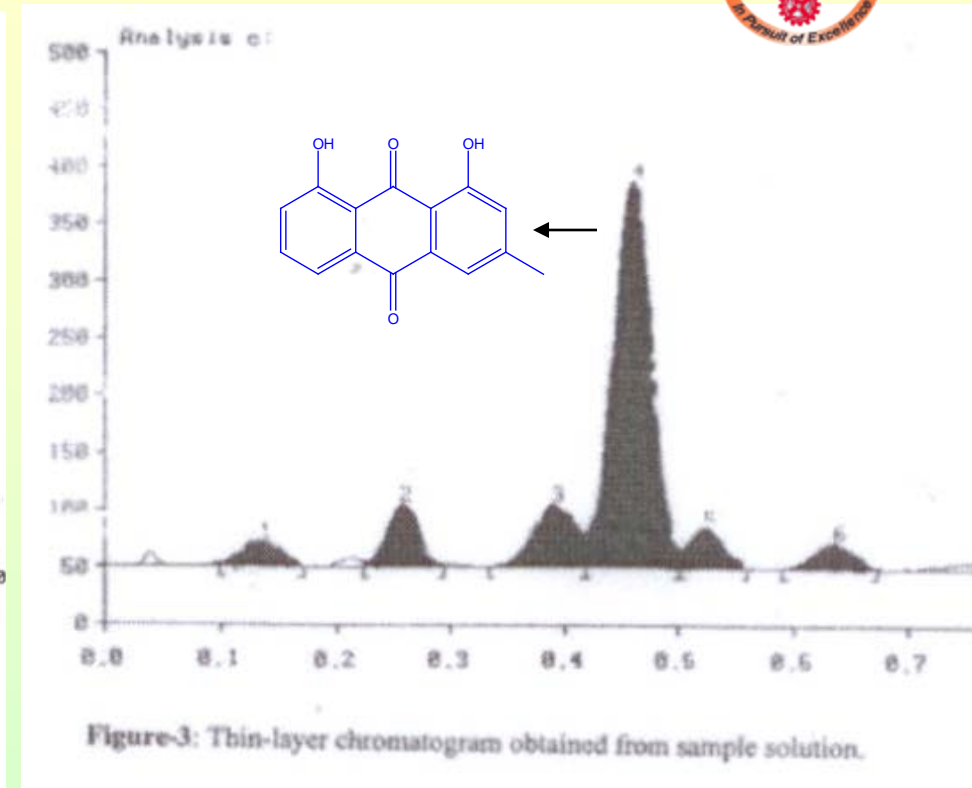
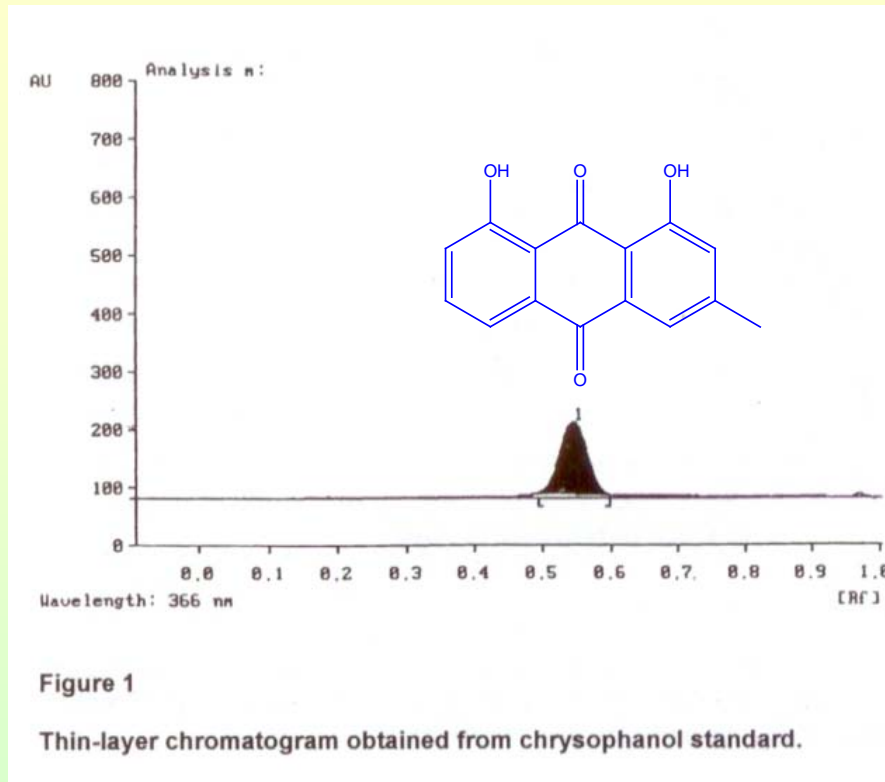
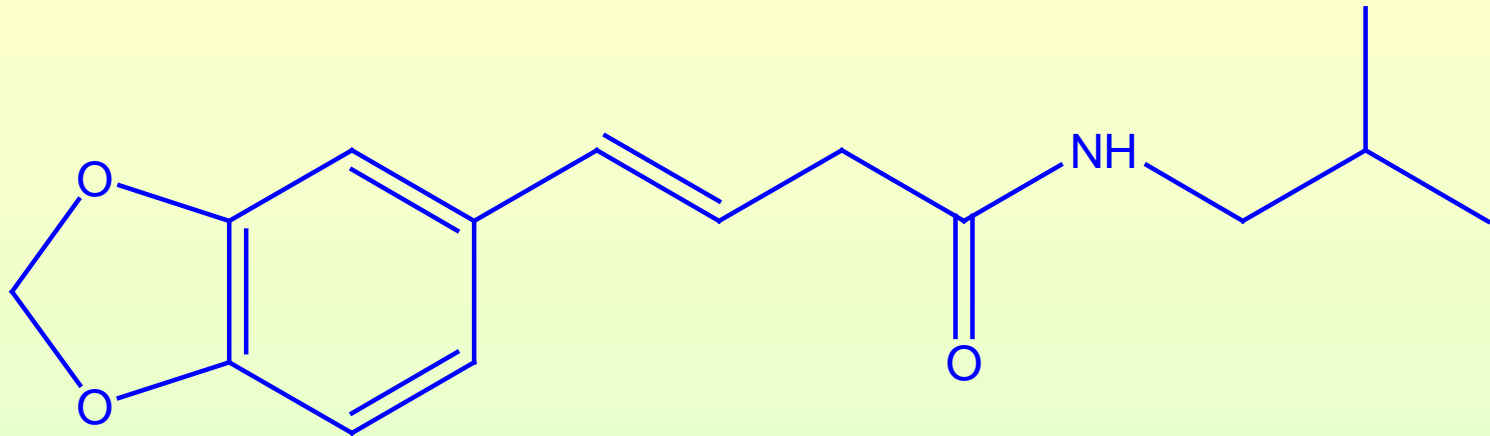


Fig:1: HPTLC chromatogram of Chrysophanol

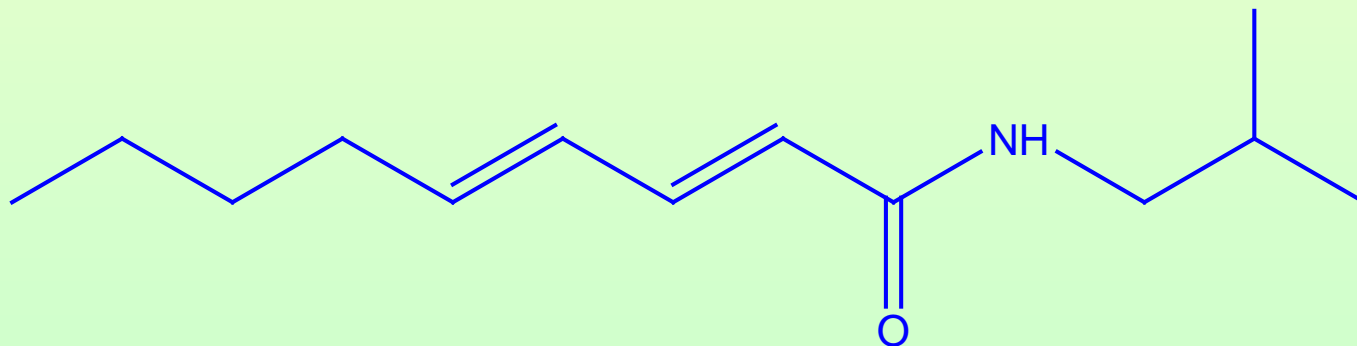
Fig:2: HPTLC chromatogram of methanolic extract of R. emodi



