ISSN 1817-7077

FAO JECFA Monographs 14

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

77th meeting 2013

World Health Organization

Food and Agriculture Organization of the United Nations

ISSN 1817-7077

FAO JECFA Monographs 14

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

77th Meeting 2013

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2014

ISBN 978-92-5-108091-7 (print)
E-ISBN 978-92-5-108092-4 (PDF)

SPECIAL NOTE

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of, or connected with, their use.

LIST OF PARTICIPANTS

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES, 77th MEETING
Rome, 4 – 13 June, 2013

Members

Dr D. Benford, Food Standards Agency, London, United Kingdom (*Vice-Chairperson*)

Dr M. DiNovi, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, United States of America (USA)

Dr D. Folmer, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

Dr Y. Kawamura, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan

Dr Madduri Veerabhadra Rao, Department of the President's Affairs, Al Ain, United Arab Emirates

Mrs I. Meyland, Birkerød, Denmark (*Chairperson*)

Dr U. Mueller, Food Standards Australia New Zealand, Barton, ACT, Australia (*Joint Rapporteur*)

Dr J. Schlatter, Zurich, Switzerland

Dr P. Sinhaseni, Community Risk Analysis Research and Development Center, Bangkok, Thailand

Mrs H. Wallin, Helsinki, Finland (*Joint Rapporteur*)

Secretariat

Ms J. Baines, Food Standards Australia New Zealand, Canberra, ACT, Australia (*FAO Expert*)

Dr G. Brisco, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr S. Cahill, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Secretariat*)

Dr R. Cantrill, AOCS, Urbana, IL, USA (*FAO Expert*)

Dr V. Carolissen, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr S. Choudhuri, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Expert*)

Mr S.J. Crossley, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

Dr V. Fattori, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Secretariat*)

Dr M. Feeley, Food Directorate, Health Canada, Ottawa, Canada (*WHO Expert*)

Dr E. Furukawa, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Secretariat*)

Dr Y. Ito, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan (*FAO Expert*)

Dr F. Kayama, School of Medicine, Jichi Medical University, Tochigi, Japan (*WHO Expert*)

Mr J. Kim, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Secretariat*)

Professor S. Rath, Department of Analytical Chemistry, University of Campinas, Campinas, São Paulo, Brazil (*FAO Expert*)

Ms M. Sheffer, Ottawa, Canada (*WHO Editor*)

Dr J.R. Srinivasan, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*FAO Expert*)

Professor I. Stankovic, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia (*FAO Expert*)

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)

Dr T. Umemura, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (*WHO Expert*)

Dr G. Wolterink, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*WHO Expert*)

Dr H.J. Yoon, Hazardous Substances Analysis Division, Ministry of Food and Drug Safety, Seoul, Republic of Korea (*WHO Expert*)

INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 77th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome on 4 - 13 June 2013. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings (flavouring agent) specifications which has been updated and modernized. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 76th meeting, are available in the new format online searchable database at the JECFA website at FAO: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

An account of the purpose and function of specifications of identity and purity, the role of JECFA specifications in the Codex system, the link between specifications and methods of analysis, and the format of specifications, are set out in the Introduction to the Combined Compendium, which is available in shortened format online on the query page, which could be consulted for further information on the role of specifications in the risk assessment of additives.

Chemical and Technical Assessments (CTAs) for some of the food additives have been prepared as background documentation for the meeting. These documents are available online at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/technical-assessments/en/.

