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HUMAN HEALTH | ENVIRONMENTAL HEALTH

VOLUME 24 • Winter Edition, 2010 | India Analytical Sciences

Merry Christmas
and a very
Happy New Year

Fresh

VOLUME 24 • Winter Edition, 2010

E D I T O R I A L

Dear customers and readers,

The world is getting closer to the end of the first decade of the 21st century. We all have experienced the eventful year 2010. The Indian economy sustained against all odds. The hosting of commonwealth was as a success with haul of gold medals.

Good monsoon supported the cultivators and farmers for better crop and agriculture yields. The industrial production is also looking better placed. The Indian consumer is getting exposures to a variety of goods. The food industry is also not an exception as quality food with nutritional values are available to the consumers. This lifestyle has also made the industry follow the international norms of food safety and consumer health.

In this winter FRESH we bring you the applications for various food items and ingredients. Please note that we have included a case study for the analysis of cholesterol in milk and milk products in faster and cheaper way. This work is the sole effort of our esteemed customer. The toxic elements analysis and furans in Chinese spices and other food materials respectively is addressed by the Indian application team.

We also include here the article based on thermal Desorber technique for the analysis of flavors and fragrances. There are numerous applications that researchers and industries can develop. We welcome any new applications based on analytical techniques from our readers to publish in Fresh.

Wishing you a "Merry Christmas & a very happy New Year, 2011"

WHAT'S Fresh inside...

The role of chromatography in determining quality of essential oils, flavors and fragrances

Introduction

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils or aetherolea, or simply as the "oil of" the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant.

They are used in perfumes, cosmetics, soap and other products, for flavoring food and drink, and for scenting incense and household cleaning products. Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction.

The ability to use these oils for holistic medicine focus on utilizing unadulterated oils that are of the highest possible quality. The main purpose of testing essential oils is that it will assure consumers that the oil is truly capable of delivering cure to certain ailments, which the oil is known to address. On the other end, consumers will be able to gain confidence that such oil is safe for use on humans without causing harm or other complications. After all, use of essential oils in aromatherapy are considered safe and effective due to the use of natural substances that lack harmful synthetic chemicals.

Purity vs Quality

Two of the most basic concepts dealt whilst testing essential oils are purity and quality. Both, these are related, so it gives an impression that they are same however, they are not. But each one plays an important role in determining whether a specific essential oil is safe to use in aromatherapy or not.

The aroma therapists and chemists utilize various information to determine the quality of the essential oil. The factors that affect the quality of the oil are : quality of the botanical material collected, the variety of the material and the place the oil is extracted from, the conditions of the habitat of the plant, the farming practices followed for cultivation, the harvesting procedure, and the choice of extraction method.

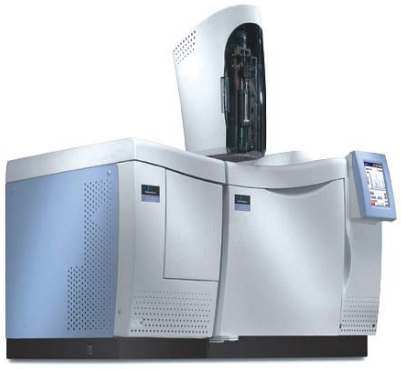
As for the purity of the essential oil, aroma therapists are interested in finding out whether it contains adulterated substances that could turn the natural oil into a toxic substance. Several factors that can dilute the oil's purity, such as blending with high grade oils with low grade ones, adding synthetic materials to enhance aroma, and blending it with synthetic oils.

Some common tests that can be performed to ensure that the oil quality before it is released into the market for human use,

- Sensory Evaluation: Prior to conducting complicated and expansive tests on essential oils, this is one of the most basic tests to perform. Visual examination can often discriminate superior oils from the inferior ones. Hence, always observe for on color, consistency, and appearance of the oil prior release in the market.
- Odor Evaluation: Each essential oils has a distinguished aroma, which are mainly responsible for the healing benefits. A trained nose can easily identify whether the oil is pure or is synthetically produced. Therefore, it would be beneficial to perform an odor test to evaluate whether the oil is adulterated or not.
- Qualitative and Quantitative Analysis: In the qualitative aspect of the test, it aims to identify the chemical constituents found in the essential oil by GC/MS. The quantitative aspect, on the other hand, determines the amount of individual constituents found in the essential oil.
- Finally a patch test: Applying the oil on a sensitive part such as back of the ear, on the wrist, behind the knee, or under the arm.

Need to analyse

Gas chromatography-mass spectrometry (GC/MS) is used extensively by the essential oil industry. Essential oils are complex natural products consisting of many components that span a wide concentration range. This complexity makes the analysis of essential oils challenging. To name a few, determining the flavor/fragrance components for chemical synthesis, what components make up this fragrance so it can be synthesized for production, investigating amount of flavor/fragrance transferred to a product, investigating problems - "bad odors or bad flavors" in a product, investigating competitive products for flavours and fragrances used in them, determining the pesticide or herbicides residues etc.



Gas Chromatography

Gas chromatography is a popular method for testing the essential oils for safety, this technique is also known as Gas Liquid Chromatography. This method is performed by identifying the chemical constituents found in the essential oil and measuring the amount of each of the components found in the oil. A representative sample is often taken from the bulk of essential oil sample, and then injected into the gas chromatograph. The volatile components are vaporized and separated on a column, which is the heart of the chromatograph.

