Talanta 84 (2011) 1342–1349

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous determination of some food additives in soft drinks and other liquid foods by flow injection on-line dialysis coupled to high performance liquid chromatography

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ARTICLE INFO

Article history:
Available online 4 March 2011

Keywords:
Food additives
Soft drink
Liquid food
On-line dialysis
High performance liquid chromatography

ABSTRACT

Flow injection on-line dialysis was developed for sample pretreatment prior to the simultaneous determination of some food additives by high performance liquid chromatography (FID–HPLC). A liquid sample or mixed standard solution (900 μL) was injected into a donor stream (5%, w/v, sucrose) of FID system and was pushed further through a dialysis cell, while an acceptor solution (0.025 mol L^{-1} phosphate buffer, pH 3.75) was held in the opposite side of the dialysis membrane. The dialysate was then flowed to an injection loop of the HPLC valve, where it was further injected into the HPLC system and analyzed under isocratic reverse-phase HPLC conditions and UV detection (230 nm). The order of elution of five food additives was acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid, respectively, with the analysis time of 14 min. On-line dialysis and HPLC analysis could be performed in parallel, providing sample throughput of 4.3 h^{-1}. Dialysis efficiencies of five food additives were in ranges of 5–11%. Linear calibration graphs were in ranges of 10–100 mg L^{-1} for acesulfame-K and saccharin, 10–250 mg L^{-1} for benzoic acid and 10–500 mg L^{-1} for caffeine and sorbic acid. Good precisions (RSD < 5%) for all the additives were obtained. The proposed system was applied to soft drink and other liquid food samples. Acceptable percentage recoveries could be obtained by appropriate dilution of the sample before injecting into the system. The developed system has advantages of high degrees of automation for sample pretreatment, i.e., on-line sample separation and dilution and low consumption of chemicals and materials.

1. Introduction

A food additive [1,2] is defined as a substance or mixture of substances, other than a basic foodstuff, which is presented in food as a result of production, processing, storage or packaging. The term does not include chance contaminants. Food additives may be divided into 6 categories [2]: preservatives (consist of antimicrobials, antioxidants and anti-browning agents), nutritional additives (include vitamins, minerals, amino acids and fibers), flavoring agents (comprise sweeteners, natural flavors, synthetic flavors and flavor enhancers), coloring agents, texturizing agents (such as emulsifiers and stabilizers) and miscellaneous additives (such as enzymes, various solvents, chelating agents and catalysts). Benzoic and sorbic acids and their salts are commonly used for food preservative. Benzoic acid and its sodium salts have long been used to inhibit microbial growth in foods. Sorbic acid and its potassium, calcium or sodium salts have been used in foods as effective inhibitors of fungi and bacteria. Acesulfame-K and saccharin are artificial sweeteners and commonly used in low-calorie foods to control calorie intake. Caffeine is miscellaneous additives and normally added in foods as stimulating agent of a central nervous system.

In many instant food products more than one additives are added, especially most of low calories soft drinks contain preservatives, artificial sweeteners and caffeine, therefore analytical methods that simultaneously determine these additives are interesting to control the maximum regulation permitted levels of individual additives for maintaining food quality and characteristic as well as promoting food safety. These methods include HPLC with UV detection [3–16], HPLC with evaporative light scattering detection [17], HPLC with spectrofluorimetric detection [18], molecular absorption spectrophotometry using multivariate [19], ion chromatography with UV detection [20,21], mixed micellar electrokinetic chromatography with UV detection [22], micellar electrokinetic capillary chromatography with UV detection [23,24], capillary electrophoresis (CE) with UV detection [25], gas chro-

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0039-9140/$ – see front matter.
doi:10.1016/j.talanta.2011.02.045

