



RESEARCH ARTICLE

ISOLATION OF PHARMACEUTICAL IMPURITIES FROM BULK DRUG PREPARATIONS

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Abstract

This article is basically based on the idea that how to identify and isolate the pharmaceutical impurities present in Active pharmaceutical ingredients (API). Generally, the impurities present in API are isolated by various Chromatographic and Non-Chromatographic method based on the nature of impurities. According to the regulatory guidelines some impurities even in trace amount/ small amount is unavoidable and there is a necessity to control them if the level of identified impurity is greater than the threshold impurities but if the identified impurity is less than the threshold impurities, we can neglect it. After the isolation and identification of impurities we characterize them by using several techniques Like NMR (Nuclear magnetic resonance), LC-MS (Liquid chromatography coupled to mass spectrometry) and etc.

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Introduction:-

The impurities in Pharmaceuticals preparations are those substances which exists with API and develop due to aging, formulation or may be during synthesis. Presence of even small amount of impurities can influence the efficacy and safety of drug.[1] According to the ICH (International Council of Harmonization) impurities defined as a "Substance that are not API itself or the excipient used to manufacture or formulate something" i.e. impurities are the those unwanted chemical that remain present within formulation. It became a crucial part to control and identify the impurities because sometimes it may cause various side effects like carcinogenic, toxic, teratogenic effects.[2] Identification, isolation and characterization become an important part of pharmaceutical industry and drug development. It is necessary to present Qualitative and Quantitative report together from drug authorities, so that customers of bulk drug production can control these impurities as per the standards, "less than threshold impurities". [3] According to ICH guidelines, FDA (Food and Drug Administration) and EMEA (European Medicine agency) identifying and characterization of all impurities present in drug substance and drug products must be evaluated if the impurities level is greater than >0.1%.[4]

Table 1:- Given below shows that some drugs have been withdrawn from the market due to presence of impurity greater than acceptance criteria and threshold impurity.

Drug name	Withdrawn	Country	Remarks
Ranitidine	2019	Worldwide	Cause cancer
Flupirtine	2018	European Union	Liver toxicity
Propoxyphene	2010	Worldwide	Increased risk of heart attacks and stroke.

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Alosetron	2000	US	Serious gastrointestinal adverse events; ischemic colitis; severe constipation
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FDA&ICH regulatory guidelines on impurity:

The various regulatory guidelines regarding impurities provided by FDA ensuring the Safety and efficacy of Pharmaceutical Products, which are prepared by ICH are as follow [5,6,7]:

1. ICH Guidelines(Q1A) (R) : “Stability testing of New Drug Substance and Product”.
2. ICH Guidelines(Q3A) (R) : “Impurities in New Drug Substance”.
3. ICH Guidelines(Q3B) : “Impurities in New drug Products”.
4. ICH Guidelines(Q3C) : “Impurities guidelines for Residua Solvent”.
5. US FDA Guidelines(NDAs): “Impurities in New drug Substances”.
6. ICH Guidelines(ANDAs) : “Impurities in New drug Substances

Table 2:- Provides the ICH threshold for control of the organic impurities in new drug substances and new drug products.

Maximum daily dose ^x	Reporting threshold ^{y,z}	Identification threshold ^z	Qualification threshold
< 2g/day	0.05%	0.1% or 1 mg per day intake (whichever is lower)	0.15% or 1 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

X= the amount of drug substance administered per day.

Y= Higher reporting thresholds should be scientifically justified.

Z= Lower thresholds can be appropriate if the impurity is unusually toxic.

