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Research Article

DISSOLUTION METHOD DEVELOPMENT FOR GASTRO RETENTIVE CONTROLLED RELEASE CEPHALAXIN TABLET

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ABSTRACT

Cephalexin is in a group of drugs called cephalosporin antibiotics and is used to fight bacteria in the body. It works by interfering with the bacteria's cell wall formation, causing it to rupture, and killing the bacteria. A controlled release tablet dissolution method was developed for evaluation of Cephalaxin CR tablet & determination of Cephalaxin is done by UV spectrophotometer. The solubility and stability of the cephalexin API was determined in ten different solutions. In that 0.1N HCl, glycine buffer pH 3.0, acetate buffer pH 4.5 and water gave good stability and the solubility. Dissolution profiling of cephalexin gastroretentive controlled release (GR CR of single batch was done with the selected media containing varying concentration of surfactants (tween 80 and Sodium lauryl sulphate- SLS). The release profile is compared with that of the control media. The media that gave discriminately faster release than that of the control were found to be 0.1N HCl with 0.75% of tween 80, With the selected media, dissolution profile was done on the three different batches of cephalexin GR CR tablets one with lesser polymer ratio and other with higher polymer ratio that that of the test batch. Only 0.1N HCl with 0.75% of tween 80 as dissolution medium was found to show good discrimination in the release profile with change in the formulation conditions. The discriminative dissolution method developed was validated for its specificity, accuracy, stability, linearity and precision and it passes all parameters.

KEY WORD: UV spectrophotometer, Cephalaxin Monohydrate, gastroretentive mucoadhesive.

INTRODUCTION:

Dissolution[1] is defined as the process by which a solid substance enters in the solvent to yield a solution. Cephalexin is in a group of drugs called cephalosporin antibiotics and is used to fight bacteria in the body. Cephalexin is a first generation cephalosporin antibacterial[2]. It works by interfering with the bacteria's cell wall formation, causing it to rupture, and killing the bacteria[3]. Cephalaxin Monohydrate [Cephalexin is (7*R*)-3-methyl-7-(α-D phenylglycylamino)-3- cephem-4-carboxylic acid monohydrate]. Cephalaxin Monohydrate is official in Indian Pharmacopoeia and the dissolution medium is water and method of analysis of Tablet is carried out by High Performance Liquid Chromatography [HPLC][4].

Other publication is of CPH – Hydrocloride salts, and its dissolution is analyzed by UV spectrophotometer and dissolution media is Water. Reviewing the literatures reveled that [proposed dissolution method using 1.2 pH HCL as medium and determination of content by UV spectrophotometer[5].

The selectivity and accurancy of spectrophotometer analysis of dissolution samples containing absorbing substance may be markedly improved by the technique of UV spectrophotometer. The essential feature of UV spectrophotometric assay is that the measured value of absorbance in equimolar solutions of analyte in dissolution medium and estimated of its content at its wavelength maximum. The purpose of applying UV spectrophotometry to the assay of an anayte is to facilitate faster analysis of the analyte at different time intervals in dissolution method at the analytical wavelength in suitable medium[5].

The commonly employed technique for analyzing the dissolution samples is by means of UV spectrophotometry. The Ultrvoilet–visible absorption spectra of many substances containing ionizable functional groups which are dependent on the state of ionization of that particular functional group and on the pH of the solution. At a particular pH, an ionizable substance has a characteristic absorption spectrum. Active ingredients do not show a significant absorption because of their poor solubility and lesser ionizing capacity[6].

The present method describes the development of new dissolution method using 1.2 pH Hcl as medium followed by determination of Cephalaxin Monohydrate content in the tablet by UV spectrophotometer[7]. This method was based on the UV Spectrum of Cephalaxin Monohydrate in 1.2 pH HCl at its absorption maximum 260 nm. The experimental condition like rpm, time intervals, medium & buffer volume and dissolution apparatus were optimized.

MATERIAL AND METHODS:

Jasco Double beam UV-Spectophotometer (Jascco V 630, Japan.) with 1 cm quartz cell, Electrolab Dissolution testing apparatus & pH meter.

REAGENTS:

0.1M Hydrochloric Acid, pH 4.0 Acetate Buffer, pH 6.8 Phosphate Buffer, pH 7.2 Phosphate Buffer, Cephalaxin Monohydrate, Purified Water & Calibrated Volumetric Glasswares.

