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Original Research Article

Quantitative estimation of sitagliptin and dapagliflozin propanediol monohydrate in synthetic mixture using 1st order derivative spectroscopy simultaneous spectrophotometric analysis

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ABSTRACT

Current research paper describes highly specific and reproducible 1st order derivative spectroscopic method for quantitative analysis of Sitagliptin which is a DPP4 inhibitors and Dapagliflozin which is SGLT2 inhibitors from its synthetic mixture. Both drugs are from Anti Diabetics class. Present analytical method was developed on Shimadzu double beam spectrophotometer equipped with UV probe 2.42 as software using methyl alcohol as solvent. Quantification of Sitagliptin was carried out at zero cross over point of Dapagliflozin that is 275 nm and for Dapagliflozin, it was achieved at 232 nm which is zero cross over point of Sitagliptin. Method shows linear response in the range of 25-125 μg/mL of Sitagliptin and 2.5-12.5 μg/mL of Dapagliflozin. Method was found to be accurate with recovery between 99.3 – 100.1 % for Sitagliptin and 98.2 – 100.7 % for Dapagliflozin. The developed method was validated as per ICH Q2 R1 guidelines and was successfully applied for quantitative analysis of synthetic mixture of Sitagliptin and Dapagliflozin.

1. Introduction

Sitagliptin (SITA) acting as Anti Diabetic agent (Dipeptidyl peptidase-4 inhibitor) which boosts post prandial insulin release, decrease Glucagon secretion and lower mean time as well as fasting blood glucose in type 2 diabetes.[1] This agent is used in combination with other oral hypoglycemic agents. Dapagliflozin (DAPA) acting as Sodium-Glucose cotransporter-2(SGLT2) inhibitors. This agent is used in combination with diet and exercise to improve glycemic control in adult with type -2 Diabetes. SGLT 2 is major transporter of glucose whose inhibition induces glucosuria and lower blood sugar in type 2 diabetes mellitus.[2] According to the clinical trial study of real-World Evidence with SGLT2i(DAPA) and DPP4i (SITA) in Type-2 Diabetes patients in Spain (NCT04149067)it shows beneficial positive effect on patient of Diabetes Mellitus (Type-2) at the close level of 5-10 mg of DAPA and 50-100 mg of SITA.[3] Best clinical effectiveness was observed at 100 mg dose of SITA and 10 mg of DAPA and hence for method development purpose dose was selected as a mixture comprising 10 mg of DAPA and 100 mg of SITA. Several analytical methods are available which can determine SITA and DAPA individually or in combination with another drug. From detailed review of literature, it was found that no analytical method is available for determination of DAPA and SITA from simulated mixture or formulation,[4–24] Furthermore UV spectrophotometric methods are more convenient with respect to operation in comparison with chromatographic methods of analysis. In

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addition, derivative spectroscopy aids virtue of specificity to the analytical method due to estimation at Zero Cross Over point (ZCP). So for the same reason derivative spectroscopy was selected as method of choice from all other multi component UV spectrometric methods.

