INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 3 | Jul - Sep - 2019
Journal Home page: www.ijpar.com

Research article *Open Access*

Stability indicating development and validation for simultaneous estimation of ciprofloxacin and fluocinolone RP-HPLC method

Sridhar Siddiraju[1]*, S.B. Bhanja[1], M. Sudhakar[1], P. Vandana[1], Dillip Kumar Jena[2]

[1]*Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Osmania University, Maisammaguda, Hyderabad, India.*

[2]*Department of Pharmaceutical Chemistry, Gayatri Institute of Science and Technology, Regeda, Gunpur, Odisha, India.*

***Corresponding Author: Sridhar Siddiraju**
Email: drss.chem@gmail.com

ABSTRACT

The objective of study was to develop simple, Accurate, precise method for the simultaneous estimation of the Ciprofloxacin and Fluocinolone in Tablet dosage form. Chromatogram was run through Std Kromasil 250 x 4.6 mm, 5µ. Mobile phase containing Buffer Ortho phosphoric acid: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% Ortho phosphoric acid buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Ciprofloxacin and Fluocinolone were found to be 2.149 min and 3.513. %RSD of the Ciprofloxacin and Fluocinolone were and found to be 0.6 and 0.5 respectively. %Recovery was obtained as 99.36% and 99.71% for Ciprofloxacin and Fluocinolone respectively. LOD, LOQ values obtained from regression equations of Ciprofloxacin and Fluocinolone were 0.13, 0.39 and 0.02, 0.06 respectively. Regression equation of Ciprofloxacin is y = 26832.x + 26137, and of y = 53678.x + 5169 Fluocinolone.

Keywords: Ciprofloxacin, Fluocinolone, RP-HPLC

INTRODUCTION

Fluocinolone Acetonide [7-15] is a glucocorticoid derivative used topically in the treatment of various skin disorders. It is usually employed as a cream, gel, lotion, or ointment. Fluocinolone Acetonide is a corticosteroid that 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. This leads to diminished eicosanoid production. Cyclooxygenase (both COX-1 and COX-2). Ciprofloxacin [1-6] is a broad-spectrum antimicrobial carboxyfluoroquinoline. the chemical structure was shown in fig.1.Ciprofloxacin has in vitro activity against a wide range of gram-negative and gram-positive microorganisms. The mechanism of action of quinolones, including ciprofloxacin, is different from that of other antimicrobial agents such as beta-lactams, macrolides, tetracyclines, or aminoglycosides; therefore, organisms resistant to these drugs may be susceptible to ciprofloxacin. The ciprofloxacin acts from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination. the chemical structure was shown in fig.1.

The literature survey reveals that there is no analytical method available for estimation of Ciprofloxacin and Fluocinolone. The reported methods available for the individual estimation of ciprofloxacin and combination with Fluocinolone [16, 17] and ciprofloxacin and other drugs hplc method [18-20] Since the lack of official high performance liquid chromatographic methods for the simultaneous estimation of Ciprofloxacin and Fluocinolone, we have planned to develop a new, simple, precise, economic and accurate Stability indicating RP-HPLC method development and validation for the estimation of Ciprofloxacin and Fluocinolone in pharmaceutical dosage form according to ICH [21-23] Guidelines.

Importance of analysis is the formulation of Ciprobiotic FC having lot of advantages for treating of Bacterial infections, Microbial infections, Inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses and other conditions. Till date no official methods developed for combination of Ciprobiotic FC. So this method is used for routine analysis of Ciprobiotic FC in industries.

EXPERIMENTAL

Materials and methods

Active pharmaceutical ingredients Ciprofloxacin and Fluocinolone were obtained as a gift sample from Spectrum pharma research solutions, Hyderabad. The pharmaceutical dosage form (Ciprobiotic FC from Emcure labs Ltd) was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai.

