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Sakshi A. Jadhav, Nikita A. Bhilare, B. Chandanshive, Gurappa K. Dyade
Department of Post Graduate in Pharmaceutical Quality Assurance, SVPM'S College of Pharmacy, Malegaon (BKII) Baramati, Pune, Maharashtra, India 413115

Amit S. Lunkad
Department of Pharmaceutical Chemistry, SitabaiThite College of Pharmacy, Shirur, Pune, Maharashtra, India 412210

Correspondence

Gurappa K. Dyade
Department of Post Graduate in Pharmaceutical Quality Assurance, SVPM'S College of Pharmacy, Malegaon (BKII) Baramati, Pune, Maharashtra, India 413115

Email:
pharmacyresearchsvpmcop@gmail.com

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Sustainable Green Analytical Chemistry: Spectrophotometric Method Development of Dapagliflozin and Omeprazole by Using Eco-friendly Solvent

Sakshi A. Jadhav, Nikita A. Bhilare, B. Chandanshive, Gurappa K. Dyade, Amit S. Lunkad

ABSTRACT

The present research's aim was to develop analytical method utilizing ecologically suitable solvent which enhances solubility of analyte, sensitivity of the method etc. An analytical method was developed for the estimation of dapagliflozin (DGZ) and omeprazole (OPZ) by using aqueous 0.1 N HCl on the UV-VIS spectrophotometer. Wavelengths 223 nm and 282.5 nm were set to measure absorbance of DGZ and OPZ respectively. The results of different spectral characteristic techniques were examined to select the parameters and the design was validated against the ICH Q 2 R1 regulatory guidelines. The linearity of the drug was determined at a concentration of 1 to 50µg/ml and 1 to 32µg/ml for DGZ and OPZ respectively. The accuracy was found within acceptable limit with standard deviation 1.1874 to 6.4984 for DGZ and 0.1401 to 0.9843 for OPZ; and the assay study data was found 99.77 % for DGZ and 97.92 % for OPZ. The stability study of the method was performed out by minute variation in the wavelength, scan speed. The developed method is rigid, robust and efficient for the estimation of DGZ and OPZ from their respective dosage form. The effort was made to develop green or eco-friendly analytical method utilizing hydrotropic solvent for water insoluble drug dapagliflozin and omeprazole.

Key words: Green method, Dapagliflozin, Omeprazole, analytical method, eco-friendly solvent, UV spectroscopy

1. INTRODUCTION

The prime objective of the present research was to use ecologically suitable solvent and to enhance the solubility of analyte. The water solubility of the therapeutically active drug is an important property as it controls its solubility, absorption, and in vivo activity; and also restricts use of organic solvent in method development.[1] Articles[2,3]is deliberately showing significance of agents like hydro tropes in solubilization of very poor water-soluble drug. The development of eco-friendly method by avoiding organic solvent could be termed as economical green method.[4] There is consistently pressure from environmental department to minimize hazardous and volatile solvent content in the waste which seriously affects environment. Use of hydrotropic solutions, supercritical fluids in the organic synthesis curbs use of organic solvent in view point of green chemistry.[5] Capability of hydro tropes to increase the water solubility of organic compounds up to 200 times is also reported. In literature review it is revealed that green FT-IR method[6], eco-friendly methods[7,8] are suitable for analytical purpose; and green analytical methods are preferred over analytical methods using harmful organic solvent for environment.[9] Drugs dapagliflozin and omeprazole were selected for this research purpose study due to their poor water solubility.

Dapagliflozin (DGZ) is a (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol [10] antidiabetic and pharmacologically sodium-glucose co-transporter 2 inhibitor that enhances urinary excretion of glucose by suppressing renal reabsorption of glucose.

Many analytical methods have been published for the estimation of DGZ alone or in combined state with other anti-diabetic agents in pharmaceutical dosage form includes lonely UV spectroscopic method [12], with other drug UV spectroscopic method[13-16], HPLC method [17,18], stability indicating HPLC [19-22], QbD bio analytical[23] UHPLC bio analytical [24], kinetic study UHPLC method[25], stability indicating UPLC method [26], UPLC DAD bio analytical[27], LC-MS/MS bio analytical method[28-30]and critical review on bio analytical[31].

