



Simultaneous determination of L-tryptophan impurities in meat products

Doo-Hee Lee¹ · Yang Hee Kim² · Mina Baek² · In Kyung Heo² · Yonguk Shin²

Received: 11 July 2022 / Accepted: 14 November 2022 / Published online: 2 January 2023
© The Author(s) 2022

Abstract

L-tryptophan has been used as a feed additive for swine and poultry and as a nutrient supplement for humans. However, some impurities in L-tryptophan have been reported as causative components of eosinophilia-myalgia syndrome. Therefore, from a safety perspective, it is important to analyze meat samples for these impurities. This study aims to develop an analytical method for the simultaneous detection of L-tryptophan impurities in meat products using LC–MS/MS. Among the various impurities, detection methods for (S)-2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid (5-hydroxytryptophan) (HTP), 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid (PIC), and 1,1'-ethylidenebistryptophan (EBT) and 2-(3-indoymethyl)-L-tryptophan (IMT) were developed. The developed method allowed simultaneous determination of these four impurities in 5 min. No interferences from the matrix were observed, and the method showed good sensitivity to each analyte. The method detection limit and limit of quantification in meat matrices were below 11.2 and 35.7 μg/kg, respectively.

Keywords L-tryptophan impurities · Eosinophilia-myalgia syndrome · LC–MS/MS · Meat products

Abbreviations

AAA	(S)-2-Amino-3-(2-((S,E)-7-methylnon-1-en-1-yl)-1H-indol-3-yl) propanoic acid, or (S)-2-amino-3-(2-((E)-dec-1-en-1-yl)-1H-indol-3-yl) propanoic acid	LC–MS/MS	Liquid chromatography—tandem mass spectrometry
EBT	1,1'-Ethylidenebistryptophan	LOQ	Limit of quantification
EMS	Eosinophilia-myalgia syndrome	MDL	Method detection limit
HIT	2-(2-Hydroxyindoline)-tryptophan	MTCA	1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid
HPLC	High-performance liquid chromatography	PAA	3-Phenylaminoalanine
HTP	(S)-2-amino-3-(5-hydroxy-1H-indol-3-yl) propanoic acid (5-hydroxytryptophan)	PIC	3A-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid
IMT	2-(3-Indoymethyl)-L-tryptophan	PTFE	Polytetrafluoroethylene
		SRM	Selected reaction monitoring
		UHPLC	Ultra high-performance liquid chromatography

Handling editor: D. Tsikas.

Doo-Hee Lee and Yang Hee Kim have contributed equally to this work.

✉ Yang Hee Kim
yanghee.kim@cj.net

¹ National Instrumentation Center for Environmental Management, Seoul National University, Seoul 08826, Republic of Korea

² Institute of Biotechnology, CJ CheilJedang, Suwon, Gyeonggi 16495, Republic of Korea

Introduction

L-tryptophan ((S)-2-amino-3-(3-indolyl)propionic acid) is an essential amino acid in animal diets and is regarded as the fourth and third limiting amino acid for the growth of swine and poultry, respectively (Jansman et al. 2010; Peganova et al. 2003; Le Floc'h and Seve 2007). L-tryptophan is primarily used as a building block for protein synthesis. In addition to its effect on protein synthesis, L-tryptophan plays a role in other physiological processes that are associated

with immune and inflammatory responses such as the synthesis of serotonin and niacin (Le Floch and Seve 2007; Sève 1999; Höglund et al. 2019; Fukuwatari and Shibata 2013).

Safety concerns regarding the use of L-tryptophan as a human dietary supplement have been raised because of its potential association with eosinophilia-myalgia syndrome (EMS). EMS is a rare condition characterized by inflammation in various organs, including the muscles, skin, and lungs. The clinical symptoms of EMS include subacute myalgia, eosinophilia in the peripheral blood, induration of skin, and chronic neuropathy; other common clinical signs include pneumonitis or pneumonia with or without pulmonary vasculitis, dyspnea, fatigue, rash, arthralgia, and edema of the limbs (Allen and Varga 2014; Aronson 2015). It has been reported that consumption of L-tryptophan human dietary supplement containing contamination caused EMS. The contamination occurred as a result of changes in the L-tryptophan manufacturing process at the Showa Denko K. K (SD) (Belongia et al. 1990; Philen et al. 1993; Blackburn 1997). The contamination was linked to the changes in the manufacturing process of SD L-tryptophan (Kilbourne 1992). When the SD L-tryptophan was analyzed using high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS), more than 60 impurities were found. Sample lot analyses and consequent epidemiological studies confirmed that 6 out of the 60 impurities were likely associated with the EMS outbreak in 1989: (1) 3-phenylaminoalanine (PAA), (2) 1,1'-ethylidenebistryptophan (EBT), (3) 2(3-indoymethyl)-L-tryptophan (IMT), (4) 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid (PIC), (5) 2-(2-hydroxyindoline)-tryptophan (HIT), and (6) (S)-2-amino-3-(2-((S,E)-7-methylnon-1-en-1-yl)-1H-indol-3-yl)propanoic acid, or (S)-2-amino-3-(2-((E)-dec-1-en-1-yl)-1H-indol-3-yl)propanoic acid (AAA) (Mayeno et al. 1990; Hill et al. 1993; Williamson et al. 1998; Klarskov et al. 2018). In addition to these 6 compounds, two additional compounds that may be related to EMS, namely 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (MTCA) and (S)-2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid (HTP), were mentioned (Das et al. 2004; Ogawa et al. 1993). HTP can be used as a substitute for L-tryptophan in physiological metabolism, and MTCA which is a decomposition product of EBT is expected as one of the EMS causative substances (Yamaguchi et al. 1991; Ito et al. 1992).

