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Optimization of Mobile Phase for Simultaneous Determination of Sweeteners, Preservatives and Dyes by UFLC

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Abstract : The mobile phase of phosphate buffer and methanol are one of the most commonly used motion phases in routine analysis. The composition and pH of the mobile phase of one of the compounds can not be used as an option for separation of the compound. Suitable mobile phase, both solvent type, composition and pH of the mobile phase are the factors that determine success in separation of the compound. The study was conducted to determine the composition and pH of the mobile phase in the development of a mixed separation method from food additives containing sodium saccharin, sodium cyclamate, sodium benzoate, potassium sorbate, tartrazine and sunset yellow. Research using high performance liquid chromatography, reversed phase with instrument: UFLC 1290 DAD (Agilent), C18 column 100 mm x 4.6 mm x 3.5 μm (Agilent). The results showed that pH and optimum mobile phase composition were 4.5 and 75: 25 (v / v). Parameter of optimization includes the capacity factor, plate number, resolution, selectivity and tailing factor meet the requirements of analysis.

Key words : UFLC, pH, Composition, Food Additives.

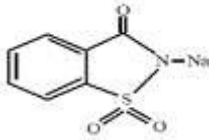
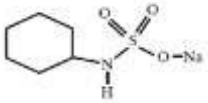
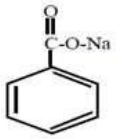
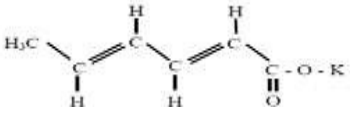
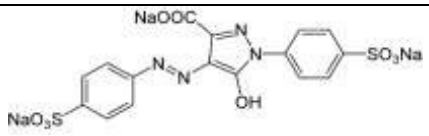
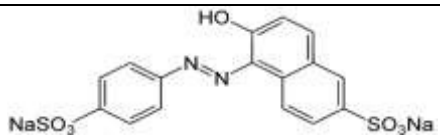
Introduction

The selection of the mobile phase can only be done by trial and error until the desired chromatogram is obtained. The mobile phase usually consists of a mixture of solvents haven excellent elution and resolution of the compounds in the sample. Elution ability and resolution are determined by the polarity of the solvent, the polarity of the stationary phase and the properties of the sample component. The normal phase, the stationary phase is more polar than the mobile phase will have an increased elution ability with an increase of the solvent polarity. The reversed phase, the stationary phase is less polar than the mobile phase will have decreased elution ability with an increase of the solvent polarity[1,2].

Optimization are an attempt to get a better separation, fast analysis, improving sensitivity and save costs. The methods development of chromatography are done so that the performance of simple, better, precise, accurate, economical, selective, sensitive, and specific[3,4].

Compounds of weak acids or weak bases can be separated by using the mobile phase of the buffer solution to improve resolution and selectivity. The mobile phase of the acid buffer solution causes the ionized bases compound to be faster eluted and the acid compound aren't ionized so that it is slower to elute, if the pH of the buffer solution are lower than the pKa of the compound (Table 1)[1,2,5]. The selected buffer solution should provide the best separation based on resolution and capacity factor, while also providing a shorter analysis time [3,4].

Table 1. List of Additives according to the European Commission and the acid dissociation constants of food additives [6,7,8,9,10]

No	Additive (E-number)	Formula	pKa
1	Sodium Saccharine (E-954)		1.8
2	Sodium Cyclamate (E-952)		1.9
3	Sodium Benzoate (E-211)		4.2
4	Potasium Sorbate (E-202)		4.8
5	Tartrazine (E-102)		9.4
6	Sunset yellow (E-110)		10.3

The mobile phase composition gives an indication of polarity. The polarity of the compound in the mobile phase provides an important role in the separation [1,2]. The composition and pH of the mobile phase of one of the compounds can not be used as an option for separation of the compound, can only be used as a separation reference. Suitable mobile phase, both solvent type, composition and pH of the mobile phase are the factors that determine success in separation of the compound[11,12,13]. The mobile phase of phosphate buffer and methanol are one of the most commonly used motion phases in routine analysis.

The determination of sodium saccharin, sodium cyclamate, sodium benzoate, potassium sorbate, tartrazine and sunset yellow can be carried out in the buffer phase of pH 4.0 to pH 6.0 with a certain organic phase composition[14,15,16,17,18,19]. The six compounds have a certain mobile phase composition, it would be better if pH optimization and composition of the mobile phase are applied.

