ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

High Performance Liquid Chromatographic method for quantization of apigenin from dried root powder of *Gmelina arborea* Linn.

Corresponding Author

SHREEDA ADHYAPAK

Department of Chemistry, Ramnarain Ruia College, Mumbai – 400019, India

Co Authors

Dr. VIDYA DIGHE[1], DHANASHRI MESTRY[1] AND NEETA SHAMBHU[1]

[1]Department of Chemistry, Ramnarain Ruia College, Mumbai – 400019, India

ABSTRACT

A simple fast and precise reverse phase high performance liquid chromatographic method is developed for the quantitative determination of apigenin, a flavonoid from dried root powder of *Gmelina arborea* Linn. Apigenin was extracted from root powder of *Gmelina arborea* Linn. by using warm methanol as the extracting solvent. The quantization of apigenin was carried out on an Inertsil ODS-3V-C_{18}, (25cm x 4.6 mm i.d., 5µm) column, using acetonitrile and distilled water in volume ratio of (45:55), as the mobile phase. The detection of the standard was carried out at 340 nm, using UV-Visible detector, which is reported to be the wavelength of maximum absorption of the standard apigenin.The proposed HPLC method was validated and applied for the quantitative determination of apigenin from *Gemlina arborea* Linn

KEYWORDS

Column liquid chromatography, Apigenin, *Gmelina arborea* Linn., Densitometry

1. INTRODUCTION

Gmelina arborea Linn is a tall tree belonging to the verbeneacae family, with young parts densely velvety tomentose[1]. Leaves are opposite, broadly ovate or elliptic-rhomboid, cordate or subtruncate, fulvous tomentose beneath[1]. It is found throughout India, from foot of North West Himalaya to Chittagong and throughout Deccan Peninsula[2].

Gmelina arborea Linn. root bark is acrid, bitter, sweet, stomachic, tonic, laxative, galactagogue and anthelmintic[1,2]. It is useful in treatment of hallucination, fever, dyspepsia, hyperdipsia, haemorrhoids, gastralgia, anasarca and burning sensation[2]. The root is an ingredient of the *dasmula* of the Vaidyas[1,2].

Some of the chemical constituents of *Gmelina arborea* Linn. roots are apiosylskimmin[3], gmelinol[4], arboreal[4], apigenin[5], gmelofuran[6].

Apigenin is found to provide protection from cancers[7, 8].

HPLC methods for the detection of apigenin from rat plasma and mouse tissues have been reported in literature[9,11]. Also reverse phase HPLC method for the determination and quantitation of apigenin in human urine after oral administration of tablet of *Chrysanthemum moriflum* extract[10] and simultaneous determination of apigenin with other flavonoids from alcoholic extract of Pigeonpea [*Cajanus cajan* (L.) Millsp.] leaves is reported in literature[12]. However, the determination and quantitation of apigenin from dried root powder of *Gmelina arborea* Linn. has not been reported in literature. In the present research work, a simple, precise and accurate HPLC method has been developed for the quantitation of apigenin from root bark of *Gmelina arborea* Linn.

2. EXPERIMENTAL

2.1. MATERIALS

2.1.1. STANDARD AND REAGENTS

Standard apigenin was procured from Sigma-Aldrich Chemie GmbH (Aldrich Division; Steinheim, Federal Republic of Germany) with 98.17% purity. Distilled water used in the present research work was obtained using a water purifying system. (Millipore, U.S.A).

All solvents of HPLC grade; Acetonitrile (purity 99%), methanol (purity 99.00%), methanol (purity 99.00%) were obtained from Qualigens Fine Chemicals, Mumbai, India.

2.1.2. PLANT MATERIAL

Gmelina arborea Linn. roots were collected as wild plant, from Karjat, Mumbai, India. A herbarium of *Gmelina arborea* Linn. was prepared in duplicate and was authenticated from Botanical Survey of India, Pune. The plant material was thoroughly washed to remove dust particles. Roots of the plant were separated and then air dried. Immediately after drying, roots were powdered using an electric mixer-grinder and sieved through a BSS mesh no. 85 sieve. The sieved powder was used for the present research work.

