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Validated UV-spectrophotometric estimation of diclazurilin API and drug product

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ABSTRACT

Three simple, precise, accurate, and economical UV spectrophotometric methods have been developed and validated for the routine estimation of diclazuril in active pharmaceutical ingredient (API) and drug product. Diclazuril was estimated at 286.2 nm in UV-spectroscopic method (Method A), 260 nm in first order derivative spectroscopy (Method B) and scanned at 300 – 273 nm in Area Under Curve method (Method C).Linearity range was found to be 2 -22 µg/ml (Correlation coefficient r^2 = 0.9996 in method A, r^2 = 0.09997 in method B and r^2 = 0.9995 in method C) in all three methods. The molar absorptivity was found to be 1.6x10^4 Lmol^{-1} cm^{-1} in method A, 2.038x10^3 Lmol^{-1} cm^{-1} in method B, and 5.5x10^4 Lmol^{-1} cm^{-1} in method C. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Diclazuril in drug product (powder). The results demonstrated that the procedure is accurate, precise and reproducible (% relative standard deviation < 1%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Diclazuril in powder dosage forms.

Keywords: Diclazuril, UV spectrophotometric, first order derivative spectroscopy, Area Under Curve (AUC).

INTRODUCTION

Diclazuril for veterinary use, chemically is (*RS*) - (4-Chlorophenyl) [2, 6-dichloro-4-(3, 5-dioxo-4, 5-dihydro-1, 2, 4,-trizin-2-(3*H*) phenyl] acetonitrile (Fig. 1) [1, 2].Diclazuril is an antiprotozoal that has been tried in AIDS patients for the management of diarrhea associated with protozoal infection. It is used in veterinary practice for the control of coccdiosis in lambs and poultry[3], there are few methods for determination of Diclazuril in animal plasma, animal feed and eggs. In literature survey analytical methods includes liquid chromatography-tandem mass spectrometry[4, 5], gas chromatography- mass spectrometry [6] and liquid chromatography [7-9] in drug substance and drug products. But, chromatographic techniques are time consuming, costly and require expertise. This paper describes a UV-Spectrophotometric, Frist Order Derivative and UAC methods for estimation of Diclazuril. In present study, simple, economical, accurate, reproducible analytical methods with better detection range for estimation of Diclazuril in API and drug products were developed. Both these developed methods were validated as per ICH guidelines [10].

MATERIALS AND METHODS

Experimental procedures:
Instrument
SHIMADZU UV-2450 PC Series Spectrophotometer (Tokyo – Japan) with two matched 1 cm quartz cells using the following spectral parameters: Scan mode: absorbance, Speed: fast and Slit width: 2 nm

Materials

Diclazuril (Batch no: 61613010)was kindly supplied by Changzhou Yabba- QH Pharmachem Colt(Ponds Co.) and assayed for purity according to the official potentiometric titration[1,2]to contain 99.80%±0.19%.Primazuril 0.2% powder B.N. 13015, each one gram was labeled to contain 2.0mg of Diclazurilmanufactured by Pharm Cure Pharmaceutical Industries for PRIMA VET.Curacoxin 0.5% B. N. 2014/1, each one gram contain 5.0 mg of Diclazuril manufactured by Arabcomed Co. N, N Dimethylformamide, laboratory reagent grade, FissionsScientific Equipment, Bishop Meadow, Southborough, LE 11 ORG, England, Code: D/3840/15 Lot: 1127285.

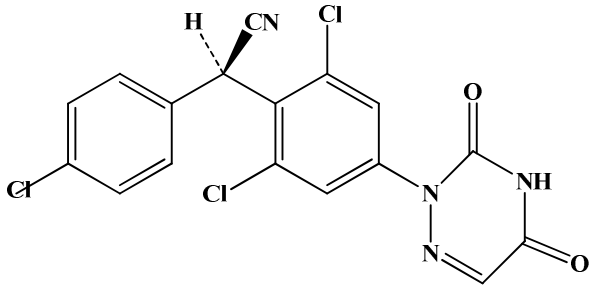


Fig.1: Diclazuril

Analytical method development

Different solvents were investigated to develop suitable UV-spectrophotometric methods for the analysis of Diclazuril dosage form. Diclazuril practically insoluble in water, alcohol, and methylene chloride, sparingly soluble in dimethylformamide. For the selection of solvent the critical employed were sensitivity of the method, ease of sample preparation, solubility of the drug, cost of the solvent and applicability of the method to various purposes. Absorbance of the Diclazuril in selected solvent at respective wavelength was determined and molar absorptivity and Sandal's sensitivity was calculated according to the standard formulae (Table 1).

