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Development and validation of the UV-Spectrophotometric method for determination of Diaveridine in bulk and in formulation.

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ABSTRACT

Background: The aim was the study to develop a technique and validate it in a basic, financially effective UV spectrophotometry strategy for the estimation of Diaveridine in bulk and pharmaceutical dosage form according to ICH rules.

Results: The λmax of Diaveridine in 0.1N HCl was viewed in 275nm. The drug follows linearity in the fixation range 5-40µg/ml with corelation coefficient value of 0.999. The proposed technique was applied to drug formulation and percent measure of drug assessed was 92.91% and was viewed as to be in good agreement with the label claim . The accuracy was checked by recovery performed at 3 unique levels i.e. 80,100,120. The percent% recovery was viewed as in the scope of 99.12-99.84. The precision of strategy is depicted by low worth of % RSD (<2).

Conclusion: In this study 0.1N HCl picked on the grounds that it was a fast device for routine examination of Diaveridine in mass and drug dose structure.

KEYWORDS: Diaveridine, U.V spectrometry, Method development, Validation, Beer's law, 0.1N HCl.

INTRODUCTION

Compounds containing pyrimidine rings assume a huge part in countless organic method because of their correlate properties. Thus, substituted 2,4 diaminopyrimidines are broadly utilized as metabolic inhibitors of pathways prompting the synthesis of proteins and nucleic acids [1] .Among these type of mixtures, diaveridine (5-[(3,4-diethoxyphenyl)methyl]-pyrimidine-2,4-diamine) is a notable biological vehicle , and furthermore, it very well may be utilized as an antiprotozoal vehicle in animal [2, 3]. Diaveridine was found act as as powerful antibacterial drug or medication due to a li to two methoxy class substituted on the benzene rings [4], further more the presence of two aromatic amino groups or the acidic hydrogen in the particles make it goes about as areas of strong acceptor [5, 6]. It has wide antibacterial activity against numerous Gram-negative and Gram-positive microscopic organisms [7]. It likewise has wonderful action against coccidian so; it is utilized in the treatment of chicken coccidiosis, fowl cholera, and pullorum [8]. Not so much work has been seen on this medication.

Accordingly, we focused to study the response of this medication with massive counter anions, for example, sulfonphthalein acidic dye. Sulfonphthaleins are one of the most fascinating groups of anionic dyes and stand out enough to be noticed; this is ascribed to their atomic design which permits arrangement of ion associate complexes with different medications, where ion pair are those buildings where the scientific species partners with oppositely charged particles to form unbiased compounds [9-12].Ion pair ligand development techniques were utilized for the visible spectrophotometric determination of different medications in the literature through their ion pairing with oppositely charged dyes to form tint complexes [13].A number of HPLC measure strategies have been accounted for the assurance of diaveridine. Writing overview uncovered that different analytical techniques, for example, HPLC and UV spectrometry techniques were also documented. In this review, endeavors were made to develop, simple and cost effective UV spectrophotometric technique utilizing a 0.1N HCl.

MATERIALS AND METHODS

INSTRUMENTS

The current work was achieved on AglientGS2881 UV-visible spectrophotometer having single beam detector order. The absorption spectra of reference and test solution were completed in a 1cm quartz cell over the radius of 200-400 nm. All chemicals of analytical grade used as it is.

PREPARATION OF STANDARD STOCK SOLUTION

Precisely weigh around 50mg of the drug and moved to 50ml of volumetric flask and liquefy in around 20ml of 0.1N HCl. Then, at that point, volume was made upto the level with 0.1N HCL.5 ml of this solution was moved to 50ml volumetric flask and diluted upto 50ml with 0.1HCL .This solution contained 100µg of drug per ml of the solution.

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PROCEDURE

Determination of wavelength of maximum absorbance (λmax):

1ml of standard stock solution was pipette out and moved to a 10ml volumetric flask .The volume was made upto the level with 0.1N HCL .This solution contained 10µg/ml of the drug. The absorbance of this solution was scanned in the UV range of 200-400nm against ethanol.

