



# Optimization and validation of HPLC–DAD method for simultaneous analysis of sweeteners, preservatives, and caffeine in sugar-free beverages

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## Abstract

In this study, the optimization and performance evaluation of a high-performance liquid chromatography with diode array detection (HPLC–DAD) method for the simultaneous determination of four sweeteners (acesulfame-potassium, saccharin, aspartame, and rebaudioside A), two preservatives (sodium benzoate and potassium sorbate), and caffeine in sugar-free drinks are presented. The separation was carried out using a gradient elution of acetonitrile and phosphate buffer (12.5 mM, pH = 3.3) on a reversed-phase column. Under the optimized conditions, the method allowed for the specific and selective simultaneous separation of all target analytes in less than 9 min. The developed HPLC–DAD method was validated and demonstrated excellent linearity (all analytical curves showed  $R^2 \geq 0.9995$ ), satisfactory accuracy (recovery values ranging between 94.1 and 99.2% in real samples), and repeatability (intra- and inter-day relative standard deviations were  $\leq 2.49\%$ ). Overall, 69 products available in the Hungarian market were successfully tested with the applied method.

**Keywords** Sweeteners · Preservatives · Caffeine · HPLC–DAD · Drinks · Rapid quantification

## Introduction

Recently, the market of diet-friendly beverages has been growing due to the fact that consumer food habits are trending towards healthier alternatives to high calorie beverages. According to the newest report of Grand View Research, Inc. [1], thanks to an average annual growth rate of 3.2%, the global diet soft drink market revenue is expected to reach USD 5.17 billion by 2025.

Diet soft drinks typically contain water, carbon dioxide, sweeteners, acidifiers, flavorings, preservatives, and antioxidants [2]. In addition, cola and energy drinks contain higher amounts of stimulant ingredients such as caffeine (CAF), thereby helping the consumer feel less tired [3]. Most sugar-free beverages are sweetened with artificial sweeteners, such as acesulfame-potassium (ACE), saccharin (SAC), aspartame (ASP), sodium cyclamate, and/or sucralose. On the

other hand, since the use of steviol glycosides (e.g., rebaudioside A (RBA) and stevioside) have been authorized in the EU as natural sweeteners [4], more and more manufacturers have been using them in their products. Among preservatives, the most significant are sodium benzoate (BEN) and potassium sorbate (SOR). Although these food additives are considered generally safe (GRAS) substances [5], the safety of their artificial variants is still debated, especially in larger quantities. For instance, Debras et al. [6] affirmed in their study that some artificial sweeteners (especially ASP and ACE) could be associated with increased cancer risk. Earlier studies also described that benzoates could cause several undesirable symptoms such as hyperactivity [7], histological changes in liver [8], or apoptosis [9]. In addition, the consumption of caffeinated soft drinks has increased in recent decades, especially among children and young people [10]. It may also be of some concern because several studies have shown that higher levels of caffeine intake can enhance its adverse effects, especially at a younger age [11].

The most widely used method for determining sweeteners, preservatives and caffeine in foods is reversed-phase HPLC with ultraviolet spectrophotometry in both laboratories and the food industry. The advantage of HPLC is that it is fast, highly efficient, accurate, and sensitive. However,

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It is important to note, that some sweeteners, such as cyclamate and sucralose, are undetectable by UV detection due to the absence of a chromophore. Ion chromatography (IC) and capillary electrophoresis (CE) methods have also been used in this area. The advantage of IC is that harmless and cheap eluents are used for the analysis instead of organic solvents. Furthermore, in the case of the EC method, the analysis time is shorter, and the solvent consumption is also lower. Other alternative methods for the determination of sweeteners are the following: flow-injection analysis, electrochemical, and spectroscopic methods. Although these methods are time-efficient, they are rather expensive and can only be used for the simultaneous determination of one or at most two analytes. Gas chromatography may be an alternative method for the analysis of sweeteners. However, this method has many drawbacks, including having a required derivatization step and a time-consuming and labor-intensive implementation [12]. From a chemical point of view, the other important thing is that, although sugar-free drinks seem to be simple matrices, they may pose significant challenges during separation by HPLC due to the complexity of their ingredients. One of the main problems is that the compounds are from different chemical groups, and they have significantly different physicochemical, electrochemical, and spectral properties. Therefore, there is a limited choice of detection systems that can detect the various compounds simultaneously in this type of drink [13]. For example, the different substances including sweeteners, preservatives, and caffeine are usually analyzed separately [14]. Therefore, the aim of the study was to optimize and validate an easy and effective HPLC–DAD method for simultaneous determination of sweeteners, preservatives, and caffeine in sugar-free beverages. This could be of great help to soft drink producers as simultaneous determination of food components is very important for food quality control and regulation enforcement. Furthermore, we also assessed the compliance of the products available on the market in Hungary with the requirements of Regulation (EC) No 1333/2008 [15] of the European Parliament and of the Council.

