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A DETAILED REVIEW ON IMPURITY PROFILE OF AN ANTI-INFLAMMATORY API

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ABSTRACT

The goal of the collection of analytical operations known as "impurity profiling" is to detect, characterise the structure of, and quantify organic and inorganic impurities as well as residual solvents in bulk pharmaceuticals and pharmaceutical formulations. Impurity control is presently a crucial problem for healthcare production. Diverse strategies, which include capillary electrophoresis, gas-liquid chromatography, high performance liquid chromatography, solid-phase extraction methods, ultraviolet spectrometry, infrared spectroscopy. Thousands of tons of pharmacologically active substances are used annually to treat or to prevent illnesses, or to help people with the stress of modern life. Non-steroidal anti-inflammatory drugs (NSAIDs) are the group most often used in human health care, since they are available without prescription for treatment of fever and minor pain. The present review addresses the use of various technique for the analysis of impurities of NSAIDs. Numerous chemical mediators, including prostaglandins, leukotrienes, and platelet-activating factor, cause both acute and chronic inflammations. A variety of activity mechanisms are used by anti-inflammatory drugs to demonstrate their effects. The most often given medications for treating inflammatory illnesses are non-steroid anti-inflammatory medicines (NSAIDs). The patients receive symptomatic relief from the NSAIDs, but they do not alter the pathogenesis of inflammation. Furthermore, due to serious adverse effects, particularly on the gastrointestinal mucosa, prolonged administration should be avoided.

KEYWORD: anti –inflammatory drugs, impurity profiling, ICH guidelines, retention time.

INTRODUCTION

❖ **IMPURITY PROFILING**

- **Impurity profiling** The ICH (International Conference on Harmonization) describes an impurity profile of a drug material as "a description of the identified and unidentified impurities present in a new drug substance."[1] The phrase "impurity profiling" refers to analytical techniques that have as their principal aims the detection, identification, structural characterization, and quantitative quantification of organic and inorganic impurities, as well as any residual solvents, in bulk pharmaceuticals and medications.[2]

- **Impurity** Any raw material in a new drug substance that is not the chemical entity described as the ingredient in the new drug substance, any ingredient in a drug product that is not the chemical entity described as the ingredient in the drug product, or any excipient in a drug product are all recognised as impurities.[3]

➢ **CLASSIFICATION OF IMPURITIES**
Impurities can be classified into the following categories:
1. Organic Impurities (process and drug related).
2. Inorganic impurities.
3. Residual solvents.

1. Organic impurities It can arise during the manufacturing process and/or storage of the new drug substance. This organic impurities can be identified or unidentified, volatile or non volatile and also include - Starting materials, By-products, Intermediates, Degradation products, Reagents, ligands and catalysts.

2. Inorganic Impurities -Inorganic impurities are usually detected and quantified using Pharmacopeial or other appropriate principles. Carryover of catalysts to the drug substance should be evaluated throughout development. These kinds of impurities can result from the manufacturing progression. These are normally known and identified and include Reagents, ligands and catalysts, Heavy metals or other residual metals,

Inorganic salts. Other materials (e.g., filter aids, charcoal).

Table No 01: Classification of residual solvents.

Solvent	Risk assessment	Example
Class I	Solvents to be avoided	Benzene (2ppm), Carbon tetrachloride (4ppm), Methylene chloride (600ppm), Methanol (3000ppm), Pyridine (200ppm), Toluene (890ppm)
Class II	Solvents to be avoided	N, N- dimethyl formamide (880ppm), acetonitrile (410ppm)
Class III	solvents with low toxic potential	Acetic acid, Ethanol, Acetone has permitted daily exposure of ≤50mg/day.