SOLUBILITY CHARACTERIZATION:

The pH solubility profile of Cephalaxin Monohydrate at room Temperature was determined. Excess Raw Material was suspended in 10 ml of series of buffers adjusted with different pH and the suspension was equilibrated by shaking in a water bath for 12 hrs. at designated temperature. Aliquots were withdrawn and filtered through 0.45 µm hydrophilic filters. The filtered solution was diluted to the working concentration and analyzed by official HPLC method. The solubility of Cephalaxin Monohydrate in presence of excipients was also in various buffer at 37±0.5^{0}C using the above procedure for their quantification by HPLC. It was revealed that solubility of Cephalaxin Monohydrate were very high in Acidic pH, in comparison with other pH buffers.

SELECTION Of DISCRIMINATIVE MEDIA:

By comparing the dissolution profile of the different media, four media were selected for discriminative dissolution study. Comparative dissolution profiling of Cephalaxin tablets was done (three different formulations)

in the selected media and from this the media giving best discrimination is taken. Validation of discriminative dissolution method was carried out.

DETERMINATION OF QUANTITATIVE PH DEPENDENT SATURATION SOLUBILITY:

Totally 10 solutions have been selected for evaluation of solubility of cephalexin. They are 0.1N HCl (pH 1.00), 0.07N HCl (pH 1.25), 0.01N HCl (pH 1.75), Simulated gastric fluid (SGF pH 2.10), Glycine buffer (pH 3.00), Acetate buffer (pH 4.50), Water (pH 5.30), Phosphate Buffer (pH 6.80), Simulated Intestinal fluid (SIF pH 6.80), Phosphate buffer (pH 7.20).

PREPARATION OF STANDARD SOLUTION:

100 mg of cephalexin working standard is dissolved and diluted to100 ml with the media in which solubility is studied. From this 5 ml is withdrawn and diluted to 250 ml with same media.

PREPARATION OF TEST SOLUTION:

Two conical flasks each containing 100 ml of the media were taken. To both the flask known amount of API of Cephalexin was added and sonicated for 30minutes. Then the sample was visually inspected for the separation of solution. If the saturation of the solution did not occur then addition quantity of API was added and the sonication was further continued. This procedure was repeated till the solution shows a sign of turbidity or opalescence which indicated the saturation point. Then the solution was filtered through 0.45µ nylon filter. The filtrate was diluted arbitrarily to match with standard concentration. This is considered as test solution. The same procedure was carried out for all the test solution. The absorbance at the λmax of 260 nm was measured for both the test and the standard solution. The quantitative pH dependent solubility of the API was calculated.

THE STUDY OF DISSOLUTION PARAMETERS:

Dissolution Parameters such as Medium Volume, rpm, time intervals and USP apparatus have been evaluated. Four different methods have been adopted to conduct using single and multiple vessel dissolution apparatus and shown below:

Method I: Volume 900 ml, 50 rpm, 37±0.5^{0}C, time 1 hr, 4 hrs, 8 hrs, 12 hrs. and Paddle Apparatus.

Method II: Volume 900 ml, 100 rpm, 37±0.5^{0}C, time 1 hr, 4 hrs, 8 hrs, 12 hrs. and Paddle Apparatus.

Method III: Volume 900 ml, 50 rpm, 37±0.5^{0}C, time 1 hr, 4 hrs, 8 hrs, 12 hrs. and Basket Apparatus.

Method IV:Volume 900 ml, 50 rpm, 37±0.5^{0}C, time 1 hr, 4 hrs, 8 hrs, 12 hrs. and Basket Apparatus.

Theoretical dissolution profile has been designed depend on pharmacokinetic parameters of drug release, absorption, elimination & reabsorption. This is considered as standard dissolution profile for calucating F1 & F2.The dissolution samples were analyzed by UV spectrophotometer and most suitable dissolution method condition were optimized and shown in Table 1.

PROCEDURE FOR DISSOLUTION OF CEPHALAXIN MONOHYDRATE GR CR TABLET:

Electrolab Dissolution Test Apparatus was set for equilibrium after adjusting dissolution parameters. Tablets are placed in dissolution medium after getting required temperatures. Dissolution testing was run continuously for 24 hrs with intermittent samples withdrawn for successive time intervals of 1 hr, 4 hrs, 8 hrs, 12 hrs, 18 hrs & 24 hrs. The drug content was estimated by UV spectrophotometer against a standard concentration and percent dissolution at 24 hrs [Q Value] and dissolution profile was computed.

STANDARD SOLUTION:

45 mg of cephalexin working standard is dissolved and diluted 100 ml in purified DM water. From this 5 ml is diluted to 100 ml with the media under study.

