KNAUER

Application Note

Determination of coumarin in cinnamon products

Category	Food analysis
Matrix	Extract from biscuits
Method	UHPLC
Keywords	Coumarin, 2H-1-Benzopyran-2-one, 1,2-Benzopyrone, cinnamon biscuits, cassia, safety control
Analytes	Coumarin
ID	**VFD0073N**, 05/11

PLATIN blue

Summary

This application note presents a gradient method using a sub-2 µm column for the determination of coumarin in cinnamon products. Applying the KNAUER PLATINblue UHPLC system, analysis time and eluent consumption could be reduced dramatically compared to the conventional HPLC method. The high speed and reliability of the method make it well-suited for routine analyses in food safety control.

Introduction

Coumarin is a naturally occurring benzo-a-pyrone compound and can be found in a large number of plants from different families including tonka beans, woodruff, lavender oil, cassia (common cinnamon source) and melilot (sweet clover). Due to its frequent use in fragrances and fragrance containing products, dermal exposure to coumarin is common. Human exposure to coumarin also occurs orally via natural foodstuffs, from pharmaceutical use, and from tobacco products.[1]

According to the Federal Institute for Risk Assessment (BfR), relatively small amounts of coumarin can already damage the liver of particularly sensitive individuals. Additionally, very high levels of coumarin administered over long periods can trigger cancer in rats and mice as revealed by animal experiments. Official food control authorities brought to light that the limited level of two milligrams coumarin per kilogram food has been exceeded considerably in some cinnamon biscuits.[2] This urged the Federal Institute for Risk Assessment (BfR) to evaluate potential health risks consumers are exposed to by the uptake of coumarin in cinnamon-containing foods and to establish a tolerable daily intake (TDI) of 0.1 mg/kg body weight.[3]

These limitations necessitate an easy method for the determination of coumarin levels especially in cinnamon containing products. Conventionally, coumarin is analysed by means of HPLC methods using a gradient elution concerning acetonitrile. These methods are often time consuming, 60 min per run are common, and are therewith an expensive choice.

In this application note, a UHPLC method for the determination of coumarin in cinnamon products in less than 4 minutes analysis time is described. A gradient elution concerning methanol is used, which is much more economic compared to the use of acetonitrile.

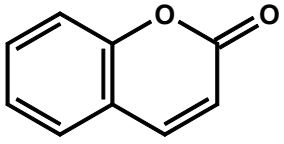

Fig. 1

Chemical structure of coumarin

Experimental sample preparation

Coumarin can be extracted easily from homogenized cinnamon product samples using an ethanol/water 70:30 (v/v) mixture as the solvent. After centrifugation and filtration, the sample is ready for analysis by HPLC or UHPLC methods. The sample was supplied by a private German food control laboratory.

Experimental preparation of standard solution

A standard solution of coumarin was prepared by weighing out the desired mass of 1mg and dissolving it in the mobile phase water/methanol 50:50 v/v to the desired concentrations. The standard solution was supplied by a private German food control laboratory.

Method parameters

Column	BlueOrchid C18 1.8 µm, 100 x 2 mm		
Eluent A	Water		
Eluent B	Methanol		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	50	50
	4.60	30	70
	4.70	5	95
	6.00	5	95
Flow rate	0.3 ml/min		
Injection volume	2 µl		
Column temperature	30 °C		
System pressure	approx. 530 bar		
Detection	UV at 278 nm (50 Hz)		
Run time	6 min		

