



Whitepaper

**CHIRAL LC & SFC
METHOD DEVELOPMENT**

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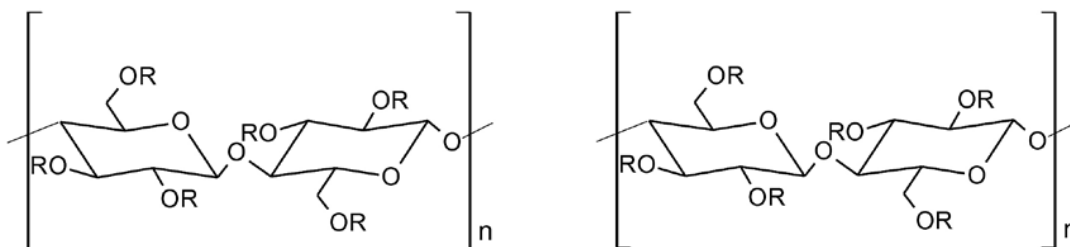
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Introduction

Analytical methods to assess the enantiomeric purity in drugs and other substances are essential in the pharmaceutical and chemical industry. The chromatographic isolation of pure enantiomers at analytical and preparative scale has become an achievable reality today.

Polysaccharides, such as cellulose and amylose, are naturally occurring polymers which incorporate various levels of chiral information in their structures. In the early 1980s a group of Japanese scientists came up with the idea of derivatising them, in order to enhance their chiral recognition abilities, and then coating them on a silica gel support [1, 2]. This important step forward has resulted in polysaccharide derivatives (figure 1) that have become the first and broadest choice of selectors to be used for liquid and supercritical fluid chromatography (LC and SFC) for analytical and preparative separations of enantiomers.



Cellulose derivatives

Cellulose derivatives

Figure 1: Chiral selectors: amylose and cellulose derivatives.

Coated Polysaccharides

YMC has successfully developed a new family of chiral products for chiral LC/SFC: CHIRAL ART Amylose-C and CHIRAL ART Cellulose-C. The silica packing material is coated with polysaccharide derivatives. A wide application range is provided with these two different selectors. They show high stability, and are also suitable for SFC and simulated moving bed (SMB) separations. These phases are fully scalable from 3 to 20 μm and find applications from analytical to preparative scale.

Table 1: Properties of coated polysaccharide chiral stationary phases.

Specifications	CHIRAL ART Amylose-C	CHIRAL ART Cellulose-C
Particle size	3, 5, 10, 20 μm	
Chiral selector	Amylose tris (3,5-dimethylphenylcarbamate)	Cellulose tris (3,5-dimethylphenylcarbamate)
USP	L51	L40
Type	Coated type	
Separation mode	NP/SFC	
Temp. range	0-40 $^{\circ}\text{C}$	
Pressure limit	300 bar / 30 MPa / 4350 psi	

As the silica packing material is coated with polysaccharide derivatives, solvents such as THF, acetone, ethyl acetate, chloroform, dichloromethane, DMSO and DMF might potentially dissolve the chiral selector itself. These solvents should be avoided in the mobile phase and the sample solvent.

Immobilised Polysaccharides

With the need to develop phases for polar compounds and separations using RP mode, a new series of immobilised polysaccharide-derived Chiral Stationary Phases (CSPs) has become available.

YMC applies an innovative technology to immobilise polysaccharide derivatives to implement the immobilised polysaccharide phases CHIRAL ART Amylose-SA, CHIRAL ART Cellulose-SB and CHIRAL ART Cellulose-SC. These form a series of chiral separation columns with high stereoselectivity. They are suitable for separations of a wide range of chiral compounds, cis-trans isomers and geometric isomers. The range of particle sizes and column dimensions available offer outstanding cost effectiveness for analytical to preparative separations.

Table 2: Properties of immobilised polysaccharide chiral stationary phase

Specifications	CHIRAL ART Amylose-SA	CHIRAL ART Cellulose-SB	CHIRAL ART Cellulose-SC
Particle size	3, 5, 10, 20 µm		
Chiral selector	Amylose tris (3,5-dimethylphenyl-carbamate)	Cellulose tris (3,5-dimethylphenyl-carbamate)	Cellulose tris (3,5-dichlorophenyl-carbamate)
Type	Immobilised type		
Separation mode	NP/ RP/ SFC		
Temp. range	0-40 °C		
pH-range	2.0 – 9.0		
Pressure limit	300 bar / 30 MPa / 4350 psi		
Usable organic solvents	<i>n</i> -hexane, <i>n</i> -heptane, chloroform, dichloromethane, <i>tert</i> -butyl methyl ether, ethyl acetate, THF, alcohols, acetonitrile etc.		

CHIRAL ART columns are currently available packed with one of the three different types of immobilised polysaccharide CSP based on high strength super-wide pore silica particles having 3, 5, 10 or 20 µm diameters. Consistent retention and selectivity parameters within the same chiral selector are obtained across particle sizes and therefore allow full scalability.

All these innovative supports are prepared by proprietary immobilisation technologies, providing the benefits of extended solvent compatibility, broad chiral recognition abilities, high chromatographic efficiency and excellent reproducibility.

Owing to their immobilised nature, these CSPs exhibit excellent solvent versatility: they can be used with mobile phases of various types, ranging from alcohol mixtures in alkanes to mobile phases containing methyl *tert*-butyl ether (MTBE), tetrahydrofuran (THF), chlorinated solvents and ethyl acetate, among others. They can be used in NP, RP and even SFC mode.

Chiral Method Development Strategy for Normal Phase

Each method development starts with the determination of the initial conditions such as the column itself and the mobile phase. This is especially demanding for chiral separations as predictions concerning the suitable selector for the target analyte cannot, as yet, be made theoretically. The first step in method screening is to screen the different CHIRAL ART columns followed by method optimisation using the column which provided the best results. In the following, examples for NP, RP and SFC method screening are shown.

Coated as well as immobilised CHIRAL ART columns can be used for normal phase screenings. However, whilst first choice eluents can be used with coated and immobilised columns, second choice eluents can only be used with immobilised columns.

First Choice Eluents

First choice eluents are mixtures of either an alkane such as *n*-hexane with 2-propanol or ethanol. Based on previous experimental data, the proportions indicated in table 3 can be taken as a starting point and can be adjusted according to the compound to be separated. The retentive behaviour of the compound will steer the adjustment of the mobile phase. With these so-called first choice data one can decide which are the best solvent(s) or solvent ratios to perform the tests.

Second Choice Eluents

If no satisfactory separation is obtained with the first choice screening of mobile phase systems, other solvents can then be considered, such as THF, dichloromethane or MTBE (table 3).

Table 3: Mobile phases for initial screening

Eluent A/B	First Choice		Second Choice*		
	<i>n</i> -hexane/ 2-propanol	<i>n</i> -hexane/ ethanol	<i>n</i> -hexane/ THF	<i>n</i> -hexane/ CH ₂ Cl ₂	<i>n</i> -hexane/ MTBE
Gradient B	10-50%	10-50%	10-50%	30-50%	30-50%
Gradient time	0-20 min	0-20 min	0-20 min	0-20 min	0-20 min

* Only applicable for immobilised columns

When dichloromethane is used for immobilised columns, a mixture of dichloromethane and alkane is suitable for weakly retained compounds. For samples having stronger interaction with the stationary phase however, addition of an alcohol into dichloromethane would be needed. EtOH has proved to be an option for its universal miscibility, but methanol is also suitable due to its lower viscosity and stronger eluting strength. Alkanes should not be combined with dichloromethane containing MeOH in order to avoid potential miscibility issues.

Additives

For basic and acidic compounds, it is often necessary to incorporate an additive in the mobile phase in order to minimise or eliminate strong interactions of an achiral nature. This type of interaction does not contribute to the enantioselective recognition but may induce broad peak shape, peak tailing and excessive retention. Therefore, basic samples may require a basic additive (such as diethylamine (DEA), butylamine, ethanolamine or ethylenediamine) and acidic compounds require the addition of an acidic additive (e.g. trifluoroacetic acid (TFA), acetic or formic acid (FA)). The percentage required is typically 0.1%.

TFA can be challenging for some coated amylose phases in regards to retention time stability and column lifetime. However, CHIRAL ART Amylose-C shows extended stability when using mobile phases containing TFA. The retention behaviour and column efficiency remain completely unaffected (figure 2). Therefore, there are no limits in terms of additive use for coated phases.

