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Development of High-Performance Thin-Layer chromatography method for quantitation of Quercetin in NORMACID SYRUP- a poly herbal formulation.

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ABSTRACT

Normacid syrup (consisting of 17 ingredients), used in the treatment of hyperacidity and gastritis. Quercetin is one of the phytoconstituent present in Normacid syrup – a poly herbal formulation. In the present study an attempt has been made to develop a HPTLC method for quantitative estimation of quercetin in dried barks used in formulations. This HPTLC method was found to be reproducible, accurate and precise and detect quercetin concentration at nanogram level. The developed HPTLC method would be an important tool in the quality control method of polyherbal formulations.

Keywords: quercetin, Gastritis, HPTLC, Normacid Syrup.

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INTRODUCTION

Gastritis is an inflammation of the stomach, and has many possible causes like excessive alcohol consumption or prolonged use of non steroidal anti-inflammatory drugs. Sometimes gastritis develops after major surgery, traumatic injury, burns or severs infections. Gastritis may also occur in those who have had weight loss surgery resulting in the banding or reconstruction of the digestive tract. Chronic causes are infection with bacteria like *Helicobacter pylori*, certain diseases such as pernicious anemia, chronic bile reflux, stress and certain autoimmune disorders. The most common symptom is abdominal upset or pain. Other symptoms are indigestion, abdominal bloating, nausea, and vomiting. A gastroscopy, blood test, complete blood count test, or a stool test may be used to diagnose gastritis. Normacid syrup is a poly herbal antacid formulation used to treat gastritis and hyperacidity which contains 17 ingredients viz., water extract of *Ficus glomerata, Fagonia arabica, Vetiveria zizinoides, Santalum album, Andrographis panniculata, Azadirechta indica, Terminalia chebula, Terminalia balarica, Embilica officinalis, Trichosanthes doica, Adhatoda vasica, Tinospora cordifolia, Fumaria officinalis,* Shauktik bhasma, Kapardika bhasma & Pravel bhasma. From which extract of *Andrographis panniculata(Kalmegh) , Fagonia arabica (Dhamaso), Fumaria officinalis (Pittapapro)* and *Trichosanthes doica (Kadu patol)* are used for the quantitative estimation of quercetin. Quercetin is a plant-derived flavonoid, specifically a flavonol, used as a nutritional supplement. Laboratory studies show it may have anti-inflammatory and antioxidant properties[3,4], and it is being investigated for a wide range of potential health benefits.[4,5] Quercetin has been shown to increase energy expenditure in rats, but only for short periods (fewer than 8 weeks)[3]. Effects of quercetin on exercise tolerance in mice have been associated with increased mitochondrial biogenesis.[4] As the literature survey clearly reveals that there is no proper analytical method available for the quantitative estimation of rutin in herbal syrups, the present study focused to develop a rapid, efficient and reproducible method for the analysis of rutin in herbal syrup by HPTLC.

MATERIALS AND METHODS

Experimental Analysis

HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). The HPTLC was carried out using a Hemilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60F254(Merck), 0.2 mm thickness. HPTLC finger printing technique is useful to identify and to check the purity of raw herbal extracts as well as finished product. Hence forth it is very useful tool in standardizing process of raw herbal extracts and finished products.

Steps involved in HPTLC analysis

- **Selection of plate and adsorbent:** Precoated aluminium plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection. The

plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography.

- **Standard solution:** An accurately weighed quantity (50 mg) of quercetin was dissolved in diluent [methanol] taken in 50ml volumetric flask. Then the volume is made up to 50ml with diluent to obtain a stock solution having 1 mg/ml concentration of rutin. The standard stock solution of rutin was diluted to prepare working standard with concentration range of 1 – 5 µg/ml.
- **Sample solution:** Accurately weighted 20 mg of extract of all plants and syrup was taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume made up to the mark with Methanol. The volume made up to the mark with Methanol.
- **Application of sample:** Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of all the extracts and marker solution also.
- **Development:** The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent Toluene: Ethyl acetate: Acetone: Formic acid (10:5:15:1) for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done.
- **Detection:** The plate was scanned at UV 366 nm and 254 nm using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of each compound which were separated on plate and data of peak area of each band was recorded.

