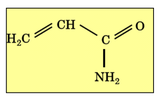
☐ What is acrylamide

Extract from National Cancer Institute website
http://www.cancer.gov/cancertopics/factsheet/Risk/acrylamide-in-food

What is acrylamide - I

Q.1 What is acrylamide

A.1 Acrylamide is a chemical used primarily for industrial purposes.



Acrylamide
(CAS 79-06-1)
M.W.:71

Q.2 Is there acrylamide in food?

A.2 Reseachers have found acrylamide in certain foods that were heated to a temperature above 120 degree Celsius.

Acrylamide concentration in foods(example)

Foods	Acrylamide conc. (ng/g)
Potato chips	117~3,770
French fries	59~5,200
Potato(Raw)	<10~50
Confectionaly	18~3,324
Cereal	11~1,057
Instant coffee	195~4,948
Toasted tea, oolong	<9~567
Roasted barley	140~578
Bottled black olive	123~1,925

Codex Alimentarius commission (2006)

What is acrylamide - II

Q.3 How does cooking produce acrylamide?

A.3 When heated to high temperature in the presence of a kind of amino acid and certain sugars can form acrylamide. High-temperature cooking methods, such as frying, baking, or broiling, have been found to produce acrylamide.

Q.4 What are health effects of acrylamide?

A.4 High levels of acrylamide in the workplace have been shown to cause neurogical damage. And studies in rodent models have found that acrylamide exposure poses a risk for several types of cancer.

The guideline to prevent and reduce formation of acrylamide in food is enacted.

Necessity of reducing acrylamide in foods

CODEX prepared "CODE OF PRACTICE FOR THE REDUCTION OF ACRYLAMIDE IN FOODS" CAC/RCP 67-2009
In Japan, Ministry of agriculture, Forestry and fisheries enforces to follow the reduction of acrylamide in food

The guidance covers three strategies for reducing acrylamide formation in particular products:
i) Raw materials;
ii) Control / addition of other ingredients; and
iii) Food processing and heating.

The measurement of acrylamide in food is necessary. Because have to compare the data which was measured before doing the reduction method and after it. Then have to evaluate the method whether useful.

Developing of new acrylamide measurement method

Generally, LC/MS, LC/MS/MS, GC/MS are used for analysis of acrylamide
But these conventional analyses are high cost (especially initial cost) and need skilled person. These methods are difficult to analysis many samples at once.

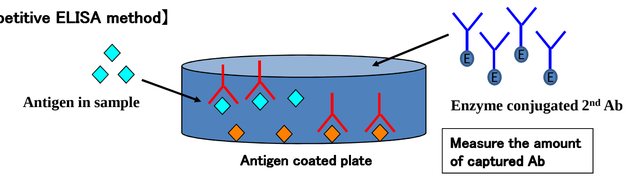
We developed the new method which is faster, easier, lower cost and measures many sample.

The strategy of acrylamide measurement

Since an antibody to acrylamide cannot be raised in animals by immunization simply using acrylamide, an acrylamide derivative (3-CTBA; 3-[(2-carbamoylethyl)thio] benzoic acid) was employed as a hapten.

3-CTBA is too small to establish the sanwich assay. So, we select Competitive ELISA method

【Competitive ELISA method】



Summary of measurement methods

① Extract Acrylamide is extracted from the test food sample into water.
Apply the extract to a solid-phase extraction cartridge
The time required:2 hours

② Derivatization reaction
3-MBA (3-Mercaptobenzoic Acid) add to "acrylamide standards" and "test food sample extracts" In this step, acrylamide is quantitatively converted to 3-CTBA by reacting with a large excess of 3-MBA
The time required:2 hours

③ Enzyme Immunoassay: EIA
Apply the "derivatized samples" to EIA
The time required:2 hours

measurement methods - 1

Extraction)

Homogenize the test food sample by a method appropriate to the nature of the food.

three consecutive off-and-on cycles of extraction at 30 sec intervals using a MILLCER (Type IFM-800DG, Iwatani Co. Ltd., Tokyo, Japan), a safety food processor like a Waring blender, is also applicable

Centrifuge the tube containing the homogenate at 3,000×g (preferably 12,000×g) for 20 min at 20-30ºC and retain the supernatant.

