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# DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF QUINONES IN *TECTONA GRANDIS LINN*

**Shailendra Rane*[1], Manish Hate[1], Prashant Hande[2], Ajitkumar BS[2] and Ajit Datar[3]**

[1]Ramnarain Ruia College, Matunga, India.

[2]Guru Nanak Khalsa College, Matunga, India.

[3]Shimadzu Analytical (India) Pvt. Ltd.

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***Corresponding Author**
**Shailendra Rane**
Ramnarain Ruia College,
Matunga, India.

## ABSTRACT

A simple, precise and accurate LC-MS/MS method has been developed for simultaneous determination of four quinones viz. 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin in leaves powder of *Tectona grandis* linn. MRM (Multiple Reaction Monitoring) transitions 223.10>223.05, 174.00>174.20, 236.20>236.05 and 268.90>269.20 were optimized on Shimadzu triple quadrupole mass spectrometer instrument (Model: LCMS-8040) for quantification of 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin respectively. Chromatographic method was developed on a Shimadzu shimpack-XR C18 column (75mm x 3.0mm x 2.2 µ) by gradient program. The proposed method was validated for linearity, accuracy, precision, recovery, limit of detection and limit of quantitation. The validated LC-MS/MS method can be used for a routine quality control analysis and simultaneous quantitation of quinones viz. 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin in *Tectona grandis* linn.

**KEYWORDS:** LC-MS/MS, quinones, 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone, emodin

## INTRODUCTION

During the past decade, there has been increasing public interest and acceptance of natural therapies in both developing and developed countries. Due to poverty and limited access to modern medicine, about 80% of the world's population, especially in the developing

countries uses herbal medicine as their source of primary healthcare.[1] In these communities, traditional medical practice is often viewed as an integral part of their culture. In the West, people are attracted to herbal therapies for many reasons, the most Important reason being that, viz. our ancestors, it is believed they will help us live healthier lives.[2] Herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products. As such, they represent a substantial proportion of the global drug market[3] Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process.

Hence with the aid of modern scientific methodology and techniques, the standardization of herbal constituents can be carried out systematically and specifically which is the aim of this research work.[4] Recently the hyphenation of HPLC with tandem mass spectrometry LC-MS/MS is widely used because of its better selectivity and sensitivity.[5, 6] Identification, separation and quantification of bio-markers in complex samples can be performed in less time as well as reduced sample preparation as compared to other analytical techniques.

Teak (Tectona grandis Linn. f.) is considered the noblest among all woods not simply because of its golden hue and wonderful texture, but even more because of its durability, strength, attractiveness, workability, and superior seasoning capacity. Teak is one of the most widely planted hardwood timber species in the world.[7]

Teak is moreover considered as a major constituent in many of the traditional medicines. The different extracts from various parts of teak shows expectorant, anti-inflammatory, anthelmintic properties. Traditionally, teak is used against bronchitis, biliousness, hyperacidity, diabetes, leprosy, astringent, and helmintiasis. In traditional medicine, a wood powder paste has been used against bilious headache and swellings. They are also used to treat swellings.[8, 9] According to Ayurveda, the teak wood is acrid, cooling, laxative, sedative to gravid uterus and useful in treatment of piles, leucoderma and dysentery. It allays thirst and possesses anthelmintic and expectorant properties.[10] *T. grandis* Linn. leaf extract is widely used in the folklore for the treatment of various kinds of wounds, especially burn

wounds.[11] Even though commonly seen, teak has not been explored much on pharmacological aspects than for its timber value. Lot of chemicals, with and without biological activity has been isolated from teak. Many anthra- and naphtho-quinones isolated from teak is reported to have biological activity. Some are 2-hydroxymethylanthraquinone, 3'-OH-deoxyisolapachol, hemitectol, 5-hydroxyl-1-4-naphthalenedione, lapachol, deoxylapachol, tectoquinone etc.

Hence, the principle of the study was to develop a simple, economic, rapid, precise standardization method and validate technique for simultaneous quantitation of quinones viz. 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin (6-methyl-1,3,8-trihydroxyanthraquinone) which is available in herbs and herbal formulation

## MATERIALS AND METHODS

***Plant material and sample preparation:*** *Tectona grandis* Linn. Leaves were collected from thane district of Maharashtra. Herbarium of *tectona grandis* linn. was prepared and authenticated from MS University (Vadodara). The leaves collected were washed under running tap water. The plant kept for drying in oven at temperature 40±2$^{o}$C. The dried leaves powder was used for further studies. 500 mg of *tectona grandis* linn. leaves powder was extracted with 10 mL of acidic methanol. The mixture was vortexed for 5 min and it was put for overnight extraction. Extract was filtered through 0.2micron syringe filter and then it was subjected to LC-MS/MS analysis.

