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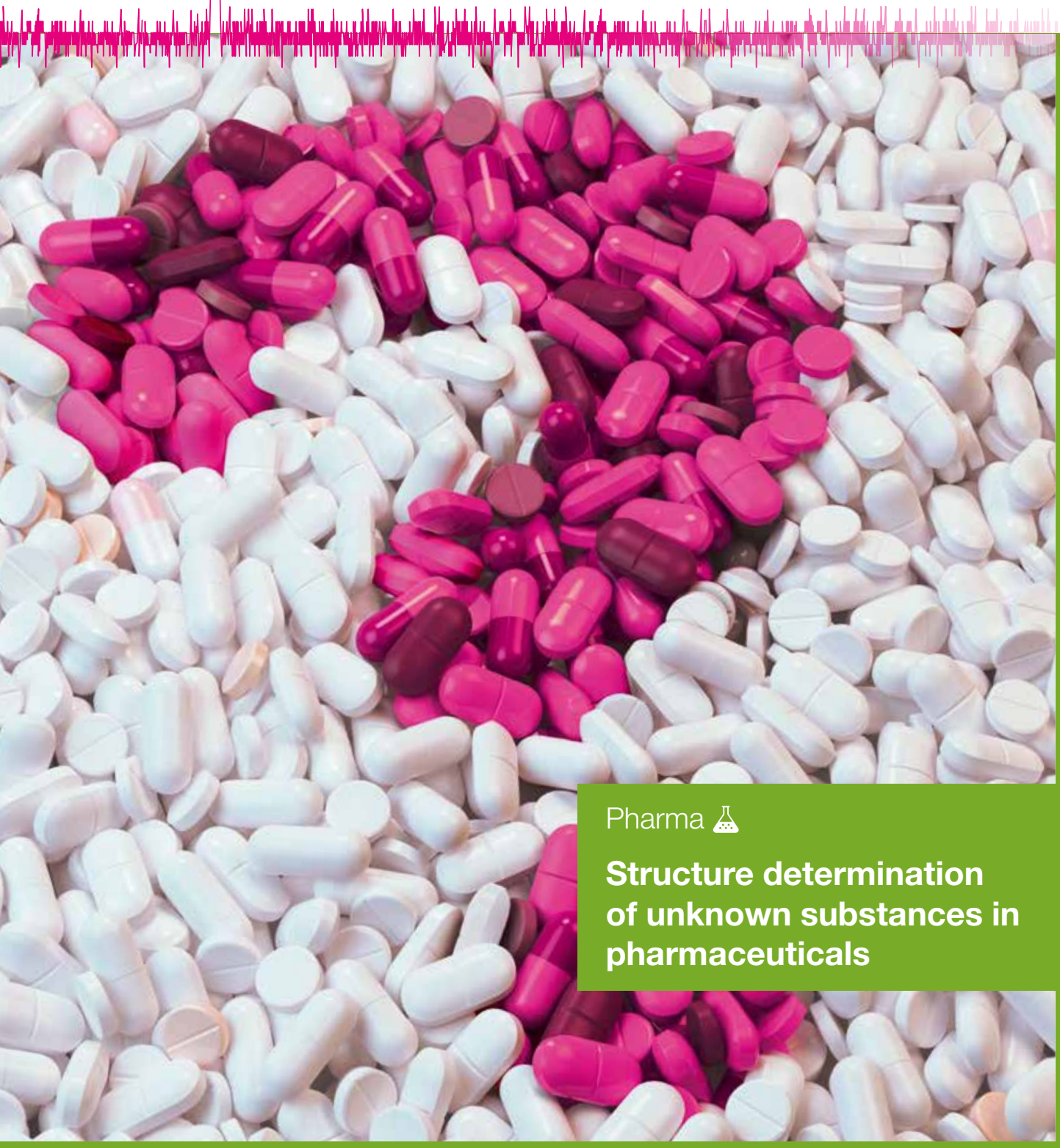

Pharma

Structure determination of unknown substances in pharmaceuticals

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Introduction

The potentially toxic effect of an unknown impurity in pharmaceutical products requires further investigation if the concentration of the impurity exceeds a certain target value [1][2]. In order to be able to perform the corresponding toxicological investigations, the chemical structure of the impurity must first be determined. The most common technique for clarifying the structure of an unknown compound is a combination of high-resolution mass spectrometry (LC-HRMS) and two-dimensional NMR spectroscopy [3][4].

