Research Article

Analysis of 31 Hydrazones of Carbonyl Compounds by RRLC-UV and RRLC-MS(/MS): A Comparison of Methods

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Aldehydes and ketones are volatile organic compounds (VOC) emitted into the atmosphere by a large number of natural and anthropogenic sources. Carbonyl compounds (CC) are atmospheric pollutants with known damaging effects for the human's health. In this work, the separation of 31 carbonyl compounds (CC) in their 2,4-dinitrophenylhydrazones form was optimized by rapid resolution liquid chromatography in 9 minutes and simultaneously detected by ultraviolet and mass spectrometry with an APCI(−) as ionization source. The mass spectra of hydrazones presented the $[M-H]^-$ ions as base peak, but the MS/MS spectra showed fragments related to different structural classes of aldehydes and ketones, representing an important tool to assist structure elucidation of unknown CC in real samples. Multiple reactions monitoring (MRM) improved the sensitivity and selectivity for the quantitation method. Analytical parameters using both UV and MS (linearity, determination coefficients, detection limits, and sensitivity) were compared. The detection methods are complementary and a powerful analytical tool for the detection and quantitation of CC in complex environmental samples.

1. Introduction

Aldehydes and ketones are volatile organic compounds (VOC) emitted to the atmosphere by a large number of natural and anthropogenic sources, such as vegetation and industrial emissions [1, 2], cigarette smoke [3, 4], and fossil fuel or vegetation burning [5, 6]. VOC photooxidation is also considered a secondary source of the emission of these compounds [6]. Carbonyl compounds (CC) affect the atmospheric chemistry of polluted areas through a series of complex routes. The induction period for the generation of photochemical smog decreases significantly with increasing concentrations of CC, due to their high reactivity, resulting in increased ozone concentration in the troposphere [7]. Certain CC can also affect human health; for example, formaldehyde is classified as carcinogenic and

acetaldehyde as probably carcinogenic by International Agency for Research on Cancer (IARC) [8, 9]. CC also occur in a large number of environmental and artificial matrixes such as natural and drinking waters and disinfected and swimming pool water. They can also be formed during the frying process of vegetable oil as byproducts of thermal degradation/oxidation [1, 10].

There is great interest to improve the detection limits of techniques for the CC determination at trace levels (ppb) for several important applications, for example, characterization of air emission from combustion process and industrial sources, air pollution, and evaluation of human exposure to toxic contaminants present in indoor and work place areas.

Many analytical techniques have been employed for the analysis of aldehydes and ketones in air as previously discussed [10], but certainly their derivatization using

2,4-dinitrophenylhydrazine (DNPH) in acidic media to form the respective hydrazones followed by the analysis using high performance liquid chromatography and UV detection (HPLC-UV) at 360 nm has been the technique of choice for CC determination [10–15]. Moreover, this methodology is currently recommended by environmental agencies including the US Environmental Protection Agency (US EPA) [14].

Regarding the analytical tools for CC determinations, significant improvement of resolution, detectability, and analytical throughput is now being achieved by using LC stationary phases with sub-2 μm particles, well known as rapid resolution or ultraperformance liquid chromatography (RRLC or UPLC) [16]. In a recent work, we optimized the conditions of rapid resolution liquid chromatography (RRLC) for determination of hydrazones and compared them with those of HPLC-UV [10]. The RRLC method allowed the determination of up to 31 atmospheric CC [17] and the monitoring of occupational exposure to formaldehyde in an institute of morphology [18].

On the other hand, HPLC-MS allowed significant improvement in quantification and positive identification of a number of carbonyls in samples. Oehme and coworkers applied this technique to investigate the fragmentation pathways of some CC [19, 20]. An ion trap mass spectrometer, following atmospheric pressure chemical ionization (APCI) and detection of negative ions, was applied for the evaluation of air samples [15, 21, 22]. This method was also applied in other works to determine CC, such as the evaluation of oxidation products formed in the reaction between pinene and OH radicals [23, 24], biological relevant aldehydes in exhaled breath [25], and carbonyl compounds in urine [26]. Electrospray ionization (ESI) [12, 26–28] or atmospheric pressure photoionization (APPI) [29, 30] was also implemented, but APCI is the most frequent ionization method used for CC detection and quantitation [15, 21, 22, 25, 31–33].

