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Journal of Chromatography A, 891 (2000) 267–273

JOURNAL OF
CHROMATOGRAPHY A

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Microwave-assisted derivatization of volatile carbonyl compounds with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine

Steffen Strassnig, Thomas Wenzl, Ernst P. Lankmayr*

Institute for Analytical Chemistry, Micro- and Radiochemistry, Technical University of Graz, Technikerstrasse 4, 8010 Graz, Austria

Received 10 April 2000; received in revised form 5 June 2000; accepted 6 June 2000

Abstract

A method for the determination of carbonyl compounds, either directly from gaseous phase or following a volatilization from liquid or solid samples after trapping on Tenax TA is presented. Following solvent desorption, the carbonyls are derivatized using *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine. The reaction is accomplished in a microwave oven using closed vessels to minimize reaction time compared to conventional methodology. The solvent for the chemical reaction was selected according to the requirements of microwave energy interaction and solubility. After gas chromatographic separation of the corresponding oximes, they are detected using electron impact mass spectrometry in single ion monitoring mode. Quantification is carried out using internal standardization with 3-fluorobenzaldehyde, resulting in limits of detection in the ppm range following the calibration graph method. The optimized conditions provide for good recoveries and fast reaction rates for the volatile carbonyls studied so far. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, GC; Microwave-assisted derivatization; Vegetable oils; Carbonyl compounds; Volatile organic compounds; Pentafluorobenzylhydroxylamine

1. Introduction

Quite a few fields of scientific and industrial applications demand a fast and accurate methodology for the determination of volatile carbonyl compounds. Depending on its composition, this class of components for example contributes to the characteristic smell of a broad range of foodstuff or consumer products [1,2]. Owing to their apparent formation in oxidation reactions, aldehydes, oxoacids and hydroxyaldehydes are often generated in food processing, or contribute important marker compounds of lipid peroxidation in analytical biochemis-

try and medicine [3–5]. Another source of carbonyl compounds is the ozonolysis of olefins and several photochemical reactions on hydrocarbons in the atmosphere. The reaction mechanisms involved as well as methods for the determination of carbonyl compounds have been investigated from the perspective of environmental and atmospheric chemistry [6,7].

Selective as well as sensitive determination of these substances in sample matrices of diverse complexity is therefore required in order to enhance both selectivity and sensitivity and to avoid losses due to volatility, chemical derivatization methods prior to chromatographic analyses have been found useful. In general, such procedures can also help to simplify the structural elucidation of compounds

*Corresponding author. Tel.: +43-316-873-8305; fax: +43-316-873-8304.

utilizing the fragmentation pattern for mass spectrometric detection.

For analysis by high-performance liquid chromatography, 2,4-dinitrophenylhydrazine (DNPH) with UV detection [8,9] or 4-(*N,N*-dimethylaminosulphonyl)-7-hydrazino-2,1,3-benzoxadiazole with fluorescence detection [10] are common as derivatizing agents.

For gas chromatographic analysis, the formation of thiazolidines with cysteamine hydrochloride as the derivatization reagent, preferably using nitrogen-phosphorous-selective detection, has been proposed, but a major limitation of this approach is insufficient reactivity with α,β -unsaturated aldehydes [11]. Therefore, pentafluorophenylhydrazine (PFBH) and *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBOA) are often preferred to form volatile derivatives with a wide variety of carbonyls. Several attempts have been made to develop methods for the determination of carbonylic compounds in ozonated water, beer or other alcoholic beverages applying pentafluorobenzoylation directly in the liquid matrix [12–14]. To increase the recovery of carbonyls and shorten analysis time PFBOA-coated solid-phase extraction (SPE) cartridges with simultaneous trapping and derivatization [15,16] or PFBOA-coated solid-phase microextraction (SPME) fibers [17] have also been proposed. Reaction products are *cis/trans*-isomers at the oxime-bond, which yield double peaks for asymmetric carbonyl compounds. In order to further decrease the limit of detection, the double bond can be selectively reduced with a pyridine/borane complex, generating *O*-pentafluorobenzylamines which produce a single peak, resulting in better selectivity and detectability [14]. A comparison of different methods of derivatization of carbonyl compounds from liquid matrices and several detection systems after gas chromatographic separation has been compiled by Stashenko et al. [18]. In a recent review, different derivatives are compared in order to demonstrate ways to gain maximum information on the structure of the original aldehydes, using mass spectrometric fragmentation patterns [19].