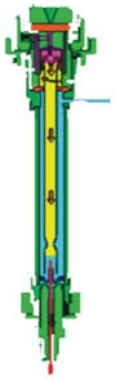
Mass Spectrometry

The components separated on the GC are identified and if necessary quantified using a mass spectrometer. The mass spec ionizes those constituents of the oil and produces a fragmentation pattern based on mass-to-charge ratio of the components and their fragments. The components are identified based on the molecular weights.

Flame ionization detector

The components separated on the column are identified on the FID. The use of GC-FID is a very traditional in the analyzing essential oils.

Sample introduction techniques :
Introducing the sample in the system could be done in a number of ways.



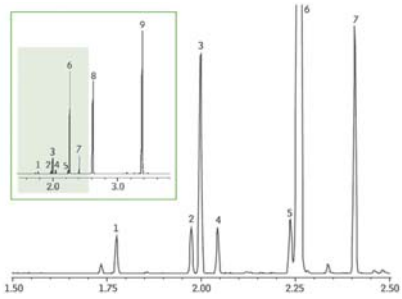
GC capillary inlet Direct liquid injection

TurboMatrix Thermal Desorber TD

TurboMatrix headspace trap

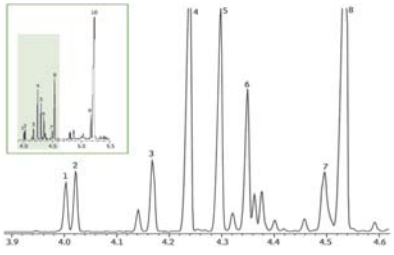
Some examples of determination by GC-MS are:

Figure 1 Analyses of essential oils, using a 10m x 0.10mm x 0.10µm df Elite-5 column.



Bergamot oil

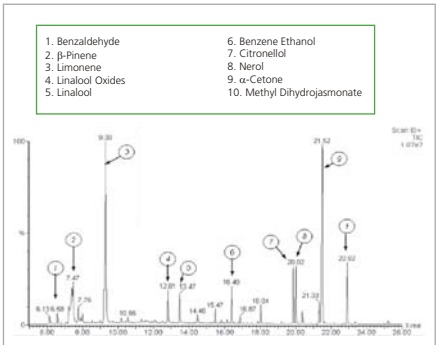
1. α-pinene
2. sabinene
3. ß-pinene
4. ß-myrcene
5. p-cymene
6. D-limonene
7. γ-terpinene
8. linalool
9. linalyl acetate



Patchouli Oil

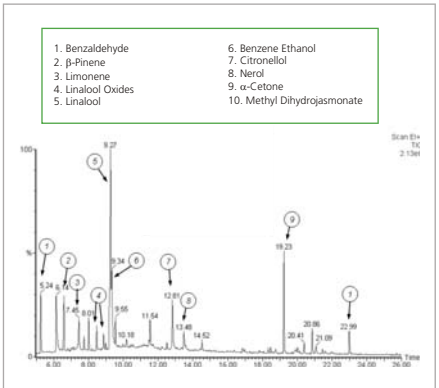
1. ß-patchoulene
2. ß-elemene
3. caryophyllene
4. α-guaiene
5. seychellene
6. α-patchoulene
7. guaiene
8. δ-guaiene
9. selinene
10. patchouli alcohol

Bar of Soap



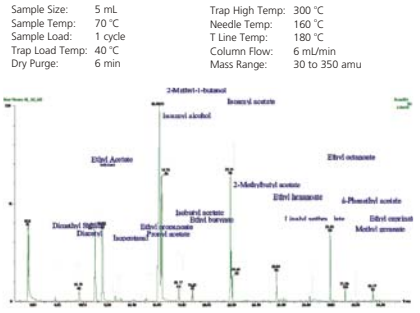
Compounds in Bar of Soap by Thermal Desorption

Body Cream

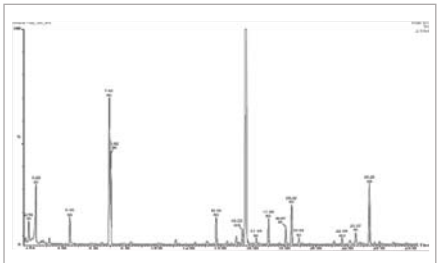


Compounds in Body Cream by Thermal Desorption

Beer flavors Green Tea flavors



Beer Flavors (Headspace Trap – GC/MS)



Flavors in Green Tea – HS-GC/MS

Discussion

Bergapten is an photoactive ingredient in bergamot oil which promotes melanogenesis, so the oil produced should be free of bergapten. This method could be used for determination of the variation volatile constituents in the oil. The main volatile constituents of P. cablin and P. frutescens could be separated and identified. This method could be used to distinguish the origin of the patchouli oil from these two species. The fingerprint study of various soaps, body oils and perfumes could be done and the variability's can be studied. There is a beer for every occasion, lagers, ales, fruit beer, wheat beer etc. Professional tasters can identify 100 different flavors in beer. The resins and oils within hops are the chief flavouring for beer, giving the distinctive bitter taste. Different yeast strains produce different subtle flavours so brewers are very protective of their personal strains. The various chemical components responsible for off flavours in beer can be determined to retain the quality of the broth. It provides qualitative and quantitative information with high sensitivities, the peak purity algorithm enables analyst to easily recognize co-eluters. The volatile and the non volatile components of tea can be determined by these techniques.

