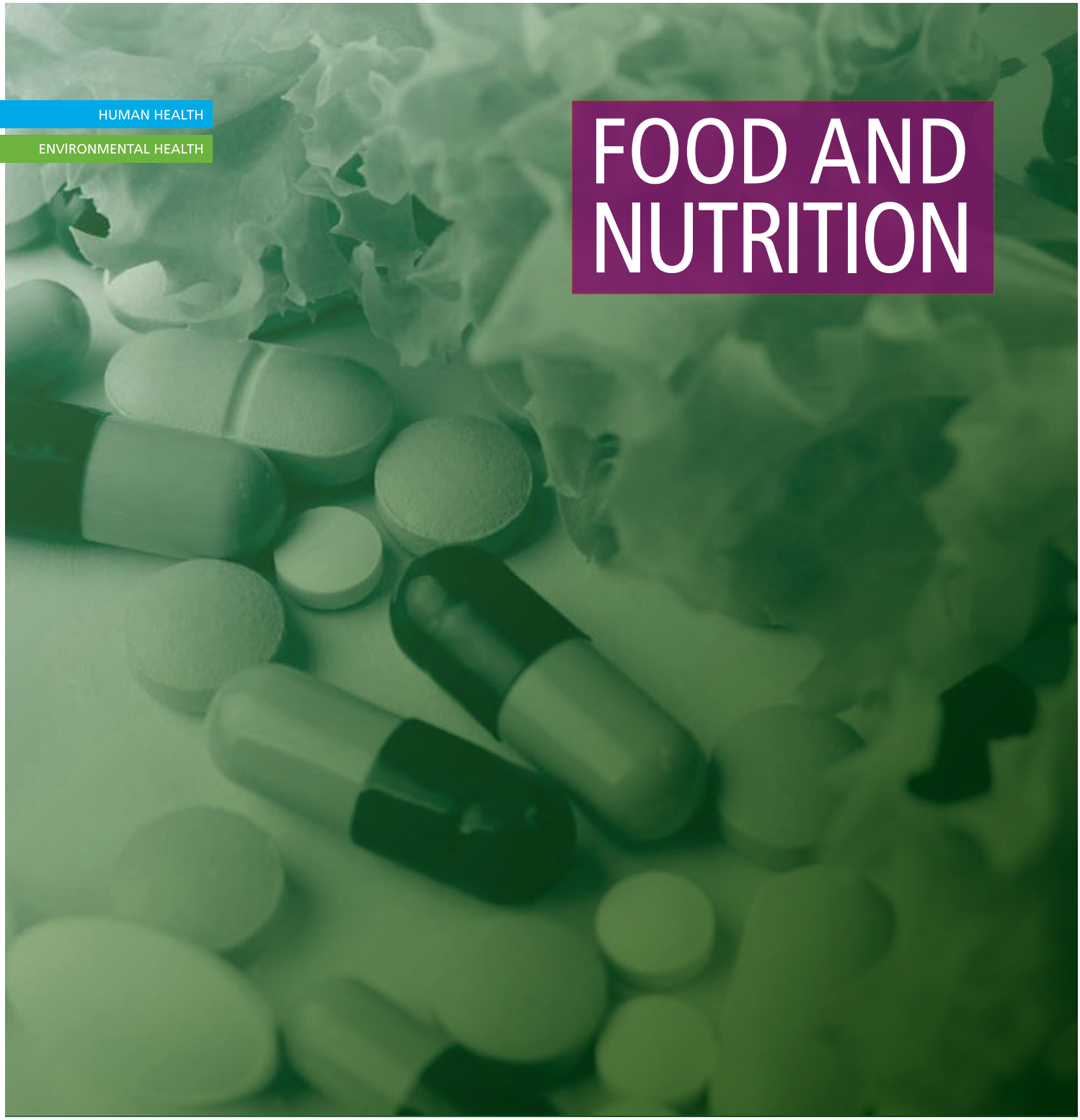
HUMAN HEALTH

ENVIRONMENTAL HEALTH

FOOD AND NUTRITION



Food and Nutrition Compendium

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FOOD AND NUTRITION

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CASE STUDY

Food

FT-IR Analysis Critical to Fast Characterisation of Unknown Contaminants in Food

Advances in analytical chemistry mean there are a now variety of techniques that can be used in the identification of unknown contaminants. The challenge for testing laboratories is to balance the use of established methods, such as IR, with more specific techniques such as LC/MS. The goal being efficient and fast sample testing. This enables clients to benefit from rapid sample throughput (from processing to analysis and final reporting), and have the ability to respond swiftly to any urgent requests for investigation.

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"Recording IR data, using the Spectrum 400 system, is a critical part of the problem solving process in characterising samples. The systems performance in mid-IR and near-IR coupled with the ability to gather data in a matter of seconds saves hours of lab work. Avoiding tedious HPTLC runs, we can now efficiently determine the next steps required for rapid identification of unknown samples."

James Neal-Kababick, Director, Flora Research Laboratories, USA.

Protecting consumer safety with FT-IR analysis

Reports of children falling ill having consumed a particular brand of butter lead the manufacturers searching to quickly identify the source of contamination. After previous investigations were unsuccessful, the analytical team at Flora Research Laboratories were tasked with identifying the contaminant. The SpectrumTM 400 IR system is the first instrument used in any forensic situation at Flora Research Laboratories. Jim Neal-Kababick, Director at Flora Research Laboratories, commented, "With data generated in seconds, it gives clarity to the problem and allows us to rapidly determine the next step to secure compound identification".

Fast, non-destructive sampling increases productivity

Stains were clearly visible on the packaging of contaminated butter. Recording the IR spectra was very quick and easy. Cutting out a section of the packaging the spectra was collected using a sampling accessory (UATR); generating results in under a minute. Comparison of spectra showed the presence of a unique band only in the contaminated packaging. Indicative of an organic compound, an appropriate GC/MS was readily selected and the contaminant identified as a chemical used in insect pheromone baits. It was concluded that prior contamination of the packaging lead to leaching of the chemical into the butter and caused consumers to fall ill. This result was confirmed within one working day, enabling remedial action to be taken swiftly.

This non-destructive technique enabled the same sample to be used for both the IR and GC/MS analysis. This becomes increasingly important for non homogeneous samples, or when sample is limited.

Company: Flora Research Laboratories

Business: Contract Testing Laboratories. Flora Research Laboratories is a leading independent full service testing laboratory specializing in the quality control and research of natural products, and dietary supplements. The birthplace of the emerging field of Phytoforensic science, Flora Research Laboratories are focused on using advanced technologies from microscopy to mass spectroscopy to protect the global food supply chain.

2009: Spectrum 400 MIR/NIR system used as the front line screening technique in all investigations.

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APPLICATION NOTE

Differential Scanning Calorimetry

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Practical Food Applications of Differential Scanning Calorimetry (DSC)

Abstract

This note describes a number of important food applications utilising the PerkinElmer DSC demonstrating the versatility of the technique as a tool in the food industry.

Introduction

Food is often a complex system including various compositions and structures. The characterization of food can therefore be challenging. Many analytical methods have been used to study food, including differential scanning calorimetry (DSC).[1] DSC is a thermal analysis technique to measure the temperature and heat flows associated with phase transitions in materials, as a function of time and temperature. Such measurements can provide both quantitative and qualitative information concerning physical and chemical changes that involve endothermic (energy consuming) and exothermic (energy producing) processes, or changes in heat capacity.

DSC is particularly suitable for analysis of food systems because they are often subject to heating or cooling during processing. The calorimetric information from DSC can be directly used to understand the thermal transitions that the food system may undergo during processing or storage. DSC is easy to operate and in most cases no special sample preparation is required. With a wide range of DSC sample pans available, both liquid and solid food samples can be studied. Typical food samples and the type of information that can be obtained by DSC are listed in Table 1. These tests can be used for both QC and R&D purposes. DSC applications are used from troubleshooting up to new product developments.

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Table 1. **Typical food samples and their application by DSC.**

Type of Samples	Type of Information
Oils, fats and spreads	Onset temp of melt/crystallisation /polymorphic behaviour/oxidation stability
Flour and rice starch	Retrogradation/gelatinization/glass transition Tg
Vegetable powders	Glass transition Tg
Pastes and gels containing polysaccharides or gums	Specific heat Cp, onset temp of melt and crystallisation
Protein	Denaturation/aggregation

In this note, several samples of food material systems are given to illustrate the versatility of DSC.

DSC of oils and fats

Using a heat-cool-heat DSC program, the onset temperature, the heat of fusion (ΔH), the identification of polymorphic behaviour and crystallisation of oils and fats can be determined. An isothermal method or scanning method with an oxygen atmosphere can also be used to determine the oxidation induction time (OIT), in which case a heat-cool-heat method is applied to hydrogenated vegetable oils. Sometimes additional information about the sample is necessary for data interpretation, as for example in combination with XRD analysis which provides information on the specific polymorphic transitions. Most triglycerides[2] exist at least in three crystalline forms, α (alpha), β' (beta-prime), and β (beta) that can be identified according to their X-ray diffraction patterns.[3]

In Figure 1 it can be observed that a α-modification is formed after a heat-cool treatment. This will be transformed into a β'-modification and after a certain time at room temperature partially to the β-modification. In Figure 2 the influence of storage time at room temperature is shown. The first heating of day 8 shows a better resolved peaks due to the transition of the less stable β' to a more stable polymorphic fraction, as it was also confirmed by XRD.

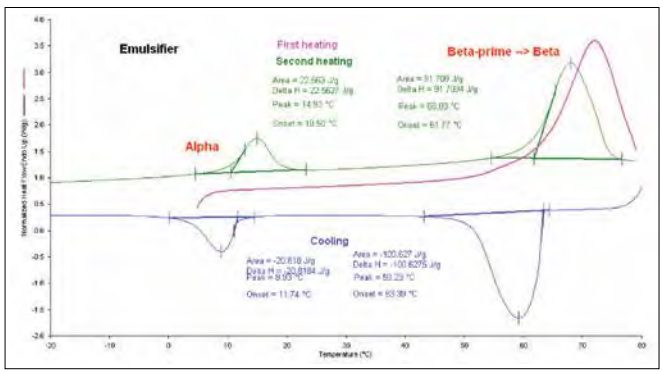

Figure 1. Heat influence on emulsifier.

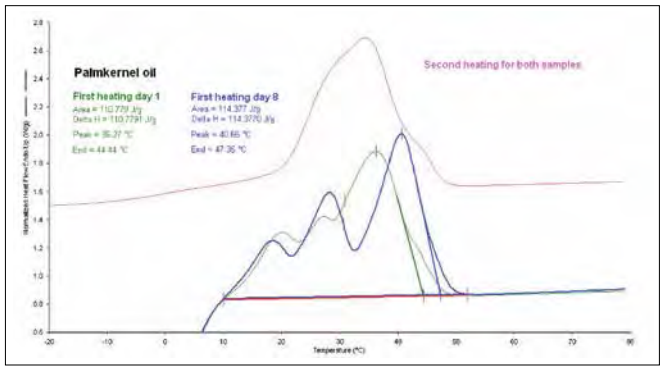

Figure 2. Time influence on palmkernel oil melting behaviour.

DSC is used to study fat phase transitions and melting range. It is one technique to explain the physical and textural properties of fats in bulk and final products. The combination of DSC and XRD is often used to identify the stable β-form, which can result in grainy mouth feel in final products.

DSC is used to compare batches of a product to study the melting behaviour indicating differences in crystallinity of the fat or composition of the end product. Different scanning rates are used to investigate the cooling effect on the crystallisation of a specific fat. The solid fat content (SFC) of a fat system can be determined over a given melting range. The solid fat content values are calculated through the partial areas of DSC heating curves usually between 5-60 °C and compared to NMR (Minispec) data.[4,5]

To study the aging of a fat or end product the sample is kept at an isothermal temperature to mimic e.g. refrigerator conditions. Comparing the DSC thermograms of a fresh sample and after a known storage time gives information on phase transitions during these storage conditions.

Other studies[6] involve tempering to investigate the influence on the final product after temperature abuse or due to transport at ambient. Tempering consisted of warming the systems up to a temperature between 15 and 30 °C and cooling down to 5 °C. These results can be correlated with the storage modulus (G').

DSC melting and crystallisation behaviour of different types of oils and fats are studied when replacing them in a product. In a factory and also at lab scale, different ingredients are added at different stages of the production process. Adding an ingredient which is not at the correct temperature can cause encapsulation of other ingredients or may stay present in the product as a particle. The filling temperature of a product is important for example to obtain the desired firmness of a product and to prevent graininess.

An AOCS[7] method can be carried out for quality control of fats to analyse these raw materials used in food products. This is a "fingerprint" method whereby the sample is melted, subsequently cooled down with a predefined scanning rate to a low temperature. After crystallisation for a specific time, a heating curve is obtained also with a predefined scanning rate.

DSC of starch samples

Starch[8,9], a major structure-forming food hydrocolloid[10], is a polymeric mixture of essentially linear (amylose) and branched (amylopectin) molecules. Small amounts of non-carbohydrate constituents (lipids, phosphorus, and proteins) present in native starch also contribute to its functionality. Starch is used as thickening agent in e.g. dry sauce bases, instant soups, mayonnaise, spreads. Starch pastes can be used as stabilizers for oil emulsions in for instance dressings.

Native starch or modified starch used in these types of food products can show different endothermic peaks in the DSC thermograms respectively, retrogradation (recrystallized amylopectin), gelatinization (50 < T < 80 °C depending on the type of starch), amylose-lipid complex (T > 100 °C) or recrystallized amylose (T > 140 °C) can be observed.

Retrogradation is only possible in processed (cooked or modified starch) materials which have been stored at lower temperatures. Retrogradation can expel water from a polymer network also known as syneresis but it can also cause dough to harden.

The hydrogen bond arrangement of amylopectin and amylose makes it difficult for water to penetrate into intact starch granules. When the water is heated the granules swell and gelatinization is observed. DSC measures the temperature at which irreversible changes occur in the granule. This process can also be observed by polarised light microscopy during heating.

The starch powders can be analysed dry to obtain information about the pure sample. Additionally, after adding a known amount of water, information is obtained about the degree of gelatinization. The level of water used is of influence on the gelatinization degree and peak shapes. Starch with low and intermediate water content can show more melting endotherms. The gelatinization information can be used to determine the temperature and time necessary for e.g. rice which is used in instant soups. If the rice has a too high amount of gelatinization left in the product, this will result in hard uncooked rice in the instant soup.

Most starches and rice products contain a lipid (fat) which can form an amylose-lipid complex. This complex can be formed during gelatinization.[1] It is also a thermo reversible complex and should show an exothermic peak on cooling. Sometimes the modification of the amylose with a lipid is performed to control the texture of the final starch.

The composition of plain rice[11] is starch (76.5%), water (12%), protein (7.5%), fat (1.9%) and minors (2.1%). An example of a native rice (Figure 3) and rice slurry (Figure 4) show the presence of retrogradation and amylose-lipid complex endotherms.

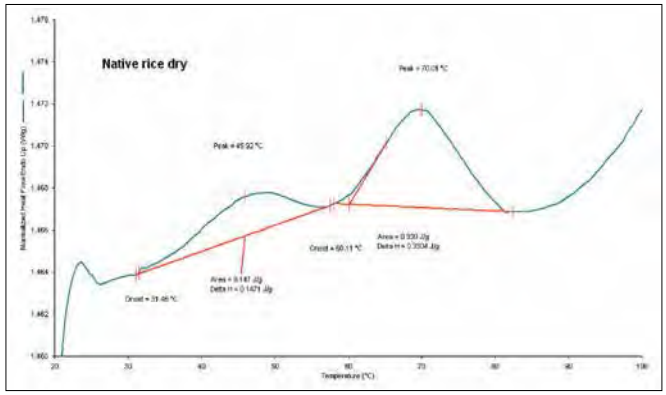

Figure 3. Native rice dry sample showing a retrogradation peak around 45 °C and a gelatinization peak around 70 °C.

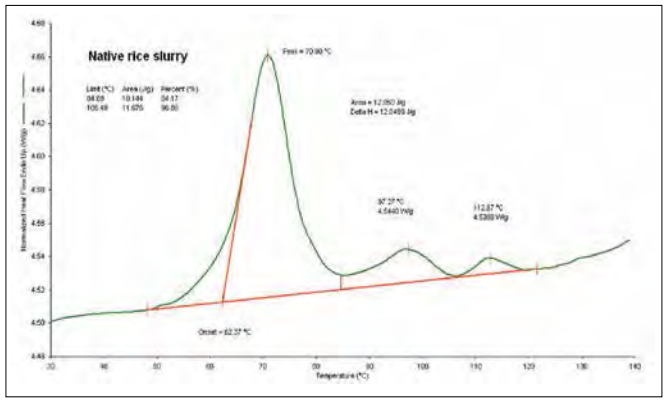

Figure 4. Native rice wet sample showing a gelatinization peak at around 70 °C and some amylose-lipid complex at 112 °C.

DSC of vegetable powders

Since food products are complex mixtures of several compounds, it is often difficult to determine their glass transition (Tg) temperatures accurately. Understanding the glass transition[12] phenomenon provides an insight into the causes of the cohesiveness of many important powders and influencing the wetability or solubility of the powder, which is important for new product development. Food material often contains water which can be present as free or bound water. The free water is related to the wateractivity (Aw). The plasticization effect of water leads to depression of the glass transition temperature causing significant changes in the physicochemical and crystallization properties during storage. Loss of physical stability by the effect of moisture and temperature will reduce flowability and increase caking tendency and, to a smaller extent, affect other physical properties such as colour. A Tg is only observed for amorphous matter. Sugars in a powder can undergo a phase transition from amorphous to crystalline at a given relative humidity during storage and thus have an effect on the glass transition temperature.

DSC is widely used to study glass transition phenomena. The effect of water as a plasticizer on Tg was studied for vegetable powders stored at different Aw values (humidity). At a higher Aw value the samples take up more water. In Figure 5 it is shown that the Tg drops to lower temperatures as the amount of water in the sample increases. The knowledge of Tg in combination with the water activity is important in predicting the physical state of the powder at various conditions, from free flowable to stickiness or phase transitions to crystalline matter.

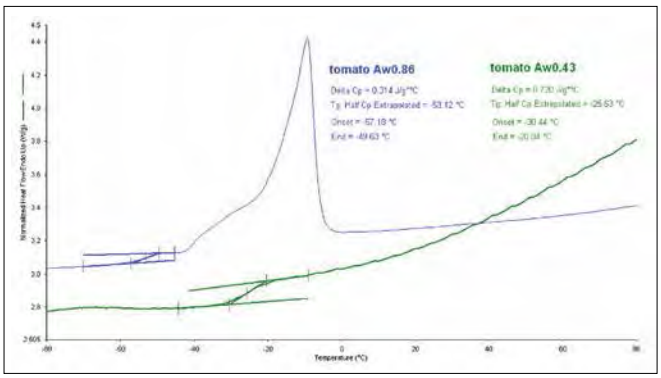

Figure 5. Water influence on Tg of tomato, the Aw 0.86 also shows an endothermic peak which is due to the melting of free water.

Proteins denaturation is also intensively studied by DSC. The influences of pH, salt and polysaccharides were investigated[13] for food proteins.

Conclusion

DSC is an essential tool to reveal the underlying phase-compositional principles of food systems. For systems with a clearly established phase-composition-functionality relation, DSC can contribute to the development of novel food products.

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FIELD APPLICATION REPORT

ICP-Mass Spectrometry

The Determination of Iodine in Food with the ELAN DRC-e ICP-MS

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Acknowledgements

The method development has been done by Fabien Bolle and Khalid Boutakhrit of the Belgian Scientific Institute of Public Health.

Introduction

Iodine is essential for the production of thyroid hormones. These hormones stimulate metabolism in the body, as well as mental growth and development. The recommended daily iodine intake is 0.1-0.2 mg, with the most common sources of iodine being fish, seafood, milk and food supplements. The determination of iodine in food has always been difficult due to the low concentrations (mg/kg), difficult sample preparation and the volatility of iodine.

This work demonstrates the ability of the ELAN® ICP-MS to measure iodine in food samples.

Experimental

Sample Preparation

Samples consisted of three certified reference materials: skimmed milk powder, cod, and mussel. Prior to weighing, the samples were mixed or slightly crushed; 0.25-0.5 g of sample was then added to PFA tubes, followed by 4.5 mL H_20 (Milli-Q) and 1 mL TMAH (25%).

After capping, the tubes were placed in a drying oven at 90 °C for 3 hours. After cooling, Milli-Q® water was added to a final volume of 10 mL. These solutions were then centrifuged at 3000 rpm for 15 minutes. If any visible particulates remained after centrifuging, the samples were then filtered. The resulting solutions can then be analyzed directly or with an extra dilution if high matrix concentrations are present.

Instrumental Conditions

The instrument used for this analysis was an ELAN DRC™ II ICP-MS. Instrumental operating parameters are shown in Table 1. All measurements were done in standard mode.

Calibration standards ranging from 5 to 20 µg/L were used, prepared in 0.5% (v/v) TMAH. After each standard and sample were analyzed, a 45 second rinse with of 0.5% TMAH was performed.

Table 1. **Instrumental Conditions.**

Spray chamber	Cyclonic
Nebulizer	Meinhard®
Sample Uptake Rate	1 mL/min
RF Power	1100 W
Plasma Gas Flow (L/min)	15
Nebulizer Gas Flow (L/min)	0.93
Auxiliary Gas Flow (L/min)	1.2
Dwell Time (ms)	50
Sweeps per reading	25
Replicates	3
Delay Time (s)	50
Wash Time (s)	45

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Results

Table 2 shows the results of the analysis, along with the certified values for the samples. These results demonstrate that iodine can be accurately recovered in standard mode, indicating that there are no common interferences present. The limit of detection (3 times the standard deviation of the blank) in this method is 0.335 µg/L in the solution and 6.7 µg/kg in the original sample.

Iodine is known to have long washout times due to its volatility. This issue is overcome by rinsing with 0.5% TMAH between standards and samples. Iodine forms a non-volatile complex in basic environments, so using the TMAH rinse greatly reduces the washout time, compared to aqueous or acidic wash solutions.

Table 2. **Results for Iodine Analysis in Food SRMs.**

	Sample	Measured Value (µg/kg)	Certified Value (µg/kg)
BCR 150	Skimmed milk (powder)	1237 ±73	1290 ±90
BCR 422	Cod	4889 ±456	4950 ±490
SRM 2977	Mussel	23540 ±750	26000 (n.c.)

Conclusion

This work demonstrates the ability of the ELAN to measure iodine in food samples using a simple digestion procedure. The problem of iodine washout was overcome by using a basic digestion medium and a basic wash between samples. The ELAN DRC operating in standard mode provided accurate results; the DRC mode was not needed due to the lack of common spectral interferences on iodine.

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APPLICATION NOTE

GC-Mass Spectrometry and Headspace Sampling

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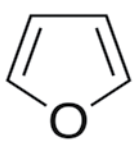
Determination of Furan in Food by Gas Chromatography-Mass Spectrometry and Headspace Sampling

Introduction

Furan is naturally occurring at low levels in many foods and drinks.[1] Furan consumption is of concern because it has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, based on studies with laboratory animals. The U.S. FDA has recently published a report on the occurrence of furan in a large number of thermally processed foods, especially canned and jarred foods, including baby foods and infant formulas. The primary source of furan in food is considered to be thermal degradation of carbohydrates, such as glucose, lactose and fructose.

Of all the foods tested in various papers, coffee contained the largest amount of furans.[1] Furan is a colorless, volatile and lipophilic organic compound. It has a molecular weight of 68 and a low boiling point (31 ˚C). Due to its high volatility, furan levels in foods are easily determined, with high accuracy, by headspace methods.

This application note will demonstrate a rapid method for the identification and quantification of furan in food samples, using gas chromatography with headspace sampling and mass spectrometry. In addition to method optimization and standard analysis, we will analyze a number of food samples for furan. We chose to test coffee containing drinks, sauces, and canned foods, as previous studies demonstrated high levels of furan in these foods. The samples were randomly collected from the local market.



Synonyms: furfuran, oxole, tetrole, divinylene oxide, oxacyclopentadiene
Formula: C_4H_4O
MW: 68.07
MP: -85.6 ºC
BP: 31 ºC

Figure 1. Structure and physical properties of furan.

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Experimental

The PerkinElmer® Clarus® 680 Gas Chromatograph, Clarus 600 C Mass Spectrometer and a TurboMatrix™ HS-40 system were used for this application. Table 1 presents the detailed operating parameters of the GC/MS and the HS system. The instrument interaction, data analysis and reporting was completed with the PerkinElmer TurboMass™ data system.

Table 1. **Detailed Instrument Conditions Used in the Determination of Furans.**

Instrument Details:	**Clarus 680 Gas Chromatograph**	
Analytical Column	PerkinElmer Elite™-624 N9316204 (60 meter, 0.32 mm i.d., 1.8 µm df)	
GC Column Flow	1.4 mL/min helium at constant flow mode	
GC Inlet Temperature	200 °C	
Split Ratio	2:1	
Oven Temperature Program	40 °C hold for 6.0 min, 20 °C/min to 110 °C and hold for 1.0 min, 70 °C/min to 250 °C and hold for 3.5 min; runtime is 20 min	
MS Parameters:	**Clarus 600 C Mass Spectrometer**	
MS Source Temperature	230 °C	
MS Interface Temperature	225 °C	
Scan Range	m/z 35-150	
Scan Time	2.5-25 min	
Multiplier	500 V	
Scans/Sec	5.56	
Headspace Parameters:	**TurboMatrix HS-40**	
Temperatures	Thermostatting Oven	60 °C
	Needle	100 °C
	Transfer Line	130 °C
Time	Injection	0.2 min
	Pressurization	0.5 min
	Withdrawal	0.2 min
	Equilibration	20 min
	Cycle	20 min
Options	Vial Vent	ON
	Shaker	ON
	Operation Mode	Constant
	Injection Mode	Time
	Hi Psi Injection	ON
PPC	Inject	35 psi
	Column/ Headspace Pressure	25 psi

Headspace is a perfect technique for sample introduction in furan analysis due to the ease of sample preparation and the limited interaction of the instrumentation with the sample matrix. Caution must be taken when setting the vial oven temperature; a high temperature can result in furan formation in the sample during analysis. To reduce this risk the method presented here uses a low incubation temperature.

Stock Solution: A stock solution of 1000 µg/mL of furan and furan-d_4 was used as the starting point for all standard solutions (SPEX CertiPrep®).

Standard Preparation:

10 µL of the stock furan solution was diluted to 10 mL in methanol to give a solution of 1 µg/mL. 20 µL of the stock furan-d_4 solution was diluted to 10 mL in methanol to give a solution of 2 µg/mL.

Calibration Curve: The volume of 1 µg/mL furan was diluted in water to achieve the final standard concentration presented in Table 2. 100 µL of furan-d_4 from 2 µg/mL stock was added to each headspace vial containing 10 mL of water resulting in an internal standard concentration of 0.02 µg/mL (20 ppb). 4 g of NaCl was added to each of the vials to decrease the miscibility of furan in water.

Preparation of Solutions:

Table 2. **Scheme Used for the Creation of a Five Level Calibration.**

Calibration Level No.	Concentration of Furan in ppb	Std Solution Added in µL	Final Vol. (mL)
1	1	10	10
2	2	20	10
3	10	100	10
4	20	200	10
5	40	400	10

*4 gm of NaCl was added to each of the headspace vials.

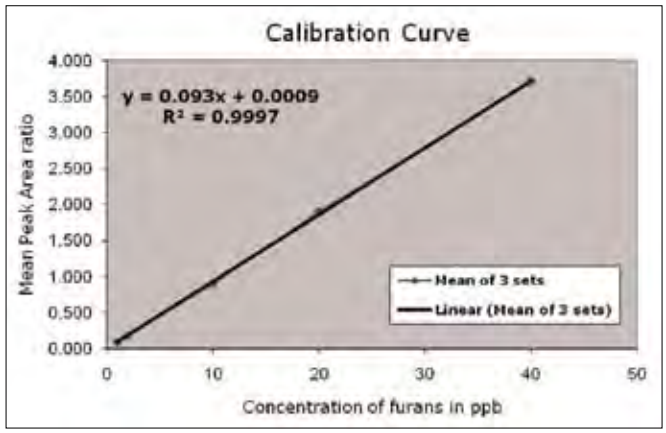

Figure 2. Calibration curve for furan.

Calibration: The MS was calibrated across the range of 1.0 to 40 ng/mL and each calibration point was run in triplicate to demonstrate the precision of the system. The average coefficient of determination for a line of linear regression was 0.9997 for furan. The calibration curve for furan is depicted in Figure 2.

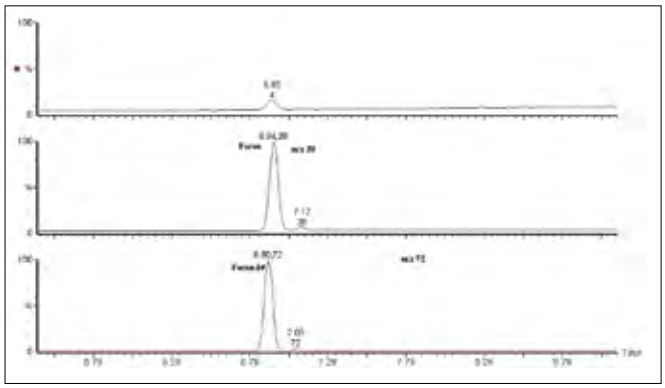

Figure 3. Example chromatogram of 40 ppb furan standard showing the total and extracted ion chromatograms as well as the extraction ion chromatogram for the furan-d_4 internal standard.

