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# LC-MS/MS Analysis of Collagen from Meat Extracts

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Data source	: poster
Year	: 2009

<b>Conditions</b>	
Column	: Inertsil Hilic (5µm, 150 x 3.0 mm I.D.)
Column Cat. No.	: 5020-07735
Eluent	: A) 10 mM ammonium acetate in $CH_3CN$
	B) 10 mM ammonium acetate in $H_2O$ (pH 6.7)
	A/B = 90/10 -4 min- 90/10 -2 min- 75/25 ,v/v
Flow Rate	: 0.5 mL/min
Detection	: LC/MS/MS (3200 <sup>TM</sup> : ESI, Positive, MRM)
Sample	: meat extract, collagen
Analyte	: Creatinine
	Hydroxyproline

# LC-MS/MS Analysis of Collagen from Meat Extracts

Anna S. F. Margues<sup>1</sup>, Helio A. Martins-Júnior<sup>1</sup>, José L. Da Costa<sup>1</sup>, Takeo Sakuma<sup>2</sup>, Daniel Lebre<sup>2</sup> and Robert Ellis<sup>2</sup>: <sup>1</sup>Applied Biosystems Brazil, Av. Do Café, 277-1° andar-Torre A, São Paulo, SP Brazil 0431-000; <sup>2</sup> Applied Biosystems/MDS Analytical Technologies, 71 Four Valley Dr., Concord, Ontario, Canada L4K 4V8

# ABSTRACT

Collagen is the most plentiful protein present in the bodies of mammals, including humans. In fact, this major structural protein makes up about 25 % of the total amount of protein in the body. Hydroxyproline is necessary for the construction of collagen. Creatinine is a break-down product of creatine phosphate in muscles. Together, these compounds determine the juiciness and tenderness of meat products. This is the first time that a LC-MS/MS method is developed to analyze creatinine and hydroxyproline from collagen extracts.

Linear calibration curves were obtained over a dynamic range of 0.05 - 1.56 µg/mL for creatinine and 0.5 - 15.6 µg/mL for hydroxyproline. Standard solutions and samples were injected in triplicate to determine analytical coefficients of variation (*Cv*). The method showed good *Cv* values over the entire dynamic range. Seven meat extract samples were analyzed with good selectivity and sensitivity. Creatinine was detected in a range of 1.56 -36.1 µg/mL and hydroxyproline 13.5 – 297.0 µg/mL. The samples were simply diluted and injected into the LC-MS/MS system, with no extraction or clean-up process needed. This analytical method can speed up the sample analyses process, which in turn, improves the whole processing of collagen products.

# INTRODUCTION

Collagen is the main protein in connective tissues of animals and is the most abundant protein (25 – 35% of the whole body protein content) in mammals. Collagen is used as gelatin in foods, adhesives, dietary supplements, cosmetic formulations, artificial skin substitutes in the management of severe burns, reconstruction of bone and many dental, orthopedic and surgical procedures. To determine the juiciness and tenderness of meats, hydroxyproline, a major amino acid in collagen, and creatinine, a break-down product of creatine phosphate in muscles are routinely measured by colorimetric methods [1,2] in the meat and leather industries in Brazil. However, these colorimetric methods require extensive sample preparation, and are subject to interference from concomitant components in complex tendon extracts. A faster and more accurate analytical method is required. In the present study, a LC-MS/MS method was developed to quantify both hydroxyproline and creatinine from meat extracts in one analysis. The meat extracts were produced by adding hydrochloric acid to tendon in factory concentration tanks. It was possible to detect and quantify both hydroxyproline and creatinine with good detection limits. These meat extracts have several uses: manufacturing of different meat products to satisfy tastes of export destinations, soup flavoring and several meat-based ready-to-serve products.

