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REACTION OF ACRYLIC ACID LEACHING FROM PACKAGING COMPONENT WITH MOXIFLOXACIN HYDROCHLORIDE AND THE STRUCTURAL ELUCIDATION OF REACTION PRODUCT BY LC-MS-MS.

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ABSTRACT

Light density polyethylene (LDPE) has excessive rewards as a primary container closure system (CCS) to store ocular dosage forms due to its distinguished performance. As a regulatory requirement.[1] and to ascertain the safety and efficacy of drug products, extractables and leachables need to be studied by means of extreme polarity of organic solvents and aqueous phase at extreme acidic and basic pH. If extractables are above AET; those need to be classified as potential leachables and to be controlled in drug product as a quality attribute. Leachables are being scrutinized by a unique method to evaluate suitability of CCS. There is high possibility of interaction between drug product components with leachables and generates the degradation product / reaction product / adduct which may not be possible to estimate using method designed for leachables and as a drug product impurity using quality attribute "related substances" of drug product. This study illustrates that only extractables and leachables study is not adequate to ascertain the quality of CCS and product safety. Moxifloxacin ophthalmic solution was selected as a drug product for this study. Acrylic acid is commonly used in adhesive of label of product, printing ink and varnish hence it is selected as a slow migrant. Acrylic acid is a small molecule hence there is a high possibility of its penetration through semi permeable containers. Acrylic acid tends to form adduct due to the presence of N=H bond. The N=H bond is predominant in the structure of moxifloxacin. The reaction of acrylic acid with moxifloxacin is confirmed using placebo and solution of moxifloxacin hydrochloride. The reaction is monitored for formation of moxifloxacin adduct.

The IUPAC nomenclature of moxifloxacin adduct is identified as "7-((4aS,7aS)-1-(2-carboxyethyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid" [Chemical Formula: $C_{24}H_{28}FN_3O_6$, Molecular Weight: 473.50] using universal LC-MS technique. The MS-MS analysis was used to provide mass spectroscopic fragments to support the confirmation of structure of moxifloxacin adduct. The product related degradation products are distinguished by various studies such as forced degradation, compatibility study, stress study and stability study of drug product. The intention of this study is to demonstrate the possibility of reaction between leachable and drug product packed in semipermeable CCS which may not be monitored as a product quality attribute and may be risk to the patient. This study helps to design the process to evaluate an anticipated degradation product / adduct to ensure drug purity, safety for patient and to design product specification well in advance during product development phase.

KEYWORDS: Active Pharmaceutical Ingredient, Ophthalmic Drug Product, Stress study at strenuous condition, Leachable, Container closure system, Label, Leaflet, Carton, Migration, Adduct, LC-MS, MS-MS.

1. INTRODUCTION

Safety of ophthalmic drug product is an utmost important due to direct exposure of impurities to human eye. Control strategy of most of the impurities in generic drug product are ensured by drug and excipient compatibility study, selecting appropriate excipients, optimizing manufacturing process, selecting appropriate CCS, based on innovator's information and providing appropriate product storage statement. Recent trends of most of the regulators across globe expect to control the unspecified impurities below 0.10% in generic medicinal products. As per regulators recent interpretation ICH Q3 guidance is designed for oral dosage forms and not applicable to ophthalmic drug products. The regulator also expects to control drug substance / product in line with the maximum levels observed in near expiry innovator product.[2] Migration of unwanted compounds into the food through the packaging is a challenge to food industry.[3,4] Pharmaceutical manufacturers are facing multiple challenges to control the leachables and those forms due to interaction between leachables and drug product components.[5] The analytical technique can be a challenge due to the limitations of sensitivity of instrument. The control of reaction product is a hard task due to the reaction between unknown leachables with drug product, impurities of drug product excipients and secondary reaction between impurities and leachable. This leads to a complex process to

identify and quality the levels. Several research articles[6-10] are available indicating efforts made by researchers to establish the degradation pathway due to leachable and various dosage forms. However, there is less attention given by researchers to assess the reaction product between leachables from secondary CCS and active component present in drug product. Researchers[11] have studied the interaction between moxifloxacin and ethylene glycol monoformate. Interaction of label components and acrylic acid with brimonidine tartrate is studied[12] in ophthalmic drug product. Stability data of moxifloxacin ophthalmic solution showed increasing in tend for unspecified impurity at RRT 1.23 hence, this impurity is selected for this invention. In this study, moxifloxacin ophthalmic solution and acrylic acid as a leachable from secondary CCS (carton) is selected as study model and to correlate the unspecified impurity at RRT 1.23. The reaction product is identified as moxifloxacin adduct.

