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Original Article

Simultaneous Estimation of Gallic Acid, Vasicine, Pterostilbene and Piperine Using RP-HPLC Method from *Ayurvedic* Proprietary Formulations

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ABSTRACT

Introduction: A simple, accurate, precise, sensitive, and validated RP-HPLC method was developed for simultaneous estimation of Gallic Acid (GA), Vasicine (VS), Pterostilbene (PTS) and Piperine (PP) from *Ayurvedic* Proprietary Formulations developed in two different dosage forms viz. Amarantha Gynorite syrup and Amarantha Gynorite capsule. **Materials and Methods:** The optimized chromatographic condition was used for separation on Agilent Zorbax SB-C_{18} (5 µm 4.6 x 250 mm), Rheodyne injector with volume of 20 µL and mobile phase consisting of Solvent A (70 percent methanol) and solvent B [10 nm Hexane sulfonic acid, Acetonitrile and Glacial Acetic acid (70:30:1)] in composition of 95: 05 v/v with isocratic elution for 40 min at flow rate of 0.5 mL/min. Quantification was carried out using a photodiode array detector at 280 nm. The method was validated in accordance with International Conference on Harmonization (ICH) guidelines. **Results and Conclusion:** No chromatographic interference from syrup and capsule excipients was analyzed. Hence, the present analytical method is applicable for simultaneous determination of GA, VS, PTS and PP in *Ayurvedic* proprietary formulations.

Keywords: Gallic Acid (GA), RP-HPLC, Piperine (PP), Pterostilbene (PTS), Vasicine (VS).

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INTRODUCTION

It has been estimated that not less than 80% of people in the world are relying on herbal medicines. Since last few decades, use of herbal medicines has been increasing for accomplishment of different therapeutic needs.[1-3] This influences research and development (R&D) sector of pharmaceutical industries to achieve desired scientific quality standards in herbal medicinal products. Medicinal activity of herbs is primarily depend upon presence of secondary metabolites or phytochemicals. Identification, isolation, quantification, purification, and structural characterization of these phytochemicals with various assessment techniques help to improve the quality of finished products.[1-3]

Amarantha Gynorite syrup and Amarantha Gynorite capsule are *Ayurvedic* proprietary formulations manufactured by Ari Healthcare Private Limited for the management of menstrual problems such as dysmenorrhea, dysfunctional uterine bleeding, hypomenorrhea, oligomenorrhea, amenorrhea, PCOS, menorrhagia, metrorrhagia and menometrorrhagia.[4-6] Both formulations contain *Saraca indica* (*Ashoka* bark) extract, *Adhatoda vasica* (*Vasa* leaf) extract, *Pterocarpus marsupium* (*Bijaka* stem) extract and *Trikatu* extract as key ingredients in combination with other ingredients. Gallic Acid (GA), Vasicine (VS), Pterostilbene (PTS) and Piperine (PP) are major marker compounds considerably responsible for pharmacological actions of both the formulations.[4-12]

Gallic acid or 3,4,5-trihydroxybenzoic acid is one of the most abundant phenolic acids of many plants responsible for anti-inflammatory, free radical scavenging and antioxidant activities.[7] Vasicine (peganine) is a quinazoline alkaloid. It is one of the major chemical compounds found in *Adhatoda vasica*. VS chemically characterized as 1,2,3,9-Tetrahydropyrrolo[2,1-b]quinazolin-3-ol. VS is reported to have antioxidant, anti-inflammatory and immunomodulator activities.[8] Pterostilbene (trans-3,5-dimethoxy-40 -hydroxystilbene) is a natural polyphenol and a dimethyl ether analog of resveratrol. Various research studies have reported that PTS is useful in the management of gynecological disorders,

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hematological diseases and vascular dysfunction.[9] Research studies showed that pterostilbene treatment significantly increases uterus and ovarian index, FSH, LH and regulates secretion of gonadotropin hormones.[10] Piperine ($C_{17}H_{19}NO_3$) is an alkaloid found in *Piper longum* and *Piper nigrum*. PP is potent bioavailability enhancer. It exerts antioxidant, antiplatelet, anti-inflammatory, antihypertensive, hepatoprotective, antithyroid and antitumor activities.[11,12]

