Journal of Liquid Chromatography & Related Technologies®, 30: 877–935, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701191151

Identification of Pharmaceutical Impurities

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Abstract: Identification of pharmaceutical impurities is a critical analytical activity in the drug development process whose goal is to fully elucidate the chemical structures of unknown pharmaceutical impurities present in either drug substances or drug products above a particular threshold. Knowledge of the chemical structure of an impurity is essential to assess its toxicological implications and to gain an understanding of its formation mechanism. Knowledge of the formation mechanism is critical for improving the synthetic chemical processes and optimizing the drug formulation to reduce or eliminate the impurity. This article reviews the current regulatory requirements for impurity identification and the chemical origins of various impurities, particularly those derived from degradation of drugs. Strategies for identification of pharmaceutical impurities are discussed followed by an overview of the critical steps and the roles of various analytical techniques, such as HPLC-DAD, LC-MS, LC-NMR, GC-MS, and NMR, in pharmaceutical impurity identification, with an emphasis on applications of mass spectrometry based hyphenated techniques. Carefully selected examples are included to demonstrate key points in impurity formation and the appropriate application of various analytical techniques.

Keywords: Pharmaceutical impurities, Hyphenated techniques, Impurity identification

INTRODUCTION

Impurity profiling and control is one of the most regulated areas in the pharmaceutical industry.[1–7] According to ICH Q3A (R) "Impurities in the New Drug Substance."[1] and ICH Q3B (R) "Impurities in the New Drug Product",[2] a drug substance impurity is "any component of the new drug substance that is not the chemical entity defined as the new drug

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substance," and a drug product impurity is "any component of the new drug product that is not the drug substance or an excipient in the drug product." In a pharmaceutical product, an impurity is first and foremost a quality issue, since it could potentially compromise the efficacy of the drug product. Secondly, impurities also cause safety concerns.[9–12] Hence, any impurity present in a drug product must be fully understood, both qualitatively (chemically) and quantitatively, and qualified, if necessary, through toxicological assessment. From a chemical perspective, pharmaceutical impurities are inevitable because no chemical reaction has 100% selectivity and no chemical compound is "rock" stable. Nonetheless, it is possible to reduce impurities via synthetic process improvement and appropriate preformulation/formulation studies. Knowing the structure of an impurity is essential for allowing assessment of its toxicological implications and for understanding its formation mechanisms, which is critical knowledge for improving the synthetic chemical process and optimizing the formulation. Impurity identification is a specialized field in the pharmaceutical industry which requires specialized analytical facilities and expertise.[13–21] The goal of this review is to provide an overview of the current regulatory requirements on impurity identification, the chemical origins of various impurities, and the strategies and roles of various analytical techniques in pharmaceutical impurity identification, with an emphasis on mass spectrometry based hyphenated techniques. The examples presented in this review are used to illustrate key points in impurity formation chemistry and applications of various analytical techniques, particularly mass spectrometry based hyphenated techniques.

REGULATORY REQUIREMENTS FOR PHARMACEUTICAL IMPURITY IDENTIFICATION

ICH guidelines[1–3] classify impurities into three categories: organic impurities, inorganic impurities, and residual solvents. These impurities can be from a variety of sources, as given in Table 1. Organic impurities are derived from drug substance synthetic processes and degradation reactions in drug substances

Table 1. Impurity classification based on ICH guidelines Q3A(R),[1] Q3B (R),[2] and Q3C[3]

Organic impurities	Starting materials
	Intermediates
	By-products
	Degradation products
	Reagents, ligands and catalysts
Inorganic impurities	Reagents, ligands and catalysts
	Heavy metals or other residual metals
	Inorganic salts
Residual solvents	Inorganic or organic liquids

Table 2. ICH reporting, identification and qualification threshold for organic impurities in drug substance[1]

Maximum daily dose[a]	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/day	0.05%	0.1% or 1.0 mg TDI[b], which ever is lower	0.15% or 1.0 mg TDI, which ever is lower
>2 g/day	0.03%	0.05%	0.05%

[a]The amount of drug substance administered per day.
[b]TDI: Total daily intake of the impurity.

and drug products. Synthetic process related impurities can be derived from starting materials, intermediates, reagents, ligands, and catalysts used in the chemical synthesis, as well as by-products from the side-reactions of the chemical synthesis. Degradation products are derived from the chemical degradation of drug substances and drug products under storage or stress conditions.

Control of the organic impurities in new drug substances is based on the Maximum Daily Dose and total daily intake (TDI) of the impurities. Table 2 provides the ICH threshold for control of the organic impurities in new drug substances.[1] Depending on whether the Maximum Daily Dose higher or lower than 2 g, organic impurities in a new drug substance at (or greater than) 0.05% or 0.1% require identification. Note that these thresholds do not apply to toxic impurities.[7,22,23] According to EMEA CHMP recommendations,[7] genotoxic impurities should be controlled based on compound-specific risk assessment. For an unstudied impurity (such risk assessment data do not exist), a threshold of toxicology concern (TTC) of 1.5 μg/day can be applied, with exceptions for aflatoxin-like, N-nitroso- and azoxy-compounds, which should be assessed based on compound-specific toxicity data.

Control of organic impurities in new drug products are outlined in Table 3 and Table 4.[2] Note that these thresholds are not the same as those for impurities in new drug substances given in Table 2. Based on the Maximum Daily Dose, the identification thresholds for organic impurities in new drug products are divided into 4 groups to give more consideration to low dose drug products. For most new drug products, the Maximum Daily Dose is between 10 mg–2 g/day, therefore, any impurities at 0.2% or greater would have to be identified.

Table 3. Reporting thresholds for impurities in new drug product[2]

Maximum daily dose	Reporting threshold
≤1 g	0.1%
>1 g	0.05%

Table 4. Identification thresholds for organic impurities in new drug product[2]

Maximum daily dose	Identification threshold
<1 mg	1.0% or 5μg TDI, which ever is lower
1–10 mg	0.5% or 20 μg TDI, which ever is lower
>10 mg–2 g	0.2% or 2 mg TDI, which ever is lower
>2 g	0.1%

Leachables are a separate class of drug product impurities, however, control of leachbales is not covered by ICH guidance.[2] Recently, the PQRI (Product Quality Research Institute) Leachables and Extractables Working Group proposed safety and qualification thresholds for leachables in OINDP (Orally Inhaled and Nasal Drug Products) to the regulatory authorities.[8] The proposed Safety concern threshold (SCT) is 0.15 μg/day, and the Qualification Threshold (QT) is 5 μg/day for an individual leachable in an OINDP. Note that proposed safety thresholds only apply to OINDP and not other drug product type (e.g., injectables, ophthalmic, etc.)

Inorganic impurities are, in most cases, introduced from the synthetic process of the drug substance (e.g., catalyst), or as impurities present in excipients. Analysis and control of inorganic impurities usually follows pharmacopoeial monographs or other appropriate procedures and will not be discussed further in this review.

Residual solvents are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products.[3] Residual solvents are divided by a risk assessment approach into three classes.

Class 1 solvents are known human carcinogens, strongly suspected human carcinogens, and environmental hazards; therefore, these solvents should be avoided in the production of drug substance, excipients, or drug products, unless their use can be strongly justified in a risk-benefit assessment. If unavoidable, the level of an individual Class 1 residual solvent should be strictly controlled below the concentration limits (for example the limit for benzene is 2 ppm).[3] Class 2 solvents are non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Class 2 solvents are controlled according to the PDEs (Permitted Daily Exposure) and Maximum Daily Dose (Option 1 and Option 2). ICH Q3C[3] provides PDEs of all Class 2 solvents. Class 3 solvents are solvents with low toxic potential to man. It is recommended that amounts of these residual solvents of 50 mg per day or less would be acceptable without justification.

For solvents for which no adequate toxicological data are available, manufacturers should supply justification for residual levels of these solvents in pharmaceutical products.

ORIGINS OF PHARMACEUTICAL IMPURITIES

Organic impurities can be originated from a variety of sources in both drug substances and drug products. Generally, the origins of impurities can be categorized into the following sources: synthetic process of the drug substance, degradation of the drug substance, container/closer and packing materials, and extraneous contaminants.

Impurities Originating from Drug Substance Synthetic Processes

Most small molecule drug substances are chemically synthesized. Chemical entities, other than the drug substance, that are involved or produced in the synthetic process can be carried over to the final drug substance as trace level impurities. These chemical entities include raw materials, intermediates, solvents, chemical reagents, catalysts, by-products, impurities present in the starting materials, and chemical entities formed from those starting material impurities (particularly those involved in the last steps of the synthesis). These impurities are usually referred to as process impurities. The goal of process impurity identification is to determine the structures and origins of these impurities. This knowledge is critical for improving the synthetic chemical process, in order to eliminate or minimize process impurities.

Process Impurities Originating from Starting Materials and Intermediates

Starting materials and intermediates are the chemical building blocks used to construct the final form of a drug substance molecule. Unreacted starting materials and intermediates, particularly those involved in the last a few steps of the synthesis, can potentially survive the synthetic and purification process and appear in the final product as impurities.[23] For example, in the synthesis of tipranavir drug substance, the "aniline" is the intermediate in the last step of the synthesis.[24] Because the similarity between the structures of the "aniline" and the final product, it is difficult to totally eliminate it in the subsequent purification step. Consequently, it appears in the drug substance at around 0.1%.

Process Impurities Originating from Impurities in the Starting Materials

Impurities present in the staring materials[25–27] could follow the same reaction pathways as the starting material itself, and the reaction products could carry over to the final product as process impurities. Knowledge of the impurities in starting materials helps to identify related impurities in the final product, and to understand the formation mechanisms of these related process impurities. An often encountered scenario involves starting materials containing structural analogs, positional isomers, etc., as trace level impurities. These impurities are difficult to separate and remove from the starting material during its manufacturing process. When the starting material is used for synthesis of a drug substance, these impurities will likely form analogs or isomers of the desired drug substance. Some real world examples of process impurities derived from impurities in starting materials were reported by Görög et al.[28–30] One such example is the presence of a 4-trifluoromethyl positional isomer in 3-trifluoromethyl-α-ethyl-benzhydrol (flumecinol), due to the presence of 4-trifluoromethylbenzene impurity in the starting material, 3-trifluoromethylbenzene. A second example involves a 2-methyl analogue present as a trace impurity in tolperisone, due to the presence of 2-methylpropiophenone in the starting material, 4-methylpropiophenone.

Process Impurities Originating from Chemical Reagents, Ligands and Catalysts

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 example, carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP), which is used as an alkylating agent in the synthesis of a ß-lactam drug substance, was observed in the final product as an impurity.[31] 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, just to name a few. It is expected that these heavy metals will appear in drug substances at trace levels when such catalysts are employed. Most heavy metals have safety concerns; therefore, specifications and analytical methods should be in place to monitor the heavy metals involved in the process.[32,33] Garrett et al.[34] recently reviewed the practices for meeting palladium specifications in drug substances. In some cases, reagents or catalysts may react with intermediates or final products to form by-products. Görög et al.[35] reported that pyridine, a catalyst used in the course of synthesis of mazipredone, reacts with an intermediate to form a pyridinium impurity. Figure 1 shows a process where t-BuOK (potassium t-butyloxide) is used to extract a proton from an intermediate and promote the coupling of two intermediates to form a drug substance. During this process, t-butyloxyl also attacks the cyclopentanol ester moiety of one intermediate to form an impurity with a molecular weight of M-12 (M being molecular weight of the intermediate).

Another example of reagent related impurities is the observation of elemental sulfur (S_8) in a batch of bulk drug. In this case, the last step of the synthesis involves $Na_2S_2O_3$ as a reducing reagent to scavenge the residual oxidant. When $Na_2S_2O_3$ is used in excessive amount, elemental

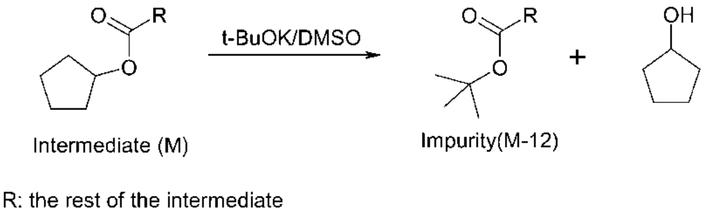


Figure 1. Formation of a process impurity (M-12) due to the side reaction between an intermediate and a reagent.

sulfur is formed, according to the proposed mechanism:

$$S_2O_3^{2-} + H^+ \longrightarrow HSO_3^- + 1/8S_8\,(\text{solid})$$

Elemental sulfur could not be eliminated by the subsequent cleaning steps; as a consequence, it appeared in the final product as process impurities.

Process Impurities Originating from By-Products of the Synthesis

The selectivity of a chemical reaction is rarely 100%, and side-reactions are common during the synthesis of drug substances. By-products from the side-reactions are among the most common process impurities in drugs. By-products can be formed through a variety of side reactions, such as incomplete reaction, overreaction, isomerization, dimerization, rearrangement, or unwanted reactions between starting materials or intermediates with chemical reagents (e.g., as shown in Figure 1) or catalysts.[35] There are numerous case studies in identification and control of by-product related process impurities in the literature.[35–43] The following three examples demonstrate typical formation pathways of by-products of drug substances. Proudfoot et al.[43] reported that isomeric by-products are formed in the course of the synthesis of nevirapine ring systems. The mechanism of formation of these isomeric impurities is through a so called Smiles rearrangement. Horvath et al.[44,45] reported a case where an over-reaction product, an epimeric impurity and a dimeric impurity were formed during the synthesis of 17α-ethinyl-17-hydroxy steroids. During preparation of LY297802 tartrate, Olsen and Baertschi[46] observed two by-products in the step from intermediate **I** to intermediate **II** due to elimination of HCl and hydroxylation, as shown in Figure 2. These by-products were able to undergo the next reaction in the synthesis in the same way as the desired intermediate, to form process impurities, Impurity 1 and Impurity 2.

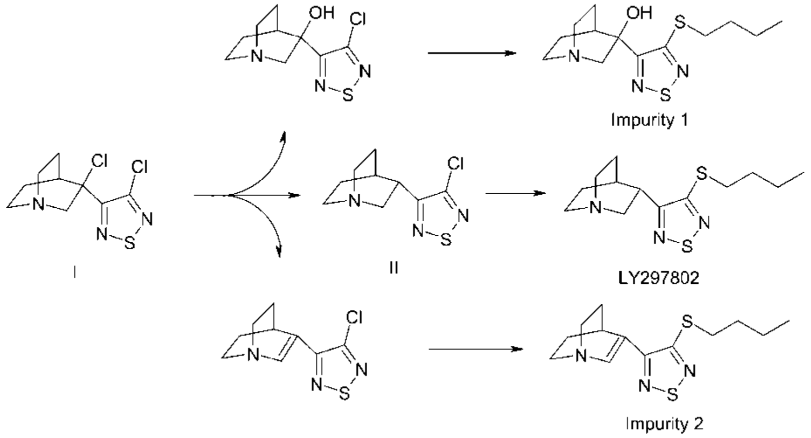


Figure 2. By-product impurities formed through side reactions during preparation of the drug substance LY297802 (adapted from ref. 46).

Process Impurities Originating from Solvents

Residual solvents are organic volatile impurities in drug substances and drug products which are used or produced in the manufacturing process of the drug substance (e.g., solvents for chemical reaction, separation, and crystallization), excipients, and drug products (e,g., solvents for wet granulation and coating). Residual solvents are expected impurities; therefore, identification of residual solvents is relatively straightforward. Recent progress in analysis of residual solvents was reviewed by Camarasu et al.[15]

Impurities Originating from Degradation of the Drug Substance

Degradation of the drug substance is one of the main sources of impurities in both bulk drug and formulated product. Degradation of the drug substance is caused by chemical instability of the drug substance under the conditions (e.g., heat, humidity, solvent, pH, light, etc.) of manufacturing, isolation, purification, drying, storage, transportation, and interactions with other chemical entities in the formulation (e.g., excipients and coating materials). Chemical stability is an inherent property of a drug substance and is a reflection of the chemical properties of all functional groups in the drug molecule. In the following sections, various impurities originating from degradations of drug substances are discussed based on their formation mechanisms.

Impurities Formed from Hydrolysis of the Drug Substance

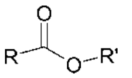
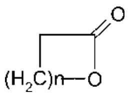
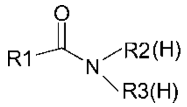
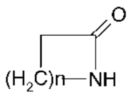
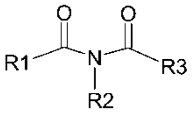
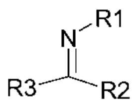
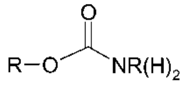
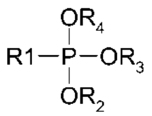
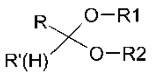
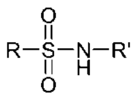
Hydrolysis of the drug substance is a chemical reaction between the drug substance and water. Obviously, the presence of water is a prerequisite for the hydrolysis of the drug substance. In a drug substance or drug product, water can be introduced as residual water from the manufacturing process of the drug or excipient, or absorbed from the environment. Most hydrolytic degradations are catalyzed by both acid and base. In some cases, it can follow other mechanisms as well. For example, it was reported that metal ions can catalyze the hydrolysis of acrylate esters and amides.[47]

A variety of functional groups are subject to hydrolysis under acidic or basic conditions.[50] Common functional groups that are susceptible to hydrolysis and some real world examples are summarized in Table 5. Depending on the structure of the drug substance, hydrolysis can either break the drug molecule into two pieces or break a cyclic structure (e.g., in lactones and lactams) into a linear structure. Esters and amides (including cyclic esters and amides) are the most common functional groups in drugs that are susceptible to hydrolysis. The hydrolysis of esters and amides is mostly catalyzed by acid and base. In most cases, drugs with these functional groups give the same hydrolytic degradation products under acidic or basic conditions. Sometimes, different hydrolytic degradation pathways can be observed. For example, penicillin undergoes different hydrolytic pathways under acidic and basic conditions to give two different primary hydrolytic degradation products, i.e., penicillenic acid under acidic condition, and penicilloic acid under basic condition, respectively.[58] Hydrolysis of carbonyl-containing functional groups, such as esters, amides, and carbamates, etc., usually takes place at the acyl-O(N) bond. Exceptions can be found where the alkyl-O(N) bond cleaves.[78] In this hydrolytic pathway, the alkyl groups are usually those functional groups that can stabilize the carbonium ion formed by alkyl-O(N) cleavage, such as a tertiary carbon or a benzyl group. Hydrolysis of the drug substance is often followed by further degradation. Primary hydrolytic degradation products can further undergo dehydration, decarboxylation, cyclization, or rearrangement, etc., to form the final degradation products.[57,78]

Impurities Formed from Oxidative Degradation of the Drug Substance

In organic chemistry, oxidation is a class of chemical reactions that leads to an increase in oxidation state of an element. In the pharmaceutical industry, oxidative degradation is confined to chemical reactions of drug substances that increase the oxidation state of C and, in some cases, N, S, P, etc., via addition of oxygen or abstraction of hydrogen. Autoxidation is the reaction of organic compounds with elemental oxygen under mild conditions (e.g., drug storage conditions). Autoxidation involves ground state elemental oxygen; thus, under normal storage conditions, it is the most important oxidative degradation pathway of drugs. Autoxidation is a free radical reaction; typically it requires a free radical initiator to start the chain reaction. Autoxidation initiators are often trace level impurities in the drug substance or excipients, such as peroxide or metal ions. These impurities

Table 5. Functional groups susceptible to hydrolysis in drugs

Functional group	Basic structure	Example drugs
Ester		Aspirin,[48] cisatracurium besylate,[51] nicergolin,[52] lovastatin,[53] and atropine[68]
Lactone		Lovastatin[53] and warfarin[69]
Amide		Acetaminophen,[49] indinavir,[54] and indomethacin[55]
Lactam		Amoxicillin,[56,57] penicillin,[58] cephalosporin,[58] bromazepam,[67] diazepam[70]
Imide		Phenobarbital[59]
Imine		Adinazolam,[60] famotidine,[61] diazepam,[70]
Carbamic ester		Benzimidazole anthelmintics,[62] zolmitriptan,[63] loratadine[77]
Phosphate	 $R1{-}P(OR_2)(OR_3){-}OR_4$	Rivastigmine,[64] triamcinolone acetonide 21-phosphate[74]
Ether	R1—O—R2	Diphenhydramine hydrochloride,[65] duloxetine[79]
Thioether	R1—S—R2	Penicillin[58]
Nitrile	$R{\equiv}N$	Danazol,[66] cimetidine[71]
Acetal/ketal		Erythromycin,[72] ECyd acetal derivatives,[73] triamcinolone[75]
Halides	R—X	Chlorambucil[76]
Sulfonamide	 $R{-}S(=O)_2{-}NH{-}R'$	Sulfamethazine[78]

can be brought into the drug product from the manufacturing process or formed from degradation of formulation components (e.g., PEG and PVP) in the drug product. Autoxidation can be self initiated because the ground state oxygen molecule is a biradical; however, this reaction is expected to be slow because the ground state of the majority of organic molecules is singlet; thus the reaction is spin-forbidden. A typical autoxidation mechanism is shown below:

$$\begin{aligned}
&\mathrm{RH} + \mathrm{ln}^{\bullet} \longrightarrow \mathrm{R}^{\bullet} + \mathrm{lnH}\,\text{(Intiation Step)}\\
&\mathrm{R}^{\bullet} + \mathrm{O_2} \longrightarrow \mathrm{ROOH}^{\bullet}\\
&\mathrm{ROO}^{\bullet} + \mathrm{RH} \longrightarrow \mathrm{ROOH} + \mathrm{R}^{\bullet}\,\text{(Propagation Step)}\\
&2\mathrm{ROO}^{\bullet} \longrightarrow \text{Non-radical molecule (Termination Step)}\\
&\mathrm{ROO}^{\bullet} + \mathrm{R}^{\bullet} \longrightarrow \text{Non-radical molecule (Termination Step)}\\
&\mathrm{R}^{\bullet} + \mathrm{R}^{\bullet} \longrightarrow \text{Non-radical molecule (Termination Step)}
\end{aligned}$$

The non-radical molecules formed in the propagation and termination steps are likely to appear in the drug substance or drug product as impurities.

