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Review

To Develop and Validate Analytical Method for Determination of Diaveridine by UV-Spectrophotometry in Pure Form

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Abstract:
A simple, accurate and selective UV-spectro-photometric method was developed for the estimation of Diaveridinein bulk and pharmaceutical dosage forms. The method was developed and validated according to International Conference on Harmonization (ICH Q2 R1) guidelines. The developed method was validated statistically with respect to linearity, range, precision, accuracy, and ruggedness, limit of detection and limit of quantization. A simple, sensitive, specific, and validated UV method has been developed for the quantitative determination of Diaveridine as API. The λmax was found to be 275nm. The linearity was found in concentration range of 5-40µg/ml with correlation coefficient 0.999. The regression equation was found to be as Y=0.0253x-0.0015. This indicated that the developed method was accurate, precise, specific, rapid and suitable for the analysis of commercial samples.

Keywords: Diaveridine, U.V spectroscopy, Validation, Beer's law, Accuracy, Precision.

INTRODUCTION

Validation of an analytical procedure is defined as a process by which it is established that the performance characteristics of the procedure meet the requirements for the intended analytical applications in laboratory studies. The main objectives of any analytical measurement are to obtain consistent, reliable and accurate data. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice [1]. The main parameters used for validation purpose are linearity and range, accuracy and precision, recovery, limit of detection and limit of quantitation. Diaveridine is chemically 2, 4-Diamino-5-veratrylpyrimidine.It is used for the coccidial infection and intestinal bacterial infection of birds [1-4]. These are used in veterinary medicine that exhibits anti-parasitic activity. Literature survey reveals that, spectro-photometric methods [5-7], HPLC [8-9], Mass spectroscopy [7] method. In the present study, an attempt has been made to develop UV Spectrophotometric method for the determination of Diaveridine in bulk form using 0.1 N HCL. The develop method was found to be simple, sensitive and reproducible.

MATERIALS AND METHODS

INSTRUMENTS

The present work was carried out on AglientGS2881 UV-Visible spectrophotometer having single beam detector configuration. The absorption spectra of reference and test solution were carried out in a 1cm quartz cell over the range of 200-400 nm.

CHEMICALS

All chemicals used are of analytical grade and recieved as gift sample from Windlas Healthcare (P) Ltd.

PREPARATION OF STANDARD STOCK SOLUTION: Accurately weigh about 50mg of the drug and transferred to 50ml of volumetric flask and

dissolved in about 20ml of 0.1M HCL .Then volume was made upto the mark with 0.1N hCL. 5 ml of this solution was transferred to 50ml volumetric flask and Volume was made upto the mark with solvent .This solution contained 100µg of drug per ml of the solution.

PROCEDURE

Determination of wavelength of maximum absorbance (ƛmax)

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

VALIDATION

LINEARITY AND RANGE:

Calibration curve demonstrates that the drug shows linear response in the range of 5 to 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.

1. 0.5,1,1.5,2,2.5,3,3.5,4 ml of standard stock solution (100µg/ml) were pipette out into a series of 10ml volumetric flask . The volumes were made upto the mark with ethanol and mixed to obtain the solutions in the concentration range of 5,10,15,20,25,30,35 and 40µg/ml of drug.
2. The absorbance of these resultant solutions were measured at 275 nm against 0.1 N HCL as a blank and a graph was plotted between absorbance obtained and the concentrations of the solution.

PRECISION:

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. Six 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 transfer into separate 10 ml volumetric flasks. Dilute all 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 measured at 275nm using 0.1N HCL as blank. Such three studies were performed within a day at 0, 3 and 6 hrs. Interval. The result obtained is summarized in the table below 3.

INTER-DAY PRECISION: Pipette out 1,2 and 3 ml working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with 0.1N HCL to get solution of concentrations 10, 20 and 30µg/ml resp ectively. Absorbance of the resultant solutions was measured at 275 nm using .1N HCl as blank. Such three studies were performed for three day at 0, 24 and 48hrs interval. The result obtained is summarized 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: 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 HCLThe result obtained is summarized in the table 6:

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.

