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High-resolution gas chromatography with nitrogen–phosphorous detection of saturated volatile aldehydes derivatized with 2-hydrazinobenzothiazole

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Abstract

An analytical method to measure highly volatile saturated aldehydes, degradation products of lipid peroxidation, was developed for fused-silica narrow-bore capillary gas chromatography with nitrogen–phosphorous detection. The carbonyl compounds were derivatized quantitatively with 2-hydrazinobenzothiazole (HBT) at room temperature to afford their corresponding water-insoluble hydrazones. The derivatives were extracted into non-polar phases and detected with high selectivity by high-resolution gas chromatography with nitrogen–phosphorous detection due to their high nitrogen content. Analyte concentration, pH and type of extraction technique were studied to determine optimal recovery conditions. The efficiencies for solid-phase extraction (SPE) on 3 ml C_{18} octadecyl-bonded phase cartridges ranged from 81.1±1.1 to 98.1±1.4% compared to 70.1±1.4 to 92.4±1.0% obtained for liquid–liquid extraction (LLE) (hexane–isopropanol, 9:1, v/v) for spiked aldehyde solutions at concentrations of 20–60 μg/ml. The recoveries from aqueous solutions at pH 2.96 were routinely 2–7% higher than those obtained at pH 5.99. SPE efficiencies were less affected by pH than LLE. Recoveries achieved by LLE showed an average decrease of 5–12% in comparison with those of SPE. The method was applied to the analysis of the volatile aldehydes generated during the thermal oxidation of olive oil at 220°C.

Keywords: Derivatization, GC; Lipid peroxidation; Aldehydes; Hydrazinobenzothiazole

1. Introduction

Lipid hydroperoxides, the first products in the complex process of lipid oxidation, are highly reactive species that decompose to form aldehydes that can be used then as indicators of lipid peroxidation [1]. Medium and short-chain aldehydes of this group are responsible for the odor of rancid fat-rich foods [2] and some have been connected with the damaging effects associated with free-radical-initiated peroxidation processes in various biological systems [3–5]. The determination of reactive aldehydes is widely used in combination with other methods (e.g. analyses of lipid hydroperoxides [6], conjugated dienes [7,8] and estimation of expired hydrocarbons [9,10]) to measure the extent of lipid peroxidation

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[11–13]. Medium- and short-chain aldehydes are reactive, volatile, partially water-soluble compounds, whose extraction from aqueous solutions, foods or biological fluids, accompanied by GC analysis with conventional detection systems [flame ionization detection (FID), thermal conductivity detection (TCD) or mass-selective detection (MS)], is marked by low recoveries and sensitivity. Chemical derivatization of these compounds and the utilization of highly selective and specific detectors have been the solution to this problem. The most common method for aldehyde analysis is derivatization with 2,4-dinitrophenylhydrazine and analysis by high-performance liquid chromatography [14–16], however, this requires strong acidic conditions which may alter the analytes of interest. Another simple and specific method involves the derivatization of aldehydes with cysteamine (2-aminoethanethiol) to form stable thiazolidines under mild conditions at room temperature and neutral pH [17,18]. However, cysteamine does not react with α,β-unsaturated aldehydes which are also found among the lipid peroxidation products. Other derivatizing agents such as morpholine [19], N-methylhydrazine [20,21] and N-benzylethanolamine [22] have also been employed for volatile aldehyde derivatization. Pentafluorophenylhydrazones from volatile aldehydes have been obtained [23] and analyzed by FID, TCD [24], electron-capture detection (ECD) and MS [24–26]. The derivatization of malondialdehyde, 2,4-pentanedione and acetoacetaldehyde with 2-hydrazinobenzothiazole (HBT) was described by Beljean-Leymarie and Bruna [27], who determined optimal conditions for the hydrazone synthesis and characterized the HBT-derivatives by means of MS (EI, 70 eV) and NMR. Nevertheless, the analytical method described [27], based on the use of a packed column fitted with NPD, does not permit a complete separation of the complex aldehyde mixtures normally generated during lipid peroxidation. Thus, the use of capillary columns for aldehyde analysis is mandatory.

