

cc: Laima Haticus

MEMORANDUM

DATE: September 11, 1986

TO: F. Kotsonis
M. Steffens✓

CC: J. Witt


FROM: P. Lai

SUBJECT: Aspartame in Carbonated Beverages

Attached is the report on the "Study of Aspartame in Carbonated Beverages." The report was submitted mainly for distribution to our major soft drink customers.

The report covers 52 weeks of the study and summarizes the conversion of aspartame under a number of pH and temperature conditions under simulated retail market conditions.

If you require any further information, please don't hesitate to contact me at extension 8190.



Peter Lai

Attachment
PL/llg
983-CDA-A

NUTRASWEET R&D
REPORT REVIEW AND RELEASE

DEPARTMENT: NUTRASWEET R&D ANALYTICAL

TITLE: STABILITY OF ASPARTAME IN BEVERAGES

DOCUMENT NUMBER: NSA-R86-002-A

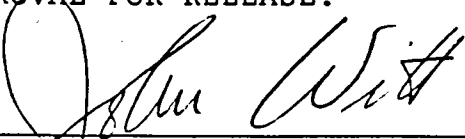
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Peter Lai

APPROVAL FOR RELEASE:


John Witt
Senior Director
NutraSweet R&D

5-13-86
DATE

NUTRASWEET RESEARCH AND DEVELOPMENT
SKOKIE, ILLINOIS

NUTRASWEET R&D ANALYTICAL REPORT

DEPARTMENT: NUTRASWEET R&D ANALYTICAL

TITLE: STABILITY OF ASPARTAME IN BEVERAGES

DOCUMENT NUMBER: NSA-R86-002-A

AUTHORS: Mia Igyarto, Peter Lai

CONTRIBUTORS: L. Lindquist, V. Mallari, E. Victorino

STABILITY OF ASPARTAME IN BEVERAGES

ABSTRACT

A study was conducted to monitor the stability of aspartame in simulated beverages in relation to the pH range(2.53 - 4.35), the temperature range (5 - 40°C) and effect of flavor.

This report describes the analytical methodologies used for monitoring the conversion products, the results of analytical data and the summary of mass balance data through 52 weeks of the study.

A complete mass balance has been achieved. The effects of pH and temperature of the medium were observed. The flavor did not affect the stability in the test range.

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STUDY OBJECTIVE

Aspartame (N-L- α -aspartyl-L-phenylalanine, 1-methyl ester) is a non-nutritive sweetener manufactured by The Nutrasweet Co. Currently aspartame (marketed as NutraSweet) is used as a tabletop sweetener, in a variety of food products, and in carbonated soft drinks. To determine what effect typical market beverage conditions have on the integrity of aspartame (α -APM), a stability study was initiated which was intended to simulate retail beverage market conditions covering a reasonable range of pH, temperature and time.

The primary goals of the study were to develop analytical methods with sufficient sensitivity to quantitate α -APM and its related conversion products when present at 0.1% of the initial APM concentration; achieve mass balance for the original α -APM through quantitative analysis of α -APM and its known conversion products; and to determine that there is no significant formation of any other compounds.

In addition, it was hoped that we could determine the α -APM chemical conversion pathway, and any trends regarding the kinetics of α -APM conversion.

This report summarizes the information generated through 52 weeks of the study.

SCOPE OF THE STUDY

The mock beverages were prepared, carbonated, and bottled in 10 ounce clear flint bottles. Samples were then stored in temperature controlled environmental cabinets at 5°, 20°, 30° and 40°C. The sampling points were chosen by use of the Arrhenius principle based on a pseudo first order kinetic conversion model.

Listed below are the concentrations of the various components used in the preparation of the simulated beverage samples.

Study No.	<u>8202</u>	<u>8203</u>	<u>8213</u>	<u>8214</u>	<u>8215</u>
pH	3.52*	2.55	3.19	3.48	4.37
Aspartame***	0.50**	0.50	0.50	0.50	0.50
80% Phosphoric Acid	--	0.83	0.83	--	--
Citric Acid	0.96	--	--	0.96	0.96
Sodium Citrate	0.28	--	0.46	0.28	1.20
Sodium Benzoate (Preservative)	0.20	--	0.20	0.20	0.20
Lemon-Lime Flavor	--	--	--	0.95	--

* - pH values listed are the pH values at time zero.

** - Concentration of each component given in units of grams/liter.

*** - The approximate initial concentration of α -APM.

SAMPLE ANALYSIS

The concentration of APM and its major conversion products was determined quantitatively as indicated in the following table.

<u>Compound</u>			<u>Assay</u> <u>By</u>
1.	α -APM	N-L- α -aspartyl-L-phenylalanine, 1-methyl ester	HPLC
2.	α -AP	N-L- α -aspartyl-L-phenylalanine	HPLC
3.	β -APM	N-L- β -aspartyl-L-phenylalanine, 1-methyl ester	HPLC
4.	β -AP	N-L- β -aspartyl-L-phenylalanine	HPLC
5.	L-Phe	L-phenylalanine	HPLC
6.	PM	L-phenylalanine methyl ester	HPLC
7.	DKP	Diketopiperazine of α -APM	HPLC
8.	PA	N-L-phenylalanyl-L-aspartic acid	HPLC
9.	L-Asp	L-aspartic acid	AAA
10.	MeOH	Methanol	GC

AAA = Amino Acid Analysis

GC = Gas Chromatography

HPLC = High Performance Liquid Chromatography

Thin Layer Chromatography was used as a back-up technique for the other methods.

These compounds were all available for use as standards and had been determined to be of sufficient purity for this study. (See Appendix 1 for appropriate structures.)

METHOD DEVELOPMENT ACTIVITIES

Due to the nature of the compounds of interest and the current state of analytical technology, it was apparent that High Performance Liquid Chromatography (HPLC) was the method of choice for the quantitation of most of the components. HPLC has been proven to be a highly sensitive technique (assuming appropriate detection response), and affords the necessary selectivity and flexibility to quantitatively monitor many compounds with similar structure and properties.

For the volatile compound, MeOH, gas chromatography (GC) was utilized; L-Asp was quantitated by amino acid analysis because of its lack of a chromophore in the UV range. Thin Layer Chromatography (TLC) was used as a back-up technique.

In order to achieve a satisfactory mass balance, a series of steps were taken to ensure that the methods have the capability to monitor each compound of interest with sufficient accuracy and sensitivity. Additionally, the method has to be rugged in order to minimize day-to-day variations between samplings.

A pilot study was conducted prior to the initiation of the stability study in order to determine that 100% mass balance (on a molar basis) was achievable, and that the presence of any components in the beverage matrices would not interfere with the accuracy of the assay.