Classification of impurities:

Various types of impurities are present in pharmaceutical Substances which are classified further into following:

1. Organic Impurity
2. Inorganic Impurity
3. Residual Impurity

Organic Impurity:

These impurities most likely to be arise during manufacturing, synthesis, storage of substance. [8,9] These may be due to the starting material impurities, by product impurities, degradation and enantiomeric impurities.[10]

Inorganic Impurity:

Inorganic impurities are mainly arising during manufacturing process, but they are generally known and identified. Inorganic impurities include heavy metal impurities, residual impurity and some filter aid impurities.[11] These impurities are quantified and detected by using pharmacopoeia standard.[8]

Residual Impurity:

These are the organic and inorganic liquids which are used as a vehicle in various manufacturing processes. They may influence the property of certain compounds during synthesis which may be hazardous for human use. So, these solvents should be control according to the acceptance criteria of ICH guidelines, FDA guidelines and pharmacopoeia standards.[12]

Classification of residual solvents:[13]

Class I:

These types of solvents are very toxic in nature, thus not used in manufacturing of drug substance. Some common solvents are shown in **Table-3**.

Table 3:- Class I solvents:

SOLVENT	SOLVENT CONC (ppm)	ADVERSE EFFECTS
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Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic

Class II:

These types of solvent are limited used in Pharmaceutical products. Some common solvents are shown in **Table-4**.

Table 4:- Class II solvents:

SOLVENTS	PREMETTED DAILY EXPOSURE (mg/day)	SOLVENT CONC (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
Hexane	2.9	290
Methanol	30	3000
Toluene	8.9	890
Dichloromethane	6.0	600

Class III:

These solvents are less toxic than other solvents. For example, Acetic acid, ethanol, butanol etc.

Class IV:

Contain various solvents like isopropyl ether, trichloroacetic acid, isooctane etc.

Sources of impurities:

It is clear that impurity can be generated from various sources from which some of them are discussed below:

Degradation Products:

Impurities can be formed while there is degradation of end product during the synthesis of drug substance. Degradation products may arise by storage and aging. Some antibiotics like Penicillin, Cephalosporins are examples of degradation products.[14]

Reagents, Ligands and Catalysts:

These types of impurities are rare. There are some sources where Penicillin and cephalosporins are described as there is a presence of trace of ampicillin polymer and hydrolyzed produced in that API.[15]

Enantiomeric Impurities:

In some drugs there is presence of single form of chiral drug, which is a chemical substance that show improved pharmacological and influence therapeutic activity, with some adverse reactions.[29]

Single isomeric drugs show less advantages for example: the pharmacokinetics profile of Levofloxacin(S-isomeric) and Ofloxacin (R-isomeric) are comparable. [16] It is shown that in some instances different enantiomers can have different effects as shown in **Table-5**

Table 5:- Pharmaceutical products and their effects due to chirality.

S. No.	Drug	Isomer	Effect
1.	Barbiturates	R-isomer	Convulsant
		S-isomer	Depressant
2.	Thalidomide	R-isomer	Anti-nausea, Sleep inducing

		S-isomer	Teratogenic
3.	Labetalol	R, R-isomer	β -Blocker
		S, R-isomer	α -Blocker
4.	Penicillamine	D-isomer	Anti-arthritis
		L-isomer	Toxic
5.	Warfarin	R-isomer	Toxic
		S-isomer	Anti-coagulant

Heavy Metals:

The main source of heavy metal impurity in water which contains Ag, Na, Mn, Cd and Mg which lead to various hydrolytic reactions in drugs. To overcome these unwanted reactions de-mineralized water and glass-lined reactors are generally used. [8,11]

Dosages Form Related impurity:

The Pharmaceutical companies perform reformulation studies, stability studies and other studies as well before marketing any product, due to instability of drug company must have to withdraw or recall their products from market [19]. For example, 0.05% Fluocinonide topical solution was recalled from USA due to degradation of active ingredient (TEVA Pharmaceutical, USA).[18]

Crystallization Related Impurity:

Polymorphism is defined as a crystal system where a substance having different crystal packaging arrangements, physical properties, solubility, crystal shapes, density, melting point, vapor pressure etc. Polymorphism influences bio pharmaceutical behavior of the drugs. Pharmaceutical organizations and companies have regulatory authorities to take interest in Polymorphism and Solvatomorphism. [20,21]

The nature of crystal structure present in material can influence the following properties given in Table-6. [22]

Table 6:- Factors affecting Crystal Structure of Impurities.

