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ENANTIOSEPARATIONS OF LIQUID CRYSTALLINE MATERIALS

ENANTIOSEPARACE KAPALNĚ-KRYSTALICKÝCH LÁTEK

Ph.D. Thesis

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The experimental work that constitutes this Ph.D. thesis was realized between September 2017 and June 2022. Results from liquid chromatography were obtained in the laboratory based at the Department of Analytical Chemistry; results from sub/supercritical fluid chromatography were obtained in the laboratory based at the Department of Physical and Macromolecular Chemistry. Both departments belong to the Faculty of Science, Charles University, Prague, Czech Republic.

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Abstract (EN)

Liquid crystalline materials are an important domain of the material science. Chiral liquid crystals (CLCs) are researched for their unique chirality-derived properties. The development of new CLCs focuses both on appropriate molecular design to fit their intended purpose and also on ways of using widely available and less expensive sources of chirality to enable cost-effective production for a potential large-scale production. Methods capable of enantioseparation are needed mainly for chiral purity control of newly synthesized CLCs and for their potential use in scaling up for preparative purpose. Nowadays, enantioselective chromatography has evolved to be the method of choice for enantioseparations.

This thesis aims to establish new approaches to chromatographic enantioseparations of CLCs by developing fast and robust enantioselective methods using two different ultra-high performance chromatographic techniques – sub/supercritical fluid chromatography (UHPSFC) and liquid chromatography (UHPLC). UHPSFC is introduced and successfully employed for the first time for this purpose. For some CLCs, high values of enantioselectivity and enantioresolution were achieved, opening the possibility of scaling up to semipreparative use. In UHPLC, two modes (reversed-phase and polar organic solvent mode) were explored for their suitability for enantioseparation of CLCs.

In past, normal-phase mode of liquid chromatography (NPLC) has been used for this purpose; all three setups (sub/supercritical fluid chromatography and two modes of liquid chromatography) were studied to evaluate their viability as greener and more versatile alternatives to the NPLC. The tested CLCs comprised of several structural types of chiral center as well as of lesser variations in their structure such as the length of alkyl residues or spacers, the halogen substitution pattern and the number of benzene rings.

All experiments were done as direct enantioseparations via the use of chiral stationary phases (CSPs); in majority of the experiments, CSPs based on derivatives of polysaccharides amylose or cellulose were used as they proved to be exceptional for enantioseparation of rod-like CLCs. Systematic studies of chromatographic behavior of CLCs were also conducted to survey the effects of column temperature, mobile phase composition, CSP and solute structure on the enantioseparation.

Abstrakt (CZ)

Kapalně-krystalické látky patří mezi významné odvětví materiálové chemie. Chirální kapalné krystaly (CLCs – z anglického chiral liquid crystals) jsou zkoumány pro jejich unikátní vlastnosti odvozené od chirality. Vývoj nových CLCs je soustředěn na molekulární design, který zajistí požadované vlastnosti látek a zároveň na hledání dostupných a levnějších zdrojů chirality, které by zajistily případnou ekonomicky výhodnou výrobu ve větším měřítku. Metody pro enantioseparace jsou potřebné především pro kontrolu chirální čistoty nově připravených CLCs a také pro eventuální využití v preparativním měřítku. Enantioselektivní chromatografie je v současnosti preferovanou metodou pro enantioseparace.

Tato závěrečná práce je zaměřena na zavedení nových postupů pro chromatografické enantioseparace CLCs skrz vývoj rychlých a robustních enantioselektivních metod v prostředí dvou různých (ultra)vysokoúčinných chromatografických technik – sub/superkritické fluidní chromatografie (UHPSFC) a kapalinové chromatografie (UHPLC). Technika UHPSFC byla použita poprvé a následně úspěšně zavedena pro enantioseparace CLCs. Pro některé CLCs byly získány tak vysoké hodnoty enantioselektivity a enantiomerního rozlišení, že je zde možnost přenositelnosti metody do semipreparativního měřítka. V UHPLC, dva módy (reverzní mód a mód s polárně organickými rozpouštědly) byly testovány z hlediska vhodnosti jejich použití pro enantioseparace CLCs.

V minulosti se pro separace enantiomerů CLCs používala téměř výhradně kapalinová chromatografie v normálním módu; všechny tři zmíněné systémy (sub/superkritická fluidní chromatografie a dva módy kapalinové chromatografie) byly zkoumány za účelem zhodnocení, zda by mohly normální mód nahradit jako univerzálnější a k životnímu prostředí šetrnější alternativy. Použité CLCs zahrnovaly především několik typů chirálního centra a dále se lišily délkou alkylových zbytků nebo spacerů, rozdílnou substitucí halogeny a počtem benzenových jader.

Všechny experimenty byly provedeny formou přímé enantioseparace za využití chirálních stacionárních fází; ve většině případů se jednalo o chirální stacionární fáze odvozené od derivátů polysacharidů amylózy nebo celulózy, jelikož se ukázaly být vhodné pro enantioseparaci tyčinkovitých CLCs. Byly provedeny i souhrnné studie chromatografického chování CLCs a byl zkoumán vliv teploty, složení mobilní fáze, použité chirální stacionární fáze a struktury analytu na enantioseparaci.

Keywords

enantioselective chromatography, liquid chromatography, sub/supercritical fluid chromatography, liquid crystals, chiral stationary phase

Klíčová slova

enantioselektivní chromatografie, kapalinová chromatografie, sub/superkritická fluidní chromatografie, kapalné krystaly, chirální stacionární fáze

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List of symbols and abbreviations

BP(s) – blue phase(s)

CLC(s) – chiral liquid crystal(s)

CS(s) – chiral selector(s)

CSP(s) – chiral stationary phase(s)

EEO – enantiomer elution order

F – volumetric mobile phase flow-rate

GC – gas chromatography

HETP – height equivalent to a theoretical plate

HPLC – high performance liquid chromatography

k – retention factor

LC(s) – liquid crystal(s)

LCD – liquid crystal display

LCM(s) – liquid crystalline material(s)

LOD – limit of detection

LOQ – limit of quantitation

MP(s) – mobile phase(s)

N – plate count

NPLC – normal-phase liquid chromatography

POSC – polar organic solvent chromatography

p-CSP(s) – polysaccharide-based chiral stationary phase(s)

RPLC – reversed-phase liquid chromatography

R_s – enantioresolution

RSD – relative standard deviation

sCO_2 – supercritical carbon dioxide

SF – supercritical fluid

SFC – sub/supercritical fluid chromatography

T – column temperature

t – retention time

u – linear velocity of the mobile phase

UHPLC – ultra-high performance liquid chromatography

UHPSFC – ultra-high performance sub/supercritical fluid chromatography

α – enantioselectivity

1. Introduction

1.1. Liquid crystals

Despite the early discovery of liquid crystals (LCs) in the late 19th century [1,2], it took almost two decades before the existence of liquid crystallinity has been widely recognized [3] and it wasn't until the second half of the 20th century that LCs got into the spotlight of both scientific research and industry, mostly thanks to the invention of the liquid crystal display (LCD) [4]. LCs are materials capable of forming a mesophase – a state of matter at the borderline between solid and liquid state. While in mesophase, the material appears to be liquid (it flows), but it also maintains orientational (sometimes also positional) order of the molecules. The ordering is neither as uniform nor as rigid as in solid phase crystal, however, it is sufficiently manifested to induce properties such as optical anisotropy (birefringence) or (anti)ferroelectricity. On the other hand, the liquid aspect of LCs provides them with the ability to rapidly reorient the molecules in case of outer influence, e.g. in response to applied electric or magnetic field [5]. These features make LCs suitable for application in all kinds of photonic and electro-optic devices, such as LCDs, optical switches and filters, spatial light modulators, etc.

LCs are divided into three classes by the nature of origin of the mesophase(s): thermotropic, lyotropic and amphotropic. Thermotropic LCs form mesophase(s) in a defined temperature span as an intermediate state between solid state and isotropic liquid when being heated/cooled. Lyotropic LCs form mesophase(s) only when dissolved in a suitable solvent at appropriate concentration range and amphotropic (amphitropic) LCs can do both. The thermotropic mesogens are divided into groups based on the overall shape of the molecules into rod-like, discotic and bent-core mesogens, see **Figure 1**. This thesis is focused on thermotropic LCs, specifically on a subclass of rod-like thermotropic LCs.

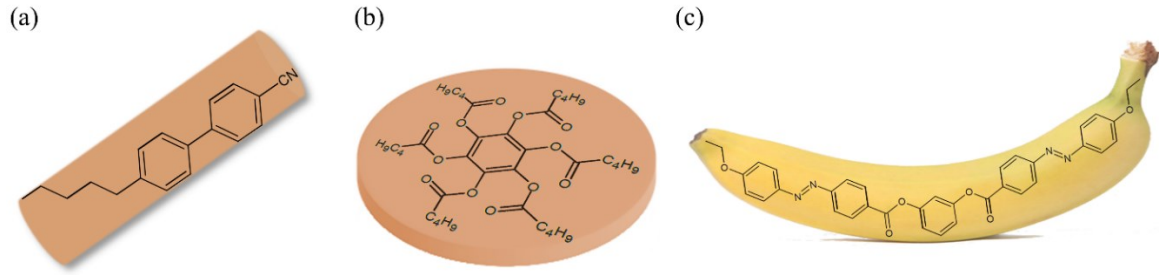

Figure 1 Examples of molecular design of (a) rod-like (calamitic), (b) discotic and (c) bent-core (also called banana-shaped) mesogens. Redrawn with inspiration from Ref. [6].

Rod-like LCs are a group of materials with a common structural motive - a rigid elongated core composed of aromatic rings with flexible chains attached at the ends of the core. The aromatic rings of the core can be linked by ester or ether bonds, connected or conjugated. For some materials the linkage might also be an azo group, meaning the materials are photosensitive and can undergo photoisomerization from *trans* to *cis* form of the azo group [7]. Resulting from altering the shape of the molecule, the switch to *cis* form also changes the chromatographic behavior of the compounds; the azo group in *cis* form is exposed and the molecule has higher dipole moment compared to the *trans* form. This usually leads to it being eluted earlier in RPLC [8].

The mesophases formed by rod-like LCs are described by the director - a vector representing statistically most probable orientation of LCs longitudinal molecular axis in space. The three most common mesophases of calamitic LCs are nematic, smectic A and smectic C, for illustration see **Figure 2**.

