Separation of Paraben Preservatives by Reversed-Phase HPLC

Application

Foods, Beverages, and Cosmetics

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Abstract

Paraben preservatives are shown to be readily and quickly analyzed using reversed-phase HPLC with a ZORBAX Eclipse XDB-C18 Rapid Resolution column.

Introduction

Preservatives are a class of chemical agents that are commonly used to prevent the growth of bacteria in foods, beverages, and cosmetics. The paraben preservatives (4-hydroxybenzoic acid esters) are among the most widely used. These preservatives were developed in the 1930's to stabilize creams.

Synthetic methyl, ethyl, and propyl parabens were developed from benzoic acid and were considered effective and economical since they were inexpensive to use as both a cosmetic and food grade preservative. It is estimated that 99% of all cosmetic and body care products contain some form of paraben preservatives. Methyl and propyl parabens are generally recognized as safe (GRAS) substances. Recently, however, this preservative system has come into question as these substances were found in cancerous tissues, especially breast tissue.

A study by the Journal of Pharmaceutical Science revealed that after receiving multiple doses of a gentamicin formula containing paraben preservatives, six infants found traces of up to 82.6% of the parabens in urine samples.

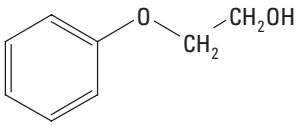
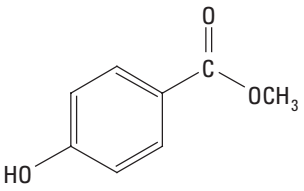
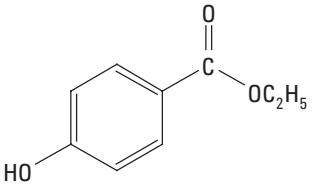
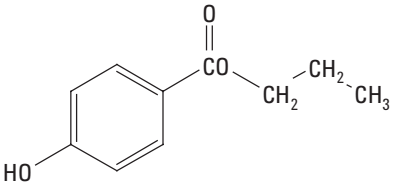
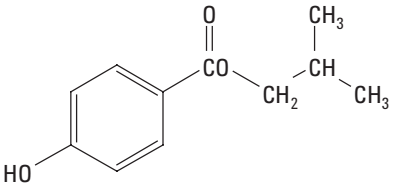
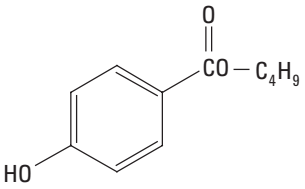
Researchers of the Department of Biology and Biochemistry of Brunel University in the United Kingdom found that the greatest concern regarding parabens focuses on their estrogen-mimicking ability in laboratory animals. In addition, 2-phenoxyethanol (2PX), a chemical substance also used as a preservative in several vaccines, is sometimes used in conjunction with parabens. Paraben mixtures have the advantages of being broad-spectrum, leading to reduced inventory levels and cost savings. It is easier to handle one liquid in reasonable quantities rather than several small quantities of powders or liquids. Phenonip, a product of Clariant Ltd, Horsforth, Leeds, United Kingdom, is a mixture of parabens in 2PX solution. This product is probably the best known of the paraben mixtures and is often copied.

Analysis of these substances at formulation and trace levels in foods and cosmetics is of great interest. HPLC is an ideal method for their separation and analysis.

Chemical Characteristics

The structures of 2PX and the parabens are depicted in Table 1. Due to their phenyl ring, these compounds are UV-detectable at extremely low concentrations. Since they have no ionic functional groups, they are considered lipophilic. Due to this lipophilicity, some accumulation in fatty tissues of the body would be expected. Parabens are slightly soluble in water, with the solubility decreasing as the ester chain length increases. For example, methyl paraben dissolves at the 0.25% (w/w) level at 20 °C while butyl paraben is soluble at the 0.02% (w/w) level. Most of the parabens are freely soluble in alcohol, acetone, ether and a number of other organic solvents. With such solubility properties, reversed-phase chromatography (RPC) is an ideal separation technique. Many reversed-phase separations of parabens are published in the chromatography literature [1–4].

Table 1. Structures and Concentrations of Preservative Compounds

2PX:	2-Phenoxyethanol (1.4 mg/mL)	
MEP:	Methylparaben (0.30 mg/mL)	
ETP:	Ethylparaben (0.07 mg/mL)	
PRP:	Propylparaben (0.04 mg/mL)	
IBP:	Isobutylparaben (0.04 mg/mL)	
BTP:	Butylparaben (0.08 mg/mL)	

Chromatographic Conditions

Column:	ZORBAX Eclipse XDB-C18 Rapid Resolution, 4.6 mm × 150 mm, 3.5 µm	
Mobile phase:	Solvent A: Water Solvent B: Methanol	
Gradient:	Time	% MeOH
	0	38
	5	38
	6	60
	16	60
	17	62
	20	38
Flow rate:	0.8 mL/min	
Temperature:	40 °C	
Detector:	UV 254 nm	
Injection volume:	5 µL	

Results and Discussion

The separation of the parabens and 2PX contained in a paraben product mix is depicted in Figure 1.

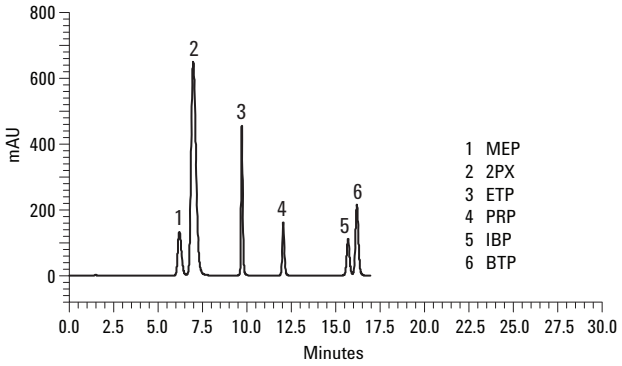


Figure 1. Separation of preservatives by reversed-phase HPLC.

Sample preparation merely involved dilution of the sample mix with methanol. All components were separated to baseline. On other columns, the separation of MEP and 2PX and IBP and BTP is usually quite difficult, especially in such a short analysis time (16 min). The method is reproducible with good separation efficiency.

Conclusion

Paraben preservatives are readily and quickly analyzed using reversed-phase HPLC with a ZORBAX Eclipse XDB-C18 Rapid Resolution column, 4.6 mm × 150 mm, 3.5 µm.

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Printed in the USA
August 16, 2005
5989-3635EN

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