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Validated UV spectroscopic method for the estimation of three marker compounds in marketed polyherbal ayurvedic formulation

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ABSTRACT

The modern methods of analysis are essential for qualitative and quantitative determination of herbal formulations and standardization as per the WHO guidelines. In present study simple and precise UV spectroscopic method was developed and validated as per ICH guidelines for the estimation of the active constituents in the polyherbal Ayurvedic. The method was developed using methanol: water (50:50) mixture as solvent. The recovery studies were performed by standard addition method. The method was found to be validated and can be used for routine analysis of herbal formulation containing withanolide, colchicine and gingerol.

Keywords: Polyherbal formulation, Withanolide, Colchicine, Gingerol

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INTRODUCTION

The polyherbal tablet formulation containing three herbs such as *Withania somnifera*, *Colchicum luteum* and *Zingiber officinalis* was selected for the study. The formulation is prescribed mainly for rheumatic conditions and vata diseases and is having a good sale in the market. The marker selection for method development based on the amount present in the herb and responsible for showing the activity. The marker compound selected were from *Withania somnifera* was *withanolide*, from *Colchicum luteum* was colchicine and from *Zingiber officinalis* was gingerol. The various methods such HPLC, HPTLC are reported for analysis of these markers in various herbal formulations[1-5].

The herbal products are usually a mixture of two or more herbs and responsible for a variety of action. The effect of herbal formulation depends on the amount of active constituent present in it. The raw material collected from the natural source may vary in the composition of active constituent responsible for the action. So it becomes necessary to quantify the active constituent in polyherbal formulation[7]. The modern method of analysis, such as chromatographic methods are costly and time consuming, where as a UV spectroscopic method is simple, rapid, sensitive, rugged, robust and gives results in a short duration of time. Since UV spectroscopic method was not reported for analysis of these markers in polyherbal formulation, the objective was to develop validated UV spectroscopic method for analysis of marketed polyherbal formulation.

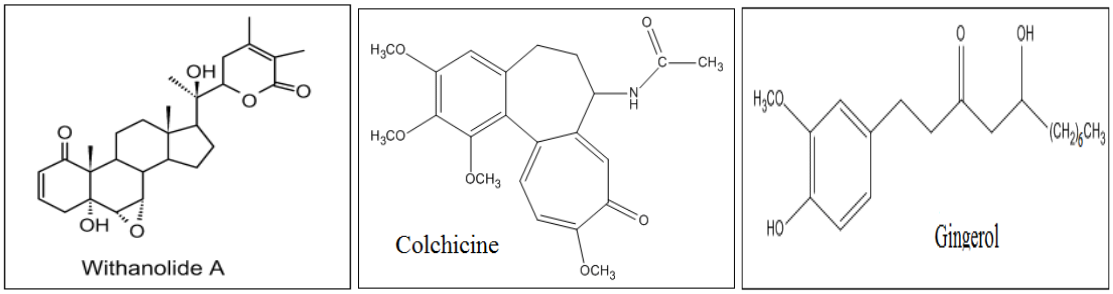

Figure 1: Chemical structure of marker

MATERIALS AND METHODS

Instrumentation-
UV-Visible Spectrophotometer single beam (model- UV mini 1240 Shimadzu, Japan) and Double beam (model- UV-1650PC Shimadzu, Japan) with matching quartz covet was used to measure absorbance.

Chemicals
Authenticated marker compounds were used for the method development. All chemicals used were of analytical grade. The marketed polyherbal formulation was used for the estimation of the drug content.

Method Development-
Preparation of standard solution-
100mg of each marker compound was dissolved in 100ml solvent methanol: water (50:50) to produce a stock solution of 1000 µg/ml.

Development of UV method for estimation of Withanolide, Colchicine and Gingerol as per ICH guidelines [8]

Table 1: Summary for the dilutions of markers

Sr.No.	Marker	Stock solution	Dilution Range	λ_{max}
1.	Withanolide	1000 µg/ml	5-30 µg/ml	223nm
2.	Colchicine	1000 µg/ml	2-10 µg/ml	247nm
3.	Gingerol	1000 µg/ml	10-60 µg/ml	279nm

Linearity- The working dilution were prepared by taking suitable aliquots of the sample from the standard and absorbance was recorded on the UV spectrophotometer. The graph of conc. V's absorbance was plotted and the regression coefficient (R^2) was calculated.

