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DISSOLUTION METHOD DEVELOPMENT AND VALIDATION OF EPLERENONE TABLETS BY UV SPECTROPHOTOMETRY

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Abstract:
The article aims at developing simple dissolution method for Eplerenone immediate release tablets by UV spectroscopy and validate as per ICH guidelines. The optimized dissolution method includes 0.1N HCl as dissolution media, apparatus as USP Type 2 Paddle, rpm as 100, temperature of dissolution media as 37±0.5ºC, dissolution volume as 500ml, dissolution time point as 1 hour, working concentration of standard and sample as 10µg/ml and a detection wavelength of 245 nm.The developed method resulted in Eplerenone exhibiting linearity in the range 2.5-20µg/ml. System precision and intra-day precision are exemplified by relative standard deviation of 0.695% and 0.933% respectively. Method was found to be rugged/inter day precise as %RSD was found to be 1.507%. Percentage Mean recovery was found to be in the range of 90-110 % by absolute method during accuracy studies. Hence it can be concluded that effective dissolution method by UV spectroscopy is developed and validated which can be applicable in various pharmaceutical industries.

Keywords: *Eplerenone, Dissolution method, UV, Validation.*

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INTRODUCTION:

The rate and extent at which the amount of drug substance is dissolved over a period of time is called dissolution. It is expressed as percentage release of drug substances present in dosage forms such as tablets, capsules, oral suspensions, transdermal patches, suppositories, semi-solid topical preparations and ointments. It describes about manufacturing reproducibility, product performance similarity and biological availability of drug from its formulation. Therefore, it is considered as one of the most quality control test of solid pharmaceutical dosage forms.

Eplerenone (**Figure 1**) is pregen-4-ene-7,21-dicarboxylic acid, 9,11epoxy-17-hydroxy-3-oxo,γ-lactone, methyl ester (7α,11α,17α)[1,3]. It has a molecular formula of $C_{24}H_{30}O_6$ and a molecular mass of 414.49. Eplerenone is the first highly selective aldosterone receptor antagonist (SARA) to effectively block aldosterone at receptor sites in body tissues, aldosterone being a component of rennin angiotensin aldosterone system [1-6]. Eplerenone is used for treatment of hypertension and heart failure [1-6]. It appears equivalent to spironolactone but is much more expensive [7].

Few analytical methods have been reported for the determination of Eplerenone in various matrices by LCMS [8-9], TLC/Densitometry [1], in bulk and formulations by UV spectroscopy [10-14] and RP-HPLC [3, 15]. UV assay methods were reported using methanol 30% in water, KH_2PO_4 (pH 2.0) and 0.05N HCl as solvent respectively. Our present study aims at developing first dissolution method for Eplerenone immediate release tablets by UV spectrophotometry and validate as per ICH guidelines.

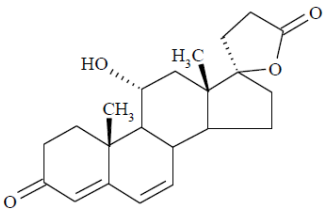

Fig. 1: Structure of Eplerenone

MATERIALS AND METHODS:

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model UV-1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. Dissolution studies were performed on USP Dissolution apparatus (Electrolab, Model: TDT-08L). An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) were used in the study.

Chemicals and Reagents

Analytically pure sample of Eplerenone with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [PLANEP] was procured from Apollo pharmacy, Hyderabad, India with label claim of 25mg. Concentrated HCl was obtained from SD Fine chemicals (Hyderabad, India). 0.22µm nylon membrane filters were purchased from Spincotech Private Limited, Hyderabad, India.

Preliminary Solubility Studies: Solubility studies were explored for Eplerenone in various solvents ranging pH of 1 to 7.5.

1.Distilledwater: 1mg of drug was added to 10ml of distilled water and found to be insoluble even after sonication. Similar solubility procedure was followed using other solvents.

2. Preparation of pH 6.8 buffer as per USP: To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 22.4ml of 0.2M NaOH solution and later made up to the volume with distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.

3. Preparation of pH 4.5 buffer as per USP: 2.99gm of sodium acetate in 1000ml volumetric flask was taken and then was added 14 ml 2N acetic acid solution which was finally made to the volume using water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute acetic acid and sodium hydroxide solutions.

4. Preparation of pH 7.5 Buffer as per USP: To 50ml of mono basic potassium phosphate solution (0.2M, 22.7g/L) in a 200ml volumetric flask, was added 37ml of 0.2M NaOH solution and made up the volume using distilled water, whose pH was checked. If desired pH was not achieved, solution was adjusted to desired pH using dilute phosphoric acid and sodium hydroxide solutions.

5. 0.1N HCl: 8.33 ml of concentrated HCl was made up to 1000ml using distilled water.

