JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Simultaneous estimation of Sildenafil citrate and Asian ginseng powder extract in Pharmaceutical dosage form – Effervescent Tablet

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ABSTRACT:

Analysis of pharmaceutical product is very important as it concerned with quality of life. Sildenafil citrate is a PDE-5 inhibitor. Asian ginseng extract is Panax Ginseng obtained from herbaceous species (Family: Araliaceae). Combination of Sildenafil citrate and Asian ginseng extract are used in treatment of erectly dysfunction. The objective is to develop simple, rapid, precise and validated RP-HPLC method for simultaneous estimation of Sildenafil citrate and Asian ginseng extract in the pharmaceutical dosage form. Chromatographic separation of Sildenafil citrate and Asian ginseng extract was carried out on BDS Hypersil C18, 250mm × 4.6mm, 5µ (particle size), Thermo scientific column using mobile phase Acetonitrile : 0.02M Phosphate buffer (pH 3) (60 : 40 v/v) & detection at 210 nm. Linearity of Sildenafil citrate and Asian ginseng extract were found to be 25 – 75 µg/ml and 2.5 – 7.5 µg/ml. The correlation coefficient was found to be 0.9992 and 0.9995 was found for Sildenafil citrate and Asian ginseng extract respectively. The % RSD for precision was found to be less than 2 % and the % recovery was found between 97-102 %. Developed & validated RP-HPLC method was found to be simple, accurate, economical, robust and reproducible. There was no interference of the excipients in the determination of drugs from pharmaceutical dosage form so it can be successfully applied for routine analysis.

Keywords: Sildenafil citrate, Asian ginseng extract, RP-HPLC method, Validation, Effervescent Tablet.

Article history:
Received 4 April 2014
Accepted 19 April 2014
Available online 13 Sept 2014

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INTRODUCTION:

Sildenafil citrate is Chemically 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine. [1] Sildenafil citrate is phosphodiesterase type-5 (PDE5) inhibitor. It protect cGMP (cyclic guanosine monophosphate) from degradation by PDE5 (cGMP-specific Phosphodiesterase type 5) in the corpus cavernosum. [2-8] The main chemical constituent of Asian ginseng extract is Ginsenoside Rg1. Asian ginseng extract is an Adaptogen (improve physical stamina and mental concentration) and Aphrodisiac (intensify sexual desire). [10] Ginsenosides are thought to be adaptogenic and are transformed into pharmacologically active substances (compound K or M4) by intestinal microorganisms. Ginsenosides act as pro-drugs for these metabolites. [13-18] Sildenafil citrate and Asian ginseng extract are commercially available in various dosage forms in individual formulation. VIP AGRA 24 is the combination of Sildenafil citrate and Asian ginseng extract. Combination of Sildenafil citrate and Asian ginseng extract are useful in erectile dysfunction. Sildenafil citrate is official in IP. [9] Sildenafil citrate is official in USP [11] & BP [12]. From Literature survey, various methods (UV, HPLC, HPTLC, GC and Colorimetric) were reported for the analysis of individual drug and in combination with other drug but no method were

reported for simultaneous estimation of Sildenafil citrate and Asian ginseng extract. Hence, the purpose of the present work was to develop and validate RP-HPLC method for simultaneous estimation of Sildenafil citrate and Asian ginseng extract in Pharmaceutical dosage form. [1-18]

MATERIAL AND METHODS

Instruments

Chromatographic measurements were performed on Shimadzu SPD-20AT having pump LC-20AT; detector SPD-20AT; BDS Hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size), Thermo scientific column. All weighing were done on AX 200. The pH meter of Chemiline, India and Ultra sonic cleaner of Toshcon (Toshniwal process instrument Pvt. Ltd., Ajmer) were used.

Chromatographic Conditions

Stationary phase: BDS Hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size), Thermo scientific.

Mobile phase: Acetonitrile : 0.02M Phosphate Buffer (pH 3) 60:40 (v/v) (pH of Phosphate Buffer was adjusted to 3.0 using 1 % Ortho phosphoric acid)
Temperature: ambient
Flow rate: 1.0 ml/min
Wave length: 210 nm.
Run time: 15 min.

Chemicals and Reagents

The bulk drug (Sildenafil citrate & Asian ginseng extract) and Pharmaceutical formulation (VIP AGRA 24 Effervescent Tablet) were obtained from Vovantis Laboratory Pvt. Ltd. Vadodara as gift samples.

