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Stability indicating simultaneous validation of Ketorolac, Phenylephrine hydrochloride and Chlorpheniramine maleate with forced degradation behavior Study by RP-HPLC in pharmaceutical dosage form

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ABSTRACT

A simple, precise, and accurate RP-HPLC method has been developed and validated for the simultaneous assay of Ketorolac, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in drops. Isocratic RP-HPLC method was developed on BDS hypersil C_{18}, (250mm×4.6mm internal diameter, 5µ particle size) using mobile phase as 0.02M Potassium Dihydrogen Phosphate (pH-3.0): Acetonitrile (80:20v/v) at a flow rate of 1.0 mL/min and the detection was carried out at 220nm using tunable absorbance detector (Waters 486). Forced degradation study was carried out by acid degradation, base degradation, thermal degradation, oxidation of the drug. The method was validated for linearity, precision, accuracy and robustness. The method was found to be linear in the concentration range of 5-15 μg/mL with correlation coefficient of 0.9995 for Ketorolac, 1.2-3.6 μg/mL with correlation coefficient of 0.9998 for Phenylephrine Hydrochloride and 2-6 μg/mL μg/mL with correlation coefficient of 0.9982 for Chlorpheniramine Maleate. Degradation products produced as a result of stress studies did not interfere with the detection of Ketorolac, Phenylephrine Hydrochloride and Chlorpheniramine Maleate, therefore, the assay can be considered to be stability indicating.

Keywords: HPLC, Ketorolac, Phenylephrine Hydrochloride, Chlorpheniramine Maleate, Validation, Forced degradation.

1. INTRODUCTION

Chlorpheniramine Maleate's (CPM) empirical formula is $C_{20}H_{23}ClN_2O_4$ and its IUPAC name is 3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine. Figure 1 shows chemical structure of CPM salt. Chemically CPM is a H-1 receptor blocker and it is an antihistamine used to relieve symptoms of allergy, hay fever, and the common cold which includes rashes, watery eyes, cough, runny nose, sneezing and itching in eyes, nose, throat, skin etc. Ketorolac's (KT) empirical formula is $C_{15}H_{13}NO_3$ and its IUPAC name is (±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid, 2-amino-2(hydroxymethyl)-1,3-propanediol. Figure 2 shows the chemical structure of KT. Chemically it's a member of the heterocyclic acetic acid derivative family and it is a non steroidal anti inflammatory drug with an efficacy close to that of the opioid family. Ketorolac acts by inhibiting the bodily synthesis of prostaglandins. It is mainly used for the short term treatment of post-operative pain as it is highly selective for the COX-1 enzyme. It is approved by the USA Food and Drug Administration and it is non-narcotic, fast acting and non-addictive. It can be administered orally or by injection. When administered systemically has demonstrated analgesic, anti inflammatory and anti-pyretic activity. Phenylephrine Hydrochloride's (PH) empirical formula is $C_9H_{13}NO_2HCl$ and its IUPAC name is (R)-1-(3-hydroxyphenyl)-2-methylamino-ethanol hydrochloride. Figure 3 shows the chemical structure of PH. Chemically it's a hydrochloride and is α-adrenoreceptor agonist which decreases nasal congestion, and increases drainage of sinus cavities and generally used as a decongestant, mydriatic, vasopressor and detumescent agent.

These drugs are official in Indian Pharmacopoeia, British Pharmacopoeia, and

United States Pharmacopoeia. Literature survey reveals that quantitative analysis of Chlorpheniramine Maleate's (CPM), Ketorolac(KT) and Phenylephrine Hydrochloride (PH) have been done separately on in combination of two and in combination of other drugs but no method is reported for the simultaneous estimation of CPM, KT, and PH in combined dosage form. The present study involved the development and validation of RP-HPLC method for the estimation of CPM, KT, and PH in combined pharmaceutical dosage form and their stability study[1-9].

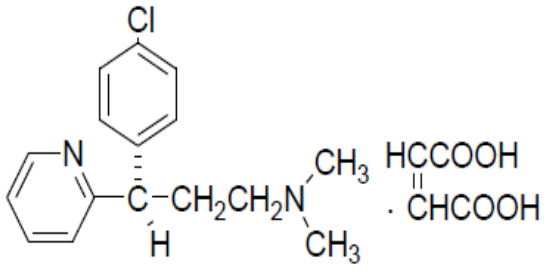

Figure - 1: Structure of Chlorpheniramine Maleate.

