# Determination of Phthalates in Liquor Beverages by Single Quadrupole GC-MS

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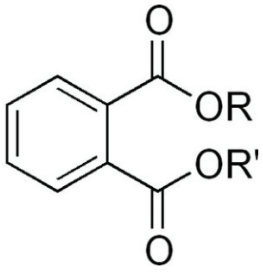
## Introduction

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 to 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 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. Phthalates are reported to cause carcinogenic, teratogenic, and mutagenic effects and constitute a health hazard to humans.




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.

This study follows the China regulation GB/T 21911-2008 for the determining of phthalates in food[1].The sample preparation procedure was optimized from GB/T 21911-2008 with the ethanol removal from liquor beverages followed by an n-hexane extraction and gas chromatography/mass spectrometry (GC-MS) 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 spirit 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 equipped with the instant connect SSL injector module (split/splitless injector) and a Thermo Scientific AS 1310 liquid autosampler. The instrument conditions are listed in Tables 1 and 2.

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Table 1. GC conditions

| | |
|---|---|
| **Column type** | Thermo Scientific™ TraceGOLD TG-35MS |
| **Column dimensions (length × i.d. × film thickness)** | 30 m × 0.25 mm × 0.25 µm |
| | P/N 26094-1420 |
| **Injector type, Temperature** | SSL, 280 °C |
| **Injection mode, Volume** | Splitless, 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 Conditions

| | |
|---|---|
| **Ionization** | 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.

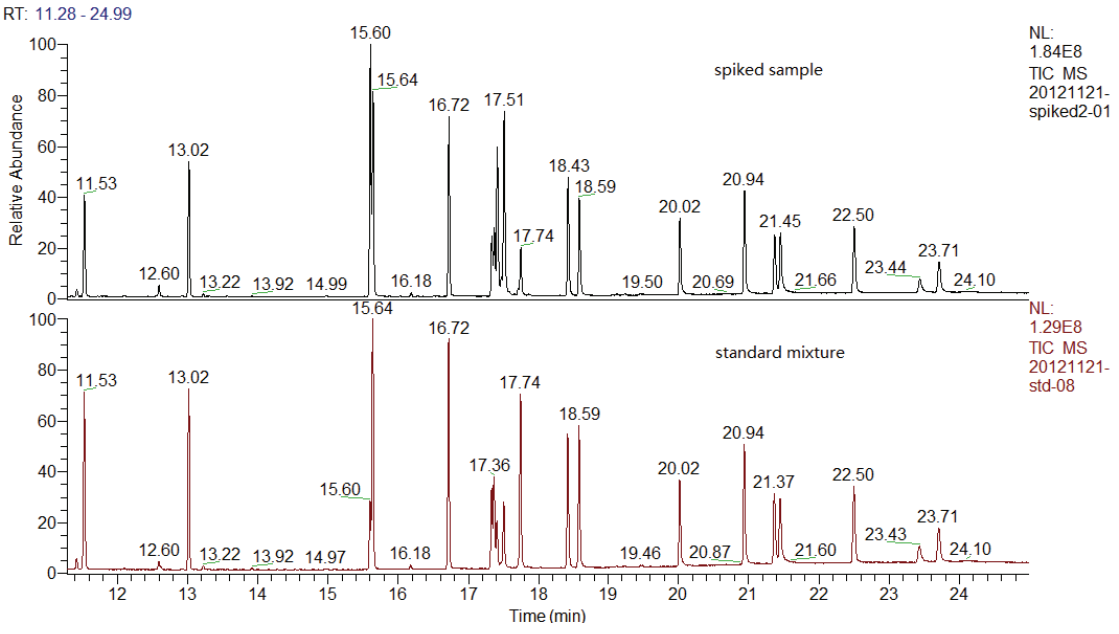



Figure1. Chromatograms of a spiked sample (top) and of the standard compounds run (bottom).

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 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 extractionis 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.