2. MATERIALS AND METHODS

2.1 Materials

HPLC grade methanol manufactured by Merck was used. Moxifloxacin hydrochloride (anhydrous) was obtained from MSN Pharma Pvt. Ltd. Generic medicinal product "Moxifloxacin ophthalmic solution 0.5%" was manufactured. The placebo was manufactured in a similar manner to that of generic drug product by excluding moxifloxacin hydrochloride in manufacturing process. Acrylic acid and Varnish manufactured by Merck were used. The ultrapure water was used as a vehicle of drug product. ACS grade ammonium acetate, acetic acid, sodium phosphate monobasic, tetra butyl ammonium hydrogen sulfate and orthophosphoric acid manufactured by Sigma-Aldrich were used.

2.2 Methods

2.2.1 HPLC Analysis

Compendial analytical procedure listed in USP monograph of "Moxifloxacin ophthalmic solution" was used. L11 packing, 250 mm x 4.6 mm, 5 µm Inertsil Phenyl HPLC column was selected. Isocratic elution with flow rate 0.9mL/minutes selected for adequate separation between the peaks. Detector wavelength as 293 nm is set for estimation. The 45°C was maintained as column temperature. The 4°C temperature selected as sampler temperature. Buffer solution was prepared by weighing 0.5 g of tetra butyl ammonium hydrogen sulfate and 1.0 g of monobasic potassium phosphate and dissolving in 1000 mL of water. 2 mL of orthophosphoric acid was added in this solution and mixed well. Mobile phase was prepared by mixing buffer solution and methanol in the ratio (60 : 40). Mobile phase used as diluent.

The method was validated as per ICH Q2 guidance. The method found specific and sensitive to estimate the degradation products well below the 0.5 times to that of reporting threshold. Sensitivity solution was prepared using moxifloxacin hydrochloride. Moxifloxacin hydrochloride ophthalmic solution (equivalent to 10 mg of moxifloxacin) was diluted to achieve as 100 ppm concentration of moxifloxacin. The commercially available Moxifloxacin ophthalmic solution was analysed. Unspecified impurity at RRT 1.23 found at the level of 0.14%. The UV spectra of unspecified impurity at RRT 1.23 closely resembles to the moxifloxacin and its specified impurities hence, unspecified impurity at RRT 1.23 is selected for this study.

2.2.2 Stress study

The stability data of drug product was correlated specifically for unspecified impurity at RRT 1.23. The forced degradation was studied in strenuous environments to generate the peak at RRT 1.23. Following degradation conditions were used to forcefully degrade the samples.

Table 1.

Type of Degradation	Degradation condition
Acid	2mL of Samples mixed with 2mL 5M HCl and heated at 80°C for 1 hour on water bath.
Base	2mL of Samples mixed with 2mL 5M NaOH and heated at 80°C for 1 hour on water bath.
Oxidative	2mL of Samples mixed with 2mL 5% hydrogen peroxide heated at 60°C for 1 hour on water bath.
Thermal	Samples was exposed in oven at 60°C for 8 days.
Light	Samples was exposed to light not less than 1.2 million lux hours for visible and integrated near ultraviolet energy of NLT 200 watt hours/ square meter.

The chromatograms obtained due to a) diluent b) placebo, c) Solution of moxifloxacin hydrochloride, d) Moxifloxacin ophthalmic solution and h) forcefully degraded solutions in glass container were evaluated. It noticed that; unspecified impurity at RRT 1.23 could not generate in any forcefully degraded sample.

2.2.3 Extractable study using primary CCS

To correlate the leachable from primary packaging component, the extractable study was undertaken at elevated temperature. The drug product was stored into glass containers and LDPE bottles without applying secondary packaging components. These samples were exposed at 60 °C for 24 hours and analysed. Unspecified impurity at RRT 1.23 not observed in stressed sample stored in both containers.

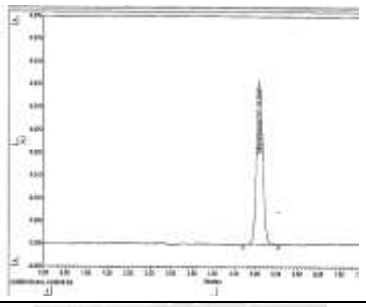
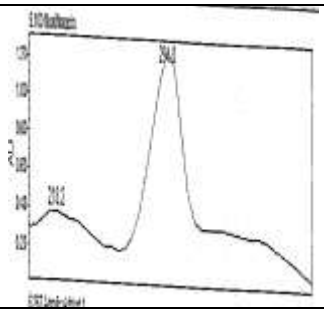
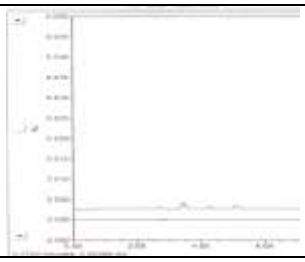
2.2.4 Extractable study using secondary CCS

