

An Application of Gas Chromatography-Mass Spectrometry (GC-MS) Fast Automated Scan/SIM Type (FASST) in Determining the Preservative Levels in Foods

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Abstract

The preservatives used in food products are strictly monitored and regulated. The present study aims to imply a sensitive and reliable analytical method to quantify two classes of preservatives (*i.e.* carboxylic acid and phenolic compounds) in food by means of gas chromatography-mass spectrometry (GC-MS), aimed at monitoring the products available within the local stores. Target analytes were derivatized via aqueous-phase isobutyl chloroformate-mediated reaction followed by dispersive liquid-liquid microextraction (DLLME) method. The quantity and quality determination of the studied samples were assured by performing FASST approach in a single run GC-MS analysis. The combination of standard addition method with sample dilution compensated the effect of sample matrix on the quantitative determination of the tested preservatives in the examined samples. The concentrations of sorbic acid (SA) were 210 µg/mL and 1000 µg/mL in soft drink and sauces samples, respectively. On the other hand, sodium benzoate (226 µg/mL) was only found in soft drink, whereas, no parabens were detected in any samples collected from the local stores.

Keywords

Food, Preservatives, GC-MS, FASST Acquisition, Chloroformate Derivatization, DLLME

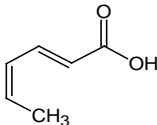
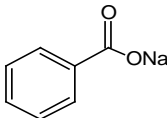
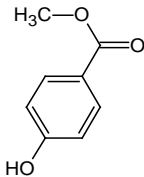
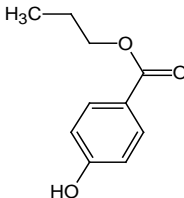
1. Introduction

Preservatives are added to many food products such as soda, juices, and sauces

to prevent decompositions resulting from microbial and alteration caused by chemical changes. The most commonly used global preservatives in food are SA, BA and their corresponding salts as well as parabens (properties of these preservatives are shown in **Table 1**) [1] [2]. Preservatives are added to extend the shelf life of unstable food products preventing the alteration in texture, flavour and color, as well as, to protect the health of consumers. However, adding high amount of preservatives could cause alteration in taste. Whereas, less than optimal amount will have little effect on microbial growth resulting in failure of preservative properties of the substance. Maximum levels for preservatives application in food products are subjected to international food law regulations [3] [4] [5] [6]. Therefore, an effective and efficient analytical method is required to assure that the amount of the added preservative is within the permitted levels. Several analytical methods have been applied in research studies to determine preservatives in foods include high performance liquid chromatography (HPLC) [7] [8], headspace gas chromatography-flame ionization detector (HS GC-FID) [9], gas chromatography-mass spectrometry [10], and miniature ion trap mass spectrometer [11].

In a previous study we optimized and validated a reliable method based on derivatisation and dispersive liquid-liquid microextraction method, followed by gas chromatography-mass spectrometry (GC-MS). Fast automated Scan/SIM Type (FASST) acquisition was applied to simultaneously determine (qualitative and quantitative analysis) two different classes of preservatives, *i.e.*, carboxylic acid and phenolic compounds in representative pharmaceuticals and cosmetics [12]. FASST is a type of mass acquisition that switches between Scan and SIM mode for a single group during a measurement. As a result, acquisition parameters for both Scan and SIM modes can be specified in the same group. Here we report on the application of the develop method to the analysis of the studied preservatives in two different food products (*i.e.*, soft drink and sauces).

Table 1. Properties of SA, SB, MP and PP.

	SA	SB	MP	PP
IUPAC	2,4-hexadienoic acid	Sodium benzoate	Methyl-4-hydroxybenzoate	Propyl-4-hydroxybenzoate
Formula	$C_6H_8O_2$	$C_7H_5O_2Na$	$C_8H_8O_3$	$C_{10}H_{12}O_3$
M.wt (gm/mole)	112.13	144.11	152.15	180.20
logK_{ow}	1.33	1.87	1.91	2.94
Structure				
CAS No.	[22500-92-1]	[532-32-1]	[99-76-3]	[94-13-3]
E-number	E-200	E-211	E-218	E-216