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

| Compound | CAS # | Abbreviation | With outethanol 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 | 106.0 | 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 | 3.38 | 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.

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.

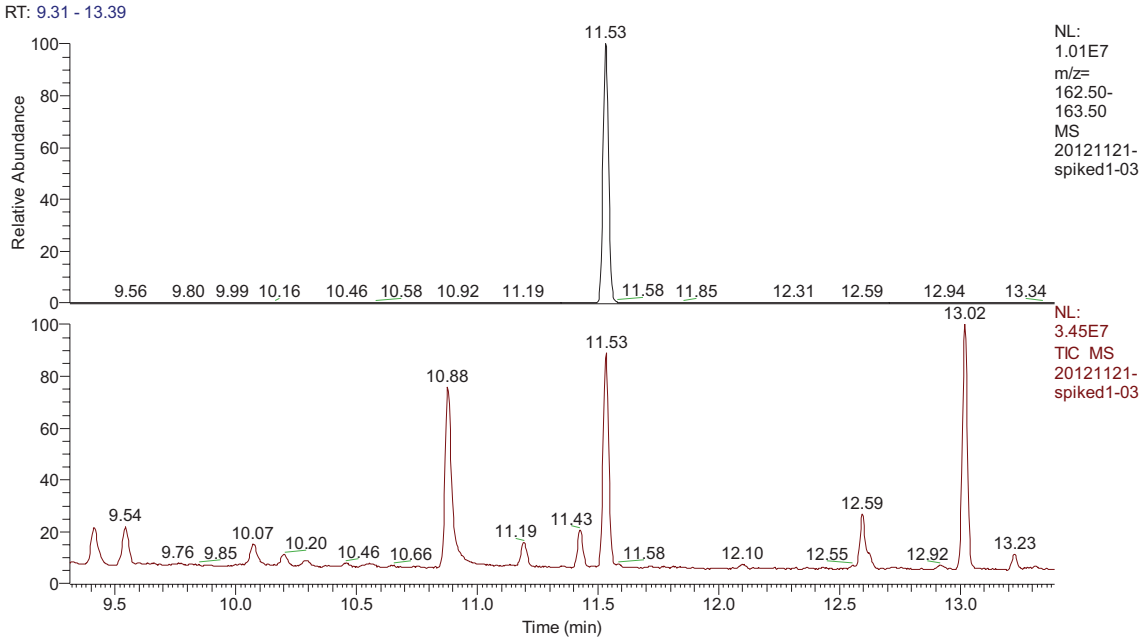



Figure 2. Dimethyl-phthalate chromatograms from spiked sample with the selective mass chromatogram (top) and the Full Scan trace (bottom) allowing the interference free peak area integration of the PAE compound.