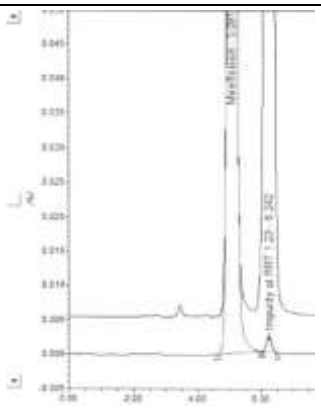
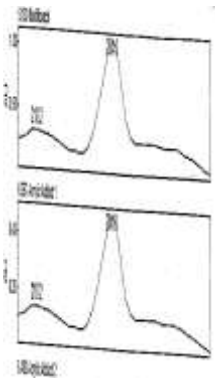
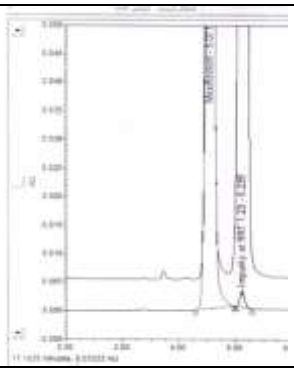
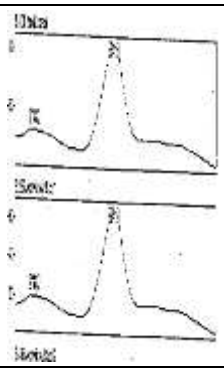
To correlate the leachable from secondary CCS a mixture of small pieces of printed label, product leaflet and product carton were mixed with placebo, solution of moxifloxacin hydrochloride and drug product. These mixtures were heated at 60 °C for 24 hours and analysed. Unspecified impurity at RRT 1.23 not observed in mixture containing placebo, printed label and drug product and mixture of product leaflet and drug product. However, it observed in mixture of carton and drug product.

2.2.5 Confirmation of adduct formation

Varnish is major component generally used in printing technology to print product information on carton. Acrylic acid is major component used in manufacturing of varnish. Hence, to check formation of unspecified impurity at RRT 1.23, placebo, solution of moxifloxacin hydrochloride and drug product were mixed with varnish and acrylic acid in glass bottle and heated at 60 °C for 24 hours. It noticed that; unspecified impurity at RRT 1.23 observed in mixture of varnish and drug product, mixture of varnish and solution of moxifloxacin hydrochloride. The unspecified impurity at RRT 1.23 also observed at significant level in mixture of acrylic acid and drug product and mixture of acrylic acid with solution of moxifloxacin hydrochloride. This study confirmed the formation of unspecified impurity at RRT 1.23 due to interaction between acrylic acid and moxifloxacin. Following Table-2 indicates the reaction sample, chromatographic output, UV spectrum of unspecified impurity at RRT 1.23 and its content.

Table 2.

Reaction sample	Chromatogram	UV spectrum of moxifloxacin & Peak at RRT 1.23	Content (in%)
a) Moxifloxacin hydrochloride standard solution (without any reaction).			N.A.
b) Placebo heated with varnish. c) Placebo heated with acrylic acid.		Peak at RRT 1.23 not observed	Not Detected

d) Solution of moxifloxacin hydrochloride heated with varnish. e) Solution of moxifloxacin hydrochloride heated with acrylic acid.			0.9 9.2
f) Drug product heated with varnish. g) Drug product heated with acrylic acid.			1.2 10.2

