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RESEARCH ARTICLE

Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Chlorzoxazone and Paracetamol in Tablet Dosage Form

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Abstract:
A simple, precise and rapid stability-indicating RP-HPLC method has been developed and validated for simultaneous estimation of Chlorzoxazone (CHL) and Paracetamol (PCM) in tablet dosage form using X Terra® C_{18} column (150 mm × 4.6 mm id, 5 µm particle size) as stationary phase, Acetonitrile: Methanol: HPLC grade water [20: 10: 70 v/v/v] as a mobile phase with flow rate of 0.7 ml/min. Quantification was achieved with Photo Diode Array detector at 270 nm. The retention time for Paracetamol and Chlorzoxazone were found to be 2.822 and 5.377 min. Chlorzoxazone and Paracetamol were exposed to acid/base hydrolytic, oxidative, thermal and photolytic stress conditions, and stressed sample were analyzed by proposed method. There were no other co-eluting, interfering peaks from excipients, impurities, or degradation products due to variable stress conditions, and the method is specific for the estimation of Chlorzoxazone and Paracetamol in the presence of degradation products. The linearity was obtained in the concentration range of 5-50 µg/ml for both drugs. The mean recoveries were 100.83 and 100.50% for CHL and PCM, respectively. The proposed method can be useful in routine quality control of bulk manufacturing and pharmaceutical dosage forms.

Keywords: Chlorzoxazone, Paracetamol, RP-HPLC, Stability indicating, Validation, Recovery.

Introduction

Chlorzoxazone (CHL) is chemically 5-chloro-3H-benzooxazol-2-one [1] (Figure-1). Chlorzoxazone (CHL) is a skeletal muscle relaxant. It acts by inhibiting multi synaptic reflexes involved in producing and maintaining skeletal muscle spasm of varied etiology [2]. Chlorzoxazone (CHL) is official in United States Pharmacopeia (USP)[3]. USP describes UV and liquid chromatography method for its estimation. Literature survey reveals Flourimetry[4], Electrochemical[5], HPLC[6-8] , GC-MS[9] methods for determination of CHL alone. Literature survey also reveals UV [10-12], HPLC [13-21], HPTLC [22] methods for the determination of CHL with other drugs combination. Paracetamol (PCM) is chemically N-(4-hydroxyphenyl) acetamide (Figure 2) also known as acetaminophen, is a popular analgesic and antipyretic drug widely used for management of pain and fever [23]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopeia (USP) and Japanese Pharmacopoeia (JP). IP [24] and JP [25] describe UV method for its estimation, IP and USP [26] describes liquid chromatography method for its estimation, while EP [27], BP [28] titration method for its estimation. Literature survey reveals UV [29-30], HPLC [31], GC [32], LC-MS [33] methods for estimation of PCM alone. Literature survey also reveals UV [34-38], HPLC [39-44] HPTLC [45] methods for determination of PCM with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of CHL and PCM in their combined synthetic mixture or dosage forms. Literature survey reveals only simultaneous equation method and RP-HPLC method for CHL and PCM in combined dosage forms [46].

Figure 1- Chemical structure of Chlorzoxazone

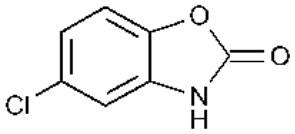
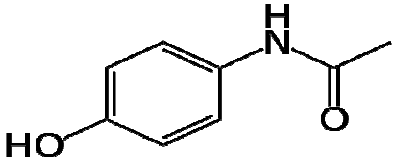


Figure 2- Chemical structure of Paracetamol



MATERIALS AND METHODS

Apparatus

RP-HPLC instrument equipped with a photodiode array detector, (Shimadzu, LC-2010$C_{HT,}$ Japan,), auto sampler, X Terra® C_{18} column (150 mm × 4.6 mm id, 5 µm particle size) and LC-solution software were used, Analytical balance (Sartorius CP224S, Germany), Triple distillation unit consisting of borosilicate glass, Digital pH meter (LI 712 pH analyzer, Eli co Ltd., Ahmadabad), volumetric flasks, Ultra sonic cleaner (Frontline FS 4, Mumbai, India)

Reagents and materials

Paracetamol (PCM) and Chlorzoxazone (CHL) were kindly supplied as a gift samples from Brussels Laboratories Pvt. Ltd, Changodar, Ahmadabad, Gujarat; Tablet samples were purchased from local pharmacy (MYOSPAZ- Labelled claim: 325 mg PCM and 250 mg CHL). HPLC grade methanol (Merck Ltd., Mumbai, India) and acetonitrile (Finar Chemicals Ltd., Mumbai, India) were used during study. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter. AR Grade Sodium hydroxide, Hydrogen peroxide, Hydrochloric acid (S. D. Fine Chemicals Ltd., Mumbai, India), Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India), Whatman filter paper no. 41. (Whatman International Ltd., England) were used during study.

