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APPLICATION NOTE 90606

Porcine gelatin peptide detection in cosmetic products and food confectionery using a TSQ Altis triple quadrupole mass spectrometer with Vanquish HPLC for halal testing application

Authors

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Keywords

TSQ Altis, High-performance liquid chromatography, Halal, Porcine gelatin, Cosmetic, Food, Confectionery, Adulteration

Goal

To establish a sensitive, fast, accurate and robust method using a LC-MS/MS for the detection of porcine-based gelatin in cosmetic and food confectionery products.

Introduction

Adulteration of cosmetic and food products has become a growing concern in the 21st century as manufacturers seeking financial gains often substitute expensive ingredients with cheaper alternatives. Contamination of products through the introduction of foreign ingredients during the manufacturing process can also contribute to questionable products being purchased by the end consumer. Such incidences are a huge problem as the authenticity of a labelled product can no longer be guaranteed, especially for consumers with health, ethical, or religious concerns. An example would be the requirement that products consumed or used by the Muslim population are halal and do not contain any non-halal ingredients, especially anything from a porcine source.

Porcine-based products are not only a major source of meat in the global food supply chain, the other non-edible parts of the animal such as the skin and bones are also processed into a very flexible chemical agent called gelatin. Gelatin consists of a mixture of peptides and proteins obtained from

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the permanent hydrolysis of collagen, the major protein in animal skin and bones. Although it can be derived from various animal by-products, it is primarily sourced from pork due to economic reasons. Known as one of the most flexible food and chemical modifiers, gelatin is translucent, colourless, brittle, and flavourless in terms of its physical properties[1]. The multiple functions of gelatin includes stabilizing, binding, thickening, emulsifying, plasticizing and foaming, resulting in a wide area of application including food processing, pharmaceutical, cosmetics, photography, and printing. Such wide applications for gelatin especially in a variety of food and cosmetic products are a huge cause of concern for the Muslim consumers. These concerns lead to the need for effective testing methodologies to detect porcine gelatin in various products.

Currently, there are two techniques that have been established for the detection of porcine gelatin. The first technique is by polymerase chain reaction (PCR), which is frequently used in species determination through the identification of animal DNA present in the sample. However, the use of PCR for detection of gelatin species origin has been proven to be extremely difficult and unreliable[2-4]. This is due to the high temperature and pressure involved during the processing of collagen in gelatin which results in near complete destruction of any DNA present. Furthermore, the PCR test cannot be used to determine the level of contamination in a gelatin sample as the detection of DNA does not correlate to the amount of protein or peptide in a sample. The second commonly used technique is a protein-based method known as ELISA (enzyme-linked immunosorbent assay). The ELISA test relies on the detection of antigens specific to proteins of the targeted species. Low sensitivity especially in complex highly processed samples and the high risk of inaccurate results from cross-reaction of the antibody used often limits the application of this test in many samples[5].

Unreliability of existing testing protocols has elevated the need for a reliable, sensitive, simple and accurate test for the detection of porcine gelatin. In this publication, we developed a testing procedure focusing on the use of triple quadrupole LC-MS/MS technology that can rapidly identify the presence of porcine gelatin through the combination of UHPLC fast separation and SRM-based targeted approach for a highly sensitive and robust solution.

Methods

Sample preparation

The sample preparation method is suitable and applicable for both food and cosmetics sample matrices. Individual cosmetic or food product samples were first homogenised and weighed into tubes consisting of 0.2 g of sample each. Hexane was added into the sample tube to remove lipids which are prevalent in certain types of samples. The samples were then dissolved in Sodium bicarbonate buffer solution and incubated overnight with the enzyme trypsin to digest the gelatin into peptide fragments suitable for mass spectrometry analysis (detailed protocol is shown in Figure 1).

