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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING METHOD FOR THE SIMULTANEOUS DETERMINATION OF SAXAGLIPTIN HYDROCHLORIDE AND DAPAGLIFLOZIN PROPENEDIOL MONOHYDRATE IN QTERN® BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

High performance liquid chromatography (HPLC) is commonly used for the separation and identification of compounds in a sample. Reversed-phase HPLC (RP-HPLC) is the most commonly used separation mechanism in liquid chromatography. This mechanism consists of a polar mobile phase and a non-polar stationary phase. The purpose of this research was to develop a simple, fast, precise and accurate RP-HPLC stability-indicating method that can be applied to the drug Qtern®, as it contains both Saxagliptin Hydrochloride and Dapagliflozin Propenediol monohydrate.

KEYWORDS: Reversed-phase HPLC (RP-HPLC), Saxagliptin Hydrochloride, Dapagliflozin Propenediol monohydrate, Chromatograms and Chromatographic conditions.

INTRODUCTION

Articles have been published conducting research on the separation and stability of Dapagliflozin and Saxagliptin individually. However, there was no research completed on a RP-HPLC method for the simultaneous separation and stability-indicating method for Dapagliflozin Propenediol monohydrate and Saxagliptin Hydrochloride in a combined pharmaceutical dosage form. Therefore, the purpose of this research was to develop a simple, fast, precise and accurate RP-HPLC stability-indicating method that can be applied to the drug Qtern®, as it contains both Saxagliptin Hydrochloride and Dapagliflozin Propenediol monohydrate. The method will be validated according to FDA and ICH guidelines.

1. MATERIALS AND METHOD

Drug: Qtern® (Each tablet contains Saxagliptin hydrochloride equivalent to 5 mg Saxagliptin and Dapagliflozin Propenediol monohydrate equivalent to 10 mg Dapagliflozin), AstraZeneca® Inc., Saxagliptin Hydrochloride, (S)-Dapagliflozin Propenediol monohydrate.

Instruments

1100 Series HPLC System with MWD (UV/VIS Detector), Agilent Technologies, UV/VIS Spectrophotometer, Hitachi.

Reagents

Triethylamine (TEA), Acetonitrile, Methanol, Deionized water, Phosphoric Acid, 85%, Glacial Acetic Acid Solution, Monobasic Potassium Phosphate, Dibasic Potassium Phosphate, Sodium Acetate, Hydrochloric Acid Solution, 12 M, Sodium Hydroxide Salt, Hydrogen peroxide 3.0%, pH buffer standard solutions for calibration: pH 4 & pH 7.

Stock Solution Preparation of Qtern®

Qtern® Film-coated tablet solution as the finished drug product is available with the concentrations of 2,000/5,000 ppm (0.2%/0.5%) of Saxagliptin Hydrochloride/Dapagliflozin Propenediol monohydrate respectively.

3. METHOD DEVELOPMENT - OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

3.1 Solubility Study

The solvent chosen for the dilution and dissolution of Qtern®, SXG, and DAP for the rest of the method was DI water.

3.2 Detector Wavelength Selection

A Hitachi UV/VIS Spectrophotometer was used to find the wavelength absorptions of SXG and DAP, Qtern®. The spectrum can be seen in Figure i. The maximum UV absorbance by SXG was at 254.0 nm and that of DAP was at 300.0 nm. A wavelength of 285.0 nm was chosen because SXG and DAP behave similarly in this area.

