



vMethod™ Application
for Food Testing

Screening and Speciation of Raw and Processed Meat Products

A Selective and Robust LC-MS/MS Method for Multiple Meat Speciation and Authentication on the QTRAP® 4500 System

Rapid and Reliable Detection of Multiple Meat Species in Food Products in a Single Injection

Si Mou¹, Lei Xiong¹, Lee Sun New², Peibin Qin³, Guo Lihai³, and Hua-Fen Liu¹

¹SCIEX Redwood City, California (USA); ²SCIEX Singapore; ³SCIEX Beijing, China

Introduction

In early 2013, horse and pig DNA were identified in beef products sold in several supermarket chains. Further testing across Europe and beyond had revealed widespread incidences of such contamination.¹ This type of contamination not only misleads the consumers, but also has health, religious, and ethical implications. In response to this, the Food Safety Authority (FSA) and Department for Environment Food & Rural Affairs (Defra) have set the threshold for undeclared meat species in meat products to 1% (w/w).² Therefore, it is imperative that analytical methods are sensitive and accurate enough to screen for the presence of meat adulteration in food products.

Traditionally, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) are used for meat speciation. PCR amplifies fragments of DNA extracted from food samples and demonstrates good sensitivity in unprocessed products. However, DNA can be easily disrupted or removed during food processing and manufacturing, thus limiting the use of PCR for processed or cooked meat products. ELISA is relatively quick and simple to perform, but has poor selectivity and is susceptible to cross-species reactivity which can lead to false positive or false negative results. Moreover, most ELISA tests lack multiplexing capabilities. Hence, LC-MS/MS provides an excellent alternative to these methodologies to identify and confirm different meat species with more accuracy and reliability.

Herein, we present a robust and sensitive LC-MS/MS method using the QTRAP® 4500 LC-MS/MS system that detects and screens pork, beef, lamb, chicken, duck and horse simultaneously in a single injection. The optimized sample preparation procedure is easy to follow and can be used for analyzing raw, cooked and processed meat products. Signature marker peptides unique to each species were identified and verified to ensure that they do not present any cross-species reactivity. Presently, this method can detect peptides from each meat species at a threshold detection limit of 1% w/w (10 mg/g) in a variety of food products.



Experimental

Sample Preparation

Meats or meat products (10 g) were frozen for 1 hour and grounded using a food processor or a coffee grinder. As an optional step, each grounded meat (1 g) was defatted with hexane and dried under a gentle flow of nitrogen. Extraction buffer was added to each defatted meat sample and the mixture was homogenized at high speed using a probe homogenizer to extract the proteins. Standard samples were prepared by combining different amounts of pork, beef, lamb, chicken, duck and horse homogenates to final concentrations of 0% and 1% (w/w) for each meat species (single-point calibration). The mixed meat homogenates (2 mL) were centrifuged and 0.4 mL of supernatant was diluted with ammonium bicarbonate buffer. Reducing reagent was added and the samples were incubated at 60°C for 1 hour. After cooling to room temperature, samples were alkylated using a cysteine blocking reagent. The modified proteins were digested with trypsin (4 to 12 hours). After which, the enzymatic activity was quenched with formic acid. Digested samples were desalted and concentrated using Agela

