Comprehensive non-targeted chemical fingerprinting of coffee silverskin extracts with MRMS

Coffee silverskin is a major coffee bean roasting by-product, which is currently underutilized and mainly discarded as industrial waste. However, silverskin is a rich source of polyphenols and other bioactive ingredients, and thus a potential feed-stock for pharmaceutical, cosmetics and food sectors.

Abstract

Coffee silverskin, a by-product of the coffee roasting process, contains a plethora of bioactive compounds, such as caffeine, lipids, chlorogenic acids, and melanoidins, which possess a considerable potential in several industrial applications. In this study, a comprehensive non-targeted chemical fingerprinting of solvent extracts of pelletized coffee silverskin residue was performed by using ultrahigh-resolution MRMS technology, giving access to the hundreds of chemical constituents, including organic acids, polyphenols, sugars, and nitrogen-containing heterocycles.

Keywords: coffee, silverskin, polyphenol, MRMS, metabolomics

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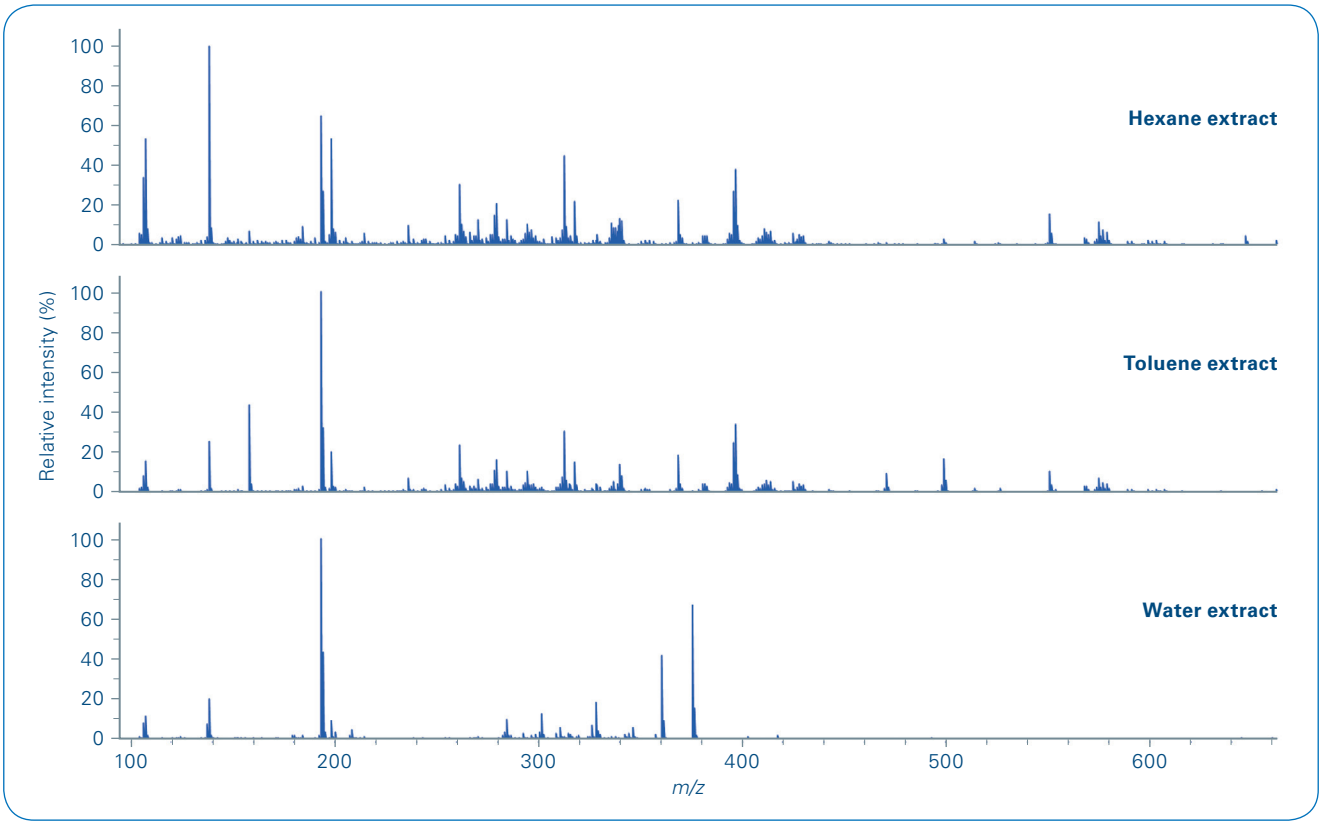


Figure 1: Selected APPI spectra in positive ion mode of coffee silverskin extracts using different extraction solvents.