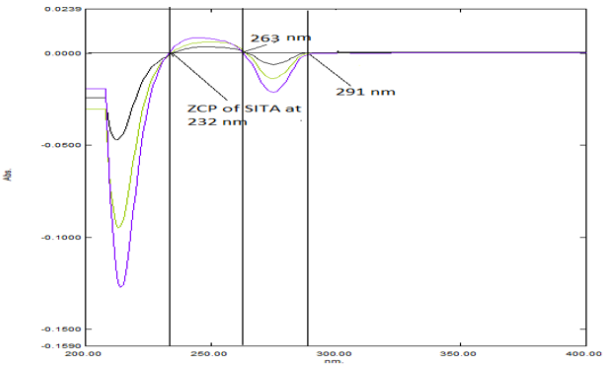

Fig. 1: Zero cross over point of SITA

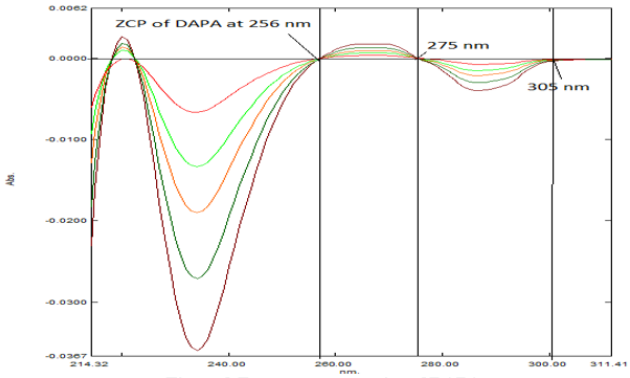

Fig. 2: Zero cross over point of DAPA

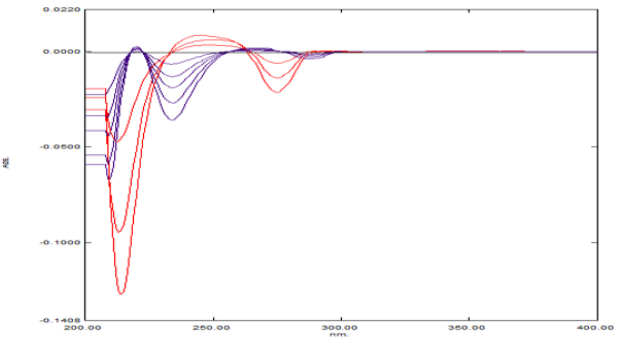

Fig. 3: Overlain D1 spectra of SITA and DAPA

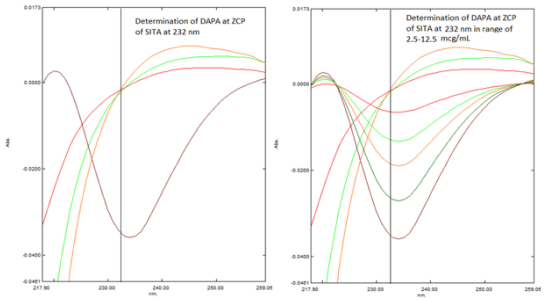

Fig. 4: Determination of DAPA at ZCP of SITA (at 232 nm)

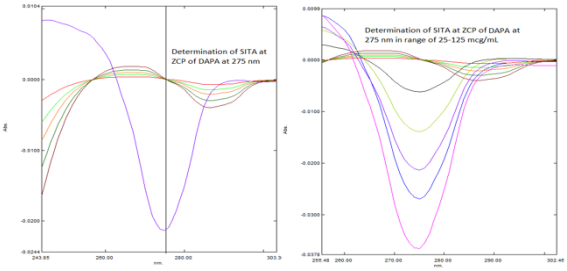

Fig. 5: Determination of SITA at ZCP of DAPA (at 275 nm)

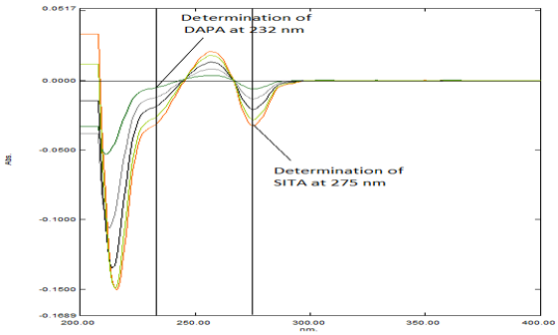

Fig. 6: D1 Spectra of standard mixture of SITA (25-125 μg/mL) and DAPA (2.5-12.5 μg/mL) for linearity study

