DETERMINING THE STRUCTURE OF IMPURITIES

Most, if not all pharmaceutical companies will have experienced a product, that has been on the market for a long time, to suddenly and unexpectedly trigger a signal for an unknown substance in the quality control (QC) laboratory approval process. Deciding on the next course of action is of the upmost importance to the company's success. Ultimately, the most important consideration is product quality and safety, however the financial impact is also a commercial priority too.

ABSTRACT

In the pharmaceutical industry, impurities can arise at numerous points in the various processes used. They could occur during research and development, but also during production of the active substances. In the latter case, it is of paramount importance that the structure of the impurity is clarified so that the triggering fault can be rectified and/or a toxicological evaluation carried out. This is the only basis on which to assess whether the batch produced can be used, or whether it requires filtration to get it to a usable state. The worst-case scenario is that the entire batch would need to be destroyed.

Furthermore, it is important to ascertain whether the impurity originates from the raw material, is process-related (a chemical byproduct) or is caused by the replacement of an item in the process. A different strategy is required on a case-by-case basis to avoid this impurity from occurring again. This complexity explains why it is so important to determine the structure in each case. However, in no way can the quality of the substance be compromised, even with the obvious time pressure. By discovering the cause at this stage, it is possible to help to avoid batches being afflicted by the same cause in the future. It is always advised that specialists be appointed so that they can recommend a remedial strategy, advice on the potential financial commitment, estimate the chance of success for the batch and calculate the expected costs.

PLANNING, PREPARATION AND CONTINGENCIES

If an impurity arises during a laboratory analysis (as part of quality control), it is essential to have a plan in place for such a situation. Being prepared for a trigger before it happens can ensure that if it does, remedial actions can be implemented in a timely fashion, with better control of the budgetary impact. Time is critical if an impurity is detected at this stage, with any significant hold-up having detrimental effects along the whole analysis and production process. Good planning, by gathering all information to even the smallest of details at each production step, helps to determine potential corrective actions and therefore increases the chance of final success.

Furthermore, consideration should be given to the possibility of gaining information from third parties. Discussions with suppliers could provide valuable insights to help more quickly and easily determine possible causes. For example, perhaps the research laboratory has information on known side effects of the synthesis.

Finally, additional important questions need to be clarified such as how many batches are affected? This might lead to the discovery that it is in fact the production process that is lacking in stability, but there may also be a temporary, unforeseen problem. Another factor to consider is how much the batch is worth and the economic cost. Destroying a batch can sometimes be a cheaper option than investing in an expensive, uncertain investigation. However, the larger the batch, the more intensively the issue needs to be pursued. Are there further conditions that need to be considered such as storage stability? A substance with a short stability substantially increases the time pressure on any further course of action, or might make further treatment impossible. Further questions to consider include how laborious is the synthesis and is it possible to isolate the origin of the impurity?

FAULT ANALYSIS

If an impurity is detected, it is recommended to carry out a fault analysis. There is a wealth of informational material and templates available describing how this can be carried out as a risk assessment. The following points should be considered:

- Have new reactants been used?
- Has there been a change in supplier?
- Have other solvents been used?
- Have reaction conditions, such as temperature, pressure, time or similar changed?
- Consider also weather influences
- Has a production component been changed?
- What is your company's incoming goods inspection process? Despite an increase in the quality of raw materials, it is always possible for the quality of the basic product to vary
- Are all parts of the production process being inspected using suitable methods, even the process materials? It can also be very expedient to view the control laboratory's analysis method in more detail. This allows the magnitude of the problem to be better understood, but also allows the understanding of the product and the analytical methods to be refreshed.
- With what analysis method did the impurity arise? Can any findings already be gained from the method? For example from the polarity, the boiling point, color, substance class, solubility or the rough mass of the unknown substance? Likewise, an estimation of quantity can be carried out even for an unknown substance. This information, and the available sample quantity, can be a limiting factor since the smaller the impurity the larger the cost of carrying out the sample preparation for successfully determining the structure.

The points discussed above demonstrate how important the preparation stage is. This gives an insight into how easy it could be to invest in the wrong corrective strategy.

