

Introduction

Raw materials are a critical part of any cell culture medium; therefore, it is of utmost importance to understand and characterize them for high-quality product. The raw material characterization (RMC) program at SAFC focuses on individual screening of raw materials both analytically and biologically. The goal of the program is to develop the best-in-class knowledge base of the raw materials used in SAFC's media formulations and their impact on performance of products.

A prioritized list of 100 "high-risk" raw materials was developed based on a risk assessment performed within SAFC. This poster will focus on the analytical screening of certain "high-risk" raw materials within the prioritized list to identify any variability and critical contaminants present. In order to achieve this, orthogonal methods were used that include ultra-high performance liquid chromatography-mass spectrometry (U-HPLC/MS) for non-volatile polar components and gas chromatography-mass spectrometry (GC/MS) for volatile non-polar materials. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was also used to identify any trace metal contamination present. In addition, the solubility of the raw materials is also tested to identify any variability within a vendor or between different vendors. The data presented will demonstrate the significance of the RMC initiative and how it helps SAFC to better understand their raw materials.

Process Flow

Figure 1 shows the process flow followed within the analytical RMC initiative. It describes how each raw material is screened and the strategy for analyzing any possible contaminant.

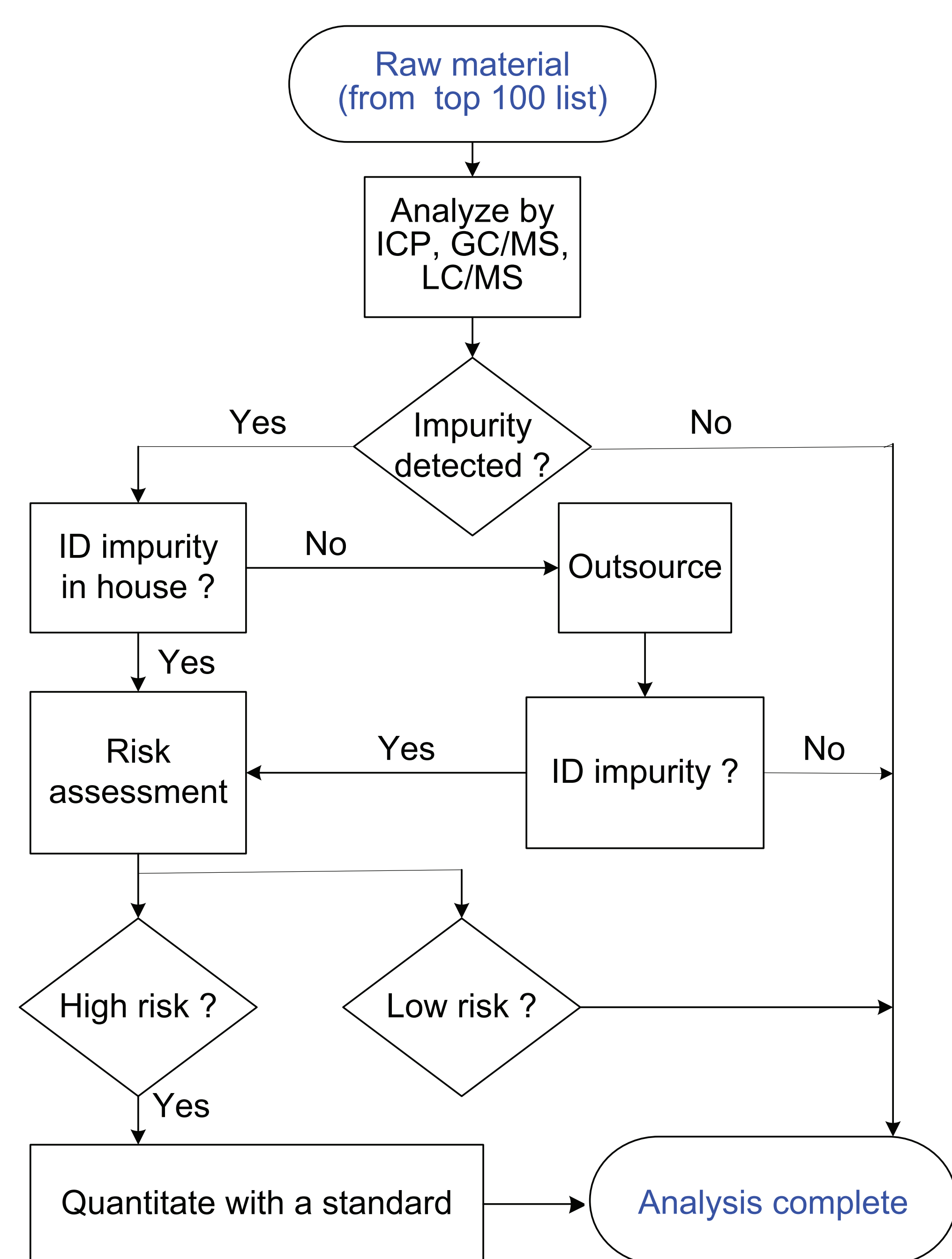


Figure 1: Analytical RMC process flow.

Amino Acids: Chemical Characterization

Amino acids are vital ingredients of any cell culture medium and hence are characterized by multiple techniques by the RMC team. Even though amino acids are polar components, they can be analyzed by GC using published derivatization methods¹. It was noticed that when orthogonal methods were used, a better insight into the characteristic detail of the amino acids was obtained. Figure 2 shows a comparison between the GC and LC techniques. Using the GC technique (A), we identified a peak with an indole ring. As L-tryptophan has an indole ring in its structure, it was not conclusive if the peak was a real contaminant or an artifact of the method. To confirm this, a neat sample of L-tryptophan was injected and characterized by LC and detected by photodiode array (B) and mass spectroscopic techniques (C). The LC results confirm the absence of any contaminant or impurity in the sample and therefore it can be concluded that the L-tryptophan samples are clean.

