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Original Article

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Correlation of results between validated in-house analysis method with new pharmacopeia monograph for analysis of Sitagliptin Phosphate API

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Abstract

Having validated analysis methods for medicinal ingredients is attractive for pharmaceutical companies. When a new molecule is introduced to the market, there is not any pharmacopeial analysis method for that. After publishing official methods, the correlation between validated in-house methods and the official one could establish the value of the in-house method. Sitagliptin phosphate is a new antidiabetic pharmaceutical ingredient and many pharmaceutical companies are trying to manufacture high-quality dosage forms using this agent. In the present study, a full validated in-house method for analysis of sitagliptin phosphate Active Pharmaceutical Ingredient (API) is presented and the method is compared with newly published United State Pharmacopeia (USP) monograph. Results show that the in-house method is correlated with the USP method with regard to assay study and even could separate and detect more probable impurities in the sample. In brief, a full analytical method validation based on USP general chapter (<1225>) was done on the developed analysis method and a calibration curve was plotted successfully with a reasonable R2 equal to 0.9993 and the equation of the curve was Y = 3.4588X +30.099. Then precision, accuracy, and robustness were studied. The mobile phases, column, column temperature, sample preparation of the solvent, as well as detector wavelength, are different in two methods. It seems that the validated method could be a valuable alternative method for USP method depending on users facilities for analysis. It seems with presenting of this method, more pharmaceutical research centers will be able to analyze sitagliptin with a high degree of assurance.

Keywords: Sitagliptin Phosphate, Analytical Method Validation, Assay, Impurities, HPLC, Pharmacopeial analytical method.

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Introduction

Sitagliptin Phosphate, previously identified as MK-0431 and marketed under the trade name Januvia, is an oral antihyperglycemic drug. Sitagliptin was approved by the U.S. Food and Drug Administration (FDA) on October 17, 2006. Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and

Table 1: in-house analysis method in comparison to USP method (USP 2006)

HPLC Column		**Mobile phase composition**		**Flow rate**		**Column temp.**	
USP Method	In-house method	USP Method	In-house method	USP Method	In-house method	USP Method	In-house method
4.6-mm*15-cm;5-μ packing L 10	4.6-mm*15-cm;5-μ packing L 1	Mobile phase: (Acetonitrile: buffer) (15:85) Buffer: 1.36 g/L monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 2.0	Mobile phase A: Dissolve 11.2 ml /L triethylamine and add 5 ml phosphoric acid, adjusted with phosphoric acid to a pH of 3.0 Mobile phase B: (Acetonitrile: Mobile phase A) (70:30)	1 ml/ min	1 ml/ min	30 ° C	Room temperature
Solvent		Sitagliptin peak retention time		Detector		Injection volume	
USP Method	In-house method	USP Method	In-house method	USP Method	In-house method	USP Method	In-house method
(Acetonitrile:dilute phosphoric acid) (5:95) Dilute phosphoric acid: 1ml/L phosphoric acid	Mobile phase B	7-8 min	11-13 min	UV 205 nm	UV 220 nm	20 μL	20 μL

