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Simultaneous Determination of Ibuprofen and Sitagliptin by HPLC

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ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), widely used over the counter drug worldwide. Co-administration of antidiabetic drugs with non-steroidal anti-inflammatory drug (NSAID) such as Ibuprofen is quite common. Rapid liquid chromatographic procedure proposed for analysis of Ibuprofen and Sitagliptin in pharmaceutical preparations using methanol: water (80:20 v/v) as a mobile phase, adjusting pH to 3.2 with UV detection at 275 nm. The advantages of this method include good and rapid separation, well resolved peaks, and only a small amount of sample is required for assay that display adequate precision. The method showed good linearity in the range of 231.25-1000 µg mL−1 for Ibuprofen and Sitagliptin with a correlation coefficient of 0.9999. The recovery of Ibuprofen and Sitagliptin was > 99.99% and > 99.96%, respectively. The proposed method may be used for the quantitative analysis of Ibuprofen and sitagliptin alone or in combination in bulk drugs and combined dosage formulations.

Keywords: *Ibuprofen, Sitagliptin, Formulation, HPLC, Analysis*

INTRODUCTION*

Ibuprofen belongs to a non-steroidal anti-inflammatory drug (NSAID) that comprises of arylpropionic acid derivatives. Chemically ibuprofen is [(RS)-2-{4-(2-methylpropyl) phenyl}] propanoic acid (figure 1). [1]. Molecular weight is 206 and structural formula is C13H18O2.[2[' It shows analgesic, anti-inflammatory, and antipyretic activity [3.4]. Sitagliptin is an oral antihyperglycemic drug that belongs of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs[5]. This drug is used either alone or in combination with other oral antihyperglycemic agents for treatment of type 2 diabetes mellitus[6]

There are a number of liquid chromatographic methods reported in the literature for the determination of Ibuprofen by HPLC [7-9] Several methods also reported for determination of Sitagliptin [10-14]. Although there are a number of methods reported for the determination of both Ibuprofen and Sitagliptin separately or in combination with other drugs [15], no method is reported for the simultaneous determination of these drugs using RP-HPLC in pharmaceutical formulations.

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METHODOLOGY

Standard bulk drug sample of Ibuprofen were supplied by Glaxo Smith Kline, tablet formulation of Ibuprofen was used including Brufen 400mg Abbott Laboratories Pakistan Ltd, Standard bulk drug sample of Sitagliptin were supplied by Highnoon Laboratories Ltd. HPLC grade acetonitrile and methanol

were obtained from RCI Labscan Limited, Darmstadt, Germany.

Instrumentation

High pressure liquid chromatography system model Shimadzu LC-20 AT, equipped with UV–visible detector model SPD-10A(V) vp, connected by CBM-102 communication Bus Module Shimadzu to Intel Pentium 4 machine with Shimadzu_ Class LC-20 Version 1:62 is used for integration and processing of chromatograms. HPLC Column Mediterranea Sea-18 of dimension 150 mm 4.6 mm i.d. 5 µm was used as the analytical column. All analysis was done at ambient temperature (25± 2 _C). The mobile phase is a mixture of methanol-acetonitrile-water (80:5:20, v/v) and using orthophosphoric acid pH was adjusted to 3.2. The flow rate was 1.0 mL.min_1 and volume of injection was 10 µL. Before using all solutions the mobile phase was sonicated for 30 min by WUC-A02H on-line degasser and filtered through 0.45-micron membrane filter, calibrated Pyrex glassware was used for the solution and mobile phase preparation. UV detection was performed at 275 nm for Ibuprofen and Sitagliptin.

Preparation of Solution

For the concentration of 1000 µgmL−1, 100 mg of the Ibuprofen and Sitagliptin was diluted to 100 mL using mobile phase. This solution was used for preparation of working solutions which were prepared by diluting the stock solutions using the same solvent to contain 31.25-1000 µgmL−1 for Ibuprofen and for Sitagliptin then filtered with 0.45-micron membrane filter. These solutions were ready to inject.