The objective of the present study was to minimize the analytical effort needed for a determination of aldehydes and ketones in either liquid or solid matrices. Carbonylic compounds are volatilized,

trapped on Tenax TA, followed by solvent desorption and microwave-assisted derivatization. The application of microwave technology is pervading inorganic and organic chemistry and has proved useful for enhancing chemical reactions, sample digestion or extraction due to its inherent ability to control energy input. An overview of the fundamentals of microwave heating mechanisms and their applications has been provided by Kingston and Haswell [20]. In the context of analysis, the recovery of PFB-oximes is determined by the completeness of the trapping step, the extraction efficiency from the sample phase as well as by the conversion percentage of the derivatization reaction itself. The derivatization efficiency mainly depends on reaction time and temperature as well as on the amount of PFBOA and the pH in the reaction medium.

The first step was to select an organic solvent for desorption of the trapped analytes from Tenax TA, with the aim of maximum recovery and suitability for the following microwave-accelerated reaction. The second part of method development was the optimization of the derivatization conditions. Thus, the parameters heating time and power, the reaction medium itself and the concentration of PFBOA were optimized to maximize the reaction efficiency. Due to potential differences in the reaction rates between the different carbonyl classes [21], several aldehydes as well as ketones were included in the optimization process. For the present studies heptanal, 2-heptanone, benzaldehyde and acetophenone were chosen as representative carbonyls.

The optimized method has been further validated applying the appropriate standard methodology [22,23] and was used for the determination of carbonyl compounds from artificially aged vegetable oils.

2. Experimental

2.1. Chemicals

O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA·HCl) was purchased from Sigma Aldrich (Vienna, Austria), methanol and acetic acid were obtained from Merck (Darmstadt, Germany), the quality was analytical-reagent grade.

All aldehydes and ketones were purchased from Merck, the quality was analytical-reagent grade. Purified air (quality 5.0) was used as oxidizing agent in the ageing process and as purge gas.

Chrompack Tenax TA (150–180 μm) was obtained from CP-Analytica (Vienna, Austria). Water was bidistilled in the laboratory prior to use.

2.2. Instrumentation and conditions of analysis

Volumes up to 100 μl were handled by means of calibrated capillaries, for larger volumes up to 1 ml Transferettes from Brandt (Wertheim/Main, Germany) were used. The microwave reactions were carried out in 11-ml screw vials with PTFE-lined screw caps (Pyrex, UK) in a CEM MDS-205 microwave oven (CEM, Matthews, NC, USA).

All GC analyses were performed using a Hewlett-Packard Model 6890 GC system equipped with a HP 7683 series injector and interfaced to a HP 5973 mass-selective detector using electron impact ionization in single ion monitoring mode. The column was a HP5-MS 30 $\text{m} \times 0.25$ mm, 0.25 μm fused-silica capillary (all from Hewlett-Packard, Waldbronn, Germany). Helium 5.0 was used as carrier gas at constant flow of 1.1 ml/min with a pressure pulse of 150 kPa for 0.5 min at injection. The gas chromatograph was programmed to keep an initial temperature of 80°C for 1 min followed by an heating rate of 8°C/min up to a temperature of 220°C and to further increase the temperature at a rate of 30°C/min to a final temperature of 300°C which was held for 2 min. Injection volume was 1 μl in splitless mode.

Data acquisition was carried out with a Hewlett-Packard Chemstation G1701BA. For further evaluation, validation and method comparison studies, Microsoft-Excel (V 7.0), the Excel-macro ValiData (V 1.04, Rohrer/Wegscheider ASA, Graz, Austria) and Statgraphics Plus 3.0 (Manugistics, Rockville, MD, USA) were used.

2.3. Standards and samples

For the optimization of the solvent desorption and microwave-accelerated derivatization reaction parameters, standard mixtures of the four carbonyls heptanal, 2-heptanone, benzaldehyde and acetophenone were applied. The final parameters were em-

ployed for the determination of aldehydes from butanal to decanal and benzaldehyde as well as 2-heptanone and acetophenone in standard mixtures from 1 to 80 μg absolute sample amount in the reaction vessels, and with 3-fluorobenzaldehyde as the internal standard.

Standard vapours of carbonyl compounds were generated by transferring defined aliquots of the standard solution into a glass tube which was connected to the carrier gas supply. The open end of the tube was coupled to a 10-ml glass cartridge filled with 0.5 g Tenax TA. For sample desorption, the glass tube was heated to 200°C and the volatilized carbonyls were transferred onto the sorbent bed employing an air flow of 50 ml/min for 30 min.