Herein, we describe the comparison of the two methods of detection of the DNPH derivatives (hydrazones) of 31 carbonyl compounds. For this purpose, sequential detection of the hydrazones using both UV and negative mode APCI-MS/MS was employed following their separation under previously optimized RRLC conditions. The choice of these chromatographic conditions and the sequential detection allowed simultaneous detection of all hydrazones in the same optimized separation conditions.

2. Materials and Methods

2.1. Reagents and Solvents. A standard solution containing 15 CC-DNPH derivatives (hydrazones of formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde, valeraldehyde, o-, m-, and p-tolualdehydes, hexaldehyde, and 2,5-dimethylbenzaldehyde) in concentrations corresponding to 15 $\text{mg}\cdot\text{L}^{-1}$ of each carbonyl compounds was purchased from Sigma (T011/IP-6A Aldehyde/Ketone-DNPH mix; Supelco). Other 16 hydrazones of selected CC (specifically, 2,3-butanedione, butanone, methacrolein, salicylaldehyde, cyclohexanone, 3-pentanone, 2-pentanone, p-methoxyacetophenone, methyl isobutyl ketone, heptanaldehyde,

octylaldehyde, nonanaldehyde, decanaldehyde, undecanaldehyde, dodecanaldehyde, and tridecanaldehyde) were synthesized via a reaction with DNPH (Aldrich) in sulfuric acid solution [34]. The solids were purified after two recrystallization steps in ethanol. The crystals were dried and used as DNPH standards, after evaluation of their purities by RRLC-UV.

Acetonitrile, methanol, tetrahydrofuran, and isopropanol (HPLC grade) were from Tedia, Brazil. Ultrapurified water was prepared through a Simplicity System (Millipore, EUA) following reversed osmosis (Rios D3, Millipore, EUA).

2.2. Standard Solutions. A standard stock solution of the 15 carbonyl-DNPH derivatives was prepared by dilution of 1.00 mL of the standard solution up to 3.00 mL using acetonitrile. Standard solutions of the 16 carbonyl-DNPH derivatives synthesized by our group were prepared by weighing appropriate masses of the synthesized solids and dissolving them in acetonitrile. Working standards were prepared by dilution of appropriate aliquots of the stock solution up to 1.00 mL using acetonitrile.

2.3. Chromatographic Separation and Detection. A rapid resolution liquid chromatography system (Agilent 1200 Series, USA) equipped with a binary pump, a degasser, an automated injector, a thermostated column compartment and an ultraviolet diode array detector (UV-DAD), and an Ion Trap SL mass spectrometer (IT-MS) (Agilent 6300 Series, USA) was used. The UV-DAD and IT-MS were connected in series through the output of the UV-DAD detector that was directly connected to the APCI source, operating in negative ion mode in the mass range of m/z 50–500.

A rapid separation (around 9.5 min in total) of the 31 hydrazones was achieved using a Zorbax Eclipse Plus C18 (50×2.1 mm; 1.8 μm , Agilent, USA) column and the previously optimized conditions [10]. Briefly, a quaternary mobile phase consisted of methanol, tetrahydrofuran (THF), isopropanol, and water. The best separation condition was obtained at 35°C of column temperature, using a multistep gradient of methanol (A) and a mixture of water (75% v/v), isopropanol (15% v/v), and THF (10% v/v) (B). The mobile phase gradient was as follows: 0–30% of (A) in 2 min, held constant during 0.5 min; a linear gradient to 80% of (A) during 2.5 min; a linear gradient of (A) to 85% during 1.5 min, held constant during 3 min. The flow rate was 0.55 $\text{mL}\cdot\text{min}^{-1}$ which is compatible with the APCI characteristics. Volumes of 3 μL were injected and an equilibration time of 1 min between successive RRLC runs was always adopted. Prior to use, all solvents were filtered using polyvinylidene fluoride membrane filters (0.22 μm , 47 mm, Millipore).

Parameters of the UV-DAD detector such as detector slit and response time that directly influence the detector signals were further optimized. A slit of 4 nm and a response time of 0.05 min gave the best responses considering peak width and form and chromatogram smooth [10]. Detection was carried out at 360 nm.

The mass spectrometer was calibrated by direct infusion of the Tune Mix Solution (APCI/APPI Calibrant Solution,

G2432A, Agilent) at a constant flow rate of 0.60 mL h^{-1} , using an automated syringe pump in APCI(-). For quantitative evaluations, the Expert Parameters Settings were used and MS parameters were optimized to improve the detection of the ion m/z 556 of the calibrant solution continuously injected by the automated syringe pump.

