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RESEARCH ARTICLE

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# Rapid detection and validated quantification of psychoactive compounds in complex plant matrices by direct analysis in real time-high resolution mass spectrometry – Application to "Kava" psychoactive pepper products

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**Rationale:** Classified by the UNODC as a top 20 plant of concern, *Piper methysticum* (also known as Kava) is being increasingly abused recreationally for its mind-altering effects. It is of significant forensic relevance to establish methods to rapidly identifyand quantify psychoactive compounds, especially those yet to be scheduled ascontrolled substances and which have exhibited various noteworthy health concerns.

**Methods:** Direct analysis in real time high-resolution mass spectrometry (DART-HRMS) demonstrated the ability to detect a range of *kavalactones* in *Pipermethysticum* derived products and plant material with no sample preparation. Inaddition, a validated method using calibration curves developed with a deuteratedinternal standard was used for the quantification of the psychoactive moleculeyangonin in various products.

**Results:** DART-HRMS detected the protonated masses of six major kavalactonesand three flavokavains in 18 commercial Kava products. A method consistent withFDA validation guidelines was established for the quantification of yangonin in thevarious complex matrices. Implementation of this method, with an LLOQ of 5 mg/mL, enabled successful quantification of yangonin in 16 Kava products.Concentrations for solid products ranged from 2.71 to 8.99 mg/g, while that forliquid products ranged from 1.03 to 4.59 mg/mL.

**Conclusions:** Rapid identification and quantification of psychoactive smallmolecules in plant material can be accomplished using a validated DART-HRMSprotocol. This work illustrates an approach to qualitative and quantitative analysesof a wide variety of complex matrices derived from plants, and demonstrates thatthe commercially available products analyzed are *P. methysticum* derived and docontain psychoactive yangonin at quantifiable levels.

## 1 | INTRODUCTION

In 2013, the United Nations Office on Drugs and Crime (UNODC) published a report assessing the challenges associated with new emerging psychoactive substances and presented a list of 20 plant-based substances of increasing concern.[1] One of these is *Piper methysticum* (also known as Kava), a seedless, slow-growing pepper shrub that is native to the South Pacific Islands and used ceremonially, medicinally, and recreationally.[2] Products of this plant are available in grocery stores and markets, are widely accessible over the internet, and can be obtained as freshly prepared beverages from Kava bars and lounges. Kava supplements remain commercially

  

available in the United States (US), typically sold as tablets and capsules. Powders, roots and extracts are other forms of Kava obtainable for purchase. While Kava consumption has been an important tradition in Southern Pacific ceremonies with a long history of safe use, it is increasingly being abused around the world for its mind-altering effects.[3] Kava is not classified as a controlled substance in the US but is regulated in several other countries.[3]

The main psycho- and pharmacologically active components of *P. methysticum* plants and derivative products are lipid-soluble kavalactones. A total of 18 have been identified, with six classified as major and which comprise 96% of the total number of kavalactones present.[4] These compounds are responsible for the anxiolytic[3,5-7] and sedative[3,6] effects associated with Kava usage.[3] Factors such as geographic source, plant maturity at time of harvest, the plant organ from which the product is derived and the presence of contaminants and impurities, have been shown to affect the concentrations of the six major kavalactones.[2,3,8-10] The highest concentration of kavalactones, particularly yangonin, has been found in the roots, rhizomes and stumps of the Kava plant, with their concentrations decreasing in the aerial parts.[2,11,12]

One of the primary concerns associated with chronic Kava use is hepatotoxicity.[2,4,6,8,13] Yangonin has a higher potency than other kavalactones,[4] and shows toxic effects at low concentrations.[8,14] Flavokavains have been proposed to also contribute to the hepatotoxicity of Kava[15] but the potentially harmful properties of these compounds have not been fully investigated. Proposed rules for a standardization code for quality control for Kava have been made, with consideration given to the health concerns associated with Kava preparation and consumer safety.[3,5,7,16,17]

Legislation prohibiting the sale and export of what are known as "non-noble" or non-medicinal Kava varieties has been drafted over the years. The success of these efforts has hinged on the availability of reliable approaches for the identification of Kava products and quantification of individual components. Techniques used have included gas chromatography (GC),[18-20] liquid chromatography/mass spectrometry (LC/MS),[21,22] high-performance[10,18,23,24] and ultra-performance[25] liquid chromatography (HPLC and UPLC) and supercritical fluid chromatography (SFC),[18,26] among others.[18,22,27-31] The approaches differ in terms of speed, sensitivity, selectivity and the extent to which they can be readily applied to both qualitative and quantitative studies, as well as the level of sample pretreatment required. Furthermore, they all exhibit varying levels of difficulty in detection and quantification of yangonin. Their facile thermal decomposition, limitations regarding separation time and peak resolution, and difficulties with complex matrix analysis are typical of the challenges encountered in the detection and quantification of kavalactones. Poor stability in certain solvent systems,[20] calibration curves with low $R^2$ values,[30] sample degradation,[22] insufficient separation[22] and *cis*/*trans* photoisomerization in aqueous and methanolic solvents[15,18,21,22,28] are complicating factors in the detection and quantification of yangonin using these methods. Therefore, there continues to be a need for the development of suitable approaches for the simultaneous identification of a product as being *P. methysticum* derived, and for the quantification of its key psychoactive component, yangonin.