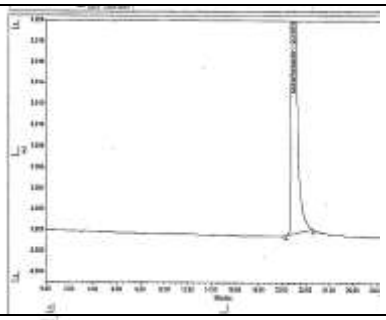
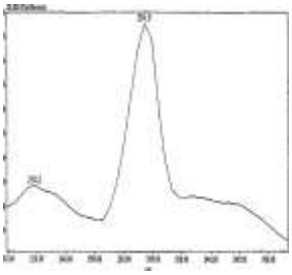
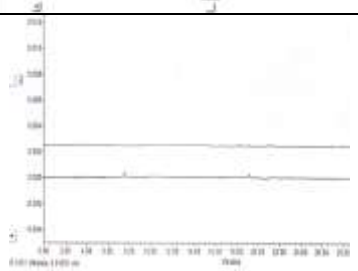
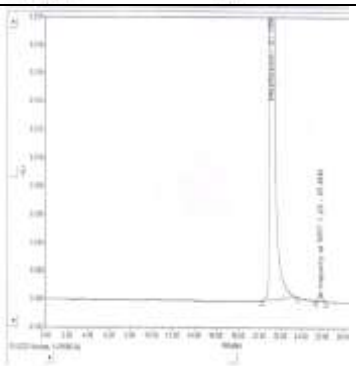
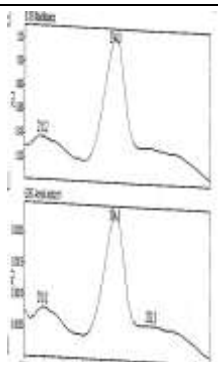
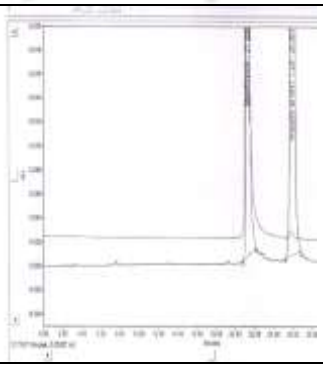
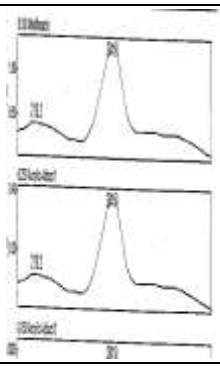
Unspecified impurity at RRT 1.23 not observed in chromatogram of mixture of placebo with varnish and acrylic acid. However, it found in chromatogram of mixture of moxifloxacin hydrochloride with varnish and acrylic acid. This impurity also seen in chromatogram of mixture of drug product with varnish and acrylic acid. UV spectrum of peak due to unspecified impurity at RRT 1.23 found comparable to the peak due to moxifloxacin. Based on this study it confirmed that moxifloxacin reacts with acrylic acid and generates moxifloxacin adduct.

2.2.6 Method development for LC-MS

Compendial procedure listed in USP monograph of "Moxifloxacin ophthalmic solution" is not compatible to LC-MS hence suitable method was developed. HPLC column L1 packing, 250 mm x 4.6 mm, 5 µm was selected. The gradient program was developed to adequately separate the peaks. Detector wavelength selected as 293 nm. The column and sampler temperature were maintained at 25°C. Mobile phase-A was prepared using ammonium acetate, water and acetic acid. Prefiltered and degassed methanol was used as mobile phase-B. Mobile phase-A was used as diluent. The method found specific, sensitive and able to discriminate product related peaks and peak due to unspecified impurity at RRT 1.23. Mixtures of placebo with varnish and acrylic acid, mixture of solution of moxifloxacin hydrochloride with varnish and acrylic acid, mixture of moxifloxacin ophthalmic solution

with varnish and acrylic acid were analyzed. The following table indicates reaction sample, chromatographic output and UV spectrum of peak of interest.

Table 3.

Reaction sample	Chromatogram	UV spectrum of moxifloxacin & Peak at RRT 1.23	Content (in%)
a) Moxifloxacin hydrochloride standard (without any reaction)			N.A.
b) Placebo heated with varnish. c) Placebo heated with acrylic acid.		Peak at RRT 1.23 not observed	Not Detected
d) Solution of moxifloxacin hydrochloride heated with varnish.			0.5
e) Drug product heated with varnish. f) Drug product heated with acrylic acid.			0.6 9.3

Unspecified impurity at RRT 1.23 not observed in chromatogram of mixture of placebo with varnish and acrylic acid. However, it observed in chromatogram of mixture of moxifloxacin hydrochloride with varnish and acrylic acid. This impurity also seen in chromatogram of mixture of moxifloxacin ophthalmic solution with varnish and acrylic acid. UV spectrum of

peak due to unspecified impurity at RRT 1.23 found comparable to the peak due to moxifloxacin. The UV spectrum of the peak due to Impurity at RRT 1.23 found same to the UV spectrum observed for the peak at RRT 1.23 in above confirmation study.

2.2.7 Estimation of molecular weight

An accurate mass was estimated using liquid chromatographic system model 1290 (Agilent Technologies, Palo Alto, CA) coupled to 6530 Q-TOF mass spectrometer equipped with a dual jet stream electrospray ionization interface. Agilent Mass Hunter software was used to control instrument and for data collection. The mass spectral behaviour obtained for the peak due to moxifloxacin and unspecified impurity at RRT 1.23 are reported in following figures.

