1.4. The Role of Impurity Profiling in Drug Research, Development and Production

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1.4.1. Impurity Profiling in Synthetic Drug Research

The use of analytical methods is of utmost importance in all phases of synthetic research and related areas (biotechnology, extraction of materials of plant and animal origin) aiming to introduce new chemical entities into the therapy [1–3]. During the gram-scale preparation of new compounds for pharmacological screening the analytical activity takes place in two main directions: (a) structure elucidation of the reaction products by spectroscopic methods, and (b) estimation of their purity (endproduct and its intermediates). When the synthetic chemist takes a sample from the reaction mixture or the crude reaction product for a rapid chromatographic (mainly TLC) test the primary aim is not yet impurity profiling. On the basis of the chromatogram it is possible to get a picture on the course of the reaction. The questions to be answered are if the reaction is completed (the spot/peak of the starting material disappears) and if so is it unidirectional under the selected conditions (one main spot/peak accompanied with minor ones or commensurable spots/peaks appear). On the basis of the data thus obtained the organic chemist is able to select the suitable reaction to reach the goal and optimise the reaction conditions.

It would be difficult to state at which point of the synthetic research real impurity profiling begins. The requirements for the purity of the samples can be quite different in various research departments. Generally speaking it is not reasonable to prepare the large number of test samples for the first pharmacological screening in highly purified form: it is predictable at this stage that the overwhelming majority of the new compounds will be dropped after the first tests and there is ample time afterwards to estimate the impurity profiles of the few materials which have been selected for further investigations. (This is even more the case with the products prepared by means of combinatorial chemistry where mixtures of large number of components are subjected to high throughput screening. The tasks of analytical chemists in this area are quite different.) It is, however, sometimes the case that organic chemists require the identification/structure elucidation of key impurities already at this stage of the research since the rational strategy for the preparation of the target molecule can only be established in possession of this information.
The drug candidates selected for further pharmacological and toxicological tests should undergo thorough analytical investigations. Generally speaking, the analytical information obtained on the selected materials should grow together with the amount of chemical and pharmacological information. After a further decision point, the organic chemists have to optimise the synthesis and purification of the materials in order that these can be scaled up to prepare material for the pharmaceutical technologists to develop the drug formulation and for the further, decisive toxicological, preclinical and clinical trials. The most important task in this cooperation between organic chemists and analysts is the estimation of the impurity profile of the potential drug substance as a function of several factors (conditions of the reaction, purification and storage, selection and quality of the starting materials, reagents, solvents, catalysts, etc.). It should be noted that in this stage of the development it is not mandatory to identify the impurities, it should be, however, assured that the same impurities occur in and the same limits apply to the batches used for these trials and for those reported in the registration documents and to be used in the therapy. For details see Section 1.5.3.3. It is, however, the general practice of drug manufacturers that impurity profiling studies even in this earlier phase of the development include the identification of the impurities above the threshold limits (0.05–0.1%) since the organic chemists and pharmaceutical technologists need this information for their work.

In the course of the investigation of the influence of the above listed factors on the impurity profile, special attention should be paid to the purity of the starting materials of the synthesis. Organic chemists usually use materials of higher purity during the gram-scale synthesis than it would be reasonable from the point of view of the economy of the production of industrial batches. In some cases, the purity of the starting material more or less determines the purity of the endproduct. For example, the reaction leading to flumecinol (3-trifluoromethyl-α-ethyl-benzhydrol) was the addition of the Grignard reagent prepared from 3-trifluoromethyl-bromobenzene to propiophenone. Any 4-trifluoromethyl-bromobenzene impurity in the reagent causes the appearance of the isomeric 4-trifluoromethyl derivative separable by packed column [4], capillary column [5] gas chromatography and high-performance liquid chromatography [6] from the main component. (See Figs. 2.6.D and E in Section 2.6.6.) Since the price of the reagent depends on its isomer content, a compromise had to be reached as regards its quality: it should be acceptable from the point of view of both drug safety and economy. It is a general rule that it is not prudent to use unreasonably pure endproduct for the preclinical studies because the same purity will be required for the clinical and industrial batches.

The estimation of the impurity profile of a drug material includes the identification of the main impurities in the intermediates in their synthesis, too. The registration documentation should contain the description of the
reasons for the presence of the impurity. In the case of a synthesis related impurity this means that it should be proved that it is really a synthesis related impurity and the mechanism of its formation should also be presented. This cannot be done without the identification of the main impurities in the intermediates.

The methodological aspects of the identification and quantitative determination of the impurities in bulk drug materials are outlined in Section 2.1 and discussed in detail in Sections 2.2–2.11. The estimation of enantiomeric impurities is the subject of Chapter 6. The identification of degradation products and determination of degradation pathways also begins already in the course of the analytical studies with the bulk drug material (stability studies under stress conditions). This aspect is outlined in the following section and discussed in detail in Chapter 5. Impurity profiling also includes the identification and quantitative determination of solvent residues and inorganic impurities. These are subjects of Chapters 3 and 4, respectively.

