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Analysis and impurity identification in pharmaceuticals

Abstract: Impurity is not a much-liked word by pharmaceutical and industry people, because they are concerned about quality. Here we discuss various impurities that might be present in API formulations. To fulfill our purpose we have compiled a variety of regulatory authorities' guidelines (i.e., ICH, WHO, and pharmacopoeias), which serve in endlessly regulating the impurities by various means. As the impurity present in a drug can affect its quality and thus its efficiency, it is therefore crucial to know about impurities. The current article reveals the different terms, regulatory control, and basic techniques (e.g., HPLC, LC-MS, TLC) that will help novices to understand, identify, and quantitatively estimate impurities and that have the advantage of profiling. This article primarily focuses on identification and control of various impurities (i.e., organic, inorganic, and genotoxic). For any of the substances, quality is the prime objective. Because impurities can alter quality, understanding the various impurities will help in producing quality products.

Keywords: analytical methods; genotoxic impurity; inorganic impurity; organic impurity; regulatory requirement in impurity profile.

DOI 10.1515/revac-2014-0001
Received January 7, 2014; accepted March 19, 2014; previously published online May 7, 2014

Introduction

The pharmaceutical world is dedicated to quality. Speaking from the customer's perspective, quality means pleasant appearance with good packaging. But in the case of pharmaceutical industries, quality means providing drug standards conforming to a variety of conditions and making profit from them. So, they should be aware of the various types of impurities and their regulation and control, which infer quality. Therefore, in this paper we have tried to summarize different types of impurities, along with their effects and limitations as given by the International Conference on Harmonization (ICH). ICH has given guidelines [ICH Q-3B (R2) 2006] for impurity in a drug, and according to ICH, it is a chemical entity, which is not defined as a drug per the Drugs and Cosmetic act and which has an impact on the purity of the active pharmaceutical ingredient or drug substance.

Every pharmaceutical manufacturer defines impurity in its own words, making it difficult to find an exact definition of impurity. In the pharma world, impurity can be identified by various terms that we will see later. Drug substances or drug products are prepared with various solvents. Remaining solvents or residual solvents that might be present in the final product often are cited as organic volatile impurities (OVI) (ICH Q-3C [R4] 2009), and the impurities associated with the inactive pharmaceutical ingredients used in formulation or as additives or adjuvants are rarely mentioned.

Bulk pharmaceutical chemicals (BPCs), can be obtained or synthesized from multiple sources and, therefore, it is very important that impurities in BCPs be carefully monitored and controlled. Recently British pharmacopoeia (BP), United State Pharmacopoeia (USP), and Indian pharmacopoeia (IP) started incorporating allowable limits of impurities present in drug substances or drug products (Kovaleski et al. 2007, Gad 2008). This article thoroughly reviews different impurities found in the pharmaceuticals by methods for isolation, extraction, and identity of possible impurities.

Impurity should be defined as *identified impurity* – an impurity available with information about the structural characterization, and *unidentified impurity* – an impurity that can be identified only with qualitative analytical values (e.g., peak area, retention time, etc.), for which structural information is not yet available.

Impurities present in new drug substances used in clinical and safety trials are covered under two aspects [ICH Q-3A (R2) 2006]. *Chemistry aspects* classify and identify impurities, generate the report for different impurities,

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list various impurities present in any substances, and give a brief discussion of analytical procedures for impurity detection. *Safety aspects* include those impurities that are present at a considerably lower amount or not present at all in a discovery of new drug substance.

Commonly used impurity terms

A number of terms have been commonly used to describe an impurity or impurities (Francis et al. 1984, McNaught and Wilkinson 1997):

- Intermediate
- Penultimate intermediate
- By-product
- Transformation product
- Interaction product
- Related product
- Degradation product
- Foreign substance
- Toxic impurity
- Concomitant component
- Ordinary impurity
- Organic volatile impurity (OVI).

Intermediate

The compounds formed in the process of synthesis for the desired product are called intermediates or reaction intermediates. They are defined as products that have undergone a partial processing and are used as raw material in a successive productive step.

Penultimate intermediate

As the name suggests, this is the compound found in the synthesis chain before the production of the desired compound. Sometimes confusion arises when the desired material is a salt of a free base or acid. In our opinion, it is inappropriate to label the free base or acid as the penultimate intermediate if the drug substance is a salt.