Chromatographic Condition

Chromatographic separation were performed using a X Terra® C_{18} column (150 mm x 4.6 mm id., 5 µm particle size) at ambient temperature, eluted with mobile phase at a flow rate of 0.7 ml/min. The mobile phase consisted of Acetonitrile: Methanol: HPLC grade water [20: 10: 70 v/v/v]. Measurements were made with an injection volume of 20 µL and UV detection at 270 nm, as both components showed reasonably good response at this wavelength.

Preparation of standard stock solution

Standard solution of CHL (50 µg/ml) and PCM (50 µg/ml) was prepared by transferring accurately weighed CHL (5 mg) and PCM (5 mg) in 100 ml volumetric flask, separately and dissolving in HPLC grade water. The solution was diluted to 100 ml with HPLC grade water in separate volumetric flask to inject in chromatographic system.

Preparation of sample solution

Twenty tablets were weighed and mass of tablet powder equivalent to 25 mg Chlorzoxazone of tablet powder and transferred to 100 ml volumetric flask containing 50 ml HPLC grade water and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with HPLC grade water. An aliquot (20 ml) was transferred in to a 100 ml volumetric flask and the volume was made up to mark with HPLC grade water to achieve a concentration of PCM (65 µg/ml) and CHL (50 µg/ml). From resulting solution an aliquot (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with HPLC grade water to achieve a final concentration of PCM (26 µg/ml) and CHL (20 µg/ml).

Forced degradation study

Acid, Alkali and Oxidative degradation

For acid, base and oxidative degradation study 25 mg of CHL and 25 mg of PCM were transferred in 50 mL volumetric flasks, separately. Volume of each flask under each condition were made up to mark with respective degradant to achieve final concentration of 500 µg/mL for both CHL and PCM, respectively. After sufficient degradation condition, each degradation sample was diluted with HPLC grade water to achieve concentration of 50 µg/ml for both CHL and PCM, respectively, which were injected in HPLC system.

For Acid degradation**,** solution of CHL and PCM were refluxed at 100 ^{0}C for 3 hr to facilitate degradation. For Alkali degradation**,** solution of CHL and PCM were refluxed at 100 ^{0}C for 5 hr to facilitate degradation. For Oxidation degradation**,** solution of CHL and PCM were refluxed at 100^{0}C for 5 hr to facilitate degradation.

Photolytic and Thermal degradation

For photo and thermal degradation 100 mg standard powder of both drugs were spread on the Petri dish in 2 mm thick layer, separately. After sufficient degradation condition, each degradation sample was dissolved and diluted with HPLC grade water to achieve 50 µg/mL concentrations of both CHL and PCM, respectively, which were injected in HPLC system.

For Photolytic degradation, standard powder of CHL and PCM were exposed to UV light at 254 and 366 nm to determine the effect of light radiation on the

stability of CHL and PCM in solid state. All samples for photo stability testing were placed in UV light for 4 days. For Thermal degradation, standard powder of CHL and PCM were heated in hot air oven to determine the effect of heat on the stability of CHL and PCM in solid state. All samples for thermal stability testing were placed in hot air oven for 7 hr at 100 ^{0} C.

ANALYSIS OF DRUGS IN TABLET SAMPLE

The response of the sample solution was measured at 270 nm under the chromatographic condition mentioned above for the quantification of CHL and PCM. The amounts of CHL and PCM present in sample solution were determined by applying values of the peak area to the regression equations of the calibration curve.

RESULTS AND DISCUSSION

Method Development

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for CHL and PCM was obtained with a mobile phase Acetonitrile: Methanol: water (20: 10: 70, v/v/v) at a flow rate of 0.7 ml/min to get better reproducibility and repeatability. Quantification was carried out at 270 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 3). Degraded samples prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method.