Omeprazole (OPZ) chemically is 6-methoxy-2- [[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole[10] and is proton pump inhibitor, suppresses secretion of gastric acid by inhibiting the enzyme system of H/K ATPase the proton pump of the gastric parietal cells. It is used in aspiration syndrome, dyspepsia, GRD, peptic ulcer and zollinger-ellison syndrome[11]. The drug OPZ is official in recently published British Pharmacopoeia[32] and Indian Pharmacopoeia[33]. Chemical structure of drug is shown in (Figure 1).

Various analytical methods have been reported for the estimation of OPZ alone or in combination with other GIT agents in pharmaceutical dosage form includes UV spectroscopic method [34], RP-HPLC methods[35-41], stability indicating HPLC method[42,43]. DOE based HPLC[44], bio analytical HPLC chromatographic method[45-47], UPLC-TOF analyzer[48], HPTLC method[49,50] and Densitometric HPTLC method.[51]

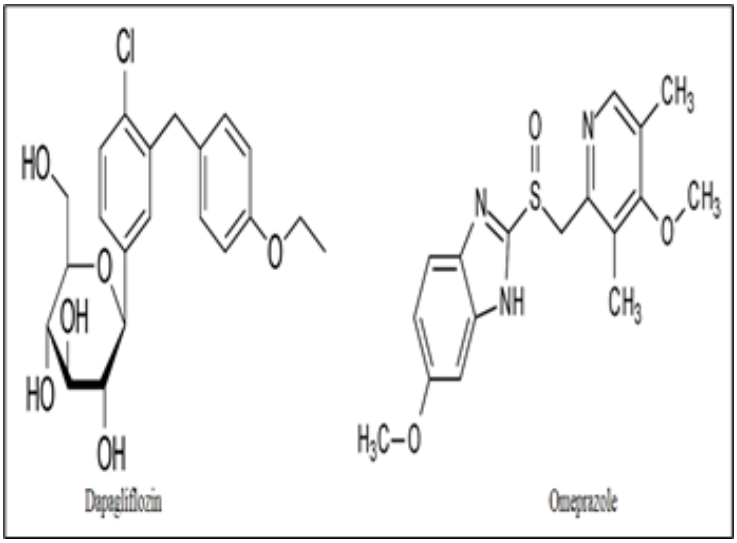

Figure 1: Chemical structure of Drug molecule

For analytical method validation ICH Q2 (R1) has given various method performance characteristics.[52,53]

2. MATERIALS AND METHODS

2.1 Instrumentation

Analysis was performed using a 10 mm matched quartz cell with a Shimadzu Double-beam UV–Visible spectrophotometer (Shimadzu, Kyoto, Japan) with a spectral bandwidth of 2 nm and an accurate wavelength of ±1 nm. The analyte was weighed and degassed using an Afcoset balance (The Bombay Burmah Trading Corpo Ltd), an electronic balance model ER 200A with an accuracy of ± 0.1 mg using a 1.8 Liter ultrasonic cleaner (Labman Scientific Instruments Chennai).

2.2 Reagents and Chemicals

Pure pharmaceutical sample of Dapagliflozin from Smruthi organic Ltd Solapur and Omeprazole from BLD pharmatech Co Hyderabad were procured as a gift samples and the marketed commercial formulation Zucapride-10mg containing DGZ and Omez- 20mg containing omeprazole were procured from local market. HCl acid AR and distilled water were utilized for preparation of solvent.

2.3 Solvent Selection

Research article[54] was focused on techniques to be adopted while selection of suitable solvent. DGZ is freely soluble in DMSO, ethanol and DMF, very slightly soluble in water. OPZ is liberally soluble in dichloromethane, chloroform, soluble in ethanol 95%, dilute aqueous solutions of acid, alkali and very slightly soluble in water. Solubility of the procured drug wasstudied in 0.1N HCl, ethanol and 0.1 N NaOH; and to understand characteristic nature of spectra each solution of known conc of analyte was scanned in UV range. The recorded spectra in these solvents are shown in (Figure 2 and 3).