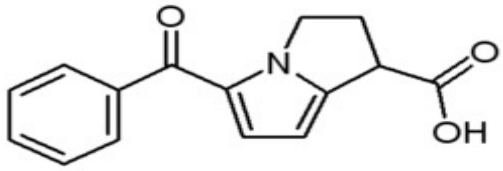

Figure - 2: Structure of Ketorolac.

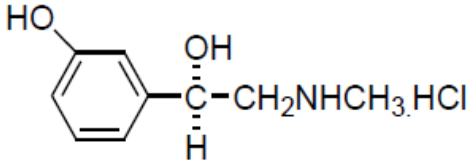

Figure - 3: Structure of Phenylephrine hydrochloride.

2. MATERIAL AND METHODS

2.1. Instruments

The liquid chromatographic system consists of Waters series M510 equipped with a tunable absorbance detector (Waters 486), HPLC pump (Waters 510), and manual injector rheodyne valve with 20 μL fixed loop. The analytes were monitored at 220 nm. Chromatographic analysis was performed on Thermo scientific BDS hypersil C_{18}, (250mm × 4.6mm internal diameter, 5µ particle size). All the drugs and chemicals were weighed on Citizen electronic balance. Chemiline India pH meter and Toshcon Ultrasonicator was used.

2.2. Chemicals and reagents

Acetonitrile and Methanol were of HPLC grade obtained from Merk Ltd., Mumbai. Water was of HPLC grade prepared by triple distillation method. Potassium Dihydrogen Phosphate, Ortho Phosphoric Acid (OPA), Sodium Hydroxide (NaOH), Hydrogen Peroxide (H_2O_2) and Hydrochloric Acid (HCl) were of AR grade and were obtained from Merck, Mumbai India. Chlorpheniramine Maleate (CPM), Ketorolac (KT) and Phenylephrine Hydrochloride (PH) reference standards obtained as gift samples from FDC limited, Mumbai. Drops containing 0.2% of Chlorpheniramine Maleate (CPM), 0.5% of Ketorolac (KT) and 0.12% Phenylephrine Hydrochloride (PH) manufactured by Laborate Pharmaceuticales India Ltd. was procured from local market.

2.3. HPLC Condition

The mobile phase consisted of 0.02M Potassium Dihydrogen Phosphate (pH-3.0): Acetonitrile (80:20v/v).The mobile phase was prepared freshly and it was sonicated by using Toshcon Ultrasonicator for 5 min before use. BDS hypersil C_{18}, (250mm×4.6mm internal diameter, 5µ particle size) was used and it was was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature. The eluent was monitored by UV detection at 220 nm. Analysis was done at flowrate of 1.0ml/min with 20µl volume of injection. All data were analyzed by using Empower 3 software.

2.4. Preparation of Mobile Phase

The mobile phase was prepared by mixing 0.02M Potassium dihydrogen Phosphate (pH-3.0) and acetonitrile in the ratio of (80:20%v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

2.5. Preparation of 0.02M Potassium dihydrogen phosphate (pH-3.0)

Take about 2.72gm potasium dihydrogen phosphate into a 1000ml beaker. Add 800ml water and dissolve. Adjust ph 3.0 of this solution with 1% Orthophosphoric acid. Make up volume upto 1000ml with water.

2.6. Preparation of standard solution

Standard stock solution of KT, PH and CPM were prepared by accurately weighing 10mg, 24mg and 40mg respectively and dissolving them separately in 100ml with methanol to prepare solution of 100μg/mL, 240μg/mL and 400μg/mL. The solutions of PH and CPM were further diluted by taking 10 ml of standard stock solution and diluted upto 100 ml with methanol separately to

prepare solution of 24μg/mL and 40μg/mL respectively.

