Determination of Enantiomeric Excess of Metolachlor from Chiral Synthesis using the Agilent 1260 Infinity Analytical SFC System

Application Note

Specialty Chemicals

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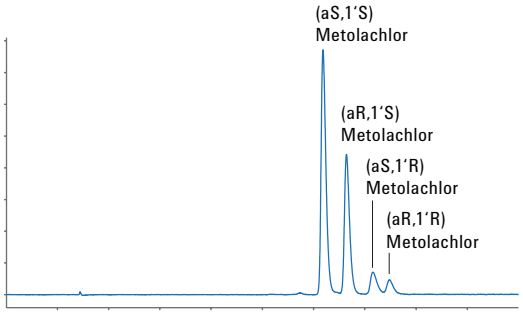
Abstract

This Application Note describes the development of a method for the separation of four stereoisomers, which were derived from a compound with two steric centers. The method was developed using an Agilent 1260 Infinity Analytical Supercritical Fluid Chromatography (SFC) System with Agilent ChemStation Method Scouting Wizard software. The developed method was used to compare the ratio of stereoisomers obtained from racemic and stereoselective syntheses.



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Introduction

The herbicide metolachlor is used worldwide in large amounts for the control of a variety of broad-leaved weeds in corn and other crops. Worldwide production exceeds 30,000 tons per year[1,2]. Metolachlor has one stereogenic center located at an asymmetrically-substituted carbon atom and an additional chiral axis. This structure means metolachlor exists as four stereoisomers (Figure 1).

The biological activity depends mainly on the configuration on the stereogenic carbon atom. About 95 % of the biological activity stems from both forms of the S-enantiomer, (aS,1′S) and (aR,1′S), which differ in spatial arrangement at the chiral axis[3]. Due to this fact, a metolchor formulation enriched with the S-enantiomers could lower the intake of the compound by the environment while maintaining the desired herbicidal effect. Large efforts were taken to replace the racemic syntheses by a stereoselective synthesis of the S-enantiomer. The final solution was found with a catalytic hydrogenation reaction driven by a chiral ferrocenyl catalyst (Figure 2)[4,5]. This synthesis enables production of metolachlor with an enantiomer excess of about 80 % in amounts greater than 50,000 tons per year. To determine the enantiomer excess and thereby the success of the chiral synthesis, all four stereoisomers must be resolved. This separation has been done by normal phase HPLC[6,7]. With the HPLC method, the four stereoisomers elute between 20 and 30 minutes from the column, and typical harmful normal phase solvents are used.

In this Application Note, we demonstrate how a method for the separation of all four stereoisomers of metolachlor can be developed using the Agilent 1260 Infinity Analytical SFC System. The developed method is able to separate the four stereoisomers within a much shorter time than typically needed for the separation using normal phase HPLC conditions. In addition, the SFC method avoids using harmful solvents.

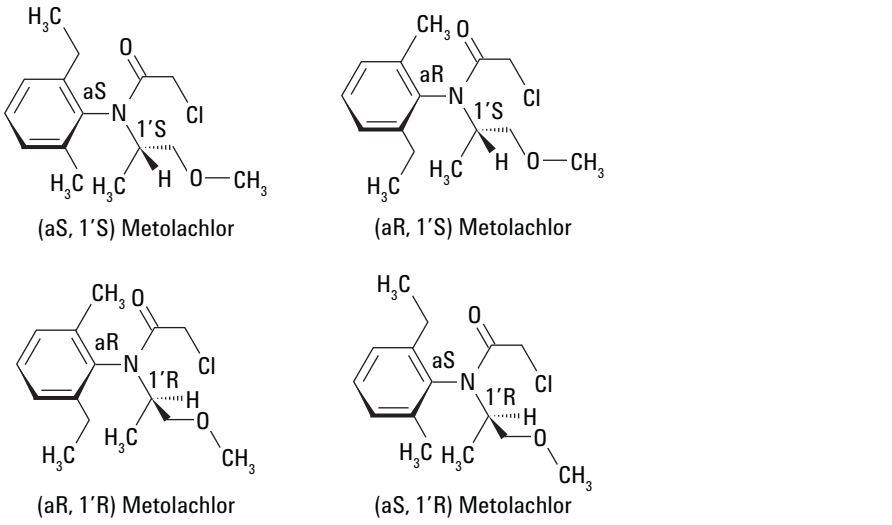

Figure 1 Stereoisomers of the pesticide metolachlor. The 1′S-enantiomers are biologically active, independent of the spatial arrangement at the chiral axis.