Methods for the analysis of L-tryptophan impurities, more specifically EMS-related substances, have been reported, and most are HPLC-based analyses. Simat et al. reported that the existence of these substances was analyzed from the pharmaceutical and feed grade of L-tryptophan by using HPLC (Simat et al. 2003). They provided the analytical method for MTCA, EBT, and IMT by using HPLC with a

UV detector, of PAA by using HPLC with an amperometric detector, and PIC by using HPLC with a fluorescence detector. The detection limit of the method was between 0.5 and 7 mg/kg. Another research group reported an HPLC analysis method for the detection of MTCA, EBT, and PAA by using UV and fluorescence detection after separating the analyte with different cartridges (PRS, SCX, C8) (Adachi et al. 2000). Recently, the EBT analysis method from dietary supplements containing L-tryptophan was reported based on LC–MS/MS (Karakawa et al. 2022).

The L-tryptophan impurities in feed-grade L-tryptophan lead to human consumption through meat products. Therefore, the potential human intake of L-tryptophan impurities by consuming meat products should be considered. In this study, a simultaneous analytical method of L-tryptophan impurities in meat products was developed using LC–MS/MS that showed higher sensitivity than the previously reported methods.

Materials and methods

Materials

(S)-2-Amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid (5-hydroxytryptophan) (HTP) and 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (MTCA) were procured from Sigma Aldrich (St. Louis USA). 3a-Hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid (PIC), 1,1'-ethylidenebistryptophan (EBT) and 2-(3-indoymethyl)-L-tryptophan (IMT) were procured from Wuxi Apptec Co., Ltd. (Tianjin, China), Carbosynth (Berkshire, UK), and TLC Pharmaceutical Standards (Ontario, Canada), respectively.

All reagents used in the analysis were of LC–MS grade and used without further purification. Ammonium formate ($\geq 99\%$ purity) and formic acid ($\geq 98\%$ purity) were procured from Sigma Aldrich (St. Louis, USA). Methanol (100% purity) and water were procured from Merck (Darmstadt, Germany).

Meat samples (pig and chicken muscle) originating from Korea, Spain, and United States were collected from a local supermarket in Korea. And other meat samples (pig muscle, skin and organs) were obtained from a slaughterhouse in Korea. The samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Sample preparation

The samples of pig muscle, pig skin, pig liver, pig kidney, pig lung, pig serum, and chicken muscle used for the spiking test and the commercial meat product were homogenized by grinding using a blender.

Table 1 Selected reaction monitoring (SRM) properties for the analysis of L-tryptophan impurities

Compound	Formula	Retention time (min)	Precursor (<i>m/z</i>)	Product (<i>m/z</i>)		Collision energy (V)	
				Quantifier ion	Qualifier ion	Quantifier ion	Qualifier ion
HTP	C ₁₁ H ₁₂ N ₂ O ₃	1.25	221.1	162.1	134.1	18.9	26.1
					204.2		11.0
PTC	C ₁₁ H ₁₂ N ₂ O ₃	1.51	221.1	130.1	158.1	30.7	20.5
					175.1		13.1
MTCA	C ₁₃ H ₁₄ N ₂ O ₂	3.73	231.1	158.1	143.1	17.0	34.6
					214.2		12.1
IMT	C ₂₀ H ₁₉ N ₃ O ₂	4.62	334.2	130.1	205.2	20.8	11.4
					217.2		11.1
EBT	C ₂₄ H ₂₆ N ₄ O ₄	4.65	435.2	157.1	214.2	41.4	28.9
					231.2		16.1

To obtain spiked samples, an appropriate amount of each standard chemical was added to the homogenized meat samples. The homogenized samples (1 g) were placed into a conical tube. Then, 80% methanol (10 mL) was added to the tube. The mixture was shaken and sonicated for 1 h. After sonication, the samples were centrifuged at 15,000 rpm for 10 min. The supernatants were taken and filtered using a 0.2 µm PTFE syringe filter.