Also in Table 3 is the percent relative standard deviation (%RSD) for each calibration point (n=3). The precision of the system across the calibration range is excellent. The chromatograms and the spectrum from the analysis of standard material are shown in Figure 3.

Table 3. **% RSD's for Three Sets of Linearity Experiment.**

Sr. Number	Number of Levels	Mean Peak Area Average Relative Response (n=3)	%RSD
1	1	0.098	10.046
2	2	0.184	8.012
3	10	0.904	1.475
4	20	1.900	0.435
5	40	3.709	1.627

The precision of the method was measured at both 0.5 and 1 ppb. The detection limit of this method is approximately 0.5 ppb (Table 4).

Table 4. **RSD Values for Detection Limit and Quantification Level.**

Sr. No.	Conc. of Furan in ppb	Furan/IS Area Ratio	Conc. of Furan in ppb	Furan/IS Area Ratio
1	0.5	0.035	1	0.102
2	0.5	0.031	1	0.097
3	0.5	0.031	1	0.106
4	0.5	0.021	1	0.103
5	0.5	0.021	1	0.096
6	0.5	0.022	1	0.093
Mean		**0.03**		**0.1**
S.D.		**0.01**		**0.0**
%RSD		**23.75**		**4.78**

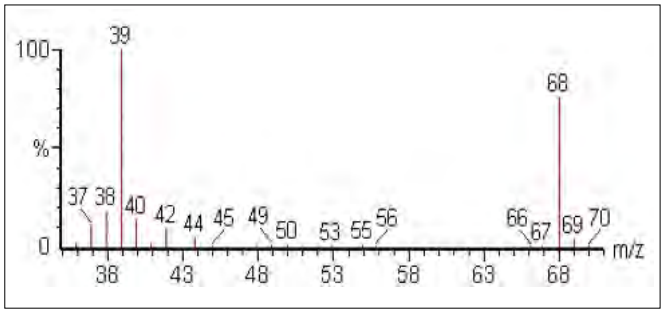

Figure 4. Full scan mass spectrum obtained experimentally for furan.

Table 5. **Method Validation Summary.**

Linearity:	1.0 ppb to 40 ppb of furan
RSD for Replicate Analysis:	for 1.0 ppb 4.78%
Detection Level:	0.5 ppb
Quantification Level:	1.0 ppb
Recovery Study:	at three levels for all the samples within 80-120%

Sample Preparation: Samples were collected from the local market. The samples included: coffee, milk, canned foods, sauces, peanut butter and apple juice (Table 6). All the samples were refrigerated before analysis. 10 mL of sample was transferred into a headspace vial; 4 g of NaCl was added to it. Milk and other viscous samples were diluted with water (1:2 or 1:4). The semi-solid samples were ground and 5 g of sample was added to headspace vials with 5 mL of saturated salt (NaCl) solution. Coffee powder was dissolved following directions on the package, and then treated like a non-viscous liquid sample.

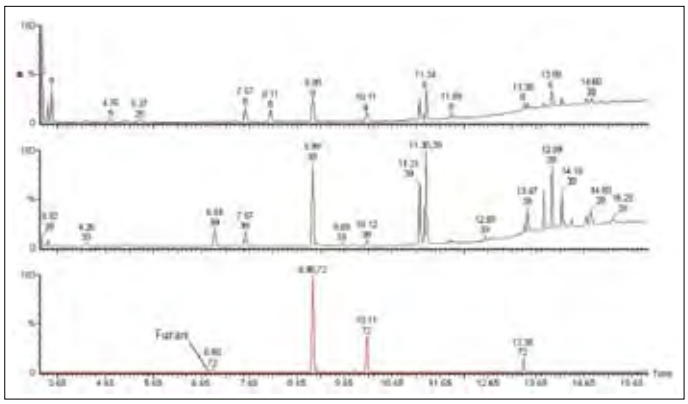

Figure 5. Experimental chromatogram from the analysis of espresso coffee with furan peak visible at 6.9 minutes.

***Table 6.* Sample Analysis Results.**

Sample No.	Sample Details	Amt. of Furan Found in ppb
Sample 1	Lab Coffee	0.67
Sample 2	Chocolate Flavored Milk (AKCF)	1.67
Sample 3	Espresso Coffee	45.18
Sample 4	Coffee Flavored Milk (AKC)	10.87
Sample 5	Cocoa Flavored Milk (AKK)	1.76
Sample 6	Energy Drink (milk based) (NAEM)	13.21
Sample 7	Brewed Coffee	36.59
Sample 8	Filtered Coffee	253.99

Method Validation:

The recovery of the method was tested with the analysis of the brewed coffee sample spiked at three different levels: 2, 5, 10 µg/L. The measured amount was 2.03, 5.44, 9.54 µg/L demonstrating that the headspace technique is quantitative in its extraction of furan from an aqueous matrix.

Results

Eight samples of common beverages were analyzed using the HS-GC/MS method developed here. The samples were chosen because they had been shown to have detectable levels of furan in the literature. Of the samples analyzed, brewed coffee was demonstrated to have the highest levels of furan, at 250 µg/L. The remaining sample results are demonstrated in Table 6.

Conclusion

This application presents a method for the determination of furans in beverages using headspace sample introduction. Headspace GC is fast, reliable and can be used for the quantification of furans in common beverages. The internal standard calibration of furan across 1-40 µg/L responded linearly. Beverages were analyzed and the level of furan determined. The furan was identified by both the retention time and the MS fragmentation pattern. The method was validated at several levels and coffee matrix recovery values were between 95-101%.

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APPLICATION NOTE

Atomic Absorption

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Determination of Lead and Cadmium in Foods by Graphite Furnace Atomic Absorption Spectroscopy

Background

Humans can be exposed to heavy metals through a variety of means, including consumption of contaminated food. Although heavy metals are usually present in foods at very low levels, long-term exposure can have negative health impacts. Two of the more important toxic elements that must be monitored are cadmium (Cd) and lead (Pb), which can enter food either through environmental processes or through contamination in processing and/or packaging. As a result, it is very important to accurately measure low levels of Cd and Pb in a variety of food matrices.

A major challenge in the analysis of food samples is the extremely low analyte levels and the very high matrix levels. For many years, graphite furnace atomic absorption spectroscopy (GFAAS) has been a reliable technique and the preferred method for this analysis, especially for the determination of Cd and Pb.

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In the past few years, a number of instrumental developments have contributed to providing more reliable results and better detection limits for trace determination of lead and cadmium by GFAAS. These include improved electrodeless discharge (EDL) and hollow cathode (HCL) lamps for increased light output, and improved wet ashing sample preparation techniques (e.g., microwave digestion).

This work will focus on the use of GFAAS for the determination of lead and cadmium in a variety of food samples.

Experimental

Atomic Absorption Instrumentation

A PerkinElmer PinAAcle™ 900H atomic absorption (AA) spectrometer (Figure 1) was used for all analyses. This instrument was equipped with a Massman-type/HGA graphite furnace and deuterium continuum source background correction, AS900 autosampler, water re-circulator system, high-speed automatic wavelength drive, automatic lamp selection, and EDL power supply. The use of cutting-edge fiber optics in the PinAAcle 900 spectrometers maximizes light throughput for improved detection levels. Syngistix™ for AA software was used and includes a Method Development module which automates the optimization of the temperature program for each element in a specific matrix (previous-generation WinLab™ for AA software would provide equivalent capabilities and results). The instrument's TubeView™ furnace camera (Figure 2) is extremely useful for the user in adjusting the pipette tip to the most appropriate depth in the graphite tube and also for monitoring any residue buildup on the platform during the progress of the analysis. The furnace camera was also used during method development of the temperature program to verify the drying steps, ensuring that sample boiling or spattering does not occur.

Figure 1. PerkinElmer's PinAAcle 900H atomic absorption spectrometer with AS900 furnace autosampler.

Figure 2. TubeView furnace camera on PerkinElmer's PinAAcle 900H, showing the optimized sampler tip depth inside the tube.

Reagents

All solutions were prepared in polypropylene volumetric flasks using ultra-pure deionized (DI) water. Other reagents used include:

1. Nitric acid Ultrapure 10 (TAMAPURE, Tama Chemicals Co., Kanagawa Japan)
2. Hydrogen peroxide: Ultrapure (30%), (Kanto Chemical Co., Tokyo, Japan)
3. Lead (Pb) and cadmium (Cd) stock solutions, 1000 mg/L (PerkinElmer). Cadmium and lead working solutions were prepared fresh daily by dilution of the cadmium and lead stock solutions with 2% (v/v) nitric acid.
4. Matrix modifier: mixtures of palladium [$Pd(NO_3)_2$] and magnesium nitrate [$Mg(NO_3)_2 \cdot 6H_20$] in 10 % nitric acid solution for Pb analysis and ammonia phosphate [$NH_4H_2PO_4$] and magnesium nitrate [$Mg(NO_3)_2 \cdot 6H_20$] in 10% nitric acid solution for Cd analysis.

Sample Preparation

The samples were conveniently and rapidly digested in a microwave oven using standard pressure vessels which were pre-cleaned by rinsing with ultrapure nitric acid prior to use.

All sample weighings were carried out in a Class 100 laminar flow cabinet. Samples of 300 mg each were accurately weighed in the digestion vessels, followed by the addition of 7 mL concentrated double-distilled nitric acid. Eight vessels were placed into the rotor and heated in the microwave oven according to the temperature program shown in Table 1.

Table 1. Temperature program for microwave.

Step	Temp (°C)	Pressure (Bar)	Ramp (min)	Hold (min)	Power (%)
1	150	30	5	10	80
2	200	30	5	10	100
3	50	30	0	20	0

The rotor was removed from the microwave oven and allowed to cool to room temperature. The vessels were carefully opened in a fume cupboard and the inner walls rinsed with DI water. The final volume of each sample was made up to 20 mL with 1 mL hydrogen peroxide and water.

GFAAS Determination of Lead and Cadmium

The wavelength and GFAAS instrument parameters for the determination of lead and cadmium are listed in Table 2. Pyrolytically coated integrated platform HGA graphite tubes were longitudinally heated. Calibrations were performed using external standards (blank, five calibration standards). All analyses were performed with triplicate firings. During an analytical series, a mid-range QC standard solution was injected every 10 analytical sample solutions to verify the calibration slope. QC readings can be plotted to monitor the trend using the software's built-in QC Charting Wizard, as shown in Figure 3. Recovery checks were carried out using system spikes on every sample.

Table 2. Instrument settings used on the PinAAcle 900H.

Element	Wavelength (nm)	Slit (nm)	Lamp Type	Lamp Current (mA)	Read Delay (sec)	Read Time (sec)
Cd	228.8	0.7	HCL	4	0.5	2.5
Pb	283.3	0.7	HCL	10	0.5	3.5

Baseline offset correction (BOC) was five seconds for all analytes.

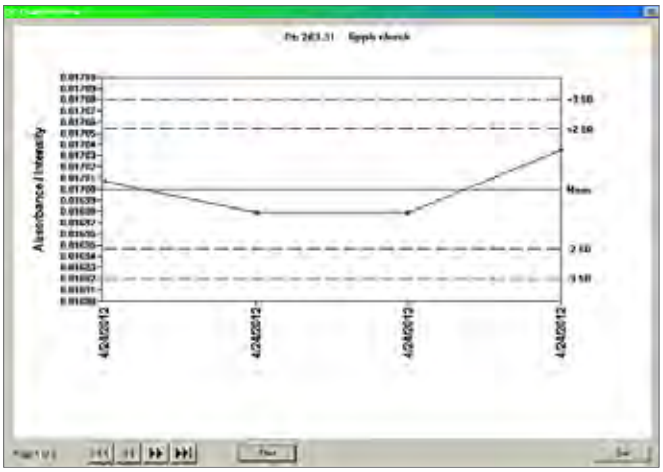

Figure 3. QC Charting of lead (Pb) QC samples.

Results and Discussion

Graphite Furnace Method Development

The aim is to use a single matrix modifier for both elements to simplify the analytical process. The main purpose of a matrix modifier is to volatilize the matrix during the pyrolysis while increasing the stability of the element. This ensures almost interference-free analysis whereby the elment is separated from the potential interference. As a result, aqueous calibration standards can be used; the method of standard additions is not required. The best results obtained in terms of peak profile and recoveries were with a 5 µL mixture of 0.06% magnesium nitrate and 0.1% palladium for Pb mixture, while a 5 µL mixture of 0.1% ammonia phosphate and 0.06% magnesium nitrate gives better peak profile and recovery for Cd. In both cases, the non-specific background was somewhat higher than without modifier. The peak profiles for Pb and Cd standards are shown in Figures 4 and 5.

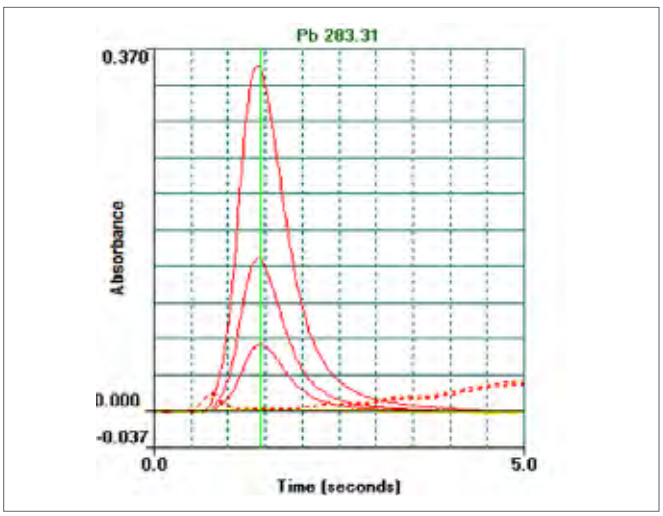

Figure 4. Overlay of spectral profiles of Pb standard solutions at 6 µg/L, 16 µg/L, and 40 µg/L.

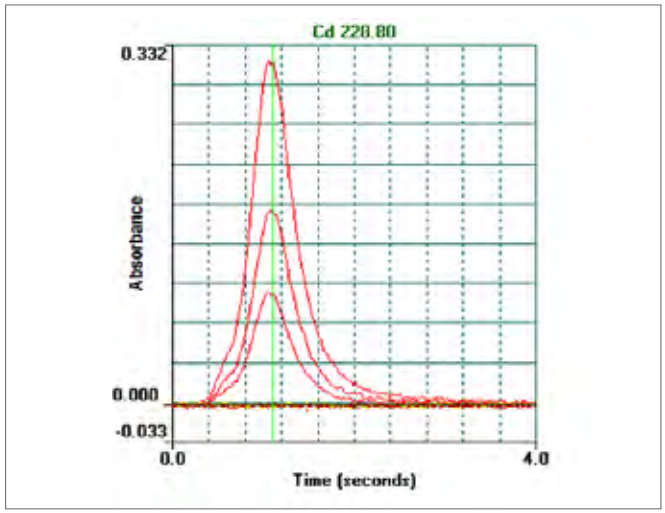

Figure 5. Overlay of spectral profiles of Cd standard solutions at 0.5 µg/L, 1 µg/L, and 2 µg/L.

With respect to the GFAAS temperature programs, the final pyrolysis and atomization temperatures adopted are listed for lead and cadmium in Tables 3 and 4.

Table 3. Furnace temperature program for lead (Pb).

Temp (°C)	Ramp (sec)	Hold (sec)	Internal Flow (mL/min)
110	5	25	250
130	15	20	250
800	10	20	250
2000	0	5	0
2600	1	3	250

Table 4. Furnace temperature program for cadmium (Cd).

Temp (°C)	Ramp (sec)	Hold (sec)	Internal Flow (mL/min)
110	5	25	250
130	15	25	250
850	10	20	250
1650	0	5	0
2600	1	5	250

Calibration Range

Lead was calibrated at 6 µg/L (ppb), 16 µg/L (ppb) and 40 µg/L (ppb) with 0.003 mg $Mg(NO_3)_2$ + 0.005 mg Pd, resulting in a calibration correlation coefficient of > 0.995, as shown in Figure 6a.

Cadmium was calibrated at 0.5 µg/L (ppb), 1 µg/L (ppb) and 2 µg/L (ppb) with 0.05 mg $NH_4H_2PO_4$ + 0.003 mg $Mg(NO_3)_2$, resulting in a calibration correlation coefficient of > 0.995, as shown in Figure 6b.

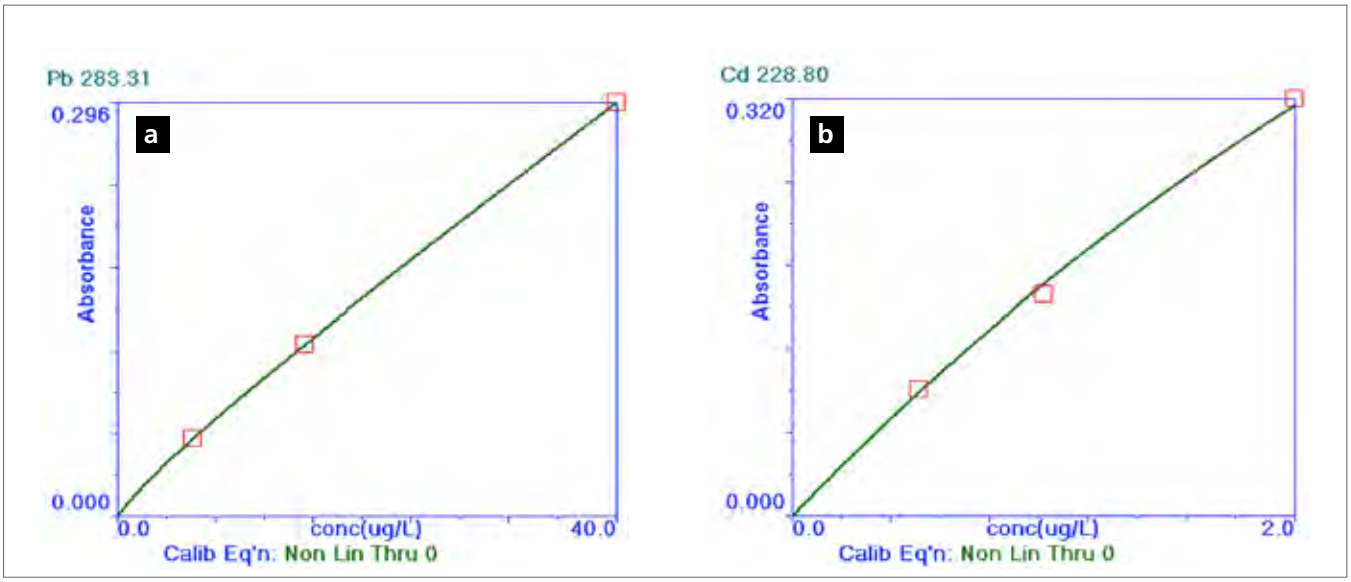

Figure 6. Calibration curves for Pb (a) and Cd (b).

Limits of Detection and Quantification

An important aspect of the method performance evaluation is the calculation of the limits of detection. The limits of detection, based on the repeated analysis of blank solutions, were calculated as instrument detection limit (IDL), while the average standard deviation of repeated analysis of sample blanks (or samples containing very low concentration of the analytes) were calculated as the method detection limit (MDL). All detection limits were obtained by analyzing 10 blank/samples each; the results are shown in Table 5.

Table 5. IDL, MDL and Linear Range using the PinAAcle 900H.

Element	IDL (µg/L)	MDL (µg/L)	Linear Range (µg/L)
Cd	0.03	0.08	2.50
Pb	0.3	0.4	100

Result and Recovery

To ascertain the accuracy of the results obtained with the developed method, spike recoveries were carried out on the samples. An 8 µg/L system spike was used for Pb, while a 0.5 µg/L system spike was performed on Cd. The results and recoveries are tabulated in Tables 6 and 7 for Pb and Cd, respectively. Both show recoveries of 100% ± 15%, well within the tolerance for complex food sample matrices.

Table 6. Sample recoveries for Pb.

Sample	Sample Result (µg/L)	Sample Spike (µg/L)	% Recovery
Milk	1.42	8.68	90.7
Mushroom	5.72	13.9	95.7
Coffee 1	2.37	9.73	92.0
Coffee 2	2.57	9.59	87.8
Soybean	4.37	12.4	99.8
Cooking Oil	1.24	9.11	98.5

Table 7. Sample recoveries for Cd.

Sample	Sample Result (µg/L)	Sample Spike (µg/L)	% Recovery
Milk	1.98	3.75	88.6
Mushroom	3.80	5.85	102
Coffee 1	1.56	2.07	103
Coffee 2	1.69	2.17	94.9
Soybean	1.97	2.44	94.9
Cooking Oil	<MDL	0.44	87.8

Conclusion

The PerkinElmer PinAAcle 900H AA spectrometer features a closed-furnace design that is sealed at both ends with easily removable bayonet-mount windows. The closed design is a prerequisite for controlled, reproducible analytical conditions. The separate, independently controlled external and internal gas streams provide maximum flexibility, tube life and sensitivity.

The PinAAcle 900H system uses enhanced power control circuitry to maintain a uniform heating rate, so no matter where a system is located, it provides outstanding and consistent performance. The PinAAcle 900H, equipped with an HGA graphite furnace, has demonstrated its capabilities in handling different digested food sample matrices. The system provides the superior sensitivity required for heavy-metal testing in food matrices, with excellent accuracy, as observed from the recovery studies.

The TubeView furnace camera is a handy tool to determine the optimized drying temperature and holding time for complete dryness prior to the pyrolysis step. Through the aid of Method Development software and TubeView, the PinAAcle 900H AA spectrometer makes it faster and easier to get from sample to results by reducing the time required for method development.

The AS900 furnace autosampler was used to ensure accurate delivery of the sample, and the probe did not require any cleaning or maintenance between analytical runs to prevent carryover from high-level samples. The autosampler can also provide automated spiking of samples for recovery checks. Results from QC samples, standards, or any sample can be plotted using the software's QC Charting Wizard. Limit ranges, means, or expected values can be included on the chart. Quality control charts can be quickly and easily prepared.

Consumables Used

Component	Description	Part Number
Cadmium lamp	Electrodeless discharge lamp (EDL)	N3050615
Lead lamp	Electrodeless discharge lamp (EDL)	N3050657
Graphite tubes	Pyrocoated graphite tubes with advanced platform	N9307834 (5-pack) N9307835 (20-pack)
	Pyrocoated graphite tubes with integrated platform	B3001262 (5-pack) B3001264 (20-pack) N9300651 (40-pack)
Autosampler cups	1.2 mL polypropylene (2000)	B0510397
Cadmium standard	1000 ppm	N9300176 (125 mL) N9300107 (500 mL)
Lead standard	1000 ppm	N9300175 (125 mL) N9300128 (500 mL)
$Mg(NO_3)_2$	1% Mg, 100 mL	B0190634
$Pd(NO_3)_2$	1% Pd, 50 mL	B0190635
$NH_4H_2PO_4$	10%, 100 mL	N9303445

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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Analysis of Butylated Hydroxytoluene in Food with Headspace Trap-GC/MS

Introduction

Butylated hydroxytoluene (BHT, 2,6-di-tert-butyl-4-methylphenol) is a common food additive. BHT is found in many types of food including butter, meats, cereals, chewing gum, baked goods, snack foods, dehydrated potatoes and beverages. It is used to preserve food odor, color and flavor. BHT is oxidized preferentially in fats or oils, protecting the foods from spoilage.

Concern exists that long-term human consumption of BHT may have potential health risks. It has undergone the additive application and review process required by the U.S. Food and Drug Administration (FDA); the committee concluded that no evidence in the available information on BHT demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, uncertainties exist requiring that additional studies should be conducted.[1] The chemical properties which make BHT an excellent preservative may also be implicated in health effects. The oxidative characteristics and metabolites of BHT may contribute to carcinogenicity. Some people may have difficulty metabolizing BHT, resulting in health and behavioral changes.

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This application note will demonstrate a fast and easy analytical technique to determine the amount of BHT in foods. Headspace (HS) sample introduction is used because it provides a means to analyze food without any sample preparation. Headspace eliminates the need for solvents and other sample-preparation steps to reduce cost and complexity of extraction. In this application note, an adsorbent trap is used to concentrate the headspace sample and increase sensitivity, allowing for low-level detection or small sample sizes.

The analysis is carried out with gas chromatography mass spectrometry (GC/MS) – this will allow us to resolve the BHT from other volatile compounds in the food matrices and provide positive identification of the BHT with mass spectral data. Calibration of the system and analysis of food samples will be demonstrated.

Experimental

The instrumental platform for this application is the TurboMatrix™ HS Trap coupled to a Clarus® 680 GC/MS, both platforms from PerkinElmer. The transfer line of the HS was directly connected to the Elite™-17ms column with a universal butt connector. The samples are heated in a sealed vial at 80 ˚C for 30 minutes to drive the BHT from the food into the headspace. Using automated headspace technology, the gas is extracted from the vial, concentrated on an adsorbent trap (PerkinElmer® Air Toxics), and injected into the GC/MS system. Table 1 shows the detailed instrumental setup parameters for the HS Trap-GC/MS system.

Table 1. **Instrument Parameters.**

Sample Introduction	**PerkinElmer TurboMatrix HS-40 Trap**
Needle Temperature	90 ˚C
Transfer Line Temperature	110 ˚C
Oven Temperature	80 ˚C
Trap Low Temperature	40 ˚C
Trap High Temperature	280 ˚C
Dry Purge (Helium)	5 min
Trap Hold Time	6 min
Desorb Time	0.5 min
Thermostatting Time	30 min
Pressurization Time	1 min
Decay Time	2 min
Column Pressure	17 psi
Vial Pressure	35 psi
Desorb Pressure	10 psi
Universal Capillary Column Connector	Part No. N9302149
Transfer Line	Fused Silica 2 m x 320 µm

Gas Chromatograph	**PerkinElmer Clarus 680 GC**
Headspace Connector	Universal Connector
Inlet Temperature	150 ˚C
Oven Program Initial Temp	50 ˚C
Hold Time 1	1 min
Ramp 1	25 ˚C/min to 280 ˚C
Hold Time 2	1.8 min
Vacuum Compensation	On
Headspace Control	On
Column	Elite-17ms 30 m x 0.25 mm x 0.25 µm
Carrier Gas	Helium
Mass Spectrometer	**PerkinElmer Clarus 600 MS**
Mass Range	45-300 u
Solvent Delay Time	0.1 min
Scan Time	0.20 sec
InterScan Delay Time	0.02 sec
Transfer Line Temperature	240 °C
Source Temperature	200 °C
Multiplier	500 V

Calibration-Standards Preparation

A 10 ng/µL standard stock solution was prepared by diluting 0.1 mL of a 1000 µg/mL BHT standard to 10 mL with methanol. 1 ng/µL, 2 ng/µL and 5 ng/µL standard working solutions were prepared by diluting 0.1 mL, 0.2 mL and 0.5 mL of a 10 ng/µL BHT standard to 1 mL with methanol. 20 ng/µL, 50 ng/µL and 100 ng/µL standard working solution was prepared by diluting 0.02 mL, 0.05 mL and 0.1 mL of a 1000 µg/mL BHT standard to 1 mL with methanol.

The working curve was prepared by injecting 1 µL of each working standard solution into headspace vials. Working calibration standards at 1, 2, 5, 10, 20, 50, and 100 ng were prepared fresh each day.

One gram of each food sample purchased at local Shanghai markets were placed into the headspace vials. All headspace vials were sealed immediately and transferred to the headspace-trap vial tray.

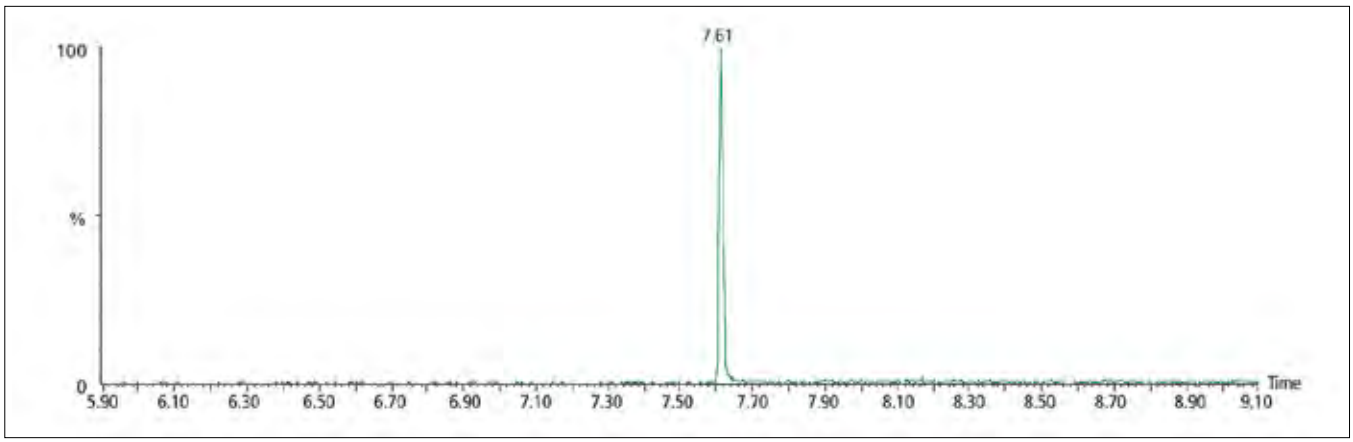

Figure 1. Example chromatogram of a 100 ng standard injection of BHT.

