Identification and determination of p-hydroxybenzoic acid, its esters and 4-methoxycinnamic acid derivatives

A-15.5

Key words

Quantitative HPTLC analysis - densitometry by absorbance - quality assurance - stability tests - process control - food analysis - pharmaceuticals - cosmetics - p-hydroxybenzoic acid - 4-methoxycinnamic acid derivatives

Scope

p-Hydroxybenzoic esters (parabens, PHB) are used as stabilizers in cosmetic preparations. Typical concentrations are 0.1 - 0.3% methylparaben and 0.05 - 0.15% propylparaben respectively. 4-Methoxycinnamic esters in a concentration of about 2% serve as light absorbing agents in sun screen and tanning lotions. Substrates of such cosmetics are usually emulsions or mixtures of waxes and oils.

Also parabens are added in concentrations of up to 0.3% to stabilize many kinds of food.

Described here is the analysis procedure for cosmetic preparations, including sample preparation.

Advantages of performing this analysis by instrumental TLC

- Easy sample preparation
- Positive identification by comparison of spectra and Rf values
- Simple procedure, short analysis time
- Low operating costs

Reagents

Acetone
Ethyl acetate
Pentane
Dichloromethane
Petrol ether b.p. 40-60°C
Diethylketone
Acetic acid

Standards:

p-Hydroxybenzoic acid	PHB
Methylparaben	MP
Propylparaben	PP
Parsol MCX (4-Methoycinnamate) (Giveaudan)	PA

Sample preparation

- Dissolve or suspend 1.0 g of the cosmetic preparation in 40 mL acetone - ethyl acetate 2:1 at 40°C. Let cool to room temperature
- Filter, rinse with 7 mL acetone - ethyl acetate 2:1
- Make up to 50 mL

Standard solutions

Method A, determination of MP and PP in process control:

Dissolve 50 mg MP + 25 mg PP in 50 mL acetone - ethyl acetate 2:1, then dilute 1:10.

Method B, determination of PHB in stability testing:

Dissolve 5 mg PHB + 50 mg MP + 25 mg PP in 50 mL acetone - ethyl acetate 2:1, then dilute 1:10.

Method C, analysis of stabilizing and light absorbing agents:

Dissolve 50 mg MP + 25 mg PP + 500 mg PA in 50 mL acetone - ethyl acetate 2:1, then dilute 1:10.

Layer

Precoated HPTLC plates silica gel MERCK 60 F 254, 20 x 10 cm

Sample application

Bandwise with CAMAG Linomat, 8 mm band length, distance between bands 4 mm, distance from side edge 22 mm, distance from bottom edge 8 mm = 13 applications.

Recommended application pattern

Method A	S5 5 µL	U1 10 µL	U2 10 µL	S5 5 µL	U3 10 µL	U4 10 µL	S5 5 µL	U5 10 µL	U6 10 µL	S5 5 µL	U7 10 µL	U8 10 µL	S5 5 µL
Methods B und C	S1 1 µL	U1 10 µL	S2 2 µL	U2 10 µL	S3 3 µL	U1 10 µL	S4 4 µL	U2 10 µL	S5 5 µL	U1 10 µL	S6 6 µL	U2 10 µL	S7 7 µL

U = unknown S = standard

Chromatography

In CAMAG Twin Trough Chamber 20 x 10 cm, without pre-equilibration

Developing solvent for parabens: pentane - dichloromethane - acetic acid 25:25:3

Developing solvent for parabens plus methoxycinnamic acid derivatives: petrol ether - diethylketone - acetic acid 88:5:12

Solvent migration distance about 40 mm (³10 min); after chromatography, dry plate 3 min with hair dryer.

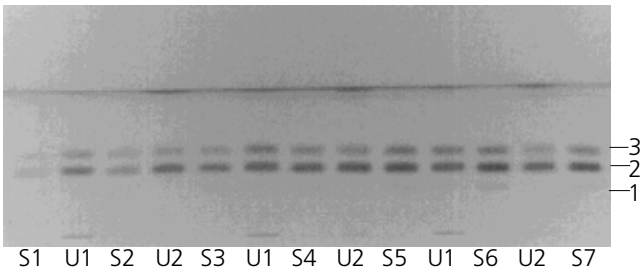

Fig. 1

PHB, MP and PP chromatographed on silica gel (method B)

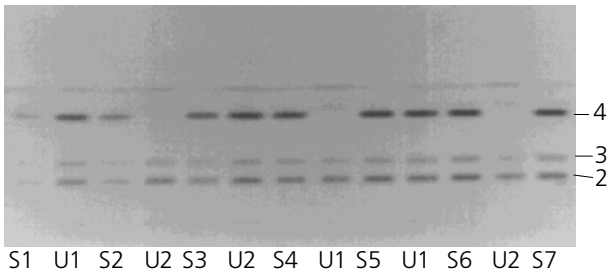

Fig. 2

MP, PP and PA chromatographed on silica gel (method C)

Densitometric evaluation

CAMAG Scanner with CATS software, deuterium lamp, scanning by absorbance at 255 nm for PHB, MP and PP, at 310 nm for PA; monochromator bandwidth 10 nm, slit dimensions 0.3 x 5 mm.