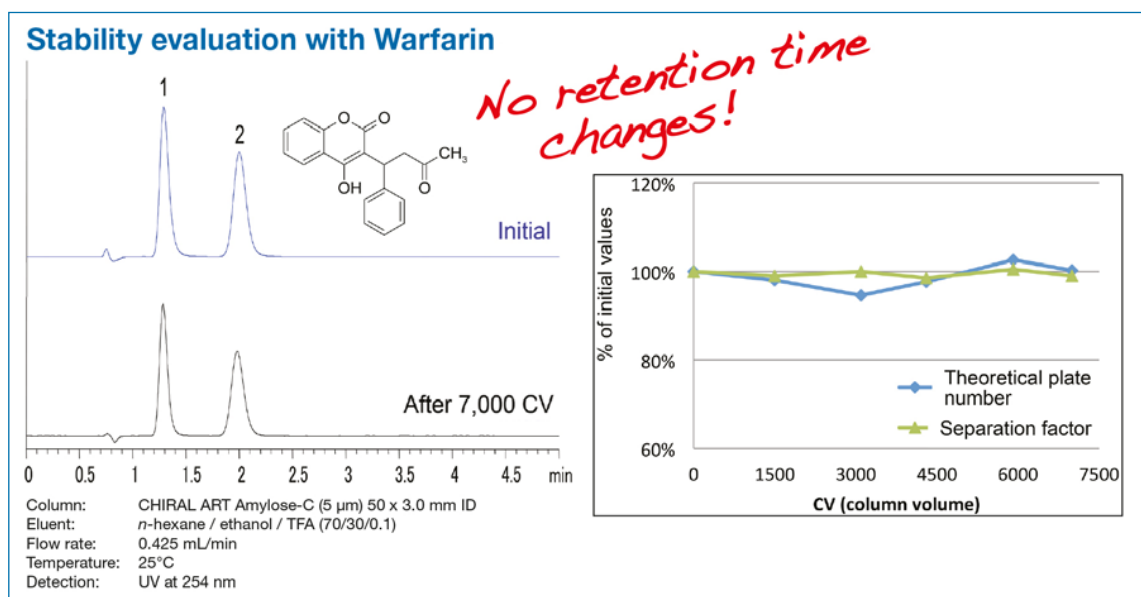


Figure 2: Stability evaluation with Warfarin

NP Example

As an example of screening in NP mode, Astaxanthin was used with the aim of separating all three enantiomers **1**, **2** and **3** with high resolution and short retention times. The two coated phases CHIRAL ART Amylose-C and Cellulose-C were applied. Additionally, CHIRAL ART Cellulose-SB as an immobilised phase with the same chiral selector as Cellulose-C was also part of the phase selection.

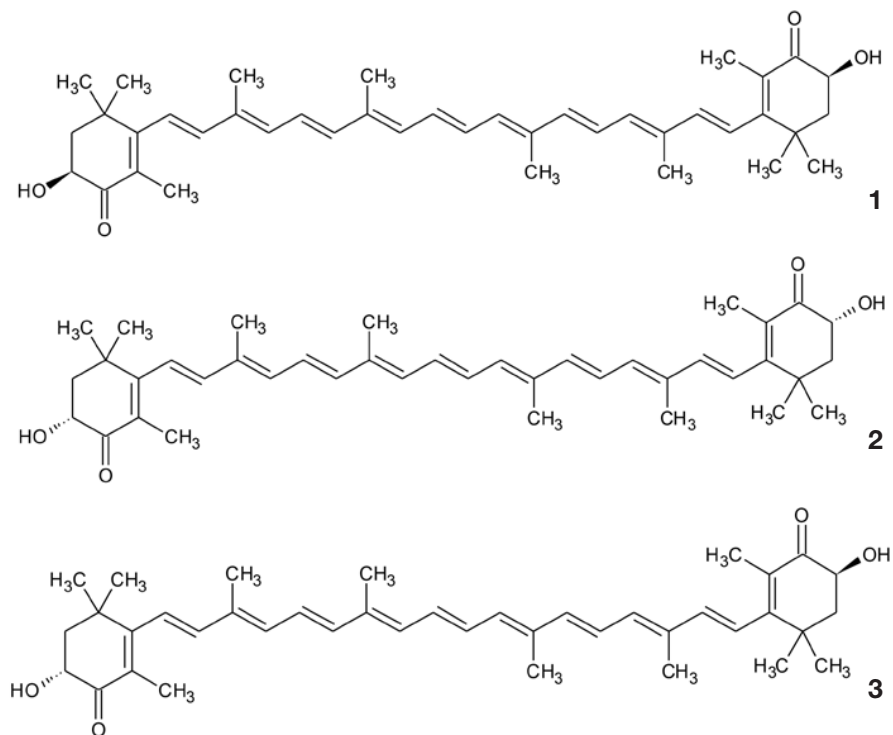


Figure 3: (3S,3'S)-all-trans-Astaxanthin (1), (3R,3'R)-all-trans-Astaxanthin (2), (3R,3'S)-all-trans-Astaxanthin (3)

In this screening there was no separation achieved with the first choice of solvents. Therefore, other solvents, such as THF, CH₂Cl₂ or MTBE, were considered for the second choice. These solvents however cannot be used with the two coated phases as they are not stable. The best result was achieved for the astaxanthin enantiomers using CHIRAL ART Cellulose-SB eluted with *n*-hexane and THF.

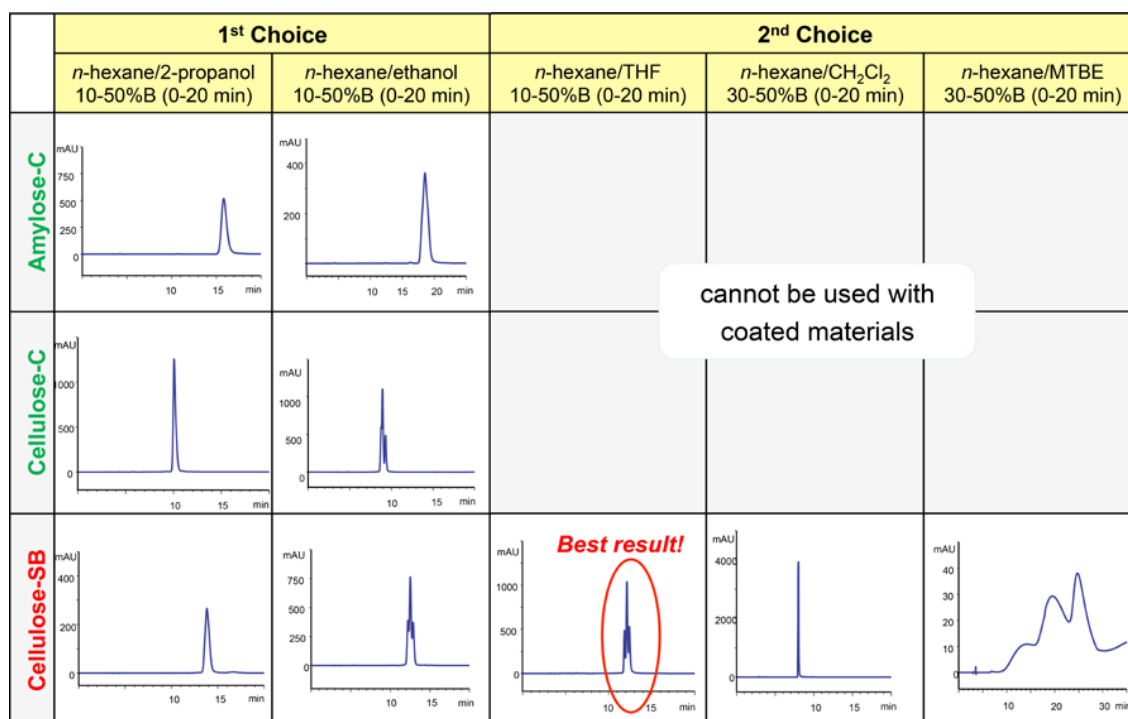


Figure 4: Primary screening of astaxanthin on CHIRAL ART Amylose-C, Cellulose-C and Cellulose-SB.

This first screening of the solvents provides an ideal starting point for further optimisation. The following aspects can be taken into account:

- Column dimension
- Gradient => isocratic
- Flow rate
- Temperature
- Injection volume
- Additives: basic: e.g. DEA, ethanolamine;
acidic: e.g. TFA, formic acid

Figure 5 provides an example of straightforward optimisation based on the result of the primary screening.

- 1.) A gradient from 10-50% B was used for initial screening.
 - 2.) This method was transferred to isocratic conditions using 15-20% lower composition than that at which the compound eluted under gradient conditions.
- Best result was *n*-hexane/THF 85/15.