It was concluded from the preliminary solubility studies that Eplerenone was found to be freely soluble in 0.1NHCl and hence was taken forward for performing stability studies.

Preparation of Stock and Working Standard Solution

10mg of Eplerenone wasaccurately weighed and taken in 100ml clean and dry volumetric flask containing 80ml ofsolvent (0.1NHCl)and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 1ml of the stock solution was pipettedout and made up to 10 ml to get a concentration 10µg/ml, treated as working standard, 100% target concentration for which UV spectrum was recorded **(Figure 2).**

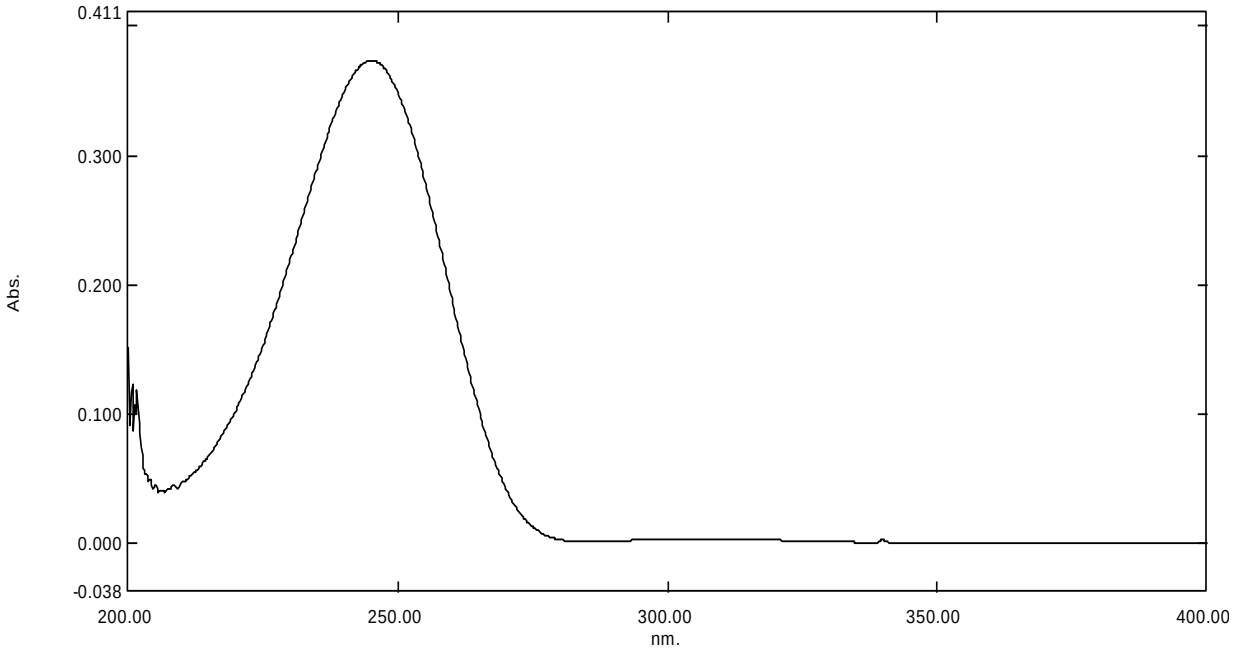

Fig.2: UV Spectrum of Eplerenone Standard

Selection of Wavelength

Suitable wavelength for the analysis was determined by recording UV spectrum in the range of 200-400 nm for 10µg/ml of Eplerenone standard as above and λmax was found to be 245nm and hence 245nm was chosen for the analysis.

Stability Studies: Both standard and sample were studied for stability by UV spectrophotometer at concentration of 10µg/ml and found to be minimum stable for 6 hours at room temperature as percentage degradation was within 2% and accordingly concluded to use this solvent for dissolution studies.

Dissolution Method Conditions: The optimized dissolution method includes the following keeping the acceptance criteria for % drug release (Q value) as greater than 85% at dissolution sampling point (Q point), 60min. Dissolution media volume was considered based on sink conditions where in dissolution media volume should be at least 3 times of saturation volume of the dose in the formulation.

Rpm : 100

Dissolution medium: 0.1N HCl

Dissolution media volume: 500mL

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Apparatus: USP Type 2 (Paddle)

Sampling time point (Q point): 60 min

Sampling volume: 10ml

Temperature: 37±0.5ºC

Working concentration of standard: 10µg/ml

Working concentration of sample: 10µg/ml

Detection wavelength: 245nm

Preparation of Stock and Working Sample Solution

One tablet (dose:25mg) was studied under above dissolution conditions for 60 minutes and dissolution sample volume of 10ml was sampled out and later filtered through 0.22µm nylon filter. First few ml of the filtrate was discarded and then from the filtrate (stock solution of sample), 2 ml was pipetted out and made up to 10ml using 0.1NHCl, to get working sample solution concentration equivalent to 10µg/ml, 100% target concentration as that of standard. UV spectrum of this solution was recorded which is shown in Figure3.

