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Estimation Of Praziquantel In Tablets By UV Visible Spectrophotometry And Its Forced Degradation Studies

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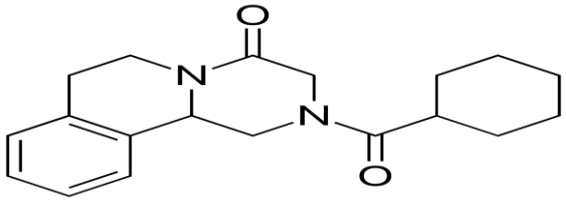
Abstract
The aim of the project is to create a simple and inexpensive UV visible spectrophotometric method that can be validated in accordance with ICH guidelines for the estimation of praziquantel in bulk and tablet dosage form. The technique used the absorbance maxima method, which is based on the measurement of praziquantel's absorbance at 261.8 nm in ethanol. According to ICH guidelines, the method was validated for linearity, precision, accuracy, LOD, and LOQ. For praziquantel, the proposed technique was found to be linear over the concentration range of 10-950 µg/ml. In accordance with ICH guidelines, it was put under various stress conditions. A stability-indicating UV visible spectroscopic method was developed for the analysis of the drug in the presence of the degradation products. It involved a 5-hour study, in which distilled ethanol was used as a solvent. The amount of degraded drug was calculated by taking absorbance at 261.8 nm.

Keywords: Praziquantel, UV visible spectrophotometry[11], Method development, Validation, Stress degradation studies.

Introduction

A drug called praziquantel is used to treat various parasitic worm infections in living things like fish, birds, amphibians, mammals, and amphibians.



Structure of Praziquantel

Method Development

Method Development involves the following steps:

Solvent selection: Solubility studies were conducted with praziquantel with various solvents. It was found that praziquantel was freely soluble in ethanol.

Preparation of standard stock solution: Standard praziquantel of 100mg was weighed, transferred to a 100ml volumetric flask and dissolved in ethanol. The flask was shaken and the volume was made upto the mark with ethanol to give a stock solution of 1000µg/ml.

Determination of maximum absorbance wavelength of 10 µg/ml solution: The stock solution of praziquantel was again diluted with distilled water to get 10 µg/ml concentrations. Absorbance was checked at various wavelengths and it was found that 261.8 nm is the maximum absorbance wavelength[9].

Selection of analytical concentration range of analytes: From the standard stock solution of praziquantel, appropriate aliquots 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 6, 7.5,9, 9.5 ml respectively were pipetted out in 10ml volumetric flasks and makeup with distilled water to obtain working standard solutions of concentrations from 10-950µg/ml. Absorbance for these solutions was measured at 261.8 nm. These concentrations were showing linear values[1].

Construction of Calibration Curve: From standard praziquantel stock solution, concentrations of 150, 300, 450, 600, 750, and 900µg/ml were prepared. The absorbance value of each solution against distilled water was 261.8 nm. From the absorbance values, a calibration curve was constructed. The regression equation and correlation coefficient (R^2) are determined from the experimental results[4,10].

Assay: Twenty tablets were weighed and individual tablet weight was determined. Label claim of praziquantel is 600 mg. Drug equivalent to 100 mg was taken from tablet powder. 100 µg/ml was taken from the tablet stock solution. Its absorbance was noted at 261.8 nm. The amount of drug in the tablet was calculated by the Regression equation method and calibration curve method.The percentage purity was calculated from the amount of drug[5,15].

Method Validation

Validation Parameters: The method validation was performed in terms of linearity, LOQ, LOD, Precision, accuracy, and ruggedness.

a) Linearity: From the standard stock solution of praziquantel, appropriate aliquots of 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,1 ml respectively were pipetted out in 10ml volumetric flasks and make up with distilled water to obtain working standard of concentrations from 8-100µg/ml. Absorbance for these solutions was measured at 261.8nm. These concentrations were showing linear values. From that absorbance value, the Regression equation and correlation coefficient (R^2) are determined[2].

b) Precision: Intra-day and inter-day precision methods are performed. Analyzing the three different concentrations of the drug three times on the same day was performed in intra-day study. Analyzing three different drug concentrations for three days a week was performed in inter-day study. Three different concentrations were LQC (lower quality control), MQC (middle quality control), HQC (Higher quality control)[3].

c) Accuracy: Recovery studies were used to evaluate the proposed method accuracy at three different levels, namely 50%, 100%, and 150%. The pre-analyzed formulation was subjected to recovery tests by adding a known quantity of the drug's standard solution in increasing manner to the sample solution the resulting solutions were then reanalyzed using recommended techniques[7].

d) Limit of detection (LOD): The limit of Detection was found by the formula method[8].

e) Limit of Quantification (LOQ): The limit of quantification was found by the formula method[8].

f) Ruggedness: Absorbance values were taken by two analysts with the same instrument and with the two instruments by the same analyst[14].

