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Optimization of Volume Void and Wavelengths at Simultaneous Determination Method Development of Sweeteners, Preservatives and Dyes by UFLC

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Abstract : Void volume is an important parameter in chromatography to determines accuracy of chromatography. Void volume which is not in accordance cause a widening or changes the symmetry of the chromatogram. Void volume is very influential at the capacity factor. Wavelength range from UV to Visible should be optimized so as not to be used from the maximum wavelength respectively. This analysis leads to the determination of the void volume and the wavelengths for the development of methods of separation of a mixture of saccharin, cyclamate, benzoic, sorbic, tartrazine and sunset yellow. This study used high performance liquid chromatography reversed phase the wavelength range of 200 nm - 470 nm, the instrument of UFLC 1290 DAD (Agilent), C18 column 100 mm x 4.6 mm x 3.5 μm (Agilent). The results showed that the optimum empty volume is 30% with a wavelength of 200, 220 and 450 nm in the mobile phase of phosphate buffer (pH 4.5) and methanol 75: 25 (v / v), flow rate of 1.0 ml / min, column temperature 30°C. Parameter of optimization includes the capacity factor, plate number, resolution, selectivity and tailing factor meet the requirements of analysis.
Keywords : UFLC, Volume Void, Wavelength, Food Additives.

Introduction

Optimization is an attempt to get a better separation, fast analysis, improving sensitivity and save costs[1,2]. The methods development of chromatography is done so that the performance of simple, better, precise, accurate, economical, selective, sensitive, and specific[3,4,5].

Void volume is the total volume of mobile phase contained in a column, an estimated 65% of the volume of the empty column[6]. Each column has a void volume that varies, depending from diameter of the particles the stationary phase of column for each product and duration of use[7,8]. Void volume is an important parameter to determine the accuracy of chromatography. Void volume describes the molecular retention. The change of void volume caused changes of the shape of chromatograms[9]. There is a correlation of void volume by a capacity factor, plate number, resolution, selectivity and tailing factor, so the void volume change will result in a change of separation efficiency [6,8,10].

Capacity factor (k') is the degree of retention of the sample component in the column. Capacity factor (k ') as the size of the columns retain sample components or the relative time of the solute in the stationary phase and the mobile phase[10]. Capacity factor as a ratio of the retention volume with the void volume of the system[6]. The capacity factor is directly proportional to the volume of the stationary phase and inversely proportional to the volume of the mobile phase or void volume. Separation of with value of k = 0 causes the

components of the compound is not retained by the stationary phase and eluted together with the solvent. K < 1 possibility of overlap with the matrix compound at t_0. The best capacity factor are 1 to 10, more extensive range are $0.5 \leq k \leq 20$. Elution with k > 20 difficult to detect compounds because of broadening[10,12].

The plate number (N) reflects the amount of time during the partitioning of compounds through the column and shows the efficiency of columns. N > 2000 stated that the two chromatograms were separated perfectly[6].

Resolution (Rs) or the separation of two adjacent chromatograms as differences in retention time of two peaks divided by the average peak width. Resolution > 1.5 indicates that the two peaks separated perfectly. Should be done until resolution of ≥ 2 for method development[10,13].

Selectivity (α) is the ability to separate two or more compounds. The complete separation of α > 1. The selectivity depends on the nature of the compound and the interaction between the surface of the compound with the stationary phase and the mobile phase, as the ratio of the capacity factors of different compounds [6]. Relationship between the resolution and selectivity, plate number and capacity factor can be illustrated as follows[14]:

$$R_s = \frac{1}{4}(\alpha - 1)\,N^{1/2}\,\frac{k}{1+k}$$

Tailing factor (Ft) was used to control the chromatography system. Increased peak asymmetry will caused the decrease in resolution, detection limit, and precision[6]. Peak symmetry ($0.9 \leq Ft \leq 1.2$) and peak tailing (Ft > 2). Peak tailing or broadening caused by factors void volume, the void volume should be arranged[10,15].