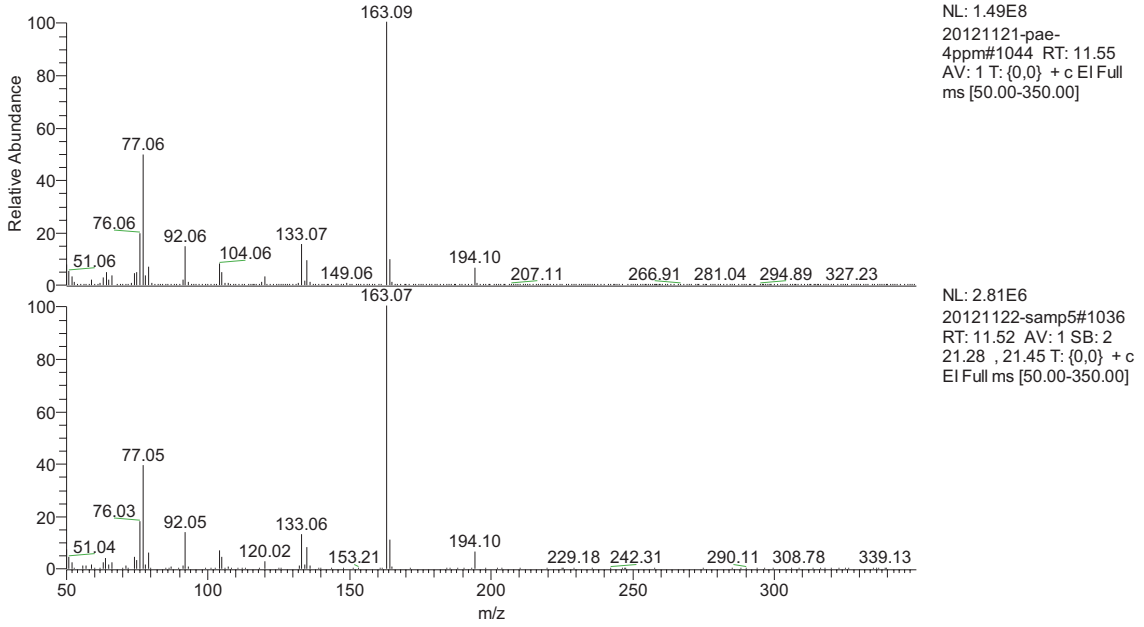



Figure 3. Dimethyl-phthalate spectra from standard (top) and sample (bottom).

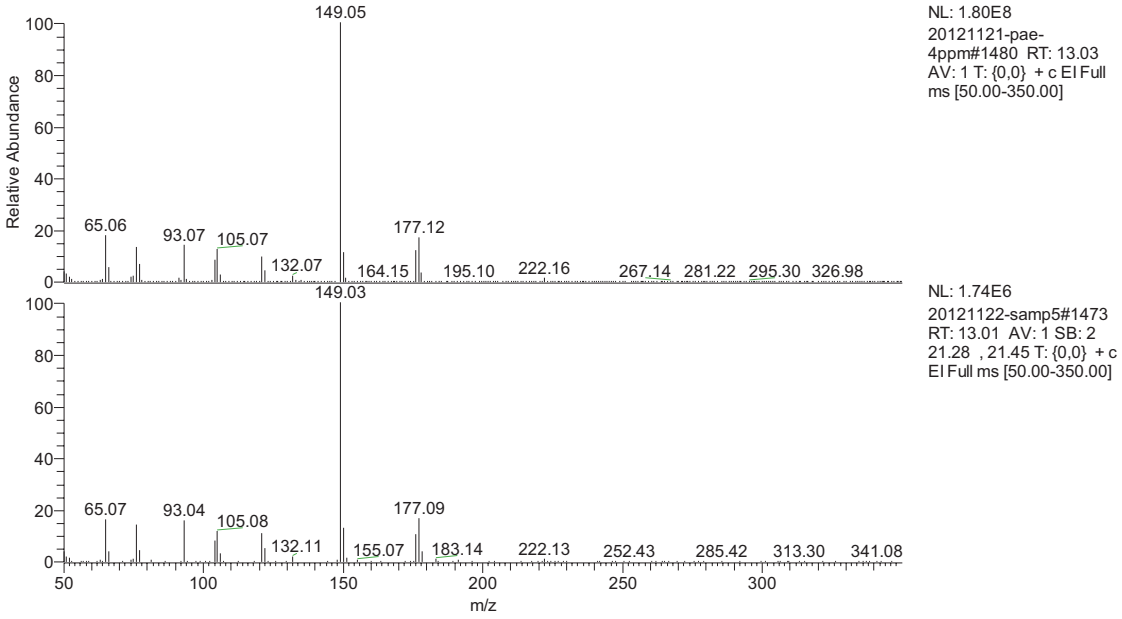



Figure 4. Diethyl-phthalate spectra from standard (top) and sample (bottom).