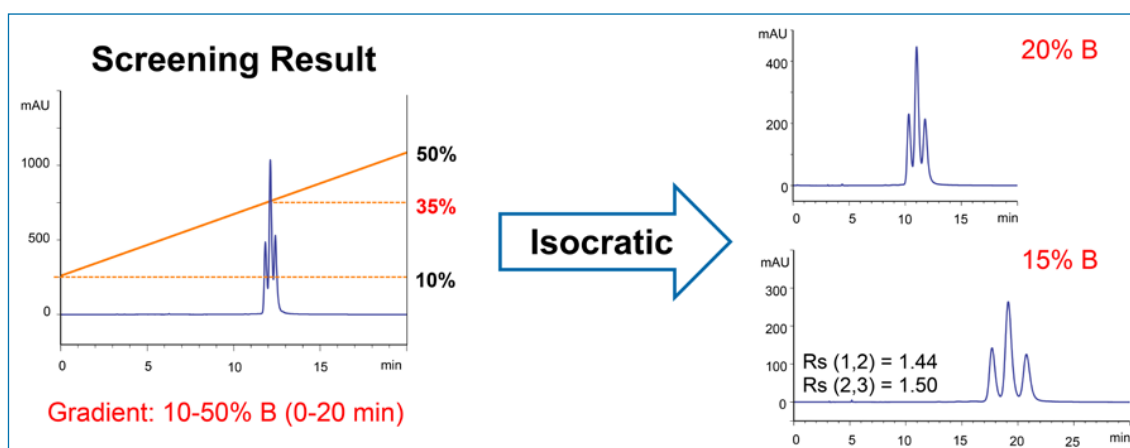


Figure 5: Method optimisation while applying isocratic condition.

Additional optimisation could be achieved by adjusting the flow rate. Changing the flow rate from 1.0 mL/min to 0.5 mL/min led to an increase in resolution. As shown in figure 6 the resolution between peak 1, 2 and 3 respectively was improved.

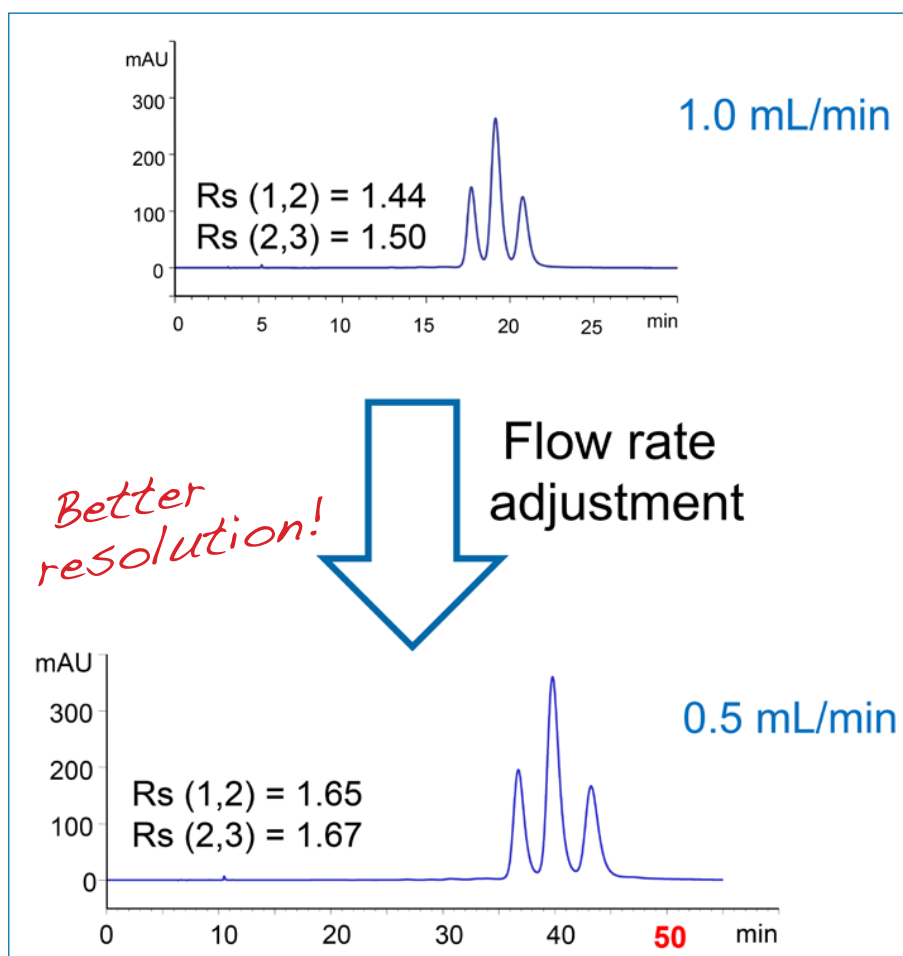


Figure 6: Method optimisation by adjusting the flow rate.

Chiral Method Development Strategy for Reversed Phase

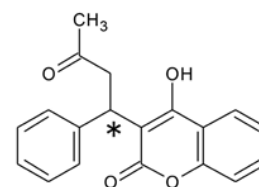
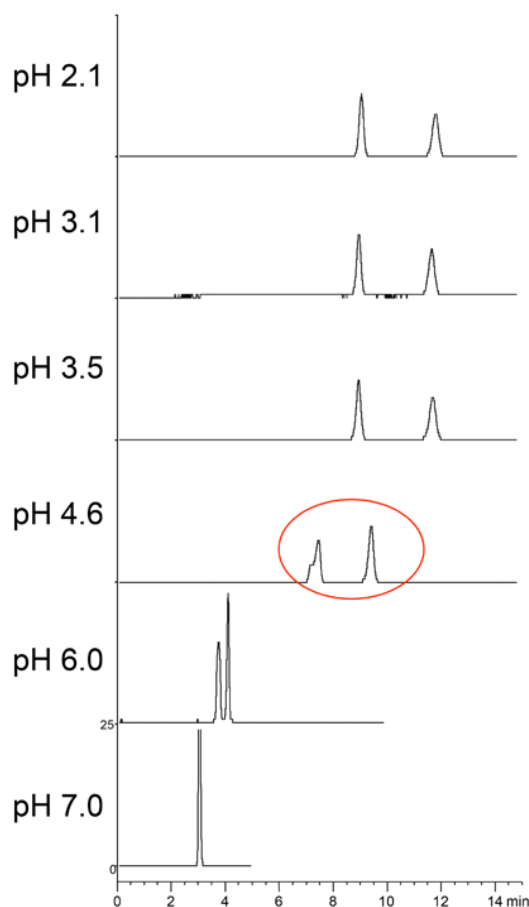
Suitable compounds for RP mode separations are hydrophilic compounds, which are hardly dissolved in NP eluents including compounds which form salts. Here, the use of coated phases is not possible anymore. Immobilised phases such as YMC's CHIRAL ART Amylose-SA, Cellulose-SB and Cellulose-SC become the first choice. The immobilised phases are universally applicable for RP, NP and SFC. Typical RP eluents, which include combinations of acetonitrile, methanol, 2-propanol or THF with an aqueous solvent, can be used with a gradient elution of 5-80% organic.

Table 4: Possible mobile phases for the RP mode

Mobile Phase Organic	pH (aq.)
Acetonitrile	Acidic (pH 2.9) 0.1% HCOOH
Methanol	Neutral (pH 6.9) 10 mM CH ₃ COONH ₄
2-Propanol	Alkaline (pH 9.0) 20 mM NH ₄ HCO ₃ adjust pH 9 with DEA
THF	

Influence of pH

For acidic and basic analytes the pH of the mobile phase plays a very important role. Experimental studies of acidic compounds such as Warfarin (pKa 5.56) show deterioration of the separation around their pKa values (figure 7).



Warfarin (pKa 5.56)

Peak shape may deteriorate at around pKa value of the compound

Figure 7: Studies under different pH values of the mobile phase.

Comparison studies of the retention factor k' for Warfarin show that a good separation can be achieved under acidic conditions for pH below 3.5 (figure 8).