Determination of cyclamate by HPLC-UV detector at 196 nm and 205 nm[16,17]; saccharin, benzoate and sorbate at 220 nm, 230 nm and 240 nm[18,19,20]; and tartrazine and sunset yellow by using HPLC-Visible at 420 nm, 450 nm and 470 nm[21,22,23,24]. A total of six compounds in the mixture has a maximum wavelength, respectively. It would be better if it were optimized wavelengths for detection of these compounds[25].

Experimental

Chemicals

HPLC grade methanol, potasium dihydrogen phosphate and ortophosphoric acid were purchased from Merck. Commercial standards of tartrazine, sunset yellow, saccharine, cyclamate, benzoate and sorbate were purchased from Sigma Aldrich. Aqua pro injection was purchased from Ekapharmindo Putramas.

Instruments

The separation and determination of sweeteners, preservatives and dyes solution were performed using an UFLC system Agilent Series 1290 equipped with diode array detection - DAD, on reverse column and auto-sampler, stationary phase as ZORBAX Eclipse Plus C18 Column (100 mm x 4.6 mm, 3.5 µm).

Preparation of phosphate buffer

Phospates buffer solution (pH 4.5) was prepared by 1,3601 g KH_2PO_4 in 1000 ml volumetric flask and adjusted the pH to 4.5 with aqueous ortophosphoric acid solution (10 mM).

Preparation of mobile phase

The mobile phase consisting of buffer (10 mM KH_2PO_4): methanol was filtered through 0.20µ membrane filter before use, degassed and was pumped from the solvent reservoir in the ratio of 75:25 v/v.

Preparation of standard stock solution

Single stock solution 1000 ppm of tartrazine, sunset yellow, saccharin, cyclamate, benzoate and sorbate were prepared by dissolving 0,0501 g; 0,0503 g; 0,0501 g; 0,0502 g; 0,0504 g; and 0,0501 g from tartrazine, saccharine, cyclamate, sunset yellow, benzoate and sorbate in 50 ml volumetric flask by aqua pro injection, respectively. Stock solution were kept in freezer at 4°C.

Working standard solutions

Standard solution containing tartrazine, saccharin, cyclamate, sunset yellow, benzoate and sorbate were 10 ppm, 1 ppm, 75 ppm, 5 ppm, 3 ppm, 6 ppm, respectively.

Experimental procedures

Solution was filtered with a 0.45 µm PTFE syringe, sonicated for 15 minutes, injected 5 mL, flow rate of 1.0 ml / min, the column temperature 30°C, the composition of phosphate buffer and methanol (75 : 25), the mobile phase pH 4.5 and void volume test of 20% - 60% and wavelengths test of 200 nm - 470 nm . 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 void volume

Differences of the print out before and after optimization void volume can be seen in Table 1 and Table 2. Chromatogram empty volume optimization results can be seen in Figure 1. The volume of voids is not optimized, it is not guaranteed that the peaks separated perfectly, as the capacity factor can not be controlled. Development of methods for UFLC obtained the best resolution and peak symmetry[10,13,15,26,27].

Table 1. Results of the print out before optimization void volume

RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.237	-	237.02415	40.51626	0.77	0.0704	1700	-	-
1.924	-	71.84729	19.10031	0.75	0.0676	6409	6.24	2.70
3.229	-	21.12481	3.04883	0.53	0.1060	5280	9.45	2.29
4.660	-	35.26659	2.36718	0.70	0.2054	2892	5.40	1.62
7.212	-	208.18743	19.41299	0.91	0.1523	12231	8.35	1.65
10.836	-	71.88071	4.17660	0.73	0.2310	12158	11.28	1.55