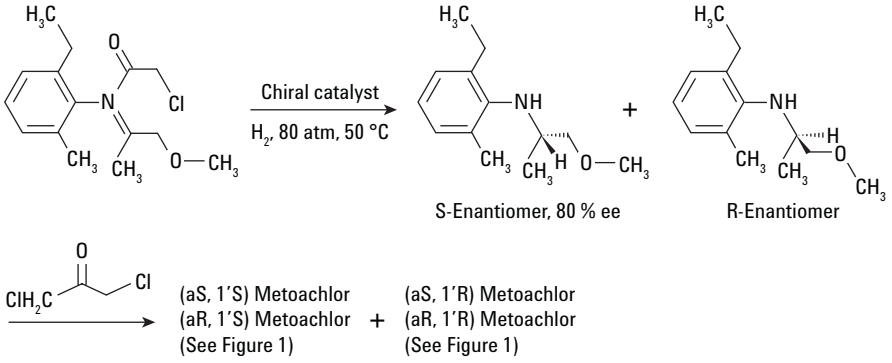

Figure 2 Stereoselective synthesis of S-metolachlor.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1260 Infinity Analytical SFC System (G4309A) comprising:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment with valve drive
- Agilent 1260 Infinity Diode Array Detector with high pressure SFC flow cell

The following additional equipment was required for automated method development with the SFC system:

- Agilent 1290 Infinity Thermostatted Column Compartments (G1316C) with valve drive
- Agilent 1200 Infinity Series 8-position/9-port Quick-Change Valves, 2x (G4230A)
- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent 1200 Infinity Series 12-position/13-port Quick-Change Valve (G4235A)
- Capillary kit for method development (p/n 5067-1595)

Instrumental setup

For solvent selection, the instrument configuration menu in OpenLAB CDS was used to cluster the SFC binary pump with a 12-position/13-port valve. The solvents were defined in the pump setup menu of OpenLAB CDS. For column selection, the instrument configuration menu in OpenLAB CDS was also used to cluster the two thermostatted column compartments, each of which were equipped with an 8-position/9-port valve. The method development capillary kit enables using up to eight columns. Details of the columns were entered in the columns database of OpenLAB CDS and configured in the column compartment menu.

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.06, with Agilent ChemStation Method Scouting Wizard, version A02.04 (G2196AA)

Columns

- Chiral Technologies, Chiralpak IA3, 4.6 × 250 mm, 3 µm
- Chiral Technologies, Chiralpak IB, 4.6 × 250 mm, 5 µm
- Chiral Technologies, Chiralpak IC, 4.6 × 250 mm, 5 µm
- Chiral Technologies, Chiralpak ID, 4.6 × 250 mm, 5 µm

Chemicals

- Metolachlor and S-Metolachlor were purchased from Sigma-Aldrich, Germany. A solution in 5 mg/mL isopropanol was used for the experiments.
- All solvents were purchased from Merck, Germany.
- Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

SFC methods

Conditions of the optimized final method are in shown in bold.

Parameter	Value
Solvent A	CO_2
Modifier B	Methanol, ethanol, isopropanol
SFC flow	3 mL/min
Isocratic elution	2.5 %, 5 %, 10 %, and 20 % modifier
Modifier	Methanol
BPR temperature	60 °C
BPR pressure	120 bar
Column temperature	35 °C
Injection volume	1 µL, fixed loop, 10-times overfill, needle wash in vial with isopropanol
Detection	220 nm/bandwidth 4 nm, reference 360 nm/bandwidth 100 nm, 10-Hz data rate

Results and Discussion

A racemic mixture of metolachlor comprising all four stereoisomers was used for a screening with four different chiral columns and three organic modifiers of increasing eluting strength; isopropanol, ethanol, and methanol. Three different isocratic conditions were used for the initial screening with 5, 10, and 20 % of the organic modifier. The screening of one column with three different solvents and three different isocratic compositions took about 90 minutes, including solvent exchange and equilibration. The most promising elution pattern obtained by the screening process was achieved on the Chiralpak IA3 column (Figure 3, other columns are not shown).

