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APPLICATION NOTE 10339

Determination of phthalates in liquor beverages by single quadrupole GC-MS

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Keywords

Environmental analysis, food safety, alcoholic beverage, phthalates, PAEs, liquor gas chromatography, ISQ 7000, single quadrupole mass spectrometry

Goal

Demonstrate a sensitive, rapid, and accurate GC-MS method to detect trace level of phthalate esters in beverages with high alcoholic content.

Introduction

Phthalates (Phthalate Acid Esters, PAEs) have widespread use in the polymer industry as plasticizers and softeners to increase the plasticity of polymer materials and their toughness and strength. They are chemically inert, have high density, low to medium volatility, high solubility in organic solvents, and are easily released in the environment during aging of polymer materials. Phthalates had been reported as functional solvents in the aromatic, essential oil, and even beverage industries. Phthalate plasticizers could also migrate from plastic containers or closures into soft drinks and alcoholic beverages.

PAEs in the environment and food chain can act as hormones, simulate the body's natural endocrine responses, interfere with the normal role of hormones, and affect the body's most basic physiological control mechanisms.[1] Phthalates are reported to cause carcinogenic, teratogenic, and mutagenic effects and constitute a health hazard to humans.

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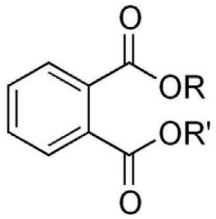

Phthalate residues in food and beverages are regulated internationally. The China Ministry of Health issued a public notice on June 1st, 2011, that phthalate esters are clearly prohibited as non-food substances for use in food. PAEs are introduced into the food chain primarily through food packaging material. Alcoholic beverages in plastic containers are a particular risk, since the containing ethanol provides a very good solubility for PAEs and is leaching the PAEs into the beverages from the plastic contact materials. The contamination risk increases with liquors having high ethanol content. On November 19th, 2012, Chinese media reported that, according to third-party testing, PAE plasticizer content in a well-known domestic liquor brand was up to 260% higher than the regulated level.

In this study, the sample preparation procedure was optimized with ethanol removal from liquor beverages followed by n-hexane extraction and gas chromatography/mass spectrometry (GCMS) detection. The method is sensitive, rapid, and accurate, and covers a wide linear range to meet the need for trace level detection of phthalate esters in different types of beverages.

Experimental conditions

Sample preparation

The sample used for this application was a white spirit, bought from a local liquor store. An accurate amount of 5.0 mL sample was transferred in a glass centrifuge tube and then heated in a boiling water bath to remove the ethanol.[2] The heating time depends on the alcoholic strength of the sample. Usually the tube was removed from the water bath with a residual volume of 2–3 mL. After cooling to room temperature, 2.0 mL of n-hexane was added, and the glass tube was shaken for extraction and left standing 5 minutes for phase separation. The supernatant was transferred to autosampler vials for analysis.

A commercial phthalate standard was used for method development. For optimization of the extraction procedure and recovery determination, one liquor sample was spiked with 4 mg/L concentration of the phthalate standard.

GC-MS instrument conditions

All measurements have been carried out using the Thermo Scientific™ ISQ Series single quadrupole GC-MS system with a Thermo Scientific™ TRACE™ 1310 GC system* equipped with the Thermo Scientific™ Instant Connect Split/Splitless (SSL) Injector and a Thermo Scientific™ AI/AS 1310 Series Autosampler. The instrument conditions are listed in Tables 1 and 2.

*Equivalent or better results are obtained with the ISQ 7000 single quadrupole GC-MS system.

Table 1. Gas chromatograph and injector conditions

Column type:	Thermo Scientific™ TraceGOLD TG-35MS column
Column dimensions:	30 m × 0.25 mm × 0.25 µm (P/N 26094-1420)
Injector type:	SSL
Injector temperature:	280 °C
Injection mode:	Splitless
Injection volume:	1 µL
Carrier gas, flow:	Helium, constant flow 1 mL/min
Oven program:	80 °C, 1 min 10 °C/min to 280 °C 280 °C, 10 min
Transfer line temperature:	280 °C

Table 2. MS system conditions

Ionization:	ExtractaBrite EI, 70 eV
Scan mode, range:	Full-scan, 50–350 Da
Acquisition rate:	0.2 s
Ion source temperature:	280 °C

Sample measurements

First, the elution order of the phthalate compounds was determined by analyzing a standard mixture at medium concentration. The spectra observed were compared with the NIST data base for identification and retention time determination.

The compound quantitation was performed by selecting the most intense and unique ions of the compounds providing selective mass chromatograms for individual peak integration.

Finally, eight commercial liquor samples from a local liquor shop were prepared by the described sample preparation method for determining possible contamination by phthalate esters.

Optimization of the liquor sample extraction

Chinese liquor typically contains between 30 to 60 vol% ethanol. Phthalate esters are highly soluble in ethanol, so the extraction of phthalate esters using n-hexane as solvent is less effective.[2] The removal of the major part of ethanol from the liquor before n-hexane extraction is necessary to avoid low recoveries.

Accurately measured 5.0 mL liquor samples were transferred into glass tubes. Then the standard solution was added to obtain a spiked solution at 0.80 mg/L concentration level. Figure 1 shows chromatograms of spiked sample and standard mixture runs. The experiment results were compared with and without ethanol removal. The results from the recovery experiment are shown in Table 3. After removal of ethanol before the extraction with n-hexane, good and consistent recoveries of the phthalate compounds in the range of 89–112% were obtained.