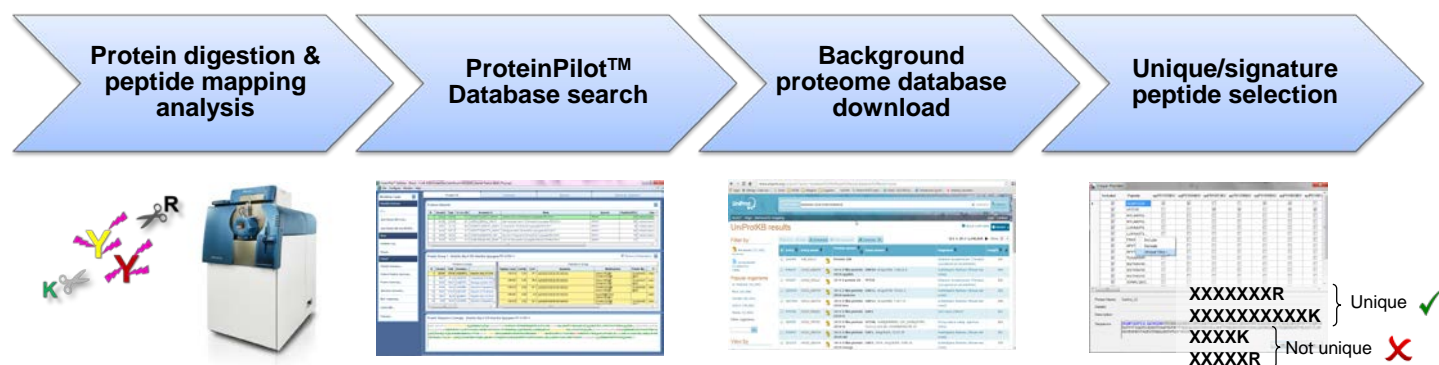


Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

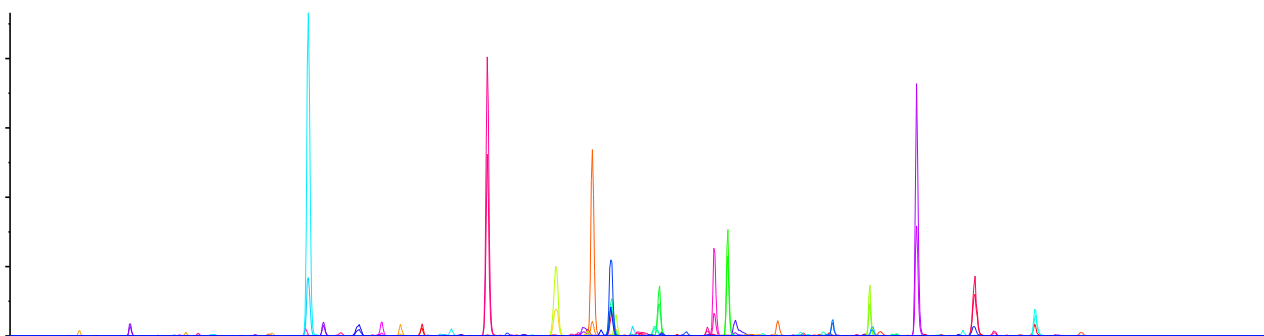


Figure 2. Extracted ion chromatograms from the LC-MS/MS analysis of raw meat mixture containing pork, beef, lamb, chicken, duck and horse (10, 20, 20, 20, 20 and 10% w/w, respectively). Multiple peaks corresponding to tryptic marker peptides are displayed.

Technologies Cleanert PEP SPE cartridges (60 mg/3 mL). The SPE eluents containing the peptides were dried and reconstituted for LC-MS/MS analysis.

LC Separation

Analytes (10 µL injection volume) were chromatographically separated using a ExionLC™ AC system equipped with a Phenomenex Kinetex C18 column (2.6 µm, 100 x 4.6 mm i.d.). A linear gradient was employed over 15 min at a flow rate of 500 µL/min using 0.1% formic acid in water and 0.1% formic acid in acetonitrile.

MS/MS Detection

Ion-dependent acquisitions (IDA) on a TripleTOF® 6600 LC-MS/MS System were performed to identify the proteins and peptides representative of pork, beef, lamb, chicken, duck and horse meats (Figure 1). The strategy for the selection of signature peptides can be found in more detail in the Results and Discussion.

Meat speciation and screening analysis was performed on a SCIEX QTRAP® 4500 system with Turbo V™ source in positive ESI mode using an ion source temperature of 650 °C. The Scheduled MRM™ algorithm was used to analyze food samples for 6 meats in a single injection by multiplexing the detection of multiple MRM transitions for unique signature peptides.

Results and Discussion

Comprehensive information of protein/peptide IDs was generated using the ProteinPilot™ software's protein database search features after LC-MS/MS analysis of digested meat samples on a TripleTOF® 6600 System (Figure 1). Selections of signature peptides for each meat species were performed using the Skyline software and NCBI Protein BLAST to ensure that the shortlisted peptides were unique and not found in other common livestock.

Signature peptides were finalized for each meat based on their: 1) specificity for each meat species; 2) uniqueness compared to the cross-species background; 3) sensitivity of detection; and 4) ability to be detected in both raw and cooked or processed meat samples.

For each meat species, two unique proteins, two unique peptides per protein, and two unique MRM transitions per peptide were chosen to ensure confidence in positive identification (Table 1). This corresponds to 24 marker peptides or a total of 48 MRM transitions representing pork, beef, lamb, chicken, duck and horse, for the simultaneous identification of multiple meat species in the same food sample (Figure 2). To monitor many MRM transitions during a single injection, the Scheduled MRM™ algorithm was employed, where each MRM transition was monitored for a short period during its expected retention time, decreasing the total number of concurrent MRM experiments during a cycle and allowing cycle time and dwell time to be maintained. This approach maximized the sensitivity for signature peptide detection and allows the method to be expanded as markers from other meats are identified.

