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Characterization and isolation of quinones from *Tectona grandis* Linn. leaves using innovative hyphenation of TLC-MS interface with LC/MS/MS system

ASMS 2015 MP 229

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Introduction

Tectona grandis Linn. (*T. grandis* Linn.) (Family - Verbenaceae) is commonly called as 'teak' (shown in Figure 1) and locally known as 'sagwan' and one of the most famous timber plants in the world. This plant is well known for its dimensional stability, extreme durability and hardness which also resist decay even when unprotected by paints and preservatives. A variety of interesting but limited compounds have been isolated and identified from *T. grandis* Linn. still more study needs to be performed especially for its color components which mainly includes quinones[1][2].The botanical classification of *T. grandis* Linn. is given in Table 1.

HPTLC technique is used for the separation of phytochemicals, followed by its identification using LC and LC/MS/MS system. Traditional introduction of sample from TLC plate to MS is cumbersome and time consuming.The new TLC-MS interface by CAMAG, Switzerland (shown in Figure 2) has made it possible to have online transfer of a fraction from TLC plate to MS. The feature of 'optimisation of method' present in LCMS-8040 (shown in Figure 3), provides qualitative confirmation of compounds w.r.t. standards.

Figure 1. Leaves of *Tectona grandis* Linn.

Table 1. Botanical classification of *T. grandis* Linn.

Kingdom	Plantae
Division	Eudicots
Class	Asterids
Order	Lamiales
Family	Verbenaceae
Genus	*Tectona*
Species	*grandis*

Method of analysis

Collection of plant material

T. grandis Linn. leaves were collected from Keshav Srushti botanical garden, Mumbai during winter season and taxonomically certified. Leaves were dried under the shed for three weeks and then powdered.

T. grandis Linn. dry leaves powder was subjected to HPTLC separation, six well separated colour bands were observed as shown in Figure 6, which subsequently underwent TLC-MS and LC/MS/MS analysis.

Procedure of sample preparation

250 mg of plant powder was taken in glass tube and then 0.1 mL of HCL was added. After mixing well, 4.9 mL of ethyl acetate was added to it and sonicated for 30 min, filtered and used for HPTLC analysis.

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Optimized conditions for HPTLC

Chromatography was performed on HPTLC silica gel 60 F_{254} pre-coated plates. 10 µL of sample was applied on the HPTLC plate with the help of CAMAG sample applicator. Chamber saturation was done using whatman no.1 filter paper for 15 minutes with respective mobile phases.

For first development PET ether (60-80) :ethyl acetate : formic acid (7:3:0.5 v/v/v) was used and developed up to 50 mm, then plate was dried in cold air. The second development was done up to 80 mm with PET ether (60-80) :ethyl acetate : formic acid (8.5:1.5:0.5 v/v/v). HPTLC chromatogram obtained from CAMAG densitometer as shown in Figure 5 and bands image obtained from CAMAG TLC visualizer as shown in Figure 6.

TLC-MS interface and LC/MS/MS conditions

Figure 2. TLC-MS interface by CAMAG

Figure 3. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Detail parameters of TLC-MS interface and LC/MS/MS are given in Table 2.

Table 2. analytical conditions of TLC-MS interface and LC/MS/MS

Column	: Not applicable
Mobile phase	: A: 0.1% formic acid in water B: acetonitrile (A:B, 30:70 v/v)
Flow rate	: 0.3 mL/min
Oven temperature	: 40 °C
MS interface	: Dual Ion Source (DUIS)
MS analysis mode	: Q3 scan mode
Polarity	: Positive and negative
MS gas flow	: Nebulizing gas 2 L/min; Drying gas 15 L/min
MS temperature	: Desolvation line 250 °C; Heat block 400 °C
TLC-MS interface N_2 gas pressure	: 4-6 bar

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Steps involved in sample analysis by TLC-MS interface

Entire procedure can be divided into three steps.

1. Developing of the TLC plate

The procedure is same as followed with any TLC experiment (Refer point 2-3). The precaution needs to be taken of using HPLC grade solvents and high purity reagents.

2. Extraction of the bands/zone of interest by using the new interface

Component mixtures, even with heavy matrix load, can be separated cost efficiently on TLC/HPTLC plates. The TLC-MS interface is then positioned on the band/zone. TLC-MS interface is operated in semi automatic mode; it means that after manual positioning of the band/zone to be extracted, the piston of the interface is lowered. The flow of the solvent can then be started, which extracts the band/zone.

The data acquisition needs to be started by direct infusion method or by Flow Injection Analysis (FIA) before the actual extraction. After the extraction is completed the cleaning procedure is to be carried out for 5 seconds and then the system is ready for the extraction of the next band/zone.

The extraction piston is lowered on the plate with a force of 20 kg to seal the zone to be extracted. Then a suitable solvent e.g. methanol and may be a buffer depending on the application, is pumped through the inlet capillary of the piston (shown in Figure 4). The typical flow rates are between 50 to 500 µL/min. The solvent is pumped through the layer and elutes the substance.