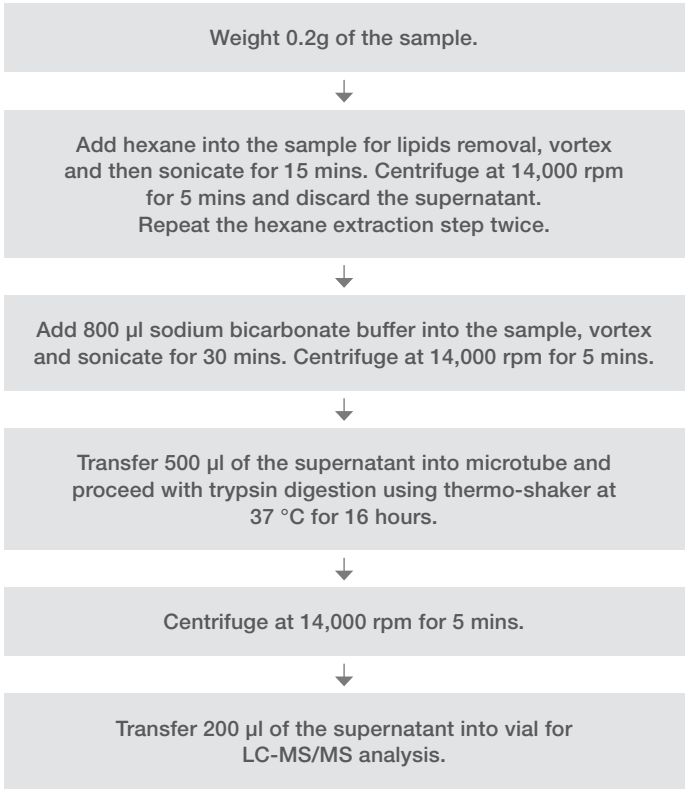


Figure 1. Sample preparation protocol for gelatin extraction from cosmetic and food confectionery samples.

Liquid chromatography (LC)

Chromatographic separation of the digested sample solution was performed on a **Thermo Scientific™ Acclaim™ PepMap™ 100 C18 LC column** with 3µm particle size and dimensions of 1.0 mm x 150 mm using a **Thermo Scientific™ Vanquish™ Flex Binary UHPLC system**. Injection volume was 2 µl. Mobile phase A was water with 0.1 % Formic Acid and mobile phase B was Acetonitrile with 0.1 % Formic Acid. Flow rate was 0.1 ml/min and column temperature was 45 °C. The gradient is shown in Table 1:

Table 1. UHPLC gradient setting

Time (min)	Flow (ml/min)	% A	% B
0	0.1	95	5
2	0.1	95	5
13	0.1	50	50
15	0.1	50	50
15.1	0.1	95	5
25	0.1	95	5

Mass spectrometry (MS)

MS analysis was carried out on a **Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer** with a heated electrospray ionization (H-ESI) probe. The ion source conditions were as follows:

Ion Source Type:	Vaporizer Temp (°C): 250
Spray Voltage: Static	Cycle Time (sec): 0.8
Positive Ion (V): 3800	Use Calibrated RF Lens: True
Negative Ion (V): 2500	Q1 Resolution (FWHM): 0.7
Sheath Gas (Arb): 20	Q3 Resolution (FWHM): 0.7
Aux Gas (Arb): 10	CID Gas (mTorr): 1.5
Sweep Gas (Arb): 1	Source Fragmentation (V): 0
Ion Transfer Tube Temp (°C): 325	Chromatographic Peak Width (sec): 30

A total of 11 unique porcine peptide markers was discovered and identified using Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer in our previous study[6] (data not shown in this article). The porcine peptide markers was then transferred into the SRM-based LC-MS/MS method. Multiple fragment ions (at least 2) giving good peak signal intensity were selected for each peptide marker to increase the confidence of detection. The SRM transitions for each of the peptide markers and optimized parameters (Retention Time min., Collision Energy, V; Polarity; Dwell Time, ms) are summarized in Table 2.

