PART 4

ASSESSMENT OF GENOTOXIC RISK: QUALITY PERSPECTIVE
CHAPTER 9

STRATEGIES FOR THE EVALUATION OF GENOTOXIC IMPURITY RISK

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9.1 INTRODUCTION

Since the publication of the European Medicines Evaluation Agency (EMEA) position paper in 2002 on limits for genotoxic impurities, it has been necessary for pharmaceutical companies to consider the potential risk posed by genotoxic impurities within their products. This has therefore driven the need to develop an effective strategy that both identifies and assesses the risk posed by any genotoxic impurity within the process used to manufacture the active pharmaceutical ingredient (API), and any subsequent change to the synthesis.

In order to synthesize APIs efficiently, it is necessary to build up the molecular structure through the combination of simple structural motifs. This typically involves the formation of carbon–carbon, carbon–nitrogen, and carbon–oxygen bonds. The current status of synthetic methodology is such that this is impractical to achieve without the use of electrophilic species that fall into the broad class of alkylating agents, and are hence potentially genotoxic.

Thus, many intrinsically reactive starting materials, intermediates, and reagents used in the synthesis of APIs are potentially genotoxic, and furthermore may present as residual impurities within the API. Although avoidance is generally considered to be the preferable option from a regulator’s perspective, there is tacit acceptance of the fact that this is generally impractical, and hence rather than avoidance, the issue becomes one of control.¹

Several organizations have published details of their approach,²–⁴ and these are discussed below; all are based on the same general principal.

- First, materials present in the synthesis are screened for potential genotoxicity, typically through the application of an appropriate structure activity relationship (SAR) process, using commercial systems such as DEREK® (Lhasa Limited, Andrew Teasdale  
Dave Elder  
Simon Fenner

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¹Genotoxic Impurities: Strategies for Identification and Control, Edited by Andrew Teasdale  
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Leeds, UK) and/or MCase® (Multicase Inc., Beachwood, OH) to predict for mutagenicity.

- Then, for materials flagging as potentially (PGI) or actually genotoxic (GI), this is followed by an evaluation of the likelihood of the material in question to carry through to the API, taking into consideration the properties of the compound in question, and the downstream process conditions.

This chapter describes this evaluation process in detail. A structured approach is defined based on the principals of quality by design (ICH Q8) and risk assessment (ICH Q9), providing an effective, robust process that identifies and addresses the risk posed by genotoxic impurities. This also examines the scope of such activities and the critical factors to consider when assessing risk. The relationship between analytical and safety testing, as well as the relative timing of such activities, is also considered.

The practical application of this process is then demonstrated in several case studies.

### 9.2 SCOPE

#### 9.2.1 General

Both the adopted EMEA guideline, and the draft FDA guideline, are stated to be only applicable to new products and those currently in clinical development. However, the principles of the assessment process are also equally applicable to the assessment of a marketed product, in circumstances where these guidelines are deemed to be retrospectively applicable.

#### 9.2.2 Impurities: API and Formulated Product

While it is likely that the scope of the assessment is focused on starting materials, raw materials, reagents, and intermediates within the synthetic process, the EMEA guideline also stipulates the need to take “probable” impurities and degradants into consideration. The guideline specifically defines the scope as probable impurities, and thus any evaluation of impurities should be framed in this context. It is impractical and unnecessary to attempt to evaluate every conceivable product derived from increasingly improbable side reactions. Further definition of “probable” is pertinent and will be explored in more detail in Section 9.3.1.

It is important to note that the guidelines also stipulate the need for consideration of any additional impurities that are observed in the formulated product.

#### 9.2.3 Degradants

Although the threat posed by genotoxic impurities originates principally from the synthetic process used for the manufacture of the API, genotoxic impurities can also be formed as a result of degradation in either the API and/or the drug product. It is thus important that the genotoxic impurity risk assessment takes into consideration information related to degradants gained during stability and/or degradation studies. This is examined in detail in Chapter 15.
9.2.4 Excipients

Established excipients are generally considered to be outside of the scope of the EMEA guideline. The principles behind this exclusion are similar to those that exclude existing marketing products, being based on safety data established through use within existing products. Furthermore, many excipients are also used in other applications, particularly food, and some are also listed on the FDA GRAS (generally regarded as safe) list. This area has been thoroughly reviewed by Brusick.

However, the guideline is considered applicable when considering novel excipients, and in such cases, the same assessment principles described in this document may also be applied.

It is recommended that compatibility is taken into consideration should there be any potential for reaction between an API and an excipient (see Chapter 15). In most instances where such incompatibilities arise, it is likely to lead to replacement of that particular excipient, or where unavoidable, a specific grade or form designed to minimize the interaction should be selected. This mirrors the approach employed during API development, in which a thorough understanding of the science is combined with a strategy to reduce or control the formation of the PGI material of concern. For example, an inerting atmosphere might be selected for the processing of liquid or semi-solid formulations, and/or addition of antioxidants made to prevent or limit oxidatively-mediated degradation processes. Similarly, the use of low moisture excipients, low moisture processing (e.g. direct compression or dry granulation), and highly protective container closures (e.g. cold-form aluminium blisters) would be recommended to preclude moisture-mediated hydrolysis processes. As with API synthetic routes, the rejection of the formulation in question and the development of an alternative one is typically the least favored option.

9.2.5 Metabolites

Neither the EMEA guideline nor the draft FDA guideline makes specific reference to potentially genotoxic metabolites of APIs. Separate guidance is available covering the approach to take for significant metabolites.

However, there may be occasions where a process intermediate, impurity, or degradant may also be a human metabolite. In such cases, the overall risk assessment should take into account total exposure, that is, to both the process related material and metabolite. The evaluation of genotoxic risk associated with metabolites is discussed in detail in Chapter 6.

9.2.6 Quality Risk Management Process

The need for quality risk management of pharmaceutical processes and procedures has been fully discussed within ICH Q9. Risk management is based on an evaluation of two key factors:

- the probability or likelihood of occurrence; and
- the severity or impact of the resulting outcome.
It is a systematic process that evaluates:

• what can go wrong;
• what is the likelihood of it going wrong;
• what are the consequences; and
• what can be done to either eliminate or control the risk.

A further factor in risk evaluation is the level of effort taken to evaluate the risk. This should be directly related to the level of the risk, as should the efforts taken to eliminate or reduce it.

