Evaluation of SFC-MS Configurations for the Analysis of Lipids, Sterols, and PAHs

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Introduction

Supercritical fluid chromatography (SFC) is a powerful separation technique, and especially for the separation of apolar compounds, it offers an interesting alternative to normal phase HPLC. Most SFC work has been performed using UV detection, although the coupling to mass spectroscopic detection can largely extend SFC applicability.

Initial experiments with the Agilent 1260 Infinity SFC/MS sytem showed that the hyphenation of SFC with MS results in high sensitivity and good mass spectral quality due to the fact that the major component of the mobile phase is CO_2, which evaporates upon entering the source, allowing for analysis at high flow rates. However, the carbon dioxide decompression and expansion after the backpressure regulator (BPR), used in all commercially available instruments, also causes cooling of the transfer line to the MS, which can result in solute deposition, baseline noise and irreproducible results.

For hyphenation of SFC with MS, different configurations can be used, including effluent splitting before or after the BPR [1]. Effluent splitting obviously reduces sensitivity and alternatives were studied. Best results in term of sensitivity, baseline stability, retention time and peak area repeatability were obtained using a configuration whereby a small (typically 0.2 mL/min) make-up flow is added before the BPR [2]. The total effluent is introduced in the MS (both ESI and APCI were tested). It was also found that heating of a part of the transfer line, just before the ionization source entrance, was needed to obtain optimal peak shape and sensitivity.

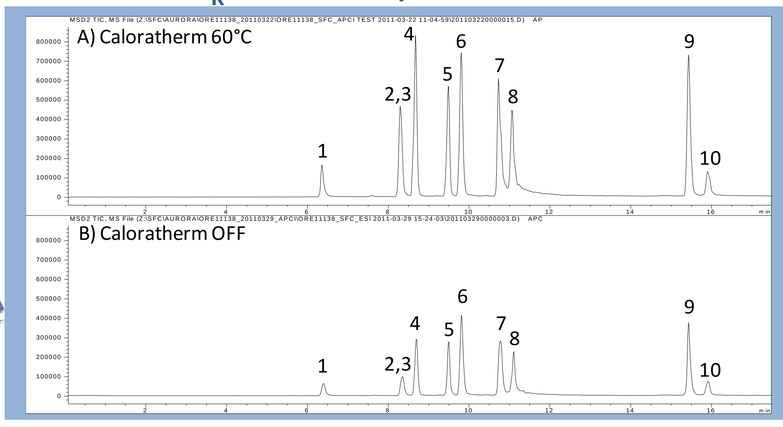
This SFC-MS configuration was used to analyze several different types of apolar solutes, including lipids, sterols, and polycyclic aromatic hydrocarbons (PAHs). These compounds are notoriously difficult to detect using LC-MS, and comparisons were made between the two techniques. In all cases, similar separation and MS conditions were used.

1 System Configuration and Initial Testing

Figure 1. SFC-MS Configuration with the Agilent 1260 Infinity analytical SFC system and the Agilent G6130A single Quadrupole MS. *Caloratherm from SST.*

Initial testing performed using 10 compound test mixture: theobromine (1), theophylline (2), cortisone (3), prednisone (4), hydrocortisone (5), prednisolone (6), suflaquinoxaline (7), sulfamerazine (8), sulfaguanidine (9), cytosine (10)

- MS scan 10X more sensitive than UV
- **MS: t_R RSD < 0.3%, Raw Peak Area in Scan Mode RSD ≤ 10%**



Heating prior to MS inlet important!