Table 1. Results of the print out after optimization void volume

RetTime [min]	k'	Area [mAU*s]	Height [mAU]	Symm.	Width [min]	Plates	Resolution	Selectivity
1.237	0.98	221.74931	41.08545	0.88	0.0726	1598	-	-
1.926	2.09	70.04002	18.06778	0.75	0.0584	6040	6.18	2.12
3.233	4.19	19.42756	2.90306	0.52	0.1080	4966	9.23	2.00
4.664	6.48	35.76808	2.42128	0.73	0.1978	3084	5.50	1.55
7.216	10.58	196.84177	18.73155	1.00	0.1597	11298	8.39	1.63
10.846	16.40	61.92334	4.05772	1.02	0.2289	12441	10.97	1.55

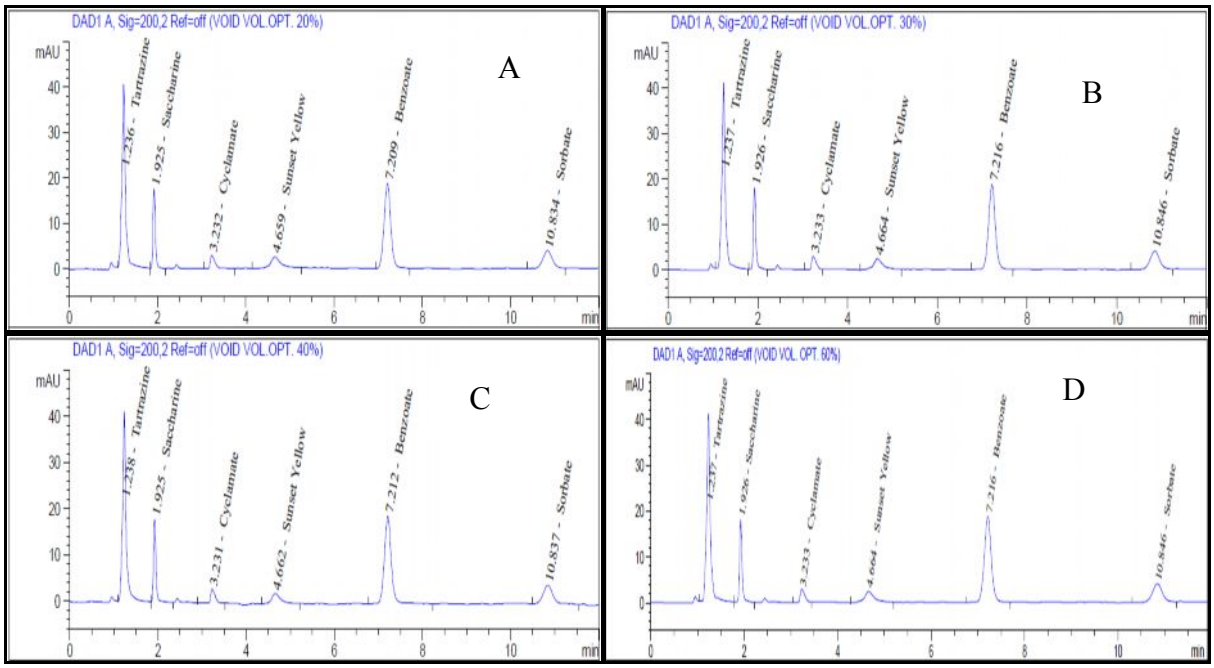
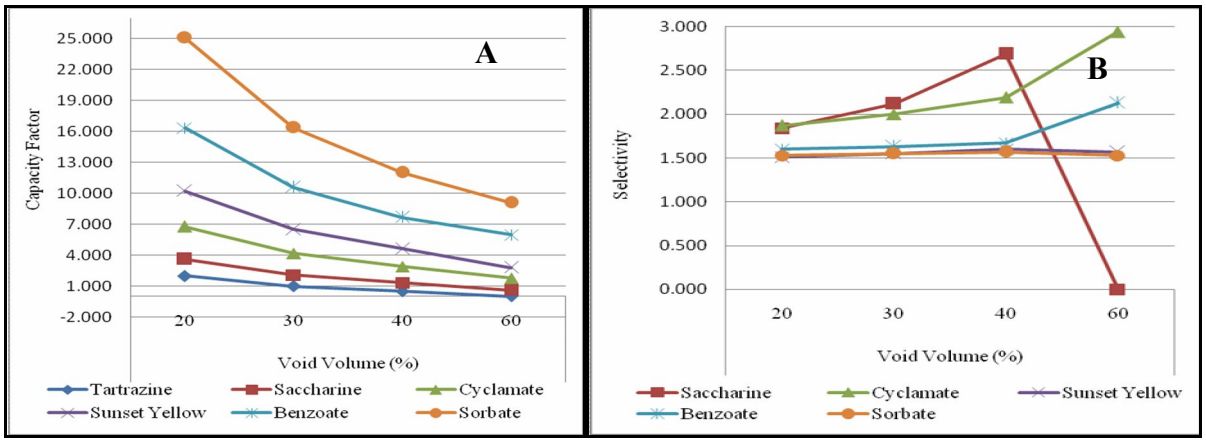


