

# Effect of Boric Acid on $\alpha$ -Hydroxy Acids Compounds in Thin Layer Chromatography

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**Abstract**—In this investigation Salicylic acid, Sulfosalicylic acid and Acetyl salicylic acid were chosen as a sample for thin layer chromatography (TLC) on silica gel plates. Bicarbonate buffer at different pH containing different amounts of boric acid was applied as mobile phase. Specific interaction of these substances with boric acid has effect on  $R_f$  in thin layer chromatography. Regular and similar trend was observed in variations of  $R_f$  for mentioned compounds in TLC by altering of percentages of boric acid in mobile phase in pH range of 8-10. Also effect of organic solvent, mixture of water/ organic solvent and organic solvent containing boric acid as mobile phase was studied.

**Keywords**—Thin layer chromatography (TLC), Aspirin, Salicylic acid, Sulfosalicylic acid, Boric acid.

## I. INTRODUCTION

ASPIRIN is one of the most widely used drugs in the world. Recently, it has been suggested to use Aspirin to prevent intravascular thrombosis, reduce Alzheimer disease, and prevent colon cancer. Aspirin is well established as a prophylactic to prevent secondary stroke [1]. It inhibits the synthesis of thromboxane  $A_2$  in platelets by irreversible acetylation of a serin residue close to the active site of cyclooxygenase, the enzyme that catalyses the formation of an unstable endoperoxide intermediate prostaglandin  $H_2$  from arachidonic acid [2].

Salicylic acid (SA) is a natural signaling molecule for activation of plant defense mechanism and is a pharmacological agent for controlling the inflammatory response in humans. It has been shown in plant cells that SA is synthesized in response to environmental injury [3] and serves as a messenger molecule to induce the expression of plant defense-related genes [4]. Acetylsalicylic acid properties was subsequently synthesized and remains one of the most commonly used anti-inflammatory drugs [5]. Recent studies have shown that SA and Aspirin also have anti-neoplastic properties [6]. Aspirin and salicylate can modulate gene transcription [7], the activity of several protein kinases, and other molecular pathways as well [8, 9]. moreover, It is very interesting for scientist study the molecular mechanisms responsible for the effect of aspirin on cell cycle and survival [10-13].

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We present here a simple unidimensional TLC system for the chromatographic behavior of Aspirin, salicylic acid, and sulfosalicylic acid. The similar patterns for  $R_f$  values were observed for  $\alpha$ -hydroxy acids in present of boric acid.

## II. MATERIALS

Acetylsalicylic acid (Aspirin) (AS), salicylic acid (SA), sulfosalicylic acid (Sulfo-SA), sodium bicarbonate chloroform methanol and ferric chloride, were products of Merck (Darmstadt, Germany).

### A. TLC Methods

All the plates were predeveloped with chloroform-methanol 1:1 (v/v) and air-dried over night, prior to use. A solution of 1% Aspirin, salicylic acid and sulfosalicylic acid (see Fig. 1) in ethanol was prepared. The sample were spotted to silica gel plate 0.1 M bicarbonate buffer at rang of pH=8-10 containing 0%, 1%, 2%, 3% and 4% of bicarbonate was chosen as mobile phase.

After elution with appropriate solvent system to a distance of 60 mm from origin, the plates was dried and position of the analytes visualized by UV and chemical detection and  $R_f$  values measured. Further,  $R_f$  values were verified separately for each compound.

### B. Effect of Mobile Phase

The same procedure (previous section) was applied with ethanol/water (1:1) containing boric acid 1%, ethanol and ethanol containing boric acid 1% as mobile phase.

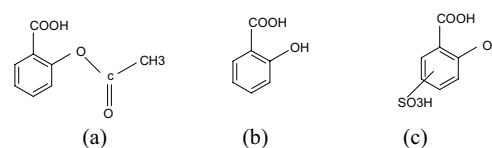


Fig. 1 Structure of Aspirin (a), Salicylic acid (b) and SulfoSalicylic acid (c)

## III. RESULT AND DISCUSSION

Thin layer chromatography (TLC) is an extremely useful technique for monitoring reaction, determination the proper solvent system for performing separations using column chromatography, and separation of wide rang of compounds like medicine, drugs and so on[14,15].

The TLC methods reported here facilitate the separation of Aspirin and salicylic and sulfosalicylic acid on silica gel.

Separation of AS, SA and sulf-SA, using elutions with simple buffer solvent containing different amount of boric acid was accomplished. The  $R_f$  changes for these compounds showed similar pattern (Fig. 2).