➢ **Sources Of Impurities**
The impurities can originate from several sources; such as;

- Crystallization-related impurities
- Stereochemistry-related impurities
- Impurities arising during storage
- Method related impurity
- Residual solvents
- Synthetic intermediates and by-products
- Functional group-related typical degradation
- Mutual interaction amongst ingredients.[5,6,7]

➢ **ANALYTICAL METHODS FOR IDENTIFICATION OF IMPURITIES-**
The impurities can be identified by following different methods like. Reference standard method - The main purpose of this method is to provides the basic information for evaluating process and product performance of drug substances, drug products, impurities, degradation products, starting materials, intermediates, and excipients.

- Spectroscopic method - The UV, IR, MS, NMR and Raman spectroscopic methods are abundantly used for the identification of impurities.

- Separation method - The separation method includes chromatographic techniques like TLC, HPTLC, HPLC, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), Electrophoresis techniques like Capillary electrophoresis, Gel permeation chromatography etc.

- Isolation method- Number of methods can be used for isolation and characterization of impurities .i.e. Solid-phase extraction methods, Liquid-liquid extraction methods, Accelerated solvent extraction methods, Column chromatography, Flash chromatography, TLC, GC, HPLC, HPTLC, Capillary electrophoresis (CE), Supercritical fluid chromatography (SFC).

- Characterization method- For characterization of impurities, different techniques are used; *HPLC-UV Studies *HPLC-MS Studies *GC-MS Studies *TLC-MS Studies *CE-MS Studies *MEKC-MS and CEC-MS Studies *HPLC-NMR Studies.[8,9]

❖ **Anti-inflammatory Drugs**
Although acetylsalicylic acid - the first of the non-steroidal anti-inflammatory drugs (NSAIDs) - has been with us since the beginning of the century, it was never so called because anti-inflammatory therapy in rheumatologically practice was not demonstrated clinically until the advent of cortisone in 1949. The first substance to be called a NSAID was phenylbutazone which was introduced in 1952. It was the dramatic reduction of swelling in inflamed joint structures induced by cortisone and other corticosteroids introduced later, and the dramatic relief of swelling and stiffness with decrease, in pain and improved mobility that led to the corticosteroids being termed the 'miracle drugs' of the time. The first 3, aspirin, phenylbutazone/oxyphenbutazone and indomethacin, did in deed demonstrably reduce joint tissue swelling but usually much less dramatically than did corticosteroids at medium or high dosage levels. The idea of reducing pain and swelling by a peripheral action in the affected tissues rather than by reducing pain centrally in the central nervous system.[10]

❖ **Mechanism of Action of Anti-inflammatory drugs.**
NSAIDs probably act in most cases by inhibiting the synthesis of prostaglandins in inflamed tissues, thereby preventing sensitisation of pain receptors to mediators of inflammation. These mediators - histamine, serotonin, kinins and free oxygen radicals - play some part in the inflammatory arthritis process, but playa variable role in different types of inflammation. Arachidonic acid is the precursor substrate for both cyclo-oxygenase (prostaglandin synthetase) and lipoxygenase enzymes, the former leading to the production of prostaglandins F, D and E, prostacyclin and the thromboxanes, the latter via the unstable intermediate 5-HPETE to 5-HETE and the leukotrienes (fig. 1). There is a reasonably good correlation between an NSAID's potency in reducing experimentally induced oedema in laboratory animals and its ability to inhibit prostaglandin production, but it is possible that immediate symptomatic relief of chronic inflammation is produced by blocking cyclo-oxygenase

