Poster #1

Natural Compound Isolation with Preparative SFC

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Sepiatec´s Prep SFC M5 system is a versatile preparative bench top unit, which can be used for chiral and achiral separations. In this work, we show the isolation of compounds from saffron *Crocus sativus L.,* performed with the Prep SFC M5 system with mass spectrometer.

Saffron, *Crocus sativus L.*

Sepiatec Prep SFC M5 with MS

The dried stigma of *Crocus sativus L.* is called saffron and the most expensive spice in the world. Besides its importance in food industry saffron also has many therapeutic properties, including antitumor, neuron protective and antidepressant effects.

Until now, there is no published SFC method for the isolation of secondary metabolites from saffron. In this work, we present a mass spectrometry coupled SFC method, which allows for the accurate measurement and identification of saffron constituents as well as high-yield mass directed collection of the desired compounds. This enables a quality assessment, which is very important for companies working in the food industry. Further, collected metabolites can be used as reference standards or for subsequent evaluation in pharmaceutical studies.

Through the use of food grade only chemicals, direct sensory analysis of the collected fractions without any clean-up is possible.

Poster #2

Implementation of a Novel Shimadzu Supercritical Fluid Chromatography Screening Platform to Support Drug Discovery Purification

Shuping Dong, William Leister
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Supercritical fluid chromatography (SFC) is a useful tool to purify medicinal chemistry samples. More and more early discovery compound purifications require the use of SFC, especially for chiral/isomers separations. To accommodate this increased workload an advanced SFC screening system was needed to handle larger numbers of samples. Desirable system attributes include sample multiplexing, robustness, reduced footprint and user friendly software.

In this poster we will detail a first its kind of Shimadzu SFC Screening System. The system was designed and built to meet our increasing demands for high throughput screening. It has had a profound impact on our standard screening process.

Poster #3

Sample Loadability on Coated and Immobilized Polysaccharide-Based CSPs

Edward G. Franklin, Gay Lowden

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Chiral stationary phases (CSPs) prepared by coating phenylcarbamate derivatives of amylose and cellulose on supporting silica gels have proven invaluable for the separation and purification of enantiomers in both high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). One notable limitation of coated polysaccharide phases is the restricted use of certain organic mobile phases, such as acetone, chloroform, ethyl acetate, tetrahydrofuran (THF), and toluene. These solvents must be avoided as they can swell or dissolve the polysaccharide and destroy columns packed with coated CSPs. Immobilized versions of the same chiral selectors have expanded the capabilities of these phases to allow for the use of such solvents in analysis and sample purification. For some applications, however, practitioners have observed important differences in selectivity and sample loading capacities between the coated and immobilized versions. Herein, three examples of chiral separations performed using HPLC and SFC are discussed with respect to how these differences may be manifested and overcome or exploited to advantage.

Poster #4

Recent SFC Applications for Daicel's DCPak® Poly(4-vinylpyridine) Achiral Stationary Phase

Dr. Weston Umstead, Chiral Technologies Inc., West Chester, PA 19380,

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It's important for a chemist to isolate and characterize the various components in a reaction mixture. Particularly in the pharmaceutical industry as a lead compound progresses through drug trials, a thorough understanding of the toxicological impact of each component is essential. Because many of these reaction impurities are not enantiomers of the lead compound, they can be isolated using achiral, reversed phase chromatography with columns such as those based on an ODS or a substituted-ethylpyridine stationary phase. Daicel has launched a new polymeric achiral stationary phase, DCPak® P4VP, which contains a unique selector, poly(4-vinylpyridine). This presentation highlights a few of the applications developed thus far using this phase, as well as a new industry application in collaboration with Pfizer Inc., located in La Jolla, California. This new application highlights the improvements in performance that can be achieved with DCPak® P4VP versus other legacy achiral columns, as well as its performance upon scaling to a preparative SFC method.