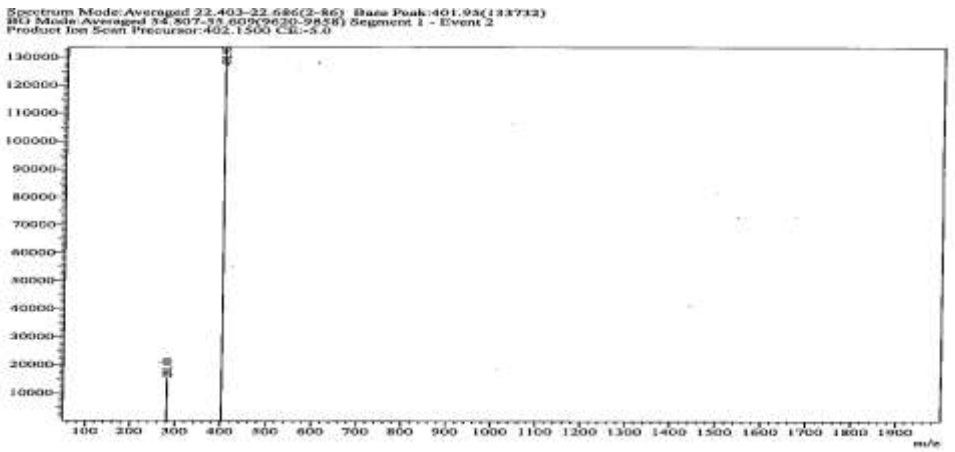


Fig-1: Mass spectra of peak due to moxifloxacin hydrochloride.

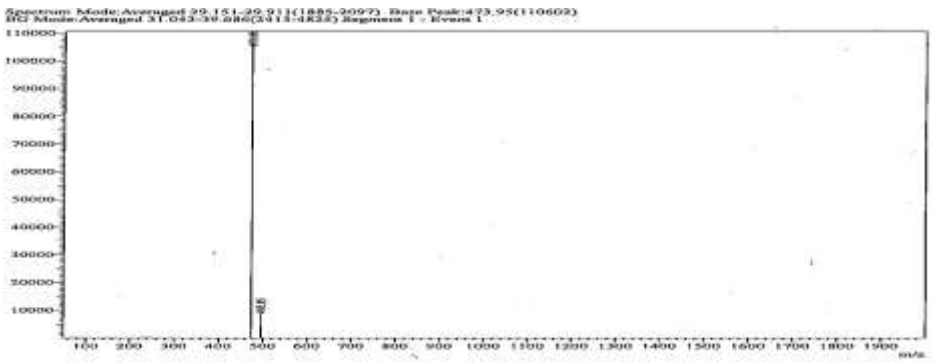


Fig-2 Mass spectral behavior of peak due to unspecified impurity at RRT 1.23.

Molecular weight of moxifloxacin found 401.95 g/mol which is corelated with theoretical molecular weight i.e. 401.43 g/mol. Molecular weight of unspecified impurity at RRT 1.23 (moxifloxacin adduct) found to be 473.95 g/mol.

2.2.8 MS-MS analysis

Chromatographic parameters used in LC-MS method (as per section 2.2.6) were used for this study. The collision energy 35V and the cell accelerator voltage 4V was applied to obtain fragmentation pattern of the precursor ion of m/z 473.95. Following figure depicts fragmentation pattern of peak of interest.

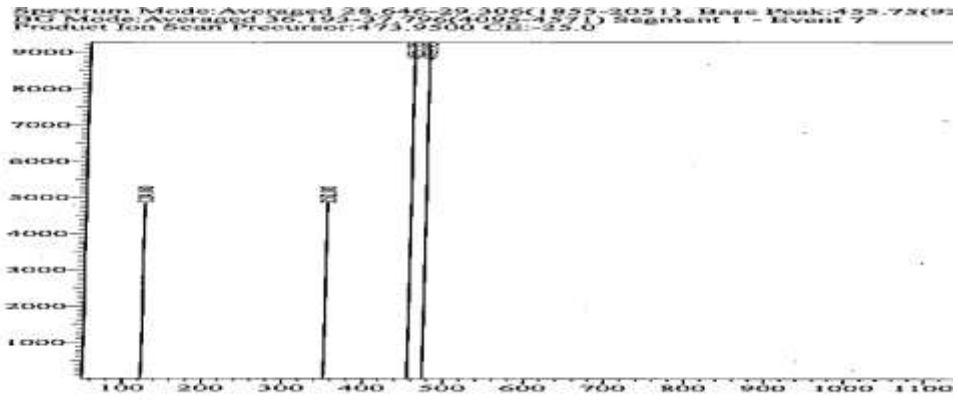


Fig 3: Fragmentated molecular weights of unspecified impurity at RRT 1.23.

Based of fragmentation of pattern of peak of interest 473.95 g/mol, 455.75 g/mol, 352.00 g/mol and 124.00 g/moles found. As a confirmatory step; the fragmentation pattern of the peak due to moxifloxacin was checked. Following figure depicts the fragmentation pattern of peak due to moxifloxacin.

