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OPTIMIZATION OF THE SEPARATION OF *cis*- AND *trans*-HYDROXYCINNAMIC ACIDS BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The conditions for the reversed-phase thin-layer chromatographic separation of eight phenolic acids (derivatives of cinnamic acid) have been investigated. Silica gel impregnated with paraffin oil and buffers with pH values in the range 3–10 were used as stationary and mobile phases, respectively. The effect of the pH changes of the mobile phase on the partition of the *trans* and *cis* isomers of the compounds is presented.

INTRODUCTION

An interest in hydroxycinnamic acids is due mainly to their occurrence in plant kingdom and their pharmacological properties[1,2], but they also play an important role in the food industry, affecting the quality and stability of several alimentary products. Different analytical chromatographic methods for this group of compounds have been described, such as paper[3], thin-layer[4,5] (TLC), high-performance liquid[6–9] (HPLC) and gas chromatography[10–12]. The TLC technique was used only for separation of *trans* isomers of several phenolic acids using mainly silica gel or its mixture with cellulose. The reversed-phase modification, and the application of binary water–organic solvent mobile phases gave satisfactory results, but not for all the compounds studied[13].

The usage of buffers of different pH values as the mobile phase is described below, and results in good separation of *cis* and *trans* isomers of hydroxycinnamic acids.

EXPERIMENTAL

Reversed-phase thin-layer chromatography (RP-TLC) investigations were carried out on glass plates covered with a 0.25-mm layer of silica gel 60 H (Merck, Darmstadt, G.F.R.) coated with paraffin oil. A suspension of 20 g of the gel in a solution of 3 g of paraffin oil in 60 cm^3 of benzene–acetone (1:1) was shaken for 10 min, and then the slurry was used to cover five 20 × 20 plates. The plates were air-dried and developed in glass tanks.

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The following buffers were used as the mobile phase: citrate–phosphate according to McIlvaine, pH 2.93, 3.32, 3.68, 4.13, 4.52, 4.97, 5.32, 5.72, 6.04, 6.44, 6.83, 7.24, 7.65, and 8.16; and Bates–Bower borate buffer, pH 8.46, 8.89, 9.28, 9.69, and 10.10.

The visualization of spots was carried out with iodine vapour.

Trans-cinnamic acids were commercial samples: *m*- and *p*-coumaric, caffeic, dihydrocaffeic and ferulic (Fluka, Buchs, Switzerland); isoferulic and sinapic (K & K Labs., Plainview, NY, U.S.A.); *o*-coumaric (Schuchardt, Munich, G.F.R.). *Cis*-*p*-coumaric acid was purchased from Aldrich-Europe (Beerse, Belgium) and other *cis* isomers were obtained *in situ* by 10-min UV irradiation (λ = 360 nm) of the spots of appropriate *trans* isomers applied on the chromatoplates.

RESULTS AND DISCUSSION

The results of chromatographic investigations are presented in Figs. 1–3 as plots of R_M values *versus* the pH values of the mobile phase, according to the theory

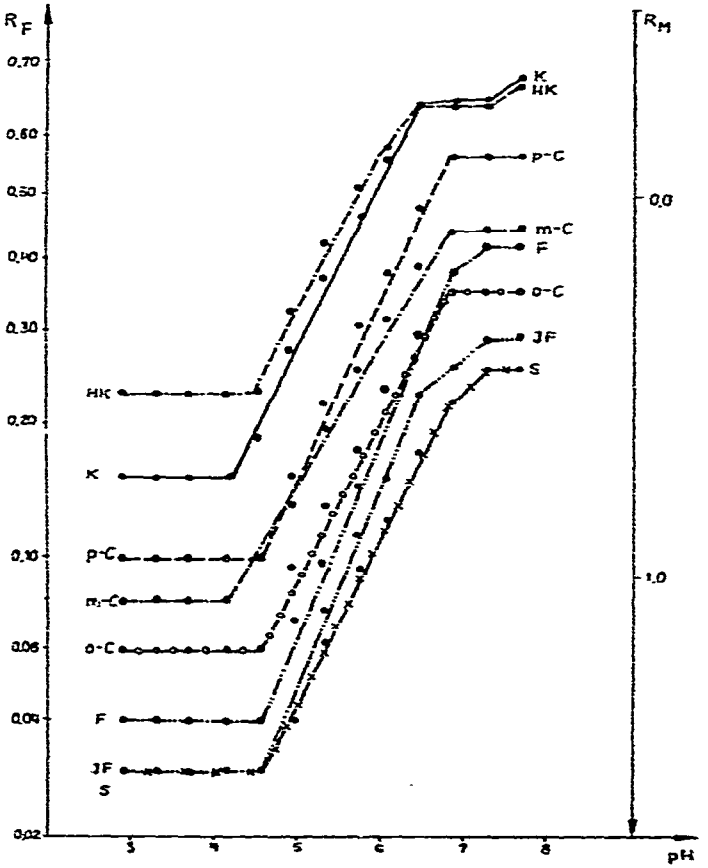


Fig. 1. R_M values of *trans*-cinnamic acids plotted against the pH of the mobile phase. (For notation of the solutes, see Table I).

