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Poster #1

Orthogonal sample analysis by a SFC/UHPLC-Hybrid System

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Introduction

Separations, which are done under SFC and UHPLC conditions are truly orthogonal due to their different separation mechanisms. They are based on the interaction of the analytes in completely different fluid media with different stationary phases. The SFC- and UHPLC-based separations of a given sample are able to deliver complementary information about the sample content. On the other hand, it is also possible to analyze the same sample for different analytes, which either perform better under SFC separation conditions or under UHPLC separation conditions to give a more comprehensive picture of the sample content.

Method

In SFC mode, separations were achieved on silica or amino columns. The used CO_2 modifier was methanol under isocratic or gradient conditions. In UHPLC mode, the system was used with a C18 column and typical reverse phase gradient elution conditions with water – acetonitrile. The switching between SFC mode or UHPLC mode was controlled by a 2positon/10port valve. For fast switching between SFC and UHPLC column a 4-column selection valve was installed in the column oven. The detection was done by a DAD. The additional mass spectrometer for compound confirmation and quantification was also connected to the 2positon/10port valve.

Results

This poster provides a detailed explanation of the setup of the used SFC/UHPLC hybrid system. As a real application example, the separation of a mixture of pesticides in SFC-mode and in UHPLC-mode will be shown and the orthogonality of the separation will be discussed. In addition, samples with water and fat soluble compounds (e.g. vitamins) will be analyzed under SFC and UHPLC conditions to demonstrate the capability of the instrument to gain comprehensive information.

Poster #2

Chiral Open Access

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Open Access HPLC is a powerful tool that can allow for fast sample purification performed by med-chemists with little to no analytical chemistry knowledge. In the past few years the Separations team at Novartis has shown that this open access process can also be utilized for achiral SFC purifications. Increasing demand for chiral purifications and for larger scale chiral purifications has created a bottleneck in the drug discovery process. Open Access SFC purification of chiral compounds is the next step towards increasing throughput and decreasing turnaround time.

The objective of our study was to demonstrate that with a limited number of method conditions a robust open access platform could be designed to resolve many of the chiral mixtures which currently require submission to a core purification lab. This was done by implementing broad gradient analytical screens to be run on multiple chiral stationary phases, using analytical retention times and time between peaks to scale up to preparative scale utilizing Open Access software, and utilizing limited conditions to simplify management of the process.

Open Access chiral SFC is achievable and our data shows that a large number of submissions for small scale chiral purifications can in fact be performed using this method. Enabling chemists to perform their own chiral purifications is the next step towards increasing efficiency in drug discovery.

Poster #3

Separation of Natural Cannabinoids by SuperSep®

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For the high efficiency and less organic solvent consumption, supercritical fluid chromatography (SFC) has been widely used for chiral separations in pharmaceutical industry. Now, the SFC application is being broadened to achiral separation and purification of natural extracts, which are often very complex mixtures. This poster presents the preparative SFC purification of cannabinoids. The process was developed using a bench-top SFC instrument and then directly scaled-up to SuperSep® 1000 with a 50 mm i.d. column for manufacturing. Cannabinoid oil extracted from cannabis plants contains many different compounds such as Δ9-tetrahydro-cannabinol (Δ9-THC), which is a well-known psychoactive compound, and cannabidiol (CBD), which has been getting a lot of attention in the last few years for health benefits. For various pharmaceutical and neutraceutical manufacturing, having an access to gram to kilogram amounts of purified cannabinoids will have great value.

Poster #4

Role of SFC in Method Development and Separation for Chiral Molecules

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Separation of racemic mixtures is important in drug development and FDA recommendation is for this to occur even at the earliest drug discovery stage. Differences in potency, toxicity and other biological properties are often observed between pure enantiomers of a molecule. At Gilead we have developed a system to separate enantiomers to support enantiomeric excess (ee) calculations and/or to process small to medium quantities of medicinal chemistry compounds synthesized during development.

Chiral separation is part art and part science and is most successful when practitioners are well versed in the methods and challenges. Gilead Research maintains a dedicated capability to provide chiral separation services. Initially all methods and separations were normal phase chromatography run on analytical and semi-preparative HPLC's. Recently we incorporated a hybrid supercritical fluid chromatography (SFC) system to our separation workflows, capable of running both scales of separation. Both the HPLC and SFC systems are set up to run a set of columns and solvents in a matrix configuration using prescribed methods to identify and optimize methods suitable for larger scale separations.

This poster presents the instrumentation and methodologies used to employ chiral separations and SFC as an important component of the drug discovery efforts at Gilead.

