DART Enables Chromatography-Free Analysis of Cinnamon Products

Abstract

The asignment of cinnamon species is of interest because of differences price and coumarin content.
This application note highlights the application of Bruker's Direct Analysis in Real Time (DART) chromatography-free ionization, in combination with two designated sampling modules, for rapid and robust analysis of solid and liquid cinnamon samples. The powerful combination of DART and QTOF mass spectrometry not only allows for the classification of unlabeled samples, but also to address adulteration or contamination, while simultaneously eliminating the need for the time consuming steps of sample preparation and chromatographic separation.

Keywords:
Sample Preparation-free; DART; QTOF; Chromatography-free; Cinnamon; Food analysis

Introduction

The spice cinnamon

Cinnamon is a spice made from the inner bark of trees from the genus *Cinnamomum*. The distinct flavor of cinnamon has brought it to widespread use as an aromatic additive in many cuisines across the world in both sweet and savory dishes, as well as a scent in fragrances and cosmetics. The spice is available in a ground powder form or as whole sticks.

Cinnamon primarily contains essential oils and other compounds, such as cinnamaldehyde, cinnamic acid, and cinnamate. Some studies have suggested that cinnamaldehyde in cinnamon has antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties, and that it might offer protection from cancer and cardiovascular disease[1].

Ilona Nordhorn and Carsten Baessmann, Bruker Applied Mass Spectrometry, Bremen, Germany

Species of cinnamon

There are two main species of cinnamon: Ceylon *(Cinnamomum verum)* and Cassia *(Cinnamomum cassia)*, both of which have different flavor, nutritional and chemical profiles. *C. verum* is considered "the true" cinnamon with a light and less bitter taste whereas *C. cassia* has a stronger, spicier flavor. Cassia is the most consumed type of cinnamon around the world, but it is considered lower quality making it a more cost-effective option for consumers.
An important compound found in many types of cinnamon is the phenylpropanoid coumarin. It occurs naturally in several plant species but can be harmful to animals and humans in large doses. Research suggests that coumarin may be hepatotoxic and carcinogenic[2].

Therefore, guidelines set by the European Food Safety Authority (EFSA) suggest the tolerable daily intake (TDI) of coumarin to be 0.1 mg coumarin/kg body weight[3].

While *C. cassia* contains significant amounts of coumarin, *C. verum* is the only Cinnamomum species that naturally contains only trace levels of coumarin. Therefore, it is recommended that Ceylon cinnamon is consumed over Cassia cinnamon for frequent cinnamon consumers[4].

Differentiating cinnamon species

Ceylon and Cassia cinnamon as raw products can be easily differentiated macroscopically by their color and layer structure sa shown in Figure 1.

With ground cinnamon, however, macroscopical differentiation is not possible. Therefore, in many commercially available cinnamon products, the species is often unclear and not declared on the label resulting in a lack of transparency for consumers.

With the prevalence of Cassia cinnamon on the market, there is a need to develop a rapid technique that can easily differentiate it from Ceylon cinnamon.

Figure 1. Whole bark sticks from Cinnamomum ceylon (left), *Cinnamomum cassia* (middle) and ground cinnamon (right). Differences in color and layer structure are immediately apparent at the whole bar sticks.

Rapid and reliable chromatography-free analysis

Food samples can be difficult to analyze because of their solid or powdered form. Because chromatographic methods are unsuited to solid form testing, products must first be extracted or dissolved into solution, adding a time-consuming sample preparation step that can introduce errors and potential contaminants.

DART is an ambient ionization technique that eliminates the need for chromatographic separation and time-consuming sample preparation. Food samples can be analyzed directly, enabling instantaneous results.

This is particularly valuable for point-of-need applications where reliable quantitative and qualitative analyses are needed quickly. Operating at ambient pressure, DART can be used in the laboratory or onsite to analyze samples in seconds rather than hours with chromatographic methods. The DART chromatography-free workflow reduces consumable usage for an improved total cost of ownership and environmental footprint.

The analysis of ground cinnamon, cinnamon sticks and processed foods containing cinnamon is presented.

