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ESTIMATION OF GLYCYRRHETINIC ACID AND GALLIC ACID IN AYURVEDIC PROPRITERY MEDICINE BY UV- SPECTROPHOTOMETRY

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Keywords:

Polyherbal churna, Glycyrrhetinic acid ,Gallic acid, UV spectrophotometer Simultaneous equation, Validation

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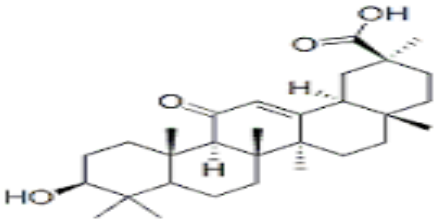
ABSTRACT

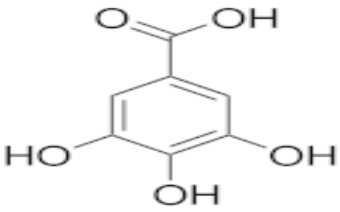
A simple, rapid, accurate, precise, and economic spectrophotometric method for simultaneous estimation of Glycyrrhetinic acid and Gallic acid in Polyherbal churna have been developed. The hydroalcoholic extract of developed churna was obtained by continuous heat extraction method. The method was validated using parameters as linearity, precision, limit of detection, limit of quantification and recovery as per ICH guidelines[9]. Method is based on solving simultaneous equation. Glycyrrhetinic acid and Gallic acid show absorbance maximum at 254nm and 273nm respectively, The concentration of Glycyrrhetinic acid and Gallic acid was found to be 1.055±0.011w/w and1.074±0.014w/w in Polyherbal churna. The samples were prepared in methanol and methods obey Beers-Lamberts law in concentration ranges employed for evaluation. Hence, the proposed method can be used for the reliable quantification of active marker compounds in crude drug and its herbal formulations.

INTRODUCTION

Polyherbal churna is well known Ayurvedic Proprietary formulation, traditionally used as Anti-diabetics[11], Anti-oxidant[12]. It consist of ingredients *Glycyrrhiza glabra*[1], *Emblica officinalis*[2],*Gymnema Sylvester*[3],*Curcuma longa*[4], *Azardichata indica*[5], *Syzygium jambulanum*[6.] For standardization of natural products, crude drugs, single chemical entities, "marker compounds", may be used as potency standards in U.V. analysis. The checking of herbal drugs used in the preparation can be checked scientifically through certain well established norms and standards through the research works.



Glycyrrhetinic acid



Gallic acid

Glycyrrhetinic acid is chemically 3b-Hydroxy-11-oxo-18b,20b-olean-12-en-29-oic acid and Gallic acid is chemically 3,4,5-Trihydroxybenzoic acid.

The *Glycyrrhiza glabra*[1] contains starches (30%), pectins, tannins, glycosides, protein, volatile oils. Glycyrrhizin (glycyrrhizic acid, glycyrrhizinate) constitutes 10–25% of liquorice root extract.It is a saponin compound (60 times sweeter than cane sugar) comprised of a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid; enoxolone) conjugated to a disaccharide of glucuronic acid. *Emblica officinalis*[2] contains Vit.c ,Gallic acid , Ellagic acid , Rutin , Quarcetin, Phyllembin, Ethy gallate, *Gymnema Sylvester*[3]contains olanane type triterpenoids saponines known as gymnemic acid .Other plant constituents are flavones , antraquinones. *Curcuma longa*[4] contains Turmeric contains 5% of volatile oil, resin,zingiberaceous starch grains & yellow coloured curcuminoids. The chief components of curcuminoids is known as curcumin. *Azardichata indica*[5] contains nimbin , nimbinene, azadirachtin, azadirachtol, azadirachtol, nimbandiol , nimbolide, quercetin, betasitosterol, n-hexacosanol, nimbocin. *Syzygium jambulanum*[6] contains Gallic acid , Ellagic acid , Rutin , Quarcetin, glucoside such as jamboline , phenolic constituents.