Table 2. Optimised SRM parameters for all 11 porcine peptide markers

Peptide Marker	Retention Time (min)	Polarity	Transition	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Dwell Time (ms)
P1	2.15	Positive	Quan	472.7	432.2	25	20.32
			Qual 1st	472.7	529.2	18	
			Qual 2nd	472.7	586.3	18	
			Qual 3rd	472.7	717.3	18	
P2	1.56	Positive	Quan	406.2	499.3	18	20.32
			Qual 1st	406.2	556.3	10	
			Qual 2nd	406.2	657.4	25	
P3	6.17	Positive	Quan	739.8	730.8	25	20.32
			Qual 1st	739.8	818.4	25	
			Qual 2nd	739.8	875.4	18	
			Qual 3rd	739.8	962.4	18	
P4	6.62	Positive	Quan	735.7	429.2	25	20.32
			Qual 1st	735.7	850.9	18	
			Qual 2nd	735.7	992.9	18	
			Qual 3rd	735.7	1051.6	18	
P5	6.88	Positive	Quan	774.9	602.6	18	20.32
			Qual 1st	774.9	978.4	25	
			Qual 2nd	774.9	1035.5	25	
P6	6.99	Positive	Quan	921.5	639.3	25	20.32
			Qual 1st	921.5	792.3	25	
			Qual 2nd	921.5	836.5	25	
			Qual 3rd	921.5	1050.5	25	
P7	7.58	Positive	Quan	1075	458.2	25	20.32
			Qual 1st	1075	697.3	25	
			Qual 2nd	1075	982.4	25	
			Qual 3rd	1075	1452.7	25	
P8	7.3	Positive	Quan	940.8	756.5	18	20.32
			Qual 1st	940.8	817.2	18	
			Qual 2nd	940.8	836.2	18	
P9	6.69	Positive	Quan	682	724.4	25	20.32
			Qual 1st	682	781.4	25	
			Qual 2nd	682	880.5	25	
			Qual 3rd	682	1024.6	25	
P10	6.81	Positive	Quan	1111	429.2	25	20.32
			Qual 1st	1111	1051.6	25	
P11	6.47	Positive	Quan	1095	429.2	25	20.32
			Qual 1st	1095	850.9	25	

Data processing

Data processing was carried out using **Thermo Scientific™ TraceFinder™ software (version 4.1)**. TraceFinder enables routine quantitation, targeted screening, and unknown screening, in one complete workflow using a single software platform.

Results and discussion

To evaluate the effectiveness of the SRM-based LC-MS/MS approach for porcine gelatin detection, a series of experiments were conducted to demonstrate robustness, sensitivity and accuracy using both standard reference gelatin material and real-world cosmetic and food samples. Comparison of reference gelatin standard from porcine and bovine sources analyzed using this method showed successful detection of all 11 porcine peptide markers in the porcine gelatin standard and negative detection of porcine markers in bovine gelatin standard (Figure 2). Good chromatographic separation of all 11 peptide markers was achieved using the Acclaim PepMap C18 column. The positive identification of porcine gelatin relies on correct matching of the retention time, the proper occurrence and alignment of all SRM transition peaks, and the correct ion ratio between the different fragment ions for the same peptide marker. Having three levels of identification criteria ensure highly reliable and accurate results even at very low detection limits. Furthermore, the use of up to 11 unique porcine peptide markers provides very high confidence by reducing the probability of a false detection.