Parameters of ionization and of the APCI interface were optimized by carrying out multiple injection of a standard mixture of hydrazones while each parameter was varied to obtain the best signal-to-noise ratio for hydrazones mass spectra.

2.4. Carbonyl-DNPH Derivatives Identification and Quantification. All hydrazones were first detected by their retention time and elution order, considering their detection by the UV-DAD detector [10].

In order to evaluate the detection limits (DL) and quantification limits (QL) of each hydrazone, calibration curves were built between the range of 1.00 and $500 \mu\text{g L}^{-1}$ with standard solutions containing all the studied hydrazones. Calibration curves were then obtained by least-squares regression of data. In this way, DL and QL were obtained by the ratio between three and ten times the signal-to-noise ratio by the angular coefficients of calibration curves, for DL and QL, respectively. Signal-to-noise ratios were estimated by standard deviations of peak areas obtained after 6 subsequent injections of the most diluted standards (1.00 , 2.00 , and $5.00 \mu\text{g L}^{-1}$) [35].

3. Results and Discussion

3.1. Optimization of MS Conditions. Instrumental parameters of the mass spectrometer were optimized by direct infusion of the calibrant solution and detection of the ion m/z 556 in negative mode. These parameters were used in all MS evaluations (Table 1).

Parameters for the acquisition of mass spectra data were optimized by carrying out multiple injections ($3 \mu\text{L}$) of a solution obtained after dilution of the Supelco hydrazones mixture to a final concentration of $50.00 \mu\text{g L}^{-1}$. Each APCI source parameter was varied in order to obtain the best signal-to-noise ratio for the deprotonated molecules, $[\text{M-H}]^-$ ions (Figure 1).

Ionization processes in the APCI interface occur in vapor phase and are strongly influenced by the vaporization temperature of the nebulizer and by the drying temperature of the source. Vaporization temperature of the APCI source was evaluated between the range of 150 and 400°C under the optimized chromatographic conditions, leading to an optimal condition at 400°C . The best APCI drying temperature was also optimized and found to be at 350°C . A nitrogen flow of 5 L min^{-1} is suggested for the RRLC flow rate (0.55 mL min^{-1}) and the default nebulizer gas pressure of 60 psi was used. Values for all the optimized APCI parameters are shown in Table 2.

3.2. Evaluation of MS/MS Spectra of Carbonyl-DNPH Derivatives. The mass spectrum of 31 hydrazones obtained in

TABLE 1: Optimized mass spectrometry parameters for the detection of hydrazones.

Parameter	Optimized value
Capillary (V)	1,000
Skimmer (V)	-33.1
Capillary outlet (V)	-107.4
Octupole 1 DC (V)	-4.4
Octupole 2 DC (V)	-1.37
Trap drive (V)	40.03
Octupole RF (V)	136
Lens 1 (V)	0.82
Lens 2 (V)	33.6
Trap drive	30
Target	30,000

APCI(-) is dominated mainly by its deprotonated molecules, $[\text{M-H}]^-$ as base peak and the MS/MS spectra hydrazones of carbonyl compounds showed fragments related to different structural classes of aldehydes and ketones, represented in Figure 2.

Specific fragmentation pathways were observed as a function of the hydrazone structure. The fragment ions of MS/MS spectra obtained for different structures and their relative abundance are summarized in Table 3. As previously shown [19, 21, 31], the observed fragments of the hydrazones are related to different structural classes of aldehydes and ketones. For example, aliphatic aldehydes, such as propionaldehyde and heptanaldehyde, showed a relatively abundant ion of m/z 163 (100%) and another of m/z 152 with a relative abundance around 50% (Figures 3(a) and 3(b)).

The MS/MS spectra of aromatic aldehydes showed characteristic fragment ion $[\text{M-H-164}]^-$ with a relative abundance of around 50%. It corresponded to an ion of m/z 121 (Figure 3(d)) in the MS/MS spectra of benzaldehyde and to an ion of m/z 135 (Figure 3(c)) in the MS/MS spectra of *o*-, *m*-, and *p*-tolualdehydes-hydrazones, which coeluted under our chromatographic conditions [10]. Benzaldehyde and substituted aromatic aldehydes such as tolualdehydes-hydrazones also exhibited the fragment ion $[\text{M-H-93}]^-$ as observed in the MS/MS spectra (m/z of 192 for benzaldehyde and m/z 206 for tolualdehydes, Figures 3(c) and 3(d)).