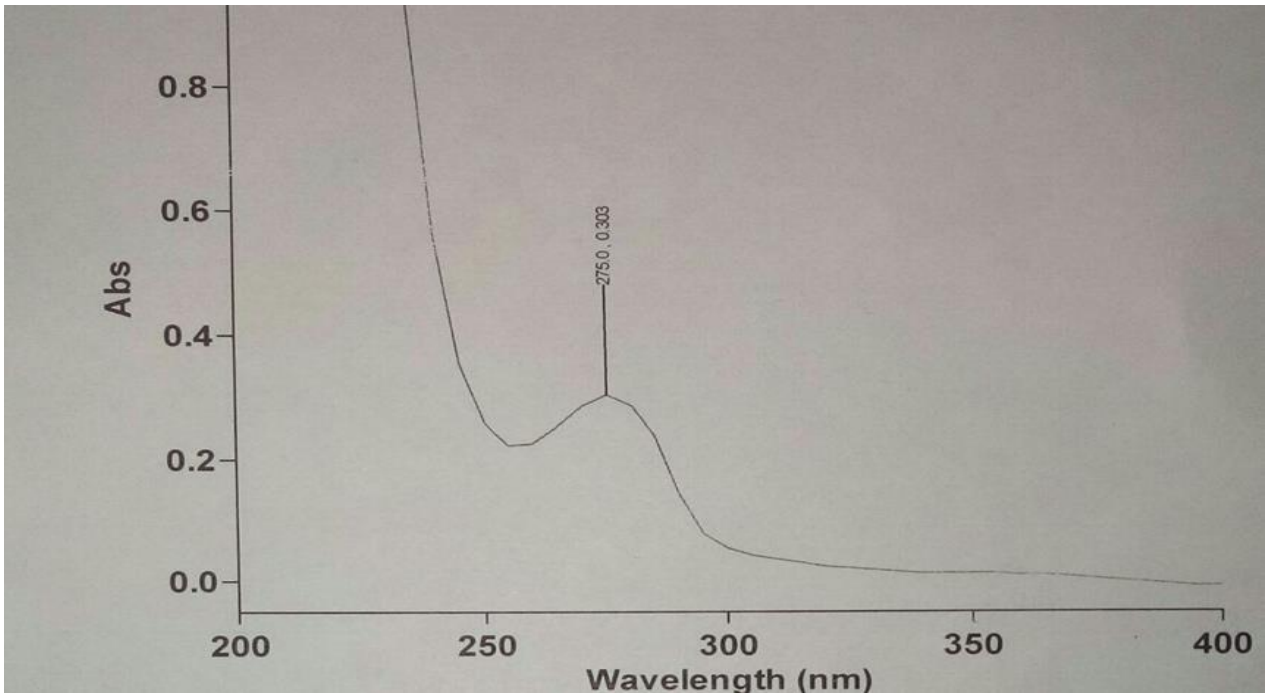

Figure 1: Scan of Diaveridine in the range of 240-400nm

VALIDATION (15,16)

REPEATABILITY: Pipette out 1 ml working solution and transfer into 10 ml volumetric flasks. Dilute it to 10 ml with 0.1N HCL to get 10µg/ml solutions. Nine separate 10 µg/ml solutions of the drug were prepared. Absorbance of the resultant solutions was measured at 275 nm using 0.1N HCl as blank. The result obtained is summarized in the table 2**.**

INTRA-DAY PRECISION: Pipette out 1, 2 and 3 ml working solution and move into separate 10 ml volumetric flasks. Dilute every one of them to 10 ml with 0.1N HCL to get solution of concentrations 10, 20 and 30µg/ml respectively. Absorbance of the resultant solutions was estimated at 275nm utilizing 0.1N HCL as blank. Such three studies were performed in no less than a day at 0, 3 and 6 hrs. Span. The outcome acquired is summed up in the table under 3.