During screening on the Chiralpak IA3 column, the four seteroisomers were not separated using methanol, which has the highest eluting strength (Figure 3A). Using ethanol, which has a lower eluting strength, separation began at a ratio of 10 %. At a ratio of 5 %, the two pairs were separated, but the individual compounds were not completely separated (Figure 3B). The weakest eluting solvent, isopropanol, separated the first and last compounds almost at baseline, and the second and third compound with a valley at 5 % under isocratic conditions (Figure 3C). The final optimization was done using these starting conditions. The final method for the separation of the four stereoisomers applied isocratic conditions with 2.5 % isopropanol and separated the four compounds between 6.16 and 7.44 minutes (Figure 4).

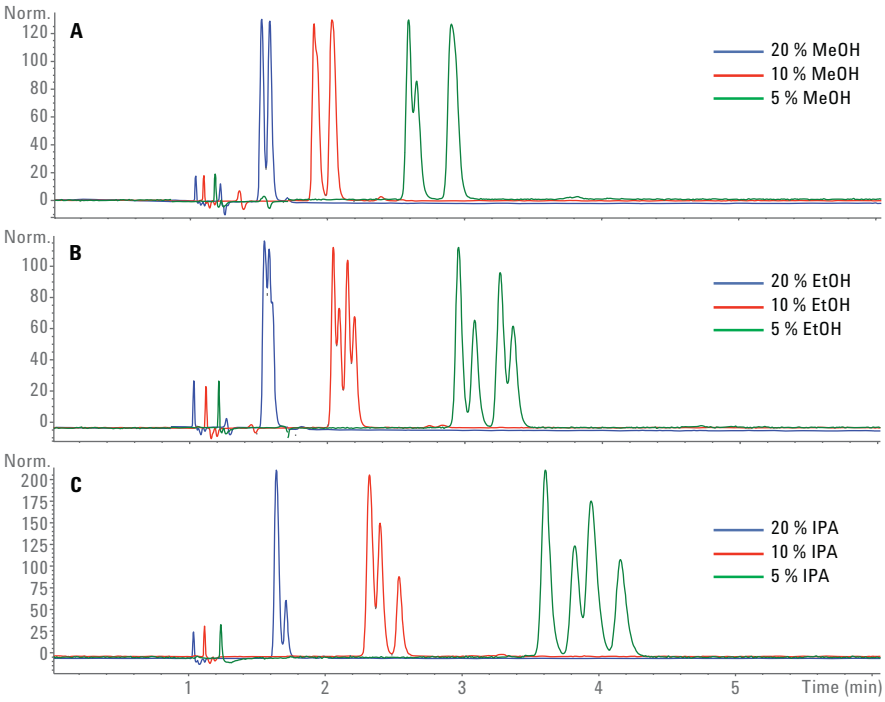

Figure 3. Separation of the four chiral metolachlor isomers with 5, 10, and 20 % of A) MeOH, B) EtOH, and C) isopropanol (IPA). As starting point for the final optimization of the separation method on column Chiralpak IA3, 5 % isocratic IPA was chosen.

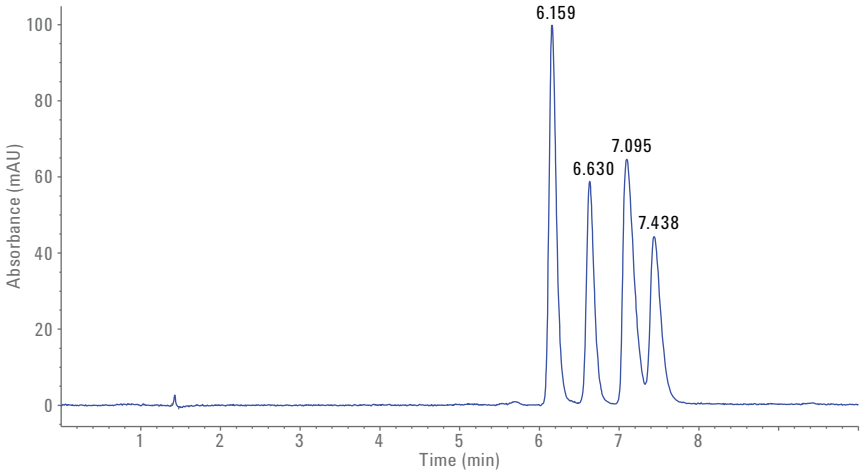

Figure 4. Separation of four stereoisomers of metolachlor with the final optimized method between 6.159 and 7.438 minutes on the Chiralpak IA3 column with 2.5 % isocratic isopropanol.