By-product

The unintentional compounds that arise during the reaction are commonly called by-products. Not all by-products can be quantified easily; hence, they present a thorny problem to the analytical chemist. A by-product can be useful and marketable or it can be considered waste.

Transformation product

This relates to an expected and non-expected product that may be formed in the reaction. Transformation products are very similar to by-products, except the term tends to connote that more is known about the reaction products than transformation products.

Interaction product

This term is slightly more comprehensive and more difficult to evaluate than by-products and transformation products in that it considers interactions occurring among various chemicals involved in reaction.

Related product

As mentioned, impurity is a word that is not well liked. So a related product actually is similar to an impurity, but active pharmaceuticals use the term related products instead, thus playing down the negativity frequently attached to the term impurity. These products can have similar chemical structure and might have standardized biological activity; however, this by itself does not provide any guarantee of effect.

Degradation product

The compounds produced due to decomposition of the material of interest or active ingredients often are referred to as degradation products.

Foreign substance

This is the material that may be present due to contamination or adulteration, not as outcomes of synthesis.

Toxic impurity

Toxic impurities might affect the biological activity, even at very low concentrations. They require identification by qualitative or quantitative means.

Concomitant component

Bulk pharmaceutical chemicals may contain concomitant components, which are geometric and optical isomers and antibiotics that are mixtures.

Ordinary impurity

An impurity having enough potency to have biological activity – even at trace level – is called an ordinary impurity.

Organic volatile impurity

A solvent that may remain in the drug substance should be considered as an organic volatile impurity (OVI).

Classification of impurities

- Organic (process and drug related)
- Inorganic
- Residual solvents
- Polymorphic
- Enantiomeric.

Organic impurities

Organic impurities come into existence during the synthesis of the active and inactive materials. They may occur during manufacturing or during storage of the materials. These impurities can be deduced from degradation reactions and ongoing synthesis in active pharmaceutical entities and drug products. Impurities generated during the synthetic process are intermediates, by-products, and reagents, as well as ligands and catalysts used in the chemical synthesis (Ahuja 1998, Qiu and Narwood 2007).

Starting materials and intermediates

These are the chemical compositions used to synthesize the desired constitute of a drug substance molecule. Starting materials and intermediates that are not reacted in the reaction, especially when the synthesis is about to complete, will remain in the final product as impurities (Muehlen 1992, Gorog 2000, Gavin and Olsen 2006). One such example is 4-aminophenol, a starting material for synthesis of paracetamol bulk drug, which might be present in final product as an impurity having a toxic effect on the liver.

According to Dir. 2001/83/EC (EMEA 2012), for biological medicinal products, "Starting materials means any substance prevailed from the human or plant or microorganisms or any alteration to the biological origins by means biotechnological cell constructs which will have tendency to formed drug product." So measures for controlling sourcing of starting materials or intermediates must be strong.

An intermediate is a substance that is produced in the reaction vessel from the starting materials and which might undergo further chemical modification to provide the final product.

By-product

As mentioned earlier, the desired product is commonly called the *main product*, and product that is unwanted but might be useful is known as a *by-product*.

Degradation product

Degradation products are the compounds formed due to chemical changes in drug products during storage. Degradants may form because of chemical interactions with other compounds or due to contaminants present in the drug substances.

In certain cases, physical degradation occurs for a variety of reasons: change in the polymorphic state of the molecule, aggregation of proteinaceous material due to heat or residual solvents, absorption of water, loss of water, and others. A degradation product can be determined by short- and long-term stability studies per ICH, for example, in treatment for common cold formulations containing acetaminophen, phenylephrine hydrochloride, and chlorpheniramine maleate. Degradation products for these formulations were isolated and found to be an addition compound of phenylephrine and maleic acid (Wong et al. 2006). The definition of degradation product in accordance with the ICH guideline is "any chemical change occur[ing] due to overreaction or over heating or changing in condition of solution, i.e., change in pH, exposure to light, etc. or reaction of final product with container or closure or excipients used in making product" [Gorog 2003, ICH Q-3A (R2) 2006].

Reagents, ligands, and catalysts

Reagents, ligands, and catalysts are seldom present in the final products (Ahuja 1998, Roy 2002). For the synthesis of the drug substance or any excipient catalysts, chemical reagents and ligands are used that can be conveyed to the concluding products as impurities in minute levels. For example, carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP) (Gorog 2003), is an alkylating agent that was observed as an impurity in the synthesis of a β lactam drug substance.

