



Supercritical Fluid Extraction & Chromatography (SFE-SFC)

The Critical Promise for a Greener Tomorrow

November 2nd, 2021

Introductions



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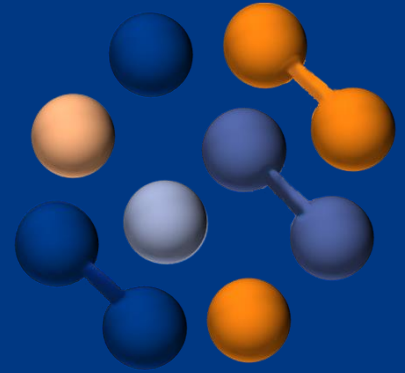
Richard Sanders

Eurofins US Food Laboratory Network
National Business Development Director

Conflicts of Interest & Transparency Statements

- We are not endorsing any specific instrument company(s) over another
- We are not representing any government, industry, and academia groups other than the Eurofins Laboratory Network
- We love food & drink, take supplements and care about the planet. Testing for life is a real thing.

Who is Eurofins Scientific



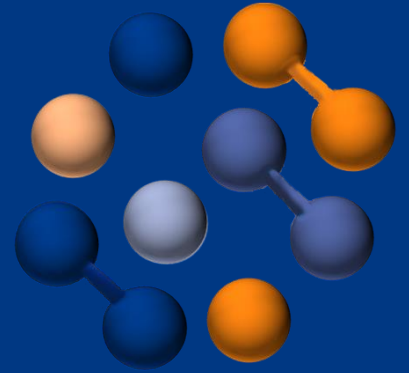
In 1987, Eurofins was founded when Gilles Martin, the current Group CEO, purchased the rights to the ground-breaking testing technology SNIF-NMR® from the University of Nantes. It uses Nuclear Magnetic Resonance to detect whether sugar has been added during the wine-making process to increase the alcohol content. Certain such additions were not detectable by any other existing method.

In the following years, the SNIF-NMR® technology applications were broadened to a wider range of products than the original wine test, such as fruit juices, natural flavours and other non-alcoholic beverages, picking up sophisticated frauds that traditional testing methods failed to detect.

Now, over 30 years later, building on its research and innovation roots since its foundation, Eurofins has continue to develop many new testing technologies that have a positive impact on day-to-day life.

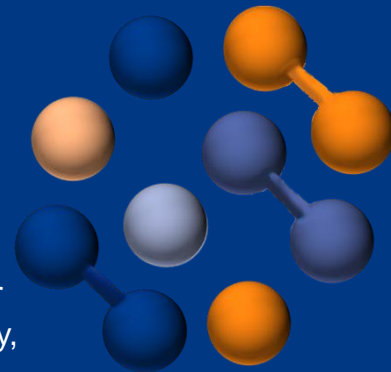
Today the Global Eurofins Group is a leading provider of analytical services

- 900 Laboratories
- 50 Countries
- 55,000 Staff
- 200,000 Analytical methods
- 10.8 million sq ft of laboratories
- 450 million tests performed each year to evaluate the safety, identity, composition, authenticity, origin, traceability, purity of biological substances and products, and clinical diagnostic services.





<https://www.eurofins.com/about-us/>



OUR VISION (*Our long-term aspiration*): To be the Global Leader in Testing for Life.

OUR MISSION (*Why we are here - the cause/purpose of our business*): To contribute to a safer and healthier world by providing our customers with innovative and high quality laboratory, research and advisory services whilst creating opportunities for our employees and generating sustainable shareholder value.

OUR VALUES (*What we stand for/what is important for us*): Customer Services, Competence and Team Spirit, Quality and Integrity

OUR SUSTAINABILITY PROMISE (*How we approach corporate sustainability*): As an organization, embedding corporate sustainability principles in our business strategy and the way we operate is an essential component in both creating long-term value and achieving our mission of contributing to a healthier and safer world.

