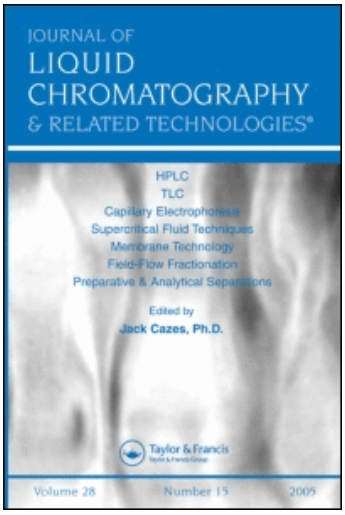

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Estimation of Uncertainty in the Analysis of Carbonyl Compounds by HPLC-UV Using DNPH Derivatization

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Abstract: This work describes the estimation of uncertainty following the "bottom-up" approach for the quantification process of thirteen carbonyl compounds, as 2,4-dinitrophenylhydrazone derivatives, by high performance liquid chromatography with ultraviolet detection (HPLC-UV). These results are compared with the ones obtained using gas chromatography-mass spectrometry (GC-MS). A study of the linear range was established and validation was performed for both methods using statistical analysis of several indicative parameters. In terms of validation data, precision (RSD $< 7.8\%$ for HPLC-UV and $<20\%$ for GC-MS), and accuracy (relative error $<8.3\%$ for HPLC-UV and $<7.3\%$ for GC-MS) were obtained under day to day conditions. The results of the estimation of uncertainty for both methods demonstrated that the contributions due to the preparation of the standard solutions are not significant. The uncertainty associated with the estimation of the compound concentrations from the calibration curves are similar for both methodology, or slightly lower when HPLC-UV is used. The cause of higher uncertainty and, especially, when GC-MS is used, is the repeatability of the measurements.

Keywords: Uncertainty, Carbonyl compounds, High performance liquid chromatography, Gas chromatography

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INTRODUCTION

Carbonyl compounds are known to have toxic and carcinogenic properties and, therefore, their presence in the environment is of great concern with regard to their adverse effects to public health and vegetation.[1] These compounds are emitted into the atmosphere by both anthropogenic sources as the combustion of organic matter,[2,3] and natural sources as the emission of plants.[4]

Compounds like ketones, aldehydes, acids, and esters have been determined in the smoke produced in the pyrolisis process of some woods.[5] Nevertheless, aldehydes and ketones have been considered of great influence in the development of texture, colour, and aroma in smoked foods. Several methods have been developed for the determination of aldehydes and ketones in the gas phase,[6,7] liquid phase[8] of the atmosphere, atmospheric aerosol,[9] and cigarette smoke.[10] However, few data can be found in the literature on the determination of carbonyl compounds in smoke from incomplete biomass combustion.[11]

In recent years, chromatographic methods including gas chromatography[12,13] and liquid chromatography[14–18] have been the most frequently reported for the determination of formaldehyde based on chemical derivatization. The most common analytical procedure used for speciation and quantification of carbonyl compounds involves reaction with an acidic solution of 2,4-dinitrophenylhydrazine (DNPH) to form the corresponding hydrazone.

The HPLC procedure has been typically preferred to the GC-MS due to its robustness and good repeatability. However, in very complex mixtures, application of the HPLC separation has potential interferences, and the determination of certain carbonyl compounds present at trace levels is difficult. For this reason, a GC-MS procedure was needed to separate a number of these carbonyl compounds.[19,20] The GC coupled with MS detection, compared to HPLC, has the advantages that positive identification of each DNPH-carbonyl compound can be achieved, as well as potentially better separation.

The present study optimises and compares the HPLC-UV and the GC-MS habitual procedures used for the determination of thirteen carbonyl compounds after 2,4-dinitrophenylhydrazine derivatization.

In chemical analysis, the first and most essential link in the traceability chain is the calibration of the measurement system with known calibrants. The uncertainty from this calibration is, therefore, one of the most important component, which is sometimes the highest contribution to the combined uncertainty of the analytical result.

In this paper, the uncertainty associated with the measurement of the sample and the calibration process by HPLC-UV, using the bottom-up approach in conjunction with in-house validation data was evaluated for the quantification process of thirteen carbonyl compounds, as 2,4-dinitrophenylhydrazone derivatives. These results are compared with the ones obtained using gas chromatography mass spectrometry (GC-MS).

EXPERIMENTAL

Reagents

The standard mixture solution of 13 carbonyl-DNPH compounds (carbonyl-DNPH mix 1) was supplied by Supelco (Bellefonte, PA, USA) and contains 20 μg/mL of each carbonyl compound in acetonitrile: acetaldehyde, acetone, acrolein, benzaldehyde, butyraldehyde, crotonaldehyde, hexaldehyde, methacrolein, *p*-tolualdehyde, propionaldehyde, valeraldehyde, and 2-butanone, except formaldehyde with 40 μg/mL. This standard was stored at 4°C and used for the preparation of working standard solutions for the HPLC and GC analysis.

Acetonitrile of HPLC grade (Merck) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) were used throughout the liquid chromatography analysis.

Instrumentation

The HPLC equipment used was a liquid chromatograph consisting of a delivery solvent L-2130 Pump supplied by Hitachi (Japan) equipped with an Autosampler ProStar 410 (Varian, USA) and a Waters Lambda-Max 481 LC variable wavelength spectrophotometric detector. Autoanalysis 2.4 (Sciware, vcerda@p01.uib.es) software was used for data acquisition. The analytical system used two columns in series, Supelcosil™ LC-18, 5 μm, 100 Å (25 cm × 4.6 mm ID) supplied by Supelco with a μBondapak C_{18} guard column, 10 μm, 125 Å (20 mm × 3.9 mm ID) supplied by Waters.