The optimized procedure was tested for the determination of volatile carbonylic decomposition products from vegetable oils, for example soybean and olive oil. A sample volume of 1 μl of the vegetable oil was heated up to 150°C for 10 min with an air flow of 50 ml/min established as oxidizing agent and carrier to transfer the volatiles onto the Tenax TA bed.

2.4. Solvent desorption from Tenax TA

Several aspects need to be considered for the selection of a suitable solvent for desorption purposes. The carbonyls should be entirely eluted with a minimum of solvent volume and the polarity of the solvent should provide for maximum reaction efficiency in a microwave field. The solvent should be miscible with water since an aqueous solution of PFBOA·HCl is used for derivatization and finally, the mixture of solvent and aqueous solution of PFBOA·HCl should be applicable for a liquid–liquid extraction of the oximes. Methanol proved to be best suited according to these objectives and was therefore used throughout the experiments. A volume of 4 ml of methanol was sufficient for the desorption from the previously described sorption tubes.

Next, the trapping efficiency and recovery of the solvent desorption step were therefore investigated. Defined amounts of a standard mixture were evaporated, trapped on Tenax TA, eluted and derivatized following the optimized procedure. The resulting signals were compared with those obtained from

directly derivatized standard mixtures of the same concentration.

2.5. Derivatization procedure

As outlined in Section 3, a central composite design was used for the optimization of the microwave-assisted derivatization reaction. These investigations led to the following experimental conditions being selected for all further experiments: 1 ml of a 5 mg/ml solution of PFBOA·HCl in bidistilled water is added to a solution of the standard components and the internal standard contained in 5 ml of methanol. The glass vials are tightly closed with PTFE-lined screw caps and are inserted in a protective covering. The vessels are placed in the microwave oven where the reaction occurs at 900 W for 20 s. After cooling of the vials to ambient, the oximes are extracted with 1 ml of toluene. Further 4 ml of water are added to increase the efficiency of the liquid–liquid extraction step. After centrifugation at 2800 g for 5 min the toluene layer is withdrawn, dried over anhydrous Na_2SO_4 and is then ready for chromatographic analysis.

3. Results

For the optimization of the microwave-assisted derivatization procedure an experimental setup based on a central composite design was applied. The effects of variation of solvent volume, reaction time at constant microwave energy and concentration of the reagent PFBOA·HCl within physically sound limits were studied. The lower boundary value for the solvent volume was chosen according to the minimum requirements for an efficient sample desorption, the upper boundary value was set according to the pressure stability limits of the reaction vessels. Subsequently the volume in the reaction vessels was varied between 4 and 6 ml. The range for reaction time was selected to be from 10 to 30 s, respectively, while the concentration of PFBOA·HCl was varied from 1 to 5 mg absolute amount in the reaction solution.

Longer reaction time and higher concentrations of PFBOA·HCl resulted in better recoveries of the derivatives. Derivative formation is more effectively

enhanced by an increase of reagent concentration than by prolonged reaction time. This effect is more pronounced for ketones than for aldehydes. The variation of the reaction volume did not significantly influence the formation of derivatives. Fig. 1 shows an estimated response surface for heptanone as a typical example.

The efficiency of the optimized method was directly compared with derivatization by means of conventional heating. The standard mixture was derivatized by both methods at three concentration levels of 70 ng, and 1.3 and 30 μg . After gas chromatographic analysis the resulting peak areas were compared using a robust linear regression method [24,25]. In contrast to conventional linear least-squares regression, which requires per definition the numerical values of one variable being free of error, such a type of regression is preferable when the values of the abscissa as well as the values of the y-axis are prone to error. Robust linear regression is useful for revealing potential differences between two series of measurements by the slope of the regression graph. A slope $\neq 1$ is indicative for a difference between the compared methods.

In Table 1 the slopes resulting from the robust regression plots are listed, indicating differences in the reaction efficiency between the two methods. Apparently, the recovery of aldehydes as well as ketones is equal or better from the optimized microwave procedure than from the conventional hotplate heating method. As the data from the conventional reference method are drawn on the abscissa, slopes >1 are indicative of better derivatization efficiency obtained by the compared method. The correlation

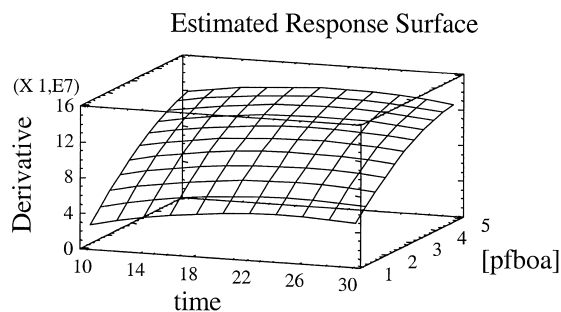


Fig. 1. Estimated response surface for 2-heptanone for the variables reaction time and reagent concentration.