***Piper longum* is standardised with respect to Dihydropiperlonguminine and pellitorine**



Dihydropiperlonguminine



Pellitorine

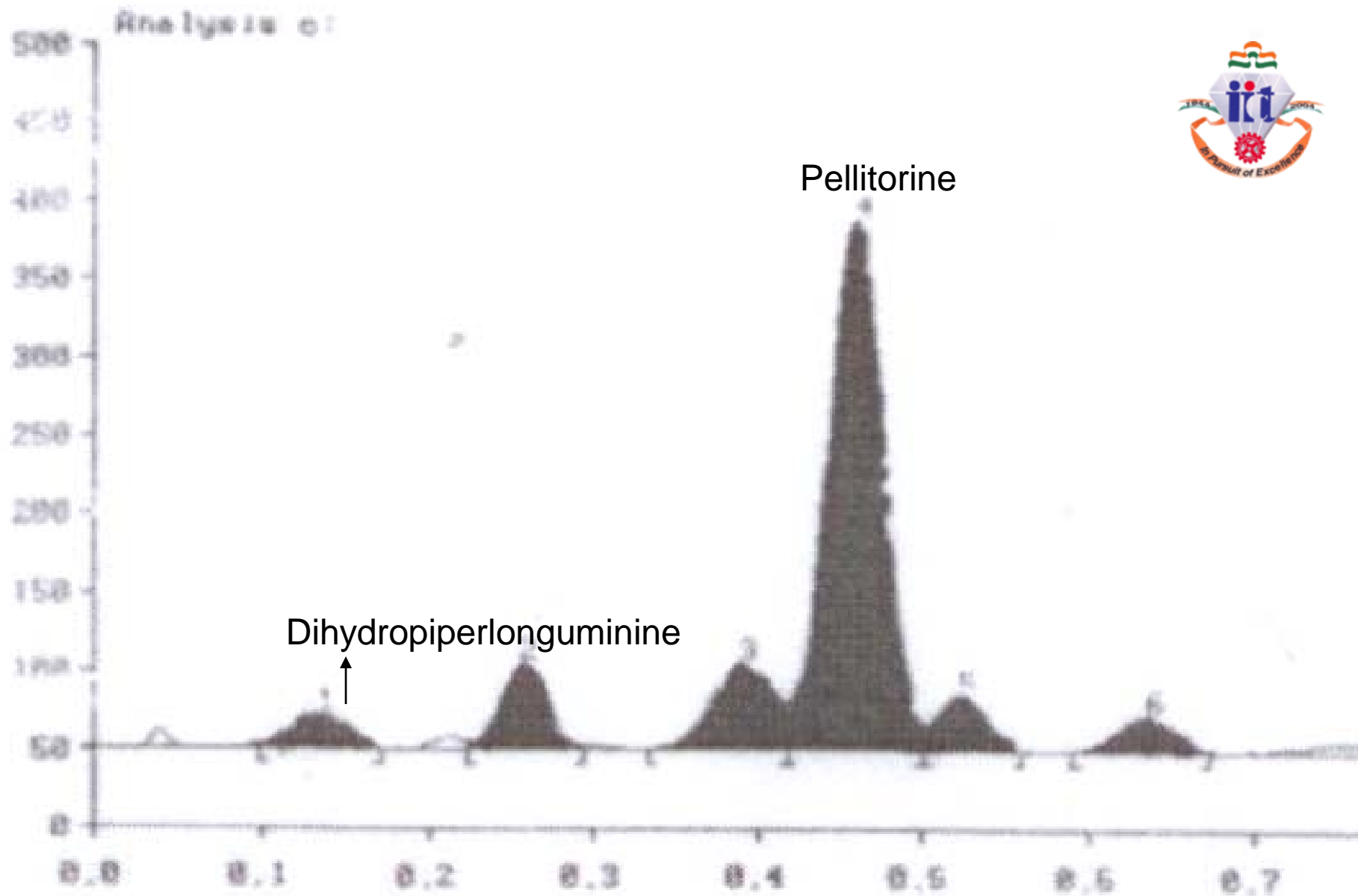
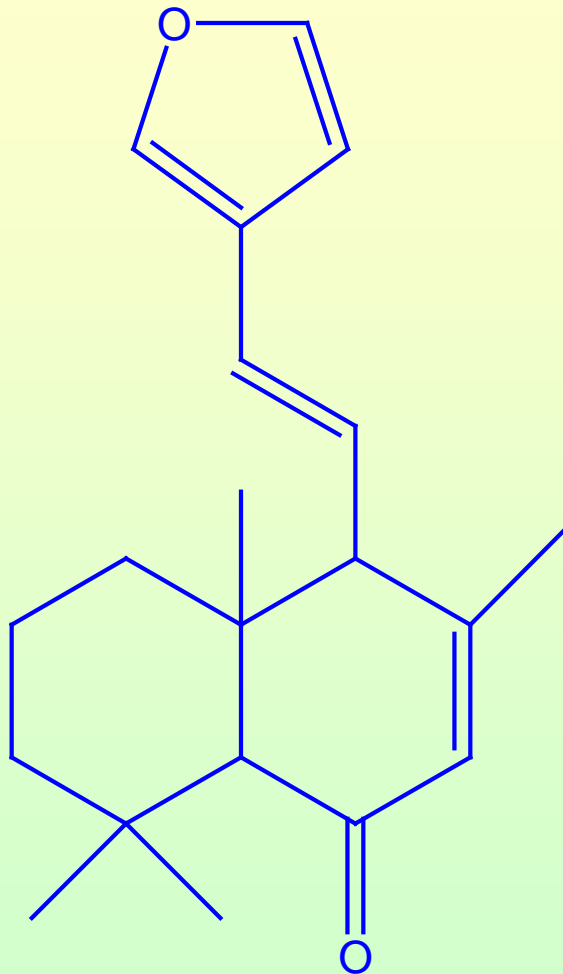


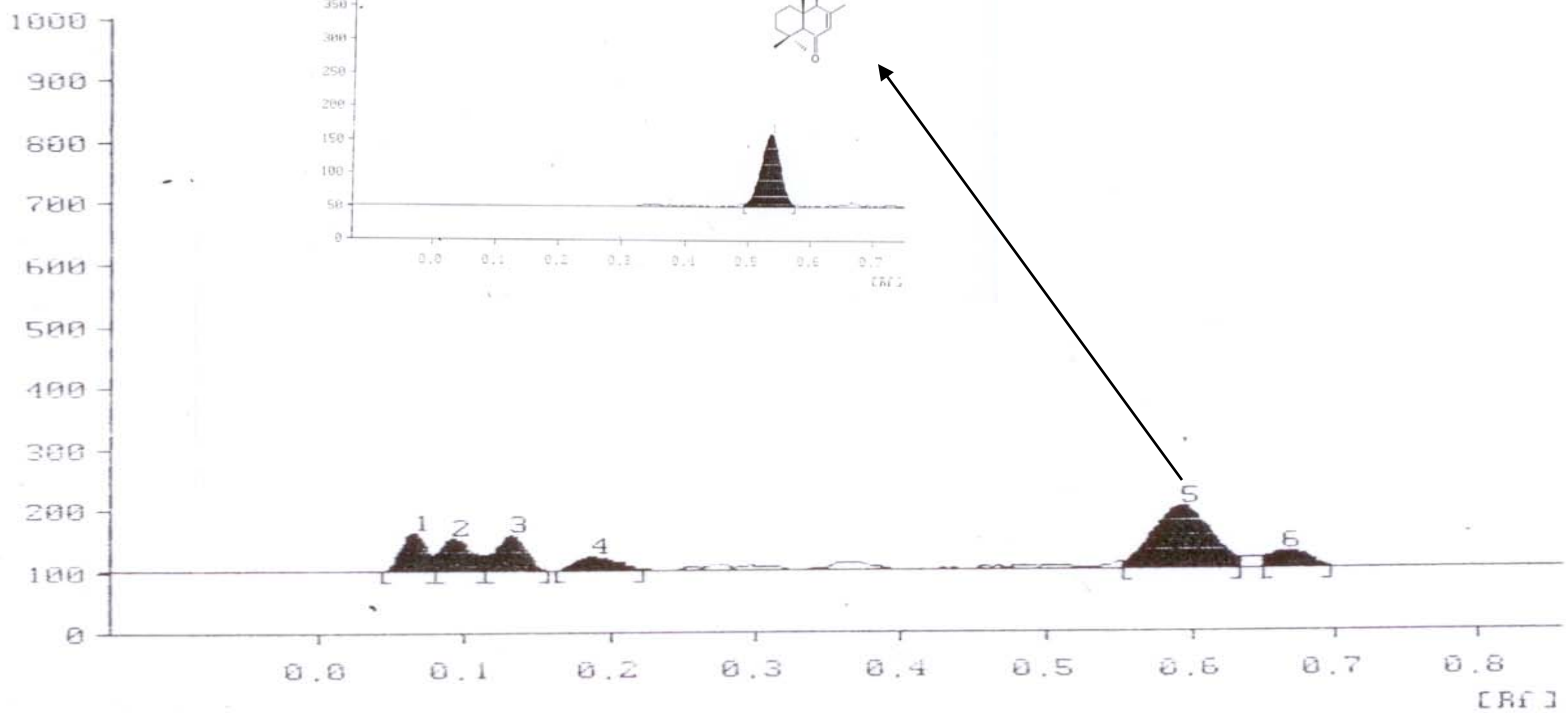
Figure-3: Thin-layer chromatogram obtained from sample solution.



Hedychium spicatum is standardized with respect to hedychenone



Hedychenone



CAMAG SOFTWARE (C) 1998

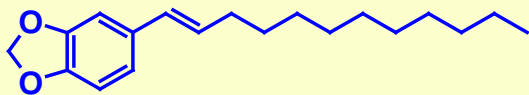
Peak #	start			max			end			area	
	Rf	H	Rf	H	[%]	Rf	H	F	[%]		
1	0.04	0.1	0.07	63.7	19.43	0.08	27.1	899.7	11.97		
2	0.08	27.1	0.09	54.0	16.47	0.11	25.3	966.2	12.86		
3	0.11	25.3	0.13	58.3	17.79	0.16	0.3	1034.9	13.77		
4	0.16	0.0	0.19	23.4	7.13	0.22	1.5	573.9	7.64		
5	0.55	13.0	0.59	101.1	30.87	0.63	17.9	3398.3	45.23		
6	0.65	18.9	0.66	27.2	8.30	0.70	2.2	640.9	8.53		
Total height =				327.6		total area =				7514.0	

Figure-2: Thin layer chromatogram obtained from sample solution.

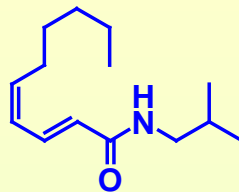


Chemical Fingerprinting

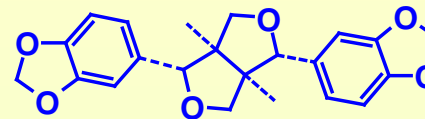
- To isolate all the possible constituents and to generate fingerprinting protocols for the same.
- Phytochemical investigation of *P. longum* led to the isolation of 11 constituents.
- Pipataline, Pellitorine, Sesamine, Brachystamide-B, Guineensine, 5-Hydroxy-7,3',4'-trimethoxyflavone, 5-Hydroxy-7,4'-dimethoxyflavone, 5,4'-Dihydroxy-7-methoxyflavone, Diaeudesmin, Piperine, Dihydropiperlonguminine.



