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Stabity indicating Dissolution Method Development for Estimation of Paracetamol & Chlorzoxazone in Combine Dosage form

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ABSTRACT

The present work concerns with development and validation of dissolution test for Paracetamol and Chlorzoxazone in combine tablets dosage form using spectrophotometric method. 0.1M HCl (pH 1.0, 900 mL) was used as dissolution medium, using a paddle apparatus, stirring rate was 50 rpm. The percent drug release was determined by UV spectrophotometric method the wavelength selected for analysis are 242.80 nm for Paracetamol and 279.80 nm for Chlorzoxazone from results it can be concluded that the method developed consists in an efficient alternative for assay of this tablets combination. The method was validated to meet requirements for a global regulatory filing which includes validation parameters as linearity, accuracy, precision, ruggedness and robustness which are as per ICH guidelines. In addition, filter suitability and drug stability in medium were demonstrated.

Keywords: Dissolution, Paracetamol, Chlorzoxazone, *In vitro* drug release, Spectrophotometry, Simultaneous equation method, Validation

INTRODUCTION

Paracetamol (PCM) is 4-hydroxyacetanilide it is official in Indian Pharmacopoeia [1]. Paracetamol is centrally and peripherally acting non opioid analgesic and antipyretic property overdose of Paracetamol can lead to hepatic necrosis or renal failure [2]. Chemical structure of PCM is given in Figure 1.

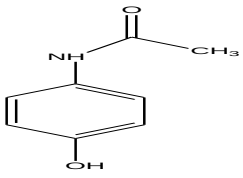

Figure 1: Chemical structure of Paracetamol

Chlorzoxazone is 2(3H)-Benzoxazolone, 5-chloro-5-chloro-2 benzoxazolinone. It is official in United States pharmacopoeia [3]. Chlorzoxazone is non-steroidal Anti-inflammatory drug and used for relief of joint stiffness in patients suffering from rheumatoid arthritis. CHZ also may reduce the release of inflammatory leukotriene [4]. Chemical structure of CHZ is given in Figure 2.

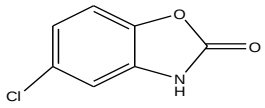

Figure 2: Chemical structure of chlorzoxazone

Various qualitative and quantitative techniques such as spectrophotometry and several method based on separation technique including HPLC have been reported in literature for this drugs alone or in combination with other drugs [5-11]. Therefore here is attempt to develop simple and reproducible dissolution method for analysis of CHZ and PCM by using spectroscopy and this method was validated as per the (ICH) guidelines [12,13]

MATERIALS AND METHODS

Paracetamol was received as a gift samples from Glenmark Pharmaceuticals Ltd. (Goa, India) and Chlorzoxazone was received as a gift samples from Flemingo Pharmaceuticals Nanded, India. The tablets of Paracetamol and Chlorzoxazone combination i.e, Myospaz tablet (PCM 325 mg+ CHZ 250 mg) are purchased from local market.

Instrumentation

Dissolution test was performed by using instrument make from ELECTROLAB (VK7025) Model (TDT-06L) [14]. It was multi-bath (n=6), in accordance to USP Pharmacopoeia.

The medium were vacuum degassed and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visible spectrophotometer (Model: UV 1800, Shimadzu] with a fixed slit width (2 nm) using 1.0 cm quartz cell was used for all absorbance measurements. Elico pH analyzer (Model: Elico 11610) was used to determine the pH of all solutions.

Media selection for stability study

stability study was perform by using four dissolution media such as distilled water, 0.1M HCl, Phosphate buffer pH (6.8), and Acetate buffers pH (5.5) this media are selected as per USP guidelines [United States Pharmacopoeia XXX, 2007]. pH meter (Elico make) was used to adjust pH of the buffers. Stock solutions of both drugs were prepared by dissolving accurately weighed 10 mg of PCM and CHZ in 100 ml of distilled water, 0.1M HCL, Phosphate buffer pH (6.8), and Acetate buffers pH (5.5) separately to obtain 100 µg/ml solutions. All the solutions were sonicated using ultrasonicater to remove air bubbles. From these solutions 1 ml of both drugs was pipette out into 10 ml volumetric flask and diluted with the same solvent system up to the mark to obtain 10 µg/ml solutions. Two sets of 10 µg/ml solutions of PCM and CHZ are prepared and stability was tested in the above prepared dissolution media at room temperature (RT) and 37^{o}C in an incubator (Thermo lab) for 48 h separately. These samples are studied at 0, 24 and 48 h interval by using a double-beam UV-visible spectrophotometer (shimadzu UV1800). The λmax and absorbance value was measured for all the solutions and deviations in the values are recorded which indicates stability in 0.1M HCl respectively. These stable dissolution Media was used for further studies is shown in Tables 1 and 2.