A gas chromatograph-mass spectrometer (Varian 3800-Varian Saturn 2000) with a 30 m × 0.25 mm ID WCOT CP-SIL-8 CB column supplied by Chrompack (The Netherlands) and equipped with an Autosampler model 8200 CX was used. Saturn GC-MS Workstation 5.3 software was used for data acquisition.

Analytical Procedures

Standard Solutions Preparation

The first standard solution of thirteen carbonyl-DNPH compounds was prepared diluting two carbonyl-DNPH mixture of 1 mL containing 13 hydrazone derivatives with acetonitrile to 5 mL in a volumetric flask. The second standard solution was prepared diluting an aliquot of 640 μL of the first standard solution to 5 mL in a volumetric flask. Finally, calibration curves were prepared pipetting the appropriate volumes (Table 5) of the

second standard solution and diluting to 1 mL with an appropriate volume of acetonitrile.

HPLC and GC Procedures

The working standard solutions were injected into the chromatographic system. The HPLC method used for the separation and determination of carbonyl-DNPH compounds consisted of a gradient elution procedure with spectrophotometric detector operating at 360 nm.

As mobile phase a mixture of acetonitrile and water with a linear gradient from 55 to 66% of acetonitrile over 21.7 min, 66% of acetonitrile for 6.6 min, a linear gradient from 66 to 75% of acetonitrile over 5 min, and 75% of acetonitrile for 8.7 min was used. The flow rate was 1 mL/min and the injection volume was 20 μL.

The GC-MS was used under the following conditions: ion source, electron impact (70 eV); transfer line temperature, 315°C; ion trap manifold temperature, 72°C; ion trap temperature, 200°C; carrier gas, He, with a flow rate of 2 mL/min; injection in splitless mode; injection volume, 2 μL. The oven temperature was programmed from 100°C, 10°C/min, until 315°C, and then held for 20.8 min. The injector temperature was 200°C. The MS analysis was carried out in the scan mode with a range of mass between 50 and 300 amu. The quantitative determination was carried out using the mass values corresponding to the molecular ions of the different carbonyl- DNPH (SIM mode).

RESULTS AND DISCUSSION

Chromatographic Separations

Chromatographic conditions were optimised to achieve a good resolution and quantification of the thirteen hidrazone derivatives by means of HPLC-UV and GC-MS. The representative chromatograms of standards are shown in Figure 1, using the final selected chromatographic conditions described in section HPLC and GC procedures.

By the HPLC-UV procedure, it is possible to achieve a satisfactory chromatographic resolution for nine of thirteen DNPH-carbonyl compounds. However, the chromatograms show two couples of unresolved peaks, acrolein + acetone and 2-butanone + butyraldehyde derivatives. The total running time was 39 min and the peaks identifications are given in Table 1.

By the GC-MS procedure, *p*-Tolualdehyde derivative was not detected due to their low sensibility, and the chromatograms show two couples of unresolved peaks, propionaldehyde + acrolein and methacrolein + butyraldehyde derivatives. However, the compounds have different ions and, therefore, the individual identification is possible. The total running time was 20 min.

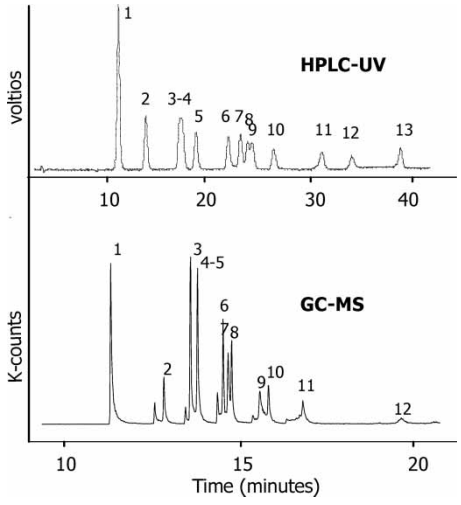

Figure 1. Chromatograms of a standard solution of carbonyl compounds derivatives by HPLC-UV and GC-MS analysis. The assignment of peaks numbers as in Table 1.

Table 1 summarizes the retention time window (RTW) and their standard deviation determined for all the compounds and the ions used for their quantification. The RTW is defined as the average of retention times, obtained from 6 replicates in the HPLC-UV method and 5 replicates in the GC-MS method.

Validation

The validation parameters studied were: response linearity, precision, and accuracy. Using an external calibration procedure, the calibration curves were carried out daily during six days for their analysis by HPLC-UV and five days by GC-MS. Besides, with the aim of studying the accuracy and precision of both methods, three different concentration levels included in the linearity range, except for benzaldehyde and hexaldehyde derivates with two different concentration levels in the GC-MS method, were repeatedly analysed each day.

Linearity

Linearity for both methods was evaluated by the calculation of a seven-point linear plot (except for the formaldehyde compound in the HPLC-UV method, with $n = 5$), based on residual standard deviation and correlation coefficient, R^2, using peak area as analytical response.

