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Dual Analyte Determination and Validation of Trace Levels Methanesulfonic Acid and Trifluroacetic Acid in Saxagliptin Drug Substance using Ion Chromatographic Technique

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ABSTRACT

A sensitive ion chromatgraphic method was developed and optimized for the determination of methanesulfonic acid and trifluroacetic acid in saxagliptin drug substance. These organic acids in lower limits act as potential impurities and causes undesirable by products. The method was developed to enhance the detection by this technique and minimizing the acquisition time by using 30 min. To prove the performance characteristic of the optimized method validation parameters performed as per the ICH guidelines requirements for selectivity, sensitivity, LOD, LOQ, linearity, precision, robustness and accuracy.

Keywords: Methanesulfonic acid, Trifluoroacetic acid, Saxagliptin drug substance, Ion chromatography, Validation

INTRODUCTION

Saxagliptin monohydrate (**Figure 1**) an oral hypoglycemic drug or antidiabetic drug classified under dipeptidylpeptidase-4 (DPP-4) inhibitor [1]. It is mostly used in combination of other drugs for the treatment of type 2 diabetes. Saxagliptin was supposed to act as inhibitor of DPP4 augment postprandial insulin secretion and emphasizes as to stimulate insulin secretion in a glucose dependent manner. This mechanism of action is expected to present low risk of hypoglycemia and may not lead to weight gain [2].

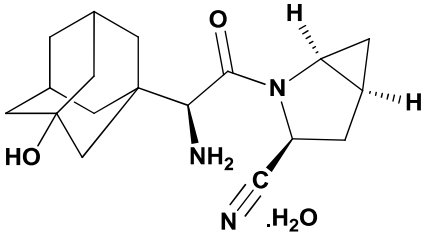

Figure 1: Structure of Saxagliptin monohydrate

During the process preparation of saxagliptin two of the genotoxic impurities i.e., methanesulfonic acid and trifluoroacetic acid were used in the basic intermediary stages [3]. These reagents may be present in the final drug substances as impurities which were believed as active reactive impurities with unwanted toxicities, including genotoxicity and carcinogenicity. Such genotoxic impurities should be controlled in final drug based on maximum daily dose [4]. The limit for daily dose fall at low in µg/ml levels, Hence conventional HPLC, GC methods may not suitable for their determination [5].

Several reports reported for their individual identification in fruit juices and wines [6-8]. These types of entities can be analyzed using ion chromatographic techniques, fewer methods reported for their analysis [9]. A method need to

be finalized where dual chemical entities identified and characterized. These two impurities were estimated in trace levels using ion chromatographic technique. Where the technique was adopted due to its sensitivity for the detection in trace level uses eco-friendly chemicals and cheaper compared to other techniques like high performance liquid chromatographic and gas chromatographic technique.

MATERIALS AND METHODS

Chemicals and reagents

Methanesulfonic acid and trifluoroacetic acid used as reference standard was purchased from sigma-aldrich. Sodium carbonate, sulfuric acid, sodium-bicarbonate was purchased from E. Merck (Mumbai, India). Water was distilled and purified with Millipore system (Millipore Corporation, India). The known related substances of saxagliptin monohdrate were prepared at *Aurobindo Pharma Ltd.* Research Centre, India were used for studies.

Instrumentation

Ionchromatography

An Ion chromatograph (Metrohm 733 compact IC Flex) with conductometric detector, metrohm 732 IC detector module and Metrohm 813 compact auto sampler or equivalent and Metrohm 762 IC interface with Metrohm IC Net 2.3 or equivalent data handling system or An Ion chromatograph (Dionex ICS 5000+) with conductometric detector and AS AP auto sampler or equivalent with Chromeleon 6.8 version or equivalent data handling system, 20 µl loop, Sartorious analytical balance and ultra-microbalances were used.

Mobile phase solution

The mobile phase was a mixture of 2.8 mm of sodium-bicarbonate and 2.2 mm of sodium carbonate in water. Filter through 0.45 µm finer porosity membrane filter. The analysis was carried out on Metrosep Asupp 5 (6.1006.530), 250 mm long, 4.0 mm i.e., 5 µm particle diameter column, maintained at ambient conditions. Mobile phase was pumped through the column at a flow rate of 0.6 ml/min. The run time for the standard and sample was 30 min. The injection volume was 20 µl. The retention time of methanesulfonic acid and trifluoroacetic acid is about 8.0 min and 15.0 min respectively. Water is used as diluent.