Figure 1. Chromatograms of void volume optimizations of 20% (A), 30% (B), 40% (C) and 60% (D) of mixed standard solution at a wavelength of 200 nm

Table 3. The results of the optimization void volum

Compounds	Void Volume (%)									
	20					30				
	k'	N	Rs	α	Ft	k'	N	Rs	α	Ft
Tartrazine	1.980	1582	0.000	0.000	1.193	0.980	1598	0.000	0.000	1.152
Saccharin	3.630	6220	6.210	1.840	1.209	2.090	6040	6.180	2.120	1.230
Cyclamate	6.780	4765	9.150	1.870	2.390	4.190	4966	9.230	2.000	1.714
Sunset Yellow	10.210	2699	5.220	1.510	1.767	6.490	3084	5.500	1.550	1.293
Benzoate	16.350	11423	8.100	1.600	1.065	10.580	11298	8.390	1.630	1.058
Sorbate	25.080	12410	10.990	1.530	1.115	16.400	12441	10.970	1.550	1.122
	40					60				
Tartrazine	0.490	1707	0.000	0.000	1.229	0.000	1006	0.000	0.000	1.150
Saccharin	1.320	6404	6.340	2.690	1.200	0.610	4850	5.810	0.000	1.230
Cyclamate	2.890	5220	9.470	2.190	1.596	1.780	2959	7.920	2.940	1.720
Sunset Yellow	4.610	2878	5.430	1.600	1.637	2.800	3562	4.410	1.570	1.300
Benzoate	7.680	12261	8.370	1.670	1.077	5.970	3839	8.990	2.130	1.160
Sorbate	12.040	12188	11.080	1.570	1.051	9.110	5066	9.160	1.530	1.230



