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Detection and Quantification of Phthalates in Liquid Food Products by GC-MS.

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ABSTRACT

The present work deals with the detection and quantification of phthalates in liquid food products. GC-MS technique was used to detect and quantify the phthalates. Linearity was obeyed in the concentration range of 0.5 to 5 mg/L. The developed method was successfully applied to the analysis of marketed liquid food products. The average recovery of the samples by this method was 102.1%. Among the phthalates generally present, Di 2-ethyl hexyl phthalate was detected in all the samples in the concentration range of 1.31 to 1.65 mg/L.

Keywords: Phthalates, Sample, GC-MS, Validation.

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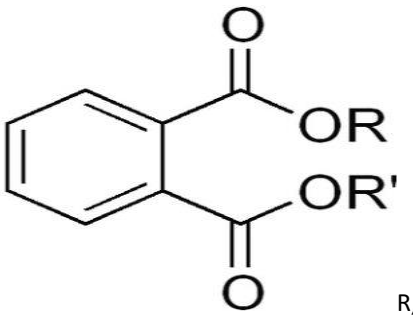
INTRODUCTION

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[1-3]. 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[4,5].

PAEs in the environment and food chain can act as hormones, stimulate the body's natural endocrine responses, interfere with the normal role of hormones and affect the body's most basic physiological control mechanisms[6-8]. Phthalates are reported to cause carcinogenic, teratogenic and mutagenic effects and constitute a health hazard to humans[9]. Phthalate plasticizers also migrate from plastic containers or closures into soft drinks and alcoholic beverages[10]. The general structure of phthalates is shown in **Figure 1**.



R, R^1–alkyl chain

Figure 1. General Structure of Phthalates

PAEs are introduced into the food chain primarily through food packaging material[11]. Alcoholic beverages in plastic containers are a particular risk, since the ethanol provides a very good solubility for PAEs which leach into the beverages from the plastic contact materials[12].

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. This study attempts to detect phthalates in liquid food samples by GC-MS technique. The method is sensitive, rapid and accurate, covers a wide linear range to meet the need for trace level detection of phthalate esters in different types of liquid samples.

EXPERIMENTAL

Sample Preparation

The samples used for this method were water, soft drinks and liquor, bought from the local stores. 10 ml of each of the above samples was taken and extracted with 10 ml of HPLC grade hexane. Then finally the supernatant was transferred for analysis. (The liquor sample was evaporated prior to extraction).Commercial phthalate standards were used for method development. The instrument conditions used are listed in Table - 1.

Method

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 chromatogram of the mixed standards is shown in **Figure 2.**

Validation of the Proposed Method

Linearity and calibration curve

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The calibration curve was constructed by taking various concentrations of mixed standards in the range of 0.5 to 5 mg/L. The solutions 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 10 phthalate esters(Di isobutyl phthalate-DIBP, Di butyl phthalate-DBP, Bis(2-methoxy ethyl) phthalate-BMOEP, Di isopentyl phthalate-DIPP, Pentyl isopentyl phthalate-IPPP, Di-n-propyl phthalate-DPP, Di hexyl phthalate-DHP, Benzyl butyl phthalate-BBP, Di 2-ethyl hexyl phthalate-DEHP, Di-n-octyl phthalate-DnOP) show a very good linear relationship in the calibration range of 0.50 to 5.00 mg/L as furnished in Table - 2.

LOD and LOQ

The LOD and LOQ values are reported in Table - 2. The determination of the limit of detection (LOD) and limit of quantification (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.

Precision and recovery

The accuracy of the method was determined by carrying out recovery studies. The average recovery of spiked samples were found to be 95-105 % . The results of precision and recovery studies are shown in Table - 3.

