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UV-spectrophotometric method development for estimation of glycyrrhetinic acid in Pratishyayghna kwath

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ABSTRACT

A simple and reproducible UV- spectrophotometric method for the quantitative determination of Glycyrrhetinic acid in Pratishyayghna kwath (PK) were developed and validated in the present work. The parameters linearity, precision, accuracy, and standard error were studies according to Indian herbal pharmacopiea. In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of Glycyrrhetinic acid in laboratory herbal formulation of Pratishyayghna kwath. Each ingredient was purchased from the local market and they were evaluated as per Indian herbal Pharmacopoeia and WHO guidelines. The % w/w content of Glycyrrhetinic acid in the laboratory preparations of Pratishyayghna kwath PK-I, PK-II, PK-III were found to be 1.27±0.03 %, 1.29±0.02 %1.25±0.04 % respecti vely. Glycyrrhetinic acid has the maximum wavelength at 204 nm and hence the UV spectrophotometric method was performed at 204 nm. The samples were prepared in methanol and method obeys Beers law in concentration ranges 10- 50µg/ml. The content of Glycyrrhetinic acid in ayurvedic formulation was determined. The results of analysis have been validated statistically and confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of Glycyrrhetinic acid in herbal formulation.

Keywords: Pratishyayghna kwath, finger printing, Glycyrrhetinic acid, UV Spectrophotometer etc.

INTRODUCTION

Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological activities, higher safety margins and lesser costs. In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is about $ 80 million[.1] The recent global resurgence of interest in herbal medicines has led to an increase in the demand for them. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Commercialization of the manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine[2]. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration, variation in composition and level of active constituents due to variation in climatic conditions. Also variation in the chemical profile of the herbal formulations is due to the factors like growing, harvesting, storage and drying processes. Therefore quality control of herbal medicines offers a host of problems. It is very important that a system of standardization is established for every plant medicine available in the market because the scope for variation in different batches of medicine is enormous[3-6].The

formulation of Pratishyayghna kwath(PK) is well known ayurvedic formulation is official in Ayurvedic text "Sar Sangarh", traditionally used for cough and cold[7] .Through it is very popular medicine, no establishment of quality control for this drug studies have been performed yet. This paper reports on instrumental methods for ensuring the Identity, Potency, Purity, Safety and efficacy of the Pratishyayghna kwath. This paper includes estimation of Glycyrrhetinic acid for different batch sample of Pratishyayghna kwath by UV spectroscopic methods. Spectroscopic studies were carried out to develop the spectrum of the formulation and validated by overlain and linearity study (Beer's law).The results of all batches were found in close proximity with each other.

The present study is an attempt to develop the fingerprinting method for Pratishyayghna kwath (PK) by spectrophotometric determination using as Glycyrrhetinic acid as a standard, which is an important and major content in formulation. The developed spectroscopic fingerprints can be used as a standard Glycyrrhetinic acid and can be used as a possible marker compound for fingerprinting of Pratishyayghna kwath.

MATERIALS AND METHODS

Procurement of crude drug

Pratishyayghna kwath consists of 7 ingredients *Glycyrrhiza glabra* (Mulethi), *Vitis vinifera* (Munnakaa), *Cordia dichotoma* (Lisoda), *Viola odorata* (Banafsha), *Saccharum officinarum* (Sugar crystal), *Piper nigrum* (Kalimarich)[8-12]. All these 7 ingredients were procured from local market and identified morphologically and microscopically and compared with standard pharmacopoeial monograph*. Sample of crude drug were also authenticated by Botany department from Saifia science collage Bhopal (M.P).

Preparation of Pratishyayghna kwath

Pratishyayghna kwath was prepared according to the procedure to the mentioned in "Sar sangarh". The ratio of ingredient has been showed in (Table 1).Each ingredients were coarsely powered individually and weighed and mixed. Then boiled 1 tola (11.66gm) of vegetable substance in coarse form with 16 parts with portable water in earthen pot until the volume reduced up to 1/4th from the initial quantity. After desirable reduction of volume, the Kwath was filtered through muslin cloth and collected in separate vessel. The residue remained above cloth was discarded[7]. The results obtained during the preparation of kwath have been tabulated in (Table 2).