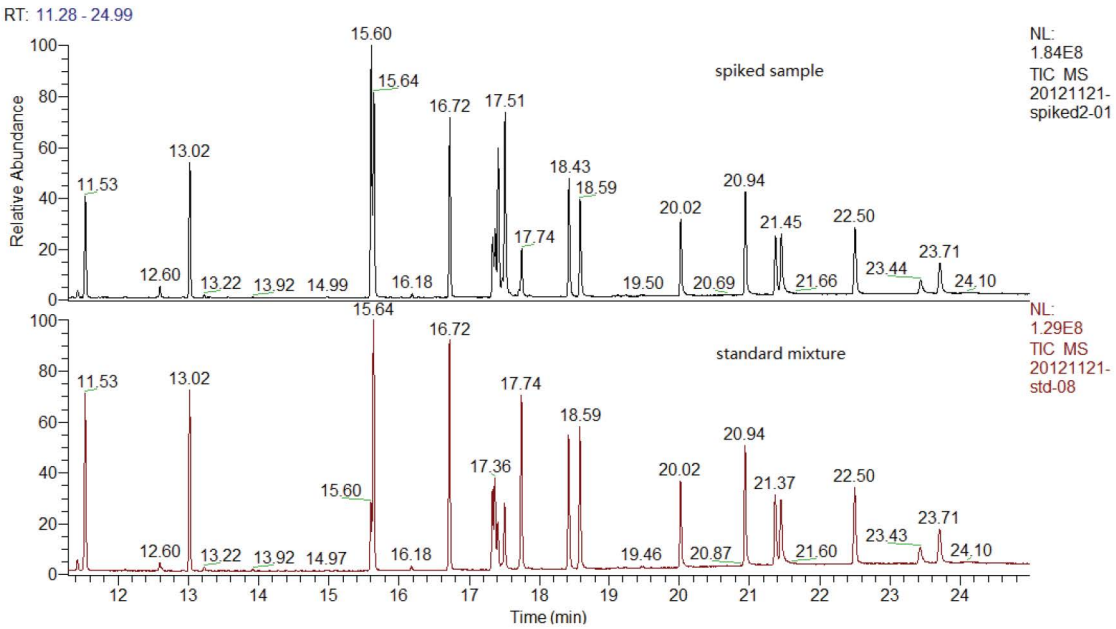

Figure 1. Chromatograms of a spiked sample at 0.8 mg/L (top) and of the mid-level standard mixture run (bottom)

Table 3. Comparison of recovery of phthalates from liquor without and with prior removal of ethanol before extraction

Compound	CAS #	Abbreviation	Without ethanol removal Recovery (%)	With ethanol removal Recovery (%)
Dimethyl phthalate	131-11-3	DMP	60.0	102.0
Diethyl phthalate	84-66-2	DEP	35.4	107.0
Diisobutylphthalate	84-69-5	DIBP	99.5	94.4
Di-n-butyl phthalate	84-74-2	DBP 1	06.0	104.0
Di-(4-methyl-2-pentyl) phthalate	146-50-9	DMPP	99.7	95.1
Di-(2-methoxy)-ethyl phthalate	117-82-8	DMEP	3.4	88.8
Diamylphthalate	131-18-0	DPP	109.0	108.0
Di-(2-ethoxy)-ethyl phthalate	605-54-9	DEEP	13.6	103.0
Dihexylphthalate	68515-50-4	DHP	104.0	101.0
Butylbenzyl phthalate	85-68-7	BBP	88.4	108.0
Di-(2-ethylhexyl) phthalate	117-81-7	DEHP	106.0	108.0
Di-(2-butoxy)-ethylphthalate	117-83-9	DBEP	83.1	104.0
Dicyclohexyl phthalate	84-61-7	DCHP	94.8	102.0
Di-n-octylphthalate	117-84-0	DNOP	103.0	106.0
Diphenyl phthalate	84-62-8	DPhP	77.1	112.0
Dinonylphthalate	84-76-4	DNP	110.0	109.0

Results

In the following, the detection of five components of the phthalate standard mixture is shown as an example of the investigated PAE compounds listed in Table 3. Although the full scan chromatograms shown in Figures 2–7 give high background signals and include the elution of many other compounds dissolved in the spirit, the selective mass traces of the major phthalate ions allow a very good selectivity for reliable peak area integration.