***Chemicals and standard solutions Preparation:*** All the chemicals used in the experiments were of LCMS grade. 2-methylanthraquinone(95.0% purity), 5-hydroxy-1,4-naphthoquinone(97.0% purity), 2-(hydroxymethyl)anthraquinone(97.0% purity), and emodin (90.0% purity) were procured from sigma Aldrich chemie (steinheim Germany). The stock solutions (1mg/mL) of each were prepared separately in methanol. From this individual stocks mix working stock solution of 10ug/mL of each standard were prepared in methanol. Standard solutions were prepared by dilution of the mixed working stock solutions.

***Instrumentation and Chromatographic Conditions***

Chromatographic development was performed on Shimadzu Nexera, UHPLC (Ultra High Performance Liquid Chromatograph) system with LC-30AD pumps, SIL-30A autosampler and CTO-20AC as column oven.LABsolutions software was used for operating the

instrument. Shimadzu LCMS-8040 model (Triple Quadrupole Mass Spectrometer) was used for optimization of MRM transitions for 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin. Analysis was performed on shimadzu, shimapack-XR, C18 column (75mm x3.0mm, 2.2 µm). The mobile phase comprising of A: 0.1% formic acid in water and B: methanol was filtered through a 0.2 µm membrane filter (Millipore) and degassed by sonication. Gradient method was used for chromatographic separation. Gradient program is given in below table1.

**Table 1.**

| Time | % Mobile phase A | % Mobile phase B |
|---|---|---|
| 0.00 | 55 | 45 |
| 2.50 | 20 | 80 |
| 5.00 | 20 | 80 |
| 5.10 | 55 | 45 |
| 7.00 | STOP | STOP |

Flow rate 0.5mL/min was used for analysis. Column oven was set at 40°C. Analysis was performed using APCI (Atmospheric Pressure Chemical Ionization) interface at positive and negative mode. Other MS parameters; Nebulizing Gas flow: 3L/min, Drying Gas flow : 5 L/min, Interface temperature : 350°C,DL temperature: 200°C and Heating Block : 200°C were used for the analysis.

## RESULTS

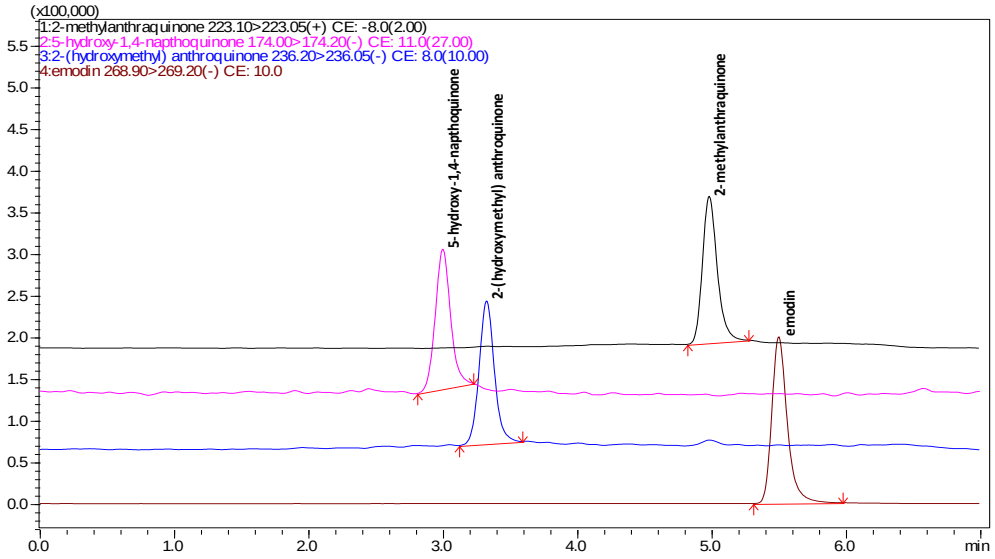
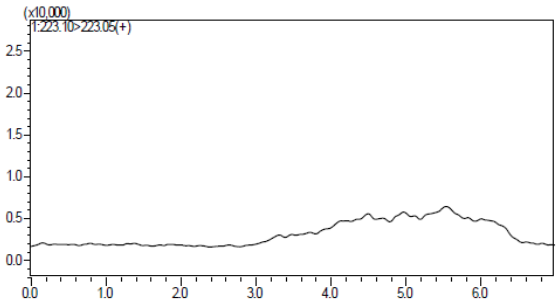
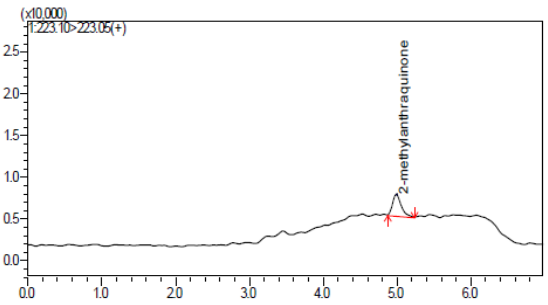


