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DETERMINATION OF QUERCETIN BY HPTLC METHOD PRESENT IN ZYMODYNE SYRUP- A POLY HERBAL FORMULATION

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ABSTRACT

Zymodyne Syrup is poly herbal formulation made by Ayrulab Herbals Pvt. Ltd., as an appetite enhancer. This formulation contains various 17 plant extract from these 6 plants namely *Cyperus rotundus, Bacopa monnieri, Glycyrrhiza glabra, Phyllanthus amarus, Asparagus racemosus,* and *Tribulus terrestris* contains Quercetin flavonoid as a chemical constitute and it can be determined by HPTLC method. The concentration of Quercetin in formulation was 0.1100 mcg/ml and in individual extracts were 0.870, 0.783, 0.782, 0.855, 0.875, 0.957 mcg/ml respectively. This study may provide useful information in searching the role of Quercetin to enhance appetite biologically.

INTRODUCTION: Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide range of biological activities, higher safety margins and lesser costs [1]. Public, academic and government interest in herbal medicines is growing exponentially due to increased incidence of the adverse drug reactions and economic burden of the modern system of medicine [2].

Herbal therapy is one of the best practices to overcome the illness [3]. Today's world anorexia and dyspepsia are two major disorders owing to stressful competitive life and fast food culture respectively [4, 5]. In India, both these problems are seen mostly in youngsters and working professionals. Zymodyne syrup, developed by Ayurlab Herbals Pvt. Ltd., Halol, Gujarat; cures both the problems as it is completely herbal formulation so avoid of side effects. Zymodyne Syrup contains various 17 plant extracts.

In present study determination of Quercetin flavonoid present in *Cyperus rotundus, Bacopa monnieri, Glycyrrhiza glabra, Phyllanthus amarus, Asparagus racemosus,* and *Tribulus terrestris* plant extracts and Zymodyne syrup by HPTLC method. However individual HPTLC work for all the plants listed above has been done. Here, for the HPTLC work there is need of Quercetin marker, methanolic extracts of plants listed above and mixture of ingredients of Zymodyne syrup formulation.

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. Development of plates, chromatograms, calibration curve are given in figures for both plant extracts and formulation. From the calibration curve determination of Quercetin in respective extracts and formulation can be done.

MATERIALS AND METHODS: HPTLC work done by using 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.

Plant extracts were obtained from Ayurlab Herbals Pvt. Ltd while syrup formulation was obtained from finished drug store of Ayurlab Herbals Pvt. Ltd., and marker namely Quercetin was procured from Shri B M Shah College of Pharmacy, Modasa. All the extracts and syrup formulation have certificate of analysis.

Steps involved in HPTLC analysis: 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. Accurately weighted each of extract as per decoction ratio in **Table 1**, was taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume made up to the mark with Petroleum ether.

TABLE 1: DECOCTION RATIO FOR ZYMODYNE SYRUP

Name of ingredients	label claim mg/10 ml	For 10 ml decoction in mg
Pueraria tuberose	1000	111.1
Emblica officinalis	1000	250.0
Phylanthus amarus	500	166.66
Asparagus racemosus	300	75.0
Zingiber officinale	200	33.33
Cyperrus rotundus	200	20.0
Tinospora cordifolia	200	20.0
Withania somnifera	200	20.0
Tribulus teristris	200	20.0
Bacopa monnieri	200	20.0
Pipper longum root	100	8.33
Pipper chaba	100	10.0
Plumbago Zeylanica	100	12.5
Convolvulus pluricaulis	100	10.0
Glycerhiza glabra	50	10.0
Pipper longum fruit	50	12.5
Acorus calamus	20	5.0

Here, we used Quercetin as a marker. 10 mg of working standard was dissolved in 10 ml of methanol to yield stock solution of 1000 µg/ml concentration. Calibration curve from 1-5 µg/spot was prepared and checked for reproducibility, linearity and validating the proposed method.

Sample application is the most critical step for obtaining good resolution for determination 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.