Table 2: Analysis data for sample 1 using in-house and USP method

Sample 1 (QC-STG-95003) (in-house method)											
Assay				**Related**			**Std RT**	**Impurities area**			**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Impurities RT**							
				1	**2**	**3**		**1**	**2**	**3**	
11.12	11.72	414.03	432.06	12.47	14.20	16.61	13.77	5213	1627	1064	75443
11.12	11.18	410.58	433.44	12.45	14.19	16.61	13.77	5224	1620	1059	73979
11.10	11.15	408.64	436.76	12.46	14.18	16.56	13.73	5224	1611	1082	75762
Average		411.08	434.09	**Average**				5220.33	1619.33	1068.33	75061.33
RSD		0.66	0.56	**RSD**				0.12	0.50	1.13	1.27
Result		77 ± 1.28	72 ± 1.84	**Each impurity: 0.007 %**				**Total impurity: 0.01 %**			
Sample 1 (QC-STG-95003) (USP method)											
Assay				**Related**			**Std RT**	**Impurities area**			**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Impurities RT**							
				1	**2**			**1**	**2**		
11.35	11.36	4866281	4605156	5.723	9.303	11.032		331	420	8149	
11.35	11.34	4885179	4613642	5.691	9.247	11.040		331	420	8276	
11.32	11.31	4921541	4614962	5.703	9.284	11.043		330	417	8265	
Average		4891000	4611253	**Average**				331	419	8230	
RSD		0.11	0.12	**RSD**				0.17	0.41	0.65	
Result		99.12%		**Each impurity: 0.004 %**				**Total: 0.007 %**			

Table 3: Analysis data for sample 2 using in-house and USP method

Sample 2 (QC-STG-95004) (in-house method)																	
Assay				**Related**						**Std RT**	**Impurities area**						**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Impurities RT**													
				1	**2**	**3**	**4**	**5**	**6**		**1**	**2**	**3**	**4**	**5**	**6**	
10.57	10.57	501.28	571.48	12.37	12.47	13.03	14.20	14.75	16.61	13.79	5213	1627	1064	1778	16468	755	73395
10.58	10.58	499.79	571.26	12.38	12.47	13.03	14.20	14.76	16.62	13.78	5224	1620	1059	1773	16249	756	73789
10.55	10.55	498.85	579.63	12.35	12.43	13.00	14.17	14.73	16.59	13.78	5224	1611	1082	1790	16633	778	72799
Average		411.08	499.98	**Average**							5220.33	1619.33	1068.33	1783	16450	763	75061.33
RSD		0.66	0.25	**RSD**							0.12	0.50	1.13	0.34	1.17	1.70	1.27
Result		**100.26 %**		**Each impurity: 0.02 %**							**Total impurities: 0.04 %**						

Sample 2 (QC-STG-95004) (USP method)							
Assay				**Related**	**Std RT**	**Impurities area**	**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Impurities RT**			
				1		**1**	
10.8500	10.8333	3746.83	4242.28	9.189	11.032	333	8149
10.8500	10.8333	3744.38	4243.73	9.126	11.040	344	8276
10.8333	10.8333	3740.75	4245.27	9.143	11.043	334	8265
Average		3743.99	4243.76	**Average**		337	8230
RSD		0.08	0.04	**RSD**		1.80	0.65
Result		**99.98%**		**Each impurity: 0.003 %**		**Total impurities: 0.003 %**	

Table 4: Analysis data for sample 3 using in-house and USP method

Sample 3 (QC-STG-95005) (in-house method)													
Assay				**Related**				**Std RT**	**Impurities area**				**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Related**									
				1	**2**	**3**	**4**		**1**	**2**	**3**	**4**	
13.27	13.27	618596	672304	12.55	12.93	12.43	16.67	38 ± 1.42	7891	2675	1427	1005	71240
13.27	13.27	625496	677553	12.56	12.93	14.23	16.67	46 ± 1.84	7941	2605	1464	983	71465
13.28	13.26	621567	673514	12.54	12.92	14.24	16.60	54 ± 1.27	7960	2636	1422	990	71824
Average		621916	6744573	**Average**					7919	2638	1420	989	71509
RSD		0.56	0.41	**RSD**					0.46	1.33	0.5	1.62	0.41
Result		**99.79%**		**Each impurity: 0.01 %**					**Total impurity: 0.02 %**				
Sample 3(QC-STG-95005) (USP method)													
Assay				**Related**				**Std RT**	**Impurities area**				**Std area**
Std RT	**Sam RT**	**Std area**	**Sam area**	**Impurities RT**									
				1	**2**				**1**	**2**			
11.02	11.07	4123.84	4638.12	4.475	9.134			11.032	236	448			8149
11.03	11.07	4122.36	4669.38	4.468	9.116			11.040	239	462			8276
11.03	11.07	4116.41	4670.04	4.466	9.134			11.043	234	451			82654
Average		4120.87	4659.18	**Average**					236	454			8230
RSD		0.10	0.39	**RSD**					1.06	1.62			0.65
Result		**99.19%**		**Each impurity: 0.004 %**					**Total impurities: 0.007 %**				

GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas (Herman et al., 2006 and Herman et al., 2005). Although many companies synthesized the Sitagliptin API and others formulated that as pharmaceutical dosage form but after 11 years from discovery of this molecule a Pharmacopeial analytical method was published for it in USP 39. Prior to the USP method, a full validated analysis method was developed by our research team which is presented in this manuscript. Also, we compared the results of both pharmacopeial and in-house methods when we tested three different batches of sitagliptin API.

Materials and Methods

In-house method validation:

The developed method is presented in Table 1 in comparison with USP monograph. A full analytical method validation based on USP general chapter (<1225>) was done on the developed analysis method. In brief, a calibration curve was plotted successfully with a reasonable R2 equal to 0.9993 and the equation of the curve was Y = 3.4588X +30.099. The range of linearity was 1-1000 ppm. Precision was studied for two different concentrations, 10 and 100 ppm, RSD was 0.62 and 0.44% (≤2). The accuracy of the method was investigated for the same concentrations as precision and error percentages were 0.55 and 1.2%, which are completely reasonable. Robustness was studied by changing mobile phase flow rate, pH and temperature and the effect of changing these factors on tailing factor were investigated. Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the method were 0.75 and 2.5 ppm, respectively.

Comparison of the assay and related substances test results using In-house and USP method:

Three different batches of sitagliptin were analyzed by both methods and results were compared with each other. Results are described in Table 2-4.

Results and discussion

The results of analysis of three samples with two methods are presented in Tables 2-4. Results of the assay for all samples are very similar. Generally, in-house method could detect more impurities in most of the samples. Although previous validated HPLC and UV spectroscopy methods were published for analysis of sitagliptin using different analysis conditions (Lavanya et al., 2013, Ravisankar et al., 2015 and Tarkase et al., 2013), there is not any published data in which results were compared to USP monograph.

Conclusion

An HPLC method was found to be simple, accurate, precise, linear, robust and specific for quantitative estimation of Sitagliptin phosphate in bulk API. Then the correlation between results of this method with newly published USP monograph was investigated. As the column, column temperature, mobile phase composition, sample preparation solvent and detector wavelength are different in two methods, it seems that in-house method could be a valuable alternative for the USP method which could be performed when the user facilities are fit with that.

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References

Herman G, Bergman A, Liu F, Stevens C, Wang A, Zeng W et al. Pharmacokinetics and pharmacodynamic effects of the oral DPP-4 inhibitor sitagliptin in middle-aged obese subjects.. J Clin Pharmacol 2006; 46 (8): 876–86.

Herman GA, Stevens C, van Dyck K, Bergman A, Yi B, De Smet M, Snyder K 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.

The United State Pharmacopeial Convention, united state pharmacopeia 2016.5854-5855.

Lavanya R, Yunoos MD. Development and Validation of RP-HPLC method for the estimation of Sitagliptin Phosphate in Bulk and its Tablet Dosage Form. J Adv Pharm Edu & Res 2013; 3(4): 475-479.

Ravisankar P, Hassain SK, Neeha SM. Novel RP-HPLC Method for Simultanious Determination of Sitagliptin and Simvastation in Bulk and Tablet Dosage Form. IOSR Journal of Pharmacy 2015; 5(8): 34-40.

Tarkase KN, Sarode MB, Gulve SA, Gawade A. Development and validation of UV spectrophotometricmethod for estimation of sitagliptin phosphate. Der Pharmacia Lettre 2013; 5 (3):315-318.