Direct analysis in real time high resolution mass spectrometry (DART-HRMS) is an ambient ionization mass spectrometric technique that provides immediate mass spectral data.[32] Samples can be analyzed directly in their native form to obtain identifying chemical fingerprint signatures, and typically, little to no sample preparation is necessary.[32,33] Successful detection of both psychoactive and non-psychoactive components of plants using DART-HRMS has been accomplished.[34-40] Although DART-HRMS has already been demonstrated to be effective in the detection of kavalactone components in *P. methysticum*,[39] it has not been reported for the quantification of any of these compounds. Given the advantages that this ambient ionization mass spectrometric approach might offer relative to conventional methods, and the increasing number of laboratories that are utilizing this technology in a forensic context, we embarked on this study to assess its utility in quantification of analytes of interest. Of particular importance for such analyses is the availability of validated methods that can be used as standard operating procedures, but very few validated protocols for the quantification of kavalactones have appeared. Reported approaches have exploited the use of LC/MS,[22] HPLC/UV[23] and SFC.[26]

Although relatively few reports on the application of DART-HRMS to the quantification of small molecules have appeared, recent studies indicate that the technique potentially has widespread applicability.[37,40-43] It provides not only a fast and efficient approach to the rapid screening and identification of constituents in plant material, but also allows for the quantification of compounds of interest. Accordingly, we demonstrate here an approach to detection of a range of kavalactones in Kava products, the development of a validated method for the quantification of yangonin, and the application of the protocol to the detection and quantification of kavalactones in an assortment of 18 commercially available Kava products.

## 2 | EXPERIMENTAL

### 2.1 | Kava products

Happy Kava Brand Micronized Powder, ISA Powdered Kava Root, Rough Cut Whole Kava Root, Kona Kava Farm Kava Root Only Capsules, Happy Kava Brand Kava Passion Flower Tincture Blend, Happy Kava Brand Kava Valerian Tincture, Root of Happiness Liquid Extract, Kavalactone Paste 55%, and Zend Kava Supplement were all purchased from Kava Dot Com (West Seneca, NY, USA). Hawaiian Kava Capsules and Hawaiian Kava Extract were purchased from Maui Medicinal Herbs (Makawao, HI, USA). Kava Kava Root Capsules were purchased from Starwest Botanicals (Sacramento, CA, USA). Kava Kava Liquid Tincture was purchased from Herbal Island (UT, USA). Kava Kava Root, Kava Kava Root Powder, and Kavalactones 70% Powder were purchased from Bouncing Bear

Botanicals (Lawrence, KS, USA). Vanuatu Ceremonial Kava Powder was purchased from Herb Stomp (Portland, OR, USA). Kava Kava Liquid Capsules were purchased from Whole Foods Market (Colonie, NY, USA). Kava 40% Powder was purchased from eBay (http://ebay.com, USA).

## 2.2 | Chemical standards

Yangonin standards were purchased as solids from Cayman Chemical (Ann Arbor, MI, USA). A yangonin-$d_3$ standard in solid form was purchased from Toronto Research Chemicals (North York, ON, CA). Ethanol was purchased from Sigma-Aldrich (St. Louis, MO, USA).

## 2.3 | DART-HRMS spectral acquisition and data processing

DART-HRMS spectra of standards, plant material, and commercially available Kava products were obtained using a DART-SVP ion source (IonSense, Saugus, MA, USA) coupled to an AccuTOF high resolution time-of-flight mass spectrometer (JEOL USA, Peabody, MA, USA) in positive ion mode. Parameters for the DART ion source were as follows: grid voltage, 250 V and heater temperature, 350°C. Settings for the mass spectrometer were: ring lens voltage, 5 V; orifice 1 voltage, 20 V; orifice 2 voltage, 5 V; and peak voltage, 600 V. Mass spectra were collected over a range of *m/z* 60–1000 at a rate of 1 spectrum per s. The helium flow rate for the DART ion source was 2 L $min^{-1}$. The mass spectrometer had a resolving power of 6000 FWHM (full width at half maximum) and a mass accuracy of 5 millimass units (mmu).

Mass spectral data calibration, spectral averaging, background subtraction, peak centroiding, and peak integration were completed using TssPro3 software (Shrader Software Solutions, Detroit, IL, USA). Polyethylene glycol (PEG 600) was used for mass calibration of all calibrators and commercial Kava products. Mass spectral analysis and isotope analysis were performed using Mass Mountaineer (RBC Software, Portsmouth, NH, USA).

Rapid screening of the Kava products to confirm the presence of the six major kavalactones was conducted for all samples. For analysis of powders, capsule contents, tinctures, liquids and pastes, the closed end of a melting point capillary was dipped into the sample, and the coated surface was held at the center of the 4 cm space between the mass spectrometer inlet and the ion source for approximately 5 s. Whole root samples were presented via tweezers and suspended between the mass spectrometer inlet and ion source. All of the samples were analyzed in replicates of five, meaning that in a single DART acquisition, five replicates of a single sample were introduced into the DART ion stream. Sampling of each replicate was performed by either dipping a new capillary tube into the solid (for powder samples) or the liquid sample, and presenting the coated surface of the tube to the open-air space between the DART ion source and MS inlet.