Stress degradation studies

Acid hydrolysis

To 15ml of stock solution (100µg/ml) of Praziquantel, 5ml of 0.5 N HCI, and 5 ml of distilled water were added and the prepared solution was refluxed for 30 min. Further required dilutions were made with distilled water and the initial absorbance was checked out then, it was kept for one hour and the absorbance was checked. This procedure was repeated for 2, 3, 4, and 5 hours and absorbance was noted. Distilled water was used as the blank[6].

Base hydrolysis

To the 15ml of stock solution (100µg/ml) of Praziquantel, 5ml of 0.5N NaOH and 5 ml of distilled water were added and the prepared solution was refluxed for 30 min. Further required dilutions were made with distilled water and initial absorbance was checked. Then it was left aside after 1, 2, 3, 4, and 5 hours. The absorbance was checked. For the blank, distilled water was used[12].

Neutral hydrolysis

To 20ml of stock solution (100µg/ml) of Praziquantel, the prepared solution was refluxed for 30 min and further dilutions were made with distilled water and absorbance was checked at 0, 1, 2, 3, 4 and 5 hours. For the blank distilled water was used[13].

Thermal hydrolysis

A Petri plate containing 10 mg of the sample was placed in an oven and set to 70 degrees Celsius for five hours. After 5 hours,1 mg of the sample was diluted with distilled water to a volume of 10 ml (100 µg/ml). From this solution, dilution was carried out to get proper concentration of 10 µg/ml. The solution was taken in a cuvette for the UV-VIS analysis. Distilled water was utilized for the blank.

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Oxidative degradation

To the 15ml of stock solution, (100µg/ml) of Praziquantel, 0.5ml of 30% hydrogen peroxide, and 5ml of distilled water were used and further dilutions were made with distilled water. These solutions were kept at room temperature for one hour then the solution was taken in a cuvette and analysed in UV spectrophotometer at 0, 1, 2, 3, 4, and 5 hours. For the blank, distilled water was used.

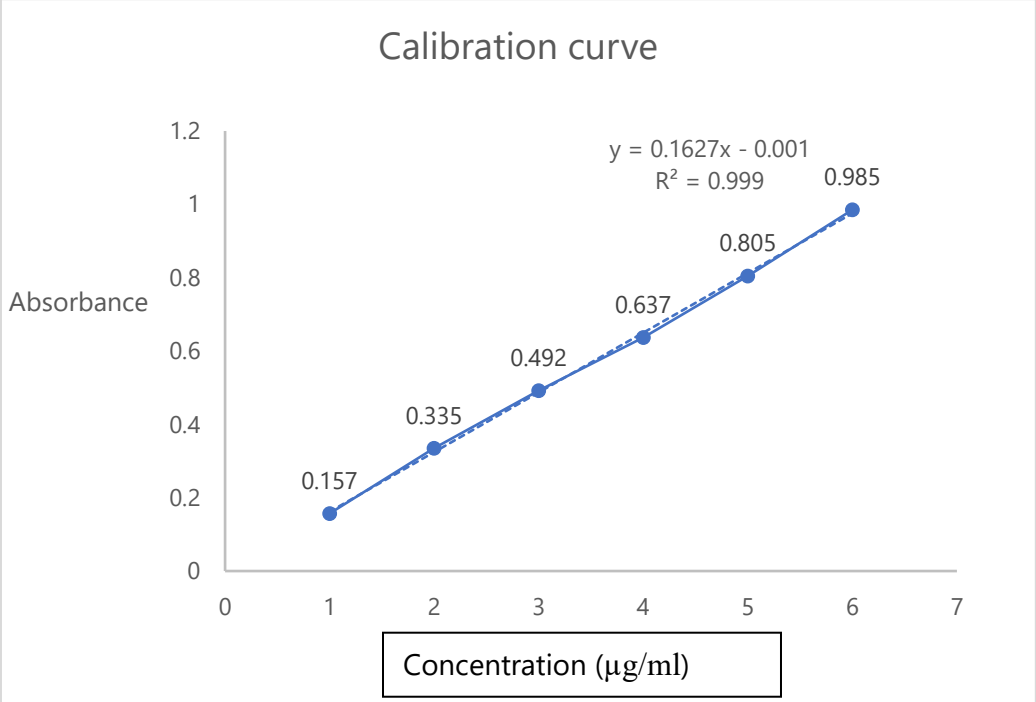
Photolytic degradation

A Petri plate containing 10 mg of the sample was placed outside in sunlight for five hours. After 5 hours, 1 mg of the sample was diluted with distilled water to a volume of 10 ml (100 µg/ml). From this solution dilution was carried out to get proper concentration of 10 µg/ml. The solution was taken in a cuvette for the UV-VIS spectrophotometer analysis. Distilled water was utilised for the blank.