## Materials and methods

### Chemicals and reagents

Each analytical standard, namely ACE-K ( $\geq 99\%$ ), SAC ( $\geq 98\%$ ), ASP ( $\geq 98\%$ ), RBA ( $\geq 96\%$ ), CAF (99%), SOR ( $\geq 99\%$ ), and BEN ( $\geq 99.5\%$ ) was purchased from Sigma-Aldrich (Hungary). HPLC grade acetonitrile was supplied by Fisher Scientific (UK). Phosphoric acid (85%) and potassium dihydrogen phosphate (p. a. grade) were from Merck (Germany). High purity deionized water with a resistivity

of 18 M $\Omega$  cm was used for the dissolution of chemicals and dilution of diet, light, and zero drinks.

### Instrumentation

Analyses of ACE-K, SAC, ASP, CAF, SOR, BEN, and RBA were performed using a LaChrome 7000 type of HPLC system (Merck Hitachi, Germany), which was composed of L-7000 pump, L-7250 autosampler, L-7350 column thermostat, and L-7450 diode-array detector. Data processing was performed using D-7000 HPLC System Manager 4.0 (HSM) software.

In addition, the fruit nectars were centrifuged using a Z206A laboratory centrifuge (Hermle, Germany) before dilution. Carbonated soft drinks were treated in a UC002BM1 ultrasound bath (Tesla, Czechoslovakia). The high purity deionized water was produced by Zeneer Power I system (Human Corporation, Korea).

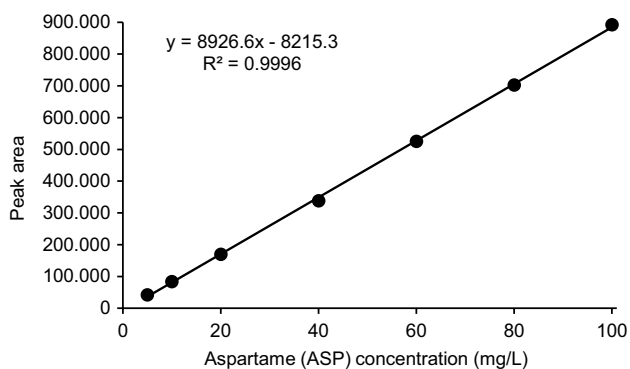
### Chromatographic conditions

Separation and quantification was carried out using a Kromasil<sup>®</sup> C18 (150 mm  $\times$  4.6 mm, 5  $\mu$ m) analytical column. The mobile phase was composed of acetonitrile (A) and phosphate buffer (B; 12.5 mM at pH 3.3). The optimized gradient elution program used for the separation of ACE, SAC, CAF, ASP, BEN, SOR, and RBA was as follows: 0 min: 5% A, 0–10 min: 50% A, held for 5 min, 15–16 min: 5% A, and held for 5 min for re-equilibration. The flow rate was 1.5 mL/min, and the injection volume was 10  $\mu$ L. The column oven temperature was set to 30 °C. The detection wavelength range in each case was 200–380 nm. All samples were filtered through 0.22  $\mu$ m PVDF membrane filters prior to HPLC analysis.

### Samples and sample preparations

In this study, 69 sugar-free (diet, light, and zero) beverages were analyzed. The samples were purchased at several supermarkets and one of the largest chains of fast-food restaurants in Hungary and were categorized as follows: soft drinks (cola, tonic, lemon, lime, orange, ginger, grape, raspberry, watermelon, apple, sour cherry, cranberry, forest fruit, blood orange, and mixed fruit flavors), fruit nectars (apple, peach, orange, pineapple, white grape, and mixed fruit flavors), iced teas (natural, lemon, and peach flavors), and energy drinks (natural, strawberry, and peach flavors). All the collected samples were stored at 5 °C in a refrigerator until analysis.

The carbonated drinks (approximately 100 mL) were sonicated for 15 min at maximum power (300 W), in order to remove the carbon dioxide. Fruit nectars were centrifuged



**Fig. 1** Linear calibration curve of aspartame by using optimized HPLC–DAD method

for 20 min at 6000 $\times$ g. Finally, the aliquots (1 mL) of all samples were diluted 5 times in H<sub>2</sub>O before HPLC analysis.

