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Research Article

ANALYSIS OF DISODIUM 5'-GUANYLATE AND DISODIUM 5'-INOSINATE AS FLAVOR ENHANCER IN FOOD SPICES BY THIN-LAYER CHROMATOGRAPHY–DENSITOMETRY

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ABSTRACT

Objective: The objective of this study was to obtain an optimum and valid method of analysis to determine the levels of disodium 5'-guanylate (DSG) and disodium 5'-inosinate (DSI) in six samples of spices.

Methods: The optimum method was obtained using silica gel 60 F_{254} as the stationary phase and isopropanol:water:25% ammonia at a ratio of 6:3:1 (v/v) as the mobile phase. The developed spots were scanned using a densitometer in absorbance mode at 260 nm. The methods were valid based on the accuracy criteria (DSG, 99.11–99.96%, and DSI, 98.56–101.05%), precision (DSG, 1.09%, and DSI, 0.49%), and linearity (DSG, r=0.9909, and DSI, r=0.9976).

Results: The results showed that the levels of DSG in samples A, B, C, D, E, and F were 0.70%, 0.79%, 0.78%, 0.99%, 1.08%, and 1.08% and those of DSI were 0.66%, 0.74%, 0.71%, 0.66%, 0.54%, and 0.67%, respectively.

Conclusion: The optimum conditions of DSG and DSI for thin-layer chromatography-densitometry were obtained with silica gel 60 F_{254} as the stationary phase, isopropanol:water:25% ammonia (6:3:1) as the mobile phase, and a maximum wavelength of about 260 nm. Validation results indicated that the accuracy of the analytical method for DSG was about 99.11–99.96% with a coefficient variation (precision) of 0.70–1.41%, while that for DSI was 98.56–101.05% with a coefficient variation of 0.23–0.75%. The correlation coefficients for the analytical method for DSG and DSI were 0.9909 and 0.9976, respectively. The results determined that the levels of DSG and DSI in samples A, B, C, D, E, and F were 0.70%/0.60%, 0.79%/0.74%, 0.78%/0.71%, 0.99%/0.66%; 1.08%/0.54%, and 1.08%/0.67%, respectively.

Keywords: Disodium 5'-guanylate, Disodium 5'-inosinate, Thin-layer chromatography-densitometry, Optimization, Validation.

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INTRODUCTION

Flavor enhancers, both natural and synthetic, are widely used in food products. In addition to monosodium L-glutamate (2-aminopentanedioate or 2-amino glutaric acid), disodium 5'-guanylate (DSG) and disodium 5'-inosinate (DSI) are frequently used in traditional Asian foods [1]. The PERMENKES 033 of 2012 guidelines has approved the use of L-glutamic acid and its salts, guanylic acid and its salts, inosinic acid and its salts, and salts from disodium 5'-ribonucleotides as flavor enhancers [2].

The salt forms of inosine monophosphate and guanosine monophosphate are potent flavor enhancers that are frequently used as additives along with monosodium L-glutamate [3]. DSG and DSI are purine nucleotides that are synthesized *in vivo* at rates consistent with physiological needs. Purine biosynthesis involves three important components: An amphibolic intermediate, purine phosphorylase, and purine nucleoside phosphorylase [4].

The amount of a food additive consumed per day should not exceed the recommended acceptable daily intake (ADI), which is the maximum amount of a food additive measured in milligrams per kilogram of body weight that can be consumed daily for life without causing adverse health effects [5]. According to Fenaroli's Handbook of Flavor Ingredients, the ADI values of DSG and DSI are about 0.07768 and 0.09053mg/kg/day, respectively.

Purine nucleotide intake that exceeds the ADI may lead to metabolic disorders, such as gout, Lesch–Nyhan syndrome, adenosine deaminase deficiency, and purine nucleoside phosphorylase deficiency [4]. Of these, gouty arthritis is an inflammatory disorder caused by excessive consumption of DSG and DSI, which results in increased uric acid levels in the blood and subsequent inflammation due to the crystallization of sodium urate in the soft tissues and joints [6].

The daily use of flavor enhancers poses a potential danger to the amount of non-essential purine nucleotides that enter the body. Therefore, research is needed to optimize and validate analytical methods to determine the levels of DSG and DSI in spices, such as thin-layer chromatography (TLC)-densitometry. In addition, a previous study identified 10 types of nucleotides in shrimp using high-performance liquid chromatography [7]. However, that study did not report the levels of DSG and DSI in the spice samples that are widely used in Asian communities. In the present study, TLC-densitometry was chosen to measure DSG and DSI levels in spices commonly used in Asian foods, as this method is simple, relatively fast, and inexpensive and can be used for the qualitative and quantitative analyses of small quantities [8,9].

