Quantitative computation and stability evaluation of phase III composition comprising sitagliptin and dapagliflozin propanediol monohydrate by RP-HPLC

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ABSTRACT
It is always quite essential to evaluate stability performance of any formulation before it enters into the commercial market or available for the clinical trial and so is the case with formulation containing sitagliptin and dapagliflozin propanediol monohydrate, which is at present in clinical phase-III trial. With the same perspective, a reverse phase high performance liquid chromatography was engineered for quantification of both components in presence of their degradation products formed during stress testing. Elution was achieved on Inertsil ODS C18 column with Methyl Nitrile (25 parts) and 0.02 M KH2PO4 buffer 0.02 M having 1 ml triethylamine with neutral pH adjusted by orthophosphoric acid (75 parts) in isocratic mode (flow rate of 1 ml/minute), while chromatogram was monitored at 210 nm. All system suitability parameters are indicative of excellent separation. Analysis of stressed samples (according to ICH Q1) showed that the household formulation showed oxidative instability and overall results of forced stress were predictive in nature. The optimized analytical technique was subjected to validation (according to ICHQ1 guideline) and was observed to be highly reproducible with greater level of specificity, and therefore can be practiced for routine quantification of sitagliptin and dapagliflozin propanediol monohydrate from proposed formulation.

INTRODUCTION
When we discuss about sitagliptin (SITA), it is chemically a trizole derivative with pyrazin moiety which exerts its physiological action which is preliminary hypoglycemic action thorough inhibition of dipeptidyl peptidase-4 system. This active chemical substance is used in combination with diet and exercise in type-2 diabetes either alone or combination with other oral hypoglycemic agents for having potential antidiabetic action (Zerilli and Pyon, 2007). On contrast, dapagliflozin propanediol monohydrate (DAPA), is chemically an oxane derivative with triol moiety and having hypoglycemic action through supression of sodium-glucose cotransporter-2 system, which is again available in combination of other oral hypoglycemic agents operating on different principle for better glycemic control (Dhillon, 2019).

Extensive literature search on SITA highlights majorly UV spectroscopy (Pathade et al., 2011; Pritam et al., 2011), high performance liquid chromatography (HPLC) (Patel et al., 2014; Peraman et al., 2013; Ramalingam et al., 2014; Sunetha et al., 2020), LC-MS (Burugula et al., 2013; Reddy et al., 2015), and HPTLC (Manasa et al., 2015; Modi et al., 2013) as analytical methods for analysis from bulk and pharmaceutical dosage form. In similar way, detailed literature review revealed UV (Jani et al., 2015; Mante et al., 2017), HPLC (Deepan et al., 2017, 2018; Sanagapatni et al., 2014; Usman et al., 2020), LC-MS (Aubry et al., 2010; El-Zaher et al., 2019), and HPTLC (Abdelrahman et al., 2020; Suma et al., 2019) method for determination of DAPA in bulk and pharmaceutical dosage form.

At present, several clinical trials and literature are suggestive of better management of glycemic control in type-II diabetes with reduction in glycated haemoglobin when SITA...
and DAPA are administered in a combination at a dose level of 10 mg of DAPA and 100 mg of SITA (Jabbour et al., 2014; Medical Professionals reference, 2012). The literature survey revealed only one reverse phase high performance liquid chromatography (RP-HPLC) method for quantification of both the components which has serious limitation. Based on above-mentioned facts, it was decided to develop and validate a new, simple, precise and accurate, stability indicating RP-HPLC method for the determination of DAPA and SITA in synthetic mixture.

**MATERIALS AND METHODS**

**Instruments and chemicals**

AGILENT (Series 1260 infinity) HPLC unit equipped with autosampler having provision of UV-VIS detector and capable of quaternary gradient operation was used to process the samples, while OPENLAB software was used to process and integrate the obtained chromatogram. The columns employed were INERTSIL ODS C₁₈ and HIBAR ODS C₁₈ having dimensions of 250 mm as length and 4.6 mm internal diameter. Mettler Toledo weighing balance was used to weigh accurate quantities of samples having sensitivity of 0.1 mg.

