HUMAN HEALTH

ENVIRONMENTAL HEALTH

PHARMA AND FOOD EDITION

Fresh

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For the Better

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WHAT'S Fresh inside...

A message from the President, Environment Health ....

Srinivas Addepalli

Dear readers,

Thanks for downloading this FRESH, Winter Edition!

For the first time we are happy to include our customer's columns. We thank to Dr. Saurabh Arora, MD, Arbro Pharma for enlighten on Metal detection in Food and beverages.

We have dedicated this edition to Pharmaceuticals and Food.

With witnessing launch of New Thermo Gravimetric Analyzer Model TGA8000, please also see very new application on pharma and food analysis.

Thanks once again for your support and making us your research partner.

I solicit your feedback on this edition.

Sincerely
Srinivas Addepalli
President, Environment Health
PerkinElmer, India

Customers voice

Featured Article from customer

Is Detection of Metals in Food & Beverages Important?

The Food and Beverage (F&B) Services market in India is expanding at a rapid pace. The compounded annual growth rate is currently 25% and is expected to remain so over the next few years. The overall F&B scenario in India has evolved dramatically over the past decade. While in the past, there were only a handful of brands to choose from; now the consumers have difficulty in choosing from the multitude of brands on offer! The F&B industry has also attracted good investments in recent years. So, with the booming F&B industry, comes the issue of food safety. In order to ensure that the F&B market keeps flourishing, it is important to focus on the quality of food offered to the consumers.

It is of the utmost importance that the food we eat and the beverages we drink are absolutely safe for human consumption. Therefore, carrying out food safety checks is a mandatory requirement for ensuring food safety. F&B need to be tested for a large number of contaminants. Of these, contaminating metals are very important, as these have a deleterious effect on health if the levels are above the specified values. Many metals act as co-factors for enzymes involved in various metabolic pathways. It follows that large quantities of contaminating metals can have an adverse effect on these metabolic pathways, leading to health problems, especially upon continuous, long-term exposure.

The issue of metal contamination came into the headlines, following lead contamination of Maggi instant noodles. Lead can be particularly harmful to young children, especially upon long-term exposure. The test results from government laboratories proved beyond doubt that Maggi was contaminated with lead over and above the permissible safety limits. However, it should be borne in mind that it is not only lead, but also various other metals like copper, arsenic, mercury, tin, cadmium, zinc, chromium and nickel, which can cause contamination of the human food chain.

The various types of metals, in particular, the heavy metals are widely distributed in our environment, and can enter our food chain though various ways. For example, heavy metals in the streams, rivers and lakes can accumulate in fish, which in turn are consumed by humans, leading to heavy metals entering the human food chain. This is only one example out of a myriad. However, regardless of the mode of entry into the food cycle, they disturb the normal functioning of the body metabolism and can accumulate in the body causing severe toxicity.

Safety limits for heavy metals recommended by FSSAI.

To protect the consumers, regulatory bodies across the world have established regulations with stringent limits on the permitted levels of heavy metals in different items of food. **The Food Safety and Standards Authority of India (FSSAI),** the apex regulatory body on food in India, has recommended safety limits for metal contaminants in F&B, which should not be exceeded. For example, there are specified upper limits for lead in beverages such as soft drinks, juices and tea; and food items like ice creams, canned fish & meat, sugar, edible oils etc. Similarly, the limits for the other metal contaminants have also been specified for F&B.

Dr. Saurabh Arora,
Managing Director,
Arbro Pharma Ltd.

In the light of the recent lead contamination controversy, the **FSSAI** has issued a new notification that has fixed, among other parameters, the maximum limits for the metals **Lead, Copper, Arsenic, Mercury, Tin, Cadmium & Zinc** in the food items **Noodles, Pastas & Macaroni** and other such items (Table 1), which had previously been classified by FSSAI under "Foods Not Specified".

Table 1: Upper limits of metal contaminants as per the new FSSAI notification

Metal contaminant	Upper limits
Lead	2.5 mg/kg
Copper	3.0 mg/kg
Arsenic	1.1 mg/kg
Mercury	1.0 mg/kg
Tin	250 mg/kg
Cadmium	1.5 mg/kg
Zinc	50 mg/kg

How are metals tested in food and beverages?

The presence of heavy metal contaminants in F&B makes it important for the food industry to ensure that their products are free from these toxic elements by regularly testing their ingredients and products for compliance with the regulatory requirements.

Testing for metals in foodstuff essentially involves the following four steps:

- **Sampling:** The objective of this step is to obtain a small and representative portion from the large sample in such a way that any subsequent test on the sample will give reproducible results.
- **Destruction of organic matter:** The commonly used methods of destruction of organic matter can be broadly grouped into wet oxidation, dry ashing and microwave digestion.
- **Separation and concentration of the metal:** Once the organic component is destroyed, the element of interest may be concentrated by applying physico-chemical methods.
- **Measurement and determination of the metal:** The concentrated element is then subjected to analytical methods to determine its actual level in the original sample of food.

The FSSAI has recommended a number of methods for testing metal conta in foodstuff, which have been approved and validated internationally by leading agencies like the USFDA and the European Food Safety Authority (EFSA) of the EU. It is important to note that approval by international agencies in various countries means that the methods have been standardized and harmonized as per global standards. Therefore, when the Indian food products are exported to these countries and retested before distribution, they will pass the quality and safety checks easily. Some of the approved methods include Atomic Absorption Spectrometry (AAS) for testing lead, cadmium, copper and zinc. Besides mercury and arsenic, the other four metals can also be estimated by colorimetric methods.

The most advanced method for testing metals, which is considered the "Gold Standard" is a combination of Microwave Digestion for sample preparation, followed by Inductively Coupled Plasma Mass Spectrometry (ICP MS) for sample analysis. ICP MS gives the advantage of analyzing all the metals at the same time with minimum manual intervention. It also offers unparalleled low detection limits, in the parts per trillion levels range, allowing for the use of smaller sample quantities which can be properly digested giving better recovery and reproducibility of results. Both AAS and ICP MS are quantitative methods and are highly sensitive and very accurate. Conclusion.

From the foregoing discussion, it is evident that testing for metal conta is a very important aspect of maintaining food safety. These heavy metals should not exceed the permissible limits in F&B, as recommended by the FSSAI.

The FSSAI is reviewing existing standards set for caffeine and toxic contaminants and residues in various food products. The regulator is also setting-up standards for imported food products, so that only safe food items are made available to the Indian citizens.

The FSSAI is also in the process of finalizing 12,000 standards for food additives and ingredients in line with global safety standards, so that the lengthy process of product approval can be averted. Currently, there are standards prescribed for Approx. 380 food articles as per FSS Act, Rules & Regulations. This will help the food business community to be more proactive to conform to the prescribed new standards, thereby alleviating the need to apply for product approval. These new FSSAI standards are in harmony with the global standards, as these follow the Codex Alimentarius Commission of the United Nation's FAO and WHO. Importantly, there is a need for greater co-ordination of the food industry players with the FSSAI, so that the best quality food reaches the consumers. This will indeed be a "win-win" situation for all stakeholders!

New Technologies

New Thermogravimetric Analyser Model TGA800

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We are very excited to introduce a high performance new TGA 8000™ Thermogravimetric Analyzer. It offers a wide range of features to enhance performance whether you're in research inventing new materials or ensuring the quality of your products, the TGA 8000 delivers great results, all day and every day.

A new TGA 8000 gives you complete control over your sample environment and delivers high throughput and reliability, even unattended. An advanced hyphenation technology works beautifully with FT-IR, MS, GC/MS, and other systems for better understanding of evolved gases. In other words, complex characterization is made simple.

Product Features:

- High sensitivity ultra micro-balance provides unsurpassed accuracy for all applications.
- Balance temp compensation minimizes drift for ultimate performance in precision.
- Wide Temp range -20C to 1200C catering to wide range of applications,
- Fast scanning rates upto 500C/min to meet growing demands and fast turn around time.
- Two integrated mass flow controllers for precise gas atomosphere control
- New Auto sampler for highest Lab through put and simplified sample loading/unloading.
- Colored illumination provides unique identification of run status.
- Power of analysis increased by coupling Hyphenated techniques such as FT-IR, MS, GC/MS.
- Intuitive, user-friendly Pyris Software that provides a wide range of standard and advanced capabilities, delivering maximum flexibility.
- iOS Application for local TGA 8000 control and an iPhone® or iPad for convenient local control and monitoring status of unit.

Pharmaceuticals

How does the sum of toxic elements that might be ingested every day compare to the new USP guidelines?

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Introduction

In 2012, the United States Pharmacopeia (USP) proposed that chapter <231> be replaced with two new chapters which outline ICP-OES and ICP-MS methods to determine a group of elemental impurities that may be found in pharmaceutical products. The new requirements have been summarized in General Chapters <232>, <233> and <2232>1-3, which are currently undergoing final revision with proposed implementation in 2018.

Chapters <232> and <2232> specify the list of elements and their permissible daily exposure (PDE) limits, based on the route of administration. A detailed summary of the method is available 4, so only a brief description will be included here.

Table 1 displays the various routes of exposure to humans and lists examples of each. The maximum PDE for each element varies by the route of administration and is shown in Table 2, based on a 50 kg person. The elements in Table 2 are divided into two categories: those that must be considered for testing in all drug products (Cd, Pb, As, Hg) and those that must be measured only if they have been added/used during the manufacturing process or are present in the raw material.

Many articles and papers have presented data from the analysis of single drug or supplements based on the recommended dosage using the new USP methodology4. However, it is common for people to take several medications and supplements daily, and the total daily exposure from all consumed pharmaceutical products should be considered.

This work looks at the sum of USP <232>/<2232> contaminants found in a hypothetical mix of commonly-consumed oral medications and supplements that a person might use daily.

Table 1. Routes of Pharmaceutical Administration and Examples.

Route of Administration	Examples
Oral	Solids, Liquids
Parenteral	Injections, Implants, Ophthalmic
Topical, Dermal	Creams
Mucosal	Nasal, Urethral
Inhalation	Aerosols, Inhalers, Gases

Experimental

Samples and Sample Preparation

Table 3 shows the oral medications and supplements used for this analysis, which represent a mix of medications that a person may take daily.

All samples were digested using

Table 2. Permissible Daily Exposure (PDE) Limits Defined in USP <232>/<2232>.

Element	Oral Daily Dose PDE (µg/day)	Parenteral Daily Dose PDE (µg/day)	Inhalation Daily Dose (µg/day)
Cadmium (Cd)	5	2	2
Lead (Pb)	5	5	5
Arsenic (As)*	15	15	2
Mercury (Hg)*	30	3	1
Iridium (Ir)	100	10	1
Osmium (Os)	100	10	1
Palladium (Pd)	100	10	1
Platinum (Pt)	100	10	1
Rhodium (Rh)	100	10	1
Ruthenium (Ru)	100	1100	3
Chromium (Cr)	11000	1100	3
Molybdenum (Mo)	3000	1500	10
Nickel (Ni)	200	20	5
Vanadium (V)	100	10	1
Copper (Cu)	3000	300	30

* = inorganic forms

PerkinElmer's Titan MPS™ microwave sample preparation system with standard 75 mL PTFE vessels. The mass of the tablets and pills used in this study ranged from 0.12 – 5.6 g. Approximately 0.25 g of each sample was added to each digestion vessel, along with 5 mL of nitric acid (70%), 1 mL of hydrochloric acid (35%), and 2 mL of hydrogen

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peroxide (30%). (Tablets weighing more than 0.25 g were crushed, and 0.25 g taken for digestion.) The samples were digested following the program in Table 4. When the digestion was complete, all samples were diluted with deionized water to a final volume of 50 mL. To stabilize mercury, 200 ppb Au was added to each sample, standard and blank.

Instrumentation

All analyses were performed on a PerkinElmer NexION® 350 ICP-MS in Collision mode, using the standard sample introduction system and conditions; the elements and masses are shown in Table 5. (Chromium oral limits in <232> were recently implemented and were not defined at the time of testing). Although most of the measured elements do not have polyatomic interferences, Collision mode was used to produce a rapid analysis (80 seconds sample-to-sample) and protect against unexpected interferences.