2. Materials and Methods

2.1. Chemicals

Methylparaben (99%, MP), propylparaben (99%, PP), sorbic acid (98%, SA), and isobutyl chloroformate (99%, iBCF) were acquired from Merck (Germany). Sodium benzoate (99%, SB), sodium hydroxide (98%, NaOH), pyridine (98%, Pyr), and chloroform (99.5%, CHCl₃) were provided by BDH (England). Ethanol and acetonitrile were purchased from Sigma-Aldrich (USA). Individual stock solutions of each target analyte at a concentration of 1000 µg/mL were prepared in distilled water and stored at 4°C. Further dilutions and mixtures were prepared on the day of analysis in distilled water.

2.2. Food Samples

Liquid (*i.e.*, soft drink) and semi-solid (*i.e.*, sauces) food samples by global brands were purchased from local Baghdad stores. An approximately 1.0 gm of sauces sample was diluted to 10 mL with deionized water and then treated with an ultrasonic bath for 10 min to disperse the aliquot. The sauces sample was then filtered to remove undissolved particles prior to analysis. Whereas, 1 mL of soft drink was diluted to 10 mL and degassed for 10 min in the ultrasonic bath at room temperature. Samples were spiked with 5 µg/mL of the corresponding mixture of the target analytes at different dilution ratio (*i.e.*, 1:50, 1:100, 1:1000, and 1:2000) to evaluate matrix effect of real samples.

2.3. Derivatization and DLLME Procedures

The applied method for preparing and extracting iBCF derivatives of the target analytes in foods was adopted from our previous study [12]. Briefly, acylation derivatisation was performed with 30 µL of iBCF, 30 µL of pyridine, and 150 µL ethanol, which was followed by DLLME approach to recover the generated derivatives with 1 mL of CHCl₃. After centrifugation, 1 µL from the organic phase was injected into the GC-MS. **Figure 1** illustrates the applied method.

2.4. GC-MS Analysis

Analyses were performed on a Gas Chromatograph (GC 2010Plus) coupled to a Mass Spectrometer (QPMS 2010Ultra), both instruments were made in Shimadzu/Japan. The detailed setup parameters for the GC-MS system are shown in **Table 2**. The GC-MS spectra were evaluated by GCMSsolution v.4 software (Shimadzu, Japan). Target analytes were searched in the National Institute of Standards and Technology (NIST) MS search v.8 library browser and verified by comparing with the authentic standards.

3. Results and Discussion

3.1. Targeted GC-MS Analysis

According to the combined derivatisation-extraction method that has been optimized in our pervious study, two families of preservatives (*i.e.*, carboxylic acid

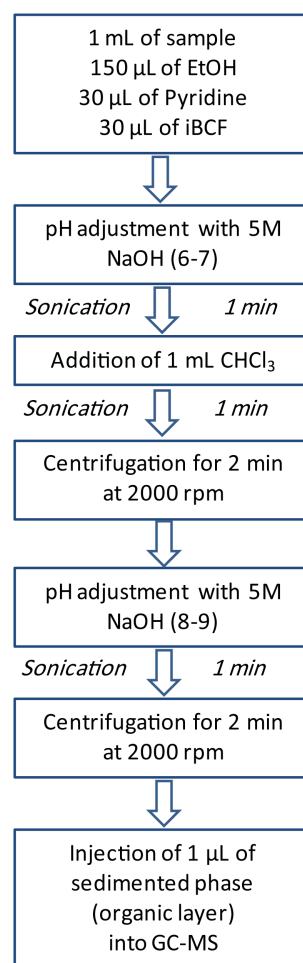


Figure 1. A scheme of the applied method [12].

Table 2. GC-MS setup parameters.

