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SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DAPAGLIFLOZIN USING HYDROTROPHIC PHENOMENONA

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ABSTRACT

The aim of the present study was to assess the feasibility of hydrotropes (Urea, N, N Dimethyl urea, Sodium benzoate, Sodium Acetate) for the development of an ecofriendly, operator-safe and cost-effective spectroscopic method and its validation for estimation of Dapagliflozin from pharmaceutical formulation. Enhancement of solubility was more than 50 and 70 % for DAPA respectively in mixed hydrotropic solution. The enhancement of solubility of DAPA in water was due to hydrotrophy phenomenon. Stability of both drugs were observed by dissolving DAPA in Sodium acetate:Urea (2M:8M) solution used as solvent. Solution of DAPA was prepared in the conc. of 10µg/ml and scanned under time scan for 30 min. Standard stock solutions and working solutions were made using serial dilution methods DAPA's peak absorbance was noted at 225.0 nm. Linearity of both drugs was established by response ratios of drugs. Recovery studies evaluated the suggested approaches' accuracy at three distinct levels, i.e. 80%, 100%, and 120%. Precision of the methods was studie/ at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. The method's specificity was evaluated in order to conclusively determine if the analyte included any components that may be anticipated to be present, such as contaminants, degradation products, and matrix components. It was determined that the suggested approach is safe,

innovative, novel, straightforward, accurate, free from pollution, exact, and it can be used effectively in the regular analysis for dapagliflozin. The offered approaches entirely achieve the goal of this study work due to their simplicity, speed, repeatability, and economy.

KEYWORDS: Validation, estimation, Dapaglifloxin, Hydrotrophy.

INTRODUCTION

Dapagliflozin is slightly soluble in water and freely soluble in methanol and acetonitrile. Furthermore, methanol and acetonitrile is ranked by the U.S. Environmental Protection Agency (U.S. EPA) as a toxic chemical as liquid or vapor and waste has to be detoxified through special chemical treatment, leading to high to very high disposal cost.[1] Later, attempts have been made to switch the media from acetonitrile/ methanol to water.[2] However, acetonitrile/ methanol are still highly toxic to humans and causes adverse effects on aquatic life from pharmaceutical formulation.[3] To the best of our knowledge, literature search from databases has not presented the evidence of using greener and less toxic solvent for the spectroscopic analysis of Dapagliflozin.[4]

The word "Hydrotropy" has been used to describe how the introduction of several additives increases the aqueous solubility of diverse substances that are weakly water-soluble.[5] Hydrotropy's mechanism is still not fully understood. In 1916, Neuberg originally established the idea of hydrotropy.[6] According to his definition, hydrotropes are metal salts of organic acids, which at fairly high concentration increase the solubility of poorly water-soluble compounds.[7]

Therefore, the aim of the present study was to assess the feasibility of hydrotropes (Urea, N, N Dimethyl urea, Sodium benzoate, Sodium Acetate) for the development of an ecofriendly, operator-safe and cost-effective spectroscopic method and its validation for estimation of Dapagliflozin from pharmaceutical formulation.

EXPERIMENTAL WORK

The DAPA's reference standard was a kind gift from a pharmaceutical business. Urea and sodium acetate were purchased from the Mumbai-based Merck Chemical Division. Commercial tablets of DAPA and were procured from the local drug market. Label claim of DAPA in tablet is 10mg respectively. Reverse Osmosis Water was used throughout the study.

Farxiga of Astra Zeneca 10mg marketed formulation was estimated by the hydrotropy**.**

Solubility: Solubility of DAPA was determined at 25±1°C. 10 milligrammes precisely weighed DAPA was introduced to several 10 ml volumetric flasks containing various solvents and shaken mechanically for eight hours. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. Filtrates were diluted suitably and analyzed spectrophotometrically against water.

Enhancement of solubility was more than 50 and 70 % for DAPA respectively in mixed hydrotropic solution. The enhancement in solubility of DAPA was due to the hydrotropic solubilization phenomenon. Results of solubility in different solvent for both the drug were shown in Table 7.1.

Table 7.1: Solubility of Drug in Different Solvents.

