WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 12, 941-957 Research Article ISSN 2278 – 4357

SIMULTENOUS ESTIMATION OF AMINO ACID AND STEROIDAL SAPOGENIN IN BULK AND HERBAL FORMULATION

Sonal More*, Dr. Varsha M. Jadhav and Dr. Vilasrao J. Kadam

Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai- 400614.

Article Received on
08 Oct. 2018,

Revised on 28 Oct. 2018,
Accepted on 18 Nov. 2018

DOI: 10.20959/wjpps201812-12811

***Corresponding Author**
Dr. Sonal More
Bharati Vidyapeeth's College of Pharmacy, C.B.D. Belapur, Navi Mumbai-400614.

ABSTRACT

The present work deals with development of HPTLC method for simultaneous estimation of l-dopa and diosgenin in *Mucuna pruriens* and *Tribulus terristris* respectively and also in a marketed herbal formulation containing *Mucuna pruriens* seeds powder and *Tribulus terristis* fruits powder as the major ingredients. Chromatographic separation of the drugs was performed on Merck TLC aluminium plates pre-coated with silica gel 60F254 as the stationary phase. The mobile phase selected was toluene: ethyl acetate: methanol: formic acid: glacial acetic acid(4: 2.5: 2:1.20:0.30 v/v/v/v/v). The sample solutions were prepared in methanol and linear ascending development was carried out in twin trough glass chamber. The two markers were satisfactorily resolved with R_f values 0.24±0.02 and 0.73±0.02 for l-dopa and diosgenin, respectively. The linear regression analysis data for the calibration plots for l-dopa and diosgenin showed good linear relationship with regression coefficient (r^2) 0.9991 and 0.998 in the concentration range of 200-1400 ng/spot and 10-70 ng/spot, respectively. The method was validated for linearity, specificity, recovery, precision, robustness as per ICH guidelines. The developed method was found to be precise, specific and reliable for the determination of l-dopa and diosgenin from these individual herbs as well as from marketed herbal formulations.

KEYWORDS: L-dopa, Diosgenin, HPTLC, Validation, Vigomax Forte tablets, Analytical method development.

INTRODUCTION

According to an estimate of the World Health Organization (WHO), about 80% of the world populations still use herbs and other conventional medicines for their fundamental health care needs. Of the 252 drugs considered as basic and essential by the WHO, 11% are exclusively

of plant origin and a remarkable number of synthetic drugs are obtained from natural precursors.[1]

This increased usage of herbal medicines is due to several reasons namely, inadequacy of conventional medicines such as side effects and ineffective therapy, abusive use of synthetic drugs resulting in adverse effects, large percentage of world‘s population does not have access to conventional pharmacological treatment and folk medicines and ecological awareness which suggest that natural products are harmless.[2] Standardization is an important tool for assessing and maintaining the quality and safety of the polyherbal formulation. Standardization reduces batch to batch variations; assure safety, efficacy, quality and acceptability of polyherbal formulations. Thin layer chromatography (TLC) and High Performance Thin layer Chromatography (HPTLC) fingerprint profiles are used for deciding the identity, purity and strength of polyherbal formulation.[3]

Mucuna pruriens is a tropical twining herb commonly known as Velvet bean belongs to the family Fabeaceae.[4] 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.[5] On treatment with *Mucuna pruriens* remarkably ameliorate 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.[6]

Tribulus terrestris Linn. (Gokshura/Gokhru) is a procumbent annual or perennial herb which belongs of Zygophyllaceae family.[7] 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.[8] Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, gluco-corticoids and progesterone.[9]

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.

Many techniques have been developed in many literatures for the estimation of L-dopa and Diosgenin individually in plant extracts or in combination with other markers. Kshirsagar VB, Deokate UA, Bharkad VB, Khadabadi SS developed a HPTLC method development for the simultaneous estimation of diosgenin and levodopa in marketed formulation.[10] Stationary phase used was silica gel G60F254, 20x10 cm TLC plate. The mobile phase consisted of Toluene: ethyl acetate: GAA: Formic acid (2:1:1:0.75 v/v/v) gave R_f values of 0.27+0.2 and 0.61+0.2 for Levodopa and Diosgenin respectively. No reagent was ultilized for the detection of diosgrnin.

The present research work deals with development of HPTLC method for standardization of herbal drugs *Mucuna pruriens*, *Tribulus terristris* and herbal formulation containing both the herbal drug powders by detection and quantification of markers l-dopa and diosgenin simultaneously. The proposed method was validated on the basis of its linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness according to ICH guidelines.

This paper reports a more precise HPTLC method inorder to standardize the individual herbs i.e *Mucuna pruriens* seeds and *Tribulus terristris* fruits as well as marketed aphrodisiac formulation Vigomax Forte tablets containing these two herbs. There is no reported HPTLC method for this marketed formulation. Toluene: Ethyl acetate : Methanol: Formic acid : Glacial acetic acid(4 : 2.5 : 2 : 1.20 : 0.30 v/v/v/v/v. The anisaldehyde sulphuric acid reagent was used for detection of diosgenin.