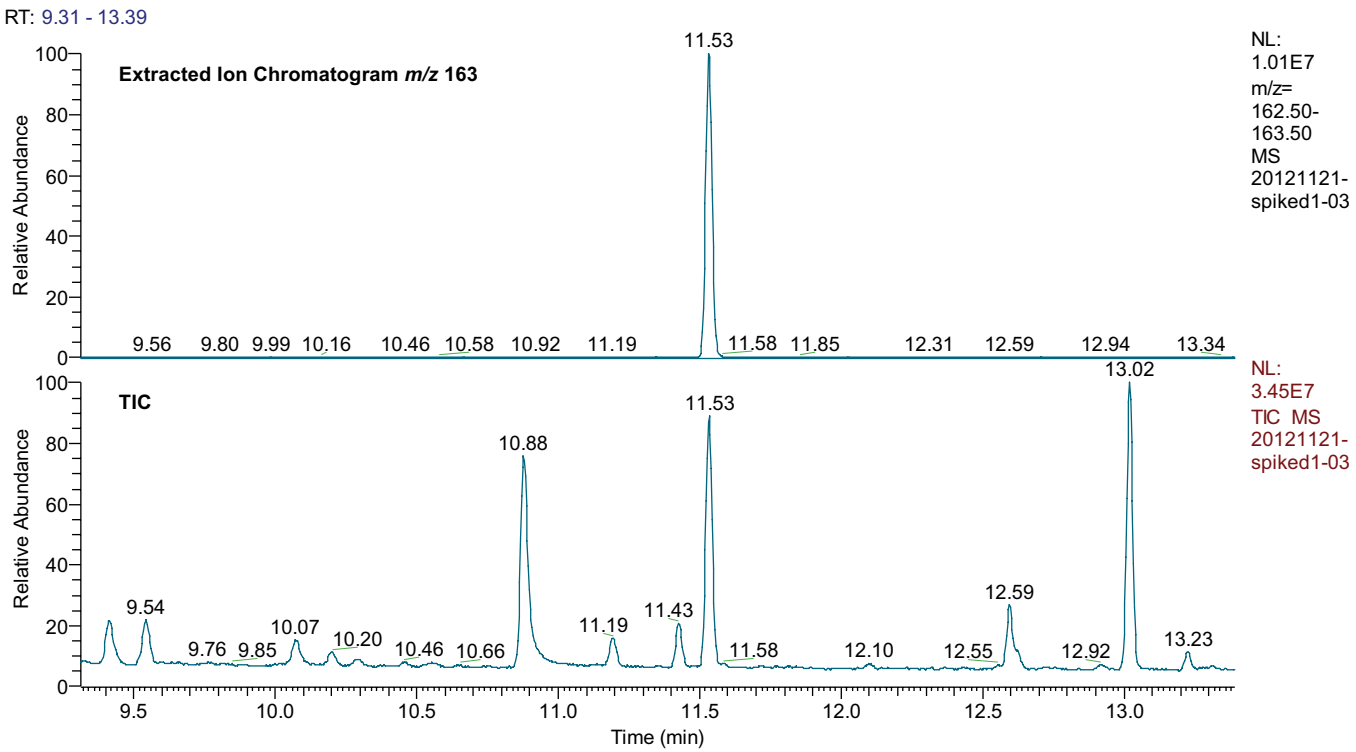

Figure 2. Dimethyl-phthalate chromatograms from spiked sample with the selective mass chromatogram (top) and the full-scan trace (bottom) allowing the interference-free peak area integration of the PAE compound

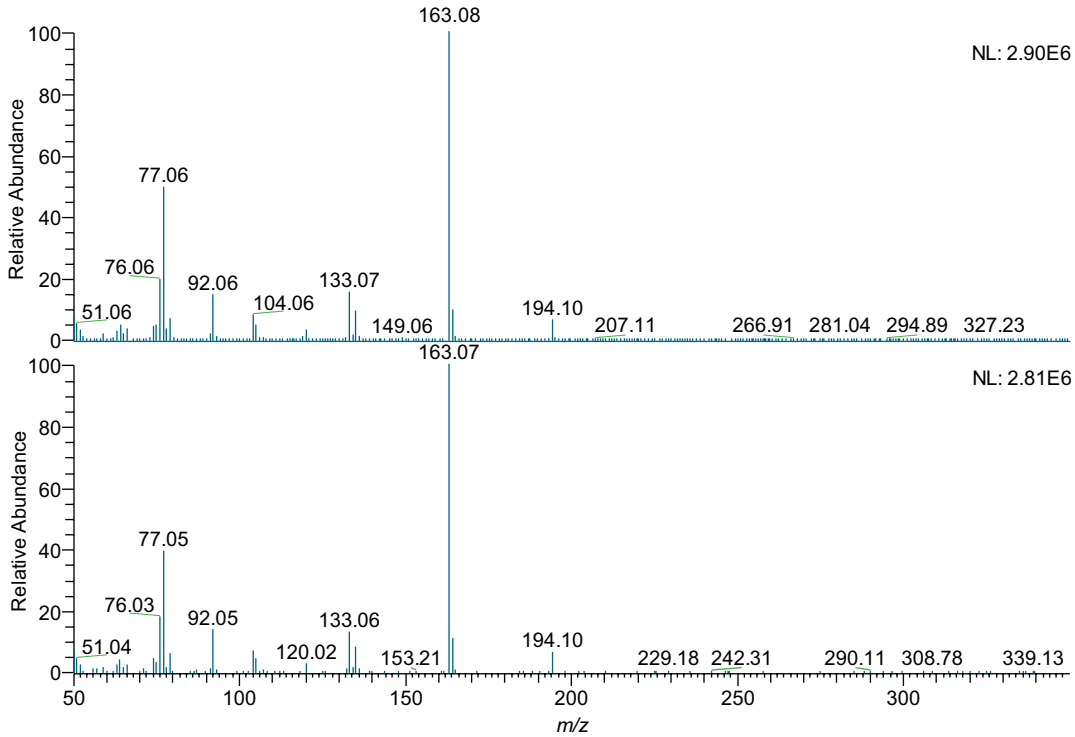

Figure 3. Dimethyl-phthalate EI spectra from standard (top) and sample (bottom)