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the prime options of phytochemical analysis with advancement of isocratic and gradient elution.[13-15] It is a sophisticated method available for estimation of GA, VS, PTS and PP individually or in combination with other molecules.[13-21] As estimation of GA, VS, PTS and PP individually is time and cost consuming by HPLC; this study was aimed towards the development of cost efficient method with less time requirement for simultaneous separation and determination of GA, VS, PTS and PP. In order to standardize Amarantha Gynorite syrup and Amarantha Gynorite capsule an attempt was made to develop simultaneous determination of four different marker compounds by using RP-HPLC method.

MATERIALS AND METHODS

Instrumentation

The chromatography was performed on water (Waters e2695 Alliance system with a 2996 PDA) HPLC instrument equipped with Photo diode Array Detector with wavelength range of 210 nm to 400 nm and empowers software. Agilent Zorbax SB-C_{18} (4.6 x 250 mm, 5 µ particle size) was used as stationary phase. Rheodyne manual injector with 100 µL capacity loop was used. Sartorius with weighing capacity of 0.1 mg - 220 g was used as analytical balance, pH meter of Labman and bath sonicator of LABTEK was used in the study.

Reagents and Materials

HPLC-grade solvents such as methanol, acetonitrile, glacial acetic acid, water and hexane-1-sulfonic acid sodium salt were purchased from Merck Life Science Private Limited, Mumbai, India. The reference standards were purchased from Natural Remedies Limited, Bangalore, India. Details of standards are mentioned in Table 1.

Amarantha Gynorite syrup and Amarantha Gynorite capsule were developed and supplied by Ari Healthcare Private Limited, Pune. Both dosage forms contain *Saraca indica* (*Ashoka* bark) extract, *Aloe vera* (*Kumari* leaf) extract, *Pterocarpus marsupium* (*Bijaka* stem) extract, *Symplocos racemosa* (*Lodhra* bark) extract, *Caesalpinie bonducella* (*Latakaranja* seed) extract, *Adhatoda vasica* (*Vasa* leaf) extract, *Carum carvi* (*Krishna-Jiraka* fruit) extract, *Apium graveolens* (*Ajamoda* fruits) extract, *Trikatu* extract as classical *Ayurvedic* formulation, *Pushyanuga Choorna* and *Tankana* and *Kasisa Bhasma*.

Preparation of Solutions

Preparation of Standard-stock Solutions

Four different standards were prepared namely Gallic Acid (GA), Vasicine (VS), Pterostilbene (PTS) and Piperine (PP). The standard stock solutions were prepared by dissolving 10 mg of each standard in 10 mL of methanol to obtain concentration 1000 µg/mL (1000 ppm).

Preparation of Working-standard Solution

From the standard stock solution 1 mL of each standards solution was pipette out and diluted with methanol up to 10 mL in volumetric flask to obtain concentration 100 µg/mL (100 ppm) as working standard solution.

Preparation of Calibration Curve

A calibration curve was established with seven different concentrations which were prepared by diluting working standard solution at concentrations ranging from 6 to 18 µg/mL. The dilutions are given in Table 2.

Preparation of Solvents

Solvent A was prepared using 70% methanol. The ratio of distill water to methanol was 30:70. Solvent B was prepared by solubilizing 4.1248 gm of hexane-1-sulfonic acid sodium salt in mill-Q water (1000 mL), and pH was adjusted with glacial acetic acid to 3.5. A mixture of hexane-1-sulfonic acid sodium salt and acetonitrile (70:30%v/v) were mixed, filtered and degassed.

Mobile Phase Preparation

Mobile phase was prepared using solvent A and B in ratio of 95:5% v/v.

Method Development

Depending on solubility, stability and suitability of standard solution, various compositions of mobile phase were performed to obtain sharp peak with good resolution. Individual standards and sample solutions were allowed to run in different mobile phase compositions to obtain better results. The optimized method was preferred because it gave sharper peak and good resolution at absorbance of 280 nm.