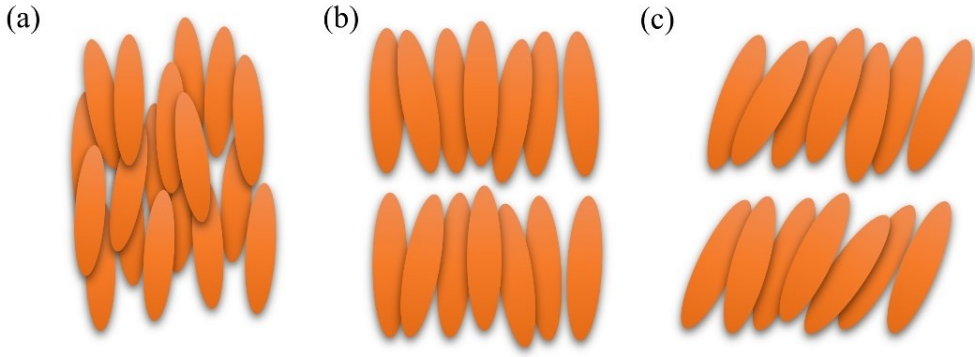

Figure 2 Illustration of (a) nematic, (b) smectic A and (c) smectic C mesophase formed by achiral rod-like mesogens. Redrawn with inspiration from [9].

1.2. Chirality and liquid crystals

For a compound to be chiral, it must be possible for it to exist in two different forms that are non-superimposable mirror images, meaning they can't be translated into one another by rotation of the whole molecule or its parts on freely rotating bonds. Chirality is induced by existence of a chiral element in the structure of the compound - in its simplest form, chirality is a result of four different substituents of a single carbon atom; based on the Cahn-Ingold-Prelog system of substituents priority [10], the two mirror forms are denoted as *R* and *S* enantiomer. The main effect of chirality is the rotation of the plane of linearly polarized light while transmitted through the sample of a chiral compound.

Chirality of liquid crystalline materials (LCMs) is gained either by incorporating a chiral center in the structure of a liquid crystal or by mixing a chiral dopant[1] with an achiral LCs[2]. In both cases, the resulting material is chiral (has a non-zero optical rotation in polarimeter) and displays mesomorphic properties related to chiral LCMs. Chirality of a mesophase is demonstrated by the existence of supramolecular helix; the helical organization created by rotation of the director. For a chiral nematic mesophase (N*, cholesteric) the director rotates continually while moving perpendicularly from the base surface across hypothetical planes. In case of a chiral smectic C mesophase (SmC*), the director performs a gradual precession motion across the individual layers of molecules. For illustration of chiral mesophases see **Figure 3**.

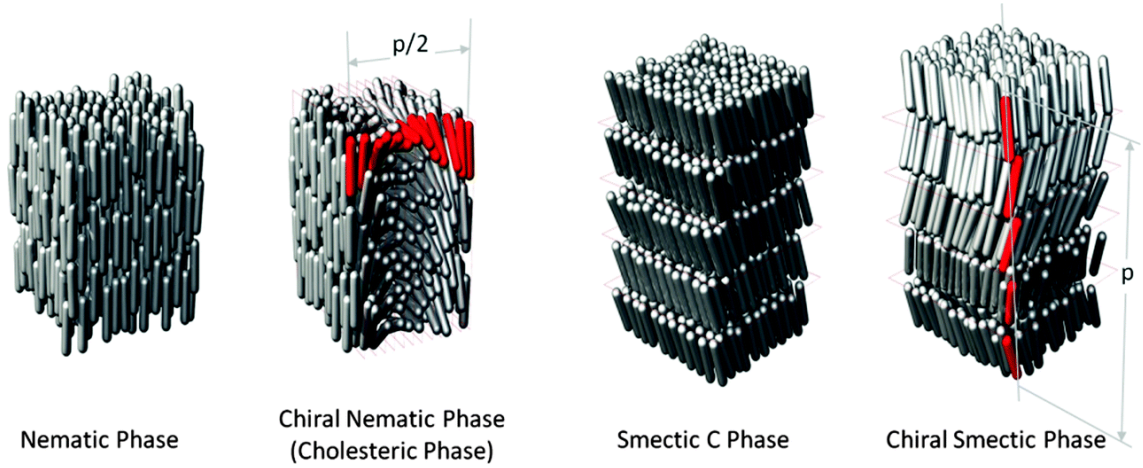

Figure 3 Differences in molecular ordering between nematic and smectic C phase and their chiral analogues. Republished with permission of Royal Society of Chemistry from Ref. [12]; permission conveyed through Copyright Clearance Center, Inc.

As with any helical structures, the helices are defined by a helical pitch (p = the distance of two points between which the helix makes one full turn). A typical chirality-dependent attribute of LCs is their ability to reflect incident light of the same wavelength as the helical pitch of their chiral mesophase; this feature is the cornerstone of their use in display technology. All chirality-induced mesomorphic properties are influenced by chiral purity of the LCM [13–15]. The helical pitch decreases with decreasing enantiomeric excess of chiral liquid crystals (CLCs); in racemic mixtures it is considered infinite [16]. On the other hand,

[1] Chiral dopant is a stable chiral compound that is miscible with the LC; the dopant itself doesn´t have to be mesogenic.
[2] A special case - spontaneous deracemization into chiral domains can be observed in mesophases of achiral bent-core mesogens [11].

CLCs of very high optical purity often exhibit interesting mesomorphic behavior. For example, mesogens forming blue phases (BPs) are of interest given sub-millisecond response time and wide viewing angle of BP, which makes them desirable for use in display technology [17]. However, the BPs manifest only for highly twisted LCs samples with sufficient enantiomeric purity [18]. Conclusively, enantiomeric ratio of any CLC should be determined before studying and discussing its chirality-related liquid crystalline properties. In case the synthesis of the CLC proceeds under conditions that may influence the configuration of the chiral center, its chiral purity must be verified as a part of the characterization process.

Aside from applications in electric and optic devices, LCs have found their use in numerous branches of chemistry. They have been used as (chiral) stationary phases in chromatographic and electrophoretic separations, aligning media for nuclear magnetic resonance, detecting surfaces for other (chiral) analytes, environments for asymmetric synthesis, etc. Their utilizations for any purpose dealing to some extent with enantiodiscrimination were recently covered in a review article [19].

1.3. Determination of enantiomeric excess

The oldest technique enabling determination of enantiomeric excess is polarimetry. A solution of a chiral compound of known concentration is put into the sample cell with defined length and linearly polarized light passes through it. The angle between the planes of polarized light before and after passing through the sample is measured and the optical purity is calculated by comparing the measured optical rotation with standard reference data. In case of CLCs, in past the researchers often had to omit the enantiomeric purity determination from the characterization process. Polarimetry couldn't be used for novel compounds as there are no data on standard optical rotation to use for reference and enantioselective chromatography wasn't as accessible in past as it is today. In some articles, the authors settled for confirmation of optical purity of starting substances/intermediate products only or relied on the purity data from the supplier [20–23]. In other works, a simple polarimetric confirmation of optical activity of the resulting material was done (without any further quantitation of enantiomeric excess) to verify that racemization was avoided [24–27]. Finally, in works published from 1990s[3], enantioselective chromatography has been used for

[3] The breakthrough works of Okamoto that led to commercial production of polysaccharide-based CSPs were published in 1984 [28,29].

determination of chiral purity of either intermediate or final products [30–45]. In all cases, the enantioseparations were performed under NPLC conditions and on polysaccharide-based chiral stationary phases (p-CSPs) with the exception of Pirkle type (*S*,*S*)-Whelk-O1 used for naphthyl propionate CLCs [46]. The dominance of p-CSPs for analysis of CLCs can be explained by their macromolecular character which is advantageous for interaction with rather bulky and rigid CLCs. In 2017, a first enantioseparation of CLCs in reversed-phased mode was published [47].

1.4. Enantioseparations

Enantioselective chromatography is a highly versatile technique for determination of the enantiomeric ratio of chiral compounds. The two enantiomers of a compound have identical physical properties; thus, it is impossible to separate them based on interactions with achiral environment, e.g. common octadecyl-silica stationary phase. Instead, chiral environment is needed to promote enantiomer-specific interactions between the enantiomers and the stationary phase. In case of separation techniques, the chiral environment is created by employing chiral selectors (CSs). CSs are molecules, that are chiral themselves and therefore may interact enantioselectively with the solute (both in attractive and repulsive manner). The attractive CS-chiral solute interactions comprise of hydrogen bonds, ion-dipole interactions, dipole-dipole interactions, π-π stacking, etc., depending on the CS and solute structure; steric hindrance or coulombic interaction between an ionic CS and a solute of the same sign are the typical repulsive interactions [48]. Chromatographic enantioseparation (separation of enantiomers in their native state) through use of a CS can be performed in two ways. The most common approach is the use of a column packed with a chiral stationary phase (CSP) created by attachment of the CS on the support particles [49]. A rare approach is to add a soluble CS into the mobile phase (MP) and perform the separation on an achiral stationary phase, such as octadecyl-silica [50].

All p-CSPs are derived from either amylose or cellulose. The two macromolecules have differently coiled helices, which usually grants them a complementarity – the enantiomer elution order (EEO) is often the opposite on amylose vs. cellulose-based CSPs [51,52]. Additionally, the derivatization of hydroxyl groups is used to create the variety of p-CSPs. Since the introduction of the polysaccharide derivatives as CSPs [28] many different variations were prepared and utilized in column chromatography. The phenylcarbamates prepared by reaction of amylose/cellulose with substituted phenylisocyanates are the most

widely used commercially available derivatives. The resulting carbamate linkages can function as both hydrogen bond donor and acceptor and are believed to be the main chiral recognition sites [53]. Among the polysaccharide phenylcarbamate derivatives, methylated, methylated/chlorinated and chlorinated CSs stand out [54]. The aromaticity of phenyl rings is not disturbed by the substitution, and they can interact in e.g. π-π stacking. However, the substituents have electron-donating or electron-withdrawing effect and can therefore influence not only the strength of interactions involving the aromatic ring, but also the interactions of the carbamate function. Lastly, the chlorine can form halogen bond with the solute, which was recently recognized as another possible enantioseparation-driving interaction [55]. The multitude of interactions offered by the p-CSPs is the reason of their success across the various modes of chromatographic separations.

The intricacy of enantiorecognition mechanisms on macromolecular CSPs often complicate and prolong the method development. In last years, there has been a considerable development in molecular docking simulations of enantioseparation [56]. In case of small molecular CSs such as in Pirkle-type CSPs, the enantiorecognition process is much simpler and has been more or less unveiled [57]. Thus, the computer docking simulations are often in accordance with the experimentally obtained results and reliable in silico search for the suitable separation conditions appears to be attainable in near future. However, the complexity of the enantioseparation mechanism on the polysaccharide-based CSPs hinders the use of computational approach for them and results in the need of experimental screening approach when developing new methods. The inscrutability of solutes' chromatographic behavior on polysaccharide derivatives stems mostly from conformational flexibility of the CSs [58]. The macromolecular helix may undergo conformational changes depending on the column temperature or the MP composition [58–60] and change the size and shape of the chiral cavities available for interaction with solutes, see **Figure 4** for illustration.

