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Formulation, HPTLC Method Development and Validation of Gallic Acid in Health Drinks

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Abstract

The present research work supports formulation, development of health drinks containing *Withania somnifera, Ocimum sanctum, Glycyrrhiza glabra, Emblica officinalis, Asparagus racemosus, Tribulus terrestris, Terminalia arjuna, Tinospora cordifolia, Chlorophytum borivilianum, Piper nigrum, Curcuma longa, Myristica fragrans* as an active ingredients. The product was developed by treating concentrates of each crude drug with purified water. A new, simple, sensitive, selective, precise and robust high-performance thin-layer chromatographic (HPTLC) method for analysis of Gallic acid from health drink was developed and validated. Pre-coated silica aluminium plate 60F-254 (20 × 10 cm) with 200 µm thickness was used as stationary phase while toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8 v/v) system was developed as a mobile phase. Spectrum analysis showed the same Rf values and spectrum pattern of standard and sample. The developed method was quite good and most sensitive for the present products.

Keywords: Polyherbal Formulation, TLC profile, Method Development, Validation, Gallic Acid, Health Drinks.

Introduction

Phytopharmaceuticals are always mixtures of many constituents and are therefore vary variable and difficult to characterize. The active principles in Phytopharmaceuticals are not always known. To prove the constant composition of herbal preparations, appropriate analytical methods have to be applied and different concepts have to be used in order to establish relevant criteria for uniformity[1]. Health drinks contain *Ashwagandha, Tulsi, Mulethi, Awala, Shatavari, Gokhru, Arjuna, Giloy, Safed musali, Kalimirchi, Haldi, Jaiphal* have been reported as nervine tonic, immunomodulatory agents, antioxidants, tonics for heart and liver, blood purifier.

Withania somnifera[2] is a tonic, abortifacient, astringent, deobstruent, nervine, aphrodisiac and sedative. It has been used in diseases such as rheumatism, leprosy and arthritis. It is used to treat general debility, arthritis, depression, chronic fatigue, insomnia, anxiety, depressed immunity, infertility and memory loss. *Myristica fragrans*[3] is aromatic, carminative, digestive, anti-inflammatory, diuretic, lactagogue, aphrodisiac, hypnotic, hallucinogenic, antispasmodic and stimulant agent. *Piper nigrum*[4] stimulates appetite, encourages peristalsis, tones the colon muscles and is a general digestive tonic. Sometimes it is used in gonorrhoea. *Tinospora cardiofolia*[5] is antiperiodic, antipyretic, alterative, diuretic, and anti-inflammatory. It is a constituent of several compound preparations. It clears out brain toxin that hinders mental activity. *Curcuma longa*[6] is also used as an anti-inflammatory agent, and remedy for gastrointestinal discomfort associated with irritable bowel syndrome, and other digestive disorders. It is currently being investigated for possible benefits in Alzheimer's disease, cancer and liver disorders. *Terminalia arjuna*[7] is mainly used in heart disease, contusions, and fractures. *Chlorophytum borivilianum*[8] is a rare divine-graced herb to offer all the effects required for achievement of health par excellence or for attaining the ultimate positive health. It treats male sexual inadequacies like oligospermia, lack of libido, impotency, etc, general debility. *Asparagus racemosus*[9] has been used in Ayurveda for various conditions. Its main use has been as a galactogogue to increase milk secretion during lactation. It is useful in nervous disorders, dyspepsia, and tumours, scalding of urine, throat infections, tuberculosis, cough bronchitis and general debility. *Tribulus terrestris*[10] is used in the treatment of urinary disorders and impotence, kidney diseases and gravel, diseases of the genito-urinary system, calculus affections, gout etc. It is also useful for diseases of the heart, and many other conditions. It is being studied as a potential herbal remedy against AIDS. *Glycyrrhiza glabra*[11] is a popular remedy for coughs, some complications of tuberculosis, and chest complaints in general, such as bronchitis. It is also highly regarded as a soothing ingredient for sore throat and laryngitis. It is also used to strengthen and balance the female reproductive system. *Ocimum Sanctu*[12] is widely used as a cough medicine as well as used to expel worms. Basil is the essential ingredient used to ease headaches. It is used in malaria, bronchitis and gastric disorders. It also lowers blood sugar levels and its powder is used for mouth ulcers. *Emblica officinalis*[13] fruit is the richest known source of vitamin 'C' and used as a diuretic, appetizer, laxative, hair dye, and shampoo etc. It cures insomnia and is healthy for hair .It is also used as a Cardio protective, useful in haemorrhage, menorrahagia, leucorrhoea, and discharge of blood from uterus. Health drinks are the liquid preparation that contains vitamins, amino acids, minerals and other dietary supplements. Heath drinks are useful[14] for body maintenance, to prevent and treat disease.