INTER-DAY PRECISION: Pipette out 1, 2 and 3 ml working solutions and move into separate 10 ml volumetric flasks. Dilute every one of them to 10 ml with 0.1N HCL to get solution of concentrations 10, 20 and 30µg/ml respectively. Absorbance of the resultant solutions was estimated at 275 nm utilizing .1N HCl as blank. Such three examinations were performed for three day at 0, 24 and 48hrs stretch. The outcome got is summed up in the table 4.

ACCURACY: Pipette out 0.5 ml working solution and transfer into 10 ml volumetric flasks. Nine such transfers are made. Spike three of the solutions with 0.4 ml of working solution and dilute each to 10 ml with 0.1N HClto get 9 µg/ml solutions.

Spike another three of the solutions with 0.5 ml of working solution and dilute each to 10 ml with 0.1N HCl to get 10 µg/ml solutions.Spike last three of the solutions with 0.6 ml of working solution and dilute each to 10 ml with 0.1N HCl to get 11 µg/ml solutions.

Absorbance of the resultant solutions was measured at 275 nm using .1N HCL as blank. The result obtained is summarized in the table 5.

SPECIFICITY:25. Specificity study was carried out by observing any interference in absorbance of drug in the presence of common excipients like starch, talc, lactose, magnesium stearate etc. Absorbance of 10 µg/ml drug solution with and without excipients was measured at 275nm using 0.1N HCL The result obtained is summarized in the table 6.

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LINEARITY AND RANGE:
Calibration curve demonstrates that the drug shows linear response in the range of 5-40 µg/ml. The linear relationship between concentration and absorbance is given by equation.
Abs = 0.0253x- 0.0015 Conc. and Correlation coefficient = 0.9999.

ESTIMATION OF DIAVERIDINE IN PURE FORM:
Weigh accurately 50 mg of the drug and dissolve it in 0.1N HCL. Make up the volume to 50 ml with 0.1N HCl in volumetric flask. Pipette out 5 ml of the solution and transfer into a 50 ml volumetric flask. Dilute it to 50 ml with .1N HCl. Pipette out 1 ml of the resultant solution and transfer into 10 ml volumetric flasks. Dilute it to 10 ml with 0.1N HCl. Absorbance of the final solution was measured at 275 nm using .1N HCl.
The above procedure was repeated for three times. The result obtained is summarized in the table 7.

ESTIMATION OF DIAVERIDINE IN PHARMACEUTICAL DOSAGE FORM:
Weigh 20 tablets and calculate the average weight. Powder those tablets.
Weigh accurately a quantity of powdered tablets containing about 50 mg of Diaveridine and transfer it into 50 ml volumetric flask. Add 35 ml 0.1N HCl and sonication for 15 minutes. Make up the volume to 50 ml, mix and filter. Dilute 5 ml of the filtrate to 50 ml with .1N HCl. Further dilute 1 ml of the resulting solution to 10 ml with 0.1N HCl. Measure the absorbance of the resulting solution at 275 nm.
The above procedure was repeated for three times. The result obtained is summarized in the table 8.

RESULT AND DISCUSSIONS
The optimum conditions for UV spectroscopy method has been established by varying the parameters one at a time keeping the other parameters fixed and observing the effects of products on the absorbance of the sample. Beer's law limits, molar absorbivity, sandal's sensitivity. The regression analysis using the method of least squares was made for the slope(b),intercept(a) and correlation coefficient (r) obtained from different concentrations are given in table.

