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Application Note

Determination of preservatives in foodstuffs and cosmetics

Category	Food
Matrix	Foodstuff / cosmetics
Method	HPLC
Keywords	Preservatives
Analytes	Sorbic acid, benzoic acid, para hydroxybenzoic acid (PHB), methylparaben (PHB-meth), ethylparaben (PHB-eth), propylparaben (PHB-prop), butylparaben (PHB-butyl), 2-methoxybenzoic acid (IS)
ID	**VFD3**, 12/07, updated 08/10

Summary

The inspection of adherence to legal limits of preservatives in foodstuffs and cosmetics can be performed by HPLC. This method describes an analysis procedure of preservatives in food and cosmetics with reversed phase HPLC in the application range of 0.05 up to 0.2 %.

Introduction

Preservatives in food and cosmetics are added to prevent any alteration or degration caused by the microbial contamination [1], and to protect the health of consumers. The common preservatives that have been widely used in cosmetics and foodstuffs are parabens or esters of 4-hydroxybenzoic acid. Using parabens in small amounts minimizes their potential health risks. Consequently, the quantitative analysis of parabens in consumer products is important. The typical concentrations of preservatives found in directly consumable commercial foodstuffs lie around 0.1 % for sorbic acid and benzoic acid, and around 0.05 % for para hydroxybenzoic acid (PHB) and PHB-ester. Several analytical methods, including gas chromatography (GC) and liquid chromatography (LC) have been reported [2-4]. Determination of the preservatives found in such extracts is accomplished through HPLC with subsequent UV detection at various wavelengths.

Experimental

Sample preparation

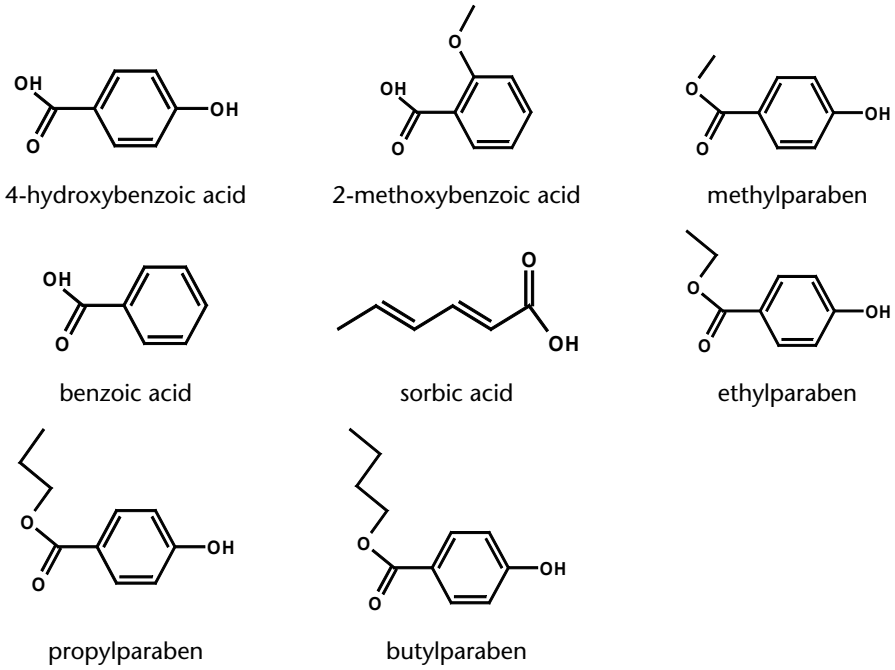
Preservatives in food or cosmetics will be extracted by means of ammonium formiate buffer and methanol (60:40, v/v) in ultrasonic bath and slight warming. The buffer solution will be adjusted at pH 4.8 by adding 5% ammonia solution. 1 to 5 g of the homogenized sample are extracted with 20 ml extraction solution in an ultrasonic bath for 10 min. 1 ml of the internal standard solution (2-methoxybenzoic acid, 1 mg/ml) is added to the extraction solution for increased accuracy of the method. The suspension is transferred to a 50 ml volume flask. Interfering additives can be removed with 1 ml Carez I (150 g/l solution of potassium hexacyanoferrate in water) and 1 ml Carez II (300 g/l zinc sulfate in water). By analyzing 1 g of the sample this is equivalent to 0.5 – 2 g preservative/kg or 0.05% – 0.2 %.