Moreover, the fabrication process of p-CSPs also influences its conformational freedom and thus enantioselectivity; differences in results were described for columns with an identical CS but produced by different manufacturers [61,62] or for p-CSPs differing in the attachment of the CS onto the support particles [63,64]. In conclusion, a purely experimental screening is still the typical approach for developing a new enantioseparation method on the p-CSPs [65].

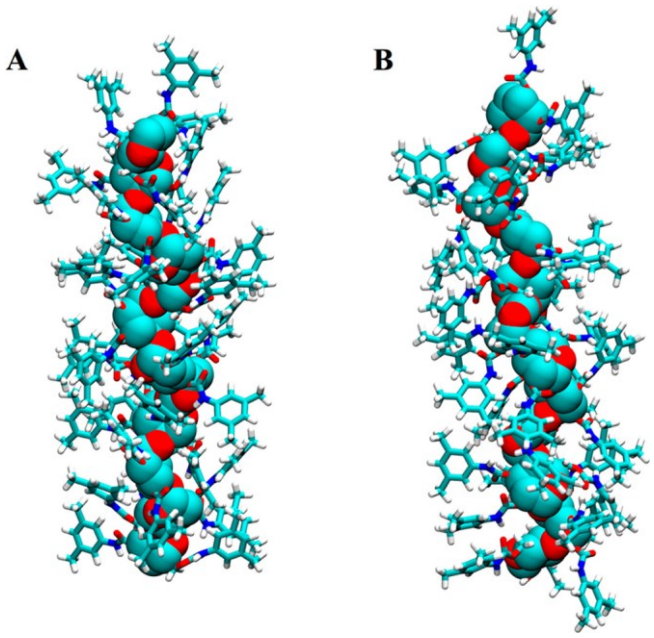

Figure 4 Average structures of amylose tris(3,5-dimethylphenylcarbamate) in (A) methanol and (B) *n*-heptane/propan-2-ol 90/10 (*v*/*v*). The backbone atoms are represented in spheres and the derivatives are represented with sticks. Hydrogen atoms are in white, carbon atoms in cyan, nitrogen atoms in blue and oxygen atoms in red. Reprinted with permission from Ref. [58] © 2017 American Chemical Society.

1.5. Sub/supercritical fluid chromatography

Chromatography in which a supercritical fluid (SF) is used as the main component of the MP is in its current form a relatively young technique. Although supercritical fluids were used for extraction since 1950s [66], they were introduced as a MP in chromatography at the turn of the 1962 [67]. At that time, however, their utilization was researched in the framework of gas chromatography (GC), meaning that they were used in their pure state as the MP and the separations were performed on GC-like instruments with GC columns, limiting the adjustment of elution power to temperature and pressure programs [68]. In 1981, sub/supercritical fluid chromatography (SFC) with capillary columns was introduced by Novotny et al. [69] and brought at the time unparalleled combination of high efficiency and applicability to thermolabile and non-volatile samples[4]. Later in 1982, two important advances ultimately paved the way for the rapid development and spread of the SFC: (a) the procedure for easy conversion of a commercial high-performance liquid chromatography

[4] Efficiency of liquid chromatography was at the time strongly limited by the instrumentation (large stationary phase particle size in combination with solvent pump insufficiency to work at higher pressures).

(HPLC) instrumentation to an SFC with carbon dioxide and subsequently (b) the option of adding a small portion of organic solvent (mostly methanol) to the MP as the MP modifier [70]. The use of modifiers enabled by moving from GC to HPLC-like instrumentation introduced a new aspect to the SFC, the possibility of programming the MP elution power in a way comparable to HPLC.

Nowadays, the possibility of separating complex samples containing both hydrophilic and hydrophobic compounds by exploiting MP gradient ranging from pure SF[5] to pure modifier has been demonstrated [71], once and for all connecting the SFC and HPLC domains. However, when neat supercritical carbon dioxide is mixed with a modifier, the critical parameters of the MP might be displaced towards values higher than the selected back-pressure and temperature. Thus, the system can no longer be considered supercritical. As a solution, different names are used in the literature depending on the portion of modifier: supercritical fluid chromatography (for < 5 % of modifier or as an umbrella term for any SF-employing chromatography), subcritical fluid chromatography [72] and convergence chromatography [73] (for > 5 % of modifier) or unified chromatography (gradient with a full transition to HPLC) [71]. In this thesis the abbreviation SFC (or UHPSFC) is used, encompassing both supercritical and subcritical state of the MP in the performed experiments.

SFC is becoming the method of choice for developing enantioseparation methods [74]. The mild conditions of noncorrosive, (almost) nonreactive sCO_2 together with small amounts of polar organic solvents is suitable for working with delicate, thermolabile or non-volatile analytes. Additionally, similarly to NPLC the SFC environment enables polar interactions that are more directional than e.g. hydrophobic effect and are therefore more desired in enantioselective chromatography [75,76] and it is often possible to replace an NPLC method with SFC [77].

In past, SFC has been successfully used for analysis of liquid crystal mixtures on C18 column [78]. Neat sCO_2 was used as the MP and the elution of strongly retained solutes was sped up by using a pressure gradient. Despite the low reproducibility due to the instrumental limitations in precise MP flow-rate control throughout the gradient, the article demonstrates some advantages of SFC. The SFC separation was fast, especially thanks to employing

[5] Supercritical carbon dioxide (sCO_2) has been established as the SF of choice in SFC, use of other substance/compound is uncommon in laboratory practice.

pressure gradient with no need of equilibration between runs, and the low UV cut-off of sCO_2 allowed monitoring of a wide range of wavelengths for solute detection and identification. Supercritical CO_2 was found to be suitable for dissolving liquid crystalline materials and mixtures.

1.6. Aim of the thesis

The main aim of this work was to develop ultra-(high)performance chromatographic methods capable of enantioseparation of CLCs in the environment of sub/supercritical fluid chromatography (SFC), reversed-phase liquid chromatography (RPLC) and polar organic solvent liquid chromatography (POSC).

In case of SFC, the aim of this work is to introduce this technique for the enantioseparations of CLCs and prove its suitability for it as it has never been used for this purpose before. The partial goals are to develop enantioseparation methods for various groups of CLCs and to draw suggestions about possible scaling up from analytical to semi-preparative SFC.

Regarding the liquid chromatography, this thesis partially follows up on the first published use of RPLC for enantioseparation of CLCs in 2017 [47] with the aim to explore its viability for other liquid-crystalline materials, i.e. those with higher molecular weight and hydrophobicity. The POSC was proposed to enable faster analysis compared to RPLC and to investigate whether the wide selection of organic solvents usable with modern CSPs grants this mode a higher flexibility in the method development.

The results from both techniques are meant to demonstrate their usefulness as viable alternatives to the NPLC that is commonly used for the chiral purity control of CLCs.

2. Theory

The results of chromatographic resolution of compounds are interpreted through several parameters. Retention of individual solutes in chromatographic system is characterized by their retention time in order of their elution as $t_{r,1}$, $t_{r,2}$, … (retention of the peak eluted as the first, second and so on). In case of enantioseparations the retention time can be denoted according to Cahn-Ingold-Prelog system by the configuration of the enantiomer as t_R and t_S (retention of the *R* and *S* enantiomer). Retention factor *k* (indexed according to the previously chosen style of designation) describes how much time the solute spends being retained by the stationary phase compared to the time spent in the MP and is calculated from the equation (1):

$$k_n = \frac{t_n - t_m}{t_m} \tag{1}$$

where t_n is the retention time of the peak and t_m is the system void time (time between injection and detection of unretained solute). The ratio of retention factors of two peaks is called selectivity (α) and serves to express how much stronger is the second eluting peak retained in the system according to the equation (2):

$$\alpha = \frac{k_2}{k_1} \tag{2}$$

where k_2 and k_1 refer to the retention factors of the peaks in question. In enantioseparation the selectivity is replaced by enantioselectivity and describes the retention factor ratio of an enantiomeric pair. Enantioselectivity value higher than 1 means that the stationary phase is able to interact differently with the two enantiomers and their peaks can be resolved in a run. The enantioresolution of the two optical isomers (R_s) is then calculated from the equation (3):

$$R_s = 1.18 \cdot \frac{(t_{r,2} - t_{r,1})}{(w_{0.5,2} + w_{0.5,1})} \tag{3}$$

where $w_{0.5,n}$ is the peak width at half of its height. However, the chromatographic enantioresolution of peaks is a result of combined effects of efficiency, retention and enantioselectivity and can be expressed by the fundamental resolution equation (4):

$$R_s = \left(\frac{\sqrt{N}}{4}\right) \cdot \left(\frac{k}{k+1}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \tag{4}$$

where N is the plate count, k is the retention factor of the second eluting peak and α is the enantioselectivity. The first, the second and the third bracket denotes the efficiency, the retention and the enantioselectivity aspect, respectively. The efficiency aspect of resolution can be usually effectively optimized by adjusting the MP flow-rate and column temperature. The column efficiency can be expressed as the plate number (N) or as the height equivalent to a theoretical plate ($HETP$) which is obtained by dividing the column length by the plate number. The separation efficiency depends on linear MP velocity (u) according to the van Deemter equation (5):

$$HETP = A + \frac{B}{u} + C \cdot u \tag{5}$$

where the A is the eddy dispersion, B is the molecular diffusion, and C is the mass transfer resistance. In practice, the linear velocity can be replaced by the volumetric flow-rate for the sole purpose of method optimization.

The retention aspect of equation (4) can be exploited to enhance the enantioresolution, however, the situation is complicated. Usually, retention can be prolonged by lowering the MP elution power or by decreasing the column temperature in liquid chromatography. However, these actions may have an unforeseeable impact as both parameters might also influence the enantioselectivity aspect in a negative or positive way. Regarding the column temperature, apart from the very rare occurrences, such as the retention increasing with the temperature [79], the lower temperatures often lead to peak broadening which cancels the positive effect of the prolonged retention on the enantioresolution, see below.

Apart from the above-mentioned factors (temperature and MP composition) often affecting the enantioselectivity aspect of equation (4), the most prominent is of course the structure of the CS itself.

3. Results and discussion

The following sections consist of combined results from the Works I-VI and of unpublished results. It is meant to give a concise account of the specificities associated with chromatographic enantioseparation of CLCs while abstaining from reproduction of the matters already discussed in the published articles (Works I-VI are attached at the end of the thesis). For the structures of the CLCs discussed in this section see **Table S1** in Supplementary Data.

3.1. Factors affecting enantioseparation of CLCs

Chiral stationary phase

Selection of the CSP is the cornerstone of successful enantioseparation. The p-CSPs were chosen based on their overall success rate throughout the history of enantioseparations and in consideration of the average structure of CLCs. Most of the experimental work was conducted with p-CSPs listed in **Table 1**.