Poster #5

Scale-up Study of a Supercritical Fluid Extraction Process for Cannabinoids Derived from *Cannabis Sativa*

Eric Kawka,
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Scale-up criteria for supercritical fluid extraction (SFE) has been studied and optimized for a variety of purposes in the food and nutraceutical industries. However, when utilizing SFE technology to extract cannabinoids from *Cannabis sativa*, data is limited. The objective of this work is to study the scale-up of the SFE process, with a specific focus on cannabinoids derived from *Cannabis sativa*. A laboratory scale SFE instrument (1000 mL extraction vessel) and a pilot scale instrument (5 L extraction vessel) are used in this study. The scale-up criterion adopted consists of maintaining solvent mass to feed mass ratio at a constant level. Mass balance and extraction efficiency data will be presented and discussed.

Poster #6

SFC Purification System Performance Evaluation of Purity and Recovery

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The overall goal in SFC purification is to meet increasing needs for high throughput and productivity, while at the same time achieving purity and recovery requirements. Although application areas may differ, user requirements are often similar with a desire for high purity and recovery of the final isolate. Purity and recovery are impacted by not only the quality of the chromatographic separation, but the attributes of the instrument design and implementation. In this poster, techniques for assessing system performance, as it relates to isolate purity and recovery of a sample test mixture, will be demonstrated. Additionally, scale-up calculations will be illustrated to reveal the influences of column diameter and flow rate, versus throughput.

Poster #7

Isolating Valuable Cannabinoids from CBD Mother Liquor using Preparative SFC

Jacquelyn Runco, John Van Antwerp

Thar Process, Pittsburgh, PA, USA

One of the most common ways of obtaining CBD isolate is by fractional distillation and crystallization. This process results in highly pure CBD crystals. The process also produces a mother liquor that contains not only a remaining high percentage of CBD, but also enriched levels of other cannabinoids that can be quite valuable as isolated compounds. Due to the toxic solvents such as pentane, hexane and acetone contaminating the mother liquor, it is sometimes discarded as waste. One solution for isolating these cannabinoids is preparative SFC. Here, the isolation of CBD, CBC, and CBG from a CBD mother liquor will be demonstrated using CO_2 and a small percentage of ethanol as the mobile phase. Preparative SFC provides a way to reclaim value from the large volumes of waste created during CBD crystallization.

Poster #8

Cannabinoid content analysis of commercial CBD products by UPC^2

Jacquelyn Runco, Jayme Kennedy

Thar Process, Pittsburgh, PA, USA

Hemp-based CBD products are now widely available as tinctures, topicals, capsules, vape oil, gummies and other products. As more and more of these products become available, consumers can be confused by the type and quality of products hitting the market. The industry has rapidly expanded, and CBD specifically has been growing in popularity, along with full-spectrum “THC-free” products.

Many of the products on the market have been shown to have incorrect or incomplete cannabinoid content label claims. Some have no active ingredients; others contain unsafe or high levels of cannabinoids like THC, that are under-reported or not listed at all. This can create a dangerous risk to patients and consumers who think they are getting a safe product for themselves or their children. Robust and reliable methodologies are required to analyze hemp-based products in order to determine or confirm potency. In this study, the robust methodology of cannabinoid content analysis by SFC will be shown. A variety of commercially available hemp-based products were purchased and analyzed using Waters ACQUITY UPC^2 System. The results were compared against label claim (where available), and statistical data is presented.

Poster #9

Chiral Sub-2 µm Particle Columns Applicated in Bioanalytical SFC-MS/MS Separating Propranolol's Phase-1 Metabolites

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Chiral recognition of drugs and their metabolites plays an important role for comprehension of pharmacodynamic effects and pharmacokinetic behaviour of chiral biological active compounds.

The β-blocker propranolol undergoes extensive phase-1- and phase-2-metabolism. R-Propranolol is preferably metabolised in human phase-1-metabolism and thus exhibits a shorter half-life [1], while S-propranolol is about 100-fold more active than R-propranolol. The metabolite 4'-hydroxypropranolol is also known to possess pharmaceutical activity. According to literature, at least CYP2D6, CYP1A2, CYP2C19 are involved in catalysing ring oxidation, side chain dealkylation and further side chain oxidation [2]. Early reports mention differences in metabolic conversion of the two enantiomers. Thus reasonably, published by the FDA and EMA in 1992 and 1993, respectively, guidelines shall ensure to consider each enantiomer of pharmaceuticals as single active compound.

