Determination of Pistacienoic Acids in *Pistacia integerrima* Stewart Ex Brandis by HPTLC and HPLC

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Simple and reproducible HPTLC and HPLC methods for the determination of pistacienoic acids in *Pistacia integerrima* were developed and are described. The HPTLC method involves separation of components by TLC on precoated Silica gel 60 F_{254} plate with a solvent system of chloroform:methanol (95:05) and detection at 220 nm in absorbance mode. The sensitivity of HPTLC method was found to be 1.0 µg and the linearity was observed in the range of 1.0 µg to 8.0 µg. The HPLC method involves separation of pistacienoic acids using mobile phase comprising of acetonitrile:water:phosphoric acid (80:20:1) and scanning the chromatogram at 220 nm using a photodiode array detector. The response was linear in the range of 1.25 µg to 10.0 µg. The proposed methods being precise and sensitive can be used for detection, monitoring and quantification of pistacienoic acids in *P. integerrima*.

Standardisation of Ayurvedic drugs and plant materials is the need of the time. Many of them do not have standard identification tests or analytical procedures to maintain their consistent quality. Several pharmacopoeias[1-3] contain monographs on plant materials that describe only the physico-chemical parameters but fail to identify and quantitate the active compounds. Hence, modern methods describing the identification and quantification of active compounds in the plant may be useful for proper standardisation of herbs and their formulations. *P. integerrima* Stewart ex Brandis, Anacardiaceae is one such plant, which is widely used in indigenous system of medicine. Its galls, formed on its leaves by hemipteris, have been reported[4-5] to have medicinal values against cough, fever, loss of appetite, nose bleeding, vomiting and stomach trouble. The galls have been found to contain mixture of chemical compounds such as essential oil[5], crystalline hydrocarbon, waxy compounds, sterols and pistacienoic acids[6]. Among the complex mixture of biologically active compounds in the plant, pistacienoic acids, can be used as analytical markers to determine the quality of plant material of different sources. A suitable sensitive and reliable quantitative high performance thin layer chromatographic (HPTLC) and high pressure liquid chromatographic (HPLC) methods have been developed for the first time for quality control determination of pistacienoic acids from *P. integerrima*.

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Around 50 g of air dried sample was ground to pass through a 20 mesh SS Sieve and 2 g from it was accurately weighed and extracted with chloroform (25 ml x 6) over a steam water bath. The extracts were filtered using laboratory grade filter paper, pooled and the solvent was evaporated over a steam water bath. The residue thus obtained was dissolved in 20 ml of chloroform and final volume was made upto 25 ml in a volumetric flask using chloroform. This solution was used further for HPTLC/HPLC analysis as per the procedure mentioned below.

A Camag HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC Scanner III and an integration Software, CATS 4.05 was used. The test sample was shaken well, 1 and 2 µl of it were applied on the TLC plate alongwith 1, 2, 4, 6 and 8 µl of standard pistacienoic acids (1 mg/ml) from about 1 cm edge of TLC plate (E.Merck, Cat. No. 5554) using a band width of 6 mm and distance between tracks 5 mm. The chromatogram was developed in chloroform:methanol -95:05 solvent system upto 80 mm under chamber saturation condition. The plate was air dried and scanned at 220 nm in the

absorbance mode. The amount of pistacienoic acids was determined using the calibration curve plotted between concentrations and area of standard. The equation for pistacienoic acids was found to be y = 1943.7x + 538.5 with a correlation coefficient of 0.9991, where y is the response in peak area and x is concentration in mg/ml.

A Water's HPLC system consisting of 510 chromatographic pump, rheodyne 7125 injector, 996 photodiode array detector and millennium v.2.10 integration software was used for the analysis of pistacienoic acids. The test sample was dried and the residue was dissolved in 25 ml of methanol and passed through 0.45 µ PVDF filter using a syringe, 20 µl of it along with 20 µl each of different concentrations of standard pistacienoic acids (0.0625, 0.125, 0.250, 0.375 and 0.50 mg/ml) were injected to HPLC using Novapack RP C18 column (3.9 x 150 mm) with a reverse phase C18 guard column. The mobile phase consisting of acetonitrile:water:phosphoric acid (80:20:1) were mixed by volume and passed through 0.45 micron filter, degassed and used. The flow rate was maintained at 1.5 ml/min. The chromatogram was scanned upto 20 min, which was detected at 220 nm. The amount of pistacienoic acids was quantified using linear regression equation of calibration graph plotted between concentration and area of standards. The equation for pistacienoic acids was found to be y = 644571x + 454265 with a correlation coefficient of 0.998 where y is the response in peak area and x is the concentration in mg/ml.