The aim of this work was to develop an accurate, specific, repeatable and robust method for the determination of Gallic acid from health drink. The proposed method was validated in compliance with ICH guidelines[15].

Results and Discussion

Development of the optimum mobile phase

The TLC procedure was optimized with a view to quantify the Health Drink. Initially Toluene: Ethyl Acetate: Methanol: Formic Acid in varying ratios was tried. The mobile phase Toluene: Ethyl Acetate: Methanol: Formic Acid (3: 3: 0.2: 0.8 v/v) gave a sharp and well-defined peak at *R*f = 0.59 and good resolution for Gallic acid. This mobile phase consisting of well defined spots was obtained when the chamber was saturated with mobile phase for 30 min at room temperature.

Calibration curves

The developed HPTLC method for estimation of Gallic acid showed a good Correlation coefficient (r^2 = 0.9992± 0.0002) in concentration range of 80– 400 ng spot−1 with respect to the peak area. The mobile phase Toluene: Ethyl Acetate: Methanol: Formic Acid (3: 3: 0.2: 0.8 v/v) gave a sharp and well-defined peak at *R*f = 0.59 and good resolution for Gallic acid.

Method validation

The %R.S.D. for repeatability of sample application (600 ng spot−1) and measurement of peak areas were found to be 0.09% and 7894.5 respectively.

The measurement of the peak area at three different concentration levels showed low values of %R.S.D. (<1%) for inter- and intra-day variation, which suggested an excellent precision of the method.

The low values of S.D., %R.S.D. obtained after introducing small deliberate changes in the developed HPTLC method indicated the robustness of the method.

The limit of detection and limit of quantification with signal-to-noise ratio of 3:1 and 10:1 were found to be 2.17 and 7.60 ng, respectively, which indicates the adequate sensitivity of the method.

The proposed method when used for extraction and subsequent estimation of Gallic acid from the formulation afforded recovery of 99.19–100.33%. Low %R.S.D. value of 0.0551 between the peak area values proved the ruggedness of the method indicating that Gallic acid is stable during the extraction procedure as well as during analysis. The peak purity of Gallic acid was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot. Good correlation (*r* = 0.9992) was obtained between the standard and the sample overlain spectra of Gallic acid.

Detection of related impurities

The spots other than the principal spot for Gallic acid from the sample solution were not more intense than the principal spot from the standard solution. The sample solution showed three additional spots at *R*f = 0.2, 0.82 and 0.90 having peak areas of 2370.5, 3092.5 and 11247.5, which were much less as compared to the standard solution.

Analysis of the prepared formulation Health Drink

A spot at *R*f = 0.59 was observed in the chromatogram of the Gallic acid extracted from Health Drink. There was no interference from the excipients and the other active components present in the herbal formulation. The % recovery of the Gallic acid from the Health Drink formulation was found to be 99.70 % and was well within the limits.

Materials and Methods

Standard Gallic acid was purchased from Loba Chemie Pvt., Ltd. Mumbai, India. *Ashwagandha, Tulsi, Mulethi, Awala, Shatavari, Gokharu, Arjuna, Giloy, Safed musali, Kalimirchi, Haldi* and *Jaiphal,* was procured from the Satara Ayurvedic Arkshala, Satara. All samples were authenticated by the Botanistfrom Y. C. College of Science, Tal. Karad, Dist. Satara (M. S.). All chemicals and reagents used were of analytical grade and were purchased from Loba Chemie Pvt. Ltd. Mumbai.

Formulation and development of Health Drink

The powdered materials (500 gram each) were treated with universal solvent water in a container for seven days at a room temperature with occasional shaking. Aqueous extract of each crude drug was obtained separately by cold maceration method. The health drink was prepared by aqueous extraction method. The developed formula for health drink was as follows-

Drugs	**Quantity**
Ashwagandha concentrate	220 mg
Tulsi concentrate	110 mg
Mulethi concentrate	110 mg
Awala concentrate	220 mg
Shatavari concentrate	110 mg
Gokharu concentrate	110 mg
Arjuna concentrate	110 mg
Giloy concentrate	110 mg
Safed musali concentrate	110 mg
Kalimirchi concentrate	55 mg
Haldi concentrate	55 mg
Jaiphal concentrate	55 mg

This formula was developed for 25 ml of health drink.