Finally, this knowledge must be transferred to the laboratory that is contracted to carry out the investigations. This gives an experienced analyst reliable support and minimizes the chance of only a partial resolution.

ANALYSIS METHOD

Choosing the most suitable testing method is critical for final success, including from a financial and time expenditure perspective. Generally, an assessment is also carried out into the chance of success. Finally, a goal should be defined, such as "do isomers need to be unravelled?" or "do structures need to be clearly identified?".

The equipment used for the testing has an impact on cost, but also on the outcome. This is briefly explained in Table 1.

The laboratory phase starts once all preparations have been clarified. A method transfer must first of all take place. It is necessary for the parameters and the result of the QC laboratory to be readjusted. The test result must be verified. Not doing so represents a culpable course of action. Product analysis methods often still exist (perhaps only in part) that are not suitable for the equipment used to determine the structure. This may concern salts, solvents, ion pair reagents etc. that have been used. Method adjustment is time-consuming and always presents a challenge. It can become difficult to ensure that the correct analytes have been clarified.

In Figure 1, a typical case of an impurity can be observed. In a high-performance liquid chromatography (HPLC) process, an additional signal is eluted in addition to the product peak. This is found outside the allowed limit and must be assessed.

DETECTOR	REQUIRED QUANTITY OF SUBSTANCE	INFORMATION GAINED	PRICE PER ANALYSIS
Infrared (IR) spectroscopy	Medium	Minimal	Minimal
Mass spectroscopy (MS) high resolution	Minimal	Medium	Medium
Nuclear Magnetic Resonance (NMR) spectroscopy	High	High	High

Table 1 Performance assessment of various spectrometers

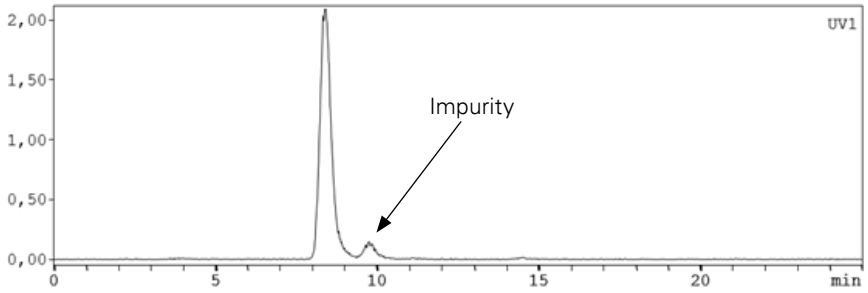


Figure 1 HPLC UV chromatogram

FAULT ASSESSMENT / OOS PROCESS

An out of specification (OOS) process typically begins in the QC laboratory. However, if a laboratory fault cannot be clearly identified, the following cascade for determining the structure is commenced. Valuable time can be lost at this stage by speculating on a solution instead of thinking and acting in parallel. For example, even a sub-problem might be overlooked. Figure 2 demonstrates this with the impurity relating to two substances. This became evident by using two-dimensional fluid chromatography testing. The impurities eluted at the same time in the quality control system. This is not uncommon in HPLC chromatography when using insufficient separating capacity (use of a gradient, unsuitable choice of column, unsuitable pH value etc). The "fault" should not necessarily be attributed to the method developer; at the time the method was developed, there may have been other requirements, needs or perhaps even no better separation phases. But it can certainly be noted that more unknown analytes than could have been expected could arise, which place special requirements on the analysis system. A suitable separation column is able to make use of the differences of molecules and to separate the substances.