Sr. No.	Name of Properties	Sr. No.	Name of Properties
1	Conductivity	5	Volume
2	Diffusivity	6	Dissolution Rate
3	Solubility	7	Density
4	Crystal Hardness	8	Melting/Sublimation Property

Isolation and characterization of impurities:

Isolation of impurities can be done by various methods depends upon the nature of the impurity. Once an impurities has been detected, it become necessary to quantify and purify .It can be done by chromatographic as well as non-chromatographic methods.[23] According to ICH if the impurity is present greater than 1% level than latest analytical tech must be used for the structure elucidation of Unknown impurities.(example: NMR,LC-MS and MALDI-TOF) for preparative isolation HPLC, Semi and automatic preparative HPLC is used for the isolation of unknown impurities.[24]

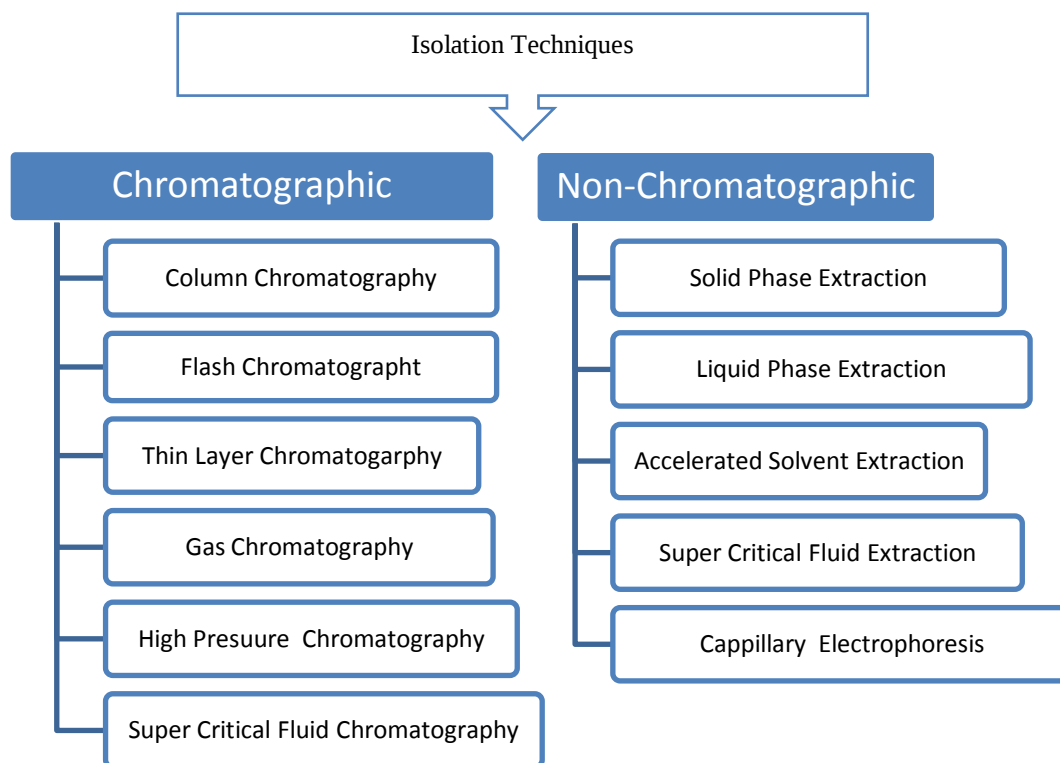


Figure 1:- Showing Various Techniques of Isolation [25,26,27,28].

A Case Study:

To perform isolation of an impurity from bulk drug substance by Preparative HPLC:

Process for Impurity Isolation

1. 200mg input sample taken for purification of impurity.
2. Sample was dissolved in minimum amount of suitable diluent.
3. Loaded the sample on preparative HPLC column and start the gradient combination of buffer and solvent and collect the fraction.
4. Selection of buffer & solvent is based on Analytical method.
5. Post fractions to be analyzed on analytical system, after reviewing the fraction.
6. If required modification in gradient, buffer or solvent can be done for separation of the impurity
7. After purification, the impurity workup is done by extraction or by desalt.

