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FORCED DEGRADATION STUDIES OF AYURVEDIC TRIPHALA FORMULATION INGREDIENTS- QUERCETIN, GALLIC ACID AND TANNIC ACID BY HPTLC METHOD

Sara Usmani[1], Shahid Husain Ansari[1], Sayeed Ahmad[1], Adil Ahmad[1], Mohamad Taleuzzaman[2]*

[1]Department of Pharmaconosy, School of Pharmaceutical Education & Research, Jamia Hamdard (Hamdard University), 110019, New Delhi, India

[2]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Maulana Azad University, Jodhpur, 342802, Rajasthan, India.

*Corresponding Author E-mail: zzaman007@gmail.com

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ABSTRACT

Ayurvedic Triphala formulation (*Phyllanthus emblica, Terminalia chebula, and Terminalia bellerica)* dried fruits. Present work has the object is to develop a simple method by HPTLC for the quantification of ingredients in Triphala formulation and performed the stability studies by this method. The developed method was validated as per ICH guidelines. Optimized the mobile phase for the individual constituents for Quercetin -Ethyl acetate: Glacial acetic acid: Formic acid: water (100:11:11:25), Gallic acid-Toluene: ethyl acetate: formic acid: methanol (4.3:4.3:1.2:0.3), Tannic acid-Toluene: acetone: formic acid (4.5:4.5:1). The Rf values 0.30 (Quercetin), 0.43 (Gallic acid) and 0.70 (Tannic acid). The linearity range is 1-5 (µg/µL) in five levels. Limit of detection (LOD) 350 ng/ µL and Limit of quantification (LOQ) 1050 ng/ µL. Stability studies performed in different conditions- like acid, base, wet heat, dry heat, freeze thaw, bench top stability and stock solution stability.

INTRODUCTION

Worldwide, demand for herbal Medicine increases day by day in developed as well as in developing countries for healthcare, reason for the popularity is because of their wide biological activities, higher safety margins and fewer costs **[**Handa 1991; Kamboj 2000**].** A major population presently suffered from life-style diseases like depression, cancer and heart troubles, key factor behind this is faulty nutrition and stress. An approach of herbal medicines is one of the appropriate practices **[**Gupta et. al., 2000**].** The developments of herbal medicine fully depend on phytochemistry or natural product chemistry research. Extraction, isolation, purification, and characterization of the constituents from the herbs or different parts of plants like a flower, seeds, fruits, leaves, etc. is a very important step for the development of herbal medicines.

Ayurveda formulation Triphala is a very important medicine practice in the ancient Indian medical system, it consists of three kinds of dried fruits from plant *Emblica officinalis, Terminalia bellirica*, and *Terminalia chebula*. The chemical constituents Quercetin ($C_{15}H_{10}O_7$) Gallic acid ($C_6H_2(OH)_3COOH$ and Tannic acid ($C_{76}H_{52}O_{46}$) are extracts from the fruits of the plant *E. officinalis, T. bellirica,* and *T. chebula* respectively, its chemical structure **[Figure -1].**

Triphala formulation is a rich source of polyphenolic bioactive compounds **[**Avula et.al 2013**].** Several therapeutic effects have been confirmed anti-inflammatory **[**Kalaiselvan 2015**],** counter the effects of mutagens and anti-cariogenic **[**Takauji et. al., 2016; Bajaj et.al., 2011**]** very effective for the gastrointestinal disease and also found effective as antineoplastic **[**Rayudu et.al., 2014; Peterson et. al., 2017**]** anti-retinopathy, anti-carcenogenic, skin lotion, creams, sun blocker **[**Sivasankar et.al.,2015; Cheriyamundath et.al.,2018; Varma et.al.,2016**],** and inhibition of protein fibrillation **[**Save et.al., 2017**]** health benefits. WHO has appointed a regulatory body IRCH (International Regulatory Cooperation for Herbal Medicines) responsible for the regulation of herbal medicines. The object of IRCH is to promote the safe use of herbal drugs and medicines. The data of the herbal ingredients produced by such authorities, share with National Drug Regulatory Authorities which strengthens the work at the national or regional level and can prevent the duplication work.

Several analytical techniques employed for the estimation and identification of chemical constituents in medicinal plants, both qualitative as well as quantitative analysis. Chromatography techniques like TLC, GC, HPLC, high-performance TLC (HPTLC), and HPLC-MS are used for separation, identification, and quantification **[**Castaldo et.al.,2002**;** Liviero et.al., 1994; Macheix et.al.,1990]. HPTLC is a very popular chromatographic technique for herbal analysis because of its rapidity, low amount sample used, and less wastage of solvent during the analysis, very popular technique for the determination of pharmacologically interesting compounds in biological matrices, different parts of plants like leaves, fruits, flowers and herbal formulations [Watanabe et. al.,2000; Singh et. al., 2005; Mishra et. al.,2005; Taleuzzaman et. al., 2017; Bhandari et.al.,2005]. Simple and precise analytical methods developed by HPLC and HPTLC are used for the nanoformulation studies [Gilani et.al.,2019; Taleuzzaman et.al.,2017].