Table 1
Slopes and correlation coefficients from orthogonal regression graphs for method comparison purposes^a

Compound	Slope	R^2
Butanal	1.83	0.992
Pentanal	1.90	0.995
Hexanal	1.90	0.995
<i>trans</i> -2-Hexenal	1.82	0.990
Heptanal	1.86	0.991
Octanal	1.79	0.991
Nonanal	1.80	0.991
Decanal	1.82	0.990
Benzaldehyde	1.82	0.990
2-Heptanone	1.68	0.997
Acetophenone	0.91	0.995

^a For two comparable methods, the resulting slopes from calibration experiments should be 1 per definition. Slopes exceeding 1, as for most analytes in this table, are indicative for a better recovery (as signal from the analytical instrument) of the compared procedure as in this case the optimized microwave derivatization method.

coefficients in Table 1 were computed by orthogonal linear least-squares regression, applying the ValiData 1.04 software. Linearity proved to be adequate for a valid method comparison.

Both, methanol and water are capable of absorbing microwave energy and can be potentially used as sole reaction media, depending on the analytical requirements of the composition of the sample matrix. In order to prove the applicability of the microwave-enhanced reaction for the derivatization of the carbonyls directly in water or alternatively in the organic solvent methanol, the recoveries and limits of detection obtained from aqueous and methanolic reaction media were additionally compared. The results are displayed in Fig. 2. With aldehydes from pentanal to nonanal, rather marginal differences between aqueous and methanolic medium were observed, while butanal and decanal show somewhat higher recovery (below 10%) rates using a methanolic reaction medium. However, reaction efficiency for the formation of ketone-oximes was significantly better in aqueous solution. The percentage difference represents the deviation of the recovery in methanolic reaction medium relative to aqueous medium which is normalized in the graph. The results of these series of experiments clearly demonstrate the suitability of both solvents as reaction media. In analytical practice, potential differences in relative

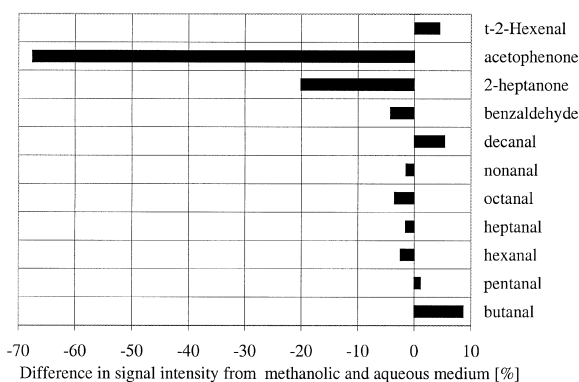


Fig. 2. Comparison of reaction efficiency for the derivatization of carbonyl compounds using PFBOA in aqueous and methanolic reaction medium. The signals obtained in aqueous medium are normalized in the diagram, while the percentual deviation of the signal intensity obtained in methanolic medium is indicated by the bars. Acetophenone and 2-heptanone exhibit reduced reaction efficiency in methanolic reaction medium.

sensitivity are bypassed by the use of the same derivatization procedure for the preparation of the calibration standards.

3.1. Trapping and solvent desorption efficiency

The recovery of carbonyl compounds from the trapping and solvent desorption step was explored by a comparison of the data obtained with a directly derivatized standard mixture with those from the signals of the PFB-oximes after volatilization, trapping on Tenax TA, solvent desorption with methanol and derivatization in the microwave oven. Standard mixtures were prepared containing 5 μg of each carbonyl compound in a volume of 4 ml methanol. Four replicates were made for both modes of sample preparation, the direct derivatization and the combination of volatilization for 30 min at 200°C in an air flow of 50 ml/min, trapping on Tenax TA followed by elution and derivatization in methanol.

The peak areas from the gas chromatographic analysis were compared by means of the analysis of variance. Significant differences of the recoveries were not observed for any of the reference components at the 95% confidence level, even without normalization of the peak areas to the internal standard.

3.2. Performance characteristics of the optimized microwave-derivatization method

An evaluation of linearity of the calibration graphs, and associated limits of detection (LODs), was performed in a range from about 1 to 60 μg absolute amount of the individual sample compounds. The data were obtained from four replicate analysis at four concentration levels. The computed regression coefficients and limits of detection are presented in Table 2. Within-day repeatability of the method was calculated from six replicate measurements at a concentration of 11 μg and is 1.6%, while the between-day repeatability (three consecutive days) equals 1%.