Products of overreaction

Products of overreaction form when reactions for the synthesis are not selective as much as necessary, so nonselective interaction at an undesired site will produce an incorrect compound. For example, the last step for the synthesis of nanodralone decanoate is the decanoylation of the 17-OH group. Enol compound 3, 17 β-dihydroxyestra-3, 5-diene disdecanoate was formed because of overreaction at the 4ene-3 oxo group site (Gorog 2000, 2003).

Contamination by organic impurities

Contamination with organic impurities is not related to a drug but might unknowingly be present in the drug. For example, for drug substances derived from plants, herbicides used to protect plants may be present, such as diquat and glyphosate, or pesticides such as carbofuran and endrin sprayed into the environment (Bauer et al. 2001).

Inorganic impurities

Inorganic impurities include filter aids, color removing agents such as charcoal, reaction rate modifiers (catalysts), ligands, and heavy metals. One example would be a catalyst used in a substitution reaction during the synthesis of the API or raw materials. Inorganic impurities might have toxic effects, so they should be removed or controlled to a minimum level. Batch-to-batch variation in impurity levels suggests that the manufacturing or synthesis process of the drug product is not controlled (Roy 2002, Basak et al. 2007, Hulse et al. 2008, ICH Q-3D 2009).

Inorganic impurities normally known and identified are as follows.

Contamination by inorganic impurities

These are unforeseen impurities found in final product. Contaminant impurities detected in drugs have been controlled in many ways. For example, previously used glass vessels for reaction are now replaced with acid/alkali resisted glass (Bauer et al. 2001). So, impurities that might be present due to leaching from glass vessel is minimized to safer levels.

Reagents, ligands, and catalysts

Reagents, Ligands and Catalysts are well defined under organic impurity of this paper. However, catalysts used in decomposition of intermediates (iodide catalysts), and monodentate ligand such as chloride ions might remain in the final product as inorganic impurities.

Residual solvents

Residual solvents in pharmaceuticals are the volatile chemicals that are produced as a result of side reactions or used in the manufacturing of API or excipients, or in the formulation [ICH Q-3C (R4) 2009]. Theoretically they can be removed from the final product but practically they can not. Therefore, it may be a vital parameter in the process for making a drug product.

Polymorphic forms

Solid material that subsists in two or more forms or in a crystalline structure is said to be polymorphic. Some organic and inorganic compounds form different crystalline structures called polymorphs or polymorphic forms. The resulting change of intermolecular interactions gives rise to different pharmacokinetic properties of medical drugs, as well as to different properties of organic and inorganic materials. Therefore, the unambiguous identification and characterization of polymorphs is very important, especially from the economic point of view. In 2006 a new crystal form of maleic acid had arisen when solution of caffeine and maleic acid (2:1) in chloroform is set aside to evaporate slowly (Day et al. 2006).

Enantiomeric impurities

To determine purity of the chiral compound term enantiomeric excess (EE) is used. These impurities present in the

drug are due to change in the critical parameter of molecules during synthesis. The following equation is used to determine enantiomeric excess (EE):

$$EE=((R-S)/(R+R))\times 100$$

where R and S stand for the individual optical isomer in the mixture (and R+S=1).

These determinations are important particularly when we are talking about efficacy of the drug, because in the case of optical isomers of a drug only one isomer has therapeutic efficacy while the rest of them have either a toxic effect or have no effect at all (Armstrong et al. 1998, Roy 2002, Gorog 2003, Qiu and Narwood 2007).

Control of impurities

According to theory, all impurities should be removed from the final product, but in practice, impurities cannot be entirely abolished from the final product. So, for a quality product, impurities should be kept within the limits. According to a study carried out for impurity, very low amount of impurities in the product should be allowed. However, in special cases, rather high quantities of impurities are permitted, for example, biotechnologically derived products that have biological activity.

Most of the bulk pharmaceutical chemicals (BPCs) are obtained from various sources. Therefore, it is crucial that impurities in BPCs be monitored and controlled very carefully.

Various controlling authorities for impurity (USP 1995, ICH Q-6A 1999, ICH Q-6B 1999) are mentioned in monographs and specifications about maximum tolerable limits.

Control of organic impurity

Most often, reduction in quantity of by-products in the reaction can be carried out by tightly controlled reaction conditions at crucial steps of the reaction to preclude a new impurity or diverging level of impurity. Another approach to reduce the quantity of impurity in the final product is to use superior quality starting materials. Likewise, the use of high-grade solvents also imparts its effort to obviate the production of by-products or any unknown entity. The thresholds for allowable organic impurities are shown in Table 1.

