International Journal of
Medicinal Chemistry & Analysis
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
www.ijmca.com
Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CHLORAMPHENICOL AND HYDROCARTISONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Chloramphenicol and Hydrocortisone in Tablet dosage form. Chromatogram was run through Std BDS C18 250 x 4.6 mm, 5m. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 50:50 was pamped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 245 nm. Retention time of Chloramphenicol and Hydrocortisone were found to be 2.136 min and 2.871 min. %RSD of the Chloramphenicol and Hydrocortisone were and found to be 0.3 and 0.5respectively. %Recovery was obtained as 99.38% and 99.83% for Chloramphenicol and Hydrocortisone respectively. LOD, LOQ values obtained from regression equations of Chloramphenicol and Hydrocortisone were 0.05, 0.14 and 0.26, 0.79 respectively. Regression equation of Chloramphenicol is y = 13708x + 17978, and y = 16395x + 1048 of Hydrocortisone. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Chloramphenicol, Hydrocartisone, RP-HPLC.

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INTRODUCTION

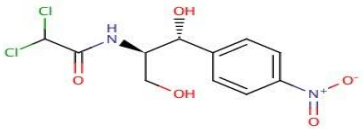
Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeias) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure, comparative laboratory data including merits / demerits should be made available.

Access this article online		
Home page: http://ijmca.com/ DOI: http://dx.doi.org/10.21276/ijmca.2019.9.2.2		Quick Response code
Received:25.07.19	**Revised**:12.08.19	**Accepted**:25.08.19

CHLORAMPHENICOL

Snonym: Chloromycetin
Chemical Formula: $C_{11}H_{12}Cl_2N_2O_5$
IUPAC Name: 2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide
Molecular Weight : Average: 323.129
Physical State: white Amorphous power
Solubility: Soluble in water (2.5 g/l) at 25° C, methanol, ethanol, butanol, ethyl acetate, acetone, DMSO, and propylene glycol (150.8 mg/ml).
Storage: Store at room temperature
Melting Point: 149-152° C
Density: 1.5 g/cm^3 (Predicted)

Fig. 1. Chemical structure of chloramphenicol



Category: Antibiotic used in bacterial infections

Mechanism of action

Chloramphenicol is lipid-soluble, allowing it to diffuse through the bacterial cell membrane. It then reversibly binds to the L16 protein of the 50S subunit of bacterial ribosomes, where transfer of amino acids to growing peptide chains is prevented (perhaps by suppression of peptidyl transferase activity), thus inhibiting peptide bond formation and subsequent protein synthesis [2].

HYDROCARTISONE

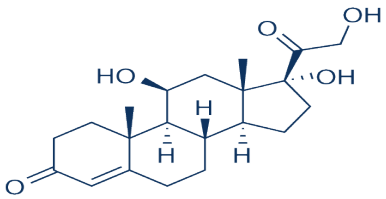
Synonym: Corticosteroid, corticoid

Chemical Formula: $C_{21}H_{30}O_5$

IUPAC Name: (1S,2R,10S,11S,14R,15S,17S)-14,17-dihydroxy-14-(2-hydroxyacetyl)-2,15-dimethyltetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadec-6-en-5-one

Weight: Average: 362.4599

Fig. 2. Chemical structure of hydrocortisone



Solubility: Soluble at 25°C in: water 0.28; ethanol 15.0; methanol 6.2; acetone 9.3; chloroform 1.6; propylene glycol 12.7; ether ~ 0.35. Soluble in concentrated sulfuric acid with intense green fluorescence

Storage: Store at room temperature

Protein binding: 95%

Metabolism: Primarily hepatic via CYP3A4

Half life: 6-8 hours

Category: Reduces inflammation

Mechanism of action

Hydrocortisone binds to the cytosolic glucocorticoid receptor. After binding the receptor the newly formed receptor-ligand complex translocates itself into the cell nucleus, where it binds to many glucocorticoid response elements (GRE) in the promoter region of the target genes. The DNA bound receptor then interacts with basic transcription factors, causing the increase in expression of specific target genes. The anti-inflammatory actions of corticosteroids are thought to involve lipocortins, phospholipase A2 inhibitory proteins which, through inhibition arachidonic acid, control the biosynthesis of prostaglandins and leukotrienes. Specifically glucocorticoids induce lipocortin-1 (annexin-1) synthesis, which then binds to cell membranes preventing the phospholipase A2 from coming into contact with its substrate arachidonic acid.

