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Comparison of extraction methods and detection systems in the gas chromatographic analysis of volatile carbonyl compounds

Elena E. Stashenko[a,*], María Constanza Ferreira[a], Luis Gonzalo Sequeda[a], Jairo René Martínez[a], Jon W. Wong[b]

[a]*Phytochemistry and Chromatography Laboratory, Chemistry Department, Industrial University of Santander, AA 678, Bucaramanga, Colombia*

[b]*Department of Environmental Toxicology, University of California, Davis, CA 95616, USA*

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Abstract

High-resolution gas chromatography (HRGC) with electron-capture detection (ECD), nitrogen–phosphorus detection (NPD), flame ionization detection (FID) or with mass spectrometry–selected ion monitoring (MS–SIM) was used in the analysis of volatile carbonyl compounds. Eighteen carbonyl compounds that are typically produced during lipid peroxidation were derivatized quantitatively with pentafluorophenylhydrazine (PFPH) at room temperature, to afford their corresponding water-insoluble hydrazones. These derivatives were extracted into non-polar phases by means of either liquid–liquid extraction (LLE) (hexane) or solid-phase extraction (SPE) on 3 ml C_{18} octadecyl-bonded phase cartridges. Detection limits of 10^{-14} and 10^{-12} mol/ml per aldehyde were achieved with the ECD and MS–SIM systems, respectively. The effects of extraction conditions on sensitivity and recovery were determined by performing parallel HRGC–ECD and HRGC–MS–SIM analyses of pentafluorophenylhydrazones of the eighteen compounds under study. Recoveries of 51.4–78.9±1.2–4.5 and 80.9–98.3±1.0–3.5% were obtained with LLE and SPE, respectively. The method was applied to the analysis of the volatile carbonyl compounds in various heated vegetable oils (corn, palm or sunflower) and to the analysis of volatile aldehydes in human urine. © 1997 Elsevier Science B.V.

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1. Introduction

The decomposition of lipid hydroperoxides is accompanied by the formation of aldehydes, which can then be used as indicators of lipid peroxidation. Medium- and short-chain aldehydes of this group are responsible for the unpleasant odour of rancid fat-rich foods [1]. These carbonyl compounds are not only end products and vestiges of lipid peroxidation processes, but also could be considered as "second toxic messengers" of the initial free-radical event [2]; some aldehydes have been linked to 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, including analyses of lipid hydroperoxides [6], conjugated dienes [7,8] and the estimation of expired

*Corresponding author.

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hydrocarbons [9,10], to measure the extent of lipid peroxidation [11–13]. Medium- and short-chain aldehydes are reactive, volatile and are partially water-soluble compounds, whose extraction from aqueous solutions, foods or biological fluids, accompanied by gas chromatographic analysis with conventional detection systems [flame ionization detection (FID), thermal conductivity detection (TCD) or mass spectrometry (MS)], is marked by poor recoveries and low sensitivity. Chemical derivatization of these compounds and the use of highly selective, specific detection systems have been the solution to this problem. The most common method for the analysis of volatile carbonyl compounds is derivatization with 2,4-dinitrophenylhydrazine and analysis by high-performance liquid chromatography [14–16]. However, the derivatization requires strong acidic conditions, which may alter the analytes of interest. Another simple and specific method involves derivatization of aldehydes with cysteamine (2-aminoethanethiol) to form stable thiazolidines under mild conditions at room temperature and neutral pH [17,18]. However, 2-aminoethanethiol 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, but none of them has led to a general method of analysis. Pentafluorophenylhydrazones from volatile aldehydes have been obtained [23] and analyzed by FID, TCD [24], electron-capture detection (ECD) and MS systems [24–26], but without achieving high sensitivity and selectivity. Malondialdehyde, derivatized with pentafluorophenylhydrazine, has been detected in femtomole quantities in biological samples, using gas chromatography (GC)–MS with negative chemical ionization [26]. The derivatization of malondialdehyde, 2,4-pentanedione and acetoacetaldehyde with 2-hydrazinobenzothiazole (HBT) was described by Beljean-Leymarie and Bruna [27]. This analytical method, based on the use of a packed column fitted with NPD, does not permit a complete separation of the complex aldehyde mixture normally generated during lipid peroxidation. The use of a fused-silica narrow-bore capillary column for GC analysis of HBT–aldehyde derivatives has solved this problem [28]. O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamines have also been used in the GC determinations of low-molecular-mass carbonyl compounds [29–31], 4-hydroxyalkenals [32], oxoacids [33,34] and other lipid peroxidation products [35,36] and of keto steroids [37]. Our survey of the GC methods currently available for the analysis of lipid peroxidation products did not find a simple method that is applicable to the detection of carbonyl compounds in complex mixtures at concentrations below pmol/ml.

In this work, we analyzed eighteen volatile carbonyl compounds as their pentofluorophenylhydrazine (PFPH) derivatives (pentafluorophenylhydrazones) using different detection systems [FID, NPD, ECD or MS–selected ion monitoring (SIM)], and compared the extraction recoveries of these derivatives from aqueous solutions, performed by liquid–liquid extraction with hexane at pH 4, and by solid-phase extraction, employing C_{18} octadecyl-bonded cartridges. Both extraction methods led to selective detection of PFPH–aldehydes. The lowest detection limits were obtained when chromatographic separation on a fused-silica narrow-bore capillary column was followed by either an ECD or an MS–SIM system.