STANDARD DISSOLUTION PROFILE: (THEORETICAL DISSOLUTION PROFILE)

Depend on Pharmacokinetic properties of drug required therapeutic effective concentration inside the body is around 110 mg. Need to maintain this concentration in body by considering elimination of drug and half life of drug. CPH is absorb completely inside the body, so accordingly dissolution profile will be as per table 2.

TABLE 1: DISSOLUTION PARAMETERS:

Medium	pH 1.2 HCL
Volume	900 ml
Temperature	37±0.5^{0}C
Rpm	50
Apparatus	USP-II, Paddle
Time Intervals	1 hr, 4 hrs, 8 hrs, 12 hrs, 18 hrs & 24 hrs
Q value	24 hrs

TABLE 2: THEORETICAL DISSOLUTION PROFILE

Time in Hrs	Amt of Drug inside body in mg	Percentage Drug Release
0	0.00	0.00
1	110.99	12.90
4	209.06	24.30
8	339.82	39.51
12	470.58	54.71
18	666.72	77.52
24	862.86	100.33

TABLE 3: DETERMINATION OF QUANTITATIVE PH DEPENDENT SATURATION SOLUBILITY

Media	pH of media	Solubility (mg/ml)
0.1N HCl	1	49.35
0.07N HCl	1.25	40.92
0.01N HCl	1.75	17.32
Simulated gastric fluid	2.1	16.12
Glycine buffer	3	14.53
Acetate Buffer	4.5	14.02
Water pH	5.3	12.41
Phosphate Buffer	6.8	18.44
Simulated Intestinal fluid	6.8	21.58
Phosphate Buffer	7.2	17.98

TABLE 4: DETERMINATION OF PH DEPENDENT SOLUTION STABILITY

Media	Stability (% of degradation)									
	0.1N HCl	0.07N HCl	0.01N HCl	SGF	Glycine Buffer	Acetate Buffer	Water	Phosphate buffer	SIF	Phosphate buffer
pH	1	1.25	1.75	2.1	3	4.5	5.3	6.8	6.8	7.2
1hr	0	0	0	--	0	1.5	0	2.1	--	3.1
2hr	0	0	0	0	0	1.5	1.1	4.7	3	4.4
4hr	0	0	0	0	0	1.5	1.5	9.5	9	10.12
6hr	0	0	0	0	0	1.6	2.4	12.5	10	15.41
8hr	0	0	0	0	0	1.7	2.9	15.5	17	18.41
10hr	0	0	0	0	0	2.1	3.1	21.7	25	20.35
12hr	0	0	0	0	0	2.9	3.4	22.5	35	26.57
24 hr	0	0	0	0	0	6	3.5	34.5	0	50.12

TABLE 5: COMPARATIVE DISSOLUTION PROFILING IN SELECTED MEDIA

Times in Hrs.	Percentage release									
	C*	0.1 N HCL	0.1 N HCl + SLS (%)				0.1 N HCL +Tween 80 (%)			
			0.25	0.5	0.75	1	0.25	0.5	0.75	1
1	23.4	11.3	25.4	23.34	25.6	8.3	10.7	16.8	9.3	10.2
2	33.1	17.5	24	27.6	26.2	14.7	11.6	14.7	13.7	15.3
4	39	26.9	34.1	24.8	36.8	23.2	18.1	17.5	21.8	20.5
6	40.7	35.1	39.6	32.7	45.1	30.5	26.8	29.4	29.2	25.6
8	43.6	42.2	46.4	41.4	50.8	37	36.1	38.5	35.6	30.3
10	46.7	47.6	51.0	47.4	57.6	41.5	48.1	48.5	42.3	39.5
12	50.2	53.2	56.4	52.2	65.3	46	53.4	59	47.7	48.9
14	54.1	58.2	62.4	61.1	74.5	51.7	67.8	65.4	52.0	55.3
16	58.5	62.8	66.1	68.9	83.9	54.9	76.7	78.7	60.0	60.1
18	62.8	68	69.5	76.8	88.2	60.8	85.8	88.4	71.2	65.4
20	67.7	72.2	74.5	81.5	92.4	65.6	93.4	94.9	71.0	75.1
22	72.7	77.8	85.7	84.4	96.3	74.5	95.2	95.2	83.4	84.6
24	78.6	82.6	93	88.3	99.1	79.4	98.23	96.2	88.8	94.2