Sample Introduction (AOC 20i, Shimadzu, Japan)	Mode	Splitless
	Injector Temperature	240 °C
	Injection Volume	1 µL
Gas Chromatograph (GC 2010Plus, Shimadzu, Japan)	Capillary Column	Shimadzu InertCap Wax (30.0 m × 0.25 mm × 0.25 µm film thickness)
	Carrier Gas	Helium (99.999%)
	Column Flow Rate	1.53 mL·min ⁻¹
	Oven Temperature Program	70 °C (held 3 min), ramped to 150 °C at 15 °C min ⁻¹ (held 2 min), and a final ramp to 240 °C at 15 °C min ⁻¹ (held 8 min)
	Run Time	21.33 min
Mass Spectrometer (QPMS 2010Ultra, Shimadzu, Japan)	Acquisition Mode	FASST
	Mass Range (m/z)	35 - 400 amu
	Solvent Delay	6.4 min
	Source Temperature	200 °C
	Transfer Line Temperature	240 °C
	Electron Energy	70 eV

and phenolic compounds) were investigated in food samples. As reported before, a tenfold increase in sensitivity has been observed for all studied analytes when compared to those analysed without derivatisation. An improvement in the chromatographic performance, *i.e.*, symmetric peak shape and responses has been observed with the applied method. The two-step derivatisation protocol was performed by applying pH adjustment to simultaneously detect the carboxylic acid and phenolic compounds in the studied samples. Compound detection was based on FASST acquisition where high sensitivity for the quantitative analysis (SIM mode) and mass spectra for unknown identification (Scan mode) can be achieved. Ions with higher abundance (higher S/N ratio) was selected for better sensitivity, whereas, ions with higher m/z ratio were chosen for better selectivity. As a result, ions monitored in FASST mode for all derivatives of target analytes were: m/z (67), 95, 140 for SA; m/z 77, (105), 122, 150 for BA; m/z 57, 121, (152) for MP; and m/z 57, 121, (138) for PP; the quantifier ions being in brackets (**Table 3**).

During the derivatisation reaction, minor derivatives with a ratio of less than 5% for all analytes and major derivatives for both classes were generated in the reaction medium. The major derivatives of MP and PP were detected as alkoxy-carbonyl esters, whereas, alkyl esters were superior for SA and BA (**Figure 2**). **Figure 3** shows the extracted ion chromatograms for the major derivatives of target preservatives that were used for the quantification analysis.

The matrix effect (ME) was evaluated accordingly to the dilution ratios mentioned in section (2.2). Percent recoveries of the target analytes from the samples were generally improved for all analytes. Sauce samples showed a higher percentage of recovery when applying 100-fold dilution, while, 1000-dilution was required to analyse soft drink samples (**Figure 4**).

Table 3. GC-MS analyses of target analytes as iBCF derivatives.

Analyte	RT	[M] ⁺ *	[M-15] ⁺	[M-28] ⁺	[M-31] ⁺	[M-56] ⁺	[M-59] ⁺	[M-72] ⁺	[M-100] ⁺	Other m/z
ES	7.81	140	125	112						97, 95 , 67, 41
IS	8.97	168 vs	153			113 [*]				95 , 67, 41
EB	9.30	150		122						105 , 77, 51
IB	10.63	ND				123 [*]				105 , 77, 56, 51
EMP	15.79	224			193 vs			152		121 , 93, 65, 41
IMP	16.43	252 vs			221				152	121, 93, 57, 41
EPP	16.50	252					193	180		138 , 121, 93, 65, 41
IPP	17.26	280 vs					221		180	138 , 121, 93, 57, 41

ES: ethyl sorbate; IS: isobutyl sorbate; EB: ethyls benzoate; EMP: ethyl methylparaben carbonate; IMP: isobutyl methylparaben carbonate; EPP: ethyl propylparaben carbonate; IPP: isobutyl propylparaben carbonate. *hydrogen migration and rearrangement; values in bold represent base peak ions; vs: very small ion peak; m/z : mass to charge ratio; ND: not detected. [M+•]: molecular ion; [M-15]: loss of methyl group (-CH₃); [M-28]: loss of ethylene group (-C₂H₄); [M-31]: loss of methoxy group (-OCH₃); [M-56]: loss of isobutyl group (-C₄H₈); [M-59]: loss of propoxy group (-OC₃H₇); [M-72]: loss of ethoxy-carbonyl group (C₃H₄O₂); [M-100]: loss of isobutoxycarbonyl group (C₅H₈O₂).

