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Purification of epigallocatechin gallate and other related polyphenols from green tea by mass-triggered fractionation

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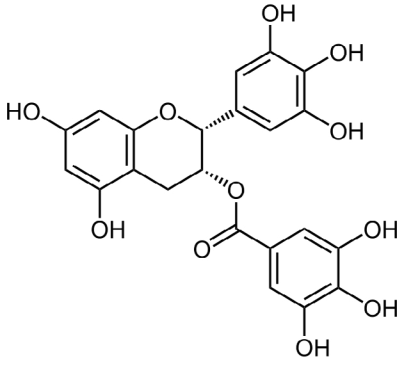
SUMMARY

Epigallocatechin gallate is one of the major metabolites in green tea material and has shown positives effects on human health in several studies. This target molecule was isolated together with three other polyphenolic compounds in a short time with an AZURA® Preparative HPLC system using mass-triggered fractionation. The number of fractions was reduced to a minimum by this technique leading to a significant decrease in past analysis time showing that mass-directed purification is the ideal method in the isolation of natural products.

INTRODUCTION

Catechins are polyphenolic metabolites that appear in plants. These molecules from the group of flavonoids gained a lot of interest over the past decades due to their antioxidant properties. Especially, epigallocatechin gallate was subjected to intensive research regarding its positive effects on human health. It can be purchased as a dietary supplement but is also available in high amounts in green tea leaves. Here, we present an easy and time-saving method for the isolation of epigallocatechin gallate and other related catechins from a green tea extract based on the technique of mass-triggered fractionation.



Structure of Epigallocatechin gallate (Catechin)

Purification of epigallocatechin gallate and other related polyphenols from green tea by mass-triggered fractionation

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RESULTS

A method for the isolation of epigallocatechin gallate from green tea extract was developed on analytical scale using an AZURA Analytical HPLC plus system and an Eurospher II C18 column (**Fig. 1**). The developed method was then transferred to the AZURA Preparative system with the ability to fractionate via molecular mass (**Fig. 2**). One fraction with the desired mass for epigallocatechin gallate (m/z 457.4; $[M-H]^-$) was collected (**Fig. 3**). In addition to this fraction, three further fractions corresponding to epicatechin, epicatechin gallate and epigallocatechin (m/z 289.2; m/z 305.2; m/z 441.4; $[M-H]^-$) were collected. The following HPLC analysis of the target fraction showed that it was possible to isolate epigallocatechin gallate with the technique of mass-triggered fractionation with a purity of >95 % (**Fig. 4**). Also, three other catechins were isolated by this method in the purity of >90 % (**Fig. A1-A3**).

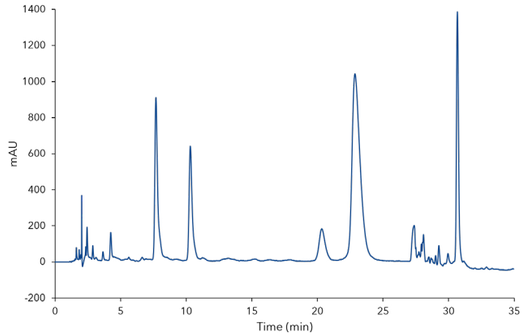


Fig. 1 Analytical chromatogram of the crude green tea extract at 220 nm; step gradient separation 10 % acetonitrile until 26 min, then 15 % acetonitrile

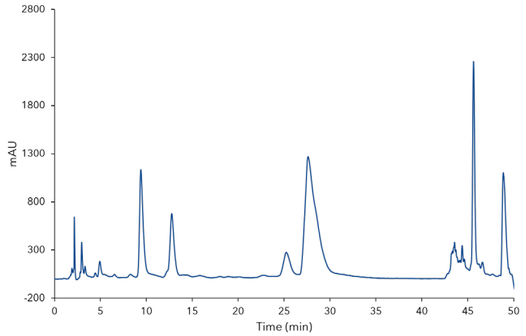


Fig. 2 UV chromatogram of a purification run for the crude green tea extract at 220 nm