Glycyrrhiza glabra used as Antidiabtic, powerful antioxidant activity, astringent ,Anti-tussive & expectorant activity, Skin lightening and skin tightening activity. *Emblica officinalis* used as Liver toni**c** , Mild laxative , Appetizer , Digestant, Antioxident. *Gymnema Sylveste* used as in hyperglycemia ,obesity, high cholesterol levels, anemia,and digestion ,useful in

hepatosplenomegaly, dyspepsia, constipation. *Curcuma longa,* used as Antidiabtic, antioxidant, antiallergic, anti-inflammatory ,antimicrobial ,aromatic, stimulant tonic,carminative. *Azardichata indica,* used as Antidiabetic , treatment of worm , jaundice , external ulcers , cardiovascular disease, malaria , rheumatism and skin disorders. *Syzygium jambulanum* used as Antidiabtic, antidiarrhoel, antioxidant , antiallergic , astringent , analgesic , anti-inflammatory, antiplaque, antimicrobial.

Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis[7] using modern analytical techniques. The method is developed in solvent Methanol. Method validation is done as per ICH guidelines.

MATERIAL AND METHODS

Apparatus:

Instrument used was an UV/Visible double beam spectrophotometer, SHIMADZU model 1800 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. An electronic analytical balance was used for weighing the sample.

Reagents and Materials:

Ayurvedic Proprietary Polyherbal churna was procured from Sahakar Medical,Islampur. All the chemicals and solvents were used of L.R. grade; standard. Glycyrrhetinic acid was procured as gift sample from GCI nutrients India pvt. Ltd. Mumbai and Gallic acid was procured as gift sample from Loba chemical Mumbai and solvent are procured from S.D. fine-chemicals limited, Mumbai.

Preparations of extract of Polyherbal churna :

The 100g of Polyherbal churna was extracted with a mixture of 95% methanol & water (75:25) at 50-60 ^{0}C in a soxhlet apparatus separately.The extract was obtained concentrated to dryness in heating mental at a temperature of 35-40 ^{0}C.The dried of the hydroalcoholic extracts weighed in a required dose and it was dissolved in known volume of distilled water, separately for further treatment.

Preparation of standard stock solution of Glycyrrhetinic acid and Gallic acid*:*

The stock solution (100µg/ml)of Glycyrrhetinic acid was prepared by dissolving accurately about 10mg of drug in sufficient quantity of methanol and then volume was adjusted to 100ml with methanol. Further series of dilution were made with methanol.

The stock solution (100µg/ml)of Gallic acid was prepared by dissolving accurately about 10mg of drug in sufficient quantity of methanol and then volume was adjusted to 100ml with methanol. Further series of dilution were made with methanol.

Calibration curve of Glycyrrhetinic acid and Gallic acid:

A series of calibrated 10ml volumetric flask was taken and appropriate aliquots of the working standard solution of Glycyrrhetinic acid and Gallic acid was withdrawn and diluted up to 10ml with methanol. The absorbance was measured at absorption maxima 254 nm and 273 nm against the reagent blank prepared in similar manner without the Glycyrrhetinic acid and Gallic acid .Absorption maxima and Beer's law limit were recorded and data that prove the linearity and obeys Bee'r law; limits were noted..

Estimation of Glycyrrhetinic acid and Gallic acid in Polyherbal churna[8]:

The appropriate aliquots from extract of Polyherbal churna was withdrawn in 10ml volumetric flask separately absorbance for aliquots of each was noted at 254nm for Glycyrrhetinic acid and at 273nm for Gallic acid. The corresponding concentration of Glycyrrhetinic acid and Gallic acid against respective absorbance value was determined by using the Glycyrrhetinic acid and Gallic acid calibration curve. The statistical analysis for checking uniformity in batches was also performed.

Table no 1: Content of Glycyrrhetinic acid and Gallic acid in Polyherbal churna

Polyherbal churna	Glycyrrhetinic acid content %w/w	1.055±0.011w/w
	Gallic acid content %w/w	1.074±0.014w/w

Validation of developed method [10]:

Linearity and range:-

The standard stock solution containing 100µg/ml each of Glycyrrhetinic acid ; was further diluted to get linearity concentration of 2-20µg/ml for Glycyrrhetinic acid and The standard stock solution containing 100µg/ml each of Gallic acid; was further diluted to get linearity concentration of 1-10µg/ml for Gallic acid. Each concentration was analyzed in triplicates. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The relation between drug and its absorbance was expressed by equation y = mx+b, where m=slope, and b= intercept.

Limit of detection and limit of quantitation:-

LOD and LOQ of the drug were derived by calculating the signal-to-noise ratio (S/N, 3.3for LOD and 10 for LOQ) using the following equation designated by ICH guidelines[9].The

residual standard deviation of regression line or standard deviation of y intercept of regression lines was used to calculate LOD and LOQ.