2.2. QUANTITATIVE ANALYSIS

2.2.1 CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was carried out with Jasco High Performance Liquid Chromatograph having Jasco, PU 980 HPLC isocratic pump, equipped with Jasco, AS – 2057 sampler and a Jasco UV- Visible detector (UV-970) variable wavelength detector. The chromatograms and data were

recorded by means of Borwin Chromatographic software 1.21
An Inertsil ODS-3V-C_{18}, (25cm x 4.6 mm i.d.,5µm) column and mobile phase comprising of acetonitrile and distilled water in volume ratio of (45:55), was used for the analysis. The system was run at a flow rate of 1.0 mL/min, 20 µL of sample was injected in the chromatographic system and the detection was done at 340nm. The proposed HPLC method was validated and applied for the quantitative determination of apigenin from *Gemlina arborea* Linn.

2.2.2. PREPARATIONS OF STOCK SOLUTION OF APIGENIN (100.00 µg/cm^3)

The stock solution of apigenin was prepared by dissolving 10.0 mg of apigenin standard in 50.0 cm^3 of warm methanol (maintained at 40^{o}C ± 2^{o}C, prior to addition) in a 100.0 cm^3 standard volumetric flask, followed by sonicating for ten minutes in an ultrasonicator and cooling it to room temperature (28^{o}C ± 2^{o}C). The contents of the flask were then diluted up to the mark with methanol.

2.2.3. PREPARATION OF WORKING STANDARD SOLUTIONS OF APIGENIN

Into a series of 10.0 cm^3 standard volumetric flask, aliquots of (100.0 µL, 200.0 µL, 400.0 µL, 600.0 µL, 800.0 µL and 1000.0 µL) were drawn from 100.0 µg/cm^3 stock solution and diluted up to the mark with the mobile phase used, to obtain a concentration range of 1.00µg/cm^3 to 10.00µg/cm^3 respectively.

2.2.4. PREPARATION OF SAMPLE SOLUTION

About 250 mg of *Gmelina arborea* Linn. root powder was accurately weighed and transferred to a stoppered test tube and 10.0 cm^3 of warm methanol (maintained at 40^{o}C ± 2^{o}C, prior to addition) was then added to it. It was then sonicated in an ultrasonicator for ten minutes and cooled to room temperature (28^{o}C ± 2^{o}C). The extract was then filtered through Whatman filter paper No. 41 (E.Merck, Mumbai, India) and the filtrate was further used as the sample solution for the assay experiment.

2.2.5. PREPARATION OF MOBILE PHASE

The mobile phase was prepared by mixing acetonitrile and double distilled water in volume ratio (45:55) and then sonicated for 10 minutes.

