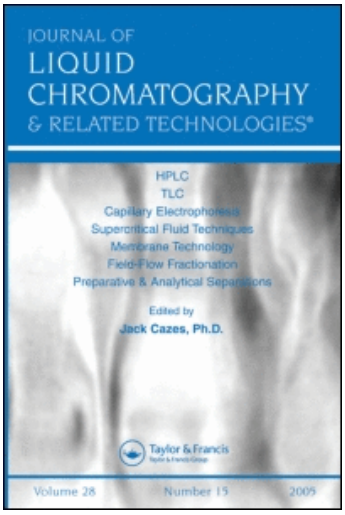

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Analysis of Hydroxybenzoic Acids by High Pressure Liquid Chromatography

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ANALYSIS OF HYDROXYBENZOIC ACIDS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

Good separation of a range of hydroxybenzoic acids was achieved by HPLC on a μBondapak phenyl column using 5% v/v acetic acid in water as the eluting solvent.

INTRODUCTION

Phenolic acids are found in most plants. They are of interest due to their involvement in the development of flavour, colour and texture in foods such as wine, fruit, vegetables and tea. Various phenolic acids also possess anti-microbial and anti-fungal activity, have pharmacological properties and can act as phytoxins [1]. Separation and identification of these compounds is usually by thin layer and paper chromatography [2]. High pressure liquid chromatography (HPLC) is a recent development that shows promise for providing more rapid analysis of phenolic acids [3-5]. Murphy and Stutte [6] have reported the most comprehensive study thus far on HPLC of phenolic acids using a reverse phase column, μBondapak C_{18} (Waters Assoc.).

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However they found it necessary to use gradient elution and a relatively complex solvent system containing butanol, methanol, acetic acid, water and ammonium acetate. In this paper we report on the resolution of hydroxybenzoic acids obtained on a μBondapak phenyl column without the use of gradient elution.

MATERIALS AND METHODS

Analyses were performed on μBondapak C_{18} and μBondapak phenyl columns (Waters Assoc.) in a Waters Liquid Chromatograph (Model No. ALC/GPC 244) using a single wavelength UV (254 nm) absorbance detector (Waters Model No. 440). The solvents examined were mixtures of acetic acid in double distilled water. Potassium chloride was also added to some solvent systems. The solvents were filtered and degassed under vacuum just prior to use. Commercially available hydroxybenzoic acids were dissolved in redistilled methanol (about 20 mg/ml) and 5-20 μl aliquots were injected onto the liquid chromatograph with the solvent flow rate at 1.5 ml/min.

RESULTS AND DISCUSSION

The solvents that produced the best resolution of the phenolic acids were 5% v/v acetic acid in water (μBondapak phenyl) and 5% v/v acetic acid plus 1% w/v potassium chloride in water (μBondapak C_{18}). Table 1 shows the data obtained in this study on both columns and also the retention data for the relevant compounds as determined by Murphy and Stutte [6]. Good resolution of phenolic acids was achieved on μBondapak phenyl and was comparable to that obtained on μBondapak C_{18} by Murphy and Stutte [6]. The use of μBondapak phenyl would be preferred as separations were obtained with a much simpler solvent system and without the use of gradient elution. Our data confirm that μBondapak C_{18} does not give good resolution with simple solvent systems.

TABLE 1

Retention Times of Hydroxybenzoic Acids Obtained by HPLC on μBondapak Phenyl and μBondapak C_{18} Columns

Compound	Retention time (min)		
	μBondapak phenyl [a]	μBondapak C_{18} [b]	C_{18} [c]
3,4,5 - trihydroxybenzoic acid (gallic acid)	3.0	2.4	2.8
3,5 - dihydroxybenzoic acid (α-resorcylic acid)	5.5	3.4	-
2,5 - dihydroxybenzoic acid (gentisic acid)	6.2	4.7	3.4
2,4 - dihydroxybenzoic acid (β-resorcylic acid)	8.1	6.1	-
4 - hydroxybenzoic acid	6.7	5.0	5.9
3 - hydroxybenzoic acid	8.1	5.7	-
2 - hydroxybenzoic acid (salicylic acid)	8.7	5.2	6.9
4 - hydroxy-3-methoxybenzoic acid (ferulic acid)	7.2	5.8	17.0

A difference in retention times of 0.5 min was found to be sufficient to give full resolution of the peaks produced by any two compounds on both columns.

Solvent systems: a - 5% v/v acetic acid in water
b - 5% v/v acetic acid + 1% potassium chloride in water
c - initial; butanol: methanol: acetic acid: water (1:5:2:92) + 18 mM ammonium acetate
Isocratic elution for 10 min, then linear gradient to final solvent system of butanol: methanol: acetic acid: water (2.4:12.5:2:83) + 18 mM ammonium acetate

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