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Spice

APPLICATION NOTE

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[1]. 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 hepatoxicity 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[2].

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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[3-6]. 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.

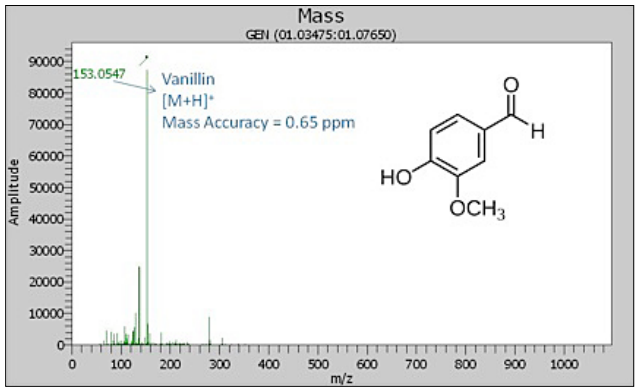

Figure 1. Mass spectra of vanilla bean extract in positive ion mode using DSA/TOF.

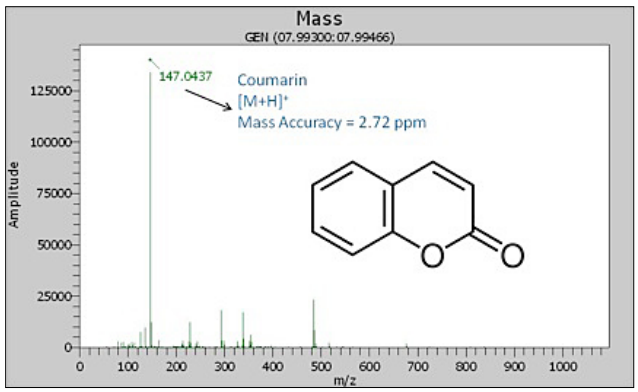

Figure 2. Mass spectra of tonka bean extract in positive ion mode using DSA/TOF.

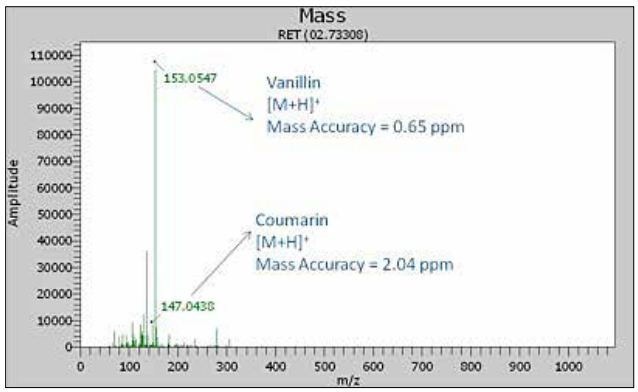

Figure 3. Mass spectra of vanilla bean extract adulterated with 2 % tonka bean extract in positive ion mode using DSA/TOF.

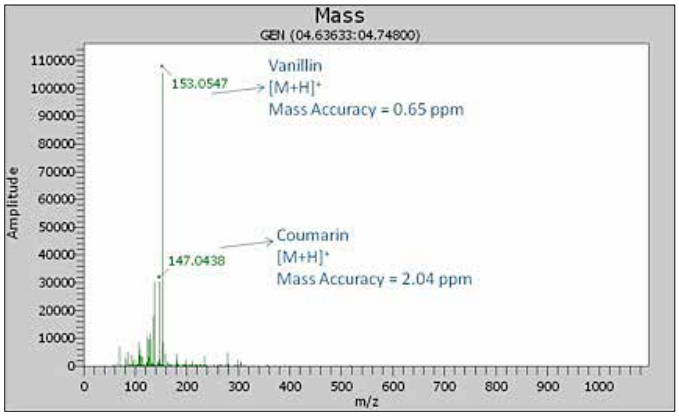

Figure 4. Mass spectra of vanilla bean extract adulterated with 10 % tonka bean extract in positive ion mode using DSA/TOF.

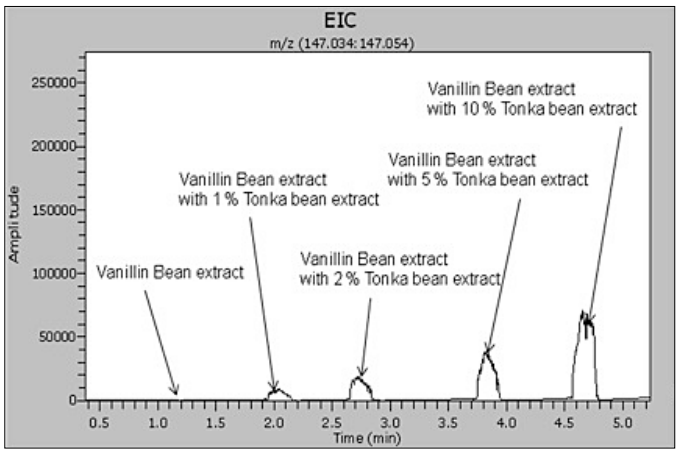

Figure 5. Extracted ion chromatogram for coumarin in vanilla bean extract with different levels of tonka bean extract.

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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Acknowledgements

The author would like to thank Robert Packer at PerkinElmer for providing a tonka bean extract sample.

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APPLICATION NOTE

Mass Spectrometry

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Rapid Differentiation Between Natural and Artificial Vanilla Flavorings for Determining Food Fraud Using AxION DSA/TOF with No Chromatography or Sample Preparation

Introduction

Vanilla is the second most expensive spice, next to saffron, and is widely used as a flavoring ingredient in the food, beverage, cosmetic, pharmaceutical and tobacco industries. Vanilla extract is the most common form of vanilla used today, and comes in 2 forms, natural & artificial. Pure vanilla 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. There are two types of artificial vanilla flavorings, which always contain vanillin that is synthesized from cheap raw material such as guaiacol, eugenol or lignin, a natural polymer found in wood; and/or ethyl vanillin that is added, which is another artificially produced vanilla compound that has three times the flavor strength of vanillin[1].

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. This drives the price of natural vanilla extract to about three to five times higher than artificial vanilla preparations. Due to quality, price concerns and economically motivated frauds, it is important to differentiate between natural and artificial forms of vanilla extracts. Apart from vanillin, natural vanilla extracts have 4-hydroxybenzaldehyde, which is absent in artificial vanilla flavorings. This compound can be used as a marker ion to rapidly differentiate between natural and artificial vanilla preparations[2].

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A variety of analytical techniques such as GC/MS, LC/MS, Headspace GC/MS, LC/UV and stable isotope ratio analysis have been used to differentiate between natural and artificial vanilla flavorings[3]. These measurement techniques are either expensive or 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 can be used for rapid differentiation between artificial and natural vanillin extracts, with no chromatography or sample preparation, and within a few seconds.

Experimental

Five natural and five artificial or imitation vanilla extracts were purchased from a local supermarket. All vanilla extracts were analyzed using the AxION 2 DSA/TOF system and required no sample preparation. 10 µl of each sample was pipetted directly onto the stainless mesh of the AxION DSA.

DSA/TOF MS Conditions:

- Corona current of 5 µA
- DSA heater temperature of 300 °C
- Auxiliary gas (N_2) pressure of 80 psi,
- Drying gas (N_2) flow of 3 l/min and drying gas (N_2) temperature of 25 °C

The AxION 2 TOF MS was run in negative ionization mode with flight voltage of 5000 V. The capillary exit voltage was set to -120 V for the analysis. Mass spectra were acquired in a range of m/z 50-700 at an acquisition rate of 5 spectra/s. Total analysis time per sample was 15 seconds. To obtain higher mass accuracy, the AxION 2 TOF instrument was calibrated by infusing a calibrant solution into the DSA source at 10 µl/min.

Results

All 10 vanilla extracts were analyzed by the AxION DSA/TOF. Figures 1 and 2 show the mass spectra for one of the natural vanilla extracts and one of the artificial vanilla extracts, respectively. The mass spectra shows the presence of vanillin in both extracts but 4-hydroxybenzaldehyde was present only in the natural vanilla extracts. This data shows that 4-hydroxybenzaldehyde can be used as a marker compound to distinguish between natural and artificial vanilla extracts using DSA/TOF. All mass measurements showed good mass accuracy with an error of less than 5 ppm.

Similar data to that shown in Figures 1 and 2 was obtained for the other four natural and two of the artificial vanilla extracts. The ingredient labels for the other two artificial vanilla extracts showed the presence of benzoic acid as a preservative. Benzoic acid has the same empirical formula as 4-hydroxybenzaldehyde and therefore it is observed, in the spectra of these extracts, at the same mass as 4-hydroxybenzaldehyde. In order to distinguish between the presence of benzoic acid and 4-hydroxybenzaldehyde in artificial vanilla extracts, fragment ions were generated using collisionally-induced dissociation (CID) at the capillary exit.

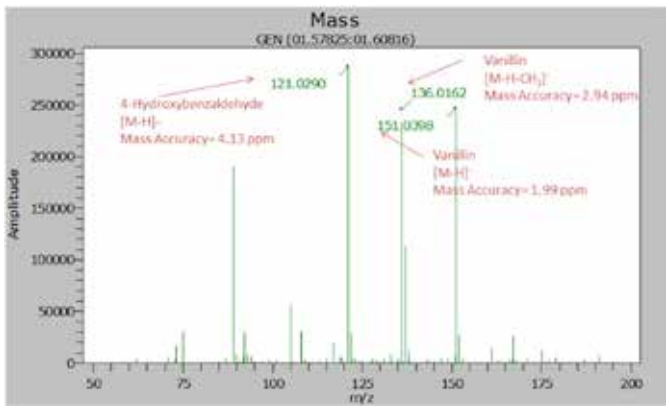

Figure 1. Mass spectra of natural vanilla extract one in negative ion mode using AxION DSA/TOF.

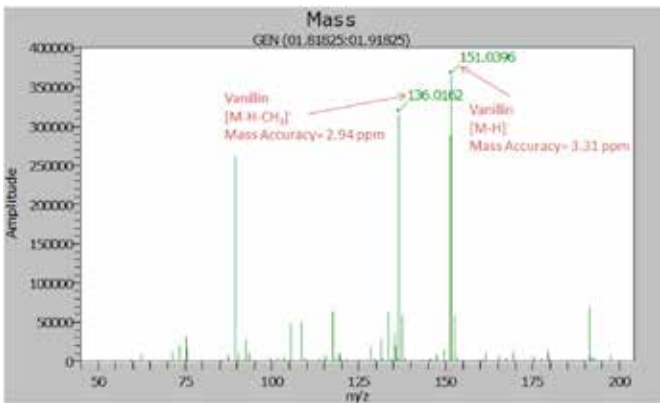

Figure 2. Mass spectra of artificial vanilla extract one in negative mode using AxION DSA/TOF.

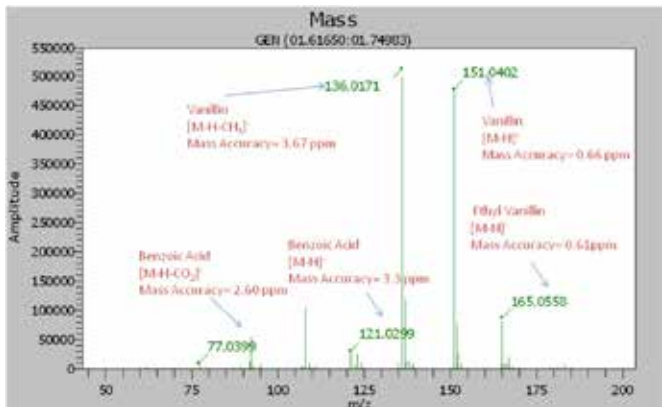

Figure 3. Mass spectra of artificial vanilla extract two in negative mode using AxION DSA/TOF.

The mass spectrum of a 10 ppm standard of benzoic acid, under these conditions, showed the presence of an ion at m/z 77.0397 Da which corresponds to $[M\text{-}H\text{-}CO_2]^-$ ion. This ion was not present however in the corresponding spectrum for a 10 ppm standard of 4-hydroxybenzaldehyde. A mass spectrum of one of the two artificial vanilla extracts is shown in Figure 3. An ion at m/z 121.0368 Da and an ion at m/z 77.0397 Da are easily identifiable confirming the presence of benzoic acid. Furthermore, an ion at m/z 165.0557 Da indicates the presence of ethyl vanillin confirming that this sample is an artificial vanilla extract.

Conclusion

This work shows the utility of the AxION DSA/TOF for rapid differentiation between natural and artificial vanilla extracts for determination of food fraud. This approach showed the presence or absence of 4-hydroxybenzaldehyde in vanillin extracts can be used to distinguish between natural and artificial vanilla extracts. The presence of benzoic acid in some of the artificial extracts can be distinguished from the presence of 4-hydroxybenzaldehyde by monitoring its characteristic fragment ion $[M\text{-}H\text{-}CO_2]^-$. The presence of ethyl vanillin in some of the artificial extracts can also be used to distinguish further between artificial and natural vanilla extracts. Mass accuracy of all measurements was less than 5 ppm with external calibration. All sample analysis time, was done with no chromatography or sample preparation, at 15 seconds per sample.

In comparison to other established techniques such as LC/MS and GC/MS, this application will improve laboratory productivity and decrease costs and analysis time.

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APPLICATION NOTE

FT-IR NIR Spectrometry

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Determination of Levels of Spice Adulteration using Near-infrared Spectroscopy

Introduction

Since the late 1800's scientists have been testing spice samples and discovering that they are adulterated. Some spices are high-value products that can be adulterated with lower-value commodities for commercial gain by unscrupulous suppliers. Some common adulterants of spices range from talc powder, ground walnut shells, cassia bark and sand, to wheat starch, saw dust, millet, buckwheat, and cornstarch. Commonly adulterated spices include garlic powder, black pepper, and cinnamon. Fourier Transform Near-Infrared Spectroscopy (FT-NIR) is shown here to be an effective and rapid technique to determine if these types of spices have been adulterated.

