



*Review Article*

## Recent approaches for impurity profiling of pharmaceuticals

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**Abstract**

Impurities must be monitored carefully to assure the quality of drugs. It is important to identify potential sources of such impurities. Selective analytical methods need to be developed to monitor them. Methodology aspects for impurity investigations are discussed along with an emphasis on understanding the origin and fate of impurities to guide decisions on process controls and specifications. Orthogonal analytical approaches for impurity investigations to provide a complete understanding of a drug substance impurity profile. Considerations for control of toxic impurities include sensitive and selective analytical methodology and determination of the process capability for removing the impurity. New impurities may be observed as changes are made in the synthesis, formulation, or production procedures, albeit for improving them. At times it is necessary to isolate and characterize an impurity when hyphenated methods do not yield the structure or when confirmation is necessary with an authentic material.

### 1. Introduction

An impure substance may be defined as any material that affects the purity of the material of interest, viz., an active pharmaceutical ingredient (API) or drug substance. Impurity control in pharmaceutical products is a primary goal of drug development. Stringent international regulatory requirements have been in place for several years as outlined in the International Conference on Harmonization (ICH) Guidelines Q3A(R), Q3B(R) and Q3C [1-3]. The purity of a drug product is in turn determined on the basis of the percentage of the labeled amount of API found in it by a suitable analytical method. Later discussion will also reveal that a drug product can have impurities that need to be monitored even though they do not affect the labeled content. Degradation product impurities in drug products are of primary concern while process-related impurities and degradation products are both necessary to control in APIs. Many potential impurities result from the API manufacturing process including starting materials, isomers, intermediates, reagents, solvents, catalysts and reaction by-products. These potential impurities should be investigated to determine process control mechanisms for their removal and the need for specification controls at appropriate points in the process. Several descriptions have appeared in the literature for impurity investigations and method development to support them [4,5]. As for impurity profiling, it is the common name of analytical activities with the aim of detecting, identifying or elucidating the structure and quantitatively determining organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations [5].

The different pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP) are slowly incorporating limits to allowable levels of impurities present in the API's or formulations. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's). Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research. A number of recent articles [17-18] have described a designed approach and guidance for isolating and identifying process-related impurities and degradation products using

Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques. Analysis has gone beyond traditional chromatographic techniques, such as HPLC and GC, in order to resolve potential impurities through efforts that embrace diverse chemical environments. This concept is known as 'orthogonality'. One such employable orthogonal technique is Supercritical Fluid Chromatography (SFC). SFC is considered a normal phase technique because it utilizes the relatively nonpolar, liquid carbon dioxide as the bulk of the mobile phase and due to higher diffusivity of the mobile phase yields greater efficiency.

## 2. Impurity profile and drug safety

The safety of drug therapy is closely related to the quality of drugs. The requirements with respect to the active ingredient content of bulk-drug materials in the various pharmacopoeias are usually in the range 98–99% [6-8]. The aim is to minimize the adverse effects of drug materials and the preparations made thereof. After establishing the pharmacological-toxicological profile of a drug substance, pharmacologists, clinicians and drug-registration authorities consider its beneficial and adverse effects to the human organism and, on the basis

of the benefit/risk ratio thus obtained, make the decision with respect to the possibility of introducing it into therapy.

## 3. Sources of impurities in drug products

In general, the various types of impurities that may be present in pharmaceutical substances can come from the following sources:

### 3.1. Synthesis-related impurities

Impurities in a drug substance or a new chemical entity (NCE) originate mainly during the synthetic process from raw materials, solvents, intermediates, and by-products. The raw materials are generally manufactured to much lower purity requirements than a drug substance. Hence, it is easy to understand why they can contain a number of components that can in turn affect the purity of the drug substance. Similarly, solvents used in the synthesis are likely to contain a number of impurities that may range from trace levels to significant amounts that can react with various chemicals used in the synthesis to produce other impurities. Intermediates are also not generally held to the purity level of the drug substance—hence the remarks made for the raw materials apply. It is not reasonably possible to theorize all by-products; as a result, any such products that may be produced in the synthesis would be hard to monitor. The “pot reactions,” i.e., when the intermediates are not isolated, are convenient, economical, and timesaving; however, they raise havoc in terms of the generation of impurities because a number of reactions can occur simultaneously. Incidentally, this problem of numerous reactions occurring simultaneously can be also encountered in single reactions where intermediate is isolated.