Both active components were received as gift samples from Cadila Healthcare Limited, Gujarat (Batch No: SA0010119 for SITA and GLS001119 for DAPA) while other solvents like Methyl alcohol, methyl nitrile, KH₂PO₄ buffer and water were of HPLC grade and procured from Merck life sciences private limited, Mumbai.

**Optimization of separation conditions**

Discrete intrinsic physico-chemical parameters of active components were studied but utmost priority was given to solubility and pKa value. Other instrumental parameters like flow rate and temperate were also studied to check effect on retention time of eluting components. Owing to the vast difference in polarity of components, ODS C₁₈ column was fixed as stationary phase which encourages the retention of nonpolar and early elution of polar component. To get accurate separation in conjunction with system suitability parameters, various elution systems were studied.

**Chromatographic conditions**

Adequate separation was achieved by utilizing methyl nitrile as organic phase in 25 parts and 75 parts of 0.02 M potassium dihydrogen phosphate buffer containing 1 ml triethylamine (TEA) having neutral pH adjusted by orthophosphoric acid (10%) as aqueous phase. The mixed elution system was screened through 0.45 µ membrane filter and was also utilized as dilution medium for components. The above-mentioned elution system was allowed flow though INERTSIL ODS C₁₈ column in isocratic mode at flow rate of 1 ml/minute with monitoring on 210 nm as wavelength where both components gave significant signal. Furthermore, suitability of this system was also assessed by system suitability parameters for which total five injections of fixed concentration was injected at 20 µl injector volume.

**Forced degradation conditions**

**Acid and base hydrolysis**

To check the stability with respect to hydrolysis by acidic condition, 5 mg of DAPA and 50 mg of SITA was treated with 5 ml of 0.1 N HCl and 4 ml of elution medium, the resulting mixture was refluxed at 60°C for half and hour which was followed by cooling of content and neutralization with 0.1 N NaOH. 0.2 ml of previous solution was further diluted to 10 ml with elution medium (Treated sample). In contrast, for base hydrolysis, 0.1 N NaOH was used as the treatment medium and 0.1 N HCl was used as the neutralizing medium. All other condition were same as that of acid hydrolysis. To compare the results, 0 hour sample and blank was prepared. 20 µl of 0 hour sample and treated sample was injected to column and comparison was made to determined % degradation.

**Oxidative hydrolysis**

Effect of oxidative stress was imparted to both drug by use of 3% hydrogen peroxide. Here in this case, 5 mg of DAPA and 50 mg of SITA was treated with 5 ml of 3% H₂O₂ and 4 ml of elution medium, the resulting mixture was refluxed at 60°C for half and hour which was followed by cooling and again volume was adjusted upto the mark. 0.2 ml of the above solution was further diluted to 10 ml with elution medium (treated sample). In similar way, 0 hour sample and blank solution was prepared and were injected on to column to assess the % degradation achieved.

**Thermal hydrolysis**

To assess impact of dry heat on the stability of DAPA and SITA, both components in preweight quantity (10 and 100 mg, respectively) were exposed to dry heat in a hot air oven at 80°C for about half hour. Treated contents were diluted upto 10 ml with mobile phase and 0.1 ml of same solution was further raised to 10 ml which was injected at 20 µl volume on to column to assess % degradation.

**Validation of optimized method**

All validation parameters are studies as per ICH Q2R1 guidelines, execution procedure of each is discussed below.

**Linearity and range**

To study linearity and range, accurately weighed quantities of 5 mg DAPA and 50 mg SITA was diluted to 10 ml with elution medium to produce stock solution containing 500 µg/ml DAPA and 5,000 µg/ml of SITA. Various aliquotes were transferred to 10 ml volumetric flask and volume of each raised to 10 ml to give mixtures having concentration of 5 + 50 µg/ml to 15 + 150 µg/ml of DAPA and SITA, respectively. Each concentration was injected at 20 µl injector volume and response obtained was plotted against concentration to observe linear regression coefficient.