Table 3. Samples Analyzed.

Oral Medications	High blood pressure
	Thyroid function
	Heart function (baby aspirin)
	Indigestion
Inhalation	Fish oil capsules
	Krill oil capsules
	Calcium + fiber, chewable
	Calcium, gummy
	Multi-vitamin, gummy
	Fiber, gummy
	Plant extract (cholesterol)

Table 4. Titan Digestion Program. Table 4. Titan Digestion Program.1

Step	Target Temp (°C)	Pressure Max (bar)	Ramp Time (min)	Hold Time (min)	Power (%)
1	150	30	5	5	60
2	200	30	5	20	90
3	50	30	1	10	0

Table 5. NexION 350 ICP-MS Measured Isotopes.

Element	Mass
Arsenic (As)	75
Cadmium (cd)	111
Copper (Cu)	63
Iridium (Ir)	193
Lead (Pb)	208
Mercury (Hg)	202
Molybdenum (Mo)	98
Nickel (Ni)	60
Osmium (Os)	192
Palladium (Pd)	105
Platinum (Pt)	194
Rhodium (Rh)	103
Ruthenium (Ru)	101
Vanadium (V)	51

Results and Discussion

The USP methodologies define the maximum daily exposure based on a person's weight, assuming a 50 kg person. The maximum analytical requirement is defined as the "J" value:

$$J = \frac{PDE}{\text{Maximum Daily Dose} \times \text{Dilution Factor}}$$

PDE = Permissible Daily Exposure
Dilution Factor = the amount the sample was diluted for analysis

More detailed explanations of the J value are available elsewhere.[1, 2, 4] For this work, the J value was calculated assuming a maximum daily dose of 10 g and a 200x dilution factor.

Method detection limits (MDLs) were determined based on five standard deviations of the digested blank. Table 6 shows the MDLs for each element, along with the J values in the measured solutions. The J values are based on a maximum PDE calculated assuming a high daily dose of 10 g of medication and supplements and a 200x dilution factor.

Tables 7 and 8 show the results for the individual medications and supplements, respectively. A comparison of the sum of all the medications with the maximum oral daily dose appears in Table 9. This comparison indicates that the total value of contaminants in the eleven analyzed medications and supplements is well below the oral permissible daily exposure limit, with the exception of As and Mo that slightly exceed the PDE. Looking at Tables 7 and 8, As mostly results from the Ca supplements, and Mo is an added component in the multi-vitamin.

Table 6. J Values and MDLs.

Element	J Value (µg/L in Solution)	MDL (µg/L in Solution)
Cd	2.5	0.005
Pb	2.5	0.002
As	7.5	0.038
Hg	15	0.003
Ir	50	0.001
Os	50	0.029
Pd	50	0.002
Pt	50	0.001
Rh	50	0.001
Ru	50	0.001
Mo	1500	0.019
Ni	100	0.006
V	50	0.011
Cu	1500	0.052

Table 7. Contaminants in Oral Medications (all units in µg/day).

Element	Blood Pressure	Thyroid	Baby Aspirin	Indigestion
Cd	0.14	0.01	ND	ND
Pb	0.24	0.07	0.41	0.03
As	1.95	0.32	0.17	0.07
Hg	ND	0.01	ND	ND
Ir	0.04	0.94	0.11	ND
Os	ND	ND	ND	ND
Pd	0.01	ND	ND	ND
Pt	0.01	ND	ND	ND
Rh	ND	0.04	0.01	ND
Ru	0.01	ND	0.01	ND
Mo	13.9	0.07	0.02	0.02
Ni	3.46	0.31	0.71	0.22
V	6.50	0.64	1.34	0.49
Cu	0.05	0.20	0.04	0.03

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Table 8. Contaminants in Oral Medications (all units in µg/day).

Element	Fish Oil Caplets	Krill Oil Caplets	Ca + Fiber Chewables	Ca Gummy	Multi-vitamin Gummy	Fiber Gummy	Cholesterol Homeopathic
	0.14	0.01	ND	ND			
Cd	ND	ND	3.55	0.61	0.10	0.02	ND
Pb	0.06	0.09	0.37	1.28	0.11	0.11	0.08
As	0.29	1.77	4.32	4.85	0.24	0.77	0.71
Hg	0.01	0.01	ND	ND	ND	ND	ND
Ir	0.07	0.04	ND	ND	ND	ND	0.18
Os	ND	ND	ND	ND	ND	ND	ND
Pd	0.02	0.01	0.01	0.09	0.09	0.16	ND
Pt	0.01	ND	ND	ND	ND	ND	ND
Rh	0.01	0.01	ND	ND	ND	ND	0.01
Ru	ND	ND	ND	ND	ND	ND	ND
Mo	0.04	0.01	0.16	6.10	167	0.53	0.71
Ni	0.30	0.36	19.3	3.29	0.95	1.03	2.67
V	0.30	0.36	10.8	13.8	1.00	1.60	0.38
Cu	0.47	0.81	2.24	6.86	0.88	7.59	0.38

Table 9. Sum of Medications and Supplements (all units in µg/day).

Element	Oral Daily Dose	Sum of Supplements and Medications
Cd	5	4.43
Pb	5	.85
As	15	15.5
Hg	30	0.03
Ir	100	1.38
Os	100	ND
Pd	100	0.39
Pt	100	0.02
Rh	100	0.08
Ru	100	0.02
Mo	3000	189
Ni	200	32.6
V	100	37.2
Cu	3000	19.6

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	0.38 mmd id (green/orange), PVC, flared, 2-stop	N0777042
Drain Tubing	1.30 mm id (gray/gray), Santoprene, 2-stop	N0777444
Internal Standard Addition Tee	Tee for on-line addition of internal standard	N0777295
Internal Standards Uptake Tubing	0.25 mm id (red/orange), PVC, flared, 2-stop	N0773111
USP Oral Element Impurities (Big 4)	Cd (25 mg/kg), Hg (15 mg/kg), Pb (5 mg/kg), As (1.5 mg/kg); 125 mL	N9304150
USP Precious Metal Impurities B (with Os)	Ir, Pd, Pt, Rh, Ru, Os (100 mg/kg); 125 mL	N9304151
USP Oral/Parenteral Elemental Impurities C	Cu (1000 mg/kg), Ni (500 mg/kg), Mo, V (100 mg/kg)	N9304153
Pure-Grade Au Standard	1000 mg/L	N9303728 (125 mL)
Autosampler Tubes	Conical, metal-fee, sterile	N0776118 (15 mL) N0776116 (50 mL)

Conclusion

When considering the elements and their maximum daily exposure limits as mandated by USP <232>/<2232>, it is necessary to account for all of the medications and supplements a person consumes daily. A variety of medications and supplements were digested with the Titan microwave digestion system and measured with the NexION 350 ICP-MS operating in Collision mode. The results demonstrate that the sum of all elements from all samples was below the maximum daily exposure level, with the exception of molybdenum (Mo) which was added to the multivitamin. This work has shown that a simple Collision mode-only method is an easy and reliable technique for measuring contaminants in accordance with USP <232>/<2232>.

References

1. General Chapter <232> Elemental Impurities – Limits: April 2015.
2. General Chapter <233> Elemental Impurities – Procedures: Apr. 2015.
3. General Chapter USP <2232> Elemental Contamination in Dietary Supplement: March 2012.
4. "Implementation of USP New Chapters <232> and <233> of Elemental Impurities in Pharmaceutical Products", PerkinElmer Inc., 2013.

Pharmaceuticals

Safety Hazard Screening by PerkinElmer DSC (Differential Scanning Calorimeter)

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Environmental concerns and legislations in Health and Safety place considerable burden on manufacturers in numerous industries to employ more complex processes to study and manage risks associated with explosions. The pharmaceutical industry is under ever increasing pressure to establish and maintain increasingly comprehensive processes to ensure safety. This is particularly relevant in its use of potentially hazardous substances and complex organic synthesis at its large scale operations. Furthermore it requires establishing safe process for synthesis, handling of precursor components, and final product (drug) that may pose unacceptable hazard to patient. Safety Hazard studies are reported in pharma synthetic intermediates and products by Ion Townsend1.

The kinetics of exothermic reactions is important in assessing the potential of materials and systems for thermal explosion. Thermal Analysis Test method provides a means for determining Arrhenius Activation Energies △E and pre-exponential factors using Differential Scanning Calorimeter, DSC. It is one of several test methods being developed by ASTM committee2 for chemical reactions (ASTM 698-11). DSC under careful conditions is used as screening technique to reveal any propensity a material may have towards significant violent exothermic decomposition reactions. Such evaluation method is to be used in conjunction with other tests to characterize the hazard potential of chemicals.

Differential Scanning Calorimetry (DSC) is being widely used in numerous chemistry fields including, but not limited to, Polymer Science, Free Drying Technology, Polymorphism studies, compatibility investigations, Purity determination, etc. Hazard screening represents a vital and important area of DSC usage.

PerkinElmer provides a solution to study such safety Hazard reactions using DSC with High Pressure capsules kit (600 psi internal pressure), and Advanced Kinetics software to predict the behavior of exothermic reactions. PerkinElmer provides both scanning and isothermal kinetics software that uses a multilinear regression equation to fit a single DSC curve at single heating rate into Arrhenius relationship to measure parameters like Activation energy, pre-exponential factor and order of reaction. Various calculation inputs can be adjusted to make the data meaningful. The reaction parameters can be used to predict the behavior of materials under two conditions namely Isothermal and Adiabatic conditions. When a kinetic analysis has been successfully performed, the program generates several outputs as follows:

Plot of isothermal calculations:

- Reaction time vs. temperature for selected degree of reaction
- Percent reacted vs. temperature for selected reaction times
- Percent reacted vs. time for selected reaction temperatures
- Multiple curve plots of the above with up to 12 selected inputs

Plots of adiabatic calculations:

- Reaction time vs. starting temperature for selected percent
- Percent reacted vs. time for selected initial temperatures for runaway reaction
- studies Reaction temperature vs. time for selected initial temperatures
- Multiple curve plots of the above with up to 12 selected inputs

Under real adiabatic conditions all heat given off during the reactions is accumulated in the system with no heat transfer with the surroundings. The accumulated heat leads to, initially very slow, and later very fast increase in the sample temperature which can result in a runaway

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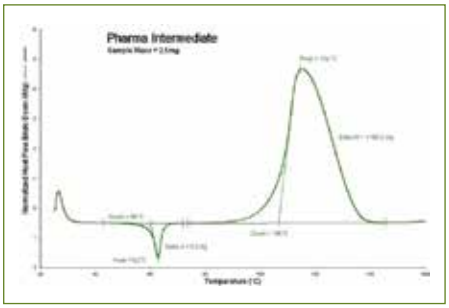
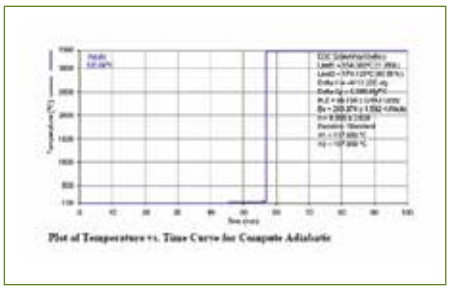
process. The rate of the temperature increase dT/dt is dependent on the heat capacity, heat release and the reaction kinetics. This approach will enable the determination of the runaway reaction time at selected temperature.

The following graph (Fig1) shows melting followed by exothermic decomposition. The low temperature and the huge exotherm which is seen at about 100C - A fast rise to the peak maximum and the low onset of this event shows that this material is quite unstable.

