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Optimization and comparison of HPLC and RRLC conditions for the analysis of carbonyl-DNPH derivatives

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ABSTRACT

Analytical conditions for the analysis of 15 carbonyl-DNPH derivatives were optimized and compared by high performance liquid chromatography (HPLC) and rapid resolution liquid chromatography (RRLC). Binary, ternary and quaternary mixtures of acetonitrile, isopropanol, methanol, tetrahydrofuran and water were evaluated under RRLC conditions employing a Zorbax Eclipse Plus C18 (50 mm × 4.6 mm × 1.8 μm) column and a Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm) column. The optimized conditions obtained employing the two RRLC columns were compared with those obtained using a Supelcosil C18 (250 mm × 4.6 mm × 5 μm; Supelco) that is designed for HPLC separation of DNPH derivatives. Chromatograms run with a Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm) column and a mobile phase composed of isopropanol, methanol, tetrahydrofuran and water led to the best separation conditions considering reduced analysis time (~6 min per run), solvent consumption rate (~2 mL per run) and resolution of propanone, acrolein and propionaldehyde hydrazones. Quantification limits and linear ranges were adequate for direct application of EPA TO-11 conditions in all sets of RRLC and HPLC conditions. The analytical method was applied in the determination of carbonyl compounds (CCs) in Niterói City, RJ, Brazil in samples that were collected during periods of 2 h. Formaldehyde (8.22–9.78 ppbv) predominated in all periods followed by acetaldehyde (1.77–3.99 ppbv) and propanone (1.89–3.26 ppbv). Heavy CCs such as butyraldehyde and benzaldehyde were also detected in most samples. Total CCs varied along the studied day. The obtained results showed that RRLC can be applied to CCs determination without any change in the conditions of sample preparation of the Method EPA TO-11.

1. Introduction

The occurrence of carbonyl compounds (CCs) in the atmosphere has fundamental interest due to their role in the oxidation of volatile organic compounds (VOCs) and in the formation of ozone in the troposphere [1,2]. Several aspects of emission, reactivity and toxicological effects of CCs were reviewed [1]. Primary sources of CCs to the atmosphere include vegetation and industrial emission, cigarette smoke [3–5] and vegetation or fossil fuel burning. Photo-oxidation of VOCs in the atmosphere represents a secondary emission source of CCs and their role in ozone formation is well known [1]. There is also concern about CCs because some of them can affect human health. Formaldehyde and acetaldehyde, for example, are considered respectively as carcinogenic and probably carcinogenic compounds by IARC. The determination of atmospheric CCs has been conducted in different places worldwide [6–17]. Different aspects of experimental conditions for the determination of CCs in the atmosphere were recently reviewed [14]. CCs were also studied in natural and drinking waters [18–20], disinfected water [21] and in pool water [22]. CCs are also formed during the frying process as by-products of vegetable oil degradation [23]. Odor-active aldehydes were also found in wines [24–26].

Several techniques have been used for the identification and determination of CCs. Derivatization with o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) followed by gas chromatography (GC) and mass spectrometry (MS) detection [24–28] or by GC–MS–MS [21] was employed in the determination of several CCs. Derivatization of CCs with pentafluorophenylhydrazine [10] followed by derivative analysis by GC–MS–SIM allowed determination of CCs with

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very low detection limits. Derivatization of formaldehyde with 3,5-bis(trifluoromethyl)phenylhydrazine followed by GC–MS or electron-capture detection (GC–ECD) was described for its determination [29]. GC–MS was also used in the determination of CCs without derivatization [23,30]. CCs were also determined by capillary electrophoresis and UV detection after derivatization with 4-hydrazinebenzoic acid [9] or with 4-hydrazinobenzene sulfonic acid [18].

Derivatization of CCs with 2,4-dinitrophenylhydrazine (DNPH) to form the corresponding 2,4-dinitrophenylhydrazone derivatives followed by their determination by high performance liquid chromatography (HPLC) became a method of choice to determination of individual CCs [31] or their mixtures in air and in different samples [5–7,11–17,19–21,32–41] possibly due to the method versatility and sensitivity. HPLC–UV [5–7,11–17,19,20,31–37] or HPLC–MS following the ionization in ESI [7,21], APCI [38–40] or APPI interfaces [40] and detection of negative ions have been used. Furthermore, derivatization with PFBHA followed by APCI–LC–MS and detection of positive ions was also used [42]. The main advantage of MS detection towards UV detection relies on its selectivity that is also improved with tandem-MS techniques [7,22]. A comparison of MS and UV detection indicates that the sensitivity of some MS methods may be comparable to or better than those of UV methods. Although there is a lack of papers comparing the performance of different HPLC–MS interfaces in the determination of CC hydrazones, APPI interfaces showed better detection limits than APCI interfaces [40].

A direct comparison of advantages and disadvantages of all these techniques and methods is not simple because many features of analytical methods are expressed in different ways. Therefore, detection limits are expressed in terms of mass or concentrations in solution or gas phase. They are also expressed by different units with respect to different analytical conditions. Furthermore, a comparison of GC and HPLC methods of CC determination is not easy because different approaches are used in each technique. Poor resolution, large analysis time and large volumes of solvent waste can be considered disadvantages of HPLC methods towards GC methods, while the need of vaporizing CC derivatives without degradation prior to GC analysis may represent a disadvantage of many GC methods.