Chromatographic Conditions

The details on optimized parameters of chromatographic conditions for estimation of GA, VS, PTS and PP are mentioned in Table 3.

Preparation of sample and test solution

Accurately 100 mg capsule powder and 5000 mg of syrup solution were weighed in 50 mL of volumetric flask. About 40 mL of methanol was added and solutions were sonicated on ultrasonic water bath for 30 min at room temperature. Then solutions were

Table 1: List of standards.

Sl. No.	Name of Standard	Batch Number	Potency (%)
1	Gallic Acid (GA)	T12K021	99.0
2	Vasicine (VS)	T22E155	98.2
3	Pterostilbene (PTS)	H220659	95.0
4	Piperine (PP)	T13D040	98.0

Table 2: Dilutions for linearity.

Sl. No.	Concentration (µg/mL)	Dilution	
1	6	1.5	25
2	8	2.0	25
3	10	2.5	25
4	12	3.0	25
5	14	3.5	25
6	16	4.0	25
7	18	4.5	25

Table 3: Optimized condition for standard solution.

Sl. No.	Parameter	Optimized condition
1	Mobile Phase	(95:05 v/v)
2	Column	Agilent Zorbax SB-C18 (5 µm 4.6 x 250 mm)
3	Flow rate	0.5 mL/min
4	Absorbance	280 nm
5	Injection Volume	20 µL
6	Column Temperature	25°C
7	Sample Temperature	25°C

allowed to cool at room temperature. Further, volume was made up to the mark with methanol. The resulting solutions were filtered with Whatman's filter paper number-41 and through 0.45 µ syringe filter. The resulting solutions were used as test solutions.

Assay % of Standard-solution from Formulations

Both the formulations (Amarantha Gynorite syrup and Amarantha Gynorite capsule) were analyzed to determine the contents of GA, VS, PTS and PP as per method described under chromatographic conditions using RP-HPLC. Every single analysis was repeated three times and results were expressed in percent assay.

Analytical Method Validation

The developed RP-HPLC method was validated as per recommendations given in International Council on Harmonization (ICH), Q2 (R^1) for validation of analytical procedure, text and methodology.[13-21] The details on parameters and procedure for analytical method validation are as follows.

Specificity

As per ICH, specificity was carried out to make sure the identity of an analyte. Specificity of method was determined by comparing retention time and chromatogram of standards such as GA, VS, PTS and PP with diluent, placebo and sample solutions (Amarantha Gynorite syrup and Amarantha Gynorite capsule).

Linearity

As per ICH guidelines, determination of linearity was performed with minimum 7 concentrations. Linearity was performed by plotting peak area against concentration of standards and finding regression coefficient (R^2).

Precision

Precision was performed in terms of system precision, method precision and intermediate precision. In system precision, the sample was analysed for six times using above mentioned procedure. Assay for each analyte was expressed in terms of % RSD. In method precision, sample was analysed for six times using above mentioned procedure. Assay for each analyte was expressed in terms of % RSD. Intermediate precision was performed on different systems, i.e., Waters e2695 Alliance system with a 2996 PDA and 2489 Ultraviolet (UV) detector by different analysts by analysing six different samples of extracts and was expressed in terms of % RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined based on the standard deviation of response and slope of calibration graph. The quantitation was done with the help of equations. LOD = 3.3 σ/ s; LOQ = 10 σ/ s. Where, σ standard deviation of response estimated based on the calibration curve and s is slope of calibration curve.

Accuracy

Accuracy should be reported as percentage recovery by assay of known added amount of analyte in the sample. Accuracy should be assessed using a minimum of 9 determinations over 3 concentration levels covering specified range. In present work, percentage of recovery was calculated by performing recovery studies in triplicates at three different concentration levels viz. 80%, 100%, 120% by adding known number of standard solutions of GA, VS, PTS and PP. These analysed samples and obtained results were compared with expected results.

Robustness

Robustness of an analytical procedure is a measurement of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability using normal usage. For assessment of the robustness of developed analytical method following parameters were deliberately changed viz. flow rate, wavelength and column temperature.