Table 1 Threshold for organic impurities [ICH Q-3A (R2)].

Maximum daily dose[a] (g/day)	Reporting threshold[b,c] (%)	Identification threshold[c]	Qualification threshold[c]
≤2	0.05	0.10% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day intake (whichever is lower)
>2	0.03	0.05%	0.05%

[a]This is the amount of drug substance administered per day.
[b]Higher reporting thresholds should be scientifically justified.
[c]Lower thresholds can be appropriate if the impurity is unusually toxic.

Control of degradation impurity

This particular impurity covers degradation products of active substance, including reaction products with excipient or container system [ICH Q-3A (R2) 2006, ICH Q-3B (R2) 2006]. Degradation products observed in stability studies performed at recommended storage conditions should be identified, qualified, and reported when the following thresholds exceeded (Table 2).

Control of inorganic impurities

Oral/parenteral concentration limits (ppm) have been proposed for 14 metals in active substances or excipients: Pt, Pd, Ir, Rh, Ru, Os, Mo, V, Ni, Cr, Cu, Mn, Zn, and Fe. Metals are divided into three classes as follows, and limits have been summarized in Table 3.

Class 1: Metals of significant safety concern

Some metals are known or suspected human carcinogens, genotoxic, and sometimes nongenotoxic carcinogens or

Table 2 Minimum degradation threshold for daily intake of drug product.

Max daily dose	Qualification threshold	Identification threshold	Reporting threshold
<1 mg	1.0% or 50 µg/TDI	1.0% or 5 µg/TDI	0.10%
1 mg–10 mg	1.0% or 50 µg/TDI[a]	0.5% or 20 µg/TDI	0.10%
>10 mg–100 mg	0.5% or 200 µg/TDI	0.2% or 2 mg/TDI	0.10%
>100 mg-1 g	0.2% or 3 mg/TDI	0.2% or 2 mg/TDI	0.10%
>1 g–2 g	0.2% or 3 mg/TDI	0.2% or 2 mg/TDI	0.05%
>2 g	0.15%	0.10%	0.05%

[a]Qualification threshold for 10 mg/day is 0.5%/200 µg TDI.

Table 3 Limits of inorganic impurities in oral and injectable (CPMP/SWP/QWP/4446/00).

Classes of metals	Oral		Injectable	
	PDE (μg/day)	Concentration (ppm)	PDE (μg/day)	Concentration (ppm)
Class 1A: Pt, Pd	100	10	10[a]	1[a]
Class 1B: Ir, Rh, Ru, Os	100[b]	10[b]	10[b]	1[b]
Class 1C: Mo, Ni, Cr, V	300	30	30[a]	3[a]
Class 2: Cu, Mn	2500	250	250	25
Class 3: Fe, Zn	13,000	1300	1300	130

[a]Separate limits for inhalation exposure to Pt, Cr (VI) and Ni.
[b]Subclass limit.
PDE, permitted daily exposure.

potential contributory agents which produce irreversible toxicity, for example, neurotoxicity or teratogenicity. A few of them produce significant but reversible toxicity, such as Ir, Pd, Pt, Ru, Rh, Os, Mo, V, Cr, and Ni.

Class 2: Metals with low safety concern

Trace metals required for nutritional purposes can be present in foodstuffs or as readily available supplements, for example, Cu and Mn.

Class 3: Metals with minimal safety concern

Metals, omnipresent in the environment or plant and animal kingdoms, as such have high tolerable toxic values for humans. The nutritional intakes of ≥ 10 mg/day is recommended. Examples are Fe and Zn.

Control of residual solvents

Various regulatory authorities have been concerned about toxicity of the residual solvent in the pharmaceutical world. At most, various pharmaceutical provide guidelines (USP 1990, BP 1996, EP 1997, EMEA 2009) for the control of residual solvents and with different categories in pharmaceuticals gives acceptance limits (Table 4). In addition, for solvents that are used in pharmaceuticals, there are only a few residual solvents that are controlled (Hu and Liu 2011). So globally there is a need for a standard guideline to be established for the control of residual solvents. Therefore, the harmonized guidelines for control of residual solvents by ICH has been released.