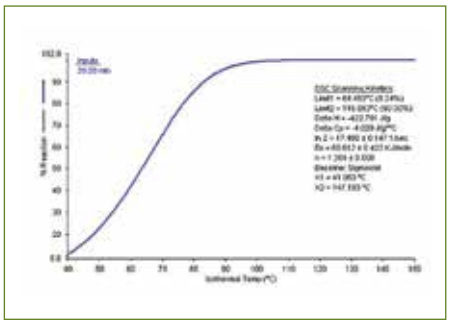
Typical Exothermic Graph of DSC for pharma intermediate Fig 1



Typical Computed Temp and Time graph adiabatically Temp % reacted…Fig 2



Typical Isothermal computed Data Temp vs % reacted… Fig 3



The above two graphs help in deriving important information about violent exothermic reaction studies related with time, temp parameters, and its impact on % reacted (Fig.3). One can generate various plots to predict the reactions progress (% reacted) and derive run away time under adiabatic conditions (Fig 2).

References:

1. Ion Townsend Journal of Thermal Analysis, Vol. 37,(1991),2031-2066.
2. ASTM E 698-11, Standard test Method for Arrhenius constants for Thermally Unstable Materials using DSC and Flynn/Wall/Ozava method.

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Pharmaceuticals

Plastic Containers Analysis as per USP 661

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The purpose of this USP 661 Chapter is to provide standards for plastic materials and components used to package medical articles (pharmaceuticals, biologics, dietary supplements and devices). Various types of plastics used for making containers are Polyethylene (HDPE & LDPE), Polypropylene (PP), Polyethylene Tetrapthalate (PET) and Polyethylene Tetrapthalate G (PETG).

Plastics are composed of a mixture of homologous polymers, having a range of molecular weights. Plastics may contain other substances such as residues from the polymerization process, plasticizers, stabilizers, antioxidants, pigments and lubricants. These ingredients added to the polymers, and those used in the fabrication of the containers, must conform to the requirements in the applicable sections of the Code of Federal Regulations, Title 21, Indirect Food Additives, or have been evaluated by the FDA and determined to be acceptable substances for the listed use.

Factors such as plastic composition, processing and cleaning procedures, surface treatment, contacting media, inks, adhesives, absorption and permeability of preservatives, and conditions of storage may also affect the suitability of plastic for a specific use.

Plastic containers are identified and characterized by IR spectroscopy and Differential Scanning Calorimetry. Standard test for analysis are provided in this chapter (USP 661) for the identification and characterization of the different types of plastics. The standards and tests provided in this chapter characterize containers and components produced from various grades and types of polymers. Stability studies have been performed to establish the expiration date of a particular dosage form in the appropriate plastics containers meeting product specifications, provided that the appropriate stability programs are expanded to include the alternative container, in order to ensure that the identity, strength, quality, and purity of the dosage form are maintained throughout the expiration period.

Typically High-Density Polyethylene (HDPE), Polypropylene (PP) and PET (Polyethylene Terephthalate) containers are being used for Pharmaceutical dosage storage.

Differential Scanning Calorimetry

As described under Thermal Analysis section Test Methods, thermogram of the plastic specimen is compared with that of USP Reference Standard (similarly determined). The limits for difference in temperature of endotherm (T_m) of specimen thermogram and that of USP Reference Standard are also specified in USP 661 and mentioned below.

Type of Plastic	T_m and T_g difference between thermogram of container specimen and USP Reference Standard
HDPE	Not more than 6.0 °C
LDPE	Not more than 8.0 °C
Homopolymer PP	Not more than 6.0 °C
Copolymer PP	Not more than 12 °C
PET	T_m and T_g not more than 9.0 and 4.0 °C respectively
PETG	T_g not more than 6.0

Perkinelmer Differential Scanning Calorimeter Model Dsc 4000

We at PerkinElmer offer high performance differential scanning calorimeter model DSC4000 to meet USP661. The DSC4000 uses well proven heat flux principle. It has very rugged robust sensor with high sensitivity. The system can be heated and cooled in linear fashion to meet regulation and covers the temp range of interest. It has built in mass

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flow controlled based gas controllers (MFC) to have tight control of inert gas.

An integrated Pyris software can perform all the calculation of onset, peak temp, peak area, compare etc., to evaluate the polymer containers. The system is also provided with required validation documents and data security is being ensured using CFR21 part 11 software protocols. All necessary training is imparted to explain the methodology used and its interpretation to provide complete solution to our customers.

From material and contaminant identification to quantitative analysis, the comprehensive Spectrum 10™ software suite allows you to focus on what matters most – Results. Designed for busy industrial or academic laboratories that require efficient operation combined with a wide range of capabilities, this comprehensive FT-IR software package facilitates data collection, processing and results generation.

Spectrum Two

For further information on products and services contact: marketing.india@perkinelmer.com

DSC4000

Infrared Spectroscopy

As described under Infrared Spectroscopy section, Test Methods, the corrected spectrum of specimen is compared with that of USP reference standard. Spectrum of specimen must exhibit absorption bands only at the same wavelengths as the spectrum of or responding USP reference standard, similarly determined.

Perkinelmer Infrared Spectrophotometer, Spectrum Two

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Food & Beverages

Determination of Residual Solvents in Flexible Packaging According to EN 13628-2:2004

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Introduction

The reference standards for food contact materials are rapidly evolving in favor of increasing consumer protection.

The Commission Regulation (EC) No. 1935/2004 is the main reference legislation in the European community. This regulation establishes that any materials that come into contact with food must not release chemicals in quantities which could:

- Pose a danger to the health of consumers
- Result in an unacceptable change in the composition of food
- Change the organoleptic properties

Part 2 of the regulation focuses the attention of food contact material producers on the need to operate in terms of quality assurance. The Commission Regulation (EC No. 2023/2006) has made it mandatory to adopt a system of Good Manufacturing Practice (GMP); with GMP referring to the set of actions to ensure a consistently high quality both in production and control process. This requires not only a deep knowledge of the materials used but also of the entire production and control process.

Flexible Packaging

In case of printed flexible packaging, Commission Regulation (EC) No. 2023/2006 Annex I prohibits the printed side of the materials to come into contact with food. Verification by GMP is also required in order to prevent any "Set-off" (process transfer of substances, from the printed side of a film to the non-printed side, due to the fact that these materials are normally produced in coils) that could ultimately transfer these chemicals onto foods.

The solvents in the inks used to print flexible packaging may represent a possible source of food contamination and therefore must be controlled.

For the determination of residual solvents from printed materials, it is highly recommended that an analytical method such as the official UNI EN 13628-2:20041 is followed. If the application of a non-official method is adopted, it requires validation by the laboratory; a task that is often long, complex and expensive.

Experimental Instrumentation

The analysis was performed using a PerkinElmer Clarus® 580 gas chromatograph equipped with a capillary column injector and an FID detector coupled to an automatic TurboMatrix™ 40 Headspace sampler. The capillary column used was a PerkinElmer Velocity-1 (30 m, 0.32 mm, 3 um – P/N N9306329).

Figure 1. Clarus 580 GC and TurboMatrix 40 Headspace sampler.

Analytical Conditions

The instrument conditions are given below:

Table 1. Instrument Conditions.

HS Conditions:	
Thermostatting Temperature	110 °C
Needle Temperature	130 °C
Transfer line Temperature	150 °C
Thermostatting Time	20 min
Pressurization Time	3 min
Injection Time	0.06 min
Pressure	21 psi
Mode	Constant
GC Conditions:	
Carrier Gas	He 1.7 ml/min
Split Ratio	1:20
Injector Temperature	230 °C
Detector Temperature FID	280 °C
Ramp 50 °C for	5 min, ramp to
	100 °C @ 5 °C/min, ramp to
	250 °C @ 10 °C/min

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Standard Preparation

Standards are prepared together as a stock mixture. Using the Total Vaporization Technique2, different levels of the calibration curves were obtained analyzing increasing amounts of the standard mixture added to the vial prior to analysis.

Table 2. Calibration Amounts.

Solvent	Level 1 mg	Level 2 mg	Level 3 mg	Level 4 mg
Ethanol	0.0065	0.0130	0.0260	0.0390
Isopropanol	0.0064	0.0128	0.0256	0.0384
MEK	0.0066	0.0132	0.2640	0.0396
Ethyl Acetate	0.0074	0.0148	0.0296	0.0444
Isobutanol	0.0065	0.0130	0.0260	0.0390
Methoxy Propanol	0.0075	0.0150	0.0300	0.0450
Ethoxy Propanol	0.0073	0.0146	0.0292	0.0438
Toluene	0.0058	0.0116	0.0232	0.0348
Butyl Acetate	0.0073	0.0146	0.0292	0.0438
m-Xylene	0.0071	0.0142	0.0284	0.0426
o-Xylene	0.0073	0.0146	0.0292	0.0438

Analytical Results

The software runs the standards/sample, calibrates the instrument and automatically produces the report. In the real world, samples can widely vary in concentration, therefore it is paramount that a high level sample does not carryover and contaminate the following samples and give false high results. The inert flow path and post sampling needle purge ensures the lowest possible carryover, producing quality results day after day. Another important area to consider is that the instrument's natural background levels are as low as possible, thus enabling ultra-low level detection when needed for those difficult analyses.

The chromatogram in Fig. 2 was obtained from the standard mixture Level 3 (blue) as compared with a blank (red) that was obtained by the analysis of an empty vial. The blank is clean and void of extraneous peaks, thus simplifying the reporting of data.

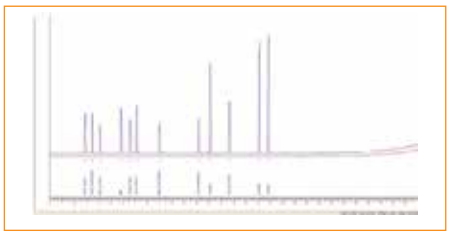

Figure 2. Chromatogram of Level 3 Standard.

Figures 3 and 4 represent the calibration curves for two example analytes: methyl acetate and toluene, both showing excellent linearity of the four calibration levels, thus enabling easy operation for the end user and improved accuracy of the results.

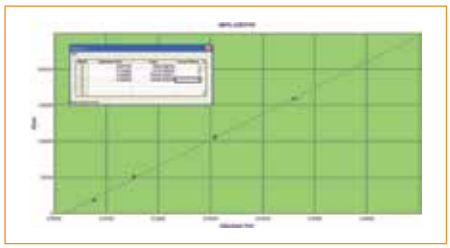

Figure 3. Methyl Acetate Calibration Curve.

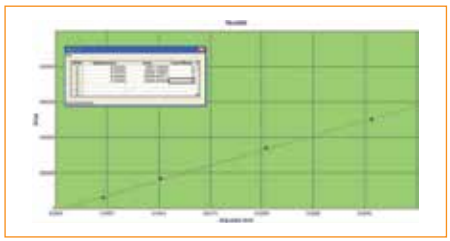

Figure 4. Toluene Calibration Curve.

Example of a Real Sample

A known Area (1 dm2) of the unknown sample is introduced into the vial and analyzed using the same analytical conditions as the standards above. The quantitative result obtained is then reported as the overall amount of solvents per m2 of material.

Figure 5 below shows the analysis of a real sample. For this sample the total content of solvent is found to be equal to 7.20 mg / m2.

Figure 5 also shows there is the presence of several unknown peaks, the one in the center is labeled "incognito." This is investigated further in the next section.

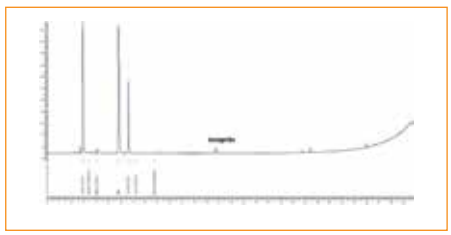

Figure 5. Chromatogram of Real Sample Showing Unknown Peak.

GC/MS

Although the standard UNI EN 13628-2:2004 requires the use of an FID detector, at times it may be necessary to identify an unknown solvent in a real sample, i.e. a solvent not included in the standard mixture. A mass spectrometer (MS) is a powerful detector for the determination of unknowns. We will use the same chromatographic system, vide supra, but coupled to a Clarus 560S MS. Figure 6 shows our target compound labeled as "incognito" at approximately five minutes into the chromatogram.