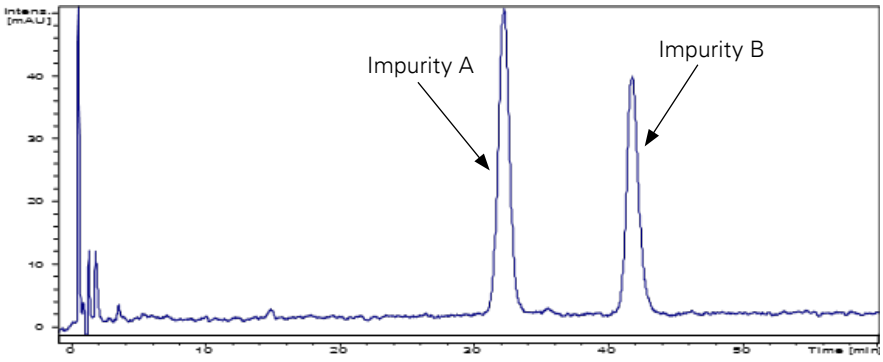


Figure 2 HPLC UV chromatogram after separation of the impurity

STRUCTURE DETERMINATION AND DATA INTERPRETATION

The impurities could be assigned to various mass spectra. In this case, the HPLC system was coupled with an ion trap mass spectrometer device. An electrospray ionization (ESI) source acted as the ion source and was operated under positive voltage. Impurity A has a molar mass of 421 dalton (Da) (see Figure 3) and impurity B has a molar mass of 405 Da (see Figure 4). In this case, more in-depth work to determine the structure should not be carried out, since this can be arbitrarily complicated. This is where specialists should take over.

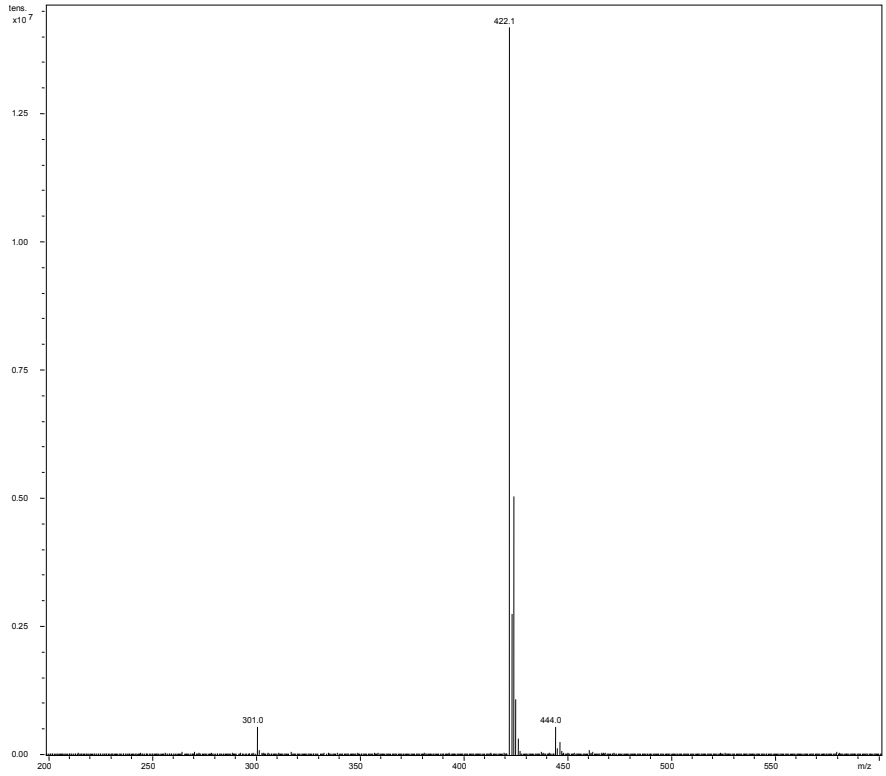


Figure 3 MS image of impurity A (proven molecular formula C20H27N3O5S)