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 was Toluene: Ethyl acetate: Acetone: Formic acid (5:2.5:7.5:0.5) [6, 7]. (Obtained from Ayur Lab Herbals Pvt. Ltd.,).

RESULTS AND DISCUSSION: HPTLC plate and 3-D image of Quercetin marker were given in **Figure 1** while chromatogram and calibration curve of Quercetin were given in **Figure 2** with peak area of marker stated in **Table 2**. HPTLC plate and 3-D image of various extracts containing Quercetin and Formulation were given in **Figure 3** and chromatograms of Quercetin containing extracts and Formulation were given in **Figure 4** with maximum R_f value and peak area stated in **Table 3**.

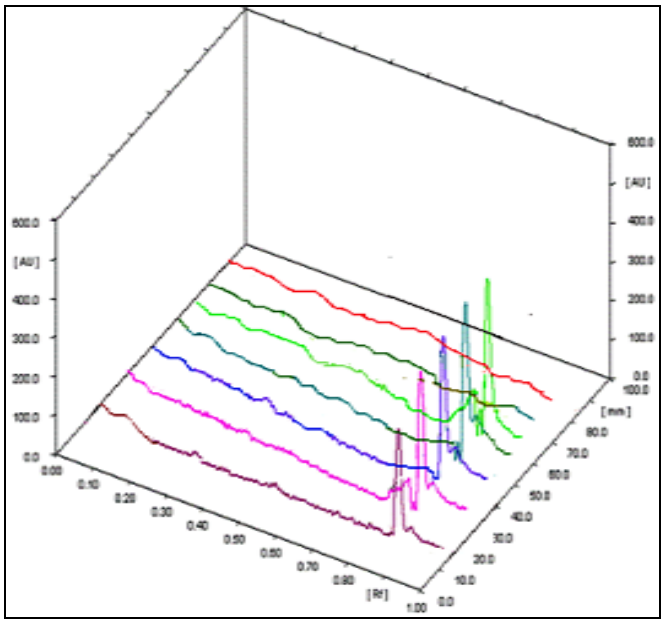
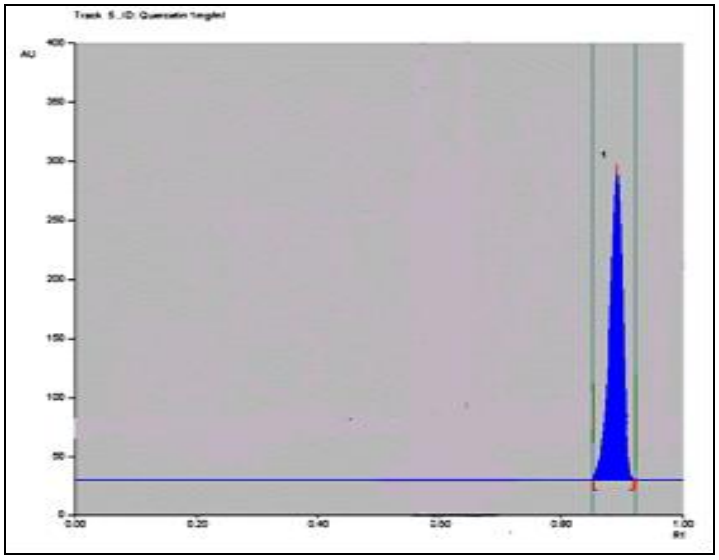


