Microsaic Systems

Easy and fast isolation of rosmarinic acid from lemon balm with mass-directed purification

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INTRODUCTION

Rosmarinic acid is a natural product widely spread over different plant families. Preparative reversed-phase chromatography was used for the mass-directed purification of rosmarinic acid from a lemon balm extract. The AZURA® Preparative HPLC System together with the 4000 MiD® mass spectrometer was showed to be well suited for this application.

The ubiquitous natural product rosmarinic acid shows antiviral, antimicrobial and anti-inflammatory characteristics. It is used in different kinds of medicinal products for example in ointments for sports injuries. Leaves of lemon balm contain a high concentration of rosmarinic acid and are therefore an interesting source for the isolation of this compound. Here, we present an effective and time-saving method for the isolation of rosmarinic acid from a lemon balm extract based on the technique of mass-directed purification.

EXPERIMENTAL

AZURA Analytical HPLC System was used for the method development consisting of a low-pressure gradient AZURA P6.1L pump, an AZURA AS 6.1L autosampler, an AZURA DAD 2.1L diode array detector equipped with a 10 mm PressureProof flow cell and an Eurosphere II 100-5 C18 150 x 4.6 mm column. The gradient method was run for 20 min at a flow rate of 1 mL/min starting with 80/20% water/acetonitrile increasing to 100 % acetonitrile over 20 min. Both eluents contained 0.1 % formic acid as an additive. The wavelength of the detector was set to 280 nm at a data rate of 20 Hz. 10 µL of the sample was injected.

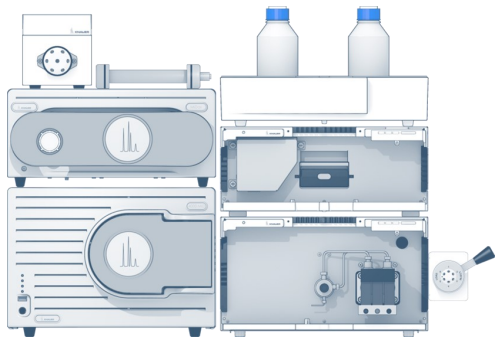

Figure 1 schematic of the Azura® - MiD® Prep system

AZURA Preparative HPLC System was used for the mass-directed purification of rosmarinic acid. The system consisted of an AZURA P 2.1L pump equipped with a 250 mL pump head and a three channel low pressure gradient (LPG) ternary module, a manual injection valve (1/8", 6 port 2 kanal) equipped with a 5 mL sample loop, an AZURA DAD 6.1L diode array detector equipped with a 3 mm PressureProof flow cell, a 4000 MiD mass spectrometer with the MiDas sampling unit, a AZURA V 2.1S equipped with a 6 port multi position valve for fractionation and an Eurospher II 100-10 C18 250 x 30 mm column. The gradient method run for 67 min at a flow rate of 21.3 mL/min under the same conditions as the analytical method described above. The wavelength of the DAD was set to 280 nm at a data rate of 10 Hz, while the mass selective detector was set to SIM mode monitoring the relevant mass of m/z 359.2. The data trace of the mass selective detector was used for fractionation via the multi-position valve. 5 mL of the crude extract obtained under sonication from dried leave material with 30 % isopropanol was injected.

ADDITIONAL MATERIAL

Software :ClarityChrom and ClarityChrom 7.4 PDA extension

Table A1: Method parameters (preparative)

Eluent A	Water + 0.1 % formic acid		
Eluent B	Acetonitrile + 0.1 % formic acid		
Gradient	Time [min]	% A	% B
	0	80	20
	67	0	100
Flow rate	21.3 mL/min	System pressure	100 bar
Run temperature	RT	Run time	67 min
Injection volume	5 mL	Injection mode	Full loop
Detection	280 nm	Data rate	10 Hz
		Time constant	0.1 s

Table A2: Method parameters (mass spectrometer analysis)

Scan mode	Interleave (Scan/SIM)
Mass range	100-400 m/z
Scan rate	1 Hz
Step	0.2
SIM	359.2 m/z
Ion mode	Negative
Gas flow	2.5 L/min

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RESULTS

A method for the isolation of rosmarinic acid from a lemon balm extract was developed on an analytical scale using an AZURA Analytical System and a Eurospher II C18 column . The UV spectra from the analysis showed the presence of many compounds with the structural motif of a phenyl acrylic acids (Fig. 2). For a time-saving isolation of the target compound, the developed method was then transferred directly to the AZURA Preparative System with the ability to fractionate via molecular mass. One fraction with a compound of the desired mass (m/z 359.2; [M-H]-) (Fig 3) was collected (Fig. 4 & Fig. 5). The following analysis of this fraction with the AZURA Analytical System showed that is was possible to isolate the target compound rosmarinic acid with the technique of mass-directed purification in a purity of >95 % (Fig. 6).

