A Comparative Forced Degradation Study of Diclofenac Sodium and Ibuprofen Using UV Spectroscopy

JAYANTI MUKHERJEE [1 *], A. RAJA REDDY[2], R. KIRAN[3], S. SRIYA[4]

[1]Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana.

[2]Associate Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana

[3]Assistant Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana. Affiliated to JNTUH

[*]Address for correspondence:

Dr Jayanti Mukherjee

Professor

CMR College of Pharmacy, Medchal, Hyderabad, Telangana, India, Pin-501401

Email Id: **jayantispandan@gmail.com**

Abstract

Forced degradation also known as "stress testing" is the process in which drug is degraded forcefully by imposing stress-inducing methods. It is a useful tool to predict stability of any Active Pharmaceutical Ingredient (API) or formulation product. These studies are performed to develop and validate stability-indicating methods as per ICH guidelines. The forced degradation studies undergo acid and base hydrolysis, photolysis, thermal and oxidative degradation. It provides a data to support identification of possible degradants; degradation pathways, intrinsic stability of the drug molecule and validation of stability indicating analytical procedure. The FDA and International Conference on Harmonization guidelines state that stress testing deliberately identifies the likely degradation products which helps in determining the inherent stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedure. Force degradation studies are regulatory requirement and scientific necessity during drug development. It has become mandatory to perform stability studies of new drug moiety before filing in registration. The FDA and ICH guidelines state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors in several conditions. Knowledge of stability of the molecule helps in selecting proper formulation and package and providing proper storage condition and shelf life, which is essential for regulatory documentation. Thus, this research helps to find the stability of diclofenac sodium and Ibuprofen drugs under stress conditions by comparing absorbance values through UV spectroscopy. The outcome of the studies showed that with elapsed time period degradation increases or in other words the stability decreases.

Keywords: *Stability indicating method, Stress testing, UV spectroscopy, Diclofenac Sodium, Ibuprofen.*

Introduction

Non-steroidal anti-inflammatory (NSAIDS) are medicines that are widely used to relieve pain and reduce inflammation. NSAIDs are typically divided into groups based on their chemical structure and selectivity: acetylated salicylates (aspirin), non-acetylated salicylates (diflunisal, salsalate), propionic acids (naproxen, Ibuprofen), acetic acids (diclofenac, indomethacin), enolic acids (meloxicam, piroxicam), anthranilic acids (meclofenamate, mefenamic acid), naphthyl alanine (nabumetone), and selective COX-2 inhibitors (celecoxib, etoricoxib). The main mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). Cyclooxygenase is required to convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids. Specifically, thromboxanes play a role in platelet adhesion, prostaglandins cause vasodilation, increase the temperature set-point in the hypothalamus, and play a role in antinociception. There are two cyclooxygenase isoenzymes, COX-1 and COX-2. COX-1 gets constitutively expressed in the body, and it plays a role in maintaining gastrointestinal mucosa lining, kidney function, and platelet aggregation. Most of the NSAIDs are nonselective and inhibit both COX-1 and COX-2 receptors. COX-1 is the prime mediator for ensuring gastric mucosal integrity and COX-2 is mainly involved in inflammation, COX-2 selective NSAIDs should provide anti-inflammatory relief without compromising the gastric mucosa.[1-5]

