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Purification of chamazulene by preparative HPLC and its scale-up

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SUMMARY

Extraction of natural components from plants that are used for medical treatments is widely applied. Often the extraction process results in an oil, such as the chamomile blue oil with many different compounds. Usually only some of these components are responsible for the medical properties of the whole extract. In chamomile oil, chamazulene is one of these medical active compounds. Here, the preparative purification of chamazulene by HPLC from chamomile blue oil is described.

INTRODUCTION

Chamomile plants are known for their medical properties, featuring anti-inflammatory, analgesic and sedative effects. These result from the various phenolic compounds found in the flowers. One of those compounds is matricin, which is converted to chamazulene during the distillation process[1, 2]. Chamazulene gives the chamomile oil, also known as "chamomile blue", its characteristic blue colour. It was shown that chamazulene alone has anti-inflammatory and antioxidant activity[3]. Different HPLC methods are established to analyze chamomile samples from different sources. Various extraction and distillation processes are described. But so far, no published preparative HPLC method for purification of chamazulene is available[2]. The present application uses preparative HPLC to purify pure chamazulene from commercially available "chamomile blue" oil.

Additional Information

Purification of chamazulene by preparative HPLC and its scale-up

RESULTS

The chamomile blue oil was diluted in methanol and water (90:10; v/v). The insoluble components were removed by filtration. The comparison of the oil sample and the chamazulene standard chromatograms revealed the chamazulene peak at a retention time of 7.6 min **(Fig. 1)**. The spectra from 190 to 400 nm of the chamomile oil sample and the standard proved that the identified peak at 7.6 min is chamazulene **(Fig. 2)**. The profiles of both spectra are nearly identical. The applied analytical method showed a baseline separation of the chamazulene peak from the nearest earlier and later eluting peaks **(Fig. 1)**. Therefore, the method was directly transferred to a semi-preparative scale. A column with the same length but larger inner diameter (4.6 to 20 mm ID), larger particles (5 to 10 qm) and an increased flow rate of 25 mL/min was used. Different sample volumes were injected. The results revealed that 1 mL sample load lead to a good separation of the chamazulene from other components in the sample **(Fig. 3)**. For the final purification 2 mL of sample were injected and the chamazulene peak was collected using a fractionation valve **(Fig. 4)**. The fraction was analysed with the analytical method and compared to the chamazulene standard. The result showed that the fractionation was successful as only the peak of chamazulene was detected **(Fig. 5)**. Quantification of chamazulene in the fraction of the 2 mL injection revealed a 100 % recovery of the chamazulene from the sample. The dilution was approximately 1:10 **(Tab. 1)**. As the purification was successful in the semi-preparative scale, the method was further scaled-up to a column with 50 mm ID. The column length was shortened from 250 to 150 mm to reduce solvent consumption and fasten the method. The method was adapted, increasing the flow rate to 150 mL/min and the injection volume to 10 mL **(Fig. 6)**. Analysis of the fraction and comparison with the chamazulene standard revealed that the fraction contained chamazulene without any other detectable impurities **(Fig. 7)**. The recovery of chamazulene was 82 % in the fraction of the 10 mL injection **(Tab. 1)**.

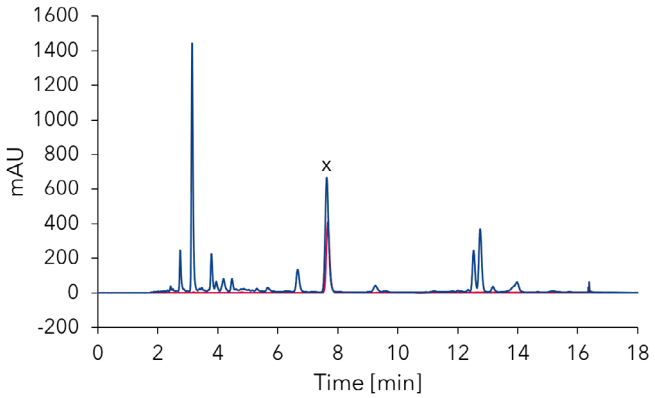

Fig. 1 Overlay chromatograms of chamazulene standard (red) and chamomile blue oil sample (blue). x – chamazulene peak; standard: 0.75 mg/mL, 1 µL injection; sample: 1:10 dilution, 1 µL injection; 1.3 mL/min.