Because yangonin is the psychoactive component of major interest in Kava plant material, a calibration curve developed using calibrators and a deuterated internal standard was used to quantify the yangonin in various Kava products. Stock solutions of 500 mg/mL yangonin and 500 mg/mL internal standard yangonin-$d_3$ were prepared by dissolving 10.00 mg in 20 mL ethanol. Serial dilutions from the yangonin stock solution were made to prepare calibrators ranging from 10 to 400 mg/mL with a final volume of 250 µL for each sample. To create a 50 mg/mL working stock solution of yangonin-$d_3$, 1 mL of the 500 mg/mL yangonin-$d_3$ stock was diluted to 10 mL. Each calibrator was spiked with 250 µL of the 50 mg/mL internal standard yangonin-$d_3$ working stock solution to bring the final volume of each calibrator to 500 µL. Therefore, the final concentration of each calibrator was diluted to half the concentration made during the serial dilution. The final concentration of the internal standard yangonin-$d_3$ was 25 mg/mL and the final calibration curve calibrators ranged from 5 to 200 mg/mL. These seven calibrators were then used to develop the calibration curve. A blank standard consisting of only ethanol was run with the calibrators. In addition, a zero calibrator containing 250 µL ethanol and 250 µL internal standard yangonin-$d_3$ was run with the blank and other calibrators. In order to follow the FDA validation guidelines, new stock solutions of 500 mg/mL yangonin, separate from the yangonin stock solution used to make the calibrators, were prepared with 10.00 mg of yangonin solid dissolved in 20 mL of ethanol. These stock solutions represented quality control (QC) sets 1 and 2. Each stock solution was used to prepare fresh QC standards each day the curve was run. Serial dilutions for the concentrations 10, 30, 160 and 350 mg/mL were prepared and 250 µL from each of these was transferred into a new 2 mL Eppendorf tube. These samples were diluted with 250 µL of yangonin-$d_3$ from the same 50 mg/mL working stock solution used to make the calibrators. This brought the QC standards to their final concentrations of 5, 15, 80 and 175 mg/mL, with the first one representing the lower limit of quantification (LLOQ), and a final volume of 500 µL. QC standards were prepared fresh from their respective stock solutions each of the three days the curve was run.

To develop reproducible and consistent measurements, a 12 Dip-it holder (IonSense, Saugus, MA, USA) was used on a linear rail system (see Figure S1, supporting information) to automate the analysis of calibrators and extracts of Kava products and plant materials. Samples were deposited onto commercially available "Dip-it tips" through a dipping method, which consisted of dipping each tip into a 2 mL Eppendorf tube containing 500 µL of sample such that the capillary tip touched the bottom of the tube, and then removing the tube and affixing it to the Dip-it holder. The height uniformity of all tips was made by visual assessment. A constant linear rail speed of 1 mm $s^{-1}$ was used.

Once validated, this method of quantification was applied to determine the levels of yangonin present in 18 Kava products comprised of a variety of roots, powders, extracts, tinctures, liquid samples, capsules and a paste. Samples that were acquired as whole roots were ground to powders to increase the surface area exposed to the solvent. Three of the four capsule products were comprised

of Kava root powder contained within a glycerin capsule, while the fourth consisted of a green gel-like material contained in the capsule. For each of the four capsule products, the contents of five randomly selected capsules were combined and mixed thoroughly. A 10 mL sample of each Kava tincture and liquid supplement was transferred into separate Falcon tubes with the tops covered with Kimwipes secured with rubber bands. The five tincture/liquid supplement samples were lyophilized for roughly 36 h. Powder samples required no sample preparation prior to the extraction process. For all root, capsule, tincture, liquid and powder samples, approximately 200 mg was extracted with 5 mL of ethanol in a 20 mL scintillation vial. After subjection to 20 min of sonication, the supernatant was transferred to a 25 mL volumetric flask. This process was repeated two more times and the extracts were pooled. After dilution with ethanol to 25 mL, 2 mL of the mixture was transferred to a 2 mL Eppendorf tube, and, after centrifugation at 1000 *g* for 5 min, 250 µL of the extract was transferred into a new 2 mL Eppendorf tube. Samples were each spiked with 250 µL of 50 mg/mL internal standard yangonin-$d_3$, which brought the final volume in each Eppendorf tube to 500 µL, and the internal standard concentration to 25 mg/mL. In order to determine if maximum extraction efficiency had been achieved, one powder sample was subjected to a fourth round of extraction and sonication, and analyzed separately using DART-HRMS. With the peak of the protonated molecular mass of yangonin at a percentage of less than 1%, it was established that three rounds of extraction and sonication were adequate for sample preparation.

Due to the observation of yangonin degradation in the calibrator samples after approximately 2 weeks, freshly prepared calibrators and QC samples were required for further analysis. To accurately quantify the yangonin concentration in each of the products, the extracts spiked with internal standard yangonin-$d_3$ were run in replicates of five throughout a freshly prepared set of calibrators and two new sets of QC samples. As was performed with the method validation, three separate runs were completed on different days.