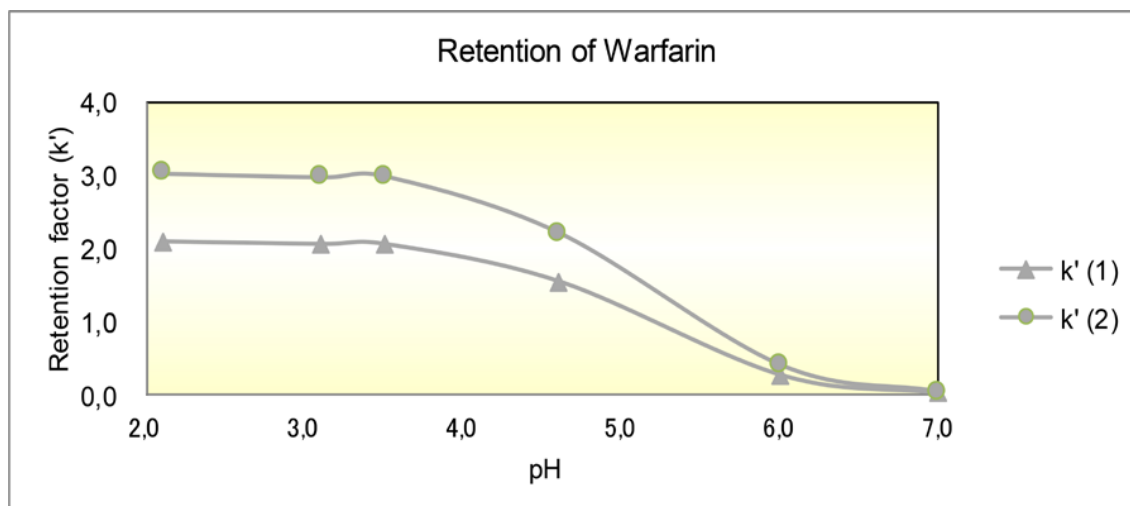


Figure 8: Retention factor (k') studies under different pH values of the mobile phase.

RP Example

In the following example, the screening of an undisclosed acidic sample from a customer is presented. CHIRAL ART Amylose-SA and CHIRAL ART Cellulose-SB were used as stationary phases. The pH of the mobile phase was adjusted as shown in figure 9. The best results were obtained using CHIRAL ART Cellulose-SB and acetonitrile with 0.1% formic acid. As shown previously, the best results could be achieved for this acidic compound under acidic conditions.

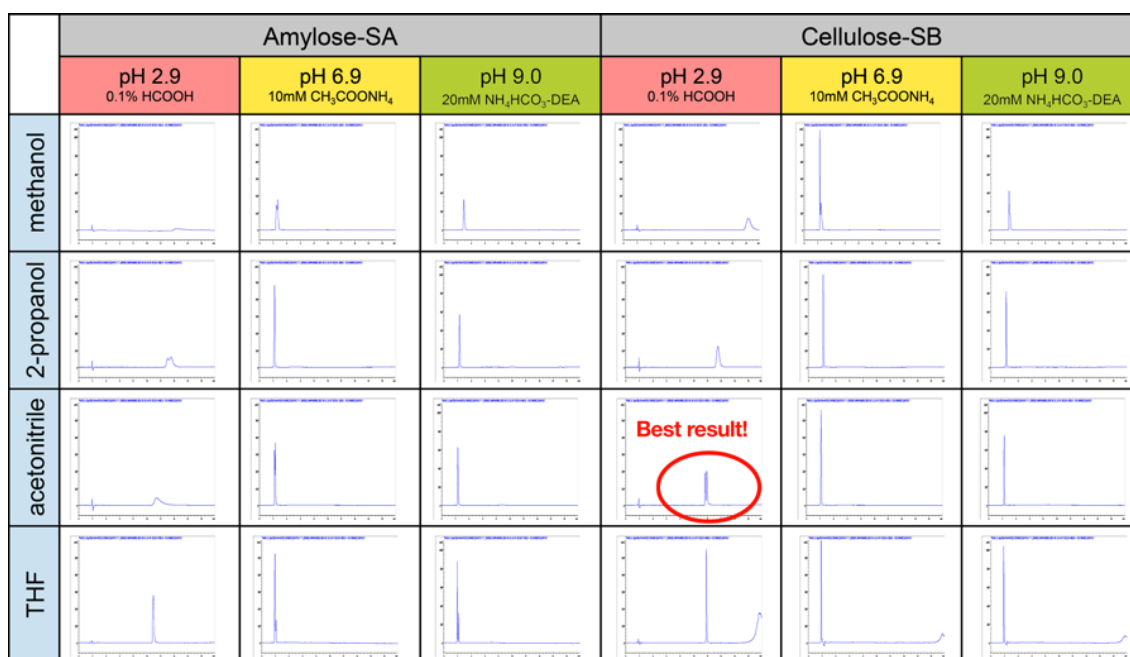


Figure 9: Method development for an undisclosed acidic sample.

This initial screening of the solvents provided an ideal starting point for further optimisation. As in the case of NP method optimisation, the following aspects should be taken into account:

- Column dimension
- Gradient => isocratic
- Flow rate
- Temperature
- Injection volume
- Additives: basic: e.g. DEA, ethanolamine;
acidic: e.g. TFA, formic acid

The method optimisation shown in figure 10 showed 40% organic content in the aqueous acidic phase (HCOOH).

- 1.) The initial gradient from 5-80% B was shown.
 - 2.) This method was transferred to isocratic conditions while using 15-20% lower composition than that at which the compound eluted under gradient conditions.
- Best result was 0.1% HCOOH/acetonitrile 60/40.

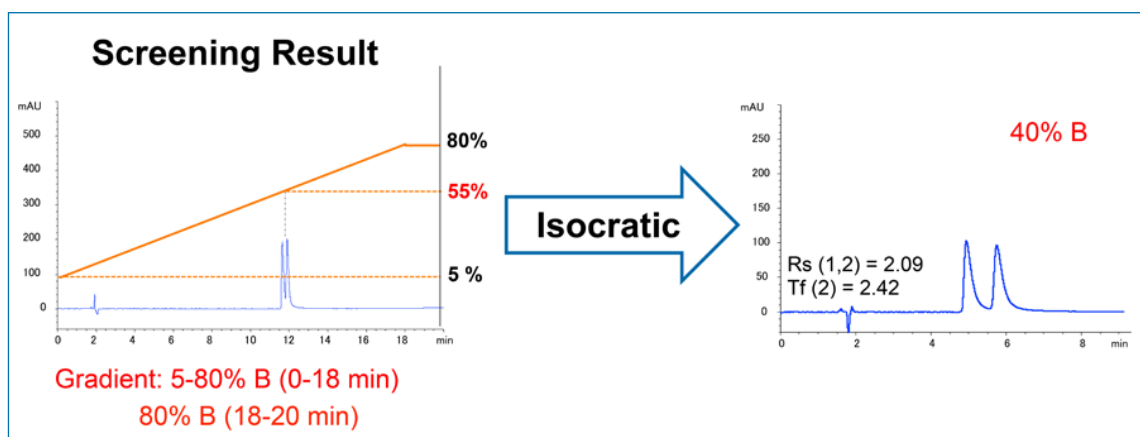


Figure 10: Method optimisation for the RP mode.

Further improvements in the separation could be achieved by changing the additive from formic acid to TFA (figure 11).