To validate the methods and to know percentage recovery a known amount of pistacienoic acids was added to about 2.0 g of fine powdered test sample in which the contents of pistacienoic acids had been estimated previously by proposed methods. The samples were extracted and analysed separately by HPTLC and HPLC as per the procedure mentioned above. The contents of pistacienoic acids were quantified using proposed methods and percentage recovery calculated. The results are provided in Table 1 and 2.

Sample preparation and development of suitable mobile phase or solvent system are two important steps in developing the analytical procedure, which becomes more significant for herbal drugs because of their complexity of chemical compounds and their affinity towards various solvents. By trying different composition of mobile phase, the desired

TABLE 1: RECOVERY OF PISTACIENOIC ACIDS FROM *P. INTEGERRIMA* USING HPTLC.

Sample	Amount of sample taken (mg) (A)	Amount of Pistacienoic acid in A (mg) (B)	Amount of Pistacienoic acid added to A (mg) (C)	Amount of Pistacienoic acid taken B+C (mg) (D)	Total Pistacienoic acids found (mg) (E)	% Recovery $\frac{E}{D} \times 100$
P. integerrima	1010	34.95	2.00	36.95	36.05	97.56
P. integerrima	1020	35.29	4.00	39.29	38.78	98.70
P. integerrima	1020	35.29	8.00	43.29	43.02	99.37

Average percentage recovery = 98.55 ± 0.92%.

TABLE 2: RECOVERY OF PISTACIENOIC ACIDS FROM *P. INTEGERRIMA* USING HPLC.

Sample	Amount of sample taken (mg) (A)	Amount of Pistacienoic acid in A (mg) (B)	Amount of Pistacienoic acid added to A (mg) (C)	Amount of Pistacienoic acid taken B+C (mg) (D)	Total Pistacienoic acids found (mg) (E)	% Recovery $\frac{E}{D} \times 100$
P. integerrima	1010	37.17	2.00	39.17	38.62	98.59
P. integerrima	1020	37.54	4.00	41.54	40.94	98.55
P. integerrima	1020	37.54	8.00	45.54	45.62	100.17

Average percentage recovery = 99.10 ± 0.92%.

resolution of pistacienoic acids with symmetrical and reproducible peaks was achieved by using chloroform:methanol (95:05) by HPTLC while for HPLC the mobile phase consisting of acetonitrile:water:phosphoric acid, (80:20:1) gave satisfactory separation and resolution. Using the proposed HPTLC methods, the migration distance of pistacienoic acids was about 37 mm while the retention time using HPLC was 4.8 min.

The calibration curves for HPTLC and HPLC were linear in the range of 1.0 to 8.0 µg and 1.25 to 10.0 µg, respectively. Further, the correlation coefficient 0.999 and 0.998 indicates good linearity between concentration and area for both the methods. The methods allow reliable quantification of pistacienoic acids and provide good resolution and separation of marker compound from other constituents of *P. integerrima*. Further recovery values of 97.56% - 99.37% (98.55% ± 0.92%) and 98.59% - 100.17% (99.10 ± 0.92%) were obtained using HPTLC and HPLC, showing excellent reliability and reproducibility of proposed methods. The values of pistacienoic acids were observed to be 3.46% and 3.68% using HPTLC and HPLC, respectively. The proposed HPTLC and HPLC methods are rapid, simple and accurate for quantitative monitoring of pistacienoic acids in *P. integerrima* and can be used for routine quality testing.

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Bioequivalence Study of Rosiglitazone Maleate 2 mg Tablets on Twelve Indian Human Male Volunteers

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Rosiglitazone is a second-generation aminopyridyl thiazolidinedione that has been observed to be a safe and effective oral glucose-lowering agent in diabetic patients. In the present study two different preparations containing 2 mg rosiglitazone were compared in twelve healthy Indian human male volunteers under fasting condition. The trial was performed in a randomized two-way crossover design with a wash out period of one week. Rosiglitazone was extracted from human plasma using liquid-liquid method of extraction. HPLC method was used to determine the plasma concentration of the drug.

Rosiglitazone is a second-generation aminopyridyl thiazolidinedione that has been observed to be a safe and effective oral glucose-lowering agent in diabetics[1]. This class of compounds has been demonstrated to lower plasma glucose by reducing insulin resistance and increasing peripheral glucose disposal[2,3]. The present study was performed to investigate bioequivalence between two preparations of 2 mg rosiglitazone maleate tablets. In order to analyze plasma samples a HPLC method was developed. This newly developed method was first validated[4,5]. The proposed

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