Introduction

Trace impurities in synthetic products that interact with human end users or may have an undesirable environmental fate are regulated by various government agencies such as FDA and EPA. As a result, impurity separations and their structural identification is an important research area in many industries, including pharmaceutical, agrochemical, foods and consumer products. Full chemical identification requires structure elucidation of the separated compound using spectroscopic techniques, mainly mass spectrometry (MS) and NMR spectroscopy. However, MS alone is often insufficient to unambiguously identify a compound, especially in the case of isomers. This often necessitates obtaining the isolated pure compounds of interest using purification procedures.

In this study it will be shown how a workflow to achieve the full structural elucidation of trace impurities can implemented using preparative supercritical fluid chromatography (SFC) to isolate trace impurities. As an example demonstrating this capability, a commercial formulation of the fungicide propiconazole will be used. (Figure 1) This fungicide has the structural potential for the existence of several stereoisomers and the propiconazole product as obtained commercially contains both isomers and also related trace impurities at approximately the 1% level. (Figure 2) Some propiconazole impurities have previously been structurally identified in the literature. The described SFC based workflow is generally applicable for impurity isolation and offers many advantages, including high speed and efficiency, fast dry-down, quick turnaround time, as well as the environmental sustainability benefit of lower solvent consumption.

Results and Discussion

150 mL of a commercial fungicide formulation containing propiconazole as the active ingredient at a concentration of 1.55% was suspended in 5% NaHCO₃ solution then extracted with dichloromethane 3 times. The combined extracts were dried over NaSO₄, filtered then concentrated. The expected amount of 2.3 g of active ingredient was contained in 4.5 g of crude extract that contained other inactive ingredients including various surfactants. These crude extracts were then analyzed using UPLC chromatography. In both achiral and chiral modes (Figure 2) trace impurities at < 1% of the amount of the original 1.55% claim amount of the active ingredient, with similar isomers to the main active ingredient, were observed. Methods were then developed to scale separations to preparative chromatography. The achiral prep chromatography was carried out using a Prep 80 instrument with a 19 mm i.d. BEH column. Analysis showed the two impurity peaks were obtained in 98% purity (Figure 3). Sufficient pure material was collected both to enable full structural assignments of the trace impurities as well as to carry out further chiral separations of each peak into their individual enantiomers. MS analysis confirmed that the two isolated impurity peaks were isobaric with each other as well as with the main ingredient suggesting that they were indeed structural isomers. 1H, 13C, 2D and NOE NMR experiments revealed that these two impurities differed from propiconazole itself by the nitrogen attachment point of the triazole moiety to the methylene group on the dioxolane ring (Figure 1). This result is clearly evident due to symmetry in the NMR. The propiconazole, being attached at the N adjacent to the other N is not symmetric, while in the impurity the N has a C atom adjacent on both sides of the nitrogen, giving symmetry to the triazole moiety and a simpler NMR. The assignment of the cis and trans isomers results from the fact that a strong NOE is seen in both a 2D NOESY NMR experiment as well as in a 1D NOE spectrum between the protons attached to carbons 11 and 14 and those on carbon 6 in the isomer assigned as cis, while this same NOE was not observed in the NMR spectrum of the isomer assigned as trans.

Having completed the full structural assignments for the two impurity peaks that can be resolved with achiral chromatography, attention was next turned to resolving the enantiomers for both the cis and trans isomers. While all four isomers can be resolved in a single run analytically on a small particle size chiral column, somewhat surprisingly, this was not the case at all upon scale up. In fact, two entirely different column chemistries were required for the separation of each enantiomeric pair. The cis isomer separated on the IC column chemistry while the trans isomer required an AD-H column. All four of the possible impurity stereoisomers were then obtained in their pure form. Chiral analysis using UPLC demonstrated that each isomer had an enantiomeric excess >98%. The cis isomer was comprised of the 2R, 4S and the 2S, 4R isomers, while the trans impurities contained the 2R, 4R and the 2S, 4S isomers. These isolated impurities do in fact possess the same structures as those found in previous study. The assignment of the absolute configurations for each of the stereoisomers was beyond the scope of this study. The capability to have all four of the impurity stereoisomers in hand, in their pure form, in sufficient supply, then allows for the unambiguous assignment of the mechanistic potencies and toxicities of each individual impurity species. This then enables improved knowledge of the efficacy and safety of the product mixture ingredients.

Conclusions

- Acquity UPC² analyses of actual product mixtures, using both chiral and achiral column chemistries, are useful for both impurity profiling and also for developing separation methods suitable for eventual scale up to preparative separations.
- Trace impurities, as in this case < 1% of a 1.55% formulation of the active ingredient, can be efficiently isolated in the amounts needed for structural elucidation and other needed studies using preparative SFC.
- The isolated impurities are able to be obtained in their pristine forms, thus enabling the facile application of HRMS and also for 1D and 2D NMR spectroscopic studies, allowing for the full structural elucidations of these trace impurities.
- Initially isolated impurities can be further separated into their enantiomeric pairs through the implementation of chiral stationary phase preparative SFC with high efficiency.
- The assigned structures for the all of the isolated impurities were in agreement with information in the prior literature.

References