2.7. Forced degradation study

2.7.1. Preparation of solution for acid degradation

Acid decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N HCl for 3 hrs. After 3 hrs solution neutralized with 2ml 0.1N NaOH and finally made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

2.7.2. Preparation of solution for basic degradation

Alkali decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N NaOH for 3.5 hrs. After 3.5 hrs solution neutralized with 2 ml of 0.1N HCL and finally made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

2.7.3. Preparation of solution for oxidative degradation

Oxidative decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml 3% H_2O_2 for 3 hrs. After 3 hrs volume made up to 10 ml with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

2.7.4. Preparation of solution for thermal degradation

Thermal decomposition study was performed by refluxing the working solution of all three drugs (1 ml) for 3 hrs at 105 ºC. After 3 hrs volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

2.7.5. Preparation of solution for UV degradation

UV degradation was performed by exposing the working solution of both drugs (1ml) to Sunlight for 4 hours. After 4 hours volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

2.8. Determination of λ max

The UV spectra of standard stock solutions of Ketorolac, Chlorpheniramine Maleate and Phenylephrine Hydrochloride taken between the wave length range of 200-400nm using methanol as blank. the λ max was found to be 248.87 nm, 208.75 nm and 216.67 nm for Ketorolac, Chlorpheniramine Maleate and Phenylephrine Hydrochloride respectively. Overlay of the three spectra taken and iso-absorptive point was selected and it was found that all three drugs show appreciable absorbance at 220 nm, so it is used for the further study.

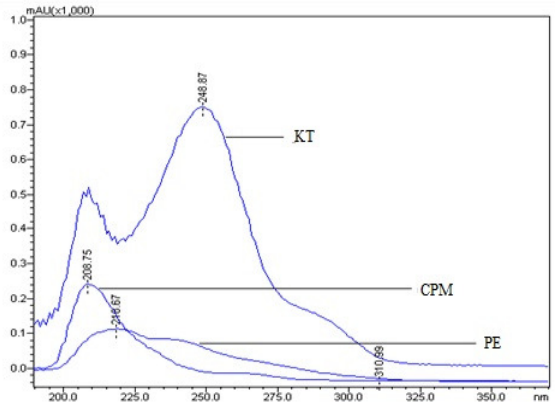

Figure - 4: Overlay absorption spectrum for Ketorolac(KT), Chlorpheniramine Maleate (CPM) and Phenylephrine Hydrochloride(PH)

2.9. Procedure of analysis

1ml from KT Standard stock solution, 1ml from PH Standard stock solution and 1ml from CPM Standard stock solution were taken and volume was make up to 10ml with Mobile phase to obtain Working standard solution containing KT (10μg/mL), PH (2.4μg/mL) and CPM (4μg/mL). For Sample stock solution, sample solution 1ml (Equivalent to 10mg KT, 2.4mg PH and 4mg CPM) was taken into a 100ml volumetric flask. 60ml methanol was added and shaken for 15 minutes. Volume was made upto 100 ml with methanol to obtain solution containing KT (100μg/mL), PH (24μg/mL) and CPM (40μg/mL). The solution was filtered with whatman filter paper-1. 1ml from Sample stock solution was taken into a 10ml volumetric flask and make up with mobile phase to obtain Working sample

solution of concentration KT (10μg/mL), PH (2.4μg/mL) and CPM (4μg/mL) respectively.

The contents of standard and sample solution were then filtered through 0.45 µm syringe filter. Chromatograms standard solution (six replicates) was recorded. A typical chromatogram of KT, PH and CPM are presented in figure 5. The retention time of KT, PH and CPM were 3.56 min, 4.92 min and 8.32 min respectively. The peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of calibration curve.

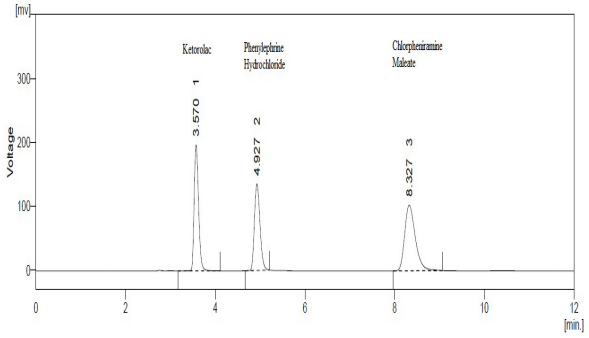

Figure - 5: Standard Chromatograms of Chlorpheniramine Maleate, Ketorolac and Phenylephrine Hydrochloride.

