Application Note: ANCCSHYPGOLDCOS

HPLC Analysis of Twenty One Preservative Compounds Found in Cosmetics Using a Thermo Scientific Hypersil GOLD Phenyl Column

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Key Words

- Hypersil GOLD Phenyl
- Cosmetics
- Preservatives
- Parabens

Abstract

This application note demonstrates the use of a Thermo Scientific Hypersil GOLD Phenyl HPLC column for the analysis of twenty one preservative compounds found in cosmetics. All analytes were retained, with thirteen showing full baseline resolution and four pairs being partially resolved. The analysis can be performed on standard HPLC instrumentation with solution conditions suitable for MS detection.

Introduction

The Hypersil GOLD® range of HPLC columns were developed to give reproducible and reliable chromatography analysis with excellent peak shape. The Hypersil GOLD Phenyl HPLC column offers excellent peak shape and alternative selectivity to the standard Hypersil GOLD HPLC column. In particular the presence of the phenyl group enhances the separation of aromatic compounds and moderately polar compounds by providing opportunity for a second mode of interaction through the phenyl ring.

Preservatives are added to cosmetic formulations to ensure that they do not carry pathogenic microorganisms and to eliminate the growth of microbes. In the USA the FDA requires that all cosmetics have to be adequately preserved and proof of this has to be prepared for every shipment. In Europe there is a list of allowed and restricted preservatives in Cosmetic Directive 76/768/EEC. It is therefore necessary for cosmetic product manufacturers to develop methods to screen cosmetics for these compounds. This analysis has become more important in recent years as there has been a rising public awareness of the additives in found in cosmetics and their health effects. In this application twenty one preservative compounds found in cosmetics are analyzed by HPLC. Some of these are aromatic and the Hypersil GOLD Phenyl HPLC column was selected to offer the best performance in separating these, in many cases, closely related compounds.

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific LC-MS grade water	W/0112/17
Thermo Scientific Pierce LC-MS grade acetonitrile	TS-511001
Fisher Scientific ammonium acetate	A/3440/50
Fisher Scientific acetic acid	A/0400/PB08

Sample Handling Equipment	
Liquid handling hardware: FinnPippette Kit 1; Includes 3 Thermo Scientific Finnpipette F2 Pipetters from 1 to 1000 µL	4700870
Vials and closures: NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific HPLC system	
Column(s):	Hypersil GOLD Phenyl 3 µm, 100 x 2.1 mm	25903-102130
Mobile phase:	(A) 5:95 acetonitrile:20 mM ammonium acetate, pH 4.45	
	(B) acetonitrile	
Gradient:	100 – 15% A over 20 minutes	
Flow rate:	0.5 mL/min	
Run time:	30 minutes (including equilibration time)	
Column temperature	30 °C	
Injection details:	2.0 µL	
UV detector wavelength:	254 nm and 214 nm	

Solutions

Standard preparation: A 1 mg/mL solution of each of the preservative compounds in methanol was diluted with water to give a final concentration of 12 µg/mL.

Data Processing	
Software:	Thermo Scientific Chromatography Software

Results

The analysis of twenty one preservative compounds commonly found in cosmetics was carried out on a Hypersil GOLD Phenyl HPLC column and the chromatograms are given in Figures 1 and 2 for detection at 254 and 214 nm respectively. Detection was carried out at these two wavelengths because some of the compounds do not absorb light at 254 nm. The chromatogram for detection at 214 nm (Figure 2) has had the baseline subtracted to correct for the large change in absorbance over the course of the gradient program. The list of compounds analyzed is given in Table 1 and the retention times and method precision for three selected compounds in six injections are given in Table 2.

Conclusions

- Twenty one preservative compounds commonly found in cosmetics are identified and separated by a Hypersil GOLD Phenyl HPLC column.
- For thirteen of the compounds baseline resolution was achieved and four pairs of compounds were partially resolved (6 and 7, 8 and 9, 11 and 12, 13 and 14).
- The analysis is reproducible with low % RSD and the mobile phase components are suitable for both MS and UV detection.