# MATERIALS AND METHODS

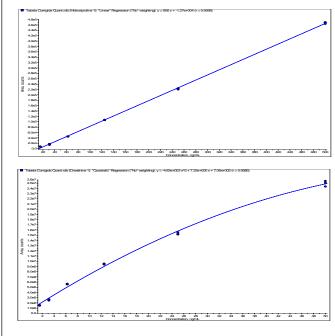
This method was developed using a Shimadzu Prominence LC system interfaced to an Applied Biosystems/MDS Analytical Technologies API 3200<sup>™</sup> LC-MS/MS system. LC separation was achieved with a GL Sciences' Inertsil HILIC column, 5-micron, 150 x 3mm, and mobile phase A = acetonitrile + 10 mM ammonium acetate and B = water + 10 mM ammonium acetate pH 6.7 at a flow rate of 0.5 mL/min. The LC gradient was: 0 – 4 min. at 10%, 10 - 25% B over 2 min, then back to 10% B for reconditioning of the column prior to analysis of the next sample. Due to high sample acidity (pH 3) the samples were diluted with a mixture of 45 mL acetonitrile, 1.25 mL of 1 M agueous ammonium acetate solution and 3.75mL of water. An aliguot of this sample was transferred to 1.7-mL auto-sampler vials for LC-MS/MS analysis using the most sensitive multiple-reaction monitoring mode (MRM).

Also to verify the method, bovine Achilles tendon collagen (0.5 g) was digested with a solution of 6 N HCl (62 mL), and boiled for 6 hours. The mixture was filtered using a 2.7-micron glass microfiber. The filtrate was transferred to a volumetric flask, and 6 N HCl was added to bring the total volume to 200 mL. An aliquot (approximately 1.7 mL) of this acidic solution was placed in a standard 1.8 mL auto sampler vial for LC/MS/MS analysis.

All quantitation data has been calculated using the IntelliQuan algorithm within Analyst® 1.5 Software (Applied Biosystems/MDS Analytical Technologies).

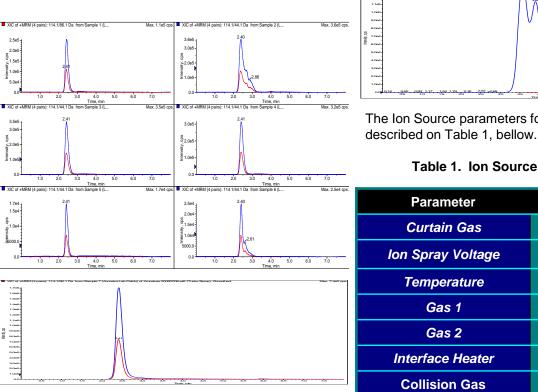
# RESULTS

Figure 1. Linear calibration curves for Hydroxyproline and Creatinine, respectively.

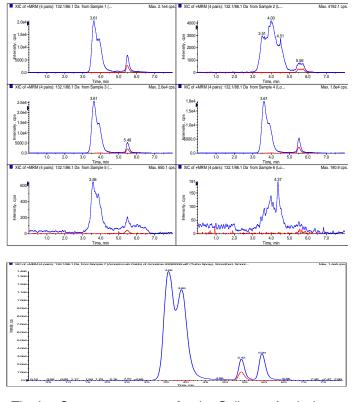


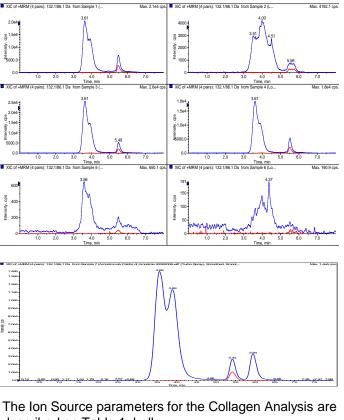
Linear calibration curves were obtained over a dynamic range of 0.5 - 15.6 µg/mL hydroxyproline. Creatinine was detected in a range of 1.56 - 36.1 µg/mL and hydroxyproline 13.5 -297.0 µa/mL.





Seven meat extract samples were analyzed with good selectivity and sensitivity. These extracts were sampled from factory tanks, which are used at the start of the meat extract concentration process. The samples were simply diluted in previously described mix and injected into the LC-MS/MS system, with no extraction or clean-up process needed. This analytical method can speed up the sample analyses process, which in turn, improves the whole processing of collagen products.





