Original Article

A Selective and Sensitive Method for the Determination of Sulfonate Ester Impurities in Saxagliptin Drug Substance by HPLC

P. Ilayaraja[1], M. Manivannan[2], P. Parthiban*[3]

[1,2,3] Department of Chemistry, Centre for Research and Development, PRIST Deemed University, Thanjavur, Tamilnadu, India.

[3] Department of Biomedical Science, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq.

Email: [3]parthiban.paramasivam@komar.edu.iq

***Corresponding Author:** P. Parthiban

Department of Chemistry, Centre for Research and Development, PRIST Deemed University, Thanjavur, Tamilnadu, India.

Department of Biomedical Science, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq.

DOI: 10.47750/pnr.2022.13.S05.322

Abstract

Saxagliptin is used in the treatment of type 2 diabetes to improve blood sugar control in combination with other drugs or as monotherapy. Sulfonate esters are formed during the synthesis of drug substances involving sulphuric acid which reacts with the alcohols thus yields alkyl and aryl esters. Sulfonate esters have the capability to undergo reaction with DNA and initiates mutagenic changes, which leads to cancerous cell generation. Therefore, the limiting of sulfonate esters in drug substances is important to the pharmaceutical products. Therefore, it is necessary to have a highly sensitive and cost effective method to monitor the sulfonate esters in the Saxagliptin drug substances. Six sulfonate esters *viz.*, methyl, ethyl and isopropyl tosylates, as well as methyl, ethyl, and isopropyl besylates were separated and quantified using the simple direct HPLC method with highest sensitivity of 0.033 ppm as limit of detection. The method was optimized with 0.10% orthophosphoric acid in Milli-Q water as mobile phase A and 100% acetonitrile as mobile phase B in gradient elution condition in Inertsil ODS 3V (250 mm x 4.6 mm x 5 µm) column at 40 °C oven temperature with a flow rate of 1.0 mL/min. The developed method is highly sensitive, accurate, linear, and precise over the range of 0.1 to 1.5ppm. The finalized procedure was confirmed by thorough validation with the International Council for Harmonisation guidelines, where all the parameters are in good agreement with the acceptance criteria.

Keywords— Sulfonates, Sulfonate esters, Saxagliptin, HPLC, Method development, Method validation.

INTRODUCTION

Saxagliptin (Figure-1) is an antidiabetic drug to treat type 2 diabetes. It is one type of dipeptidyl peptidase-4 (DPP-4) inhibitor [1-4] that works by impacting the act of natural hormones in the body called incretins. Incretins reduces plasma sugar, mostly through maximising insulin segregation in the pancreas and then lowering the sugar production by liver. By consuming Saxagliptin, GLP-1 (Glucag on like peptide-1) and GIP (glucose-dependent insulinotropic polypeptide) levels rise to 2 to 3 times since this is specific of DPP4 hindrance. Sulfonate esters [5] form (Figure 2) through the reaction of sulfonic acid with alcohols. Sulfonic acid is used as counter-ions during the formation of acid-addition salts, and as process chemical and catalyst as well in the of drug synthesis. Hence, the presences of sulfonates esters are possible in Saxagliptin but there is no specific HPLC method[6-11] reported for the estimation of sulfonates esters in Saxagliptin. Since the alkyl and aryl sulfonate esters[12] are potential genotoxic impurities, it is very unsafe to the human consumption. Hence it is essential to develop a sensitive as well as simple procedure for the quantification of sulfonate esters (Figure 3) in Saxagliptin by single HPLC method. Accordingly, the present research was focused to develop a simple, cost-effective and selective method for the analysis of multiple sulfonate esters without derivatization procedure.