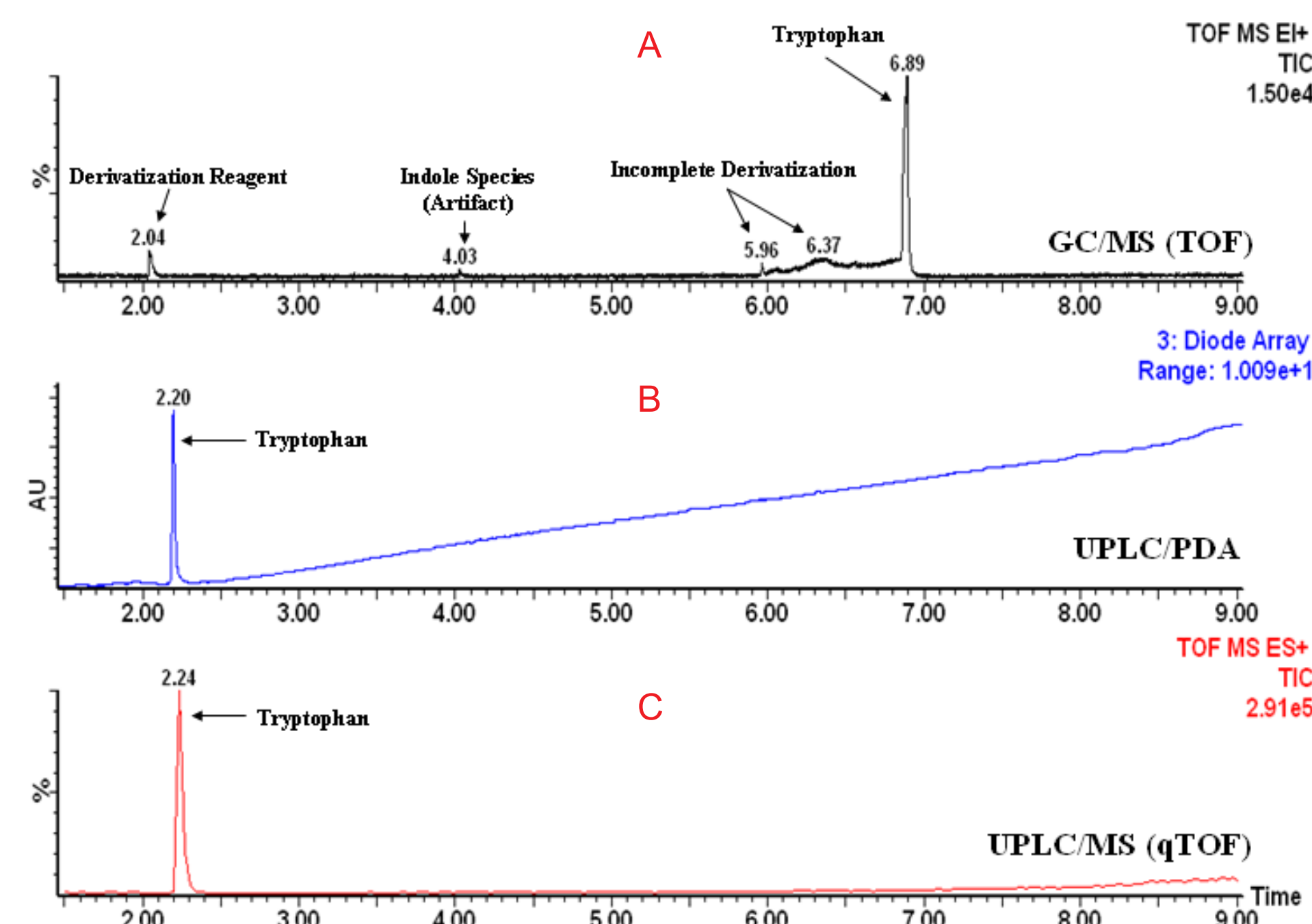


Figure 2: Tryptophan analysis comparing GC/MS (A) with LC/PDA (B) and LC/MS (C) techniques.

Table 1 shows a summary of amino acid findings using three different methods, as evident most of the amino acids are free of contaminants, the table also emphasizes the usage of orthogonal methods to characterize raw materials.

Amino Acid	# of lots	GC/MS	LC/MS	ICP	Findings
L-Lysine.HCl	12		X		No inter- or intra-vendor variability, most likely an artifact (to be confirmed)
L-Cystine.2HCl	9		X	X	Vendor 1 lots have higher concentration of Fe, Ni and Cr. Vendor has confirmed the usage of HCl in stainless steel tanks that might leach steel into the product; LC peaks likely an artifact (to be confirmed)
L-Tryptophan	9	X			Indol based impurity (<3.2%), LC/MS confirmed the sample to be pure, clean by LC-complete
L-Phenylalanine	9				No concerns-complete
L-Tyrosine Disodium Salt	6				No concerns-complete
L-Glutamic Acid Anhydrous	6	X			Possible cyclization in GC, clean by LC-complete
L-Valine	6		X	X	All material show higher S levels. Vendor has confirmed the usage of a fermentation process with H ₂ SO ₄ and NaHSO ₃ . LC showed an unknown peak in all lots-peak identity to be confirmed
L-Cysteine	3				No concerns-complete
L-Asparagine	9				No concerns-complete

Table 1: Summary of amino acid findings using GC/MS, LC/MS and ICP methods.

