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THE SEPARATION OF SOME PRESERVATIVES BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT. Rapid separation of 18 synthetic preservatives on the silica gel chemically modified HPTLC plates (RP-18F_{254s}, RP-18wF_{254s}, CN F_{254s} and Nano-SIL Diol/UV_{254}) has been achieved with methanol-water as mobile phase. The development distance was 8 cm in all cases. Identification of the separated preservatives was performed by UV light at λ=254 nm. Potassium sorbate, sodium benzoate and ascorbic acid were identified in some soft drinks after sample preparation by solid phase extraction on C18 column.

Keywords: *chromatographic separation, chemically modified silica gel, TLC, preservatives*

INTRODUCTION

Preservatives are substances commonly added to various foods and pharmaceutical products in order to prolong their shelf life. The addition of preservatives to such products is essential for avoiding alteration and degradation by microorganisms during storage. Preservatives are classified into two main classes: *antimicrobial preservatives* and *antioxidants.*

Antimicrobial preservatives are introduced in the preparations to kill or to inhibit the growth of micro-organisms inadvertently introduced during manufacture or use. Antimicrobial preservatives are classified into two main sub-groups: antifungal preservatives and antibacterial preservatives. Antifungal preservatives include compounds such as benzoic and ascorbic acids and their salts, and phenol compounds such as methyl, ethyl, propyl and butyl p-hydroxybenzoate (parabens). Antibacterial preservatives are compounds such as quaternary ammonium salts, alcohols and other phenols.

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Antioxidants are added to the pharmaceutical products to prevent deterioration by oxidation. Antioxidants are classified into three groups. The first group is known as true antioxidants, or anti-oxygen and they inhibit oxidation by reacting with free radicals blocking the chain reaction. Examples are alkylgallates butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and tocopherols. The second group consists of reducing agents, substances with lower redox potentials than the drug. Examples are ascorbic acid and potassium and sodium salts of sulphurous acid. The third group consists of antioxidant synergists which usually have little antioxidant effect themselves but probably enhance the action of antioxidants in the first group. Examples of antioxidant synergists are citric acid, editic acid and its salts, lecithin and tartaric acid.

Usually combinations of preservatives are used in pharmaceuticals, cosmetics, biological samples and foods, to prevent alteration and degradation of the product formulations. It is known that most of preservatives may be harmful to the consumers due to their potency to induce allergic contact dermatitis [1, 2]. Allergic reactions to foods represent a prominent, actual and increasing problem in clinical medicine [3].

Preservatives in common use include sorbic acid and its salts, benzoic acid and its salts, p-hydroxybenzoic acid methyl ester (methyl paraben), p-hydroxybenzoic acid ethyl ester (ethyl paraben,), p-hydroxybenzoic acid propyl ester (propyl paraben), p-hydroxybenzoic acid butyl ester (butyl paraben) and salicylic acid. The structures of 18 usually preservatives are listed in Figure 1.

A variety of analytical methods for determining preservatives have been reported. However, because the additives can be present in combinations, chromatographic methods are often used for their selective individual or joint determination.

High–performance liquid chromatography (HPLC) is often preferred for the determination of mixture of additives in food, cosmetics, and pharmaceuticals. The simultaneous determination of sweeteners, preservatives and colorings in soft drinks was done by HPLC with UV detection [4]. Also, this chromatographic technique is the most common analytical procedure for detecting and quantifying sorbic and benzoic acids in foods and beverages [5, 6]. In view of the complexity and diversity foodstuffs composition, most of preservatives (benzoic, sorbic and salicylic acid) are usually analysed by RP-HPLC with an appropriate sample preparation procedure [7-9]. A novel ion chromatographic method was a beneficial alternative to conventional HPLC for simultaneous separation and determination of artificial sweeteners (sodium saccharin, aspartame), preservatives (benzoic acid, sorbic acid), caffeine, theobromine and theophylline in food and pharmaceutical preparations [10].