Precision- Precision of the analytical method was studied by multiple measurement of homogeneous sample; 7 replicates of 30**,** 6 and 50 µg/ml solution of withanolide, colchicine and gingerol respectively were prepared and absorbance was recorded. The intra-day and inter-day precision was used to study the variability of the method. The absorbance of precisions dilution was recorded after a time interval of 6hrs and 24 hrs. SD and % RSD were calculated.

Ruggedness & Robustness – Ruggedness of the method was determined by measuring absorbance of precision dilutions on the single beam UV mini 1240. SD and % RSD between single beam and double beam were calculated. For the robustness study dilution was prepared in 0.1M HCl and absorbance was recorded.

Accuracy-
1. For Withanolide
Alkaloid from Withania somnifera- The powder material was moistened with distilled water mixed with lime and treated with ammonia solution. The lime combines with acids, tannins and other phenolic substances and set free the alkaloids. The liberated free alkaloid bases were extracted with chloroform. The above chloroform extract was concentrated and then shaken with aqueous acid and allowed to separate. Alkaloid salts are now in the aqueous

liquid phase while many impurities remain behind in the organic liquid. The aqueous liquid was collected and treated with dilute ammonia; the liberated free alkaloid extracted with chloroform, which was further evaporated to give pure alkaloid. [9-10].The extract obtained used for accuracy study.

Preparation of test solution- 10mg extract dissolved in 100 ml of solvent to produce 100ppm solution.

Procedure – Accuracy study was performed by the standard addition method at 3 different levels (50%, 100% and 150%). The amount of test solution was kept constant (1ml) and standard solution volume varied at 3 different levels (0.05, 0.1, 0.15 ml). The % recovery was calculated.

2. For Colchicine

Extraction of colchicine from tablet-

The tablet powder equivalent to 100mg of colchicine was exhaustively extracted with ethanol. Alcoholic extract was concentrated and dried to syrupy residue. The residue was dissolved in water to precipitate the insoluble fats and resins. The filtered aqueous extract was then repeatedly extracted with chloroform and digested with lead carbonate. It was filtered, evaporated to a small volume and further extracted with chloroform. The chloroform was distilled off and the amorphous colchicine was recovered after solvent evaporation was crystallized from ethyl acetate as pale yellow needles [9-10].

Preparation of test solution- 10mg of colchicine extract was weighed and dissolved in above mentioned solvent system and volume made up to 100ml. The resultant solution was used for the accuracy study.

Procedure **–** Accuracy study was performed by the standard addition method at 3 different levels (50%, 100%, 150%). The volume of test solution was kept constant (0.4ml) and volume of standard was varied (0.02, 0.04, 0.06 ml). The resultant solution was diluted upto 10ml and absorbance was recorded at 247nm on UV spectrophotometer against reference. The % recovery was calculated.

3. For Gingerol

For accuracy study gingerol was extracted from tablet by the solvent extraction method. Tablets were crushed and powder equivalent to 100mg of gingerol extracted with 95% ethanol to give alcoholic extract. Solvent was evaporated by vaccum distillation to give thick pasty mass. Then thick pasty mass suspended in water. The suspended oleoresin was extracted with solvent ether, which was further evaporated to dryness to give total ginger oleoresin [10].

Preparation of test solution- 10mg extract dissolved in 10 ml of solvent to produce 1000ppm solution.

Procedure – Accuracy study was performed by the standard addition method at 3 different levels (50%, 100%, 150%). The amount of test solution was kept constant (0.6ml) and standard solution volume varied at 3 different levels (0.3, 0.6, 0.9ml). The absorbance was recorded at 279nm on UV spectrophotometer against reference. The % recovery was calculated.

RESULTS

Determination of λ_{max}

The dilute marker solution was scanned in the range of 200-400nm to determine absorption maxima. The λ_{max} was found to be 223nm, 247nm, 279nm for Withanolide, Colchicine and Gingerol respectively.