EXPERIMENTAL

All the chemicals and reagents used were of AR & HPLC grade from Merck and Rankem.

Preparation of buffer

2.72 g of Potassium dihydrogen phosphate was accurately weighed and dissolved in 1000mL of HPLC grade water to make phosphate buffer of pH 4.7. The pH of phosphate buffer was adjusted to pH 3 with the help of 0.1N NaOH & 1% Orthophosphoric acid.

Preparation of mobile phase

A degassed mixture of Acetonitrile and 0.02M Phosphate buffer pH 3 in the ratio of 60:40 (v/v) was prepared and the mixture was filtered through 0.45 µ membrane filters and it was degassed.

Preparation of Standard Stock Solution of Sildenafil citrate

An accurately weighed quantity of standard Sildenafil citrate (50 mg) was transferred to 100 ml volumetric flask and volume was made up to mark with the mobile phase to get 500 µg/ml of sildenafil citrate.

Preparations of Standard Stock Solution of Asian ginseng extract (Ginsenoside Rg1)

An accurately weighed quantity of standard Ginsenoside Rg1 (5 mg) was transferred to 100 ml volumetric flask and volume was made up to mark with the mobile phase to get 50 µg/ml of Ginsenoside Rg1.

Preparation of Calibration curve

From above solution of Sildenafil citrate pipette out 0.5, 0.75, 1, 1.25, 1.5 ml (50%, 75%, 100%, 125%, 150% respectively) of aliquots to the 10 ml volumetric flasks from 500 µg/ml of Sildenafil citrate stock solution and make up the volume to mark with mobile phase to get final concentration 25, 37.5, 50, 62.5, 75 µg/ml respectively. From above solution of Asian ginseng extract pipette out 0.5, 0.75, 1, 1.25, 1.5 ml (50%, 75%, 100%, 125%, 150% respectively) of aliquots to the 10 ml volumetric flasks from 50 µg/ml of Sildenafil citrate stock solution and make up the volume to mark with mobile phase to get final concentration 2.5, 3.75, 5, 6.25, 7.5 µg/ml respectively. Representative chromatogram of calibration curve for Sildenafil citrate (25-75 µg/ml) & Asian ginseng extract (2.5-7.5 µg/ml) is shown in figure 2.

Procedure for analysis of tablet formulation

Total 20 tablets were accurately weighed and triturated with glass mortar and pestle. Powder equivalent to 50 mg of Sildenafil citrate was weighed and transferred in a 100 ml volumetric flask and mobile phase was added. It was sonicated for 20 minutes and final volume was made to the mark with mobile phase. The solution was filtered through Membrane filter. The sample solution was assayed as per proposed method and % assay was calculated.

Validation of RP-HPLC Method

Method validation was performed following ICH guidelines. The proposed method has been extensively validated in terms of linearity, accuracyand precision, limit of detection and limit of quantification.

Linearity

Aliquots of standard solutions of sildenafil citrate and Ginsenoside Rg1 in range 25 - 75 µg/ml and 2.5 - 7.5 µg/ml respectively, was prepared from working standard solution and injected to system with stated chromatographic conditions and analysed. The graph of peak area obtained versus respective concentration was plotted. The mean area with its standard deviation and % relative standard deviation of peak were calculated. Calibration curve were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves. The linear regression equation of Sildenafil citrate was y = 107.49x - 53.306 (R^2= 0.9992) and Ginsenoside Rg1 y = 249.21x – 15.775 (R^2= 0.9995).

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was verified as repeatability, intra-day, inter-day and reproducibility.

1. Repeatability

It is precision under the same condition (same analyte, same apparatus, same identical reagent, short interval of time) is known as repeatability. Accurately 50 µg/ml concentration of Sildenafil citrate and 5 µg/ml of Asian ginseng extract (Ginsenoside Rg1) were prepared and analyzed for 6 times and their area & % RSD were measured.

2. Intraday precision

Variation of results within same day is called intraday precision. Solutions containing 25, 50, 75 µg/ml sildenafil citrate were analysed at 3 different times on the same day and % RSD was calculated.

Solutions containing 2.5, 5.0, 7.5 µg/ml ginsenoside Rg1 were analysed at 3 different times on the same day and % RSD was calculated.