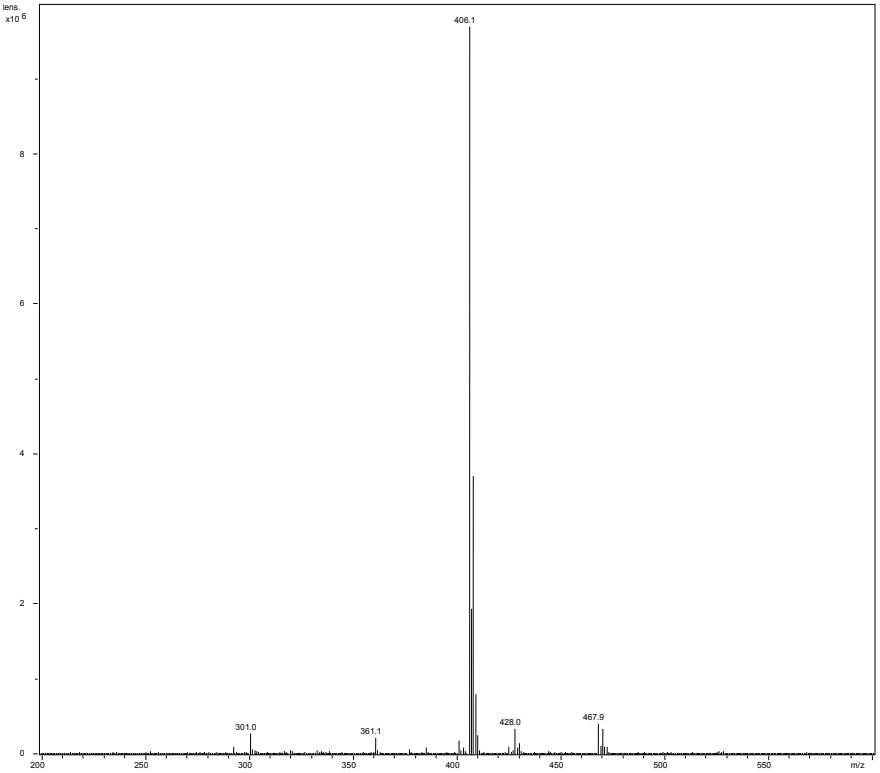


Figure 4 MS image of impurity B (proven molecular formula C20H27N3O4S)

Based on the gathered data, the analyst must now generate a structure proposal. It's clear that the reliability of this is dependent on the quality of the existing data. However, it is very important to also have an analyst on hand who has the necessary expertise and is in a position to survey the issue comprehensively. Principally, however, the process to determine the structure can be broken down into five essential points, as represented in Figure 5. These generally occur serially and hardly ever achieve a time reduction from synergetic effects. The process applies regardless of where it occurred; it can be applied both in research and in production.

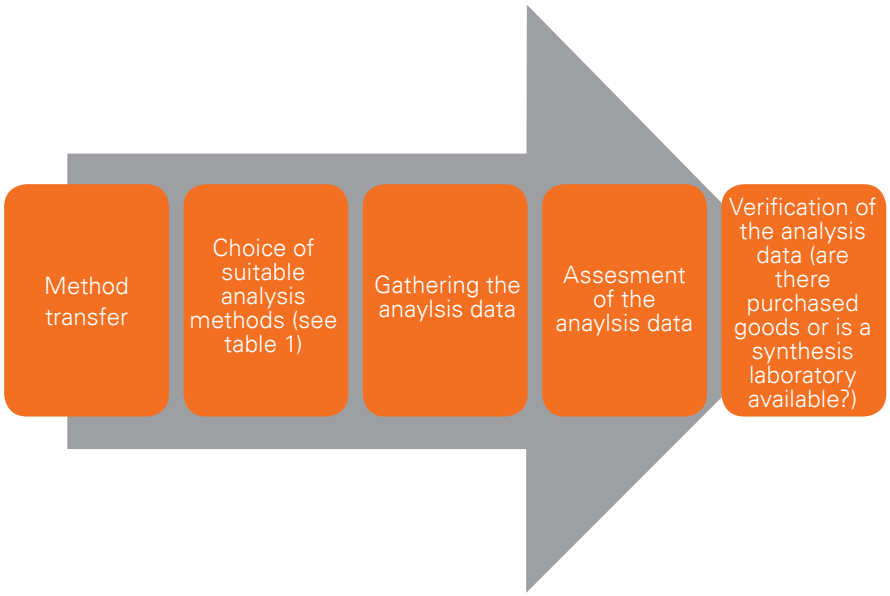


Figure 5 Chronological sequence of structure determination

Realistically, time frames range from a few weeks to several months but here are estimations of the time required for each stage:

Method transfer:	up to a week
Choice of analysis method:	one or two days
Gathering data:	from one week to two months
Evaluating the data:	up to a week
Verification:	up to a week

If, at the end of the process, the cause of the signal is identified, the source of entry must be determined. Any anomalous process parameters found must be counteracted. Standard Operating Procedures (SOPs) must be adjusted accordingly. Personnel need to be trained appropriately. The system may need to be adjusted and operated with lower variances. If a supplier quality problem arises, urgent redress must be sought. Possible in-house resources used in this context must not be forgotten.

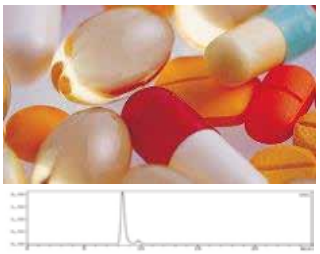
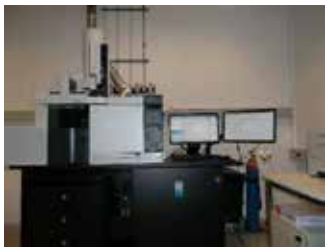
PROCESS EXAMINATION

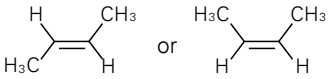
When the quality of a process material (filter, pipes, connectors, plugs etc.) is insufficient, another investigation could be helpful (e.g. an extractable study; to avoid the replacement of an item being accompanied by another problem). Here, too, a discussion with the supplier is necessary.

Furthermore, the methods' specifications should be reviewed. A recommended and proactive approach is that at the time of developing the actual quality control method, potential problems that may arise should be considered in parallel. For example, a mass spectrometer is hard to operate with a mobile phase that contains an ion pair reagent. An NMR spectrometer requires greater substance quantities (possibly still a substance that requires purification). If, here in the mobile phase, there is a fluid chromatography salt (to buffer or increase the separation performance), the expected expenditure in the preparation of the sample is considerable. In addition, the recommendation can only state that performance reserves should be considered as early as possible. A separation system that just about meets the specification, reaches its performance limit much more quickly. The additional price of a higher performance method should be assessed against the possible costs. Taking the example of the two-dimensional separation using HPLC, it is possible to determine what expenditure may be necessary to answer the question of what kind of substance the "unknown" is.

SUMMARY

Figure 6 shows how wide-ranging the subject of impurity is.

OCCURRENCE OF IMPURITY	STRATEGY	STRUCTURE ELUCIDATION	CONSIDERATION
			

• Where was the impurity determined?
• What analysis method was used (can conclusions be drawn as to the impurity)?
  • Polarity
  • Boiling point
  • Color
  • Solubility
  • Mass (of the molecule)
• Can the finding (Out Of Expectation, OOE) be reproduced?
	• Risk analysis
	• Are there incoming goods
	• inspections?
	• Was there a change of:
	  • Supplier?
	  • Quality of products used?
	  • Reaction conditions?
	  • Has anything on the system been replaced?
	• Are there known byproducts from the synthesis?
	• Investigation of the used analytical method?
	• Investigation of materials used?
	• Have there been unusual influences (e.g. weather)?
	• How much time is available for clarification (e.g. stability of the product)?
	• How much are the goods worth?
		• Method transfer
		• Preparing a suitable method (change of analysis technology required?)
		• What information is required (how precise does the result have to be)?
		 
			• Could the polluter be determined?
			  • Raw material?
			  • Process condition?
			• Is a toxicological assessment necessary?
			• Do processes need to be adjusted (Corrective and Preventive Action CAPA plan)?
			• Update Standard Operating Procedures

CONCLUSION

When an impurity arises, it is not always possible to foresee the complexity of the problem. This article should be viewed as a 'guideline' in how to anticipate, plan and proactively react to such an occurrence in a timely, cost-effective and commercially-focused manner, with the ultimate objective of producing a safe and compliant pharmaceutical substance.

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