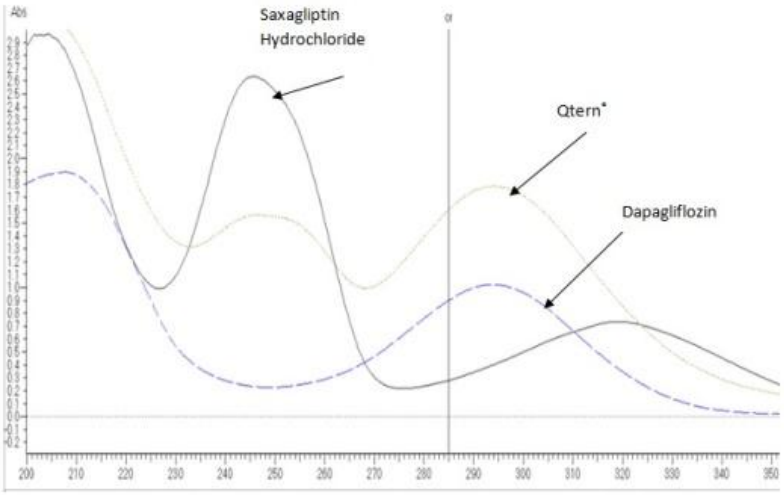

Figure i: UV/VIS spectrum of Saxagliptin Hydrochloride (solid line), Dapagliflozin maleate (dashed line), and Qtern® (dotted line) using a Hitachi UV/VIS Double Beam Spectrophotometer, Model U-2900.

3.3 Column Selection

It is very important to choose a stable column so the developed method becomes rugged and reproducible.

Qtern® with a SXG/DAP concentration of 200/500 ppm was injected into the 5 test columns.

The column that produced the best data was column manufactured by Supelco. This C_{18} column gave the highest number of theoretical plates, the lowest average tailing factors, and good resolution.

3.4 Identification of Compounds

Qtern® has 2 active ingredients. To identify which peak is SXG and which is DAP, their raw materials were dissolved in DI water, injected individually, and compared with the chromatogram of Qtern®. The chromatogram of Qtern®, used as the standard, is seen in Figure ii.

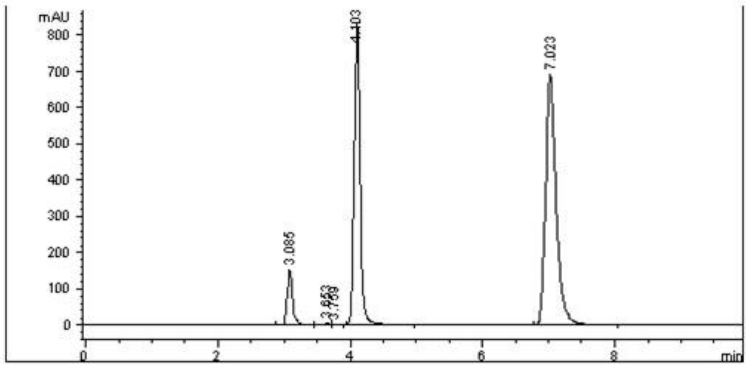

Figure ii: Chromatogram of a 200/500 ppm Qtern® injection used as the standard.

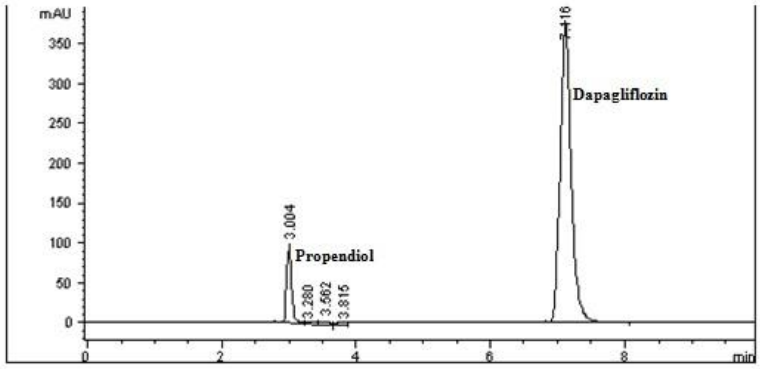

Figure iii: Chromatogram of Dapagliflozin Propendiol raw material dissolved in DI

As seen in Figure iii, Dapagliflozin Propenediol monohydrate produces 2 major peaks. Further tests were required to identify both peaks.

It was concluded that the first peak was Propene diol and the second Dapagliflozin Propenediol monohydrate.

3. 5 Mobile Phase Study

Organic solvent as ACN, while the inorganic solvent dibasic potassium phosphate buffer set at a pH of 7.0 was selected. The sample concentration of 200/500 ppm SXG/DAP was used.

The first mobile phase composition tested was 50:50 ACN/buffer followed by 25:75 ACN/buffer.