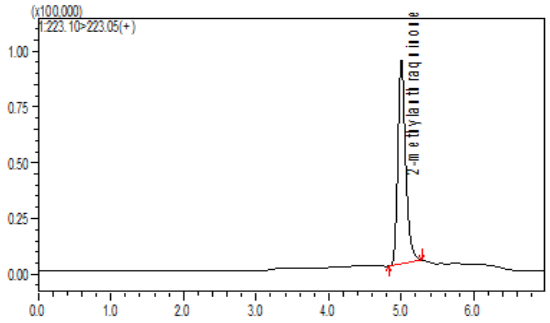

**Figure1: Overlay mass chromatograms of 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin standard mixture**



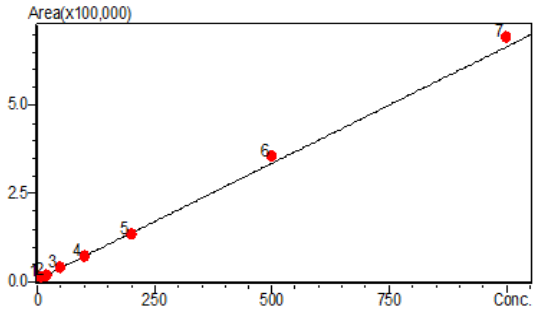

**2-methylanthraquinone _blank run**




**2-methylanthraquinone _ 20 ng mL$^{-1}$**

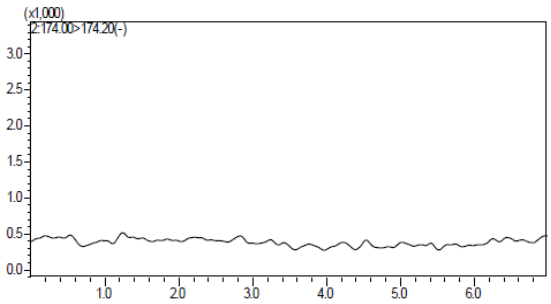



**2-methylanthraquinone _1000 ng mL$^{-1}$**

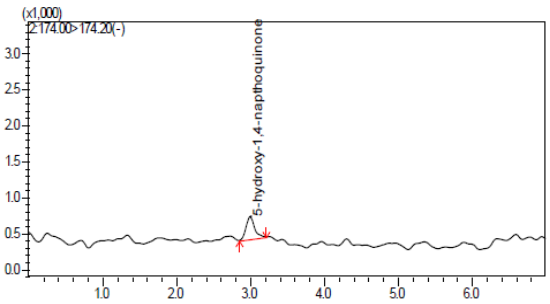



**Linearity_ (20-1000 ng mL$^{-1}$)**

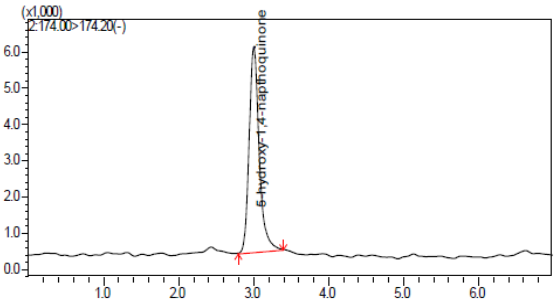
**Figure2: 2-methylanthraquinone mass chromatograms and linearity graph**