### Figure 3. Samples Analyzed for Hydroxyproline.

#### Table 1. Ion Source Parameters.

r	Value
as	25.0 psi
ltage	5000.0 V
re	550.0 °C
	60.0 psi
	40.0 psi
ater	ON
as	Medium

#### Table 2. Optimized MRM transitions and lens settings

MRM Method and Instrument Voltages							
Name	Q1	Q3	DP	EP	CEP	CE	СХР
Creatinine	114.1	44.0	26	7	10	27	4
	114.1	86.0	26	7	10	15	4
lydroxyproline	132.1	86.0	26	7	10	19	4
	132.1	68.0	26	7	10	25	4

The MRM transitions (m/z: mass-to-charge ratios for precursor, Q1 and fragment ions, Q3) are listed for creatinine and hydroxyproline as well as the critical lens voltages in Table 2, above. DP, declustering potential (V): EP entrace potential (V): CEP collision cell entrace potential (V); CE (collision energy in eV); CXP, collision cell exit potential (V). The first ion pairs  $m/z = 114.1 \rightarrow 44.0$  and  $132.1 \rightarrow 86.0$  were used for quantification, and the second ion pairs were used for confirmation by comparing intensity ratios.

Table 3 ( $\rightarrow$ ):

Quantitation data for

Creatinine (m/z =

114.1→44.0)

Standard solutions containing

1 – 6 of creatinine and

hydroxyproline were

acetonitrile + 10% water + 10 mM ammonium acetate. The

blank solvent was also run.

5-μL aliquots were run in

triplicates.

All the data points were used

(shown in "Used Record" column and integration was

done automatically (no

manual modification was done

as shown in the "Record

Modified" column.

Table 4 ( $\rightarrow$ ):

Quantitation

data for

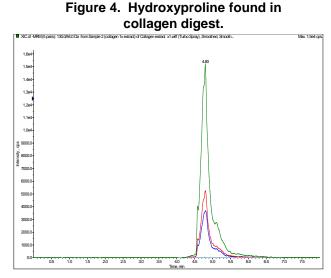
Hydroxyproline

(m/z = 132.1 →

86.0)

prepared in the initial mobile phase composition, i.e., 90%

6 different concentration poins



Bovine Achilles tendon collagen (0.5 g) was digested with a solution of 6 N HCl (62 mL), and boiled for 6 hours. The mixture was filtered using a 2.7-micron glass microfiber. The filtrate was transferred to a volumetric flask, and 6 N HCl was added to bring the total volume to 200 mL.