### 3.2. Formulation-related impurities

These impurities may be :-

#### 3.2.1 Method Related

eg- diclofenac sodium ampoule – indoline-2 one(impurity) is formed in the production of parenteral dosage form if it is terminally sterilized by autoclave because concentration of the impurity in the ampoule exceeds the limit of the raw material in the BP.

#### 3.2.2 Environmental related

Exposures to adverse temperatures. Eg - vitamin products in liquid formulations Expose to light (UV light). Ergometrine injections shows complete degradation when kept 42 hrs in direct sunlight.

Humidity e.g., Aspirin, Ranitidine & other hygroscopic products.

**3.2.3 Dosage form related:** In general , liquid dosage form are very much susceptible to both degradation and microbiological contamination. In this regard , water content , pH of solution, mutual interaction of ingredient and the primary container are critical factor.

**3.3. Degradation-related impurities:** A number of impurities can be produced because of API degradation or other interactions on storage, Eg : Nicotinamide presence in a formulation containing 4 vitamins (nicotinamide, pyridoxine, riboflavin and thiamine) causes degradation of thiamine to a substandard level within a 1-year shelf life of Vitamin B-complex injections. Therefore, it is very important to conduct stability studies to predict, evaluate, and ensure drug product

safety<sup>[9]</sup>. Stability studies include evaluation of stability of API, preformulation studies to evaluate compatibility of API with the excipients to determine its stability in the formulation matrix, accelerated stability evaluations of the test or final drug product, stability evaluation via kinetic studies and projection of expiration date, routine stability studies of drug products in marketed, sample or dispensed package under various conditions of temperature light, and humidity.

The stability studies under various exaggerated conditions of temperature, humidity, and light can help us determine what potential impurities can be produced by degradation reactions<sup>[10]</sup>.

**3.3.1 Kinetic studies:** Most of the degradation reactions of pharmaceuticals occur at finite rates and are chemical in nature. These reactions are affected by conditions such as solvent, concentration of reactants, temperature, pH of the medium, radiation energy, and the presence of catalysts. The order of the reaction is described by the manner in which the reaction rate depends on the concentration of reactant. The degradation of most pharmaceuticals can be classified as zero order, first order, or pseudo-first order, even though they may degrade by complicated mechanisms, and the true expression may be of higher order or be complex and noninteger.

#### 4. Classification of impurities

For each API there should be an impurity profile describing the identified and unidentified impurities present in a typical batch. The impurity profile is normally dependent upon the process or origin of the API<sup>1</sup>.

According to ICH guidelines,<sup>1</sup> impurities associated with API's are classified into the following categories:

- a. Organic impurities (Process and Drug-related)
- b. Inorganic impurities
- c. Residual solvents

**a. Organic Impurities:** Organic impurities may arise during the manufacturing process and/or storage of the drug substance. They may be identified or unidentified, volatile or non-volatile, and these include the starting material, intermediates, degradation products, by-products and reagents, ligands and catalyst used at different stages of synthesis of API and drug products. These are described as follows:-

**Starting materials or intermediates:** These are the most common impurities found in every API unless a proper care is taken in every step involved throughout the multi-step synthesis. Although the end products are always washed with solvents, there are always chances of having the residual unreacted starting materials unless the manufacturers are very careful about the impurities. In paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for some one manufacturer or be an intermediate for the other.

**By-products:** In synthetic organic chemistry, getting a single end product with 100% yield is very rare; there is always a chance of having by-products. By-products from the side reactions are among the most common process impurities in drugs<sup>11, 12</sup>. By-products can be formed through a variety of side reactions, such as incomplete reaction, overreaction, isomerization, dimerization, rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts<sup>13</sup>.