## 3 | RESULTS

### 3.1 | Confirmation of kavalactones in Kava products using DART-HRMS

cThe study was initiated by confirming previous results[39] that showed DART-HRMS to be a suitable method for reliable detection of kavalactones. The DART-HRMS spectrum of a representative Kava product is displayed in Figure 1. The spectrum showed a number of peaks consistent with the protonated $[M + H]^+$ molecular weights of the six major kavalactones (i.e. desmethoxyyangonin ($[C_{14}H_{12}O_3 + H]^+$; *m/z* 229.087), kavain ($[C_{14}H_{14}O_3 + H]^+$; *m/z* 231.102), dihydrokavain ($[C_{14}H_{16}O_3 + H]^+$; *m/z* 233.118), yangonin ($[C_{15}H_{14}O_4 + H]^+$; *m/z* 259.097), methysticin ($[C_{15}H_{14}O_5 + H]^+$; *m/z* 275.092) and dihydromethysticin ($[C_{15}H_{16}O_5 + H]^+$; *m/z* 277.108)). Protonated $[M + H]^+$ peaks associated with flavokavain B ($[C_{17}H_{16}O_4 + H]^+$; *m/z* 285.113), flavokavain C ($[C_{17}H_{16}O_5 + H]^+$; *m/z* 301.108) and flavokavain A ($[C_{18}H_{18}O_5 + H]^+$; *m/z* 315.123), the three major flavokavains present in Kava, were also observed. We have confirmed the identity of these six kavalactones and several flavokavain compounds in previous work using authentic standards.[39] The DART-HRMS analysis was followed by investigation of the chemical profiles of the 18 commercially available Kava products displayed in Figure 2. The DART mass spectra of these samples are illustrated in Figure 3, with the mass measurements and relative intensities for the kavalactone and flavokavain compounds also available (see Tables S1 and S2, supporting information). For each product, the full spectrum in the *m/z* range of 60-800 is presented in Figure 3. All of the masses of the protonated kavalactones were prominently featured in each spectrum, which indicated that the products were likely to have all been derived from *P. methysticum*. While the relative intensities of the various kavalactone peaks differed between products, this was not unexpected given the differences in processing methods for each product. Of note was the observation that the most abundant kavalactone in 14 of the 18 product mass spectra was yangonin, the primary psychoactive component of interest.

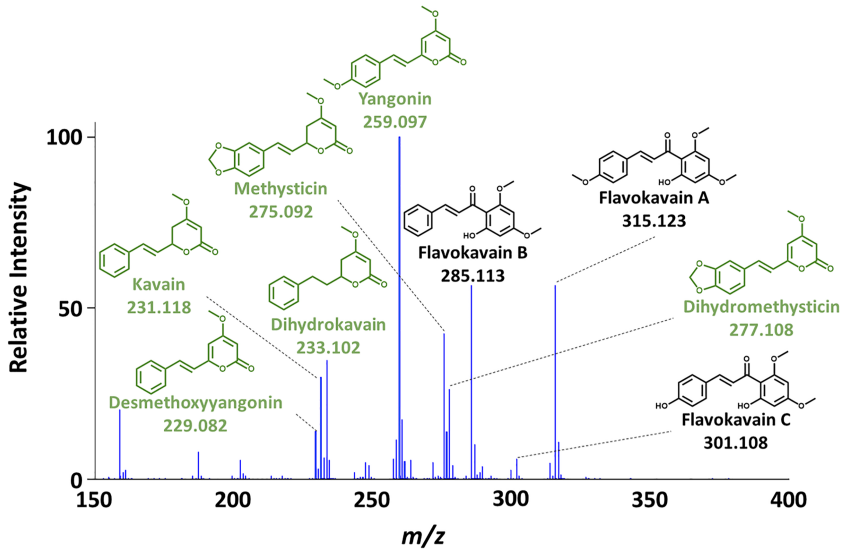


**FIGURE 1** DART-HRMS spectrum of a representative Kava product demonstrating the presence of peaks consistent with the protonated molecular weights of the six major kavalactones and three flavokavains [Color figure can be viewed at wileyonlinelibrary.com]


**FIGURE 2** Eighteen commercially available Kava products, including capsules, roots, pastes, tinctures and powders [Color figure can be viewed at wileyonlinelibrary.com]

