Automated identification of phenolic compounds in foods by using library search approach

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Introduction

Phenolic compounds have great importance in the nutritional, organoleptic and commercial properties of plant-derived food and beverages. Furthermore, their consumption has been associated with positive health benefits such as antioxidant, antiviral, antiallergenic, cardioprotective, and anticarcinogenic effects. The health effects of polyphenols depend on the amount consumed and their bioavailability. Detailed knowledge of the phenolic composition of food and beverages will contribute to a better understanding of their influence on biological properties. A method for the automated and robust identification of phenolic compounds in foods based on accurate mass and library search has been developed.

Methods

Chromatographic separation. *HPLC* Agilent 1100 series (Santa Clara, CA) with Phenomenex Synergi Fusion-RP 100A, 50x2 mm (2.5 µm) *column & precolumn.*

- flow rate 0.5 mL/min (use of splitter)
- injection volume 5 µL
- column temperature 35 ºC
- solvent A: water; solvent B: acetonitrile both with 0.1% (v/v) formic acid
- gradient: 1-100% B, 0-9 min; 100-1% B 9-9.5 min

MS conditions. *MS-instrument:* micrOTOF-Q™ ESI-Qq-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).

- positive and negative ion polarity ESI
- nebulizer 2 bar; dry gas flow 7 L/min; dry gas heater 200ºC
- spectra rate 1Hz
- acquisition in automated MS/MS mode (1 precursor ion)
- scan range 50-800 m/z
- mass scale calibrations with sodium formate acetate clusters

Library generation. MS and MS/MS mass spectra of 39 phenolic compounds (all commercial standards) were added to the spectral library using DataAnalysis™ and LibraryEditor™ software (Bruker Daltonik, GmbH). Chemical information was collected from company and public websites.

Sample preparation. *Juices:* filtered and diluted (1:1). *Phenolic concentrates* (obtained at low temperature from vegetables rich in phenolic compounds): mixed with methanol for 30 min, filtered and diluted.

Results

We have built up a library of common phenolic compounds using retention time (RT), accurate MS and MS/MS spectra. Conditions were optimized to detect phenolic acids, flavonoids and their glycosides. During spectra review for library generation, special emphasis was on the mass accuracy and isotopic pattern reflected in the sigma-value for both MS and MS/MS spectra. As proof of principle, a blueberry sample was spiked with a mixture of nine phenolic compounds. Subsequently, fruit juice and phenolic concentrate real samples were analyzed. Library search was performed against the created ESI-Qq-TOF and an existing ion trap library, containing more than 1500 spectra. The automated library search method comprises recalibration, peak detection and settings for the library search itself. The method combines the search of RT, MS and MS/MS spectra improving identification of compounds considerably while reducing ambiguities and false positive hits.

The base peak chromatogram (*BPC*) of spiked blueberry juice as well as the extracted ion chromatograms (*EIC*) of the identified compounds are shown in *figure 1.1.*

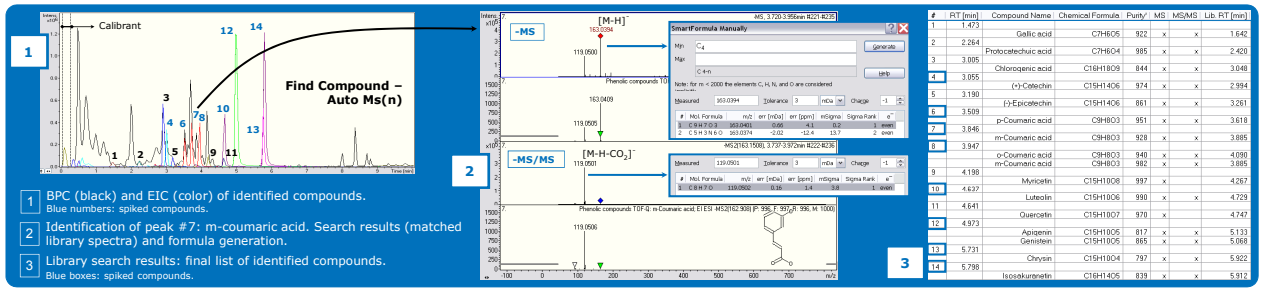

Fig. 1 Blueberry juice spiked with chrysin, apigenin, luteolin, catechin, genistein, isosakuranetin, m, o and p-coumaric acids analyzed in negative polarity ESI

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A representative compound and its search results are shown in *figure 1.2*. The identification of the compounds can be confirmed using their mass accuracy and isotopic pattern information for formula generation. The final list of identified compounds in the juice is shown in *figure 1.3*. The quality of the identification is given by an effective purity score reflecting a combined score of MS and MS/MS data as well as acquisition parameters (max. value = 1000). Peaks #9 *myricetin* and #11 *quercetin* were identified by RT and MS only due to acquisition in AutoMSn with one precursor ion.

Even low-abundance compounds can be identified using the library search approach as it is demonstrated in *figure 2.*

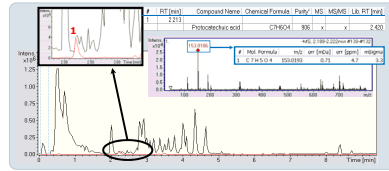


Fig. 2 Cowberry juice (**C**). BPC (black), EIC (red) and identification result of a low-abundance compound

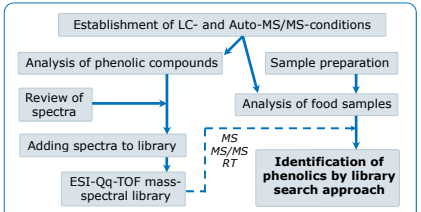
	A			B			C			D		
	Purity	1	2	Purity	1	2	Purity	1	2	Purity	1	2
Catechin				947	X	X	924	X	X			
Chlorogenic acid	950	X	X				932	X	X	999	X	X
Epicatechin										990	X	X
Gallic acid				923	X	X				863	X	X
Myricetin				986	X	X				812	X	X
Naringenin							872	X	X			
Protocatechuic acid							906	X	X	901	X	X
Quercetin				969	X	X	901	X	X	861	X	X
	E			F			G					
	Purity	1	2	Purity	1	2	Purity	1	2			
Caffeic acid							1000	X	X			
Catechin	997	X	X									
Chrysin				884	X	X	710	X				
Ferulic acid							965	X	X			
Gallic acid	998	X	X	980	X	X						
Myricetin	850	X	X									
p-Coumaric acid							940	X	X			
Protocatechuic acid							883	X	X			
Quercetin	899	X	X	860	X	X	863	X	X			

Identified by library search:
1: *MS*
2: *MS/MS*

Table. 1 Identified compounds in the analyzed samples. Juices: apple (**A**), red grape (**B**), cowberry (**C**), blueberry (**D**). Concentrates: green tea (**E**), grape (**F**), propolis (**G**)

Table 1 shows the summary of all samples analyzed in ESI negative mode. Compounds were identified by the library search with good purity scores as well as by formula generation.

Summary



Library development and identification workflow

Conclusions

- A library of dietary phenolic compounds was successfully created using data acquired with a micrOTOF-Q mass spectrometer.
- The proposed LC-Auto/MS/MS method is fast, sensitive, enabling the characterization of even low-abundance compounds, and provides mass accuracy and true isotopic pattern for both precursor and product ions.
- Positive identifications of dietary phenolics were obtained in a variety of samples analyzed under the same conditions from those of the reference spectra.

HPLC-ESI-Qq-TOF