S. No.	Solvents	Solubility DAPA
1	Water	-
2	Hot water	-
3	Cold water	-
4	2M Sodium acetate	-
5	8M Urea	-
6	2M Sodium Citrate	-
7	2M Sodium Benzoate	-
8	2M Sodium acetate:8M Urea (1:1)	+
9	2M Sodium acetate:2M Sodium Benzoate (1:1)	-
10	2M Sodium acetate:2M Sodium Citrate (1:1)	-
11	8M Urea:2M Sodium acetate (1:1)	-
12	8M Urea:2M Sodium Benzoate (1:1)	-
13	8M Urea:2M Sodium Citrate (1:1)	-
14	2M Sodium Citrate:8M Urea (1:1)	-
15	2M Sodium Citrate:2M Sodium Benzoate (1:1)	-
16	2M Sodium Citrate: 2M Sodium acetate (1:1)	-
17	2M Sodium Benzoate:8M Urea (1:1)	+
18	2M Sodium Benzoate: 2M Sodium acetate (1:1)	-
19	2M Sodium Benzoate:2M Sodium Citrate (1:1)	-

Selection of Solvent System: DAPA was scanned in various hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Sodium acetate: 8M Urea solution (50:50 W/V) was found to be most appropriate because:

- Drug is soluble in it (50 and 70 %)
- Drug is stable in it
- Drug exhibit good spectral characteristics in it.
- Sodium acetate:Urea solution have no interference with the λ_{max} of drug.

Establishment of Stability Profile: Stability of both drugs were observed by dissolving DAPA in Sodium acetate:Urea (2M:8M) solution used as solvent. Solution of DAPA was prepared in the conc. of 10µg/ml and scanned under time scan for 30 min. Spectra of both drugs under time scan shows that of both drugs are stable in mixed hydrotropic solution.

Linearity Range and Calibration Graph

Preparation of Standard Stock Solution (Stock-A): Standard stock solutions were made by dissolving 100 mg of drug in 80 mL of a mixed hydrotropic solution containing 2M sodium acetate and 8M urea (1:1), separately. The flask was then sonicated for about 10 minutes to solubilize the drug and achieve a concentration of 1000 g/ml (Stock-A) for both pharmaceuticals. Volume was then increased using a combination of hydrotropic chemicals to appropriate amount.

Preparation of Sub Stock Solution (Stock-B): A portion of 2.5 ml were removed from DAPA's standard stock solution A with the use of a pipette, put into individual 25 ml volumetric flasks, and diluted to 25 ml with RO Water to provide a concentration of 100 g/ml. (Stock-B).

Preparation of Working Standard Solution: Aliquots of 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml and 5.0 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in Volumetric flask, 10 ml and RO Water was used to get the volume up to 10 ml. This gave the solutions of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml respectively for DAPA.

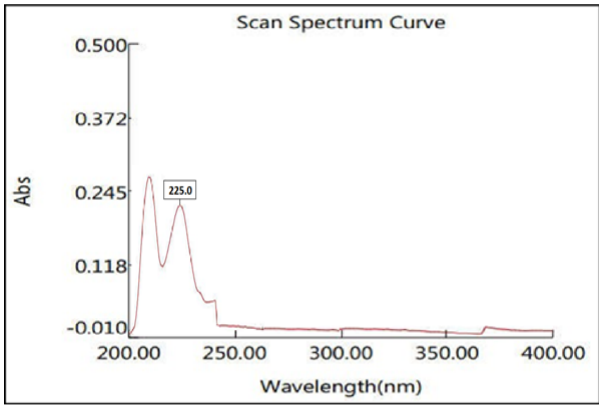

Figure 7.1: Determination of λmax.

Selection of Wavelength for Linearity: Separate solutions containing 10 g/ml of DAPA were made. From 200 nm to 400 nm, the solution was scanned in spectrum mode. DAPA's peak absorbance was noted at 225.0 nm, respectively. In the concentration range of 10–50

g/ml at their respective maxima, DAPA demonstrated linearity. Absorbance versus concentration was shown on a calibration curve. To study the linearity of DAPA the selected wavelength λ_{max} is 225.00nm.

Table 7.2: Linearity of DAPA At λmax = 225.0 nm.