Results and Discussion

The instrument calibration included seven calibration levels in the working curve; the response of this calibration curve was linear (Table 2). Additionally, the method is precise throughout the calibration range, as demonstrated by the relative standard deviation of 3.2% at the calibration limit (1 ng, n=5) and 1.9% at 10 ng (n=5).

Figure 1 is an extracted ion chromatogram, of *m/z* 205, from the analysis of a 100 ng BHT standard. Figure 2 demonstrates the spectral data of BHT which matches exactly the fragmentation of BHT in the NIST® spectral library.

Following the calibration of the system, five food samples were analyzed: a cracker, powdered coffee creamer, instant noodles, sausage, and tea leaves. The BHT concentrations are quantified (Table 3). BHT concentration in the food samples analyzed here was below the quantitation limit of 1 ng/g. It can be seen in Figure 3 (Page 4) that the BHT peak is easily identified in the sample analysis. Each sample was analyzed in triplicate – the area reproducibility achieved (Table 3) demonstrates that the method remains very precise, even below the quantitation limit.

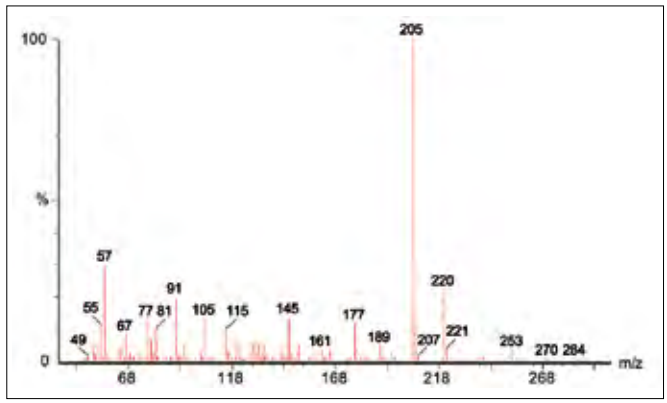

Figure 2. Background subtracted spectra from the analysis of a BHT standard.

***Table 2.* Calibration Table for BHT.**

Name	Retention Time (min)	Quantifier Ion	Qualifier Ion 1	Qualifier Ion 2	%RSD (n=5 at 1 ng)	%RSD (n=5 at 10 ng)	r^2
BHT	7.60	205	220	57	3.2	1.9	0.9980

***Table 3.* %RSD of BHT in Food Samples.**

Sample	BHT (ng/g) in 1 g of Sample	BHT (ng/g) in 1 g of Duplicate	BHT (ng/g) in 1 g of Triplicate	Values Mean (ng/g)	%RSD
Crackers	0.65	0.55	0.54	0.58	10.5
Coffee Creamer	0.66	0.73	0.69	0.69	5.1
Instant Noodles	0.67	0.67	0.70	0.68	2.5
Sausage	0.67	0.53	0.56	0.59	12.6
Tea Leaves	0.62	0.54	0.53	0.56	8.8

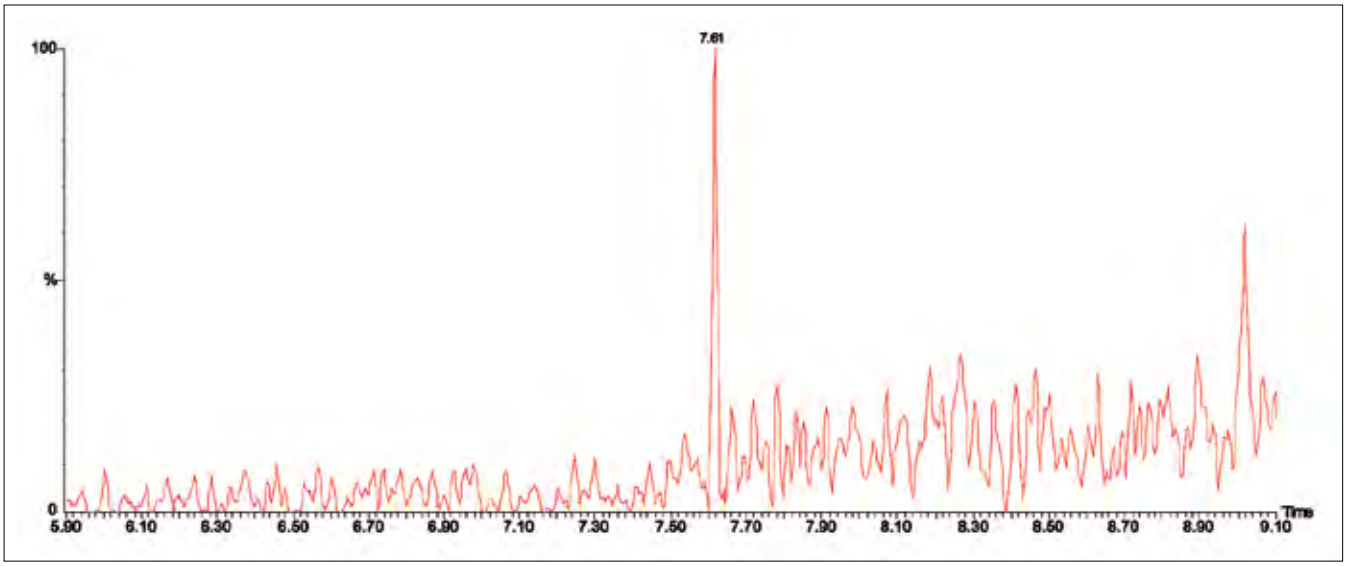

Figure 3. Resultant chromatogram from the analysis of instant noodles for BHT.

Conclusion

BHT is a common food additive used to prevent spoilage. Analysis of BHT is needed for both food quality and safety reasons. Food is often a complicated sample matrix which is time consuming to prepare and analyze. This method uses headspace technology to virtually eliminate sample preparation and reduce the cost and labor of the analysis. In addition to eliminating sample preparation, the method is both sensitive and precise as demonstrated by the analysis of standard reference materials and a variety of food samples. The throughput of the system is further improved by the Clarus 680 GC/MS with a fast-cooling GC oven, further improving throughput and productivity. The MS data provides positive confirmation of BHT in sample matrices.

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APPLICATION NOTE

Atomic Absorption

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The Determination of Toxic, Trace, and Essential Elements in Food Matrices using THGA Coupled with Longitudinal Zeeman Background Correction

Introduction

Ingestion of trace elements from food can be linked to nutrition, disease, and physiological development. Whether they are needed for proper nutritional value or contain toxic elements, the presence of major and minor elements in food needs to be verified to help determine health effects for the consumer. Contamination of food products may result from metals present during cultivation and/or processing. Acute or chronic exposure to heavy metals can lead to damaged nervous system function and have detrimental effects on vital organs. Food safety laboratories performing these analyses are often high-throughput facilities and require a detection tool that is efficient and cost effective.

Unlike flame atomic absorption spectrophotometry (FAAS) where the ground state atoms quickly diffuse into surrounding air, graphite furnace atomic absorption spectrophotometry (GFAAS), being a total consumption technique, offers the ability to dry and atomize the entire pipetted sample in a more controlled environment within the graphite tube. This significantly increases sensitivity and provides superior detection limits with microliter (µL) sample volumes. Only ICP-MS can provide the same level of detection as GFAAS, however GFAAS is more cost efficient, simpler to operate and has fewer laboratory facility requirements.

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The PerkinElmer® PinAAcle™ 900T atomic absorption spectrophotometer (Figure 1) uses the unique transversely heated graphite atomizer (THGA) design which provides a uniform temperature profile over the entire length of the graphite tube, unlike longitudinally heated systems which have only a small constant temperature zone in the center of the tube (Figure 2). The transverse design also provides exceptionally fast heating. A direct benefit of the fast, uniform temperature distribution in the THGA tube is a major reduction or elimination of condensation interferences caused by cooler temperatures at the tube ends. This significantly improves analytical accuracy and reduces memory effects. The PinAAcle 900T spectrometer has the ability to provide full implementation of the Stabilized Temperature Platform Furnace™ (STPF) technique which is paramount in providing nearly interference-free analysis. This eliminates the need for the method of additions and allows for direct calibration with simple aqueous standards, independent of the sample matrix. The curved, integrated platform inside of the THGA tube provides the ability to determine any element (including refractory elements) under full STPF conditions.

Figure 1. PerkinElmer PinAAcle 900T atomic absorption spectrophotometer.

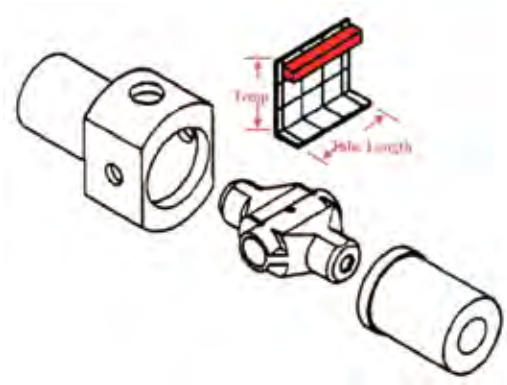

Figure 2. Uniform temperature distribution profile with THGA tube and heating assembly.

Experimental Conditions

Sample Preparation

Five different NIST® Standard Reference Material (SRM) food samples, which represent a typical cross-section of food types for human consumption, were chosen. The foods included non-fat milk powder (dairy), spinach (vegetable), mussel tissue (shellfish), bovine muscle (meat), and corn bran (grain).

The samples were digested using a Multiwave™ 3000 microwave digestion system equipped with a Rotor 16HF100. Details of the procedure are provided in Table 1. Approximately 0.5 g of each SRM was digested in duplicate with 5 mL of nitric acid (HNO_3) (Fisher Optima grade) and 2 mL of hydrogen peroxide (H_2O_2) (Fisher Optima grade) in pre-cleaned HF100 PTFE vessels. The 16-position rotor was equipped with a pressure/temperature sensor to monitor a single sample to ensure the program was running properly. In addition, an external infrared (IR) sensor measured the temperature for every location in the rotor throughout the analysis. All samples were completely digested and diluted to a final volume of 50 mL with deionized water (≥18 MΩ). Samples were further diluted as necessary in the same manner. Preparation blanks, consisting of the acid mixture, were taken through the same digestion and preparation process as the samples and analyzed accordingly.

Table 1. **Microwave digestion heating program for all six NIST® food SRMs and preparation blanks.**

Step	Power (W)	Ramp (min)	Hold (min)	Fan
1	500	1	4	1
2	1000	5	5	1
3	1400	5	10	1
4 (cooling)	0	—	15	3

Instrumentation

All data were generated with the PinAAcle 900T atomic absorption spectrophotometer in graphite furnace mode and based on a 20 µL sample + 5 µL modifier injection volume (Table 2 – Page 3). For arsenic (As) and selenium (Se), an end-capped tube (Part No. B3000653) was employed instead of the standard, open-ended tubes (Figure 3 – Page 3). End-capped tubes provide a longer atom residence time, thereby improving sensitivity. If desired, the type of tube can easily be switched and does not require major changes to the existing furnace program. End-capped tubes can be used for all elements except for refractory elements such as V, Ti, Mo, etc. With these high-temperature elements, there is little to no increase in sensitivity, but there is an increase in memory/carryover (Figure 4 – Page 3). Electrodeless discharge lamps (EDLs) were used for As (Part No. N3050605) and Se (Part No. N3050672) to provide lower detection limits and better light output compared to standard hollow cathode lamps (HCLs).

***Table 2.* GFAAS conditions for the analysis of trace metals in food.**

Analyte	Wavelength	Matrix Modifier	Tube Type	Lamp
As	193.7	$Pd/Mg(NO_3)_2$	End-Capped	EDL
Cd	228.8	$PO_4/Mg(NO_3)_2$	Standard	HCL
Cr	357.9	$Mg(NO_3)_2$	Standard	HCL
Cu	324.8	$Pd/Mg(NO_3)_2$	Standard	HCL
Fe	248.3	$Mg(NO_3)_2$	Standard	HCL
Mn	279.5	$Pd/Mg(NO_3)_2$	Standard	HCL
Ni	232.0	$Pd/Mg(NO_3)_2$	Standard	HCL
Pb	283.3	$PO_4/Mg(NO_3)_2$	Standard	HCL
Se	196.0	$Pd/Mg(NO_3)_2$	End-Capped	EDL

Figure 3. Standard THGA tube (left) (Part No. B3000641) and end-capped THGA tube (right) (Part No. B3000653).

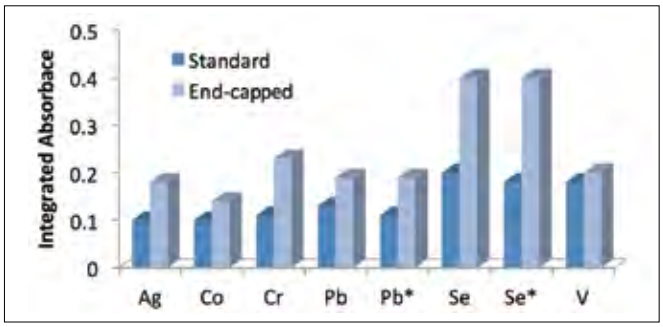

Figure 4. Comparison of integrated absorbances for end-capped vs. standard THGA tubes. *Denotes use of the recommended matrix modifier.

Furnace Program

All elements except for selenium (Se) used a conventional program that included two drying steps, a pyrolysis or pretreatment step to remove unwanted matrix components, an atomization step with gas-stop conditions, and a brief clean-out step (Table 3). The Se program was modified to include pre-reduction of the Pd modifier using hydrogen. This results in lower background levels during the atomization step, reducing the potential for vapor-phase interferences that could degrade data quality (Table 4).

***Table 3.* Conventional furnace program, using argon, for the analysis of trace metals in food products using GFAAS.**

Step	Temp (˚C)	Ramp Time (s)	Hold Time (s)	Internal Flow (mL/min)	Gas Type
1	110	1	30	250	Normal
2	140	15	35	250	Normal
3	1200	10	40	250	Normal
4*	2100	0	4	0	Normal
5	2450	1	3	250	Normal

**Read step*

***Table 4.* Furnace program incorporating an alternate gas type (Ar/H_2) for the analysis of Se.**

Step	Temp (˚C)	Ramp Time (s)	Hold Time (s)	Internal Flow (mL/min)	Gas Type
1	120	1	40	250	Special
2	1000	10	30	250	Special
3	110	1	30	250	Normal
4	140	15	40	250	Normal
5	1200	10	45	250	Normal
6*	2100	0	5	0	Normal
7	2450	1	3	250	Normal

**Read step*

The PinAAcle 900T spectrometer's TubeView™ furnace camera (Figure 5) was used to check the position of the pipette tip and in optimizing the drying times and temperatures for the food matrix.

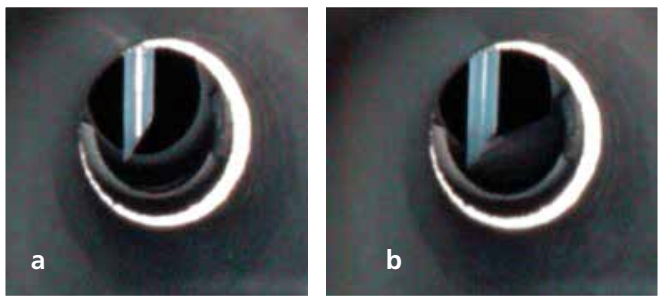

Figure 5. TubeView furnace camera captures of AS 900 autosampler pipette in graphite tube during sample deposition.

Results

Calibration

Calibration standards were chosen to ensure all samples fell within range. A linear calibration equation, forced through zero, was used and the calibration statistics showed excellent correlation (Table 5). The instrument design and software provided for easy implementation, selection and use of the special Ar/H_2 gas for the determination of Se. Preparation blanks were determined to contain negligible levels as compared to reported data.

Table 5. **Calibration data for the detection of trace elements in food.**

Analyte	Wavelength	Matrix Modifier	Tube Type	Lamp
As	193.7	$Pd/Mg(NO_3)_2$	End-Capped	EDL
Cd	228.8	$PO_4/Mg(NO_3)_2$	Standard	HCL
Cr	357.9	$Mg(NO_3)_2$	Standard	HCL
Cu	324.8	$Pd/Mg(NO_3)_2$	Standard	HCL
Fe	248.3	$Mg(NO_3)_2$	Standard	HCL
Mn	279.5	$Pd/Mg(NO_3)_2$	Standard	HCL
Ni	232.0	$Pd/Mg(NO_3)_2$	Standard	HCL
Pb	283.3	$PO_4/Mg(NO_3)_2$	Standard	HCL
Se	196.0	$Pd/Mg(NO_3)_2$	End-Capped	EDL

Standard Reference Materials

Quantitative results for the five different SRMs are shown in Tables 6-10. Recovery data is based on the mean reference value provided by NIST®. All of the experimental values with concentrations sufficient for this technique had recoveries of 90-110% and/or fell within the confidence limit given by NIST®. Even at the very low level of lead and selenium in the non-fat milk powder reference material, with a solution concentration of 0.158 µg/L and 1.26 µg/L respectively, the PinAAcle 900T spectrometer was able to achieve an excellent recoveries with respect to the reference range.

Non-specific background, caused by high concentrations of concomitant molecules, small particles, or smoke that may absorb or scatter light from the light source, is the most common type of interference. This can be noteworthy in high-matrix samples such as food digests. Longitudinal Zeeman background correction is especially suited to correct for high or structured background. The absorption and background signal are measured with the same light source and at the same wavelength, with identical resolution. This ensures an exceptionally accurate, background-corrected measurement. In addition, with the longitudinal Zeeman design of the PinAAcle 900T spectrometer, the need for an optical polarizer is eliminated. Compared to the transverse Zeeman designs of other systems, this provides a greater than 50% increase in light throughput. The accuracy of the method incorporating longitudinal Zeeman background correction is shown in Tables 6-10 with the recoveries of trace metals from food reference materials.

Standard programs with the recommended matrix modifiers were used to successfully analyze arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and lead (Pb). A custom program was successfully used to analyze Se. The custom analysis of Se in WinLab32™ for AA software indicates the ability of the system to easily implement an alternate gas with a custom AS 900 autosampler sequence whereby the modifier was pipetted and furnace steps 1 and 2 were performed; then the sample plus diluent were added and furnace steps 3 through 7 were run.

Table 6. **Analysis of NIST® 1549 Non-Fat Milk Powder.**

Analyte	Reference Value (mg/kg)	Experimental Value (mg/kg)	Recovery (%)
Cd	0.0005 ±0.0002	*	
Cu	0.7 ±0.1	0.627	90
Pb	0.019 ±0.003	0.0158	83
Mn	0.26 ±0.06	0.268	103
Se	0.11 ±0.01	0.126	115

**Concentration was not sufficient for this technique.*

Table 7. **Analysis of NIST® 1570a Spinach Leaves.**

Analyte	Reference Value (mg/kg)	Experimental Value (mg/kg)	Recovery (%)
Cd	2.89 ±0.07	2.61	90
Cu	12.2 ±0.6	12.5	102
Pb	(0.20)	0.208	104
Mn	75.9 ±1.9	74.8	99
Ni	2.14 ±0.10	2.21	103

The value in parentheses ( ) in the "Reference Value" column is not a certified value – it is included for information only.

Table 8. **Analysis of NIST® 2976 Mussel Tissue.**

Analyte	Reference Value (mg/kg)	Experimental Value (mg/kg)	Recovery (%)
As	13.3 ±1.8	13.1	98
Cd	0.82 ±0.16	0.869	106
Cr	0.50 ±0.16	0.501	100
Cu	4.02 ±0.33	3.97	99
Fe	171.0 ±4.9	182	106
Pb	1.19 ±0.18	1.07	90
Mn	33 ±2	34.2	104
Ni	0.93 ±0.12	0.958	103
Se	1.80 ±0.15	1.74	97

Table 9. **Analysis of NIST® 8414 Bovine Muscle Powder.**

Analyte	Reference Value (mg/kg)	Experimental Value (mg/kg)	Recovery (%)
Cd	0.013 ±0.011	0.0162	125
Cu	2.84 ±0.45	2.69	95
Fe	71.2 ±9.2	70.9	99
Pb	0.38 ±0.24	0.334	87
Mn	0.37 ±0.09	0.343	93
Ni	0.05 ±0.04	0.0631	126

Table 10. **Analysis of NIST® 8433 Corn Bran.**

Analyte	Reference Value (mg/kg)	Experimental Value (mg/kg)	Recovery (%)
Cd	0.012 ±0.005	*	
Cu	2.47 ±0.40	2.61	106
Fe	14.8 ±1.8	14.8	100
Pb	0.14 ±0.034	0.120	86
Mn	2.55 ±0.29	2.44	96
Ni	0.158 ±0.054	0.158	100

**Concentration not sufficient for this technique.*

Conclusion

The analysis of the various SRM materials clearly demonstrates the ability of the PinAAcle 900T spectrometer to generate quality data incorporating THGA tubes, longitudinal Zeeman background correction and STPF conditions for interference-free, accurate quantitation. In addition, the ability of the Multiwave 3000 system to perform complete dissolution in a closed-vessel microwave digestion was shown. The overall agreement of the results compared to the various SRM matrices demonstrates the versatility and ruggedness of the Multiwave 3000 digestion system using a single program with the same reagents for several food types. This work demonstrates the ability of the PinAAcle 900T spectrometer to quantitate in the low-ppb range, thereby reducing the need for an ICP-MS to successfully measure this suite of elements in foods. The PinAAcle 900Z (Longitudinal Zeeman Furnace only) spectrometer can also be used for this application.

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APPLICATION NOTE

ICP – Mass Spectrometry

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The Determination of Toxic, Essential, and Nutritional Elements in Food Matrices Using the NexION 300/350 ICP-MS

Introduction

The elemental and dynamic range of inductively coupled plasma-mass spectrometry (ICP-MS) makes it ideally suited for the analysis of food materials. The ultratrace detection limits of ICP-MS permit the determination of low-level contaminants such as Pb, As, Se, and Hg, while the macro-level nutritional elements such as Ca, Mg, K, and Na can be quantified using the extended dynamic range capability of ICP-MS which provides 9-orders of magnitude. However, there are still a number of challenges to overcome, which makes the routine analysis of foods difficult unless the sample dissolution procedure is well thought out and instrumental conditions are optimized for complex sample matrices.

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For example, the wide variety of edible products available means that a highly diverse range of matrices must be brought into solution for ICP-MS analysis. These complex acid-digested matrices, which are a combination of dissolved carbohydrates, fats, and proteins, can pose major problems for any ICP-MS because of the potential for blocking of the interface cones and/or deposition on the quadrupole ion deflector (QID). For this reason, if instrument design does not account for high-matrix samples, long-term stability can be severely compromised.

In addition to signal drift, digested food matrices can also cause major spectral complications. The sample's organic components, together with macro minerals, can combine with elements present in the digestion acid and/or the plasma argon to form polyatomic interferences. For example, chloride ions (at mass 35) combine with the major argon isotope (mass 40) to produce the argon chloride interference $^{40}Ar^{35}Cl^+$, which interferes with arsenic at mass 75. Another example is the argon dimer ($ArAr^+$), which forms from the plasma gas and exists at the same masses as the major selenium isotopes. In addition, the major isotope of chromium at mass 52 is overlapped by $^{40}Ar^{12}C^+$, $^{35}Cl^{17}O^+$, and $^{35}Cl^{16}OH^+$ interferences generated by the sample matrix and the plasma gas. As a result, these kinds of spectral interferences have made the determination of both trace and macro elements in food samples extremely challenging.

To overcome these issues, a NexION® 300X ICP-MS (PerkinElmer, Inc., Shelton, CT) was used for the analysis of various food substances, focusing on toxic and typical essential and macro elements in a group of NIST® (Gaithersburg, MD) standard reference materials (SRMs).

Experimental

Six different NIST® SRM food samples that represent a typical cross-section of the types of foods for human consumption were chosen for the evaluation. The foods included spinach leaves (leafy vegetable), corn bran (grain), wheat flour (grain), bovine muscle (meat), mussel tissue (shellfish), and milk powder (dairy product). The samples were brought into solution with a Multiwave™ 3000 microwave digestion system. Details of the sample digestion procedure are shown in Table 1.

Sample Preparation

Approximately 0.5-0.6 g of each SRM was digested in duplicate with 5 mL of nitric acid (Fisher Optima HNO_3) and 2 mL of hydrogen peroxide (Fisher Optima H_2O_2) in precleaned PTFE HF-100 microwave sample vessels. The filled vessels were placed on a 16-position rotor with an internal p/T sensor positioned in one of the samples to monitor the pressure and temperature inside the sample container. In addition, an external IR sensor provided the temperatures for each individual sample in the tray. The digestion program consisted of 30 min of heating and 15 min of cooling, as shown in Table 1. All the SRM samples were completely dissolved, resulting in clear solutions that were diluted to a final volume of 50 mL with deionized water. No further sample dilutions were necessary. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Preparation blanks, consisting of the acid mixture, were taken through the same microwave digestion program as the samples.

Table 1. **Microwave Digestion Heating Program for All Six NIST® Food SRMs.**

Step	Power (W)	Ramp (min)	Hold (min)
1	500	1	4
2	1000	5	5
3	1400	5	10
4 (cooling)	0	—	15

Instrumental Conditions

All data in this study were generated under normal operating conditions on a NexION 300X ICP-MS using an autosampler. The instrumental operating conditions are shown in Table 2.

Table 2. **ICP-MS Instrumental Operating Conditions for this Application.**

Component/Parameter	Type/Value/Mode
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Cones	Nickel
Plasma gas flow	18.0 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.98 L/min
Sample uptake rate	300 µL/min
RF power	1600 W
Total integration time	0.5 (1.5 seconds for As, Se, Hg)
No. of replicates per sample	3
Universal Cell Technology™*	KED mode

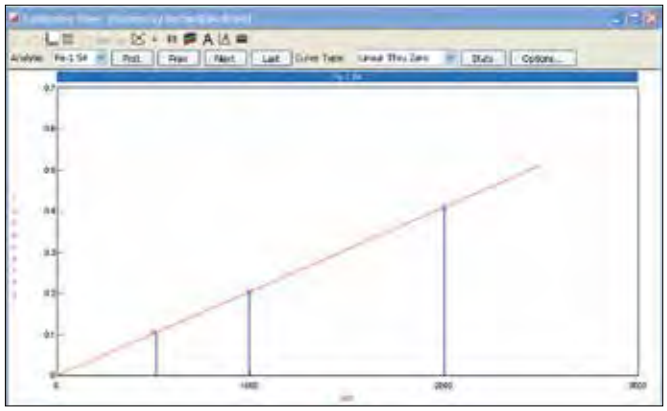
**PerkinElmer, Inc.*

Calibration

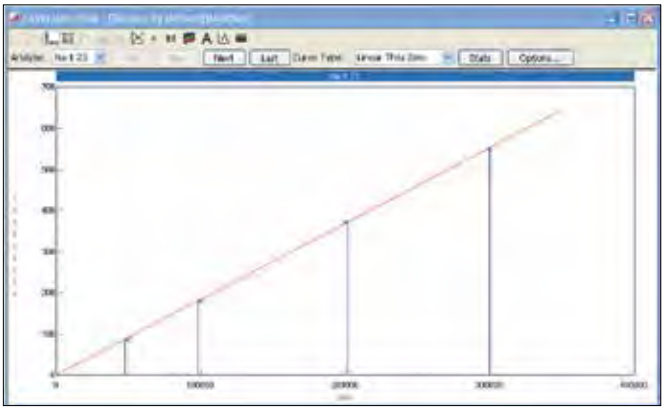
Multielement calibration standards, representing all the analytes covered by the six NIST® SRMs, were made up from PerkinElmer® Pure single and multielement standards and diluted into 10% HNO_3. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. However, it is important to mention that each food SRM was certified for a slightly different group of elements. For that reason, quantitation was only carried out on the analytes that had reference values. Calibration standard ranges were based on whether the analyte was expected to be a high-level, nutritional element like potassium (K) or sodium (Na), a low/medium-level essential element like manganese (Mn) or iron (Fe), or a trace/ultratrace contaminant such as lead (Pb) or mercury (Hg). Depending on the certificate value of the analytes, five different calibration ranges were made up to cover the complete range of elements being determined. They were:

- High-level nutritional analytes: 0-300 ppm
- Medium-level essential analytes: 0-20 ppm
- Low-level essential analytes: 0-2 ppm
- Trace-level contaminants: 0-200 ppb
- Ultratrace-level contaminants: 0-20 ppb

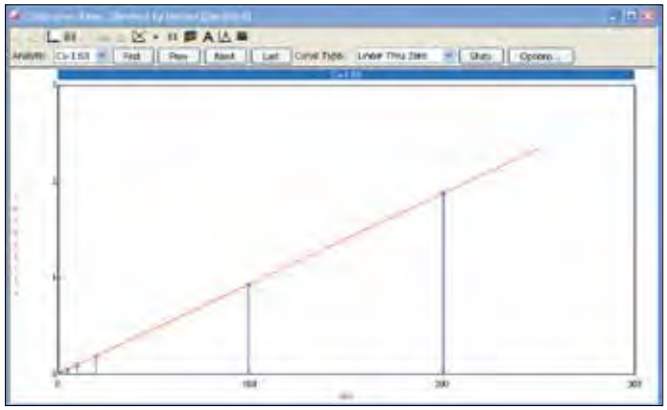
Figure 1 shows representative calibration curves for each range.