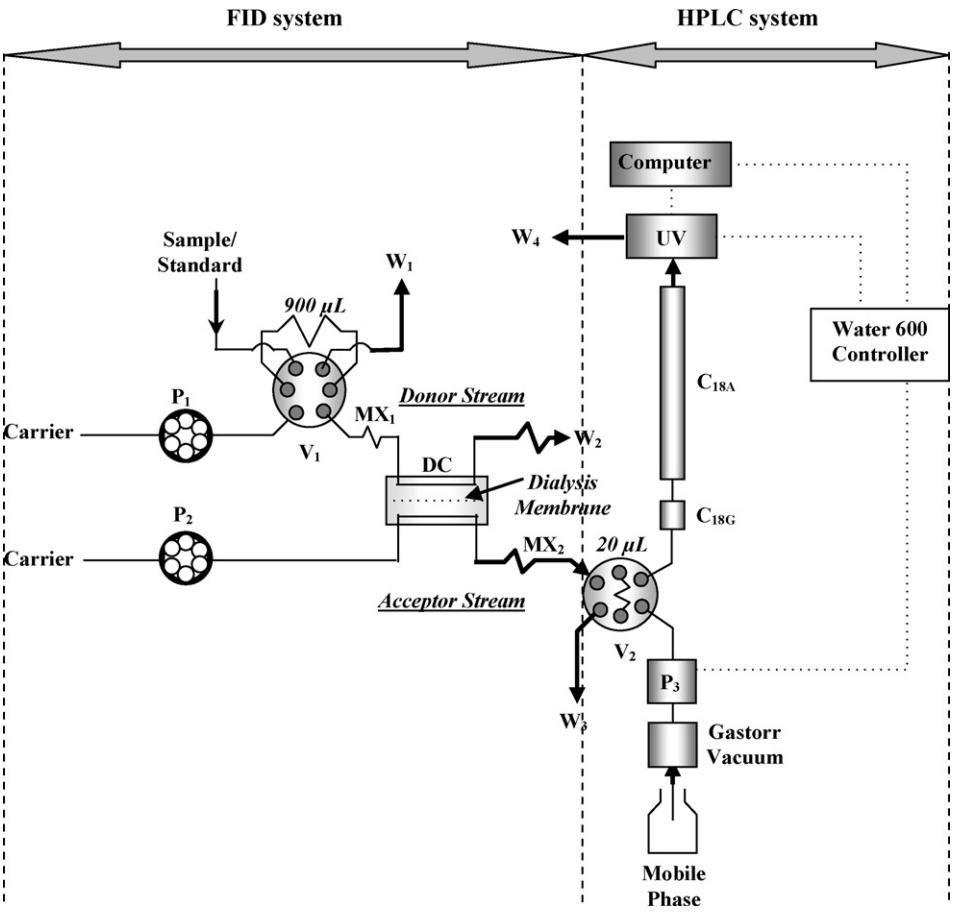

Fig. 1. A manifold of the FID–HPLC system used for the determination of some food additives; flow rate of carrier of donor and acceptor streams: 0.2 mL min^{-1}, P_1 and P_2: peristaltic pumps 1 and 2, P_3: a HPLC pump, V_1: a manual-rotary injection valve, V_2: a HPLC manual-rotary injection valve, DC: dialysis cell, MX_1: a mixing coil 1 (12 cm × 0.8 mm i.d.), MX_2: a mixing coil 2 (7.5 cm × 0.8 mm i.d.), C_{18A}: a C_{18} analytical column, C_{18G}: a C_{18} guard column, UV: a photodiode array detector and W_1, W_2, W_3 and W_4: wastes 1, 2, 3 and 4.

matography (GC) with flame ionization detection [26–28], flow injection analysis (FI) with UV detection [29–31], FI–GC with flame ionization detection [32] and electrokinetic FI–CE with UV detection [33]. The most popular method used for the simultaneous determination of food additives in food samples is HPLC [34], which needs a proper sample pretreatment to homogenize, extract, clean-up and concentrate the analytes from the complexity of matrix interferences in food samples.

Several sample pretreatment techniques for food additive analysis such as filtration or dilution or centrifugation [3,4,6,7,9,13–18], off-line dialysis [5], liquid–liquid extraction [8,11,12], solid phase extraction [8,10,12,17,34] and steam distillation [12] are performed prior to HPLC analysis in order to remove particulate matter and minimize the matrix interferences from food samples and to prevent this particles and matrices to interfere the identification and quantification of analytes, damaging the pumping or injection system or clogging the column. Conventional or off-line dialysis [5,35] is a simple process in which small solute molecules diffuse from a high concentration solution to a low concentration solution across a semi-permeable membrane until equilibrium is reached. When the porous membrane selectively allows smaller solutes to pass while retaining larger species, dialysis can effectively be used as a clean-up process. Therefore, for food analysis, the conventional dialysis sample pretreatment has been applied for removing or reducing the high molecular weight molecules (such as proteins and fatty matters), suspended particulate matters and other matrices in food samples from the low molecular weight molecules, especially food additive molecules. However, the conventional dialysis procedure is usually tedious, time-consuming and consumed large amounts of sample, reagent and materials. On-line dialysis seems to be a good choice for HPLC, which is simple, quick and inexpensive. This technique is operated by continuously feed liquid food sample on donor side of the dialysis membrane while solution in acceptor side is flowed or stopped.

Although, many analytes of low molecular weight molecules in various food samples such as organic acids in wine [36] or milk [37], sugars and organic acids in foods and beverages [38], amino acids in foods, beverages and feedstuffs [39], amino acids, sugars and organic acids in grape juices and wines [40] and food colors in sugar rich foods [41] were simultaneously determined by on-line dialysis coupled to HPLC system, but there was no report on the simultaneous determination of preservatives, artificial sweeteners and/or caffeine by this system. Therefore, in this work, the development of a FID–HPLC system for the simultaneous determination of some food additives (acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid) in some soft drinks and other liquid foods is reported. This proposed system offered a simple, convenient and low consumption sample pretreatment system. Additional advantages, the dialysis pretreatment should prolong life-time of the expensive HPLC columns by

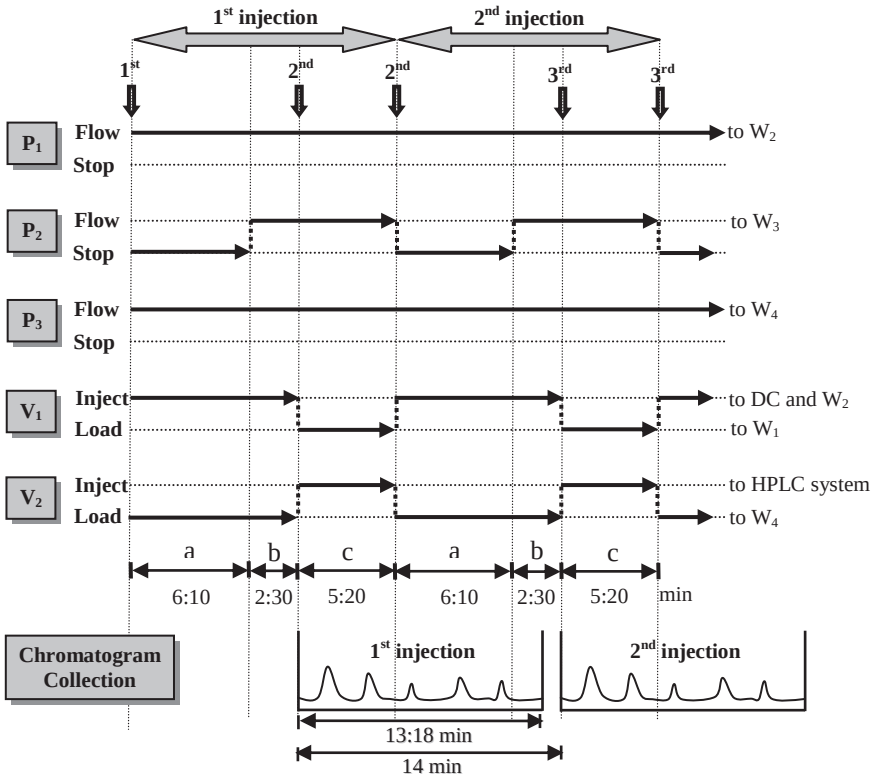

Fig. 2. A schematic of timing diagram for the operation cycle of a FID–HPLC system: P_1 (donor stream): continuously operate along the operation, P_2 (acceptor stream): control to stop during dialysis period and flow to fill the dialysate into sample loop of the HPLC valve, P_3 (HPLC pump): continuously flow along the operation, V_1 (FID valve position): control to inject and load standard/sample (900 μL) into the donor stream, V_2 (HPLC valve position): control to inject and load (20 μL) dialysate in the acceptor stream into HPLC system and time durations: a, dialysis time; b, traveling time of dialysate to HPLC loop; c, cleaning time of both acceptor and donor streams.

preventing the particulate and some large molecules to enter the column.

