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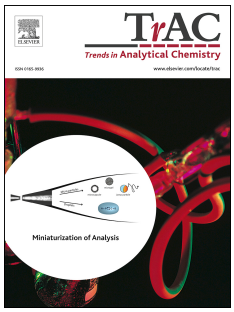

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Stereoselective LC-MS/MS methodologies for environmental analysis of chiral pesticides

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Abstract

Chiral pesticides can exert stereospecific toxicity in contaminated environmental compartments. Therefore, measuring pesticides at the enantiomeric level is essential to assess the risk posed to exposed organisms, including humans. In recent years, there has been rapid progress on the development and application of stereoselective liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies for monitoring pesticides in the environment. Coupling chiral LC separations with MS/MS detection enables trace enantiomeric determination of pesticides in complex environmental matrices. The intent of this review is to provide an up-to-date synopsis on recent advances of stereoselective LC-MS/MS methodologies for pesticide analysis. Key aspects of these methodologies discussed include sample storage and extraction method, stationary phases for separation, multi-residue separations, and method of quantitation. Finally, future trends in this rapidly growing field of analytical chemistry research are outlined.

Key words: pesticide; chiral; enantiomer; LC-MS/MS; polysaccharide; environment; multi-residue; fungicide; herbicide; insecticide

1. Introduction

Increasing pressure on food production has resulted in the continued development and use of pesticides. Their persistence and mobility has seen them detected throughout the environment including soils, sediments, plant matter, river water and ground water [1-7]. The potential negative long-term health impacts to exposed organisms, including humans, remain largely unknown [8-10]. Consequently, legislation is in place to control the use and exposure to pesticides. For example, the European Union's Drinking Water Directive stipulates a limit of 0.1 µg L^{-1} for individual pesticides in water for human consumption. However, an important consideration for environmental pollutants such as pesticides which is not currently addressed in legislation, is chirality. This is despite of a high percentage of them being chiral (e.g., a compiled list of 1,693 pesticides identified 482 (28 %) as chiral [11]).

Chiral compounds can exist in the form of enantiomers which have identical chemical structures but different spatial arrangements around the stereogenic centre and are thus non-superimposable stereoisomers (Figure 1). Enantiomers have identical physico-chemical properties, however they often exhibit different pharmacokinetics and pharmacodynamics that can result in enantiomer dependent toxicity [12-14]. For example, *in vitro* tests with *Phythophthora infestans* and *Pythium ultimum* found the fungicide *R*-metalaxyl to be ~1,000 times more active that *S*-metalaxyl [15]. Toxicity testing has found stereoselective toxicity for a number of pesticides including the insecticides profenofos and fonofos towards *Daphnia magna* and *Ceriodaphnia dubia* [16]. The insecticide fipronil has also been found to exhibit stereoselective toxicity towards *C. dubia* [17]. In this study *S(+)*-fipronil was found to be 5 times more toxic than *R(-)*-fipronil during acute exposure. Despite enantiomers of chiral pesticides differing in their toxicity, they are normally produced as a racemic mixture (i.e., equimolar concentration of individual enantiomers) [18].

Stereoselective degradation of chiral pesticides has been observed in soils, sediments, water and plants [19-22], which can lead to the enrichment of the more or less toxic enantiomers. This process can be driven biotically (e.g., by bacterial action) and abiotically (e.g., by chemical reaction). To demonstrate, in anaerobic sediments *S(+)*-fipronil was preferentially degraded over *R(-)*-fipronil [23].

A racemic enantiomeric fraction (0.55) was converted to 0.10-0.11 within 8 days. On the other hand, *S*-metalaxyl (inactive enantiomer) was found to degrade faster than *R*-metaxyl in aerobic soil with pH <4 and in anaerobic soils [24]. However, enrichment was found to proceed in either direction and was dependent on the specific soil conditions. Furthermore, racemization is also possible whereby one enantiomer is converted to another. For example, racemization of the fungicide triadimefon has been observed in water [25] and in soil [26]. Li et al., [26] reported the conversion of *S(+)*-triadimefon to *R(-)*-triadimefon in sterile soils, and was pH dependant. Nevertheless, enrichment (or racemization) of enantiomers of varying toxicity in environment compartments requires their quantitation as separate entities.

Gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE) have all previously been used to separate chiral pesticides at the enantiomeric level [23, 27, 28]. CE is the least popular method for environmental analysis as it is not routinely coupled to mass spectrometry (MS). In GC-MS and LC-MS methods, the most popular means of enantiomer separation is direct, using chiral stationary phases. Here separation is reliant on the 3 point model whereby 3 points of contact between the enantiomer and chiral stationary phase are needed to achieve chiral recognition [29]. A review of ~500 pesticides by Alder et al. [27] concluded that LC-MS is superior to equivalent GC-MS systems due to its wider scope of study and superior sensitivity. The shortcomings of GC-MS are its inability to analyze samples of low volatility, high polarity, or thermal instability. Furthermore, the general trend of new pesticides on the market becoming more polar in nature makes LC-MS the popular choice for analysis [30]. The improved specificity and sensitivity offered by tandem mass spectrometers (MS/MS) such as triple quadrupole is essential for environmental analysis. Therefore, the aim of this review is to appraise up-to-date stereoselective LC-MS/MS methodologies for the determination of chiral pesticides in environmental samples.

2. Pesticide stability in collected samples

Sampling is a fundamental process in the determination of pollutants in the environment. It is potentially the largest source of error yet it often receives little attention [31, 32]. An important consideration during collection and storage of biologically active matrices such as environmental samples is analyte stability [33, 34]. In particular, chiral analytes have the potential to undergo stereoselective transformation during sampling and storage.