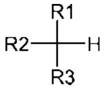
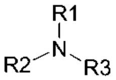
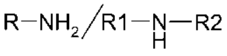
Autoxidation can be catalyzed by heavy metals. These metals usually have two readily accessible oxidation states differing by one unit (e.g., $\mathrm{Cu^{2+}/Cu^{+}}$ and $\mathrm{Fe^{3+}/Fe^{2+}}$). Peroxides react with both the upper and lower oxidation states of these metals to generate more free radicals.[80] As a result, metal ions can greatly enhance the effectiveness of peroxides as initiators.

$$\begin{aligned}
&\mathrm{Fe^{2}} + \mathrm{ROOH} \longrightarrow \mathrm{Fe(OH)^{2+}} + \mathrm{OR}^{\bullet}\\
&\mathrm{Fe(OH)^{2+}} + \mathrm{ROOH} \longrightarrow \mathrm{Fe^{2+}} + \mathrm{HOH} + \mathrm{ROO}^{\bullet}
\end{aligned}$$

Drug substance oxidative degradation can also occur through an electron transfer mechanism to form reactive anions or cations. Amines, sulfides, and phenols are susceptible for electron transfer oxidation to give N-oxides, hydroxylamine, sulfones, sulfoxides, etc.[84] C=C double bonds may react with hydrogen peroxide and peroxy acid to form epoxides via a similar ionic mechanism.[81] In addition, heavy metals such as $\mathrm{Fe^{3+}}$ and $\mathrm{Cu^{2+}}$ can also be directly involved in the oxidation of drug substances through an electron transfer mechanism.

To some extent, the susceptibility of a drug substance to oxidative degradation can be predicted from the functional groups in its chemical structure. Functional groups with a labile hydrogen, such as benzylic carbon, allylic carbon, tertiary carbon, or α-positions of a hetero atom[100] are often susceptible to oxidation to form hydroperoxide, hydroxide, or ketone.[84] Functional groups without a labile hydrogen, such as a tertiary amine or thioether, can undergo oxidative degradation to form N-oxide[86] or sulfoxide.[88] Common functional groups in drugs that are sensitive to oxidation and examples of drugs that contain these functional groups are summarized in Table 6.

Table 6. Functional groups susceptible to oxidative degradation in drugs

Functional group	Basic structure	Example drugs
Benzyl	Ph-CH_2-	Tipranavir[83] methoxamine hydrochloride,[89] impipramine hydrochloride[90]
Allylic	-CH=CH-CH_2-	Tetrazepam,[82] Reserpine,[85]
Tertiary C		Tipranavir[83]
Olefins	C=C	L-tryptophan[87]
Phenol	Ar-OH	Epinephrine[91]
Alcohol	R-OH	Lovastatin[92]
Ether	R-O-R′	Ragaglitazar[98]
Thioether	R-S-R′	Pergolidemesylate,[93] fluphenazine enanthate,[94] tipredane[99]
Tertiary Amine		Pipamperone[86], dibucaine hydrochloride,[96] raloxifene hydrochloride[97]
Primary/secondary amine	 $R-NH_2/R1-\underset{H}{N}-R2$	Brinzolamide[95]

Abstraction of protons from functional groups, such as CH_2-CH_2, CH-OH, or CH-NH in the drug molecule, to form unsaturated bonds C=C, C=O or C=N increases the oxidation state of C, and N; therefore, dehydrogenation is also an oxidative degradation. Dehydrogenation can occur in both solid state and in liquid phase. Typical types of dehydrogenation include aromatization, conversion of alcohols to ketones or aldehydes, conversion of amine to imine or nitriles, etc. For example, L-1,4-cyclohexadiene-1-alanine hydrate degrades to L-phenylalanine in solid state through dehydrogenation;[101] nifedipine can convert to dehydrogenated degradation products in solution within a few hours under photo irradiation.[102]

Impurities Formed from Photolytic Degradation of the Drug Substance

When exposed to light, many types of organic molecules can undergo photochemical reactions and form photolytic degradation products.[103] There are two main types of photochemical reactions that are relevant to degradations of drugs. The first type is a non-oxidative photochemical reaction, which includes light induced isomerization, cyclization, dimerization, rearrangement, hydrolysis, decarboxylation, and homolytic cleavage of X-C hetero bonds, such as halogen bond, ether bond, N-alkyl bond in amine (dealkylation or deamination), SO_2-C bond, etc. Under light irradiation, *cis-/trans* isomerization and dimerization are very common in drug molecules that contain a C═C double bond.[104–106,108] Other types of photochemical degradations of drugs, such as light induced hydrolysis of halogen,[107] rearrangement,[109,111] dealkylation,[110] and cyclization[112] were also reported.

Photo-oxidative degradation is the other type of photolytic degradation of drugs. Photo-oxidative degradation products can be formed from either a triplet oxygen ($^3O_2$) or a singlet oxygen ($^1O_2$) mechanism, depending on the electronic state of the oxygen molecule. The former requires sensitization and formation of a free radical of the drug molecule, which then reacts with a triplet oxygen molecule to form a peroxide. Singlet oxygen molecules react with unsaturated bonds such as alkenes, dienes, polynuclear aromatic hydrocarbons, etc., to form photo-oxidative degradation products. Depending on the structure of the drug molecule, singlet oxygen oxidation can take place through three pathways, i.e., [2 + 2] cycloaddition, [4 + 2] cycloaddition, and "ene" reaction, as illustrated in Figure 3. For example, the C═C double bond in thiothixene undergoes photo-oxidative degradation through a [2 + 2] cycloaddition mechanism.[113] Heterocyclic unsaturated rings (e.g., thiazole, oxazole, and pyrazole, etc.) are known to be sensitive toward light irradiation. For instance, an oxazole containing drug undergoes photolytic degradation through a proposed [4 + 2] cycloaddition

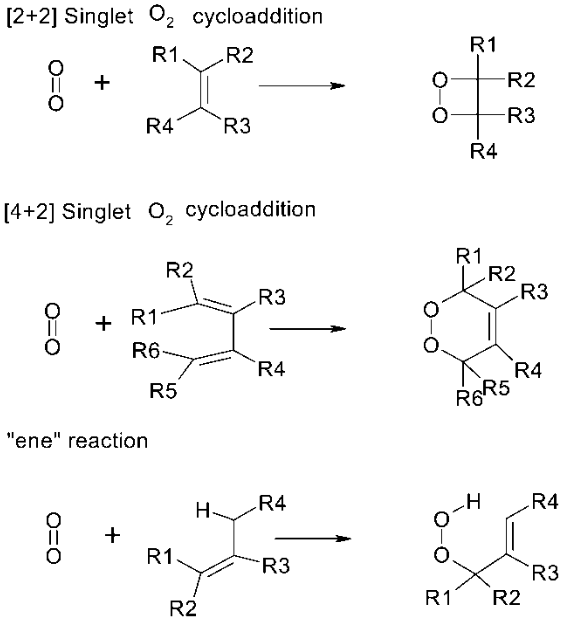


Figure 3. Singlet O_2 oxidative degradation pathways of drugs that contain unsaturated bonds.

mechanism.[114] An example of an "ene" reaction was discussed by Boccardi.[115] Usually, the peroxides formed through these reactions are not stable and are just intermediates to the final photolytic degradation products.

Although rare, photolytic degradation of drugs can also take place through a reduction mechanism. A typical example of such degradation is the reduction of the nitro group to a nitroso group in nifedipine.[102]

Impurities Formed through Isomerization and Oligomerization of the Drug Substance

Isomerization and oligomerization are common degradation pathways of drugs. Formation of isomers and oligomers of a drug substance can occur through a variety of mechanisms; therefore, it is worthwhile to have a separate overview of the isomerization and oligomerization of drugs.

Commonly seen isomerizations include photo-induced *cis*/*trans* isomerization of drugs with a C=C double bond,[104,105] other asymmetric double bonds (e.g., C=N-OH and C=N-NH_2)[116–118] and racemization or epimerization of drug substance with chiral centers. Drug molecules with chiral centers can undergo racemization and epimerization under light,[119] heat,[119] acidic,[120] and basic[121] conditions. Aso et al.[122] found that gamma cyclodextrins can facilitate the epimerization and racemization of carbenicillin. Rearrangement is also a source of isomeric impurities. For example, fenoprofen calcium can degrade to a mixture of isomeric biphenyls via a photo-Fries rearrangement.[111] In the presence of water, FK506 can readily epimerize to form tautomeric impurities.[123]

In addition to the photo-cycloaddition of alkenes discussed above, other types of dimerization or oligomerization can also occur. For example, thiols can dimerize under oxidative conditions to form disulfides;[124] indoles can dimerize under acidic conditions;[125] nalidixic acid dimerizes through a thermo-decarboxylation pathway,[126] and losartan dimerization is induced by moisture and acid.[127] It was reported that formaldehyde, which is a potential impurity in some excipients (e.g., PEG and PVP), can react with primary or secondary amine drug substance to form a cross-linked dimer with an addition of CH_2 group in between.[128] The highly electrophilic carbonyl carbon of β-lactam antibiotics (e.g., ampicillin and ceftazidime)[129] can readily oligomerize in drug formulations.[130,131] The oligomerization degradation of 5-ASA (5-aminosalicylic acid) was studied by Jensen et al.[132] 5-ASA dimer and tetramer were observed in an aqueous solution with the presence of Fe(II)/EDTA via autoxidation; a trimer was formed at pH 6.5 using hypochlorite as the oxidant; while a second trimer was the major degradation product in distilled water.

Impurities Formed through Interaction between Drug Substance and Excipients or Packaging Materials

Another important source of drug product impurities is the chemical interactions of formulation components. Impurities formed via drug-excipient interactions, excipient-excipient interactions, and drug-packaging interactions (e.g., drug-capsule shell interactions) fall into this category. Doelker et al.[133] reviewed the shell/content interactions in gelatin capsules. In a gelatin capsule product, migration of drug or excipient to the capsule shell or the shell component into the fill solution causes interactions of the formulation with the gelatin shell. A typical example of drug-capsule shell interaction is the degradation of topotecan through interaction with ammonia in the hard shell encapsulation.[134] Excipient-excipient interaction is the chemical reaction between excipients in the formulation; therefore, the interaction product is not related to the drug substance. Nonetheless, according to the impurity definition given by ICH guidelines,[2] excipient-excipient interaction products are drug product impurities because they are neither the drug substance nor an excipient in the formulation. However, excipient-excipient interaction products are not covered by ICH Q3B(R), and are usually not required to be reported in regulatory submissions. Based on the author's experiences, excipient-excipient interactions could also cause formulation stability issues; thus, they could potentially impact the quality and safety of the drug product.

Drug-excipient interactions can occur between drug substances and excipients, between drug substance related impurities/degradants and excipients, and between drug substances and impurities in excipients. The latter case is, in fact, the most common type of drug excipient interaction. One of the most often occurring drug-excipient interactions in drug formulations involves oxidative reactions induced by trace level oxidative impurities in the excipients, such as peroxides, aldehydes, and heavy metals. The origins of these oxidative impurities are either process impurities from manufacturing of excipients or oxidative degradation products of the excipients.[84] For example, Jartauer et al.[135] observed an order of magnitude increase of the N-oxide degradation product of raloxifene hydrochloride in drug tablets in the presence of two excipients, povidone and crospovidone, due to the existence of peroxide impurities in these excipients. The oxidative interactions take place in accordance with the same mechanisms as was discussed in the oxidative degradation section; thus, there is no need for further discussions here.

The other common drug-excipient interaction pathway is nucleophilic addition of primary and secondary amines to unsaturated bonds in the excipients or impurities in the excipients. One such reaction is the so called Maillard reaction. The Maillard reaction is a class of reactions between amines and reducing sugars. The mechanism of the Maillard reaction was studied and was reviewed by a number of authors.[136–139] The initial steps of the Maillard reaction involve nucleophilic attack by an amine on the reducing sugar, followed by dehydration and Amadori rearrangement.[139] The Maillard reaction of lactose and fluxetine HCl produces a primary degradant, glycosylamine, and an Amadori rearrangement product.[136a]

Formation of pyrrole derivatives through a Maillard reaction between reducing sugars and primary amines via a dehydrated intermediate of the reducing sugar, such as 3-deoxyglucosone from glucose was reported.[136b,137] It was reported that such a reaction can also occur between a phenyl amine and a reducing sugar, as in the case of mesalamine (Figure 4).[138]

Primary and secondary amines can undergo similar reactions with formaldehyde, or other aldehydes, in the drug formulation to form hemiaminal and subsequent secondary degradation products, such as imine and dimers, etc.[140]

The other type of nucleophilic addition is the reaction between primary or secondary amines and activated olefins, such as α,β-unsaturated carbonyl compounds. A classic example is the reaction between seproxetine and maleic acid through 1,2 and 1,4 addition mechanisms (the latter is also known as the Michael addition).[139]

Another type of drug-excipient interaction proceeds via a nucleophilic substitution mechanism. Arguably, hydrolysis, esterification (reactions of alcohol and acid, e.g., carboxylic acid and sulfonic acid to form an ester), acid-base reaction (e.g., reaction between carboxylic acid and amine to form an amide), transesterification (exchange of the alkoxy group of an ester by another alcohol to form another ester), and acyl substitution of amine (exchange of alkoxy group of an ester by an primary or secondary amine to form an amide) can all be classified as nucleophilic substitution reactions.

Hydrolysis as a source of pharmaceutical impurities has been discussed above. Hydrolytic degradation products of drug substance in a formulation can be considered as a drug-excipient interaction product, because water is often an impurity in some commonly used excipients. For instance, lactose

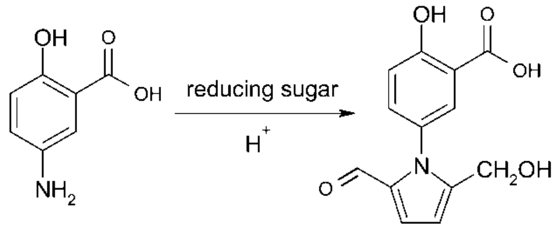


Figure 4. Formation of a pyrrole derivative through Maillard reaction between mesalamine and reducing sugars.

can contain 4.5–5.5% water, and hydroxypropyl methyl cellulose can contain up to 10% water.[50] Because ester, alcohol, carboxylic acid, and amine are common functional groups in drug molecules and excipients, nucleophilic substitutions other than hydrolysis are also an important source of drug-excipient interaction products. For example, in a SEDDS (Self Emulsified Drug Delivery System) formulation of tipranavir, tris (one of the excipients in the formulation) interacts with the lactone moiety of a primary degradation product of the drug molecule to form an amide impurity during storage.[141] Another example is the reaction between duloxetine with the enteric coatings HPMCAS (hydroxypropyl methylcellulose acetate succinate) and HPMCP (hydroxypropyl methylcellulose phthalate) to form succinamide and phthalamide, respectively, during stress testing or long term stability studies.[142]

Impurities Derived from Container/Closer/Packaging Materials

Leachables are drug product impurities leached from container/closer/packaging components. Leachables can be found in a variety of drug products, including OINDP (Orally Inhaled and Nasal Drug Products), injectables, solid dosage forms, etc.; they include both organic and inorganic chemical entities.[143] Organic leachables can be monomers or oligomers of the polymeric material, or additives, cross-linking/curing agents, antioxidants, plasticizers, pigments, lubricants, and mould release agents, etc., that are used in the manufacture of the container/closer/packaging materials.[144] Labels, inks, and adhesives associated with the drug product container/closer/packaging systems can also leach impurities into the drug product. Identification of leachables can be a significant analytical challenge for some dosage forms. For example, in an MDI (metered dose inhaler), the rubber and plastic components of the metering valve are in direct, constant contact with the formulation, which is primarily a propellant, such as a CFC (chlorofluorocarbon) or more ozone friendly HFA (hydrofluoroalkane), which are all good organic solvents. It is anticipated that these plastic and rubber valve components in the MDI will leach various organic chemical entities into the drug product. Fortunately, information on potential leachables may be obtained from the known ingredients of the rubber and plastic materials, as well as the fabrication process of the valve. For example, thiurams, dithiocarbamates, and mercaptobenzothiazoles are commonly used sulfur-containing curing agents in rubber manufacturing;[145] hence, they are potential leachables in the drug product where sulfur cured rubber is used. PBT (polybutylene terephthalate) is a widely used polyester plastic in medical device and MDI valve components. PBT oligomers and other residuals or degradants (as shown in Figure 5) can be leached from the valve components fabricated from this material.[146]

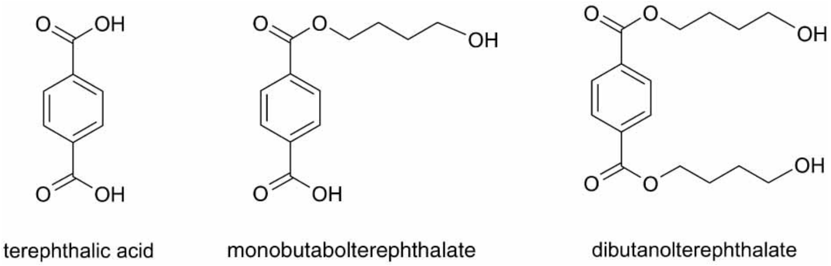

terephthalic acid monobutabolterephthalate dibutanolterephthalate

Figure 5. Examples of leachables derived from the PBT plastic component in a metered dose inhaler.

Contaminations

Extraneous or environmental contaminants are also sources of impurities. Contamination can occur during manufacturing, handling, storage, and transportation of the drug substance or the drug product. Although, from a regulatory point of view,[1,2] contamination is a GMP issue, rather than an impurity issue, technically, identification of a contaminant is similar to identification of any unknown impurities. However, because of the negative nature of contamination, it is rare to see a case study for identification of contaminants in pharmaceutical products in the literature.