25:75 ACN/buffer run was conducted an added concentration of 25 mM TEA. The resulting chromatogram is seen in Figure iv.

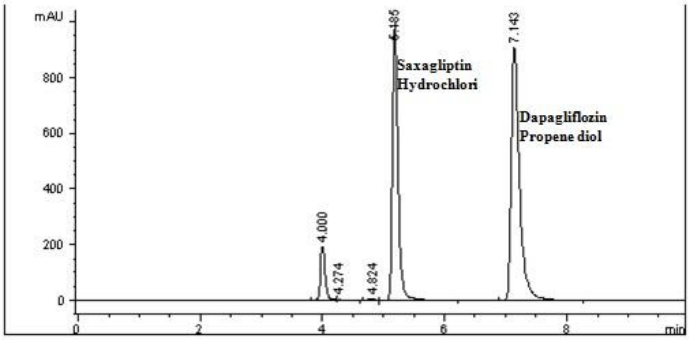

Figure iv: Chromatogram of Qtern® with a mobile phase concentration of 25:75 ACN/buffer with TEA.

Conditions: Isocratic elution, mobile phase 25:75 ACN/dibasic potassium phosphate buffer pH 7.0 with 25 mM TEA, flow rate 1.0 mL/min, ambient temperature, detection wavelength at 285 nm, 15 µL injection volume.

The next couple of tests used a ratio of 30:70 ACN/buffer with TEA and 20:80 ACN/buffer with TEA. Since a solvent strength of 25% ACN gave the highest number of theoretical plates while maintaining acceptable tailing factors, the ratio of 25:75 ACN/buffer with TEA was used as the preferred mobile phase composition for the rest of the method development.

3.6 pH Study

Three buffers were prepared and tested at a ratio of 25:75 ACN/buffer with TEA. The first was a dibasic potassium phosphate buffer with 25 mM TEA set at pH 7.0. The second buffer was a sodium acetate buffer with 25 mM TEA set at pH 5.0. The final buffer tested was a monobasic potassium phosphate buffer with 25 mM TEA set at pH 2.9. In this study, only the type of buffer was altered to see the affect on retention times, tailing factors, theoretical plate numbers, and resolution on the active ingredients of Qtern®.

As a result, the dibasic potassium phosphate buffer set at a pH of 7.0 was chosen for the rest of method development and validation because it produced a high number of theoretical plates, a resolution greater than 2 for both active ingredients, and an acceptable tailing factor.

3.7 Organic Modifier Concentration Study

Study was done to optimize the concentration of TEA in the buffer. In addition to 25 mM TEA, 30 mM and 35 mM concentrations of TEA in the pH 7.0 buffer were tested to see the effects on peak shape.

As the concentration of TEA increases, the tailing factors of SXG and DAP decrease. For this method, a concentration of 30 mM TEA added to the buffer was selected.

3. 8 Determination of Nominal Concentration

Five sample solutions with SXG/DAP concentrations ranging from 100/250 ppm to 500/1250 ppm from stock solution of 2,000/5,000 ppm were run in order to determine the nominal concentration.

Each concentration was injected into the HPLC. The peak areas of the sample were determined and recorded in Table i. A plot of peak area versus concentration was created and the resulting linear regressions can be seen in Figure v. The correlation coefficient of SXG was found to be 0.9990, while that of DAP gave a correlation coefficient of 0.9991. The 200/500 ppm concentration was chosen to be nominal and was used for the rest of method development.

Qtern® Film-coated tablet Solution			
Saxagliptin Hydrochloride		Dapagliflozin Propenediol monohydrate	
Concentration (ppm)	Peak Area	Concentration (ppm)	Peak Area
100	3576	250	4759
200	6750	500	8986
320	11054	800	14890
400	13327	1000	18043
500	16553	1250	22514

Table i: Sample concentrations and resulting peak areas for nominal concentration study.