LC-MS/MS analyses of raw and cooked (pan-fried) meat mixtures were performed to evaluate the thermal stability of the marker peptides. As shown in Figure 3, each meat marker peptide was detected without significant changes in sensitivity before (raw) and after cooking.

To demonstrate that signature peptide signals were linear in response to increasing meat concentrations, calibration curves for each peptide were generated over a wide dynamic range (0 to 100% w/w) with good reproducibility in combined meat matrix. For all meat species tested (pork, beef, lamb, chicken, duck and horse), MRM transitions were linear over a broad dynamic range with correlation coefficient values of over 0.99 for both MRM transitions. Figures 4 and 5 show examples of pork and beef with good linear response in meat matrix.

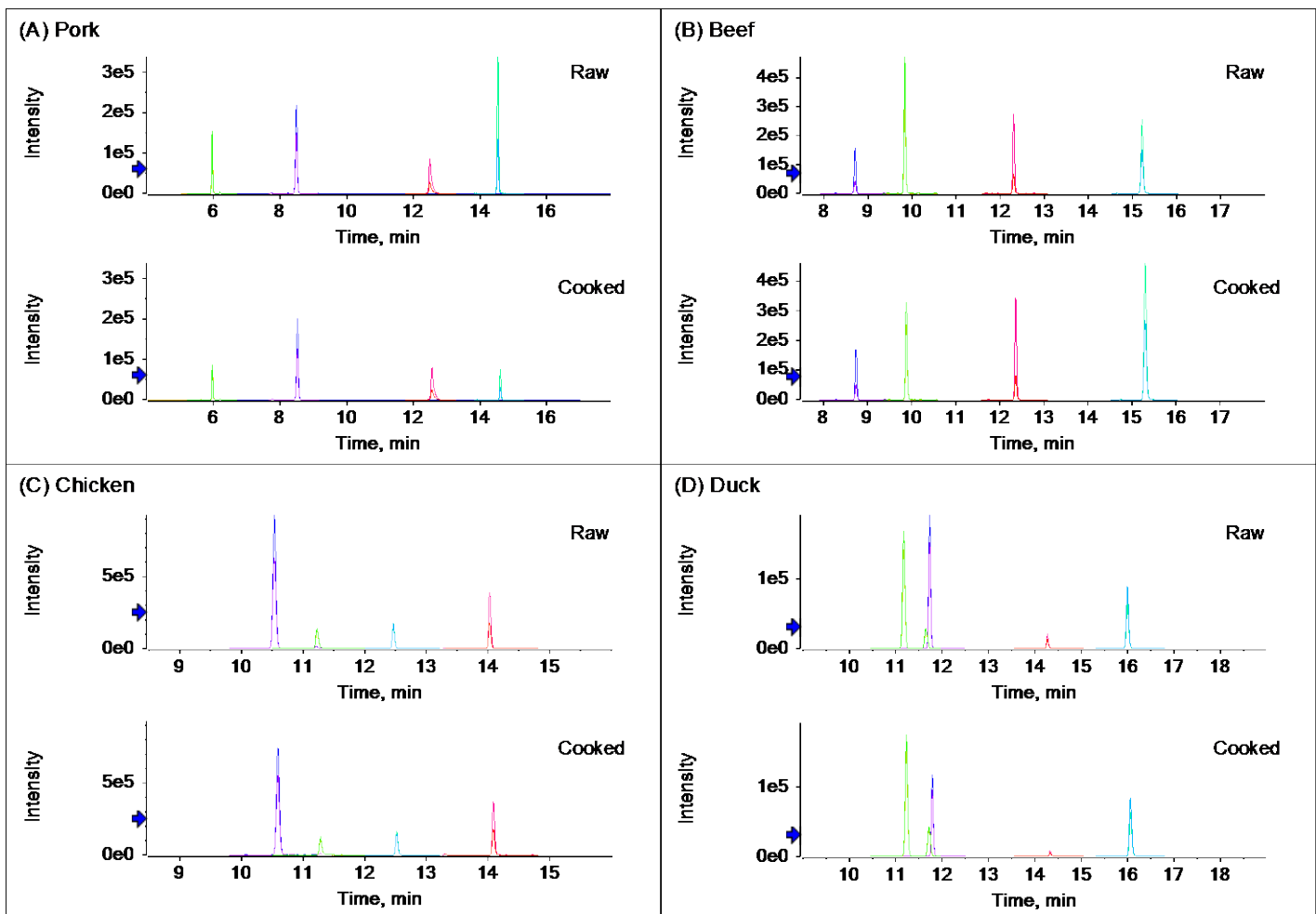


Figure 3. Extracted ion chromatograms (XIC) from the LC-MS/MS analysis of raw (top) and cooked (bottom) meat mixture containing pork, beef, chicken, duck and lamb (data not shown).