IDENTIFICATION OF PHARMACEUTICAL IMPURITIES: THE STRATEGIES AND THE ROLES OF VARIOUS ANALYTICAL TECHNIQUES

Identification of impurities is an analytical activity aiming to elucidate the chemical structures and the possible mechanisms of formation of unknown impurities observed during various phases of the drug development process. Because of the complexity and diversity of the impurities in both their origins and properties, the identification strategies are determined by the specific situations. For ultimate efficiency, experiments at every step of the process should be carefully designed, based on the information obtained at the previous steps. Depending on the nature and complexity of a particular impurity, identification of an impurity could involve just a simple retention time matching exercise using an authentic standard, or a multidisciplinary team effort which could last from weeks to months. The strategies in impurity identification are also inevitably affected by the availability of modern analytical capabilities and expertise in a specific laboratory. The process outlined in Figure 6 illustrates the overall strategy used in the author's laboratory for identification of unknown impurities. This approach emphasizes the efficient use of mass spectrometry based hyphenated

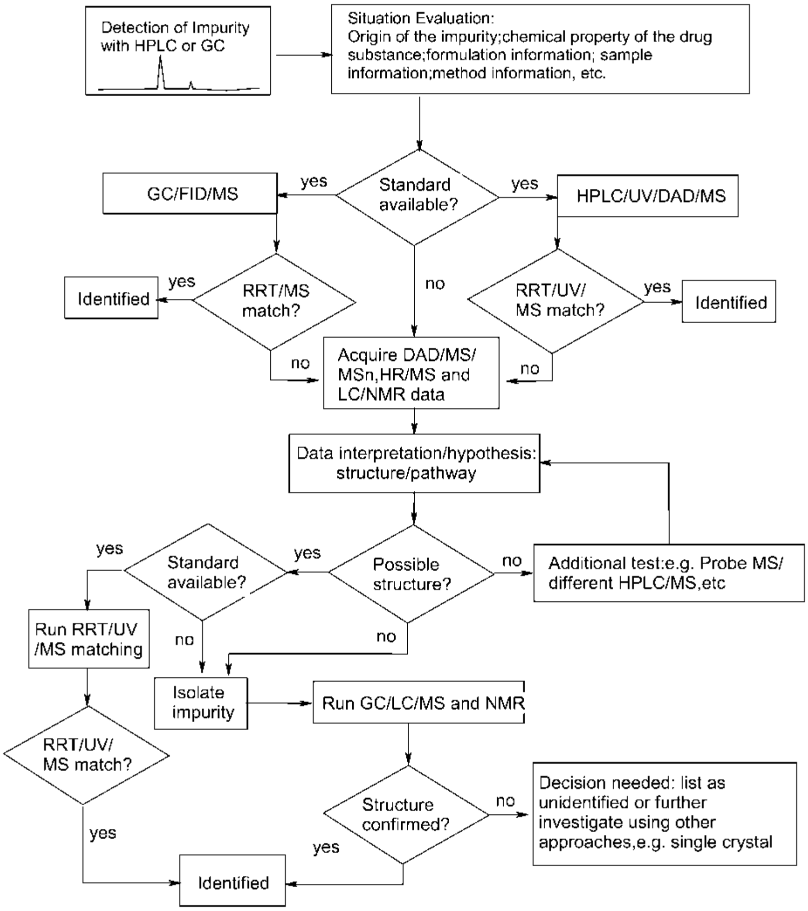


Figure 6. Flow-chart for identification of unknown impurities in drugs.

techniques, guided by the chemical knowledge about the impurity obtained prior to, or during the identification process. In the following sections, basic steps/considerations and the roles of various analytical techniques in the process of unknown impurity identification are discussed and illustrated with real world examples.

Detection of Unknown Impurities

Detection of an unknown impurity is the first step in impurity identification. Typically, unknown impurities are observed during analysis of intermediates or drug substances for process control or at release, during pharmaceutical development (such as excipient compatibility studies), or during stress studies and formal stability studies of the drug substances and drug products using various chromatographic techniques. Typically, the chromatographic technique is TLC (Thin layer chromatography), HPLC (High performance liquid chromatography), GC (Gas chromatography), CE (Capillary electrophoresis), CEC (Capillary electrochromatography), or SFC (Supercritical fluid chromatography). If identification of the unknown could help chemists or formulators to better understand the chemical process or the stability of the formulation, or address a regulatory concern, a decision for identification is made.

Situation Evaluation

Once the decision for identification is made, the nature and the origin of the impurity should be assessed, based on when, where, and how the unknown is initially observed. Depending on the assessment of the impurity, some or all of the following information, such as the structure of the drug substance, the synthetic scheme, impurities in the starting material, known process impurities, formulation ingredients, and the analytical method that is used for the initial detection of the unknown impurities should be obtained. The chemical properties of the drug substance should be evaluated based on its known structure. Chemical properties such as proton affinity, polarity, and volatility are important information for choosing which hyphenated techniques to use for the initial identification. Functional groups present in the drug substance molecule should be reviewed, as they may give clues to potential degradations. By evaluating the situation and gathering all the information, a plan on how to approach the problem can be formulated.

Identification of Impurities with Authentic Standards

The simplest situation for impurity identification is when an authentic standard of the impurity is available. This situation is more common during the early stage chemical development process, where starting materials, reagents, intermediates, or expected by-products are present as impurities, either in intermediates or in the final product. Authentic standards of these materials should be available, either in the chemistry laboratory or in the reference standard unit. The structural analysis laboratory should have a database to document the structures and chromatographic properties of all the materials used in the synthetic process and the expected by-products. If the initial evaluation indicates that the observed impurity falls into this category, the impurity identification turns into a confirmation practice. This can be achieved by three runs (sample, standard, and the spiked sample) using at least two orthogonal chromatographic and spectroscopic techniques.

An excellent combination of such techniques is HPLC-DAD and HPLC-MS, or GC-FID and GC-MS for volatile impurities. Taking HPLC-DAD and LC/MS as an example, one can compare the RRT (relative retention time), UV spectra, and mass spectra (sometimes with MS/MS or MS^n). A match of these 3 or 4 dimensional data of the sample and authentic standard can be considered definitive for the structure confirmation.

Identification of Unknown Impurities using Hyphenated Techniques

If the unknown does not match with any known impurities, more spectroscopic data are needed. Because of the complexity of pharmaceutical samples and trace level of the impurity that needs to be identified, the most useful analytical techniques for identification of unknown impurities are hyphenated chromatographic and spectrometric techniques, such as HPLC-DAD, LC-MS, LC-NMR, and GC-MS. These techniques are capable of dealing with trace levels of impurities, at or below the ICH identification thresholds.[1−3] In the pharmaceutical business, an absolute unknown impurity is very rare. One always knows the structure and chromatographic and spectroscopic properties of the drug substance. By comparing the chromatographic and spectroscopic properties of the drug substance with those of the unknown impurity, one can obtain a great deal of information about the structure of the unknown. In most cases, it is possible to propose a structure for the unknown impurity after a systematic investigation with these hyphenated techniques.

The Role of HPLC-DAD

Although HPLC-DAD is not as informative as LC-MS or NMR in structure elucidation, sometimes it can provide diagnostic information about the structure of the impurity. For drug related impurities, if the UV spectrum of an unknown is identical to that of the drug substance, it is likely that the impurity has the same chromophore as the drug substance. In this case, the modification in the impurity molecule could be too remote from the chromophore to cause any significant impact on the UV spectrum. When the UV spectrum of the impurity has a similar pattern, but the absorption maximum shifted, this implies that the chromophore is probably still the same, but the chemical environment of the chromophore has been changed, as demonstrated by Görög et al.[147] in identifying positional isomers of norgestrel hydroxylated impurities. The UV absorbance maximum shift allowed differentiation of the phenolic positional isomers. On the other hand, if a drug substance related impurity has a different UV spectrum from the drug substance, it is an indication that the chromophore has been modified in the impurity. In some cases, the UV spectrum can also be used to differentiate target impurity peaks from other components in the sample. For example, during the identification process of leachable impurities derived from PBT plastic components in an MDI drug product, (structures of some of the leachables are shown in Figure 5), the PBT related leachables were readily picked up from other drug and excipient related peaks in a complicated HPLC-UV chromatogram of the drug product, because terephthalic acid and other terephthalic acid derivative leachables have the same distinctive UV spectrum.[146]

The Role of LC-MS

LC-MS is the most widely used hyphenated technique for unknown impurity identification for the following reasons: First, modern API (Atmospheric pressure ionization) techniques allow the direct transfer of a majority of the HPLC methods used in process analysis, impurity profiling, or stability studies to LC-MS. Second, modern API techniques are suitable for ionization of a majority of the pharmaceutically relevant compounds. Third, the sensitivity of mass spectrometry allows direct investigation of trace level impurities, at or below the ICH identification thresholds,[1–3] which eliminates the need for impurity isolation or sample enrichment of trace impurities. In addition, an on-line PDA (photo diode array) detector can be readily integrated into the LC-MS system, which enables the acquisition of both UV and MS data in the same LC-MS run. After a step-by-step investigation using various LC-MS techniques, it is often possible to propose a plausible structure(s) for an unknown impurity. In the following sections, various aspects of LC-MS techniques and their applications in unknown impurity identification are discussed.

Compatibility of HPLC Methods with LC-MS

One of the key factors that contributes to the success of LC-MS in pharmaceutical analysis is that API (Atmospheric pressure ionization) techniques can tolerate large amounts of solvent introduced into the ion source of a mass spectrometer from the HPLC. This feature allows the direct transfer of HPLC methods used in impurity profiling, process control, or stability testing to LC-MS. However, one must be aware, when transferring an HPLC method to LC-MS, that the modifiers or buffers used in the HPLC method must be compatible with LC-MS. It has been demonstrated that nonvolatile buffers, such as phosphate, sulfate, borate, citrate, and octane sulfonate suppress the ionization of the analyte[148] and cause maintenance issues for the mass spectrometer, due to deposition of salts. Therefore, if the original HPLC method uses such involatile modifiers or buffers, it must be redeveloped using LC-MS compatible volatile modifiers. Commonly used LC-MS compatible modifiers include formic acid, acetic acid, ammonium formate, ammonium acetate, ammonium bicarbonate, ammonium hydroxide, and volatile ion-pair reagents such as TFA and HFBA (Hexafluorobutyric acid), etc. TFA is one of the chromatographers preferred modifiers because it has a low pKa and improves peak shapes and resolution through ion-pairing with the analyte; however, when using TFA for LC-MS, TFA could potentially suppress the signal of the impurity ions due to ion-pairing with basic impurities.[149] If TFA does affect the LC-MS sensitivity, a number of approaches can be considered to alleviate the problem. One approach is to inject a so called "TFA fix" post column.[150] Alternatively, one can use a different modifier, such as formic acid or acetic acid, to replace TFA, or use a different ionization technique.[148]

Ionization Techniques for LC-MS

ESI (Electrospray ionization)[151] and APCI (Atmospheric pressure chemical ionization)[152] are the most used API techniques for LC-MS analysis of small molecules. ESI can be used for molecules with medium polarity to ionic compounds, while APCI is better suited for weak to medium polar compounds. For the majority of pharmaceutical relevant compounds, ESI and APCI are almost interchangeable. ESI and APCI are soft ionization techniques and generate similar mass spectra for small molecules. In the positive ion mode, the mass spectrum usually features a protonated molecule $[M + H]^+$, and, in many cases, along with adduct ions such as $[M + Na]^+$, $[M + K]^+$, $[M + NH_4]^+$, or $[M + H + \text{solvent or modifier}]^+$. For quantitative applications, adduct ion formation could be viewed as a negative feature because it can be considered as a dispersion of the analyte resulting in lower sensitivity. For qualitative analysis, adduct ions can serve as a confirmation of the protonated molecule because of the distinctive mass differences between the adduct ion and the protonated molecule. Adduct ions are particularly useful when the signal is low and there are interferences from the background or other components in the sample. In negative ion mode, molecules with a COOH group or other acidic groups can form $[M\text{-}H]^-$ ion and adduct ions. When running in negative ion mode, TFA should not be used as a modifier because it would suppress negative ion formation from the analyte. Even residual TFA in the system from previous analyses can cause significant ion suppression. If needed, the system should be flushed with a solvent containing ammonium hydroxide to facilitate the removal of TFA.

More recently, another API technique APPI (Atmospheric pressure photo ionization) was introduced.[152] APPI uses a Krypton lamp which emits photons at 10.0 and 10.6 eV. These photon energies are sufficient for ionization of most analytes, but are lower than the ionization potentials of common reverse phase solvents such as water (12.62 eV) and methanol (10.85 eV); consequently, the background in an APPI spectrum is significantly reduced, compared to that in an ESI or APCI spectrum.[153] Another advantage of APPI is that it works better for relatively non-polar compounds than ESI and APCI. Ion formation under APPI can follow two mechanisms:

photoionization to form radical ion ($M^{+\cdot}$) or photo induced chemical ionization to form protonated molecules ($[M + H]^+$). To facilitate the formation of protonated molecules, a dopant (acetone or toluene) is used. With a dopant, an APPI mass spectrum appears similar to a typical ESI or APCI spectrum. Occasionally, however, a unique APPI mass spectrum can be observed from some compounds. Figure 7 shows the ESI and APPI spectra of nifedipine, where ESI gives a typical spectrum, while APPI generates a more complicated spectrum. The protonated molecule of nifedipine is not the base peak in the APPI spectrum; instead, the two dominant peaks are two photolytic degradation products of nifedipine, NDNIF (nitroso dehydronifidepine) and DNIF (dehydronifedipine).[154,155] Although the details are not clear, the formation of the two photolytic degradation products of nifedipine during the APPI process may be related to the photo-instability of nifedipine.

Formation of adduct ions of analyte with cations or anions can be considered as an ionization process and is usually referred to as cation or anion (negative ion) attachment ionization. Among others, chloride attachment[156–159] has been demonstrated as a useful ionization technique for LC-MS analysis of certain non-polar or weakly polar pharmaceutical relevant compounds, such as glycerides,[160–163] sugars or sugar moiety containing compounds,[164,165] and compounds that contain nitro groups[166] or boron atoms, all of which exhibit strong electron affinity. Norwood and Qiu[166] studied the application of chloride attachment APCI LC-MS in identification of impurities in a nitroglycerin ointment. Nitroglycerin and the two dinitroglycerin impurities are neutral compounds and do not ionize well under either ESI or APCI conditions. Using chloride attachment, two

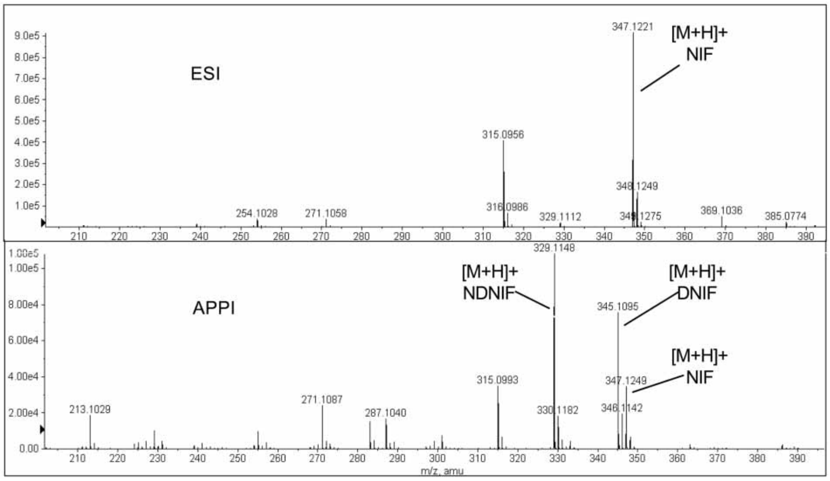


Figure 7. Comparison of the ESI and APPI mass spectra of nifedipine (NIF).

dinitroglycerin impurities are effectively ionized to form $[M + Cl]^-$ at m/z of 217. Nitroglycerin (the drug substance) and lactose (an excipient in the ointment) can also form strong chloride attachment ions at m/z 262 and m/z 377, respectively.

Negative ion attachment with other chemical species has also been used in pharmaceutical analysis. Quang et al.[167] reported the use of formate anion attachment for detection of neutral molecules in rat plasma and demonstrated that this technique can be validated and used for quantitation.

In the past few years, a number of new atmospheric pressure ionization techniques were introduced. Among them, AP-MALDI (Atmospheric pressure--matrix assisted laser desorption ionization),[168] in addition to biopolymer applications, has been demonstrated as a useful technique in small molecule analysis in either on-line or off-line mode.[169–173]

DESI (Desorption electrospray ionization)[174] is a new atmospheric pressure ionization technique for analysis of surfaces. In DESI, charged droplets from an electrospray are directed onto a sample surface and cause desorption ionization of the analyte on the surface. Pharmaceutical samples such as tablets[175] or impurity spots on TLC plates[176] can be analyzed directly within a few seconds. Liquid samples, such as urine, blood, or pharmaceutical solutions, can be deposited on a neutral surface (e.g., Teflon or PMMA (polymethylmethacrylate)) and then analyzed using DESI.[177] DART (Direct analysis in real time)[178] is another new atmospheric pressure ionization technique for direct analysis of molecules from surfaces. DART exposes the sample to a stream of electronically or vibronically exited gas (helium or nitrogen), which cause the ionization of the analyte. It works similarly to DESI for solid surface analysis;[179,180] however, DART can also analyze gas and liquid samples.[178] The advantages of DESI and DART over ESI are simplicity (no need for sample preparation) and speed (seconds) for surface analysis, and the ability to analyze solid samples (e.g., tablets) directly. The disadvantage is that it is difficult to couple these techniques to HPLC, although automated sampling from TLC plates has been reported.[176]

H/D Exchange LC-MS

From a regular LC-MS experiment, the molecular weight of the unknown impurity can be obtained from the protonated molecule $[M + H]^+$. When the protic solvents (e.g., water and methanol) used in LC-MS experiments are changed to deuterated solvents (e.g., D_2O and CH_3OD), the observed protonated molecule changes to a deuterated molecule $[M' + D]^+$ (M' stands for the molecular weight of the analyte after all exchangeable protons are fully exchanged to deuterium). The difference between M and M' indicates the number of exchangeable protons in the analyte[181–183] because, during the LC-MS experiment with deuterated solvents, these protons are exchanged to deuteriums. The number of exchangeable protons can be readily calculated from the mass shift of the molecular ion (Δm) before and after the exchange:

$$n = \Delta m - 1$$

where n is the number of the exchangeable protons, $\Delta m = m/z$ (deuterated molecule) – m/z (protonated molecule)

The benefit of such experiments is obvious. Knowing the number of exchangeable protons in an unknown impurity can be critical for elucidation of the unknown structure. Exchangeable protons are those protons that are attached to hetero-atoms in a molecule. Commonly seen exchangeable protons include those in OH, NH_2, NHR, COOH or SH functional groups. When conducting an H/D exchange experiment, at least one of the solvents in the mobile phase must be a deuterated solvent; the others must be either deuterated or aprotic. Note that the modifiers used in the mobile phase, such as TFA or acetic acid, usually do not need to be replaced with deuterated analogues because their relative amounts are small.

The following example (Figure 8) demonstrates the usefulness of H/D exchange in unknown impurity identification. The m/z of the protonated molecule of the drug and an unknown impurity are m/z 534 and m/z 506, respectively. The deuterated molecules, after H/D exchange, are m/z 535 and m/z 510, respectively. These results indicate that the drug substance has no exchangeable protons, while the unknown impurity has 3 exchangeable protons. Further examination of the isotopic pattern showed that the Br in the drug substance is no longer present in the impurity. Through a simple calculation, it is clear that the impurity and the drug substance are related as the following:

$$m/z\,506\,(\text{impurity}) = m/z\,534\,(\text{drug substance}) - 78\,(\text{Br}) + 17\,(\text{OH}) + 2 \times 16\,(\text{O})$$

Without further experimentation, one can conclude that this unknown is formed through hydrolysis of Br and oxidation to form two OH groups, which adds three exchangeable protons to the molecule.

It is worthwhile to mention that, under certain conditions, the H/D exchange can be incomplete, which can cause confusion in calculating the numbers of exchangeable protons. For example, if the sample is dissolved in a protic solvent, a shortly retained impurity may not have enough time to completely exchange on-line. This problem can be solved by using a deuterated solvent to dissolve the sample. Residual water or protic solvents can be a problem if the purity of the deuterated solvents is not high enough or the system is not completely flushed with deuterated mobile phase prior to the H/D exchange experiments. The exchange can also be incomplete if the desolvation/nebulizing gas used for API LC/MS is “wet”. This is particularly a concern when using “house” nitrogen as the desolvation/nebulizing gas during periods of high relative humidity. An easy solution to this problem is

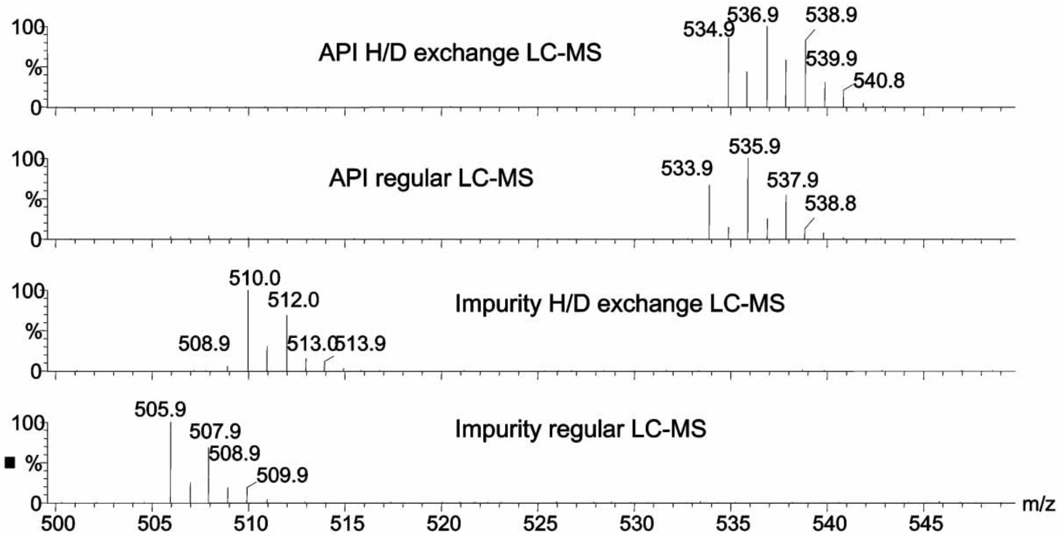


Figure 8. Mass spectra of a drug substance from regular LC-MS and H/D exchange LC-MS (top two panels), and mass spectra of an unknown impurity from regular LC-MS and H/D exchange LC-MS (bottom two panels).

either to install a water filter in the house nitrogen gas line before it gets to the mass spectrometer, or simply buy a high purity N_2 cylinder or liquid N_2 dewar just for H/D exchange experiments (Moisture in the desolvation/nebulizing gas does not affect regular LC-MS).

