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Determination of Potential Synthetic Adulteration of PDE-5 Inhibitors in Herbal Formulation by RP-HPLC Method

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ABSTRACT

A Simple accurate and validated RP-HPLC method for Determination of Potential Synthetic Adulteration of PDE-5 inhibitors in herbal formulation was developed. Chromatographic separation was operated with Methanol: Water (60:40 v/v) at a flow rate of 1 ml/min on Agilent C18 column (4.6 mm I.D. × 100 mm, 5 µm), using 232 nm as detection wavelength. The retention time for Sildenafil Citrate, Tadalafil and Testosterone Propionate was found to be 2.35 min, 1.75 min and 6.25 min respectively. The method was linear over the concentration range of 10-60 µg/ml and coefficient correlation were found to be 0.9973, 0.9947 and 0.9927 respectively. The LOD values for Sildenafil Citrate, Tadalafil and Testosterone Propionate were found to be 0.753, 0.775 and 0.544 µg/ml and LOQ values were 2.282, 2.350 and 1.649 respectively. This method was validated for intraday precision, interday precision, repeatability, robustness and ruggedness. This method can be successfully be applied for determination of synthetic adulterants.

Keywords: Adulteration; PDE-5 inhibitors; Herbal formulation; Sildenafil Citrate

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INTRODUCTION

The use of herbal medicines, food supplements or nutraceuticals are gaining popularity all over the world. The reason for this increased popularity is that manufacturers and marketers deliver the message that all dietary supplements and herbal Medicines are free from all sorts of side effects and the general people believe on that perception. Almost 80% of modern drug inventory is contributed by Herbal medicinal plants. But addition of undeclared synthetic drugs in herbal medicines and dietary supplements were recently reported from different scientific and monitoring investigations. Serious toxic-effects to health can occur due to these hidden drugs. Recently action was taken by US-FDA against such kind of Alternative medicines, counterfeit , adulterated and

dangerous dietary supplements [1]. Illegal addition of metformin and Sitagliptin was detected in some Ayurvedic medicines and dietary supplements by FDA. Patients life is at risk it was suggested by several studies that reported hidden synthetic medicines, metals, or other toxic substances in high concentrations [2]. 24% of total 2600 Chinese herbal medicines were found to contain at least one synthetic medicine [3]. A meta-analysis showed that herbal medicines contained heavy metals which are toxic for human health [4].

Dietary supplements or conventional foods or natural products with hidden drugs and harmful chemicals are now emerging trend all over the world.

Some kind of products are usually promoted and sold as pure natural or 100% natural products in some under-developing countries, as they have no specific rules and controlling system and authority for dietary supplements. Products which are adulterated have been used for the improvement of sexual dysfunction, weigh reduction, body building etc. United States Food and Drug Administration already identified, reported and displayed public notification against six such kinds of products in their website between January to March 2014 [5]. The increasing figure indicates that the number of adulterations of herbal drugs and dietary supplements is increasing drastically so FDA identified and issued public warning against such kind of counterfeit and adulterated products.

One or two or up to five of the following hidden chemicals: sildenafil, sulfoaildenafil, dimethylsildenafil, dimethylacetyldenafil, hydroxyhomosildenafil, hydroxythiohomosildenafil, vardenafil, noracetildenafil, dapoxtine, tadalafil, and aminotadalafil were found by FDA on analysis of products that were promoted as natural and dietary supplements for increasing sexual power or energy or body building contained [5].

Not only dietary supplements and sexual stimulants, many other products of traditional herbal medicines were reported to be adulterated with various types of hidden synthetic chemicals having different pharmacological activities. In traditional herbal medicines sold as 100% pure, naturally originated and free from side-effects many types of therapeutic synthetic agents as adulterants are added in Various Herbal Formulations such as presence of glucocorticoids (dexamethasone, betamethasone, prednisolone, cortisone acetate, hydrocortisone), non-steroidal antiinflammatory drugs (diclofenac, phenylbutazone, ibuprofen), antihypertensive agents (amlodipine, valsartan, clonidine, metoprolol, chlorthiazide [6].

