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Research Article

STABITY INDICATING DISSOLUTION METHOD DEVELOPMENT FOR ESTIMATION OF PARACETAMOL AND CHLORZOXAZONE IN COMBINE DOSAGE FORM

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ABSTRACT

The aim of this work was to develop dissolution test method for Paracetamol and Chlorzoxazone in combination tablet. The dissolution established conditions were 900 mL of 0.1M HCl pH 1.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The assay was performed by spectrophotometry for the better conditions stirring speed of 50 rpm, is used .Ahead of results it can be concluded that the method developed consists in an efficient alternative for assay of dissolution for tablets. The method was validated to meet requirements for a global regulatory filing which includes linearity, precision, accuracy robustness and ruggedness. In addition, filter suitability and drug stability in medium were demonstrated.

KEYWORDS:*Dissolution study of Paracetamol and Chlorzoxazone, In vitro release, Spectrophotometry, Q-Analysis Method, Validation.*

INTRODUCTION

Paracetamol (PCM) chemically is 4-hydroxyacetanilide[1]. Paracetamol acts by complex and includes the effects of both the peripheral (COX inhibition) and central (COX serotonergic descending neuronal pathway, L-arginine/NO Pathway, cannabinoid system) antinociception processes and redox mechanism [2]. Paracetamol is well tolerated drug and produces few side effects from the gastrointestinal tract. Chemical structure of PCM is given fig .1.

Chlorzoxazone Chemically is 2(3H)-Benzoxazolone,5-chloro-5-chloro-2 benzoxazolinone[3].

Chlorzoxazone acts by inhibiting multi synaptic reflexes involved in producing and maintaining skeletal muscle spasm of varied aetiology.It acts on the spinal cord by depressing reflexes. CHN a synthetic compound, inhibits antigen-induced bronco spasms.CHN inhibits degranulation of mast cells. Subsequently preventing the release of histamine and slow-reacting substance of anaphylaxis(SRS-A), mediators of type-1 allergic reactions. CHZ also may reduce the release of inflammatory leukotrienes[4]. CHZ is given fig.2.

Literature survey revealed that various analytical technique such as spectrophotometric technique [5-8]. Several method based on separation technique including HPLC[9-11], have been reported. The method was validated as per the International Conference on Harmonization (ICH) guidelines [12,13].

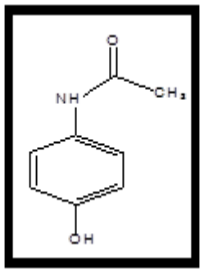

Fig. 1: chemical structure of Paracetamol

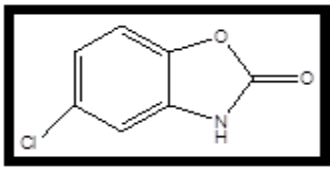

Fig. 2: chemical structure of Chlorzoxazone

MATERIALS AND METHODS

Materials:

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.

Instrumentation:

Dissolution test was performed in a ELECTROLAB (VK7025) Model(TDT-06L)[14]dissolution apparatus, multi-bath (n=6), in accordance to USP Pharmacopoeia generalmethod. The medium were vacuum degassed under in house vacuum and weremaintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visiblespectrophotometer (Model:UV 1800,Shimadzu] with a fixed slit width (2 nm)using1.0 cm quartz cell was used for all absorbance measurements. Elico pH analyzer(Model: Elico 11610) was used to determine the pH of all solutions.

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Q-Analysis Method:

Stability indicating dissolution media selection for dissolution study:

In stability study nine dissolution media were selected and prepared such as distilled water, 0.1M HCl, Phosphate buffer pH (6.8), and Acetate buffers pH (5.5) as per USP guidelines [United States Pharmacopoeia XXX, 2007]. The pH of the buffers was adjusted using Elico make pH meter. Stock solutions of PCM and CHZ were prepared by dissolving accurately weighed 10 mg of both drugs 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 dissolve the drug. From these solutions 1 ml 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°C in an incubator (Thermo lab®) for 48 hrs separately. These samples are studied at 0, 24 and 48 hrs interval by using a double-beam UV-visible spectrophotometer (shimadzu UV1800) connected to UV probe software. 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 Medias are used for further dissolution studies of both the drugs.