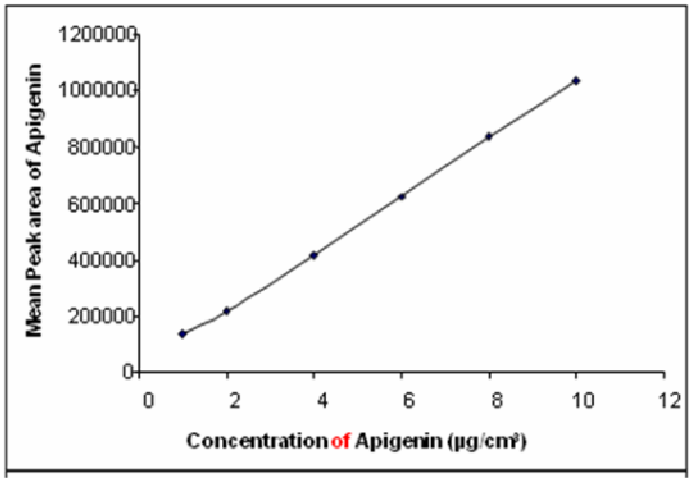
3. METHOD VALIDATION

3.1. LINEARITY OF DETECTOR RESPONSE

Standard solutions of apigenin of concentrations 1.00µg/cm^3, 2.00µg/cm^3, 4.00µg/cm^3, 6.00µg/cm^3, 8.00µg/cm^3 and 10.00µg/cm^3 were prepared and 20µL of each of the standard solutions of apigenin in the concentration range of 1.00 µg/cm^3 to 10.00 µg/cm^3 were injected into the chromatographic system under optimized chromatographic conditions. The chromatograms were recorded and the peak areas of apigenin for each injected concentration of apigenin, were noted.
The procedure was repeated three times. The densitograms were recorded and the peak areas of apigenin were noted for each concentration of the working standard solutions of apigenin, applied in triplicate. A graph of mean peak area values of apigenin (Y-axis), against the corresponding concentrations of apigenin (X-axis), was plotted and is shown in Figure 1. The graph shows that the response is linear in the concentration range of 1.00µg/cm^3 to 10.00µg/cm^3 for apigenin with correlation coefficient of 0.9995, intercept as 21897.15 and the slope as 101375.39.

Graph 1

Plot of mean peak area of apigenin against corresponding concentration of apigenin (µg/cm^3).



3.2. LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined at signal to noise ratios of 3:1 and 10:1 respectively. The values of LOD and LOQ by using the developed method were estimated as 0.01 µg/cm^3 and 0.30 µg/cm^3 respectively.

3.3. *SYSTEM SUITABILITY*

System suitability was determined by injecting, working standard solution of apigenin of concentration 5.00µg/cm^3, five times into the chromatographic system under the optimized chromatographic conditions. The chromatograms were recorded and the peak area values and the retention times of apigenin were noted for each injected concentration of apigenin.

The values of mean peak areas of apigenin for the applied concentration, standard deviation and the percent relative standard deviation were calculated. The values of percent relative standard deviations for the peak areas and retention times of apigenin were found to be 0.46 and 0.07 respectively, which are below 2, which show that the system is suitable for the chromatographic analysis.

3.4. *PRECISION*

The precision for the method was carried out by determining instrumental precision, intra-assay precision and intermediate precision. Instrumental precision was studied by replicate (n=10) injections of the same concentration (5.00 µg/cm^3) of apigenin standard solution. Intra-assay precision was evaluated by analysis of six replicate injections of freshly prepared sample solutions of same concentration, on the same day. The intermediate precision of the method was evaluated by analysis of six replicate injections of sample solutions of same concentration, on three different days. The results were expressed as percentage R.S.D of peak area of apigenin.

The values of percent R.S.D of peak area for instrumental precision, intra-assay precision and intermediate precision are given in Table 1. The values of percent R.S.D of peak area for instrumental precision, intra-assay precision and intermediate precision were less than 2, indicating that the proposed method is precise.

Table 1
***Method validation data for the quantitation of apigenin from the root bark of Gmelina arborea* Linn. by HPLC.**

Parameter	Results
Linear range [µg/cm^3]	1.00 to 10.00
Correlation coefficient [r]	0.9995
Limit of Detection (LOD) [µg/cm^3]	0.01
Limit of Quantitation (LOQ) [µg/cm^3]	0.30
Instrumental precision (*n = 10*) [Percent R.S.D.]	0.32
Intra assay precision (*n=6*) on the same day. [Percent R.S.D.]	0.59
Intermediate precision [Mean percent R.S.D. for three successive days.]	0.92

3.5. APPLICATION OF VALIDATED METHOD FOR THE QUANTITATION OF APIGENIN FROM Gmelina arborea LINN.

The quantitation of apigenin was done using above validated HPLC method. The optimized chromatographic conditions were set on the HPLC system and the system was monitored to attain a stable base-line. Twenty microlitre of solution was injected into the chromatographic system under the optimized chromatographic conditions.

