# Simultaneous Determination of Six Food Additives in Drinks by High Performance Liquid Chromatography Coupled to Diode Array Detector Detection

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**Summary**: A reversed-phase high performance liquid chromatographic method for the successful separation and determination of 6 synthetic food additives (aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow) was developed. A EclipseXDB-C18 column (250×4.6 mm I.D.; 5 μm) was used and the mobile phase contained methanol and 0.02 mol/L ammonium acetate (pH 6.0) (30:70, v/v) was pumped at a flow rate of 0.7 mL/min at room temperature. Successful separation conditions were obtained for all the compounds using an optimized gradient elution within 10 min. The diode array detector was used to monitor the food additives at 230 nm. The method was thoroughly validated, detection limits for all substances varied between 0.03 and 1.35 μg/mg, the intra-day precision (as RSD) ranged from 1.57% to 4.72 %, the inter-day precision (as RSD) was between 2.05 % and 4.18 %. Satisfactory recoveries, ranging from 90.00 % to 109.87 %, were obtained. The proposed system was applied to drink samples.

Key words: Food additives; HPLC-DAD; Drinks.

## Introduction

In recent years, concern about the importance of food and diet quality has been growing, especially due to the increase in the incidence of diseases that was directly or indirectly related to nutrition habits. As a natural outcome, analysis of food additives came into focus, especially for the assessment of their harmful potentials and quantitative or qualitative value of risks related to their use [1]. A food additive is defined as a substance or mixture of substances, which are generally added to processed foods for a specific purpose such as prevention of spoilage, conservation or fortification of color, flavor, texture, or control of pH, moisture, crispness etc. However, the excessive use of food additives could lead to adverse effects such as metabolic acidosis, convulsions and hyperpnoea in humans [2, 3]. Food additives may be divided into preservatives, flavoring agents, food colorants and so on. Aspartame, acesulfame potassium and sodium saccharin are artificial sweeteners and commonly used in low-calorie foods to control calorie intake. Tartrazine and sunset yellow have been used to make food more attractive and appetizing, they are widely used to compensate for the loss of natural colors of food. Benzoic acid has long been used to inhibit microbial growth in foods. The use of food additive in different kinds of foods is strictly controlled by legislation and harmonized [4]. In many food products more than one additives are added, especially most of low calories drinks contain preservatives, artificial sweeteners and food colorants. Therefore, it is necessary to develop an effective and reliable analytical method that simultaneously determines these additives to monitor the food additive levels for the assurance of food safety.

Different liquid chromatography methods have been reported so far for determination of some food additives in food. These methods include HPLC with UV detection [3, 5-9], ion chromatography with UV detection [10], molecular absorption spectrophotometry using multivariate [11], gas chromatography (GC) with flame ionization detection [12, 13], flow injection analysis (FI) with UV detection [14, 15] and so on. The most popular method used for the simultaneous determination of food additives in food samples is HPLC [16], which needs a proper sample pretreatment to homogenize, extract, cleanup and concentrate the analytes from the complexity of matrix interferences in food samples. Although, many analytes of low molecular weight molecules in various food samples such as organic acids, sugars, amino acids, food colors were determined by on-line dialysis coupled to HPLC system, but there was no report on the simultaneous determination of preservatives, artificial sweeteners and food colorants by this system. Therefore, in this work, the development of a RP-HPLC system for the simultaneous determination of some food additives (aspartame, acesulfame potassium, benzoic acid,

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sodium saccharin, tartrazine and sunset yellow). The food additives basic information is listed in Table-1.

## Results and Discussion

### *Optimization Conditions of HPLC System*

In order to obtain a good separation of six food additives (aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow), short analysis time, less solvent consumption and high sensitivity, the HPLC conditions used in this work were optimized. Factors such as UV absorption wavelengths, mobile phase, HPLC column temperatures and flow rates of mobile phase were optimized to obtain high efficiency and sensitivity.

Normally, the characteristic absorption wavelength was chosen as the test wavelength to quantitative analysis of some compounds in liquid chromatography method. In our work, UV absorption wavelengths of aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow on peak area responses were studied in the range of 200-600 nm. The result showed the wavelength at 230 nm was selected as characteristic absorption wavelength in order to compromise the absorption sensitivity of all analytes, because all analytes have enough absorption sensitivity at 230 nm.

