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Research Article

Development of High Performance Thin Layer Chromatography Method for Quantitation of Gallic Acid 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. Gallic acid 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 gallic acid in dried fruits used in formulations. This HPTLC method was found to be reproducible, accurate, precise and detect gallic acid at nanogram level. The developed HPTLC method would be an important tool in the quality control method of poly herbal formulation.

Keywords: HPTLC, Polyherbal, Gallic acid, Gastritis

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INTRODUCTION¹⁻²¹

The stomach normally secretes acid that is essential in the digestive process. This acid helps in breaking down the food during digestion. When there is excess production of acid in the stomach, it results in the condition known as acidity or APD. Normally, there are some protective mechanisms against acid, in the stomach and proximal intestine. But an imbalance between the protective mechanisms and the level of acid secretion can cause acidity^{1,2}. Gastritis is an inflammation of the lining of the stomach, and has many possible causes.^{3,4} The main acute causes are excessive alcohol consumption or prolonged use of nonsteroidal anti-inflammatory drugs such as aspirin or ibuprofen. Sometimes gastritis develops after major surgery, traumatic injury, burns, or severe 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, primarily *Helicobacter pylori*. Certain diseases, such as pernicious anemia, chronic bile reflux, stress and certain autoimmune disorders can cause gastritis as well. The most common symptom is abdominal upset or pain. Other symptoms are indigestion, abdominal bloating, nausea, and vomiting. Some may have a feeling of fullness or burning in the upper abdomen.^{6,7,8,10} Pain in the upper abdomen is the most common symptom. Bloating and a feeling of fullness or burning in the upper abdomen is also signs of moderate gastritis. Severe gastritis presents pallor, sweating, rapid heart beat, feeling faint or short of breath,

severe chest or stomach pain, vomiting large amounts of blood, or bloody or dark, sticky, foul-smelling bowel movements.¹⁵⁻²⁰ Tests for blood cell count, *H. pylori*, and pregnancy; and liver, kidney, gallbladder, and pancreas functions, may be ordered. Urinalysis may be used, or a stool sample taken, to look for blood in the stool. X-rays may be ordered, as well as ECGs. If none of these tests are able to be used for diagnosis, the patient may be recommended to a gastroenterologist. An endoscopy may be performed, where a flexible probe with a camera on the end is sent into the stomach to check for stomach lining inflammation and mucous erosion. At the same time, a stomach biopsy may be taken to test for gastritis and a variety of other conditions.¹⁵⁻²⁰ Normacid syrup is a polyherbal formulation and works as a natural antacid. Antacid perform a neutralization reaction, i.e. they buffer gastric acid, raising the pH to reduce acidity in the stomach. When gastric hydrochloric acid reaches the nerves in the gastrointestinal mucosa, they signal pain to the central nervous system. This happens when these nerves are exposed, as in peptic ulcers. The gastric acid may also reach ulcers in the esophagus or the duodenum.

2.14.2 Dosage

Syrup: 10 ml twice a day or as directed by physician

2.14.3 Indications

Gastritis
Acidity
Hyperacidity

The Only True Acid Normaliser. As Normacid not only neutralizes the excess acid but also normalizes acid secretion.

2.14.4 Composition of Normacid syrup

Name of ingredient	Parts Used	Label claim mg/10ml
Adumber	Bark	40
Dhamasa	Whole Plant	30
Khas	Root	5
Chandan	Wood	5
Kalmegh	Whole Plant	6
Neem	Bark	8
Harde	Bark	20
Behda	Bark	15
Amla	Fruit	20
Kadupatol	Whole Plant	8
Ardusi	Flower	8
Gaduchi	Whole Plant	8
Pittapapdo	Whole Plant	8
Shaukik bhasma	-----	25
Kapardika bhasma	-----	25
Praval bhasma	-----	10

MATERIALS AND METHOD

HPTLC finger-printing of raw material and finished product and quantitative determination of gallic acid.