In the context of an assessment of risk posed by genotoxic impurities, the threshold of toxicological concern (TTC) effectively establishes a limit in terms of defining “acceptable risk.” The requirement is therefore to assess the likelihood of a genotoxic impurity exceeding this threshold in API/DP, and where a significant likelihood is identified, to provide adequate assurance of its effective control. Such a process is described below.

A key aspect of the process is its multidisplinary nature. For such a process to be efficient, it must necessarily draw on the collective skills of personnel from disciplines, such as chemistry, toxicology, formulation, and analytical.

### 9.3 GI RISK ASSESSMENT PROCESS

The process begins with the evaluation of the synthetic route for postulated and/or known impurities. This is followed by structural assessment of agreed “probable” impurities, along with other route materials and reagents where appropriate, hazard classification, quantification and/or safety testing, risk assessment and finally establishment of control measures where required.

The evaluation process is represented schematically in Figure 9.1.

The process should be flexible; each API/DP synthesis has its own distinctive features, and, where appropriate, the ordering of the steps described may be changed; however, the overall process should generally remain the same.

There is a clear link between the assessment of risk and the permitted level for a genotoxic impurity. Any such evaluation should therefore take into account the phase of development, the intended dose and likely clinical trial study duration. Permissible limits are based on the “staged TTC” principle. Limits cited in the EMEA Q&A Supplement\textsuperscript{12} to the main guideline are given in Table 9.1.

The EMEA guideline also states that values higher than the TTC may be acceptable under certain conditions, including short-term exposure, for treatment of a life-threatening condition, when life expectancy is less than 5 years or when there is greater exposure from other sources such as food.

It is recommended that a permitted limit, for example staged TTC, is established in advance of instigating the formal evaluation, with the caveat that this limit will change dependant on both time (duration of clinical phase) and dose (absolute level of exposure).
Identification of Potential Impurities in Drug Substance and Drug Product –
Review the synthetic process – including starting materials / reagents / intermediates / known impurities + drug substance and product degradants

**Step 1**
Conduct SAR evaluation (DEREK / Mcase)

**Step 2**

**Step 3**
Structural Alert?

No
- Classified as non-genotoxic – treat as a general impurity

Yes

**Step 4**
Assessment of Risk of Potential Carry over of Impurities – Evaluate risk of carryover at levels of concern into DS/DP – Does the impurity pose any significant risk of carryover?

No
- No further action

Yes

**Step 5**
Quantification
- Analyse level of impurity

OR

Safety Testing
- Perform appropriate genotoxicity test
  - Typically Ames test

**Step 6**
Finalise Risk assessment
- Is the impurity genotoxic? Is the level >TTC?

Non-genotoxic
- Treat as a general impurity

Genotoxic / level < TTC
- Suitable for clinical use

Genotoxic / level > TTC

**Step 7**
Define strategy to achieve acceptable limits
Options:
1/ Modification of synthetic process.
2/ Additional genotoxicity testing (typically in vivo)

Figure 9.1 GI risk assessment—Process flow diagram.
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9.3.1 Step 1: Evaluation of Drug Substance and Drug Product Processes for Sources of Potentially Genotoxic Impurities

The responsibility for this step is likely to fall to the chemists and analysts responsible for the design and development of the API synthetic process, with additional input from pharmaceutical development groups who can comment on issues arising from stability and degradation studies, as well as excipient compatibility.

An evaluation of the synthetic route, focused on starting materials, intermediates, reagents, catalysts and solvents is carried out to identify materials which could possibly survive the process and present in the API as impurities. It should also include consideration of other potential impurities that may arise from the synthetic route, particularly in the final stages. These could include related substances of the API or intermediates, through to materials derived from interactions between reagents and solvents. However, care should be exercised when considering the scope of impurities to be included in the assessment. The EMEA guideline contains the following advice:

As stated in the Q3A guideline, actual and potential impurities most likely to arise during the synthesis, purification and storage of the new drug substance should be identified, based on sound scientific appraisal of the chemical reactions involved in synthesis, impurities associated with raw materials that could contribute to the impurity profile of the new drug substance, and possible degradation products. This discussion can be limited to those impurities that might reasonably be expected based on scientific knowledge of the chemical reactions and conditions involved.

Such impurities could include, for example, a regioisomer of an alerting intermediate that does not react as per the main component in a cyclization reaction, leaving the regioisomer to potentially pass unreacted into the API. Other examples

<table>
<thead>
<tr>
<th>TABLE 9.1 Adopted Allowable Daily Intakes (µg/day) for GIs during Clinical Development, a Staged TTC Approach Depending on Duration of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>ADI * (µg/day)</td>
</tr>
<tr>
<td>for different duration of exposure</td>
</tr>
<tr>
<td>normally used in clinical development</td>
</tr>
</tbody>
</table>

<sup>a</sup> Probability of not exceeding a 10<sup>−6</sup> risk is 93%.

<sup>b</sup> Probability of not exceeding a 10<sup>−5</sup> risk is 93%, which considers a 70-year exposure.

<sup>c</sup> Other limits (higher or lower) may be appropriate and the approaches used to identify, qualify and control ordinary impurities during development should be applied.

ADI, allowable daily intake.
include side reactions between sulfonic acids and alcohols to yield sulfonate esters (although such reactions can be very effectively controlled—see Chapter 14).

Potentially genotoxic impurities that might be present in API generally fall into the following categories:

- Unreacted contributory materials or intermediates with alerting substructures that have survived processing (e.g. unreacted nitroaromatic in a nitrogen heterocycle API, due to incomplete hydrogenation or a positional isomer unable to cyclize).
- Substances closely related to contributory materials, intermediates, or the API itself that contain an alerting structural motif (e.g. a chloroalkyl analogue of a hydroxyalkyl containing API following treatment with hydrochloric acid during processing).
- Unrelated substances formed by combinations of solvents and reagents with each other or with contributory materials or intermediates (e.g. isopropyl tosylate formed as a result of isopropanol damp hydroxylated intermediate being used in a reaction with tosyl chloride).

This is fully aligned with the tenets of ICH Q9, which focuses on the probability of an event occurring, combined with an evaluation of the impact of the event occurring, leading to a consideration of the risk posed. The magnitude of the risk is therefore related to the probability of the PGI being present. Hence, the greatest risk is posed by those agents used in the late stages of the API synthesis that possess well-established alerting structural motifs, and these should be the main focus of the evaluation.