Benefits of DART-QTOF-MS

Figure 2. JumpShot DART source coupled to an impact II VIP

Experimental

Analysis of cinnamon sticks and cereals: Tweezer module

Store-bought whole cinnamon sticks labeled *C. cassia* and *C. verum* and cereals with cinnamon were investigated.
The samples were introduced into the helium stream via the Tweezer module (Figure 3A) which includes a self-closing laboratory tweezer. The Tweezer module allows the presentation of the sample accurately for analysis.

Analysis of grounded cinnamon and herbal tea: DIP-it module

Eleven ground cinnamon products commercially available in local markets and supermarkets were investigated. On six of these products, the species was mentioned on the packaging: Ceylon (3), Cassia (1), Pedang (1) and Ceylon-Cassia Mix (1). The other five samples had no species information. Furthermore, a herbal tea (Chai tea) was prepared according to the product instructions. Both, the ground cinnamon samples and the herbal tea were analyzed using DIP-it tips.
A DIP-it tip is a glass stick with a closed lower end (Figure 3B). It can be used to analyze liquids and powders by dipping directly into the sample. The upper end of the tip has an adapter that allows users to connect the tip to a pipette for ease-of-use.
For automated analysis, the DIP-it tips were placed into the DIP-it tip holder module.

Instrumentation

All analyses were carried out using a JumpShot DART source coupled to an impact II VIP quadrupole time-of-flight (QTOF) mass spectrometer (both Bruker). Helium was used as the ionization gas and all analyses were carried out in positive ionization mode. Applied heater temperatures are summarized in Table 1.
The software MetaboScape® (Bruker) was used for PCA analysis.

Table 1. DART source temperature.

Sample	Heater temperature/ °C
Cinnamon sticks	300
Herbal tea	350
Cereal	350
Ground cinnamon	425