Poster #5

Separation of EPA and DHA in an Industrial Scale Simulated Moving Bed with Supercritical Fluid as the Desorbent

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In this study, the separation of ethyl ester of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from esterified fish oil was conducted on a large scale simulated moving bed (SMB) with supercritical fluid of carbon dioxide and ethanol as the desorbent and irregular bare silica as the adsorbent. An eight-column SF-SMB with 80 mm in diameter from JOPE Co. is employed and divided into three sections with 2/3/3 configuration. In this SF-SMB, the recycling of carbon dioxide is conducted by depressurization and condensation, so that the fourth section in the SMB is eliminated to increase the productivity. In this study, the supercritical state for the chromatography was controlled at 120~130 bar and 50 C with ethanol as the cosolvent. A two-step method was developed to separate EPA from the fish oil. The polyunsaturated fatty acids (PUFA) are preliminary separated from the esterified fish oil by using 5 wt% of ethanol as cosolvent. The EPA in the PUFA is further separated by a second operation of the SF-SMB with 2.5 wt% of ethanol. The purity of EPA in the final product is 97.2% of EPA with 64.1% of recovery. That the use of carbon dioxide and ethanol as cosolvent avoids the use of water as the mobile phase can greatly prevent the hydrolysis of esterified fatty acid and simplify downstream processing to reduce the operating cost. This study demonstrates that the SF-SMB can provide a safer and cost effective alternative to produce EPA with low content of DHA.

Poster #6

SFC Without Additives - An Imidazole Based Stationary Phase Designed for the Task

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SFC is a green chromatographic technique that can provide high resolution separations for both analytical and preparative applications. However many classes of compounds are difficult to chromatograph without the use of additives such as trimethylamine or diethyl amine. This is especially true for mixtures of amines. It would be desirable to chromatograph amine mixtures without the use of additives. At ES Industries we have developed an imidazole based stationary phase that provides superior peak performance for amine containing compounds. We will present several examples use this imidazole based stationary phase for the separation of amine mixtures. We will also present comparison data on commonly used SFC stationary phases using the selected amine mixtures.

Poster #7

Two-Dimensional Separation for Surfactants using SFC-LC-MS

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SFC has several advantages such as superior separation of wide range of compounds and high-throughput and low column pressure drop due to low viscosity and high diffusivity of the mobile phase. Further, SFC is suitable for multi-dimensional chromatography setups such as SFC-SFC and SFC-LC etc. The needs of multi-dimensional separation system have increased because of their capability of delivering excellent separation in complex sample using multiple columns that have a different separation mode.

Surfactant analysis is one of difficult application due to their diversity and the complexity of sample matrices. HPLC is commonly used for surfactant analysis. However, it is difficult to separate anionic, cationic, nonionic and amphoteric surfactants on a single HPLC column simultaneously. In particular, nonionic surfactants contain various molecular species which have distribution of polyethyleneoxide (PEO)/polypropyleneoxide (PPO) and alkyl groups. Separation of nonionic surfactants depending on PEO/PPO and alkyl groups separations may occur concurrently on a reversed-phase column in HPLC, thus, achieving complete separation of the surfactant mixture is extremely difficult. In this study, we developed SFC-LC-MS two-dimensional separation system and applied it to comprehensive analysis of surfactants.

First, several different columns were tested to select appropriate one for separation of nonionic surfactants in SFC and LC system. As a result, 3-HP column (Nacalai Tesque, Japan) in SFC condition showed the best separation depending on number of moles of the ethyleneoxide and FC-ODS column (Shimadzu, Japan) in LC condition only achieved separation depending on alkyl groups. Therefore, we connected these two columns in series, water/methanol-mixed solvent was supplied after SFC column and all the elute is directly introduced into LC column. Using this system, we successfully achieved good separation in nonionic surfactants (PEO alkyl ether (PEO AE)/PPO and PEO alkyl ether (PPO/PEO AE) series). Additionally, we confirmed that adding small amount of salt (ammonium formate) to the modifier improved separation of positional isomers in iso-PEO AE and PPO/PEO AE series. Finally, this system was applied to the comprehensive analysis of anionic, cationic, nonionic and amphoteric surfactants. We have achieved simultaneous separation of nonionic and ionic surfactants and classification of alkyl chain length within 75 min. in a single run.

Poster #8

Development of a Unique Chiral Column Cleaning Method

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SFC is typically used in the pharmaceutical industry to separate racemic mixtures containing a target compound and often reaction side products and process impurities. which can get trapped on the column degrading it's performance. Degraded performance can be indicated by loss of resolution, distorted peak shapes, and/or an increase in column pressure. When any of these conditions become too significant to use the column for efficient separations, the column is deemed "dead" or "unusable" and it is necessary to replace the column, or find an efficient way of cleaning the columns to remove the trapped material. We have developed a unique cleaning process using several unconventional solvents and have used it to recover a number of "dead" columns.

