Synergistic strategies for impurity and contaminant monitoring within healthcare industries

Dr Catherine Frankis, Reading Scientific Services Ltd (RSSL)

The importance of impurity and contaminant analysis

Ensuring a product is free from unwanted contaminants and impurities is paramount in providing safe and effective healthcare products, whether these are pharmaceutical drugs or over-the-counter treatments. These issues can arise for several reasons including manufacturing process challenges, changes in raw materials or unexpected reactions of previously well characterised molecules within new formulation types. Additionally, the existence of fraudulent products can endanger patients' lives from the absence of an active ingredient or the unconsidered substitution of cheaper agents designed to 'bulk out' or ensure positive test results against rudimentary analysis. Within three case studies, key considerations when planning analytical strategies in this area are highlighted and discussed.

Techniques and considerations

Chemical analyses in many forms have long been used to ensure the provenance, reliability and safety of products, however one analytical technique alone seldom provides sufficient information to fully understand the identity of an impurity or contaminant, let alone the reason for its appearance. Possible techniques for investigation include chromatography (HPLC, GC, LC-MS), spectroscopy (NMR, FTIR, UV/Vis) and basic wet-chemistry methods. An issue will often require the holistic consideration of data from multiple techniques to gain a better picture of what, why, how and if possible, when the impurity/contamination first arose.

It is important to consider sample preparation strategies when planning investigations, especially when the use of spectroscopic techniques is planned, or where instrument sensitivity is of concern. A range of techniques can be employed, building up in complexity, depending on the relative concentration of the impurity/contaminant and the nature of other ingredients present. Some analytical techniques lend themselves better to in-depth analysis of a single component in a complex matrix, while others require an isolated sample of high purity to provide best results. Conversely, it can also be of value to consider a sample on the analytical data collected from the sample in its whole ('bulk') state, where an analytical fingerprint of all compounds present can be assessed, either visually or statistically, to aid non-targeted impurity/contaminant screening.

Case Study: Identification of an unknown impurity in a pharmaceutical product

Routine quality control (QC) analysis, commonly chromatography-based, can reveal unexpected peaks, which may indicate the presence of an unknown impurity. In these cases, it is crucial to understand the nature of the impurity to assess the toxicological impact on the product and to allow generation of a root cause hypothesis. The concentration of an unknown impurity in these instances may be low and so techniques which give superior sensitivity are often employed in the first instance. An analytical HPLC method can be transferred to high accuracy LC-UV-MS in order to determine a mass ion for the unknown impurity, and therefore also a molecular formula, considering the presence of common adduct forms (e.g. sodium, ammonium). The isotope ratio of certain atoms will also give rise to distinctive patterns in the mass spectra; for example the natural abundance of ^{35}Cl and ^{37}Cl will indicate the number of chlorine atoms present within an impurity structure. However, once generated, a molecular formula can give rise to a number of structural isomers. On some occasions, LC-MS/MS can be used to look for tell-tale fragments, but in other instances, techniques that provide inherent structural information, such as NMR, can be harnessed. Consideration of observed splitting patterns and coupling constants observed within the ^{1}H NMR spectrum can confirm the structural identity of an unknown compound (*Figure 1*). As a final confirmation step, once an impurity molecule has been identified, it can be analysed using the original HPLC method and the observed retention time will agree with the original unknown peak.

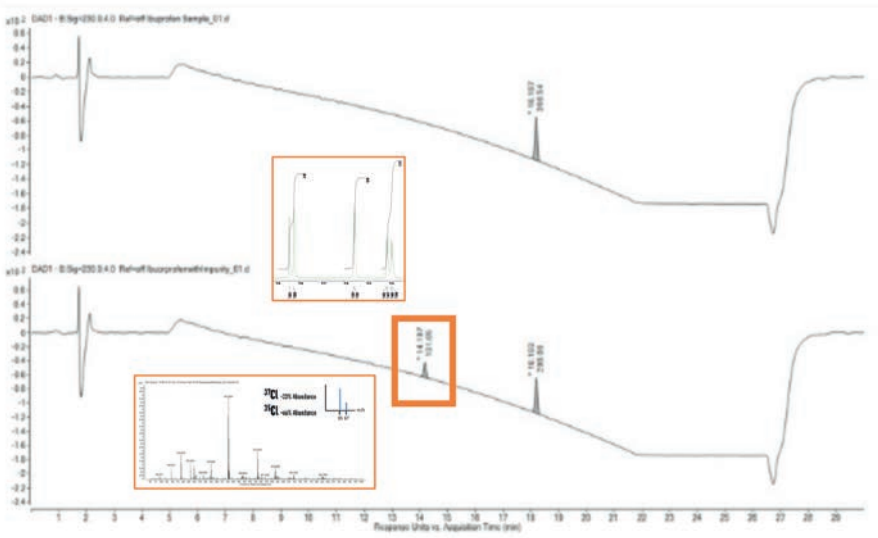

Figure 1: LC-UV chromatograms acquired from control (upper) and test (lower) samples, with impurity peak highlighted. Inset: LC-MS and NMR data used to aid structure elucidation.