The 4-week pilot study consisted of four sets of carbonated solutions. The samples included pH 2.5 phosphate buffer samples with and without aspartame, and pH 3.4 citrate buffer samples with lemon-lime flavor also with and without aspartame. The buffer solutions without APM were analyzed as blanks to ascertain whether there would be any detectable matrix interferences. The initial concentration of α -APM was 500 mcg/mL.

The samples were analyzed initially and then placed in 5° (for study control purposes) and 40°C environmental cabinets. Analysis was performed weekly for 4 weeks. Results of the pre-study verified the appropriateness of the methods in that mass balance (on a molar basis) was achieved in the samples and that no chromatographic interference was observed.

ANALYTICAL METHOD CHARACTERIZATION

The validation statistics for the quantitative methods are summarized below. The HPLC and GC validations were based on the analysis of twelve samples (2 analysts x 6 independent sample preparations and analyses). The validation of the amino acid analysis method was based on analysis of eighteen samples (3 analysts x 6 samples).

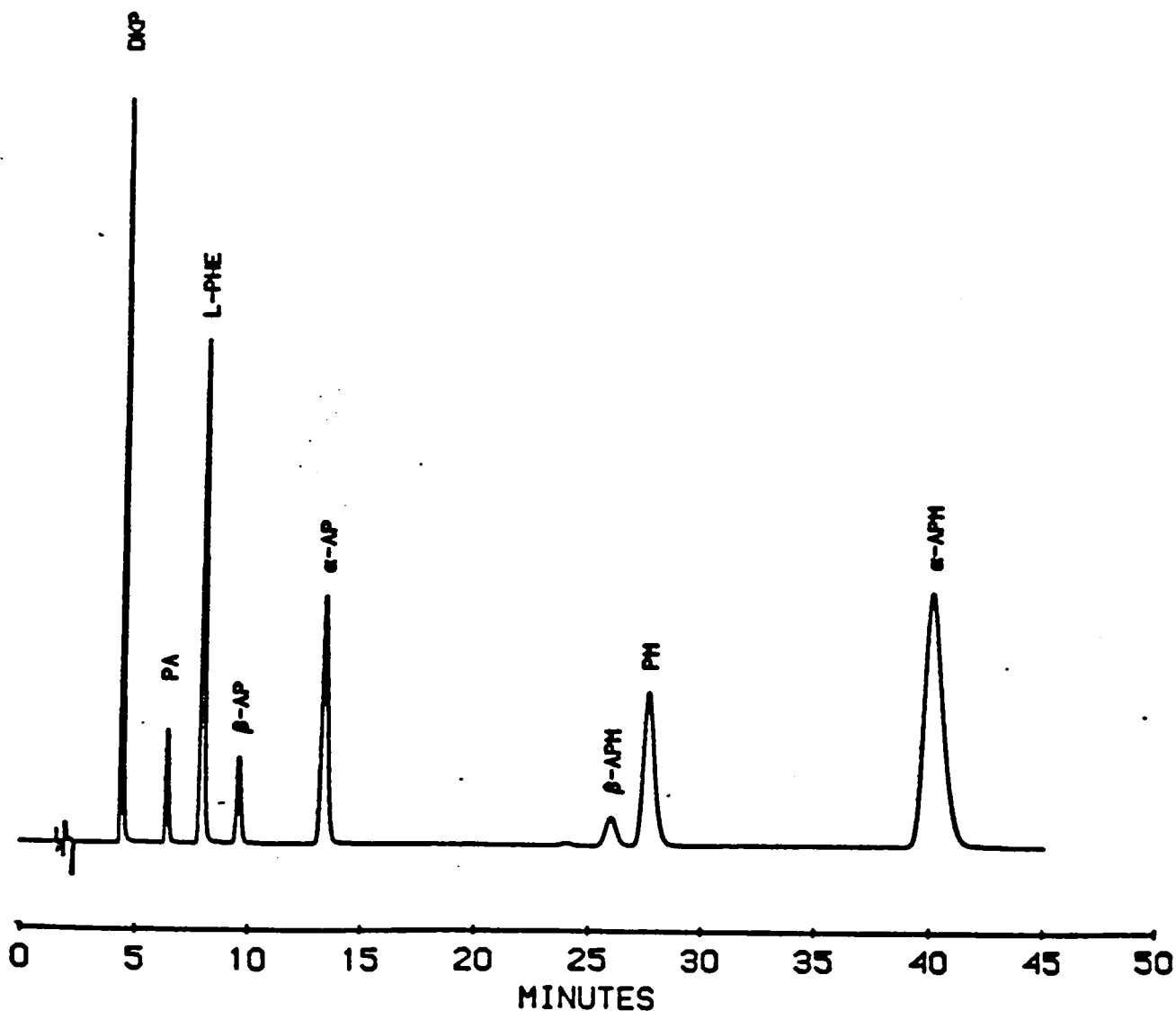
Assay	Compound	Recovery (Accuracy)	Standard Deviation (Precision)	MDQ**
HPLC	α-APM	100.5%	1.4%	0.4 mcg/mL
	β-APM	100.2%	1.9%	0.3 mcg/mL
	α-AP	100.2%	1.4%	0.2 mcg/mL
	β-AP	100.8%	1.2%	0.1 mcg/mL
	DKP	100.4%	1.5%	0.1 mcg/mL
	L-Phe	102.1%	2.4%	0.1 mcg/mL
	PM	101.3%	1.8%	0.3 mcg/mL
	PA	99.1%	0.9%	0.1 mcg/mL
GC	MeOH	104.2%	4.3%	0.8 ppm
AAA	L-Asp	99.0%	0.7%	0.04 mcg/mL

** MDQ = minimum detectable quantity. Estimate was based on the response calculation at two times the noise level.

The methods were shown to be specific in that there was no observed interference from the matrix or any of the other APM related compounds. The following is a chromatogram of the standard mix and a representative chromatogram of one of the initial samples.

Monitoring of the physical characteristics of the samples was also performed. All samples were checked for color, odor, particulation and sedimentation. Bottles were also checked visually. No perceptible changes were noted.