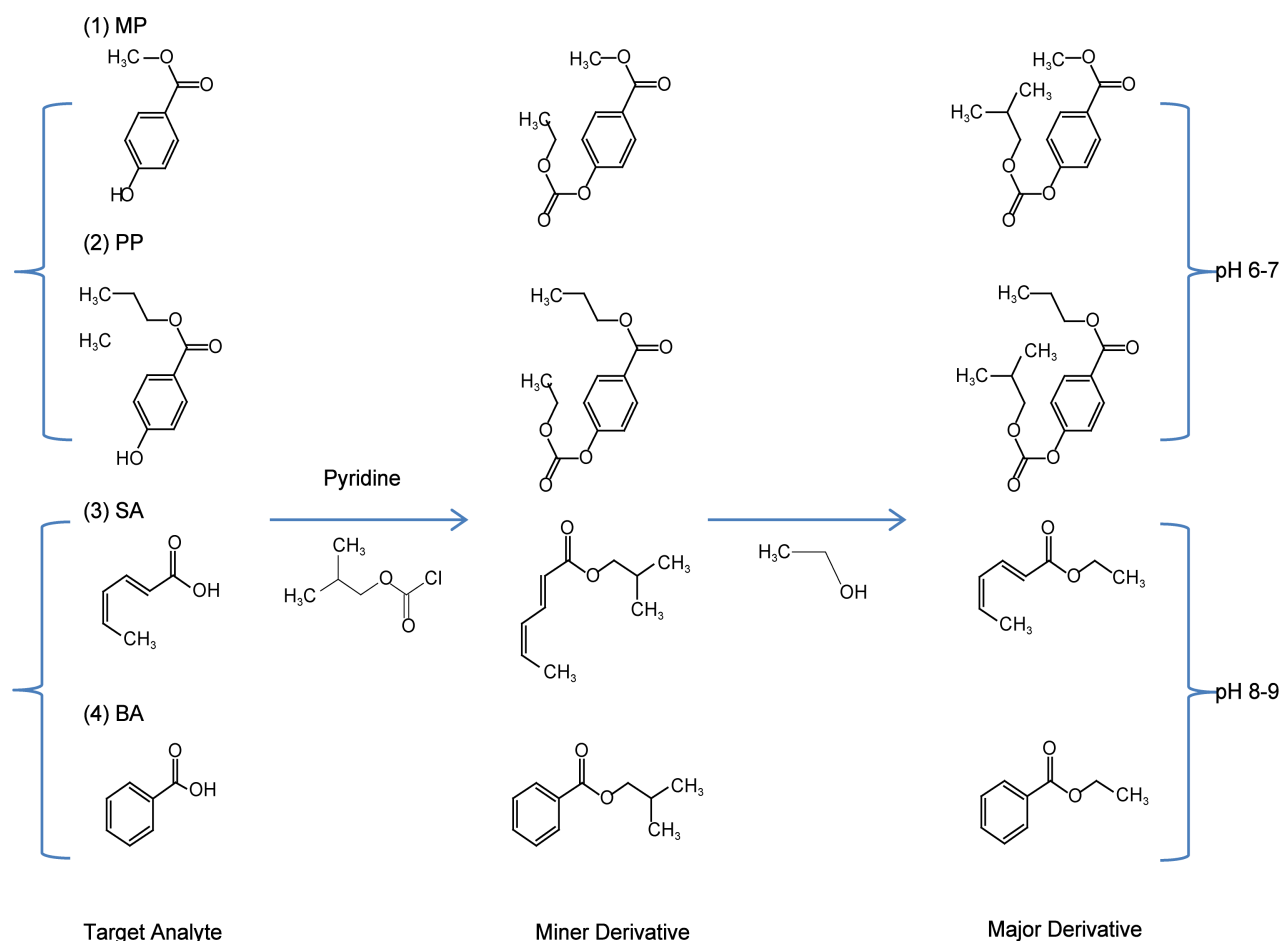


Figure 2. Overall scheme of iBCF derivatisation of four target compounds at two pH medium aqueous system: (1) MP; (2) PP; (3) SA; (4) BA.