### System suitability test

To ensure the adequate performance of the HPLC system, peak retention time, capacity factor ( $k'$ ), selectivity ( $\alpha$ ), resolution ( $R$ ), and peak asymmetry ( $A_s$ ) were evaluated by injecting three replicates using a mixture standard solution at a concentration of 20 mg/L. Acceptance criteria were based upon following:  $k'$  should be equal to or greater than 1,  $\alpha$  should be greater than 1,  $R$  should be equal to or greater than 1.5, and  $A_s$  should be between 0.8 and 1.2. The acceptance criteria were chosen according to the study reported by Burana-osot et al. [16] and the regulatory methods found in chapter of 2.2. 46. of Ph. Eur.6.0. [17]

### Analytical method validation

The developed HPLC–DAD method for the simultaneous separation of sweeteners, preservatives, and CAF was evaluated in terms of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

For the linearity study, seven levels of working solutions within the ranges of 5–80 or 5–100 mg/L were prepared by serial dilution of a 1 mg/mL stock solution. H<sub>2</sub>O was used to prepare both the stock solution and the working solutions. Linear least-squares regression was applied to analyze the standard curves of each analyte, and the determination coefficient ( $R^2$ ) of the regression formula was used to validate the linearity. Figure 1 presents of one of the analyzed compounds. Precision was assessed by analyzing three different concentrations (5, 20, and 60 mg/L) of mixed standard solution, measured on the same day (intra-day) and for three consecutive days (inter-day). The accuracy of the method was determined by a standard addition (spiking) technique. Known amounts of the working solution containing analytes

were added to soft drinks (FD39, FD21, FD14, FD05, FD41, FD28, and FD16), and the results were calculated as percent recovery (%) by comparing the amount of analyte in the spiked and nonspiked samples. Moreover, DAD was used to check the peak purity to confirm that there were no co-eluting compounds. The LOD and LOQ were calculated based on the standard deviation of the y-intercept ( $\sigma$ ) and slope ( $m$ ) of the calibration curve obtained from linear regression. The LOD and LOQ values were calculated according to Eqs. (1) and (2)

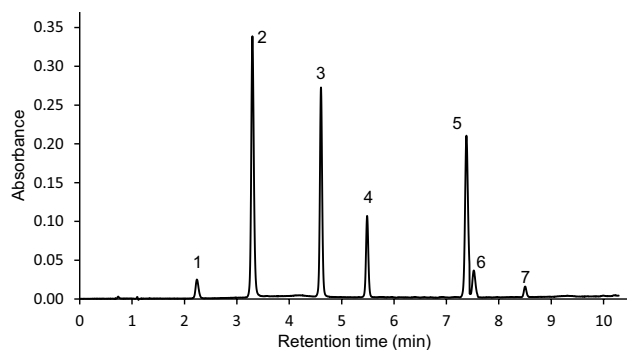
$$LOD\left(\frac{mg}{L}\right) = \frac{3.3 \times \sigma}{m} \quad (1)$$

$$LOQ\left(\frac{mg}{L}\right) = \frac{10 \times \sigma}{m} \quad (2)$$

## Results and discussion

### Optimization of HPLC–DAD separation

When developing the HPLC–DAD method, we originally chose isocratic separation due to its cost effectiveness, simplicity and the added benefit of avoiding the need for re-equilibration with the initial mobile phase composition between subsequent injections. The initial chromatographic conditions and mobile phase composition were chosen to be similar to those used by Serdar and Knežević [18]. The optimization was performed on a 150 mm long C18 column (Kromasil<sup>®</sup>) with an internal diameter of 4.6 and a particle size of 5  $\mu$ m. The mobile phase consisted of 15% acetonitrile and 85% phosphate buffer (pH = 3.3) with a flow rate of 1.0 mL/min. However, these chromatographic conditions did not provide satisfactory separation. Although the decrease of acetonitrile percentage (10%) in the mobile phase increased the retention of the peaks, the obtained values, especially for the first eluted peak (ACE: 0.44), were not acceptable. The separation time was also longer and some peaks were very distant from each other. Moreover, BEN and SOR were not separated from each other. It is well known that better  $k'$  values may be achieved by changing the mobile phase composition [19]. Therefore, we decided to use a gradient method to increase the efficiency of the chromatographic separation. Four linear-gradients were run to determine the adequate solvent domain in four separate measurements. During elution, the original acetonitrile concentration (5%) was increased to 70, 60, 50, and 40% respectively in 10 min using a flow rate of 1 mL/min. Satisfactory separation was observed when the concentration of acetonitrile increased from 5 to 50% in 10 min. However, since it



**Fig. 2** Obtained chromatogram of the 20 mg/L standard mixture at 202 nm wavelength by using optimized HPLC–DAD method. ACE (1), SAC (2), CAF (3), ASP (4), BEN (5), SOR (6), and RBA (7)

took about 15 min to separate all the target analytes from each other, the flow rate was increased to 1.5 mL/min, which allowed to reduce the total running time and the cost of analyzing the samples. As shown in Fig. 2, under the optimized condition, all target analytes were separated from each other in less than 9 min and the analysis was completed within in 21 min. The final program of gradient elution was reported in Sect. 2.3. All in all, one of the main advantages of our proposed method is that it is faster than those reported in previous studies [20–22].