Table No.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	242.60	0.956	242.60	0.965	242.60	0.947	0.95037
0.1M HCL	242.80	0.774	242.80	0.768	242.80	0.758	3.103229
Buffer (6.8)	243	0.754	243	0.769	243	0.741	1.890945
Acetate Buffer(5.5)	243	0.978	243	0.976	243	0.977	0.102354

Table No.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	279.80	0.241	279.80	0.252	279.80	0.281	7.353729
0.1M HCL	278.80	0.136	278.80	0.146	278.80	0.142	3.544523
Buffer (6.8)	279.80	0.110	279.80	0.110	279.80	0.120	4.811252
Acetate Buffer (5.5)	279.80	0.286	279.80	0.225	279.80	0.345	12.6925

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 10µg/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 Hclusing 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.

Following the above procedure the absorbance of solution were recorded at 279.80nm(CHZ) and 274.05nm (Isobestic point) by using 0.1N HCL as blank. The dissolution studies were performed triplicate

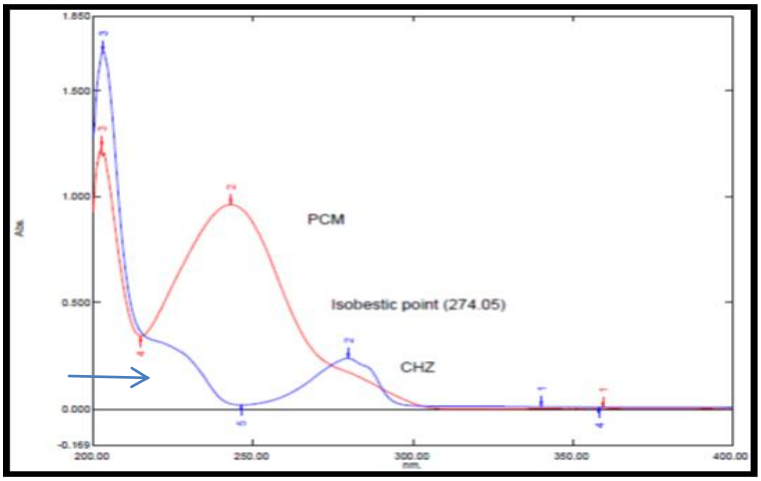

Fig.3: Overlain Spectra of PCM and CHZ

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/ml and 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 Figure 4 and 5. The calibration curves of both the drugs are shown in Figure 4 & 5.

Table No.3: Calibration curve of PCM

Conc (µg/ml)	Absorbance PCM(λmax=274.50)
5	0.099
10	0.202
15	0.309
20	0.406
25	0.498
30	0.611
35	0.691
40	0.812

Table No. 4: Calibration curve of CHZ

Conc. (µg/ml)	Absorbance 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

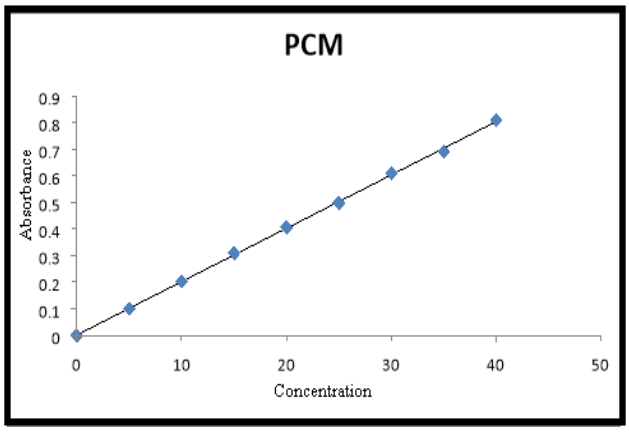

Fig.4: Linearity Curve of PCM

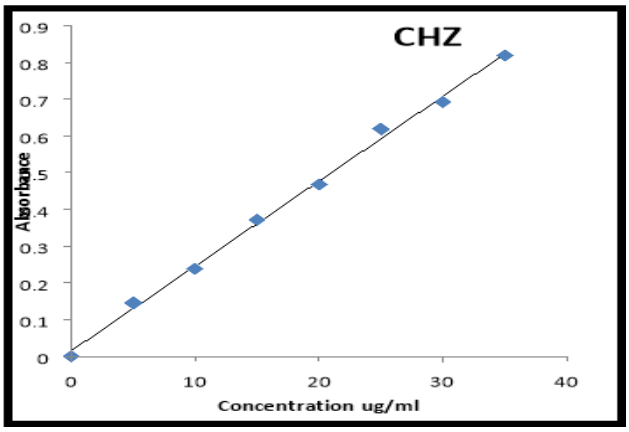

Fig. 5: Linearity Curve of CHZ

Dissolution Study Of Paracetamol and Chlorzoxazone by Q Analysis 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 900ml of 0.1N Hcl at 37± 0.5⁰c at 50rpm 10ml sample were withdrawn at the intervals of 5,15,30,45,60,75min. Sampling was carried out and every time replaced with fresh 10ml with 0.1N Hcl. The absorbance of solution were recorded at 242.80nm and 279.80nm using 0.1N Hcl as blank. The dissolution studies were performed in triplicate(n=3).