Table 1. Chemistry of the used p-CSPs. The Trefoil columns used in UHPSFC were 50 × 3.0 mm I.D. with 2.5µm fully porous particles. The Chiralpak columns used in UHPLC were 100 × 3.0 mm I.D. with 1.6µm fully porous particles.

Technique	*Trade name*	*Chemistry of chiral selector*
UHPSFC	Trefoil AMY1	Amylose tris(3,5-dimethylphenylcarbamate)
	Trefoil CEL1	Cellulose tris(3,5-dimethylphenylcarbamate)
	Trefoil CEL2	Cellulose tris(3-chloro-4-methylphenylcarbamate)
UHPLC	Chiralpak IA-U	Amylose tris(3,5-dimethylphenylcarbamate)
	Chiralpak IG-U	Amylose tris(3-chloro-5-methylphenylcarbamate)
	Chiralpak IB-U	Cellulose tris(3,5-dimethylphenylcarbamate)

The typical character of the chiral moiety of CLCs (hydrogen and one/two alkyls as the substituents of the asymmetric carbon, absence of hydroxyl groups or any other hydrogen bond donors) in combination with their hydrophobic and non-ionizable nature and rigidity encourages the use of p-CSPs, whose complexity offers a wide range of possible enantiospecific interactions. The helical macromolecular structure of p-CSPs entails a

complex spatial layout that (as opposed to the other CSP classes) puts emphasis on the overall shape of the larger solutes/their chiral moiety and their fit into the chiral grooves along the polymer chain [53]. In Work III a screening of several CSPs in UHPSFC was done and the superiority of the p-CSPs was demonstrated. In particular, a devastating loss of enantioselectivity was observed when switching from 3,5-dimethylphenylcarbamate of amylose (polysaccharide) to 3,5-dimethylphenylcarbamate of maltodextrin (oligosaccharide) highlighting the importance of p-CSPs' macromolecular character for the enantiorecognition of the CLCs [80].

In UHPLC, only p-CSPs were tested so far and were sufficient to achieve baseline separation of all rod-like CLCs with the exception of C1 and C2 from group A (**Table S1** in Supplementary Data), for which only partial resolution was obtained, see below. Comparing the results from Chiralpak IA-U and Chiralpak IG-U, all compounds from groups A, B and C showed higher retention and enantioselectivity on the chlorinated CSP [81,82]. Chiralpak IB-U brought the lowest enantioselectivity values along with the weakest retention, for illustration see **Figure 5**.

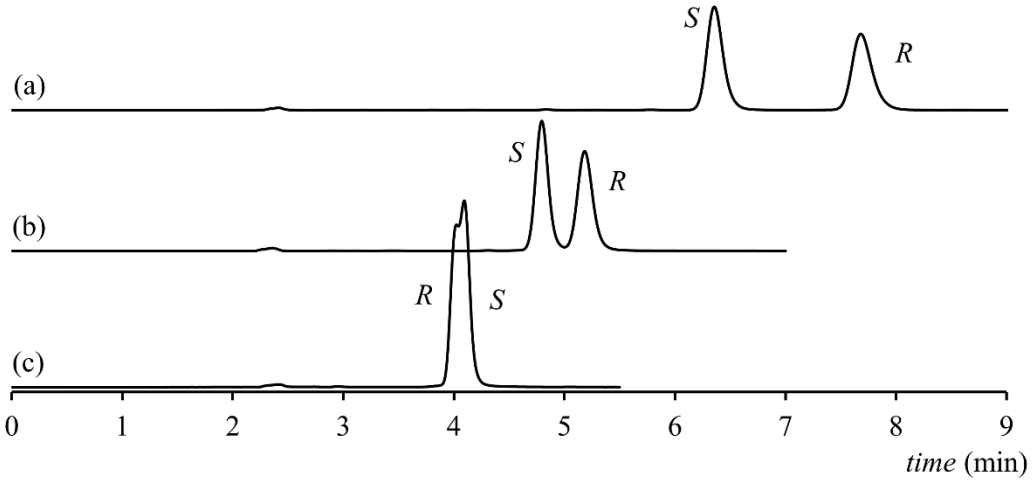

Figure 5 Enantioseparation of 3FF[2o] on (a) Chiralpak IG-U, (b) Chiralpak IA-U and (c) Chiralpak IB-U. MP 95/5 (*v*/*v*) methanol/acetonitrile, temperature 40 °C, flow-rate 0.20 $mL \cdot min^{-1}$. Similar trend across the three CSPs was found for all compounds from the group B and with various MP compositions. Unpublished results.

Both in UHPLC and UHPSFC, the reversal of EEO was observed when comparing results from amylose- and cellulose-based CSPs. In UHPSFC, it was found for majority of the analytes on AMY1 vs. CEL1 column; in UHPLC, the opposite EEO was found for materials

from group B on Chiralpak IA-U vs. Chiralpak IB-U. This is a common occurrence with amylose- and cellulose-based CSs [83–85].

The importance of a proper screening when testing different CSPs can be demonstrated on the example of BCl 6/10 in UHPLC, see **Figure 6**. Initially, after analyzing the racemate in pure acetonitrile, it may appear that Chiralpak IA-U could have higher enantiorecognition ability for the analyte as there is nearly baseline resolution despite much shorter retention time. However, after testing out MP with 2-propanol, it turns out that Chiralpak IG-U has a much higher enantioselective potential for this compound and the excessively high enantioresolution can be exploited to obtain an ultrafast analysis when employing tetrahydrofuran (a very strong eluting solvent) as a cosolvent to acetonitrile.

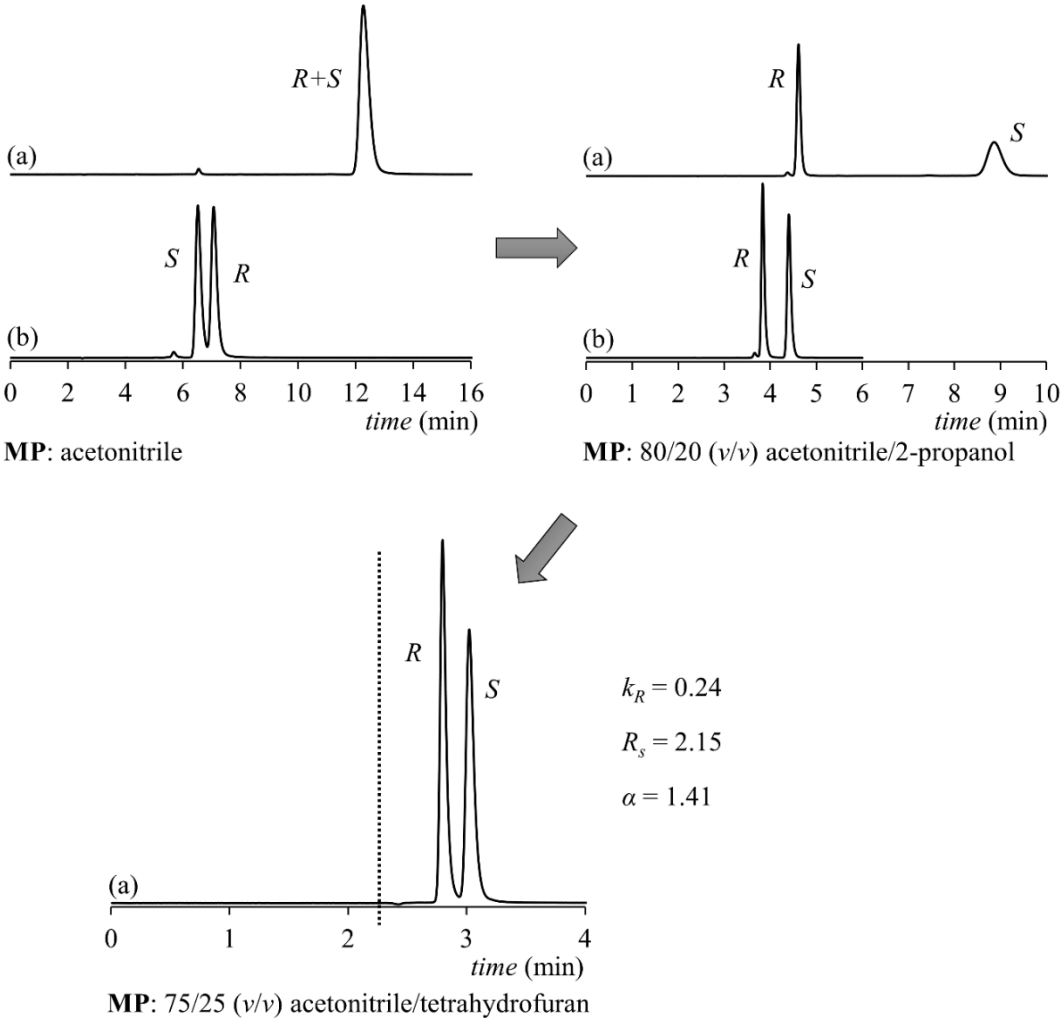

Figure 6 Chromatogram of BCl 6/10 measured on (a) Chiralpak IG-U and (b) Chiralpak IA-U with various MP compositions. Temperature 40 °C, flow-rate 0.20 mL·min^{-1}. Dashed line represents the system void time. These results are parts of the Work II [86] and Work VI [81].

Mobile phase composition

In direct enantioselective chromatography, changing the MP components or their ratio is the first step of the method optimization. The CLCs are hydrophobic and their solubility in alcohols is also very limited. The samples for UHPLC were prepared by dissolving the materials in acetonitrile and it was also the first tested MP. Pure acetonitrile and pure methanol are the two typical screening MP in POSC enantioseparations as they may offer complementarity for some analytes [87]. Use of pure methanol lead to disproportionally stronger retention for CLCs and use of methanol-rich MPs was found to be a good next step in method development in case the retention of a CLC is too weak in pure acetonitrile (k < 2) and there is no enantioresolution. For example, the partial enantioresolution achieved in methanol on Chiralpak IG-U was the best result so far obtained for compounds C1 and C2 (group A) in either UHPLC or UHPSFC, see **Figure 7**.