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Method

Samples of pure cinnamon, garlic powder, and black pepper were measured along with samples of some pure adulterants, namely talc, corn starch, and millet. Adulterated samples of the spices were prepared to demonstrate the applicability of the method. Near-infrared spectra were recorded using a PerkinElmer Frontier™ FT-NIR spectrometer equipped with a NIRA II sampling accessory. The powders were placed into petri dishes and placed on the NIRA II sample spinner accessory for the FT-NIR. Spinning the sample during data collection balanced out any inhomogeneity within the sample. Spectra were collected using 32 scans, at 8 cm^{-1} resolution, from 10,000 cm^{-1} to 4,000 cm^{-1}. Multiple independent spectra were collected for each of the spice types in order to build a model representing expected variation for that sample type. A model was created using a Soft Independent Modelling by Class Analogy (SIMCA) approach and also Adulterant Screen™ to discover if adulterated spice samples could be distinguished from a pure sample.

Results

FT-NIR spectra obtained for the pure spices are shown in Figure 1. The spectra of these materials in the near-infrared region have broad peaks showing first overtones and combination bands of the fundamental vibrations in the mid-infrared region of the spectrum. The replicate spectra measured for these materials were used to develop a SIMCA model that could be used for raw material identification or quality testing.

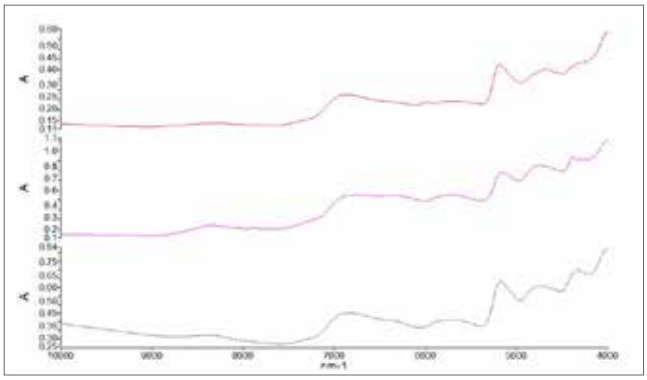

Figure 1. Spectra of the pure spices : Cinammon (red), Garlic Powder (purple), and Black Pepper (green).

The three-dimensional representation of this SIMCA model is shown as Figure 2, showing clear separation of the different classes of materials in the model for the pure materials. Using this model it would be easily possible to classify an unknown sample of any of these pure spices.

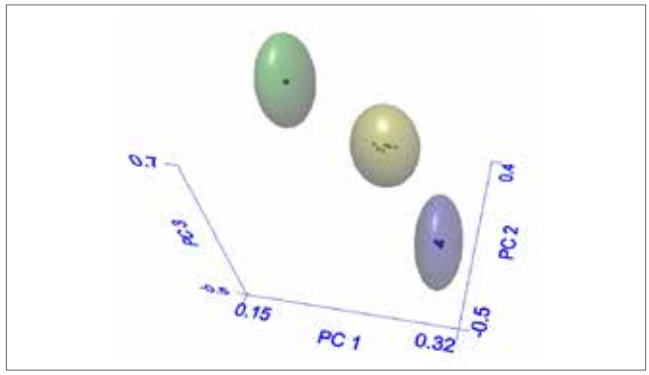

Figure 2. SIMCA model for pure spice samples: Cinnamon (top left), Black Pepper (middle), and Garlic Powder (bottom right).

FT-NIR spectra of three of the adulterants commonly encountered within spice samples are shown in Figure 3. Corn starch and millet have similar broad spectra to those observed in the spectra of the pure spices. Talc has some unique, sharp spectral features that can make detection of its use easier.

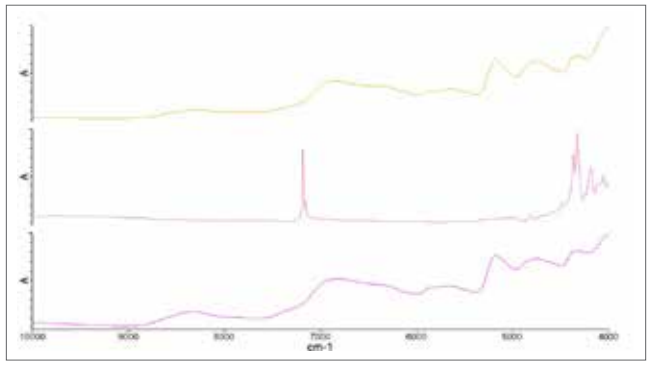

Figure 3. Spectra of the adulterants in this study: Corn Starch (top), Talc (middle), Millet (bottom).

A pure garlic powder sample was adulterated with a 10 % spiked amount of talc in order to test both the SIMCA model and an Adulterant Screen method. The spectrum of the pure garlic powder and the 10 % talc-spiked sample are shown in Figure 4. The spectral features due to the talc are highlighted in this Figure, showing clear differences from the pure sample.

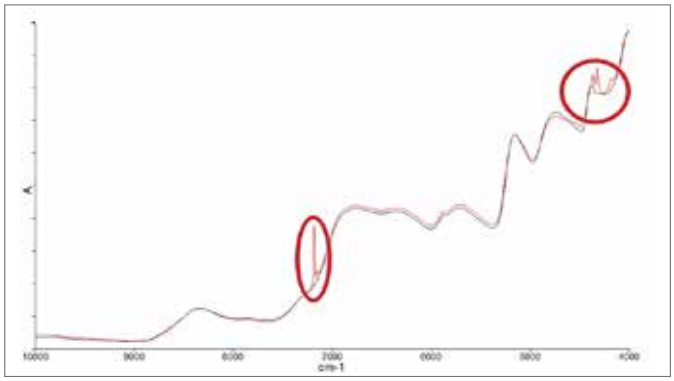

Figure 4. FT-NIR overlay of garlic powder spectrum with talc adulterant.

A Spectrum Touch™ method with a simple user interface was created to include both the SIMCA model and the Adulterant Screen method for the garlic powder. The spiked sample was tested against these methods to determine whether the adulteration would be detected. The results' screen is shown as Figure 5.

Figure 5. Example results for garlic Adulteration Screen with SIMCA from Spectrum Touch.

If the spectrum being measured does not conform to the rest of the spectra in the model then Verify™ (using the SIMCA algorithm) will fail the material. In this case, the spiked sample fails the Verify test. However, Verify can only inform the user that the sample has failed and cannot give any indication as to the adulterant present. In this case, the Adulterant Screen method also generates a fail result, but Adulterant Screen is able to inform the user which type of adulterant is present, correctly identified as talc.

A cinnamon sample was similarly spiked with an adulterant, in this case 10 % of corn starch. The Near-infrared spectra of cinnamon and corn starch are very similar and the differences cannot be seen easily in the absorbance spectra. However, applying a second derivative processing to the data does highlight the small differences as shown in Figure 6.

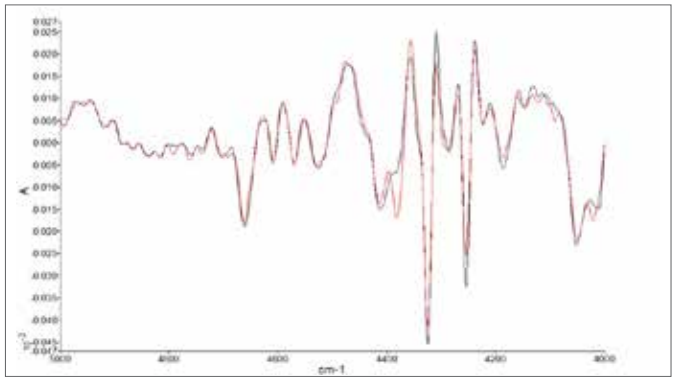

Figure 6. Second derivative spectra of pure cinnamon (black) and cinnamon spiked with Corn Starch (red).

These small differences are sufficient for the detection of corn starch as an adulterant. The same mathematical approach, as used for the garlic powder samples, was deployed for this spiked sample. Again, the Verify (SIMCA) and Adulterant Screen both generated fail results. Once the results are generated, it is possible to look at them in greater detail. Figure 7 shows the detailed results from Adulterant Screen.

Figure 7. Example results from Spectrum Touch highlighting the 'Adulterant Screen details' view for a cinnamon sample adulterated with 10% corn starch.

Not only does Adulterant Screen correctly identify the adulterant, it will also give a quantitative estimate of the level of adulterant present. This sample is reported as having corn starch present at 9.986 % for a 10 % spiked sample.

For the final spice sample in this study, black pepper, a series of dilutions were made using millet in order to generate a quantitative calibration for these mixtures. The mixtures ranged from pure black pepper containing no millet, up to 100 % millet. The spectra were measured, pre-processed using the second derivative format, then used as calibration standards within the Spectrum Quant™ package using a Partial Least Squares (PLS) algorithm. The calibration line generated is shown as Figure 8.

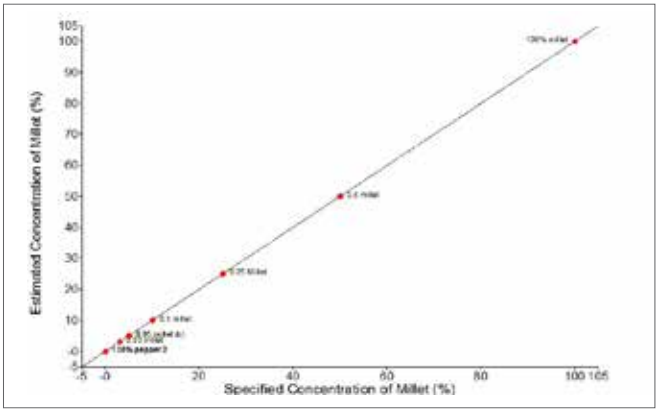

Figure 8. PLS Calibration for millet in black pepper.

The calibration shows excellent correlation between the specified and estimated concentrations of the mixtures. A small number of validation samples were used to test the quantitative method.

These same validation spectra were tested using an Adulterant Screen method for black pepper. Creating the Adulterant Screen method simply required spectra of the pure spice and the spectrum of the pure adulterant to be scanned and input into the method. Quantitative standards are not required. The spiked samples correctly failed the Adulterant Screen test. Table 1 shows the quantitative results obtained for the Validation samples using both Quant (PLS) and Adulterant Screen.

Table 1. Quantitative estimates of adulterant levels in Validation samples.

Sample	Adulterant Screen	PLS Prediction
3 % Millet	3.370	2.9834
10 % Millet	9.924	10.01
25 % Millet	22.781	24.997

These results show good levels of accuracy and a correlation between the Quant and Adulterant Screen results. The Adulterant Screen method did not require the preparation and measurement of calibration standards, thus speeding up the method development. If cases of new adulterants are uncovered, then updating the Adulterant Screen method would simply require the spectrum of that adulterant to be scanned and added to the method. A PLS method for a new adulterant would require complete re-calibration with a new set of standards.

Conclusion

FT-NIR with an Adulterant Screen is an effective way to detect adulteration of spice samples. A Verify (SIMCA) model provides sample identities with high levels of confidence and can detect when the sample does not conform to the model spectra. Additionally, Adulterant Screen can detect and identify the adulterants and predict concentrations without the need for lengthy calibration processes.

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APPLICATION NOTE

Atomic Absorption

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Analysis of Total Mercury in Chinese Spice Mixtures using Flow Injection Cold Vapor Atomic Absorption Spectrophotometry

Introduction

Spices are often used as dietary components to improve color, aroma, palatability and acceptability of food. Most spices are fragrant, aromatic and pungent.[1] Spices and herbs, grown widely in various regions of the world, have been used for several purposes since ancient times – in folk medicine as antiscorbutic, antispasmodic, tonic, carminative agents against bronchitis, ulcers and as diuretics, depuratives and vermifuges. Also, some species are used as tea flavoring agents in several regions.

Natural food spices such as pepper and mustard have been reported to contain significant quantities of some trace metals.[2] These trace metals in spices and medicinal plants play a vital role as structural and functional components of metallo proteins and enzymes in living cells.[1] Heavy metals have important positive and negative roles in human life. Some of the heavy metals are considered essential including iron, zinc and copper,[3] whereas some other metals, like mercury, have toxic roles in biochemical reactions in our body. Mercury is distributed throughout the environment in a number of different forms – it exists mainly as elemental mercury vapor in the atmosphere, while most of the mercury found in water, sediments, soil, plants, and animals is in the inorganic and organic forms (for example methylmercury) of the element.[4] The habitual addition of mercury-contaminated spices

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to food may result in accumulation of this toxic metal ion in human organs.[1] Developing fetuses are the most sensitive to the toxic effects of methyl mercury – it has been proven that children who are exposed to methyl mercury before birth may be at increased risk of poor performance on neuro-behavioral tasks, such as those measuring attention, fine motor function, language skills, visual/spatial abilities and verbal memory.[5] Contamination with mercury may be accidental (e.g. contamination of environment during plant cultivation) or deliberate (in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies).

The India and China regions have a high diversity of plants used as spices, herbs, and traditional medicines. Several spices and herbs are either produced on small farmlands or naturally grow in different regions. There is often little information available about the safety of those plants and their products with respect to mercury contamination. Due to significant amounts of spices consumed, it is important to know the mercury content in these products.

The objective of this work is two-fold: (1) to accurately analyze the total mercury levels that may be present in some major spice brands available in the local markets in China, by using cold vapor atomic absorption spectrophotometry (CVAAS); (2) to cross-reference these measured levels to the recommended limit specified by the U.S. FDA.

Experimental

The determination of mercury by flow injection-cold vapor atomic absorption spectrophotometry (FI-CVAAS) was performed using a PerkinElmer® FIAS-400 system (Shelton, CT, USA), connected to a PerkinElmer AAnalyst™ 800 Atomic Absorption Spectrophotometer equipped with the intuitive WinLab32™ for AA (Version 6.5) software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance (Figures 1 and 2).
A PerkinElmer high-energy mercury electrodeless discharge lamp (EDL) was used as the line source. The mercury absorbance was measured at 253.7 nm. The flow injection system consists of a six-port injection valve, two peristaltic pumps and a gas/liquid separator. A quartz cell with a path length of 160 mm and a diameter of 7 mm was used as the atomizer. Tygon® pump tubings were used to deliver sample and reagents as well as to remove waste from the gas/liquid separator. The optimized instrumental parameters along with sample and reagent flow rates, the concentration of reagents, argon stripping gas etc. are given in Table 1 (Page 3). The flow-injection program followed for the analysis of mercury is shown in Table 2 (Page 3).

A Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion. This is an industrial-type oven which is equipped with various accessories to optimize sample digestion. The samples were digested in the 8XF100 rotor using eight 100 mL high-pressure vessels made of PTFE-TFM protected with individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to the conventional PTFE. This vessel has a working pressure of 60 bars (870 psi) and temperatures of up to 260 °C.

Figure 1. PerkinElmer FIAS 400 Flow Injection System for atomic spectroscopy.

Figure 2. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

Figure 3. PerkinElmer/Anton-Paar Multiwave 3000 Microwave Sample Preparation System.

Table 1. **Optimized experimental conditions of FIAS 400 and AAnalyst 800.**	
Element	Hg
Wavelength	253.7 nm
Slit	0.7H nm
Mode	AA
Calibration	Linear through zero
Lamp	EDL
Current	185 mA
Standards	2.5, 5.0 & 10.0 µg/L
Correlation Coefficient	0.9999
Spike	1.0 µg/L
Read Time	15.0 sec
Carrier Solution	3% (V/V) HCl
Carrier Flow Rate	9-11 mL/min
Reductant	0.2% $NaBH_4$ in 0.05% NaOH
Reductant Flow Rate	5-6 mL/min
Carrier Gas	Argon
Carrier Gas Flow	50-60 mL/min
Sample Volume Injected	500 µL

Table 2. **Flow injection program used for mercury analysis.**				
Step	**Time (sec)**	**Pump 1 Speed (rpm)**	**Pump 2 Speed (rpm)**	**Valve**
Prefill	15	100	120	Fill
1	10	100	120	Fill
2	15	120	0	Inject, Read
3	0	0	0	Fill

Standards, Certified Reference Materials

PerkinElmer NIST® traceable mercury single-element calibration standards for atomic spectroscopy were used as the stock standard for preparing the working standards. Working standards were prepared by serial volume/volume dilution in polypropylene vials (Sarstedt®, Germany) which contained 5% volume/volume nitric acid and 1-2 drops (about 25 µL) of 5% weight/volume potassium permanganate from Merck® (Darmstadt, Germany) in order to ensure preservation of the element in solution. ASTM® Type I water (from a Millipore® filtration system, Millipore® Corporation, Billerica, Massachusetts, USA) acidified with Suprapur® nitric acid used for preparing the diluent for standards was from Merck®. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pippetting solutions. NIST® 1573e certified reference material for trace metals in tomato leaves and GBW 09101 certified reference material for trace metals in human hair were used to validate the method developed.

A single-element ICP standard for mercury in nitric acid (Spex. Certiprep.®, New Jersey, USA), prepared at midpoint of the calibration curve, was used as the quality control (QC) check standard.

Reagent Preparation

Carrier solution: Prepared by adding 15.0 mL of Suprapur® hydrochloric acid (Merck®) and making up to 500 mL in Class I standard flask, by using ASTM® Type I water.

Reducing agent: Dissolved 0.25 g of Suprapur® sodium hydroxide (Merck®) and 1 g of Suprapur® sodium boro-hydride in ASTM® Type I water, and made up to 500 mL in Class I standard flask. All the samples prepared in boro-silicate vessels were stabilized by the addition of 1-2 drops of a 5% weight/volume potassium permanganate solution.

Sample and Certified Reference Material Preparation

Four powdered spices and herb-mixture samples of famous brands available in the local markets in China (five-spice mix, ground szechuan, five-spice powder and curry powder), were bought from a Chinese supermarket and were used without any pre-treatments. ~0.5 g of each sample, accurately weighed in duplicate, was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3. The digested samples were diluted with 5% nitric acid and made up to 20 mL in Class I borosilicate standard flasks. The certified reference materials (CRMs) were also digested in a similar manner. Sample blanks were also prepared in a similar manner with each batch of digestion.

Table 3. **Program used for the digestion of spices and herbs with Multiwave.**				
Sequence	**Power**	**Ramp Time (min)**	**Hold Time (min)**	**Fan**
1	1200	15	15	1
2	0		15	3
Weight Taken	~500 mg	HNO_3	5.0 mL	
H_2O_2	1.0 mL	Rate	0.5 bar/sec	
Pressure	55 Bars			

Prior to the analyses, the flows of the 0.2% weight/volume sodium borohydride reductant and 3% volume/volume hydrochloric acid carrier solution was adjusted and set at 5-6 mL/minute and 9-11 mL/minute respectively. The argon gas flow was set at about 50-60 mL/minute. The waste flow from the gas/liquid separator was adjusted to the rate such that the liquid leaves the gas/liquid separator effectively, without any of it getting into the transfer tubing to the

quartz cell. The sensitivity was checked using a 10 µg/L mercury standard solution. 500 µL sample volume was used in every analysis. The results were obtained using peak height calculation with 2 seconds BOC (baseline offset correction) time and 19-point peak smoothing algorithm. Each result was calculated as a mean of two replicate determinations.

Results and Discussions

Problems in the digestion of samples for mercury determination are volatility, mobility, and adsorption on the walls of the containers, as well as possibility of contamination.[6] The spice samples contain a number of organic substances of different types and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution and, on the other hand, disturb spectrochemical measurements. The complete decomposition of organic matter could be achieved only under vigorous conditions such as excess of acid mixtures, high temperature and pressure, and long digestion times. In addition, oxidative conversion of all mercury species to mercury (II) in the sample is an elementary step prior to analysis.[7] Thus, digestion of the sample for mercury determination is carried out in an oxidizing environment using a combination of nitric acid and hydrogen peroxide and by utilizing closed vessel digestion to avoid loss of mercury. The oxidative conversion of all mercury species to inorganic mercury is further ensured by the addition of potassium permanganate. On the other hand, there is no need to decompose silicates because mercury cannot form natural silicate minerals due to its large ionic radius. Further preservation of mercury has been done as per the U.S. EPA method for the preservation of lower concentrations of mercury by maintaining an overall concentration of 200 ppb of gold in sample solutions.[8] The commonly observed interference caused by volatile nitrogen oxide was minimized by using a combination of nitric acid and hydrogen peroxide. However, as our experience shows, preservation using gold will help to hold the mercury in solution so that there is no need to analyze the samples immediately after digestion. Allowing some time (with occasional shaking) before analysis (~1 hour) will help to remove all dissolved nitrogen oxide, if present. The instrumental calibration was performed using four points, including the blank. Prior to starting sample analysis, the method detection limit (MDL) was measured using seven reagent blanks and then multiplying the standard deviation with the student t value of 3.14 for a confidence interval of 98% (Table 4).

Table 4. **Method detection limit (MDL).**

Analyte	MDL (µg/kg)
Hg	0.8

The good agreement between the certified value and the result (Table 5) obtained using calibration with aqueous standards suggests that the hydride generation AA technique is quite independent from matrix effects.

Table 5. **Analysis of certified reference materials.**

Metal	NIST® 1573e		GBW 09101	
	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)
Hg	0.034	0.030 ± 0.003	1.06 ± 0.28	1.31 ± 0.11

Furthermore, the excellent spike-recovery results (Table 7 – Page 5) showed that the matrix is not contributing to the final analytical results even at extremely lower concentrations. The long-term stability of the analyte signal has been monitored by analyzing quality control (QC) check standards prepared at the midpoint of calibration, at precise intervals (Table 6 – Page 5). The excellent QC recoveries, with a variation of less than 10%, usually prescribed by the regulatory bodies, showed that there is no drift in analyte signal during the course of analysis. There was virtually no difference between the QC standard which was performed immediately after calibration and the QC standard which was analyzed at the end of analysis with a time difference of three hours.

The extremely low detection limits obtained (Table 4) further demonstrated that the FIAS 400 system can be used together with the AAnalyst 800 to analyze extremely lower concentrations of mercury in difficult matrices.

Table 6. **Results of quality control (QC) check standard recoveries.**

Analyte	Prepared Concentration (µg/L) QC Standard	Measured Concentration (µg/L) QC Standard (analyzed at 11:55 am)	% QC Recovery	Measured Concentration (µg/L) QC Standard (analyzed at 15:00 pm)	% QC Recovery
Hg	5.0	4.89	98	4.93	99

Table 7. **Results of post digestion spike recoveries.**

Analyte	Spiked Concentration ion (µg/L)	Measured Concentration (µg/L) of Ground Szechuan	Measured Concentration (µg/L) of spiked Ground Szechuan (I)	% Recovery	Measured Concentration (µg/L) of spiked Ground Szechuan (II)	% Recovery
Hg	1.0	0.322	1.27	94.8	1.32	100

Table 8. **Results of spices and herbs sample analysis.**

Analyte	Five-Spice Powder	Five-Spice Mixture	Ground Szechuan	Curry Powder
Hg (µg/Kg)(FI-CVAAS)	15	16	13	3.0
Hg (µg/Kg)(ICP-MS)*	7.0	38	11	4.0

**Different set of similar samples measured at a different site.*

Conclusions

An accurate and reliable microwave-assisted sample-pre-treatment procedure for the determination of mercury in spices using FI-CVAAS is described. Application of concentrated nitric acid along with hydrogen peroxide for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by determined values of mercury in various certified reference materials. Toxicity of medicinal spices and herbs is of much greater concern today than ever before. In recent years, much emphasis is being laid on the toxic metal content of spices and herbs, as several western countries have banned many ayurvedic drugs based on their heavy-metal content exceeding the permissible limits.[9] A cursory look at results (Table 8) shows that the level of mercury did not exceed the permissible limits of 1 mg/kg specified by the U.S. FDA in any of the samples analyzed. The results confirmed that the determination of mercury after acid solubilization of spice mixture samples by microwave digestion can be performed by FI-CVAAS without any interference and the same has been cross checked by analyzing a different set of similar samples with ICP-MS (Table 8) analysis. The slight variations are due to the fact that the analyses were done on a different set of similar samples and not on the same samples.

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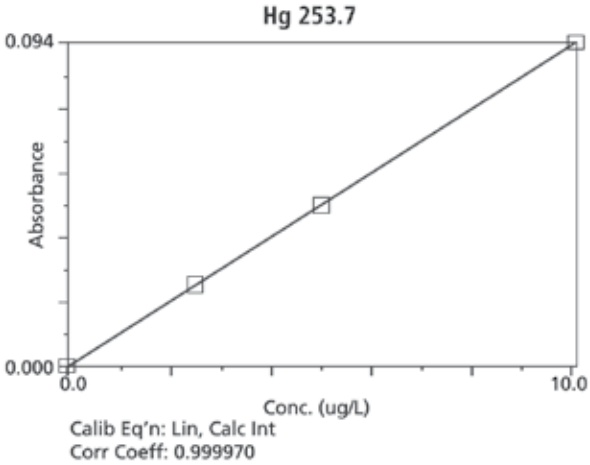
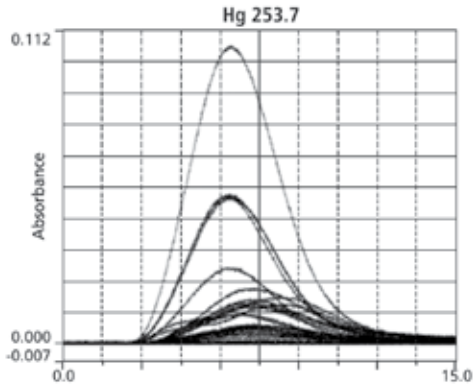
Appendix I. Calibration Curve and Atomization Profile





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APPLICATION NOTE

Atomic Absorption

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Analysis of Arsenic, Cadmium and Lead in Chinese Spice Mixtures using Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

Spices are dried parts of plants. Together with herbs, these plants grow widely in various regions of the world and have been used for several purposes since ancient times. Most are fragrant, aromatic and pungent and are used for culinary purposes to improve color, aroma, palatability and acceptability of food.[1] In addition, they are also used in folk medicine as antiscorbutic, antispasmodic, tonic, carminative agents against bronchitis, ulcers and as diuretics, depuratives and vermifuges. Also, some species are used as tea-flavoring agents in several regions. Natural food spices, such as pepper and mustard, have been reported to contain significant quantities of some heavy metals, including cadmium, lead and arsenic. Exposure to trace and heavy metals above the permissible affects human health and may result in illness to human fetus, abortion and preterm labor, as well as mental retardation to children. Adults also may experience high blood pressure, fatigue and kidney and neurological disorders.[1] Contamination with heavy metals may be accidental (e.g. contamination of environment during plant cultivation) or deliberate – in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies. Spices and herbal plants may contain heavy-metal ions over a wide range of concentrations.[2,3]

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India and China have a high diversity of plants used as spices, herbs, and traditional medicines. Several herbs and spices are either produced on small farmlands or naturally grow in different regions. There is often little information available about the safety of those plants and their products in respect to heavy metal contamination. Due to the significant amount of spices consumed, it is important to know the toxic metal contents in these spices.[4]

The objective of this work is two-fold: (1) to accurately analyze the levels of toxic heavy metals like lead, cadmium and arsenic that may be present in some major spice brands available in the local markets in China, by using graphite furnace atomic absorption spectrophotometry (GFAAS); (2) to cross-reference these measured levels to the recommended limits specified by the U.S. FDA.

Experimental

The measurements were performed using a PerkinElmer® AAnalyst™ 800 Atomic Absorption Spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA (Version 6.5) software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in this spectrophotometer provide outstanding signal-to-noise ratios. This solid-state detector is also highly efficient at low UV and high wavelengths at one time. It also features longitudinal Zeeman-effect background correction for graphite furnace analysis. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube. This eliminates the memory effect inherent with high-matrix sample analysis. The THGA features an integrated L'vov platform which is useful in overcoming potential chemical interference effects common to the GFAAS technique. High-energy electrodeless discharge lamps (EDLs) were used for all the elements.

Figure 1. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

A Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion (Figure 2). This is an industrial-type microwave oven which is equipped with various accessories to optimize the sample digestion. The samples were digested in the 8XF100 rotor using eight 100 mL high-pressure vessels made of PTFE-TFM protected with its individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to the conventional PTFE. This vessel has a working pressure of 60 bars (870 psi) and temperatures of up to 260 ˚C.

Figure 2. PerkinElmer/Anton-Paar Multiwave 3000 Microwave Sample Preparation System.