Relatively recent development of liquid chromatographic columns using sub-2-μm particles and ultra-high-pressure chromatographs allowed significant improvements of resolution, analysis speed, detection sensitivity and reduction of solvent waste. Particles size also made achieving lower plate heights over a wider range of higher linear velocities, resulting in better resolution and sensitivity as well as reduced analysis time. Rapid resolution liquid chromatography (RRLC) has been reviewed by Mazzeo et al. [43] and it has gained large analytical importance in several areas [44–47].

This study was initially focused on the comparison of analytical conditions for 15 carbonyl-DNPH derivatives obtained by conventional HPLC and RRLC. In order to compare chromatographic conditions, they were optimized and some analytical features such as resolution, detection limits, quantification limits, linearity and analysis time were evaluated. Initial steps of experimental work were conducted with mobile phases composed of binary mixtures of acetonitrile and ultrapure water that are traditionally used for CC separation.

However, the recent lack of acetonitrile in the world market, stimulated us to study and evaluate other alternatives of mobile phases such as binary, ternary and quaternary mixtures of acetonitrile, methanol, tetrahydrofuran, isopropanol and water. Chromatographic conditions were studied and optimized under these conditions and some analytical features were evaluated. Standard solutions prepared in acetonitrile were used in order to keep the same and widely used conditions of sample preparation described by US-EPA [32]. The best separation chromatographic conditions are presented and discussed here. Data obtained by application of the best analytical conditions in the determination of atmospheric CCs in Niterói City, RJ, Brazil are also presented here. To our knowledge, this is the first application of RRLC for the determination of carbonyl-DNPH derivatives. It is also the first study of atmospheric CCs in Niterói City.

2. Materials and methods

2.1. Reagents and solvents

A standard solution containing 15 carbonyl-DNPH derivatives (hydrazones) in concentrations corresponding to 15 mg L^{-1} of individual carbonyl compounds was purchased from Sigma (MO, USA). Acetonitrile (ACN), methanol (MeOH), tetrahydrofuran (THF) and isopropanol (IsopropOH) (all HPLC grade) were purchased from Tedia, Brazil. Ultra-purified water was prepared through a Simplicity System (Millipore, EUA) following distillation.

2.2. Standard solutions

A standard stock solution of the 15 carbonyl-DNPH derivatives was prepared by dilution of 1.00 mL of the primary standard solution up to 3.00 mL with ACN. Working standards solutions were prepared by dilution of appropriate aliquots of the stock solution to 1.00 mL with ACN.

2.3. Chromatographic analysis

HPLC experiments were carried out in an Agilent 1100 Series (USA) instrument and RRLC experiments of RRLC were performed in an Agilent 1200 Series (USA) instrument. Both chromatographic systems consisted of a binary pump, a degasser, an automated injector, a thermostated column compartment and an UV-DAD detector, which were all controlled by Agilent ChemStations. Detector parameters such as detector slit and response time that directly influence RRLC-UV-DAD detector signals were further optimized. A slit of 4 nm and a response time of 0.05 min led to the best responses considering peak width and form.

Chromatographic conditions (mobile phase composition, flow rate, temperature and injection volume) were studied and optimized with four HPLC columns and two RRLC columns. Injection volumes of 10 μL were usually employed but they were reduced to 3 μL when the narrowest RRLC column was used to avoid column overloading. The best RRLC separations were obtained at 35 °C except where indicated. An equilibration time of 1 min between successive HPLC or RRLC runs was always adopted. Details of column characteristics and dimensions and of instruments used in each set of experiments are summarized in Table 1. Part of the experiments was conducted with mobile phases composed of binary mixtures of ACN and ultrapure water. Other alternatives of mobile phases that are binary, ternary or quaternary mixtures of ACN, MeOH, THF, IsopropOH and water were also evaluated. All solvents were degassed in an ultrasonic bath before use.

2.4. Carbonyl-DNPH derivatives identification and quantification

All hydrazones were detected in 360 nm. Hydrazones were identified by retention times, elution order and their absorption spectra. In some cases, the retention times were compared with those of true standards acquired from Sigma–Aldrich (USA). In order to evaluate detection limits (DLs) and quantification limits (QLs) of hydrazones, calibration solutions containing all studied hydrazones

Table 1
Summary of columns and instruments characteristics used in this study.

Column characteristics	Column dimensions (mm × mm × μm)	Chromatographic technique	Instrument	Acronym of column and chromatographic technique[a]
Vydac C18 (210TP54)	250 × 4.6 × 5	HPLC	Agilent 1100 Series	V-HW
Zorbax 300 SB C18	250 × 4.6 × 5	HPLC	Agilent1100 Series	Z-HW
Supelcosil C18	250 × 4.6 × 5	HPLC	Agilent1100 Series	S-HW
Zorbax XDB C18	150 × 2.1 × 5	HPLC	Agilent1100 Series	Z-HN
Zorbax Eclipse Plus C18	50 × 4.6 × 1.8	RRLC	Agilent 1200 Series	Z-RW
Zorbax Eclipse Plus C18	50 × 2.1 × 1.8	RRLC	Agilent 1200 Series	Z-RN

[a] H = HPLC; R = RRLC; W = wide columns (4.6 mm); N = narrow columns (2.1 mm).

ranged in concentrations between 2.00 and 500 μg L^{-1}. The equations of the calibration curves were obtained by the least-squares regression method and were used to estimate these parameters. DLs and QLs were obtained by dividing respectively three and ten times the signal to noise ratios by the angular coefficients of calibration curves. Signal to noise ratios were estimated by the standard deviations of peak areas obtained after six subsequent injections of the 2.00 or 5.00 μg L^{-1} standards solutions [48].