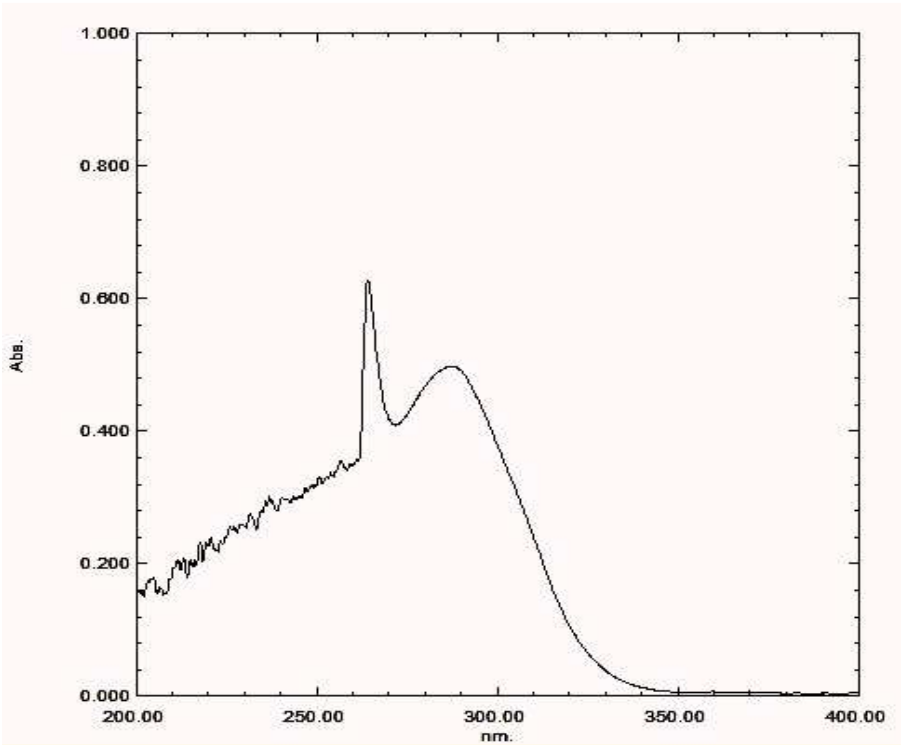


Fig.2: zero- order spectrum of 10 µg/ml diclazuril at λ_{max}286.2 nm in dimethylformamide

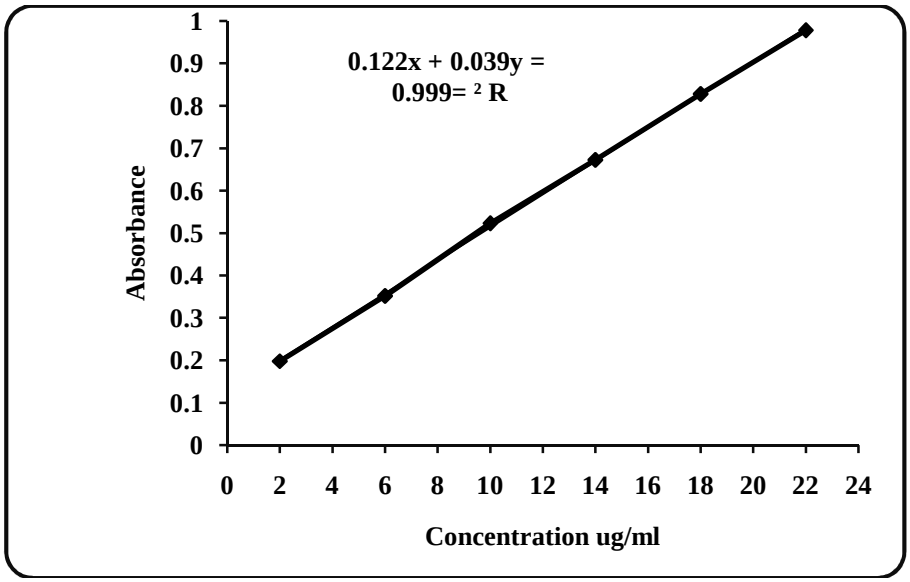
Calibration standards

Accurately about 100 mg of the drug substance was weighed and dissolved in dimethylformamide and the volume is made up to the volume 100 ml to give standard stock solution (1000µg/ml) and from this solution 10 ml of sample was transferred in to separated 100 ml volumetric flask and volume was made up to the mark 100 ml with dimethylformamide to get concentration 100µg/ml as a second stock. Aliquots of standard stock solution were

pipetted out and suitably with dimethylformamide to get the final concentration of 2,4,6,8,10,12,14,16,18,20, and 22 µg/ml of standard stock solution.