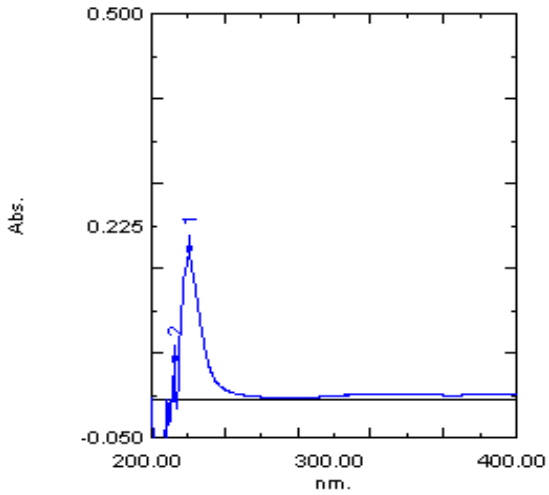

Figure 2: UV spectrum of Withanolide

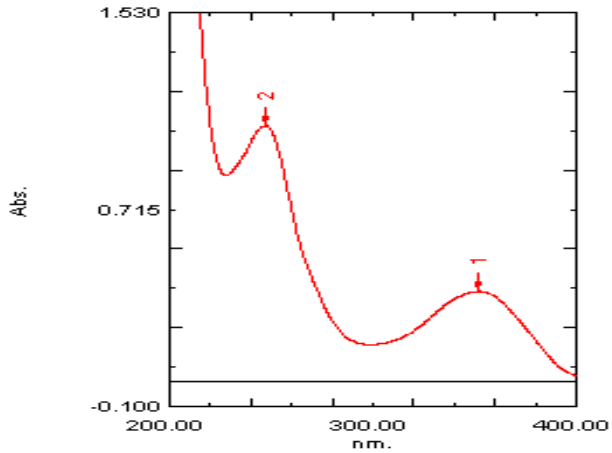

Figure 3: UV spectrum of Colchicine

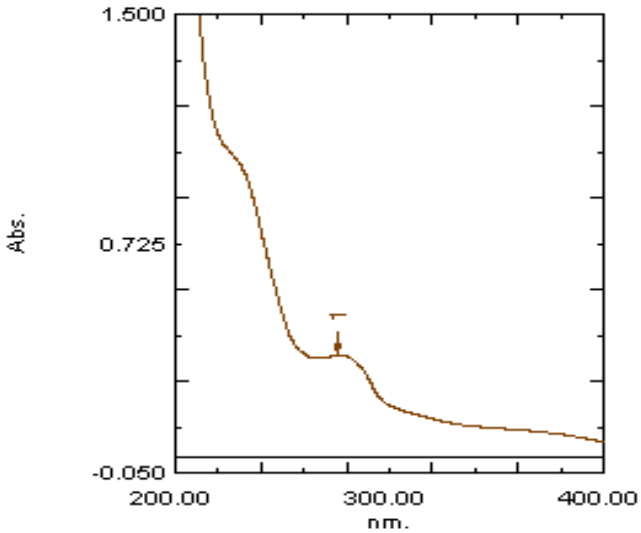

Figure 4: UV spectrum of Gingerol

Preparation of Calibration curve

The different dilutions for the each marker was prepared across the range for each and absorbance was measured. The calibration curve was plotted as concentration versus absorbance.