The standardized technique was applied to the analysis of volatile carbonyl compounds present in a lipid system (heated vegetable oils) and in an aqueous biological fluid (human urine). Lipid peroxidation was induced in vegetable oils (corn, palm or sunflower) by heating them to 250°C for 1 h or by 2-min irradiation in a microwave oven (800 W). Urine samples were obtained from ten healthy young volunteers in order to monitor the aldehyde content before and after consumption of an alcoholic beverage (beer).

2. Experimental

2.1. Chemicals and reagents

The aldehydes (97–99%) were obtained from Aldrich (Milwaukee, WI, USA). Analytical-grade hexane, dichloromethane and pesticide-grade methanol (J.T. Baker, Phillipsburg, NJ, USA) were used for extraction and preparation of the extracts and standard solutions. Pentafluorophenylhydrazine

(97%), employed as a derivatizing agent, and [$^2H_6$]acetone (acetone-d_6; 99%), used as an internal standard, were purchased from Aldrich. 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 oils (palm, corn and sunflower) and beer ("Aguila", Bavaria, 4% alcohol content) were purchased from the local market. Urine samples were obtained from ten healthy volunteers (men, 20–25 years old).

2.2. Gas chromatographic analysis

High-resolution (HR) GC analysis of the pentafluorophenylhydrazones was performed on a Hewlett-Packard (HP) (Palo Alto, CA, USA) 5890A Series II gas chromatograph that was equipped with a split/splitless injector (250°C, split ratio 1:30) and ECD (^{63}Ni), FID or NPD systems operated at 280, 250 and 240°C, respectively. Chromatographic data were processed with an HP ChemStation 3365-II (Hewlett-Packard). The cross-linked fused-silica capillary columns used were a DB-1 (J&W Scientific, Folsom, CA, USA) (50 m×0.25 mm I.D.), coated with polydimethylsiloxane (0.25 μm phase thickness) and an HP-5 (HP; 60 m×0.20 mm I.D.), coated with 5% phenyl-polymethylsiloxane (0.33 μm phase thickness). For both columns, the oven temperature was programmed from 100°C (5 min hold) at 2.5°C/min to 200°C (10 min hold) and then to 250°C at 5°C/min. Helium (AGA, 99.995%) was used as a carrier gas (inlet pressure, 200 kPa) with a linear velocity of 35.5 cm/s for both columns. An argon–methane (9:1) mixture was employed for ECD as a make-up gas at a flow-rate of 60 ml/min. Air and hydrogen flow-rates were 300 and 30 ml/min for FID, and 100 and 5 ml/min for NPD, respectively. Nitrogen was used as a make-up gas at flow-rates of 30 ml/min (FID) and 28 ml/min (NPD). 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 an HP 5972 mass-selective detector with an HP MS ChemStation data system was used for MS identification of the GC components. The column used was an HP-5MS cross-linked fused-silica capillary column (50 m×0.25 mm I.D.) that was coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The oven was programmed as described above in Section 2.2. The helium inlet pressure was 180 kPa, with a linear velocity of 38.7 cm/min (split valve, 30 ml/min). 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. For quantitative analysis, the signals were recorded by SIM at m/z 155, 182 and 224, which were key-fragments for PFPH–aldehyde derivatives and the PFPH–acetone-d_6 standard, respectively. The dwell time used for the monitored ions was fixed at 100 s.

2.4. Chemical derivatization

The procedure followed for the preparation of the pentafluorophenylhydrazones from volatile aldehydes was as described elsewhere [23–25]. The derivatives were characterized by IR and MS.

2.5. Calibration plot for PFPH–aldehyde derivatives

Calibration solutions of the aldehydes under study were prepared in methanol at low (0.175, 1.75 and 17.5 pmol/ml, 0.175 and 1.75 nmol/ml) and at high (1.75 and 17.5 nmol/ml; 0.175 and 1.75 μmol/ml) concentration levels. A 10-μl volume of the internal standard (PFPH–acetone-d_6 derivative) was added to 1 ml of the standard aldehyde solutions at low and high concentration levels, respectively (0.205 and 20.5 μmol/ml in methanol). Relative response factors for the ECD or MS–SIM systems were calculated for two different concentration levels using the integrated peak area ratio of analyte (PFPH–aldehydes) to internal standard and the respective concentrations. Detection limits for ECD, NPD, FID or

MS–SIM systems were obtained for a signal–noise ratio of five.

2.6. Recovery efficiency of aldehyde from aqueous solutions

For liquid–liquid extraction (LLE), each aldehyde was spiked at two different concentrations (1.75 or 17.5 nmol/ml) in phosphate-buffered saline (PBS) solutions and derivatized at room temperature over 3 h with 1 ml of PFPH solution (45 mg/ml in 0.1 *M* HCl) and was extracted three times with 3 ml of hexane in capped test tubes. The extracts were concentrated to 1 ml under nitrogen flow at room temperature and a 10-μl volume of the internal standard (20.5 μmol/ml PFPH–acetone-d_6 derivative in methanol) was added. A 1-μl volume of the extract was injected onto the HRGC–ECD or HRGC–MS–SIM system and was analyzed for PFPH–aldehyde derivatives. The experiments were repeated three times.