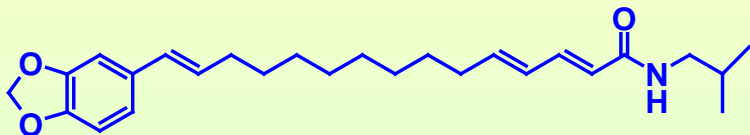
Pipataline



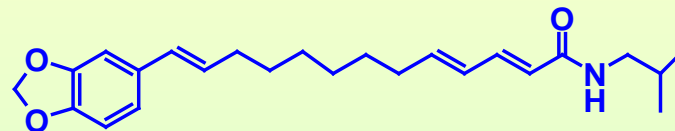
Pellitorine



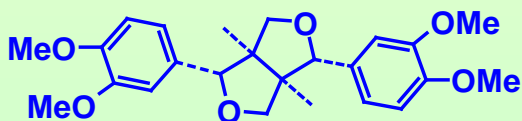
Sesamin



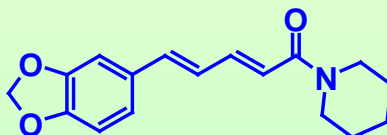
Brachystamide - B



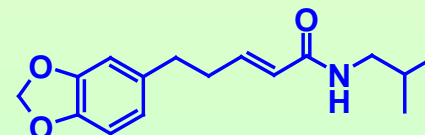
Guineensine



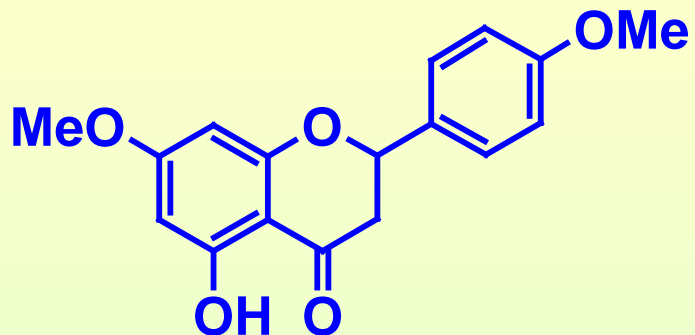
Diaeudesmin



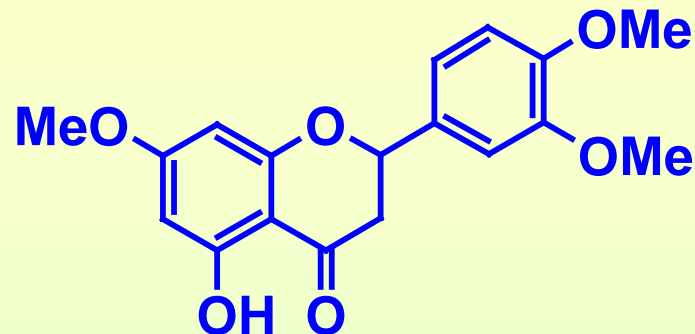
Piperine



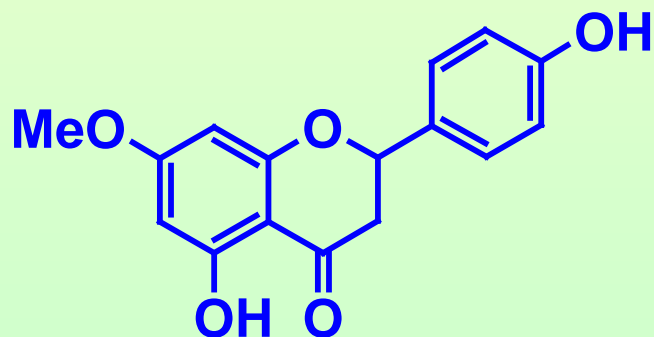
dihydropiperlonguminine



5-hydroxy-7,4'-dimethoxyflavone

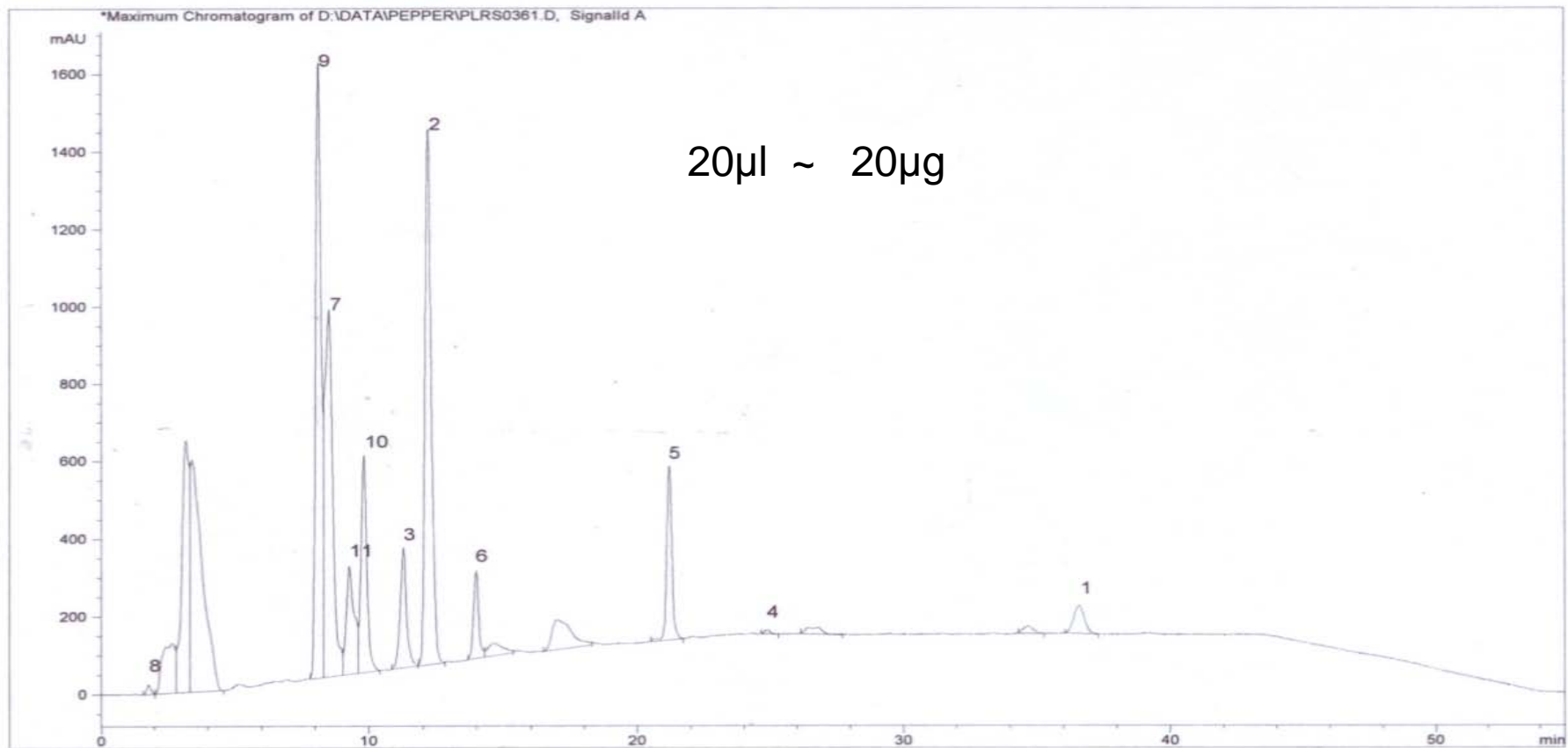


5-hydroxy-7,3',4'-trimethoxyflavone



5,4'-dihydroxy-7-methoxyflavone

1: HPLC chromatogram of methanol extract (1mg/ml) of *P. longum*.



1. Pipataline. 2. Pellitorine; 3. Sesamine; 4. Brachystamide B; 5. Guineensine;
 6. 5-Hydroxy-7, 3', 4'-Trimethoxyflavone; 7. 5-Hydroxy-7, 4'- dimethoxyflavone.
 8. 5, 4'-Dihydroxy-7-methoxyflavone; 9. Diaeudesmin; 10. Piperine;
 11. Dihydropiperlonguminine.

HPLC assisted chemobiological standardisation of α -glucosidase-I
Enzyme inhibitory constituents from *Piper longum* Linn-An Indian
Medicinal plant

P.V. Srinivas, A.K. Tiwari, V.U.M. Sarma, V. Anuradha, T. Hari Babu,
D. Krishna Rao, I.A. Khan and J. Madhusudana Rao*