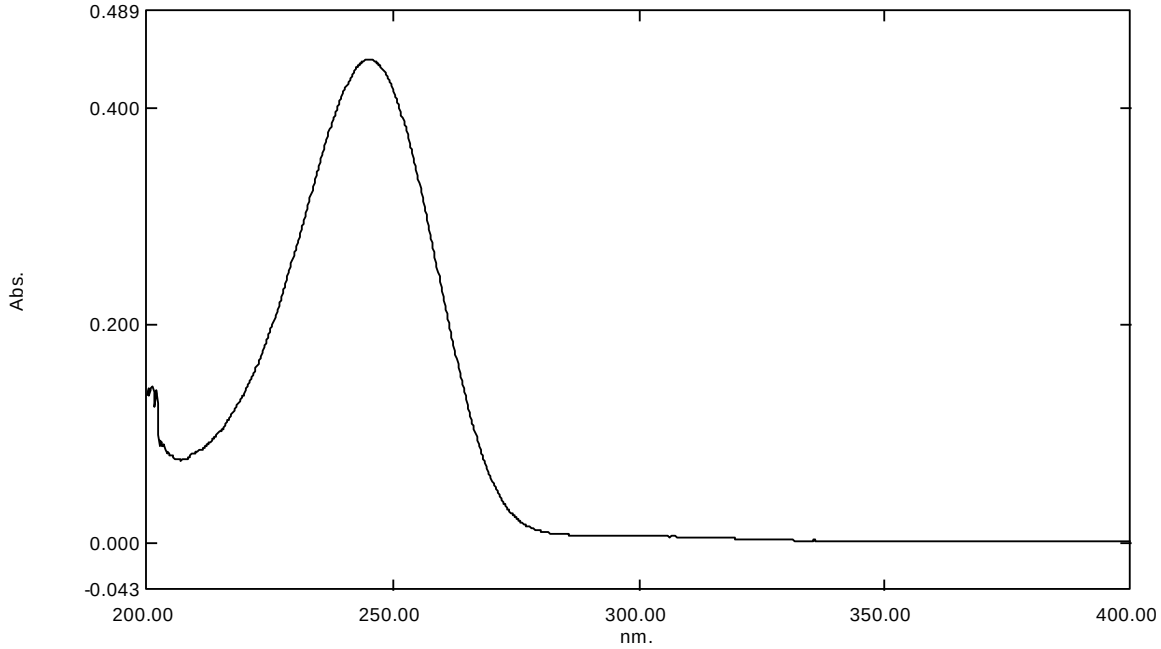

Fig.3: UV spectrum of sample

% Drug release (Q value) was calculated using the formula as below:

$$\frac{\text{Absorbance of sample x Concentration of standard X100}}{\text{Average absorbance of standard x Concentration of sample}}$$

Method Validation:

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application[16]. UV spectrophotometric dissolution method developed was validated according to International Conference on Harmonization (ICH) guidelines. The method was validated for the parameters like specificity, linearity, accuracy, system precision, intra-day precision and inter-day precision / intermediate precision/ruggedness.

Precision

System Precision

Six replicate recording of absorbance at 245 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates method is system precise. System precision results are tabulated below (**Table 1**).

Table 1: System Precision Results of Eplerenone

n	Absorbance
1	0.337
2	0.375
3	0.374
4	0.373
5	0.379
6	0.372
Average	0.375
SD	0.002607
%RSD	0.69538

Method Precision

Method precision was determined by performing dissolution studies of sample under the test of (i) Repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision or ruggedness) performed during 2 consecutive days by two different analysts at working concentration.

Repeatability (Intraday precision)

Repeatability was performed by conducting dissolution studies on six tablets on the same day and recording of absorbance at 245 nm of every dissolution sample at working concentration and calculating % RSD of % drug release at 60 minutes. % drug release was greater than 85 and % RSD was found to be less than 2, which indicate that the dissolution method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (**Table 2**).

Table 2: Intraday Precision Results of Eplerenone

n	Sample absorbance	% Drug release
1	0.4	101.33
2	0.416	105.38
3	0.408	103.36
4	0.421	106.65
5	0.415	105.13
6	0.42	106.4
Average	-	104.708
SD	-	2.024
% RSD	-	1.933

Intermediate Precision (Inter day Precision/Ruggedness)

Dissolution studies were performed on six tablets by different analysts on two consecutive days and % RSD of percentage drug release was calculated and was found to be less than 2, which indicate the method developed is inter day precise/rugged (**Table 3**).