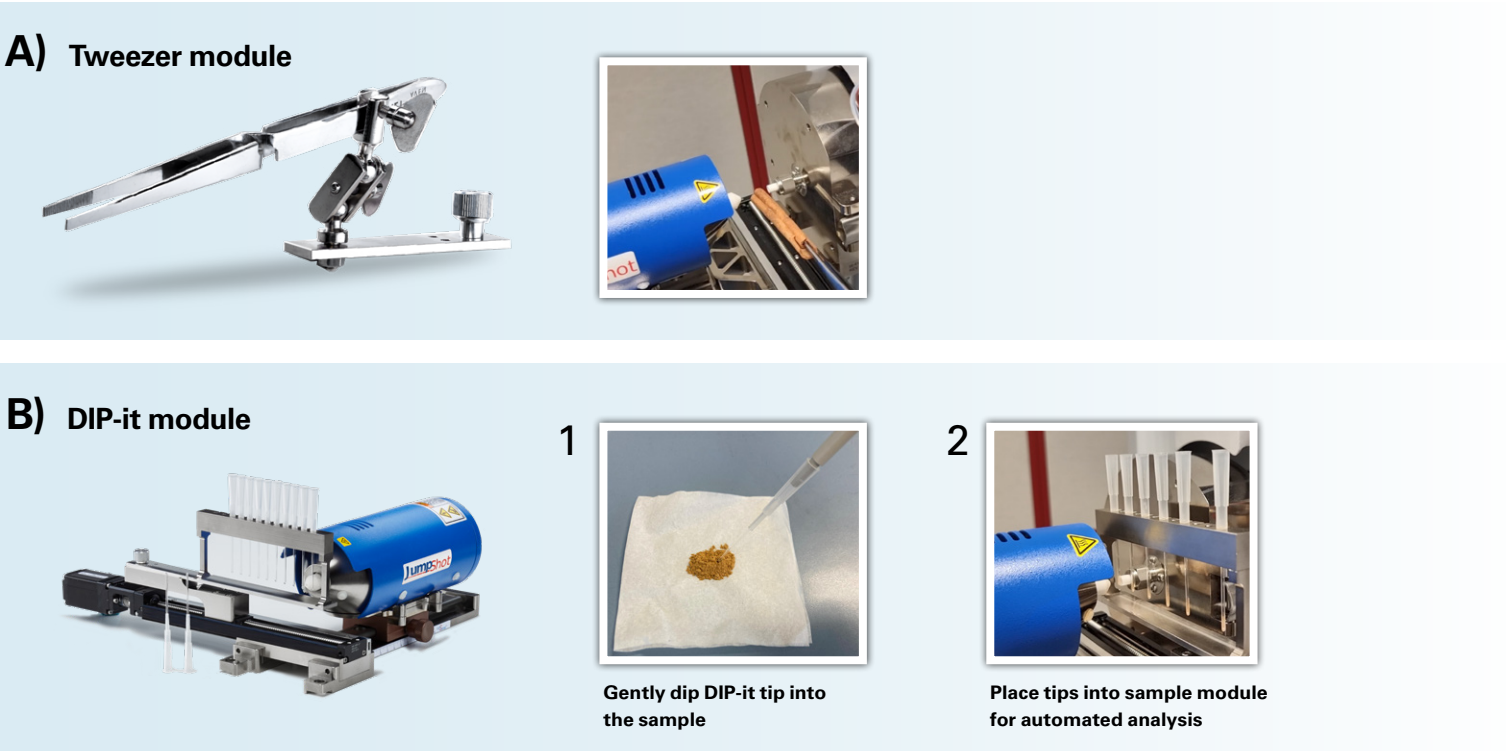

Figure 3. DART sample introduction options: Tweezer module (A) and DIP-it module (B).

Results

Figure 4 shows the acquired mass spectra for the investigated cinnamon samples. For the cinnamon sticks, the mass spectrum for the Cassia cinnamon stick was dominated by an intense signal at *m/z* 147.044 for coumarin, as expected. No coumarin was detected in the Ceylon cinnamon stick. Coumarin was detected in both, the herbal tea and cereal. Further typical compounds found in the samples were cinnamaldehyde and methyl cinnamate.