Journal of Ethnopharmacology, 108(2006) 445-449

Limits of Detection : 0.005-0.001 $\mu\text{g/ml}$ 1-5 ng/ml

Limits of quantification: 0.01-0.05 $\mu\text{g/ml}$ 10-50 ng/ml

Plants & Marker Compounds Selected

- Piper longum-
- Piper nigrum
- Piper chaba

Dihydropiperlonguminine

Piperine

Guineensine

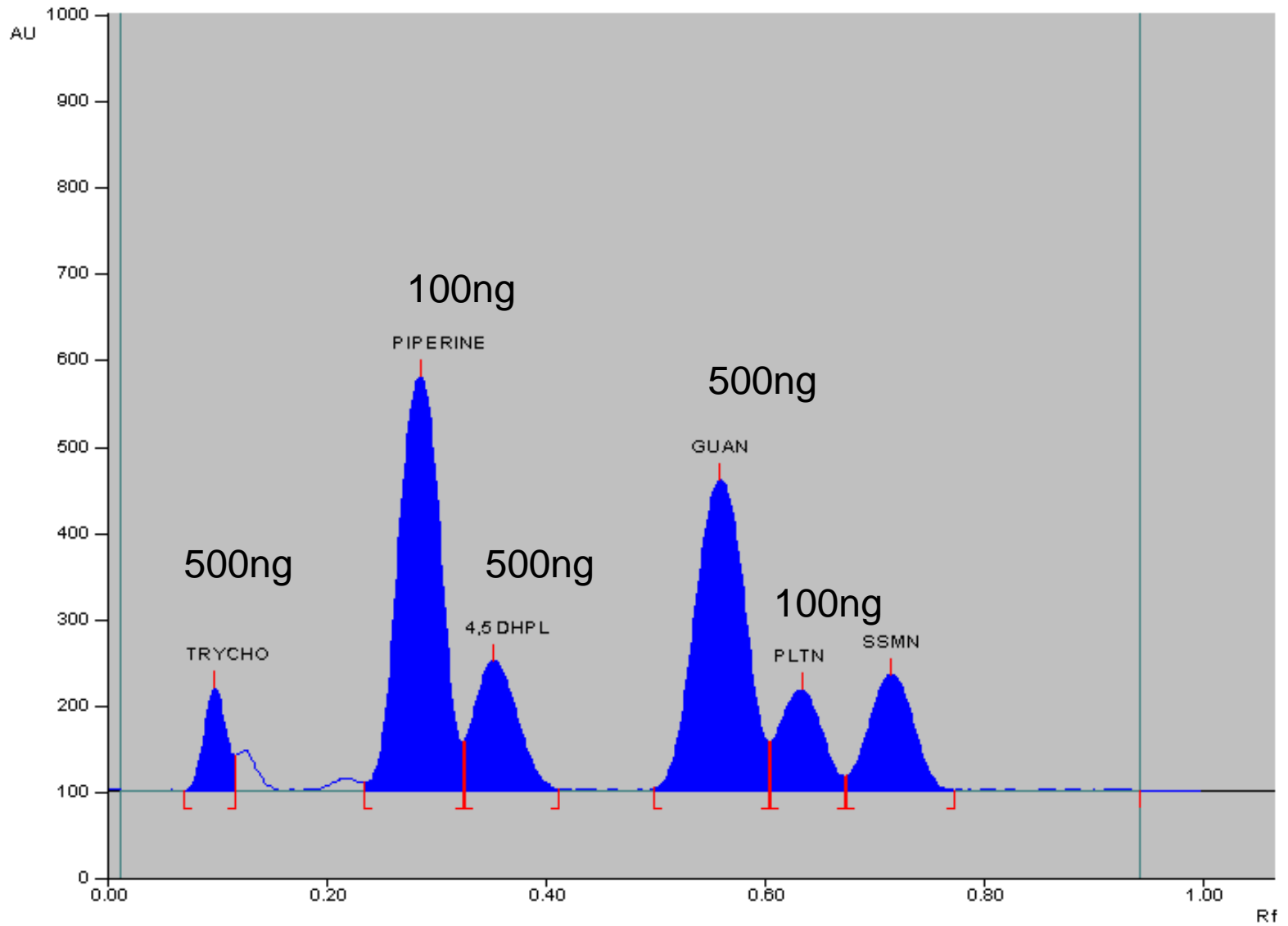
Pellitorine

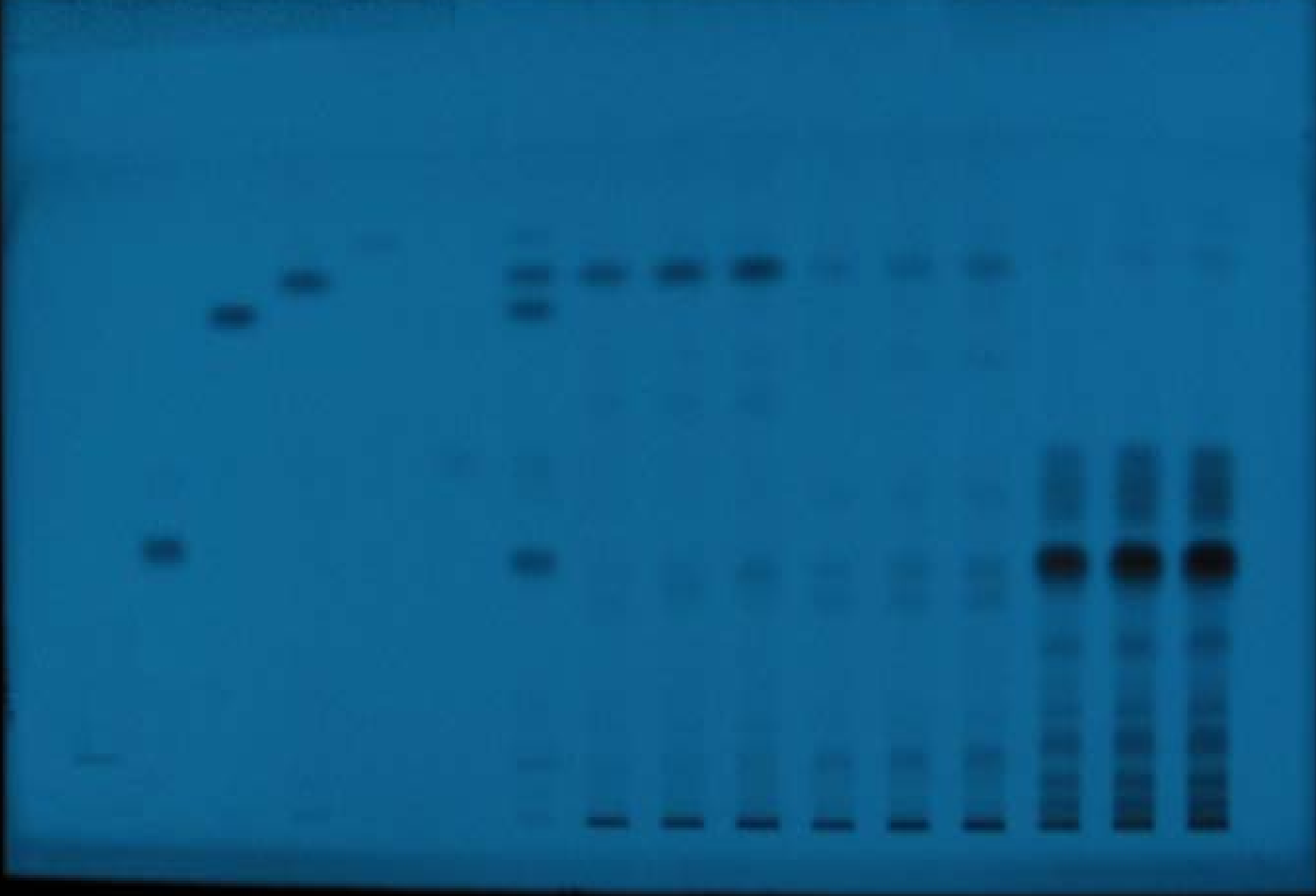
Trichostachine

4. Chamundy's Herbal Formulation for Migrane

- | | |
|----------------------|------------|
| 1. Piper nigrum - | Piperine |
| 2. Nicotina tobacum- | Nicotine |
| 3. Moringa olefera - | Tryptophan |

Track 1 ,ID:





Detection Limits in HPTLC

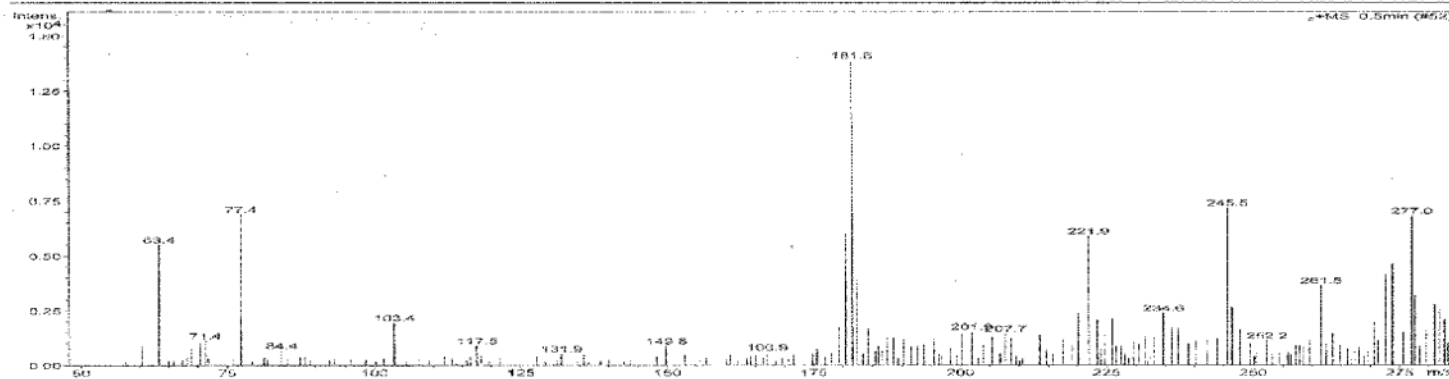
HPTLC/MS study of 4.5-Dihydropiperlonguminine [50-1000ng/track].

Display Report - Selected Window Selected Analysis

Analysis Name: 24951146.d
Method: Copy of dp.MS
Sample Name: 4,5 dhpimn
Analysis Info: 200ng

Instrument: LC-MSD-Trap-SL
Operator: Administrator

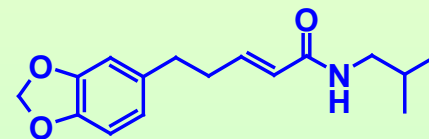
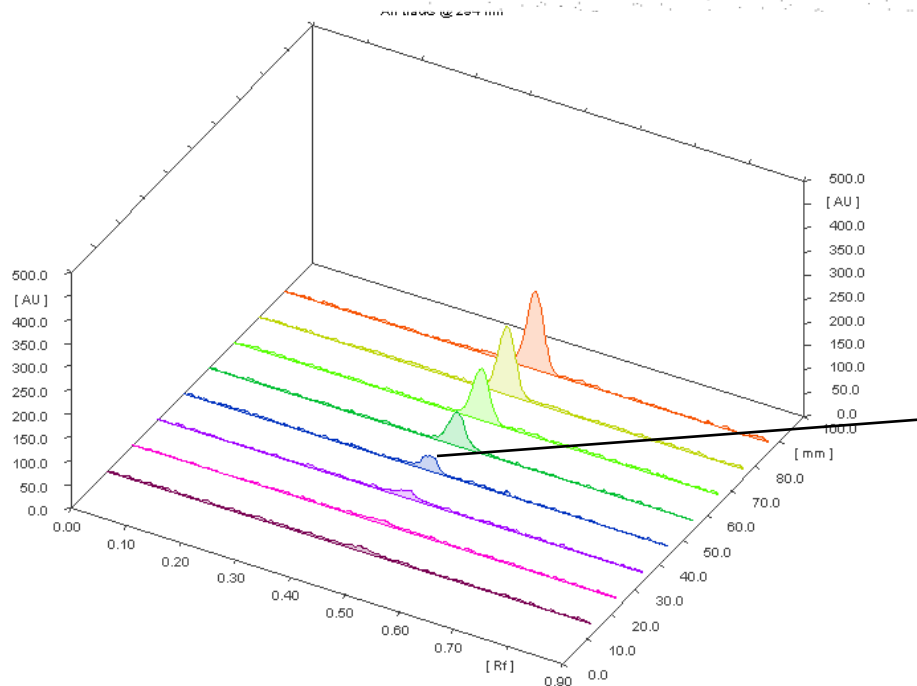
Print Date: 05/16/11 16:24:0
Acq. Date: 05/24/11 7
16:21:44



MSD Trap Report v2

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Agilent Technologies



M+ 275, 200ng

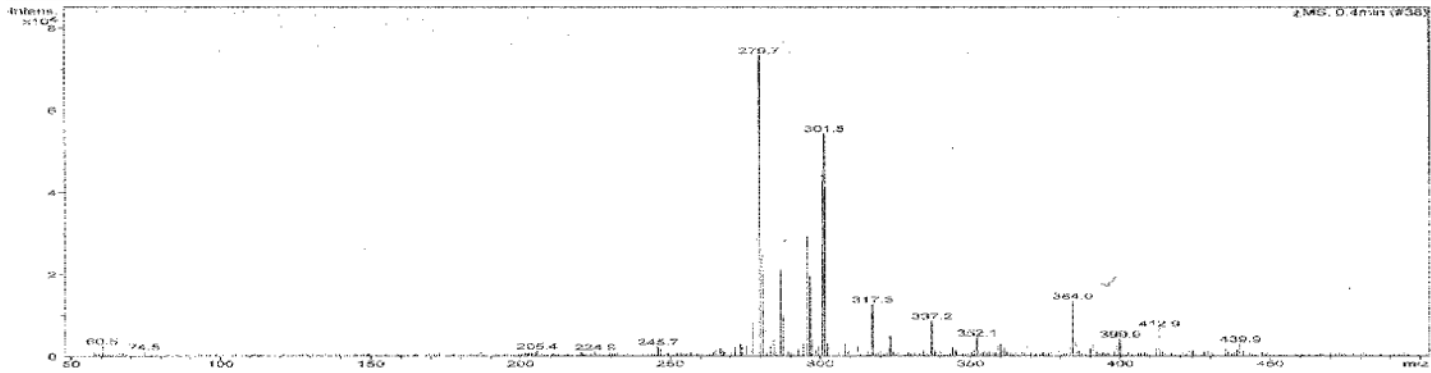
HPTLC/MS study of Guineensine[50-1000ng/track].