Experimental

Chemicals and Instruments

The materials used are methanol grade HPLC (E. Merck), potassium dihydrogen phosphate anhydrous (E. Merck), orthophosphoric acid (E. Merck), aqua pro injection (Ekapharmindo Putramas) sodium saccharin, sodium cyclamate, sodium benzoate, potassium sorbate, tartrazine, and sunset yellow (Sigma Aldrich).

Instruments used include: a set of UFLC (Agilent 1290 Infinity Diode Array Detector), Zorbax Eclipse Plus C-18 (100 x 4.6 mm, 3.5 µm), digital pH meter, sonicator (Bransonic) and vacuum pump (Boeco).

Preparation of mobile phase

The phosphate buffer solution was prepared from potassium dihydrogen phosphate anhydrous 10 mM by adding orthophosphoric acid 10 mM. The composition of the mobile phase are adjusted from a mixture of phosphate buffers and methanol.

Working standard solutions

Single stock solution of tartrazine, sunset yellow, saccharin, cyclamate, benzoate and sorbate were prepared at a concentration of 1000 ppm, respectively. The working solution was prepared by diluting the stock solution so as to obtain a mixture of tartrazine, saccharin, cyclamate, sunset yellow, benzoate and sorbate at 10 ppm, 1 ppm, 75 ppm, 5 ppm, 3 ppm, 6 ppm, respectively.

Experimental procedures

Standard solution was filtered with a 0.45 µm PTFE syringe, sonicated for 15 minutes, injected 5 µL, flow rate of 1.0 ml/min, the column temperature 30^{o}C, void volume of 30% and wavelengths of 200 nm, 220 nm and 450 nm; mobile phase pH test of 4.0; 4.3; 4.5 and 4.7; composition of phosphate buffer and methanol test of 73 : 27 ; 75 : 25 and 77 : 23. Subsequently been selected the conditions that give optimum results with the parameters of capacity factor, plate number, resolution, selectivity and tailing factor.

Results and Discussion

Optimization of pH mobile phase

The pH mobile phase optimization results obtained data in the form of retention time (Rt), capacity factor (k'), theoretical plate number (N), resolution (Rs), selectivity (α) and tailing factor (Tf). The effect of buffer pH on optimization parameters can be seen in Table 2 and Figure 1.

Table 2 and Figure 1 gives information that the separate compounds are poor in the phosphate buffer phase phases of pH 4.7; because the sodium benzoate resolution is smaller than that allowed (Rs ≥ 2)[1,2]. This provides information of ionization of sodium benzoate (pKa = 4.2) in the buffer phase of pH 4.7; so that retention time becomes faster and resolution are poor (Figure 1). Sodium saccharin and sunset yellow have tailings and sodium cyclamate undergoing fronting of phosphate buffer pH 4.7 (Table 2). This gives information about the ionization of sodium saccharin (pKa = 1,8), sodium cyclamate (pKa = 1.9) and sunset yellow (pKa = 10.3) so that the chromatograms of sodium saccharin and sunset yellow have tailings, whereas sodium cyclamate undergoes fronting (Figure 1).

Table 2. Effect of pH buffer to parameter optimization

Compounds	Wavelengths (nm)	pH of posphate buffer											
		4,0						4,3					
		Rt	k'	N	Rs	α	Tf	Rt	k'	N	Rs	α	Tf
Tartrazin	450	1.255	1.010	1701	-	-	1.911	1.238	0.990	1132	-	-	1.127
Saccharin	220	2.010	2.230	4771	6.220	2.200	1.886	2.000	2.210	5014	5.570	2.240	1.892
Cyclamate	200	3.473	4.570	3107	8.020	2.050	2.357	3.454	4.540	2581	7.540	2.060	1.056
Sunset Yellow	450	4.824	6.740	2606	14.280	6.650	1.784	4.738	6.600	3585	15.060	6.690	2.109
Benzoate	220	11.367	17.240	4627	12.590	2.560	1.420	8.728	13.000	4585	9.610	1.970	1.067
Sorbate	220	14.683	22.560	6364	4.710	1.310	1.839	12.653	19.300	5109	6.400	1.480	1.373
		4.5						4.7					
Tartrazin	450	1.217	0.950	941	-	-	1.381	1.184	0.900	1196	-	-	1.595
Saccharin	220	2.002	2.210	4986	5.750	2.330	1.976	1.980	2.180	5230	6.540	2.420	2.039
Cyclamate	200	3.470	4.570	3003	8.000	2.060	1.740	3.437	4.510	3332	8.370	2.070	0.872
Sunset Yellow	450	4.747	6.620	3852	15.160	6.950	2.212	4.614	6.400	4736	16.900	7.110	2.164
Benzoate	220	7.366	10.820	3835	6.750	1.640	0.946	5.204	7.350	4160	1.980	1.150	1.010
Sorbate	220	11.341	17.200	4848	7.040	1.590	1.072	8.612	12.820	4670	8.230	1.740	1.106