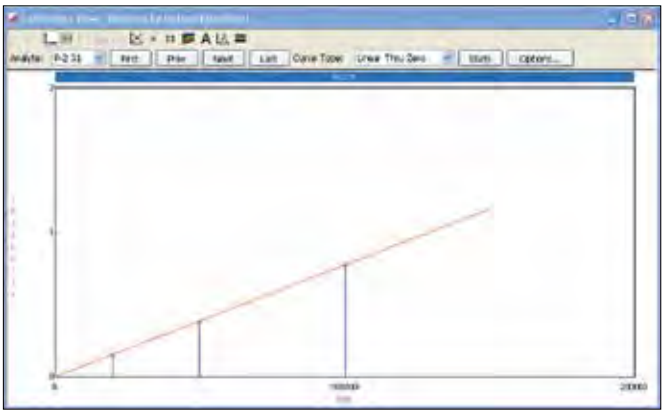
^{54}Fe Correlation Coefficient = 0.99997.



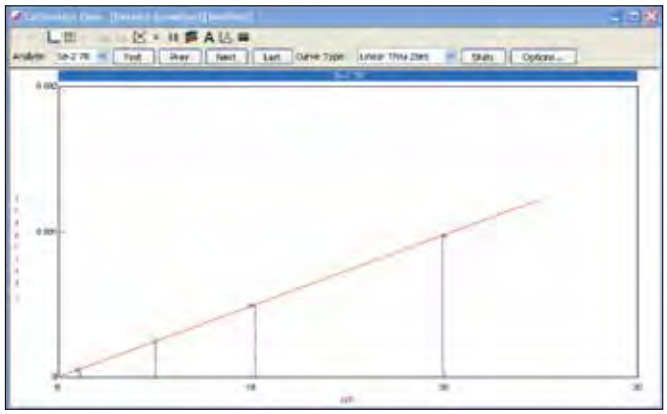
^{23}Na Correlation Coefficient = 0.99996.



^{63}Cu Correlation Coefficient = 0.99999.



^{31}P Correlation Coefficient = 0.99999.



^{78}Se Correlation Coefficient = 0.99995.

Figure 1. Calibration curves for ^{23}Na (0-300 ppm), ^{31}P (0-20 ppm), ^{54}Fe (0-2 ppm), ^{63}Cu (0-200 ppb) and ^{78}Se (0-20 ppb).

In addition to the analyte elements used for the multielement calibration, the standards, blanks, and samples were also spiked on-line using a mixing tee with a solution of ^{6}Li, Sc, Ge, In, and Tb for internal standardization across the full mass range. Acetic acid was added to the internal standard solution to compensate for residual carbon leftover from the sample digestion.

Results

Quantitative results for two sample preparations of six NIST® SRMs (Corn Bran, Bovine Muscle, Mussel Tissue, Milk Powder, Wheat Flour, and Spinach Leaves) are shown in Tables 3-8, respectively. All elements in every sample were determined with kinetic energy discrimination (KED) mode using helium as the collision gas. Figures in parentheses ( ) in the reference value column are not certified values, but are included for information purposes only. The data show very good agreement with the certified values, especially for the elements that suffer from known spectral interferences. The elements that are outside the specified limits are mostly the ones that are well recognized as environmental contaminants, which have probably been impacted by the sample preparation procedure.

Food samples are complex acid-digested matrices and can create major problems for some ICP-MS systems because of deposits on the interface cones and on the ion optics caused from high concentrations of dissolved solids. For this reason, long-term stability can be poor. However, the triple cone interface and the quadrupole ion deflector design of the NexION guarantee exceptional long-term stability. For six hours, food samples with high concentrations of dissolved solids were analyzed and a quality control (QC) sample was read every 5 samples. Figure 2 shows the long-term stability over 6 hours.

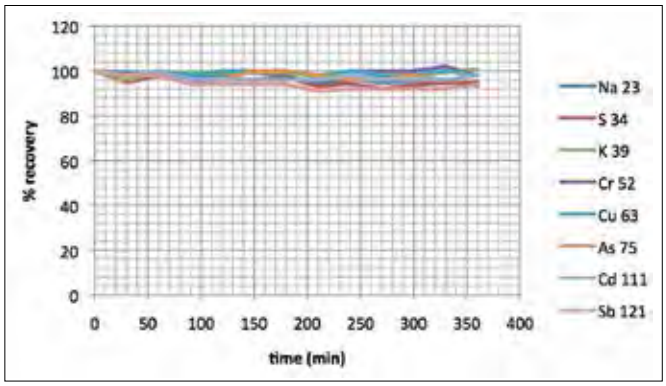


Figure 2. % Recovery of several analytes in the QC standard during 6-hour analysis.

Conclusion

The ICP-MS system used in this study is well suited for the analysis of complex digested food materials. The agreement between experimental and certified results for six NIST® food SRMs demonstrates that the NexION 300X ICP-MS can effectively measure various food samples. In addition to removing interferences, the NexION 300X allows the determination of macro-level nutritional elements in the same analysis run as lower-level elements, without having to dilute the samples. Instrument design characteristics eliminate deposition on the ion optics, leading to long-term stability in high-matrix samples while permitting trace levels to be accurately measured.

Table 3. **Analysis of NIST® 8433 Corn Bran using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	2.8 ±1.2	3.2
Na	23	430 ±31	399
Mg	26	818 ±59	787
Al	27	1.01 ±0.55	1.15
P	31	171 ±11	158
S	34	860 ±150	738
K	39	566 ±75	548
Ca	44	420 ±38	434
V	51	0.005 ±0.002	0.005
Cr	52	(0.11)	0.08
Fe	54	14.8 ±1.8	13.7
Mn	55	2.55 ±0.29	2.53
Co	59	(0.006)	0.005
Ni	60	0.158 ±0.054	0.143
Cu	63	2.47 ±0.40	2.54
Zn	66	18.6 ±2.2	17.0
As	75	(0.002)	<0.006
Se	78	0.045 ±0.008	0.056
Sr	88	4.62 ±0.56	4.56
Mo	98	0.252 ±0.039	0.255
Cd	111	0.012 ±0.005	0.013
Sn	118	–	0.015
Sb	121	(0.004)	0.003
Ba	137	2.40 ±0.52	2.26
Hg	202	0.003 ±0.001	0.005
Pb	208	0.140 ±0.034	0.122
Tl	205	–	<0.0001
Th	232	–	<0.00008
U	238	–	<0.00002

Table 4. **Analysis of NIST® 8414 Bovine Muscle using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	0.6 ±0.4	0.4
Na	23	2100 ±80	2000
Mg	26	960 ±95	960
Al	27	1.7 ±1.4	1.6
P	31	8360 ±450	7250
S	34	7950 ±410	6820
K	39	15170 ±370	14180
Ca	44	145 ±20	143
V	51	(0.005)	0.006
Cr	52	0.071 ±0.038	0.092
Fe	54	71.2 ±9.2	71.2
Mn	55	0.37 ±0.09	0.44
Co	59	0.007 ±0.003	0.014
Ni	60	0.05 ±0.04	0.05
Cu	63	2.84 ±0.45	2.81
Zn	66	142 ±14	140
As	75	0.009 ±0.003	0.011
Se	78	0.076 ±0.010	0.11
Sr	88	0.052 ±0.015	0.081
Mo	98	0.08 ±0.06	0.08
Cd	111	0.013 ±0.011	0.013
Sn	118	–	0.14
Sb	121	(0.01)	0.01
Ba	137	(0.05)	0.04
Hg	202	0.005 ±0.003	0.003
Pb	208	0.38 ±0.24	0.34
Tl	205	–	0.002
Th	232	–	<0.00008
U	238	–	<0.00002

Table 5. **Analysis of NIST® 2976 Mussel Tissue using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	–	27.5
Na	23	(35000 ±1000)	35000
Mg	26	(5300 ±500)	4800
Al	27	(134 ±34)	149
P	31	(8300)	6900
S	34	(19000)	16000
K	39	(9700 ±500)	9700
Ca	44	(7600 ±300)	7400
V	51	–	0.87
Cr	52	(0.50 ±0.16)	0.50
Fe	54	171.0 ±4.9	190
Mn	55	(33 ±2)	40
Co	59	(0.61 ±0.02)	0.67
Ni	60	(0.93 ±0.12)	0.87
Cu	63	4.02 ±0.33	3.91
Zn	66	137 ±13	145
As	75	13.3 ±1.8	16.4
Se	78	1.80 ±0.15	2.52
Sr	88	(93 ±2)	79
Mo	98	–	0.56
Cd	111	0.82 ±0.16	0.88
Sn	118	(0.096 ±0.039)	0.103
Sb	121	–	0.011
Ba	137	–	0.61
Hg	202	0.061 ±0.0036	0.058
Pb	208	1.19 ±0.18	1.06
Tl	205	(0.0013)	0.003
Th	232	(0.011 ±0.002)	0.012
U	238	–	0.22

Table 6. **Analysis of NIST® 1549 Milk Powder using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	–	2.1
Na	23	4970 ±100	4700
Mg	26	1200 ±30	1170
Al	27	(2)	0.7
P	31	10600 ±200	10500
S	34	3510 ±50	3290
K	39	16900 ±300	16500
Ca	44	13000 ±500	12800
V	51	–	0.003
Cr	52	0.0026 ±0.0007	<0.003
Fe	54	1.78 ±0.10	1.98
Mn	55	0.26 ±0.06	0.26
Co	59	(0.0041)	0.005
Ni	60	–	0.013
Cu	63	0.7 ±0.1	0.6
Zn	66	46.1 ±2.2	46.7
As	75	(0.0019)	<0.006
Se	78	0.11 ±0.01	0.17
Sr	88	–	3.7
Mo	98	(0.34)	0.37
Cd	111	0.0005 ±0.0002	<0.002
Sn	118	–	<0.002
Sb	121	(0.00027)	<0.001
Ba	137	–	0.83
Hg	202	0.0003 ±0.0002	<0.0007
Pb	208	0.019 ±0.003	0.019
Tl	205	–	<0.0001
Th	232	–	<0.00008
U	238	–	<0.00002

Table 7. **Analysis of NIST® 8436 Wheat Flour using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	–	0.62
Na	23	16.0 ±6.1	17.0
Mg	26	1070 ±80	1030
Al	27	11.7 ±4.7	11.8
P	31	2900 ±220	2330
S	34	1930 ±280	1460
K	39	3180 ±140	2950
Ca	44	278 ±26	262
V	51	0.021 ±0.006	0.026
Cr	52	0.023 ±0.009	0.053
Fe	54	41.5 ±4.0	41.4
Mn	55	16.0 ±1.0	15.1
Co	59	0.008 ±0.004	0.007
Ni	60	0.17 ±0.08	0.17
Cu	63	4.30 ±0.69	4.18
Zn	66	22.2 ±1.7	20.6
As	75	(0.03)	0.01
Se	78	1.23 ±0.09	1.22
Sr	88	1.19 ±0.09	1.19
Mo	98	0.70 ±0.12	0.72
Cd	111	0.11 ±0.05	0.11
Sn	118	–	0.032
Sb	121	–	0.002
Ba	137	2.11 ±0.47	2.04
Hg	202	0.0004 ±0.0002	<0.0007
Pb	208	0.023 ±0.006	0.35
Tl	205	–	<0.0001
Th	232	–	0.001
U	238	–	0.001

Table 8. **Analysis of NIST® 1570a Spinach using the NexION 300 ICP-MS.**

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	37.6 ±1.0	37.3
Na	23	18180 ±430	17350
Mg	26	(8900)	8600
Al	27	310 ±11	200
P	31	5180 ±110	4810
S	34	(4600)	4400
K	39	29030 ±520	26600
Ca	44	15270 ±410	15040
V	51	0.57 ±0.03	0.58
Cr	52	–	1.63
Fe	54	–	265
Mn	55	75.9 ±1.9	77.9
Co	59	0.39 ±0.05	0.37
Ni	60	2.14 ±0.10	1.97
Cu	63	12.2 ±0.6	11.6
Zn	66	82 ±3	80
As	75	0.068 ±0.012	0.081
Se	78	0.117 ±0.009	0.21
Sr	88	55.6 ±0.8	58.1
Mo	98	–	0.39
Cd	111	2.89 ±0.07	2.83
Sn	118	–	0.027
Sb	121	–	0.007
Ba	137	–	5.8
Hg	202	0.030 ±0.003	0.028
Pb	208	(0.20)	0.16
Tl	205	–	0.018
Th	232	0.048 ±0.003	0.045
U	238	(0.155 ±0.023)	0.154

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APPLICATION NOTE

Liquid Chromatography

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Safeguarding Food from Pesticides by UHPLC After Extraction with the QuEChERS Method

Introduction

The detection of pesticides in food matrices, such as fruit and vegetables, requires an ever increasing use of new technologies. Chromatographic techniques, both gas (GC) and liquid (LC), offer a suitable means of addressing the analytical requirements. They enable screening to be carried out rapidly both in gaseous and liquid phases – on samples prepared using the "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged, Safe) system, a fast and effective multi-residue extraction method.

Employing UHPLC (Ultra High Performance Liquid Chromatography) with a PDA (Photo Diode Array) detector offers considerable advantages over standard LC analysis. Indeed, it provides a very fast means of monitoring phytodrugs which are permitted in concentrations of up to 10 µg/Kg. The use of a PDA detector, together with the development of a library of spectra, enables each substance to be analyzed and identified correctly.

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Figure 1. Flexar FX-10 UHPLC.

Experimental phase

The study also includes the extraction of the samples using a standardized approach known as QuEChERS, which is an official, AOAC-compliant method for determining pesticide residues in samples of fruit and vegetables (Multi-residual pesticides) as per the European standard EN-15662.

Extraction procedure using the QuEChERS method

Place 15 g of homogenized sample in a 50 mL extraction tube containing 1.5 g of sodium acetate and 6 g of magnesium sulphate.

- Add 15 mL of an acetonitrile solution containing 1% glacial acetic acid. Add the internal standard, if required.
- Agitate vigorously for at least 1 minute and centrifuge for 1 minute at > 1,500 g.
- Transfer 1 mL of the supernatant (equivalent therefore to 1 g of sample) into a 2 mL extraction tube containing 50 mg of PSA and 150 mg of magnesium sulphate. Agitate vigorously for at least 30 seconds and then centrifuge for 1 minute at > 1,500 g.
- Take the clear supernatant and place in a vial for injection into the UHPLC system, or dilute it first, if necessary, with an appropriate solvent.

The analysis is carried out using a UHPLC system with Photo Diode Array detection.

Preparation of standards

Nine mixtures of different pesticides were used, starting with individual solutions of each in acetone at a concentration of 200 ppm. From each of these mixtures, diluting as required in 1:1 acetonitrile:water, 6 standard solutions were prepared at concentrations of 10, 40, 100, 400, 2,000 and 4,000 ppb. Chromatograms for each of the mixtures are shown in Figure 3.

***Table 1.* HPLC instrumental conditions.**

UHPLC System	PerkinElmer Flexar FX-10
Column	C18, 100 mm x 3 mm ID, 1.8 µm
Injection Volume	20 µL
Mobile phase	A) o-phosphoric acid 0.1% v/v B) Acetonitrile
Gradient	Equilibrium 3' 75% A – 25% B
Total analysis time: 16 minutes	Step 1: 2 min 60% A – 40% B curve -2 Step 2: 3 min 50% A – 50% B curve 2 Step 3: 2 min 40% A – 60% B curve 2 Step 4: 3 min 25% A – 75% B curve 3 Step 5: 2 min 15% A – 85% B curve 2 Step 6: 2 min 5% A – 95% B curve 2 Step 7: 2 min 5% A – 95% B
Back pressure	From 7000 psi (Step 1) to 4500 psi (Step 7)
Flow rate	0.65 mL/min for all steps
Wavelength	210 nm
Column temperature	40 °C

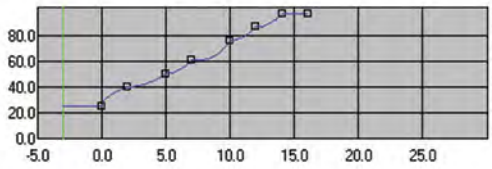

Figure 2. Pump gradient profile (solvent B).

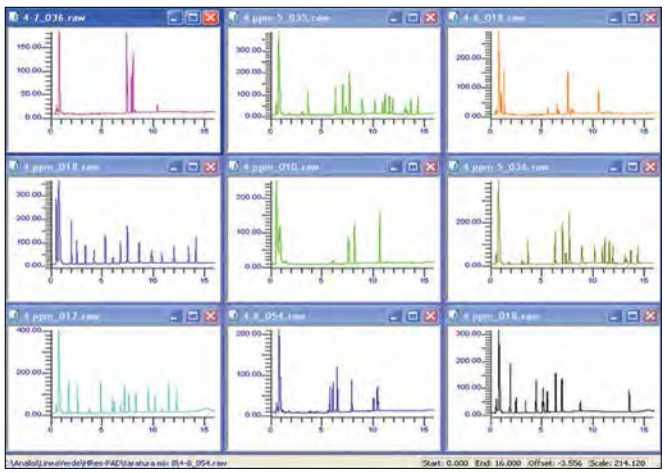

Figure 3. Pesticides standard solutions.

The calibration was linear over the range 10-4,000 ppb with a correlation value r^2 in the range 0.999-0.9999 for all components (see Appendix 1), and Figure 4 illustrates an example calibration curve for a component chosen at random (highlighted in blue).

Table 2. **List of compounds analyzed.**

Cyflutryn	Chiorpropham	Chlorpyrifos-Methyl	Fenbuconazol	Sethoxydim
Cymoxanil	Acrinathrin	Hexaconazole	Pencycuron	Iprovalicarb
Deltamethrin	Triadimefon	Cycloxidim	Thiacloprid	Methiocarb
Dicloran	Fludioxonil	Cyromazine	Pyraclostrobin	Difenoconazole
Indoxacarb	Azoxystrobin	Napropamide Fenarimol	Folpet	Metribuzin
Iprodion	Boscalid	Fenhexamid	Captan	Phomix
Metalaxyl	Diflubenzuron	Fenpyroximate	Hexythiazox	Fenamidone
Primicatb	Bitertanol	Tebuconazol	Phosalone	Pyriproxyfen
Tolcoflos Methyl	Buprofezin	Imidacloprid	Dimethomorph isomer mixture E+2 isom	Fenazaquin
Chlorothalonil	Cypermethrin isomer mixture	Etridiazole	Clomazone beta	Dithianon
Cyproconazole	Diclofuanid	Flufenoxuron	Chlorthal-dimethyl	Famoxadone
Cypronidil	Fluazifop-Buthyl	Oxyfluorfen	Aclonifen	Oxamyl
Lambda- Cythalothrin	Azinphos-Methyl	Phenamifos	Endusulfan sulphate	Carbosulfan
Benalaxyl	Azinphos-Ethyl	Propachlor	Fenvalerate	Cyazofamid
Pyrimethanil	Pirimphos-Ethy	Propaquizafop	Etofenprox	Propham
Thiram	Prochloraz	Pymetrozine	Phenmedipharm	Benfuracarb
Malathion	Fluvalinate isomer mixture	Quizalofop-p-ethyl	Lenacil	Teflubenzuron
Linuron	Fenoxaprop	Tetraconazole	Sethoxydim	

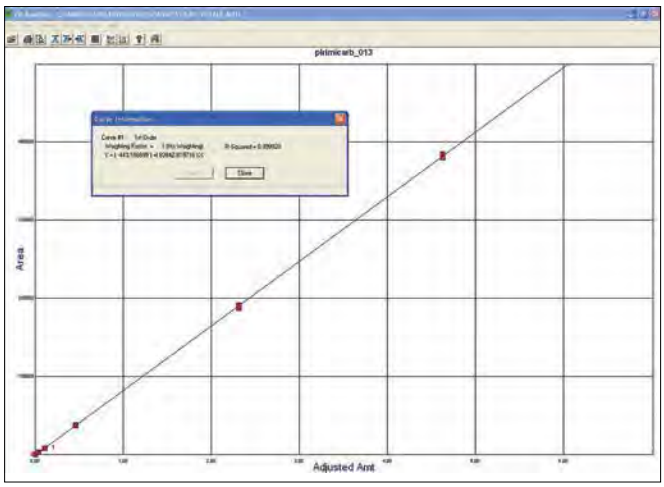

Figure 4. Calibration curve.

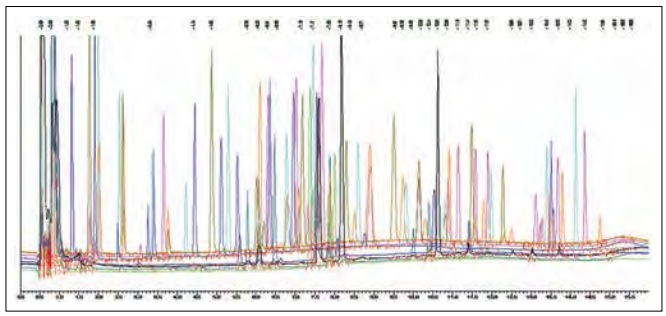

Figure 5. Overlay of all 90 pesticides.

UV spectrum identification

This method involves the identification of the individual components using 3 different libraries obtained at different concentrations:

High Concentration: 4,000 ppb

Medium Concentration: 400 ppb

Low Concentration: 40 ppb

Thus, it is always possible to optimize the identification as a function of the concentration of the sample.

An example identification based on the spectrum obtained (Figure 6) using the appropriate library is described below.

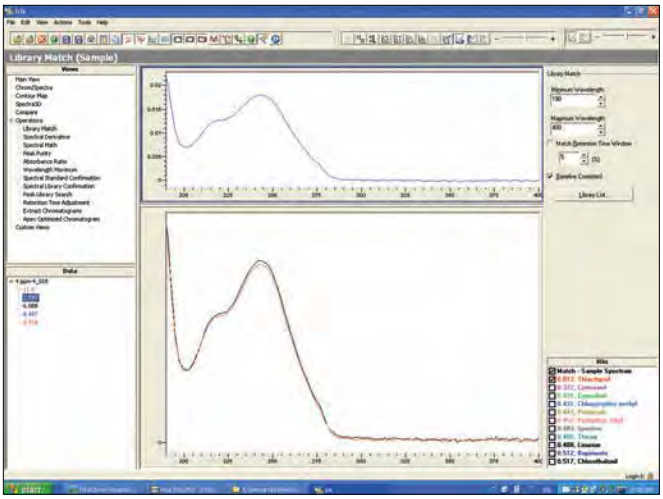

Figure 6. Identification using spectral library.

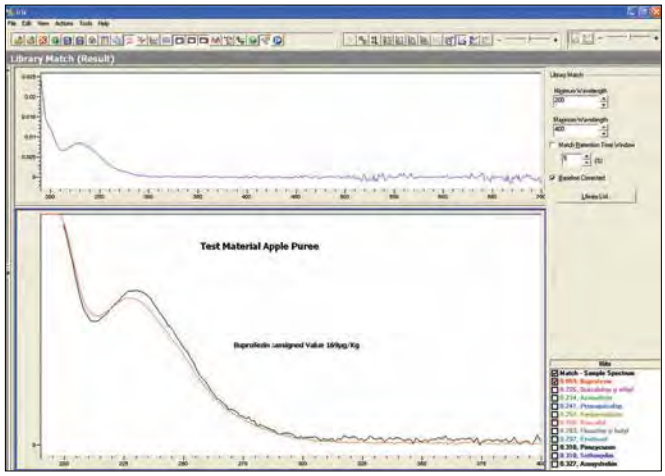

Figure 8. Spectral profile identification of apple puree test material.

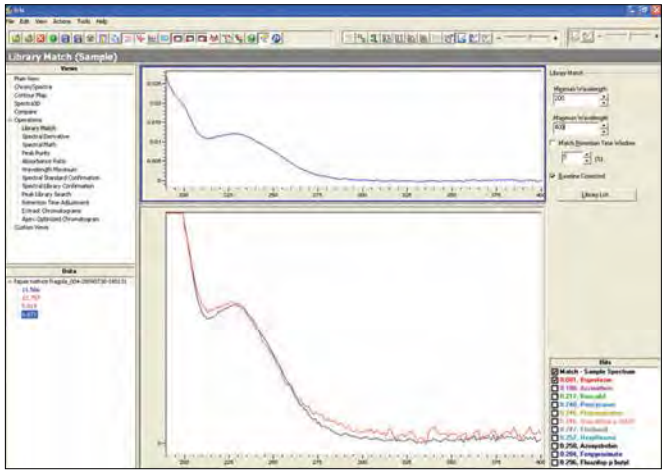

Figure 9. Spectral profile identification.

Analysis of a certified "Strawberry" sample

To demonstrate the effectiveness of the method, a certified sample of strawberries was analyzed (FAPAS® Ref. Number T1986 Strawberry Purée) containing Buprofezin. The substance was identified correctly as shown below (150 µg/kg).

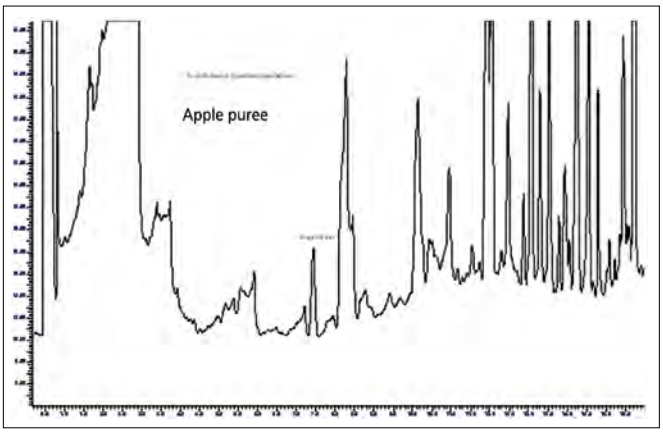

Figure 7. Chromatogram of apple puree test material.

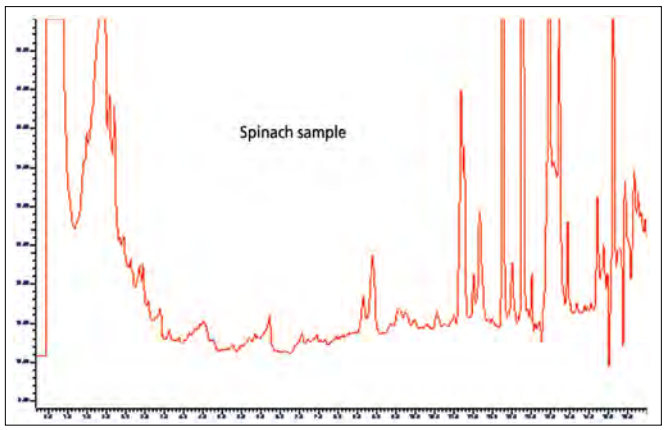

Figure 10. Chromatogram of spinach sample.

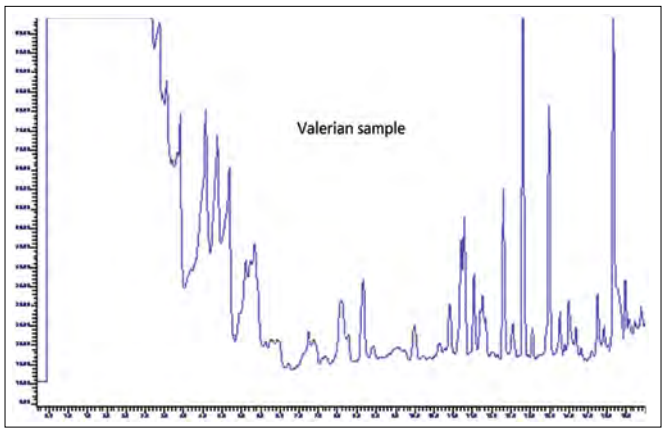

Figure 11. Chromatogram of valerian sample.

Conclusion

This project involved the analysis of over 90 pesticides which are commonly used in treating many species of fruit and vegetables. They were not determined by GC due to incomplete separation and low sensitivity.

The chromatographic analysis obtained with UHPLC enables the pesticides under investigation to be characterized with high precision identification and good sensitivity over the range 40 µg-4,000 kg in less than 16 minutes. In addition the use of the QuEChERS extraction system facilitates very rapid sample preparation. Given that a real sample will be unlikely to contain all 90 pesticides, it is not the primary aim of this method to achieve a baseline separation of every single one. The objective of using this method is to provide a rapid and reliable method of analysis which combines UHPLC with fast sample preparation. Furthermore, identification via a library of UV spectra created under the same UHPLC analytical conditions ensures that the individual compounds will be identified accurately.