Results And Discussions

Linearity of Praziquantel

S. No	Conc (µg/ml)	Absorbance ±SD	%RSD
1	8	0.0168 ±0.0001	0.5
2	9	0.0189 ± 0.0001	0.5
3	10	0.0212 ± 0.0001	0.4
4	20	0.0350 ± 0.0001	0.33
5	30	0.0520 ± 0.0001	0.1
6	40	0.0580 ± 0.0015	1.6
7	50	0.0725 ± 0.0010	1.4
8	60	0.087 ± 0.00108	1.26
9	70	0.0925 ± 0.00152	1.6
10	80	0.108 ± 0.00109	1.01
11	90	0.1224 ± 0.00118	0.97
12	100	0.136 ± 0.00244	1.8



Calibration curve of Praziquantel

Regression and Analytical parameters

S. No	Parameter	Result
1	Maximum absorbance wavelength (nm)	261.8
2	Molar absorptivity (L/mol.cm)	335.263
3	Linearity Range (µg/ml)	8-950

4	Sandell's sensitivity(µg/cm^2)	0.909
5	LOD (µg/ml)	9.9
6	LOQ (µg/ml)	30
7	Regression equation	Y = 0.0011x-0.001
8	Slope	0.0011
9	Intercept	-0.001
10	Correlation coefficient	0.999

Precision studies of Praziquantel

S. NO	**Sample**	**Intra-day (%RSD)**	**Inter-day (%RSD)**
	Praziquantel		
1	LQC (100 µg/ml)	1.86	1.86
2	MQC (450 µg/ml)	1.42	1.989
3	HQC (900 µg/ml)	1.42	0.957

Recovery Studies[16]

Sl. No	**Name of the drug**	**Amount of Sample (µg/ml)**	**Recovery level**	**Amount of drug added (µg/ml)**	**Total amount found(µg/ml) ± SD**	**% Recovery**	**% RSD**
1	Praziquantel	450	50% 100% 150%	225 450 675	651.84 868.14 1070.67	96.57 96.46 95.17	1.31 0.94 0.27

Assay Studies

Drug	**Label claim**	**Amount found**	**%Recovery**	**%RSD**
praziquantel	600 mg	576.36 mg	96.06	0.84

Ruggedness

S. NO	**Sample**	**Same instrument different analysts (%RSD)**	**Same analyst different Instruments (%RSD)**
	praziquantel		
1	LQC	0.70	1.088
2	MQC	0.9	0.952
3	HQC	0.39	0.48

Degradation studies

Acid degradation

Hours	**Absorbance**	**Concentration (µg/ml)**	**%Degradation**
0	0.109	100	0%
1	0.094	87.05	12.95
2	0.080	73.76	26.24
3	0.045	42.35	57.65
4	0.030	29.05	75.95
5	0.015	15.29	84.71

Base Degradation

Hours	**Absorbance**	**Concentration (µg/ml)**	**%Degradation**
0	0.109	100	0
1	0.095	88.10	14.9
2	0.088	81.020	18.98
3	0.059	55.10	44.9
4	0.032	30.61	69.39
5	0.021	20.40	79.6

Neutral degradation

Hours	Absorbance	Concentration (µg/ml)	%Degradation
0	0.109	100	0
1	0.101	92.92	7.08
2	0.079	81.41	18.59
3	0.078	71.68	28.32
4	0.056	52.21	47.8
5	0.046	43.36	56.64

Photolytic degradation

Hours	Absorbance	Concentration (µg/ml)	%Degradation
0	0.109	100	0
1	0.098	90.45	9.55
2	0.090	82.84	17.15
3	0.070	65.08	34.91
4	0.062	57.47	42.52
5	0.041	38.87	61.12

Thermal degradation

Hours	Absorbance	Concentration (µg/ml)	%Degradation
0	0.109	100	0
1	0.098	90.26	01.74
2	0.050	46.89	52.11
3	0.041	38.92	61.08
4	0.030	28.30	71.70
5	0.024	22.99	77.01

Oxidative Degradation

Hours	Absorbance	Concentration (µg/ml)	%Degradation
0	0.109	100	0
1	0.106	97.34	2.66
2	0.097	89.37	10.63
3	0.088	81.40	18.60
4	0.086	76.97	23.03
5	0.069	63.69	36.30

Conclusion

The proposed technique is easy to use, rapid, linear, accurate, precise, reproducible, and economical. It is useful for figuring out how much praziquantel is in a certain pharmaceutical dosage form.

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