The MS/MS spectra of unsaturated aldehydes showed the fragment ion $[\text{M-H-47}]^-$ due to the loss of HNO_2 after ionization. For example, the MS/MS spectra of crotonaldehyde showed the fragment of m/z 202, with an approximate relative abundance of 40% (Figure 3(e)). This ion also showed a similar abundance in the MS/MS spectra of aromatic aldehydes, as observed for isomers of tolualdehyde-DNPH (m/z 252) and benzaldehyde-DNPH (m/z 238).

Typical fragments of α,β -unsaturated aldehydes corresponded to ions of $[\text{M-H-31}]^-$ and $[\text{M-H-17}]^-$, this one due to a neutral loss of NH_3 [19]. These fragments were also observed in the MS/MS spectrum of crotonaldehyde-DNPH (m/z 218 and 232, resp.). Derivatives of α -hydroxylated- and dicarbonyl-CC hydrazones showed MS/MS spectra and similar fragmentation profiles, which are characterized by

TABLE 2: Evaluated parameters for optimum response of hydrazones derivatives by APCI/MS.

Parameter	Evaluated range	Optimum condition
Corona needle	2,000–20,000 nA	10,000 nA
Vaporization temperature	150–400°C	400°C
Drying temperature	250–350°C	350°C
Ion charge control	50,000–130,000	70,000
Maximum accumulation time	100–600 ms	200 ms

FIGURE 1: Evaluation of selected parameters for optimization of MS conditions by the average signal of CC derivatives: (a) corona needle (nA); (b) vaporization temperature (°C); (c) drying temperature (°C); (d) ion charge control; and (e) maximum accumulation time (ms).

TABLE 3: Major fragments (m/z) of MS² spectrum formed by fragmentation of the precursor $[M-H]^-$ ions of the hydrazones.

Hydrazone	$[M-H]^-$	Fragment ions of $[M-H]^-$ (m/z)									
		152	163	179	182	$[M-H-17]^-$	$[M-H-30]^-$	$[M-H-31]^-$	$[M-H-47]^-$	$[M-H-93]^-$	$[M-H-164]^-$
Formaldehyde	209	xx	xxx	xx							
Acetaldehyde	223	xx	xxx	xxx			X				
2,3-Butanedione	265	xx		xx			X			xxx	
Acetone	237	xx		xx			Xxx			x	
Acrolein	235	x	xxx	xx			Xx			xx	
Propionaldehyde	237	xx	xxx	xx		x	Xx			x	
Crotonaldehyde	249	xxx	xx	xx		x		xx		xx	
Butanone	251	xx		xx			Xxx			x	
Methacrolein	249	xx	xxx	xx		x		x		xx	
Butyraldehyde	251	xx	xxx	xx			xx			x	
Salicylaldehyde	301	x	xx		xxx					xx	
Benzaldehyde	285	x	xxx	x			x			xx	
Cyclohexanone	277	x		x			xxx			x	
2- and 3-pentanone	265	xx		xx			xxx			x	
Isovaleraldehyde	265	xx	xxx	xx			xx			x	
Valeraldehyde	265	xx	xxx	xx			xx			x	
p-Methoxyacetophenone	329	x		x						xxx	
o-, m-, and p-tolualdehydes	299	x	xxx	xx						xxx	
Methyl isobutyl ketone	279	xx		x		x	xxx			x	
Hexaldehyde	279	xx	xxx	xx			x			x	
Dimethylbenzaldehyde	313		xx	x						xxx	
Heptanaldehyde	293	xx	xxx	xx		x	xx			xx	
Octanaldehyde	307	xx	xxx	xx			x			x	
Nonanaldehyde	321	xx	xxx	xx			xx			xx	
Decanaldehyde	335	xx	xxx	xx		x	xx			xx	
Undecanaldehyde	349	xx	xxx	xx			xx			xx	
Dodecanaldehyde	363	xx	xxx	xx			xx			xx	
Tridecanaldehyde	377	xx	xxx	x			xx			xxx	

Relative abundance: xxx: 100%; xx: 50%; x: ≤20%.

xxx: fragment ion selected for SRM.

FIGURE 2: Schematic representation of the precursor deprotonated molecule ($[M-H]^-$) and main fragments formations for aldehydes and ketones hydrazones.