Table-1 Quantity of Each Ingredient of PK

S.No	Common Name	Parts	Ayurvedic system	In metric system
1.	Mulethi	Roots	1 Tola	11.66 gm
2.	Munnakaa	Fruits	1 Tola	11.66 gm
3.	Lisoda	Fruits	1 Tola	11.66 gm
4.	Adusa patr	Leaves	1 Tola	11.66gm
5.	Banafsha	Leaves	1 Tola	11.66 gm
6.	Kalimarich	Fruits	6 Masa	5.83 gm
7.	Mishri	Sugar crystal	6 Masa	5.83 gm
8.	Gulbanafsha	Flowers	1 Tola	11.66 gm
9.	Water	-	16 Tola	186.56 gm

Table-2 Observation obtained during the preparation of PK

S.No	Parameter	Observation
1.	Initial qty of churna for kwath preparation (gm)	11.66±0.078
2.	Total qty of water (ml)	186.56±0.024
3.	Temp during preparation of Kwath ^{0}C	97±0.707
4.	Total time for 1/4th reduced (min)	45±0.707
5.	Total qty of Kwath obtained (ml)	45±0.282

Chemicals

All the chemicals and solvents were used of A.R. grade, standard Glycyrrhetinic acid was procured from Yuccoo Enterprises, Mumbai.

Preparation of Standard Stock Solution of Glycyrrhetinic acid: - Standard Glycyrrhetinic acid (100 mg) was dissolved in 100 ml of methanol to prepare stock solution with concentration of 1000µg/ml.

Preparation of Dilution:
For the preparation of calibration curve a series of dilution with concentration of 10, 20, 30, 40, 50µg/ml were prepared by taking aliquots of 0.1, 0.2, 0.3, 0.4, 0.5ml of stock solution (1000µg/ml) and diluted up to 10 ml with methanol in 10 ml volumetric flask. The absorbance of Glycyrrhetinic acid standard solutions was measured at 204 nm against methanol as blank. Calibration curve was plotted between absorbance and concentration.

Preparation of Sample Solution:-
Weighed 11.66 gm Pratishyayghna churna and extracted with 186.56ml methanol for 45 min by decoction method. Filter the extract and concentrated the methanolic extract till semisolid mass is obtained. Then it dried to obtain 0.820 mg extract. Taken 100 mg extract was dissolved in 100 ml methanol to preparation sample stock solution. For determination of concentration of Glycyrrhetinic acid in formulation taken 0.2 ml stock solution in 10 ml volumetric flask and make the volume with methanol. Then it was filtered through a syringe filter and analysed with UV Spectroscopy[13].

Experimental
Calibration curve from standard solution of Glycyrrhetinic acid was prepared and with the help of this curve the content of Glycyrrhetinic acid from Pratishyayghna kwath was estimated. The method was validated for precision and accuracy.

Calibration curve of Glycyrrhetinic acid in Pratishyayghna kwath
A series of calibrated 10ml volumetric flask were taken and appropriate aliquots of the working standard solution of Glycyrrhetinic acid were withdrawn and diluted up to 10ml with methanol the absorbance was measured at absorption maxima 204 nm, against the blank reagent prepared in similar manner without the Glycyrrhetinic acid. The absorption maxima and Beer's law limit were recorded and data that prove the linearity and obey Beer's law limit were noted. The linear correlation between these concentrations (x-axis) and absorbance(y-axis) were graphically presented and slope(b), intercept(a), and correlation coefficients (r2) were calculated for the linear equation (Y=bx+a) by regression using the methanol as a blank solution. Table-3