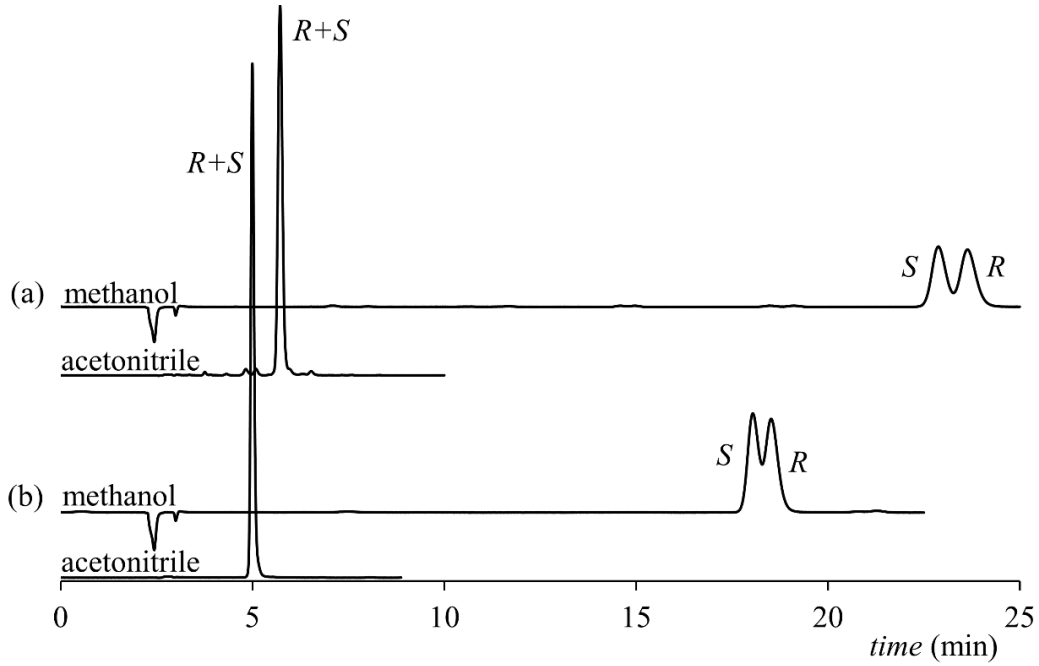

Figure 7 Analysis of racemic materials C1 (a) and C2 (b) in neat organic solvents. Column Chiralpak IA-U, temperature 40 °C, flow-rate 0.20 mL·min^{-1}. Unpublished results.

Addition of alcohol to acetonitrile in liquid chromatography [88] or to sCO_2 in SFC [89] often improves peak shape and/or efficiency and affects enantioselectivity. Methanol and 2-propanol were always tested as the MP components in all UHPLC and UHPSFC experiments with CLCs and the mixed MPs were found to yield better efficiency (and thus often enantioresolution, too) compared to pure solvents, for illustration see **Figure 8**. Ethanol was

tested for some of the CLCs, however, the results obtained with it were similar to those obtained with methanol or 2-propanol and did not surpass them.

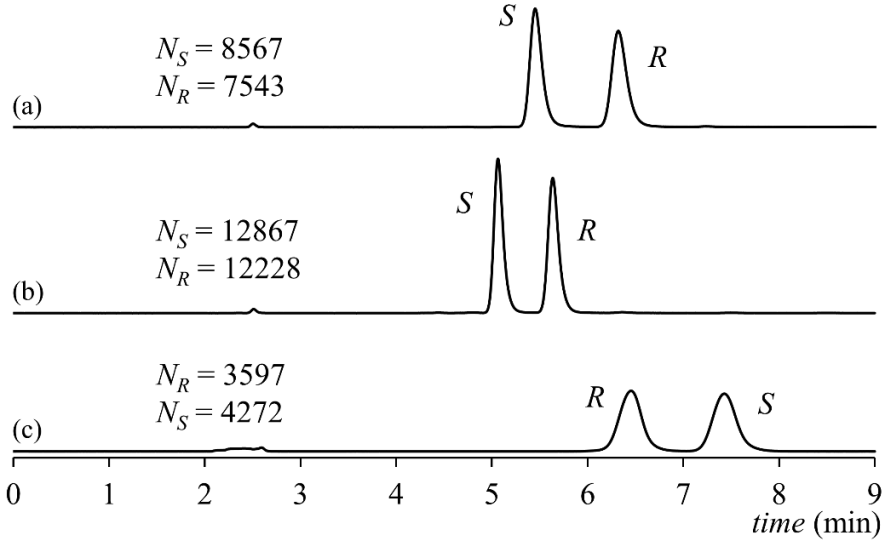

Figure 8 Column efficiency for 12PHB[2] AL in (a) pure acetonitrile, (b) 97.5/2.5 (*v*/*v*) acetonitrile/2-propanol and (c) pure 2-propanol. Column Chiralpak IA-U, temperature 40 °C, flow-rate 0.20 mL·min^{-1}. Chromatograms were obtained as part of the Work II [86].

When shifting from acetonitrile-rich to alcohol-rich MPs the U-shaped retention trends were observed for all CLCs from groups A, B and C regardless of the used alcohol. In the Work II, linear alcohols ethanol, 1-propanol and 1-butanol were tested as well and the retention weakened along with increasing alkyl length of the alcohol, see **Figure 9**.

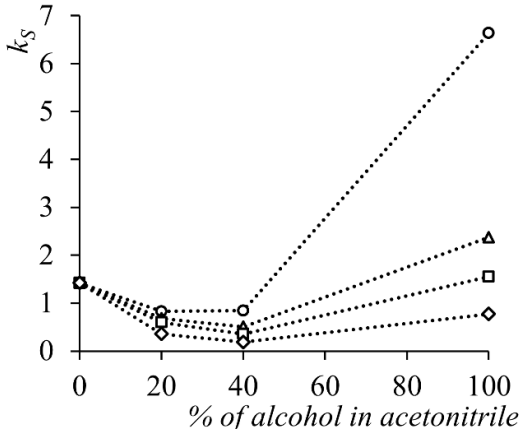

Figure 9 Effect of the linear alcohol alkyl length and its amount in the MP on the retention factor of the *S* enantiomer (k_S) of 12PHB[2] AL measured for methanol (○), ethanol (△), 1-propanol (□) and 1-butanol (◇). Column Chiralpak IA-U, temperature 40 °C, flow-rate 0.20 mL·min^{-1}. The chart was plotted using results obtained in the Work II [86]. The dotted lines are to guide the eyes only and do not represent the complete retention trends.

Given the complex nature of polysaccharide derivatives, the retention of solutes on them corresponds to mixed-mode behaviour (the solute retention is usually a result of multiple retention mechanisms/types of interactions). For aromatic solutes, U-shaped retention curves were observed in acetonitrile-alcohol MPs [90–92] indicating a gradual transition from one dominating retention mechanism to another. Specifically, it is believed to be shifting from more polar interactions (such as hydrogen bonds) in acetonitrile to less polar interactions (such as π-π interactions) in the alcohols.

Interestingly, the change of the dominant retention mechanism causing the U-shaped retention trends may or may not lead to a change of the EEO. For compounds of the group B, despite the decrease or complete loss of enantioselectivity in the MPs corresponding to the weakest retention, there was no reversal of the EEO, see **Figure 10**. On the other hand, for the lactic acid-based CLCs from the group C the EEO was inversed in ethanol, 1-/2-propanol and 1-butanol compared to acetonitrile and methanol [86]; this phenomenon was in detail studied in Work VI [81].

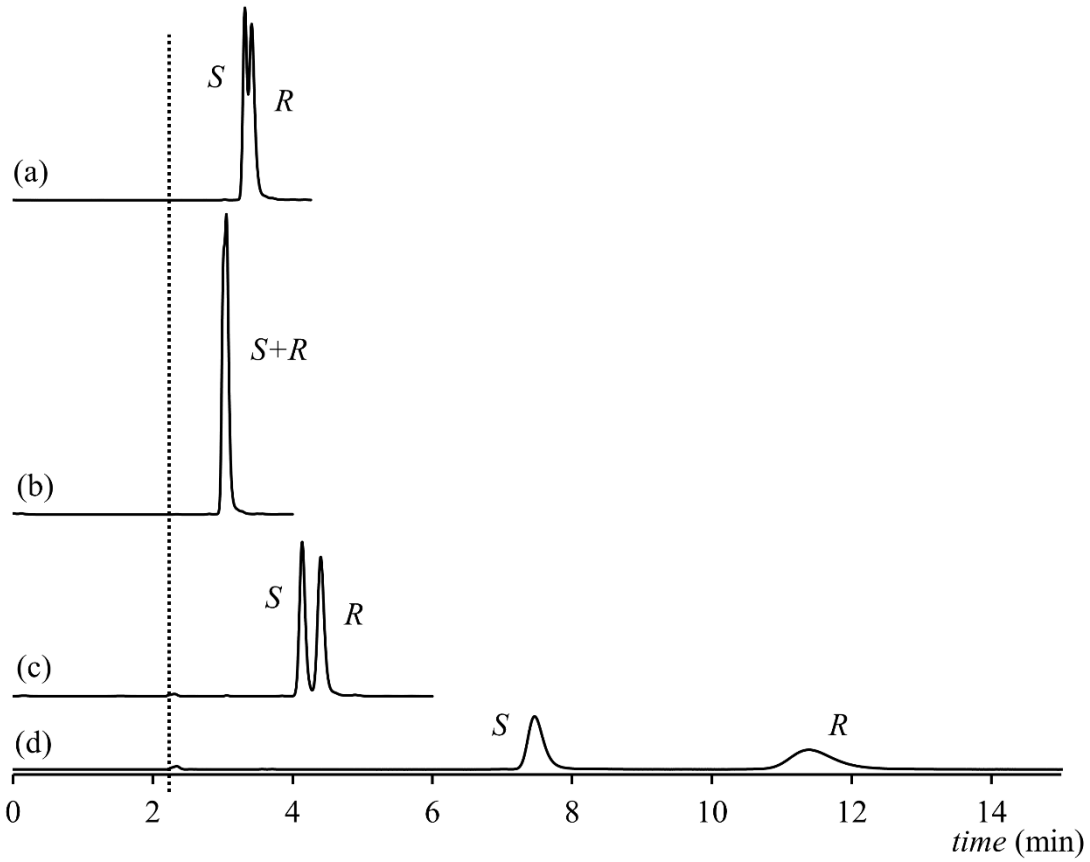

Figure 10 Chromatograms of the compound 6FF[2o] in (a) pure acetonitrile, (b) 80/20 (*v*/*v*) acetonitrile/methanol, (c) 20/80 (*v*/*v*) acetonitrile/methanol and (d) pure methanol. Column

Chiralpak IA-U, temperature 40 °C, flow-rate 0.20 mL·min^{-1}. The EEO was unchanged in corresponding experiments with ethanol and 2-propanol, too. Unpublished results.

Apart from alcohols, it is possible to add water to acetonitrile (moving from POSC to RPLC-like conditions). Increase in enantioselectivity was observed for all CLCs from group B and C accompanied by a steep increase in retention which limited the RPLC experiments to acetonitrile-rich MPs (maximum of 10 vol. % of water) [81,82,86]. The presence of water induces hydrophobic effect as one of the CSP-solute interactions responsible for retention, which is considerable given the hydrophobic nature of the CLCs.