## System suitability test

Before validation experiments, we checked whether the applied HPLC system is capable of providing data of acceptable quality. For this purpose, a standard mixture solution with a concentration of 20 mg/L was used. Table 1 presents the values obtained using the optimized method. Although the gradient program proved to be more efficient compared to the isocratic separation, the drawback of this method is that complete baseline separation between BEN and SOR was not achieved. However, an R with a value of 1.46 was acceptable because it produced 97% separation. In addition, purity analysis of the BEN and SOR peaks showed no sign of overlap and many cases, these preservatives were present individually or absent in the examined products. As shown in Table 1, all other parameters were within the appropriate ranges. Thus, the method was considered acceptable for the validation process.

## HPLC method validation

To verify that a method is effective and reliable for measurement, the validation of HPLC procedures are crucial. The results of the performance characteristics of the optimized HPLC–DAD method are in Table 2. Overall, we observed good linearity with high determination coefficient values ( $R^2 \geq 0.9995$ ), which unequivocally corroborated that the applied HPLC method is adequate for the quantification of ACE, SAC, CAF, ASP, BEN, SOR, and RBA in sugar-free

**Table 1** Results of system suitability tests of optimized HPLC method

Parameters	ACE	SAC	CAF	ASP	BEN	SOR	RBA
Retention time (min)	2.29	3.32	4.64	5.48	7.31	7.48	8.50
RSD% of the retention time	0.42	0.29	0.31	0.38	0.34	0.28	0.39
Capacity factor ( $k'$ )	1.03	1.96	3.16	3.92	5.68	5.74	6.58
Selectivity ( $\alpha$ )	–	1.91	1.62	1.24	2.78	2.85	1.16
Resolution (R)	–	10.7	15.4	10.6	19.49	1.46	10.1
Asymmetry ( $A_s$ )	1.12	1.10	1.05	1.03	1.19	1.03	1.09

**Table 2** Results of validation of proposed HPLC–DAD method for simultaneous analysis sweeteners, preservatives, and caffeine

Parameters	ACE	SAC	CAF	ASP	BEN	SOR	RBA
$A_{\max}$ (nm)	227	202	273	202	230	260	202
Linearity range (mg/L)	5–80	5–80	5–80	5–100	5–80	5–100	5–100
Slope	9 840	34 806	8 283	8 927	13 772	31 907	1 286
Intercept	– 2 694	– 39 717	– 5 505	– 8 215	– 139	– 17 755	– 255
Determination coefficient ( $R^2$ )	0.9995	0.9997	0.9996	0.9996	1.0000	0.9998	0.9998
LOD (mg/L)	1.73	1.58	1.56	1.60	0.46	1.29	1.18
LOQ (mg/L)	5.23	4.79	4.74	4.85	1.39	3.91	3.56
Purity factor	0.9998	0.9993	0.9991	0.9996	0.9994	0.9999	0.9989
Recovery (%)	94.8	95.5	97.0	94.1	96.3	99.2	98.6

beverages. Table 2 also presents the LOD and LOQ values. In this case, sensitivity was found to be satisfactory towards each analyte since the LOQs were lower than the lowest standard concentration tested in the dynamic interval of the calibration curve, except for ACE-K. However in this case, the LOQ value obtained was close to the lowest concentration of the calibration curve as well. The data also showed that the recovery values were adequate for the intended purposes. The lowest recovery was noted for ASP (94.1%), while the highest for SOR (99.2%) in the spiked samples. The peak-purity assessment demonstrated the absence of co-eluting substances, confirming that the method is adequate for the simultaneous analysis of ACE, SAC, CAF, ASP, BEN, SOR, and RBA in diet, light, and zero drinks. Table 2 shows the peak-purity index for all analytes in the standards. In addition, the peak purity for the analytes found in the samples ranged as follows: ACE: 0.9964–0.9998, SAC: 0.9985–0.9990, CAF: 0.9984–0.9989, ASP: 0.9988–0.9992, BEN: 0.9964–0.9992, SOR: 0.9975–0.9998, and RBA: 0.9876–0.9954.