Poster #9

Purification of regioisomers, insoluble intermediates and complex chiral mixtures using chiral SFC

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During the course of drug discovery process in pharmaceutical industry medicinal chemists face challenging separation problems due to the presence of very similar regioisomers, insoluble intermediates and complex chiral mixtures in their reaction products. In this poster we will discuss how analytical chemists help them to find solutions for their purification problems using chiral SFC. Several purification examples will be discussed using their partial structures.

Poster #10

Understanding the Injection Parameters of A New SFC Autosampler

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Introduction

Traditional SFC systems use fixed loop injection design. This means that loop size needs to be changed to address a wide range of injection volume when using the full loop mode. When partial loop mode is used, injection precision is often compromised. To address this, a new injection principle called feed injection is developed and it enables flexible injection from 0.1uL to 90uL without the need to change loops and with excellent injection precision.

With the new feed injection principle, two new injection parameters are introduced, "Feed Speed" and "Overfeed Volume". Feed speed controls how fast the sample is injected into the column. Overfeed volume is the additional solvent injected into the column to minimize sample loss. In this work, we study the effect of these two parameters on SFC chromatographic performance, e.g. efficiency, retention time, peak area. A Design of Experiment (DoE) method was used to identify optimal injection parameters. The results also provide guidelines for injection optimization for specific applications.

Method

A 1260 Infinity II SFC system was used in the study. Feed speed was varied from 100 uL/min to 1000 uL/min. Overfeed volume was varied from 0 to 4 uL. Different injection volumes were also used from 0.1 uL to 10 uL. A 4.6 x 150 mm, 5 µm silica column was used using methanol as the modifier under isocratic condition. Different combinations of feed speed, overfeed volume and injection volume were used and the chromatographic performance (e.g. retention time, peak area, efficiency, tailing factor) was measured.

Results

The results suggested that in isocratic elution, higher feed speed generally gives better peak efficiency. Higher overfeed volume can improve the peak area recovery but can also decrease efficiency. DoE data analysis shows that there are interactions between the injection parameters. For general applications, optimal injection parameters are chosen at 400 uL/min feed speed that gives a good compromise between efficiency and pressure, and 4 uL overfeed volume that gives a good compromise between sample recovery and peak efficiency. It is recognized that specific applications may have different optimization goals. The results obtained in this study should provide a guideline for injection optimization.

Poster # 11

Matrix effects in SFC-MS and LC-MS for biological samples

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Matrix effects are often an important concern of chromatography-MS bioanalytical methods, as they can negatively influence method accuracy, precision and sensitivity. Due to the presence of endogenous entities from the matrix eluting at the same time as the target compounds, the ionization can be altered, thus leading to ion suppression or enhancement. Matrix effects have been largely studied in liquid chromatography (LC) and several solutions, such as modifying the selectivity of the analytical method, improving the sample preparation or using a stable isotopically labelled internal standard, have been highlighted to overcome this issue. On the contrary, matrix effects have been scarcely studied in SFC-MS but the few articles dealing with this topic tend to emphasize the ability of the technique to decrease matrix effects compared to LC [1-3].

The goal of the present study is to perform a comprehensive evaluation and comparison of matrix effects in RPLC-MS vs. SFC-MS. For this sake, 40 and 38 drugs were analyzed in urine and plasma, respectively, and the matrix effects were calculated using the Matuszewski approach [4]. Two different sample preparations, a selective (SPE) and a non-selective (dilute and shoot for urine and protein precipitation for plasma), were also compared. Finally, three different stationary phase chemistries were used in SFC-MS to evaluate the impact of selectivity on the occurrence of matrix effects.

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Poster # 12

Quantitative determination of choline and acetylcholine in biological fluid sample using automated online SFE-SFC-MS/MS

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Choline, a structural element of cell membranes, and acetylcholine, known as a neurotransmitter, are both familiar compounds in the field of bioanalysis. Since acetylcholine is biosynthesized from choline in the body from choline, it is possible to estimate a quality of internal activity by monitoring both of these compounds. Current analytical methodology for quantitative determination with complex sample matrices such as biological liquids and tissues generally requires offline sample preparation procedure that is followed by a chromatographic analysis. The sample preparation and the following manual transfer to the analytical instrument are generally tedious, time-consuming and affording uncertainty and they interfere with the enhancement of efficiency of lab work and the increase of experimental throughput. An innovative new concept of online SFE-SFC has been introduced that greatly reduces a sample preparation time and a quantitative variability associated with manual procedures. This new technique provides fully automated sample preparation followed by chromatographic separation of target compounds using supercritical fluid. When online SFE-SFC is employed, extracted compounds from a sample matrix are introduced into an analytical column for separation without any human intervention. At the first step we determined choline and acetylcholine in a rat cerebrospinal fluid sample using ordinary SFC-MS/MS with direct injection of liquid sample. All necessary fundamental evaluations including column scouting procedure were carried out. Using the cyano-group bonded silica-based column under carefully optimized conditions, a linearity, a repeatability, a LOQ and a LOD were calculated and successful results were obtained. Then we tried automated online SFE-SFC-MS/MS with a dried filter paper sample (like DBS, dried blood spot) that was prepared for online SFE and is paid attention due to its easy sample handling feature. Online SFE-SFC-MS/MS provided improved sensitivity especially for acetylcholine that showed apparently small concentration compared to that of choline in rat cerebrospinal fluid and a reliable applicability of Online SFE-SFC-MS/MS was confirmed.