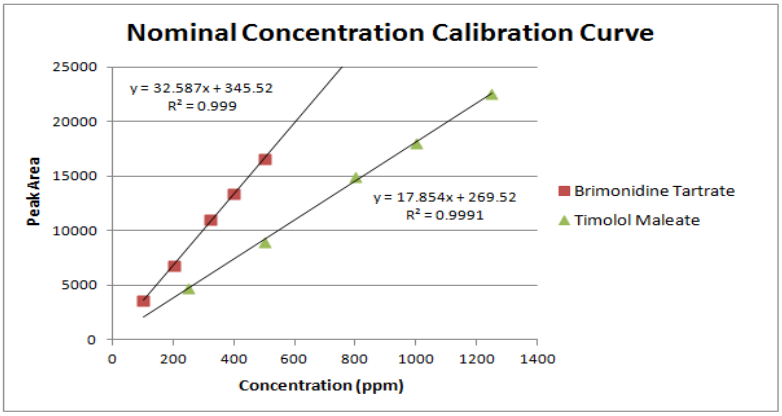

Figure v: Linear regression plot and equation used for nominal concentration determination of Qtern®.

Conditions: Isocratic elution, mobile phase 25:75 ACN/dibasic potassium phosphate buffer pH 7.0 with 30 mM TEA, flow rate 1.0 mL/min, ambient temperature, detection wavelength at 285 nm, 15 µL injection volume.

4. METHOD DEVELOPMENT - FORCED DEGRADATION STUDY

4.1 Saxagliptin Hydrochloride Forced Degradation

Before the degradation study was run, a control sample was prepared and injected in order to calculate percent degradation of the degraded sample. The control concentration of 200 ppm was prepared. The peak area of the SXG control was found to be 4010.

4.1.1 Heat Degradation Study

Heat degradation studies were carried out by heating samples of SXG solution at 75 °C for 24 hours. 1,000 ppm stock solution of SXG was taken in a test tube. The sample was heated and cooled. The solution of final concentration of 200 ppm of SXG was made.

After injection and review of the resulting chromatogram, it was found that no degradation occurred (because the structure of SXG is highly aromatic and conjugated).

4.1.2 Photolysis Study

UV degradation studies were executed by taking a sample of SXG raw material and exposing it to UV light at a wavelength of 254 nm for 24 hours. 20.0 mg of SXG raw material was weighed and placed on a watch glass. The watch glass was placed under the UV lamp for 24 hours. The sample was removed and 200 ppm solution of SXG was made. Then the solution was vortexed and transferred into an HPLC vial for injection. The chromatogram was reviewed and, once again, no degradation of SXG occurred.

4.1.3 Acid Degradation Study

Acid degradation studies were carried out by adding HCl to a solution of SXG. A volume of 2.00 mL of 3.0 M HCl was added to 2.00 mL of 1,000 ppm stock solution of SXG and placed on a heating block set at 75 °C for 24 hours. After heating, the acidic solution was neutralized with sodium hydroxide and cooled, a final concentration of 200 ppm for SXG was prepared. The solution was vortexed, filtered and then transferred into an HPLC vial for injection.

The percent degradation was calculated to be 0.35%. As predicted, this means that SXG is very stable in acidic conditions.

4.1.4 Base Degradation Study

Base degradation studies were carried out by adding sodium hydroxide to a solution of SXG. A volume of 2.00 mL of 3.0 M NaOH was added to 2.00 mL of 1,000 ppm stock solution of SXG inside a test tube. Then the test tube was and placed on a heating block set at 75 °C

and was heated for 24 hours. After heating, the basic solution was neutralized with HCl and cooled. The solution of final concentration of 200 ppm for SXG was prepared. The solution was vortexed, filtered and then transferred into an HPLC vial for injection.

The percent degradation was calculated to be 1.45%. As expected, SXG is also very stable in basic conditions.

4.1.5 Oxidation Study

Oxidation studies were carried out by adding hydrogen peroxide (concentration of 3%, H_2O_2) to a solution of SXG. A volume of 2.00 mL H_2O_2 was added to 2.00 mL of 1,000 ppm stock solution of SXG inside a test tube. Then the test tube was vortexed for a few seconds and placed on a heating block set at 75°C, heated for 24 hours and cooled. The solution of 200 ppm for SXG was prepared. The solution was vortexed, filtered and then transferred into an HPLC vial for injection.