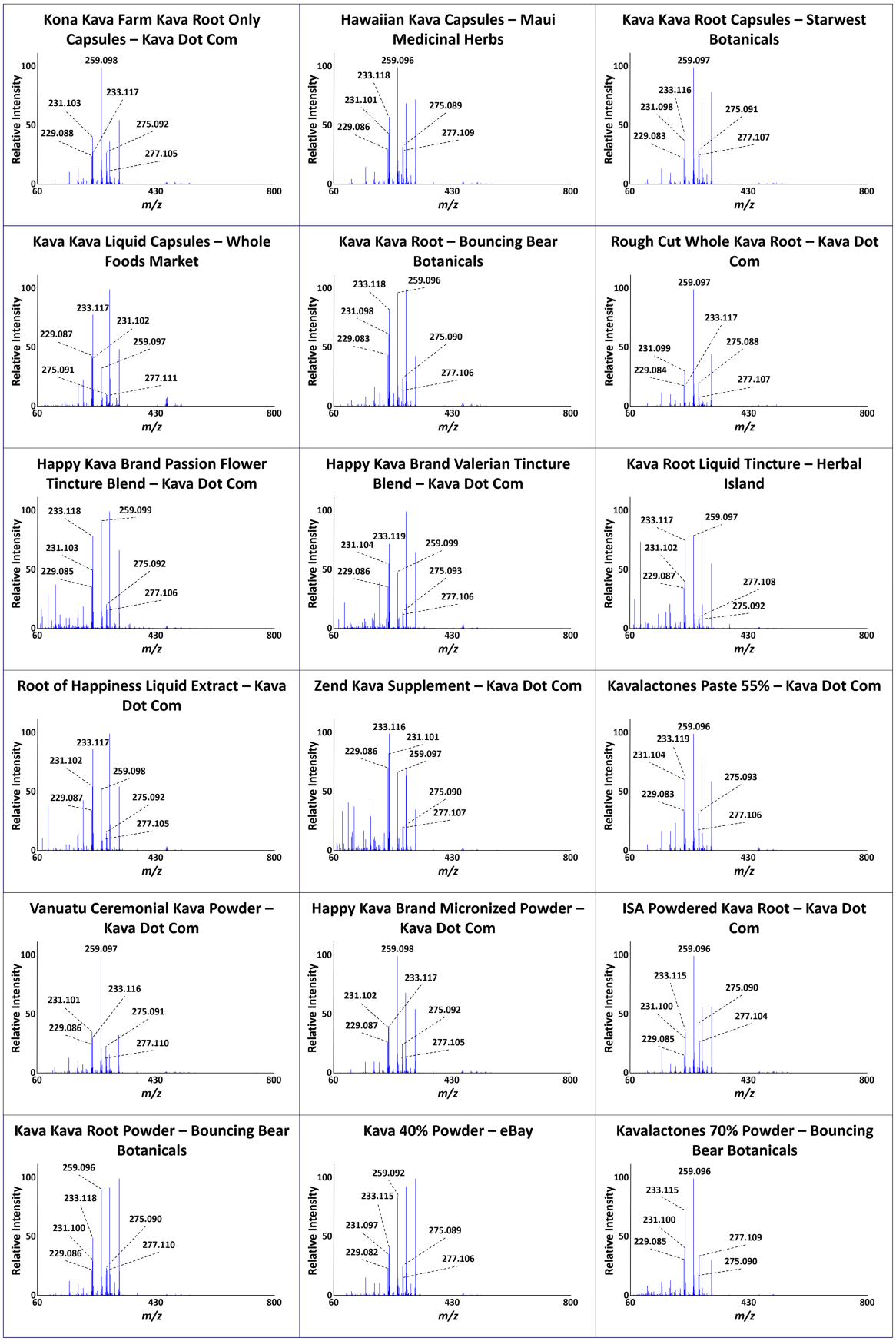


**FIGURE 3** DART-HRMS spectra for the 18 Kava products, featuring all primary kavalactones and demonstrating that all the products were likely developed from Kava plant material [Color figure can be viewed at wileyonlinelibrary.com]

### 3.2 | Peak integration of analyte and internal standard

DART-HRMS allows for the peak integration of individual samples based on selected *m/z* values. Although yangonin and yangonin-$d_3$ have nominal *m/z* values of 259 and 262 respectively for $[M+H]^+$, the corresponding isotope *m/z* values of 260 and 263 were used instead. This was done to avoid the often observed overloading of the yangonin $[M+H]^+$ peak in the non-isotopically-labeled samples, which leads to inaccurate peak area ratios. The ratio of the yangonin and yangonin-$d_3$ isotope peaks, illustrated in Figure 4, was preserved in all calibrators and Kava study samples, and could therefore be reliably used to develop a calibration curve to confirm the yangonin concentration in quality control standards (QCs), and accurately determine the unknown yangonin content in Kava products.

### 3.3 | Validation of a DART-HRMS protocol for quantification of yangonin

The US Food and Drug Administration (FDA) has established guidelines for the development and validation of bioanalytical methods.[44] The process of protocol validation for the quantification of yangonin using DART-HRMS was conducted in accordance with these guidelines and included conformance to the recommendations on range of linearity, selectivity and sensitivity, limits of quantification (LOQ) and detection (LOD), and reproducibility. Calibration curves developed with a blank calibrator (no analyte or internal standard), a zero calibrator (blank calibrator with internal standard) and seven non-zero calibrators that covered the range of quantification, were developed and run on three separate days of analysis. The blank and zero calibrators did not interfere with the analyte peak of interest, and the blank calibrator had an internal standard response that did not exceed 5% of the average responses in the curve calibrators and QCs. Sensitivity is defined by the lowest non-zero calibrator (5 mg/mL) on the calibration curve which has an analyte response of greater than five times that of the zero calibrator. It was determined to be the lower limit of quantification (LLOQ). Four QCs consisting of the LLOQ, low (L: defined as three times the LLOQ), mid (M: defined as mid-range) and high (H: defined as high-range) concentrations from a minimum of five replicates in at least three runs were completed. For a run to be considered valid, non-zero calibrators should be ±15% of nominal (theoretical) concentrations, except at the LLOQ where the calibrators should be ±20% of the nominal concentrations in each validated run. Additional requirements include, but are not limited to, 75% and a minimum of six non-zero calibrator levels should meet the previous guideline, and ≥67% of QCs should be ±15% of the nominal values. At each QC level, >50% of the QCs should be ±15% of their nominal concentrations.[44]