Sample	Peak	Peak	Concent-	Use	Record	Calculated	Accuracy
Name	Area	Height	ration	Record	Modified	Concentration	(%)
	(counts)	(cps)	(µg/mL)			(μg/mL)	. ,
Point 6-1	1.48E+06	1.72E+05	1.56	~		1.08	69.5
Point 6-2	1.51E+06	1.77E+05	1.56	~		1.12	71.9
Point 6-3	1.55E+06	1.81E+05	1.56	~		1.17	75.1
Point 5-1	2.47E+06	2.77E+05	3.13	~		2.48	79.2
Point 5-2	2.64E+06	2.95E+05	3.13	~		2.72	87.0
Point 5-3	2.69E+06	3.01E+05	3.13	~		2.79	89.4
Point 4-1	5.59E+06	5.81E+05	6.25	✓		7.07	113.0
Point 4-2	5.59E+06	5.84E+05	6.25	<b>√</b>		7.08	113.0
Point 4-3	5.67E+06	5.89E+05	6.25	✓		7.19	115.0
Point 3-1	9.40E+06	9.11E+05	12.50	✓		13.20	105.0
Point 3-2	9.53E+06	9.12E+05	12.50	<ul> <li>✓</li> </ul>		13.40	107.0
Point 3-3	9.46E+06	9.05E+05	12.50	~		13.30	106.0
Point 2-1	1.52E+07	1.34E+06	25.00	~		23.70	94.7
Point 2-2	1.52E+07	1.35E+06	25.00	~		23.80	95.4
Point 2-3	1.56E+07	1.36E+06	25.00	√		24.50	98.1
Point 1-1	2.54E+07	1.88E+06	50.00	√		52.40	105.0
Point 1-2	2.44E+07	1.93E+06	50.00	1		48.00	96.0
Point 1-2 Point 1-3	2.44E+07 2.50E+07	1.93E+06 1.92E+06	50.00 50.00	✓ ✓		48.00 50.80	<u>96.0</u> 102.0
Point 1-3	2.50E+07	1.92E+06	50.00	<ul> <li>✓</li> </ul>		50.80	102.0
Point 1-3 Sample	2.50E+07 Peak	1.92E+06 Peak	50.00 Concent-	✓ Use	Record	50.80 Calculated	102.0 Accuracy
Point 1-3	2.50E+07 Peak Area	1.92E+06 Peak Height	50.00 Concent- ration	✓ Use		50.80 Calculated Concentration	102.0
Point 1-3 Sample Name	2.50E+07 Peak Area (counts)	1.92E+06 Peak Height (cps)	50.00 Concent- ration (μg/mL)	Use Record	Modified	50.80 Calculated Concentration (μg/mL)	102.0 Accuracy (%)
Point 1-3 Sample Name Point 6-1	2.50E+07 Peak Area (counts) 7.63E+03	1.92E+06 Peak Height (cps) 6.57E+02	50.00 Concent- ration (μg/mL) 15.6	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3	102.0 Accuracy (%) 136.0
Point 1-3 Sample Name Point 6-1 Point 6-2	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02	50.00 Concent- ration (μg/mL) 15.6 15.6	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4	102.0 Accuracy (%) 136.0 137.0
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3	102.0 Accuracy (%) 136.0 137.0 136.0
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6 31.3	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 21.4 21.3 28.1	102.0 Accuracy (%) 136.0 137.0 136.0 90.0
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03	50.00 Concentration (μg/mL) 15.6 15.6 15.6 31.3 31.3	Use Record V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 21.4 21.3 28.1 31.4	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 1.35E+03	50.00 Concentration (μg/mL) 15.6 15.6 15.6 31.3 31.3 31.3	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 5-3 Point 4-1	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 1.35E+03 3.59E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6 31.3 31.3 31.3 62.5	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 4-1 Point 4-2	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.52E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 1.35E+03 3.59E+03 3.51E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6 31.3 31.3 31.3 62.5 62.5	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 4-1 Point 4-2 Point 4-3	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.52E+04 4.51E+04	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 1.35E+03 3.59E+03 3.51E+03 3.47E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 31.3 31.3 31.3 62.5 62.5 62.5	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.9 96.7
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.52E+04 4.51E+04 1.07E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 1.35E+03 3.59E+03 3.51E+03 3.47E+03 8.16E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 31.3 31.3 31.3 62.5 62.5 62.5 125.0	Use Record	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-3 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1 Point 3-2	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.74E+04 4.53E+04 4.53E+04 4.51E+04 1.07E+05 1.06E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 3.59E+03 3.51E+03 3.47E+03 8.16E+03 7.90E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6 31.3 31.3 31.3 62.5 62.5 62.5 62.5 125.0 125.0	Use Record V V V V V V V V V V V V V V V V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0 124.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8 99.6
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.52E+04 4.51E+04 1.07E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 3.59E+03 3.51E+03 3.47E+03 8.16E+03 7.90E+03 7.85E+03	50.00 Concent- ration (μg/mL) 15.6 15.6 15.6 31.3 31.3 31.3 62.5 62.5 62.5 62.5 125.0	Use Record V V V V V V V V V V V V V V V V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0 124.0 125.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-1 Point 5-2 Point 5-3 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1 Point 3-2 Point 3-3	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.63E+03 1.42E+04 1.74E+04 1.74E+04 4.53E+04 4.53E+04 4.51E+04 1.07E+05 1.06E+05 1.07E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 3.59E+03 3.51E+03 3.47E+03 8.16E+03 7.90E+03	50.00 Concentration (μg/mL) 15.6 15.6 15.6 31.3 31.3 62.5 62.5 62.5 62.5 125.0 125.0	Use Record V V V V V V V V V V V V V V V V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0 124.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8 99.6 99.9
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-2 Point 5-3 Point 5-3 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1 Point 3-2 Point 3-3 Point 2-1	2.50E+07 Peak Area (counts) 7.63E+03 7.63E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.53E+04 4.51E+04 1.07E+05 1.06E+05 1.07E+05 2.24E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 3.59E+03 3.59E+03 3.47E+03 8.16E+03 7.90E+03 7.85E+03 1.74E+04	50.00 Concentration (μg/mL) 15.6 15.6 15.6 31.3 31.3 62.5 62.5 62.5 125.0 125.0 125.0 250.0	Use Record V V V V V V V V V V V V V V V V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0 124.0 125.0 248.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8 99.6 99.8 99.6 99.9
Point 1-3 Sample Name Point 6-1 Point 6-2 Point 6-3 Point 5-3 Point 5-3 Point 5-3 Point 4-1 Point 4-2 Point 4-3 Point 3-1 Point 3-2 Point 3-3 Point 2-1 Point 2-2	2.50E+07 Peak Area (counts) 7.63E+03 7.72E+03 7.72E+03 1.42E+04 1.74E+04 1.60E+04 4.53E+04 4.53E+04 4.52E+04 4.51E+04 1.07E+05 1.06E+05 1.07E+05 2.24E+05 2.21E+05	1.92E+06 Peak Height (cps) 6.57E+02 6.04E+02 6.04E+02 6.68E+02 1.22E+03 1.32E+03 3.59E+03 3.51E+03 3.51E+03 3.47E+03 8.16E+03 7.90E+03 7.85E+03 1.74E+04	50.00 Concentration (μg/mL) 15.6 15.6 15.6 31.3 31.3 62.5 62.5 62.5 62.5 125.0 125.0 125.0 250.0	Use Record V V V V V V V V V V V V V V V V V V V	Modified	50.80 Calculated Concentration (μg/mL) 21.3 21.4 21.3 28.1 31.4 30.0 60.6 60.5 60.4 125.0 124.0 125.0 248.0 245.0	102.0 Accuracy (%) 136.0 137.0 136.0 90.0 101.0 95.9 97.0 96.9 96.7 99.8 99.6 99.8 99.6 99.9 99.2 99.2