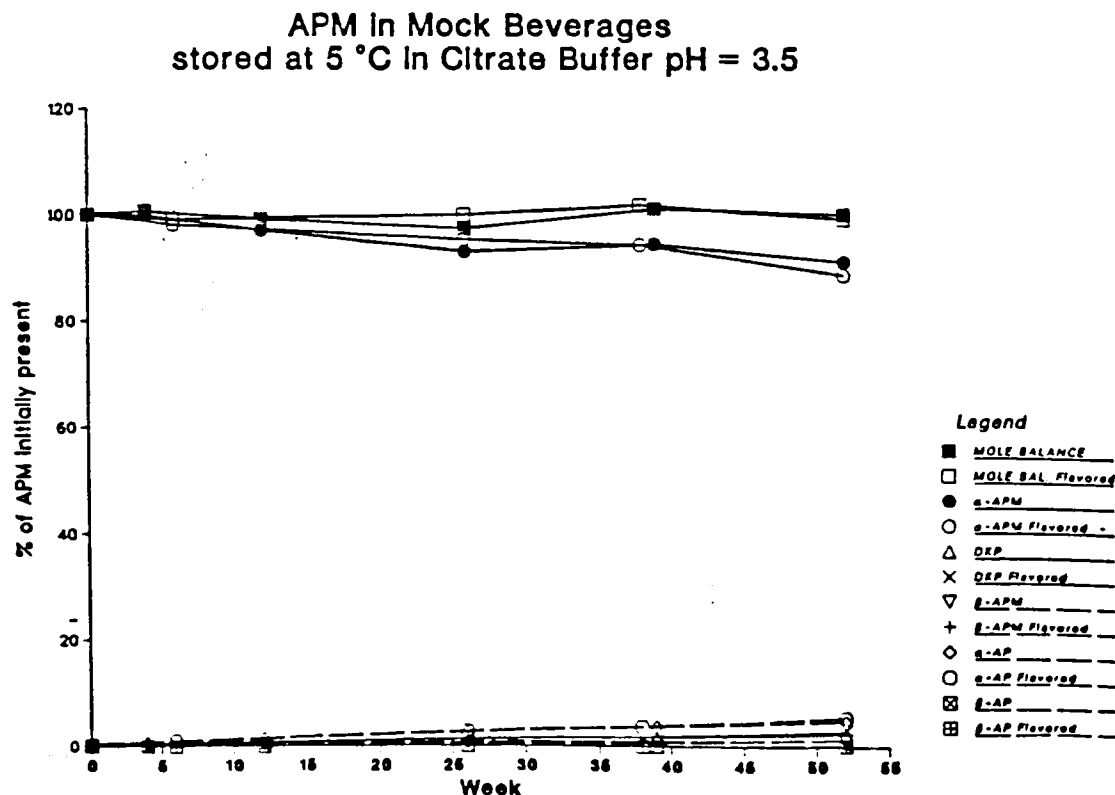
HPLC Chromatogram



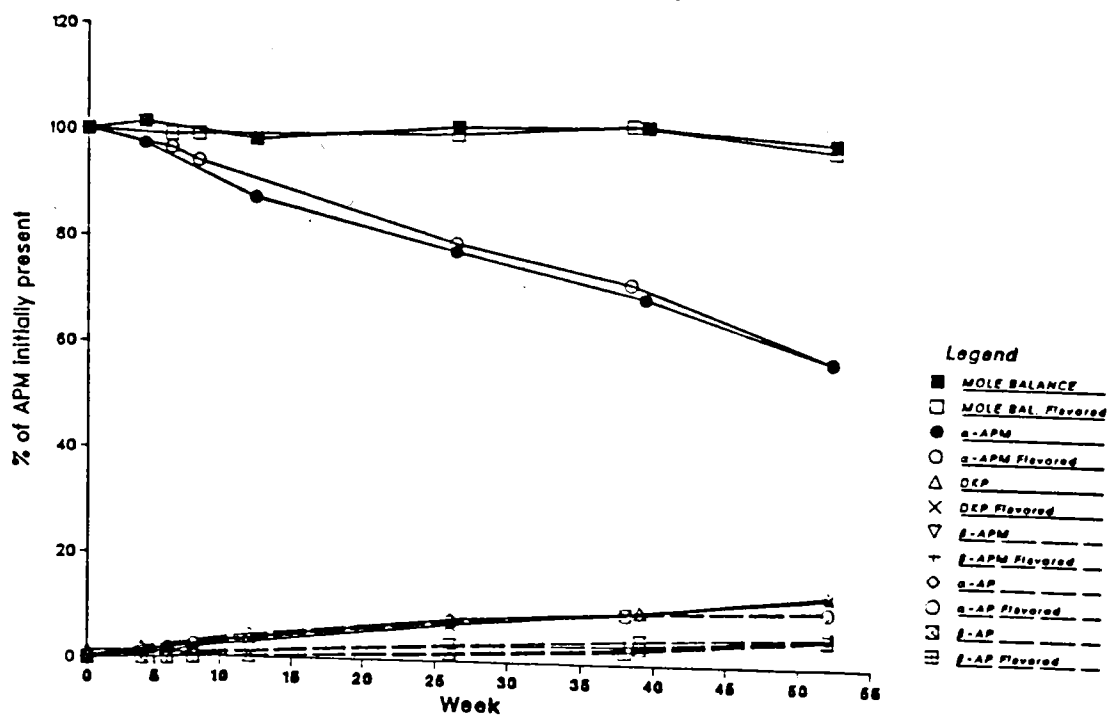
DATA PRESENTATION

At each time and temperature in each study, three bottles were sampled and two HPLC injections were made from each. Therefore six measurements were obtained. If a measurement was below detection limit, it was set to zero. In the modeling and graphs, the average of the six measurements was used. The amount of L-Phe-containing material is calculated on these averages.

These averages are listed in Appendix 2, first as concentrations (micromoles per mL) and then as a mole percentage of the initial α -APM. Representative graphs of the data from studies 8202 (pH 3.52) and 8214 (pH 3.48 with lemon-lime flavor) at 5° and 20°C are presented in the following pages. The calculation for micromoles was made using the measured concentration of each compound as (mcg/mL) and the respective molecular weights (see Appendix 3).



APM in Mock Beverages
stored at 20 °C in Citrate Buffer pH = 3.5



MOLE BALANCE ASSESSMENT

The analysis examines both the actual amount of L-Phe-containing material and this amount relative to the initial amount (mole balance closure). The amount of L-Phe-containing material is defined to be the sum (in units of micromoles/milliliter) of the following compounds: α -APM, α -AP, β -APM, β -AP, DKP, PM, L-Phe and PA.

The mole balance closure at a given time and temperature condition for a given study is defined as:

$$100 \times \frac{\text{amount of L-Phe-containing material}}{\text{initial amount of L-Phe-containing material}}$$

The mole balance closure is a traditional and well-understood measure of quantification of material, and it is useful because its reference point of 100% is independent of amounts or concentrations that may vary from study to study or laboratory to laboratory.