For each run of the calibration curve, all calibrators in the range of 5–200 mg/mL and all QC replicates at each QC level were within acceptable percentages of their nominal (theoretical) concentrations. All calibration curves had an $R^2$ value of ≥0.9990. The method developed here was considered validated once three runs that met the FDA guidelines were completed. The calibration curves and QCs are shown in Figure 5 and the raw data are available in Figure S2 (supporting information). With the success of three validated runs, the protocol was considered acceptable for application to study samples with unknown concentrations of yangonin.

### 3.4 | Quantification of yangonin in commercial Kava products

As yangonin is the psychoactive kavalactone of primary concern in Kava products and plant material, it is important to develop a protocol for the quantification of this compound that can be applied to a variety of sample types. Extracts of the products that were subsequently spiked with internal standard yangonin-$d_3$ were analyzed using DART-HRMS along with the calibrators and QC sample sets. The peak area ratio of the yangonin $[M+H]^+$ isotope peak (*m/z* 260.100) to the yangonin-$d_3$

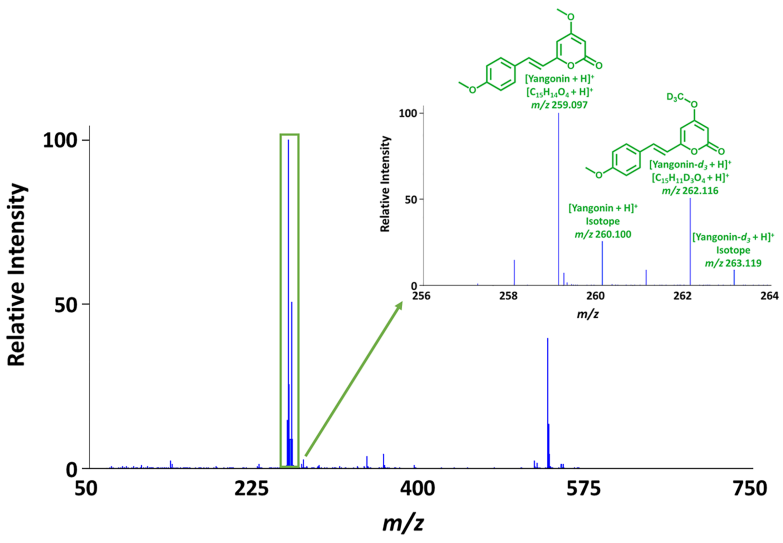


**FIGURE 4** DART-HRMS spectrum demonstrating the ratios between the yangonin and yangonin-$d_3$ peaks and the yangonin and yangonin-$d_3$ isotope peaks, all of which are in protonated form [Color figure can be viewed at wileyonlinelibrary.com]