The peak area of the degraded SXG sample was 3978. When compared to the control, the percent degradation was calculated to be 0.798%. Once again, SXG is found to be very stable.

4.2 Dapagliflozin Propenediol monohydrate Forced Degradation

The control was prepared to create a final concentration of 500 ppm. The peak area of the DAP control was found to be 4252.

4.2.1 Heat Degradation Study

Heat degradation studies were carried out by heating samples of DAP solution at 75 °C. A final concentration of 500 ppm of DAP was prepared. After injection and review of the resulting chromatogram, it was found that no degradation occurred.

4.2.2 Photolysis Study

UV degradation studies were executed by taking a sample of DAP raw material and exposing it to UV light at a wavelength of 254 nm for 24 hours. A final concentration of 500 ppm of DAP was prepared. The chromatogram was reviewed and, once again, no degradation of DAP occurred.

4.2.3 Acid Degradation Study

Acid degradation studies were carried out by adding 1.0 M HCl to a solution of DAP. A final concentration of 500 ppm of DAP was prepared, the sample was injected. The percent degradation was calculated to be 5.82%, which is within the desired range of degradation.

4.2.4 Base Degradation Study

Base degradation studies were carried out by adding various concentrations (1.0M, 0.1M and 0.05M) of NaOH to a solution of DAP.DAP is found to be unstable in first two conditions.

DAP degraded with 0.05 M NaOH for 1 hour gave a degradation of 5.58% with an area of 4014.

4.2.5 Oxidation Study

Oxidation studies were carried out by adding various concentrations of H_2O_2 (3%, 0.5%) to a solution of DAP. The conditions of 3% was found to be too harsh for DAP.

Oxidation test was run the concentration of the H_2O_2 solution was 0.5%. The peak area of the new degraded sample was 3842. When compared to the control, the percent degradation was calculated to be 9.64%, which is within the desired range of degradation.

4.3 Mixed Degradation Study

1.0 mL from each of the acidic, basic, and oxidation samples, of both SXG and DAP, that produced less than 10% degradation were mixed in a test tube vortexed and injected.

After analyzing the chromatogram, it was found that SXG was co-eluting with a degradant. To solve this issue, the solvent strength was reduced to 20% ACN to increase the separation between the degradant and the peak. Figure vi shows the resulting chromatogram.

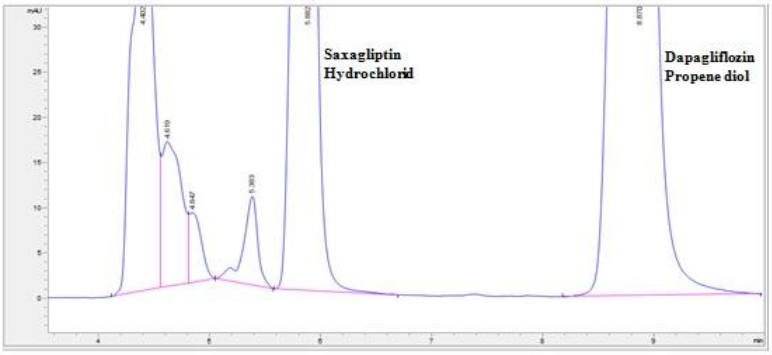

Figure vi: Zoomed in chromatogram of the optimized mixed degradation study.

Conditions: Isocratic elution, mobile phase 20:80 ACN/dibasic potassium phosphate buffer pH 7.0 with 30 mM TEA, flow rate 1.0 mL/min, ambient temperature, detection wavelength at 285 nm, 15 µL injection volume.

5. METHOD VALIDATION

5.1 System Suitability Test

The system suitability test is a necessary part of any analytical procedure. This evaluates to see if the system being used can produce reliable results. The FDA recommends the following for the separation performance criteria:

1. **Resolution** > 2
2. **Tailing Factor** 0.9 ≤ T_f ≤ 2.0
3. **Column Efficiency theoretical plates** > 2,000
4. **% RSD for Retention Time** ≤ 1%
5. **% RSD for Peak Area** ≤ 1%
6. **% Drift** ≤ 2%

System suitability was tested by preparing two standard solutions of Qtern®, Standard 1 and Standard 2. Both standards had the nominal concentrations of 200/500 ppm for SXG/DAP. Then the system was evaluated by injecting Standard 1 six times and Standard 2 twice with the optimized chromatographic conditions.

