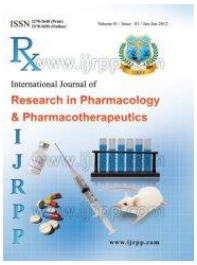
International Journal of Research in Pharmacology & Pharmacotherapeutics



IJRPP |Vol.11 | Issue 4 | Oct -Dec - 2022
Journal Home Page: www.ijrpp.com

Research Study *Open Access*

Evaluation of stereoselective dissolution of racemic ondansetron by hplc method

K. Selvakumar[1]*, J. Joysa Ruby[2]

[1]Asst. Professor, Department of Pharmaceutical Quality Assurance, Acharya & BM Reddy College of Pharmacy, Bengaluru
[2]Asst. Professor, Department of Pharmaceutics, Acharya & BM Reddy College of Pharmacy, Bengaluru.

Corresponding Author: Dr. K. Selvakumar

ABSTRACT

Pharmaceutical excipients such as cellulose (CL) and cyclodextrin (CD) derivatives are chiral in nature. Currently, these derivatives are also used as chiral stationary phases (CSPs) in liquid chromatography. The stereoselectivity of these derivatives towards enantiomers that may modulates the release of chiral drugs. Based on this, it has been hypothesized that, the stereoselectivity of the enantiomers with the CL and CD excipients may alter the release of chiral drug. Hence, the present study aimed to investigate the influence of CL and CDs on stereoselective release of formulation containing racemic ondansetron (rac-OND). In this study, formulation containing rac-OND and selected CLs; carboxymethylcellulose (CMC), hydroxypropyl methylcellulose (HPMC), and microcrystalline cellulose (MC) and CDs; beta-cyclodextrin (β-CD) and hydroxypropyl β-cyclodextrin (HCD) was prepared. Subsequently, these formulations were evaluated for enantioselective release by HPLC method. The results revealed that the formulations containing microcrystalline cellulose and hydroxypropyl methylcellulose showed slightly different release of the two enantiomers at the end of the dissolution profile. In addition, the effect of enantioselective interaction was investigated by changing content of chiral excipient and pH (1.5, 4.6 and 7.4) to support the enantioselectivity obtained on dissolution.

Keywords: **Ondansetron, Pharmaceutical excipients, Stereoselectivity, Liquid chromatography**

INTRODUCTION

Drug chirality influences drug delivery pattern because a single enantiomer may have improved solubility, dissolution, and stability when compare to their counterparts. A majority of the excipients employed in pharmaceutical dosage forms including cellulose, amylose, cyclodextrin and chitosan, etc optically pure form. These optically pure molecules may interact differently with enantiomers of a chiral drug and form stereoisomers. The latter will have different physicochemical properties from the original chiral molecule. The interaction of the enantiomers with the excipient may lead to differential rate of delivery in biological system. One of the to-be- released enantiomers favourably undergoes interaction with the chiral excipient resulting in the preferential diffusion of it[7].

The use of chiral selective dissolution testing is recommended for certain drugs that exist in racemic form in order assess the performance of a product, both *in vitro* and *in vivo*[2]. In the literature, there are different studies have been reported from the viewpoint of role of pharmaceutical excipients in chiral drug delivery. Few reports have been summarized as follows. For instance, Solinis et al. 2002, carried out the studies on release of salbutamol and ketoprofen enantiomers from hydroxypropylmethylcellulose K100M matrices containing two types of cellulose derivatives. The authors concluded that stereoselectivity is dependent on the amount of chiral excipient in the formulation.[8], evaluated *in vitro* dissolution of salbutamol from formulation containing various chiral excipients such as