This poster provides details of enantioseparation of propranolol, 4'-hydroxypropranolol, 5'-hydroxypropranolol, 7'-hydroxypropranolol, N-desisopropylpropranolol and 2-hydroxy-3-naphthalen-1-yloxypropanoic acid.
4'-, 5'-, 7'-Hydroxymetabolites, N-desisopropylpropranolol and 2-hydroxy-3-naphthalen-1-yloxypropanoic acid were used as reference substances. For proof of concept biological samples were analysed and containing phase-1-metabolites were successfully chirally discriminated.
Analyses were carried out on an Agilent 1260 SFC System coupled to an Agilent Triple Quadrupole 6495. As chiral stationary phase sub-2 µm tris-(3,5-dimethylphenyl) carbamylated cellulose, namely Chiralpak IB-U (100 mm x 3 mm I.D, 1.6 µm), were utilized. Furthermore, a chiral guard cartridge was coupled in series to broaden the spectrum of chirally separated analytes. Detailed SFC-system settings and method parameters are presented and discussed.

Chiral SFC was found applicable for fast enantiodiscrimination of diverse types of analytes within minutes or even seconds in biological analysis [3]. Highly sensitive mass detectors, easily hyphenated to SFC, enable high resolution mass detection for trace analysis in metabolism studies and therapeutic drug monitoring as well as doping control or forensic investigations.

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Poster #10

Effect of different make-up solvent compositions on ionization in supercritical fluid chromatography-mass spectrometry

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The interest in ultra-high performance supercritical fluid chromatography (UHPSFC), especially in its hyphenation with mass spectrometry (MS), is constantly growing. Electrospray ionization is the preferable option for analysis of a wide range of analytes, usually coupled to UHPSFC via pre-back pressure regulator splitting interface. The addition of make-up solvent is commonly used to support the ionization. However, the effect of various make-up solvent compositions on ionization of compounds with different physicochemical properties has not been thoroughly studied so far. The aim of this study was the optimization of parameters of SFC-MS hyphenation and the exploration of the effect of composition of the mobile phase and the make-up solvent on ionization and MS response.

Seventy compounds covering wide range of physicochemical properties including molecular weight from 126.72 to 759.94, logP -2.22 – 10.96, and pKa 0.52 – 17.59 were analyzed to investigate this phenomenon using UHPSFC-MS system in both ionization modes. CO_2-based mobile phase was modified with (i) methanol and (ii) 10 mmol/L ammonia in methanol. The effect of 45 make-up solvents including ammonium hydroxide, ammonium formate, ammonium acetate, formic acid, acetic acid, citric acid, and water in various concentrations was tested using both mobile phases and 3 stationary phases including Torus Diol, Viridis HSS C18 SB, and BEH 2-ethylpyridine. Not only that this enabled the elution of all compounds, but also the comparison of the behavior of the same compounds on different stationary phases when the only variable changed was the retention time. The single quadrupole mass spectrometry was used for the first part of the study. In the next step, triple quadrupole mass spectrometry Xevo TQ-XS will be applied to compare the role of make-up solvent composition on different MS platforms.

The study was supported by the Project of Specific Research, SVV No. 260412, GA UK project No. 1828218, and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000465) co-funded by ERDF.

Poster #11

Carbon dioxide expanded liquid as a powerful solvent for the extraction of quercetin from quince fruit

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Supercritical fluid extraction (SFE) is an approach commonly used for the extraction of nonpolar active compounds from various matrices. To change polarity, mass transfer, extraction power, and analytes solubility, the organic solvent is very often added to non-polar CO_2. Depending on the amount of added organic solvent, SFE, SFE with modifier, CO_2 expanded liquid extraction (CO_2-XLE), and pressurized liquid extraction are recognized. The neat CO_2 is not an optimal choice to extract polar compound, thus CO_2-XL in mixture with organic solvent such as methanol, ethanol, isopropanol, is very often used as an extraction solvent in this case. To improve the extraction recovery, the parameters including temperature, pressure, and type of extraction could be also tuned.

In this study, we optimized the method for the extraction of quercetin from quince fruit. At first, the effect of following parameters was tested using simple Placket Burman model. Ethanol with 0 – 20% water was chosen as a green organic solvent added to CO_2 in ratio 10 – 90 %. The temperature was tested in the range 30 – 80 °C, and pressure in the range 100 – 300 bar. After its evaluation, the water amount in ethanol was finally set up on 20% due to low effect of this parameter and for all other tested parameters, he tested ranges were narrowed. After the solvent, temperature, and pressure optimization by DoE, 4 different flow rates (1, 2, 3, and 4 mL/min) were explored to find the final extraction conditions.