Poster #13

Recent Advances in Preparative Supercritical Fluid Chromatography for Achiral Purifications

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Abstract

Preparative supercritical fluid chromatography (SFC) is an established and widely used technique for the chiral separations in the pharmaceutical industry. Reversed-phase high performance liquid chromatography (RP-HPLC), which is a complementary separation technique to SFC, has been the more generally applicable technique for achiral purifications and impurity isolations. Preparative RP-HPLC coupled to mass spectrometry (MS) has been especially useful for low level impurity isolations to enable their structural characterization. Recent developments in mass-directed SFC technology have expanded the use of preparative SFC for the purification of achiral mixtures and the isolation of low level impurities. In this presentation, we illustrate some of our most recent examples from the use of analytical SFC-MS plus preparative SFC for successful achiral SFC purifications of complex mixtures and low level impurity isolations. These examples are intended to inspire further advances in the use of preparative SFC technology for challenging purifications, including very polar compounds and larger biomolecules.

Poster #14

Aging of columns for supercritical fluid chromatography packed with eight different stationary phases

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The interest in ultra-high performance supercritical fluid chromatography (UHPSFC) separations is constantly growing. Most of UHPSFC separations in pharmaceutical and bioanalytical field benefits from use of methanol and other alcohols as modifiers of the mobile phase. While use of modified mobile phase has many advantages, it may also lead to the silyl-ether formation that causes shifts in retention and changes in selectivity over time. Moreover, a small amount of additional additive in the mobile phase is often necessary for SFC separations of ionizable compounds. The interactions between additives and stationary phase can be strong and also can generate changes in retention times over the life-time of the column, in particular after switching between different additives as well as while using additive-free mobile phase.

In this study, we analyzed about seventy different pharmaceuticals using UHPSFC. The set of analytes contained compounds with acidic, basic, and neutral properties. Eight different stationary phases were evaluated: BEH 2-EP, BEH, CSH PFP and HSS C18, Torus Diol, Torus DEA, Torus 2-PIC, Torus 1-AA. All 3.0 x 100 mm columns were packed with 1.7 μm particles in except for HSS C18 column that was packed with 1.8 μm particles. The first four columns are typical representatives of UHPSFC columns packed with bonded silica or hybrid silica particles. The other four columns feature the new generation of UHPSFC columns in which the aging due to silyl-ether formation should be substantially reduced. The separations were accomplished using methanol together with the most common and generic additives 0.1 % ammonium hydroxide and 10 mmol/L ammonium formate in methanol. The separations of all compounds using the entire set of 8 columns were repeated four times over a period of one year, specifically in 3, 9, and 12 months after the first column application. The changes in chromatographic performance were monitored and the retention time shifts for analytes in different columns were compared. Afterwards, the columns were regenerated using procedure recommended by the manufacturer, i.e. rinsing the column with water, isopropanol-water mixture, and isopropanol. The effect of the regeneration process was also evaluated.

The study was supported by the Project of Specific Research, SVV No. 260412/2017, and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

Poster #15

Impurity screening in achiral using UHPLC/SFC switching system and study on sensitivity improvement for SFC

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Organic impurities are produced when an active pharmaceutical ingredient (API) is synthesized. They consist of the API starting material, by-products, intermediate, ligand and degradation products. There are various chemical actions that cause impurity formation such as hydrolysis, oxidation and photolysis. Risk assessment of impurities included in API is very important for drug development. HPLC is generally used for measuring organic impurities, but SFC will give additional chance to separate those impurities due to its different chromatographic separation. On the other hand, many candidate conditions should be examined and this process requires extensive method development. Therefore, a quicker and simplistic system for determining the optimized analytical conditions has been needed. An UHPLC/SFC switching system can be used as a scouting system with multiple columns switching between mobile phases under both LC and SFC conditions. Thus, a variety of analytical conditions can be investigated conveniently. If the SFC method is the best way to separate impurities, it is more ecofriendly and can save on running cost. However, SFC analysis commonly has larger noise levels than HPLC. To analyze low level impurities with SFC, it is desirable to reduce noise or increase the signal of them. We will demonstrate separations of impurities using an UHPLC/SFC switching system which automatically switches analytical modes between UHPLC and SFC in a single sequence. In addition, we investigated the effect of SFC condition on the baseline noise.