**Degradation products:** Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. However, degradation products resulting from storage or formulation to different dosage forms or aging are also common impurities in the medicines. The degradation of penicillins and cephalosporins is a well-known example of degradation products. The presence of a  $\beta$ -lactam ring as well as that of an  $\alpha$ -amino group in the C6/C7 side chain plays a critical role in their degradation<sup>14, 15</sup>.

**Reagents, ligands and catalysts:** These chemicals are less commonly found in API's; however, in some cases they may pose a problem as impurities. It has also been found that the presence of certain chemicals such as triethylamine has a degradative effect on the product. Ampicillin trihydrate samples having triethylamine content of 2000 ppm to 4000 ppm (determined by visual color method developed by Gist- Brocades, Delft, Holland) were found to be stable under accelerated stability testing. However, the product showed appreciable degradation when triethylamine content became 7000 ppm<sup>15</sup>.

Chemical reagents, ligands and catalysts used in the synthesis of a drug substance can be carried over to the final products as trace level impurities. For e.g. carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP), which is used as an

alkylating agent in the synthesis of a  $\beta$ -lactam drug substance, was observed in the final product as an impurity. Many chemical reactions are promoted by metal based catalysts. For instance, a Ziegler-Natta catalyst contains titanium, Grubb's catalyst contains ruthenium and Adam's catalyst contains platinum. In some cases, reagents or catalysts may react with intermediates or final products to form byproducts. For e.g., Pyridine, a catalyst used in the course of synthesis of mazipredone, reacts with an intermediate to form a pyridinium impurity<sup>13</sup>.

#### b. Inorganic Impurities:

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

- Reagents, ligands and catalysts
- Heavy Metals or other residual metals
- Inorganic salts
- Other materials (filter aids, charcoal)

**Reagents, ligands and catalysts:** The chances of having these impurities are rare however, in some processes; these could create a problem unless the manufacturers take proper care during production.

**Heavy metals:** The main sources of heavy metals are the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place and water used in the processes. These impurities of heavy metals can easily be avoided using demineralized water and glass-lined reactors.

**Filter aids, Charcoal:** The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants, and in many cases, activated carbon is also used. The regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminations.

**c. Residual solvents:** Residual solvents are organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents<sup>3</sup> are classified as follows:

**Class 1 solvents:** Solvents to be avoided in pharmaceutical products Known human carcinogens, strongly suspected human carcinogens and environmental hazards. Example was shown in **Table 1**

**Class 2 solvents: Solvents to be limited in pharmaceutical products:** Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. solvents suspected of other significant but reversible toxicities. Example was shown in **Table 2**

**Class 3 solvents: Solvents with low toxic potential:** Solvents with low toxic potential to man; no health -based exposure limit is needed.

These solvents are less toxic in acute or short term studies and negative in genotoxic studies. The amount of these residual solvents of 50mg or less would be acceptable. Examples : Acetic acid, Acetone, Anisole, 1-Butanol, 2-Butanol etc.

**Class 4 solvents: Solvents for which No adequate toxicological data was found:** The solvents may be of interesting to manufacturers of excipients, drug substances or drug products. But there was no adequate toxicological data on which to base a Permitted Daily Exposure was found. Examples: 1,1-Diethoxy propane,1,1-Dimethoxy propane,2,2-Dimethoxy propane, Isooctane etc.

**Table 1: Solvents to be avoided in pharmaceutical products**

Solvent	Concentration limit (ppm)	concern
Benzene	2	Carcinogen
CCl <sub>4</sub>	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethane	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

**Table 2: Solvents to be limited in pharmaceutical products**

Solvent	Permitted Daily Exposure a (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethane	18.7	1870

#### 4. Others

**Enantiomeric impurities:** There are many drugs in which only single enantiomer is active. In such cases the inactive enantiomer is considered as an impurity, e. g. In pilocarpine only Dextro form is active, here levo form is considered as an impurity.

