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Standardization of 'Lavagandi Vati:' A HPLC Approach

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Abstract:
Herbal drug preparations are supposed to be produced with high"Quality**".** Quality affect all the properties of the final product which makes it optimal suitable for its intended or in formulations. Reproducable quality is a goal, which is, among others, achieved by the process of standardization. Lavangadi vati is official in Ayurvedic formulary of India (2003b) and it is the most common formulas used for Cough, Dyspnoea and Asthma (Kasa, Svasa) in Ayurvedic medicine. It comprised of the fruits of six medicinal important plants, *Eugenia caryuphyllus, Piper nigrum, Terminalia belerica, Acacia catechu and Acacia Arabica*.The present study is an attempt to The fingerprint method for quality control of Lavangadi Vati developed by simple high-performance liquid chromatography (HPLC) determination using gallic acid as a standard, which is as an important and major content in formulation. RP-HPLC methods for determination of gallic acid from the fruits of ***Terminalia belerica, Acacia catechu and Acacia Arabica*** and LV-I, LV -II and LV -III, marketed formulations (ML-I) have been developed. A C-18 LUNA (5 micron 25 cm×4.6 mm) column from Phenomenex a binary gradient high- pressure liquid chromatograph (Shimadzu HPLC class VP series) with two LC–10 AT VP pumps, variable wavelength programmable UV/Visible SPD 10 AVP were used. The standard curve for gallic acid was linear over the investigated range (10–100 μg/mL) with a percent relative standard deviation (% R.S.D.) of less than 2% based on three successive readings (Table 3.20, figure 3.3). A correlation coefficient (R^2) is suggested that the developed HPLC method had an excellent linearity over the concentration range of 10–100 μg/m of gallic acid. Under the developed HPLC conditions, the limit of quantitation was determined to be 1.578 and limit of detection was found to be 0.478 after three successive injections of the sample.
The concentration of gallic acid present in raw material is found to be 7.612± 0.289% w/w in *Terminalia belerica*, 0.012±0.669% w/w in ***Acacia catechu and*** 5.42±0.493% w/w ***Acacia Arabica.*** Gallic acid content in three identical laboratory batch of **Lavangadi Vati** LV-I, LV-II and LV-III, was found to be 1.561±0.338%, 1.564±0.693% and 1.562±0.438% w/w respectively. Obtained results were compared with marketed formulation.
Key Words: Gallic acid, Lavangadi Vati, HPLC, Ayurvedic Formulation, Fingerprinting.

1. Introduction:

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and

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agreeing upon technical standards, standardization is a tool in the quality control process [1]. According to WHO, standardization and quality control of herbals include identification of crude drug based on the use of major chemical constituents as markers. Standardization of herbal product drugs, single chemical entities, "marker compounds," may be used as potency standards in high performance liquid chromatography (HPLC) analysis. Using well-characterized marker compounds, conventional pharmaceutical manufacturing criteria for assay and content uniformity may be applied. These marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes, and obtain stability profiles [2, 3,]. HPLC analysis for marker compounds may provide additional information in the form of "chromatographic fingerprints [5]. The present paper is an effort to develop the quality control parameter of Lavangadi Vati by HPLC determination using gallic acid as an internal standard. Lavangadi vati is official in Ayurvedic formulary of India (2003b) and it is the most common formulas used for Cough, Dyspnoea and Asthma (Kasa, Svasa) in Ayurvedic medicine. It comprised of the fruits of six medicinal important plants, *Eugenia caryuphyllus*, *Piper nigrum*, *Terminalia belerica*, *Acacia catechu and Acacia Arabica*. The present study is an attempt to develop the chromatographic fingerprint method for Lavangadi Vati by High-performance liquid chromatographic method using gallic acid as a standard, which is as an important and major content in formulation. The RP-HPLC analysis which is a simple, precise, and accurate method and can be considered as one of the quality control method for routine analysis of Lavangadi Vati

2. Experimental

2.1 Materials

Dried herbs for Lavangadi Vati were procured from local market Ujjain (M.P), India and identified on the basis of morphological and microscopical characters and compared with standard Pharmacopoeial Monograph [7,8]. All the solvents were used of HPLC Grade. Standard gallic acid (98%) was procured from Lancaster England.

2.2 Apparatus

A C-18 LUNA (5 micron 25 cm×4.6 mm) column from Phenomenex a binary gradient high-pressure liquid chromatograph (Shimadzu HPLC class VP series) with two LC–10 AT VP pumps, variable wavelength programmable UV/Visible SPD 10 AVP were used.

2.3 Chromatographic conditions The mobile phase consisted acetonitrile: ethyl acetate (70:30). The flow rate was 1.2 ml/min. The wavelength of detection was 264nm.The column temperature was ambient and the injection volume was 10 µl.