1.4.2. Impurity Profiling in the Production of Bulk Drugs

The analytical activities related to the estimation of impurity profiles do not come to an end after the R&D phase of the introduction of a new drug. It is essential to ensure that no new impurities appear in the course of the scaling up procedure and the quantity of the impurities in the bulk drug material identified during the synthetic research phase remain below the specification limits. For this reason the analytical control of all steps of the scaling up procedure is of key importance. It can happen that a new impurity appears during the scaling up or even more typically the quantity of an impurity which was detected but not identified during the synthetic research period since it was in the low 0.01% range reaches or exceeds the threshold limit where its identification is mandatory. In such a situation the structure elucidation of the impurity is an important task in order that in possession of its structure the technologists can make the necessary steps to avoid its formation or at least reduce its quantity.

More or less the same applies to the cooperation of drug analysts and technologist in the course of the production of the bulk drug material in the routine scale. Even under the strictly controlled conditions of a carefully validated technology the possibility of changes in the impurity profile similar to those described in the preceding paragraph cannot be excluded. As described earlier the impurity profile depends on many factors and even minor changes in one of them can result in considerable changes and the analytical chemist should be prepared to give a rapid answer to any questions of this kind arising during the production of the bulk drug. To be able to do so it is also important to estimate the impurity profile of the (key) intermediates in the synthesis and
build in the necessary steps into the in-process-control protocol in order that the origin of a new impurity in the endproduct of a multistep synthesis can be rapidly identified.

The great importance of carefully controlling the effects of even minor changes in the technology on the impurity profile and as a consequence of this on drug safety can be characterised by a scandal related to the questionable purity of certain industrial batches of L-tryptophan. This amino acid which is said to have health benefits is widely used as a dietary supplement. At the end of 1989 an epidemic broke out referred to as eosinophilia-myalgia syndrome (EMS) which affected thousands of the consumers of L-tryptophan killing over 30 of them. Careful investigation revealed that these adverse effects occurred only with some batches of L-tryptophan produced by a single manufacturer prior to the outbreak of the epidemic. This manufacturer produced L-tryptophan by fermentation. It became clear that in the course of the production of these batches a new strain of Bacillus amyloliquefaciens had been introduced and at the same time the amount of activated charcoal had been reduced in one of the purification steps. The removal of the suspected batches from the market essentially stopped the epidemic. An extremely wide and intense research was launched to investigate the impurity profile of the suspected batches. With the aid of mainly HPLC, HPLC/MS, HPLC/MS/MS and NMR studies several minor impurities were detected and identified [7–10]. Although this research is still going on [11,12] and the picture is still not completely clear from the epidemiological point of view; some of the impurities, among them 3-(phenylamino)alanine, 1,1'-ethylidenebis(tryptophan), 2-(3-indolylmethyl)-L-tryptophan and 2-(3-indolyl)-L-tryptophan were found to be associated with EMS. Quite recently similar studies were carried out with melatonin, too, high doses of which also cause EMS-like symptoms. Some of the impurities found were structural analogues of the above mentioned EMS-related impurities [13].

1.4.3. Impurity Profiling in the Research and the Production of Drug Formulations

The identification, structure elucidation and quantitative determination of impurities and degradation products are of prime importance in the course of all phases of research, development and production of drug formulations. The close relationship between impurities and degradation products is discussed in Section 5.1. The pharmaceutical technologist should have a clear picture about the impurity profile of the bulk drug material used for the development of the formulation in order to be able to differentiate between synthesis-related impurities and degradation products. In such a way the stability indicating nature of
formulation can be established. These studies indicate which of the impurities in the bulk drug material are of degradation product type. The increase of these is expected during the stability studies while the synthesis-related impurities are likely to remain constant.

The pharmaceutical technologists should also be aware of the results of the preliminary stability studies carried out with the bulk drug material under stress conditions (see Section 5.2). Since these investigations can be considered to be part of the preformulation studies [14], in some drug companies these are carried out by the pharmaceutical formulation group while in some others this is part of the responsibilities of the analytical group. These studies reveal the degree of possible sensitivity of the drug molecule to heat, light, humidity, acidic, basic or oxidative conditions (including the investigation of the stability vs. pH profile), traces of metal ions, etc. On the basis of these studies some of the real degradation products of the drug formulation can be predicted while some others may be identified during the course of the stability tests with the formulations.

