Evaluation of an LC-MS/MS-based analytical method for acrolein detection in local food products

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Abstract

Although there have been many studies on the analysis methods, production mechanism, and toxicity of acrolein in foods, there is a lack of analysis data for various foods, especially vegetables and fruits. This study is significant in detecting acrolein by distinguishing more than 100 food samples into four matrices using a validated acrolein analysis method developed. Analytical method of acrolein was established by solid phase extraction (SPE) with high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). Linearity (R2), limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision for analytical method of acrolein, were > 0.99, 0.14–1.73 ug/kg, 0.43–5.24 ug/kg, 82.12 –119.30 % and 0.52–12.11 RSD % of intra, inter-day.

Food products were classified into a total of 4 matrixes by characteristic such as fatty solid, non fatty solid (with high and low water content) and fatty liquid. Acrolein was detected in 89 out of 102 foods such as agricultural food, meet products, sea foods and processed foods resulting in a detection rate of 96.08%.

Objective

Acrolein (2-propenal) is a structurally simple α, β-unsaturated aldehyde; a low molecular weight, highly reactive substance present in the environment, water, and food. Among food varieties, acrolein has been extensively studied in lipid-rich foods. Acrolein is formed when food is cooked; for example, acrolein can form in fats when glyceride/glycerol are heated, while in proteins, amino acids such as methionine and threonine become a source of acrolein when heated. As a toxic substance, acrolein expresses its toxicity by binding to biomacromolecules in the human body. Acrolein in association with biomacromolecules results in oxidative stress, mitochondrial dysfunction, or abnormal immune responses. Acrolein is very difficult to analyze directly due to its high instability. For example, it tends to be polymerized in water and forms various kinds of adduction with thiol groups of amino acids and proteins, nucleic acids, and other cellular components This study presents the validation results of LC-MS/MS analysis of acrolein derivatized from food samples using the linearity, limit of detection (LOD), limit of quantification (LOQ), recovery (%), accuracy (%), and precision (%) applied using the criteria of the Codex guidelines

Meaterial & Method

- Reagent

The standard (STD; acrolein-2,4-dinitrophenylhydrazine, purity 99%) and the internal standard (IS; cyclohexanone-2,4-dinitrophenylhydrazine, purity 99%) were both of analytical quality, and were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Syringe filters (30 mm, 0.22 μm) were purchased from Advantec (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Solid phase extraction (SPE) Bond Elut C18 cartridges (500 mg, 6 mL, 40 μm) were acquired from Agilent Technologies, Inc. (Santa Clara, CA, USA).

- Samples and preparation of DNPH derivatives

Food products purchased in each region were categorized into a total of four matrices: 1) solid or semi-solid with a moisture content of 60% or more, 2) solid or semi-solid with a moisture content of 20% or less, 3) solid or semi-solid with a fat content of 10% or more, and 4) fatty liquid. To validate the acrolein-detection analysis, apple, sweet potato, beef, and perilla oil were used as the representative samples of each matrix, respectively. Considering these characteristics, acrolein was derivatized with a 2,4-dinitrophenylhydrazine (DNPH) reagent that specifically reacts to aldehyde groups and ketone groups to increase molecular weight and reduce volatility, thus ensuring the accuracy of the analysis method.

- Method validation assurance1. Preparation of working solution

For each of the four matrices mentioned above—represented by apple, sweet potato, beef, and perilla oil, respectively—a calibration curve was generated by analyzing a series of standard solutions of acrolein-2,4,DNPH at five concentrations (5, 10, 20, 50, and 100 μg/L). In addition, the standard product was mixed with 200 μg/L IS (cyclohexanon-2,4-DNPH) to determine the recovery rate, which was one of the validation parameters. All standard mixtures were injected in triplicate to obtain calibration curves. According to the Codex guidelines, the method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), recovery (%), accuracy (%), and precision (%). The linear relationship between the concentration of benzyl chloride and the IS (benzyl chloride-d7) relative to the chromatographic peak area of the analyte was shown by the square of the correlation coefficient (R2) of each calibration curve. LOD and LOQ (mg/kg) were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively.

- Statistical analysis

LC-MS/MS data were acquired and analyzed using HP ChemStation software (Hewlett Packard, Sunnyvale, CA, USA) and a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). All experiments were performed in triplicate, and the results were presented as mean ± standard deviation.

Table 1. Operating conditions for LC-MS/MS

Parameters	Condition
Instrument	LC-MS/MS (SCIEX API 3200)
Column	Agilent Zorbax 300SBC18 column (1.0 mm x 50 mm, 1.8 mm particle size)
Mobile phase (gradient elution)	A : aq. Formic acid (0.1%) B : Acetonitril.Formic acid (0.1%)
Mobile phase Flow rate	1.5 ml/min
Column temperature	45℃
Injection volume	5 ul
APCI ionization source	Negative mode
Ion source temperature	600 ℃
Nubulizer gas	55 psi
Auxiliary gas	55 psi
Nebulizer current	-3 uA
The gradient elution condition	% B (0–2 min), 5%, B (2–4 min), 5-95% B (4-5 min), 95%, B (5-6 min), 5% B
MRM SCAN MODE	[ACROLEIN-DNPH] qualifying ion : m/z 235 → 46 (-48V) m/z 235 →157 (-16V) m/z 235 →65 (-24V) [CYCLOHEXANONE-DNPH] qualifying ion : m/z 277 → 247 (-18V) m/z 277 →151 (-6V) m/z 277 →230 (-15V)