UV-Spectrophotometric method (Method A)

For the selection of analytical wavelength, 10µg/ml working standard solution was prepared by appropriate dilution of standard stock solution and blank solution in dimethylformamide was used. This solution was scanned in the spectrum mode from 400 nm to 200 nm. From the spectra of drug (Fig. 2) λ_{max} of Diclazuril 286.2 nm was selected for the analysis. A calibration curve was plotted by taken the absorbance against the concentration of standard stock solutions (Graph 1). By using the calibration curve, the concentration of sample solution can be determined.



Graph 1: UV calibration curve of diclazuril

First order derivative spectroscopy (Method B)

In this method, 10µg/ml working standard solution was prepared by appropriate dilution of standard stock solution in dimethylformamide and it used as blank solution. This solution was scanned from 400nm to 200nm wavelength ranges and the first order derivative spectra were obtained at n= 1, a sharp peak at 260 nm (Fig.3). The absorbance difference at n=1 (dA /dλ) was calculated, which is directly proportional to the concentration of the standard stock solution. A calibration curve was plotted by taking the absorbance difference (dA /dλ) against the concentration of standard stock solutions (Graph 2). By using the calibration curve, the concentration of the sample solution can be determined.

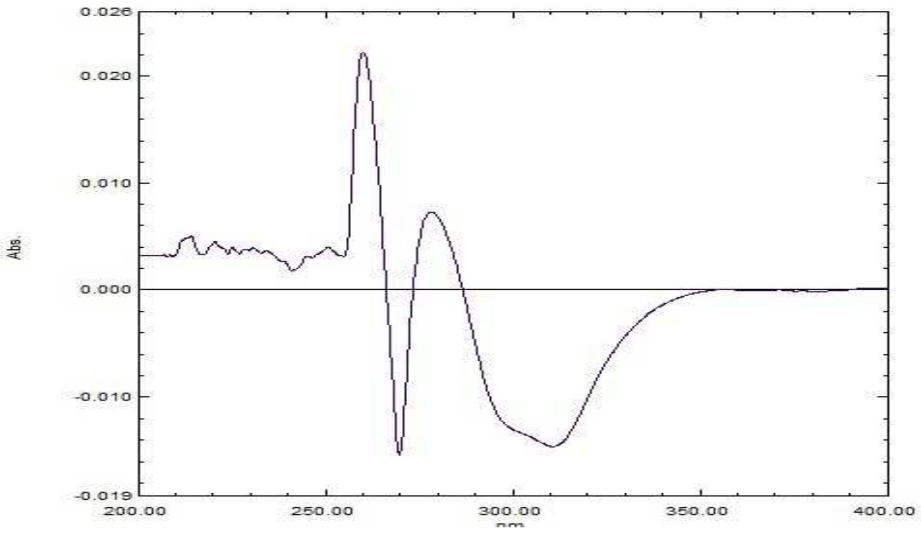
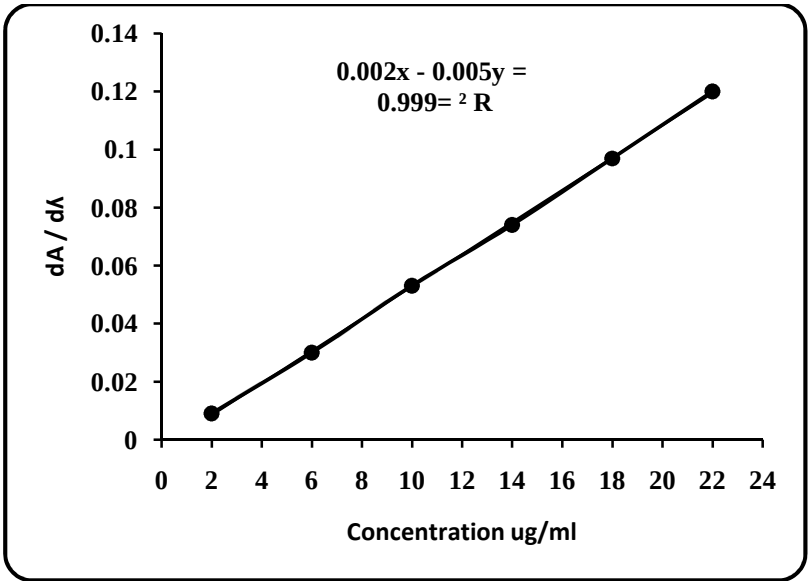