FIGURE 3: MS/MS spectra obtained from the $[M-H]^-$ ion fragmentation of selected hydrazones: (a) propionaldehyde; (b) heptanaldehyde; (c) o-, m-, and p-tolualdehydes; (d) benzaldehyde; (e) crotonaldehyde; (f) salicylaldehyde; (g) acetone; and (h) cyclohexanone.

TABLE 4: Parameters of the studied hydrazones obtained by RRLC-MS/MS and RRLLC-UV-DAD: molecular weights (MW), MS/MS transitions (m/z to m/z), retention times, equations of analytical curves, determination coefficients, detection limits, and ratio between the detection limits.

Hydrazones	MW (g mol ⁻¹)	MS/MS ions (m/z)	Retention time (min)	Equations of analytical curves ^a		Determination coefficient		Detection limits (pg)		Ratio of DLs MS/UV
				MS/MS	UV	MS/MS	UV	MS/MS	UV	
Formaldehyde	210	209/163	2.13	$A = 1.810^2 C + 8.910^3$	$A = 7.510^{-2} C - 7.010^{-2}$	0.9714	0.9999	30.9	1.62	19.0
Acetaldehyde	224	223/163	2.81	$A = 5.210^2 C + 6.110^3$	$A = 5.410^{-2} C + 4.110^{-2}$	0.9966	0.9998	5.97	2.73	2.19
2,3-Butanedione	266	265/218	3.03	$A = 7.510^2 C + 7.810^3$	$A = 3.610^{-2} C + 5.810^{-2}$	0.9975	0.9998	6.18	2.70	2.29
Acetone	238	237/207	3.57	$A = 1.410^3 C + 1.310^4$	$A = 3.810^{-2} C + 8.510^{-2}$	0.9961	0.9999	11.1	3.21	3.46
Acrolein	236	235/163	3.85	$A = 7.410^2 C + 2.410^3$	$A = 4.410^{-2} C + 1.210^{-1}$	0.9982	0.9999	9.96	6.12	1.63
Propionaldehyde	238	237/163	4.03	$A = 1.410^3 C + 1.210^4$	$A = 4.010^{-2} C + 6.210^{-2}$	0.9963	0.9999	14.01	3.75	3.75
Crotonaldehyde	250	249/152	4.59	$A = 1.210^3 C + 5.510^3$	$A = 3.310^{-2} C + 6.010^{-2}$	0.9988	0.9998	3.81	4.41	0.86
Butanone	252	251/221	4.71	$A = 7.610^3 C + 1.110^4$	$A = 2.510^{-2} C + 3.910^{-2}$	0.9925	0.9997	6.84	3.33	2.06
Methacrolein	250	249/163	4.80	$A = 5.410^2 C + 1.410^4$	$A = 3.710^{-2} C + 6.110^{-3}$	0.9991	0.9999	8.40	2.25	3.74
Butyraldehyde	252	251/163	4.84	$A = 1.710^3 C + 1.710^4$	$A = 3.410^{-2} C + 4.910^{-2}$	0.9954	1.0000	11.5	3.21	3.59
Salicylaldehyde	302	301/182	4.97	$A = 9.110^2 C + 7.210^2$	$A = 1.710^{-2} C + 5.610^{-3}$	0.9984	0.9999	4.77	3.21	1.49
Benzyaldehyde	286	285/163	5.20	$A = 1.410^3 C + 1.510^4$	$A = 2.310^{-2} C + 1.110^{-2}$	0.9916	0.9999	4.53	5.22	0.86
Cyclohexanone	278	277/247	5.25	$A = 1.110^2 C + 4.510^3$	$A = 1.810^{-2} C + 1.610^{-3}$	0.9671	0.9998	18.3	5.52	3.32
2- and 3-pentanone	266	265/235	5.28	$A = 4.210^3 C + 3.010^4$	$A = 6.210^{-2} C + 2.510^{-2}$	0.9971	0.9999	9.93	0.84	11.7
Isovaleraldehyde	266	265/163	5.33	$A = 2.210^3 C + 2.610^4$	$A = 2.810^{-2} C + 6.210^{-2}$	0.9847	0.9998	5.64	1.92	2.92
Valeraldehyde	266	265/163	5.41	$A = 2.010^3 C + 1.310^4$	$A = 2.810^{-2} C + 3.410^{-2}$	0.9991	0.9999	3.51	4.05	0.87
p-Methoxyacetophenone	330	329/282	5.55	$A = 2.310^3 C + 1.810^4$	$A = 9.810^{-3} C - 8.410^{-3}$	0.9959	0.9996	7.95	4.59	1.73
Tolualdehydes	300	299/163	5.63	$A = 4.610^3 C + 4.110^4$	$A = 6.010^{-2} C + 1.510^{-2}$	0.9962	1.0000	4.50	1.65	2.73
Methyl isobutyl ketone	280	279/249	5.69	$A = 2.510^3 C + 2.410^4$	$A = 2.710^{-2} C + 3.610^{-2}$	0.9958	0.9999	7.92	2.91	2.73
Hexaldehyde	280	279/163	5.82	$A = 1.710^3 C + 1.610^4$	$A = 2.410^{-2} C + 8.110^{-2}$	0.9945	0.9995	2.13	6.90	0.31
Dimethylbenzaldehyde	314	313/266	5.96	$A = 1.810^3 C + 1.110^4$	$A = 1.710^{-2} C + 1.410^{-3}$	0.9928	1.0000	6.90	5.43	1.27
Heptanaldehyde	294	293/163	6.15	$A = 1.610^3 C + 7.210^3$	$A = 2.410^{-2} C + 8.310^{-3}$	0.9992	0.9999	10.7	7.35	1.45
Octanaldehyde	308	307/163	6.48	$A = 3.710^2 C + 2.610^3$	$A = 4.710^{-3} C + 4.510^{-2}$	0.9863	0.9986	4.65	8.46	0.55
Nonanaldehyde	322	307/163	6.83	$A = 1.310^3 C + 5.810^3$	$A = 1.710^{-2} C + 1.510^{-2}$	0.9969	0.9999	5.67	5.16	1.09
Decanaldehyde	336	335/163	7.21	$A = 1.210^3 C + 9.010^3$	$A = 1.610^{-2} C + 3.710^{-2}$	0.9981	0.9999	8.70	2.70	3.21
Undecanaldehyde	350	349/163	7.62	$A = 9.410^2 C + 3.410^3$	$A = 1.310^{-2} C + 1.510^{-2}$	0.9997	0.9994	8.07	5.61	1.44
Dodecanaldehyde	364	363/163	8.10	$A = 6.210^2 C + 5.710^3$	$A = 1.010^{-2} C + 3.510^{-2}$	0.9877	0.9978	9.00	6.00	1.50
Tridecanaldehyde	378	377/163	8.70	$A = 6.110^2 C + 5.210^3$	$A = 1.110^{-2} C + 3.210^{-2}$	0.9886	0.9984	9.00	6.00	1.50