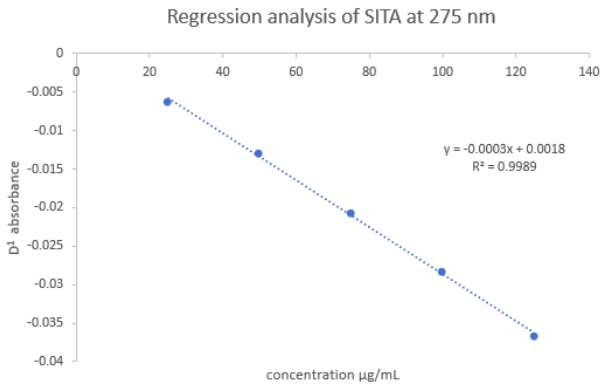

Fig. 7: Regression analysis of SITA (25-125 μg/ml) at 275 nm

Table 1: Preparation of solutions for accuracy studies

preparation of solutions for Accuracy studies			
Unspiked	**50%**	**100%**	**150%**
Placebo	Placebo	Placebo	Placebo
	Weigh accurately 2.5 mg of DAPA and 25 mg of SITA in 10 ml calibrated volumetric flask	Weigh accurately 5 mg of DAPA and 50 mg of SITA in 10 ml calibrated volumetric flask	Weigh accurately 7.5 mg of DAPA and 75 mg of SITA in 10 ml calibrated volumetric flask
Contents diluted up to 10 ml calibrated volumetric flask with Methyl Alcohol and filter the solution (A) Take 1 ml of this filtrate and dilute up to 10 ml with Methyl alcohol (B)			
	To achieve final concentration, 1 ml of solution B diluted up to 10 ml with Methyl Alcohol (2.5+25 μg/ml)	To achieve final concentration, 1 ml of solution B diluted up to 10 ml with Methyl Alcohol (5+50 μg/ml)	To achieve final concentration, 1 ml of solution B diluted up to 10 ml with Methyl Alcohol (7.5+75 μg/ml)

Table 2: Linearity data of sita

Sr. No.	**Concentration (μg/mL)**	**Mean (D^1 abs.) + S.D.**	**R.S.D. (%)**
1.	25	-0.0062 ± 0.00007	1.2031
2.	50	-0.0131 ± 0.00012	0.9118
3.	75	-0.0208 ± 0.00012	0.6081
4	100	-0.0284 ± 0.00014	0.5160
5.	125	-0.0367 ± 0.00008	0.2177

Table 3: Linearity data of DAPA

Sr. No.	**Concentration (μg/mL)**	**Mean (D1 abs.) + S.D.**	**R.S.D. (%)**
1.	2.5	-0.0057 ± 0.00004	0.8534
2.	5.0	-0.0128 ± 0.00011	0.8879
3.	7.5	-0.0192 ± 0.00012	0.6378
4	10	-0.0268 ± 0.00016	0.618
5.	12.5	-0.0326 ± 0.00011	0.3568

Table 4: Repeatability data of SITA at 275 nm (n= 5 determinations)

	concentration (μg/mL)				
Sr. no.	**25**	**50**	**75**	**100**	**125**
1	-0.0063	-0.0133	-0.0208	-0.0285	-0.0368
2	-0.0062	-0.0133	-0.021	-0.0286	-0.0367
3	-0.0063	-0.013	-0.0206	-0.0285	-0.0368
4	-0.0062	-0.0131	-0.0208	-0.0282	-0.0366
5	-0.0061	-0.0131	-0.0208	-0.0286	-0.0368
Mean	-0.0062	-0.0131	-0.0208	-0.0284	-0.0367
S.D.	0.00007	0.00012	0.00012	0.00014	0.00008
R.S.D. (%)	1.2031	0.9118	0.6081	0.5160	0.2177

Table 5: Repeatability data of DAPA at 323 nm (n= 5 determinations)

	concentration (μg/mL)				
Sr. no.	**2.5**	**5**	**7.5**	**10**	**12.5**
1	-0.0057	-0.0130	-0.0193	-0.0265	-0.0328
2	-0.0058	-0.0129	-0.0192	-0.0266	-0.0327
3	-0.0057	-0.0128	-0.019	-0.0268	-0.0326
4	-0.0058	-0.0128	-0.0193	-0.0269	-0.0328
5	-0.0057	-0.0127	-0.0192	-0.0266	-0.0325
Mean	-0.0057	-0.0128	-0.0192	-0.0266	-0.0326
S.D.	0.00004	0.00011	0.00012	0.00016	0.00011
R.S.D. (%)	0.8534	0.8879	0.6378	0.6158	0.3568