NSAIDs have well-known adverse effects affecting the gastric mucosa, renal system, cardiovascular system, hepatic system, and hematologic system. Gastric adverse effects are likely due to the inhibition of COX-1, preventing the creation of prostaglandins that protect the gastric mucosa. The damage is more likely in a patient that has a prior history of peptic ulcers. Since it is COX-1 specific, the use of COX-2 selective NSAIDs is a lower-risk alternative. Renal adverse effects are caused due to COX-1 and COX-2 NSAIDS facilitating the production of prostaglandins that play a role in renal hemodynamics[6]. In a patient with normal renal function, inhibition of prostaglandin synthesis does not pose a large problem; however, in a patient with renal dysfunction, these prostaglandins play a greater role and can be the source of problems when reduced via NSAIDs. Complications that can occur include acute renal dysfunction, fluid and electrolyte disorders, renal papillary necrosis, and nephrotic syndrome/ interstitial nephritis[7]. Cardiovascular adverse effects can also be increased with NSAIDS uses; these include myocardial infarction, thromboembolic events, and atrial fibrillation. Diclofenac seems to be the NSAID with the highest reported increase in adverse cardiovascular events. Hepatic adverse effects are less common; NSAID-associated risk of hepatotoxicity (raised aminotransferase levels) is not very common, and liver-related hospitalization is very rare. Among the various NSAIDs, diclofenac has a higher rate of hepatotoxic effects. Hematologic adverse effects are possible, particularly with nonselective NSAIDs due to their anti-platelet activity[8]. This anti-platelet effect typically only possesses a problem if the patient has a history of GI ulcers, diseases that impair platelet activity (hemophilia, thrombocytopenia, von Willebrand, etc.), and in some perioperative cases. Other minor adverse effects include anaphylactic reactions that involve the skin and pulmonary systems, like urticaria and aspirin-exacerbated respiratory disease.[9,10]

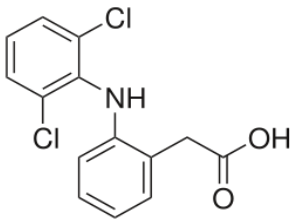
Aims and Objectives:

The present study was aimed to develop an assessment stability profile of two NSAIDs Diclofenac Sodium and Ibuprofen based on the forced degradation studies using UV Spectrophotometer. These two NSAIDs having the same solubility profile, mode of action, indications and are frequently used medicines to treat osteoarthritis and rheumatoid arthritis. The main purpose of forced degradation testing studies is to investigate stability-related properties of an API and to develop an understanding of the degradation products and pathways. Knowledge of the stability of molecule helps in selecting proper formulation and package as well as providing proper storage conditions and shelf life, which is essential for regulatory documentation. This testing may involve the drug substance alone and in simple solutions/suspensions to validate the analytical procedures. For development and validation purposes, it is appropriate to limit exposure and end the studies if extensive decomposition occurs. These studies should also be used to evaluate the susceptibility of the drug substance to hydrolyze across a wide range of pH values. Based on research, we found there is no literature on comparative degradation studies of both these drugs so it could help in detailed study of degradation and stability pattern of NSAIDs using UV- Spectroscopy.

Diclofenac Sodium

Diclofenac is a medicine that reduces swelling (inflammation) and pain. The chemical name of Diclofenac Sodium is 2-[(2,6-Dichlorophenyl) amino] benzene acetic acid sodium salt. This drug is soluble in water, phosphate-buffered saline, ethanol, dimethyl formamide, dimethyl sulfoxide, methanol and acetone. Diclofenac inhibits cyclooxygenase-1 and cyclooxygenase-2, the enzymes responsible for the production of prostaglandin (PG_2) which is the precursor to other PGs. The molecules have broad activity in pain & inflammation and the inhibition of their production is the common mechanism linking each effect of diclofenac[11]. It is completely absorbed from GI tract but likely undergoes significant first pass metabolism with only 60% of the drug reaching systemic circulation unchanged. Many topical formulations are absorbed percutaneously and produce clinically significant plasma concentrations. Absorption is dose proportional over the range of 25-150 mg. Diclofenac distributes to the synovial fluid reaching peak concentration 2-4 hour after administration. Diclofenac is over 99.7% bound to serum proteins, primarily albumin[12]. It undergoes limited binding to lipoproteins as well as with 1.1% bound to HDL, 0.3% to LDL, and 0.15% to VLDL. Diclofenac undergoes oxidative metabolism to hydroxy metabolites as well as conjugation to glucuronic acid, sulfate, and taurine. Diclofenac is used to treat mild to moderate postoperative or post-traumatic pain, in particular when inflammation is present, and is effective against menstrual pain and endometriosis[13]. It is often used to treat chronic pain associated with cancer. Some common adverse effects of Diclofenac sodium are indigestion, gas nausea, vomiting, stomach pain, diarrhea, headache, drowsiness, itching, stuffy nose, increased blood pressure, swelling or pain in arms or legs[14].