Optimized Chromatographic Condition:

Stationary phase :Thermo scientific BDS hypersil C_{18} (250mm × 4.6mm, 5µ).

Mobile phase :Potassium dihydrogen phosphate (pH 3.0):acetonitrile (80:20)

Flow rate :1.0 ml/min

Run time (min) :12min

Detection :At 220 nm

Injection (volume) :20µl

2.10. Method validation procedure

The developed method was validated for the parameters listed in ICH guidelines [10-13].

2.10.1. Linearity

The method was linear in the range of 5-15 μg/mL, 1.2-3.6 μg/mL and 2-6 μg/mL for KT, PH and CPM respectively. The linear correlation coefficient for KT, PH and CPM were found to be 0.9995, 0.9998 and 0.9982 respectively, and are recorded in table 2, 3 and 4. Calibration curve of KT, PH and CPM was obtained by plotting the peak area ratio versus the respective concentrations (Figure 6, 7 and 8).

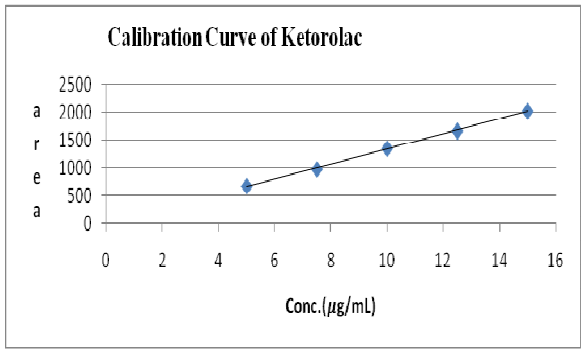

Figure - 6: Calibration curve of Ketorolac.

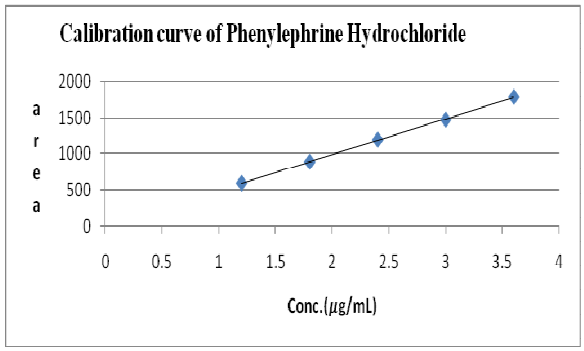

Figure - 7: Calibration curve of Phenylephrine Hydrochloride.

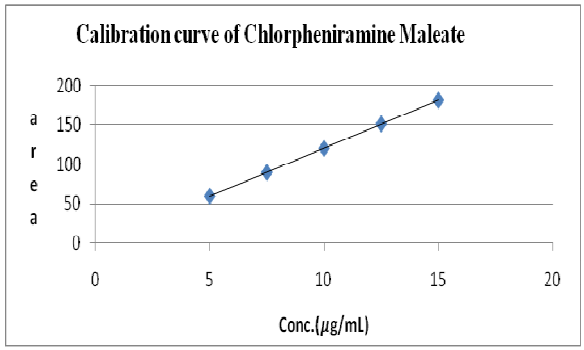

Figure 8: Calibration curve of Chlorpheniramine Maleate

Table - 1: System suitability of proposed method

Parameters	Ketorolac	Phenylephrine Hydrochloride	Chlorpheniramine Maleate
Theoritical plates	6206	7199	6146
Resolution	-	6.561	10.348
Asymmetry	1.458	1.419	1.500
Retention time	3.570 min	4.927 min	8.327 min

Table - 2: Linearity results of Ketorolac		
Linearity Level	**Concentration**	**Area**
I	5 µg/ml	678.62
II	7.5 µg/ml	982.20
III	10 µg/ml	1356.26
IV	12.5 µg/ml	1668.86
V	15 µg/ml	2026.14
Correlation coefficient		0.9996