What is Workup?

Workup is a process by which we convert the impurity in its physical form or we can say that its stable form which can be solid, liquid, etc.

Procedure for workup:

1. The first step is to obtain the Active Pharmaceutical Ingredient of the Drug.
2. Then an Analytical HPLC along with a Preparative HPLC is set up.
3. Before operating any system, the system is purged with water to remove any previous adsorbed analyte.
4. This is done for at least 10-15 minutes.
5. A prep-HPLC is set up by selecting the desired column and purged with water through the channels and pumps to clean any previous residues and similarly for the Analytical HPLC.

Monitoring using analytical HPLC Technique:

All the fractions were monitored in the analytical HPLC. Firstly purged with water for 10-15 minutes and then conditioned with the organic solvents and buffer.

Preparation of Buffer (pH-2.5):

Add 2g of Sodium Perchlorate Monohydrate to 2000 ml of Water and adjust the pH with dil. OPA.

Preparation of Mobile Phase:

Mix 2000ml of buffer and 500 ml of Methanol

Organic Solvent:

Acetonitrile (Gradient-Grade) Approx. 1ml of the sample of interest was taken in HPLC vials and checked for its retention time in the Analytical HPLC. The peak of interest of impurity-A for Drug-Cabozantinib Tablets lies between 21 to 23 minutes of the run time.

Chromatographic parameter:

Column used; Zorbax Eclipse XDB C8 5micrometer (150X4.6) mm, Detector Wavelength; 210nm, Sample Tray Temperature; 25degree Celsius, Column Temperature; 30degree Celsius, Run Time; 65 min, Flow Rate; 1.5ml/min, Injection Volume; 10microliter

Gradient used for analytical hplc:

Time	Mobile Phase (%)	ACN (%)
00	40	60
20	25	75
35	5	95
60	5	95
65	40	60

Experimental Work For Isolation Of Impurity-Afor Drug-Cabozantinib Tablets:**Impurity isolation using prep-hplc:**

The column was used; YMC Pack ODS-A, with dimensions of 500mmx30mm and particle size of 15µm, 12nm. The column was washed with water for 10-15 minutes.

Preparation of Buffer (pH = 2.25):

5 ml of ortho-Phosphoric Acid were added to 5000 ml of water.

Organic Solvent:

Acetonitrile (Gradient-Grade)

Gradient Used For Prep-Hplc:

Flow Rate: 30ml/min, Detector Wavelength: 278 nm, Pressure: 70 bar

For Input Load 1:

S. No.	Time (min)	Buffer	ACN
1.	0-30	100 %	0%
2.	30-60	90%	10%
3.	60-90	80%	20%
4.	90-120	60%	40%
5.	120-150	50%	50%
6.	150-172	10%	90%

Input Load-1:

Before start any isolation, we should know about the impurity i.e. its retention time, solubility, Chemical nature (polar or Non polar) etc. So, the very first step is analyzing the API by Analytical HPLC. So, for that the pure Active Pharmaceutical Ingredient i.e. API was mixed with its diluent or in those solvent in which it does not get converted into its another formor we can also say thatit does not get degraded. After solubilize the pure API, it run into the HPLC, gradient was set and as a result chromatogram was observed. From which we get to know about that peak which should be isolated. The peak of interest was marked in the figure is currently 5.80% (17.5 min peak) pure in the mixture. We have to pure this impurity from 5.80% pure to above 90% pure according to ICH.