**Repeatability**

For adjudging repeatability of method, each concentration form range was injected for five times and each level is observed for relative standard deviation (RSD).

**Limit of detection and quantitation (LOD/LOQ)**

Detection limit as well as quantification limit was calculated statistically by utilization of regression data from repeatability. Standard deviation of standard error (σ) and average of slope (S) were used to process LOD and LOQ.
**Accuracy**

As it refers to the % recovery of analyte in presence of excipients, it was practiced by spiking of placebo with standard at 50%, 100%, and 150% of target concentration. Each concentration was injected for three times and % recovery was calculated on the basis of observed area at each spiking level (by utilization of linear regression analysis).

**Method precision**

This parameter was studies by injecting individual concentration that represents overall range are studies on same day and between days and for the same mixture of DAPA and SITA that represents overall range (8 + 2, 16 + 4, and 24 + 6 μg/ml) were analyzed on the same day at different time interval for intraday precision and different day for interday precision. Each concentration was analyzed for three times and was monitored for RSD at each level.

**Robustness**

To study the effect of minor variations of method parameters on to the results, operation parameters like temperature, pH of mobile phase and flow rate was deliberately altered within acceptable range and effect of the same was observed on the system suitability parameters as retention time and % assay, which was also monitored for relative standard deviation.

**Ruggedness**

This parameters refers to the method repeatability and was studied by utilizing the different column having same specification and to be more precise HIBAR ODS C_{18} column was employed and difference in the area obtained from both was monitored through student t-test.

**Assay of synthetic mixture**

Weight accurately 10 mg of DAPA, 100 mg of SITA with placebo in 10 ml calibrated volumetric flask. Contents diluted up to 10 ml calibrated volumetric flask with methyl alcohol and filter the solution. 1 ml of master stock solution further dilute up to 10 ml with mobile phase. 1 ml filtrate dilute up to 10 ml with mobile phase (10 + 100 μg/ml). Resulting solution was chromatographed in triplicates and mean observed area was statistically transformed by linear regression equation to get total % of DATA and SITA in synthetic mixture.

**RESULTS AND DISCUSSION**

**Optimized chromatographic conditions**

Initially trials were initiated using combination of methyl nitrile and water in equal proportion but distorted peak without any sort of separation were observed. As separation was not observed in the previous case, methyl alcohol was introduced to the system but with the same only DAPA was detected. In all the cases, flow rate was 1.2 ml/minute. All the results were monitored at wavelengths as 210, 224, and 267 nm. Due to previous observed problems, 0.02 M KH_{2}PO_{4} (0.02 M) containing 1 ml TEA and pH adjusting to 7 by orthophosphoric acid in equal volumes was used as elution medium. Here, both the peak were detected but still were eluting closely to each other. So in the next trial the proportion of buffer was increased and flow rate was further reduced to 1 ml/minute. Even the solution of DAPA and SITA were also prepared in the mobile phase. When method was operated using later discussed chromatographic conditions, well resolved peak of DAPA and SITA was observed at 2.89 and 16.41 minutes, respectively (Fig. 1). The detection wavelength was kept at 210 nm as linearity and peak shape for both the components were perfect at this wavelength. All the system suitability parameteres were within United States Pharmacopoeia guidelines with RSD of less than 1. All the system suitability parameteres have been highlighted in Table 1. The newly developed method was found to be superior to reported method (Gupta and Mishra, 2021) by far degree as they had employed gradient elution with potassium phosphate monobasic buffer pH (3.0) as mobile phase A while methanol and acetonitrile in the ratio of (60:40 v/v) as a mobile phase.

![Figure 1. Chromatogram of mixture of DAPA and SITA under optimized chromatographic conditions.](image)

<table>
<thead>
<tr>
<th>Table 1. System suitability parameters for optimized RP-HPLC method.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>DAPA</td>
</tr>
<tr>
<td>SITA</td>
</tr>
</tbody>
</table>

Figures indicated are mean of five similar determinations.