Different mobile phases were tested to find the correct eluent composition for satisfactory resolution of the analytes. The chromatographic behavior of analytes was investigated with the following isocratic mobile phases: Acetonitrile-0.02 mol/L ammonium acetate (30:70, v/v) and methanol-0.02 mol/L ammonium acetate (30:70, v/v), the total runtime was extended and the peak shapes were worse, and some peaks of the five food additives were overlapped for the former mobile phase. The runtime was shorter using the latter mobile phases and the peaks of the analytes were completely separated and the shapes were symmetrical in Fig. 1. Therefore, mobile phase methanol and 0.02 mol/L ammonium acetate (30:70, v/v) was selected for the further experiments.

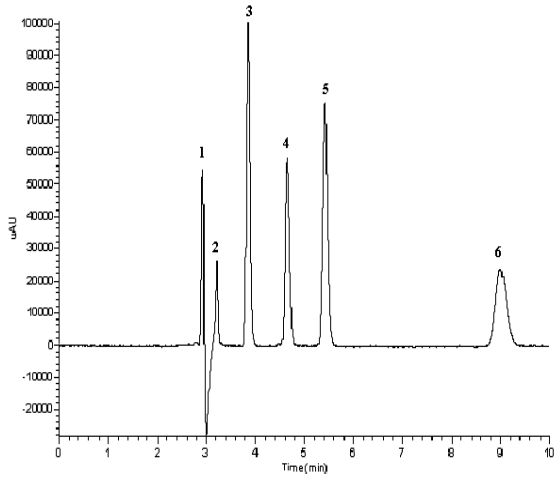



Fig. 1: The chromatograms of the six food additives at 230 nm wavelength scan with HPLC-DAD method in the optimum HPLC conditions (1. Aspartame 2. Acesulfame potassium 3. Benzoic acid 4. Sodium saccharin 5. Tartrazine 6. Sunset yellow).

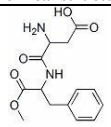
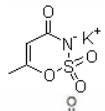
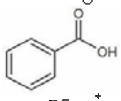
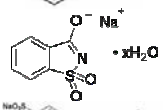
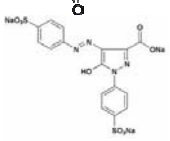
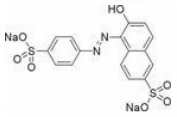
Column temperatures and flow rates of mobile phase ranges of 20-40 $^{o}$C and 0.5-1.5 mL/min, respectively, were investigated. It could be noted that the retention time of each food additive and the analysis time slightly decreased when column temperatures and flow rates of mobile phase increased, while complete separation of all analytes was achieved, a column temperature at 25 $^{o}$C was selected for all future works and the flow rate of 0.7 mL/min was chosen which gave shorter analysis time.

### *Validation of the Method*

Calibration equations of mixed standard solutions, correlation coefficient, linear range, the limits of detection (LOD) and the limits of quantification (LOQ) for the analytes are presented in Table-2. As shown in Table-2, the limits of detection (LOD) and the limits of quantification (LOQ) for the analytes were 0.03-1.35 and 0.15-4.29 mg/kg, respectively.

The precision experiments resulted in good RSDs for both intra-day and inter-day precision. The intra- and inter-day values of retention times and RSD (%) are described in Table-3.

Table-1: Basic information about molecular formula, MW, UV absorption wavelengths and chemical structure of aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow.

| Compound | Molecular formula | MW | UV absorption wavelengthes (nm) | Chemical structure |
|---|---|---|---|---|
| **Aspartame** | $C_{14}H_{18}N_2O_5$ | **294.31** | **258 428** |  |
| **Acesulfame potassium** | $C_4H_4KNO_4S$ | **201.23** | **257 428** |  |
| **Benzoic acid** | $C_7H_6O_2$ | **122.13** | **230** |  |
| **Sodium saccharin** | $C_7H_4O_3NSNa{\cdot}2H_2O$ | **205.17** | **228** |  |
| **Tartrazine** | $C_{16}H_9N_4O_9S_2Na_3$ | **534.37** | **228 269** |  |
| **Sunset yellow** | $C_{16}H_{10}N_2Na_2O_7S_2$ | **452.38** | **236 313 483** |  |

The accuracy of the analytical method was evaluated using the recovery test. The developed method resulted in satisfactory recoveries for the sample 2, ranging from 90% to 109.87%. The obtained mean recoveries are shown in Table-4.

