RESEARCH ARTICLE

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Development of Simultaneous Estimation Method for Paracetamol and Chlorzoxazone in Marketed Formulation by Validated UV-Spectrophotometric and RP-HPLC Method

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Abstract: A simple, selective, rapid, precise and economical UV spectrophotometric method and reverse phase high performance liquid chromatography method has been developed for the simultaneous estimation of paracetamol and chlorxazone from pharmaceutical formulation. The primary method was based on UV-spectrophotometric determination of paracetamol and chlorzoxazone, using simultaneous equation method. Its absorbance measurement at 282.5 nm (λ_{max} of chlorzoxazone) and 248.0 nm (λ_{max} of paracetamol) in methanol and the linearity was obtained in the range of 5 – 25 µg/ml for paracetamol and chlorzoxazone. The secondary method was based on RP- HPLC separation of the paracetamol and chlorzoxazone in reverse phase mode. Linearity was obtained in the concentration range of 100-500 µg/ml for paracetamol and 50-250 for the chlorzoxazone. Both these methods have been successively applied to pharmaceutical formulation and were validated according to ICH guidelines. The developed methods are validated in terms of specificity, selectivity, accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability.

INTRODUCTION

It is necessary to find the concept of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage for treatment.

Many such combinations are available in market; one of such formulations is combination of paracetamol (PCM), chlorzoxazone (CHZ) due to their synergistic effects, quick relief, multiple actions, tolerability, and patient acceptance.

Paracetamol (N-(4-hydroxyphenyl) acetamide, is a para-aminophenol derivative Paracetamol is an analgesic-antipyretic agent. It is effective in treating mild to moderate pain such as headache, neuralgia and pain of musculo-skeletal origin. It has weak anti-inflammatory effects.

They are both recognized by the same enzyme, which is called cyclo-oxygenase (COX). Cyclo-oxygenase serves as a pain activator, amplifying the degree of pain experienced in order to let the body know that there is a problem. It is this enzyme that is responsible for the biosynthesis of prostoglandins. By reducing the amount of prostaglandin available for synthesis, paracetamol helps relieve headache pain by reducing the dilation of the blood vessels that cause the pain. [1-3]

Chlorzoxazone (CHZ), (5-chloro-2(3H)-benzoxazolone), It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal disorders. [4, 5]

MATERIALS AND METHODS

Paracetamol and Chlorzoxazone was obtained as gift sample from Win Medicare Pvt. Ltd., Delhi, India. All other chemicals/solvents used were HPLC grade and collected from local Market.

UV- Spectrophotometry Method

Standard stock solutions of 100 µg.ml^{-1} were prepared by dissolving 10 mg of each in 100 ml of methanol. From these stock solutions, working standard solutions having concentration 10 µg.ml^{-1} each were prepared by appropriate dilutions. They were scanned in the wavelength range of 400–200 nm and the overlain spectrum was obtained. Two wavelengths 282.5 nm (λ_{max} of chlorzoxazone) and 248.0 nm (λ_{max} of paracetamol) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration range of 30– 70 µg.ml^{-1}, paracetamol and 20-60 for chlorzoxazone. The absorptivity coefficients of each drug at both wavelengths were determined. [6]

HPLC Method

Stock solution was prepared by dissolving 10 mg of paracetamol and chlorzoxazone in 100 ml volumetric flask with methanol:water in the ratio of 80:20. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. The optimal composition of mobile phase was determined to be TEA buffer:methanol in the ratio of 55:45 v/v. The flow rate was set to 1 ml.min^{-1} and UV detection was carried out at 240 nm.

From the above stock solutions, dilutions were made in the concentration range of 100–500 µg.ml^{-1} of paracetamol and 50-300 chlorzoxazone, respectively. A volume of 20 µl of each sample was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area ratios of analyte to internal standard vs. the corresponding drug concentration.

Analysis of Tablet Formulation

The commercial tablet formulations of paracetamol and chlorzoxazone are in the ratio of 500:250 Based on this fact

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Table 1: Data Analysis of Tablet Formulations

Parameters	UV Spectrophotometry		HPLC	
	Paracetamol	Chlorzoxazone	Paracetamol	Chlorzoxazone
Label Claim	500	250	500	250
*Drug content	99.95	98.40	99.22	99.41
± S. D.	0.039	0.112	0.155	0.154
% R.S.D.	0.127	0.121	0.159	0.156

* Value for Drug content (%) are the mean of five estimations; S.D. is standard deviation and R.S.D. is relative standard deviation

Table 2: Recovery Studies

UV Spectrophotometry			HPLC		
Excess Drug	*Recovery	% R.S.D.	Excess Drug	*Recovery	% R.S.D.
Paracetamol					
80	99.37	0.2953	80	99.37	0.9405
100	99.72	0.2026	100	100.11	0.0115
120	99.51	0.0672	120	100.58	0.9702
Chlorzoxazone					
80	99.99	0.2953	80	100.32	1.1238
100	99.83	0.1036	100	99.33	0.0232
120	99.99	0.1165	120	98.80	1.1243

* Recovery is mean of three estimations

Table 3: Summary of Repeatability, Precision and Ruggedness

Parameter	UV Spectrophotometry		HPLC	
	Paracetamol	Chlorzoxazone	Paracetamol	Chlorzoxazone
Repeatability	1.52	0.09	0.62	0.57
Precision				
Intra-day	1.07	0.13	0.29	0.43
Inter-day	0.17	0.14	0.56	1.55
Ruggedness				
Analyst 1	0.68	0.52	0.37	0.87
Analyst 2	0.32	0.58	0.34	1.56

six mixed standards were selected for quantitative analysis, which gave satisfactory results. Stock solution was prepared in the same manner. Further dilutions were made to prepare the mixed standard of desired concentration.