2. Experimental

2.1. Instruments

The instruments used in this work were similar to our previous FID–HPLC system for the determination of some organic acids in wine [36]. The FID–HPLC system (Fig. 1) consisted of two peristaltic pumps, P_1 (Masterflex C/L 60 RPM, model 77120-62, Cole-Parmer, USA) and P_2 (EYELA, model MP-3, Tokyo Rikakikai, Tokyo, Japan), a 6-port manual-injection valve (V_1) (Model V-451, Upchurch Scientific, USA), a home-made dialysis cell (DC) and a HPLC system (Waters 600E, Water Corporation, Milford, MA, USA). All tubings for assembling the FID system, except pump tubing, were 0.8 mm i.d. PTFE tubing (Upchurch Scientific, USA). A home-made DC unit was made of two acrylic plates (15 cm length, 4.8 cm width and 1 cm height), engraving for donor and acceptor channels (with each of 350 mm length, 1.5 mm width, 0.75 mm depth). The two channels were separated by a cellulose dialysis membrane (molecular weight cut off (MWCO) of 12,000–14,000 Da, Spectra/Por®, Houston, TX, USA), which was cutting to be a sheet of 2.5 cm wide and 13 cm long and thoroughly washed with water before use.

The HPLC system consisted of a Gastorr vacuum, a Waters 600E pump (P_3), a Waters 600 controller, a Rheodyne 7725i manual-injection valve (V_2) with a 20 μL sample loop and a Waters 2996 photodiode array (PDA) detector. The Empower PDA software (Water Corporation, Milford, MA, USA) was used to control the system, record the chromatograms and evaluate for peak areas and retention times. The guard column (5 μm particle size, 10 mm length, 4.6 mm i.d.) and analytical column (5 μm particle size, 250 mm length, 4.6 mm i.d.) were an Aquasil C_{18} column (Thermo Fisher Scientific, USA).

2.2. Chemicals and solutions

All chemicals and solvents used were analytical reagent and/or HPLC grades. Ultrapure water (18.2 MΩ cm^{-1} quality) obtained from the water purification system (Elga Elgastat Maxima HPLC, England) was used for the preparation of all solutions. Stock standard solutions (10,000 mg L^{-1}) of five food additives were prepared by dissolving acesulfame-K (99%, Fluka), caffeine anhydrous (99%, Fluka), potassium benzoate (99%, Fluka), potassium sorbate (99%, Fluka) and sodium saccharin (97%, Fluka) in water. These solutions were stored in a brown glass bottle and kept at 4 °C. Mixed working standard solutions of five food additives were prepared daily by appropriate dilution of the stock solutions in water. The HPLC mobile phase contained a mixture (5:30:65, %v/v) of acetonitrile (99.8%, BDH), methanol (99.99%, Fisher Scientific) and 0.025 mol L^{-1} potassium dihydrogen orthophosphate (99.99%, Fisher Scientific) buffer pH 3.75. The pH was adjusted with 1.0 mol L^{-1} phosphoric acid (85%, BDH) to obtain pH of 3.75. The isocratic elution conditions were employed. Before used, the mobile phase was vacuum-filtered through a 0.45 μm nylon membrane filter and degassed in an ultrasonic bath for 15 min.

All of the soft drink and other liquid food samples were purchased at a local supermarket. These sample solutions were mixed thoroughly, degassed in an ultrasonic bath for 15 min and followed by appropriate dilution with water before injected into the system. Food samples containing suspended solids were vacuum-filtered through a 0.45 μm nylon membrane filter before diluted and injected into the FID–HPLC.

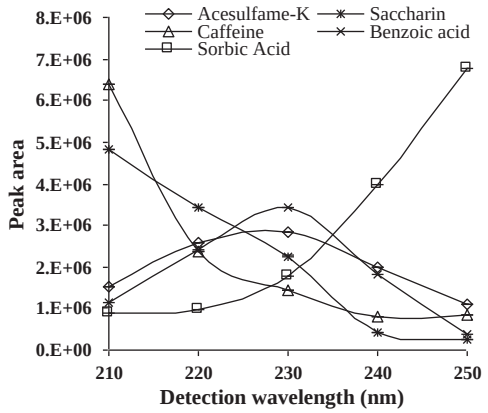

Fig. 3. Effect of detection wavelength on peak area of the chromatogram of five food additives (50 mg L^{-1} of each analyte).

2.3. FID–HPLC manifold and procedures

A schematic diagram of the manifold configuration is shown in Fig. 1, which was combined the FI on-line dialysis (FID) and HPLC systems. Firstly, the HPLC system (P_3) was started to operate. Eluent was pumped through the column to clean the system. The donor (P_1, flow rate of 0.2 mL min^{-1}) and acceptor (P_2, flow rate of 0.2 mL min^{-1}) solutions of the FID system were flowed to fill all tubings and channels. Three consecutive operation times of one operation cycle (14 min) comprised of a dialysis time of a standard or sample, a traveling time of the dialysate to HPLC loop and a cleaning time of the FID–HPLC system is illustrated in Fig. 2. For the first injection, a mixed standard or sample solution (900 μL) at V_1 was injected into the donor stream and then it was propelled to the DC, where small solute molecules in the donor solution were dialysed through the dialysis membrane into the acceptor solution for a dialysis time of 6 min 10 s. In order to enhance the sensitivity, the donor stream was continuously flowed while the acceptor stream was stopped during the dialysis period. The dialysate zone containing food additives was filled into a sample loop (20 μL) of the HPLC valve (V_2), with a suitable traveling time of 2 min 30 s. Then, it was further injected into the HPLC system and analyzed under normal HPLC conditions, using an isocratic mobile phase, a reversed-phase (C_{18}) analytical column and the PDA UV detector. The chromatogram was recorded for 13 min 18 s, which was within one operation cycle of the FID system (Fig. 2).