Instrumentation and chromatographic conditions

The analysis was performed on a high performance liquid chromatography system consists of waters 2695 with 2996 module Photo Diode Array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed by using Empower2 software. The chromatographic separation was performed on Kromasil C_{18} column (150mm x 4.6mm x 5µ).The flow rate was kept at 1ml/min. The column temperature was maintained at 30°C.The mobile phase was made of 0.01N Ortho phosphoric acid buffer and Acetonitrile in 55:45 ratio had gave acceptable retention time and good resolution between Ciprofloxacin and Fluocinolone. The method was optimized at 230nm. Data acquisition and processing was performed by using empower2 system software. The run time was taken as 10min. All the determinations are carried out at an ambient temperature.

Preparation of Standard stock solutions

Accurately weighed 7.5mg of Ciprofloxacin, 3.75mg of Fluocinolone and transferred to 10ml and 100ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (750µg/ml of Ciprofloxacin and 37.5µg/ml Fluocinolone) 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (75µg/ml of Ciprofloxacin and 3.75µg/ml of Fluocinolone)

Preparation of Sample stock solutions

5 gm cream were weighed and the average weight of 5gm cream was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (750µg/ml of Ciprofloxacin and 37.5µg/ml of Fluocinolone). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (75µg/ml of Ciprofloxacin and 3.75µg/ml of Fluocinolone)

Preparation of buffer

Buffer: (0.1% Ortho phosphoric acid buffer)

1 ml of Ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Method validation

The method was validated according to ICH guidelines. The different validation characteristics which were performed are following: Linearity, accuracy, Precision, limit of detection, limit of quantification, robustness and the stability indicating capability.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Ciprofloxacin (75ppm) and Fluocinolone (3.75ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity

The linearity of the method is determined by preparing three individual series of solutions in the range of Ciprofloxacin (18.5-112.5µg/ml) and Fluocinolone (0.9375-5.625g/ml). The obtained peak areas are plotted against concentration.

Preparation of linearity solutions

Accurately weighed 7.5mg of Ciprofloxacin, and 3.75mg of Fluocinolone and transferred to three 25ml volumetric flasks separately. 10ml of diluent was added to flasks and sonicated for 15mins. Flasks were made up with diluent and labeled as Standard stock solution 1, 2 and 3.

From three stock solutions pipette out 0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml, 1.50ml into 10ml volumetric flak to get 25%,50%, 75%, 100%, 125%, 150% of standard solutions.

Precision

Method precision (repeatability)

The method precision/ repeatability can be determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated.

Intermediate precision

The intermediate precision can be determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, %Relative standard deviation, %assay were calculated. The results obtained were within the acceptance criteria.

Accuracy

Accuracy is tested by the standard addition method at three different levels 50, 100 and 150%. The percentage recoveries of Ciprofloxacin and Fluocinolone present in the pharmaceutical dosage form were calculated.

Preparation of 50% Spiked Solution

From the formulation 5ml of solution was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 50% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% standard stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution

From the formulation 10ml of solution was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 100% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution

From the formulation 15ml of solution was taken into a 50ml volumetric flask and made up with diluents followed by filtration with HPLC filters and labeled as Accuracy 150% Sample stock solution. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Standard stock solution was spiked and made up with diluents.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) of Ciprofloxacin, and Fluocinolone were determined by calibration curve method. Solutions of Ciprofloxacin, and Fluocinolone were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration

Method robustness

The robustness can be determined by varying the following parameters:

Robustness of the developed method was determined by making small deliberate changes in flow rate (±0.1ml/min), column temperature (±5%), organic mobile phase ratio (±10%), along with the optimized method.