Standards, Chemicals and Certified Reference Materials

PerkinElmer single-element calibration standards for Atomic Spectroscopy were used as the stock standards for preparing the working standards. Working standards were prepared by serial volume/volume dilution in polypropylene vials (Sarstedt®, Germany)

ASTM® Type I water (from a Millipore® filtration system – Millipore® Corporation, Billerica, Massachusetts, USA) acidified with 0.2% Suprapur® nitric acid, from Merck® (Darmstadt, Germany) was used as the calibration blank and for all dilutions. Chemical modifiers were prepared from stock solutions, by diluting with acidified Millipore® water and were added automatically to each standard, blank and sample by the AS-800 autosampler, an integral part of the AAnalyst 800. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pippetting solutions.

NIST® 1573e certified reference material for trace metals in tomato leaves, GBW 10016 certified reference material for trace metals in tea leaves, and GBW 09101 certified reference material for trace metals in human hair were used to validate the method developed. Multi-element ICP standards for trace-metal ions in nitric acid (Spex. Certiprep.®, New Jersey, USA), prepared at concentrations of the midpoint of the calibration curves for different elements, were used as quality control (QC) check standards.

Sample and Certified Reference Material Preparation

Four branded powdered spice and herb samples available in China (five-spice mix, ground szechuan, five-spice powder and curry powder), bought from a local Chinese supermarket, were used for the analysis.

Approx. 0.5 g of each sample, accurately weighed in duplicate, was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3 (Page 4). The digested samples were diluted with 0.2% HNO_3 and made up to 20 mL in polypropylene vials. The certified reference materials were also digested in a similar manner.

Plastic bottles were cleaned by soaking with 10% volume/volume HNO_3 for at least 24 hours and rinsed abundantly in de-ionized water before use. The instrumental conditions for furnace experiments are given in Table 1, and the graphite furnace temperature programs are listed in Appendix I (Page 6). Heated injection at 90 °C was used for all the furnace experiments. Pyrolytically-coated graphite tubes with integrated platforms were used. The autosampler cups were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO_3 acid before use. Five-point calibration curves (four standards and one blank) were constructed for all the metal ions and the calibration-curve correlation coefficient was ensured to be better than 0.999 before the start of the sample analysis.

Table 1. **Optimized experimental conditions of AAnalyst 800.**

Element	Cd	Pb	As
Wavelength (nm)	228.8	283.3	193.7
Slit (nm)	0.7	0.7	0.7
Mode	AA-BG	AA-BG	AA-BG
Calibration	Linear with calculated intercept	Linear with calculated intercept	Linear with calculated intercept
Lamp	EDL	EDL	EDL
Current (mA)	230	440	380
Standards (µg/L)	0.2, 0.5, 1.0	5.0, 10, 25	5.0, 10, 25
Correlation Coefficient	0.9997	0.9991	0.9992
Read Time (sec)	5	5	5
Measurement	Peak Area	Peak Area	Peak Area
Injection Temp (°C)	90	90	90
Sample Volume (µL)	20	20	20
Matrix Modifier	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.005 mg Pd and 0.003 mg $Mg(NO_3)_2$
Modifier Volume (µL)	5	5	5

Table 2. **Program used for the digestion of spices and herbs with the Multiwave.**

Sequence	Power	Ramp Time (min)	Hold Time (min)	Fan
1	1200	15	15	1
2	0		15	3
Weight Taken	~500 mg	HNO_3	5.0 mL	
H_2O_2	1.0 mL	Rate	0.5 bar/sec	
Pressure	55 Bars			

Results and Discussions

The validity of the developed method has been ensured by incorporating various quality control (QC) checks and analysis of certified reference materials (CRMs). The agreement between the certified values and the measured values were excellent, which demonstrates the accuracy of the generated calibration as well as the overall accuracy of the developed method (Table 3 – Page 4). The slightly higher values obtained for Cd in NIST® CRM 1573e is due to the fact that the container of the CRM was opened a few years ago and the reference material was left in a polyethylene bag where contamination for Cd had occurred. This was confirmed by analyzing the same CRM using an ICP-MS (PerkinElmer ELAN® DRC-e) where a value of 2.37 µg/g was obtained. The QC standard gave excellent recovery with a variation of less than 10% usually prescribed by the regulatory bodies (Table 4 – Page 4). There was virtually no difference between the QC standard which was performed immediately after calibration and the QC standard which was analyzed at the end of the analysis with a time difference of more than three hours. This shows the long-term stability of the instrument. Method detection limits (MDLs) were calculated (Table 5 – Page 4) based on the standard deviation of seven replicates of the reagent blanks (student t-value of 3.14 for a confidence interval of 98%). These limits were obtained under routine operating conditions, and this is not reflective of the optimum detection limits achievable by the system. The extremely lower detection limits obtained show the capability of the AAnalyst 800 spectrometer in analyzing difficult matrices at the measured concentrations.

Table 3. **Analysis of certified reference materials.**

Analyte	NIST® 1573e		GBW 09101		GBW 10016	
	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)
Pb	*	–	3.83 ± 0.18	3.83 ± 0.54	1.5 ± 0.2	1.3 ± 0.5
Cd	1.52	2.24	0.072 ± 0.010	0.075 ± 0.01	0.062 ± 0.01	0.06 ± 0.02
As	0.112	0.15	0.198 ± 0.023	0.23 ± 0.03	0.09 ± 0.01	0.07 ± 0.02

**Not a reference element*

Table 4. **Results of QC recovery studies.**

Analyte	Prepared Concentration (µg/L) QC (analyzed at 10:00 am)	Measured Concentration (µg/L) QC (analyzed at 10:00 am)	% QC Recovery	Prepared Concentration (µg/L) QC (analyzed at 1:30 pm)	Measured Concentration (µg/L) QC (analyzed at 1:30 pm)	% QC Recovery
Pb	12.5	12.6	101	12.5	12.6	101
Cd	0.50	0.50	100	0.50	0.50	100
As	12.5	12.1	97	12.5	13.3	106

Table 5. **Method detection limits (MDLs).**

Analyte	MDL (µg/kg)
Pb	9
Cd	2
As	6

Table 6. **Analysis of spices and herbs samples using GFAAS.**

Analyte (µg/g)	Five-Spice Mix	Ground Szechuan	Five-Spice Powder	Five-Spice Powder Duplicate	Curry Powder	Curry Powder Duplicate
Pb	2.42	4.48	6.30	6.93	1.21	1.22
Cd	0.25	0.07	0.25	0.23	0.38	0.35
As	0.12	0.07	0.16	0.15	0.14	0.14

Table 7. **Analysis of spices and herbs samples using ICP-MS.***

Analyte(µg/g)	Five-Spice Mix	Ground Szechuan	Five-Spice Powder	Curry Powder
Pb	2.72	4.69	6.04	1.28
Cd	0.11	0.06	0.27	0.26
As	0.19	0.16	0.18	0.31

**Different set of similar samples*

Conclusions

An accurate and reliable microwave-assisted sample pretreatment procedure for the determination of arsenic, cadmium and lead in spices using GFAAS is described. The spices contain a number of organic substances of different stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution and this also disturbs spectrochemical measurements.[5] Application of concentrated HNO_3 along with hydrogen peroxide for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by determined values of the analytes in various CRMs. Toxicity of medicinal spices and herbs is of much greater concern today than ever before. In recent years, much emphasis is being laid on toxic-element contents, as several European Union countries have banned many varieties of ayurvedic drugs. The results in Table 6 and 7 (Page 4) show that the levels of arsenic, cadmium and lead in all the samples analyzed were well within the permissible limits of 10, 0.3 and 10 mg/kg respectively, as specified by the U.S. FDA. The results confirmed that the determination of arsenic, cadmium and lead after acid solubilization of spice-mixture samples by microwave digestion can be performed by GFAAS without any interference and the same has been cross-checked by analyzing a different set of similar samples with ICP-MS analysis (using a PerkinElmer ELAN DRC-e ICP-MS). The good agreement of the values obtained with standard ICP-MS and GFAAS analysis further confirmed the accuracy of the method developed.

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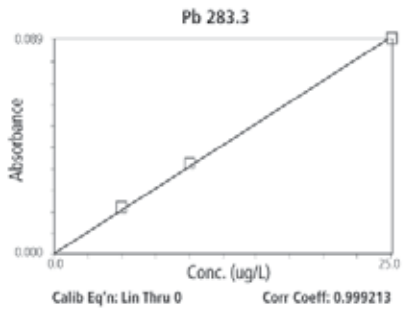
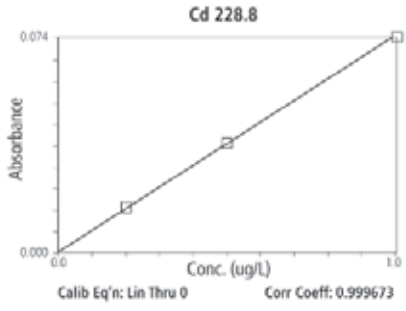
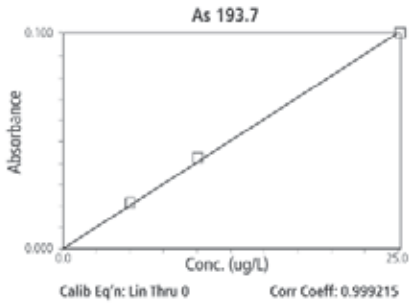
Appendix I. Graphite Furnace Temperature Program.

Element	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Cd	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	500	10	20	250	Argon
	4	1500	0	5	0	Argon
	5	2450	1	3	250	Argon
As	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	1200	10	20	250	Argon
	4	2000	0	5	0	Argon
	5	2450	1	3	250	Argon
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	Argon
	5	2450	1	3	250	Argon

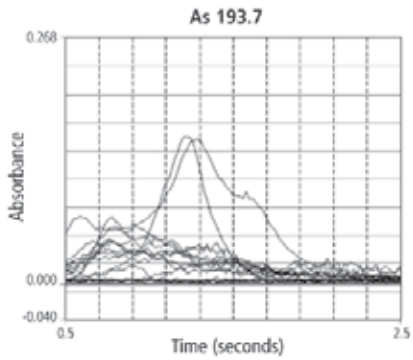
Appendix II. Calibration Graphs for Different Analytes.

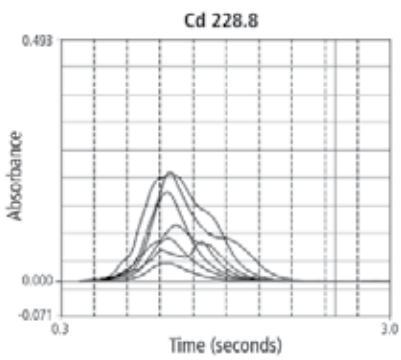


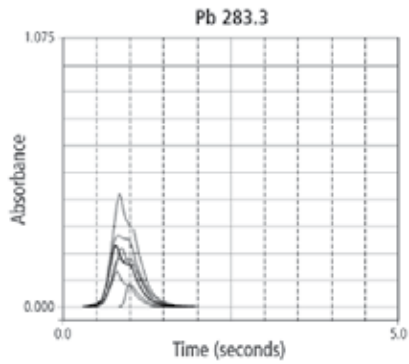




Appendix III. Atomization Profiles of Lower Standard and Some Samples.







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APPLICATION NOTE

Atomic Absorption

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Quantification of Essential Metals in Spice Mixtures for Regulatory Compliance Using Flame Atomic Absorption Spectrophotometry

Introduction

Foods, together with water, provide the major proportion of the total daily intake of trace elements by humans. Spices and vegetables are some of the most common foods in the human diet around the world. Besides polluted soil and water, foods can also be contaminated with trace metals by the introduction of mechanized farming, the increasing use of chemicals, food processing and packaging, etc. In order to minimize adverse impact, it is important to measure and continuously monitor the levels of trace elements in various kinds of food materials. Trace element food composition data are also important for both consumers and health professionals. In recent years, food labeling legislation has enforced this requirement. Trace element determination in complex matrices, such as food, often requires sample preparation prior to determination by instrumental techniques.[1]

Cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) are all essential elements, not only for mammals, but also for plants. They play important roles in many biological processes including carbohydrate and lipid metabolism.[2] For example, a daily copper intake of 1.5 - 2.0 mg is essential and copper at nearly 40 ng/mL is required for normal metabolism of many living organisms.[3] However, copper at higher levels is toxic to the circulatory system and kidneys. The trace element content of food items for all the essential elements mentioned above must be controlled on a daily basis.

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There is an increasing need to monitor the essential element levels in food samples at ever decreasing concentrations. For this purpose, very sensitive, yet rapid and inexpensive methods are necessary. The quantification of trace metals in food samples has routinely been carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES),[4] inductively coupled plasma mass spectrometry (ICP-MS),[5,6] graphite furnace atomic absorption spectrophotometry (GFAAS)[7] and flame atomic absorption spectrophotometry (FAAS).[8,9,10] Compared with other techniques, FAAS has the characteristics of good precision and simplicity with lower cost and minimum operator proficiency. The objective of this work is two-fold: (1) to accurately analyze, using FAAS, the levels of essential metals (in particular: cobalt, copper, manganese, nickel and zinc) that may be present in some major spice brands available on the market; (2) to cross-reference the measured levels to the recommended maximum tolerable daily intake limits specified by the U.S. Department of Agriculture (USDA).

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in this spectrophotometer provide outstanding signal-to-noise ratios. The solid-state detector is also highly efficient at low UV and longer wavelengths at the same time. The instrumental conditions for flame experiments are given in Table 1 (Page 3). A high-sensitivity nebulizer (Part No. N3160144) was used and the read time was 3.0 seconds for all samples. The signal type was AA and the calibration equation was linear through zero.

Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

A microwave sample preparation system was used for the digestion of spice samples and the certified reference material (CRM). This is an industrial-type microwave oven which is equipped with various accessories to optimize the sample digestion. The samples were digested using 100 mL high-pressure vessels made of PTFE. The sample digestion was performed in accordance with the program given in Table 2 (Page 3), as per U.S. Environmental Protection Agency (EPA) Method 3052.

Standards, Samples and Certified Reference Material Preparation

Single-element PerkinElmer Pure Calibration Standards for atomic spectroscopy were used as the stock standards for preparing the working standards (Part Nos. Co: N9303766; Cu: N9300183; Mn: N9303783; Ni: N9300177; Zn: N9300178). Working standards were prepared by serial volume/volume (v/v) dilution in 50 mL conical free-standing polypropylene vials (Part No. B0193234). Four-point calibration curves (three standards and one blank) were constructed for each individual metal ion and their calibration curve correlation coefficients (r^2) were better than 0.999 before the start of the sample analysis (Appendix I – Page 5).