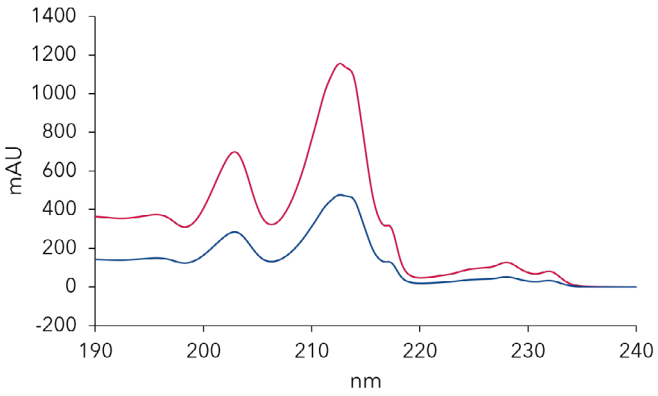

Fig. 2 Spectrum chamazulene standard (red) and chamomile oil sample (blue) at 7.6 min.

RESULTS

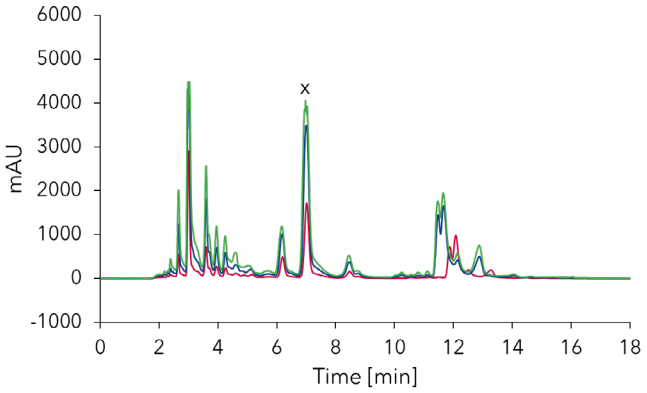

Fig. 3 Overload study. Chromatograms of different injection volumes on semi-preparative column: red 200 µL, blue 500 µL, green 1000 µL. x – chamazulene peak; chamomile oil 1:10 dilution; C18 20 x 250 mm, 10 µm, 25 mL/min.

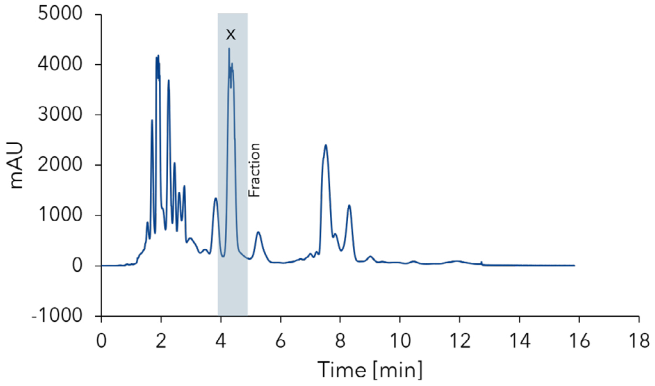

Fig. 6 Chromatogram chamazulene purification. Collected fraction highlighted in grey. 10 mL injection volume; x – chamazulene peak; C18 50 x 150 mm; 10 µm; 150 mL/min.

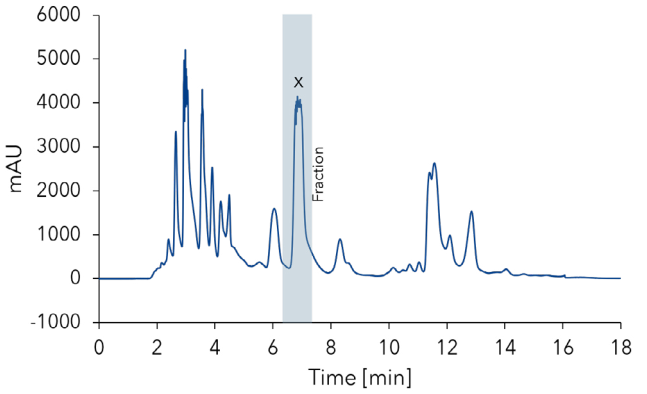

Fig. 4 Chromatogram chamazulene purification. Collected fraction highlighted in grey. 2 mL injection volume; x – chamazulene peak; C18 20 x 250 mm; 10 µm; 25 mL/min.