The European Union (EU) regulates 26 flavor and fragrance allergens. Twenty-four of these allergens are amenable to analysis by GC/MS. Some of these allergens are present at appreciable levels in essential oils used in aromatherapy.

The use of sample-introduction techniques such as HS and TD enable the analysis of raw materials in products that cannot be performed by extraction. This also prevents loses and solvent interferences associated with solvent extraction. These techniques require less maintenance as it prevents the matrix from entering in to the GC column. The components of interest could be easily concentrated to enhance detection. The detection limits could be further enhanced by using HS-trap.

Although GC-FID techniques provide a good starting point for the analysis, nowdays GC-MS is extensively used for qualitative identification of the components. Essential oils are complex mixtures containing, monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes and diterpenenoids. GC/MS offers a wide spectral library for comparison making identification convenient. Identification can be further improved by comparing spectra's from flavor and fragrances library.

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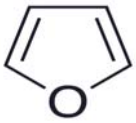
Furans in food by Gas Chromatography-Mass spectrometry and Headspace sampling

Introduction

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

Furan is a colorless, volatile and lipophilic organic compound. It has a molecular weight of 68 and a low boiling point (31 °C). Due to its high volatility, furan levels in foods are easily determined, with high accuracy, by headspace methods.

This application note will demonstrate a rapid method for the identification and quantification of furans, in food samples, using gas chromatography, with headspace sampling and mass spectrometer. The samples were chosen at random from the local market.



Synonyms: furfuran, oxole, tetrole, divinylene oxide, oxacyclopentadiene
Formula: C4H4O
MW: 68.07
MP: -85.6°C
BP: 31°C

Structure and properties of furan

Experimental conditions

The PerkinElmer® Clarus 680 Gas Chromatograph, Clarus 600 C Mass Spectrometer and a TM HS-40 system was used for this application. The analytical column used was PerkinElmer Elite -624 (60 meter, 0.32 mm i.d., 1.8 µm df) at a flow rate of 1.4 ml/min helium at constant flow mode. The GC inlet temperature was 200 °C and the oven temperature programme was 40 °C hold for 6.0 min, 20 °C/min to 110 °C and hold for 1.0 min, 70 °C/min to 250 °C and hold for 3.5 min and the runtime is 20 minutes. The MS source temperature was 230 °C, MS interface temperature 225 °C, Scan range m/z 35-150, Scan time 2.5-25 min. The vials were thermostatted at 60 °C for 20 minutes. The needle and the headspace transfer line were maintained at 100 °C and 130 °C respectively.

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

Stock Solution

A stock solution of 1000 µg/mL of furan and d4-furan was used as the starting point for all standard solutions (SPEX CertiPrep).

Standard preparation

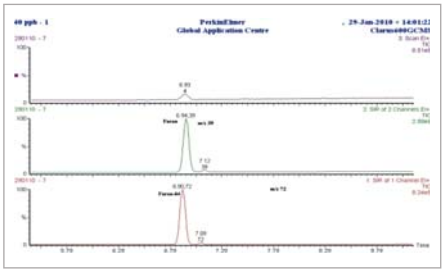
Ten µl of the stock furan was diluted to 10 ml in methanol to give a solution of 1 µg/ml. 20 µl of the stock d4-furan solution was diluted to 10 ml in methanol to give a solution of 2 µg/ml.

Calibration curve

Varying volumes of 1 µg/ml furan was diluted in water to achieve the final standard concentration to get 1, 2, 10, 20 and 40 ppb of furan. 100 µL of d4-furan from 2 µg/ml stock was added to each headspace vial containing 10 ml of water resulting in an internal standard concentration of 0.02 µg/mL (20 ppb). Four g of NaCl was added to each of the vials to decrease the miscibility of furan in water. The results of the calibration curve are as in table no.1. The chromatogram and MS spectrum for furan in shown in figure no. 1

Calibration

The FID was calibrated across the range of 1.0 to 40 ng/mL, each calibration point was run in triplicate to demo-nstrate the precision of the system. The average coefficient of determination for a line of linear regression was 0.9997 for furan. The calibration curve for furan is depicted in Figure 2.



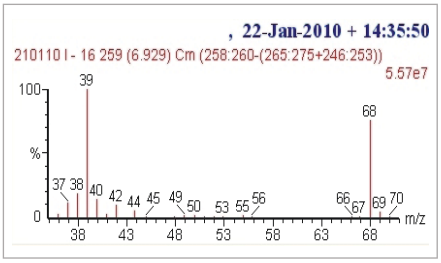


Figure 1: Example chromatogram and MS spectrum for furan.

