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Rapid Profiling and Authentication of Cinnamon Samples Using Ambient Ionization (DART) and Single Quadrupole Mass Spectrometry

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AIM

Demonstrate the utility and ease-of-use of DART coupled to the ACQUITY® QDa® Detector for the authentication of ground cinnamon and cinnamon sticks based on *Cinnamomum* species.

APPLICATION BENEFITS

Using direct analysis of samples in combination with simple mass detection allows:

- Rapid screening
- Ambient ionization
- Little to no sample preparation
- No chromatography needed
- Ease of use
- Spice profiling

WATERS SOLUTIONS

ACQUITY QDa Detector

MassLynx® MS Software

KEYWORDS

Spices, cinnamon, *C. verum*, coumarin, *C. cassia*, *C. burmannii*, *C. loureiroi*, mass detection, authenticity, DART, ambient ionization, spice profiling, adulteration

INTRODUCTION

Cinnamon is a popular spice used for cooking and baking, plus is also used as an herbal medicine. Cinnamon is derived from the dried inner bark of the *Cinnamomum* tree. There are four species of cinnamon commercially sold for consumption: *C. verum* (Ceylon), *C. cassia* (Chinese), *C. burmannii* (Korintje), and *C. loureiroi* (Saigon). Each variety has a distinct flavor and aroma profile resulting from the essential oil in the cinnamon tree bark.

Due to its popularity, cinnamon is one of the most commonly adulterated spices on the market. *C. verum* is considered the "true cinnamon" and it is more expensive than the other varieties. This leads to adulteration of cinnamon, or even substitution with a lower priced variety.

While there are subtle taste and odor differences between the species of cinnamon, there is an important difference in chemical makeup of the cinnamon varieties. *C. verum* is known to be the only species of cinnamon to naturally contain low levels of coumarin.[1] Coumarin is a compound of concern as it is regarded as a hepatotoxic compound. It produces a sweet smell that made it a popular food additive before it was banned due to its potential toxicity. Therefore substitution of *C. verum* with *C. cassia*, *C. burmannii*, and *C. loureiroi* cinnamon can be concerning.

Typically, analysis to determine cinnamon species is performed using a variety of methods that include sample extraction and chromatographic separation steps. Direct Analysis in Real Time (DART) is an ambient ionization technique[2] that eliminates the needs for time-consuming sample preparation and chromatographic separation. In this application note, we describe a novel approach that employs DART and Waters® ACQUITY QDa Detector for the simple and rapid analysis of cinnamon samples to determine species origin. Whole stick and ground cinnamon samples were successfully analyzed using this DART-MS technique and species identifications on store bought cinnamon samples are provided.

EXPERIMENTAL

DART conditions

Ionization mode:	+
Temp.:	450 °C
Sampling speed:	1.0 mm/sec
Grid voltage:	350 V

MS conditions

MS system:	ACQUITY QDa (Performance Option)
Ionization mode:	+
Cone voltage:	5 V
Mass range:	50 to 500 amu
Acquisition speed:	2 Hz

Sample analysis

A variety of cinnamon samples were purchased from local retailers. Cinnamon of varying price points were acquired for this sample analysis. Ground cinnamon (12 samples) and whole cinnamon sticks (3 samples) were tested. By examining the labels and researching manufacturers websites, it was determined that known samples of *C. verum*, *C. cassia*, *C. burmannii*, and *C. loureiroi* were available in the ground cinnamon testing group. However, eight of the remaining ground cinnamon samples were vague on their label claims, and as a result were treated as unknown samples during the analysis.

Cinnamon sticks were broken into smaller pieces prior to analysis and held in front of the heated helium DART beam with a pair of tweezers to perform sampling. The ground cinnamon samples were introduced onto the QuickStrip cards (see Figure 1) by dipping a cotton swab into the sample and then rubbing the swab over the mesh screen of the card's sampling area. Most of the ground cinnamon fell through the screen, but enough of a residue was left behind for analysis. Sampling in this manner maintained the cleanliness of the ACQUITY QDa source.