Stereoselective LC-MS/MS methodologies reported in the literature store liquid samples such as river water prior to extraction and instrumental analysis at -20 ˚C (Table 1). Alternatively samples are filtered (0.45 μm), adjusted to pH 1.5 and stored at 4 ˚C [35]. These approaches are adopted to mitigate any potential stereospecific (and non-stereospecific) changes due to microbial activity. On the other hand, care must be taken that the change to pH does not induce any abiotic processes which cause pesticide loss or racemization. Similar to liquid samples, the common approach to store solid samples prior to analysis is at temperatures of -20 ˚C or -40 ˚C (Table 1). Alternatively solid samples have been air dried and stored in the dark at 4 ˚C or at room temperature [36]. However, air drying samples (instead of lyophilizing) can increase the risk of sample contamination. Li et al. [37] investigated the stability of the chiral insecticide flufiprole and flufiprole-amide in a variety of matrices including rice, rice straw, water and soil stored at -20 ˚C. Samples were spiked at environmentally relevant parts-per-billion (ppb) concentrations levels and analysed monthly. It was found that no significant changes to the overall concentration or enantiomeric distribution were observed for either analyte.

The potential influence of abiotic processes on stereoisomeric composition of chiral pesticides during sample storage and processing also needs considered. Racemization of malathion, phenthoate and fenpropathrin has been found in methanol, ethanol and water [25]. The extent of racemization was affected by both temperature and pH, with this process proceeding more rapidly at neutral pH than pH 5.8. It was proposed that racemization occurred via proton exchange at the stereogenic centre [25]. The synthetic pyrethroids cypermethrin and cyfluthrin have also been found to be unstable in sterile water. Isomer conversion at the αC resulted in the stereoisomer converting to an epimer at rates of

0.050 and 0.044 day^{-1}, respectively [16]. On the other hand both *cis*-bifenthrin and permethrin were stable. Such information is important to consider during sample storage and is often not investigated (or reported) during development and validation of new methodologies (Table 1).

3. Extraction and clean-up methods

Several extraction and clean-up methods are utilized for chiral pesticides in environmental samples prior to stereoselective LC-MS/MS (Table 1). Although extraction techniques should not be stereoselective in nature, it is important they achieve adequate recovery (and reproducibility) of pesticides from complex environmental matrices whilst reducing matrix interferences prior to instrumental analysis. The most popular method of extraction and clean-up is the quick, easy, cheap, effective, rugged and safe (QuEChERS) technique combined with (dispersive) solid phase extraction (SPE) [38-43]. Such techniques are well established and have been discussed in detail elsewhere [44, 45]. Therefore, only the most up-to-date and alternative sample extraction methods are discussed here.

Contemporary extraction methods in the literature report the use of microextraction techniques [35, 36, 46-48] (Table 1). Combining matrix solid phase dispersion (MSPD) and dispersive liquid-liquid microextraction (DLLME) is proposed as a method to reduced matrix interferences and improve sensitivity [49, 50]. Zhao et al. [48] optimized MSPD-DLLME for the determination of 8 chiral pesticides (log K_{OW} 1.7-4.3) in soil and sediment at the enantiomeric level. For MSPD 0.1 g of soil or sediment was blended with 0.4 g of C18 sorbent and packed into an empty SPE cartridge. Analytes were eluted in methanol and dried prior to the addition of 5 mL water ready for DLLME. 960 µL of acetonitrile as the dispersing solvent and 550 µL dichloromethane as the extraction solvent was injected rapidly into the aqueous sample [48]. Following 1.6 min sonication the homogenized emulsion was centrifuged. The extraction solvent was removed and evaporated to dryness before reconstitution in mobile phase and enantioselective LC-MS/MS analysis. Overall method recovery

ranged from 62-95 % for sediments and soils, with RSDs ≤10 % [48]. DDLME has also been successfully applied for the extraction of six chiral pesticides in river water and wastewaters [46].

Supramolecular solvents are proposed as an alternative means of extracting pesticides from environmental samples. Supramolecular solvents are nano-structured water immiscible liquids comprising three-dimensional amphiphilic aggregates [51]. They are suitable for the extraction of polar analytes due to the polarity of the amphiphile head groups as well as the high concentration of amphiphiles in the solvent (0.1-1 mg μL^{-1}) [35]. Caballo et al. [36] adopted supramolecular solvent-based microextraction (SUSME) for the determination of mecoprop (log K_{OW} 3.1) and dichlorprop (log K_{OW} 3.4) in water and soil. In this study, reverse aggregates of dodecanoic acid was used for microextraction. Water was added to dodecanoic acid dissolved in tetrahydrofuran causing the assembly of dodecanoic acid and formation of oily droplets (at pH <4) [35]. The proposed mechanism of extraction into the supramolecular solvent is via hydrogen bonding and dispersion interactions between the hydrophobic moieties of mecoprop and dichloroprop and the hydrocarbon chain of dodecanoic acid. The less dense supramolecular extract (approximately 270 µL) was removed, dried and 100 mM acetate buffer pH 5 added for back-extraction. The aqueous extract was separated from insoluble matrix extracts and solid dodecanoic acid by centrifugation prior to LC-MS/MS analysis. Analyte recoveries using the SUSME technique was 73-80 % (RSDs ≤4 %) in water samples and 66-83 % (RSDs ≤6 %) in soils samples [35, 36].

