Science Together

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

Jan Wendrich, Yannick Krauke, Lilit Avagyan, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net

## SUMMARY

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® Prep System together with the 4000 MiD mass spectrometer was showed to be well suited for this application.

## INTRODUCTION

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.

## 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 (**Fig. 1**). The UV spectra from the analysis showed the presence of many compounds with the structural motif of a phenyl acrylic acids. 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; $[M-H]^-$) was collected (**Fig. 2** & **Fig. 3**). 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. 4**).

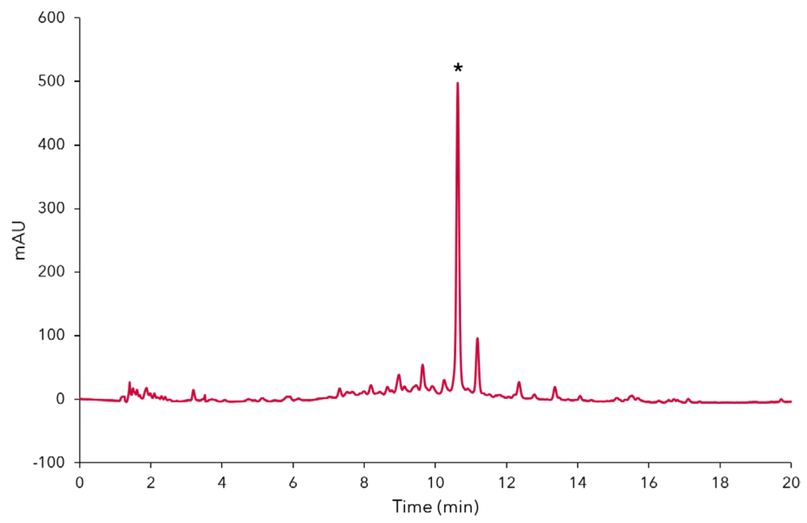



**Fig. 1** Analytical chromatogram of the crude lemon balm extract at 280 nm; gradient separation 20 %-100 % acetonitrile, *rosmarinic acid peak

