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Application News

No.L463

High Performance Liquid Chromatography

Analysis of Histamine and Tyramine Using Prominence Amino Acid Analysis System

Putrefactive non-volatile amines, histamine and tyramine, are formed through decomposition of histidine and tyrosine, respectively, due to the action of microorganisms. When ingested food such as processed products and red-fleshed fish such as tuna, bonito, mackerel, etc., contain a large amount of histamine, food poisoning symptoms such as fever, hives, and palpitations may appear. There are also reported cases of food poisoning associated with fermented foods such as wine and cheese. Further, tyramine can also strengthen the toxicity of histamine, and has been reported as a causative agent in food-associated migraine.

Although there are no specific histamine-related regulations in Japan, in other countries, including the United States and the EU, Codex (International Food Standards) regulatory limits for histamine have been established for fish and fishery products.

As tyramine and histamine, like amino acids, contain an amino group, fluorescence detection is possible using derivatization with ortho-phthalaldehyde (OPA). Here, we introduce an example of analysis of tyramine and histamine using the Prominence Amino Acid Analysis System, in which detection is conducted using post-column fluorescence derivatization. Mobile phase and reagent kits, which are available for this application, contain the required mobile phases and reaction reagent solution, thus eliminating the tedious preparation of mobile phase. Moreover, as sample pretreatment consists only of filtering and dilution for this application, analysis can be conducted without complicated processing.

Analysis of Standard Solution

The analytical conditions that were used are shown in Table 1, and the chromatogram obtained from analysis of a standard solution of histamine and tyramine (each at 10 mg/L) is shown in Fig. 1. The standard solution was prepared by dissolving these in pH 2.2 sodium citrate buffer solution. For the mobile phases, the mobile phase B and C of the Amino Acid Mobile Phase kits (Na type) were used, and analysis was conducted using gradient elution. Also, because the elution positions vary depending on the mobile phase pH, use of a carbon dioxide gas trap is suggested when conducting analysis.

Table 1 Analytical Conditions

Column	: Shim-pack Amino-Na (100 mm L. × 6.0 mm I.D.)	
Ammonia Trap	: Shim-pack ISC-30 / S0504Na (50 mm L. × 4.0 mm I.D.)	
Mobile Phase	: Amino Acid Mobile Phase Kits (Na type, Mobile Phase B and C)	
Time Program	: Time (min)	B. Conc. (%)
	0	80
	15.00	65
	15.01	0
	20.00	0
	20.01	80
	25.00	Stop
Flowrate	: 0.6 mL/min	
Column Temp.	: 60 °C	
Reagent	: Amino Acid Reagent Kits	
Flowrate of Reagent	: 0.2 mL/min	
Reaction Temp.	: 60 °C	
Detection	: RF-20Axs, Ex at 350 nm, Em at 450 nm	
Injection Vol.	: 10 µL	

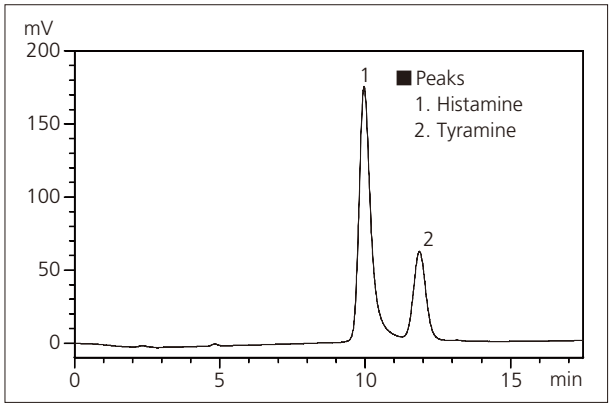

Fig. 1 Chromatogram of Histamine and Tyramine Standard Solution (Each 10 mg/L)

Linearity

Fig. 2 shows the linearity of histamine and tyramine using a concentration range from 0.1 mg/L to 100 mg/L. Excellent linearity was obtained with a coefficient of determination greater than R^2=0.9998 for both substances.