In UHPSFC, addition of alcohols significantly improved peak shape and lead to a decrease of retention. CLCs were too strongly retained on the p-CSPs and their elution from the column was impossible with neat sCO_2 as the MP. For materials from the group B, it was sufficient to add 5-10 vol. % of cosolvent to acquire successful analysis in less than 10 minutes [93,94]. However, CLCs from the group C were even more retained on all p-CSPs, e.g. 30 vol. % of 2-propanol were needed to elute 10 ZBDL in under 10 minutes; for methanol it was even 40 vol. % [86]. For all the compounds analyzed in UHPSFC, enantioselectivity was either very similar or slowly decreased with increasing of the alcohol fraction in the MP; in contrast to UHPLC, no EEO reversals induced by the volume fraction of alcohols in the MP were observed. However, changes of EEO were found for CLCs from the group C when switching between various alcohols (and their mixtures) [86]. The experiments were limited to maximum of 50 vol.% of cosolvent, so it is impossible to tell whether alcohol-rich MPs would bring any intriguing results similarly to UHPLC.

Mobile phase flow-rate

To determine optimal flow-rate for following UHPLC experiments, a set of lactate-derived CLCs was analyzed within 0.10-0.80 mL·min^{-1} flow-rate range and in addition with extremely low flow-rates of 0.05 and 0.03 mL·min^{-1}, see **Table S2** in Supplementary Data. The volumetric flow-rate range recommended by the column manufacturer is 0.20-0.80 mL·min^{-1}. The flow-rates below the suggested lower limit were included to demonstrate the coherence with van Deemter theory, specifically the presence of B-branch of *HETP*/*F* curve where the efficiency is controlled by the B-term, see **Figure 11**.

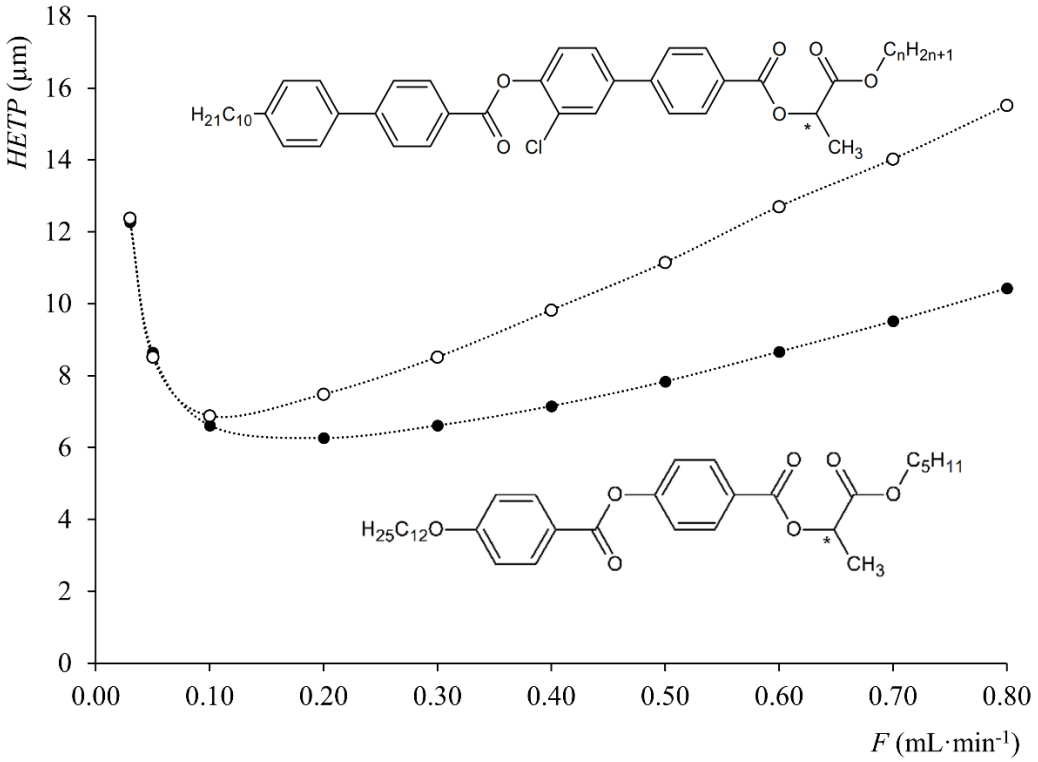

Figure 11 The influence of MP flow-rate on the column efficiency displayed as the HETP of the first eluted enantiomer of 12PHB^2 AL (●) and 10 ZBDL (○). Measured on Chiralpak IA-U, MP 85/15 acetonitrile/2-propanol (*v*/*v*), temperature 40 °C. Unpublished results.

For the CLCs, HETP was found to be increasing along with flow-rate except for the lowest tested flow-rates demonstrating the B-term (molecular diffusion) is small and only leads to peak broadening with extremely low MP velocity. This is attributed to small diffusivity of CLCs in RPLC solvents, which could be explained by their considerable size and low polarity [95]. The efficiency of enantioseparation on macromolecular CSPs is usually governed by the C-term of van Deemter equation (except for very small flow-rates) as it accounts for slow adsorption–desorption process [96]. The steepness of the C-term branch of the van Deemter curve roughly correlates with retention of solutes [97], the steepest rise is observed for the strongest retained 10 ZBDL, the slightest for the least retained 12 PHB^2 AL. Correspondingly, the more retained solutes have the curve minima at lower flow-rates compared to less retained compounds.

For all racemic mixtures of CLCs, the differences in *HETP*/*F* curves of the *R* and *S* enantiomers were due to increased C-term of the late eluting enantiomers and can be interpreted as a result of slower adsorption-desorption due to the need of steric adjustments

to fit into chiral cavities of the CSPs [98], see **Figure 12**. For all following UHPLC measurements of CLCs, the flow-rate 0.20 mL·min^{-1} was chosen as it is the lowest value within the range recommended by the column manufacturer and presents a compromise between separation efficiency and analysis time.

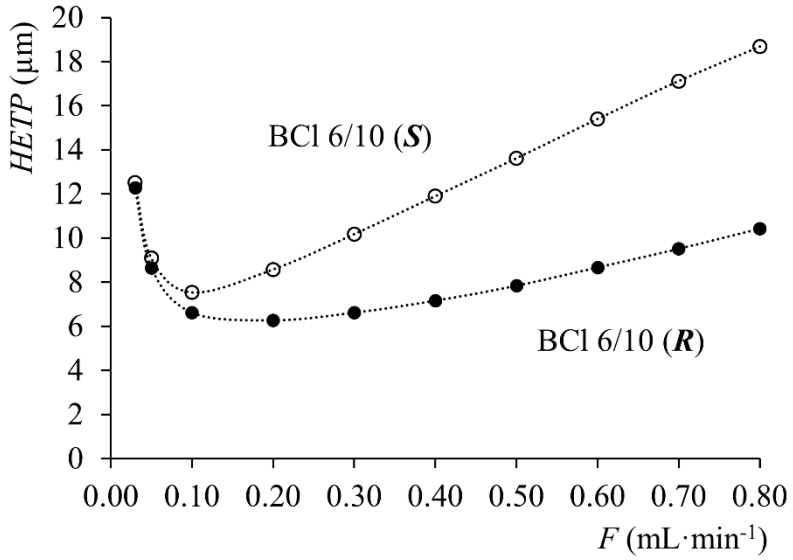

Figure 12 Effect of the MP flow-rate on the column efficiency displayed as the HETP of the first eluting *R* (●) and the second eluting *S* (○) enantiomer of BCl 6/10. Column Chiralpak IA-U, MP 85/15 acetonitrile/2-propanol (*v*/*v*), temperature 40 °C. Unpublished results.

Column temperature

The temperature is one of the separation conditions that affects the resolution in chromatography. However, its optimization in enantioselective chromatography is more complicated than flow-rate optimization. The effect of temperature on the peak resolution comprises two aspects: the enantioselectivity and the efficiency. The thermodynamical analysis of enantioseparation is discussed in Work VI [81], for the purpose of this section it is sufficient to note that enantioselectivity might increase, decrease or stay constant depending on the column temperature and thus influence the enantioresolution through the enantioselectivity aspect of equation (4). However, the efficiency aspect is usually very significant in chromatographic enantioseparations and the plate number increases steeply with increasing temperature [99]. For the CLCs the decrease of temperature was found to have disastrous effect on the peak shape in UHPLC, especially for the late eluting enantiomer, for illustration see **Figure 13**.

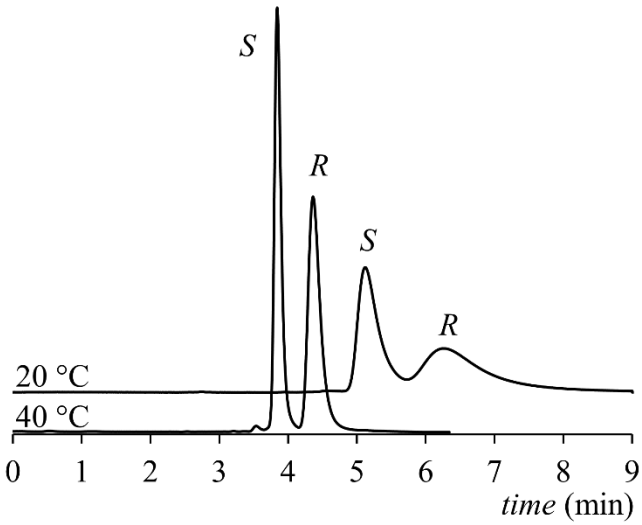

Figure 13 Loss of enantioresolution for compound 5HH[2o] on the Chiralpak IA-U column with decrease of temperature. Enantioselectivity did not change with the temperature and the deterioration was caused by the peak broadening alone. Pure acetonitrile was used as the MP, flow-rate 0.20 mL·min^{-1}. Unpublished results.

The reduce in efficiency can be so significant as to result in a decline of enantioresolution despite the simultaneous rise of enantioselectivity as is illustrated on the example of BCl 6/10, see **Figure 14**.