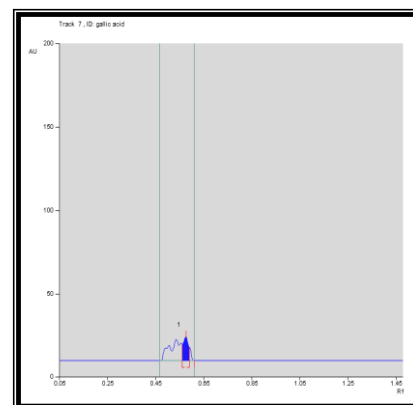
HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). The HPTLC was carried out using a Hemilton 100 µl HPTLC syringe, Camag Linomat V automatic 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

RESULT AND DISCUSSION

Determination of Gallic acid by HPTLC



HPTLC plate of Gallic acid



HPTLC chromatogram of Gallic acid

Track 1: 1 µg/ml of Gallic acid marker; Track 2: 2 µg/ml of Gallic acid marke; Track 3: 3 µg/ml of Gallic acid marker; Track 4: 4 µg/ml of Gallic acid marke; Track 5: 5 µg/ml of Gallic acid marker; Track 6: 6 µg/ml of Gallic acid marker; Track 7: 7 µg/ml of Gallic acid marker

very useful tool in standardizing process of raw herbal extracts and finished products.

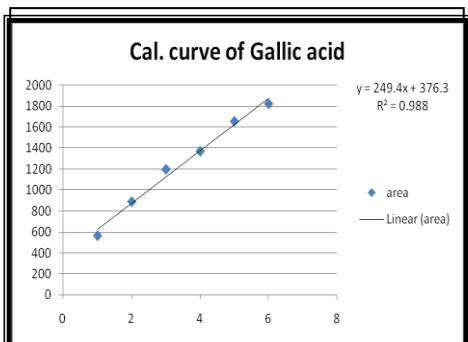
Steps involved in HPTLC analysis

- **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.
- **Sample solution:** Accurately weighted 20 mg of extract of *Adhatoda vasica* root 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 for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done. The mobile phase or solvent system for all the raw herbs, raw ingredients, marker compound which is given in the Table 1.

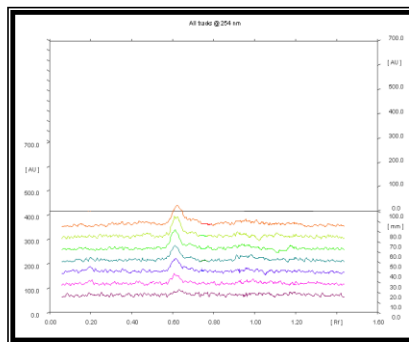
Table 1. Solvent system for plant, extract of Normacid Syrup

Sr No	Sample	Solvent system
1	Gallic acid, Neem, Amla, Behda, Harde	Toluene: Ethyl acetate: Formic acid: Methanol (6:6:1.6:0.4)

- **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.



$\mu\text{g/ml}$	Area
1	566.3
2	890.3
3	1197.3
4	1369.5
5	1653.9
6	1820.1

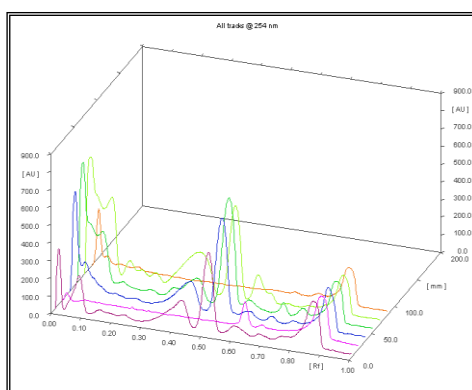
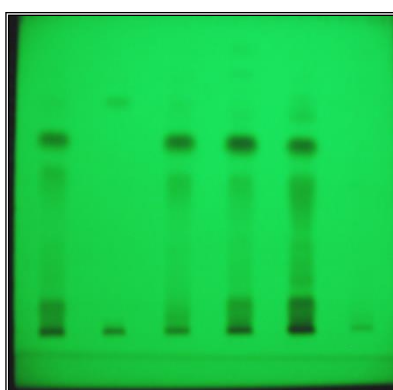


Calibration curve for Gallic acid

3D image of the Gallic acid

The peak areas of Gallic acid for (1 $\mu\text{g/ml}$ -7 $\mu\text{g/ml}$) concentration were recorded. Calibration curve was prepared by plotting peak areas of Gallic acid against concentration. The results showed linearity and correlation coefficient within the range of concentration (1 $\mu\text{g/ml}$ -