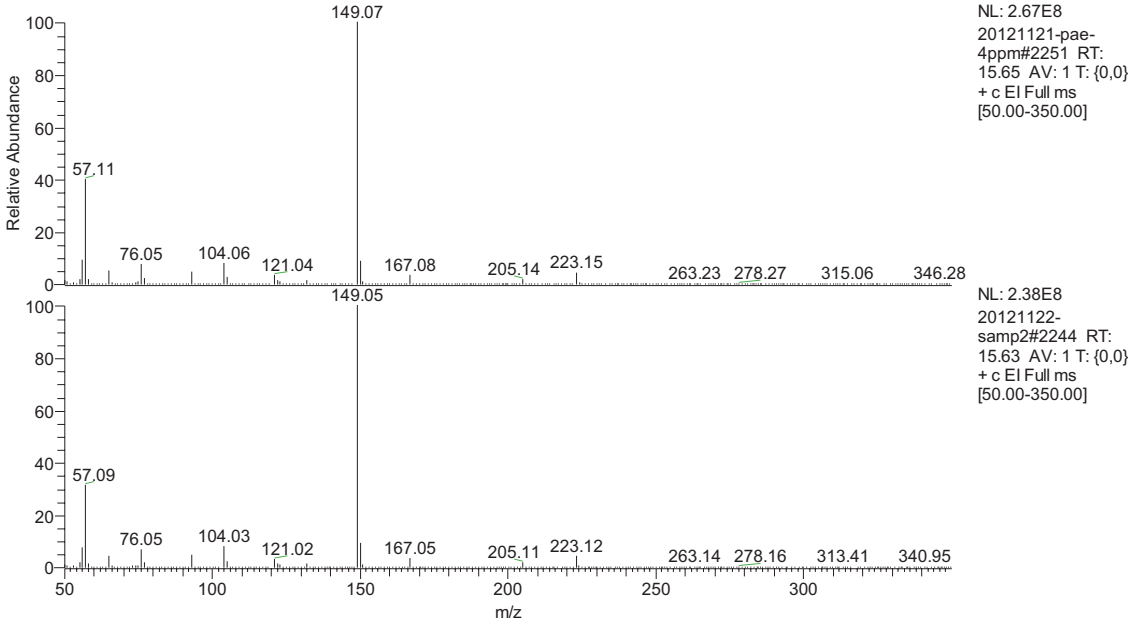



Figure 5. Di-isobutyl-phthalate spectra from standard (top) and sample (bottom).