Analysis in Formulation

Twenty tablets of one brand of Ibuprofen and Sitagliptin were accurately weighed, grinded to create a fine powder. Calculated amount of powder of brand of ibuprofen was weighed, which was corresponding to 100 mg of Ibuprofen and transferred to a separate 100 mL volumetric flask and was dissolved in the mobile phase i.e. methanol-water, 80:20 v/v and filtered using a membrane filter of pore size 0.45 µ. The sample solution was further diluted to desire concentrations i.e. 31.25-1000 µgmL−1 and then used for the analysis.

RESULTS AND DISCUSSION

Method development and optimization

To select the optimal chromatographic conditions, different C18 stationary phases have been tried. Best separation, adequate resolution short retention time and symmetric peak of IBU and Internal Standard were achieved by Mediterranean Sea-18 of dimension 150 mm 4.6 mm i.d. 5 µm. To investigate appropriate wavelength for determination of Ibuprofen and Sitagliptin, we scanned solution of both drugs by UV-visible spectrophotometer. It was observed that the maximum absorbance of drug was obtained at 275 nm. Initially methanol and water were tried in different ratio o, at the ratio f 90:10 (v/v), the peak was not symmetrical. When mobile phase methanol-water, 80:20 v/v was used the drug showed typical peak nature and symmetry. To select the optimum mobile phase pH range 2.5 to 4.0 were investigated, excellent performance was achieved at pH 3.2 adjusted with orthophosphoric acid. Total run time was less than 7 min; short analysis times are essential for routine analysis.

Method validation

According to US Pharmacopeia and ICH guidelines, using a range of parameters (including system suitability, selectivity, specificity, accuracy test, linearity, precision, robustness, ruggedness, sensitivity, limit of detection and quantification) the developed method was validated.

Specificity and selectivity

To ascertain the specificity of the proposed method in presence of pharmacopoeial impurities, no peak of excipient was seen in

chromatogram, which authenticates that the method can be applied productively to dosage formulation and the method demonstrated excellent resolutions. Figure 5 represents typical chromatograms of drugs.