Appendix 1

Pesticide	Linear regression coefficient (r^2)
Pirimicarb	0.999920
Clopyralid	0.999705
Imazamox	0.999616
Chloridazon	0.999908
Imidacloprid	0.99988
Cymoxanil	0.999779
Thiram	0.999832
Pyrimethanil	0.999775
Thiacloprid	0.999885
Lenacil	0.999699
Metribuzin	0.995980
Cyprodinil	0.999512
Benfuracarb	0.998758
Carbosulfan	0.999448
Bupirimate	0.999270
Metalaxyl	0.999944
Prochloraz	0.997694
Propaclor	0.999848
Propham	0.999334
Clomazone	0.999958
Buprofezin	0.999455
Dichloran	0.999696
Cyproconazol	0.999116
Dimethomorph	0.998258

Pesticide	Linear regression coefficient (r^2)
Methiocarb	0.999662
Phenmedipham	0.998339
Linurom	0.999913
Fludioxonil	0.999184
Cyazofamid	0.999129
Fenazaquin	0.999951
Azinphos methyl	0.998249
Captan	0.999921
Fenamiphos	0.999767
Iprovalicarb	0.999892
Azoxystrobin	0.999666
Triadimefon	0.999645
Fenhexamid	0.999473
Tebuconazol	0.999608
Boscalid	0.997999
Tetraconazole	0.999703
Sethoxydim	0.999757
Napropamid	0.999802
Chlorpropham	0.999489
Fenamidone	0.999874
Hexaconazole	0.999090
Dithianon	0.995886
Fenbuconazol	0.999163
Diflubenzuron	0.999441

Appendix 1 continued

Pesticide	Linear regression coefficient (r^2)
Iprodion	0.999230
Chlorothalonil	0.999785
Fenoxaprop-p-ethyl	0.999658
Bitertanol	0.998290
Malathion	0.998745
Folpet	0.999858
Azinphos ethyl	0.998583
Etridiazol	0.996639
Aclonifen	0.998505
Dichlofluanid	0.999348
Benalaxyl	0.999051
Difenconazol	0.999642
Chlorthal dimethyl	0.999152
Famoxadone	0.999825
Pyraclostrobin	0.997821
Chlorpyriphos methyl	0.999293
Toclofos methyl	0.998704
Pencycuron	0.999220
Pirimiphos ethyl	0.998005
Endosulfan sulfate	0.998667

Pesticide	Linear regression coefficient (r^2)
Phosalone	0.998413
Teflubenzuron	0.999864
Cycloxidim	0.999844
Indoxacarb	0.991326
Quizalofop-p-ethyl	0.997600
Propaquizafop	0.996202
Pyriproxyfen	0.998287
Pyriproxyfen	0.998287
Oxyfluorfen	0.997626
Fluazifop-p-butyl	0.997439
Flufenoxuron	0.998968
Hexythiazox	0.993939
Fenpyroximate	0.997778
Cyfluthrin	0.999320
Lambda cyhalothrin	0.993716
Deltamethrin	0.999384
Fenvalerate	0.999085
Acrinathrin	0.999826
Fluvalinate	0.996263
Etofenprox	0.996979
Phoxim	0.998382

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APPLICATION BRIEF

Atomic Absorption

Determination of Nickel in Fats and Oils

Scope

Triglyceride-based vegetable fats and oils can be transformed through partial or complete hydrogenation to fats and oils of greater molecular weight. The hydrogenation process involves sparging the oil at high temperature and pressure with hydrogen in the presence of a catalyst, typically a powdered nickel compound. Atomic Absorption Spectrometry is commonly used to estimate the amount of nickel left in the vegetable oils.

Typical Analytical Procedure

Materials and Methods

The following reagents and equipment are used for the measurement:

- Atomic absorption spectrometer
- Nickel metal
- Conc. nitric acid
- Conc. hydrochloric acid
- Double distilled water

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Solution Preparation

Preparation of nickel standard solution: Weigh accurately 1.00 g of nickel metal into a clean beaker. Add 25 mL aqua regia (3 mL concentrated hydrochloric acid: 1 mL concentrated nitric acid) and heat until it dissolves. Transfer the solution into a one-liter volumetric flask and dilute to volume with distilled water. This is the 1000 mg/L nickel standard solution.

Preparation of the working standards: Transfer 10 mL of 1000 mg/L standard solution into a 100-mL volumetric flask and dilute to volume with distilled water. This is the 100 mg/L standard solution. Prepare 1.0, 2.0, 3.0 and 4.0 mg/L of the nickel standard solutions by transferring 1, 2, 3 and 4 mL of this 100 mg/L nickel standard solution in different 100-mL volumetric flasks and dilute to volume with distilled water.

Preparation of the stock sample solution: Weigh accurately 1.9094 g of fat in a clean beaker. Add 10 ml of 1:1 hydrochloric acid. The contents are heated to boiling, then add 10 mL of 1% dimethylglyoxime in ethyl alcohol to acidify the fat in hot condition, stirring thoroughly. 1:1 ammonia is added in slight excess and stirred. The final contents are transferred into a separating funnel. The aqueous phase is separated into a 100-mL volumetric flask. The fat is washed with 25 mL of hot distilled water and the aqueous phase is taken into the volumetric flask. This is repeated twice to extract total amount of nickel from the fat sample. Add 5 mL of concentrated hydrochloric acid to the flask and dilute to volume with distilled water.

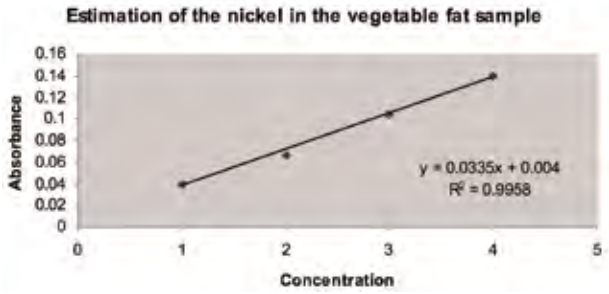
Analysis Procedure

- Element - Ni
- Flame – air-acetylene flame
- Slit width – 0.15 nm
- Wavelength – 232.1 nm
- Detector gain – 575 V
- Lamp current – 7.0 mA
- BGC – off
- Mode – concentration mode
- Type of fit – least squares

Aspirate the nickel standard solutions of 1, 2, 3, 4 mg/L into air-acetylene flame to create the calibration curve and then analyze the samples. The absorbance and concentration readings of the standard and sample solutions are below.

Results

Calibration Curve for the Standards



Std no.	Conc. in mg/L	Absorbance
1	1.0	0.040
2	2.0	0.067
3	3.0	0.105
4	4.0	0.139

Sample Data

Sample no.	Sample name	Absorbance	Conc. in mg/L
1	Fat solution	0.035	1.03
2	Fat solution	0.035	1.03

Calculations

$$\% \text{ of nickel in the sample} = \frac{100 \times [\text{Ni}] \text{ in mg/L}}{[\text{sample}] \text{ in mg/L}}$$

Report: The nickel content in the sample is 0.54%.

References

W.J. Price, J.T.H. Roos and A.F. Clay, Rapid determination of nickel in edible fats by atomic-absorption spectrophotometry, Analyst, 1970, 95, 760-762.

ASTM Method Ref. E-63-86, IS 504, 1989.

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APPLICATION NOTE

Differential Scanning Calorimetry

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Investigating the Destabilization of Solid Emulsions Using Differential Scanning Calorimetry (DSC)

Introduction

Emulsions are systems that consist of two or more liquid phases that are partially or completely immiscible with one liquid being dispersed in the other in the form of droplets. Emulsions constitute an important product class in various industries including the food, chemical and pharmaceutical industries. Emulsions are inherently unstable since the two immiscible liquids have a tendency to phase separate over time in order to minimize the thermodynamically unfavorable interaction between the two or more molecular species. As such, the kinetic stability of emulsions that is their ability to resist phase separation due to destabilization processes such as flocculation, coalescence, or Ostwald ripening is of key importance to manufacturers.

There are a number of methods available to determine the stability of emulsions, with each method having certain advantages and disadvantages. Light scattering is a very common method to determine the stability of emulsions, because it can be used to assess changes in the droplet size distribution of the emulsion. Unfortunately, light scattering does not yield information about differences in composition of individual particles in the emulsions, and therefore, it cannot give specific information on emulsion

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stability if the sample contains chemically diverse structures aside from the emulsion droplets such as, polymer aggregates. Furthermore, light scattering does not yield information about whether the breakdown of an emulsion is due to flocculation (i.e. the aggregation of several droplets to form large flocs) or coalescence (i.e. the merging of several droplets into a larger one). Optical microscopy can in some cases be used to distinguish between flocculation and coalescence, but it is limited to the observation of larger particles (> 500 nm). Moreover, under the microscope and in the absence of specialized staining techniques, air bubbles may appear similar to coalesced oil droplets; and thus, give the researcher false information on the stability of the sample. In contrast, differential scanning calorimetry (DSC) can be used to detect the presence of coalesced emulsion particles, because of a shift in crystallization temperatures. Moreover, DSC is sensitive to the chemical nature of the material and thus the stability of emulsion droplets may be assessed in samples that contain other colloidal particles. Finally, the presence of air droplets will not affect the measurement unless it causes thermal conductivity to be significantly reduced.

In order to understand why DSC is able to assess emulsion stability, it is necessary to understand why alterations in crystallization temperature of emulsions occur when the size of emulsion droplets changes, a phenomena that is related to the degree of supercooling required to induce solidification. Supercooling describes the phenomena of cooling a material below its crystallization point without it becoming solid (Sato and Garti 1988). Supercooling occurs since substantial energy expenditure or activation energy is required to form the initial solid crystal seeds in the liquid. If the magnitude of the activation energy, required for crystal formation, is sufficiently high compared to the thermal energy of the system, crystallization will not occur, even though the transition is thermodynamically favorable. The existence of an energy barrier is also due to the fact that very small crystals are thermodynamically unstable and re-dissolve. Crystallization can therefore only occur if crystals have grown large enough to be stable; otherwise the material will remain in a supercooled state (Sato and Garti 1988). In a supercooled state, the material, which in the case of an emulsion is generally a lipid, may form crystals under two conditions. Firstly, if the temperatures are substantially lower than the crystallization temperature of the bulk lipid, the energy barrier may be overcome allowing the lipid to crystallize. Secondly, the lipid may come in contact with solid impurities in the liquid that may serve as a crystallization template; in which case less activation energy is required for crystallization.

All naturally occurring lipids contain some amount of impurities that can function as a crystal template, and only a small amount of impurities are needed to initiate crystal formation in large volumes of samples. For example, in 1 ml of lipid, numerous impurities can be found that are able to act as nuclei. However, if that 1 ml of lipid is emulsified into 1,000,000 small droplets then only a handful of droplets will contain impurities, while the other droplets are void of them (Figure 1). Thus, if a lipid is emulsified into smaller and smaller emulsion droplets, then less and less volume of the lipid will be in contact with nuclei that are able to lower the required activation energy. As a result, smaller droplets will increasingly crystallize at lower temperatures because majority of the lipid droplets will not contain any nuclei. Conversely, if the oil droplets coalesce and form larger droplets, then increasing volume of lipid will be in contact with nuclei and will; therefore, crystallize at higher temperatures. In other words, an emulsion containing droplets with small droplet sizes crystallize at low temperatures while emulsions containing highly coalesced droplets crystallize at higher temperature (McClements 1999; Helgason, Awad et al. 2008).

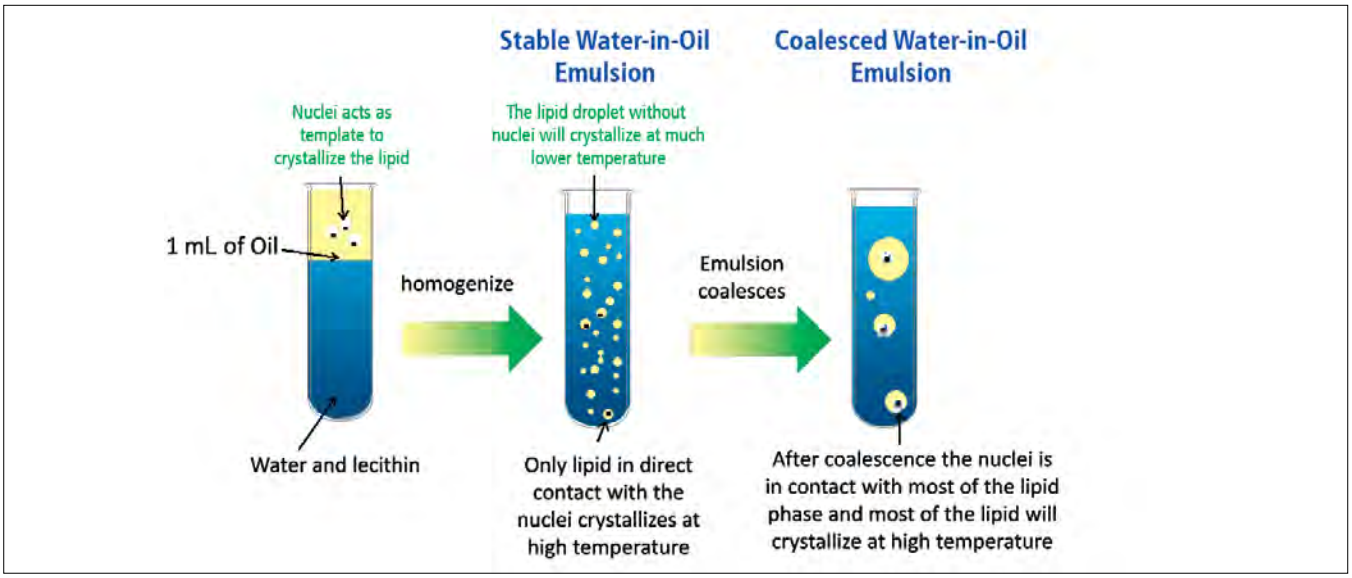

Figure 1. Schematic drawing of the nucleation mechanism in oil-in-water emulsions, and the impact of coalescence.

The temperature at which the crystallization occurs can thus be used to follow the coalescence of emulsified lipid. Most lipids will crystallize above -50 °C. Therefore, a DSC with a two-stage cooling unit is well suited to accurately follow this process. In this application note, we present an example of thermal analytical measurements that may be used by manufacturers and researchers to assess stability of emulsions, particularly those that contain solidified droplets that are obtained by cooling a hot emulsion below the crystallization temperature of the lipid. Many manufacturers have encountered stability issues when attempting to manufacture emulsions that contain solid lipid droplets.

Materials and Methods

A lipid phase [10% (w/w) octadecane] was fully melted by heating to 40-45 °C. The lipid phase was then mixed with a 3% lecithin solution [Alcolec® PC 75, obtained as a gift from Lipoid LLC (Newark, NJ, USA)] held at 40-45 °C in 10 mM citrate buffer. An emulsion premix was made using a hand-held high-speed blender at 100% power for 1 min (Homogenizer Standard Unit, Labworld-Online.com, Straufen, Germany). The hot emulsion premix was homogenized with a thermostated Microfluidizer® 3 times at 10,000 psi (Microfluidics, Newton, MA, USA). This process yielded droplets with z-average sizes of 170.9 ±0.8 nm and polydispersity index of 0.13 ±0.02. Finally, the solution was placed in a refrigerator set at 5 °C to initiate crystallization of the lipid.

Acetic acid was used to titrate the samples to pH 2.8, 3.1, 3.7, 4, and 5. Between 8-10 mg of emulsion was placed in aluminum pans and hermetically sealed. Empty pans were used as a reference for the emulsion samples. Each sample was first heated to 40 °C at 10 °C/min to melt the lipid, and then cooled to 1 °C at 10 °C/min in a differential scanning calorimeter (DSC 8000, PerkinElmer, Shelton, CT, USA). All samples were measured 1 day after pH had been adjusted. Nitrogen 20 mL/min was used as purge gas.

Results and Discussion

At high pH, the surfactant lecithin stabilizes the emulsion droplet because of its high electronegative surface charge. When two droplets come into close proximity, the negative charges on the interface of the droplets will cause an electrostatically driven repulsion causing the droplets to remain separated. When the pH is decreased, the lecithin increasingly loses its electronegativity, which leads to a destabilization of the emulsions since the electrostatic repulsion is no longer sufficient to prevent droplets from coming into contact due to Brownian motion. The pH-induced reduction of surface charges was therefore used to demonstrate how a DSC could be used to investigate stability of emulsions, particularly those that undergo a liquid-solid transition.

An emulsion that contains solidified instead of liquid droplets is often prone to destabilization. At a low pH, the solid particles have a tendency to quickly aggregate, since there are firstly insufficient repulsive forces present between the individual droplets allowing the solid lipid droplets to touch each other. Secondly, the transition from liquid to solid is often accompanied by increases in surface area (since imperfect crystals are formed) causing the surfaces to be insufficiently covered by protective surfactant. Thus, the lipid phases of two solid lipid droplets may be in direct contact, causing them to merge together when the emulsion is heated. As a consequence, many solid lipid emulsions coalesce upon melting. In turn, the coalesced droplets upon cooling crystallize at higher temperature than the uncoalesced particles, a fact that is visible in DSC experiments.

The crystallization onset temperature of octadecane in a bulk phase is 28 °C; therefore, if there is any coalescence, the particles will crystallize close to or slightly below 28 °C. Emulsified droplets on the other hand will crystallize at temperatures far below the crystallization temperature of an octadecane bulk phase. This is demonstrated in Figure 1, where the emulsion was first heated, and then upon cooling at 10 °C/min, crystallization temperatures of 11.37 ±0.18 and 11.32 ±0.23 °C at pH 5 and 4 respectively, are observed. The low crystallization temperatures indicate that the oil remained dispersed in small droplets despite the heating/cooling cycle as long as the pH was sufficiently high (> pH 4). Conversely, at pH 3.7 two crystallization peaks, can be observed; the first one at 25.43 ±0.01 °C and the second at 11.86 ±0.08 °C. The presence of two distinct crystallization peaks indicates that some of the emulsion droplets had begun to coalesce when the emulsion was heated. However, not all the droplets crystallized at the higher temperature indicating that some of the droplets did not coalesce and remained stable. At pH 3.1 and 2.8 only one crystallization peak at 25.22 ±0.01 and 25.24 ±0.06 °C, respectively, were visible. This indicates that at pH 3.1 and 2.8, the emulsion had undergone extensive coalescence due to the fact that the droplets' lipid surfaces had been able to come into direct contact with each other causing a merging upon heating.

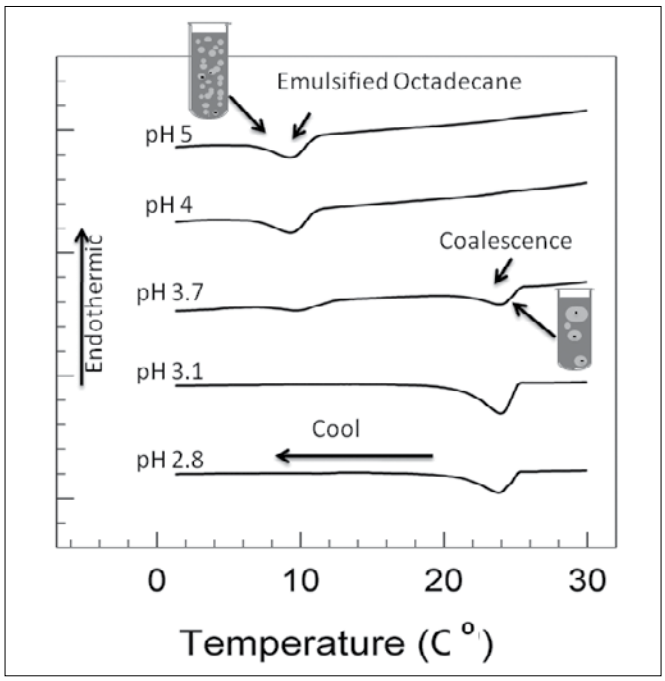

Figure 2. DSC thermographs during cooling of octadecane emulsions, stabilized with lecithin at various pH values.

Conclusion

The brief results shown demonstrate that DSC is an extremely useful method to assess the degree of coalescence in samples, without having to conduct light scattering or microscopic experiments. In combination with microscope or light scattering (dynamic or static) methods, a detailed understanding of ongoing processes responsible for a deterioration of an emulsion's quality can be gained. It should be noted that lipids with relatively sharp crystallization behavior needs to be used, since otherwise the crystallization becomes too complex and it will not be possible to distinguish between coalesced crystallization and crystallization of emulsified lipids. Moreover, high heating and cooling rates can amplify signals obtained from low volume or dilute samples. This method can also be used to investigate emulsions with water as a dispersed phase and oil as the continuous phase (water-in-oil emulsions). In that case, water would crystallize at a different temperature depending on how small the water droplets are. Moreover in multiple emulsions such as water-in-oil-in-water, bulk water and internal phase water could be distinguished.

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APPLICATION BRIEF

ICP - Mass Spectrometry

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The Elemental Analysis of Grains with the NexION 300/350 ICP-MS

Introduction

Trace elemental analysis of grains can provide associations between air pollution sources and soil variables. The elements themselves are distributed unevenly throughout the cereal grain, with the germ and the outer layers having the highest concentrations. Therefore, the elemental analysis requires the ability to measure both trace and high levels.

The elemental capabilities and dynamic range of inductively coupled plasma mass spectrometry (ICP-MS) make it ideally suited for the analysis of food materials. The ultratrace detection limits of ICP-MS permit the determination of low-level contaminants, such as Pb, As, Se, and Hg, while the macro-level nutritional elements, such as Ca, Mg, K, and Na, can be quantified using the extended dynamic range capability of ICP-MS which provides the ability to measure concentrations over nine orders of magnitude. However, there are still a number of challenges to overcome, including complex sample matrices, high levels of dissolved solids, and interferences. With the proper ICP-MS instrumental conditions and design, all of these issues can be overcome, allowing for the successful analysis of food samples, as described elsewhere[1]. This work will focus on the analysis of grains.

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Experimental

Sample Preparation

NIST® 8433 Corn Bran and NIST® 8436 Wheat Flour were used in this work. Approximately 0.5-0.6 g were digested in duplicate with 5 mL of nitric acid (Fisher Scientific™, Optima grade) and 2 mL of hydrogen peroxide (Fisher Scientific™, Optima grade) in pre-cleaned PTFE microwave sample vessels. The digestion program consisted of 30 min of heating and 15 min of cooling, as shown in Table 1. All samples were completely dissolved, resulting in clear solutions that were diluted to a final volume of 50 mL with deionized water. No further sample dilutions were necessary. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Preparation blanks, consisting of the acid mixture, were taken through the same microwave digestion program as the samples.

Table 1. Microwave Digestion Program.

Step	Power (W)	Ramp (min)	Hold (min)
1	500	1	4
2	1000	5	5
3	1400	5	10
4 (cooling)	0	—	15

Instrumental Conditions

All data in this study were generated under normal operating conditions on a PerkinElmer NexION® 300/350X ICP-MS using an autosampler. The instrumental operating conditions are shown in Table 2.

Table 2. ICP-MS Instrumental Operating Conditions for this Application.

Parameter	Value
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Cones	Nickel
Plasma gas flow	18.0 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.98 L/min
Sample uptake rate	300 µL/min
RF power	1600 W
Total integration time	0.5 (1.5 seconds for As, Se, Hg)
Replicates per sample	3
Universal Cell Technology™*	Collision mode

*PerkinElmer, Inc.

Calibration

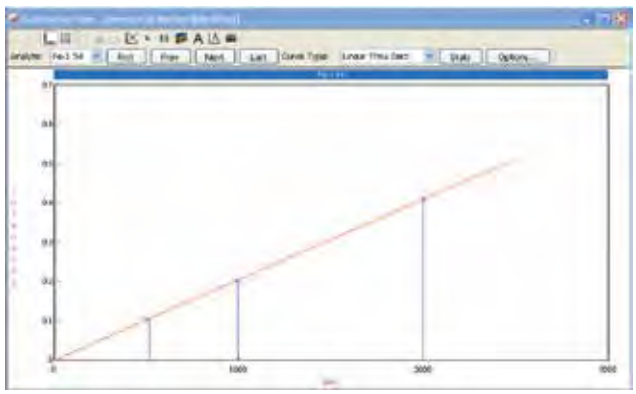
Multielement calibration standards, representing all the analytes in the SRM, were made up from PerkinElmer Pure single and multielement standards and diluted into 10% HNO_3. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Calibration standard ranges were based on whether the analyte was expected to be a high-level nutritional element like potassium (K) or sodium (Na), low/medium-level essential element like manganese (Mn) or iron (Fe), or trace/ultratrace contaminant such as lead (Pb) or mercury (Hg).

Depending on the certified value of the analytes, five different calibration ranges were made up to cover the complete range of elements being determined:

- High-level nutritional analytes: 0-300 ppm
- Medium-level essential analytes: 0-20 ppm
- Low-level essential analytes: 0-2 ppm
- Trace-level contaminants: 0-200 ppb
- Ultratrace-level contaminants: 0-20 ppb

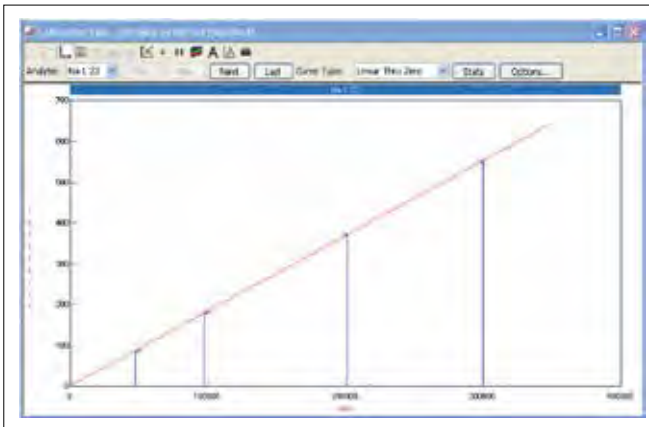
Figures 1 to 5 show representative calibration curves for each range.

In addition to the analyte elements used for the multielement calibration, the standards, blanks, and samples were also spiked on-line using a mixing tee with a solution of ^{6}Li, Sc, Ge, In, and Tb for internal standardization across the full mass range. Acetic acid was added to the internal standard solution to compensate for residual carbon left over from the sample digestion.



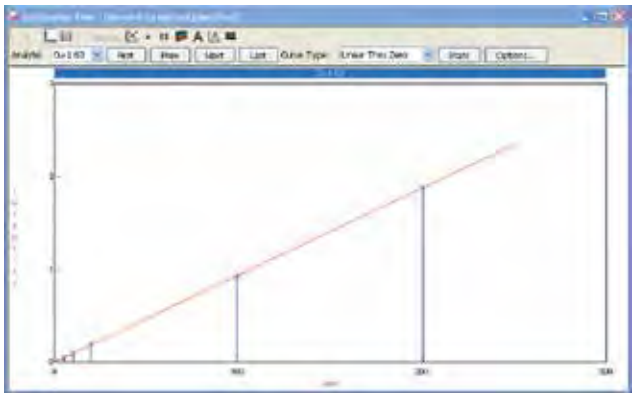
^{54}Fe Correlation Coefficient = 0.99997.

Figure 1. Calibration curves for ^{54}Fe (0-2 ppm).



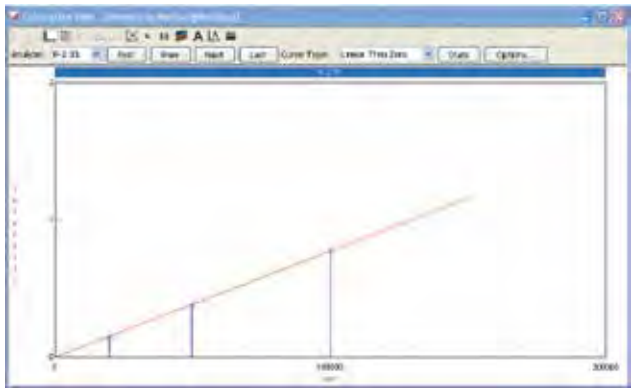
^{23}Na Correlation Coefficient = 0.99996.

Figure 2. Calibration curve for ^{23}Na (0-300 ppm).



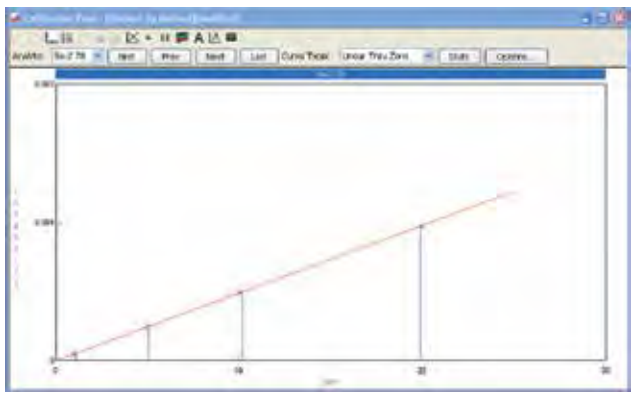
^{63}Cu Correlation Coefficient = 0.99999.

Figure 3. Calibration curve for ^{63}Cu (0-200 ppb).