3. Interday precision

Variation of results amongst the days is called intraday precision. Solutions containing 25, 50, 75 µg/ml sildenafil citrate were analysed at 3 different days and % RSD was calculated.

Solutions containing 2.5, 5.0, 7.5 µg/ml ginsenoside Rg1 were analysed at 3 different days and % RSD was calculated.

Accuracy

It is defined as 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. It is measure of exactness of analytical method.

Accuracy should be expressed as % recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

Accuracy should be established across the specified range of the analytical procedure.
It was determined by calculating the recovery of sildenafil citrate & ginsenoside Rg1 by standard spiking method. The mean % recovery is shown in table 1.

LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. Limit of detection can be calculated using following equation as per ICH guidelines.

LOD = 3.3 × N/S

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

LIMIT OF QUANTITATION (LOQ)

It is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. Limit of quantification can be calculated using following equation as per ICH guidelines.

LOQ = 10 × N/S

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULT AND DISCUSSION

A reliable chromatographic method was developed for simultaneous estimation of Sildenafil citrate and Asian ginseng extract in Pharmaceutical dosage form by RP-HPLC. For RP-HPLC method different mobile phases were tried and the mobile phase containing Acetonitrile and 0.02M phosphate buffer (pH 3) (60:40, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 5.15 min and 7.63 min for Sildenafil citrate and Asian ginseng extract respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

Results were found to be linear in the concentration range of 25-75 mg/mL for Sildenafil citrate and 2.5–7.5 mg/mL for Asian ginseng extract. The correlation coefficients for the plots were 0.9992 for Sildenafil citrate and 0.9995 for Asian ginseng extract. The proposed method was also evaluated by the assay of commercially available tablets containing Sildenafil citrate and Asian ginseng extract. The % assay was found to be 99.5394 ± 0.4182 for Sildenafil citrate and 103.3661 ± 1.0321 for Sildenafil citrate (mean ± S.D., n = 3). The method was found to be accurate and precise, as indicated by recovery

studies and % RSD not more than 2. The summary of validation parameters of proposed HPLC method is given in Table 2.

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Sildenafil citrate and Asian ginseng extract in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The developed method can be used for routine quantitative estimation of Sildenafil citrate and Asian ginseng extract in pharmaceutical preparation. The mobile phase Acetonitrile : 0.02M Phosphate buffer pH 3.0 (60 : 40, v/v) was found to be ideal for the simultaneous estimation of Sildenafil citrate and Asian ginseng extract. The elution was as followed for Sildenafil citrate (Rt – 5.15) and Asian ginseng extract (Rt - 7.63). The mean % recovery for Sildenafil citrate was found to be 99.69 % and for Asian ginseng extract it was found to be 99.77 %. The values of % recovery and standard deviation show that the proposed method was simple, reproducible, accurate and precise.

ACKNOWLEDGEMENT

The authors are highly thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing all the facilities to carry out the work.

FIGURES & TABLES

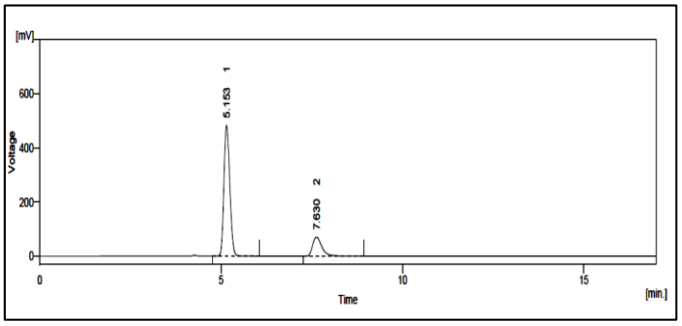

Figure 1: Representative chromatogram obtained for standard mixture containing Sildenafil citrate (50 µg/ml) and Ginsenoside Rg1 (5 µg/ml)

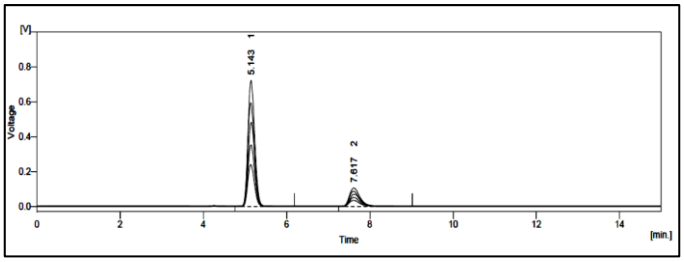

Figure 2: Representative chromatogram of calibration curve for Sildenafil citrate (25-75 µg/ml) and Ginsenoside Rg1 (2.5-7.5 µg/ml)