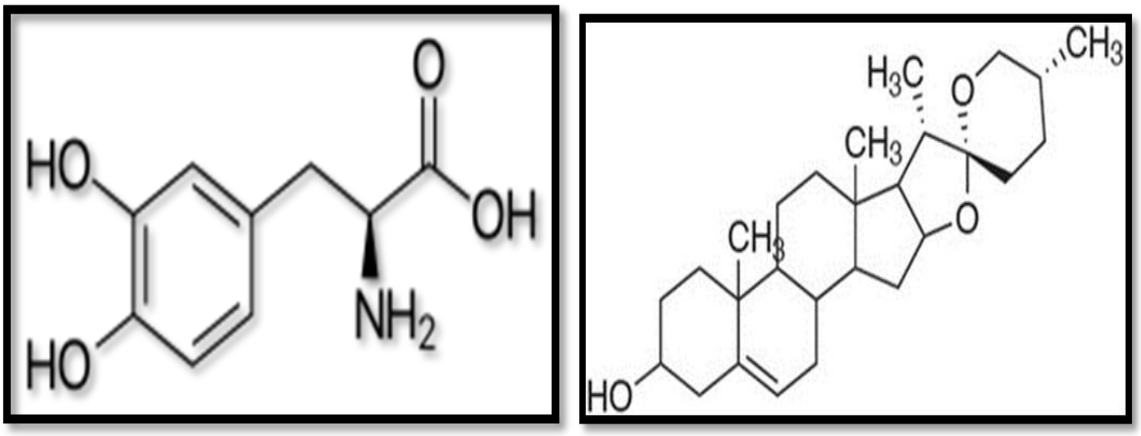

Figure 1: Structure of L-dopa and diosgenin.

MATERIALS AND METHOD

Procurement of Crude drugs

The powdered form of crude drugs namely, *Mucuna pruriens* seeds and *Tribulus terristris* fruits used in the study were procured from local market of Thane, Maharashtra, India. The crude drugs were authenticated by botanist, Dr. H. M. Pandit, Mumbai Specimen no. ssm p 1816505 and ssm p1816510 for *Mucuna pruriens* seeds powder and *Tribulus terrsitris* fruits powder respectively.

Procurement of Marketed formulation

VIGOMAX FORTE tablets (Charak Pharma Pvt.Ltd) was purchased from local market of Thane (west), Maharashtra, India**.**

Standards and Reagents

All the chemicals of LR grade were procured from S.D. fine chemicals, Mumbai, Maharashtra, India. Analytical standards of L-dopa and Diogenin were purchased from Yucca Enterprises, Mumbai.

Instrumentation

Chromatographic separation was attained on HPTLC plates using Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with 100µl Hamilton syringe. TLC scanner 3 with win CATS software was used for detection of samples.

Preparation of Standard Stock Solutions

10 mg of l-dopa and diosgenin were accurately weighed and transferred to individual volumetric flasks of 10 ml each. Volume was made upto 10 ml with methanol to obtain concentration of 1000μg/ml.

Preparation of Working Solutions

Working solutions were prepared from standard solutions of l-dopa and diosgenin. 1 ml of each solution was transferred to separate 10 ml volumetric flask and volume was made upto 10ml with methanol to get concentration in the range of 100μg/ml. Solutions for calibration curve were prepared by diluting the stock solutions so that application of 10μl volume gave a series of spots covering the range of 200-1400 ng/spot (200,400,600,800,1000,1200,1400 ng/spot) for l-dopa and 10-70 ng/spot (10,20,30,40,50,60,70 ng/spot) for diosgenin.

Preparation of Sample Solutions

A. Sample preparation for *Mucuna pruriens* and *Tribulus terristris* extracts

10 mg of the individual extracts were weighed and diluted with methanol in 10 ml volumetric flask up to the mark. The resulting sample solutions were filtered through 0.45μ filter. 10 μl of filtered solution was then applied as triplicate on TLC plate and then subjected for development and scanning.

B. Preparation of sample solution of Vigmomax forte tablets

Tablets were triturated and powder equivalent to 2gm was weighed and extracted with 30 ml of methanol by maceration method using rotamantle for 30min. The solution was further cooled and filtered to get methanolic extract. 0.1ml was withdrawn and diluted upto 10ml with methanol.

HPTLC method development

Optimization of chromatographic conditions

The samples were spotted in the form of bands (6 mm width) with a Hamilton microliter syringe (100 µL) under a controlled nitrogen stream using a Cammag Linomat V sample applicator The slit dimension was kept at 5 mm × 0.45 mm and a scanning speed 10 mm/s. Precoated TLC silica gel aluminium Plates 60 F254 (20 cm × 10 cm, 250 m thickness, Merck, Darmstadt, Germany) were utilized. The chromatographic ascending development was performed using a mixture of mobile phase toluene: ethyl acetate: methanol: formic acid: glacial acetic acid (4: 2.5: 2: 1.20: 0.30 v/v/v/v/v) to a migration distance of 80 mm. The chamber was previously saturated for 15 min at temperature 25±2°. The volume applied on each track was 10 μl. After development, plates were dried undercurrent of air at room temperature. The densitometric scanning was performed using Camag TLC scanner III operated using win CATS software (V 1.44 CAMAG). For quantification of l-dopa, the plates were directly scanned in the UV mode with the deuterium source set at 280nm.The spot corresponding to l-dopa were observed at 0.24±0.02. For diosgenin, the dried plates were dipped into the anisaldehyde-sulphuric acid detection reagent and dried for 10 minutes under hot air followed by placing in an oven at 105°C for 10 minutes. The plates were scanned within 10 minutes using densitometric scanner III with Win CATS software (Camag) in source set at 430 nm. The spots corresponding to diosgenin were observed at 0.73±0.02.

Calibration curves for L-dopa and Diosgenin

Serial dilutions were made in the concentration range of 20-140 µg/ml and 1- 7 µg/ml for l-dopa and diosgenin, respectively. Aliquot of above solutions (10µl) were applied with the band width of 6 mm, in triplicate on TLC plate (10×10 cm) to obtain a concentration range of 200-1400ng/spot for l-dopa and 10-70 ng/spot for diosgenin. Peak area for each band was recorded. Separate calibration curves were obtained by plotting a graph of peak area vs. concentration of l-dopa and diosgenin.