At an appropriate point in the development of an API, the risk assessment should also include consideration of materials arising from degradation during manufacture or on long term storage of the API or its formulated product. This review may be based on a combination of factors, including scientific knowledge and in silico predictions of the typical degradation pathways of the API and formulated product based on the chemical structure and literature precedent. The conduct of such assessments is described in detail in Chapter 15.

Another factor for consideration when defining the scope of the evaluation is the level of an impurity and the extent of characterization. It was confirmed through the EMEA Q&A supplement to the main guideline that no action is required for any unidentified impurity below the ICH identification threshold. Hence, there is no requirement to identify and assess every impurity observed within the API and the resulting formulated product, the threshold for identification being defined by ICH Q3A/B.

Having agreed a list of materials, which might comprise structurally contributing raw materials, intermediates, known process impurities, other probable process impurities, as well as significant degradation products, these should then be subjected to a formal structural assessment for genotoxicity.

9.3.2 Step 2: Structural Assessment

Both EMEA and FDA draft guidelines recognize the use of structural assessment as a valid means by which an assessment of genotoxic potential can be made. The use of in silico systems is generally recommended; the most commonly applied are the
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Structural Alerts for Mutagenicity

Group 1: Aromatic Groups

- N-Hydroxyaryls
- N-Acylated aminoaaryls
- Aza-aryl N-oxides
- Aminoaryls and alkylated aminoaaryls

Group 2: Alkyl and Aryl Groups

- Aldehydes
- N-Methylols
- N-Nitrosoamines
- Nitro Compounds
- Carbamates (Urethanes)

Group 3: Heteroatomic Groups

- Epoxides
- Aziridines
- Michael-reactive Acceptors
- Alkyl Esters of Phosphonates or Sulfonates
- Halo-alkenes
- Primary Halides (Alkyl and aryl-CH₂)

Legend: A = Alkyl, Aryl, or H
Halogen = F, Cl, Br, I
EWG = Electron withdrawing group (CN, C=O, ester, etc)

Figure 9.2 Structural alerts.

commercial packages DEREK® and MCase®; often used in tandem, these are described in more detail in Chapter 4. Evaluations are primarily focused on mutagenicity (Ames positive) due to this being recognized as the most appropriate indicator for direct interaction with DNA.

Smaller organizations may find these systems to be prohibitively expensive, in which case a simple system based on Ashby and Tennant alerts¹⁵ (see Fig. 9.2) is available.

In addition to using commercial databases, some organizations have developed their own in silico systems utilizing in-house data that allow for further refinement of predictions.
9.3.3 Step 3: Classification

Once a structural assessment has been completed, each impurity should be categorized according to its genotoxic hazard. The five-class classification scheme, defined by Mueller et al., has been widely adopted for this purpose, and this is shown in Table 9.2.

It is important to be aware that the SAR evaluation procedures can only be as good as the databases and rule sets that underpin the systems. It is known that there are deficiencies in the models for some compound classes, for example those relating to anilines and heteroaromatic amines. It may be advisable to treat these cases individually, with the option to consider safety testing (Ames test) where this is deemed necessary.

Although in silico systems are comprehensive in terms of the compound classes covered, there are nevertheless examples of classes that are not covered and for which there is no closely related data in the underlying database. This point was made by Dobo et al. in respect of heteroaromatic nitro compounds. Hence, it is important for the recipients of the SAR output to scrutinize the findings. If an impurity has no flags for genotoxicity, but is used in the process as an electrophile, then it would be prudent to seek expert judgment with respect to the strength of the underlying dataset.

Evaluation of genotoxic risk can also be augmented by data derived from within the public domain. Included within these is TOXNET, a searchable database provided by the U.S. Library of Medicine. This provides access to a series of databases through a common portal:

- HSDB: Hazardous Substances Databank;
- CCRIS: Chemical Carcinogenesis Research Information System; and
- IRIS: Integrated Risk Information System)

that provide an excellent source of safety data for many common chemicals. Another related system is the Berkeley database. Indeed, as described in Chapter 5, it is often

<table>
<thead>
<tr>
<th>Classification</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Class 1</td>
<td>Known genotoxic carcinogens, based on evidence of carcinogenicity derived from animal/human carcinogenicity studies</td>
</tr>
<tr>
<td>Class 2</td>
<td>Impurities known to be genotoxic (mutagenic) with unknown carcinogenic potential. Genotoxic potential typically identified by a positive response in an Ames test.</td>
</tr>
<tr>
<td>Class 3</td>
<td>Impurities judged to be alerting for genotoxicity by SAR analysis, but for which no data on genotoxic potential exist.</td>
</tr>
<tr>
<td>Class 4</td>
<td>Impurities judged to be alerting for genotoxicity by SAR analysis, but for which the alert in question is common to one also present in the parent API. Provided that the API has been adequately assessed for mutagenicity and shown to negative, such impurities can be considered qualified and controlled as a normal impurity</td>
</tr>
<tr>
<td>Class 5</td>
<td>Impurities for which there is no identified structural alerts and no additional data to suggest the existence of any mutagenic potential. Such impurities are controlled as normal impurities</td>
</tr>
</tbody>
</table>
possible with common reagents to locate sufficient safety data to allow genotoxic risk to be assessed on a compound specific basis rather than simply applying the TTC. This is defined as an allowable approach within the recent FDA guideline.  

9.3.4 Step 4: Assessment of Risk of Potential Carry over of Impurities

Once impurities with a potential genotoxic safety concern have been identified by the SAR evaluation process, the next step is to consider the likelihood of a particular impurity being present in the isolated API, often referred to as impurity fate mapping.

The impurities under consideration are by nature often highly reactive, hence their removal during downstream processing is facilitated by this intrinsic activity. Acidic and/or basic work-up conditions frequently encountered in manufacturing processes may lead to decomposition and/or removal of the material of concern. Similarly, other reagents used in downstream processing may react with the material, rendering it less reactive and thereby “safe.” Factors that contribute to removal of such impurities are reviewed in depth in the following section.

Such impurity fate assessments have largely been based on the theoretical knowledge and experience of the evaluating chemist. Unfortunately, however compelling the arguments developed, they have often been viewed as nonquantitative from a regulatory perspective. Thus, in many cases, there is a need to provide further analytical data to substantiate the impurity fate assessment. Hence, a quality by testing (QbT) approach is adopted rather than a quality by design (QbD) approach.