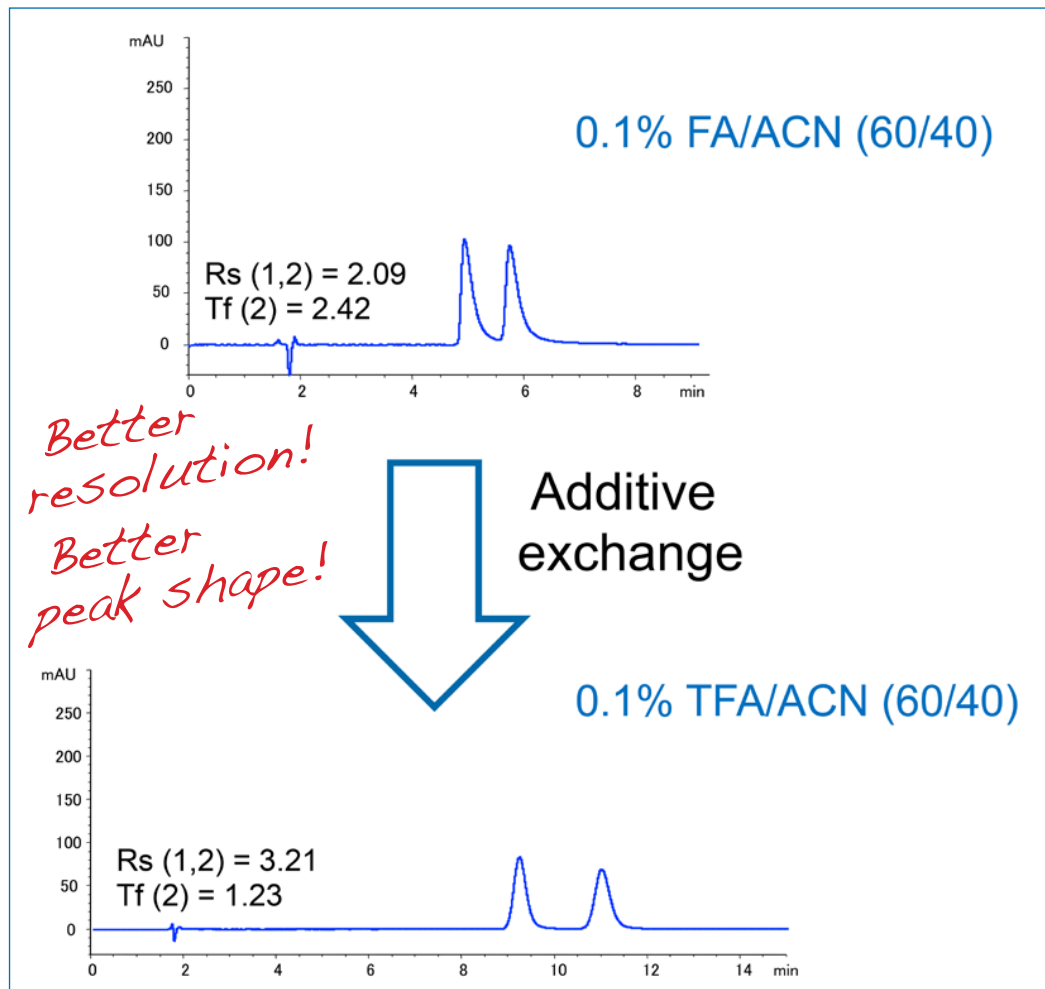


Figure 11: Method optimisation by change of additive.

Method developments for NP and RP mode have been presented using analytes with different characteristics. The strategy presented, which has been developed using know-how and experience, follows two steps. The first step is the choice of the initial conditions such as the selection of column type and mobile phase. The second step leads to method development by exploiting the influence of flow rate, pH-values, isocratic conditions and different additives.

Chiral Method Development Strategy for SFC

A pure substance can be liquid, solid or gaseous depending on pressure and temperature. Within a state diagram there is one critical point beyond which there is a phase which is neither liquid nor gas, it is called supercritical fluid (table 5). Supercritical fluids have lower viscosities and high diffusion coefficients. This makes Supercritical Fluid Chromatography suitable for high-throughput separations.

For laboratories overwhelmed with solvent disposal and time-consuming solvent cleanup, SFC is a beneficial alternative to purification and analytical applications.

The principles of SFC are similar to those of liquid chromatography, however SFC typically uses carbon dioxide as the main mobile phase. SFC is essentially a normal-phase chromatographic technique with inherent high speed and efficiency due to its mobile phase. As a high pressure liquid, or supercritical fluid, using CO₂ is an excellent solvent due to its low viscosity and high diffusivity which are needed in today's demanding purification labs and it is also a sustainable solvent since it can be reused after being recovered from other industrial processes.

SFC excels at separating and purifying chiral compounds and natural products because it's faster, uses much less solvent, and overall it is a less expensive and "greener" method than high pressure liquid chromatography (HPLC) for chiral separations.

Table 5: Properties of supercritical fluid compared to gas and liquid

	Diffusion coefficient [cm ² /s]	Viscosity [g/cm·s]
Gas	10 ⁻¹	10 ⁻⁴
Supercritical fluid	10 ⁻³	10 ⁻³
Liquid	10 ⁻⁵	10 ⁻²

CHIRAL ART Columns in SFC mode

All CHIRAL ART columns can be used under SFC conditions. One even has the choice between standard LC columns that are SFC compatible or columns exclusively dedicated to SFC mode.

First and Second Choice Eluents

Based on a significant quantity of experimental data, the proportions indicated in table 6 can be taken as a starting point and can be adjusted according to the compound to be separated. The retentive behaviour of the compound will drive the adjustment of the mobile phase. With this so-called first choice data one can decide which are the best solvent(s) or solvent ratios to perform the tests.

Alcohols (methanol, ethanol and 2-propanol) are typically used as first choice solvents in combination with CO₂. All CHIRAL ART columns whether coated (CHIRAL ART Amylose-C, Cellulose-C) or immobilised (CHIRAL ART Amylose-SA, Cellulose-SB or Cellulose-SC) are suitable.

Second choice solvents such as acetonitrile, THF and MTBE can also be used (see Table 6), but again with the restriction that only immobilised columns such as CHIRAL ART Amylose-SA, Cellulose-SB or Cellulose-SC can be used. Samples dissolvable in alcohol are most applicable for SFC.

Table 6: Mobile phases for initially screening in SFC mode

	First Choice			Second Choice*		
Eluent A	CO ₂	CO ₂	CO ₂	CO ₂	CO ₂	CO ₂
Eluent B	MeOH	EtOH	2-propanol	MeOH/ACN (50/50)	MeOH/THF (50/50)	MeOH/MTBE (50/50)
Gradient elution	10-50% modifier					

*only for immobilised column

SFC Example

Figure 13 provides an example of the first choice screening of Flavanone (figure 12), an enantiomeric flavonoid. Here, the best result was achieved with a coated CHIRAL ART Amylose-C column using CO₂/ethanol 80/20.

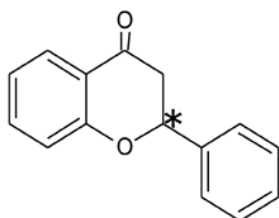


Figure 12: Flavanone

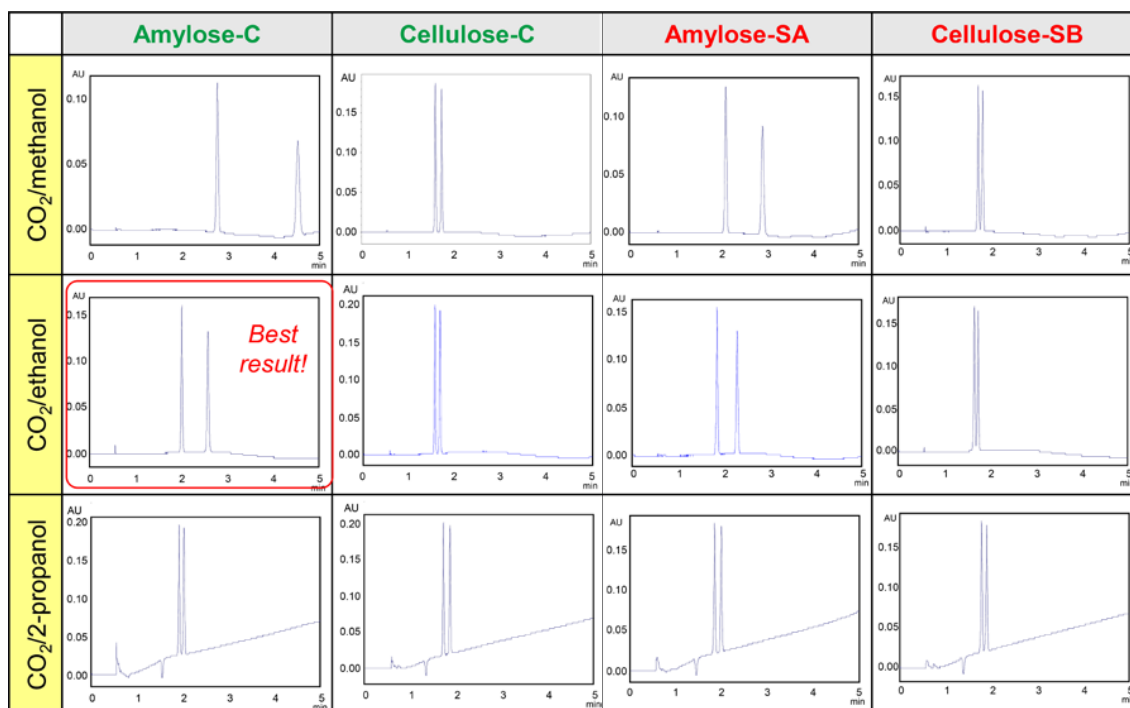


Figure 13: First choice screening on CHIRAL ART Amylose-C, Cellulose-C, Amylose-SA and Cellulose-SB.

The first choice screening provides an ideal starting point for further optimisation. The following aspects can be taken into account for method optimisation in SFC mode:

- Column dimension
- Gradient => isocratic
- Flow rate
- Temperature
- Injection volume
- Backpressure
- Additives: basic: e.g. DEA, ethanolamine;
acidic: e.g. TFA, formic acid

Scaling Up to Preparative Scale

SFC also plays an important role when scaling up to preparative scale. For Flavanone a CHIRAL ART Amylose-C column (5 µm, 250 x 20 mm ID) was used for HPLC. In comparison a SFC dedicated Amylose-C column (5 µm, 250 x 20 mm ID) was used for the preparative SFC. Figure 14 shows that for both methods, high values of 100% enantiopurity for fraction 1 and almost 100% for fraction 2 are obtained. Also, the recovery rates are comparably high.