The results from the system suitability test are recorded in Table ii and iii for SXG and DAP. Percent relative standard deviations (%RSD) were calculated and were under acceptable criteria. Percent drift was also calculated for both the peak areas and retention times by following the equations in Figure vii.

$$\%\ Drift = \frac{A1 - A2}{A1} * 100$$

A1 = Average peak area of the injections of Standard 1

A2 = Average peak area of the injections of Standard 2

$$\%\ Drift = \frac{T1 - T2}{T1} * 100$$

T1 = Average peak retention time of the injections of Standard 1

T2 = Average peak retention time of the injections of Standard 2

Figure vii: Equations for percent drift of peak area and retention time for system for system suitability testing.

Finally, column efficiency and tailing factors were evaluated. The data shows that all the theoretical plate numbers were greater than 2,000 while all the tailing factors fell between the required ranges. Therefore, all parameters passed the system suitability testing guidelines.

Table ii: System suitability test results for Saxagliptin Hydrochloride in Qtern® Film-coated tablet

Saxagliptin Hydrochloride								
Standard 1	**Retention Time**	**Retention Time %RSD**	**Retention Time % Drift**	**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	**Peak Area % Drift**
Injection 1	3.963	0.153%	0.029%	0.999	8201	6155	0.847%	0.550%
Injection 2	3.965			0.994	7985	6099		
Injection 3	3.953			0.997	8283	6138		
Injection 4	3.954			0.983	7940	6051		
Injection 5	3.952			0.987	7932	6018		
Injection 6	3.951			0.994	8277	6097		
Standard 2	**Retention Time**	**Retention Time %RSD**		**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	
Injection 1	3.959	0.054%		0.990	7961	6097	0.875%	
Injection 2	3.956			0.988	8198	6022		

Table iii: System suitability test results for Dapagliflozin maleate in Qtern® Film-coated tablet

Dapagliflozin Propenediol monohydrate								
Standard 1	**Retention Time**	**Retention Time %RSD**	**Retention Time % Drift**	**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	**Peak Area % Drift**
Injection 1	6.87	0.163%	0.022%	1.253	8689	8432	0.604%	0.137%
Injection 2	6.869			1.264	8701	8406		
Injection 3	6.845			1.259	8640	8329		
Injection 4	6.848			1.245	8648	8415		
Injection 5	6.848			1.244	8646	8378		
Injection 6	6.853			1.231	8659	8305		
Standard 2	**Retention Time**	**Retention Time %RSD**		**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	
Injection 1	6.859	0.041%		1.265	8352	6097	0.875%	
Injection 2	6.855			1.268	8340	6022		

5.2 Solution Stability

A sample solution of Qtern® with 200/500 ppm SXG/DAP concentration was prepared, and then the solution was injected under the optimized chromatographic conditions. The data from this injection was considered to be the initial time of 0 hours and injected again 24, 48, and 72 hours later to test its stability. After all the results were obtained, each peak area was compared to the initial injection at time 0 and the percent change was calculated. Table iv shows the results for the solution stability tests. The acceptance criterion for solution stability is that the % Change should be less than 2% throughout the study. Therefore, the diluted solution of Qtern® is stable since SXG and DAP both had less than a 2% change.

Table iv: Solution stability results for Qtern® over a period of 72 hours.

Qtern® Film-coated tablet Solution				
	Saxagliptin Hydrochloride		**Dapagliflozin Propenediol monohydrate**	
Time (hours)	Peak Area	% Change of Peak Areas	Peak Area	% Change of Peak Areas
0	7225	-----	9898	-----
24	7222	0.042%	9852	0.465%
48	7218	0.097%	9933	0.354%
72	7215	0.138%	9924	0.263%

5.3 Specificity

Specificity is the ability of the method to produce pure peaks. SXG and DAP must be separated from their impurities and degradants.