The mole balance closures ranged from 96 percent to 104 percent, over all five stability studies. The ranges and average closures for each study are as follows:

Study Number	Number of Time - Temperature Points	Mole Balance Closure (percent)		
		<u>Minimum</u>	<u>Average</u>	<u>Maximum</u>
8202	25	97.8	100.5	103.3
8203	26	97.9	100.8	102.2
8213	25	96.8	99.8	102.5
8214	24	96.6	100.0	104.4
8215	22	95.9	99.8	103.9

The following table lists the initial, minimum, average and maximum values of the amount of L-Phe-containing material, along with the coefficient of variation.

Study Number	Number of Time- Temperature Points*	Amount of L-Phe-containing material (umol)			
		<u>Minimum</u>	<u>Average</u>	<u>Maximum</u>	<u>Initial</u>
8202	24	1.680	1.728	1.776	1.724
8203	25	1.654	1.703	1.731	1.705
8213	24	1.681	1.734	1.780	1.747
8214	23	1.671	1.728	1.805	1.737
8215	21	1.674	1.745	1.818	1.754

* - Because the mole balance is reported for only post-initial conditions, while the L-Phe-containing material is reported for each sampling point, the numbers of time and temperature combinations are one greater for each stability study than those in the previous table.

CONCLUSIONS

In all cases, the mass balance calculated on the initial molar concentration of α -APM was acceptable (96-104% of the theoretical). Therefore, we are able to account for essentially all of the α -APM through the quantitation of the components we have indicated. This includes samples in which less than 10% of the initial α -APM is remaining.

Trends in the rate of α -APM conversion followed the expected pattern, in that the rate increased with an increase in temperature and a decrease in pH. This is limited to the range tested. It is important to note that studies 8202 and 8214 (pH ~3.5 with and without lemon-lime flavor) exhibited the same rate of conversion and the same pattern of conversion product formation. This indicates that flavor does not have any effect on the integrity of APM in these solutions.

The following chart indicates the conversion pathway by which α -APM ultimately reaches its constituent amino acids L-Aspartic Acid and L-Phenylalanine. The data generated by this stability study supports this pathway.

APPENDIX

APPENDIX 1 - Structures

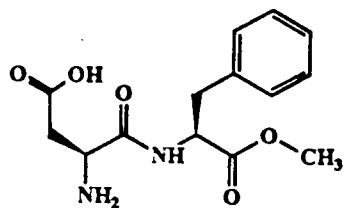
APPENDIX 2 - Table of mole percents and concentrations

APPENDIX 3 - Molecular weights

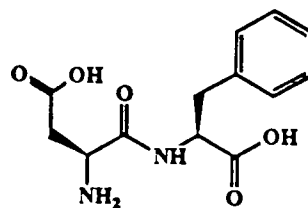
APPENDIX 4 - Conversion pathway

APPENDIX 1 - Structures

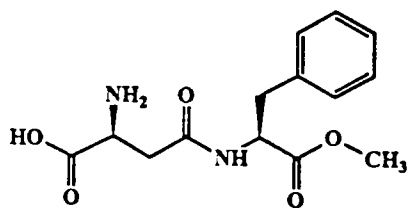
Structures



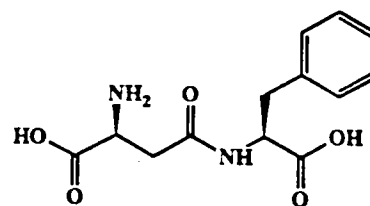
L,L-alpha-APM



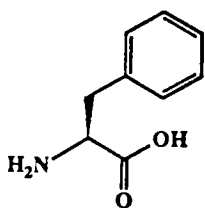
L,L-alpha-AP



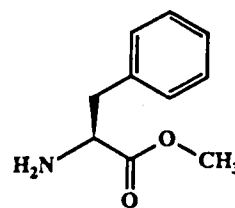
L,L-beta-APM



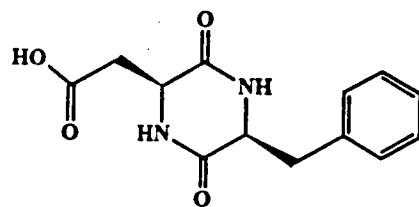
L,L-beta-AP



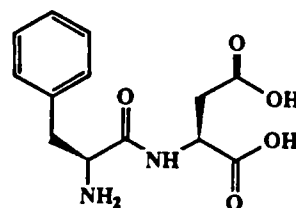
L-Phe



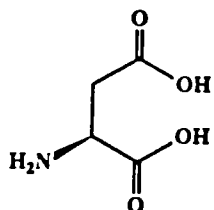
L-PM



L,L-DKP of APM



L,L-PA



L-ASP

CH₃OH

MeOH

APPENDIX 2 - Table of mole percents and concentrations

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : % of APM initially present