Poster #16

Enantiomeric separation of chiral scaffolds and cores used in drug discovery by SFC and HPLC

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Successful therapeutic intervention often requires chiral medicines due to the chirality of many molecules in biological systems. Potency, efficacy and safety can be highly dependent on the stereochemical geometry of the molecules and therefore determining the biological profile of enantiomers in the early stages of drug discovery is important for successful optimization towards clinical candidates. Early stages of lead discovery and optimization reactions often yield racemic mixtures that lead to questions about the relative activity of the individual isomers. Fortunately, chiral stationary phases can be used with HPLC and/or SFC to purify the individual isomers, enabling establishment of structure activity relationships for the individual enantiomers.

This work demonstrates screening of several privileged scaffolds and cores by SFC and HPLC. Compound classes screened include thiazoles, indoles, triazines, benzothiazoles, biphenyls, leucolines, dihydroquinazolinones, and pyranoquinolones. Results from screening 45 compounds from eight different classes showed that the majority (>90%) of chiral compounds from early drug discovery HTS screens can be separated into their individual enantiomers by HPLC and/or SFC using only three chiral stationary phases. By using complementary phases, an orthogonal approach towards separation can be applied to improve the success rate of chiral separations.

Poster # 17

CHIRAL/ACHIRAL ANALYSIS OF NATURALLY OCCURRING CANNABINOIDS USING A NEW SUB-2 µM CHIRAL STATIONARY PHASE WITH ULTRA HIGH PERFORMANCE SFC-MS

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The interest in the medical use of Cannabis Sativa L. is steadily increasing because of its therapeutic efficacy towards a wide variety of ailments, and its unique chemistry, characterized by the presence of cannabinoids that are concentrated in the female inflorescence. The fiber type of Cannabis Sativa L. is cultivated in Europe for textile production or for food (seeds, flour, and oil), and has a low concentration of psychoactive (-)-Δ9-trans-tetrahydrocannabinol ((-)-Δ9-THC) that is typically less than 0.2%. The main cannabinoid in the fiber type of Cannabis Sativa L. is (-)-cannabidiol ((-)-CBD) but there is also (-)-cannabidivarin ((-)-CBDV), cannabigerol (CBG), cannabinol (CBN) and the racemic cannabichromene (rac-CBC), each having various therapeutic actions. The analysis of the original composition of plant material is necessary for phenotype determination and quality control of medicinal cannabis used in therapeutic treatments.

The presence of natural racemic compounds (rac-CBC) in plant extract was investigated using Chiral Stationary Phases (CSPs) in enantioselective "e" Ultra High Performance Supercritical Fluid Chromatography (eUHPSFC). All of the analyses were performed using a new UHPSFC-compatible chiral column developed in the Sapienza University laboratory in collaboration with Regis Technologies. Kinetic evaluation using van Deemter plots showed excellent kinetic performance for the 100x4.6 mm column packed with 1.8 µm on a modified low-dispersion Waters UPC^2 system. Indeed, using a 90:10 CO_2/MeOH mixture as mobile phase and TSO (trans-Stilbene oxide) as a probe, efficiencies up to 290,000 N/m measured on the first enantiomer at flow rate = 4.0 mL/min, and more than 278,000 N/m on the second enantiomer (flow rate = 3.7 mL/min) were observed . The Chiral Stationary Phase (CSP) Whelk-O1 allowed resolution of the racemic compound cannabichromene (rac-CBC) in plant extract and the synthetic racemic (+/-)-Δ9-trans-tetrahydrocannabinol. Good separation, in terms of chemio- and enantio- selectivity, was obtained with high resolution for all cannabinoids, and their acid forms, under isocratic conditions.

Poster # 18

Comprehensive evaluation of a sub-2 micron chiral stationary phase: characterization, applications and ultrafast analyses

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In the last decade, technological progress has led to the development of stationary phases on increasingly smaller silica particles, and instrumentation (UHPLC/UHPSFC) with low dead volumes and the ability to reach very high pressures. These innovations have allowed higher column efficiencies, greater resolution, reduced analysis times, and lower eluent consumption. Many chiral stationary phases (CSPs) are available in particle sizes down to 3 µm, and until recently, none were available in particle sizes smaller than 3 µm. Chiral Stationary Phases (CSPs) are now moving towards sub-2µm diameter particles in order to realize the benefits of greater separation power and faster analysis times.