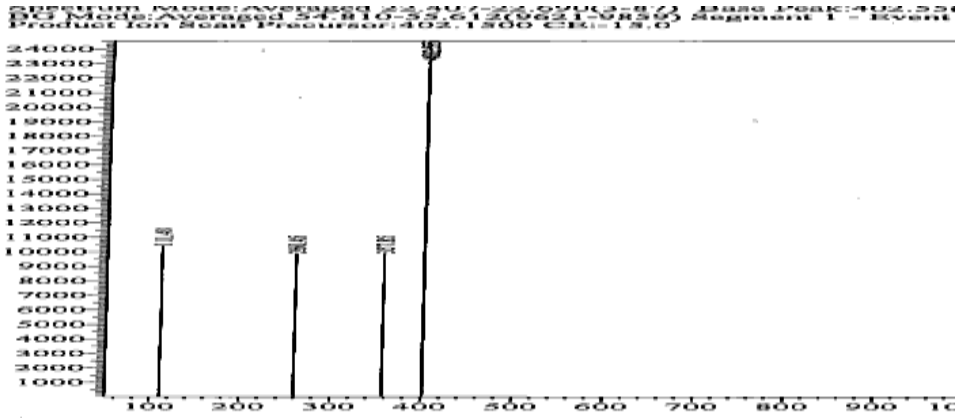
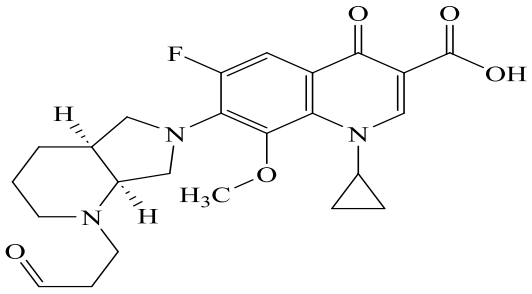
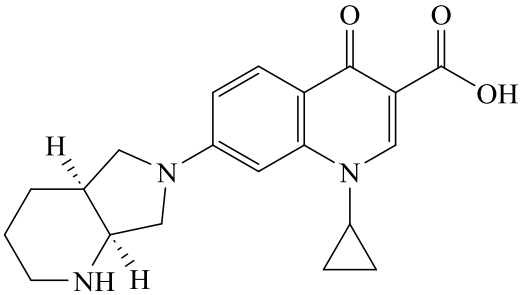


Fig-4: Fragmented molecular weight of the peak due to moxifloxacin.

Based of fragmentation of peak due to moxifloxacin 402.55 g/mol, 357.85 g/mol, 260.45 g/mol and 111.40 g/moles found. This confirms that the fragmentation pattern of unspecified impurity at RRT 1.23 is correct. The structure of principle two fragments of unspecified impurity at RRT 1.23 is depicted below.



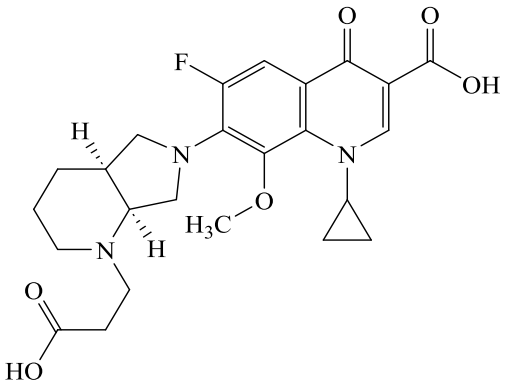
1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-((4a*S*,7a*S*)-1-(3-oxopropyl)octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-1,4-dihydroquinoline-3-carboxylic acid
Chemical Formula: $C_{24}H_{28}FN_3O_5$
Molecular Weight: 457.50



1-cyclopropyl-7-((4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
Chemical Formula: $C_{20}H_{23}N_3O_3$
Molecular Weight: 353.42

2.2.9 Isolation of moxifloxacin adduct and its characterization

The preparative HPLC method was optimized to separate the moxifloxacin adduct. 200 mL moxifloxacin ophthalmic solution and 200 mL varnish containing acrylic acid was mixed and exposed at 60 °C for 24 hours. The resultant solution was injected in preparative HPLC in several replicates. The fraction containing impurity of interest was collected. Each isolate was again analysed by compendia HPLC method to ensure the content of unspecified impurity at RRT 1.23. Enough fraction was dried using lyophilizer. The solid material was further characterized using H-NMR, IR, UV and mass spectrometer. Finally, the structure of unspecified impurity at RRT 1.23 (moxifloxacin adduct) was elucidated below.