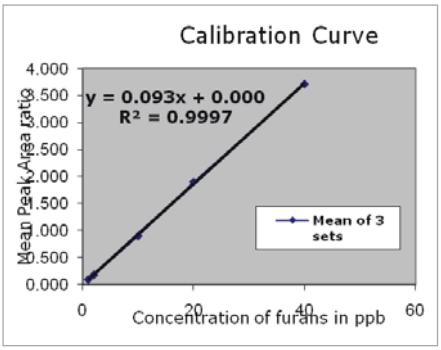


Figure 2. Calibration curve for furan

Sr. No.	No. of levels	Mean Peak area Average Relative Response (n=3)	%RSD
1	1	0.098	10.046
2	2	0.184	8.012
3	10	0.904	1.475
4	20	1.900	0.435
5	40	3.709	1.627

Table 1. % RSD's for three sets of linearity experiment.

Summary of method validation experiment	
Linearity:	1.0 ppb to 40 ppb of furan
RSD for replicate analysis:	for 1.0 ppb 4.78%
Detection level:	0.5 ppb
Quantification level:	1.0 ppb
Recovery study:	at three levels for all the samples 80-120%

Sample preparation

Samples were collected from the local market. Headspace sample preparation is relatively very easy. Ten ml of sample was transferred into a headspace vial; 4 g of NaCl was added to it. Milk and other viscous were diluted. The semi solid samples were ground and 5g of sample was added to headspace vials 5ml of saturated salt solution: Coffee powder was dissolved following directions on the package, and then treated like a non-viscous liquid sample. The chromatogram and MS spectrum of furan n sample is shown in figure no.3

Method Validation

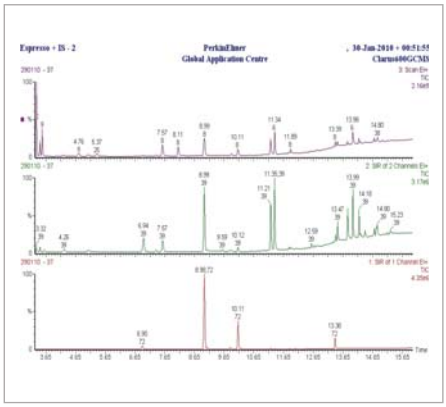
The recovery of the method was tested with the analysis of the brewed coffee sample spiked at 3 different levels: 2, 5, 10 ug/L. The measured amount was

2.03, 5.44, 9.54 ug/L demonstrating that the headspace technique is quantitative in its extraction of furan from an aqueous matrix.

Results

Eight samples of common beverages were analyzed using the HS-GC method developed here. Of the samples analyzed, brewed coffee demonstrated to have the highest levels of furan, at 250 ug/L.

Sample No.	Sample details	Amount of Furan in ppb
Sample 1	Lab Coffee	0.67
Sample 2	Chocolate Flavoured milk (AKCF)	1.67
Sample 3	Espresso Coffee	45.18
Sample 4	Coffee flavoured milk (AKC)	10.87
Sample 5	Cocoa flavoured milk (AKK)	1.76
Sample 6	Energy drink (milk based) (NAEM)	13.21
Sample 7	B-coffee	36.59
Sample 8	Filter coffee	253.99



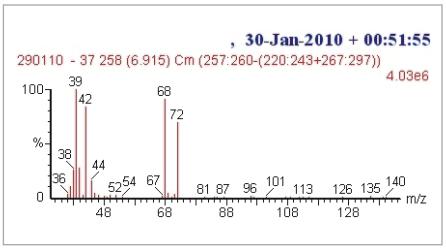


Figure 3 Chromatogram and MS spectrum of furan peak in sample.

Conclusion

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

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Case study on estimation of Cholesterol by FTIR spectrometry

Abstract

A method was developed for analysis of cholesterol in whole milk powder and commercial liquid milk products. Analysis was carried out using Fourier Transformed infrared Spectroscopy (FTIR). Sample preparation was by saponification with methanolic potassium hydroxide followed by extraction with ether. The quantitative estimation was calculated for the particular peak of cholesterol (2937 cm^{-1}).

A corroborative study was also performed by HPLC method which shows excellent correlations within the two methods. High Performance Chromatographic employed a nonaqueous medium reversed chromatographic system. The chromatography system included a carbon – 18 column with a hexane/isopropanol mobile phase and detection at 205 nm.

Introduction

Cholesterol is a structure containing 27 carbons, commonly found as a in cell membrane. Biologically it is an important precursor of bile acid, provitamin D3 and several steroidal hormones. Accurate determination of cholesterol is important due to its close correlation to the occurrence of coronary heart disease.

Hence determination of cholesterol in various foods is important for two reasons. The first is the more obvious concern for health about the atherogenic role of excess plasma cholesterol and the influence of diet on plasma lipids. The second reason deals with regulatory aspects of food labeling.

High Performance Liquid Chromatography (HPLC) has been extensively used for analysis of lipids in foods. Analysis of steroids, especially cholesterol routinely has been accomplished by gas liquid chromatography (GLC). Recently

cholesterol analysis was done by HPLC through the ultra-violet (UV) absorbing derivative. Enzymatic methods have been promulgated for steroid analysis, but problems result when other sterols are present, because the enzymes attach a common sterol site.

But all the methods described above require longer time and also involves cost. Analysis by GLC takes a longer time and analysis by HPLC consumes costly HPLC grade solvents as mobile phase. The method here reported allows for direct determination of cholesterol without using any costly solvents and it is also less time taking.