Stability: All international guidelines on standardization and quality control relates to establishment and demonstration of stability, shelf-life and storage conditions. Stability testing provide evidence for the quality and purity of drug substance as well drug products changes with time under the impact of various environmental factors such a temperature, humidity, oxidation, light, pH and moisture content, etc. The different dosage forms of pharmaceutical products require performing the stability studies at different stressed condition like acidic, basic, oxidation, freeze thaw etc. with object to finalize the self-life of the formulation [Bankoti et. al., 2012; Moolakkadath et.al., 2020; Stolarczyk et.al., 2010]. HPTLC fingerprinting chromatogram, assay procedures, physical tests, sensory tests and other procedures are used for the stability studies for the medicinal product. In the case of herbal formulations the variation of the content should not exceed ± 5% of the declared assay value for the proposed shelf-life and in case of the herbal medicinal product have herbal substances or herbal preparation where constituent of known therapeutic activity are known, the proposed shelf-life ±10% **[**Anonymus. 2000**].** Stability studies is confirmed the degradation pathways of drug substances and drug products, make clear the structure of degradation product, find out the intrinsic stability of a drug substance in a formulation **[**Reynolds et. al., 2002**].**

Terminalia chebula, Embellica officinalis and Terminalia bellerica (Gaertn.) Roxb is a very well known and generally used plant in ayurvedic formulation due to its therapeutic effects. Commercially there is a large consumption of these drugs not only in India but throughout the world. Triphala itself is a formulation that is added as an ingredient in various formulations. The aim of the research is to study the stability testing of quercetin in *E. officinalis*, gallic acid in *T. chebula*, and tannic acid in *T. bellerica* following official

MATERIAL AND METHODS:

Plant Material: The crude drugs (fruits) - *Embellica officinalis*, *Terminalia chebula* and *Terminalia bellerica* (Gaertn.) Roxb is purchased from the local market, kharibaoli, Old Delhi, authenticated by the taxonomist. The drugs were dried in shade, powdered to

coarse and were kept in air-tight containers individually, away from moisture. The powdered drugs were standardized for the following parameters (macroscopy, foreign organic matter, organoleptic properties).

Chemical, Solvents, and Reagents: Quercetin, Gallic acid and, Tannic acid were procured in analytical grade, LR grade solvents from "Chemie labs" were used. For high-performance thin-layer chromatography, the distilled water is used wherever water is mentioned.

Extraction of plant material: Extraction was carried out using a 70% solution of hydro alcohol (ethanol). The extraction was done after performing extractions in various solvents depending upon polarity. The highest amount of residue was found to be in an alcoholic solvent. Hence, a further experiment was carried out in Ethanol. The dried sample of each selected drug was powdered into a coarse particle size. The pattern of extraction was studied in each grade by extracting 2gm of the powdered sample under reflux with 100 ml ethanol at 70ºC for 2 hrs. Each extract was filtered, concentrated to dryness, and weighed. From that residue, 1mg was dissolved in 1ml of ethanol. TLC analysis of the samples was done.

CHROMATOGRAPHIC METHOD

Thin Layer Chromatography

The quantitative thin layer chromatography and fingerprint profiling were done using pre-coated silica gel TLC plates (E. Merck) of 200-micrometer thickness; the number and position of bands were observing the plate under ultraviolet light. A large number of solvent systems with varying compositions were tried for developing the fingerprint profiles. CAMAG TLC scanner was used for analytical studies. The samples were applied using CAMAG automatic TLC was sampled by TLC sampler 4 and were developed in CAMAG twin through the chamber using appropriate solvents. The plates were dried and an image of TLC chromatography was taken under 284nm UV light using CAMAG Reprostar scanner, peaks were recorded. The amount of the desired component was calculated from the standard of the respective marker.

Calibration Curve of Standards: The known amount of standard solution was spotted on TLC plates in increasing order. The spots were developed in the solvent system followed by heating at 100º C, the spots were scanned in the TLC scanner set at respective wavelengths. The average area under the curve was calculated and the standard plot was constructed.

TLC Densitometric Estimation: The known amount of test solution of quercetin, gallic acid and, tannic acid was applied on the plate along with the band of standards. The plate was developed and scanned amount of quercetin, gallic acid, and tannic acid was calculated in the test solution from the mean AUC values of the peaks corresponding to marker using its calibration curve.