Mass Analyzers for LC-MS

The quadrupole mass analyzer is very popular for LC-MS, due to its relative simplicity and relatively low cost. The primary information available from a LC-MS experiment using a single quadrupole analyzer is the molecular weight of the unknown impurity. Molecular weight is the most important structural information required for unknown structure elucidation. By comparing the molecular weight of the unknown with that of the drug substance, one can often gain some understanding of the nature of the unknown. Many impurities can be tentatively identified just based on their molecular weights. For example, impurities with molecular weights of $M + 16$, $M + 32$ or M-2, where M is the molecular weight of the drug substance, are very likely oxidative degradation products formed through addition of one or two oxygen atoms, or by dehydrogenation. Another important use of single quadrupole analyzer in impurity identification is as a mass-selective triggering device coupled with a prep-HPLC system for isolation and enrichment of unknown impurities.[184,185]

Triple quadrupole (QqQ)[186] instruments are the most widely used mass spectrometers for small molecule LC-MS. A triple quadrupole mass spectrometer is comprised of two separate quadrupole mass analyzers (Q1 and Q3) and an RF (radio frequency) only quadrupole (q2, note that newer instrument may use a hexapole or octapole or other device as the collision cell, yet the name of the triple quadrupole still remains.) which is used as a collision cell. This type of instrument can provide, not only molecular weight, but also structural information using MS/MS (mass spectrometry/mass spectrometry, also known as tandem mass spectrometry) scan modes. MS/MS scan modes on a triple quadrupole instrument include product ion scan, precursor ion scan and neutral loss scan. Product ion scan generates fragments (product ions) which are crucial in structural elucidation.[141] Precursor scan gives information on all molecules that can generate a common fragment ion; hence, it is very useful in looking for homologs in a complex sample. Neutral loss scan gives information on all molecules that have a common neutral fragment. For unknown impurity elucidation, the most useful MS/MS technique is clearly the product ion scan. The molecular ion (e.g., $[M + H]^+$) of the impurity of interest can be mass selected by the first quadrupole and then fragmented in the collision cell. The induced fragments are then mass analyzed using the second quadrupole analyzer. Since the structure of the drug substance is known, by comparing the resulting CID (Collision induced dissociation) spectrum of the drug substance with that of the impurity, one can find common ions and different ions from the drug substance and the impurity. The common ions indicate the common substructure of the impurity and the drug substance, whereas the different ions indicate the portion of the structure that is modified in the impurity.

An example of the application of MS/MS in unknown impurity identification was discussed by Qiu[141] recently. By studying the MS/MS spectra of two unknown impurities observed during the stability study of tipranavir drug product, the following structural information was obtained: 1) the two unknowns are isomers because their molecular weights (721) and MS/MS spectra are identical; 2) the unknown impurities are drug related and contain common substructures with the drug substance, because common fragment ions were observed in the MS/MS spectrum of the drug substance and those of the unknowns (e.g., m/z 347 and m/z 201, etc.); 3) it is likely that the impurities are related to a formulation excipient, tris (MW = 121), because the appearance of the fragment ions at m/z 122 and m/z 104. Consequently, the MS/MS data demonstrated that the unknowns are likely a pair of isomeric drug-tris interaction products.

The quadrupole ion trap is another commonly seen tandem mass analyzer for LC/MS.[187] There are two types of ion trap mass analyzers, termed 3D[188] and linear.[189,190] The main difference between them is that a 3D ion trap uses a three dimensional radio-frequency quadrupolar field, while a linear ion trap confines ions along the axis of a quadrupole analyzer using a two dimensional radio-frequency field. Consequently, a linear ion trap has a large trapping volume, which leads to a greater dynamic range. Ion trap instruments compete with triple quadrupole instruments as the most popular LC-MS technique for tandem mass spectrometry. The advantage of ion trap over triple quadrupole instrument is that ion trap instrument can achieve multiple stage tandem mass spectrometry (MS^n, tandem in time), which greatly increases the amount of structural information obtainable for a given molecule, while a triple quadrupole instrument can only achieve MS/MS because of the physical limitation of tandem in space mass spectrometry. The ion trap instrument also has certain disadvantages. First, it is impossible to perform triple quadrupole-type precursor ion scan and neutral loss scan experiments using an ion trap. Second, ion trap tandem mass spectrometry has a discriminating effect for low mass fragments (also known as the "one third rule"). Third, the dynamic range of ion trap is not as great as that of the triple quadrupole for quantitation applications, mainly because of space charging effects.

Quadrupoles and ion traps are low resolution analyzers. They can only provide low mass resolution data (typically unit mass resolution), which means only the nominal masses of the molecular or fragment ions are achievable. Accurate mass measurement that leads to accurate determination of the molecular formula of the protonated molecule or the chemical formula of fragment ions is obviously valuable in identifying an unknown molecule. This can be done with high resolution mass analyzers.

For decades, magnetic sector double or triple focusing mass analyzers were the primary technique for exact mass measurement.[191–193] In a magnetic sector instrument, among others issues, high resolution comes with a cost in sensitivity, which makes it difficult for on-line LC-MS high resolution trace impurity analysis. Although magnetic sector instruments are still being used for high resolution EI (electron ionization), FAB (fast atom bombardment) and even GC/MS, they have rapidly lost ground in competition with TOF (time-of-flight), FT-ICR (Fourier transform-ion cyclotron resonance) and FT-Orbitrap for high resolution LC-MS, LC-MS/MS, and LC-MS^n analysis of trace level impurities.

Time-of-flight (TOF) is one of the most widely used mass analyzers for accurate mass measurement using LC-MS. In a TOF analyzer, the mass of an ion is determined based on the time it takes to reach a detector through an evacuated flight tube; therefore, physically, there is no limit on the molecular size. Because of this feature, TOF is an ideal mass analyzer for large biomolecules that are ionized by MALDI (Matrix assisted laser desorption ionization); however, low resolution TOF was never a real player in small molecule analysis because it does not have any significant advantage over the quadrupole analyzer in low resolution mass spectrometric analysis of small molecules. In the past decade, the resolving power of the TOF analyzer has increased dramatically from several hundred to 15,000 (FWHM), largely because of advances in reflectron TOF technologies.[194–196] The newer generation LC-TOF can routinely generate data with an mass accuracy of 3 ppm or better.

Quadrupole-TOF[197–199] is a hybrid instrument that combines a quadrupole mass analyzer, a collision cell, and a high resolution TOF analyzer. On the one hand, it is similar to a triple quadrupole instrument in the way it performs a product ion scan; on the other hand, it is better than a triple quadrupole for qualitative analysis because it offers accurate mass measurement of both full scan data and MS/MS data. Quadrupole-TOF LC-MS systems provide a less expensive alternative of FT-MS for accurate mass measurement.

At this time, FT-ICR[200] is still the mass analyzer of choice for achieving ultimate resolving power and mass accuracy. In FT-ICR, the m/z values of ions are obtained by Fourier-transform of the frequencies of coherent cyclotron motion of ions undergoing RF (radio frequency) excitation in a magnetic field. Because the frequencies are acquired with high accuracy, the corresponding m/z values are also highly accurate. The following two examples illustrate the usefulness of the ultra high mass resolution in small molecule structure elucidation. In example 1, an unknown impurity was observed partially co-eluting with the drug substance. The low resolution ESI mass spectra of the unknown and the drug substance are shown in Figure 9. The dominant ion in the ESI mass spectrum of the unknown has the exact same m/z value as the sodium adduct ion of the drug substance (m/z 797.5). Is the unknown a diastereomer of the drug substance with a preference to form a sodium adduct ion, or is it a different impurity? FT-MS data demonstrated that the exact mass of the unknown is 797.3100, which corresponds to a molecular formula $C_{39}H_{50}N_6O_8SCl$ with 0.1 ppm error, while

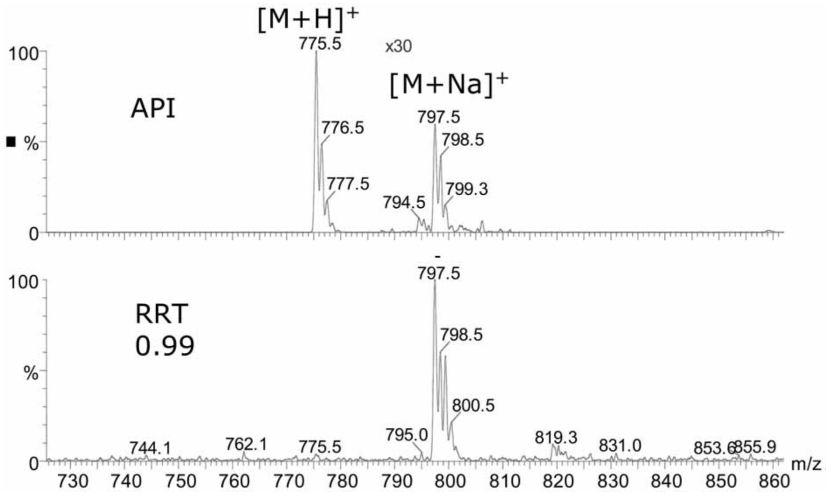


Figure 9. ESI mass spectra of a drug substance (upper panel) and an unknown impurity that partially co-elutes with the drug substance at around 0.1% level (bottom panel).

the sodium adduct of the drug substance has an accurate mass of 797.3302, which corresponds to a molecular formula of $C_{40}H_{50}N_6O_8SNa$ with 0.2 ppm error. In order to separate the two ions, the following resolving power is required:

$$R = m/\Delta m = 797/0.0207 = 38502$$

Although this is a routine operating resolving power for an FT-ICR-MS mass spectrometer, at the moment, no commercial LC-TOF or LC-Q-TOF instrument can achieve this.

In the second example, the ultra high resolving power of the FT-ICR analyzer allows separation of the isotopic peaks, ^{13}C, ^{33}S, and ^{15}N, in the ESI mass spectrum of tetramethylthiuram monosulfide (Figure 10), which normally appear at $[M + H + 1]^+$ as a single peak in a low resolution or high resolution TOF spectrum. It is impossible to obtain this kind of fingerprint information of individual elements using a TOF analyzer.

CID MS/MS or MS^n experiments can be performed within the ICR cell using Sustained off-resonance irradiation (SORI).[201] SORI experiments involves pumping collision gas in and out of the ICR cell. Since this process alone takes several seconds, this conventional FT-ICR technique is not quite compatible with on-line trace impurity LC-MS^n analysis. Furthermore, FT-ICR-MS is notorious for the cost of both initial purchase and instrument maintenance. It is not surprising that this technique has not been considered as a routine analytical technique, and was only used in a small number of research groups until the introduction of hybrid FT-ICR

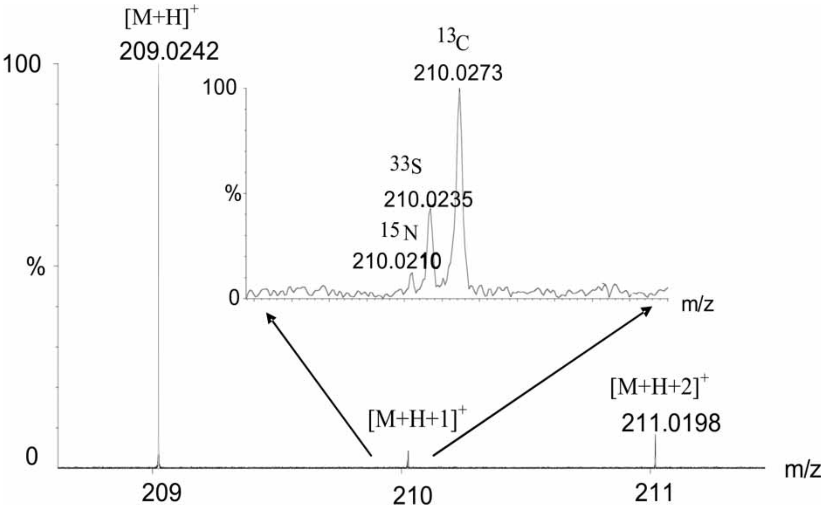


Figure 10. ESI-FT-ICR mass spectrum of tetramethylthiuram monosulfide acquired at 250,000 resolution power (FWHM).

instruments[202] in 2003. The first commercially available hybrid FT-MS consists of a linear ion trap as the front end, and a 7 Tesla FT-ICR as a high resolution analyzer. With this arrangement, the CID and all MS^n experiments are done in the linear ion trap, and the FT-ICR is just used to determine the accurate masses of molecular or product ions. The major benefits of the hybrid configuration include that it eliminates the often tedious tuning process of the conventional FT-ICR instrument, and makes the hybrid FT-ICR technique much more user friendly than the conventional FT-ICR instruments. As a result, accurate mass measurements of trace impurities can be routinely achieved using hybrid FT-ICR LC-MS. More recently, other hybrid FT-ICR instruments were introduced to the market. These instruments combine a quadrupole and a collision cell with the FT-ICR analyzer and have a Qq-FT-ICR configuration,[203] which is similar to a Qq-TOF instrument, but with much higher resolving power and accuracy. Although the quadrupole portion of the instrument cannot be operated independently, this kind of instrument also offers ease of use and compatibility with separation techniques in a similar way as the hybrid linear ion trap FT-MS. In summary, hybrid FT-ICR technologies have transformed the FT-ICR into a routine analytical tool; however, the cost of these instruments is still a limiting factor for the spread of this technology. In 2005, a new concept FT-MS, the OrbiTrap, was introduced.[204,205] This technology fits in between hybrid FT-ICR and Q-TOF in terms of both cost and performance. It is anticipated that more and more applications of OrbiTrap in unknown impurity identification will be forthcoming.[206]

The Role of LC-NMR

One of the challenges in identification of pharmaceutical impurities at levels around the ICH identification threshold[1–3] is to acquire NMR data. Due to the relatively poor mass sensitivity of NMR techniques, the unknown impurity typically needs to be isolated for off-line NMR characterization. This process is often labor intensive and time consuming. The combination of HPLC with NMR resembles the idea of other hyphenated techniques such as HPLC-DAD or HPLC-MS; therefore, the potential application of LC-NMR for unknown impurity identification is obvious. However, because of the low sensitivity of NMR, it is not as straightforward to perform an LC-NMR experiment as it is to perform an LC-MS experiment. One of the hurdles to overcome is the HPLC method compatibility with NMR. Protic solvents such as water and alcohol need to be replaced with deuterated solvents to avoid solvent suppression. Unlike for LC-MS, mineral acid buffers are preferred for LC-NMR, and organic modifiers need to be avoided.[207] To deal with the sensitivity issue of online LC-NMR, stop-flow or loop-collection approaches are used to increase the observation time for the trace impurity peak of interest.[208] In recent years, improvements have been made in many areas of the LC-NMR technique, such as better RF systems for multiple solvent suppression, new coil designs, and reduction in flow-cell volume to increase on-line sensitivity, as well as availability of high field NMR for LC-NMR. Feinberg[218] demonstrated that it is possible to obtain 1D proton NMR for an impurity at 0.1% level using LC-NMR. Although there are still difficulties to performing on-line ^{13}C analysis, LC-NMR has been playing an increasing role in unknown impurity identification.[209–217]

The Role of GC-MS

In pharmaceutical impurity analysis, HPLC based techniques (e.g., HPLC-DAD and HPLC-MS) can analyze the majority (at least 80%, based on the author's experience) of relevant organic compounds. GC based techniques (e.g., GC-FID or GC-MS)[219–224] play a major role in analysis of the remaining 20% of organic compounds. These compounds are either not suitable for HPLC analysis (e.g., volatile compounds), or not suitable for ionization by API techniques (e.g., non-polar compounds). Electron ionization (EI) is a better ionization technique for non-polar compounds. EI uses an electron beam produced through thermionic emission from a heated wire filament. The electrons are accelerated through an ionization chamber in the ion source towards an anode and impact analyte molecules in the gas phase, which causes the analyte to ionize and cleave to form a radical ion (e.g., molecular ion $M^{+\cdot}$) and fragment ions. EI is a "hard" ionization technique; subsequently, an EI spectrum can contain many fragment ions. Because of this inherent feature, it is usually not necessary to acquire MS/MS data with EI, although GC-MS tandem mass spectrometers do exist. On the other hand, extensive fragmentation in EI often results in the absence of the molecular ion in the EI spectrum, which adds difficulty to the determination of the molecular weight of the unknown. For this reason, CI (Chemical ionization) and other so called soft ionization techniques have been developed. Under CI, the analyte is ionized by chemical ion-molecule reactions during collisions in the source. This is a less energetic procedure than electron ionization and the ions produced are protonated molecules $[M + H]^+$. These ions are often relatively stable, tending not to fragment as readily as ions produced by electron ionization. CI is still being used in many areas;[225] however, the importance of CI GC-MS in pharmaceutical impurity identification has decreased significantly due to the routine availability of API based LC-MS techniques.

As previously discussed, residual solvents are expected impurities. Identification and quantitation of residual solvents and other organic volatile impurities are usually accomplished by GC-FID or GC-MS methods. Applications of HS (headspace) GC-FID or GC-MS have become routine practice in many laboratories.[226] GC-FID and GC-MS, with on-line sample preparation, are also applied to residual solvent identification and quantitation, such as PAM-GC-MS (Purge and membrane).[227] SPME (Solid phase micro extraction)-GC-FID or GC-MS,[224,228] and TD (Thermo desorption)-GC-MS.[229,230] One of the advantages of EI-MS is that the appearance of the EI (70eV) mass spectrum is not instrument dependent. This feature makes it possible to compile the EI mass spectra of known structures into a searchable spectral library. Currently, EI spectral libraries with over 200,000 EI spectra are commercially available. Identification through library matching has been demonstrated to be valid and has been recognized by pharmaceutical regulatory agencies such as the FDA.[231]

Other Hyphenated Techniques

Besides the hyphenated techniques discussed above, there are other hyphenated techniques that have been demonstrated to be useful in impurity profiling and identification, but have not been widely applied. These techniques include: CE-MS (Capillary electrophoresis-mass spectrometry),[231–234] CEC-MS (Capillary electrochromatography-mass spectrometry),[235–237] SFC-MS (Supercritical fluid chromatography-mass spectrometry),[238,239] and GC-FTIR.[240,241]

CE-MS, CEC-MS, and SFC-MS combine unique features in separation mechanism of CE, CEC, and SFC with the mass spectrometry; therefore, they are excellent complimentary hyphenated techniques to GC-MS and HPLC-MS. For more details on these techniques and their application in impurity profiling and identification, readers are directed to review articles in these areas.[232,236,242,243]

Confirmation of the Hypothesis when a Standard can be Obtained

A tentative structure proposal based on LC-MS data eventually requires confirmation with an orthogonal spectroscopic technique. Once a proposed structure is achieved, the feasibility of obtaining a standard should be evaluated. If a standard can be obtained through chemical synthesis or commercial sources, a confirmation can be achieved by the procedure discussed in the section IDENTIFICATION OF IMPURITIES WITH AN AUTHENTIC STANDARD. Besides the process impurities mentioned in that section, identification of leachables is another case where authentic standards can be readily available because leachables are typically small molecule ingredients used in the manufacturing of the packaging/container/closure systems (e.g., rubber or plastic). Sometimes, authentic standards can be purchased from commercial sources. This process can be demonstrated using the example given in Figure 11. In this case, an unknown impurity was observed in the mesalamine tablet drug product.