This sample was injected in an HPLC instrument equipped with a DAD. The SXG and DAP degraded peaks were evaluated further to determine their purity. Seven spectra were used to calculate the purity of SXG, a purity factor of 999.998 out of 1000.000 was found. Usually, the desired purity factor to pass specificity is above 990.

Peak purity was evaluated for the forced degraded DAP also. Eighteen spectra were used to calculate the purity of DAP, a purity factor of 1000.000 out of 1000.000 was found.

5.4 Method Robustness

Robustness is the measure of the developed method's capability to be unaffected by small variations in method parameters.

PARAMETERS	VARIATION MADE	RESULT
Solvent strength	±2%.	Both SXG and DAP stay within FDA tailing factor and column efficiency criteria.
Buffer pH	± 0.2	Both SXG and DAP stay within FDA tailing factor and column efficiency criteria.
Wavelength selection (285 nm)	± 2	Both SXG and DAP stay within FDA tailing factor and column efficiency criteria.

Table v: Robustness

5.5 Limit of Detection & Limit of Quantification

A stock solution of 2/5 ppm SXG/DAP Qtern® was prepared for the determination of LOD and LOQ. After preparation, each concentration was injected and analyzed to find its signal-to-noise ratio. The LOD, with the signal-to-noise ratios of 3.5 and 3.8 for SXG and DAP respectively, was determined to be at the low concentration of 0.08/0.20 ppm SXG/DAP Qtern®. In addition, the LOQ, with the signal-to-noise ratios of 10.5 and 10.1 for SXG and DAP respectively, was found to be at 0.24/0.60 ppm SXG/DAP in Qtern®.

Since the LOQ is such a low concentration, results may vary widely due to inaccuracy of detector response. Therefore, to confirm that the LOQ is 0.24/0.60 ppm for SXG/DAP, the LOQ concentration was injected six times and the %RSD of the peak areas were calculated. The criteria at such low concentrations is that %RSD must be less than or equal to 10%. SXG had a %RSD of 4.709% while DAP had a %RSD of 3.660%.

5.6 Linearity

Five concentrations of standard solutions were made for testing linearity. Each standard sample was prepared in triplicate and injected the average peak area of each concentration was calculated. The average peak areas were then plotted versus concentration. The linearity plot for Qtern® can be seen in Figure viii. The correlation coefficients for SXG and DAP were 0.9992 and 0.9996 respectively. These results satisfy the criteria of the linearity validation study.

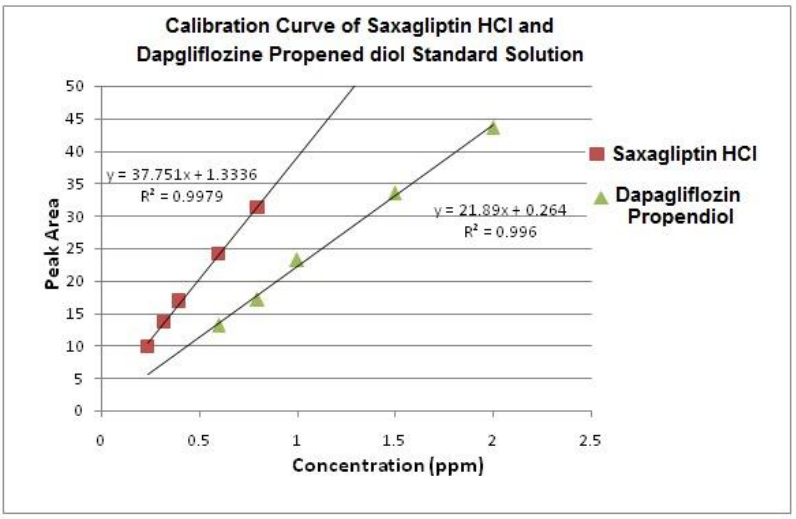

Figure viii: Peak area vs. concentration plot of Saxagliptin Hydrochloride and Dapagliflozin maleate for linearity study.

5.7 Accuracy

Three different concentrations of Qtern® are needed for this study: 80%, 100% and 120% of the nominal concentration. Each sample was prepared in triplicate and injected.

Once all the peak areas were analyzed, the average peak area of each concentration was calculated. The smallest recovery was 98.71%, while the highest was 101.10%. Therefore, all the concentrations met within the desired percent recovery range for the accuracy study.