2.5. Sampling and sample analysis

Air sampling followed the Method EPA TO-11 [32]. Briefly 60 L of air were collected with SPE cartridges impregnated with 2,4-dinitrophenylhydrazine (Waters, USA) and connected in series with a KI ozone denuder (Waters, USA). Samples were collected in the Valonguinho Campus of Federal Fluminense University located in a mixed commercial-residential neighborhood of Niterói City Center. Sampling point faced an eleven lane traffic system. Samples were collected 5 m above ground in open area located at around 200 m of Guanabara Bay margin. Samples were collected in intervals of 2 h (from 08:00 to 21:00) in May 21, 2009 in six sampling periods (8:10–10:10, 10:15–12:15, 12:20–14:20, 14:25–16:25, 16:30–18:30 and 18:35–20:50 h). Hydrazones were extracted from the SPE cartridges by the addition of acetonitrile (5 mL) [32] in reverse direction of sampling. Aliquots of the final solutions were transferred to 2 mL vials and analyzed under the best analytical conditions. Atmospheric concentrations of CCs were calculated considering sampled air volume, mean temperature during sampling (25 °C) and pressure (1 atm).

3. Results and discussion

3.1. Optimization of HPLC separation conditions for the 15 hydrazones

A HPLC instrument (Agilent 1100 Series) was used in this part of the study and four HPLC columns were evaluated (Table 1). The separation of the 15 hydrazones was initially evaluated in a Vydac C18 (250 mm × 4.6 mm × 5 μm) (V-HW) column and a Zorbax 300 SB C18 (250 mm × 4.6 mm × 5 μm; Agilent) (Z-HW) column. No satisfactory sets of conditions for appropriate separation of hydrazones were obtained with both columns and they were discarded for posterior study. A Zorbax Eclipse XDB C18 (150 mm × 2.1 mm × 5 μm; Agilent) (Z-HN) column with the optimized gradient of ACN:water shown in Table 2 allowed satisfactory separation of the 15 studied hydrazones except of those derived of o-, m- and p-tolualdehydes that were only partly resolved. A total analysis time of 26 min was obtained at 28 °C after injection of 5 μL.

A Supelcosil C18 (250 mm × 4.6 mm × 5 μm; Supelco) (S-HW) column, which is designated for the studied separation, showed the best performance among the HPLC columns. A satisfactory resolution of the studied compounds, except of propanone and acrolein hydrazones, was obtained at 30 °C. A mobile phase flow rate of 1.5 mL min^{-1} and a binary elution gradient of ACN (A) and water (B) were used. The gradient was as follows: 65% of A held for 10 min, increased linearly to 66.5% of A during 3 min. An analysis time of 12.5 min was obtained. Fig. 1 shows a typical chromatogram of the 15 hydrazone mixture under these conditions. Although propanone and acrolein hydrazones were not well separated, their resolution obtained under these conditions is comparable or even better than those presented elsewhere [49]. Isomeric tolualdehyde hydrazones were not well resolved as previously observed [49].

Table 2
Optimized gradient used for separation of 15 hydrazones by HPLC. Column Zorbax Eclipse XDB C18 (150 mm × 2.1 mm × 5 μm; Agilent) (Z-HN). T = 28 °C; injected volume = 5 μL.

Time (min)	ACN (%)	Water (%)	Flow rate (mL/min)
0	32	68	1.5
10	32	68	1.5
15	46	54	1.5
20	46	54	1.5
25	60	40	1.6
26	60	40	1.6

3.2. Optimization of RRLC separation conditions for the 15 hydrazones

All subsequent steps of this study were focused on optimizing the separation of the 15 studied hydrazones under RRLC. An Agilent RRLC 1200 Series instrument and two RRLC columns (Table 1) were used. The starting point for the optimization of RRLC separations

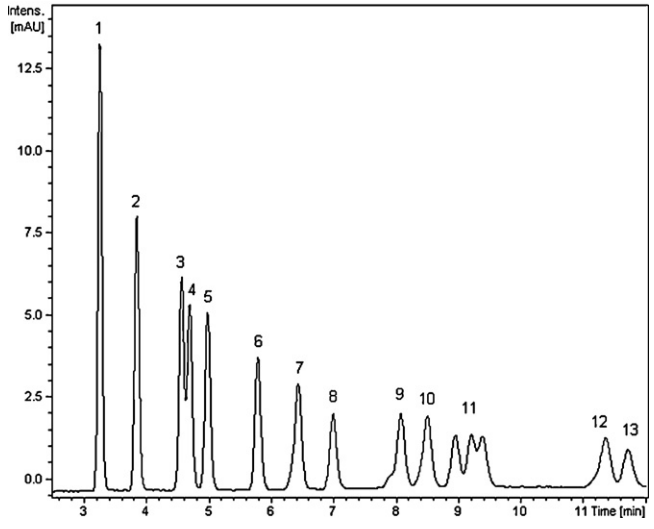

Fig. 1. Chromatogram of a mixture of 15 DNPH derivatives (100 μg L^{-1}) obtained by HPLC, with detection at 360 nm. Column Supelcosil C18 (250 mm × 4.6 mm × 5 μm; Supelco) (S-HW), T = 28 °C and gradient of ACN:water. (1) Formaldehyde; (2) acetaldehyde; (3) propanone; (4) acrolein; (5) propionaldehyde; (6) crotonaldehyde; (7) butyraldehyde; (8) benzaldehyde; (9) isovaleraldehyde; (10) valeraldehyde; (11) o-, m-, p-tolualdehydes; (12) hexaldehyde; (13) 2,5-dimethylbenzaldehyde.