RESULT AND DISCUSSION

Table 1 Peak table of chromatogram of Quercetin, extracts and formulation

	Peak	Max. Rf	Peak Area	Quercetin mcg/ml
Normacid	1	0.88	21292.6	4.10
Marker	1	0.86	16523	----
Kalmegh	1	0.85	15141.7	3.36
Dhamas	1	0.86	15155.9	3.22
Kadupatol	1	0.89	14134.7	3.42
Pittapapdo	1	0.87	16564.1	3.10

The peak areas of Quercetin for (1µg/ml-5µg/ml) concentration were recorded. Calibration curve was prepared by plotting peak areas of Quercetin against concentration. The results showed linearity and correlation coefficient within the range of concentration (1µg/ml-5µg/ml) The best fitting liner equation was Y= 516.7X+633. (R2 = 0.988). There was good correlation between peak area and the corresponding concentration of Quercetin as shown in figure of calibration curve of Quercetin. Stationary phase Silica Gel TLC plate and mobile phase toluene: ethyl acetate: acetone: formic acid (5:2.5:7.5:0.5) had given good separation of Quercetin at R_f = 0.86. The calibration curve of Quercetin was found to be linear dependent on the concentration against area. The best fitting line equation was $y_=$ 516.7X+633. R^2 = 0.988; indicated good linearity between concentration and peak area in table 5.9. Quercetin content in the methanol extracts of Kalmegh, Dhamas, Kadupatol, Pittapapdo and Normacid

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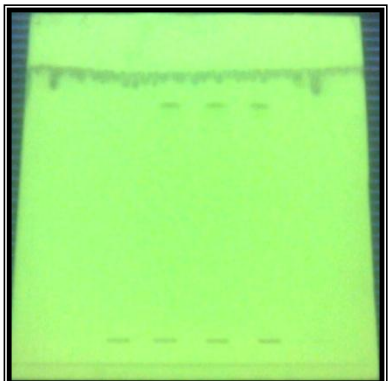
syrup by the proposed HPTLC method was 3.36, 3.22, 3.42, 3.10 and 4.10 mcg/ml respectively. The detection of the Quercetin band in the sample extract solution was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard of Quercetin, using the Camag TLC scanner 3.



µg/ml	Area
1	1222.2
2	1653.5
3	2125.7
4	2864.3
5	3200

Fig. 1 HPTLC Plate of Quercetin

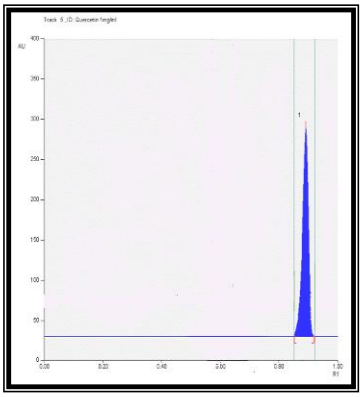
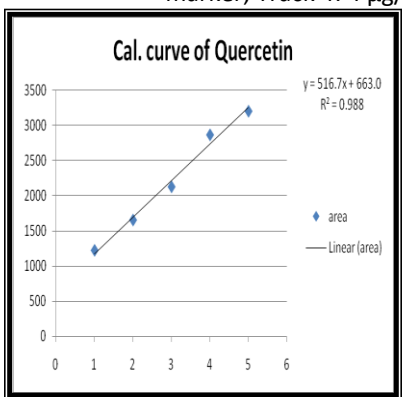


Fig. 2 HPTLC chromatogram of Quercetin

[Track 1: 1 µg/ml of Quercetin marker; Track 2: 2 µg/ml of Quercetin marker; Track 3: 3 µg/ml of Quercetin marker; Track 4: 4 µg/ml of Quercetin marker; Track 5: 5 µg/ml of Quercetin marker]