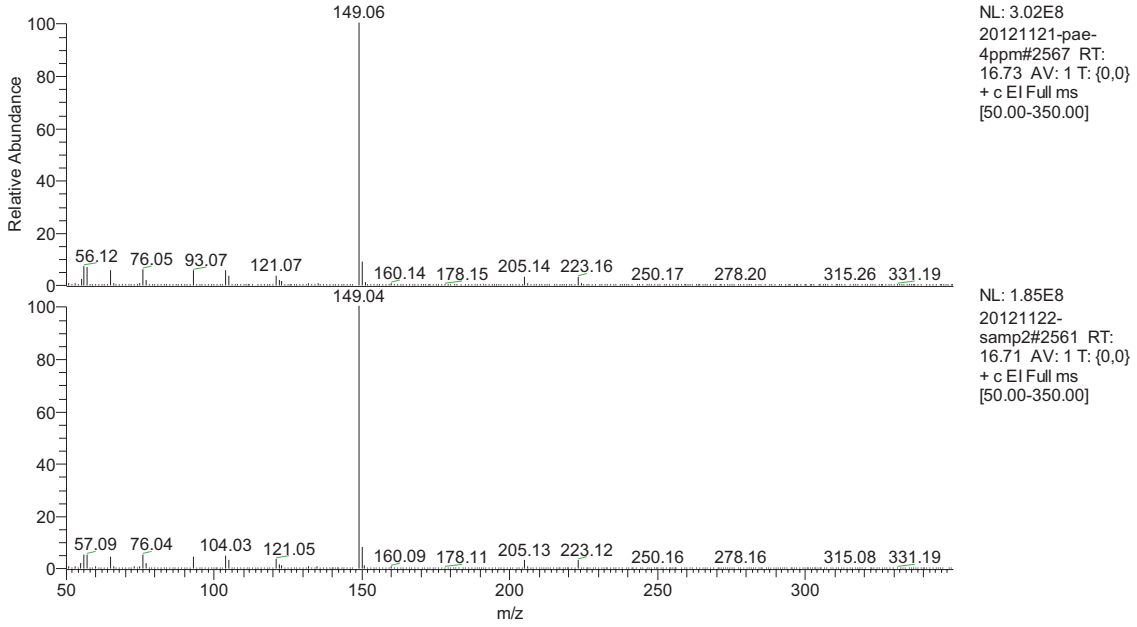



Figure 6.Dibutyl-phthalate spectra from standard (top) and sample (bottom).

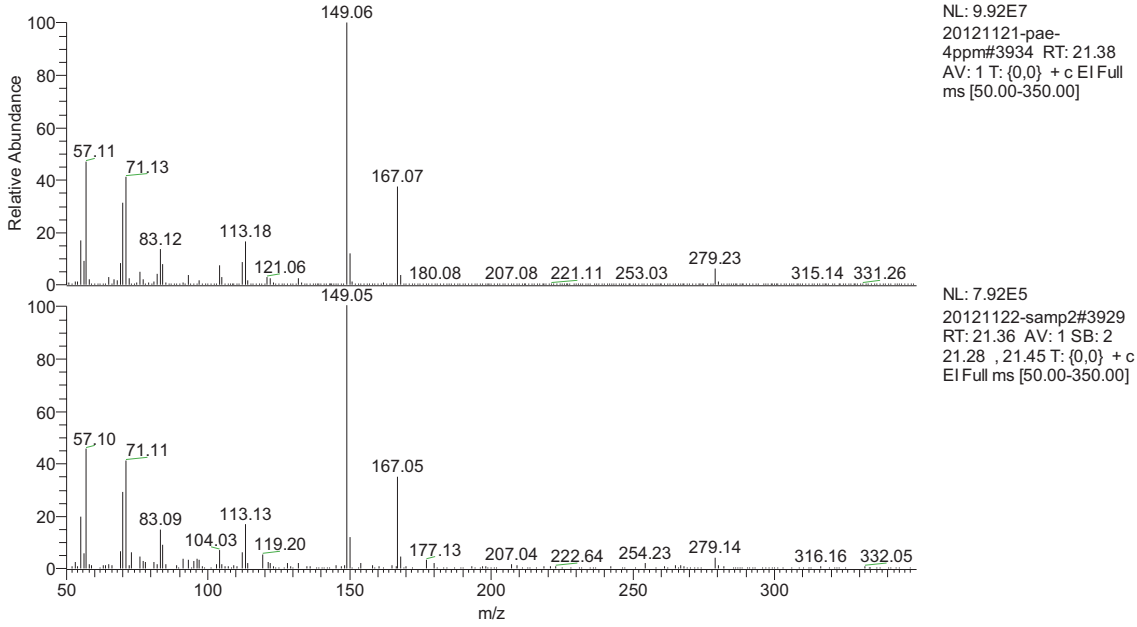



Figure 7. Di-(2-ethylhexyl) phthalate spectra from standard (top) and sample (bottom).