3. **Residual Solvents** -Solvents are inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance.[4]

metabolism and a more permanent effect by blocking   lipoxygenase metabolism.[11,12,13]
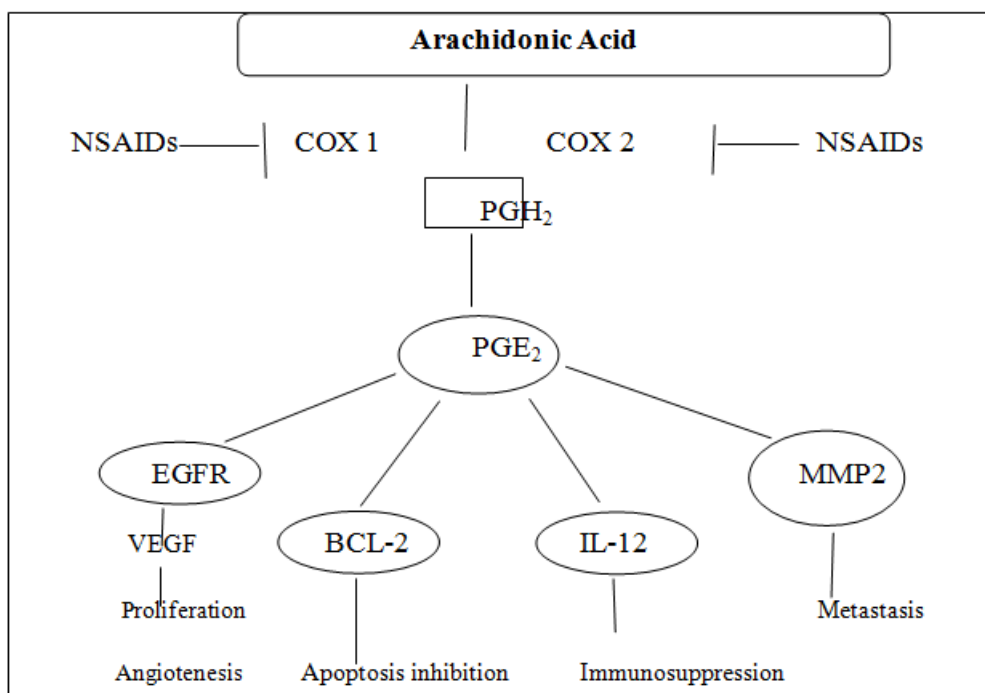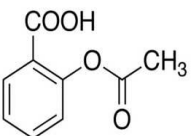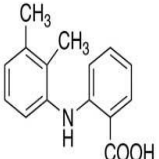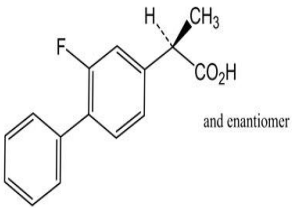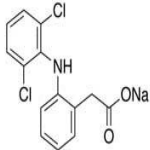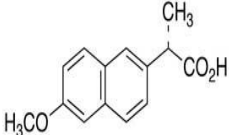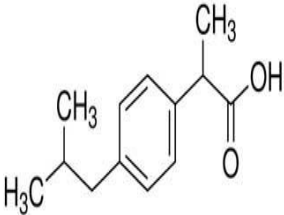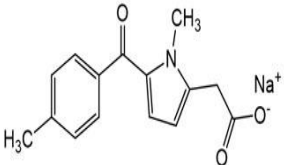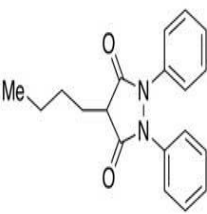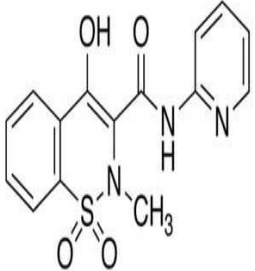
Fig. NO. 01 .Biosynthesis of Prostaglandin
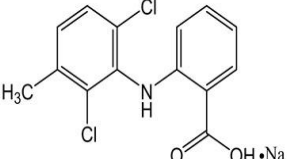
❖ Impurities of Anti-inflammatory drugs[10]
Table No 02: Impurities of NSAID.