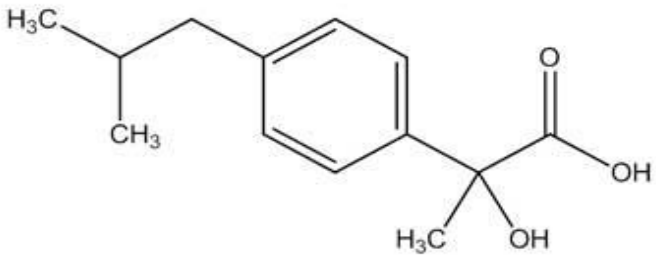
Structure of Diclofenac Sodium



Ibuprofen

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). It works by reducing hormones that cause inflammation and pain in the body. The chemical name of Ibuprofen is (RS)-2-(4-(2-methyl propyl) phenyl) propanoic acid[15]. This drug is practically insoluble in water and soluble in most organic solvents like ethanol (66.18 g/100 mL at 40^{o} C for 90% EtOH), methanol, acetone and dichloromethane. Ibuprofen is a non-selective COX inhibitor and hence, it inhibits the activity of both COX-1 and COX-2[16]. The inhibition of COX-2 activity decreases the synthesis of prostaglandins involved in mediating inflammation, pain, fever, and swelling while the inhibition of COX-1 is thought to cause some of the side effects including GI ulceration[17]. When Ibuprofen is administered immediately after a meal, there is a slight reduction in the absorption rate but there is no change in the extent of the absorption. The apparent volume of distribution of ibuprofen is of 0.1 L/kg. Ibuprofen dosage is more than 99% bound to plasma proteins and purified albumin, binding appears to be saturable and becomes non-linear at concentrations exceeding 20 mcg/ml[18]. Ibuprofen is rapidly metabolized and bio transformed in the liver to the formation of major metabolites which are the hydroxylated and carboxylated derivatives. The daily limit for ibuprofen is 1200mg[19]. It is used to reduce fever and treat pain or inflammation caused by many conditions such as headache, toothache, back pain, arthritis, menstrual cramps, or minor injury. Some of adverse effects of ibuprofen are nausea or vomiting, constipation or diarrhea, indigestion (dyspepsia) or abdominal pain[20,21].

Structure of Ibuprofen:



Materials and Methods:

Instrumentation: UV Spectrophotometer (Shimadzu UV-1601), Quartz Cuvettes, Arklite Ultraviolet lamp (Genuine Electricals), Weighing Balance: Pioneer OHAIUS (Item PA214C)

Materials: Diclofenac Sodium, Ibuprofen, ethanol, 0.1 N Hydrochloric acid, 0.1 N Sodium Hydroxide, 0.3% Hydrogen Peroxide and distilled water.

Preparation of standard solutions of concentration 10µg/ml:

Weighed accurately 100 mg of drug samples of both Diclofenac sodium and Ibuprofen in 100 ml volumetric flasks respectively. Then both the samples were diluted with ethanol until it forms clear solution and then made upto 100 ml with distilled water. A solution of 10 ml from each sample solutions were pipetted in separate 100 ml volumetric flasks and making up the volume to 100ml with distilled water. Further a solution of 10 ml of Stock-1 solutions were diluted to100 ml of distilled water to prepare Stock-2.

Wavelength Selection: The wavelength maxima (λ_{max}) of Diclofenac Sodium was obtained at 275 nm and the wavelength maxima (λ_{max}) of Ibuprofen was obtained at 225 nm.

Forced degradation studies: Both the drug sample solutions were analyzed for a period of 5 hours to find out the stability of both the solutions during analysis. The results demonstrated that Ibuprofen and Diclofenac sodium both exhibit changes in absorbance values in different time intervals indicating the degradation of the drugs as inevitable in the forced degradation studies continued for 5 hours. The results of forced degradation studies for both the drugs were shown in **Table 2 and 3**.

Acid degradation: In this test, an amount of 2ml of prepared 0.1N Hydrochloric acid solution was added to 10 ml of both stock-2 drug solutions. The absorbance readings were recorded in every one hour up to 5 hrs.

Alkaline degradation: Here 10 ml of both stock-2 drug solutions were taken and to each of the solutions 2ml of the prepared 0.1N Sodium Hydroxide solution was added. The absorbance readings were recorded in every one hour up to 5 hrs.