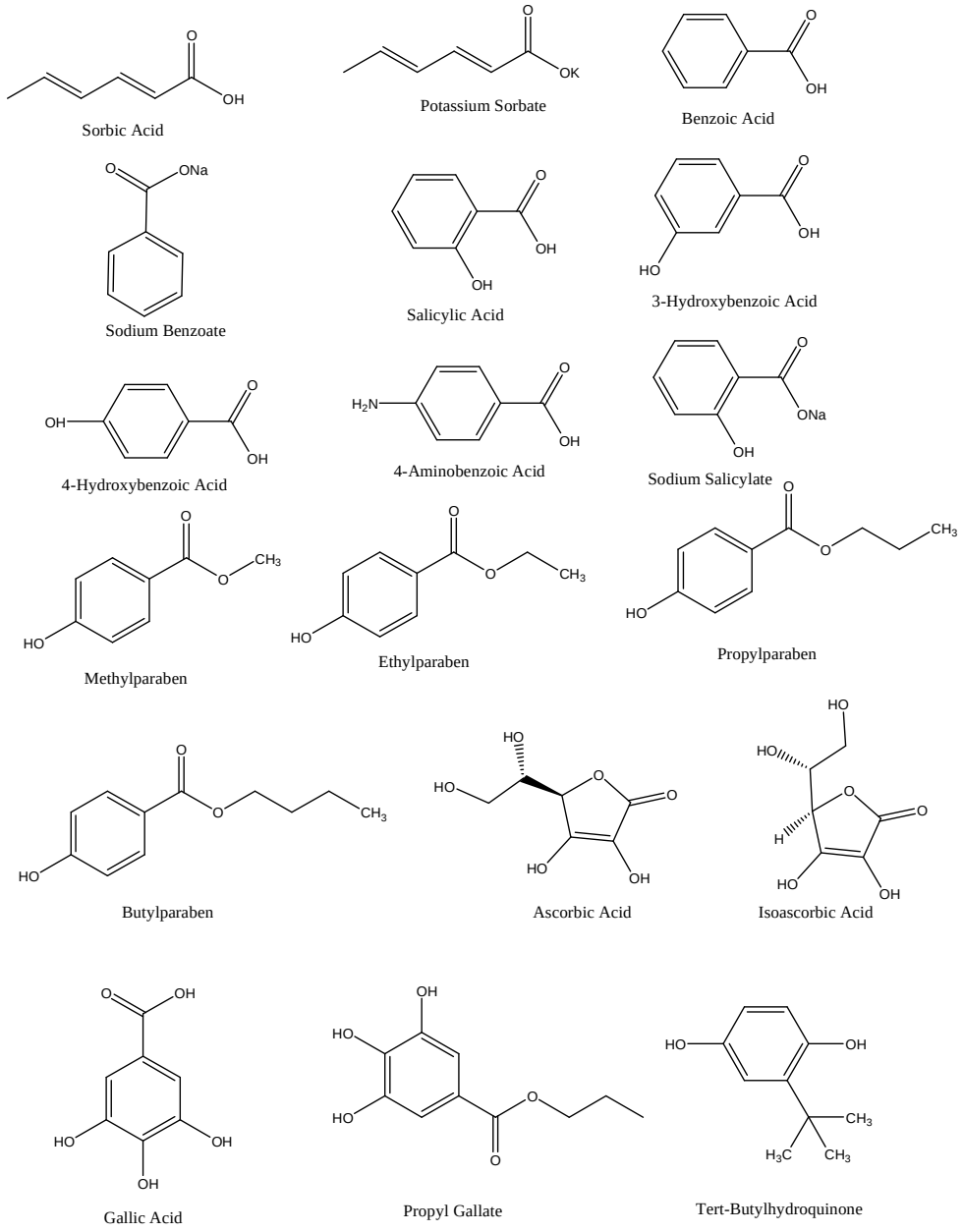

Figure 1. The structures of some usually synthetic preservatives.

Also, HPLC is a reliable method for simultaneous determination of phenolic preservatives such as parahydroxybenzoic acid, methyl, ethyl, propyl, butyl and pentyl parahydroxybenzoates from gelatin and vacant capsules for medicinal use [11]. Recently, liquid chromatography time-of-fight mass spectrometry (HPLC/TOF-MS) was developed to identification and quantification of 18 synthetic preservatives in beverages [12].