Table 1: Media Selection of PCM

Medium	0 HOUR		24 HOUR		48 HOUR		% CV
	λ max (nm)	Absorbance	λ max (nm)	Absorbance	λ max (nm)	Absorbance	
Distilled water	242.6	0.956	242.6	0.965	242.6	0.947	0.95037
0.1M HCL	242.8	0.774	242.8	0.768	242.8	0.758	3.10323
Buffer (6.8)	243	0.754	243	0.769	243	0.741	1.89095
Acetate Buffer -5.5	243	0.978	243	0.976	243	0.977	0.10235

Table 2: Media Selection of CHZ

Medium	0 HOUR		24 HOUR		48 HOUR		% CV
	λ max (nm)	Absorbance	λ max (nm)	Absorbance	λ max (nm)	Absorbance	
Distilled water	279.8	0.241	279.8	0.252	279.8	0.281	7.35373
0.1M HCL	278.8	0.136	278.8	0.146	278.8	0.142	3.54452
Buffer (6.8)	279.8	0.11	279.8	0.11	279.8	0.12	4.81125
Acetate Buffer -5.5	279.8	0.286	279.8	0.225	279.8	0.345	12.6925

Simultaneous equation method

An accurately weighed quantity of PCM and CHZ (10 mg) each were transferred in 100 ml volumetric flask, dissolved in sufficient quantity of 0.1 N HCL. The volume was made up to the mark with 0.1 N HCL to get the concentration 100 µg/ml. An aliquot (1 ml) of this solution was diluted with 0.1 N HCL in a 10 ml volumetric flask up to mark to get final concentration 10 µg/ml. The standard solution of PCM and CHZ were scanned in the range of 200-400 nm in 1.0 cm cell against 0.1 N HCL using UV spectrophotometer (Shimadzu, Japan) and spectra was recorded to determine the λmax of both the drugs. Figure 3 shows the overlain spectra of PCM and CHZ drugs.

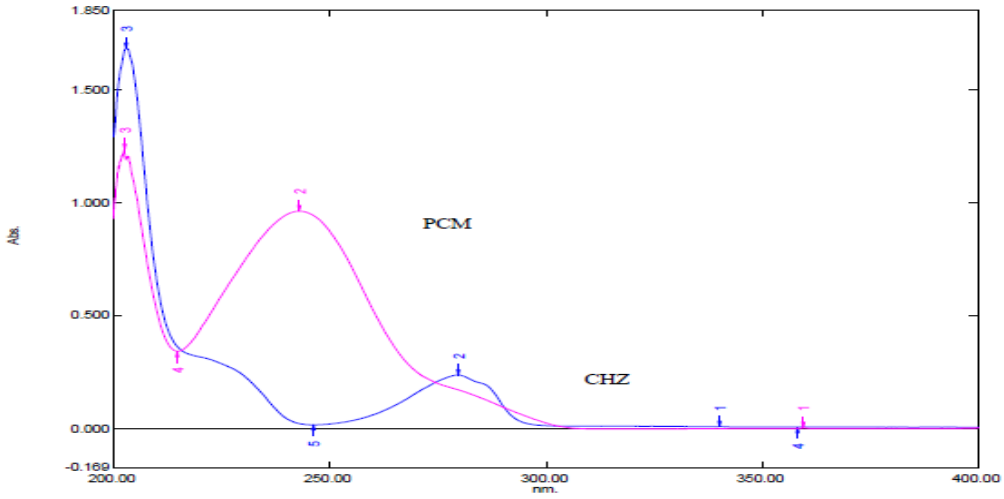

Figure 3: Overlain Spectra of PCM and CHZ

Preparation of standard solutions and calibration curve

From the stock solution of PCM and CHZ (100 µg/ml), sample solutions of PCM were prepared in the concentration range of 2 µg/ml to 12 µg/mland 5 µg/ml to 35 µg/m for CHZ by transferring appropriate volume to 10 ml of volumetric flask and making up the volume with 0.1N HCL.

