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Thin-Layer Chromatography on Silica Gel G of some Phenol Carboxylic Acids

by

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Polyamide thin-layer chromatography has been particularly used for the separation of phenolic compounds (1–6), almost always with aliphatic solvents. Silica gel G was also used as adsorbent for the aromatic hydroxy-carboxylic acids (7–9); a mixture of six of them (10) was successfully resolved. Aromatic solvent systems were generally used, i. e. benzene or toluene. Of a mixture of nine phenol carboxylic acids studied, we separated eight on silica gel G Merck by using aliphatic solvent mixtures instead of benzene or toluene. The phenolic acids studied were chlorogenic, gallic, protocatechuic, caffeic, o- and p-coumaric, ferulic, vanillic and cinnamic acids.

Standard solutions of the cited acids were prepared by dissolving 20 mg of each in 10 ml of ethanol 95% (conc. 27 μ l). Developments of the chromatograms were accomplished using the following mixtures:

- 1) Chloroform-Methanol-H₂O-85% Formic acid (80 : 13 : 2 : 5; organic layer)
- 2) Chloroform-Methanol-Formic acid 85% (85 : 10 : 5)
- 3) Isopropyl alcohol - NH₃ 28 Bé - H₂O (8 : 1 : 1)

The detection of the acids occurred with an aqueous 2% solution FeCl₃ acidified with 1 ml of 2N HCl according to BATE-SMITH and WESTALL (11). 10 × 15 cm plates were coated with 2 g silica gel G Merck in 5 ml of water. The layers were hand-made, that is to say, the slurry was simply poured into plates and evenly distributed by tapping and shaking. The coated plates were allowed to dry for 20 min. at room temperature and for 60 min. at 110° C. 1 μ l of each standard solution was applied along a line 1,5 cm above the bottom edge. The solvent was allowed to run a distance of about 12–13 cm in 30 min. The R_f values of phenolic acids on silica gel G layers are shown on table 1

Table 1
R_f values of phenol carboxylic acids on silica gel G Layers

Acid	Solvent			Color reaction with FeCl ₃
	1	2	3	
Chlorogenic	0,06	0,07	—	green olive
Gallic	0,13	0,11	—	grey violet
Protocatechuic	0,33	0,27	—	blue green
Caffeic	0,39	0,35	0,03	grey brown
p-Coumaric	0,55	0,50	0,47	rot brown
o-Coumaric	0,56	0,54	0,38	orange
Ferulic	0,61	0,56	—	lilac
Vanillic	0,64	0,60	—	yellow brown
Cinnamic	0,67	0,67	0,53	bright yellow

The solvent mixture 1 separates the chlorogenic, gallic, protocatechuic, caffeic, p-coumaric, ferulic and cinnamic acids, while the solvent system 2 separates the chlorogenic, gallic, protocatechuic, caffeic, ferulic, vanillic, cinnamic, and either o- or p-coumaric acids.

Satisfactory resolution of o- and p-coumaric acids was also obtained with the solvent system 3. This method has been used by us to recognise the phenolic acids in musts and wines (Table 2).

Table 2
Phenolic acids recognized in wines by thin-layer chromatography on kieselgel G.

Acids	Wines			
	Merlot (<i>V. vinifera</i>)	Raboso (<i>V. vinifera</i>)	Clinton (<i>V. labrusca</i>) × <i>V. riparia</i>)	Baco (Folle Blanche × <i>V. riparia</i>)
Chlorogenic	+	abs.	+	+
Gallic	+	+	+	+
Protocatechuic	+	+	abs.	+
Caffeic	+	+	abs.	+
p-Coumaric	+	+	+	+
o-coumaric	abs.	abs.	abs.	abs.
Ferulic	+	+	abs.	+
Vanillic	abs.	abs.	abs.	abs.
Cinnamic	abs.	abs.	abs.	abs.

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