MATERIALS AND METHODS

Preparation of Standard stock solutions

Accurately weighed 25 mg of Chloramphenicol, 12.5 mg of Hydrocortisone and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Chloramphenicol and 50µg/ml of Hydrocortisone)

Preparation of Sample stock solutions

Take 5 gm of ointment (**XENICOL-H**) transferred into a 25 ml volumetric flask, add 10ml of Diluent, stirr for 40min on magnetic stirrer and made up to mark with methanol and then It was centrifuged for 20 min. Then the supernatant liquid was collected and filtered using 0.45µm filters using (Millipore, Milford, PVDF) (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

Preparation of Sample working solutions (100% solution)

2.5 ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent. (100µg/ml of Chloramphenicol and 50µg/ml of Hydrocortisone)

Preparation of buffer

0.1% OPA Buffer: 1 ml of Conc Ortho Phosphoric acid was diluted to 1000 ml with water.

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Chromatographic conditions

Mobile phase: 50% 0.1% OPA buffer: 50% Acetonitrile

Flow rate: 1 ml/min

Column: BDS C_{18} (4.6 x 250mm, 5µm)

Detector wave length: 245nm

Column temperature: 30°C

Injection volume: 10µL

Run time: 6 min

Diluent: Water and Acetonitrile in the ratio 50:50

VALIDATION

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Chloramphenicol (100ppm) and Hydrocortisone (50ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined [3].

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific [4].

Precision

Preparation of Standard stock solutions

Accurately weighed 25 mg of Chloramphenicol, 12.5 mg of Hydrocortisone and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

Preparation of standard working solutions (100% solution)

1 ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml Chloramphenicol of and 50µg/ml of Hydrocortisone)

Preparation of sample stock solutions

Take 5gm of ointment (**XENICOL-H**) transferred into a 25 ml volumetric flask, add 10ml of diluent, stirr for 40min on magnetic stirrer and made up to mark with methanol and then It was centrifuged for 20 min. Then the supernatant liquid was collected and filtered using 0.45µm filters using (Millipore, Milford, PVDF) (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

Preparation of sample working solutions (100% solution)

2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Chloramphenicol and 50µg/ml of Hydrocortisone)

Linearity

Preparation of standard stock solutions

Accurately weighed 25 mg of Chloramphenicol, 12.5 mg of Hydrocortisone and transferred to individual 10 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

25% Standard solution: 0.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml (25µg/ml of Chloramphenicol and 12.5 µg/ml of Hydrocortisone)

50% Standard solution: 0.5 ml each from two standard stock solutions was pipetted out and made up to 10 ml. (50µg/ml of Chloramphenicol and 25µg/ml of Hydrocortisone)

75% Standard solution: 0.75 ml each from two standard stock solutions was pipetted out and made up to 10 ml. (75µg/ml of Chloramphenicol and 37.5µg/ml of Hydrocortisone)

100% Standard solution: 1.0 ml each from two standard stock solutions was pipetted out and made up to 10 ml. (100µg/ml of Chloramphenicol and 50µg/ml of Hydrocortisone)

125% Standard solution: 1.25 ml each from two standard stock solutions was pipetted out and made up to 10 ml. (125µg/ml of Chloramphenicol and 62.5µg/ml of Hydrocortisone)

150% Standard solution: 1.5 ml each from two standard stock solutions was pipettede out and made up to 10 ml (150µg/ml of Chloramphenicol and 75µg/ml of Hydrocortisone)

Accuracy

Preparation of standard stock solutions

Accurately weighed 25 mg of Chloramphenicol, 12.5 mg of Hydrocortisone and transferred to individual 10 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as standard stock solution 1and 2. (1000µg/ml of Chloramphenicol and 500µg/ml of Hydrocortisone)

Preparation of 50% Spiked Solution

0.5 ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution:

1.5 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.

Robustness

Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit [5].

LOD sample preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Chloramphenicol, Hydrocortisone, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample preparation

0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Chloramphenicol, Hydrocortisone, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent [6].