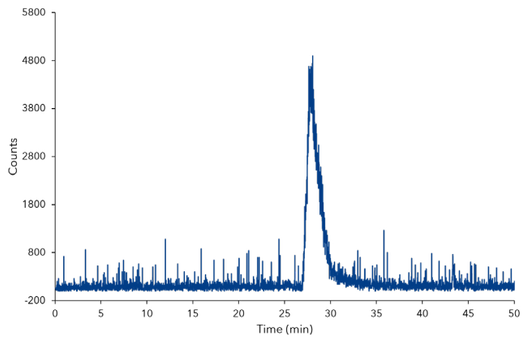


Fig. 3 SIM (single ion monitoring) chromatogram of a purification run for the target mass of m/z 457.4

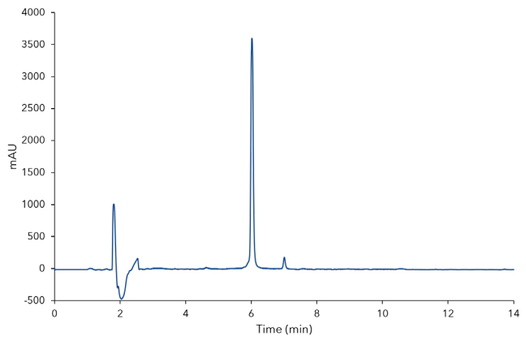


Fig. 4 Analytical chromatogram of the third fraction containing epigallocatechin gallate (m/z 457.4; $[M-H]^-$); linear gradient separation 5 %-50 % acetonitrile

MATERIALS AND METHOD

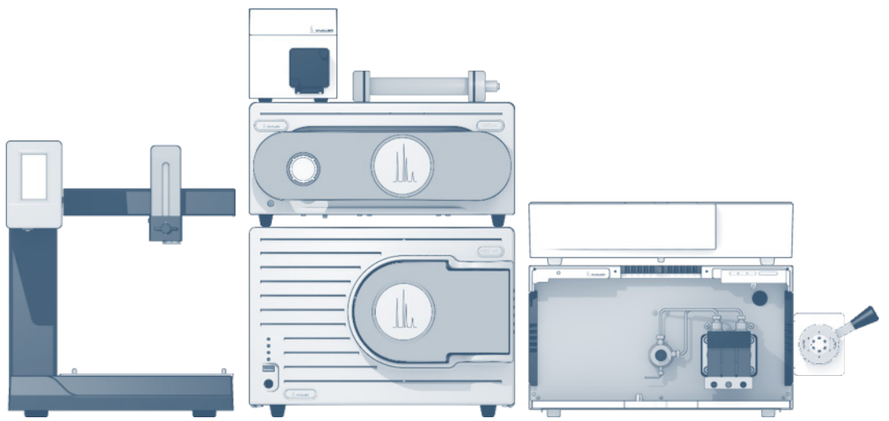
AZURA Analytical HPLC Plus system was used for the method development. Method optimization on this analytical system led to a step gradient, which was used for the isolation of polyphenols from green tea.

AZURA Preparative HPLC system was used for the mass-directed purification of epigallocatechin gallate. 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 position) equipped with a 5 mL sample loop, an AZURA UVD 2.1S detector equipped with a 3 mm flow cell, a 4000 MiD mass spectrometer with the MiDas sampling unit, a Foxy R1 fraction collector and an Eurospher II 100-5 C18 150 x 20 mm column. The step gradient method run for 50 min at a flow rate of 18.9 ml/min with the following composition: 0 min 10 %B, 40 min 10 %B, 40.1 15 %B, 50 min 15 %B, with 0.1% formic acid in water (A) and acetonitrile (B) as eluents. The wavelength of the detector was set to 220 nm at a data rate of 10 Hz, while the mass selective detector was set to negative SIM mode monitoring the masses of m/z 289.2, 305.2, 441.4, 457.4.

The green tea extract was prepared by sonification of ground green tea leaves with 75 % ethanol for 60 min, followed by filtration and the dilution in a ration of 1:1 with water.