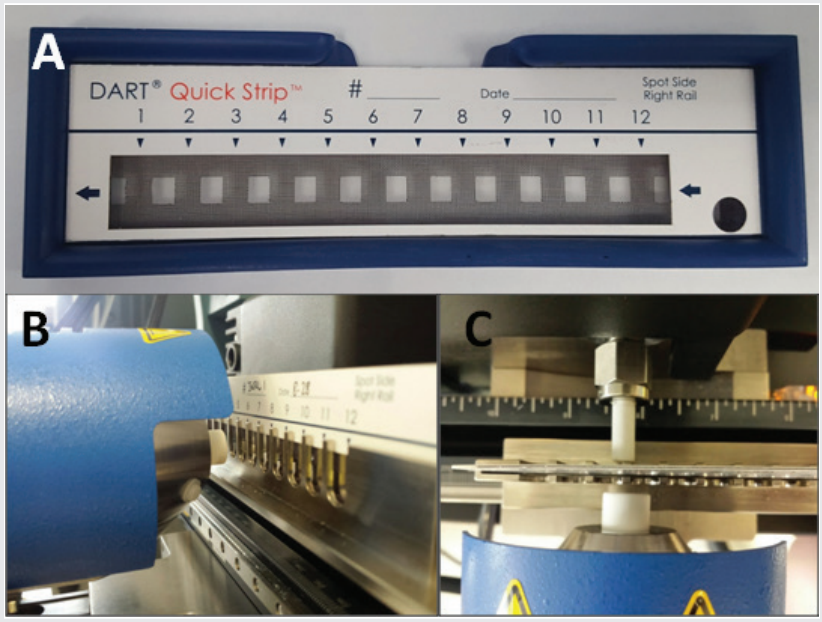

Figure 1. A. 12 spot QuickStrip card used for sampling; B. QuickStrip card automatically being moved into the heated helium ionization beam; and C. Ceramic tube pulling ions into the ACQUITY QDa source (top down look at the DART source).

RESULTS AND DISCUSSION

CINNAMON STICK ANALYSIS

Of the three cinnamon stick samples available for analysis, one was of the *C. verum* species, and two were of the *C. burmannii* species. Representative mass spectra of each species are presented in Figure 2. The spectra are not complex, but the zoomed insets of Figure 2 highlight the important features. The distinguishing feature between the two species analyzed in stick form is the ratio of cinnamaldehyde (*m/z* 133) to coumarin (*m/z* 147). *C. verum* is known to contain very small levels of coumarin, which was verified by this analysis. In contrast, *C. burmannii* is known to contain high levels of coumarin, again verified by the DART-MS analysis of the cinnamon stick.