Table 1. RTW (retention time window) for HPLC-UV and RTW and MS data for GC-MS

HPLC-UV		GC-MS		
Compound	RTW (S.D.) (min)	Compound	Quantification (m/z)	RTW (S.D.) (min)
1. Formaldehyde-2,4-DNPH	11.41 (0.21)	1. Formaldehyde-2,4-DNPH	79	12.22 (0.04)
2. Acetaldehyde-2,4-DNPH	14.20 (0.31)	2. Acetaldehyde-2,4-DNPH	79	13.54 (0.03)
3–4. Acrolein-2,4-DNPH + acetone-2,4-DNPH	17.71 (0.42)	3. Acetone-2,4-DNPH	238	14.17 (0.01)
		4–5. Propionaldehyde-2,4-DNPH + acrolein-2,4-DNPH	238 236	14.37 (0.02)
5. Propionaldehyde-2,4-DNPH	19.30 (0.45)			
6. Crotonaldehyde-2,4-DNPH	22.55 (0.54)	6. 2-Butanone-2,4-DNPH	252	14.99 (0.01)
7. Methacrolein-2,4-DNPH	23.78 (0.58)	7. Methacrolein-2,4-DNPH	173	15.17 (0.01)
8–9. 2-Butanone-2,4-DNPH + butyraldehyde-2,4-DNPH	24.73 (0.52)	8. Butyraldehyde-2,4-DNPH	252	15.23 (0.01)
		9. Crotonaldehyde-2,4-DNPH	202	16.08 (0.02)
10. Benzaldehyde-2,4-DNPH	26.78 (0.76)	10. Valeraldehyde-2,4-DNPH	206	16.20 (0.01)
11. Valeraldehyde-2,4-DNPH	31.32 (0.98)	11. Hexaldehyde-2,4-DNPH	83	16.97 (0.01)
12. *p*-Tolualdehyde-2,4-DNPH	34.62 (0.85)	12. Benzaldehyde-2,4-DNPH	286	19.50 (0.02)
13. Hexaldehyde-2,4-DNPH	38.83 (0.79)			

Linearity of the HPLC-UV method was examined over a range of 0.031–0.729 mg/L for all the compounds, except formaldehyde, which shows a linear range of 0.061–0.500 mg/L. In the GC-MS method, the formaldehyde has a linear range from 0.500 to 1.941 mg/L, while for the rest of the compounds in this study was from 0.250 to 0.971 mg/L.

The similarity of slopes and intercepts of the standard curves obtained day to day were checked by means of a Student t-test. The application of this test was significant at the $\alpha = 0.05$ significance level and a pooled slope and a pooled intercept have not been able to be calculated. From the obtained results, the uncertainty for each one of the standard curves obtained day to day was estimated. Tables 2 and 3, show the linear regression parameters for each one of the compounds and for those calibration curves that led to a higher uncertainty.

Limits of Detection and Quantification

Limits of detection (LODs) and limits of quantification (LOQs) were calculated as 3- and 10-fold the residual standard deviation divided by the slope for the calibration curves tested under day to day conditions.[21] Tables 2 and 3 summarize the LODs and LOQs obtained using those calibration curves that led to higher uncertainty. For the HPLC-UV method, the values of limits of detection obtained oscillate between 0.023 and 0.126 mg/L. For GC-MS method, with the exception of the formaldehyde with a LOD of 0.145 mg/L, the rest of the aldehydes and ketones present very close detection limits, oscillating between 0.057 and 0.085 mg/L.

Precision and Accuracy

Precision expressed as relative standard deviation, RSD (%), and accuracy estimated as relative error, Er (%), for the three concentration levels studied inside the range of linearity of the curves for each one of the compounds are shown in Tables 2 and 3.

As might be expected, the highest RSD values were obtained at the lowest concentration levels, close to the LOQ, for both methods. The values found were lower for the compounds analysed by HPLC-UV (2.4–7.8%) than by GC-MS, which showed RSD ranging between 4.9 and 11% (except crotonaldehyde with a 20% RSD).

As in the above case, the relative errors were lower for HPLC-UV, ranging between 0.01 and 4.4% except for 2-butanone + butyraldehyde with 8.3%. The compounds determined by GC-MS present values lower than 5.5% except for crotonaldehyde (7.3%).

Table 2. Validation data for HPLC-UV method for those calibration curves that led to a higher uncertainty

Compound	Intercept	S_a	Slope	S_b	$S_{y/x}$	R^2	Concentration levels (mg/L)[a]	Mean (mg/L)	RSD (%)	Relative error (%)	LOD (mg/L)	LOQ (mg/L)
Formaldehyde*	−0.049	0.666	53.3	2.08	0.720	0.995	0.061	0.061	4.8	0.01	0.040	0.135
							0.279	0.272	1.3	2.4		
							0.500	0.492	1.6	1.6		
Acetaldehyde	−0.593	0.315	41.2	0.714	0.442	0.998	0.139	0.137	2.4	1.9	0.032	0.107
							0.369	0.367	1.8	0.5		
							0.610	0.608	1.1	0.4		
Acroleine + acetone	−0.583	0.349	33.8	0.395	0.489	0.999	0.279	0.273	2.4	2.0	0.043	0.145
							0.737	0.733	1.8	0.6		
							1.221	1.214	1.1	0.6		
Propionaldehyde	−0.473	0.173	31.4	0.392	0.243	0.999	0.139	0.136	3.2	2.5	0.023	0.077
							0.369	0.370	1.4	0.3		
							0.610	0.605	1.5	0.8		
Crotonaldehyde	0.183	0.289	24.0	0.655	0.406	0.996	0.139	0.138	3.4	0.8	0.051	0.169
							0.369	0.366	2.9	0.8		
							0.610	0.615	1.4	0.8		
Methacrolein	0.112	0.207	16.1	0.470	0.291	0.996	0.139	0.135	4.3	3.1	0.054	0.180
							0.369	0.370	3.0	0.4		
							0.610	0.608	3.1	0.3		