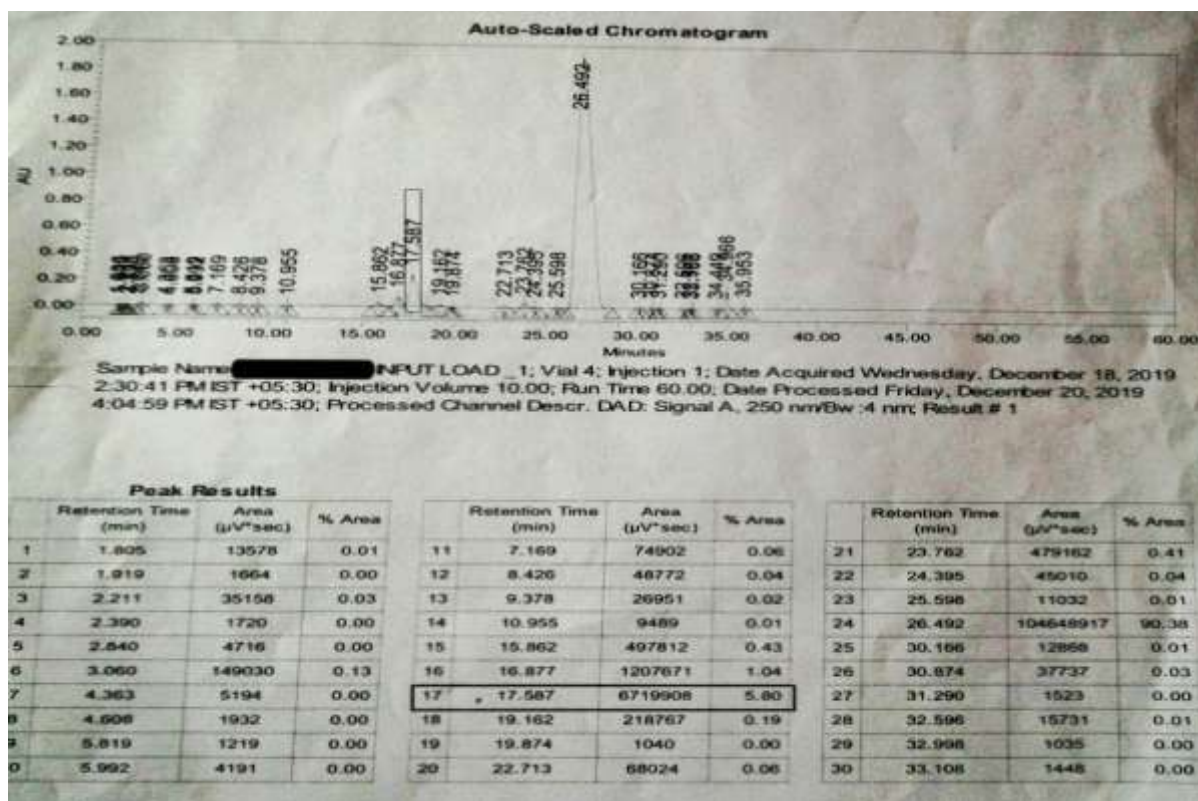


Figure 2:- Chromatogram of INPUT LOAD 1 Showing an impurity having retention time (RT) 17.5 Minute, this impurity is only 5% pure.

Other HPLC Chromatogram for Load 1, Fraction 17; the impurity is 38.68% pure:

As seen from the chromatograms of load 1 fraction 16 contains 26.19% pure impurity, fraction 17 contains 38.68% pure impurity and fraction 18 contains 39.11% pure impurity. So, to isolate the pure form of impurity these fractions were mixed and again loaded into the HPLC but this time it will be considered as a reloading. Now from the reloading 1, fraction no. 5 to 14 contained high % area of impurity, so these fractions were mixed and reloaded into the HPLC using the same gradient and wavelength.

Workup Data:

Workup is the process in which we convert the impurity into its physical form or its stable form. It is done by two ways; Work Up is of mainly two types;

1. Workup by Desalting
2. Workup by Extraction

Workup by Desalting:

In this process, concentrated pure aqueous fraction was loaded through injector in Preparative HPLC column, after that flush it with water for 40 to 60 mins. Then a combination of water and solvent was flushed through Prep. HPLC column to elute the impurity. This technique is used for polar compounds. Eluted impurity shall be dried on Rotavapor.