All dilutions were scanned in wavelength range of 200 nm to 400 nm. The absorbance was plotted against the respective concentrations to obtain the calibration curve of both the drugs. The UV spectra for the linearity of both the drugs are shown in Figures 4, 5 and Tables 3, 4.

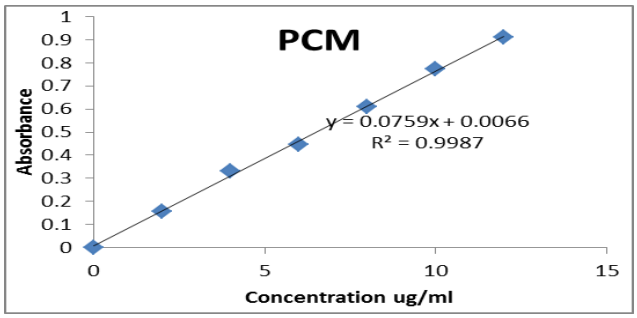

Figure 4: Linearity curve of PCM

Table 3: Calibration curve of PCM

Conc	Absorbance
µg/ml	PCM(λmax=242.80)
2	0.157
4	0.332
6	0.447
8	0.611
10	0.772

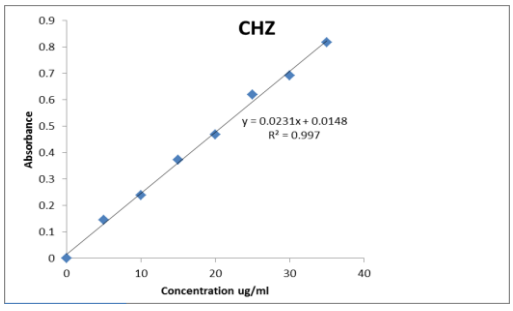

Figure 5: Linearity curve of CHZ

Table 4: Calibration curve of CHZ

Conc.	Absorbance
µg/ml	CHZ(λmax=279.80)
5	0.145
10	0.238
15	0.372
20	0.468
25	0.619
30	0.692
35	0.818

Dissolution study of Paracetamol and chlorzoxazone from tablets using simultaneous equation method

The release of kinetic of Paracetamol and Chlorzoxazone from tablets was studied by conducting dissolution tests. Dissolution tests performed using USP type 2 dissolution apparatus and 900 ml of 0.1N HCL at 37 ± 0.5°C at 50 rpm 10 ml sample were withdrawn at the intervals of 5, 15, 30, 45, 60, 75 min. Sampling was carried out and every time replaced with fresh 10 ml with 0.1N HCL. The absorbance of solution were recorded at 242.80 nm and 279.80 nm using 0.1N HCL as blank. The dissolution studies were performed in triplicate (n=3) Figure 6 and Table 5.

Table 5: Calculation by simultaneous equation method

Sr No.	Sampling	Absorbance		Percentage released (%)	
	Time (Min)	PCM (242.80 nm)	CHZ (279.80 nm)	PCM	CHZ
1	5	0.131	0.045	9	10
2	15	0.275	0.098	20	26
3	30	0.46	0.152	34	43
4	45	0.958	0.256	70	76
5	60	1.335	0.325	99.55	99.08
6	75	1.306	0.32	96	97

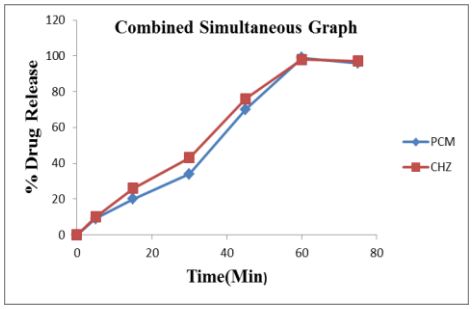

Figure 6: Simultaneous equation Graph

Validation parameters

Validation of the proposed methods was carried out for its linearity & Range, Accuracy, Specificity and Precision according to ICH guidelines.

Linearity

For the determination of linearity, sample solutions of different concentrations were prepared for PCM and CHZ. The absorbance of the above solutions was measured at 242.80 nm and 278.80 nm respectively for PCM and CHZ. Correlation coefficient was calculated by plotting a graph of absorbance *vs.* concentration.

Precision

The precision was determined by studying the intermediate precision and repeatability. The percentage relative standard deviation (%RSD) was calculated. Repeatability to check the degree of repeatability of the methods, suitable sample solutions were prepared and statistical evaluation was carried out. Six times repeatability was performed with tablets formulation is shown in Table 6.