*Application of the Developed Method to Real Samples*

The simultaneous proposed method was applied to the analysis of four soft drink samples. Each sample was analysed according to the procedure described above. The chromatograms of all samples with HPLC-DAD method in the optimum HPLC conditions are showed in Fig. 2, the results obtained are summarized in Table-5. The results indicated all samples contained tartrazine. In addition to sample 4, other samples did not contain benzoic acid. Sample 2 contained four food additives in addition to benzoic acid, sample 3 contained only aspartame and tartrazine, sample 4 contained aspartame, benzoic acid and tartrazine. Therefore, the proposed RP-HPLC method is useful for simultaneous determination of food additives.

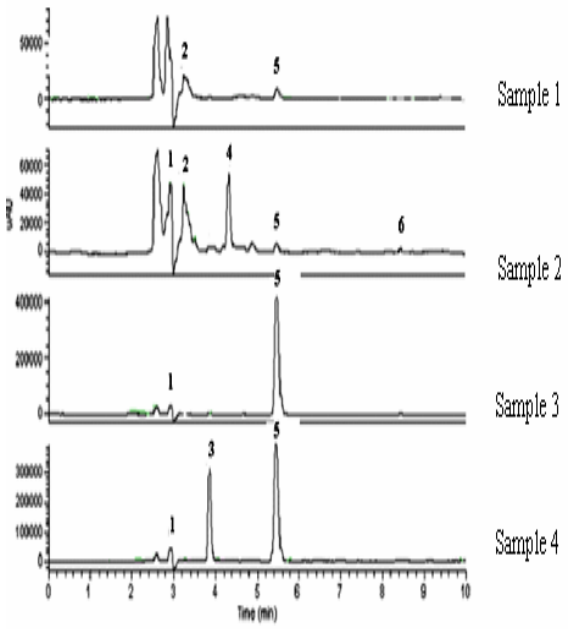



Fig. 2: The chromatograms of all samples with HPLC-DAD method in the optimum HPLC conditions (1. Aspartame 2. Acesulfame potassium 3. Benzoic acid 4. Sodium saccharin 5. Tartrazine 6. Sunset yellow).

Table-2: Linearity and detection limits for the analyte.

| Compound | Regression equation* | Correlation coefficient ($r^2$) | Linear range (µg/mg) | LOD (µg/mg) | LOQ (µg/mg) |
|---|---|---|---|---|---|
| Aspartame | Y=91988x+23547 | 0.9986 | 0.8-50 | 1.35 | 4.29 |
| Acesulfame potassium | Y=60888x-20451 | 0.9952 | 1.5-21 | 0.43 | 1.42 |
| Benzoic acid | Y=343332x-98975 | 0.997 | 0.15-28 | 0.03 | 0.15 |
| Sodium saccharin | Y=248835x-85170 | 0.997 | 0.05-15 | 0.03 | 0.17 |
| Tartrazine | Y=374411x-130093 | 0.9973 | 0.4-40 | 0.12 | 0.40 |
| Sunset yellow | Y=277332x-113416 | 0.9957 | 0.5-35 | 0.18 | 0.62 |

* Y= peak area, X= concentration of compound (µg/mg).

Table-3: Intra- and inter-day precision values for acesulfame potassium, sodium saccharin, aspartame, benzoic acid, tartrazine and sunset yellow.

| Compound | Intra-day precision (n=6, mean) | | | Inter-day precision (n=18, mean) |
|---|---|---|---|---|
| | Day 1 RSD (%) | Day 2 RSD (%) | Day 3 RSD (%) | RSD (%) |
| Aspartame | 1.97 | 2.84 | 2.67 | 2.05 |
| Acesulfame potassium | 3.75 | 4.87 | 3.84 | 4.18 |
| Benzoic acid | 1.57 | 3.61 | 3.59 | 3.27 |
| Sodium saccharin | 4.57 | 1.98 | 3.40 | 3.59 |
| Tartrazine | 3.24 | 4.15 | 4.72 | 3.47 |
| Sunset yellow | 2.71 | 4.92 | 3.65 | 4.16 |

Table-4: Determination results of the recovery and accuracy of the method (n=6).