Degradation behaviour

Singh and Bakshi, in their article [47] on stress testing suggested a target degradation of 20-80 % of establishing the stability nature of assay method, as even intermediate degradation product should not interfere at any stage of drug analysis. Although conditions used for force degradation were adjusted to achieve degradation in the range of 20-80 %, this could not achieve in some cases even after exposures for a prolonged duration. Results of degradation behaviour of CHL and PCM under various conditions are depicted in table 2. HPLC studies on CHL (50 µg/mL), PCM (50 µg/mL) under different stress condition suggested the following degradation behaviour.

Figure 4, 5, 6, 7 and 8 show acidic, alkaline, oxidative, photolytic and thermal degradation, respectively of CHL and PCM. It was found that there were no co-eluting peaks of degradation product(s) / impurities with the original peaks of CHL and PCM.

Method validation

The %RSD values of inter-day (0.35-1.45 % and 0.21-1.36 %) and intra-day (0.33-0.63 % and 0.32-1.10 %) for CHL and PCM, respectively, reveals that the proposed method is precise (Table 5). LOD values for CHL and PCM were found to be 0.23µg/ml and 0.39 µg/ml, respectively and LOQ values for CHL and PCM were found to be 0.76 µg/ml and 1.27 µg/ml respectively (Table 5). These data show that the proposed method is sensitive for the determination of CHL and PCM.

The recovery experiment was performed by the standard addition method. The recoveries obtained were 100.83 ± 0.62 % and 100.5 ± 0.51 % for CHL and PCM, respectively (Table 3). The low value of standard deviation indicates that the proposed method is accurate.

Specificity

The specificity of the method was ascertained by analyzing standard solution for sample CHL and PCM. The peak purity index was found to be 1.000 for both standard solution of CHL and PCM respectively and for the sample solution of CHL and PCM peak purity index was 0.9991 and 0.9996. The above results suggest that proposed method was specific for the simultaneous estimation of CHL and PCM. Peak purity spectra of standard and sample solution of CHL and PCM are shown in figure 9 – 12.

Assay:

The proposed validated method was successfully applied to determine CHL and PCM in tablet dosage form. The result obtained for CHL and PCM was comparable with the corresponding labelled amounts (Table 4). The RP-HPLC chromatogram for CHL and PCM in sample was recorded and is shown in Figure 13.

CONCLUSION

Based on the results obtained from the analysis of forced degraded samples using the described method, it can be concluded that there is no other co-eluting peak with the main peaks and the method is specific for the estimation of CHL and PCM in the presence of degradation product(s) / impurities. The proposed method was found to be linear in the concentration range of 5-50 µg/mL for both CHL and PCM with co-efficient of correlation, (r^2) 0.9990 and 0.9987, respectively. The result of the analysis of tablet formulation by the proposed method was highly reproducible and reliable and it is in good agreement with the label claim of the drug. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of CHL and PCM in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in their combined dosage form. Although no attempt was made to identify the degradation products, the described method can be used as a stability-indicating method for the assay of CHL and PCM in their combination drug products.

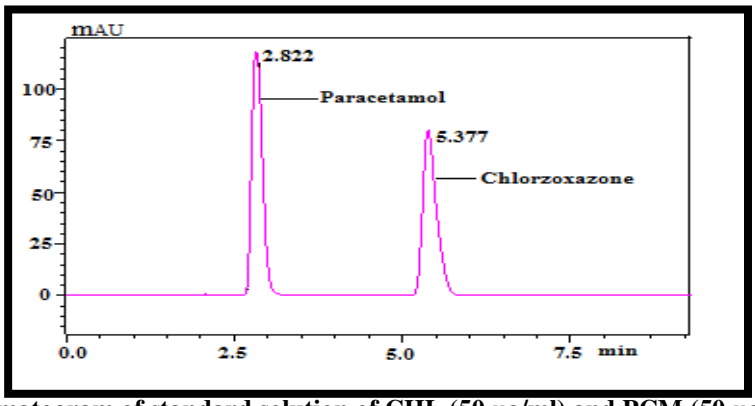


Figure: 3 Chromatogram of standard solution of CHL (50 µg/ml) and PCM (50 µg/ml) at 270 nm