Introduction

Coffee and other caffeinated drinks are the most consumed beverages in the world. The highest consumption per capita occurs in Finland, where about 10 kg of roasted coffee beans are consumed per person a year. Coffee is enjoyed mainly due to its taste, habit and its stimulating effect, caused mainly by caffeine, while it contains no essential nutrients. Moderate coffee consumption has also been associated with the reduced risk of neurodegenerative diseases, like Parkinson's disease, type II diabetes, and some types of cancer [1]. Coffee silverskin (CS) is the thin outermost layer of the coffee bean and the only by-product obtained from the coffee roasting process. Over 10 million tons of coffee is roasted globally every year [2], leading to the estimated CS production of about 200,000 tons. Despite the huge availability of this feedstock, its current utilization is very limited, and it is mostly used as a solid fuel or soil fertilizer. However, CS is a rich source of polyphenols, lipids, and other bioactive compounds, and thus its revalorization has gained more attention recently [3,4]. The most interesting compounds include chlorogenic acids (CGAs) and melanoidins, which could find use in pharmaceutical, cosmetics, food, and techno-chemical sectors [5-7]. Compounds from CS can be recovered by various methods such as hot water or solvent extraction. Due to the chemical complexity of CS extracts, rapid and sensitive analysis methods for their characterization are desired. In this work, we employed ultrahigh-resolution Magnetic Resonance Mass Spectrometry (MRMS) technology for comprehensive, non-targeted chemical fingerprinting of the solvent extracts of CS.

Materials and Methods

Solvent extraction

The coffee silverskin pellets, kindly provided by Meira roastery (Helsinki, Finland), were subjected to continuous Soxhlet extraction. The solvents used were chloroform, dichloromethane, hexane, toluene, acetone, acetonitrile, methanol, ethanol, and water (HPLC grade). Both, non-polar and polar solvents were used to assess their ability to extract different types of compounds from the silverskin pellets. Prior to the mass spectrometric analysis, the obtained extracts were further diluted with methanol (for negative-ion ESI) or a methanol/toluene mix (10:1, v/v) (for positive-ion APPI), to the approximate concentration of 50 - 100 µg/mL.

MS analysis

All mass spectrometric analyses were performed on a solarix 12T XR MRMS instrument (Bruker Daltonik GmbH, Bremen, Germany), equipped with a dynamically harmonized ICR cell (ParaCell). All samples were directly infused to an Apollo-II ESI and APPI ion source by using a syringe pump (flow rate of 6.7 µL/min for APPI and 2 µL/min for ESI). The ions were detected at the *m/z* range of 92 - 2000 with a mass resolving power of ~530,000 at *m/z* 300 (transient length 1.05 s). Two hundred single scans were co-added for each mass spectrum and processed in magnitude mode. The instrument was externally calibrated by sodium trifluoroacetate (NaTFA) clusters. Furthermore, internal mass recalibration was accomplished with a custom-made calibration list, containing known analytes from different compound classes. For the elemental formula search (SmartFormula), the following parameters were used: mass error ≤ 0.8 ppm; relative intensity ≥ 0.1%; signal-to-noise (S/N) ratio ≥ 5.0; H/C ratio ≤ 3; DBE ≤ 80; elemental formula $^{1}H_{1-200}{}^{12}C_{1-100}{}^{16}O_{1-25}{}^{14}N_{1-5}{}^{32}S_{1-2}$.

Data processing and structural annotations

The mass spectra were processed using DataAnalysis 5.0 (Bruker Daltonik GmbH, Bremen, Germany). Further analysis and structural annotations were accomplished by using MetaboScape 5.0 (Bruker Daltonik GmbH, Bremen, Germany) with CompoundCrawler database search engine.

MS analysis

Ultrahigh-resolution MRMS represents an unparalleled analysis tool for non-targeted chemical fingerprinting of natural extracts and other complex mixtures, giving access to hundreds or even thousands of analytes in a single measurement. When combined with different ionization techniques such as electrospray ionization (ESI) or atmospheric pressure photoionization (APPI), both polar and non-polar analytes can be detected.