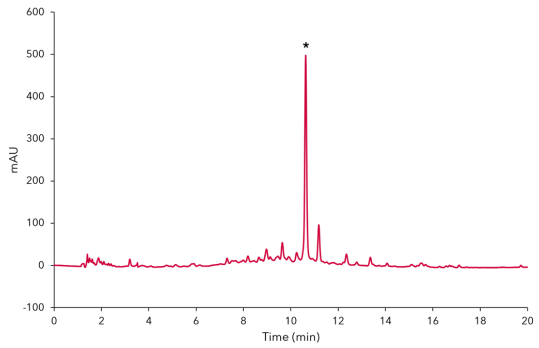

Figure 2. Analytical chromatogram of the crude lemon balm extract at 280 nm; gradient separation 20 %-100 % acetonitrile, *rosmarinic acid peak. .

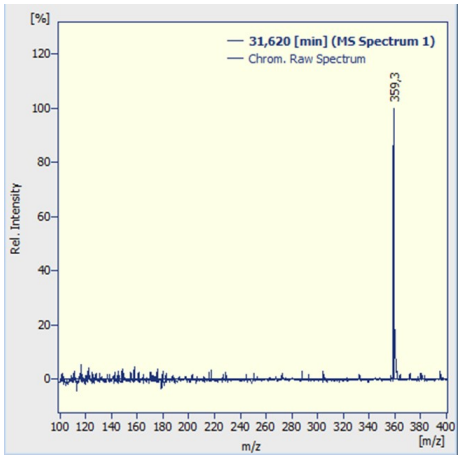

Figure 3. Mass Spectrum of rosmarinic acid ([M-H]-) 359.3 m/z

ACKNOWLEDGEMENTS

Microsaic would like to thank Knauer for all the information provided in this application note. Please visit www.knauer.net for more details.

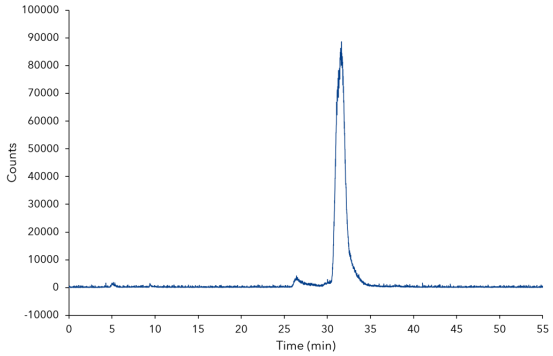

Figure 4. SIM (single ion monitoring) chromatogram of a purification run for the target mass of m/z 359.2. .

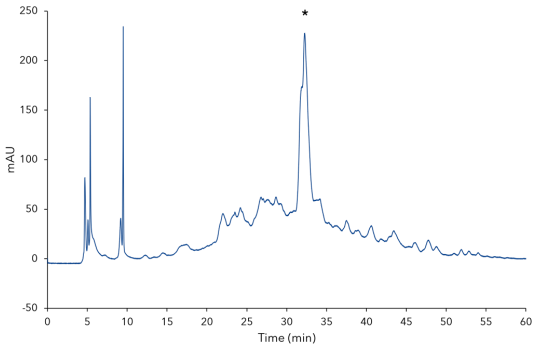

Figure 5. UV-Chromatogram of a purification run for the crude lemon balm extract at 280 nm, *rosmarinic acid peak

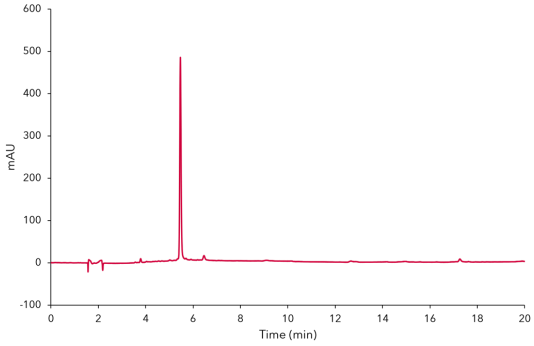

Figure 6: Analytical chromatogram of the fraction containing rosmarinic acid at 280 nm, isocratic separation 50/50 water/acetonitrile.

CONCLUSIONS

Rosmarinic acid was the main metabolite of the extracted lemon balm material. This target molecule was isolated in a short time with an AZURA Preparative HPLC system using the technique of mass-directed purification. By this, the number of fractions was reduced to one leading to a significant decrease of analysis time.