Table 2 and Figure 1 show that all compounds are well separated on phosphate buffer pH 4.0, but the capacity factor of potassium sorbate is above the maximum limit (Table 2). A very large capacity factor shows that the analysis time is longer. Potassium sorbate does not ionize then the partition in the mobile phase are

poor. Sodium cyclamate undergoes tailings with a tailings factor value of 2.357 (Table 2). This information as an indication of ionization of sodium cyclamate at phosphate buffer phase pH 4.0 causes partition in the p hase of motion are poor then the chromatogram form into tailings.

The compounds are well separated in the mobile phase of the phosphate buffer pH 4.3 and pH 4.5 and meet the requirements[1,2]. Tailings occur at sunset yellow (pKa = 10.3), both in the mobile phase of phosphate buffer pH 4.3 and pH 4.5. This shows that yellow sunset ionization causes partitions in the mobile phase to be poor. The potassium sorbate capacity factor at pH 4.3 (k = 19.30) was greater than pH 4.5 (k = 17.20), so the analysis at pH 4.5 was better than at pH 4.3. Therefore, the phosphate buffer solution used for the study should be at pH 4.5.

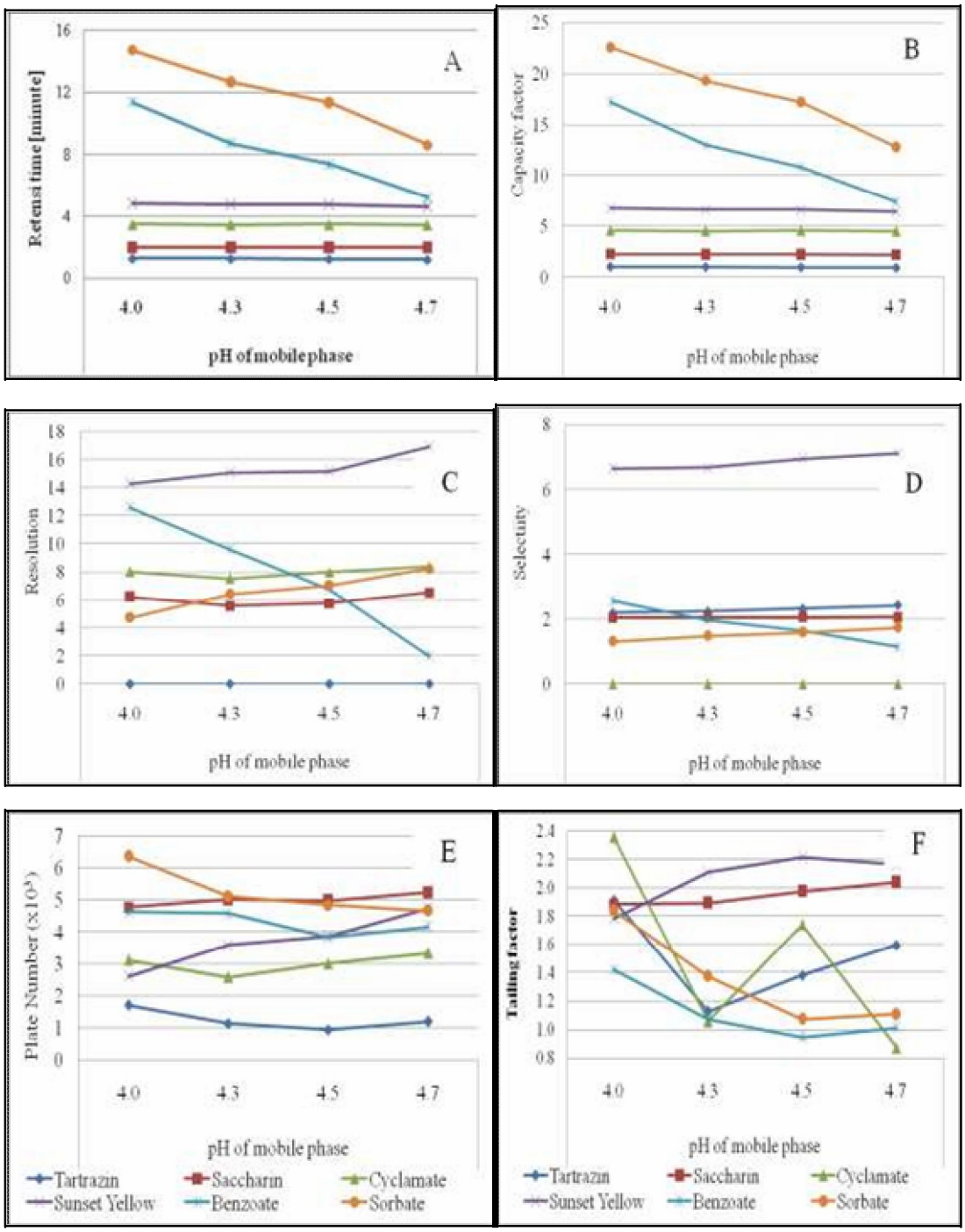


Figure 1. Effect of mobile phase pH on the retention time (A), capacity factor (B), resolution (C) selectivity (D) plate number (E) and tailing factor (F)

The best results used the mobile phase of phosphate buffer: methanol (pH = 4.5) and were in the range performed by previous researchers[14,15,16,17,18,19]. Differences in pH occur due to the organic mobile phase, the type of buffer used and the components of the compound to be separated are different. Chromatogram pH optimization results of the mobile phase can be seen in Figure 2.