While the dialysate of the first injection was injected into the HPLC system, the second injection was loaded into the sample loop of the FID valve (V_1). After a period of 5 min 20 s for cleaning of the donor and acceptor lines, the second injection was started. When a chromatographic separation of the first injection was ended (with an analysis time of 13 min 18 s), the dialysate of the second injection was injected into the HPLC system and the third injection was loaded into the FID system. This parallel operation helped increase sample throughput. Under the selected conditions described above, the total analysis time for one injection was 14 min, resulting in an injection throughput of approximately 4.3 chromatograms per hour.

3. Results and discussion

3.1. Optimization conditions of HPLC system

In order to obtain a good separation (with peak resolution; $R \geq 1.5$) of five food additives (acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid), short analysis time, less solvent consumption and high sensitivity, the HPLC conditions used in this work were optimized. Factors such as mobile phase concentrations, pH of mobile phase, HPLC column oven temperatures and flow rates of mobile phase were studied to compromise the separation efficiency, analysis time, column lifetime and solvent consumption, while UV absorption wavelengths were optimized to obtain high sensitivity.

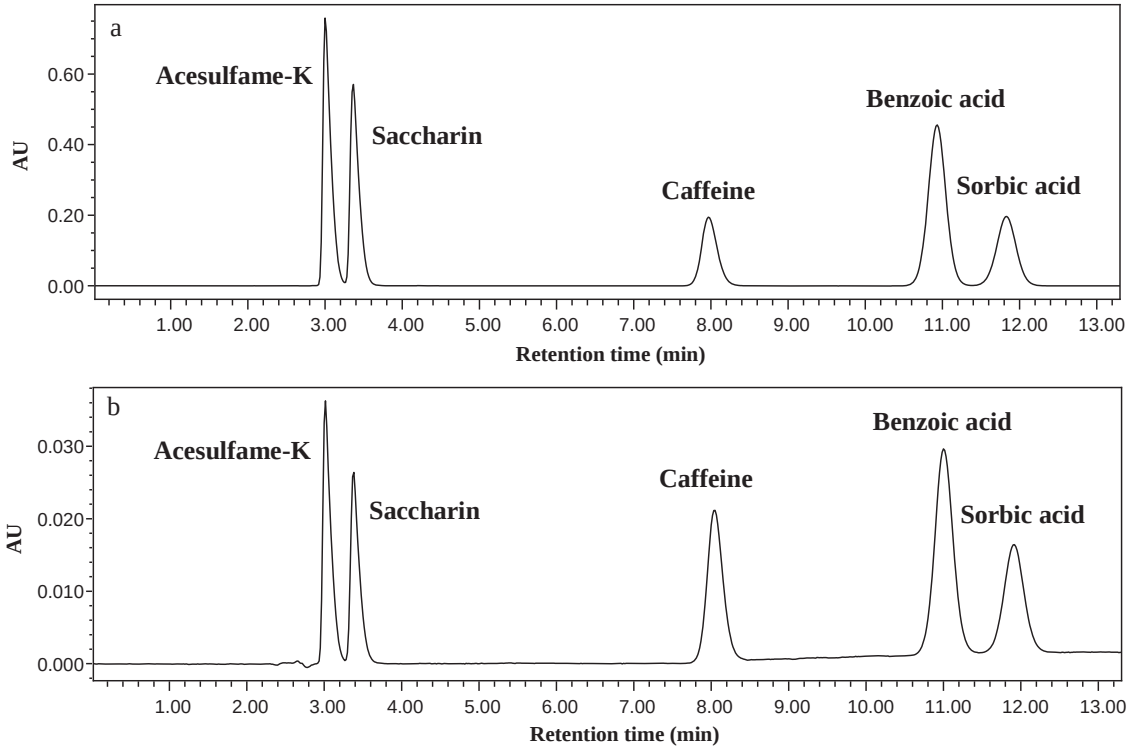

Fig. 4. Chromatograms of a mixed standard solution containing all food additives at 100 mg L^{-1}: (a) by direct injection to HPLC system and (b) by FID–HPLC system.

Table 1
Calibration data of the food additives determination by direct injection to HPLC and FID–HPLC.

Analyte	Range (mg L^{-1})	Linear equation ($y = ax + b$)	r^2	% RSD[a] ($n = 3$)	Detection limit[b] (mg L^{-1})	Quantitation limit[c] (mg L^{-1})	% dialysis[d]
Direct injection to HPLC							
Acesulfame-K	10–500	$y = 51{,}596x + 268{,}659$	0.9993	0.2–5.1	1.1	3.6	-
Saccharin	10–500	$y = 43{,}765x + 117{,}849$	0.9999	0.1–6.1	1.2	4.0	-
Caffeine	10–500	$y = 28{,}161x - 10{,}313$	1.0000	0.3–6.2	0.7	2.3	-
Benzoic acid	10–500	$y = 76{,}130x - 191{,}312$	0.9998	0.1–6.5	0.6	2.0	-
Sorbic acid	10–500	$y = 35{,}671x - 12{,}879$	1.0000	0.4–6.4	0.2	0.6	-
FID–HPLC							
Acesulfame-K	10–100	$y = 2814x - 8940$	0.9992	0.2–3.8	0.1	0.3	6
Saccharin	10–100	$y = 2229x - 8661$	0.9979	0.1–3.5	0.1	0.3	5
Caffeine	10–500	$y = 3169x + 2868$	0.9998	0.4–3.6	0.3	1.0	11
Benzoic acid	10–250	$y = 5062x - 8377$	0.9993	0.7–5.3	1.8	6.0	7
Sorbic acid	10–500	$y = 2752x + 10{,}182$	0.9997	0.1–3.6	0.4	1.3	8

[a] Relative standard deviation of different concentrations in the calibration range.
[b] Calculated from three times standard deviation of the response of the lowest concentration of analyte in a sample that can be detected, divided by the slope of the calibration graph [43].
[c] Calculated from ten times standard deviation of the response of the lowest concentration of analyte in a sample that can be detected, divided by the slope of the calibration graph [43].
[d] % dialysis of each acid = (slope of calibration graph by FID–HPLC/slope of calibration graph by direct injection to HPLC) × 100.