Degradation studies [7]

Oxidation

To 1 ml of stock solution of Chloramphenicol and Hydrocortisone, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml & 50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation studies

To 1 ml of stock s solution Chloramphenicol and Hydrocortisone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. .The resultant solution was diluted to obtain 100 µg/ml&50µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Chloramphenicol and Hydrocortisone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 100µg/ml & 50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry heat degradation Studies

The standard drug solution was placed in oven at 105°C for 1h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml & 50µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo stability studies

The photochemical stability of the drug was also studied by exposing the 100µg/ml & 500µg/ml solution to UV light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml & 50µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample..

Neutral degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hr at a temperature of 60º. For HPLC study, the resultant solution was diluted to 100µg/ml & 50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Table 1. Drugs Used

S.No	NAME	SUPPLIER
1	Chloramphenicol and Hydrocrtisone	Syntho pharmaceuticals pvt.LTD

Table 2. Instruments Used

S.No	NAME	SUPPLIER (MODEL)
1	Hplc	Waters (2695)
2	P^H meter	Adwa (ad 1020)
3	Ultra sonicator	BVK enterprises,
4	Centrifuse	Sku (4001)
5	Magnetic stirrer	Labman (MS-300)

Table 3. Chemical Specifications

S.No	NAME	SUPPLIER
1	Orthophosphoric acid (opa)	Rankem
2	Acitonitrile	Rankem
3	Methanol	Rankem
4	Glacial acetic acid	Rankem

RESUITS AND DISCUSSION

Table 4. System suitability parameters for Chloramphenicol and Hydrocortisone

S.No	Chloramphenicol			Hydrocortisone			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.136	4072	1.26	2.871	5291	1.04	4.9
2	2.138	4050	1.25	2.884	5245	1.04	5.0
3	2.141	4097	1.29	2.887	5211	1.03	4.9
4	2.141	4113	1.29	2.888	5206	1.04	4.9
5	2.152	4256	1.25	2.897	5329	1.04	5.1
6	2.156	4201	1.28	2.906	5602	1.05	4.9

Table 5. Linearity table for Chloramphenicol and Hydrocortisone

Chloramphenicol		Hydrocortisone	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
25	357852	12.5	206433
50	710674	25	411367
75	1066814	37.5	624758
100	1413656	50	813559
125	1712085	62.5	1016001
150	2061319	75	1239025

Table 6. System precision table of Chloramphenicol and Hydrocortisone

S.No	Area of Chloramphenicol	Area of Hydrocortisone
1	1413897	806035
2	1413857	802128
3	1432179	815003
4	1423883	822985
5	1414668	812649
6	1416429	813085
Mean	1419152	811981
S.D	7419.7	7270.8
%RSD	0.5	0.9

Table 7. Repeatability table of Chloramphenicol and Hydrocortisone

S.No	Area of Chloramphenicol	Area of Hydrocortisone
1	1404237	818339
2	1408593	807309
3	1410216	806301
4	1417199	809717
5	1410533	810108
6	1410626	811970
Mean	1410234	810624
S.D	4181.2	4291.8
%RSD	0.3	0.5

Table 8. Intermediate precision table of Chloramphenicol and Hydrocortisone

S.No	Area of Chloramphenicol	Area of Hydrocortisone
1	1400243	798035
2	1395857	791138
3	1380011	805003
4	1403883	808288
5	1404668	798649
6	1396429	808576
Mean	1396849	801615
S.D	9020.1	6868.0
%RSD	0.6	0.9

Table 9. Accuracy table of Chloramphenicol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.61	99.21	99.38%
	50	49.51	99.02	
	50	49.57	99.15	
100%	100	99.44	99.44	
	100	98.43	98.43	
	100	99.77	99.77	
150%	150	148.02	98.68	
	150	150.60	100.40	
	150	150.44	100.29	

Table 10. Accuracy table of Hydrocortisone

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.97	99.86	99.83%
	25	25.05	100.18	
	25	24.90	99.60	
100%	50	50.01	100.01	
	50	49.92	99.84	
	50	49.87	99.74	
150%	75	74.70	99.60	
	75	75.64	100.85	
	75	74.10	98.80	

