A Streamlined Workflow For The Profiling Of Impurities Using High Resolution Accurate Mass Spectrometry

ABSTRACT

Impurity profiling is a key analytical workflow used within many stages of the drug discovery pipeline. It is used to identify, characterize and quantify impurities in Active Pharmaceutical Ingredients (API) and pharmaceutical products.

In this study we utilise high resolution accurate mass spectrometry to obtain the impurity profiles for a pharmaceutical product containing the active ingredients of Paracetamol and Dextromethorphan.

INTRODUCTION

Impurities within a pharmaceutical product carry cause for concern as they can lead to side effects, toxicity and/or impact the drug's activity. Therefore this process is required to ensure consumer safety and product quality.

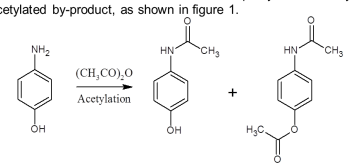
Regulatory authorities such as the US Food and Drug Administration (F.D.A), European Medicines Agency (E.M.A) and The International Conference on Harmonisation (I.C.H) have standardized levels at which impurities need to be identified and qualified. The I.C.H has four working guidelines for impurity characterization, Q3A – Q3D covering Impurities in New Drug Substances, New Drug Products, Guideline for Residual Solvents and Guideline for Elemental Impurities. [1-3]

The type of impurities are classified into four groups; organic impurities, inorganic impurities, residual solvents and genotoxic.

Organic impurities occur through the manufacturing and/or storage process of a new drug substance. Organic Impurities include:

| Raw materials | Reaction intermediates | By products | Excipients | Reagents, ligands and catalysts |

In the production of Paracetamol the final step in the synthesis is acetylation of p-aminophenol. Therefore in the final product it is possible to find this intermediate as an impurity. In the acetylation process it is possible to synthesize the di-acetylated by-product, as shown in figure 1.



p-aminophenol **Paracetamol** **Di-acetylated by-product**

Figure 1. The synthesis of Paracetamol from the intermediate p-aminophenol

Inorganic impurities typically result from the manufacturing process and are known and identified and include:

| Reagents, ligands and catalysts | Heavy metals or other residual metals |
| Inorganic salts | Other materials (e.g. filter acids, charcoal) |

The third class of impurities are residual solvents which are used in the preparation of solutions and/or suspensions when the pharmaceutical product is manufactured. These are sub-categorized into three classes; Class 1, class 2 and class 3. These cover solvents to be avoided, limited and with low toxic potential, respectively.

Traditionally, impurity profiling is performed using liquid chromatography with analog detection like UV/Vis and Diode Array. These techniques are effective when using very high concentrations to detect impurities within the specified levels. However low level impurities can be missed if they co-elute with several other peaks making identification difficult and/or they do not have a good chromophore.

Coupling of LC-UV to high resolution accurate mass spectrometry (HRAMS) systems is becoming more routine. This allows the acquisition of the impurity profile and provide the identity and structure of the detected impurities. To aid the process of identification and elucidation software is very important as manual interpretation is time consuming. In this study we look at a new software platform called ImpurityPilot™. ImpurityPilot™ allows the user to profile, identify, and structurally elucidate the potential impurities.

MATERIALS AND METHODS

Sample Preparation: The pharmaceutical product was supplied in two forms; with and without the Active Pharmaceutical Ingredients (API). The product was supplied as a solution with a simple dilution with the mobile phase performed for LC-MS/MS analysis

UHPLC Conditions: A Shimadzu Nexera LC system with an UV/Vis detector in line was used (wavelength 254nm). A Phenomenex Kinetex C18, 100x2.1mm, 2.6µm column at 40° C with a gradient of mobile phase A of 10mM ammonium formate in water and mobile phase B 10mM ammonium formate in acetonitrile/water (95:5) was used at a flow rate of 700µL/min. The injection volume was set to 15µL. A 14 minute gradient elution profile was utilized with a total runtime of 18 minutes.