HPTLC Method Validation[11]

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

Linearity

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 was obtained by plotting peak area Vs concentration of standard and finding regression coefficient(r^2).

Specificity

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix. The specificity of the method was ascertained by comparing the R_f value and the peak purity was assessed by comparing the spectrum of standard l-dopa and diosgenin with sample.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. As per the ICH guidelines precision should be performed at three different levels: Lower Quality Control (LQC), Medium Quality Control (MQC) and Higher Quality Control (HQC). Three replicates of Quality Control (QC) samples of l-dopa and diosgenin at three different concentration levels (200, 800, 1400 ng/spot for l-dopa and 10, 40, 70 ng/spot for diosgenin) i.e. LQC, MQC and HQC, respectively, were analyzed on three different days to determine inter-day precision. Intra-day precision was ascertained by taking three replicates of each concentration level (LQC, MQC

and HQC) at three different times on a same day. % RSD for each concentration level was calculated.

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. This was done by spiking the extract of drugs as well as marketed formulations with 80%, 100% and 120% of standard solutions of l-dopa and diosgenin each separately. The experiment was performed in triplicate; mean recovery and % RSD were calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated, under the stated experimental conditions. LOQ is the lowest amount of analyte which can be detected and estimated quantitatively with required accuracy and precision. LOD and LOQ for l-dopa and diosgenin by this method were evaluated by using slope of the final calibration curve (mean calibration curve obtained from the responses of three calibration curves) and the standard deviation of the Y-intercept value obtained from three calibration curves as described in ICH guidelines.

LOD = 3.3 X Standard deviation of the y-intercept

Slope of the calibration curve

LOQ = 10 X Standard deviation of the y-intercept

Slope of the calibration curve

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. Following the introduction of small changes in the amount of mobile phase was varied over the range of ± 5%. The saturation time of development chamber was varied by ± 5 min. The robustness of the

method was determined at two concentration levels (200, 1400 ng/spot) for l-dopa and (10,70 ng/spot) for diosgenin.

RESULTS AND DISCUSSION

The mobile phase toluene: ethyl acetate: methanol: formic acid: glacial acetic acid (4: 2.5: 2:1.20:0.30 v/v/v/v) was optimized for separation. The R_f values of l-dopa and diosgenin were found to be 0.24 ± 0.2 and 0.73 ± 0.2 respectively. Figure 2 demonstrates the densitogram of l-dopa and diosgenin at optimized chromatographic conditions.

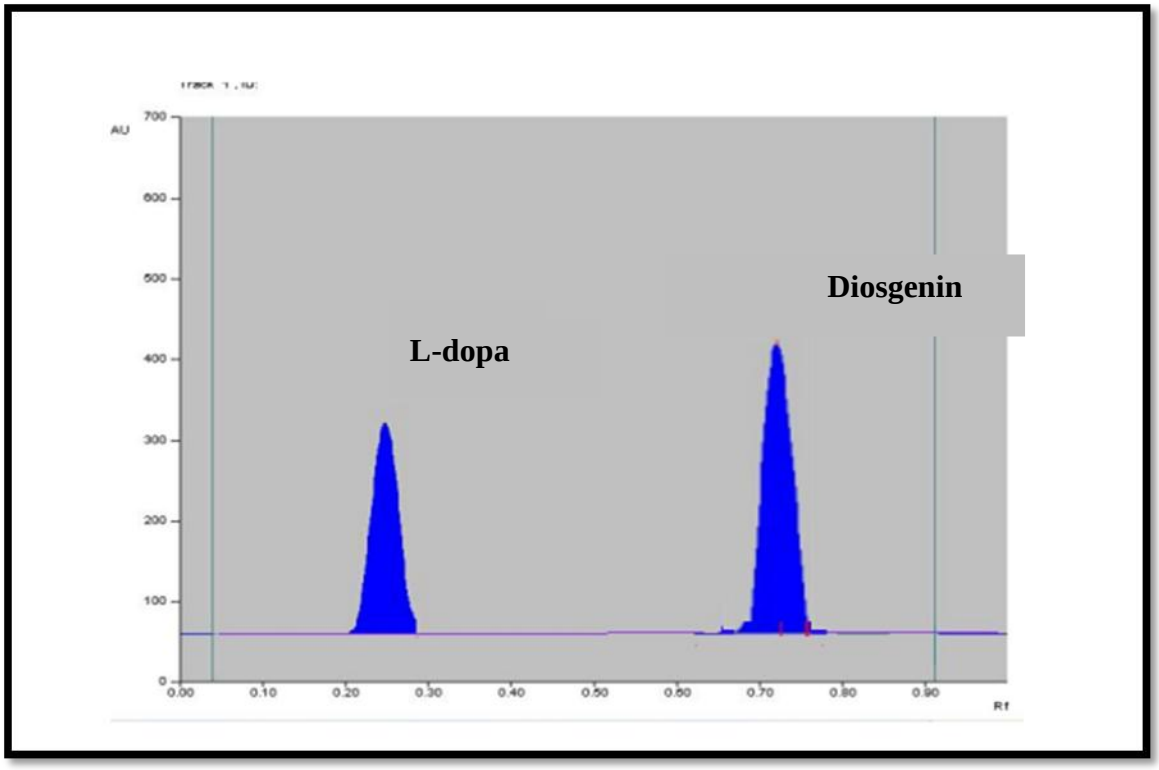

Figure 2: Densitogram of both marker compounds at optimized chromatographic condition.

HPTLC Method Validation

Specificity

When the spectra of standard l-dopa and diosgenin were overlaid or compared with the spectra of individual drug extracts and marketed formulation extract, it was observed that the other constituents present in the extracts did not interfere with the peaks of l-dopa and diosgenin as shown in Figure 5.4 and 5.5 respectively. Therefore the method was found to be specific.