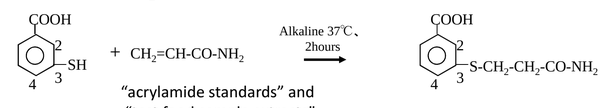
To the conditioned cartridge, apply a 1.0 mL aliquot of the supernatant in then elute with 3.0 mL distilled water.

Extracted by MILLCER

measurement methods - 2

Derivatization

【*Derivatization reaction*】



3-MBA (3-Mercaptobenzoic Acid) add to "acrylamide standards" and "test food sample extracts"

37℃, 2hours

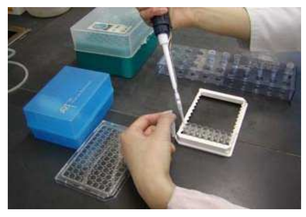
After the incubation, add "Sample Dilution Buffer" to each tubes

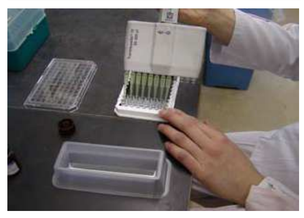
measurement methods - 3

EIA

↓Dispensing "derivatized samples (50μL/well)
↓Dispensing "Rabbit Anti-3-CTBA Antibody" (50μL/well)
(Standing for 1 h at 20-30°C)
↓ **wash** 6 times
↓ **Dispensing Enzyme-conjugated Goat Anti-rabbit IgG Antibody.**(100μL/well)
(**Standing for 30 min at 20-30°C**)
↓ **Wash**x6 times
↓ **Dispensing Enzyme Substrate** (100μL/well)
(**Standing for 30 min at 20-30°C**)
↓ **Dispensing Stopping Solution**(100μL/well)
↓ **Measurement of absorbance**
450 nm (the primary wavelength) and 610-650 nm (the secondary wavelength)

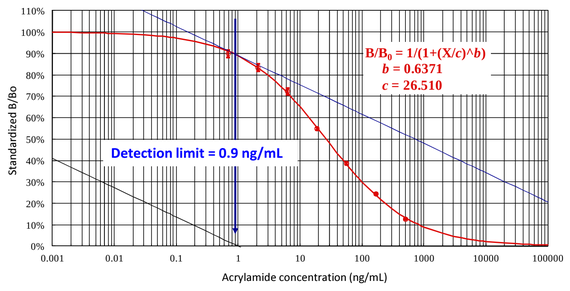
Calculation of the acrylamide concentration





Calibration curve and Detection limit

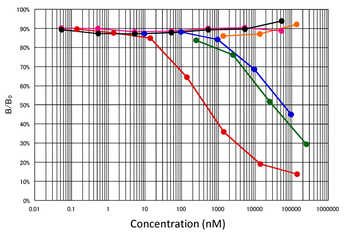
Calibration curve and Detection limit (the 12 times average)



$$B/B_0 = \frac{\text{Absorbance for "acrylamide standards (0.69-500 ng/mL)" or "test food sample extracts"}}{\text{Absorbance for null concentration of "acrylamide standard (0 ng/mL)"}}$$

Cross reactivity

Cross reactivity of Acrylamide's structural analogue

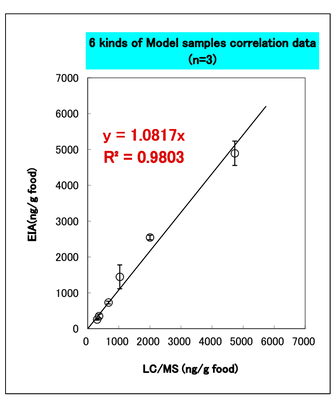


CH_2=CH-CO-NH_2	Acrylamide
CH_2=C(CH_3)-CO-NH_2	Metacrylamide
CH_2=CH-CN	Acrylonitrile
CH_2=CH-COOH	Acrylic acid
HOOC-CH=CH-COOH	Maleic acid
HOOC-CH=CH-CO-NH_2	Maleamic acid

Cross reactivity (Acrylamide : 100%)