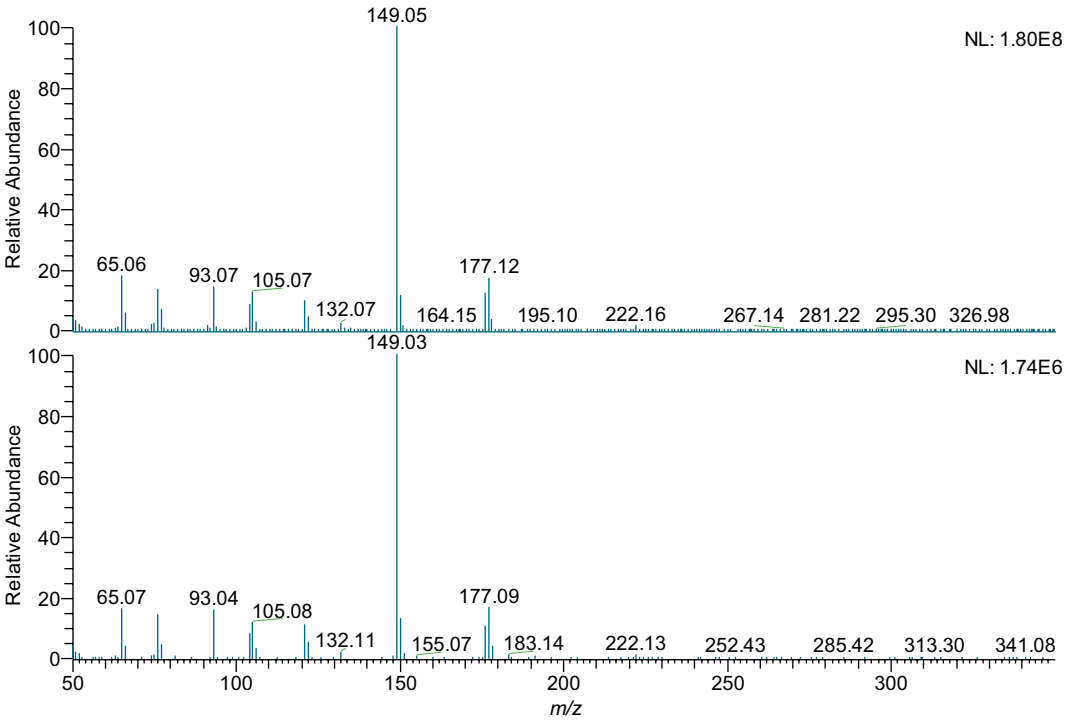

Figure 4. Diethyl-phthalate EI spectra from standard (top) and sample (bottom)

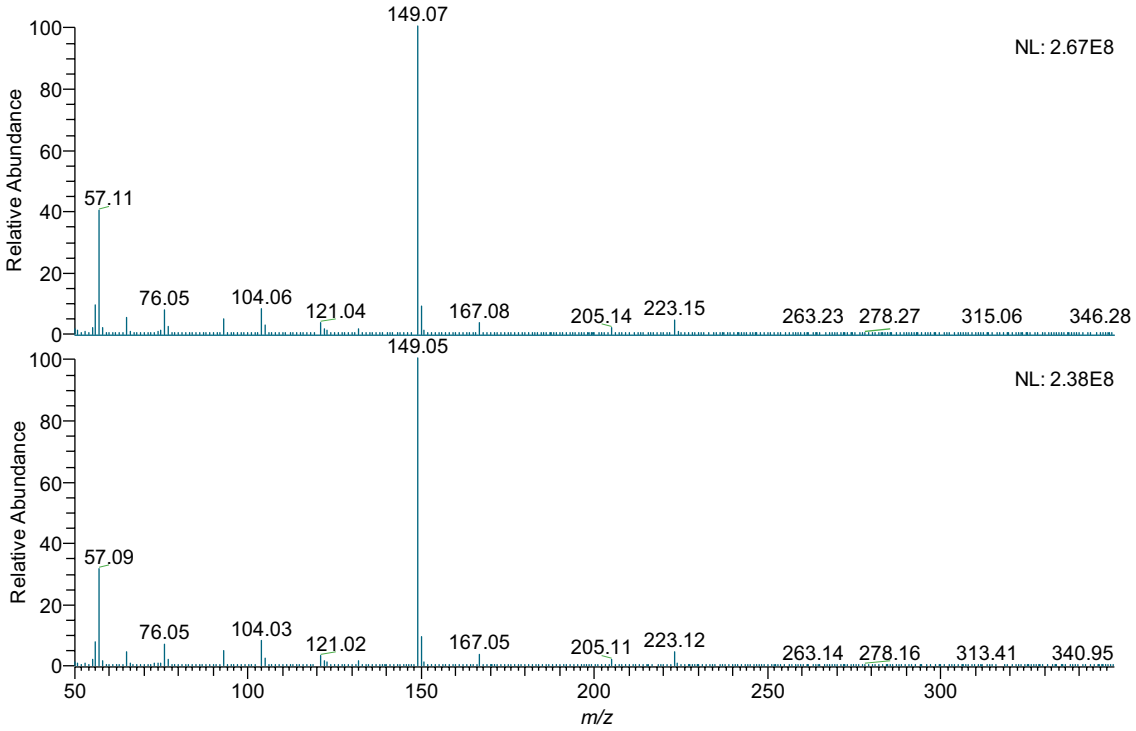

Figure 5. Di-isobutyl-phthalate EI spectra from standard (top) and sample (bottom)