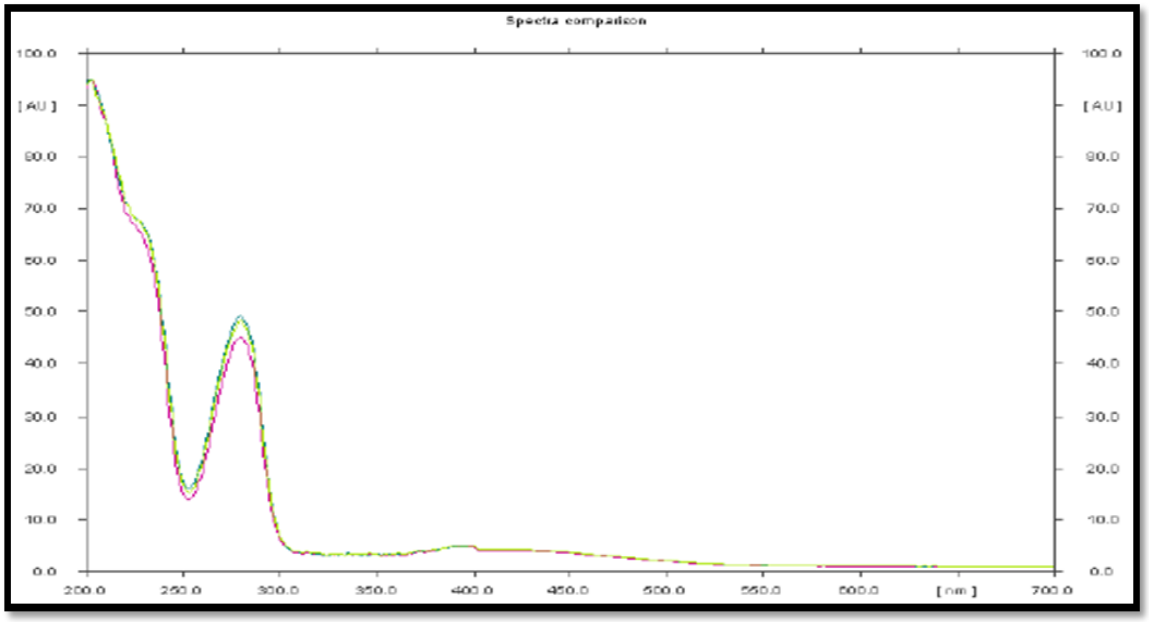

Figure 3: Overlain Spectra of Standard l-dopa and l-dopa from Methanolic Extract of *Mucuna pruriens seeds* and Marketed formulation.

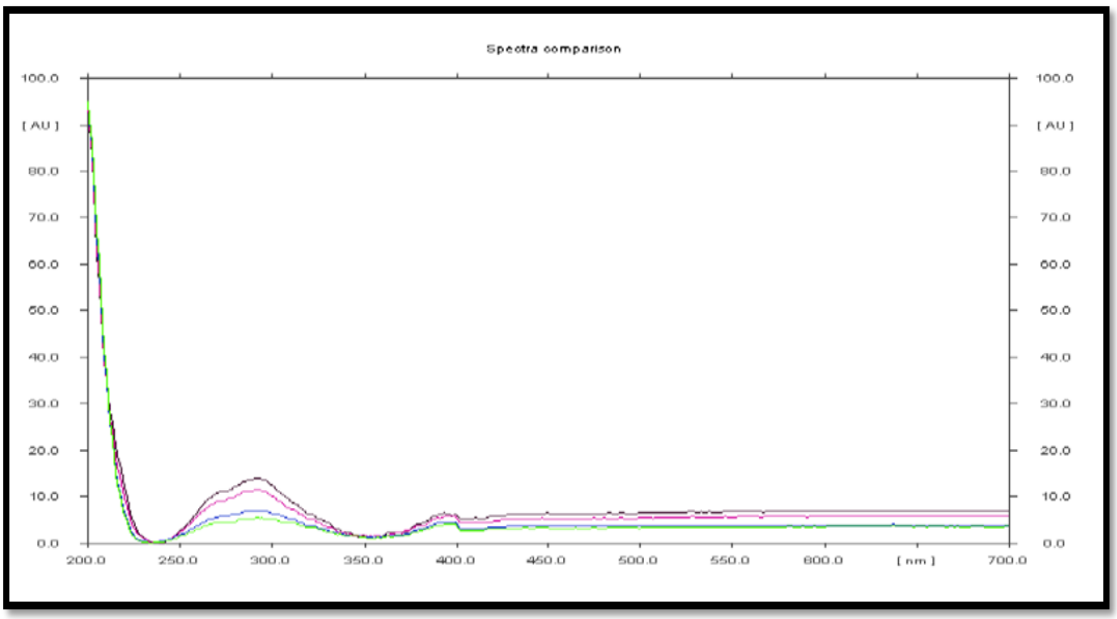

Figure 4: Overlain Spectra of Standard diosgenin and diosgenin from Methanolic Extract of *Tribulus terristris fruits* and Marketed formulation.

Linearity

Linear relationship was obtained by plotting drug concentration against peak area for each compound l-dopa and diosgenin showed linear response in the concentration range of 200-1400 ng/spot and 10-70ng/spot, respectively (Figure 5.1 and 5.2). The linearity was validated by the high value of correlation coefficients. The results are tabulated in table 1.