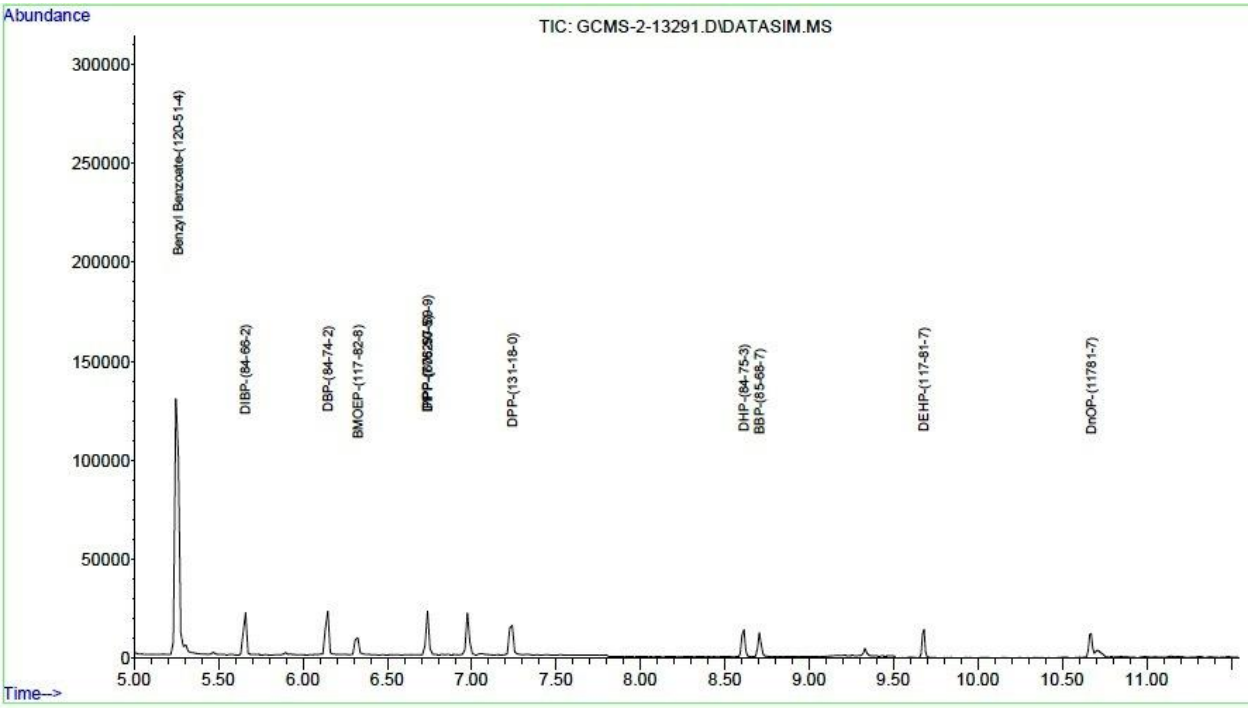

Figure 2. Chromatogram of Mixed Standard

Sample Analysis

The six commercial liquid samples were prepared by the described sample preparation method for determining possible contamination by phthalate esters(sample 1,2-water, 3,4- soft drinks, 5,6- liquor samples). The concentrations of phthalate ester residues present in the commercial liquid samples were analyzed by using the above described method and the concentrations of phthalate residues found are shown in Table - 4.

DEHP was found to be present in all the analyzed samples. The mass spectrum of DEHP standard is shown in **Figure 3** and the spectrum of all the samples are shown in **Figures 4 to 9**.