We also analyzed intra- and inter-day variability to assess the precision and accuracy of the optimized method (Table 3). The percent relative standard deviations (RSD%) were below 2.49% for repeatability of both intra- and inter-day precision studies. In addition, the intra- and inter-day

accuracy of different analytes varied from 95.5 to 113 and 96.2 to 109%, respectively.

In general, the results of the performance characteristics revealed that the optimized HPLC–DAD method was adequately precise, accurate, and reliable for the simultaneous measurement of these seven analytes present in sugar-free drinks.

### Analysis of samples

The optimized method was successfully applied for the analysis of ACE, SAC, CAF, ASP, BEN, SOR, and CAF in sugar-free drinks (Fig. 3).

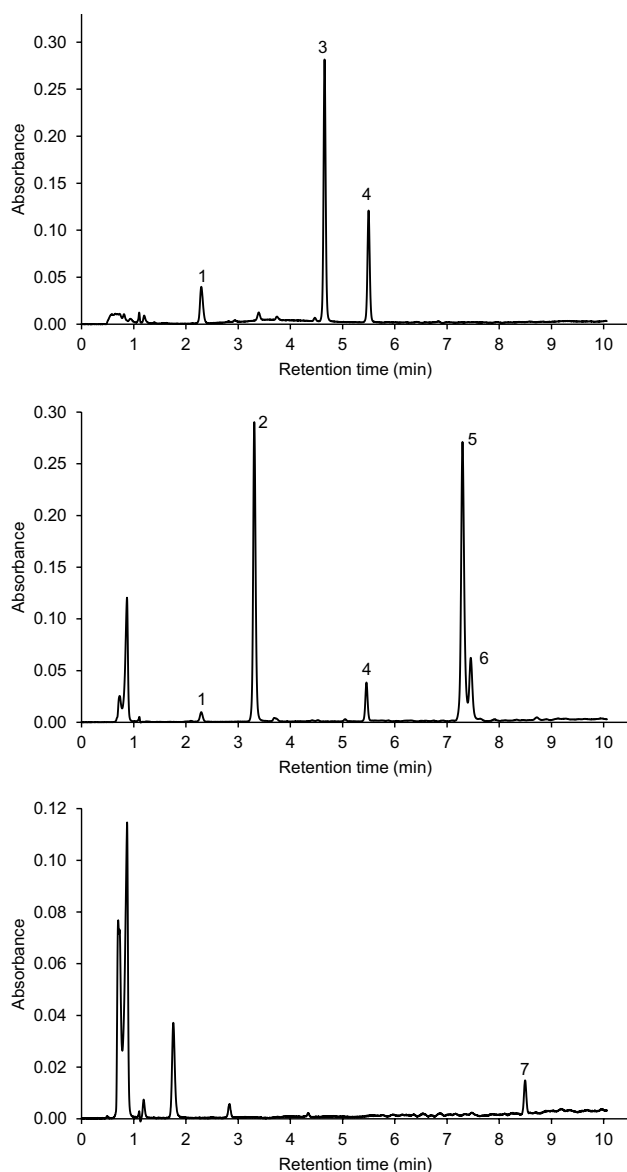
Among the sweeteners analyzed, ACE was the most frequently detected in sugar-free drinks (62 out of 69 samples) at concentrations between  $16.0 \pm 0.18$  and  $238 \pm 5.82$  mg/L, while RBA ( $78.6 \pm 0.36$ – $103 \pm 2.41$  mg/L) was only found in four samples. ASP ( $42.2 \pm 0.12$ – $490 \pm 6.44$ ) and SAC ( $12.3 \pm 0.14$ – $86.0 \pm 2.06$ ) were identified in 22 and 9 samples respectively. Overall, the results obtained for SAC were below those found in Bangladesh [23] and Brazil [24]. At the same time, studies in Turkey [13] and Poland [25] revealed similar results to ours studies for ACE and ASP. BEN and SOR were detected and quantified simultaneously in 11 samples and separately in 10 samples.