For solid-phase extraction (SPE), C_{18} octadecyl SPE cartridges (500 mg sorbent mass/2.8 ml column volume) were rinsed in succession with dichloromethane, methanol, deionized water and PBS. The spiked aldehyde samples, 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 5 ml of hexane. The extracts were concentrated under nitrogen flow at room temperature to 1 ml and a 10-μl volume of the internal standard (20.5 μmol/ml PFPH–acetone-d_6 derivative in methanol) was added. A 1-μl volume of the extract was injected onto the HRGC–ECD or HRGC–MS–SIM system. The extraction procedures were repeated three times.

2.7. Analysis of volatile aldehydes in heated vegetable oils

A 2-ml volume (ca. 1.5 g) of vegetable oil (palm, corn or sunflower) was placed in a capped test tube and heated for 1 h to 250°C in a silicone oil bath. After cooling, each sample was derivatized at room temperature with a 2-ml volume of PFPH solution (0.22 *M* in 0.1 *M* HCl) over 3 h. For LLE, PFPH–aldehyde derivatives were extracted (from the aqueous layer) three times with 2 ml of hexane and the extract was separated and concentrated to 1 ml under nitrogen flow at room temperature. For SPE, the separated aqueous layer was loaded onto the SPE cartridge and the PFPH–aldehyde derivatives were eluted with 2 ml of hexane and concentrated to 1 ml, as described above. A 10-μl volume of the internal standard (20.5 μmol/ml PFPH–acetone-d_6 in methanol) and anhydrous sodium sulphate (to remove moisture) were added to the extract. A 1-μl volume of the extract was injected onto the HRGC–ECD or HRGC–MS–SIM system for PFPH–aldehyde derivative quantitation.

A 2-ml volume (ca. 1.5 g) of vegetable oil (palm, corn or sunflower), placed in a capped test tube, was put in a conventional microwave oven (Kendo, MO-124, Japan, 800 W, 2.45 GHz) and irradiated at full power for 2 min. After cooling the tube, carbonyl compounds present in the oil were derivatized with PFPH. The pentafluorophenylhydrazones were then extracted and analyzed as described above. All experiments, including blank analyses, were repeated three times.

2.8. Analysis of volatile aldehydes from human urine

Urine samples (blanks) were taken from ten fasted young volunteers (all men, 20–25 years old). Each volunteer ingested 1.8 l of beer over a period of 1 h, without consuming any other foods. Subsequently, urine samples were taken every 2 h and were analyzed as described below.

A 5-ml volume of urine was derivatized at room temperature with 1 ml of PFPH solution (0.22 *M* in 0.1 *M* HCl) over 2 h. The mixture was loaded onto the SPE cartridge and the PFPH–aldehyde derivatives were eluted with 5 ml of hexane and then were concentrated to 1 ml under nitrogen flow at room temperature. A 10-μl volume of the internal standard (0.20 μmol/ml PFPH–acetone-d_6 in methanol) and anhydrous sodium sulphate were added to the extract. A 1-μl volume of the extract was injected onto the HRGC–ECD or HRGC–MS–SIM system for PFPH–aldehyde derivative quantitation.

3. Results and discussion

Relative retention times (t_{RR}), relative response factors (RR_f) (calculated using the acetone–d_6-PFPH

derivative as an internal standard), and the detection limits obtained with the various detection systems (FID, NPD, ECD, MS–SIM) for the pentafluorophenylhydrazones of the eighteen carbonyl compounds under study are given in Table 1. Due to the high fluorine content of PFPH–aldehyde derivatives, the highest sensitivity was obtained for ECD, which detected aldehydes at concentrations of 1.60–1.7 fmol/ml. Lower sensitivity levels were obtained with MS–SIM (0.74–2.54 pmol/ml), followed by NPD (1.00–1.55 nmol/ml) and FID (15.04–23.05 nmol/ml) detection systems. The NPD system was selective, but was not a very sensitive technique. All detection limits were calculated at a signal–noise of five (split ratio 1:30, 1 μl injection volume). The calibration curves obtained for the high (1.75 nmol/ml to 1.75 μmol/ml) and low (0.175 pmol/ml to 1.75 nmol/ml) concentration levels of the PFPH–aldehyde derivatives presented good linearity (R^2= 0.997–0.999).

Although nonanal and *trans*-2-octenal, decanal and *trans*-2-nonenal PFPH-derivatives were not baseline resolved on the DB-1 (50 m×0.25 mm, 0.25 μm), HP-5 (30 m×0.25 mm, 0.25 μm) and HP-5 (60 m×0.20 mm, 0.33 μm) columns connected to the FID, NPD or ECD systems, their "separation" could be performed on the same columns using MS–SIM at $M^{+\cdot}$ *m/z* 322 and 306, and at $M^{+\cdot}$ *m/z* 336 and 320, respectively. Thus, these compounds can be quantified in the complex mixture using the HRGC–MS–SIM system.

