

## Mass Spectrometry

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## Rapid Detection of Vanilla Bean Extract Adulteration with Tonka Bean Extract with No Chromatography or Sample Preparation

### Introduction

Vanilla is the second most expensive spice, and is widely used as a flavoring ingredient in the food, beverage, cosmetic, pharmaceutical and tobacco industries. Pure vanilla bean extract is made by soaking at least 13.35 ounces of vanilla beans in a gallon solution containing a minimum of 35% ethyl alcohol in water.

The production of vanilla beans is quite expensive, since it is a very labor intensive process and harvesting takes place two to three years after planting. Due to this, the price of natural vanilla bean extract is quite expensive. It is quite often adulterated with cheaper tonka bean extract, which smells and tastes like vanilla bean extract due to the presence of a compound called coumarin<sup>1</sup>. Since coumarin is absent in vanilla bean extracts, it can be used as a marker compound to detect its adulteration with tonka bean extracts.

Tonka bean extract is banned for human consumption by the FDA due to its adverse health effects caused by the presence of coumarin. Coumarin is banned in foods based on histological evidence of hepatotoxicity in animal experiments. Coumarin is toxic to the liver and kidneys and causes thinning of the blood. This is particularly dangerous for people taking blood thinning drugs because the interaction of coumarin and blood thinners can increase the likelihood of bleeding<sup>2</sup>.

A variety of analytical techniques such as GC/MS, LC/MS, LC/UV, headspace GC/MS and stable isotope ratio analysis have been used to characterize vanilla bean extracts and detect its adulteration<sup>3-6</sup>. These measurement techniques are either expensive, time consuming, or both, and require extensive method development and sample preparation. In this work, we demonstrated that the AxION® Direct Sample Analysis™ (DSA™) system integrated with the AxION® 2 Time-of-Flight (TOF) mass spectrometer (DSA/TOF) can be used to detect contamination of vanilla bean extracts with tonka bean extracts with no chromatography or sample preparation, and within a few seconds.

## Experimental

Both vanilla bean extracts and tonka bean extracts were purchased from a local supermarket. They were mixed in different proportions to simulate the adulteration of vanilla extracts with tonka bean extracts at different levels. Both extracts and their mixtures were analyzed using the DSA/TOF system with no sample preparation. 10 µl of each sample was pipetted directly onto the stainless mesh of the AxION DSA system. The DSA/TOF experimental parameters are given in Table 1. Total analysis time per sample was 15 seconds. To obtain higher mass accuracy, the AxION 2 TOF instrument was calibrated externally by infusing a calibrant solution into the DSA source at 10 µl/min.

Table 1. The experimental parameters used with DSA/TOF.

Parameter	Value
DSA Heater Temperature	300 °C
Corona Current	5 µA
TOF Acquisition Mode	Pulse
TOF Polarity	Positive Ion Mode
TOF Flight Voltage	-8000 V
Capillary Exit Voltage	90 V
Mass Range	50-1000 Da
Acquisition Rate	10 spectra/s

## Results

Both vanilla and tonka bean extracts and their mixtures were directly analyzed by DSA/TOF, with no sample preparation. Figure 1 and Figure 2 show the mass spectra for vanilla and tonka bean extract in positive ion mode using DSA/TOF, respectively. The mass spectra show that the main compounds, vanillin and coumarin were present in vanilla and tonka bean extract, respectively. The data shows that coumarin can be used as a marker compound to determine the adulteration of vanilla extract with tonka bean extract using DSA/TOF. This is supported further by data in Figure 3 and Figure 4, which shows the

presence of coumarin in a vanilla bean extract sample adulterated with 2 % and 10 % tonka bean extract, respectively. Figure 5 shows the extracted ion chromatogram of coumarin in vanilla bean extract with different amounts of tonka bean extract. The data showed that coumarin was absent in unadulterated vanilla bean extract and the response for coumarin increases with the increase in tonka bean extract amount in the vanilla bean extract. This confirmed that the adulteration of vanilla bean extract with tonka bean extract can be detected by the presence of coumarin. All mass measurements showed good mass accuracy with an error of less than 5 ppm.

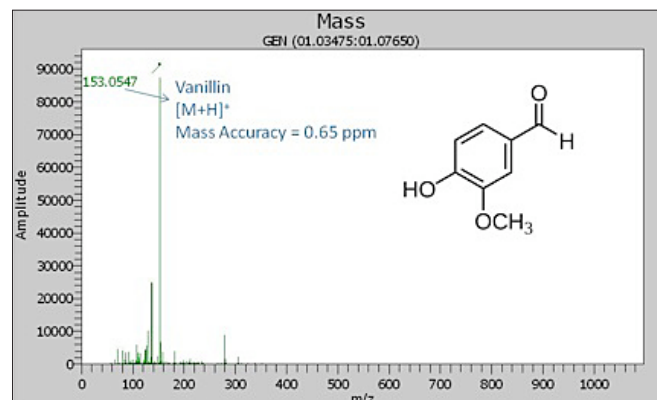


Figure 1. Mass spectra of vanilla bean extract in positive ion mode using DSA/TOF.