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/. Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org

TABLE OF CONTENTS

Table of contents	v
List of participants	vii
Introduction	ix
Specifications for certain food additives	1
Advantame	3
Aluminium silicate	11
Calcium aluminium silicate	15
Calcium silicate	19
Glucoamylase from *Trichoderma reesei* expressed in *Trichoderma reesei*	23
Glycerol ester of gum rosin	27
Glycerol ester of wood rosin	31
Mineral oil (medium viscosity)	37
Modified starches	39
Nisin	41
Octenyl succinic acid modified gum Arabic	45
Paprika extract	49
Phytase from *Aspergillus niger* expressed in *A. niger*	55
Potassium aluminium silicate	59
Potassium aluminium silicate–based pearlescent pigments, Type I	61
Potassium aluminium silicate–based pearlescent pigments, Type II	65
Potassium aluminium silicate–based pearlescent pigments, Type III	69
Silicon dioxide, amorphous	73
Sodium aluminosilicate	77
Analytical Methods	81
Determination of residual solvents in annatto extracts (solvent-extracted bixin and norbixin)	83
Phosphorous, calcium, magnesium and aluminium determination by Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES)	85
Measurement of minerals and metals by Inductively Coupled Plasma –Atomic Emission spectrophotometric (ICP-AES) Technique	86
Carbon number at 5% distillation point	89
Annex 1: Summary of recommendations from the 77th JECFA	91
Annex 2: Further information required or desired	94

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for eight food additives and these are presented in this publication:

Advantame (N, T)
Aluminium silicate (R, T)
Calcium aluminium silicate (R, T)
Calcium silicate (R, T)
Glucoamylase from *Trichoderma reesei* expressed in *Trichoderma reesei* (N)
Glycerol ester of gum rosin (R, T)
Glycerol ester of wood rosin (R)
Mineral oil (medium viscosity) (R)
Modified starches (R)
Nisin (R)
Octenyl succinic acid modified gum Arabic (R, T)
Paprika extract (R)
Phytase from *Aspergillus niger* expressed in *A. niger* (R)
Potassium aluminium silicate (R)
Potassium aluminium silicate–based pearlescent pigments, Type I (N)
Potassium aluminium silicate–based pearlescent pigments, Type II (N)
Potassium aluminium silicate–based pearlescent pigments, Type III (N)
Silicon dioxide, amorphous (R, T)
Sodium aluminosilicate (R, T)

In the specifications monographs that have been assigned a tentative status (T), there is information on the outstanding data and a timeline by which this information should be submitted to the FAO JECFA Secretariat.

The tentative specifications for glycerol ester of tail oil rosin (GETOR) (INS 445(ii)) were withdrawn since no data was submitted and the JECFA Secretariat was informed that the compound was no longer supported by the previous data sponsor

Editorial changes to specifications

The following specifications monographs were amended editorially and only the online edition of the Joint Compendium is revised:

Specifications monographs	***INS***	***Description of changes***
Monosodium L-glutamate	621	The test for pH should read *(1 in 20 soln)*
Sodium percarbonate		Method of assay: error in the formula corrected

ADVANTAME
(TENTATIVE)

New tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-5 mg/kg body weight was established at the 77th JECFA (2013).

Information required on:

- *Suitability of the head space GC method (using appropriate dissolution solvent) for determination of residual solvents published in the "Combined Compendium of Food Additives Specifications, Vol. 4" and data, in a minimum of 5 batches, using the method,*
- *An alternative/improved HPLC method for the assay of advantame and acid of advantame using a standard curve,*
- *Additional data and analytical methods for determination of palladium and platinum,*
- *Information on the purity and availability of the commercial reference standards used in the assay of advantame and acid of advantame*

SYNONYMS INS No. 969

DEFINITION Advantame is manufactured by *N*-alkylation of aspartic acid portion of aspartame (L-α-aspartyl-L-phenylalanine methylester) with 3-(3-hydroxy-4-methoxyphenyl) propionaldehyde produced by selective catalytic hydrogenation from 3-hydroxy-4-methoxycinnamaldehyde. The product is purified through re-crystallisation and dried.