This poster describes the development and characterization of a brush-type (Pirkle type) CSP with the Whelk-O1 selector[1] that is covalently bonded on 1.8 µm Fully Porous Particles (FPP)[2] using the classic Pirkle synthetic method[3]. Performance of columns containing the same selector with different internal diameters ranging from 4.6 - 2.1 mm, and different lengths (from 15cm down to 1cm) was evaluated to compare performance and provide an overview of the transition from HPLC to near-UHPLC to UHPLC/UHPSFC. Kinetic performances were evaluated through van Deemter analysis, and resulted in efficiencies close to 300'000 plates/m on the first eluted enantiomer of trans-Stilbene Oxide (rac-TSO) on the 1.8 µm CSP. An "ultra short" narrow-bore column with a length of 10 mm and diameter of 3.0 mm provided a separation in only 0.91 seconds, and maintained a resolution value higher than 2.0 for rac-TSO.

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Poster # 19

SFC Analysis of Nutraceuticals Based on SFC Optimized Stationary Phases

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Reversed-phase HPLC is widely used for separation and analytical analysis of many nutraceuticals mixtures. Unfortunately, there are mixtures that are not well separated by HPLC leading to incomplete analytical results. An alternative separation technique maybe required such as SFC (supercritical fluid chromatography) to effect a complete separation of many mixtures. In addition, SFC can be utilized as an orthogonal separation technique to HPLC for many separations. SFC provides many unique features including producing high pressure/high speed separations. These features suit SFC well to utilizing columns packed with small particles and SFC optimized stationary phases. It is the purpose of the work to develop high performance columns that have been engineered to specifically for SFC. We will provide examples and applications on how the chromatographer can benefit from these types of stationary phases using the high performance columns. We will demonstrate how these SFC columns can provide for the high resolution separations over a wide variety flow rate conditions and mobile compositions.

Poster #20

Unique Chemically Modified Carbohydrate Based Chiral Stationary Phases to Improve Chiral Separations

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The chromatographic separation of chiral compounds is an important tool in the search for new pharmaceutical entities. Both HPLC and SFC separations of chiral chemicals are important tools for analytical determination and preparative isolation of enantiomeric mixtures. Existing chiral stationary phases can separate a many chiral mixtures. Many of these chiral stationary phases are based on chemically modified carbohydrates. However, even with the existing chemically modified carbohydrates stationary phases there are still many enantiomeric mixtures that are difficult to separate limiting the ability to characterize and purify chemical mixtures containing chiral compounds. In this study we are chemically modifying carbohydrates, such as cellulose, chitin, cyclodextrins and amylose with functional groups that have not been routinely employed. Chemical modifications of the carbohydrates include halogenated, aromatic and hetero-aromatic functional groups. We will present information on the chiral separation characteristics and overall separation capabilities for these chemically modified carbohydrate based chiral stationary phases.

Poster # 21

The Development of Unique SFC Stationary Phases that Utilize Advanced Particle Technologies

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Both Reversed-phase HPLC and SFC is widely used for separation of many chemical compounds. A majority of these separations are based on ODS type columns. However, retention and separation of various compounds have proven to be a challenge. Many of these types of compounds are unretained, poorly retained or unseparated on most conventional ODS reversed-phase columns, even when these ODS column are packed with highly efficienct sub 2 particles. Fortunately, to deal with these types of analytes we can employ alternative modes of chromatography that use unique stationary phases containing polar groups, organic bases, fluorinated groups and other non-hydrocarbon functional groups. These columns can to be used in SFC chromatography. SFC uses supercritical CO2 along with an organic modifier such as methanol. These unique stationary phases are bonded to support materials that utilize advanced particle technologies. It will be demonstrated that the combination of unique stationary phases bonded to advanced particle technologies will improved separations and add flexibility to operating conditions.

Poster #22

Switching System of SFE/Prep SFC with MS Detector

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A mass spectrometer provides mass information of analytes, and generally provides high sensitivity combined with selective analysis, compared with conventional optical detectors. In recent years, it has been used as the method of selective detection for target analytes and identification of impurities in the development of pharmaceuticals and in a variety of other industries.

Supercritical fluid extraction (SFE) enables fast and efficient extraction using a supercritical fluid that has the specific characteristics of highly diffusivity, permeability, and solubility. Carbon dioxide (CO_2) has been widely used as an extraction solvent in SFE because it easily achieves a supercritical state under moderate conditions (supercritical temperature; 31ºC, supercritical pressure; 7.4 MPa). SFE with supercritical CO_2 has advantages of easy post extraction handling, lower solvent costs, and automation by device control.

We developed a switching system combining SFE with preparative SFC and an MS detector, which provides extraction, preparation and purification with MS trigger in a single system. In this presentation, we will introduce the application of extraction from coffee beans with preparative separation and purification of caffeine using this system.

Poster # 23

High Sensitivity Fluorescence Detector for Supercritical Fluid Chromatography

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Fluorescence detection has been widely used as one of a practical detection method in HPLC analysis. It enables the selective detection of fluorescent substances, with sensitivity that can be up to 1000 times greater than with UV detection.