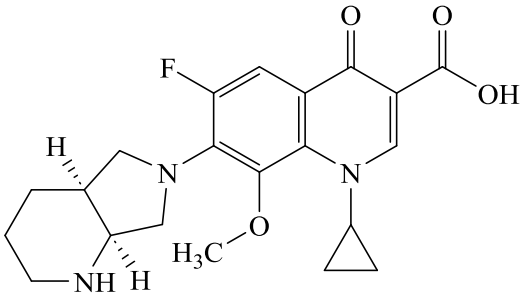


7-((4a*S*,7a*S*)-1-(2-carboxyethyl)octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
Chemical Formula: $C_{24}H_{28}FN_3O_6$
Molecular Weight: 473.50

3. RESULTS AND DISCUSSION

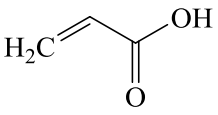
The stability data of moxifloxacin ophthalmic solution was trigger point and referred as a source information for characterization of moxifloxacin adduct. Stability data indicated that the amount of unspecified impurity at RRT 1.23 showed increasing in trend on time till product shelf life. To get information of impurity profile root cause of formation of unspecified impurity at RRT 1.23 was investigated. Moxifloxacin and Moxifloxacin ophthalmic solution related impurities were discriminated by forced degradation study using moxifloxacin hydrochloride, placebo and moxifloxacin ophthalmic solution. Finally, moxifloxacin adduct (reaction product) was discriminated based on forced degradation data, stability data and interaction study of CCS component with moxifloxacin and moxifloxacin ophthalmic solution. As a part of further invention, an interaction of moxifloxacin ophthalmic solution with packaging component was ascertained using reaction study of moxifloxacin hydrochloride and moxifloxacin ophthalmic solution with secondary packaging component, varnish and acrylic acid. The data indicated that the interaction is possible between acrylic acid and moxifloxacin. The adduct formation is possible due to Azo Michel addition reaction followed by dehydration reaction between acrylic acid and Moxifloxacin.[13,14] The azo-Michael addition reaction, an extension of the Michael addition reaction, involves the conjugate addition of nitrogen nucleophiles resulting in the synthesis of beta amino carbonyl compounds. In moxifloxacin having **"R-NH-R"** group, nitrogen forms nucleophiles and CH_2=CH_2 alkyl group acrylic acid attached to nitrogen group of moxifloxacin and formed moxifloxacin acrylate adduct, this reaction matches with Azo-Michael addition reaction. The molecular mass of reaction product was further confirmed by LC-MS and MS-MS. Further unspecified impurity at RRT 1.23 was purposefully generated using a reaction between

moxifloxacin hydrochloride and moxifloxacin ophthalmic solution with varnish containing acrylic acid. The impurity was further separated using preparative HPLC and characterized adequately using various spectral techniques. Table-2 and Table-3 depicts the similarity of UV spectral behavior of moxifloxacin adduct with moxifloxacin. Figure-1 depicts the mass spectra of moxifloxacin adduct and moxifloxacin. The control of product related impurities is ascertained by compendial procedure; however, identification and quantification of such reaction product is necessary and can be ensured referring to the study design used in this invention. Researchers have studied the formation of moxifloxacin adduct due to interaction of moxifloxacin with ethylene glycol monoformate. However, this invention provides the information of formation of another moxifloxacin adduct with same molecular weight and elutes at same retention time having different structure due to interaction of secondary packaging component i.e. acrylic acid with moxifloxacin. Formation of moxifloxacin adduct is depicted below.

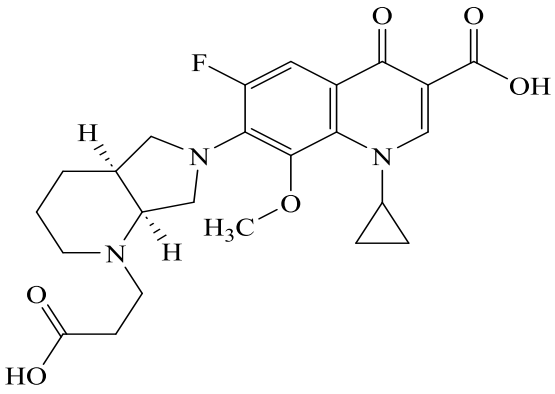


Chemical Formula: $C_{21}H_{24}FN_3O_4$
Molecular Weight: 401.44

+



Chemical Formula: $C_3H_4O_2$
Molecular Weight: 72.06



7-((4a*S*,7a*S*)-1-(2-carboxyethyl)octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid
Chemical Formula: $C_{24}H_{28}FN_3O_6$
Molecular Weight: 473.50

4. CONCLUSION

In finished dosage form of moxifloxacin ophthalmic solution, formation of moxifloxacin adduct due to interaction between moxifloxacin and ethylene glycol monoformate is possible. This invention indicates that; there is possibility of formation of another moxifloxacin adduct due to interaction between acrylic acid (leachable form secondary container closure system) and moxifloxacin. This study further concludes that; these two moxifloxacin adducts has same molecular weight but structurally different. The compendial analytical procedure reported in USP monograph can estimate each moxifloxacin adduct. However, simultaneous estimation using compendial procedure is not possible since; both these adducts elutes at same retention time. The study design of this invention benefits to understand reaction product due to possible migrant with the product matrix packed in semipermeable container closure system. Extractable and leachable studies provides information about anticipated non-reactive leachable; however, there are less chances of understanding of reaction products. This study design can help development scientists well in advance during product development phase and to select appropriate container closure system for all types of liquid drug products to be packed in semi permeable containers. This study design further provides information about systematic approach to be followed during selection of semi migrant / non migrant secondary container closure system, selection of quality of varnish and quality of printing components so that; patient safety can be ascertained during development phase itself and avoids commercial product recalls.