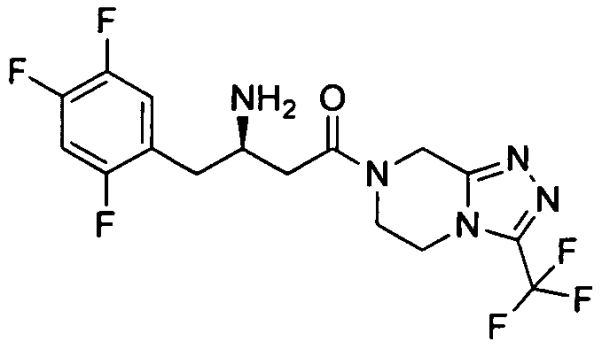

Fig. 1. Structure of Sitagliptin

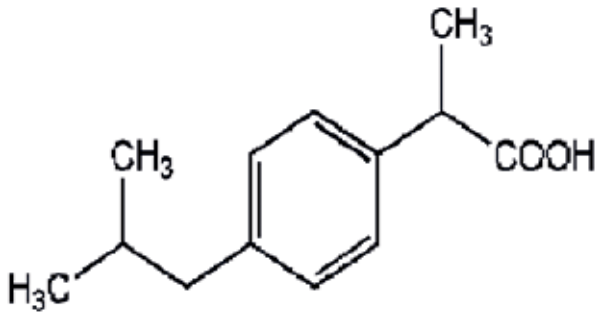

Fig. 2. Structure of Ibuprofen

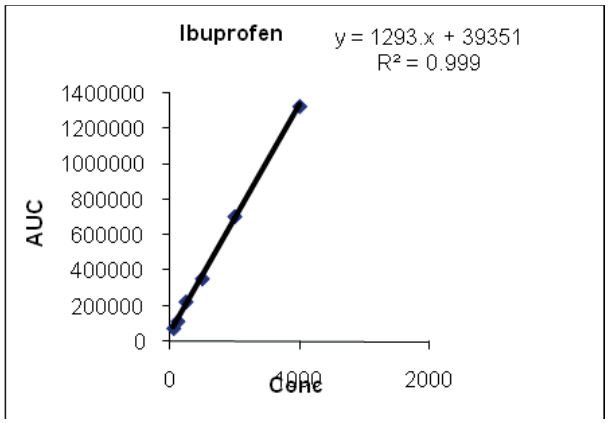

Fig. 3. Linearity of Ibuprofen

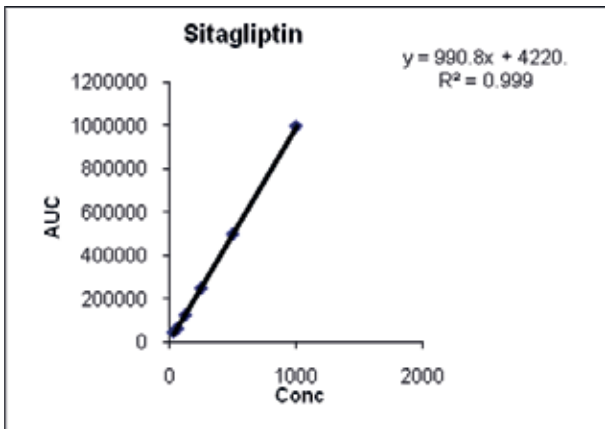

Fig. 4. Linearity of Sitagliptin

Table 1. Regression Statitics

Drug	Conc(µg mL^{-1})	Regression Equations	r2
Ibuprofen	31.25-1000	y = 1293.x + 39351	0.999
Sitagliptin	31.25-1000	y = 990.8.x + 4220	0.999

Table 2. Accuracy of Ibuprofen and Sitagliptin in Formulation

Drugs	Conc injected µg mL^{-1}	Conc found µg mL^{-1}	% Recovery	%RSD
Ibuprofen	250	249.99	99.99	0.11
	500	499.99	99.99	0.36
	1000	1000.0035	100.0035	0.1

Drugs	Conc injected µg mL^{-1}	Conc found µg mL^{-1}	% Recovery	%RSD
Sitagliptin	250	249.9	99.96	0.45
	500	500.05	100.01	0.002
	1000	1000.009	100.0009	0.32

Table 3. Interday Intraday Precision of Ibuprofen and Sitagliptin in API

Drugs	Conc injected µg mL^{-1}	% RSD	% Recovery
Ibuprofen	31.25	0.00082	100.002
	62.5	0.00052	101.001
	125	0.00026	99.98
	250	0.00033	99.99
	500	0.00008	99.99
	1000	0.00004	100.0035
Sitagliptin	31.25	0.0004	99.98
	62.5	0.0002	100.002
	125	0.0006	99.95
	250	0.0001	99.96
	500	0.0003	100.01
	1000	0.00021	100.0009

System suitability and Linearity

Parameter of system suitability are peak height, theoretical plates of the column, mass distribution ratio (capacity factor), retention time and resolution as summarized in table 1. Linearity was determined in the range 31.25-1000 µg mL−1 for both the drugs. Concentration of Ibuprofen and Sitagliptin versus peak area was subjected to least square linear regression analysis. A linear regression line was obtained with correlation coefficient (R2 > 0.999). The regression equations for active and serum (Figure 3 & 4) were shown in Table 2.

Accuracy and Precision

Method accuracy was evaluated as the percentage of recovery of known amounts of Ibuprofen and sitagliptin of the pharmaceutical formulation. It is performed at concentration that was 50%, 100% and 150%. Each sample was injected five times and result range was 99.99-100.003% for ibuprofen and 99.96-100.01, compiled in Table 3, high recovery specifies that the method has a high degree of accuracy. By repeatability i.e. intra-day precision and intermediate precision i.e.inter-day precision of the projected method was determined. It was expressed as relative standard deviation i.e. RSD. Six different concentrations of Ibuprofen and Sitagliptin in the linear range were analyzed in the same day (intra-day precision) and two consecutive days (inter-day precision); every sample was injected five times. Both intra- and inter-day RSD values were in the range 0.00004-0.00082 for Ibuprofen and 0.0001-0.0006 for Sitagliptin confirming excellent precision as shown in Table 4. The results were insignificant and indicated no noteworthy deference in interday and intraday precision.

Limit of detection and limit of quantification

The limits of detection i.e. LOD (calculated by

least concentration of Ibuprofen and Sitagliptin) and quantification i.e. LOQ were determined from the calibration curve. The LOD and LOQ were 1.44 ng mL−1 and 4.3 ng mL−1 for the Ibuprofen and for Sitagliptin it was 1.2 ng mL−1 and 2.2 ng mL−1respectively.