HRAMS Conditions: A SCIEX TripleTOF® 5600+ high resolution accurate mass (HRAMS) LC-MS/MS system with the DuoSpray™ source and Electrospray Ionization (ESI) probe was used.

Software: Analyst® TF 1.7 control software and ImpurityPilot™ *beta* software were used.

RESULTS

To achieve the profiling of the pharmaceutical product Information Dependent Acquisition (IDA) was utilized to simultaneously acquire high resolution MS and MS/MS data. As part of the IDA strategy a couple of real time acquisition filters were employed; Dynamic Background Subtraction (DBS) and the Real-Time Multiple Mass Defect Filter (RT-MMDF). Both strategies are used to help acquire MS/MS spectra on ions of interest and not background ions. Figure 2 shows the MS spectra for one identified impurity of Dextromethorphan (loss of CH_2).

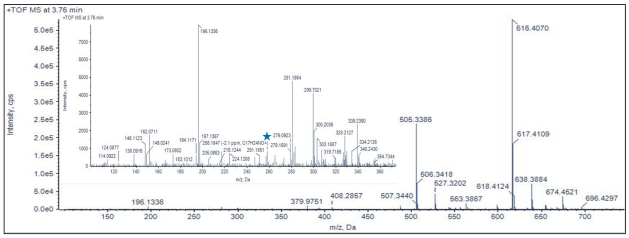


Figure 2. MS spectrum at a retention time of 3.76 minutes. Inset MS spectrum zoomed between *m/z* 100-370

Figure 2 shows the difficulty faced by standard IDA acquisition. In this case the impurity is at very low intensity and outside of the top 15-20 most abundant *m/z* features. This results in no MS/MS spectrum being acquired in standard IDA mode. Therefore an additional IDA acquisition strategy is required to collect MS/MS, this is where the RT-MMDF comes in.

Mass defect filtering has been shown to be a powerful tool in identifying compounds that are related in structure and composition to a parent molecule. Traditional mass defect filtering is performed post MS data acquisition to identify the related compounds. This is followed by a second injection to collect the MS/MS spectra for these compounds of interest.

The unique feature of the real-time MMDF is it being applied during acquisition in the IDA logic. This allows the prioritization of MS/MS acquisition on related compounds and then the normal IDA logic is applied to collect MS/MS on all other candidate ions. Figure 3 shows the benefit of using the RT-MMDF algorithm.

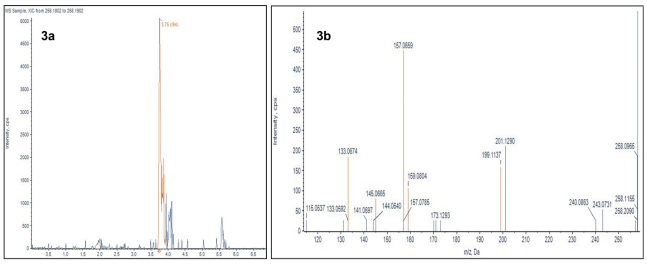


Figure 3. 3a. Extracted ion chromatogram for the loss of CH_2 impurity for Dextromethorphan. **3b**. MS/MS triggered using the RT-MMDF.

Figure 3a shows the chromatographic peak for the identified impurity of the loss of CH_2. It is a low level impurity at 0.1% of the parent. When running under standard IDA acquisition logic monitoring the top 15 candidate ions MS/MS was not acquired. As shown in figure 3b, this is the MS/MS spectrum acquired when the RT-MMDF is being utilised in the acquisition logic. The MS/MS is of good quality and sensitivity to allow comparison to the parent MS/MS and to identify the impurity. Table 1 shows the impurities for Dextromethorphan comparing standard IDA to RT-MMDF acquired data.