An alternative sample extraction method based on the use of magnetic SPE utilizing multi-walled carbon nanotubes (MWCNTs) has been reported by Zhao et al., [47]. MWCNTs represent an emerging adsorbent comprising tubular graphite sheets. The incorporation of nanoparticles of magnetic properties (e.g., Fe_3O_4) into their structure enables easy phase separation, overcoming the limitations of MWCNTs used in conventional SPE mode (e.g., blockages and loading time). In the study by Zhao et al. [47], amino functionalized MWCNTs were synthesized to increase the hydrophilicity of nanotubes which improves dispersion in water and analyte contact time. For the analysis of water samples (200 mL), 75 mg of the synthesized composite was added and subject to shaking at 150 rpm for 12 min for complete analyte adsorption. The MWCNTs were then separated

from the water using a magnet and dispersed in acetonitrile for analyte desorption. Acetonitrile was evaporated to dryness and reconstituted in mobile phase ready for instrumental analysis. A total of 18 chiral pesticides including triazole fungicides, organophosphate insecticides, phenoxyalkanoic acid herbicides, phenylpyrazole and neonicotinoid insecticides were successfully extracted. The studied pesticides encompassed a range of chemical properties. For example, dinotefuran is considered hydrophilic with a log K_{OW} -0.6 whereas profenofos is comparatively hydrophobic with a log K_{OW} 4.7. The optimized protocol achieved analyte recoveries in the range 80-106 % (RSDs ≤13 %) for a variety of environmental matrices including river waters, wastewaters, soils and sediments.

4. Stereoselective LC-MS/MS methods

4.1. Polysaccharide stationary phases

Reported stereoselective LC-MS/MS methods all adopt chiral stationary phases to achieve direct enantioseparations (Table 1). Among commercially available chiral stationary phases, polysaccharide phases are popular due to their high selectivity, sensitivity and reproducibility [39, 42, 52], and it is reported that 95 % of chiral compounds have been resolved by polysaccharide phases [53]. These macromolecular chiral selectors are either amylose or cellulose based. However, cellulose and amylose in their native state results in poor resolution and peak broadening due to slow diffusion of analytes through the polymer network [39]. To overcome this shortcoming, derivatives of amylose and cellulose were synthesized. This involves the reaction of hydroxyl functional groups with appropriate reagents to produce derivative forms such as cellulose or amylose tris-(3,5-dimethylphenylcarbamate) (Figure 2). Such derivatives have a high number of chiral centres in the ordered polysaccharide backbone, as well as the phenyl ring and carbamate groups providing sites for π-π and hydrogen bonding interactions. Both the backbone structure and derivative group influence enantioseparations. To demonstrate, Pan et al. [42] compared cellulose and amylose with the same derivative in their structure (tris-(3,5-dimethylphenylcarbamate)) for the separation of the fungicide zoxamide. The amylose based stationary phase provided superior resolving ability over cellulose for this pesticide. On the other hand, different chiral recognition was observed between two amylose

derivatives ((3,5-dimethylphenylcarbamate) and (5-chloro-2-methylphenylcarbamate)) due to the nature and location of substituents. The wide variety of derivative polysaccharide phases now available make them ideal for separation of chiral pesticides generally, which encompass a broad range of physicochemical properties (e.g., log K_{OW}). However, it remains difficult to predict enantiomer separation on different stationary phases based on their structure. Therefore, screening multiple stationary phases followed by considerable method optimization (i.e., mobile phase conditions) needs undertaken for chiral pesticides not previously separated.

Stereoselective LC-MS/MS methods operate in high performance liquid chromatography mode with particle sizes of 3 or 5 μm (Table 1). These methods utilize reversed phase conditions as normal phase is generally incompatible with MS detection. Both methanol and acetonitrile are common organic modifiers with the latter having the greater eluting strength. Mobile phase additives are also added to help achieve satisfactory separation and sensitivity. Ammonium salts (formate, bicarbonate etc) and volatile acids (e.g., acetic and formic) are popular due to their compatibility with MS (i.e., thermally labile). Buffered mobile phases at pH 2 to 5 are preferred for the analysis of both basic and acidic pesticides (Table 1). However, it is suggested that the use of ammonium acetate or ammonium hydrogen carbonate buffered at high pH (8-9) could provide better enantioselectivity on polysaccharide columns whilst achieving comparable sensitivity to low pH mobile phases [54]. Temperature can also play an important role in enantioseparations. Reducing temperature can improve enantioresolution in enthalpy driven separations. The opposite is true for entropy driven separations. In the study by Pan et al. [42] enantioresolution of zoxamide was found to significantly reduce with increasing temperature in the range 25-40 ˚C. However, it has been found that the effect of temperature on separation is unpredictable and needs investigated on a case by case basis [54].

The most popular stationary phase for pesticide analysis is cellulose tris-(3,5-dimethylphenylcarbamate) [38, 40, 46, 48, 55-57] (Table 1). This well-established phase has been successful for the separation of pesticides representing a range of physicochemical properties. For example, a mobile phase consisting 90:10 acetonitrile: water was used to separate epoxiconazole (fungicide) enantiomers (log K_{OW} 3.6) within 5 min [55]. On the other hand a mobile phase

209 comprising acetonitrile: 0.1 % formic acid 40:60 achieved separation of metalaxyl (log K_{OW} 1.7) [48].
210 The main interactions between the analytes and stationary phase which influence separation are
211 proposed to be hydrogen bonding, π-π, dipole-dipole stacking and steric interactions, and hydrophobic
212 interactions [54]. These interactions are sensitive to the organic component of the mobile phase.
213 Increasing mobile phase organic content weakens interactions between the analyte and stationary
214 phase reducing retention time similar to that observed with conventional achiral methods. Other
215 polysaccharide derivatives utilized in the separation of chiral pesticides include the amylose
216 derivatives tris-(3 chloro-5 methylphenyl carbamate [47], tris-(5 chloro 2 methylphenyl carbamate)
217 [42], tris-(3,5 dimethylphenylcarbamate) [39, 58, 59] and cellulose derivatives tris-(3,5-
218 dichlorophenylcarbamate) [52], tris-(4 chloro 3 methylphenylcarbamate) [37, 43], tris-
219 (methylbenzoate) [41] (Figure 2, Table 1). The growing range of polysaccharide derivative phases
220 now available increases the capability to separate previously unresolved pesticides as well as further
221 exploring the opportunity of multi-residue enantioseparations.