Oxidative degradation: In this study, a volume of 10 ml of both stock-2 sample solutions were taken and then 2ml of prepared 0.3% Hydrogen Peroxide was added to both the solutions. The absorbance readings were recorded in every one hour up to 5 hrs.

Photolytic degradation: A measured volume of 10 ml of both stock-2 sample solutions were taken in a beaker and kept under UV light for 5 hrs and observed the absorbance in every hour for 5 hours at a stretch.

Thermal degradation: A measured volume of 10 ml of both stock-2 sample solutions were taken in a beaker and both the solutions were heated in water bath at 40-60^{o}C for 5 hrs and observed the absorbance readings for every one hour.

RESULTS AND DISCUSSION

Table 1: Absorbance readings prior to Forced Degradation Studies

S No.	Drugs	Absorbance Readings
1.	Diclofenac Sodium	0.331
2.	Ibuprofen	0.279

Table 2: Forced Degradation Studies of Diclofenac Sodium

Duration	% Acid Degradation	% Alkaline Degradation	% Oxidative Degradation	% Photolytic Degradation	%Thermal Degradation
5 Hour	64.1	87.61	47.7	24.77	9.36
4 Hour	56.9	55.3	44.0	27.0	5.1
3 Hour	49.2	52.3	40.2	34.0	2.5
2 Hour	48.1	49.4	35.1	33.7	2.1
1 Hour	33.0	46.5	31.5	22.5	0.5
0 Hour	22.2	43.5	21.8	17.5	1.32

Table 3: Forced Degradation Studies of Ibuprofen

Duration	% Acid Degradation	% Alkaline Degradation	% Oxidative Degradation	% Photolytic Degradation	%Thermal Degradation
5 Hour	85.6	78.2	67.7	55.4	35.4
4 Hour	81.4	72.2	63.4	52.9	33.2
3 Hour	79.5	71.7	58.6	47.7	28.5
2 Hour	77.0	69.8	52.7	39.6	21.0
1 Hour	72.8	57.2	36.6	35.6	17.3
0 Hour	48.9	50.7	28.1	14.3	11.4