MATERIALS AND METHODS

Materials

The samples for analysis were six different brands of food seasoning products containing flavor enhancers that were obtained from traditional markets and supermarkets in Jakarta, Indonesia.

Chemical materials

DSG and DSI were purchased from Cheil Jedang Indonesia (Jakarta, Indonesia), and isopropanol, 25% ammonia, 1-butanol, methanol, chloroform, formic acid, and distilled water were obtained from Merck KGaA (Darmstadt, Germany).

Equipment

The equipment used in this study included analytical scales (Radwag Balances and Scales, Radom, Poland), a densitometer (TLC Scanner 3; CAMAG Scientific Inc., Muttenz, Switzerland), a personal computer equipped with the "Wincats" application (CAMAG Scientific Inc.), a chromatography chamber, sonicator, a silica gel chromatography plate (60 F_{254}; Merck KGaA), volume syringe, capillary pipe, filter paper (no. 41; Whatman's, Maidstone, UK), and various glass tools.

Methods

Optimization of analytical conditions

The mobile phase was done with the following compositions:

a. Isopropanol:water:25% ammonia (7:2:1)
b. 1-Butanol:water:25% ammonia (86:14:1)
c. Methanol:chloroform:formic acid (5:5:1)
d. Isopropanol:water:25% ammonia (6:2:2)
e. Isopropanol:water:25% ammonia (6:3:1).

The results obtained from the variation of the mobile phase composition were compared, and the most optimum analytical conditions were assessed based on the retardation factor (Rf) values and good standard mixed solution chromatogram separation based on a resolution value of >1.5. Maximum wavelength optimization was required to obtain the maximum peak of the analysis. The maximum wavelength was determined with the winCATS program by entering a wavelength range of 200–780 nm using deuterium and a tungsten lamp on the "Spectral-Scanner 3" menu.

Validation of analytical methods

A linearity equation, obtained from the calibration curves of DSG and DSI, was used to calculate the factors of linearity of the line, which had a correlation coefficient of >0.999 [10]. The solution for the calibration curve was obtained from a dilution of the main solution with a concentration of 1000 µg/mL. A calibration curve was determined using concentrations of 150, 200, 250, 300, 450, and 500 µg/mL for DSG and 150, 200, 250, 300, 350, 400, and 450 µg/mL for DSI. The limits of detection and quantitation were determined from the calibration curve equation [10]. Selectivity was determined by comparing the analytical results of the samples containing contaminants, one kind of compound, other foreign compounds, or placebo carriers without no other compounds [11].

For accuracy and precision testing, 750 mg of the sample powder was weighed and transferred to three 25-mL flasks (flasks 1, 2, and 3). Three different concentrations of standard solution (150, 300, and 450 ppm) were made. Flask 1 contained 3.75 mL of 150 ppm standard solution, flask 2 contained 7.5 mL of 300 ppm standard solution, and flask 3 contained 11.25 mL of 450 ppm standard solution. Standard solutions of low, medium, and high concentrations were obtained from the calibration curves. The standard solutions were mixtures of the standardized DSG and DSI. Afterward, aquadest was added to each flask to a total volume of 25 mL. A 1-µl aliquot of the test solution at each concentration was analyzed 6 times.

Sample preparation

About 3 g of each of the six samples tested in this study was dissolved with aquadest in a 100-mL flask to a concentration of 30,000 ppm. The samples were then sonicated for 15 min and filtered through Whatman's no. 41 filter paper.

Determination of sample level

All samples were prepared in the same manner. Briefly, 1 µl of each sample was analyzed under optimal analytical conditions. The experiment was repeated 3 times. The calculation of the sample concentration was carried out using a linear regression equation obtained from the obtained calibration curve.

RESULTS

Optimization of analysis conditions

Each standardized solution was added to an activated silica gel plate, eluted with the appropriate mobile phase (i.e., 6:3:1 of isopropanol-water-25% ammonia) and analyzed with a densitometer at a wavelength of 260 nm. Selection of the mobile phase was based on the separation resolution value of both compounds (i.e., 1.56), which met the requirements of good separation (Figs. 1 and 2).