222

223 **4.2. Multi-residue methods**

224 One of the greatest challenges of stereoselective separations is the ability to perform multi-residue
225 separation of analytes exhibiting a range of chemical properties. Such methods are important for
226 environmental monitoring and risk assessment, especially considering that synergistic effects are
227 possible for exposed organisms [60, 61]. Only a limited number of studies report the simultaneous
228 separation of ≥8 pesticides at the enantiomeric level [38-40, 47, 48] (Table 1).

229 Li, et al. [38] screened several polysaccharide stationary phases for the simultaneous separation of 8
230 triazole fungicides (tetraconazole, fenbuconazole, epoxiconazole, diniconazole, hexaconazole,
231 triadimefon, paclobutrazol, myclobutanil). Cellulose tris-(3,5 dimethylphenylcarbamate), cellulose
232 tris-(3-chloro-4-methylphenylcarbamate), amylose tris-(3,5 dimethylphenylcarbamate) and amylose
233 tris-(5-chloro-2-methylphenylcarbamate) were screened against a range of reverse phase mobile phase
234 conditions. This included different organic modifiers (both acetonitrile and methanol). Successful

separation (R_S ≥1.5) of all 8 fungicides simultaneously was achieved using cellulose tris-(3,5-dimethylphenylcarbamate). The mobile phase consisted of 45:55 2 mM ammonium acetate: acetonitrile operated isocratically for 25 min [38]. Similar conditions were used for the simultaneous separation of 8 different fungicides (epoxiconazole, diniconazole, hexaconazole, paclobutrazol, myclobutanil, metalaxyl), herbicides (napropamide) and insecticides (isocarbophos) [48]. A mobile phase consisting 60:40 0.1 % formic acid: acetonitrile (isocratic) achieved satisfactory separation (R_S ≥1.5) of all target analytes using the cellulose tris-(3,5-dimethylphenylcarbamate) stationary phase. However, a total run time of 70 min was required due to the diverse nature of that studied pesticides (log K_{OW} 1.7-4.3). This comparatively long run time highlights the challenge of developing multi-residue chiral methods under isocratic conditions.

Nevertheless, Zhao et al. [47] separated (R_S ≥1.5) a total of 18 pesticides at the enantiomeric level under isocratic conditions (Figure 3). In this excellent study a new generation amylose tris-(3-chloro-5-methylphenylcarbamate) stationary phase was used for the first time and challenged with a diverse range of fungicides (e.g., difenoconazole log K_{OW} 4.4), herbicides (e.g., napropamide log K_{OW} 3.3) and insecticides (e.g., dinotefuran log K_{OW} -0.6). To establish the best mobile phase conditions for multi-residue separation, ammonium acetate and formic acid concentrations, organic modifiers (acetonitrile and methanol) and their proportion, flow rate and column temperature were all optimized. Acetonitrile was found to give better enantioresolution than methanol. Methanol being a protic solvent could disrupt hydrogen bonding between the analyte and stationary phase in some cases. On the other hand, ammonium acetate concentration had little impact to separation but was important to optimize to achieve maximum ionization and sensitivity. Formic acid content also played an important role in sensitivity as well as separation, but also in peak shape as it reduced tailing. The final conditions using a 250 x 4.6 mm column with 5 μm particle size were 47:53 5 mM ammonium acetate containing 0.05 % formic acid: acetonitrile. The flow rate was 0.6 mL min^{-1} and the column temperature 30 ˚C. The total run time was 55 min which is offset by the large number of pesticides separated simultaneously.

Alternatively, both He et al. [40] and Li et al. [39] have used mobile phase gradients for stereoselective separation of multiple pesticides. For example, He et al. [40] used the cellulose tris-(3,5-dimethylphenylcarbamate) stationary phase with 2 mM ammonium acetate (mobile phase A) and acetonitrile. Starting conditions of 50:50 A:B were maintained for 15 min before the organic phase was increased to 80 %. The method had a 6 min equilibration period and a total run time of 25 min. A total of 10 pesticides were baseline resolved with $R_S \geq 1.5$. The proposed method offered considerably shorter retention times of pesticides in common with Zhao et al., [48] (metalaxyl, epoxiconazole etc), albeit smaller column diameter (2 mm vs. 4.6 mm) and particle sizes (3 µm vs. 5 µm) were used which can contribute to this. Nevertheless, gradient elution provides another option for achieving multi-residue chiral separations by LC-MS/MS at comparatively shorter run times. Li et al. [39] reported that an isocratic run time of 55 min was reduced to 35 min by adopting a gradient programme.

4.3. Method of quantitation

Triple quadrupole MS/MS detectors offer excellent sensitivity and specificity for environmental analysis with detection limits in the low or sub-ppb range for both liquid and solid matrices (Table 1). However, a well-known drawback of LC-MS/MS for environmental analysis is the loss of sensitivity due to quenching of signal strength during ESI [62]. On the other hand, this can also lead to signal enhancement in some cases [37, 40, 41, 57]. Furthermore, suppression (or enhancement) of signal strength can be stereoselective and significant in some cases. To demonstrate, Zhang et al. [57] reported 71 % suppression of *(-)-cis*-epoxiconazole in tea leaves. In contrast *(+)-cis*-epoxiconazole was suppressed by 53 %. To account for these interferences deuterated surrogates can be used. From the collated methodologies two authors reports the use of deuterated surrogates in their methods [35, 36]. Deuterated surrogates of mecoprop and dichloprop (mecoprop-d6 and dichlorprop-d6) have been used in the analysis of soil as well as ground water and river water [35, 36]. Whereas, metalaxyl and epoxiconazole have been quantified in soils and sediments using the surrogates metalaxyl-d6 and epoxiconazole-d4, respectively [48].