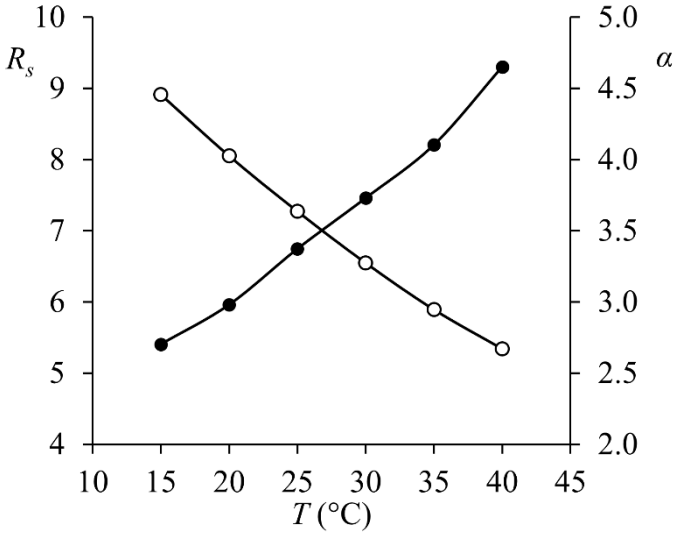

Figure 14 Increase of enantioresolution (R_s, ●) along with increasing temperature (T) despite the decrease of enantioselectivity (α, ○). MP 60/40 (*v*/*v*) acetonitrile/2-propanol, column Chiralpak IG-U, flow-rate 0.20 mL·min^{-1}. The chart was plotted using results obtained in Work VI [81].

In UHPSFC, the effect of temperature was examined as a part of the Work I and the highest temperature was found to offer the best enantioresolution. In all of the experimental work both in UHPSFC and UHPLC, the temperature of 40 °C was set as the default column temperature because it yields the best attainable column efficiency while being the upper limit for p-CSPs that were employed in the following research.

3.2. Work I

P. Vaňkátová, K. Kalíková, A. Kubíčková, Ultra-performance supercritical fluid chromatography: A powerful tool for the enantioseparation of thermotropic fluorinated liquid crystals, *Analytica Chimica Acta* **1038** (2018) 191-197.

The first study is focused on introduction of UHPSFC as a chromatographic technique suitable for enantioseparation of CLCs. It is the first published use of an SFC setup for this purpose and baseline separation of all racemates was achieved. It was demonstrated that the type of cosolvent (specifically methanol vs. 2-propanol) has interesting impact on the enantioseparation. Apart from influencing overall retention (methanol being stronger eluent than 2-propanol) different trend of enantioselectivity was observed for the two alcohols regarding solutes' structure. In overall, 2-propanol was found to be beneficial for resolution of compounds with shorter alkyloxy spacer and two laterally substituted fluorine atoms, while methanol yielded better results for materials with longer spacer. The analytes studied in this work were from the group B, specifically a set of twenty CLCs with chiral center derived from 2-octanol (general abbreviation rX_1X_2[2o]). The published version of the Work I is attached at the end of the thesis, see Annexes.

3.3. Work II

P. Vaňkátová, A. Kubíčková, M. Cigl, K. Kalíková, Ultra-performance chromatographic methods for enantioseparation of liquid crystals based on lactic acid. *The Journal of Supercritical Fluids* **146** (2019) 217-225.

After the successful use of UHPSFC for resolution of CLCs with chiral center derived from 2-octanol, a different set of analytes was put together; this time a set of CLCs with chiral center based on lactic acid from the group C. Lactic acid is a popular source of chirality for liquid crystalline materials as it is cheap and abundant. Five CLCs were analyzed in UHPSFC and after that in UHPLC; baseline enantioseparation of all analytes was achieved using both techniques. MP composition-induced inversions of EEO were discovered in both setups. The UHPLC offered faster analysis with all five CLCs separated to the baseline in under 6 minutes which corresponds to retention factor $k_S \approx 1.13$, resp. 0.51 of the peak of the late eluting *S* enantiomer of the most retained analyte and the least retained analyte, respectively.

On the other hand, MP compositions yielding enantioselectivity $\alpha > 1.5$ and enantioresolution $R_s > 4$ were found for every compound in UHPSFC, indicating possible scale-up to semipreparative SFC. The published version of the Work II is attached at the end of the thesis, see Annexes.

3.4. Work III

P. Vaňkátová, D. Folprechtová, K. Kalíková, A. Kubíčková, D.W. Armstrong, E. Tesařová, Enantiorecognition ability of different chiral selectors for separation of liquid crystals in supercritical fluid chromatography; critical evaluation. *Journal of Chromatography A* **1622** (2020) 461138.

Given the two previous successful uses of UHPSFC, a set of structurally various CLCs (from groups A to D) was applied to conduct a survey into enantioselective potential of several CSPs in the environment of UHPSFC. Three derivates of polysaccharides, one oligosaccharide derivate and four modified macrocyclic glycopeptides were employed as the CSPs. All CLCs were analyzed on each of the column with a variety of MPs also including MPs with acidic/basic additives. Among the tested CSPs, the polysaccharide derivatives were perceived to be superior for the CLCs most probably due to their macromolecular character that enhances the steric aspect of CSPs' enantioselectivity for bulky solutes such as CLCs. The published version of the Work III is attached at the end of the thesis, see Annexes.

3.5. Work IV

P. Vaňkátová, T. Šrolerová, A. Kubíčková, K. Kalíková, Fast UHPLC enantioseparation of liquid crystalline materials with chiral center based on octanol in reversed-phase and polar organic mode. *Monatshefte für Chemie-Chemical Monthly* **151** (2020) 1235-1240.

As part of the research into the effect of CLC's molecular design on its mesomorphic properties ten new *S* enantiomers and corresponding racemic mixtures belonging to the group B were synthesized. The compounds are analogues to those used in Work I (and partly in Work III) except that their chiral center is derived from 3-octanol instead of 2-octanol (general abbreviation rX_1X_2[3o], both sets of CLCs ([2o] and [3o]) were used in this work. This was the first time this structural type of CLCs was separated under RPLC conditions and this work mostly focused on the enhancing effect of water on enantioseparation. The published version of the Work IV is attached at the end of the thesis, see Annexes.

3.6. Work V

M. Urbańska, **P. Vaňkátová**, A. Kubíčková, K. Kalíková, Synthesis, characterisation and supercritical fluid chromatography enantioseparation of new liquid crystalline materials. *Liquid Crystals* **47** (2020) 1832-1843.

In this work the method was developed including assessment of some of the method validation parameters such as limit of detection (LOD), limit of quantitation (LOQ), linearity and precision. The ten new CLCs from the group B that were analyzed by UHPLC in Work IV (general abbreviation rX_1X_2[3o]) were chosen to demonstrate the reliability of UHPSFC methods. Given the results from Work III, a cellulose-based CSP was chosen for the enantioseparation as the enantiomers were found to elute in *R*, *S* order (opposite to the amylose-based CSP). This EEO is advantageous as the chiral CLCs synthesized from octanol are predominantly the *S* enantiomers. The published version of the Work V is attached at the end of the thesis, see Annexes.

3.7. Work VI

P. Vaňkátová, A. Kubíčková, K. Kalíková, How mobile phase composition and column temperature affect enantiomer elution order of liquid crystals on amylose tris (3-chloro-5-methylphenylcarbamate) as chiral selector. *Electrophoresis* **42** (2021) 1844-1852.

The analytes with chiral center based on lactic acid from the group C that was studied in Work II and in addition compound Z 12/* from the group A were used to conduct a thorough investigation into the influence of the column temperature and the MP composition on their chromatographic behavior. This study was following up on the interesting phenomenon of EEO switch observed in Work II. Reversals of EEO are intriguing from the point of view of enantioseparation mechanism but are also highly interesting for the method development as well. It is advantageous if the EEO can be programmed to fit the target application of a developed method. In this work both temperature- and MP-induced EEO reversals were described. The published version of the Work VI is attached at the end of the thesis, see Annexes.

3.8. General remarks

With respect to the results obtained throughout the experimental work that comprise this thesis, some general conclusions on enantioseparation of CLCs can be drawn that can be used as suggestions for the future method development in both UHPLC and UHPSFC:

a. Polysaccharide-based CSPs should be the first choice when selecting the CSP - amylose derivatives were found to be more successful in both techniques compared to cellulose derivatives.
b. Chlorinated p-CSPs might offer higher enantioselectivity compared to their non-chlorinated counterparts.
c. The highest temperature allowed by the column manufacturer should be set as the default for the initial screenings.
d. Decreasing MP flow-rate might help to enhance enantioresolution (at the cost of longer analysis time).
e. Adding methanol or 2-propanol as cosolvents to acetonitrile (in UHPLC) and to sCO_2 (in UHPSFC) might yield interesting enantioselectivity – the two alcohols might also assure complementarity in terms of EEO. Use of ethanol is not expected to bring results interesting enough to justify its use, especially regarding its higher cost.
f. At least two or three different MPs should be tested for every CSP in the initial screening as there is no single universal MP to ascertain enantioselective potential of a CSP towards a solute and enantioselectivity varies with the MP composition.
g. The use of acidic/basic additives will probably bring diminutive changes in results on p-CSPs and can be omitted in the method development.

Additionally, in UHPLC small amount of water may significantly enhance enantioselectivity (at the cost of longer analysis time). Given the neutral and hydrophobic nature of CLCs, water has been used only in small amount (<20 %) and in pure state (no buffers). Switching from acetonitrile-rich to alcohol-rich MP might be helpful for analytes that are too weakly retained in neat acetonitrile.

In UHPSFC, decreasing the back-pressure may enhance enantioresolution, although retention is prolonged due to decreased solvation power of the MP. The low UHPSFC system pressure opens the possibility of tandem coupling of two CSPs to help separate poorly resolved enantiomers. This approach has never been tested for the enantioseparation of CLCs yet and might be a part of future experiments.

4. Conclusion

Two ultra-high performance chromatographic techniques were successfully used for enantioseparation of various chiral liquid crystals. For both supercritical fluid chromatography and liquid chromatography, the results were published in impacted scientific journals and demonstrated suitability of the techniques for the analysis of this type of compounds. The tested analytes comprised variations in the chiral center structure, halogen substitution, the number of benzene rings and the length of the terminal alkyls or alkyloxy spacer. The majority of experiments were done employing polysaccharide-based chiral stationary phases. The effect of column temperature and mobile phase flow-rate on enantioseparation of CLCs was found to be as follows: increased temperature and decreased flow-rate yield better results in terms of column performance in both UHPSFC and UHPLC. In some of the studies, acidic and basic additives were added to the mobile phase and were found to have no noteworthy effect on the enantioseparation on p-CSPs given the non-ionizable nature of the CLCs. Small amounts of alcohols added to acetonitrile (in UHPLC) or sCO_2 (in UHPSFC) reduce the retention times while improving the peak shape. At the same time, their presence might influence the enantioselectivity of the CSPs and lead to mobile phase-induced reversals of enantiomer elution order which were described in both UHPLC and UHPSFC.