Study no.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8202	5	0	100.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.7
8202	5	4	99.8	0.0	0.7	0.0	0.4	0.0	0.0	0.0	0.0	
8202	5	12	97.1	0.3	1.4	0.0	0.7	0.0	0.0	0.0	0.1	2.0
8202	5	26	93.2	0.7	2.6	0.0	1.3	0.0	0.0	0.0	0.0	4.3
8202	5	39	94.6	1.0	3.8	0.1	1.8	1.0	0.3	0.0	0.4	5.7
8202	5	52	91.0	1.3	4.8	0.2	2.4	0.5	0.1	0.0	0.6	31.2
8202	20	4	97.3	0.6	1.9	0.0	1.4	0.4	0.0	0.0		
8202	20	12	87.0	1.4	4.4	0.3	3.8	1.2	0.2	0.0	1.5	8.0
8202	20	26	77.4	3.1	8.0	1.6	8.0	2.4	0.7	0.0	2.8	16.3
8202	20	39	69.1	4.6	9.8	3.2	10.0	3.5	1.6	0.1	9.8	22.3
8202	20	52	57.7	5.8	10.8	5.4	13.7	3.6	2.4	0.1	5.5	7.6
8202	30	2	95.7	0.8	2.2	0.1	2.3	1.0	0.1	0.0	0.9	6.6
8202	30	4	89.4	1.5	3.8	0.3	4.2	1.7	0.2	0.0		
8202	30	12	69.1	3.7	7.2	2.6	11.5	4.1	1.5	0.1	5.6	19.9
8202	30	16	62.2	5.4	8.0	4.4	14.4	4.9	2.4	0.1		
8202	30	26	44.7	6.7	8.0	8.6	20.1	5.8	5.2	0.3	9.7	36.6
8202	30	39	29.7	8.5	6.9	14.5	24.7	6.3	8.8	0.5	12.5	49.9
8202	30	52	19.0	7.9	5.5	18.2	28.9	5.3	12.5	0.8	16.6	62.2
8202	40	1	93.2	0.9	2.2	0.5	3.2	1.5	0.0	0.0	1.5	6.6
8202	40	2	85.0	1.7	3.7	0.6	6.1	2.8	0.4	0.0	3.0	
8202	40	4	72.5	3.6	5.5	2.1	11.3	5.1	1.4	0.1	5.1	17.2
8202	40	6	60.9	4.7	6.0	4.0	16.3	6.3	2.9	0.1	8.7	26.1
8202	40	8	51.3	6.0	6.0	6.1	19.3	7.1	4.4	0.2	9.3	33.3
8202	40	12	34.8	6.4	5.2	10.0	25.7	7.7	8.3	0.4	16.0	44.1
8202	40	16	26.2	8.9	4.5	13.3	29.8	8.0	11.9	0.7	19.1	50.7
8203	5	0	100.0	0.0	0.7	0.0	0.2	0.0	0.0	0.0	0.0	2.1
8203	5	4	98.1	0.0	2.3	0.0	0.7	0.0	0.0	0.0		
8203	5	12	94.8	0.4	4.9	0.0	1.4	0.6	0.0	0.0	0.6	5.2
8203	5	26	88.1	0.6	9.0	0.1	2.7	1.0	0.1	0.0	0.9	11.4
8203	5	39	82.0	0.9	12.3	0.3	3.5	1.8	0.4	0.0	1.3	15.0
8203	5	52	77.7	1.1	15.3	0.4	4.7	1.7	0.4	0.0	1.8	20.3
8203	20	4	91.2	0.7	5.9	0.1	2.8	1.4	0.1	0.0		
8203	20	12	72.2	2.1	13.9	0.9	8.1	3.8	0.9	0.1	4.4	21.2
8203	20	26	49.6	3.2	20.2	3.6	15.5	6.0	3.1	0.4	7.5	37.5
8203	20	39	35.2	3.6	21.8	6.8	18.8	7.0	5.2	0.8	10.0	48.5
8203	20	52	25.3	3.4	21.4	9.9	24.2	7.0	8.0	1.4	14.1	62.5
8203	30	2	85.9	0.9	6.5	0.2	4.2	2.7	0.3	0.0	2.6	12.9
8203	30	4	74.7	1.9	10.7	0.7	8.0	4.8	0.8	0.1		
8203	30	8	54.4	3.4	14.8	2.8	14.5	7.5	2.7	0.4	8.6	
8203	30	12	40.8	4.2	16.3	5.5	20.1	9.2	5.3	0.8	13.1	41.1
8203	30	16	29.0	4.1	15.5	8.2	23.5	9.7	7.6	1.2		

NOTE: A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : % of APM initially present

Study no.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8203	30	26	13.4	3.1	12.4	13.6	30.1	9.7	14.9	2.6	20.5	66.1
8203	30	39	4.5	1.8	8.0	18.3	31.4	7.9	21.4	4.6	25.4	75.3
8203	40	1	82.8	1.1	6.4	1.1	6.0	4.2	0.1	0.0	4.3	12.9
8203	40	2	66.0	2.4	9.7	1.2	11.0	7.6	1.5	0.2	7.7	
8203	40	4	44.3	4.2	12.1	4.0	19.0	11.7	4.7	0.7	13.3	36.4
8203	40	6	29.2	4.7	11.5	7.3	25.7	13.4	8.9	1.3	21.4	49.0
8203	40	8	18.6	4.4	9.8	10.2	28.3	13.8	12.8	2.1	21.0	56.2
8203	40	12	7.5	3.3	6.6	13.9	31.8	12.4	20.8	3.6	30.7	68.6
8203	40	16	3.3	2.0	4.7	15.4	32.4	10.4	27.4	5.0	37.4	76.1
8203	40	26	0.0	0.4	3.0	14.6	29.9	5.6	39.5	6.8	37.6	83.5
8213	5	0	100.0	0.0	-0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0
8213	5	6	96.6	0.1	1.4	0.0	0.7	0.0	0.0	0.0		
8213	5	26	93.0	0.7	4.0	0.1	1.9	0.5	0.1	0.0	0.5	6.1
8213	5	38	91.3	0.8	5.2	0.1	2.4	1.3	1.1	0.0	0.7	7.3
8213	5	52	84.8	1.9	7.5	0.5	4.0	1.3	0.2	0.0	1.0	10.1
8213	20	6	90.6	0.8	3.5	0.1	2.5	1.0	0.1	0.0		
8213	20	8	88.9	1.1	4.3	0.2	3.2	1.2	0.1	0.0		
8213	20	16	81.8	2.5	7.5	0.8	6.6	2.3	0.5	0.0		
8213	20	26	69.6	4.0	9.9	2.2	9.2	3.3	1.1	0.1	3.8	21.3
8213	20	38	59.4	5.1	11.9	4.1	13.1	4.8	2.8	0.2	5.4	29.3
8213	20	52	45.7	6.7	12.7	7.7	17.1	5.1	3.7	0.3	8.3	37.7
8213	30	2	91.1	0.9	3.3	0.1	3.2	1.5	0.1	0.0	1.4	6.1
8213	30	4	84.0	1.8	5.0	0.5	5.8	2.7	0.4	0.0		
8213	30	6	77.8	2.7	6.5	1.1	8.0	4.2	0.7	0.0		
8213	30	8	70.4	3.8	7.4	1.9	10.2	4.4	1.2	0.1	5.3	19.0
8213	30	16	51.7	6.3	9.4	5.9	18.5	6.6	3.9	0.2		
8213	30	26	31.8	7.6	8.5	11.8	23.7	7.4	7.4	0.6	12.6	50.5
8213	30	38	18.4	7.3	6.7	16.6	28.7	8.0	12.7	1.0	16.4	61.5
8213	30	52	8.4	6.6	4.5	20.9	31.3	5.8	17.6	1.7	23.0	71.8
8213	40	1	89.5	0.0	2.9	0.2	4.2	2.1	0.2	0.0	2.2	7.0
8213	40	2	80.0	2.2	4.8	0.8	7.8	4.1	0.6	0.0		
8213	40	4	63.5	4.4	6.6	2.9	14.4	6.9	2.2	0.1	8.1	22.5
8213	40	6	49.8	5.4	6.9	5.1	18.5	8.2	3.9	0.2	11.7	30.7
8213	40	12	23.9	7.2	5.2	11.9	28.0	9.7	10.6	0.8	20.0	51.6
8213	40	16	15.0	7.9	4.1	14.9	32.2	9.0	16.3	1.2	23.4	62.5
8214	5	0	100.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0	0.0	0.3
8214	5	6	98.1	0.0	1.0	0.0	0.5	0.0	0.0	0.0		
8214	5	26	95.5	0.5	2.8	0.0	1.4	0.3	0.1	0.0	0.4	4.0
8214	5	38	94.5	0.7	3.8	0.1	1.8	0.8	0.8	0.0	0.5	5.5
8214	5	52	88.5	1.4	5.5	0.3	3.0	0.8	0.1	0.0	0.7	7.6
8214	20	6	96.4	0.0	1.8	0.0	1.2	0.0	0.0	0.0		