For pharmaceutical production, organic solvents invariably remain present in the processes. The pharmaceutical industry is one of the largest users of organic solvents per amount of the final product (Slater et al. 2006, Smith and Webb 2007, Katarzyna and Andrzej 2010). The synthesis of an active or inactive pharmaceutical ingredient usually requires large amounts of solvent and sometimes during the drug product formulation process, as well as during the formulation process methylene chloride, is used as solvent in large amounts for coating process. Residual solvents are placed in following classes based on their toxic effects to human health.

Table 4 Limits of initially controlled residual solvents in pharmacopoeias.

Organic volatile impurities	Limit (ppm)			
	USP 22 3rd edition	BP (1993)	EP 3rd edition	ChP 1995 edition
Chloroform	50	50	50	50
Benzene	100	100	100	100
1,4-Dioxane	100	100	100	100
Dichloromethane	100	100	100	100
Trichloroethene	100	100	100	100
Acetonitrile	–	50	50	–
Pyridine	–	100	100	100
Toluene	–	–	–	100

Class 1 solvents: solvents to be avoided

Solvents in class 1, due to their known carcinogenicity and hazardousness to environment should not be utilized in the manufacturing of active and inactive materials, or drug products. Even so, in any circumstances, if we can avoid use of this class of solvents, they should be limited in the final product as shown in Table 5.

Class 2 solvents: solvents to be limited

Solvents listed in Table 6 might be less toxic than class 1 solvents, but because of their inherent toxicity they should be limited as PDEs. This class is very much higher than class 1.

Table 5 Solvents in pharmaceutical products that should be avoided (ICH Q3-C).

Solvent	Concentration limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 6 Solvents in pharmaceutical products that should be limited (ICH Q-3C).

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Dichloromethane	6.0	600
N,N-imethylformamide	8.8	880
1,4-Dioxane	3.8	380
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene[a]	21.7	2170

[a]Usually 60% m-xylene, 14% p-xylene, 9% o-xylene with 17% ethyl benzene.

Class 3 solvents: solvents with low toxic potential

Solvents in this class have low toxic potential to humans, as these solvents have PDEs of 50 mg or more per day.

Class 3 solvents which should be limited by GMP are as under (per ICH Q3C):

1-Butanol Methyl acetate	1-Pentanol	Ethanol
Heptanes	1-Propanol	Propyl acetate
Acetone Isobutyl acetate	Tert-Butylmethyl ether	Ethyl acetate
2-Butanol 3-Methyl-1-butanol	Methyl isobutyl ketone	Ethyl ether
Anisole Isopropyl acetate	2-Methyl-1-propanol	2-Propanol
Acetic acid	Ethyl format	Cumene
Methyl ethyl ketone	Pentane	Formic acid
Butyl acetate	Dimethyl sulfoxide	

Other class: solvents for which no adequate toxicological data was found

This class lists additional solvents for which no adequate toxicological data available to generate a PDE. Some examples are (ICH Q-3C 2009)

Isooctane	1, 1-Dimethoxymethane	Petroleum ether
Methyl isopropyl ketone	1, 1-Diethoxypropane	Trifluoroacetic acid
2, 2-Dimethoxypropane	Isopropyl ether	
Methyltetrahydrofuran	Trichloroacetic acid	

Control of genotoxic impurities

Existing ICH Q-3 guidelines do not provide acceptable toxicological limits of genotoxic impurities in active pharmaceuticals. Determination of genotoxic effects of impurities without any data is very difficult for assessing impurity. Most of the pharmaceutical and other concerned industries accept the approach of threshold of toxicological concern (TTC). This approach gives an acceptable risk value (a TTC value of 1.5 µg/day intake) for intake of genotoxic impurity for most pharmaceuticals.

Various classes of genotoxic impurities are as follows (McGovern and Jacobson-Kram 2006):

- Class 1: Impurities known to be genotoxic (mutagenic) and carcinogenic
- Class 2: Impurities known to be genotoxic (mutagenic) but with unknown carcinogenic potential
- Class 3: Alerting structure, unrelated to the parent structure and of unknown genotoxic (mutagenic) potential
- Class 4: Alerting structure, related to the parent API
- Class 5: No alerting structure or indication of genotoxic potential.

During clinical trials some data that signifies the allowable daily intake is summarized in Table 7 (EMEA 2006, McGovern and Jacobson-Kram 2006).

Table 7 Permissible limit for daily intake of genotoxic impurities on clinical development.