Work I marked the first published enantioseparation of CLCs in UHPSFC. In Work II similar UHPSFC separation conditions were applied for resolution of structurally different CLCs and the analytes were also resolved in UHPLC. In Work III the superiority of CSPs based on polysaccharide derivatives for enantioseparation of CLCs in UHPSFC was demonstrated by comparing several commonly used CSPs. Work IV exemplified the merit of water and RPLC conditions for the enantioseparation of CLCs. In Work V an UHPSFC method for chiral purity control of novel CLCs with chiral center derived from 3-octanol was developed and several of the validation parameters were evaluated. Work VI is a comprehensive study into the influence of column temperature and mobile phase composition on enantioseparation of CLCs and focused on EEO reversals. Aside from the main gist of these works, all of them also included discussions on the effect of analytes' structure on their chromatographic behavior.

All studies that comprise this thesis demonstrated the potential of SFC, POSC and RPLC environments as alternatives to the so far preferred normal phase mode of liquid

chromatography. All techniques proved to be appropriate for the purpose of analytical enantioseparation methods usable for determination of the enantiomeric excess. Hopefully, some research into the possibility of scaling up to semipreparative setup of enantioselective SFC for the purpose of purifying enantiomers will be conducted in future.

Confirmation of participation

P. Vaňkátová, K. Kalíková, A. Kubíčková, Ultra-performance supercritical fluid chromatography: A powerful tool for the enantioseparation of thermotropic fluorinated liquid crystals, *Analytica Chimica Acta* **1038** (2018) 191-197. **80%**

P. Vaňkátová, A. Kubíčková, M. Cigl, K. Kalíková, Ultra-performance chromatographic methods for enantioseparation of liquid crystals based on lactic acid. *The Journal of Supercritical Fluids* **146** (2019) 217-225. **70%**

P. Vaňkátová, D. Folprechtová, K. Kalíková, A. Kubíčková, D.W. Armstrong, E. Tesařová, Enantiorecognition ability of different chiral selectors for separation of liquid crystals in supercritical fluid chromatography; critical evaluation. *Journal of Chromatography A* **1622** (2020) 461138. **40%**

P. Vaňkátová, T. Šrolerová, A. Kubíčková, K. Kalíková, Fast UHPLC enantioseparation of liquid crystalline materials with chiral center based on octanol in reversed-phase and polar organic mode. *Monatshefte für Chemie-Chemical Monthly* **151** (2020) 1235-1240. **70%**

M. Urbańska, **P. Vaňkátová**, A. Kubíčková, K. Kalíková, Synthesis, characterisation and supercritical fluid chromatography enantioseparation of new liquid crystalline materials. *Liquid Crystals* **47** (2020) 1832-1843. **40%**

P. Vaňkátová, A. Kubíčková, K. Kalíková, How mobile phase composition and column temperature affect enantiomer elution order of liquid crystals on amylose tris (3-chloro-5-methylphenylcarbamate) as chiral selector. *Electrophoresis* **42** (2021) 1844-1852. **80%**

I confirm that the information given above is true, complete and accurate.

Prague, 5th of August 2022

…….

RNDr. Anna Kubíčková, Ph.D.

List of publications

P. Vaňkátová, K. Kalíková, A. Kubíčková, Ultra-performance supercritical fluid chromatography: A powerful tool for the enantioseparation of thermotropic fluorinated liquid crystals, *Analytica Chimica Acta* **1038** (2018) 191-197.
DOI: 10.1016/j.aca.2018.07.001 IF_{2018}= 5.256

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DOI: 10.1080/02678292.2019.1599454 IF_{2019}= 3.125

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DOI: 10.1002/elps.202000350 IF_{2021}= 3.535

Awarded Best Student Paper for September 2021, editors' choice.

T. Vojtylová-Jurkovičová, **P. Vaňkátová**, M. Urbańska, V. Hamplová, D. Sýkora, A. Bubnov, Effective control of optical purity by chiral HPLC separation for ester-based liquid crystalline materials forming anticlinic smectic phases. *Liquid Crystals* **48** (2021) 43-53.
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Conference contributions

P. Vaňkátová, Supercritical fluid chromatography – a new tool for the optical purity control of chiral liquid crystals XXII Conference on Liquid Crystals - Chemistry, Physics and Applications, 22nd Conference on Liquid Crystals 17th-21st of September 2018 Jastrzębia Góra, Poland. Oral presentation

P. Vaňkátová, A. Bubnov, K. Pomeisl, D. Pociecha, V. Hamplová, Photosensitive reactive mesogens with lateral substitution in vicinity of azo group XXII Conference on Liquid Crystals - Chemistry, Physics and Applications, 22nd Conference on Liquid Crystals 17th-21st of September 2018, Jastrzębia Góra, Poland. Poster presentation

P. Vaňkátová, Use of ultra-performance chromatographic methods for the evaluation of enantiomeric excess of chiral liquid crystals, 15th International Students Conference 'Modern Analytical Chemistry' 19th-20th of September 2019, Prague, Czech Republic. Oral presentation

P. Vaňkátová, K. Kalíková, M. Cigl, A. Kubíčková, Ultra performance chromatographic methods for optical purity control of chiral liquid crystals, 30th of June-5th of July 2019 Wroclaw, Poland, *Jury Award - 3rd place in Best Poster Presentation Award*. Poster presentation

P. Vaňkátová, Use of polar organic and reversed-phase mode for UHPLC enantioseparation of liquid crystals., 16th International Students Conference 'Modern Analytical Chemistry' 17th-18th of September 2020, Prague, Czech Republic. Oral presentation

P. Vaňkátová, Chiral chromatography for analysis of liquid crystals and their precursors, 23rd Conference on Liquid Crystals 18th-22nd of October 2021, Karpacz, Poland. Invited oral presentation

P. Vaňkátová, M. Urbańska, A. Kubíčková, K. Kalíková, Enantioselective chromatography for chiral purity control of ester-based fluorinated liquid crystals, International Liquid Crystal Conference 24th-29th of July 2022, Lisbon, Portugal. Poster presentation

P. Vaňkátová, A. Kubíčková, K. Kalíková, Versatility of liquid chromatography for characterization and study of liquid crystals, International Liquid Crystal Conference 24th-29th of July 2022, Lisbon, Portugal. Poster presentation

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Supplementary Data

Table S1. Structures of CLCs used in Works I-VI and other experimental studies.

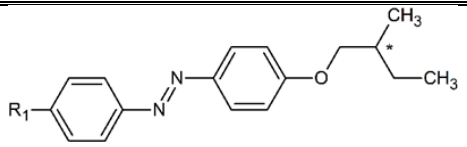
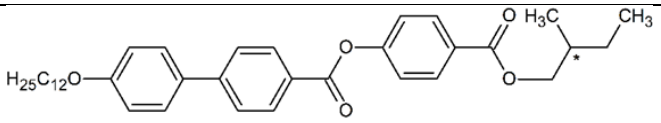
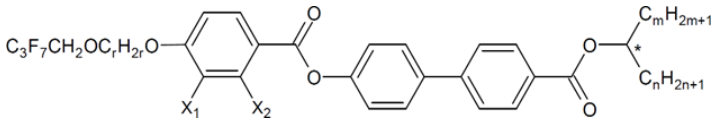
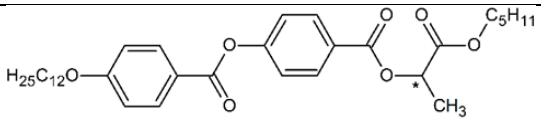
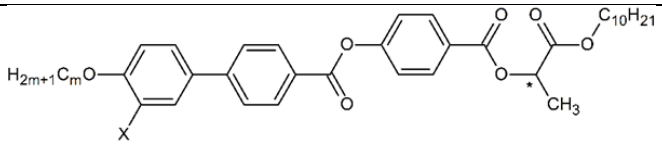
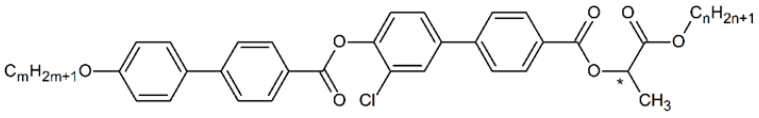
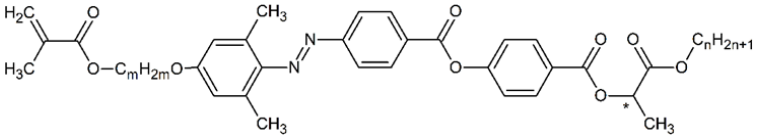
Group	*Abbrev.*		*Structure*
A	C1	$R_1=C_8H_{17}$	
	C2	$R_1=OC_8H_{17}$	
	Z 12/*	-	
B	rX$_1$X$_2$[2o], rX$_1$X$_2$[3o], rX$_1$X$_2$[2h]	$X_{1,2}$=H/F r=2-7 [2o]=m=1,n=6 [3o]=m=2,n=5 [2h]=m=1,n=4	
C	12 PHB^{2}AL	-	
	ZL 12/10	m=12, X=H	
	BCl 6/10	m=6, X=Cl	
	9 ZBBL	m=9, n=4	
	10 ZBBL	m=10, n=4	
	11 ZBBL	m=11, n=4	
	10 ZBDL	m=10, n=10	
D	MDA 6/6	m=6, n=6	
	MDA 6/10	m=6, n=10	
	MDA 10/6	m=10, n=6	
	MDA 10/10	m=10, n=10	

Table S2. HETP values (μm) obtained for the first eluting enantiomer of the lactic acid-based CLCs in MP flow-rate range (F, mL·min^{-1}). The columns are denoted in the observed enantiomer elution order of each compound. Measured on Chiralpak IA-U, MP 85/15 (*v*/*v*) acetonitrile/2-propanol, temperature 40 °C.

F	12PHB^2 AL	ZL 12/10	BCl 6/10	10 ZBBL	10 ZBDL
0.80	8.86	12.66	10.42	13.85	15.52
0.75	8.39	12.08	9.88	13.23	14.60
0.70	8.14	11.58	9.51	12.71	14.02
0.65	7.83	11.08	9.09	12.06	13.32
0.60	7.57	10.46	8.66	11.37	12.70
0.55	7.26	9.84	8.28	10.88	11.93
0.50	6.86	9.14	7.83	10.11	11.15
0.45	6.55	8.67	7.56	9.60	10.51
0.40	6.38	8.27	7.15	9.19	9.92
0.35	6.23	7.76	6.86	8.60	9.29
0.30	6.08	7.29	6.61	8.09	8.52
0.25	5.96	6.92	6.45	7.67	8.06
0.20	5.94	6.57	6.26	7.29	7.48
0.15	6.13	6.43	6.23	7.01	7.00
0.10	6.78	6.47	6.61	7.05	6.88
0.05	8.92	8.41	8.64	8.72	8.51
0.03	12.65	11.80	12.27	12.37	12.37

Annexes

P. Vaňkátová, K. Kalíková, A. Kubíčková, Ultra-performance supercritical fluid chromatography: A powerful tool for the enantioseparation of thermotropic fluorinated liquid crystals, *Analytica Chimica Acta* **1038** (2018) 191-197.

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