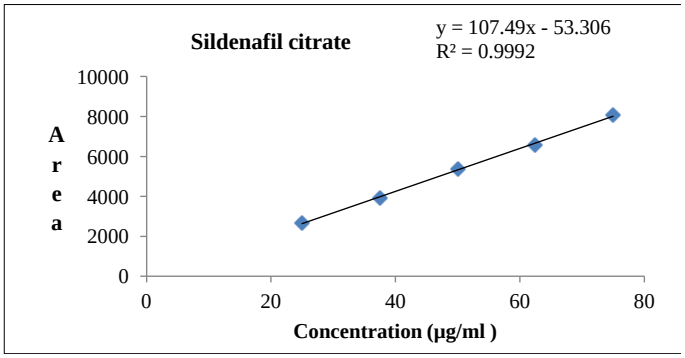

Figure 3: Calibration curve for Sildenafil citrate

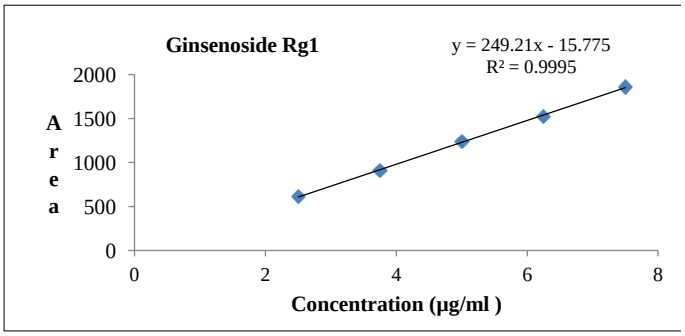

Figure 4: Calibration curve for Ginsenoside Rg1

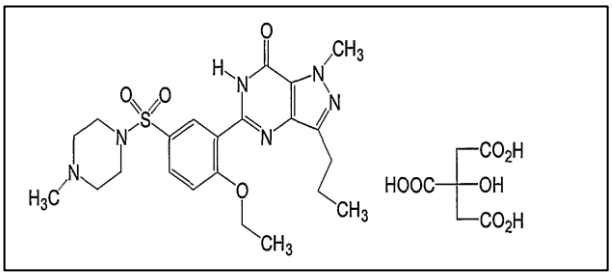

Figure 5: Structure of Sildenafil citrate

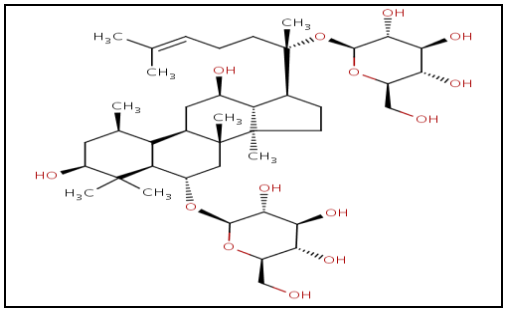

Figure 6: Structure of Ginsenoside Rg1 (Asian ginseng extract)

Table 1: Accuracy for Sildenafil citrate and Asian ginseng extract

Drug	Level (%)	Test amount (µg/ml)	Spiked STD amount (µg/ml)	Total amount recovered (µg/ml)	% Mean recovery ± SD. (n=3)
Sildenafil citrate	80	25	20	19.93823	99.69118 ± 0.92343
	100	25	25	24.89177	99.56711 ± 0.64842
	120	25	30	29.88686	99.62280 ± 0.40880
Asian ginseng extract	80	2.5	2	1.99549	99.77487 ± 1.31038
	100	2.5	2.5	2.52381	100.95261 ± 0.87290
	120	2.5	3	2.99835	99.94518 ± 1.51499