^{31}P Correlation Coefficient = 0.99999.

Figure 4. Calibration curve for ^{31}P (0-100 ppm).



^{78}Se Correlation Coefficient = 0.99995.

Figure 5. Calibration curve for ^{78}Se (0-20 ppb).

Results

Quantitative results for two sample preparations of the NIST® 8436 Wheat Flour and NIST® 8433 Corn Bran reference materials are shown in Tables 3 and 4. All elements in every sample were determined with Universal Cell operating in Collision mode using helium as the cell gas. Figures in parentheses ( ) in the Reference Value column are not certified values but are included for information purposes only. The data show very good agreement with the certified values, especially for the elements that suffer from known spectral interferences. The elements that are outside the specified limits are mostly the ones that are well recognized as environmental contaminants, which have most likely been impacted by the sample preparation procedure.

Table 3. Analysis of NIST® 8436 Wheat Flour using the NexION 300/350 ICP-MS.

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	–	0.62
Na	23	16.0±6.1	17.0
Mg	26	1070±80	1030
Al	27	11.7±4.7	11.8
P	31	2900±220	2330
S	34	1930±280	1460
K	39	3180±140	2950
Ca	44	278±26	262
V	51	0.021±0.006	0.026
Cr	52	0.023±0.009	0.053
Fe	54	41.5 ±4.0	41.4
Mn	55	16.0±1.0	15.1
Co	59	0.008±0.004	0.007
Ni	60	0.17±0.08	0.17
Cu	63	4.30±0.69	4.18
Zn	66	22.2±1.7	20.6
As	75	(0.03)	0.01
Se	78	1.23±0.09	1.22
Sr	88	1.19±0.09	1.19
Mo	98	0.70±0.12	0.72
Cd	111	0.11±0.05	0.11
Sn	118	–	0.032
Sb	121	–	0.002
Ba	137	2.11±0.47	2.04
Hg	202	0.0004±0.0002	<0.0007
Pb	208	0.023±0.006	0.35
Tl	205	–	<0.0001
Th	232	–	0.001
U	238	–	0.001

Table 4. Analysis of NIST® 8433 Corn Bran using the NexION 300/350 ICP-MS.

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	2.8±1.2	3.2
Na	23	430±31	399
Mg	26	818±59	787
Al	27	1.01±0.55	1.15
P	31	171±11	158
S	34	860±150	738
K	39	566±75	548
Ca	44	420±38	434
V	51	0.005±0.002	0.005
Cr	52	(0.11)	0.08
Fe	54	14.8±1.8	13.7
Mn	55	2.55±0.29	2.53
Co	59	(0.006)	0.005
Ni	60	0.158±0.054	0.143
Cu	63	2.47±0.40	2.54
Zn	66	18.6±2.2	17.0
As	75	(0.002)	<0.006
Se	78	0.045±0.008	0.056
Sr	88	4.62±0.56	4.56
Mo	98	0.252±0.039	0.255
Cd	111	0.012±0.005	0.013
Sn	118	–	0.015
Sb	121	(0.004)	0.003
Ba	137	2.40±0.52	2.26
Hg	202	0.003±0.001	0.005
Pb	208	0.140±0.034	0.122
Tl	205	–	<0.0001
Th	232	–	<0.00008
U	238	–	<0.00002

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 300/350X ICP-MS to effectively measure macro-level nutritional elements in the same analysis run as lower-level elements, without having to dilute the samples. The agreement between experimental and certified results for NIST® 8436 Wheat Flour and NIST® 8433 Corn Bran demonstrates the accuracy of the analysis. Instrument design characteristics eliminate deposition on the ion optics, leading to long-term stability in high-matrix samples, while permitting trace levels to be accurately measured.

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APPLICATION NOTE

ICP - Mass Spectrometry

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Characterization of Silver Nanoparticles in Dietary Supplements by Single Particle ICP-Mass Spectrometry

Introduction

A nanopartcile is defined as a small object, between 1 and 100 nanometers in size, that behaves as a whole unit with respect to its transport and properties. Because of their small size and large surface area, nanoparticles can exhibit different chemical and physical properties from the bulk material. Nanoparticles have found their way into a large number of consumer products. As of 2013, it is estimated that there are over 1300 different consumer products which feature nanoparticles. Silver nanoparticles (AgNPs) are the most frequently found element in all varieties of consumer products (>23%)[1]. Manufacturers of consumer products use AgNPs due primarily to their known antimicrobial properties. Because of their very small size, AgNPs have high surface areas yielding high reaction rates, increasing the efficacy of silver as an antimicrobial.

The use of colloidal and nano silver is directly marketed to the public in such forms as odorless clothing, mildew-resistant shower curtains, food containers and food cutting boards and are even being promoted for direct human consumption as dietary supplements to fortify one's immune system.

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Even as silver nanoparticles have become a valuable weapon in microbial warfare, there are increasing concerns over AgNPs release into the environment and their impact on plant and aquatic organisms. Other studies have shown that AgNPs can kill liver and brain cells in laboratory rats. On the nano scale, AgNPs can easily penetrate into organs and cells[2].

To better understand the fate of AgNPs in our environment, several analytical techniques are usually needed to measure the following key characteristics: particle concentration, composition, shape, size, size distribution, as well as dissolution and agglomeration tracking. Inductively coupled plasma mass spectrometry (ICP-MS) is a very sensitive, element-specific technique for studying elements at environmentally relevant concentrations. Recently, the capabilities of this technique have been extended to single particle analysis mode (SP-ICP-MS)[3, 4], a unique operating mode that allows the differentiation between ionic and particulate forms of prescribed elemental composition entering the plasma source. The resulting data allows for the determination of particle concentration, size, size distribution and dissolved concentration at very low levels, making SP-ICP-MS the conventional technique assessing the fate of engineered nanoparticles in diverse environmental matrices.

Experimental

Instrumental Conditions

Silver nanoparticles, which are suspended in aqueous samples, are introduced into the plasma in exactly the same manner as dissolved solutions with a standard nebulizer and spray chamber combination. However, the data acquisition parameters are very different. Each small nanoparticle entering the plasma is ionized into a very short burst of ions. To accurately measure that burst of ions, the quadrupole and measurement electronics of the instrument must be able to acquire data in the double digit µsec range.

Figure 1 shows a real-time signal that is produced from a single nanoparticle's cloud of ions. In this study, the instrument is continuously acquiring data using a 50 µsec dwell time with no settling time between readings; this eliminates the chances of missing a particle or partially integrating a particle signal, improving particle counting and sizing information. The PerkinElmer NexION® 350D ICP-MS in single particle mode routinely generates up to six million data points per minute of analysis time (operated at 10 µsec dwell time).

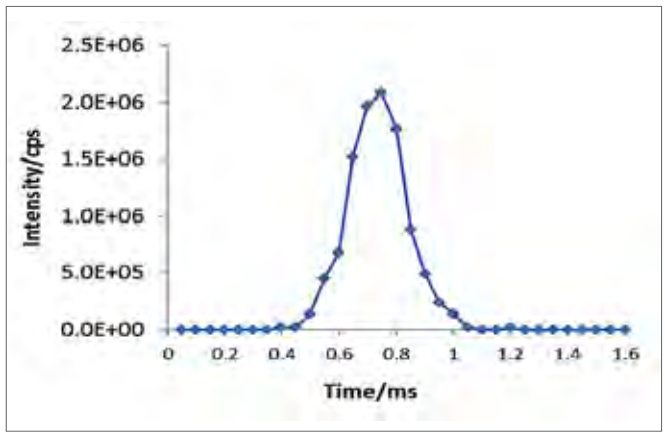

Figure 1. Signal of a single silver nanoparticle captured by SP-ICP-MS. Data acquired at 50 µsec dwell time and zero quadrupole settling time.

SP-ICP-MS data processing is based on the ability to count every particle going through the plasma source, and the ability to distinguish between particles and dissolved metal. When analyzing dissolved elements with an ICP-MS, the resulting signal is essentially a steady-state signal (Figure 2a). When analyzing a dilute suspension of nanoparticles, however, the signal is markedly different, showing ion bursts or plumes from individual nanoparticles (Figure 2b).

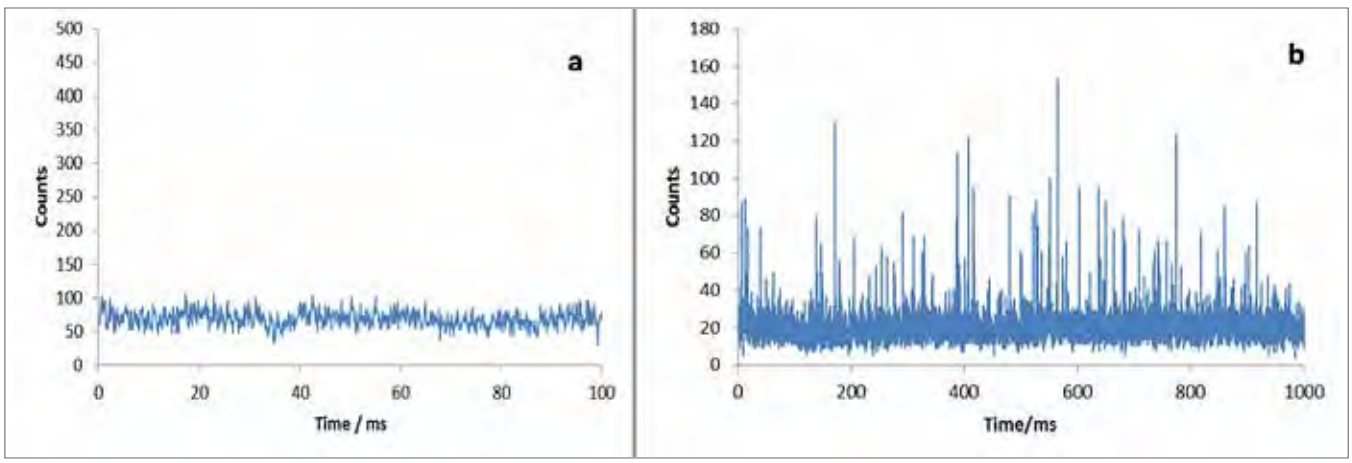

Figure 2. (a) Continous signal from dissolved ionic silver; (b) raw data of sample containing AgNPs.

The Syngistix™ Nano Application Module (PerkinElmer, Shelton, CT), an extension of Syngistix for ICP-MS software, calculates the metal solution concentration (µg/L), the concentration of particles (particles/mL), the size and size distribution of the particles, shows data acquisition in real time, and a size distribution histogram of the sample (Figure 3).

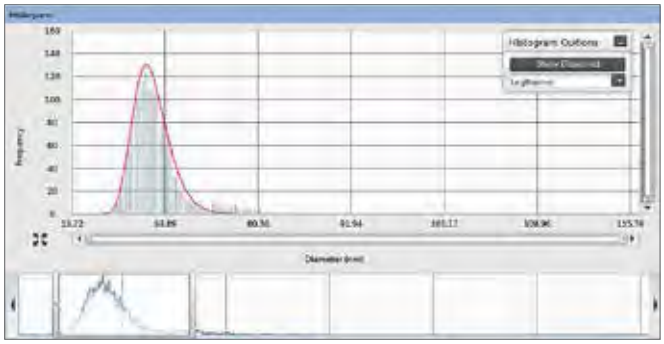

Figure 3. The Results panel of the Syngistix Nano Application Module for SP-ICP-MS, showing size distribution histogram and adjustable integration window at the bottom.

The amount of analyte actually entering the plasma is a fraction of the amount aspirated. This ratio, known as the transport efficiency, is an integral part of particle concentration calculation[5]. AgNP suspensions of known size and concentration are available from several commercial sources. For size and transport efficiency calculations, this work used silver nanoparticle suspensions of 20 nm, 50 nm and 80 nm nominal sizes in citrate buffer from Ted Pella, Inc. (Redding, CA). For the ionic or dissolved silver determinations, a calibration curve from 0.5 to 5 µg/L was constructed from PE Pure (PerkinElmer, Shelton, CT) primary silver standard.

All SP-ICP-MS measurements were made with a NexION 350D ICP-MS (PerkinElmer, Shelton, CT) operating in Standard (no gas) mode and with the Syngistix Nano Application Module. The Syngistix Nano Application Module leads the user through all the components of SP-ICP-MS data collection in an intelligent workflow manner. All measurements were made per the instrumental conditions shown in Table 1.

Table 1. Instrumental Operating Parameters and Conditions

Parameter	Condition
Instrument	NexION 350D ICP-MS in Standard Mode
Nebulizer	ESI PFA Concentric
Spray Chamber	Baffled Cyclonic, Glass
Injector	2.0 mm id Quartz
Power	1600 W
Aux Flow	1.1 L/min
Neb Gas Flow	1.05 L/min
Sample Uptake Rate	0.4 mL/min
Silver Isotope	107 AMU
Dwell Time	50 µsec
Quad Settling Time	Zero
Sampling Time	120 sec

Sample Preparation

Reference silver nanoparticles and the three commercially available dietary supplement samples were placed in an ultrasonic bath for more than five minutes to help ensure that the particles were evenly distributed in the solutions and to minimize agglomeration. The samples were serially diluted with laboratory Type I deionized (DI) water into polyethylene 50 mL sample tubes. The samples and reference solutions were diluted such that the particle concentration was approximately 200,000 particles/mL. This dilution allows measurements to be taken on individual particles and minimizes particle coincidence.

Figure 4. Three commercially available dietary supplements containing silver nanoparticles used in this work.

Results

SP-ICP-MS produces different kinds of information about an unknown sample. While acquiring the data, the ICP-MS signal produced can be viewed in real time. This provides instant information on the sample being analyzed and helps indicate if furthur dilution is necessary to avoid particle coincidence. After the data is collected, it can be reviewed in a number of ways. Graphically, size data are shown as a size distribution histogram. An adjustable integration window with slider bars allows one to choose a slice of the size distribution data for statistical evaluation. Data is also tabulated in a list of scrolling results which shows information on each sample such as: most frequent size, mean size, particle concentration, dissolved concentration and other information about the particle counts. This tabulated data is instantly updated to reflect the slice of data selected with the adjustable window and the type of histogram data fitting used (Gaussian, LogNormal, or Maximum).

Figures 5 and 6 show the size distribution histograms for AgNPs in Sample #1 and Sample #2. Both are using the LogNormal fitting algorithm, as shown by the solid line.

The Sample #1 data show a clear distribution of particles around 15 nm, and the Sample #2 data show a distribution of particles around 33 nm.

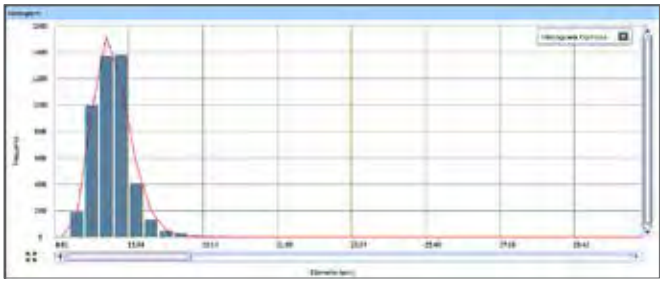

Figure 5. Sample #1 size distribution histogram from Syngistix Nano Application Module.

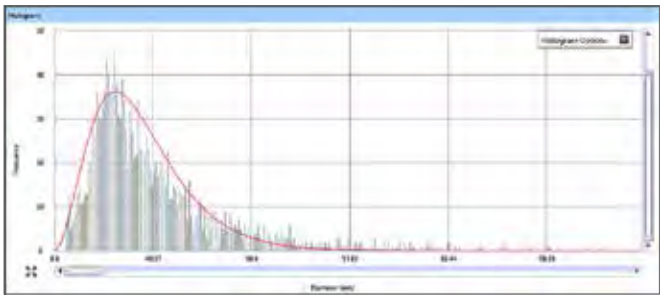

Figure 6. Sample #2 size distribution histogram from Syngistix Nano Aplication Module.

The tabulated data for the samples are given in Table 2. Besides the size distrubution results, the Syngistix Nano Application Module gives the particle concentration and the dissolved concetrations in the samples. The concentration results shown in Table 2 are corrected for the laboratory's dilution. The Ted Pella™ 50 nm AgNP standard was run as control sample check. The most frequent size of 48 nm is in good agreement with the given value of 50 nm, and the measured particle concentration is in good agreement with the value given by the manufacturer of 2.5 E+10 particles/mL.

Table 2. Results for the SP-ICP-MS Analysis of Silver in Dietary Supplement Samples

Sample ID	Mean Size (nm)	Most Frequent Size (nm)	Particle Concentration (particles/mL)	Dissolved Concentration (mg/L)
Sample #1	14	14	3.0 E+10	9.5
Sample #2	39	33	2.1 E+9	21.9
Sample #3	53	59	2.7 E+10	48.1
Ted Pella™ Ag 50 nm	43	48	2.8 E+10	-

Conclusion

Using PerkinElmer's Syngistix Nano Application Module along with the superior ultra-fast data acquisition electronics of the NexION 350 ICP-MS, silver nanoparticles were measured in three commercially available dietary supplements.

Single particle ICP-MS allows for the differentiation and quantitation between the dissolved ionic and particulate fractions of the analyte. In a single analysis, particle composition, concentration, size and size distribution can be directly determined.

The use of the SP-ICP-MS technique has been extended to other elements – and beyond consumer products – into food analyses, biological fluids and to the study of the fate of nanoparticles in the environment.

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APPLICATION NOTE

Liquid Chromatography

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The Qualitative and Quantitative Analysis of Water-Soluble B Vitamins by HPLC-PDA in Various Multivitamin Tablets

Introduction

Vitamins are essential nutritional elements in a human diet. Though we are able to synthesize certain vitamins, such as Vitamin D when exposed to sunlight, we mainly depend on our diet to supply our vitamins.

Since Ancient Egypt, humans have recognized that eating certain foods guarded us from certain diseases. But only in 1912 did Polish biochemist Kazimierz Funk establish the term *"Vitamine"*, derived from the compound words *Vital* and *Amine,* together meaning *amine of life.* Today, the term *"Vitamin"* is described as an organic compound serving as a vital nutrient that an organism requires in limited amounts.[1]

The food industry routinely fortifies many foods with vitamins to enhance their nutritional value and to help with deficiencies in dietary requirements. However, to meet legal requirements, the manufacturers must label their products in accordance to the specific regulations pertaining to the country in which the products are sold and consumed.

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In 2009, the United States Pharmacopeial Convention introduced the USP Dietary Supplements Compendium (DSC) – an industry-directed resource, including regulatory guidance and reference tools. In the U.S., the Food and Drug Administration (FDA) regulates all dietary supplement products and manufacturing practices under 21 CFR (Code of Federal Regulation), Part 111.[2,3] In Europe, the main EU legislation for vitamins and minerals in food supplements is Directive 2002/46/EC.[4]

Considering the above, this application focused on providing a robust chromatographic method for the separation of eight water-soluble B vitamins in vitamin supplement tablets using HPLC with photodiode array (PDA) detection. Due to the diverse absorptivity range amongst these vitamins, multiple analytical wavelengths were used to provide the best overall response. Method conditions and performance data, including linearity and repeatability, are presented.

Experimental

Hardware/Software

For chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the Altus A-10 solvent/sample module, integrated vacuum degasser, A-10 column module and Altus A-10 PDA detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 chromatography data software (CDS) platform.

Method Parameters

The HPLC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee™ SPP C18 2.7 µm, 3.0 x 100 mm (Part# N9308410)
Mobile Phase:	Mobile Phase A: 5 mM ammonium formate (NH_4 formate) buffer; pH 4.8 – 4.9 Mobile Phase B: Acetonitrile (ACN) Solvent program:

	Time	Flow	%A	%B	%C	%D	Curve
1		0.60	100.00	0.0	0.0	0.0	
2	2.00	0.60	100.00	0.0	0.0	0.0	6
3	8.00	0.60	70.00	30.0	0.0	0.0	6
4	10.00	0.60	70.00	30.0	0.0	0.0	6
5	10.01	0.60	100.00	0.0	0.0	0.0	6

Analysis Time:	10 min.; 6-minute injection delay time between injections
Flow Rate:	0.6 mL/min. (max. pressure: ~2300 psi/~153 bar)
Oven Temp.:	40 °C
Detection:	Altus A-10 PDA; Wavelengths used for calibration/quantitation: 214 nm: for B3, B3', B6, B7, B9, and B12 267 nm: for vitamins B1 and B2
Injection Volume:	3 µL
Sampling (Data) Rate:	10 pts./sec

Solvents, Standards and Samples

All solvents and diluents used were HPLC-grade and filtered via 0.45-µm filters.

Vitamin B standards were obtained from Sigma-Aldrich Inc® (St Louis, MO). These included: thiamine HCl (B1), riboflavin (B2), niacin (nicotinic acid; B3), niacinamide (B3; labeled "B3'" to distinguish it from niacin), pyridoxine (B6), biotin (B7), folic acid (B9) and cyanocobalamin (B12). The structures of these vitamins are shown in Figure 1.

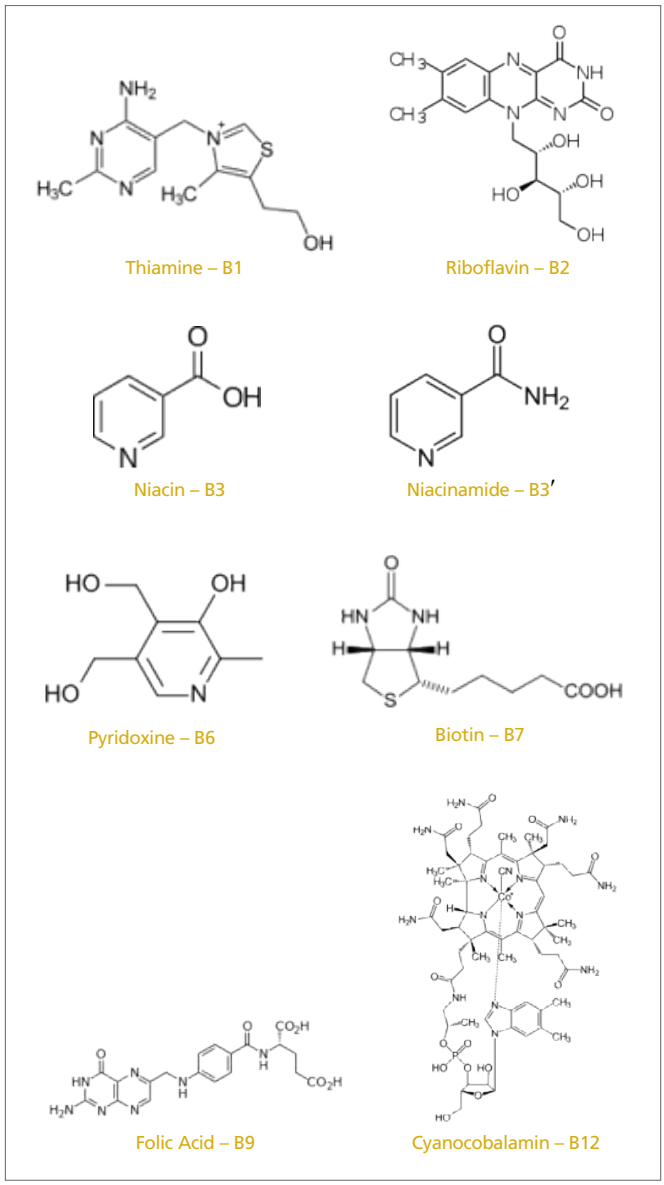

Figure 1. Chemical structures of B vitamins.

As the concentration of individual B vitamins in dietary supplements varies widely from one vitamin to another, two sets of standards were prepared. The first, working standard 1 (WS1), was used for demonstrating chromatographic separation and retention time (RT) reproducibility. It was prepared by weighing out 80 - 320 µg/ml of each of the eight vitamins, depending on the vitamin's relative absorptivity. The second, working standard 2 (WS2), was used for calibration/linearity. It was prepared by weighing individually selected amounts for each vitamin, depending on its expected concentration range in typical dietary supplements.

For both WS1 and WS2, the eight vitamins were weighed out and transferred into individual 50-mL volumetric flasks, to which 30 mL of water was added. Additionally, for vitamins B9, B2 and B7, to improve solubility, 0.5 mL of 0.1N NaOH was added to the corresponding flasks. All flasks were sonicated for five minutes and then filled to the mark with water. Equal volumes of the eight respective stock standards were then added together to produce each of the two WS solutions. For WS1, the resulting concentration for the eight vitamins was between 10 - 40 µg/ml. For WS2, the vitamins ranged in concentration from 0.9 to 525 µg/mL, depending on the vitamin. For calibration, all subsequent concentration levels were prepared from WS2.

All individual stock solutions and working standards were stored at 4.0 °C when not in use.

The multivitamin tablet samples, Multivitamin X and Multivitamin Y, were purchased from a local store. For each sample, a multivitamin tablet was transferred into an individual 50-mL volumetric flask and 30 mL of water was then added. The flask was then stoppered and placed on a heater/stirrer and heated at 40 °C while stirring for 15 min. Once the tablet was completely dissolved, each solution was sonicated for 10 min and then filled to the mark with HPLC-grade water. To remove any small particles, all standards and samples were filtered through 0.45 µm filters prior to injection.

Results and Discussion

Using the optimized chromatographic conditions described above, Figure 2 shows the HPLC separation of the eight B vitamins in WS1, at both 214 and 267 nm. All eight B vitamins are well resolved. The unknown peak at about 1.5 minutes did not interfere with the separation of the B vitamins and was not further characterized.

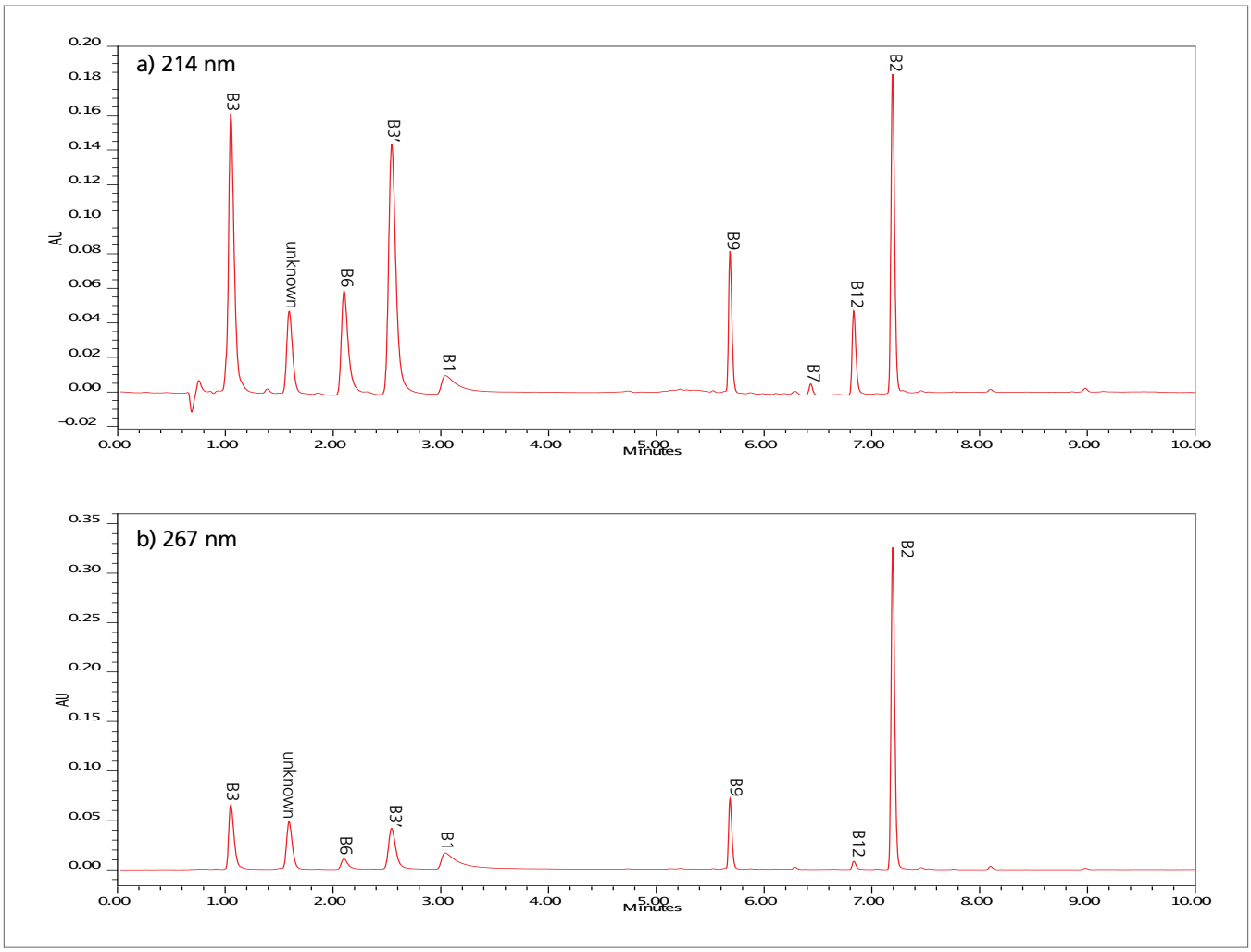

Figure 2. Chromatogram of the vitamin B working standard 1 (WS1); wavelength: a) 214 nm and b) 267 nm.