3.3. Samples

An illustrative demonstration of the suitability of the overall procedure is given with the application of the analysis of volatile carbonylic degradation products from the heating of a vegetable oil. The conditions of analysis were set as described in Section 2. Fig. 3 shows a representative GC–MS chromatogram of soybean-oil recorded in scan-mode and reconstructed from the four ion traces mentioned below. With all compounds used in this study, the most abundant fragment is m/z 181, representing the pentafluorobenzyl-ion. Further characteristic ions are 239 (saturated aldehydes), 250 (2-unsaturated aldehydes) and 253 (saturated ketones). Similar chro-

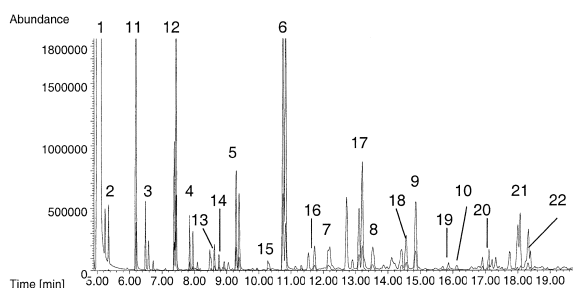


Fig. 3. Reconstructed chromatogram (ion traces m/z 181, 239, 250, 253) of volatiles from heated soybean-oil after pentafluorobenzylation using the optimized microwave heating method. The annotation of peak identity corresponds to the PFB derivatives of: (1) PFB-*O*-reagent peak, (2) acetaldehyde, (3) propanal, (4) butanal, (5) pentanal, (6) hexanal, (7) heptanal, (8) octanal, (9) nonanal, (10) decanal, (11) acetone, (12) 2-propenal, (13) 2-butenal, (14) 2-pentanone, (15) 2-pentenal, (16) 2-hexenal, (17) 2-heptenal, (18) 2-octenal, (19) 2-nonenal, (20) 2-decenal, (21) 2,4-decadienal, (22) 2-undecenal.

matograms could be obtained from the oxidative degradation of olive oil and other vegetable oils.

4. Conclusion

A universally applicable method for the determination of volatile carbonyl compounds in either gaseous, liquid or solid sample matrices has been developed. Carbonyl compounds are volatilized, trapped on Tenax TA, desorbed with methanol and derivatized by means of a microwave-accelerated derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine. Compared with the classical derivatization methodology, reaction time is substantially reduced by a factor 50–100, which is an argument in favour of the application of a microwave field for a fast and efficient energy transfer. This is supported by the selection of an appropriate dipolar solvent as reaction medium as well as by the dipolar character of reagent and sample molecules. Robust regression techniques are applied to provide a means of comparison of classical methodology with the microwave-accelerated method.

Additional emphasis has been given to an investigation of trapping and elution efficiency which could potentially affect the overall performance of the procedure. It could be demonstrated that the

Table 2
Performance characteristics of the optimized microwave method^a

	R^2	LOD (μg)
Butanal	0.994	1.21
Pentanal	0.997	1.12
Hexanal	0.999	1.36
Heptanal	0.998	1.29
Octanal	1.000	1.12
Nonanal	0.999	1.37
Decanal	0.998	1.52
Benzaldehyde	1.000	1.59
2-Heptanone	0.999	1.29
Acetophenone	0.995	3.75
<i>trans</i> -2-Hexenal	0.998	1.30

^a Coefficients of correlation and limits of detection are determined from a calibration from 1 to 60 ng absolute amount per carbonyl compound in the reaction vessel.

recoveries show no significant difference from 100%.

Since most literature published so far on derivatization of carbonyl compounds with PFBOA-HCl deals with the use of aqueous sample matrices, reaction efficiencies in water and methanolic medium were compared. The recovery of PFB-oximes originating from aldehydes was not altered by solvent composition, while reaction efficiency for ketones is higher in the aqueous reaction medium. The different magnitude of absorptivity for microwave irradiation of the two solvents, caused by their dielectric properties, may explain these results.

The performance of the optimized microwave accelerated derivatization technique with respect to limits of detection is comparable for all analytes included in the test mixture except for acetophenone. Linearity of calibration graphs as well as reproducibility and repeatability are within acceptable limits.

Finally, this procedure has been used to demonstrate its suitability on the example of the analysis of volatile carbonylic compounds originating from complex sample matrices such as oxidatively degraded vegetable oils.

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