FIG. 1: HPTLC PLATE OF STANDARD QUERCETIN (254 NM) & 3-D IMAGE OF QUERCETIN

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



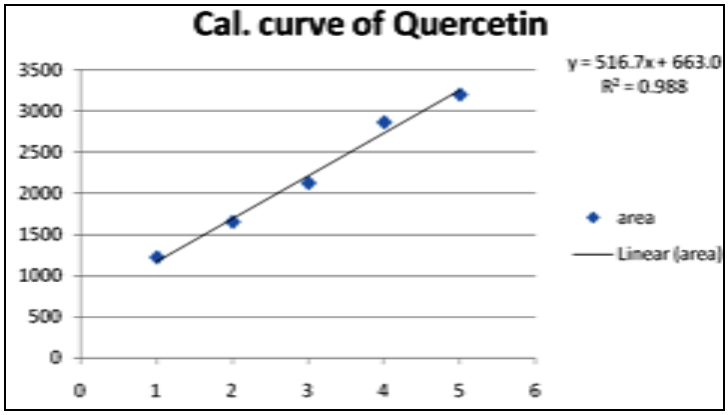
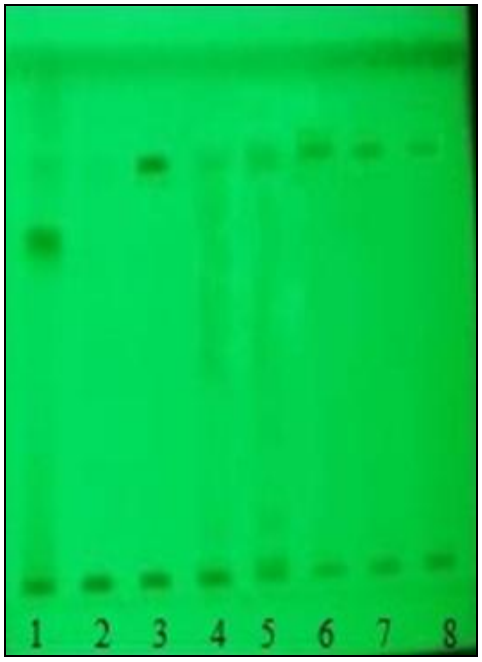


FIG. 2: DENSITOMETRIC CHROMATOGRAM & CALIBRATION CURVE OF QUERCETIN

TABLE 2: CALIBRATION CURVE OF QUERCETIN

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



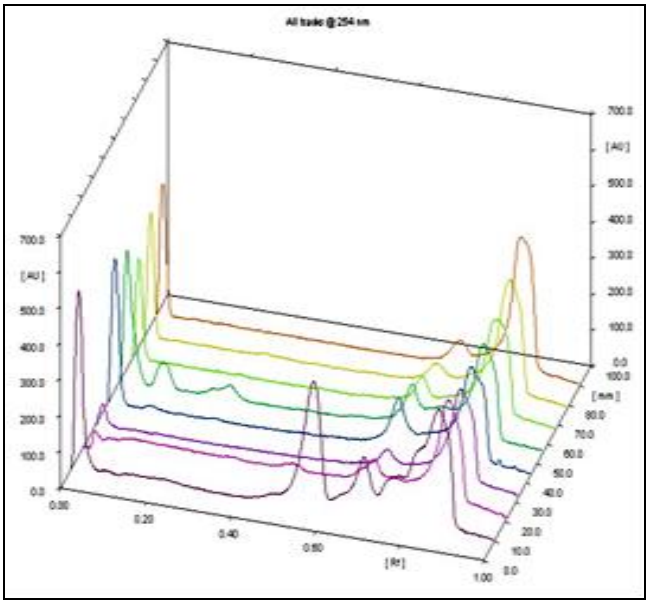
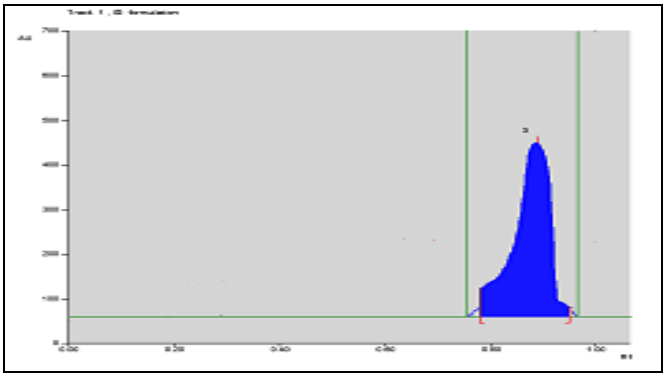