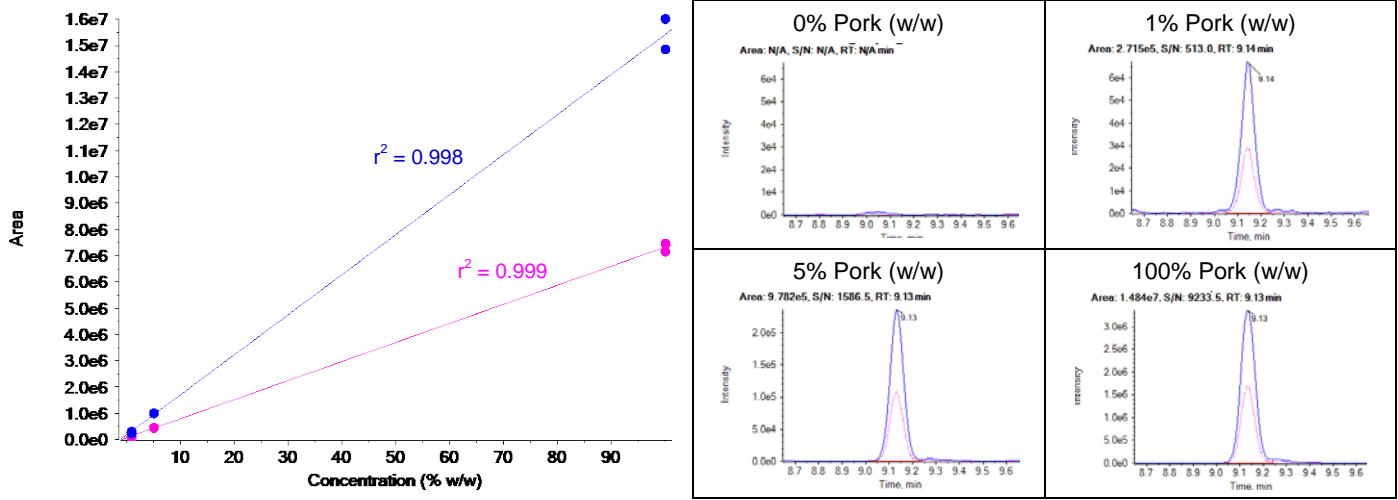


Figure 4. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw pork (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