**Table 3** Intra- and inter-day precision of sweeteners, preservatives, and caffeine

Component	Nominal (mg/L)	Intra-day precision			Inter-day precision		
		Measured (mg/L)	RSD (%)	Accuracy (%)	Measured (mg/L)	RSD (%)	Accuracy (%)
ACE	5	5.03	0.04	101	4.99	0.42	99.8
	20	20.1	0.91	100	19.9	0.76	99.3
	60	60.0	2.17	100	59.4	0.19	98.9
SAC	5	5.47	0.25	109	5.37	0.52	107
	20	19.3	0.40	96.5	19.2	0.31	96.2
	60	58.6	1.92	97.7	58.5	0.94	97.5
CAF	5	5.37	1.46	107	5.25	0.10	105
	20	19.7	0.81	98.7	19.6	0.16	97.9
	60	60.1	2.49	100	59.0	0.12	98.4
ASP	5	5.60	0.84	111	5.46	0.46	109
	20	19.8	1.25	99.2	19.6	0.41	98.2
	60	57.3	1.44	95.5	58.0	0.02	96.7
BEN	5	5.65	0.39	113	5.55	0.20	109
	20	19.9	0.15	99.3	19.7	0.27	98.5
	60	60.0	0.11	100	59.6	0.12	99.4
SOR	5	5.48	0.52	110	5.41	0.49	108
	20	20.5	0.76	98.6	19.3	0.02	96.4
	60	59.1	0.18	98.4	58.8	0.53	97.9
RBA	5	5.23	0.49	105	5.09	0.08	102
	20	19.9	0.33	99.5	19.5	0.28	97.6
	60	60.3	0.36	100	59.3	0.14	98.8

Data are presented as mean values; n = 3





**Fig. 3** Chromatograms of some real samples under optimized conditions at 202 nm. ACE (1), SAC (2), CAF (3), ASP (4), BEN (5), SOR (6), and RBA (7)

Overall, the concentrations of BEN and SOR ranged from 22.2 to 150 mg/L (mean value:  $112 \pm 0.88$  mg/L) and from 49.7 to 278 mg/L (mean value:  $168 \pm 1.20$  mg/L), respectively. In Portugal, Lino and Pena [22] found that BEN was a more commonly used as a preservative than SOR. The values measured by the authors were between 91 and 172 mg/L with an average concentration of  $158 \pm 39$  mg/L for BEN, while for SOR was measured in the range of 78 to 350 mg/L with the average amount being  $172 \pm 96$  mg/L. CAF was detected in 24 samples at concentrations between  $32.5 \pm 0.16$  and  $328 \pm 0.32$ . As expected, energy drinks contained the most CAF from among the analyzed caffeinated beverages, with an average value of  $310 \pm 1.56$  mg/L.

In their study, Al-Bratty et al. [26] showed that the CAF content of commercial energy beverages available in the Saudi Arabian Market ranged between 208 and 337 mg/L with an average value of 297 mg/L. Colas from the carbonated soft drink group showed CAF content in the range of  $86.6 \pm 0.61$ – $110 \pm 2.11$ . These findings were similar to those obtained by Grembecka and Szefer [27]. The lowest CAF levels were measured in iced teas. The concentrations obtained from the measurements are provided in Table 4 and expressed in mg/L.

The results were also evaluated in comparison to the maximum permitted concentration provided in Regulation (EC) No 1333/2008 of the European Parliament and of the Council [15]. This regulation controls the use of BEN and SOR in beverages, setting a limit of up to 150 mg/L and 300 mg/L, respectively. Moreover, this regulation states that the maximum level of SOR can only be 250 mg/L in combination with BEN. In the case of artificial sweeteners, the maximum permitted concentration for ACE, ASP, and SAC are 350 mg/L, 600 mg/L, and 80 mg/L, respectively. In the EU, there is no legal limit for the maximum amount of CAF in beverages. However, the Consumer Information Regulation (EU) 1169/2011 [28], which came into force in December 2014, states that beverages (energy drinks) with an added caffeine content of more than 150 mg/L must be labeled as “High caffeine content”. As shown in Table 4, the measured concentrations of all compounds were in compliance with the requirements of the EU regulation with the exception of steviol glycosides. In this case, the maximum permitted level is 80 mg/L. However, a higher RBA level was measured in two samples (FD38 and FD40). A similar observation was also reported by Diviš et al. [14].

Similarly to previous observations in literature [14, 29], the results of qualitative analysis were not in agreement with the specifications on the labels in some cases. In two of these cases, we did not detect ASP even though it was represented on the label of products FD09 and ED01. Additionally, the label of product FD04 indicated the presence of SOR, even though it was not possible to identify in the sample.

