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DEVELOPMENT OF VALIDATED UV SPECTROPHOTOMETRIC STABILITY INDICATING METHOD FOR ESTIMATION OF GALLIC ACID IN BULK FORM

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ABSTRACT

A novel simple, reliable, rapid and accurate UV spectrophotometric stability indicating method (SIM) was developed for estimation of gallic acid from bulk powder. The study was carried out at 220 nm. Gallic acid has shown linear absorbance over the concentration range of 4-20 µg/Ml with R^2 value 0.999. This $y = 0.061\,x + 0.016$ was used for determination of concentrations of test solution. The proposed method was validated as per ICH Q2 (R1) guidelines for various parameters eg. Prcision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and linearity and range. Results of validation study has shown compliance with criteria of ICH guidelines. Relative standard deviation was found less than 2% and recovery was found in range 98.6- 100.63 %. Method was very sensitive as LOD and LOQ were found to be 0.147 µg/mL and 0.447 µg/mL respectvely. Stability indicating potential was studied by anlysing sample subjected to various stress conditions like hydrolysis, oxidation, photodegradation and thermal degradtions. Proposed method reveals that gallic acid was found to be unstable at hydrolytic and oxidative stress conditions.

KEY WORDS

Gallic acid, ICH guidelines, Stability indicating method, Stress conditions, Validation

INTRODUCTION:

All pharmaceutical substances un-avoidably contain impurities and the role of ethical pharmaceutical industry is to define an impurity profile that is acceptable for the intended use of a given drug, without compromising its therapeutic safety and efficacy [1,2]. The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a lower dose than expected. Therefore, it is essential to know the purity profile and behavior of a drug substance under various environmental conditions which could be possible by stability testing [3,4].

ICH defined stability indicating assay methods (SIAM) as, quantitative analytical methods that are based on structural, chemical or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured [5,6].

SIAM can also be defined as “An analytical method that accurately quantitates the active ingredients without interference from the degradation products, process impurities, excipients or other potential impurities” [5].

Validation of a method indicates to establish documented evidence that the system is doing what its purpose to do. Validation is necessary when a method or a procedure is going to be used by a manufacturing

company or to be published in any Pharmacopoeias. The validated assay methods will be more accurate, precise and reproducible [7].

Stress testing of the drug can help to identify the degradation products which can help to establish degradation pathways and the intrinsic stability of the molecule and validate stability indicating power for analytical procedures used [8].

Stress testing is performed by exposing drug substances and drug products to extreme conditions, such as pH, photolysis, oxidation and temperature, over a very short time period. It also referred to as forced degradation studies [9,10].

basic criteria for new method development of SIAM: [11]

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper stability indicating analytical procedure for the drug may not be available in the literature due to patent regulations.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Stability indicating assay method provides the support to track the quality of the product from time to time.

Gallic acid:

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is a natural phenolic antioxidant compound widely distributed in herbal plants, fruits and vegetables. Structurally gallic acid has phenolic groups that serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the phenolic structure [12]. The interest in gallic acid is due to its pharmacological activities such as radical scavenging, antimutagenic, anticarcinogenic and antioxidant. It has potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated and these include cardiovascular diseases, cancer, neurodegenerative disorders and aging [13]. The phenolics are also of interest in food, cosmetic and pharmaceutical industries, as substitutes for synthetic antioxidants.

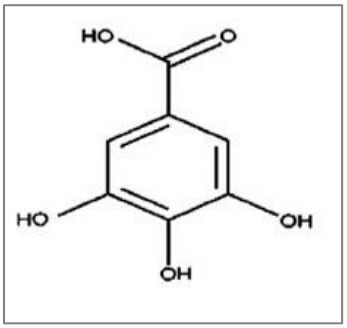
Gallic acid is promising phytochemical and active ingredient of most of the herbal formulations. Many analytical methods like UV spectrophotometric,RP-HPLC, TLC, and HPTLC have beendeveloped for simultaneous estimation of gallic acid and other active ingredients of polyherbal formulations. Despite the remarkable use of gallic acid there was no single SIAM UV spectrophotometric method reported in the literature [14-16]. With the consideration current scenario, it was planned to develop simple rapid and economic validated UV spectrophotometric SIAM for estimation of gallic acid in bulk form.