LOD = 3.3 x D/S

LOQ = 10 x D/S

Where, D=Standard deviation of y intercept of regression lines & S =Slope of calibration curve.

Recovery studies:-

It was carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed sample and percentage recoveries were calculated.

Precision:-

The precision of the method was determined by carrying analysis of binary mixture of Glycyrrhetinic acid and Gallic acid on same day (intra day precision) and on consecutive days (inter day).

Ruggedness study:-

It expresses the precision within laboratories variations like different analyst. Ruggedness of the method was assessed by spiking the standard 3 times with different analyst by using same equipment.

RESULT AND DISCUSSION

The proposed method was validated as per ICH guideline[9]. Method discussed in present work provides convenient and accurate way for simultaneous analysis of Glycyrrhetinic acid and Gallic acid.

Glycyrrhetinic acid and Gallic acid obeys Beer Lambert's law in concentration range 2-20µg/ml and 1-10µg/ml at the λ_{max} 254nm and λ_{max} 273nm. The correlation coefficiant (R^2) was calculated, where the (R^2) value 0.999 for Glycyrrhetinic acid and 0.994 for Gallic acid indicates the good linearity between the concentration and absorbance. The estimation of Glycyrrhetinic acid and Gallic acid in MMC was carried out.

The concentration of Glycyrrhetinic acid and Gallic acid present in raw material was found to be 1.056w/w and 1.074w/w respectively in MMC.