Table 2 shows the extraction efficiencies of the PFPH–aldehyde derivatives in spiked samples, obtained for two concentrations (1.75 and 17.5 nmol/ml). SPE showed higher recovery and reproducibility (80.9–98.3±1.0–3.5%) in comparison with the LLE method (51.4–78.9±1.0–4.5%). The recoveries were higher for short-chain saturated aldehydes (C_1–C_5) than for their medium-chain homologues (C_6–C_{11}). This trend was more marked for LLE than for SPE. The extraction efficiencies obtained by LLE for α,β-unsaturated aldehydes showed an average decrease in

Table 1
Relative retention times (t_{RR}), relative response factors (RR_f) and detection limits of PFPH-derivatized carbonyl compounds

PFPH derivative	t_{RR}[a]	S.D.	R.S.D.	Detection limits[b]				RR_f[c]			
				ECD (fmol/ml)	MS–SIM (pmol/ml)	NPD (nmol/ml)	FID (nmol/ml)	ECD		MS–SIM	
								I[d]	II[e]	I	II
Methanal	0.64	$6.6\cdot10^{-3}$	1.03	16.20	1.00	1.01	19.28	1.05	1.05	1.01	1.05
Ethanal	0.86	$8.6\cdot10^{-3}$	1.00	16.20	0.80	1.01	19.45	0.99	1.01	1.60	1.67
Acetone	1.01	$3.5\cdot10^{-3}$	0.35	16.00	0.85	1.01	19.78	0.98	0.82	1.70	1.64
Propanal	1.05	$2.0\cdot10^{-3}$	0.19	16.30	0.84	1.00	20.00	0.95	0.90	1.72	1.73
Acrolein	1.13	$1.1\cdot10^{-2}$	0.97	16.60	1.31	1.25	20.54	1.80	1.81	1.52	1.63
Butanal	1.27	$4.4\cdot10^{-3}$	0.37	17.60	1.46	1.22	21.15	0.72	0.90	2.47	2.56
Isopentanal	1.39	$8.0\cdot10^{-3}$	0.57	16.30	1.00	1.06	19.64	1.00	1.01	1.98	2.19
Crotonaldehyde	1.46	$1.0\cdot10^{-2}$	0.68	16.00	1.00	1.04	19.30	1.13	1.50	1.58	1.67
Pentanal	1.50	$9.1\cdot10^{-3}$	0.60	16.00	1.00	1.03	19.21	1.09	1.01	1.98	2.20
Hexanal	1.71	$4.1\cdot10^{-3}$	0.24	16.30	0.94	1.05	18.50	1.11	0.94	2.61	2.75
trans-2-Hexenal	1.88	$1.8\cdot10^{-2}$	0.95	17.00	0.99	1.00	18.11	1.02	0.97	2.10	2.22
Heptanal	1.91	$6.5\cdot10^{-3}$	0.34	17.20	0.85	1.00	18.24	1.10	1.05	2.61	2.75
Octanal	2.10	$3.7\cdot10^{-3}$	0.17	16.50	0.76	1.01	15.11	1.36	1.70	3.32	3.48
Nonanal	2.31	$7.0\cdot10^{-3}$	0.30	16.60	0.74	1.00	15.04	1.54	1.47	2.66	2.80
trans-2-Octenal	2.32	$1.8\cdot10^{-2}$	0.77	16.90	1.60	1.55	21.10	1.32	1.27	1.76	1.74
Decanal	2.58	$4.5\cdot10^{-3}$	0.17	17.20	2.32	1.55	22.00	0.60	0.70	1.24	1.43
trans-2-Nonenal	2.59	$8.4\cdot10^{-3}$	0.32	16.90	2.54	1.52	23.05	1.25	1.36	1.14	1.26
Undecanal	2.93	$4.0\cdot10^{-3}$	0.13	16.90	1.69	1.51	22.01	0.51	0.58	0.51	0.64

[a] Mean value (n=10). t_{RR} was determined on the HP-5 column.
[b] Detection limits determined for a signal-to-noise ratio of five.
[c] Response factors, measured relative to PFPH–acetone-d_6, which was used as an internal standard.
[d] Concentration range from 0.175 pmol/ml to 1.75 nmol/ml.
[e] Concentration range from 1.75 nmol/ml to 1.75 μmol/ml.

Table 2
Comparison of the extraction efficiencies (%) of PFPH-derivatized volatile carbonyl compounds

PFPH derivative	Recovery (%)[a]			
	Carbon 1 compound concentration in spiked solutions			
	1.75 nmol/ml		17.5 nmol/ml	
	LLE	SPE	LLE	SPE
Methanal	78.9±4.50	96.4±1.82	77.4±4.1	98.3±1.70
Ethanal	73.9±3.53	96.0±1.30	75.4±3.8	97.3±1.72
Propanal	75.0±2.01	95.0±1.00	74.8±2.3	96.4±1.29
Acetone	75.0±2.00	96.9±1.60	75.8±2.9	96.4±1.29
Acrolein	61.5±3.32	93.4±1.30	62.3±3.5	94.8±1.10
Butanal	70.8±1.01	92.0±1.20	71.3±1.2	97.6±2.00
Isopentanal	74.0±1.31	91.1±1.10	75.0±1.4	95.0±1.60
Crotonaldehyde	68.0±2.50	90.1±1.60	68.2±3.0	92.1±1.20
Pentanal	73.8±1.60	95.0±1.48	75.0±1.6	94.7±1.55
Hexanal	66.6±2.00	92.4±1.20	67.5±1.7	90.4±1.00
trans-2-Hexenal	63.3±2.00	87.8±1.60	65.4±2.2	89.2±1.80
Heptanal	72.1±3.00	93.8±1.60	72.4±3.3	91.8±1.60
Octanal	72.0±1.51	92.5±1.30	72.4±1.4	90.5±1.10
trans-2-Octenal	60.0±2.50	86.7±1.70	61.0±2.8	85.7±1.90
Nonanal	60.0±1.50	90.1±3.55	59.7±1.7	88.8±3.20
trans-2-Nonenal	54.0±4.00	82.8±2.40	53.5±3.8	81.9±2.10
Decanal	55.5±2.00	83.9±3.10	56.5±2.1	84.5±2.10
Undecanal	52.3±2.00	87.9±1.80	51.4±2.5	80.9±1.40

[a] $n=3$, mean±S.D.

recovery of 10–20%, in comparison with their saturated analogues. For SPE, this difference was not so noticeable (Table 2). Generally, LLE showed an average reduction in recoveries of 15–25% relative to SPE.