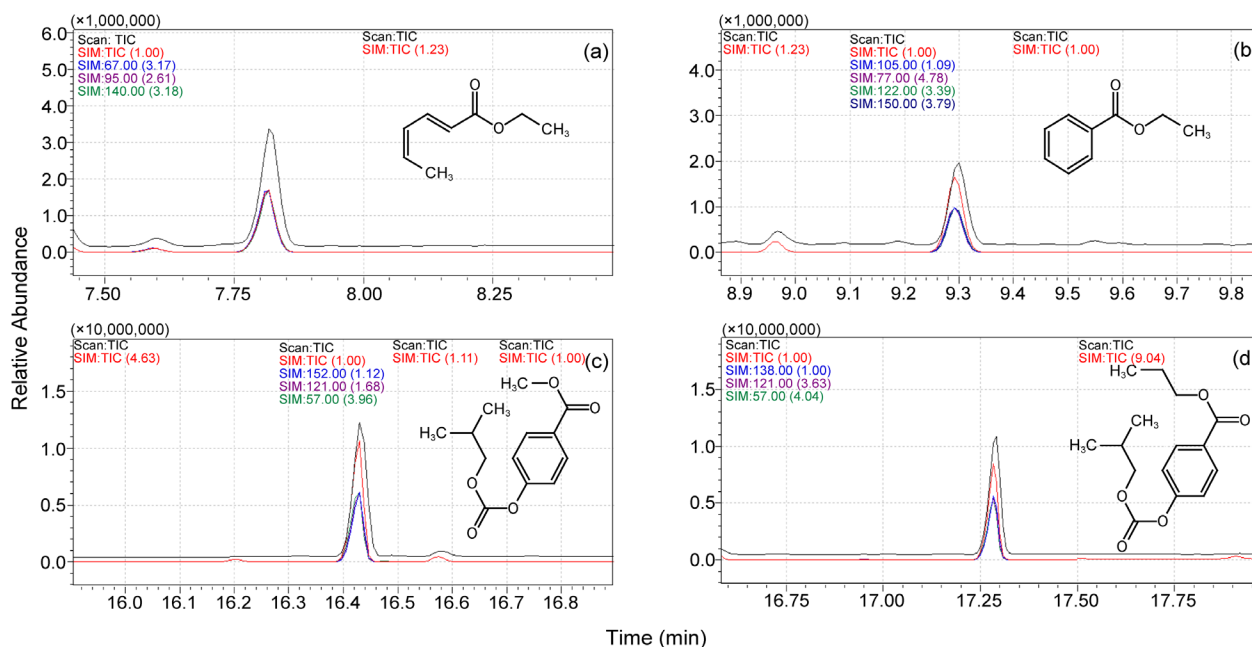


Figure 3. GC-MS (FASST) ion chromatograms of derivatised: SA (a); BA (b) characterized as ethyl esters; MP (c); and PP (d) characterized as isobutoxycarbonyl esters.

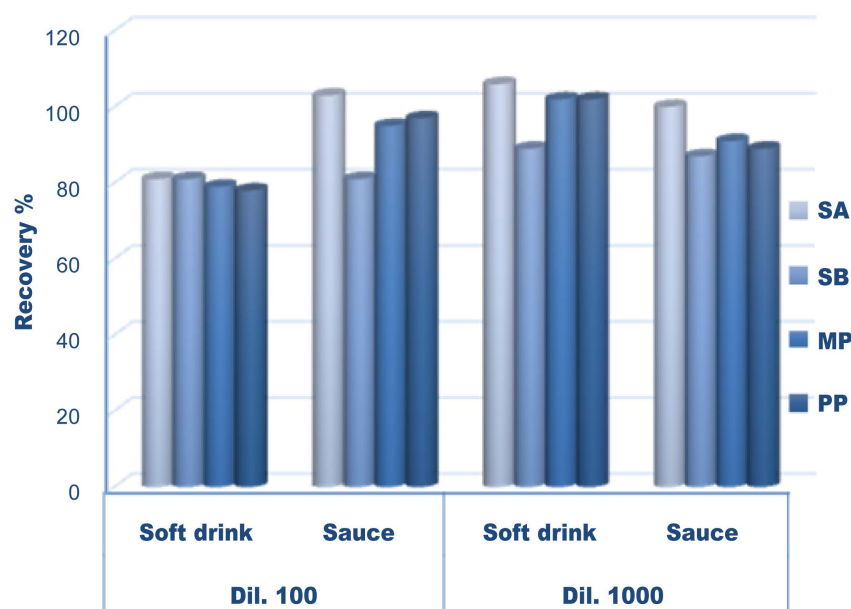


Figure 4. Recovery of target preservatives (SA, BA, MP, and PP) spiked into food samples after dilution of 1:100 and 1:1000.