NOTE: A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : % of APM initially present

Study no.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8214	20	8	93.9	0.6	2.4	0.1	1.8	0.6	0.0	0.0		
8214	20	26	78.9	2.8	7.5	1.2	6.9	2.1	0.6	0.0	2.3	15.0
8214	20	38	71.8	3.6	9.5	2.5	9.6	3.5	1.8	0.0	3.4	20.9
8214	20	52	57.9	5.5	10.5	5.2	13.1	3.6	2.3	0.1	5.4	28.6
8214	30	2	94.1	0.7	2.4	0.1	2.4	1.0	0.1	0.0	1.0	4.8
8214	30	4	88.5	1.4	3.8	0.4	4.5	1.8	0.2	0.0		
8214	30	6	83.2	2.0	5.1	0.7	6.4	2.4	0.5	0.0		
8214	30	8	77.0	2.8	5.9	1.3	7.9	3.1	0.7	0.0	3.5	14.9
8214	30	16	61.3	5.0	7.9	4.2	14.6	4.7	2.5	0.1		
8214	30	26	44.7	6.4	8.2	9.1	20.7	5.9	5.3	0.3	9.1	39.8
8214	30	38	30.5	7.6	7.0	13.7	25.1	6.6	9.2	0.5	13.0	51.6
8214	30	52	17.5	8.4	5.0	18.2	28.5	5.3	12.8	0.9	17.5	62.8
8214	40	1	97.1	0.0	2.3	0.1	3.3	1.5	0.1	0.0	1.4	6.5
8214	40	2	85.6	1.6	3.7	0.5	5.9	2.7	0.4	0.0		
8214	40	4	72.6	3.2	5.3	2.0	11.1	4.8	1.3	0.1	5.2	18.4
8214	40	6	60.0	4.5	5.9	3.7	15.1	6.0	2.6	0.1	8.2	25.1
8214	40	12	36.2	7.2	5.3	9.5	24.2	7.8	7.5	0.4	14.7	43.9
8214	40	16	25.4	8.0	4.4	12.4	28.5	7.5	11.7	0.6	18.8	56.6
8215	5	0	100.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	1.5
8215	5	6	100.3	0.0	0.6	0.0	0.4	0.0	0.0	0.0		
8215	5	26	97.2	0.2	1.9	0.0	1.1	0.0	0.0	0.0	0.1	3.0
8215	5	38	95.7	0.3	2.5	0.0	1.4	0.4	0.4	0.0	0.0	3.9
8215	5	52	92.7	0.4	3.7	0.0	2.3	0.0	0.0	0.0	0.1	4.9
8215	20	6	98.6	0.0	2.1	0.0	1.8	0.1	0.0	0.0	0.0	
8215	20	28	85.0	1.1	6.8	0.5	6.3	0.5	0.3	0.0	0.6	13.8
8215	20	38	79.5	1.4	8.7	1.0	8.3	0.7	0.6	0.0	1.0	16.3
8215	30	2	96.8	0.2	1.6	0.0	1.9	0.0	0.0	0.0	0.4	2.7
8215	30	4	91.5	0.0	2.8	0.1	3.4	0.0	0.1	0.0		
8215	30	6	89.8	0.7	4.0	0.2	5.1	0.5	0.2	0.0		
8215	30	8	84.3	0.9	4.8	0.4	6.2	0.7	0.3	0.0	0.9	11.0
8215	30	16	74.5	1.9	7.9	1.5	11.8	0.9	1.1	0.0		
8215	30	26	62.7	2.9	10.3	3.6	17.2	1.1	2.1	0.1	2.6	30.2
8215	30	38	50.6	3.1	11.4	6.5	21.9	1.5	3.9	0.1	3.8	40.5
8215	30	52	37.4	3.8	11.1	10.5	26.6	0.8	5.3	0.4	5.6	50.4
8215	40	1	99.6	0.0	1.6	0.0	2.6	0.1	0.0	0.0	0.6	4.6
8215	40	2	91.4	0.6	2.9	0.2	4.5	0.5	0.2	0.0		
8215	40	4	82.1	1.2	4.9	0.6	8.6	1.0	0.6	0.0	1.6	13.6
8215	40	6	75.8	1.6	6.5	1.3	12.6	1.1	1.2	0.0	2.3	18.0
8215	40	12	55.7	3.5	8.4	4.5	20.8	1.4	3.2	0.1	4.6	35.7
8215	40	16	45.3	4.6	8.6	7.1	26.8	1.2	5.4	0.2	5.4	45.2

NOTE: A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : micromoles per milliliter