Table 11. Sensitivity table of Chloramphenicol and Hydrocortisone

Molecule	LOD	LOQ
Chloramphenicol	0.05	0.14
Hydrocortisone	0.26	0.79

Table 12. Robustness data for Chloramphenicol and Hydrocortisone

S.No	Condition	%RSD of Chloramphenicol	%RSD of Hydrocortisone
1	Flow rate (-) 0.9ml/min	0.7	0.7
2	Flow rate (+) 1.1ml/min	1.2	0.9
3	Mobile phase (-) 55B:45A	0.4	0.9
4	Mobile phase (+) 45B:55A	1.4	0.5
5	Temperature (-) 25°C	0.8	0.6
6	Temperature (+) 35°C	0.5	0.3

Table 13. Assay Data of Chloramphenicol

S.No	Standard Area	Sample area	% Assay
1	1413897	1404237	98.85
2	1413857	1408593	99.16
3	1432179	1410216	99.27
4	1423883	1417199	99.76
5	1414668	1410533	99.29
6	1416429	1410626	99.30
Avg	1419152	1410234	99.27
Stdev	7419.7	4181.2	0.29
%RSD	0.5	0.3	0.3

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Table 14. Assay Data of Hydrocortisone

S.No	Standard Area	Sample area	% Assay
1	806035	818339	100.68
2	802128	807309	99.33
3	815003	806301	99.20
4	822985	809717	99.62
5	812649	810108	99.67
6	813085	811970	99.90
Avg	811302	810624	99.73
Stdev	7270.8	4291.8	0.5280
%RSD	0.9	0.5	0.5

Table 15. Degradation data

Type of degradation	Chloramphenicol			Hydrocortisone		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	1345260	94.70	5.30	752625	92.60	7.40
Base	1350964	95.10	4.90	763925	93.99	6.01
Peroxide	1358280	95.61	4.39	785272	96.61	3.39
Thermal	1382912	97.35	2.65	796053	97.94	2.06
Uv	1394984	98.20	1.80	801877	98.66	1.34
Water	1406318	99.00	1.00	809203	99.56	0.44

Table 16. Summary

Parameters		Chloramphenicol	Hydrocortisone	LIMIT
Linearity Range (µg/ml)		25-150µg/ml	12.5-75 µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		13708	16395	
Intercept(c)		17978	1048.	
Regression equation (Y=mx+c)		y = 13708x + 17978	y = 16395x + 1048.	
Assay(%mean assay)		99.27%	99.73%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.5	0.9	NMT 2.0%
Method precision %RSD		0.3	0.5	NMT 2.0%
Accuracy %recovery		99.38%	99.83%	98-102%
LOD		0.05	0.26	NMT 3
LOQ		0.14	0.79	NMT 10
Robustness	**FM**	0.7	0.7	%RSD NMT 2.0
	FP	1.2	0.9	
	MM	0.4	0.9	