\( R_t = \) Retention time; \( A_s = \) Peak asymmetry factor; \( N = \) Number of theoretical plates; \( R_s = \) Resolution; SD = Standard deviation.
phase B. The condition itself is too complex and the flow of elution was kept at 1.5 ml/minute which gives rise to too much column back pressure. Furthermore, the major con of the reported method is that, both DAPA and SITA have the same concentration range, which is quite contrary to the fixed combination dose, and hence the newly developed method outsmarts the reported method in all the segments.

**Forced degradation study**

When optimized method was applied for evaluating the stability and behaviour of active components by forced degradation study (acid hydrolysis, base hydrolysis, oxidative stress, and thermal stress), the optimized method was found to be stability indicating as it is able to separate all the degradation products in the presence of active ingredients. No degradation products found to interfere with estimation of DAPA and SITA in stressed samples (Figs. 2–4). Even the stress condition applied for the study were found to be optimim as, % degradation observed was predictive in nature (below 20%). % degradation observed for each condition is highlighted in Table 2.

**Validation of analytical method**

The proposed optimized RP-HPLC method was studied for validation parameters according to the ICH Q2R1 guidelines.

![Figure 2. Chromatogram of treated sample with 0.1 N Hydrochloric acid (acid hydrolysis).](image)

![Figure 3. Chromatogram of treated sample with 0.1 N NaOH (base hydrolysis).](image)

![Figure 4. Chromatogram of treated sample with 3% hydrogen peroxide (oxidative stress).](image)
Table 2. Summary of forced degradation studies.

<table>
<thead>
<tr>
<th>Stress conditions</th>
<th>Type of sample</th>
<th>Area observed DAPA</th>
<th>Area observed SITA</th>
<th>% Degradation DAPA</th>
<th>% Degradation SITA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid hydrolysis</td>
<td>0 hour sample</td>
<td>4,260,771</td>
<td>17,087,836</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Treated sample</td>
<td>3,567,915</td>
<td>18,986,932</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base hydrolysis</td>
<td>0 hour sample</td>
<td>4,499,039</td>
<td>26,918,993</td>
<td>18.24</td>
<td>15.38</td>
</tr>
<tr>
<td></td>
<td>Treated sample</td>
<td>3,678,030</td>
<td>22,778,090</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>0 hour sample</td>
<td>40,812,761</td>
<td>44,860,783</td>
<td>12.2</td>
<td>22.31</td>
</tr>
<tr>
<td></td>
<td>Treated sample</td>
<td>35,833,659</td>
<td>34,850,773</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal stress</td>
<td>0 hour sample</td>
<td>3,060,766</td>
<td>45,229,827</td>
<td>8.03</td>
<td>11.24</td>
</tr>
<tr>
<td></td>
<td>Treated sample</td>
<td>2,815,802</td>
<td>40,149,994</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0 hour sample = Sample prepared without imposing any stressed condition; Treated sample = sample prepared with stressed conditions.

Figure 5. Overlaid chromatogram of mixtures representative of linearity study.

Figure 6. Regression analysis of DAPA (For linearity studies).

Figure 7. Regression analysis of SITA (for linearity studies).
and each validation parameter was found to be in accordance with guidelines. Method was found to be linear with linear regression co-efficient of 0.9983 and 0.999 over the range of 5–15 µg/ml of DAPA and 50–150 µg/ml SITA, respectively (Figs. 5–7). Method