Study No.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8202	5	0	1.719	0.000	0.003	0.000	0.002	0.000	0.000	0.000	0.000	0.012
8202	5	4	1.716	0.001	0.012	0.000	0.006	0.000	0.000	0.000	0.000	0.034
8202	5	12	1.669	0.006	0.024	0.000	0.013	0.000	0.000	0.000	0.001	0.073
8202	5	26	1.601	0.011	0.044	0.001	0.022	0.000	0.001	0.000	0.000	0.098
8202	5	39	1.626	0.018	0.065	0.002	0.030	0.016	0.004	0.000	0.008	0.536
8202	5	52	1.565	0.023	0.083	0.003	0.040	0.008	0.002	0.000	0.011	0.137
8202	20	4	1.672	0.010	0.032	0.000	0.024	0.006	0.000	0.000	0.026	0.280
8202	20	12	1.496	0.024	0.076	0.006	0.066	0.021	0.003	0.000	0.048	0.384
8202	20	26	1.330	0.054	0.138	0.027	0.138	0.042	0.013	0.000	0.094	0.130
8202	20	39	1.187	0.080	0.169	0.054	0.172	0.060	0.027	0.001	0.015	0.114
8202	20	52	0.991	0.100	0.186	0.092	0.236	0.062	0.041	0.002	0.097	0.343
8202	30	2	1.645	0.013	0.038	0.001	0.039	0.017	0.001	0.000	0.166	0.629
8202	30	4	1.537	0.027	0.065	0.006	0.072	0.029	0.004	0.000	0.215	0.857
8202	30	12	1.187	0.063	0.124	0.046	0.198	0.070	0.026	0.001	0.285	1.070
8202	30	16	1.070	0.093	0.138	0.075	0.247	0.085	0.041	0.002	0.025	0.114
8202	30	26	0.768	0.115	0.138	0.148	0.346	0.100	0.089	0.004	0.051	0.296
8202	30	39	0.510	0.147	0.119	0.250	0.425	0.108	0.151	0.009	0.088	0.449
8202	30	52	0.327	0.136	0.094	0.312	0.496	0.091	0.215	0.014	0.149	0.572
8202	40	1	1.602	0.015	0.037	0.009	0.055	0.026	0.001	0.000	0.275	0.759
8202	40	2	1.461	0.030	0.064	0.010	0.105	0.048	0.007	0.000	0.328	0.871
8202	40	4	1.246	0.062	0.094	0.035	0.194	0.088	0.025	0.001	0.000	0.035
8202	40	6	1.047	0.081	0.104	0.069	0.279	0.108	0.049	0.002	0.010	0.087
8202	40	8	0.882	0.103	0.104	0.104	0.332	0.122	0.076	0.004	0.015	0.193
8202	40	12	0.598	0.111	0.090	0.172	0.441	0.133	0.143	0.008	0.022	0.254
8202	40	16	0.450	0.154	0.077	0.228	0.512	0.138	0.205	0.012	0.030	0.343
8203	5	0	1.690	0.000	0.011	0.000	0.004	0.000	0.000	0.000	0.075	0.359
8203	5	4	1.658	0.000	0.038	0.000	0.012	0.000	0.000	0.000	0.127	0.634
8203	5	12	1.603	0.007	0.083	0.001	0.024	0.010	0.001	0.000	0.169	0.820
8203	5	26	1.489	0.010	0.152	0.002	0.046	0.017	0.002	0.000	0.238	1.056
8203	5	39	1.386	0.016	0.207	0.004	0.059	0.030	0.007	0.000	0.044	0.218
8203	5	52	1.313	0.019	0.259	0.007	0.079	0.028	0.007	0.001	0.145	0.695
8203	20	4	1.541	0.011	0.100	0.001	0.047	0.024	0.002	0.000	0.222	0.014
8203	20	12	1.220	0.035	0.235	0.016	0.136	0.065	0.015	0.002	0.075	0.359
8203	20	26	0.839	0.054	0.341	0.061	0.262	0.101	0.052	0.007	0.127	0.634
8203	20	39	0.595	0.060	0.369	0.114	0.318	0.118	0.088	0.014	0.169	0.820
8203	20	52	0.427	0.057	0.361	0.167	0.409	0.118	0.134	0.023	0.238	1.056
8203	30	2	1.452	0.015	0.110	0.003	0.070	0.045	0.004	0.000	0.044	0.218
8203	30	4	1.263	0.032	0.181	0.012	0.136	0.081	0.014	0.002	0.145	0.695
8203	30	8	0.919	0.058	0.251	0.047	0.245	0.127	0.046	0.006	0.222	0.014
8203	30	12	0.690	0.072	0.276	0.093	0.340	0.156	0.090	0.014	0.000	0.000
8203	30	16	0.489	0.070	0.262	0.139	0.397	0.164	0.129	0.020	0.000	0.000

NOTE : A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : micromoles per milliliter

Study No.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8203	30	26	0.227	0.053	0.210	0.231	0.508	0.164	0.252	0.045	0.347	1.117
8203	30	39	0.077	0.030	0.134	0.309	0.531	0.134	0.362	0.077	0.429	1.272
8203	40	1	1.400	0.019	0.108	0.018	0.102	0.071	0.002	0.001	0.072	0.218
8203	40	2	1.115	0.041	0.165	0.020	0.185	0.128	0.025	0.003	0.129	
8203	40	4	0.748	0.071	0.205	0.067	0.321	0.198	0.079	0.011	0.224	0.616
8203	40	6	0.494	0.080	0.194	0.124	0.434	0.226	0.150	0.022	0.362	0.828
8203	40	8	0.315	0.075	0.165	0.173	0.479	0.234	0.216	0.035	0.355	0.951
8203	40	12	0.126	0.055	0.112	0.235	0.537	0.210	0.352	0.062	0.519	1.159
8203	40	16	0.055	0.033	0.080	0.261	0.547	0.175	0.463	0.084	0.632	1.286
8203	40	26	0.000	0.008	0.051	0.247	0.506	0.094	0.667	0.115	0.635	1.412
8213	5	0	1.737	0.000	0.007	0.000	0.004	0.000	0.000	0.000	0.000	0.000
8213	5	6	1.677	0.002	0.024	0.000	0.012	0.000	0.000	0.000	0.008	0.105
8213	5	26	1.615	0.013	0.070	0.001	0.033	0.009	0.001	0.000	0.012	0.127
8213	5	38	1.585	0.015	0.091	0.002	0.043	0.022	0.019	0.000	0.017	0.176
8213	5	52	1.473	0.033	0.131	0.008	0.070	0.023	0.004	0.000		
8213	20	6	1.573	0.013	0.061	0.001	0.043	0.017	0.001	0.000		
8213	20	8	1.544	0.019	0.074	0.003	0.056	0.022	0.002	0.000		
8213	20	16	1.421	0.043	0.130	0.014	0.114	0.040	0.009	0.000		
8213	20	26	1.209	0.069	0.172	0.038	0.160	0.058	0.020	0.001	0.066	0.369
8213	20	38	1.031	0.089	0.207	0.072	0.227	0.083	0.049	0.003	0.094	0.509
8213	20	52	0.794	0.116	0.221	0.133	0.296	0.089	0.065	0.005	0.144	0.655
8213	30	2	1.582	0.015	0.057	0.002	0.056	0.026	0.002	0.000	0.024	0.107
8213	30	4	1.458	0.031	0.088	0.009	0.101	0.046	0.007	0.001		
8213	30	6	1.352	0.047	0.114	0.018	0.139	0.072	0.012	0.001		
8213	30	8	1.223	0.066	0.129	0.033	0.178	0.077	0.020	0.001	0.092	0.331
8213	30	16	0.897	0.110	0.163	0.102	0.321	0.115	0.068	0.004		
8213	30	26	0.553	0.131	0.147	0.204	0.411	0.129	0.129	0.010	0.219	0.878
8213	30	38	0.319	0.127	0.116	0.289	0.498	0.140	0.221	0.018	0.284	1.068
8213	30	52	0.146	0.114	0.078	0.363	0.543	0.101	0.306	0.030	0.400	1.246
8213	40	1	1.554	0.000	0.051	0.003	0.074	0.037	0.003	0.000	0.038	0.121
8213	40	2	1.389	0.038	0.084	0.014	0.135	0.071	0.011	0.001		
8213	40	4	1.102	0.077	0.115	0.050	0.250	0.120	0.037	0.002	0.140	0.391
8213	40	6	0.865	0.094	0.119	0.088	0.322	0.142	0.068	0.004	0.204	0.533
8213	40	12	0.416	0.124	0.090	0.207	0.487	0.168	0.184	0.014	0.347	0.896
8213	40	16	0.260	0.137	0.070	0.259	0.559	0.157	0.283	0.021	0.407	1.085
8214	5	0	1.729	0.000	0.005	0.000	0.003	0.000	0.000	0.000	0.000	0.005
8214	5	6	1.696	0.000	0.017	0.000	0.009	0.000	0.000	0.000		
8214	5	26	1.652	0.009	0.048	0.001	0.025	0.005	0.001	0.000	0.007	0.069
8214	5	38	1.634	0.012	0.066	0.001	0.032	0.015	0.014	0.000	0.009	0.096
8214	5	52	1.530	0.024	0.094	0.005	0.052	0.014	0.002	0.000	0.012	0.132
8214	20	6	1.666	0.000	0.031	0.000	0.021	0.001	0.000	0.000		