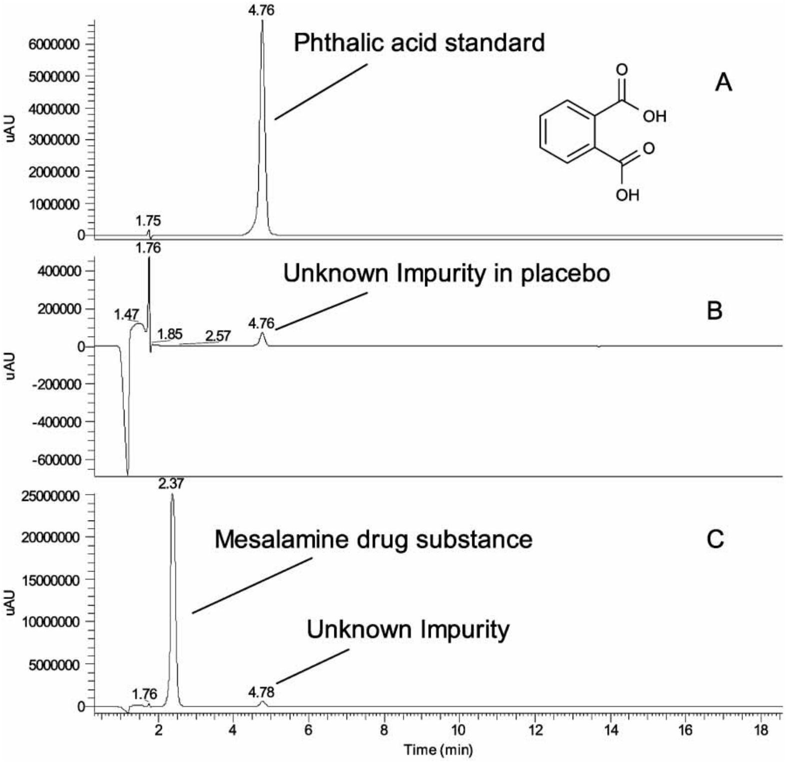


Figure 11. HPLC-UV chromatograms of mesalamine drug product (C), Placebo (B), and a phthalic acid standard (A).

Since it is also observed in the placebo, this impurity is not related to the drug substance. LC-MS results demonstrated that it is likely one of the 1,2, 1,3, and 1,4-phthalic acid isomers. Based on the preliminary results, three standards were purchased. Among them, the 1,2-phthalic acid isomer standard matches the RRT, UV spectrum, and LC-MS data of the unknown. Consequently, the unknown is identified as 1,2-phthalic acid.[146]

If a standard is not available through commercial sources, and if the difficulty to synthesize the impurity outweighs the challenge to isolate it either from the bulk drug or a stressed sample, a preparative isolation method needs to be developed. The subsequent isolate then needs to be fully characterized using NMR.

Isolation of the Impurity for NMR Characterization

Preparing a suitable sample for NMR characterization (mg level with high purity) is often the most laborious and time consuming part of the whole process of unknown impurity structure elucidation. This is mainly because the level of the target impurity in the pharmaceutical product could be very low (0.1% or lower) and the matrix could be complex (e.g., crude synthetic mixture or a drug formulation). Thus, if it is feasible, efforts should always be made to enrich the unknown impurity and simplify the matrix before starting the isolation.

For process related impurities, one should always check if this is a starting material, intermediate, or any reagent used in the synthetic process and, if it is, then the identification turns into a confirmation of known impunity. Such conformation can be readily achieved by retention time and mass spectral matching, as described above. Side-reactions are expected from any chemical process; thus, a process impurity can also be a by-product from a side-reaction. If the impurity is tentatively identified by LC-MS and a mechanism of formation is proposed, it is often possible for the chemist to find a way to chemically synthesize the by-product. In this case, a chemical approach (synthesis) may be more favorable than an analytical approach (isolation). For example, when the unknown impurity (M-12) shown in Figure 1 was tentatively identified using LC-MS, the impurity was then synthesized followed by retention time matching and NMR confirmation using the synthesized material.[146] If there is not an easy way to synthesize the unknown, then isolation may be the only viable alternative.

For identification of degradation products, forced degradation is a very useful tool. By stressing the drug at various conditions, one can not only obtain information about the formation mechanism of the unknown, but also an enriched sample for isolation and identification. If the degradation chemistry (plausible structure, mechanism of formation, etc.) is established, a preparative forced degradation should be conducted[141] to further enrich the sample for isolation. Preparative forced degradation is a study that aims at maximizing the yield of the unknown degradation product for preparative isolation. In comparison, regular forced degradation is usually conducted at generic conditions and with a reasonable degradation rate (normally around 10% of the drug substance.[244] Figure 12 presents an example where a photolytic degradation product was observed in a regular light stress study. Based on the LC-MS results, it was proposed that this photolytic degradation product would be formed through singlet oxygen [2 + 2] cycloaddition. A preparative forced degradation study was then performed using Rose Bengal as a sensitizer to facilitate the singlet oxygen oxidation under light irradiation. As a result, the yield of the desired impurity increased from less than 1% of the drug substance in the regular light stress study to 65%.[141]

Leachables are also drug product impurities. For obvious reasons, it is very undesirable to isolate leachables directly from a drug product formulation. When dealing with leachables, the best practice is to correlate the leachables to the so-called extractables. Extractables are chemical entities that can be extracted in the laboratory from the same materials used for drug product packaging/container/closer systems. Therefore, extractables are potential leachables. If a leachable impurity is identified as the same as an extractable peak, efforts should be made to isolate the extractable derived from the packaging/container/closer material. It is important to mention that leachables are usually related to the formulation of the packaging/container/closer material (e.g., plasticizers, curing agents, anti-oxidants, or

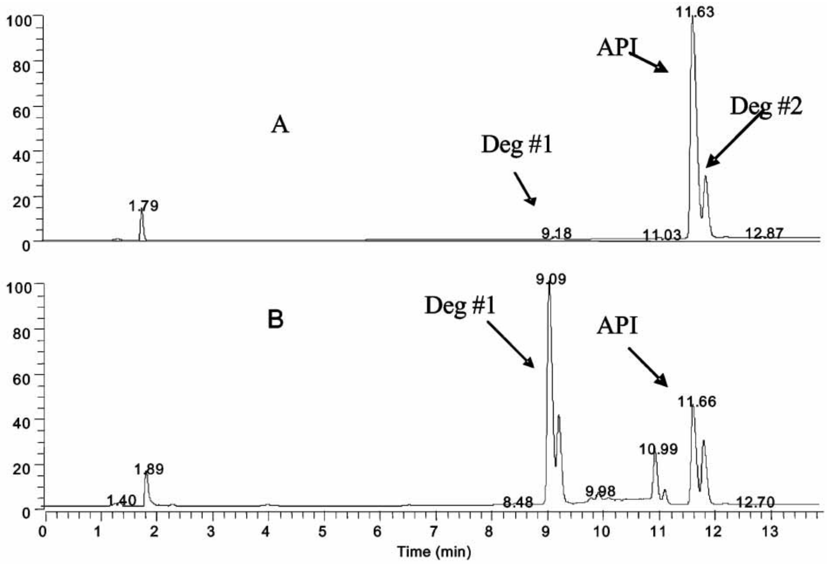


Figure 12. HPLC-UV chromatograms of a drug substance after normal photo stress study (A) and after preparative forced degradation study (B).

monomers and oligomers of the polymeric material); consequently, there is a good chance that leachables are known compounds.

After a suitable sample is prepared for isolation, an isolation technique needs to be selected. Commonly used preparative isolation techniques include TLC (Thin layer chromatography),[245] Flash Chromatography,[246] SPE (Solid phase extraction),[247] LLE (Liquid-liquid extraction),[248] ASE (Accelerated solvent extraction),[249] preparative HPLC,[250] and preparative SFC (Supercritical fluid chromatography).[251] LC and flash chromatography are simple to use and cost effective. SPE and LLE can be used either as a sample enrichment tool or an isolation tool. It is also very common to use either LLC or SPE to purify the isolate generated from other isolation techniques, e.g., desalting. LLC and ASE are often used for preparation of leachable/extractable impurities. LLC can be facilitated by sonication or reflux. ASE is programmable and, therefore, is good for automation and batch processing. While every technique has its pros and cons, the choice of the method is often determined by the availability of the technique and analyst's personal experience and expertise. These techniques can either be used separately or combined. For example, during preparative forced degradation and isolation of the tipranavir alcohol and tris adducts, unknown 1 and unknown 2, we found that, after complete degradation of tipranavir alcohol, only about 10% of the total degradation products were unknown 1 and unknown 2; the majority of the degradation products were a series of dehydration and decarboxylation isomers. We also found that it is not productive to isolate the unknowns directly from the crude mixture of degradation products using preparative HPLC. Hence, a two-step isolation approach was developed. In the first step, flash chromatography was used to separate the dehydration and decarboxylation isomers from the unknown isomers. Figure 13 shows the extracted ion chromatogram at m/z 722 ($[M+H]^+$ of the unknowns) of the 4th fraction from the flash chromatography, compared to that of a stability sample. LC-MS demonstrated that this flash chromatographic fraction contained only the isomers of the target unknown impurities. In the second step, a preparative HPLC method was developed based on the drug product impurity method. About 5 mg of the unknown 1 and 2 mixture was isolated at about 85% purity. With this sample, NMR was able to elucidate the unknown structures as a pair of E/Z isomers.[141] We found later that other isomer peaks shown in Figure 13 are also E/Z isomers with the double bonds in different side chains.

The Role of NMR in Identification of Unknown Impurities

NMR (Nuclear magnetic resonance) is probably the most powerful analytical tool for small molecule structure elucidation and confirmation, provided that a sufficient amount of high purity sample is available (mg level). The aim of this section is to provide a brief overview of the role of NMR in the overall strategy

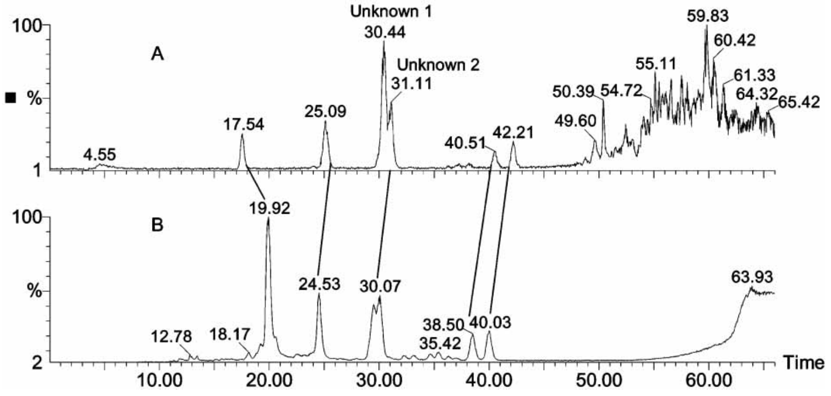


Figure 13. Extracted ion chromatograms of m/z 722 of a tipranavir drug product sample (A) and Fraction VI of synthetic mixtures by flush chromatography (B).

for impurity identification outlined in Figure 6. For details on theoretical and practical aspects of NMR, the readers are directed to more dedicated review articles and book chapters in this area.[252,253]

In principle, the total connectivity of all H and C atoms in the molecule can be established through a series of NMR experiments. The most common and useful NMR experiments in unknown impurity elucidation include 1-D (one-dimensional) and 2-D (two-dimensional) ^{1}H (homonuclear), and 1-D and 2-D ^{13}C (heteronuclear) experiments. In some special cases, ^{15}N,[254–256] ^{19}F[257–259] ^{2}H[260,261] and ^{31}P[262] can also be useful. 1-D ^{1}H experiments can reveal a proton's chemical environments through chemical shifts, the relative number of protons through integrals, the relationship of protons to neighboring protons through peak splitting patterns, and information on proton exchange in the molecule. 2-D ^{1}H NMR reveals connectivity between protons. The simplest 2-D ^{1}H experiment is COSY (Correlation spectroscopy).[263] COSY experiments provide connectivity information on neighboring protons through chemical bonds. In the COSY spectrum of nifedipine (A portion is shown Figure 14), the four aromatic protons H2, H3, H4, and H5 are correlated to each other through bond to form symmetric off-diagonal signals. Among them, the three groups of strong cross peak signals are caused by the J couplings of the neighboring (3 bonds coupling) protons, H2-H3, H3-H4, and H4-H5, respectively, as indicated by the three boxes in Figure 14. Note that the distance (i.e., the numbers of bonds) between two coupling protons that gives the strongest J coupling varies in different molecules. TOCSY (Total correlation spectroscopy)[264] is a similar experiment to COSY, but it also gives information on long range proton connectivity (over 4 bonds) to show individual spin systems. In addition to cross bond connectivity provided by COSY and TOCSY, spatial proton-proton correlation information can be acquired by

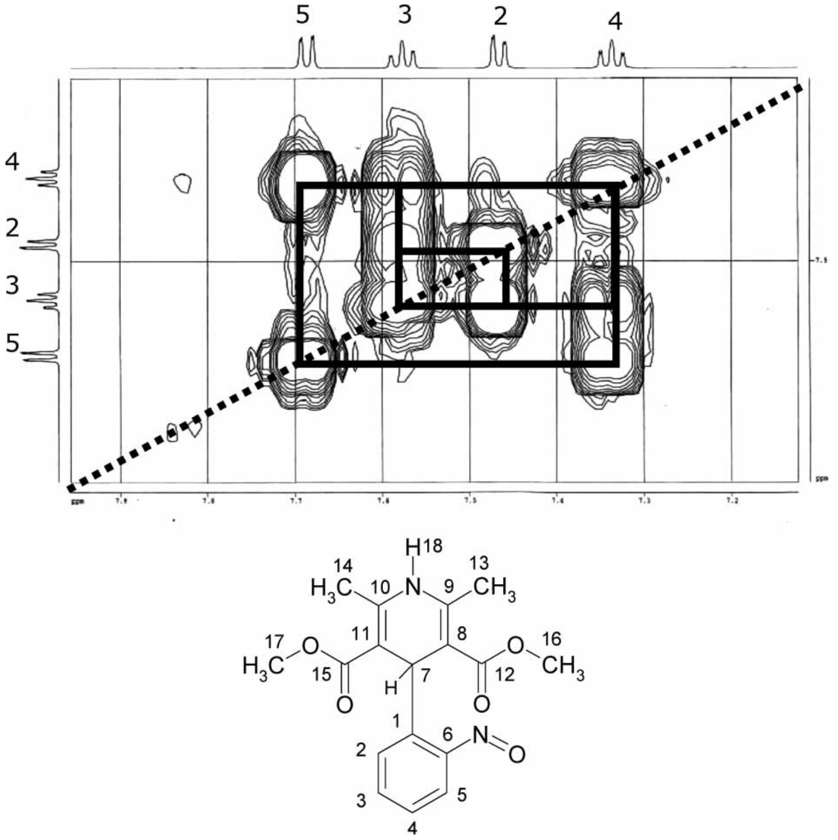


Figure 14. Chemical structure and a COSY spectrum of nifedipine.

NOESY (Nuclear overhauser enhancement spectroscopy)[265] and ROESY (Rotating-frame nuclear overhauser enhancement spectroscopy)[266] experiments. Protons within 4 angstroms through space in a molecule can correlate to each other and give cross peak signals in the NOESY spectrum. This feature allows the assignment of protons that are close in space and, therefore, is very useful for elucidation of isomeric impurities.

Proton NMR is significantly more sensitive than carbon NMR, so NMR characterization always starts with proton experiments. When proton data are not sufficient, ^{13}C experiments are needed. A ^{1}H decoupled 1-D ^{13}C spectrum shows a peak for every unique carbon atom in the molecule. Like the 1-D proton spectrum, symmetric carbons give a single peak. The chemical shifts of carbon atoms follow similar rules as for proton chemical shifts, except these are usually 15–20 times greater than the corresponding proton chemical shifts. A ^{13}C spectrum is not quantitative; however, it usually contains clues about the numbers of carbons. For example, a signal that contains two symmetric carbons usually appears larger than a single carbon signal. Extra structural information can be obtained through some modified 1-D ^{13}C experiments. These techniques include APT (Attached proton test),[267] DEPT (Distortionless enhancement by polarization transfer),[268] and INEPT (Insensitive nuclear enhancement by polarization transfer).[269] One of the features of these experiments is that they are phase sensitive, i.e., methyl and methine carbons give positive signals, while methylene carbons give negative signals (note that phase assignment is arbitrary). In an APT spectrum, the quaternary carbon gives a negative signal as do methylene carbons. In INEPT and DEPT spectra, the quaternary carbon signal is suppressed. Connectivity between heteronuclears (e.g., between carbon and proton) can be achieved using 2-D heteronuclear experiments. Among them, HMQC (Heteronuclear multiple quantum coherence)[270] and HMBC (Heteronuclear multiple bond correlation)[271] are the most useful for small molecule structure elucidation. HMQC provides connectivity information between a carbon and the protons directly attached to it (1 bond correlation), while HMBC provides correlation information between a carbon and protons that are not directly attached to it. Usually, only the ^{1}H-^{13}C correlations within 2 to 4 bonds are observed in an HMBC experiment. The correlation intensity generally follows the order $^{3}J_{C\text{-}H} > {}^{2}J_{C\text{-}H} > {}^{4}J_{C\text{-}H.}$ This technique is very useful for assignment of quaternary carbons. Taking the quaternary carbons C12/15 of nifedipine as an example, C12/15 is 3 bonds away from H16/17, two bonds away from H7, and four bonds away from H8 and H13/14, consequently, a strong HMBC signal was observed between C12/15 and H16/17($^{3}J_{C\text{-}H}$), and weaker signals were observed between C12/15 and other proton groups respectively ($^{2}J_{C\text{-}H}$ and $^{4}J_{C\text{-}H}$), as shown in Figure 15. C12/15 is five bonds or more from those aromatic protons; therefore, no HMBC signals between them were observed.

By assigning all protons and carbons using various NMR techniques, a structure, or at least a candidate structure, can be proposed. If this is consistent with the tentative structures proposed based on the results of the on-line techniques, the unknown can be considered identified. If the NMR results are inconsistent with the mass spectral results, an investigation should be conducted. Potential factors that could affect the tests results of both techniques should be considered, such as sample preparation, sample purity and quantity, contamination, chemical stability, solvent effects, etc.

Although it is rare, unsuccessful elucidation of an unknown impurity with both mass spectrometry and NMR data can happen. When this happens, one should look into whether other orthogonal techniques can provide any useful information. For example, FTIR is not routinely used in unknown identification; however, sometimes it may provide useful information, as demonstrated by Alsante and colleagues.[272] Single crystal analysis is mostly used for stereochemistry confirmation of drug substance, but it can be used for structure elucidation of an impurity if a single crystal of

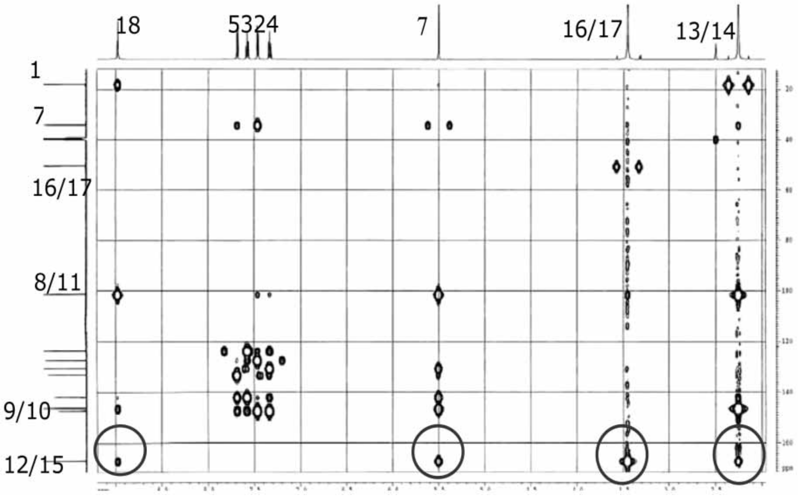


Figure 15. An HMBC spectrum of nifedipine.

the impurity is obtained. This approach was used by Vasu Dev et al.[273] in identification of one of the docetaxel impurities. Note that regulatory agencies do allow unsuccessful identification in a new drug application (NDA) if efforts have been made to the identification, as stated in ICH Q3A (R) "When identification of an impurity is not feasible, a summary of the laboratory studies demonstrating the unsuccessful efforts should be included in the application."[2]

CONCLUSIONS

Identification of unknown pharmaceutical impurities is a critical analytical activity in the drug development process. The goal of this activity is to elucidate the chemical structures and mechanisms of formation of unknown impurities observed at various pharmaceutical developmental stages. For early development projects, this information is essential in assessing the toxicological implications of the impurity and improving the chemical and pharmaceutical development process to minimize or eliminate the impurity. For late phase product that is intended for marketing application, identification of unknown impurities, at or above the identification thresholds, is a regulatory requirement. As described in this review, the origin and formation mechanisms of pharmaceutical impurities are complex; thus, an understanding of the chemistry of impurity formation is critical for a successful identification. The strategies for identification of unknown impurities are determined by the specific situation and the availability of modern analytical techniques and expertise in a structural analytical laboratory. While every analytical technique mentioned in this article can play an important role in identification of a specific unknown impurity, in general, mass spectrometry based hyphenated techniques have been the most widely used and most powerful techniques. NMR is the most definitive analytical tool for structure elucidation and, in many cases, it is required for definitive structure elucidation. Due to the sensitivity issue of NMR, isolation of the unknown impurity for NMR characterization remains a vital part of the overall identification strategy.