Active ingredients and excipients in pharmaceuticals can decompose in a variety of ways, including hydrolysis, oxidation or photochemical processes. Furthermore, the ingredients can react with each other and form adducts [5]. In addition, unknown impurities are usually embedded in a very complex product matrix. The structure determination of unknown impurities in pharmaceuticals therefore represents an analytical challenge.

Procedure

The general procedure for structure determination will be described here using the example of an unknown impurity in a drug containing opioids. The process can be divided into six steps:

1. The first step is to implement the customer's analytical method and identify the individual components of the preparation (active ingredients, excipients and impurities) by comparing the respective retention times and UV spectra in the HPLC-UV chromatogram.

2. In a second step, a few micrograms of the impurity are isolated using analytical HPLC (see HPLC-UV chromatogram in figure 1). This tiny amount of impurity is used for the subsequent LC-HRMS analysis and also serves as a reference substance for any method developments (see step 5).

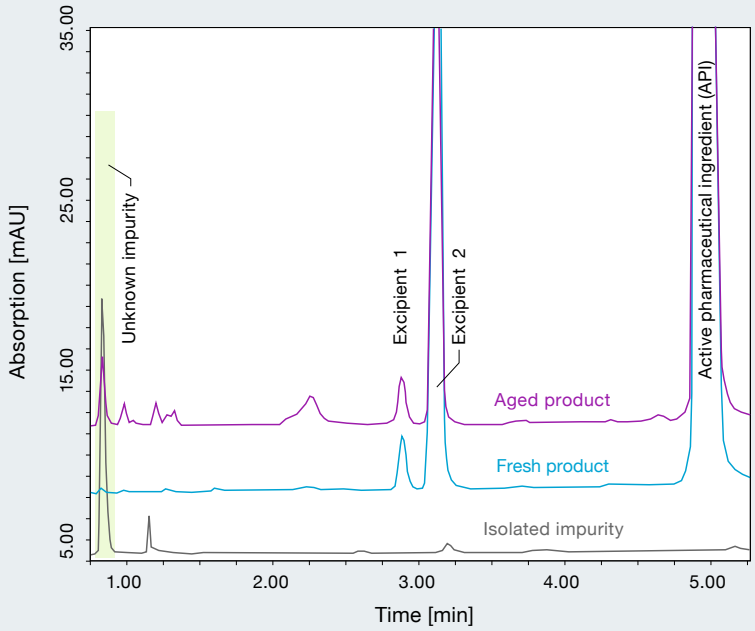
3. A high-resolution mass spectrum (LC-HRMS) of the isolated impurity is recorded. This allows the determination of possible elemental compositions of the unknown impurity. Thanks to the knowledge of the individual ingredients of the preparation and the resulting possible decomposition reactions, the number of mathematically conceivable elemental compositions of the impurity can be limited. Structural proposals can now be postulated.

4. The next step is to perform an MS/MS analysis of the isolated impurity (see MS/MS fragment spectrum in Figure 2). Possible elemental compositions can also be calculated for the measured individual fragments of the impurity. The comparison of the detected fragments with the expected fragments of a postulated structure allows checking their plausibility. In the ideal case, a structure valid with high probability can already be postulated at this point in time.

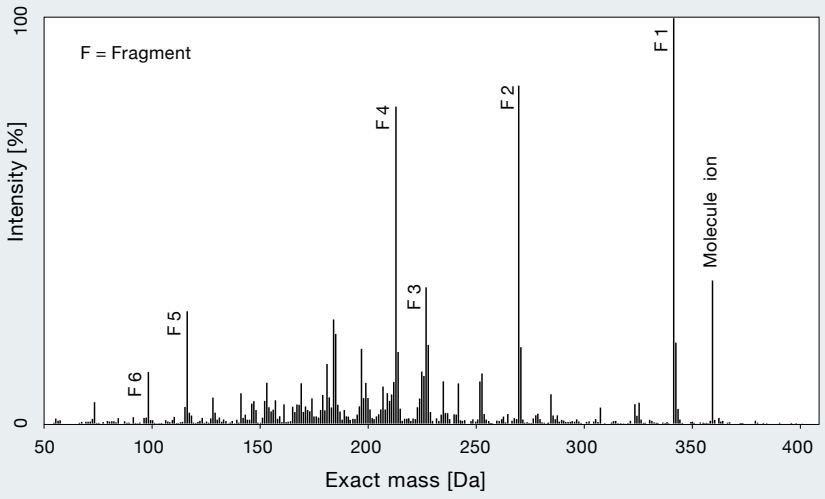
5. Fifthly, a method for the isolation of larger amounts of impurities by semi-preparative HPLC is being developed. The amount of impurity required for 2D-NMR measurements (approx. 3 - 5 mg) is then isolated from the drug preparation. Depending on the concentration of the unknown impurity in the product, quite large amounts of the drug are required.