Properties of Gallic acid:

Chemical formula: $C_7H_6O_5$

Density: 1.70 g/cm^3

Average Molar mass: 170.12 g/mol



Structure of Gallic acid

MATERIALS AND METHODS:

apparatus: Shimadzu 1601 double beam UV-Visible spectrophotometer with 10mm matched quartz cell was used for measurement of absorbance. All weighings were done on single pan balance Dhona 200 D. Analytical grade (AR) chemicals and reagents were used in the study.

methods

stock solutions: (1000 µg/ml)

An accurately weighed 100 mg of pure gallic acid was transferred to 100 mL volumetric flask and 30 mL water was added to it. Solution was shaked for 5 minutes to solubilize compound and final volume was made upto mark with water.

working standard solutions:

Appropriate aliquots were withdrawn from the stock solution and diluted up to 10 mL with water to obtain standard solutions of different concentrations (4,8,12,16 and 20 µg/mL).

selection of wavelength:

Working standard solution of concentration 10 µg/ml was scanned using spectrophotometer within UV range i,e 200–400 nm against water as a blank. Wavelength 220 nm was selected for measurement of the

absorbance. The UV spectrum of gallic acid as shown below in Fig. 1.

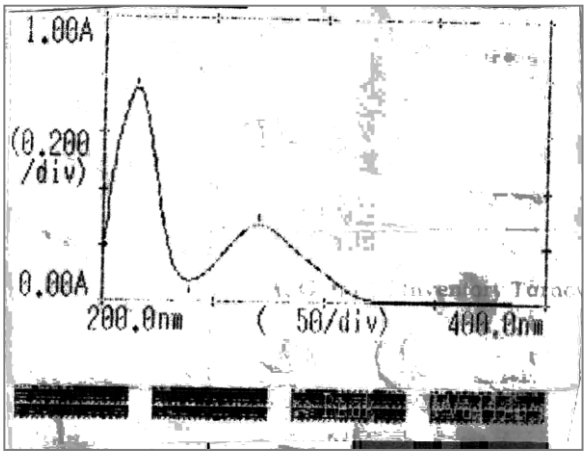


Fig 1. Spectrum of gallic acid

linearity study:

Absorbance of each working solution was measured at the selected wavelength 220 nm. Calibration curve was plotted by taking absorbance on ordinate and concentration on abscissa as shown below in fig. 2

Table 1. Observations of linearity study

Sr.No.	Concentration of GA µg/ml	Absorbance at 220 nm
1.	0.000	0.000
2.	4.000	0.274
3.	8.000	0.515
4.	12.000	0.752
5.	16.000	1.013
6.	20.000	1.228

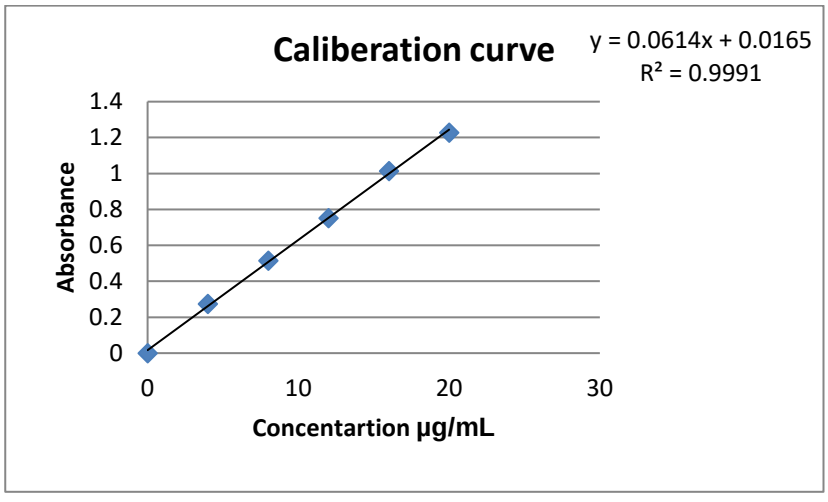


Fig 2. Calibration curve of Gallic acid

Formula:

Concentration of test solutions were calculated by using slope and intercept equation obtained from the calibration curve of gallic acid.

Slope and intercept equation: **y= m x + c**

Where, y is y axis value i.e. Absorbance of test solution;
x is x axis value i.e. Concentration of test solution

$$y=0.061\,x+0.016$$

$$x=\frac{y-0.016}{0.061}$$

preparation of bulk powder:

An accurately weighed quantity of gallic acid was mixed with excipients which are commonly used in formulations. This mixture was well triturated and uniformly mixed. Mixture was packed in air tight container.