RESULTS

The composition of mobile phase in RP-HPLC method was optimized by testing different solvent compositions of varying polarity, column chemistry, column temperature and pH of mobile phase. Significant efforts were taken to obtain maximum results by using present method, which produced highly symmetrical peaks and showed prominent resolution between each standard and formulations. The scanning wavelength of 280 nm was selected to provide comparable results. At this wavelength all analytes showed optimum response. The details are mentioned in Figures 1, 2 and 3.

Specificity Assessment

Specificity of RP-HPLC method was demonstrated by separation of analyte from other potential components such as placebo, diluent and other active constituent of formulation. A volume of 20 µL of potential components was injected and chromatogram was recorded. Peaks of these components were not found at retention time of 4.2 min, 6.8 min, 21.1 min and 23.8 min of GA, VS, PTS and PP; respectively. Hence, present method was considered as specific on specificity parameters. The details are mentioned in Figures 4 and 5.

Linearity Assessment

The calibrations graph was linear with concentration range of 6-18 µg/mL. The linear regression data for calibration curves, Limit of Detections (LOD) and Limit of Quantifications (LOQ) were presented in Table 4 and Figure 6.

Precision Assessment

A volume of 20 µL of standard solution from 100 µg/mL of stock solution was injected under optimized chromatographic condition to evaluate system suitability, method precision and intermediate precision. An intermediate precision was performed on two different systems. The percentage of Relative Standard Deviation (RSD), USP tailing and plate count were found within acceptance limit. The details are mentioned in Tables 5, 6 and 7.

Repeatability Assessment

The repeatability of proposed method was ascertained by injecting five replicates of 100 µg/mL concentration and calculating peak area by proposed RP-HPLC method. From this peak area % RSD was calculated. The details are mentioned in Table 8.

Accuracy Assessment

The accuracy study was carried out by spiking known number of standards into placebo solution at 80%, 100% and 120% of working concentration, respectively. The overall recovery percent were calculated. The details are mentioned in Table 9.

Robustness Assessment

The robustness of RP-HPLC method was evaluated by analyzing the system suitability parameters by varying flow Rate (±2%), detection wavelength (±2%) and column temperature flow rate (± 2%) and wavelength (±5%). By alteration of these parameters, no change was observed in % RSD of peak area. The change in the retention time was found to be significant. The details are given in Table 10.

DISCUSSION

Market is flooded with *Ayurvedic* proprietary medicines indicated for menstrual irregularities/PCOS. These Ayurvedic medicines are available in various dosage forms such as tablet, capsule, granules, syrup, and many others. Among these dosage forms, syrup-based formulations are being preferred by consumers. Most of these *Ayurvedic* syrup formulations are prepared using crude herbs. During manufacturing process, only water-soluble components of crude herbs are available, whereas those components which are not soluble in water will not be available in the final formulation. This may impact efficacy of the product.

In order to make a comprehensive formulation, Amarantha Gynorite Syrup and Amarantha Gynorite Capsule are prepared using standardized herbal extracts. Standardization of polyherbal *Ayurvedic* medicines has become very essential as it makes sure

Table 4: Linear regression data for calibrations curve (*n* = 7).

Sl. No.	Parameter	Results			
		Gallic Acid	Vasicine	Pterostilbene	Piperine
1	Linearity Range (µg/mL)	6 -18	6 -18	6 -18	6 -18
2	Correlation coefficient (R^2)	0.9996	0.9986	0.9983	0.9966
3	y-intercept	43523	31738	49458	56028
4	Slope	187318	134123	176218	236730
5	LOD	0.3125	0.5759	0.6499	0.9150
6	LOQ	0.9471	1.7450	1.9694	2.7728

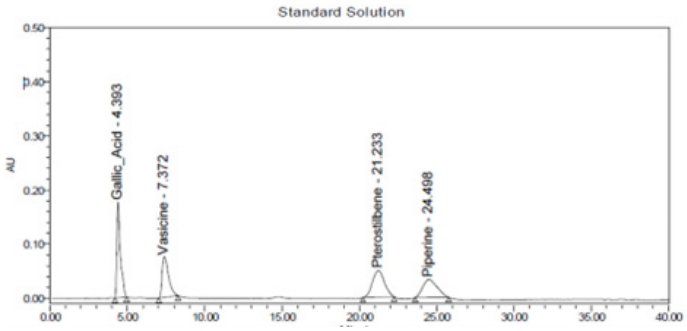

Figure 1: Chromatogram of standard solution.