Display Report - Selected Window Selected Analysis

Analysis Name: 24051147.d
Method: Copy of dip MS
Sample Name: guineensine
Analysis Info: 50ng

Instrument: LC-MSD-Trap-SL
Operator: Administrator

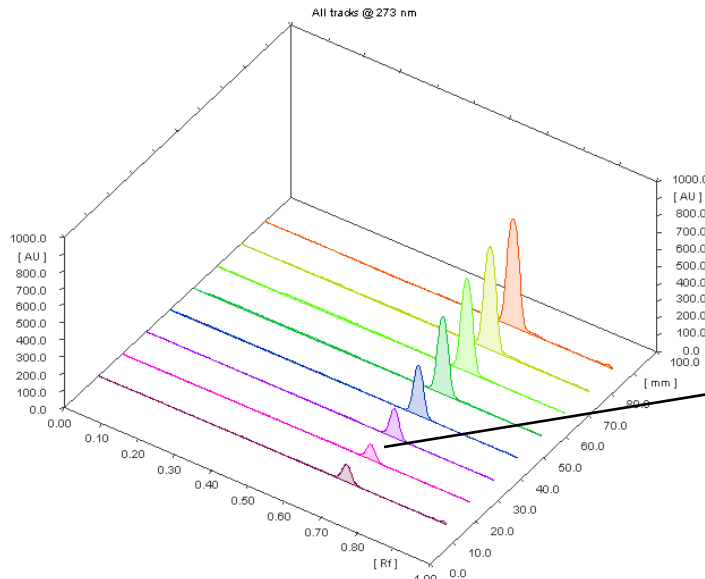
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16:33:59



MSD Trap Report v2

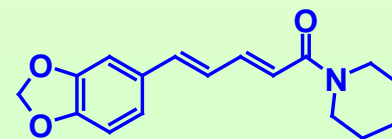
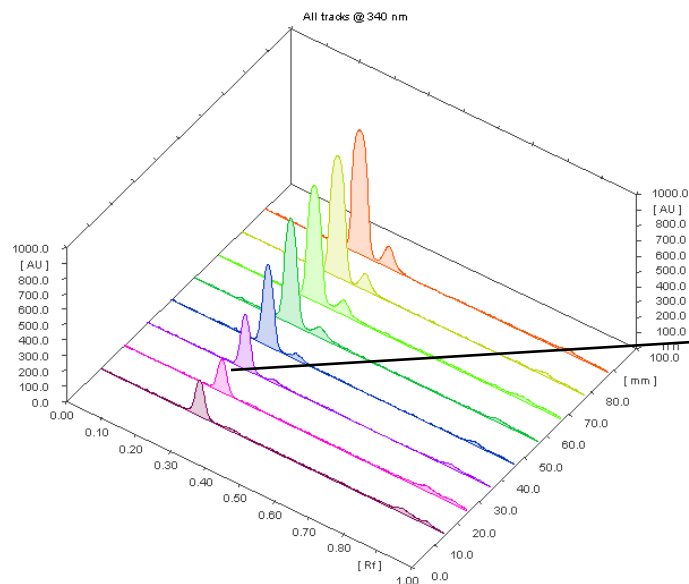
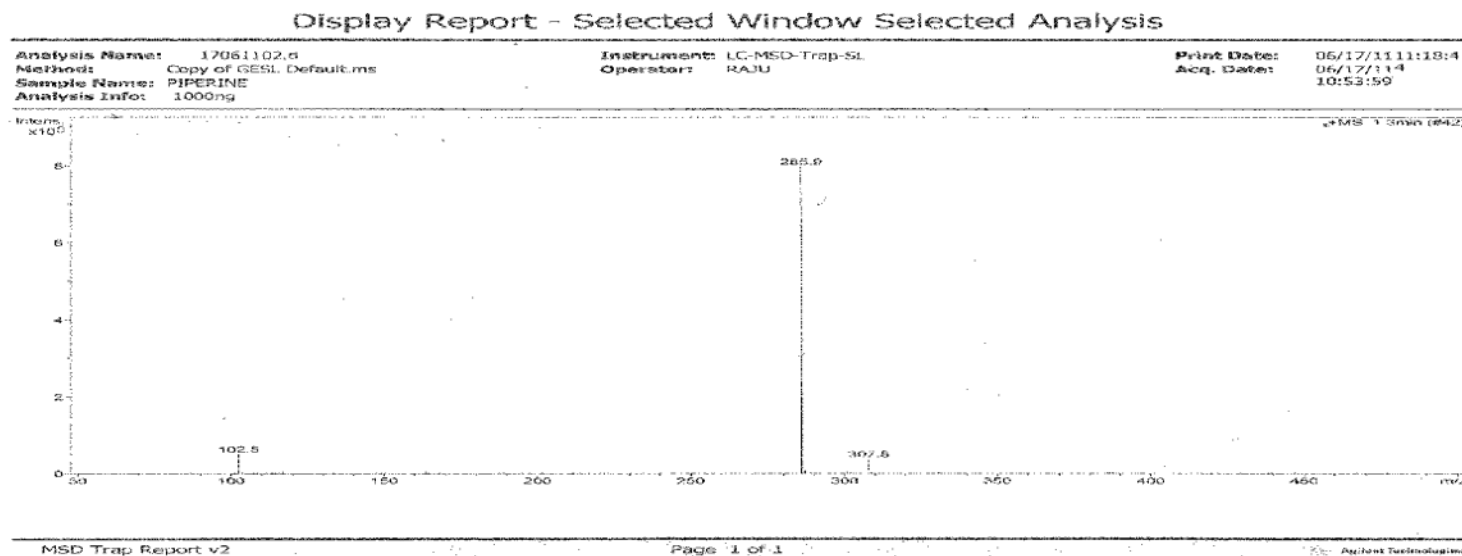
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Agilent Technologies



M+ 383, 50ng

HPTLC/MS Study of Piperine [50-1000ng/track].

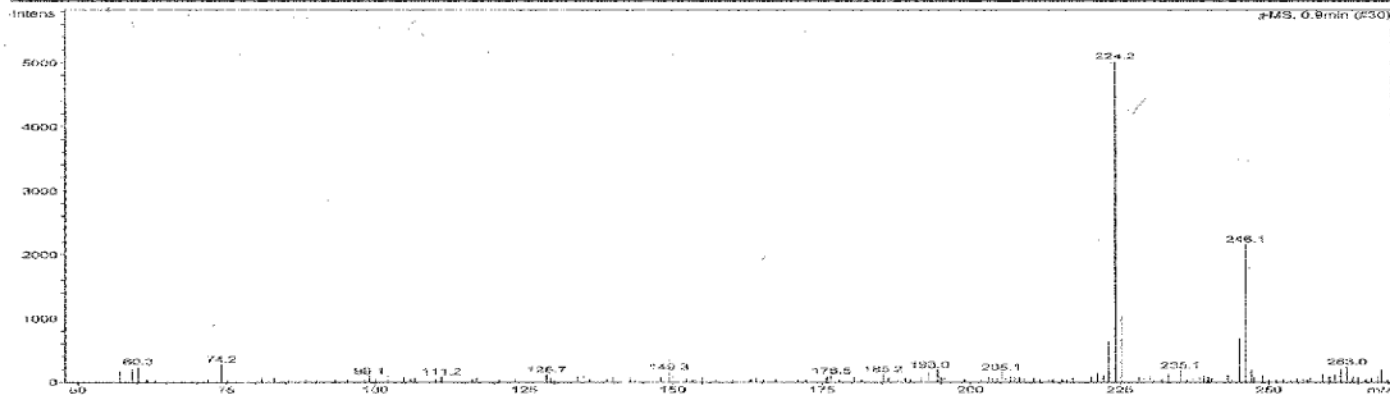


M+ 285, 50ng

HPTLC/MS Study of Pellitorine [50-1000ng/track].