ACKNOWLEDGMENTS

The author gratefully acknowledges Mr. Scot Campbell, Dr. Alice Granger, Mr. Scott Pennino, Mr. Keith McKellop, Dr. Cindy Qin, Mr. Walter Davidson, Dr. Carl Busacca, Dr. Anjan Saha, and Mr. Diego Cobice for their technical contributions, and Mr. James Mullis, Mr. Scot Campbell and Dr. Zhanna Yuabova for help in reviewing the manuscript.

REFERENCES

1. ICH Q3A (R): Impurities in New Drug Substance. 2002.
2. ICH Q3B (R): Impurities in New Drug Product. 2003.
3. ICH Q3C and ICH Q3C(M): Residual Solvents. 2002.
4. FDA draft guidance: Non-clinical studies for development of pharmaceutical ingredients. 2002.
5. ICH Q6A Specifications: Test procedures and Acceptance Criteria for New Drug Substances and New Drug products: Chemical Substances. **2000**.
6. EMEA CPMP note for Guidance on specification limits for residuals of metal Catalysis. 2002.
7. EMEA CHMP draft guidance on the limits of Genotoxic Impurities (CPMP/SWP/5199/02). 2004.
8. Recommendations. PQRI leachables and Extractables WorkShop. December 2005, Bethesda, Maryland; p. 7.
9. Bundgaard, H.; De Weck, A.L. Role of amino-reactive impurities in acetylsalicylic acid allergy. Intl. Arch. Allergy Appl. Immunol. **1975**, *49* (1–2), 119–124.
10. Bundgaard, H. Pharmaceutical aspects of penicillin allergy: polymerization of penicillins and reactions with carbohydrates. J. Clin. Hosp. Pharm. **1980**, *5* (2), 73–96.
11. Quaglia, M.G.; Farina, A.; Donati, E.; Cotechini, V.; Bossu, E. Determination of MPTP, a toxic impurity of pethidine. J. Pharm. Biomed. Anal. **2003**, *33* (1), 1–6.
12. Cegg, S.; Safe, S.; Crocker, J.F.S. Identification of a toxic impurity in commercial diphenylamine. J. Envir. Sci. Health, Part B: Pest. Food Contam. Agric. Wastes **1981**, *B16* (2), 125–130.
13. Görög, S. Identification and determination of impurities in drugs. In *Prog. Pharm. Biomed. Anal*, *4*; Elsevier: Amsterdam, 2000; 748.
14. Ahuja, S.; Alsante, K. *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*, *5*; Academic Press: San Diego, 2003, 341.

15. Camarasu, C.; Madichie, C.; Williams, R. Recent progress in the determination of volatile impurities in pharmaceuticals. Trends Anal. Chem. **2006**, *25* (8), 768–777.
16. Rahman, N.; Azmi, S.N.H.; Wu, H. The importance of impurity analysis in pharmaceutical products: an integrated approach. Accred. Qual. Assur. **2006**, *11* (1–2), 69–74.
17. Kauffman, J.S. Identification and risk-assessment of extractables and leachables. Pharm. Technol. **2006** (Suppl), S14, S16–S18, S20–S22.
18. Grekas, N. Organic impurities in chemical drug substances. Pharm. Technol. Eur. **2005**, *17* (10), 24, 26, 28, 30, 32.
19. Glazkov, I.N.; Bochkareva, N.L.; Revel'skii, I.A. Determination of organic impurities in pharmaceutical preparations. J. Anal. Chem. **2005**, *60* (2), 107–118.
20. Norwood, D.L.; Qiu, F. Strategies for the analysis of pharmaceutical excipients and their trace level impurities. Amer. Pharm. Rev. **2004**, *7* (5), 92, 94, 96–99.
21. Jiben, R. Pharmaceutical impurities-A mini review. AAPS PharmScitech. **2002**, *3* (2) article 6.
22. Dobo, K.L.; Greene, N.; Cyr, M.O.; Caron, S.; Ku, W.W. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Regul. Toxicol. Pharmacol. **2006**, *44* (3), 282–293.
23. Okafo, G.; Tolson, D.; Monte, S.; Marchbank, J. Analysis of process impurities in the basic drug SB-253149 using capillary electrophoresis and on-line mass spectrometric detection. Rapid Comm. Mass Spectrom. **2000**, *14* (23), 2320–2327.
24. Turner, S.R.; Strohbach, J.W.; Tommasi, R.A.; Aristoff, P.A.; Johnson, P.D.; Skulnick, H.I.; Dolak, L.A.; Seest, E.P.; Tomich, P.K.; Bohanon, M.J.; Horng, M.; Lynn, J.C.; Chong, K.; Hinshaw, R.R.; Watenpaugh, K.D.; Janakiraman, M.N.; Thaisrivongs, S. Tipranavir (PNU-140690): A potent, prally bioavailable nonpeptidic HIV protease inhibitor of the 5, 6-dihydro-4-hydroxy-2-pyrone sulfonamide class. J. Med. Chem. **1998**, *41* (18), 3467–3476.
25. Gavin, P.F.; Olsen, B.A.; Wirth, D.D.; Lorenz, K.T. A quality evaluation strategy for multi-sourced active pharmaceutical ingredient (API) starting materials. J. Pharm. Biomed. Anal. **2006**, *41* (4), 1251–1259.
26. Sheldon, E.M.; Downar, J.B. Development and validation of a single robust HPLC method for the characterization of a pharmaceutical starting material and impurities from three suppliers using three separate synthetic routes. J. Pharm. Biomed. Anal. **2000**, *23* (2–3), 561–572.
27. Muehlen, E. Impurities in starting materials and drugs. Pharmazeut. Ind. **1992**, *54* (10), 837–41.
28. Görög, S.; Laukó, A.; Herényi, B. Estimation of impurity profiles in drugs and related materials. J. Pharm. Biomed. Anal. **1988**, *6*, 697–705.
29. Görög, S.; Herényi, B.; Renyei, M. Estimation of impurity profiles of drugs and related materials. Part 9: HPLC investigation of flumecinol. J. Pharm. Biomed. Anal. **1992**, *10* (10–12), 831–835.
30. Görög, S. (ed.); Identification and determination of impurities in drugs. In *Prog. Pharm. Biomed. Anal*, *4*; Elsevier: Amsterdam, 2000, 12–13.
31. Li, H.; Sluggett, G.W. Development and validation of a sensitive GC-MS method for the determination of trace levels of an alkylating reagent in a ß-lactam active pharmaceutical ingredient. J. Pharm. Biomed. Anal. **2005**, *39*, 3–4.
32. Murty, A.S.R.K.; Kulshresta, U.C.; Rao, T.N.; Talluri, M.V.N.K. Determination of heavy metals in selected drug substances by inductively coupled plasma—mass spectrometry. Indian J. Chem. Tech. **2005**, *12* (2), 229–231.

33. Lewen, N.; Mathew, S.; Schenkenberger, M.; Raglione, T. A rapid ICP-MS screen for heavy metals in pharmaceutical compounds. J. Pharm. Biomed. Anal. **2004**, *35* (4), 739–752.
34. Garrett, C.E.; Prasad, K. The art of meeting palladium specifications in active pharmaceutical ingredients produced by Pd-catalyzed reactions. Adv. Synth. Catal. **2004**, *346* (8), 889–900.
35. Görög, S. (ed.); *Steroid Analysis in the Pharmaceutical Industry*; Ellis Horwood: Chichester, 1989; 181–211.
36. Naegele, E.; Moritz, R. Identification of minor by—products in the antibiotic drug amoxicillin with accurate mass measurements using ESI-TOF and ion trap MRM. LC-GC Europe **2005**, 7–8.
37. Blachut, D.; Wojtasiewicz, K.; Czarnocki, Z. Some pyridine derivatives as "route-specific markers" in 4-methoxyamphetamine (PMA) prepared by the Leuckart method. Foren. Sci. Intl. **2005**, *152* (2–3), 157–173.
38. Reddy, K.V.S.R.K.; Babu, J.M.; Mathad, V.T.; Eswaraiah, S.; Reddy, M.S.; Dubey, P.K.; Vyas, K. Impurity profile study of repaglinide. J. Pharm. Biomed. Anal. **2003**, *32* (3), 461–467.
39. Laniewski, K.; Vageroe, M.; Forsberg, E.; Forngren, T.; Hagman, G. Complementary use of gas chromatog.-mass spectrometry, gas chromatography-atomic emission detection and NMR for identification of pharmaceutically related impurities of unknown structures. J. Chrom. A **2004**, *1027* (1–2), 93–102.
40. Gunawardana, G.; Childress, C.; Tripp, M.; Zhang, X.; West, P. The identification of 1,6′- and 1,3″-di-N-(L-4-amino-2-hydroxybutyryl) derivatives of kanamycin as synthetic byproducts of amikacin. J. Antibiot. **1997**, *50* (10), 887–889.
41. Italia, A.; Dosi, L.; Schiavi, M. Improved high-performance liquid chromatographic resolution of the geometric isomers of 6-hydroxy-4-(1-hydroxy-1-methylethyl)-1-cyclohexene-1-ethanol and byproducts with ß-cyclodextrin. J. Chrom. **1991**, *553* (1–2), 15–19.
42. Bogdan, K.W.; Jean, L.H.; Clifford, W.C. Magnesium meerwein-ponndorf-verly-oppenauer reaction. The origin of an impurity in PDA-641 batches. Org. Proc. Res. Devel. **1998**, *2*, 407–411.
43. Proudfoot, J.R.; Patel, U.R.; Campbell, S.J. A novel smiles rearrangement gives access to the a-ring pyridine isomers of the nevirapine ring systems. J. Org. Chem. **1993**, *58* (25), 6996–7000.
44. Horvath, P.; Balogh, G.; Brlik, J.; Cseshi, A.; Dravecz, F.; Halmos, Zs.; Lauko, A.; Renyei, M.; Varga, K.; Görög, S. Estimation of impurity profiles of drugs and related materials part 16: identification of the side products of the ethinylation step in the synthesis of contraceptive gestogens. J. Pharm. Biomed. Anal. **1997**, *15*, 1343–1349.
45. Görög, S. (ed.) Identification and determination of impurities in drugs. In *Prog. Pharm. Biomed. Anal*, *4*; Elsevier: Amsterdam, 2000, 10.
46. Olsen, B.A.; Baertschi, S.W. Strategies for investigation and control of process- and degradation related impurities. In *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*; Ahuja, S., Alsante, K.M., (eds.), Academic Press: New York, 2003, 89–117.
47. (a) Mortellaro, M.A.; Bleisch, T.J.; Duerr, B.F.; Kang, M.S.; Huang, H.; Czarnik, A.W. Metal ion-catalyzed hydrolysis of acrylate esters and amides by way of their conjugate addition product. J. Org. Chem. **1995**, *60*, 7238–7246; (b) Thakur, A.B.; Morris, K.; Grosso, J.A.; Himes, K. Megnsium and Kinetics

of metal ion mediated degradation of fosinopril sodium. Pharm. Res. **1993**, *10*, 800–809.

48. Florey, K. (ed.), *Analytical Profiles of Drug Substances*; Academic Press: New York, 1979; Vol. 8, 30.
49. Florey, K. (ed.), *Analytical Profiles of Drug Substances*; Academic Press: New York, 1974; Vol. 3, 39.
50. Waterman, K.C.; Adami, R.C.; Alsante, K.M.; Antipas, A.S.; Arenson, D.R.; Carrier, R. Hydrolysis in pharmaceutical formulations. Pharm. Devel. Technol. **2002**, *7* (2), 113–146.
51. Zhang, H.; Wang, P.; Bartlett, M.G.; Stewart, J.T. HPLC determination of cisatracurium besylate and propofol mixtures with LC-MS identification of degradation products. J. Pharm. Biomed. Anal. **1998**, *16* (7), 1241–1249.
52. Ahmad, A.K.S.; Kawy, M.A.; Nebsen, M. First derivative ratio spectrophotometric, HPTLC-densitometric, and HPLC determination of nicergoline in presence of its hydrolysis-induced degradation product. J. Pharm. Biomed. Anal. **2002**, *30* (3), 479–489.
53. Alvarez-Lueje, A.; Pastine, J.; Squella, J.A.; Nunez-Vergara, L.J. Assessment of the hydrolytic degradation of lovastatin by HPLC. J. Chilean Chem. Soc. **2005**, *50* (4), 639–646.
54. Jancic, B.; Medenica, M.; Ivanovic, D.; Malenovic, A. Evaluation of a liquid chromatographic method for analysis of indinavir and degradation products arising from hydrolysis of its amide bond. Chromatographia **2005**, *62* (5/6), 233–238.
55. Archontaki, H.A. Kinetic study on the degradation of indomethacin in alkaline aqueous solutions by derivative ultraviolet spectrophotometry. Analyst **1995**, *120* (10), 2627–2634.
56. Qureshi, S.Z.; Qayoom, T.; Helalet, M.I. Simultaneous spectrophotometric and volumetric determinations of amoxycillin, ampicillin and cloxacillin in drug formulations: reaction mechanism in the base catalysed hydrolysis followed by oxidation with iodate in dilute acid solution. J. Pharm. Biomed. Anal. **1999**, *21* (3), 473–482.
57. Naegele, E.; Moritz, R. Structure elucidation of degradation products of the antibiotic amoxicillin with ion trap MSn and accurate mass determination by ESI TOF. J. Am. Soc. Mass Spectrom. **2005**, *16* (10), 1670–1676.
58. Basaez, L.; Vanysek, P. Transport studies of ß-lactam antibiotics and their degradation products across electrified water/oil interface. J. Pharm. Biomed. Anal. **1999**, *19* (1–2), 183–192.
59. Plessas, Ch.T. Alkaline hydrolysis of phenobarbital. Chim. Chron. **1972**, *1* (2), 109–114.
60. Stemm, N.L.; Skoug, J.W.; Robins, R.H. Gradient high-performance liquid chromatographic assay for degradation products of adinazolam mesylate in a sustained-release tablet formulation. Pharm. Res. **1995**, *12* (5), 738–745.
61. Junnarkar, G.H.; Stavchansky, S. Isothermal and nonisothermal decomposition of famotidine in aqueous solution. Pharm. Res. **1995**, *12* (4), 599–604.
62. Ragno, G.; Risoli, A.; Ioele, G.; De Luca, M. Photo- and thermal-stability studies on benzimidazole anthelmintics by HPLC and GC-MS. Chem. Pharm. Bull. **2006**, *54* (6), 802–806.
63. Rao, B.M.; Srinivasu, M.K.; Sridhar, G.; Kumar, P.R.; Chandrasekhar, K.B.; Islam, A. A stability indicating LC method for zolmitriptan. J. Pharm. Biomed. Anal. **2005**, *39* (3–4), 503–509.

64. Bretnall, A.E.; Hodgkinson, M.M.; Clarke, G.S. Micellar electrokinetic chromatography stability indicating assay and content uniformity determination for a cholesterol-lowering drug product. J. Pharm. Biomed. Anal. **1997**, *15* (8), 1071–1075.
65. Karaisz, K.G.; Snow, N.H. The use of solid-phase microextraction/gas chromatography-mass spectrometry for the determination of degradation products of volatile and semivolatile compounds. J. Microcol. Sep. **2001**, *13* (1), 1–7.
66. Gadkariem, E.A.; El-Obeid, H.A.; Abounassif, M.A.; Ahmed, S.M.; Ibrahim, K.E.E. Effects of alkali and simulated gastric and intestinal fluids on danazol stability. J. Pharm. Biomed. Anal. **2003**, *31* (4), 743–751.
67. Panderi, I.; Archontaki, H.; Gikas, E.; Parissi-Poulou, M. Acidic hydrolysis of bromazepam studied by high performance liquid chromatography, Isolation and identification of its degradation products. J. Pharm. Biomed. Anal. **1998**, *17* (2), 327–335.
68. Wang, E.; Yu, Z.; Li, N. Investigations of hydrolysis kinetics of atropine sulfate by electroanalysis at the water/nitrobenzene interface. J. Electroanal. Chem. **1992**, *334* (1–2), 195–201.
69. O'Neal, J.S.; Schulman, S.G.; Van der Giesen, W.F.; Roomer, A.C.J. Alkaline hydrolytic lability of some hydroxy- and methoxycoumarins and related anticoagulants. Intl. J. Pharmaceut. **1982**, *12* (4), 355–359.
70. Yang, S.K.; Tang, R.; Yang, T.J.; Pu, Q.L.; Bao, Z. 2-(Methylamino)-5-chlorobenzophenone imine: A novel product formed in base-catalyzed hydrolysis of diazepam. J. Pharm. Sci. **1996**, *85* (7), 745–748.
71. Buur, A.; Bundgaard, H. Kinetics of the acid-catalyzed hydrolysis of cimetidine. Acta Pharm. Nord. **1989**, *1* (6), 337–342.
72. Yudi, L.M.; Baruzzi, A.M.; Solis, V. Quantitative determination of erythromycin and its hydrolysis products by cyclic voltammetry at the interface between water and 1, 2-dichloroethane. J. Electroanal. Chem. **1993**, *360* (1–2), 211–219.
73. Nomura, M.; Shuto, S.; Matsuda, A. Synthesis of the cyclic and acyclic acetal derivatives of 1-(3-C-ethynyl-ß-D-ribo-pentofuranosyl)cytosine, a potent antitumor nucleoside. Design of prodrugs to be selectively activated in tumor tissues via the bio-reduction-hydrolysis mechanism. Bioorg. Med. Chem. **2003**, *11* (11), 2453–2461.
74. Kripalani, K.J.; Cohen, A.I.; Weliky, I.; Schreiber, E.C. Metabolism of triamcinolone acetonide 21-phosphate in dogs, monkeys, and rats. J. Pharm. Sci. **1975**, *64* (8), 1351–1359.
75. Florey, K. (ed.), *Analytical Profiles of Drug Substances*; Academic Press: New York, **1972**, Vol. 1, 411.
76. Florea-Wang, D.; Haapala, E.; Mattinen, J.; Hakala, K.; Vilpo, J.; Hovinen, J. Reactions of N,N-Bis(2-chloroethyl)-p-aminophenylbutyric acid (Chlorambucil) with 2′-deoxyadenosine. Chem. Res. Toxicol. **2003**, *16* (3), 403–408.
77. El Ragehy, N.A.; Badawey, A.M.; Khateeb, S.Z.-El. Stability indicating methods for the determination of loratadine in the presence of its degradation product. J. Pharm. Biomed. Anal. **2002**, *28* (6), 1041–1053.
78. Doerr, R.C.; Parris, N.; Parks, O.W. Determination of sulfanilic acid in the presence of sulfanilamide and some sulfa drugs by reversed-phase ion-pair high-performance liquid chromatography. J. Chrom. **1980**, *196* (3), 498–500.
79. Baerschi, S.W.; Jansen, P.J. Stress testing: a predictive tool. In *Pharmaceutical Stress Testing*; Baerschi, S.W., (ed.), Taylor & Francis, 2005; 37.
80. Gould, E.S. *Mechanism and Structure in Organic Chemistry*; Holt, Rinehart and Winston: New York, 1959, 709.

81. Boccardi, G. Oxidative susceptibility testing. In *Pharmaceutical Stress Testing-Predicting Drug Degradation*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 220.
82. Boccardi, G. Oxidative susceptibility testing. In *Pharmaceutical Stress Testing-Predicting Drug Degradation*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 215.
83. Hovorka, S.W.; Hageman, M.J.; Schoneich, C. Oxidative degradation of a dulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone in aqueous/organic cosolvent mixtures. Pharm. Res. **2002**, *19* (4), 538–545.
84. Waterman, K.C.; Adami, R.C.; Alsante, K.; Hong, J.; Landis, M.S.; Lombardo, F.; Roberts, C.J. Stabilization of pharmaceuticals to oxidative degradation. Pharm. Devel. Technol. **2002**, *7* (1), 1–32.
85. Awang, D.V.C.; Dawson, B.A.; Girard, M.; Vincent, A.; Ekiel, I. The product of reserpine autoxidation. J. Org. Chem. **1990**, *55*, 4443–4448.
86. Trabelsi, H.; Hassen, I.E.; Bouabdallah, S.; Bouzouita, K.; Safta, F. Stability indicating LC method for the determination of pipamperone. J. Pharm. Biomed. Anal. **2005**, *39* (5), 914–919.
87. Boccardi, G. Oxidative susceptibility testing. In *Pharmaceutical Stress Testing-Predicting Drug Degradation*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 225.
88. Baertschi, S.W.; Alsante, K.M. Stress testing: The chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 89.
89. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 103.
90. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 102.
91. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 95.
92. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 93.
93. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 90.
94. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 89.
95. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 72.
96. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 71.
97. Hartauer, K.; Arbuthnot, G.; Baerschi, S.W.; Johnson, R.; Luke, W.; Pearson, N.; Richard, E.; Tsang, P.; Wiens, R. Influence of peroxide impurity in povidone and crospovidone on the tablet stability of raloxifene hydrochloride, indentification and control an oxidative degradation product. Pharm. Dev. Tech. **2000**, *5* (3), 303–319.
98. Malmstroem, J.; Hansen, L.; Ryager, A.; Olsen, H. High speed development work by preparation of an "in-hand" library of NMR-quantified degradation products. J. Pharm. Sci. **2005**, *94* (11), 2549–2567.
99. Görög, S. (ed.), *Identification and Determination of Impurities in Drugs*; Elsevier: Amsterdam, 2000; 493.