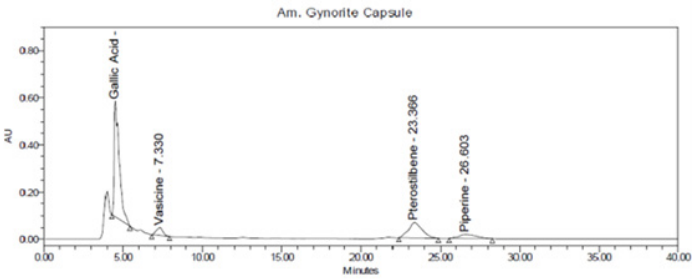

Figure 2: Chromatogram of Amarantha Gynorite capsule.

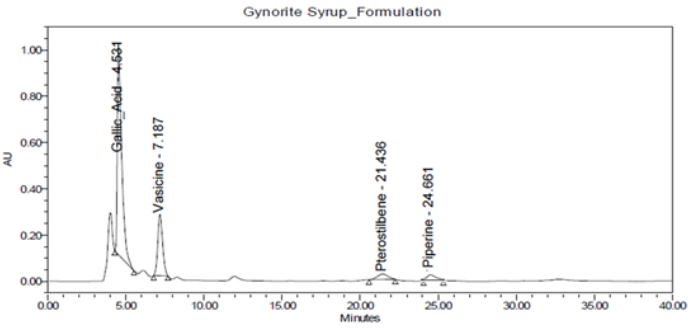

Figure 3: Chromatogram of Amarantha Gynorite syrup.

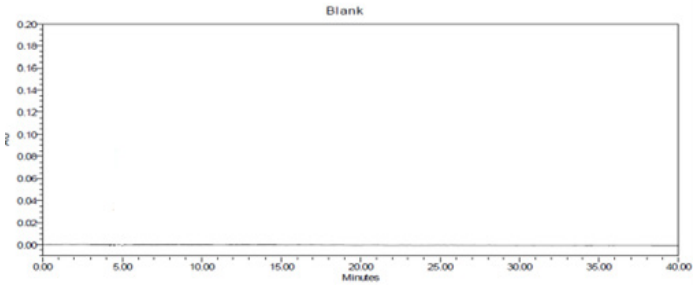

Figure 4: Chromatogram of blank.

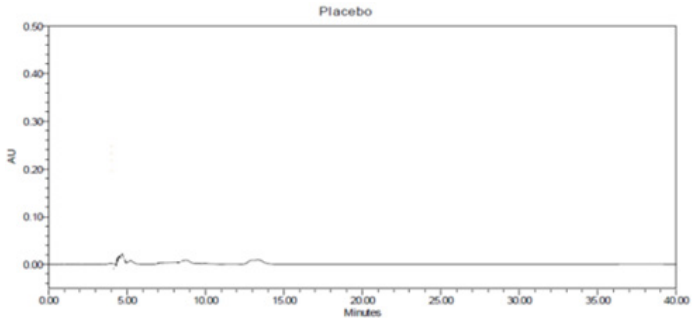

Figure 5: Chromatogram of placebo.

about the quality of product; broadly it covers qualitative and quantitative part of analysis[13-21]. Qualitative and quantitative analysis of polyherbal formulation is extremely intricate and it also involves lots of time, and various resources including infrastructure, man power, consumables, etc. Through this research, a single RP-HPLC method which is simple, cost and time effective for analysis of various marker compounds including Gallic Acid (GA), Vasicine (VS), Pterostilbene (PTS) and Piperine (PP) from Amarantha Gynorite Syrup and Amarantha Gynorite Capsule is developed.