ASTM® Type I water (Millipore® Corporation, Billerica, Massachusetts, U.S.) acidified with 0.2% nitric acid (Tamapure®,TAMA Chemicals, Japan) was used as the calibration blank and for all dilutions. NIST® 1568a CRM for Trace Metals in Rice Flour was used to validate the method. Quality control (QC) check standards were prepared at the calibration curve midpoint for each individual element. Three branded powdered spice and herb samples available in India (coriander powder, ginger powder, and black pepper powder) were bought from a supermarket to be analyzed.

Approximately 0.5 g of each sample or CRM was accurately weighed in duplicate, then transferred to a digestion vessel. The digested samples were brought up to 25 mL in polypropylene vials with 0.2% HNO_3. All sample vessels were cleaned by soaking with 10% v/v HNO_3 for at least 24 hours and rinsed abundantly in de-ionized water before use.

Table 1. **Optimized experimental conditions of PinAAcle 900T.**

Analyte	Co	Cu	Mn	Ni	Zn
Wavelength (nm)	240.73	324.75	279.48	232.00	213.86
Slit (nm)	0.2	0.7	0.2	0.2	0.7
Lamp Current (mA)	30	15	20	25	15
Calibration Stds (mg/L)	0.25, 0.5, 1.0	0.16, 0.32, 0.64	0.125, 0.5, 1.0	0.5, 1.0, 2.0	0.06, 0.12, 0.24
r^2	0.9995	0.9999	0.9998	0.9994	0.9999
QC Std (mg/L)	0.50	0.32	0.50	1.0	0.12
Lamp Type	HCL	HCL	HCL	HCL	HCL
Lamp Part No.	N3050118	N3050121	N3050152	N3050145	N3050191

Table 2. **Program used for the digestion of spices and herbs.**

Sequence	1	2
Power (watts)	1000	0
Ramp Time (min)	10	0
Hold Time (min)	10	20
Weight Taken (mg)	~500	
H_2O_2 (mL)	1.0	
HNO_3 (mL)	7.0	
Temp (˚C)	180	

Results and Discussions

The role of the sample introduction system is of paramount importance in optimizing the short-term stability of signals. Furthermore, the best sensitivity can be achieved by careful optimization of flame conditions. WinLab32™ for AA software comes with a unique "Optimize Gas Flows" feature which helps to achieve the best possible sensitivity. This is an extremely important feature to assist in the analysis of low-level analytes in high matrix samples. The new easy-fit sample introduction system with the inert polymer spray chamber and high-precision nebulizer maximizes short-term stability and sensitivity even for the high acid matrix samples like the one used here. This is evident from the excellent percent relative standard deviation (%RSD) values obtained for low concentration standard signals (Table 3).

Table 3. **Precision at midpoint of calibration for 10 replicate readings (%RSD).**

Analyte	% RSD	Concentration (mg/L)
Co	0.8	0.50
Cu	0.7	0.32
Mn	0.9	0.50
Ni	0.7	1.0
Zn	0.9	0.12

The agreement between the certified values of the CRM and the measured values were good, demonstrating the accuracy of the generated calibration, as well as the overall accuracy of the developed method (Table 4). Method detection limits (MDLs) were calculated (Table 5) based on the standard deviation of seven replicates of the reagent blank (Student's t-value = 3.14, ρ = 0.02) and took into consideration the dilution factor of the samples. These limits were obtained under routine operating conditions. The extremely low detection limits obtained show the ability of the PinAAcle 900T spectrometer to analyze difficult matrices at the measured concentrations.

Table 4. **Analysis of certified reference material by FAAS.**

	NIST® 1568a Rice Flour	
Analyte	Certified Value (µg/g)	Measured Value (µg/g)
Cu	2.4 ± 0.3	2.30 ± 0.7
Mn	20.0 ± 1.6	20.0 ± 0.2
Zn	19.4 ± 0.5	18.8 ± 0.1

**Co and Ni were not reference elements.*

Table 5. **Typical method detection limits (MDLs) for the analysis of essential elements in spices.**

Analyte	MDL (mg/L)
Co	0.50
Cu	0.45
Mn	0.15
Ni	0.30
Zn	0.10

Post-digestion recovery studies were carried out and the results are summarized in Table 6. The recoveries obtained for post-digestion spikes easily met the U.S. EPA guideline of ±15%. However, it is known that incomplete mineralization of samples during the microwave digestion process may cause difficulty in transferring analytes into solution and disturbances in the spectrochemical measurements.[11]

The spice analysis results are summarized in Table 6. For example, the maximum tolerable daily intake limit specified by the USDA for copper is 10.0 mg. The results (Table 7) show that the level of Cu is not going to exceed the maximum tolerable daily intake limits specified by the USDA, assuming that no one would be consuming ≥ 300 g of the spice mixture on a daily basis.

Conclusions

Methods were developed for the accurate determination of Co, Cu, Mn, Ni, and Zn in spice mixtures using the PinAAcle 900T atomic absorption spectrometer in the FAAS mode and microwave digeston. The results confirmed that the determination of copper, manganese, cobalt, nickel and zinc in spices, after acid solubilization by microwave digestion, can be performed by FAAS on the PinAAcle 900T without any interferences. The PinAAcle 900H (Flame and Deuterium Furnace) and PinAAcle 900F (Flame only) spectrometers can also be used for this application.

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Table 6. **Post-digestion spike recovery study – concentrations are based on the diluted solutions (two replicates (n=2) were performed for each sample).**

Analyte	Sample Recovery (%)		Spike Level (mg/L)
	Black Pepper Powder	Ginger Powder	
Co	90	96	0.5
Cu	101	106	0.16
Mn	94	87	0.13
Ni	90	90	0.5
Zn	102	105	0.06

Table 7. **Concentration of metals in spice and herb samples using FAAS compared to USDA guidelines (two replicates (n=2) were performed for each sample and sample duplicate).**

Analyte	USDA Regulatory Level (mg)	Black Pepper Powder (mg/kg)		Coriander Powder (mg/kg)		Ginger Powder (mg/kg)	
		Sample	Duplicate	Sample	Duplicate	Sample	Duplicate
Co	*	0.80	0.82	0.65	0.75	1.5	1.3
Cu	10.0	32.3	33.3	13.9	13.8	5.9	5.6
Mn	11.0	185	189	145	145	770	745
Ni	*	4.1	4.0	1.7	1.4	2.3	2.1
Zn	40.0	39	36	74	71	50	51

**No USDA reference value for specified tolerable upper intake level was found.*

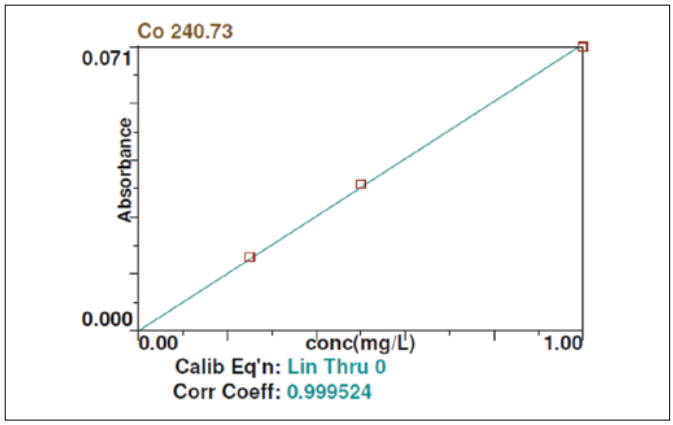

Figure 2. Calibration curve for the detection of Co using FAAS.

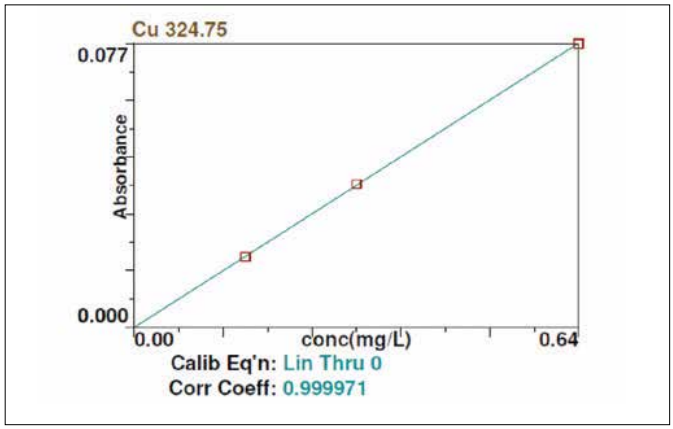

Figure 3. Calibration curve for the detection of Cu using FAAS.

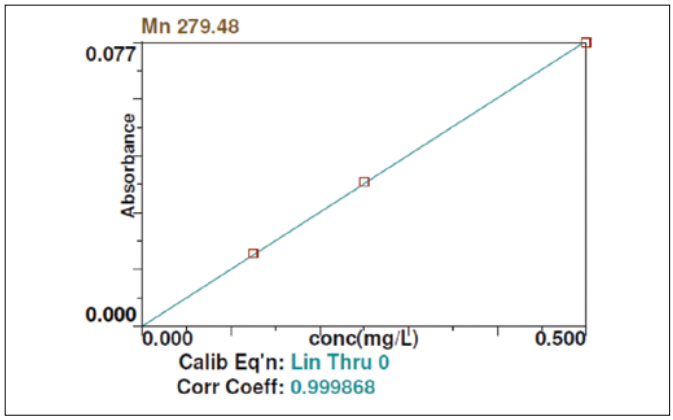

Figure 4. Calibration curve for the detection of Mn using FAAS.

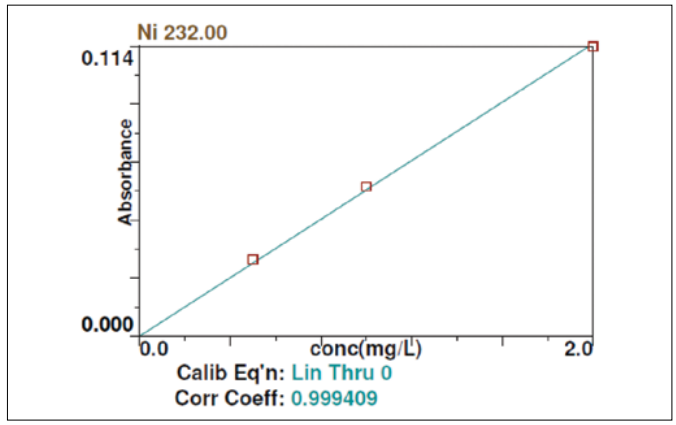

Figure 5. Calibration curve for the detection of Ni using FAAS.

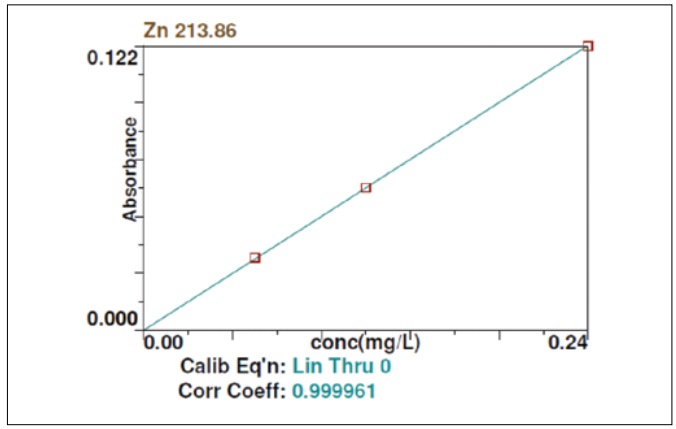

Figure 6. Calibration curve for the detection of Zn using FAAS.

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APPLICATION NOTE

Atomic Absorption

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Analysis of Pb, Cd and As in Spice Mixtures using Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

The toxicity and effect of trace heavy metals on human health and the environment has attracted considerable attention and concern in recent years. With an inherent toxicity, a tendency to accumulate in the food chain and a particularly low removal rate through excretion,[1] lead (Pb), cadmium (Cd) and arsenic (As) cause harm to humans even at low concentrations. Exposure to trace and heavy metals above the permissible level affects human health and may result in teratogenicity (reproductive effects). Individuals may also experience high blood pressure, fatigue, as well as kidney and neurological disorders.

Spices, the dried parts of plants, grow widely in various regions of the world, are produced either on small farmlands or naturally grown, and have been used for several purposes since ancient times. Most are fragrant and flavorful and are used for culinary purposes to improve the quality of food.[2] Natural food spices, such as pepper, have been reported to contain significant quantities of some heavy metals, including Pb, Cd and As. Contamination with heavy metals may be accidental (e.g. contamination of the environment during plant cultivation) or deliberate – in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies. Spices and herbal plants may contain heavy metal ions over a wide range of concentrations.[3,4] There is often little information available about the safety of those plants and their products in respect to heavy metal contamination. Due to the significant amount of spices consumed, it is important to know the toxic metal concentrations in them.[5]

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The major challenge in performing spice analyses is the extremely low analyte concentrations combined with high matrix levels. Graphite furnace atomic absorption spectrophotometry (GFAAS) has been one of the more reliable techniques used for many years and is therefore the preferred analytical method. The use of longitudinal Zeeman background correction and the use of matrix modifiers provides accurate results and low detection limits in samples with high matrices. This makes GFAAS an indispensible tool in carrying out such analyses.

The objective of this work is two-fold: (1) to use GFAAS to accurately analyze the levels of Pb, Cd, and As present in some major spices commonly available on the market; and (2) to cross reference these measured levels to the recommended limits specified by the U.S. FDA.

Experimental Conditions

Instrumentation

The measurements were performed using a PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA software running under Microsoft® Windows™ 7, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance (Figure 1). The high-efficiency optical system and solid-state detector used in the PinAAcle 900T spectrometer provide outstanding signal-to-noise ratios. The longitudinal Zeeman-effect background correction for graphite furnace analysis provides accurate background correction without the loss of light that usually occurs in transverse Zeeman systems. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube, eliminating memory effects and potential interferences that may occur with high-matrix sample analysis. Pyrolytically coated THGA tubes with end caps (Part No. B3000655) were used for all measurements. The optimized instrumental conditions for furnace experiments are given in Table 1 (Page 3), and the graphite furnace temperature programs are listed in Appendix I (Page 5). A heated injection was used for lead; it can also be used for cadmium and arsenic.

Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

A microwave sample preparation system was used for the microwave-assisted digestion. This is an industrial-type microwave oven, which is equipped with various accessories to optimize the sample digestion. The samples were digested using ten 100 mL high-pressure vessels made of PTFE. The microwave digestion program is listed in Table 2 (Page 3).

Standards, Chemicals and Certified Reference Materials

PerkinElmer Pure single-element calibration standards for Pb, Cd, and As were used as the stock standards for preparing the working standards (Part Nos. Pb: N9300128; Cd: N9300107; As: N9300102). Working standards were prepared by serial volume/volume (v/v) dilution in polypropylene vials (Part Nos. B0193233 15 mL Conical; B0193234 50 mL Conical Freestanding) ASTM® Type I deionized water (Millipore® Corporation, Billerica, Massachusetts, U.S.) acidified with 0.2% nitric acid (HNO_3) (Tamapure®, TAMA Chemicals, Japan) was used as the calibration blank and for all dilutions. 30% hydrogen peroxide (H_2O_2) (Kanto Chemicals, Tokyo, Japan) was used for digestion along with nitric acid.

Matrix modifiers were prepared from 10% $NH_4H_2PO_4$ (Part No. N9303445), 1% Mg as $Mg(NO_3)_2$ (Part No. B0190634) and 1% Pd (Part No. B0190635) stock solutions, by diluting with the 0.2% HNO_3 made above. Matrix modifiers were added automatically to each standard, blank and sample by the AS 900 autosampler, an integral part of the PinAAcle 900T spectrometer.

NIST® 1568a Certified Reference Material (CRM) for Trace Metals in Rice Flour was used to validate the method. Quality control (QC) check standards were prepared at the calibration curve midpoint concentration for each individual element.

Sample and Certified Reference Material Preparation

Plastic bottles were cleaned by soaking with 10% v/v HNO_3 for at least 24 hours and rinsed abundantly in deionized water before use. The polypropylene autosampler cups (Part No. B3001566) were soaked in 20% nitric acid overnight to minimize sample contamination, and thoroughly rinsed with 0.5% HNO_3 acid before use. Five-point calibration curves (four standards and one blank) were constructed for each analyte and the calibration curve correlation coefficient was examined to ensure a value better than 0.998 before the start of the sample analysis (Appendix II – Page 6).

Five branded powdered spice samples available in local supermarkets in India (coriander powder, ginger powder, asafetida, black pepper powder and red chili powder) were analyzed. Approximately 0.5 g of each sample or CRM, accurately weighed in duplicate, was transferred to the vessel of the microwave digestion system and the sample digestion method (Table 2) was performed in accordance with U.S. Environmental Protection Agency (EPA) Method 3052. The digested samples were diluted with 0.2% HNO_3 and brought up to 25 mL in polypropylene vials.

Table 1. **Optimized experimental conditions of PinAAcle 900T.**

Analyte	**Pb**	**Cd**	**As**
Wavelength (nm)	283.3	228.8	193.7
Lamp Type	EDL	HCL	EDL
	(Part No. N3050657)	(Part No. N3050115)	(Part No. N3050605)
Standards (µg/L)	5, 10, 15, 20	0.5, 0.75, 1.0, 2.0	10, 20, 30, 40
Correlation Coefficient	0.9991	0.9996	0.9989
Read Time (sec)	3	5	3
Injection Temp (˚C)	90	20	20
Matrix Modifier (mg)	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$	0.005 mg Pd and 0.003 mg $Mg(NO_3)_2$
Modifier Volume (µL)	5		
Slit Width (nm)	0.7		
Sample Volume (µL)	20		
Measurement Type	Peak Area		
Calibration	Linear through zero		

Table 2. **Program used for the digestion of spices and herbs.**

Sequence	**1**	**2**
Power (watts)	1000	0
Ramp Time (min)	10	0
Hold Time (min)	10	20
Weight Taken (mg)	~500	
H_2O_2 (mL)	1.0	
HNO_3 (mL)	7.0	
Temperature (˚C)	180	

Results and Discussions

In the analysis of complex samples with the graphite furnace, obtaining reproducible results can be a challenging task as one has to deal with analytes present at the low µg/L ppb level and the potential for matrix interferences. With Stabilized Temperature Platform Furnace (STPF) conditions (including: pyrolytically coated graphite tubes, platform atomization, rapid heating, internal gas stop during atomization, fast signal processing and Zeeman background correction) the analysis of low level analytes in spice mixtures is an easy task.

The graphite furnace sample introduction system is of paramount importance in optimizing the short-term stability of signals. The PinAAcle 900T spectrometer uses a unique built-in camera to monitor sampler tip alignment and sample introduction into the graphite tube. With the TubeView™ furnace camera, it is simple to position the AS 900 autosampler sample capillary tip at the correct depth inside the tube so as to achieve highly reproducible pipetting.

Table 3. **Analysis of certified reference material by GFAA.**

Analyte	NIST® 1568a Rice Flour	
	Certified Value (µg/g)	Measured Value (µg/g)
Pb	<0.010	0.0093
Cd	0.022 ±0.002	0.020 ±0.004
As	0.29 ±0.03	0.24 ±0.02

Table 4. **Estimated method detection limits (MDLs).**

Analyte	MDL (µg/L)
Pb	9.5
Cd	2.35
As	9.5

Table 5. **Spike recovery studies.**

Analyte	Sample	Post-digestion Recovery (%)	Spike Level (µg/L)	Pre-digestion Recovery (%)	Spike Level (µg/L)
Pb	Coriander powder	91	5.00	111	5.00
	Ginger powder	90		*	
Cd	Coriander powder	104	0.625	119	1.00
	Ginger powder	96		*	
As	Coriander powder	108	8.33	100	5.00
	Ginger powder	106		*	

**Pre-digestion spike recovery not performed*

The developed method has been validated by incorporating various QC checks and by the analysis of a CRM. The agreement between the certified and measured values was good (Table 3), which demonstrates the accuracy of the direct calibration being used as well as the overall accuracy of the developed method. Method detection limits (MDLs) were calculated (Table 4) based on the standard deviation of seven replicates of the reagent blank (t-value = 3.14) and took into account the dilution factor for the samples. The low method detection limits obtained show the capability of the PinAAcle 900T spectrometer in analyzing difficult matrices at the measured concentrations.

Incomplete mineralization of samples during the microwave-digestion process, due to high organic content, may cause difficulty in transferring analytes into solution. These impurities may also affect the analytical measurements.[6] Application of concentrated HNO_3 along with H_2O_2 for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by the determination of the values of the analytes in the CRM (Table 3). Both a pre-digestion and an automatic post-digestion recovery study were performed. The recoveries obtained for the pre-digestion spike easily meet the U.S. EPA recovery guidelines of ±30% and indicate that neither analyte loss nor contamination was of major concern throughout the analytycal procedure (Table 5). The recoveries obtained for the post-digestion spike indicate that no matrix interference towards the analyte signal was seen. The results of the sample analysis are summarized in Table 6. The level of arsenic, cadmium and lead in all the samples analyzed were well within the permissible limits of 10, 0.3 and 10 mg/kg respectively, as specified by the U.S. FDA.

Table 6. **Analysis of spices and herbs samples using GFAAS (µg/kg).**

Analyte	U.S. FDA Limit	Replicate	Coriander Powder	Ginger Powder	Asafetida	Black Pepper Powder	Red Chili Powder
Pb	10,000	Sample	455	551	124	9.8	286
Cd	300	Sample	48	89	8.2	25	57
		Duplicate	47	92	8.2	28	59
As	10,000	Sample	20	49	41	49	<MDL
		Duplicate	24	42	38	32	<MDL

Conclusions

A complete method for the accurate determination of Pb, Cd and As in various spice mixtures using the PinAAcle 900T atomic absorption spectrophotometer in the GFAAS mode was developed. The results confirmed that the determination of arsenic, cadmium and lead in spice mixtures, after acid solubilization by microwave digestion, can be performed by GFAAS without any interference. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

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Appendix I – Graphite Furnace Temperature Program

Table 7. **Furnace temperature program for Pb.**

Analyte	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	–
	5	2450	1	3	250	Argon

Table 8. **Furnace temperature program for Cd.**

Analyte	Step	Temp (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Cd	1	110	10	30	250	Argon
	2	130	15	30	250	Argon
	3	500	15	35	250	Argon
	4	1500	0	3	0	–
	5	2450	1	3	250	Argon

Table 9. **Furnace temperature program for As.**

Analyte	Step	Temp (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
As	1	110	5	30	250	Argon
	2	130	20	30	250	Argon
	3	800	15	40	250	Argon
	4	1200	15	30	250	Argon
	5	2200	0	5	0	–
	6	2450	1	3	250	Argon

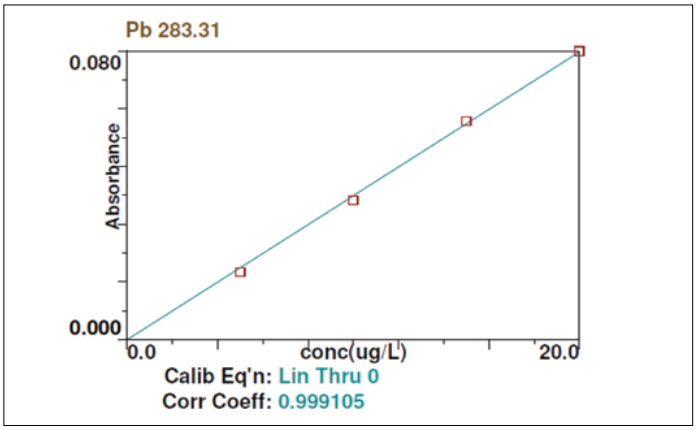

Figure 2. Calibration curve for the determination of Pb using GFAAS.

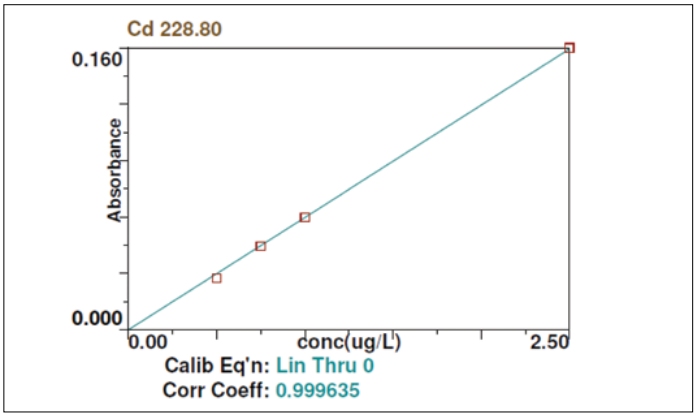

Figure 3. Calibration curve for the determination of Cd using GFAAS.

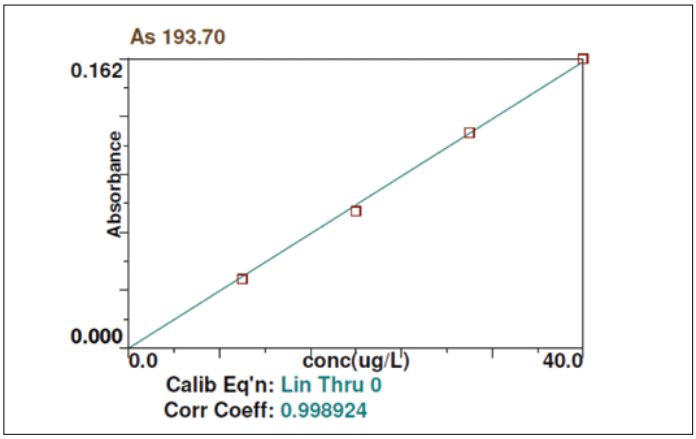

Figure 4. Calibration curve for the determination of As using GFAAS.

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APPLICATION NOTE

UHPLC

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Analysis of Ginsenosides in Ginseng Root with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

The root of the panax genus plant (also called Ginseng) has been used as an herbal medicine in Asia for over two thousand years for its purported various health benefits, including (but not limited to), antioxidant, anticarcinogenic, anti-inflammatory, antihypertensive and anti-diabetic. The pharmacologically active compounds behind the claims of ginseng's efficacy are ginsenosides; their underlying mechanism of action although not entirely elucidated appears to be similar to that of steroid hormones. There are a number of ginseng species, and each has its own set of ginsenosides. In fact, more than forty different ginsenosides have been identified. Ginsenosides are a diverse group of steroidal saponins with a four ring-like steroid structure with sugar moieties (Figure 1); they are found exclusively in ginseng plants and are in higher concentration in their roots. There are two main groups of ginsenosides: the panaxadiol group or Rb1 group that includes Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3; and the panaxatriol group or Rg1 group that includes Rg1, Re, Rf, Rg2 and Rh1.

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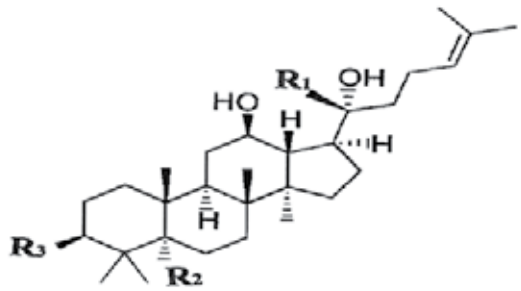
Qualitative and quantitative analytical techniques for the analysis of ginsenosides are in demand to ensure quality control in ginseng root processing, as well as for the study of their metabolism and bioavailability. This application note presents a robust liquid chromatography method to simultaneously test seven ginsenosides. Method conditions and performance data including precision, accuracy and linearity are presented. The method is applied to a panax ginseng (Korean Ginseng) root capsules and the types of ginsenosides are confirmed.



Ginsenoside	R3	R2	R1
Rb1 group	-O-Glc$_{(2-1)}$Glc	-H	-O-Glc$_{(6-1)}$Glc
Rg1 group	-OH	-O-Glc	-O-Glc

Glc Glucopyranoside (β-D-Glucose).
Subscripts indicate the carbon in the glucose rings linking the two carbohydrates.

Figure 1. Molecular structure of ginsenosides.