Suppressor solution

Transfer carefully 2.8 ml of sulfuric acid to 1000 ml of water. [For Metrohm system] (or)

Transfer carefully 4.0 ml of sulfuric acid to 4000 ml of water. [For Dionex system]

Stock solution A

Accurately weigh and transfer about 100 mg each of methanesulfonic acid reference standard into 100 ml clean dry volumetric flask, containing 60 ml of diluent and swirl well. Make up to 100 ml with diluent.

Stock solution B

Accurately weigh and transfer about 100 mg each of Trifluoroacetic acid reference standard into 100 ml clean, dry volumetric flask through sides, containing 60 ml of diluent and swirl well. Make up to 100 ml with diluent.

Standard solution

Transfer exactly each 5 ml of Stock solution A and Stock solution B into a 100 ml clean, dry volumetric flask and make up with diluent. Further dilute 2 ml of this solution to 100 ml with diluent.

Sample solution

Accurately weigh and transfer about 100 mg of saxagliptin monohydrate reference sample into 100 ml clean dry volumetric flask and add 30 ml of diluent shake gently to dissolve and make up to 100 ml with diluent.

Procedure

Inject 20 µl of diluent, standard solution and sample solution into the chromatograph and record the chromatogram. Examine the diluent chromatogram and no interference peak should be observed at the retention times of methanesulfonic acid trifluoroacetic acid. Integrate peak due to methanesulfonic acid trifluoroacetic acid only (RT of methanesulfonic acid is at 8.0 min and trifluoroacetic acid is at 15.0 min).

RESULTS AND DISCUSSION

Method development

Determination of methanesulfonic acid trifluoroacetic acid in ppm levels which are strongly retained in the drug using ion chromatography was the main objective of this work. Initial trials were made on instrument with suppressor ion chromatographic mode. Where mobile phase used was 3.2 mm sodium carbonate and 1.0 mm of sodium-bicarbonate and column 'Metrosep A Supp 5' with packing material as polyvinyl alcohol with quaternary ammonium groups used as stationary phase. As saxagliptin monohydrate, methanesulfonic acid and trifluoroacetic acid were soluble in water; solutions were prepared using water as diluents and injected in IC. The retention time methanesulfonic acid standard is about 7.2 min and trifluoroacetic acid merging with unknown peak was observed. But, both of the peaks were found to be very close to each other. For the better resolution the method was optimized. In another trial 0.25 mm sodium carbonate and 4.0 mm of sodium-bicarbonate was used where methane sulfonic acid observed at about 9.0 min and trifluoroacetic acid is about 20.0 min but the diluent has observed negative peak at the retention time of trifluoroacetic acid.

Better resolution and peak shapes obtained when the concentration of sodium carbonate was increased to 2.2 mm and bicarbonate decreased to 2.8 mm at ambient temperature. Result shown to have better resolution of 8.0 and 15.0 min corresponding to two peaks.

Method validation

In order to determine the methanesulfonic acid and trifluoroacetic acid saxagliptin monohydrate drug substance, the method was validated as per the ICH guidelines. Individually in terms of specificity, LOD, LOQ, linearity, accuracy and precision of sample solution.

Specificity

To prove the selectivity of the method, it is necessary to evaluate a retention time of each impurities present in the drug substances [10]. To identify analyte, each solution was prepared individually the retention time of each analyst, each solution was prepared as per the methodology. Further the sample solution was prepared by spiking known related substances of saxagliptin monohydrate drug substance at about 0.10 %w/w and injected as per procedure and conform the no co-elution of peaks from the sample matrix. The chromatogram of each analyte clearly shows that the methanesulfonic acid and trifluoroacetic acid peaks were well resolved from that of saxagliptin monohydrate drug substance, related substance of the saxagliptin monohydrate and blank solution which indicated that the method is selective for determination of methanesulfonic acid and trifluoroacetic acid in saxagliptin monohydrate (**Table 1**). An overlay chromatogram of diluent, standard solution and sample solution spiked with known amount related impurities of saxagliptin monohydrate **Figures 2-4**.