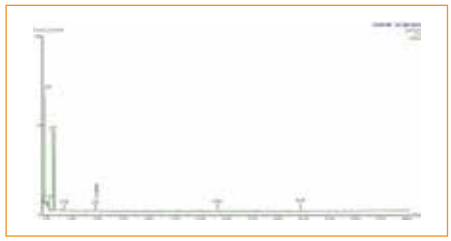

Figure 6. GC/MS Chromatogram of Sample Shown in Figure 5.

A mass spectrum of the unknown peak can easily be obtained by clicking on the peak. To assist in the identification of this unknown, the resulting mass spectrum was searched against a NIST mass spectra library that contains over 200,000 compounds. The NIST library software has selected the following solvent, 3-methyl heptane, as a possiblibility in Figure 7.

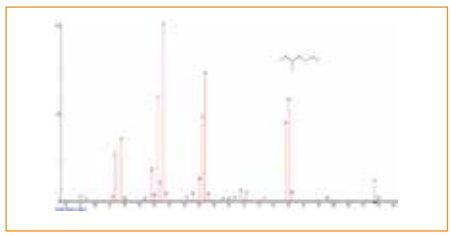

Figure 7. NIST Library Search Match of Peak Labeled "Incognito."

Conclusion

The Clarus 580 GC and TurboMatrix HS system can easily and accurately quantify the amount of residual solvents according to the official method EN13628-2:2004.

References:

1. Uni En 13628-2:2004 Packaging - Flexible Packaging Material - Determination Of Residual Solvents By Static Headspace Gas Chromatography - Part 2: Industrial Methods.
2. Static Headspace-Gas Chromatography Theory and Practice by B. Kolb, L. Ettre, 1997 p. 142 Wiley-VCH.

Food & Beverages

Accurate and Rapid Determination of Arsenic Speciation in Apple Juice

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Introduction

In the past several years, concern about the presence of arsenic (As) in apple juice has grown greatly due to its publicity in the popular media. Arsenic can enter apple juice either naturally through environmental uptake by the apple trees or anthropogenically through the use of pesticides and/or contamination during processing. Regardless of how it enters the juice, the presence of arsenic is a concern, especially since apple juice is commonly consumed by children.

In 2013, the U.S. FDA proposed an action level of 10 µg/L inorganic arsenic in apple juice, following the U.S. EPA regulated limit of 10 µg/L As in drinking water. If samples read at or above this level, they should undergo speciation analysis to determine which forms of the arsenic are present. It is possible that in the future, the action level for arsenic in apple juice will decrease due to the susceptibility of children.

Arsenic can be divided into two classes: inorganic and organic. While inorganic arsenic is toxic, the organic forms typically found in apple juice are considerably less toxic. Therefore, it is important to distinguish and measure the various forms of arsenic in apple juice as opposed to just monitoring the total arsenic concentration.

This work builds on our previous study of arsenic species in apple juice1 by incorporating several improvements to the methodology and exploring the analysis more deeply

Experimental

Sample Preparation

Seven apple juice samples were purchased at local grocery stores and filtered through 0.45 µm filters prior to analysis. Analyses were performed on undiluted samples.

All quantitative measurements were carried out against external calibration curves ranging from 0.1 – 15 µg/L. This range was chosen to both give accurate results at low concentrations, yet also include the action level (10 µg/L). The following reagents were used for preparation of standards: As3 1000 ppm in 2% HCl (Spex CertiPrep), As5 1000 ppm in 2% HNO3 (PerkinElmer), Dimethylarsinic acid 98% (Sigma), Monosodium methylarsonate 99.0% (Chem Service). All calibration standards were prepared in the aqueous component of the mobile phase.

Instrumental Conditions and Parameters

All analyses were performed with a PerkinElmer Altus™ HPLC system coupled to a PerkinElmer NexION® 350D ICP-MS. Details of the HPLC and ICP-MS method conditions are shown in Tables 1 and 2 and were based on our previous work1. Since any chloride present in the apple juice samples did not cause arsenic interferences, Standard mode was used for analysis, although Reaction mode could also be used. All data collection and analysis was done with Waters® Empower® 3 Software. The following reagents were used for mobile phase preparation: 1-octanesulfonic acid, sodium salt (98%, Sigma-Aldrich), malonic acid (99%, Acros Organics), and methanol (Optima grade, Fisher Scientific).

Results and Discussion

From our previous work, all apple juice samples were found to contain both forms of inorganic arsenic (As3 and As5) and one form of organic arsenic (dimethylarsinic acid, DMA). A second form of organic arsenic (monomethylarsonic acid, MMA) was only found in a few samples. Given that both forms of inorganic arsenic were present in all juices, the pH of all samples in this

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study was measured. The pH ranged from 3.4 – 3.6, which suggests that As3 and As5 are stable at these pHs. Measurement of apple juice samples before and after nine days of storage in a refrigerator confirmed species were stable: the percent differences between results were within 7% for all species present at significant levels to be quantified accurately. With the stability of As species established at the acidic pH of apple juice, a 1 µg/L mixed standard was prepared in mobile phase, which has a similar pH as apple juice, and analyzed daily over eight days. It was observed that the relative abundance of the species did not change, confirming the stability of all As species in slightly acidic conditions. The implication is that the calibration standards can be prepared in the mobile phase without worrying about the relative abundance of the species changing.

Table 1. Altus HPLC Conditions

Parameter	Condition
Column	C18, 4.6 x 250 mm, 5 µm
Mobile Phase	Octanesulfonic Acid (2 mM) + Malonic Acid (2 mM) + Methanol (1%)
pH	4.0 (adjusted with 10% NH4OH)
Flow Rate	1.5 mL/min
Separation Scheme	Isocratic
Column Temperature	50 °C
Injection Volume	20 µL
LC Vials	Plastic, 1.5 mL

To examine any matrix effects of the undiluted apple juice on the chromatography, the chromatogram of an apple juice sample was compared to those of a spiked apple juice sample and a standard prepared in mobile phase, as shown in Figure 1. These chromatograms indicate that the apple juice matrix has almost no effect on the chromatography, with only a very slight retention time shift seen for As3. This demonstrates the robustness of the separation method and confirms that apple juice samples can be analyzed undiluted, thus simplifying sample preparation.

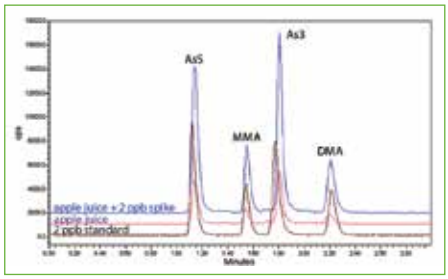

Figure 1. Chromatograms of a 2 µg/L mixed standard, undiluted apple juice, and undiluted apple juice spiked with 2 µg/L of all arsenic species.

Table 2. NexION 350D ICP-MS Conditions

Parameter	Condition
Nebulizer	Glass Concentric
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Nebulizer Flow	Optimized for < 2% oxides
Mode	Standard
Isotope	^{75}As
Dwell Time	500 ms
Sampling Rate	2 points/second

With the chromatography established, calibration standards were run, and the seven apple juice samples analyzed. All species yielded calibration curves with r2 > 0.999. Figure 2 shows overlaid chromatograms of the blank (i.e. mobile phase) and five lowest calibration standards. Based on our previous studies, we expect arsenic to be present at low levels; therefore, multiple low-level standards were run for accuracy at low concentrations. To accurately measure concentrations at the action level (10 µg/L), additional higher-level calibration standards were also run (5, 10, 15 µg/L) but are not shown in Figure 2, since the low-level standards would not be visible.

After the calibration curves were established, the seven apple juice samples were measured, with the results appearing in Table 3. These results indicate that all samples have arsenic concentrations significantly below the action level of 10 µg/L. It is interesting to note that the inorganic arsenic is always present at levels greater than the organic arsenic, which is consistent with our previous study which analyzed different commercial apple juice samples1.

Since there are no certified reference materials for arsenic species in apple juice, spike recovery studies were used to assess the accuracy of this method. Each sample was spiked at two different levels: 2 µg/L (to represent typical low levels) and 10 µg/L (the action level). Table 4 shows the spike recovery results, which were all within ± 10%, demonstrating the accuracy of the methodology.

Table 3. Results for Apple Juice Samples (all units in µg/L)

Sample	As5	As3	MMA	DMA	Sum
1	0.94	0.52	< DL	0.49	1.95
2	0.76	0.64	< DL	0.38	1.78
3	1.50	1.10	0.09	0.33	3.02
4	0.73	0.19	0.05	0.20	1.17
5	0.82	0.98	0.56	0.43	2.79
6	0.32	0.75	0.09	0.48	1.64
7	0.75	1.1	0.18	0.42	2.45

With the accuracy established, both short- and long-term stability of the methodology were examined. Short-term stability was evaluated by looking at consecutive injections of the same sample. Figure 3 shows an overlay of seven consecutive injections of one of the apple juice samples (Sample 5) over 30 minutes, along with the concentrations and relative standard deviations (RSDs) for all As species. The low RSDs indicate good short-term stability, with the lowest-concentration species (DMA) having the highest RSD, as expected.

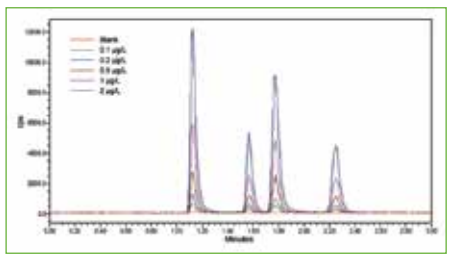

Figure 2. Chromatograms of low-level calibration standards.

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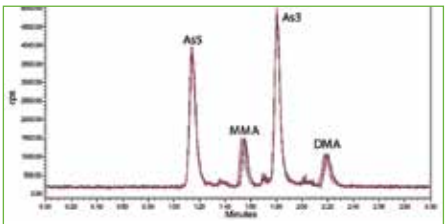

	As5	As3	MMA	DMA
Conc (ppb)	0.85	0.98	0.57	0.4
Std. Dev.	0.02	0.02	0.01	0.03
RSD	2.6%	2.5%	1.7%	5.8%

Figure 3. Overlay of seven consecutive injections of Apple Juice Sample 5, along with the associated concentrations and RSDs.

Long-term stability was evaluated by measuring 2 and 10 µg/L check standards every thirty minutes during an eight-hour analysis of all the apple juice samples. The check standard levels were selected to represent both low (i.e. typical) and elevated (i.e. action levels) concentrations of arsenic. Figure 4 shows both stability plots, where each reading has been normalized to the first measurement. Both plots indicate exceptional stability, with all measurements being within ± 6% of the initial reading.

Detection limits can be determined in a variety ways, each giving different results. In general, chromatographic detection limits are a function of many factors, including the injection volume, baseline noise, and peak shape. The commonly-accepted way of determining chromatographic detection limits is to find the concentrations (for a given injection volume) which produce peaks that are three times the amplitude of the baseline noise. Figure 5 shows a chromatogram from a 20 µL injection of standard containing arsenic species ranging from 0.02 to 0.07 µg/L. These peaks are clearly visible above the baseline and can be integrated, indicating that they are near the detection limit.

Since detection limits are not an issue for the measurement of As in apple juice (given that the action level is 10 µg/L), smaller injection volumes can also be used. Smaller injection volumes have two main benefits: extending column lifetime and producing taller, narrower peaks. Since sharper peaks result from lower injection volumes for the same concentration, we found that detection limits do not suffer proportionally to the volume reduction. Likewise, if lower detection limits are desired, larger injection volumes can be used. However, effects of the juice matrix on the chromatography may be more pronounced. Larger injection volumes will also shorten column lifetimes and produce broader peaks.

The work presented here was accomplished without using an internal standard. The good reproducibility of individual injections (Figure 3) and excellent long-term stability (Figure 4) eliminate the need for an internal standard. However, if the use of an internal standard is desired, arsenobetaine (AsB) can be used. As shown in Figure 6, AsB elutes after the DMA, so its presence will not interfere with the peaks of interest.