Experimental

Seven stock standard solutions of each ginsenoside at 1 mg/mL concentration were prepared by dilution with 70:30 methanol/water (diluent), followed by one minute vortex. A working standard of 0.14 mg/mL was prepared by mixing together 0.5 mL of each of the stock solution.

Precision was evaluated with eight injections of the working standard. Linearity was determined across a range of 7 µg/mL to 140 µg/mL. To assess the accuracy of the method, purified water was spiked with the working standard to obtain a solution with 7 µg/mL ginsenosides. About 3 g of a panax ginseng powder from a popular brand capsules was transferred into 50 mL volumetric flask, 30 mL of diluent was added followed by about a minute vortexing and 30 min. sonication. The solution was then centrifuged at 5000 RPM for 10 min. and the supernatant was collected and set aside. 15 mL of diluent was added in the remaining precipitate followed by vortexing, sonication and centrifugation similar to that described above. This latter supernatant was collected and added to the first collected supernatant in a 50 mL volumetric flask; the solution was brought volume with diluent, mixed well and filtered with a 0.2 µm nylon membrane prior to testing.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector served as a platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) column.

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume, partial loop mode
	350 µL mixer volume; injector wash and carrier: water
Injection:	2 µL
PDA Detector:	Scanned from 190-400 nm, recording setting 203 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) at 45 °C, Part No. N9308402
Mobile Phase:	A: water
	B: acetonitrile

Time (min)	Flow rate (mL/min)	B %	Curve
2.5	0.4	30-35	1
3.5	0.4	35-50	1

	3 minutes equilibration after each run
	(HPLC grade solvent and ACS grade reagent)
Sampling Rate:	5 pt/s
Software:	Chromera® Version 3.0

Results and Discussion

The optimal flow rate of this method was determined to be 0.4 mL/min. at 45 °C, the pressure stabilized around 5150 PSI (355 bar) and all the peaks eluted within six minutes. A representative chromatogram of the standards solution and the Korean ginseng tested are in Figure 2 and 3. Excellent method performance was achieved: the linearity of the analysis had a R-squared of not less than 0.997 for each ginsenoside and a precision (relative standard deviation %RSD) with values ranging from 0.6% to1.2%. The spiked purified water tested had an average recovery of 99.9% with values ranging from 91.2% to108.0% (Figure 4). Details of the method performance and results of the panax ginseng and spiked sample tested are presented in Table 2.

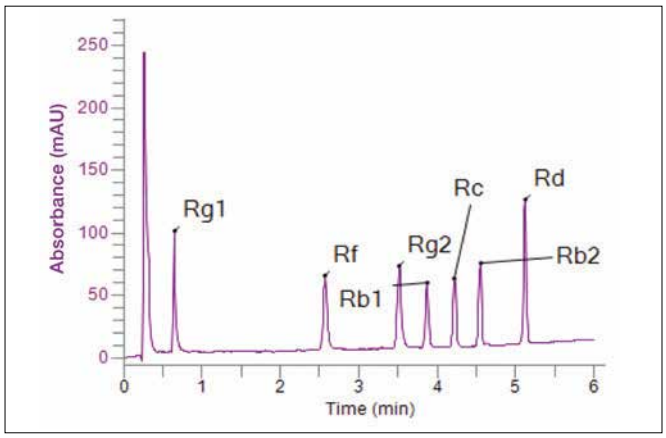


Figure 2. Chromatogram from the analysis of a standard.

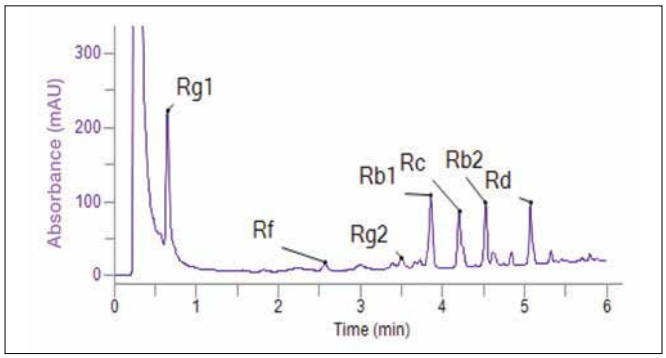


Figure 3. Chromatogram from the analyses of panax ginseng.

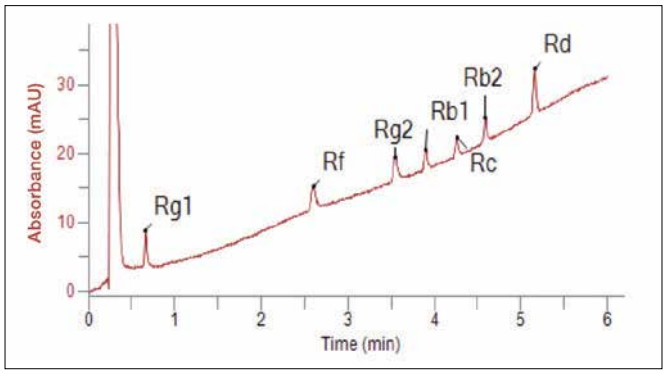


Figure 4. Chromatogram from the analyses of 7 ppm ginsenosides in water.

Table 2. **Precision, linearity, accuracy and samples.**

Compound	%RSD n = 8	r^2	Range (µg/mL)	Korean Ginseng (mg/g)	7 ppm Spiked Water
Rg1	0.9	0.9997	7 - 140	13	97.5
Rf	0.6	0.9971	7 - 140	1	91.2
Rg2	1.2	0.9983	7 - 140	1	98.7
Rb1	1.1	1	7 - 140	10	102.1
Rc	1.2	0.9994	7 - 140	10	100.3
Rb2	1.0	0.9996	7 - 140	7	101.4
Rd	1.2	0.9997	7 - 140	4	108.0
Avg/Tot.	**1.0/NA**	**0.9988/NA**	**NA**	**NA/46**	**99.9/NA**

NA = Not Applicable

Conclusion

The seven ginsenosides were well resolved within six minutes. The method was shown to be linear with R-squared ≥ 0.997, precise with %RSD ≤1.2 and accurate with a recovery averaging 99.9%. The Korean ginseng capsule tested has 46 mg/g of ginsenosides. PerkinElmer's Flexar FX PDA detector provides rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3-D and contour maps, which are powerful tools that give insight to the information content of a 3-D photodiode array chromatogram. The spectra library search function allowed the storage of standard peaks spectra that could later be used for peak identification confirmation in the sample.

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Note: This application note is subject to change without prior notice.

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APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Polyphenols in Saffron Petal Extracts with UHPLC/UV/MS

Introduction

Saffron is one of the most expensive spices by weight and is savored around the world. It is derived from the flowers of the crocus plant *Crocus sativus,* which is mostly cultivated in the Mediterranean and Middle East regions. Saffron has recently received additional focus for its naturally-containing carotenoids and polyphenols, both of which have been reported to have nutraceutical/medicinal value, particularly for their antioxidant and anti-inflammatory characteristics[1].

With the above-mentioned interest in mind, this particular work focused on the characterization of the major polyphenolic compounds that are naturally present in saffron petal extract, using UHPLC (ultra-high pressure liquid chromatography) and both UV/Vis and ESI-MS (electrospray ionization mass spectrometer) detectors. The UV/Vis detector was used for the initial detection of the chromatographically-separated analytes while the complimentary ESI-MS was primarily used for analyte identification/confirmation.

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Experimental

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade.

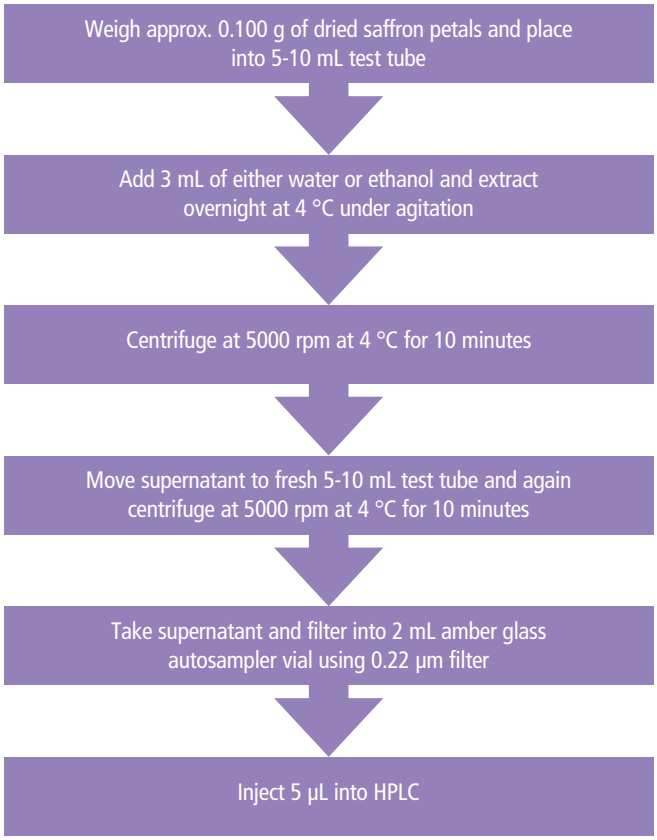
Formic acid, used as a solvent modifier, was obtained from Sigma-Aldrich®, St. Louis, MO.

All standards were obtained from Polyphenols Laboratories AS, Sandnes, Norway. All standards were diluted in 90:10 methanol: water with 0.1 % formic acid.

Saffron petal extracts were obtained from Agenzia per lo Sviluppo, Azienda Speciale CCIAA, L'Aquila, Italy.

Sample Preparation

The saffron petal extracts were prepared using the following procedure:



Hardware/Software

For all chromatographic separations, a PerkinElmer Flexar™ UHPLC system was used, including an FX-15 pump, FX UHPLC autosampler, UHPLC UV/Vis detector, Flexar SQ 300 MS detector, vacuum degasser and column oven. All instrument control, analysis and data processing was done via PerkinElmer Chromera™ software.

Method Parameters

The HPLC method and SQ 300 MS detector parameters are shown in Table 1 and Table 2, respectively.

Table 1. HPLC Method Parameters.

HPLC Conditions					
Column	Zorbax RRHD Extended C18 3x150 mm, 1.8 µm				
Mobile Phase	A = water with 0.1 % formic acid B = methanol with 0.1 % formic acid				
	Step	**Time [min]**	**A%**	**B%**	**Curve**
	0	4	95	5	Isocratic
	1	12	35	65	-1.5 (convex)
	2	2	20	80	1 (linear)
Analysis Time	14 min				
Flow Rate	0.45 mL/min.				
Oven Temp.	25 °C				
Detection	1) Flexar UV/Vis; Wavelength: 270 nm 2) Flexar SQ 300 MS				
Injection Volume	5 µL				

Table 2. SQ 300 MS Conditions.

Interface	ESI
Polarity	Negative
Scan	TIC (200-800 amu)
Drying Gas Temperature	320 °C
Drying Gas Flow Rate	12 L/min
Nebulizer Gas Pressure	85 psi
Capillary Exit	-300 eV
Skimmer	-30 V

Results and Discussion

Figures 1 a/b and 2 a/b show the UV chromatogram (λ= 270 nm) and the ESI-MS TIC (total ion chromatogram) for both ethanolic and aqueous saffron extracts, respectively. As can be seen, using either extraction procedure, the chromatograms showed the presence of many analytes, demonstrating that either of the two extraction procedures is equally effective. A number of these analytes were expected to be free or glycosylated flavonoids.

Some of the analytes were identifiable through the combination of chromatographic retention times and mass-spectrum analysis, while others, to be properly identified, would require a more thorough literature review and a comparison with pure standards.

Being particularly interested in the discrimination between two flavonoids having similar MWs, we focused our analysis on kaempferol sophoroside and quercetin rutinoside, two glycosylated compounds, both having a parent MW of 610. The UV chromatographic results of a 2 ppm standard mix of quercetin rutinoside and kaempferol sophoroside are shown in Figure 3.

Using the before-mentioned chromatographic conditions employed, the peak of quercetin rutinoside had a retention time of about 5.20 minutes, while that of kaempferol soforoside had a retention time of 4.75 minutes. These align quite well with labeled peaks in Figures 1 a/b and 2a/b. However, for more definitive identification, more information is needed. This is where the MS spectral results play a key role.

As these two compounds have very similar MWs, the basic mass spectrum analysis and their identification via just the molecular ion weight can be misleading. Taking advantage of both the information in an on-line database (www.phenol-explorer.com) and ChemSketch, also available on the web, it was possible to analyze the structure of molecules having similar molecular weight (MW), develop an understanding of how they fragment and, then, derive the mass spectrum that would be expected by ESI-MS.

As shown in Figures 4a and 5a, by molecularly analyzing both quercetin rutinoside and kaempferol-3O-sophoroside, fragmented at the 3O-glycosidic bond or between the two glucose molecules, it allowed us to determine the most likely ESI-MS fragmentations and, thereby, target fragments with corresponding weights in the actual mass spectral results.

Thereupon, for both quercetin rutinoside and kaempferol sophoroside, respectively, Figures 4 b/c and 5 b/c show the MS spectral results for both ethanolic and aqueous extracts. These results closely matched the expected fragmentations derived from ChemSketch. The combined chromatographic and mass spectral results allowed for easy interpretation and clear confirmation of the targeted analytes.

Similarly, using the same analytical procedure, it is possible to identify other polyphenols present in the chromatographic separation. By way of example, the expected fragmentations and the mass spectral results obtained for soforoside quercetin and kaempferol rutinoside via both ethanolic and aqueous extracts are shown in Figures 6 and 7, respectively.

Combined, these results provided a definitive characterization of the targeted analytes present in the two saffron extracts. Such composite information is often crucial in the qualitative analysis of complex molecules, such as polyphenols.

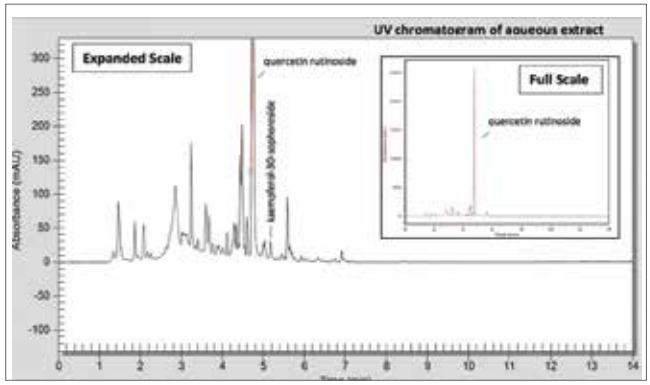


Figure 1a. UV chromatogram of the ethanolic extract of saffron petals; UV at 270 nm.