Results

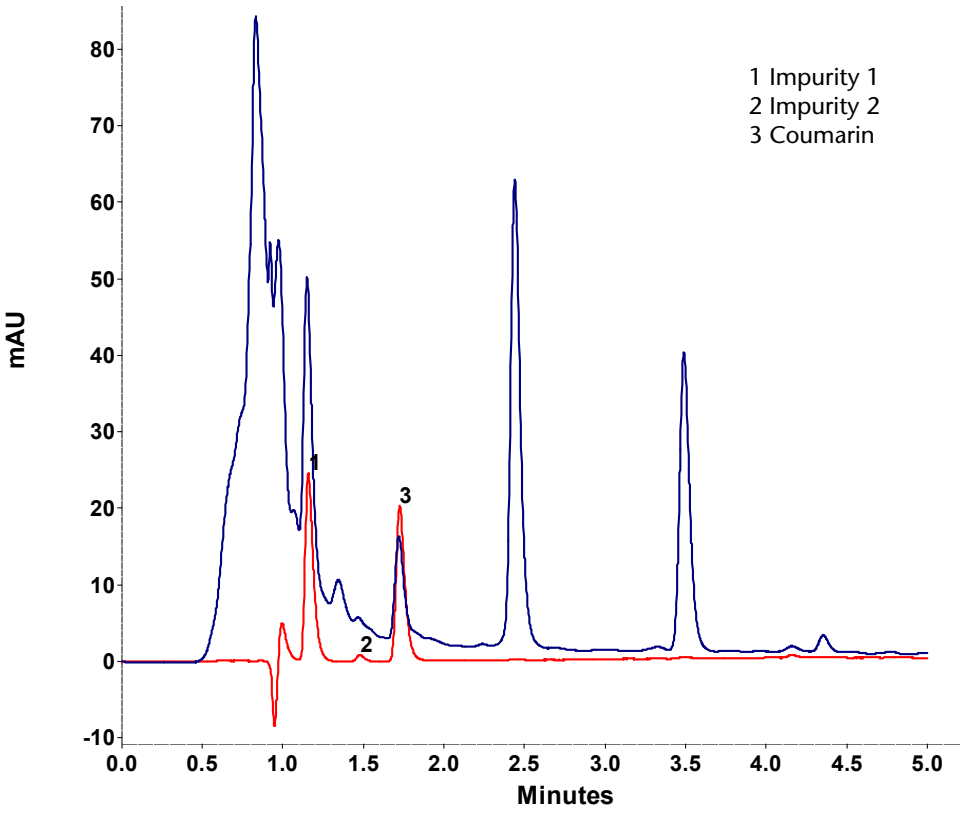

Fig. 2

Separation of coumarin mixtures (red: standard, blue: sample cookies)

Using a KNAUER PLATINblue UHPLC system and a BlueOrchid C18 1.8 µm column, coumarin was successfully separated from impurities and the biscuit-extract matrix in less than 4 min (Fig. 2), more than 5x faster than the conventional HPLC gradient method. Additionally, saving is 92 % concerning the eluent and 78 % concerning the analysis time per run applying the UHPLC method presented in this application note.
Retention time reproducibility of the UHPLC method lies in the range of less than 0.3 % RSD (n = 8) what underlines its robustness and reliability.

Method performance

Retention time precision*	< 0.3 % RSD
Peak area precision*	< 3 % RSD

*repeatability calculated over 8 replicate runs

Conclusion

In this Application note, a UHPLC method for the fast determination of coumarin in cinnamon products is presented. It becomes obvious, that the advantages of applying a PLATINblue UHPLC system and BlueOrchid sub-2 µm columns show outstanding results.
From an economic point of view, saving is 92 % concerning the eluent and 78 % concerning the analysis time per run. Equilibration time is also minimized by using a short column with small inner diameter what saves again time and also eluent.
As a conclusion, the high speed analysis of coumarin in cinnamon products illustrates how food quality analyses can benefit from sub-2 µm columns like BlueOrchid in combination with a PLATINblue UHPLC system in terms of faster separations, higher resolution, higher sensitivity and reduced mobile phase consumption. All these facts show that the presented method is well suited for the routine analysis in food safety control.

References

1 Carlton, Betsy D.: Coumarins, Encyclopedia of Toxicology (Second Edition) Pages 674-676 (2005)

2 Federal Institute for Risk Assessment (BfR), Frequently Asked Questions about coumarin in cinnamon and other foods (Updated FAQs, of 2006-10-30)

3 Federal Institute for Risk Assessment (BfR), Deutsche Lebensmittel-Rundschau, 103. Jahrgang, Heft 10, Pages 480 - 487 (2007)

Physical properties of recommended column

BlueOrchid C18 use hydrophobic interactions for separation mechanism and offers an extended pH range for analysis of acidic, basic and neutral analytes in reversed phase mode. All BlueOrchid phases feature exceptional peak symmetry and resolution. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.

Stationary phase	BlueOrchid C18 1.8 µm
USP code	L1
Pore size	175 Å
Pore volume	0.98 ml/g
Specific surface area	320 m^2/g
Particle size	1.8 µm
Form	spherical
Surface area	320 m^2/g
% C	19
Endcapping	yes
Dimensions	100 x 2 mm
Order number	10BI181BOE

Recommended instrumentation

This application requires the PLATINblue binary high pressure gradient UHPLC system equipped with degasser, autosampler, column thermostat, and PDA detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
PLATINblue UHPLC-System	A69420
PLATINblue Pump P-1	
PLATINblue Pump P-1 with Degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue Detector PDA-1	
PDA-1 flow cell (10 mm, 2 µl)	
PLATINblue modular eluent tray	
PLATINblue CG Data system	
PLATINblue CG PDA license	
PLATINblue stainless steel capillary kit	

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