Fig.3: first derivative spectrum of 10 µg/ml diclazuril showing amplitude at 260 nm in dimethylformamide



Graph 2: Frist derivative calibration curve of diclazuril

Area Under Curve method (Method C)

This method is applicable when there is no sharp or when broad spectra obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve [11] and concentration. For the selection of analytical wavelength, 10µg/ml working standard solution was prepared by appropriate dilution of standard stock solution in dimethylformamide and it used as blank solution. This solution was scanned in the spectrum mode from 400nm to 200nm (Fig.4). From the spectra of drug, area under curve in the range of 300 – 273nm was selected for the analysis. The calibration curve was plotted as AUC against concentration of standard stock solution (Graph 3). By using the calibration curve, the concentration of the sample solution can be determined.

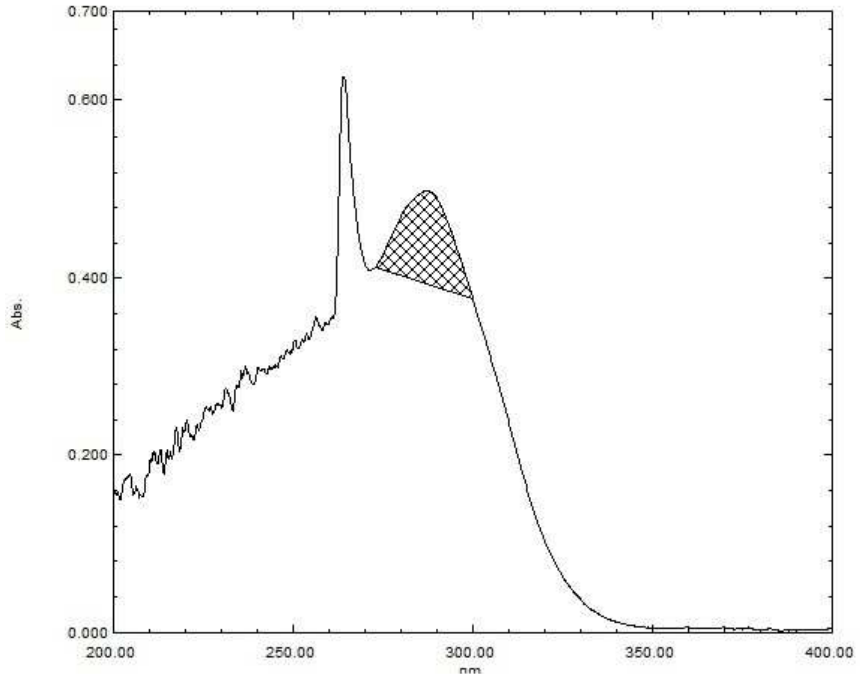
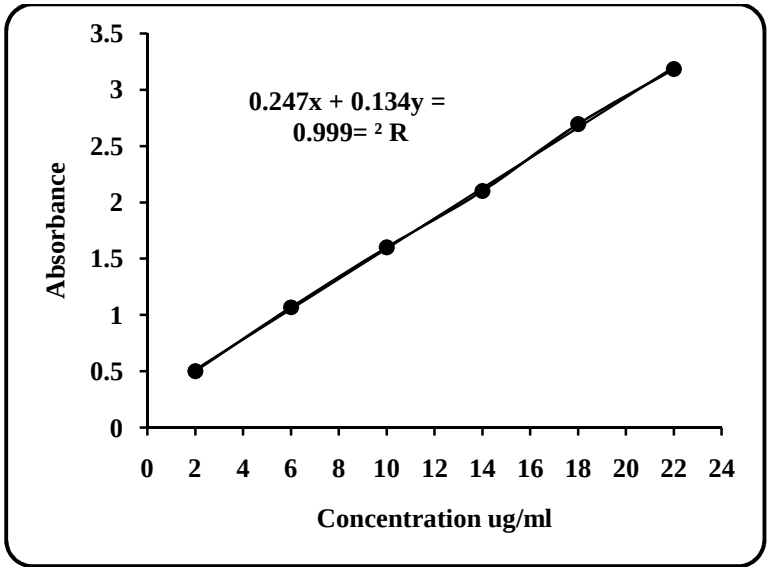