High-Performance Thin-Layer Chromatography (HPTLC): It is a sophisticated instrumental technique that worked based on the thin layer chromatography with a better analysis performance. Development of chromatogram by a simple and precise method with software-controlled evaluation. The qualitative and quantitative analysis of herbal substance or formulation by HPTLC based on scientific facts as well as standardized methods. HPTLC meets all quality requirements of today's analytical labs, even in a fully regulated environment (camag.com).

Validation:

Method Validation

The new method developed by HPTLC, developed method was validated by parameters like specificity, sensitivity, accuracy, precision, repeatability, and robustness. Follow the International Conference on Harmonization (ICH) guidelines Q2 (R1).

Specificity

Carried out experiments (N=6) sample to establish the specificity of the developed method and confirm the zero interferences at the retention factor value (Rf) of individual ingredients quercetin, gallic acid and, tannic acid in formulation compare with the chromatogram of standard respective substances.

Sensitivity

The numerical value of the limit of detection (LOD) and limit of quantification (LOQ) determine by the developed method and it indicates the sensitivity of the method. A series of standard solutions (N=6) prepared of

individuals ingredients, experimented to establish the value of LOD and LOQ.

Linearity and Calibration Curves

Prepared a calibration curve at five concentration levels, develop linearity of the method. Solutions of the standard prepared in ethanol have concentrations range of 1-5 µg/mL of individual ingredients. Experimented, on TLC plate applied a fixed volume to get the final concentration in range of 50–1000 ng/spot, 50–1050 ng/spot and 200–600 ng/spot for quercetin, gallic acid and tannic acid respectively. Developed the calibration curve by taken peak areas obtained in Y-axis versus corresponding concentrations in X-axis, treated by linear least-square regression analysis.

Accuracy:

Experimented to evaluate the accuracy of the method by recovery studies at three levels. Consider three different levels of standard drugs 50, 100, and 150% each has (N=6).

Precision

Precision estimated by performing the experiments in terms of intra-day and inter-day precisions. Performed Intra-day precision, three different levels covering low, medium and higher concentration within the calibration range solutions for six-time each of ingredients. Determine inter-day precision value by analyzing sample solutions at three levels covering low, medium, and higher concentrations over seven days (n=6). Calculate the values of CV and SD by evaluating the peak area.

Robustness

Developed method analyzed to know about the robustness, conduct experiment on by small changes in mobile phase composition and its volume, chamber saturation time, and a slight change in the solvent migration distance; the effects on the results were examined. Calculate the value of SD and CV by evaluating the peak area at a concentration level of 3µg/ml in triplicate.

Stress Degradation Studies:

 Acid stability- 5ml of 1N HCl was added to the mixture of 2gm of the powdered drug was extracted with a hydro alcoholic solution for 2 hrs and filtered. The filtrate is concentrated to dryness and weighed. The sample was made by taking 0.1mg of extract in 1ml ethanol. The analysis was done after 18 hrs of sample preparation.

 Basic stability-5ml of 4N NaOH was added to the mixture of 2gm of the powdered drug (till pH 11) which was further extracted with a hydro alcoholic solution for 2hrs and filtered. The filtrate is concentrated to dryness and weighed. The sample was made by taking 0.1mg of extract in 1ml ethanol. The analysis was done after 18 hrs of sample preparation.

 Oxidation stability-1ml (3%) hydrogen peroxide was added to the mixture of 2gm of powdered drug which was further extracted with 70% hydro alcoholic solution for 2 hrs filtered, concentrated to dryness and weighed. the analysis was performed after 18hrs of sample preparation.

 Wet heat stability-2gm of the powdered drug was refluxed with a hydro alcoholic solution on a water bath for 3hrs at 70ºC. The solution was filtered, concentrated to dryness and weighed. The analysis was done after 18 hrs of sample preparation.

 Dry heat stability: 2gm of the powdered drug was kept in a hot air oven at 80ºC for 4hrs and then refluxed on a water bath for 2 hrs solutions is filtered, concentrated to dryness and weighed. The analysis was done after 18 hrs of sample preparation.

 Freeze-thaw stability: 2gm of the powdered drug was refluxed with 100 ml hydro alcoholic solution for 2 hrs extract was filtered, concentrated to dryness and weighed. The residue is reconstituted with ethanol (10ml). From these three concentrations of the high, medium, and low is prepared. The volume is made up to 5 ml. These samples are frozen at -20ºC for 24hrs and thawed unassisted for the next 24 hrs. This cycle was repeated 3 times before analysis. The analysis is done after 18hrs of sample preparation.

 Bench top stability: 2gm powdered drug is extracted with a hydro alcoholic solution for 2 hrs at 70ºC. The extract is filtered, concentrated to dryness and weighed. The residue is reconstituted with ethanol (10ml). The prepared sample is kept at room temperature for at least 24 hrs. The analysis is done after 18 hrs of sample preparation

 Stock solution stability: 2gm of the powdered drug was extracted with a 100ml hydro alcoholic solution for 2hrs at 70ºC. The extract

was filtered, concentrated to dryness and weighed. The residue was reconstituted with ethanol and volume was made up to 10ml. The stock solution is then frozen at -20ºC for 7days and subsequently kept for 6 hrs at room temperature. The analysis was done after 18 hrs of sample preparation.