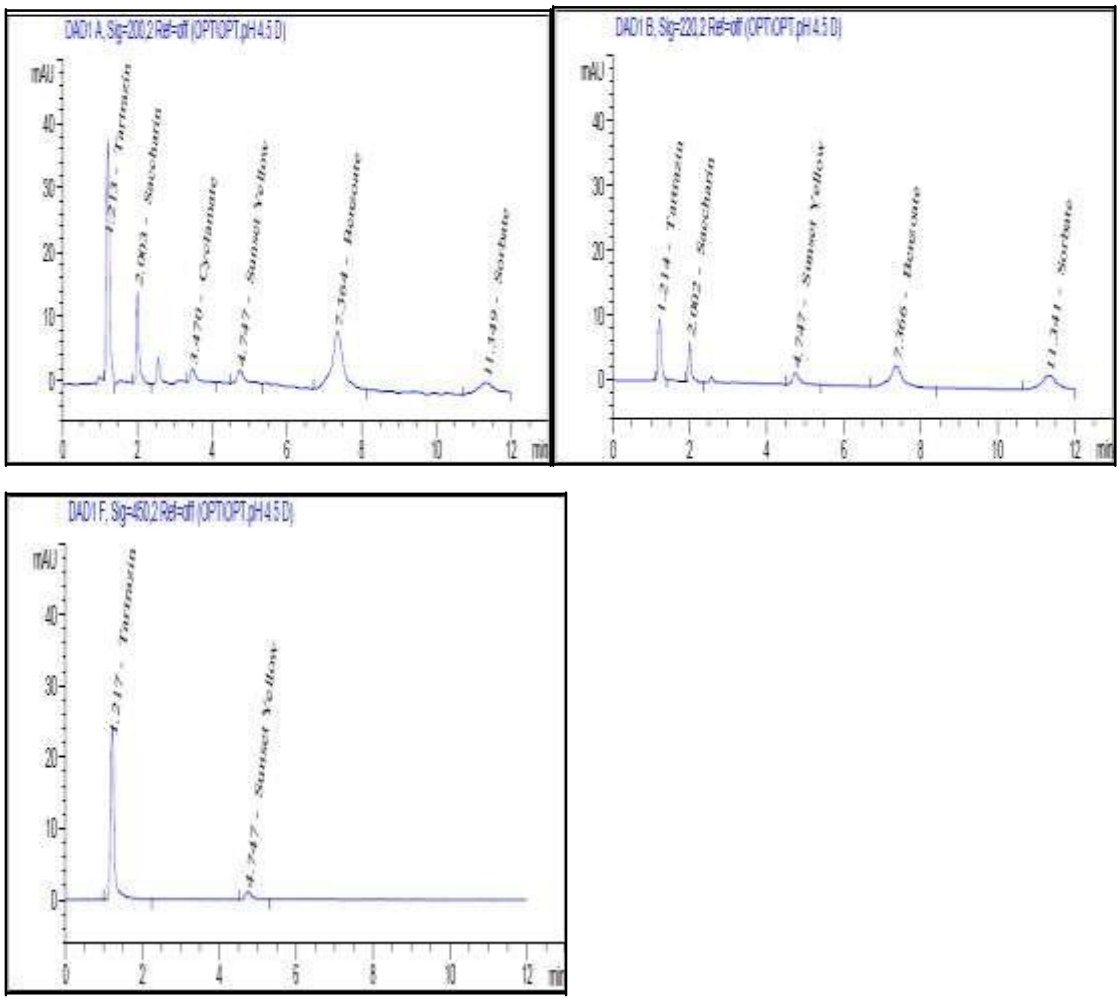


Figure 2. Chromatogram of the results of the mobile phase pH optimization

Optimization of mobile phase composition

The optimized mobile phase is phosphate buffer pH 4.5 and methanol at composition of 73: 27; 75: 25 and 77: 23 (v/v). The effect of mobile phase composition on the optimization parameter in the form of data (Table 3) and Figure (Figure 2).

Table 3. Effect of mobile phase composition on optimization parameters

Compounds	Wavelengths (nm)	Mobile Phase Composition of Phosphate Buffer-Methanol																	
		73 : 27						75 : 25						77 : 23					
		Rt	k'	N	Rs	α	Tf	Rt	k'	N	Rs	α	Tf	Rt	k'	N	Rs	α	Tf
Tartrazin	450	1.169	0.880	1092	-	-	1.959	1.239	0.990	1483	-	-	1.430	1.273	1.040	2826	-	-	2.493
Saccharin	220	1.829	1.940	5757	5.650	2.210	1.311	1.948	2.130	6193	6.040	2.150	1.269	2.099	2.370	6725	8.340	2.270	1.192
Cyclamate	200	3.054	3.900	4900	9.010	2.020	2.102	3.292	4.280	4906	9.310	2.010	1.941	3.573	4.730	5486	9.980	2.000	1.740
Sunset Yellow	450	3.755	5.030	3547	13.130	5.740	1.649	4.724	6.580	3727	15.870	6.660	1.565	6.111	8.810	4193	20.400	8.440	1.611
Benzoate	220	6.631	9.640	11072	11.280	1.920	1.020	7.431	10.920	11632	9.240	1.660	1.024	8.355	12.410	11931	6.510	1.410	1.045
Sorbate	220	9.807	14.740	12474	10.510	1.530	1.040	11.188	16.950	12734	11.160	1.550	1.041	12.789	19.520	12738	11.660	1.570	1.048

Figure 3 shows that the retention time in the phosphate buffer phase phosphate composition pH 4.5 and methanol 77: 23 are slower than in the 75: 25 and 73: 27 compositions. The methanol composition is increasingly causing the capacity factor, the number of theoretical plates, the resolution and the selectivity experienced Decrease, except for resolution and selectivity of benzoate.

Figure 3 shows that the capacity factor of benzoate (pKa = 4.2) decreases due to increased solubility as the methanol fraction increases. Benzoate is not fully ionized in phosphate buffer phase pH 4.5. Resolution and selectivity of benzoate increased, indicating that the separation of benzoate from the mixture was more perfect. Decreased capacity factors of saccharin, cyclamate, sorbate, tartrazine and sunset yellow lead to decreased resolution and selectivity. This is due to the decrease in the viscosity of the mobile phase, not the increase in the solubility of the compounds in methanol because the compounds are ionized in the polar phase.