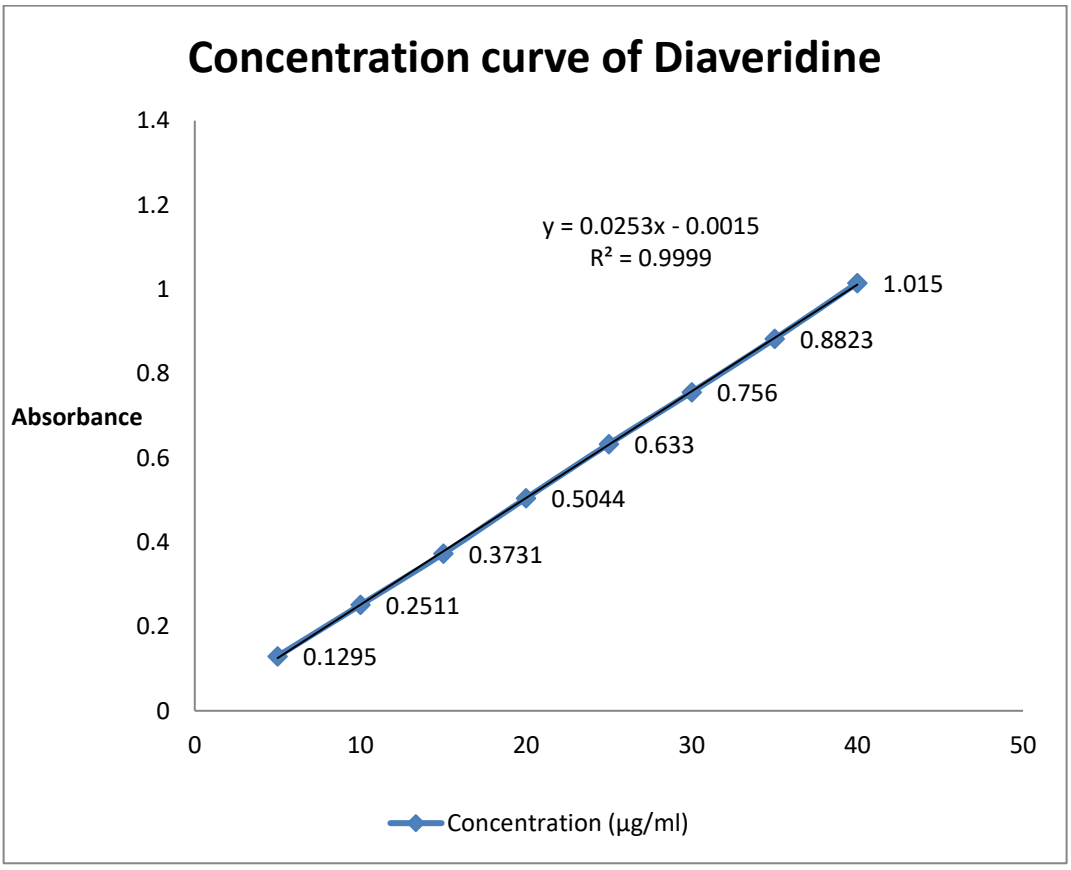

Figure 2: Calibration curve of Diaveridine

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Table1: **The absorbance, E 1% 1cm, Absorptivity and molar absorptivity values of Diaveridine in different concentration at ƛmax=275nm.**

Sr. No	Conc (µg/ml)	Absorbance	Absorbance/ Conc. (A/C)	E(1%,1cm) (A/C x 10000)	Absorptivity (E(1%,1cm)/10)	Molecular Absorptivity
1	5	0.1295	0.0259	259.00	25.9	6741.69
2	10	0.2511	0.0251	251.10	25.11	6536.05
3	15	0.3731	0.0248	248.74	24.87	6473.58
4	20	0.5044	0.0252	252.2	25.22	6564.69
5	25	0.6330	0.0253	253.20	25.32	6590.72
6	30	0.756	0.0252	252.00	25.20	6559.48
7	35	0.8822	0.0252	252.05	25.20	6559.48
8	40	1.015	0.0253	253.75	25.37	6603.73
Mean				**252.75**	**25.73**	**6578.67**

Table 2: Repeatability values of Diaveridine at ƛmax=275nm.

Nominal Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	Mean Conc.(µg/ml)	SD	%RSD
10	0.2511 0.2516 0.2520 0.2497 0.2524 0.2485 0.2514	10.21 10.24 10.26 10.12 10.28 10.10 10.22	10.24	0.0687	0.67

TABLE 3: Intra –Day Precision values of Diaveridine at ƛmax=275nm.