To decrease the analysis time by reducing the polarity of mobile phase, the concentrations of acetonitrile and phosphate buffer (0.025 mol L^{-1}, pH 3.25) in mobile phase were firstly optimized in the range 0–7% (v/v) and 63–70% (v/v), respectively, while the following conditions were kept constant: methanol, 30% (v/v); column oven temperature, 30 °C; flow rate of mobile phase, 1.0 mL min^{-1} and detection wavelength, 225 nm. It is clear that the higher of acetonitrile concentration, the lower peak resolution ($R < 1.5$) of benzoic acid and sorbic acid and the shorter retention time of each food additive were obtained. Eluent containing acetonitrile and phosphate buffer (0.025 mol L^{-1}, pH 3.25) of 5% (v/v) and 65%, respectively, which provided elution time within 17 min was chosen for further study.

Because of the pK_a of benzoic acid and sorbic acid are 4.21 and 4.76 and the Aquasil C_{18} column should be used at pH > 2.0, therefore the effect of pH of mobile phase in the range 2.5–4.0 was investigated that both acids are in the undissociated form. It was found that the total analysis time and the retention time of caffeine, benzoic acid and sorbic acid decreased with the increase of pH of the mobile phase. Poor resolution between benzoic acid and sorbic acid was observed at pH 2.5 ($R = 0$) and pH 3.0 ($R = 0.5$). At pH 4.0, long peak tailing of caffeine and sorbic acid was observed. A pH of mobile phase at 3.75 was therefore selected for all subsequent studies with the analysis time of 16 min.

Column oven temperatures and flow rates of mobile phase ranges of 30–40 °C and 1.0–1.2 mL min^{-1}, respectively, were investigated. It could be noted that the retention time of each food additive and the analysis time slightly decreased when column oven temperatures and flow rates of mobile phase increased, while complete separation ($R > 1.5$) of all analytes was achieved. In order to avoid deterioration of the stationary phase (recommended operating temperature of the column was <60 °C), a column temperature at 30 °C was selected for all future works and the flow rate of 1.2 mL min^{-1} was chosen which gave shorter analysis time of 13 min 18 s.

Effect of UV absorption wavelengths on peak area responses was then studied in the range of 210–250 nm (Fig. 3). It could be noticed that higher peak responses were observed at 210 nm for saccharin and caffeine, 230 nm for acesulfame-K and benzoic acid and 250 nm for sorbic acid. The wavelength at 230 nm was selected in order to compromise the sensitivity of all analytes. Although using PDA detector the different wavelengths may be set for different analytes, the single wavelength was used because it provides convenient operation of the system and enough sensitivity was achieved. The compromised detection wavelength of 230 nm is also useful for the HPLC system that has only single wavelength detector.

Under the HPLC conditions used as described above, the analysis of a mixed standard solution of the additives by direct injection to the HPLC system gave a chromatogram as shown in Fig. 4a, with an analysis time of 13 min 18 s. The order of elution was acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid, with retention times (t_R) of 3.000 ± 0.001, 3.360 ± 0.001, 8.030 ± 0.002, 11.050 ± 0.005 and 11.970 ± 0.005 min, respectively. Table 1 summarizes calibration data of different food additives under the selected conditions.

3.2. Optimization of the conditions of FID–HPLC system and interference study

The FID–HPLC system (Fig. 1) and the operational procedure as described in Section 2.3 were used. The conditions required for achieving high dialysis efficiency, good sensitivity, good reproducibility and suitable timing of an operation cycle for the FID system were investigated such as traveling times of dialysate to HPLC loop (at V_2), injection volume of standard/sample at V_1, concentration of solutions to be used as donor and acceptor streams.

According to our previous study on the dialysis efficiency [36], the flow rate of 0.2 mL min^{-1} for donor and acceptor streams and the pore size of the dialysis membrane of 12,000–14,000 Da were adopted for all subsequent studies. Firstly, the traveling time of dialysate to HPLC loop (at V_2) was optimized over the range 30–180 s, while the following parameters were kept constant: sample volume at V_1, 400 μL; dialysis time, 2 min 50 s; cleaning time of the donor and acceptor lines, 2 min 40 s; carrier solution of donor and acceptor streams, water. A traveling time of 150 s (2 min 30 s) provided a good sensitivity and reproducibility and was selected for further experiments. The effect of injection volume at V_1 over the range 300–1000 μL was then investigated. Sensitivity increased drastically with the increase of sample volume and leveled off at about 900 μL. An injection volume of 900 μL (with 6 min 10 s of dialysis time) was selected as it provided good sensitivity, reproducibility and dialysis efficiency. This obtained dialysis time of the proposed FID system is much shorter than an off-line dialysis used in the previously studied by Nuengchamnong [5], which was found that 15 h of the dialysis time was needed to reach the equilibrium using a dialysis cellophane tubing (150 mm × 27 mm i.d. and a MWCO of 12,000–14,000 Da) and with sample consumption of 5–10 mL.

Table 2
Food additive contents (mg L^{-1}; $n = 3$) in soft drink and other liquid food samples, as determination by the FID–HPLC system (percentage recoveries were obtained by spiking into the sample with mixed standard solution containing 25 and 40 mg L^{-1} of acesulfame-K and saccharin and 40 and 150 mg L^{-1} of caffeine, benzoic acid and sorbic acid).

