Precolumn *o*-Phthalaldehyde Derivatization and Reversed-Phase Liquid Chromatography of *S*-Methylmethioninesulfonium in Satsuma Mandarin Juice

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Experiments were conducted to determine the amount of *S*-methylmethioninesulfonium (MMS) in Satsuma mandarin juice using a precolumn *o*-phthalaldehyde (OPA) derivatization and reversed-phase liquid chromatography. Favorable analytical conditions that allowed MMS analysis were achieved by using OPA/3-mercaptopropionic acid (MPA) as a derivatization agent with fluorescence detection (excitation at 330 nm, emission at 450 nm). AC18 reversed-phase column with 5 μm particle size and a length of 250 mm was used. Resolution of MMS/OPA/MPA derivative was accomplished with a linear gradient eluent (30 mM sodium acetate buffer solution, pH 7.3, and 70% (v/v) methanol). Quantitative analysis of MMS by the internal standard method using β-alanine gave highly reproducible result with a coefficient of variation less than 3%. Recovery of MMS added to juice samples was 105%. MMS content in Satsuma mandarin juice was 28.2 μM.

Key words: *S*-methylmethioninesulfonium, Satsuma mandarin, precolumn derivatization, *o*-phthalaldehyde, fluorescence detection, reversed-phase HPLC.

S-Methylmethioninesulfonium (MMS) is widely distributed in nature and has been reported as a constituent of Satsuma mandarin, tomato, sweet corn, green tea, milk, soybean, asparagus, and cabbage. MMS has considerable biological and medicinal interest. It is a precursor of dimethylsulfide (DMS), which is the characteristic component of the off-flavor produced by heating Satsuma mandarin juice (1, 2). Shimoda et al. reported on a method that determined MMS in Satsuma mandarin juice. Satsuma mandarin juice was injected directly into a GC injection port heated at 200℃. DMS produced by heat degradation of MMS in the injection port was quantitatively analyzed by using a flame photometric detector. MMS content was calculated from the DMS amount (3). Kovatcheva described a method to determine MMS in plant products using an amino acid analyzer (4). Ohtsuki et al. reported MMS in the extracts of various kinds of teas, such as green teas, black teas, and oolong teas, by the use of an amino acid analyzer (5, 6).

Recently, the determination of amino acids by reversed-phase liquid chromatography (RPLC) in combination with precolumn *o*-phthalaldehyde (OPA) derivatization and fluorescence detection has gained wide popularity for its sensitivity, speed, comparative simplicity, and lower cost (7, 8).

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Classically, amino acids have been separated by automatic amino acid analyzers that involve postcolumn derivatization. Both ion exchange and RP packing have been used in the analysis of amino acids. However, the RP mode now appears to be the method of choice. C18 columns are widely used on separation of amino acids.

Precolumn, off-line derivatization is often used in LC because it does not impose any restrictions on the chromatographic system in terms of the mobile phase composition, reaction temperature, duration, etc. However, postcolumn, on-line derivatization does impose restrictions on the mobile phase composition, reaction temperature, etc. Also, postcolumn, on-line derivatization requires a special reactor of small volume to avoid band broadening (9).

In this paper, we use the precolumn off-line derivatization method and C18 column RPLC method for the determination of MMS in Satsuma mandarin juice.

Experimental

Reagents

(a) *Sodium acetate trihydrate, sodium borate.*——Special grade (Wako Pure Chemical Industries Ltd. Osaka, Japan).

(b) *Methanol.*——LC grade (Wako).

(c) *OPA.*——Biochemical grade (Wako).

(d) *S-Methylmethioninesulfonium chloride (MMS-CL).*——Greater than 99% pure (Tokyo Kasei Kogyo Co. Ltd. Tokyo, Japan).

(e) *β-alanine (β-Ala), L-glutamine (Gln), and 3-mercaptopropionic acid (MPA).*——(Tokyo Kasei Kogyo).