Based on the data, all CS extracts were highly complex with up to ~4600 and ~2200 spectral features detected with ESI negative ion mode and APPI positive ion mode, respectively. Figure 1 shows selected APPI spectra for three different solvents. The most abundant compounds detected with APPI in positive ion mode included different acids, di- and triterpenoids, sterols, phenolic acids, and nitrogen heterocycles (Figure 2). The most abundant compound detected with APPI was caffeine ($C_8H_{10}N_4O_2$), which was present in both non-polar and polar

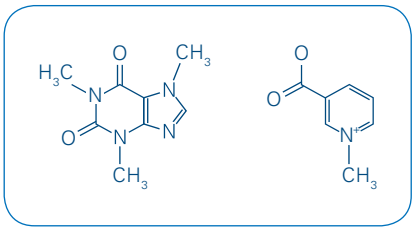


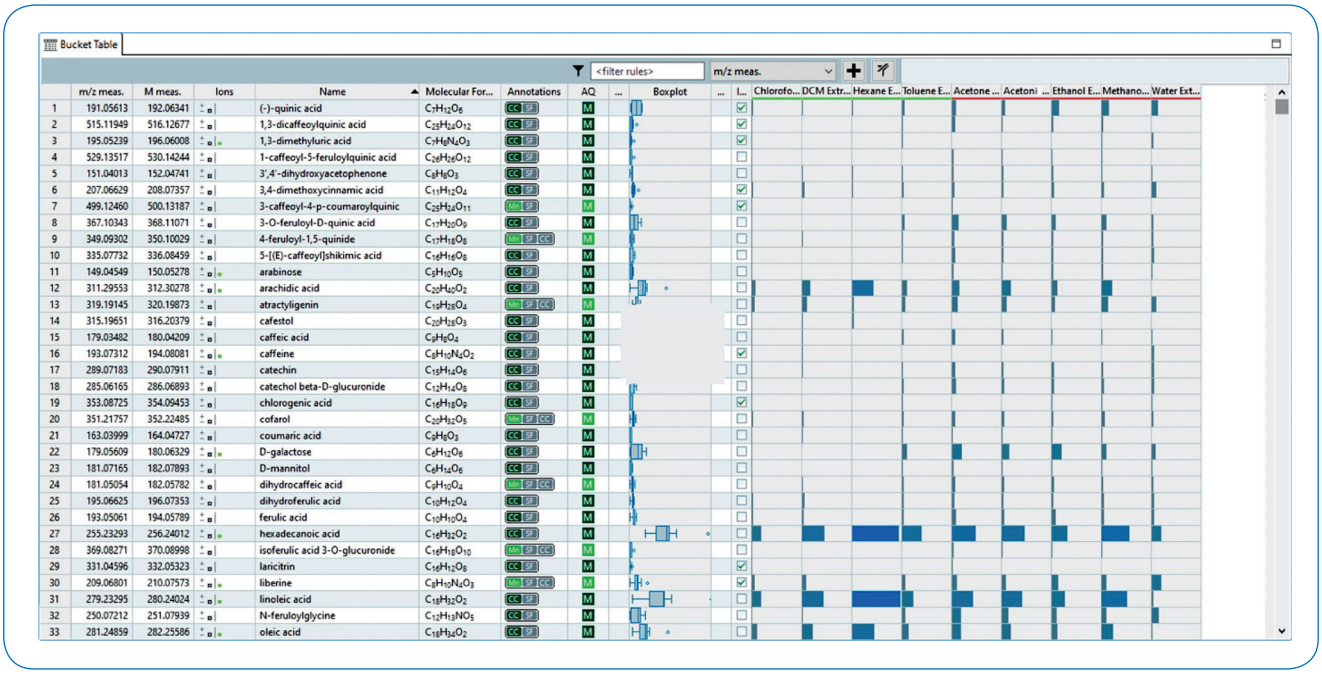
Figure 3: Example of nitrogen-containing alkaloids found in coffee silverskin extracts: caffeine (left), trigonelline (right).