FAST TECH

- Introduction to Super Critical Fluid (SF)
- SFC, GC, HPLC A Brief History
- A Name To Remember

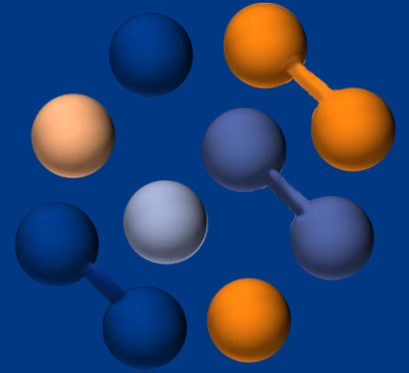
BETTER TECH

- Green Chemistry
- Method Overview
- Poster Session



UNCOMPROMISING TECH

- Development Guidelines & Accreditations
- How my samples compare
- Codes To Enter

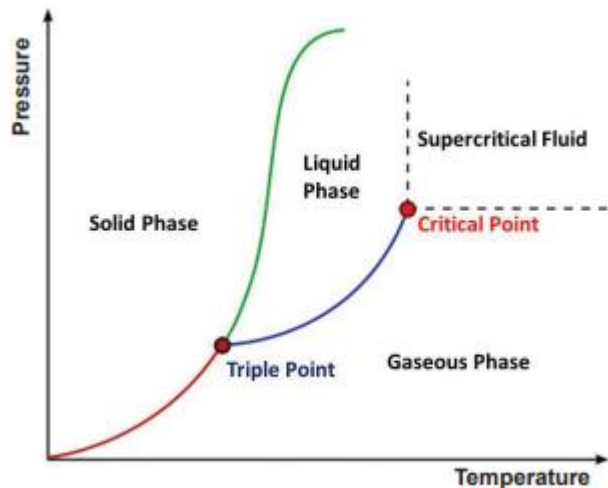


Part 1

FAST TECH

What Do These Three Have In Common





	Rate of Diffusion (cm ² /s)	Density (g/cm ³)	Viscosity (g/cm · s)
Gas	10 ⁻¹	10 ⁻³	10 ⁻⁴
Supercritical Fluid	10 ⁻⁴ – 10 ⁻³ Liquid Like	0.2-0.8 Liquid Like	10 ⁻⁴ Gas Like
Liquid	10 ⁻⁵ - 10 ⁻⁶	1	10 ⁻²

High diffusivity and density
with low viscosity

=

Faster flow rates
with lower pressure

Dissolve better, move faster with less pressure concern

HOURS TURNED INTO MINUTES

Compound	T _c (C)	P _c (Mpa)
NH ₃	132	11.28
CO ₂	31	7.38
N ₂ O	36	7.24
H ₂ O	374	22.06
Propane (C ₃ H ₈)	97	4.25
Hexane (C ₆ H ₁₄)	234	2.97

CO₂ Is The Choice

Low T_c and P_c
Non toxic
Non flammable
Abundant Supply
Easy Removal
GRAS

A brief history of the unifying chromatography

The late 1960's was the dawn of commercially available chromatography units. Three main systems were being discussed in the international academic world. Each system was characterized by the mobile phase of the system.

- Gas Mobile Phase Chromatography
- Supercritical Fluid State Mobile Phase Chrom
- Liquid Mobile Phase Chromatography



Where the chromatography family split

- GC 1967 First commercially available integrated system
- SFC 1967-1970 Delays due to factor error, first generation engineering issues
- HPLC 1968 First commercially available integrated system



*Also making its debut in 1967 – the Science Tricorder
Coincidence Probably not.....*

James Lovelock - Amazon Rainforest - Supercritical Thought



Lovelock's GC Went to Mars on Viking 2 Mission



Lovelock's Gaia hypothesis – where Earth is described as a self-regulating system

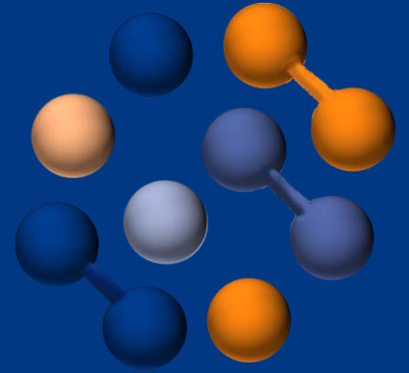
It's Easy Being Green




Lovelock first theorized using CO₂ as a supercritical chromatographic mobile phase in 1958

Part 2

BETTER TECH





GREEN CHEMISTRY
A U.S. EPA Program

www.epa.gov/greenchemistry

EPA
United States
Environmental Protection
Agency

The 12 Principles of Green Chemistry

- Prevent Waste
- Design Safer Chemicals and Products
- Design Less Hazardous Chemical Syntheses
- Use Safer Solvents/ Reaction Conditions
- Increase Energy Efficiency
- Use Renewable Feedstocks
- Design Chemicals and Products that Degrade After Use
- Minimize the Potential for Accidents
- Analyze in Real Time to Prevent Pollution
- Use Catalysts, Not Stoichiometric Reagents
- Maximize Atom Economy
- Avoid Chemical Derivatives

U.S. EPA
Green Chemistry Program
Washington, DC 20460

www.epa.gov/greenchemistry
email: greenchemistry@epa.gov

SFC using CO₂ meets many of the EPA Green Chemistry objectives

Non toxic
Non flammable
Abundant Supply
Easy Removal
GRAS
95 % - 98% Less Solvents*
Reduced Exposure*

*Dichloromethane, chloroform, hexane, DMSO, conc. KOH



OUR MISSION

Our international gatherings promote environmentally sustainable chemical R&D by bringing together thought leaders to present the latest research and industrial accomplishments in SFC and SFE.