The identity of peak of apigenin in the sample solution was confirmed by comparing the chromatogram of the sample (Figure 1) with that of the apigenin standard solution having retention time as 7.80 (Figure 2). The retention time of apigenin in the sample solution containing apigenin was found to be 7.80 minutes.

Amount of apigenin present in the sample solution was determined from the calibration curve by using the peak area of apigenin in the sample solution.

To ascertain the repeatability of the method, the assay experiment was repeated seven times. Mean amount of apigenin was found to be 0.1622 mg/g

Figure 1
A typical chromatogram of methanolic extract of root powder of *Gmelina arborea* Linn.

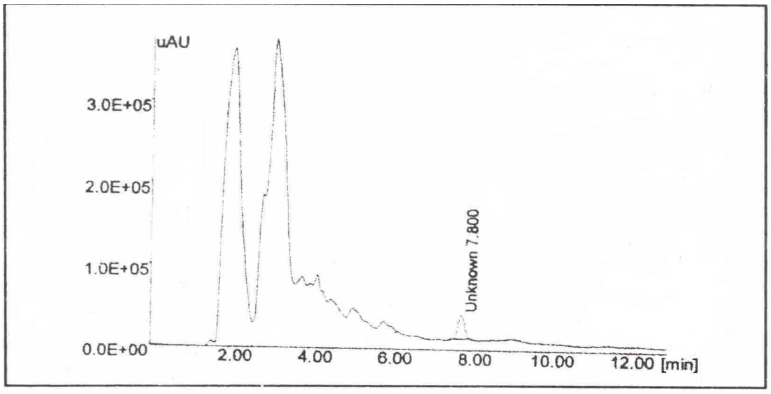
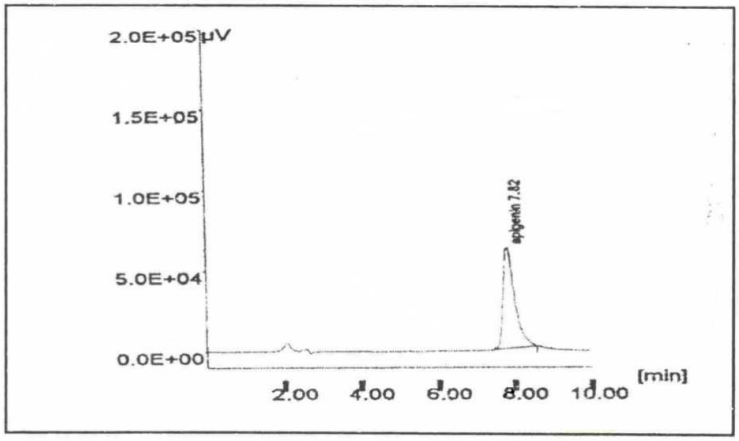


Figure 2
A typical chromatogram of apigenin of concentration 4.0 µg/cm^3



3.6. *ACCURACY*

The accuracy of the method was established by performing recovery experiments, using the standard addition method, at three different levels. About 250 mg of powdered roots of *Gmelina arborea* Linn., was accurately weighed into each of the four stoppered test tubes. Known amounts (0.00 mg, 0.02 mg, 0.04 mg and 0.06 mg) of powdered apigenin standard was added in solution form to each of the stoppered test tube respectively and 10.00 cm^3, warm methanol (maintained at 40^{o}C $\pm$ 2^{o}C, prior to addition) was added to each of the test tube, followed by sonicating for ten minutes in an ultrasonicator and cooling it to room temperature (28^{o}C $\pm$ 2^{o}C). The contents of the test tube were filtered separately and each solution was analyzed seven times, under optimized chromatographic conditions. Value of percentage recovery for apigenin was then determined. The results of the recovery experiment are given in Table 2. The value of percentage recovery of apigenin lies in the acceptable limits (98.0 % to 102.0 %), indicating good accuracy of the method.

Table 2
Recovery of apigenin from the root bark of *Gmelina arborea* Linn.