There would be considerable value from an industry and regulatory perspective in defining a standardized approach to such assessments. It should be possible to assess fate at least semi-quantitatively based on factors linked to the impurity’s physicochemical properties (and taking in to account those of the API and intermediates), and the process conditions employed in the route of manufacture to the API. Pierson et al. have suggested that an assumption could be made of a 10-fold reduction per synthetic stage. In many cases, this would suffice and indeed may even be a cautious estimate of the risk. However, in certain circumstances, for example an unreactive genotoxic reagent or intermediate used in a “telescoped” process (no isolations between stages), this may be too simplistic. For this reason, a more quantitative approach, based on actual process conditions and the physicochemical properties of the genotoxic impurity in question, has been sought, and is outlined below.

A number of contributory factors have been defined that should be taken into account for such an assessment; these are described below.

9.3.4.1 Reactivity  As already described, many of the PGIs/GIs that are likely to be of concern are intrinsically reactive. For example, acyl halides are compounds that are so reactive that there would be little practical value in monitoring their presence in the outcome of the reaction. Moreover, should there be any residual analyte; this would be effectively eliminated through procedures such as an aqueous quench or even a simple water wash of the resulting product.

Even in processes where there is a likelihood that some residue may remain in an intermediate, for many such compounds, there is a high probability of reaction
in a subsequent process stage. Consider, for example, an alkyl halide used early in a synthesis; should a further alkylating agent be used downstream in the process, any residual quantities of the initial alkyl halide are highly likely to be consumed in the latter stage (see Fig. 9.3). In this process, any residual allyl bromide remaining after stage 1 may carry through into stage 3 where, if still present, it would be expected to react to produce the allyl analogue of the desired product.

On the basis of chemical reactivity, it is proposed that genotoxic compounds could be placed into one of three categories (see Table 9.3).
9.3.4.2 Solubility—Isolated Stages  Many of the reagents/intermediates that are highly electrophilic (and hence often genotoxic) are introduced into the synthetic process at those stages specifically designed to optimize the yield and product quality. A critical factor in most processes is that reactants are physically able to react with one another; in practical terms, this is best achieved by the reactants being in solution. By inference, this means that the genotoxic reactant in question is likely to be highly soluble in the reaction solution selected for the process. Thus, should the process concerned result in the isolation of the product as a solid, then the genotoxic reactant should remain in the reaction mother liquors and thus be removed, provided the deliquoring process is efficient. This can be augmented further by washing the cake with a solvent in which the genotoxic reagent is freely soluble and the product is not. Often isolation may involve some form of solvent replacement; where this is the case, the solubility of the genotoxic reagent should be evaluated in the replacement solvent in order to define the appropriate purge factor.

9.3.4.3 Volatility  A number of the genotoxic materials likely to be encountered within a typical synthetic process are volatile, including low molecular weight alkyl halides, aldehydes, and nitrogen or sulphur haloethyl “mustards.” Distillation is frequently used to lower or completely remove the volume of reaction solvent present, and this can be effective in reducing or eliminating any residual genotoxic material, dependant on the volatility of the genotoxin relative to the boiling point of the solvent.

9.3.4.4 Ionizability  Aromatic amines are perhaps the most obvious example of potentially genotoxic materials that contain an ionizable group. The majority of APIs and some intermediates will be potentially ionizable. Where this is the case, and there is a suitable difference in the ionisability of the genotoxin of concern and the matrix in which it is potentially present, it should be possible to reduce the level of the former by manipulation of the pH of the aqueous phase and extraction into an organic solvent.

Reduction of a nitro compound to an amine is an example where such a process would be very effective. Although they generally possess a common genotoxic metabolic intermediate (a nitrenium ion), the nitro compound in question may be found to genotoxic, whereas its amine analogue may not. In such circumstances, the removal of the nitro precursor is desired, particularly if the nitro compound

### TABLE 9.3 Genotoxic Compounds classified based on Reactivity

<table>
<thead>
<tr>
<th>Reactivity class</th>
<th>Genotoxic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly reactive(^a)</td>
<td>Epoxides/aldehydes/sulfonate esters/acyl halides/aziridines/hydrazines</td>
</tr>
<tr>
<td>Moderate reactivity</td>
<td>N or S mustards/Michael reactive acceptors/halo-alkenes, primary halides</td>
</tr>
<tr>
<td>Low reactivity</td>
<td>Amino aryls, nitro compounds, purines or pyrimidines, carbamates</td>
</tr>
</tbody>
</table>

\(^a\) Susceptible to attack by a wide range of potential nucleophiles.

NB: This table is intended to be used simply as a guideline. Chemical reactivity should be evaluated on a case-by-case basis for each process.
concerned is unreactive in the downstream process, and there is the potential for it to carry through the process in its original form. Where there is a need to remove excess nitro compound, this can be achieved very effectively through a liquid/liquid extraction by employing a two-phase system with an acidic aqueous layer. Any nonionizable nitro compound will reside in the organic layer, whereas the ionized amine will reside in the aqueous layer. The organic layer would then be discarded, and amine then simply back extracted in a new organic solvent layer following basification.

In the synthesis of the common painkiller paracetamol, the penultimate stage involves the reduction of 4-nitrophenol to the corresponding amine. The reaction mixtures are extracted with toluene, basified, and the amine is extracted.

In addition to classical liquid/liquid extractions, solid-phase extraction (SPE) can also be employed. By exploiting both the physical properties (ionisability) and chemical properties (polarity), this technique may confer an advantage over liquid/liquid extraction. The variety of stationary phases available, which can separate analytes according to different chemical properties, is another favorable characteristic of SPE. Most stationary phases are based on silica, and increasingly, this has been modified by attachment of a specific functional group. Modifying functional groups include:

- hydrocarbon chains of varying length (for reversed phase SPE);
- quaternary ammonium or amino groups (for anion exchange); and
- sulfonic acid or carboxyl groups (for cation exchange).

The main drawback of solid phase extraction has been its practicality at a manufacturing scale.

9.3.4.5 Chromatography  The technique of chromatography offers a range of options to remove or reduce a potential genotoxic impurity from API or an intermediate. Techniques range from simple “filtration” through a silica bed to preparative liquid chromatography.