Bucket Table

<filter rules> m/z meas.

	m/z meas.	M meas.	Ions	Name	Molecular For...	Annotations	AQ	...	Boxplot	...	I...	Chloroform	DCM_Coffe...	Toluene_Ext...	hEXANE_Co...	Ethanol_Cof...	Methanol_C...	Water_Coffe...	aCETONE_C...
1	330.21892	330.21947		16-O-methylcafestol	$C_{21}H_{30}O_3$	Mn Sf Cc	M												
2	399.36232	398.35505		24-methylenecholesterol	$C_{28}H_{46}O$	Mn Sf	M												
3	153.05462	152.04734		3',4'-dihydroxyacetophenone	$C_8H_8O_3$	Cc Sf	M												
4	208.07307	208.07361		3,4-dimethoxycinnamic acid	$C_{11}H_{12}O_4$	Cc Sf	M												
5	139.07528	138.06800		3-ethylcatechol	$C_8H_{10}O_2$	Cc Sf	M												
6	159.06784	159.06838		6-methoxyquinoline	$C_{10}H_9NO$	Cc Sf	M												
7	411.36212	410.35484		7-dehydrostigmasterol	$C_{29}H_{46}O$	Mn Sf Cc	M												
8	430.38057	430.38112		alpha-tocopherol	$C_{29}H_{50}O_2$	Cc Sf	M												
9	321.20610	320.19882		atractyligenin	$C_{19}H_{28}O_4$	Mn Sf Cc	M												
10	316.20336	316.20391		cafestol	$C_{20}H_{28}O_3$	Cc Sf	M												
11	554.43265	554.43320		cafestol palmitate	$C_{36}H_{58}O_4$	Mn Sf Cc	M												
12	181.04959	180.04232		caffeic acid	$C_9H_8O_4$	Cc Sf	M												
13	194.07986	194.08041		caffeine	$C_8H_{10}N_4O_2$	Cc Sf	M												
14	111.04403	110.03675		catechol	$C_6H_6O_2$	Cc Sf	M												
15	291.23196	290.22469		coffeediol	$C_{19}H_{30}O_2$	Cc Sf	M												
16	426.38551	426.38606		cycloartenol	$C_{30}H_{50}O$	Cc Sf	M												
17	298.19269	298.19324		dehydrocafestol	$C_{20}H_{26}O_2$	Mn Sf Cc	M												
18	296.17705	296.17760		dehydrokahweol	$C_{20}H_{24}O_2$	Mn Sf Cc	M												
19	183.06516	182.05788		dihydrocaffeic acid	$C_9H_{10}O_4$	Mn Sf Cc	M												
20	289.25260	288.24532		ent-kaur-16-en-19-ol	$C_{20}H_{32}O$	Cc Sf	M												
21	194.05737	194.05791		ferulic acid	$C_{10}H_{10}O_4$	Cc Sf	M												
22	314.18782	314.18836		kahweol	$C_{20}H_{26}O_3$	Cc Sf	M												
23	552.41691	552.41746		kahweol palmitate	$C_{36}H_{56}O_4$	Mn Sf Cc	M												
24	275.27339	274.26611		kaurane	$C_{20}H_{34}$	Cc Sf	M												
25	210.07477	210.07532		liberine	$C_8H_{10}N_4O_3$	Mn Sf Cc	M												
26	124.03923	123.03196		nicotinic acid	$C_6H_5NO_2$	Cc Sf	M												
27	414.38559	414.38614		sitosterol	$C_{29}H_{50}O$	Cc Sf	M												
28	396.37506	396.37561		stigmastan-3,5-diene	$C_{29}H_{48}$	Mn Sf Cc	M												
29	412.36999	412.37054		stigmasterol	$C_{29}H_{48}O$	Cc Sf	M												
30	181.07200	180.06473		theophylline	$C_7H_8N_4O_2$	Cc Sf	M												
31	138.05483	137.04755		trigonelline	$C_7H_7NO_2$	Cc Sf	M												

Figure 2: A table showing the most abundant compounds detected in the coffee silverskin extracts with APPI in positive ion mode and their relative abundance variation across different samples (different extraction solvents).