Table 1: Specificity values were clubbed with method precision data of both methanesulfonic acid and trifluoroacetic acid

S. No.	Sample	Methanesulfonic acid (µg/g)	Trifluoroacetic acid (µg/g)
1	Method Precision-1	990	1017
2	Method Precision-2	982	1021
3	Method Precision-3	979	1006
4	Method Precision-4	983	1009
5	Method Precision-5	979	1023
6	Method Precision-6	984	1048
7	Specificity-1	1017	990
8	Specificity-2	991	1023
9	Specificity-3	997	995
Method Precision	Mean	983	1021
	SD	4	15
	% RSD	0.4	1.5
Specificity	Mean	1002	1003
	SD	14	18
	% RSD	1.4	1.8
Over all Mean		989	1015
Over all Standard Deviation		12	17
Over all % RSD		1.2	1.7

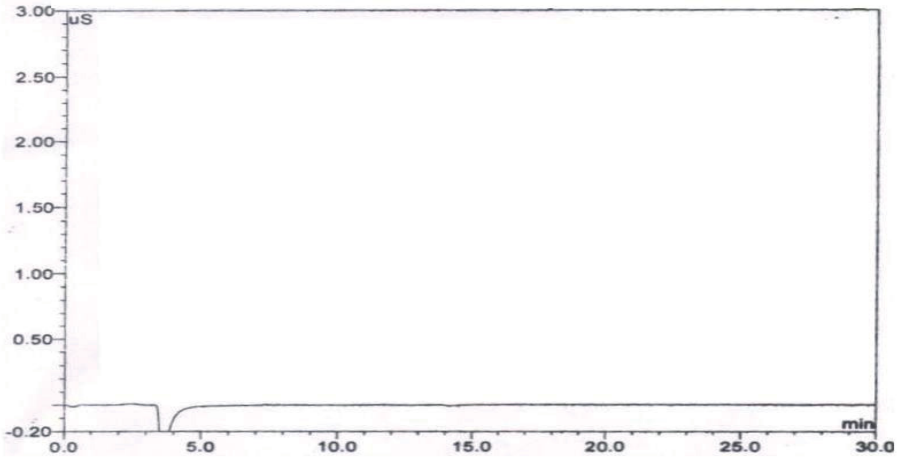

Figure 2: Diluent chromatogram

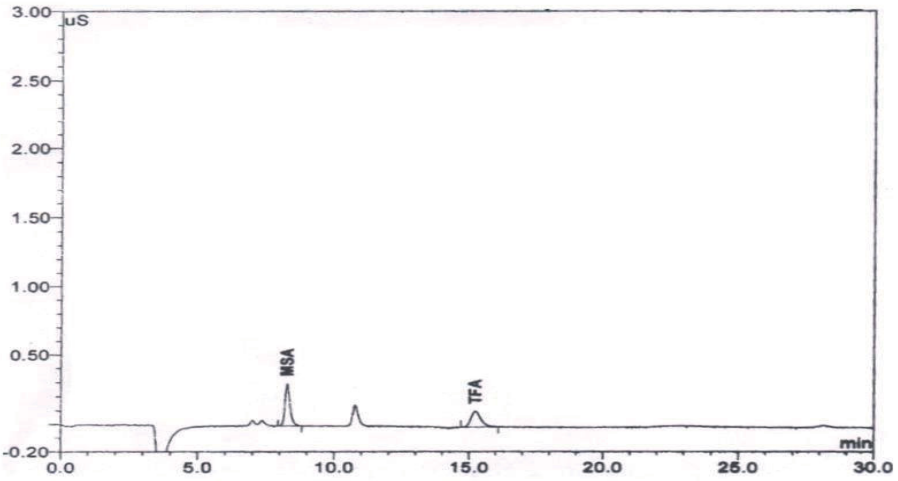

Figure 3: Chromatogram of methanesulfonic acid and trifluoroacetic acid standards