3.2. Application to Real Samples

The applicability of the method to real samples was evaluated by the determination of the target analytes to accurately quantify the target preservatives in the selected samples (*i.e.*, soft drink and sauce). The target analytes were determined according to the standard addition method where real samples spiked at three concentration levels (0.5, 1.0, and 5.0 $\mu\text{g/mL}$). Each sample was analyzed in triplicate, and the mean values of the concentration of SA were 210 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ in soft drink and sauce samples, respectively. On the other hand, BA (226 $\mu\text{g/mL}$) was only detected in soft drink, whereas, parabens were not found in any sample collected from the local stores. The added preservatives that are labelled on the sample must not exceed the maximum allowed limit. As reported in food survey of benzoates and sorbates in soft drinks, 150 $\mu\text{g/mL}$ is the maximum permitted level for BA, whereas, 300 $\mu\text{g/mL}$ for SA (as a single additive) and 250 $\mu\text{g/mL}$ (as a combined additive with BA) are the maximum allowed level [13]. Benzoate is largely used in beverage industry because of the high concentration of fructose corn syrup in soft drinks including water-based, flavoured, and carbonated drinks [14]. As a result, benzoate containing soft drinks account for the highest human consumption in the world. Also, chemical changes can occur to BA due to the presence of other chemical ingredients in a sample resulting in the generation of carcinogenic benzene. Therefore, food control systems are essential for imported and exported foods to ensure health and safety of domestic consumers, and to maintain consumer confidence in the food system.

3.3. Non-Targeted GC-MS Analysis

In addition to the main objective targeted analysis of the commonly used preservatives in food, non-targeted approach of the applied samples was achieved

by only a single run. In another word, the key benefit of this multi-analysis approach was the non-targeted analysis that allowed screening of unknown substances in the samples without authentic standards. The chromatogram of **Figure 5** verifies that the used method can be applied for quantitative and monitoring analysis. The ingredients (citric acid, malic acid) found in soft drinks were putatively characterised as ethyl derivatives (**Figure 6**).

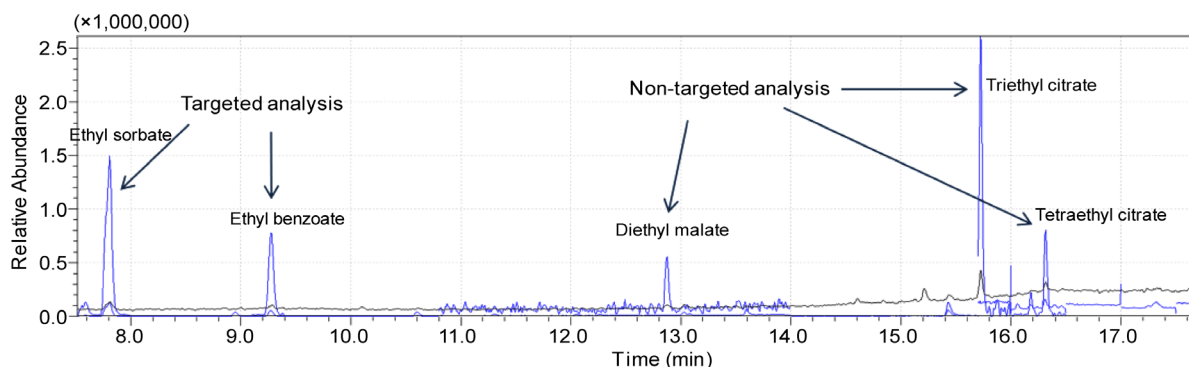


Figure 5. Resultant ion chromatogram from the GC-MS (FASST) analysis of soft drink for the added ingredients.