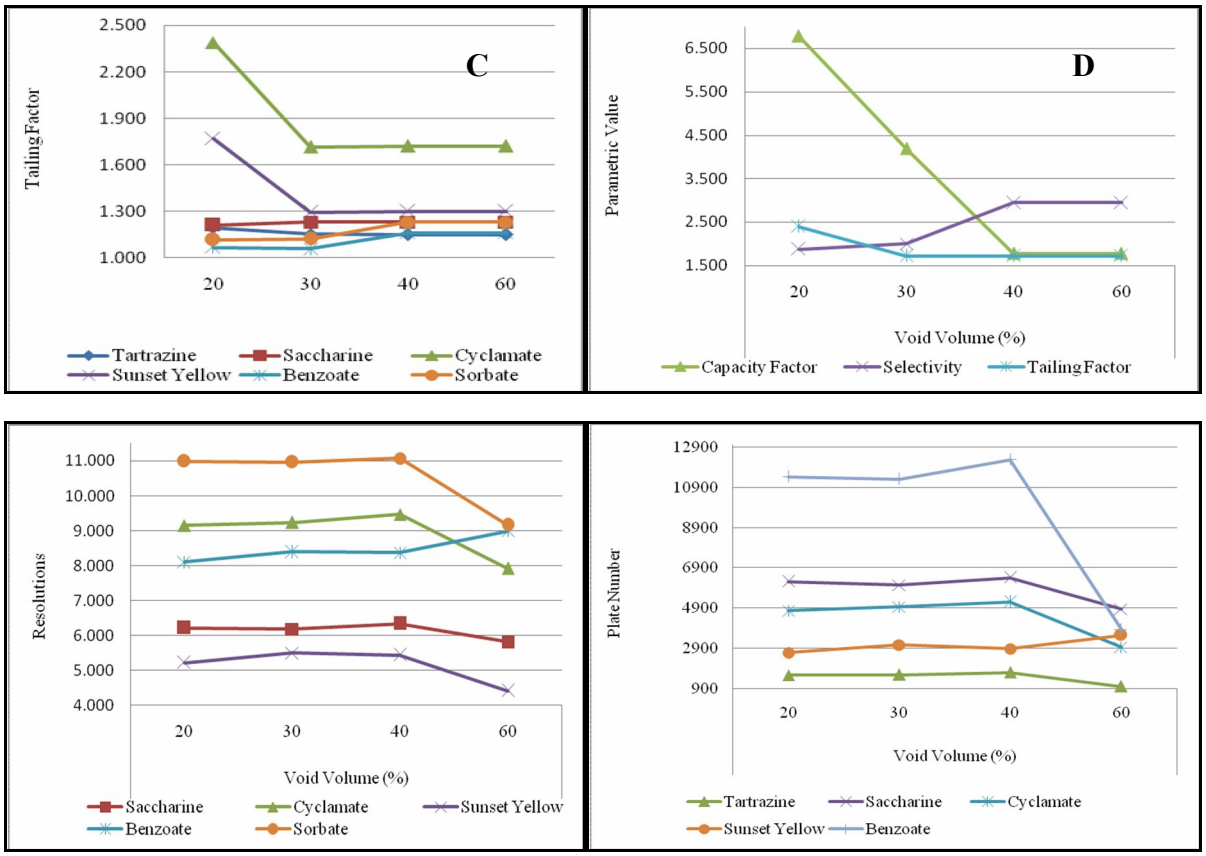


Figure 2. Void volume relationship with capacity factor (A), selectivity (B), tailing factor (C and D) resolutions (E) and plate number (F)

The results of voids volume optimization can be seen in Table 3. Table 3 gives information that capacity factor, selectivity and tailing factors is influenced of voids volume. The decrease of capacity factor (Figure 2 A) and the an increase of the selectivity (Figure B) because the larger the voids volume. The decrease of capacity factor shows that the solubility of the compound in the mobile phase was better. The increase of the volume void causing a decrease of the capacity factor and tailing factor and increasing the selectivity of cyclamate (Figure 2D). Capacity factor (Figure 2A), selectivity (Figure 2B), tailing factor (Figure 2C), resolution of (Figure 2E) and the plate number (Figure 2F) was influenced the void volume. The majority of compounds having plate number of > 2000, resolution of > 2.0 and selectivity of > 1.5 (Table 3). This reflects that chromatograms of all compounds are perfectly separated[6].