Structual analog	IC_{50} (nM)	$1/IC_{50}$ (nM)	Cross reactivity
Acrylamide	430	0.00233	100%
Metacrylamide	60000	0.00002	0.72%
Acrylonitrile	30000	0.00003	1.43%
Acrylic acid	>1000000	<0.000001	<0.04%
Maleic acid	>1000000	<0.000001	<0.04%
Maleamicacid	>1000000	<0.000001	<0.04%

Correlation with LC/MS method -1

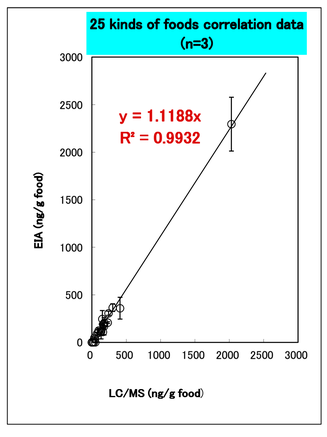


Model Sample	LC/MS (ng/g food)	EIA Mean±SD (ng/g food)
①	4,733	4,894 ± 340
②	2,002	2,546 ± 63
③	1,031	1,446 ± 333
④	667	729 ± 38
⑤	364	351 ± 55
⑥	303	256 ± 15

(※LC/MS accuracy±5%)

Model sample ①~⑥ were prepared by CALBEE Inc.

Correlation with LC/MS method -2



Foods	Main ingreadient	Acrylamide concentration (ng/g foods) LC/MS	EIA
Biscuit A	Wheat	182	206 ± 30
Biscuit B	Wheat	171	186 ± 41
Biscuit C	Wheat	N.D.	N.D.
Biscuit D	Wheat	164	192 ± 15
Biscuit E	Wheat	75	92 ± 15
Cracker F	Wheat	34	47 ± 7
Cracker G	Wheat	410	359 ± 115
Sticks of dough	Wheat	243	306 ± 21
Bread	Wheat	49	N.D.
Doughnut	Wheat	28	N.D.
Corn snack	Corn	93	103 ± 28
Cornflakes	Corn	19	N.D.
Fried rice cracker	Rice	99	125 ± 25
Mashed potatoes	Potato	43	33 ± 9
Potato snack	Potato	2031	2296 ± 283
French fries	Potato	230	208 ± 9
sweetened sweet potato fries	Sweet potato	162	110 ± 30
Unrefined sugar	Sugar cane	151	246 ± 89
cocoa	cacao	178	169 ± 92
Black chocolate	cacao	134	115 ± 78
Broiled Tofu	Soybean	<6	N.D.
Fried Tofu	Soybean	<6	N.D.
Toasted tea leaf	Tea leaf	303	365 ± 40
Black tea leaf	Tea leaf	<6	N.D.
Green tea leaf	Tea leaf	<6	N.D.

N.D.: not detected

Advantage of EIA method

	ELISA	Conventional method
Initial cost	Inexpensive(Plate reader)	Expensive(GC/MS, LC/MS, LC/MS/MS)
Running/maintenance cost	Easy and low cost	Expensive・skilled worker
Measurment	Easy	Complicated
Analyze time	Short	Long
Sensitivity	ng/mL(μg/kg)	ng/mL(μg/kg)
Accuracy	±20%	±5%
Notes	Analyse by myself	Analysis contract service
Analysys cost	3,000JPY/Sample	30,000JPY/Sample
Total analysis time	6hours	One week

Conclusion-1

I. we established a competitive enzyme immunoassay.
II. The limit of detection of this immunoassay was 0.9ng/mL.
III. The measuring range were 3ng/mL to 200ng/mL.
IV. The cross reactivity of structural analogues were that acrylonitrile was 1.4%, methacrylamide was 0.7%, acrylic acid, maleic acid and maleamic acid were under 0.04%.
V. The correlation coefficient between the measured value by instrumental analysis and by ELISA were 0.98 to 0.99 (R^2 value).
VI. This method can measure 24 samples at the same time and finish within 4.5 hours.

Conclusion - 2

Finally, we developed simple and easy analysis for acrylamide using enzyme immunoassay.

Now we attempt to increase the sensitivity of this method and to use for environmental chemistry.