	m/z meas.	M meas.	Ions	Name	Molecular For...	Annotations	AQ
1	191.05613	192.06341		(-)-quinic acid	$C_7H_{12}O_6$	CC SF	M
2	515.11949	516.12677		1,3-dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	CC SF	M
3	195.05239	196.06008		1,3-dimethyluric acid	$C_7H_8N_4O_3$	CC SF	M
4	529.13517	530.14244		1-caffeoyl-5-feruloylquinic acid	$C_{26}H_{26}O_{12}$	CC SF	M
5	151.04013	152.04741		3',4'-dihydroxyacetophenone	$C_8H_8O_3$	CC SF	M
6	207.06629	208.07357		3,4-dimethoxycinnamic acid	$C_{11}H_{12}O_4$	CC SF	M
7	499.12460	500.13187		3-caffeoyl-4-p-coumaroylquinic	$C_{25}H_{24}O_{11}$	Mn SF	M
8	367.10343	368.11071		3-O-feruloyl-D-quinic acid	$C_{17}H_{20}O_9$	CC SF	M
9	349.09302	350.10029		4-feruloyl-1,5-quinide	$C_{17}H_{18}O_8$	Mn SF CC	M
10	335.07732	336.08459		5-[(E)-caffeoyl]shikimic acid	$C_{16}H_{16}O_8$	CC SF	M
11	149.04549	150.05278		arabinose	$C_5H_{10}O_5$	CC SF	M
12	311.29553	312.30278		arachidic acid	$C_{20}H_{40}O_2$	CC SF	M
13	319.19145	320.19873		atractyligenin	$C_{19}H_{28}O_4$	Mn SF CC	M
14	315.19651	316.20379		cafestol	$C_{20}H_{28}O_3$	CC SF	M
15	179.03482	180.04209		caffeic acid	$C_9H_8O_4$	CC SF	M
16	193.07312	194.08081		caffeine	$C_8H_{10}N_4O_2$	CC SF	M
17	289.07183	290.07911		catechin	$C_{15}H_{14}O_6$	CC SF	M
18	285.06165	286.06893		catechol beta-D-glucuronide	$C_{12}H_{14}O_8$	CC SF	M
19	353.08725	354.09453		chlorogenic acid	$C_{16}H_{18}O_9$	CC SF	M
20	351.21757	352.22485		cofarol	$C_{20}H_{32}O_5$	Mn SF CC	M
21	163.03999	164.04727		coumaric acid	$C_9H_8O_3$	CC SF	M
22	179.05609	180.06329		D-galactose	$C_6H_{12}O_6$	CC SF	M
23	181.07165	182.07893		D-mannitol	$C_6H_{14}O_6$	CC SF	M
24	181.05054	182.05782		dihydrocaffeic acid	$C_9H_{10}O_4$	Mn SF CC	M
25	195.06625	196.07353		dihydroferulic acid	$C_{10}H_{12}O_4$	CC SF	M
26	193.05061	194.05789		ferulic acid	$C_{10}H_{10}O_4$	CC SF	M
27	255.23293	256.24012		hexadecanoic acid	$C_{16}H_{32}O_2$	CC SF	M
28	369.08271	370.08998		isoferulic acid 3-O-glucuronide	$C_{16}H_{18}O_{10}$	Mn SF CC	M
29	331.04596	332.05323		laricitrin	$C_{16}H_{12}O_8$	CC SF	M
30	209.06801	210.07573		liberine	$C_9H_{10}N_4O_3$	Mn SF CC	M
31	279.23295	280.24024		linoleic acid	$C_{18}H_{32}O_2$	CC SF	M
32	250.07212	251.07939		N-feruloylglycine	$C_{12}H_{13}NO_5$	CC SF	M
33	281.24859	282.25586		oleic acid	$C_{18}H_{34}O_2$	CC SF	M

Figure 4: A table showing the most abundant compounds detected in the coffee silverskin extracts with ESI in negative ion mode and their relative abundance variation across different samples (different extraction solvents).