Table - 3: Linearity of Phenylephrine Hydrochloride.		
Linearity Level	**Concentration**	**Area**
I	1.2 µg/ml	600.67
II	1.8 µg/ml	891.37
III	2.4 µg/ml	1195.69
IV	3.0µg/ml	1471.25
V	3.6 µg/ml	1786.23
Correlation coefficient		0.9998

Table - 4: Linearity results of Chlorpheniramine Maleate		
Linearity Level	**Concentration**	**Area**
I	2 µg/ml	867.91
II	3 µg/ml	1219.82
III	4 µg/ml	1732.94
IV	5 µg/ml	2128.26
V	6 µg/ml	2492.55
Correlation coefficient		0.9982

Table - 5: Results of Accuracy

Sample	**Accuracy**	**Standard Drug** (µg/ml)	**Formulation** (µg/ml)	**% of recovery**	**S.D.**	**% RSD**
KT	80%	4	5	100.66	0.835	0.83
	100%	5	5	100.58	1.479	1.47
	120%	6	5	100.96	0.986	0.99
PH	80%	0.96	1.2	98.45	0.904	0.92
	100%	1.2	1.2	101.83	1.499	1.47
	120%	1.44	1.2	98.51	1.272	1.29
CPM	80%	8	10	98.05	0.576	0.59
	100%	10	10	98.42	0.507	0.51
	120%	12	10	101.36	0.516	0.51

2.10.2. Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed Drop solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For all the three drugs, recovery was performed in the same way. The recovery studies were performed in triplicate and results are recorded in table 5. This standard addition method was performed at 80%, 100%, 120% level and the percentage recovery was calculated. Percent recovery was within the range of 100.58 to 100.96 for KT, 98.45 to 101.83 for PH and 98.06 to 101.36 for CPM which indicates that the method was accurate.

2.10.3. Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated and the results are shown in table 6. The %RSD for KT, PH and CPM were found to be 0.38%, 1.36% and 1.28 % respectively. From the data obtained the developed RP-HPLC method was found to be precise. For interday and intraday precision three different concentrations (50%, 100% and 150% of analyte) of standard solutions were injected on same day and three consecutive days in three replicates and results were recorded in table 7 and 8.

2.10.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and quantification were calculated using standard deviation of response and slope of the calibration curve and results are recorded table 9. The LOD for KT, PH and CPM was found to be 0.452 µg/ml, 0.064 µg/ml and 0.362 µg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ for KT, PH and CPM was 1.368 µg/ml, 0.193 µg/ml and 1.096 µg/ml.

Table - 6: Results of Precision

Injection	Area of KT	Area of PH	Area of CPM
Injection 1	1344.205	1210.414	1717.518
Injection 2	1332.100	1177.060	1702.094
Injection 3	1333.400	1178.186	1703.826
Injection 4	1337.420	1204.412	1682.135
Injection 5	1340.059	1206.763	1663.984
Injection 6	1331.094	1212.879	1720.929
Average	1336.380	1198.286	1698.414
S.D.	5.1183	16.2726	21.7493
% RSD	0.38	1.36	1.28

Tabel - 7: Result of Interday Precision

Conc. (µg/ml)			Area			% RSD		
KT	PH	CPM	KT	PH	CPM	KT	PH	CPM
5	1.2	2	671.330	592.572	848.035	0.10	0.47	1.20
10	2.4	4	1340.204	1200.814	1717.451	0.81	1.49	0.36
15	3.6	6	2017.376	1774.653	2569.568	0.35	0.36	0.80

Table - 8: Result of Intraday Precision

Conc. (µg/ml)			Area			% RSD		
KT	PH	CPM	KT	PH	CPM	KT	PH	CPM
5	**1.2**	**2**	667.388	591.773	850.161	1.05	0.76	1.71
10	**2.4**	**4**	1345.494	1196.328	1696.912	0.63	1.60	1.63
15	**3.6**	**6**	2010.803	1776.198	2555.209	0.96	0.65	1.80

Table - 9: Results of LOD and LOQ			
Parameter	**KT (µg/ml)**	**PH (µg/ml)**	**CPM (µg/ml)**
LOD	0.452	0.064	0.362
LOQ	1.368	0.193	1.096

2.10.5. Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like flow rate, mobile phase ratio and pH of buffer and the result were recorded in table 10. It was observed that there were no marked changes in chromatograms and % relative standard deviation was found below 2%, which demonstrated that the developed RP-HPLC method is robust.