Validation

1. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 100 to 500 µg/ml and 50 to 250 µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve and the standard calibration curve of the drugs. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration

2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

3. Precision

a. Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

b. Intermediate Precision

i. Day to Day
ii. Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out.

4. Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and/or concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical

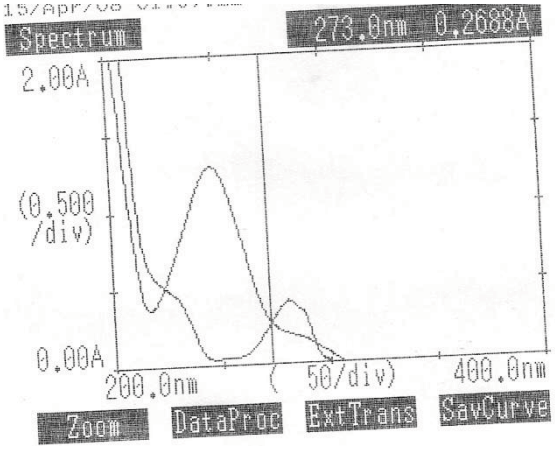

Figure 1: Overlay spectra of paracetamol and chlorzoxazone

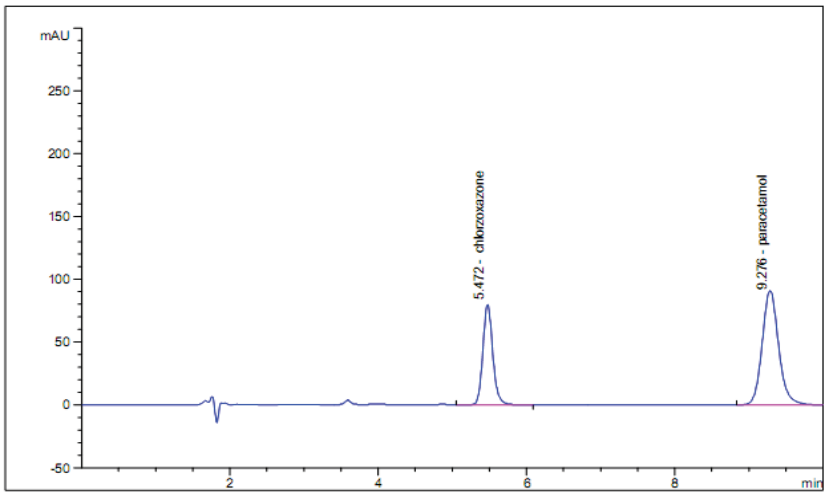

Figure 2: Chromatogram of paracetamol and chlorzoxazone

plates, area under curve and percentage content of paracetamol and chlorzoxazone were studied.

RESULTS AND DISCUSSION

Paracetamol and Chlorzoxazone showed maximum absorbance at 248 nm and 282 nm, respectively. 273 nm was selected as a detection wavelength as the absorbance shown by all the two components was at the detectable range.

UV spectrophotometric and HPLC methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation of paracetamol and chlorzoxazone, in tablet dosage forms. For UV spectrophotometric method, linearity was obtained in concentration range of 30– 70 $\mu g.ml^{-1}$ for paracetatmol and 20-60 $\mu g.ml^{-1}$; with regression 0.9975 and 0.998, intercept – 0.009 and 0.004 and slope 0.0456 and 0.005 for Paracetamol and Chlorzoxazone, respectively. Recovery was in the range of 99 – 101 %; the value of standard deviation and % R.S.D. were found to be <2 %; shows the high precision of the method.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate drugs and internal standard. Mobile phase and flow rate selection was based on peak parameters, run time etc. The system with TEA Buffer:Methanol in the ratio of 55:45 v/v with 1 $ml.min^{-1}$ flow rate is quite robust. The optimum wavelength for detection was 273 nm at which better detector response for drugs was obtained. The average retention times for paracetamol and chlorzoxazone was found to be 9.276±0.03 and 5.472±0.03 min, respectively. According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system.

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 100 – 500 $\mu g.ml^{-1}$ for paracetamol and 50-300 for chlorzoxazone with regression 0.9982 and 0.9996, intercept – 114.31 and – 305.32 and slope 50.565 and 60.58 for paracetamol and chlorzoxazone respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 98 –100 %.

Sample – to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that

there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values reported that proposed methods provides acceptable intra – day and inter – day variation of paracetamol and chlorzoxazone.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions; the % R.S.D. was found to be less than 2 %.

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of paracetamol and chlorzoxazone in tablet dosage form by internal standardization method. Hence, it can be conveniently adopted for the routine quality control analysis in the combination formulations. As the drug combination is available in market, hence, work was toward development of an analysis.

CONCLUSION

It was concluded that new simple, sensitive simultaneous estimation method was developed for estimation of paracetamol and chlorzoxazone in marketed formulation by validated UV-Spectrophotometric and RP-HPLC method.

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