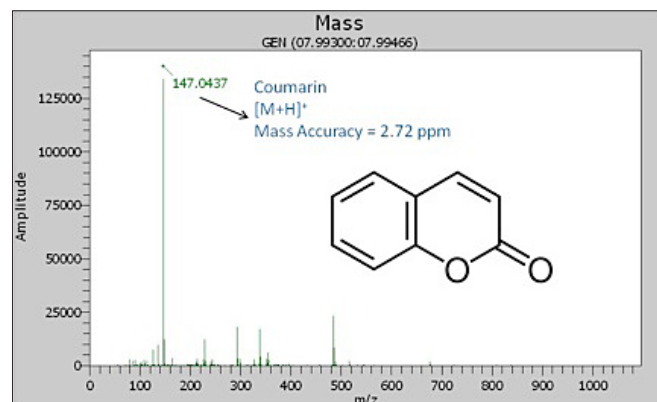


Figure 2. Mass spectra of tonka bean extract in positive ion mode using DSA/TOF.

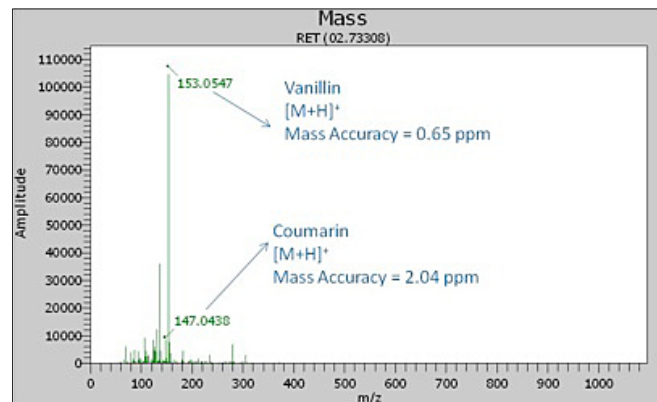


Figure 3. Mass spectra of vanilla bean extract adulterated with 2 % tonka bean extract in positive ion mode using DSA/TOF.

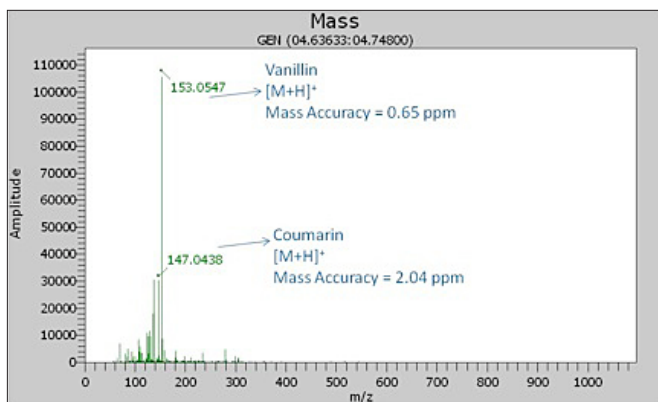


Figure 4. Mass spectra of vanilla bean extract adulterated with 10 % tonka bean extract in positive ion mode using DSA/TOF.

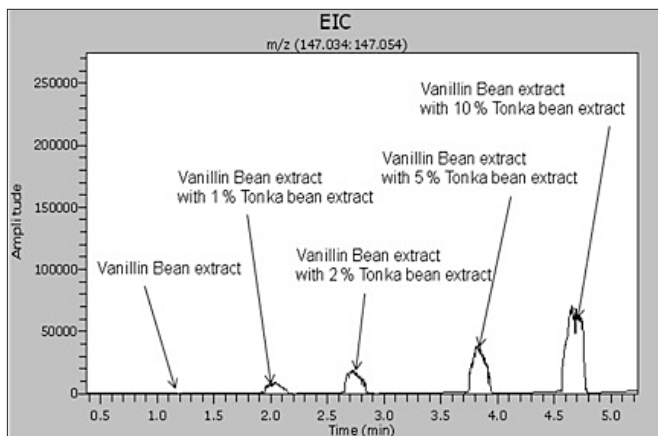


Figure 5. Extracted ion chromatogram for coumarin in vanilla bean extract with different levels of tonka bean extract.

## Conclusion

This work shows the utility of the DSA/TOF for rapid detection of adulteration of vanilla bean extract with tonka bean extract for the determination of food fraud. Our work showed that the presence of coumarin, as a marker compound, in vanilla bean extract can be used to detect its adulteration with tonka bean extract. The mass accuracy of all measurements was less than 5 ppm with external calibration. All samples analysis time, with no chromatography or sample preparation, was 15 seconds per sample. In comparison to other established techniques such as LC/MS, GC/MS and LC/UV and stable isotope ratio analysis, DSA/TOF will improve laboratory efficiency, decrease expenses and testing time.

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