2-Butanone + butyraldehyde	0.322	0.409	13.7	0.464	0.574	0.994	0.279	0.256	6.5	8.2	0.126	0.420
							0.737	0.733	4.9	0.6		
							1.221	1.190	3.3	2.5		
Benzaldehyde	−0.380	0.209	18.6	0.474	0.294	0.997	0.139	0.136	4.6	2.6	0.047	0.0158
	−0.292	0.210	18.6	0.476	0.295	0.997	0.369	0.362	3.3	1.8	0.048	0.0159
							0.610	0.606	1.9	0.7		
Valeraldehyde	−0.685	0.302	21.6	0.684	0.424	0.995	0.139	0.133	6.1	4.4	0.059	0.196
							0.369	0.360	2.3	2.4		
							0.610	0.596	4.5	2.3		
p-Tolualdehyde	−0.358	0.203	14.9	0.459	0.284	0.995	0.139	0.135	7.8	2.9	0.057	0.191
							0.369	0.367	3.7	0.5		
							0.610	0.602	2.5	1.3		
Hexaldehyde	−0.634	0.311	19.0	0.652	0.324	0.995	0.139	0.138	6.6	1.1	0.051	0.170
	−0.682	0.288	19.0	0.652	0.404	0.994	0.369	0.364	5.3	1.2	0.064	0.213
							0.610	0.604	3.4	1.1		

Linear regression evaluated with n = 7 concentration values, except * with n = 5.

[a]Run-to-run (n = 2) on day-to-day (n = 6) samples analysed.

S_a: Standard deviation of the intercept; S_b: Standard deviation of the slope; $S_{y/x}$: The residual standard deviation.

Table 3. Validation data for GC-MS method for those calibration curves that led to a higher uncertainty

Compound	Intercept · 10^{-5}	$S_a \cdot 10^{-4}$	Slope · 10^{-5}	$S_b \cdot 10^{-4}$	$S_{y/x} \cdot 10^{-4}$	R^2	Concentration levels (mg/L)[a]	Mean (mg/L)	RSD (%)	Relative error (%)	LOD (mg/L)	LOQ (mg/L)
Formaldehyde	−5.84	7.63	15.3	5.82	7.41	0.993	0.737	0.758	8.9	2.8	0.145	0.483
							1.221	1.189	3.8	2.6		
							1.700	1.693	1.5	0.4		
Acetaldehyde	− 0.92	1.32	6.15	2.01	1.28	0.995	0.369	0.369	6.1	0.2	0.062	0.208
	−1.60	1.66	7.63	2.53	1.61	0.994	0.610	0.616	2.5	0.9	0.063	0.211
							0.850	0.849	2.4	0.2		
Acroleine	− 1.80	1.81	5.75	2.58	1.30	0.992	0.369	0.381	5.2	3.4	0.068	0.226
	−0.12	1.60	5.71	2.44	1.55	0.991	0.610	0.607	5.5	0.6	0.081	0.271
							0.850	0.848	3.8	0.2		
Acetone	−1.73	2.67	12.3	4.08	2.59	0.994	0.369	0.376	4.9	2.1	0.063	0.210
	−3.31	3.28	13.6	5.01	3.19	0.993	0.610	0.593	3.4	2.9	0.070	0.234
							0.850	0.859	2.8	1.0		
Propionaldehyde	−1.16	1.68	6.64	2.56	1.63	0.993	0.369	0.389	7.7	5.5	0.074	0.245
							0.610	0.617	3.6	1.1		
							0.850	0.850	3.0	0.05		
Crotonaldehyde	−0.78	1.54	5.90	2.35	1.49	0.992	0.369	0.342	20	7.3	0.076	0.253
							0.610	0.599	11	1.9		
	−1.33	1.64	7.08	2.51	1.59	0.994	0.850	0.821	7.4	3.4	0.068	0.225

Methacrolein	−0.86	1.15	5.87	1.76	1.12	0.995	0.369	0.380	11	3.2	0.057	0.190
	−1.28	1.37	5.72	2.09	1.33	0.993	0.610	0.627	5.0	2.7	0.070	0.232
							0.850	0.862	4.4	1.4		
Butyraldehyde	−0.92	1.16	4.88	1.77	1.13	0.993	0.369	0.375	5.8	1.8	0.069	0.231
							0.610	0.618	7.1	1.2		
	−1.20	1.10	4.88	1.69	1.07	0.994	0.850	0.861	3.7	1.3	0.066	0.220
2-Butanone	−0.82	1.81	6.83	2.76	1.76	0.992	0.369	0.377	6.5	2.4	0.077	0.257
							0.610	0.608	3.4	0.4		
							0.850	0.851	2.3	0.2		
Benzaldehyde	− 1.03	1.43	2.21	1.78	4.79	0.987	0.610	0.632	7.2	3.5	0.065	0.217
							0.850	0.855	4.6	0.6		
Valeraldehyde	−1.05	1.41	4.85	2.16	1.37	0.990	0.369	0.375	6.9	1.8	0.085	0.283
							0.610	0.608	3.5	0.4		
	−0.30	0.83	3.16	1.27	0.81	0.992	0.850	0.857	2.6	0.8	0.077	0.256
Hexaldehyde	−1.39	1.79	2.69	2.24	0.60	0.986	0.610	0.612	4.4	0.3	0.067	0.223
	−0.60	0.57	1.81	0.81	0.41	0.992	0.850	0.830	5.6	2.4	0.068	0.225

Linear regression evaluated with n = 7 concentration values.
[a]Run-to-run (n = 2) on day-to-day (n = 5) samples analysed.

Estimation of Measurement Uncertainty

The main approaches to calculate uncertainty, which have been proposed up to date, are the "bottom-up" and "top-down" methods. The first approach considers the division of the analytical method into its steps and the identification, quantification, and combination of all uncertainty sources. The "bottom-up" method was proposed by ISO in order to quantify uncertainty in physical measurements and was subsequently adapted by Eurachem.[22,23] On the other hand, the "top-down" approach uses validation data and data from proficiency testing schemes to estimate the uncertainty of the method.[24] A disadvantage of the latter method compared with the "bottom-up" method is that no information about the variation of uncertainty is available and no corrective actions can be performed on critical steps of analysis.