| Compound | Blank (µg/mg) | Added (µg/mg) | Detected (µg/mg) (n=6) | Mean recovery (%) (n=6) | RSD (%) |
|---|---|---|---|---|---|
| | | 20 | 44.54 | 102.12 | 3.28 |
| Aspartame | 24.12 | 25 | 49.01 | 99.56 | 3.25 |
| | | 30 | 54.53 | 101.36 | 3.71 |
| | | 45 | 106.30 | 107.24 | 4.11 |
| Acesulfame potassium | 58.04 | 60 | 123.96 | 109.87 | 4.97 |
| | | 70 | 131.11 | 104.39 | 3.57 |
| | | 0.05 | 0.049 | 98.00 | 2.43 |
| Benzoic acid | 0.00 | 0.10 | 0.101 | 101.00 | 1.95 |
| | | 1.50 | 1.45 | 96.66 | 3.82 |
| | | 3.50 | 8.05 | 98.29 | 2.16 |
| Sodium saccharin | 4.61 | 5.00 | 9.55 | 98.80 | 3.66 |
| | | 5.5 | 10.21 | 101.82 | 3.38 |
| | | 3.50 | 7.91 | 99.43 | 2.79 |
| Tartrazine | 4.43 | 4.50 | 8.99 | 101.33 | 4.04 |
| | | 5.50 | 9.77 | 97.09 | 2.20 |
| | | 1.00 | 2.20 | 92.00 | 4.39 |
| Sunset yellow | 1.28 | 1.30 | 2.45 | 90.00 | 4.74 |
| | | 1.50 | 2.68 | 93.33 | 3.88 |

Calculated by subtracting the total amount after spiking from the amount in the sample 2 before spiking. Data were expressed as means of six experiments.

Table-5: Determination results of aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow all samples (n=6) (µg/mg)

| Sample | Aspartame | Acesulfame potassium | Benzoic acid | Sodium saccharin | Tartrazine | Sunset yellow |
|---|---|---|---|---|---|---|
| Sample 1 | Not detected | 29.32 | Not detected | Not detected | 5.19 | Not detected |
| Sample 2 | 24.12 | 58.04 | Not detected | 4.61 | 4.43 | 1.28 |
| Sample 3 | 6.93 | Not detected | Not detected | Not detected | 82.92 | Not detected |
| Sample 4 | 11.98 | Not detected | 48.43 | Not detected | 78.90 | Not detected |

## Experimental

*Instrumentation*

pH measurements of the mobile phase were done by using a digital type pH meter (Mettler Toledo, S20 SevenEasy, Columbia, USA). The analytes were carried out employing an on-line coupling between DAD detection and RP-HPLC. The chromatographic system consisted of an online Finnigan Surveyor degasser, a Finnigan Surveyor Pump Plus (Thermo Finnigan, MA, USA), a diode array UV detection (DAD) system (Thermo Finnigan, MA, USA). A EclipseXDB-C18 column (250×4.6 mm I.D.; 5 µm pore size; Agilent Technologies, Inc., Palo Alto, CA) was used for the analysis, preceded by a C18 guard column (12.5 × 4.6 mm I.D.; 5 µm pore size; Agilent Technologies, Inc., Palo Alto, CA).

*Chemicals and Reagents*

Aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow came from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). An Ultra-pure Water System (SG Ultra Clear system, Wasseraufbereitung und Regenerierstation GmbH, Germany) was used to produce ultra pure water with specific conductivity down to 0.055 µS/cm for the analysis of HPLC.

Mixed stock solutions of aspartame, acesulfame potassium, benzoic acid, sodium saccharin, tartrazine and sunset yellow were prepared at concentrations of 400 μg/mL in methanol; 12.5 mL mixed stock solutions were transferred into 50 mL volumetric flasks and diluted with mobile phase (30% of methanol and 70% 0.02 mol/L ammonium acetate) to volume to yield mixed standard solutions. Then, 0. 5, 1.00, 5.00, 10.00, 20.00 and 40 μg/mL of the mixed standard solutions were prepared to yield a series of working solutions for the calibration curves. Mixed stock solutions were stored at 4 $^{o}$C. Working solutions were prepared fresh daily by diluting mixed stock solutions with mobile phase and filtered through a 0.45 μm micro-filter prior to analysis.

*Chromatographic Conditions*

The HPLC isocratic mobile phase which consisted of methanol and 0.02 mol/L ammonium acetate (pH 6.0) (30:70, v/v) was pumped at a flow rate of 0.7 mL/min at room temperature (25 $^{o}$C) [17]. The sample injection volume was 20 μL. The UV detection was operated at 230 nm. The total analysis time was 10 min. Six injections were performed for each sample.