Workup by extraction:

Concentrated pure aqueous fraction was extracted with suitable solvent like Dichloromethane, chloroform, ethyl acetate etc. in separating funnel. The aqueous layer and the organic layer are separated. The organic layer was further dried on Rotavapor.

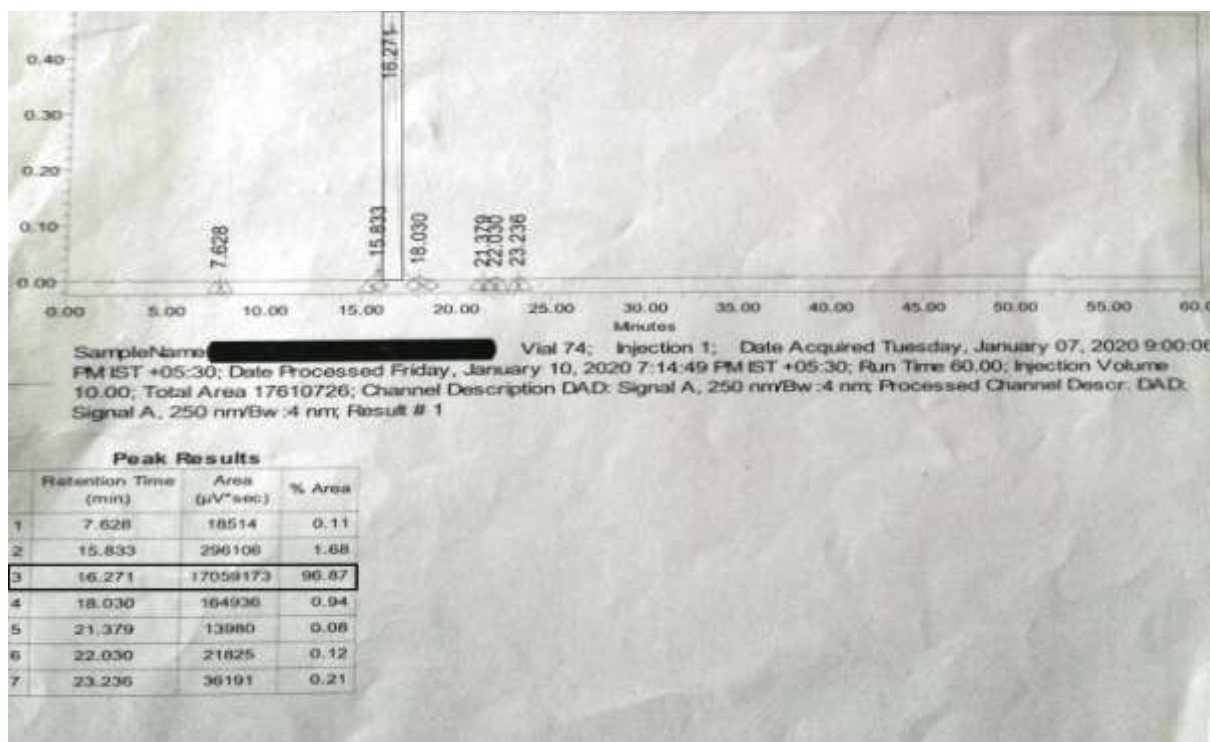


Figure 3:- Chromatogram showing that impurity is now 96% pure after collection of various pooled fraction and Workup.

Results:-

Initially the Impurity-A was 5.88 % pure. By the use of HPLC (prep and analytical), it is possible to purify the impurity more than 90%. This was achieved by loading and reloading the samples, after the reload15 the impurity A was 96% pure. About 200 mg impurity was isolated after workup, Thiswas sufficient for the characterization of impurity by LC-MS, NMR etc.

Conclusion:-

Now-a-days, impurity profiling is mandatory to know the impurity present in API so that the impurity can be controlled at early stage for the safety and efficacy purpose. Isolation and characterization of impurity is used for gathering information and evaluating relevant data which provide biological safety which disclose the need and opportunity of impurity profiling in pharmaceutical organization. Isolation, identification and purification are important aspects of pharmaceutical research and development.

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