Analysis validation

Linearity test

Based on the calibration curve, the linear regression equation value of DSG was 3944x+1390 with a correlation coefficient of 0.9909, while that of DSI was 24144x+1365 with a correlation coefficient of 0.9976 (Figs. 3 and 4).

The correlation coefficient was <0.999 because of the less sensitive spots which were measured, so a greater concentration was required. Further research is needed to obtain linearity that meets the test requirements.

Limits of detection and quantitation

The smallest and largest concentrations of DSG and DSI that could still be detected by linear regression equation were 45.12 and 150.41 µg/mL and 23.93 and 79.77 µg/mL, respectively.

Selectivity testing

The selectivity test was performed by comparing the chromatogram spots and Rf that were obtained from standard solution analysis and the eluted sample solution. Selectivity was also assessed on the basis of relative retention (α). In this study, the Rf values of DSG and DSI from the standard solution and the obtained samples were 0.48 and 0.72, respectively. The value of α was 1.86 where the requirement was α>1.0.

Accuracy testing

The average accuracy of DSG was 99.11–99.96% with a standard deviation of 1.76–4.27%, while that for DSI was 98.56–101.05% with a standard deviation of 1.76–2.31%. This result satisfies the criteria of precision with a value of 98–102% (Tables 1 and 2).

Precision testing

In the precision test, the KV values of DSG and DSI were 0.70–1.41% and 0.23–0.70%, respectively. These results met the requirements of a KV value of ≤2% (Tables 1-4).

Determination of sample concentrations

a. Sample A: DSG, 0.70%, and DSI, 0.66%
b. Sample B: DSG, 0.79%, and DSI, 0.74%
c. Sample C: DSG, 0.78%, and DSI, 0.71%
d. Sample D: DSG, 0.99%, and DSI, 0.66%
e. Sample E: DSG, 1.08%, and DSI, 0.54%
f. Sample F: DSG, 1.08%, and DSI, 0.67%.

DISCUSSION

The calculation of line linearity parameters for DSG and DSI showed that the calibration curve equation line of each test substance did not meet the requirements. The correlation coefficient was <0.999 because of the less sensitive spotting measurements, and thus, greater concentrations were required. Further research is needed to obtain linearity that meets the test requirements.

In regard to the limits of detection and quantitation, the results obtained for each test substance show that the response obtained was still significant and the results were considered as precise.

Table 1: Accuracy and precision test result of DSG

Concentration (µg/mL)	Measured area* (mm^2)	Actual area** (mm^2)	Measured concentration (µg/mL)	% UPK	% Average of UPK	SD	% KV
152.1	4966.9	2372.2	153.15	100.69			
	4948.5	2349.1	151.65	99.71			
	4928.2	2335.7	150.79	99.14			
	4983.7	2330.7	150.47	98.93	99.11	1.76	1.17
	4979.8	2289.1	147.78	97.16			
	4994.6	2332.7	150.60	99.01			
304.2	5283.4	2688.7	308.07	101.27			
	5245.7	2646.3	303.21	99.68			
	5272.8	2680.3	307.11	100.96			
	5269.0	2616.0	299.74	98.53	99.47	4.27	1.41
	5289.1	2598.4	297.73	97.87			
	5277.6	2615.7	299.71	98.52			
456.3	6023.7	3429.0	454.35	99.57			
	6076.4	3477.0	460.71	100.97			
	6029.9	3437.4	455.47	99.82			
	6112.2	3459.2	458.35	100.45	99.96	3.17	0.70
	6098.7	3408.0	451.57	98.96			
	6106.1	3444.2	456.37	100.01			

DSG: Disodium 5'-guanylate, SD: Standard deviation, response detector (AU)

Table 2: Accuracy and precision test result of DSI

Concentration (µg/mL)	Measured area* (mm^2)	Actual area** (mm^2)	Measured concentration (µg/mL)	% UPK	% Average of UPK	SD	% KV
150.9	695.7	294.5	149.38	98.99			
	696.8	294.0	149.12	98.82			
	697.1	293.6	148.92	98.69			
	696.6	295.2	149.73	99.23	98.96	1.76	0.23
	698.5	295.2	149.73	99.23			
	695.6	293.9	149.07	98.79			
301.8	782.7	381.5	298.05	98.76			
	785.8	383.0	299.22	99.15			
	780.1	376.6	294.22	97.49			
	785.6	384.2	300.16	99.46	98.56	2.24	0.75
	781.5	378.2	295.47	97.90			
	782.6	380.9	297.58	98.60			
452.7	875.4	474.2	459.38	101.48			
	872.8	470.0	455.32	100.58			
	872.1	468.6	453.96	100.28			
	875.7	474.3	459.48	101.50	101.05	2.31	0.50
	875.9	472.6	457.83	101.13			
	875.3	473.6	458.80	101.35			