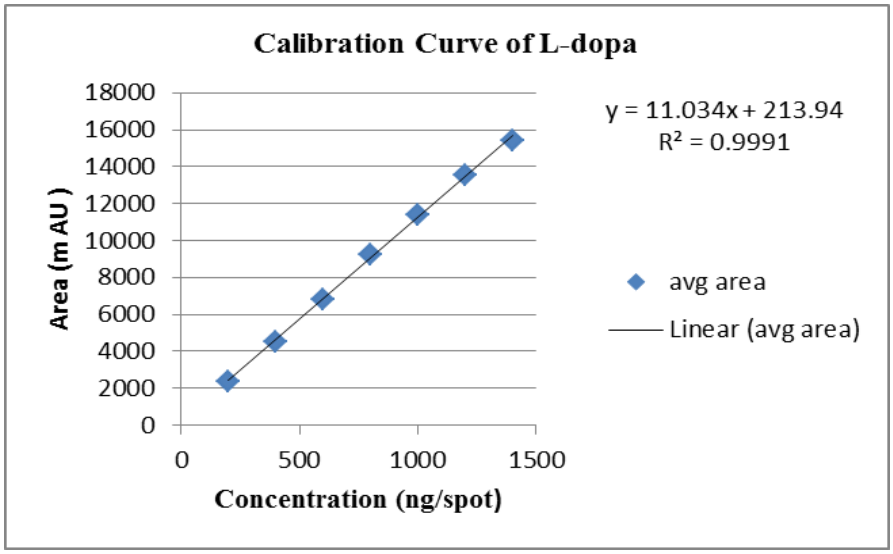

Figure 5.1: Calibration curve of L-dopa.

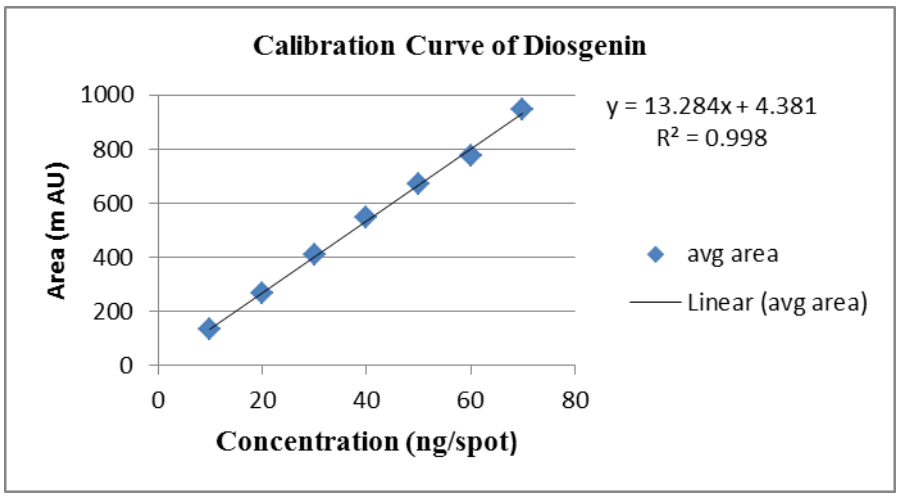

Figure 5.2: Calibration curve of Diosgenin.

Table 1: Linear regression data for calibration curves of l-dopa and diosgenin.

Parameters	L-dopa	Diosgenin
Linearity range	200-1400 ng/spot	10-70 ng/spot
Regression equation	y=11.034+213.94	y=13.284x+ 4.381
Correlation coefficient (r^2±S.D.)	0.9991	0.998
Slope (mean ± S.D.)	11.034	13.284
Intercept (mean± S.D.)	213.94	4.381

Precision

Intraday precision is used to describe the variation of the method, at three different concentration levels within the same day while interday precision is for variation between different days. The % RSD values for both intraday and interday precision were found within acceptable limits as shown in Table 2 respectively.

Table 2: Intra-day and Inter-day precision results.

Intra-day precision				Inter-day precision	
Marker	**Concentration (ng/spot)**	**Standard Deviation**	**% RSD**	**Standard Deviation**	**% RSD**
L-dopa	200	27.63	1.19	35.75	1.51
	800	140.34	1.51	115.64	1.24
	1400	116.86	0.75	275.08	1.79
Diosgenin	10	1.57	1.09	2.77	1.96
	40	3.90	0.712	6.04	1.15
	70	11.65	1.25	8.00	0.86

Accuracy

As per ICH, a minimum of three concentrations were selected and each concentration was analyzed in triplicate.

This way, accuracy was performed and calculated for nine determinations over a specified range. Recovery of l-dopa and diosgenin from each of the three formulations and individual herbal extracts was checked by spiking a known quantity of standards (i.e. 80%, 100% and 120% of the quantified amount) to the test samples. Analysis of these samples was done in triplicate using HPTLC. The results obtained for accuracy of l-dopa and diosgenin are demonstrated in table 3.1 and 3.2 respectively.

Table 3.1: Accuracy data for l-dopa.

Drug/ Formulation	**Level of Recovery (%)**	**Amount of marker present (ng)**	**Amount of marker added (ng)**	**Total amount of marker (ng)**	**Amount of marker recovered (ng)**	**Recovery (%)**	**Mean Recovery (%)**
Mucuna pruriens	80	970	776	1746	1759.06	100.74	100.24
	100	970	970	1940	1939.91	99.99	
	120	970	1164	2134	2134.41	100.01	
Vigomax Forte tablet	80	212.09	169.67	381.76	390.38	102.25	101.38
	100	212.09	212.09	424.18	422.63	99.63	
	120	212.09	254.50	466.59	477.20	102.27	

Table 3.2: Accuracy data for diosgenin.