Table 6: Intraday and inter day precision data of SITA (n = 3 determinations)

Concentration (μg/ml)	Intraday Mean + SD	%RSD	Inter-Day Mean + SD	%RSD
25	-0.0062 ± 0.00007	1.2031	-0.0062 ± 0.00006	1.0200
75	-0.0208 ± 0.00012	0.6081	-0.0208 ± 0.00013	0.6509
125	-0.0367 ± 0.00008	0.2177	-0.0367 ± 0.00008	0.2437

Table 7: Intraday and inter day precision data for DAPA (n = 3 determinations)

Concentration (μg/ml)	Intraday Mean + SD	%RSD	Inter-Day Mean + SD	%RSD
2.5	-0.0057 ± 0.00004	0.8534	-0.0055 ± 0.00004	0.8842
7.5	-0.0192 ± 0.00012	0.6378	-0.0192 ± 0.00010	0.5300
12.5	-0.0326 ± 0.00011	0.3568	-0.0327 ± 0.00010	0.3349

Table 8: Accuracy data of SITA and DAPA by derivative spectroscopy method

Level of spiking	Total placebo (mg)	Amount of std. drug added (μg/ml)		Amount of drug recovered (μg/ml)		% Rsecovery	
		SITA	DAPA	SITA	DAPA	SITA	DAPA
Unspiked		-	-	-	-	-	-
50 %		25	2.5	24.88 ± 0.111	2.48 ± 0.004	100.1 ± 0.47	99.0 ± 0.18
100 %		50	5	49.67 ± 0.008	5.03 ± 0.212	99.3 ± 0.06	100.7 ± 0.42
150 %		75	7.5	75.66 ± 0.607	7.36 ± 0.004	100.1 ± 0.60	98.2 ± 0.78

Table 9: Assay of synthetic mixture by validated 1st order derivative spectroscopic method

Drug	Amount taken (μg/mL)	Amount recovered (μg/mL)	% Assay
SITA	125	124.99 ± 0.6	100.0 ± 0.6
DAPA	12.5	12.6 ± 0.7	100.6 ± 0.7

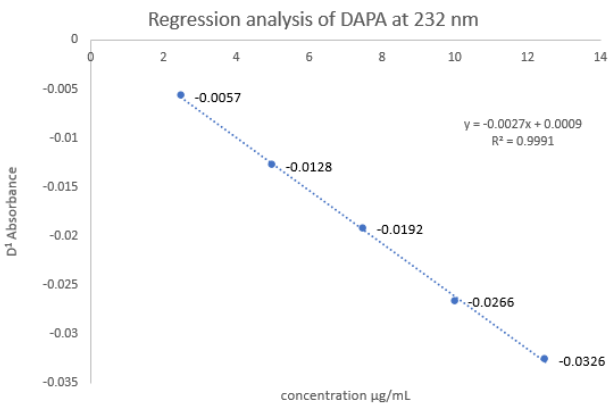

Fig. 8: Regression Analysis of DAPA (2.5-12.5 μg/ml) at 232 nm

2. Materials and Methods

2.1. Materials

DAPA (99.98% pure) and SITA (99.96% pure) were obtained as gift sample for research purpose from, Cadila Healthcare Ltd., Sanand. Methyl alcohol (LR grade) was purchase from S.D. fines.