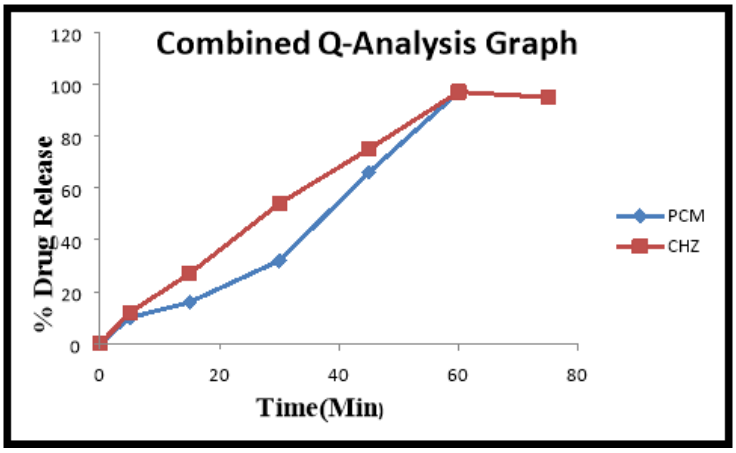

Fig. 6:Combine Q Analysis Graph

Table No.5: Calculation by Q-Analysis method

S. NO.	Sampling Time (Min)	Absorbance		PercentageReleased(%)	
		PCM(274.05nm)	CHZ(279.80nm)	PCM	CHZ
1	5	0.135	0.052	10	12
2	15	0.228	0.101	16	27
3	30	0.442	0.185	32	54
4	45	0.902	0.254	66	75
5	60	1.313	0.322	99.09	99.07
6	75	1.271	0.312	997.84	97.80

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 279.80 nm and 274.05 nm respectively for PCM and CHZ. A graph of absorbance vs. concentration is plotted and correlation coefficient was calculated.

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. Repeatability was performed for six times with tablets formulation.

Table No.6: Data of Precision

Mean		Standard deviation		Coefficient of variation		Standard error	
Interday							
PCM	**CHZ**	**PCM**	**CHZ**	**PCM**	**CHZ**	**PCM**	**CHZ**
99.02	99.0	0.009	0.110	0.009	0.1115	0.005	0.080
Intraday							
99.78	99.6	0.535	0.484	0.536	0.4860	0.308	0.279

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 Table 2 and Table 3. 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.

Ruggedness:

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

Table No.7: Data of Accuracy

Level of % Recovery	Amt. Present (mcg/tab)		Amt. of standard added (mcg/tab)		Total Amt. Recoverd (mcg)		% Recovery	
	PCM	CHZ	PCM	CHZ	PCM	CHZ	PCM	CHZ
80	36	28	28.8	22.4	64.8	50.4	99.64	100.61
100	36	28	36	28	72	56	100.07	100.51
120	36	28	43.2	33.6	79.2	61.6	99.78	98.98

Table No.8: Data of Ruggedness

Analyst1		Analyst2	
PCM	**CHZ**	**PCM**	**CHZ**
100.2	99.99	100.2	99.99

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 35µg/ml for CHZ. Calibration curve of PCM and CHZ pure drug is shown in figure 4 and 5.

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CONCLUSION

Dissolution method was developed and validated for PCM & CHZ tablets using UV spectrophotometric method. The method was validated according to ICH guidelines which include accuracy, precision, specificity, linearity, and analytical range. Stability and solubility of both the drugs in different media i.e., water, 0.1M Hcl, phosphate buffer, Acetate buffer was studied. Dissolution conditions were 900 ml of 0.1N Hcl as dissolution medium at 37±0.5 °C; using USP apparatus II at a stirring rate of 50 rpm for 1hr were selected for study Thus, the proposed dissolution method and analytical method can be applied successfully for the Quality control of PCM and CHZ in marketed tablets.

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