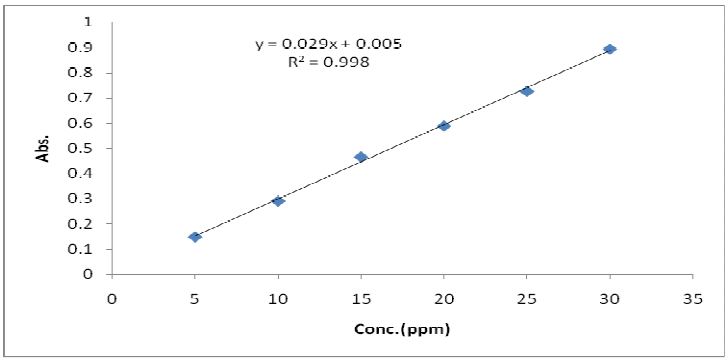

Figure 5: Calibration curve withanolide

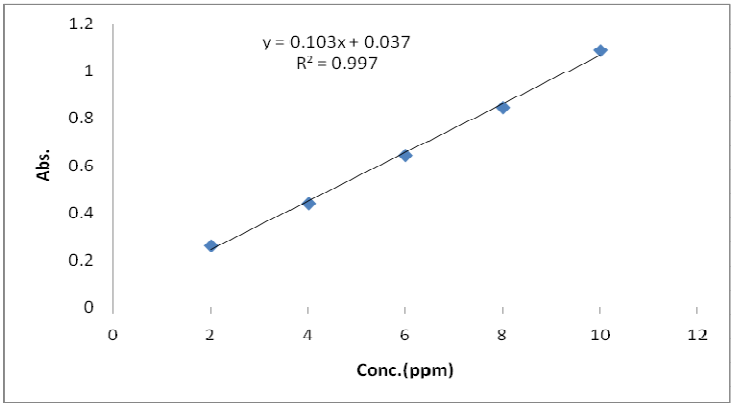

Figure 6: Calibration curve of colchicine

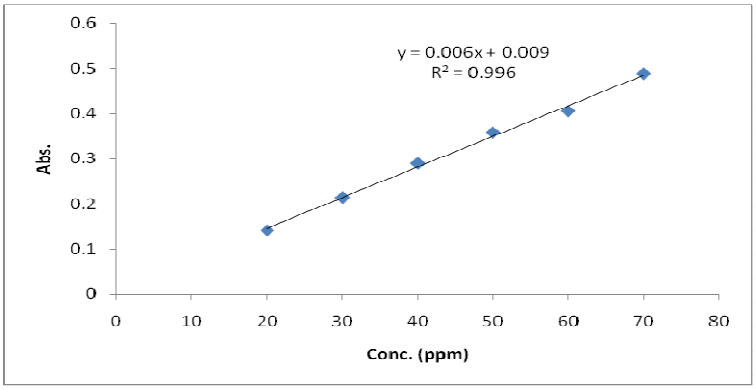

Figure 7: Calibration curve of gingerol

The develop method was validated as per ICH guideline.
The result for validation parameters

1. Linearity

Table 2: Observation for the linearity

Sr. No.	Parameters	Results		
		Withanolide	Colchicine	Gingerol
1.	Linearity			
	Range	5-35 µg/ml	2-10 µg/ml	20-70 µg/ml
	Regression	0.998	0.997	0.996
	Slope	0.02941	0.1032	0.006
	Intercept	0.005347	0.03723	0.009
	Line Equation	y = 0.02941x + 0.005347	y = 0.1032x + 0.03723	y=0.006x+0.009
2.	LOD	0.123 µg/ml	0.078 µg/ml	1.4 µg/ml
3.	LOQ	0.37 µg/ml	0.23 µg/ml	4.5 µg/ml

2. Precision

Table 3: Precision, ruggedness and robustness data

Sr. No.	Parameter	Withanolide		Colchicine		Gingerol	
		SD	% RSD	SD	% RSD	SD	%RSD
1.	Precision	0.0011	0.123	0.0024	0.38	0.0027	0.76
2.	Intraday Precision	0.0029	0.33	0.0029	0.45	0.0028	0.78
3.	Interday Precision	0.0042	0.46	0.0033	0.51	0.0030	0.84
4.	Robustness	0.0033	0.37	0.0031	0.49	0.0031	0.88
5.	Ruggedness	0.0031	0.33	0.0039	0.49	0.0029	0.83

Accuracy

The accuracy was reported in form of percent recovered amount in triplicate and SD.

Table 4: Observation for recovery study

Sr.No.	Marker	Amount taken (µg/ml)		Level	% Recovery ±SD (n=3)
		Std.	Test		
1.	Withanolide	5	10	50%	96.00±0.160
		10	10	100%	96.88±0.115
		15	10	150%	97.42±0.15
2.	Colchicine	2	4	50%	96.57±0.257
		4	4	100%	96.35±0.137
		6	4	150%	98.49±0.072
3.	Gingerol	30	60	50%	98.87±0.142
		60	60	100%	96.43±0.160
		90	60	150%	95.16±0.73

The accuracy of the method was found to be for withanolide 96.76%, for colchicine 97.13% and for gingerol 96.82%**.**

Estimation of drug content in marketed formulation-

Table 5- Quantitative estimation of active bio-marker in formulation by UV.

Sr. No.	Marker	Concentration in mg/tablet (n=3)	Theoretical Concentration in mg/tablet [11-12]
1.	**Withanolide**	0.3mg	0.1-0.33
2.	**Colchicine**	1.25mg	0.5-1.3
3.	**Gingerol**	6.5mg	5.8-8.5

The drug content was observed within the label claim.

CONCLUSION

The develop method was validated according to the ICH guidelines. The method was simple, precise, robust, rugged and accurate and can be used for the routine analysis of polyherbal formulation.

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