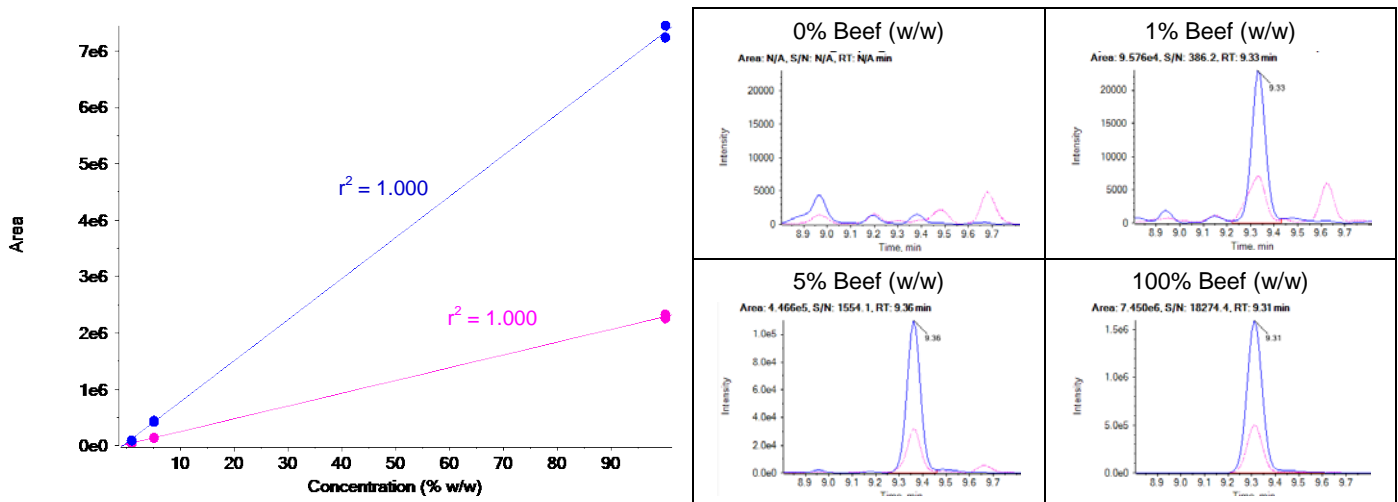


Figure 5. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw beef (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

The 1% (w/w) detection threshold limit of meat species in the combined meat matrix was verified on a SCIEX QTRAP® 4500 system by analyzing the 0% and spiked 1% (w/w) meat species in meat matrix. All marker peptides for each meat species were reliably detected at 1% spiked and no interference signals were observed in the background matrix (0%). Figures 5 and 6 show example XICs of quantifier ion (Protein_1.Peptide_A1) for each

meat in 0% and 1% (w/w) samples, demonstrating high sensitivity and reliability of detection. It's worth noting that 0.1% (w/w) detection threshold limit of meat can also be achieved with a SCIEX QTRAP® 6500+ system (data not shown).

To verify the effectiveness of the method for detecting meat contamination or adulteration, various raw and processed food products purchased from supermarkets were screened. As an

example in Figure 7, no significant pork marker peptides were detected in the halal certified products. Pork was tested positive only in products that had this meat labeled as one of the ingredients.

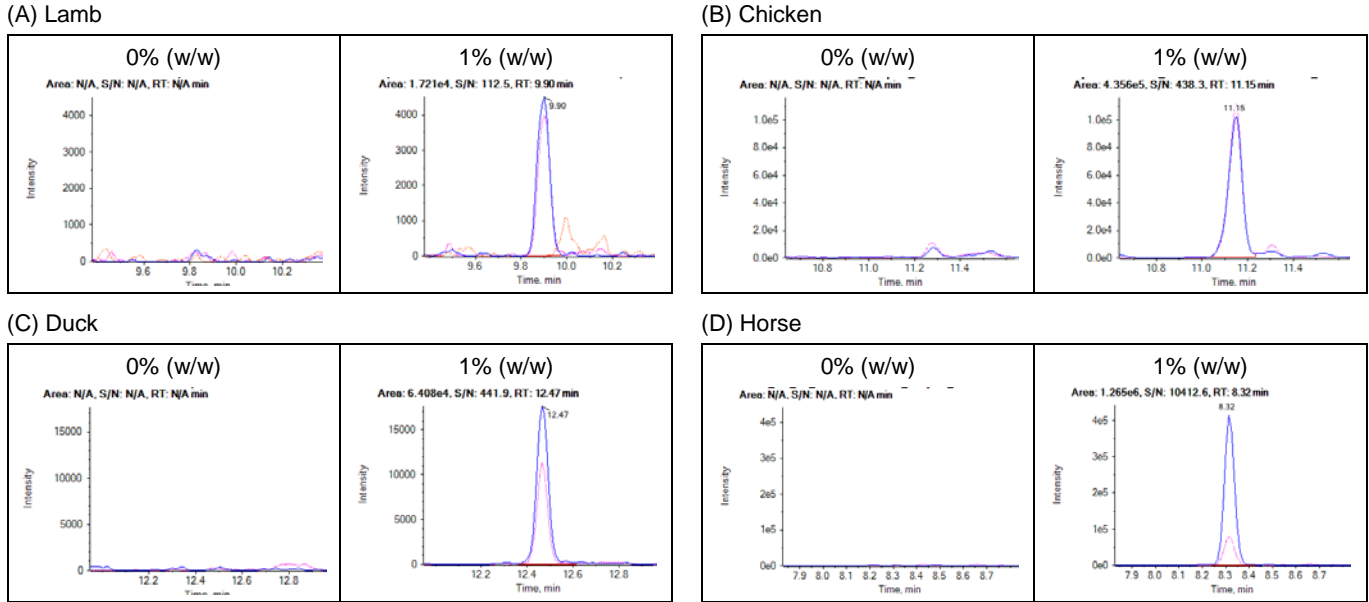


Figure 6. XICs of Protein_1.Peptide_A for 0 and 1% (w/w) of lamb, chicken, duck and horse in combined meat matrix (refer to Figure 5 for detection of pork and beef at 0 and 1% w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for each marker peptide.