of Soczewiński and Jusiak[14]. For all the investigated *trans*-cinnamic acids (Fig. 1) the relationships R_M = f(pH) are linear within the pH range 4.52–6.44. For the pH range where the undissociated species are predominant (pH 2.93–4.13), the R_M values are independent of the pH of the mobile phase. The same phenomenon is observed above pH 7, where the monoionized form markedly prevails. From Figs. 1 and 2 it follows that all these acids have satisfactory R_M values in the range from −0.25 to 1.0 in buffers of pH 5.5–7.

From Fig. 1 it follows that satisfactory separation of three monohydroxy *o*-, *m*- and *p*-coumaric acids may be achieved. At pH 6.44–7.24 the difference between the R_M values for different substitution of the hydroxyl group in the benzene ring is 0.17. Similarly, the best separation of isomeric hydroxymetroxycinnamic acids (ferulic and isoferulic acids) is possible with ΔR_M = 0.29 at pH 7.

Dihydrocaffeic acid at lower pH values is readily extracted into the mobile phase, while above the pH 6.44, after ionization of the carboxyl group, the R_M values of caffeic and dihydrocaffeic acids are very similar.

After UV irradiation of the spots of *trans*-cinnamic acids applied on the plates,

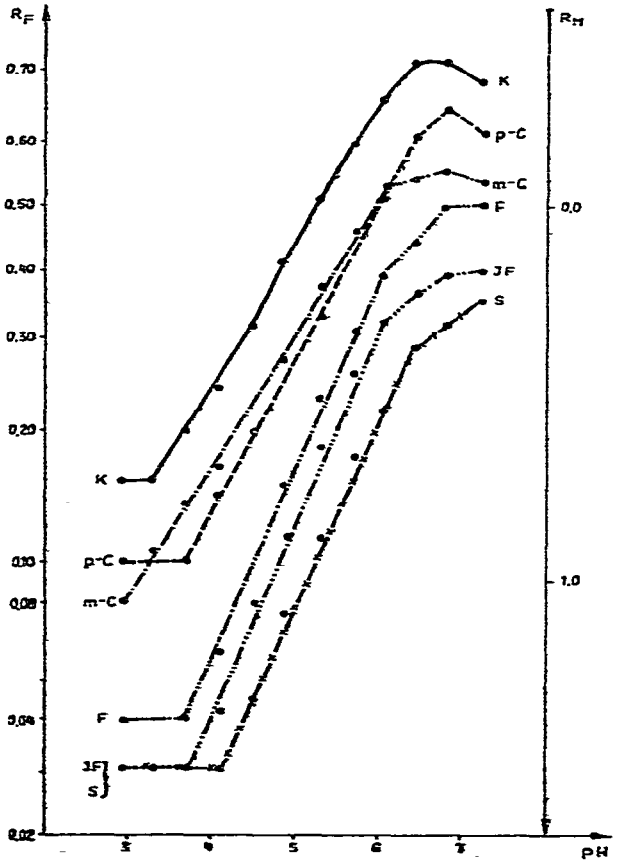


Fig. 2. R_M values of *cis*-cinnamic acids plotted against the pH of the mobile phase. (For notation of the solutes, see Table I).