Rf-values and absorption maxima

	Substance		UV_{Max}	A (R_F)	B (R_F)	C (R_F)
1	p-Hydroxybenzoic acid	PHB	258	---	0.32	---
2	Methylparaben	MP	257	0.45	0.45	0.32
3	Propylparaben	PP	257	0.58	0.58	0.46
4	Parsol MCX	PA	309	---	---	0.78

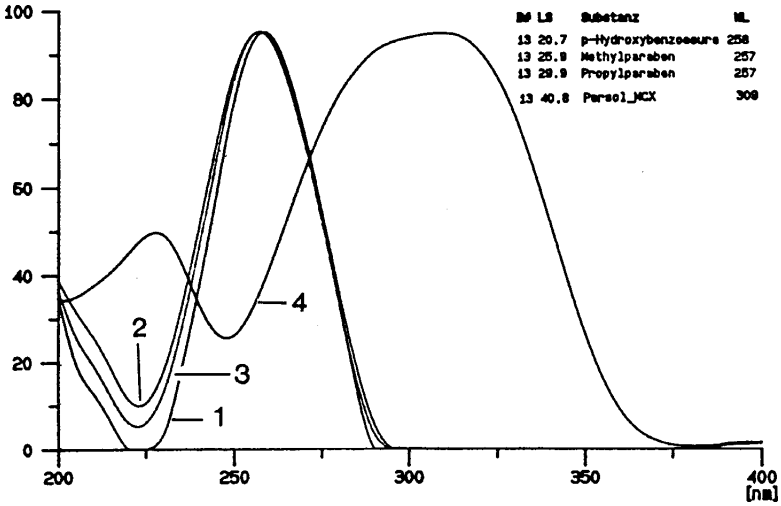

Fig. 3 UV spectra of free p-hydroxybenzoic acid PHB (1), methylparaben MP (2), propylparaben PP (3) and Parsol PA (4); the spectra 1-3 are typical for PHB esters but differences between the individual ones are very small. The individual PHB esters are identified by their Rfs.

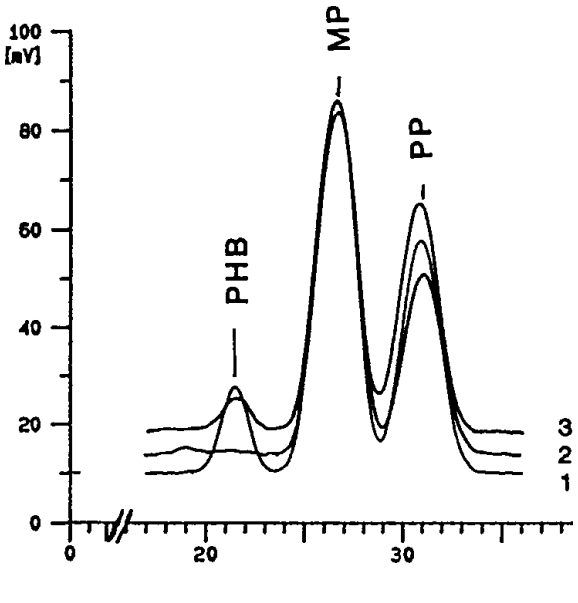

Fig. 4

Densitogram curves according to methods A and B: (1) standard solution,
(2) extract from a night cream, (3) extract from a body lotion.

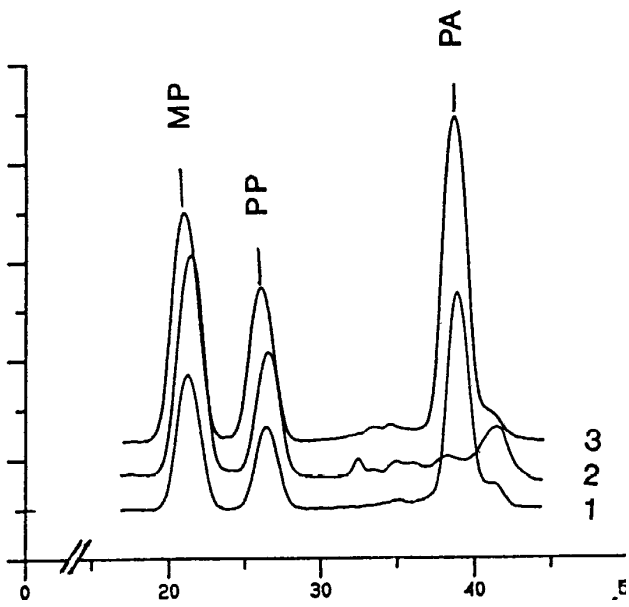

Fig. 5

Densitogram curves of standard solution C (1), of an extract from a night cream (2), and of a tanning lotion containing Parsol MCX (3)