Accelerated Study:

The samples of powdered drugs and formulation are separately kept in the stability chamber of room no. 110, school of pharmaceutical education and research, Jamia Hamdard, Delhi, India. The storage conditions for accelerated stability are 40 ºC and RH is 75%. Testing of samples is done at 0, 3 and 6 months after they are kept in provided conditions. Extraction of 2 gm of each is done under reflux on a water bath with a 100 ml hydro alcoholic solution for 2 hrs at 70ºC. Each extract was filtered concentrated to dryness and weighed. The analysis is done after 18 hrs of sample preparation.

Real-Time Stability Testing: (For Crude Drugs)

In these samples of whole crude drugs are kept at room temperature. The storage condition varies between 15 ºC and RH 75% to 31 ºC and RH 77% during the period of study. Testing of samples of crude drugs is taken out 0, 3 and 6 months. Each plant drug is powdered. The extraction of 2gm of drug of the powdered drug is done with a hydro alcoholic solution for 2hrs at 70 ºC. Each extract is filtered, concentrated to dryness and weighed. The analysis is done after 18 hrs of sample preparation.

EXPERIMENTAL:

Standard solution: A standard solution was prepared by dissolving 1mg of individual standard drug quercetin, gallic acid and tannic acid in 0.5 ml ethanol and making volume up to 1ml (1mg/ml).The Rf value founded 0.30, 0.43 and 0.70 for quercetin, gallic acid and, tannic acid respectively.

Sample application: A uniform volume of 1, 2, 3, 4, 5µl of standard and 4µl test solution were applied separately using CAMAG automatic TLC sampler in the form of a 6mm band on pre-coated silica gel 60F254 TLC plates of uniform thickness of 200 µm.

Development of the plate and visualization

The plate was developed in the appropriate solvent system to a distance of 80% of the plate in a twin trough chamber with a pre-saturation time of 10 min. The plate was air-dried and the image of the TLC chromatogram was taken under 254 nm ultraviolet light using CAMAG Reprostar 3. Run the all standard solution of calibration curve with extract sample of individual constituients. *Phyllanthus Embellica* (Quercetin)-Densitogramin **(Figure-1)** showing the percentage of quercetin in the extract (2.82%) **[Table-2].** *Terminalia Chebula (*Gallic acid)-Densitogram in **(Figure-2)** showing the percentage of gallic acid in the extract (0.29%) **[Table-4].** *Terminalia bellerica* (Tannic acid): Densitogram in **(Figure-3)** showing the percentage of tannic acid in the extract (3.62%) **[Table-6].**

Result and Discussion:

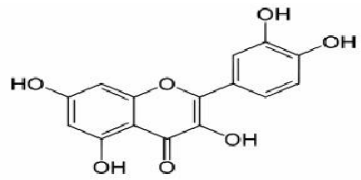
Validation

The developed method validated, performed validation parameter accuracy-analysis in three levels calculated as a percentage of recovery result pass the acceptance criteria, precision in three-level concentration taken sample (N=6) and robustness analysis performed taken each sample (N=6) results in **[Table-1].**

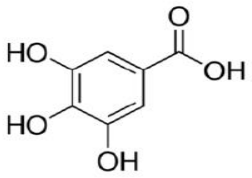
Stability studies:

Estimation of Quercetin in forced degradation studies.

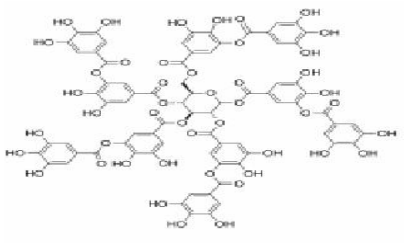
The percentage content of quercetin was calculated from the standard curve by considering the area under the curve, 2.82 % in the coarse powder of *P. emblica* Degradation of quercetin in acid medium by 2.38%, in basic medium 1.72 %, in oxidation condition the percentage of reduction is 2.42 %, in wet heat condition reduced to 1.86 %, in dry heat condition it found 1.65%. The content of quercetin in bench-top stability studies found 2.77%, in accelerated stability studies condition it found 2.25% (three months) and 1.01% (six months). Real-time stability the percentage of quercetin in coarse powder on 3 months and 6 months storage under normal conditions at room temperature was found to be 0.79%, 0.6% respectively. The reduction in content of quercetin was found to be 2.03%, 2.22% respectively **[Table-3] and [Chromatogram Fig.4 & 5].**



[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one]