In this work, we extend the research described by Beljean-Leymarie and Bruna [27] to include the analysis of seven volatile aldehydes (C_1–C_5, *iso*-C_5, C_6) as HBT derivatives by capillary GC coupled with NPD. We also compared the extraction efficiencies of HBT–aldehyde derivatives from aqueous solutions at pH values of 2.96 and 5.99, performed by liquid–liquid extraction (LLE) with hexane–isopropanol (9:1, v/v) and by solid-phase extraction (SPE), employing C_{18} octadecyl-bonded cartridges. Both methods led to selective detection of HBT–aldehyde derivatives with NPD after chromatographic separation on a fused-silica narrow-bore capillary column. The technique was applied to the analysis of volatile aldehydes in heated olive oil.

2. Experimental

2.1. Chemicals and reagents

The aldehydes (97–99%) were obtained from Aldrich (Milwaukee, WI, USA). Analytical-grade hexane, isopropanol and ethanol (J.T. Baker, Phillipsburg, NJ, USA) were used for extraction and preparation of the extracts and standard solutions. HBT (Eastman Kodak, Rochester, NY, USA) was employed as a derivatizing agent and 2,4-pentanedione (98%) (purchased from Aldrich, Milwaukee, WI, USA) was used as an internal standard. SPE cartridges (3 ml, C_{18} octadecyl-bonded phase) were obtained from Varian Sample Preparation Products (Harbor City, CA, USA). All other chemicals, reagents and solvents were of analytical grade. Vegetable oil (cold-pressed olive oil, *Extra virgin*) was purchased from the local market.

2.2. Gas chromatographic analysis

High-resolution gas chromatography (HRGC) analysis of the HBT–aldehyde derivatives was performed on a Hewlett-Packard (HP) (Palo Alto, CA, USA) 5890A Series II gas chromatograph equipped with a split/splitless injector (split ratio 1:10) and a nitrogen–phosphorus detection (NPD) system, both operated at 250°C. GC peak areas were integrated with a HP-3390A integrator. The column used was a DB-5 (J&W Scientific, Folsom, CA, USA) cross-linked fused-silica capillary column (30 m×0.25 mm I.D.), coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The oven temperature was programmed from 100°C (10 min hold) to 250°C, at 20°C min^{-1}. Helium was employed as a carrier gas (inlet pressure 80 kPa) with a linear

velocity of 35.5 cm s^{-1}. Flow-rates of air, nitrogen and hydrogen for NPD were 120, 30.5 and 4 ml min^{-1}, respectively. The injections were made using the air-plug, hot-needle mode and the injection volume was 1 μl.

2.3. GC–MS analysis

A Hewlett-Packard 5890A Series II gas chromatograph interfaced to a HP 5972 mass-selective detector with a HP MS ChemStation Data system was used for MS identification of the GC components. The column used was a HP-5MS cross-linked fused-silica capillary column (30 m×0.25 mm I.D.) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The oven was programmed as described in Section 2.2. The helium inlet pressure was 78 kPa, with a linear velocity of 38.7 cm min^{-1} (split 10 ml min^{-1}). The injector temperature was kept at 250°C and the volume injected was 1 μl. The temperatures of the ionization chamber and of the transfer line were 180 and 285°C, respectively. The electron beam energy was 70 eV. Mass spectra and reconstructed chromatograms were obtained by automatic scanning in the mass range m/z 50–400 at 2.2 scan s^{-1}.

2.4. Preparation and characterization of the HBT–aldehyde derivatives

To 0.5 g (0.003 mol) of HBT dissolved in 50 ml of 0.1 *M* HCl, ca. 1 ml of each aldehyde under study was added. The aldehydes were added slowly, dropwise, stirring the reaction mixture at room temperature for 20 min. The HBT-derivatized aldehydes precipitated readily. The reaction mixture was then filtered and each precipitate was washed with 0.1 *M* HCl and then dried. The products were recrystallized from 95% ethanol. White, needle-shaped crystals were obtained and characterized by means of mass spectrometry (EI, 70 eV), and ^{1}H and ^{13}C NMR. $C^{2}HCl_{3}$ solutions of the HBT-derivatized aldehydes were run on a Bruker AMX600 NMR spectrometer, using the solvent peaks (7.24 and 77.0 ppm) to assign the chemical shift scales. The 2,4-pentanedione–HBT derivative, used in this study as an internal standard, was obtained and characterized as described elsewhere [27].