Point 1-3 4.70E+05 3.73E+04 500.0 🔽 🏹 504.0 101.0

Table 5 (right): Statistical Results of Calibration Curves for Creatinine (green) and Hydroxyproline (orange)

Triplicate injections of blank and 6 solutions of different concentrations were made. Except for the lowest concentrations, we have good accuracy  $(\pm 15\%)$ .

## Table 6 (below):

Quantitation data for creatinine (green) and hydroxyproline (orange) based on calibration curves made for Table 5.

Triplicate measurements were made for each sample, and averaged to obtain calculated concentrations

Sample Name	Creatinine Peak Area (counts)	Creatinine Calculated Concentration (μg/mL)	Hydroxyproline Peak Area (counts)	Hydroxyproline Calculated Concentration (μg/mL)
Lot 176-1, semi-concentrated	2,970,000	3.20	75,500	92.2
Lot 176-2, semi-concentrated	8,120,000	11.00	168	30.9
Lot 176-1, concentrated	4,420,000	5.32	58,200	74.1
Lot 176-2, concentrated	4,110,000	4.85	57,800	73.7
Lot 176-1 broth	166,000	< 1.56	1,330	14.7
Lot 176-2 broth	325,000	<1.56	226	13.5
Sample lab broth	20,600,000	36.10	271,000	297.0

# CONCLUSIONS

and quantitation limits within 8-min chromatographic run.

### REFERENCES

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### TRADEMARKS/LICENSING

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# MP 267

Creatinine							
Expected	Sample	# Values	Mean	Standard	% Cv	Accuracy	
Concentration	Name	Used	value	Deviation		%	
1.56	Point 6	3 of 3	2.13	0.006	0.260	136.5	
3.13	Point 5	3 of 3	2.99	0.166	5.560	95.4	
6.25	Point 4	3 of 3	6.05	0.010	0.160	96.8	
12.50	Point3	3 of 3	12.47	0.020	0.160	99.8	
25.00	Point 2	3 of 3	24.55	0.213	0.870	98.2	
50.00	Point 1	3 of 3	50.25	0.328	0.660	100.5	
		Нус	droxyprol	ine			
Expected	Sample	Number of	Mean	Standard	% Cv	Accuracy	
Concentration	Name	Values Used	value	Deviation		%	
15.60	Point 6	3 of 3	11.25	0.440	3.912	72.1	
31.30	Point 5	3 of 3	26.64	1.639	6.155	85.1	
62.50	Point 4	3 of 3	71.13	0.675	0.949	113.8	
125.00	Point3	3 of 3	132.60	1.055	0.796	106.1	
250.00	Point 2	3 of 3	240.20	4.485	1.867	96.1	
500.00	Point 1	3 of 3	504.18	22.397	4.442	100.8	

We demonstrated that it is possible to quantify both creatinine and hydroxyproline in meat extracts with good detection

This LC/MS/MS method can replace the traditional colorimetric method used in the meat and leather industry. This method offers faster analysis time and more accurate data compared to the colorimetric method.