Gas chromatography (GC), with or without derivatization is also employed for the selective determination of food preservatives [13, 14] in beverages [15], soft drinks, yogurts and sauces [16]. Capillary electrophoresis method was developed to determine simultaneously artificial sweeteners, preservatives and dyes used as additives in carbonated soft drinks [17, 18]. A microemulsion electrokinetic chromatographic (MEEKC) method was used to separate seven preservatives (four parabens -methyl, ethyl, propyl and butyl, sorbic acid, benzoic acid, and dehydroacetic acid) which are commonly used as additives in various food products such as drinks, soy sauces and wines [19]. This method was successfully applied also for separation and determination of parabens (methyl, ethyl, propyl, and butyl *p* -hydroxybenzoate) in cosmetic products [20, 21].

The determination of preservatives using conventional methods like HPLC, GC and capillary electrophoresis (CE), some time can be difficult because of high-cost instruments and time-consuming pretreatment technique separations. Besides, equipments for these techniques are not available for small laboratories due their high cost.

Thin-layer chromatographic procedure (TLC) was successfully used for separation of preservatives in cosmetics [22-24], in foods and beverages [25-27] and minimal sample preparation is necessary.

The purpose of this work was to investigate potential use of TLC (on different stationary phases) for rapid separation and identification of preservatives from carbonated soft drinks.

RESULTS AND DISCUSSION

The obtained R_f values of studied preservatives and mobile phases used for different HPTLC plates are listed in Table 1. As we can see from this table, the sorbic, benzoic, salicylic, ascorbic acids and salts like potassium sorbate and sodium benzoate (preservatives founded usually together in foods) were accurately separated on CN F_{254s} plates and Nano-SIL Diol/UV_{254} plates. The best stationary phases for the separation of potassium sorbate, sodium benzoate and ascorbic acid (found in mixture in majority of soft drinks), are silica gel RP-18wF_{254s} and silica gel CN F_{254s}.

Table 1. R_f values of some preservatives on Silica gel HPTLC plates

Compounds	R_f values Silica gel HPTLC plates			
	RP-18F_{254s} Mobile phase MeOH:H_2O (6:4, v/v)	**RP-18wF_{254s} Mobile phase MeOH:H_2O (1:1, v/v)**	**CN F_{254s} Mobile phase MeOH:H_2O (3:7, v/v)**	**Nano-SIL Diol/UV_{254} Mobile phase MeOH:H_2O (4:6, v/v)**
1. Sorbic Acid	0.39	0.45	0.40	0.63
2. Potassium Sorbate	0.41	0.44	0.40	0.67
3. Benzoic Acid	0.43	0.47	0.29	0.70
4. Sodium Benzoate	0.48	0.53	0.54	0.73
5. Salicylic Acid	0.37	0.48	0.22	0.58
6. 3-Hydroxybenzoic Acid	0.68	0.61	0.46	0.72
7. 4- Hydroxybenzoic Acid	0.68	0.58	0.44	0.67
8. 4-Aminobenzoic Acid	0.76	0.61	0.41	0.76
9. Sodium Salicylate	0.64	0.67	0.62	0.70
10. Methylparaben	0.44	0.34	0.21	0.45
11. Ethylparaben	0.31	0.25	0.15	0.38
12. Propylparaben	0.20	0.17	0.10	0.27
13. Butylparaben	0.11	0.10	0.05	0.15
14. Ascorbic Acid	0.77	0.82	0.86	0.87
15. Isoascorbic Acid	0.73	0.80	0.86	0.84
16. Gallic Acid	0.81	0.76	0.64	0.72
17. Propyl Gallate	0.55	0.41	0.21	0.44
18. Tert-Buylhydroquinone	0.44	0.33	0.14	0.37

In order to separate and identify preservatives from some commercially available soft drinks, the samples (20 mL) were passed through the C18 column for the solid phase extraction at the rate of approximately 0.5 mL/ minute. The column was conditioned prior to use by washing with methanol (5 mL) and water (5 mL). After retention of the interested compounds from the sample, the column was washed with water (10 mL) and the adsorbed preservatives were eluted with 2 mL methanol. The resulted samples were analyzed by HPTLC using mixtures of methanol:water (1:1 v/v, for RP-18wF_{254s} plates and 3:7 v/v, for CN F_{254s} plates) as mobile phase. The obtained chromatograms are shown in Figure 2.