Forced degradation studies

Oxidation

To 1 ml of stock solution of Ciprofloxacin, and Fluocinolone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 75µg/ml and 3.75µg/ml of all components 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 ssolution Ciprofloxacin, and Fluocinolone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 75µg/ml and 3.75µg/ml of all components 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 Ciprofloxacin, and Fluocinolone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 75µg/ml and 3.75µg/ml of all components 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 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 75µg/ml and 3.75µg/ml of all components and10µl were inject d 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 24µg/ml, 40µg/ml and 100µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 75µg/ml and 3.75µg/ml of all components 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 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 75µg/ml and 3.75µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSIONS

Development and optimization of HPLC method

The present work was focused to develop a stability indicating RP-HPLC method for the simultaneous estimation of Ciprofloxacin, and Fluocinolone in pharmaceutical dosage form. The solubility of the active pharmaceutical ingredient was checked in different solvents like methanol, water, acetonitrile and in different ratios but finally the standard is soluble in water: (50:50) so it was

chosen as a diluent. The different mobile phases like Acetonitrile and water, Water and methanol and Acetonitrile and Ortho phosphoric acid buffer were used in compositions with a flow rate of 1ml/min but the peak resolution, retention time and tailing factor were not satisfactory, so at last 0.1% Ortho phosphoric acid and Acetonitrile was selected as a buffer at flow rate of 1ml/min. Initially ODS®"(150mm x 4.6mm x 5µ) and " BDS" (150mm x 4.6mm x 5µ) columns with different temperatures like 30, 35, 40, 45°C were used but the retention time, run time and peak resolution were not exact and the problem was get rid by using kromosil® C_{18} column (250mm x 4.6mm x 5µ) kept at 30°c with a run time of 10 minutes. Finally the method was optimized by altering the mobile phase composition / ratio and the optimized wavelength of three drugs Ciprofloxacin, and Fluocinolone was found to be at 230nm.

Forced degradation studies

The stability studies were conducted by exposing the dosage form to different stress conditions like acid, base, peroxide, thermal, light and water. It was found that the dosage form was slightly degraded in acid, base peroxide and thermal conditions but stable in photolytic and hydrolytic conditions. Chromatograms were shown in Fig 2.

System suitability parameters

The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times were 2.149min and 3.513min for Ciprofloxacin, and Fluocinolone, plate count was >2000, peak tailing was <2 and the %RSD of peak areas of six injections were ≤ 2% (Table 1). Hence the proposed method was successfully applied to routine analysis without any problems. Chromatograms were shown in Fig 4.

Linearity range

The linearity range was in the interval of Ciprofloxacin (18.5-112.5µg/ml) and Fluocinolone (0.9375-5.625µg/ml), respectively. These were represented by a linear regression equation as follows: y (Ciprofloxacin) = 26832.x + 26137. (r^2=0.999) and y (Fluocinolone) = 53678.x + 5169. Regression line was established by least squares method and correlation coefficient (r^2) for Ciprofloxacin and Fluocinolone were found to be greater than 0.999. Hence the curves established were linear (Table 2). Chromatograms were shown in Fig 5.

Precision

Six replicates injections at the same concentration were analyzed on same day and two different days for verifying the variation in the precision and the % RSD for Ciprofloxacin, and Fluocinolone were within acceptable limit of ≤2. Hence the method is reproducible on different days with different analyst and column. This indicates that the method is precise (Table 3).

Accuracy

The percentage recoveries for Ciprofloxacin, and Fluocinolone were found to be 99.36% and 99.71% respectively (Table 4, 5). The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

Limit of detection (LOD) and limit of quantification (LOQ)

The determined values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values for Ciprofloxacin were found to be 0.13 and 0.39µg/ml and Fluocinolone were found to be 0.02 and 0.06µg/ml µg/ml respectively (Table 6).

Robustness

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Ciprofloxacin, and Fluocinolone solution (Table 7). After this the results obtained were compared with that of optimized method. It was confirmed that by the deliberate changes in the parameters there was no any significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor.