Analytical method

Chromatographic separation of the samples was performed on a UHPLC Vanquish (Thermo Scientific, USA) system using a Cortecs C8 (2.1 mm × 150 mm, 1.6 µm) (Waters, USA). The column oven was operated at 45 °C. Mobile phase A consisted of 10 mM ammonium formate and 0.1% formic acid in the water, and mobile phase B consisted of 0.1% formic acid in methanol. An optimized gradient elution with mobile phase A and mobile phase B (0–0.1 min, 10% B, 0.35 mL/min; 0.1–2.0 min, 30% B, 0.35 mL/min; 2.0–3.5 min, 100% B, 0.35 mL/min; 3.5–5.0 min, 100% B, 0.35 mL/min; 5.0–7.0 min, 10% B, 0.4 mL/min) were used.

A TSQ Altis triple quadrupole mass spectrometer (Thermo Scientific, USA) equipped with a heated electrospray ionization (H-ESI) ion source was used. This instrument was coupled to the UHPLC system. The source parameters of optimized mass spectrometry were positive ion 3500 V, sheath gas 50 Arb, aux gas 10 Arb, sweep gas 1 Arb, ion transfer tube temperature 325 °C, and vaporizer temperature 350 °C. Trace Finder 4.1 software (Thermo Scientific, USA) was used for the data analysis. Selected reaction monitoring (SRM) transitions were monitored to quantify the analytes. The SRM parameters are described in Table 1.

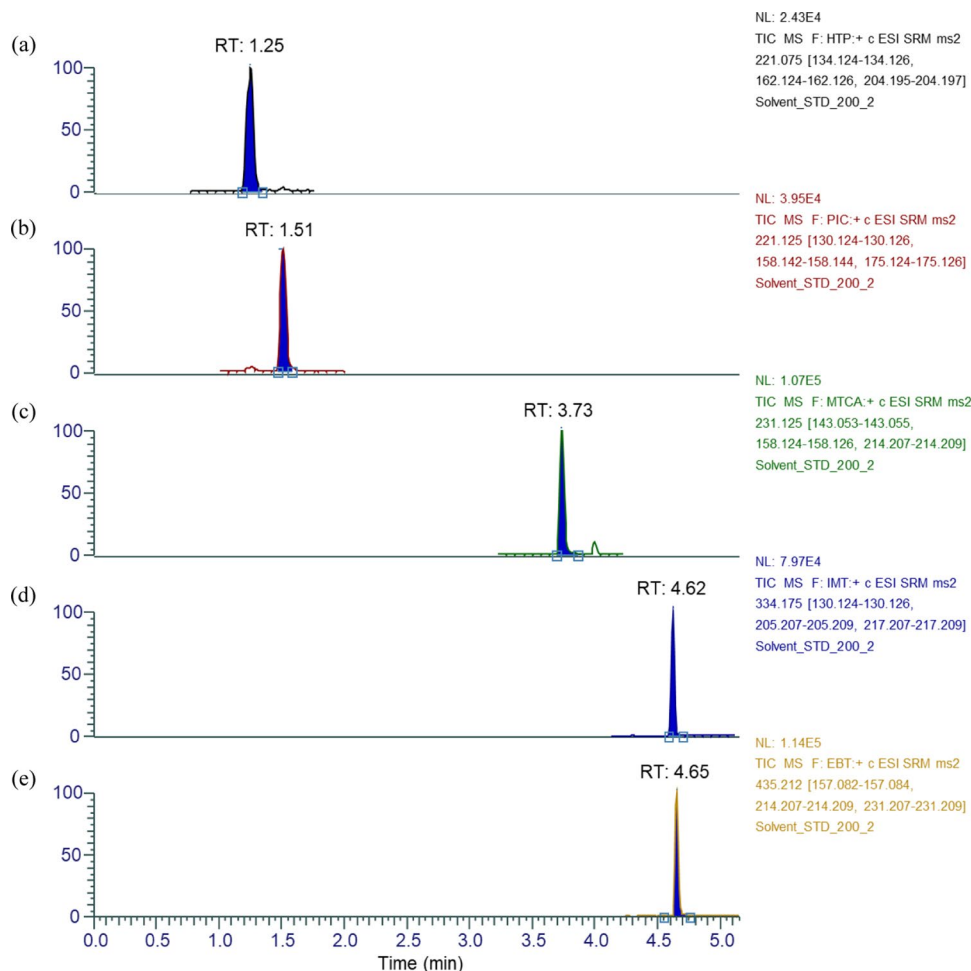
Method performance evaluation

The analytical method developed in this study was validated by referring to official guidance (ICH 2005; EC 2002, 2021). The performance of the analytical method was evaluated considering the specificity, method detection limit (MDL), limit of quantification (LOQ), interday precision, recovery, and matrix effect.

The linearity of the method was evaluated with calibration curves prepared in matrices by spiking analytes in pig muscle, pig skin, pig liver, pig kidney, pig lung, pig serum, and chicken muscle. The regression equation and coefficient of each calibration curve were calculated.