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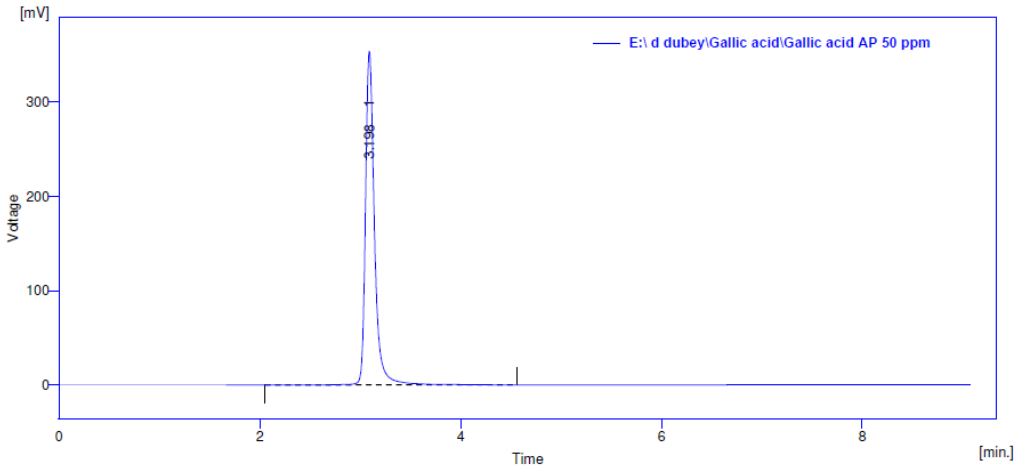

Figure 1 HPLC Chromatogram of gallic acid

2.4 Preparation of standard solution of gallic acid

The stock solution of gallic acid was prepared by dissolving 10.0 mg in 100.0 mL methanol, creating a 100 µg/mL solution. This solution was diluted with the solvent as needed to prepare different standard solutions (10, 20, 30, 40, 50,--------90, 100µg/mL).

2.5 Preparation of calibration curve of gallic acid

Standard solutions (2, 4, 6, 8, 10,--------18, 20µg/mL), each in three replicates, were injected into the system. The method of linear regression was used for data evaluation. Peak area ratios of standard compounds were plotted against theoretical concentrations of standards. Linearity was expressed as a correlation coefficient. (Table 1, figure 2).

Table 1 Range of linearity

Concentration (µg/mL)	Gallic acid Peak area (mean and S.D.)
10	304.25±0.986
20	671.78±0.681
30	896.14±0.634
40	1263.43±0.432
50	1412.12±0.526
60	1796.23±0.236
70	2256.27±0.864
80	2521.43±0.651
90	2770.12±0.196
100	3442.12±0.298

Mean ± S.D. (n = 3).

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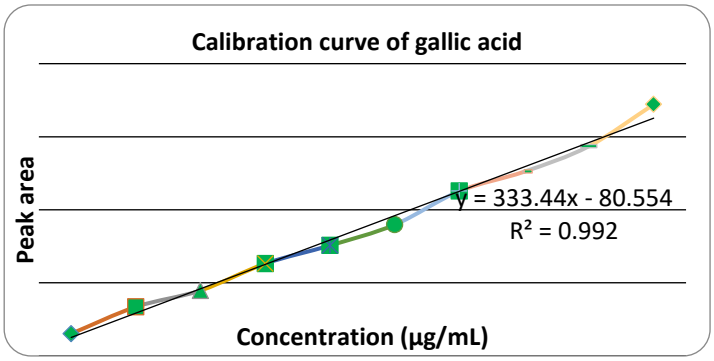

Figure 2 Calibration curve of gallic acid

2.6 Preparation of extract of Lavangadi Vati

The Lavangadi Vati (1gm) was refluxed with 60 ml methanol for 90 min. and filtered. The marc was re reflux with 40 ml of methanol for another 1hours. Filter and the filtrate were combined. The methanol extract was concentrated under vacuum till the semisolid mass is obtained. The residue was dissolved in 75 ml methanol and filtered through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected in 100 ml volumetric flask and volume was made with methanol.

The same procedure was performed for each batch of Lavangadi Vati, one marketed formulation M-I and M-II and separately powdered *Terminalia belerica, Acacia catechu and Acacia Arabica* and solution (100 ml) of their extract were prepared.

2.7 Method Validation

The method was validated for linearity, precision, accuracy, limit of detection, limit of quantification. (Table 2)

Limit of detection and limit of quantitation

The LODs and LOQs under the present HPLC-UV method were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. Standard solution containing gallic acid as a reference compounds was diluted to a series of appropriate concentrations with methanol and an aliquot of the diluted solution was injected into HPLC for analysis.