Results

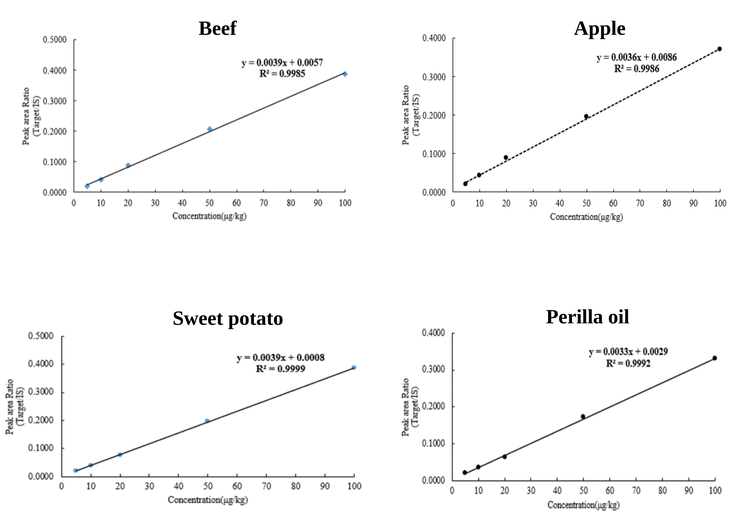

Figure 2. Calibration curve for four representative sample

Table 2. Calibration equations, linearity (R^2), limit of detection (LOD), and limit of quantification (LOQ) of acrolein (ARC).

Matrix		STD	Calibration equation	Linearity	LOD	LOQ
State of food	contents	(μg/L)		(R^2)	(μg/kg)[1]	(μg/kg)[2]
Solid or semi solid	More than 10% fat content	ACR (1–100)	y = 0.0039x + 0.0057	0.9985	1.728	5.237
Solid	More than 60% water content	ACR (1–100)	y = 0.0036x + 0.0086	0.9986	0.814	2.467
Solid	Less than 20% water content	ACR (1–100)	y = 0.0039x + 0.0008	0.9999	0.648	1.963
Liquid	Fat oil	ACR (1–100)	y = 0.0033x + 0.0029	0.9992	0.141	0.428

[1]The signal-to-noise ratio (S/N) of the LOD is 3.3.

[2]The signal-to-noise ratio (S/N) of the LOQ is 10.

Table 4. Comparison of accuracy and precision (CV) for acrolein detection from the fatty solid matrix.

Matrix		Concentration (μg/L) contents	Intra-day (n = 3)		Inter-day (n = 3)	
State of food	Contents		Accuracy (%)[1]	Precision (%)[2]	Accuracy (%)[1]	Precision (%)[2]
Solids or semi-solids	More than 10% fat content	5	110.634	5.985	119.298	8.022
		10	112.522	6.064	118.877	15.665
		20	114.101	4.811	114.654	5.301
		50	115.793	5.223	111.712	4.9143
		100	110.437	3.098	110.007	5.732

[1]Accuracy (%) = [1-(mean concentration of measured standard solution-concentration of spiked sample) /concentration of spiked sample] × 100.

[2] Precision (%) = (standard deviation/mean) × 100.

Table 4. Intra-laboratory acrolein detection method study results for apple samples.

Concentration (mg/kg)	Parameter	Laboratory A	Laboratory B	Laboratory C
10	Accuracy (%)	109.603	102.489	104.419
	CV (%)	11.451	0.066	5.14
20	Accuracy (%)	98.302	103.515	97.818
	CV (%)	2.022	0.838	3.421
30	Accuracy (%)	110.081	101.4667	97.445
	CV (%)	2.503	0.32	0.545

Conclusion & Discussion

LC-MS/MS chromatograms of acrolein derivatives and method optimization

Acrolein-2,4-DNPH standard and cyclohexanon-2,4-DNPH internal standard were used to analyte acrolein**.** the retention times of acrolein-2,4-DNPH and cyclohexanon-2,4-DNPH were 4.34 and 4.63. The fragment ion of acrolein-2,4-DNPH used in this study was m/z 157.9. The MS/MS parameters Decluster Potential (DP), Entry Potential (EP), Collision Energy (CE), and Collision Cell Exit Potential (CXP) were optimized to measure the maximum intensity of detection.

Table 1. Optimal MS/MS parameters, including precursor ions, product ions, declustering potential (DP), entrance potential (EP), collision energies (CE), and collision cell exit potential (CXP).

Carbonyl-DNPH	Retention time (min)	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
Acrolein	4.37	236.18	157.9	-45	-8.5	-16	-11
Cyclohexanone	4.36	278.26	246.9	-38	-6.5	-24	-9

Linearity, LOD, LOQ, precision, and accuracy were evaluated as elements of intra-laboratory validation, and precision and accuracy were evaluated by inter-laboratory validation at the two institutions. An interlaboratory study was conducted to ensure the objectivity of the acrolein detection. The analysis method was performed in two external laboratories containing similar equipment by analyzing the same sample, which was prepared by spiking low, medium, and high concentration of standards and an internal standard. As a result, the accuracies of the two samples were greater than 97.445% and less than 116.134%, and the coefficients of variation were all less than 11.451%. The developed method was validated according to the Codex guidelines (CAC/GL 71-2009). As a result, the derived results for R2 (>0.99), accuracy (80 - 120%), precision (<15%), LOD (<0.2 mg/kg), and LOQ (<0.6 mg/kg) showed that they satisfied the Codex guidelines

Reference

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