2.2. Instrument and experimental conditions

Spectrophotometric analysis was performed on shimadzu UV-1800 double beam spectrophoto-meter having path length of 1 cm matched pair of quartz cells. Obtained spectra of SITA and DAPA were derivatized 1^{st} order using UV probe 2.42 as software at delta λ of 10 nm

2.3. Preparation of master stock solution

For the method development purpose, 10 mg of SITA was weighed and diluted to 10 mL (1000 μg/mL) and was further diluted to give final concentration of 250 μg/mL. In similar way 50 mg of DAPA was weighed and diluted to 100 ml (500 μg/mL) and was further diluted to give final concentration of 25 μg/mL.

2.4. Selection of analytical wavelength

The working standards of SITA (25-125 μg/ml) and DAPA (2.5-12.5 μg/ml) were prepared in 10 ml volumetric flask using methyl alcohol as a solvent. They were scanned in the UV range of 200 – 400 nm and D^0 spectra is recorded by UV spectrophotometer. All the D^0 spectra of SITA and DAPA were transformed into D^1 spectra with the help of UV probe 2.42 software. For confirmation of D^1 spectra of SITA and

DAPA, $D^0$and D^1 spectra of the same were overlapped.

2.5. Preparation of solutions for analytical method validation

2.5.1. Preparation of solution for linearity and range

To check linearity of method, SITA was prepared in the concentration range of 25-125 μg/ml and DAPA was prepared in the range of 2.5-12.5 μg/ml from master stock solution in 10 ml volumetric flask. When D^1 Absorbance was plotted against concentration, non-linearity was observed above 150 μg/ml for SITA and above 20 μg/ml for DAPA so final range for validation was selected at mixture containing 25-125 μg/ml for SITA and 2.5-12.5 μg/ml for DAPA. All prepared solutions were scanned between 200-400 nm and all spectra were derivatized to 1^{st} order. D^1 absorbance were obtained at selected wavelength and mean D^1 absorbance was plotted against concentration (To get mean D^1 absorbance, procedure was repeated for five times)

2.5.2. Intermediate precision (Repeatability)

To adjudge the repeatability of analytical method, solution of linearity studies were analyzed for five time with same conditions. Mean D^1 absorbance was recorded at all concentration for SITA and DAPA and were observed for relative standard deviation.

2.6. Method precision

Method precision was determined by performing intraday and interday precision. Mixture that represents overall range (2.5+25, 7.5+75 and 12.5+125 μg/mL) were analyzed on same day at different time interval for intraday precision. Mixture that represents overall range (2.5+25, 7.5+75 and 12.5+125 μg/mL) were analyzed on different days for interday precision.

2.7. Accuracy study

Accuracy of analytical method was adjudged by spiking of placebo with standard solution. Mixture containing 100 mg of directly compressible lactose, 2mg of talc and 2 mg of magnesium stearate was selected as placebo and was spiked at 50, 100 and 150% of target concentration (5+50 μg/mL) (Table 1). Placebo (un spiked) was analyzed at given wavelengths for any possible interference. Each spiked concentration was analyzed for three times and mean % recovery was observed at each spiked level.

2.8. Solvent stability

Solvent stability was determined by scanning the same solution prepared in selected solvent (methyl alcohol) at 3 different time interval that is at 0 hour, 6 hours and 24 hours. Mixture of 12.5+125 μg/ml solution of SITA and DAPA in methyl alcohol were scanned at selected time interval and characteristics of spectra were compared (λ_{max}).

2.9. Assay

As the proposed synthetic mixture is having dose of 10 mg of DAPA and 100 mg of SITA, was mixed with selected placebo, and diluted appropriately to give mixture containing 125 μg/ml of SITA and 12.5 μg/ml of DAPA. This mixture was scanned between 200-400 nm and was derivatized to 1^{st} order. D^1 absorbance was measured at selected wavelengths and were transformed to concentration with help of linear regression equation. This mixture was analyzed for three times and mean % assay was drawn.