Calibration curve for Quercetin

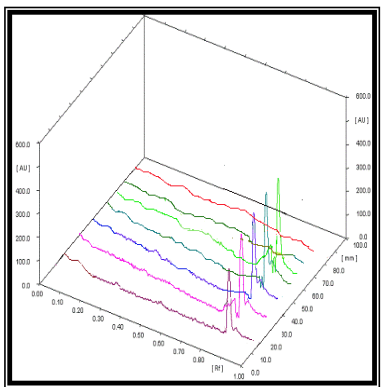


Fig. 5.3 (c) 3D-image of the Quercetin

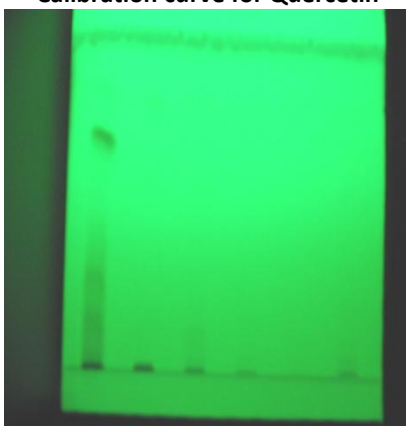


Fig. 3 HPTLC plate of Quercetin

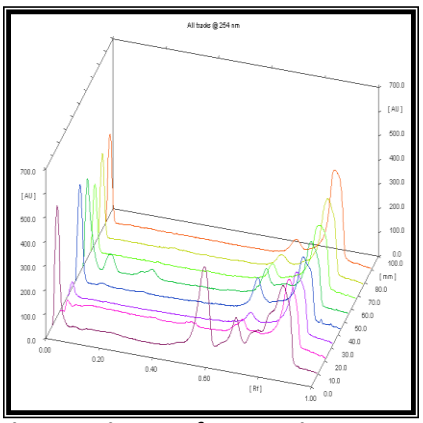


Fig. 4 3-D image of Quercetin extract and formulation extract and formulation

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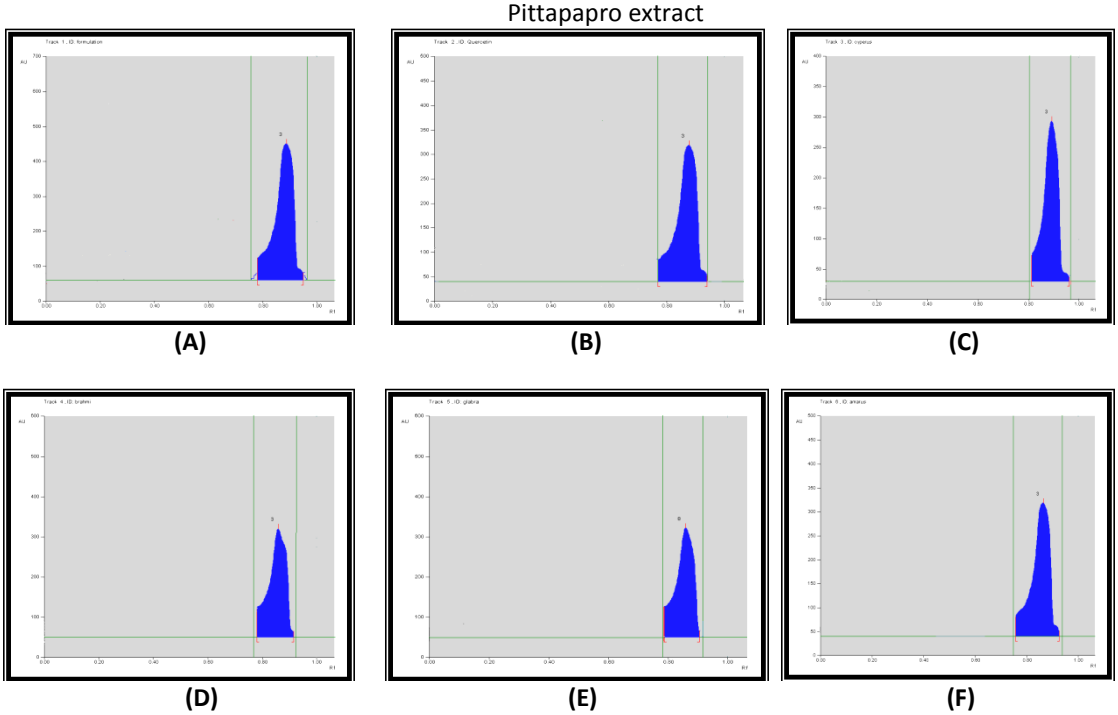
Track 1: 10 µg/ml of Normacid Syrup; Track 2: 4 µg/ml of Quercetin marker; Track 3: 10 µg/ml Kalmegh extract; Track 4: 10 µg/ml of Dhamas extract; Track 5: 10 µg/ml of Kadupatol extract; Track 6: 10 µg/ml of Pittapapro extract



(A) (B) (C)

(D) (E) (F)

A.Track 1: Chromatogram of Normacid Formulation; B. Track 2: Chromatogram of Quercetin marker; C. Track 3: Chromatogram of Kalmegh extract; D. Track 4: Chromatogram of Dhamas extract; E. Track 5: Chromatogram of Kadupatol extract; F. Track 6: Chromatogram of Pittapapro extract

Fig. 5 Chromatograms of Quercetin, extracts and formulation.

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