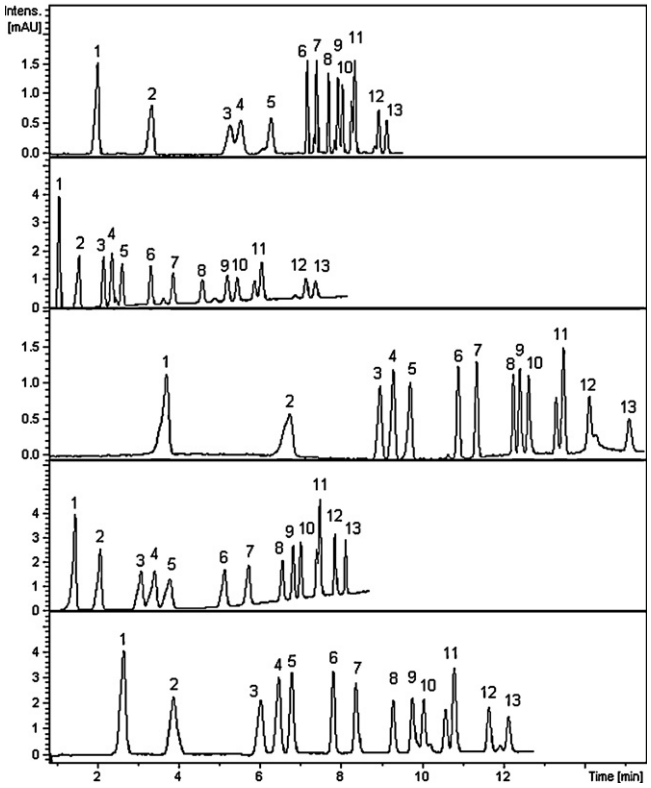

Fig. 2. Chromatograms of a mixture of 15 DNPH derivatives (250 μg L^{-1}) obtained by RRLC, with detection at 360 nm. Column Zorbax Eclipse Plus C18 (50 mm × 4.6 mm × 1.8 μm; Agilent) (Z-RW). Gradients of (a) ACN:water; (b) ACN:water:THF; (c) MeOH:water:THF; (d) MeOH:water:THF:IsopropOH; (e) Water:THF:IsopropOH. Peak identification: hydrazones of (1) formaldehyde; (2) acetaldehyde; (3) propanone; (4) acrolein; (5) propionaldehyde; (6) crotonaldehyde; (7) butyraldehyde; (8) benzaldehyde; (9) isovaleraldehyde; (10) valeraldehyde; (11) o-, m-, p-tolualdehydes; (12) hexaldehyde; (13) 2,5-dimethylbenzaldehyde.

with ACN:water mobile phases was obtained by a software (Agilent) that estimated RRLC conditions from the best HPLC conditions obtained with the S-HW column and this pair of solvents. Other parameters that directly affect chromatographic separation such as column temperature, injection volume, mobile phase composition and flow rate were further studied and optimized. As stated below the recent lack of acetonitrile in the world market stimulated us to investigate hydrazone separation with other solvent systems by RRLC.

Table 3 summarizes the best conditions achieved using the Zorbax Eclipse Plus C18 (50 mm × 4.6 mm × 1.8 μm) (Z-RW) column and different mobile phases. The corresponding chromatograms are shown in Fig. 2. An analysis time of 9.5 min was obtained using a ACN:water gradient and a constant flow rate of 3.0 mL min^{-1} (Table 3 and Fig. 2a). Gradients of ACN:water:THF containing low percentages of THF were developed from these conditions. The presence of THF in mobile phase improved the resolution of propanone and acrolein hydrazones as previously observed [50]. The best set of conditions (Table 3) resulted in an analysis time of 7.5 min (Fig. 2b).

Acetonitrile was completely replaced for methanol, but MeOH:water mobile phases in all proportions and conditions were unable to separate propanone and acrolein hydrazones. Addition of THF to this mobile phase improved their resolution resulting in a chromatogram that was completed in 15.5 min (Fig. 2c) with a flow rate of 2.3 mL min^{-1} of a MeOH:water:THF ternary mobile phase (Table 3). Next, a small percentage of IsopropOH was added to the ternary mobile phase to improve solvent miscibility. The best separation of the 15 hydrazones with a MeOH:water:THF:IsopropOH mobile phase was obtained in 8.5 min with a flow rate of 1.5 mL min^{-1} (Table 3 and Fig. 2d). The polarity of this quaternary mixture indicated the possibility of using MeOH free mobile phases, that is ternary mixtures of water, THF and IsopropOH (Table 3). The best separation of the 15 hydrazones was completed in 13 min with a water:THF:IsopropOH mobile phase and a flow rate of 0.8 mL min^{-1} (Fig. 2e).