Amino Acids: Physical Characterization

Amino acids are also being characterized based on their solubility in a neutral medium. The goal of this experiment is to qualify and quantify differences in raw material solubility limits and to evaluate any inter- or intra-vendor differences. To measure this, a weight of raw material suitable for maximum solubility at neutral pH and ambient temperature was dissolved in 100mL of a buffer solution. Nephelometric Turbidity Unit (NTU) readings were measured (pre-filtration) to confirm the complete dissolution of the raw material; specifically, the maximum amount of raw material in solution should produce an NTU value not exceeding 3.50. For temperature-dependent stability testing, solutions with raw material amounts corresponding to 95%, 75%, and 50% of the maximum soluble amount of that raw material lot were sterile-filtered through a 0.2 µm filter. These solutions were stored at 2-8 °C, 37 °C and ambient temperature and were observed daily for a period of 60 days, or until the material precipitated.

Table 2 shows data for three amino acids from multiple vendors. As evident from the table, there are clear inter- and intra-vendor differences. This variability is to be further assessed by clarifying the vendor's manufacturing site and processes.

Vendor	Lot No.	Solubility in 100 ml of a neutral buffer		
		Lysine.HCl (g)	Cystine.2HCl (mg)	Tyrosine.2Na (mg)
A	1	5.0	12.4	175.1
	2	12.5	35.0	185.8
	3	10.0	35.2	185.5
B	1	15.0	30.3	185.8
	2	12.5	50.8	195.2
	3	12.0	48.2	200.7
C	1	60.0	32.0	N/A
	2	60.0	35.2	N/A
	3	60.0	32.8	N/A
D	1	60.0	N/A	N/A
	2	60.0	N/A	N/A

Table 2: Summary of solubility findings on multiple lots of three amino acids.

Fatty Acids

Fatty acids are non-polar components and are primarily amenable to GC, so GC was the core method used to analyze fatty acids. To date, multiple lots of DL-α-lipoic acid, linoleic acid and cholesterol have been analyzed and Table 3 summarizes these findings. To identify any metal contaminant present, the samples are currently being analyzed by ICP.

Fatty Acid	# of lots	GC	ICP	Findings
DL-α-lipoic acid	9			Ongoing
Linoleic Acid	3	X		Ongoing
Cholesterol	3	X		Ongoing

Table 3: Summary of findings on multiple lots of three fatty acids.

Figure 3 shows a GC chromatogram of linoleic acid of three different lots. Linoleic acid elutes at 10.73 minutes, and the inset picture shows a closer view of the impurity peaks at retention times ranging from 13.80 minutes to 14.30 minutes. The figure shows that for Lot 2, the percentage of contaminant is the highest; Lot 1 and Lot 3 show the purity of linoleic acid very close to its reported purity of 99.0%. The contaminant peaks have been identified as isomers of octadecatrienoic acid.