Assay:

An accurate weight 100 mg of the sample was transferred to 100 ml volumetric flask. Small volume of water was added into it and shake for 5 minutes to dissolve Gallic acid. Excipients were filtered out and filtrate was diluted to 100 ml with water. Aliquots about 0.1 ml were taken in three different 10 ml volumetric flask and volume was made up to the mark with water. Absorbance of each solution was recorded at determined λ_{max} and the content of the gallic acid in a sample was found out.

validation:

Precision: The precision of the proposed method was ascertained by actual determination of three replicates of fixed concentration of the drug within the Beer's range and finding out the percentage purity by the proposed method. Standard deviation and % RSD were calculated for the results [7,17-19].

Accuracy: To ascertain the accuracy of the proposed methods, recovery studies were carried out by the standard addition method at three different levels (80%,100 % & 120 %) of concentration of test solution of the bulk powder [7,17-19].

Linearity and range study: Accurately weighed quantity of sample was diluted to obtain concentration in the range of 80-120 % of test concentration. Absorbance of each solution was recorded at 220 nm. It is found that sample obeys linearity over 80 -120 % of test concentration as shown in Figure [7].

sensitivity: Sensitivity of the proposed method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated using formulae [7,17-19]

LOD = $3.3 \times \frac{\sigma}{s}$

LOQ = $10 \times \frac{\sigma}{s}$

where 'σ' is the standard deviation (n=5) taken as a measure of noise,

and 's' is the slope of the corresponding calibration curve.

Table 2. Result of assay of gallic acid

Sr. No.	Wt. of sample in mg	Absorbance at 220 nm	Conc. (µg/ml)	% Drug	Estimation
1.	100	0.625	9.952	99.52	
2.	100	0.632	10.06	100.63	
3.	100	0.622	9.904	99.04	
			Mean	**99.73**	
Statistical values for n=3			**SD**	**0.815**	
			%RSD	**0.817**	

Table 3. Result of precision study

Sr. No.	Wt. of sample in mg	Absorbance At 220 nm	Conc. (µg /ml)	% Drug Estimation
1.	100	0.632	10.063	100.63
2.	100	0.626	9.968	99.68
3.	100	0.636	10.12	101.2
4	100	0.618	9.86	98.6
5	100	0.634	10.13	101.3
6	100	0.629	10.04	100.4
			Mean	**100.30**
Statistical values for n=6			**SD**	**1.020**
			%RSD	**1.016**

Table 4. Result of accuracy study

Sr.No	Sample %	Concentration (µg/mL)		% Recovery	Statistical Analysis
		Pure	Laboratory Mixture		
1	S1: 80	8	10	98.50	Mean: 98.91
2	S1:80	8	10	98.90	SD: 0.425
3	S1:80	8	10	99.35	%RSD: 0.429
4	S2:100	10	10	99.62	Mean: 99.39
5	S2:100	10	10	99.45	SD: 0.254
6	S2:100	10	10	99.12	%RSD: 0.255
7	S3:120	12	10	100.2	Mean: 99.82
8	S3: 120	12	10	99.75	SD: 0.345
9	S3:120	12	10	99.52	%RSD: 0.345

Table 5. Observation table of Linearity and Range Study

Sr. No.	%Label claim	Absorbance at 220 nm
1.	80%	0.510
2.	90%	0.562
3.	100%	0.635
4.	110%	0.678
5.	120%	0.750

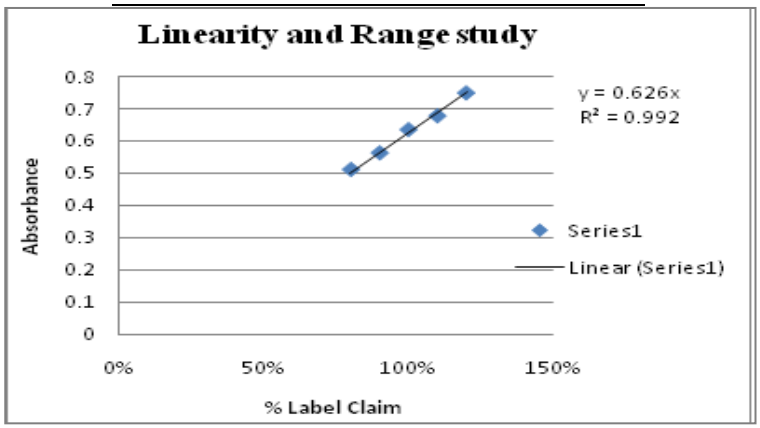


Fig 3. Linear curve of laboratory mixture

forced degradation study

Pure gallic acid was subjected to various stress condition. UV spectrum of each stressed sample was recorded and compared with standard to check stability of the compound. Absorbance of sample solutions were measured at 220 nm to determine the % degradation.