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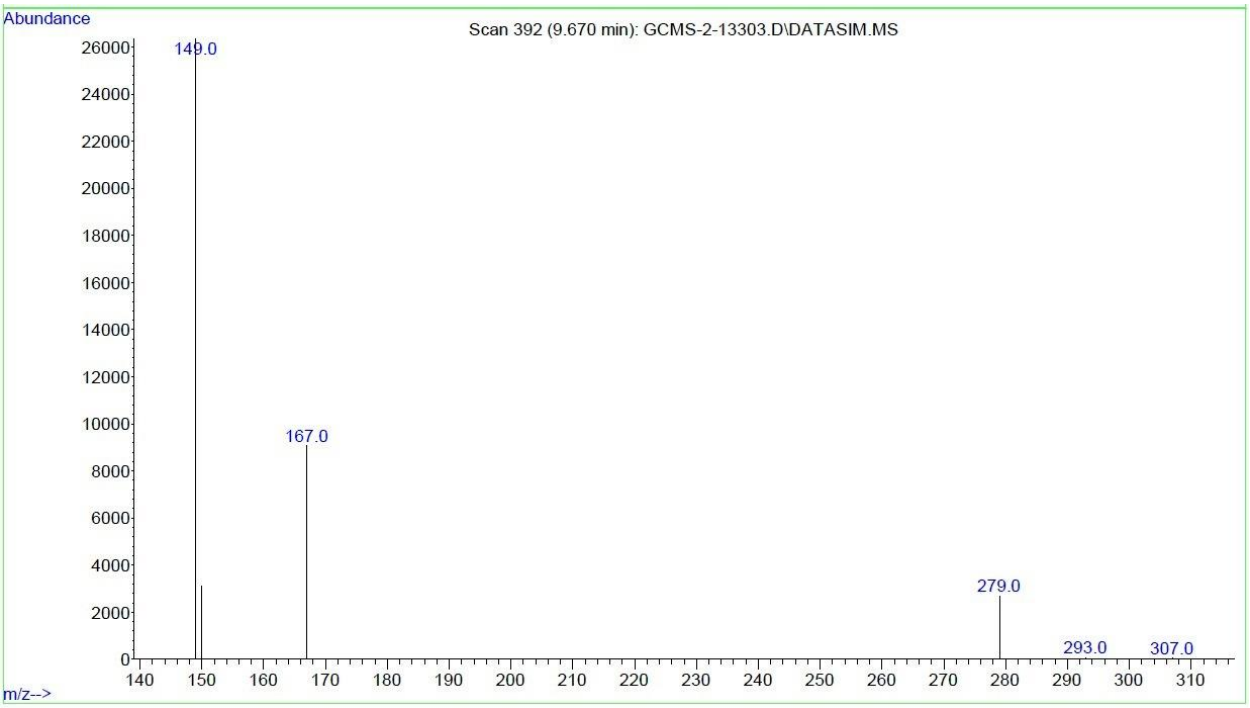

Figure 3. Mass Spectrum of DEHP (Standard)