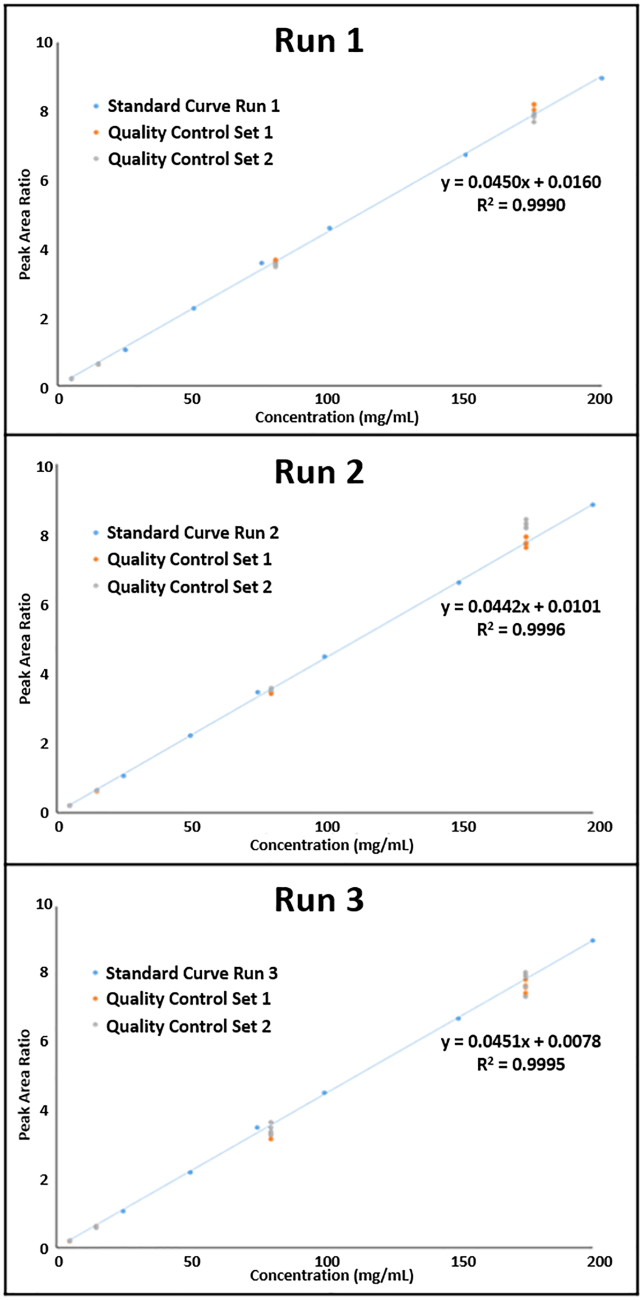


**FIGURE 5** Standard curves for yangonin quantification obtained from DART-HRMS data. All curves were developed with yangonin calibrators and yangonin-$d_3$ as an internal standard, with quality control samples to demonstrate precision and accuracy within and between runs [Color figure can be viewed at wileyonlinelibrary.com]

$[M + H]^+$ isotope peak (*m/z* 263.119) was calculated to determine the concentration of yangonin in the extracts based on the calibration curve associated with that analysis. These extract concentrations were then used to determine the original concentration of yangonin in the Kava products. For an extract concentration to be considered accurate, it must fall within the linear range of quantification defined by the calibration curve. Sixteen of the 18 products analyzed had extract concentrations that fell within the curve range, and two tincture products had extract concentrations that fell just below the curve range. These latter products included the Kava Root Liquid Tincture and the Zend Kava Supplement and were considered to have yangonin levels that were below the LLOQ (BQL). The average yangonin concentrations determined for the remaining products are listed in Table 1. The yangonin concentrations for the solid samples, consisting of capsules, pastes, powders and roots, ranged from 2.709–8.994 mg per gram of Kava material. As for the tincture samples, the yangonin concentrations only ranged from 1.031–4.586 mg per mL of Kava product. The concentrations of the tincture products were determined first by determining the concentration of yangonin in the dried material, and then converting it into units of mg/mL based on the amount of liquid product that was lyophilized.

## 4 | DISCUSSION

In their 2016 Technical Report on the safety of Kava consumption, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations emphasized several data gaps and various needs required for assessment of the safety of this plant.[3] This study of Kava-derived products and plant material has addressed several of these by providing a straightforward analytical method for the determination of the composition of kavalactones and flavokavains in Kava-derived materials, and quantifying the major component of interest, yangonin.

There are a variety of Kava product types available for consumers to purchase. Therefore, any method developed for the analysis, identification and quantification of these materials must be applicable to as wide a range of potential samples as possible. Product types can include bulk plant material such as leaves, stems, and roots, or they can be in forms that are easier to consume, including powders, extracts, tinctures, capsules and pastes. In addition to the range of products, the composition of each product varies from one vendor to another, depending upon the manner in which it is processed. Protonated masses for all six major kavalactones, which are 4-methoxy-2-pyrones that contain either phenyl or styryl groups at the $6^{th}$ position, were prominent in all 18 of the Kava products tested. In 11 of these, yangonin represented the base peak. The quantification of yangonin was only accurate in 16 samples since the yangonin concentrations for the Kava Root Liquid Tincture and the Zend Kava Supplement fell just below the LLOQ. Flavokavains A, B and C were also readily detected and featured prominently in the spectra of all the samples. Although a method was not developed for the quantification of these compounds in the study reported here, DART-HRMS demonstrates potential application to the analysis and quantification of these flavokavains as well. The product with the highest yangonin concentration was the Kavalactones Paste 55%, with a concentration of almost 9 mg/g. The 55% designation is not based on our analysis, but was provided by the vendor. The two root products also appeared to have high concentrations of yangonin, with levels greater than 7 mg/g, in comparison with the other products. The tinctures, on the other hand, all had relatively low concentrations of yangonin, with all

**TABLE 1** Yangonin concentrations measured in commercially available *P. methysticum* products

| Product type | Product | Vendor | Average conc. | SD | Units |
|---|---|---|---|---|---|
| Capsule | Kona Kava Farm Kava Root Only Capsules | Kava Dot Com | 8.379 | 0.2944 | mg/g |
| | Hawaiian Kava Capsules | Maui Medicinal Herbs | 5.672 | 0.2632 | mg/g |
| | Kava Kava Root Capsules | Starwest Botanicals | 5.562 | 0.1007 | mg/g |
| | Kava Kava Liquid Capsules | Whole foods market | 2.855 | 0.06478 | mg/g |
| Paste | Kavalactone paste 55% | Kava Dot Com | 8.994 | 0.3659 | mg/g |
| Powder | Happy Kava Brand Micronized Powder | Kava Dot Com | 7.706 | 0.5263 | mg/g |
| | Vanuatu Ceremonial Kava Powder | Herb Stomp | 6.749 | 0.3638 | mg/g |
| | Kava 40% Powder | eBay | 5.841 | 0.2017 | mg/g |
| | ISA Powdered Kava Root | Kava Dot Com | 4.262 | 0.2480 | mg/g |
| | Kava Kava Root Powder | Bouncing Bear Botanicals | 3.802 | 0.06305 | mg/g |
| | Kavalactones 70% Powder | Bouncing Bear Botanicals | 2.709 | 0.1811 | mg/g |
| Root | Rough Cut Whole Kava Root | Kava Dot Com | 8.270 | 0.1527 | mg/g |
| | Kava Kava Root | Bouncing Bear Botanicals | 7.072 | 0.08969 | mg/g |
| Tincture | Root of Happiness Liquid Extract | Kava Dot Com | 4.586 | 0.4505 | mg/mL |
| | Happy Kava Brand Passion Flower Tincture Blend | Kava Dot Com | 1.645 | 0.02853 | mg/mL |
| | Happy Kava Brand Valerian Tincture Blend | Kava Dot Com | 1.031 | 0.01468 | mg/mL |
| | Kava Root Liquid Tincture | Herbal Island | BQL | - | - |
| | Zend Kava Supplement | Kava Dot Com | BQL | - | - |