Fig. 2 indicates effect of boric acid concentration in the mobile phase on the  $R_f$  values of AS, SA, sulf-SA. This effect is due to specific interaction and complex formation of  $\alpha$ -hydroxy acid compounds with boric acid. The  $R_f$  values for SA and sulf-SA decrease with increasing of boric acid concentration in the mobile phase. A possible explanation of this fact is that salicylic acid and sulfosalicylic acid can form complex with boric acid. Aspirin has no complex with boric acid, so  $R_f$  value variations for Aspirin showed different behaviors respecting to salicylic acid and sulfosalicylic acid. The  $R_f$  values of salicylic acid and sulfosalicylic acid proved to be lower if these compounds were developed with mobile phase containing more percentage of boric acid.

We also calculated capacity factor ( $K'$ ) and selectivity factor ( $\alpha$ ) for each experimental point (Table I). Fig. 3 indicates selectivity factor ( $\alpha$ ) for each experimental point. This Fig. clearly demonstrates optimum percentage of boric acid in buffer solution for this separation is 1%. Like  $R_f$ , the selectivity factor changes for these compounds showed similar pattern with increasing amount of boric acid in the buffer (compare with Fig. 2).

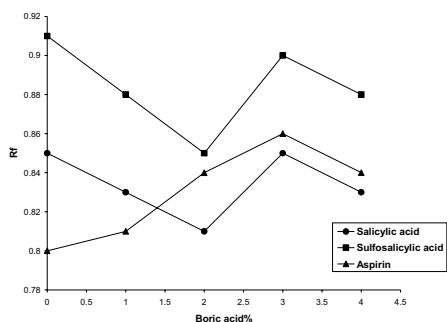


Fig. 2 The  $R_f$  values of AS, SA, sulf-SA versus different boric acid concentration

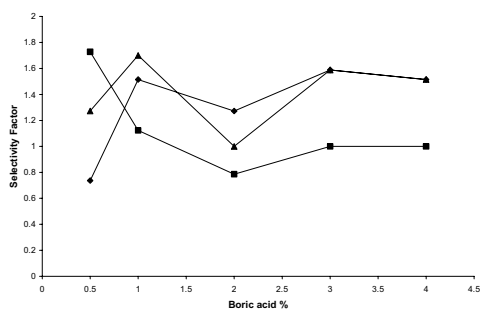


Fig. 3 Selectivity factor ( $\alpha$ ) of  $\diamond$  SA, sulf-SA,  $\blacksquare$  AS, SA  $\blacktriangle$  AS, sulf-SA versus different boric acid concentration

TABLE I  
CAPACITY FACTOR ( $K'$ ) AND SELECTIVITY FACTOR ( $\alpha$ ) FOR EACH EXPERIMENTAL POINT

Boric acid%	$K'$ salicylic acid	$K'$ sulfosalicylic acid	$K'$ aspirin	$\alpha$ salicylic acid> sulfosalicylic acid	aspirin, $\alpha$ salicylic acid	$\alpha$ aspirin sulfosalicylic acid
0	0.176	0.091	0.250	1.94	1.42	2.75
0.5	0.052	0.071	0.091	0.73	1.73	1.27
1	0.200	0.132	0.224	1.51	1.12	1.70
2	0.220	0.176	0.176	1.27	0.79	1.00
3	0.176	0.111	0.176	1.58	1.00	1.59
4	0.200	0.132	0.200	1.51	1.00	1.51

Effect of organic solvent as mobile phase was investigated. Above procedure was applied with ethanol/water (1:1) containing boric acid 1%, ethanol and ethanol containing boric acid 1% as mobile phase for salicylic acid spot (Fig. 4). It was observed that with increasing of percentage of water in the mixture of mobile phases, the  $R_f$  value would increase. In addition, Fig. 4 indicates that presence of boric acid in ethanol cause of tailing of salicylic acid peak.

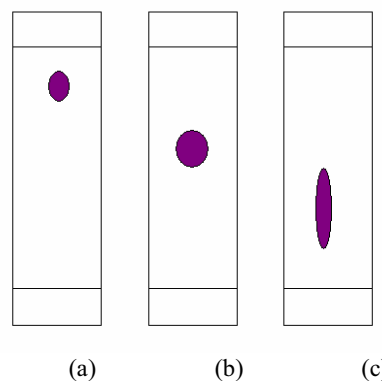


Fig. 4 Effect of organic solvent as mobile phase (a) ethanol/water (1:1) containing boric acid 1%, (b) ethanol and (c) ethanol containing boric acid 1%

#### IV. CONCLUSION

The proposed TLC method can be employed in the screening profiling of Aspirin and deacetylated impurities. This method is simple, rapid and inexpensive. Our study demonstrated that in the presence of boric acid in mobile phase Aspirin and its deacetylated metabolite show two different behavior in silica gel TLC plate.

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