Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

# A NORMAL PHASE HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF TWO TRITERPENOIDS LUPEOL AND BETA-AMYRIN FROM *Caesalpinia bonducella* Linn. AND *Coccinia indica* WIGHT & ARN.

**SHREEDA ADHYAPAK* AND VIDYA DIGHE**

**Department of Chemistry, Ramnarain Ruia College, Matunga, Mumbai 400019, Maharashtra, India. Email:shreeda_adhyapak@yahoo.com**

*Received: 18 Oct 2013, Revised and Accepted: 09 Nov 2013*

**ABSTRACT**

Objective: A normal phase high performance thin layer chromatography (HPTLC) method has been developed and validated for simultaneous quantitative determination of two triterpenoids, lupeol and beta-amyrin from seed kernel powder of *Caesalpinia bonducella* Linn., from fruit powder and root powder of *Coccinia indica* Wight & Arn.

Method: The analysis was performed on TLC aluminium plates pre-coated with silica gel $60F_{254}$. Linear ascending development was carried out in twin-trough glass chamber saturated with suitable mobile phase. The densitometric scanning was done after derivatization at λ=580nm. The accuracy of the developed HPTLC method was checked by carrying out the recovery experiment at three different levels, by using standard addition method.

Result: The detector response was linear for concentrations ranging from 0.03µg/band to 0.18µg/band for lupeol and 0.02µg/band to 0.18µg/band for beta–amyrin with correlation coefficient of 0.999 for both the components. The method was precise as the value of percent relative standard deviation was found to be less than 2.

Conclusion: The proposed HPTLC method was successfully applied for the simultaneous determination of lupeol and beta-amyrin in all the three plant samples.

**Keywords:** Lupeol; Beta amyrin; *Caesalpinia bonducella* Linn.; *Coccinia indica* Wight & Arn.; Iodine pre derivatization technique.

## INTRODUCTION

*Caesalpinia bonducella* Linn. is used as a folklore medicine for treatment of diabetes. The tribal people of India use it for controlling blood sugar. The seeds are reported to possess anti-diabetic or hypoglycaemic activity [1]. The seeds kernels also possess antipyretic, anti-diuretic, antibacterial, antiviral and anti-estrogenic activity. The roasted seeds are made into coffee for treating diabetes [2,3]. Shushruta prescribed this plant as a potherb for curing swellings [2].

*Coccinia indica* Wight & Arn. commonly known as 'Tondali' is used as a vegetable. The parts like fruits, leaves, roots of *Coccinia indica* Wight & Arn. have been widely used in treatment of diabetes mellitus [4]. The various extract of fruit and root juice has been reported to cure dysentery, vomiting, mouth ulcers and bronchitis, asthma and gastrointestinal disturbances [4].

Both the plants have high medicinal value with important phyto-chemicals present in them. The two common triterpenoids present in the selected parts of both the plants are lupeol and beta-amyrin [5,6].

Lupeol is reported to reduce blood glucose by reducing the activity of alpha-amylase [7]. It also shows anti-protozoal, anticancer, chemo preventive and anti-inflammatory properties [7].

Beta-amyrin is reported to have antihyperglycemic activity [8]. It has been reported to exhibit various other pharmacological activities *in vitro* and *in vivo* conditions against various health-related conditions, including conditions such as inflammation, microbial, fungal, and viral infections and cancer cells. Beta-amyrin is involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides [9].

Hence, a high performance thin layer chromatographic method has been developed to quantitate these two triterpenoids so as assess the quality of the medicinal plants.

In the literature [10] determination of lupeol and beta-amyrin in epicuticular wax of cabbage (*Brassica oleracea* Linn.) by using HPTLC has been reported. The method was developed on HPTLC Silica gel 60 $F_{254}$ plates using n-hexane: ethyl acetate (5.0 : 1.0 v/v) as mobile phase. The post derivatization was done by using anisaldehyde-sulphuric acid reagent.

A HPTLC method [11] for identification of an isomer of lupeol i.e. epi-lupeol and beta amyrin from resins and various valuable medicinal plants has been reported. The silica gel 60 $F_{254}$ plates were coated with 10 % aqueous solution of silver nitrate. The mobile phase used was n-hexane: dicholoromethane: methanol in volume ratio of 10: 10:1. The post derivatization was done by spraying anisaldehyde-sulphuric acid reagent on to the developed plate.