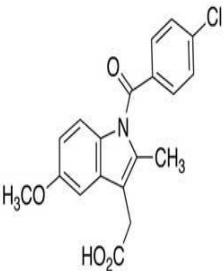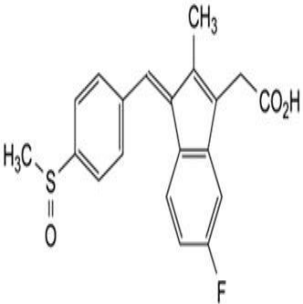| Sr.No	Drug Name	Impurities.
1.	**Aspirin** 	Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, Impurity F, Acetylsalicylic Acid-d4,Acetylsalicylic Acid-d3, Acetylsalicylic Acid-d7, Acetylsalicylic Anhydride-d8.
2.	**Mefenamic Acid** 	Impurity A, Impurity B, Impurity C, Impurity D, 3-Chloro-2-methylaniline , 2-Chloro-5-iodobenzoic Acid, 3-Carboxy Mefenamic Acid, 3-Carboxy Mefenamic Acid Acyl-β-D-glucuronide, 3-Hydroxymethyl Mefenamic Acid Acyl-β-D-glucuronide, Mefenamic Acyl-β-D-glucuronide.
3.	**Meclofenamic Acid** 	2-Fluorobenzoic Acid-d4, 3-Hydroxymethyl Meclofenamic Acid-d4, Meclofenamic Acid-d4, Meclofenamate Sodium.
4.	**Flurbiprofen**	Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, 3',4'-

		Dihydroxy Flurbiprofen, 3',4'-Dimethoxy α-Desmethyl Flurbiprofen, Flurbiprofen Axetil, Flurbiprofen Sulfate, Flurbiprofen Acyl-β-D-glucuronide-d3, 4'-Hydroxy Flurbiprofen-d3.
5.	**Diclofenac**	Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, Impurity F, Diclofenac Methyl Ester, Diclofenac Diethylamine, N-Nitroso-Diclofenac.
6.	**Naproxen**	Impurity A, Impurity B, Impurity C, Impurity D ,Impurity E, Impurity F, , Impurity G, , Impurity H, Impurity I, Impurity J, Impurity K, Impurity L, Impurity M, Impurity N , Impurity O, Isopropyl 2-hydroxy-2-(6-methoxynaphthalen-2-yl)acetate, rac-5-Bromo Naproxen, 1-Deoxy-1-(octylamino)-D-glucitol.
7.	**Ibuprofen**	Impurity A, Impurity B, Impurity C, Impurity D ,Impurity E, Impurity F, , Impurity G, , Impurity H, , Impurity I, Impurity J, Impurity K, Impurity L, Impurity M, Impurity N , Impurity O, Impurity P, Impurity Q, Impurity R, α-Methyl-4-propylphenylacetic Acid , 2-(4-n-Propylphenyl)propionic Acid, Ibuprofen Lysinate, Ibuprofen 1,4-Sorbitan Ester, race 2-(tert-Butyldimethylsilyloxy) Ibuprofen-d6.
8.	**Tolmetin**	Amtolmetin Guacil-d3, Tolmetin-d3, Tolmetin-d3 Ethyl Ester.