Sample no.	Food material	Concentration found and % recovery (concentration added; mg L^{-1})									
		Acesulfame-K		Saccharin		Caffeine		Benzoic acid		Sorbic acid	
		mg L^{-1} [a]	%Rec[a]	mg L^{-1}	%Rec	mg L^{-1}	%Rec	mg L^{-1}	%Rec	mg L^{-1}	%Rec
1	Diet cola brand 1	188 ± 3	88 ± 2 (25)	ND[b]	100 ± 2 (25)	120 ± 3	108 ± 2 (40)	325 ± 5	108 ± 3 (40)	ND	107 ± 1 (40)
2			98 ± 2 (40)		103 ± 2 (40)		103 ± 3 (150)		103 ± 1 (150)		106 ± 1 (150)
3	Diet cola brand 2	166 ± 4	99 ± 4 (25)	ND	97 ± 2 (25)	91 ± 3	97 ± 2 (40)	373 ± 7	106 ± 2 (40)	ND	106 ± 1 (40)
4			95 ± 3 (40)		103 ± 2 (40)		98 ± 2 (150)		107 ± 1 (150)		104 ± 1 (150)
5	Diet cola brand 3	127 ± 3	95 ± 3 (25)	ND	96 ± 3 (25)	139 ± 4	97 ± 2 (40)	266 ± 9	108 ± 1 (40)	ND	112 ± 1 (40)
6			97 ± 2 (40)		102 ± 2 (40)		101 ± 3 (150)		103 ± 2 (150)		108 ± 2 (150)
7	Diet cola brand 4	227 ± 6	92 ± 3 (25)	ND	106 ± 4 (25)	ND	98 ± 3 (40)	ND	108 ± 1 (40)	276 ± 2	107 ± 1 (40)
8			96 ± 3 (40)		101 ± 2 (40)		92 ± 1 (150)		110 ± 2 (150)		109 ± 2 (150)
9	Diet green tea	46 ± 1	91 ± 1 (25)	ND	98 ± 3 (25)	134 ± 2	105 ± 4 (40)	ND	106 ± 1 (40)	ND	94 ± 4 (40)
10			102 ± 2 (40)		102 ± 1 (40)		106 ± 4 (150)		111 ± 2 (150)		100 ± 4 (150)
11	Regular green tea	ND	112 ± 2 (25)	ND	101 ± 2 (25)	97 ± 2	111 ± 3 (40)	ND	107 ± 1 (40)	ND	101 ± 1 (40)
12			110 ± 2 (40)		102 ± 2 (40)		101 ± 3 (150)		99 ± 2 (150)		101 ± 2 (150)
13	Grape juice with aloe-gelatin	ND	89 ± 1 (25)	ND	87 ± 1 (25)	ND	102 ± 3 (40)	260 ± 6	90 ± 4 (40)	ND	81 ± 1 (40)
14			109 ± 2 (40)		99 ± 1 (40)		91 ± 2 (150)		101 ± 3 (150)		115 ± 4 (150)
15	Chinese mustard pickle liquid	ND	128 ± 4 (25)	ND	133 ± 4 (25)	ND	84 ± 4 (40)	1628 ± 82	137 ± 7 (40)	ND	106 ± 1 (40)
16			123 ± 2 (40)		121 ± 2 (40)		81 ± 1 (150)		119 ± 2 (150)		111 ± 1 (150)
17	Japanese soy sauce	ND	111 ± 3 (25)	ND	138 ± 2 (25)	ND	80 ± 2 (40)	1523 ± 31	120 ± 4 (40)	ND	95 ± 1 (40)
18			111 ± 4 (40)		132 ± 2 (40)		85 ± 2 (150)		128 ± 2 (150)		111 ± 1 (150)

[a] Average value ± standard deviation of triplicate results.
[b] ND, not detected.

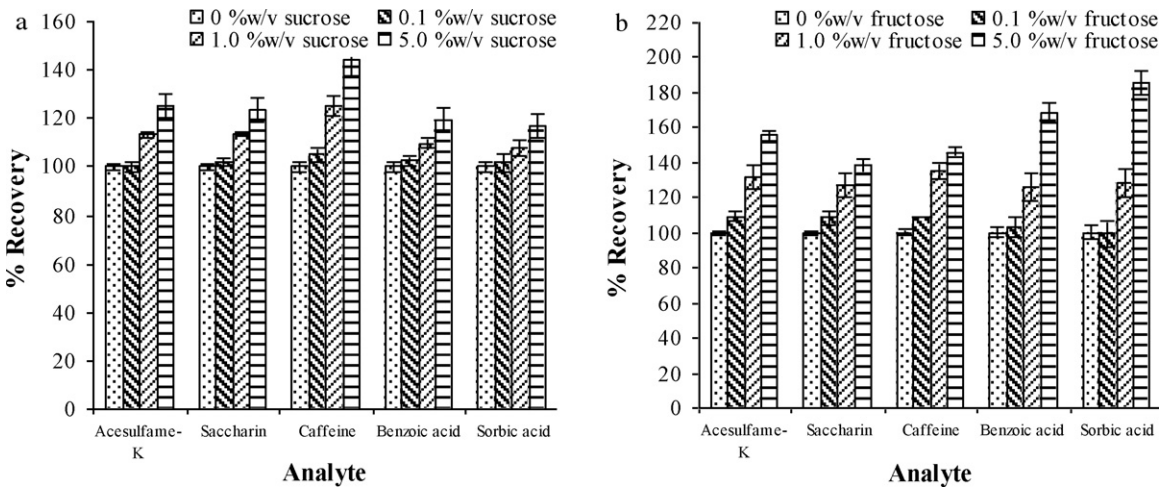

Fig. 5. Interference study of: (a) sucrose and (b) fructose on recoveries of five food additives; percentage recoveries were obtained by spiking interferences into a mixed standard solution containing 250 mg L^{-1} of each additive. Water was used as donor and acceptor solutions.

Some compounds such as aspartame, sucrose, sucralose, fructose, citric acid, pigment and protein, which might be commonly found in regular and diet soft drinks, grape juice, Chinese mustard pickle liquid and soy sauce, were tested for interference under the above selected FID–HPLC conditions. These compounds could possibly interfere in determining the target food additives or affected the dialysis efficiency or might damage the HPLC column. Proteins [37,38] (rich in soy sauce and Chinese mustard pickle liquid) and pigment [36] (rich in grape juice, Chinese mustard pickle liquid and soy sauce) were high molecular weight molecules and could be removed or reduced by dialysis membrane with a MWCO of 12,000–14,000 Da. Fructose and aspartame were found to elute at t_R of 3.72 ± 0.002 and 7.01 ± 0.001 min while sucrose, sucralose and citric acid were not retained under these conditions.

Sucrose (rich in grape juice, Chinese mustard pickle liquid and soy sauce) and fructose (rich in regular green tea, grape juice and soy sauce) could affect sensitivities and/or recoveries of the determining target food additives. Therefore, the effects of sucrose (with a molecular weight close to sucralose) and fructose were investigated at concentrations of those approximately found in the samples. In this study, sucrose (0–5%, w/v) (Fig. 5a) and fructose (0–5%, w/v) (Fig. 5b) were added into a mixed standard solution of 250 mg L^{-1} of each analyte and the solution was injected into the FID–HPLC system under the above selected conditions. It was found that sucrose and fructose concentration of higher than 0.1% (w/v) gave increase in percentage recoveries of all analytes. Both the interferences might enhance the dialysis of the analytes, so the positive interfering was observed.