Preparative chromatography is typically performed in normal phase mode, that is the use of a polar (typically silica) stationary phase and a nonpolar mobile phase (organic solvent system). The reason for the use of normal phase mode (most analytical chromatography is now performed in reverse phase mode) is due to the practical need to isolate the compound in question—normal phase using volatile solvents that are easily removed. Reverse phase can be used, but the difficulty in removing aqueous-based solvent systems limits the applicability, although it is possible to employ freeze drying as a means of removal. Another potential alternative is Super-critical fluid chromatography (SFC); this has the advantage of a readily removable eluent (CO$_2$).

Preparative HPLC is a now a standard technique within the pharmaceutical industry for the reduction or removal of impurities, with multi-kilo capability present in the larger companies, as well as a range of contract manufacturers. Improvements in the quality and range of stationary phases, as well as the supporting hardware, have greatly increased the scope of this technique, and there are few separations that cannot be achieved in this fashion.
The removal of a GI can be considered a subset of the standard chromatographic challenge of impurity removal, and typically will be approached in the same way. Additional considerations for GIs may exist, for example, “what is the stability of the PGI?” or “is there any risk of producing additional PGIs?” from the systems being considered. Indeed, with respect to the first consideration, Welch et al.\textsuperscript{19} (Merck USA) have published work on the removal of an unwanted oxime via its high reactivity to a packing material; the resin/packing material can simply be stirred with the reaction solution, or cycled though a preparative column.

Bandichhor et al.\textsuperscript{20} reported the purification of rizatriptan, a serotonin 5-HT receptor agonist. A genotoxic dimer impurity generated in the synthesis could not be removed to an acceptable limit of mass fraction 0.01% by conventional processes, such as fractional crystallization and recrystallization. A reverse phase method was developed using careful pH and ionic strength modification to increase the selectivity between the rizatriptan and the genotoxic dimer. The genotoxic dimer was strongly retained on the column, and the loadability optimized to give maximum productivity without any appreciable breakthrough of the genotoxic dimer into the product. Here, the retention of the rizatriptan was kept to a minimum, and the genotoxic dimer washed off between injections. The authors reported a decrease in the level of the genotoxic dimer from ca. 40,000 ppm to 40–80 ppm (yield $>95\%$). Full details of the method are available in the paper.

\subsection*{9.3.4.6 Recrystallization} Perhaps one of the most effective ways in which to remove impurities from API or intermediates, including those that are potentially genotoxic, is recrystallization. This involves selecting a solvent in which the API or intermediate is highly soluble when hot and virtually insoluble when cold. The impure API or intermediate is dissolved in the smallest practical volume of the solvent at an elevated temperature. The hot solution is then typically filtered to remove any impurities insoluble in the hot solvent for clinically destined API as a GMP against extraneous solid contamination. The filtered solution is then allowed to cool under carefully controlled conditions until the product crystallizes out of the cooling solvent. Impurities that are more soluble in the cold solvent remain in solution. The product is then isolated by filtration, leaving impurities in the filtrate (mother liquors).

The process can be further refined through the introduction of seed crystals (previously isolated product material); although typically used to determine or modify a specific property, for example morphic form, it can help to improve the selectivity of the recrystallization.

\subsection*{9.3.4.7 Other Techniques} As well as the techniques described above, there are a variety of other “niche” techniques that can be applied. Two examples are activated charcoal and resins.

\textit{Activated Charcoal} Activated charcoal is used in a variety of industries, including the water industry and alcoholic beverage (e.g. vodka, rum) industry to remove a range of impurities. Activated charcoal is, however, a complex material in terms of its physicochemical properties, and the effect of charcoal is very difficult to predict. In some circumstances, and often in combination with a recrystallization process, it can prove to be very effective in removing certain species, particularly colored impurities.
**Scavenger Resins**  Polymer scavengers are functionalized polymers that are designed to react with and bind reagents and by-products. The concept is analogous to that of other extraction and partitioning techniques, the genotoxic impurity of interest binds to the resin and can thus be removed by filtration as the desired product remains in solution. Such resins have found widespread applicability in the combinatorial chemistry arena.

Some of the types of resin available and their potential applicability are described in Table 9.4.

<table>
<thead>
<tr>
<th>Scavenger (functional group)</th>
<th>Structure</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzaldehyde</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges nucleophiles including primary amines, hydrazines</td>
</tr>
<tr>
<td>Isocyanate</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges nucleophiles including amines</td>
</tr>
<tr>
<td>Amine</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges acid chlorides, sulfonyl chlorides and miscellaneous electrophiles</td>
</tr>
<tr>
<td>Thiophenol</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges alkylating agents e.g. alkyl halides</td>
</tr>
<tr>
<td>Trisamine</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges acid chlorides, sulfonyl chlorides and miscellaneous electrophiles</td>
</tr>
<tr>
<td>Hydrazide</td>
<td><img src="image" alt="Structure" /></td>
<td>Scavenges Aldehydes</td>
</tr>
</tbody>
</table>
A recent publication examined the potential use of such resins to remove sulfonate esters. The successful removal of methyl sulfonate esters was reported; however, related ethyl and isopropyl esters were only partially removed. Nevertheless, the authors concluded that the use of such resins showed some potential, and suggested that this could be extended to other classes of genotoxins, for example alkyl halides.

A drawback to date of such polymer-based resins has been their stability in aggressive organic solvents such as tetrahydrofuran (THF). Leaching of the monomer has been observed, hence a procedure to remove potentially genotoxic impurities may lead to the potential contamination of the product with another material. Depending on the nature of the monomer in question, this could introduce a bigger problem, and this factor has largely precluded the use of such resins in large-scale synthetic processes.

9.3.4.8 Overall Quantification of Risk  In order to make a quantitative assessment of the level of carryover of a particular material into an API or downstream intermediate, a number of mitigating criteria have been selected and are defined in Table 9.5. For each mitigating criteria, a purge factor can then be selected according to the characteristics of the material under consideration. The numerical scale has been developed to link individual process steps to the physicochemical properties of the individual impurity in question. Each factor is scored (high–low) in terms of its ability to purge the impurity, thus the higher the score, the greater the likelihood that the impurity would be purged from the process.