Table 6: Tablets formulation

Method	Mean		Standard deviation		Coefficient of variation		Standard error	
Interday								
	PCM	**CHZ**	**PCM**	**CHZ**	**PCM**	**CHZ**	**PCM**	**CHZ**
	99.07	99.5	0.425	0.189	0.428	0.1902	0.245	0.137
Intraday								
	99.81	99.8	0.775	0.691	0.776	0.6928	0.447	0.398

n = 3, SD = Standard deviation, CV = Coefficient variation, SE = Standard error

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 50, 100 and 150% of the test concentration as per ICH guidelines for both the drugs. The recovery study was performed in triplicate at each level. The result of the recovery studies for the two brands is reported in Tables 1 and 2. The absorbance of the standard solutions of 50%, 100% and 150% at 279.80 nm and 274.05 nm for PCM and CHZ respectively were measured. From this, individual recovery and mean recovery values were calculated is shown in Table 7.

Ruggedness

Ruggedness of the method is determined by carrying out the analysis by two different analysis and the respective dissolution values are calculated Table 8.

Table 7: Individual recovery and mean recovery values

Method	Level of % Recovery	Amt. Present (mcg/tab)		Amt. of standard added (mcg/tab)	Total Amt. Recovered (mcg)	% Recovery		
		PCM	**CHZ**	**PCM**	**PCM**	**CHZ**	**PCM**	**CHZ**
	80	36	28	28.8	64.8	50.4	100.49	99.39
	100	36	28	36	72	56	99.11	99.27
	120	36	28	43.2	79.2	61.6	99.23	99.09

Table 8: Dissolution values

Method	Analyst1		Analyst2	
	PCM	**CHZ**	**PCM**	**CHZ**
	99.13	99.24	99.56	99.29

Mean ± S.D. (n=3)

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in the method parameters. The different parameters studied for analysis was PH, temperature, revolution per minute (R.P.M.) Filters and molarity of HCL. The solution containing 35.6 mcg/ml of PCM and 8.4 mcg/ml were analyzed under different condition as above and results are represented in Tables 9-11.

1. Temperature changes

Table 9: Temperature changes

Temperature	Level	Time of Dissolution(Min)		% Drug Release	
		PCM	CHZ	PCM	CHZ
32	-5	50	50	100.2	99.42
37	0	60	60	99.59	99.55
42	5	90	90	99.41	99.42

2. Molarity of HCL

Table 10: Molarity of HCL

HCL	Level	Amt. Of drug release (µg/ml)		% drug release	
		PCM	CHZ	PCM	CHZ
0.05	-5	35.26	28.11	97.21	97.2
0.1	0	36	28	99.92	99.96
0.5	5	35.88	28.82	98.58	98.84

Mean ± S.D. (n=3) PCM 98.57 ± 1.355028 Mean ± S.D. (n=3) CHZ 98.66 ± 1.38814

3. Filters

Table 11: Filters

Filters	Amt. of drug release		% Release	
	PCM	CHZ	PCM	CHZ
0.2 µm	36.21	28.29	99.92	99.96
0.45 µm	36	28	99.98	99.91
Whatmann	36.56	28.62	99.93	99.98

Mean ± S.D. (n=3) PCM 99.94 ± 0.032146 Mean ± S.D. (n=3) CHZ 99.95 ± 0.036056

RESULTS AND DISCUSSION

Determination of λmax the UV spectra for the linearity of both the drugs (PCM & CHZ) are shown in Figure 3. Beer's law is obeyed in concentration range of 2 to 12 µg/ml for PCM and 5 to 35 µg/ml for CHZ. Calibration curve of PCM and CHZ pure drug is shown in Figure 4 and 5.

CONCLUSION

Dissolution method was developed and validated for Paracetamol and Chlorzoxazone tablets using UV spectroscopy method. This method was validated as per ICH guidelines which include validation parameters such as accuracy, precision, specificity, linearity, and analytical range. Stability and solubility of both the drugs was studied in different media such as water, 0.1M HCL, phosphate buffer, Acetate buffer was studied. For Dissolution study 0.1N HCL was selected as dissolution medium (900 ml) at temperature 37 ± 0.5°C; using USP apparatus II stirring rate of dissolution was 50 rpm for 1 h. Thus, the proposed dissolution method can be applied successfully for the Quality control of Paracetamol and Chlorzoxazone in marketed tablets.

ACKNOWLEDGEMENT

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