3,4,5-trihydroxybenzoic acid



Decagalloyl glucose

Figure -1 Chemical structure

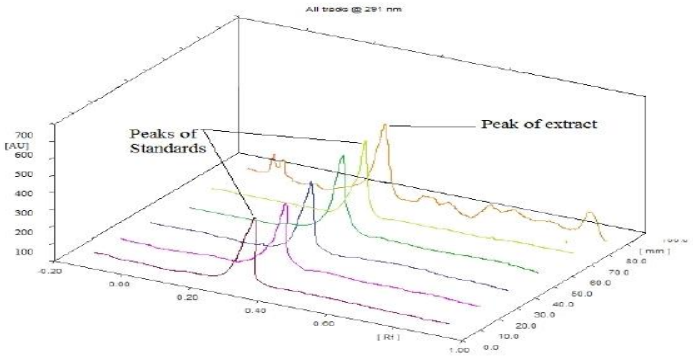


Figure-2: Densitogram showing peaks of standard with extract (Quercetin)

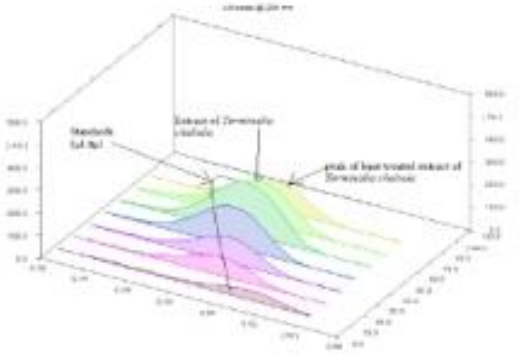


Figure 3: Densitogram showing peaks of standard and extract (Gallic acid)

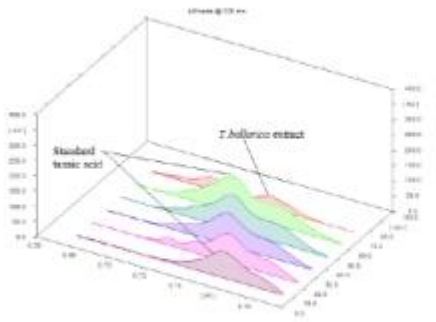


Figure 4: Densitogram showing peaks of standard and extract (Tannic acid)

Table 1:Method development and validation

Parameter	**Phyllanthus Embellica (Quercetin)**		**Terminalia Chebula (Gallic acid)**		**Terminalia bellerica (Tannic acid)**	
Solvent system	Ethyl acetate: Glacial acetic acid: Formic acid: water (100: 11: 11: 25)		Toluene: ethyl acetate: formic acid: methanol (4.3:4.3:1.2:0.3)		Toluene: acetone: formic acid (4.5:4.5:1)	
Scanning wavelength	300 nm		284 nm		291 nm	
TLC plates	Pre-coated plates of silica gel 60 F254 with a uniform thickness of 200µm.		Pre-coated plates of silica gel 60 F254 with a uniform thickness of 200µm.		Pre-coated plates of silica gel 60 F254 with a uniform thickness of 200µm.	
Calibration Curve	1-5 (µg/ µL) Y= 20.74x-27.85 R^2=0.99		1-5 (µg/ µL) Y= 1272x-481.9 R^2=0.98		1-5 (µg/ µL) Y= 7.54x-85.38 R^2=0.98	
R_f	0.30		0.43		0.70	
Accuracy(N=6) SD CV	 0.05-0.06 1.9-1.2		 0.05-0.07 1.6-1.2		 0.04-0.05 1.6-1.0	
Precision(N=6)	**Intra-batch**	**Inter-batch**	**Intra-batch**	**Inter-batch**	**Intra-batch**	**Inter-batch**
SD **CV**	**0.03-0.11** **1.58-2.79**	**0.03-0.12** **1.54-0.54**	**0.06-0.07** **3.5-1.77**	**0.05-0.04** **2.6-1.21**	**0.06-0.02** **3.14-0.63**	**0.04-0.05** **2.35-1.3**
Robustness (N=6)	**Mobile phase composition**					
	5.16-2.87		**4.13-2.73**		**3.76-0.86**	
SD **CV**	**0.04-0.02**		**0.15-0.10**		**0.71-0.70**	
	Saturation time(10±5min)					
	2.89-2.24		**2.56-2.18**		**2.56-2.15**	
	0.03-0.02		**0.09-0.08**		**0.09-0.07**	
	Activation temperature (110 ±5°C)					
	1.89-1.24		**1.56-1.18**		**1.76-1.15**	
	0.06-0.04		**0.09-0.06**		**0.09-0.06**	