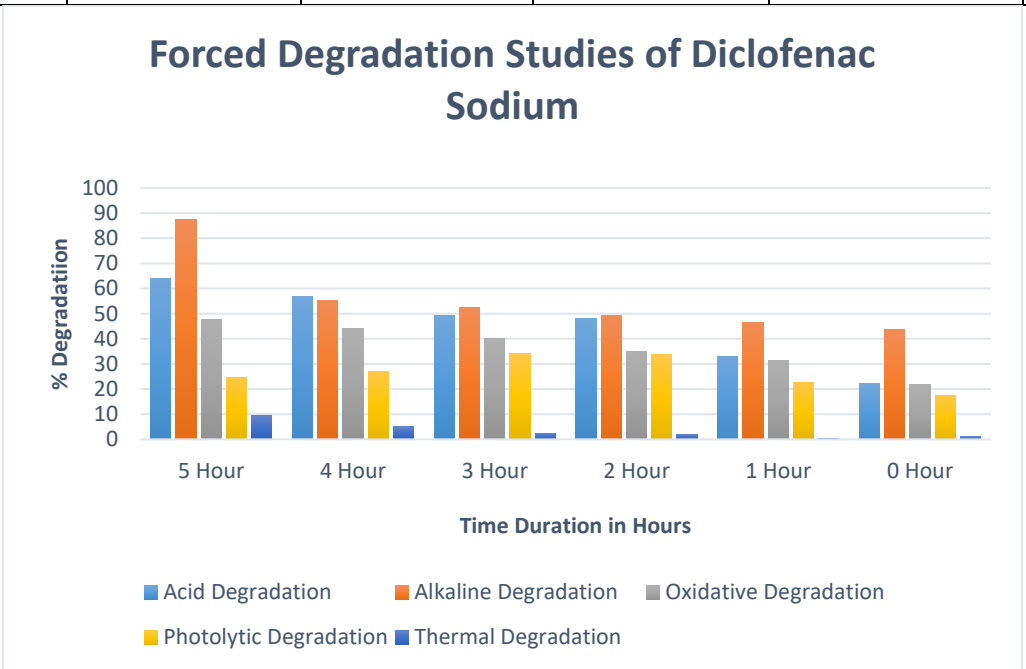


Fig 1: Bar diagram showing % Degradation of Diclofenac Sodium

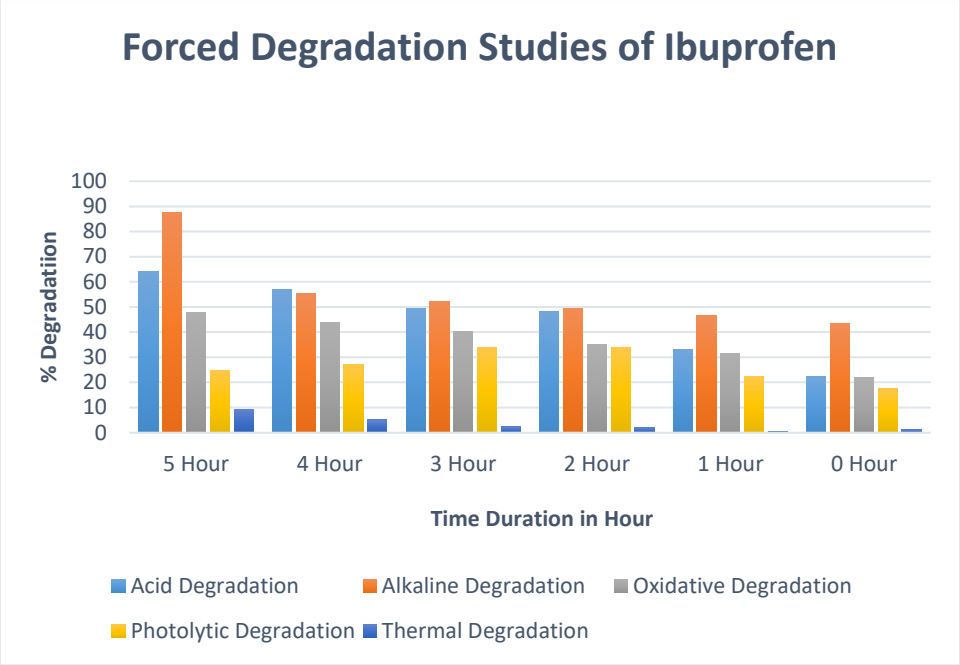


Fig 2: Bar diagram showing % Degradation of Ibuprofen

TABLE 4: % Drug degradation of Diclofenac Sodium after the 5th hour of the study

S No.	Stress Condition	Absorbance	% Drug Degradation	%Drug Remaining
1.	Acidic	0.212	64.1%	35.9%
2.	Alkaline	0.290	87.61%	12.39%
3.	Oxidative	0.157	47.7%	52.3%
4.	Thermal	0.082	24.77%	75.23%
5.	Photolytic	0.031	9.36%	90.64%

TABLE 5: %Drug degradation of Ibuprofen after the 5th hour of the study

S No.	Stress Condition	Absorbance	% Drug Degradation	%Drug Remaining
1.	Acidic	0.238	85.63%	14.37%
2.	Alkaline	0.218	78.27%	21.73%
3.	Oxidative	0.189	67.75%	32.25%
4.	Thermal	0.154	55.44%	44.56%
5.	Photolytic	0.098	35.43%	64.57%

CONCLUSION

The forced degradation study clearly explained about the degradation pattern of the two NSAIDS under the different stress conditions. We can conclude from Table 4 that the degradation of diclofenac sodium is more than 87% in alkaline condition, followed by extent of degradation in acidic and oxidative condition with the values of 64.1% and 47.7% respectively. Whereas in case of Ibuprofen, highest degradation rate of 85.63% was observed

in acidic stress condition, 78.27% in alkaline followed by 67.75% in oxidative state as shown in Table 5. The amount of degradation is least in photolytic stress condition for both the drugs. This study throws the light on the stability profile of the two most commonly used NSAIDS, diclofenac sodium and ibuprofen under the similar set of stress conditions. And the readings of this stability study would be of help to a scientist in designing any formulations concerning these widely used NSAIDS.