Assay

The Content of Ciprofloxacin, and Fluocinolone in the pharmaceutical dosage form were found by using the developed method. The percentage purity of Ciprofloxacin, and Fluocinolone were found to be 99.17% and 99.06% and %RSD values for Ciprofloxacin, and Fluocinolone in were within limit of ≤2.

Forced degradation studies

The forced degradation studies were conducted and all the parameters for Ciprofloxacin, and Fluocinolone were within the limits. Ciprofloxacin, and Fluocinolone has shown significant sensitivity towards the treatment of HCl, NaOH and peroxide solutions. The drugs gradually undergone degradation with time and prominent degradation was observed. Ciprofloxacin, and Fluocinolone were stable under forced thermal degradation, photolytic and neutral degradations. From the degradation studies, Peak purity test results derived from PDA detector, confirmed that the Ciprofloxacin, and Fluocinolone peaks were homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 95.33%. Chromatograms were shown in Fig 2 and purity plots were shown in Fig 3.

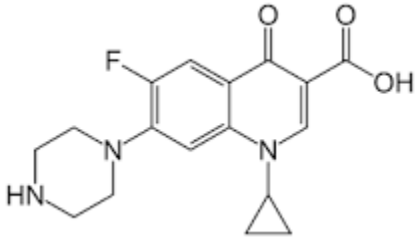

Figure 1: Structure of Ciprofloxacin

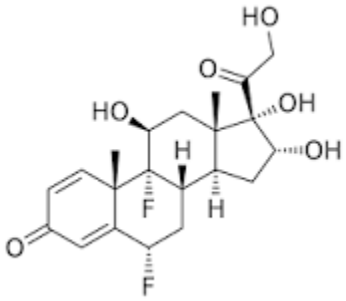

Figure 2: Structure of fluocinolone

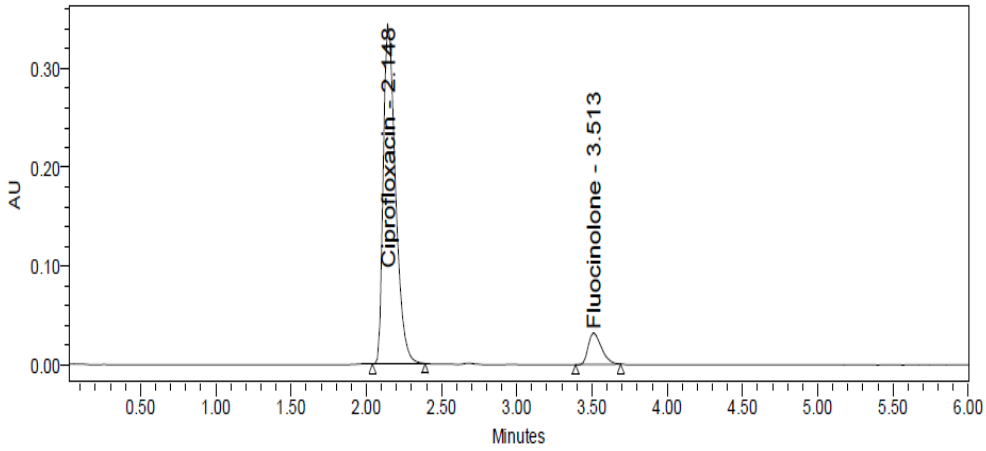

Figure 3. Standard chromatogram of Ciprofloxacin and Fluocinolone

Table 1: System suitability parameters for Ciprofloxacin and Fluocinolone.

S no	Ciprofloxacin			Fluocinolone			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.146	3316	1.42	3.509	7199	1.37	8.6
2	2.148	3443	1.40	3.513	7353	1.38	8.6

3	2.148	3429	1.40	3.513	7232	1.36	8.5
4	2.148	3390	1.40	3.518	7344	1.36	8.5
5	2.149	3354	1.38	3.518	7381	1.36	8.6
6	2.150	3407	1.40	3.518	7450	1.35	8.7

Table 2: Linearity table for Ciprofloxacin and Fluocinolone.