Materials and Methods

Samples

Milk samples were from commercial sources and stored at 4^0C until needed for analysis.

Solvents

Hexane, isopropanol and carbon tetrachloride were HPLC grade. Diethyl ether was purchased without ethanol preservative and checked to ensure it was peroxide free. Petroleum ether (bp 30 to 60^0) was used as received. The 10% NaCl (vol/vol) was prepared as needed while the 2.0 (N) methanolic KOH was prepared daily.
Standards

(a) Standard cholesterol:

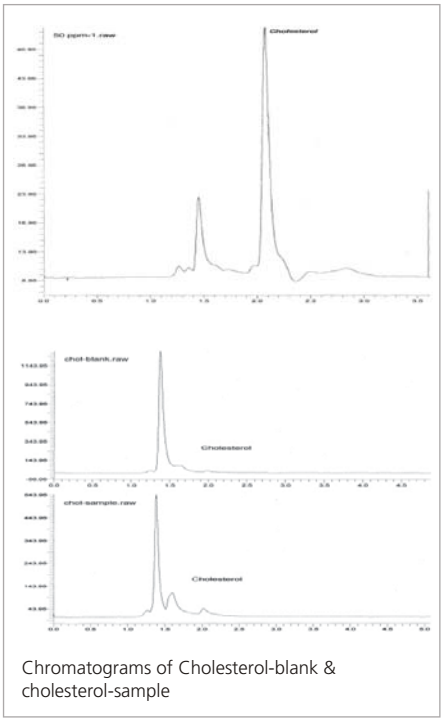
Standard cholesterol (5-α-cholestan-3-β-ol, chromatography grade; from Hi Media) solution was prepared from stock solution (100 ppm solution). The stock solution was used to prepare working standard solution containing 10, 30 and 50 ppm cholesterol in carbon tetrachloride (HPLC grade).

(b) Analysis by High performance Liquid Chromatograph (HPLC):

One gram of milk powder was taken to a 250 ml round bottom flask, little MeOH was added to prevent clumping.

PerkinElmer Flexar HPLC system

50 ml of freshly prepared 2.0 (N) KOH in MeOH was added to it and refluxed for 30 mins. The warm solution was transferred to a 250 ml separatory funnel. The round bottom flask was washed with 2x 25 ml of distilled water and the washings are taken into the separatory funnel. This solution was cooled to room temperature and 10 ml of 10% NaCl was added. The unsaponified fraction was extracted twice with two 100 ml portions of (1:1) diethyl ether and petroleum ether. The ether phases were collected and washed with 0.5 (N) NaOH and repeatedly with distilled water until neutral using Phenolphthalein as indicator. Finally the extract was evaporated to dryness using rotary vacuum evaporator. Final volume was made upto 10 ml in carbon tetrachloride (HPLC grade). For measurement by HPLC, 2 ml of this solution was evaporated to dryness again and volume was made upto 2 ml with the HPLC mobile phase ie., 0.1% isopropanol in n-hexane and measurement was done.



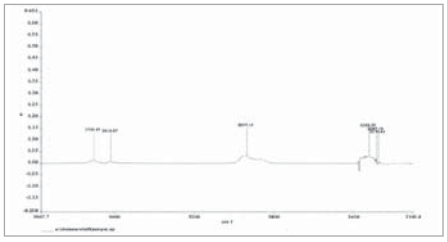
Chromatograms of Cholesterol-blank & cholesterol-sample

In the similar manner another 1g sample was saponified and extracted after spiking 20 ppm cholesterol standard in it. The extract was divided in two parts and measured using both FTIR and HPLC technique.

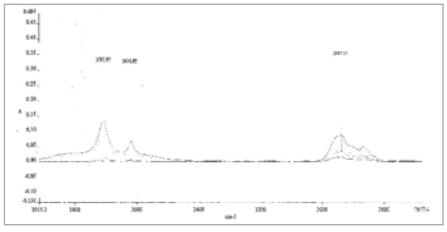
(c) Measurement by Fourier Transform Infra Red Spectrometer:

PerkinElmer Spectrum 100 FTIR spectrometer with 10mm path cell

The FTIR measurements were done using a Perkin Elmer Spectrum 100 model spectrometer in the frequency range 4000 – 400 cm^{-1}. The quantitative method was adopted by using the baseline method. This method automatically corrects the cell absorbance, reflection losses and eliminates possible errors.
The solutions were taken in 10 mm path length cell and scanned from 3100 – 2800 cm^{-1} range. Positive correlations among the peak 2937 cm^{-1} of different concentrations have been observed.



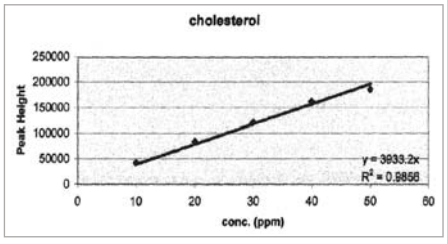
IR spectrum of the sample1

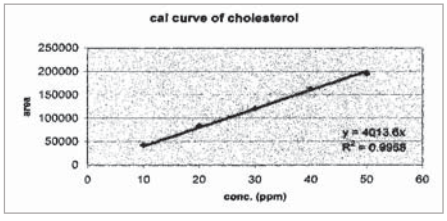


IR spectrum of sample 2

(d) Measurement by HPLC :

The HPLC measurements were done using a Perkin Elmer Series 200 pump and Series 200 UV/Vis variable wavelength detector, a Brownlee C-18 column. The HPLC mobile phase is IPA/Hexane (0.1:99.9 vol/vol flowing at 2 ml/min.). Measurement was done at 205 nm. Injection volume was 20 µl.