gamma cyclodextrin, heptakis (2,6 di-Omethyl)–beta cyclodextrin, sulfobutyl-beta-cyclodextrin, HPMC and egg albumin. The formulation containing gamma cyclodextrin provided significant stereoselectivity throughout the dissolution profile. The release of eutomer R-salbutamol was higher than that of the distomer S-salbutamol from the gamma cyclodextrin tablets.[9], studied the enantioselective *in vitro* release of propranolol. The influence of the method of polymer synthesis, drug to polymer ratio, pH, and temperature on the release of two enantiomers was determined. The study revealed that release of eutomer can be controlled via means of formulation. The distomer was retained in the dosage form.[11], evaluated stereoselective dissolution of racemic ketoprofen from formulation containing chiral excipients such as sodium alginate, β-cyclodextrin, and hydroxymethylpropylcellulose (HPMC). In this work, the influenze of pH in stereoselective dissolution was tested. The authors reported that the release of ketoprofen enantiomers from formulation containing HPMC matrix at pH 7.4 is stereoselective. This research indicated that choosing appropriate chiral excipient and other formulation conditions affect the drug release. Based on the literature survey, it is concluded that the development of enantioselectively controlled release (ESR) dosage form of a racemate by retarding the other isomer is an approach towards the formulation of chiral drugs. For the drugs where only one of the forms (R or S) is active, the use of a stereoselective dissolution test is recommended for the development and ESR[2].,[1] stated that the enantioselectively controlled release of the desired enantiomer from a racemate while retarding the other isomer provides a novel route toward practical applications of biologically chiral molecules. Such processes may maximize the efficacy while minimizing the dosage and frequency of chiral compounds.

Ondansetron (OND) is a 5-hydroxytryptamine type 3 (5-HT3) receptor antagonists. OND is used for the prevention and treatment of nausea and vomiting. OND possesses one stereogenic center in the carbazol ring and exists in two enantiomeric forms (R- and S-). R-OND, the eutomer, is a highly selective and more potent than S-counterpart (S-OND)[5,3]. Hence, R-OND exhibit good therapeutic effect. Enantioselectively controlled release (ESR) of the desired enantiomer from a racemate by retarding the other isomer is an approach towards the formulation of chiral drugs. Hence, the present study was focused on the development of ESR dosage form to release pharmacologically active R-OND enantiomer by retarding counterpart by using commonly used pharmaceutical excipients.

MATERIALS AND METHODS

Materials

The active pharmaceutical ingredient OND was procured from Yarrow Chem Products, Mumbai. Solvents and reagents for HPLC analysis HPLC water, acetonitrile, methanol, nhexane, isopropylalcohol, diethylamine, triethylamine. All other chemicals, excipients were collected from ooja Scientific Bengaluru, Subra Scientific, Bengaluru, Research Lab Fine Chem Industries. The solvents used for HPLC analysis were HPLC grade.

Preformulation Studies

Fourier Transform Infrared (FT-IR) Analysis

The FT-IR spectra of the samples were recorded on an FT-IR spectrophotometer (Tensor 27, Bruker opics, Mumbai). The spectrum for each sample was recorded over than 450 -4000 cm^{-1}. About 5 mg of sample was mixed with 100mg of KBr and compressed into pellet using a hydraulic press. All spectra were corrected against the reference spectrum of KBr pellet.

DSC Analysis & X-Ray Diffraction Analysis

Differential scanning calorimetry (DSC) and X-Ray Diffraction analysis was performed for pure OND.

Chromatographic condition

The chromatographic separation of OND enantiomers was carried out on a Daicel Chiral Pak AS-3R (150 mm x 4.6 mm i.d., 3µm) connected with a Daicel Chiral Pak AS-3R guard cadridge. The mobile phase consisted of MeOH/water/diethylamine. Prior to use, the mobile phase was degassed for 15 min in an ultrasonic bath and vacuum filtered through 0.45µm membrane filter (Gelman Science, India). An injection volume of the sample was 20µL. The HPLC system was used in an air conditioned laboratory atmosphere (20±2ºC).

Stock and working standard solutions

Stock standard solutions of racemic OND at 1000 µgmL-1 were prepared using mixture of MeOH and water in 80:20 v/v and stored at 4ºC protected from light. The stock solutions of (RS)-OND further diluted with the mobile phase to give a series of standard mixtures having a final concentration in the range of 4-20 µg mL-1. The solution prepared for the optimization procedure comprised of (RS)-OND, at 8 µg mL^{-1}.