2.10.6. Specificity

The specificity of proposed method is justified by the chromatograms of blank, placebo, standard and sample solutions under same chromatographic conditions shown in figure 9. The placebos did not interfere in determination of KT, PH and CPM in commercial drop. Specificity of the developed method was also evaluated by applying different stress conditions (oxidation, acid, base, thermal and photolytic) to KT, PH and CPM drop.

2.10.6.1. Degradation Study

From the results of forced degradation studies showed that these components does not remained intact under stressed conditions and hence special storage conditions should be provided for the dosage form. The specificity studies showed that the principle peaks were well resolved (peak purity 99.99%) and free from any interference from the degradation product. The stress conditions were applied and degraded products of all three drugs are compared and showed in table 10 and chromatograms are in figure 10. From the stress studies it is concluded that substantial degradation of KT, PH and CPM occurred in acid, basic, oxidative thermal and photolytic stress conditions. The degradation products (impurities) in addition to percent degradation under acid, base, oxidation, thermal and photolytic stresses have unique retention times (RT) to acidic stress (6 impurities, RT: 2.46 min, 2.74 min, 3.25 min, 4.17 min, 5.64 min and 7.56 min), basic stress (8 impurities, RT: 1.97 min, 2.39 min, at 2.747 min, 4.08 min, 4.21 min, 5.56 min, 6.68 min and 10.15 min), oxidative stress (8 impurities, RT: 2.21 min, 2.64 min, 6.22 min, 7.10 min, 9.34 min, 9.83 min, 11.44 min and 11.75 min), thermal stress (6 impurities, RT: 2.42 min, 2.82 min, 6.41 min, 6.94 min, 10.49 min and 10.75 min) and photolytic stress (5 impurities, RT: 6.35 min, 7.39 min, 9.34 min, 9.54 min and 10.76 min). Degradation studies justified the method specificity for its intended application.

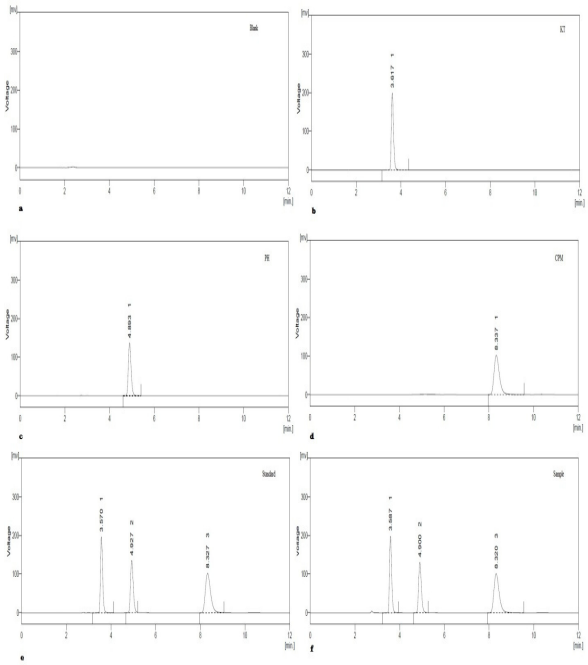

Figure - 9: Chromatograms of (a) blank, (b) KT, (c) PH, (d) CPM, (e) standard mixture and (f) sample mixture.