A $C_{18}$ RP-HPTLC method [12] has been reported for separation and identification of isomeric triterpenoids. The mobile phases used were ethyl acetate: acetonitrile (3.0: 2.0 v/v) and acetone: acetonitrile (5.0:1.0 v/v). The plates were further derivatized with anisaldehyde sulphuric acid reagent.

However, no HPTLC method is reported for the simultaneous quantification of lupeol and beta-amyrin from seed kernel powder of *Caesalpinia bonducella* Linn., from fruit powder and root powder of *Coccinia indica* Wight & Arn.

Thus, in present research work, a simple, rapid, precise and accurate HPTLC method has been developed and validated using International Conference on Harmonization (ICH) guidelines for simultaneous determination and quantification of lupeol and beta-amyrin from seed kernel powder of *Caesalpinia bonducella* Linn. from fruit powder and root powder of *Coccinia indica* Wight & Arn.

## MATERIALS AND METHODS

### Experimental Reagents

HPLC grade n- Pentane (purity 99.8%), ethyl acetate (purity 99.0%), n-propanol (purity 99.9%), methanol (purity 98.9%), anisaldehyde (AR grade), glacial acetic acid (AR grade) and concentrated sulphuric acid (98%) were procured from E. Merck (India).

Iodine crystals were obtained from Loba Chemie, Mumbai.

The pre-coated Silica 60 $F_{254}$ TLC plates were obtained from E. Merck (India).

**Reference Standard**

Reference standards lupeol (purity 99.7%) and beta-amyrin (purity 99.3%), were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany).

**Plant material**

The seed kernels of *Caesalpinia bonducella* Linn. and fruits and roots of *Coccinia indica* Wight & Arn. were procured from Keshav Shrushti, Bhayander, Thane.

The herbarium was prepared and was authenticated from the Botanical Survey of India (BSI), Pune, with Voucher no. SHSHACAB1 and SHSHACOG2.dated: 10/11/2010. A duplicate copy of herbarium is preserved in Chemistry Department of Ramnarain Ruia College.

Both the plant materials were washed with water to remove soil particles, dried in shade, finely powdered and then sieved through BSS mesh size 85 and stored in an airtight container at room temperature (28 ± 2° C).

**Preparation of solutions**

**Preparation of stock solution of standard lupeol (1000.0µg/ml)**

The stock solution of lupeol was prepared by dissolving 10.0 mg of lupeol standard in 5.0ml of n-propanol in a 10.0 ml standard volumetric flask. It was then sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of lupeol. The contents were then diluted up to the mark with n-propanol to obtain a solution of 1000.0µg/ml of lupeol.

**Preparation of stock solution of standard beta-amyrin (1000.0µg/ml)**

The stock solution of beta-amyrin was prepared by dissolving 5.0 mg of beta-amyrin standard in 2.0 ml of n-propanol in a 5.0 ml standard volumetric flask. It was then sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 5.0 minutes for complete dissolution of beta-amyrin. The contents were then diluted up to the mark with n-propanol to obtain a solution of 1000.0µg/ml of beta-amyrin.

**Preparation of working standard solutions of lupeol and beta-amyrin (10.0µg/ml)**

0.1 ml of stock solution of lupeol and beta-amyrin (1000.0µg/ml) were taken in two separate 10.0 ml volumetric flasks. The volumes of both the volumetric flasks were made up to 10.0ml with n-propanol. The working standard solutions of lupeol and beta-amyrin each with concentration of 10.0µg/ml were thus prepared.

**Preparation of sample solution**

About 500 mg of dried seed kernel powder of *Caesalpinia bonducella* Linn. was accurately weighed and transferred to 100.0 ml stoppered conical flask and 20.0 ml of n-propanol was added to it.

About 500 mg of dried fruit powder of *Coccinia indica* Wight & Arn. was accurately weighed and transferred to 100.0 ml stoppered conical flask and 20.0 ml of n-propanol was added to it.