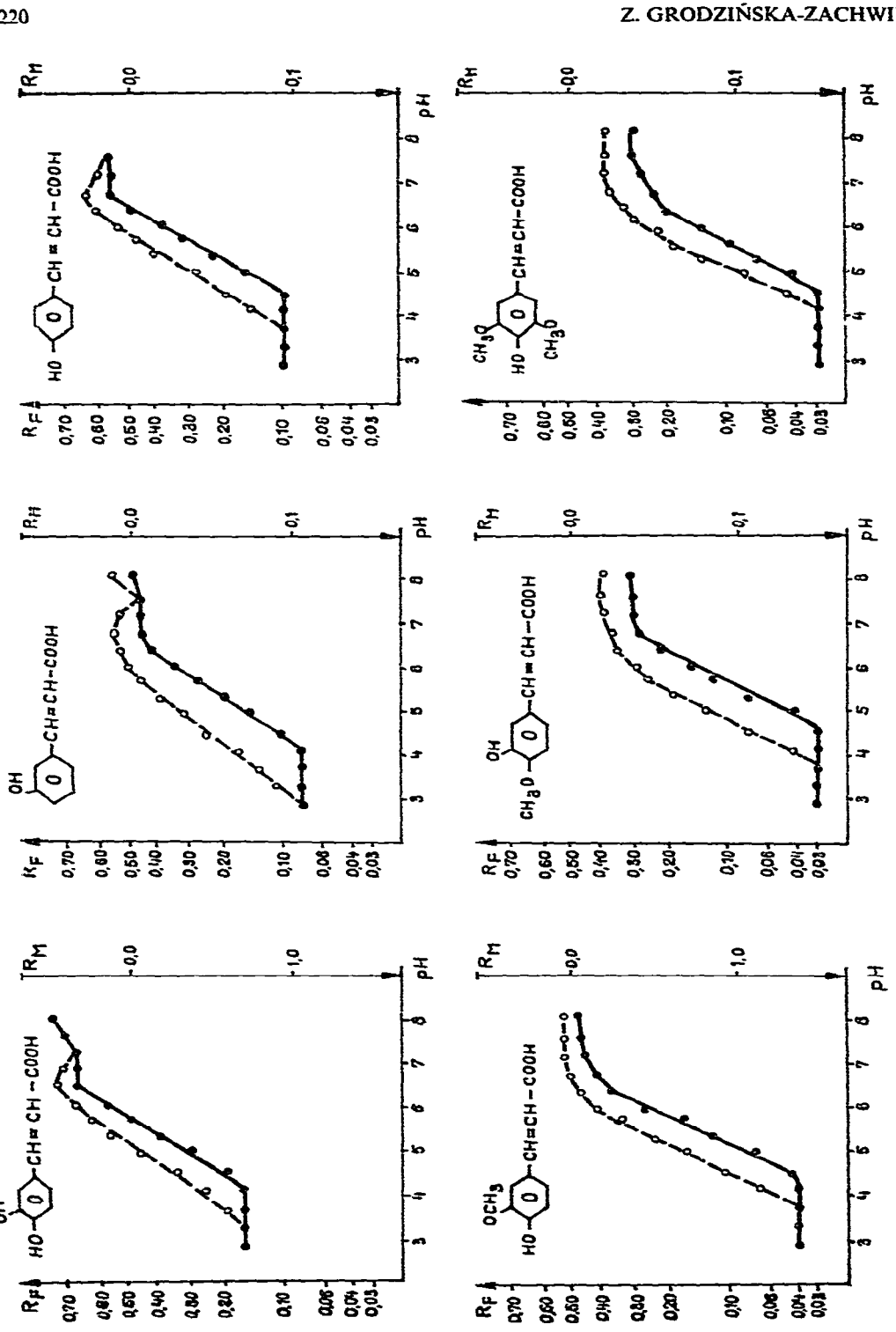
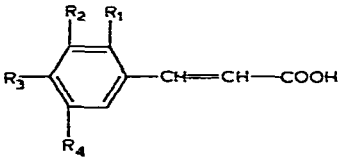


Fig. 3. R_M values of *cis*- and *trans*-cinnamic acids plotted against the pH of the mobile phase for individual cinnamic acids (○, *cis* isomers; ●, *trans* isomers).

TABLE I

INVESTIGATED COMPOUNDS



Code	*Solute*	R_1	R_2	R_3	R_4	pK_a
A	cinnamic acid	H	H	H	H	4.57
o-C	*o*-coumaric acid	OH	H	H	H	4.87
m-C	*m*-coumaric acid	H	OH	H	H	4.53
p-C	*p*-coumaric acid	H	H	OH	H	4.86
K	caffeic acid	H	OH	OH	H	4.78
F	ferulic acid	H	OCH_3	OH	H	4.86
IF	isoferulic acid	H	OH	OCH_3	H	4.86
S	sinapic acid	H	OCH_3	OH	OCH_3	4.86
HK	dihydrocaffeic acid	3,4-dihydroxyphenylpropionic acid				4.96

and subsequent development of these plates in the buffers, two spots were visible on chromatograms for each compound (with the exception of *o*-coumaric acid). The spots with lower R_M values corresponded to the standards of *trans* isomers, while those with higher R_M values were ascribed to the *cis* isomers, taking into account literature report[7] and parallel development with the standard of *cis*-*p*-coumaric acid. Linear relationships of R_M *versus* pH values for *cis* isomers (Fig. 2) were observed in the pH range 4.13–6.00. Owing to the lower pK_a values of these compounds in comparison with the *trans* isomers the increse of the R_M values begins at lower pH values than for these last compounds. The *cis* isomers of hydroxycinnamic acids show the best mutual separation in buffers of pH 4.5–5 and 6.5–7, and those of hydroxymethoxycinnamic acids at pH 5.5–7.

The separation of *cis* from *trans* isomers for the investigated compounds is presented in Fig. 3. A good selectivity was obtained using buffers in the pH range 4.5–6.5. Below pH 4 and above pH 7 the R_M values of *cis* and *trans* isomers are close or identical.

When the plates were developed in buffers of pH greater than 8 the spots began to separate again, reaching the maximum ΔR_M at pH 8.5–9.9, but in all buffers above pH 8.5 the tailing of spots was observed.

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