(f) *L-Aspartic acid (Asp), L-glutamic acid (Glu), L-asparagine monohydrate (Asn), L-serine (Ser), glycine (Gly), L-threonine (Thr), and L-arginine monohydrochloride (Arg).*——Analytical grade (Takara Kohsan Co. Ltd. Tokyo, Japan).

All Satsuma mandarins were purchased from local markets.

Apparatus

(a) *Elgastat UHQ Purification System.*——Used in the preparation of buffer solutions and standard samples (Elga Ltd. Lane End, U.K.).

(b) *CN-type membrane filters.*——Used for sample preparation, 0.45 μm pore size (Advantec Toyo Corp., Tokyo, Japan).

(c) *LC system.*——Consisted of 2 Model LC-6AD LC pumps, a Model SCL-6B system controller for gradient programming, a Model SIL-6B automatic sample injector to add reagents or dilute samples, CTO-6A column oven with a preheater, and a Model DGU-1A degasser for degassing of mobile phases with helium (Shimadzu, Kyoto, Japan).

(d) *Spectrofluorometer.*——Model RF-535, used to routinely monitor fluorescence (Shimadzu). Measurements were made at an excitation wavelength of 330 nm and an emission wavelength of 450 nm, using a 12 μL flow cell and a xenon lamp. Chromatographic data were recorded and processed by a Model Chromatopac C-R4A system (Shimadzu).

(e) *Reversed-phase LC column.*——Inertsil ODS-2 250×4.6 mm, 5 μm (Gasukuro Kogyo Inc., Tokyo, Japan).

(f) *Mini-guard column.*——OS-type 10×4.6 mm, 5 μm (Gasukuro Kogyo Inc.).

Standard Preparation

Satsuma mandarins were reamed. Juice was passed through a 20 mesh sieve and

adjusted to pH 8.7. Juice was heated in an autoclave at 120℃ for 10 min to eliminate MMS. Aliquots of 0, 5, 10, 20, and 30 μL 2 mM MMS-Cl were added to separate 1 mL portions of MMS-free juice containing 10 μL 100 mM β-Ala (internal standard), respectively. Standard samples were centrifuged at 1000 × *g* for 20 min. Supernatants were passed through a membrane filter and analyzed.

Sample Preparation

Sample preparation was the same as standard preparation, excluding the MMS-free treatment and MMS-Cl addition. Prepared Juice containing MMS was analyzed with the OPA derivatization method.

Precolumn Derivatization of MMS with OPA

Preparation of OPA-MPA derivatization reagent.——A 50 mg portion of OPA was dissolved in 4.5 mL methanol, then 50 μL MPA and 0.5 mL 0.1 M sodium borate buffer (pH 9.5) were added. The reagent mixture was kept in the dark at 4℃. Fresh mixtures were prepared each week (8).

Derivatization procedure by automatic sample injector.——Methanol (0.2 mL), 0.2 mL 0.1 M sodium borate buffer (pH 9.5), and 50 μL OPA-MPA derivatization reagent were added to 50 μL MMS standard or 50 μL juice sample in 2 mL vials with PTFE (polytetrafluoroethylene) screw-caps. After thorough mixing and a reaction time of 1.7 min, 1 μL of the resulting solution was injected. All of these processes were automated using an automatic sample injector.

Chromatography and Quantitation

Chromatographic conditions.——Gradients were prepared by mixing 2 mobile phases, A and B. Mobile phase A was 30 mM sodium acetate, pH 7.3, with water. Mobile phase B was 70% methanol (v/v). Both mobile phases were degassed with helium sparking before use. Linear gradient was from 0% mobile phase B. Mobile phase B increased to 5% for 10 min, then increased from 5 to 15% for 50 min, and then from 15 to 25% for 10 min. An additional step to 100% B for 20 min was used to flush retained comoponents from column. Program was then returned to 100% A for 15 min to regenerate column. Flow-rate was 1.0 mL/min. Temperature of column was maintained at 40℃.