Posteriorly, hydrazone separation was studied by RRLC using a Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm) (Z-RN) column. Table 4 summarizes the optimized conditions and the corresponding chromatograms are shown in Fig. 3. A mobile phase composed of ACN:water (Table 4) allowed the best separation of the 15 hydrazones in 6 min at 26 °C with a flow rate of 0.75 mL min^{-1} (Fig. 3a). As pointed before ACN:water:THF mobile phases containing low percentages of THF (Table 4) improved the resolution of propanone and acrolein hydrazones. The best conditions led to an analysis time of 6.5 min (Fig. 3b). Acceptable separations of the 15 hydrazones using this column and MeOH/water mobile phases were also not possible. The best set of conditions obtained with MeOH/water/THF mobile phases (Table 4) and a flow rate of 0.55 mL min^{-1} allowed their complete separation in 11 min (Fig. 3c). Addition of IsopropOH to the mobile phase (Table 4) allowed the best chromatographic separation in 6 min using a MeOH:water:THF:IsopropOH mobile phase and a flow rate of 0.55 mL min^{-1} (Fig. 3d). A MeOH free mobile phase composed of water:THF:IsopropOH (Table 4) allowed the best separation of the 15 hydrazones in 8 min with a flow rate of 0.35 mL min^{-1} (Fig. 3e).

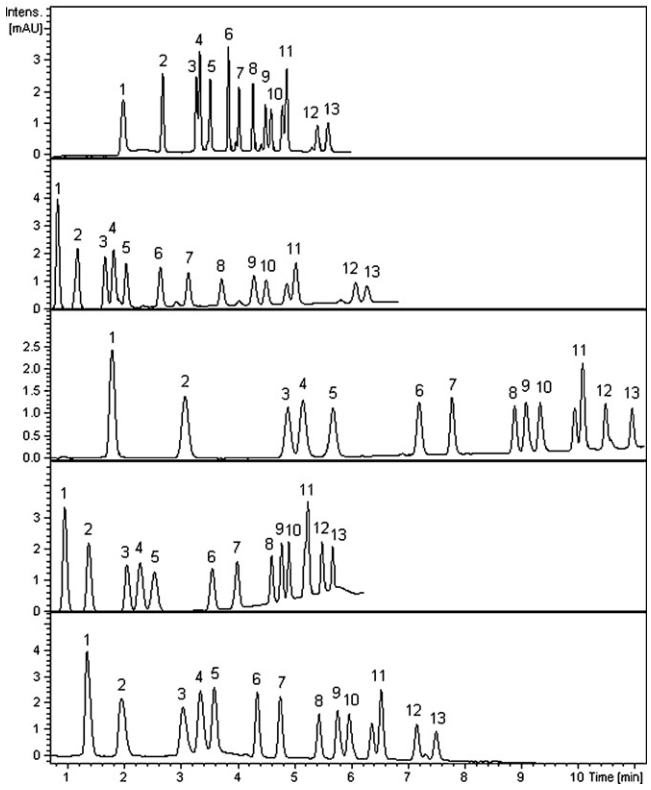

Fig. 3. Chromatograms of a mixture of 15 DNPH derivatives (250 μg L^{-1}) obtained by RRLC, with detection at 360 nm. Column Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm; Agilent) (Z-RN). Gradients of (a) ACN:water; (b) ACN:water:THF; (c) MeOH:water:THF; (d) MeOH:water:THF:IsopropOH; (e) Water:THF:IsopropOH. Peak identification: hydrazones of (1) formaldehyde; (2) acetaldehyde; (3) propanone; (4) acrolein; (5) propionaldehyde; (6) crotonaldehyde; (7) butyraldehyde; (8) benzaldehyde; (9) isovaleraldehyde; (10) valeraldehyde; (11) o-, m-, p-tolualdehydes; (12) hexaldehyde; (13) 2,5-dimethylbenzaldehyde.

Table 3
Summary of RRLC optimized gradients obtained using the Zorbax Eclipse Plus C18 (50 mm × 4.6 mm × 1.8 μm) (Z-RW) column.

Mobile phase solutions		Optimized gradient (%A and analysis time)	Flow rate (mL/min)	Analysis time (min)	Chromatogram
A	B				
ACN	Water	32% A (0–2 min); linear gradient to 35% A (2–3 min); 35% A (3–5 min); linear gradient to 40% A (5–6 min); linear gradient to 45% A (6–6.5 min); linear gradient to 70% A (6.5–7 min); 70% A (7–9.5 min)	3.0	9.5	Fig. 2a
ACN:water (60:40, v/v)	Water:ACN:THF (60:30:10, v/v/v)	15% A (0–0.8 min); linear gradient to 93% A (0.8–7.5 min)	2.5	7.5	Fig. 2b
MeOH:water (95:5, v/v)	Water:MeOH:THF (90:5:5, v/v/v)	30% A (0–5.5 min); linear gradient to 70% A (5.5–12 min); 70% A (12–15.5 min)	2.3	15.5	Fig. 2c
MeOH	Water:IsopropOH:THF (75:15:10, v/v/v)	30% A (0–3.5 min); linear gradient to 80% A (3.5–8 min); 60% A (8–8.5 min)	1.5	8.5	Fig. 2d
IsopropOH:water (90:10, v/v)	Water:THF (95:5, v/v))	30% A (0–4.5 min); linear gradient to 42% A (4.5–6 min); linear gradient to 56% A (6–13 min)	0.8	13.0	Fig. 2e

3.3. General evaluation of data

An overall and critical comparison of different optimized RRLC conditions indicated that all mobile phase compositions led to a better separation of propanone, acrolein and propionaldehyde hydrazones (Figs. 2b–e and 3b–e) than those found using ACN:water mobile phases under RRLC (Figs. 2a and 3a) and HPLC (Fig. 1). Partial overlapping of propanone and acrolein hydrazones peaks could be overcome in several sets of chromatographic conditions after addition of THF to the mobile phases that improved their resolution. This fact can be observed when Figs. 2a and b or 3a and b are compared. THF showed to be crucial in allowing baseline separation of propanone, acrolein and propionaldehyde hydrazones when MeOH:water mobile phases were studied. However, it was observed that high THF concentrations tended to reduce retention times of the heaviest hydrazones leading consequently to poor resolutions.