9.	**Indomethacin**	Impurity A, Impurity B, Impurity C, Impurity D ,Impurity E, Impurity F, , Impurity G, , Impurity H, , Impurity I, Impurity J, Indomethacin Isopropyl Ester, O-Desmethyl Indomethacin-d4, Indomethacin-d4 Methyl Ester, α-Glucametacin-d4.
10.	**Sulindac**	Impurity A, Impurity B, Impurity C, Sulindac Acyl-β-D-Glucuronide, Sulindac Sulfone Acyl-β-D-Glucuronide, Sulindac Sulfide-d6, Sulindac Sulfide Methyl Ester, sulindac sulfone.
11.	**Phenylbutazone**	Impurity A, Impurity B, Impurity C, Impurity D, N-Acetylaminoisonitrosoacetanilide,1-Acetyl-2-phenylhydrazine,Oxyphenbutazone,nCaproylhydrazobenzene,2-(1,2-Diphenylhydrazine-1-carbonyl)-2hydroxyhexanoic Acid.
12.	**Piroxicam**	Impurity A, Impurity B, Impurity C, Impurity D ,Impurity E, Impurity F, Impurity G, Impurity H, Impurity I, Impurity J, Impurity K, Impurity L, N-(6-Methyl-2-pyridyl)-4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxamide 1,1-Dioxide, N-(2-Pyridyl)oxamic Acid, 1,3-Dipalmitoyl-2-chloropropanediol-d5, Piroxicam-d3 O-β-D-Glucuronide, 2-Chloro-N-(pyridin-2-yl)acetamide, 2-(1,1-Dioxido-3-oxobenzo[d]isothiazol-2(3H)-ylN-(pyridin-2-yl)acetamide.

1. Aspirin Chemically, aspirin (ASP) is known as 2-acetyloxybenzoic acid (acetylsalicylic acid). ASP is frequently employed to treat fever, inflammatory diseases, and mild to moderate discomfort. For the measurement of ASP impurities in ASP and DPY capsules, an internal LC gradient technique was created using an Inertsil ODS-3 C18, 250 4.6 mm, 5 mm column with a mobile phase made up of 0.01 M Na2HPO4, pH set to 2.5, and orthophosphoric acid as mobile phase-A. Acetonitrile served as mobile phase-B. Salicylic acid (impurity-C), salsalate (impurity-E), 4-hydroxybenzoic acid (impurity-A), 4-hydroxyisophthalic acid (impurity-B), and unknown were isolated using this technique impurities of ASP and Dipyridamole capsule.[15,16,17]
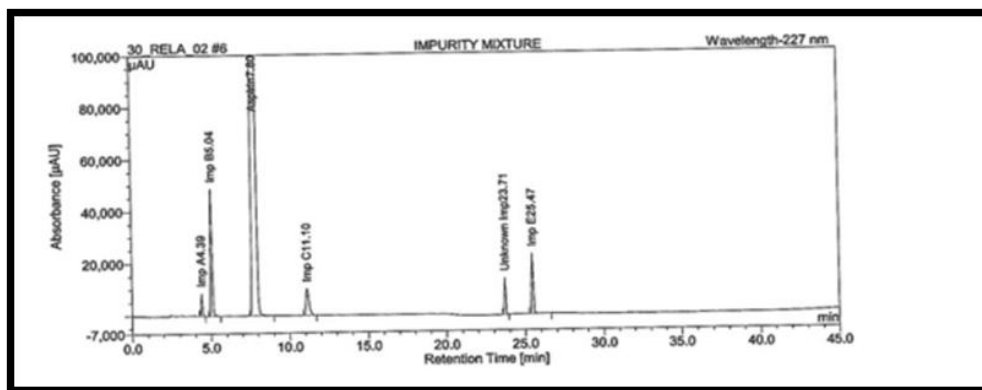

Fig: No. 02: Typical chromatogram of aspirin and impurities.