Quantitation.——Fluorescence detection was made at an excitation wavelength of 330 nm and an emission wavelength of 450nm. Fluorescence response was measured at sensitivity dial setting of High (H), response dial setting of Medium (M), and attenuation dial of 32 on the detector. Peak areas of MMS derivative and β-Ala derivative (internal standard) were obtained using the data processor, Chromatopac C-R4A. Linearity was plotted for the ratio of peak area (MMS derivative-β-Ala derivative) as a function of MMS concentration. Analytic concentration was quantitated by comparing peak area of MMS derivative with that of β-Ala derivative (internal standard).

MMS recovery experiments.——Aliquots of 5 or 10 μL 2 mM MMS-Cl were added to 1 mL orange juice containing 10 μL 100 mM β-Ala as internal standard, respectively. Duplicate samples were analyzed.

Results and Discussion

The primary purpose of this paper is the RPLC determination of MMS in Satsuma

mandarin juice by precolumn OPA derivatization and fluorescence detection. Derivatization of MMS and separation of the derivative can be accomplished efficiently using the described system. Satsuma mandarin juice contains a large amount of free amino acid. Figure 1 shows a typical chromatogram of MMS-OPA-MPA derivative and amino acid-OPA-MPA derivatives from the Satsuma mandarin juice. Peak intensities of amino acid-OPA-MPA derivatives were very strong compared with peak intensity of the MMS-OPA-MPA derivative. The pH of mobile phase A (30 mM sodium acetate buffer) must be carefully maintained, because the retention time of the MMS-OPA-MPA derivative is extremely pH sensitive when compared to its amino acid-OPA-MPA derivatives. Identification of a peak of the MMS-OPA-MPA derivative was confirmed by the disappearance of the peak on the alkaline heat-treated juice, and the appearance at the same position of a peak by addition of MMS-Cl to the MMS free juice. The regression equation obtained by a least square method was $Y=7.212\times10^{-4}X+3.511\times10^{-4}$, where Y is the peak area ratio, MMS derivative-β-Ala derivative (internal standard), and X is the MMS concentration in μM.

Linearity was good ($\gamma=0.9996$). MMS content of Satsuma mandarin juice was 28.2 μM. Coefficient of variation (CV) was 2.78% for 5 measurements. Recovery of MMS (added as MMS-Cl, 10 or 20 μM) from spiked Satsuma juice averaged 104.9% for 4 measurements. CV of recovery was 0.77%. Limit of detection for MMS-Cl aqueous solution was approximately 0.1 μM. MMS contents of additional 2 samples of Satsuma mandarin juice measured by the present method were 24.8 and 30.3 μM, respectively.

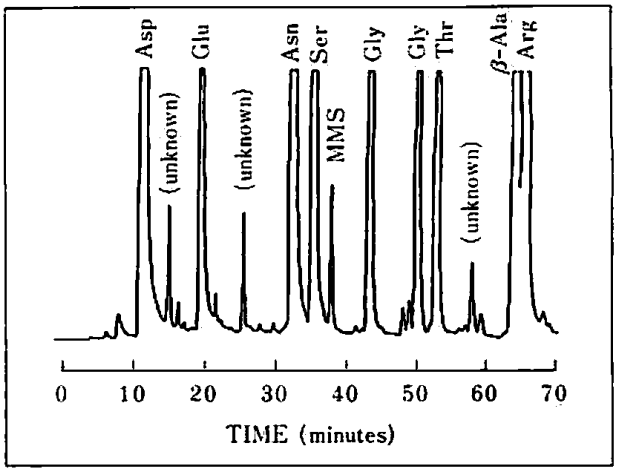

Figure.1. Typical chromatogram of MMS-OPA-MPA derivative and amino acid-OPA-MPA derivatives from the Satsuma mandarin juice. Abbreviations: Asp=L-aspartic acid; Glu-L-glutamic acid; Asn=L-asparagine; Ser=L-serine; MMS=S-methylmethloninesulfonium; Gly=glycine; Thr=L-threonine; β-Ala=β-alanine (internal standard); Arg=L-arginine.

The present results demonstrate that OPA-based MMS analysis can be used for Satsuma mandarin juice.

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