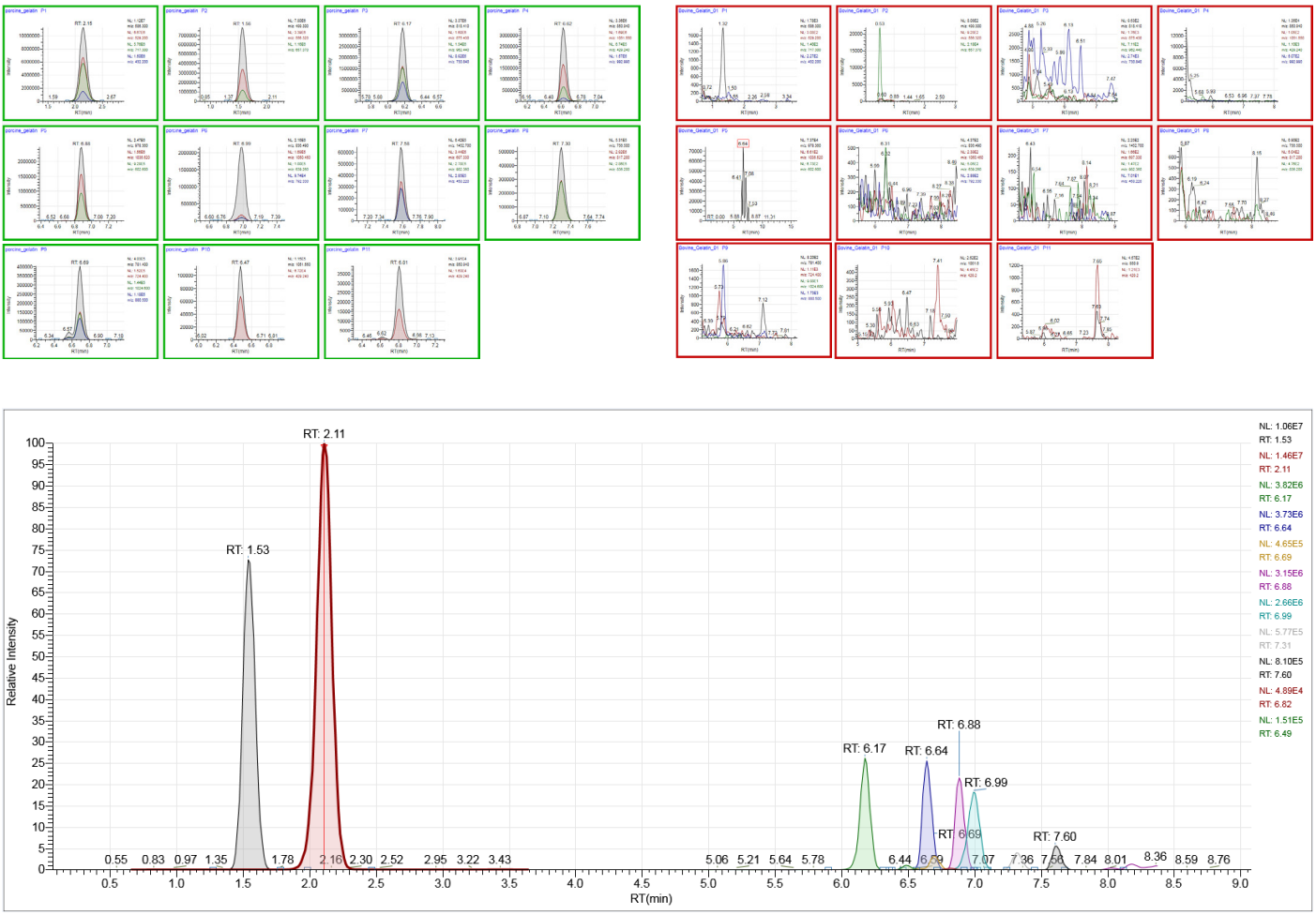


Figure 2. Detection of all 11 porcine peptide markers were observed for porcine gelatin standard with excellent signal intensity and good peak shape (top left). None of the peptide markers were detected in the bovine gelatin standard (top right). The bottom panel showed good chromatographic separation of all peptide markers at concentration of 10µg/mL porcine gelatin standard.

Further method validation matrices to determine the performance of the method in different matrix conditions was performed by spiking porcine gelatin standard to simulate contamination levels of varying degree (spiked solution concentration of 0.01 %, 0.05 %, 0.1 %, 0.5 %, 1.0 % and 5.0 %) into 2 separate sample. Hair moisturizer cream which is an oil-based matrix sample was analysed and showed negative detection for all porcine peptide markers without spiking (Figure 3). When the same sample was spiked with porcine gelatin standard, all 11 porcine peptide markers were detected, even in the lowest concentration of 0.01 % spiked sample. The method achieved excellent linear calibration curve with r2 value > 0.99 as demonstrated for marker P1 in Figure 4. Inter-day analysis of the samples also showed good reproducibility with area % RSD of 15 % or less for different concentration levels. Similar results were also obtained for the spiked facial peeling gel sample, which is a water-based gel matrix (Figure 5).