In India,

- Substitution of the herbs is the need of the hour with more than 300 medicinal plants becoming red listed.
- Substitution of herbs achieved many goals though basic idea was to provide similar therapeutic effect as that of original drug.
- It provided a greater scope for the physician to utilize herbs that are easily available, cost effective and most appropriate for the clinical condition.
- Suppliers are illiterate and not aware about their spurious supply.
- Major reasons are confusion in name, non-availability and lack of knowledge about authentic plant.
- Nearly list of 30 common substituents has been found out which are commonly adulterated in herbal products [7,8].

Extensive literature review revealed that no method is available for detection of synthetic adulterants (Sildenafil Citrate, Tadalafil and Testosterone Propionate) [9-21] in a single RP-HPLC method, so an attempt was made to develop a simple and validated method for simultaneous detection of these drugs in herbal formulation.

EXPERIMENTAL SECTION

Instrumentation

Model: Cyberlab 1600 EX

Column: Inertsil C18 (4.6 mm I.D. × 100 mm, 5 μm)

Injector: Rheodyne 7725i

Pump: EX 1600 HP

Detector: UV Detector (Deuterium)

Reagents and Materials

Reagents and materials are given in Table 1.

Table 1. Reagents and materials used

Reagents and Materials	Supplier
Sildenafil Citrate	Cadila Pharmaceuticals, Ahemdabad
Tadalafil	Zydus Cadila, Ahemdabad
Testosterone Propionate	Glenmark, Mumbai
Methanol	Molychem, Mumbai
Water	Molychem, Mumbai

Selection of Wavelength

Using appropriate dilution of standard stock solution, the three solutions were scanned separately in order to get results. All the solutions were scanned between 200-400 nm using UV-Visible spectrophotometer. Wavelength was selected from the overlay spectra of above solutions. The overlay spectrum for selection of wavelength is given in Figures 1 and 2.

Preparation of Standard Stock Solution

Accurately 10 mg of Sildenafil Citrate, Tadalafil, Testosterone Propionate were weighed separately and transferred to three different 10 mL volumetric flask. The volume was made up to the mark with methanol. All the solutions were ultrasonicated for 20 minutes on ultra-sonicator. From this solutions 1 ml was pipetted out and diluted up to 10 ml with methanol to give a final solution containing 100 μg/mL of Sildenafil Citrate, 100 μg/mL of Tadalafil and 100 μg/mL of Testosterone propionate respectively. Then filtered through 0.45 μm 47 mm membrane filter paper.

Preparation of Standard Solution for Ternary Mixtures of Sildenafil Citrate, Tadalafil and Testosterone Propionate

Accurately 10 mg of all three drugs was weighed and transferred into same 10 mL volumetric flask. Volume was made up to mark with methanol. The solutions were ultrasonicated for 20 minutes on ultra-sonicator. Then filtered through 0.45 μm membrane filter paper.

Selection of Mobile Phase

Ternary mixture containing 10 μg/mL of Sildenafil citrate, 10 μg/mL of tadalafil and 10 μg/mL of Testosterone propionate were injected into the HPLC system and run in different solvent systems. Method development was

started with 100% methanol but poor resolution was found between peaks of all the drugs. The mobile phase was then taken as 100% water. But all three peaks got merged into one another. In order to optimize the better peak separation and resolution, Ratio of water and methanol was altered logically. Finally, the mobile phase contains water: methanol (40:60, v/v) with flow rate 1 mL/min. was selected.

Preparationo Mobile Phase

HPLC grade water and methanol were ultrasonicated for 20 minutes on ultrasonicator and then filtered through 0.45 µm 47 mm membrane filter paper. Mobile phase was prepared by mixing 40 mL of water with 60 mL of methanol and again the mixture was sonicated for 20 min on ultra sonicator.

Chromatographic Separation

Standard solutions of Sildenafil citrate, tadalafil, and testosterone propionate were injected in column with 20 µL micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Water: Methanol (40:60 v/v) which was previously degassed. The flow rate was set to 1 mL/min. and detection was carried out at wavelength 232 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc. were recorded using software.

Validation of Analytical Method

Linearity

For mixture containing all three drugs appropriate aliquots were pipetted out into a series of 10 mL volumetric flasks. The volume was made up to the mark with Mobile phase to get a set of solutions for Sildenafil citrate having concentration range having 10, 20,30,40,50 and 60 µg/mL, for Tadalafil having concentration range 10, 20,30,40,50 and 60 µg/mL and Testosterone propionate having concentration range 10, 20,30,40,50 and 60 µg/mL.