2.5. Calibration plot for HBT–aldehyde derivatives

Calibration solutions of the HBT-derivatized aldehydes under study were prepared in hexane–isopropanol (9:1, v/v) at concentrations of 0.1, 1, 10, 25, 50 and 100 μg/ml. A 150-μl volume of the internal standard, 2,4-pentanedione–HBT derivative [154.7 μg/ml in hexane–isopropanol (9:1, v/v)], was added to 1 ml of each calibration solution of HBT-derivatized aldehydes. Relative response factors for NPD were calculated using the integrated peak area ratio of analyte (HBT–aldehydes) to internal standard and the respective concentrations.

2.6. Recovery efficiency of aldehydes from aqueous solutions

Two 1 m*M* phosphate-buffered saline (PBS) solutions were prepared at pH values of 2.96 and 5.99, by adjusting the pH with 6 *M* HCl. An aldehyde stock solution (2000 μg/ml) was prepared by adding 400 mg of each aldehyde to 200 ml of ethanol. From this stock solution six working aldehyde solutions were prepared in PBS at pH values of 2.96 and 5.99, respectively, at concentrations of 1, 10, 20, 40, 60 or 100 μg/ml. An HBT solution was made by dissolving 384 mg (2.3 mmol) of HBT in 200 ml of PBS at pH 3.0. To a 1-ml volume of each working aldehyde solution, 3 ml of PBS at pH 2.96 or 5.99, respectively, and 2 ml of HBT-derivative solution (1.92 mg/ml) were added. The mixture was stirred at room temperature for 5–10 min. For LLE, the resulting mixture was extracted three times with 1 ml of hexane–isopropanol (9:1, v/v) in capped test tubes.

The extract was concentrated to 1 ml under nitrogen flow at room temperature and a 150-μl volume of the internal standard [154.7 μg/ml 2,4-pentanedione–HBT derivative in hexane–isopropanol (9:1, v/v)] was added. A 1-μl volume of the extract was injected on the HRGC–NPD system and analyzed for HBT–aldehydes. For SPE, C_{18} octadecyl SPE cartridges (500 mg sorbent mass/2.8 ml column volume) were rinsed in succession with hexane–isopropanol (9:1, v/v), methanol, deionized water and PBS. The spiked aldehyde samples at pH 2.96 or 5.99, derivatized as described in Section 2.4, were then loaded on the cartridges connected to a vacuum manifold (J.T. Baker SPE-24G) and eluted

with 3 ml of hexane–isopropanol (9:1, v/v). The extract was concentrated under nitrogen flow to 1 ml and a 150-μl volume of the internal standard [154.7 μg/ml 2,4-pentanedione–HBT derivative in hexane–isopropanol (9:1, v/v)] was added. A 1-μl volume of the extract was injected onto the HRGC–NPD system.

2.7. Analysis of volatile aldehydes from heated olive oil

A 100-ml volume of olive oil (cold-pressed) was heated at 220°C for 2 h. To 2 ml of the heated oil, 5 g of NaCl, 3 ml of PBS solution at pH 2.96 and 2 ml of HBT-derivative solution (1.92 mg/ml) were added. The mixture was stirred for 20 min. The separated aqueous layer was loaded into the SPE cartridge and the HBT–aldehyde derivatives were eluted with 3 ml of hexane–isopropanol (9:1, v/v) and concentrated to 1 ml as described in Section 2.6 and a 150-μl aliquot of the internal standard [154.7 μg/ml 2,4-pentanedione–HBT derivative in hexane–isopropanol (9:1, v/v)] was added. A 1-μl volume of the extract was injected onto the HRGC–NPD system for HBT–aldehyde derivative quantitation.

3. Results and discussion

Capillary GC coupled with NPD was used to analyze the HBT-derivatized saturated volatile aldehydes; a typical chromatogram is shown in Fig. 1. Relative retention times (t_{RR}) and relative response factors (RRf), related to the 2,4-pentanedione–HBT derivative, used as an internal standard, are given in Table 1. The HBT-derivatized aldehydes were characterized by their mass spectra (EI, 70 eV), ^{1}H NMR and ^{13}C NMR. The spectroscopic data appear in Tables 1–3. ^{1}H NMR peak assignments were consistent with peak areas and the peak splittings predicted for the proposed chemical structure. All ^{13}C NMR peaks agreed with the relative intensities and positions expected for the proposed structures [28]. HBT–aldehyde derivatives possess specific fragmentation patterns in mass spectra (EI, 70 eV). The proposed pathways for the formation of key ions in their mass spectra are presented in Fig. 2.