**Identification and qualification thresholds of impurities & Limits:** The International Conference on Harmonisation addresses questions relating to impurities as follows :

Q1A (R) stability testing of new drug substances and products

Q3A (R) impurities in drug substances

Q3B (R) impurities in drug products

Q3C impurities: residual solvents

Q6A specifications: test procedures and acceptance criteria for new drug substances and new drug products; chemical substances.

**Limits for impurities:** According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level, is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. According to ICH, the maximum daily dose qualification threshold to be considered is as follows as shown in **Table 3**.

As can be seen from the data in **Table 4**, ICH treats the degradation products slightly differently than impurities even though for all intents and purposes the degradation products are impurities.

**Table 3: Drug substance impurities thresholds**

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤ 2g/day	0.05%	0.10% or 1.0 mg/day intake (whichever is less)	0.15% or 1.0 mg/day intake (whichever is less)
≥ 2g/day	0.03%	0.05%	0.05%

**Table 4: Thresholds for Reporting of Degradation Products in New Medicinal Products**

Maximum Daily Dose	Threshold
<1g	0.1%
> 1 g	0.05%

**Selective analytical methodologies:** A variety of methods are available for monitoring impurities. The primary criterion is the ability to differentiate between the compounds of interest. This requirement reduces the availability of methods primarily to spectroscopic and separation methods or a combination thereof.

**Spectroscopic methods:** The following spectroscopic methods can be used:

- Ultraviolet (UV)
- Infrared (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry (MS)

UV at a single wavelength provides minimal selectivity of analysis; however, with the availability of diode array detectors (DAD), it is now possible to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.

**Infrared spectrophotometry:** Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. However, low-level detectability is frequently a problem that may require more involved approaches to circumvent the problem.

**Nuclear magnetic resonance spectroscopy:** Nuclear magnetic resonance spectroscopy provides fairly detailed structural information on a molecule and is a very useful method for characterization of impurities; however, it has limited use as a quantitative method because of cost and time considerations.

**Mass spectrometry:** Mass spectrometry provides excellent structural information, and, based on the resolution of the instrument, it may provide an effective tool for differentiating molecules with small differences in molecular weight. However, it has limited use as a quantitative technique because of cost and time considerations.

In summary, IR, NMR, and MS are excellent techniques for characterization of impurities that have been isolated by any of the techniques discussed above. UV has been found to be especially useful for analyzing most samples with high-pressure liquid chromatography. This combination is commonly used in pharmaceutical analysis.

### Separation methods

The following separation methods can be used:

- Thin-layer chromatography (TLC)
- Gas chromatography (GC)
- High-pressure liquid chromatography (HPLC)
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

Except for CE, all these techniques are chromatographic methods. CE is an electrophoretic method that is frequently lumped with the chromatographic methods because it shares many of the common requirements of chromatography. However, it is not strictly a two-phase separation system — a primary requirement in chromatography. Hyphenated methods such as GC–MS, LC–MS, GC–LC–MS, LC–MS–MS, etc. are all discussed later in this chapter.

A broad range of compounds can be resolved using TLC by utilizing a variety of different plates and mobile phases. The primary difficulties related to this method are limited resolution, detection, and ease of quantification. The greatest advantages are the ease of use and low cost.

**Gas chromatography** is a very useful technique for quantification. It can provide the desired resolution, selectivity, and ease of quantification. However, the primary limitation is that the sample must be volatile or has to be made volatile by derivatization. This technique is very useful for organic volatile impurities.

**High-pressure liquid chromatography** is frequently casually referred to as high-performance liquid chromatography today. Both of these terms can be abbreviated as HPLC, and they are used interchangeably by chromatographers. This is a useful technique with applications that have been significantly extended for the pharmaceutical chemist by the use of a variety of detectors such as fluorescence, electrometric, MS, etc.

**Capillary electrophoresis** is a useful technique when very low quantities of samples are available and high resolution is required. The primary difficulty is assuring reproducibility of the injected samples.