Standard Conc. (mg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
10	0.158	0.157	0.157	0.158	0.159	0.157
20	0.304	0.303	0.304	0.304	0.304	0.303
30	0.454	0.453	0.454	0.453	0.454	0.453
40	0.614	0.614	0.612	0.614	0.614	0.613
50	0.75	0.747	0.75	0.748	0.749	0.748
Correlation Coefficient (r^2)	0.999	0.999	0.998	0.998	0.998	0.998
Slope (m)	0.015	0.015	0.015	0.015	0.015	0.015
Intercept (c)	0.002	0.002	0.003	0.002	0.003	0.002

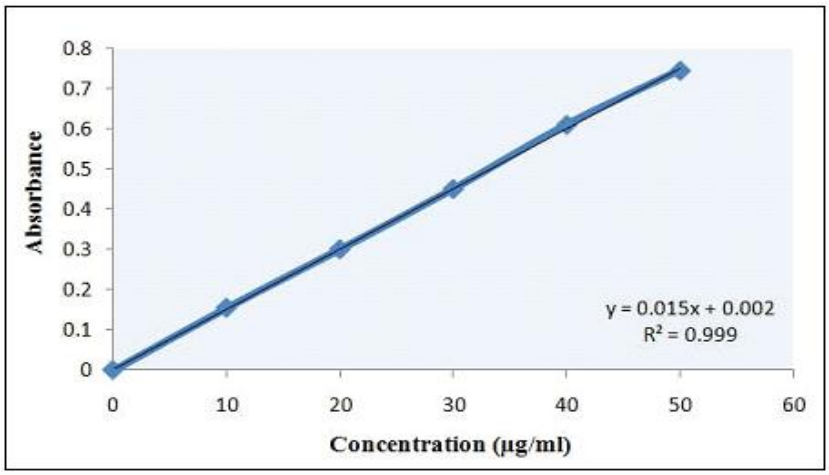

Fig. 7.2: Calibration Curve of DAPA.

Method Validation

A_1: Linearity: Linearity of both drugs was established by response ratios of drugs. Response ratio of drug was calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Table 7.3: Response Ratio of DAPA.

S. No.	Conc. (µg/ml)	ABS	Response Ratio
1	0	0	0
2	10	0.158	0.016
3	20	0.304	0.015
4	30	0.454	0.015
5	40	0.614	0.015
6	50	0.749	0.015

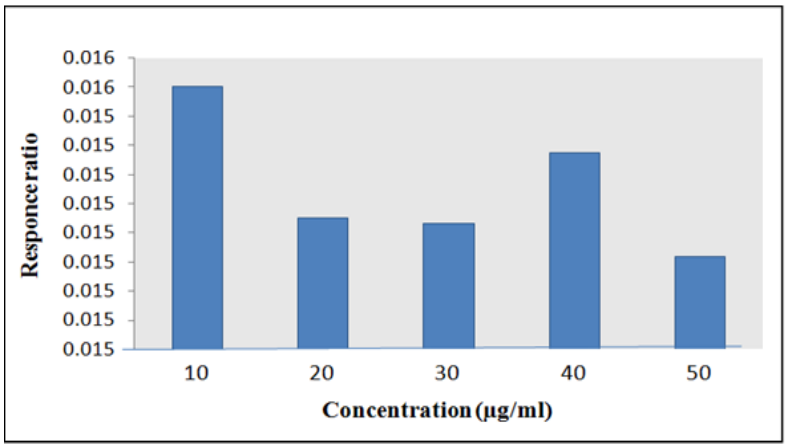

Fig 7.3: Response ration data for linearity.

B_1: Accuracy: Recovery studies evaluated the suggested approaches' accuracy at three distinct levels, i.e. 80%, 100%, and 120%. In order to conduct the recovery tests, preanalysed tablet solutions were mixed with a specified quantity of DAPA standard solution. The answers that emerged were then re-analyzed using the suggested techniques. To determine if the additional drug sample could be recovered, the whole analytical process was redone. This recovery analysis was carried out three times with five different concentration levels.

Table 7.4: Recovery study of DAPA (80% level).

DAPA Tablet (mg/ml)	Std. DAPA Added (mg/ml)	Rep-1		Rep-2		Rep-3		DAPA % Mean
		DAPA Found	% Found	DAPA Found	% Found	DAPA Found	% Found	
2.5	2	1.93	96.01	1.86	92.51	1.97	98.01	95.51
5	4	3.92	97.76	3.65	91.01	3.6	89.76	92.84
7.5	6	5.65	94.01	5.65	94.01	5.6	93.17	93.73
10	8	7.83	97.76	7.82	97.63	7.6	94.88	96.76
12.5	10	9.65	96.41	9.87	98.61	9.68	96.71	97.24
							Mean*	95.216
							SD*	0.102
							% RSD*	1.698

* Mean of 3 replicate and 5 concentrations

Table 7.5: Recovery study of DAPA (100% level).