About 500 mg of dried root powder of *Coccinia indica* Wight & Arn. was accurately weighed and transferred to 100.0 ml stoppered conical flask and 10.0 ml of n-propanol was added to it. All the three flasks were then shaken at 80 rpm, on a conical flask shaker at room temperature (28ºC + 2ºC). The contents of the each flask were filtered through Whatman No.41 filter paper (E. Merck, Mumbai, India) and the filtrates were further used as the sample solution for the assay experiment. The sample solutions were then filtered through 0.45µm filter paper before analysis.

**Pre-Derivatization Reagent**

Iodine vapours were used as pre-derivatizing agent. Iodine vapours were generated by heating iodine crystals in a closed flat bottom beaker. The TLC plate with applied bands of standards and sample solutions was then exposed to these iodine vapours for 10 minutes. The excess of iodine was removed by heating the plate at 100 ºC for 10 minutes. This pre-derivatized plate was then used for development.

**Preparation of mobile phase**

The mobile phase used in the present research work for simultaneous quantification of lupeol and beta-amyrin was prepared by mixing n-pentane and ethyl acetate in the volume ratio of 8.0: 2.0. The mobile phase was vortexed for 2 minutes for proper mixing of solvents. During development of each plate, a fresh mobile phase was prepared.

**Post-Derivatization Reagent**

The anisaldehyde-sulphuric acid reagent was used as post derivatizing reagent. 10.0 ml of sulphuric acid ($H_2SO_4$) was added to an ice cooled mixture of 170.0 ml of methanol and 20.0 ml of glacial acetic acid. To this solution 1.0 ml of anisaldehyde solution was added with consistent shaking.

**Chromatography**

Chromatography was performed on 10.0 cm x 10.0 cm TLC aluminium plates, precoated with 200 µm layers of Silica gel $60F_{254}$ (E. Merck, Mumbai, India). Working standard solutions of lupeol and beta-amyrin, sample solutions of dried seed kernel powder of *Caesalpinia bonducella* Linn. and of dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn. were applied on the same plate as 8mm bands, 6mm apart from each other and 10mm from bottom edge of the plate, under a continuous supply of nitrogen by means of a Camag Linomat V Semi-automatic TLC sample applicator with a 100 µL syringe (Hamilton, Bonaduz, Switzerland).

After application, pre-derivatisation step was done by exposing the plate to iodine vapours for 10 minutes. The pre-derivatized plate was developed vertically ascending in a twin-trough glass chamber (Camag, Switzerland) saturated with mobile phase comprising of n-pentane: ethyl acetate in volume ratio of 8.0: 2.0. The optimized chamber saturation time for the mobile phase was 20 minutes at room temperature (28 ± 2ºC). The chromatographic run length was 90mm from the bottom edge of the plate. After development, the plate was derivatized by dipping the developed plate in anisaldehyde sulphuric acid reagent for 2 seconds. The plate was then air dried and heated at 110ºC for 10 minutes. The densitometric scanning was performed at λ=580 nm in absorbance/reflectance mode using CAMAG TLC scanner IV with Win CATS software version 1.4.6.

**Method validation**

**Linear working range lupeol and beta-amyrin**

Linearity was evaluated by applying 10.0 µg/ml concentration of mixture of lupeol and bet-amyrin with volumes of 2µL, 3 µL, 4µL, 6 µL, 8 µL, 10 µL, 12 µL, 14 µL, 16 µL and 18 µL on TLC plate to obtain concentrations of 0.02µg/band to 0.18µg/band of lupeol and beta-amyrin.

The peak areas obtained from densitograms for each applied concentration of lupeol and beta-amyrin were noted.

The calibration curves standards were obtained by plotting graphs of mean peak areas of each standard vs. corresponding concentrations. The results listed in Table 1.0, shows that within the concentration range indicated, there was a good correlation between mean peak area and concentration of standards.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The Limit of Detection (LOD) is defined as a peak, whose signal-to-noise (S/N) ratio is 3:1. The Limit of Quantification (LOQ) is defined as a peak, whose signal-to-noise (S/N) ratio is 10:1. The results are listed in Table 1.