	Duration of exposure				
	≤1 month	>1–3 months	>3–6 months	>6–12 months	>12 months
Allowable daily intake (μg/day) for all phases of development	120	40	20	10	1.5
	or	or	or	or	
Alternative maximum based on percentage of impurity in API	0.5%	0.5%	0.5%	0.5%	

Isolation and characterization of impurities

A number of methods can be used for isolating and characterizing impurities. The application of any given method depends on the nature of the impurity, in other words, its structure, physical and chemical properties, and availability (the amount present in the original material from which it must be isolated). The following methods may be useful in this context:

- Extraction
- Chromatography
- Preparative separations.

Extraction

Extraction is one of the most useful methods for isolation of an impurity. For this the following methods can be helpful:

- Liquid/solid extraction
- Supercritical fluid extraction
- Liquid/liquid extraction or solvent extraction.

Liquid/solid extraction or solid-phase extraction (SPE)

Solid-liquid extraction allows soluble components to be removed from solids using a solvent. The same principle is applied here to choose a solvent for dissolving the impurity of interest present in the solid matrix. For example, if we want to determine salt in sand, we would simply use water to dissolve it and filter the solution, which on evaporation will produce salt in a reasonably pure form. If, conversely, other water-soluble impurities were present in the sand, then it would be necessary to select a different solvent or it would be necessary to manipulate the solution further.

It is noticed that when we are talking about the impurities that are already present in pharmaceuticals, it will be harder to isolate the impurity in its pure form. We have to use an organic solvent or mixtures of organic solvents to deal with the impurity. Moreover, organic solvents are volatile in nature, so we can evaporate them under low temperature to get a concentrated product.

Solid-phase extraction (SPE) (Thurman and Mills 1998, Fritz 1999, Dobo et al. 2006) is normally done with the use of cartridges and disks, available with a variety of stationary phases.

Normal phase SPE

The theory involved in normal phase SPE generally require mid- to nonpolar solvent mixtures (e.g., n-hexane, methylene dichloride, acetic acid, diethyl ether, etc.), a polar substrate (e.g., drug molecule, excipients, etc.) and a polar stationary phase. For the normal phase, various stationary phase materials are used. One of them is silica, which can be modified further with polar heads (e.g., Si-C4-CN, Si-C4-NH2, etc.). Other adsorbents used are florisil, alumina, etc.; the mechanism involved in retention of substrate in normal phase SPE is principally the interaction with a polar analyte functional group and polar heads in the stationary phase.

Reversed phase SPE

The mechanism involved in reversed phase SPE requires a polar mobile phase (e.g., methanol, ethanol, water, etc.) or a semi-polar solvent mixture and a nonpolar stationary phase. In the reverse phase SPE modified silica is used as the stationary phase, in other words alkyl- or aryl-bonded silicas (Si-C-18, Si-C-8, Si-C-4, and Si-C-Ph).

Ion exchange SPE

The main rationale of the ion exchange SPE is to separate oppositely charged ions in a solution. Different types of exchangers have been used to separate the charged moieties. Commercially available ion exchangers contain resinous parts having amine or quaternary ammonium groups or other ionic groups for the separation of anionic or cationic compounds. The retention mechanism for the analyte is at the exchanger surface for the diffusion of ions. This depends on the concentration of the solution and the degree of cross linking of ion exchangers.

Anion exchange SPE

Material used in anion exchange SPE for the stationary phase is having a positively charged group (e.g., an aliphatic quaternary amine group or amino group). Positively charged groups such as quaternary amines are strong bases that will draw anionic molecules into the solution and strongly attach to the exchanged group. As it strongly binds to the anionic group, it is termed a strong anion exchanger (SAX). Because of its strong binding capacity, it is generally used when recovery of anion is no longer required. However anions that can be displaced by another anion shall be eluted by changing the pH of the solution.

The stationary phase containing amino group, used in the normal phase SPE, can be used as a weak anion exchanger (WAX). The advantage of WAX utilization for separation of species is that we can isolate and recover strong as well as weak anions.

Cation exchange

The materials used for cation exchange are high molecular weight cross-linked polymers having carboxylic, phenolic, or aliphatic sulfonic acid groups. Among these groups sulfonic acid pulls in cationic species strongly present in solution and so is termed a strong cation exchanger (SCX). Moreover, materials containing a carboxylic or phenolic group that is a weak anion can be used as weak cation exchanger (WCX). By the use of WCX, strong and weak cations can be isolated and recovered easily.