*Measured area=Standard addition of the sample, **Actual area=Standard area of the addition, DSI: Disodium 5'-inosinate, SD: Standard deviation, Response Detector (AU)

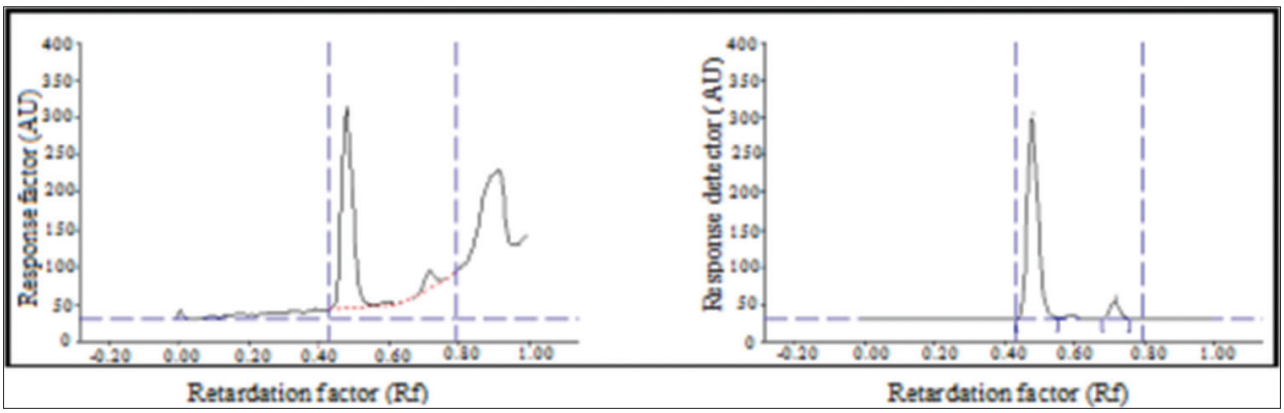

Fig. 1: A chromatogram from the separation analysis of both compounds using silica gel 60F_{254} plate with a mobile phase of (6:3:1) isopropanol:water:25% ammonia and analyzed at a wavelength of 260 nm

The selectivity test results had an α-value of 1.86, which met the requirement of α>1.0. Thus, the analytical conditions of both test substances were considered as good with selective separation potency.

The results of accuracy and precision testing showed that the methods had met the accuracy and precision requirements (% UPK, 98–102%, and % KV, ≤2.0%) [10,12,13].

Table 3: DSG Levels of the six spice samples

Sample	Sample concentration (µg/mL)	Area (mm^2)	Measured concentration (µg/mL)	Level (%)	Amount per pack (mg)	Amount of daily consumption based on ADI (mg/60 kg body weight/day)
A	30126	2206.6	207.05	0.70	63/9 g	1.05
		2281.5	226.04			
		2183.5	201.19			
	Average		211.43			
B	30090	2327.2	237.75	0.79	86.9/11 g	1.45
		2307.6	231.14			
		2365.6	247.36			
	Average		238.75			
C	30160	2368.6	248.12	0.78	156/20 g	0.29
		2349.6	243.31			
		2244.2	216.58			
	Average		236			
D	30762	2594.7	305.45	0.99	247.5/25g	4.14
		2599.4	306.64			
		2592.5	304.89			
	Average		305.66			
E	30175	2859	372.46	1.08	864/80g	14.4
		2661.9	322.49			
		2506	282.96			
	Average		325.97			
F	30226	2653	320.23	1.08	10800/kg	180
		2690.7	329.79			
		2703.1	332.91			
	Average		327.64			