As per the above developed formula, crude drugs concentrates were mixed together with purified water and homogeneous product was developed. Prepared heath drink was visually observed for its general appearance and colour. A slightly viscous yellow coloured health drinks were prepared. Aqueous extract of each crude drug was obtained separately by cold maceration method[16] (WHO Quality control). The health drink was prepared by aqueous extraction method. Each concentrate of different crude drugs was mixed together and was treated with purified water for 7 days with occasional shaking at room temperature and homogeneous product was developed.

TLC profile

TLC plates were developed by using mobile phase as toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8 v/v). Rf value was calculated by the ratio of the distance traveled by the spot to the distance traveled by the solvent.

For TLC, silica G was used as a coating material. The TLC plates were prepared by applying silica G on glass plate by pouring method that is, a measured amount of slurry of silica was poured on glass plate which is kept on a level surface. The plate was then tipped back and forth to spread the slurry uniformly over the surface. Dried the plate at room temperature for 30 min then activate the plate at 110°C for thirty minutes in an oven.

The sample of health drinks and standard Gallic acid were applied by using capillary tubes then again dry the plate at room temperature. The plate was then placed in a development chamber. The bottom of the chamber was covered up to nearly one mm by the solvent system after solvent has traveled one half to two thirds the length of the plate. Plates were removed and dried at room temperature. The positions of the spots were determined. The Rf value of the spots were determined.

HPTLC [17] Method development

Instrumentation and chromatographic conditions:

The samples were spotted in the form of bands of width 6 mm with a Camag µl syringe on precoated silica health drink aluminium plate 60F254 (20 cm×10 cm) with 200 µm thickness (E. Merck, Germany) using a Camag Linomat V (Switzerland) sample applicator.

Calibration curve of Gallic acid

A stock solution of standard gallic acid (40 µg/mL) was prepared by transferring 4 mg of gallic acid, accurately weighed, into a 100 mL volumetric flask, dissolving in 50 mL methanol. It was then sonicated for 10 minutes and the final volume of the solutions was made up to 100 mL with methanol to get stock solutions containing 40 µg/mL.

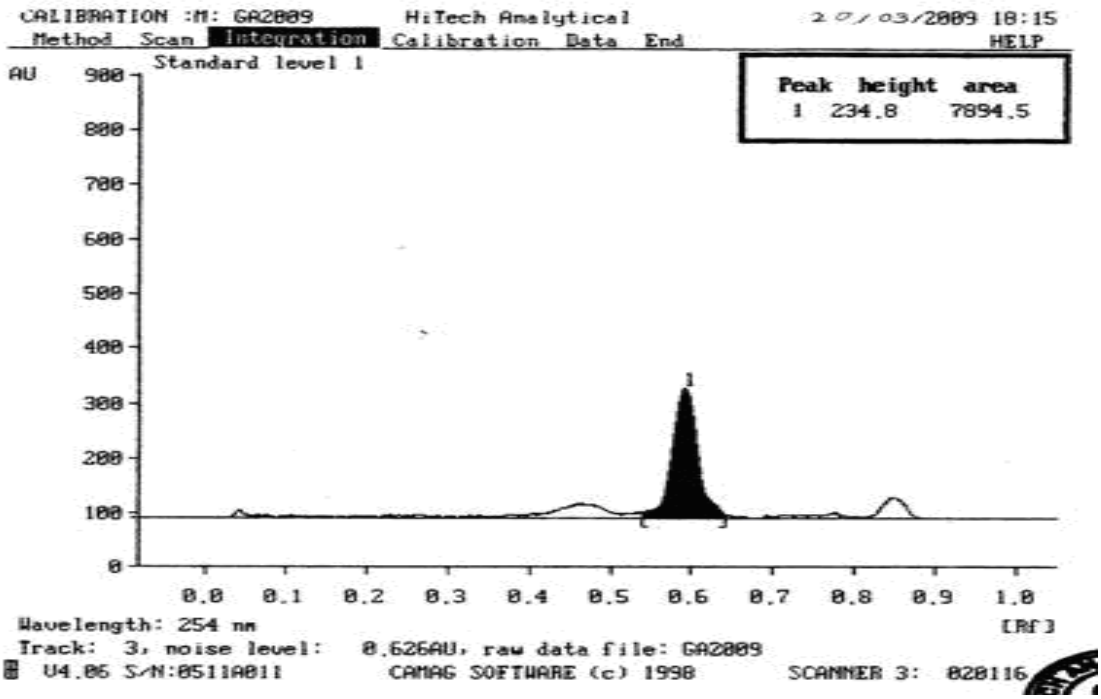

Figure 1:- Chromatogram of Standard Gallic Acid

The calibration curve from 80- 400 ng/ spot was prepared and checked for reproducibility prepared and checked for reproducibility, linearity and validating the proposed method. The data of peak areas plotted against the corresponding concentrations were treated by least-square regression analysis.