Where deuterated surrogates are not available or cost prohibitive, quantitation is by external calibration prepared in extracted matrix (Table 1). Matrix matching between prepared samples and calibration standards accounts for signal suppression during ESI. However, this approach has limitations as it may not be possible to obtain a blank matrix (not containing the pesticide of interest) for calibration preparation, and is time consuming. Furthermore, composition of the blank may not be the same as the samples analysed, particularly in monitoring studies. This has been observed with different apple varieties [62]. For example, signal suppression during ESI of the non-chiral insecticide aldicarb (extracted using QuEChERS) varied by 42 % between 5 different apple varieties. Repeatability between apples of the same variety was 4 %. This observation provides an extra challenge in obtaining representative information during monitoring studies using this quantitation approach, particularly if stereoselective signal suppression is observed.

5. Conclusion and future trends

The determination of chiral pesticides using stereoselective LC-MS/MS is a rapidly growing field of analytical chemistry research. A total of 18 validated methods are reported in the mainstream scientific literature, all of which were published since 2012 (Table 1). These reported methods achieve adequate sensitivity of pesticide enantiomers (e.g., ppb levels) for environmental monitoring (Table 1). Current trends focus on the development of methods capable of multi-residue separations. This requires considerable investment as predicting (multi-residue) chiral separations is often not possible and a number of stationary and mobile phases need screened and optimized. Nevertheless, the factor limiting the widespread use of stereoselective LC-MS/MS during routine pesticide monitoring is sample run time. Multi-residue methods often require run times ≥30 min, and in some cases >60 min (Table 1). Gradient mobile phase conditions have been used to reduce run times [40]. However, columns capable of ultra-high performance liquid chromatography performance (i.e., sub 2 μm particle sizes) in terms of run time and column efficiency would be beneficial. Nevertheless, supercritical fluid chromatography (SFC) has shown great promise for stereoselective separation of pesticides in short run times (≤9 min) [63-66]. Supercritical fluids integrate the advantages of both

gas states and liquid states [64, 67]. SFC is normally operated in normal phase mode with CO_2, forming the main component of the mobile phase. The addition of an organic modifier to the mobile phase increases solvent strength to elute and analyze relatively polar analytes. Due to the viscosity and diffusivity of CO_2, analytical method run times are considerably reduced whilst achieving improved separation of chiral enantiomers [64, 66]. However, SFC has not been explored for multi-residue pesticide analysis at the enantiomeric level to date.

The majority of stereoselective methods reported in the literature focus on parent pesticides, and do not incorporate pesticide metabolites/breakdown products which are often chiral themselves. This is a consequence of limited knowledge on their transformation pathways under environmental conditions (and a resultant lack of reference standards available for such compounds). It is anticipated that multi-residue methods used for environmental monitoring will become more dynamic, such that they can perform non-target or qualitative screening as well as targeted quantitative determinations simultaneously. This is reliant on the use of high resolution mass spectrometers such as time-of-flight or Orbitrap mass spectrometers of suitable sensitivity. A better understanding of the breakdown pathways and products of chiral pesticides will aid risk assessments. Furthermore, the inclusion of achiral pesticides into such methods is recommended to reduce the need of running multiple methods to encompass a full suite of pesticides during monitoring. It is also expected that these stereoselective methods will have wider applicability and can support a range of applications in the future. For example, wastewater based epidemiology has previously been utilized to estimate human exposure to pesticides at the community level, through consumption of contaminated foodstuffs [68-70]. Information at the enantiomeric level could provide further insight into human exposure to chiral pesticides, particularly if metabolites are also studied.

Declarations of interest

None

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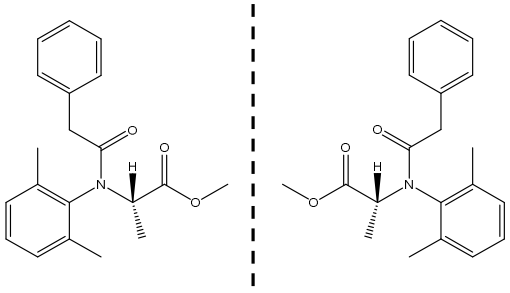
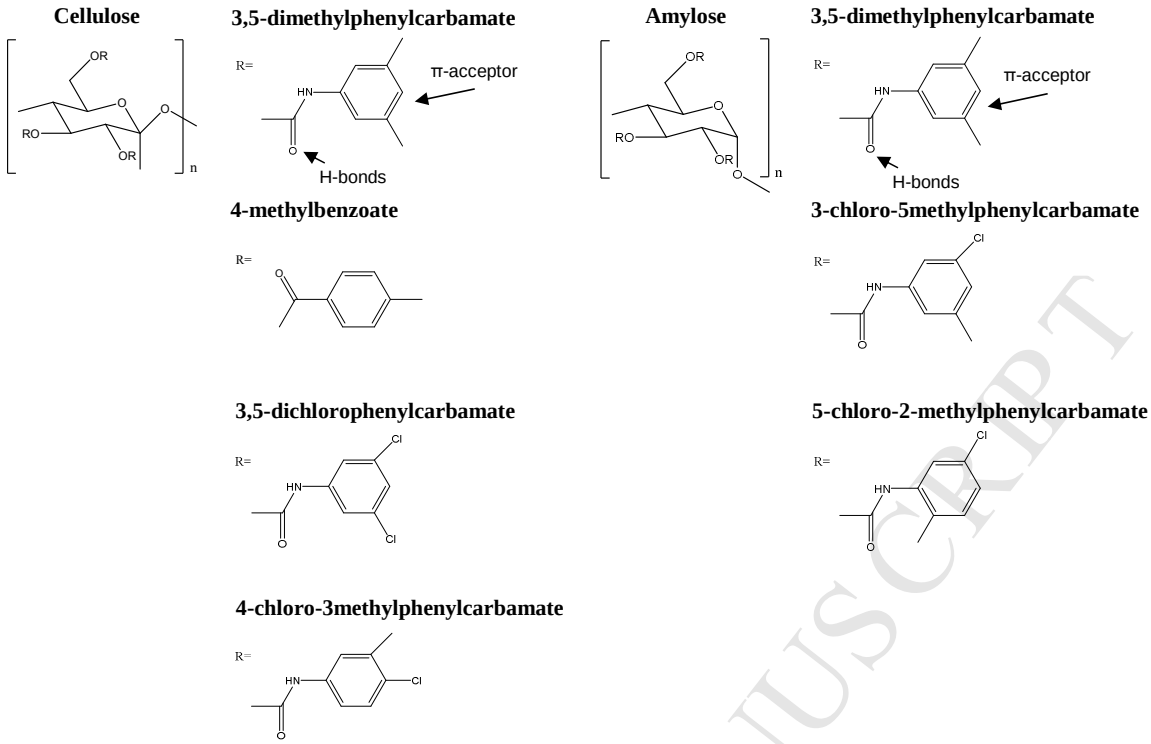