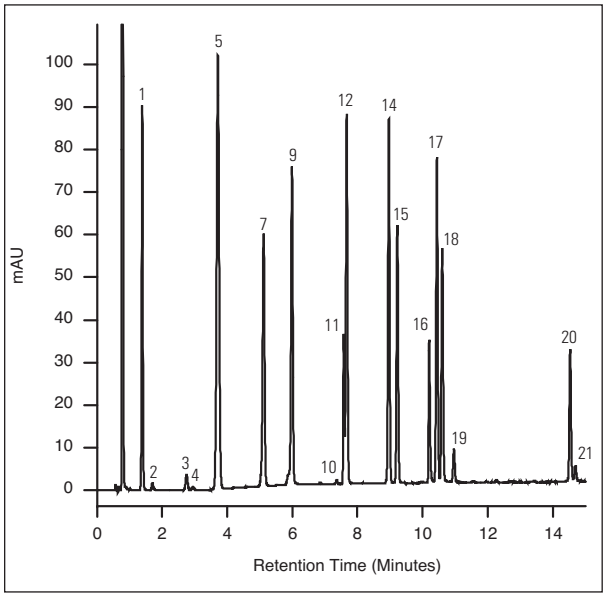

Figure 1: Chromatogram for twenty one preservative compounds found in cosmetics separated on an Hypersil GOLD Phenyl 3 µm, 100 x 2.1 mm column at 254 nm (compounds 6, 8 and 13 do not absorb at 254 nm)

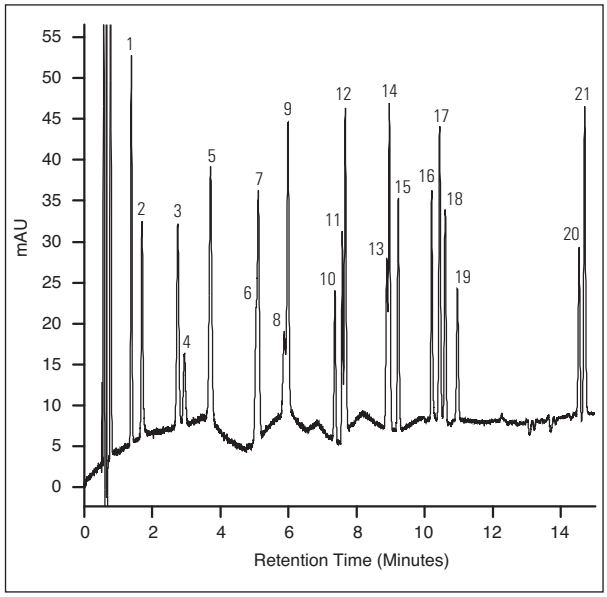

Figure 2: A chromatogram for twenty one preservative compounds found in cosmetics separated on a Hypersil GOLD Phenyl 3 µm, 100 x 2.1 mm column at 214 nm (all the compounds can be detected at this wavelength). A blank chromatogram is subtracted from the data and the raised baseline is due to absorption from mobile phase components

	Compound		Compound
1	4-hydroxybenzoic acid	12	Ethylparaben
2	Salicylic acid	13	Dichlorobenzyl alcohol
3	Benzoic acid	14	Isopropylparaben
4	Benzyl alcohol	15	Propylparaben
5	Sorbic acid	16	Chlorhexidin
6	Phenoxyethanol	17	Isobutylparaben
7	p-Anisic acid	18	Butylparaben
8	Dehydroacetic acid	19	o-Phenylphenol
9	Methylparaben	20	Triclocarban
10	Chlorphenisin	21	Triclosan
11	Hexamidine 2-hydroxyethansulfonate		

Table 1: List of preservative compounds analyzed

Peak	t_R/min	% RSD
5	3.73	0.00
19	11.03	0.13
20	14.51	0.08

Table 2: Average retention time and method precision (% RSD) for six replicate injections for three of the twenty one compounds

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