Case Study: Nitrosamines – analytical strategies for mechanistic investigations

In recent years, the pharmaceutical industry has become concerned about the formation of nitrosamines within products, which can be formed either intramolecularly or by reaction of selected functional groups with low level nitrate and nitrite compounds, commonly found in a broad range of excipient materials. As such, there is a need to screen raw materials for the presence of nitrate and nitrite at sub-ppm levels. HPIC (ion chromatography) with conductivity detection is a popular method for anion screening and recent advances in hardware and consumables available now mean that detection levels as low as 1 ppb can be achieved in solution. In certain materials, however, high levels of chloride may interfere with nitrite determination, and in such instances alternative detection methods, such as UV-visible absorption or MS, can be used (*Figure 2*). It's also important to consider the effect of excipient properties on the performance of the analytical method; for example, water-soluble polymeric excipients (e.g. copovidone) can collect on the column and affect the method performance without suitable dilution and/or column clean-up procedures. Overall, this

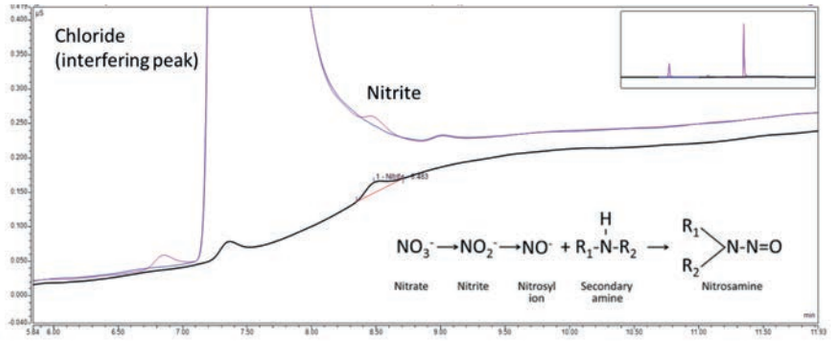

Figure 2: HPIC chromatograms acquired for nitrite standard, test material and spiked sample. Presence of chloride peak may affect accuracy of method and alternative detection would be recommended.

analysis allows manufacturers to understand which products are more susceptible to nitrosamine formation and risk assess accordingly.

Case Study: Detection of counterfeit pharmaceutical products

The presence of fraudulent healthcare products in the open market is a serious concern for manufacturers and regulators alike, jeopardising brand reputation and costing companies millions in lost revenue; most crucially, they pose a serious risk to patient safety. The identification and subsequent analytical investigations play a major role in tracking down and bringing perpetrators to justice. Analytical assessments often work up in complexity, focussing first on any obvious differences in packaging or printing, either visually or using light microscopy. Spectroscopy-based techniques, such as FTIR and Raman, are also used to compare dyes and adhesives present on suspect products with those used on their genuine counterparts. More advanced techniques can also be used to understand microstructure similarity; for example, X-ray tomography can assess the uniformity of tablet coating. In terms of chemical analysis, non-targeted methods of profiling the product itself should be implemented, allowing conclusions to be drawn regarding similarity of the suspect sample to the genuine, as well as the presence of any potentially harmful compounds. Techniques should be carefully selected to ensure both organic and inorganic components are profiled, for example a combination of energy dispersive X-ray spectroscopy and NMR or FTIR could be employed (*Figure 3*). On occasions, the chemical difference between a genuine and fraudulent product could be subtle, however it is still possible that a product has been manufactured with lower grade excipient material, meaning the resulting product will not pass high regulatory standards. In these cases, specific differences in materials may need to be targeted; for example, the difference in pharmaceutical grade cellulose and a lower quality material can be identified by looking at degree of crystallinity, either using solid state NMR or powder XRD.

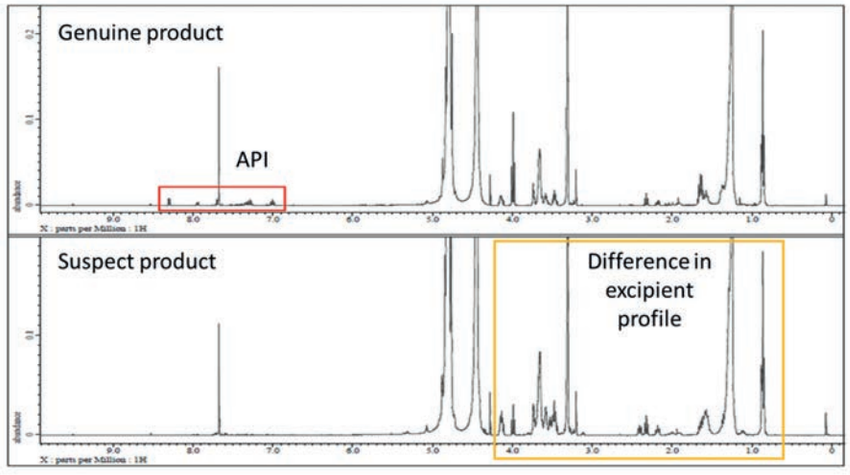

Figure 3: ^{1}H NMR spectra of genuine and suspected fraudulent healthcare product, showing absence of API signals but also differences in excipient profile.

Conclusion

The identification of impurities and contaminants, whether inherent to the matrix or the result of external factors, are critical in assuring continued patient safety. One technique alone will seldom provide all the necessary information to either fully elucidate, or to allow the necessary risk assessment to be undertaken. The list of analytical techniques in the modern laboratory environment can be extensive, so it is important to consider whether any particular technique will provide sufficient selectivity or sensitivity, or whether additional sample preparation steps need to be implemented prior to analysis. Finally, when assessing the impact of a contamination or possible fraudulent product, it is necessary to harness non-targeted methodologies to ensure no potentially harmful components to patients are missed.

About the author

Dr Catherine Frankis is a Senior Scientist II at RSSL who works across the Functional Ingredients and Investigative Analysis teams, specializing in ion exchange chromatography and nuclear magnetic resonance spectroscopy. Frankis works on projects covering a wide variety of issues, including contamination, adulteration, and the development and validation of new analytical methods within the food, pharmaceutical, and cosmetic industries. In 2010, Frankis joined RSSL after completing her PhD in the synthesis and characterisation of stereoselective biopolymer initiators at the University of Bath, where she used a variety of analytical characterisation and thermal property techniques.