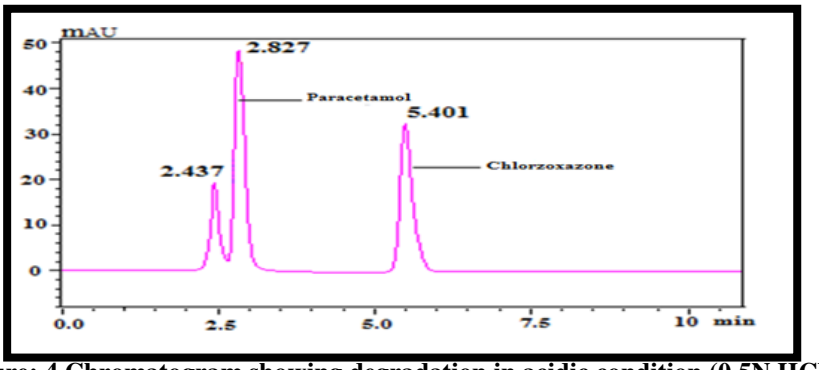


Figure: 4 Chromatogram showing degradation in acidic condition (0.5N HCL)

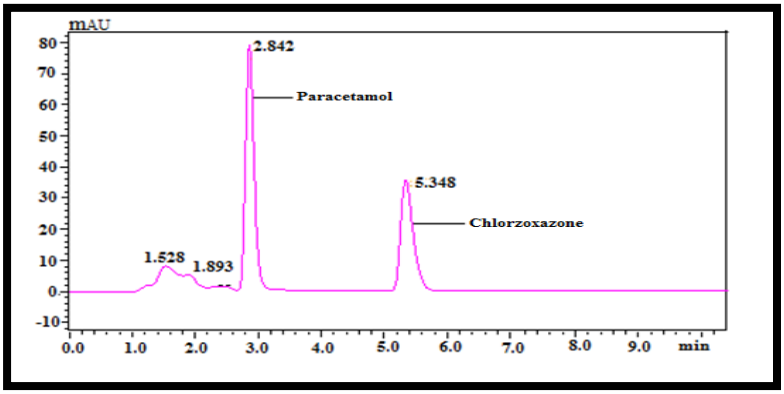


Figure: 5 Chromatogram showing degradation in alkali condition (0.1N NaOH)

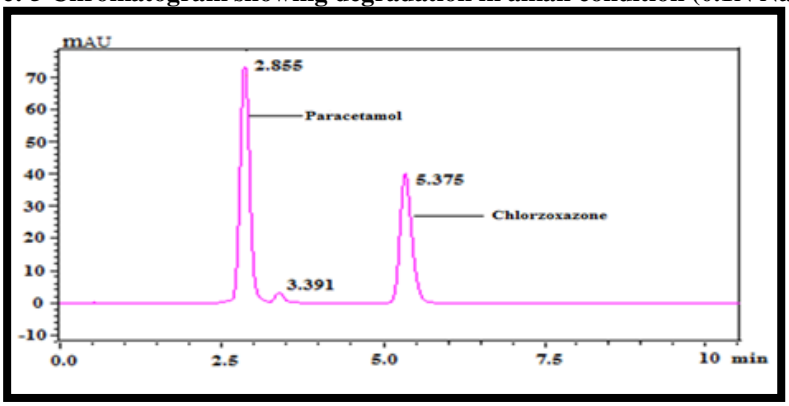


Figure: 6 Chromatogram showing degradation in oxidative condition (5% H_2O_2)