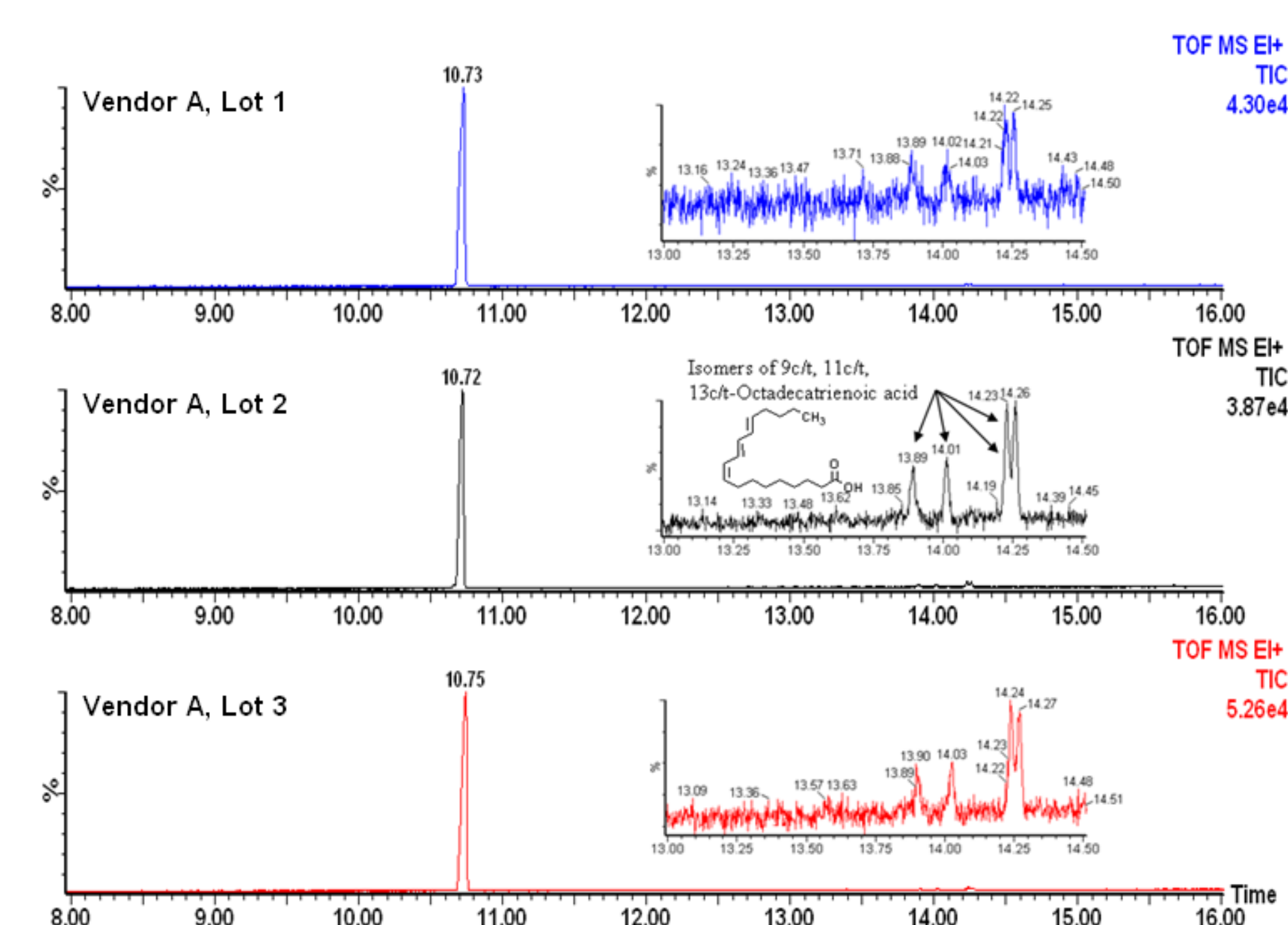


Figure 3: Comparison of three lots of linoleic acid by GC/MS method.

Figure 4 shows comparison of three lots of cholesterol, the peak at retention time 8.61 minutes corresponds to cholesterol. For Lot 2, a peak at retention time 8.45 minutes corresponds to desmosterol, an analog of cholesterol. This contaminant will be assessed for risk in cell culture media and will be quantified with a known standard.

