The Applicability of HPLC for the Analysis of Salt Additives

Akihiro Koga, Yasushi Niino and Yutaka Yoshifuji

Sea Water Science Research Laboratory, The Salt Industry Center of Japan,
4-13-20 Sakawa, Odawara, Kanagawa 256-0816, JAPAN

Currently, table salt containing additives such as flavour enhancers and minerals, in addition to anticaking agents, is commercially available. As flavour enhancing additives, usually organic or amino acids or salts of organic acids are used. Examples of added organic acids are malic acid and citric acid, those of salts of organic acids are calcium citrate and calcium lactate, and those of amino acids are L-glutamic acid monosodium salt and L-lysine chloride monohydrate. We studied the applicability of the HPLC method to the analysis of these salt additives. The HPLC calibration curve was a straight line (organic acids: 0-60 mg/l and amino acids: 0-400mg/l), and NaCl had no effect on the measurement. Consequently, these results support the applicability of the method to the measurement of salt additives.

1. INTRODUCTION

Marketing will make headway in Japan, as the regime of monopolization is abrogated, and competition will be further intensified. In this regard new commodities have been developed such as table salt containing various constituents, which now appear on the market. Currently, table salt containing additives such as flavour enhancers, in addition to anticaking agents, is commercially available. Such commodities will probably increase in number. Therefore a method for the determination of these salt additives must be established. We report here the progress regarding amino acids and organic acids. High-performance liquid chromatography (HPLC) has been used to measure these organic constituents (1, 2), therefore we studied the applicability of the HPLC measure these additives in salt. In the analysis of salt, sodium chloride is the matrix, which may exert various effects on the column's state, the shape of the peak and the time of elution. Therefore, the applicability of HPLC for such analysis must be studied. We investigated the influence of the matrix and the conditions for measurement and established a method for measuring amino acids and organic acids.

2. MEASUREMENTS

The methods for measuring amino acids and organic acids by means of HPLC were studied. For the measurement of amino acids, we studied the "AccQ. Tag amino acid analysis method" developed by Waters Corporation. This method involves the derivatization of an amino acid and its separation by passing it through a column. Organic acids were measured by HPLC with the aid of the phosphoric acid eluent developed by Muratani et al. (ref no.3). The measurement method is described below.

Table 1 The solutions for the HPLC eluent

EluentA	Sodium Acetate Trihydrate 1 19 wt%
	Phosphonic Acid 6-7wt%
	Triethylamine 1-2wt%
	Sodium Azide 0.1wt%
	Water 72-73wt%
EluentB	Acetonitrile
EluentC	MilliQ Water

2. 1. Measurement of amino acids (AccQ·Tag amino acid analysis method)

In the "AccQ - Tag am amino acid analysis method " the only preconditioning is the derivatization which is carried out by mixing some reagents, which is strong point of this easy method. The reagents were prepared and the measurement of glutamic acid monosodiurn salt was carried out as described below.

The derivatization reagents are as follows:

1. AccQ. Fluor Reagent 2A (AQC: N-Hydroxysuccinimidyl-6-aminoquinolinyl carbamate)
2. AccQ. Fluor Reagent Diluent 2B (Acetonitrile, Dehydrated)
3. AccQ. Fluor Borate Buffer

First the "AccQ . Fluor Reagent" is prepared as follows. One ml of reagent (2) (acetonitrile) was added to a vial containing reagent (1) (AQC). This solution. was vortexed by VORTEX-GENIE to dissolve the AQC. This vial was then put into a small polyethylene bag and soaked in a water bath at 55^{0}C for ten minutes to completely dissolvethe AQC was. This solution was called "AccQ. Fluor reagent", and was used for the derivatized as follows.

Table2 The condition of HPLC for amino acids analysis	
Column	AccQ-Tag Amino Acid Analysis Column
Flow rate	1 ml/min
UV	254 nrn
Injection Volume	10 µl
Temperature	37 ^{0}C

Twenty µ1 of the sample solution was asp~ by a micropipette and introduced do the vial, and then 170 µ1 of borate buffer was added. The solution in the vial was vortexed. Ten µ1 of AccQ. Fluor reagent prepared previously was added to the vial and then vortexed. This solution was left to stand for one minute.