Chromatogram of the drugs was performed with UV detector at 232 nm. Peak areas were recorded for all the peaks. Standard calibration curves for Sildenafil citrate, tadalafil and testosterone propionate were Plot separately with concentration vs. the respective peak area as shown in figures respectively (Figures 3-5).

Limit of detection and limit of quantitation

Calibration curve was repeated six times and the standard deviation of the intercepts was calculated (Table 2). Then LOD and LOQ were calculated as follow:

$$\text{LOD}=3.3\ \text{X}\frac{SD}{Slope\ of\ calibration\ curve}$$

$$\text{LOQ}=10\ \text{X}\ \frac{SD}{Slope\ of\ calibration\ curve}$$

Where, SD=Standard Deviation of intercepts of calibration curves.

Precision

Repeatability: Standard solution containing Sildenafil Citrate (10 µg/mL), Tadalafil (10 µg/mL) and Testosterone propionate (10 µg/mL) were injected six times and area of peak were measured and % RSD was calculated.

Intra day precision: Standard solution containing Sildenafil Citrate (10 µg/mL), Tadalafil (10 µg/mL) and Testosterone propionate (10 µg/mL) were analyzed six times on the same day and % RSD was calculated.

Inter day precision: Standard solution containing Sildenafil Citrate (10 µg/mL), Tadalafil (10 µg/mL) and Testosterone propionate (10 µg/mL) were analysed six times on the different day and % RSD was calculated.

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. Robustness was performed by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 1% variation in volume of mobile phase and ± 0.1 mL/min. flow rate of mobile phase. The solution containing 10 µg/mL of Sildenafil Citrate, 10 µg/mL of Tadalafil and 10 µg/mL of Testosterone Propionate were injected into sample injector of HPLC three times.

Ruggedness

Ruggedness was performed by keeping all the Experimental parameters same except it was done by two different analysts. Each of analyst prepared individually solution containing 10 µg/mL of Sildenafil Citrate, 10 µg/mL of Tadalafil and 10 µg/mL of Testosterone Propionate and they were injected into sample injector.

Extraction Technique

Method of extraction of sildenafil citrate, Tadalafil and Testosterone Propionate from herbal formulation:

The drug was freely soluble in methanol and water. Thus, for the extraction of the drug, a mixture of methanol: water (50:50) was used. Powdered material was subjected to double extraction with a total of 50 ml of methanol: water (50:50) solution. The powdered material was suspended in 25 ml of methanol: water (50:50) solution and the suspension was sonicated for 20 min. The suspension was then centrifuged at 3000 rpm for 15 min and the supernatant was collected. The sediment left was collected and re-dispersed in 25 ml of fresh extraction media and same extraction procedure was followed and other 25 ml was obtained. Both of them were combined to obtain 50 ml solution.

RESULTS AND DISCUSSION

Precision

Repeatability data: Repeatability data for sildenafil citrate, tadalafil and testosterone propionate are given in Tables 3 and 4.

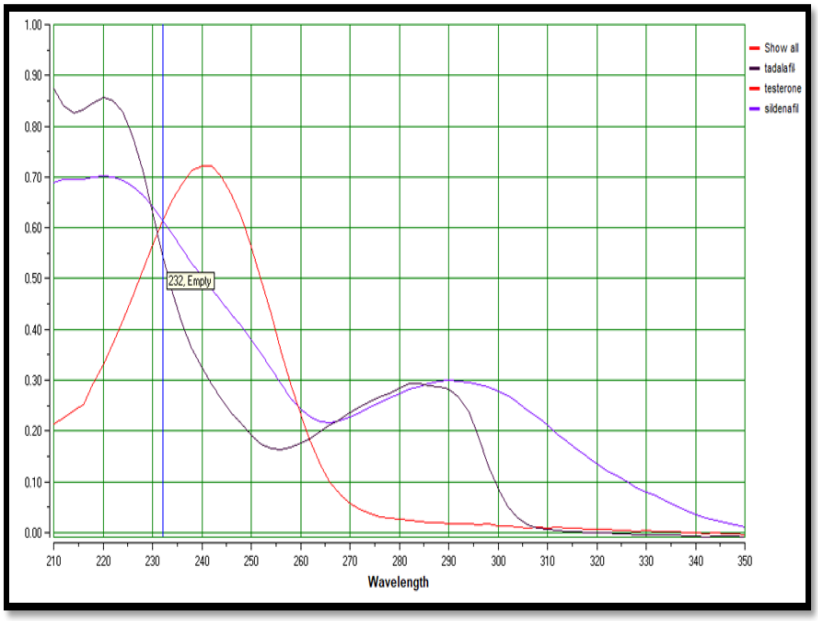

Figure 1. Overlay UV spectrum of sildenafil citrate, tadalafil and testosterone propionate