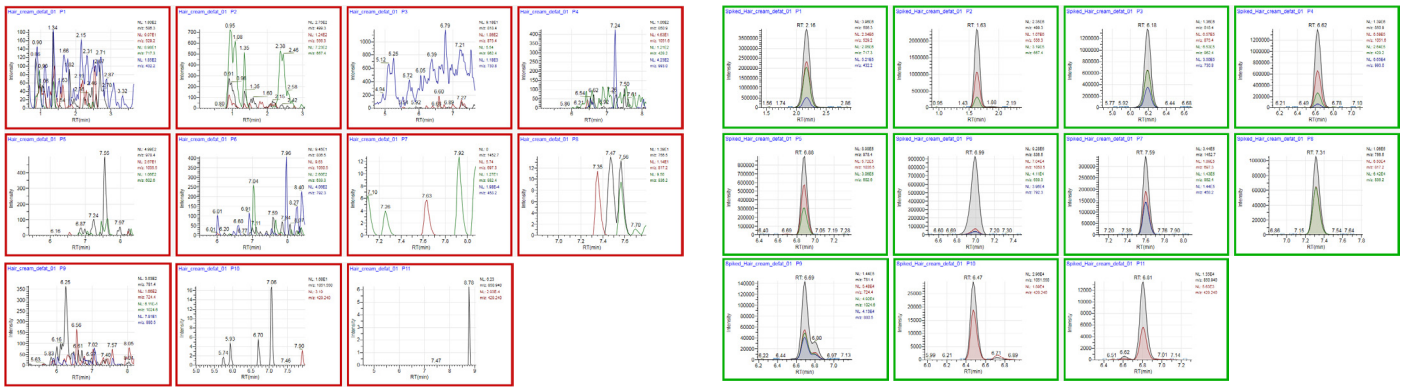
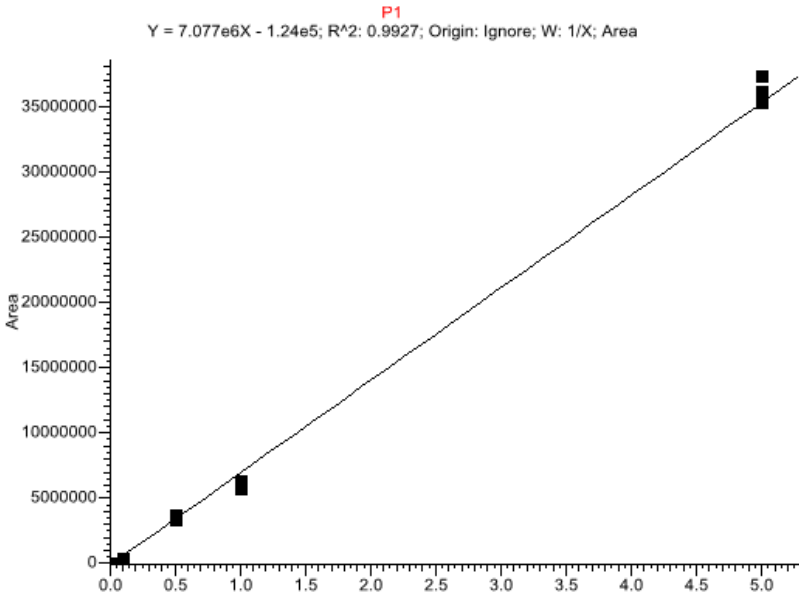
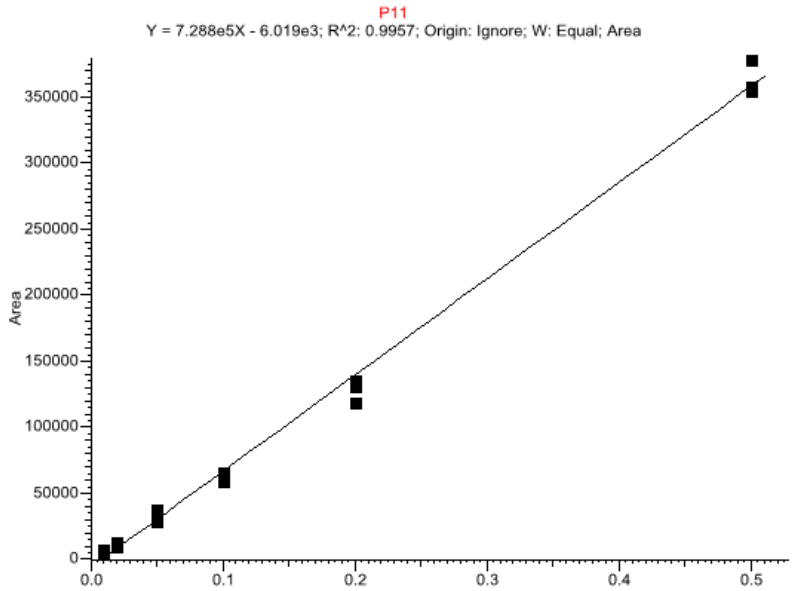


Figure 3. Comparison between hair moisturizer cream sample without spiking and with sample at 0.01 % porcine gelatin standard spike level.