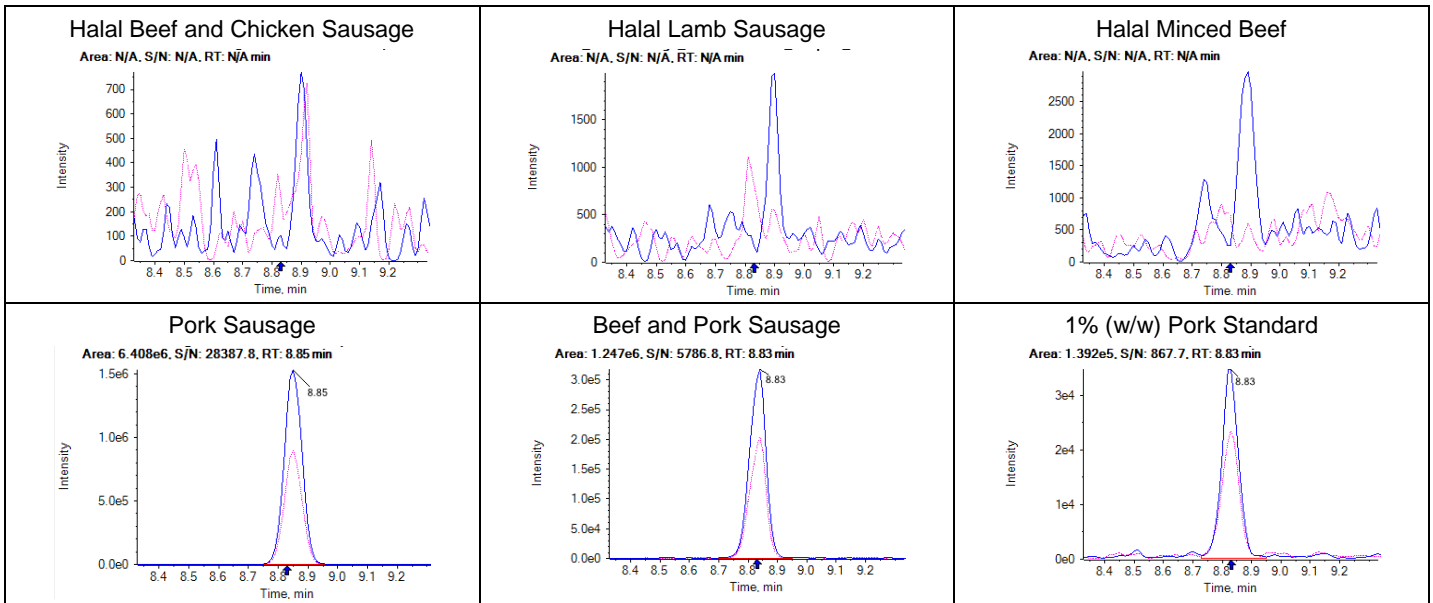


Figure 7. XIC of Pork.Protein_1.Peptide_A in commercial sausage products. Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for the marker peptide.

Summary

We have developed an LC-MS/MS-based meat speciation method for screening meat adulteration at 1% (w/w) for pork, beef, lamb, chicken, duck and horse. This method identifies MRM transitions corresponding to unique peptides for each meat species, and multiplexes their detection into a single injection. Unlike PCR and ELISA, the method is applicable to both unprocessed and processed meat matrices, providing high specificity and sensitivity in a single analysis. In addition to 1% meat adulteration screening on a SCIEX QTRAP® 4500 system, the method also demonstrates good linear responses at different meat concentrations in meat matrix, indicating its potential capability for relative quantitation. The vMethod package includes an easy-to-follow and robust sample preparation procedure, an optimized LC-MS/MS acquisition method, established templates

for data processing and reporting to facilitate the rapid detection and identification of meat adulteration or contamination in food products.

References

1. Department for Environment Food & Rural Affairs (2013). Processed beef products and horse meat. Retrieved from <https://www.gov.uk/government/news/processed-beef-products-and-horse-meat>
2. S. Wearne. Setting a threshold for contamination of processed meat products with undeclared meat species (2014). Retrieved from <https://www.food.gov.uk/sites/default/files/multimedia/pdfs/board/fsa-140104v1.pdf>