acid degradation: accurately weighed 10mg GA was taken in three 10 ml volumetric flask and dissolved in 0.1 N, 1N and 5 N HCL. Final volume was made up to 10 ml with respective acids. These solutions were refluxed for 8 hours at 80 ^{0} c. Aliquots about 0.1 ml were withdrawn from each flask, neutralized and diluted to 10 ml with water. UV spectrum was recored for each solution and compared with spectrum of pure Gallic acid [9,20].

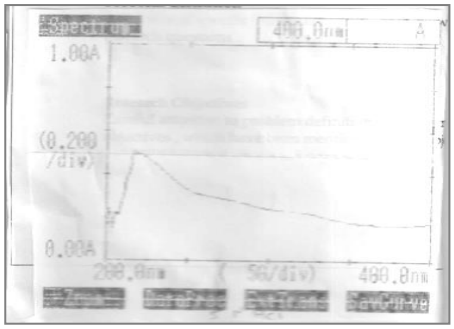


Fig 3. UV Spectrum of gallic acid sample in 5N HCL

Alkali degradation: Accurately weighed 10 mg compound was taken in three 10 ml volumetric flask and

dissolved in 0.1 N, 1N and 5 N NaOH. Final volume was made up to 10 ml with respective alkali. These solutions were refluxed for 8 hours at 80^0c. Aliquots about 0.1 ml were withdrawn from each flask, neutralized and diluted to 10 ml with water. Diluted solutions were scanned under 200-400 nm to record UV spectrum and compared with UV spectrum of pure gallic acid (9,20).

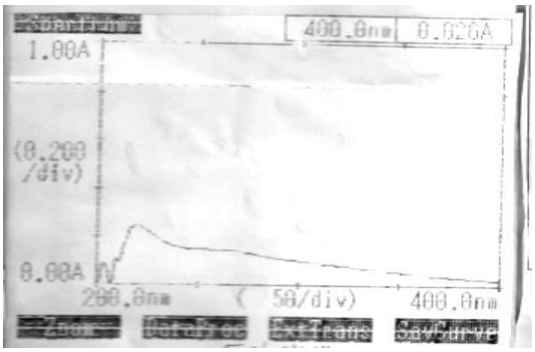


Fig 4. UV Spectrum of gallic acid sample in 5N NaOH

Thermal degradation: A specific amount of compound was taken in a cleaned Petridish and placed in an oven at 60^0 c for 8 hours. Sample withdrawn for 4 hours interval was transferred to different 10 ml volumetric flask and volume adjusted with water. Aliquots about 0.1 ml were withdrawn from each stock and diluted to 10 ml with water. Diluted solutions were scanned under 200-400 nm to obtain UV spectra which further compared spectrum of pure Gallic acid [11,20].

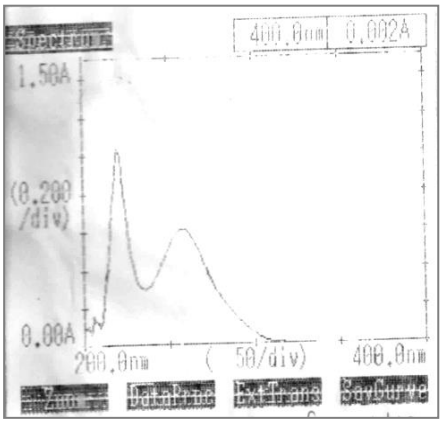


Fig 5. UV Spectrum thermal degraded sample after 8 hr

photodegradation: A specific amount of compound was taken in a cleaned petridish and exposed to sunlight for 8 hours. Sample withdrawn for 4 hours interval was transferred to different 10 ml volumetric flask and volume adjusted with water. Aliquots about 0.1 ml were withdrawn from each stock and diluted to 10 ml with water. Diluted solutions were scanned under 200-400 nm to obtain UV spectra which further compared spectrum of pure gallic acid [20,21].