Incomplete separation of hydrazones derived of the three isomeric tolualdehydes was a major drawback in both RRLC and HPLC optimized conditions. This separation is a difficult task since these aldehydes and their derivatives share very similar polarities. Partial separations of these hydrazones in two groups of tolualdehyde hydrazones were obtained with ACN:water:THF mobile phases in both RRLC columns (Figs. 2b and 3b). MeOH:water:THF (Figs. 2c and 3c) and water:THF:IsopropOH (Figs. 2e and 3e) mobile phases allowed separations of two groups of tolualdehyde hydrazones in both RRLC columns. Some asymmetric peaks were observed when the Z-RW column was employed (Fig. 2c and d) and some small peaks were observed in several RRLC chromatograms (Figs. 2b and d and 3b and d, possibly because the isomeric struc-

Table 4
Summary of RRLC optimized gradients obtained using the Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm) (Z-RN) column.

Mobile phase solutions		Optimized gradient (%A and analysis time)	Flow rate (mL/min)	Analysis time (min)	Chromatogram
A	B				
ACN	Water	30% A (0–3 min); linear gradient to 38% A (3–4 min); 38% A (4–4.5 min); linear gradient to 60% A (4.5–6 min)	0.75	6.0	Fig. 3a
ACN:water (60:40, v/v)	Water:ACN:THF (60:30:10, v/v/v)	15% A (0–0.5 min); linear gradient to 38% A (0.5–6 min)	0.60	6.5	Fig. 3b
MeOH:water (95:5, v/v)	Water:MeOH:THF (90:5:5, v/v/v)	40% A (0–2 min); linear gradient to 50% A (2–5 min); linear gradient to 90% A (5–11 min)	0.55	11.0	Fig. 3c
MeOH	Water:IsopropOH:THF (75:15:10, v/v/v)	30% A (0–2 min); linear gradient to 80% A (2–5 min); linear gradient to 82.5% A (5–6 min)	0.55	6.0	Fig. 3d
IsopropOH:water (90:10, v/v)	Water:THF (95:5, v/v)	30% A (0–1.5 min); linear gradient to 40% A (1.5–2.5 min); linear gradient to 60% A (2.5–6.5 min); 60% A (6.5–8 min)	0.35	8.0	Fig. 3e

Table 5
Comparison of analysis times, mobile phase and ACN consumption rates (mL per run) obtained using each set of optimized conditions.

Columns (mm × mm × μm)	Mobile phase composition	Analysis time (min)	Consumption rates (mL per run)	
			Mobile phase	ACN
Supelco (250 × 4.6 × 5)(S-HW)	ACN:water	12.5	21.0	13.7
Zorbax (150 × 2.1 × 5) (Z-HN)	ACN:water	26.0	40.5	17.6
Zorbax (50 × 4.6 × 1.8) (Z-RW)	ACN:water	9.5	31.5	13.5
	ACN:water:THF	7.5	21.3	9.1
	MeOH:water:THF	15.5	35.7	0
	MeOH:water:THF:IsopropOH	8.5	14.3	0
	Water:THF: IsopropOH	13.0	11.2	0
Zorbax (50 × 2.1 × 1.8) (Z-RN)	ACN:water	6.0	6.8	2.7
	ACN:water:THF	6.5	4.5	2.0
	MeOH:water:THF	11.0	6.1	0
	MeOH:water:THF:IsopropOH	6.0	3.9	0
	Water:THF: IsopropOH	8.0	3.2	0

tures of derivatives are not well resolved in C18 columns, as previously observed [51].

A comparison of some parameters (analysis time, mobile phase and ACN consumption rates) obtained under RRLC and HPLC optimized conditions is shown in Table 5. All aspects of optimized conditions observed by conventional HPLC using the S-HW column were better than those obtained using the Z-HN column. For example, analysis time and mobile phase consumption found using the S-HW column were nearly the half of those found with the Z-HN column. Therefore, all comparisons of RRLC and HPLC considered only data and conditions obtained with the S-HW column.

Analysis time by RRLC using the Z-RW column varied between 7.5 and 15.5 min (Fig. 2). The shortest time (7.5 min) was obtained with a mobile phase composed of ACN:water:THF followed by those found with MeOH:water:THF:IsopropOH (8.5 min) and ACN:water (9.5 min) mobile phases. These conditions allowed an increase of method throughput when compared with that obtained by conventional HPLC using the S-HW column. The other two mobile phases showed analysis time (13.0 or 15.5 min) longer than that obtained by HPLC using the S-HW column (12.5 min) (Figs. 1 and 2).