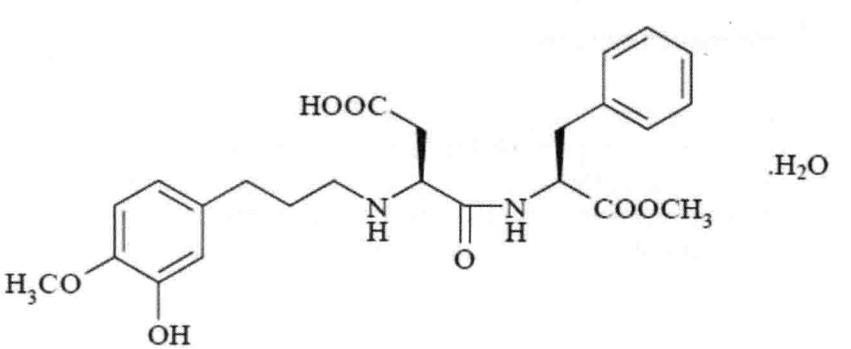
Only the following solvents may be used for the production: methanol and ethyl acetate.

Chemical names (3S)-3-[3-(3-hydroxy-4-methoxyphenyl)propylamino]-4-[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid hydrate, *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl]-L-phenylalanine 1-methyl ester, monohydrate

C.A.S. number 714229-20-6

Chemical formula $C_{24}H_{30}N_2O_7 \cdot H_2O$

Structural formula



Formula weight 476.52

Assay	Not less than 97.0% and not more than 102.0% on the anhydrous basis
DESCRIPTION	White to yellow powder
FUNCTIONAL USES	Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very slightly soluble in water, sparingly soluble in ethanol
Infrared spectrum	The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.

PURITY

Water (Vol. 4)	Not more than 5% (Karl Fischer)
Residue on ignition (Vol. 4)	Not more than 0.2% (use 5 g of the sample)
N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-asparty]-L-phenyl-alanine (acid of advantame)	Not more than 1% See description under TESTS
Other related substances	Not more than 1.5% (expressed as acid of advantame) See description under TESTS
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between -45° and -38°(0.2% solution in ethanol)
Residual solvents	Methanol: Not more than 500 mg/kg Ethyl acetate: Not more than 500 mg/kg See description under TESTS
Palladium	Information required
Platinum	Information required
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (acid of advantame)

Determination of *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine by HPLC (Tentative)

Mobile phase:
Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 100 ml of acetonitrile to 900 ml of this solution, mix well, and sonicate for about 5 min.
Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 600 ml of acetonitrile to 400 ml of this solution, mix well, and sonicate for about 5 min.

Preparation of Standard Solution: To prepare the standard stock solution of acid of advantame, accurately weigh about 100 mg of acid of advantame standard (ANS9801-acid Standard Reagent, available from Ajinomoto Co., Inc., Japan) and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml, in order to prepare acid of advantame standard solution, pipet 2 ml of standard stock solution and add a mixture of water and acetonitrile (7:3 v/v) to make an exact volume of 20 ml.

Preparation of Sample Solution: Accurately weigh about 100 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml.

HPLC conditions:

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm), GL Sciences, or equiv.
Column temperature:	50°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (9:1 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (2:3 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV detector at 210 nm
Run Time:	80 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	85	15
30.0	85	15
55.0	75	25
75.0	0	100
80.0	0	100
80.1	85	15
90.0	85	15

Calculate the content (%) of acid of advantame using the following formula:

Acid of advantame (%) = $[(W_S \times C_S)/W_T] \times [A_{T1}/A_S] \times [1/100]$

where
A_{T1} is peak area of acid of advantame from the sample solution;
A_s is peak area of acid of advantame from the standard solution;
W_T is weight (g) of advantame;
W_S is weight (g) of acid of advantame standard reagent; and
C_S is purity (%) of acid of advantame standard reagent.

Other related substances Calculate from the results of the Test for acid of advantame using the following formula:

Total content of other related substances (%) = $[(W_S \times C_S)/W_T] \times [A_{T2}/A_S] \times 1/100$

where
A_{T2} is total peak area other than advantame and other than acid of advantame from the sample solution. The peak areas below the quantitation limit (i.e., 0.02%) are not used in the calculation.
A_S is peak area of acid of advantame from the standard solution;
C_S is purity (%) of acid of advantame Standard Reagent;
W_T is weight (g) of advantame; and
W_S is weight (g) of acid of advantame Standard Reagent.