Ciprofloxacin		**Fluocinolone**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
18.5	573607	0.9375	58370
37.5	1013070	1.875	107153
56.25	1512375	2.8125	159128
75	2057156	3.75	208479
93.75	2551123	4.6875	255086
112.5	3033852	5.625	304766

Table 3: Determination of repeatability and intermediate precision

	Repeatability		**Intermediate precision**	
S. No	**Area of Ciprofloxacin**	**Area of Fluocinolone**	**Area of Ciprofloxacin**	**Area of Fluocinolone**
1.	1948996	197187	1920349	195898
2.	1932825	195181	1910129	193141
3.	1928925	196651	1922819	192835
4.	1930814	196271	1912307	194966
5.	1943153	195177	1921095	196239
6.	1915727	194577	1919771	194134
Mean	1933407	195841	1917745	194536
S.D	11642.5	1012.6	5204.4	1410.0
%RSD	0.6	0.5	0.3	0.7

Table 4: Determination of Accuracy of Ciprofloxacin

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	37.5	37.07502	98.87	
	37.5	37.02367	98.73	99.36%
	37.5	37.18828	99.17	
100%	75	73.77814	98.37	
	75	74.08017	98.77	
	75	75.35771	100.48	
150%	112.5	112.1417	99.68	
	112.5	112.5559	100.05	
	112.5	112.6694	100.15	

Table 5: Determination of Accuracy of Fluocinolone

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	3.75	1.871111	99.79	99.71%
	3.75	1.918691	102.33	
	3.75	1.860846	99.25	

100%	3.75	3.681872	98.18
	3.75	3.725893	99.36
	3.75	3.764475	100.39
150%	3.75	5.527209	98.26
	3.75	5.634068	100.16
	3.75	5.604913	99.64

Table 6: Sensitivity table of Ciprofloxacin and Fluocinolone

Molecule	LOD	LOQ
Ciprofloxacin	0.13	0.39
Fluocinolone	0.02	0.06

Table 7: Robustness data for Ciprofloxacin and Fluocinolone.

S.no	Condition	%RSD of Ciprofloxacin	%RSD of Fluocinolone
1	Flow rate (-) 1.1ml/min	0.7	0.7
2	Flow rate (+) 1.3ml/min	0.3	0.3
3	Mobile phase (-) 35B:65A	0.7	0.5
4	Mobile phase (+) 45B:55A	0.9	0.3
5	Temperature (-) 25°C	0.1	0.4
6	Temperature (+) 35°C	0.4	1.3

Table 8: Degradation Data of Ciprofloxacin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.34	0.815	0.968
2	Alkali	2.84	0.036	0.318
3	Oxidation	1.75	0.312	0.417
4	Thermal	0.81	0.160	0.301
5	UV	0.73	0.226	0.382
6	Water	0.56	0.177	0.315

Table 9: Degradation Data of Fluocinolone

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.67	3.577	7.793
2	Alkali	2.90	0.801	1.069
3	Oxidation	1.59	0.764	1.318
4	Thermal	0.45	0.697	0.986
5	UV	0.44	1.621	1.948
6	Water	0.42	0.801	1.089

CONCLUSION

A new, simple, rapid and precise stability indicating high performance liquid chromatographic method was developed for the simultaneous estimation of Ciprofloxacin and Fluocinolone in pharmaceutical dosage form. Hence this method can be applied for the estimation of Ciprofloxacin and Fluocinolone in drug testing laboratories and pharmaceutical industries.

Acknowledgements

The authors were thankful for Spectrum pharma research solutions, Hyderabad for providing Ciprofloxacin and Fluocinolone reference standards as a gift sample to carry out the research work.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

REFERENCES

[1]. http://www.drugbank.ca/drugs/DB00331.