**Supercritical fluid chromatography** offers some of the advantages of GC in terms of detection and HPLC in terms of separations, in that volatility of the sample is not of paramount importance. This technique is still evolving, and its greatest application has been found in the extraction of samples.

### Hyphenated methods

The following hyphenated methods can be used effectively to monitor impurities :

- GC–MS
- LC–MS

- LC–DAD–MS
- LC–NMR
- LC–DAD–NMR–MS
- LC–MS–MS

**Isolation methods:** Mostly the chromatographic techniques are used for isolation of impurities along with non-chromatographic techniques are also rarely used.

It is often necessary to isolate impurities because the instrumental methods mentioned above are not available or further confirmation is needed. For example, when hyphenated methods such as LC–MS are not suitable or do not provide unambiguous characterization, it may be necessary to isolate impurities for further confirmation of structure or for conducting toxicity studies.

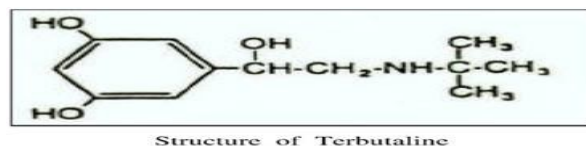
The following methods have been used for isolation of impurities:

- |                                       |                                      |
|---------------------------------------|--------------------------------------|
| ▪ Solid-phase extraction              | ▪ Column chromatography              |
| ▪ Flash chromatography                | ▪ Supercritical fluid chromatography |
| ▪ Thin-layer chromatography           | ▪ Capillary electrophoresis          |
| ▪ Gas chromatography                  | ▪ Supercritical fluid extraction     |
| ▪ High-pressure liquid chromatography | ▪ Accelerated solvent extraction     |
| ▪ Liquid–liquid extraction            |                                      |

Isolation should be initiated based on simple extraction or partition methods. It may be possible to extract impurities selectively on the basis of acidity, basicity, or neutrality. The

extraction process usually involves liquid–liquid extraction, where one phase is an aqueous solution and the other is an organic phase that is nonpolar.

**A case study:** A case study is presented below relating to monitoring impurities in terbutaline sulfate (it is sold as a racemate).



**HPLC methods:** The first step in this process was to review all potential sources of impurities in terbutaline.

**Synthesis:** starting materials, solvents used, intermediates, theorize potential by-products.

**Formulation:** solvents used, potential interaction products, any potential degradation products.

**Stability:** potential degradation products or reaction products that may be produced because of thermal, hydrolytic, oxidation, or photochemical reactions.

A careful assessment revealed that there could be 13 potential impurities in terbutaline (for chemical names, see legends in Figs. 1 and 2) that must be resolved. HPLC was clearly indicated as the preferred methodology of choice, based on physicochemical properties of terbutaline<sup>[10]</sup>.

**Achiral impurities:** All of the potential impurities were classified into four groups to assist the method development:

- Dihydroxyphenyl compounds with t-butylamino side chain
- Cyclized dihydroxyphenyl compounds with basic N in the ring
- Dibenzoyloxyphenyl compounds with no t-butylamino side chain
- Dihydroxyphenyl compounds with no t-butylamino side chain

Two HPLC methods were developed to resolve all achiral impurities with the same C-8 column with 3- $\mu$ m particle size<sup>[10]</sup>.

System 1 (suitable for degradation products and less likely synthetic impurities): 0.005 mol 1-octanesulfonic acid in water:tetrahydrofuran:methanol (75:11:14).

System 2 (suitable for dibenzyloxyphenyl compounds; starting material and intermediates): water:tetrahydrofuran:acetonitrile:acetic acid:triethylamine (500:465:35:5:2).