Table 3: Intermediate Precision / Ruggedness results of Eplerenone

n	Day 1 % Drug release	Day 2 % Drug release
1	101.33	93.91
2	105.38	93.05
3	103.36	95.64
4	106.65	93.48
5	105.13	94.56
6	106.4	95.21
Average	104.708	94.3
SD	2.024	1.007758
% RSD	1.933	1.068

Linearity

Standard solutions of Eplerenone at different concentrations level (25%, 50%, 75%, 100%, 125%, 150%, 175% and 200%) were prepared. Calibration curve (**Figure 4**) was constructed by plotting the concentration of drug versus absorbance at 245 nm. The results show an excellent linear relationship between absorbance and concentration of drug within the concentration range of 2.5-20µg/ml (**Table 4**). The correlation coefficient was found to be 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 2.5-20µg/ml.

Accuracy

Accuracy was determined by means of recovery experiments by the determination of % mean recovery of dissolution sample by absolute method at three different levels 50, 100% and 150%. At each level, three determinations were performed. **Table 5** represents percent % mean recovery**.** Individual recovery and % mean recovery was found to be greater than 85% at 60 minutes, which indicates good recovery values and hence the accuracy of the method developed. **Table 6** summarizes the validation parameters about the developed dissolution method.

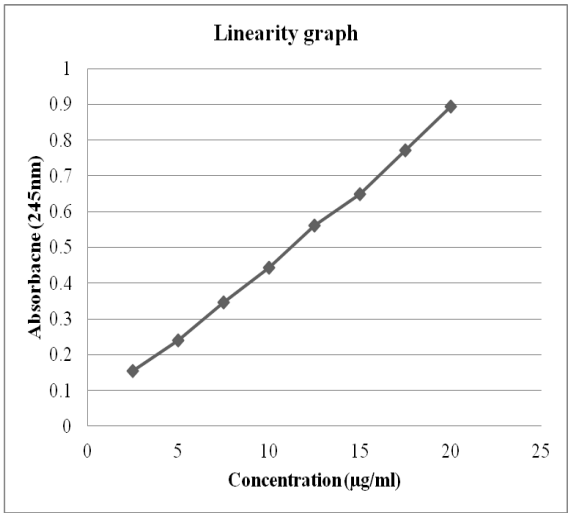

Fig.4: Calibration of Eplerenone

Table 4: Calibration Data for Eplerenone

%Level	pipette out volume from stock (100µg/ml)	Actual concentration (µg/ml)	Absorbance (245nm)
25	0.25ml to 10	2.5	0.154
50	0.5ml to 10	5	0.241
75	0.75ml to 10	7.5	0.347
100	1ml to 10	10	0.443
125	1.25ml to 10	12.5	0.561
150	1.5ml to 10	15	0.65
175	1.75ml to 10	17.5	0.772
200	2ml to 10	20	0.893
Regression Coefficient			**0.999**
Slope (m)			**0.042166667**
Intercept (c)			**0.03325**
Regression Equation			**y=0.0421x+0.03325**

Table 5: Results of Accuracy Studies for Eplerenone

% Level	Sample absorbance	% Recovery	% Mean Recovery
50	0.228	99.58	**96.086**
50	0.217	94.78	
50	0.215	93.9	
100	0.442	96.52	**95.43**
100	0.432	94.34	
100	0.437	95.43	
150	0.647	93.25	**95.55**
150	0.661	95.27	
150	0.681	98.15	

Table 6: Optical Characteristics and Validation Parameters of Eplerenone

Parameters	Results
Detection wavelength (nm)	245
Beer's Law limits (µg/ml)	2.5-20
Regression equation (y = mx+c)	y=0.04216x+0.03325
Correlation coefficient	0.999
Slope (m)	0.0421
Intercept (c)	0.03325
(% RSD) System precision	0.695
(% RSD) Intra-day precision	1.933
(% RSD) Inter-day precision	1.06
Accuracy (% Mean Recovery)	
50 % Level	96.086
100 % Level	95.43
150 % Level	95.55

Specificity

Blank (0.1N HCl) had zero absorbance at all wavelengths from 200-400nm while standard solution exhibited UV spectrum, hence the method is said to be specific for the analyte of interest.

CONCLUSION:

A simple dissolution method by UV spectrophotometry method was developed and validated for the estimation of Eplerenone in immediate release tablets as per ICH guidelines. The optimized method uses 0.1N HCl as a solvent and dissolution medium, and detection wavelength of 245 nm. The developed method resulted in Eplerenone exhibiting linearity in the range 2.5-20µg/ml. System precision and intra-day precision are exemplified by relative standard deviation of 0.695% and1.933% respectively. Method was found to be rugged as precision was found to be 1.068%. Accordingly it is concluded that the developed dissolution method by UV spectrophotometry is simple, accurate, precise, linear and rugged and therefore the method can be employed for the routine dissolution analysis of Eplerenonein tablets in various pharmaceutical industries.

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