Display Report - Selected Window Selected Analysis

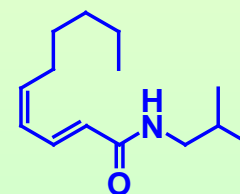
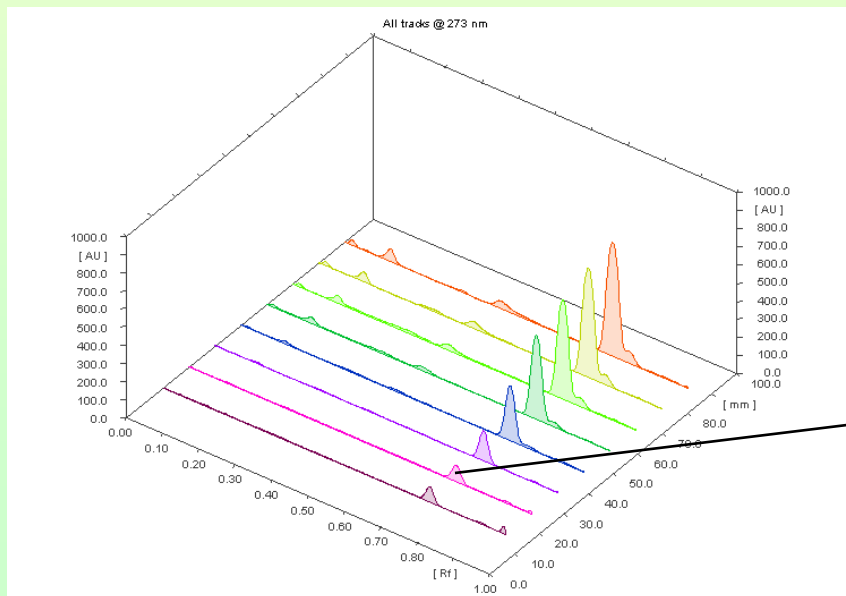
Analysis Name: 240S1135.d Instrument: LC-MSD-Trap-SL Print Date: 06/16/11 16:21:03
Method: Copy of dip.MS Operator: Administrator Acq. Date: 05/24/11 14:56:53
Sample Name: pellitorine-1
Analysis Info: 50ng per track



MSD Trap Report v2

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M+ 223, 50ng

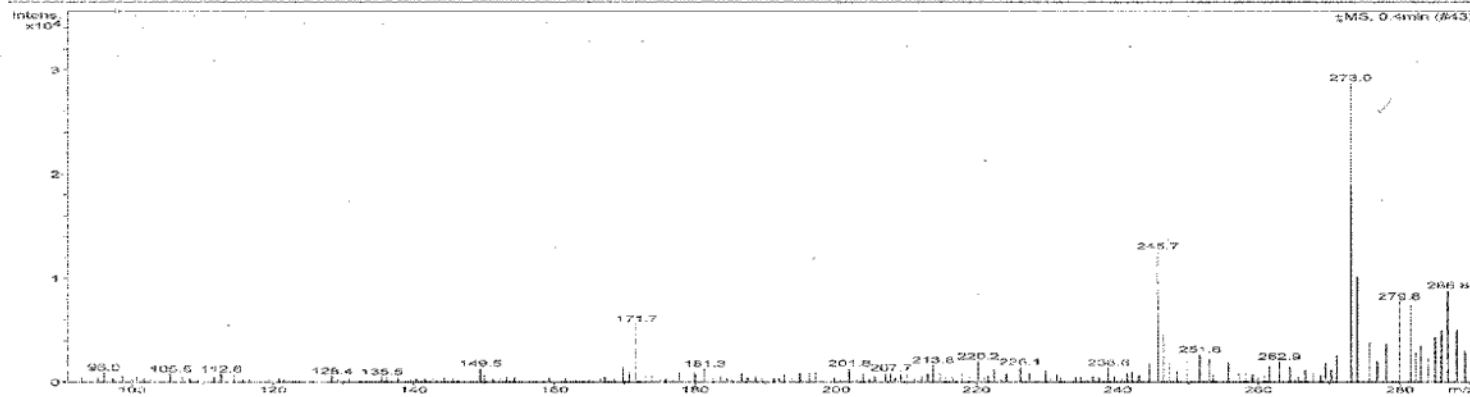
HPTLC/MS Study of trichostachine [50-1000ng/track].

Display Report - Selected Window Selected Analysis

Analysis Name: 24051142.d
Method: Copy of dip.MS
Sample Name: trichostachine
Analysis Info: 50ng

Instrument: LC-MSD-Trap-SL
Operator: Administrator

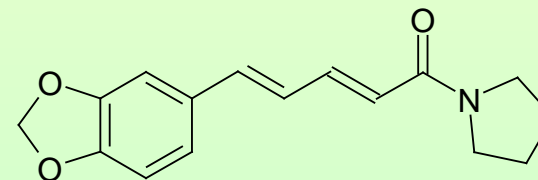
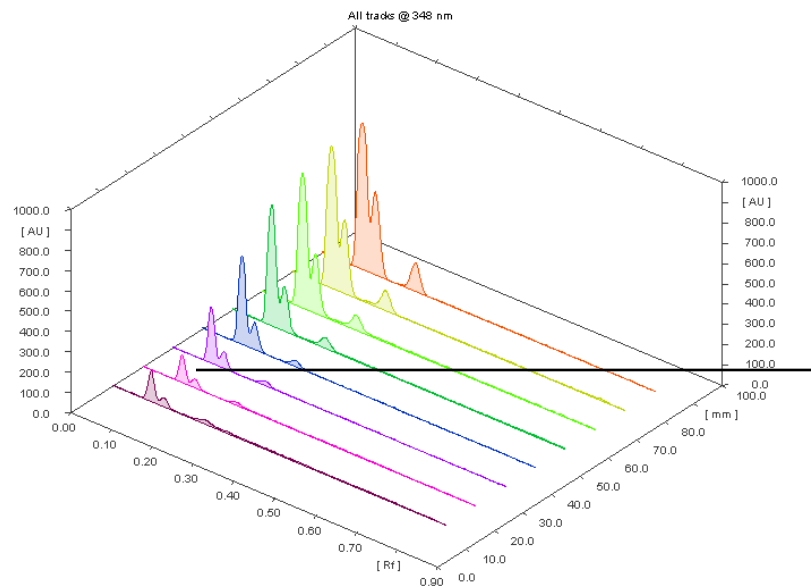
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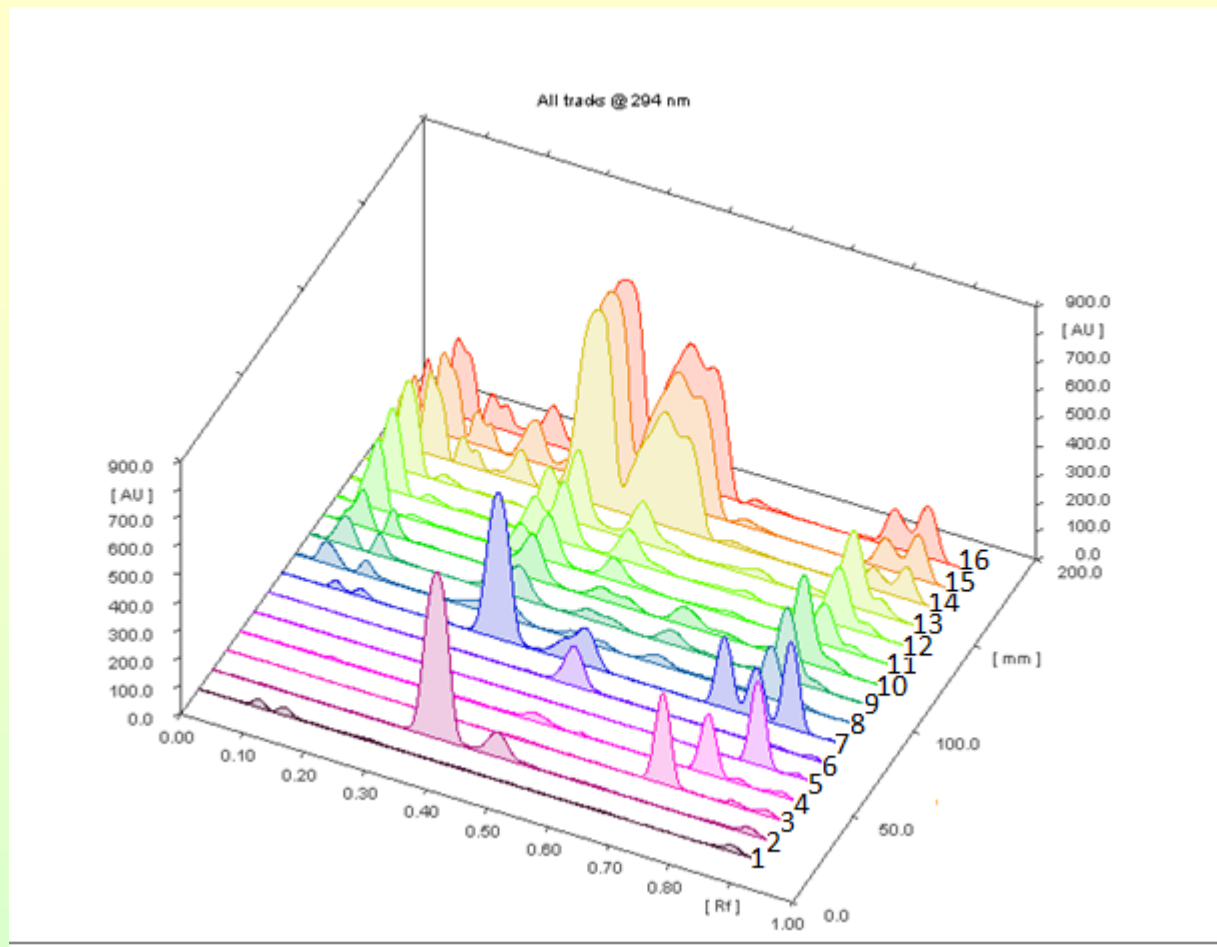
MSD Trap Report v2