Fig.4: Area under curve spectrum of 10 µg/ml diclazuril showing at 300nm – 273nm in dimethylformamide



Graph 3: Area under curve calibration curve of diclazuril

Analytical Validations

Specificity and Selectivity

Diclazuril solutions (10µg/ml) were prepared in dimethylformamide along with and without common excipients [aerosol and lactose monohydrate].All the solutions were scanned in the range 400-200 nm and checked for in absorbance at respective wavelength. In a separate study, drug concentration of 10µg/ml was prepared independently from pure drug stock and commercial sample stock in selected media and analyzed (N=6) t-test at 95% level was performed to compare the means absorbance (Table 1).

Table 1: Optical characteristics, statistical data of the correlation and validation parameters for Diclazuril by UV spectroscopy, first order derivative & AUC method

Parameters	Method A	Method B	Method C
ƛ max	286.2 nm	260.0 nm	300.0 – 273.0 nm
Molar absorptivity (L mol^{-1} cm^{-1})	$1.6x10^4$	$2.038x\ 10^3$	$5.5x10^4$
Sandal sensitivity (µcm^{-2} /0.001 abs unit)	$2.20x10^{-3}$	$1.83x10^{-3}$	$6.90x10^{-3}$
Regression equation (Y= a+ bc)			
Slope (b)	0.039	0.005	0.135
SD of slope	0.000478	0.00447	0.00234
S E of slope	0.000368	0.00436	0.00153
Confidence limit of slope	0.038087 – 0.040128	0.005492 – 0.005671	0.1300 01 – 0.13849
Intercept (a)	0.122	-0.0027	0.247
SD of Intercept	0.0004821	0.000623	0.08100
S E of Intercept	0.005075	0.000603	0.02133
Confidence limit of Intercept	0.1084 – 0.136579	-0.00444 - -0.00109	0.18849 – 0.30584
Correlation coefficient (r)	0.9996	0.9997	0.9995
SE of (r)	0.00615	0.00073	0.02561
Validation parameters			
Selectivity and Specificity (t-test) (2.228)	0.175	1.15	0.491
Accuracy(Mean ± RSD %)	99.845 ± 0.867 %	99.0 ± 0.957 %	99.916 ± 0.862%
Linearity (µg /ml)	2.0 -22.0 µg /ml	2.0 -22.0 µg /ml	2.0 -22.0 µg /ml
Limit of detection [LOD]	0.825 µg /ml	0.891 µg /ml	0.726 µg /ml
Limit of quantitation [LOQ]	2.500 µg /ml	2.700 µg /ml	2.200 µg /ml
Robustness(mean% recovery ±S.D)	100.11% ± 0.545	100.08% ± 0.416	100.4% ± 0.583

Y = bC + a where C is the concentration of Dicalzuril in µg /ml and Y is absorbance unit.
(Each value is a result of six separate determinations), [c] 95% confidence limit.

Precision

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solution and analyzed (N=6). Inter-day and intra-day variation and instrument variation were taken to determine intermediate precision of the proposed methods (N=6). The % relative standard deviation of the predicted concentrations from the regression equation was taken as precision (Table 2).

Table 2: results of intermediate precision study

Concentration (in µg / ml)	Inter-day repeatability % RSD (N = 6)			Intra-day repeatability(N = 6) Mean ±% RSD	Inter-instrument* Repeatability(N = 6) Mean ± % RSD
	Day 1	Day 2	Day 3		
Method A					
2	0.1783	0.1566	0.1134	100.11± 0.439%	100.06 ± 0.653
12	0.5992	0.3214	0.3188	100.08 ± 0.306 %	100.08 ± 0.257
22	0.9778	0.7210	0.5379	100.23 ± 0.601%	100.04 ± 0.206
Method B					
2	0.009	0.005	0.001	100.21 ± 0.619%	100.10± 0.276%
12	0.064	0.051	0.035	100.16 ± 0.421%	99.98 ± 0.383%
22	0.120	0.110	0.009	100.44 ± 0.472%	99.99 ± 0.299%
Method C					
2	0.500	0.328	0.155	100.00± 0.228%	100.14 ± 0.229%
12	1.850	1.365	0.998	100.15 ± 0.507%	100.12 ± 0.310%
22	3.184	2.367	1.581	100.08 ± 0.285%	100.20± 0.227%