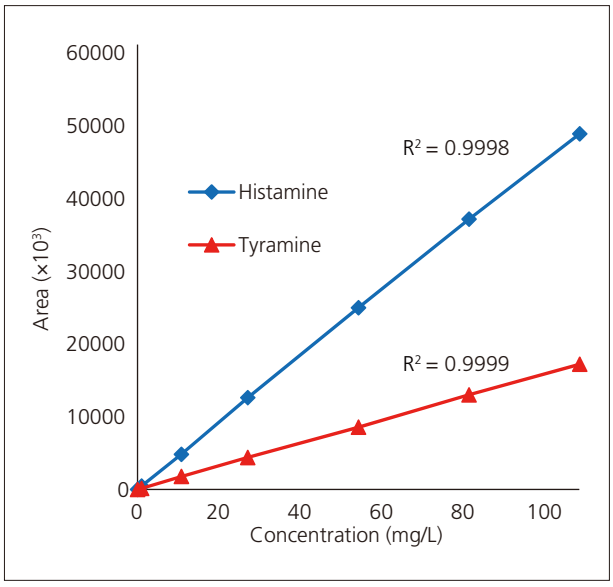

Fig. 2 Linearity of Histamine and Tyramine (0.1 – 100 mg/L)

Repeatability

Table 2 and 3 show the relative standard deviations (n=6) of retention time and peak area obtained in repeat analysis of a mixed histamine and tyramine standard solution (each 1 mg/L). Limits for histamine differ depending on the country, but in Codex, for example, the limit associated with decomposition in fish and fishery products is 100 mg/kg. Good repeatability has been obtained at a concentration of about 1/100 of the criterion.

Table 2 Repeatability of Peak Area and Retention Time of Histamine

	R.t (min)	Peak Area
1st	9.962	433,724
2nd	9.983	431,874
3rd	9.967	441,528
4th	9.962	429,887
5th	9.972	439,560
6th	9.993	434,818
Ave	9.973	435,232
%RSD	0.12	1.03

Table 3 Repeatability of Peak Area and Retention Time of Tyramine

	R.t (min)	Peak Area
1st	11.844	153,458
2nd	11.871	155,582
3rd	11.858	155,848
4th	11.855	154,509
5th	11.882	151,206
6th	11.911	153,960
Ave	11.87	154,094
%RSD	0.20	1.09

Analysis of Food

Figures 3 to 8 show examples of analysis of commercial fish sauce, wine, and soy sauce. Pretreatment consisted of preparing a 10-fold dilution using pH 2.2 sodium citrate buffer solution, and filtering through a 0.2 µm pore diameter membrane filter. As for the red wine and white wine, both were spiked with histamine and tyramine at 50 mg/L each.

