KNAUER

Application Note

► Fast analyses of additives in soft drinks by minimal sample preparation

Category	Food analysis
Matrix	Soft drinks
Method	HPLC
Keywords	Preservatives, sweeteners
Analytes	Ascorbic acid, acesulfam K, saccharin, caffeine, aspartame, sorbic acid, benzoic acid
ID	**VFD0009N**, 10/08, updated 05/11

Summary

A direct analysis of additives in soft drinks with a short sample preparation is demonstrated. The method involves sweeteners and preservatives.

Introduction

In the last year's soft drinks with low calories became more and more importance by the consumers. The addition of preservatives in this case is especially required.[1] Both of them, sweeteners and preservatives are highly polar molecules which causes problems in extraction-methods. Therefore the best choice is a direct injection without any extraction steps. To realize adequate retention times the use of a reversed phase with polar embedded groups, that allows a high amount of water in the eluent is essential. [2]

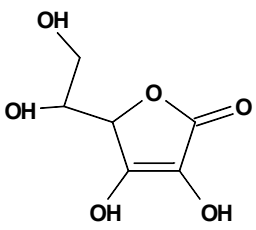
Experimental sample preparation

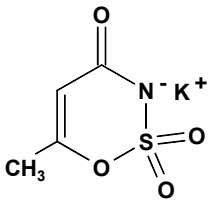
Soft drink probes can be easily treated in an ultrasonic bath to remove the carbon dioxide for 2 minutes. After filtering through a 0.45 µm syringe filter the sample is ready to inject.

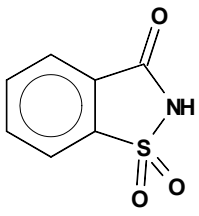
Experimental preparation of standard solution

All standard solutions were prepared with 100% buffer. A preliminary standard (V1) was prepared by weighing out 50 mg of ascorbic acid and 25 mg each of the other standards compounds in a 50 ml flask. The final standard (level 3) was created by adding 10 ml of the V1 solution to the second flask and adjusting the volume to 100 ml with buffer. The level 2-standard was created by adding 4 ml of level 3-standard in a 10 ml flask and adjusted to 10 ml with buffer. The same operation was done with 2 ml for level 1-standard. The final concentration of each compound in the three levels was as follows:

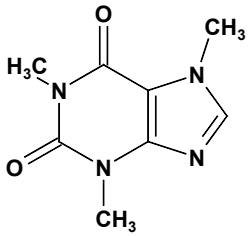
	level 1	level 2	level 3
Ascorbic acid	0.02 mg/ml	0.04 mg/ml	0.10 mg/ml
other compounds	0.01 mg/ml	0.02 mg/ml	0.05 mg/ml



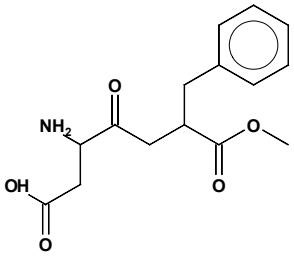




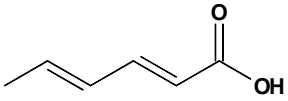
Ascorbic acid (1)



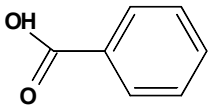
Acesulfam K (2)



Saccharin (3)



Caffeine (4)



Aspartame (5)

Sorbic acid (6)

Benzoic acid (7)

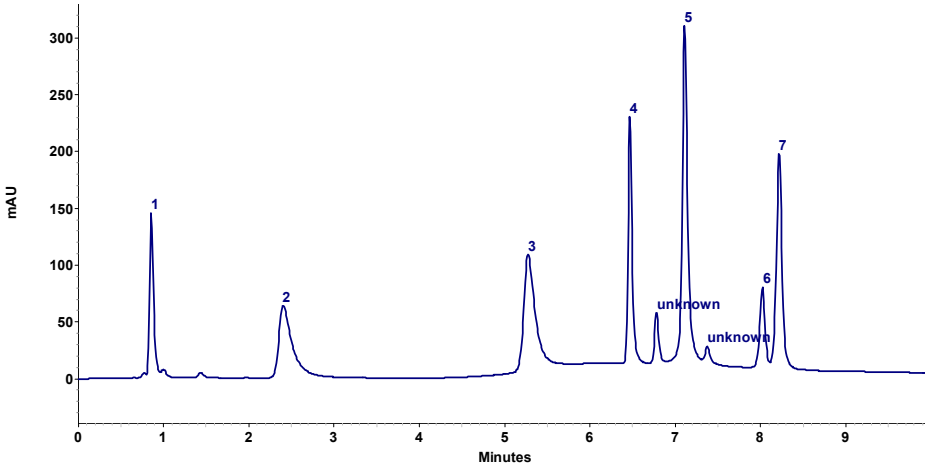


Fig. 1

Chromatogram of the level 2 standard.