S-(+)-Benalaxyl **R-(-)-Benalaxyl**

Figure 1. Structure of benalaxyl enantiomers



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469 Figure 2. Derivatives of cellulose and amylose chiral selectors previously used for the separation of
470 pesticide enantiomers

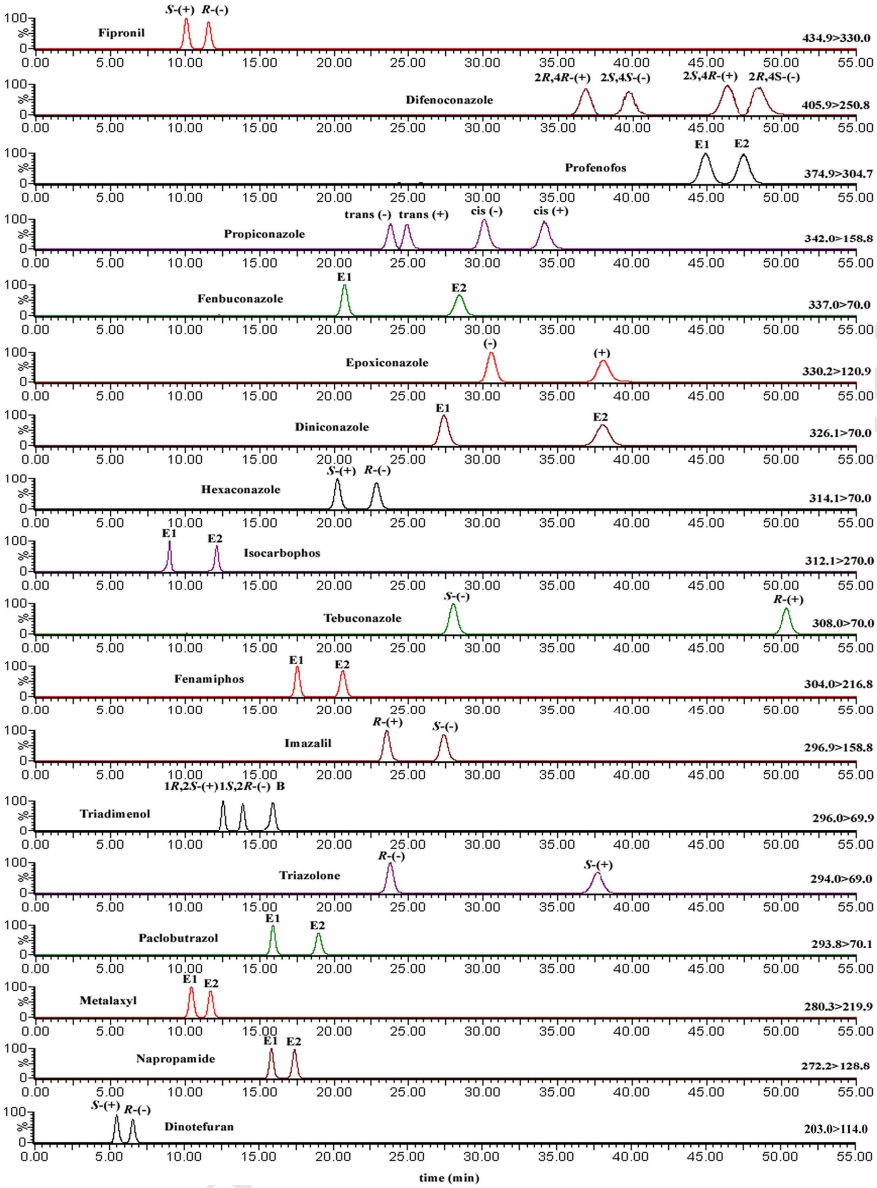

Figure 3. Multi-residue stereoselective LC-MS/MS chromatograms of chiral pesticides separated using immobilized amylose tris-(3-chloro-5-methylphenylcarbamate) (250 x 4.6 mm, internal diameter 5 µm) maintained at 30 °C with a mobile phase consisting 5 mM ammonium acetate + 0.05 % formic acid : acetonitrile 47:53 at 0.6 mL min^{-1} (reproduced with permission, [47]).