**Instrument 1:SHIMADZU UV-2450 PC Series Spectrophotometer*
Instrument 2: UNICAM UV 300 Spectrophotometer

Accuracy

As a part of determining accuracy of the proposed methods, different levels of drug concentrations were prepared from independent stock solution and analyzed (N=6). Accuracy was assessed as the standard deviation and percentage relative standard deviation studies were found to be satisfactory (Table 1). To give additional support to accuracy of the developed assay method, standard addition method was done. The percent recovery of the added API was calculated as,

$$\% \text{ Recovery} = [(C_y - C_u) / C_a] \text{ X } 100$$

Where C_y is the total drug concentration measured after standard addition, C_u is the drug concentration in formulation and C_a is the drug concentration added to the formulation (Table 3).

Table 3: results of standard addition method

Method	Concentration of drug in formulation (µg /ml)	Concentration of pure drug added (µg /ml)	% level of pure drug added	Total concentration of drug found (µg /ml)	% Analytical recovery ± SD
Method A	10	2	20	11.98	99.77±0.01
	10	12	120	22.12	101.21±0.14
	10	20	200	30.24	102.05±0.01
Method B	10	2	20	12.02	100.23±0.01
	10	12	120	21.90	99.80±0.53
	10	20	200	29.88	99.00±0.07
Method C	10	2	20	12.10	100.25±0.12
	10	12	120	22.21	101.10±0.10
	10	20	200	30.11	102.00±0.04

Linearity

To establish linearity of the proposed method, six separate series of solutions of the drug (2 -22 µg/ml) were prepared from the stock solution and analyzed.

Limit Of Detection (LOD) and Limit Of Quantitation (LOQ)

The LOD and LOQ of the Diclazuril by proposed methods were determined by using calibration standard. LOD and LOQ were calculated as 3.3σ /S and 10σ /S, respectively, where S is slope of the calibration curve and σ is standard deviation of y-intercept of regression equation(Table 1).

Robustness

Robustness of the proposed method was determined by stability of the Diclazuril at room temperature for 8 hours. The different concentrations were prepared in dimethylformamide.Mean percentage recovery was determined(Table 1).

ESTIMATION OF DICLAZURIL FROM DRUG PRODUCT

The validity of the suggested methods was assessed by applying the standard addition technique by adding Diclazuril to the previously analyzed drug products. Statistical comparison of the results obtained by the proposed method with those obtained by HPLC reported method [12-13] showed that the recommended procedures are simpler and sensitive without any loss of accuracy or precision (Table 3, 4).

Table 4: Analysis of Diclazuril in its drug products by the proposed (method A, method B, and method C) methods and compared with the reported HPLC method

Parameter	Primazuril 0.2% powder				Curacoxin 0.5% powder			
	Method A	Method B	Method C	Reported HPLC[12]	Method A	Method B	Method C	Reported HPLC[13]
Mean*	97.2	96.7	95.8	94.5	99.43	99.23	99.05	98.82
± RSD%	0.551	0.611	0.264	0.388	1.11	0.48	0.521	0.609
Variance	0.302	0.373	0.069	0.151	1.23	0.23	0.272	0.372
SE	0.224	0.249	0.107	0.092	0.453	0.272	0.212	0.248
t-test (2.228)[a]	1.23	1.56	1.99		2.1	1.91	1.23	
F-test (5.1)[a]	2.00	2.47	2.19		3.3	1.61	1.36	

** Average of six different experiments. [a]Theoretical values.*

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for the analysis of Diclazuril in its drug product. Absorbance maxima of Diclazuril at 286.2 nm (Method A) and in the first order derivative spectra, sharp peak in 260 nm (Method B) were selected for the analysis. Method C was area under curve (AUC) and the wavelength range for quantitation was 300 – 273 nm. Linearity for detector response was observed in the concentration range of 2 -22 µg/ml for all three methods.Percentage label claim for Diclazuril in powder analysis, by all the three methods, was found to be in the range of 95.80% to 99.43%. Standard deviation and correlation coefficient for powder, by all the methods, was found to be less than ± 1.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percentage analytical recovery for Diclazuril, by all the methods, was found in range of 99.00% to 102.05% values of standard.

CONCLUSION

In summary, the proposed methods were less time consuming, rapid, accurate, precise and inexpensive and can be used for routine analysis of Diclazuril in API and drug products. The sample recoveries in powder and oral suspension were in good agreement with their respective label claims and suggested non-interference of excipients in the estimation of drug products.

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