	MP	1.4	0.5	
	TM	0.8	0.6	
	TP	0.5	0.3	

Fig. 3. System suitability Chromatogram

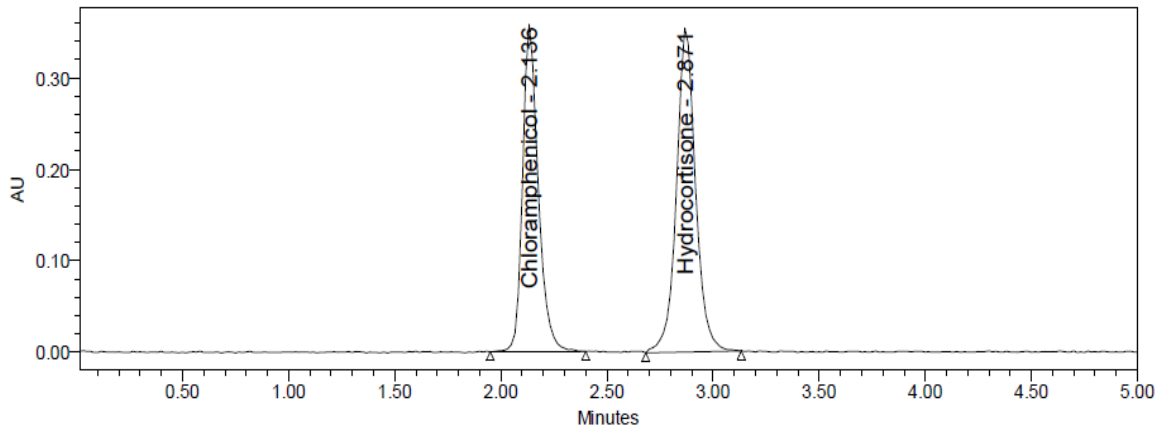


Fig. 4. Chromatogram of Sample

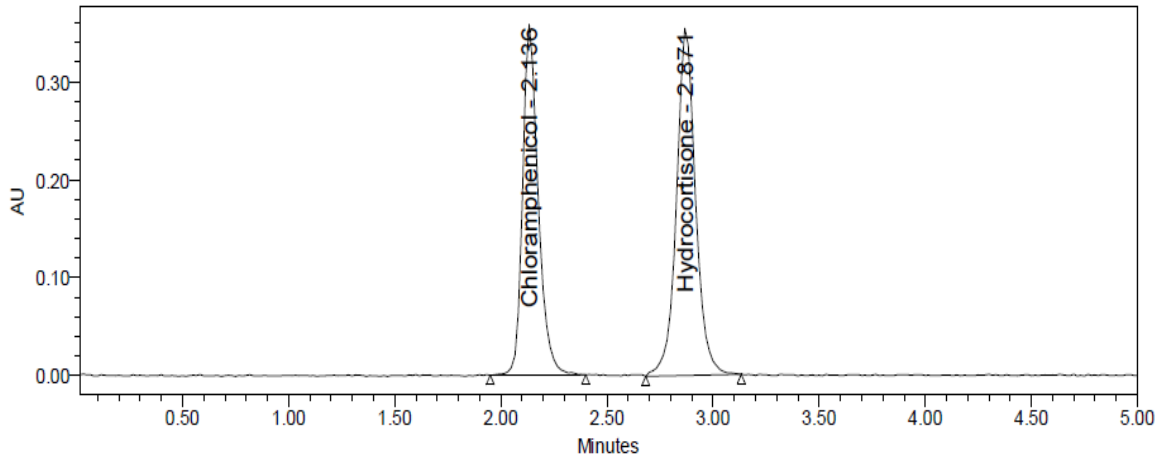


Fig. 5. Linearity 100% Chromatogram of Chloramphenicol and Hydrocortisone Injection-1