**System suitability**

System suitability was carried out to verify that resolution and reproducibility of the system were acceptable for the analysis. System suitability test was carried out by applying standard solutions of lupeol with concentration of 0.1µg/band and beta-

amyrin with concentration of 0.05µg/band on same TLC plate in six replicates under specified chromatographic conditions. The chromatograms were recorded and the values of percent relative standard deviations of peak area and retardation factor of standards were taken as an indicator of system suitability and were less than 2, indicating that the method is suitable for analysis.

**Precision**

The method was validated in terms of repeatability and intermediate precision.

The repeatability was evaluated in triplicates by applying n-propanolic extract of all three samples on TLC plate on the same day, under the specified chromatographic conditions. The peak areas of lupeol and beta-amyrin were recorded and assessed.

The intermediate precision of the method was evaluated by analyzing the sample solution in triplicate on three different days, under the specified chromatographic conditions. The peak areas of lupeol and beta-amyrin were recorded and assessed.

The precision results were expressed as percentage relative standard deviations of peak areas of lupeol and beta-amyrin. The results listed in Table 2, indicates that the proposed method is precise and reproducible.

**Standard stability**

The stability of standard lupeol and beta-amyrin solutions were determined by comparing the peak areas of standard solution of lupeol and beta-amyrin at different time intervals, for a period of minimum 48 hrs, at room temperature. Standard solutions of lupeol with concentration of 0.10µg/band and beta-amyrin with concentration 0.05µg/band, were applied as bands on same TLC plate at intervals of 0, 12, 24, 48 hrs and analyzed under the specified chromatographic conditions.

The results showed that the peak areas of lupeol and beta-amyrin almost remained unchanged (values of percent relative standard deviation were less than 2) over a period of 48 hrs, and no significant degradation was observed within the given period, indicating the stability of standard solutions of lupeol and beta-amyrin for minimum period of 48 hrs.

**Assay**

The developed and validated HPTLC method was used for quantitation of lupeol and beta-amyrin from seed kernel of *Caesalpinia bonducella* Linn. and from fruit powder and root powder of *Coccinia indica* Wight & Arn. 20 µL of n-propanolic extract of seed kernel of *Caesalpinia bonducella* Linn., 10 µL of n-propanolic extract of fruit powder of *Coccinia indica* Wight & Arn. and 20 µL of n-propanolic extract of root powder of *Coccinia indica* Wight & Arn. were applied as bands on the TLC plate. The plate was developed and scanned under the specified chromatographic conditions. The chromatograms were recorded. To check the repeatability of the method, assay experiment was repeated seven times and the values of mean standard deviation (S.D.) and percent relative standard deviation (%R.S.D.) were calculated. The results of assay experiment are shown in Table 3. The amounts of lupeol and beta-amyrin present in each sample solution were determined from the calibration curve, by using the peak areas of lupeol and beta-amyrin in the sample.

**Table 1: Method validation data**

| Parameters | Observations | |
|---|---|---|
| | **Lupeol** | **Beta-amyrin** |
| Linear Working Range (µg/band) | 0.03 – 0.18 | 0.02 – 0.18 |
| Correlation coefficient (r) | 0.999 | 0.999 |
| Limit of Detection (LOD) (µg/band) | 0.01 | 0.01 |
| Limit of Quantification (LOQ) (µg/band) | 0.03 | 0.02 |
| Stability of standard solution | 48 hrs. | 48 hrs. |

**Table 2: Results for precision of the developed HPTLC method**

| Parameters | % R.S.D | |
|---|---|---|
| | **Lupeol** | **Beta-amyrin** |
| **Repeatability (n=3)** | | |
| Seed kernel powder of *Caesalpinia bonducella* Linn. | 1.07 | 0.98 |
| Fruit powder of *Coccinia indica* Wight & Arn. | 0.87 | 0.89 |
| Root powder of *Coccinia indica* Wight & Arn. | 0.87 | 0.90 |
| **Intermediate precision (n=9)** | | |
| Seed kernel powder of *Caesalpinia bonducella* Linn. | 1.06 | 0.99 |
| Fruit powder of *Coccinia indica* Wight & Arn. | 0.84 | 0.89 |
| Root powder of *Coccinia indica* Wight & Arn. | 0.90 | 0.91 |