Optimized method used CO_2/EtOH + 20% H_2O (10/90, v/v) at 66 °C, 223 bar, and 4 mL/min flow rate. In 30 min, it provided extraction of quercetin in 30 minutes with recoveries 120 ng with 22% RSD (n=9, 3 different days) per 0.5 g of sample.

This method will be used for the isolation of quercetin from different varieties of quince fruit to compare them in the term of quercetin level.

The study was supported by the project STARSS reg. no.: CZ.02.1.01/0.0/0.0/15_003/0000465 funded by ERDF.

Poster #12

A new strategy for improved method transfer and scale up in SFC

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A new and alternate strategy for retention matching due to the, usually unavoidable, varying pressure drop under the SFC run, will be presented. This strategy involves adjusting the mass fraction co-solvent in such a way that the apparent retention factor, obtained under varying conditions of local pressure/density in the column, exactly matches the retention factor of the reference system.

We developed a simple simulation software based on empirical retention modelling using data from twelve different solutes eluting under varying experimental conditions. With these models we can calculate the apparent retention factor for SFC systems using any amount of co-solvent, pressure and temperature. The simulations accounted as well for axial density and linear velocity gradients.

The pressure drop over the column can increase or decrease under a chromatographic run. The former can be the case, under method transfer in order to increase resolution in an analytical separation problem, for example scaling down (to smaller particles sizes) from SFC to UHPSFC. The is the case for a standard scale up of an analytical SFC system to a larger particle-size preparative system.

The strategy will be demonstrated by firm theory, but we also derive a rule of thumb to empirically utilize the strategy without the need for complex calculations. The new approach was shown to only marginally affect the selectivity and does not require any adjustments of the back-pressure.

Poster #13

Peak distortions in SFC due to the mobile phase composition

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The interest for SFC has increased in recent years thanks to development of new and improved instruments together with significant theoretical development allowing for more robust operation. However, SFC is still considerably more complex than LC, which we believe can limit its use.

In SFC, one of the most important factors to consider is the mobile phase, which usually contains pressurized CO_2 as main eluent and a polar co-solvent, added to enhance the elution strength. For polar solutes, often the case for charged solutes, the addition of an additive is also necessary to achieve acceptable performance. Due to the compressibility of the mobile phase the conditions inside the column will vary with pressure and temperature and even isocratic conditions experience gradients of volumetric flow rate, volumetric co-solvent fractions, density, temperature and pressure. To quantify these gradients, actual conditions inside the column needs to be understood.

The co-solvent fraction in the eluent generally has a large impact on solute retention. In addition, it may also adsorb to the stationary surface and compete with the solute for the available stationary phase space. Depending on the relative retention of the solutes and the perturbation peak of the co-solvent severe peak deformations may appear. Here, this deformation is going to be explained and demonstrated for several different commonly used co-solvents, on different columns and for several different compounds. For charged compounds as in LC other additives are also required to achieve acceptable peak shapes. Here we will demonstrate that this additive can also generates the above-mentioned peak deformations as well as complicated multilayer adsorption. This multilayer formation most probably a kosmotropic effect, this will in detail be explained. This effect is in accordance with the Hofmeister series or lyotropic series that amines are kosmotroptic and used for enhancing hydrophobic interactions, consider the protein salting-out using ammonium sulfate.

To top this off here the popular addition of water in the eluent will be discussed. We start off by considering the importance of elution mode. Here we will show why gradient elution is much more robust than isocratic elution. This will be demonstrate using the peptide Gramicidin separation with water added to the eluent. Here we will demonstrate using statistical modelling that water addition in the co-solvent is the most important factor for controlling the retention. In this case, it competes for the stationary phase surface. Controlling and understanding this is crucial knowledge for conducting method developed in a QC environment. Addition of water to the eluent is very complicated and, in some cases, it acts as a powerful additive but in some other co-solvent fractions result in HILIC-like retention mechanism. This phenomenon is dependent also on the selection of column material as will be demonstrated here.