Drug/ Formulation	Level of Recovery (%)	Amount of marker present (ng)	Amount of marker added (ng)	Total amount of marker (ng)	Amount of marker recovered (ng)	Recovery (%)	Mean Recovery (%)
Tribulus terristris	80	68.66	54.92	123.58	122.0	98.71	100.03
	100	68.66	68.66	137.32	138.84	101.10	
	120	68.66	82.39	151.05	151.46	100.27	
Vigomax Forte tablet	80	10.64	8.51	19.15	19.25	100.51	101.77
	100	10.64	10.64	21.28	21.89	102.86	
	120	10.64	12.76	23.40	23.86	100.96	

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were found to be 48.38 ng/spot and 146.63 ng/spot for L-dopa and 0.24 ng/spot and 0.73 ng/spot for Diosgenin, respectively.

Robustness

Robustness of the developed HPTLC method was evaluated by making deliberate changes in the mobile phase composition and saturation time. Two concentrations were spotted for each of the marker in triplicate.

200ng/spot and 1400 ng/spot of l-dopa and 10 ng/spot and 70 ng/spot of diosgenin were the concentrations selected for analysis. Table 5.9 summarizes the results obtained. It was found that % RSD value for all the results is less than 2%. Hence, the method was found to be robust.

Table 5: Robustness results for L-dopa and Diosgenin

Parameter	Deviation	% RSD			
		L-dopa		Diosgenin	
		200ng	1400ng	10ng	70ng
Saturation time	+5minutes	1.56	0.64	0.50	0.44
	- 5minutes	0.78	1.43	0.63	0.77
Mobile phase volume	+ 5%	0.39	0.66	1.15	1.05
	- 5 %	0.58	0.97	0.99	1.17

Analysis of Herbal extracts and marketed formulations

The developed method was applied for detection and quantification of l-dopa and diosgenin in the individual drugs, *Mucuna pruriens* and *Tribulus terristris* as well as marketed formulations containing these drugs. The peaks for l-dopa and diosgenin were observed at R_f values 0.24±0.02 and 0.73±0.02, respectively in the densitogram of the extracts. There was

no interference from other compounds present in the extracts. The amount of l-dopa and diosgenin present in the individual drugs as well as marketed formulations was calculated by using linear regression equation.

Table 6: L-dopa and Diosgenin content in Herbal extracts and formulation.

Herbal extracts/Formulation	% w/w content	
Vigomax Forte Tablets	L-dopa	Diosgenin
	0.318	0.015
Mucuna pruriens	0.970	
Tribulus terristris	0.068	