6. Two-dimensional NMR spectroscopy (^{1}H, ^{13}C, COSY, NOESY, HSQC, HMBC) can be used to check the plausibility of the proposed structure. Furthermore, possible double bonds, residual groups (R1 - R4) and the absolute configuration of stereo centres can be determined (see structure of the opioid in figure 3).

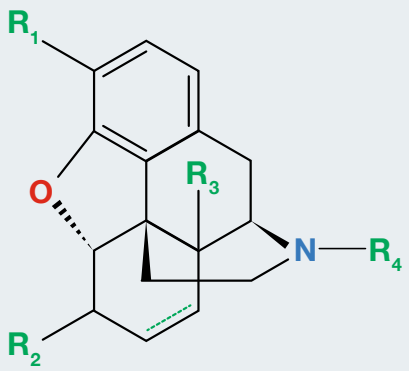
1 HPLC-UV-Chromatogram



2 MS/MS-Fragment-Spectrum



3 Postulated structure of the unknown opioid



Using a combination of high-resolution mass spectrometry (elemental composition, structural fragments) and two-dimensional NMR spectroscopy (functional groups, binding within the molecule, stereo configuration) as well as UV spectra, the postulated structure of an unknown impurity can usually be confirmed.

Optionally, in addition to the structure, the mechanism of impurity formation in pharmaceuticals can be investigated:

7. To study the reaction mechanism of the formation of a (now known) impurity, fresh and aged product is analysed and compared by HPLC-UV/MS and/or GC-MS. The differences found and the presence of small molecules (building blocks, catalysts) can provide information on the path of the formation of the impurity. Binary mixtures of active ingredient and excipients are also incubated with each other under selected reaction conditions to accelerate the formation of the impurity. In this way, excipients involved in the formation or impurities from the excipients can be identified

8. From the reaction mechanism (e.g. impurities from excipients) and the reaction conditions (e.g. temperature or humidity), the customer can define corrective and preventive actions (CAPA's) to optimise the manufacturing process of the product in order to prevent the formation of the impurity completely in the best case.

On the one hand, the elucidation of the reaction mechanism underpins the previously postulated structure of the impurity, on the other hand it can help to define appropriate actions to prevent the formation of the impurity. It is therefore highly recommended to determine not only the structure of an unknown impurity but also its path of formation.

The investigation of a possible reaction mechanism (step 7) can also help to clarify the structure of an impurity itself. If the substances involved in the formation of the impurity and the reaction conditions are known, conclusions can usually be drawn about the structure. In addition, under the right reaction conditions, the formation of the impurity can be accelerated, which may facilitate its isolation in step 5. Project-specifically, it can therefore be useful to include investigations regarding the reaction mechanism (step 7) between steps 4 and 5.

General approach (step 1-6)

Unknown impurity

1. Implementation of customer method:
· Reproduction HPLC-UV chromatogram
· Identification API/excipients/impurity

2. Small-scale purification for LC-HRMS:
· Isolation of a few micrograms of impurity
· Reference for MS analysis and large-scale purification

3. LC-HRMS Analysis:
· Determination of elemental composition
→ Postulation of structural proposals

4. MS/MS-Fragment-Analysis:
· Analysis of individual molecule fragments
→ Review of the postulated structural proposals

5. Purification for 2D-NMR:
· Method development for semi-preparative HPLC
· Isolation of a few milligrams of impurity

6. 2-Dimensional NMR-Analysis:
· Review of the structural proposals for plausibility
· Identification of the remaining groups (R1 – R4)
· Determination of the absolute configuration

Contamination formation & process optimization (step 7-8)

Confirmed structure

7. Reaction mechanism:
· Mechanisms of impurity formation
· Binary mixtures of active ingredient and excipients
· Comparison of fresh and aged product

Possible reaction mechanism

8. Process optimization:
· Definition of CAPA's by the customer for prevention of the impurity formation

Conclusion

The structure determination of unknown impurities from pharmaceuticals is a multi-stage process and requires sophisticated analytical methods such as high-resolution mass spectrometry. The basic schema for structure determination must be individually adapted for each pharmaceutical product and requires extensive analytical know-how. This includes detailed knowledge of decomposition processes of pharmaceutically active substances and possible side reactions with excipients. It is therefore advisable to develop a suitable strategy for the structure determination of unknown impurities in pharmaceuticals at an early stage. □

References

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