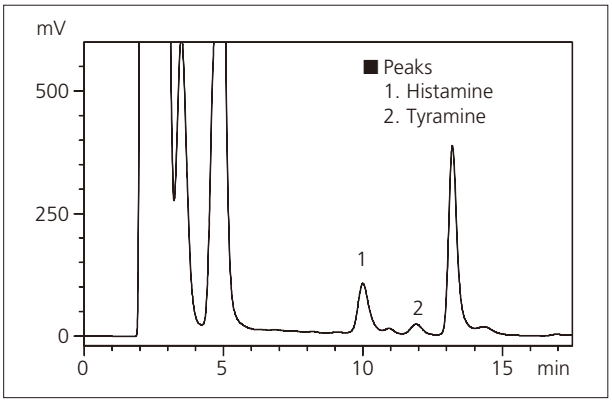

Fig. 3 Chromatogram of Fish Sauce A

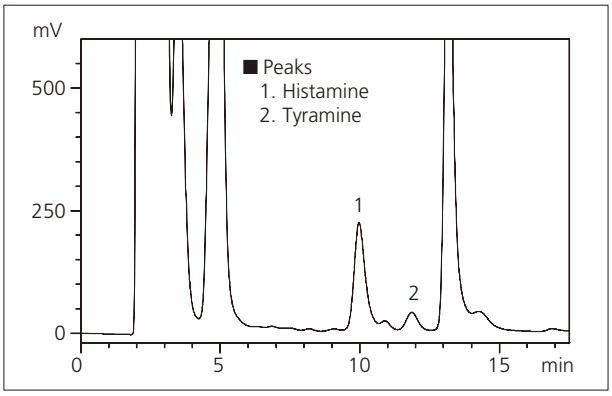

Fig. 4 Chromatogram of Fish Sauce B

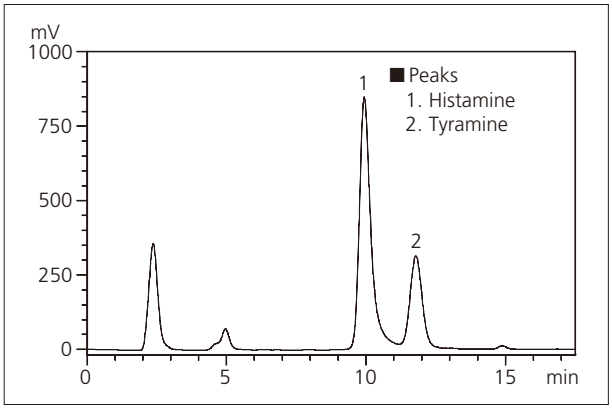

Fig. 5 Chromatogram of Red Wine

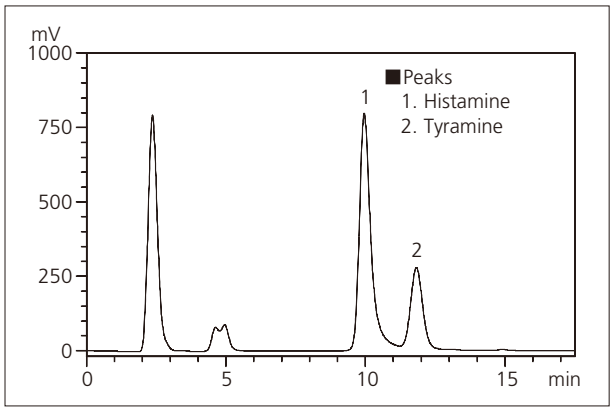

Fig. 6 Chromatogram of White Wine

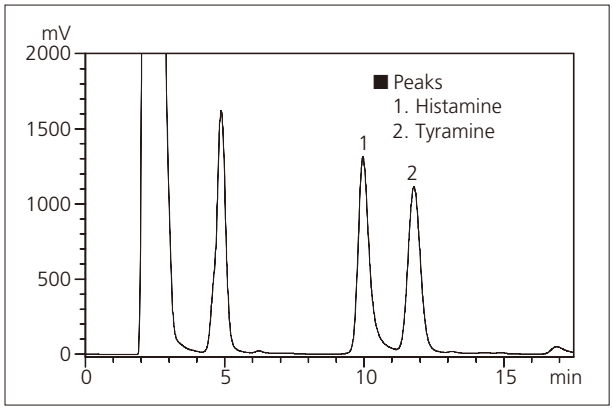

Fig. 7 Chromatogram of Soy Sauce A

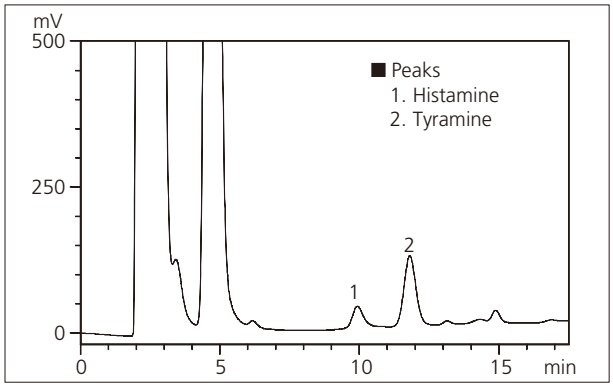

Fig. 8 Chromatogram of Soy Sauce B

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