Experimental

Preparation of standard solution

Stock solutions of each of the standards (sorbic acid (E200-203), benzoic acid (E210-213), 4-hydroxybenzoic acid (PHB, E218-E219), methylparaben (PHB-met, E218-219), para hydroxybenzoic acid ethyl ester (PHB-eth, E214-215), para hydroxybenzoic acid propyl ester (PHB-prop, E216-2117), para hydroxybenzoic acid butyl ester (PHB-but) and 2-methoxybenzoic acid (ISTD) were prepared in water/methanol (60:40, v/v) at a concentration of 1 mg/ml. Identification of the substances present is made through their spectrums (see fig. 1 and 2) and retention times. This is of advantage because a variety of the sample's components also absorb at 235 nm, such as artificial sweeteners and antioxidants. The chromatographic conditions (eluent concentration, flow rate, temperature) were optimized in such a way that the preservatives analyzed could be separated as fast as possible in one run. The concentration range for the calibration was 5 mg/l to 100 mg/l.

Chemical structures



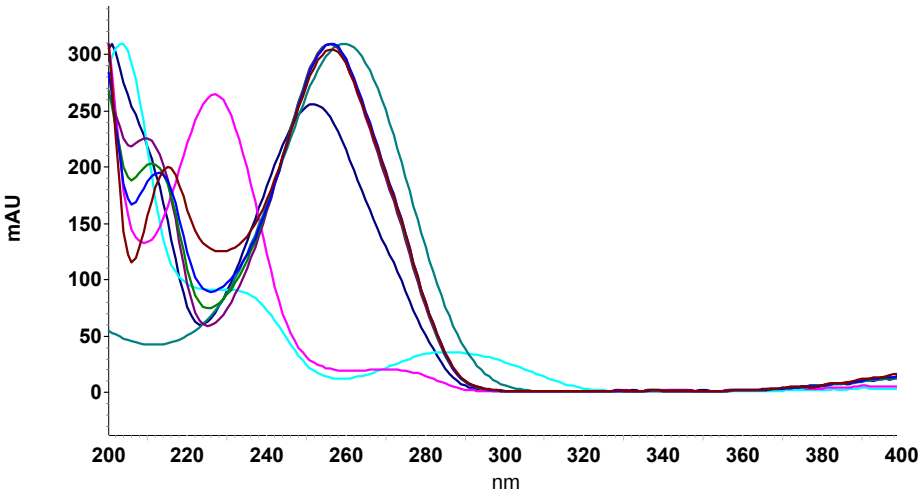


Fig. 1

Wavelength spectrum of analyzed preservatives

Method Parametersp

Column	Eurospher 100-5 C8, 125 x 4 mm		
Eluent A	A: Ammonium formiate buffer/methanol 50:20		
Eluent B	B: Ammonium formiate buffer/methanol 50:70		
Gradient	Ammonium formiate buffer: 0.4 ml formic acid and 0.8 ml ammonia (25%) filled with water to 1l		
	Time (min)	**% A**	**% B**
	0.00	100	0
	10.00	0	100
	12.00	0	100
	12.02	100	0
	15.00	100	0
Flow rate	1.2 ml/min		
Injection volume	20 µl		
Column temperature	40 °C		
System pressure	approx. 85 bar		
Detection	UV at 255 nm or λ program: 0.00 min 255 nm 1.80 min 235 nm 2.90 min 255 nm		
Run time	12 min		

Results

All of the standards gave very good linear calibration curves with regression coefficients (r^2) of 0.9998 or better. This is caused by the method of internal standard. The variation coefficient is listed in table 1. The determined working range for analyzing food and cosmetic samples is 0.05 up to 0.5 %. By way of the spectrum check feature, an additional assurance of the sample values obtained was possible. This feature is useful for confirming the identity of each preservative peak, particularly when analyzing foodstuff and cosmetic matrices in which additional components that coelute with the preservatives of interest are common. Such coelution can be simply detected with the peak purity function during spectrum acquisition. For the investigated food sample (liver sausage) the assay results are listed in table 2.