The method detection limit and limit of quantitation were determined by analyzing seven samples with concentrations near the expected limit of detection. The standard deviation of seven samples was simply multiplied by the correct Student's *t* value. The *t* value for six degrees of freedom and a

Fig. 1 LC–MS/MS (SRM) chromatogram of L-tryptophan impurities **a** 5-hydroxytryptophan, **b** 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid, **c** 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid, **d** 2-(3-indoymethyl)-L-tryptophan, and **e** 1,1'-ethylidenebistryptophan standards at 200 $\mu\text{g}/\text{mL}$



99% confidence level is 3.14. Therefore, the MDL and LOQ were calculated to be $3.14 \times \text{sd}$ (standard deviation) and $10 \times \text{sd}$ (standard deviation), respectively.

The interday precision was determined using a meat matrix spiked with analytes at the levels of 20 ng/mL . It was determined by analyzing the sample on seven consecutive days.

Recovery was evaluated at three fortified levels of analytes within the linear range of the calibration curve. The percentage of recovery was calculated as the concentration determined by the following equation:

$$\text{Recovery (\%)} = 100 \times \frac{\text{measured concentration}}{\text{fortified concentration}}$$

The matrix effects of the analysis were assessed by comparing the peak area of the standards in solvent and matrix. It was evaluated according to the following equation:

$$\text{Matrix effect (\%)} = 100 \times \frac{\text{peak area (matrix)}}{\text{peak area (solvent)}} - 100.$$

Results and discussion

Specificity

The chromatogram of the compounds is shown in Fig. 1. No significant interfering peak in the LC–MS/MS chromatogram was observed under the developed analysis conditions. Individual peaks for each analyte were separated well with a run time of less than 5 min.

Linearity

The linearity of the analysis was observed through the calibration curves of each analyte obtained using the matrices spiked with the analytes. Linear regression analysis was conducted by plotting the peak area and concentration of the analyte. The linear range of the method is summarized

Table 2 Linear range, coefficient of determination (R^2), method detection limit (MDL), and limit of quantification (LOQ) for L-tryptophan impurities in meat matrices

Compound	Meat matrix	Linear range (ng/mL)	R^2	MDL ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)
HTP	Pig muscle	2–50	1.0000	2.4	7.8
	Pig skin	2–50	1.0000	1.5	4.7
	Pig liver	2–50	0.9999	2.4	7.6
	Pig kidney	2–50	0.9999	2.9	9.4
	Pig lung	2–50	0.9990	3.0	9.4
	Pig serum	2–50	1.0000	1.2	3.7
	Chicken muscle	2–50	0.9998	3.5	11.1
PIC	Pig muscle	2–200	0.9999	1.5	4.9
	Pig skin	1–50	0.9999	2.0	6.4
	Pig liver	5–200	1.0000	1.9	6.2
	Pig kidney	5–200	0.9996	4.1	13.0
	Pig lung	10–200	0.9999	2.6	8.4
	Pig serum	1–100	0.9999	1.7	5.4
	Chicken muscle	2–100	0.9999	2.8	8.9
MTCA	Pig muscle	2–100	0.9999	1.1	3.5
	Pig skin	2–100	1.0000	2.3	7.5
	Pig liver	1–50	0.9999	4.3	13.7
	Pig kidney	1–50	1.0000	3.3	10.5
	Pig lung	2–50	1.0000	1.4	4.6
	Pig serum	1–100	1.0000	1.6	5.2
	Chicken muscle	1–50	1.0000	2.3	7.5
IMT	Pig muscle	2–100	1.0000	2.8	8.9
	Pig skin	1–50	0.9999	1.0	3.2
	Pig liver	1–50	0.9999	2.7	8.5
	Pig kidney	1–50	1.0000	2.4	7.8
	Pig lung	1–50	0.9998	2.3	7.3
	Pig serum	1–50	1.0000	3.0	9.5
	Chicken muscle	2–50	1.0000	2.7	8.7
EBT	Pig muscle	1–50	0.9999	2.3	7.4
	Pig skin	1–100	0.9997	2.3	7.4
	Pig liver	1–50	1.0000	11.2	35.7
	Pig kidney	1–50	1.0000	6.8	21.7
	Pig lung	1–50	0.9999	4.6	14.5
	Pig serum	1–50	0.9999	1.4	4.3
	Chicken muscle	2–50	1.0000	5.6	17.8

in Table 2. The parameters of calibration for each analyte showed very good linearity, with a mean regression coefficient (R^2) above 0.99.

Method detection limit and limit of quantification

The matrix-matched standard solutions of HTP, PIC, EBT, IMT, and MTCA were prepared by adding appropriate amounts of each compound to various meat matrices. The method detection limit (MDL) and limit of quantification (LOQ) of five analytes were calculated based on the seven samples of concentrations near the expected MDL (5 ng/mL). The MDL and LOQ of the analytes are summarized in

Table 2. The MDL and LOQ of this analytical method were lower than 11.2 and 35.7 $\mu\text{g/kg}$, respectively. Although the analytical method developed to detect L-tryptophan impurities in meat products, it showed a lower detection limit than the previous data in less complex matrices such as a finished product of L-tryptophan and dietary supplement (Adachi et al. 2000; Simat et al. 2003; Karakawa et al. 2022).