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Applint Technologies

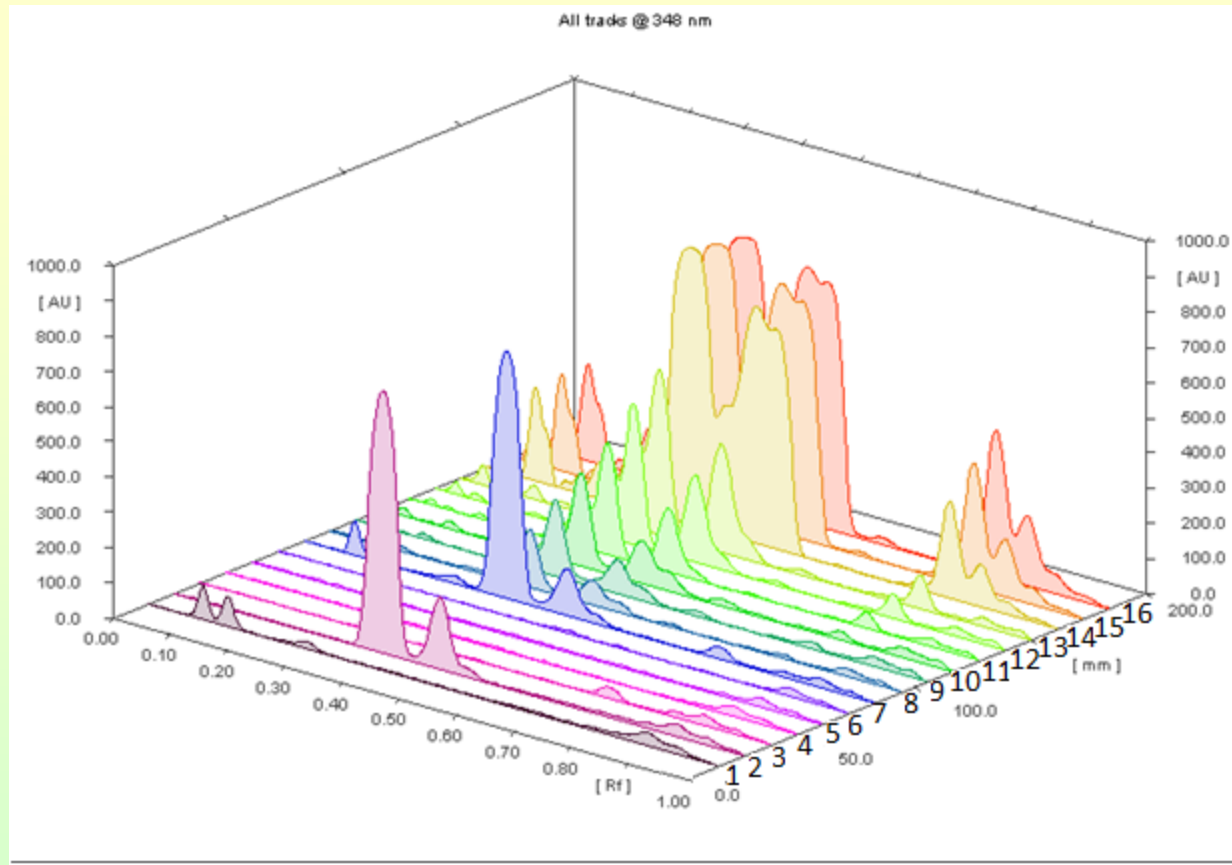


M⁺ 271, 50ng



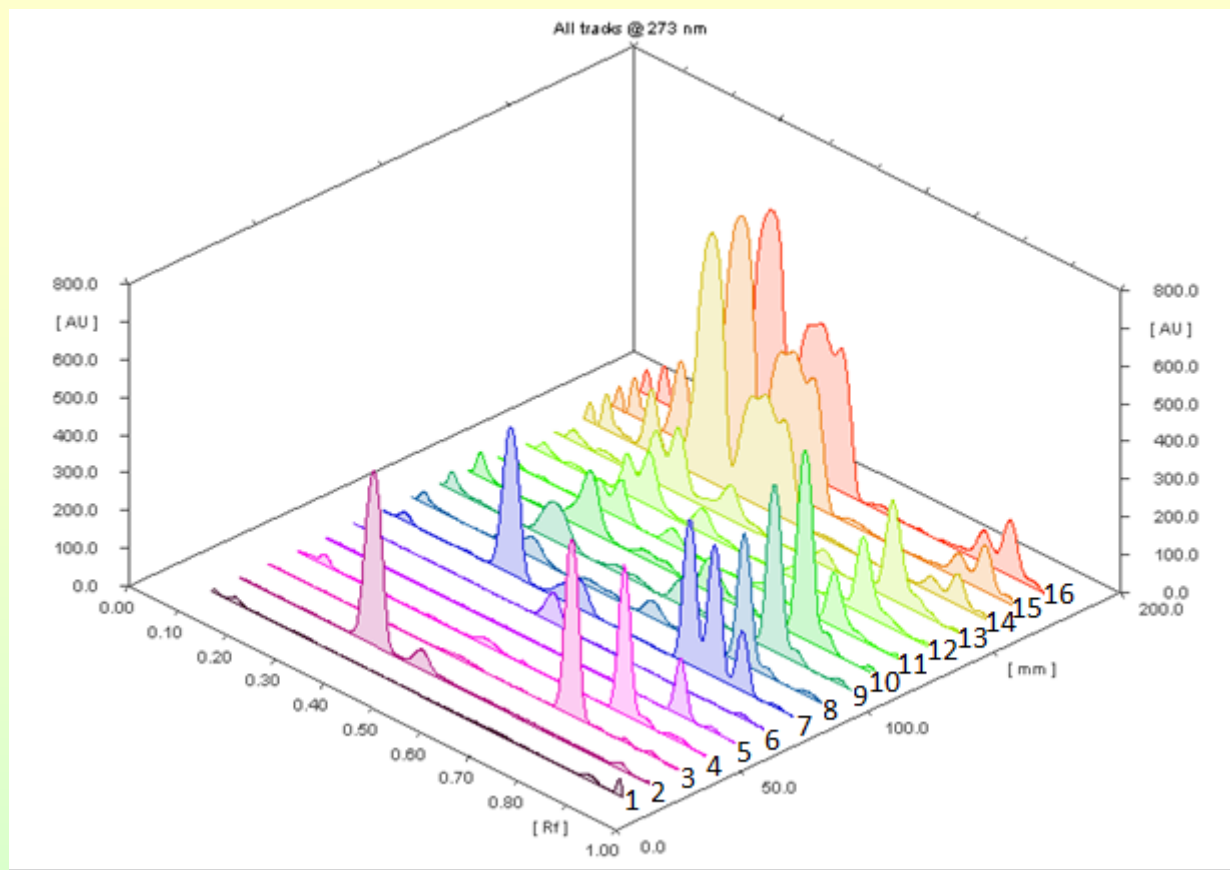
Track-6 4, 5-Dihydropiperlongamunine
Track-7 Standard mix.

Overlaid chromatograms of individual standards (track 1-6) with that of methanolic extracts of *Piper longum*,(track 8-10) *Piper chaba*(track 11-13) and *Piper nigrum*(track 14-16).



**Track-1 Trichostachine. Track-2 Piperine.
Track-7 Standard mix.**

Overlaid chromatograms of individual standards (track 1-6) with that of methanolic extracts of *Piper longum*,(track 8-10) *Piper chaba*(track 11-13) and *Piper nigrum*(track 14-16).

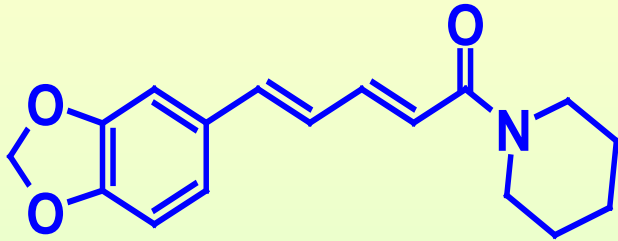


**Track-3 Pellitorine. Track- 4 Guineesine.
Track-7 Standard mix.**

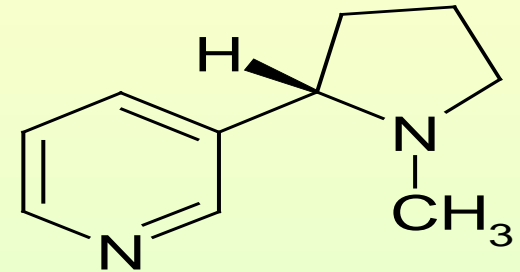
Overlaid chromatograms of individual standards (track 1-6) with that of methanolic extracts of *Piper longum*,(track 8-10) *Piper chaba*(track 11-13) and *Piper nigrum*(track 14-16).