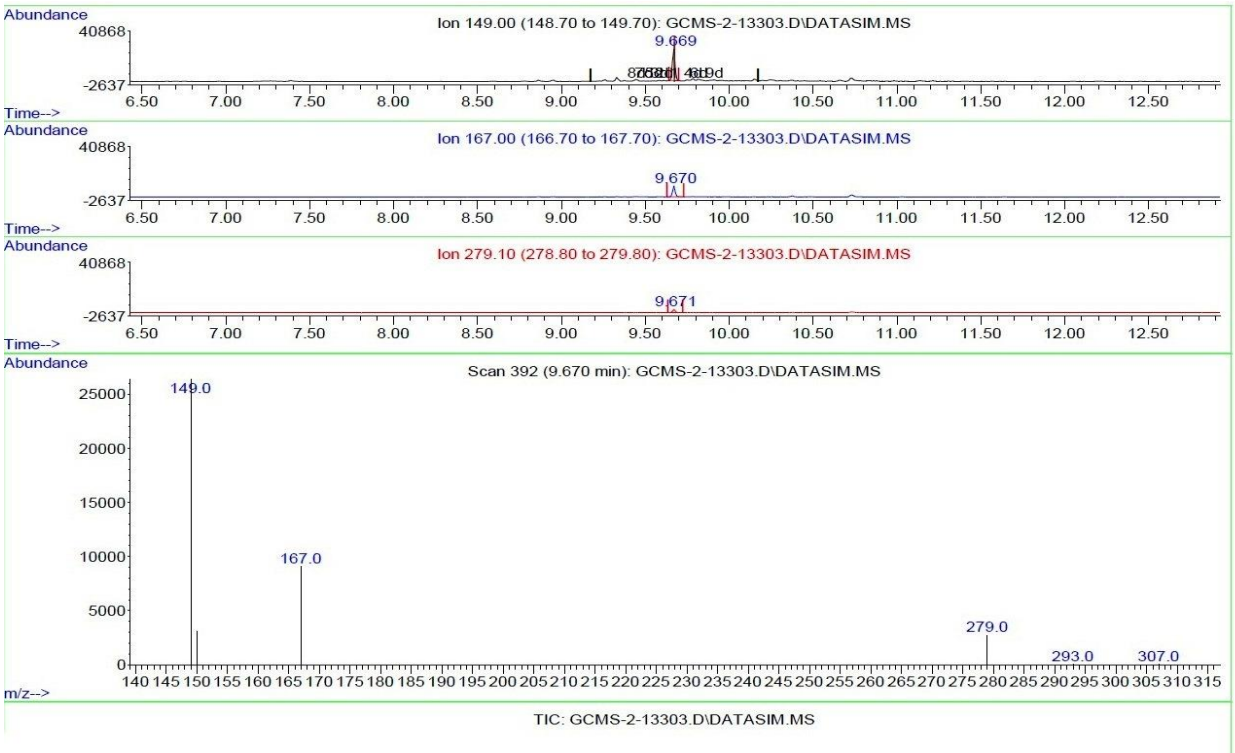

Figure 4. Mass Spectrum of Sample -1

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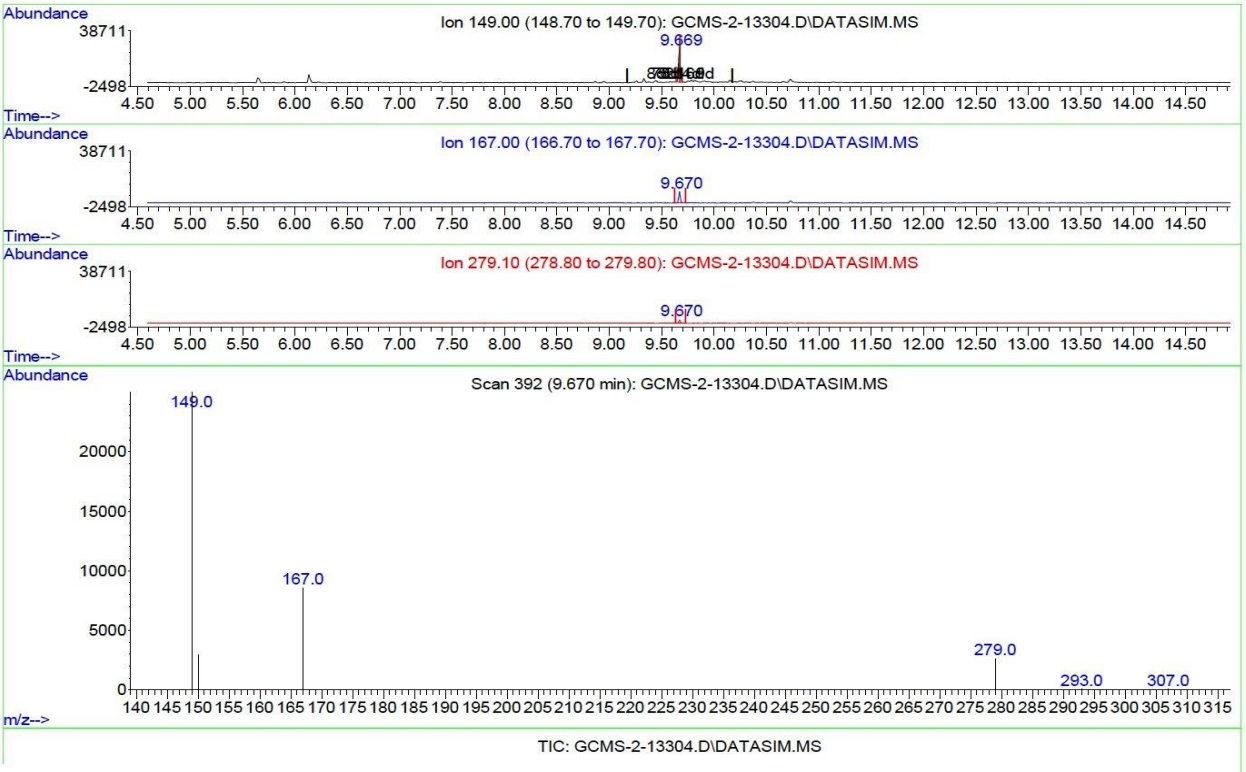