NOTE : A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

MOLE BALANCE REPORT FOR ASPARTAME AND ITS CONVERSION PRODUCTS IN BEVERAGES

Unit : micromoles per milliliter

Study No.	Temperature C	Time (week)	alpha-APM	beta-APM	alpha-AP	beta-AP	DKP	PM	L-Phe	PA	L-Asp	Methanol
8214	20	8	1.624	0.010	0.042	0.001	0.030	0.010	0.001	0.000		
8214	20	26	1.363	0.048	0.129	0.021	0.119	0.037	0.010	0.000	0.040	0.259
8214	20	38	1.241	0.063	0.164	0.044	0.166	0.060	0.031	0.000	0.059	0.362
8214	20	52	1.001	0.096	0.181	0.090	0.226	0.063	0.039	0.001	0.093	0.494
8214	30	2	1.627	0.011	0.041	0.001	0.042	0.017	0.001	0.000	0.018	0.082
8214	30	4	1.530	0.024	0.066	0.006	0.077	0.032	0.004	0.000		
8214	30	6	1.438	0.034	0.089	0.013	0.111	0.042	0.008	0.000		
8214	30	8	1.332	0.049	0.101	0.023	0.137	0.053	0.013	0.000	0.060	0.258
8214	30	16	1.060	0.086	0.137	0.072	0.253	0.081	0.044	0.002		
8214	30	26	0.772	0.110	0.142	0.157	0.358	0.102	0.091	0.005	0.158	0.689
8214	30	38	0.528	0.132	0.122	0.237	0.434	0.113	0.159	0.009	0.224	0.892
8214	30	52	0.303	0.145	0.087	0.315	0.492	0.092	0.221	0.016	0.302	1.085
8214	40	1	1.679	0.000	0.039	0.002	0.058	0.025	0.002	0.000	0.025	0.113
8214	40	2	1.480	0.027	0.064	0.009	0.102	0.047	0.007	0.000		
8214	40	4	1.256	0.056	0.092	0.034	0.193	0.082	0.022	0.001	0.090	0.319
8214	40	6	1.038	0.078	0.102	0.064	0.260	0.103	0.045	0.002	0.142	0.434
8214	40	12	0.625	0.124	0.091	0.165	0.418	0.135	0.129	0.007	0.255	0.759
8214	40	16	0.439	0.138	0.076	0.214	0.493	0.130	0.202	0.011	0.325	0.978
8215	5	0	1.749	0.000	0.003	0.000	0.003	0.000	0.000	0.000	0.000	0.026
8215	5	6	1.754	0.000	0.011	0.000	0.007	0.000	0.000	0.000	0.001	0.052
8215	5	26	1.699	0.003	0.033	0.000	0.020	0.000	0.000	0.000	0.000	0.068
8215	5	38	1.673	0.004	0.044	0.000	0.025	0.007	0.007	0.000	0.003	0.086
8215	5	52	1.621	0.007	0.065	0.000	0.039	0.000	0.000	0.000	0.000	
8215	20	6	1.724	0.000	0.037	0.000	0.032	0.001	0.001	0.000	0.011	0.242
8215	20	28	1.487	0.020	0.119	0.009	0.110	0.008	0.005	0.000	0.018	0.285
8215	20	38	1.390	0.025	0.152	0.017	0.145	0.012	0.010	0.000	0.008	0.047
8215	30	2	1.693	0.004	0.029	0.000	0.032	0.000	0.001	0.000		
8215	30	4	1.601	0.001	0.049	0.002	0.060	0.001	0.002	0.000		
8215	30	6	1.570	0.012	0.070	0.004	0.089	0.008	0.003	0.000		
8215	30	8	1.475	0.016	0.083	0.007	0.109	0.012	0.005	0.000	0.015	0.192
8215	30	16	1.303	0.034	0.139	0.026	0.207	0.016	0.019	0.000		
8215	30	26	1.096	0.050	0.180	0.064	0.302	0.020	0.037	0.001	0.045	0.529
8215	30	38	0.885	0.055	0.200	0.114	0.383	0.026	0.068	0.002	0.067	0.708
8215	30	52	0.654	0.066	0.194	0.183	0.465	0.013	0.093	0.006	0.097	0.882
8215	40	1	1.742	0.000	0.029	0.000	0.045	0.001	0.001	0.000	0.010	0.080
8215	40	2	1.598	0.011	0.051	0.003	0.079	0.009	0.003	0.000		
8215	40	4	1.435	0.021	0.085	0.011	0.150	0.017	0.010	0.000	0.028	0.239
8215	40	6	1.325	0.029	0.114	0.023	0.220	0.019	0.021	0.001	0.040	0.316
8215	40	12	0.974	0.060	0.146	0.078	0.364	0.024	0.056	0.002	0.081	0.624
8215	40	16	0.792	0.081	0.150	0.124	0.468	0.022	0.095	0.003	0.095	0.791

NOTE : A ZERO REPRESENTS A VALUE BELOW DETECTION LIMIT

APPENDIX 3 - Molecular weights .

Molecular Weights

Compound	Molecular Weight
α -APM	294.3
α -AP	280.3
β -APM	294.3
β -AP	280.3
L-Phe	165.2
PM	179.2
DKP	262.3
PA	280.3
L-Asp	133
MeOH	32

APPENDIX 4 - Conversion pathway