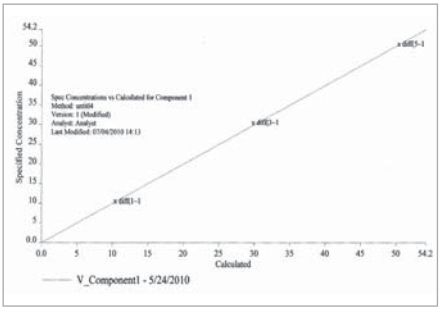




Results and Discussion

A. From the FTIR spectra the following points were noted:
Presence of cholesterol was characterized by large O-H stretching absorption band at 3618 cm^{-1}, characteristic peak for cholesterol at 2937 cm^{-1}.

Calibration curves were drawn considering three components:

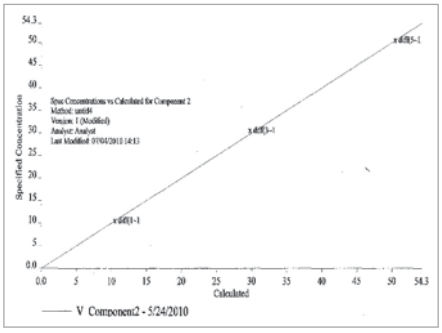


Component 1: 3000 – 2916 cm^{-1}

Slope: 10.0; Intercept: 1.7803;
Correlation : 0.9999

Components	
Name:	Component 1
Units	
Peak Ratio:	No
Calculation Type:	Area
Start:	3000.00
End:	2916.00
Baseline Corrected:	No
Calibration Line	
Slope:	10.0140
Intercept:	1.7803
Correlation:	0.9999
Standard Error:	0.4509
StdErr of Prediction:	0.9575

Standard	Value	Residual
diff (1~1	10.0000	-0.1891
diff (3~1	30.0000	0.3681
diff (5~1	50.0000	-0.1790

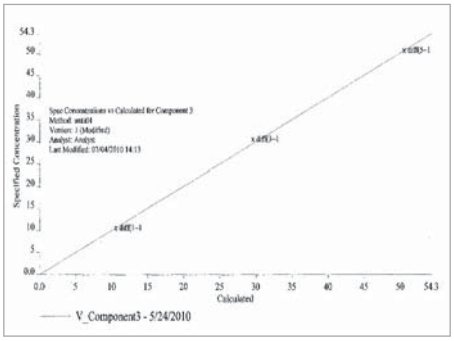


Component 2: 2916 – 2882 cm^{-1}

Slope: 29.54; Intercept: 2.0357;
Correlation: 0.9998

Components	
Name:	Component 2
Units	
Peak Ratio:	No
Calculation Type:	Area
Start:	2916.00
End:	2882.00
Baseline Corrected:	No
Calibration Line	
Slope:	29.5434
Intercept:	2.0357
Correlation:	0.9998
Standard Error:	0.5979
StdErr of Prediction:	1.2709

Standard	Value	Residual
diff (1~1	10.0000	-0.2530
diff (3~1	30.0000	0.4884
diff (5~1	50.0000	-0.2351



Component 3: 2880 – 2834 cm^{-1}
Slope: 28.9194; Intercept: 1.7558;
Correlation: 0.9996

Components	
Name:	Component 3
Units	
Peak Ratio:	No
Calculation Type:	Area
Start:	2880.00
End:	2834.00
Baseline Corrected:	No
Calibration Line	
Slope:	28.9194
Intercept:	1.7558
Correlation:	0.9996
Standard Error:	0.8104
StdErr of Prediction:	1.7254

Standard	Value	Residual
diff (1~1	10.0000	-0.3471
diff (3~1	30.0000	0.6614
diff (5~1	50.0000	-0.3143

The above set data shows a better correlation for the component 1 (3000 – 2916 cm^{-1}).

The spiked sample shows a concentration of 20.353 ppm of cholesterol ie., with a recovery of 101.76%.

B. From the HPLC study the sample shows 20.3 ppm ie., with a recovery of 101.5% of cholesterol.

The two methods were evaluated for accuracy and precision. Linearity was evaluated by a serial dilution of standards by both techniques.

Conclusion

Spectroscopy is one of the major analytical tools for analyzing cholesterol qualitatively as well as quantitatively. An FTIR spectrum is a very sensitive, reliable and less time consuming technique for cholesterol analysis. The quantitative analysis was performed for three consecutive peaks for cholesterol. The results are in strong agreement with the existing HPLC method. Although the method was used for analysis of cholesterol in milk powder, the same method may be extended for analysis of cholesterol in other commodities also like egg, fish, meat etc.

Acknowledgment

The author is grateful to Prof. Barun Gupta. Without his continuous guidance and blessings it would not have been possible to go ahead.