Calibration curve for OND enantiomers

The calibration curve for OND enantiomers was assessed at five concentration levels in the range of 4-20 µg mL^{-1} for OND. The calibration curve was plotted using the linear least squares regression procedure.

Drug Content analysis

The enantiomeric content analysis of formulation containing Racemic OND was determined by proposed HPLC method.

HPLC screening of enantioselectivity of cyclodextrin and cellulose derivatives on OND racemic mixture

Screening of OND enantiomers selectivity on selected cyclodextrin and cellulose derivatives was carried by using

HPLC method. The experiments were carried out by incorporating chiral mobile phase additives (CMPA) such as cyclodextrins (β-cyclodextrin, hydroxypropyl β-cyclodextrin) and cellulose derivatives (microcrystalline cellulose, carboxymethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, ethylcellulose and hydroxyethyl cellulose) into mobile phase consisting of different compositions of water, ACN and MeOH. The chromatographic separation was carried out by using Phenomenex C_{18} analytical column (150 × 4.6 mm, 5µm) connected to a Phenomenex C_{18} guard cadridge (4 mm x 3 mm i.d., 5 µm). The sample composition for HPLC screening is listed in Table 1.

Table 1. Composition of OND Sample

(±) OND-CE-I category	Drug	Cellulose derivatives
S1	(±)-OND	Carboxymethylcellulose
S2	(±)-OND	Hydroxypropyl cellulose
S3	(±)-OND	Hydroxypropyl methyl cellulose
S4	(±)-OND	Microcrystalline cellulose
(±) OND-CE-II category		Cyclodextrin derivatives
F5	(±)-OND	Beta-cyclodextrin
F6	(±)-OND	Hydroxypropyl β-cyclodextrin
OND-ACE-III category		
F7	(±)-OND	Eudrogit

In vitro enantioselective dissolution of OND-chiral matix tablets

The *in vitro* enantioselective dissolution studies was carried out to compare the release of both enatiomers (R-OND and S-OND) from the selected chiral excipients and to observe the enantiomeric release pattern. The *in vitro* enantioselective dissolution study was performed using the dialysis bag method (USP apparatus 1) [Amatya et al., 2013]. The dialysis bag will be dipped into the receptor compartment containing the dissolution medium of buffer solution of pH 1.5 (KCl-HCl buffer), 4.6 (acetate buffer) and 7.4 (phosphate buffer) was stirred continuously at 100 rpm maintained at 37° C. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh medium. The OND enantiomeric peaks was measured by HPLC method.

FT-IR Analysis

The FT-IR spectra for OND was shown in Fig. 1. The FT-IR interpretation results were shown in Table 2

Table 2. FT-IR interpretation of OND

Wave number (cm^{-1})	Bond
3405	O-H stretch, H-bonded
2934	C-H stretch
2869	C-H stretch
1623	C=O stretch
1577	C-C stretch (in rings)
1481	C-C stretch (in rings)
1420	C-C stretch (in rings)
1346	C-H rock
1279	C-O stretch
1196	C-N stretch
1090	C-N stretch
960	=C-H bend

DSC Analysis

Differential scanning calorimetry (DSC) analysis was performed for pure OND. The obtained thermogram for OND was shown in **Fig.** 2.

Thermal profile of OND exhibited two endothermic peaks at the range of 53-140°C due to vaporization of water and 190-200°C which was its shift in melting point.

X-Ray Diffraction Analysis

X-Ray powder diffraction (XRD) patterns of OND, was recorded. The diffractogram of OND was shown in the **Fig. 3**

The diffractogram of OND shows major characteristic peaks at 2θ at 22.66°, 23.52°, 27.44°, and 29.59° indicating the high degree of crystallinity of OND, its crystal lattice constant α corresponds to 3.92, 3.78, 3.25 and 3.02 respectively.