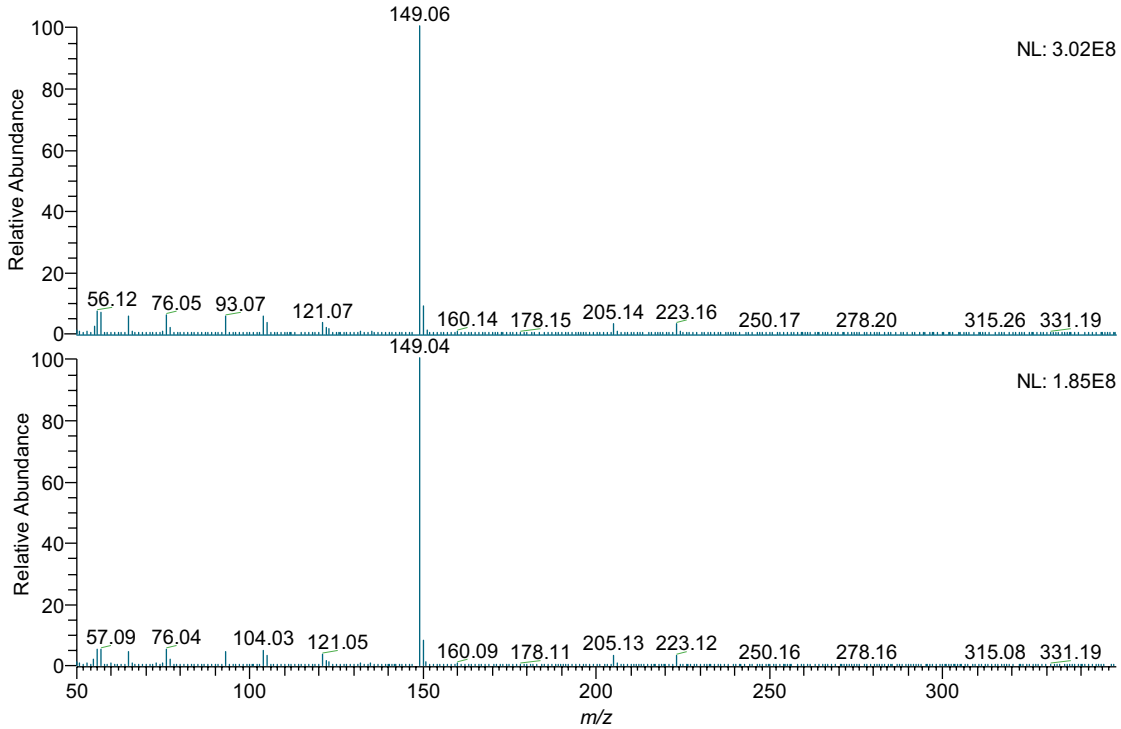

Figure 6.Dibutyl-phthalate EI spectra from standard (top) and sample (bottom)

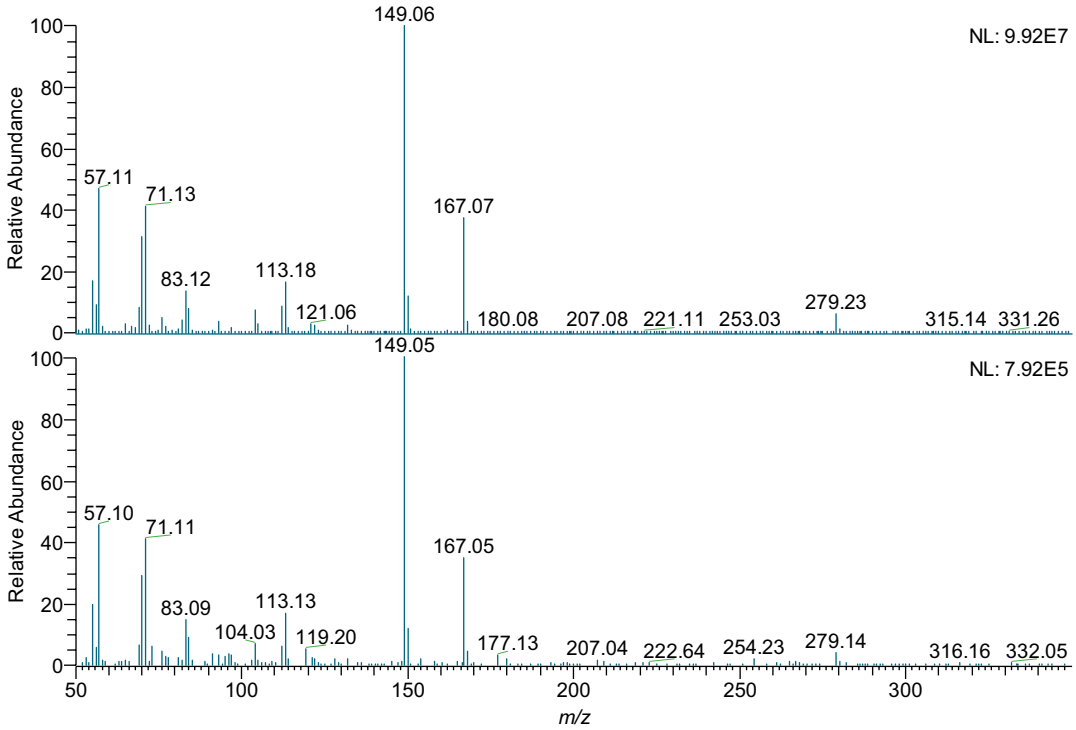

Figure 7. Di-(2-ethylhexyl) phthalate EI spectra from standard (top) and sample (bottom)

The mass spectra shown in Figure 8 are taken for comparison to confirm the compound identity from the analysis of the spiked sample and the standard run.