Table 3. Robustness data of optimized RP-HPLC method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Level of changes</th>
<th>DAPA</th>
<th>SITA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tr Assay</td>
<td>Tr Assay</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.1 ml/minute</td>
<td>2.81</td>
<td>99.61 ± 0.639</td>
</tr>
<tr>
<td></td>
<td>0.9 ml/minute</td>
<td>3.00</td>
<td>99.90 ± 0.417</td>
</tr>
<tr>
<td>Temperature</td>
<td>28°C</td>
<td>2.89</td>
<td>99.65 ± 0.581</td>
</tr>
<tr>
<td></td>
<td>32°C</td>
<td>2.86</td>
<td>99.63 ± 0.134</td>
</tr>
<tr>
<td>pH of mobile Phase</td>
<td>6.8 unit</td>
<td>2.89</td>
<td>99.81 ± 0.911</td>
</tr>
<tr>
<td></td>
<td>7.2 unit</td>
<td>2.89</td>
<td>99.81 ± 0.650</td>
</tr>
</tbody>
</table>

Table 4. Ruggedness of the developed RP-HPLC method.

<table>
<thead>
<tr>
<th>Change in Parameter</th>
<th>Result</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in column</td>
<td>t-test</td>
<td></td>
</tr>
<tr>
<td>Inertsil ODS C_{18}</td>
<td>0.0068 (DAPA)</td>
<td>No significant difference</td>
</tr>
<tr>
<td>Hibar ODS C_{18}</td>
<td>0.0094 (MON)</td>
<td></td>
</tr>
</tbody>
</table>

Results are mentioned at 95% confidence interval.

Table 5. Summary of validation parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DAPA</th>
<th>SITA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity and range</td>
<td>5–15 µg/ml</td>
<td>50–150 µg/ml</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 314,703 × −75,487</td>
<td>Y = 456,837 × −291,840</td>
</tr>
<tr>
<td>Standard error (%)</td>
<td>75,487 (2.64%)</td>
<td>291,840 (0.68%)</td>
</tr>
<tr>
<td>Repeatability (RSD)</td>
<td>0.77–1.16</td>
<td>0.09–0.28</td>
</tr>
<tr>
<td>Accuracy (% recovery)</td>
<td>98.79–99.65</td>
<td>99.64–100.48</td>
</tr>
<tr>
<td>Intraday precision (RSD)</td>
<td>0.22–0.34</td>
<td>0.13–0.16</td>
</tr>
<tr>
<td>Interday precision (RSD)</td>
<td>0.33–0.43</td>
<td>0.05–0.28</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robust</td>
<td>Robust</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>Rugged</td>
<td>Rugged</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Specific</td>
</tr>
</tbody>
</table>

Figure 8. Assay of synthetic mixture (DAPA and SITA, 10 and 100 µg/ml, respectively).
was found to be robust and rugged as there is no significant change in results (retention time and % assay) by minor variation in method parameters, the same highlighted in Tables 3 and 4. Summary of all validation parameters is highlighted in Table 5. When optimized and validated method was applied for quantitative analysis of synthetic mixture containing DAPA and SITA (Fig. 8), the method gave accurate results with % of DAPA and SITA as 99.43 and 99.41, respectively.

CONCLUSION

As the proposed combination of DAPA and SITA is in clinical phase III, no analytical method is available yet for simultaneous quantitative expression of both the components from synthetic mixture. The proposed stability indicating RP-HPLC method not only separates and quantify the components from synthetic mixture with at most accuracy but also gives idea about the stability of mentioned components under variety of conditions as per ICHQ1 guidelines. It can be concluded that both the components are highly susceptible to oxidative stress and base hydrolysis, and hence formulation of both can be made and packaging material can be employed in such a way that it can be protected from exposure to atmospheric oxygen and exposure to moisture. Finally, method was successfully validated as per ICHQ2R1 guidelines and applied for determination of DAPA and SITA from synthetic mixture.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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AUTHOR CONTRIBUTIONS

From execution of research point of view, Pinak Patel is responsible for designing of overall experiment, data acquisition, communication with team members, and preparation of manuscript along. Yesha Patel, Jigna Bhatt, and Binny Mehta are responsible for in depth review of literature and preparing each chemicals/ solution and data analysis. Krunal Detholia has a specific role to play for statistical analysis the raw data.

REFERENCES


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