Due to the results obtained with the eighteen standard carbonyl compounds (Table 1), the NPD and FID systems were not used for further analysis of complex mixtures. Thus, we performed the determination of volatile carbonyl compounds in heated vegetable oils and in human urine, using PFPH derivatization, LLE or SPE, and quantitation was performed using the HRGC–ECD or HRGC–MS–SIM system.

Vegetable oils (palm, corn and sunflower) were heated in capped test tubes to 250°C for 1 h, or by microwave (MW, 800 W) irradiation for 2 min. The volatile carbonyl compounds, formed in the oils by thermally induced peroxidation, were extracted by LLE or SPE and were derivatized, as described in Section 2.7. Triplicate experiments and blank analyses (oils without heating) were carried out for each measurement. Fig. 1 shows a typical gas chromatogram of the aldehydes (as pentafluoro-

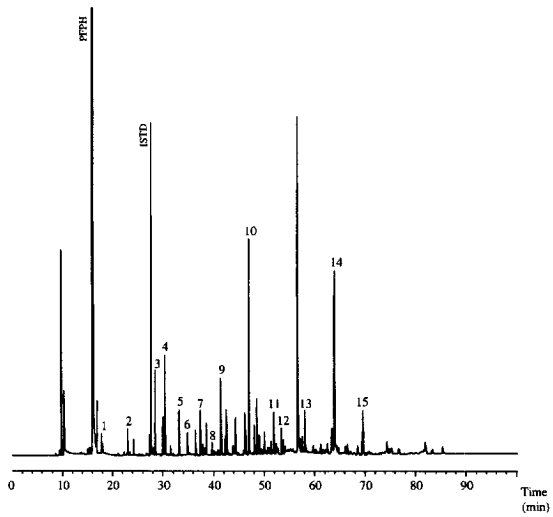

Fig. 1. GC profile obtained on the HP-5 (60 m×0.20 mm, 0.33 μm) column fitted with ECD, of the carbonyl compounds (as PFPH derivatives) present in the sunflower oil heated for 1 h at 250°C. Internal standard, acetone-d_6–PFPH derivative. 1=methanal, 2=ethanal, 3=acetone, 4=propanal, 5=acrolein, 6=butanal, 7=isopentanal, 8=crotonaldehyde, 9=pentanal, 10=hexanal, 11=*trans*-2-hexenal, 12=heptanal, 13=octanal, 14=nonanal and 15=decanal.