The HPLC eluent was prepared as follows. The types of solutions for use as eluent are listed in Table 1. Eluent

A, which is made by Waters Corp., is a mixture of various solutions. Eluents A and C were mixed in the ratio of 1: 10 (volume). Then eluent B was added to this solution to a concentration of 2%. The whole solution was used as the HPLC eluent.

The conditions for HPLC analysis of amino acid are shown in Table 2.

In order to measure glutamic acid monosodium salt we obtained the calibration curve and investigated the effects of sodium chloride on the measurement. Acids must be added depending on the salt additive investigated, therefore, the effects of acids were likewise studied.

2.2. Measurement of organic acids

Organic acids that are present in table salts as salt additives include malic acid and citric acid. In the method described below an aqueous solution of the sample is directly used for HPLC, as no preconditioning is needed.

Table3 The condition Of HPLC for organic acids analysis	
Column	SHISEIDO C18 UG120 6mm diam. x 150mm
Flow rate	1 ml/min
UV	210 nm
Injection Volume	50 µl
Temperature	35 ^{0}C

The conditions for HPLC analysis are shown in Table 3. The eluent was prepared by adding phosphoric acid to a 10 mM potassium phosphate monobasic solution (pH=2.3). Malic acid (0.1g) was dissolved in 100ml water. Exactly 5, 10, 20 and 50 ml each of this solution was added to water to make 100 ml. The citric acid standard solution was prepared in the same manner. We made a calibration curve using these solutions. Then the properties of this curve and the effects of sodium chloride were studied.

3. RESULTS AND DISCUSSION

3.1. Measurement of amino acids

The eluent was prepared. The motion of chromatogram was confirmed by varying the ratio of mixed solution A (eluent A:C = 1:10) and acetonitrile (B). The chromatogram of glutamic acid is shown in Fig.1 as a function of acetonitrile concentration in the eluent. As the concentration of acetonitrile increased, the elution time decreased and the peak became sharp. Because the polarity of acetonitrile is low, the polarity of the entire argent in lowered with an increase of the concentration of acetonitrile and the elution time decreased. However, when the eluent containing 3% acetonitrile was used, the peaks overlapped because the elution time was very short. Based on these results, we decided that the ratio of acetonitrile in the eluent be 2%, because the peaks appear within a short time without overlapping The calibration curve of aqueous glutamic solution is shown in Fig.2. A good correlation (R^2=.9953) is observed, therefore, the measurement of glutamic acid in water is possible. Then, we investigated whether the quantity of sodium chloride exerts some effects on the measurement. Sodium chloride was added to aqueous glutamic acid solution (0-20%). The difference in measurement results was within 0.002%. We could not find any distinct change in the measurement results, with the increase or decrease quantity of sodium chloride. Consequently, we concluded that sodium chloride has no effect on the measurement.

"ClHIYODA SALT' or "ENRICH,", which are example of Japanese table salts, contains a salt of carbonic acid, an insoluble compound. Acid must added to dissolve this salt. Therefore, we have to confirm the effects of acid on the measurement by HPLC. As sample, aqueous glutamic acid solution (0.02%) was used. Six mol/l hydrochloric acid was added to these solutions in steps (1 - 3ml). The results of measurements using these solutions are shown in Table 4. It was found that hydrochloric acid has no effect on the measurement by HPLC. Therefore, dissolution of the salt of carbonic acid by hydrochloric acid poses no problem. After the addition of hydrochloric acid (6M 0.5ml) and the dissolution of the salt of carbonic acid, the volume of the solution was increased to 500 ml.
Based on the results obtained, using table salts available commercially the quantitative analysis of glutamic acid monosodium salt was carried out. We

Table 4 The value of measurements by changing the quantities of hydrochloric acid

The concentration of 6M HCl (%)	The amount of glutamic acid (%)
0.0	0.010
0.1	0.010
0.2	0.010
0.3	0.010