[2]. Benoit Viollet, Bruno Guigas, Nieves Sanz Garcia, Jocelyne Leclerc, Marc Foretz, and Fabrizio Andreelli, cellular and molecular mechanisms of Ciprofloxacin: An overview, Clincal Science (London), 122(6), 2012, 253–270.

[3]. K. D. Tripathi, Essentials of Medical Pharmacology, Jaypee brother's medical publishers (P) LTD, 6, 254-255.

[4]. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2, 2010, 1657-1658.

[5]. Steele, John W.; Faulds, Diana; Goa, Karen L. "Ciprofloxacin". Drugs & Aging. **3**(6), 1993, 532–555. doi:10.2165/00002512-199303060-00007.

[6]. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P (January 2012). "Fluocinolone, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors". 2012

[7]. "http://www.drugbank.ca/drugs/DB0903832.

[8]. Abdul-Ghani MA, DeFronzo RA. "Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus". Endocr Pract 14(6), 2008, 782–90,2010.

[9]. Nair S, Wilding JP ,"Sodium glucose cotransporter 2 inhibitors as a new treatment for diabetes mellitus". 95(1), 2012, 34–42.

[10]. "https://www.drugs.com/sfx/Fluocinolone-side-effects.html"

[11]. "http://www.rxlist.com/jardiance-drug/overdosage-contraindications.html" Terashima, H; Hama, K. "Effects of a new aldose reductase inhibitor on various tissue in vitro". J Pharamacol Exp Ther. **229,** 1984, 226–230.

[12]. Ramirez, Mary Ann; Borja, Nancy L. "Ciprofloxacin: An Aldose Reductase Inhibitor for the Treatment of Diabetic Neuropathy". Pharmacotherapy. **28**(5), 2008, 646–655. doi:10.1592/phco.28.5.646.

[13]. Authors unspecified: Schedules of controlled substances: placement of Fluocinolone into schedule V. Final rule. Fed Regist. 28, 2005, 70(144), 43633-5. [PubMed:16050051]

[14]. Su TZ, Feng MR, Weber ML: Mediation of highly concentrative uptake of Fluocinolone by L-type amino acid transport in Chinese hamster ovary and Caco-2 cells. J Pharmacol Exp Ther. 2005 313(3), 2005, 1406-15. Epub 15. [PubMed:15769862]

[15]. Li Z, Taylor CP, Weber M, Piechan J, Prior F, Bian F, Cui M, Hoffman D, Donevan S: Fluocinolone is a potent and selective ligand for alpha (2) delta-1 and alpha (2) delta-2 calcium channel subunits. Eur J Pharmacol. 30, 2011, 667(1-3), 80-90. doi: 10.1016/j.ejphar.2011.05.054. Epub 2011 Jun 1. [PubMed:21651903]

[16]. Jatin R Gohil, et al., rp-hplc method development and validation for the simultaneous estimation of Ciprofloxacin sodium and Fluocinolone sodium in parenteral preparation.

[17]. Prasanna Pradhan et al., rp-hplc method development and validation for simultaneous estimation of Ciprofloxacin and Fluocinolone in their combined dosage form.

[18]. K.vinod kumar et al., rp-hplc method development and validation for simultaneous estimation of sulbactam and Ciprofloxacin in dosage form and in plasma. International Journal of Pharma and Bio Sciences

[19]. B. Dhandapani et al., RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ciprofloxacin and Sulbactam in Parenteral Preparation

[20]. Madhuri a. Hinge et al., development and validation of high performance liquid chromatographic method for simultaneous estimation of Ciprofloxacin sodium and sulbactum sodium in combined dosage form.

[21]. ICH Stability testing of new drug substances and products (Q1AR). International conference on harmonization, IFPMA, Stability Testing of New Drug Substances and Products: Geneva 2000.

[22]. ICH, Q1A (R2) Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, 2003.

[23]. ICH, Q1B Stability Testing: Photo stability testing of New Drug Substances and Products, International Conference on Harmonization, Geneva, 1996.