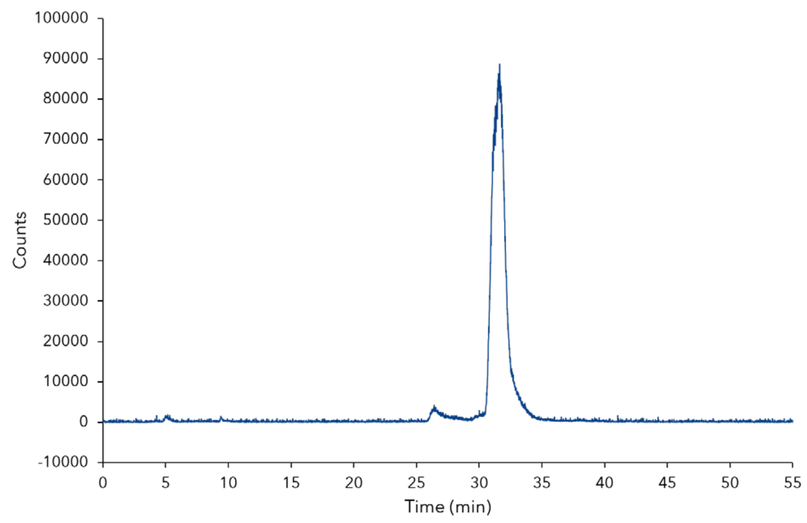



**Fig. 2** SIM (single ion monitoring) chromatogram of a purification run for the target mass of m/z 359.2

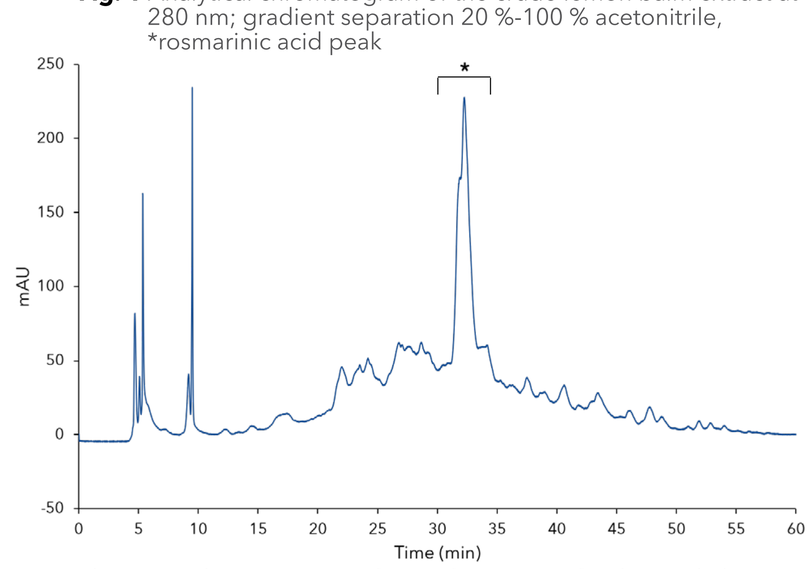



**Fig. 3** UV-Chromatogram of a purification run for the crude lemon balm extract at 280 nm, *rosmarinic acid peak

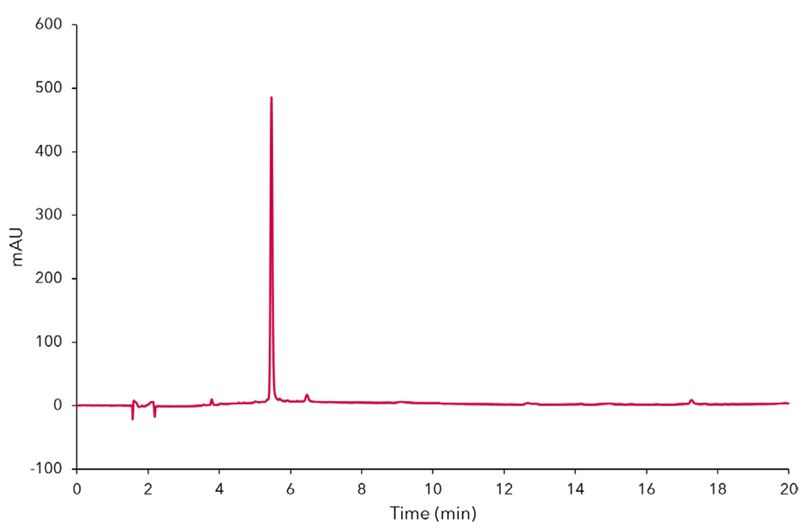
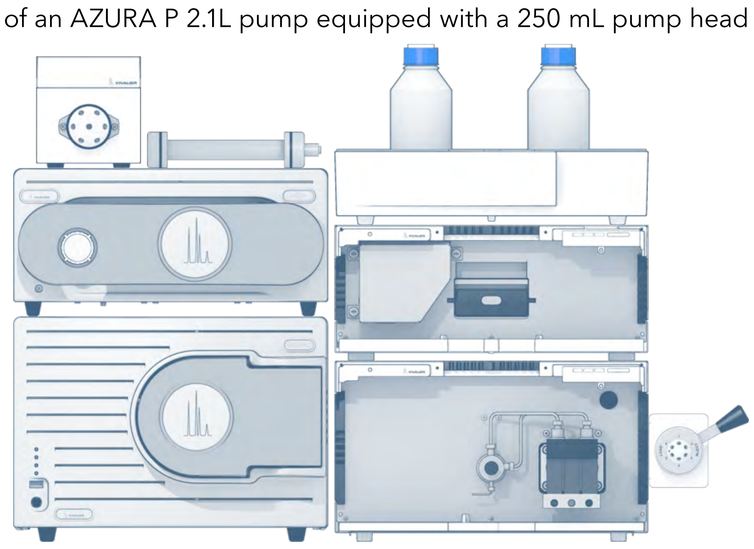



**Fig. 4** Analytical chromatogram of the fraction containing rosmarinic acid at 280 nm, isocratic separation 50/50 water/acetonitrile

## MATERIALS AND METHODS

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.

