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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF L-DOPA AND DIOSGENIN FROM MARKETED APHRODISIAC FORMULATION

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ABSTRACT

The present work deals with development of HPLC method for simultaneous estimation of L-dopa and Diosgenin in marketed aphrodisiac formulation containing Mucuna pruriens seed and Tribulus terristris fruit powder as the ingredients. Chromatographic separation of the drugs was carried out on a Hemochrom Instil [(C18 (250 X 4.6 mm, 5 µm)] using the mobile phase ACN: 0.1%orthophosphoric acid in water (20:80 v/v), pumped at the flow rate of 0.6ml/min with UV-detection at 216nm. The two markers were satisfactorily resolved with retention time 3.781 and 5.255 for L- dopa and Diosgenin, respectively. The method was validated and shown to be linear for L-dopa and Diosgenin.The correlation coefficients for L-dopa and Diosgenin are 0.9954 and 0.9961 respectively. The developed method was found to be rapid, simple, specific and reliable for the determination of L-dopa and Diosgenin from these marketed aphrodisiac formulation.

KEYWORDS: L-dopa, Diosgenin, HPLC, Validation, ICH Guidelines.

INTRODUCTION

Medicinal herbs are moving from fringe to conventional use with the increased number of people seeking cure and health approaches, being free from side effects caused by synthetic chemicals. From past few decades, herbal drugs have been achieving importance because of the extensive chemical diversity that they offer. This has led to astounding increase in the demand for herbal medicines in the last two decades and a necessity has been felt for

ensuring the quality, safety and efficacy of herbal drugs.[1] According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs.[2] Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles.[3] Use of newer modern techniques like High Performance Thin Layer Chromatography (HPTLC), High Pressure Liquid Chromatography (HPLC), UV-Visible Spectrophotometry, etc. have assisted providing more reliable results for quantitative and qualitative analysis of herbal drugs and formulations.[4]

Marketed aphrodisiac formulation of VIGOMAX FORTE tablets from brand name Charak Pharma Pvt.Ltd. has composition as follows Ashwagandha (*Withania somnifera)* Root-200mg, Kaucha beej(*Mucuna pruriens)*Seeds-200mg,Salep (*Orchis latifolia)* Root-200mg, Gokhru(*Tribulus terrestris)* fruit-225mg, Safed musli (*Chlorophytum arundinaceum*) Tuber-100mg , Erand mool (*Ricinus communis)* Root-150mg.

Mucuna pruriens is a tropical twining herb commonly known as Velvet bean belongs to the family Fabeaceae.[5] In history, *Mucuna pruriens* has been used as an efficacious aphrodisiac and is still used to raise libido in both men and women due to its dopamine inducing properties.[6] On treatment with *Mucuna pruriens* remarkably improves psychological stress and seminal plasma lipid peroxide levels along with enhanced sperm count and motility and also restored the levels of SOD (Super Oxide dismutase), catalase, GSH (Glutathione) and ascorbic acid in seminal plasma of infertile men which was found to be low before the treatment.[7]

Tribulus terrestris Linn. (Gokshura/Gokhru) is a procumbent annual or perennial herb which belongs of Zygophyllaceae family.[8] A large amount of prospective active components have been identified in gokhru, including steroidal saponins such as diosgenin, it is procured by hydrolysis of crude saponins isolated from gokhru.[9] Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, gluco-corticoids and progesterone.[10]

Many techniques have been developed in many literatures for the estimation of L-dopa and Diosgenin individually or in combination with other markers. However, there is no HPLC method reported for the simultaneous determination of these markers in combined formulation according to my knowledge. The aim of this developed method is to widen RP-

HPLC method with UV detection for the simultaneous estimation of L-dopa and Diosgenin in an aphrodisiac formulation.

MATERIALS AND METHODS

Materials

Standard L-dopa and Diosgenin were procured from Yucca enterprises Mumbai, India. All the chemicals used in the experiment were of analytical grade procured from S.D. Fine Chemicals, Pvt. Ltd. Mumbai India.

Marketed herbal formulations

The marketed aphrodisiac herbal formulation namely Vigomax Forte tablets by Charak Pharmaceuticals were procured from local pharmacy stores.

HPLC Method Development

Preparation of standard solution

Each marker weighed accurately 10 mg (L-dopa and Diosgenin) was transferred individually in two volumetric flasks and volume was made up to the 10 ml with 0.1 N HCl and methanol respectively, to obtain 1000 ppm solutions. These were used as stock solution and stored in refrigerator.