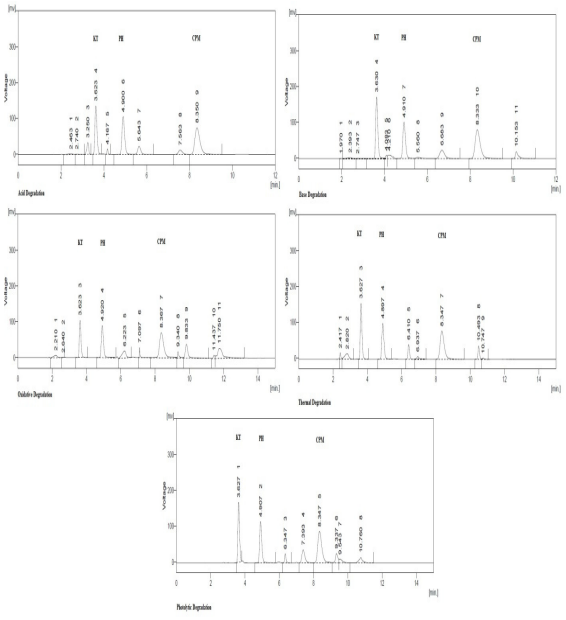

Figure - 10: Chromatograms of (a) Acid Degradation, (b) Base Degradation (c) Oxidative Degradation, (d) Thermal Degradation, (e) Photolytic Degradation.

Table - 10: Results of Robustness

Condition	Variation	Average Area			% RSD		
		KT	PH	CPM	KT	PH	CPM
Flow rate	0.8 min	1362.585	1220.612	1743.002	0.22	1.08	0.10
	1.2 min	1312.063	1171.433	1670.100	0.27	1.68	0.79
Mobile phase	Buffer: Methanol 82:18	1292.211	1153.720	1651.123	0.36	1.08	0.36
	Buffer: Methanol 78:22	1380.259	1234.985	1763.561	0.36	1.06	0.36
pH	3.2	1352.210	1221.481	1725.361	0.95	0.40	0.85
	2.8	1314.021	1175.803	1680.022	0.26	0.91	0.29

Table - 10: Stability study results

Type of degradation	Drug	Peak Area of Standard	Conditions	Peak area			
				Standard		Sample	
				Area	% Deg.	Area	% Deg.
Acid degradation	KT	1375.914	3 hours at Room Temperature	948.859	31.04	917.320	33.33
	PH	1188.235		896.059	24.59	922.452	22.37
	CPM	1703.483		1229.258	27.84	1241.959	27.09
Base degradation	KT	1375.914	3.5 hours at Room Temperature	1183.122	14.01	1214.977	11.70
	PH	1188.235		868.124	26.94	903.220	23.99
	CPM	1703.483		1359.600	20.19	1342.088	21.22
Oxidative degradation	KT	1375.914	3 hours at Room Temperature	1104.251	19.74	1112.322	19.16
	PH	1188.235		744.19	37.37	820.753	30.93
	CPM	1703.483		1194.536	29.88	1231.006	27.74
Thermal degradation	KT	1375.914	3 hours at 105^0C	1046.853	23.92	1062.598	22.77
	PH	1188.235		842.347	29.11	861.52	27.50
	CPM	1703.483		1358.621	20.24	1312.323	22.96
Photolytic degradation	KT	1375.914	4 hours in direct Sun light	1139.95	17.15	1117.34	18.79
	PH	1188.235		1002.518	15.63	1017.514	14.37
	CPM	1703.483		1505.403	11.63	1473.915	13.48

3. RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with Thermo scientific BDS hypersil C_{18}, 250mm×4.6mm internal diameter, 5µ particle size or equivalent column and mobile phase comprising of Acetonitrile : Buffer (0.02M potassium dihydrogen phosphate) pH 3.0 with orthophosphoric acid (80:20v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 220nm based on peak area. The retention time for Ketorolac, Chlorpheniramine Maleate and Phenylephrine Hydrochloride were found to be 3.56 min, 4.92 min and 8.32 min, respectively.

The optimized method was validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported. The method was found to be linear in the concentration range of 5–15 μg/mL with correlation coefficient of 0.9995 for Ketorolac, 2–6 μg/mL with correlation coefficient

of 0.9982 for Chlorpheniramine Maleate and 1.2–3.6 μg/mL with correlation coefficient of 0.9998 for Phenylephrine Hydrochloride. The results of recovery study (100.58% for Ketorolac, 101.83% for Phenylephrine Hydrochloride and 98.42% for Chlorpheniramine Maleate) suggest that the method has good recovery. The precision of the proposed method was carried in terms of the repeatability. The low% RSD (<2) values of 0.38%, 1.36% and 1.28% variation for Ketorolac, Phenylephrine Hydrochloride and Chlorpheniramine Maleate, respectively, reveals that the proposed method is precise. The LOD and LOQ values for Ketorolac were found to be 0.452µg/ml and 1.368µg/ml, for Phenylephrine Hydrochloride were 0.064µg/ml and 0.193µg/ml and for Chlorpheniramine Maleate were 0.362µg/ml and 1.096µg/ml. The results of robustness in the present method showed no significant changes. The results of analysis of drop indicated that no interference due to common excipients was observed with the developed method. Degradation studies justified the method specificity for its intended application. Therefore, the proposed method can be used for routine analysis of three drugs in their combined pharmaceutical dosage form.