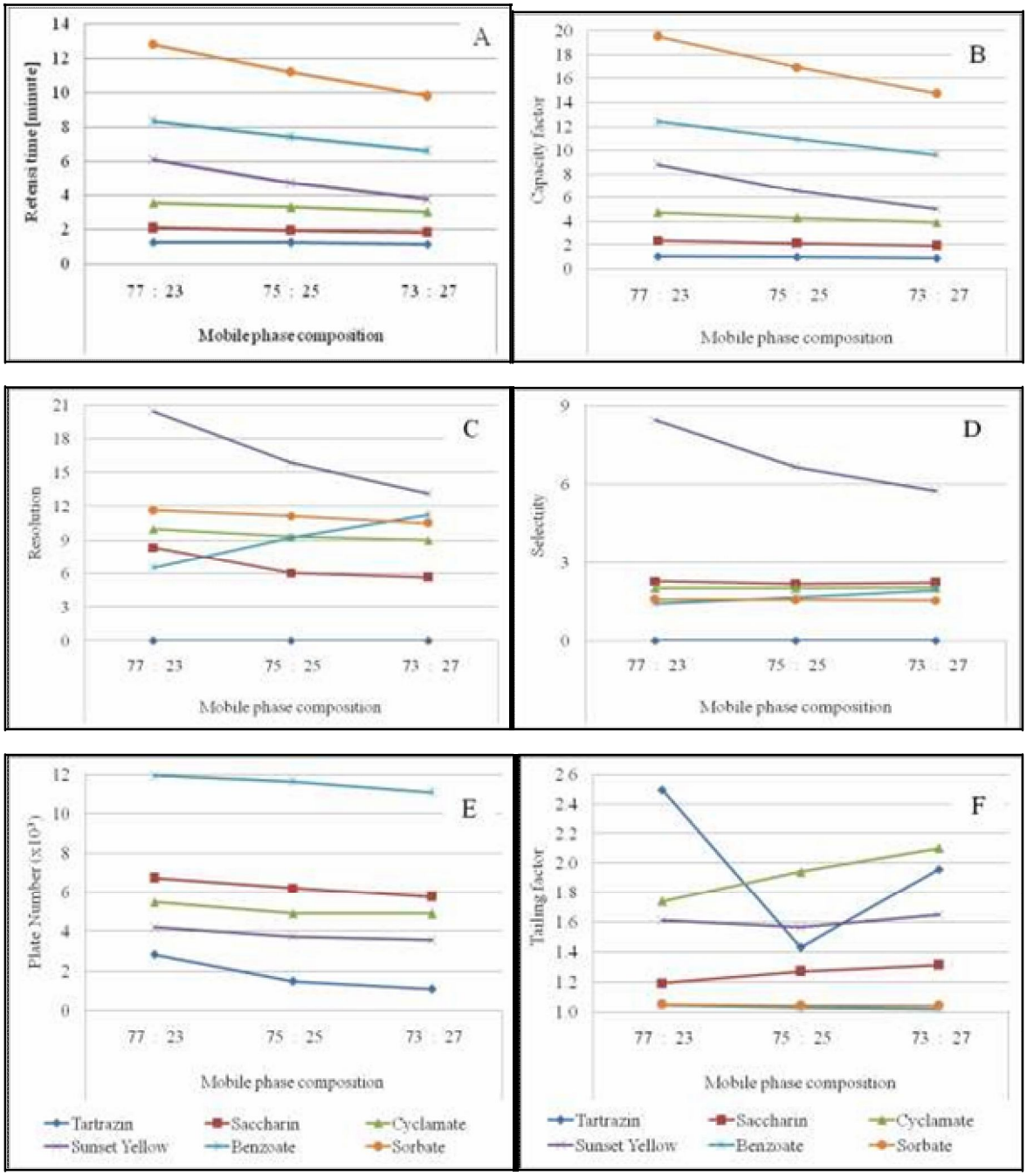


Figure 2. Effect of mobile phase composition of phospat buffer-methanol (v/v) on the retensi time (A), Capacity factor (B), resolution (C) selectivity (D) plate number (E) and tailing factor (F)

The results of the study met the requirements of optimization parameters[1,2], but cyclamate and tartrazine had tailings in phosphate buffer phase pH 4.5 and methanol at a ratio of 73: 27 and 77: 23 (v/v). Cyclamate and tartrazine can not partition in the mobile phase of the comparison. Therefore, the analysis is better done in phosphate buffer: methanol 75: 25 (v/v). The optimized chromatogram can be seen in Figure 3.