2. Mefenamic Acid-Mefenamic acid (MEF) is 2-[(2,3-dimethylphenyl)amino]benzoic acid (1). It is an anthranilic acid derivative and a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs). It is used in mild to moderate pain including headache, dental pain, post operative, postpartum pain and dysmenorrheal. TLC-densitometric method and RP-HPLC-DAD method, were developed and validated for the simultaneous determination of mefenamic acid (MEF) and its two toxic impurities, benzoic acid (BA) and 2,3-dimethylaniline (DMA). In the proposed TLC-densitometric method a developing system consisting of chloroform: acetone:acetic acid:ammonia solution(70:30:2:2, v/v/v/v) was used, TLC aluminum plates 60 F254 was used as a stationary phase and the separated bands were UV-scanned at 225 nm.[24,25,26]
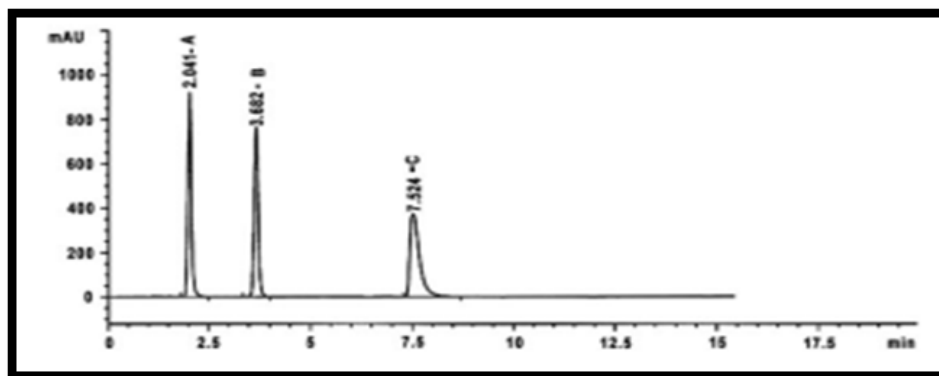
Fig: No. 03: HPLC chromatogram of (a) benzoic acid, (b) 2, 3-dimethylaniline, (c) mefenamic acid.

3. Diclofenac Diclofenac (DIC) (2-[(2,6-dichlorophenyl)amino]phenyl acetic acid is an essential non-steroidal anti-inflammatory drug (NSAID) which is clinically prescribed for the treatment of inflammatory disorders, such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis. Development and Validation of RP-HPLC Method for Simultaneous Determination of Diclofenac Potassium and its Process Related Impurities was carried out by using 150×4.6 mm, i.d., 5 µm C-18 column with prepared mobile phase-A consisting 800:200 (v/v) of 0.01 M ammonium acetate adjusted pH 5.3 with acetic acid and acetonitrile and mobile phase-B consisting 200:800 (v/v) of 0.01 M ammonium acetate adjusted pH 5.3 with acetic acid and acetonitrile.[27,29,30,31]