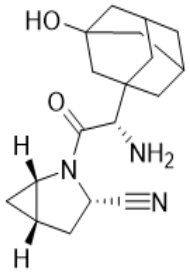


Figure 1. *Saxagliptin structure*

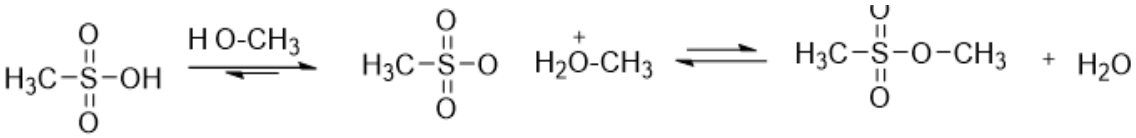
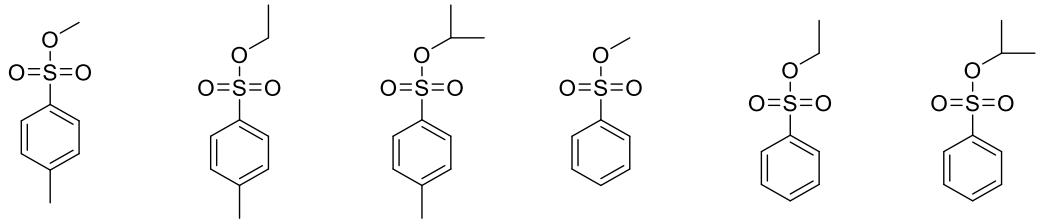


Figure 2. *Scheme of sulfonate ester formation*



MT: Methyl tosylate, ET: Ethyl tosylate, IPT: Isopropyl tosylate, MB: Methyl besylate, EB: Ethyl besylate, IPB: Isopropyl besylate.

Figure 3. *Sulfonate esters structures*

MATERIALS AND METHODS

Analytical reagent grade high pure orthophosphoric acid, acetonitrile and methanol were procured from Rankem Chemicals (Hyderabad, India). HPLC grade water was obtained from Spectrochem. The high pure sulfonate ester standards were procured from Sigma Aldrich.

Instrumentation

The liquid chromatographic analysis was performed on Agilent 1200 HPLC System with Empower 3 chromatography data system consist of a quaternary pump, degasser, variable wavelength/diode array detectors, an auto sampler, and column compartment with temperature control facility.

Chromatographic Conditions

The required peak symmetry and selectivity were achieved on Inertsil ODS 3V (250 x 4.6) mm, 5 µm stationary phase with 0.10% orthophosphoric acid in water as eluent A and acetonitrile as eluent B in gradient elution. Column oven temperature was maintained at 40 °C with a flow rate of 1.0 mL/min using 20 µL injections. The gradient elution was as follows: time (min)/%A: 0/65, 52/65, 20/50, 45/15, 50/15, 52/65, and 60/65 with detector wavelength of 220 nm.

Standard preparation

Each 5mg of all sulfonate esters were transferred in to individual flasks and diluted to 100 mL with diluent (stock-1). Then 5ml of stock-1 solution is diluted to 100 mL (stock-2). Further, 5mL of the stock-2 solution was diluted to 100 mL (stock-3). Finally, 1 mL of stock-3 solution was diluted to 10 mL. The resulting solutions contain 1.0 ppm of sulfonate ester with respect to the test concentration of 12.5 mg/mL.

Test solution preparation

125 mg of Saxagliptin sample was added in to a 10 mL standard flask and sonicated to dissolve and made up to the volume with diluent. The sample solution was filtered and injected.

Method development and optimization:

The method was developed by multiple verifications such as stationary and mobile phase selections, column oven temperature and organic modifiers selections. Based on trials, the method was finalized using Inertsil ODS 3V (250 x 4.6) mm, 5 µm as stationary phase and 0.10% orthophosphoric acid in water as eluent A and acetonitrile as eluent B in gradient elution (time/%A: 0/65, 5/65, 20/50, 45/15, 50/15, 52/65 and 60/65; column oven temperature: 40°C; flow rate: 1.0 mL/min; detector wavelength: 220 nm; injection volume: 20 µL). Further, the method was verified by optimizing different buffer strengths, flow rates and column oven temperatures (25 to 45°C). The symmetrical peak shape was achieved at higher pH of 9.0. The developed method's chromatograms were captured and reproduced in Figures 4-7**.**