FIG. 3: HPTLC PLATE AND 3D IMAGE OF QUERCETIN CONTAINING EXTRACTS & FORMULATION

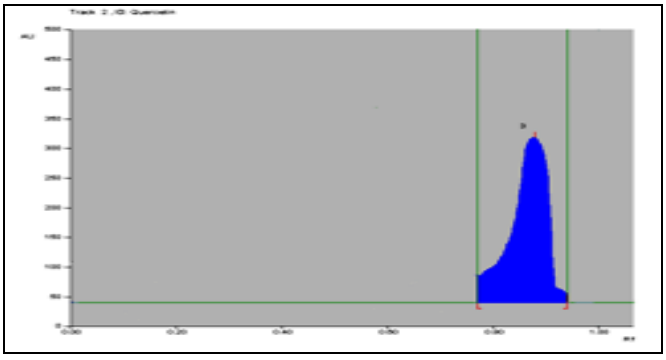
[Track 1: 10 µg/ml of Zymodyne Formulation; Track 2: 4 µg/ml of Standard Quercetin; Track 3: 10 µg/ml of *C. rotundus* extract; Track 4: 10 µg/ml of *B. monnieri* extract; Track 5: 10 µg/ml of *G. glabra* extract; Track 6: 10 µg/ml of *P. amarus* extract; Track 7: 10 µg/ml of *A. racemosus* extract; Track 8: 10 µg/ml of *T. terrestris* extract]

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). There was good correlation between peak area and the corresponding concentration of Quercetin as shown in Figure 2. 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.

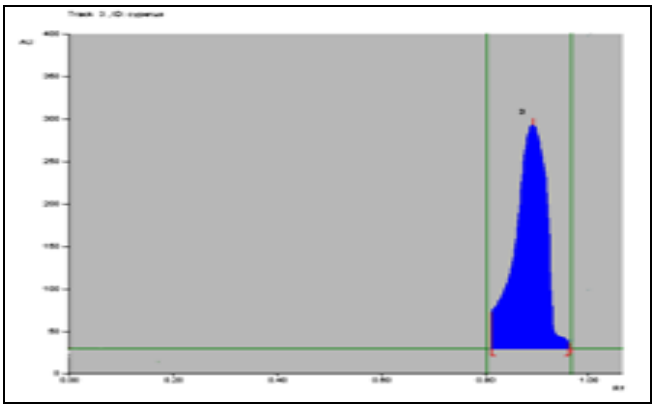
The detector response/calibration curve of Quercetin was found to be linear dependent on the concentration against area. The best fitting line equation was $y_=$ 516.7X+633. R^2 = 0.988, indicated good linearity between concentration and peak area. Quercetin content in the extracts of formulation, *Cyperus rotundus, Bacopa monnieri, Glycyrrhiza glabra, Phyllanthus amarus, Asparagus racemosus,* and *Tribulus terrestris* and by the proposed HPTLC method was 0.1100, 0.870, 0.783, 0.782, 0.855, 0.875, 0.957 mcg/ml respectively. The detection of the Quercetin band in the sample extract solution was confirmed by superimposing overlaying the UV absorption spectrum of the sample with that from the reference standard of Quercetin.



