DEVELOPMENT OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR QUANTITATION OF β -SITOSTEROL 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. β - Sitosterol 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 β - Sitosterol in dried plant parts used in formulations. This HPTLC method was found to be reproducible, accurate, precise and detect β - Sitosterol at nanogram level. The developed HPTLC method would be an important tool in the quality control method of poly herbal formulation.

Keywords: HPTLC, Polyherbal, β - Sitosterol, Gastritis

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 are 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.

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

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RESULT AND DISCUSSION

Determination of β - Sitosterol by HPTLC

HPTLC plate of β -sitosterol

HPTLC chromatogram of β -sitosterol

Track 1: 1 $\mu\text{g/ml}$ of β -sitosterol marker; Track 2: 2 $\mu\text{g/ml}$ of β -sitosterol marker; Track 3: 3 $\mu\text{g/ml}$ of β -sitosterol marker; Track 4: 4 $\mu\text{g/ml}$ of β -sitosterol marker; Track 5: 5 $\mu\text{g/ml}$ of β -sitosterol marker.

$\mu\text{g/ml}$	Area
1	1753.4
2	2341.2
3	2863.7
4	3312.6
5	3762.4

Calibration curve for β -sitosterol

3 D- image of the β -sitosterol

The peak areas of β -sitosterol for (1 $\mu\text{g/ml}$ -5 $\mu\text{g/ml}$) concentration were recorded. Calibration curve was prepared by plotting peak areas of β -sitosterol 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 β -sitosterol as shown in figure of Calibration curve for β -sitosterol. The best fitting liner equation was $y = 498.9X + 1309$. ($R^2 = 0.996$)

**HPTLC plate of β -sitosterol
extracts and formulation**

**Three-D image of β -sitosterol
extract & formulation**

Track 1: 10 $\mu\text{g/ml}$ of Normacid Formulation; **Track 2:** 4 $\mu\text{g/ml}$ of β -sitosterol marker; **Track 3:** 10 $\mu\text{g/ml}$ of Adumber extract; **Track 4:** 10 $\mu\text{g/ml}$ of Galo extract

A. Track 1: Chromatogram of Normacid Formulation; **B. Track 2:** Chromatogram of β -sitosterol marker; **C. Track 3:** Chromatogram of Adumber; **D. Track 4:** Chromatogram of Galo
Chromatograms of β -sitosterol, extracts and formulation.

Table 2: Peak table of chromatogram of β -sitosterol, extracts and formulation.

	Peak	Max. R _f	Area	β -sitosterol mcg/ml
Normacid	1	0.61	2389.4	2.25
β -sitosterol	1	0.60	4005.9	-----
Adumber	1	0.63	3278.1	3.41
Galo	1	0.61	5287.9	1.76

Stationary phase Silica gel TLC plate and mobile phase hexane: ethyl acetate (1:1) had given good separation of β -sitosterol at $R_f = 0.60$. The calibration curve of β -sitosterol was found to be linear dependent on the concentration against area. The best fitting line equation was $y = 498.9X + 1309$. $R^2 = 0.996$ indicated good linearity between concentration and peak area in table 5.11. β -sitosterol content in the methanolic extract of Adumber, Galo and Normacid syrup by the proposed HPTLC method was found to be 2.25, 3.41 and 1.76 mcg/ml respectively. The identity of the β -sitosterol band in the sample extract solution was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard of β -sitosterol, using the Camag TLC scanner 3.

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