Fluorescence detection can also be applied to non-fluorescent substances with the use of derivatization, with many derivatization agents becoming commercially available in recent years. Therefore, this technique provides the advantages of highly sensitivity and selectivity to many different classes of compound, and broadens the range of applications in many industries. However, fluorescence detection has not been used in SFC analysis due to the difficulty of developing a suitable high pressure flow cell.

We developed a fluorescence detector with a flow cell specifically constructed for operation at the high pressures required for SFC. This fluorescence detector is included in our newer SFC system (SFC-4000 series). In this presentation, we will introduce an application for the analysis of several compounds using SFC separation with fluorescence detection.

References

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Poster # 24

Fast Quantitative Analysis of Four Rodenticides Using Agilent Infinity II SFC Coupled with Single Quadruple MS

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Anticoagulant rodenticides are widely used and can contaminate food and environment. Human and pets may get accidentally poisoned. Quantitative analysis of these rodenticides in food, environment and biological matrices are important for both environmental monitoring and basic science research. Currently, HPLC and GC are the two common separation methods. Difenacoum and brodifacoum have two chiral centers and it has been reported the ratio of brodifacoum diastereomeric pairs varies in different rat tissues. Here, we developed a fast SFC-MS method for monitoring four rodenticides: warfarin, coumatetralyl, difenacoum and brodifacoum. The diastereomeric pairs of difenacoum and brodifacoum were baseline resolved. Linearity, precision, accuracy and limit of detections were determined for the method. The linearity and precision for variable injections using Agilent Feed-injection technology were also evaluated.

Poster # 25

Development of SFE-SFC/MS system by integrating a novel splitter

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On-line coupling of extraction and chromatographic separation allows the entire analysis to be performed in a closed system. Therefore, online systems are useful for labile and volatile compounds; automated online processes also reduce sample contamination. Development of an online system can solve the problems associated with analytical procedures.

In this study, an online supercritical fluid extraction (SFE)-supercritical fluid chromatography (SFC) with mass spectrometry (MS) was applied using supercritical fluid carbon dioxide ($SCCO_2$) and modifier as the medium. A supercritical fluid (SF) possesses features like low viscosity and high diffusivity, which provide rapid processing. Particularly, carbon dioxide (CO_2) is commonly used as a medium because it is easy to handle due to its lower critical temperature and pressure, and the polarity changes upon adding a polar organic solvent. Online coupling of SFE and SFC enables rapid analysis in addition to improving the analysis process. However, there are some disadvantages associated with online coupling of SFE and SFC. When targeting a heterogeneous solid sample like food, sample dilution is difficult, which leads to overloading and high contamination in the separation and detection steps. In addition, it has been reported that when targeting polar compounds that require a solvent with high elution power for the extraction, peak broadening and distortion occurred during separation, with poor retention on the tip of the column during extraction. To overcome these limitations of SFE-SFC, extracts should be branched prior to the separation section to avoid the inflow of large amounts of extract. Therefore, we proposed a novel splitter using two high-precision back pressure regulators (BPRs), and used it as an interface between SFE and SFC. According to the differential pressure of BPRs, extracts were successfully split and introduced into SFC. Additionally, pesticide standards have shown good linearity of calibration curves at an arbitrary introduction rate. Finally, we attempted to analyze pesticides spiked in agricultural products using SFE-SFC/MS integrated with the splitter. Numerous pesticides were successfully and reliably analyzed on complex food matrices.

Poster # 26

Investigation of the need of using actual operational conditions in analytical SFC separations as compared to the set operational conditions

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The need to determine the actual operational conditions, instead of merely using the set operational conditions, was investigated for in packed supercritical fluid chromatography (SFC) by design of experiments(DoE) using a most important type of compounds, pharmaceutical basics, as models. We know from the work by Guiochon et al this is necessary in preparative SFC but what about analytical SFC?

The actual values of temperature, pressure, and methanol levels were recorded and calculated from external sensors, while the responses in the DoE were the retention factors and selectivity. A Kromasil CelluCoat column was used as the stationary phase, carbon dioxide containing varying methanol contents as the mobile phase, and the six racemates of alprenolol, atenolol, metoprolol, propranolol, clenbuterol, and mianserin were selected as model solutes. For the retention modeling, the most important term was the methanol fraction followed by the temperature and pressure. Significant differences ($p < 0.05$) between most of the coefficients in the retention models were observed when comparing models from set and actual conditions. The selectivity was much less affected by operational changes, and therefore was not severely affected by difference between set and actual conditions. The temperature differences were usually small, maximum ±1.4◦C, whereas the pressure differences were larger, typically approximately +10.5 bar. The set and actual fractions of methanol also differed, usually by ±0.4 percentage points. A cautious conclusion is that the primary reason for the discrepancy between the models is a mismatch between the set and actual methanol fractions. This mismatch is more serious in retention models at low methanol fractions. The study demonstrates that the actual conditions should almost always be preferred.