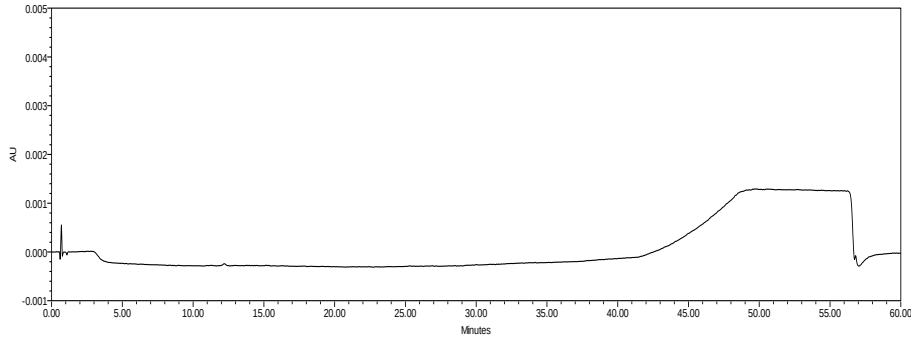


Figure 4. *Blank chromatogram*

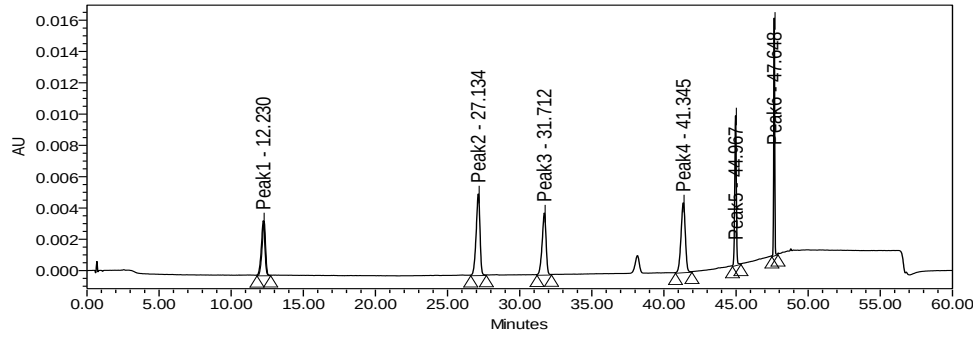


Figure 5: *Standard chromatogram (1 ppm)*

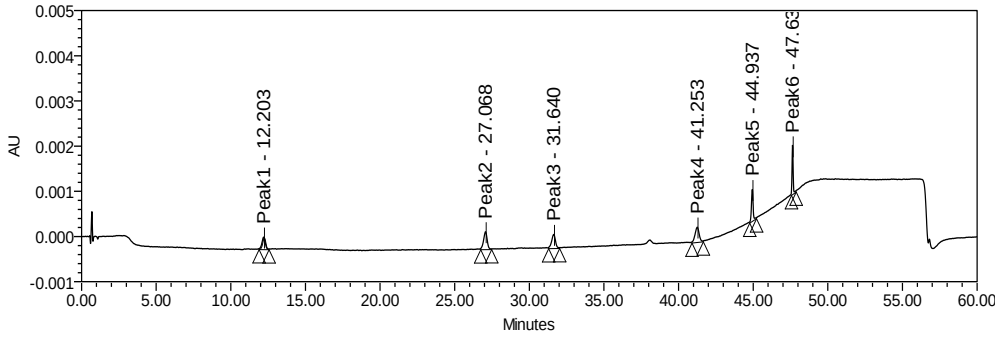


Figure 6: *LOQ solution chromatogram (0.1 ppm)*

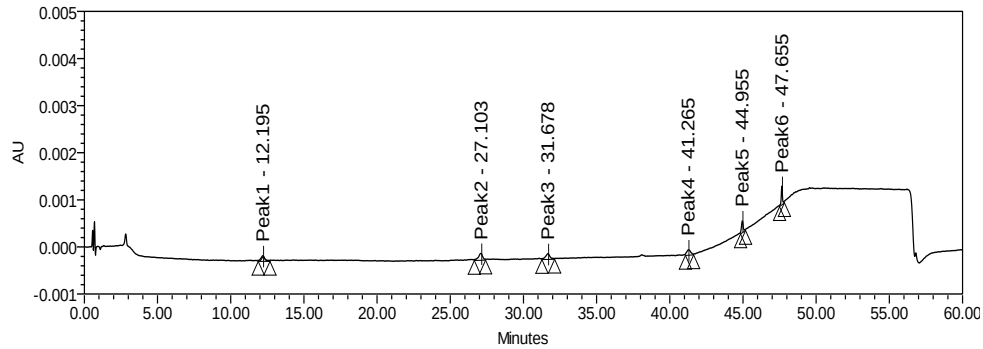


Figure 7: *LOD solution chromatogram (0.03 ppm)*

RESULTS AND DISCUSSION

Method validation

The finalised HPLC method of the sulfonates estimation in Saxagliptin was validated in accordance with ICH guidelines. The following validation parameters, namely system suitability, specificity, system precision, method precision, intermediate precision, recovery, robustness, linearity, LOQ, LOQ precision, LOD and range were determined.