Table 3
Volatile carbonyl compounds found in heated vegetable oils

PFPH derivative	Concentration[a] (μg/ml)								
	Corn oil			Sunflower oil			Palm oil		
	0 h[b]	1 h	MW[c]	0 h	1 h	MW	0 h	1 h	MW
Methanal	$0.01\pm3.1\cdot10^{-3}$	$0.12\pm2.5\cdot10^{-3}$	$0.10\pm3.8\cdot10^{-3}$	N.D.	$0.09\pm3.2\cdot10^{-3}$	$0.10\pm3.8\cdot10^{-3}$	$0.12\pm2.5\cdot10^{-3}$	$0.26\pm5.3\cdot10^{-3}$	$0.29\pm5.4\cdot10^{-2}$
Ethanal	$0.03\pm3.5\cdot10^{-3}$	$0.07\pm4.9\cdot10^{-3}$	$0.05\pm5.3\cdot10^{-3}$	$0.01\pm3.2\cdot10^{-3}$	$0.05\pm5.0\cdot10^{-3}$	$0.08\pm5.9\cdot10^{-3}$	$0.05\pm5.6\cdot10^{-3}$	$0.55\pm1.5\cdot10^{-2}$	$0.50\pm1.6\cdot10^{-2}$
Acetone	$0.02\pm3.4\cdot10^{-3}$	$0.39\pm2.3\cdot10^{-3}$	$0.19\pm2.9\cdot10^{-3}$	N.D.	$0.20\pm2.1\cdot10^{-3}$	$0.25\pm2.8\cdot10^{-3}$	N.D.	$1.24\pm4.5\cdot10^{-2}$	$1.20\pm4.7\cdot10^{-2}$
Propanal	$0.18\pm2.6\cdot10^{-3}$	$0.41\pm2.8\cdot10^{-3}$	$0.39\pm3.5\cdot10^{-3}$	N.D.	$0.22\pm2.0\cdot10^{-3}$	$0.25\pm2.8\cdot10^{-3}$	N.D.	$1.27\pm4.6\cdot10^{-2}$	$1.20\pm5.6\cdot10^{-2}$
Acrolein	N.D.	$0.10\pm3.8\cdot10^{-3}$	N.D.	N.D.	$0.09\pm4.2\cdot10^{-3}$	N.D.	N.D.	$0.02\pm1.3\cdot10^{-3}$	N.D.
Butanal	$0.03\pm4.1\cdot10^{-3}$	$0.10\pm2.1\cdot10^{-3}$	$0.05\pm3.7\cdot10^{-3}$	N.D.	$0.09\pm1.8\cdot10^{-3}$	$0.10\pm1.9\cdot10^{-3}$	$0.12\pm2.5\cdot10^{-3}$	$0.28\pm5.7\cdot10^{-3}$	$0.21\pm6.7\cdot10^{-3}$
Isopentanal	$0.02\pm2.6\cdot10^{-3}$	$0.15\pm4.9\cdot10^{-3}$	$0.08\pm5.3\cdot10^{-3}$	N.D.	$0.08\pm3.0\cdot10^{-3}$	$0.10\pm3.3\cdot10^{-3}$	$0.11\pm1.8\cdot10^{-3}$	$0.32\pm5.6\cdot10^{-3}$	$0.31\pm6.6\cdot10^{-3}$
Crotonaldehyde	$0.01\pm3.5\cdot10^{-3}$	$0.07\pm3.5\cdot10^{-3}$	$0.03\pm3.7\cdot10^{-3}$	$0.01\pm2.7\cdot10^{-3}$	$0.06\pm4.0\cdot10^{-3}$	$0.10\pm5.0\cdot10^{-3}$	$0.05\pm3.8\cdot10^{-3}$	$0.15\pm6.0\cdot10^{-3}$	$0.13\pm6.0\cdot10^{-3}$
Pentanal	$0.02\pm4.5\cdot10^{-3}$	$0.38\pm1.8\cdot10^{-3}$	$0.30\pm1.9\cdot10^{-3}$	N.D.	$0.21\pm1.0\cdot10^{-3}$	$0.30\pm2.0\cdot10^{-3}$	$0.17\pm2.7\cdot10^{-3}$	$0.65\pm1.0\cdot10^{-2}$	$0.61\pm2.0\cdot10^{-2}$
Hexanal	$0.07\pm3.1\cdot10^{-3}$	$1.43\pm2.6\cdot10^{-3}$	$1.35\pm3.6\cdot10^{-2}$	N.D.	$0.89\pm2.4\cdot10^{-3}$	$0.95\pm3.4\cdot10^{-3}$	$0.09\pm1.9\cdot10^{-3}$	$2.57\pm6.2\cdot10^{-2}$	$2.54\pm6.7\cdot10^{-2}$
trans-2-Hexenal	N.D.	$0.11\pm2.2\cdot10^{-3}$	N.D.	N.D.	$0.10\pm3.4\cdot10^{-3}$	N.D.	N.D.	$0.17\pm5.2\cdot10^{-3}$	N.D.
Heptanal	$0.03\pm1.2\cdot10^{-3}$	$0.05\pm1.4\cdot10^{-3}$	$0.03\pm1.8\cdot10^{-3}$	N.D.	$0.03\pm1.5\cdot10^{-3}$	$0.50\pm2.5\cdot10^{-3}$	N.D.	N.D.	N.D.
Octanal	N.D	$0.11\pm9.1\cdot10^{-3}$	$0.04\pm9.1\cdot10^{-3}$	N.D.	$0.09\pm5.6\cdot10^{-3}$	$0.10\pm6.7\cdot10^{-3}$	N.D	$0.02\pm1.0\cdot10^{-3}$	$0.01\pm3.0\cdot10^{-3}$
Nonanal	$0.02\pm2.8\cdot10^{-3}$	$0.82\pm7.0\cdot10^{-2}$	$0.75\pm7.8\cdot10^{-2}$	N.D.	$0.78\pm8.1\cdot10^{-3}$	$0.90\pm8.9\cdot10^{-3}$	N.D	$0.93\pm3.2\cdot10^{-2}$	$0.89\pm4.2\cdot10^{-2}$
Decanal	$0.01\pm2.0\cdot10^{-3}$	$0.27\pm1.3\cdot10^{-2}$	$0.13\pm1.9\cdot10^{-2}$	N.D.	$0.20\pm6.1\cdot10^{-3}$	$0.21\pm6.9\cdot10^{-3}$	N.D	$0.72\pm7.8\cdot10^{-3}$	$0.68\pm7.9\cdot10^{-3}$
Total	$0.45\pm4.5\cdot10^{-3}$	$4.58\pm7.0\cdot10^{-2}$	$3.49\pm7.8\cdot10^{-2}$	$0.02\pm3.2\cdot10^{-3}$	$3.18\pm8.1\cdot10^{-3}$	$3.94\pm8.9\cdot10^{-2}$	$0.71\pm5.6\cdot10^{-3}$	$9.15\pm6.2\cdot10^{-2}$	$8.57\pm6.7\cdot10^{-2}$

[a] Mean value±S.D. ($n=3$).
[b] Heating time, h.
[c] Microwave heating (800 W, 2 min).
N.D.=Not detected.

phenylhydrazones) formed in heated sunflower oil and isolated by SPE. The amounts of various aldehydes present in the heated and irradiated vegetable oils under study are given in Table 3. Just as in the case of spiked aqueous solutions, the extraction of aldehydes from vegetable oils showed better reproducibility and recoveries with SPE than with LLE. The data shown in Table 3 correspond to SPE. Propanal, butanal, pentanal, hexanal and heptanal were among the most abundant aldehydes in the heated oils. The concentration of low-molecular-mass carbonyl compounds in the oils after 1 h of convectional heating was highest in palm (9.15 μg/ml), followed by corn (4.58 μg/ml) and sunflower (3.18 μg/ml) oils. It is worth noticing that the rapid (2 min) heating of vegetable oils by microwave irradiation led to roughly as much aldehyde content as that obtained by convectional heating to 250°C for 1 h (Table 3). While the total aldehyde content in the MW-heated palm oil was 8.57 μg/ml, in the corn and sunflower oils, the total of low-molecular-mass carbonyl compounds were found at concentrations of 3.49 and 3.94 μg/ml, respectively. These results illustrate potential applications of the described analytical procedure in areas such as the quality control of vegetable oils and other fat-rich products, the monitoring of the efficiency of antioxidants added to fat-rich foods and the optimization of food preparation processes.