Figure 5. Mass Spectrum of Sample -2

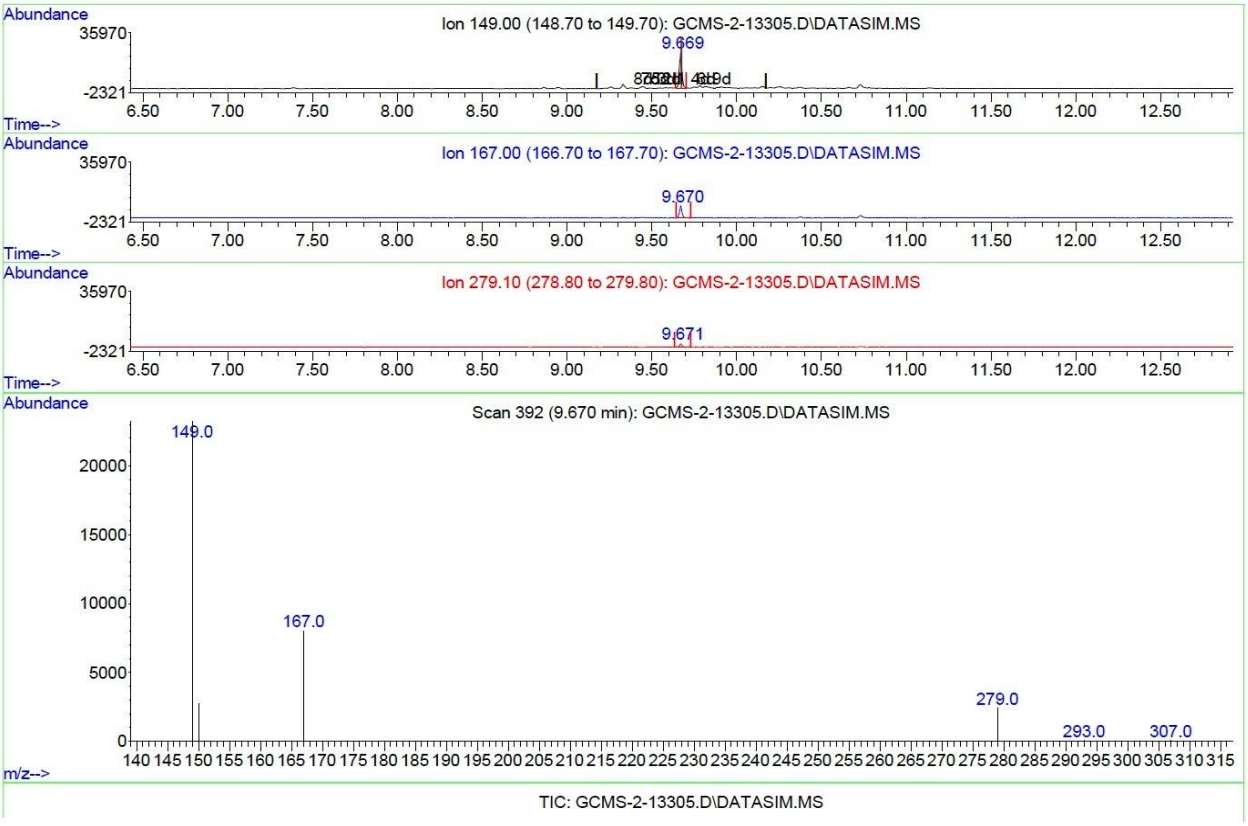

Figure 6. Mass Spectrum of Sample -3

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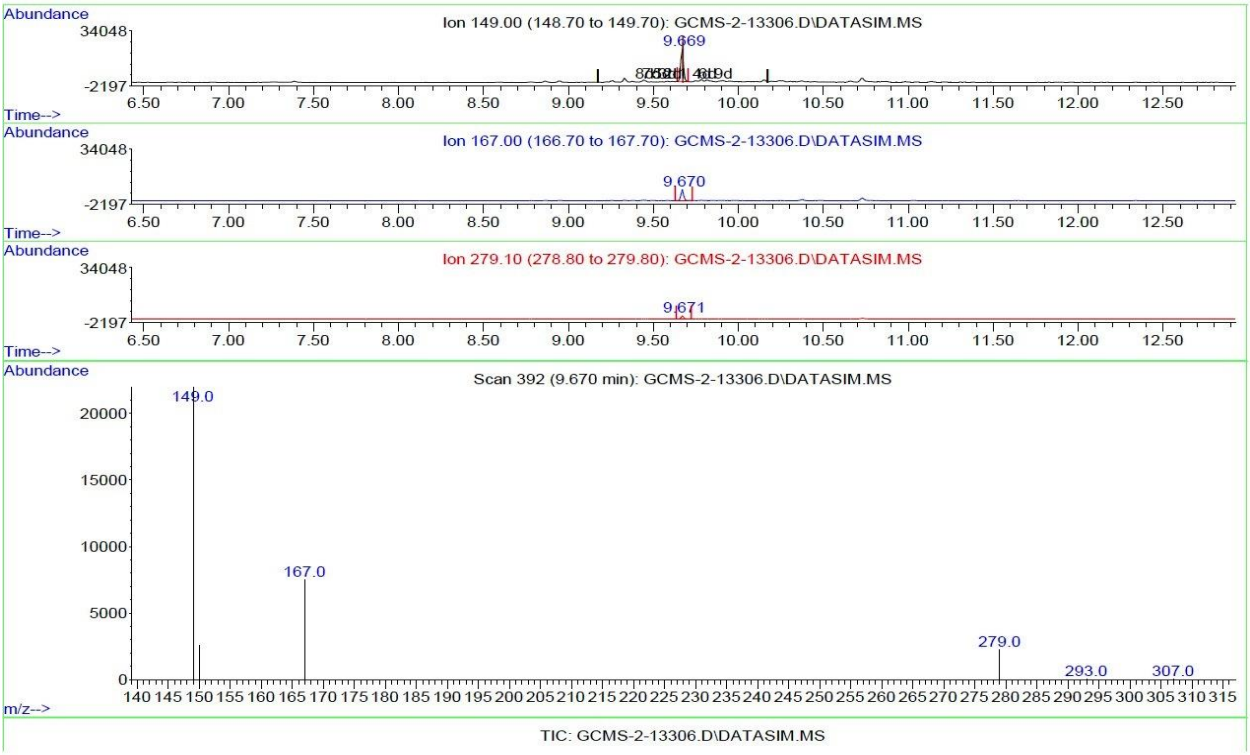