Calibration curve for OND enantiomers

The calibration curve for OND enantiomers was assessed at five concentration levels in the range of 4-20 µg mL^{-1} for OND (approximately 20-200 % of the nominal range of the analyte). The calibration curve was plotted using the linear least squares regression procedure. The obtained mean (n=6) regression equations were y = 0.484x – 0.132 and y = 0.489x – 0.142 for S-OND and R-OND respectively. Correlation coefficients were found to be more than 0.998 for both enantiomers. The obtained calibration curves are presented in **Fig. 4a and b**.

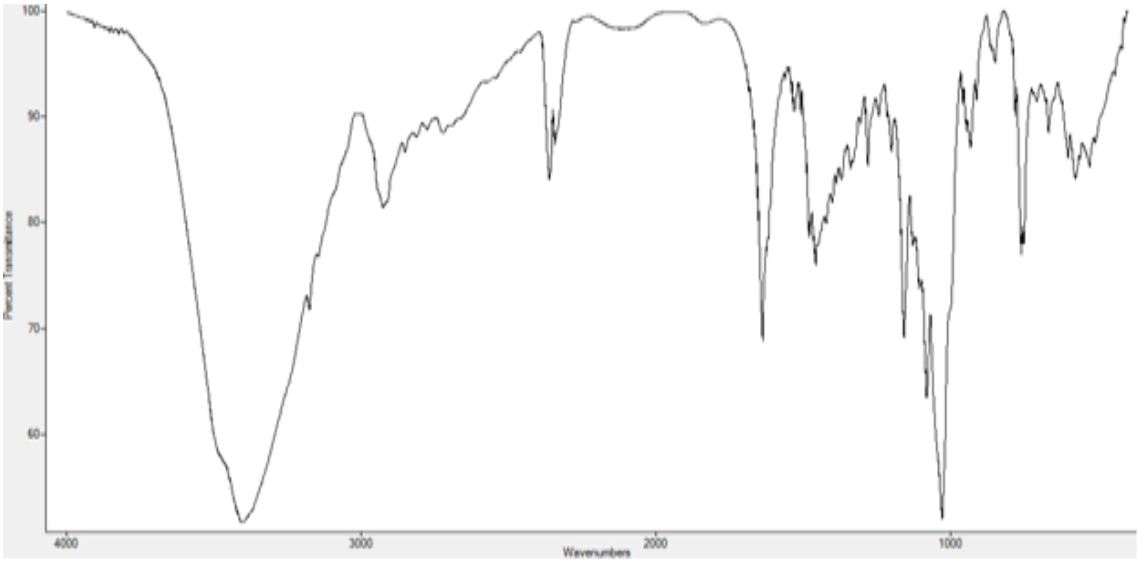


Fig.1 FT-IR spectra for OND

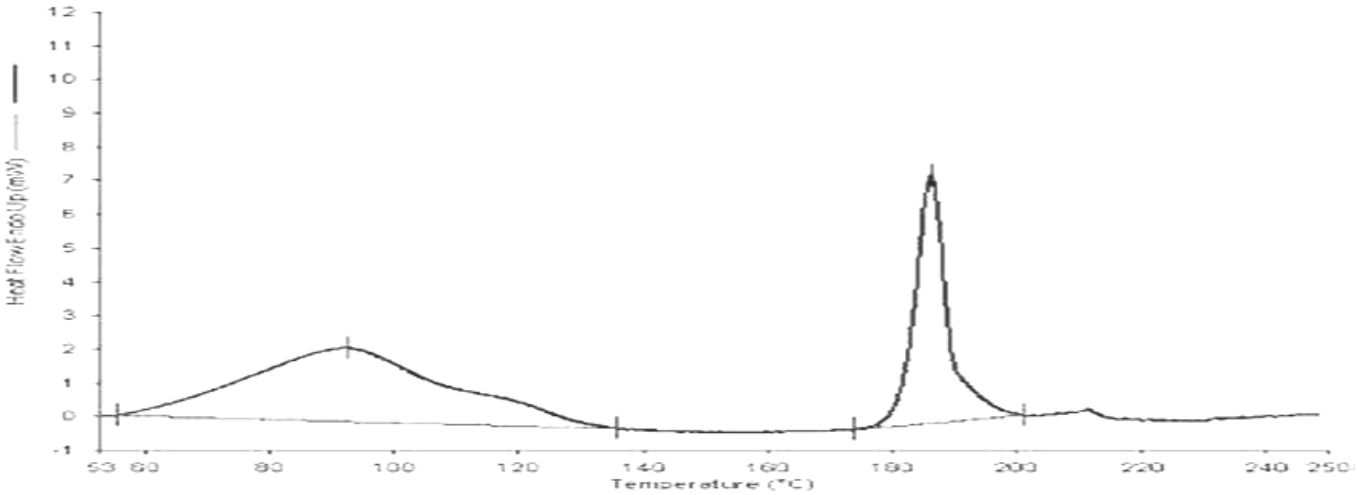


Fig 2: DSC of OND

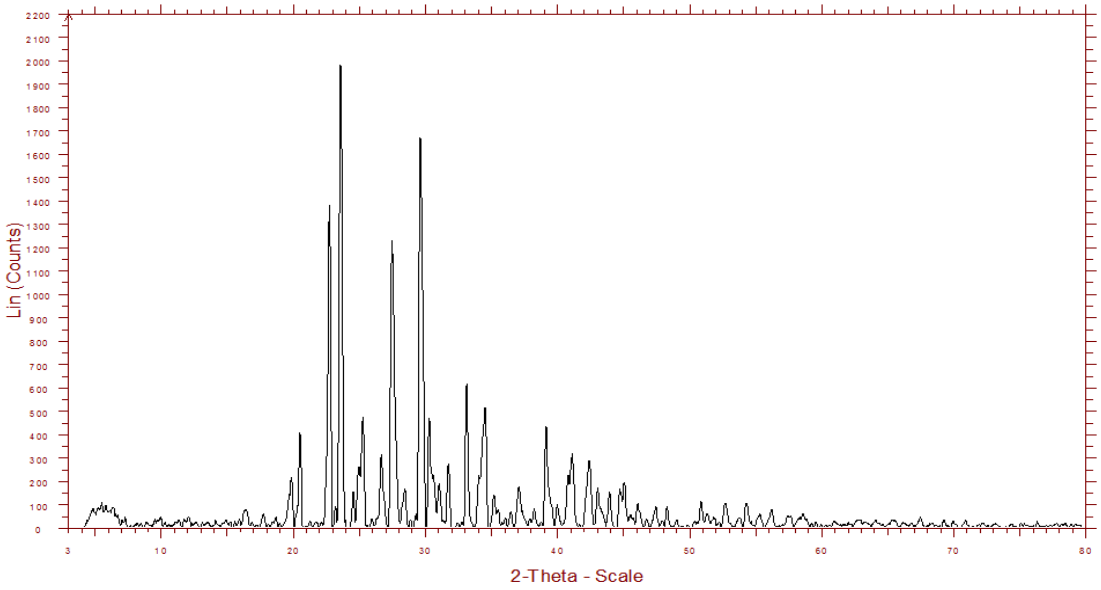


Fig. 3: XRD of OND

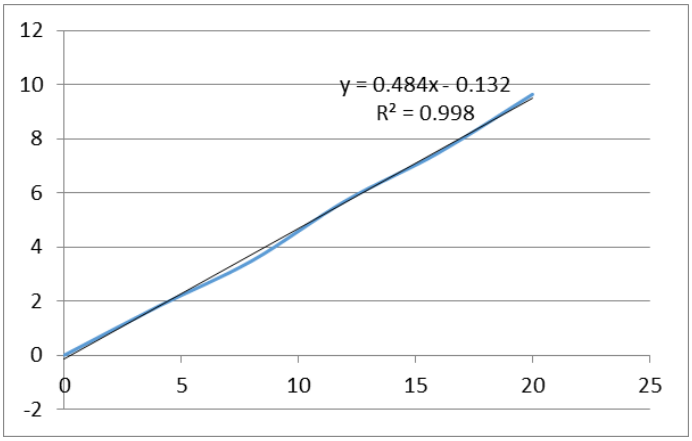


Fig. 4a Calibration graph of S-OND

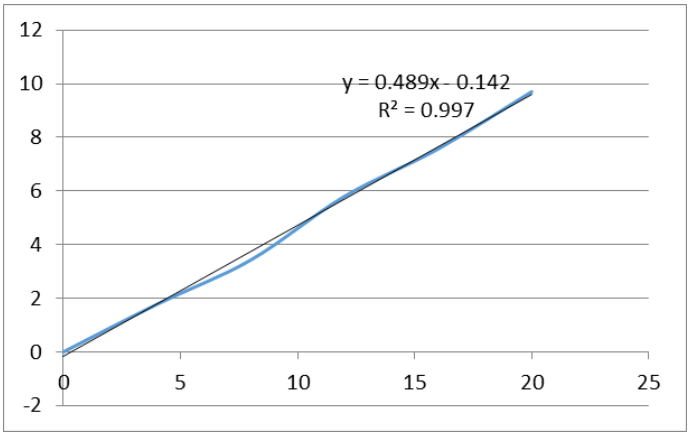


Fig. 4b Calibration graph of R-OND

HPLC screening of enantioselectivity of cyclodextrin and cellulose derivatives on OND racemic mixture