5.8 Method Precision

Six different samples of Qtern®, each with the nominal concentration of 200/500 ppm SXG/DAP, were prepared and injected. The results can be seen in Table vi. The %RSD for SXG and DAP were 0.549% and 0.848% respectively. These results meet the criteria for method precision.

Table vi: Peak area results of six sample preparations of Qtern® for method precision study.

Qtern® Film-coated tablet Solution								
	Saxagliptin Hydrochloride				Dapagliflozin Propenediol monohydrate			
Sample Preparation	Peak Area	Average	Standard Deviation	Peak Area %RSD	Peak Area	Average	Standard Deviation	Peak Area %RSD
1	7269	7245	39.80	0.549%	10060	10062	85.28	0.848%
2	7187				9945			
3	7232				10040			
4	7217				10019			
5	7295				10194			
6	7268				10116			

5.9 Injection Precision

One sample of Qtern®, with the nominal concentration of 200/500 ppm SXG/DAP, was prepared and was injected six times. The results can be seen in Table vii. The %RSD for SXG and DAP were 0.859% and 0.808% respectively. These results meet the criteria for injection precision.

Table vii: Peak area results of six injections of Qtern® for injection precision study.

Qtern® Film-coated tablet Solution								
	Saxagliptin Hydrochloride				Dapagliflozin Propenediol monohydrate			
Injection	Peak Area	Average	Standard Deviation	Peak Area %RSD	Peak Area	Average	Standard Deviation	Peak Area %RSD
1	7468	7420	63.77	0.859%	10316	10406	84.10	0.808%
2	7306				10456			
3	7401				10294			
4	7413				10431			
5	7452				10512			
6	7480				10425			

5.10 Intermediate Precision

Six samples of Qtern® at the nominal concentration of 200/500 ppm SXG/DAP were prepared and injected. The data is found in Table viii. The %RSD for SXG and DAP were 0.884% and 1.354% respectively. These results met the criteria for intermediate precision.

Qtern® Film-coated tablet Solution								
	Saxagliptin Hydrochloride				Dapagliflozin Propenediol monohydrate			
Sample Preparation	Peak Area	Average	Standard Deviation	Peak Area %RSD	Peak Area	Average	Standard Deviation	Peak Area %RSD
1	7159	7239	63.96	0.884%	10155	10065	136.24	1.354%
2	7243				9942			
3	7215				10170			
4	7319				9973			
5	7191				10232			
6	7308				9916			

Table viii: Peak area results of six preparations by Aktham Mestareehi of Qtern® for intermediate precision study.

6. CONCLUSION

A reversed-phase high performance liquid chromatography stability-indicating method has been developed and validated for Qtern® and the raw materials Saxagliptin Hydrochloride and Dapagliflozin Propenediol monohydrate. This method used an isocratic elution technique with a run time of 10 minutes. The mobile phase was composed of a mixture of ACN and a dibasic potassium phosphate buffer with 30 mM TEA set at a pH of 7.0. The organic solvent to buffer ratio was 20:80, therefore making the method cost efficient with minimal organic waste. The optimized HPLC conditions for this developed method are as follows:

Column: Supelco Discovery C18 (250 x 4.6 mm), 5µm particle size
Mobile Phase: 20:80 ACN/dibasic potassium phosphate buffer pH 7.0 with 30 mM TEA
Flow Rate: 1 mL/min
Wavelength: UV set to 285 nm
Temperature: Ambient
Injection Volume: 15 µL

The stability of SXG and DAP was analyzed through different stress conditions. SXG was found to be highly stable. This method was validated using ICH and FDA guidelines by performing system suitability, solution stability, specificity, method robustness, LOD, LOQ, linearity, accuracy, and precision studies. For future work, the developed method may be applied as a quality control procedure to the finished drug product, Qtern®, or to drug products containing Saxagliptin Hydrochloride or Dapagliflozin Propenediol monohydrate. Future research may also involve improvements to this method by using a DAD set to the maximum absorption wavelengths of both SXG and DAP. Overall, this developed stability-indicating method is simple, fast, and reliable.

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