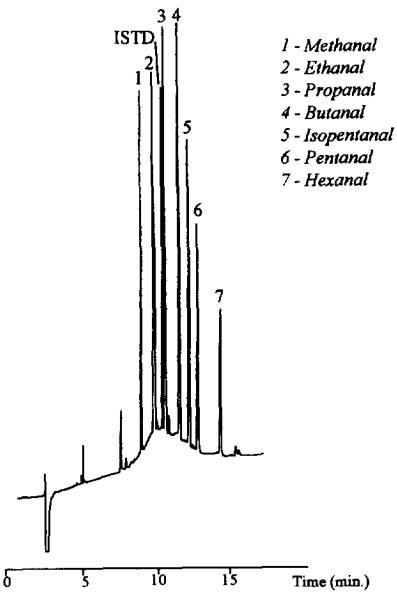

Fig. 1. Typical gas chromatogram of the HBT-derivatized aldehydes (10 μg/ml standard mixture) obtained on the DB-5 (30 m×0.25 mm, 0.25 μm) column, NPD (Attn. 5). ISTD: 2,4-pentanedione–HBT derivative.

For each HBT aldehyde derivative, triplicate GC runs of six calibration solutions (0.1, 1, 10, 25, 50 and 100 μg/ml) in hexane–isopropanol (9:1, v/v) were made. The calibration curves for HBT–aldehyde derivatives presented good linearity (R^2= 0.997–0.999). The GC determination of the volatile saturated aldehydes as their HBT derivatives is a rapid and efficient technique. Aldehyde derivatization, performed quantitatively at room temperature, is followed by hydrazone separation with high resolution on the 30 m narrow-bore (0.25 mm) capillary column in just 14.5 min (see Fig. 1).

The extraction efficiencies of HBT-derivatized aldehydes using a variety of analyte concentrations, pH and different extraction techniques are shown in Table 4. The efficiencies for SPE ranged from 81.1±1.1 to 98.1±1.4% compared to 70.1±3.1 to 92.4±1.0% obtained by LLE, for spiked aldehyde solutions at concentrations from 20 to 60 μg/ml. For lower concentration levels (1 or 10 μg/ml), both SPE and LLE showed an average decrease in recovery of 15%. For all concentrations, the recoveries from spiked aqueous solutions at pH 2.96

Table 1
Chromatographic and mass spectrometric parameters of HBT–aldehyde derivatives

HBT–Aldehyde	M_r	t^a_{RR}	σ	R.S.D.	RRf[b]	$(R^2)^c$	Characteristic ions in EI mass spectra
Methanal	177	0.85	8.5E−3	1.00	1.57	0.997	*177*[d], 176, 150, 149, 105, 108, 122, 78
Ethanal	191	0.94	6.8E−3	0.72	1.18	0.999	*176*, 149, 191, 105, 150, 108, 122, 123
Propanal	205	1.03	4.7E−3	0.46	1.17	0.999	*176*, 149, 205, 150, 105, 108, 123, 122
Butanal	219	1.11	1.9E−3	0.17	1.32	0.998	*176*, 149, 150, 219, 105, 108, 123, 122
Isopentanal	233	1.14	9.6E−3	0.84	1.24	0.997	*176*, 150, 149, 233, 105, 136, 108, 123
Pentanal	233	1.24	7.8E−3	0.63	1.28	0.997	*176*, 150, 149, 233, 105, 123, 136, 108
Hexanal	247	2.16	5.7E−3	0.26	1.45	0.998	*176*, 150, 149, 247, 105, 108, 123, 122

[a] Mean value ($n=10$).
[b] Response factors, measured relative to the 2,4-pentanedione–HBT derivative, used as an internal standard.
[c] Regression coefficients after multipoint calibration ($n=6$).
[d] Base peak in mass spectra, followed by characteristic ions (m/z) in decreasing order of their abundances.