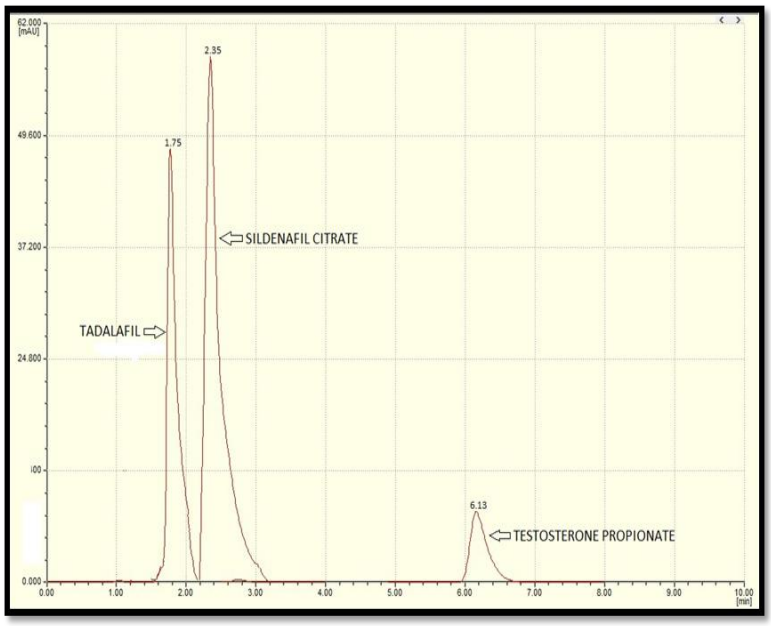

Figure 2. Chromatogram of sildenafil citrate, tadalafil and testosterone propionate in methanol and water (60:40)