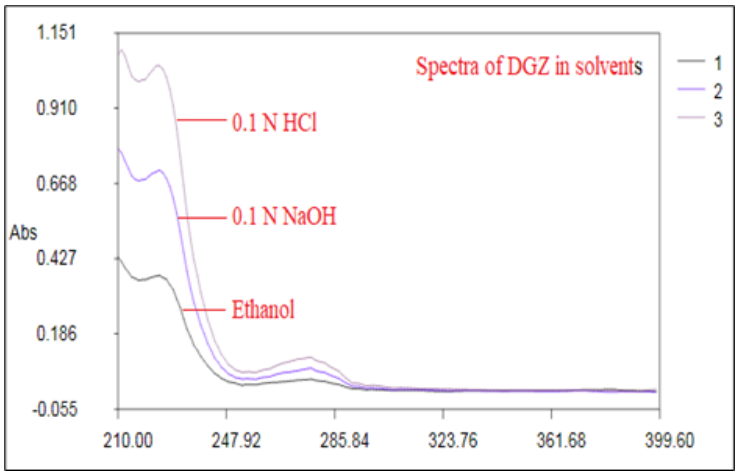

Figure 2: UV-VIS spectra of Dapagliflozin in selection of solvent study

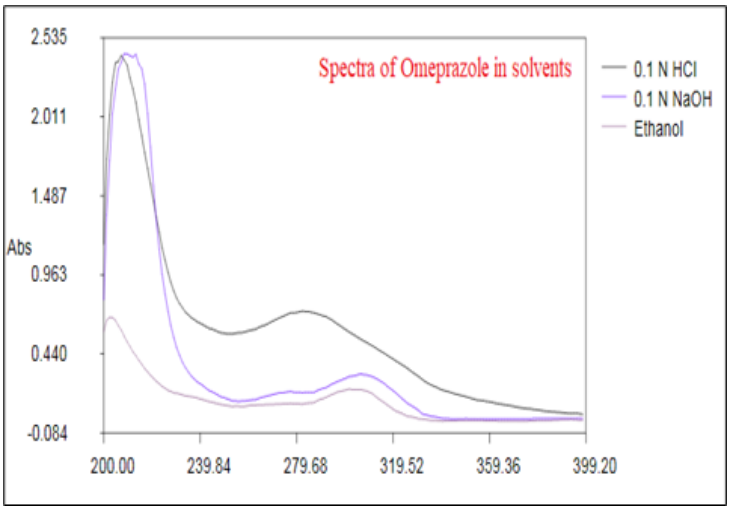

Figure 3: UV-VIS spectra of Omeprazole in selection of solvent study

2.4 Preparation of Stock Solutions and Standard Solutions

10 mg of pure DGZ drug and OPZ were accurately and separately weighed; and transferred into separate 50 ml volumetric flask. Dissolved into 0.1 N HCl solution and volume was made to 50 ml with solvent. Working standard solution of DGZ and OPZ 20µg/ml was obtained by diluting aliquot of stock solution.

2.5 Selection of Wavelength and Conc Range

From UV spectra it was found that DGZ and OPZ have measurable absorbance at 223 and 282.5 nm. From the nature of spectra working conc. range 1 to 50µg/ml and 1 to 12mcg/ml (µg/ml) was selected in solvent for DGZ and OPZ respectively. Also, drug solution was prepared simulated to marketed formulation. The observations discussed above lead us to select and use the main points listed in Table 1; the process has been validated against ICH guidelines and by analyzing as per industry standards.