Table 2
^{13}C Chemical shifts for representative HBT–aldehyde derivatives

Carbon position	Assignment (ppm)	
	HBT–ethanal	HBT–pentanal
2	168.20	168.80
3a	150.20	150.10
4	126.00	125.90
5	121.80	121.70
6	121.30	121.30
7	118.00	117.70
7a	130.10	130.00
10	143.70	148.20
11	18.41	32.04
12	–	28.59
13	–	22.26
14	–	13.84

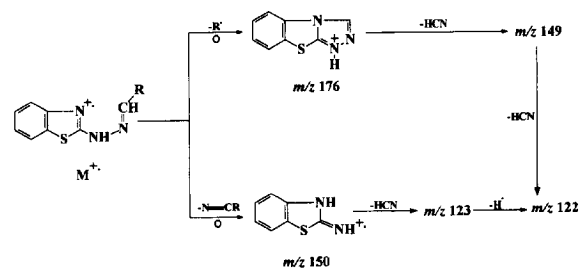

Fig. 2. MS fragmentation patterns of HBT–aldehyde derivatives.

were on the average 2–7% higher than those obtained at pH 5.99, although SPE efficiency was less affected by pH than was LLE. LLE showed an average reduction in recoveries of 5–12% relative to

Table 3
^1H Chemical shifts for HBT–aldehyde derivatives

Proton position position	Assignment (ppm)					
	HBT–ethanal	HBT–propanal	HBT–butanal	HBT–iso pentanal	HBT–pentanal	HBT–hexanal
4	7.629	7.622	7.663	7.663	7.626	7.664
5	7.290	7.281	7.291	7.291	7.293	7.291
6	7.115	7.112	7.122	7.120	7.111	7.109
7	7.491	7.487	7.627	7.471	7.489	7.476
8	1.562	1.564	1.606	1.610	1.600	1.600
10	7.275	7.281	7.291	7.291	7.258	7.291
11	2.028	2.358	2.314	2.219	2.330	2.329
12	–	1.146	1.606	1.891	1.540	1.541
13	–	–	0.984	0.980 0.969	1.40	1.346
14	–	–	–	0.980 0.969	0.933	1.346
15	–	–	–	–	–	0.899

Table 4
Comparison of the extraction efficiencies (%) of HBT-derivatized volatile aldehydes

HBT–aldehyde	Recovery (%)[a]											
	Aldehyde concentration in spiked solutions											
	1 μg/ml		10 μg/ml		20 μg/ml		40 μg/ml		60 μg/ml		100 μg/ml	
	LLE	SPE	LLE	SPE	LLE	SPE	LLE	SPE	LLE	SPE	LLE	SPE
Methanal	77.1±4.0[b]	80.2±3.0	79.3±5.1	92.9±2.2	86.2±1.4	97.1±1.7	92.4±1.0	98.1±1.4	91.1±4.1	97.1±2.0	90.3±3.1	97.8±2.1
	70.2±3.0[c]	76.2±2.0	75.1±3.1	89.3±3.1	84.1±1.0	96.4±2.2	90.1±2.0	98.0±0.5	89.5±1.6	97.0±1.0	87.1±1.4	96.2±1.2
Ethanal	75.5±3.4	77.9±4.0	76.9±6.0	93.4±2.9	82.4±1.1	96.6±1.0	91.7±1.9	97.2±1.6	89.5±2.6	96.5±0.9	91.4±2.8	96.4±0.3
	71.3±2.6	75.1±5.6	73.2±2.1	87.5±4.1	80.2±2.0	95.1±1.2	88.4±0.7	97.1±1.0	88.8±1.0	95.1±2.2	86.2±2.0	96.1±1.0
Propanal	70.6±4.3	78.8±3.0	77.1±3.2	90.1±3.4	80.1±1.2	93.0±2.0	90.0±2.5	95.1±2.1	90.1±1.4	94.1±2.1	90.1±2.2	95.6±1.2
	68.3±1.2	74.1±2.1	70.9±3.1	89.1±2.0	77.8±2.4	92.3±2.5	85.9±4.0	94.9±2.0	87.4±2.1	93.6±2.4	88.1±2.5	95.1±2.0
Butanal	71.7±3.1	78.1±2.9	74.3±4.1	85.6±4.6	79.6±2.4	89.3±1.6	86.9±3.1	92.2±1.0	89.2±2.1	95.1±1.5	90.0±2.6	95.3±2.6
	65.4±3.1	73.9±3.8	73.4±0.9	78.9±4.0	77.9±3.0	90.0±3.1	83.3±2.9	91.8±2.1	86.2±2.0	93.5±1.6	87.4±1.9	93.4±1.6
Isopentanal	70.5±2.8	77.9±3.4	72.2±5.6	83.7±5.0	75.4±1.9	87.1±2.8	84.4±2.9	89.1±2.4	85.2±1.9	92.1±1.4	85.3±1.2	90.1±3.4
	64.3±4.1	74.6±3.6	70.1±4.1	79.4±2.5	73.2±2.0	86.2±1.1	82.7±2.4	90.0±1.5	80.9±4.1	91.9±2.0	82.4±2.8	90.0±1.2
Pentanal	66.1±3.4	70.5±6.9	69.9±6.0	84.1±4.6	72.2±2.3	83.3±1.9	81.6±3.1	85.2±3.6	82.6±2.1	90.4±1.6	80.1±3.2	89.9±2.8
	59.2±9.1	66.9±5.1	64.9±5.1	80.7±2.4	70.6±3.4	82.1±2.0	79.4±4.1	84.5±2.4	80.1±3.4	88.4±2.5	78.1±4.0	90.0±1.0
Hexanal	60.9±6.1	71.6±2.5	66.3±5.6	80.2±3.1	70.1±3.1	81.1±1.1	79.9±3.2	87.1±2.4	80.9±1.9	90.5±2.1	79.4±2.8	88.1±2.1
	59.9±7.0	68.5±3.2	61.9±4.1	77.7±4.0	69.2±4.0	78.9±2.4	76.9±3.5	85.4±1.5	77.9±2.2	89.0±1.5	76.9±3.0	87.9±2.0