Preparation of working solution: Working solutions were prepared from the stock solutions. Solution of each markers having concentration 100 ppm were prepared from the 1000 ppm stock solution. From these further dilutions were made using methanol to get solutions of 15-45 ppm and 2-14 ppm for L-dopa and Diosgenin respectively.

Preparation of sample solution: Tablets were triturated in a mortar and pestle to obtain fine powder. Powder equivalent to 2 gm was extracted with 30 ml methanol using reflux condenser for 2 hours and filtered through Whatmann filter paper no. 41. The final volume was made upto 30 ml with methanol.0.1 ml of above extract was diluted to 10ml with methanol and used for further analysis.

HPLC METHOD DEVELOPMENT

Chromatographic conditions: The optimized mobile phase was ACN: 0.1% Orthophosphoric acid (20:80) and flow rate was kept 0.6 mL/min, column temperature was set at 40 °C. Retention time of L-dopa and Diosgenin found for this mobile phase was 3.781 ± 0.2 min. and 5.255 ± 0.2 min., respectively.

HPLC method validation[11]

The developed method was validated as per ICH guidelines Q2 (R1) for parameters such as linearity, specificity, precision, accuracy, Limit of detection, Limit of quantitation and robustness.

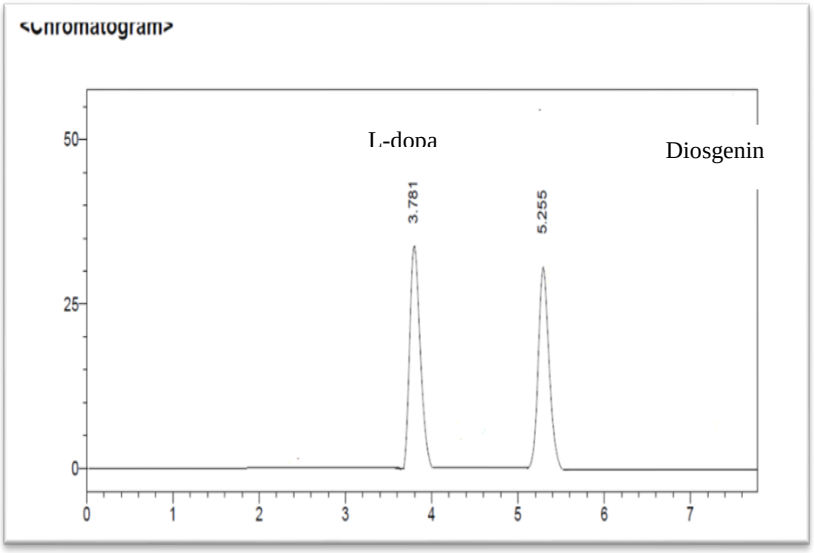


Fig. 1: HPLC chromatogram of combined solution of L-dopa and Diosgenin obtained using optimized mobile phase.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. It is analyzed by plotting the area against concentration of analyte and the results of test are evaluated by calculation of regression coefficient (r^2).

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present in the analyte. It is performed to ensure the identity, purity and quantitation of maker compound from the formulation under examination. It is determined by comparing the UV spectra and retention time of the standards with the components of interest obtained from extract of marketed formulation.

Analysis of Marketed Formulation

The amount of L-dopa and Diosgenin present in Vigomax Forte tablets was calculated using linear regression analysis. Quantification of markers was done by performing HPLC analysis

of test solutions i.e 10 ppm concentration extract according to the developed method. The area obtained for each marker from formulation extract was extrapolated on respective calibration curve of that marker. Analysis was performed in triplicates.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the authorized conditions. Three replicates of Quality Control (QC) samples of L-dopa and Diosgenin at three different levels (15, 30, 45ppm) and (2, 8, 14ppm) i.e. low quality control (LQC), mid-quality control (MQC) and high quality control (HQC) respectively, were analyzed at three different times on a same day (intra-day precision) and on three different days (interday precision).

Accuracy (Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery of L-dopa and Diosgenin from formulation was checked by spiking a known quantity of standards at three concentration level (i.e. 80%, 100%, and 120% of the quantified amount) to the test samples in triplicate using HPLC. In this form, recovery was calculated for nine determinations over a specified range and mean recovery was calculated.