Table 1: Selected critical parameter for UV-VIS analytical method of DGZ and OPZ

Parameter	Selected variables for DGZ	Selected variables for OPZ
Wavelength range	400-200 nm	400-200 nm
Wavelength	223 nm	282.5 nm
Solvent	0.1 N HCl	0.1 N HCl
Scan speed	Fast	Fast
Sampling interval	± 0.2 nm	± 0.2 nm

2.6 Experimental Method for Estimation

Analytical simple calibration curve method was found suitable for the estimation of the both drugs individual formulation/dosage form. The calibration curve method comprises use of the stock solution to prepare the series of six standard solutions within the working concentration range and record the measured absorbance at the selected wavelength; it is based on the calibration curve diagram, i.e. the relationship between absorbance and concentration. The best linear relationship between concentration and absorbance was determined from triplicate calibration curves prepared from different stock solutions. Used the regression equation Y = mX + c (where m is the slope and c are the intercept) to calculate the sample concentration. Also, spectrophotometers quantitative method was utilized to know the conc. of sample/formulation solution.

2.7 Validation of the Method

In order to obtain an analysis plan for this method, the main options must meet the effectiveness of the analysis method. To comply with these ICH guidelines, Q2 R1 is used to examine effectiveness by significance. Procedure validated according to ICH guidelines.[52,53]

2.8 System Suitability

System suitability study was conducted to demonstrate the appropriateness of the designed procedure under consideration for the analytical method. Six replicates each of DGZ and OPZ working standard solutions having conc 10µg/ml were prepared separately and absorbance was recorded; SD and % RSD of the response were calculated. Stability of the solution was also studied by bench top stability at laboratory temp.

DGZ and OPZ activity levels were prepared in 6 replicates at a concentration level of 10µg/ml and absorbance was recorded; SD and %RSD of responses were calculated. Chemical stability was also examined by benchtop stability at laboratory temperature.

2.9 Linearity

The linearity of an analytical method is studied by obtaining response i.e. absorbance which should be directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range from 1 to 50µg/ml (DGZ) and 1 to 12µg/ml (OPZ) and scanned from 400 to 200 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at 223 and 282.5 nm for DGZ and OPZ respectively in spectrum order. Microsoft office excel software is the tool, was used to obtain the standard regression

curve and its analysis as slope, intercept, and correlation coefficient.

2.10 Assay of Formulation

Content of drug i.e. active ingredient in the formulation was carried out by proposed method and process was validated by determining statistical parameters.

2.10.1 Estimation of Formulations by Calibration Curve Method

Weighed and powdered the tablets; weighed out the tablet powder equal to 10 mg DGZ and transferred to a 50 ml volumetric flask. Dissolved in 0.1N HCl solvent and bring to volume with solvent. Further solution was filtered through filter paper Whatman No 40 and aliquot of filtered solution was diluted to obtain sample solution. Solution was scanned in the range of 400 to 200 nm, recorded absorbance of sample solution at 223 nm in spectrum order.

Similar process was followed for estimation of OPZ from the dosage form and for recording absorption wavelength 282.5 nm was set in the spectrum mode. Obtained absorbance was utilized to calculate unknown conc of formulation; and results are statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

2.10.2 Accuracy and Precision

The analytical method's accuracy is expressed as the closeness of an agreement between test result and true result. Recovery study is the process to check accuracy and performed by i.e. standard addition method; diluted sample solution of DGZ and OPZ was prepared separately and standard solutions added in 80,100 and 120% proportionate to the sample solution. Three replicates at each of these three levels were obtained and absorbance recorded; then % of conc, SD and RSD of replicates were calculated.

To ascertain precision of the method, process was carried out by performing assay of tablet six times; also,interday and intraday precision was studied to achieve the reproducibility in result.

2.10.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD, LOQ of DGZ, OPZ by this proposed method were determined using calibration graph method and formula 3.3σ/s and 10 σ/s was used to obtain LOD and LOQ respectively where σ is the standard deviation of calibration curve and s is the slope of regression line.

2.10.4 Robustness and Ruggedness

It is measure of capability of any analytical procedure to remain unaffected by small but deliberate variations in method parameter.