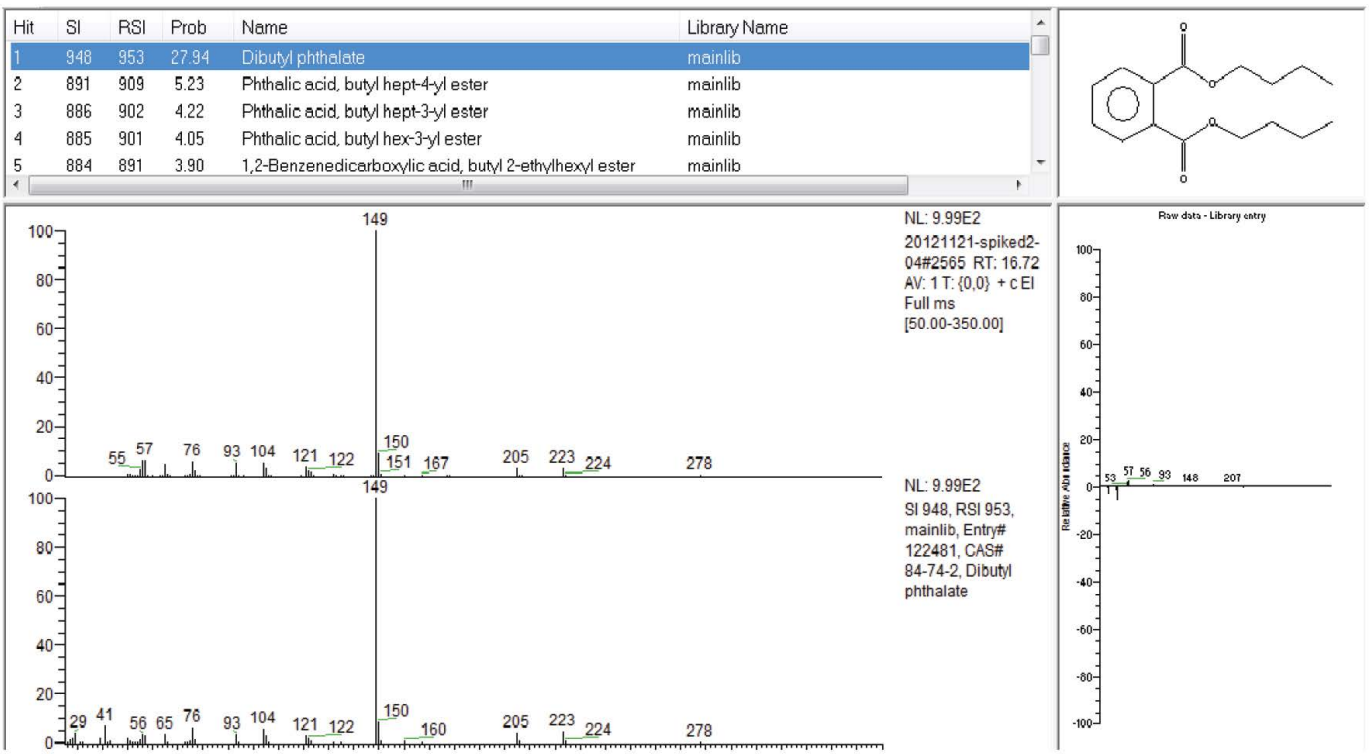

Figure 8. Comparison of spectra between the spiked sample (top) and NIST library (bottom) indicating excellent spectral match value.
Similarity Index = SI, Reversed Search Index = RSI

Quantitation

A series of matrix spiked samples with five different concentrations was prepared in the range of 0.10 to 4.00 mg/L. The samples were injected in sequence from low to high concentration. The peak areas were used to plot linear regression responses and average R^2 values of 0.999 were obtained for all PAE compounds. The results for 15 phthalate esters show a very good linear relationship in the full calibration range of 0.10 to 4.00 mg/L (Figure 10).

The dinonyl-phthalates (DNP) posed a special analytical challenge. The DNPs typically consist of a mixture of technical C9-isomers that cannot be chromatographically separated using a single dimension GC column. Hence the response obtained of DNP is distributed to individual isomers. The integration of the unresolved DNP chromatographic peak needs to be performed over a wider but constant retention-time range, as shown in Figure 9. This way, a linear calibration range for DNP of 0.40 to 4.00 mg/L could be achieved, allowing for accurate quantitation of this compound.

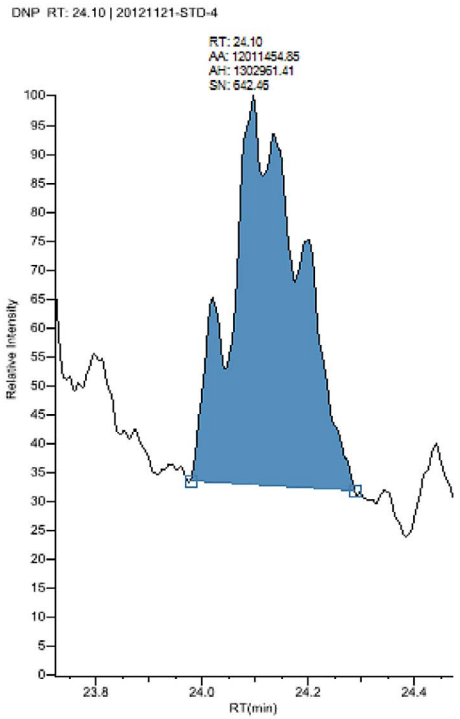

Figure 9. Quantitation peak of the unresolved DNP isomers over a set retention time range using the TraceFinder software (extracted mass 149 *m/z*)

Sensitivity

The determination of the limit of detection (LOD) and limit of quantitation (LOQ) were based on the characteristic extracted ion mass chromatograms with a peak signal-to-noise ratio S/N ≥ 3 for LOD, and S/N ≥10 for LOQ, as given in Table 4. For the individual phthalate compounds.

Table 4. Phthalate quantitation: linear range with limit of detection (LOD) and limit of quantification (LOQ), average R^2 0.9990

Compound name	Retention time [min]	Quantitation ion [*m/z*]	Linear range [mg/L]	Correlation coefficient R^2	LOD [µg/L]	LOQ [µg/L]
DMP	11.53	163	0.1–4.0	0.9994	0.1	0.3
DEP	13.02	149	0.1–4.0	0.9999	0.1	0.3
DIBP	15.64	149	0.1–4.0	0.9981	0.1	0.3
DBP	16.72	149	0.1–4.0	0.9986	0.1	0.3
DMPP	17.33/17.36	149	0.1–4.0	0.9993	0.2	0.6
DMEP	17.74	59	0.1–4.0	0.9984	0.2	0.6
DPP	18.43	149	0.1–4.0	0.9996	0.1	0.3
DEEP	18.59	72	0.1–40	0.9996	0.1	0.3
DHP	20.02	149	0.1–4.0	0.9990	0.1	0.3
BBP	20.94	149	0.1–4.0	0.9998	0.2	0.6
DEHP	21.37	149	0.1–4.0	0.9969	0.2	0.6
DBEP	21.45	149	0.1–4.0	0.9993	0.5	1.5
DCHP	22.50	149	0.1–4.0	0.9985	0.2	0.6
DOP	23.43	149	0.1–4.0	0.9998	0.5	1.5
DPhP	23.70	225	0.1–4.0	0.9988	0.2	0.6
DNP	24.0-24.4	149	0.4–4.0	0.9983	50	150

Figures 10-1 and 10-2 show the calibration curves of 16 PAE compounds.