Analysis time by RRLC using the Z-RW column varied between 6.0 and 11.0 min (Fig. 3) increasing method throughput in all optimized conditions when compared to that obtained by HPLC using the S-HW column (12.5 min) (Fig. 1). MeOH:water:THF mobile phase allowed a less significant analysis time reduction because hydrazone separation was completed in 11.0 min by RRLC. However, all other conditions obtained by RRLC using the Z-RN column led to more significant analysis time reduction because the separations were completed in 6.0–8.5 min. As a consequence, method throughput under RRLC can be almost doubled relative to conventional HPLC.

In liquid chromatographic analysis solvent waste that is a function of mobile phase consumption rate, should be considered in method development and application. RRLC separations using the Z-RW column led to mobile phase consumption rates (11.2–31.5 mL per run) that are comparable to that found by HPLC using the S-HW column (21.0 mL per run). However, dramatic reductions of mobile phase consumption rates (between 3.2 and 6.8 mL per run) were observed with the narrowest Z-RN column. Furthermore, addition of THF and/or IsopropOH to the mobile phases helped to reduce mobile phase consumption rates when both RRLC columns were used (Table 5).

ACN consumption rates were compared and showed to be different under HPLC and RRLC. In fact, a reduction of ACN consumption to zero was found when ACN free mobile phases were used. A reduction of ACN consumption rate of almost 4 mL per run was observed when HPLC optimized conditions using the S-HW (13.7 mL per run) and the Z-HN column (17.6 mL per run) were compared. Using RRLC, the Z-RW column led to the largest ACN consumption rates that varied between 9.1 (ACN:water:THF mobile phase) and 13.5 mL per run (ACN:water mobile phase). This value is comparable to that found by HPLC using the S-HW. The Z-RN column led to ACN consumption rates between 2.0 (ACN:water:THF mobile phase) and 2.7 mL per run (ACN:water mobile phase). Addition of THF to the mobile phases reduced ACN consumption rates using RRLC.

The columns and the conditions of separation were compared considering some criteria: analysis time (min), mobile phase and

Table 6
Scores for comparison of columns and optimized conditions according to different criteria: analysis time (min), mobile phase and ACN consumption rates (mL per run), peak symmetry, resolutions of hydrazones derived of propanone, acrolein and propionaldehyde (C_3 CC) and resolution of tolualdehyde hydrazones.

Columns	Mobile phases	Time (min)	Peak symmetry	Consumption rates[a]		Resolution of hydrazones		Final scores
				Mobile phase	ACN	C_3 CC	Tolualdehyde	
Supelco (250 × 4.6 × 5)(S-HW)	ACN:water	1	2	0	0	0	3	6
Zorbax (50 × 4.6 × 1.8) (Z-RW)	ACN:water	2	1	0	0	0	2	5
	ACN:water:THF	2	2	0	1	3	2	10
	MeOH:water:THF	0	0	0	3	3	2	8
	MeOH:water:THF:IsopropOH	2	1	1	3	3	0	10
	Water:THF: IsopropOH	1	3	1	3	3	2	13
Zorbax (50 × 2.1 × 1.8) (Z-RN)	ACN:water	3	3	2	2	0	1	11
	ACN:water:THF	3	3	3	2	2	2	15
	MeOH:water:THF	1	3	2	3	3	2	14
	MeOH:water:THF:IsopropOH	3	3	3	3	3	0	15
	Water:THF: IsopropOH	2	3	3	3	3	2	16

[a] mL per run.

ACN consumption rates (mL per run), peak symmetry, resolutions of hydrazones derived of propanone, acrolein and propionaldehyde and resolution of tolualdehyde hydrazones. Scores ranging from 0 to 3 were attributed to these criteria (Table 6). The conditions obtained using the Z-HW column were not included in this comparison because they were worse than those obtained using the S-HW column as pointed before. Comparison made in both Tables 5 and 6 indicates that RRLC has several advantages over HPLC such as reduction of analysis time and reductions of mobile phase and ACN consumption rates (mL per run). Moreover, the incomplete separation of propanone and acrolein hydrazones represented a major drawback under HPLC conditions. An advantage of this set of HPLC conditions over all other conditions is the (partial) separation of tolualdehyde hydrazones (Fig. 1).

A comparison of RRLC optimized conditions indicates that the Z-RN column presents several advantages when compared to the Z-RW column, such as reductions in analysis time and in consumption rates of ACN and mobile phase. As consequence almost all sets of optimized conditions using the Z-RN column were very well ranked (Table 6). Water:THF:IsoproOH and MeOH:water:THF:IsoproOH gradients were considered the best suited for separation of CC hydrazones. The Z-RN column and the quaternary gradient (MeOH:water:THF:IsopropOH) were chosen to sample evaluation because they allowed the fastest chromatograms and consequently the highest throughput method. Finally, these results indicate that RRLC methods represent a viable alternative that show several comparative advantages over HPLC methods of determination of CCs.