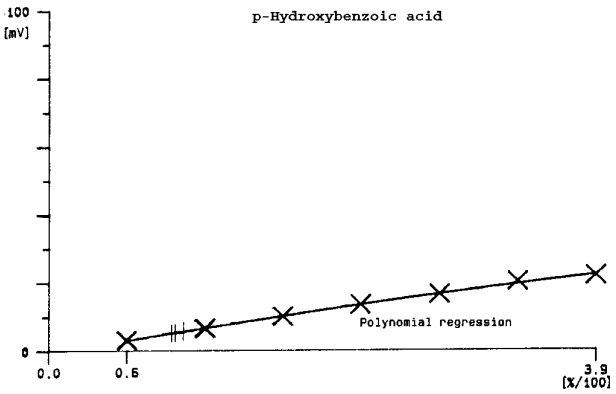

Fig. 6 Calibration function for p-hydroxybenzoic acid in the range from 0.5 to 3.5 mg/g (0.05 - 0.35%) method B

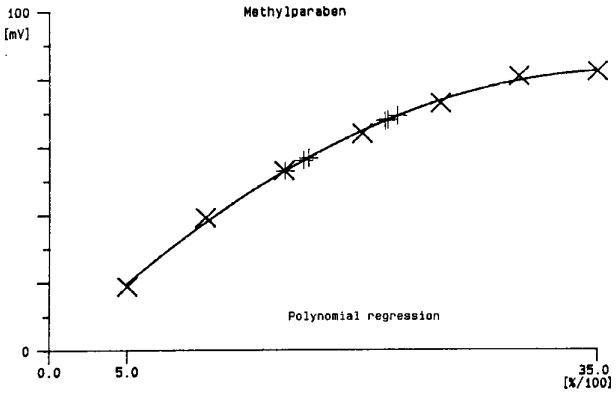

Fig. 7 Calibration function for methylparaben in the range from 5.0 to 35.0 mg/g (0.5 -3.5%) method B

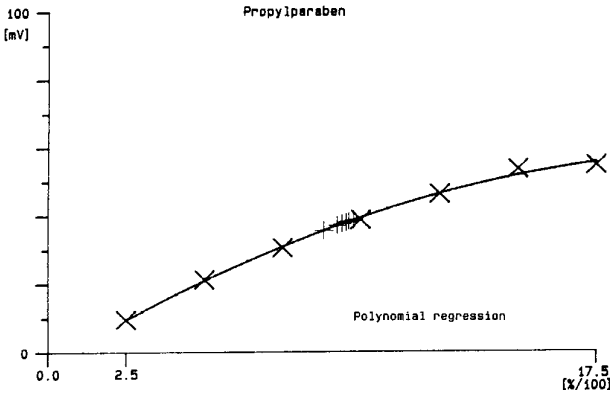

Fig. 8 Calibration function for propylparaben in the range from 2.5 to 17.5 mg/g (0.25 - 1.75%) method B

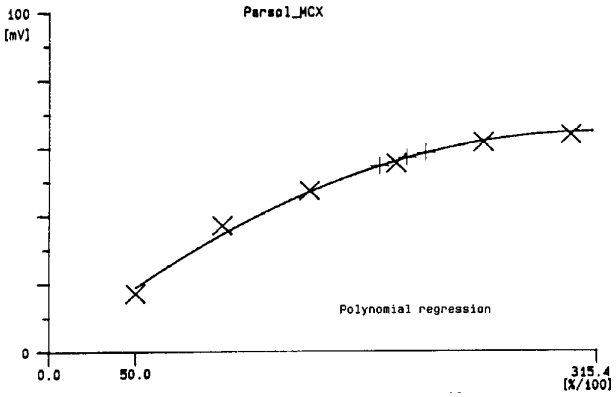

Fig. 9 Calibration function for Parsol MCX in the range from 50 to 350 mg/g (5.0 - 35.0%) method C

Remark

Method precision: depending on kind of sample and component 2 - 6%

Recovery: depending on kind of sample and component 87-100%

Literature

G. Zimmermann: (TLC as a tool in the analysis of cosmetic products) presented at the 8th Symposium of the German Association for Scientific and Applied Cosmetics, Hamburg, November 1989