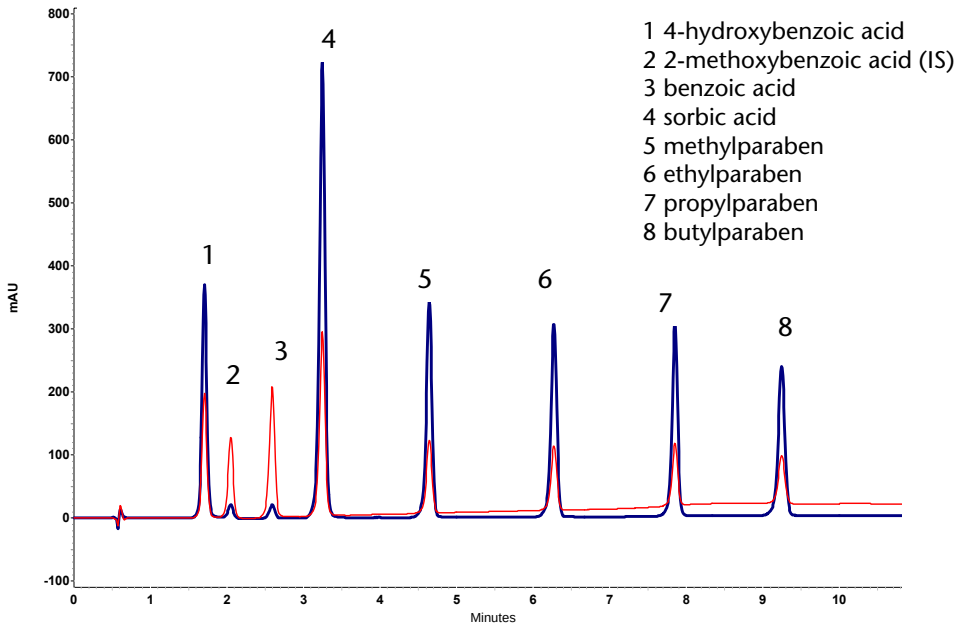


Fig. 2

Separation of preservative standard at different wavelength (255 nm blue, 235 nm red)

Table 1
Variation coefficient for Method of internal standard

Substance	Coefficient [%]
sorbic acid	1.39 %
benzoic acid	1.75 %
para hydroxybenzoic acid (PHB)	1.62 %
para hydroxybenzoic acid methylester (PHB-meth)	2.15 %
para hydroxybenzoic acid ethylester (PHB-eth)	2.04 %
para hydroxybenzoic acid propylester (PHB-prop)	3.59 %
Para hydroxybenzoic acid butylester (PHB-but)	5.32 %

Table 2
Assay results for food

Substance	t_R (min)	Amount [g/kg]	LOD (mg/l)
sorbic acid	3.187	0.89	0.25
benzoic acid	2.575	0.67	1.40
PHB	1.692	0.35	0.50
PHB-meth	4.608	0.16	0.52
PHB-eth	6.266	-	0.57
PHB-prop	7.716	-	0.61
PHB-but	9.142	0.21	0.75

Method performance

Limit of detection	ng range (S/N = 3)
Linearity (r^2)	0.99981-0.99992
Linearity range	0.25 to 50 mg/l
Retention time precision*	< 1 % RSD
Peak area precision*	< 2 % RSD

* repeatability calculated over 5 replicate runs

Conclusion

HPLC is powerful tool for analyzing preservatives in a wide range of consumer products. An uncomplicated and fast analysis of preservatives can be carried out using a short Eurospher 100-5 C8 column.

References

1 J. E. Foulke "A fresh look at food preservatives" in FDA Consumer (October 1993), US. Food & Drug Administration

2 L. Gagliardi, D. Orsi, P. Chimenti, R. Porra, and D. Tonelli, Anal.Sci., 2003, 19, 1195

3 L. Labat, E. Kummel, and J.P. Dubost, J. Pharm. Biomed. Anal., 2000, 23, 763

4 J.P.Rauha, H. Salomies, and M.Aalto, J. Pharm. Biomed. Anal., 1996, 15, 287

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Physical properties of recommended column

The endcapped, less hydrophobic Eurospher C8 packing material can be universally used in different application areas. The stationary phase is stable in a pH range from 2 – 8.5.

Stationary phase	Eurospher 100-5 C8
USP code	L7
Pore size	100 Å
Pore volume	0.9 ml/g
Specific surface area	350 m^2/g
Particle size	5 µm
Form	spherical
% C	7
Endcapping	yes
Dimensions	100 x 4 mm
Order number	10DE081ESJ

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.

Description	**Order No.**
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with LPG and degasser	A5313
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
ChromGate PDA License for Detector 2600	A1459

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