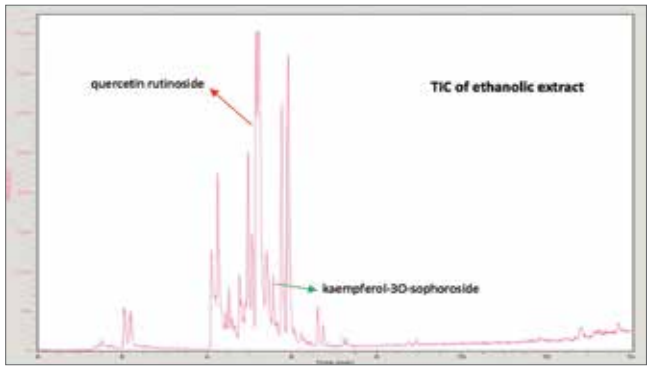


Figure 1b. TIC (total ion chromatogram) of the ethanolic extract of saffron petals. The mass spectra of the highlighted peaks are shown in detail in Figures 5 and 6.

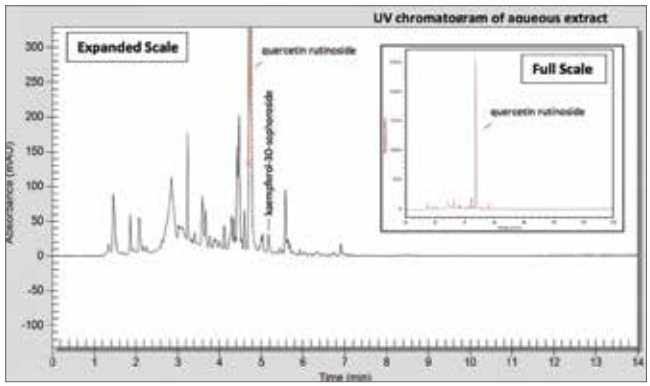


Figure 2a. UV chromatogram of the aqueous extract of saffron petals; UV at 270 nm.

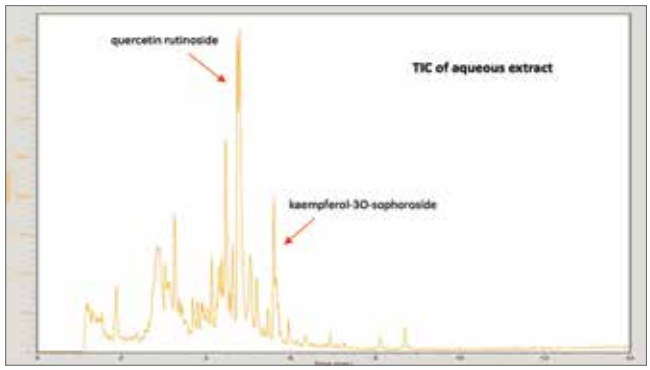


Figure 2b. TIC chromatogram of the aqueous extract of saffron petals.

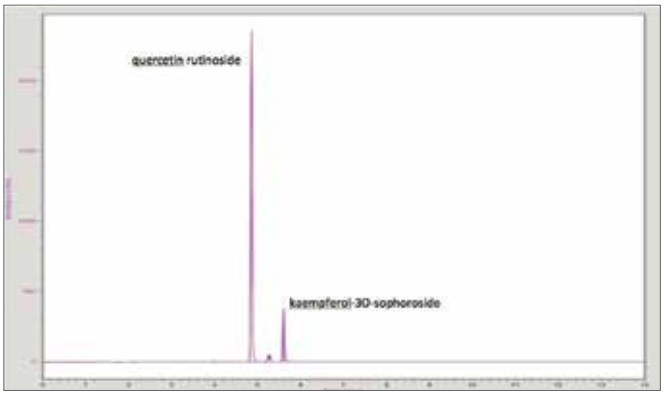


Figure 3. Chromatogram of quercetin rutinoside and kaempferol-3O-sophoroside standard mix (2 ppm each); UV at 270 nm.

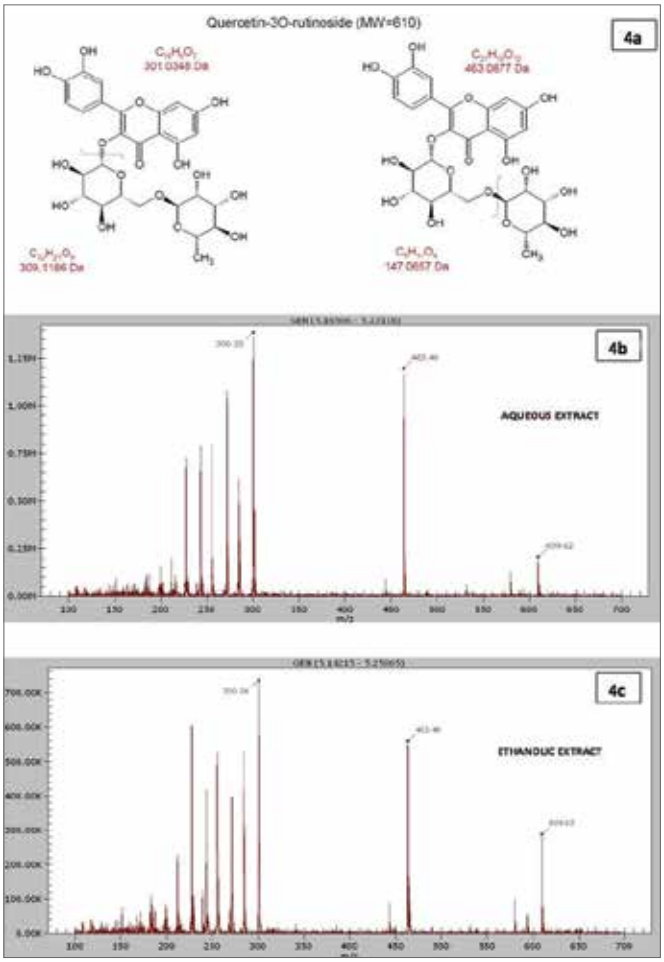


Figure 4. Figure 4a shows the most likely ESI-MS fragmentations for quercetin-3O-rutinoside. Figures 4b and 4c show the actual mass spectra obtained from the chromatographic analysis of the aqueous and ethanolic extracts, respectively.

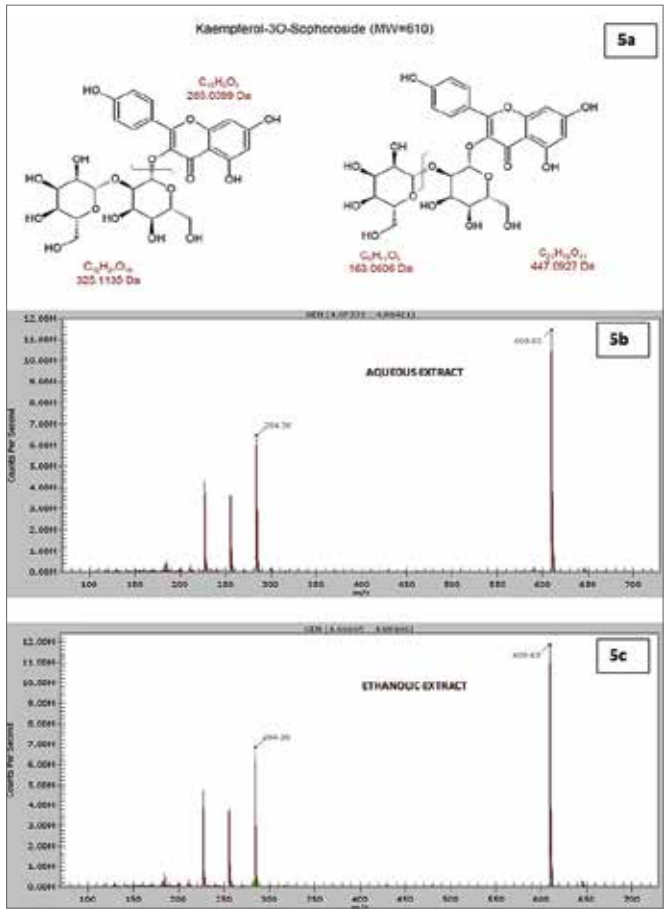


Figure 5. Figure 5a shows the most likely ESI-MS fragmentations for kaempferol-3O-sophoroside. Figures 5b and 5c show the actual mass spectra obtained from the chromatographic analysis of the aqueous and ethanolic extracts, respectively.

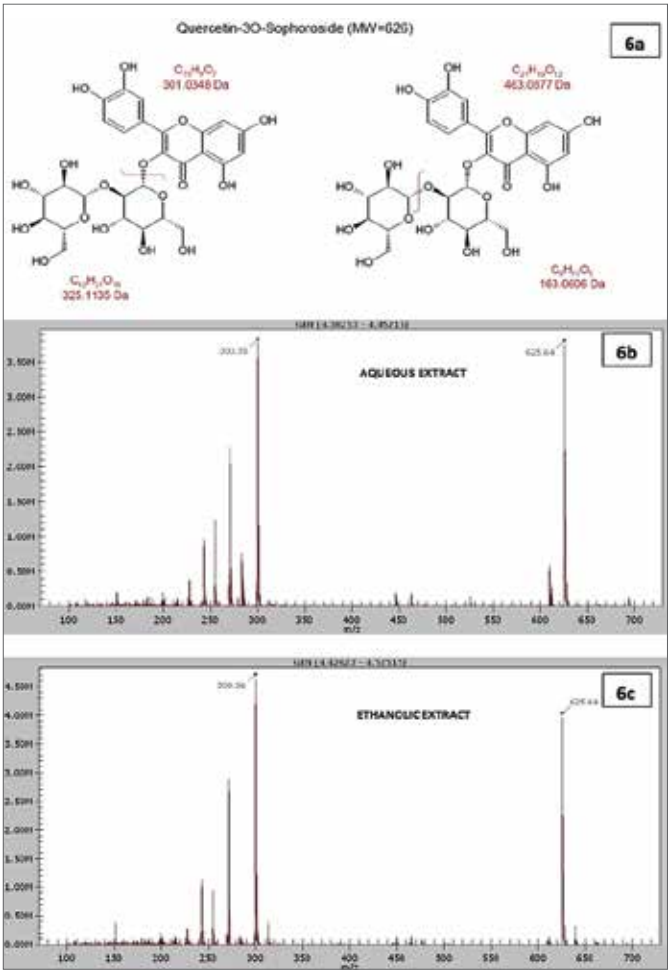


Figure 6. Figure 6a shows the most likely ESI-MS fragmentations for quercetin-3O-sophoroside. Figures 6b and 6c show the actual mass spectra obtained from the chromatographic analysis of the aqueous and ethanolic extracts, respectively.

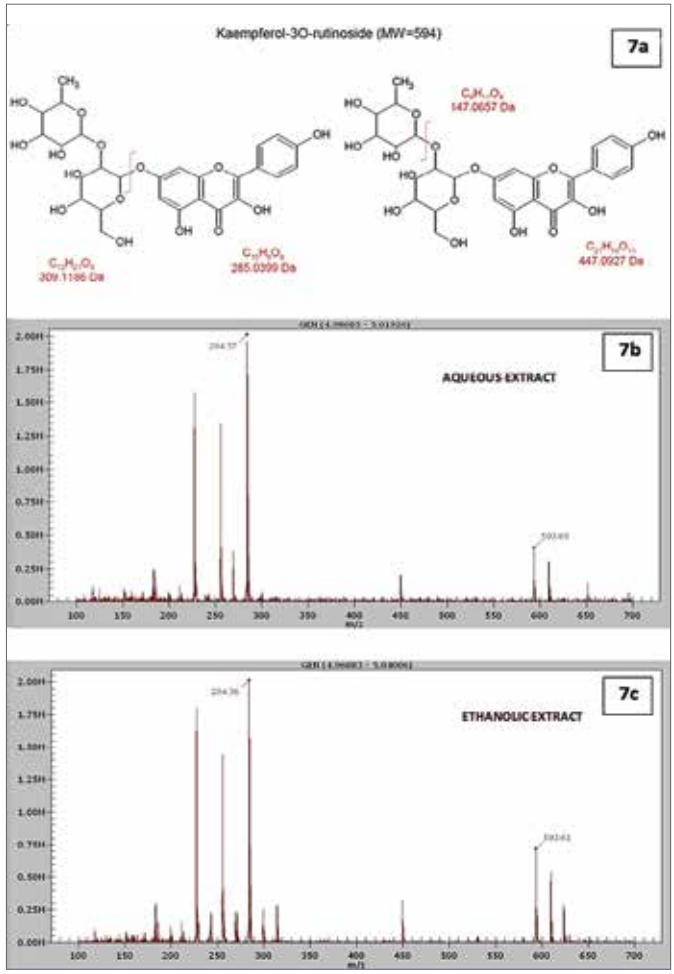


Figure 7. Figure 7a shows the most likely ESI-MS fragmentations for kaempferol-3O-rutinoside. Figures 7b and 7c show the actual mass spectra obtained from the chromatographic analysis of the aqueous and ethanolic extracts, respectively.

Conclusion

Combining chromatographic results, projected fragmentation ions (via software, such as ChemSketch) and mass spectral results allowed for the definitive identification of four polyphenols naturally found in saffron petal extract. This was based on the chromatographic retention times of standards, the mass of the molecular ion and the primary fragment ions that were obtained.

Regarding quercetin rutinoside and kaempferol soforoside, the results clearly demonstrate the ability of definitively associating the resulting spectra with one of the two flavonoids, even though both have the same parent MW.

For quercetin sophoroside and kaempferol rutinoside, the corresponding fragment ions obtained from these molecules provided clear confirmation of compound identity.

In summary, this analytical approach allows for the conclusive identification of primary polyphenols in saffron petal extract.

Acknowledgements

PerkinElmer would like thank the Agenzia per lo Sviluppo, Azienda Speciale CCIAA, Italy for supplying the saffron petal extracts.

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