^a A = peak area (arbitrary units); C = concentrations ($\mu\text{g L}^{-1}$).

FIGURE 4: Chromatograms of a mixture of ((a) and (c)) CC-DNPH derivatives from T011/IP-6A Supelco and ((b) and (d)) hydrazones of selected CC synthesized ($200 \mu\text{g L}^{-1}$) by RRLC-UV-DAD method ((a) and (b)) and by RRLC-APCI-MS/MS method ((c) and (d)) (peak identification: hydrazones of 1: formaldehyde; 2: acetaldehyde; 3: acetone; 4: acrolein; 5: propionaldehyde; 6: crotonaldehyde; 7: butyraldehyde; 8: benzaldehyde; 9: isovaleraldehyde; 10: valeraldehyde; 11: o-, m-, and p-tolualdehyde; 12: hexaldehyde; 13: 2,5-dimethylbenzaldehyde; 14: 2,3-butanedione; 15: butanone; 16: methacrolein; 17: salicylaldehyde; 18: cyclohexanone, 3-pentanone, and 2-pentanone; 19: p-methoxyacetophenone; 20: methyl isobutyl ketone; 21: heptanaldehyde; 22: octylaldehyde; 23: nonanaldehyde; 24: decanaldehyde; 25: undecanaldehyde; 26: dodecanaldehyde; 27: tridecanaldehyde).

an intense fragment of m/z 182 and a low abundance of fragment of m/z 179 [25] as observed in the MS/MS spectrum of salicylaldehyde-DNPH (Figure 3(f)) that shows an ion of m/z 182 as main fragment ion.