DSG: Disodium 5'-guanylate, ADI: Acceptable daily intake

Table 4: Results of DSI level on six samples of food spices

Sample	Sample concentration (µg/mL)	Area (mm^2)	Measured Concentration (µg/mL)	Level (%)	Amount per pack (mg)	Amount of daily consumption based on ADI (mg/60 kg body weight/day)
A	30126	886.9	198.02			
		887.1	197.94	0.66	59.4/9 g	0.99
		881.2	200.38			
	Average		198.78			
B	30090	887.5	197.77			
		800.7	233.72	0.74	81.4/11 g	1.36
		803.2	232.69			
	Average		221.40			
C	30160	832.6	220.51			
		814	228.21	0.71	142/20 g	2.37
		892.5	195.7			
	Average		214.80			
D	30762	892.7	195.62			
		835.8	219.18	0.66	165/25 g	2.75
		890.1	196.69			
	Average		203.83			
E	30175	978.3	160.16	0.54		
		973.2	162.28		432/80 g	7.2
		961.7	167.04			
	Average		163.16			
F	30226	820.6	225.48	0.67		
		880.6	200.63		6700/kg	111.67
		927.8	181.37			
	Average		202.49			

DSG: Disodium 5'-guanylate, ADI: Acceptable daily intakec

The determined levels of DSG and DSI in the six samples were not in agreement with the recommended levels in Fenaroli's Handbook of Flavor Ingredients of 0.07768 and 0.09053mg/kg/day, respectively.

CONCLUSION

On the basis of these results, it can be concluded that (a) the optimum conditions of DSG and DSI for TLC-densitometry were obtained with

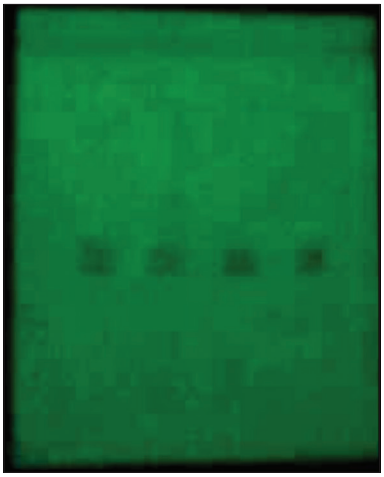

Fig. 2: Elution result of the standard mixed solution with a mobile phase of isopropanol:water:25% ammonia (6:3:1)

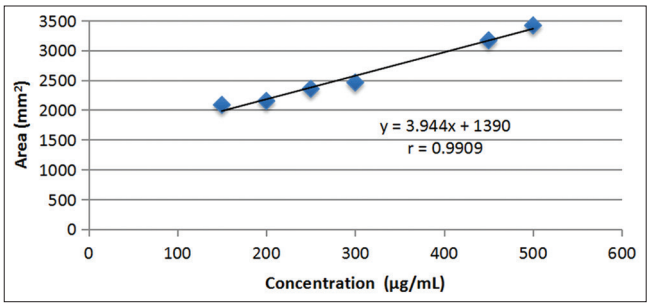

Fig. 3: Calibration curve of disodium 5'-guanylate

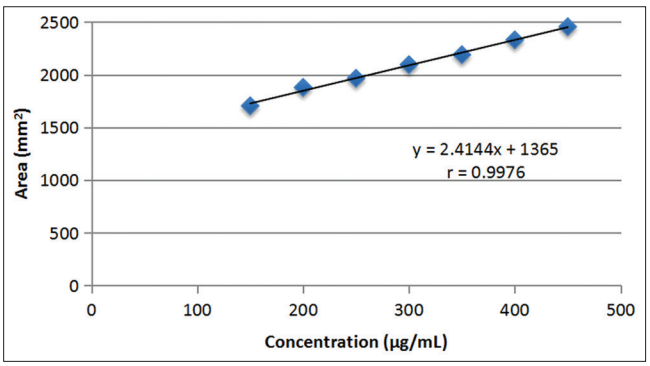

Fig. 4: Calibration curve of disodium 5'-inosinate

silica gel 60 F_{254} as the stationary phase, isopropanol:water:25% ammonia (6:3:1) as the mobile phase, and a maximum wavelength of about 260 nm. (b) Validation results indicated that the accuracy of the analytical method for DSG was about 99.11–99.96% with a coefficient variation (precision) of 0.70–1.41%, while that for DSI was 98.56–101.05% with a coefficient variation of 0.23–0.75%. The correlation coefficients for the analytical method for DSG and DSI were 0.9909 and 0.9976, respectively. (c) The results determined that the levels of DSG and DSI in samples A, B, C, D, E, and F were 0.70%/0.60%, 0.79%/0.74%, 0.78%/0.71%, 0.99%/0.66%; 1.08%/0.54%, and 1.08%/0.67%, respectively.

CONFLICTS OF INTEREST

All authors have none to declare.

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