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Analysis of arsenic, cadmium and lead in CHINESE SPICE MIXTURES

Using Graphite Furnace Atomic Absorption Spectrophotometry

Introduction

Spices and herbs are grown widely in various regions of the world and have been used for several purposes since ancient times. Most are fragrant, aromatic and pungent and are used for culinary purposes to improve color, aroma, palatability and acceptability of food (1). In addition, they are also used in folk medicine as antiscorbutic, antispasmodic, tonic, as carminative agents etc. Also, some species are used as tea flavoring agents in several regions. Natural food spices, such as pepper and mustard, have been reported to contain significant quantities of some heavy metals, including cadmium, lead and arsenic. Exposure to trace and heavy metals above the permissible affects human health and may result in illness to human fetus, abortion and preterm labor, as well as mental retardation to children. Adults also may experience high blood pressure, fatigue and kidney and neurological disorders (1). Contamination with heavy metals may be accidental (e.g. contamination of environment during plant cultivation) or deliberate – in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies (2, 3). India and China have a high diversity of plants used as spices, herbs, and traditional medicines. Several herbs and spices are either produced on small farmlands or naturally grow in different regions. There is often little information available about the safety of those plants and their products in respect to heavy metal contamination. Due to the significant amount of spices consumed, it is important to know the toxic metal contents in these spices (4). The objective of this work is two-fold: (1) to accurately analyze the levels of toxic heavy metals like lead, cadmium and arsenic that may be present in some major spice brands available in the local markets in China, by using graphite furnace atomic absorption spectrophotometer (GFAAS); (2) to cross-reference these measured levels to the recommended limits specified by the U.S. FDA.

Analytical Challenges

The instrument which is selected to perform this task should have the best detection limits possible as the regulatory agencies set limits are

extremely low. The spices and herbs samples are usually rich in matrix elements and hence an excellent back ground correction technique should be in place. Long term and short term stability of the signals measured should be some other minimum requirements just like any other analysis.

Experimental

The measurements were performed using a PerkinElmer® AAnalyst™ 800 Atomic Absorption Spectrophotometer (Shelton, CT, USA) equipped with the intuitive WinLab32™ for AA (Version 6.5) software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance. The high-efficiency optical system and solid-state detector used in this spectrophotometer provide outstanding signal-to-noise ratios. This solid-state detector is also highly efficient at low UV and high wavelengths at one time. It also features longitudinal Zeeman effect background correction for graphite furnace analysis. The use of a transversely heated graphite atomizer (THGA) provides uniform temperature distribution across the entire length of the graphite tube. This eliminates the memory effect inherent with high-matrix sample analysis. A Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) was used for the microwave assisted digestion. This is an industrial-type microwave oven which is equipped with various accessories to optimize the sample digestion.

Sample and Certified Reference Material Preparation

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

used for the analysis. Approx. 0.5 g of each sample, accurately weighed in duplicate, was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done. The digested samples were diluted with 0.2% HNO3 and made up to 20 mL in polypropylene vials. The certified reference materials were also digested in a similar manner. Heated injection at 90 ˚C was used for all the furnace experiments. Four-point calibration curves (three standards and one blank) were constructed for all the metal ions and the calibration curve correlation coefficient was ensured to be better than 0.999 before the start of the sample analysis.

Results and Discussions

The validity of the developed method has been ensured by incorporating various quality control (QC) checks and analysis of certified reference materials (CRMs). The agreement between the certified values and the measured values were excellent, which demonstrates the accuracy of the generated calibration as well as the overall accuracy of the developed method. The QC standard gave excellent recovery with a variation of less than 10% usually prescribed by the regulatory bodies. There was virtually no difference between the QC standard which was performed immediately after calibration and the QC standard which was analyzed at the end of the analysis with a time difference of more than three hours. This shows the long-term stability of the instrument. Method detection limits (MDLs) were calculated based on the standard deviation of seven replicates of the reagent blanks (student t-value of 3.14 for a confidence interval of 98%). These limits were obtained under routine operating conditions, and this is not reflective of the optimum detection limits achievable by the system. The extremely lower detection limits obtained show the capability of the AAnalyst 800 spectrometer in analyzing difficult matrices at the measured concentrations.

Conclusions

An accurate and reliable microwave-assisted sample pretreatment procedure for the determination of arsenic, cadmium and lead in spices using GFAAS is described. The spices contain a number of organic substances of different stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution and this also disturbs spectrochemical measurements (5). Application of concentrated HNO3 along with hydrogen peroxide for mineralization of spices and herbs leads

to the complete digestion of samples, which is proven by determined values of the analytes in various CRMs. Toxicity of medicinal spices and herbs, is of much greater concern today than ever before. In recent 5 years, much emphasis is being laid on toxic-element contents, as several European Union countries have banned many varieties of ayurvedic drugs. The results show that the levels of arsenic, cadmium and lead in all the samples analyzed were well within the permissible limits of 10, 0.3 and 10 mg/kg respectively, as specified by the U.S. FDA. The results confirmed that the determination of arsenic, cadmium and lead after acid solubilization of spice-mixture samples by microwave digestion can be performed by GFAAS without any interference and the same has been cross-checked by analyzing a different set of similar samples with ICP-MS analysis (using a PerkinElmer ELAN DRC-e ICP-MS). The good agreement of the values obtained with standard ICP-MS and GFAAS analysis further confirmed the accuracy of the method developed.