Specificity

Specificity of this method was performed by spiking all the sulfonate esters in the sample and the retention time of each component was separately determined by individual injections also monitoring the peak purity of each component in spiked sample. The Saxagliptin and the six sulfonate esters peak were found to be homogenous from the peak purity results.

Linearity and range

The linearity was performed from 0.1 to 1.5 ppm concentration using sulfonate esters standard solution. The standard solutions were prepared from the respective sulfonate esters high pure standard by diluting in to respective concentrations levels. The method was linear from 0.1 to 1.5 ppm, which indicates the developed method's very high working range, and thus it can be utilized to quantify even trace level of sulfonate esters. Correlation co-efficient and regression were calculated for each sulfonate esters response against respective level concentrations and the linearity graph was found to be satisfactory.

Precision

System precision was executed by injecting the six sulfonate esters standard in six replicate injections and the RSD for the area of each sulfonate esters peak was found to be <0.97%. It indicates the selected method's suitability to determine the sulfonate esters in Saxagliptin. Method precision analysis was executed by preparing the sample in six replicate preparations from the same batch sample by spiking all the six sulfonate esters in 100% level concentration and injected in duplicate injections. All the sulfonate esters content were calculated for each preparation and %RSD was kept as acceptance criteria. Intermediate precision was performed in different days with different instruments by performing the content of sulfonate esters and cumulative %RSD for the content values have been calculated and it was found to be <1.0%. Based on the method and intermediate precisions results, the developed method was found to be reproducible in any laboratory.

Accuracy

Accuracy is the closeness of agreement between the experimental and observed values. Accuracy was performed by preparing the sample by spiking known quantities of sulfonate esters from LOQ level to 150% in three different replicate preparations. The obtained recovery results were calculated in the form of accuracy and all the results fall between 80 and 120%.

Robustness

The strength of an analytical methodology to remain intact by small, but intentional variations in method parameters is its robustness. Robustness of the method clearly defines its reliability during usage. It is important to have robustness in validation parameters to support the method transfer from one site to other site or to execute it in other laboratories. Here it is evaluated with deliberate changes in buffer strength (range: 0.08 to 0.12%), flow rate (range: 0.80 to 1.20 mL/min), and column oven temperature (range: 30 to 40°C) on the final method conditions. System suitability parameters and sulfonate esters content variation between control and modified conditions were used as a tool to verify the robustness parameters. The control and robustness conditions data were compared and found to be meeting the acceptance criteria.

Hence, this research proposes a simple and high throughput HPLC chromatographic method, capable of separating all sulfonate esters from Saxagliptin and quantifying with highest sensitivity of 0.1 ppm quantification level. The developed method's validation results are reported in Tables 1-4. The developed method for the separation and determination of PGIs was validated as per ICH guidelines.

***Table 1**: System suitability and system precision*

System suitability parameter	MB	EB	IPB	MT	ET	IPT
USP Tailing	0.98	0.99	0.98	1	1.02	1.01
USP Theoretical plates	17361	22451	20543	21565	24954	23584
%RSD n=6	0.71	0.23	0.65	0.33	0.45	0.66

***Table 2**: Linearity*

Parameters	MB	EB	IPB	MT	ET	IPT
Linearity range (ppm)	0.110-1.54	0.106-1.52	0.098-1.52	0.102-1.52	0.103-1.52	0.101-1.52
Correlation co-efficient	0.9997	0.9998	0.9997	0.9996	0.9997	0.9995
Regression co-efficient	0.9987	0.9988	0.9986	0.9985	0.9988	0.9983
% Y Intercept	1.23	2.1	1.81	1.66	1.39	1.41
LOQ	0.11	0.106	0.098	0.102	0.103	0.101
LOD	0.036	0.035	0.032	0.034	0.034	0.033