476 **Table 1. Stereoselective LC-MS/MS methodologies for chiral pesticides in environmental matrices**

Group	Target(s)	Matrix	Sample collection and storage	Extraction & clean up	Stationary phase & column dimensions	Mobile phase conditions and run time	Ionization source & detector	Calibration method	R_S	Signal suppression (%)	Recovery (%)	LOD (ppb)	Ref.
Fungicides, insecticides & herbicides	Metalaxyl, epoxiconazole, myclobutanil, hexaconazole, napropamide & isocarbophos	River water (200mL), wastewater (200mL)	Stored in amber bottles @ -20 °C. Filtered (0.45 µm)	SPE + DLLME	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 x 4.6 mm i.d., 5 µm	0.1 % FA: ACN (60:40 v/v) @ 0.6 mL/min. 30 °C. 10 µL injection. 70 min.	ESI+ MS/MS	Matrix matched	≥1.5	-49 to -3	83-103	0.1-0.5	[46]
Fungicides, insecticides & herbicides	Triadimenol, hexaconazole, metalaxyl, napropamide, isocarbophos, epoxiconazole, paclobutrazol, diniconazole, triazolone, fenamiphos, imazalil, difenoconazole, fenbuconazole, profenofos, fipronil, tebuconazole, dinotefuran & propiconazole	Soil (2g), sediment (2g), river water (200mL), wastewater (200mL)	Dried @ 35 °C, sieved (154 µm) & stored @ -20 °C in the dark.	SLE + MSPE (m-MWCNTs-NH_2)	Amylose tris-(3-chloro-5methylphenylcarbamate) 250 x 4.6 mm i.d., 5 µm	ACN: 5 mM NH_4OAc + 0.05% FA (53:47 v:v) @ 0.6 mL/min. 55 min.	ESI+/- MS/MS	Matrix matched	≥1.5	-50 to 33	81-106	0.1-0.6	[47]
Fungicides, insecticides & herbicides	Myclobutanil, hexaconazole, metalaxyl, napropamide, isocarbophos, epoxiconazole, paclobutrazol & diniconazole	Soil (0.1g), sediment (0.1g)	Air dried, sieved (154 µm) & stored @ -20 °C in the dark.	MSPD-DLLME	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 x 4.6 mm i.d., 5 µm	0.1 % FA: ACN (60:40 v/v) @ 0.6 mL/min. 30 °C. 10 µL injection. 70 min.	ESI+ MS/MS	Matrix matched + Internal standard (deuterated surrogates)	≥1.5	-33 to 4	62-95	0.2-1.5	[48]
Fungicides	Benalaxyl & benalaxyl acid	Soil (10g)	Samples collected from laboratory study & stored @ -20 °C	SLE	Cellulose tris-(3,5-dichlorophenylcarbamate) 250 x 4.6 mm i.d.	ACN:H_2O:FA (90:10:0.1, v/v/v) @ 0.5 mL/min. 20°C. 10 min.	ESI+ MS/MS	Matrix matched	NR	NR	81-104	1.5-5.6	[52]
Fungicides	Zoxamide	Soil, fruits & vegetables (10g), water (10mL)	Samples collected from laboratory study and stored @ -20 °C	QuEChERS & dSPE	Amylose tris-(5-chloro-2-methylphenylcarbamate) 150 × 2 mm i.d., 3 µm	ACN: H_2O (70:30 v/v) @ 0.5 mL/min. 25 °C. 2 µL injection. 3.5 min.	ESI+ MS/MS	Matrix matched	>1.5	0 to 121	90-117	0.5*	[42]
Insecticides	Flufiprole & flufiprole-amide	10g (vegetables), 5g (soil)	Samples collected from field study and stored @ -20 °C. Stability in solvent & matrix matched standards	QuEChERS & SPE	Cellulose tris-(4-chloro-3-methylphenylcarbamate) 150 × 2.0 mm i.d., 3 µm	ACN:0.1 % FA (65:35 v/v) @ 0.25 mL/min. 25 °C. 1 µL injection. 7 min.	ESI+ MS/MS	Matrix matched	≥2.7	-19 to 17	84-107	<2	[37]