Figure 7. Mass Spectrum of Sample -4

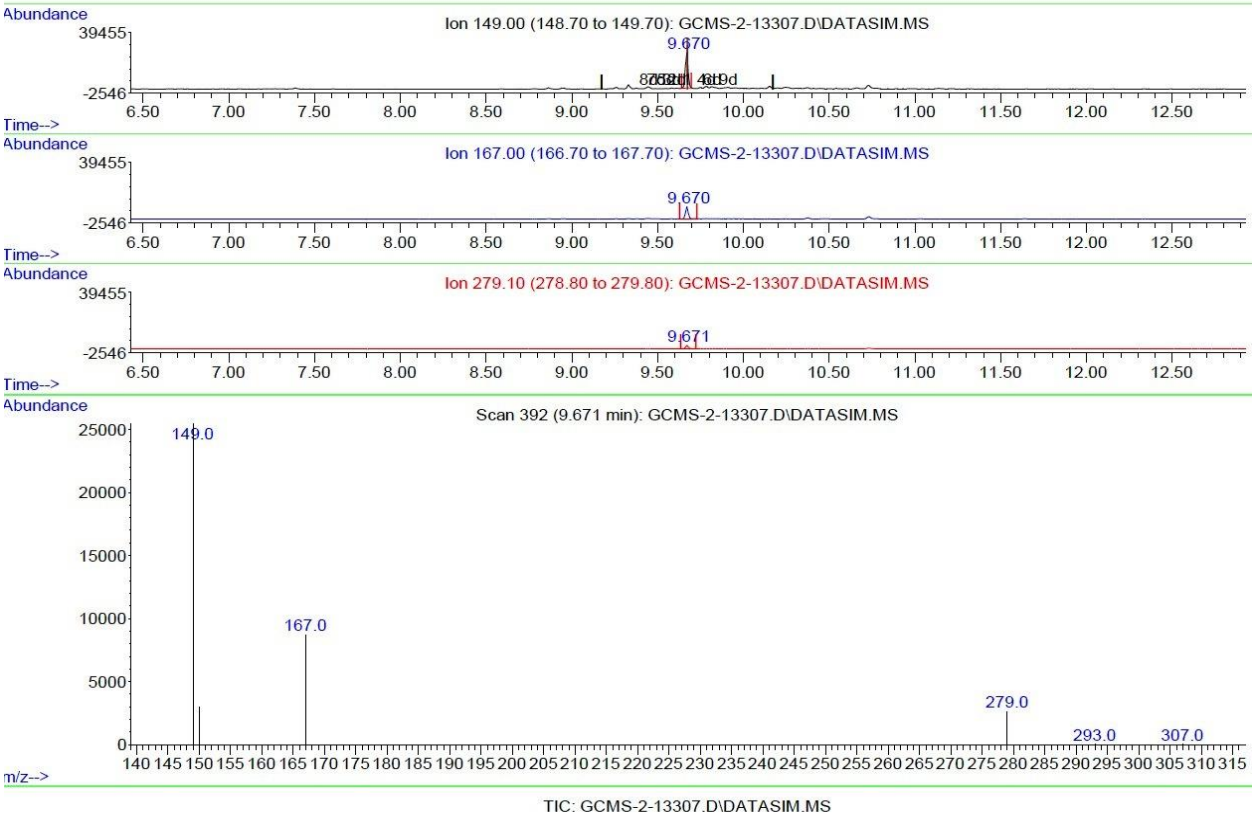

Figure 8. Mass Spectrum of Sample -5