% Porcine in Hair Moisturizer Cream	% RSD of area (n=3)
0.01	12.94
0.05	10.05
0.1	2.6
0.5	4.12
1	5.9
5	2.67

Figure 4. Example of inter-day reproducibility data for marker P1 of porcine gelatin standard spiked into hair moisturizer cream sample from concentrations of 0.01 % to 5 %.



% Porcine in Facial Gel	%RSD of area (n=6)
0.01	4.35
0.02	7.76
0.05	11.21
0.1	5.5
0.2	6.57
0.5	3.59

Figure 5. Example of inter-day reproducibility data for marker P3 of porcine gelatin standard spiked into facial peeling gel sample from concentrations of 0.01 % to 0.5 %.

In order to determine the accuracy of the method against existing testing methodology, a comparative study between the SRM-based LC-MS/MS method and ELISA method was conducted on various off- the-shelf gelatin-based products. The cosmetic and food confectionery products and the respective test result are summarised in Table 3. Positive correlation between the LC-MS/MS method and ELISA method were observed for most gelatine-based samples. This observation confirmed the viability of the LC-MS/MS method for detection of porcine gelatine in various cosmetic and food matrixes. Furthermore, 2 cosmetic samples (Hair Conditioner - Brand O and Hair Moisturizer Cream - Brand M) which returned inconsistent result using the ELISA method were confirmed to be negative for porcine peptides using the LC-MS/MS method. The successful analysis of such challenging cosmetic products consisting of lipid-based matrix is an important demonstration as many cosmetic and food products contain heavy lipid content which can often affect the confident analysis of such samples.

Table 3. Comparative analysis of various cosmetic and food confectionery products between ELISA and LC-MS/MS methodology.

No.	Tested samples	ELISA	SRM-based LC/MS
Cosmetic			
1	Facial Moisturizer Gel (Brand K)	Positive	Positive
2	Hydrolysed Collagen (Brand U)	Positive	Positive
3	Pork Gelatin from Skin (Brand S)	Positive	Positive
4	Face Serum (Brand ES)	Negative	Negative
5	Face Toner (Brand ES)	Negative	Negative
6	Facial Peeling Gel (Brand MC)	Negative	Negative
7	Hair Conditioner (Brand O)	Inconsistent	Negative
8	Hair Shampoo (Brand O)	Negative	Negative
9	Whitening Cream (Brand BS)	Positive	Positive
10	Hair Moisturizer Cream (Brand M)	Inconsistent	Negative
11	Facial Gel (Brand E)	Positive	Positive
12	Foundation Face Serum (Brand H)	Negative	Negative
13	Face Cream (Brand T)	Negative	Negative
14	Foundation Facial Cream (Brand WG)	Negative	Negative
15	Beauty Jelly Collagen Supplement (Brand J)	Positive	Positive
16	Cosmetic Beauty Drinks (Brand M)	Positive	Positive
Food Confectionery			
17	Gummy Candy (Brand P)	Positive	Positive
18	Marshmallow (Brand P)	Positive	Positive
19	Marshmallow (Brand T)	Negative	Negative
20	Marshmallow (Brand RM)	Positive	Positive
21	Marshmallow (Brand E)	Positive	Positive
22	Gummy Candy (Brand H)	Negative	Negative
23	Jelly (Band B)	Positive	Positive
24	Jelly (Band W)	Positive	Positive
25	Milk Candy (Brand R)	Positive	Positive
26	In house Reference Material marshmallow (negative)	Negative	Negative
27	In house Reference Material marshmallow (positive)	Positive	Positive

*positive = Porcine gelatin detected, *negative = Porcine gelatin detected, *inconsistent = Porcine gelatin detection is not consistent

Conclusion

In this study, a sensitive, robust and reliable LC-MS/MS method for the detection of porcine gelatin in cosmetic and food confectionery products has been demonstrated. The method can confidently detect the presence of porcine gelatin peptides in various sample matrixes down to 0.01 % of contamination level through multiple confirmation criteria. By combining a simple sample preparation procedure and rapid SRM-based LC-MS/MS approach, this method allows for rapid identification with high accuracy and can be adopted by testing laboratories to complement existing testing protocols.

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