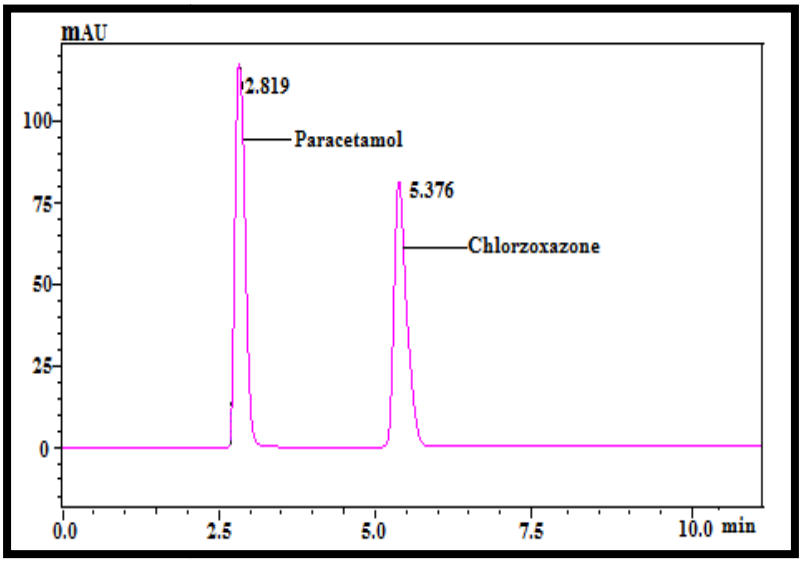


Figure: 7 Chromatogram showing degradation in photolytic condition (UV light)

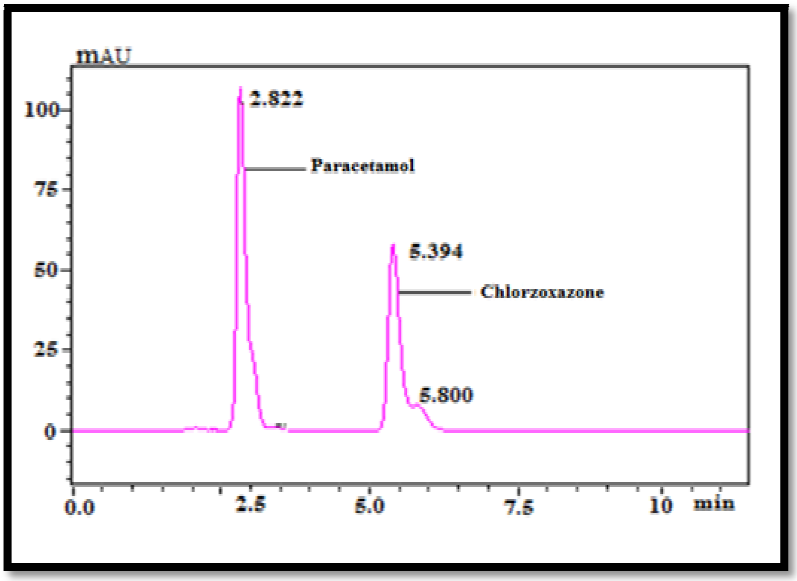


Figure: 8 Chromatogram showing after exposure of drug to thermal condition

Table 1 System suitability parameters of chromatogram for CHL and PCM

Parameters	CHL± RSD (n = 6)	PCM ± RSD (n = 6)
Retention time (min)	5.377 ± 0.56	2.822 ± 0.49
Tailing factor	1.310 ± 1.48	1.280 ± 1.82
Theoretical plates	3023 ± 1.42	2481 ± 1.13
Resolution	3.888 ± 0.23	