Figure 3 shows the overlay of 20 replicate WS1 injections, demonstrating exceptional reproducibility. The retention time % RSDs for all eight vitamins were < 0.3% (0.142% for riboflavin).

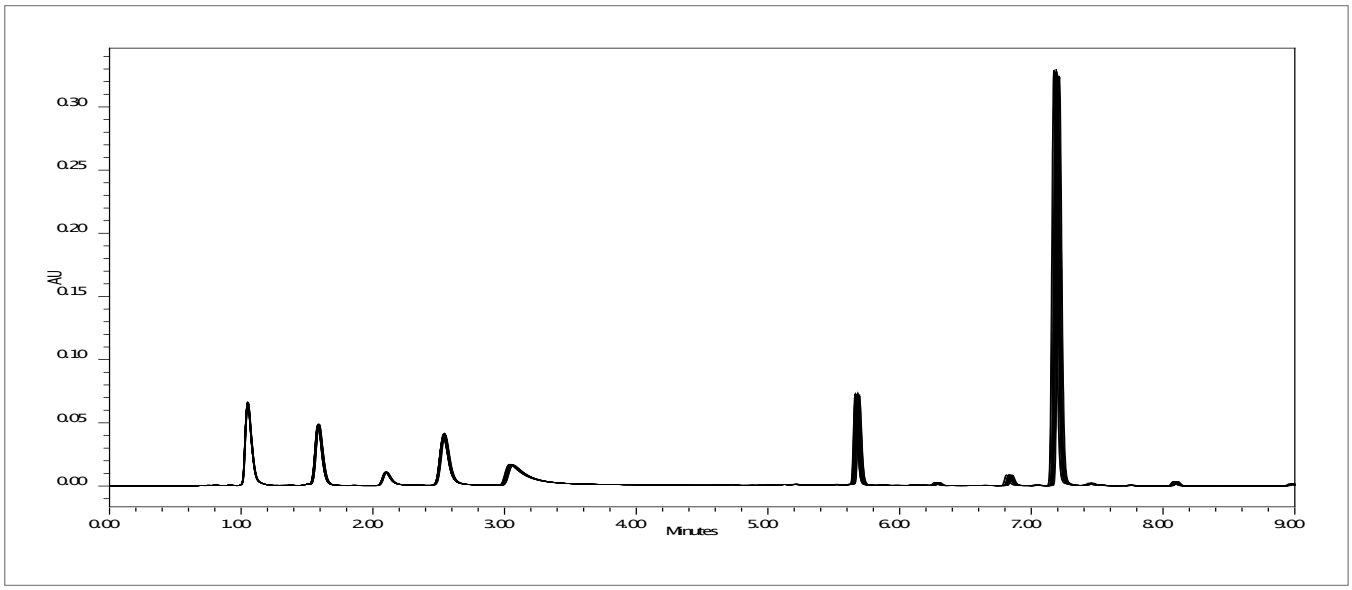

Figure 3. Overlay of 20 replicates of the WS1 vitamin B standard; wavelength: 267 nm.

Table 2 shows the concentration at each calibration level for each of the eight vitamins. Figure 4 shows the calibration results for four example vitamins. All B vitamins displayed linear fits, with most resulting in R^2 values > 0.999 (n = 3 at each level).

Table 2. Concentration (µg/mL) of B vitamins in WS2, for calibration/linearity.

Vitamins	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
B1	2.64	8.79	17.58	26.38	35.17	43.96	52.75
B2	2.66	8.88	17.75	26.63	35.50	44.38	53.25
B3	2.65	8.83	17.67	26.50	35.33	44.17	53.00
B3′	26.23	87.42	174.83	262.25	349.67	437.08	524.50
B6	3.13	10.42	20.83	31.25	41.67	52.08	62.50
B7	0.54	1.79	3.58	5.38	7.17	8.96	10.75
B9	0.91	3.04	6.08	9.13	12.17	15.21	18.25
B12	0.04	0.15	0.29	0.44	0.58	0.73	0.88

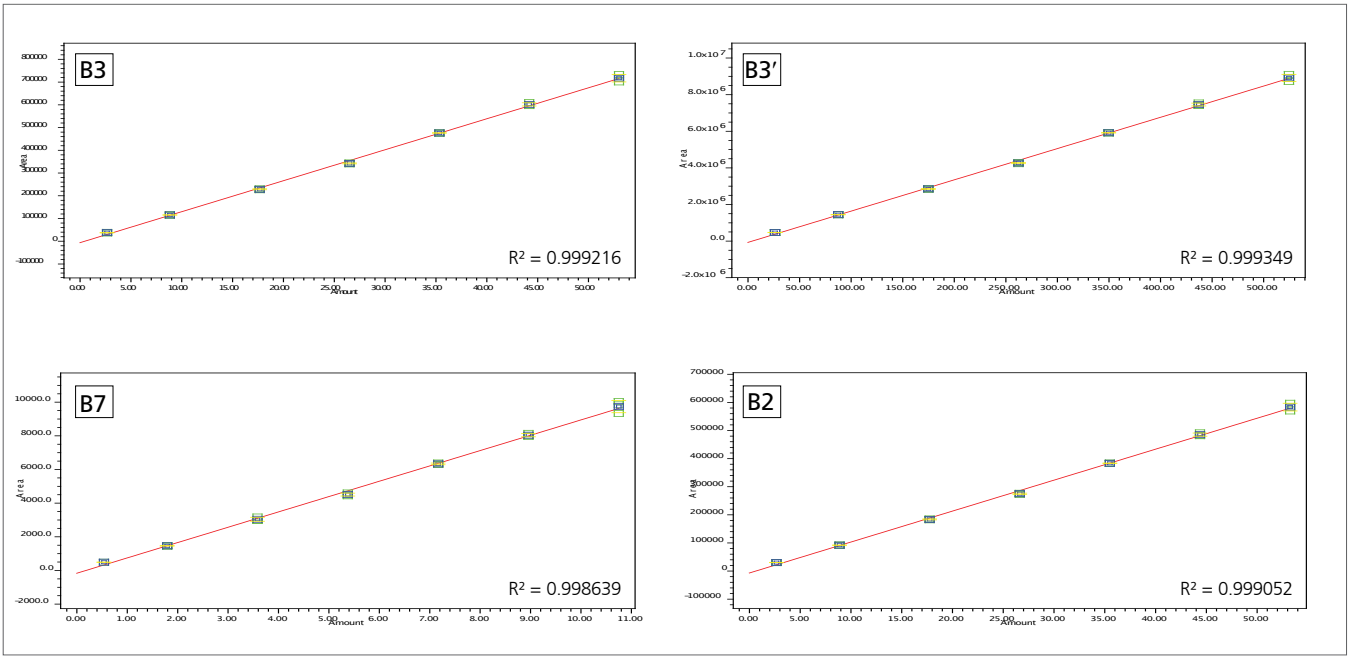

Figure 4. Calibration plots of 7-level calibration set for the vitamins B3, B3′, B7 and B2.

Using the same chromatographic conditions, the two multivitamin tablets, Multivitamin X and Multivitamin Y, were then analyzed. The chromatographic results are shown in Figures 5 and 7-9, displayed at 267 and 214 nm for each of the two samples. Comparing the chromatograms of both of these samples with the WS2 standard mix, it appears that both samples have a similar vitamin B profile. Their presence was further confimed by UV spectral matching, except for vitamins B7 and B9, due to their very low concentration in the samples.

For both samples, the large initial peak eluting very near the void volume was expected to be primarily vitamin C (ascorbic acid). As vitamin C was not the focus of this application, this peak was not further characterized.

Per Figure 5, for Multivitamin X, although a peak eluted at the expected retention time for vitamin B9 (folic acid), the UV spectra for that peak did not match the expected spectra for vitamin B9 (Figure 6). As there was no positive spectral confirmation of vitamin B9 in either sample, the amount of vitamin B9 could not be calculated.

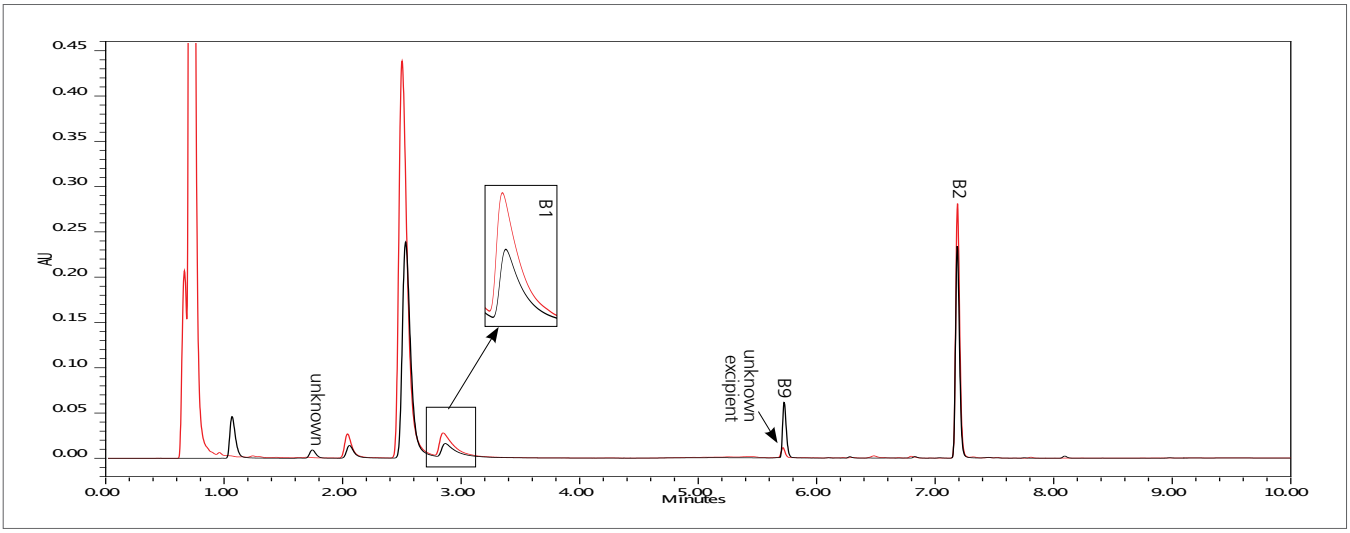

Figure 5. Overlaid chromatograms of Multivitamin X (red) with WS2 (black); wavelength: 267 nm. For optimal sensitivity, only vitamins B1 and B2 were quantitated at 267 nm.

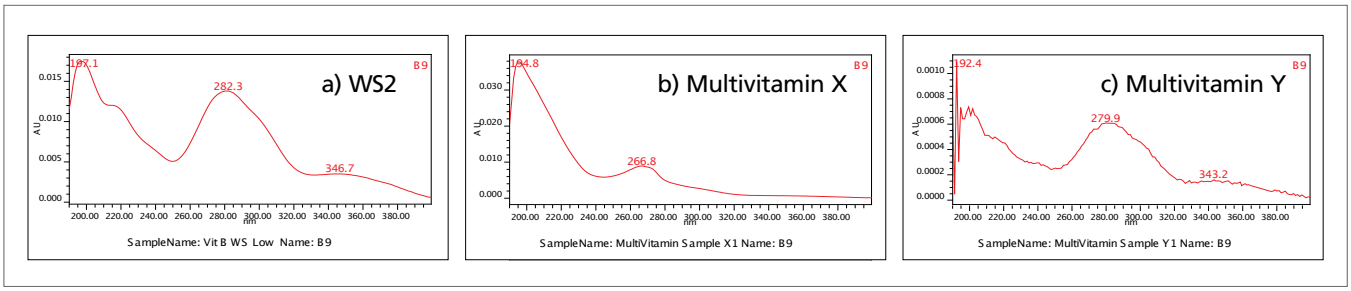

Figure 6. UV spectra captured at the expected elution time for vitamin B9: a) WS2, b) Multivitamin X and c) Multivitamin Y.

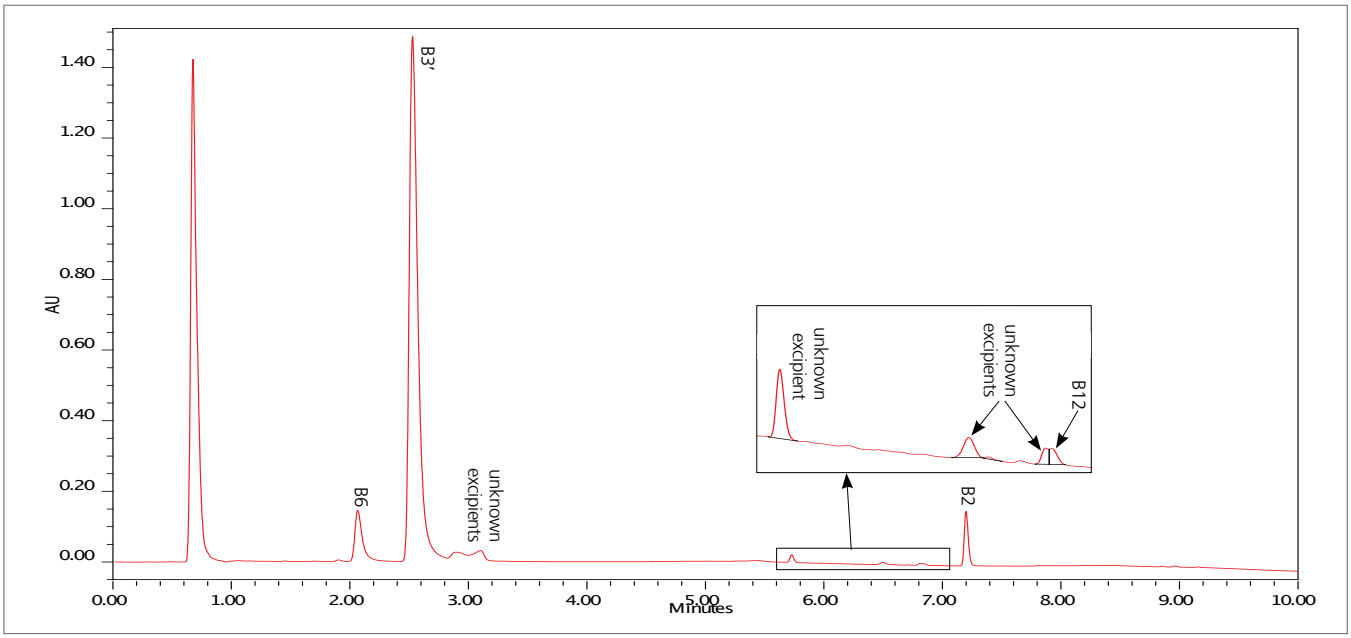

Figure 7. Single chromatogram of Multivitamin X; wavelength: 214 nm. For optimal sensitivity, only vitamins B6, B3' and B12 were quantitated at 214 nm. Vitamins B3 and B7 were not detected..

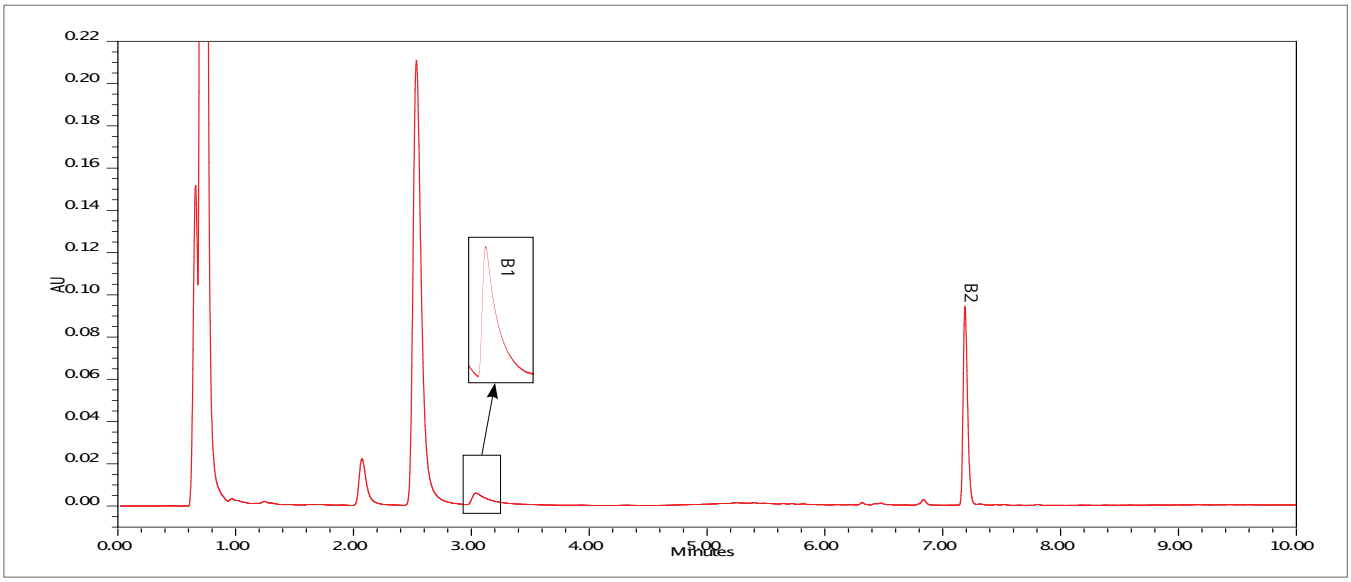

Figure 8. Single chromatogram of Multivitamin Y; wavelength: 267 nm. For optimal sensitivity, only vitamins B1 and B2 were quantitated at 267 nm.

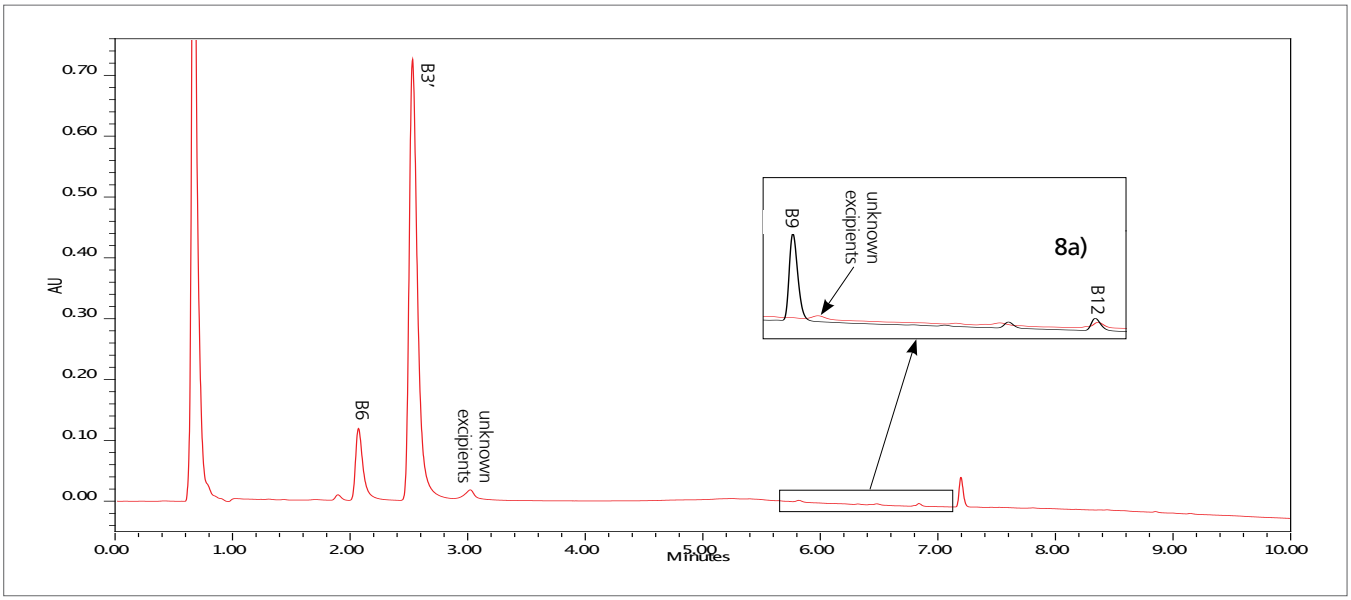

Figure 9. Chromatogram of Multivitamin Y; wavelength: 214 nm. For optimal sensitivity, only vitamins B6, B3' and B12 were quantitated at 214 nm Vitamins B3 and B7 were not detected. The insert (8a) highlights the region in which vitamins B9 and B12 were expected. Multivitamin Y (red), and WS2 (black) were overlaid to show the expected elution times of vitamins B9 and B12. In this region, only vitamin B12 was positively identified and quantitated.

Table 3. Quantitative results for Mutivitamin X and Y.

Multivitamin X:		
Vitamins	**Label claim per serving (µg) (serving size: 1 tablet)**	**Actual results (µg) (based on avg. of 2 indivdual tablets) (3 replicate injections per tablet)**
B1 – Thiamine HCl	1,500	2,237
B2 - Ribolfavin	1,700	1,680
B3 – Niacin (Nicotinic Acid)	0	0
B3' – Niacinamide	20,000	23,625
B6 - Pyridoxine	2,000	2,738
B7 - Biotin	30	ND*
B9 – Folic Acid	400	ND*
B12 - Cyanocobalamin	6	7
Multivitamin Y:		
Vitamins	**Amount per serving (µg) (serving size: 1 tablet)**	**Actual results (µg) (based on avg. of 2 indivdual tablets) (3 replicate injections per tablet)**
B1 – Thiamine HCl	1,500	1,218
B2 - Ribolfavin	1,700	518
B3 – Niacin (Nicotinic Acid)	0	0
B3' – Niacinamide	10,000	10,711
B6 - Pyridoxine	2,000	2,341
B7 - Biotin	30	ND*
B9 – Folic Acid	400	ND*
B12 - Cyanocobalamin	6	11

** Not detected: as vitamins B7 and B9 were not spectrally confirmed, identification was not established and, therefore, amounts could not be calculated.*

Table 3 shows the quantitative results for each multivitamin sample as compared to the label claim. This was based on the average of two analyzed tablets per sample and three replicates per injection. The daily recommended dose for each of these products is one tablet.

For both tablets, the calculated amounts for vitamins B3'and B6 were all close to label claim. Vitamin B3 was shown to be absent in both tablets, also as per label claim. For vitamins B1, though the amount found in Multivitamin Y was closer to label claim, the amount found in Multivitamin X was about 1.5-fold higher than label claim. For vitamin B2, the amount found in Multivitamin X was close to label claim; however, the amount found in Multivitamin Y was about 3-fold lower than label claim. For vitamins B12, though the amount found in Multivitamin X was close to label claim, the amount found in Multivitamin Y was about 2-fold higher than label claim.

The descrepencies from the label claim noted above were certainly of interest but could not be explained. As the sample preparation of these two tablet samples involved merely solubilizing in water followed by filtering through a 0.45 µm filter, there is of course the distinct possibility that some of the vitamin B content in these tablets was indeed other than claimed.

Regarding vitamin B7 (biotin), due to the combination of the low concentration and low-level excipient interference, the amounts could not be calculated.

Conclusion

The results obtained confirm the applicability of this method for the effective and robust analysis of B vitamins in dietary supplement tablets. All eight vitamins are well separated in under eight minutes.

Except for vitamins B7 and B9, the amount of each vitamin B found in two analyzed multivitamin tablets was easily quantitated and any excipients present did not interfere with the peaks of interest. Other than the noted exceptions, the determined amounts were close to the label claim.

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APPLICATION NOTE

Liquid Chromatography

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Fast Analysis of Fat-Soluble Vitamins Using Flexar FX-10 and Chromera CDS

Introduction

Vitamins are essential nutrients and used in numerous systems throughout the human body. Vitamins are divided into two groups: water-soluble (B-complex and C) and fat-soluble (A, D, E and K). Water-soluble vitamins have limited retention in the body and need regular replacement. Fat-soluble vitamins are stored in the liver and fatty tissues, allowing accumulation and consumption over time.

The determination of vitamin content in foods is important in quality, labeling and marketing. Europe and the United States require food products to be clearly labeled with nutritional information; the vitamin content is required on the label.

Supplements and functional foods are intended to provide health benefits to consumers–these products often contain vitamins. Vitamins, D and E are commonly marketed in supplements because of their role in calcium absorption of bones and antioxidant properties, respectively.

A reliable and fast determination of fat-soluble vitamins in food and supplement products is important in complying with regulations and controlling the quality and safety of products. This application note will present a fast and robust method for the determination of fat-soluble vitamins using UHPLC. An optimized separation of vitamins A acetate, K2, D2, D3, E succinate, E acetate and K1 using the PerkinElmer® Flexar™ UHPLC system is presented. Calibration of the UHPLC system and analysis of the vitamins in a commercial supplement will be presented.

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Experimental

The PerkinElmer Flexar FX-10 UHPLC system was used throughout this application. A 1.5 µm particle, 50 mm length, 2.1 mm id, C18 column provided ample resolution and short run times. This column required an operating pressure of approximately 8000 psi resulting in a mobile phase flow rate of approximately 0.7 mL/min. A Flexar UV/Vis detector was operated at wavelengths specific to each vitamin, see Table 1. The instrument interaction, data analysis, and reporting was completed with the PerkinElmer Chromera® data system. The detailed operating parameters of the UHPLC system are presented in Table 1.

Table 1: Detailed analytical conditions for the Flexar LC.

Instrument	Flexar FX-10	
Autosampler	Flexar UHPLC	
Detector	Flexar UV-Vis	
Column	Grace Vision HL B C18, 100 mm x 1.5 µm, 2.1 mm i.d.	
Column temperature	40°C	
Detector wavelength	**Time (min)**	**Wavelength (nm)**
	0.00	325
	2.40	248
	3.60	265
	4.50	285
	6.20	248
	7.50	325
Injection volume	2 µL Fixed loop	
Flow rate	0.7 mL/min	
Mobile phase	A: Water, pH adjusted to 3.0 w/ o-phosphoric acid:Methanol (1:1)	
	B: Acetonitrile	

Gradient program

Type	**Time**	**Flow (min)**	**% A (mL/min)**	**% B**	**Curve**
Equil	3.0	0.8	20	80	0
Run	3.5	0.8	20	80	1
Run	1.0	0.8	0	100	1
Run	2.0	0.8	0	100	1
Run	1.0	0.8	20	80	1

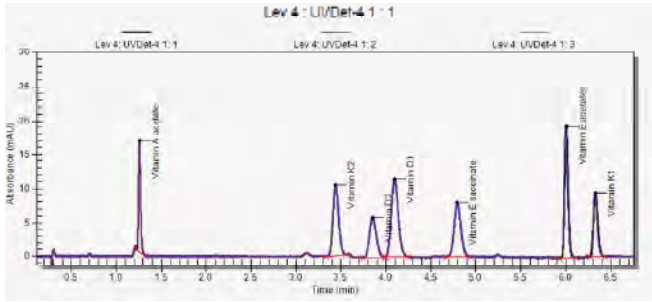

Figure 1: Overlaid chromatogram of three replicates analyses of calibration standard level 4.

Standard preparation (stock): The standards were procured from Chromadex. 10 mg of Vitamin A acetate, Vitamin K2, Vitamin D3, Vitamin E succinate and Vitamin E acetate were dissolved in water and acetonitrile (2:8) to give a solution of 1000 µg/mL (ppm). 5 mg of Vitamin D2, Vitamin K1 were dissolved in water and acetonitrile (2:8) to give a solution of 500 µg/mL each. These stock solutions were further diluted to create the calibration levels presented in Table 2.

Table 2: Detailed dilution scheme for preparation of calibration standards.

Cal level no.	**Concentration in µg/mL**						
	A	K2	D2	D3	E-s	E-a	K1
1.	0.5	2	2	2	20	20	2
2.	1.0	4	4	4	40	40	4
3.	1.5	6	6	6	60	60	6
4.	2.0	8	8	8	80	80	8
5.	2.5	10	10	10	100	100	10
6.	3.0	12	12	12	120	120	12

The UV detector was calibrated with a linear response across the range of 0.5-3.0 µg/mL for vitamin A; 2-12 µg/mL for vitamins K and D; and 20-120 µg/mL for vitamin E, the details are presented in Table 2.

Table 3: Calibration data for the determination of fat-soluble vitamins.

Vitamin	r^2
A	0.9997
K2	0.9989
D2	0.9999
D3	0.9999
E-s	0.9996
E-a	0.9997
K1	0.9994

Sample preparation: The developed method was used to determine the fat-soluble vitamin content of commercially available formulations. The samples were crushed, weighed and sonicated in 10 mL of (2:8) water : acetonitrile. All samples were filtered through 0.2 micron nylon filters. Furthermore, 1 mL of the sample solution was diluted to 5 mL. Finally, 1 mL of the latter solution is then further diluted to 10 mL and injected.

Discussion

Commercially available supplements were analyzed for fat-soluble vitamin content. Using the method developed here, the analytical run was 7.5 minutes with an elution order of Vitamin A acetate, Vitamin K2, Vitamin D2, Vitamin D3, Vitamin E succinate, Vitamin E acetate, and Vitamin K1. All the peaks were well resolved and the resolution between the closest eluting pair is 1.7. The conventional HPLC method, with a 50-minute gradient run, was reduced to a 6.5-minute run with the FX-10. This method has also decreased the solvent used per sample to 5.25 mL. When compared to conventional HPLC, this directly translates into solvent savings of approximately 45 mL per sample, or a reduction of 90% in solvent usage and waste generation.