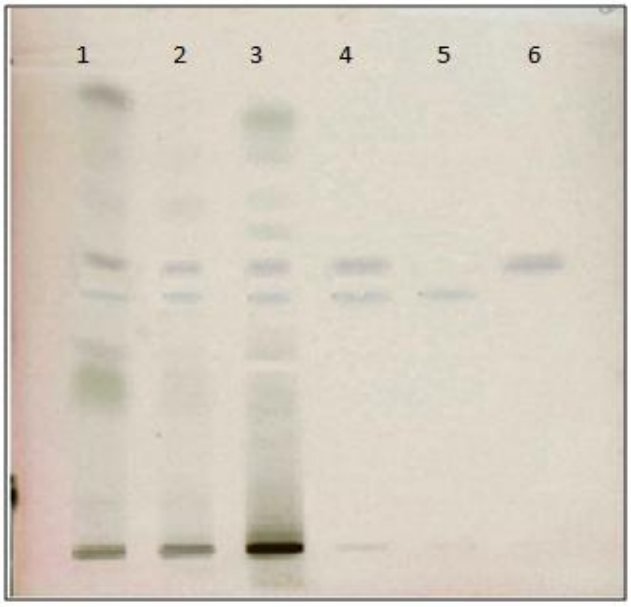


**Fig. 1: Developed TLC plate showing separation lupeol and beta-amyrin at λ= 580m**

Track 1 shows separation in n-propanolic extract of dried seed kernel powder of *Caesalpinia bonducella* Linn.; Track 2 shows separation in n-propanolic extract of fruit powder of *Coccinia indica* Wight & Arn; Track 3 shows separation in n-propanolic extract of root powder of *Coccinia indica* Wight & Arn.; Track 4 shows separation of mixture of standard lupeol and beta-amyrin; Track 5 represents standard lupeol and Track 6 represents standard beta-amyrin.

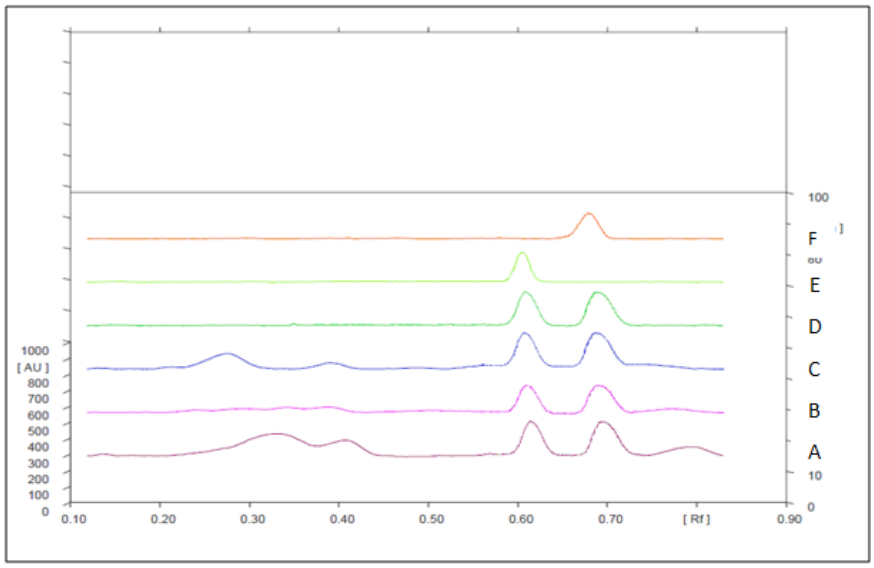


**Fig. 2: Overlay chromatogram of standard lupeol and beta amyrin and all three samples at λ=580 nm**

Figure 2 shows overlay chromatograms of seed kernel powder of Caesalpinia bonducella Linn. (A); fruit powder of Coccinia indica Wight & Arn (B); root powder of Coccinia indica Wight & Arn.(C); mixture of standard lupeol and beta-amyrin (D); standard lupeol (E) and standard beta-amyrin (F).

**Accuracy**

The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels.

About 500 mg of dried seed kernel powder of *Caesalpinia bonducella* Linn. was accurately weighed and to it known amounts of standard lupeol (50µg, 75µg, 100µg) and beta-amyrin (30µg, 60µg, 90 µg) were added, and extracted using n-propanol as mentioned in sample preparation.