Poster #14

Evaluation of Supercritical Fluid Chromatography for the analysis of Novel Psychoactive Substances (NPS) - regioisomeric phenidine derivatives

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A detailed evaluation of the use of Supercritical Fluid Chromatograph (SFC) for the analysis of 33 Novel Psychoactive Substances (NPS) based on phenidine is reported (see Figure 1). NPS designer drugs are analogues of controlled substances that are designed to produce effects similar to the controlled substances they mimic. The rate at which such substances are appearing poses significant issues for forensic laboratories with respect to identification and quantification, as validated analytical methods and reference standards are not usually available.

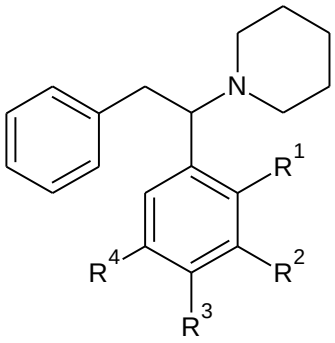

Figure 1 Structure of Diphenidine (R^1, R^2, R^3 and R^4 = H)

Though the supply and production of phenidine derivatives is now controlled in the United Kingdom by the Psychoactive Substances Act (2016), the global prevalence of these compounds still raises considerable legal and analytical challenges in the forensic identification of these materials. Phenidine derivatives have been implicated in a number of fatalities in Europe and are encountered in both tablet and powder forms. Analytical differentiation of regioisomers of NPS is a significant issue in forensic drug analysis, because, in most cases, legal controls are placed on only one or two of the conceivable NPS regioisomers and hence require a forensic scientist to show unequivocally that a sample

Poster #15

Separation of Highly Polar Compounds by SFC (EFLC) With Accurate-Mass Q-TOF LC/MS Detection and Database and Library Search Identification

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Screening of river water samples to get an overview of organic contaminants released to the environment has become increasingly popular. These screens typically use high-resolution liquid chromatography/mass spectrometry (HR-LC/MS). However, analysis by LC/MS is not easy because highly polar analytes do not show good retention on reversed phase columns and therefore often coelute with inorganic salts, which leads to ion suppression and insufficient limits of detection.

Typical SFC usage follows a rule of thumb that doubling modifier halves retention. This suggests decreased benefit from increases in modifier composition and emotionally limits modifier to 55-60% of mobile phase composition despite ever increasing solvent strength. Nonetheless, metabolomic studies have shown highly polar or even ionic compounds may require modifier concentrations above 90%. Such high modifier techniques fall in the realm of enhanced fluidity liquid chromatography (EFLC).

This work demonstrates separating highly polar compounds on different columns by modifier gradients continuing up to 95%. A test mix of 46 highly polar compounds is separated and the effluent was then analyzed with an accurate-mass Q-TOF LC/MS ionized by an Agilent Jet Steam source. The identification was performed by database search based on accurate mass. Finally, a spiked river water sample is analyzed directly after dilution with modifier.

Poster #16

Rapid Analysis of Plants-Derived Glucosylceramides and Steryl Glucosides under Supercritical Fluid Chromatography Condition

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Supercritical fluid chromatography (SFC) is increasing in use in the analytical and preparative separation field due to the high-throughput advantages together with ecological aspects.

Glucosylceramides (GlcCer) are major sphingolipid, which can be found in variety of plants, such as konjac, soybean, corn (maize), wheat, rice, and so on. Oral intake of GlcCer is known to be effective for improving skin barrier function [1]. When extracting GlcCer from plants, steryl glucosides (SGs) are often included as impurities, which are difficult to isolate. Therefore, SGs are often found in GlcCer containing botanical extracts. The ingredients of GlcCer are often identified in high performance liquid chromatography (HPLC) condition by using octadecyl group bonded silica gel column. However, the peak of GlcCer derived from various plants especially from konjac (kGlcCer) often co-eluted with SGs in such HPLC condition, which makes it difficult to qualitatively identify.