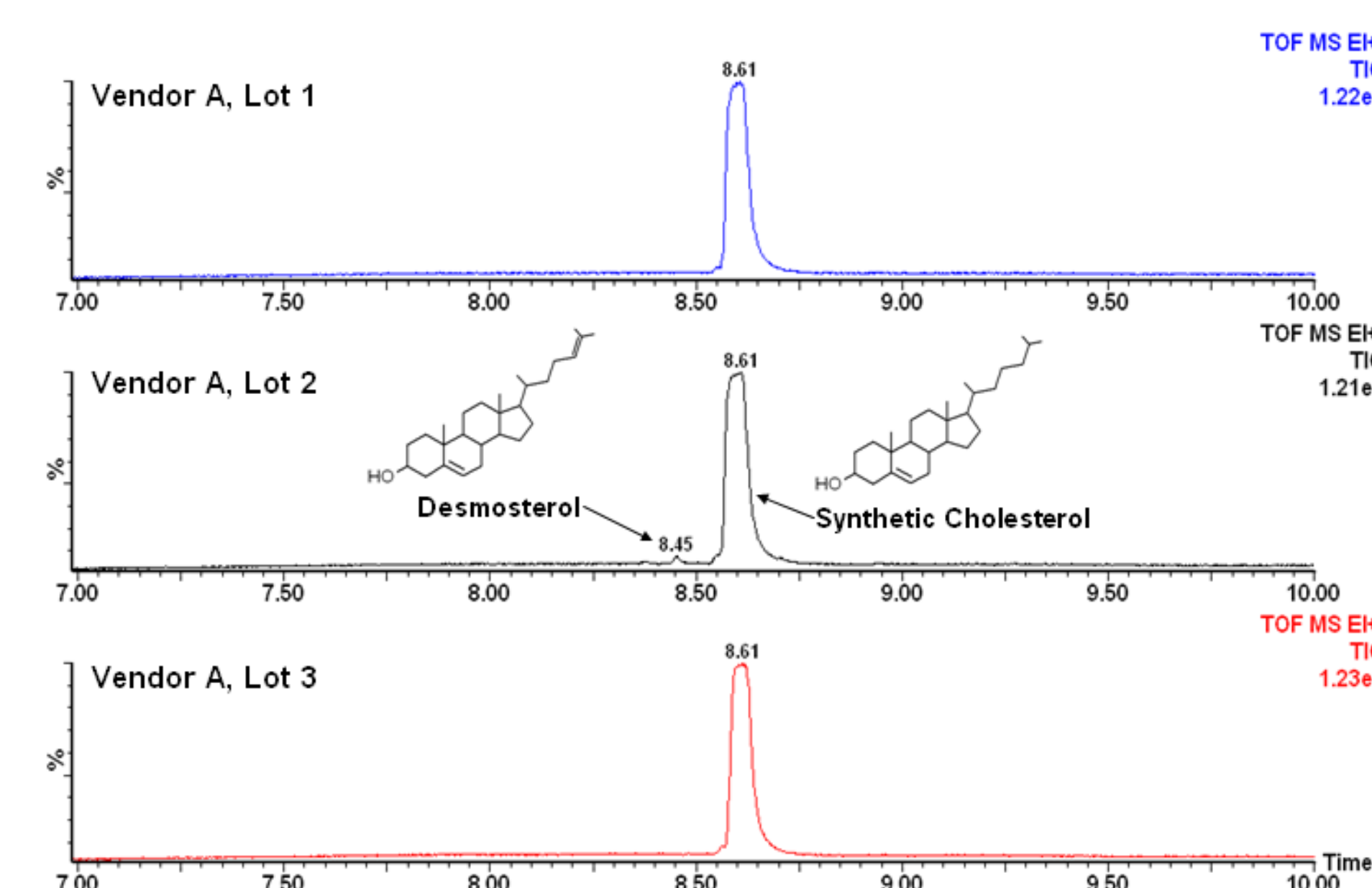


Figure 4: Comparison of three lots of cholesterol by GC/MS method.

Conclusion

The studies presented within this poster demonstrate SAFC's commitment to develop a clear understanding of raw materials that are a critical part of our finished products. The orthogonal methods cited to characterize raw materials have proven to be robust and reliable for the intended purpose. The solubility experiment described for amino acids illustrates a significant difference in solubility limits of amino acids and establishes intra- and inter-vendor variability. This program has helped SAFC get better insight into their suppliers' manufacturing processes. This is a long term initiative within the organization and the most important goal through the program is to develop a better understanding of raw materials to deliver superior products of the finest quality.

References

1. Mohabbat, T., Drew, B., *J Chromatography B*. 2008 Vol. 862; Issues 1-2; p86-92

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