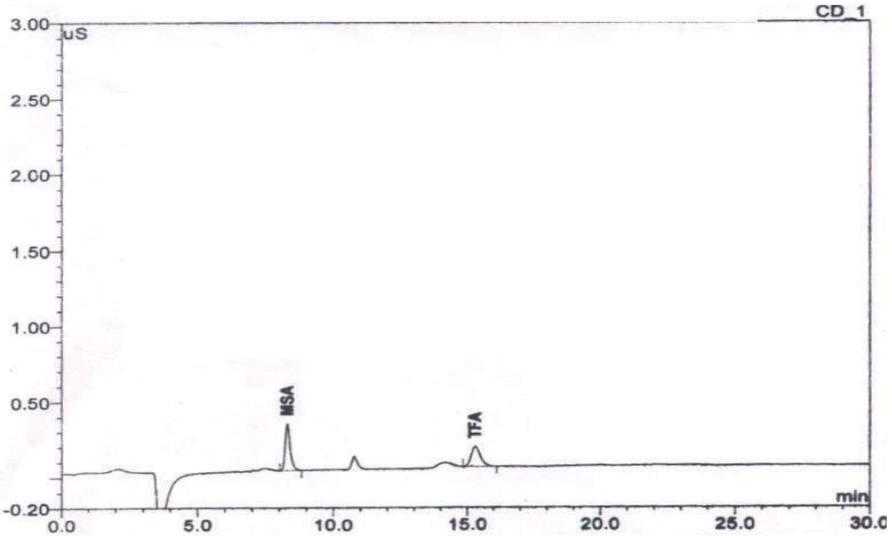

Figure 4: Chromatogram saxagliptin monohydrate drug substance spiked with all known related substances including methanesulfonic acid and trifluoroacetic acid spiked sample

LOD and LOQ

The sensitivity of the method was evaluated by constructing a linearity curve. The solutions of different concentrations of methanesulfonic acid and trifluoroacetic acid solutions were prepared from a lower concentration level of 0.183 µg/ml to a higher concentration level of 1.501 µg/ml corresponding to methanesulfonic acid and 0.300 µg/ml to a higher concentration level of 1.527 µg/ml corresponding to trifluoroacetic acid. The slope (S) and residual standard deviation (SD) were determined from the linearity curve. By using a slope (S) and residual standard deviation (SD) the limit of quantification and limit of detection of the method was arrived, which is being one of the three approaches described in ICH guidelines [11-13].

The formula used for the determination of LOQ and LOD were 10 × STEYX/SLOPE and 3.3 × STEYX/SLOPE respectively. The predicted LOQ and LOD levels for methanesulfonic acid were 0.183 µg/ml and 0.061 µg/ml and for trifluoroacetic acid 0.300 µg/ml and 0.100 µg/ml respectively.

To prove the predicted levels of LOQ and LOD values are precision and these levels can be easily quantify in the sample without any ambiguity. The solutions were prepared at the predicted concentration of LOD and LOQ levels, and analyzed for six times. The data of six-replicated injection for LOQ and LOD is tabulated in the **Table 2**.

Table 2: Precision data of LOD and LOQ of methanesulfonic acid and trifluoroacetic acid

Injection ID	Methanesulfonic acid		Trifluoroacetic acid	
	LOD (Area Count)	LOQ (Area Count)	LOD (Area Count)	LOQ (Area Count)
1	0.375	1.007	0.584	1.301
2	0.360	1.022	0.546	1.370
3	0.336	0.959	0.552	1.504
4	0.338	1.005	0.477	1.394
5	0.385	1.018	0.504	1.417
6	0.296	1.002	0.536	1.472
Mean	0.348	1.002	0.533	1.410
SD	0.032	0.023	0.038	0.073
% RSD	9.2	2.3	7.1	5.2
Concentration (µg/mL)	0.061	0.183	0.100	0.300

Linearity

The detector response was established by preparing a series of diluted solutions of methanesulfonic acid were 0.183 µg/ml to 1.501 µg/ml and for trifluoroacetic acid 0.300 µg/ml to 1.527 µg/ml respectively as per the methodology. Each solution was injected into the ion chromatography and measured the response and concentration of the solutions. From the area response of the analyte and concentration of the linear regression line plotted was constructed. From the linear regression line, the correlation coefficient of the regression line was found to be 0.9993 and 0.9985 respectively. The statistical analysis of linear regression line was evaluated and is summarized in **Tables 3** and **4** linearity plot of concentration of methanesulfonic acid vs. area response is shown in the **Figure 5.**

Table 3: Linearity data showing the concentration of Methanesulfonic acid and area response of each concentration

S. No.	Concentration (µg/mL)	Area (Area Counts)
1	0.183	1.002
2	0.250	1.603
3	0.500	3.170
4	0.750	4.903
5	1.001	6.652
6	1.251	7.929
7	1.501	9.631
Slope		6.493
Intercept		0.058
STEYX		0.136
Correlation Coefficient		0.9993