Fig: No. 04. Reverse phase HPLC chromatogram Diclofenac impurities(Diclofenac Potassium, Oxindole, Impurity-A, Impurity-D, Alcohol analog, Benzaldehyde analog).
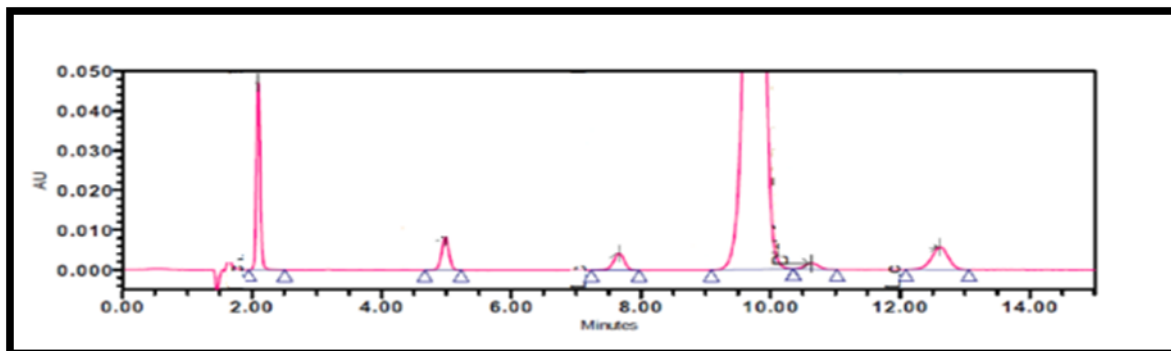
4. Naproxen Naproxen [(S)-2-(6-methoxynapthalen-2-yl)propanoic acid] is a non-steroidal anti-inflammatory drug used in reduction of pain, fever, inflammation and treatment of rheumatoid arthritis, degenerative joint disease, ankylosing spondylitis, acute gout and primary dismenorrea. The HPLC analysis was performed on an Agilent 1100 series LC system, Agilent Technologies Inc., Santa Clara, CA, USA. Hypersil ODS column (4.6 mm × 100 mm, 5 m) and mobile phase consisting of a mixture of sodium acetate trihydrate (pH 4.7; 0.04 M) – methanol (60:40, v/v), UV detection at 254 nm, flow rate of 1.5 mL/min was used for resolution of all the impurities i.e. 2-(6-methoxynaphthalen-2-yl)acrylic acid.[33,34,35]
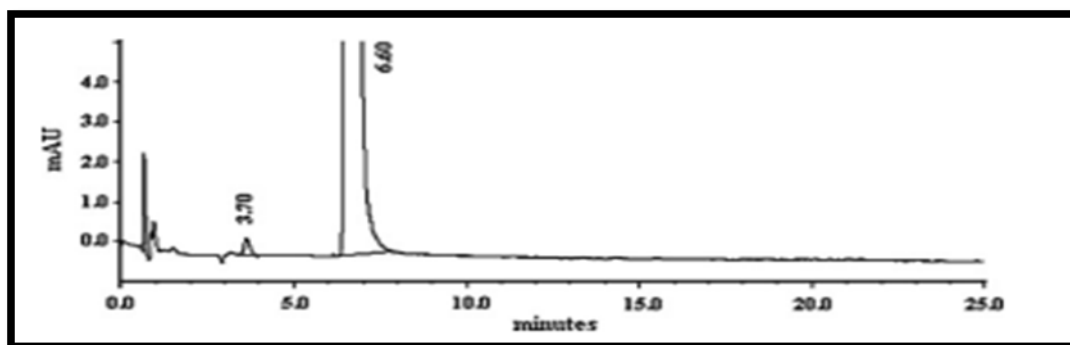

Fig: No.05 HPLC chromatogram of Naproxen & acrylic acid impurity.

5. Ibuprofen Ibuprofen has many impurities: A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R. The main impurity is Ibuprofen impurity B: 2-(4-butylphenyl) propionic acid is considered a degradation product, β-Isomer, Guaifenesin, Dioxylonone. HPLC gradient method is developed for the determination of related substances by using Acetonitrile HPLC grade, Potassium dihydrogenate phosphate-AR grade as mobile phase, with column C18, 150×4.6mm, 5µm(Devolosil ODS or equivalent)& detected at wave length 220 nm.[34,35]

6. Indomethacin -Indomethacin ([1-(4-chlorobenzoyl)-5-methoxy-2- methylindol-3-yl]acetic acid

it has been widely used observed when investigated with compendial methods. in musculoskeletal and joint disorders such as rheumatoid arthritis. It may also be used in inflammation, pain and in the oedema. y, an HPLC–UV method was by Merck developed which allowed for the separation and quantification of the possible process impurities 2–9 from indomethacin. HPLC separation of all impurities from indomethacin. Conditions: isocratic HPLC, stationary phase: Nucleosil 120-5, C , 250 mm 18 34.6 mm ID; mobile phase: 75 (v/v) methanol and 25 (v/v) 0.2% phosphoric mobile phase was acid, flow rate 1.5 ml/min, UV detection at 320 nm.[36,37,38,39]
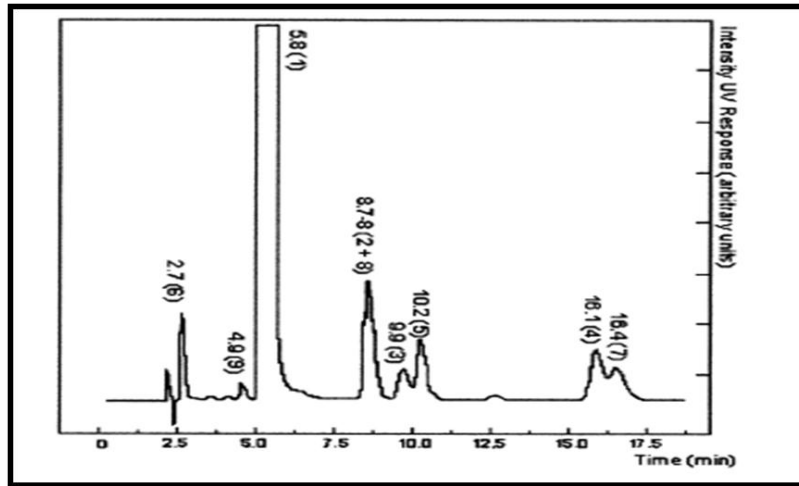
Fig: No. 07 HPLC separation of all impurities from indomethacin.