Fungicides	Pyrisoxazole	10g (vegetables & fruit), 5g (soil)	assessed @ -20 °C 2 kg strawberries from 8 randomly selected sites, homogenized & stored @ -20 °C	QuEChERS & dSPE	Cellulose tris-(4-methylbenzoate) 150 × 2.0 mm i.d., 3 µm	MeOH: H_2O (70:30 v/v) @ 0.35 mL/min. 30 °C. 10 min.	ESI+ TOF/MS	Matrix matched	≥2.4	-51 to 108	64-100	0.2-1	[41]
Fungicides	Metalaxyl, myclobutanil, paclobutrazol, diniconazole, hexaconazole, triadimefon, epoxiconazole, tetraconazole, famoxadone, & fenbuconazole	Fruit and vegetables (10g)	135 (various) samples collected from several markets	QuEChERS & dSPE	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 × 2.0 mm i.d., 3 µm	2 mM NH_4OAc:ACN gradient @ 25 °C. 5 µL injection. 25 min.	ESI+ MS/LIT	Matrix matched	>1.5	-35 to 138	70-120	0.05-1*	[40]
Insecticides	Isocarbophos & isocarbophos oxon	Soil and rice (5g), water (100mL)	Samples collected from laboratory study & stored @ -20 °C	QuEChERS & SPE	Amylose tris-(3,5-dimethylphenylcarbamate) 150 × 2 mm i.d., 3 µm	ACN with 0.1 % FA: 0.1% FA solution @ 0.3 mL/min. 5 µL injection. 11 min.	ESI+ MS/MS	Matrix matched	NR	-13 to 6	90-103	0.1-0.5	[58]
Herbicides	Mecoprop & dichlorprop	Soil (0.8g)	Air dried , sieved (2mm) & stored @ 4 °C in the dark	SUSME	α-CD permethylated 200 × 4 mm i.d., 5 µm	MeOH:100 mM FA/NH_4HCO_2 (pH 4.0) (65:35 v/v) @ 0.5 mL/min. 25 °C. 40 µL injection. 13 min.	ESI- MS/MS	Internal standard (deuterated surrogates)	NR	NR	66-83	0.03	[36]
Fungicides & insecticides	Cis-epoxiconazole & indoxacarb	Soil (5g), teas (2g), infusion (3g)	15 random samples collected from field study, sieved & stored @ -18 °C	USE & SPE	Cellulose tris (3,5-dimethylphenylcarbamate) 150 × 2 mm i.d., 3 µm	0.2% TFA and 2 mM NH_4HCO_2 aqueous solution: MeOH (25:75 v/v) @ 0.4 mL/min. 40 °C. 20 min.	ESI+ QTOF/MS	Matrix matched	NR	-71 to 100	61-130	<1.4	[57]
Herbicides	Mecoprop & dichlorprop	River & ground water	Grab samples in dark glass containers, filtered (0.45 µm), adjusted to pH 1.5 & stored @ 4 °C.	SUSME	α-CD permethylated 200 × 4 mm i.d., 5 µm	MeOH:100 mM FA/ NH_4HCO_2 (pH 4.0) (65:35 v/v) @ 0.5 mL/min. 25 °C. 40 µL injection. 13 min.	ESI- MS/MS	Internal standard (deuterated surrogates)	≥1.9	NR	73-80	0.001-0.004	[35]
Herbicides, insecticides & fungicides	Indoxacarb, benalaxyl, carfentrazone-ethyl, quizalofop-ethyl, isocarbophos, fenamiphos, simeconazole, napropamide & paclobutrazol	Soil (10g) & river water (100mL)	15 random soil samples collected from field study @ varying depths (0-30 cm), air dried, sieved (2 mm) & stored in the dark. Grab samples of river water collected.	QuEChERS & dSPE/SPE	Amylose tris-(3,5-dimethylphenylcarbamate) 150 × 4.6 mm i.d., 5 µm	ACN:2 mM NH_4OAc in water (gradient) @ 0.45 mL/min. 25 °C. 10 µL injection. 35 min.	ESI+ MS/MS	Matrix matched	≥1.5	-10 to 19	78-106	<1.8	[39]
Fungicides	Myclobutanil	Cucumber and soil (10g)	15 random samples collected from greenhouse study @	QuEChERS & SPE	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 × 4.6 mm i.d., 5 µm	ACN:H_2O (70:30 v/v) @ 0.5 mL/min. 40 °C. 10 µL injection. 10 min.	ESI+ MS/MS	Matrix matched	NR	-32 to 55	>50	0.6-1.0	[56]

			varying time intervals (0-15 cm depths for soils).									
Fungicides	Tetraconazole, fenbuconazole, epoxiconazole, diniconazole, hexaconazole, triadimefon, paclobutrazol, & myclobutanil	Soil (10g) & water (100mL)	Samples collected from field study & stored in the dark @ -20 °C.	QuEChERS & dSPE/SPE	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 × 4.6 mm i.d., 5 µm	ACN:2 mM NH_4OAc in water (55:45 v/v) @ 0.45 mL/min. 25 °C. 10 µL injection. 25 min.	ESI+ MS/MS	Matrix matched	≥1.5	2 to 10	76–108 (soil), 81–107 (water)	<1	[38]
Fungicides	Epoxiconazole	Grape & soil (10g)	Samples collected from field study @ different time intervals. 2 kg of grape & 8 random soil sampling points collected. Stored @ -20 °C.	USE & SPE	Cellulose tris (3,5-dimethylphenylcarbamate) 150 x 2.0 mm, i.d., 3 µm	ACN:H_2O (90:10 v/v) @ 0.3 mL/min. 10 µL injection. 5 min.	ESI+ MS/MS	Matrix matched	NR	69 to 89	76-92	5	[55]
Fungicides	Benalaxyl, furalaxyl & metalaxyl	Vegetables & fruits (15g)	-	QuEChERS & dSPE	Cellulose tris-(4-chloro-3-methylphenylcarbamate) 150 x 2 mm i.d., 3 µm	ACN:0.1 % FA solution (45:55 v/v) @ 0.2 mL/min. 5 µL injection. 25 min.	ESI+ MS/MS	Matrix matched	≥1.7	-10 to 10	81-96	0.3	[43]
Insecticides	Isocarbophos	Soil (5g)	Samples collected from laboratory study @ different time intervals & stored @ -40 °C	QuEChERS	Amylose tris-(3,5-dimethylphenylcarbamate) 150 x 2.1 mm i.d., 3 µm	ACN:2 mM NH_4OAc solution + 0.1 % formic acid (60:40 v/v) @ 0.3 mL/min. 10 µL injection. 5 min.	ESI+ MS/MS	Matrix matched	NR	10 to 18	89-97	5	[59]

Key: ACN, Acetonitrile; CD, Cyclodextrin; FA, formic acid; LOD, limit of detection; *LOQ, limit of quantification; MeOH: methanol; MS/MS, tandem mass spectrometry; MSPD, matrix solid phase dispersion; NH_4HCO_2; ammonium formate; NH_4OAc, ammonium acetate; QuEChERS, quick, easy, cheap, effective, rugged and safe method; SPE, solid phase extraction; dSPE, dispersive solid phase extraction; MSPE, magnetic solid phase extraction; m-MWCNTs-NH_2, magnetic amino modified multiwalled carbon nanotubes; SLE, solid liquid extraction; SUSME, supramolecular solvent-based microextraction; DLLME, dispersive liquid-liquid microextraction; TFA: trifluoroacetic acid; TOF/MS, time-of-flight mass spectrometer; USE, ultrasonic solvent extraction; NR, not reported; ESI, electrospray ionization; R_S, resolution

- Progress on development of stereoselective LC-MS/MS pesticide methods reviewed
- Possible enantiomer changes by (a)biotic processes during sample storage overlooked
- Polysaccharide derivative phases offer wide scope for pesticide separations
- Stereoselective LC-MS/MS methods for multi-residue analysis now being developed
- Lack of deuterated surrogates and metabolites available for monitoring studies