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.

CONCLUSIONS

A simple and sensitive spectro-photometric method for quantitative determination of Diaveridine in either pure form or in pharmaceutical dosage form was developed.

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 97.63±0.75 and 99.34±0.50 respectively.

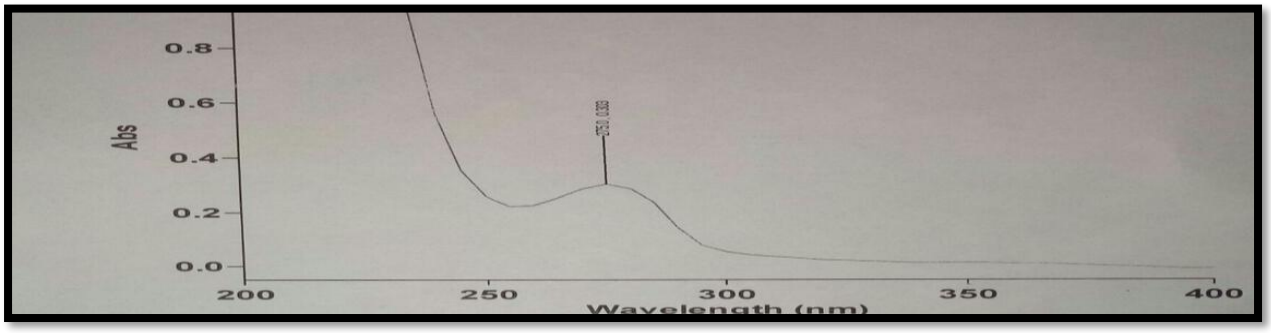

Figure 1:Scan of Diaveridine in the range of 240-400nm

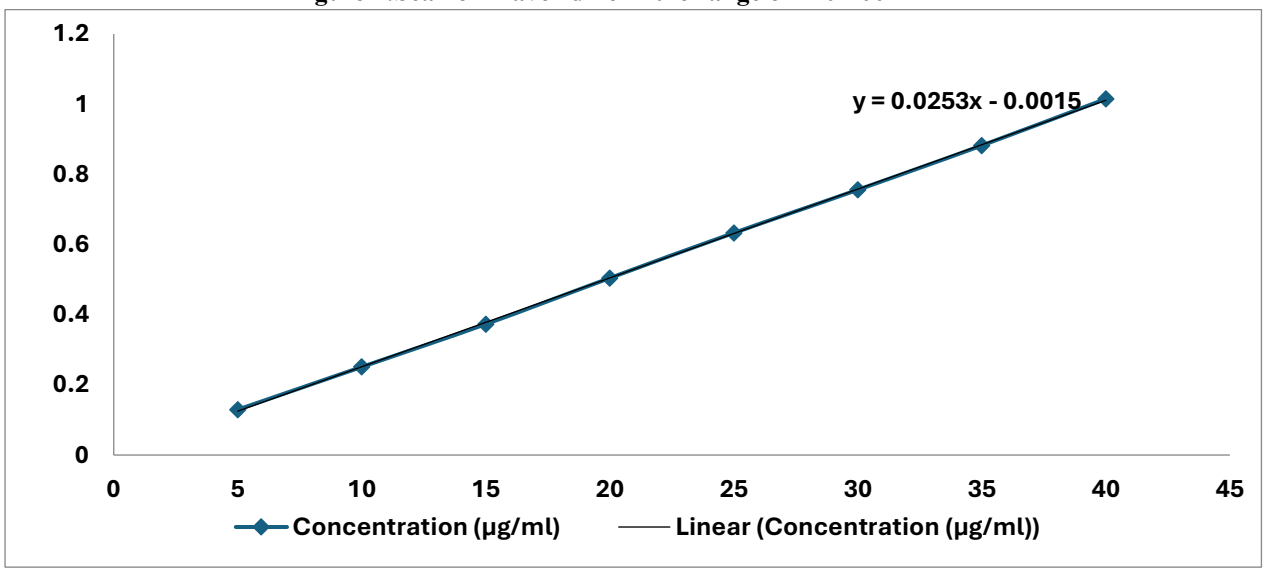

Figure 2: Calibration curve of Diaveridine

Sr. No	Conc (µg/ml)	Absorbance	Absorbance/ Conc. (A/C)	Absorptivity (E(1%,1cm)/10)	Molecular Absorptivity ($1mole^{-1}cm^{-1}$)
1	5	0.1295	0.0259	25.9	6741.69
2	10	0.2511	0.0251	25.11	6536.05
3	15	0.3731	0.0248	24.87	6473.58
4	20	0.5044	0.0252	25.22	6564.69
5	25	0.6330	0.0253	25.32	6590.72
6	30	0.756	0.0252	25.20	6559.48
7	35	0.8822	0.0252	25.20	6559.48
8	40	1.015	0.0253	25.37	6603.73
Mean				**25.73**	**6578.67**

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

Parameters	Observation
Beer's Law Limit(µg/ml)	5-45µg/ml
Molar Absorptivity ($1mole^{-1}cm^{-1}$)	6578.67
Sandell's Sensitivity(µg/cm^2/.001 absorbance unit)	39.5×10^{-3}
Regression equation (y=mx+c) **Slope(m)** **Intercept(c)**	Y=0.0253X-0.0015 0.0253 0.0015
Correlation coefficient(r^2)	0.9999

Optical parameters for Diaveridine

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	10.21 10.24 10.26 10.12 10.28 10.10	10.20	0.0687	0.67

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

Nominal Conc.(µg/ml)	Absorbance			Observed Conc.(µg/ml)			Mean Conc.(µg/ml)	SD	%RSD
	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 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	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 4: Inter-Day Precision 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

TABLE 5: Accuracy 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 6: Specificity values of Diaveridine at ƛmax=275nm.