REFERENCES

1. *Guidance for Industry ANDA's*. Container Closure System for Packaging Human Drugs and Biologics, May 1999.
2. *Guidance for Industry ANDAs*: Impurities in Drug Products, November 2010.
3. I.S. Arvanitoyannis, L. Bosnea, Migration of substances from food packagingmaterials to foods, *Crit. Rev. Food Sci. Nutr,* 2004; 44: 63–76.
4. K.V.D. Houwe, A.V. Heyst, C. Evrard, J.V. Loco, F. Bolle, F. Lynen, E.V. Hoeck, Migration of 17 photoinitiators from printing inks and cardboard into packaged food - results of a belgian market survey, *Packag. Technol. Sci*, 2016; 29: 121–131.
5. Gagandeep Sing, Dujuan Lu, Chongming Liu, Danny Hower, Analytical challenges, and recent advances in the identification quantification of extractables and leachables in pharmaceutical and medicinal products. *TrAC Trends in analytical Chemistry*, August 2021; 141: 116266.

6. Noemí Dorival-García, Sara Carillo, Christine Ta, Dominic Roberts, Kate Comstock, Simon Lofthouse, Elena Ciceri, Kyle D'Silva, Gerald Kierans, Christian Kaisermayer, Anna Lindeberg, and Jonathan Bones; Large-Scale Assessment of Extractables and Leachable in Single-Use Bags for Biomanufacturing; *Anal. Chem*, 2018; 90: 9006-1015.
7. Cheryl L. M. Stults, Jaromir Mikl, Oliver Whelehan, Bradley Morrical, William Duffield, and Lee M. Nagao; A Risk-Based Approach to Management of Leachable Utilizing Statistical Analysis of Extractables; *AAPS Pharm Sci Tech*, April 2015; 16(2).
8. Holm R, Elder DP. Analytical advances in pharmaceutical impurity profiling. Eur, *J Pharm Sci*, 2016; 87: 118-135.
9. Thomas H Broschard, Susanne Glowienke, Uma S Bruen, Lee M Nagao, Andrew Teasdale, Cheryl L M Stults, Kim L Li, Laurie A Iciek, Greg Erexson, Elizabeth A Martin, Douglas J Ball, Assessing safety of extractables from materials and leachable in pharmaceuticals and biologics – current challenges and approaches. *Regulatory Toxicology and Pharmacology*, 2016; 81: 201-211.
10. Paskiet D, Jenke D, Ball D, Houston C, Norwood DL, Markovic I. the product Quality Research Institute (PQRI) leachable and extractables working group initiatives from parenteral and ophthalmic drug product (PODP). *PDA J. Pharm Sci Technol*, 2013; 67: 430-447.
11. Ramarao Gollapalli, Gagandeep Sing, Alejandro Blinder, Jeremiah Brittin, Arijit Sengupta, Bikash Mondal, Milan Patel, Biswajit Pati, James Lee, Amit Ghode Mahesh Kote, Identification of an Adduct Impurity of an Active Pharmaceutical Ingredient and Leachable in an Ophthalmic Drug Product Using LC-QTOF. *Journal of Pharmaceutical Sciences*, October 2019; 108(10): 3187-3197.
12. Dr. Sandeep Zokande, Dr. Kavita Inamdar and Amit Kale, Characterization of Specific Potential Degradation Products due to Interaction of Leachable from Product Label in Container Closure System of Ophthalmic Drug Product Containing Brimonidine Tartrate, *World Journal of Pharmacy and Pharmaceutical Sciences*, 12(4): 1600-1607.
13. Ana P. Esteves, Marília E. Silva, Lígia M. Rodrigues, Ana M.F. Oliveira-Campos, Radim Hrdina, Aza-Michael reactions with vinyl sulfones and Amberlyst-15 as catalyst, *Tetrahedron Letters*, 17 December 2007; 48(51): 9040-9043.
14. Aymeric Genest, Daniel Portinha, Etienne Fleury, François Ganachaud, The aza-Michael reaction as an alternative strategy to generate advanced silicon-based (macro)molecules and materials, *Progress in polymer science*, September 2017; 72: 61-110.