***Table 3**: Accuracy*

Parameters	MB	EB	IPB	MT	ET	IPT
Recovery LOQ	95.6	97.8	98.8	96.3	95.9	95.3
Recovery 50%	98.6	99.5	97.9	100.1	98.3	100.5
Recovery 100%	98.8	100.3	99.7	99.3	98.2	99.8
Recovery 150%	100.1	99.8	100.5	100.8	99.6	98.7

***Table 4**: Precision*

Method precision	MB	EB	IPB	MT	ET	IPT
Average Content	1.03	1.02	1.03	0.98	0.99	0.97
%RSD	0.43	0.54	0.32	0.45	0.53	0.27
Intermediate precision	1.01	1.02	1.00	1.01	1.01	1.01
%RSD	0.23	0.22	0.37	0.45	0.32	0.27
Cumulative %RSD	0.63	0.49	0.65	0.72	0.69	0.88

CONCLUSION

The disclosed and validated RP-HPLC procedure relies on conventional HPLC with high sensitivity and good throughput for estimating the sulfonate esters in Saxagliptin. This procedure is a direct analysis with the usage of easy operational procedures. In addition, this method can be applied to the other drug substances through simple modifications in order to support the sample matrix issues.

Disclosure statement

There are no conflicts of interest, according to the authors.

REFERENCES

1. Golightly LK, Drayna CC, McDermott MT: Comparative clinical pharmacokinetics of dipeptidyl peptidase-4 inhibitors. Clin Pharmacokinet. 2012; 51(8):501-514.
2. Dave DJ. Saxagliptin: A dipeptidyl peptidase-4 inhibitor in the treatment of type 2 diabetes mellitus. J Pharmacol Pharmacother. 2011; 2:230–235
3. Cobble, M.E., Frederich, R. Saxagliptin for the treatment of type 2 diabetes mellitus: assessing cardiovascular data. Cardiovasc Diabetol 2012. 11, 6.
4. Konya H, Yano Y, Matsutani S, Tsunoda T, Ikawa T, Kusunoki Y, Matsuo T, Miuchi M, Katsuno T, Hamaguchi T, Miyagawa J, Namba M. Profile of saxagliptin in the treatment of type 2 diabetes: focus on Japanese patients. Ther Clin Risk Manag. 2014;10:547-558
5. David E, Kevin LF, Jeffrey NL, Rodney P, David R, Lesley S, Andrew T. An Approach to Control Strategies for Sulfonate Ester Formation in Pharmaceutical Manufacturing Based on Recent Scientific Understanding, Organic Process Research & Development 2012 16 (11), 1707-1710
6. Jin B, Guo K, Zhang T, Li T, Ma C. Simultaneous Determination of 15 Sulfonate Ester Impurities in Phentolamine Mesylate, Amlodipine Besylate, and Tosufloxacin Tosylate by LC-APCI-MS/MS. J Anal Methods Chem. 2019; 4059765.
7. ICH (1994) Q2 (R1) Validation of Analytical Procedures: Text and Methodology. European Medicines Agency, London, UK, p. 1-15.
8. ICH (1996) Q2B Guideline Validation of Analytical Procedures Methodology: Comments for its application. p: 71-76.
9. Scheeren LE, Marcolino AIP, Adams AIH, Rolim CMB. Stability indicating RP-LC-PDA method for the quantitative analysis of saxagliptin in pharmaceutical dosage form. Braz J Pharm Sci. 2015; 51:461–466.
10. Gaikwad DD, Patel SG, Waman SA, Jadhav SL, Dhobale SM. Method Development and Validation of Saxagliptin Hydrochloride by RP-HPLC Method. Bull. Env. Pharmacol. Life Sci., 2020; 9:22-28.
11. Bailey F. Applications of high-performance liquid chromatography in the pharmaceutical industry. J Chromatogr A, 1976; 122:73-84.
12. Elder DP, Teasdale A, Lipczynski AM. Control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients (APIs). J Pharm Biomed Anal. 2008; 46(1):1-8.