In possession of suitable analytical methods and the above listed data regarding the possible degradation mechanisms of the drug material the pharmaceutical technologists can begin compatibility studies which are also to be carried out under strict analytical control including the estimation of the impurity profiles. The aim of these studies is to find possible interactions between the active ingredient(s), excipients, antioxidants, etc. As a result of these new impurities can be found. As an example of the immediate adverse effect of excipients the study of Dijkstra and Dekker [15] is mentioned for the stabilisation of the solutions of prednisolone sodium phosphate or dexamethasone sodium phosphate with their oxidisable dihydroxyacetone-type side chain at position 17. They detected impurities by HPLC when sodium metabisulphite was used as an antioxidant. As seen in Fig. 1.4.A, the impurities were found to be products of an addition reaction between the conjugated double bond system in ring A (Δ^1 double bond) and the antioxidant. In the case of prednisolone

![Figure 1.4.A.](image-url)

**Figure 1.4.A.**
Reaction between prednisolone sodium phosphate and sodium metabisulphite (from Ref. [15])
sodium phosphate both epimers, the 1α- and 1β-sulphonates were separated and identified; with dexamethasone sodium phosphate only the 1β-derivative was detected.

The stability studies of the experimental dosage forms are in the center of the investigations aiming to find the most suitable dosage form with optimal stability and bioavailability. At the beginning of these studies when several variants of the aimed formulation are the subject of the investigation, the main goal is the detection, identification and quantitative determination of degradation products formed under stress conditions. This is the most rapid and most economical way to obtain the data necessary for the selection of the most suitable composition for the formulation. Some of the degradation products are usually identical with those found during the above mentioned stress stability studies of the bulk drug material, but some others can be new and these should be characterised to some extent in order to be able to make a good decision. (It should be mentioned that some of the degradation products found under stress conditions will not occur under the milder conditions of the long-term or accelerated stability studies.) After the selection of the final composition of the dosage form, in the course of scaling up and industrial level production of the formulation the latter forms of the stability studies come to the fore. For details the reader is referred to Section 5.2, the ICH Guidelines [16] and the book of Carstensen [17].

As for the methodological problems of profiling impurities and degradation products in drug formulations it can be stated that in the case of relatively simple solid dosage forms and injectables with not too low active ingredient content there are no particular difficulties as compared with the profiling of bulk drug materials. The situation is, however, much more difficult when solid dosage forms with low active ingredient content or formulations containing complicated matrices (ointments, creams, suppositories, oil-injectables) are investigated as shown for steroid formulations of this kind by Kirschbaum and Cohen [18]. In Sections 5.3–5.5 several practical examples are presented for the identification, structure elucidation and quantitative determination of degradation products among others in pharmaceutical formulations.

1.4.4. Impurity Profiles in Drug Registration: Legal Aspects

As described in the preceding sections of this chapter, the impurity profile of a drug is influenced by several factors, the synthetic route, reaction conditions, source and quality of the starting materials, reagents and solvents used during the synthesis, the purification steps, conditions of crystallisation, drying, distillation and storage of bulk drug materials and drug formulations. For this reason the estimation of impurity profiles of drugs is an excellent means by
which the drug authorities can control the level of the manufacturing process. The number of impurities and their quantities are the best indication of the quality of the drug product. The proportion of identified impurities among the detected (qualified) ones and the standards of the analytical (chromatographic and spectroscopic) dossier describing their detection, identification/structure elucidation and determination enables the authorities to judge the level of analytical activities at the manufacturer. The comparison of the impurity profiles of several batches from the same manufacturer provides a good indication for the constancy of the manufacturing process while the comparison of samples originating from different manufacturers can give a clear picture about the differences between their purity and the levels of the manufacturing procedures.

The comparison between the impurity profiles of drug samples from different manufacturers furnishes at the same time information about the synthesis route used by the different companies. Certain impurities can be considered to be indicators of a certain synthesis pathway (often called “synthesis markers”) even if they are detected at a much lower level than required by the drug authorities. Due to the very high confidentiality of analytical works done in this area very little if any has been published on these legal aspects of drug impurity profiling, when some companies try to make use of analytical data in providing evidence for the illegal use of their patented synthesis route by another company.

One of the rare published studies where the results of the comparison of the impurity profile of a drug originating from different sources is described is in the recent paper by Lehr et al. [19]. In this study the impurity profile of 22 lots of trimethoprim bulk drug substance from five different manufacturers in three countries were compared. Using a gradient RP-HPLC system they detected two major impurities which are not separated and detected by the TLC system of USP 24 [20]. The structures of the two impurities were determined by HPLC/MS and NMR spectroscopy after preparative HPLC separation. The chromatograms of five characteristic examples from among the 22 lots are shown in Fig. 1.4.B. The total quantity of impurity I and II in the various lots reached 2.1% in one of the lots while it was absent in some others. The “fingerprint-like” character of the chromatograms in Fig. 1.4.B is evident and certainly betrays the different synthetic/purification procedures used by the various manufacturers.
Figure 1.4.B.
HPLC profiles of trimethoprim drug substance from various manufacturers. Column: Beckman Ultrasphere ODS, 5 μm, 4.6 × 250 mm; gradient elution A: 0.25% triethylamine and 1.1% formic acid in water (pH 5.8), B: acetonitrile. 0–10 min 10% B, 20–30 min 25% B, 35–45 min 40% B, 50–60 min 80% B. Flow rate 1.0 ml/min. UV detector 272 nm (from Ref. [19])
References