Table 4. Spike Recoveries for Apple Juice Samples (all results expressed as %)

	2 µg/L Spike Recoveries				10 µg/L Spike Recoveries			
Sample	As5	As3	MMA	DMA	As5	As3	MMA	DMA
1	92	100	93	91	99	104	96	94
2	96	102	99	93	95	102	94	90
3	97	106	100	95	98	103	97	94
4	98	109	99	95	101	109	101	100
5	99	106	96	95	99	105	98	95
6	99	104	101	95	102	107	99	95
7	97	104	98	96	101	107	101	98

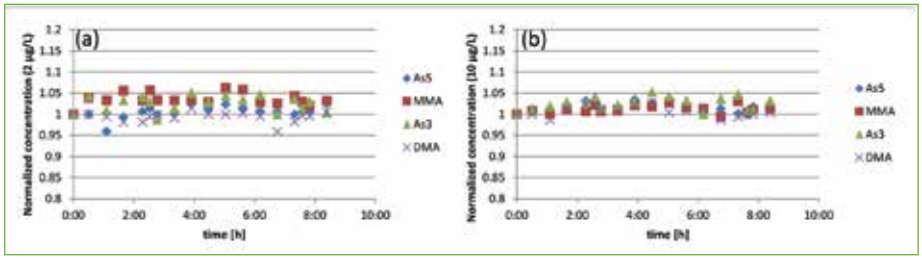

Figure 4. Stability of (a) 2 µg/L and (b) 10 µg/L As check standards run every 30 minutes during an 8-hour run of apple juice samples.

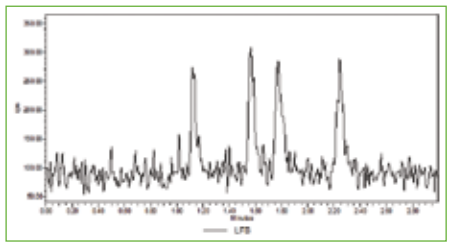

Figure 5. Chromatogram of arsenic species near the detection limit with a 20 µL injection: As5=20 ng/L; MMA=70 ng/L; As3=40 ng/L; DMA=70 ng/L.

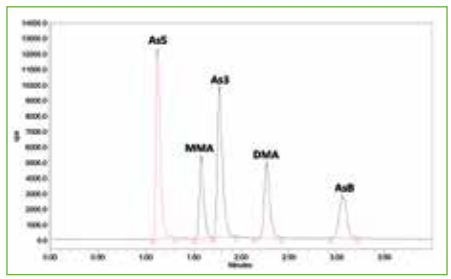

Figure 6. Chromatogram of a mixed arsenic standard containing 2 µg/L of each species, including arsenobetaine (AsB).

Conclusions

This work has demonstrated the rapid and accurate measurement of arsenic species in apple juice. By using reversed-phase chromatography with a cation-

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pairing reagent, the elution order of the species is reversed compared to the more traditional anion exchange chromatography. The separation is faster than traditional methods with associated benefits of shorter run time and taller, narrower peaks, enabling lower levels to be measured. Sample preparation is simplified as only filtration is required. Commercial apple juice samples were analyzed by this methodology and all found to contain significantly lower arsenic concentrations than the action level. The methodology allows separation of As species in a three-minute run time and was shown to be accurate at both low and high concentrations through spike recovery studies. In addition, the methodology produces excellent short- and long-term stability.

Consumables Used

Component	Part Number
Column: C18, 4.6 x 250 mm, 5 µm	N8145326
Autosampler Vials, clear, 1.5 mL (package of 100, with caps)	N9301736
Disposable Syringes, 10 mL, Luer-Lock (package of 100)	2542893
PTFE Syringe Filters, 0.45 µm, 25 mm (package of 100)	2542927
PEEK Tubing, 0.007" ID x 1/16" OD (5 feet)	N9302678
PEEK Finger Tight Fittings	9920513

Consumables Used

Component	Part Number
PEEK Solvent Filter, 10 µm	N8122249
PEEK In-line Filter, 10 µm	N8122250
Nebulizer Connector for HPLC	WE024372
Connector for Peristaltic Pump Tubing to PEEK Tubing	N8122258
Finger Tight Connector for 1/16" OD PEEK Tubing	9920513

References

1. Neubauer, K., Perrone, P., Reuter, W., "Determination of Arsenic Speciation in Apple Juice by HPLC/ICP-MS", PerkinElmer Application Note, 2012.

Food & Beverages

Analysis of Plant Materials for Toxic and Nutritional Elements with the NexION 350 ICP-MS

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Introduction

Plants primarily serve as a food substance, being an important source of nutrients. However, toxic elements can also be found in plants, primarily through uptake from the soil, water, and fertilizer. Therefore, it is important to measure both the nutritional and toxic elemental content of plants and plant materials. Several challenges arise in the elemental analysis of plants. First, because both toxic and nutritional elements must be measured, a wide dynamic range is required. Plants are complex biological entities which require sample preparation, usually consisting of homogenization followed by digestion in order to break down the complex matrix and extract the elements. Despite these steps, matrix-induced spectral interferences still persist which could cause false readings, especially for the toxic elements. Therefore, Collision or Reaction Cell technology has to be used to remove the interferences One plant species which is gaining considerable interest in the U.S. is cannabis (i.e. marijuana) since its use has been legalized in several states, both for recreational and medicinal purposes through inhalation and consumption in food products. With its increased use, interest in the toxic and mineral element content has also risen.

An additional challenge of cannabis analysis in the U.S. is legally attaining samples, since it is illegal in some states. However, hops are a generally accepted surrogate for cannabis due to its similar chemical and physical properties.

This work discusses the analysis of hops (as a surrogate for cannabis) for both toxic and nutritional elements with ICP-MS.

Experimental

Sample Preparation

Hops were purchased from a commercial store and chopped into small pieces, both to homogenize the sample and expose more surface area for increased digestion efficiency. The Titan MPS™ microwave sample preparation system with the standard 75 mL PTFE vessels was used for digestion, following the program in Table 1. Each vessel contained 0.25 g of plant material, 5.0 mL of concentrated nitric acid, 5.0 mL water, and 3.0 mL of 30% hydrogen peroxide. After digestion, the samples were diluted to 50 mL with deionized water, along with the addition of gold (Au) to stabilize mercury (200 µg/L Au in the final solution).

Table 1. Titan MPS Microwave Digestion Program

Step	Target Temp (°C)	Pressure, Max (bar)	Ramp Time (min)	Hold Time (min)	Power
1	150	30	5	5	60
2	200	30	5	20	90
3	50	30	1	10	0

Instrumental Conditions

All analyses were performed on a PerkinElmer NexION® 350 ICP-MS with the standard sample introduction components and conditions. The elements and analysis mode used are shown in Table 2. The internal standards were added on-line via a mixing tee. The final concentration introduced to the instrument were 10 mg/L Sc, 5 mg/L Ge, and 0.1 mg/L Rh, In, Tb in 10% methanol and 1% nitric acid. Using both Standard and Collision modes, the analysis time was 100 seconds per sample.

Results and Discussion

Table 3 shows the average results for two digestions of hops. To test the accuracy, pre-digestion spikes were added for those elements present at less than 50 mg/kg. The spike levels were 20 mg/L for all elements, except Hg, which was spiked at 2 mg/L. All spike recov-

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eries were within 15% of the added amounts, further validating the methodology.

Table 2. Elements and Analysis Mode

Element	Mass	Mode
Beryllium (Be)	9	Standard
Boron (B)	11	Standard
Sodium (Na)	23	Collision
Magnesium (Mg)	24	Collision
Aluminum (Al)	27	Collision
Phosphorus (P)	31	Collision
Sulfur (S)	34	Collision
Potassium (K)	39	Collision
Calcium (Ca)	44	Collision
Vanadium (V)	51	Collision
Chromium (Cr)	52	Collision
Manganese (Mn)	55	Collision
Iron (Fe)	56	Collision
Cobalt (Co)	59	Collision
Nickel (Ni)	60	Collision
Copper (Cu)	63	Collision
Zinc (Zn)	66	Collision
Arsenic (As)	75	Collision
Selenium (Se)	78	Collision
Strontium (Sr)	88	Collision
Molybdenum (Mo)	95	Collision
Cadmium (Cd)	111	Collision
Tin (Sn)	118	Standard
Antimony (Sb)	121	Standard
Barium (Ba)	137	Standard
Mercury (Hg)	202	Standard
Thallium (Tl)	205	Standard
Lead (Pb)	208	Standard
Thorium (Th)	232	Standard
Uranium (U)	238	Standard

Table 3. Results for Analysis of Hops

Element	Experimental (mg/kg)	% Recovery
Be	0.00	86
B	27.7	106
Na	13.2	113
Mg	3617	---
Al	10.8	108
P	6580	---
S	2001	---
K	34358	---
Ca	10936	---
V	0.04	101
Cr	0.23	96
Mn	17.3	113
Fe	58.6	---
Co	1.33	105
Ni	2.27	107
Cu	6.27	90
Zn	31.8	122
As	0.03	100
Se	0.20	102
Sr	17.9	99
Mo	0.93	102
Cd	0.02	97
Sn	0.28	96
Sb	0.01	96
Ba	18.1	94

Conclusions

This work has demonstrated the ability of the NexION 350 ICP-MS, combined with a Titan MPS microwave, to effectively analyze hops (as a surrogate for cannabis) for both nutritional and toxic elements. Analyses are accomplished in both Collision and Standard modes and require only 100 seconds per sample. The accuracy of the applied method was previously validated by analyzing a variety of NIST™ plant materials1.

References

1. Bosnak, C., Pruszkowski, E., "The Determination of Toxic, Essential, and Nutritional Elements in Food Matrices Using the NexION 300/350 ICP-MS", PerkinElmer Application Note.

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	0.38 mm id (green/orange), PVC, flared, 2-stop	N0777042
Drain Tubing	1.30 mm id (gray, gray), Santoprene, 2-stop	N0777444
Internal Standard Addition	Tee Tee for on-line addition of internal standard	N0777295
Internal Standard Uptake Tubing	0.25 mm id (red/orange), PVC, flared, 2-stop	N0773111
Multielement Standard Solution	100 mg/L Ag, Al, As, Ba, Ve, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb,	108
Se, Sn, Sr, Tl, V, Zn	N9301721 (125 mL)	---
Multielement Salt Solution	1000 mg/L Ca, Mg, Na, K	N9307805 (125 mL)
Mercury Solution	10 mg/L Hg	N9300253 (125 mL)
Internal Standard Solution	Sc (100 mg/L), Ge (50 mg/L), and In, Rh, Tb (1 mg/L)	N9308592 (125 mL)
Pure-Grade Au Standard	1000 mg/L	N9303728 (125 mL)
Autosampler Tubes	Conical, metal-free, sterile	N0776118 (15 mL) N0776116 (50 mL)

Food & Beverages

Analysis of Micronutrients in Fruit Juice Using FAST Flame Sample Automation for Increased Sample Throughput

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Introduction

Consumers select fruit juice because it is a tasty, convenient beverage and generally understood to be a more nutritious alternative to carbonated beverages. For 100% juice products, the nutrition content of the original fruit itself is well known, which translates to the expected nutritional value of the final juice product. Detailed labeling is required on food products; for consumers, any comparative variance can be a strong incentive to choose one product over another. In an effort to appeal to consumers and address market needs, many juice products may also be fortified with micronutrients to boost or add to what is already present naturally.

For food manufacturers and processors, it is imperative that there is a means to quantify the content of food products, including micronutrients, for both safety and quality reasons, along with regulatory label-claim requirements. Screening raw materials for elemental contaminants prior to use and then confirming the micronutrient content of the final product are two basic examples of the benefits of analytical testing. Accurate and precise analysis can also help improve the production process by utilizing the analytical data generated and employing statistical analysis to maximize nutrient yield or production volume where appropriate.