Robustness and Ruggedness

Robustness was performed by making minor variations in the percentage of mobile phase (methanol -water) wave length, pH and flow rate. Therefore, samples were injected repeatedly five times under small variations in each of the parameter. When a parameter was changed ± 0.2 % (in flow rate that was 1 ml min-1), ± 0.2% (pH 3.2), and ± 5 % wave length from its optimum condition, the shifting in retention time of ± 0.2% was also observed that evaluated as insignificance. The method proved to be fairly stable (Table 5).Ruggedness of our proposed method was determined by two different operators and two consecutive days. All parameters were compared, the developed method did not show any remarkable difference in calculated results from acceptable limits in precision, but the area under curve of peak was affected with change of wavelength.

CONCLUSION

The method is accurate and precise as indicated from the recovery study and the reasonable retention times (< 10 minutes). It can be concluded that the proposed HPLC method has great promise for the simultaneous determination of Ibuprofen and Sitagliptin in pharmaceutical formulations.

REFERENCES

1. Hardman JG, Limbird LE, Gilman AG. Goodman & Gilman's the pharmacological basis of therapeutics. 10th ed. New York, USA: McGraw-Hill. 2001.

2. National Center for biotechnology Information. PubChem Compound Database; CID=3672, https://pubchem.ncbi.nlm.nih.gov/compound/3672 (accessed Oct. 15, 2015).

3. Davies NM. Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin Pharmacokinet. 1998;34:101–154.

4. Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology. 2009;17:275–342.

5. Herman GA; et al . "Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses". Clin Pharmacol Ther. 2005; 78 (6): 675–88.

6. National Prescribing Service (August 2010). "Sitagliptin for Type 2 Diabetes". Retrieved 27 August 2010.

7. Ravisankar, S., Vasudevan, M., Gandhimathi, M., & Suresh, B. . Reversed-phase HPLC method for the estimation of acetaminophen, ibuprofen and chlorzoxazone in formulations. Talanta. 1998; 46(6), 1577-1581.

8. Farrar, H., Letzig, L., & Gill, M. . Validation of a liquid chromatographic method for the determination of ibuprofen in human plasma. Journal of Chromatography B. 2002; 780(2), 341-348.

9. Battu, P. R., & Reddy, M. S. . RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in tablets. Asian J. Research Chem. 2009; 2(1), 70-72.

10. Dubala, A., Khatwal, R., Kosaraju, J., Meda, V., & Samanta, M. . Bioanalytical method development and validation of sitagliptin phosphate by RP-HPLC and its application

to pharmacokinetic study. Int. J. Pharm. Pharmaceut. Sci; 2012; 4(2), 691-694.

11. Shyamala, M., Mohideen, S., Satyanarayana, T., NarasimhaRaju, C., Suresh, K. P., & Swetha, K. . Validated RP-HPLC for simultaneous estimation of sitagliptin phosphate and metformin hydrochloride in tablet dosage form. American Journal of PharmTech Research. 2011; 2(1): 93-101.

12. Pulla, R. P., Sastry, B. S., Prasad, Y. R., & Raju, N. A. . Simultaneous Estimation of Metformin HCl and Sitagliptin Phosphate in Tablet Dosage Forms by RP-HPLC. Research Journal of Pharmacy and Technology. 2011; 4(4): 646-649.

13. Phaneemdra, D., V. Venkatesh, and N. Ramarao. "Simultaneous estimation of simvastatin and sitagliptin by using different analytical methods." International Journal of Advances in Pharmaceutical Analysis. 2012; 2(1);19-23.

14. Tang, Y., Li, X., Wen, N., Sun, X., & Yu, M.. RP-HPLC determination of the content and the related substances of sitagliptin phosphate. Chinese Journal of Pharmaceutical Analysis. 2009; 29(8):1370-1372.

15. Patil, S. L., Jayant R. B, and Chetan M. B. "Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Determination of Sitagliptin and Metformin in Tablet Dosage Form." Pharma and Biomed. Sci. 2013; 4(2):590-596.