Supercritical fluid extraction

In the field of supercritical fluid extraction (SFE) (Hedrick et al. 1992, Wai and Laintz 1994, Simpson 2000, McHugh and Krukonis 2008) various researchers proposed the use of supercritical carbon dioxide (CO_2) as an extractant for separating various components.

The procedure involved in SFE is very convenient for novices. A sample thimble is used to handle a sample through which supercritical fluid is pumped. The extraction of the soluble compounds is allowed to take place as the supercritical fluid passes into a collection trap through a restricting nozzle. After passing through the nozzle, it is recompressed by venting in the collection trap for future use. The material left behind in the collection trap is the product of the extraction. Characteristics of gases normally using SFE are given in Table 8.

Table 8 Solvents for SFE.

Solvent	Pressure (atm)	Temperature (°C)	Density (g/ml)
n-Pentane	33.6	196.6	0.232
CO_2	72.9	–	0.448
NH_3	111.3	132.3	0.24

Liquid/liquid extraction or solvent extraction

In liquid-liquid extraction components are separated based on their solubility in two slightly miscible or completely immiscible solvents, where mass transfer occurs at the interface and components separate by their affinity to the solvents (Qiu and Narwood 2007, Aguilar and Cortina 2008).

Partition coefficient plays an important role in this extraction process, by which the amount of solute that is distributed between two immiscible solvents a and b can be easily found:

$$K_d = C_a / C_b$$

where, K_d is the distribution coefficient or partition coefficient.

C_a is the concentration of component in solvent a.

C_b is the concentration of component in solvent b.

By the use of this technique the solution containing impurity can be concentrated and thus the impurity can be easily detected.

Chromatography

Most recently, organic drug substance impurities are measured using chromatographic procedures, as they give more accurate results. These procedures should involve a separation mode that allows for the resolution of impurities from the drug substance and a detection mode that allows for the accurate measurement of impurities.

Owing to the polar and nonvolatile nature of most compounds used as medicinal drugs, reversed-phase HPLC is the most common technique for monitoring the drug substance and its impurities. GC is also used, particularly for residual solvents, and capillary electrophoresis (CE) has been introduced in more recent times. Some older methods use thin-layer chromatography (TLC), but use of this methodology for the quantitative measurement of impurities is not common.

HPLC-MS or HPLC-NMR

The most common technique for monitoring impurities is HPLC with UV detection. Quantification of impurities is achieved by reference standards, when available, or by area percent or height percent relative to the parent compound (Lee and Kerns 1999, Kostiainen et al. 2003). Important application for impurity identification with HPLC is by the use of MS as detector.

Recently, HPLC-MS has become the popular technique for structural elucidation and confirmation of impurities. During the synthesis it is necessary to identify the various types of impurities for maintaining the quality of the product. Because of its selectivity, sensitivity, and compatibility with LC, LC-MS and LC-NMR have become absolutely necessary analytical techniques for the analysis of impurities present in various drugs and drug products and have become the first choice method. As it provides some structural information about fragments, empirical formula, and molecular weight, it has become a popular and advantageous method for the impurity analysis.

Coupling of LC and NMR (Treiber 1987, Albert 2002) has recently attracted research because of reduction in tedious preparative steps and substantially acquires higher efficiency and precision when handling complex mixtures.

Thin-layer chromatography (TLC)

For isolation and purification of compounds, TLC has gained importance because of its simplicity and utility. No major equipment is required, and the method of development is relatively easy (Sherma and Fried 1991, Ahuja 2003, Smith and Webb 2007). The primary limitation is the small number of theoretical plates that are obtained with this method as compared to GC or HPLC.

Detection frequently is performed visually or by UV (e.g., 366 nm). The fluorescence-quenching substances absorbing UV light in the short-wavelength region also can be detected if the layer is impregnated with a fluorescent substance. Iodine vapors can help detect most organic substances. A number of techniques can be used to recover the sample from the plate. The most simple and convenient method for obtaining the desired material is scraping the sorbent from the adsorbent site and shifting it to an extraction vessel, where different solvents are used for extraction of a compound.

Capillary electrophoresis (CE)

CE is not used much for impurity identification, but it offers the advantage that CE procedures can be employed when HPLC procedures have failed to measure the impurities adequately. CE is particularly important for the separation of chiral compounds that have closely related structures.

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