Table 2: Regression analysis data and summary of validation parameters

Sr. No.	VALIDATION PARAMETERS	RESULTS		STANDARD VALUES
		Sildenafil citrate	Ginsenoside Rg1	
1	Linearity & Range	25 – 75 µg/ml	2.5 – 7.5 µg/ml	---
2	Regression Equation	y = 107.49x - 53.306	y = 249.21x - 15.775	---
3	Slope	107.49	249.21	---
4	Intercept	53.306	15.775	---
5	Correlation Coefficient	0.9992	0.9995	≥ 0.999
6	Precision (% RSD)			
7	Repeatability	0.5890	0.5326	≤ 2.0 %RSD
8	Intraday precision	0.5283 - 0.6719	0.9082 - 1.8634	
9	Interday precision	0.3487 - 0.6058	0.7355 - 1.5681	
10	Mean % Recovery	99.69118	99.77487	98 – 102 %
11	Specificity		Specific	
12	LOD (µg/ml)	0.5206	0.1366	---
13	LOQ (µg/ml)	1.5776	0.4141	---
14	Robustness	Complies		≤ 2.0 %RSD

Table 3: Analysis of Sildenafil citrate and Asian ginseng extract by proposed method

Brand Name	Label Claim		% Mean assay ± S.D. (n=3)	
	Sildenafil citrate	Asian ginseng extract	Sildenafil citrate	Asian ginseng extract
VIP AGRA 24	100 mg	10 mg	99.5394 ± 0.4182	103.3661 ± 1.0321

REFERENCES

1. Badwan AA, Nabusli L, Al-omari MM, Daraghmeh N and Ashour M. Analytical profiles of drug substances and excipients, The Jordanian Pharmaceutical Manufacturing Corporation, Amman, Jordan, volume-27: 339-376.
2. Wass JH and Stewart PM. Oxford text book of Endocrinology and Diabetes, Oxford university press, 2nd edition: 1452-1454.
3. Eardley I. Pathophysiology of erectile dysfunction, The British journal of diabetes and vascular disease, July 2002: 272-276.
4. Dean RC and Lue TM. Physiology of penile erection and pathophysiology of erectile dysfunction, NIHRA Manuscripts, November 2005; 32(4): 379.
5. Salamon M (May 2013) Erectile dysfunction: symptoms and treatment (http://www.livescience.com/34724-erectile-dysfunction-disorder-symptoms-treatment.html) Accessed on March 2014.
6. Hardman JG and Limbird LE. Goodman Gilman's the Pharmacological Basis of Therapeutics, McGraw - Hill Companies Inc., 10th Edition (2001): 1007.
7. Sildenafil drug description (March 2014) (http://canadapharm24.com/order-viagra-online-en.html) accessed on March 2014.
8. Webb DJ, Freestone S, Allen MJ and Muirhead GJ. Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist, American Journal of Cardiology, March 4, 1999; 83 (5A): 21C–28C.
9. Indian Pharmacopoeia, Government of India, Ministry of Health & Family Welfare, Published by The Indian Pharmacopoeia Commission, Ghaziabad, 2014, Volume 3: 2725.

10. Dewick PM. Medicinal natural product: A biosynthetic approach, John Wiley & Sons Ltd, 2nd edition: 222-224.
11. US Pharmacopoeia-27, National Formulary-22, 2004, United States Pharmacopoeial Convention Inc., Asian edition: 2005-2009.
12. British Pharmacopoeia, The Department of Health & Social Services and Public Safety, Published by British Pharmacopoeia Commission, 2009, Volume 3: 6953, 7023, 7196.
13. Holland D, Ginseng (http://www.herballegacy.com/Holland_Chemical.html) accessed on March 2014.
14. Santa Cruz Biotechnology Inc. (2007-2014) (http://www.scbt.com/datasheet-205708-ginsenoside-rg1.html) accessed on March 2014.
15. Evans WC. Trees & Evans: Pharmacognosy, Elsevier Publication, 15th edition: 295-297.
16. Ginseng drug description, (2014) Web MD Inc. (http://www.emedicinehealth.com/drug-ginseng/article_em.htm#whatis) accessed on March 2014.
17. Ginseng, Panax Overview Information (2005-2014) Web MD, LLC. (http://www.webmd.com/vitamins-supplements/ingredientmono-1000-GINSENG,%20PANAX.aspx?activeIngredientId=1000&activeIngredientName=GINSENG %20PANAX) accessed on March 2014.
18. Sigma Aldrich Co. LLC. (2014) (http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant profiler/panax-ginseng.html) accessed on March 2014.