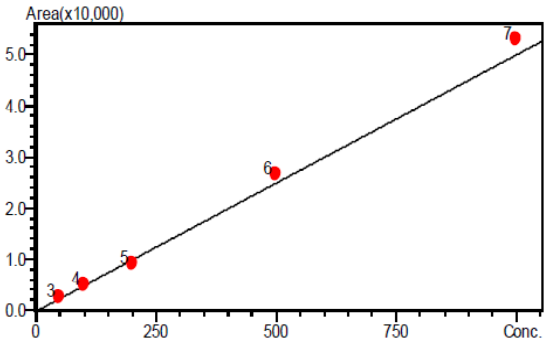

**5-hydroxy-1,4-naphthoquinone _blank run**




**5-hydroxy-1,4-naphthoquinone _50 ng mL$^{-1}$**

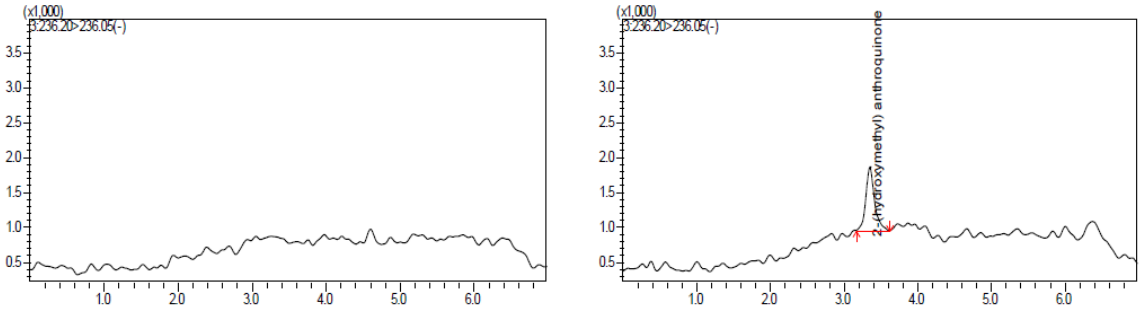



**5-hydroxy-1,4-naphthoquinone _1000 ng mL$^{-1}$**

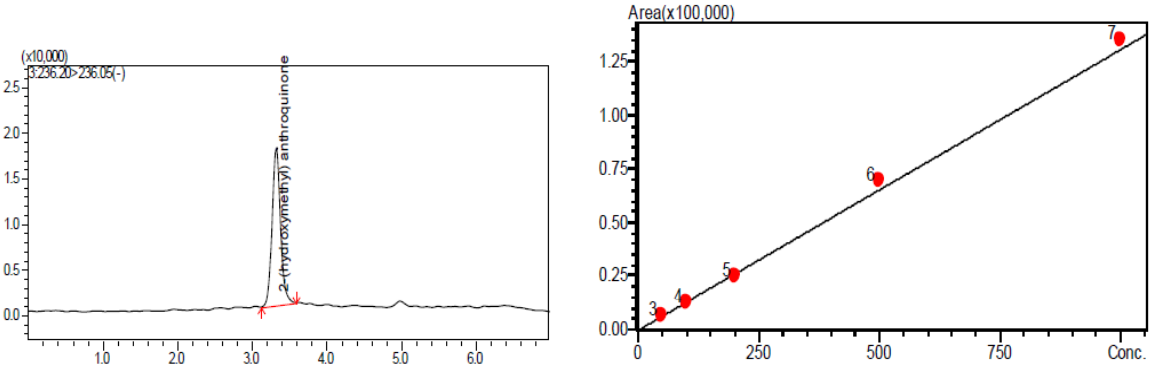



**Linearity_ (50-1000 ng mL$^{-1}$)**

**Figure3: 5-hydroxy-1,4-naphthoquinone mass chromatograms and linearity graph**

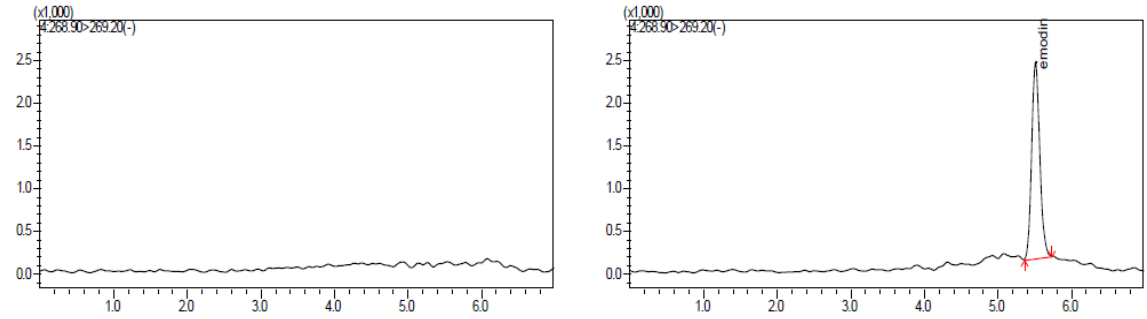



**2-(hydroxymethyl) anthraquinone _blank run 2-(hydroxymethyl) anthraquinone _50 ng mL$^{-1}$**

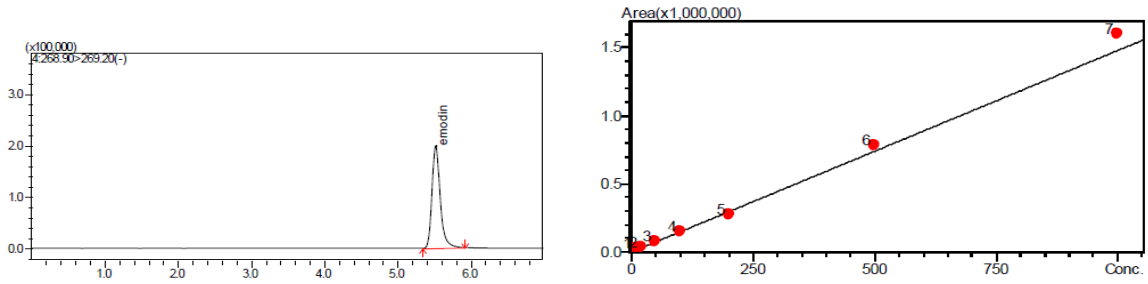