[a] $n=4$, mean±S.D.
[b] pH 2.96.
[c] pH 5.99.

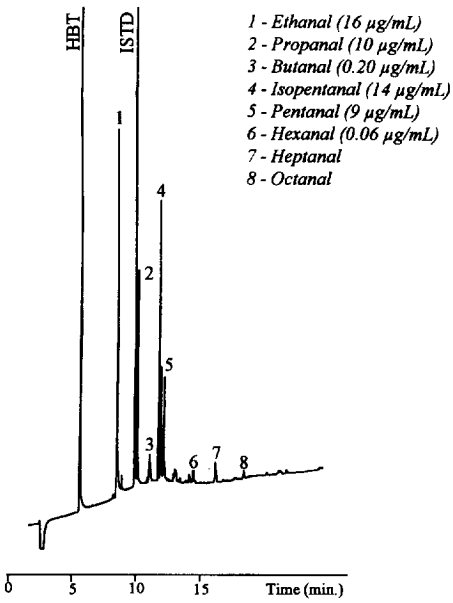

Fig. 3. GC profile obtained on the DB-5 (30 m×0.25 mm, 0.25 μm) column fitted with NPD, of the aldehydes (as HBT derivatives) present in the olive oil heated at 220°C for 2 h. ISTD: 2,4-pentanedione–HBT derivative.

SPE. The differences in SPE and LLE efficiencies were much more significant at low concentrations (1 or 10 μg/ml).

The standardized method was applied to the determination of volatile aldehydes present in olive oil heated at 220°C for 2 h. Fig. 3 shows a typical chromatographic profile of the aldehydes (as their HBT-derivatives) found in the heated olive oil. Ethanal (16.0 μg/ml), propanal (10 μg/ml), isopentanal (14.0 μg/ml) and pentanal (9.0 μg/ml) were detected among the principal volatile aldehydes in the heated olive oil. This example corroborates that the analytical method developed is selective and rapid and can be applied successfully to the quality control of vegetable oils, or other fat-rich food products.

4. Conclusions

Volatile aldehydes, typical products of lipid peroxidation, derivatized quantitatively at room temperature with HBT formed stable, water-insoluble, trinitrogenous organosulfur compounds, which can be selectively and sensitively determined by capillary chromatography, using narrow-bore columns fitted with NPD. The established method is highly selective and versatile, because other instrumentation tools can also be used. As HBT-derivatized aldehydes are polar and relatively low-volatile compounds, their analysis can be carried out using high-performance liquid chromatography with an UV detector. The specific fragmentation patterns of HBT–aldehyde derivatives and the presence of sulfur in the molecules allows analysis by HRGC–MS in SIM-mode at m/z 176, 149 and m/z 150, or by HRGC–flame-photometric detection, respectively. Thus, the method described in this work can be successfully applied to the monitoring of lipid peroxidation products in food, biological fluids or other analytes of interest in environmental toxicology.

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