DAPA Tablet (mg/ml	Std. DAPA Added (mg/ml)	Rep-1		Rep-2		Rep-3		DAPA % Mean
		DAPA Found	% Found	DAPA Found	% Found	DAPA Found	DAPA Found	
2.5	2.5	2.47	98.41	2.5	99.61	2.43	96.81	97.61
5	5	4.87	97.21	4.4	87.81	4.38	87.41	90.81
7.5	7.5	7.4	98.54	7.49	99.74	7.36	98.01	98.76
10	10	9.65	96.41	9.7	96.91	9.73	97.21	96.84

12.5	12.5	12.4	99.13	12.46	99.61	12.35	98.73	99.15
							MEAN*	**96.634**
							SD*	**0.094**
							% RSD*	**1.807**

* Mean of 3 replicate and 5 concentrations

Table 7.6: Recovery study of DAPA (120% level).

DAPA Tablet (mg/ml	Std. DAPA Added (mg/ml)	Rep-1		Rep-2		Rep-3		DAPA % Mean
		DAPA Found	% Found	DAPA Found	% Found	DAPA Found	DAPA Found	
2.5	3	2.6	86.34	2.68	89.01	2.6	86.34	87.23
5	6	5.65	94.01	5.87	97.67	5.5	91.51	94.39
7.5	9	8.69	96.45	8.6	95.45	8.77	97.34	96.41
10	12	11.87	98.84	11.8	98.26	11.83	98.51	98.53
12.5	15	14.83	98.81	14.93	99.47	14.77	98.41	98.89
							MEAN*	**95.09**
							SD*	**0.087**
							% RSD*	**1.375**

* Mean of 3 replicate and 5 concentrations

C_1: Precision: Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables.

C_1-1: Repeatability

Table 7.7: Repeatability of DAPA.

Replicate	Concentration Found					
	10	**20**	**30**	**40**	**50**	
Replicate-1	9.96	19.99	29.96	39.87	49.83	
Replicate-2	9.91	19.96	29.9	39.86	49.9	
Replicate-3	9.9	19.94	29.85	39.96	49.89	
Replicate-4	9.9	19.97	29.96	39.99	49.96	
Replicate-5	10	19.99	29.92	39.92	49.93	
Mean	9.938	19.968	29.924	39.926	49.92	
% Mean	99.368	99.834	99.745	99.814	99.821	**99.716**
S.D.	0.045	0.022	0.047	0.057	0.05	**0.044**
% R.S.D.	0.449	0.107	0.155	0.142	0.099	**0.19**

C_1-2: Intermediate Precision

C_1-2.1: Day-to-Day Variation

Table 7.8: Day-to-Day Variation of DAPA.

	Concentration Found					
Replicate	**10**	**20**	**30**	**40**	**50**	
Day -1	9.9	19.96	29.9	39.9	49.86	
Day -2	9.96	19.99	29.92	39.88	49.88	
Day -3	9.9	19.88	29.96	39.99	49.9	
Mean	9.92	19.953	29.935	39.66	49.922	
% Mean	99.101	99.716	99.751	99.126	99.825	99.504
S.D.	0.036	0.058	0.032	0.06	0.021	0.041
% R.S.D.	0.036	0.058	0.032	0.06	0.021	0.041

C_1-3: Reproducibility

Table 7.10: Reproducibility of DAPA.

Replicate	**Concentration Found**					
	10	**20**	**30**	**40**	**50**	
Replicate-1	9.99	19.9	29.9	39.9	49.88	
Replicate-2	9.96	19.88	29.88	39.96	49.9	
Replicate-3	9.97	19.9	29.92	39.88	49.88	
Replicate-4	9.9	19.99	29.92	39.86	49.86	
Replicate-5	9.88	19.92	30	39.85	49.88	
Mean	9.943	19.924	29.929	39.901	49.893	
% Mean	99.418	99.618	99.762	99.751	99.784	**99.667**
S.D.	0.048	0.044	0.047	0.045	0.015	**0.041**
% R.S.D.	0.478	0.215	0.153	0.11	0.029	**0.197**

Analysis of Tablet sample

20 commercial DAPA pills were weighed and ground into a fine powder, and the resulting mixture was dissolved in a 10 ml volumetric flask, equating to 50 mg of DAPA. After sonicating the flask for around 10 minutes to solubilize the drug included in the powdered tablet, the volume was then raised to the necessary amount with hydrotropic solution. 4 ml of the sodium acetate and urea solution were added after that. Filtration was carried out using Whatman Filter Paper No. 41 after sonication. In order to achieve the final concentrations of both medications in the usable range, filtrate was collected and further diluted with RO Water. At certain wavelengths, the absorbances of the final dilutions were measured, and the concentrations were determined using the calibration curve method. Five instances of the process were completed.