100. Alsante, K.M.; Hatajik, T.D.; Lohr, L.L.; Santafianos, D.; Sharp, T.R. Solving impurity/degradation problems: case studies. In *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*; Ahuja, S., Alsante, K., (eds.), Academic Press: New York, 2003; 380.
101. Ressler, C. The solid state dehydrogenation of L-1,4-cyclohexadiene-1-ananine hydrate to L-phenylalanine. J. Org. Chem. **1972**, *37* (19), 2933–2936.
102. Qiu, F. Identification of degradation products from small molecule drug substance and drug product using modern analytical techniques, Proc. 2nd Ann. Forced Degrad. Studies, Washington, DC, Feb. 15–17, 2005.
103. Kopecky, J. *Photochemistry: A Visual Approach*; VCH Publishers, Inc., 1992*, 57–73*, 191–211.
104. Frith, R.G.; Philipou, J. Application of clomiphene photolysis to assay based on analysis of the derived phenanthrenes. J. Chromatogr. **1986**, *367*, 260–266.
105. Allwood, M.C.; Plane, J.H. The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. Intl. J. Pharm. **1986**, *31* (1–2), 1–7.
106. Kumar, N.; Windisch, V.; Ammon, H.L. Photoinstability of some tyrphostin drugs: chemical consequences of crystallinity. Pharm. Res. **1995**, *12* (11), 1708–1715.
107. Fasani, E.; Albini, A. Photostability stress testing. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 304.
108. Kopecky, J. *Organic Photochemistry: A Visual Approach*; VCH Publishers: New York, 1992, 64.
109. Fasani, E.; Albini, A. Photostability stress testing. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 309.
110. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 112.
111. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 90.
112. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 99.
113. Baertschi, S.W.; Alsante, K.M. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis: **2005**, 105.
114. Baertschi, S.W.; Draper, J.R.; Jansen, P.J.; Smith, W.K. Introduction to oxidative stress testing, The 3rd Annul Forced degradation Studies, Best Practices for the Pharmaceutical Industry, Feb. 27–March 1, 2006, Short Hills, NJ.
115. Boccardi, G. Oxidative susceptibility testing. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 224.
116. Fabre, H.; Ibork, H.; Lerner, D.A. Photodegradation kinetics under UV light of aztreonam solutions. J. Pharm. Biomed. Anal. **1992**, *10*, 645–650.
117. Werner, D.A.; Bannerfond, G.; Fabre, H.; Mandrou, B.; Simeon de Buochberg, M. Photodegradation paths of cefatoxim. J. Pharm. Sci. **1988**, *77*, 699–703.
118. Quillian, M.A.; McCurry, B.E.; Hoo, K.H.; McCalla, D.R.; Weitekunas, S. Identification of the photolysis products of nitrofurazone irradiated under laboratory illumination. Canad. J. Chem. **1987**, *65*, 1128–1132.
119. Brittain, H.G. (ed.), *Analytical Profiles of Drug Substances and Excipients, 1999*; Academic Press, 1999; Vol. 26, 87.

120. Florey, K. (ed.), *Analytical Profiles of Drug Substances*; Academic Press: New York, 1975, *4*, 399.
121. Florey, K. *Analytical Profiles of Drug Substances*; Florey, K., (ed.), Academic Press: New York, 1989; Vol. 18, 141.
122. Aso, Y.; Yoshioka, S.; Takeda, Y. Epimerization and racemization of some chiral drugs in the presence of cyclodextrin and liposomes **1989**, *37* (10), Chem. Pharm. Bull.
123. Akashi, T.; Nefuji, T.; Yoshida, M.; Hosoda, J. Quantitative determination of tautomeric FK506 by reversed-phase liquid chromatography. J. Pharm. Biomed. Anal. **1996**, *14*, 339–346.
124. Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 122.
125. Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 114.
126. Grubb, P.E. Nalidixic acid. In *Analytical Profiles of Drug Substances*; Florey, K., (ed.), Academic Press: New York, 1979; Vol. 8, 382.
127. Zhao, Z.; Wang, Q.; Tsai, E.W.; Qin, X.Z.; Ip, D. Identification of losartan degradates in stressed tablets by LC/MS and LC/MS/MS. J. Pharm. Biomed. Anal. **1999**, *20*, 129–136.
128. Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 68.
129. Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 56–57.
130. Wu, Y. The use of liquid chromatography-mass spectrometry for the identification of drug degradation products in pharmaceutical formulations. Biomed. Chromatogr. **2000**, *14*, 384–396.
131. Smith, G.B.; Bezeny, G.C.; Douglas, A.W. Stability and kinetics of degradation of imipenem in aqueous solution. J. Pharm. Sci. **1990**, *79*, 732.
132. Jensen, J.; Cornett, C.; Olsen, C.; Tjørnelund, E.J.; Hansen, S.H. Identification of major degradation products of 5-aminosalicylic acid formed in aqueous solutions and in pharmaceuticals. Intl. J. Pharmaceut. **1992**, *88*, 177–187.
133. Doelker, E.; Vial-Bernasconi, A.C. Shell/content interactions in gelatin capsules a critical evaluation of their effects on the drug availability. STP Pharma. **1988**, *4* (4), 298–306.
134. Patel, K.; Kearney, A.S.; Palepu, N.R. Investigation of new degradation products arising from the encapsulation of an oil-based suspension formulation of topotecan. Intl. J. Pharmaceut. **1997**, *151*, 7–13.
135. Jartauer, K.J.; Arbuthnot, G.N.; Baertschi, S.W.; Johnson, R.A. Influence of peroxide impurities in povidone and crospovidone on the stability of raloxifene hydrochloride in tablets: identification and control of an oxidative degradation product. Pharm. Devel. Technol. **2000**, *5* (3), 303–310.
136. (a) Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 74–75; (b) Nissl, J.; Pischetsrieder, M.; Klein, E.; Severin, T. Binding of maillard products to proteins: formation of pyrrole carbimines. Carbohydr. Res. **1995**, *270*, C1–C5.

137. Miyata, S.; monnier, V. Immunohistochemical detection of advanced glycosylation end products in diabetic tissues using monoclonal antibody to pyrraline. J. Clin. Invest. **1992**, *89*, 1102–1112.
138. Kaczanowski, M.J.; Williams, T.D.; Trombley, K.F.; Redman-Furey, N.L. Compositions containing 5-amino-2-hydroxybenzoic acid and a reducing sugar. U.S. Pat. Appl. Publ. US2006/0046973, March 2**,** 2006.
139. Wirth, D.D.; Baertschi, S.W.; Johnson, R.A.; Maple, S.R.; Miller, M.S.; Hallenbeck, D.K.; Gregg, S.M. Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. J. Pharm. Sci. **1998**, *87* (1), 31–39.
140. Baertschi, S.W.; Alsante, K. Stress testing: the chemistry of drug degradation. In *Pharmaceutical Stress Testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 77.
141. Qiu, F. Identification of drug excipient interaction products using a stress testing assisted approach. Amer. Pharmaceut. Rev. **2006**, *9* (6), 142–145.
142. Baerschi, S.W.; Jansen, P.J. Stress testing: a predictive tool. In *Pharmaceutical Stress testing*; Baertschi, S.W., (ed.), Taylor & Francis, 2005; 38–39.
143. Waterman, K.; Sanga, S.V. Review of glass types available for packaging parenteral solutions. J. Parent. Drug Assn. **1979**, *33*, 61–64.
144. Norwood, D.L.; Nagao, L.; Lyapustina, S.; Munos, M. Application of modern analytical technologies to the identification of extractables and leachables. Amer. Pharmaceut. Rev. **2005**, *8* (1), 78–87.
145. Fusco, J.V.; Hous, P. Butyl and halobutyl rubbers. In *Rubber Technology*, 3rd edn.; Morton, M., (ed.), Chapman and Hall, 1995; 295.
146. Unpublished data.
147. Görög, S.; Bihari, M.; Csizér, É.; Dravetz, F.; Gazdag, M.; Herényi, B.; Renyei, M. Estimation of impurity profiles of drugs and related materials part 14: the role of HPLC/diode-arry UV spectroscopy in the identification of minor components (impurities, degradation products, metabolites) in various matrices. J. Pharm. Biomed. Anal. **1995**, *14*, 85–92.
148. King, R.; Bonfiglio, R.; Fernandez-Metzler, C.; Miller-Stein, C.; Olah, T. Mechanistic investigation of ionization suppresion in electrospray ionization. J. Am. Soc. Mass Spectrom. **2000**, *11*, 942–950.
149. Mallet, C.R.; Lu, Z.; Mazzeo, J.R. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. Rapid Comm. Mass Spectrom. **2004**, *18*, 49–58.
150. Han, J.; Sheng, L.; Yang, Z.; Xiang, B.; An, D. Tryptic mapping of recombinant human growth hormone by microbore liquid chromatography/electrospray ionization mass spectrometry. Zhongguo Yaoke Daxue Xuebao **1999**, *30* (4), 269–274.
151. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M. Electrospray ionization for mass spectrometry of large biomolecules. Science **1989**, *246* (4926), 64–71.
152. (a) Carroll, D.I.; Dzidic, I.; Stillwell, R.N.; Haegele, K.D.; Horning, E.C. Atmospheric pressure ionization mass spectrometry: corona discharge ion source for use in liquid chromatograph - mass spectrometer-computer analytical system. Anal. Chem. **1975**, *47* (14), 2369–2373; (b) Robb, D.B.; Covey, T.R.; Bruins, A.P. Atmospheric pressure photoionization: an ionization method for liquid chromatography-mass spectrometry. Anal. Chem. **2000**, *72* (15), 3653–3659.
153. Hanold, K.A.; Fischer, S.M.; Cormia, P.H.; Miller, C.E.; Syage, J.A. Atmospheric pressure photoionization. 1. General properties for LC/MS. Anal. Chem. **2004**, *76* (10), 2842–2851.

154. Grundy, H.S.; Kherani, R.; Foster, R.T. Photostability determination of commercially available nifedipine oral dosage formulation. J. Pharm. Biomed. Anal. **1994**, *12* (12), 1529–1535.
155. Ozaltin, N. Application of micellar electrokinectic capillary chromatography for the determination of nifedipine and its degradation product in pharmaceutical preparations. Pharm. Anal. **2003**, *36* (2), 371–387.
156. Parker, C.E.; Yamaguchi, K.; Harvan, D.J.; Smith, R.W.; Hass, J.R. Liquid chromatography-chloride-attachment negative chemical ionization mass spectrometry. J. Chromatogr. **1985**, *319* (3), 273–283.
157. Tannenbaum, H.P.; Roberts, J.D.; Dougherty, R.C. Negative chemical ionization mass spectrometry. Chloride attachment spectra. Anal. Chem. **1975**, *47* (1), 49–54.
158. Zhao, X.; Yinon, J. Identification of nitrate ester explosives by liquid chromatography-electrospray ionization and atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A **2002**, *977* (1), 59–68.
159. Zhu, J.; Cole, R.B. Formation and decompositions of chloride adduct ions, [M + Cl]-, in negative ion electrospray ionization mass spectrometry. J. Amer. Soc. Mass Spectrom. **2000**, *11* (11), 932–941.
160. Marai, L.; Kuksis, A.; Myher, J.J.; Itabashi, Y. Liquid chromatography chloride attachment negative chemical ionization mass spectrometry of diacylglycerol dinitrophenylurethanes. Biol. Mass Spectrom. **1992**, *21* (11), 541–547.
161. Kato, Y.; Tanikawa, K.; Matsumura, K. Analysis of sugars and sugar alcohols by LC/APCI-MS. Kuromatogurafi **1992**, *13* (5), 245–246.
162. Kuksis, A.; Marai, L.; Myher, J.J. Reversed-phase liquid chromatography-mass spectrometry of complex mixtures of natural triacylglycerols with chloride-attachment negative chemical ionization. J. Chromatogr. **1991**, *588* (1–2), 73–87.
163. Stuebiger, G.; Pittenauer, E.; Allmaier, G. Characterization of castor oil by on-line and off-line non-aqueous reverse-phase high-performance liquid chromatography-mass spectrometry (APCI and UV/MALDI). Phytochem. Anal. **2003**, *14* (6), 337–346.
164. Liang, H.R.; Takagaki, T.; Foltz, R.L.; Bennett, P. Quantitative determination of endogenous sorbitol and fructose in human nerve tissues by atmospheric-pressure chemical ionization liquid chromatography/tandem mass spectrometry. Rapid Comm. Mass Spectrom. **2005**, *19* (16), 2284–2294.
165. Prome, D.; Prome, J.C.; Puzo, G.; Aurelle, H. Mass spectrometry of oligosaccharides by chloride-attachment reactions: the origin of fragment loss. Carbohydr. Res. **1985**, *140* (1), 121–129.
166. Norwood, D.L.; Qiu, F. Strategies for the analysis of pharmaceutical excipients and their trace level impurities. Amer. Pharm. Rev. **2004**, *7* (5), 92, 94, 96–99.
167. Quang, C.; Kocan, G.; Tang, D.; Fast, D.M.; Michael, S.M. Development of an LC-ESI-MS/MS Method with Formate Anion Attachment for detecting Neutral Molecules in Rat Plasma. Amer. Lab. **2006**, 26.
168. Laiko, V.V.; Baldwin, M.A.; Burlingame, A.L. Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. Anal. Chem. **2000**, *72* (4), 652–657.
169. Daniel, J.M.; Laiko, V.V.; Doroshenko, V.M.; Zenobi, R. Interfacing liquid chromatography with atmospheric pressure MALDI-MS. Anal. Bioanal. Chem. **2005**, *383* (6), 895–902.
170. Salo, P.K.; Salomies, H.; Harju, K.; Ketola, R.A.; Kotiaho, T.; Yli-Kauhaluoma, J.; Kostiainen, R. Analysis of small molecules by ultra thin-layer

chromatography-atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. J. Amer. Soc. Mass Spectrom. **2005**, *16* (6), 906–915.

171. Pihlainen, K.; Grigoras, K.; Franssila, S.; Ketola, R.; Kotiaho, T.; Kostiainen, R. Analysis of amphetamines and fentanyls by atmospheric pressure desorption/ionization on silicon mass spectrometry and matrix-assisted laser desorption/ionization mass spectrometry and its application to forensic analysis of drug seizures. J. Mass Spectrom. **2005**, *40* (4), 539–545.
172. Cui, M.; McCooeye, M.A.; Fraser, C.; Mester, Z. Quantitation of lysergic acid diethylamide in urine using atmospheric pressure matrix-assisted laser desorption/ionization ion trap mass spectrometry. Anal. Chem. **2004**, *76* (23), 7143–7148.
173. Daniel, J.M.; Ehala, S.; Friess, S.D.; Zenobi, R. On-line atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. Analyst **2004**, *129* (7), 574–578.
174. Takats, Z.; Wiseman, J.M.; Gologan, B.; Cooks, R.G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science **2004**, *306* (5695), 471–473.
175. Leuthold, L.A.; Mandscheff, J.-F.; Fathi, M.; Giroud, C.; Augsburger, M.; Varesio, E.; Hopfgartner, G. Desorption electrospray ionization mass spectrometry: direct toxicological screening and analysis of illicit ecstasy tablets. Rapid Comm. Mass Spectrom. Volume Date **2005, 2006**, *20* (2), 103–110.
176. Van Berkel, G.J.; Kertesz, V. Automated sampling and imaging of analytes separated on thin-layer chromatography plates using desorption electrospray ionization mass spectrometry. Anal. Chem. **2006**, *78* (14), 4938–4944.
177. Kauppila, T.J.; Wiseman, J.M.; Ketola, R.A.; Kotiaho, T.; Cooks, R.G.; Kostiainen, R. Desorption electrospray ionization mass spectrometry for the analysis of pharmaceuticals and metabolites. Rapid Comm. Mass Spectrom. **2006**, *20* (3), 387–392.
178. Cody, R.B.; Laramee, J.A.; Durst, H.D. Direct analysis in real time (DART). Anal. Chem. **2005**, *77* (8), 2297–2302.
179. Pierce, C.Y.; Barr, J.R.; Woolfitt, A.R.; Moura, H.; Thompson, H.A.; Massung, R.F.; Cody, R.B.; Fernandez, F.M. Strain identification of the category B agent Coxiella burnetii by DART TOF-MS and multivariate pattern recognition. Abstr., 232nd ACS National Meeting, San Francisco, CA, Sept. 10–14, 2006.
180. Morlock, G.; Schwack, W. Determination of isopropylthioxanthone (ITX) in milk, yoghurt and fat by HPTLC-FLD, HPTLC-ESI/ MS and HPTLC- DART/ MS. Anal. Bioanal. Chem. **2006**, *385* (3), 586–595.
181. Liu, D.Q.; Hop, C.E.C.A.; Beconi, M.G.; Mao, A.; Chiu, S.-H.L. Use of on-line hydrogen/deuterium exchange to facilitate metabolite identification. Rapid Comm. Mass Spectrom. **2001**, *15* (19), 1832–1839.
182. Liu, D.Q.; Hop, C.E.C.A. Strategies for characterization of drug metabolites using liquid chromatography-tandem mass spectrometry in conjunction with chemical derivatization and on-line H/D exchange approaches. J. Pharm. Biomed. Anal. **2005**, *37* (1), 1–18.
183. Kamel, A.M.; Zandi, K.S.; Massefski, W.W. Identification of the degradation product of ezlopitant, a non-peptidic substance p antagonist receptor, by hydrogen deuterium exchange, electrospray ionization tandem mass spectrometry (ESI/MS/MS) and nuclear magnetic resonance (NMR) spectroscopy. J. Pharm. Biomed. Anal. **2003**, *31* (6), 1211–1222.

184. Xu, R.; Wang, T.; Isbell, J.; Cai, Z.; Sykes, C.; Brailsford, A.; Kassel, D.B. High-throughput mass-directed parallel purification incorporating a multiplexed single quadrupole mass spectrometer. Anal. Chem. **2002**, *74* (13), 3055–3062.

185. Rosentreter, U.; Huber, U. Optimal fraction collecting in preparative LC/MS. J. Combin. Chem. **2004**, *6* (2), 159–164.

186. Yost, R.A.; Enke, C.G. Selected ion fragmentation with a tandem quadrupole mass spectrometer. J. Am. Chem. Soc. **1978**, *100* (7), 2274–2275.

187. (a) Stafford, G. Ion trap mass spectrometry: a personal perspective. J. Am. Soc. Mass Spectrom. **2002**, *13* (6), 589–596; (b) Stafford, G. Ion traps provide high-throughput analysis. R&D (Cahners) **2001**, *43* (11), 49.

188. Bier, M.; Yang, C. From 2D to 3D to 2D: the evolution of the Paul trap. Abstr, 35th Central Regional Meeting of the American Chemical Society, Pittsburgh, PA, October 19–22, 2003; 45.

189. Schwartz, J.C.; Kovtoun, V.V.; Senko, M.W. Two-dimensional quadrupole ion trap operated as a mass spectrometer. U.S. Pat. Appl. Publ. 2005; 13; Cont.-in-part of U.S. Ser. No. 357,712.

190. Schwartz, J.C.; Senko, M.W.; Syka, J.E.P. A two-dimensional quadrupole ion trap mass spectrometer. J. Am. Soc. Mass Spectrom. **2002**, *13* (6), 659–669.

191. Johnson, E.G.; Nier, A.O. Angular aberrations in sector-shaped electromagnetic lenses for focusing beams of charged particles. Phys. Rev. **1953**, *91*, 10–17; Nier, A.O. Natl. Bur. Stand. Circ. (U.S.) **1953**, *522*, 29–36.