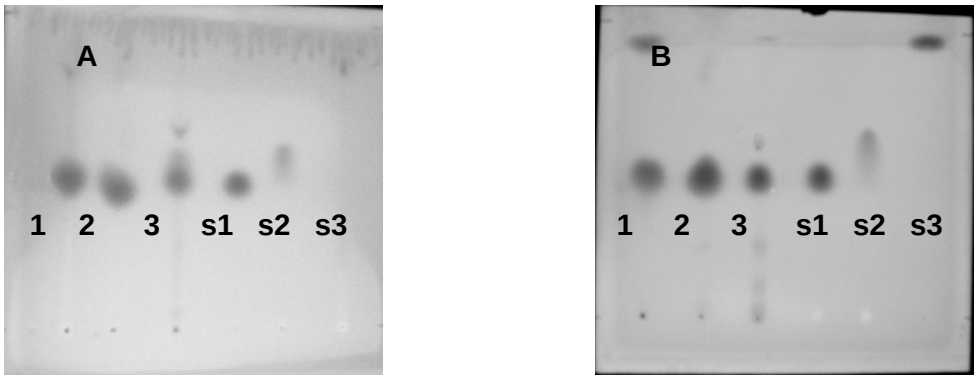

Figure 2. Chromatograms of soft drinks on RP-18wF_{254s} silica gel plates (A) and CN F_{254s} silica gel plates (B) obtained using methanol:water as mobile phase.(1. juice Adria-oranges; 2. juice Giusto natura-tropical and 3. juice Frutti Fresh-pink, s1-standard of potassium sorbate, s2-standard of sodium benzoate, s3-standard of ascorbic acid).

As we can see from chromatograms, using solide-phase extraction on C18 column for sample preparation and TLC method for identification, the preservatives from soft drinks were separated and identified. On RP-18wF_{254s} silica gel plates only potassium sorbate was identified in analysed soft drinks. On CN F_{254s} plates we identified potassium sorbate and ascorbic acid in Adria-orange juice, potassium sorbate in Giusto natura-tropical juice and potassium sorbate and sodium benzoate in Frutti Fresh-pink juice.

CONCLUSIONS

The retention behavior of 18 preservatives has been investigated by TLC on different stationary phases. The best separation was achieved for parabens (which can be found in mixtures in pharmaceuticals and cosmetics) for all types of studied HPTLC plates. The sorbic, benzoic, salicylic, ascorbic acids and salts like potassium sorbate and sodium benzoate (preservatives founded usually together in foods) were accurately separated on CN F_{254s} plates and Nano-SIL Diol/UV_{254} plates.

Ascorbic acid, potassium sorbate and sodium benzoate can be identified from soft drinks using TLC method after sample preparation by solid-phase extraction on C18 column.

EXPERIMENTAL SECTION

The preservatives sorbic acid, potassium sorbate, benzoic acid, sodium benzoate, salicylic acid, 3-hydroxybenzoic acid, 4- hydroxybenzoic acid, 4-aminobenzoic acid, sodium salicylate, methylparaben, ethylparaben,

propylparaben, butylparaben, ascorbic acid, isoascorbic acid, gallic acid, propyl gallate and tert-butylhydroquinone of analytical grade were obtained from Merck or Fluka. The samples analyzed (soft drinks) are commercially available.

Thin-layer chromatography was performed on 10x10 cm glass HPTLC plates coated with silica gel RP-18F_{254s}, RP-18wF_{254s}, CN F_{254s} and Nano-SIL Diol/UV_{254}.

The standard solutions (1 mg mL^{-1}) were prepared in methanol and 2µL of them were applied manually on the plates as spots. Different mixtures of methanol:water (6:4 v/v for RP-18F_{254s} plates, 1:1 v/v for RP-18WF_{254s} plates, 3:7 v/v for CN F_{254s} plates and 4:6 v/v for Nano-SIL Diol/UV_{254} plates) were used as mobile phases. The plates were developed on a distance of 8 cm in a normal chamber (Camag) in all cases. After development the plates were dried in air and inspected under UV illumination at λ=254 nm.

From soft drinks (Adria-oranges, Giusto natura-tropical and Frutti fresh-pink grapefruit) preservatives were isolated using solid-phase extraction on C18 column and identified by RP-TLC on 10x10 cm HPTLC plates coated with silica gel RP-18wF_{254s} and silica gel CN F_{254s}.

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