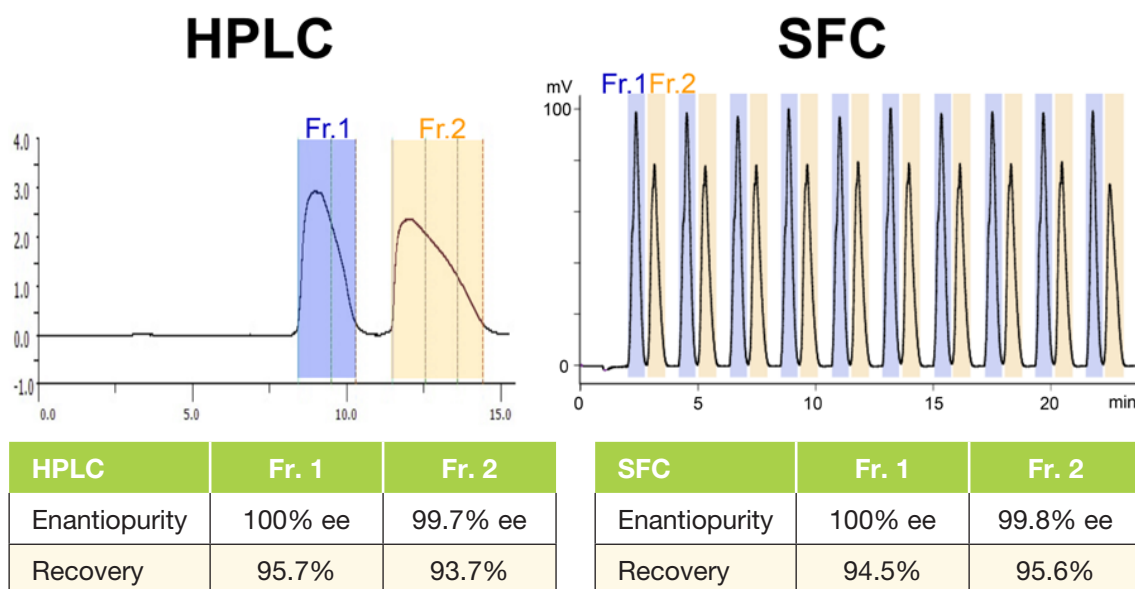


Figure 14: Method transfer from HPLC to SFC (stacked injections)

Effectiveness of SFC Separation

The advantages of SFC separation compared to HPLC become apparent when considering the productivity. It is 100% higher with 340 mg product/h for fraction 1 than for fraction 1 achieved by HPLC (table 7). The same is true for fraction 2. Together with the low solvent consumption this makes SFC very effective for preparative chromatography.

Table 7: Comparison of HPLC and SFC for preparation of Flavanone

HPLC				SFC	
Column: 250 x 20 mm ID		Fr. 1	Fr. 2	Fr. 1	Fr. 2
Enantiopurity	[%ee]	> 99.9	99.7	> 99.9	99.8
Recovery	[%]	95.7	93.7	94.5	95.6
Productivity	[mg product / h]	172	169	340	344
Fraction volume	[L solvent / g product]	1.15	2.88	0.39	0.57
Solvent consumption	[L solvent / g product]	7.0		2.0	

+100% →

-75% →

-70% →

Advantages of SFC Separation

- Productivity per unit time almost twice that of HPLC
- Evaporation of solvent from fractions easier
- Fraction volume is about 25% that from HPLC separation
- Decrease of solvent consumption of about 70% is achievable

Example of a Chiral Method Development for LC-MS

A new state-of-the-art LC-based chiral separation for Spiroxamine that can be coupled to LC-MS/MS, in the background of analyses of residues of Spiroxamine at trace concentrations, was developed. Therefore, this meant a preference for RP conditions should be investigated. Further, the demand was to perform the separation of the four isomers in less than 30 minutes. It was intended to first develop a separation application using LC-UV and then to transfer the application to LC-MS/MS.

Spiroxamine is a systemic fungicide, which was brought to the market by Bayer CropScience. The substance is a mixture of diastereomers A and B each of which consists of 2 enantiomers giving 4 enantiomers in total, A1, A2, B1 and B2 (figure 15).

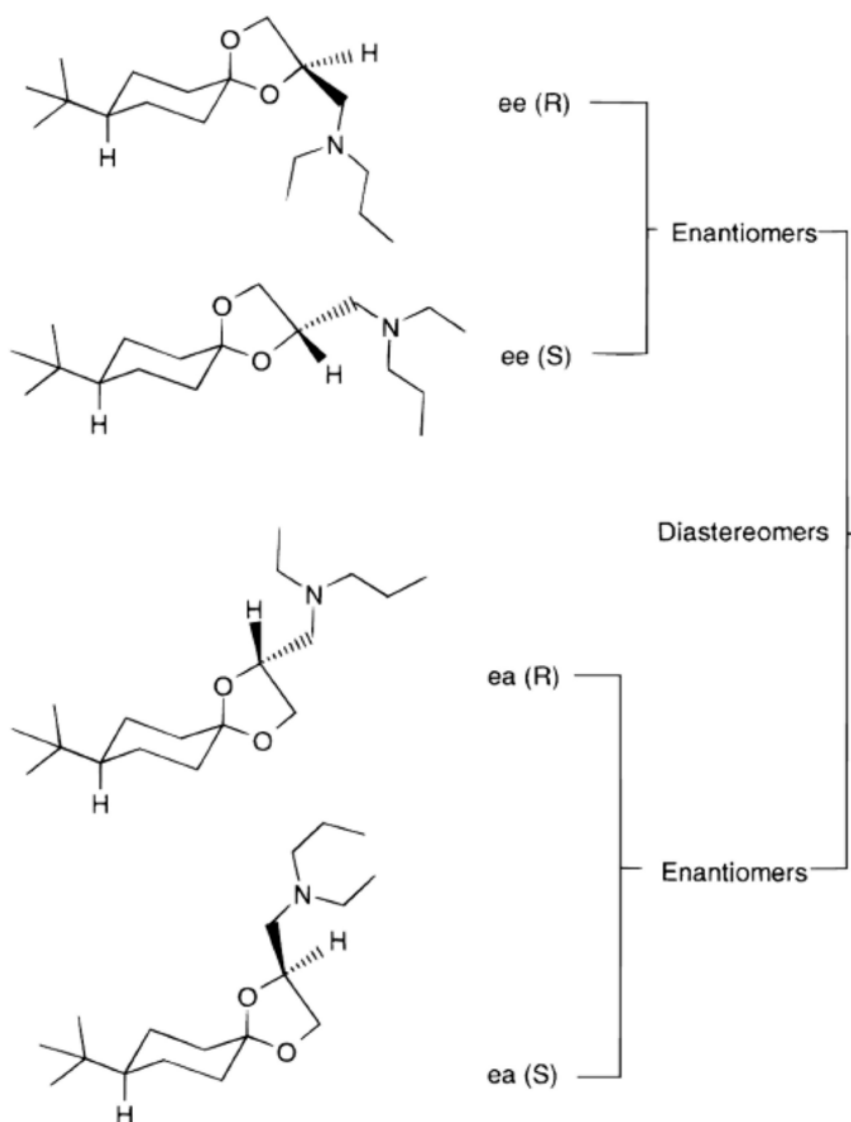


Figure 15: Isomers of Spiroxamine [3], diastereomer A: $\log Pow = 2.79$ (at 20 °C), diastereomer B: $\log Pow = 2.92$ (at 20 °C), pKa value = 6.9.

Chiral RP Screening

To overcome the requirements of RP conditions, all immobilised YMC chiral phases, namely CHIRAL ART Amylose-SA, Cellulose-SB and Cellulose-SC, were tested with RP eluents. A sufficient separation could only be obtained on a CHIRAL ART Amylose-SA column (figure 16). A particle size of 5 μm and a dimension of 250 x 4.6 mm were used for each column.