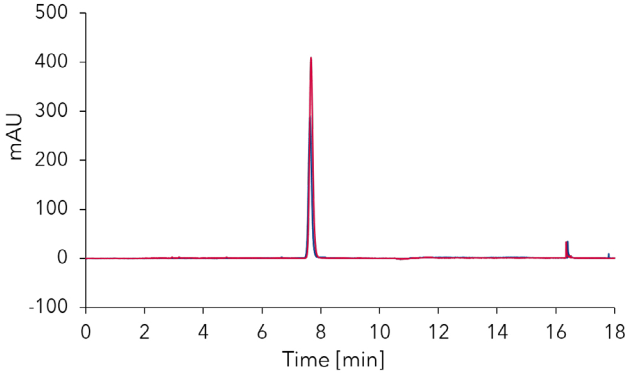

Fig. 7 Overlay fraction from Fig. 6 and chamazulene standard (0.75 mg/mL) on analytical column.

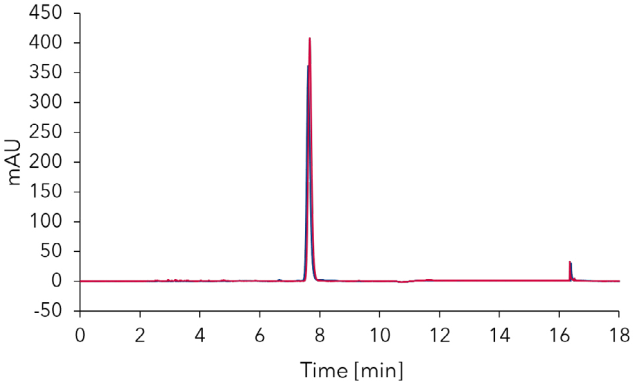

Fig. 5 Overlay fraction from Fig. 4 in blue and chamazulene standard (0.75 mg/mL) in red on analytical column.

Tab. 1 Quantification of chamazulene concentration in the fractions of 2 and 10 mL injection from purification

	2 mL injection	10 mL injection
Chamazulene concentration sample [mg/mL]	1,240	1,048
Total amount chamazulene injected [mg]	2,48	10,48
Chamazulene concentration fraction [mg/mL]	0,133	0,101
Fraction volume [mL]	19	85
Total amount chamazulene in fraction [mg/mL]	2,526	8,619
Recovery [%]	101,80	82,20

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SAMPLE PREPARATIONS

The chamazulene standard (Sigma Aldrich) was dissolved in methanol to desired concentrations for calibration curve and filtered (0.45 µm). A 5-point calibration curve was calculated with following concentrations in triplicates: 0.015, 0.150, 0.375, 0.750 and 1.500 mg/mL chamazulene. Blue chamomile essential oil "Kamillen Öl Blau" was purchased by manufacturer/distributor ASAV Apoth.Serv.Arzneim.Vetr.GmbH; PZN 06984428; 2 mL. The oil was diluted in a ratio of 1:10 with methanol and water (90:10; v/v). The insoluble fraction was removed by filtration through a 0.45 µm filter.

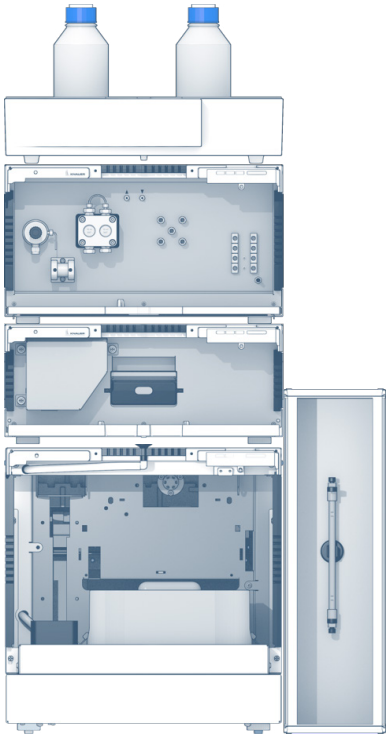
CONCLUSION

A preparative reverse phase method was developed for the purification of chamazulene from chamomile blue oil sample. The chamazulene was purified in high purity (approximatly 100 %) and with high recovery (> 100 %) in semi-preparative scale (20 mm ID column). The high recovery can be explained by error propagation during the measurements. The chamazulene was identified in the sample and fraction by comparing retention time and spectra with the chamazulene standard. To increase the throughput, the method was adapted to a 50 mm ID column with the same particle size as the semi–preparative column. The pump was easily upgraded to higher flow rates by replacing the 100 mL pump head with a 250 mL pump head. From the 10 mL injected sample 82 % chamazulene were recovered by fractionation. The recovery could be increased by enlarging the fraction window, but could lead to impurities in the fraction. After the development of a reverse phase method for preparative HPLC purification of chamazulene from chamomile blue oil, an alternative normal phase HPLC method could be interesting. Normal phase separations are often used in industrial scale due to better solubility for some samples and easier evaporation of the used solvents.