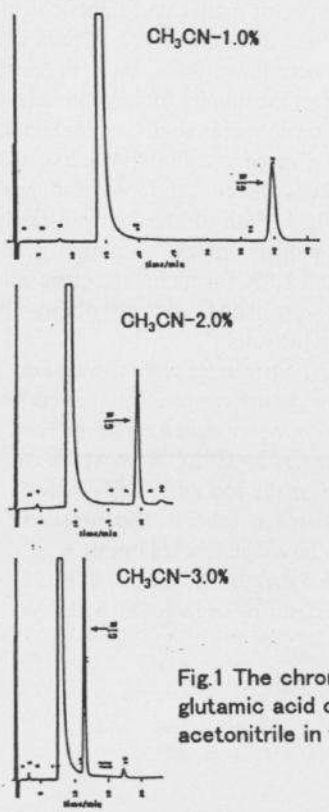

Fig.1 The chromatogram of glutamic acid changing of acetonitrile in the eluent

Table 5 The concentrations of glutamic acid in the table salts

Samples	The value of measurements(%)	The indicated quantities(%)
AJISHIO	10.3	10
CHIYODASALT	0.49	0.5
ENRICH	1.3	1.0

used the following table salts: "AJISHIO" (the amount of added glutamic add monosodium salt according to the label: 10%), "ENRICIT' (1.0%),"CHIYODA SALT' (0.5%). Ten g of each of these samples was dissolved and added to water to make 500 ml. Two sample solutions were prepared for each salt. Calcium carbonate and magnesium carbonate were added to "CHIYODA SALT", and calcium carbonate was added to "ENRICH". We prepared these 500 ml. solutions after salt of carbonate was dissolved in 0.5 ml of hydrochloric acid (6M). As "AJISHO" contained much glutamic acid monosodium salt, the solution was diluted to one-tenth its original volume with distilled water. These solutions were derivatized and injected into the HPLC. The peak area was plotted against the concentration of glutamic add, and the quantity of glutamic add monosodium salt was measured. The results and the values on the product labels are given in the table 5. The measured values are very close to the values appearing on the labels.

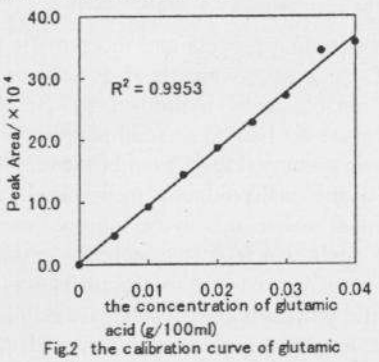

Fig.2 the calibration curve of glutamic acid

3.2. Measurements of organic acid

The chromatogram of malic acid standard solutions is shown in Figs.3-a and b. A difference in the chromatogram appeared between DL-malic acid and L, malic acid. In the chromatogrrn of L-Malic acid, we found a small peak at 6.8min. besides the main peak at 3.9min. The chromatogram of "TSUKEMONOEN", which is one of the Japanese table salts containing malic acid and citric acid, is shown in Fig.3-c. These chromatograms show that other peaks appeared besides those of malic acid or citric acid. As there were no other salt additives present besides these, we thought that some impurities were present.
There are three processes for manufacturing malic acid: the sampling process from fruit, the zymotic process and the synthetic process. Only L-malic acid can be formed by

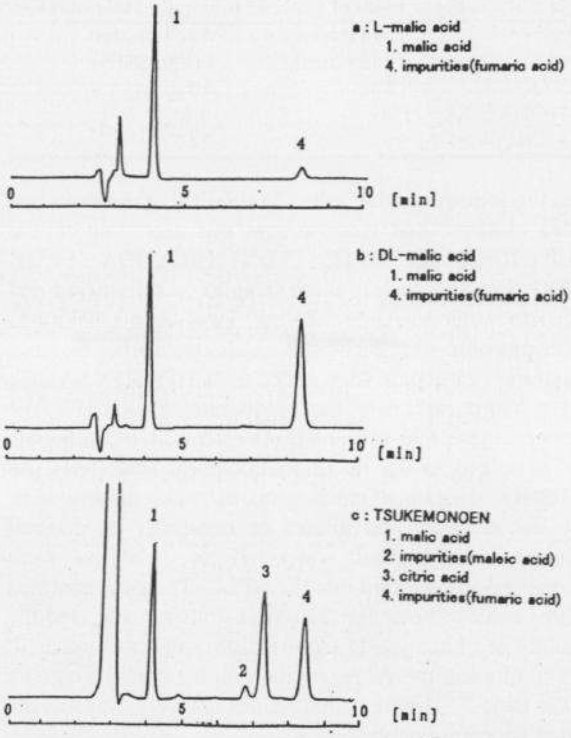

Fig.3 The chromatogram of organic acids

means of the sampling process and the zymotic process. However, DL-malic acid, which is an equivalent mixture of D- and L-malic acids, is formed by the synthetic process. DL-malic acid used as food additive is formed by the synthetic process (Fig.4) from benzene. Therefore, if malic acid as the final product is incompletely purified, it is possible that maleic acid in the intermediate process and fumaric acid, which is a secondary product, are present. The reagent used this measurement was formed by the synthetic process. Consequently, we estimated that the peaks besides those of malic and citric adds represent maleic add and fumaric acid.