The individual standards and samples were run in different mobile phases. From different mobile phases optimized mobile phase was chosen with detection absorbance 280 nm. A satisfactory separation and usual resolutions peak were observed at optimized conditions and at detection wavelength 280 nm. Finally, a cost-efficient RP-HPLC method was developed with less time requirement for simultaneous separation and determination of GA, VS, PTS and PP. This new isocratic RP-HPLC was found

Table 5: System suitability parameter.

Sl. No.	Name of Standard	Retention Time (RT)	Mean Peak Area	% RSD	USP Tailing	Plate Count
1	Gallic Acid	4.393	2785223.6	0.78	1.02	25698
2	Vasicine	7.372	2304964.6	0.46	1.16	361032
3	Pterostilbene	21.23	2950334.4	1.06	1.06	541186
4	Piperine	24.49	2542287.6	1.27	1.12	189866

Table 6: Method precision parameter.

Sl. No.	Name of Standard	% RSD
1	Gallic Acid	0.524
2	Vasicine	1.121
3	Pterostilbene	0.645
4	Piperine	1.159

Table 7: Intermediate precision parameter.

Sl. No.	Name of Standard	% RSD for System-1	% RSD for System-2	Overall % RSD
1	Gallic Acid	0.524	1.471	1.002
2	Vasicine	1.121	0.964	1.042
3	Pterostilbene	0.645	1.596	1.105
4	Piperine	1.159	0.982	1.071

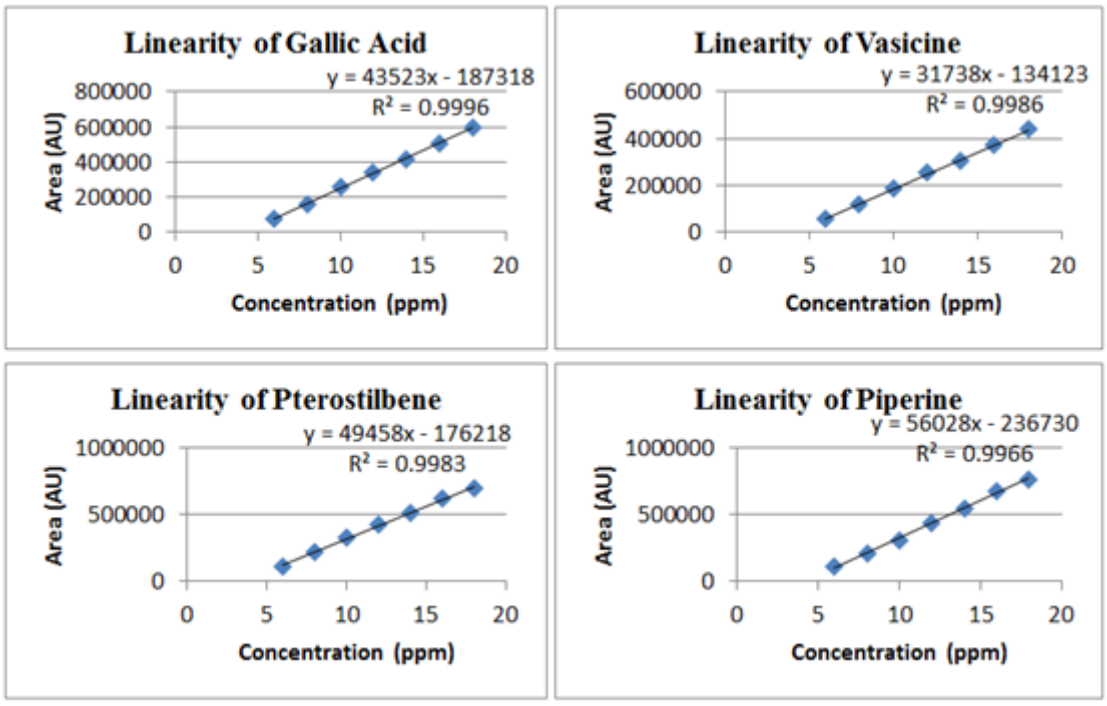

Figure 6: Linearity graph of GA, VS, PTS and PP.

to be economical, simple, precise, accurate and reproducible. It was suitable for qualitative and quantitative analysis of GA, VS, PTS and PP.