This study was directed to the separation of kGlcCer and SGs in SFC condition. We compared the chromatographic performance of various commercially available SFC columns. When DAICEL DCpak PBT® column [2] was used, SG eluted later than kGlcCer, and their peaks were totally separated. In addition, SFC technique enabled the high throughput analysis compared with conventional HPLC method because it took ca. 20–40 mins by using HPLC, which is ca. 4 to 10 times longer than SFC method. As in case of kGlcCer, the peaks of GlcCer extracted from various plants are totally separated with that of SGs. These results indicate that DCpak PBT column under SFC condition achieved rapid and qualitative analyses of various plants derived GlcCer.

References:

[1] K. Mukai, et al. *Jpn. Pharmacol Ther.* **2018**, *46*, 781–799.
[2] K. Nagai, et al. *J. Chromatogr. A* **2018**, *1549*, 85–92.

Poster #17

Minimizing Matrix Effects in SFE-SFC-MS

William Hedgepeth, Yuka Fujito, Shimadzu, USA

Matrix effects can have a significant impact on the measurement of analytes in mass spectrometry and are typically manifested as ion suppression. ESI and positive mode are more subject to this effect than APCI and negative mode. Matrix effects can be calculated by comparing recoveries of the target analyte with and without the sample matrix.

In this particular study, the analysis of vitamin D2 (Ergocalciferol) in dog food was studied. Dog food contains a large number of compounds including corn, various meals, fat, vitamins, minerals and food dyes. Vitamin D2 was found to suffer from a significant matrix effect when analyzed by SFE-SFC-MS/MS, providing recoveries that were approximately 5 per cent of a spiked sample that contained no dog food matrix. Various parameters including the use of an extraction modifier, inclusion of additional columns, and adjustment of SFC gradient strength were studied to determine if the matrix effect could be minimized. Results of these studies will be presented in the poster.

Poster #18

Practical applicability of sequential analysis of reverse phase liquid chromatography (RPLC) and supercritical fluid chromatography (SFC) with an LC/SFC switching system

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Today, RPLC is the most popular separation mode in HPLC, but, normal phase liquid chromatography (NPLC) is often chosen in the case of enantiomer separation. Nonetheless, RPLC may provide better separation, so it is ideal that considering both separation modes and choose one. If MS detection is required, e.g. bioanalysis, RPLC is chosen unqualifiedly due to the compatibility with MS.

CO_2 is used as the main solvent in the most case of SFC. It works as a less polar solvent, but also has compatibility with MS because of nonflammable. However, it is almost immiscible with H_2O. Consequently, unwanted existence of H_2O may cause not only peak shifting, deteriorated peak shape, but also hardware trouble. In the meantime, H_2O is commonly used in the most case of RPLC.

We have studied to do automatic switching between these two trade-off separation modes alternately within a single chromatograph. Here, we report its validity through the repeatability of retention time and accuracy of peak area.

At this time, we used methanol solution of trans-stilbene oxide in the same vessel for both of RPLC and SFC. For RPLC, the mobile phase was 10 mmol/L ammonium hydrogen carbonate in water : methanol and the column was CHIRALPAK IC-3 (3 um, 150 mm × 4.6 mm, Daicel). For SFC, the mobile phase was CO_2 : methanol and the column was CHIRALPAK IC-3/SFC (3 um, 50 mm × 4.6 mm, Daicel). The separation mode was switched 3 times automatically between RPLC and SFC.

Overlaid chromatograms are almost identical in each mode. This switching system meets a preliminary purpose of suggesting which separation mode is more preferable.

Poster #19

Analysis of Volatile Compounds by SFC/MS with Novel Polymer-Based Column

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The separation technique of both analytical and preparative scale for the volatile compounds (flavor, aroma, fragrance compounds) are in high demand due to their wide range of usage in various kind of industrial fields. SFC is capable of high speed, high peak capacity separation and also has an advantage in preparative purification due to mobile phase condition. However, there was no commercially available column which is suitable for the separation of volatile compounds in SFC because it is limited to use of silica-based column due to pressure, swelling and shrinkage tolerance issues This study demonstrated capabilities of SFC as separation technique for volatile compounds using highly cross-linking styrene divinylbenzene (SDVB) polymer-based column for SFC. In this study, 23 compounds with various types of functional groups were selected as target compounds to exhaustively evaluate the retention and separation behavior.