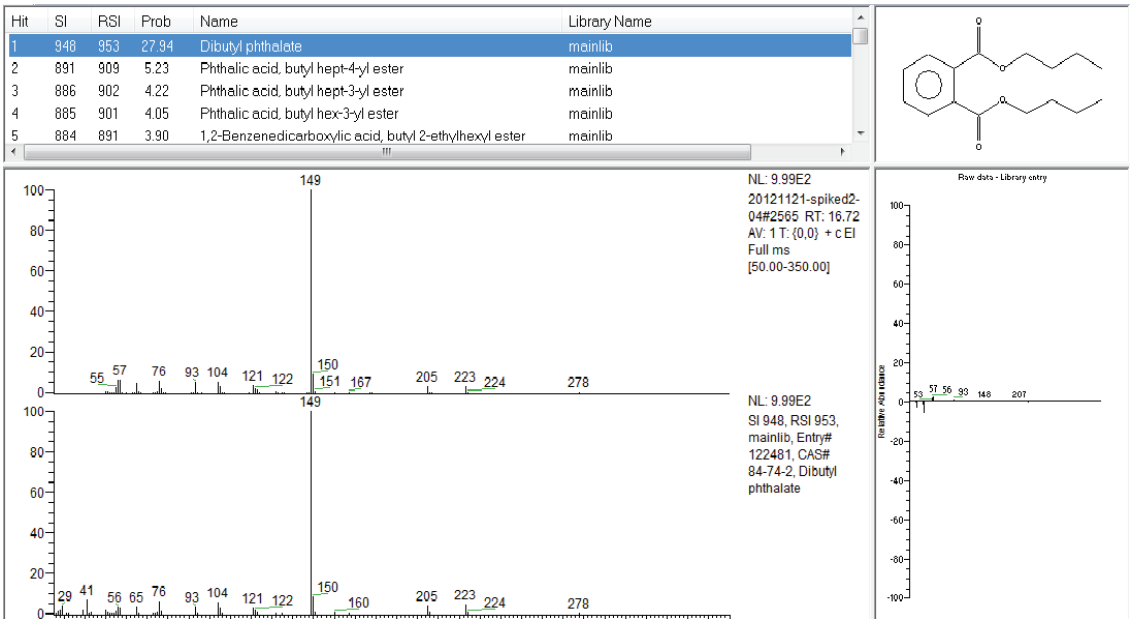



Figure 8. Comparison of spectra between the spiked sample (top) and NIST library (bottom) indicating a high match factors (Similarity Index SI, Reversed Search Index RSI).

## Quantitation

A series of matrix spiked samples with five different concentrations was prepared in the range of 0.10 to 4.00 mg/L of the standard solution. The samples were injected in sequence from low to high concentration. The peak areas were calculated for the standard curve with linear regression of very good precision with an average $R^2$ value of 0.999 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.

The dinonyl-phthalates (DNP) create a special analytical challenge. The DNPs typically consist of a mixture of technical C9-isomers. Hence the response of DNP is distributed to individual isomers. The integration of the unresolved DNP peaks needs to be performed over a wider but constant retention-time range, as shown in Figure 9 from the applied Thermo Scientific™ TraceFinder™ software data processing. We could achieve a linear calibration range for DNP of 0.40 to 4.00 mg/L.