BQL: Below the lower limit of quantification

being ≤5 mg/g, and with two of these samples being below the limit of quantification. The six powder samples ranged from low yangonin concentrations of less than 3 mg/g, to concentrations greater than 8 mg/g. Finally, the three capsules containing Kava powder had higher yangonin concentrations than the gel capsule, which had a concentration of only 2.855 mg/g. While the results presented here represent one sample trial, a second sample trial was completed and the results were comparable (data not shown). There appeared to be no overarching trend in the yangonin concentrations of these products, other than the tincture samples having lower concentrations than most of the other products. It is important to note that the concentrations reported here are only for the compound yangonin, and therefore they are not representative of the overall kavalactone content of the products, nor do they reflect the concentrations of the other kavalactone and flavokavain compounds. According to the DART mass spectra displayed in Figure 3 and the mass measurements (see Table S1, supporting information), most of the samples had low relative abundances of methysticin and dihydromethysticin, with moderate relative abundances of desmethoxyyangonin, kavain and dihydrokavain. As previously noted, the yangonin $[M + H]^+$ ion was the base peak in 14 of the 18 products. Based on spectra (Figure 3) and the corresponding mass measurement data (Table S2, supporting information), four of the five tinctures, the liquid capsules and one root product had the flavokavain B $[M + H]^+$ ion as the base peak. The flavokavain C $[M + H]^+$ ion was observed at low relative intensities (<15%), while the flavokavain A $[M + H]^+$ ion relative intensity ranged from 30.4% to 100.0% in the powder samples (i.e. Kava 40% Powder and Kava Kava Root Powder).

Although a variety of techniques have been applied to qualitative and quantitative studies of kavalactones in Kava products, thermal decomposition, low sensitivity and limitations regarding separation time and peak resolution have proven troublesome in Kava analysis.[18-20] Near-infrared reflectance spectroscopy (NIRS) and FTIR with attenuated total reflectance (ATR) have demonstrated advantages including non-destructiveness, high speed and sensitivity, and involve minimal to no sample preparation for analysis and quantification of major kavalactones.[20,29,30] However, the poor stability of yangonin in certain solvent systems and calibration curves with low $R^2$ values for several major kavalactones remain problematic with these methods.[20,30] A validated SFC method was developed for the quantification of major kavalactones in Kava root powders and extracts, but difficulties in resolving several of the kavalactones were encountered.[18,26] While multiple HPLC methods for quantification of kavalactones such as normal phase (NP) HPLC, reversed-phase (RP) HPLC and HPLC/UV have been reported with varied success,[18,20,23] long run times, toxic solvents, lengthy sample preparation steps, and difficulties in separating kavalactone peaks due to oily complex matrices, complicate data analysis.[18,20,23] A protocol using HPLC/UV to quantify the six major kavalactones and several flavokavains was recently published as the first fully validated method to accomplish these quantifications in a single run.[23] Another protocol using RP-LC with atmospheric pressure chemical ionization tandem mass spectrometry succeeded in validation of quantification of major kavalactones in food supplements, except for yangonin, which underwent degradation.[22] It is demonstrated here that DART-HRMS can be used to circumvent a number of these challenges. However, when using a mass analyzer that is not configured with MS/MS analysis, it is necessary to take the additional step of confirming, by an independent method, the identities of compounds by comparing their fragmentation patterns with those of authentic standards, and/or by GC retention time comparisons.

## 5 | CONCLUSIONS

It is of significant forensic relevance to establish a method to rapidly identify and quantify the presence of psychoactive compounds listed on the UNODC's top 20 list of plant drugs of concern, one of which

is *Piper methysticum* (Kava). We not only demonstrate the application of DART-HRMS for the rapid screening of kavalactones in a variety of commercially available *Piper methysticum* products and plant material, but also validate the usage of DART-HRMS to quantify yangonin, the psychoactive component of Kava, in a wide variety of Kava product types. With the use of a deuterated internal standard and protonated isotope peak integration, quantification of yangonin using DART-HRMS proved to be simple and effective. The approach illustrates how method validation using DART-HRMS can be accomplished for analysis of other complex plant matrices, including additional plants on the UNODC's plants of concern list, such as salvinorin A in *Salvia divinorum* and hordenine in *Sceletium tortuosum*.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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