Conclusions

An improved determination of vitamins using UHPLC is demonstrated in this application. Using a Flexar FX-10 UHPLC system in combination with a small particle (1.5 µm) LC column, five fat-soluble vitamins were rapidly determined in commercial vitamin supplements.

In comparison to conventional HPLC, this UHPLC method offers over 6-times faster chromatography while reducing solvent consumption by 90%. This not only improves productivity, but greatly reduces operating costs and the environmental footprint of the analysis.

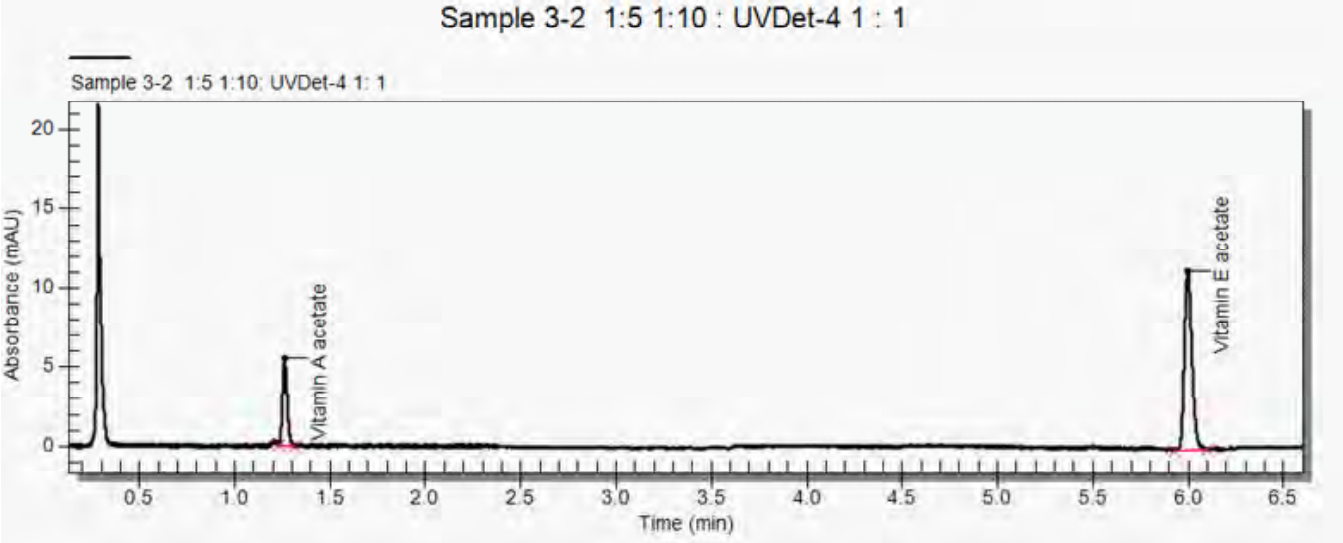

Figure 4: Example chromatogram of the analysis of fat-soluble vitamins in a commercial formulation.

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APPLICATION NOTE

Liquid Chromatography

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Analysis of Capsaicin and Dihydrocapsaicin in Chili Peppers Using the PerkinElmer Altus HPLC System with PDA Detection

Introduction

Capsaicinoids are the compounds that produce the pungency, aroma and flavor of chili peppers. The two most abundant capsaicinoids in chili peppers are capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin. Combined, these two make up close to 90% of the most pungent varieties of capsaicinoids, with capsaisin making up about 71%.[1] The capsaicin content of peppers is one of the parameters that determine their commercial quality. The amount of capsaicin can vary, depending on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit in the plant.[2]

Besides their widespread uses in foods, capsaicinoids are increasingly being used as the active component in pharmaceuticals and have been used as an analgesic against arthritis pain and inflammation.[3] They have also been reported to be active against neurogenic inflammation (as in pepper sprays) and have shown protective effects against high cholesterol levels and obesity.[4, 5]

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Considering the increased use of capsaicinoids in both foods and pharmaceuticals, there is an increasing demand for their accurate quantitation as part of monitoring the quality of chili peppers. In this regard, this application focuses on the extraction, HPLC separation and quantitation of capsaisin and dihydrocapsaicin in two store-bought chili pepper powders. Method conditions and performance data, including linearity and repeatability, are presented.

Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer Altus™ HPLC System was used, including the A-10 solvent/sample module with integrated vacuum degasser, A-10 column module and A-10 PDA detector. All instrument control, analysis and data processing was performed using the Waters® Empower® 3 chromatography data software (CDS) platform.

Method Parameters

The HPLC method parameters are shown in Table 1.

Table 1. HPLC Method Parameters.

HPLC Conditions	
Column:	PerkinElmer Brownlee™ Validated C18 3 µm, 4.6 x 100-mm (Part# N9303552)
Mobile Phase:	Solvent A: Water Solvent B: Methanol Solvent Program:
Analysis Time:	4.0 min.
Flow Rate:	1.0 mL/min. (3000 psi)
Oven Temp.:	25 °C
Detection:	Altus A-10 PDA Excitation: 222 nm
Injection Volume:	10 µL
Sampling (Data) Rate:	10 pts./sec

	Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D
1	Initial	1.0	20.0	80.0	0.0	0.0
2	4.0	1.0	20.0	80.0	0.0	0.0

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters.

The capsaicinoid standards were obtained from Sigma Aldrich, Inc® (Allentown, PA) and consisted of capsaicin and dihydrocapsaicin. A stock 200-ppm standard was made using methanol as diluent. The lower level standards were then prepared from this stock solution.

Two chili powder samples were purchased at a local store. They were labeled *Cayenne1* and *Jalapeño1*. Each chili powder was prepared by adding 2.0 g into 20-mL methanol. The solutions were placed in a water bath at 60 °C for 2 hours.[6] After cooling to room temperature, the samples were then centrifuged at 5000 rpm for five min. The supernatants were then filtered, diluted 1:1 with methanol and injected.

Prior to injection, all calibrants and sample extracts were filtered through 0.45-µm filters to remove small particles.

Results and Discussion

Figure 1 shows the chromatographic separation of the 100-ppm capsaicinoid standard (100-ppm Std) using the optimized conditions described above. The analysis time was under four minutes.

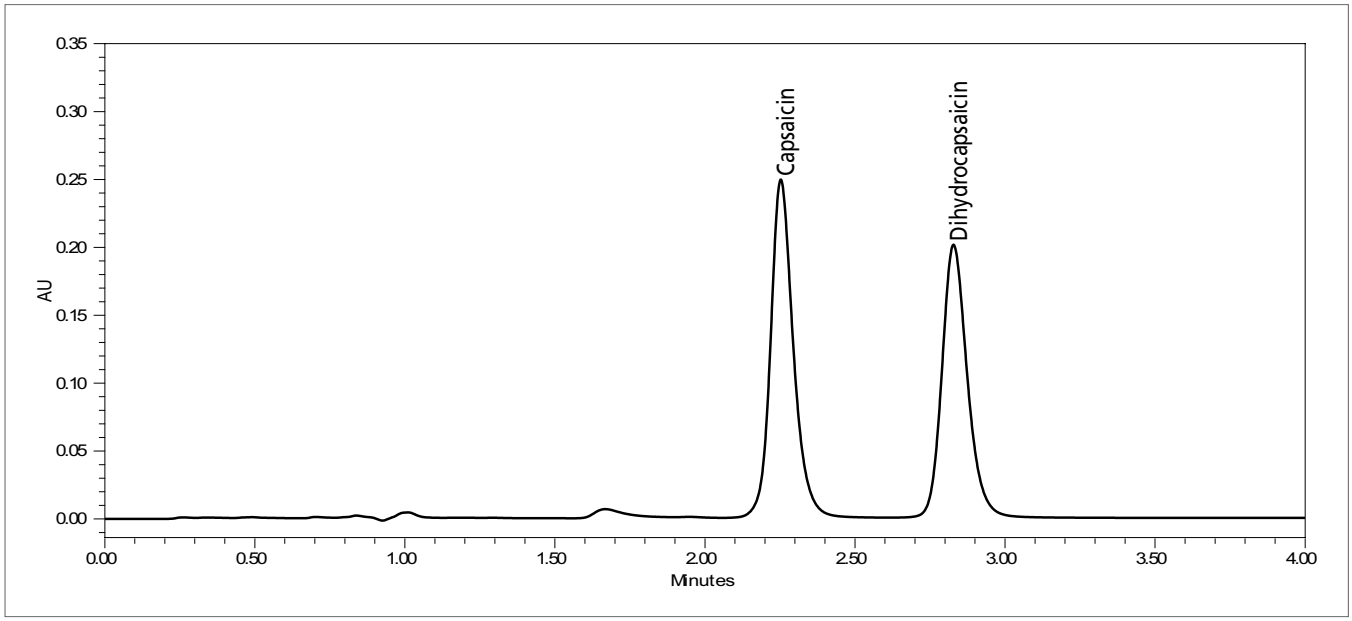

Figure 1. Chromatogram of the 100-ppm capsaicinoid standard (100-ppm Std).

Figure 2 shows the overlay of 10 replicate 100-ppm Std injections, demonstrating exceptional reproducibility. The retention time %RSD for capsaicin was 0.067%.

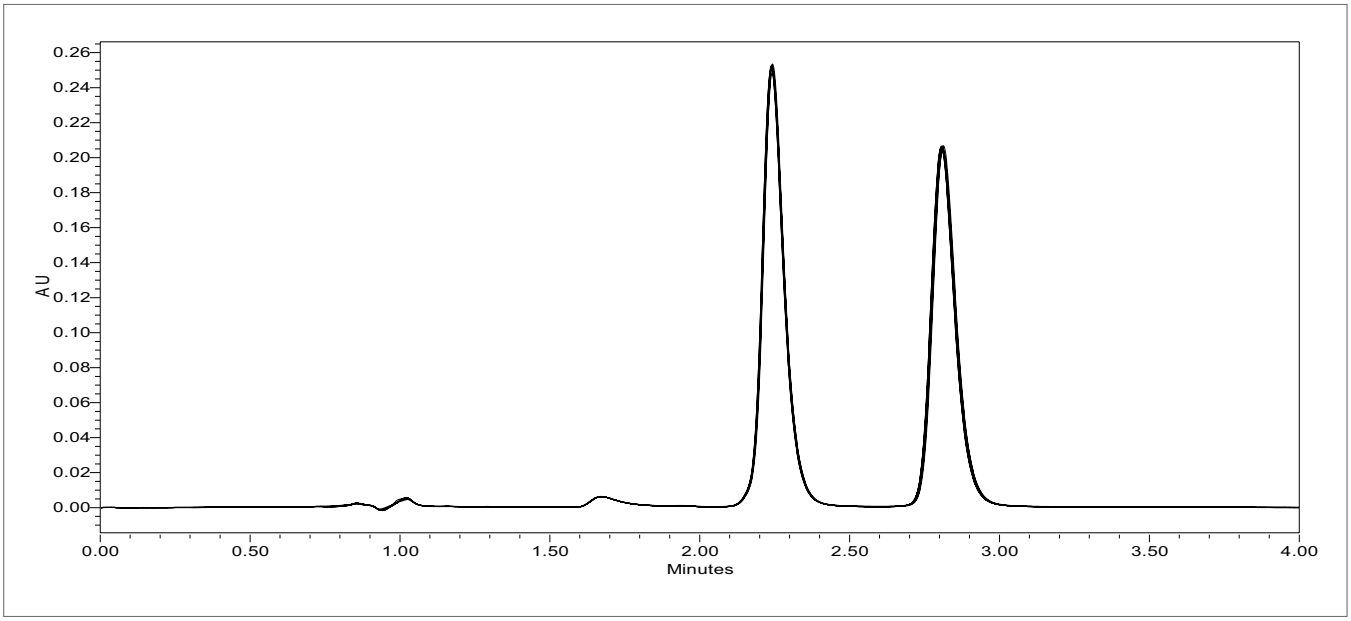

Figure 2. Overlay of 10 replicates of the 100-ppm Std.

Figure 3 and Figure 4 show the calibration results for capsaicin and dihydrocapsaicin over a concentration range of 1 to 100 ppm. Both capsaicinoids followed a linear (1st order) fit and had R^2 coefficients > 0.999 (n = 3 at each level).

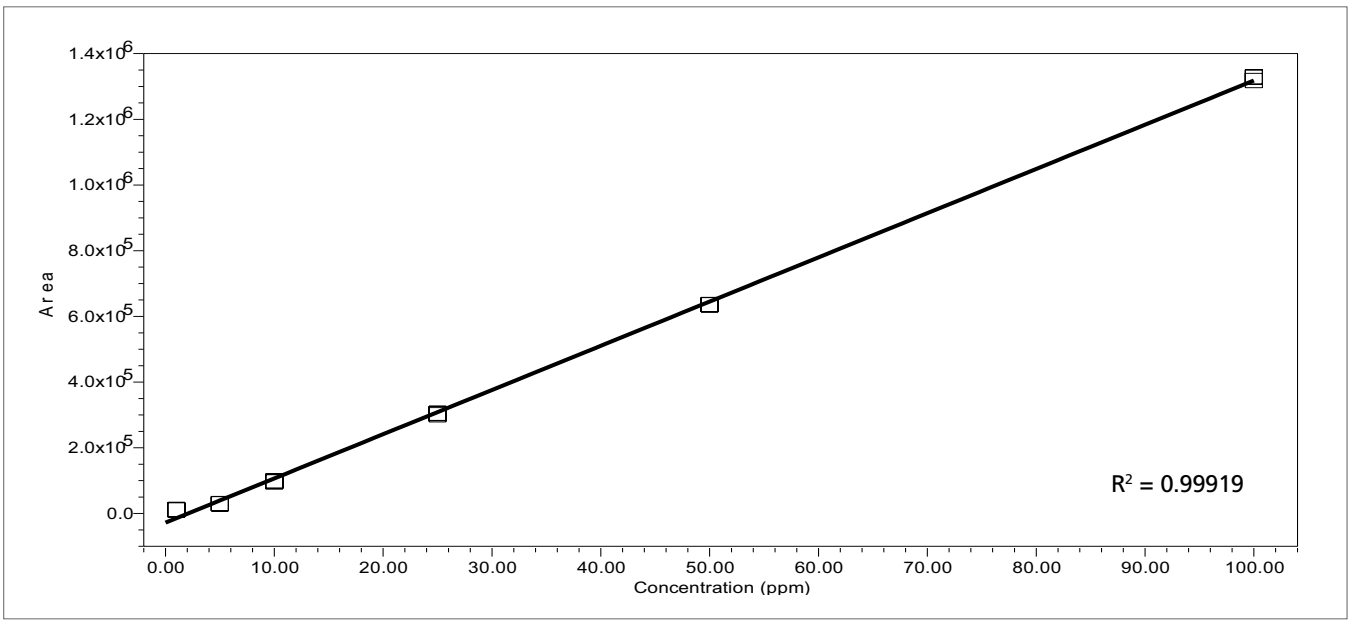

Figure 3. Results of 6-level calibration set for capsaicin.

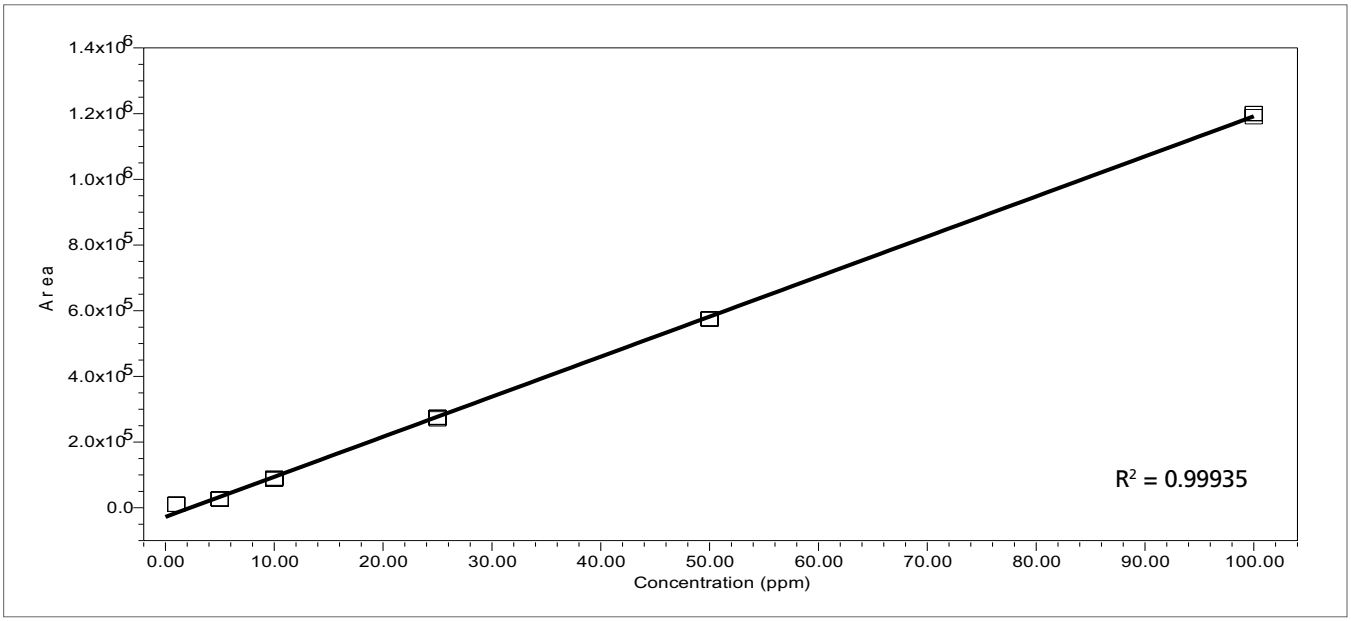

Figure 4. Results of 6-level calibration set for dihydrocapsaicin.

Using the same chromatographic conditions, two chili pepper powder samples were then analyzed: *Cayenne1* and *Jalapeño1*. The chromatographic results are shown in Figure 5. Comparing the chromatograms of these chili pepper samples with the 100-ppm Std, it can be observed that both samples contain capsaicin and dihydrocapsaicin.

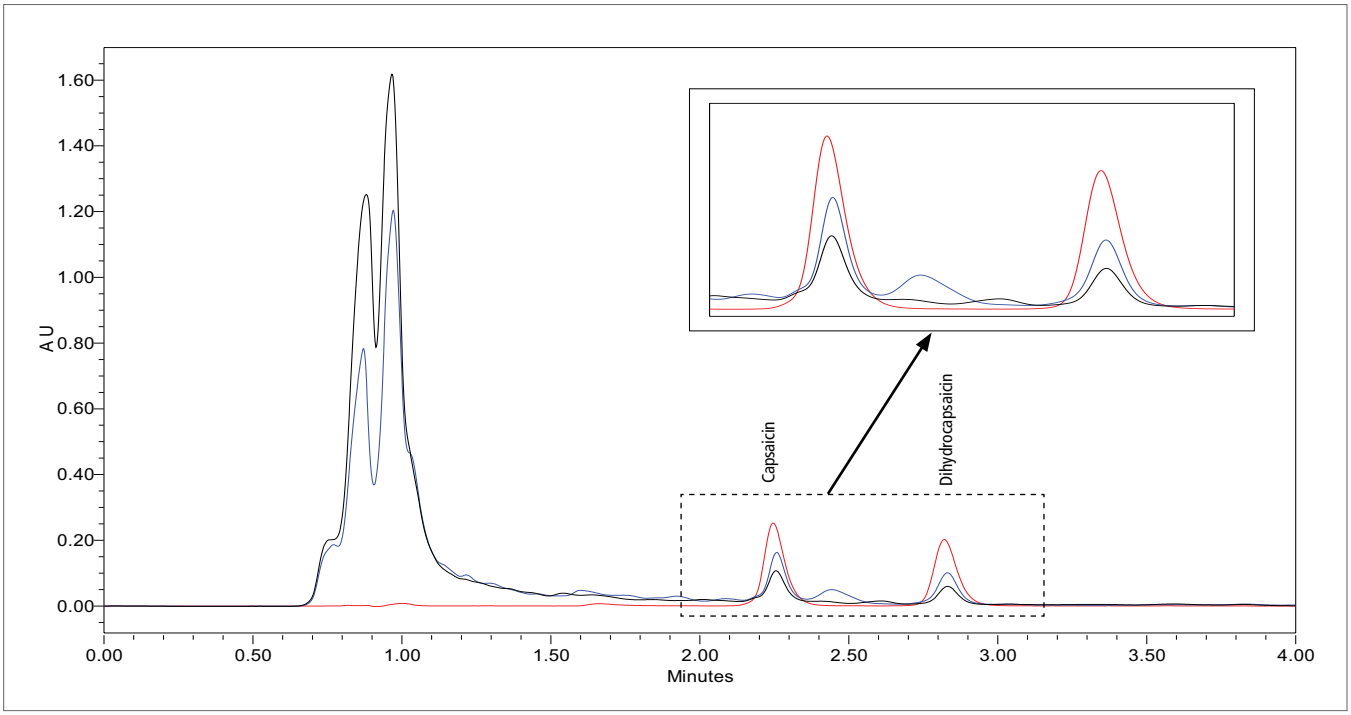

Figure 5. Overlaid chromatograms of Cayenne1 (blue), Jalapeño1 (black), and 100-ppm Std (red).

Based on the standard calibration plots and the 20-fold sample dilution during sample preparation, the quantitative results for each chili powder sample are shown in Table 2. The Cayenne1 pepper powder was shown to contain approximately two times more capsaicin and dihydrocapsacin than the Jalapeño1 pepper powder. The results from this experiment correspond with the Scoville scale, used to measure the pungency of peppers, where cayenne peppers are rated significantly more pungent than jalapeño peppers.[7]

Table 2. Quantitative Results.

Pepper Powder	Capsaicin (µg/g)	Dihydrocapsaicin (µg/g)
Cayenne1	1030	771
Jalapeño1	694	428

Conclusion

This work has demonstrated the effective chromatographic separation and quantitation of two capsaicinoids using a PerkinElmer Altus HPLC System with PDA detection. The results exhibited very good retention time repeatability as well as excellent linearity over the tested 1-100 ppm concentration range. Although a PDA detector was used for possible component characterization/confirmation, a UV detector could also be used for this analysis.

From a food quality perspective, there is an ever growing emphasis on food monitoring. This is especially the case pertaining to capsaicinoids for their use as active components in pharmaceutical products. With this in mind, the results of this work also demonstrated the effective and robust analysis of capsaicinoids in two store-bought chili pepper powders, identifying the particular analytes contained in each of the two samples, as well as comparing their profiles, both chromatographically and quantitatively.

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APPLICATION NOTE

Atomic Absorption

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The Determination of Minerals and Metals in Multi-Mineral/Multi-Vitamin Tablets by Flame Atomic Absorption Spectroscopy

Introduction

There are many mineral dietary supplements available in today's marketplace to ensure that mineral deficiencies do not occur in one's diet. The mineral content of these products must be verified for quality control (QC) purposes. Furthermore, the Nutritional Labeling and Education Act of 1990 mandates accurate labeling of all food supplements sold in the U.S. which means accurate testing of the products is mandatory. In many labs, this task is accomplished by the technique of flame atomic absorption spectroscopy (FAAS). FAAS has the advantages of lower initial cost, low cost per analysis, and requires less operator training than many other trace elemental techniques. The objective of this work is to demonstrate the applicability of FAAS using the PerkinElmer® PinAAcle™ 900T to accomplish this task. Seven elements are determined in two commercially available multi-mineral tablets, a NIST® Standard Reference Material, and a commercial reference material which simulates a mixed food diet.

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Experimental

Instrumentation

The PinAAcle 900T flame and longitudinal Zeeman furnace atomic absorption spectrometer controlled by WinLab32™ for AA software, running under Microsoft® Windows® 7, was used for all analyses (PerkinElmer, Inc., Shelton, CT). A high-sensitivity nebulizer (Part No. N3160112) with a spacer was employed. Single-element Lumina™ hollow cathode lamps (HCLs) were used and the instrumental operating conditions for each element in this application are shown in Table 1. A four-second integration time and three replicates were used for all elements.

Figure 1. PinAAcle 900T atomic absorption spectrometer.

Sample and Standard Preparation

One NIST® SRM 3280 Multi-vitamin/Multi-mineral Tablet and two over the counter multi-vitamin/multi-mineral tablets were coarsely ground. Nominally, 0.75 g were weighed out and rinsed into a 1000 mL volumetric flask containing about 20 mL of ASTM® Type I deionized water (>16MΩ • cm). Four mL of HCl (trace metal grade), two mL of HNO_3 (trace metal grade), and a magnetic stirring bar were then added to the flask. This sample digestion solution was stirred for approximately one hour and warmed, not to boiling, on a hot plate. Deionized water was then added to the volumetric mark after removal of the magnetic stir bar. The sample digestion solution was then filtered through Whatman® 46 paper (passes < 8 micron) to remove waxy undissolved material, presumably from the tablet's coating. The filtrate was then analyzed, after appropriate dilution, against single-element aqueous standards (PerkinElmer Pure) for elemental quantitation. A Certified Reference Material Mixed Food Diet (CRM-MFD) (High Purity Standards, Charleston, SC) was prepared by dissolution, following the manufacturer's instructions, to an appropriate concentration for individual elements.

All tablets also contained about 2 mg/tablet of silicon, much of which may have remained undissolved. For an accurate measurement of the silicon, HF (trace metal grade) and plastic volumetric flasks should be employed for the dissolution.

For calcium and magnesium determinations, $La(NO_3)_2$ (reagent grade) was added to all standards, blank and samples to a concentration level of 0.2% to remove chemical inferences from other matrix elements. For potassium determinations, CsCl (reagent grade) was added to all test solutions to a concentration level of 0.2% as an ionization buffer.

Results

All elements showed acceptable calibration criteria. The results obtained for the FAAS analyses of the multi-vitamin/multi-mineral tablets are shown in Table 2. The values are corrected for laboratory sample dissolution steps and dilutions. The commercial tablets were calculated and reported in mg/tablet as this was the unit shown and guaranteed on each label. All elements were found to be within the certified range for the NIST® 3280 tablet. Both commercial tablets showed good correlation to labeled quantity guaranteed by the manufacturer.

***Table 1.* PinAAcle 900T Instrumental Parameters.**

	Element						
Parameter	**Ca**	**Mg**	**K**	**Fe**	**Zn**	**Cu**	**Mn**
Wavelength (nm)	422.7	285.2	766.5	248.3	213.9	324.8	279.5
Slit Width (nm)	0.7	0.7	0.7	0.2	0.7	0.7	0.2
Oxidant Flow (L/min)	10	10	10	10	10	8.7	10
C_2H_2 Flow (L/min)	2.5	2.5	2.5	2.2	2.5	2.0	2.5
Working Range (mg/L)	5	1	4	4	1	4	2
HCL Part Nos.	N3050114	N3050144	N3050139	N3050126	N3050191	N3050121	N3050145
Standard Part Nos.	N9303763	N9300179	N9303779	N9303771	N9300178	N9300183	N9303783

Table 2. **Corrected FAAS results for one certified reference material and two commercial multi-vitamin/multi-mineral samples.**

	NIST® 3280 (mg/g)			Commercial Tablet 1 (mg/Tab)			Commercial Tablet 2 (mg/Tab)		
Element	**Certified**	**Found**	**SD**	**Label**	**Found**	**SD**	**Label**	**Found**	**SD**
Ca	110.7	107	0.8	162	163	0.6	200	211	0.6
Mg	67.8	69.1	0.2	100	107	0.4	100	109	0.4
K	53.1	53.8	0.3	80	83.8	0.4	80	90.7	0.4
Fe	12.35	12.9	0.05	18	19.1	0.08	n/a	0.23	0.006
Zn	10.15	10.1	0.02	15	15.5	0.04	15	15.2	0.03
Cu	1.4	1.42	0.01	2	2.19	0.01	2	2.04	0.01
Mn	1.44	1.48	0.01	2	1.91	0.01	2	2.04	0.01

The results for the FAAS analysis of the Certified Reference Material – Mixed Food Diet from High Purity Standards – are given in Table 3. All elements were found to be within 5% of the certified value, showing excellent agreement with the reference material.

Table 3. **Mixed Food Diet CRM results using aqueous standards and FAAS on a PinAAcle 900T.**

	CRM-MFD (mg/L)		
Element	**Certified**	**Found**	**SD**
Ca	40	39.8	0.2
Mg	12	11.8	0.03
K	160	159	0.005
Fe	0.8	0.802	0.005
Zn	0.3	0.290	0.001
Cu	0.06	0.061	0.001
Mn	0.2	0.191	0.003

Conclusions

The mineral content of multi-vitamin/multi-mineral tablets must be determined in order to ensure the quality of commercial dietary supplements. This method demonstrates the ability of the PinAAcle 900T flame atomic absorption system to accurately measure minerals in both commercial tablets and food simulation diets. It is an efficacious method that allows for cost effectiveness and ease of use, with less operator training than other analytical methods. The PinAAcle 900H (Flame and Deuterium Furnace) and PinAAcle 900F (Flame only) spectrometers can also be used for this application.

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