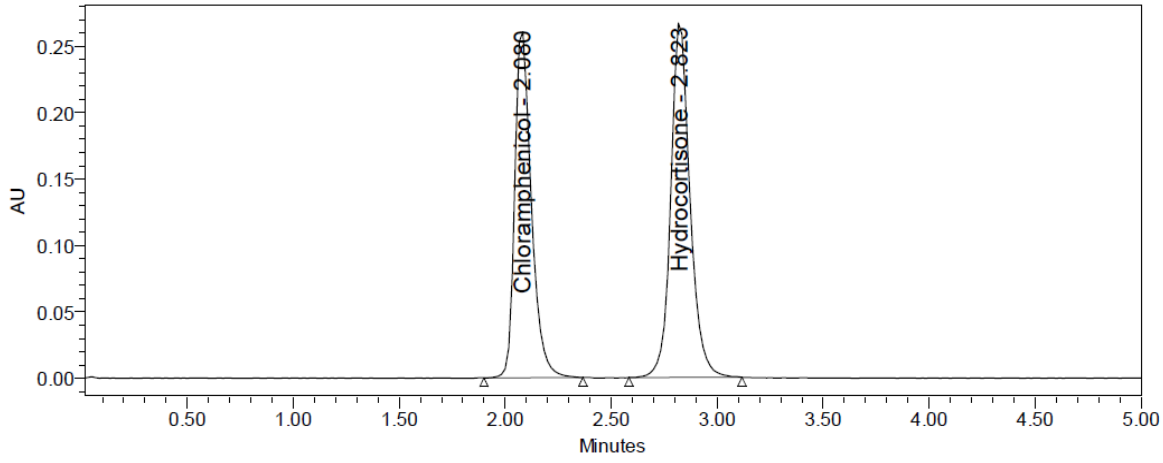


Fig. 6. LOD Chromatogram of Standard

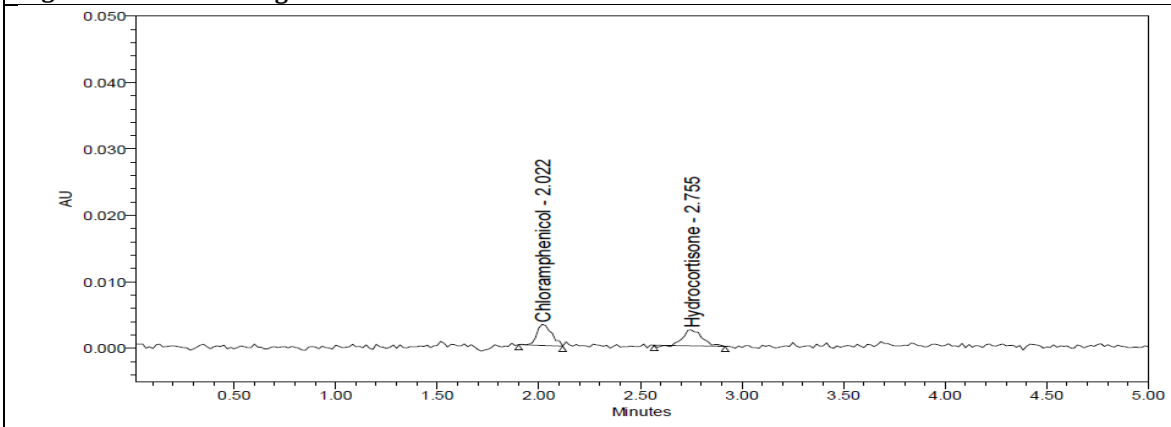


Fig. 7. Thermal degradation chromatogram

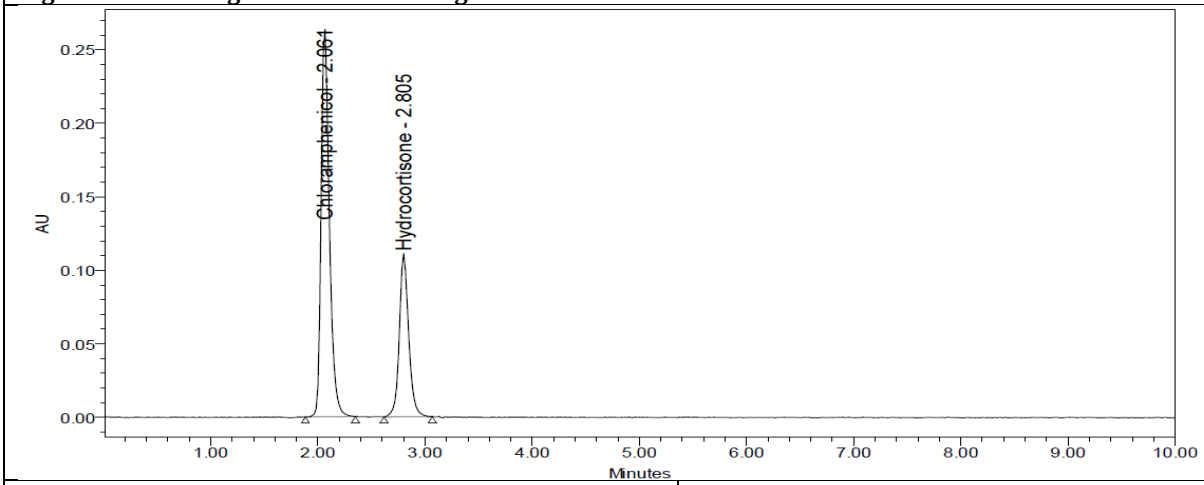


Fig. 8. Calibration curve of Chloramphenicol

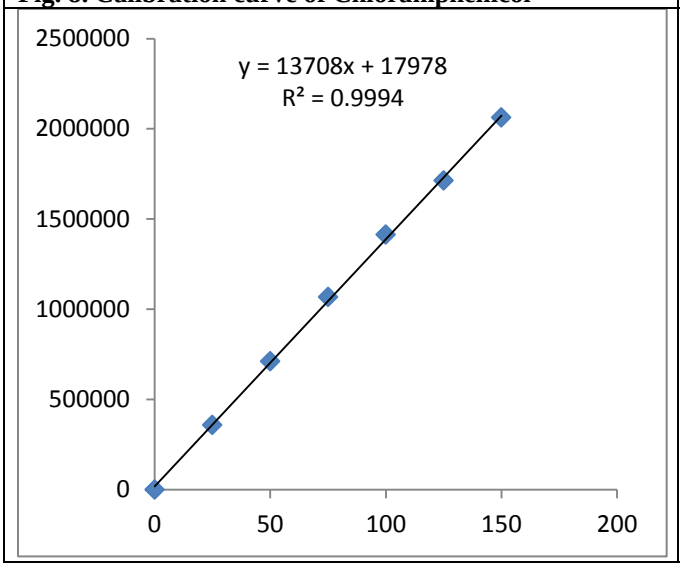
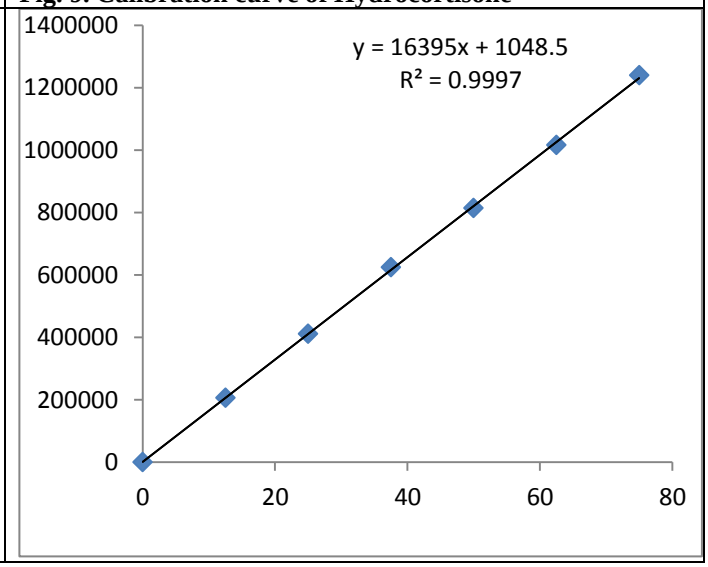


Fig. 9. Calibration curve of Hydrocortisone



System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Specificity

Retention times of Chloramphenicol and Hydrocortisone were 2.136 min and 2.871 min respectively was not found the interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Six linear concentrations of Chloramphenicol (25-150µg/ml) and Hydrocortisone (12.5-75µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Chloramphenicol was y = 13708x + 17978 and of Hydrocortisone was y = 16395x + 1048. Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 0.9% respectively for Chloramphenicol and Hydrocortisone. As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.3% and 0.5% respectively for Chloramphenicol and Hydrocortisone. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision)

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.6% and 0.9% respectively for Chloramphenicol and Hydrocortisone. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.38% and 99.83% for Chloramphenicol and Hydrocortisone respectively.

Robustness

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay

XENON Pharmaceuticals (**XENICOL-H**), bearing the label claim Chloramphenicol 10mg, Hydrocortisone 5mg. Assay was performed with the above formulation. Average % Assay for Chloramphenicol and Hydrocortisone obtained was 99.27% and 99.73% respectively.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Chloramphenicol and Hydrocortisone in Tablet dosage form. Retention time of Chloramphenicol and Hydrocortisone were found to be 2.136min and 8.871min. % RSD of the Chloramphenicol and Hydrocortisone were and found to be 0.3 and 0.5 respectively. % Recovery was obtained as 99.38 % and 99.83% for Chloramphenicol and Hydrocortisone respectively. LOD, LOQ values obtained from regression equations of Chloramphenicol and Hydrocortisone were 0.05, 0.14 and 0.26, 0.79 respectively. Regression equation of Chloramphenicol is y = 13708x + 17978, and y = 16395x + 1048. of Hydrocortisone. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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