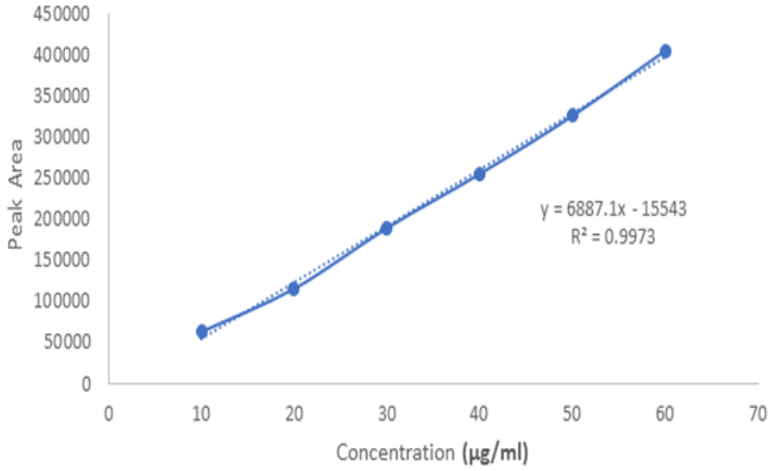

Figure 3. Calibration curve of sildenafil citrate

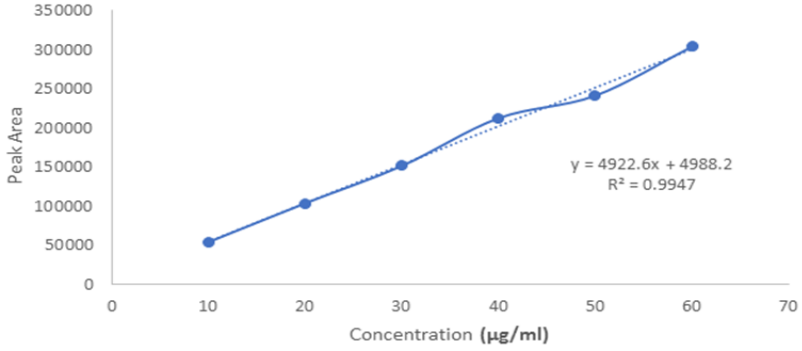

Figure 4. Calibration curve of tadalafil

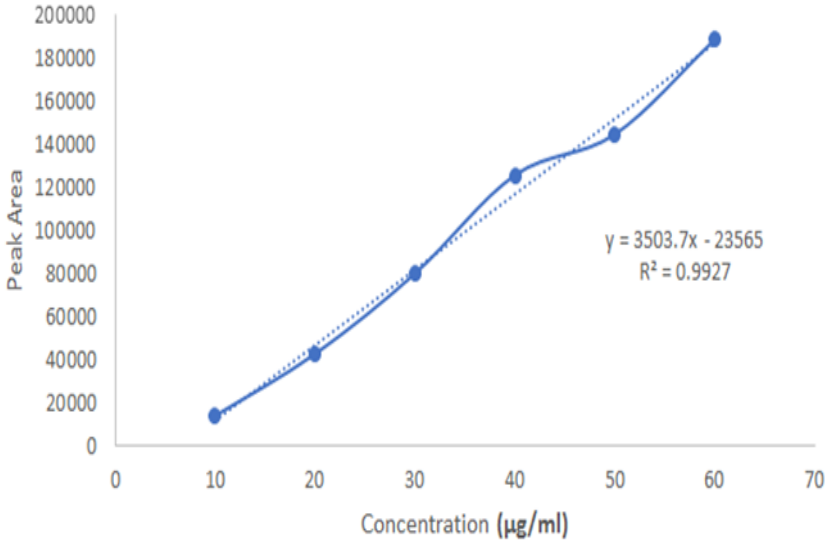

Figure 5. Calibration Curve of Testosterone Propionate

Table 2. LOD and LOQ data for sildenafil citrate, tadalafil and testosterone propionate

Drug	LOD (µg/ml)	LOQ (µg/ml)
Sildenafil Citrate	0.753	2.282
Tadalafil	0.775	2.35
Testosterone Propionate	0.544	1.649

Table 3. Repeatability data for sildenafil citrate, tadalafil and testosterone propionate

Sr. No.	Concentration (µg/ml)	Sildenafil Citrate		Tadalafil		Testosterone Propionate	
		Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)
1	30	188558	99.7	80983.3	101.5	150172.7	99.5
2	30	187612.4	99.2	79946	100.2	149116.2	98.8
3	30	186288.5	98.5	80504.5	100.9	150625.4	99.8
4	30	189692.8	100.3	79626.9	99.8	152738.4	101.2
5	30	188179.8	99.5	80345	100.7	153493.1	101.7
6	30	186855.9	98.8	79387.5	99.5	151832.9	100.6

Table 4. Statistical validation of repeatability

Drug	Mean label claim (%)	Standard Deviation	% RSD	Standard Error
Sildenafil Citrate	99.33	0.647	0.651	0.264
Tadalafil	100.43	0.742	0.738	0.302
Testosterone propionate	100.26	1.095	1.092	0.447

Intra-day Precision Data

Intra-day precision data for sildenafil citrate, tadalafil and testosterone propionate are given in Tables 5 and 6.

Table 5. Intra-day precision data for sildenafil citrate, tadalafil and testosterone propionate

Sr. No.	Concentration (µg/ml)	Sildenafil Citrate		Tadalafil		Testosterone Propionate	
		Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)
1	30	188558	99.7	80983.3	101.5	150172.7	99.5
2	30	187612.4	99.2	79946	100.2	149116.2	98.8
3	30	186288.5	98.5	80504.5	100.9	150625.4	99.8
4	30	189692.8	100.3	79626.9	99.8	152738.4	101.2
5	30	188179.8	99.5	80345	100.7	153493.1	101.7
6	30	186855.9	98.8	79387.5	99.5	151832.9	100.6

Table 6. Statistical validation of intra-day precision data*

Drug	Mean label claim (%)	Standard Deviation	% RSD	Standard Error
Sildenafil Citrate	99.33	0.647	0.651	0.264
Tadalafil	100.43	0.742	0.738	0.302
Testosterone propionate	100.26	1.095	1.092	0.447

*n=6

The results have shown that the recovery of Sildenafil Citrate, Tadalafil and Testosterone Propionate were in the range of 98.00 % to 102.00 %. The relative standard deviation was less than 2 %. These results demonstrate that this method was repeatable and precise.