7. Phenylbutazone- Phenylbutazone is an anti-inflammatory, antipyretic, and analgesic activities. It is known to be effective especially in the treatment of ankylosing spondylitis. Degraded PBZ results in several types of impurities where benzidine is one of the impurities which is known to be a GTI. HPLC method was validated for impurities detection carried out by using buffer (potassium dihydrogen phosphate): Acetonitrile: methanol (30:50:20 v/v/v) at pH 3.5 in isocratic mode as mobile phase.[41]

Table No: 03 Validated impurities of Anti-inflammatory drugs.

Sr.no.	Drug	Name of impurity	Retention time (min.)
1.	Aspirin	Imp-A	4.30
		Imp-B	5.04
		Imp-C	11.10
		Imp-E	25.47
		Imp-I	23.72
2.	Mefenamic acid	Benzoic acid	2.041
		Dimethylaniline	3.682
3.	Diclofenac sodium	Oxindole	1.75
		Impurity A	2.25
		Benzaldehyde	4.7
		Alcohol analog	7.55
		Impurity D	10.01
4.	Naproxen	Acrylic acid A	3.50
		Acrylic acid B	6.60
5.	Ibuprofen	B-Isomer	0.74
		Guaifenesin	0.85
		Dioxylonone	1.84
		2-(4-Isobutyryl phenyl)propionic acid	2.01
6.	Indomethacin	1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]- 2. acetic acid methylate	8.8
		[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]- acetic acid ethylate	9.9
		[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]- acetic acid tert. butylate	16.1
		4-chlorobenzoic acid 2-[1-(4-chlorobenzoyl)-5methoxy-2-methylindol-3-yl]acetylj-N-[4-methoxy- - 6 phenyl)-hydrazide	10.2
		5-methoxy-2-methyl-3-indoleacetic acid	2.7
		[1-(4-chlorobenzoyl)-5-methoxy-2,3-dimethylindole	16.4
		1-(3,4-dichlorobenzoyl)-5-methoxy-2-methylindol-3yl]acetic acid	8.7

		4-chlorobenzoic acid N9-(4-chlorobenzoyl)-N-(4 methoxyphenyl)-hydrazide	4.9
6.	**Phenylbutazone**	benzidine	2.046

CONCLUSION

Recently, impurity profiling of pharmaceutical products has attracted significant attention because impurities can be detri-mental to human health and can adversely affect the quality of pharmaceuticals. Currently, various methods are available for impurity profiling and other hyphenated techniques. In this review we have discussed the present state of the art of HPLC for determination of impurities of some important anti-inflammatory agent in brief. Impurities present in pharmaceuticals can originate from many sources. They can stem from starting materials, can be introduced or formed during synthesis or can be caused from excipients or due to degradation. As they can be the cause of undesirable pharmacological effects, their content is liable to the control of regulatory authorities. Accordingly, the pharmaceutical impurities must be declared in the range as low as 0.01–0.10% relative to the API.

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