Residual solvents Determine by GC using the following conditions:
(Tentative)
Preparation of Sample Solution: Accurately weigh about 0.1 g of advantame, and add 1-butanol to make exactly 10 ml.

Preparation of Standard Solution: Accurately weigh 0.1 g methanol, and add 1-butanol to make exactly 50 ml (stock solution 1). Accurately weigh 0.1 g ethyl acetate, and add 1-butanol to make exactly 50 ml (stock solution 2). Pipet and transfer 2.5 ml each of stock solution 1 and stock solution 2 into a 50-ml volumetric flask, and add 1-butanol to make exactly 50 ml (mixture stock solution). Pipet and transfer 2 ml of mixture stock solution into a 20 ml volumetric flask, and add 1-butanol to make exactly 20 ml.

GC conditions:

Column:	DB-WAX (30 m x 0.53 mm i.d., 1.0 µm), J & W Scientific, or equiv.
Column temperature:	40° – 7 min - 80°/min - 220° – 5 min
Detector:	Flame-ionization detector
Carrier gas:	Helium
Carrier gas flow rate:	36 cm/sec (2.8 ml/min)
Injection volume:	1 µl
Detector temperature:	220°
Injector temperature:	120°

Calculation: Calculate the content (µg/g) of each residual solvent using the following formula:

Content of methanol or ethyl acetate (µg/g) = $[W_S \times C_S/W_T] \times [A_T/A_S] \times 10$
where
A_T is a peak area of methanol or ethyl acetate from the sample solution;

A_S is a peak area of methanol or ethyl acetate from the standard solution;
W_S is weight (mg) of methanol or ethyl acetate in the standard solution;
W_T is weight (mg) of advantame sampled;
C_S is purity (%) of methanol or ethyl acetate; and
10 is correction factor for dilution.

METHOD OF ASSAY (Tentative)

Determine by HPLC using the following conditions:

Mobile phase:
Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 250 ml of acetonitrile to 750 ml of this solution, mix well, and sonicate for about 5 min.

Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 500 ml of acetonitrile to 500 ml of this solution, mix well, and sonicate for about 5 min.

Preparation of Internal Standard: Accurately weigh about 40 mg of benzoic acid and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Preparation of Standard Solution: Accurately weigh about 40 mg of advantame reference standard (available from Ajinomoto Co., Inc., Japan), dissolve in a mixture of water and acetonitrile (7:3 v/v) to make 50 ml. Pipet 10 ml of this solution, add 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Preparation of Sample Solution: Accurately weigh about 40 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml. Pipet 10 ml of this solution, add 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

HPLC conditions:

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm) GL Sciences, or equiv.
Column temperature:	40°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (75:25 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (50:50 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV detector at 280 nm
Run Time:	55 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
20	100	0
50	0	100
55	0	100

Calculate the content (%) of advantame using the following formula:

Advantame (%) = $[W_S/W_T] \times [Q_T/Q_S] \times [(100 - W_{std} - S_{std})/(100 - W_{smp} - S_{smp})] \times 100$