Uncertainty can be expressed in two different forms, the so called standard and expanded uncertainties. The uncertainty evaluation for the results of a measurement leads to the standard uncertainty, u(x), which is expressed as a standard deviation. If the standard uncertainty is derived from different sources of uncertainty, it is referred to as combined standard uncertainty, the individual components are expressed as standard uncertainties $u(y_i)$. The expanded uncertainty U(x) provides an interval within which the value of the measurand is believed to lie with a higher level of confidence: $X = x \pm U(x)$. U(x) is obtained by multiplying u(x), the combined standard uncertainty, by a coverage factor k, $U(x) = ku(x)$.[23] The choice of the factor k is based on the level of confidence desired. For an approximate level of confidence of 95%, k is 2.

We consider here uncertainty estimations using information from in-house validation results of the analytical HPLC-UV and GC-MS processes of thirteen carbonyl compounds after 2,4-dinitrophenylhydrazine derivatization. It implies that no real estimate of complete reproducibility is known, but that only an estimate of the intermediate between-day precision is available. The estimated bias is an overall bias, which is a combination of the laboratory bias and the method bias. To separate the method bias from the laboratory bias inter-laboratory is required.

The evaluation of the total uncertainty of an analytical result should comprise all the sources of uncertainty that contribute to the analytical result. If we only consider the uncertainty of the quantification process, the sources of uncertainty can be limited to the measurement of the signal from the sample and the calibration of the instrument. This uncertainty u(CC), is a combination of the uncertainties associated to the preparation of the standard solutions used to plot the calibration curve u(std), the transformation of the chromatographic signals in concentrations by interpolation in a calibration curve u(cal), and the reproducibility of the measurements, u(rep). The combined uncertainty in terms of relative uncertainty can be calculated with the expression:

$$u_{rel}(CC) = \sqrt{u_{rel}^2(std) + u_{rel}^2(cal) + u_{rel}^2(rep)} \quad (1)$$

where each term of the sum is the relative standard uncertainty associated to each source identified above.

Uncertainty Associated with the Preparation of the Calibration Standard Solutions u(std)

$$u_{rel}(std) = \sqrt{u_{rel}^2(fs) + u_{rel}^2(ss) + u_{rel}^2(ccs)} \quad (1.1)$$

It is estimated for each compound, being a combination of the uncertainty derived from the preparation of the first, $u_{rel}(fs)$, and second standard solutions, $u_{rel}(ss)$, and from the preparation of the calibration curve, $u_{rel}(ccs)$, (seven concentration levels for HPLC-UV and GC-MS methods, except the formaldehyde with 5 levels in HPLC-UV) by diluting the second standard solution.

The concentration of the first standard solution is given by dilution up 5 mL (V_{f1}) of the standard mixture solution of 13-carbonyl-DNPH and the second standard solution by the volume (V_{p1}) taken with a pipette from the first standard solution and the volume (V_{f2}) filled up in the second dilution.

The standard uncertainty associated to these steps can be obtained as:

$$u_{rel}(fs) = \sqrt{u_{rel}^2(sm) + u_{rel}^2(V_{f1})} \quad (1.1.1)$$

$$u_{rel}(ss) = \sqrt{u_{rel}^2(V_{p1}) + u_{rel}^2(V_{f2})} \quad (1.1.2)$$

The relative uncertainty associated with the mass of each standard $u_{rel}(sm)$ was estimated, using the data from the certificate of standard mixture solution, as standard deviation divided by functional gravimetric concentration. See Table 4.

The uncertainty associated with dilution volume was estimated of the tolerance of the volumetric flask used and assuming a triangular distribution.[23] The uncertainty associated with the volume taken with micropipettes was calculated as the quadratic sum of uncertainties associated with micropipette calibration (u_{mc}) and repeatability (u_{rep}).

The u_{mc} was evaluated as the accuracy, mean error relative (%), given by the manufacturer. In this case it has an interval without any specification on the distribution type neither of the level of trust, for what we suppose a triangular distribution, and it is necessary to divide for root of 6. The u_{rep} was evaluated as the standard deviation obtained using a gravimetric method. The repeatability is obtained for all volumes, by dispensing 10 measures with a single pipette and a single tip. Table 4 shows the data used for the

Table 4. Volumetric material and carbonyl-DNPH compounds used for preparing standards.

Micropipettes		Standard mixture solution of 13-carbonyl-DNPH		
Error (%)[a] (μl)	SD[b](μl)	Compound	Functional gravimetric conc.(mg/L)	SD
1.80 (20)	0.28 (30)	Acetaldehyde-2,4-DNPH	20.05	0.05
0.80 (50)	0.36 (50)	Acetone-2,4-DNPH	20.00	0.04
0.80 (100)	0.39 (80)	Acrolein-2,4-DNPH	19.94	0.02
	0.24 (100)	Benzaldehyde-2,4-DNPH	19.98	0.03
		Butyraldehyde-2,4-DNPH	20.01	0.04
		Crotonaldehyde-2,4-DNPH	20.18	0.03
		Formaldehyde-2,4-DNPH	40.01	0.06
0.8 (200)	0.66 (200)	Hexaldehyde-2,4-DNPH	19.97	0.07
0.7 (500)	0.94 (400)	Methacrolein-2,4-DNPH	20.17	0.03
0.6 (1000)	1.31 (640)	*p*-Tolualdehyde-2,4-DNPH	20.00	0.04
	1.24 (1000)	Propionaldehyde-2,4-DNPH	19.99	0.03
		Valeraldehyde-2,4-DNPH	20.08	0.05
		2-Butanone-2,4-DNPH	20.01	0.02

[a]The values in parenthesis are the volumes in which the relative error value is established by manufactures.