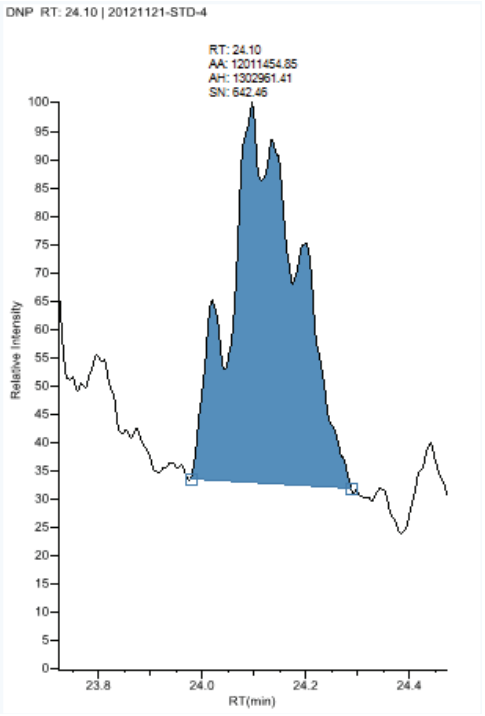


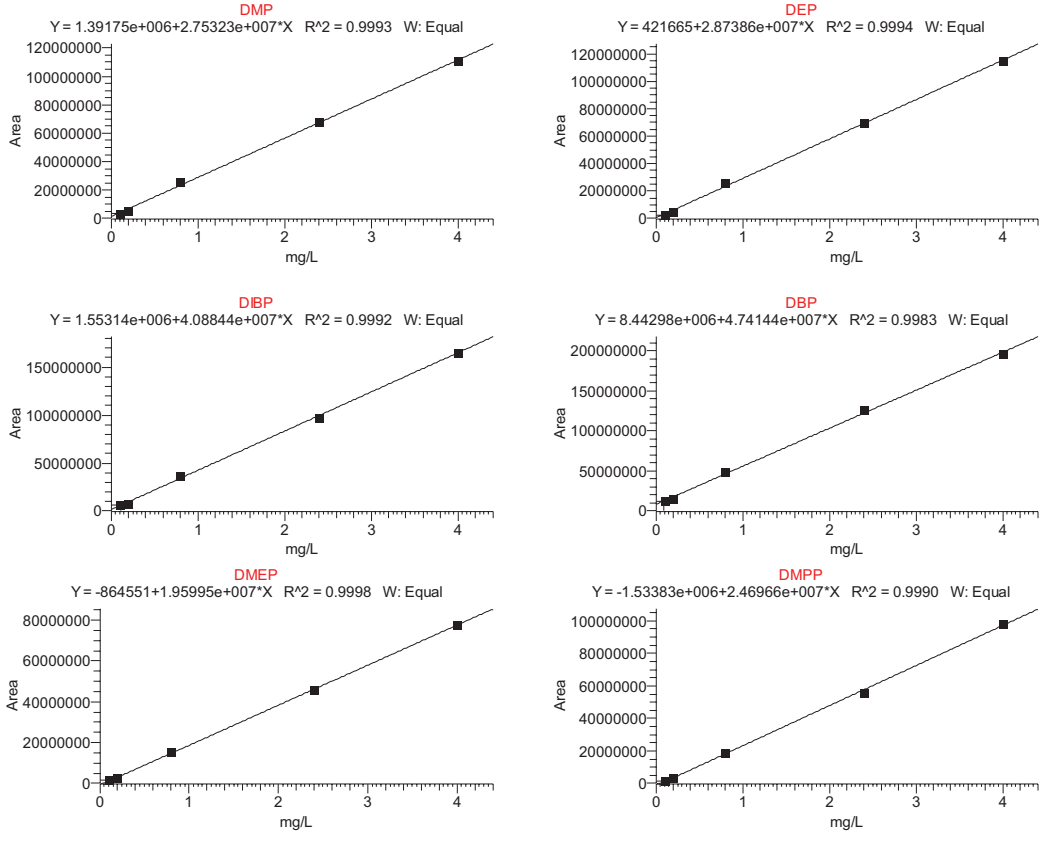

Figure 9. Quantitation peaks of the unresolved DNP isomers over a set retention time range using the TraceFinder quantitation software.

### 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. Figure 10 shows the calibration curves of 16 PAE compounds.

Table 3. Phthalate Quantitation - Linearrange withlimit of detection (LOD) andlimit 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 | 0.1-4.0 | 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 |