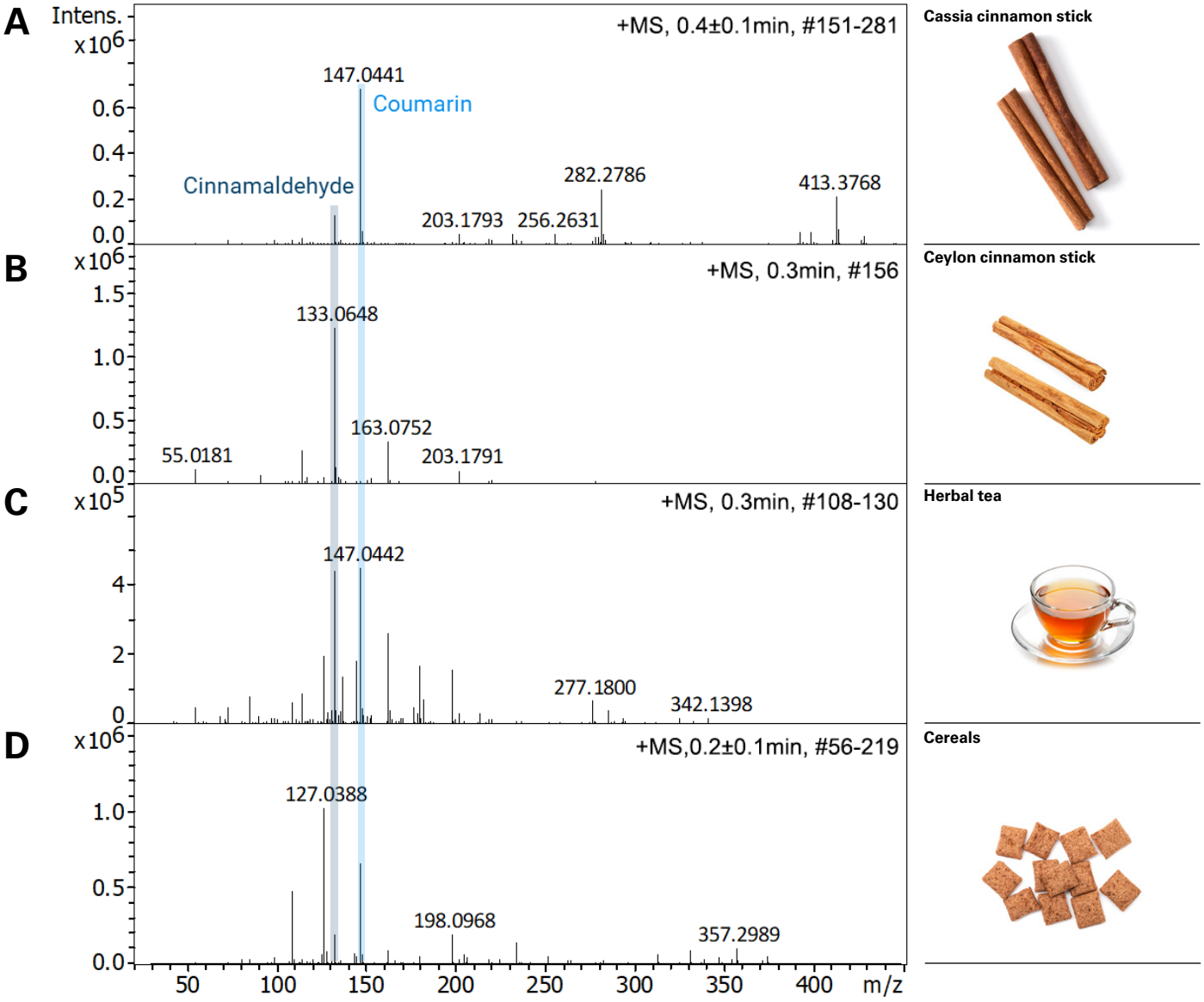

Figure 4. Results of the analysis of Cassia (A) and Ceylon (B) cinnamon sticks, as well as herbal tea (C) and cereal (D) by DART-QTOF-MS.

Acquisition of MS/MS spectra

Using Bruker's QTOF mass spectrometers, initial annotation of compounds can be made based on the accurate mass and isotopic pattern supported by the high mass accuracy and mass resolution.
For further annotation confidence, tandem mass spectrometry MS/MS capabilities of the QTOF-MS instruments covering data-dependent acquisition (DDA) and data-independent acquisition (DIA) modes allow for the generation of fragment information. In this study, full MS scans and MS/MS spectra were acquired alternately so that information on both the spectral fingerprint of the sample and compound structure are obtained obtained in a single measurement. MS/MS spectra for coumarin and cinnamaldehyde received from the analysis of cinnamon sticks are presented in Figure 5.