Void volume of 20% gives a capacity factor of 25.08 (Table 3) for sorbate, exceed the maximum extent permitted, but chromatogram of sorbic not tailing. This shows there is still the partition of sorbate with the mobile phase. However, cyclamate and sunset yellow experienced tailings (Table 3), indicating the partition with the mobile phase was poorly. Research is not recommended in the void volume of 20% (Table 3) for the elution of the compounds difficult to detected[10,11]. Void volume of 40% gives a capacity factor (k') of 0.490 (Table 3) for tartrazine, this showed an overlap with solvent[10,11]. Cyclamate and sunset yellow also experienced a tailings (Table 3), this indicates the partition cyclamate less well with the mobile phase. Void volume of 60% gives the capacity factor (k') of 0,000 to tartrazine, this showed an overlap with solvent[10,11]. The plate number (N = 1006) for tartrazine and selectivity (α = 0.00) for saccharin (Table 3) reflect on a compound partitions between the two phases through the column and the column efficiency is very bad[6] (Figure 2A), so the chromatogram separation of tartrazine and saccharin is not perfect, column not selective for the saccharin (Figure 2B). Cyclamate also experienced a tailings (Table 3), this indicates that the partition of cyclamate by the mobile phase poorly[6]. The Capacity factor (k') ranges of 0.980 to 16.400 obtained when the void volume 30% (Table 3), are in the allowed range, the analysis should be done on the void volume 30%.

Optimization of wavelengths

Determination of cyclamate by HPLC at wavelengths of 196 nm and 205 nm[16,17]. UFLC-DAD can only detect a minimum at a wavelength of 200 nm. Cyclamate was detected at a wavelength of 200 nm, are in the range of previous studies by a factor of tailings that are in the allowed range (Ft = 1.714). Therefore, the analysis should be performed at a wavelength of 200 nm.

The solvent absorptions of phosphate buffer pH 4.5 and methanol at a ratio of 75: 25 occurs on the retention of 0.714 minutes - 1.058 minutes at a wavelength of of 200 nm, so that tartrazine and saccharin can not be analyzed due to retention of 0.941 minutes and 1.589 minutes (Figure 3).

Determination of saccharin, benzoic and sorbic been performed by HPLC-UV at wavelengths of 220 nm, 230 nm and 240 nm[18,19,20]. The results showed that the analysis can be performed at a wavelengths of 220-240 nm. Analysis of tailings factor (Figure 4A) and height peaks (Figure 4C) of saccharin was smaller and higher at a wavelength of 220 nm. Therefore, the analysis of saccharin, benzoate and sorbate should be performed at a wavelength of 220 nm.

Determination of tartrazine and sunset yellow by using HPLC at wavelengths of 420 nm, 450 nm and 470 nm[21,22,23,24]. These results showed that the analysis can be performed at wavelengths of 420 nm - 470 nm. Analysis of tailings factor (Figure 4B) and peak height (Figure 4D) for tartrazine and sunset yellow at wavelengths of 440 nm - 470 nm showed that the analysis performed better at wavelengths of 450 nm. Chromatogram of optimization results for the wavelength can be seen in Figure 5.

