THE APPLICATION OF 2D HPLC TO ADDRESS THE NEED TO INVESTIGATE THE CHIRAL PROFILE OF ACTIVE INGREDIENTS AND THEIR METABOLITES IN ENVIRONMENTAL FATE AND METABOLISM STUDIES

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ABSTRACT

The recently published guidance from EFSA on risk assessments for active substances that contain stereoisomers provides recommended approaches to address and assess data requirements for active substances with the same molecular formula but different three-dimensional orientations. One approach is to study different isomers of an active ingredient and individually assess each isomer, but this approach is both costly and time consuming, requiring the synthesis of the individual isomers. Chromatography is a constantly evolving technique and while the concept and application of 2D-HPLC has been around for a number of decades, recent improvements in equipment and column technology allows the routine use of 2D-HPLC in a reliable and robust manner. The use of 2D-HPLC to couple reverse phase profiling methods with reverse phase chiral methods along with tandem mass spectrometry – with or without radiolabelled analysis – enables the investigation of chiral profiles of agrochemicals and their metabolites without the need for separate isomer studies or extensive sample isolation, workup, and subsequent chiral chromatography. This presentation discusses the practical application of 2D reverse phase-chiral separations and their potential to become a routine approach to analysis in environmental fate and metabolism studies when considering the data requirements to fulfil the new EFSA guidance.

BACKGROUND

An estimated 25% of active substances (a.s.) contain at least one asymmetric centre, current EFSA guidance requires that enough information should be obtained to characterise the risk to the environment resulting from stereoisomer behavior in plants, animals and non-target species. Registration of active substances require this information and the formation and effects of stereo-active active substances and their metabolites should be investigated to assess their biological activity. This requires assessment of stereoisomer composition in a number of matrices across plant, animal and fate studies. Typically preferential isomer transformation and/or interconversion of isomers would be determined and significant changes in isomeric ratio would be assessed. Traditionally this would have involved isolating peaks from reverse phase analysis of sample extracts, sample work-up and subsequent analysis using a chiral method. These chiral methods were often incompatible with reverse phase HPLC and risked interconversion of isomers on work-up or suffered from quantitative losses on work-up resulting in inaccurate information for risk assessment.

INSTRUMENTATION

2D-HPLC has been utilised for novel separations for many years and the automation of the technique is now commonplace. HPLC column manufacturers have developed a much larger range of reverse phase (RP) compatible chiral columns in recent years and this allows the combination of typical profiling HPLC methods with chiral analysis. This presents a number of advantages when investigating the stereoisomer profile of an a.s. and its metabolites, namely;

- Chiral analysis directly relates to the profiling method
- No additional sample manipulation is required, this minimises procedural losses
- Interconversion of isomers is minimised
- Can easily be interfaced with LC-MS for confirmatory analysis
- Throughput is maximised

2D-HPLC Enables the investigation of stereiosomers without extensive sample work-up

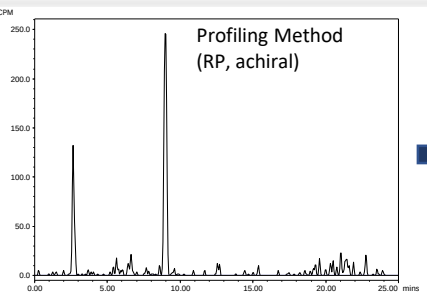

Fig. 1 RP achiral profiling HPLC method showing the parent compound at *ca* 9 minutes.

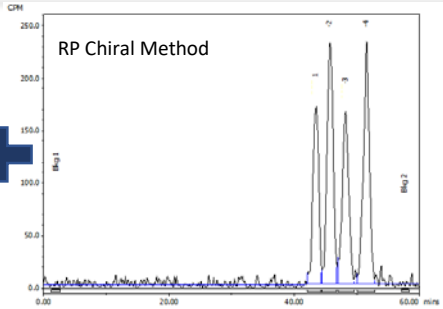

Fig. 2 RP chiral separation of the parent compound.

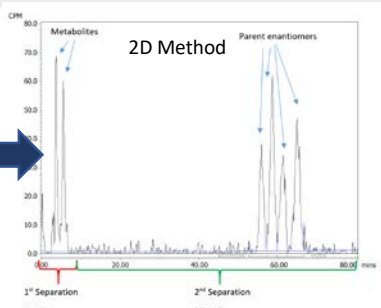

Fig. 3 2D RP chiral separation of the parent compound, the 9 minute peak was automatically "heart cut" to the chiral column.

CONCLUSIONS

The coupling of reverse phase chiral methods with reverse phase achiral methods via 2D-HPLC analysis allows the accurate investigation of the isomer profile of active substances and their metabolites without the need for time consuming and potentially detrimental sample workup. Furthermore, advancements in column and HPLC technology allows the routine application of such separations in regulatory studies.

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