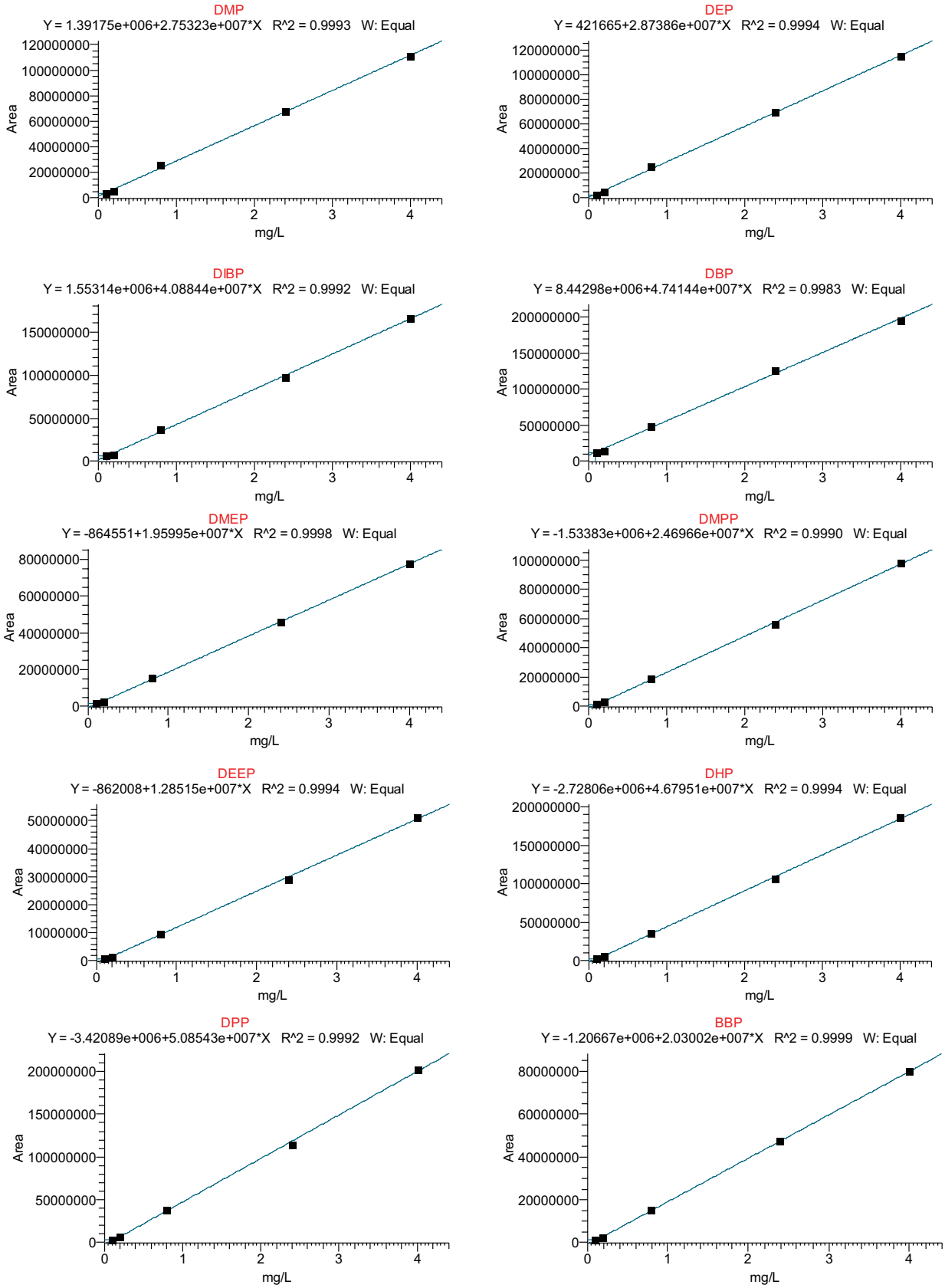

Figure 10-1. Calibration curves of 16 PAEs

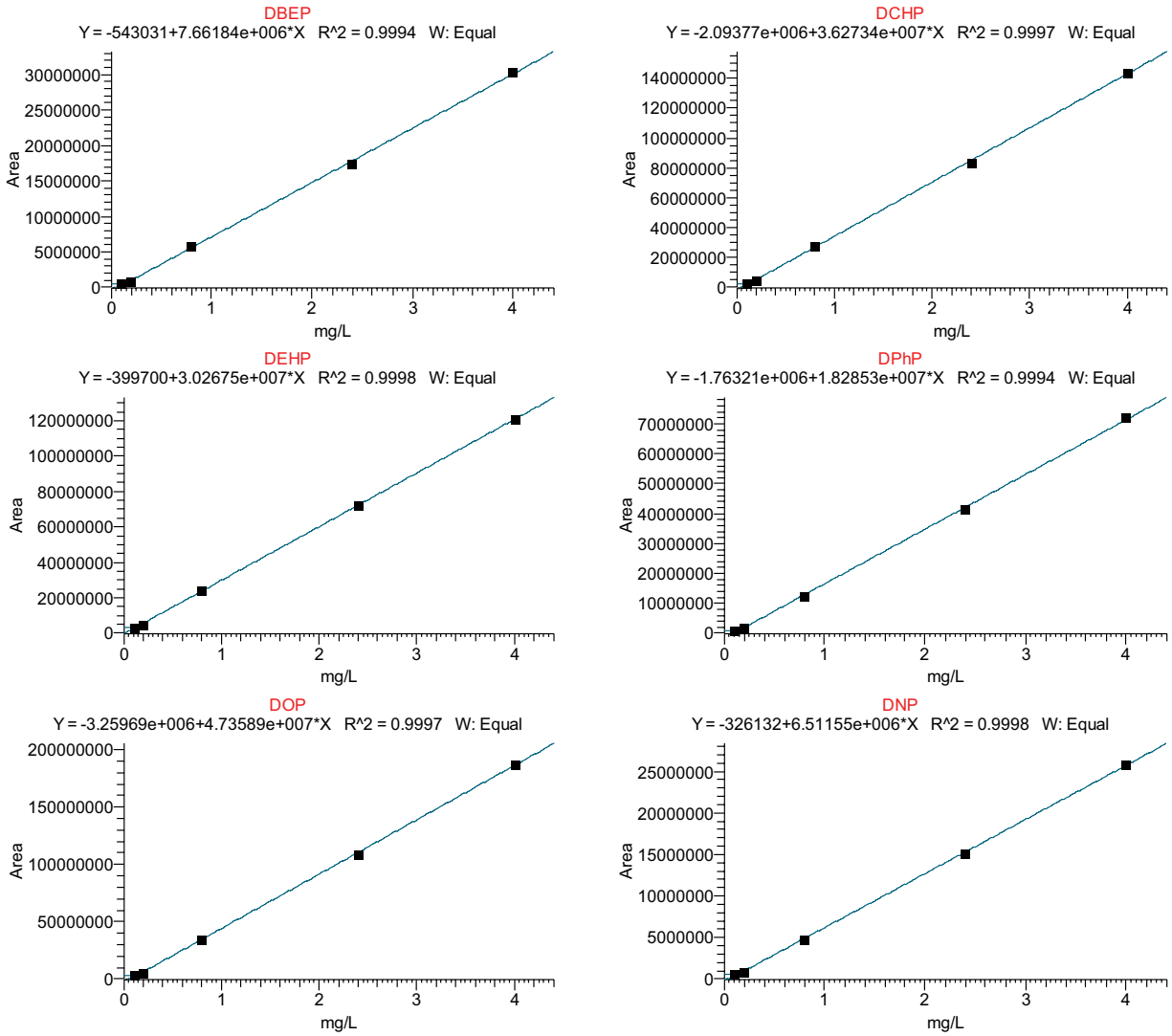

Figure 10-2. Calibration curves of 16 PAEs

Method precision and determination of recovery at trace level

The measured liquor samples were spiked by two low concentration levels at 0.1 and 0.3 mg/L, and measured five times at each level. The results show that the average recovery even at trace level was 83.2–110%, and the relative standard deviation range (RSD, n=5) was 1.3 to 8.4%. The recovery and precision data results are shown in Table 5.

Eight samples of commercially available liquor brands were analyzed using the above described method. The concentrations of phthalate ester residues found are shown in Table 6. The samples tested showed that DIBP, DBP, DEHP are prevalent, and DEHP was found in all the analyzed white wine samples.

Table 5. Method recovery and precision data at trace level (average recovery 103%)

Compound name	Spike level 0.1 mg/L		Spike level 0.3 mg/L	
	Recovery %	RSD %	Recovery %	RSD %
DMP	95.0	5.4	99.0	4.7
DEP	103.0	5.5	108.0	2.2
DIBP	101.0	2.0	101.0	3.2
DBP	107.0	6.6	101.0	1.3
DMPP	105.0	3.3	107.0	5.7
DMEP	86.3	5.3	83.2	3.4
DPP	109.0	6.0	104.0	1.6
DEEP	103.0	4.1	104.0	3.2
DHP	104.0	4.6	109.0	3.7
BBP	110.0	3.6	103.0	3.7
DEHP	102.0	1.4	105.0	4.1
DBEP	104.0	5.0	108.0	4.6
DCHP	103.0	4.1	103.0	3.6
DOP	105.0	5.8	104.0	2.6
DPhP	108.0	4.2	109.0	1.8
DNP	107.0	8.4	101.0	5.4

Table 6. The phthalate ester concentration in eight commercial liquor samples (mg/L)

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
DMP	ND	0.303	ND	ND	0.005	ND	ND	0.025
DEP	ND	ND	ND	ND	0.011	ND	ND	ND
DIBP	ND	1.526	ND	1.373	0.106	ND	ND	ND
DBP	ND	1.024	0.045	0.656	0.133	ND	0.469	0.064
DMPP	ND	ND	ND	ND	ND	ND	ND	ND
DMEP	ND	ND	ND	ND	ND	ND	ND	ND
DPP	ND	ND	ND	ND	ND	ND	ND	ND
DEEP	ND	ND	ND	ND	ND	ND	ND	ND
DHP	ND	ND	ND	ND	ND	ND	ND	ND
BBP	ND	ND	ND	ND	ND	ND	ND	ND
DEHP	0.086	0.029	0.010	0.236	0.014	0.006	0.017	0.016