This optimized method was applied to analyze metolachlor obtained from a stereoselective synthesis, which had an enantiomeric excess of the S-enantiomers (Figure 5). The (aS,1′S) and (aR,1′S) enantiomers elute at 6.18 and 6.64 minutes as the main compounds in the mixture. The (aS,1′R) and (aR,1′R) enantiomers elute at 7.16 and 7.48 minutes as the minor components. The enantiomeric excess of the S-enantiomers was calculated from the peak areas to be about 78 % (Table 1). The peak areas obtained from the racemic metolachlor sample showed a 50:50-% ratio of the S- and R-enantiomers, whereas one of the stereoisomers of the chiral axis, aS or aR, was preferred (Table 1).

A statistical evaluation with 10 injections of the racemic and the S-selective mixture showed retention time RSDs typically below 0.2 % and area RSDs typically at 1 % (Table 1).

Conclusion

This Application Note demonstrates the development of a method for the chiral separation of a compound, the pesticide metolachlor, with more than one chiral center and, hence, more than two enantiomers. The final method was used to determine the enantiomeric excess of the product from stereoselective synthesis. The developed method had a run time of 10 minutes. In contrast, the typical normal phase HPLC method takes at least 30 minutes. The SFC method is about three-times faster than a classically used normal phase method. In addition, compared to the normal phase method, the SFC does not use harmful solvents such as *n*-hexane. The obtained retention time RSD values were below 0.2 %, and area RSD values were about 1 %.

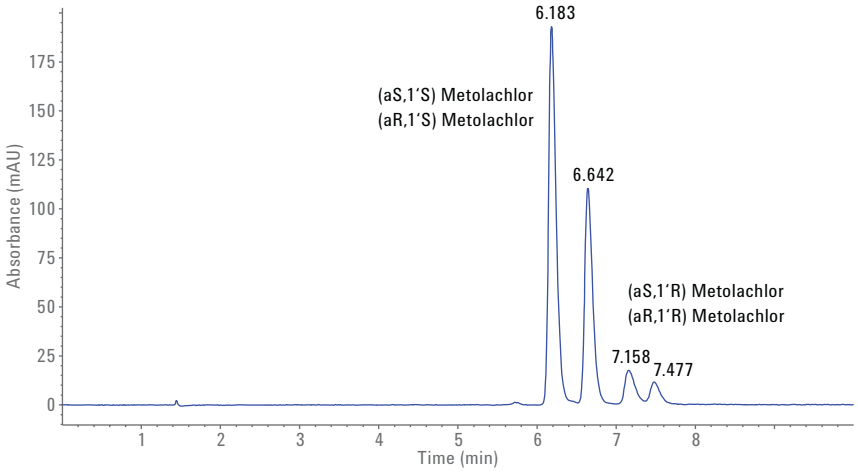

Figure 5. Separation of four stereoisomers of metolachlor synthesized by a steroslecitve synthesis. The major components, the S-enantiomers, elute at 6.183 and 6.642 minutes. The enantiomeric excess of the S-enantiomer is about 78 %.

Table 1. Statistical evaluation of the separation of four metolachlor stereoisomers from a racemic and S-selective synthesis, and relative determination of the enantiomeric composition.

		Racemic synthesis			S-selective synthesis		
	Retention time	Retention time RSD	Area RSD	Area %	Retention time RSD	Area RSD	Area %
Peak 1	6.183	0.25	1.06	30.77	0.20	0.90	55.35
Peak 2	6.642	0.22	1.05	19.24	0.19	0.97	33.66
Peak 3	7.158	0.16	1.06	30.08	0.18	1.93	6.70
Peak 4	7.477	0.17	1.04	19.91	0.16	1.34	4.29

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Published in the USA, October 15, 2017
5991-5618EN

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