Hence, if a material is identified three steps from API, given the characteristics of the material concerned, and the nature of the three downstream processing stages, an overall purge factor can be assigned by multiplying the purge factors arising from

<table>
<thead>
<tr>
<th>Nature of Process</th>
<th>Purge factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactivity—compound class</td>
<td>Highly reactive = 100</td>
</tr>
<tr>
<td></td>
<td>Moderate reactivity = 10</td>
</tr>
<tr>
<td></td>
<td>Low reactivity = 1</td>
</tr>
<tr>
<td>Solubility</td>
<td>Freely soluble = 10</td>
</tr>
<tr>
<td></td>
<td>Moderately soluble = 3</td>
</tr>
<tr>
<td></td>
<td>Sparingly soluble/insoluble = 1</td>
</tr>
<tr>
<td>Volatility</td>
<td>Boiling point &gt;20°C below that of solvent = 10</td>
</tr>
<tr>
<td></td>
<td>Boiling point +/- 10°C of solvent = 3</td>
</tr>
<tr>
<td></td>
<td>Boiling point &gt;20°C of solvent or in-volatile = 1</td>
</tr>
<tr>
<td>Ionisability</td>
<td>Ionization potential of GI significantly different to API/matrix = 10</td>
</tr>
<tr>
<td>Chromatography</td>
<td>Chromatography—GI elutes prior to API = 100</td>
</tr>
<tr>
<td></td>
<td>Chromatography—GI elutes after API = 10</td>
</tr>
<tr>
<td></td>
<td>Others—evaluated on an individual basis</td>
</tr>
<tr>
<td>Recrystallization</td>
<td>Factor = 10</td>
</tr>
<tr>
<td>Others, e.g. resins</td>
<td>Evaluated on a individual basis</td>
</tr>
</tbody>
</table>
each separate stage. Based on this value, a decision can be made as to what if any further action may be required.

Where a purge factor of greater than 10,000 is assigned, the likelihood of the genotoxic impurity in question being present in API at ppm levels is very low. Unless the daily API dose were 1 gram per day or greater, this theoretical evaluation alone should provide sufficient justification to take no further action, with no specific controls considered necessary.

For purge factors between 100 and 10,000, additional verification of the purge capabilities of the process should be considered. This would typically take the form of spiking studies.

If a purge factor less than 100 is assigned, formal process controls should be considered in addition to spiking studies. This will typically reflect the scenario where the genotoxic impurity in question is introduced or formed in the final or penultimate bond forming step.

The use of a semi-quantitative assessment process such as that described would shift the focus of effort to those genotoxic impurities with a high likelihood of being present in API, and hence could pose a significant risk to patient safety.

A number of case studies that examine the utility of such an approach are outlined below in Section 9.6.

**9.3.5 Step 5: Further Evaluation**

Having compiled an initial list of potential impurities, retained those that are known or suspected genotoxins, and evaluated which of these is likely to be present in API based on the material characteristics, origin in the process, and ability to survive the process intact, a shortlist of remaining materials is produced.

There are now two ways by which the risk of such potential genotoxic impurities may be mitigated.

1. **Safety testing**: Demonstrating that a material is nongenotoxic will allow it to be addressed under ICH Q3A/B.

2. **Analytical testing**: Demonstrating that a material is below the permitted TTC or staged TTC limit.

Which approach to take will depend on the unique nature of the project and the impurity concerned, and may be influenced by factors such as the availability of pure samples of the material of concern, and/or availability of appropriate analytical methodology with which to determine levels.

**9.3.5.1 Safety Testing** For any impurity identified as being potentially genotoxic (based on SAR evaluation) and assessed as having a high likelihood of carry-over into the API, the next step is often to carry out *in vitro* safety testing.

If *in vitro* testing is selected, it is recommended that the synthesized or isolated impurity is tested for mutagenicity as an individual impurity. However, where this is impractical, then spiked samples or batches of material that contain elevated levels of the impurity of concern may be tested. The latter approach is not generally encouraged by regulatory authorities, and in such cases, an early dialogue with the relevant regulatory authority is recommended.
It is generally accepted that a single point bacterial mutation assay, such as the Ames test, has the necessary sensitivity and specificity to detect nonthresholded genotoxic chemicals.

However, some structural groups, such as carbamates, are known to be inefficiently detected in bacterial genotoxicity tests. In such situations, a mammalian cell assay (e.g. in vitro mouse lymphoma TK assay to detect chromosomal aberrations or chromosome damage in cultured human lymphocytes) should be considered. Such testing is examined in detail in Chapter 3.

A positive result in one or more of these tests is generally sufficient evidence to define the impurity as genotoxic, in which case it will be then necessary to adopt the appropriate TTC approach. Occasionally, a thresholded mechanism can be argued based on available safety data. If an impurity is found to be negative, it is considered nongenotoxic (qualified for genotoxicity), and can then be treated as a normal impurity under ICH Q3A/B.13,14

The genotoxic potential of in vitro positive materials may be further evaluated in vivo in order to establish the biological relevance of the in vitro findings; this is examined in detail in Chapter 3.

9.3.5.2 Quantification of Level Present For potential GIs that have been assessed as having a reasonable likelihood of being present in API at levels of concern, it may be appropriate to attempt to determine the level, in parallel with, or instead of the safety testing described above. The level of concern will be set by the staged TTC for the phase of development, which is related to the maximum clinical dose and the maximum duration of the proposed trial(s). This in turn will have an effect on the choice of analytical technique.

- Choice of technique?: The nature of the impurity (analyte), the characteristics of the API or intermediate (matrix) and the level to be determined will influence the detection technique employed. Many organizations have developed specific strategies for refining such selections, and an example strategy is presented in Chapter 11.

- Where in the process to test?: Testing may be performed on upstream intermediates, API, or drug product as appropriate. It is often desirable to test as close as possible to the point of introduction of a GI/PGI into the process. This approach may permit standard techniques such as HPLC with UV detection to be used, if this is allied to spiking experiments demonstrating the removal in the downstream process. While development laboratories may be equipped with more sensitive techniques suitable for analysis at the low ppm level, manufacturing quality control laboratories are unlikely to have such facilities. In addition, there would be resistance to outsourcing these more esoteric analyses, since the goal of the modern quality control laboratory is a rapid turnaround of a minimum set of controls.

- Quantitative assay or limit test: Both types of methods are used in the analysis of genotoxic impurities. Quantitative tests are useful to furnish data for process development and to support further process modifications to reduce or more
consistently control levels of a PGI. Having established a validated process, limit tests are likely to be favored for routine QC testing.

Limit tests are also more likely to be applied to upstream testing at an intermediate stage, where they are used in conjunction with demonstrated evidence of further reduction through processing.