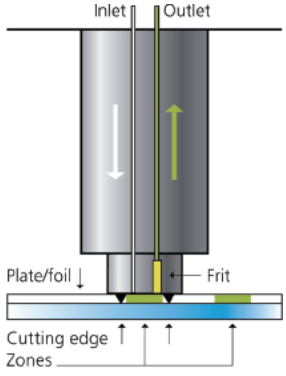

Figure 4. The schematic of extraction piston

3. The MS or MS/MS analysis using ESI, APCI and DUIS mode

The eluted substance from the band/zone is passed through the frit to avoid silica particles from entering into the MS or its ionization source. By switching the valve the zone is directly connected to MS through one of its ionization sources (ESI, APCI and DUIS). The use of ionization source and the selection of solvents depend upon the nature of the substance under evaluation.

HPTLC bands were online transferred to LC/MS/MS system with the help of TLC-MS interface for determination of m/z values. Since, ionization tendencies of these components were not known, mass spectrometric analysis was performed using DUIS mode. In case of DUIS mode, both Electro Spray Ionisation (ESI) and Atmospheric Pressure Chemical Ionisation (APCI) are used simultaneously and hence polar to slightly mid-polar molecules can be analysed in the single run.

Full scan was performed in both positive and negative polarities simultaneously. This enables determination of m/z of HPTLC spots.

Later HPTLC bands were collected using same interface in HPLC vials. It was then subjected to Nexera UHPLC coupled with LCMS-8040 (Shimadzu Corporation, Japan) for MS/MS fragmentation.

LCMS-8040 has a feature of 'optimisation of method' in which the mass spectrometer selects the best product ion(s) for given precursor ion and optimises MS voltage parameters for selected transition. During this optimization step, precursor ions were subjected to series of collision energies sequentially (0 V to -100 V in positive mode and from 0 V to +100 V in negative mode).

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Results

HPTLC and LC/MS/MS analysis

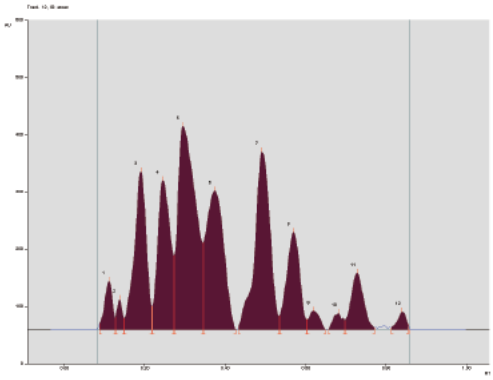

Figure 5. HPTLC chromatogram (540nm)

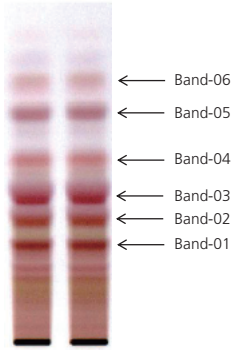

Figure 6. HPTLC image in visible light

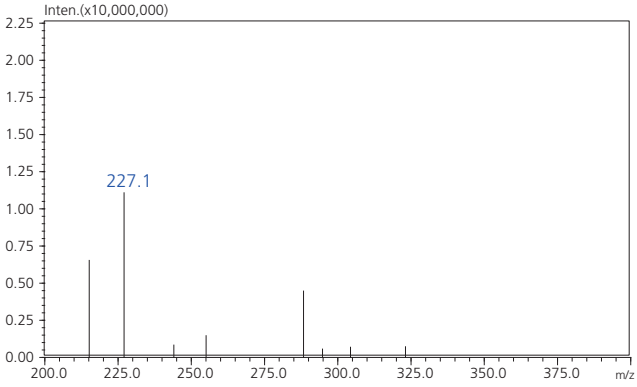

Figure 7. Mass spectrum of band - 03 (m/z 227)

Table 3. HPTLC peak table

Peak	Start Position (Rf)	Start Height (AU)	Max Position (Rf)	Max Height (AU)	Max %	End position (Rf)	End height (AU)	Area (AU)	Area %
1	0.06	20.1	0.07	45.3	2.46	0.08	37.8	729.6	1.21
2	0.08	38.1	0.12	122.5	6.67	0.13	31.7	3820.5	6.35
3	0.13	82.4	0.15	128.9	7.02	0.16	31.4	2455.1	4.08
4	0.16	62.4	0.20	259.4	14.12	0.23	43.9	8249.6	13.71
5	0.23	45.5	0.26	286.9	15.62	0.29	30.7	10008.3	16.63
6	0.29	131.1	0.31	348.6	18.98	0.36	88.7	12714.0	21.12
7	0.36	88.7	0.39	200.7	10.93	0.43	0.2	6819.4	11.33
8	0.44	0.2	0.50	250.0	13.61	0.53	35.0	8607.2	14.30
9	0.53	35.7	0.57	122.6	6.68	0.60	34.3	4722.5	7.85
10	0.60	34.7	0.62	53.3	2.90	0.65	6.9	1612.9	2.68
11	0.66	6.5	0.67	18.6	1.01	0.69	6.0	449.4	0.75