Table 4: Linearity data showing the concentration of Trifluoroacetic acid and area response of each concentration

S. No.	Concentration (µg/mL)	Area (Area Counts)
1	0.300	1.410
2	0.509	2.565
3	0.764	3.983
4	1.018	5.048
5	1.273	6.686
6	1.527	7.685
Slope		5.518
Intercept		0.072
STEYX		0.146
Correlation Coefficient		0.9985

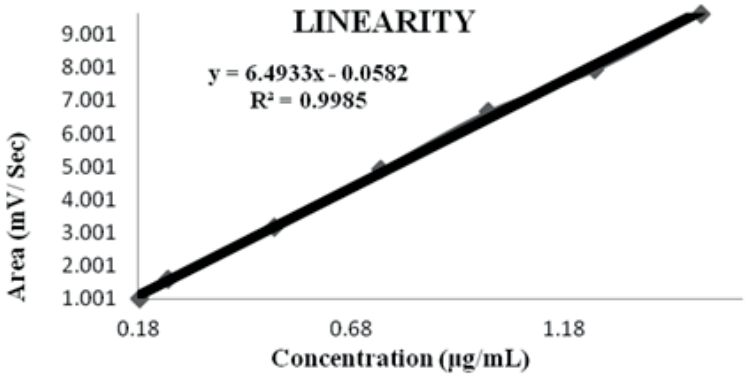

Figure 5: Linearity plot of concentration of methanesulfonic acid vs. area response

Accuracy

The recovery or accuracy of the method was tested by adding the methanesulfonic acid and trifluoroacetic acid to the saxagliptin monohydrate drug substance at three different concentration levels. These concentrations were prepared by adding methanesulfonic acid and trifluoroacetic acid to the saxagliptin monohydrate drug substance at about 183 µg/g(LOQ-MSA), 300 µg/g(LOQ-TFA), 500 µg/g 1000 µg/g and 1500 µg/g. Sample solutions were prepared in triplicate for each concentration, injected into the IC system and calculate the amount of methanesulfonic acid and trifluoroacetic acid present. The mean recovery was found to be for LOQ Level (183 µg/g,300 µg/g) is 100.2% (MSA), 89.5% (TFA) and mean recovery was found to be for 50%, 100% and 150% of specification level (1000 µg/g) is 99.9% (MSA), 100.0% (TFA) for methanesulfonic acid and trifluoroacetic acid respectively. The results are summarized in **Tables 5 and 6.**

Table 5: The recovery data of methanesulfonic acid in saxagliptin monohydrate

Concentration/Sample ID	Amount Added (µg/g)	Amount Recovered (µg/g)	Recovery (%)	Statistical Analysis	
LOQ Level Sample-1	183	179	97.8	Mean	100.2
LOQ Level Sample -2	182	182	100.0	SD	2.45
LOQ Level Sample -3	182	187	102.7	% RSD	2.4
Methanesulfonic acid (50% to 150% level)					
50% level Sample -1	495	502	101.4	Mean	103.0
50% level Sample -2	494	507	102.6	SD	1.83
50% level Sample -3	498	523	105.0	% RSD	1.8
100% level Sample -1	975	962	98.7	Mean	97.7
100% level Sample -2	975	947	97.1	SD	0.90
100% level Sample -3	996	968	97.2	% RSD	0.9
150% level Sample -1	1471	1466	99.7	Mean	99.1
150% level Sample -2	1491	1483	99.5	SD	0.87
150% level Sample -3	1477	1449	98.1	% RSD	0.9
Overall Statistical Analysis					
Mean		99.9			
SD		2.64			
% RSD		2.6			
95 % Confidence Interval		2. 0			

Precision

System Precision, method precision and intermediate precision were performed using methanesulfonic acid and trifluoroacetic acid standard solution was prepared as per the methodology. In system precision methanesulfonic acid and trifluoroacetic acid solution was injected into the system for six replications and calculated the percentage relative standard deviation of replicate injections (**Table 6**). In method precision, the sample solution of saxagliptin solution of the same batch substance was prepared in six times as per methodology. The six preparations of the sample solutions were separately injected to the chromatogram and evaluate the repeatability to the test method by calculating the content of the methane sulfonic acid and trifluoroacetic acid in the sample solution for the six preparations and the relative standard deviation. The amount of methanesulfonic acid and trifluoroacetic acid and its percentage relative deviation were tabulated in **Tables 7 and 8**.