Standard sample preparation steps



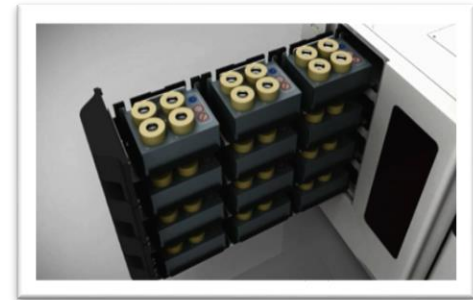
SFE sample preparation



Significant reduction in analyte degradation elements due to minimized exposure to air, light, reagents, heat, transfer steps etc.

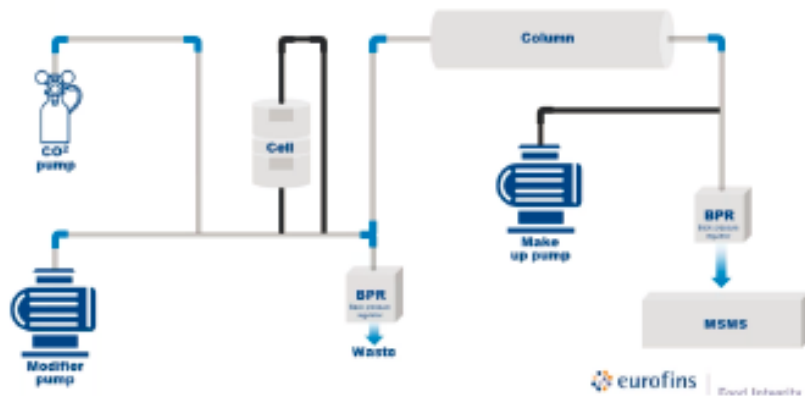
References: Photo comparison of sample preparation and Nexera UC sample preparation from Supercritical Fluid Extraction/Chromatograph System brochure

- Collect an aliquot of finely ground / prepared sample
- Add water, stable isotope internal standard and alcohol
- Combine the slurry with a water absorbing material
- Transfer to an extraction cell, load on instrument for analysis



Shimadzu Nexera UC (SFE/SFC) System

- CO₂ pump
- Quadratic modifier pump
- Isocratic makeup pump
- Extractor
- Column oven
- 2 Back pressure regulators



AB Sciex 6500 plus MSMS with APCI source

First Wave of Matrices and Analytes

Infant Formula Powder

Supplements

Tablets

Liquids

Powders

Soft-gels



Vitamin A Acetate + cis A Acetate

Vitamin A Palmitate + cis A Palmitate

Vitamin E (alpha Tocopherol)

Vitamin E Acetate

Vitamin E Succinate

Vitamin D2 + pre D2

Vitamin D3 + pre D3

Vitamin K1

Vitamin K2-MK4

Vitamin K2-MK7

Table 1. Result Comparison of a Vitamin Gel Capsule

Analysis Method	HPLC-FLD	SFE-SFC-MSMS, i-Amylose-1 Column	SFE-SFC-MSMS, 1-AA Column
n	20	11	12
Trans Vitamin K2-MK7 Average Result (mcg/g)	245	252	244
RSD	7.4	7.4	7.4

Table 2. Analysis of Locally Purchased Supplements, n=3 for Each Test

Sample	Total Vitamin K2-MK7 (<i>cis+trans</i>) (mcg/g)		Trans Vitamin K2-MK7 (mcg/g)	
	X	RSD	X	RSD
Chewable tablet	18.7	1.0	0.433	5.2
Capsule 1	55.4	4.5	2.16	3.2
Capsule 2	42.7	3.9	1.73	2.9