Under these screening conditions, it was observed that OND enantiomeric peaks were co-eluted and did not result any enantiomeric peak separation. This might be attributed to poor affinity of the OND enantiomers to cyclodextrin or difficulty in inclusion of the analyte into the chiral cavity. On the other hand, partial enantioselectivity of OND enantiomers peaks were observed with cellulose derivatives such as microcrystalline cellulose and hydroxypropyl methylcellulose. Hence, cellulose derivatives has been selected as chiral excipient for further evaluation. The result of the study was summarized in **Table 3**.

HPLC separation of OND enantiomers using polysaccharide based chiral stationary phase

HPLC separation of OND enantiomers was carried out as per the method reported by Valliappan and Selvakumar, 2017. The chiral separation was performed on Chiralpak AS-3R analytical column (150 mm × 4.6 mm i.d., 3 µm). The mobile phase containing solvent mixture methanol/water/diethylamine (85/15/0.1% v/v/v). Solvent mixtures are delivered at 1.5mLmin^{-1} flow rate, and enantiomeric peaks were detected at 222 nm.

Table 3. Summary of enantioselective screening of OND enantiomers by HPLC using cyclodextrin and cellulose derivatives as chiral mobile phase additive (CMPA)

#	Experimental condition	CMPA	Stationary phase	Observation
1.	**Mobile phase** Different compositions of water, ACN and MeOH.	β-cyclodextrin, Hydroxypropyl β-cyclodextrin	Phenomenex C_{18} analytical column (150×4.6 mm, 5µm)	OND enantiomeric peaks were co-eluted and did not result enantiomeric peak separation
2.	**Mobile phase** Different compositions of water, ACN and MeOH with cellulose derivatives	Microcrystalline cellulose, carboxymethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose	Phenomenex C_{18} analytical column (150×4.6 mm, 5µm)	The partial enantioselectivity of OND enantiomers was observed with microcrystalline cellulose and hydroxypropyl methylcellulose.

Preparation of OND matrix tablets

Based on the results obtained during enantioselective screening, it was observed that there was a partial enantioselectivity of OND enantiomers was observed with microcrystalline cellulose and hydroxypropyl methylcellulose. Hence, microcrystalline cellulose and hydroxypropyl methylcellulose were selected a chiral excipient to prepare OND matrix tablets. The tablets containing OND and cellulose derivative were prepared by wet granulation method.