Quantitative assays are usually applied at the final isolated API, as they provide a measure of true levels of the PGI/GI that would be administered in the drug product. Since the staged TTC concept for acceptable levels of PGIs/GIs is routinely applied during clinical stages of development, a quantitative test is generally desirable since acceptable levels vary as the clinical program develops. However, limit tests may be appropriate at the API or DP stage if this figure is well below the staged TTC control level.

9.3.6 Step 6: Overall Risk Assessment

Once analytical and/or safety test data are available, these are used to finalise the risk assessment.

Possible outcomes include;

- A PGI returns a negative Ames test result and thus no longer requires control as a genotoxic impurity, but defaults to ICH Q3 levels of control.\(^\text{13,14}\)
- A PGI returns a positive Ames test result, but analytical testing demonstrates adequate process control over levels, that is level well below appropriate TTC limit.
- Analytical data demonstrates that a PGI/GI is below a current staged TTC, but above future dose duration levels. In such circumstances, this may necessitate a modification of the process to reduce or eliminate the impurity in question. If the material is potentially genotoxic rather than a known genotoxin, the expedient of safety testing, with the possibility of a negative Ames test result, would remove the need for further process development and analytical control at trace levels.
- Analytical and safety data reveal an Ames positive material above a staged TTC level for a planned clinical study.\(^*\) In such a scenario, it is likely that the material in question would need to be reprocessed, unless a compelling case could be made for the benefit of the treatment over the risk posed (see ICH S9\(^2\) for example). In most cases, the process would need to be redeveloped to bring levels of the genotoxin in question within the TTC for the envisaged marketed product dose and duration.

This is not meant to be an exhaustive list, but serves to illustrate some of the potential outcomes and likely courses of action in each case.

\(^*\) Should it be discovered that the level of a GI is above permitted levels in material currently used in clinical trials, then this may lead to suspension of the trial and expedited reporting under 15-day rules to regulatory authorities.
It should be recognized that the evaluation of genotoxic risk is an iterative process, and needs to be updated in line with any process related changes and/or emerging information relating to impurities and/or degradants in drug substance (or drug product). Other factors such as a change in the trial duration, trial population, and/or dose may also require a review of the risk assessment.

### 9.3.7 Further Evaluation of Risk–Purge (Spiking) Studies

Alongside the theoretical evaluation of risk described above, there is often the need to examine this experimentally through the conduct of appropriate purging or spiking experiments. This is most likely to be required where a moderate to high risk of potential carryover into the API has been defined. Spiking refers to the practice of adding in a fixed quantity, or spike, of the material to be followed in order to have a quantifiable baseline. Purging refers to the aim of defining the extent to which the material in question is purged out of the downstream material, or API.

Pierson et al. reported on a generic approach to the assessment, testing strategies and analytical assessments of genotoxic impurities in API, encompassing the use of spiking experiments. Their approach was influenced by the point in the synthetic process at which the potential genotoxic impurity was introduced or identified. Introduction or identification of a source of a GI in the final stage of the API was defined as the worst-case scenario, and in most cases, such an outcome would necessitate the introduction of specifications and analysis. However, they stated that omission of routine controls could be justified if supported by purge studies where these conclusively demonstrated absence of the material of concern from the API.

If a potentially genotoxic impurity was introduced in the penultimate step, but was shown to be below the proposed TTC limit for the final API, they also proposed that there should be no further action. This was demonstrated with reference to a recent project in which an alkylating agent (substituted benzyl bromide) was formed in the penultimate step, and a limit of 20 ppm was assigned (based on the TTC, taking account of the dose). Testing of the isolated final intermediate revealed levels of only 2 ppm; hence, it was concluded that there was no need for a specification or testing of the API.

The authors also proposed that where the potential genotoxic impurity was introduced three to four stages upstream from the API, then a risk assessment based on chemistry rationale and/or spike/purge experiments would be required to evaluate the ability of the downstream process to remove or reduce the impurity to acceptable levels. If these evaluations indicated that carry through to API was likely, then controls in the API would be required.

Finally, if the potential genotoxic impurity was introduced greater than three to four stages upstream from the API, then risk assessment based on chemistry rationale alone was typically required.

The use of purge studies was further examined by Liu et al. who reported on the analytical control strategy for five potential genotoxic impurities in a novel oncology product, pazopanib hydrochloride (see Fig. 9.4—GIs highlighted as boxed items). They described the approach in the terms of quality by design (QbD), with levels of genotoxic impurities being thought of as critical quality attributes (CQAs).
Each of the impurities in question was spiked at elevated levels (as high as 5% in some cases) into the process. Impurity fate mapping was then used to demonstrate the serial reductions of levels in downstream products. For example, the stage 1 product contained 670 ppm of compound (ii), Stage 2 product 23 ppm, while Stage 3 product, intermediate grade API and final API contained less than 1.7 ppm of (ii). This enabled them to focus on upstream control in starting materials or intermediates, and avoided the need for control in the drug substance. The attractiveness of this approach is that it permits control limits to be set at higher levels, with the assurance that downstream purging will reduce the levels of materials of concern to acceptable levels. It also allows the control strategy to be based on less sophisticated and sensitive analytical methods, which are more aligned to a routine quality control environment.

### 9.4 CONCLUSION

The need to adequately assess the risk posed by GIs, and to limit the level present in API/DP is clearly established in the EMEA\(^7\) and draft FDA\(^8\) guidelines. It is the
opinion of the authors of this chapter that the most effective way to achieve this is to establish control strategies based on a combination of semi-quantitative assessment, allied to analytical results and data from appropriate purging studies. Such an approach should ensure that any actual GI related risk is clearly identified and managed.

9.5 ACKNOWLEDGMENTS

The authors would like to thank Dr Stephen Smith (AstraZeneca) for the provision of advice and data relating to the chromatographic removal of GIs.

9.6 CASE STUDIES

9.6.1 Case Study 1

The following example from the synthesis of omeprazole (see Fig. 9.5) is presented to demonstrate how the fate of a genotoxic nitroaniline starting material was assessed. Using the scoring system defined in Table 9.5, the following factors were assigned:

9.6.1.1 Calculated Risk

Reactivity Based on the relative ease of the nitro reduction stage, the nitroaniline (I) was categorized as highly reactive, and therefore a factor of 100 was applied. A further factor of 10 was applied to the subsequent cyclization step, giving an overall reactivity factor of 1000.