4. CONCLUSION

A simple, precise, accurate and rapid method was developed for simultaneous estimation of Ketorolac, Phenylephrine Hydrochloride and Chlorpheniramine Maleate from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims. Hence, this method can be easily and conveniently adopted for routine analysis of Ketorolac, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in pure form and its dosage form.

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5. REFERENCES

1. Kumar A, Sharma A, Nair A and Saini G. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Nimesulide, Phenylephrine Hydrochloride, Chlorpheniramine Maleate and Caffeine Anhydrous in Pharmaceutical Dosage Form. **Acta Poloniae Pharmaceutica - Drug Research.** 2012; 69(6): 1017-1022.
2. Patel NP, Samanthula G, Shrigod V, Modh SC. and Chaudhari JR. RP-HPLC Method for Determination of Several NSAIDs and Their Combination Drugs. Chromatography **Research International.** 2013; 2013: Article ID 242868, 13 pages.
3. Sanchaniya PM, Mehta FA and Uchadadiya NB. Development and Validation of an RP-HPLC Method for Estimation of Chlorpheniramine Maleate, Ibuprofen, and Phenylephrine Hydrochloride in Combined Pharmaceutical Dosage Form, **Chromatography Research International.** 2013; Article ID 424865, 6 pages.
4. Palled M, Karagane S, Mane A, Bhat A and Shinde P. Analytical Method Development and Validation of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide in Tablet Dosage Form by RP- HPLC. **International Journal of Pharmaceutical Science Invention.** 2013; 2(2): 9-15.
5. Bandelwar R, Nikam A and Sawant S. Analytical Method Development and Validation of Phenylephrine Hydrochloride, Chlorpheniramine Maleate, Paracetamol and Caffeine in Bulk Drug and Tablet Dosage Form by RP-HPLC. **Indo American journal of Pharmaceutical Research.** 2013; 3(2): 4330-4338.
6. Patel KB, Thula KC and Maheshwari DG. Stability Indicating HPLC Method for simultaneous estimation of Ciprofloxacin and Phenylephrine in Pharmaceutical Dosage Form. **Pharmacophore.** 2014; 5(2): 262-272.
7. Khairnar DA, Chaudhari CS and Anantwar SP. Method Development and Validation of Ketorolac Tromethamine n Tablet Formulation by RP-HPLC Method. **International Journal of Pharmaceutical Sciences and Research.** 2014; 5(9): 3696-3703.
8. Prathap B, Dey A, and Rao GHS. Analytical Method Development and Validation for simultaneous estimation of Febuxostat and Ketorolac in Bulk and Pharmaceutical Dosage Form in Rat Plasma by RP-HPLC. Indo **American Journal of Pharmaceutical Research.** 2014; 4(4): 1717-1729.
9. Ali A, Ahmed M, Mahmud T, Qadir MA. Nadeem K and Saleem A. Stability-indicating High-performance Liquid Chromatography Method for Simultaneous Determination of Aminophylline and Chlorpheniramine Maleate

in Pharmaceutical Formulations. **Indian Journal of Pharmaceutical Sciences.** 2015; 77(5): 515-521.

10. FDA. **Guidelines on General Principles of Process Validation.** 1987.
11. Lambert J. **Validation Guidelines for Pharmaceutical Dosage Forms.** Health Canada/ Health Products and Food Branch Inspectorate. 2004; 7-15.
12. Nash RA and Watcher AH**. Pharmaceutical Process Validation an International Third Edition Revised and Expanded,** Marcel Dekker Inc., New York. 2003; 760-792.
13. **ICH Guidance on analytical Method Validation** In: proceedings of International Conference of Harmonization, Geneva: 1996.