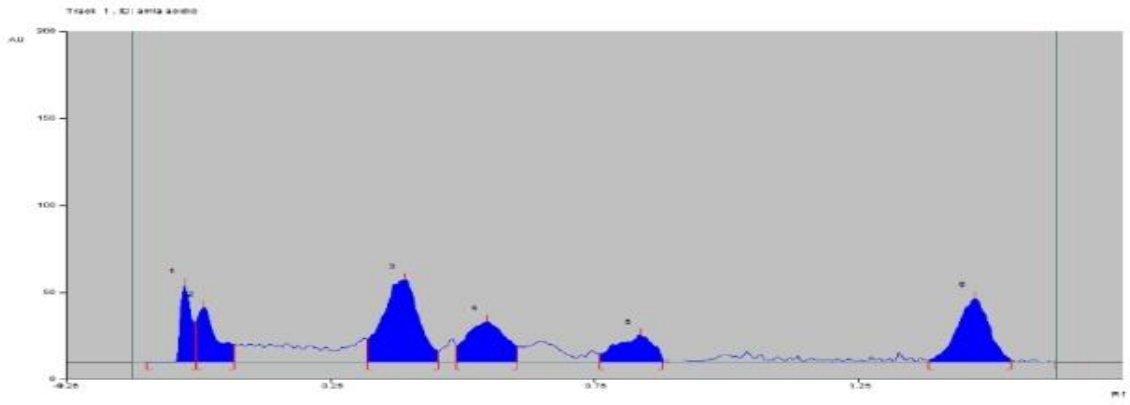


Figure 5: Acid stability (Peak no 3) Quercetin

Table-2: Percentage of Quercetin in *P.Embellica* extracts

Substance	AUC	Estimated content of Quercetin in spotted volume (nanogram)	Quercetin content in dried fruit %
Standard 1	1369	64.664	-
Standard 2	5766	276.67	-
Standard 3	10552	507.43	-
Standard 4	15742	757.67	-
Standard 5	20377	981.15	-
Phyllanthus emblica	8599.3	413.28	2.82

Table-3: Content of Quercetin in different stress conditions

Stability test	AUC	Estimated content of Quercetin in spotted volume (nanogram)	Quercetin content in dried residue (mg)	Percentage of quercetin in dried fruit
Acidic stability	14091.1	66.59	8.87	0.44
Basic stability	3451	165.05	22.006	1.1
Dry hat stability	3598	172.03	23.52	1.17
Wet heat stability	2952	140.99	19.26	0.96
Bench-top stability	8453	406.22	55.51	2.77
Oxidation stability	1265	59.65	8.15	0.40
Accelerated (3M)	1769	83.95	11.47	0.57
Accelerated (6M)	3070.7	146.68	20.04	1.01
Real Time Stability Study (3M)	2430	115.822	15.82	0.79
Real Time Stability Study (6M)	1855	88.09	12.03	0.6

Table 4: Percentage of Gallic acid in extract of *T. chebula*

Substance	AUC	Estimated content of gallic acid in spotted volume (nanogram)	Gallic acid content in dried fruit %
Standard 1	1018	56.27	-
Standard 2	2163.6	273.82	-
Standard 3	2704	376.44	-
Standard 4	4652.5	746.46	-
Standard 5	6134.3	1027.85	-
T.chebula extract	4974.1	3.531	0.29

Table-5: Content of Gallic acid in different stress conditions.

Sample	AUC	Estimated content of gallic acid in spotted volume (ng)	Gallic acid content in dried residue (mg)	% Gallic acid in dried fruit
Acidic	12188.8	9.2	1.50	0.08
Basic	21972.1	16.89	2.75	0.14
Dry heat	23024.3	17.72	2.89	0.15
Wet heat	20298.5	15.57	2.54	0.13
Benchtop	4974.1	3.513	5.76	0.29
Oxidation	2304	1.43	5.23	0.28
Freeze-thaw first dilution	6769	4.92	0.8	0.04
Freeze-thaw second dilution	5334	3.81	0.62	0.03
Freeze-thaw third dilution	3909	2.69	0.44	0.02
Accelerated Stability Study (3M)	6412.7	4.662	0.76	0.04
Accelerated Stability Study (6M)	4762.7	3.365	0.54	0.03
Real – Time Sttabilty Study (3M)	2411.7	1.517	0.24	0.0123
Real – Time Sttabilty Study (6M)	1201.8	0.565	0.092	0.0146

Table-6: Percentage of Tannic acid in extract of *T. bellerica* (Gaertn.) Roxb

Substance	AUC	Estimated content of gallic acid in spotted volume (nanogram)	Tannic acid content in dried fruit %
Standard 1	1735.5	218.85	-
Standard 2	2429.8	310.93	-
Standard 3	2885.2	473.3	-
Standard 4	3290.7	525.1	-
Standard 5	3771.1	598.4	-
T.bellerica extract	3431	443.71	3.62

Estimation of Gallic acid in forced degradation studies.