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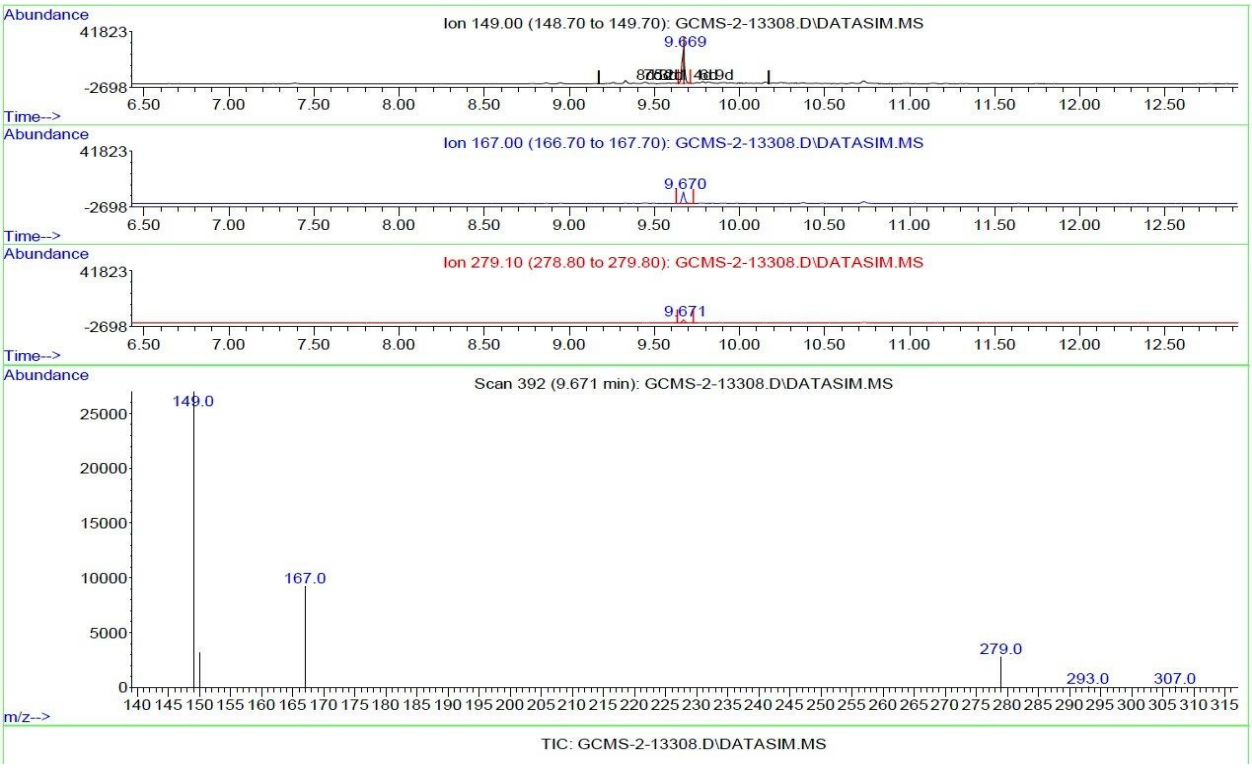