ACKNOWLEDGEMENT

We are thankful to the Department of Pharmaceutical Analysis of CMR College of Pharmacy for providing the facilities to carry out this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Phillips WJ, Currier BL. Analgesic pharmacology: II. Specific analgesics. J Am Acad Orthop Surg. 2004 Jul-Aug;12(4):221-33.
2. Dawood MY. Primary dysmenorrhea: advances in pathogenesis and management. Obstet Gynecol. 2006 Aug;108(2):428-41.
3. Shekelle PG, Newberry SJ, FitzGerald JD, Motala A, O'Hanlon CE, Tariq A, Okunogbe A, Han D, Shanman R. Management of Gout: A Systematic Review in Support of an American College of Physicians Clinical Practice Guideline. Ann Intern Med. 2017 Jan 03;166(1):37-51.
4. Oyler DR, Parli SE, Bernard AC, Chang PK, Procter LD, Harned ME. Nonopioid management of acute pain associated with trauma: Focus on pharmacologic options. J Trauma Acute Care Surg. 2015 Sep;79(3):475-83.
5. Zacher J, Altman R, Bellamy N, Brühlmann P, Da Silva J, Huskisson E, Taylor RS. Topical diclofenac and its role in pain and inflammation: an evidence-based review. Curr Med Res Opin. 2008 Apr;24(4):925-50.
6. van den Bekerom MPJ, Sjer A, Somford MP, Bulstra GH, Struijs PAA, Kerkhoffs GMMJ. Non-steroidal anti-inflammatory drugs (NSAIDs) for treating acute ankle sprains in adults: benefits outweigh adverse events. Knee Surg Sports Traumatol Arthrosc. 2015 Aug;23(8):2390-2399.
7. May JJ, Lovell G, Hopkins WG. Effectiveness of 1% diclofenac gel in the treatment of wrist extensor tenosynovitis in long distance kayakers. J Sci Med Sport. 2007 Feb;10(1):59-65.
8. Barkin RL. Topical Nonsteroidal Anti-Inflammatory Drugs: The Importance of Drug, Delivery, and Therapeutic Outcome. Am J Ther. 2015 Sep-Oct;22(5):388-407.
9. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971 Jun 23;231(25):232-5.
10. Chaiamnuay S, Allison JJ, Curtis JR. Risks versus benefits of cyclooxygenase-2-selective nonsteroidal antiinflammatory drugs. Am J Health Syst Pharm. 2006 Oct 01;63(19):1837-51.

11. 12.Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010 Apr;24(2):121-32.
12. Whelton A. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiologic foundations and clinical implications. Am J Med. 1999 May 31;106(5B):13S-24S.
13. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci. 2013;16(5):821-47.
14. Sriuttha P, Sirichanchuen B, Permsuwan U. Hepatotoxicity of Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review of Randomized Controlled Trials. Int J Hepatol. 2018; 2018:5253623.
15. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C: Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherthrombosis? Meta-analysis of randomised trials. BMJ. 2006 Jun 3; 332(7553):1302-8.
16. Solomon DH, Avorn J, Sturmer T, Glynn RJ, Mogun H, Schneeweiss S: Cardiovascular outcomes in new users of coxibs and nonstroidal antiinflamatory drugs : high-risk subgroups and time course of risk. Arthritis Rheum. 2006 May;54(5):1378-89.
17. FitzGerald GA, Patrono C: The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med. 2001 Aug 9;345(6):433-42.
18. Bushra R, Aslam N: An overview of clinical pharmacology of Ibuprofen. Oman Med J. 2010 Jul;25(3):155-1661. doi: 10.5001/omj.2010.49.
19. Rainsford KD: Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology. 2009 Dec;17(6):275-342. doi: 10.1007/s10787-009-0016-x. Epub 2009 Nov 21.
20. Tracy TS, Hall SD: Metabolic inversion of (R)-Ibuprofen. Epimerization and hydrolysis of Ibuprofenyl-coenzyme A. Drug Metab Dispos. 1992 Mar-Apr;20(2):322-7.
21. Rao P, Knaus EE: Evolution of nonsteroidal anti-inflammatory drugs (NSAIDS): cyclooxygenase (COX) inhibiton and beyond. J Pharm Sci. 2008 Sep 20;11(2):81s-110s.