The percentage content of gallic acid was calculated from the standard curve by considering the area under the curve, 0.29% the coarse powder of *T. chebula*. Degradation of gallic acid in acid medium found 0.08%, in basic medium 0.14 %, in oxidation condition the percentage of reduction is 0.28%, in wet heat condition reduced to 0.13 %, in dry heat condition it found 0.15 %. The content of gallic acid in bench-top stability studies found 0.29%, different freeze-thaw conditions content of gallic acid found in first dilution 0.04%, the second dilution 0.03%, the third dilution 0.02%, in accelerated stability studies condition it found 0.04% (three months) and 0.03% (six months). Real-time stability the percentage of gallic acid in coarse powder on 3 months and 6 months storage under normal conditions at room temperature was found to be 0.0123%, 0.0046% respectively **[Table-5] and [Chromatogram Fig. 6 & 7].**

Estimation of Tannic acid in forced degradation studies.

The percentage content of tannic acid was calculated from the standard curve by considering the area under the curve, 3.62% in the coarse powder of *T. bellerica*. Degradation tannic acid in acid medium found 3.57 %, in basic medium 1.07 %, in oxidation condition the percentage reduced to 1.05%, in wet heat condition reduced to 1.34 %, in dry heat condition it found 2.77 %. The content of tannic acid in bench-top stability studies found 3.92%, different freeze-thaw conditions content of tannic acid found in first dilution 1.64%, the second dilution 1.65%, the third dilution 1.77%, in accelerated stability studies condition it found 2.19 % (three months) and 1.60% (six months). Real-time stability the percentage of tannic acid in coarse powder on 3 months and 6-month storage under normal conditions at room temperature was found to be 1.91%, 0.9% respectively **[Table-7] [Chromatogram Fig. 8 & 9].**