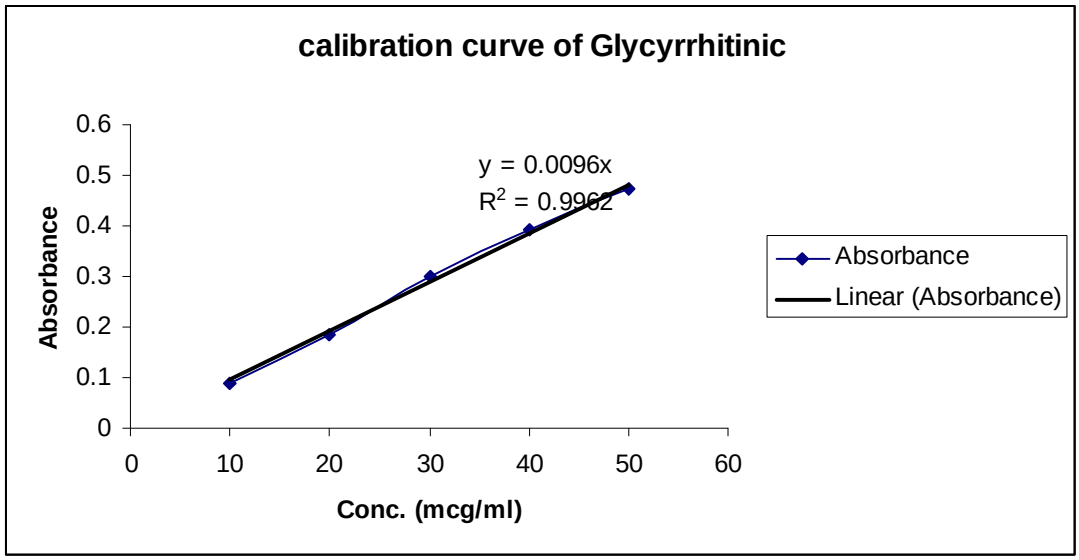
Validation parameters of Glycyrrhetinic acid in PK Table-3

S.NO.	Parameters	Value
1.	Absorption maxima	204 nm
2.	Beer's law limit	10-50ug/ml
3.	Regression equation(y=bx+a)	0.009x-0.003
4.	Intercept(a)	- 0.003
5.	Slope(b)	0.009
6.	Correlation coefficients(r2)	0.997
7.	Precision (n=6, % RSD)	0.17
8.	Accuracy (%)	99.03

Observation of UV Spectroscopy of Glycyrrhetinic acid

S.NO.	Concentration(µg/ml)	Absorbance
1.	10	0.087
2.	20	0.185
3.	30	0.301
4.	40	0.391
5.	50	0.475

Calibration Curve of Glycyrrhetinic acid



Estimation of glycyrrhetinic acid in Pratishyayghna kwath

S.NO	Formulation	Glycyrrhetinic acid% w/w
1.	Pratishyayghna Kwath	1.27±0.03
2.		1.29±0.02
3.		1.25±0.04

(Mean% ±SD, n=3)

RESULTS AND DISCUSSION

The method involves measurement of UV absorbance at 204nm for Glycyrrhetinic acid corresponding to the absorption maxima of the herbal formulations. The absorbance characteristics showed that Glycyrrhetinic acid obeys Beer Lambert's law within the concentration range 10-50 µg/ml at the λ-max of 204nm with the regression value of 0.997 and calibration equation Y = 0.009x-0.003. The % w/w content of Glycyrrhetinic acid in the Laboratory preparations of Pratishyayghna Kwath PK-I, PK-II and PK-III were found to be1.27±0.03 %, 1.29±0.02 %1.25±0.04 % respectively. Almost similar results were obtained for two days of all three samples which indicating the reproducibility of the method.

CONCLUSION

The developed method was found to be accurate, simple, precise and rapid. It can therefore be applied for routine analysis of Glycyrrhetinic acid in polyherbal formulations containing Glycyrrhetinic acid.

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