Accuracy and precision

The accuracy and precision of the method were assessed by a recovery test conducted by analyzing the known quantities of each compound added to the matrix-matched sample. As

Table 3 Recovery and relative standard deviation (RSD) of the L-tryptophan impurities in meat matrices at four fortified levels of 5, 10, 20, and 50 ng/mL

Compound	Fortified level (ng/mL)	Pig muscle		Pig skin		Pig liver		Pig kidney		Pig lung		Pig serum		Chicken muscle	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
HTP	5	102.97	0.52	100.17	0.10	108.83	1.16	101.65	1.02	98.08	1.02	99.73	0.43	106.66	0.88
	10	105.02	1.77	103.79	1.63	105.37	0.81	102.00	1.00	105.24	1.31	100.86	1.73	100.91	0.48
	20	104.15	0.73	98.63	2.30	99.94	2.58	101.59	0.99	109.87	0.17	100.96	1.32	102.84	1.16
PIC	50	98.32	0.60	105.73	1.30	98.86	1.13	101.45	0.25	108.55	2.64	98.75	0.93	97.74	0.79
	5	105.49	0.70	105.33	0.42	99.69	0.84	108.87	2.09	90.77	2.70	99.92	1.24	110.19	1.58
	10	107.18	0.75	106.17	2.98	95.55	0.61	97.55	1.66	95.63	0.84	105.13	1.13	97.01	0.72
MTCA	20	112.73	0.46	107.72	1.39	97.98	1.71	96.99	2.57	92.91	1.93	102.58	2.17	99.05	0.73
	50	104.55	1.92	102.81	2.12	100.78	1.18	95.94	0.39	97.13	3.08	99.94	1.61	96.66	2.11
	5	106.62	0.31	101.39	0.36	98.33	1.08	101.74	0.83	99.19	0.62	100.43	1.03	99.40	1.69
IMT	10	108.59	0.20	101.49	0.89	95.84	0.79	98.49	1.74	103.21	3.59	96.39	1.83	97.56	2.53
	20	92.62	0.10	97.43	1.39	93.77	0.89	93.55	2.01	99.47	1.25	99.22	1.31	99.67	3.61
	50	95.17	1.03	106.45	0.51	101.20	2.63	103.88	1.46	98.46	0.85	97.91	0.63	100.74	0.66
EBT	5	103.96	1.11	108.42	0.42	97.09	1.23	115.93	0.28	97.68	2.15	92.83	0.48	101.14	1.33
	10	108.26	0.59	103.79	2.16	101.33	1.28	104.91	2.36	106.45	1.53	92.10	1.83	102.55	3.24
	20	102.39	2.18	105.46	2.58	90.81	2.21	103.84	2.73	95.34	0.78	95.78	0.22	95.70	2.11
EBT	50	105.69	1.32	110.20	1.96	99.16	0.54	100.66	2.30	102.26	0.64	98.16	1.02	98.37	0.83
	5	110.65	0.74	107.44	1.10	98.43	2.78	99.99	1.67	96.13	1.48	99.79	0.96	99.84	1.31
	10	111.28	0.68	103.56	1.25	94.83	5.63	102.68	1.83	98.63	0.51	97.61	0.31	101.96	5.38
EBT	20	107.57	1.02	107.17	0.26	97.34	4.93	89.28	0.59	96.61	4.63	97.38	2.40	96.31	4.45
	50	102.88	0.65	107.62	1.07	100.02	0.63	102.29	0.58	102.72	1.75	98.43	0.32	94.82	3.28

shown in Table 3, the accuracy was expressed by the recovery percentage, and precision was expressed by the relative standard deviation (% RSD).

The matrix-matched sample was spiked with HTP, PIC, EBT, IMT, and MTCA at 4 different concentrations (5, 10, 20, 50 ng/mL). Table 3 presents the recovery and RSD of the test. The recovery of the analysis ranged from 92.6 to 112.7% for pig muscle, from 97.4 to 110.2% for pig skin, from 90.8 to 108.8% for pig liver, from 89.3 to 115.9% for pig kidney, from 90.8 to 109.9% for pig lung, from 98.8 to 105.1% for pig serum, and from 94.8 to 110.2% for chicken muscle.

For the interday precision, the test solution with the same concentration (20 ng/mL) was analyzed on seven consecutive days (Table 4). The interday precision ranged from 1.29 to 3.35% for pig muscle, from 1.32 to 3.77% for pig skin, from 2.26 to 4.55% for pig liver, from 1.65 to 4.44% for pig kidney, from 2.46 to 6.35% for pig lung, from 0.70 to 1.20% for pig serum, and from 1.39 to 4.96% for chicken muscle.

Generally, the acceptable range of mean recoveries at the relevant concentration level should be 70–120% and the precision $\leq 20\%$ RSD (EC 2021). For all other matrices, the range of mean recoveries at the fortified concentration level was 80–120%, and the precision was less than 10% RSD. Therefore, it was clear that the accuracy and precision of the proposed method for determining L-tryptophan impurities in the meat products were within the expected ranges indicating the high accuracy and precision of the method.