[b]The values in parenthesis are the volumes in which the standard deviation was established by successive check deliveries found by weighing.

calculation of these terms. When the volume taken is different to the tabulated value, the error and standard deviation are obtained by interpolation.

The uncertainty associated to the preparation of the calibration curve is calculated for each concentration level as:

$$u_{rel}(ccs) = \sqrt{u_{rel}^2(V_{p2}) + u_{rel}^2(V_{p3})} \qquad (1.1.3)$$

where: V_{p2} is the volume taken with micropipette from the second standard solution and V_{p3} is the acetonitrile volume taken with micropipette for diluting to 1 mL. Table 5 shows the values of V_{p2} used for preparing each calibration point.

Uncertainty Associated with the Calibration Curve u(cal)

The uncertainty derived from the estimation of the compound concentration from the calibration curve is estimated by applying the expression for the

Table 5. Data for calculating the uncertainty associated with the standards preparation

Method	Compound	C(mg/L)	$V_{p2}(\mu L)$
HPLC-UV	Formaldehyde	0.061	30
	The remaining compounds	0.172	84
		0.279	136
		0.389	190
		0.500	244
		0.031	30
		0.139	136
		0.250	244
		0.369	360
		0.489	478
		0.610	596
		0.729	712
GC-MS	Formaldehyde	0.500	244
	The remaining compounds	0.737	360
		0.979	478
		1.221	596
		1.458	712
		1.700	830
		1.941	948
		0.250	244
		0.369	360
		0.489	478
		0.610	596
		0.729	712
		0.850	830
		0.971	948

linear regression of least squares of residuals:

$$u(cal) = \frac{1}{b}\sqrt{S_{y/x}^2 \cdot \left(\frac{1}{n}\right) + (x_0 - \bar{x})^2 \cdot S_b^2} \tag{1.2}$$

where b is the slope of the calibration curve, $S_{y/x}$ the residual standard deviation, x_0 the compound concentration at each concentration level studied, $\bar{x}$ represents the mean of all the standards used for calibration curve, S_b the standard deviation of the slope of the calibration curve and n the number of concentration values of the linear regression.

Uncertainty Associated with the Precision u(rep)

In order to estimate the uncertainty associated to the precision, two aliquots of each concentration level were analysed in repeatability conditions during six and five days for HPLC-UV and GC-MS, respectively. This uncertainty is given by:

$$u(rep) = \frac{SD}{\sqrt{r}} \tag{1.3}$$

where SD is the standard deviation and r the number of replicates of each sample when analysed in routine analysis. We have considered $r = 1$, because in routine analysis, samples will be determined only once.

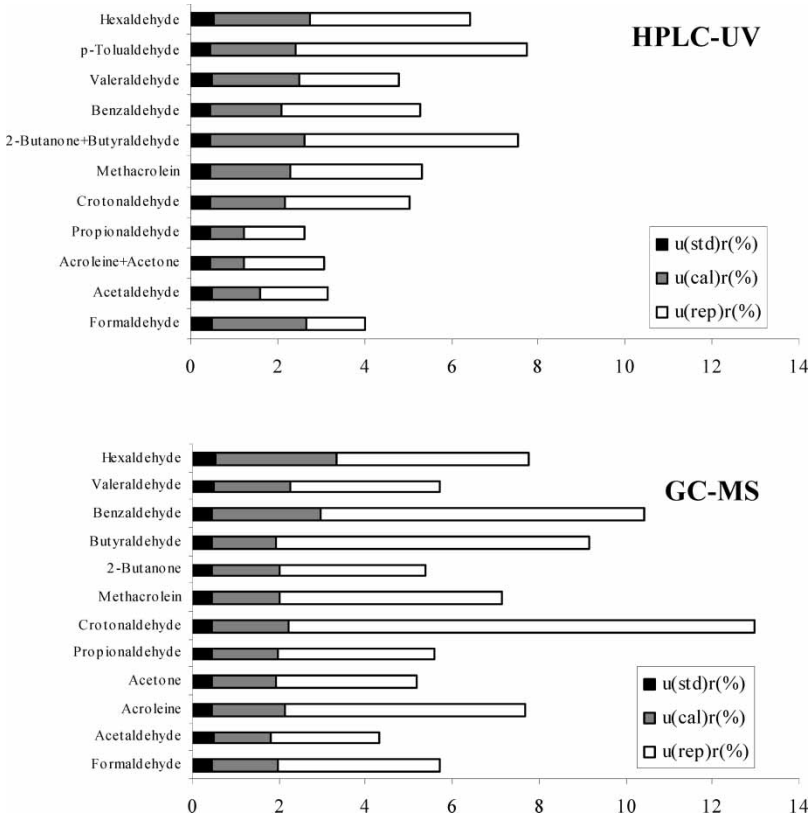

Figure 2. Diagram of the partial relative uncertainties for all the compounds analysed by HPLC-UV and GC-MS for the intermediate level of concentration.

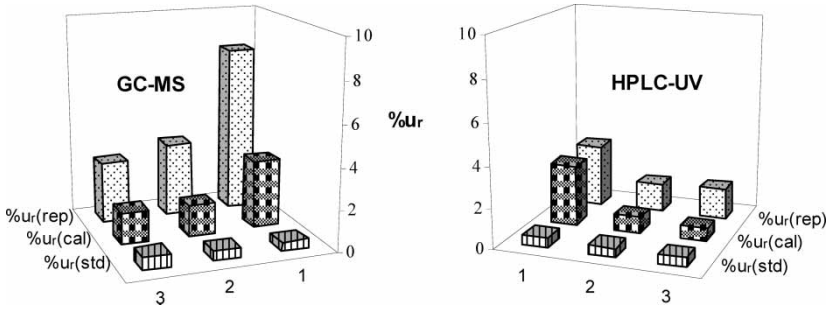

Figure 3. Diagram of the different uncertainty components for each concentration level of Propionaldehyde.