In vitro enantioselective dissolution of OND-chiral matix tablets

The dialysis bag was dipped into the receptor compartment containing the dissolution medium of buffer solution of pH 1.5 (KCl-HCl buffer), 4.6 (acetate buffer) and 7.4 (phosphate buffer) was stirred continuously at 100 rpm maintained at 37°C. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh medium. The dissolution experiments for all the formulations were carried out as per the

method reported by[11]. The prepared tablets in the basket were placed in 900ml of dissolution medium equilibrated to 37±0.5 ℃. Samples (5 ml) were withdrawn from the from the dissolution vessel and replaced with fresh dissolution medium using auto sampler at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 min. The collected samples were filtered and the quantification of OND enantiomers was determined by HPLC method. It was observed that the release of (R- and S-) OND is affected by changing the pH.

CONCLUSION

OND possesses one stereogenic center in the carbazol ring and exists in two enantiomeric forms (R- and S-). R-OND, the eutomer, is a highly selective and more potent 5-HT3 antagonist which shows approximately eightfold higher activity than S-counterpart (S-OND). OND is commercially marketed as racemic dosage forms though the R-OND exhibit good therapeutic effect. The development stereoselective dosage form that favours the release of R-OND by retarding the S-OND from a racemic dosage form helps in acheiving better therapeutic efficacy.

ACKNOWLEDGEMENT

The authors are grateful to Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, Karnatka for providing financial assistance through research grant to carry out the research work.

REFERENCES

1. Huli Yu, Xueyong Y, Junya L, Jianping D, Youping W, Materials established for enantioselective release of chiral compounds. Industrial & Engineering Chemistry Research, 2016 55 (21), 6037-6048.
2. Mukesh, Overview on chirality and applications of stereo-selective dissolution testing in the formulation and development work. Dissolution Technologies, 2003 pp.16-20.
3. Patil PA, Kothekar MA. Development of safer molecules through chirality. Indian J Med Sci. 2006 Oct;60(10):427-37.
4. Shen Q, Wang L, Zhou H, Jiang HD, Yu LS, Zeng S. Stereoselective binding of chiral drugs to plasma proteins. Acta Pharmacol Sin. 2013 Aug;34(8):998-1006.
5. Smith HS, Cox LR, Smith EJ. 5-HT3 receptor antagonists for the treatment of nausea/vomiting. Ann Palliat Med. 2012 Jul;1(2):115-20.
6. Solinís MA, de la Cruz Y, Calvo B, Hernández RM, Gascón AR, Goñi I, Gurruchaga MD, Pedraz JL. Release of salbutamol sulphate and ketoprofen enantiomers from matrices containing HPMC and cellulose derivatives. Chirality. 2002 Nov;14(10):806-13.
7. Somagoni JMohan, Eaga CM, and Madhsudan RY. Chirality and its Importance in Pharmaceutical Field- An Overview, International Journal of Pharmaceutical Sciences and Nanotechnology, 2009, 4, pp. 309-316.
8. Srichana T, Suedee R. Evaluation of stereoselective dissolution of racemic salbutamol matrices prepared with commonly used excipients and 1H-NMR study. Drug Dev Ind Pharm. 2001 May;27(5):457-64.
9. Suedee R, Srichana T, Martin GP. Evaluation of matrices containing molecularly imprinted polymers in the enantioselective-controlled delivery of beta-blockers. J Control Release. 2000 May 15;66(2-3):135-47.
10. Urbaniak B, Milanowski B, Lulek J, Kokot ZJ. Study of ofloxacin enantiomers dissolution from selected solid dosage forms using high performance capillary electrophoresis method. Acta Pol Pharm. 2017 May;74(3):955-968.
11. Valliappan K, Kannan K, Manavalan R and Muralidharan, C. Evaluation of stereoselective dissolution of racemic ketoprofen from formulations containing chiral excipients. Indian Journal of Pharmaceutical Sciences. 2003 65. 253-259.
12. Yuan S, Peng Q, Palczewski K, Vogel H, Filipek S. Mechanistic studies on the stereoselectivity of the serotonin 5-HT1A receptor. Angew Chem Int Ed Engl. 2016 Jul 18;55(30):8661-5.