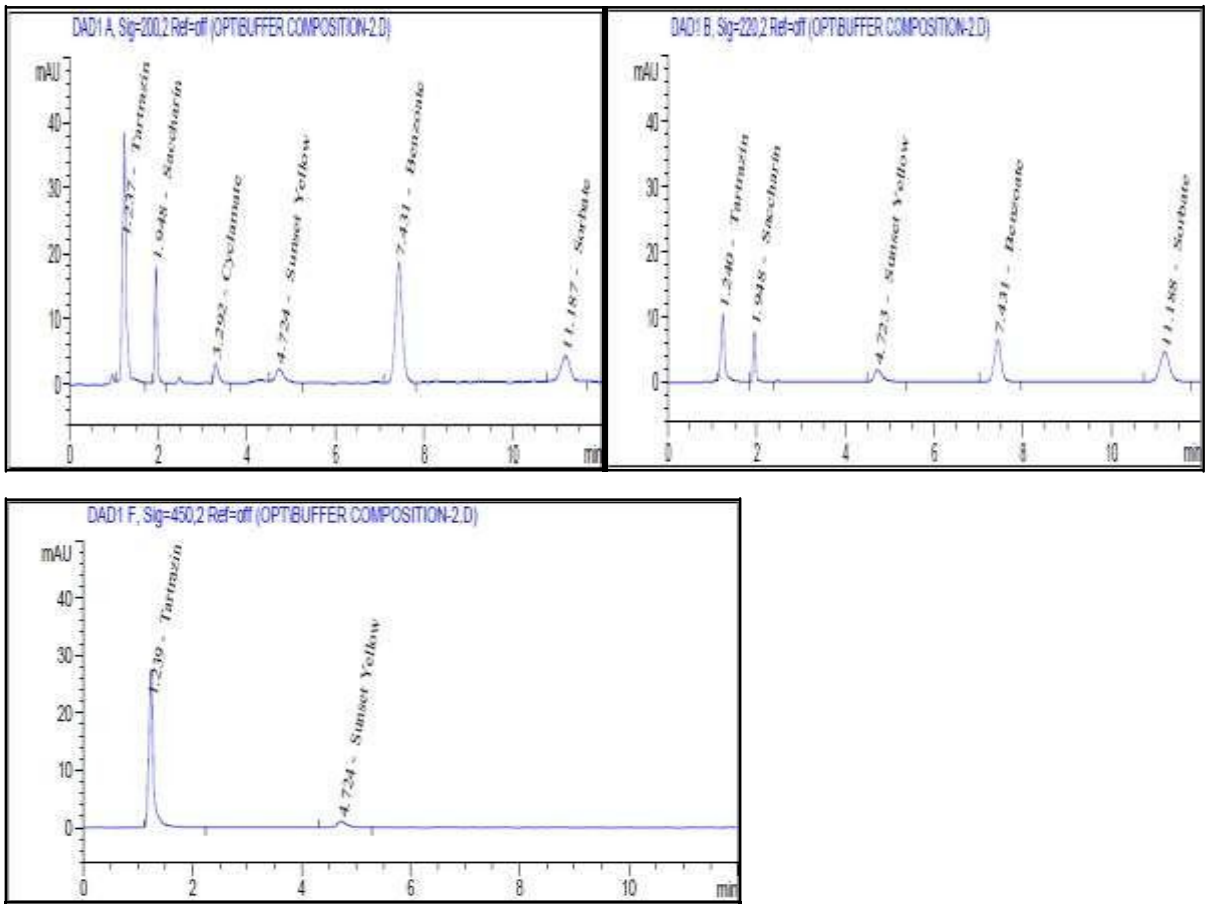


Figure 3. Results of chromatograms from the optimization of mobile phase composition

Conclusion

The optimization result of the method development was obtained that the optimum mobile phase composition was 75: 25 (v/v) with pH 4.5 at the wavelength of the analysis of 200, 230 and 450 nm; flow rate 1.0 ml/min, column temperature 30^{o}C. The optimization parameters include capacity factor, theoretical plate number, resolution, selectivity and tailing factors meeting the requirements of the analysis

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