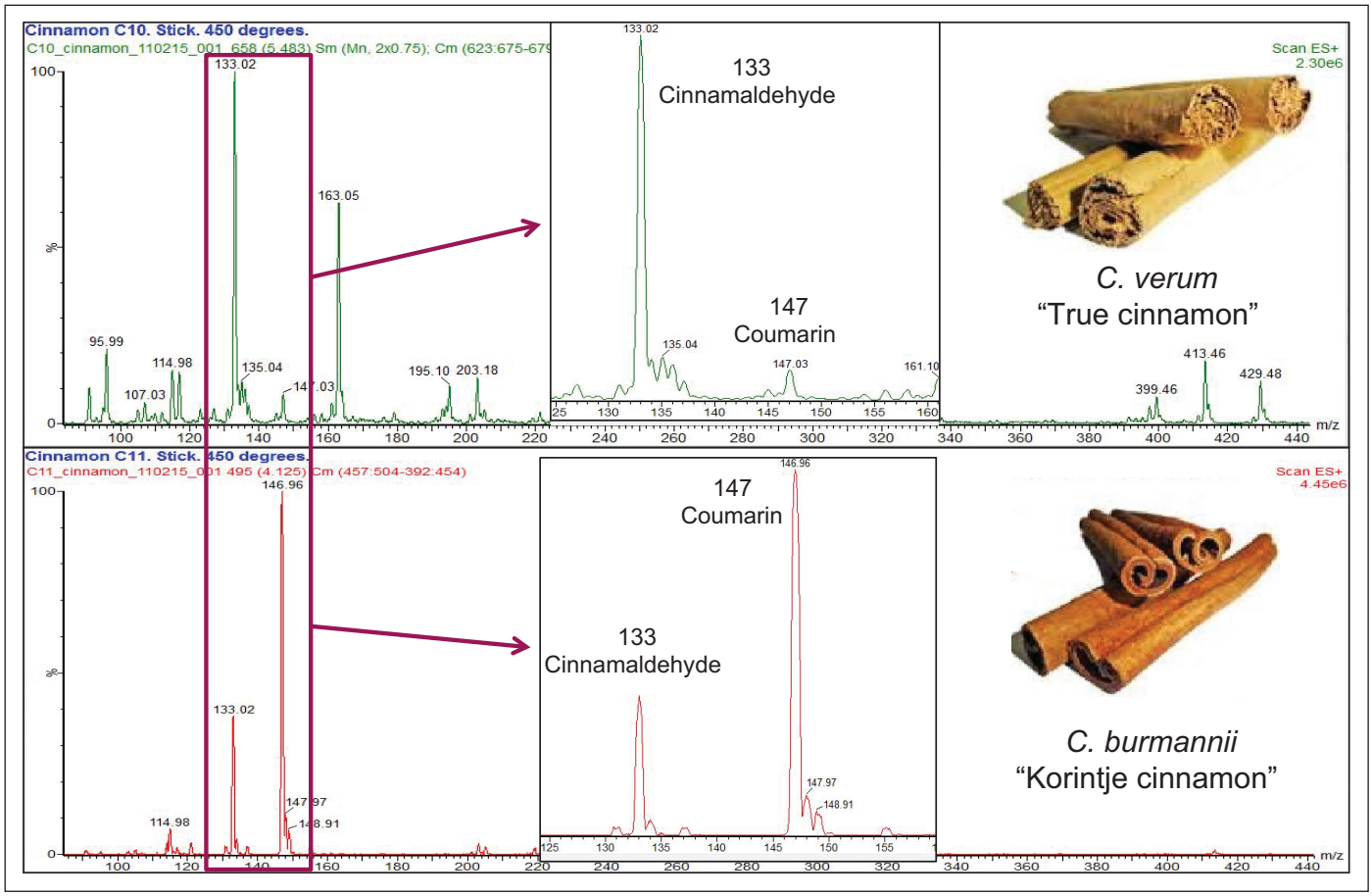

Figure 2. DART-MS analysis of whole cinnamon sticks. Mass spectrum for C. verum (top); mass spectrum for C. burmannii (bottom).

GROUND CINNAMON ANALYSIS

Analysis of ground samples of the different cinnamon species was also performed to determine if the ground spices could be distinguished by species as well. Ground spice analysis was performed by rubbing the sample onto a 12 position QuickStrip card. Analysis of the entire 12 sample card was performed in less than 5 minutes. Among the 12 ground cinnamon samples purchased, four were labeled with the derived cinnamon species. The remaining samples were treated as unknowns. Similar to the cinnamon stick analysis, the mass spectra were not complex, although there were distinguishing features present. Figure 3 shows the spectra obtained from the DART-MS analysis of the four known ground cinnamon samples. The Ceylon sample contained strong cinnamaldehyde and methyl cinnamate (*m/z* 163) peaks, but did not contain the presence of coumarin. The *C. burmannii* and *C. loureiroi* species both contained coumarin as the dominant peak, a smaller cinnamaldehyde peak, and only the *C. loureiroi* contained substantial amounts of methyl cinnamate. The *C. cassia* sample contained cinnamaldehyde as the dominant peak, and it also contained smaller levels of coumarin and methyl cinnamate. The DART-MS analysis allowed the unique characteristics for the four ground cinnamon samples to be distinguished from one another.