Figure 9. Mass Spectrum of Sample -6

Table 1. GC-MS Instrument Conditions

Instrument used	Agilent GC-7890A, MS-5975C or Equivalent
Column	DB-5 ms,20m×0.25µm× 0.25µ
Acquisition mode	SIM/scan
Injection mode	Pulse split
Split ratio	1:05
Liner & liner volume	Single tapered with wool,~1ml
Injection volume	1µl
Injection temperature	280^{0}C
Interface temperature	310^{0}C
Ion source temperature	230^{0}C
Flow rate	1ml/min
Carrier gas	Helium
Temperature program	Initial temperature =150^{0}C, hold for 10 min, raise at 20^{0}C/min to 250^{0}C for 2 min; 30^{0}C/min to 300^{0}C hold for 12 min.

Table 2: Results of Validation

Phthalate	Retention time(min)	Quantitation ion(m/z)	Linearity (mg/L)	Correlation coefficient r^2	LOD (mg/L)	LOQ (mg/L)
DIBP	5.656	149	0.5-5	0.9994	0.1	1.0
DBP	6.141	149	0.5-5	0.9991	0.2	1.3
BMOEP	6.358	59	0.5-5	0.9992	0.1	1.0
DIPP	6.753	149	0.5-5	0.9993	0.1	1.0
IPPP	6.969	149	0.5-5	0.9992	0.1	1.0
DPP	7.268	149	0.5-5	0.9994	0.2	1.3
DHP	8.595	149	0.5-5	0.9995	0.1	1.0
BBP	8.702	149	0.5-5	0.9990	0.1	1.0
DEHP	9.670	149	0.5-5	0.9996	0.1	1.0
DnOP	10.664	149	0.5-5	0.9999	0.1	1.0

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Table 3. Results of Precision and Recovery

Phthalate	Recovery %	Precision(% RSD)
DIBP	102.0	0.104
DBP	105.0	0.213
BMOEP	95.0	0.542
DIPP	103.0	0.555
IPPP	101.0	0.612
DPP	102.0	0.149
DHP	103.0	0.512
BBP	104.0	0.069
DEHP	101.0	0.172
DnOP	105.0	0.324

Table 4. Results of Sample Analysis

Phthalate	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
DIBP	ND	ND	ND	ND	ND	ND
DBP	ND	ND	ND	ND	ND	ND
BMOEP	ND	ND	ND	ND	ND	ND
DIPP	ND	ND	ND	ND	ND	ND
DEHP	1.52mg/L	1.50 mg/L	1.32 mg/L	1.31 mg/L	1.47 mg/L	1.65 mg/L
DPP	ND	ND	ND	ND	ND	ND
DHP	ND	ND	ND	ND	ND	ND
BBP	ND	ND	ND	ND	ND	ND
IPPP	ND	ND	ND	ND	ND	ND
DNOP	ND	ND	ND	ND	ND	ND

CONCLUSION

This study aims to determine the phthalate plasticizers residues in liquid food samples. The sample preparation method for liquid samples was quick and easy to accomplish using hexane as extraction solvent which provided constant and high recoveries even at trace level. The GC-MS measurement method is highly accurate as demonstrated with precise calibrations and spiked liquid samples.

The 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 determination of phthalate plasticizers using the proposed GC-MS method is very sensitive and accurate. It is easy to perform, rapid and covers a wide linear range to meet the need for trace level detections of PAEs in liquid food samples.

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