3. Result and Discussion

3.1. Selection of analytical wavelength

Three different ZCP at 232 nm, 263 nm and 291 nm were observed in overlain D^1spectra of SITA (Figure 1). Three different ZCP at 256 nm, 275 nm and 305 nm were observed in overlain D^1spectra of DAPA (Figure 2). For determination of analytical wavelength D^1 spectra of SITA and DAPA were overlapped (Figure 3). But there is very less difference between absorbance values of DAPA at 291 nm and hence difficulty in quantifying the same. Discussed problem can be eliminated at 232 nm, where the D^1 absorbance values of DAPA are linear with significant difference (Figure 4). In similar way at ZCP of DAPA, linearity was observed only at 275 nm for SITA (Figure 5). So 232 nm and 275 nm were selected as analytical wavelength for quantitative determination of DAPA and SITA respectively.

3.2. Analytical method validation

All validation parameters were studied as per ICH guidelines.[25,26]

3.3. Linearity and range

When D^0spectra of SITA was taken between 25 - 150 μg/mL, non-linearity was observed over 150 μg/mL. So, linearity for SITA was observed between 25 - 150 μg/mL. for method development purpose range was selected between 25 - 125 μg/mL (based on beer — lambert's law). In similar way D^0spectra of DAPA was taken between 2.5 - 20 μg/mL, but non-linearity was observed over 20 μg/mL. So, linearity for DAPA was observed between 2.5 - 20 μg/mL. and for method development purpose range was selected between 2.5 - 12.5 μg/mL (based on beer — lambert's law). So final range for validation was selected at mixture containing 25 - 125 μg/ml for SITA and 2.5-12.5 μg/ml for DAPA (Figure 6). When calibration curve was plotted for given concentration range (Figures 7 and 8),

value of linear regression coefficient was found to be 0.9989 for SITA and 0.9991 for DAPA. Regression equation was found to be y = 0.0003 X + 0.0018 for SITA and y = 0.0027 X + 0.0009 for DAPA. Linearity data for both drugs is shown in Tables 2 and 3

3.4. Repeatability

When all mixtures were analyzed at all concentration, calculated relative standard deviation at each level was found to be less than 2 so that method was found to be repeatable over the range of 25 - 125 μg/ml for SITA and 2.5 - 12.5 μg/ml for DAPA. Repeatability data are shown in table 4 and 5 for SITA and DAPA respectively.

3.5. Method precision

For determining inter day and intraday precision, % RSD was monitored at selected concentration level which was found to be less than 2 so method was found to be precise for estimation of SITA and DAPA. Data for intermediate precision are given in Tables 6 and 7 for SITA and DAPA respectively.

3.5.1. Accuracy study

Spiked placebo with standard solution at 50, 100 and 150% level was analyzed for % recovery which was found within 98 to 102, so method was found to be accurate (Table 8).

3.5.2. Solvent stability

As the λ_{max} wasstable over period of 24 hrs, the solvent was found to be suitable and drug was found to be stable.

3.5.3. Assay

When prepared synthetic mixture was analyzed by developed and validated method, % assay was found to be 100.0 ± 0.6 for SITA and for 100.1 ± 0.7 DAPA (Table 9)

4. Summary and Conclusion

The 1^{st} order derivative spectroscopic method was developed and validated as per ICH Q2 R1 guidelines and was successfully applied for determination of SITA and DAPA from its synthetic mixture. Present method was found to be economical in terms of cost and time. Commonly used excipient didn't interfere in estimation of SITA and DAPA so method was found to be specific. Method was also found to be repeatable and precise.

5. Acknowledgment

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6. Author contributions

1. Shivani Jani- Collection of all the data after the completion of the research work and the preparation of manuscript was done by Shivani Jani.
2. Rashmi Shukla- After designing the project, execution of project was carried out by Rashmi Shukla.
3. Pinak Patel- The design of the study from choosing the drug to choosing the method was done by Pinak Patel.
4. Binny Mehta- After completion of the study, analysis of the data obtained from UV was done by Binny Mehta.
5. Krunal Detholia- During the study, synthetic mixture preparation according to the dose of the drugs was done by Krunal Detholia.

7. Source of Funding

None.

8. Conflict of Interest

None.

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