Inter-day Precision Data

Inter-day precision data for sildenafil citrate, tadalafil and testosterone propionate are given in Tables 7 and 8.

Table 7. Inter-day precision data for sildenafil citrate

Sr. No.	Concentration (µg/ml)	Sildenafil Citrate		Tadalafil		Testosterone Propionate	
		Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)	Area (AUC)	Label Claim (%)
1	30	186288.5	98.5	80983.3	101.5	150323.6	99.6
2	30	188558	99.7	80424.8	100.8	151531	100.4
3	30	191016.7	101.1	79626.9	99.8	153191.2	101.5
4	30	190827.5	100.9	78430.1	98.3	153493.1	101.7
5	30	188179.8	99.5	80743.9	101.2	151229.2	100.2
6	30	192340	101.7	79148.2	99.2	150625.4	99.8

Table 8. Statistical validation of Inter-day precision data

Drug	Mean label claim (%)	Standard Deviation	% RSD	Standard Error
Sildenafil Citrate	100.23	1.198	1.195	0.489
Tadalafil	100.13	1.248	1.246	0.509
Testosterone propionate	100.53	0.875	0.87	0.357

*n=6.

The results have shown that the recovery of Sildenafil Citrate, Tadalafil and Testosterone Propionate were in the range of 98.00 % to 102.00 %. The relative standard deviation was less than 2 %. These results demonstrate that this method was repeatable and precise.

Robustness

Data of Robustness for sildenafil citrate, tadalafil and testosterone propionate are given in Tables 9 and 10.

Table 9. Robustness Data for Sildenafil Citrate

Change in flow rate							
		Sildenafil Citrate		Tadalafil		Testosterone Propionate	
Flow rate (ml/min.)	Level	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %
0.9	-1	2.39	101.7	1.77	101.14	6.16	100.48
1	0	2.36	100.42	1.74	99.42	6.1	99.51
1.1	1	2.32	98.72	1.72	98.28	6.07	99.02
Change in % of Methanol in mobile phase							
% of methanol in mobile phase	Level	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %
59	-1	2.31	98.29	1.73	98.85	6.06	98.85
60	0	2.34	99.57	1.76	100.57	6.12	99.83
61	1	2.38	101.27	1.78	101.71	6.19	100.97

Table 10. Statistical validation of robustness data

	Change in flow rate				Change in % of Methanol in mobile phase			
Drug	Mean Rt (min.)	SD	%RSD	Std Error	Mean Rt (min.)	SD	%RSD	Std Error
Sildenafil Citrate	2.356	1.495	1.49	0.863	2.343	1.495	1.499	0.863
Tadalafil	1.743	1.44	1.445	0.831	1.756	1.44	1.434	0.831
Testosterone propionate	6.11	0.743	0.743	0.428	6.123	1.061	1.062	0.612

The parameters included flow rate and percentage of Methanol in the mobile phase. The results have shown that the recovery of Sildenafil Citrate, Tadalafil and Testosterone Propionate were in the range of 98.00 % to 102.00 % and Also % Relative Standard deviation was less than 2%. These results demonstrate robustness of this method over the specified range.

Ruggedness

Data of ruggedness for sildenafil citrate, tadalafil and testosterone propionate are given in Tables 11 and 12.

Table 11. Ruggedness Data for Sildenafil Citrate

Change in analyst						
	Sildenafil Citrate		Tadalafil		Testosterone Propionate	
Analyst	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %	Retention time (min.)	Recovery %
1	2.37	100.85	1.77	101.14	6.16	100.48
2	2.36	100.42	1.76	100.57	6.18	100.81

Table 12. Statistical validation of robustness data

	Different Analyst			
Drug	Mean Rt (min.)	SD	%RSD	Std Error
Sildenafil Citrate	2.36	0.304	0.302	0.215
Tadalafil	1.76	0.403	0.399	0.285
Testosterone propionate	6.17	0.233	0.231	0.164

Under same conditions, two different analysts performed the experiment and the results have shown that the recovery of Sildenafil Citrate, Tadalafil and Testosterone Propionate were in the range of 98.00 % to 102.00 % and also %Relative Standard deviation was less than 2%. These results demonstrate ruggedness of this method over the specified range

CONCLUSION

Analysis of Herbal Formulation

Using the extraction technique mentioned above, 5 herbal formulations were obtained from different parts of saurashtra region and were analysed using the developed method after applying extraction technique (Table 13). It was found that 1 out of 5 herbal formulation was adultered with one of the synthetic adulterants.