Nominal Conc.(µg/ml)	Absorbance			Observed Conc.(µg/ml)			Mean Conc.(µg/ml)	SD	%RSD
	0hrs	3hrs	6hrs	0hrs	3hrs	6hrs			
10	0.2511	0.2509	0.2507	10.18	10.14	10.10	10.14	0.04	0.39
20	0.5044	0.5039	0.5033	20.21	20.17	20.15	20.17	0.030	0.14
30	0.7560	0.7557	0.7554	30.15	30.13	30.09	30.12	0.030	0.101
								Mean	0.21

TABLE 4: Inter-Day Precision values of Diaveridine at ƛmax=275nm.

Nominal Conc. (µg/ml)	Absorbance			Observed Conc.(µg/ml)			Mean Conc. (µg/ml)	SD	%RSD
	0hrs	24hrs	48hrs	0hrs	24hrs	48hrs			
10	0.2513	0.2508	0.2498	10.20	10.17	10.14	10.17	0.03	0.29
20	0.5042	0.5032	0.5021	20.17	20.12	20.08	20.12	0.045	0.22
30	0.7557	0.7548	0.7540	30.25	30.21	30.16	30.20	0.045	0.14
								Mean	0.217

TABLE 5: Accuracy values of Diaveridine at ƛmax=275nm.

Recovery at %	Nominal Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	% Recovery
80	9=5+4	0.2487	8.89	99.12
80	9=5+4	0.2491	8.92	99.20
80	9=5+4	0.2496	8.85	99.09
100	10=5+5	0.2513	9.95	99.82
100	10=5+5	0.2519	9.82	99.72
100	10=5+5	0.2521	9.89	99.77
120	11=5+6	0.2551	10.89	99.76
120	11=5+6	0.2557	10.93	99.87
120	11=5+6	0.2560	10.91	99.84
			Mean	99.57±0.80

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TABLE 6: Specificity values of Diaveridine at ƛmax=275nm.

Nominal Conc. (µg/ml)	Without Excipients		With Excipients		%Interference
	Absorbance	Observed Conc.(µg/ml)	Absorbance	Observed Conc.(µg/ml)	
10	0.2512	10.01	0.2611	9.88	1.74
10	0.2517	10.09	0.2622	9.92	1.20
10	0.2496	9.91	0.2617	9.91	1.21
10	0.2520	10.11	0.2608	9.81	1.28
10	0.2498	9.94	0.2610	9.84	1.82
				Mean	1.45

TABLE 7: Estimation of Diaveridine in pure form.

S.N	Absorbance	Conc.(µg/ml)	DilutionFactor	Content(mg)	Weight Taken (mg)	% Assay
1	0.2519	9.96	5000	49.83	50	99.66
2	0.2513	9.94	5000	49.71	50	99.42
3	0.2510	9.93	5000	49.65	50	99.30
					Mean±SD	99.46±0.14

TABLE 8: Estimation of Diaveridine in pharmaceutical Dosage form

Sr. No.	Absorbance	Conc. (µg/ml)	Dil. Factor	Content (mg)	Weight Taken (mg)	% Assay
1	0.2346	9.28	5000	46.40	50	99.24
2	0.2349	9.29	5000	46.46	50	99.36
3	0.2351	9.30	5000	46.50	50	99.42
					Mean±Sd	99.34±0.50

CONCLUSIONS

A simple and sensitive spectrophotometric method for quantitative determination of Diaveridine in either pure form or in pharmaceutical dosage for was developed and validated as per ICH. [27]

Diaveridine showed maximum absorbance at 275 nm in .1N HCl solution. It has linear response in the entire range of 5 to 40µg/ml with correlation coefficient of 0.9999.The linear regression equation obtained is Abs = 0.0253x- 0.0015Conc. The method has good precision within 2% and average accuracy as 98.41±0.70. No significant interference was observed in the absorbance of the drug in the presence of common excipients. The method was statistically validated according to ICH. The summary of validation parameters is shown in the table below.

When the method was employed for the quantitative determination of .1N HCl in pure as well as tablet dosage form, the assay values found were 99.46±0.14 and 99.34±0.50 respectively.

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