Solubility The nitroaniline is freely soluble in the solvents employed within the process, and thus any residual amounts remaining after completion of the reaction would be expected to be removed in the mother liquors. A factor of 10 was therefore applied. No other purge factors were deemed applicable. Therefore, the total purge factor is 10,000 × 10 = 10,000. Based on which, it would be anticipated that levels would be <100 ppm.

![Diagram of synthesis of omeprazole](image)

*Figure 9.5 Synthesis of omeprazole intermediate III.*
9.6.1.2 **Experimental Results**  No nitroaniline (<5 ppm) was observed in multiple batches of the isolated cyclised product; these results show a clear and accurate correlation with the theoretical prediction.

9.6.2 **Case Study 2**

The second case study is presented as an example of a potentially genotoxic impurity, nitropyridine (A), within a starting material, again with reference to the synthesis of omeprazole (see Fig. 9.6). If present within the starting material, there was a concern that nitropyridine (A) might carry-over in the downstream process, hence a risk assessment was undertaken.

![Chemical structures](image)

**Figure 9.6**  Synthesis of omeprazole potassium salt.
9.6.2.1 Calculated Risk  This is a three-step reaction; however, none of the intermediates are isolated.

Reactivity  The nitropyridine (A) is unreactive under the reaction conditions employed for all three steps; hence a purge factor of 1 is applied, since it is unlikely that it will be removed as a result of reaction in the downstream process.

Solubility  The nitropyridine (A) is highly soluble in the solvents employed in the stages described. For a process where there are no isolations, it is therefore expected that the nitropyridine (A) would remain within the process liquors. Therefore, for stages 1 and 2, the purge factor is assigned as 1 based on solubility, meaning that no reduction in level is expected.

Vollatility  The nitropyridine is not volatile, hence a purge factor of 1 is again assigned, since very little will be removed as a result of any of the solvent removal or exchange processes involved.

The total purge factor to the coupled sulphide stage is calculated as $1 \times 1 \times 1 = 1$; hence no reduction in the level of nitropyridine is predicted.

Experimental Results  In spiking experiments performed at a level of 3000 ppm nitropyridine (A) spiked into the starting alcohol, a level of approximately 2000 ppm was found in the stage 2 product (III). This is considered to validate the theoretical assessment that predicted no significant reduction in levels of nitropyridine in this process.

However, when the level of nitropyridine (A) was assessed in the isolated potassium salt, less than 1 ppm was detected. Due to its high solubility, the nitropyridine (A) remained in the reaction mother liquors following crystallisation of the desired product.

9.6.3 Case Study 3

In the third case study, based on the synthesis of a fluoroaryl amine, the impurities of concern were genotoxic sulfonate esters that could be potentially formed during the process, and in particular during the final crystallization stage (see Figure 9.7).

Figure 9.7  Simplified representation of the synthetic process used to manufacture the fluoroaryl amine API.
The potential carry over of these materials into the final API was a matter of concern; hence a risk assessment was performed.

9.6.3.1 Calculated Risk

Reactivity: Under the reaction conditions employed, the sulfonate esters (MMS, EMS, and IMS) are all very reactive; therefore, a purge factor of 100 would be applied, as it is very likely that they would be removed as a result of reaction in the downstream process. Indeed, some data are available to assess relative chemical reactivity, in particular, the Swain-Scott $s$ constants, which provide an assessment of the sensitivity of an electrophilic substrate to nucleophilic attack. The sulfonate esters (MMS, EMS, and IMS) have different $s$ values: MMS (0.83) > EMS (0.67) > IMS (0.29). In this instance, because of the existence of specific data relating to reactivity, the reactivity purge factor was adjusted to allow for these differences. The individual adjusted purge factors are therefore 83 (MMS), 67 (EMS), and 23 (IMS).

Solubility: The sulfonate esters are highly soluble in the solvents employed in the final stage described. Thus, the purge factor assigned is 10.

Volatility: All of the sulfonate esters have low volatility, with boiling points (MMS [202°C], EMS [213°C], and IMS [220°C]) greater than 20°C above that of the mixed solvent used (ethyl acetate [77°C]/acetone [56°C]/iso-octane [99°C]) for the crystallization. Therefore, the assigned purge factor is 1.

No other purge factors were deemed applicable. Therefore, the total purge factors for each species were calculated as:

$$PF_{MMS} = 830$$
$$PF_{EMS} = 670$$
$$PF_{IMS} = 230$$

To examine the purge capacity of the process, Cimarosti et al. crystallized the API with methane sulfonic acid (MSA) containing elevated levels (up to 5× the specification limits) of the three sulfonate esters (MMS [2500 ppm], EMS [2600 ppm], and IMS [1200 ppm]). In addition, extra quantities of the three esters (MMS [1600 ppm], EMS [2200 ppm], and IMS [2900 ppm]) were added to the mother liquors just prior to filtration.

Based on the levels spiked, the following maximum levels would be expected:

$$MMS = 4100/830 = 5 \text{ ppm}$$
$$EMS = 4800/670 = 7 \text{ ppm}$$
$$IMS = 4100/230 = 17 \text{ ppm}$$

9.6.3.2 Experimental Results: Initially, immediately after deliquoring, the residual levels of the sulfonate esters were inversely proportional to their $s$ values, i.e. the most reactive ester had the lowest levels of residual ester present in the API. Thereafter, the levels of residual sulfonate ester in all cases was less than the LOD of the method (<1 ppm) (Table 9.6).
These results again strongly support the use of the predictive tool, having successfully predicted the efficient removal of all three sulfonate esters in the process described.

REFERENCES


TABLE 9.6 Levels of Residual MMS, EMS, and IMS in API (Based on Cimarosti et al.24)

<table>
<thead>
<tr>
<th></th>
<th>MMS</th>
<th>EMS</th>
<th>IMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swain–Scott Reactivity Index (s)</td>
<td>0.83</td>
<td>0.67</td>
<td>0.29</td>
</tr>
<tr>
<td>Relative purge factors</td>
<td>3.61</td>
<td>2.91</td>
<td>1.00</td>
</tr>
<tr>
<td>Wash volumes of ethyl acetate</td>
<td>MMS (ppm)</td>
<td>EMS (ppm)</td>
<td>IMS (ppm)</td>
</tr>
<tr>
<td>Deliquoring (0)</td>
<td>1</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1</td>
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