Simultaneous Determination Of Sodium Benzoate And Sodium Cyclamate In Soft Drink Using High Performance Liquid Chromatography

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Abstract

Background: Sodium benzoate and sodium cyclamate are foods additives generally used as preservative and artificial sweeteners, respectively. Monitoring of sodium benzoate and sodium cyclamate need appropriate analytical method. Objective: The main objective of this study was to gain the optimum conditions for simultaneous determination of sodium benzoate and sodium cyclamate in soft drink by High Performance Liquid Chromatography. Methods: Measurement of the additives was performed in µ-Bondapak RP18 column using methanol:phosphate buffer of pH 4 (42:58) as mobile phase with flow rate of 1 mL/minute. Results: It has shown that correlation coefficients (r) of sodium benzoate and sodium cyclamate was 0.9997 and 0.9991 respectively. Recoveries of the two analyst were acceptable. Conclusions: Five out of nine samples tested contained sodium cyclamate higher than the recommended concentration. All sodium benzoate containing samples were within the recommended concentration.

Keywords*: sodium benzoate, sodium cyclamate, soft drink, high performance liquid chromatography*

Abstrak

Pendahuluan: Natrium benzoat dan natrium siklamat adalah bahan pengawet dan pemanis yang umum ditambahkan ke dalam makanan dan minuman. Monitoring penggunaan kedua bahan ini memerlukan metode yang memenuhi persyaratan uji kualitatif dan kuantitatif. Tujuan: Tujuan penelitian ini adalah memperoleh kondisi optimum pada penentuan kadar natrium benzoat dan natrium siklamat dalam minuman ringan secara simultan dengan kromatografi cair kinerja tinggi (KCKT). Metode: Metode ini menggunakan kolom *µ-Bondapak RP18*, fase gerak metanol:dapar fosfat pH 4 (42:58) dengan laju alir 1 mL/menit. Hasil: Uji linieritas menunjukkan koefisien korelasi (r) natrium benzoat dan natrium siklamat masing-masing 0,9997 dan 0,9991. Uji perolehan kembali kedua analit ini memenuhi persyaratan. Kesimpulan: Lima dari 9 sampel yang diuji mengandung natrium siklamat melebihi batas yang diijinkan. Sedang kadar natrium benzoat dalam semua sampel memenuhi persyaratan.

Kata kunci: natrium benzoat, natrium siklamat, minuman ringan, kromatografi cair kinerjatinggi

BACKGROUND

Food additive is a substance or a mixture of substances in food but it isn't part of the raw material (BPOM, 2003). Food additives are used to maintain the quality of foods by improving texture, taste, stability also preserve the food or drink (BPOM, 2003). Sodium benzoate (Figure 1 (a)) is used as preservative, while sodium cyclamate (Figure 1 (b)) is an artificial sweetener in foods (BPOM, 2004). The Permitted concentration of sodium benzoate and sodium cyclamate in soft drink are 600 mg/Kg and 3 g/Kg, respectively (BPOM-RI, 1999). These two substances are mostly used in soft drink, as favorite beverage of non-alcoholic and carbonated or none carbonated. Hence, monitoring of sodium benzoate and sodium cyclamate needs appropriate analytical methods for quality control purposes.

To date, the cyclamate analysis is still carried out using conventional gravimetric method. (Horwitz-, 2000). This technique takes a long time, tedious and the high risk of analysis error. Chromatographic technique offers more practical and easy method with a relatively shorter time consuming for analysis. One of the chromatographic methods is High Performance Liquid Chromatography (HPLC). Previous studies have reported the HPLC method for sodium cyclamate determination (Novelina, 2009). The HPLC method is also often used for determination of sodium benzoate

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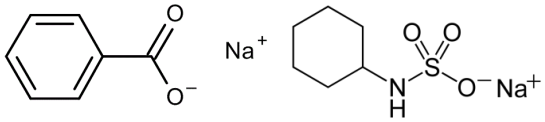
(Hayun & Aziza, 2004). On the other hands the determination of sodium cyclamate and sodium benzoate simultaneously, especially in Indonesian soft drinks, has not been reported.

The aim of this study was to obtain the optimum conditions for simultaneous determination of sodium benzoate and sodium cyclamate in soft drink samples from supermarket in Surabaya using HPLC.



(a) **(b)**

Figure 1. Molecule structures of sodium benzoate and sodium cyclamate

MATERIALS AND METHODS

Materials

Sodium Benzoate ≥ 99% purity (SIGMA), Sodium cyclamate *Pharm. grade* (Bernofarm), methanol pro HPLC (E. Merck), bi-distillated water (Ikapharmindo), distillated water (Brataco), KH_2PO_4 99,5% (SIGMA), dan H_3PO_4 99% (E. Merck). Nine sodium benzoate and sodium cyclamate containing samples were collected from a supermarket located in Surabaya.

Instrument

High Performance Liquid Chromatography Agilent 1100 Series, with µ-Bondapak RP 18 column completed with Diode Array Detector (DAD), Spectrophotometer UV-Vis Lambda EZ 201 Perkin Elmer, pH meter Fisher accumet model 230 A.