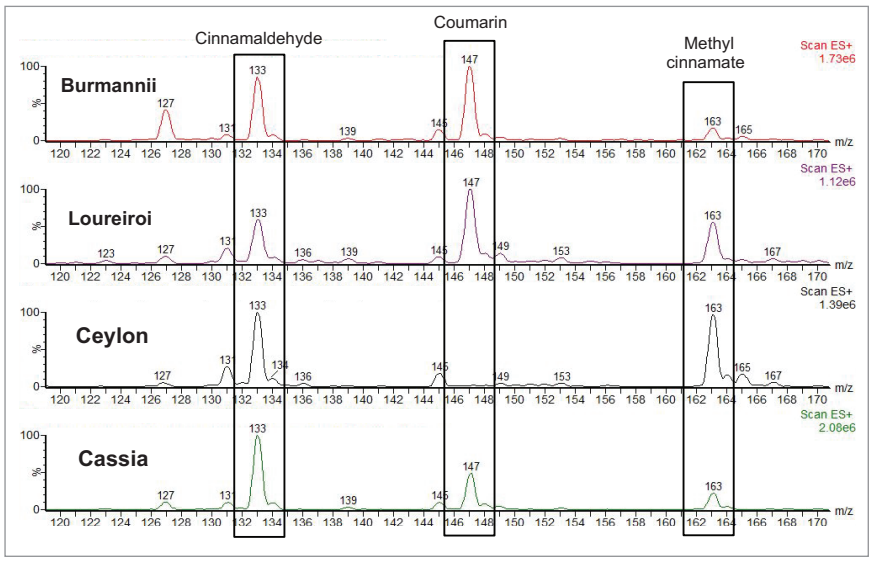

Figure 3. DART-MS analysis of four known ground cinnamon species.

Table 1 lists the species identifications made using the DART-MS analysis for all of the cinnamon samples. Of the 12 ground cinnamon samples tested, besides the sample used as the known high quality reference sample, only one other ground cinnamon indicated it may contain *C. verum* (Ceylon). This was sample C5, and it was identified as a "Ceylon mix" because the mass spectra contained large cinnamaldehyde and methyl cinnamate peaks, in agreement with Ceylon cinnamon, but it also contained a mid-sized coumarin peak. Since Ceylon cinnamon is the only species to contain high levels of methyl cinnamate, this suggests that this sample could be a mix of Ceylon cinnamon and a cheaper coumarin containing cinnamon. The species of the remainder of the ground cinnamon samples were easily identified based upon the characteristics of their mass spectra.

Sample	Form	Species Identification
C1	ground	*C. burmannii*
C2	ground	*C. burmannii*
C3	ground	*C. cassia*
C4*	ground	*Ceylon*
C5	ground	*Ceylon mix*
C6*	ground	*C. Loureiroi*
C7	ground	*C. burmannii*
C8*	ground	*C. burmannii*
C9	ground	*C. burmannii*
C10*	stick	*Ceylon*
C11*	stick	*C. burmannii*
C12*	stick	*C. burmannii*
C13	ground	*C. cassia*
C14	ground	*C. burmannii*
C15*	ground	*C. cassia*

*Table 1. Identifications of the unknown cinnamon samples using DART-MS analysis. * Indicates that the species of the sample was known prior to analysis, as determined from manufacturer information.*

CONCLUSIONS

The cinnamon analysis demonstrated that DART coupled to the ACQUITY QDa Detector can be applied to the analysis of both whole and ground spice samples. The analysis of up to 12 samples can be performed in less than 5 minutes, providing a rapid screening technique. DART-MS analysis was also able to determine the unique characteristics between the known reference samples that could be used to easily and rapidly distinguish cinnamon species of the unknown samples. This technique could easily be utilized for authenticity or food safety applications.

References

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