- *Freezing can occur without heating.*
- *Better MS reproducibility than without Caloratherm heating device.*

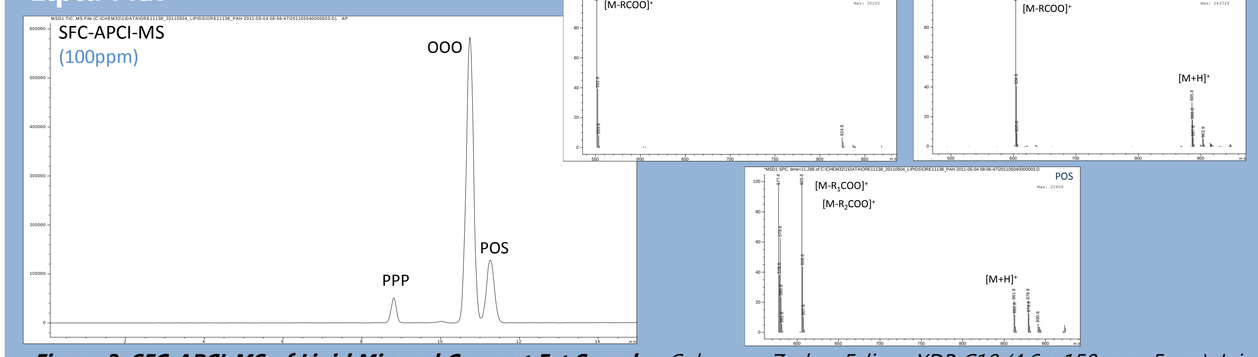
Figure 2. SFC-APCI-MS with and without Caloratherm heating.
Column = Zorbax RX-SIL (4.6 x 250 mm, 5 µm), Injection = 5 µL, Flow Rate = 2.0 mL/min, Outlet P = 120 bar, SF = CO_2, Modifier = MeOH with 20 mM NH_4COOH w. 2%H_2O Gradient = 0-20 min: 5-40% modifier, Column Temp = 40°C, Caloratherm = 60°C, Make-up Flow = MeOH at 0.2mL/min, Detection = UV 254 nm and MS Scan 80-400 amu. MS = APCI, Capillary V = +3000V, Corona I = 4.0 µA, Drying Gas = 12.0 mL/min at 325°C, Nebulizer = 50psig, Vaporizer = 350°C.

2 Lipids and Coconut Fat

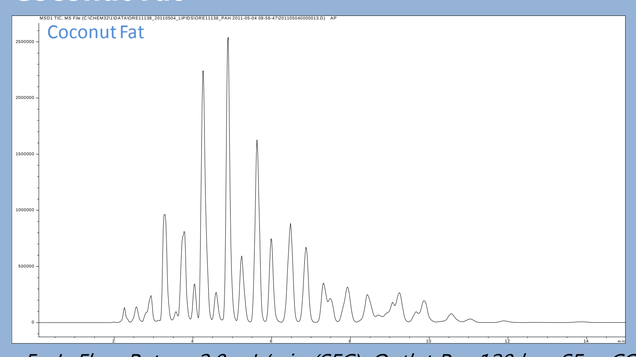
A lipid mixture containing PPP, OOO, and POS was separated using LC-MS and SFC-MS. The same MS settings were used in both cases.

- **SFC-MS separation of Coconut Fat gives excellent results**

Lipid Mix



Coconut Fat



Comments:

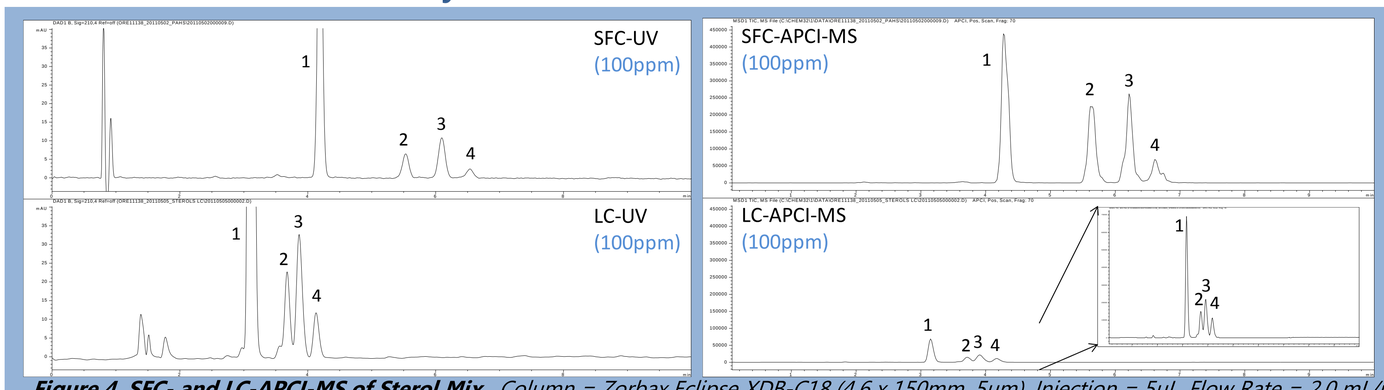
- *The lipid mix was unable to be detected using LC-MS (Data not shown)*
- *Separation power of SFC provided excellent separation of Coconut Fat sample*
- *Spectral quality is good, with the predominant ion of $[M-RCOO]^+$*

Figure 3. SFC-APCI-MS of Lipid Mix and Coconut Fat Sample. *Column = Zorbax Eclipse XDB C18 (4.6 x 150 mm, 5 µm), Injection = 5 µL, Flow Rate = 2.0 mL/min (SFC), Outlet P = 120 bar, SF = CO_2, Modifier =MeOH w. 2% H_2O, Gradient = 0-15 min: 5-50%, Temp = 40°C, Caloratherm = 60°C, Make-up = MeOH at 0.2mL/min. MS = scan 450-1000amu Capillary V = +3000V, Corona I = 4.0 µA, Drying Gas = 10mL/min at 325°C, Nebulizer = 50 psig, Vaporizer = 350°C.*

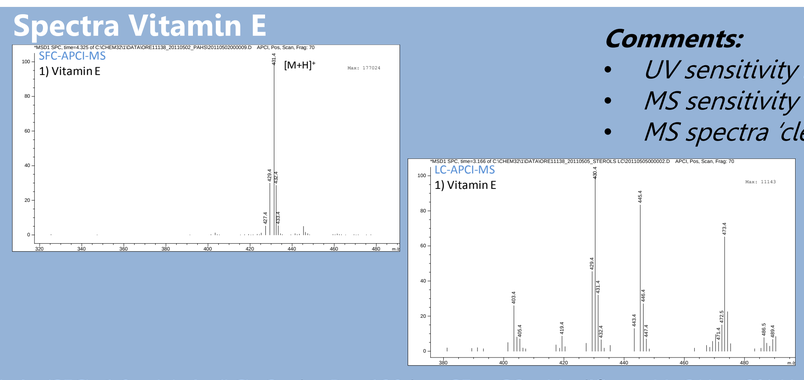
3 Sterols

A sterol mixture [vitamin E (1), cholesterol (2), stigmasterol (3), and β-sitosterol (4)] was separated using LC-APCI-MS and SFC-APCI-MS. The same MS settings were used in both cases.

- **Similar UV sensitivity obtained; however, SFC-MS much more sensitive than LC-MS**



Spectra Vitamin E



Comments:

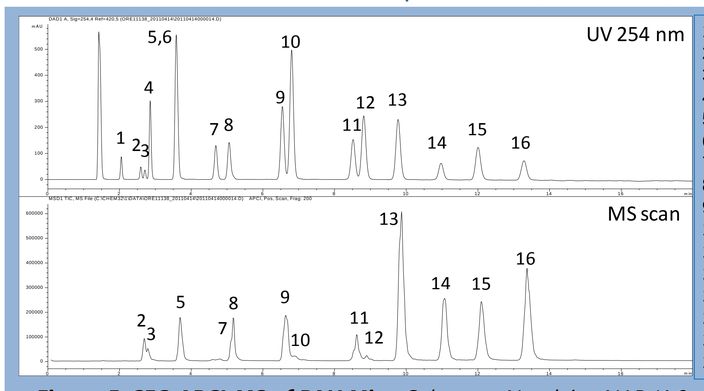
- *UV sensitivity similar between LC and SFC*
- *MS sensitivity much better with SFC-MS*
- *MS spectra 'cleaner' with SFC-MS*