Table 13. Summary of validation parameters for sildenafil citrate, tadalafil and testosterone propionate by developed method

Parameters		Sildenafil	Tadalafil	Testosterone
Retention time (min.)		2.35	1.75	6.13
Resolution		Resolution between SC and Tad is 1.69		Resolution between SC and Testosterone propionate is 13.58
Linearity (µg/ml)		Oct-60	Oct-60	Oct-60
Correlation Coefficient (r^2)		0.9973	0.9947	0.9927
Slope		6887.1	4922.6	3503.7
LOD		0.753	0.775	0.544
LOQ		2.282	2.35	1.649
Repeatability (%RSD) *n=6		0.651	0.738	1.092
Intraday precision (%RSD) *n=6		0.651	0.738	1.092
Interday precision (%RSD) *n=6		1.195	1.246	0.87
Robustness (Mean Rt ± SD)	Change in flow rate	2.356 ± 1.490	1.743 ± 1.445	6.11 ± 0.743
	Change in and of MeOH	2.343 ± 1.495	1.756 ± 1.440	6.123 ± 1.061
Ruggedness (Mean Rt ± SD)		2.365 ± 0.304	1.765 ± 0.403	6.17 ± 0.233

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REFERENCES

[1] TM Burton. FDA moves against alternative diabetes treatments. Wall Street Journal. **2013**.

[2] DM Marcus; Grollman AP. *New Engl J Med*. **2002**, 347, 2073-2076.

[3] E Ernst. *J Inter Med*. **2002**, 252, 107-13.

[4] E Ernst; J Thompson. *Clin Pharmacol Ther*. **2001**, 70, 497-504.

[5] U.S. Food and Drug Administration-U.S. department of health and human service. Tainted sexual enhancement products public notifications. **2014.**

[6] J Haneef; M Shaharyar; A Husain. *Drug Test Anal.* **2013**, 5, 607-13.

[7] O Prakash; AK Jyoti; P Kumar; NK Manna. *J Med Plants Stud*. **2013**, 1(4), 127-132.

[8] B Poornima. Adulteration and substitution in herbal drugs a critical analysis. *Int J Pharmaceutical Sci Res*. **2010**, 1(1), 8-12.

[9] AA Savaliya; RP Shah; B Prasad; S Singh. *J Pharm Biomed Anal*. **2010**, 52(3), 406-409.

[10] TS Reddy; AS Reddy; PS Devi. *J Planar Chromat.* **2006**, 19(112), 427-431.

[11] MEA Hamid. *J Liq Chrom Relat Tech*. **2006**, 29(4), 591-603.

[12] P Zou; SSY Oh; P Hou; MY Low; HL Koh. *J Chrom A*. **2006**, 1104(1), 113-122.

[13] Y Shibayama; T Higashi; K Shimada; A Odani; A Mizokami; H Konaka; E Koh; M Namiki. *J Chrom B*. **2009,** 877(25), 2615-2623.

[14] Y Cai; TG Cai; Y Shi; XL Cheng; LY Ma; SC Ma; RC Lin; W Feng. *J Liq Chrom Relat Tech*. **2010**, 33(13), 1287-1306.

[15] MH Guermouche; K Bensalah. *J Pharm Biomed Anal*. **2006,** 40(4), 952-957.

[16] F Song; A El-Demerdash; SJSH Lee. *J Pharm Biomed Anal*. **2012**, 70, 40-46.

[17] G Carlucci; P Palumbo; P Iuliani; G Palumbo. *Biomed Chrom.* **2009**, 23(7), 759-763.

[18] S Baba; M Fujioka; Y Shinohara; T Furuta. *J Chromatogr B Biomed Sci Appl*. **1985**, 337, 205-212.

[19] Y You; CE Uboh; LR Soma; F Guan; X Li; Y Liu; JA Rudy; J Chen; D Tsang. *J Chrom A*. **2011**, 1218(26), 3982-3993.

[20] R Gonzalo-Lumbreras; MA García-Miguens; R Izquierdo-Hornillos. *J Pharm Biomed Anal*. **2005**, 38(4), 757-762.

[21] C Lu; M Wang; J Mu; D Han; Y Bai; H Zhang. *Food Chem*. **2013**, 141(3), 1796-1806.