The method was also employed in the quantitation of carbonyl compounds present in human urine (ten young healthy volunteers). A typical GC profile of the hexane extract from urine is shown in Fig. 2. The amounts of the various volatile carbonyl compounds found in the urine samples obtained before and after consumption of an alcoholic beverage (1.8 l of beer) are given in Table 4. We attributed the large standard deviations to metabolic differences within the group of volunteers. The relative standard deviation ($n=3$) for a single individual was below 3%. The total aldehyde content, quantified in urine 2, 4 and 6 h after the consumption of beer, decreased by roughly 40% in comparison with their concentration in blank samples obtained from fasted volunteers. In contrast with the other aldehydes found in urine, the concentration of ethanal increased markedly (1.18 μg/ml) 2 h after the ingestion of beer. Afterwards, the ethanal content decreased progressively from 0.65 (4 h) to 0.47 μg/ml (6 h), together with the other aldehydes present in urine. This example provides evidence that the analytical method developed here is selective, sensitive and rapid, and can be applied successfully to the quantitative analysis of metabolites in complex biological matrices. The PFPH derivatization is a one-step procedure that is more advantageous than closely related methods, such as those based on derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (two-step procedures), because it permits higher sensitivities in the determination of carbonyl compounds in urine [38].

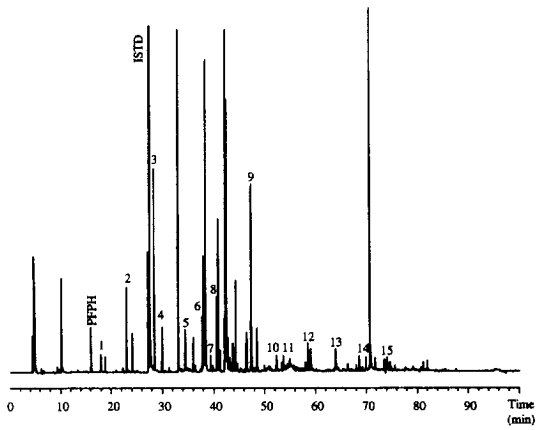

Fig. 2. GC profile obtained on the HP-5 (60 m×0.20 mm, 0.33 μm) column fitted with ECD, of the carbonyl compounds (as PFPH derivatives) present in a hexane extract of urine, obtained from a healthy young volunteer who ingested 1.8 l of beer 2 h before the urine specimen was collected. Internal standard, acetone-d_6–PFPH derivative. 1=methanal, 2=ethanal, 3=acetone, 4=propanal, 5=butanal, 6=isopentanal, 7=crotonaldehyde, 8=pentanal, 9=hexanal, 10=*trans*-2-hexenal, 11=heptanal, 12=octanal, 13=nonanal, 14=decanal and 15=undecanal.

4. Conclusions

We have compared the performance of two extraction methods and four different detection systems in the analysis of PFPH derivatives of carbonyl compounds using fused-silica narrow-bore column chromatography. Higher reproducibility and recoveries of volatile aldehydes from spiked aqueous solutions were obtained by SPE (80.9–98.3%) than

Table 4
Volatile carbonyl compounds found in human urine

PFPH derivative	Concentration (μg/ml)[a]			
	Blank	2 h[b]	4 h	6 h
Methanal	0.13±0.09	0.18±0.10	0.11±0.05	0.08±0.07
Ethanal	0.61±0.84	1.18±0.42	0.65±0.39	0.47±0.61
Acetone	2.21±1.99	2.02±1.25	1.44±0.82	1.27±0.92
Propanal	0.70±0.50	0.61±0.36	0.50±0.35	0.44±0.21
Butanal	0.37±0.26	0.47±0.27	0.02±0.02	0.02±0.02
Isopentanal	0.51±0.29	0.32±0.14	0.22±0.08	0.32±0.34
Crotonaldehyde	0.25±0.12	0.22±0.06	0.13±0.08	0.32±0.28
Pentanal	0.71±0.47	0.74±0.38	0.38±0.27	0.13±0.20
Hexanal	1.66±1.10	1.67±0.66	0.85±0.62	0.47±0.50
trans-2-Hexenal	0.07±0.06	0.02±0.03	0.01±0.03	0.86±0.99
Heptanal	0.07±0.05	0.05±0.04	0.07±0.10	0.10±0.29
Octanal	0.18±0.21	0.09±0.05	0.15±0.21	0.12±0.16
Nonanal	0.21±0.23	0.15±0.05	0.13±0.03	0.03±0.11
Decanal	0.16±0.23	0.11±0.22	0.04±0.02	0.03±0.04
Undecanal	0.33±0.16	0.20±0.08	0.13±0.06	0.16±0.14
Total	8.17±1.99	8.03±1.25	4.76±0.82	4.80±0.99