**2-(hydroxymethyl) anthraquinone _1000 ng mL$^{-1}$ Linearity_ (50-1000 ng mL$^{-1}$)**

**Figure4: 2-(hydroxymethyl) anthraquinone mass chromatograms and linearity graph**

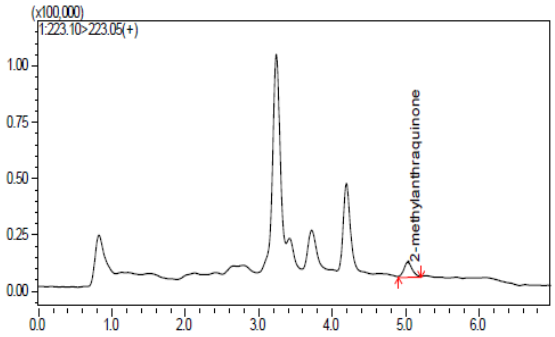



**Emodin _blank run** **Emodin _10 ng mL$^{-1}$**

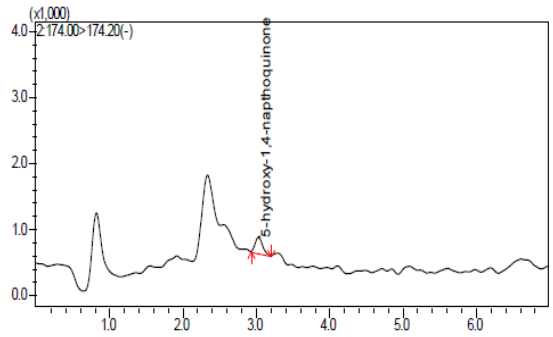



**Emodin _1000 ng mL$^{-1}$** **Linearity_ (50-1000 ng mL$^{-1}$)**

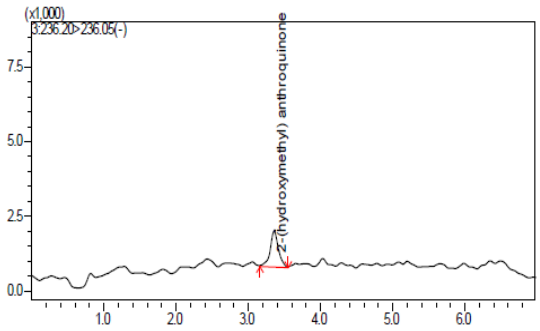
**Figure5: Emodin mass chromatograms and linearity graph**