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The LOD and LOQ are expressed as

$LOD = 3.3\ \sigma/S$

$LOQ = 10\ \sigma/S$

Where, σ = Standard deviation of response, S = Slope of the calibration curve both of them are obtained from the calibration curve of the individual maker compound.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate alterations in method parameters and provides an indication of its reliability during normal usage. Robustness of the analytical method was evaluated by making intentional changes in Flow rate (± 0.2 mL/min), mobile phase composition and absorbance(λ max±2). Two concentrations of each maker (i.e. 15 and 45 ppm) for L-dopa and (2 and 8 ppm) for Diosgenin were analyzed in triplicate in order to ensure that the method is robust.

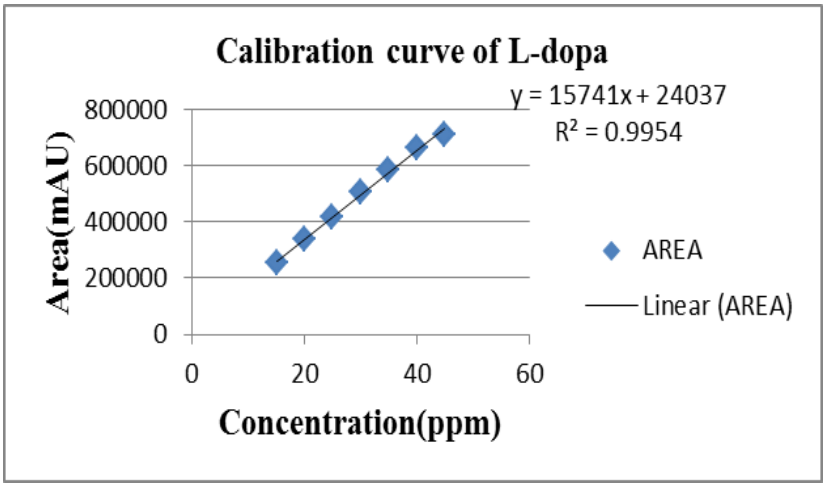
RESULTS AND DISCUSSION

Linearity

L-dopa and Diosgenin showed linear responses in the concentration rage of 15 ppm to 45 ppm and 2 ppm to 14 ppm respectively (figure 3). The linearity was validated by the high value of correlation coefficients. The results are tabulated in Table 1.

Table. 1: Linear regression data obtained from calibration curve of L-dopa and Diosgenin.

Parameter	L-dopa	Diosgenin
Linearity Range (ppm)	15 – 45 ppm	2 – 14 ppm
Equation of regression line	y=15741x + 24037	Y = 74475x - 31805
Coefficient of correlation (r2)	0.9954	0.9961
Slope	15741	74475
Intercept	24037	31805



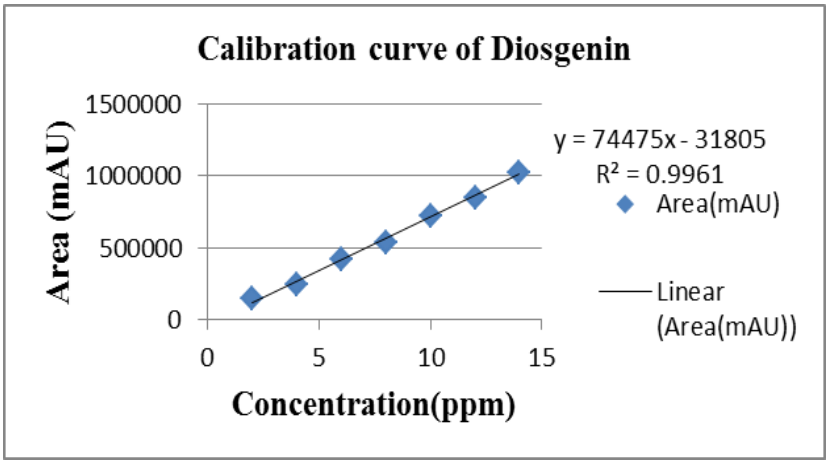


Fig. 2: Calibration curve of L-dopa and Diosgenin obtained using HPLC.

Specificity

The developed method was found to be specific because there were no interferences of any other herbal constituents at retention time of Levo-dopa i.e. 3.778 ± 0.2 min and for Diosgenin i.e.5.265 ± 0.2 min as depicted by Fig. 3.