[a] Mean±S.D. ($n=10$).
[b] Time after consumption of 1.8 l of beer.

by LLE (51.4–78.9%). Detection limits as low as 10^{-14} and 10^{-12} mol/ml were reached with the ECD and MS–SIM detection systems, respectively, at a signal–noise ratio of five (split ratio 1:30, injection volume 1 μl). According to these results, this analytical method can be recommended for determining volatile carbonyl compounds. This method starts with the derivatization of carbonyl compounds to pentafluorophenylhydrazones, followed by SPE of these derivatives and GC analysis on narrow-bore capillary columns with either ECD (highest sensitivity) or MS–SIM (better selectivity) detection systems. This method constitutes a reliable and versatile technique, which 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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References

[1] R. Fussle, S. Bhakdi, A. Sziegoleit, J. Tranum-Jensen, T. Kranz, H.J. Wellensiek, J. Cell Biol. 91 (1981) 83.
[2] H. Esterbauer, H. Zollner, Free Radic. Biol. Med. 7 (1989) 197.
[3] B.N. Ames, M.K. Shigenaga, T.M. Hagen, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7915.
[4] B. Halliwell, Haemostasis 1 (1993) 118.
[5] R.J. Collier, Bacterial Rev. 39 (1975) 54.
[6] H.J. Helbock, P.A. Motchnik, B.N. Ames, Pediatrics 91 (1993) 83.
[7] S. Banni, M.G. Salgo, R.W. Evans, F.P. Corongill, B. Lombardi, Carcinogenesis 11 (1990) 2053.
[8] A.E. Holley, T.F. Slater, Free Radic. Res. Commun. 15 (1991) 51.
[9] A. Cailleux, P. Allain, Free Radic. Res. Commun. 18 (1993) 323.
[10] P.A. Sobotka, M.D. Brottman, Z. Weitz, A.J. Birnbaum, J.L. Skosey, E.J. Zarling, Free Radic. Biol. Med. 14 (1993) 643.
[11] H. Tamura, T. Shibamoto, Lipids 26 (1991) 170.
[12] S.E. Ebeler, S.H. Hinrichs, A.J. Clifford, T. Shibamoto, Anal. Biochem. 205 (1992) 183.

[13] C.D. Rosiers, M.J. Rivest, M.J. Boily, M. Jette, A. Carrobe-Cohen, A. Kumar, Anal. Biochem. 208 (1993) 161.
[14] L.J. Papa, L.P. Turner, J. Chromatogr. Sci. 10 (1972) 747.
[15] S.J. Selim, J. Chromatogr. 136 (1977) 271.
[16] K. Kuwata, M. Uebori, Y.J. Yamasaki, J. Chromatogr. Sci. 10 (1979) 264.
[17] K. Umano, T. Shibamoto, J. Agric. Food Chem. 35 (1987) 14.
[18] A. Yasuhara, T. Shibamoto, J. Assoc. Off. Anal. Chem. 72 (1989) 749.
[19] K. Umano, T. Shibamoto, J. Agric. Food Chem. 35 (1987) 909.
[20] A. Yasuhara, J. Dennis, T. Shibamoto, Anal. Chem. 72 (1989) 749.
[21] A. Yasua, T. Shibamoto, J. Chromatogr. 547 (1991) 291.
[22] E. Kennedy, R. Hill, Anal. Chem. 54 (1982) 1739.
[23] Y. Hoshika, Y. Takata, J. Chromatogr. 120 (1976) 379.
[24] Y. Hoshika, G. Muto, J. Chromatogr. 152 (1978) 224.
[25] M. Tomita, T. Okuyama, J. Chromatogr. 526 (1990) 174.
[26] H.C. Yeo, H.J. Helbock, D.W. Chyu, B.N. Ames, Anal. Biochem. 220 (1994) 391.
[27] H. Beljean-Leymarie, E. Bruna, Anal. Biochem. 173 (1988) 174.
[28] E.E. Stashenko, J.W. Wong, J.R. Martínez, A. Mateus, T. Shibamoto, J. Chromatogr. A 752 (1996) 209.
[29] K. Kobayashi, M. Tanaka, S. Kawai, J. Chromatogr. 187 (1980) 413.
[30] K. Kobayasi, E. Fukui, M. Tanaka, S. Kawai, J. Chromatogr. 202 (1980) 93.
[31] K. Kobayashi, M. Okada, Y. Yasuda, S. Kawai, Clin. Chim. Acta 133 (1983) 223.
[32] F.J.G.M. van Kuijk, D.W. Thomas, R.J. Stephens, E.A. Dratz, Biochem. Biophys. Res. Commun. 139 (1986) 144.
[33] G. Hoffmann, L. Sweetman, J. Chromatogr. 421 (1987) 336.
[34] G. Hoffmann, S. Aramaki, E. Blum-Hoffmann, W.L. Nyhan, L. Sweetman, Clin. Chem. 35 (1989) 587.
[35] A. Loidl-Stahlhofen, G. Spiteller, Biochim. Biophys. Acta 1211 (1994) 156.
[36] A. Mlakar, G. Spiteller, Biochim. Biophys. Acta 1214 (1994) 209–220.
[37] K.T. Koshy, D.G. Kaiser, A.L. Van Der Slik, J. Chromatogr. Sci. 13 (1975) 97.
[38] X.P. Luo, M. Yazdanpanah, N. Bhooi, D.C. Lehotay, Anal. Biochem. 228 (1995) 294.