Method validation

Precision

Repeatability of the sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng spot−1 of Gallic acid) and was expressed in terms of percent relative standard deviation (%R.S.D.). The intra- and inter-day variation for the determination of Gallic acid was carried at three different concentration levels of 400, 600 and 800 ng spot−1.

Table 1: Intra and Inter day precision of HPTLC method (n = 6)

Amount (ng spot^{-1})	Intraday precision		Inter day Precision	
	S.D.	% RSD	S.D.	% RSD
400	1.50	0.023	1.51	0.062
600	1.55	0.058	1.68	0.065
300	1.48	0.061	2.31	0.035

Robustness of the method

By introducing small changes in the mobile phase composition, mobile phase volume, duration of mobile phase saturation and activation of prewashed TLC plates with methanol, the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 600 ng spot−1 and the %R.S.D. of peak areas was calculated.

Table 2: Robustness of the HPTLC Method (n =3,600 ng/spot)

Parameters	S.D.	% RSD
Mobile phase composition	1.49	0.425
Mobile phase Volume (18,20,22 ml)	1.59	0.315
Duration of Saturation (20,30,40 min)	1.32	0.284
Activation of prewashed TLC plates (2.5 and 7 min)	1.31	0.167

Limit of detection and limit of quantification

LOD is the amount of applied sample producing a peak area which is equal to the sum of mean blank area and three times standard deviation.

LOQ is the amount of applied sample producing a peak area which is equal to the sum of mean blank area and ten times its standard deviation.

Recovery studies

The pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard Gallic acid and the mixtures were reanalyzed by the proposed method. The experiment was conducted six times. This was done to check for the recovery of the Gallic acid at different levels in the formulations.

Table 3: Recovery Studies (n = 6)

Excess drug added to analyte (%)	Theoretical content (ng)	Amount Found (ng)	Recovery (%)	% RSD
0	400	397.98	99.49	0.234
50	600	598.91	99.81	0.163
100	800	802.71	100.33	0.985
150	1000	991.97	99.19	0.115

Ruggedness

A solution of concentration 1000 ng spot−1 was prepared and analyzed on day 0 and after 6, 12, 24, 48 and 72 h. Data were treated for %R.S.D. to assess ruggedness of the method.

Specificity

The specificity of the method was ascertained by analyzing the Standard drug and Health Drink. The spot for Gallic acid in the sample was confirmed by comparing the *R*f values of the spot with that of the standard. The peak purity of the Gallic acid was assessed by comparing the spectra at three different levels, viz. peak start (S), peak apex (M) and peak end (E) positions of the spot.

Detection of related impurities

The related unknown impurities were determined by spotting higher concentrations of the Gallic acid. Gallic acid solution was prepared at a concentration of 2000 µgmL−1 in methanol, and this solution was termed as sample solution. One milliliter of the sample solution was diluted to 40mL with methanol and this solution was termed as standard solution (50µgmL−1). 2 µl of both the standard (100 ng spot−1) and the sample solution (4000 ng spot−1) were applied on HPTLC plate and the chromatograms were run.

Analysis of Gallic acid in Health Drink

An accurately weighed quantity of Health Drink equivalent to about 100 ng of Gallic acid, i.e., 8.5 g of Health Drink was extracted with 25mL methanol by sonication for 30 min. This extract was centrifuged at 12,000 rpm for 15 min at 4^0C. The supernatant was filtered and the filtrate was dried to constant weight at room temperature. The residue was redissolved in 5 mL of methanol. 6 µl of the filtered solution was applied on the TLC plate followed by development and scanned. The analysis was repeated in triplicate.