Expanded Uncertainties and their Contributions

Figure 2 shows the partial relative uncertainty calculated for each identified source for all the compounds analysed by both methods. It can be observed, that the most influencing factor in the combined uncertainty is associated to the repeatability of the measurements. In general, GC-MS analysis shows a

Table 6. Combined relative uncertainty (%) at different concentration levels for HPLC-UV and GC-MS methods

	HPLC-UV			GC-MS		
Compound	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Formaldehyde	18	2.6	2.6	9.7	4.1	2.1
Acetaldehyde	5.2	2.0	1.6	6.8	2.9	2.8
Acroleine +acetone	3.7	2.0	1.3	—	—	—
Acroleine	—	—	—	6.8	5.8	4.2
Acetone	—	—	—	5.9	3.6	3.3
Propionaldehyde	4.3	1.7	1.7	8.8	3.9	3.4
Crotonaldehyde	7.5	3.4	2.2	19	11	7.3
Methacrolein	8.3	3.6	3.5	12	5.4	4.8
2-Butanone + butyraldehyde	10	5.4	3.7	—	—	—
2-Butanone	—	—	—	7.5	3.7	2.9
Butyraldehyde	—	—	—	6.7	7.4	4.1
Benzaldehyde	7.7	3.6	2.4	—	7.9	4.9
Valeraldehyde	9.6	3.1	4.7	8.2	3.9	3.2
p-Tolualdehyde	9.9	5.7	3.9	ND	ND	ND
Hexaldehyde	12	4.3	3.1	—	5.4	5.7

The concentration of levels 1, 2 and 3 for both methods are given in Tables 2 and 3.

greater uncertainty level than HPLC-UV analysis and is affected by the level in which the precision has been obtained. As shown in Figure 3, this component increases as the concentration decreases.

The contribution of the uncertainty associated to the preparation of the standard solutions is not significant compared with the contribution of the uncertainties associated to the calibration curve and to the precision, and is non-dependent of the concentration level considered.

Finally, the contribution of the uncertainty associated to the calibration curve is comparable for both methods and, as may be expected, presents maximum values for low concentration levels close to the LOQ.

Table 6 shows the combined relative uncertainty at the different concentration level for both methods. The highest uncertainties can be associated to GC-MS analysis and for the lowest concentration levels, with mean values of 9.14%, 5.39%, and 4.08% in comparison with those obtained for HPLC-UV analysis, 8.72%, 3.39%, and 2.79%, for each concentration level considered.

In order to provide a 95% level of confidence for the final results, the expanded uncertainties are obtained by multiplying the combined uncertainty by a coverage factor $k = 2$. Therefore, the final results for the determination of carbonyl compounds by HPLC-UV and GC-MS, for the lowest concentration level where the uncertainties are greater, would be expressed as shown in Table 7.

Table 7. Expanded uncertainties for the lowest concentration level of DNPH-derivatives for HPLC-UV and GC-MS methods

Compound	HPLC-UV C (mg/L)	GC-MS C (mg/L)
Formaldehyde	0.06 ± 0.02	0.74 ± 0.14
Acetaldehyde	0.14 ± 0.01	0.37 ± 0.05
Acroleine + acetone	0.28 ± 0.02	—
Acroleine	—	0.37 ± 0.05
Acetone	—	0.37 ± 0.04
Propionaldehyde	0.14 ± 0.01	0.37 ± 0.06
Crotonaldehyde	0.14 ± 0.02	0.37 ± 0.14
Methacrolein	0.14 ± 0.02	0.37 ± 0.08
2-Butanone + butyraldehyde	0.28 ± 0.06	—
2-Butanone	—	0.37 ± 0.06
Butyraldehyde	—	0.37 ± 0.05
Benzaldehyde	0.14 ± 0.02	—
Valeraldehyde	0.14 ± 0.03	0.37 ± 0.06
p-Tolualdehyde	0.14 ± 0.03	ND
Hexaldehyde	0.14 ± 0.03	—

CONCLUSIONS

The methodologies based on the derivatization of carbonyl compounds with 2,4-dinitrophenylhidrazine and the later analysis of those derivatives by HPLC-UV and GC-MS, are appropriate for the determination of these compounds in complex samples, as the smoke coming from the combustion of agricultural debris.

Considering the time being invested in the chromatographic process, the GC-MS presents remarkable advantages. In fact, to reach similar resolutions half of that time is needed than that in HPLC-UV. Besides, the possibility of quantifying compounds that present poor separations, together with the potentiality of giving information about the nature of the components present in the sample, make the GC-MS especially appropriate for the analysis of carbonyl compounds present in smoke samples.

However, when comparing the validation parameters, the HPLC-UV methodology offers better characteristics than the GC-MS, as can be seen with the precision and the relative errors that are obtained in the determination of the different carbonyl compounds. On the other hand, the detection limits reached in HPLC-UV are usually lower than those obtained by GC-MS, especially for the compounds of lower molecular weight.

As for the calculated uncertainties, it is observed that the contribution due to the preparation of the standard solutions is not significant. The uncertainty associated with the estimation of the compound concentrations from the calibration curves are similar for both methodologies or slightly lower when HPLC-UV is used. The cause of higher uncertainty and, especially when GC-MS is used, is the repeatability of the measurements.