Sample preparation

Sample was agitated in ultrasonic bath for 15 minutes for liberation of CO_2 in the sample. Two milliliter of sample (A, C, E, H, I) or 3.0 mL of sample (F, G) was diluted quantitatively with bi-distillated water in 10 mL volumetric flash. Moreover, 2.0 mL of sample (B, D) was diluted quantitatively in 25 mL volumetric flash to obtain certain concentration. The diluted sample was filtered with Whatman filter paper of 0.2 µm pores before being injected into HPLC apparatus.

Operational conditions

The sodium benzoate and sodium cyclamate standard concentrations range were 10 to 100 ppm and 1200 to 6000 ppm, respectively. A mixture of methanol and phosphate buffer of pH 4.0 ± 0.05 was used as isocratic mobile phase. The optimum operational condition of HPLC was as follow: flow rate of mobile phase was 1 mL/minute, column pressure and temperature was 220 bar and 30 °C, respectively. The loop volume was 20 µL.

RESULTS AND DISCUSSION

The spectra profile of sodium benzoate and sodium cyclamate showed that the maximum absorbance of sodium benzoate and sodium cyclamate were at 222 nm and 203 nm, respectively (Figure 2).

Their spectra were crossing at the wavelength of 243 nm. This wavelenght was then used as the wavelength detection of the two subtance examined.

Various composition of methanol and phosphate buffer of pH 4.0 were used to obtain a good resolution (Rs) between sodium benzoate and sodium cyclamate.

The methanol and phosphate buffer of pH 4.0 ± 0.05 (42:58) separated the sodium benzoate peak (t_R of 2.86 minutes) from the sodium cyclamate peak (t_R of 6.50 minutes) by resolution (Rs) of 14.5 (Figure 3). This value fulfilled the acceptability for resolution that should be more than 1.5 for complete separation of two peaks (Watson D.G., 2012).

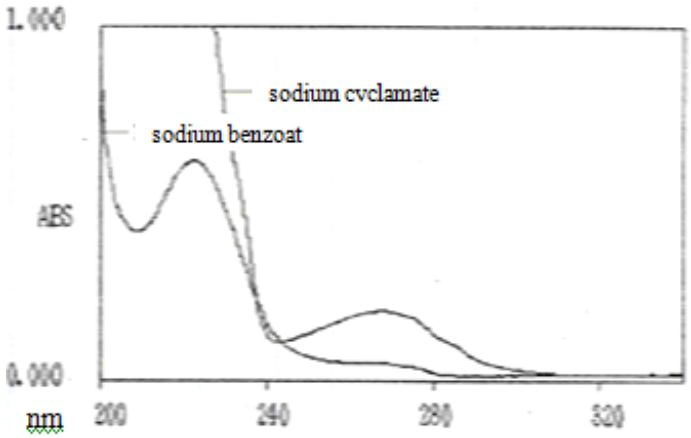


Figure 2. Overlay spectra of sodium benzoate (100 ppm) and sodium cyclamate (1200 ppm)

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Purity factor of sodium benzoate and sodium cyclamate standard peaks were 999.99 and 999.35, respectively. It can be concluded that sodium benzoate and sodium cyclamate peaks chromatogram were pure and not overlapping one and the other or contaminated by another trace substance peak.

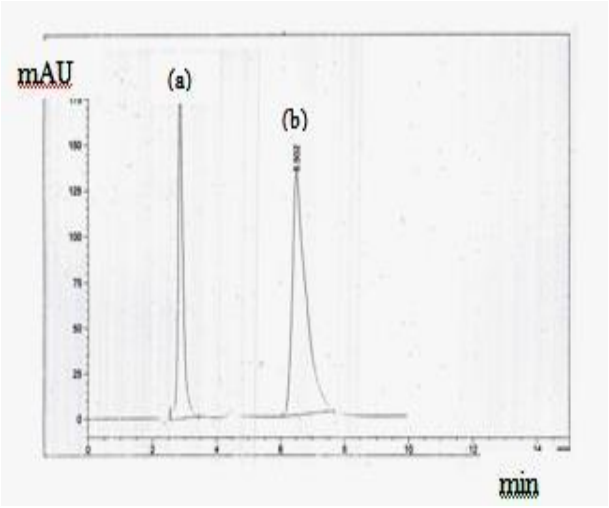

Figure 3. Chromatogram of 10 ppm sodium benzoate standard (a) and 1200 ppm sodium cyclamate standard (b)

The chromatogram of sample C was showed in Figure 4. Based on the match factor (MF) spectra of the sodium benzoate and sodium cyclamate in the samples with their standard spectra (Table 1), the sodium benzoate and the sodium cyclamate peaks in the sample were identical with standard.