TABLE 6: SYSTEM PRECISION AND METHOD PRECISION OF CEPHALEXIN IN 0.1N HCl WITH 0.75% TWEEN 80

Sr. No.	System precision	Method Precision
	Absorbance	
1	0.4438	93.3
2	0.4403	93.1
3	0.4429	92.6
4	0.4450	92.9
5	0.4414	94.4
6	0.4423	94.5
Average	0.4426	93.47
% RSD	0.380	0.852

TABLE 7: STABILITY OF CEPHALEXIN STANDARD AND TEST SOLUTIONS IN 0.1N HCl WITH 0.75% TWEEN 80

Time (Hrs)	Standard solution		Test solution	
	Absorbance	% of degradation	Absorbance	% of degradation
Initial	0.4465	0	0.6436	0
1	0.4452	0.29	0.6421	0.23
2	0.4451	0.31	0.6415	0.33
4	0.4445	0.45	0.6408	0.44
6	0.4442	0.52	0.6403	0.51
8	0.4436	0.65	0.6395	0.64
10	0.443	0.78	0.639	0.71
12	0.4428	0.83	0.6387	0.76
24	0.44	1.46	0.6381	0.85

TABLE 8: LINEARITY OF CEPHALEXIN IN 0.1N HCl WITH 0.75% TWEEN 80

Conc. In PPM	Absorbance
0	0
1	0.0224
5	0.0957
10	0.1892
20	0.3773
30	0.5422
40	0.745
50	0.9343
60	1.0912
70	1.2800
80	1.4748
100	1.8445
slope	0.0184
Y- Intercept	0.0033
Regression coefficient	0.9998

TABLE 9: ACCURACY OF THE METHOD

Recovery level	Cephalexin in mg(in 0.1N HCl with 0.75% tween 80)				
	Added	Recovered	% recovery	mean	%RSD
80%-1	600.19	595.47	99.21		
80%-2	599.79	595.16	99.23		
80%-3	599.87	597.62	99.62	99.36	0.19
100%-1	749.68	742.52	99.04		
100%-2	749.15	746.82	99.69		
100%-3	749.84	743.65	99.17	99.30	0.28
120%-1	900	902.37	100.26		
120%-2	899.5	906.88	100.82		
120%-3	899.91	906.26	100.71	100.60	0.24

RESULTS AND DISCUSSIONS:

The pH dependent saturation solubility and stability of Cephalexin API was conducted in 10 selected buffer solutions. The results are tabulated in Table 3 and 4. The phosphate buffer pH 6.8, 7.2 and SIF (pH 6.8) have sufficient solubility but their stability is poor as the percentage of degradation is more than 3% within 2 hrs. So these buffers are rejected. The SGF requires a HPLC determination and hence it is found not suitable. Hence, the remaining buffers 0.01N HCl, 0.1 N HCL, 0.07 N HCL glycine buffer, acetate buffer and water were found to be suitable media and they fall well the physiological pH range. Out of these four media 0.1 N HCL was taken as a dissolution media.With 0.1 N HCL dissolution studies were carried out and the results are tabulated in Table 5.

The dissolution method developed by considering media as an important factor for cephalexin GR CR tablets was validated for its specificity, precision, stability, linearity and accuracy. The placebo has not given a peak or absorbance at 260nm. Moreover the sample, standard and the placebo spiked with the standard gave λ_{max} of 260 nm. Hence, this method is specific to cephalexin. The system precision was determined and was found to be good with % RSD of 0.380. The method precision was also good with percentage relative standard deviation of 0.852% and it fell well with the acceptance criteria. The results are given in Table 6.

The cephalexin standard and sample solution in the developed media was found to be stable for about 24 hrs. The results are given in Table 7. The cephalexin standard solution in the discriminative media was found to be linear over a concentration range of 1 to 100 RPM. The slope if the linear line is 0.0184, Y intercept is 0.0033, which shows that line passes through the origin and the regression coefficient is 0.9998 which falls well with in the acceptance criterion. The results were given in Table 8. The range of the method was found good between lower (5µg/ml) with % RSD of 0.95 and higher concentration (425µg/ml) with % RSD of 0.33. These results were given in Table 9.

CONCLUSION:

With apparatus I (basket type) RPM 100, dissolution medium volume 900ml, only 0.1 N HCl with 0.75% tween 80 as dissolution medium was found to show good discrimination in the release profile with change in the formulation condition i.e retardation in profile with decrease in polymer ration and extension in profile with increase in polymer ratio. Thus, a discriminative dissolution method was developed for cephalexin GR CR tablets with 0.1 N HCl with 0.75% tween 80 as the discriminative media. This method was validated for its specificity, accuracy, stability, linearity and precision and it passes all the parameters.

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