## Conclusions

The present investigation resulted in the optimization of a gradient HPLC–DAD analysis method for the simultaneous separation of four sweeteners, two preservatives and caffeine, which was validated in terms of system suitability, linearity, precision, accuracy, the limit of detection, and limit of quantification. The optimized method is rapid, precise, accurate, and easy to apply, making it suitable for the quantification of ACE, SAC, CAF, ASP, BEN, SOR, and RBA in sugar-free beverages. Moreover, the separation time of all target analytes was short (<9 min). Therefore, the proposed analytical method

**Table 4** Concentrations of sweeteners, preservatives, and caffeine in real samples measured by optimized HPLC–DAD

Samples	Content of analyte (mg/L)						
	ACE	SAC	CAF	ASP	BEN	SOR	RBA
FD01 <sup>a</sup>	148 ± 0.68	–	97.2 ± 0.25	109 ± 0.26	–	–	–
FD02 <sup>a</sup>	110 ± 0.61	–	86.6 ± 0.61	203 ± 2.01	–	–	–
FD03 <sup>a</sup>	151 ± 0.75	–	98.7 ± 0.56	113 ± 0.32	–	–	–
FD04 <sup>a*</sup>	39.9 ± 0.45	–	88.9 ± 1.43	344 ± 4.54	–	n.d	–
FD05 <sup>a</sup>	40.3 ± 0.41	–	93.2 ± 0.28	352 ± 3.05	–	–	–
FD06 <sup>a</sup>	146 ± 0.87	–	95.7 ± 0.34	112 ± 0.29	–	–	–
FD07 <sup>b</sup>	34.9 ± 0.77	–	110 ± 2.11	437 ± 7.95	–	–	–
FD08 <sup>b</sup>	34.5 ± 0.12	–	106 ± 0.54	490 ± 6.44	–	–	–
FD09 <sup>b</sup>	39.2 ± 0.68	–	104 ± 1.89	n.d	–	–	–
FD10 <sup>b</sup>	142 ± 2.33	–	92.2 ± 1.74	109 ± 2.79	–	–	–
FD11 <sup>c</sup>	91.4 ± 0.83	–	124 ± 0.82	–	–	–	–
FD12 <sup>d</sup>	98.9 ± 0.98	–	132 ± 1.46	–	–	–	–
FD13 <sup>e*</sup>	145 ± 1.50	–	–	224 ± 2.72	150 ± 1.80	–	–
FD14 <sup>e</sup>	–	41.8 ± 0.81	–	116 ± 0.84	–	–	–
FD15 <sup>f*</sup>	78.3 ± 0.60	–	–	71.6 ± 0.97	22.2 ± 0.16	49.7 ± 0.84	–
FD16 <sup>f</sup>	196 ± 1.66	–	–	–	–	197 ± 2.10	–
FD17 <sup>f</sup>	86.0 ± 2.06	30.0 ± 0.35	–	–	–	183 ± 3.77	–
FD18 <sup>g</sup>	238 ± 5.82	–	–	–	–	–	–
FD19 <sup>g</sup>	182 ± 3.47	–	–	–	–	157 ± 2.39	–
FD20 <sup>h</sup>	41.3 ± 1.03	–	–	–	–	–	–
FD21 <sup>h</sup>	60.6 ± 0.68	–	–	–	–	–	–
FD22 <sup>i</sup>	16.8 ± 0.18	27.5 ± 0.39	–	–	–	–	–
FD23 <sup>i</sup>	17.3 ± 0.08	28.2 ± 0.07	–	–	–	–	–
FD24 <sup>i</sup>	14.3 ± 0.28	22.8 ± 0.05	–	–	–	–	–
FD25 <sup>i</sup>	19.0 ± 0.06	12.3 ± 0.14	–	–	–	–	–
FD26 <sup>i</sup>	16.0 ± 0.11	25.9 ± 0.45	–	–	–	–	–
FD27 <sup>j</sup>	35.5 ± 0.14	75.2 ± 0.69	–	37.2 ± 0.08	120 ± 0.06	153 ± 0.22	–
FD28 <sup>j</sup>	112 ± 0.09	–	–	113 ± 0.69	119 ± 1.00	–	–
FD29 <sup>j</sup>	–	–	–	153 ± 3.03	120 ± 2.15	–	–
FD30 <sup>k</sup>	–	65.3 ± 0.15	–	42.2 ± 0.12	–	–	–
FD31 <sup>l</sup>	44.8 ± 0.70	–	–	–	–	–	–
FD32 <sup>l</sup>	42.8 ± 0.57	–	–	–	–	–	–
FD33 <sup>l</sup>	45.9 ± 0.27	–	–	–	–	–	–
FD34 <sup>m</sup>	55.3 ± 0.20	–	–	51.9 ± 0.26	128 ± 0.40	172 ± 0.76	–
FD35 <sup>n</sup>	47.7 ± 1.35	–	–	58.9 ± 1.22	–	148 ± 2.19	–
FD36 <sup>o</sup>	66.3 ± 0.29	–	–	58.9 ± 0.23	–	118 ± 0.63	–
FD37 <sup>ö</sup>	–	–	–	–	–	–	80.2 ± 1.14
FD38 <sup>ö</sup>	–	–	–	–	–	–	103 ± 2.41
FD39 <sup>ö</sup>	–	–	–	–	–	–	78.6 ± 0.36
FD40 <sup>ö</sup>	–	–	–	–	–	–	83.3 ± 0.77
FD41 <sup>p</sup>	65.7 ± 0.88	–	–	70.2 ± 0.33	115 ± 2.24	199 ± 3.21	–
FD42 <sup>p</sup>	68.8 ± 0.76	–	–	67.1 ± 0.54	128 ± 1.99	193 ± 2.36	–
FD43 <sup>p</sup>	68.4 ± 0.21	–	–	65.2 ± 0.22	127 ± 0.36	199 ± 1.21	–
FD44 <sup>q</sup>	59.5 ± 0.40	–	–	–	–	200 ± 3.51	–
FD45 <sup>q</sup>	77.5 ± 0.40	–	–	–	129 ± 2.67	87.9 ± 0.76	–
FD46 <sup>q</sup>	69.0 ± 0.69	–	–	–	–	234 ± 1.03	–
FD47 <sup>q</sup>	63.8 ± 2.15	–	–	–	–	232 ± 0.92	–
FD48 <sup>r</sup>	140 ± 0.13	–	–	–	–	200 ± 2.73	–
FN01 <sup>s</sup>	42.9 ± 0.47	–	–	–	–	–	–