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Out of six bands, details of LC/MS/MS identification and characterization of band-03 are given here.
The third band (Rf - 0.26) on HPTLC plate (Refer Table 3), gave precursor ion of m/z 227 in full scan Q3 mode (shown in Figure 7). For this precursor ion, at CE value -45 V, major fragments with m/z 57, 136, 159, 163 and 186 were obtained. These product ions are characteristic fragments of naphthoquinone, 'Deoxylapachol' which was further confirmed by injecting this standard under same condition and matching its fragmentation pattern. Probable fragmentation pattern of deoxylapachol is predicted and shown in Figure 8. Similarly other native quinones in *T. grandis* Linn leaves like emodin, tectoquinone etc. can be identified and confirmed.

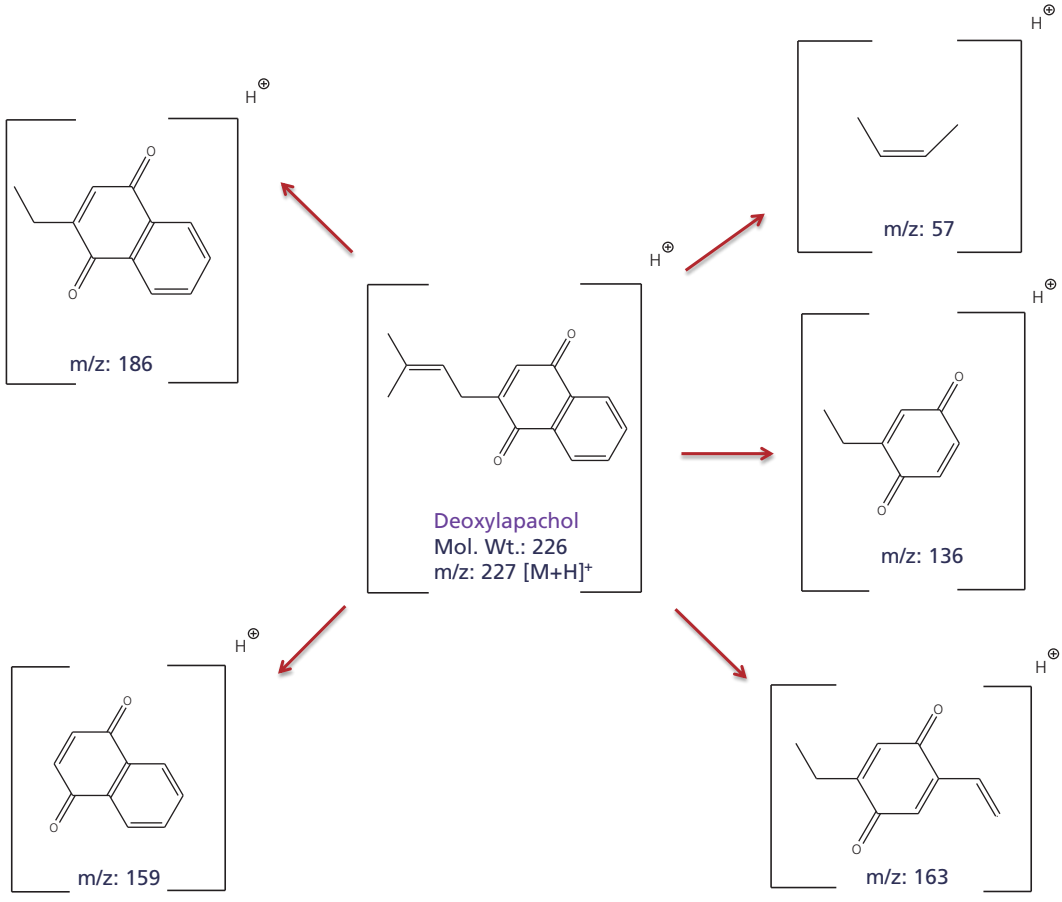

Figure 8. Probable fragmentation pathway of deoxylapachol (m/z 227) in DUIS (+) triple quadrupole mass spectrometry

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Conclusion

- The successful and innovative hyphenation of TLC-MS interface from CAMAG with Shimadzu LC/MS/MS system has enabled quick, convenient and precise online introduction of bands from TLC plate to MS.
- Rf values by TLC analysis along with the m/z and fragmentation patterns by LC/MS/MS analysis gives confirm identification and characterization of compounds.
- Hyphenation of TLC-MS interface with LC/MS/MS system, opens new possibilities into analysis of pharmaceuticals, clinical as well as natural products.

References

[1] Naira Nayeem, M.Karvekar, The International Journal of Pharmacology, Vol.8(1) Jan 8, (2010).
[2] Lacret, Rodney, Varela Rosa, Journal of Chemical Ecology, Vol.37(2011), 1341-1348.

First Edition: May, 2015

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