In order to encounter for these interferences, sucrose solutions of different concentrations (0, 5 and 10%, w/v) was used as the donor solutions, while the acceptor solution was water. It was found that the addition of sucrose into donor solution could slightly reduce interfering effect and provided better reproducibility. Therefore, 5% (w/v) sucrose was selected for all further works because of easy dissolution of the chemical.

The effect of acceptor solution was also studied using phosphate buffer (0.025 mol L^{-1}, pH 3.75) which was used as an eluent of HPLC system for adjusting ionic strength [42]. In this study, mixed standard solutions of 250 mg L^{-1} of acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid were spiked with diet cola brand 1 (dilution 5 folds) (Fig. 6a) and diet green tea (dilution 5 folds) (Fig. 6b) and then injected into the FID–HPLC system. It was found that the similar percentage recoveries were obtained with the use of phosphate buffer or water as acceptor solution. However,

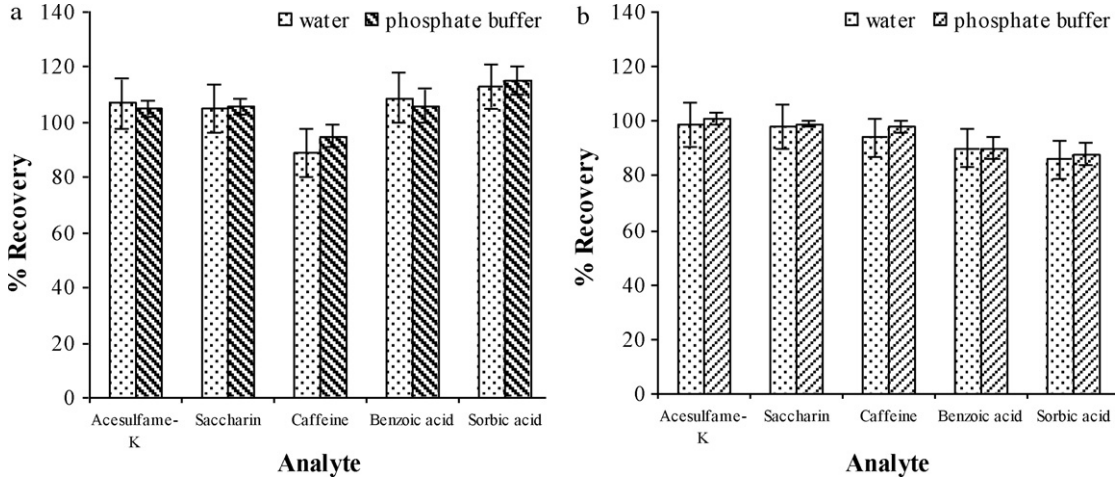

Fig. 6. Effect of water and phosphate buffer (0.025 mol L^{-1}, pH 3.75) acceptor solutions on recoveries of five food additives while donor solution was 5% (w/v) sucrose by spiking (a) diet cola brand 1 (dilution 5 folds) and (b) diet green tea (dilution 5 folds) with a mixed standard solution to obtain 250 mg L^{-1} of each additive.

phosphate buffer acceptor solution provided better reproducibility. Therefore, phosphate buffer (0.025 mol L^{-1}, pH 3.75) was selected for all further works. It was indicated that this proposed system was not suitable for the analysis of samples with high sucrose and/or fructose concentrations, excepted the samples should be adequately diluted.

3.3. Calibration data of five food additives by FID–HPLC

Chromatogram of five food additives obtained from FID–HPLC system is depicted in Fig. 4b. The order of elution is the same with that of the direct injection to HPLC system, but the sensitivity was lower by about 20 times. Calibration plots were linear in the range of 10–100 mg L^{-1} of acesulfame-K and saccharin, 10–500 mg L^{-1} of caffeine and sorbic acid and 10–250 mg L^{-1} of benzoic acid (Table 1). Slopes of the calibration graphs were smaller than direct injection to HPLC system. However, limit of detection (LOD) (3 SD) and limit of quantitation LOQ (10 SD) as calculated following the definition described in [43] were similar for both systems (Table 1). From the ratio of slopes of the calibration graphs obtained from FID–HPLC system to those obtained from the direct injection to HPLC system, the percentage dialysis could be calculated to be in the range of 5–11%. The FID–HPLC provided on-line dilution of sample about 9–20 folds. Precisions obtained from triplicate injections of the mixed standard solution of food additives were in ranges of 0.1–5.3%.

3.4. Applications to food analysis

The proposed method was applied to determine five food additives in eighteen samples of commercial regular and diet soft drinks and other liquid foods. The mixed standard solution of all the target additives was spiked into all samples at different concentration levels (25 and 40 mg L^{-1} of acesulfame-K and saccharin and 40 and 150 mg L^{-1} of caffeine, benzoic acid and sorbic acid). Samples were diluted different folds (5–30 folds) with water to obtain concentration of analytes suitable for the determination by the developed system and low concentration of interfering substances, which lead to good recoveries obtained. Each sample solution was analyzed in triplicate.

The results obtained are summarized in Table 2, with percentage recoveries found in ranges of 88–112% for all soft drinks (with less matrix interferences) and 81–138% for the analysis of other liquid foods. The results indicated that diet cola contained artificial sweetener, preservative and caffeine, diet green tea contained artificial sweetener and caffeine, regular green tea contained only caffeine and other liquid food samples analyzed in this work contained only preservative. Although Saccharine was not found in all samples, it was still of interested because it was sometimes used in Thai pickle samples. We demonstrated that the proposed FID–HPLC method is useful for simultaneous determination of food preservatives. One dialysis membrane in the FID system could be repeatedly used for more than 300 analyses.