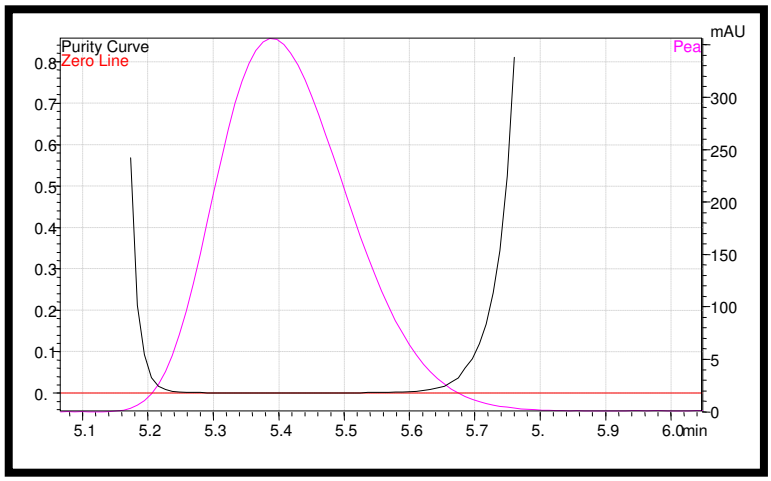


Figure 9 Peak Purity spectra of CHL standard

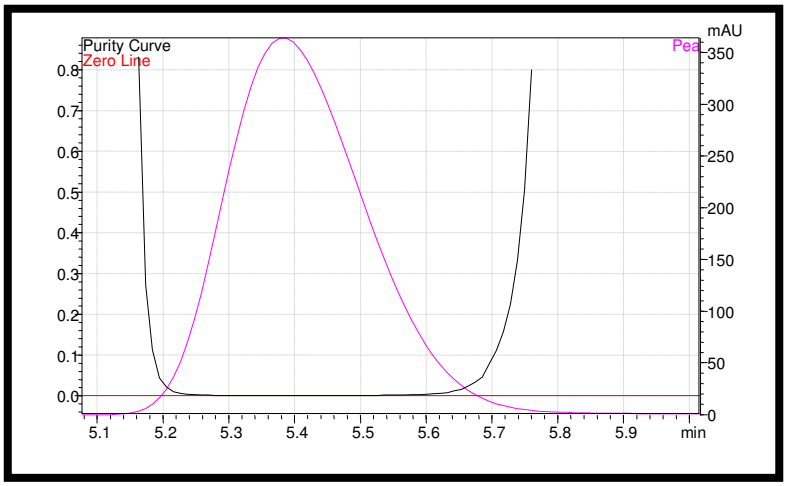


Figure 10 Peak Purity spectra of CHL sample

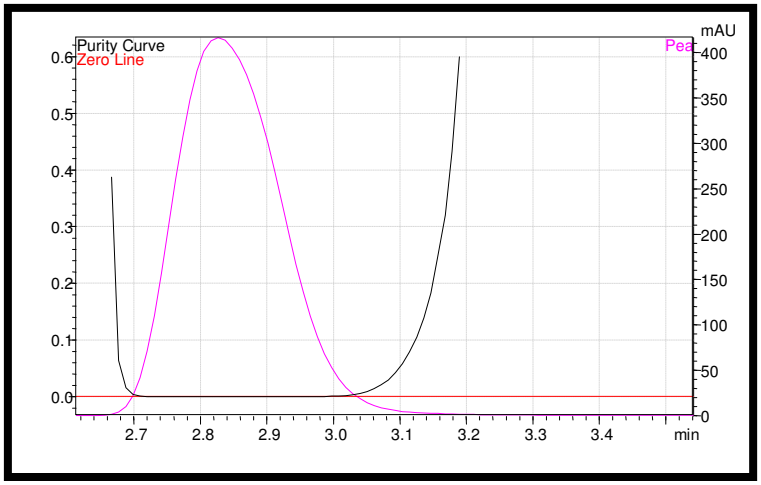


Figure 11 Peak Purity spectra of PCM standard

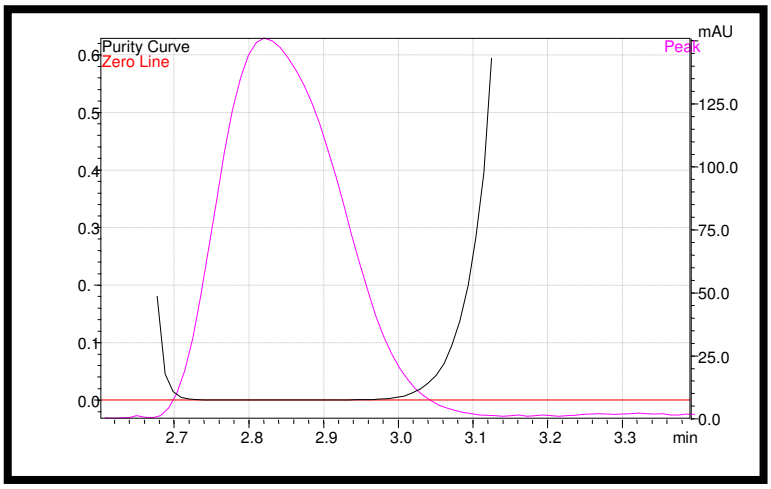


Figure 12 Peak Purity spectra of PCM sample

Table 2 Results of force degradation study

Sr. No.	Stress condition/ duration /state	Impurity formed	Retention time (min)	% Degradation
1	Acidic/ 0.5 N HC1/ 100°C/3 hr/ solution	Impurity A	2.437	
		Paracetamol	2.827	12.15%
		Chlorzoxazone	5.401	20.43%
2	Alkaline/ 0.1 N Na0H/ 100°C/ 5 hr/ solution	Impurity A	1.528	
		Impurity B	1.893	
		Paracetamol	2.842	11.86%
		Chlorzoxazone	5.348	9.79%
3	Oxidative/ 3% H_20_2/ 100°C/ 5 hr/ solution	Impurity A	3.391	
		Paracetamol	2.855	10.19%
		Chlorzoxazone	5.375	12.38%
4	Neutral/water/100°C/ 5 hr/solution	Paracetamol	2.832	-
		Chlorzoxazone	5.352	-
5	UV light/ 4 days/ solid	Paracetamol	2.819	1.13%
		Chlorzoxazone	5.376	0.68%
6	Thermal/ 100°C/ 7 hr/ solid	Impurity A	5.80	
		Paracetamol	2.822	6.17%
		Chlorzoxazone	5.394	17.31%

Table 3: Recovery data for the proposed method (n = 3)

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
CHL	I	20	50	100.41 ± 0.79
	II	20	100	101.21 ± 0.68