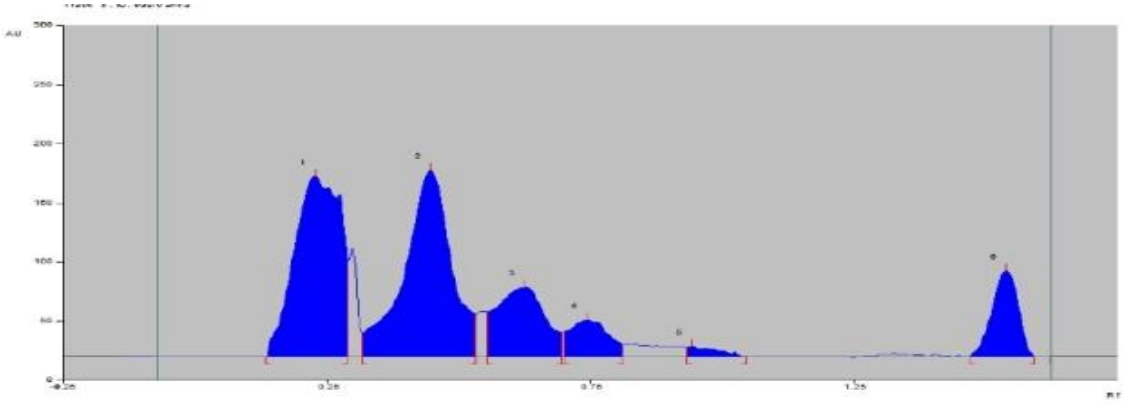


Figure 6: Base stability (Peak no.1) Quercetin

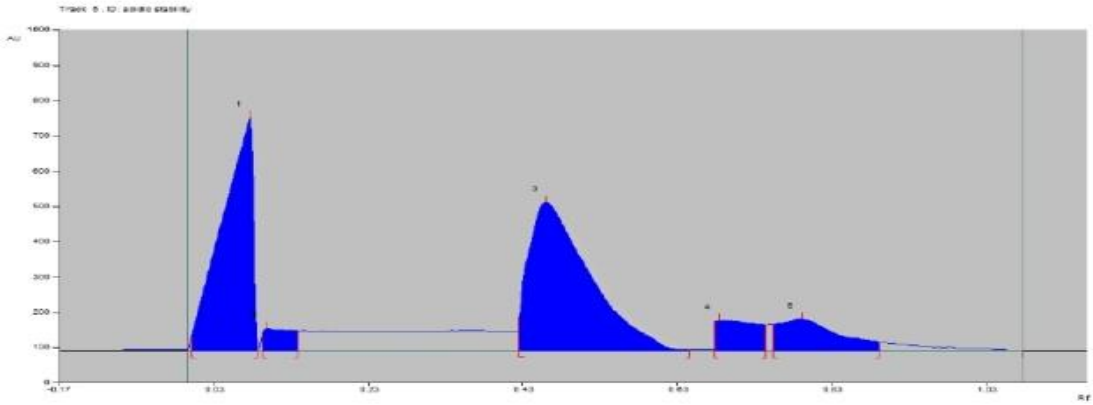


Figure 7:Acid Stability (peak 3) of Gallic acid

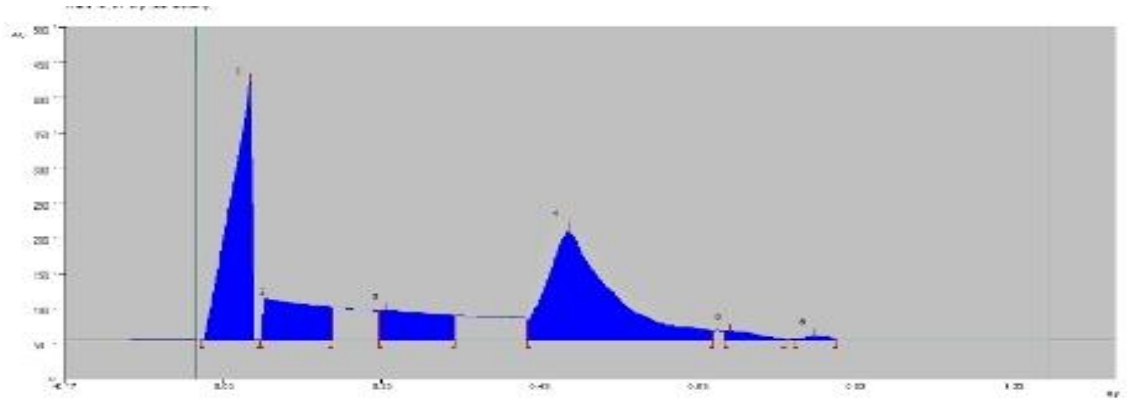


Figure 8: Base stability (Peak No.6) Gallic acid

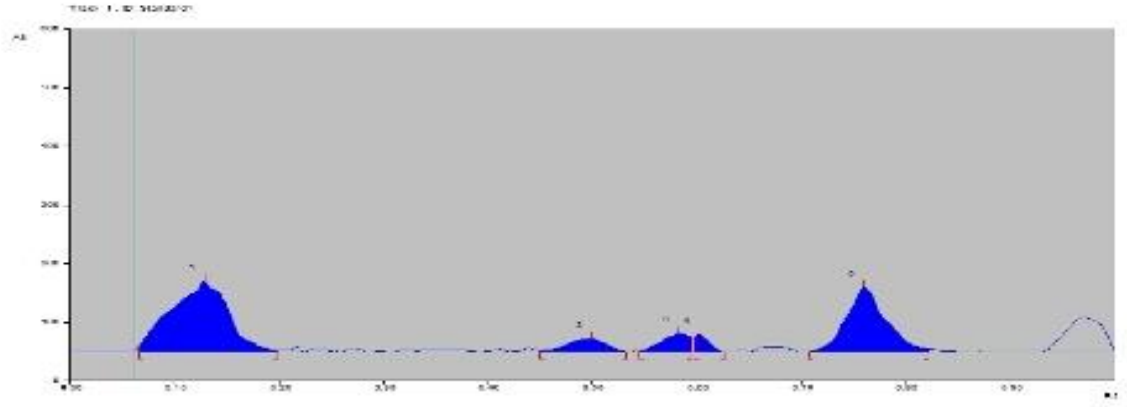


Figure 9: Acid stability (Peak No.5) Tannic acid

Table-7: Content of Tannic acid in different stress condition

Substance	AUC	Estimated content of tannic acid in spotted volume (ng)	Estimated content of tannic acid in died residue (mg)	Tannic acid content in dried fruit %
Acidic	3348.4	432.77	71.40	3.57
Basic	1067	130.19	21.47	1.07
Dry heat	2625	36.81	55.57	2.77
Wet heat	1313.8	162.92	26.88	1.34
Benchtop	3675.5	476.15	78.56	3.92
Stock solution	2427.8	310.67	51.25	2.56
Oxidation	1047	127.54	21.04	1.05
Freeze-thaw 3	1712	215.742	35.50	1.77
Freeze thaw 2	1600	200.88	33.14	1.65
Freeze thaw 1	1585	198.89	32.81	1.64
Accelerated 3 month	3290	425.015	43.91	2.19
Accelerated 6 month	2429.8	310.93	32.12	1.60
Real – Time Sttabilty Study (3M)	2885.2	371.32	38.37	1.91
Real – Time Sttabilty Study (6M)	1131	138.67	18.49	0.9

CONCLUSION:

A simple reproducible method developed for the estimation of quercetin, gallic acid, and tannic acid. The content of the ingredients in the ayurveda Triphala formulation quantified with the developed method, the stability studies of each ingredients Quercetin, Gallic acid and Tannic acid content studied in different conditions. The result of the analysis helpful for the determination of the storage condition of the herbal formulation, the sensitivity of the constituents in different conditions. The optimized method validated as per the ICH guideline.

Conflict of Interest: Authors declare no conflict of interest.

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