Chamundy's Herbal Formulation for Migrane

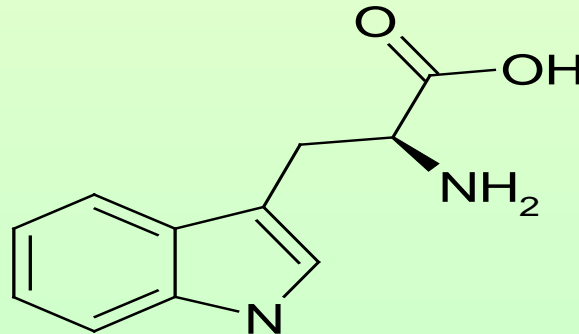
Detection limits of Marker Compounds



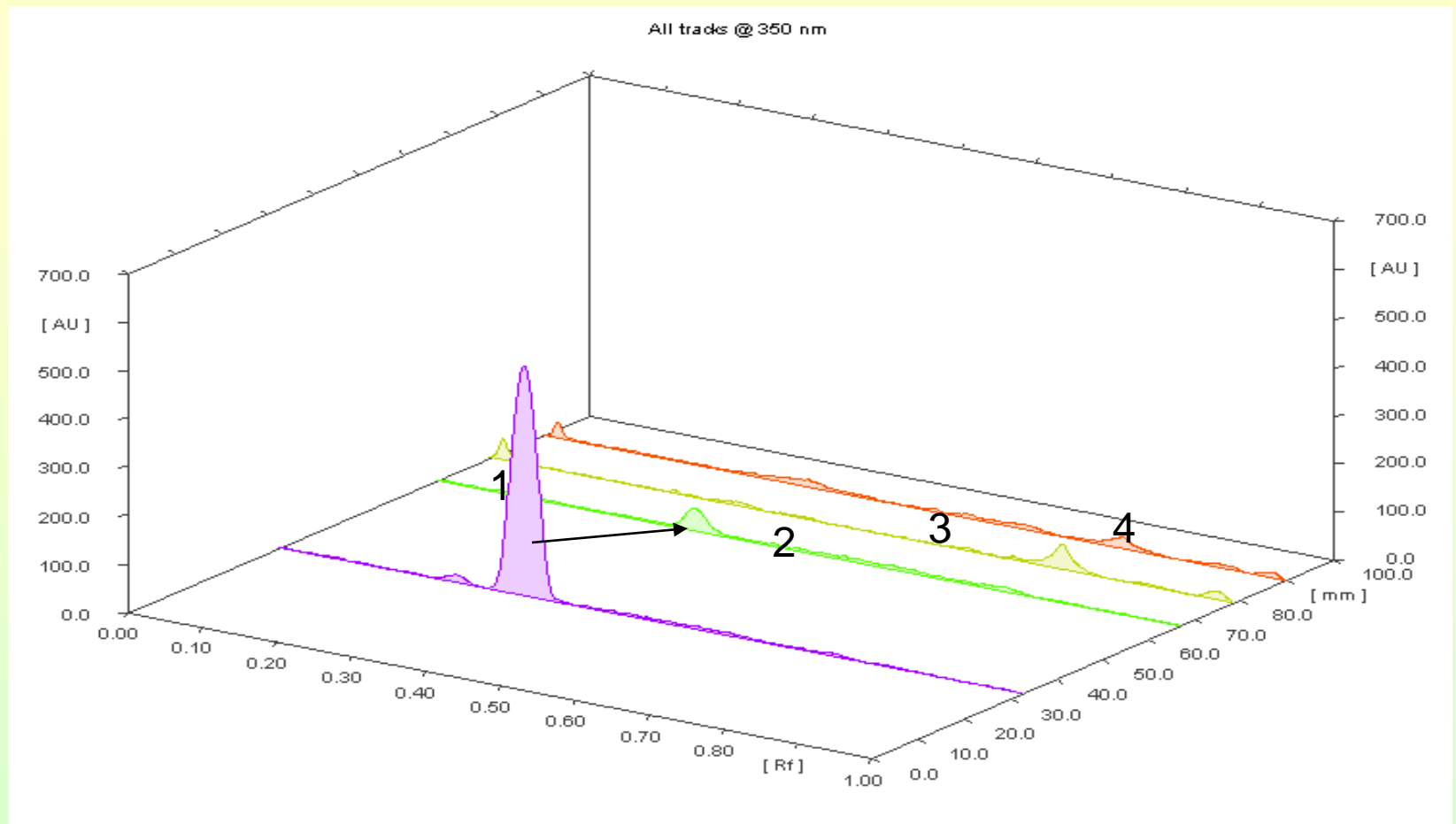
1. Piper nigrum
Piperine - 50ng



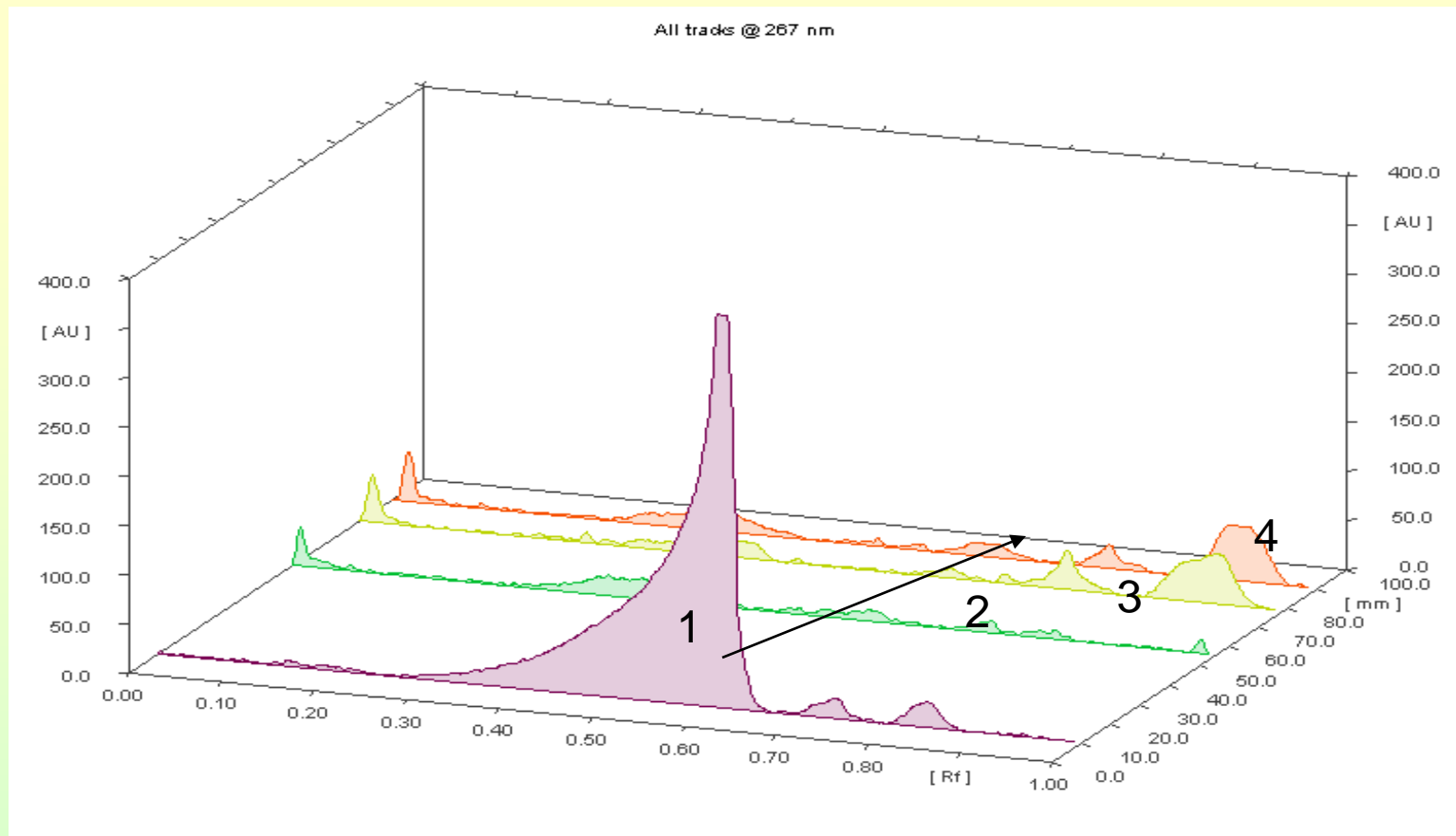
2. Nicotina tobacum
Nicotine – 500ng



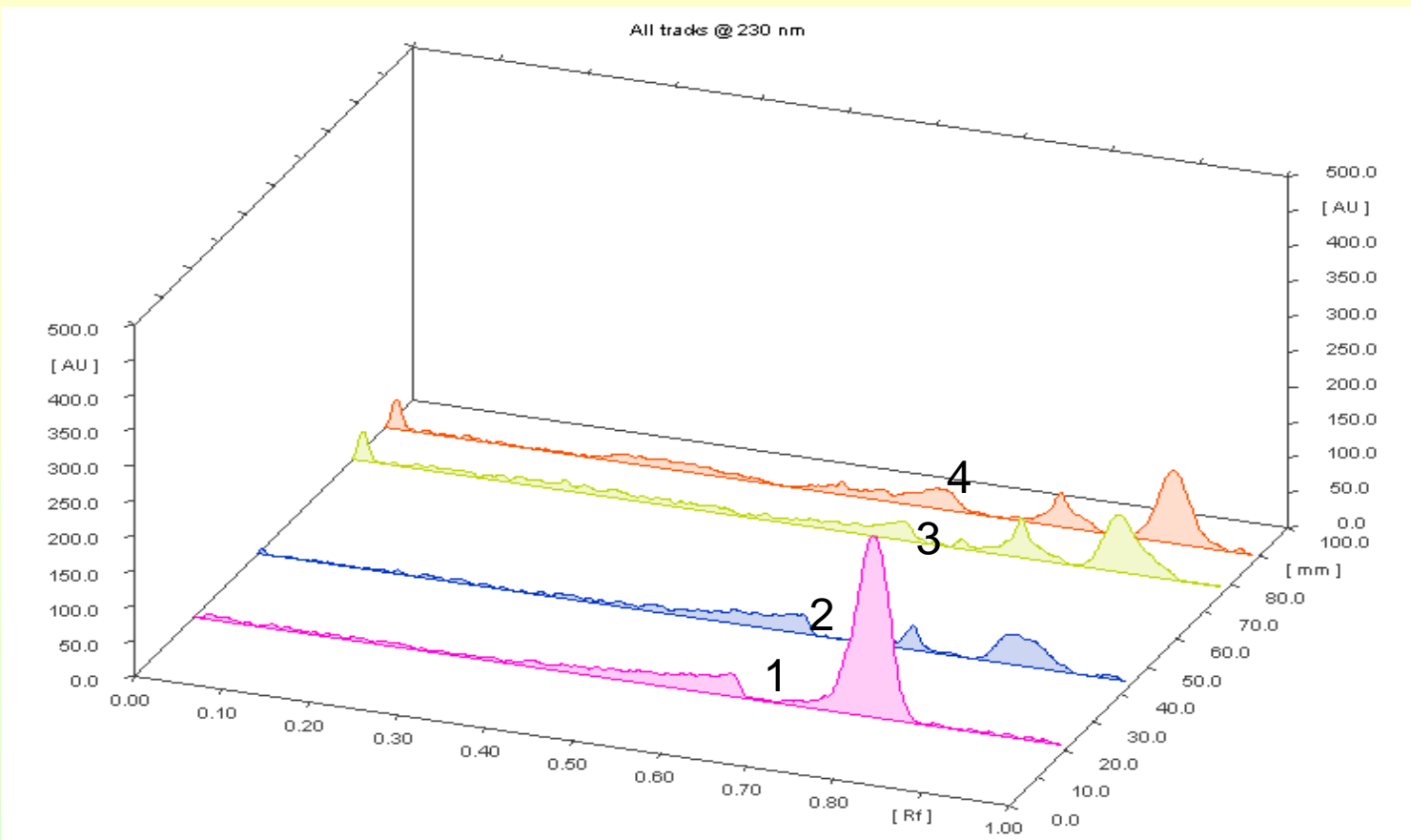
3. Moringa oleifera
Tryptophan – 500ng



1. Piperine - 50ng
2. Piper nigrum extract - Piperine detected
3. Chamundy's Formulation-1 – Piperine not detected
4. Chamundy's Formulation-2 - Piperine not detected



1. Nicotine - 500ng
2. Nicotina tobacum extract – Nicotine detected
3. Chamundy's Formulation-1-Nicotine detected
4. Chamundy's Formulation-2-Nicotine detected



1. Tryptophan – 500ng
2. Moringa oleifera extract – Tryptophan detected
3. Chamundy's Formulation – Tryptophan detected
4. Chamundy's Formulation - Tryptophan detected

Conclusion

1. Piperine, Pellitorine, Dihydropiperlonguminine could be detected in Piper Extracts with the HPTLC detection limits
2. Tryptophan and Nicotine could be detected both in individual extracts and Chamundy's formulations.
Piperine could be detected in the individual extract but not in Chamundy's formulations

Future work:

Efforts are under way for determining the Quantification Limits and improve the sample preparation method for detection Limits using HPTLC/MS



THE FACILITIES

STANDARDIZATION

- 1. HPTLC, AMD & DOCUMENTATION SYSTEM**
- 2. HPTLC/MS**
- 3. TLC-FID**

GOLDEN JUBILEE BLOCK
NATURAL PRODUCTS LABORATORY



www.iictindia.org



Thank you