	III	20	150	100.88 ± 0.38
PCM	I	26	50	99.86 ± 0.25
	II	26	100	100.12 ± 0.44
	III	26	150	101.54 ± 0.86

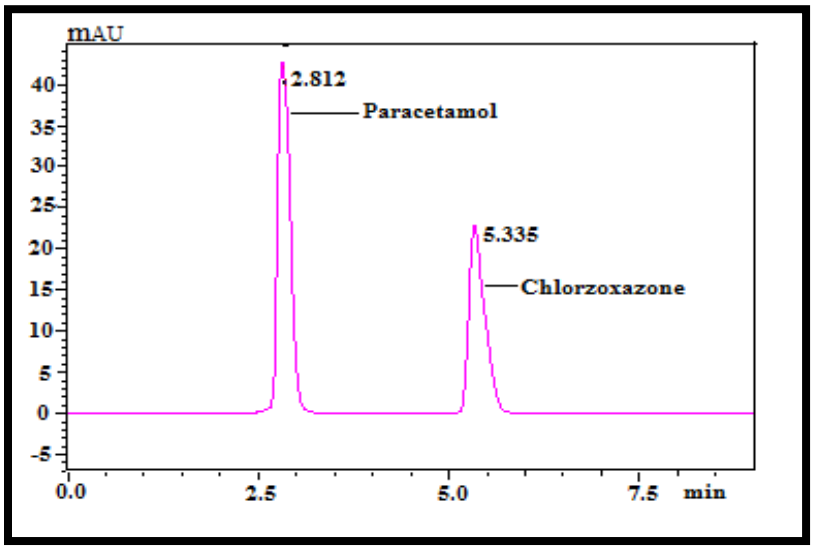


Figure 13: Chromatogram of sample solution of CHL (20µg/ml) and PCM (26 µg/ml) at 270 nm

Table 4 Assay results of tablet containing CHL and PCM by Proposed Method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim (n = 6)	
	CHL	PCM	CHL	PCM	CHL	PCM
1	250	325	250.14	326.68	100.06±0.95	100.52±0.60

TABLE: 5 Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Parameters	RP- HPLC Method	
	Chlorzoxazone	Paracetamol
Wavelength (nm)	270	270
Beer's law limit (µg /ml)	5-50	5-50
Regression equation (y = a + bc) Slope (b) Intercept (a)	y = 13,199.91x + 8,148.06 13,199.91 8,148.06	y = 17,588.96x + 20,116.80 17,588.96 20,116.80
Correlation coefficient (r^2)	0.9990	0.9987
Repeatability (% RSD n = 6)	0.50	0.46
LOD (µg/ml)	0. 23	0.39
LOQ (µg /ml)	0. 76	1. 27
Precision (% RSD, n =3) Inter-day Intra-day	 0. 35 – 1.45 0. 33– 0.63	 0. 21 – 1. 36 0.32 –1.10
Accuracy ± S.D (n =3)	100.83 ± 0.62	100.50± 0.51
Assay ± S. D. (n = 6)	100.06±0.95	100.52 ±0.60

RSD = Relative standard deviation. LOD = Limit of detection. LOQ = Limit of quantification. S.D. = standard deviation

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