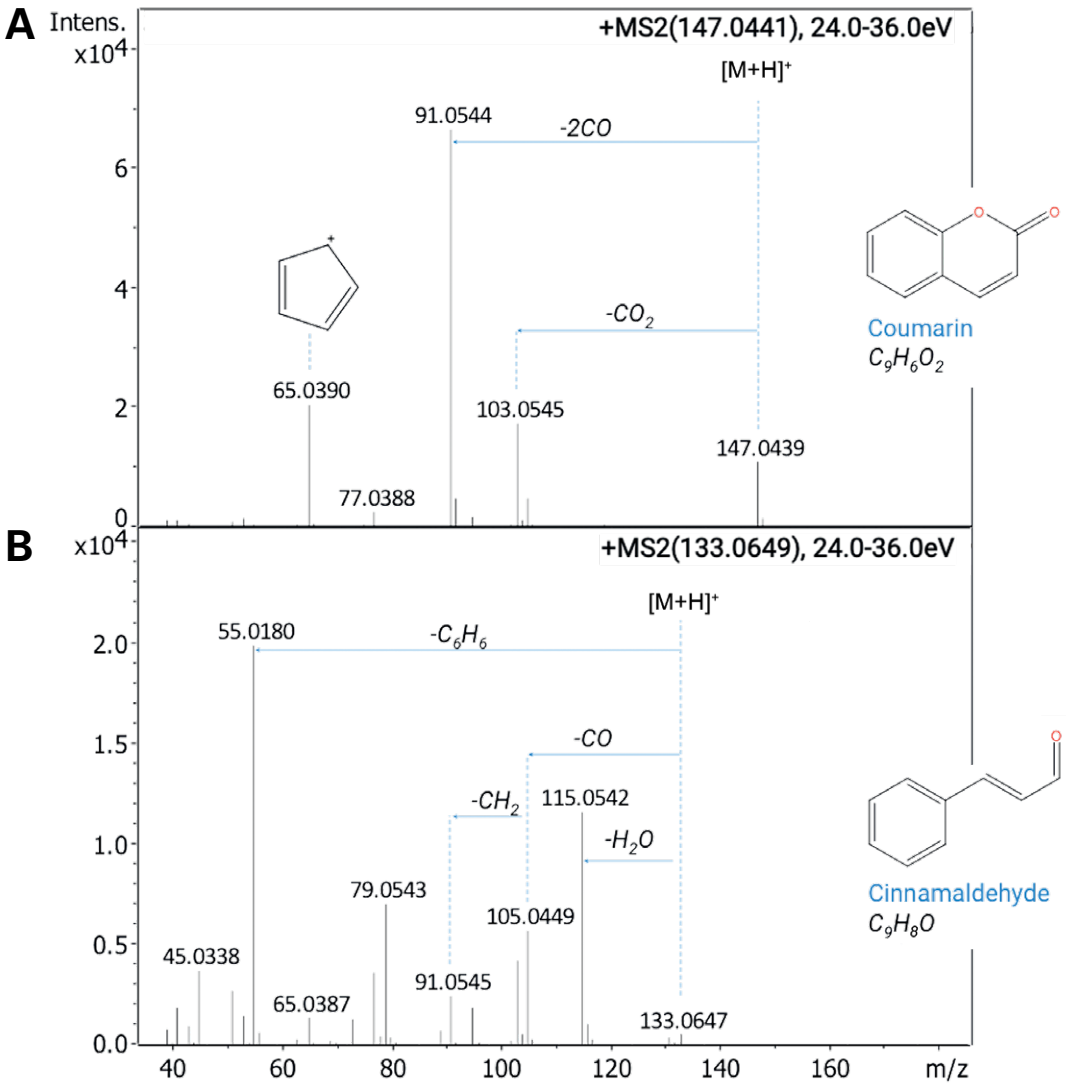

Figure 5. MS/MS spectra for coumarin (A) and cinnamaldehyde (B) acquired with DART-QTOF-MS/MS from cinnamon sticks.

Differentiation of ground cinnamon samples

Commerically available ground cinnamon products with and without species labeling were analyzed directly with the DART-HRMS setup. The results suggest that none of the unlabeled samples are pure Ceylon cinnamon, as the acquired mass spectra all show a high abundant signals for coumarin. Furthermore, principal component analysis (PCA) revealed distinctive patterns in the data. The three Ceylon samples clustered closely together, while the unknown samples were positioned near the Cassia and Pedang samples (Figure 6).

This method shows the capability of chromatography-free DART-HRMS for rapid screening analysis without any sample preparation. It not only enables unlabeled sample identification, but also addresses adulteration or contamination.