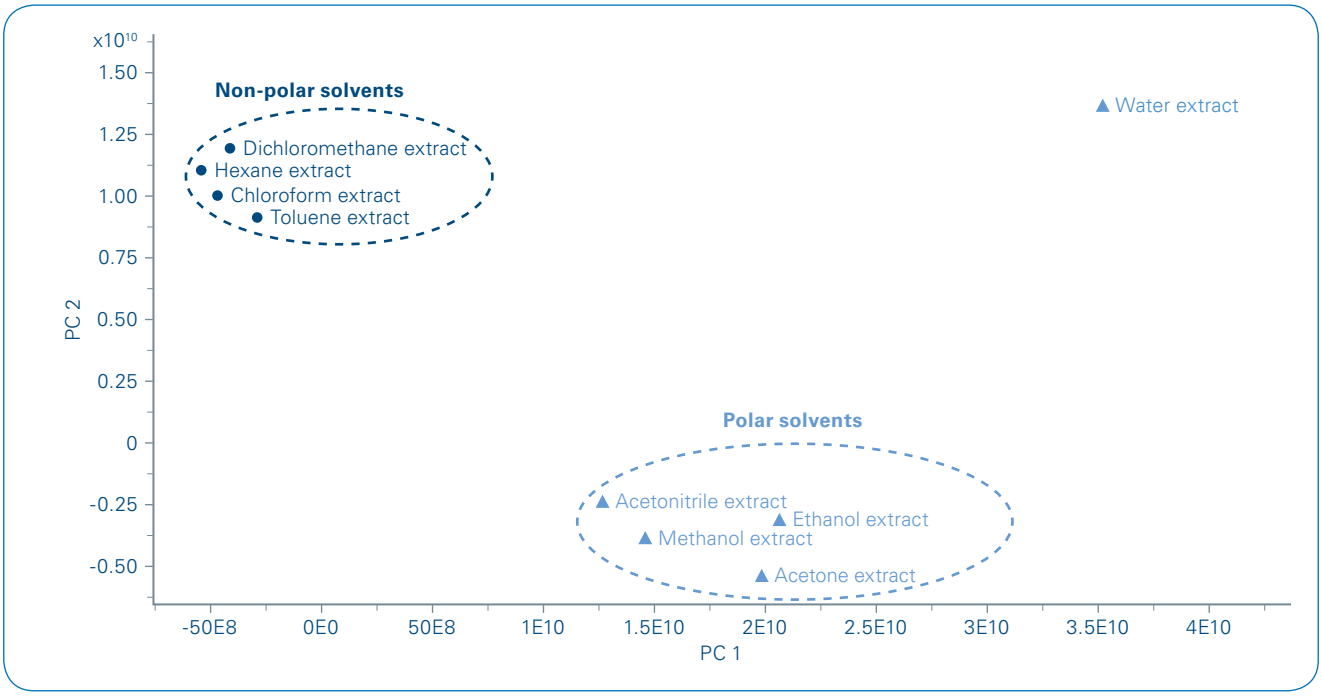


Figure 5: PCA analysis (scores plot) of the coffee silverskin extracts based on ESI data in negative ion mode. Non-polar and polar solvents are clearly separated in the scores plot while water stands out of the two groups.

solvents. The other nitrogen-containing heterocycles were 6-methoxyquinoline, liberine, theophylline and trigonelline, all naturally occurring alkaloids in coffee beans (Figure 3). The other abundant compounds included cafestol, kahweol, and their dehydro-forms, which are diterpenoids that have been associated with a variety of pharmacological effects of coffee. This confirms that CS is a rich source of valuable bioactive compounds. Since APPI does not efficiently ionize some of the more polar, aliphatic or alicyclic compounds, complementary data were acquired from the extracts by using ESI in negative ion mode. The

most abundant compounds detected with ESI included different acids, carbohydrates, and their derivatives (Figure 4). Among fatty acids, linoleic (C18:2), palmitic (C16:0), oleic (C18:1), and arachidic (C20:0) acids were detected. A plethora of chlorogenic acids (i.e. quinic and caffeic acids and their esters) were also observed. Carbohydrates (e.g. galactose and arabinose) were more enriched in the polar solvents.

To assess the overall impact of different solvents on the chemical composition of extracts, two-dimensional principal component analysis (PCA) of the ESI data was performed in MetaboScape. Figure 5 depicts the PCA scores plot, showing that non-polar as well as polar solvents are grouped and clearly separated from each other, while water stands out of the two solvent types, mainly due to higher content of nitrogen-containing analytes. Therefore, by choosing an appropriate solvent, specific types of compounds can be recovered from coffee silverskin for possible further applications.

Acknowledgements

Meira Ltd. (Helsinki, Finland) is thanked for providing the coffee silverskin sample for this study.

Conclusion

- Ultrahigh-resolution MRMS represents a powerful tool for non-targeted chemical fingerprinting of complex mixtures, exemplified here for coffee silverskin extracts. Very simple sample preparation protocols, unparalleled data acquisition speed, and confident assignment of chemical formulae for hundreds or even thousands of analytes in a single mass spectrum are the key analytical benefits of this technology.

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