Poster # 27

SFC Applications for the Cannabis Industry

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Recently, the legal landscape for cannabis has changed considerably within the United States and for many other countries. The US cannabis industry needs a wide range of chromatographic methodologies to fit within the new landscape. Analytical testing for the different cannabinoids is often the first chromatographic methodology that is developed by company that is new to the industry and this testing is typically a liquid chromatography application. Terpenes are another class of compounds that are of interest to the cannabis industry. Analytical testing for terpenes is often accomplished with gas chromatography. Pesticide analysis is currently (or soon will be) legally required for commercial cannabis products. Pesticide testing is often done by gas or liquid chromatography with mass spectrometry. Preparative liquid chromatography has a role in the cannabis industry for isolating individual components and for concentrating similar compounds. The unique properties of SFC make it a suitable platform for these chromatographic methodologies.

The work presented here will demonstrate SFC-UV and SFC-MS applications for the cannabis industry. Examples of analytical methodology for purity / potency and identification of cannabinoids will presented. SFC applicability for analytical methodology of terpene and pesticide analysis will be discussed and examples will be presented.

Poster # 28

Achiral SFC: No C18 Equivalent, No Problem

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For a several years, a large amount of research has been conducted in the search for a universal achiral SFC column. This universal column would be the SFC equivalent to the C18 column for reverse phase chromatography. These efforts have evaluated long lists of probes, many forms of column chemistry and employed sophisticated statistical treatment of screening data. However, to date there is still no magic universal column for SFC.

A universal column for achiral SFC applications would be convenient but does SFC need to have a universal column? Chiral chromatography in the pharmaceutical industry can be credited with shaping the entire SFC industry into its current position. Chiral chromatography does not have a universal column. SFC has excelled in this separation science niche because it is particularly effective at screening multiple columns and different eluent compositions. The same approach can be applied to achiral chromatography. SFC can be effectively applied to achiral applications when a limited set of columns are known to be applicable for the diverse range of compounds suitable for SFC.

The work presented here will discuss the development of achiral applications for SFC. The focus will be on compounds relevant to the pharmaceutical as well as growing cannabis industry. Columns typically utilized for normal phase, reverse phase and chiral applications will be evaluated for use their applicability in achiral SFC applications. The effect on achiral selectivity due to changes in co-solvents and modifiers will also be addressed.

Poster # 29

The Impact of Supercritical Fluid Chromatography on Pharmaceutical Analytical and Purification

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Supercritical Fluid Chromatography (SFC) is a chromatographic technique similar to HPLC, but uses a supercritical fluid as the main component of the mobile phase. SFC has lower viscosity and higher linear velocity compared to traditional HPLC, which can increase run speed and separation power. Carbon dioxide is the most commonly used supercritical fluid in SFC as it is inexpensive and easy to obtain. A great benefit to SFC is that it is “green” compared with other chromatographic techniques.

In this poster, a few examples will be shown to demonstrate the impact of SFC technology in the purification process, especially on chiral separations in support of discovery medicinal chemistry.

Poster # 30

Development of polymer based novel stationary phases for supercritical fluid chromatography

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Supercritical fluid chromatography (SFC) has great advantages over conventional chromatography such as liquid chromatography and gas chromatography, as it has a low viscosity allowing for high diffusivities and limited pressure drop. The retention and separation mechanism in SFC is likely to depend on a combination of mobile phases and the stationary phases (SPs).[1]

We have concentrated on a novel achiral polymer-based SP and launched poly(butylene terephthalate) based one as DAICEL DCpak® SFC-A in 2014, which possesses excellent column efficiency and molecular recognition ability.[2]

We consider that this unprecedented molecular recognition may be attributed to macromolecular effect, which promoted us to develop various polymer-based SPs and evaluate their performance in SFC.

Recently, we developed novel poly(4-vinylpyridine) (P4VP) based achiral SP.[3] P4VP SP provides excellent "molecular shape" recognition with better column efficiency for non-polar to polar analytes than commercially available 2-ethylpyridine (2EP) SP, probably due to the multiple interactions between pyridyl groups in the SP and analytes. Moreover, P4VP SP shows significantly better durability than commercially available 2EP. P4VP SP provides good peak symmetry for basic and acidic samples without any additives, such as acids, bases and salts. P4VP SP has been commercially available as DAICEL DCpak® SFC-B since last year.

In this study, some interesting application data by DAICEL DCpak® SFC-A and SFC-B and their characteristics will be presented.

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