3. RESULTS AND DISCUSSION

The development process consists of several steps; Solvent and sample selection are important. The use of environmentally friendly solvents has attracted great attention due to their low cost, convenience and environmental friendliness. The substance to be analyzed must have satisfactory solubility in the chosen solvent. The chemical structure and physicochemical property of the drug are available in the literature to guide the use of the appropriate solvent in this method. Solubility of DGZ and OPZ was performed in each solvent; and in 0.1 N HCl solvent both drugs were shown appreciable, measurable and stable absorbance as compare to other solvent.

3.1 System Suitability

The absorbance of six replicate of standard solutions (10 and 8 µg/ml) are shown in Table 2. The calculated SD of DGZ and OPZ were found within acceptable limits and therefore meet the system suitability requirements and appropriateness of the method.

Table 2: System suitability study of DGZ and OPZ

Sr. No	Conc in µg /ml	Absorbance of DGZ	Conc in µg /ml	Absorbance of OPZ
1	10 µg /ml	0.3098	8 µg /ml	0.2831
2	10 µg /ml	0.2808	8 µg /ml	0.3212
3	10 µg /ml	0.3090	8 µg /ml	0.3121
4	10 µg /ml	0.2852	8 µg /ml	0.3198
5	10 µg /ml	0.3098	8 µg /ml	0.3215
6	10 µg /ml	0.2975	8 µg /ml	0.3204
	SD	0.01535	SD	0.003914

3.2 Linearity

The overlay spectra acquired in linearity study was reported in Figure 4 and 5 and the designed calibration curve of both analytes was found to be linear in the selected conc. range as shown in Figure 6. The regression equation of line and its parameters slope, r2 value and intercept are tabulated in Table 3, which proved the linear relationship between amount of drug in solution and obtained response.