Figure 4. SFC- and LC-APCI-MS of Sterol Mix. *Column = Zorbax Eclipse XDB-C18 (4.6 x 150mm, 5µm), Injection = 5µL, Flow Rate = 2.0 mL/min (SFC), 1.0 mL/min (LC), Outlet P = 120 bar, SF = CO_2, Modifier = MeOH w. 2% H_2O (Isocratic at 5%), MP= ACN/Isopropanol (5:4) isocratic, Temp = 40°C (SFC), 50°C (LC), Caloratherm = 60°C (SFC), Make-up = MeOH @ 0.2mL/min (SFC). MS = scan 350-500amu., Cap V = +3000V, Corona I = 4.0 µA, Drying Gas = 12 mL/min @325°C, Neb = 50 psig, Vap = 350°C.*

4 PAHs

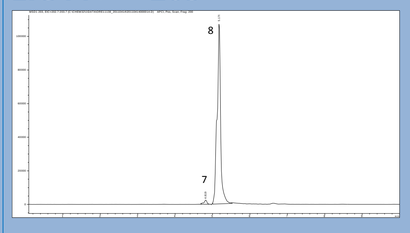
A PAH mixture containing 16 PAHs at 100ppm was separated using SFC-APCI-MS.

- Do not observe naphthalene, fluorene, or phenanthrene in MS (UV only.)

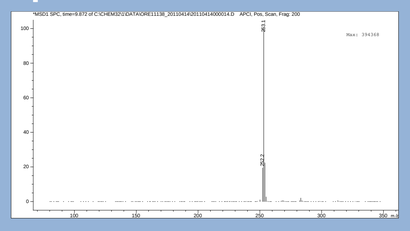


1	Naphthalene (128 g/mol)
2	Acenaphthylene (152 g/mol)
3	Acenaphthene (154 g/mol)
4	Fluorene (166 g/mol)
5	Anthracene (178 g/mol)
6	Phenanthrene (178 g/mol)
7	Fluoranthene (202 g/mol)
8	Pyrene (202 g/mol)
9	Benzo(a)anthracene (228 g/mol)
10	Chrysene (228 g/mol)
11	Benzo(k)fluoranthene (252 g/mol)
12	Benzo(b)fluoranthene (252 g/mol)
13	Benzo (a)pyrene (252 g/mol)
14	Dibenzo(a,h)anthracene (278 g/mol)
15	Indeno(1,2,3-cd)pyrene (276 g/mol)
16	Benzo(g,h,i,)perylene (276 g/mol)

EIC M+ = 203



Spectra Peak 13



Comments:

- *LC-APCI-MS LOD > 5 µg (No data shown)*
- *Similar UV response for Peaks 7 and 8 in SFC-DAD*
- *MS responses different (See EIC), probably due to structural differences*
- *Excellent spectral quality*
- *M+1 ion obtained for all PAHs*

Figure 5. SFC-APCI-MS of PAH Mix.. *Column = Nacalai π-NAP (4.6 x 150mm, 5µm), Injection = 5µL, Flow Rate = 2.0 mL/min, Outlet P = 120 bar, SF = CO_2, Modifier = MeOH w. 2% H_2O, Gradient = 0 -20 min: 5-30%, Temp = 40°C, Caloratherm = 60°C, Make-up = MeOH @ 0.2 mL/min. MS = scan 80-350 amu, Cap V = +3000V, Corona I = 4.0 µA, Drying Gas = 7.5 mL/min @ 325°C, Neb = 50 psig, Vap = 450°C.*

[1] Aurigemma and FarrellJ. Chromatogr A. 1217 (2010) 6110-6114
[2] Agilent Technical Note [5990-7972EN]

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