Table 6: The recovery data of trifluoroacetic acid in saxagliptin monohydrate

Concentration/Sample ID	Amount Added (µg/g)	Amount Recovered (µg/g)	Recovery (%)	Statistical Analysis	
LOQ Level Sample-1	300	258	86.0	Mean	89.5
LOQ Level Sample -2	299	271	90.6	SD	3.14
LOQ Level Sample -3	299	275	92.0	% RSD	3.5
Trifluoroacetic acid (50% to 150% level)					
50% level Sample -1	501	482	101.4	Mean	103.0
50% level Sample -2	499	520	102.6	SD	1.83
50% level Sample -3	504	522	105.0	% RSD	1.8
100% level Sample -1	985	979	98.7	Mean	97.7
100% level Sample -2	985	963	97.1	SD	0.90
100% level Sample -3	1007	991	97.2	% RSD	0.9
150% level Sample -1	1487	1492	99.7	Mean	99.1
150% level Sample -2	1507	1515	99.5	SD	0.87
150% level Sample -3	1493	1484	98.1	% RSD	0.9
Overall Statistical Analysis					
Mean		100.0			
SD		2.59			
% RSD		2.6			
95 % Confidence Interval		2. 0			

Table 7: System precision data (Methanesulfonic acid) of different day of analysis

Injection	Day-1 Area(µS*min)	Day-2 Area(µS*min)	Day-3 Area(µS*min)	Day-4 Area(µS*min)
Injection-1	6.535	5.654	6.508	6.771
Injection-2	6.730	5.620	6.520	6.798
Injection-3	6.708	5.757	6.517	6.753
Injection-4	6.449	5.632	6.532	6.802
Injection-5	6.665	5.790	6.493	6.724
Injection-6	6.686	5.720	6.461	6.996
Mean	6.629	5.696	6.505	6.807
SD	0.112	0.07	0.025	0.097
% RSD	1.4	1.2	0.4	1.4

Table 8: System precision data (Trifluoroacetic acid) of different day of analysis

Injection	Day-1Area (µS*min)	Day-2 Area (µS*min)	Day-3 Area (µS*min)	Day-4 Area (µS*min)
Injection-1	5.062	4.414	5.268	4.891
Injection-2	5.071	4.429	5.259	4.898
Injection-3	5.013	4.641	5.250	4.832
Injection-4	4.917	4.375	5.233	4.828
Injection-5	5.048	4.439	5.264	5.012
Injection-6	5.013	4.535	5.073	5.069
Mean	5.021	4.472	5.225	4.922
SD	0.056	0.098	0.075	0.098
% RSD	1.1	2.2	1.4	2.0

Intermediate precision was evaluated by using same lot of saxagliptin monohydrate sample which was used in method precision and prepared the sample solutions as per the method precision by changing the different lot of the column, system, day, analyst and inject the solution as per methodology. The content of methanesulfonic acid and trifluoroacetic acid in each sample solution was calculated and evaluated the relative standard deviation for the same [14,15]. The overall relative standard deviation was calculated by clubbing the method precision and intermediate precision data and the results are summarized in **Tables 9 and 10**.

Table 9: The method precision and intermediate precision data for Methanesulfonic acid in saxagliptin monohydrate drug substances and its statistical data

Sample	Methanesulfonic acid content (µg/g)	
	Method Precision	**Intermediate Precision**
1	990	936
2	982	946
3	979	986
4	983	947
5	979	943
6	984	927
Mean	983	948
SD	4	20
% RSD	0.4	2.1
95% Confidence Interval (CI)	4	21

Table 10: The method precision and intermediate precision data for Trifluoroacetic acid in saxagliptin monohydrate drug substances and its statistical data

Sample	Trifluoroaceticacid content (µg/g)	
	Method Precision	**Intermediate Precision**
1	1017	896
2	1021	924
3	1006	860
4	1009	982
5	1023	862
6	1048	885
Mean	1021	902
SD	15	46
% RSD	1.5	5.1
95% Confidence Interval (CI)	16	48

CONCLUSION

A rapid and sensitive ion chromatography method was developed, optimized and validated for the determination of methanesulfonic acid and trifluoroacetic acid. The results of various validation parameters demonstrated that the method is specific, linear, precise and accurate in saxagliptin monohdrate drug substance.

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