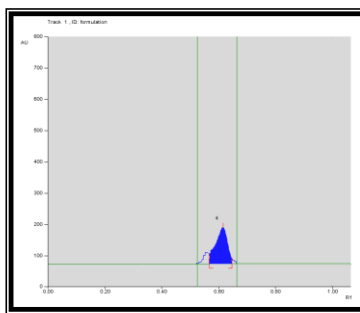
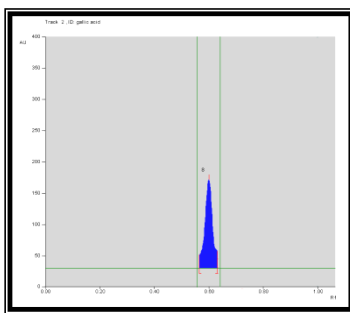
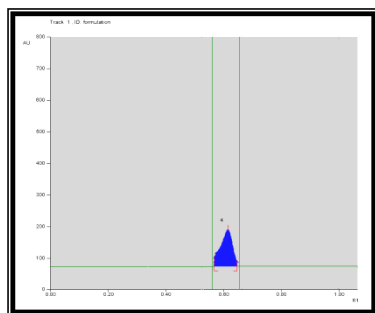
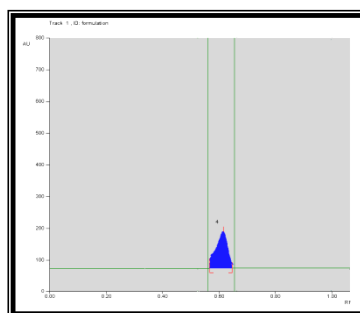
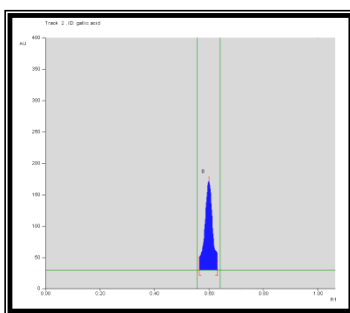
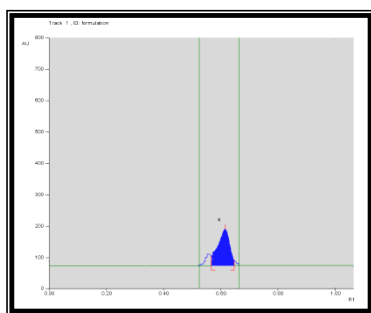
5 $\mu\text{g/ml}$). There was good correlation between peak area and the corresponding concentration of Gallic acid as shown in figure of Calibration curve for Gallic acid. The best fitting liner equation was $y = 249.4X + 376.3$ ($R^2 = 0.988$)



HPTLC plate of Gallic acid containing extract & formulation

Three-D image of Gallic acid containing extract and formulation.

Track 1: 10 $\mu\text{g/ml}$ of Normacid Formulation; Track 2: 4 $\mu\text{g/ml}$ of Gallic acid marker; Track 3: 10 $\mu\text{g/ml}$ of Amla extract; Track 4: 10 $\mu\text{g/ml}$ of Behda extract; Track 5: 10 $\mu\text{g/ml}$ of Harde extract; Track 6: 10 $\mu\text{g/ml}$ of Neem extract



Chromatograms of Gallic acid, extract and formulation.

A. Track 1: Chromatogram of Normacid Formulation; B. Track 2: Chromatogram of Gallic acid marker; C. Track 3: Chromatogram of Amla extract; D. Track 4: Chromatogram of Behda extract; E. Track 5: Chromatogram of Harde extract; F. Track 6: Chromatogram of Neem extract

Table 2. Peak table of chromatogram of Gallic acid, extracts and formulation

	Peak	Max. Rf	Peak Area	Gallic acid mcg/ml
Normacid	1	0.58	1840.6	2.35
Gallic acid	1	0.60	2253.9	---
Amla	1	0.59	1096.2	2.1
Behda	1	0.60	1902.5	2.4
Harde	1	0.59	1096.2	2.8
Neem	1	0.60	1902.5	2.4

SUMMARY AND CONCLUSION:

Stationary phase Silica gel TLC plate and mobile phase toluene: ethyl acetate: formic acid: methanol (6:6:1.4:0.6) had given good separation of Gallic acid at $R_f = 0.60$. The calibration curve of Gallic acid was found to be linear dependent on the concentration against area. The best fitting line equation was $y = 249.4X + 376.3$. R^2 0.988 indicated good linearity between concentration and peak area in table 5.10. Gallic acid content in the methanolic extract of Amla, Behda, Harde, Neem and Normacid formulation by the proposed HPTLC method was found to be 2.1mcg/ml, 2.4 mcg/ml, 2.8mcg/ml, 2.4mcg/ml and 2.35 mcg/ml respectively. The identity of the Gallic acid band in the sample extract solution was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard of Gallic acid, using the Camag TLC scanner 3.

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