About 500 mg of dried fruit powder of *Coccinia indica* Wight & Arn. was accurately weighed and to it known amounts of standard lupeol (50µg, 75µg, 100µg) and beta-amyrin (60µg, 80µg, 100µg) were added, and extracted using n-propanol as mentioned in sample preparation.

About 500 mg of dried root powder of *Coccinia indica* Wight & Arn. was accurately weighed and to it known amounts of standard lupeol (30µg, 60µg, 90 µg) and beta-amyrin (40µg, 60µg, 80µg,) were added, and extracted using n-propanol as mentioned in sample preparation.

Each of the three different levels containing sample solution and standard were applied in seven replicates on the same plate. The plate was then developed and scanned under the specified chromatographic conditions, as described earlier. The lupeol and beta-amyrin contents were quantified by the proposed method and the percentage recovery was calculated.

**Table 3: Results of Assay and Accuracy**

| Parameters | Observations | |
|---|---|---|
| | Lupeol | Beta-amyrin |
| **Assay (µg/g)** | | |
| Seed kernel powder of *Caesalpinia bonducella* Linn. | 110.95 | 64.67 |
| Fruit powder of *Coccinia indica* Wight & Arn. | 382.43 | 316.88 |
| Root powder of *Coccinia indica* Wight & Arn. | 30.72 | 64.78 |
| **Percent Recovery** | | |
| Seed kernel powder of *Caesalpinia bonducella* Linn. | 99.17 | 99.66 |
| Fruit powder of *Coccinia indica* Wight & Arn | 99.82 | 98.35 |
| Root powder of *Coccinia indica* Wight & Arn. | 99.55 | 99.79 |

**DISCUSSION**

In present research work, a simple HPTLC method has been developed for simultaneous quantitation of lupeol and beta-amyrin from seed kernel of *Caesalpinia bonducella* Linn. and from fruit powder and root powder of *Coccinia indica* Wight & Arn.

During HPTLC analysis, several different mobile phases were tried for separation of lupeol and beta-amyrin from other phyto-chemicals present in seed kernel of *Caesalpinia bonducella* Linn. and in fruit powder and root powder of *Coccinia indica* Wight & Arn. Good separation was achieved with the mobile phase comprising of n-pentane: ethyl acetate: in the volume ratio of 8.0: 2.0.

A pre-derivatization technique using iodine vapours was used to separate the isomeric compounds lupeol and beta-amyrin.

Since, the phyto-chemicals, lupeol and beta-amyrin showed no UV and visible sensitivity on plate, the plate was post-derivatized further with anisaldehyde-sulphuric acid reagent. The identity of the bands of lupeol and beta-amyrin was confirmed by comparing their $R_f$ values in sample with those of reference standard. Figure 1 shows typical HPTLC plate showing separation of lupeol and beta-amyrin standards. The derivatized plate was scanned for lupeol and beta-amyrin at λ=580nm as these compounds showed maximum absorbance at λ=580nm. The $R_f$ values for lupeol and beta-amyrin were 0.60 and 0.69 respectively.

In present research work, a simple HPTLC method has been developed for simultaneous quantitation of lupeol and beta-amyrin from dried seed kernel powder of *Caesalpinia bonducella* Linn., from dried fruit powder and dried root powder of *Coccinia indica* Wight & Arn.

In literature [10], qualitative analysis for separation of lupeol and beta-amyrin was attempted on HPTLC silica gel $60F_{254}$ plates, using mobile phase *n*-hexane-ethyl acetate (5.0:1.0, *v*/*v*). Post derivatization was carried out using anisaldehyde-sulphuric acid reagent. The identification of lupeol and beta-amyrin was done by visually comparing the colour of lupeol and beta-amyrin after derivatization. No significant $R_f$ value difference was observed. The reported method was not able to resolve the isomeric compounds lupeol and beta-amyrin.

A HPTLC method [11] for identification of an isomer of lupeol i.e. epi-lupeol and beta amyrin from resins and various valuable medicinal plants was done on silica gel 60 $F_{254}$ plates coated with 10 % aqueous solution of silver nitrate. The mobile phase used was n-hexane: dicholoromethane: methanol in volume ratio of 10: 10:1. The post derivatization was done by spraying anisaldehyde-sulphuric acid reagent on to the developed plate. This method has not been able to resolve isomer of lupeol i.e. epi-lupeol and beta amyrin. However, the present developed method uses a simple pre-derivatization technique using iodine vapours prior to development of TLC plate with mobile phase to separate lupeol and beta-amyrin.