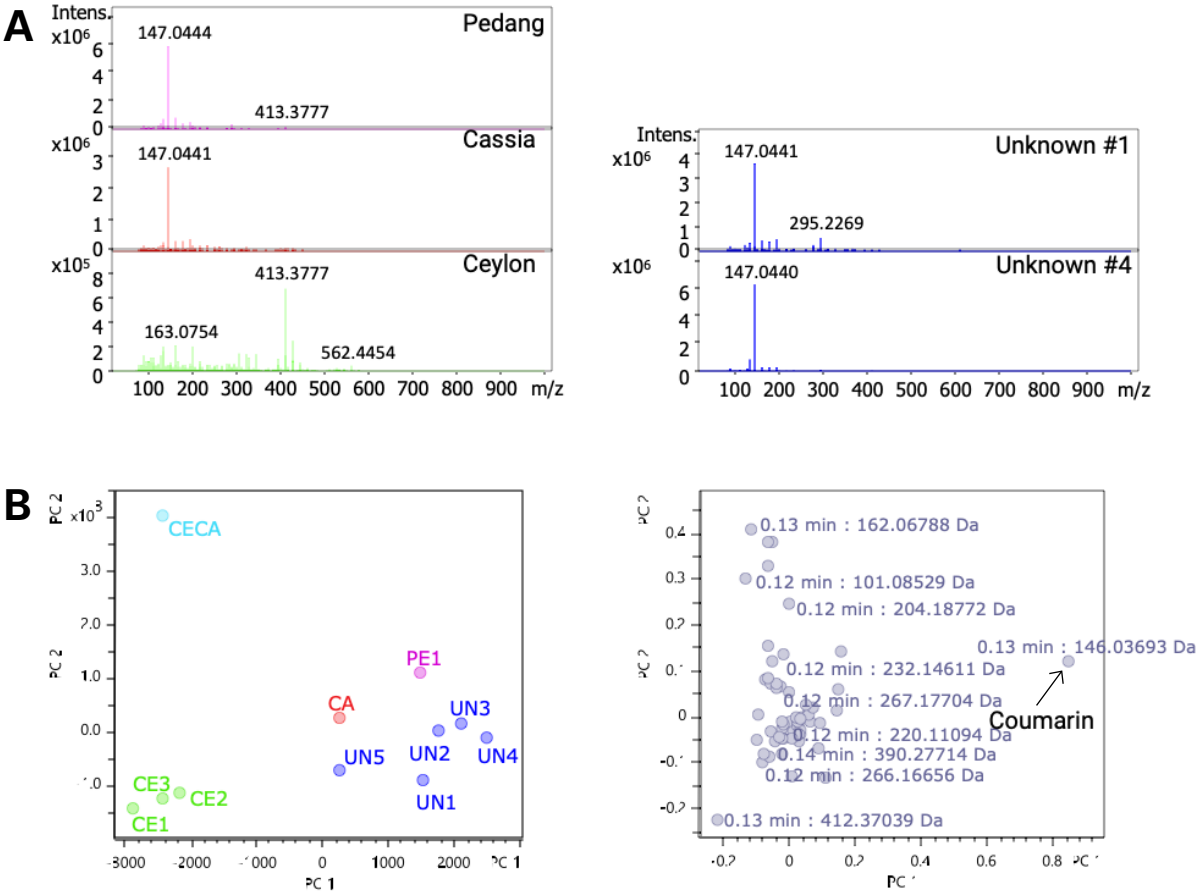

Figure 6. Mass spectra for the different ground cinnamon samples (A). Results of PCA analysis obtained in MetaboScape (B). Sample labels: Ceylon (CE), Cassia (CA), Pedang (PE), Ceylon-Cassia Mix (CECA), Unknown (UN).

Summary

We here presented the application of DART ionization in combination with Quadrupole Time of Flight Mass Spectrometry for the rapid analysis of solid and liquid cinnamon samples including ground cinnamon, cinnamon sticks, herbal tea and cereals. The samples were analyzed directly in their solid or liquid state without any sample preparation. In combination with the chromatography-free analysis, this can lead to a noticeable reduction in use of consumables and solvents. Due to the use of a QTOF mass spectrometer, full MS scans and MS/MS spectra were acquired alternately in a single run so that both the spectral fingerprint of the sample and structural information for labeling the compounds were obtained. With this method, coumarin and further typical compounds like cinnamaldehyde and methyl cinnamate were detected, enabling a confident differentiation of the two cinnamon species *Cinnamomum cassia* and *Cinnamomum ceylon*. The results demonstrate the suitability of chromatography-free DART-HRMS to be applied for the identification of unlabeled cinnamon samples. Furthermore, the detection of possible adulteration or contamination is enabled by the non-targeted screening approach.

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Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

marketing.bams.emea@bruker.com - www.bruker.com

linkedin.com/company/brukerappliedmassspec

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