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Program used for the digestion of samples

Sequence	Power	Ramp Time (min.)	Hold Time (min.)	Fan
1	1200	15	15	1
2	0		15	3

Method detection limits (MDLs)

Analyte	MDL (µg/kg)
Pb	9
Cd	2
As	6

Results of analysis of spices and herbs using GFAAS

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

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Detailed application note can be viewed at http://las.perkinelmer.com/content/ApplicationNotes/APP_ChineseSpicesbyGFAA.pdf

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Events updates

USP-PerkinElmer Knowledge Seminar on

"Impurities analysis in Pharmaceuticals"

- A success story

Dr. Todd Cecil explaining at Chandigarh

This year between19th and 22nd October 2010 PerkinElmer India joined hands with United States Pharmacopia (USP) for knowledge seminar on "Impurities analysis in Pharmaceuticals". With this PerkinElmer India achieved another landmark in its endeavor to be market centric and reposition it as a knowledge partner. It was for the first time PerkinElmer India partnered with USP or for that matter any regulatory body for the joint knowledge seminar.

As we all know pharma is a regulated market and all pharma customers are keen on tracking and implementing all new regulations by USP and FDA, as a good major part of Indian pharma is engaged in exports. USP has announced new chapters 231 and 232 for elemental impurities in pharmaceuticals. Implementation of this chapter involves use of ICP-MS technology. With new ICP-MS NexION™ 300 PerkinElmer is ready to serve the pharmaceutical industry complying to regulations.

The topic was "Impurities analysis in Pharmaceuticals – USP perspective." Which is a very important for the pharmaceuticals who are manufacturing and complying to the export regulations.

Dr. Todd Cecil (USP, USA) and Mr. Vijay Aswani (PKI, India) were the speakers and kept the entire audience interested with interactive sessions. The selected locations were Chandigarh, Hyderabad and Mumbai. Chandigarh recorded 30 participants, Hyderabad 58 and Mumbai with 75 participants.

Mr. Vijay Aswani at Mumbai

Hyderabad event was attended by 58 participants

Events updates

PerkinElmer at P-MEC CPhI 2010 India Exhibition

PerkinElmer (India) Pvt. Ltd. has taken one more step to focus on the pharmaceutical segment in India; by participating in the CPhI 2010 India exhibition which was concluded on 3rd December 2010. This was one of the big shows with more than 300 companies participated in. We were placed at the P-MEC hall no. 5.

PerkinElmer solutions to Pharmaceutical industries in every process from the drug discovery to packaging were the attraction to many visitors from the industry. This year the theme was "Be compliant be confident" which was most suited to the situation. Unlike the conventional display of the routine instruments we emphasized on the new solutions for pharmaceutical industries.

Hyphenated system with STA6000 coupled with Spectrum 100FTIR, Differential scanning calorimeter DSC8000 and new NexION™300 ICPMS were displayed. The PerkinElmer stall was nicely designed and well illuminated by our designer Ideas 4 U. The exhibition started at 10:00am on 1st December 2010. PerkinElmer booth was inaugurated by Dr. Pramod Dalvi - Sr. General Manager QC operations; Wattson Pharmaceuticals India Pvt. Ltd.

He was welcomed by Mr. Mahadeo Karnik-Director Finance PerkinElmer (India) Pvt. Ltd. Dr. P.S. Jain briefed about the new products and solutions to the Chief guest. There were many visitors at the booth showed keen interest in the solutions. The sales and service teams were busy in explaining the systems and solutions displayed. The response towards the preventive maintenance kits, One Source services was encouraging.

Business managers from the other regions were also participated in the exhibitions and attended the visitors.

Dr. Dalvi Inaugurating the booth

Dr. Jain explaining the NexION™300 ICPMS to the Chief Guest

More visitors

CPhI 2010 was a successful event at the end of the year with the clear message to industry was PerkinElmer for the better.

Events updates

PerkinElmer at the Symposium on "Essential oils, flavors and fragrances chemistry and industry"

The Chromatographic society of India had organized one day symposium on" Essential oils, flavors and fragrances chemistry and industry "at Indian Institute of Technology, Bombay, Powai, Mumbai. This was the first of its kind event where the industry and academicians came together for cultivating more innovations in this segment.

Many leading cosmetic and perfumery industries participated in the symposium and exchanged their views to grow the demand as the improvement in the lifestyle of Indians.

The presence of PerkinElmer was very well noticed by many academicians and industry partners. The major attraction of this symposium was the technical presentations and innovations. The chromatography is a major technique used by this industry across the globe but some of the Indian counterparts are still following the conventional ways. Hence every one was curious about this session.

Dr. Padmaja Prabhu presented the applications for various cosmetic products analysis with the GCMS HS and ATD technologies; which can be used without the sample preparations. During the symposium PerkinElmer also had hosted a small booth with solutions to this industry.

After the presentations we received good visitors enquiring about more information about the technology. The organizers appreciated the participants and sponsorer in the hands of Prof. Devang Khakkar-Director IIT Bombay by awarding memento and certificate.

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