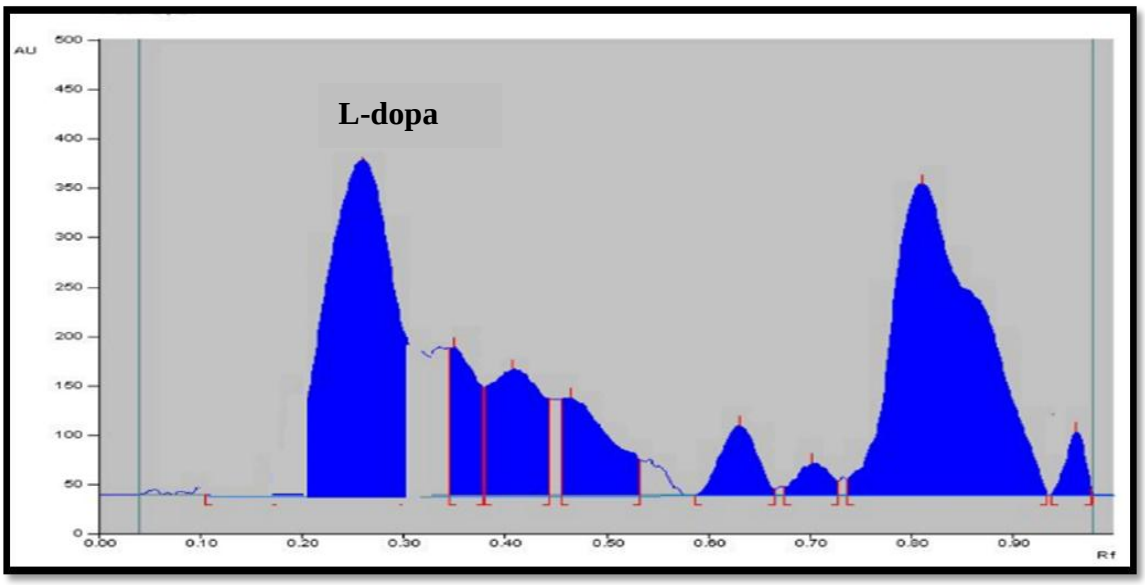

Figure 6: Densitogram of methanolic extract of *Mucuna pruriens.*

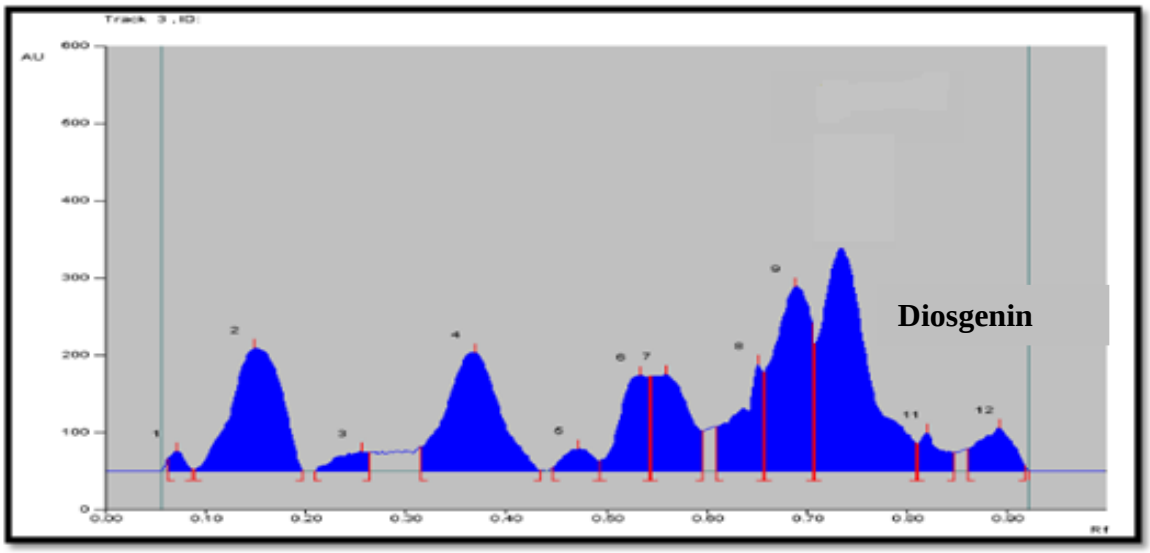

Figure 7: Densitogram of methanolic extract of *Tribulus terristris.*

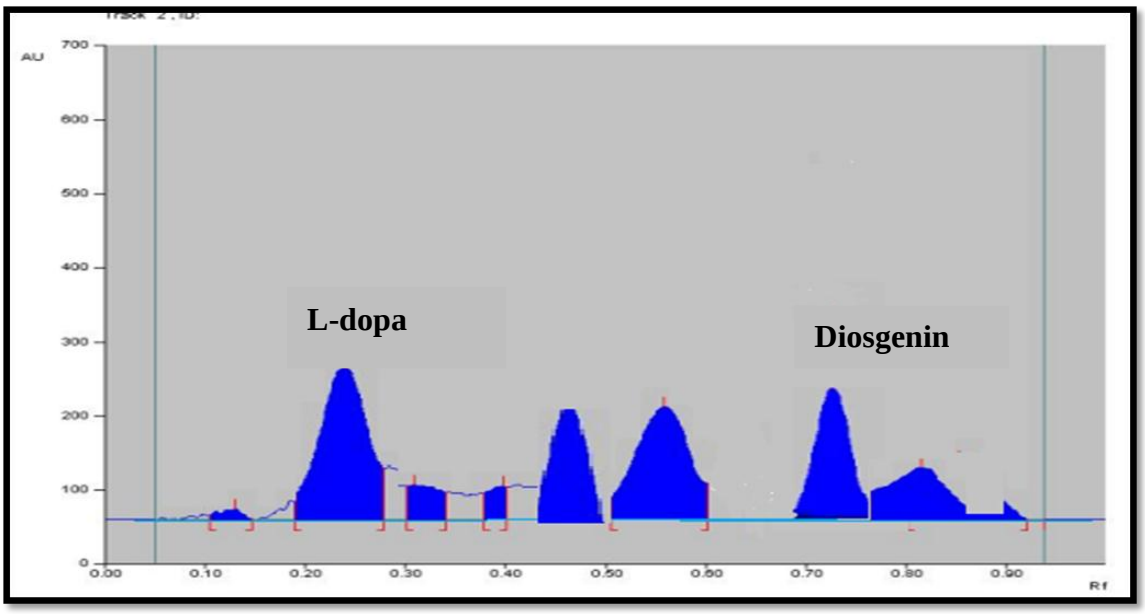

Figure 8: Densitogram of methanolic extract of Vigomax forte tablet.

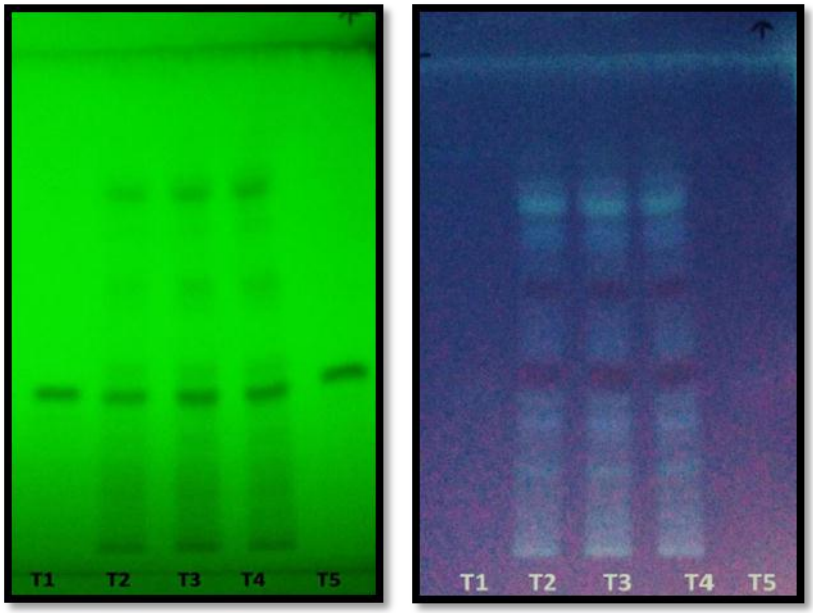

Figure 9: HPTLC fingerprinting profile of extract of *Mucuna pruriens* extract at 254nm and 366nm T1, T5 - Standard L-dopa, T2 to T4 – Extract used for quantification of L-dopa.

Figure 10: HPTLC fingerprinting profile of extract of *Tribulus terristris* after derivatizing using Anisaldehyde sulphuric acid reagent.