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 7: Estimation of Diaveridine in pure form.

REFERENCES:

1. Tanabe A, Kondo Y, Toriumi T. Effect of sulfaquinoxaline and diaveridine on leucocytozoon infections in chickens. 1985 Dec;86 (6): 411-5.
2. Yoshimura H. Mutagenicity of the coccidiostatdiaveridine in the Salmonella

microsome assay. Mutat Res 261:149-52 (1991).

3. Diaveridine[USAN:INN:BAN]https://chem.nlm.nih.gov /chemidplus/sid/0005355168.
4. Ono T, Sekiya T, et al. The genotoxicity of diaveridine and trimethoprim. Environ ToxicolPharmacol. 1997 Sep;3(4):297-306. PMID:21781790.
5. Risk MS, Mohamed GG, Tamam AA. Spectrophotometric determination ogdistigmine bromide, diaveridinehcl and tetrahydrozolinehcl with DDQ reagent. Int.J.Res.Pharm.Chem. 2013;3:168-183.
6. Gehad G Mohamed, EmanYousry Frag. Spectrophotometric Determination of Distigmine Br, CyclopentolateHCl and DiaveridineHCl via charge Transfer Complex with TCNQ & TCNE reagents. Iran J Pharm Res 2015;14(3):701-14.
7. Haoshiluo, yingli, Chong Zhang. Simultaneous determination of trimethoprim and Diaveridinein Tissues of chicken, and fish by hydrophilic interaction Liquid Chromatography- Tandem Mass spectroscopy. Food analytical Methods 2013 May 24.
8. Yunpyo Kang, Jin Yu, Yoonyoung Huh. Development of high performance liquid chromatography-ultraviolet detection method for screening mebendazole, diaveridine, and tolfenamic acid in animal based food samples.2013 april10.
9. Yongjun Li, shijin Bu. Determination of sulphachlorpyrazine-diaveridine residues by high performance liquid chromatography in broiler edible tissues. Journal of Veterinary Medical science 77(12) July 2015.
10. Text on validation of Analytical Procedures Q2 [R1] in I.C.H. Harmonised Tripartite guidelines.

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