In another reported method[12] $C_{18}$ RP-HPTLC plates prewashed with acetone were used for separation of common plant triterpenoids. The mobile phases used were ethyl acetate: acetonitrile (3.0:2.0 v/v) and acetone: acetonitrile (5.0:1.0 v/v). Both the methods separated lupeol and beta-amyrin and other triterpenoids with different functional groups. The plates were further derivatized with anisaldehyde-sulphuric acid reagent. However, in the present research work, the separation of lupeol and beta-amyrin was done on normal phase TLC Silica gel 60 $F_{254}$ plates.

The developed method for the separation of lupeol and beta-amyrin in the present research work is advantageous as compared to the reported methods as it is able to resolve lupeol and beta-amyrin on normal phase TLC plates and hence is cost effective.

The developed HPTLC method has been validated for quantitation of lupeol and beta-amyrin from seed kernel powder of *Caesalpinia bonducella* Linn. and fruit powder and root powder of *Coccinia indica* Wight & Arn. using ICH guidelines.

## CONCLUSION

A simple HPTLC method has been developed for simultaneous quantitation of lupeol and beta-amyrin from n-propanolic extracts of seed kernel powder of *Caesalpinia bonducella* Linn. and fruit powder and root powder of *Coccinia indica* Wight & Arn. The developed HPTLC method was validated as per ICH guidelines and was found to be simple, precise, sensitive and accurate.

## REFERENCES

1. Rahmatullah M, Jahan R, Khatun MA, Jahan F, Azad AK, Anwarul Bashar ABM, et al. A Pharmacological evaluation of Medicinal Plants used by folk medicinal practitioners of station Purbo Para village of jamalpur Sadar Upzila In Jamalpur district Bangladesh. Am.-Eurasian J Sustain Agric 2010; 4(2): 170-195.
2. Quality standards of Indian Medicinal Plants. New Delhi: Indian Council for Medicinal and Research; 2003. P. 25 – 33. (Vol 2).
3. Kannur DM, Hukkui VI, Akki KS. Antidiabetic activity of *Caesalpinia bonducella* seed extracts in rats. Fitoterapia 2006; 77: 546-549.
4. Gunjan M, Gautam K, Jha AK, Mishra U: Pharmacognostic and antihyperglycemic study of *Coccina indica*. Int J Phytomed 2010; 2: 36-40.
5. Prabhakar P, Doble M. A target based Therapeutic Approach towards Diabetes mellitus using medicinal plants. Current Diabetes Reviews 2008; 4: 291 – 308.
6. Khare CP. Encyclopedia of Indian Medicinal Plants, Rational Western Therapy. Ayurvedic and other Traditional usage, Botany. Springer Publications; 2004. P. 155 – 156.
7. Gallo M, Sarachine MJ: Biological activites of Lupeol. Int J Biomed Pharma Sci 2009; 3(1): 46 – 66.
8. Santos FA, Frota JT, Arruda BR. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum*in mice. Lipids Health Dis. 2012; 11: 98.
9. Francisco AO, Mariana HC, Fernanda RC, Roberto CP, Regilane MS. Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. J Ethnopharma 2005; 98: 103-108.
10. Martelanc M, Vovk I, Simonovska B. Determination of three major triperpenoids in epicuticular wax of cabbage (*Brassica oleraceae* L.) by high performance liquid chromatography with UV and mass spectrometric detection. J Chromatogr A 2007; 1164: 145 – 152.
11. Hernandez-Vazquez L, Palazon J, Mangas S, Navarro-Ocaña A. Valuable medicinal plants and resins: Commercial phytochemicals with bioactive properties. Ind Crop Prod 2010; 31: 476-480.
12. Martelanc M, Vovk I, Simonovska B. Separation and identification of some common isomeric plant triterpenoids by thin-layer chromatography and high-performance liquid chromatography. J Chromatogr A 2009; 1216: 6662-6670.