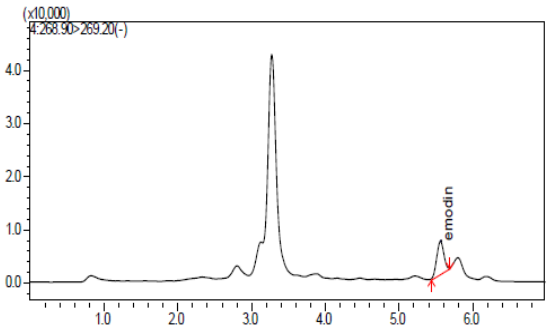

**2-methylanthraquinone**




**5-hydroxy-1,4-naphthoquinone**




**2-(hydroxymethyl) anthraquinone**




**Emodin**

**Figure 6: Chromatograms of *Tectona grandis* Linn. leaves extract.**

**Method validation summary**

***Limit of Detection (LOD) and Limits of Quantitation (LOQ):*** The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ of 2-methylanthraquinone was 6.0 ng $mL^{-1}$ and 20.0 ng $mL^{-1}$, 5-hydroxy-1,4-naphthoquinone was 18.0 ng $mL^{-1}$ and 50.0 ng $mL^{-1}$, 2-(hydroxymethyl) anthraquinone was 15.0 ng $mL^{-1}$ and 50.0 ng $mL^{-1}$ and for emodin was 0.8 ng $mL^{-1}$ and 2.0 ng$mL^{-1}$ respectively.

***Linearity:*** The experiment was performed five times and the mean was used for the calculations. The data was analyzed by linear regression least squares fitting. The statistical data obtained is given in Table 2.

**Table 2.**

| Parameters | 2-methylanthraquinone | 5-hydroxy-1,4-naphthoquinone | 2-(hydroxymethyl) anthraquinone | Emodin |
|---|---|---|---|---|
| **Linearity range[ng mL-1]** | 20-1000 | 50-1000 | 50-1000 | 10-1000 |
| **Slope [m][1]** | 671.776 | 49.4435 | 131.947 | 1479.52 |
| **Intercept [c ][1]** | 5883.30 | -248.895 | -396.033 | 810.248 |
| **Correlation Coefficient [$R^2$]** | 0.998 | 0.994 | 0.994 | 0.995 |
| **LOD [ng $mL^{-1}$] [2]** | 6 | 18 | 15 | 0.8 |
| **LOQ [ng $mL^{-1}$] [2]** | 20 | 50 | 50 | 2 |
| **Intraday precision (n=5 COV)** | 0.81 | 0.77 | 0.63 | 0.48 |
| **Inter day precision (n=5 COV)** | 0.48 | 0.59 | 0.75 | 0.56 |

[1] $y = mx + c$ equation, where $y$ is peak area, $m$ is the slope, $x$ is the concentration, and $c$ is the intercept.

[2] LOD (Level of Detection) and LOQ (Level of Quantitation) were calculated based on S/N ratio using LABSolutions software, Shimadzu.

***Recovery:*** Three replicates at 100 ng $mL^{-1}$, 200 ng $mL^{-1}$, and 500 ng $mL^{-1}$ concentration for 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin were prepared for recovery determination. The mean recovery for 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin acid were 95%, 96%,92% and 98% respectively.

***Assay:*** The developed HPLC method was used for simultaneous determination of 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin from leaves powder of *tectona grandis* linn. The sample working solution (10 µL) was injected and the area of these four quinones were measured and quantitated against the calibration curve. The retention time 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin in sample solution was 5.00, 3.00, 3.34 and 5.52 mins respectively.

The mean assay value of 2-methylanthraquinone was found to be 1.845 ug per 500 mg of plant powder with % RSD as 1.136 , mean assay value of 5-hydroxy-1,4-naphthoquinone was found to be 1.181 ug per 500 mg of plant powder with % RSD as 0.98, mean assay value of 2-(hydroxymethyl) anthraquinone was found to be 2.065 ug per 500 mg of plant powder with % RSD as 1.558 and mean assay value of emodin was found to be 0.774 ug per 500 mg of plant powder with % RSD as 1.141

***Precision and Accuracy:*** The intra-day and inter-day precision was used to study the variability of the method. The % RSD for intra-day and inter-day precision for 2-methylanthraquinone were 0.81 and 0.48, respectively, 5-hydroxy-1,4-naphthoquinone were 0.77 and 0.59 respectively, 2-(hydroxymethyl) anthraquinone were 0.77 and 0.59 respectively and emodin were 0.63 and 0.75 respectively.

## CONCLUSION

The application of a simple, rapid and accurate LCMSMS method for the simultaneous quantitation of quinones viz. 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin in leave powder of *Tectona grandis* linn.. The method was validated to track the active principles in the complex mixture of herbal ingredients. The method could be extended for the marker-based standardization of other herbal product containing quinones viz. 2-methylanthraquinone, 5-hydroxy-1,4-naphthoquinone, 2-(hydroxymethyl) anthraquinone and emodin.

The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials.

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