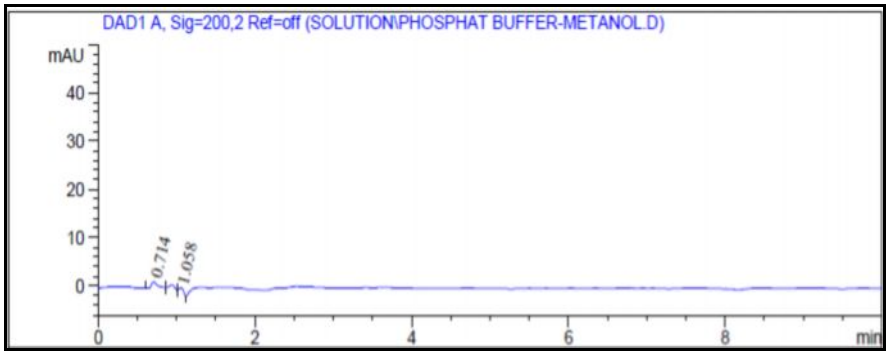
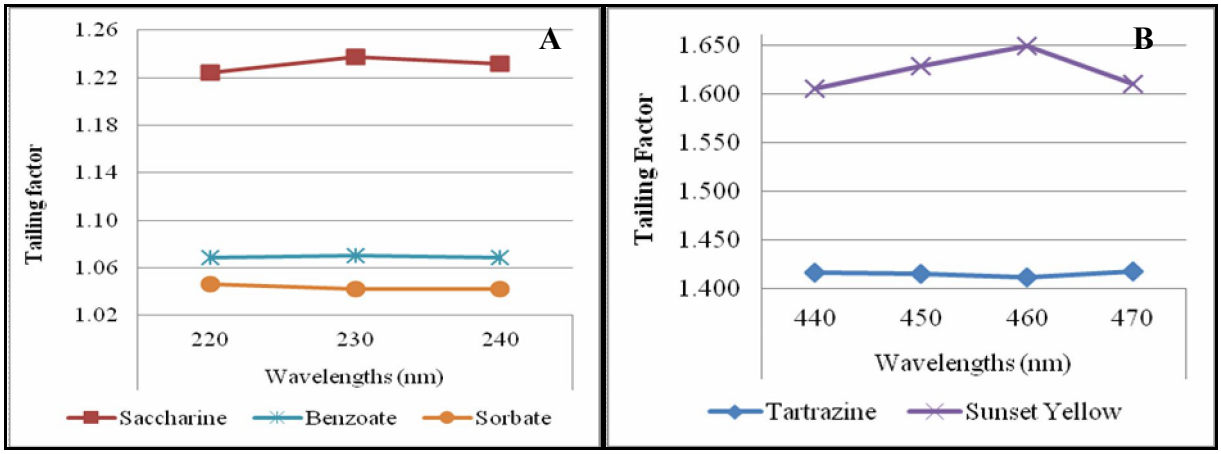