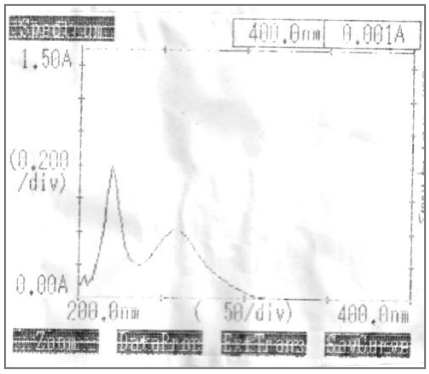


Fig 6. UV Spectrum of photodegraded sample after 8 hr

Oxidative degradation:

Accurately weighed 10 mg GA was taken in two 10 ml volumetric flask. One ml of methanol was added in each to dissolve the drug. Then 0.3 % v/v and 3% v/v H_2O_2 was added separately to make volume up to the mark. Thereafter solutions were kept for refluxing for 8 hours at 80 °C in water bath. 100 µl portions from each solution were withdrawn and further diluted to 10 ml with distilled water. The resulting solution was scanned in the range of 200 - 400 nm against blank prepared by the same way [9,20].

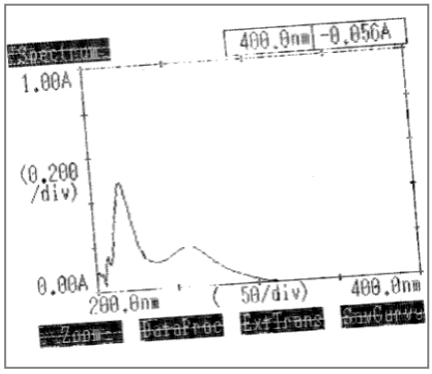


Fig 7. UV Spectrum of gallic acid in Hydrogen peroxide 0.3% v/v

Table 6. Summary of forced degradation study

Sr. no	Stress Conditions	Strength,Time and Temperature	Absorbance (mean for n=5) 220 nm	Concentration of gallic acid µg/mL	Degrdation % w/w	%RSD for n=5
1	Acidic degrdation	5 N HCL (for 8 hrs at 80^0c)	0.524	8.25	17.5	1.03
2	Alkaline degrdation	5 N NaOH (for 8 hrs at 80^0c)	0.320	4.45	55.5	0.99
3	Oxidative degradation	0.3 % H_2O_2 (for 8 hrs at 80^0c)	0.443	7.15	28.5	0.79
4	Thermal degradation	60^0c for 8 hrs	0.654	10.15	No degradation	0.657
5	Photolytic degradation	Sunlight for 8 hrs	0.631	10.02	No degradation	0.634

RESULT AND DISCUSSION

A simple, sensitive and appreciable stability indicating UV spectrophotometric method has been developed for estimation of Gallic acid in bulk sample. the absorption maxima 220 nm was selected for the study. Absorbance of standard solutions in the concentration range of 4-20 µg/ml was found linear with R^2 value 0.998. The result was shown in table 1.

The method was fast and economical and it was also selective and sensitive for the desirable range. Proposed method was validated as per ICH guidelines for precision, accuracy, linearity and sensitivity. Results of study of the aforesaid validation parameters were found satisfactory as statistical values being in range. The method was found precise and reproducible with % RSD value 0.762 as shown in table 3. The accuracy of method was assured by standard addition method and estimating percentage recovery. The method was found accurate and remain unaffected from interfering substances. The results are shown in table 4. Sensitivity of method was determined from linearity equation $y = 0.061\ x + 0.016$. LOD and LOQ were found to be 0.147 µg/mL and 0.447 µg/mL respectively.

Stability indicating capability of proposed method was assured by forced degradation study. The study was carried out by subjecting gallic acid to various stress conditions e.g. Acidic and basic pH, oxidative, thermal and photo degradations. UV spectrum of acidic, alkaline and oxidative stressed sample were not match with spectrum of pure Gallic acid. Therefore, it could be concluded that Gallic acid underwent acid, alkali and oxidative degradation. However, UV spectrum of thermal and photolytic stressed sample found identical to standard spectrum indicating thermal and photo stability of gallic acid at 80 °c up to 8 hrs. The spectrum of individual sample at various stress conditions are shown in above figures. Degradation in alkaline condition (5 N NaOH) was found to be 55.5 % w/w. Significant degradation occurred at acidic (5 N HCL) and oxidative 0.3 % v/v H_2O_2 condition with % degradation 17.5 % w/w and 28.5 % w/w respectively. Summary of forced degradation study was shown in table 5.

CONCLUSION:

The proposed analytical method was validated as ICH Q2 guidelines. It was found simple, rapid, precise, accurate, sensitive and linear. The present study has also proven stability indicating potential of method. This proposed UV spectrophotometric SIM can be used in routine analysis of gallic acid.

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