192. Gross, M.L.; Chess, E.K.; Lyon, P.A.; Crow, F.W.; Evans, S.; Tudge, H. Triple-analyzer mass spectrometry for high-resolution MS/MS studies. Intl. J. Mass Spectrom. Ion Phys. **1982**, *42* (4), 243–254.

193. Kunihiro, F.; Kanmei, Y.; Naito, M.; Itagaki, Y. The evaluation of compact-size high-resolution MS/MS system with an added small electric sector. Intl, J. Mass Spectrom. Ion Phys. **1983**, *46*, 151–154.

194. Doroshenko, V.M.; Cotter, R.J. Ideal velocity focusing in a reflectron time-of-flight mass spectrometer. J. Am. Soc. Mass Spectrom. **1999**, *10* (10), 992–999.

195. Niehuis, E.; Heller, T.; Feld, H.; Benninghoven, A. High-resolution TOF secondary ion mass spectrometer. Springer Proc. Phys. **1986**, *9*, (Ion Form. Org. Solids), 198–202.

196. Moskovets, E.V. Enhancement of the reflectron TOF focusing energy range. Appl. Phys. B: Photophys. Laser Chem. **1993**, *B56* (2), 123–129.

197. Morris, H.R.; Paxton, T.; Panico, M.; McDowell, R.; Dell, A. A novel geometry mass spectrometer, the Q-TOF, for low-femtomole/attomole-range biopolymer sequencing. J. Protein Chem. **1997**, *16* (5), 469–479.

198. Hoyes, J.; Bateman, R.; Bordoli, R.; Carruthers, R.; Gilbert, A.; Langridge, J. Automated recording of MS and MS/MS spectra during a single HPLC separation on a Q-TOF mass spectrometer. Adv. Mass Spectrom. **1998**, *14*.

199. Kagi, N. Application example of hybrid MS/MS apparatus Q-TOF. J. Mass Spectrom. Soc. Japan **1999**, *47* (3), 203–205.

200. Comisarow, M.B.; Marshall, A.G. Fourier transform ion cyclotron resonance [FT-ICR] spectroscopy. Chem. Phys. Lett. **1974**, *25* (2), 282–283.

201. Gauthier, J.W.; Trautman, T.R.; Jacobson, D.B. Sustained off-resonance irradiation for collision-activated dissociation involving Fourier transform mass spectrometry. Collision-activated dissociation technique that emulates infrared multiphoton dissociation. Anal. Chim. Acta **1991**, *246* (1), 211–225.

202. Diehnelt, C.W.; Peterman, S.M.; Budde, W.L. Liquid chromatography-tandem mass spectrometry and accurate m/z measurements of cyclic peptide cyanobacteria toxins. Trends Anal. Chem. **2005**, *24* (7), 622–634.

203. O'Connor, P.B.; Pittman, J.L.; Thomson, B.A.; Budnik, B.A.; Cournoyer, J.C.; Jebanathirajah, J.; Lin, C.; Moyer, S.; Zhao, C. A new hybrid electrospray fourier transform mass spectrometer: design and performance characteristics. Rapid Comm. Mass Spectrom. **2006**, *20* (2), 259–266.

204. Hu, Q.; Noll, R.J.; Li, H.; Makarov, A.; Hardman, M.; Cooks, R.G. The orbitrap: a new mass spectrometer. J. Mass Spectrom. **2005**, *40* (4), 430–443.

205. Makarov, A.; Denisov, E.; Kholomeev, A.; Balschun, W.; Lange, O.; Strupat, K.; Horning, S. Performance evaluation of a hybrid linear ion trap/orbitrap mass spectrometer. Anal. Chem. **2006**, *78* (7), 2113–2120.

206. Thevis, M.; Sigmund, G.; Schiffer, A.-K.; Schaenzer, W. Determination of N-desmethyl- and N-bisdesmethyl metabolites of sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. Eur. J. Mass Spectrom. **2006**, *12* (2), 129–136.

207. Feinberg, T.N. Hyphenated characterization techniques. In *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*; Ahuja, S., and Alsante, K.M., (eds.), Academic Press: New York, 2003; 355.

208. Peng, S.X. Hyphenated HPLC-NMR and its application in drug discovery. Biomed. Chromatogr. **2000**, *14*, 430–441.

209. Novak, P.; Tepes, P.; Fistric, I.; Bratos, I.; Gabelica, V. The application of LC-NMR and LC-MS for the separation and rapid structure elucidation of an unknown impurity in 5-aminosalicylic acid. J. Pharm. Biomed. Anal. **2006**, *40* (5), 1268–1272.

210. Alsante, K.M.; Boutros, P.; Couturier, M.A.; Friedmann, R.C.; Harwood, J.W.; Horan, G.J.; Jensen, A.J.; Liu, Q.; Lohr, L.L.; Morris, R.; Raggon, J.W.; Reid, G.L.; Santafianos, D.P.; Sharp, T.R.; Tucker, J.L.; Wilcox, G.E. Pharmaceutical impurity identification: A case study using a multidisciplinary approach. J. Pharm. Sci. **2004**, *93* (9), 2296–2309.

211. Guan, K.; Alves-Santana, D.; Segmuller, B.; Weber, B.; Weber, J. Method development and LC-MS and LC-NMR impurity characterizations of RWJ 54428. Abstr., 218th ACS National Meeting, New Orleans, Aug. 22–26, 1999.

212. Sharman, G.J.; Jones, I.C. Critical investigation of coupled liquid chromatography-NMR spectroscopy in pharmaceutical impurity identification. Magn. Reson. Chem. **2003**, *41* (6), 448–454.

213. Cummings, P.G.; Offen, P.; Olsen, M.A.; Kennedy-Gabb, S.; Zuber, G. LC/MS LC/ NMR, FTIR: an integrated approach to impurity identification in pharmaceutical development formulation. Amer. Pharm. Rev. **2003**, *6* (3), 88, 90, 92.

214. Crowe, E.A.; Roberts, J.K.; Smith, R.J. 1H and 19F LC / NMR: application to the identification of impurities in compounds of pharmaceutical interest. Pharm. Sci. **1995**, *1* (2), 103–105.

215. Mccrossen, S.D.; Bryant, D.K.; Cook, B.R.; Richards, J.J. Comparison of LC detection methods in the investigation of non-UV detectable organic impurities in a drug substance. J. Pharm. Biomed. Anal. **1998**, *17* (3), 455–471.

216. Yokoyama, O.; Kishi, N.; Ohe, H.; Tanaka, M.; Asakawa, N. Fundamental process for LC-NMR and its applications. Chromatography **1998**, *19* (4), 262–263.

217. Potts, B.C.M.; Albizati, K.F.; Johnson, M.O.; James, J.P. Application of LC-NMR to the identification of bulk drug impurities in GART inhibitor AG2034. Magn. Reson. Chem. **1999**, *37* (6), 393–400.

218. Feinberg, T.N. Hyphenated characterization techniques. In *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*; Ahuja, S., and Alsante, K.M., (eds.), Academic Press: New York, 2003; 341–357.

219. Cole, J.; Phillips, E.; Conoley, M. High-throughput residual solvent analysis by GC-MS. LCGC N. Amer. **2005** (*Suppl.*), 68.

220. Rocheleau, M.; Titley, M.; Bolduc, J. Measuring residual solvents in pharmaceutical samples using fast gas chromatography techniques. Can. J. Chrom. B: Anal. Technol. Biomed. Life Sci. **2004**, *805* (1), 77–86.

221. Liao, H.; Wang, Z.; Zhu, R.; Xu, K. Determination of residual solvents in somatostatin by capillary gas chromatography. Yaowu Fenxi Zazhi **2005**, *25* (4), 452–454.

222. Mulligan, K.J.; McCauley, H. Factors that influence the determination of residual solvents in pharmaceuticals by automated static headspace sampling coupled to capillary GC-MS. J. Chrom. Sci. **1995**, *33* (1), 49–54.

223. Morello, D.R.; Meyers, R.P. Qualitative and quantitative determination of residual solvents in illicit cocaine HCl and heroin HCl. J. Foren. Sci. **1995**, *40* (6), 957–963.

224. Camarasu, C.C.; Mezei-Szuts, Ma.; Varga, G.B. Residual solvents determination in pharmaceutical products by GC-HS and GC-MS-SPME. J. Pharm. Biomed. Anal. **1998**, *18* (4,5), 623–638.

225. Vajta, S.; Le Moing, J.P.; Rovei, V.; Strolin Benedetti, M. Determination of low plasma concentrations of 3-methoxy-4-hydroxyphenylethylene glycol using gas chromatography-negative-ion chemical ionization mass spectrometry. J. Chrom. **1985**, *343* (2), 239–247.

226. Mulligan, K.J.; McCauley, H. Factors that influence the determination of residual solvents in pharmaceuticals by automated static headspace sampling coupled to capillary GC-MS. J. Chrom. Sci. **1995**, *33* (1), 49–54.

227. Ojala, M.; Poutanen, M.; Mattila, I.; Ketola, R.A.; Kotiaho, T.; Kostiainen, R. Analysis of residual solvents in pharmaceuticals with purge-and-membrane mass spectrometry. Rapid Comm. Mass Spectrom. **2000**, *14* (11), 994–998.

228. Camarasu, C.C. Headspace SPME method development for the analysis of volatile polar residual solvents by GC-MS. J. Pharm. Biomed. Anal. **2000**, *23* (1), 197–210.

229. Hashimoto, K.; Urakami, K.; Fujiwara, Y.; Terada, S.; Watanabe, C. Determination of residual solvents in pharmaceuticals by thermal desorption- GC/MS. Anal. Sci. **2001**, *17* (5), 645–648.

230. Urakami, K.; Saito, Y.; Fujiwara, Y.; Watanabe, C.; Umemoto, K.; Godo, M.; Hashimoto, K. Determination of residual solvents in bulk pharmaceuticals by thermal desorption/ gas chromatography/mass spectrometry. Chem. Pharm. Bull. **2000**, *48* (12), 1894–1897.

231. Fed. Reg. 42:10412-10437; FDA (draft 2001). Guidance for Industry: mass spectrometry for confirmation of the identity of animal drug residuals.

232. Visky, D.; Jimidar, I.; Van Ael, W.; Vennekens, T.; Redlich, D.; De Smet, M. Capillary electrophoresis-mass spectrometry in impurity profiling of pharmaceutical products. Beerse Belg. Electrophoresis **2005**, *26* (7–8), 1541–1549.

233. Okafo, G.; Tolson, D.; Monte, S.; Marchbank, J. Analysis of process impurities in the basic drug SB-253149 using capillary electrophoresis and on-line mass spectrometric detection. Rapid Comm. Mass Spectrom. **2000**, *14* (23), 2320–2327.

234. Hsieh, F.Y.L.; Cai, J.; Henion, J. Determination of trace impurities of peptides and alkaloids by capillary electrophoresis-ion spray mass spectrometry. J. Chrom. A **1994**, *679* (1), 206–211.

235. Ding, J.; Barlow, T.; Dipple, A.; Vouros, P. Separation and identification of positively charged and neutral nucleoside adducts by capillary

electrochromatography-microelectrospray mass spectrometry. J. Am. Soc. Mass Spectrom. **1998**, *9* (8), 823–829.

236. Lane, S.J. CEC/MS. In *Progress in Pharmaceutical and Biomedical Analysis, 4.* (*Identification and Determination of Impurities in Drugs*); Elsevier: Amsterdam, 2000; 359–381.
237. Görög, S. Steroids. In *Progress in Pharmaceutical and Biomedical Analysis 4.* (*Identification and Determination of Impurities in Drugs*); Elsevier: Amsterdam, 2000; 712–731.
238. Zhao, Y.; Woo, G.; Thomas, S.; Semin, D.; Sandra, P. Rapid method development for chiral separation in drug discovery using sample pooling and supercritical fluid chromatography-mass spectrometry. J. Chrom. A **2003**, *1003* (1–2), 157–166.
239. MacKay, G.A.; Reed, G.D. The application of capillary SFC, packed column SFC, and capillary SFC-MS in the analysis of controlled drugs. J. High Resol. Chrom. **1991**, *14* (8), 537–541.
240. Laniewski, K.; Wannman, T.; Hagman, G. Gas chromatography with mass spectrometric, atomic emission and Fourier transform infrared spectroscopic detection as complementary analytical techniques for the identification of unknown impurities in pharmaceutical analysis. J. Chrom. A **2003**, *985* (1–2), 275–282.
241. Schrader, W.; Geiger, J.; Hoffmann, T.; Klockow, D.; Korte, E.-H. Application of gas chromatography-cryocondensation-Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry to the identification of gas phase reaction products from the α-pinene/ozone reaction. J. Chrom. A **1999**, *864* (2), 299–314.
242. Altria, K.D. Determination of drug related impurities by capillary electrophoresis. In *Identification and Determination of Impurities in Drugs*; Görög, S., (ed.), Elsevier: Amsterdam, 2000; 323.
243. Gyllenhaal, O. Supercritical fluid chromatography in determination of drug related impurities by capillary electrophoresis. In *Identification and Determination of Impurities in Drugs*; Görög, S., (ed.), Elsevier: Amsterdam, 2000; 382.
244. Baertschi, S.W.; Alsante, K.M. Stress testing: frequently asked questions. InBaertschi, S.W., (ed.), Taylor & Francis, 2005; 460.
245. Kochana, J.; Wilamowski, J.; Parczewski, A. TLC profiling of Impurities of 1-(3,4-Methylenedioxyphenyl)-2-nitropropene an Intermediate in MDMA Synthesis. Influence of Sample Preparation Methods and Conditions. J. Liquid Chrom. & Rel. Technol. **2004**, *27* (15), 2463–2470.
246. Still, W.C.; Kahn, M.; Mitra, A. Rapid chromatographic techniques for preparative separation with moderate resolution. J. Org. Chem. **1978**, *43* (14), 2923.
247. Kochana, J.; Wilamowski, J.; Parczewski, A. SPE-TLC profiling of impurities in 1-(3,4-methylenedioxyphenyl)-2-nitropropene, an intermediate in 3,4-methylenedioxy-methamphetamine (MDMA) synthesis. Chromatographia **2004**, *60* (7/8), 481–484.
248. Peng, S.X.; Henson, C.; Strojnowski, M.J.; Golebiowski, A.; Klopfenstein, S.R. Automated high-throughput liquid-liquid extraction for initial purification of combinatorial libraries. Anal. Chem. **2000**, *72* (2), 261–266.
249. Blanchard, A.J.; Alsante, K.M.; Nickerson, B.; Snyder, K.D. Extraction of low level impurities from tablets using accelerated solvent extraction. Abstr. 222nd ACS National Meeting, Chicago, IL, August 26–30, 2001.
250. Reddy, K.V.S.R.K.; Babu, J.M.; Kumar, P.A.; Chandrashekar, E.R.R.; Mathad, V.T.; Eswaraiah, S.; Reddy, M.S.; Vyas, K. Identification and

characterization of potential impurities of donepezil. J. Pharm. Biomed. Anal. **2004**, *35* (5), 1047–1058.

251. Cretier, G.; Neffati, J.; Rocca, J.L. Preparative LC and preparative SFC: two complementary techniques in the fractionation of an impurity from a major component. J. Chrom. Sci. **1994**, *32* (10), 449–454.
252. Tarkanyi, G. NMR spectroscopy. *Progress in Pharmaceutical and Biomedical Analysis, 4. Identification and Determination of Impurities in Drugs*; 2000, 562–574.
253. Lohr, L.L.; Sharp, T.R.; Alsante, K.M.; Hatajik, T.D. Isolation and identification of process related impurities and degradation products from pharmaceutical drug candidates: Part II. The roles of NMR and mass spectrometry. Amer. Pharm. Rev. **2001**, *4* (3), 104, 106, 108, 110, 112–113.
254. Wawer, I.; Pisklak, M.; Chilmonczyk, Z. 1H, 13C, 15 N NMR analysis of sildenafil base, citrate (Viagra) in solution, solid state and pharmaceutical dosage forms. J. Pharm. Biomed. Anal. **2005**, *38* (5), 865–870.
255. Burgueno-Tapia, E.; Mora-Perez, Y.; Morales-Rios, M.S.; Joseph-Nathan, P. 1H, 13C and 15 N NMR assignments of phenazopyridine derivatives. Magn. Reson. Chem. **2005**, *43* (3), 256–260.
256. Vogna, D.; Marotta, R. Advanced oxidation chemistry of paracetamol. UV/H2O2-induced hydroxylation/degradation pathways and 15 N-aided inventory of nitrogenous breakdown products. J. Org. Chem. **2002**, *67* (17), 6143–6151.
257. Navratilova, H. Enantiomeric analysis of (3S,4R)-4-(4-fluorophenyl)-3-hydroxymethyl-1-methylpiperidine by ^{19}F NMR spectroscopy. Magn. Reson. Chem. **2001**, *39* (11), 727–730.
258. Mistry, N.; Ismail, I.M.; Farrant, R.D.; Liu, M.; Nicholson, J.K.; Lindon, J.C. Impurity profiling in bulk pharmaceutical batches using 19F NMR spectroscopy and distinction between monomeric and dimeric impurities by NMR-based diffusion measurements. J. Pharm. Biomed. Anal. **1999**, *19* (3–4), 511–517.
259. Crowe, E.A.; Roberts, J.K.; Smith, R.J. 1H, 19F LC/NMR: application to the identification of impurities in compounds of pharmaceutical interest. Pharm. Sci. **1995**, *1* (2), 103–105.
260. Farrant, R.D.; Cupid, B.C.; Nicholson, J.K.; Lindon, J.C. Investigation of the feasibility of directly-coupled HPLC-NMR with 2H detection with application to the metabolism of N-dimethylformamide-d7. J. Pharm. Biomed. Anal. **1997**, *16* (1), 1–5.
261. Aso, Y.; Yoshioka, S.; Terao, T. Effect of the binding of water to excipients as measured by 2H-NMR relaxation time on cephalothin decomposition rate. Chem. Pharm. Bull. **1994**, *42* (2), 398–401.
262. Martino, R.; Gilard, V.; Desmoulin, F.; Malet-Martino, M. Fluorine-19 or phosphorus-31 NMR spectroscopy: A suitable analytical technique for quantitative in vitro metabolic studies of fluorinated or phosphorylated drugs. J. Pharm. Biomed. Anal. **2005**, *38* (5), 871–891.
263. Hurd, R.E. Gradient-enhanced spectroscopy. J. Magn. Reson. **1990**, *87* (2), 422–428.
264. Braunschweiler, L.; Ernst, R.R. Coherence transfer by isotropic mixing: application to proton correlation spectroscopy. J. Magn. Reson. **1983**, *53* (3), 521–528.
265. Jeener, J.; Meier, B.; Backmann, P.; Ernst, R.;. J. Chem. Phys. **1979**, *71*, 4546–4563.

266. Leeflang, B.R.; Kroon-Batenburg, L.M.J. CROSREL: full relaxation matrix analysis for NOESY and ROESY NMR spectroscopy. J. Biomol. NMR **1992**, *2* (5), 495–518.
267. Shoolery, J.N. Carbon-13 NMR spectral assignments simplified by APT (attached proton test) technique. Varian Inst. Applic. **1983**, *17* (1), 30.
268. Pegg, D.T.; Bendall, M.R. Two-dimensional DEPT NMR spectroscopy. J. Magn. Reson. **1983**, *55* (1), 114–127.
269. Rinaldi, P.L.; Baldwin, N.J. Selective excitation and sensitivity enhancement of proton resonances by polarization transfer from low gamma quadrupolar nuclei. J. Magn. Reson. **1985**, *61* (1), 165–167.
270. Zeng, B.; Pollack, R.M.; Summers, M.F. Conformational analysis via heteronuclear correlation NMR spectroscopy. J. Org. Chem. **1990**, *55* (8), 2534–2536.
271. Kessler, H.; Schmieder, P.; Koeck, M.; Kurz, M. Improved resolution in proton-detected heteronuclear long-range correlation. J. Magn. Reson. **1990**, *88* (3), 615–618.
272. Alsante, K.M.; Hatajik, T.D.; Lohr, L.L.; Santafianos, D.; Sharp, T.R. Solving impurity/degradation problems: case studies. In *Handbook of Isolation and Characterization of Impurities in Pharmaceuticals*; Ahuja, S., Alsante, K.M., (eds.), Academic Press: New York, 2003; 378.
273. Vasu Dev, R.; Moses Babu, J.; Vyas, K.; Sai Ram, P.; Ramachandra, P.; Sekhar, N.M.; Mohan Reddy, D.N.; Srinivasa Rao, N. Isolation and characterization of impurities in docetaxel. J. Pharm. Biomed. Anal. **2006**, *40* (3), 614–622.

Received November 20, 2006
Accepted November 30, 2006
Manuscript 6980K