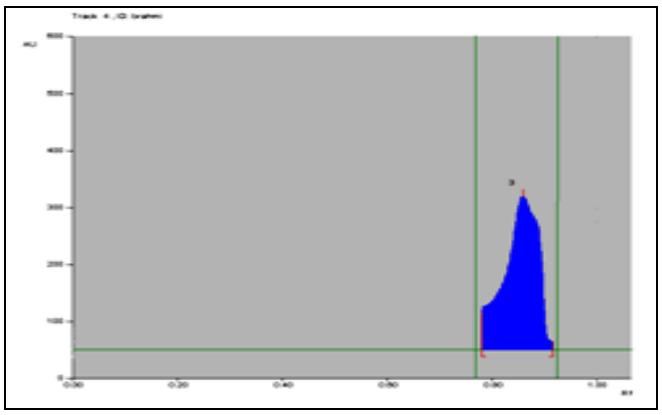
ZYMODYNE FORMULATION



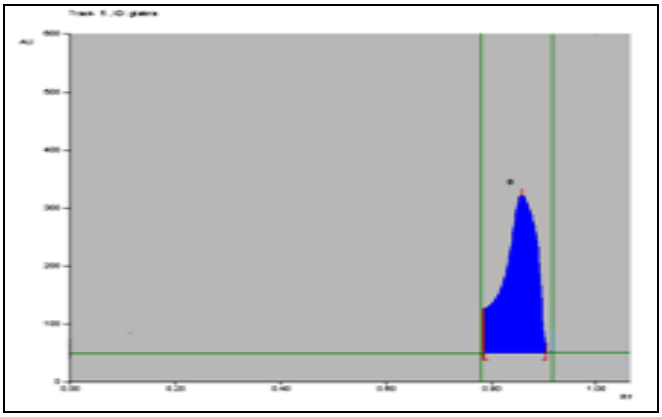
QUERCETIN MARKER



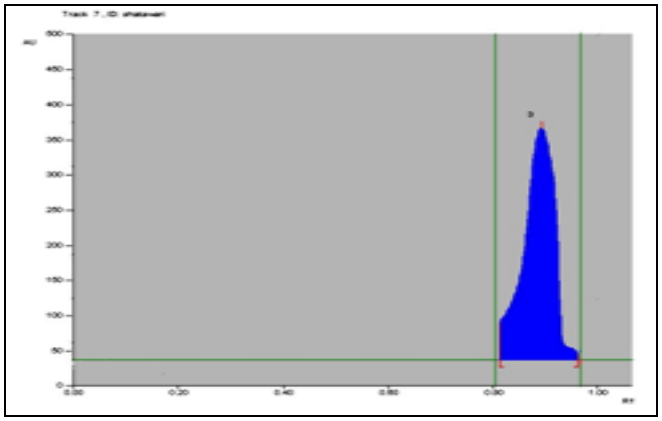
***C. ROTUNDUS* EXTRACT**



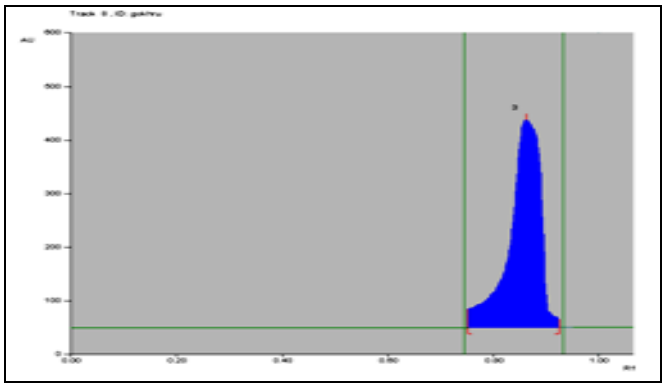
***B. MONNIERI* EXTRACT**



***P. AMARUS* EXTRACT**



***A. RACEMOSUS* EXTRACT**



***T. TERRESTRIS* EXTRACT**

FIG. 4: CHROMATOGRAMS OF QUERCETIN CONTAINING EXTRACTS AND ZYMODYNE SYRUP

TABLE 3: HPTLC FOR QUERCETIN IN PLANTS AND FORMULATION

Track	Max. Rf	Peak Area	Conc. (mcg/ml)
Zymodyne Syrup	0.88	21292.6	0.1100
Quercetin	0.86	16523	---
C. rotundus	0.85	16841.7	0.870
B. monnieri	0.86	15155.9	0.783
G. glabra	0.89	15134.7	0.782
P. amarus	0.87	16564.1	0.855
A. racemosus	0.84	16948.4	0.875
T. terrestris	0.88	18537.9	0.957

CONCLUSION: From the above data we can determine Quercetin in Zymodyne syrup and its Quercetin containing plant extracts. These data will be helpful for us to search the role of Quercetin to enhance appetite.

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