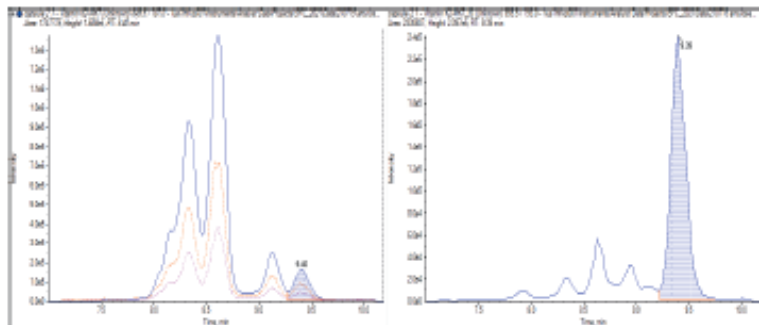


Figure 3. Analysis of trans vitamin K2-MK7 in a vitamin capsule by SFE-SFC-MSMS. Lux i-Amylose-1 column. Internal standard on right.

Detection and quantitation are performed by MSMS with an APCI source. In the workflow of this method, the sample is initially analyzed using the Torus 1-AA column for vitamin K1, K2-MK4, K2-MK7, D2 and D3. The presence of vitamin K2-MK7 *cis* isomers are readily seen. The sample is then reanalyzed with the Lux-i-Amylose-1 column to resolve the vitamin K2-MK7 *trans* isomer from the *cis* isomers.

Table 1. Analysis of CRMs by SFE-SFC-MSMS

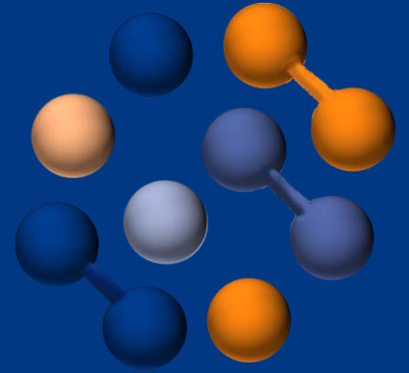
CRM	Vitamin	N	Average Result (mcg/g)	NIST range (mcg/g)
NIST 1869	E Acetate	16	183	157-191
	E	16	63.2	50.6-61.2
	A Acetate	12	12.3	9.8-12.4
	A Palmitate	12	17.3	14.2-20
	K1	12	1.33	1.04-1.40
NIST 3280	E Acetate	12	19500	17900-24900
	D2	12	6.40	6-11.2
	K1	12	21.1	20.6-25

Table 2. Comparison of SFE-SFC-MSMS Method Results to Official Methods

Sample Type	Vitamin	SFE-SFC-MSMS Average Result (mcg/g)	SFE-SFC-MSMS RSD	Official Method Average Result (mcg/g)	Official Method RSD	% of Official Method Result
Vitamin Capsule	E Succinate	35200	3.5	36200	2.6	97.4
	A Acetate	1560	4.9	1620	2.8	95.9
Breakfast Cereal	E+E Acetate	1220	3.8	1230	3.0	99.0
	A Palmitate	12.7	3.5	12.9	3.5	98.3
Vitamin Softgel	K1	3140	3.9	3350	5.4	93.7
	K2-MK4	2380	5.7	2230	6.5	107
	K2-MK7	244	7.4	245	7.4	99.8
Vitamin Tablet	D3	115	4.7	108	6.3	106
Vitamin Softgel	D3	140	4.4	131	5.0	107
Vitamin Tablet	E Acetate	31100	3.2	32100	1.6	96.9
	A Acetate	936	5.4	943	5.7	99.3
Infant Formula	E Acetate	183	3.1	180	1.9	102
	E	63.2	4.3	55.9	1.4	113
	A Acetate	12.3	2.0	11.3	0.9	107
	A Palmitate	17.3	2.9	17.4	0.9	98.0
	K1	1.33	2.5	1.32	0.8	101

Part 3

UNCOMPRIMISING TECH



Eurofins Food Chemistry Testing (EFCT) followed AOAC International Guidelines for Standard Method Performance Requirements

(SMPRs®) are a unique and novel concept for the analytical methods community. SMPRs are voluntary consensus standards, developed by stakeholders, that prescribe the minimum analytical performance requirements for classes of analytical methods

ISO/IEC 17025 Accreditation

Mini validation

Product Method Transfer Validation

Custom Approach – Let's Have a Conversation



Analyte	SampleKinect NIMS Code	Eurofins On-Line (EOL) ComLIMS Code
Vitamin A	FAST_VITA	FS1BC
Vitamin D	FAST_VITD	FS1BJ
Vitamin E	FAST_VITE	FS1BK
Vitamin K	FAST_VITK	FS1BL





Thank You!

www.eurofinsUS.com/food