Matrix effects

Matrix effect has been known as a major concern in quantitative LC–MS analysis because it affects the accuracy precision, and sensitive of the method (Zhou et al. 2017). Especially, a high concentration of nonvolatile materials solute in an extracted sample from the biological products caused the ionization suppression when ESI was used as an ionization technique (King et al. 2000). Due to the complexity of the meat matrix, the matrix effect of the developed method was evaluated by comparing the peak area of the solvent and the matrix-matched standard. The calculated matrix effects for each of the analytes in the meat matrices were summarized in Fig. 2. The percentage of the matrix effect ranged from – 17.4 to 4.7 for pig muscle, from – 8.1 to 9.7 for pig skin, from – 13.8 to 28.7 for pig liver, from – 2.8 to 23.4 for pig kidney, from – 19.0 to 4.6 for pig lung, from – 15.0 to 7.8 for pig serum, and from – 25.9 to 13.5 for chicken muscle.

Overall, the matrix effects ranged from – 20 to +20%, which could be considered to be not significant (EC 2021), with the exception of MTCA in pig liver, IMT in pig kidney, and EBT in chicken muscle, for which the matrix effect ranged from – 25.9 to 28.7%. Without complicated purification to remove biological compound during the sample

Table 4 Recovery and relative standard deviation (RSD) of the L-tryptophan impurities in meat matrices at a fortified level of 20 ng/mL (interday precision)

Compound	Fortified level (ng/mL)	Pig muscle		Pig skin		Pig liver		Pig kidney		Pig lung		Pig serum		Chicken muscle	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
HTP	20	102.63	1.29	95.68	3.77	100.90	2.26	101.23	2.58	104.00	6.35	101.47	1.20	103.14	1.39
PIC	20	112.81	1.58	106.00	2.44	99.18	2.62	99.80	4.44	93.97	2.46	101.05	1.20	98.37	2.22
MTCA	20	100.99	3.25	99.78	1.32	93.70	4.18	92.82	2.38	94.29	3.38	96.90	0.77	99.10	3.04
IMT	20	103.53	3.35	107.42	1.72	88.14	4.55	102.86	1.65	94.64	4.11	94.11	0.92	92.99	3.68
EBT	20	106.86	3.05	109.78	1.89	101.38	3.10	85.87	4.45	95.09	4.88	97.52	0.70	95.05	4.96

Fig. 2 Matrix effects of the method for L-tryptophan impurities in seven food matrices (pig muscle, pig skin, pig liver, pig kidney, pig lung, pig serum, and chicken muscle). It was assessed by comparing the peak area of each standard in solvent and matrix

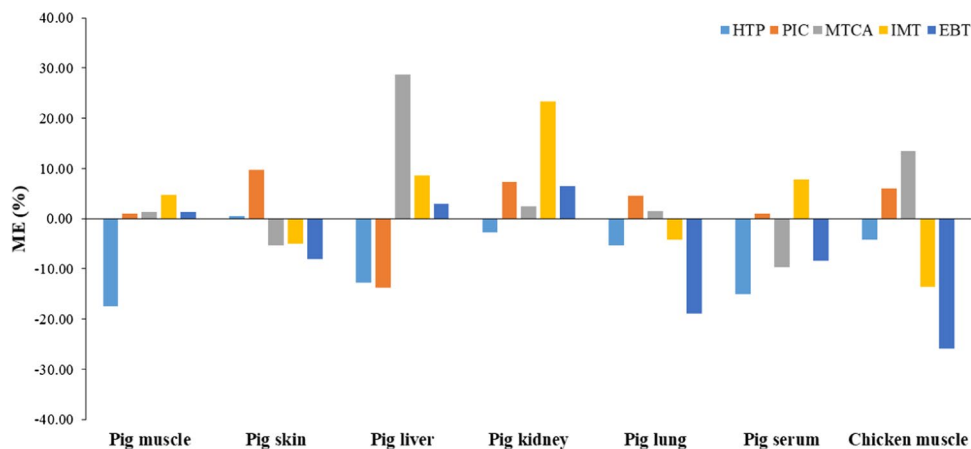


Table 5 Residue of the L-tryptophan impurities in commercial meat products

	Country of origin	HTP ($\mu\text{g}/\text{kg}$)	PIC ($\mu\text{g}/\text{kg}$)	MTCA ($\mu\text{g}/\text{kg}$)	IMT ($\mu\text{g}/\text{kg}$)	EBT ($\mu\text{g}/\text{kg}$)
Pig muscle	Republic of Korea	N.D.	46.6	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	62.4	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	38.5	N.D.	N.D.	N.D.
	Spain	N.D.	115.5	N.D.	N.D.	N.D.
	United States	N.D.	227.7	N.D.	N.D.	N.D.
Pig skin	Republic of Korea	N.D.	30.5	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	25.4	N.D.	N.D.	N.D.
Pig liver	Republic of Korea	N.D.	193.6	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	441.2	5.3	N.D.	N.D.
Pig kidney	Republic of Korea	N.D.	356.5	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	322.2	5.4	N.D.	N.D.
Pig lung	Republic of Korea	N.D.	331.8	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	447.6	N.D.	N.D.	N.D.
Chicken muscle	Republic of Korea	N.D.	55.0	N.D.	N.D.	N.D.
	Republic of Korea	N.D.	41.9	N.D.	N.D.	N.D.