While ICP-OES is generally favored in a multi-element analytical environment, the cost savings, simplicity and speed of a flame atomic absorption (AA) system provides an attractive alternative. However, measuring multiple elements by flame AA requires each sample to be analyzed individually for each element, which impacts the speed advantage of flame AA.

To address the speed issue, a fast, high-throughput sample automation system can be used. Although samples still need to be analyzed multiple times, the analysis time per sample is significantly reduced, thus increasing sample throughput compared to manual sample introduction. In addition, an automated sample introduction system increases the precision of the analysis and frees the chemist to perform other tasks.

This work will focus on the analysis of micronutrients in a variety of commercial juice products using flame AA coupled with a highthroughput sample automation system.

Experimental

Samples and Sample Preparation

With the tremendous variety of juice and juice blends available on the market, samples were selected to be representative of commonly available and purchased juices. Only samples that were made from 100% juice (as accepted under current labeling guidelines) were selected, though this still meant that in many cases the juice was reconstituted from concentrate. The samples analyzed represent two different brands of apple juice and orange juice, two different varieties of grape juice, a pomegranate juice, and a vegetable-fruit juice blend. The analytical elements selected are representative of micronutrients that commonly appear on product labels.

Juice samples were subjected to minimum sample preparation with only nitric acid added to bring the acidity to 2%. Samples were split, and the elements of interest were spiked into one set of the split samples.

Instrumental Conditions

All analyses were performed on a PerkinElmer PinAAcle™ 900T atomic absorption spectrometer operating in flame mode using a FAST Flame 2 sample automation accessory. The elements

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Table 1. PinAAcle 900 Instrument and Analytical Conditions

Element	Cu	Fe	Mg	Zn	Mn	K	Na	Ca
Mode	Absorption	Absorption	Absorption	Absorption	Absorption	Emission	Emission	Absorption
Wavelength (nm)	324.75	248.33	285.21	213.86	279.48	766.49	589.00	422.67
Slit (nm)	0.7	0.2	0.7	0.7	0.2	0.2	0.2	0.7
Acetylene Flow (L/min)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.7
Air Flow (L/min)	10	10	10	10	10	10	10	10
Burner Head Rotation	0°	0°	45°	0°	0°	45°	45°	45°
Acquisition Time (sec)	1	1	1	1	1	1	1	1
Replicates	3	3	3	3	3	3	3	3
Sample Flow Rate (mL/min)	6	6	6	6	6	6	6	6
Intermediate Standard (mg/L)	1	5	20	2	1	200	200	100
Auto-Diluted Calibration								
Standards (mg/L)	0.05 0.1 0.2 0.5 1	0.25 0.5 1 2.5 5	0.5 1 2 5 10	0.1 0.2 0.5 1 2	0.05 0.1 0.2 0.5 1	5 10 50 100 200	5 10 25 50 100	5 10 25 50 100
Calibration Curve Type	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero	Non-Linear Through Zero

of interest and instrument conditions for the analysis of the juice samples are outlined in Table 1. A high-sensitivity nebulizer was used with the standard spray chamber, along with a 10 cm burner head. External calibrations were performed using a single intermediate standard made in 2% HNO3 /deionized water which was then diluted in-line using the capabilities of the FAST Flame 2 accessory. To control ionization during the analysis of potassium (K), sodium (Na), and calcium (Ca), La2O3 was added to the solutions, standards, and diluent at a concentration of 0.5% by weight.

The FAST Flame 2 accessory is a combination of high-speed autosampler, peristaltic pump and switching valve which provides quick sample turnaround with fast rinse-out, short signal stabilization times and no sample-to-sample memory effect. FAST Flame 2 rapidly fills a sample loop via vacuum and then switches to inject the sample loop while the autosampler moves to the next sample. This scheme removes the time delay associated with self-aspiration or peristaltic pumping and eliminates the long rinse-in and rinse-out times as a result of autosampler movement and flushing, resulting in complete sample-to-sample analytical times as short as 15 seconds.

The ability of the FAST Flame 2 accessory to mechanically pump the sample during injection allows for ideal optimization of nebulizer and flame conditions, eliminates variability due to changes in sample viscosity, dissolved solids, and tubing length, and also provides long-term sample flow stability. The in-line dilution capability allows the analyst to create a single intermediate standard and then lets the FAST Flame 2 accessory automatically generate all calibration standards in-line as required. In addition, the instrument can identify QC over-range samples and then utilize the in-line dilution capability to automatically re-run a sample that falls outside the calibration range at an increased dilution factor, bringing the signal within the calibration range and providing accurate measurement along with a successful QC check.

Results and Discussion

The calibration curves for individual elements were created from a single intermediate standard with the in-line dilution capabilities of the FAST Flame 2 accessory preparing the final standards in real-time. Calibration results are shown in Table 2. The excellent correlation for the calibration standards demonstrates the value of the automatic in-line sample and standard dilution capabilities.

The independent calibration verification recoveries ensure that the calibration is valid and that the creation of standards via the dilution system is accurate. The analytical results of the juice samples are shown in Figure 1.

The juice samples displayed a fairly consistent concentration of elements with a few exceptions. The largest deviation was the Ca concentration in Orange Juice B, which was labelled as "Calcium Fortified" where the amount of Ca present was an order of magnitude greater than the other juices, verifying the label claim. Levels of K

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and Mg are consistent across all the juice samples, while Na was moderately variable with the vegetable-fruit juice blend having significantly higher levels than the other samples. It is also worth noting that the two grape juice varieties and the vegetable-fruit juice blend had higher concentrations of Mn than the other juices. This elemental distribution highlights how the different balance of nutrients in the raw fruit can translate to the nutrients present in the final product and how monitoring and measuring these can be critical for product quality and labeling accuracy.

Because of the wide range of elements among the samples, the same dilution factor was not always applied to all the samples for the same element. Table 5 shows the dilution factors which were automatically determined and performed in-line with the FAST Flame accessory.

Table 2. Calibration Results

Element	Correlation Coefficient	ICV Concentration (mg/L)	Measured ICV (mg/L)	ICV (% Recovery)
Cu	0.99999	0.500	0.508	102
Fe	0.99997	2.50	2.56	102
Mg	0.99998	10.0	10.3	103
Mn	0.99961	0.500	0.503	101
Zn	0.99954	1.00	1.00	100
K	0.99900	100	91.8	91.8
Na	0.99979	20.0	20.8	104
Ca	0.99998	50.0	47.4	94.8

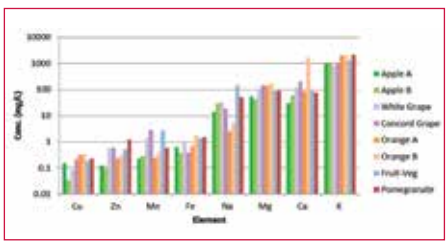

Figure 1. Results from analyses of juice samples.

Table 3. In-Line Dilution Factors

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Apple A	2	2	5	2	2	30	2	3
Apple B	2	2	5	2	2	30	2	3
White Grape	2	2	5	3	2	30	2	5
Concord Grape	2	2	5	5	2	30	2	5
Orange A	2	2	10	2	2	30	2	3
Orange B	2	2	10	2	2	30	2	20
Fruit-Vegetable	2	2	8	3	2	30	4	3
Pomegranate	2	2	8	2	2	30	2	3

To assess any possible matrix effects from the various juices, all samples were spiked with all elements at the levels shown in Table 4; the resulting spike recoveries appear in Figure 2. The recoveries of nearly all the sample spikes are within 10% of the calculated values for all elements and did not require per-sample matrix matching. However, there were two recovery values that exceeded 110% for K (Concorde Grape and Orange B), a result of the spike levels (91.9, 95.1 mg/kg, respectively) being significantly lower than the actual K concentrations in the samples (about one-tenth the amount). In all cases, the spike concentrations were established prior to analysis, and therefore, were not ideal. Nevertheless, excellent recoveries were observed. The Ca spike recovery for Orange B is not reported because of the excessively high Ca concentration in this juice. For all remaining elements and samples, the simple spiking and rapid sampling resulted in accurate analysis with good spike recovery, an absolute minimum of labor, and almost no sample preparation.

The addition of the FAST Flame 2 sample automation accessory reduced the number of standards the analyst needed to make from six (one intermediate and five final standards) to a single intermediate standard, with a commensurate reduction in human error during standard preparation. The measured concentrations of K, Mg, Mn, Na, and Ca in the samples varied enough to fall outside the calibration curve, but the in-line dilution capability of the FAST Flame 2 accessory allowed real-time dilution of these samples so that the absorbance fell within the calibration curve, producing accurate analyses. The ability of FAST Flame 2 to react to the over-range samples and auto-dilute the samples accurately and consistently without interaction from an analyst saves time and eliminates additional sample handling and lengthy re-prep.

Comparing typical autosampler performance, the total analytical time for each sample is dramatically reduced, while sample throughput is increased by nearly 4X with the use of the FAST Flame 2 accessory. The sample turnaround was reduced by 45 seconds while still maintaining the advantages of fully automated sample analysis, sample dilution, and calibration standard preparation. FAST Flame 2 retained the full automation benefits and still maintained a speed advantage even when compared with manual operation of the AA.

These results validate the accuracy and value of fruit juice analysis via flame AA along with the speed and increased productivity available from the PinAAcle and the FAST Flame 2 sample automation accessory.

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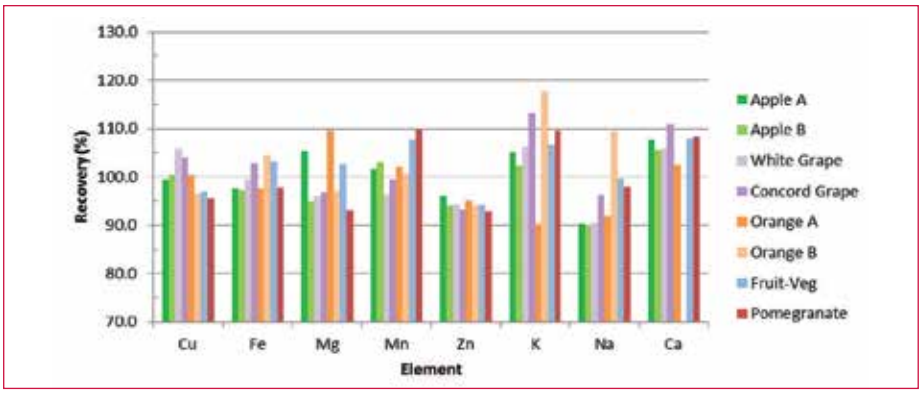
Table 4. Pre-Digestion Spike Levels (all units in mg/kg)

Sample	Cu	Fe	Mg	Mn	Zn	K	Na	Ca
Apple A	0.494	0.494	4.94	0.494	0.494	94.1	94.1	37.7
Apple B	0.508	0.508	5.08	0.508	0.508	92.0	92.0	36.8
White Grape	0.500	0.500	5.00	0.500	0.500	90.4	90.4	36.2
Concord Grape	0.475	0.475	4.75	0.475	0.475	91.9	91.9	36.8
Orange A	0.502	0.502	5.02	0.502	0.502	93.2	93.2	37.3
Orange B	0.484	0.484	4.84	0.484	0.484	95.1	95.1	38.0
Fruit-Vegetable	0.486	0.486	4.86	0.486	0.486	89.1	89.1	35.6
Pomegranate	0.479	0.479	4.79	0.479	0.479	95.8	95.8	38.3



Conclusion

This work has demonstrated the ability of the PinAAcle 900 AA spectrometer to reliably and effectively analyze a variety of fruit juice samples for Cu, Fe, Mg, Mn, Zn, K, Na, and Ca over a wide range of concentrations. Using the FAST Flame 2 sample automation accessory along with the PinAAcle 900 minimizes user errors when performing dilutions and making calibration standards while increasing throughput and productivity for the laboratory. (Equivalent results would also be obtained with the PinAAcle 500 AA spectrometer). The same analyses can also be done without the use of a FAST Flame accessory when analyzing smaller sample batches or the auto-dilution needs are not required.