**Table 4** (continued)

Samples	Content of analyte (mg/L)						
	ACE	SAC	CAF	ASP	BEN	SOR	RBA
FN02 <sup>s</sup>	86.2 ± 0.59	–	–	–	–	–	–
FN03 <sup>s</sup>	45.0 ± 0.18	–	–	–	–	–	–
FN04 <sup>s</sup>	42.5 ± 0.54	–	–	–	–	–	–
FN05 <sup>s</sup>	44.7 ± 0.18	–	–	–	–	–	–
FN06 <sup>t</sup>	58.9 ± 0.84	–	–	–	–	–	–
FN07 <sup>t</sup>	130 ± 0.58	–	–	–	–	–	–
FN08 <sup>t</sup>	82.8 ± 0.55	–	–	–	–	–	–
FN09 <sup>t</sup>	98.8 ± 0.64	–	–	–	–	–	–
IC01 <sup>*ty</sup>	31.5 ± 0.23	–	48.6 ± 0.34	–	64.6 ± 0.18	73.8 ± 0.07	–
IC02 <sup>*ty</sup>	50.5 ± 0.91	–	47.8 ± 0.69	–	63.7 ± 1.06	72.9 ± 0.13	–
IC03 <sup>ty</sup>	203 ± 4.31	–	54.9 ± 0.83	–	–	–	–
IC04 <sup>ty</sup>	32.7 ± 0.50	–	52.5 ± 0.89	–	–	–	–
IC05 <sup>ty</sup>	55.5 ± 0.28	–	39.5 ± 0.10	–	–	–	–
IC06 <sup>u</sup>	23.3 ± 0.24	–	54.2 ± 0.96	–	–	–	–
IC07 <sup>v</sup>	66.5 ± 0.41	–	32.5 ± 0.16	–	–	–	–
IC08 <sup>v</sup>	65.4 ± 0.54	–	35.3 ± 0.15	–	–	–	–
ED01 <sup>w</sup>	172 ± 0.37	–	328 ± 0.32	n.d.	–	–	–
ED02 <sup>x</sup>	27.0 ± 0.14	–	319 ± 3.98	–	–	–	–
ED03 <sup>y</sup>	49.7 ± 0.70	–	297 ± 1.46	–	141 ± 0.91	276 ± 2.50	–
ED04 <sup>z</sup>	49.1 ± 0.36	–	296 ± 1.23	–	143 ± 0.79	278 ± 0.18	–

Data are presented as mean values, n=3; different letters represent different kinds of products of the same brand; n.d.—not detected; – means that manufacturers did not use the target analyte in their products

*SD* soft drinks, *FN* fruit nectars, *IC* iced drinks, *ED* energy drinks

\*Products from fast food restaurant

is mainly recommended for routine analysis or for application in laboratories where advanced analytical equipment such as HPLC–MS, UHPLC–DAD, and UHPLC–MS are not available. A total of 69 samples were analyzed using the proposed method. In some cases, however, the results obtained did not match the information on the label, suggesting that more effective quality control is needed in this area.

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**Data availability statement** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors have no conflicts of interest to be declared.

**Compliance with ethics requirements** This article does not contain any studies with human participants or animals performed by any of the authors.

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