4. Conclusion

A flow injection on-line dialysis sample pretreatment coupled to HPLC system was developed for the simultaneous separation and determination of acesulfame-K, saccharin, caffeine, benzoic acid and sorbic acid in one run. This system has advantages of high degrees of automation in sample pretreatment, on-line sample separation and dilution, good sample clean-up for prolongation the life-time of the expensive HPLC column, low consumption of chemicals and materials and short analysis time. The proposed system has been applied to analysis of real sample of regular and diet soft drinks and other liquid foods with satisfactory results. Interfering effect could be reduced by dilution the sample before injection into the system.

Acknowledgements

The authors gratefully acknowledge financial support from Thailand Research Fund (TRF) and the Commission on Higher Education (CHE) of Thailand. We thank Miss Benjaporn Pramote and the staff of Chemistry Department, Naresuan University for their assistance.

References

[1] T.E. Furia, CRC Handbook of Food Additives, vol. I, second edition, CRC Press, Inc., Florida, 1968.
[2] A.L. Branen, P.M. Davidson, S. Salminen, Food Additives, Marcel Dekker, Inc., New York, 1990.
[3] E.C. Demiralay, G. Ozkan, Z. Guzel-Seydim, Chromatographia 63 (2006) 91.
[4] C.M. Lino, A. Pena, Food Chem. 121 (2010) 503.
[5] N. Nuengchamnong, Simultaneous analysis of some food additives by high performance liquid chromatography after sample pretreatment using dialysis. A thesis submitted for the degree of Master of Science in Chemistry, Chiang Mai University, Thailand, 1998.
[6] H.M. Pylypiw Jr., M.T. Grether, J. Chromatogr. A 883 (2000) 299.
[7] S.A.V. Tfouni, M.C.F. Toledo, Food Control 13 (2002) 117.
[8] F.J.M. Mota, I.M.P.L.V.O. Ferreira, S.C. Cunha, M. Beatriz, P.P. Oliveira, Food Chem. 82 (2003) 469.
[9] I. Garcia, M.C. Ortiz, L. Sarabia, C. Vilches, E. Gredilla, J. Chromatogr. A 992 (2003) 11.
[10] I. Techakriengkrai, R. Surakarnkul, J. Food Comp. Anal. 20 (2007) 220.
[11] B. Saad, Md.F. Bari, M.I. Saleh, K. Ahmad, M.K.M. Talib, J. Chromatogr. A 1073 (2005) 393.
[12] I.M.P.L.V.O. Ferreira, E. Mendes, P. Brito, M.A. Ferreira, Food Res. Int. 33 (2000) 113.
[13] H. Wang, K. Helliwell, X. You, Food Chem. 68 (2000) 115.
[14] A.M. Di Piedra, V. Cavrini, D. Bonazzi, L. Benfenati, Chromatographia 30 (1990) 215.
[15] N. Dossi, R. Toniolo, S. Susmel, A. Pizzarello, G. Bontempelli, Chromatographia 63 (2006) 557.
[16] W.S. Tsang, M.A. Clarke, F.W. Parrish, J. Agric. Food Chem. 33 (1985) 734.
[17] A. Wasik, J. McCourt, M. Buchgraber, J. Chromatogr. A 1157 (2007) 187.
[18] K. Wrobel, K. Wrobel, J. Chromatogr. A 773 (1997) 163.
[19] M.A. Cantarelli, R.G. Pellerano, E.J. Marchevsky, J.M. Camina, Food Chem. 115 (2009) 1128.
[20] Q.C. Chen, J. Wang, J. Chromatogr. A 937 (2001) 57.
[21] Y. Zhu, Y. Guo, M. Ye, F.S. James, J. Chromatogr. A 1085 (2005) 143.
[22] M.C. Boyce, J. Chromatogr. A 847 (1999) 369.
[23] C.O. Thompson, V.C. Trenerry, B. Kemmery, J. Chromatogr. A 694 (1995) 507.
[24] I. Pant, V.C. Trenerry, Food Chem. 53 (1995) 219.
[25] R.A. Frazier, E.L. Inns, N. Dossi, J.M. Ames, H.E. Nursten, J. Chromatogr. A 876 (2000) 213.
[26] H.J. Lin, Y.M. Choong, J. Food Drug Anal. 7 (4) (1999) 291.
[27] C. Dong, W. Wang, Anal. Chim. Acta 562 (2006) 23.
[28] C. Dong, Y. Mei, L. Chen, J. Chromatogr. A 1117 (2006) 109.
[29] J.F. Garcia-Jimenez, M.C. Valencia, L.F. Capitan-Vallvey, Anal. Chim. Acta 594 (2007) 226.
[30] J.F. Garcia-Jimenez, M.C. Valencia, L.F. Capitan-Vallvey, Anal. Lett. 39 (2006) 1333.
[31] R. Lucena, S. Cardenas, M. Gallego, M. Valcarcel, Anal. Chim. Acta 530 (2005) 283.
[32] M. Gonzalez, M. Gallego, M. Valcarcel, J. Chromatogr. A 848 (1999) 529.
[33] F. Han, Y.Z. He, L. Li, G.N. Fu, H.Y. Xie, W.E. Gan, Anal. Chim. Acta 618 (2008) 79.
[34] A. Zygler, A. Wasik, J. Namiesnik, Trends Anal. Chem. 28 (2009) 1082.
[35] N.C. van de Merbel, J. Chromatogr. A 856 (1999) 55.
[36] O. Kritsunankul, B. Pramote, J. Jakmunee, Talanta 79 (2009) 1042.
[37] M.C. Wei, C.T. Chang, J.F. Jen, Chromatographia 54 (2001) 601.
[38] E. Verette, F. Qian, F. Mangani, J. Chromatogr. A 705 (1995) 195.
[39] D. Heems, G. Luck, C. Fraudeau, E. Verette, J. Chromatogr. A 798 (1998) 9.
[40] C. Linget, C. Netter, D. Heems, E. Verette, Analysis 26 (1998) 35.
[41] G.M. Greenway, N. Kometa, R. Macrae, Food Chem. 43 (1992) 137.
[42] K. Grudpan, J. Jakmunee, P. Sooksamiti, Talanta 49 (1999) 215.
[43] M. Swartz, I.S. Krull, Analytical Method Development and Validation, Marcel Dekker, Inc., New York, 1997.