The injection volume 20 µL was loaded for quantitative analysis and to ensure quality and safety of formulations. The retention time was found to be 4.40 min for GA, 7.37 min for VS, 21.23 min for PTS and 24.98 min for PP. The optimized method was validated as per ICH guidelines. In specificity studies, no interference peak was observed in placebo, blank and both the formulations. Linearity for range of 6-18 µg/mL with correlation coefficient was found to be 0.9996, 0.9986, 0.9983 and 0.9966 for GA, VS, PTS and PP, respectively. Precision of proposed RP-HPLC method was carried out in terms of system suitability studies, method precision, intermediate precision and repeatability. The % RSD was found to be within the acceptance criteria i.e., < 2.0%.

During accuracy recovery studies, the overall recovery percent were found to be 100.16% for GA, 100.44% for VS, 100.69% for PTS and 101.03% for PP; respectively. The LOD, LOQ values were found to be 0.3125 µg/mL and 0.9471 µg/mL for GA; 0.5759 µg/mL and 1.7450 µg/mL for VS, 0.6499 µg/mL and 1.9694 µg/mL for PTS, and 0.9150 µg/mL and 2.772 µg/mL for PP; respectively.

Table 8: **Repeatability of standard solution.**

Sl. No.	Concentration	Peak Area of Standard Solutions			
		GA	VS	PTS	PP
1	100	2795852	2309238	2957946	2559864
2		2796556	2292583	2895912	2546379
3		2789514	2315689	2956128	2491567
4		2797215	2312745	2966452	2535816
5		2746981	2294568	2975234	2577812
Mean		2785223.6	2304964.6	2950334.4	2542287.6
SD		21599.10	10667.74	31357.08	32411.85
% RSD		0.78	0.48	1.06	1.27

Table 9: **Accuracy Recovery Study.**

Standard Solutions	Recovery Level	Peak Area	% Recovery	Average % Recovery	Overall Recovery
Gallic Acid	80% - 01	153264	99.24	99.67	100.16
	80% - 02	154156	99.57		
	80% - 03	154925	100.20		
	100% - 01	254834	100.12	100.68	
	100% - 02	256891	100.57		
	100% - 03	254326	101.37		
	120% - 01	339726	100.36	100.12	
	120% - 02	337451	100.14		
	120% - 03	335423	99.54		
Vasicine	80% - 01	116375	101.03	100.54	100.44
	80% - 02	115253	98.76		
	80% - 03	118851	101.84		
	100% - 01	182530	100.77	99.07	
	100% - 02	182651	101.85		
	100% - 03	182856	100.95		
	120% - 01	258815	101.28	101.72	
	120% - 02	258752	102.10		
	120% - 03	257926	101.78		

Standard Solutions	Recovery Level	Peak Area	% Recovery	Average % Recovery	Overall Recovery
Pterostilbene	80% - 01	221623	101.91	101.40	100.69
	80% - 02	219834	101.19		
	80% - 03	219681	101.12		
	100% - 01	319869	101.74	101.34	
	100% - 02	321968	100.400		
	100% - 03	323981	101.89		
	120% - 01	421888	98.38	99.35	
	120% - 02	424172	99.74		
	120% - 03	424932	99.92		
Piperine	80% - 01	230823	99.89	100.56	101.03
	80% - 02	235870	100.93		
	80% - 03	237447	100.87		
	100% - 01	304171	101.80	101.35	
	100% - 02	304473	100.94		
	100% - 03	302701	101.32		
	120% - 01	441351	101.88	101.10	
	120% - 02	431293	99.56		
	120% - 03	441279	101.87		

Table 10: Robustness Assessment.