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.



## CONCLUSION

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 past analysis time.

Additional information

VPH0067
© KNAUER Wissenschaftliche Geräte GmbH

Science Together

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

## ADDITIONAL RESULTS

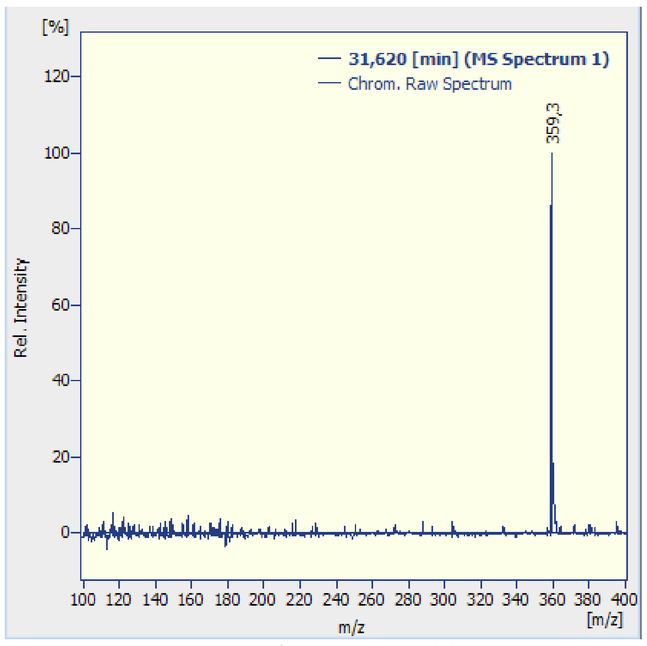



**Fig. A1** Mass spectrum of rosmarinic acid ([M-H]$^-$).

## ADDITIONAL MATERIALS AND METHODS

**Tab. 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 wavelength | 280 nm | Data rate | 10 Hz |
| | | Time constant | 0.1 s |

**Tab. 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 |

**Tab. A3** System configuration & data (analytical system)

| **Instrument** | **Description** | **Article No.** |
|---|---|---|
| Pump | AZURA P 6.1L | APH34GA |
| Autosampler | AZURA AS 6.1L | AAA00AA |
| Detector | AZURA DAD 2.1L | ADC01 |
| Flow cell | PressureProof Flow cell 10 mm 10 µl | AMC38 |
| Column | Eurospher II 100-5 C18 with precolumn, Vertex Plus Column 150 x 4.6 mm | 15VE181E2J |
| Software | ClarityChrom<br>ClarityChrom 7.4.2 - PDA extension | A1670<br>A1676 |

**Tab. A4** System configuration & data (preparative system)

| **Instrument** | **Description** | **Article No.** |
|---|---|---|
| Pump | AZURA P 2.1L 250 mL<br>AZURA LPG module for Pump P 2.1L | APE20LA<br>AZZ00AB |
| Injection | AZURA V 2.1S Valve 6 Port 2 Position | A1359 |
| Sample loop | 5 mL Sample loop | A0586-2 |
| Detector | AZURA DAD 6.1L | ADC11 |
| Flow cell | PressureProof flow cell 3 mm 2 µL | AMB18 |
| Mass spectrometer | 4000 MiD with MiDas | A66900 |
| Fractionation | AZURA V 2.1S Valve 6 Port Multiposition | AWA10BC |
| Software | ClarityChrom<br>ClarityChom PDA Extension<br>ClarityChom MS Extension<br>ClarityChrom FRC control module | A1670<br>A1676<br>A1679<br>A1682 |
| Column | VertexPlus AX Column 250x30 mm Eurospher II 100-10 C18 | 25QE181E2N |