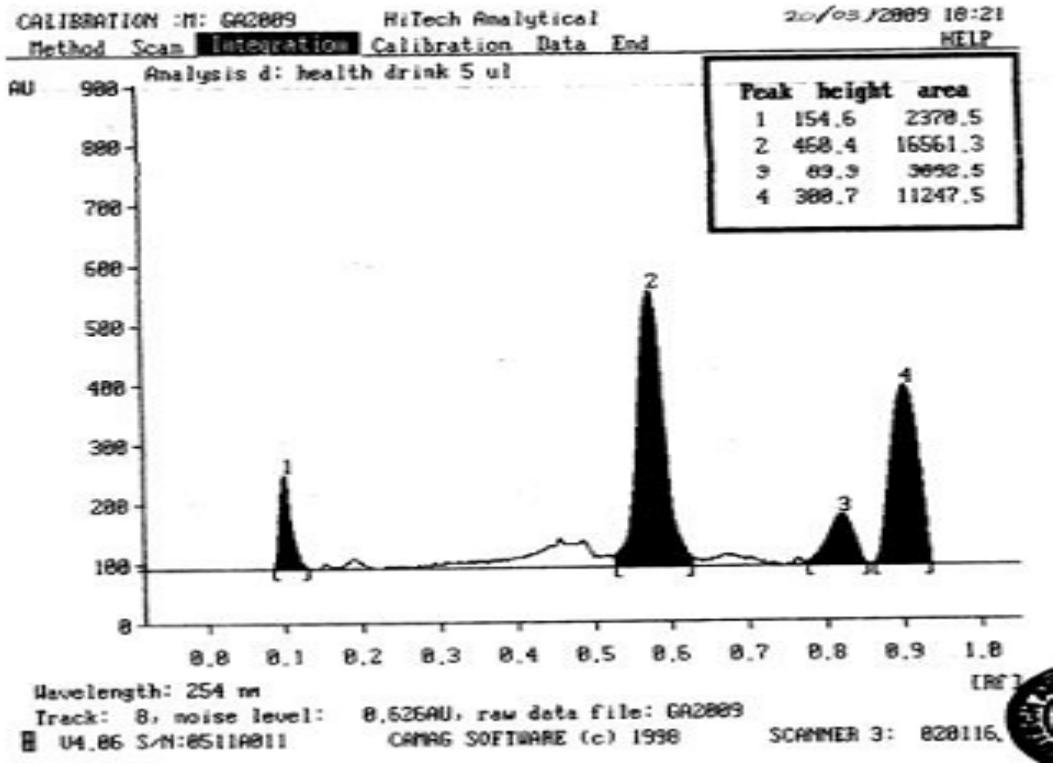

Figure 2:- Chromatogram of Sample-Health Drink

Conclusion

The developed HPTLC technique is a precise, specific, accurate and robust for the determination of Gallic acid. Statistical analysis proves that the method is reproducible and selective for the analysis of Gallic acid. Since the proposed mobile phase effectively resolves Gallic acid, the method can be used for qualitative as well as quantitative analysis of Gallic acid in Health Drink and to detect the related impurities to establish alkaloidal components content in products grown in different climatic conditions. Further the proposed method can be extended to study the degradation of Gallic acid under different stress conditions, as per the recommendations of ICH guidelines.

References

[1] Farmsorth, NR Aketala O, Binhealth drink AS and Soijarto DD Guo, World Health Organization., **1985**, 63, 965.
[2] Monograph: *Withania Somnifera, Alternat. Med. Rev*., **2004**, 9 (2), 211.
[3] Varro E. Tyler, Lymm R. Brady- James E., Roberts "Texbook of Pharmacognosy", 8th eds., Verghese Company pp. 137, **1981.**
[4] Trease and Evan`s: "Phamacognosy" 14th eds, Sunder`s Publication, pp. 500, **1997**.
[5] Singh SS, Pandey SC and Srivastava S., *Ind J Pharmacol*, **2003,** 35, 83.
[6] [Chattopadhyay I, et al., *Curr Sci*., **2004,** 87 (1), 44.
[7] Kokate CK, Purohit AP and Gokhale SB "Texbook of Pharmacognosy", 40th eds., Nirali Prakashan Pune, pp. 93-99, **2008**.
[8] Kokate CK, Purohit AP and Gokhale SB "Texbook of Pharmacognosy", 31st eds Nirali Prakashan Pune, pp. 518, 559, 593, **2005**.
[9] Velavan S, Nagulendran KR, Mahesh R and Hazeena Begum V., *Pharmacognosy Magazine*, **2007,** 3 (9), 26.
[10] Trease and Evan`s: Phamacognosy 14th eds., Sunder`s Publication, pp. 41, 504, **1997**.
[11] http://openmed.nic.in.
[12] Naram KU. Health foods. *Processed Fd. Ind.* **2000,** 3 (1): 118- 120.
[13] Q2A, ICH, Q2A (R1). Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, **2005**.
[14] WHO Quality control of herbal drug. Determination of Extractive value. Geneva. 32 **1998**.
[15] Wagnar H and Bladt S., "Plant drug Analysis". A Thin Layer Chromatography Atlas, 2nd eds., Sringer (INDIA) Pvt. Ltd. New Delhi, 216, 225, 260, 332, 594, **2004**.