Consumables

Red/Red PVC Pump Tubing	09908585
Black/Black PVC Pump Tubing	09908587
Autosampler Tubes	B0193233 (15 mL) B0193234 (50 mL)
Ca Hollow Cathode Lamp	N3050114
Cu Hollow Cathode Lamp	N3050121
Fe Hollow Cathode Lamp	N3050126
Mg Hollow Cathode Lamp	N3050144
Mn Hollow Cathode Lamp	N3050145
Zn Hollow Cathode Lamp	N3050191
Pure-Grade Ca Standard (10,000 mg/L)	N0691581 (125 mL) N9303764 (500 mL)
Pure-Grade Cu Standard (1000 mg/L)	N9300183 (125 mL) N9300114 (500 mL)
Pure-Grade Fe Standard (1000 mg/L)	N9303771 (125 mL) N9300126 (500 mL)
Pure-Grade K Standard (10,000 mg/L)	N9304121 (125 mL) N9304120 (500 mL)
Pure-Grade Mg Standard (1000 mg/L)	N9300179 (125 mL) N9300131 (500 mL)
Pure-Grade Mn Standard (1000 mg/L)	N9303783 (125 mL) N9300132 (500 mL)
Pure-Grade Na Standard (10,000 mg/L)	N9304124 (125 mL) N9304123 (500 mL)
Pure-Grade Zn Standard (1000 mg/L)	N9300178 (125 mL) N9300168 (500 mL)

Food & Beverages

Chromium Speciation in Drinking Water by LC-ICP-MS

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Introduction

With the growing concern of pollutants in the environment, more focus has been placed on identifying not only the total concentration of metals, but also the states in which they exist. Many elements can exist in various forms, either with different oxidation states or associated with various organic compounds or other elements. The toxicity and environmental impact of elements can vary depending in which form(s) they exist.

One element which has received considerable attention is chromium (Cr), which can exist in two different oxidation states: trivalent (Cr3) or hexavalent (Cr6). While trivalent chromium is an essential nutrient, hexavalent chromium is toxic. As a result, knowing the concentration of hexavalent chromium in environmental systems and samples which will be consumed is more important than knowing the total chromium concentration. This is especially true for drinking water.

Currently, the United States Environmental Protection Agency (U.S. EPA) only regulates the total chromium levels in drinking water and has set the limit at 100 ppb. However, the State of California set more aggressive limits for drinking water in 2014, both in regulating total chromium at 50 ppb and setting a MCL (maximum contaminant level) specific for hexavalent chromium at 10 ppb, with a public health goal of 20 ppt for Cr6.

This work describes the use of LC-ICP-MS to measure both trivalent and hexavalent chromium in drinking waters, with the goal of presenting a suitable methodology for analyzing the range of concentrations encompassed by the new legislation.

Experimental

Two alternative methodologies are widely employed for the separation of Cr3 and Cr6: ion exchange and ion pairing chromatography. The ion pairing method described in a previous application note 1 is still widely used and has been thoroughly validated 2, is fast, robust, and suitable for multielemental speciation 3. For this work, we opted for ion exchange on a short column with increased retention of species to maintain peak shape and separation at larger injection volumes utilized for lower detection limits.

Even though very acidic media can be employed to achieve separation of chromium species 4, the use of neutral media is preferred for equilibration-dependent methods for effective complexation of Cr3 by EDTA. Furthermore, the redox potential of the chromate/Cr3 redox couple is strongly pH dependent. Neutral pH has been demonstrated previously to provide suitable conditions for stability of chromium species when equilibration with EDTA was employed 1. After initially testing the effect of pH on separation efficiency, we optimized the method utilizing neutral pH in order to build on the established complexation ability and species stability at this pH.

Samples and Reagents

Tap water samples were collected locally and internationally, and bottled water samples were purchased from a local grocery store. Bottled water does not strictly fall under the U.S. EPA or CDPH (California Department of Public Health) regulations (it is regulated by the Food and Drug Administration) but is included here to provide a wide range of drinking water samples to gauge the effectiveness of the methodology.

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Chromium standards were made from stock solutions of trivalent and hexavalent chromium (PerkinElmer, Spex, respectively). Themobile phase was made from high purity nitric acid (GFS Chemicals), ammonium hydroxide (Fisher Scientific), and the dipotassium salt of ethylenediaminetetraacetic acid dihydrate (Sigma-Aldrich). Although the mobile phase can also be prepared from ammonium nitrate directly, it was found that using nitric acid and ammonium hydroxide produced lower chromium backgrounds. To prepare the mobile phase, 0.875 mL high-purity nitric acid and 202 mg of EDTA were added to 1L deionized water (18.2 MΩ-cm) and the pH was adjusted with 10% (v/v) ammonia hydroxide.

All quantitative measurements were made against external calibration curves with standards prepared in mobile phase.

Samples were diluted 2-fold (i.e. 1+1) in mobile phase. To allow time for the Cr3 to complex with the EDTA, all standards and samples were allowed to equilibrate for a minimum of 3 hours at room temperature prior to analysis, although equilibration time can be substantially reduced by heating the solutions5. Standards were prepared daily, as they were found not to be consistently stable for more than 24 hours at room temperature.

Plastic vials were used uncapped in order to avoid the possibility of introducing contamination from the cap. Since samples are prepared just prior to analysis, there is no need to cap the vials.

Instrumental Conditions

All analyses were run on a PerkinElmer Altus™ UPLC System, fitted with a 250 µL stainless steel expansion loop, 30 µL stainless steel needle, and 250 µL syringe, coupled to a PerkinElmer NexION® 350D ICP-MS. The syringe draw rate for sample uptake was set to 500 µL/min. The instrumental parameters are shown in Tables 1 and 2. All analyses were performed in Reaction mode using ammonia as the cell gas to remove any carbon- and chlorine-based interference at $^{52}Cr^{+}$. Interference by $^{12}C^{40}Ar+$ and $^{35}Cl^{16}OH^{+}$ can be effectively reduced by using ammonia as a reaction gas without loss of analyte sensitivity.6 All data analysis and processing was done with Waters® Empower® 3 Software.

Table 1. Altus UPLC System Conditions.

Parameter	Value
Column	Hamilton PRP-x100, 4.1 x 50 mm, 5 µm
Mobile Phase	"14 mM NH_4NO_3 (from HNO_3 + NH_4OH) + 0.5 mM EDTA"
pH	7.0 (adjusted with 10% NH_4OH)
Flow Rate	1.2 mL/min
Separation Scheme	Isocratic
Column Temperature	30 °C
Injection Volume	200 µL
LC Vials	Plastic, 1.5 mL

Table 2. NexION 350D ICP-MS Conditions.

Parameter	Value
Nebulizer	Glass Concentric
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Nebulizer Flow	Optimized for < 2% oxides
Mode	Reaction
Cell Gas	NH3 @ 0.5 mL/min
RPq	0.50
Isotope	Cr52
Dwell Time	1000 ms
Sampling Rate	1 point/second

Results and Discussion

Figure 1 shows the chromatogram of a mixed Cr standard. Both species are well separated and elute within five minutes, allowing for short run times. Additionally, the peaks elute well after the void volume (0.4 min). Initial calibration curves were established with standards from 0.04 -50 ppb for Cr3 and from 0.008–10 ppb for Cr6, where the upper levels were chosen as the MCLs for Cr6 and total chromium (for the Cr3 curve). Under the selected instrumental conditions, the peaks are acquired with sufficient precision (30 pts per peak for the smallest peaks) for reproducible integration. All calibration curves gave R2 values greater than 0.999, indicating the linearity of the method up to the levels of chromium set by the legislation.

Since the samples analyzed in this study read well below the MCLs, the calibration range was reduced to 10/2 ppb Cr3/6 to more accurately evaluate samples and spikes. Figure 2 shows the calibration curves used for the evaluation of samples, which gave R2 > 0.9999 for both Cr3 (Figure 2a) and Cr6 (Figure 2b). Also shown are the concentrations calculated when the standards themselves are evaluated with the calibration curve (generally within 4% of stated value). The largest relative deviations are observed for the lowest concentrations, as expected, but in absolute terms these deviations are very small (4 ppt - lowest two Cr6 standards, Figure 2b). The slopes of the calibration curves for Cr3 and Cr6 (parameter B in Figures 2a and 2b) match very closely (1% difference), demonstrating that the species do not interconvert, and that complexation of Cr3 with EDTA is quantitatively complete.

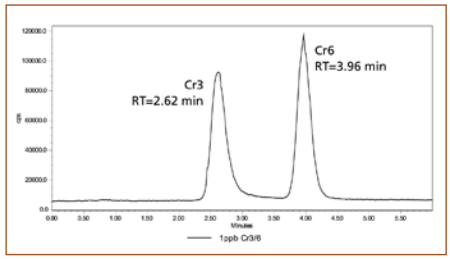

Figure 1. Chromatogram of 1 ppb Cr3/6 acquired with the instrumental and LC conditions stated in Tables 1 and 2.

The LC vials are critical to preserving the species, as it has been found that vials of different material or from different suppliers assist in reducing Cr6 to Cr31. The vials used in this work (listed in

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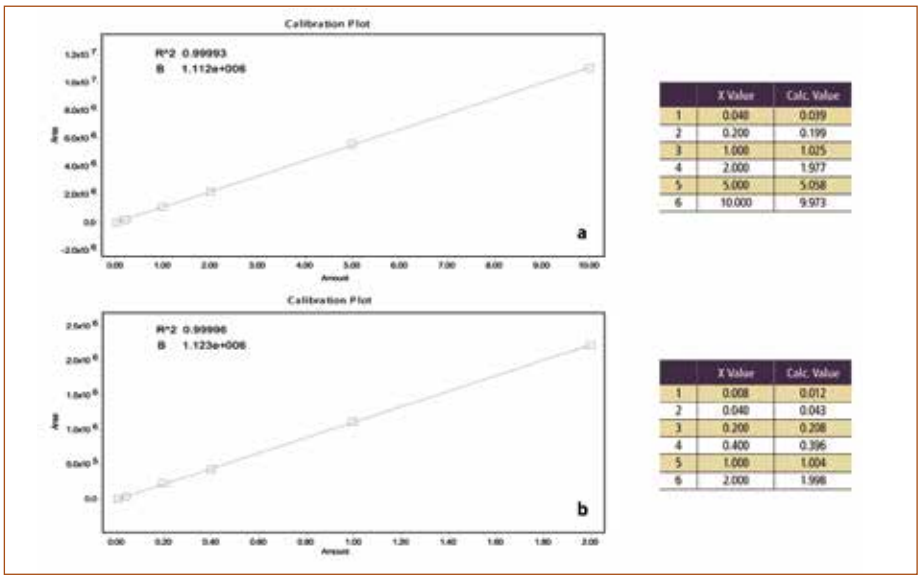

	X Value	Calc. Value
1	0.040	0.039
2	0.200	0.199
3	1.000	1.025
4	2.000	1.977
5	5.000	5.058
6	10.000	9.973

	X Value	Calc. Value
1	0.008	0.012
2	0.040	0.043
3	0.200	0.208
4	0.400	0.396
5	1.000	1.004
6	2.000	1.998

Figure 2. Calibration curves and details for Cr3 (a) and Cr6 (b).

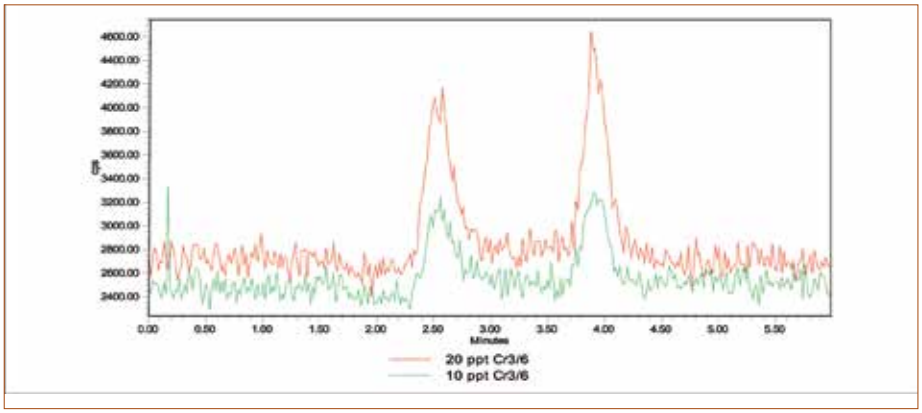

Figure 3. Chromatogram overlay of low level chromium solutions (0.010 and 0.020 ppb Cr3/6 mixed standards).