CONCLUSION

Epigallocatechin gallate is one of the major metabolites in green tea. This target molecule was isolated together with three other polyphenolic compounds in a short time with an AZURA Preparative HPLC system using the technique of mass-triggered fractionation. The number of fractions was reduced to a minimum by this technique leading to a significant decrease in past analysis time.



ADDITIONAL RESULTS

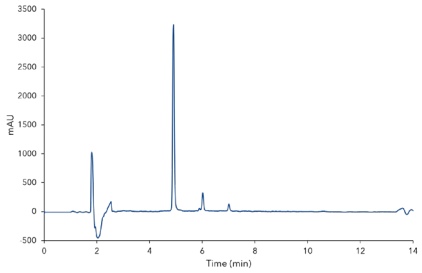


Fig. A 1 Analytical chromatogram of the first fraction containing epigallocatechin (m/z 305.2; $[M-H]^-$); linear gradient separation 5 %-50 % acetonitrile.

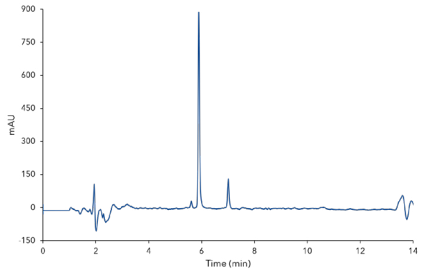


Fig. A 2 Analytical chromatogram of the second fraction containing epicatechin (m/z 289.2; $[M-H]^-$); linear gradient separation 5 %-50 % acetonitrile.

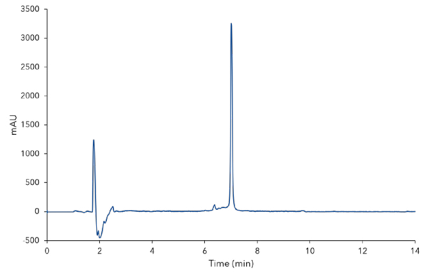


Fig. A 3 Analytical chromatogram of the fourth fraction containing epicatechin gallate (m/z 441.4; $[M-H]^-$); linear gradient separation 5 %-50 % acetonitrile.

ADDITIONAL MATERIALS AND METHODS

Tab. A1 Method parameters (preparative)

Eluent A	Water + 0.1% formic acid		
Eluent B	Acetonitrile		
Gradient	Time (min)	%A	%B
	0	90	10
	40	90	10
	40.1	85	15
	50	85	15
Flow rate	18.9 mL/min	System pressure	120 bar
Column temperature	RT	Run time	50 min
Injection volume	500 µL	Injection mode	-
Detection wavelength	220 nm	Data rate	10 Hz
		Time constant	0.1 sec

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 ClarityChrom 8.1 - PDA extension	A1670 A1676

Tab. A2 Method parameters (mass spectrometer)

Scan mode	SIM (Single Ion Monitoring)
Scan rate	1 Hz
Step	0.2
SIM	289.2 m/z, 305.2 m/z, 441.4 m/z, 457.4 m/z
Ion mode	Negative
Gas flow	2.5 l/min

Tab. A4 System configuration & data (preparative system)

Instrument	Description	Article No.
Pump	AZURA P 2.1L AZURA LPG module for Pump P 2.1L	APE20LA AZZ00AB
Injection	AZURA V 2.1	A1359
Sample loop	5 ml sample loop	A0586-2
Detector	AZURA UVD 2.1S	ADA00
Flow cell	Semi-preparative UV flow cell 3 mm, 2 µl	A4042
Mass spectrometer	4000 MiD with MiDas	A66900
Fractionation	Fraction collector Foxy R1	A59100
Column	Eurospher II 100-5 C18, Column 150x20 mm	15JE181E2J
Software	PurityChrom 5.9.69 PurityChrom Upgrade to full version PurityChrom MS license	A2650 A2652 A2655

RELATED KNAUER APPLICATIONS

VPH0067 - Easy and fast isolation of rosmarinic acid from lemon balm with mass-directed purification