Newly developed SDVB column under the SFC mobile phase condition showed overall excellent and substantial improved retention ($k > 1.6$) than conventional silica-based columns ($k > 0.3$). In addition, it was also able to retain esters (hydroxy acetate, pentyl butylate, methyl salicylate) and non-polar terpenes (limonene, pinene) that did not show sufficient retention in any other commercially available silica-based columns (NH_2, C18, and phenyl columns). Aldehydes, which were not detected in the NH_2 column owing to adsorption, were also successfully detected. Retention and separation in the SDVB column are expected to occur based on the π-π interaction between the benzene rings at the surface of the substrate and the double bond or the aroma ring of the analytes. This separation mechanism is the same as that of the phenyl column, but the retention and separation was much better than that of phenyl column. In the SDVB column, the specific surface area of the gel is about 1.6 times of that of the phenyl column. It is suggested that the magnitude of the specific surface area of the SDVB column contribute to the retention. Moreover, hydrophobic interaction also likely occurs at the same time in the SDVB column since separation dependent on the alkyl chain length was observed, and it was more efficient than that in the C18 and phenyl columns. These results indicate that SFC is capable of multiple separation for a wide variety of volatile compounds by using polymer-based column, and applicable to separation techniques for both analytical and preparative scale.

Poster #20

Chiral Separation of the Germicide Diniconazole

Matthew Przybyciel, ES Industries, USA

Diniconazole is a broad spectrum triazole germicide used to prevent plant diseases such as rust and smut. Diniconazole is a chiral chemical with one chiral center and commercial Diniconazole sold as an enantiomer mixture of R, S Diniconazole enantiomers. It has reported that R (-) Diniconazole has higher bactericidal activity, while S (+) Diniconazole shows higher plant growth regulator activity. In many cases it is important to know if environmental factors have altered the ratio of R and S Diniconazole. SFC Chiral chromatography and mass spec detection has been used as a tool to study the different ratios of Diniconazole in various plant materials. For their study they used a ChromegaChiral CCA to resolve the two Diniconazole enantiomers – chromatogram for this separation is shown. The two enantiomers are baseline resolved, however they are closely separated. We have discovered that a better separation can be achieved by using a newly developed ChromegaChiral stationary phase for a high resolution separation of Diniconazole enantiomers.

Poster #21

SFC Isolation of THCA and CBDA from Cannabis using a New Developed Chromatography Column

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At ES Industries we have developed a new phase and SFC chromatography column optimized for SFC separations and isolation of Tetrahydrocannabinolic acid (THCA) and Cannabidiolic acid (CBDA) from Cannabis. This new column/phase - Chromega NP-III has similar separation characteristics to 2-Ethyl pyridine a stationary phase and column traditionally used for separation and isolation of THCA and CBDA, however Chromega NP-III is able to rapidly separate both THCA and CBDA using minimal amount of ethanol as modifier solvent for CO2 mobile phase used in SFC chromatography. Traditional 2-ethyl pyridine column require high levels of ethanol to obtain similar separations to the new Chromega NP-III column. For this presentation examples showing the superior separation performance of the new Chromega NP-III column will shown.

Poster #22

Separation and Quantitation of Seven Cannabinoids using SFC-MS/MS.

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Cannabis has been used as a medicinal plant since ancient times. A wide range of analytical methods have been published for both qualitative and quantitative analysis of cannabinoids in plants or biological matrices. Liquid chromatography (LC) and gas chromatography (GC) are the two common separation techniques. Only limited researches have been reported with supercritical fluid chromatography (SFC). SFC coupled to a triple quadrupole mass spectrometer can provide fast separation and sensitive detection. Herein, we describe the development of a fast SFC-MS/MS method for the analysis of seven cannabinoids: tetrahydrocannabivarin (THCV), (−)-trans-Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), cannabidiol (CBD), cannabigerol (CBG), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (11-COOH-THC) and cannabinol (CBN).

Poster #23

Chiral.cloud – a tool enabling the comparison of chiral columns enantioselectivity and chiral separations library exchange

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As the need for appropriate quality control methods enabling enantiomer excess determination is still growing, supercritical fluid chromatography (SFC) has established itself as an efficient method for chiral separations as well as for impurity profiling. Enantioselectivity of liquid chromatography (LC) and SFC methods differs due to different solvent interactions, but SFC mode usually offers increased separation efficiency and enantioselectivity. However, the unexplained mechanism of SFC chiral separations does not allow simple predictions of chromatographic behavior.