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REFERENCES

1. Bakeas, E.B.; Argyris, D.I.; Siskos, P.A. Carbonyl compounds in the urban environment of Athens, Greece. Chemosphere **2003**, *52* (5), 805–813.
2. Grosjean, D. Atmospheric chemistry of toxic contaminants. 2. Saturated aliphatics - acetaldehyde, dioxane, ethylene-glycol ethers, propylene-oxide. J. Waste Mgmt. Assoc. **1990**, *40* (11), 1522–1531.
3. Deandrade, J.B.; Pinheiro, H.L.C.; Andrade, M.V. The formaldehyde and acetaldehyde content of atmospheric aerosol. J. Braz. Chem. Soc. **1995**, *6* (3), 287–290.

4. Nondek, L.; Rodier, D.R.; Birks, J.W. Measurement of sub-ppbv concentrations of aldehydes in a forest atmosphere using a new HPLC technique. Environ. Sci. Technol. **1992**, *26* (6), 1174–1178.
5. Hedberg, E.; Kristensson, A.; Ohlsson, M.; Johansson, C.; Johansson, P.-A.; Swietlicki, E.; Vesely, V.; Wideqvist, U.; Westerholm, R. Chemical and physical characterization of emissions from birch wood combustion in a wood stove. Atmos. Environ. **2002**, *36* (30), 4823–4837.
6. Lehmpuhl, D.W.; Birks, J.W. New gas chromatographic electron-capture detection method for the determination of atmospheric aldehydes and ketones based on cartridge sampling and derivatization with 2,4,6-trichlorophenylhydrazine. J. Chromatogr. A **1996**, *740* (1), 71–81.
7. Tanner, R.L.; Meng, Z. Seasonal-variations in ambient atmospheric levels of formaldehyde and acetaldehyde. Environ. Sci. Technol. **1984**, *18* (9), 723–726.
8. Keuken, M.P.; Schoonebeek, C. Simultaneous determination of c1-c2 aldehydes and organic-acids in aqueous atmospheric samples by high-performance liquid-chromatography. Int. J. Environ. Anal. Chem. **1989**, *35* (4), 227–239.
9. Kiss, G.; Varga, B.; Puchony, Z.V.; Gelencsér, A.; Krivácsy, Z.; Hlavay, J. Sample preparation of atmospheric aerosol for the determination of carbonyl compounds. Talanta **1999**, *48* (4), 755–762.
10. Dong, J.-Z.; Moldoveanu, S.C. Gas chromatography-mass spectrometry of carbonyl compounds in cigarette mainstream smoke after derivatization with 2,4-dinitrophenylhydrazine. J. Chromatogr. A **2004**, *1027* (1–2), 25–35.
11. Schauer, J.J.; Kleeman, M.J.; Cass, G.R.; Simoneit, B.R.T. Measurement of emissions from air pollution sources. 3. C-1-C-29 organic compounds from fireplace combustion of wood. Environ. Sci. Technol. **2001**, *35* (9), 1716–1728.
12. Koziel, J.; Jia, M.; Khaled, A.; Noah, J.; Pawliszyn, J. Field air analysis with SPME device. Anal. Chim. Acta **1999**, *400*, 153–162.
13. Strassnig, S.; Wenzl, T.; Lankmayr, E.P. Microwave-assisted derivatization of volatile carbonyl compounds with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine. J. Chromatogr. A **2000**, *891* (2), 267–273.
14. Michels, J.J. Improved measurement of formaldehyde in water-soluble polymers by high-performance liquid chromatography coupled with post-column reaction detection. J. Chromatogr. A **2001**, *914* (1–2), 123–129.
15. Kempter, C.; Pötter, W.; Binding, N.; Kläning, H.; Witting, U.; Karst, U. Tailor-made derivatizing agents for carbonyl compounds using liquid chromatography. Anal. Chim. Acta **2000**, *410* (1–2), 47–64.
16. Kempter, C.; Karst, U. Determination of carbonyl compounds in waters using triazine-based hydrazine reagents and liquid chromatography. Analyst **2000**, *125*, 433–438.
17. Zurek, G.; Karst, U. 2,4-dinitro-3,5,6-trideuterophenylhydrazones for the quantitation of aldehydes and ketones in air samples by liquid chromatography-mass spectrometry. J. Chromatogr. A **2000**, *869* (1–2), 251–259.
18. Jachmann, N.; Karst, U. Synthesis and application of 4-(N,N-dimethylaminosulfonyl)-7-N-methylhydrazino-2,1,3-benzoxadiazole (MDBDH) as a new derivatizing agent for aldehydes. Fresenius J. Anal. Chem. **2001**, *369* (1), 47–53.
19. Velikonja, S.; Jarc, I.; Zupancic-Kralj, L.; Marsel, J. Comparison of gas-chromatographic and spectrophotometric techniques for the determination of formaldehyde in water. J. Chromatogr. A **1995**, *704* (2), 449–454.
20. Uralets, V.P.; Rijks, J.A.; Leclercq, P.A. Syn-anti isomerization of 2,4-dinitrophenylhydrazones of volatile carbonyl-compounds in capillary gas chromatographic-mass spectrometric analyses. J. Chromatogr. **1980**, *194* (2), 135–144.

21. Miller, J.; Miller, J. *Statistics and Chemometrics for Analytical Chemistry*, 4th Edn.; Pearson Education Limited, Publisher: Prentice Hall, 2000.
22. ISO. *Guide to the Expression of Uncertainty in Measurement*; International Standards Organisation: Geneva, 1993.
23. EURACHEM Guide. *Quantifying Uncertainty in Analytical Measurement,* 2nd Edn.; 2000.
24. Analytical Methods Committee, The Royal Society of Chemistry. Uncertainty of measurement: implications of its use in analytical science. Analyst **1995**, *120*, 2303–2308.

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