MATERIALS AND METHODS

Tab. 2 Configuration analytical system

Instrument	Description	Article No.
Pump	AZURA P 6.1L LPG 10 mL/min, sst	APH34EA
Autosampler	AZURA AS 6.1L, 700 bar	AAA00AA
Detector	AZURA DAD2.1L	ADC01
Flow cell	Light guide 10mm / 2 µL / 50 bar	AMC19XA
Thermostat	AZURA CT2.1	A05852
Column	Eurospher II 100-5 C18, 250 x 4.6 mm ID	25VE181E2J
Software	ClarityChrom 8.1 – workstation, autosampler control included	A1670
Software	ClarityChrom 8.1 - PDA extension	A1676



Tab. 3 Analytical method - pump parameters

Eluent A	H_2Odd		
Eluent B	Acetonitrile		
Flow rate	1.3 mL/min		
Pump program	Time (min)	%A	%B
	0.00	10	90
	10.00	10	90
	10.02	0	100
	16.00	0	100
	16.02	10	90
	22.00	10	90

Tab. 4 Analytical method - method parameters

Column temperature	25 °C
Injection volume	5 µL
Injection mode	Partial loop
Detection	UV 245 nm / 285 nm / 3D 190 – 400 nm
Data rate	20 Hz

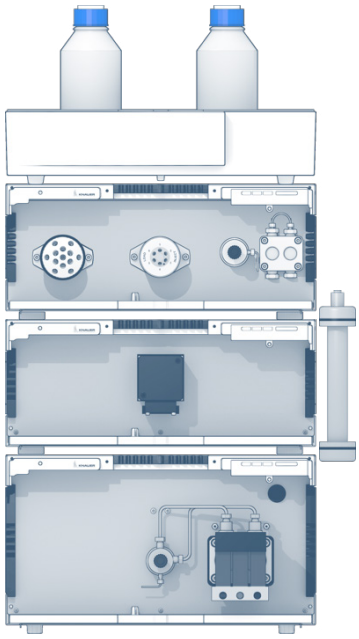
MATERIALS AND METHODS

Tab. 5 Configuration preparative system

Instrument	Description	Article No.
Pump	AZURA P 2.1L, 100 mL/min sst	APE20KA
Ternary LGP module	AZURA LPG ternary module for Pump P 2.1L	AZZ00AB
Pump head	Pump head 250 mL/min, sst	A4021-1
Detector	UVD 2.1L	ADA01XA
Assistant	Left: 6 Mpos,1/8"", sst Middle: 6Port2Pos,1/16", sst Right: P4.1S, 50 mL, sst	AYEKEABR
Flow cell	3 µL; 1/16"	A4069
Column	Eurospher II 100-10 C18, 250 x 20 mm ID	25PE181E2N
Column	Eurospher II 100-10 C18, 150 x 50 mm ID	15OE181E2N
Software	PurityChrom®Basic	A2650

Tab. 6 Preparative methods - pump parameters

Eluent A	90:10 Acetonitrile:H_2Odd (v/v)					
Eluent B	100 % Acetonitrile					
	ID 20 mm column			ID 50 mm column		
Flow rate	25 mL/min			150 mL/min		
Pump program	Time (min)	%A	%B	Time (min)	%A	%B
	0.00	100	0	0.00	100	0
	8.00	100	0	5.45	100	0
	8.02	0	100	5.47	0	100
	14.00	0	100	11.45	0	100
	14.02	100	0	11.47	100	0
	18.00	100	0	18.00	100	0



Tab. 7 Preparative methods - method parameters

	ID 20 mm column	ID 50 mm column
Column temperature	ambient	ambient
Injection volume	1 mL, 2 mL	10 mL
Injection mode	Injection loop	Injection loop
Detection	UV 245 nm	UV 245 nm
Data rate	2 Hz	2 Hz

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[1] Srivastava, J. K., Gupta, S. Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. Mol Cell Pharmacol 1(3), 138 (2009).

[2] Haghi, G., Hatami, A., Safaei, A., Mehran, M. Analysis of phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV. Res Pharm Sci 9(1), 31-7 (2014).

[3] Salihovic, M., Sapcanin, A., Spirtovic-Halilovic, S., Zavrsnik, D. DFT structural analysis of chamazulene. Bull Chem Technol Bosnia Herzegovina 46, 27–32 (2016).