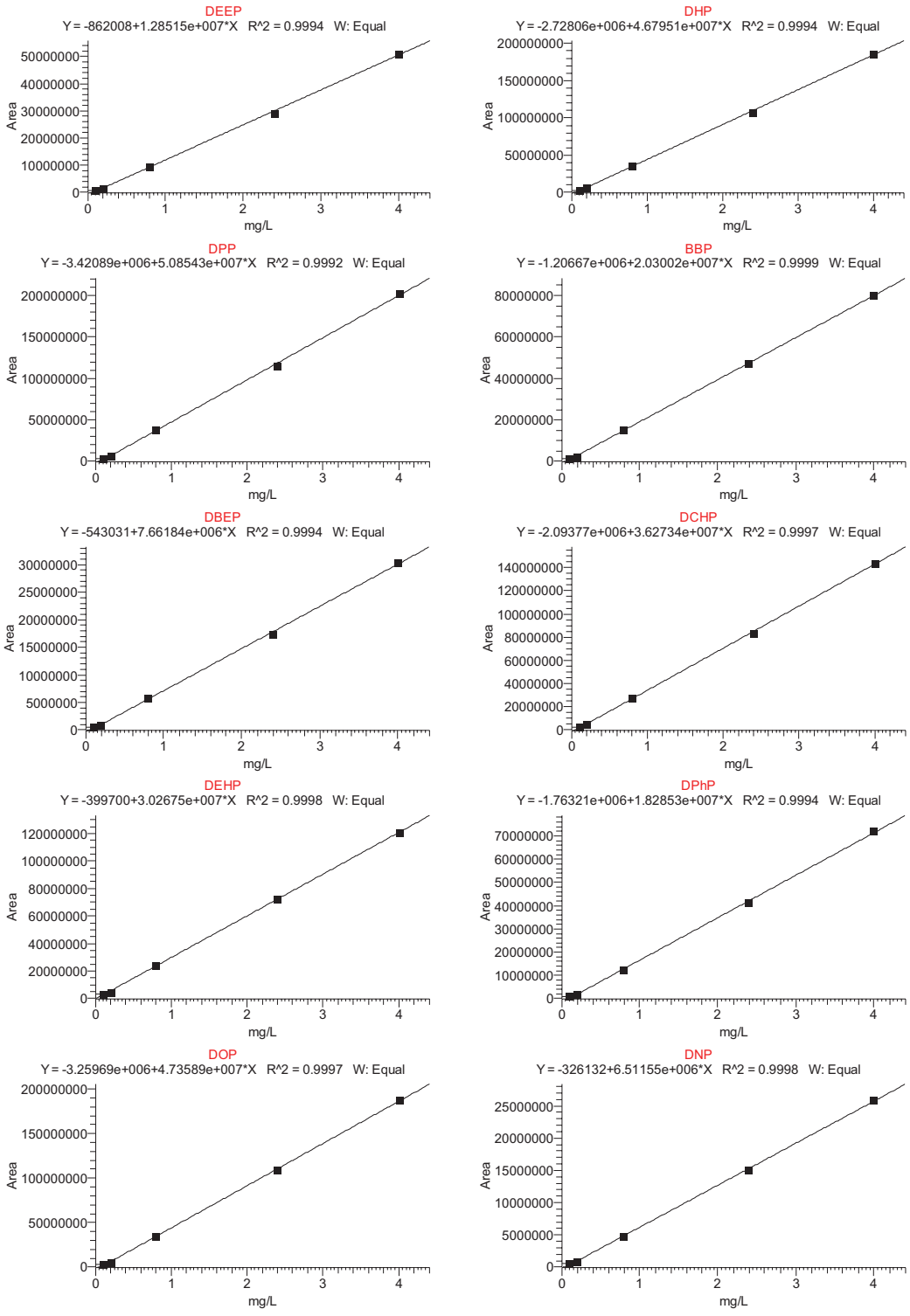



Figure 10. Calibration curves of 16 PAEs.

Table 5. Method recovery and precision data at trace level (avg. 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 |

Note: ND = not detected

### 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.

## Conclusions

In this study for the determination of phthalate plasticizer residues in liquor, the ISQ Series GC-MS met the special testing requirements set by the China method GB/T 21911-2008 for determining phthalates in food.

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

The ISQ Series GC-MS method setup 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 provided the precise, fast, and interference-free quantitative determination.

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

The described determination method for phthalate plasticizers using the ISQ Series GC-MS 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.

## References


[1] China method GB/T 21911-2008 for the "Determination of phthalates in food".

[2] Shao, Dongliang, Determination of Phthalate Ester Residues in White Spirit by GC-MS, Chemical analysis and meterage, 19(6) (2010) 33-35.