***Precision and accuracy*:** The method was validated for precision and accuracy, by performing the recovery studies at three levels by adding known amount of gallic acid extract of Lavangadi Vati, of which the gallic acid content have been estimated previously. The data were obtained and recovery was calculated (Table 3).

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Table 2: Validation Parameter of gallic acid

S.No.	Parameter		Gallic acid
1	Retention time		3.198min
2	Beer's Law limit		10-100μg/ml
3	Regression equation (y= bx+a)		y= 333.4x – 80.55
4	Intercept (a)		80.55
5	Slope (b)		333.4
6	Correlation coefficients (r^2)		r^2 = 0.992
7	Limit of quantification(LOQ)		1.578μg/ml
8	Limit of detection(LOD)		0.519μg/ml
9	Recovery Studies	Precision	0.293
		Standard Error	0.220
		Accuracy (%)	99.63

Table 3 Recovery study

S.no.	Amount of tannic acid (μg/ml)			RSD%	SE	Recovery%
	Sample	Added	Estimated			
1	100	50	149.43±0.673	0.450	0.274	99.62
2	100	100	199.24±0.497	0.249	0.203	99.62
3	100	150	249.14 ±0.449	0.180	0.183	99.66
Mean				**0.293**	**0.220**	**99.63**

Mean ± SD of six determinations, **RSD** =Relative Standard Deviation,**SE =** Standard Error

2.7 Estimation of gallic acid

The appropriate aliquots from extract of each batch of Lavangadi Vati, its one marketed formulations and separately ***Terminalia belerica, Acacia catechu and Acacia Arabica*** were withdrawn in 10 ml volumetric flask separately. The corresponding concentration of gallic acid against respective peak areas value was determined using the gallic acid calibration curve (Table 4).

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Table 4: Gallic acid content (% w/w) in Lavangadi Vati (Mean ± SD of 6 determinations)

S. No.	Formulations and crude drugs		gallic acid content % (w/w)	Standard error
1	*Terminalia belerica*		7.612± 0.289	0.117
2	***Acacia catechu***		0.012±0.669	0.273
3	***Acacia Arabica***		5.42±0.493	0.201
6	**Lavangadi Vati**	LV-I	1.561±0.338	0.138
7		LV–II	1.564±0.693	0.283
8		LV–III	1.562±0.438	0.179
9		ML-I	1.239±0.473	0.193

3. Results And Discussion

The fingerprint method for quality control of **Lavangadi Vati** is developed by simple high-performance liquid chromatography (HPLC) determination using gallic acid as a standard, which is important and major content in formulation. RP- HPLC methods for determination of gallic acid from the *Emblica officinalis*, *Terminalia belerica*, *Terminalia chebula*, *Cyperus rotundus* and *Embelica ribes* and **Lavangadi Vati** have been developed. The acetonitrile: ethyl acetate (70:30) was selected to obtain a rapid and simple assay method for gallic acid with a reasonable run time, suitable retention time and the sharpness of the peak. The chromatogram of gallic acid under experimental condition showed a single peak of the drug at 3.198 min (Figure 1).

The standard curve for gallic acid was linear over the investigated range (10–100 μg/mL) with a percent relative standard deviation (% R.S.D.) of less than 2% based on three successive readings (Table1, figure 1). A correlation coefficient (R^2) is suggested that the developed HPLC method had an excellent linearity over the concentration range of 10–100 μg/m of gallic acid.Under the developed HPLC conditions, the limit of quantitation was determined to be 1.578 and limit of detection was found to be 0.478 after three successive injections of the sample. (Table 2).

The concentration of gallic acid present in raw material is found to be 7.612± 0.289% w/w in *Terminalia belerica*, 0.012±0.669% w/w in ***Acacia catechu and*** 5.42±0.493% w/w ***Acacia Arabica.*** Gallic acid content in three identical laboratory batch of **Lavangadi Vati** LV-I, LV-II and LV-III, was found to be 1.561±0.338%, 1.564±0.693% and 1.562±0.438% w/w respectively. Marketed formulation of Bhuvnesvara vati ML-I showed gallic acid concentration to be 1.239±0.473% (Table 3.).

In order to obtain precision and accuracy the recovery study was performed at three levels by adding known amount of gallic with pre-analysed sample of gallic in **Lavangadi Vati**. The experiment was repeated three times and result are cited in Table 4, which prove reproducibility of the result.

The HPLC method developed for the estimation of gallic acid is a simple, rapid and precise for the routine estimation of **Lavangadi Vati**. The method was validated by statistical analysis and recovery studies. As **Lavangadi Vati** is a good source of gallic acid, these findings can be used

as routine chromatographic fingerprinting method for the standardization of the raw materials of the **Lavangadi Vati** as well as finished formulation.

4. Acknowledgement:
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