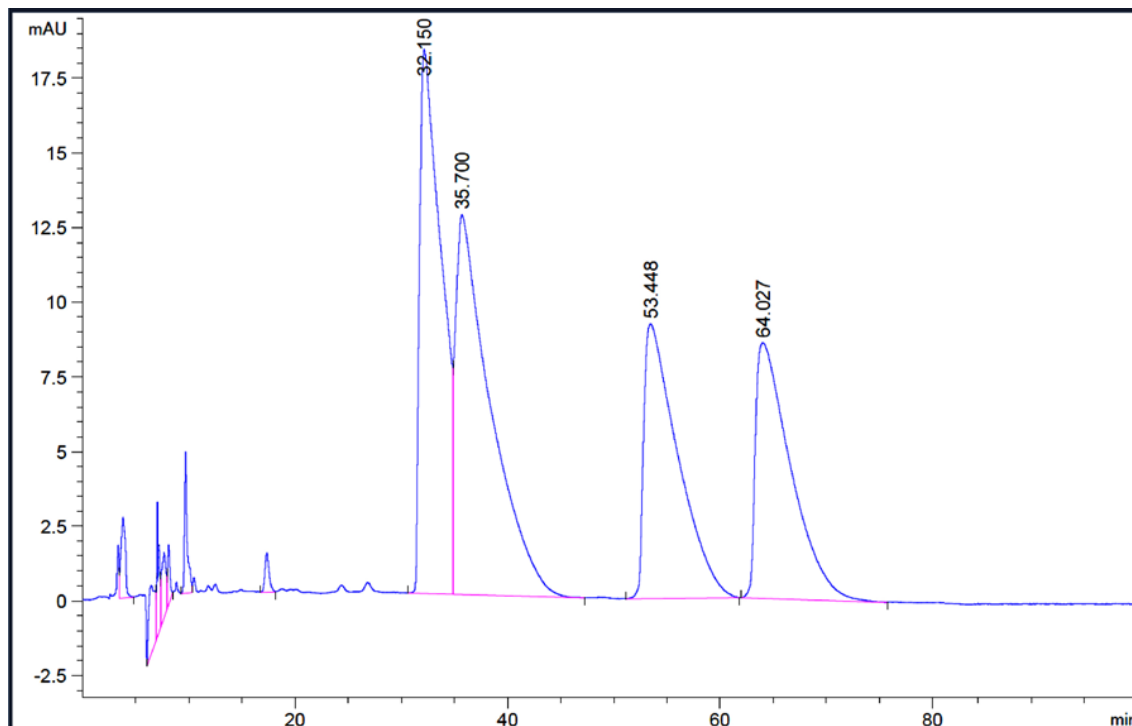


Figure 16: Result of chiral phase screening of Spiroxamine using CHIRAL ART Amylose-SA, 5 μm .

Separation in less than 20 Minutes

In order to achieve the aim of a separation in less than 30 minutes, the column length and ID were reduced. In addition, 3 μm particles were used instead of 5 μm to increase the resolution.

The ionisation in MS detection can be improved by using an additive, but the addition of acetic or formic acid was found not useful as no retention could be observed. However, the addition of diethylamine resulted in an even better peak shape.

It was not only possible to separate all 4 of the isomers from each other, but also the separation time could be reduced to just 20 minutes. Therefore, all requirements could be fulfilled.

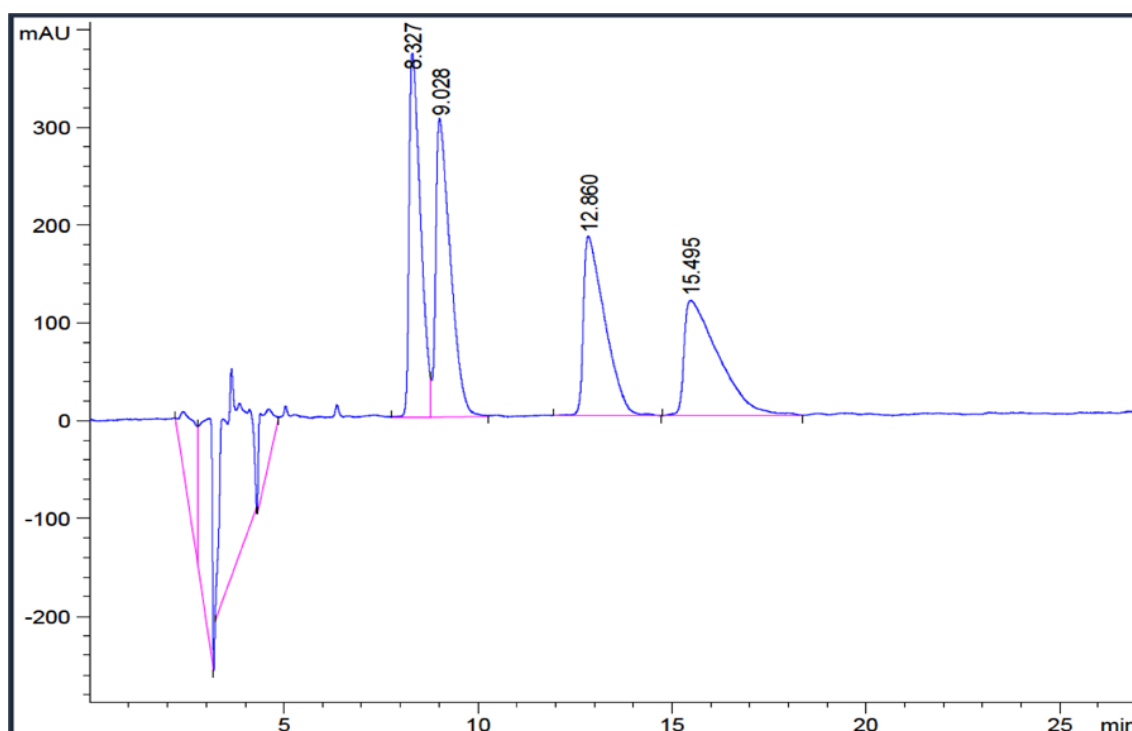


Figure 17: Optimised method for Spiroxamine using CHIRAL ART Amylose-SA, 3 μ m

Column:	CHIRAL ART Amylose-SA 3 μ m (150 x 3.0 mm ID)
Part No.:	KSA99S03-1503WT
Eluent:	water/ethanol/DEA (27.5/72.5/0.1)
Flow rate:	0.25 ml/min
Temperature:	30 °C
Detection:	UV at 210 nm
Injection:	10 μ L (10 mg/mL)

Transfer from LC-UV to LC-MS/MS

The application then was transferred to the LC-MS/MS instrument consisting of an Agilent 1290 UPLC system (binary and isocratic pump), a CTC autoinjector (Axel Semrau) and a Sciex API6500 high-end triple-quadrupole mass spectrometer.

Diethylamine used in the UV-application had to be substituted by a 10 mM ammonium carbonate solution (pH 9.5). The diethylamine strongly influenced the ionisation process and “quenched” the MS-signal by about 99%.

To improve the ionisation 1% formic acid in methanol/water 50/50 was introduced post-column into the eluent flow coming from the chiral column (“change” of pH value from weak alkaline to weak acidic protonating Spiroxamine; pK 6.9).