DBEP	ND	ND	ND	ND	ND	ND	ND	ND
DCHP	ND	ND	ND	ND	ND	ND	ND	ND
DOP	ND	ND	ND	ND	ND	ND	ND	ND
DPhP	ND	ND	ND	ND	ND	ND	ND	ND
DNP	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected

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Conclusions

In this study, a complete workflow for the determination of phthalates plasticizer residues in highly alcoholic beverages has been validated to deliver high recovery and sensitivity for reliable routine quantitation.

The sample preparation method for alcoholic beverages was quick and easy to accomplish. Using n-hexane as extraction solvent provided consistently high recoveries after removal of most methanol, even at trace level. The ISQ Series GC-MS system* measurement method is highly accurate as demonstrated with precise calibrations and spiked liquor samples.

The ISQ Series GC-MS system method set-up using full-scan has good usability, provides the necessary high sensitivity, and delivers the complete spectrum information for identification and confirmation of a wide variety of possible phthalate ester contaminations by comparison with the NIST mass spectral library. The peak area integration on the uniquely selective PAE compound ions permits a precise, fast, and interference-free quantitative determination.

The routine quantitation of commercial samples is easily accomplished using TraceFinder software, which allows the quantitation of the coeluting DNP isomers with the same high precision as the other PAE compounds under investigation.

The described procedure for phthalate plasticizers using the ISQ Series GC-MS system* is very sensitive and accurate. It is easy to perform, rapid, and covers a wide linear range to meet the need for trace level detection of PAEs in beverages.

*Equivalent or better results are obtained with the ISQ 7000 single quadrupole GC-MS system.

References

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