Method parameters

Column	ProntoSIL 120-3 C18 ace EPS 100 x 2 mm			
Eluent A	20 mM KH_2PO_4 (adjusted to pH 3)			
Eluent B	acetonitrile			
Gradient	**Time [min]**	**% A**	**% B**	**Flow rate [ml/min]**
	0.00	100	0	0.40
	1.50	100	0	0.40
	5.00	65	35	0.40
	8.50	65	35	0.40
	10.00	100	0	0.70
	15.00	100	0	0.70
	16.00	100	0	0.40
Injection volume	5 µl			
Column temperature	40 °C			
System pressure	approx. 88 bar for 0.4 ml/min			
Detection	UV at 220 nm			
Run time	8.5 min (16 min incl. regeneration)			

Results

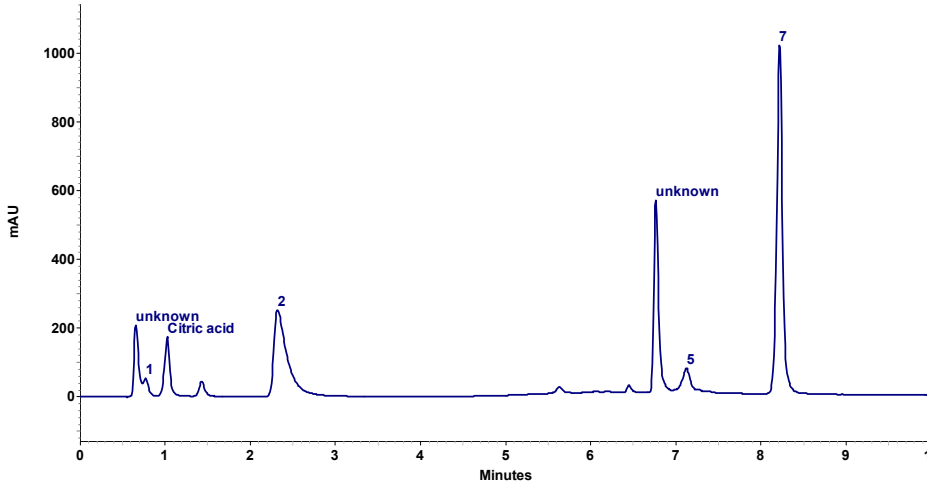


Fig. 2

Chromatogram of direct injection of a lemon soft drink

Substance	t_R [min]	mg/l	LOD [mg/l]
Ascorbic acid	0.87	0.014	0.00013
Acesulfam K	2.44	0.085	0.00017
Saccharin	5.31	-	-
Caffeine	6.46	-	-
Aspartam	7.11	0.007	0.008
Sorbic acid	8.03	-	-
Benzoic acid	8.22	0.129	0.0010

Conclusion

A separation of soft drink additive with easy sample preparation is accomplished by reversed-phase HPLC using the ProntoSIL C18 ace EPS column and Smartline HPLC. It could be appear a separation of molecules with different polarities and a runtime less than 12 minutes.

References

1 Pressemitteilung Landesbetrieb Hessisches Landeslabor 4. April 2008

2 Ron Lewis, LC Varian Application Note Nr. 23

Physical properties of recommended column

ProntoSIL C18 ace APS belongs to the new group of stationary RP-supports with polar embedded groups. This phase can be used with 100% water us eluent. In addition, it offers a maximum of hydrophobicity combined with a maximum of polar selectivity. For the separation of basic and acidic groups these supports exhibit an enhanced polar selectivity.

Stationary phase	ProntoSIL 120-3 C18 ace EPS
USP code	L1
Pore size	120 Å
Pore volume	1.06 ml/g
Specific surface area	323 m^2/g
Particle size	3 µm
Form	spherical
Surface area	300 m^2/g
% C	18.5
Endcapping	yes
Dimensions	100 x 2 mm
Order number	10BF18APSG

Recommended instrumentation

This application requires a binary gradient HPLC system (low pressure or high pressure gradient configuration) equipped with degasser, autosampler, column oven, and multi-wavelength UV detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
Smartline Pump 1050, incl. 10 ml pump head	A50353-1
Smartline Manager 5050 with LPG and degasser	A5333
SmartMix static mixer	A5351
Autosampler 3950	A5005-1
Smartline Column Oven 4050	A5300
Smartline UV Detector 2600	A5200
10 mm flow cell	A4061
ChromGate software	A1493

Author

Dr. Elmar Tepper, Columns and Applications Department, KNAUER

Contact information

Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
14163 Berlin, Germany

Tel: +49-(0)30-809727-0
Fax: +49-(0)30-8015010
E-Mail: info@knauer.net
Internet: www.knauer.net