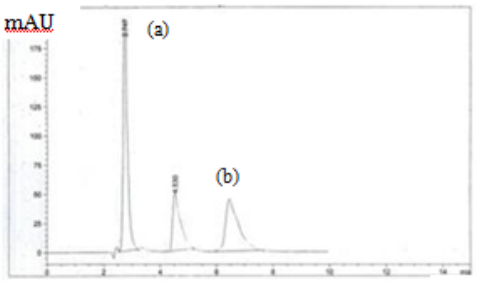

Figure 4. Chromatogram of sodium benzoate (a) and sodium cyclamate (b) in sample C

Linearity of sodium benzoate in the concentration range of 10 - 100 ppm was good. Regression equation of the sodium benzoate was y = 25.45 x + 9.39 (r = 0.9997, Vxo = 0.40%).

Moreover, coefficient correlation of the sodium cyclamate in concentration range of (1200 - 6000) ppm was 0.9991, with regression equation of y = 0.4845 x + 52.40, (Vxo = 4.9%).

Average recoveries of the sodium benzoate and sodium cyclamate standard using standard addition method were (102.28 $\pm$ 2.28)% and (101.62 $\pm$ 1.58)% (Table 2 and Table 3), respectively. These recoveries were fulfilled the AOAC recommendation (92 - 105 %) (AOAC, 2002).

Application of the HPLC method in nine samples with three replicates showed that five of nine samples contained sodium cyclamate higher than the recommended concentration, while all sodium benzoate containing sample within the recommended concentration (Table 4).

Table 1. The match factor of sodium benzoate and sodium cyclamate samples with their standard

Sample	Match Factor*	
	sodium benzoate	sodium cyclamate
A	999.8770	999.9791
B	999.8986	999.9265
C	999.8180	999.9488
D	999.7893	999.6465
E	999.8761	999.9788
F	999.7734	999.9663
G	999.8097	999.9495
H	999.7988	999.8685
I	999.8094	999.9077

*By electronic integrator of HPLC Agilent 1100 series software

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Table 2. Recovery of sodium benzoate standard in sample C

Replicate	Standard added (ppm)	Total obtained (ppm)	Sodium benzoate sample (ppm)	Recovery (%)
1	50.50	110.4	60.29	99.23
2	50.50	112.4	60.29	103.19
3	50.50	109.6	60.29	97.64
1	60.60	121.2	60.29	101.51
2	60.60	119.3	60.29	97.38
3	60.60	121.9	60.29	101.67
1	80.80	143.5	60.29	102.98
2	80.80	139.9	60.29	98.53
3	80.80	141.1	60.29	100.01
		Average		102.28
		SD		2.28
		CV		2.22 %

Table 3. Recovery of sodium cyclamate standard in sample C

Replicate	Standard added (ppm)	Total obtained (ppm)	Sodium cyclamate sample (ppm)	Recovery (%)
1	310.6	1464	1156	99.16
2	310.6	1470	1156	101.09
3	310.6	1467	1156	100.13
1	372.6	1529	1156	100.11
2	372.6	1528	1156	99.84
3	372.6	1536	1156	101.99
1	414.0	1579	1156	102.17
2	414.0	1559	1156	97.34
3	414.0	1566	1156	99.03
		Average		101.62
		SD		1.58
		CV		1.56 %

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Table 4. Concentrations of sodium cyclamate and sodium benzoate in sample

Kode Sample	Sample Performance	Sodium cyclamate/bottle	Sodium cyclamate/Kg	Sodium benzoate/bottle	Sodium benzoate/Kg
A	Soft drink 200 mL/bottle	4087 mg/200 mL	20435 mg/Kg*	66.20 mg/200 mL	331 mg/Kg
B	Syrup 300 mL/bottle	6125 mg/300 mL	20417 mg/Kg	323.6 mg/300 mL	1078 mg/Kg
C	Soft drink 190 mL/bottle	1008 mg/190 mL	5305 mg/Kg*	56.30 mg/190 mL	296 mg/Kg
D	Syrup 630 mL/bottle	724.1 mg/630 mL	1149 mg/Kg	574.2 mg/630 mL	911 mg/Kg
E	Soft drink 200 mL/bottle	2898 mg/200 mL	14490 mg/Kg*	67.18 mg/200 mL	335 mg/Kg
F	Tea 200 mL/bottle	236.2 mg/200 mL	1181 mg/Kg	55.91 mg/200 mL	279 mg/Kg
G	Soft drink 180 mL/bottle	321.6 mg/180 mL	1787 mg/Kg	49.67 mg/180 mL	275 mg/Kg
H	Soft drink 175 mL/bottle	2504 mg/175 mL	14308 mg/Kg*	93.63 mg/175 mL	535 mg/Kg
I	Soft drink 200 mL/bottle	3131 mg/200 mL	15655 mg/Kg*	37.60 mg/200 mL	188 mg/Kg

Note: * = Samples that were containing sodium cyclamate more than permitted concentration.
Assumption: syrup B or D were diluted ten times before consumption

CONCLUSION

The optimum condition for simultaneous determination of the sodium benzoate and sodium cyclamate by HPLC was selective, linear, accurate and precise.

The concentration range of the sodium benzoate and sodium cyclamate in samples, were 188–1078 mg/Kg and 1149–20435 mg/Kg, respectively. Some of these samples contain sodium cyclamate more than permitted concentration. Nevertheless, all sodium benzoate containing samples were within the recommended concentration.

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