Aldehydes and ketones hydrazones can be differentiated by their different fragmentation pathways. Ketones showed low or no formation of the fragment of m/z 163 and an ion of m/z 152 of high relative abundance, as shown in the MS/MS spectra of acetone and cyclohexanone (Figures 3(g) and 3(h), resp.). Ketones also showed a highly abundant fragment of $[M-H-30]^-$, as shown in the spectra of acetone (m/z 207) and cyclohexanone (m/z 247) (Figures 3(g) and 3(h), resp.). This ion was also found in the mass spectra of aldehydes, but with a relative low abundance.

Although the fragmentation pathways evaluation represents an important tool to achieve the structure elucidation of unknown CC-hydrazones, the most abundant and specific ions are fundamental for quantitative analysis using single reaction monitoring (SRM) techniques when the predominant transitions gave rise to the most intense signals, improving the sensitivity for quantitative measurements.

3.3. Comparison of RRLC-APCI-MS/MS Method with RRLC-UV-DAD Method for Quantification of Carbonyl-DNPH Derivatives. Table 3 shows the MS/MS transitions chosen for the MRM method. In general, the most predominant transitions were used. The regression plots and some parameters of the analytical curves obtained by RRLC-APCI-MS/MS and RRLC-UV-DAD method with detection at 360 nm as described in our previous work [10] are also shown and compared in Table 4. The chromatograms of the two groups of CC, 15 CC-DNPH derivatives from T011/IP-6A Supelco and 16 hydrazones of selected CC synthesized, obtained by RRLC-UV-DAD and RRLC-APCI-MS/MS method are shown in Figure 4.

The analytical curves showed different linear ranges for each hydrazone and detection technique. In general, the RRLC-APCI-MS method showed a linear range between 1 and 200 $\mu\text{g L}^{-1}$ and the RRLC-UV-DAD method between 2 and 500 $\mu\text{g L}^{-1}$.

The sensitivity of a method is evaluated by the regression slope, but in this case the values were not comparable due to high magnitudes of signals obtained by RRLC-APCI-MS/MS that was five orders higher (1.1×10^2 to 4.6×10^3) than RRLC-UV-DAD method (4.7×10^{-3} to 7.5×10^{-2}). Both methods showed determination coefficients closer to the unity indicating a good adherence to a linear model, but the RRLC-UV-DAD method showed values closer to 1 (0.9978 to 1.0000) than RRLC-APCI-MS method (0.9671 to 0.9997).

The detection limits of the hydrazones obtained by RRLC-APCI-MS/MS varied between 2.13 and 30.9 pg and were approximately two or three times higher than those of RRLC-UV-DAD method, which were in the range of 0.84 to 8.46 pg. A closer evaluation of the ratios between these DLs shows that the most discrepant differences occur for formaldehyde and 2- and 3-pentanones.

However, despite the higher sensitivity of UV detection, the major advantage of MS² detection relies on method

selectivity because this technique allows the identification of other CC-hydrazones, besides the studied ones here, considering their fragmentation.

Our results indicate that the application of RRLC-APCI-MS/MS and RRLC-UV-DAD method for determination of carbonyl compounds in environmental samples after derivatization to hydrazones may lead to comparable results for quantitation considering DL and QL. For example, considering typical processing conditions for the analysis of air samples (volume of sampled air 60 L and extract dilution, 5 mL) the detection limits correspond to values between 0.23 and 0.02 $\mu\text{g m}^{-3}$ using UV detection and to values between 0.86 and 0.06 $\mu\text{g m}^{-3}$ using the SRM-MS detection.

Moreover, the detection techniques can also be used as complementary ones, once apparently the UV detection allows better detection limits and a wide linearity to be achieved and the mass spectrometry detection (MS²) confers a higher selectivity to the analytical method, besides being an excellent tool for identifying other hydrazones and for confirmation of those detected and quantified with UV.

4. Conclusions

In our work specifically, RRLC-UV-DAD was more sensitive for detection of hydrazones than RRLC-APCI-MS/MS, which, on the other hand, offers several advantages such as the positive identification of CC in samples by examining the fragmentation pattern of hydrazones, even without analytical standards. The detection limit (0.71–10.3 $\mu\text{g L}^{-1}$) of this technique is slightly worse than that of RRLC-UV-DAD method, but it could also allow the quantification of carbonyl in air samples, for example. The rapid resolution liquid chromatography system allowed significant improvements of resolution, throughput, and low detection limits. The simultaneous evaluation of hydrazones using both methods APCI-MS/MS and UV-DAD raised up a suitable analytical methodology for the analysis of CC in complex environmental mixtures.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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