Table 7.11: Analysis of Tablet Formulation of DAPA.

DAPA	Replicate 1		Replicate 2		Replicate 3	
Conc. Present (µg/ml)	Conc. Found (µg/ml)	% Conc. Found	Conc. Found (µg/ml)	% Conc. Found	Conc. Found (µg/ml)	% Conc. Found
10	9.99	99.81	9.96	99.51	9.93	99.21
20	19.96	99.76	19.86	99.26	19.79	98.91
30	29.86	99.51	29.79	99.28	29.66	98.84
40	39.46	98.64	39.66	99.14	39.75	99.36
50	49.13	98.25	49.46	98.91	49.88	99.75

DISCUSSION

Eco friendly Method development and Validation for the estimation of Dapagliflozin using hydrotropic phenomena was done.

Linearity: The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and the results of linearity are reported in table 8.1.

Table 8.1: Results of Linearity of DAPA.

Parameter	DAPA
Concentration (µg/ml)	1-5
Correlation Coefficient (r^2)*	0.999
Slope (m)*	0.015
Intercept (c)*	0.002

*Value of six replicate

Specificity: The method's specificity was evaluated in order to conclusively determine if the analyte included any components that may be anticipated to be present, such as contaminants, degradation products, and matrix components.

Accuracy: The accuracy and dependability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method. Result of recovery study shown in table 8.2.

Table 7.7: Results of Recovery Study.

% LEVEL	DAPA (% Mean ± SD*)
80%	95.21±0.111
100%	96.63±0.094
120%	95.09±0.087

***** Value of three replicate and three concentrations

Precision: Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. Result of precision shown in table 8.3.

Table 8.3: Results of Precision.

Parameter	DAPA (% Mean ± SD*)
Repeatability	99.716±0.044
Intermediate precision	
Day to day precision	99.504±0.041
Analyst to analyst	99.514±0.059
Reproducibility	99.667±0.040

* Value of five replicate and five concentrations

Assay of Tablet: The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interference of excipient in the estimation of drugs.

Table 7.10: Analysis of Tablet Sample.

Label Claim (mg)	DAPA*(10mg) % Assay
Replicate 1	99.19
Replicate 2	99.22
Replicate 3	99.21
Mean	99.20
SD	0.015
% RSD	0.015

*Average of three Determination

CONCLUSION

Modern pharmaceuticals intended for human consumption must adhere to strict standards and rules laid out by the relevant authorities. Only through scientific quality control of a product's

quality can the effectiveness and safety of medical goods be guaranteed. Determine how much of a drug's constituents are present in a formulation that is being marketed is the art and science of pharmaceutical analysis. It is regarded as the implementation of techniques required to ascertain and gauge the substance's identification, potency, quality, and purity. Therefore, the creation of testing methods for drug composition is constantly needed, making the quality control laboratory the foundation of the pharmaceutical businesses. In the current research, an effective effort was made to estimate three newly-marketed hypertension combos using spectrophotometry and a hydrotropic substance. The approach was created via experimentation on the basis of an extensive literature review and was validated by statistical sample settings. Throughout the whole experiment, a Shimadzu UV/VIS double beam-double detector spectrophotometer was used. (Model-1700 series). The result shows the correctness, simplicity, speed, and precision of the developed approach. Thus, rather than processing of extraction using organic solvent separately, they may be employed for routine analysis of Dapagliflozin in bulk medicine and tablet dosage form. By combining agents with lower concentrations, it may be possible to decrease the high total concentration of hydrotropic agents required to result in a moderate improvement in solubility. Since spectrophotometry does not employ organic solvents, it does not have to worry about issues like residual toxicity, volatility-related errors, pollution, expense, etc. Thus, it was determined that the suggested approach is safe, innovative, novel, straightforward, accurate, free from pollution, exact, and it can be used effectively in the regular analysis for dapagliflozin. The offered approaches entirely achieve the goal of this study work due to their simplicity, speed, repeatability, and economy.

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