Level	Wt of sample * (mg)	Amount present in sample (mg)	Wt of std added (mg)	Mean amount of apigenin found (mg)**	Percent recovery [%]
0	250.7	0.1622	0.00	0.0388	98.58
1	250.2	0.1622	0.02	0.0590	
2	250.9	0.1622	0.04	0.0791	
3	250.4	0.1622	0.06	0.0978	

** Sample : Dried root Gmelina arborea Linn.*
** * Mean amount of apigenin found.*

4. RESULTS AND DISCUSSION

The HPLC methods reported in literature[9,11], were carried out for the determination of apigenin from rat plasma and mouse tissues respectively. The HPLC method reported in literature[10] was carried out for the determination of apigenin from human urine after administration of alcoholic extract of *Chrysanthemum morifolium* tablets. The HPLC method reported in literature was carried for simultaneous determination of apigenin and other flavonoids[12].

The mobile phase used in the present research work for quantitation of apigenin from methanolic, dried root powder extract of *Gmelina arborea* Linn. is also relatively simpler as compared to the mobile phase used in the above reported methods. [9-12].

The HPLC method reported in literature [12] was carried out for simultaneous determination of apigenin and other flavonoids from alcoholic extract of Pigeonpea [*Cajanus cajan* (L.) Millsp.] leaves, using HIQ-SIL-C_{18} V column. The retention time for apigenin was found to be 52.0 minutes while in the present research work, where Inertsil ODS-3V-C_{18},(4.6 mm i.d.,5µm.) column of length 250 mm was used, the retention time was found to be 7.80 minutes which is relatively less than the reported method [12].

The method used in the present research work was also found to be sensitive to measure the concentration as low as 0.01 µg/cm^3, whereas in the reported method [12], the detection limit was 0.03 µg/cm^3.

The column used in the present research work, comprised of octadecyl bonded to silica phase. Due to the length of the column, (250.0 mm) and small particle size of silica (5.0 µm), a good resolution of different components of root powder of *Gmelina arborea* Linn. was obtained.

5. CONCLUSION

The HPLC method used in the present research work for the quantitation of apigenin from root bark of *Gmelina arborea* Linn. was found to be simple, sensitive and accurate. It can be used for the routine quality control analysis and quantitative determination of apigenin from *Gmelina arborea* Linn.

REFERENCES

1. P.C. Sharma, M.B. Yelne and T.J. Dennis, Database on Medicinal Plants Used in Ayurveda. 3, 217-228, (2001).
2. K.M. Nadkarni, Indian Materia Medica, Bombay Popular Prakashan, 2, 584-585, (1999).

3. P. Satyanarayana, P. Subrahmanyam, R. Kasai, O. Tanaka, Phytochem., 24(8), 1862-1863, (1985).
4. A.S.R. Anjaneyulu, R.L. Ramachandra, C. Subrahmanyam, Tet. Lett., 22, 2179-2182, (1972).
5. C.J. Krishna, P. Singh, R.T. Pardasani, Planta Med., 32(1), 71-75, (1977).
6. K.C. Joshi, P. Singh, Phytochem., 47, 4719-4722, (1978).
7. D.M. Lepley, L. Boyong, D.F. Birt and J.C. Pelling, Carcinogenesis, 17(11), 2367-2375, (1996).
8. R. Torkin, J.F. Lavoie, R.K. David, H. Yeger. Mol. Cancer Ther., 4, 1-11, (2005).
9. Gran D, Jo B, Bo P, Romanova D, and Vachalkova A. J. Chromatography. 870(1-2), 463-467, (2000).
10. Li LP, Jiang HD. Journal of Pharmaceutical and Biomedical Analysis. 41(1), 261-265, (2006).
11. Cai H, Raynaud D, Steward WP, Gescher AJ. Biomed Chromatogr. Feb.28; (2006).
12. Zu YG, FU YJ, Liu W, Hou CL, and Kong Y. Chromatographia, May 4; (2006).