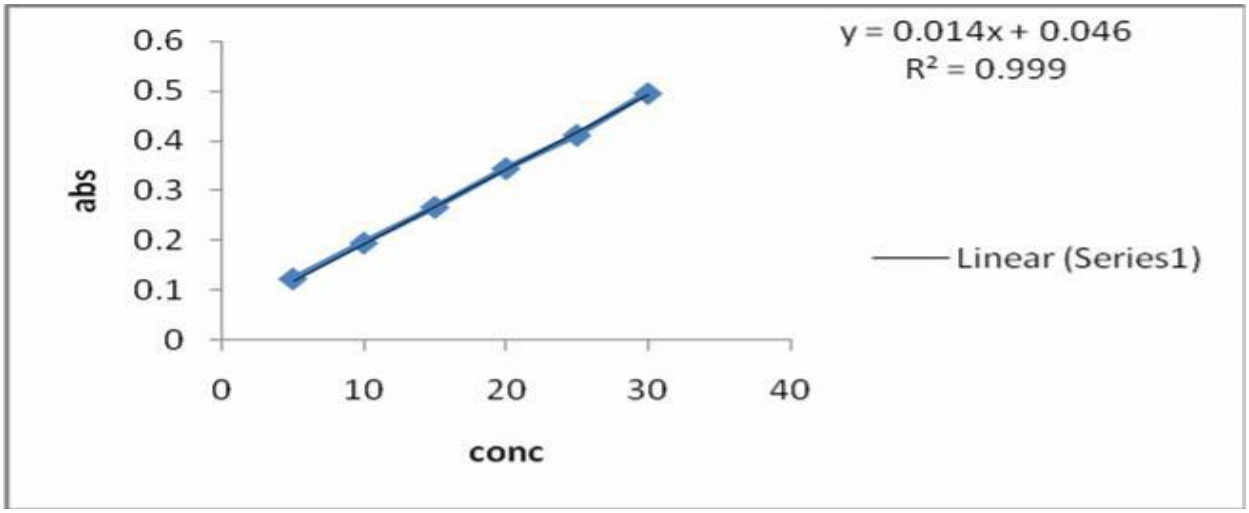


Fig.1. Calibration curve of Glycyrrhetinic acid

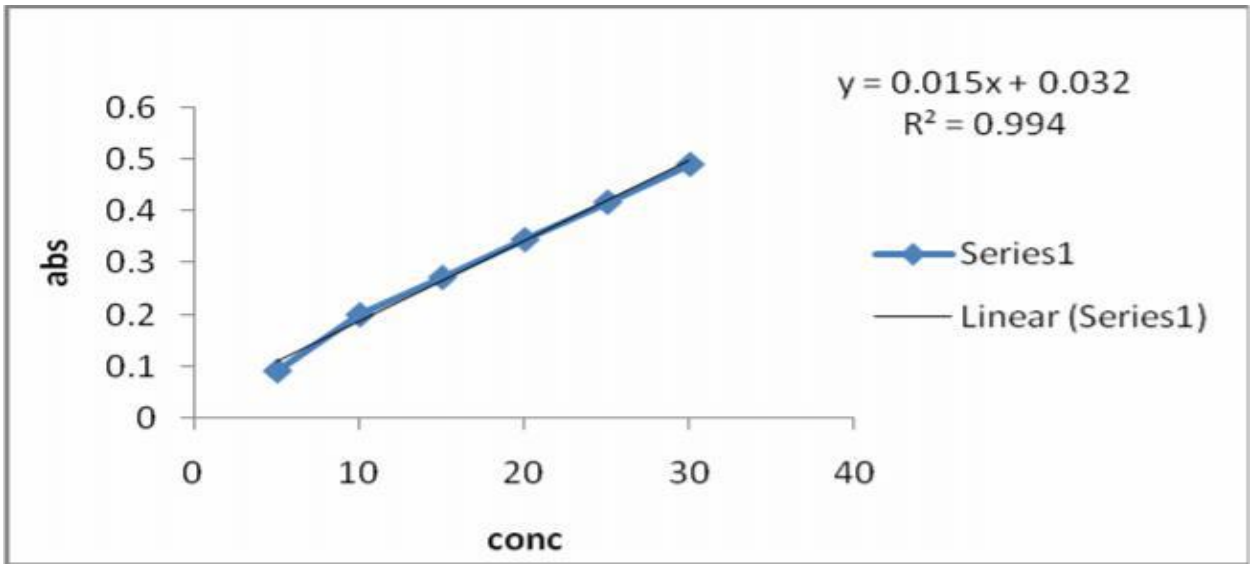


Fig.2. Calibration curve of Gallic acid

Table no 2. Result of Validation parameters

Parameters	Glycyrrhetinic acid	Gallic acid
Detection wavelength	254nm	273nm
Linearity range	2-20 µg/ml	1-10 µg/ml
Slope	0.014	0.015
Intercept	0.046	0.032
Correlation coefficient	0.999	0.994
Regression equation(y =a+ bc)	Y=0.014x-0.046	Y=0.015x-0.032
Limit of detection	0.13	1.53
Limit of quantitation	0.39	2.21

Precision is determined by studying the interday and intraday precision. In both intra and inter day precision study for both the methods % RSD are not more than 2.0% indicates good repeatability and intermediate precision.

Table no 3. Interday and Intraday precision

Parameters	Intraday *		Interday*	
	Glycyrrhetinic acid	Gallic acid	Glycyrrhetinic acid	Gallic acid
Mean	99.86	99.99	98.90	99.84
SD	0.1798	0.2042	0.2763	0.1798
%RSD	0.1802	0.2044	0.2897	0.1803

***Average of three determinations**

Table no 4 . Recovery studies

Level of % recovery	%mean recovery*		SD*		%RSD*	
	Glycyrrhetinic acid	Gallic acid	Glycyrrhetinic acid	Gallic acid	Glycyrrhetinic acid	Gallic acid
80	100.33	101.92	0.0702	0.1113	0.070	0.1092
100	98.33	101.61	0.4252	0.1345	0.4324	0.1279
120	98.46	101.33	0.3082	0.0929	0.3131	0.0914

***Average of three determinations**

Table 5: Ruggedness study

Polyherbal churna	Drug	%Amount found ± S.D*
Analyst 1	Glycyrrhetinic acid	100.33
	Gallic acid	101.92
Analyst 2	Glycyrrhetinic acid	99.86
	Gallic acid	101.33

***Average of three determinations**

CONCLUSION

Development and validation of spectrophotometric method for the estimation of Glycyrrhetinic acid and Gallic acid in Polyherbal churna could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations.After the drug is approved, pharmaceutical validation and development is necessary to ensure that the drug product will meet pharmaceutical standards for identity, strength, purity, stability, evaluation

safety and efficacy. It provides strength and certain assurance of quality products. UV analysis is most useful for quantitative estimation of target molecules in herbal products.UV detection of such compound is primary screening for further analysis of same by chromatoraphical technique.

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for simultaneous estimation of Glycyrrhetinic acid and Gallic acid in Polyherbal churna .

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