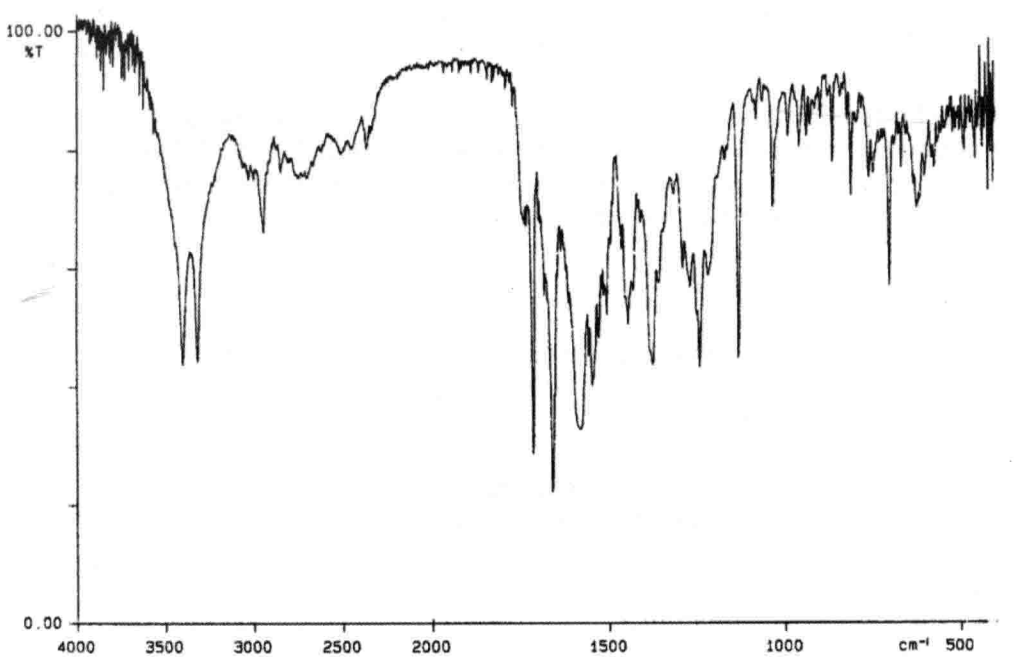
where

Q_T is ratio of the peak area of advantame to that of the internal standard from the sample solution;
Q_S is ratio of the peak area of advantame to that of the internal standard from the standard solution;
S_{std} is residual solvent content (%) of advantame reference standard;
S_{smp} is residual solvent content (%) of advantame sample;
W_S is weight (g) of advantame reference standard sampled;
W_T is weight (g) of advantame sample sampled;
W_{std} is water content (%) of advantame reference standard sample determined by Karl Fischer (Vol.4);
W_{smp} is water content (%) of advantame sample determined by Karl Fischer (Vol.4); and
100 is correction factor

See Appendix B for example of chromatogram obtained using the method.

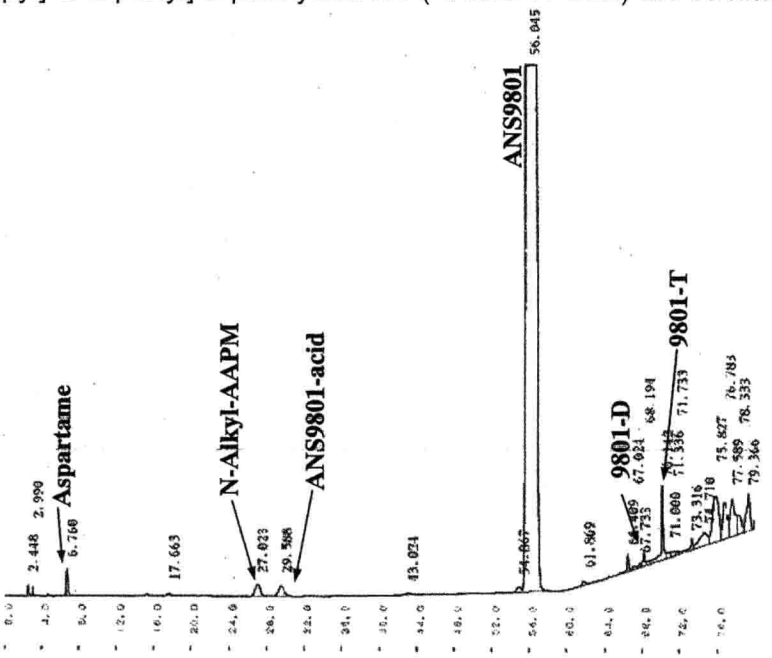
Appendix A

IR spectrum of advantame standard (Ajinomoto Co., Inc.)



Appendix B

Representative chromatogram for advantame (ANS9801) and N-[N-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid) at 210 nm.



Other identified compounds:

- L-α-aspartyl-L-phenylalanine methylester (Aspartame)
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid);
- *N*-[*N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-α-L-aspartyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (N-Alkyl-AAPM);
- N-[*N*-[3-(3-hydroxy-4-methoxyphenyl)pentyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-D); and
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)heptyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-T);