Figure 3. Chromatogram wavelengths 200 nm of phosphat buffer-metanol solution



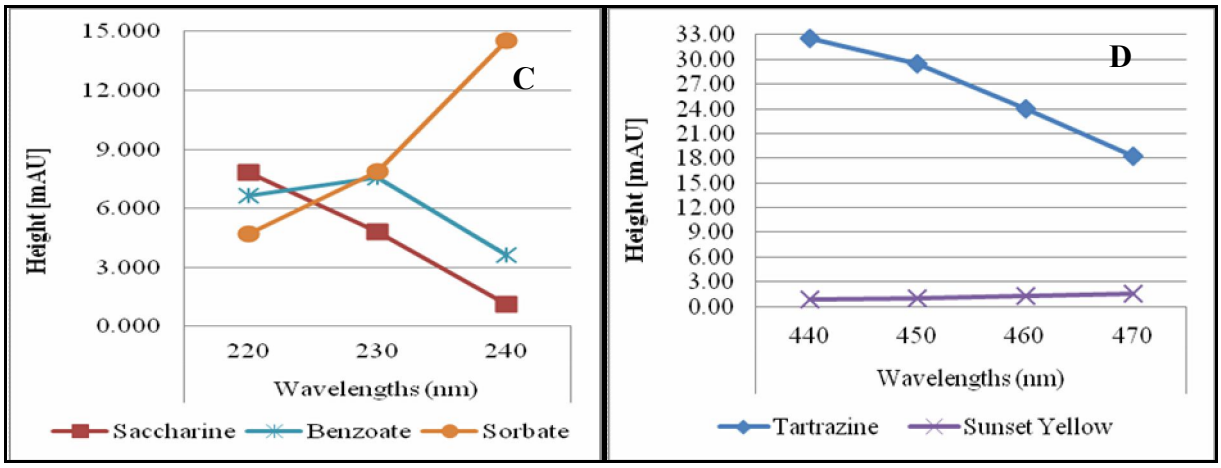
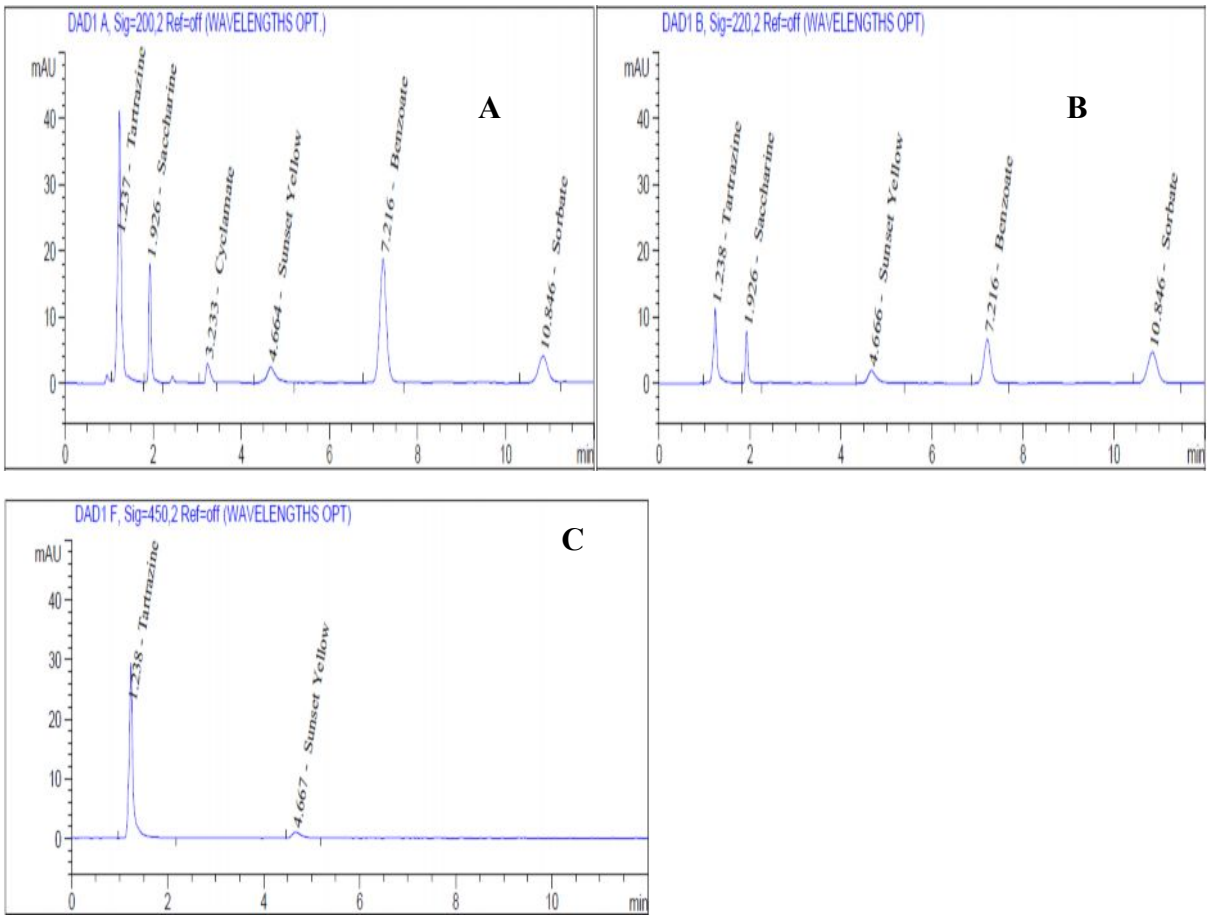


Figure 4. Wavelengths relationship with tailing factor (A, B) and Peak Height (C, D) of saccharine, benzoate, sorbate, tartrazine and sunset yellow



Fuigure 5. Chromatograms wavelengths optimization 200 nm (A), 220 nm (B) and 450 nm (C) of mixed standard solution

Conclusion

The results of the optimization method that the development of the optimum void volume is 30% with a wavelength analysis of 200, 230 and 450 nm in the mobile phase pH 4.5 phosphate buffer and methanol 75:25 (v / v), flow rate of 1.0 ml/min, column temperature 30^{o}C. Parameter optimization; capacity factor, the plate number, resolution, selectivity and tailing factor meets the requirements analysis.

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