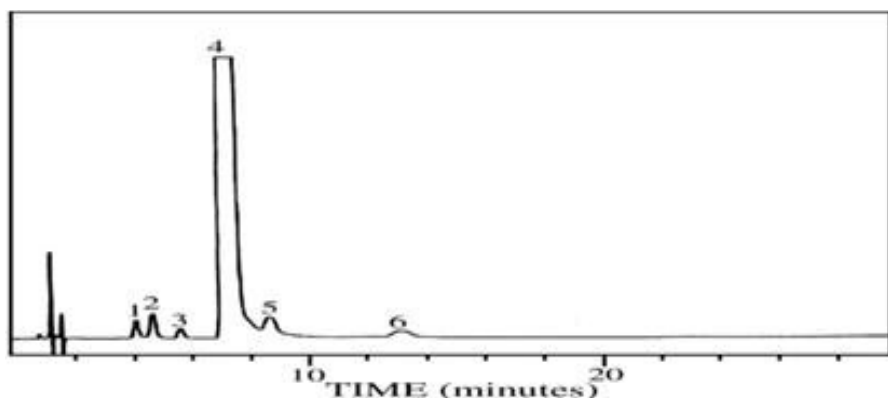
In summary, System 2 was designed primarily for quality control of API. Since no impurities were found in the API with System 2, the quality of drug product for QC and stability studies can be monitored using System 1 only.

### Chiral impurities

The L-isomer of terbutaline is 3000 times more potent as a relaxant of tracheal smooth muscle than the D-isomer [19].

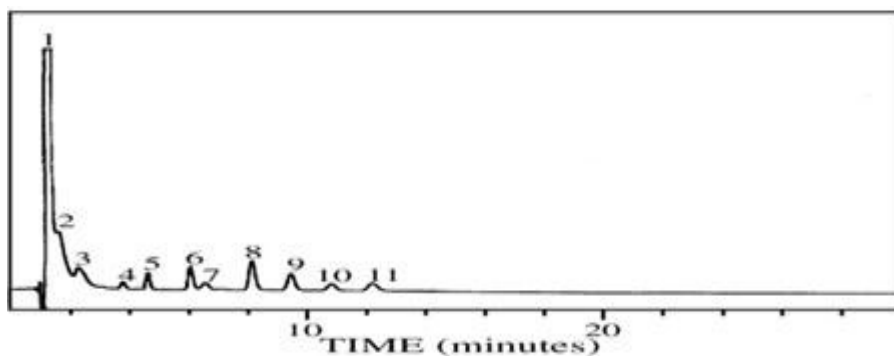
1. The isomers can be resolved on AGP column with 0.003 M tetrapropyl–ammonium bromide solution adjusted to pH 7.0.
2. Capillary electrophoresis can be used to resolve enantiomers with a background electrolyte that contains  $\beta$ -cyclodextrin or heptakis (2,6-di-O-methyl)- $\beta$ -cyclodextrin.

**Fig. 1. Resolution of potential degradation products**



1=3,5-dihydroxyacetophenone, 2=3,5 dihydroxybenzaldehyde, 3=2-t-butyl-4,6,8-trihydroxy-tetrahydroisoquinoline, 4=terbutaline, 5=3,5-dihydroxy- $\alpha$ -t-butylaminoacetophenone, 6=3,5-dihydroxybenzoic acid, ethyl ester.

**Fig. 2. Resolution of potential dibenzyloxyphenyl impurities**



1=terbutaline, 2=solvent, 3=solvent, 4= $\alpha$ -[(t-butylamino)methyl]-3,5-dibenzyloxybenzyl alcohol, 5= $\alpha$ -methyl-3,5-dibenzyloxybenzyl alcohol, 6=3,5-dibenzyloxyacetophenone, 7= $\alpha$ -[(benzyl-t-butylamino)methyl]-3,5-dibenzyloxybenzyl alcohol, 8=3,5-dibenzyloxy-2,6-dibromoacetophenone, 9=3,5-dibenzyloxy-1'-bromoacetophenone, 10=3,5-dibenzyloxy-2,6, $\alpha$ -tribromoacetophenone, 11=1'-benzyl-t-butylamino-3,5-dibenzyloxyacetophenone.

### 5. Conclusions

To assure quality of drug substances and drug products, it is important to give a careful consideration as to what constitutes impurities for a given case and proceed carefully to design a program to achieve the desired results. It is believed that the discussion included herein would be helpful in developing such a program.



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