T1 & T5-Standard diosgenin, T2, T3, T4-Extract of *Tribulus terristris*

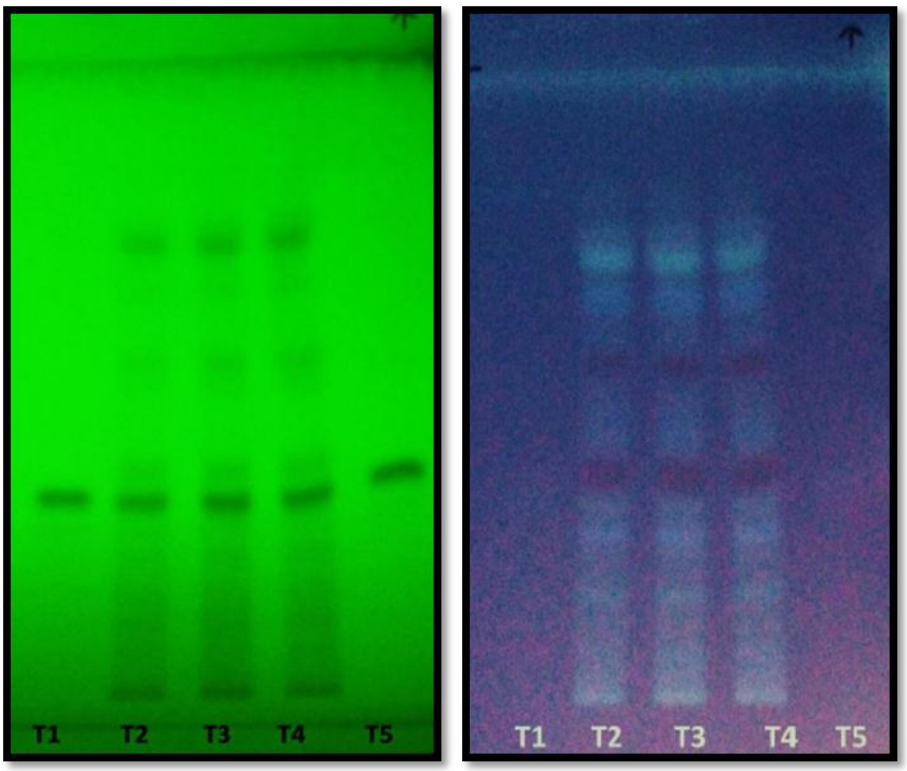

Figure 11: HPTLC fingerprinting profile of extract of Vigomax Forte tablets at 254nm and 366nm

T1 T2 T3 T4 T5

Figure 12: HPTLC fingerprint profile of extract of Vigomax Forte tablets after derivatization using anisaldehyde for diosgenin.

T1 & T5-Standard l-dopa and diosgenin,T2, T3, T4-Extract of Vigomax Forte tablet

CONCLUSION

Standardization is necessary for ensuring the quality and efficacy of the herbal raw materials. The analytical techniques have been proved as a valuable tool for standardization of herbal materials. The objective of the research work was to perform standardization of two drugs *Mucuna pruriens* and *Tribulus terristris* which have noted aphrodisiac activity. In market, herbal medicines are available containing combination of these two drugs for treating sexual disorders in men.

Standardization was carried out by development of simple, robust, accurate, precise HPTLC techniques for simultenous estimation of marker compounds l-dopa and diosgenin. The developed methods were validated as per ICH guidelines in terms of linearity, specificity, precision, accuracy, limit of detection, limit of quantitation and robustness. The developed and validated method was applied for standardization of the drugs *Mucuna pruriens* and *Tribulus terristris* as well as marketed formulations containing these drugs.

The developed method was found to be simple, rapid, precise and accurate and the developed method can be utilized as a routine quality control tool for the aforesaid materials.

REFERENCES

1. Kulkarni KM, Patil LS, Khanvilkar VV, Kadam VJ. Fingerprinting techniques in herbal standardization. J Journal of Pharm Research, 2014; 4(2): 1049-1062.
2. Raghavendra HL, Yogesh HS, Gopalakrishna B, Chandrashekhar VM, Sathishkumar B.P. An Overview of Herbal Medicines. *Int. J. Pharm, Sci*, 2009; 1: 1-20.
3. Choudhary N, Sekhon BS. An overview of advances in the standardization of herbal drugs. *Journal of Pharmaceutical Education and Research*, 2011; *2*(2): 55-70.
4. Vermal SC, Vashishth E, Singh R, Pant P, Padhi MM. A Review On Phytochemistry And Pharmacological Activity Of Parts Of Mucuna Pruriens Used As An Ayurvedic Medicine. World Journal Of Pharmaceutical Research, 2014; 3(5): 138-158.
5. Giuliano F, Allard J. Dopamine and male sexual function. European urology, 2001; 40(6): 601-608.
6. Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN, Tiwari SC. Mucuna pruriens reduces stress and improves the quality of semen in infertile men. Evidence – Based Complementary and Alternative Medicine, 2010; 7(1): 137-44.
7. Sharma PC, Yelne MB, Dennis TJ, Joshi A, Billore KV. Database on medicinal plants used in Ayurveda and Sidha. New Delhi: CCRAS, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India, 2000; 3: 229.
8. Gupta PK, Nagore DH, Kuber VV, Purohit SU. A validated RP-HPLC method for the estimation of diosgenin from polyherbal formulation containing Tribulus terrestris Linn. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(4): 91-94.
9. Mehrafarin A, Qaderi A, Rezazadeh S, Naghdi BH, Noormohammadi G, Bioengineering of important secondary metabolites and metabolic pathways in fenugreek (Trigonella foenum-graecum L.). J Med Plants, 2010; 9: 1–18.
10. Kshirsagar VB, Deokate UA, Bharkad VB, Khadabadi SS developed a HPTLC method development for the simultaneous estimation of diosgenin and levodopa in marketed formulation. Asian J Res Chem, 2008.
11. ICH harmonised tripartite guideline validation of analytical procedures: Text and methodology q2 (r1).