Standard Solutions	Parameter condition	RT	Mean Area	SD	% RSD	Average % RSD
Gallic acid	**Flow Rate (± 0.2)**					
	0.3 mL/min	5.53	2315946	9365.75	0.39	0.80
	0.5 mL/min	4.42	2285871	12128.27	0.52	
	0.7 mL/min	3.85	2333997	50555.8	1.47	
	Detection Wavelength (± 2.0 nm)					
	278 nm	4.61	2395871	26632.26	1.11	0.79
	280 nm	4.42	2285871	12128.27	0.52	
	282 nm	4.59	2362428	16843	0.71	
	Column Temperature (± 5.0°C)					
	20°C	4.60	2151774	31319.2	1.45	0.95
	25°C	4.42	2285871	12128.27	0.52	
	30°C	4.387	2142126	18762.11	0.87	
Vasicine	**Flow Rate (± 0.2)**					
	0.3 mL/min	8.85	1219238	13664.92	1.12	0.78
	0.5 mL/min	7.37	1238759	6785.26	0.55	
	0.7 mL/min	5.99	1215711	11729.21	0.69	
	Detection Wavelength (± 2.0 nm)					

Standard Solutions	Parameter condition	RT	Mean Area	SD	% RSD	Average % RSD
	278 nm	7.21	1248768	15346.96	1.23	0.89
	280 nm	7.37	1238759	6785.26	0.55	
	282 nm	7.14	1233708	11234.36	0.91	
	Column Temperature (± 5.0°C)					
	20°C	7.215	1128597	18297.73	1.62	1.23
	25°C	7.37	1238759	6785.26	0.55	
	30°C	7.14	1177325	17804.28	1.51	
Pterostilbene	**Flow Rate (± 0.2)**					
	0.3 mL/min	27.71	2052411	12611.14	0.61	1.15
	0.5 mL/min	21.23	1984869	24979.75	1.25	
	0.7 mL/min	19.68	1962876	31329.55	1.59	
	Detection Wavelength (± 2.0 nm)					
	278 nm	22.63	1974435	31794.35	1.61	1.32
	280 nm	21.23	1984869	24979.75	1.25	
	282 nm	21.97	1974917	22004.62	1.11	
	Column Temperature (± 5.0)					
	20°C	21.38	1872245	18345.22	0.98	0.98
	25°C	21.23	1984869	24979.75	1.25	
	30°C	20.70	1906682	13612.2	0.71	
Piperine	**Flow Rate (± 0.2)**					
	0.3 mL/min	31.75	2553685	29619.92	1.15	1.05
	0.5 mL/min	24.49	2541818	25934.82	1.02	
	0.7 mL/min	22.34	2532589	24889.49	0.98	
	Detection Wavelength (± 2.0nm)					
	278 nm	25.82	2538589	26285.9	1.03	0.97
	280 nm	24.49	2541818	25934.82	1.02	
	282 nm	25.04	2567650	22432.97	0.87	
	Column Temperature (± 5.0)					
	20°C	25.17	2452065	38719.79	1.58	1.39
	25°C	24.49	2541818	25934.82	1.02	
	30°C	24.32	2452065	38719.79	1.57	

The method was found to be robust with deliberate change of ±2% in flow rate, wavelength, and column temperature.

The quantity of GA, VS, PTS and PP were found to be 0.45% and 0.36%, 1.45% and 0.76%, 2.70% and 2.40% and 0.31% and 0.09% in Amarantha Gynorite syrup and Amarantha Gynorite capsule, respectively. Absence of interference peak at respective retention time indicated that the developed method can be used for routine analysis for both the *Ayurvedic* proprietary formulations. During the study, low cost, faster analysis, satisfactory precision, accuracy and robustness were the main features of RP-HPLC method.

CONCLUSION

A novel, isocratic RP-HPLC method for qualitative and quantitative analysis of various marker compounds including GA, VS, PTS and PP from Amarantha Gynorite Syrup and Amarantha Gynorite Capsule was developed. The method is economical, simple, precise, accurate and reproducible.

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CONFLICT OF INTEREST

All the authors have substantially contributed in the concept, design, literature search and analysis and interpretation of the study data. All the authors have substantially contributed in preparation, editing and review of the manuscript. No author of the study has any conflict of interest.

ABBREVIATIONS

FSH: Follicle Stimulating Hormone; **g:** Gram; **LH:** Luteinizing Hormone; **mm:** Millimeter; **nm:** Nanometer; **PCOS:** Polycystic Ovary Syndrome; **ppm:** Parts Per Million; **v/v:** Volume by Volume; **µl:** Microliter.

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