*N.D.: not detected

preparation step, the matrix effect of the developed method was an acceptable range in most cases.

Application

The applicability of the developed method was evaluated by analyzing the L-tryptophan impurities in different meat products. Several kinds of commercially available meat products sold in a local supermarket and slaughterhouse in Korea were analyzed by using LC-MS/MS under the developed analytical method. As shown in Table 5, HTP, IMT and EBT were not detected in any sample tested. PIC and MTCA were detected in some of the samples. A very small amount of MTCA was detected in pig liver and kidney. PIC was detected in most of the samples, and relatively high levels of PIC were detected in organs such as the liver, kidney, and lung.

Up to now, no analytical report regarding the L-tryptophan impurities that are related to EMS in meat products as well as food or feed product has been published. Developed method in this study is the first study that analyzes L-tryptophan impurities in the biological matrices. Furthermore, the analytical result indicated that the proposed method was effective and feasible to detect L-tryptophan impurities in meat products. However, there was no information available on how much L-tryptophan and L-tryptophan-derived impurities have been fed to pigs and chickens. Therefore, a further study is necessary to observe the residue of L-tryptophan impurities in meat products after feeding a known amount of L-tryptophan and L-tryptophan impurities to an animal.

Conclusion

The safety of L-tryptophan has been under intense debate for several decades. The outbreak of EMS in 1989 was first considered to be a direct effect of L-tryptophan but was later determined to originate from impurities in the batches produced by a particular manufacturer. The causative substances of EMS-associated L-tryptophan were identified by several researchers. In this study, we developed an advanced analytical method for the detection of EMS-related L-tryptophan impurities in meat products.

Although the analytical method was developed to detect L-tryptophan impurities in meat matrices, we provided simple, faster and higher sensitivity compared to the previously reported method that used HPLC coupled with different kinds of detectors such as UV, fluorescence and amperometric detectors. Therefore, this method is applicable for the analysis of L-tryptophan impurities in meat matrices and is simple, sensitive, rapid, reliable, and accurate.

Author contributions YHK and IKH contributed to the conception and design of the study; DL participated in the methodology design; DL and MB performed the experiment; DL and YHK wrote the main manuscript text; YS revised the manuscript. All authors reviewed the manuscript.

Data availability The authors declare that the data supporting the findings of this study are available within the article.

Declarations

Conflict of interest YHK, MB, HIK, and YS are employed by CJ CheilJedang (Suwon, South Korea).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Adachi J, Asano M, Ueno Y (2000) Tetrahydro-beta-carboline-3-carboxylic acids and contaminants of L-tryptophan. *J Chromatogr A* 881(1–2):501–515. [https://doi.org/10.1016/S0021-9673\(00\)00197-7](https://doi.org/10.1016/S0021-9673(00)00197-7)
- Allen JA, Varga J (2014) Eosinophilia-myalgia syndrome, eosinophilic fasciitis, and related fasciitis disorders. Springer, New York, pp 1523–1535
- Aronson JK (2015) Meyler's side effects of drugs: the international encyclopedia of adverse drug reactions and interactions, 16th edn. Elsevier, Amsterdam, p 114
- Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, Dunnette SL, Pirie PL, MacDonald KL, Osterholm MT (1990) An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N Engl J Med* 323(6):357–365. <https://doi.org/10.1056/NEJM199008093230601>
- Blackburn WD (1997) Eosinophilia myalgia syndrome. *Semin Arthritis Rheum* 26(6):788–793. [https://doi.org/10.1016/S0049-0172\(97\)80022-4](https://doi.org/10.1016/S0049-0172(97)80022-4)
- Das YT, Bagchi M, Bagchi D, Preuss HG (2004) Safety of 5-hydroxy-L-tryptophan. *Toxicol Lett* 150(1):111–122. <https://doi.org/10.1016/j.toxlet.2003.12.070>
- European Commission (2002) Implementing Council Directive 96/23/EC concerning the performance of analytical methods and interpretation of results, Off J Eur Commun L221/8
- European Commission (2021) Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring purposes. SANTE/2020/12830-rev. 1, 24 Feb 2021
- Fukuwatari T, Shibata K (2013) Nutritional aspect of tryptophan metabolism. *Int J Tryptophan Res* 6(Suppl 1):3–8. <https://doi.org/10.4137/IJTR.S11588>
- Hill RH, Caudill SP, Philen RM, Bailey SL, Flanders WD, Driskell WJ, Kamb ML, Needham LL, Sampson EJ (1993) Contaminants in L-tryptophan associated with eosinophilia myalgia syndrome. *Arch Environ Contam Toxicol* 25(1):134–142. <https://doi.org/10.1007/BF00230724>
- Höglund E, Øverli Ø, Winberg S (2019) Tryptophan metabolic pathways and brain serotonergic activity: a comparative review. *Front Endocrinol* 10:158. <https://doi.org/10.3389/fendo.2019.00158>
- ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1)
- Ito J, Hosaki Y, Torigoe Y, Sakimoto K (1992) Identification of substances formed by decomposition of peak E substance in tryptophan. *Food Chem Toxicol* 30(1):71–81. [https://doi.org/10.1016/0278-6915\(92\)90139-c](https://doi.org/10.1016/0278-6915(92)90139-c)
- Jansman AJM, van Diepen JTM, Melchior MB (2010) The effect of diet composition on tryptophan requirement of young piglets. *J Anim Sci* 88(3):1017–1027. <https://doi.org/10.2527/jas.2008-1627>
- Karakawa S, Nakayama A, Ohtsuka N, Sato K, Smriga M (2022) Detection of impurities in dietary supplements containing L-tryptophan. *Amino Acids* 54:835–839. <https://doi.org/10.1007/s00726-022-03125-9>
- Kilbourne EM (1992) Eosinophilia-myalgia syndrome: coming to grips with a new illness. *Epidemiol Rev* 14:16–36. <https://doi.org/10.1093/oxfordjournals.epirev.a036085>
- King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T (2000) Mechanistic investigation of ionization suppression in electrospray ionization. *J Am Soc Mass Spectrom* 11(11):942–950. [https://doi.org/10.1016/S1044-0305\(00\)00163-X](https://doi.org/10.1016/S1044-0305(00)00163-X)
- Klarskov K, Gagnon H, Boudreault PL, Normandin C, Plancq B, Marsault E, Gleich GJ, Naylor S (2018) Structure determination of disease associated peak AAA from L-Tryptophan implicated in the eosinophilia-myalgia syndrome. *Toxicol Lett* 282:71–80. <https://doi.org/10.1016/j.toxlet.2017.10.012>
- Le Floc'h N, Seve B (2007) Biological roles of tryptophan and its metabolism: potential implications for pig feeding. *Livest Sci* 112(1–2):23–32. <https://doi.org/10.1016/j.livsci.2007.07.002>
- Mayeno AN, Lin F, Foote CS, Loegering DA, Ames MM, Hedberg CW, Gleich GJ (1990) Characterization of "peak E," a novel amino acid associated with eosinophilia-myalgia syndrome. *Science* 250(4988):1707–1708. <https://doi.org/10.1126/science.2270484>