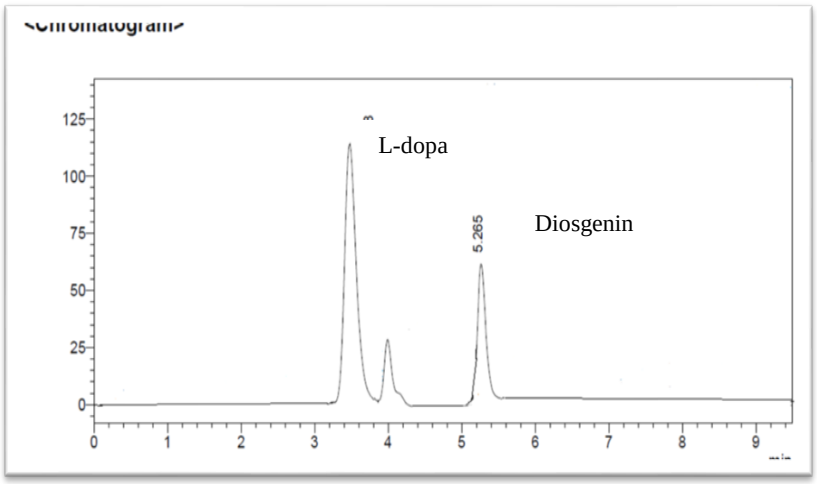


Fig. 3: Chromatogram of extract of Vigomax Forte tablets.

Analysis of Marketed Formulation

Table 2: Content of makers in marketed formulation.

Formulation	% w/w content	
Vigomax Tablets	L-dopa	Diosgenin
	0.037	0.011

Precision: The statistical analysis of the result proved that % relative standard deviation (RSD) of the peak area obtained was less than 2%. Hence, the developed method was found to be precise.

Table. 3: Intra-day and Inter-day Precision of L-dopa and Diosgenin.

Intraday				Interday	
Marker	**Concentration (ppm)**	**Standard Deviation**	**% RSD**	**Standard Deviation**	**% RSD**
L-dopa	15	2646.798	1.076	1575.374	0.628
	30	2654.145	0.524	5471.072	1.063
	45	1499.906	0.210	6024.274	0.840
Diosgenin	2	666.489	0.531	1860.338	1.476
	8	8834.443	1.6513	4994.607	0.941
	14	6275.354	0.542	14585.62	1.251

Accuracy

Table. 4: Results of accuracy for L-dopa.

Formulation	Level of recovery (%)	Theoretical content of marker (ppm)	Amount of marker recovered (ppm)	%Recovery	Average % recovery
Vigomax Forte Tablets	80	45.43	45.48	100.11	100.11
	100	50.48	50.75	100.53	
	120	55.52	55.35	99.69	

Table. 5: Results of accuracy for Diosgenin.

Formulation	Level of recovery (%)	Theoretical content of marker (ppm)	Amount of marker recovered (ppm)	%Recovery	Average % recovery
Vigomax Forte Tablets	80	13.39	13.50	100.82	99.75
	100	14.88	14.75	99.12	
	120	16.36	16.25	99.32	

LOD and LOQ: LOD and LOQ of L-dopa were found to be 0.36 and 1.104 respectively and that of Diosgenin were found to be 0.109 and 0.332 respectively.

Robustness: The method was found to be robust as the statistical data indicate that % RSD of the peak areas obtained was less than 2%.

Table. 6: Robustness results for Levo-dopa and Diosgenin.

Parameter	Deviation	% RSD			
		Levo-dopa		Diosgenin	
		Area		Area	
		15ppm	45ppm	2ppm	14ppm
Flow rate (mL/min)	0.4	0.403	0.395	0.820	0.107
	0.8	0.245	0.032	0.776	0.204
Mobile phase composition	15 : 85	1.024	0.247	0.500	0.813
	25 : 75	1.386	0.948	0.969	0.081
λ max	214	0.201	0.211	0.500	0.813
	216	1.386	0.948	0.969	0.081

The validated method was successfully established for evaluation of Vigomax Forte tablets. L-dopa and Diosgenin showed good resolved peaks with selected and optimized mobile phase. This can be used as a standard technique for routine, rapid and accurate quantitative determination of L-dopa and Diosgenin in the marketed formulations. This validated method quantified both the markers from Vigomax Forte tablets. This method is validated according to the guidelines provided in ICH Q2 (R1) in terms of linearity, specificity, precision, limit of detection, limit of quantification, accuracy and robustness. This validated HPLC method can be used to evaluate both the markers from any formulation.

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