*Preparation of Real Samples*

All of the drink samples were purchased at a local supermarket. These sample solutions were degassed thoroughly in an ultrasonic bath for 20 min. An aliquot of 10 mL was transferred to a 10 mL polypropylene centrifuge tube, a 20 min centrifugation at 10000 rpm was applied, and the resulting supernatant was carefully taken and filtered through a 0.45 mm micro-filter.

*Method Validation*

Several criteria were used to evaluate the method, including the sensitivity, the linearity, the reproducibility and the recovery of the method. The sensitivity of the method was evaluated via the limits of detection (LOD) and quantification (LOQ), which were determined according to ICH recommendations, based on the SD of the response and the slope. For LOD, the ratio of the SD of γ-intercepts of regression lines (σ) to the slope was multiplied by 3.3, whereas it was 10 for LOQ. Slope and s values were calculated from the pooled data obtained from linearity experiments. In addition to the calculation method, evaluation of LOD and LOQ based on signal to noise ratio was also used to confirm the values. The signal to noise ratio was taken as 3.3 for LOD and 10 for LOQ. The linearity of the method was evaluated via the correlation coefficients ($r^2$) of the calibration curves. The method precision included intra-day and inter-day precision experiments, the intra-day precision was determined by analysising an independently sample solution using the proposed method on the same day. The inter-day precision was determined by analysising an independently sample solution on three successive days. The recoveries of the analytes from spiked food samples were obtained by comparing with spiked standard aqueous samples extracted under the same conditions, which were investigated at low, medium and high concentrations, respectively, according to the calibration curve ranges.

**Conclusion**

An efficient and accurate HPLC analytical method for the simultaneous determination of six permitted synthetic food additives in a single run by high performance liquid chromatography-diode array detection was developed, the separation of mixtures of such additives was successfully under optimized conditions using methanol and 0.02 mol/L ammonium acetate (pH 6.0) (30:70, v/v) as mobile phase. This proposed method has shown good operational stability and gave reliable and reproducible results with simple sample pretreatment operation.

**References**

1. O. Nafiz Can, G. Arli and Y. Lafci. *Journal of Separation Science*, **34**, 2214 (2011).
2. A. L. Branen, P. M. Davidson and S. Salminen, *Food Additives*, Marcel Dekker, Inc., New York (1990).
3. E. C. Demiralay, G. Ozkan and Z. Guzel-Seydim, *Chromatographia*, **63**, 91 (2006).
4. *National Standard of the People's Republic of China GB 2760*, Standards Press of China, Beijing (1996)
5. C. M. Lino and A. Pena, *Food Chemistry*, **121**, 503 (2010).
6. S. A. V. Tfouni and M. C. F. Toledo, *Food Control*, **13**, 117 (2002).
7. I. Garcia, M. C. Ortiz, L. Sarabia, C. Vilches and E. Gredilla, *Journal of Chromatography* A, **992**, 11 (2003).
8. I. Techakriengkrai and R. Surakarnkul, *Journal of Food Composition and Analysis*, **20**, 220 (2007).

9. B. Saad, Md. F. Bari, M. I. Saleh, K. Ahmad and M. K. M. Talib, *Journal of Chromatography* A, **1073**, 393 (2005).
10. N. Dossi, R. Toniolo, S. Susmel, A. Pizzarello and G. Bontempelli, *Chromatographia*, **63**, 557 (2006).
11. M. A. Cantarelli, R. G. Pellerano, E. J. Marchevsky and J. M. Camina, *Food Chemistry*, **115**, 1128 (2009).
12. C. Dong and W. Wang, *Analytica Chimica Acta*, **562**, 23 (2006).
13. C. Dong, Y. Mei and L. Chen, *Journal of Chromatography* A, **1117**, 109 (2006).
14. J. F. Garcia-Jimenez, M. C. Valencia and L. F. Capitan-Vallvey, *Analytica Chimica Acta*, **594**, 226 (2007).
15. J. F. Garcia-Jimenez, M. C. Valencia and L. F. Capitan-Vallvey, *Analytical Letters*, **39**, 1333 (2006).
16. R. Lucena, S. Cardenas, M. Gallego and M. Valcarcel, *Analytica Chimica Acta*, **530**, 283 (2005).
17. Q. H. Yan, L. Yang, H. R. Zhang and L. Y. Niu, *Journal of Liquid Chromatography and Related Technologies,* **35**, 268 (2012).