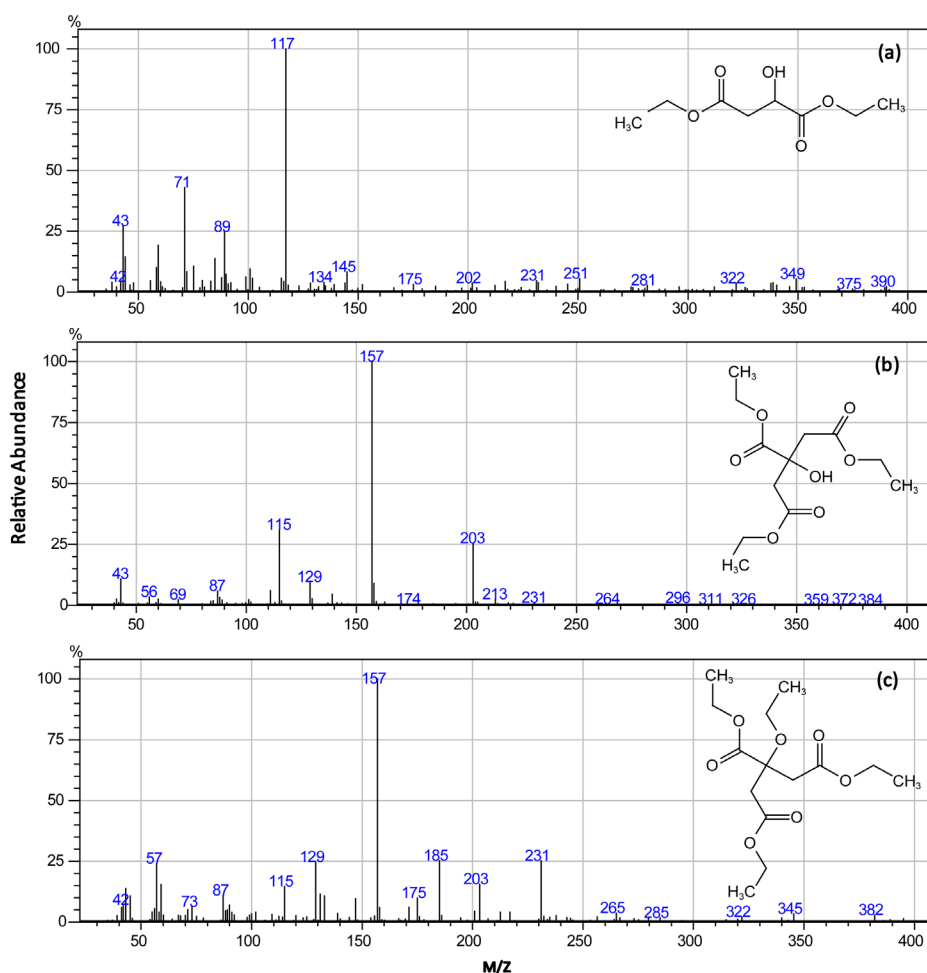


Figure 6. Mass spectra for three ingredients found in soft drink samples that putatively identified as (a) diethyl malate, (b) triethyl citrate, and (c) tetraethyl citrate at RT of 12.87 min, 15.72 min, and 16.31 min, respectively.

In comparison to previous studies, the applied method demonstrated the convenient usage of isobutyl chloroformate derivatisation to qualitative and quantitative analysis of various ingredient compounds present in different food matrices by GC-MS in FASST mode. FASST acquisition allows improved sensitivity and selectivity of analytes as well as minimizes sample matrix interferences. The application of this method along with appropriate standard addition with sample dilution approach proved that matrix effects were strongly diminished for two classes of preservatives in two different matrices of real samples, and therefore will find a wide-range application for routine analysis in food control laboratories.

4. Conclusion

The method based on derivatization-DLLME followed by GC-MS FASST for the simultaneous determination of the commonly used preservatives (*i.e.*, SA, BA, MP and PP) in food samples has been applied. The present study demonstrated the capability of performing this multicomponent analytical method conveniently in laboratory-based quality control.

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