We carried out the following experiment to identify the impurities. Aqueous solutions of malic acid, citric acid, maleic acid and fumaric acid were prepared and subjected

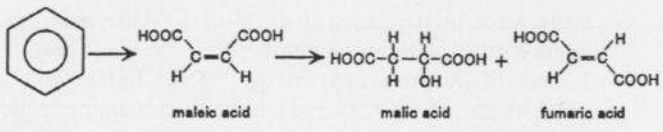

Fig.4 synthesis process of malic acid

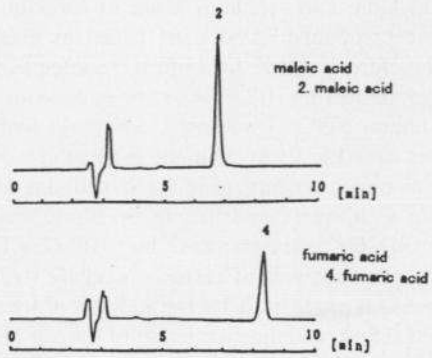

Fig.5 The chromatogram of maleic acid and fumaric acid

to HPLC. The results are shown in Fig.5. The peaks besides those of malic and citric adds were in agreement with the peaks of maleic acid and fumaric acid; therefore, maleic and fumaric acids are present in "TSUKEMONOEN", in addition to malic and citric acid as salt additives.

However, the peaks of maleic and fumaric acids can be separated from those of malic and citric acids by adjusting the pH of the eluent and the temperature of the column. Therefore, these impurities have no effects on the measurements. The quantities of maleic and fumaric acids in "TSUKEMONOEN" were determined to be 2ppm and 0.5ppm, respectively. Since these adds exist in small quantities, they do not effect the quality of the table salt.

Using malic acid and citric acid solution containing sodium chloride (2%), we made a calibration curve that shows the relationship between concentration and peak area, which is shown in Fig.6. Both solutions gave straight lines in the concentration range 1-50mg/l. The reproducibility is 1.3% and 1.0% for malic and citric acid respectively. Then, we estimated the possibility of measuring these acids in table salts.

Measurements of malic and citric acids were carried out using "TSUKEMONOEN" as the sample. Ten g of each of the acids was dissolved in water to make 500 ml The calibration curve was obtained by HPLC from which we determined the quantity of malic and citric acids in this sample. The results are shown in Table 6. The measured values were almost the same as the labeled ones; consequently we concluded that this method of HPLC analysis is effective for measuring organic salt additives.

4. SUMMARY

We studied the applicability of the HPLC method to the analysis of salt additives such as amino acids and organic acids. The concentrations of glutamic acid monosodium salt which is one of the amino acids, were determined using the "AccQ·Tag amino acid analysis method" developed by Waters Corporation. Aqueous glutamic acid solutions, of which the concentrations of sodium chloride and hydrochloric acid were changed, were measured. It was found that these had no effect on the measured values. The HPLC peak area was plotted against the concentration of glutamic acid, and a straight calibration curve was obtained. The concentration of glutamic acid in the table salts was determined, and it was found that measured values were almost the same as the labeled ones.

Besides, we attempted the measurement of organic acids such as magic acid and citric acid by means of HPLC. Peaks representing impurities appeared in the chromatogram of DL-malic acid. We thought that, these peaks could be ascribed to maleic acid and fumaric acid. However, since they exist in small amounts, they do not effect the quality of the table salts. The amount of malic acid and citric acid determined in "TSUKEMONOEN" which is one of the Japanese table salts, were in agreement with the labeled values.

Based on the results obtained, we conclude that the HPLC analysis method is effective for measuring organic salt additives.

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