Nine selected chiral stationary phases based on tris(3,5-dimethylphenylcarbamate) derivatives of cellulose and amylose were evaluated in this study using 37 pairs of enantiomers. The evaluated parameters included asymmetry factor, peak width at half height, capacity factor, and resolution. Primary screening was carried out with ten different mobile phases

varying in modifiers and additives followed by the final evaluation using three best performing mobile phases. Apart from the evaluation, these data also served as initial data set for chiral separation library. Chiral separation libraries can be used to better estimate the separation of groups of drugs. The developing of machine learning algorithms or deep neural networks to predict enantioseparations is becoming state-of-the art approach due to more accurate predictions. Large set of data is necessary for development of such methods, and they are usually not publicly available. Moreover, these methods do not explain the mechanism behind the prediction, since they are operating as "black box" where the input is introduced by the operator and the algorithms delivers the results. Another disadvantage is caused by batch reproducibility and column equivalency which are relatively common problems of the column chemistry.

Chiral.cloud aims to gather more information about various columns and their batches and to correlate them. This enables to obtain separation data allowing the comparison of columns selectivity and relative success to separate particular enantiomer. Subsequently, this can facilitate faster chiral method development. The algorithm modeling the predictions for large variability of molecules will get more precise with higher amount of processed data. Therefore, chiral.cloud is based on exchange principle which allows users to upload chosen molecule separation in exchange for the separation data for another molecule.

Keywords: chiral screening; column equivalence; supercritical fluid chromatography; polysaccharide enantioselective stationary phases; column evaluation

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Poster #24

Multivariate approach to on-line supercritical fluid extraction – supercritical fluid chromatography - mass spectrometry method development

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On-line supercritical fluid extraction (SFE) - supercritical fluid chromatography (SFC) – mass spectrometry (MS) allows for analyte extraction, separation, and detection in a single flow path, offering a significant decrease in total analysis time. Although rapid extraction and separation can be achieved, limited examples for the use of SFE-SFC-MS for quantitative analysis have been reported. This is likely due to the inherent complexity of the system. Here, we systematically evaluated user-definable variables in the system by response surface methodology (RSM) to establish a basis for method development in determining analytes of varying polarity and classes across sample matrices of varying retentivity to elevate this platform to a more user-friendly and accessible technology.

Silica, amino, and C18 stationary phases were used to mimic the interaction properties of various sample matrices. Initially, a two-level half factorial design was used to screen for the most influential factors and their interactions in on-line SFE-SFC-MS for each 'manufactured' matrix. Factors of interest include extraction pressure, extraction temperature,

static and dynamic extraction times, co-modifier concentration, and flow rate. Significant factors were further optimized by Central Composite Design.

In each matrix, the chromatographic efficiency, peak area, and peak width were monitored for analytes of varying retention time, Log P, pKa, and monoisotopic mass. Using the statistical analysis software, DesignExpert 11, more than 250 models or equations were generated for the experiments performed. Based on the models generated for each monitored response in each matrix, assumptions were made as to which extraction parameters required further optimization by RSM. Matrix mimics were verified using real sample matrices. From the models, general conclusions were also made that the mimic and real samples were comparable although not identical. The most comparable models were found for amino-functionalized silica as a representative of protein powder. The observed significant differences among the mimics versus the real samples may be a reflection of matrix interferences in the real samples. Overall, modifier concentration, extraction pressure, and dynamic extraction time had the most significant effect on measured responses. For the silica phase and amino phase, flow rate had added influence on responses, while static extraction time influenced response in C18. Extraction temperature had no significant effect. Based on the results of the factor screening, a Central Composite Design was performed to optimize the factors of significance in each matrix. The suggested optimal extraction parameters were confirmed for each of the analytes (n=3). Peak areas ranged from 53000 – 55000000 with RSD 2 – 14%. Peak widths ranged 0.030 – 0.240 min with RSD < 1 – 4%. The resulting methods will provide researchers with the basic tools needed to effectively develop robust methods for on-line SFE-SFC-MS for the extraction, separation, detection, and quantification of analytes in a single analysis for multiple applications in different complex matrices.