Impurity Name	Formula	MS/MS IDA	MS/MS MMDF	MS - % Area of Parent
Parent	C18H25NO	Yes	Yes	-
Loss of CH2	C17H23NO	No	Yes	0.1%
Ketone Formation	C18H23NO2	Yes	Yes	0.3%
Oxidation	C18H25NO2	No	Yes	<0.1%

Table 1. Identified Impurities for Dextromethorphan from ImpurityPilot™ *beta* software

As shown in table 1, when using IDA acquisition the two lowest identified impurities at 0.1% did not have MS/MS spectra. When the RT-MMDF was added into the IDA logic we find all impurities have MS/MS spectra. Table 2 shows the identified impurities for Paracetamol with a comparison of IDA MS/MS to MMDF MS/MS.

Impurity Name	Formula	MS/MS IDA	MS/MS MMDF	MS - % Area of Parent
Parent	C8H9NO2	Yes	Yes	-
Loss of C_2H_2O (4-aminophenol)	C6H7NO	Yes	Yes	9.1
Paracetamol Dimer	C16H18N2O4	Yes	Yes	0.5
Loss of O	C8H9NO	No	Yes	0.1
Acetylation	C10H11NO3	No	Yes	0.1
Methylation	C9H11NO2	Yes	Yes	2.8
Oxidation	C8H9NO3	Yes	Yes	0.2

Table 2. Identified Impurities for Paracetamol from ImpurityPilot™ *beta* software

For Paracetamol the two lowest identified impurities at 0.1% did not have MS/MS spectra under standard IDA acquisition. When the RT-MMDF was added into the acquisition all impurities have MS/MS spectra.

The next step once MS/MS has been acquired for all impurities is to perform structural elucidation.

ImpurityPilot™ *beta* software has a second section in the results space, which is the interpretation tools. This takes any potential impurity and allows the user to perform structural elucidation and in-silico fragmentation to match the experimental MS/MS to the theoretical fragmentation of the proposed structure. In figure 4 an example is shown for the intermediate impurity of Paracetamol; p-aminophenol.

The parent structure is imported and modified to achieve the proposed impurities structure. Once this is completed the structure is *in-silico* fragmented and the theoretical fragments compared to the experimental MS/MS spectrum. If the structure matches the MS/MS then all fragments will be assigned with good mass accuracy. In this case all of the major fragments are assigned and we can verify them as highlighted.

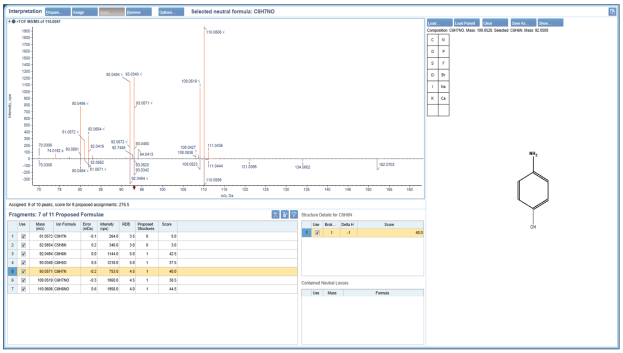


Figure 4. ImpurityPilot™ *beta* - Structural elucidation and *in-silico* fragmentation matching to MS/MS

Once this process is completed we have identified and given structures to all possible impurities. Finally batch to batch correlation to the original fully characterized product can be performed.

CONCLUSIONS

A novel workflow combining the TripleTOF® 5600+, the Real-Time Multiple Mass Defect Filter and ImpurityPilot™ *beta* software is a streamlined workflow for impurity profiling. The identification has been achieved for all impurities at or above the I.C.H minimum limit of 0.05%.

REFERENCES

1 I.C.H Harmonized Triplicate Guideline, Impurities In New Drug Substances Q3A(R2), Current Step 4 version dated 25 October 2006

2 I.C.H Harmonized Triplicate Guideline, Impurities In New Drug Products Q3B(R2), Current Step 4 version dated 2 June 2006

3 I.C.H Harmonized Triplicate Guideline, Impurities: Guideline For Residual Solvents Q3C(R2), Current Step 4 version dated 4 February 2011

TRADEMARKS/LICENSING