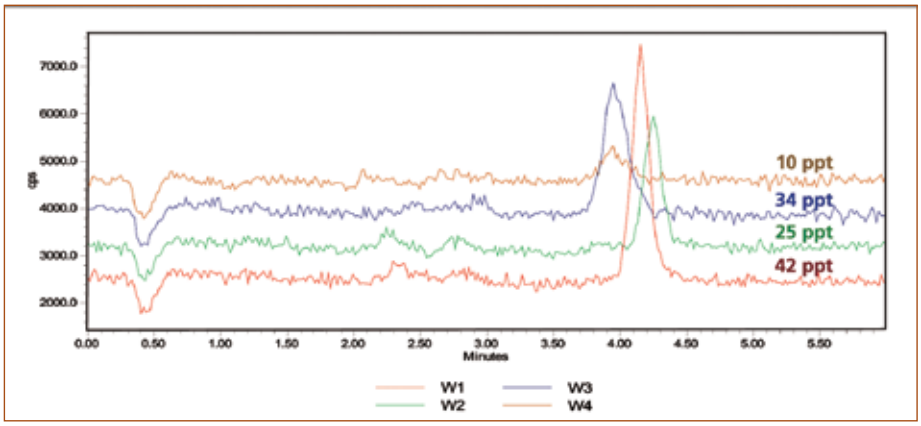

Figure 4. Chromatograms for several waters samples (with y-axes offset for clarity). All samples contain only detectable levels of Cr6. The raw analysis results (i.e., not corrected for dilution) for Cr6 are indicated on the chromatograms.

the Consumables Used table at the end) did not promote reduction of Cr6 to Cr3. These vials were used directly from the box and did not require any cleaning or pretreatment procedures.

The chromatograms of a 10 and 20 ppt mixed standard are shown in Figure 3. Both peaks are clearly visible above baseline and can be quantitated, indicating that under these conditions, detection limits are less than 10 ppt for each species. If lower levels are desired, larger injection volumes can be used, or the dilution ratio can be reduced by diluting samples with concentrated mobile phase. The injection volume and dilution ratio chosen here were found to be optimal for best detection while maintaining peak shape, peak separation, and ease of operation.

With the separation established, a variety of drinking water samples were analyzed, with the results appearing in Table 3. It is interesting to note that none of the samples contained Cr3, yet all had low levels of Cr6.

Table 3. Quantitative results for a variety of drinking water samples, corrected for dilution.

Sample	Cr3 (ppb)	Cr6 (ppb)
W1	---	0.084
W2	---	0.050
W3	---	0.068
W4	---	0.020
W5	---	0.046
W6	---	0.080
W7	---	0.076
W8	---	0.080

The chromatograms of several water samples are shown in Figure 4, demonstrating that the drinking water matrices do not affect the ability to measure low levels of Cr6. The Cr6 concentrations shown in Figure 4 are those read by the instrument and have not been corrected for the 2x dilution.

While for most samples shifts in retention time are small (see Figure 4), it was noted that in some samples, the Cr6

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peak shifted to longer retention times by up to 30 seconds, compared to the standards. This peak shift is caused by the matrix composition of the water sample. To test the effect of salt concentration on retention time, solutions of varying concentrations of sodium chloride (up to 1000 mg/L), were diluted 2-fold with mobile phase and spiked with 1 ppb mixed Cr3/6. As seen in Figure 5, the retention time for both chromium species is only marginally affected by the sodium chloride content, indicating the robustness of the method towards elevated salt levels. Other matrix components are therefore causing the larger retention time shifts of Cr6 in some drinking water samples. However, these were not investigated further as they do not affect the ability to measure Cr6 in water. Any change in retention time can be accounted for in the Empower® 3 Software by expanding the peak search window or manually assigning the retention time when reviewing the data. Alternately, higher dilution factors may be employed to minimize retention time shifts. Results for samples with detectable Cr6 at 5-fold dilution agreed well with the data from 2-fold analysis, showing that higher dilutions can be used in cases where lowest method detection limits are not required. An associated benefit is lower sample loading on the column, which contributes to longer column lifetime. To determine the accuracy of the results, all samples were spiked with 0.5 ppb of both chromium species. All spikes recovered within 10% of the target values (as shown in Figure 6), indicating the accuracy of the method.

With the separation, ruggedness, and quantitative accuracy of the method established, both short- and long-term stability were investigated. For short-term stability, one of the water samples (W7) was analyzed eight times within one hour. Figure 7 shows the resulting Cr6 concentrations, with all readings normalized to the first measurement. With concentrations varying by less than + 4%, the short-term stability of the methodology is demonstrated.

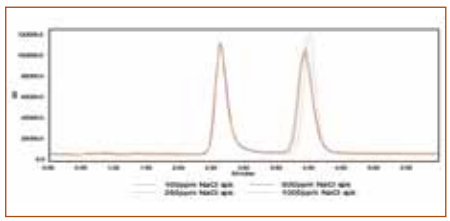

Figure 5. Method robustness: effect of salt concentration (100 – 1,000 ppm NaCl) on chromatography.

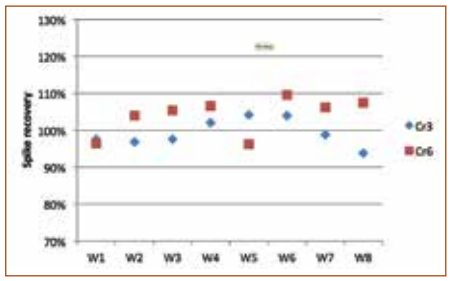

Figure 6. Recoveries for 0.5 ppb Cr3/6 spikes on all water samples.

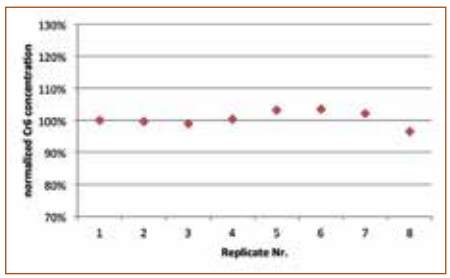

Figure 7. Repeated analysis of one sample (W8) over one hour.

Long-term stability of the analysis was explored by running all of the water samples continuously over 14 hours, with a check standard (Cr3=2 ppb and Cr6=0.4 ppb) run every 30 minutes. Figure 8 shows a plot of the check standard, with all results normalized to the initial reading. Variations of less than 6% for both species prove the stability of the standards, instrumentation and methodology.

Despite the long-term stability of standard solutions, another factor which must be considered is the stability of the species in the samples. Since water samples contain multiple components, it is possible that chromium interconversion may occur over time in the samples. Reanalyzing water samples 11 hours after their initial analysis (and 15 hours after sample preparation) showed that all samples were stable (raw results agreed within 5 ppt) for this time frame, except one sample (W5) where the Cr6 concentration decreased and Cr3 appeared. Spiking this sample also showed interconversion of the species over time. Since only this sample showed interconversion, it suggests that something in the sample is reducing the Cr6 to Cr3. Since the check standards did not show species interconversion (Figure 8), the possibility that the vial is causing the conversion is eliminated.

Although the methodology and instrumentation are stable over long runs, it is therefore recommended to prepare samples in smaller batches to minimize the possibility of species interconversion. Another option is to chill the autosampler tray (4-10°C) to slow the chemistry of interconversion.

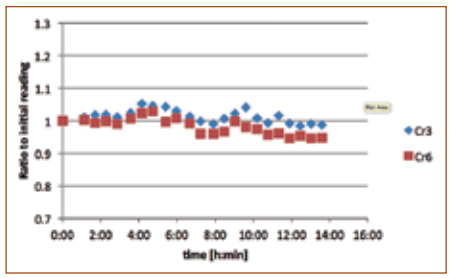

Figure 8. Data for check standard (2/0.4 ppb Cr3/6) run intermittently during drinking water analysis demonstrates long-term stability.

Conclusion

This work has shown that the Altus UPLC System together with the NexION 350D ICP-MS can be used successfully for chromium speciation at the levels relevant to recent, more stringent legislation. The presented method separates Cr3 and Cr6 within a 6-minute run, measures less than 10 ppt (20 ppt in the sample), and covers a wide linear range up to at least 50 ppb.

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The optimum procedure uses a 2-fold sample dilution with mobile phase for sufficient complexation of Cr3 with EDTA, reduced retention time shifts caused by sample matrix, adequate peak shape and resolution, and the convenience of using identical solutions for sample dilution and the mobile phase.

The method is rugged with a relatively large salt tolerance (up to at least 1000 ppm NaCl) and is accurate for both chromium species as demonstrated by spike recoveries for a wide variety of drinking water samples. Both short-term (one hour) and long-term (14 hours) stability have been demonstrated, ensuring collection of high-quality data over long run times.

References

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Consumables Used

Component	Part Number
Autosampler Vials, clear, polypropylene, 1.5 mL (package of 100 with caps)	N9301736
PEEK Tubing, 0.007" ID x 1/16" OD (5 feet)	N9302678
PEEK Solvent Filter, 10 µm	N8122249
Nebulizer Connector from UPLC	WE024372
Connector for Peristaltic Pump Tubing to PEEK Tubing	N8122258
Finger Tight Connector for 1/16" OD PEEK Tubing	9920513

Food Value and Analysis chain: PerkinElmer, Perten and Torian solution fit

Inputs / breeders	Production / Growers	Procurements, Grain traders & Storage	Manufacturing / Processing	Finished product And Packaging	Retailing
Quality test	Quality test	Quality test	Quality test	Quality test Labelling	Out of Lab Check
Seed, Fertilizers and Farm Equipment	Farmers Cooperatives and private companies	Warehouses, cold storage and silos	Grading, sorting, milling	packing, and Labeling	Retail shops, Malls, cash and carry
IM 9500	AM 5200	DA 7300	IM 9500	PE FTIR-Packaging	IM 9500
Falling Number	IM 8800	IM 8800	Falling Number	Protein Analysis: CHNSO	Lambda UV
Glutomatic	Clarus GC		IM 8800	Fat Analysis: Clarus SQ8GCMS	PE FTIR-Packaging
DA 7250	AA, ICP, MS		AM 5200	Mineral: AA, ICP, MS	TRIDION™-9
DA 7300			DSC & STA	pesticides: Clarus SQ8GCMS	
AM 5200			DMA		
Clarus GC			TGA		
AA, ICP, MS					

Regulatory Agency

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News and Event Update

ASIA LABEX is an international exhibition on analytical, research, diagnostic, laboratory instruments & consumables. PerkinElmer had an opportunity to meet many of customers and they were amazed to see PerkinElmer participation in exhibitions from Aug 25-27, 2015, New Delhi.

It's been few year since PerkinElmer showcased all the product line, the objective of this Exhibition was to enhance the level of customer connect, brand recall, reinforcing brand PerkinElmer as innovator and elevate employee satisfaction level.

India Lab Expo- Analytica Anacon
India Lab Expo and Analytica Anacon India is India's one of largest and most important platform for the analysis, laboratory-technology and biotechnology market with more than 300 exhibitors and 8500+ visitors. It offers unique platform for exhibitors to display latest techniques in all application sectors.

Co-organizers: IAIA – Indian Analytical Instruments Association
Inauguration Ceremony: Exhibition was inaugurated by Dr. Akun Sabharwal, Controller of Drugs – Telangana State on 8th Oct, 2015 at 10:00 am in the august presence of all exhibitors.

This exhibition attracted various QC/QA Managers, Scientific Officers, Research Scholars, R&D Scientists, Lab Directors, Purchase Managers.

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