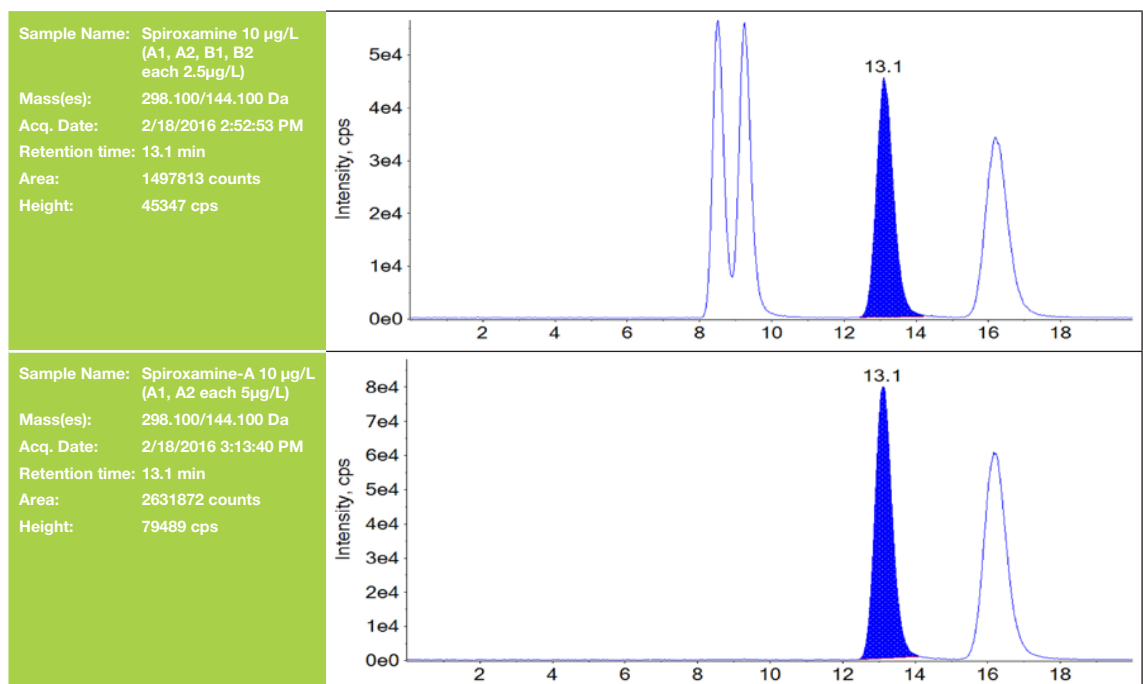
Final Set-up

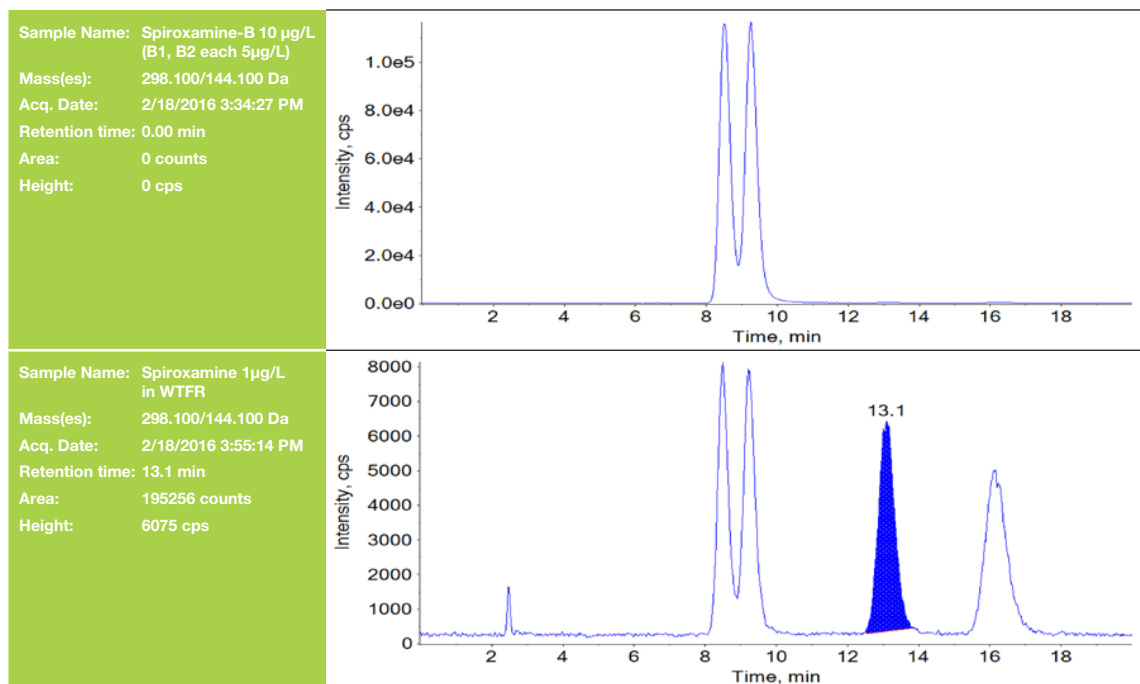
Final conc. at LOQ: 1 µg/L spiroxamine = 0.54 µg/L A-Isomer; 0.46 µg/L B-Isomer
 = 0.27 µg/L A1 enantiomer
 = 0.27 µg/L A2 enantiomer
 = 0.23 µg/L B1 enantiomer
 = 0.23 µg/L B2 enantiomer

HPLC Column:	CHIRAL ART Amylose-SA, 3 µm (150 x 3 mm ID)
Eluent:	A/B (25/75) A: water/ethanol 9/1 + 10 mM ammonium carbonate (pH ~9.5) B: water/ethanol 1/9 + 10 mM ammonium carbonate
Flow:	0.3 mL/min
Injection:	1 µL
Iso-pump:	post-column make-up via T-piece with 0.3 mL/min 1% formic acid in methanol/ water 50/50.
MS-MS conditions:	Multiple-reaction-monitoring (MRM) mode in ESI positive, MRM 298-144 for quantitation and MRM 298-100 for confirmation.

Example Chromatogram with Enantiomer A1 at LOQ in Grape Matrix Extract

Table 8: Determination of enantiomer A1 in grape matrix extract, 4th repetition of each chromatogram [4]





Chromatographic Performance Parameters

HPLC Column:	CHIRAL ART Amylose-SA
Evaluated Sample:	10 µg/L spiroxamine (mixtures of 4 enantiomers)
Column length:	150 mm
Colum ID:	3 mm
Particle Size d_p :	3 µm
$V_{d\ col}$:	742.1 µl
V_{loop} :	1 µl
$V_{capillaries}$:	5.7 µl
V_{total} :	748.7 µl
Flow:	300 µL/min
$t_{d\ cal.}$:	2.496 min

theor. Plates N:
$$N = 5.54 \left(\frac{t_R}{w_{0.5}} \right)^2$$

Peak capacity n:
$$n = 1 + \frac{\sqrt{N_{max}}}{4} \ln(1 + k_{max})$$

Capacity Factor: should be >3 best: 4-10
 Separation Factor: k'_2/k'_1 always >1
 Peak Resolution: 0.0 = co-eluting
 0.6 = 12% peak overlapping
 1.0 = 2% peak overlapping
 1.5 = baseline separated

Analyt = Order of Elution	t_R [min]	k' -Value	W_H [min]	W_H [s]	St.Dev. _o [s]	Theor. Plates N	Plate Height H [µm]	plates/m N/m	Separation Factor $a_{n,n+1}$	Peak Resolution $RS_{n,n+1}$	k'_{max} u. N_{max}	Peak capacity n
Spiroxamine enantiomere B1	8.50	2.41	0.3385	20.31	8.628	3494	42.9297	23294			k'_{max}	29
Spiroxamine enantiomere B2	9.24	2.70	0.3570	21.42	9.099	3712	40.4083	1.12	1.22	5.49		
Spiroxamine enantiomere A1	13.10	4.25	0.5080	30.48	12.948	3685	40.7064	1.57	4.47	N_{max}		
Spiroxamine enantiomere A2	16.19	5.49	0.6708	40.25	17.098	3228	46.4698	1.29	2.71		3712	

Chromatographic characteristics: Peak capacity n 29, theor. plates/m 24747

Conclusions

Chiral method development for NP, RP and SFC has been presented in this paper using analytes with different characteristics. The strategy for development follows two steps. The first step is the choice of initial conditions such as the selection of column type and mobile phase. Therefore, potential solvents for first and second choice are introduced with the appropriate gradients. These results provide a good starting point for the further optimisation of the method. The next step leads to method development by optimising the influence of flow rate, pH-values, isocratic conditions and different additives. Each separation mode, whether NP, RP or SFC, has the need for a different screening approach, allowing the use of either both chiral column types, coated and immobilised, or just immobilised columns. For each mode a screening example has been presented with a specific substance to demonstrate the practical procedure, e.g. for Astaxanthin in NP mode or Flavanone in SFC mode.

For acidic and basic analytes the choice of an additive can be significant. Experimental studies of acidic compounds such as Warfarin (pKa 5.56) show deterioration of the separation around their pKa values. Therefore, the pH-value of the mobile phase plays an important role for method development and has to be adjusted accordingly.

The advantages of both coated and immobilised chiral CSPs are also been demonstrated for SFC mode. The extremely high productivity in combination with low solvent consumption makes SFC very attractive. The advantages of SFC separations compared to HPLC become apparent when considering the productivity at preparative scale.

Literature

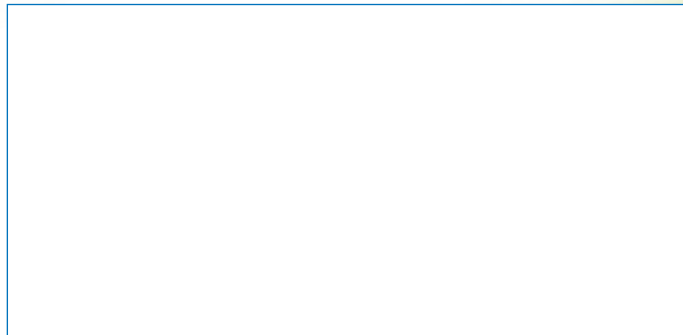
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