- Ogawa Y, Adachi J, Tatsuno Y (1993) Accumulation of 1-methyl-tetrahydro-B-carboline-3-carboxylic acid in blood and organs of rat. A possible causative substance of eosinophilia-myalgia syndrome associated with ingestion of L-tryptophan. *Arch Toxicol* 67:290–293. <https://doi.org/10.1007/BF01974349>
- Peganova S, Hirche F, Eder K (2003) Requirement of tryptophan in relation to the supply of large neutral amino acids in laying hens. *Poult Sci* 82(5):815–822. <https://doi.org/10.1093/ps/82.5.815>
- Philen RM, Hill RH Jr, Flanders WD, Caudill SP, Needham L, Sewell L, Sampson EJ, Falk H, Kilbourne EM (1993) Tryptophan contaminants associated with eosinophilia-myalgia syndrome. *Am J Epidemiol* 138(3):154–159. <https://doi.org/10.1093/oxfordjournals.aje.a116841>
- Sève B (1999) Physiological roles of tryptophan in pig nutrition. In: Huether G, Kochen W, Simat TJ, Steinhart H (eds) *Tryptophan, serotonin, and melatonin*, vol 467. Springer, Boston
- Simat TJ, Kleeberg KK, Müller B, Sierts A (2003) Contamination of commercially available L-tryptophan by related substances. *Eur Food Res Technol* 216(3):241–252. <https://doi.org/10.1007/s00217-002-0646-3>
- Williamson BL, Johnson KL, Tomlinson AJ, Gleich GJ, Naylor S (1998) On-line HPLC-tandem mass spectrometry structural characterization of case-associated contaminants of l-tryptophan implicated with the onset of eosinophilia myalgia syndrome. *Toxicol Lett* 99(2):139–150. [https://doi.org/10.1016/s0378-4274\(98\)00223-9](https://doi.org/10.1016/s0378-4274(98)00223-9)
- Yamaguchi Y, Tsunoda J, Suda T, Miura Y, Shioiri-Nakano K, Kasahara T (1991) Effect of synthesized constituents in the L-tryptophan product on the differentiation of eosinophils and the induction of IL-6: a possible cause of eosinophilia-myalgia syndrome. *Biochem Biophys Res Commun* 178(3):1008–1013. [https://doi.org/10.1016/0006-291x\(91\)90992-g](https://doi.org/10.1016/0006-291x(91)90992-g)
- Zhou W, Yang S, Wang PG (2017) Matrix effects and application of matrix effect factor. *Bioanalysis* 9(23):1839–1844. <https://doi.org/10.4155/bio-2017-0214>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.