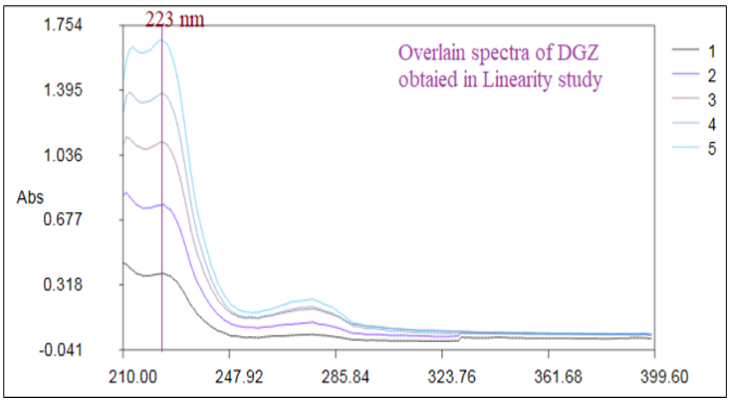

Figure 4: UV-VIS overlain spectra of DGZ in linearity study

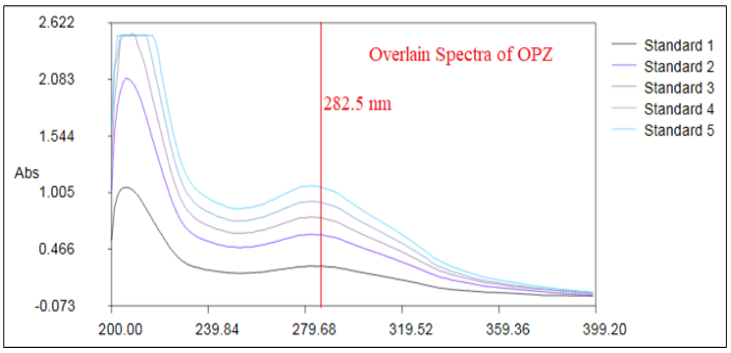

Figure 5: UV-VIS overlain spectra of OPZ in linearity study

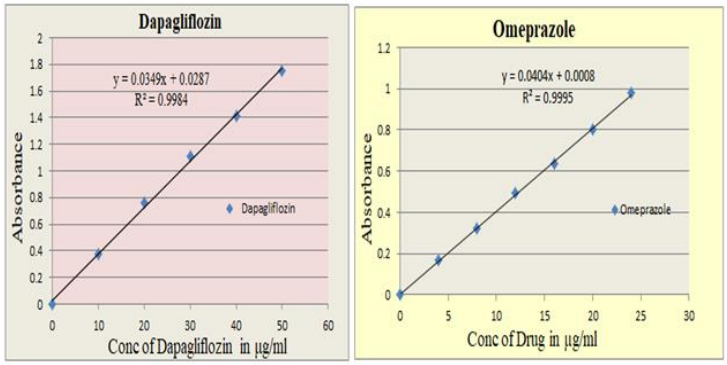

Figure 6: Calibration curve of Dapagliflozin and omeprazole

Table 3: Parameters of regression equation obtained in Microsoft excel office

Parameters	DGZ	OPZ
Detection wavelength	223 nm	282.5 nm
Beer's law limit (µg/ml)	1 – 50 µg/ml	1 – 24µg/ml
Correlation coefficient (r^2)	0.9984	0.9995
Regression equation (y = mx + c)	Y = 0.0349 X + 0.0287	Y= 0.0404 + 0.0008

3.3 Assay

The assay was performed and processed by calibration curve method. From the obtained spectra of dosage form, calculated % of nominal conc. and SD. The obtained data was found within acceptable limits are summarized in Table 4. The results indicated applicability of the method for estimation of formulation.

Table 4: Results of assay of formulation by proposed method

Formulation	Drug	Label Claim (mg/Tablet)	Amount found/mg; n=6	Drug Content %	Std Deviation	% RSD
Zucapride-10	DGZ	10 mg	9.977 mg	99.77 %	3.0372	3.0442
Omez-20	OPZ	20 mg	19.58 mg	97.92 %	1.1352	0.41942

3.4 Accuracy and Precision

The results of accuracy are summarized in Table 5, the obtained results were consistent, within acceptable limit; and methods accuracy was justified by calculating % drug content. The precision study included multiple assays of solutions performance as per guidelines; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarized in Table 5.

Table 5: Results of accuracy and precision

Sr. No	Parameter	Level of study	Data Title	Obtd. Data	S.D.	RSD
1	Precision study of DGZ	Intraday Precision	Mean of Abs n= 6	106.08 %	5.4982	5.1827
		Interday precision		102.96 %	1.2055	1.1689
2	Accuracy study of DGZ	80 %	% Purity	106.30 %	4.2659	4.0131
		100 %		98.49 %	1.1874	1.2055
		120 %		103.99 %	6.4984	6.2489

Sr. No	Parameter	Level of study	Data Title	Obtd Data	S.D.	RSD
1	Precision study of OPZ	Intraday Precision	Mean of Abs n= 6	102.428	0.9843	0.9612
		Interday precision		94.374	0.6442	0.6821
2	Accuracy study of OPZ	80%	% Purity found	102.89	0.5835	0.5755
		100%		103.4912	0.1401	0.1354
		120%		103.76	0.6101	0.627

3.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of DGZ and OPZby the proposed method was found within acceptable limit.

3.6 Robustness and Ruggedness

Study of robustness was carried out and capacity of designed analytical procedure to measure drug was remain unaffected by small but deliberate variations in method parameter like variation in the wavelength ± 2 nm, variation in the solvent strength by ± 0.1 %. The analytical method was found rugged during the stage of development; similarly, the result was achieved by performing the analysis by different analyst.

4. CONCLUSION

The analytical method was designed with environmentally friendly, easily available aqueous 0.1 N HCl solvent. Dapagliflozin and Omeprazole were estimated from their respective formulation by the proposed method and satisfactory results were obtained. This method's reproducibility in the result; and obtained results data within acceptable limits given in the pharmacopoeia is confirmed validity of the method. So, this validated method is economical, precise, accurate, robust and reproducible hence can be routinely used for estimation of both the drugs from the dosage form.

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