3.4. Evaluation of analytical features of the optimized sets-ups of analytical conditions

DLs and QLs of hydrazones were evaluated by HPLC considering the separation obtained for the S-HW column and ACN:water mobile phase and by RRLC with respect to all sets of optimized conditions for the Z-RW and Z-RN columns (Table 7). Calibration lines of the evaluated hydrazones showed very good linear relationships with standard solutions concentrations, as expressed by their correlation coefficients (>0.998) in the studied range. DLs shown in Table 7 were expressed in ppbv of CC (nL of CC per L of air) taking into account the Ideal Gas Equation, sampling and extraction conditions (Section 2.5), and temperature and pressure during sampling. The DLs obtained by HPLC conditions varied between 0.04 and 0.13 ppbv. These values are comparable to the values of 0.07–0.09 ppbv found by Pal et al. [15] and to values compiled or found by Carvalho et al. [6], which varied widely depending of the CC and of the study. A comparison of DLs obtained by RRLC and HPLC (Table 7) showed that all DLs obtained by RRLC using the Z-RW column varied between 0.01 and 0.25 ppbv. The worst and widest range of DLs was obtained using MeOH:water:THF mobile phase possibly as a consequence of some baseline fluctuation. DLs obtained using the Z-RN column varied between 0.01 and 0.09 ppbv with a range of values lower than a magnitude order and with the largest DLs corresponding to the lightest CCs. These DLs are comparable with those previously obtained by very sensitive methods such as GC–MS–SIM [10] and HPLC–ESI–MS [7].

The UV detector responds to concentration. Thus analytical DLs found from analytical curves are expressed in concentrations ($\mu g\,L^{-1}$ or $nmol\,L^{-1}$). The injected mass of CCs corresponding to these DLs can be calculated by multiplying the DLs ($\mu g\,L^{-1}$) and the injected volumes in the Z-RN or Z-RW columns (3 or 10 μL respectively). Masses as low as 0.5–10 pg were found using the Z-RN column while a wider range of 2.5–70 pg was found using the Z-RW column. The most sensitive conditions, with DLs ranging between 1.2 and 4.0 pg were found using the Z-RN with a MeOH:water:THF:IsoproOH mobile phase that depending of the

Table 7
Detection limits (DLs) (ppbv and pg) of the carbonyl compounds obtained under optimized conditions by HPLC using the Supelco (250 mm × 4.6 mm × 5 μm—S-HW) column and by RRLC conditions using the Zorbax Eclipse Plus C18 columns (50 mm × 4.6 mm × 1.8 μm—Z-RW and 50 mm × 2.1 mm × 1.8 μm—Z-RN).

Hydrazones derived of	S-HW	Z-RW			Z-RN					Ranges
	ACN/water	ACN/water	ACN/water/THF	MeOH/water/THF	MeOH/water/THFIsopropOH	THF/water/IsopropOH	ACN/water	ACN/water/THF	MeOH/water/THF	MeOH/water/THFIsopropOH	THF/water/IsopropOH	
Formaldehyde	0.10[a] (15)[b]	0.07 (9.6)	0.15 (23)	0.14 (21)	0.02 (2.6)	0.02 (2.9)	0.04 (1.9)	0.03 (1.4)	0.07 (3.2)	0.06 (2.4)	0.05 (2.4)	0.02–0.15 (1.4–23)
Acetaldehyde	0.04 (9.0)	0.06 (12)	0.10 (22)	0.11 (23)	0.02 (4.1)	0.02 (4.0)	0.05 (3.0)	0.05 (3.3)	0.05 (3.1)	0.04 (2.4)	0.04 (2.5)	0.02–0.11 (2.4–23)
Propanone	0.08 (21)	0.09 (24)	0.09 (24)	0.09 (25)	0.06 (17)	0.02 (6.5)	0.09 (7.1)	0.04 (3.2)	0.03 (2.6)	0.01 (1.2)	0.03 (2.4)	0.01–0.09 (1.2–25)
Acrolein	0.06 (18)	0.08 (24)	0.09 (24)	0.12 (33)	0.04 (10)	0.02 (6.9)	0.07 (5.7)	0.04 (3.2)	0.03 (2.6)	0.01 (1.2)	0.03 (2.6)	0.01–0.12 (1.2–33)
Propionaldehyde	0.12 (33)	0.07 (19)	0.08 (23)	0.12 (35)	0.02 (6.4)	0.03 (8.4)	0.05 (4.4)	0.03 (2.7)	0.03 (2.3)	0.02 (1.9)	0.02 (1.7)	0.02–0.12 (1.7–35)
Crotonaldehyde	0.05 (18)	0.06 (21)	0.12 (40)	0.22 (74)	0.05 (17)	0.04 (12)	0.04 (4.1)	0.03 (3.1)	0.04 (4.6)	0.01 (1.3)	0.04 (4.2)	0.01–0.22 (1.3–40)
Butyraldehyde	0.13 (45)	0.06 (22)	0.09 (33)	0.09 (31)	0.03 (11)	0.04 (13)	0.06 (6.3)	0.02 (1.9)	0.01 (1.2)	0.04 (4.0)	0.03 (3.7)	0.01–0.09 (1.2–40)
Benzaldehyde	0.12 (63)	0.05 (26)	0.03 (16)	0.13 (68)	0.03 (17)	0.03 (17)	0.06 (9.7)	0.03 (5.1)	0.03 (5.1)	0.01 (1.2)	0.02 (3.1)	0.01–0.13 (1.2–68)
Isovaleraldehyde	0.07 (30)	0.08 (34)	0.06 (24)	0.12 (53)	0.05 (21)	0.01 (5.7)	0.08 (9.8)	0.03 (3.6)	0.02 (2.5)	0.01 (1.2)	0.01 (0.5)	0.01–0.12 (0.5–53)
Valeraldehyde	0.10 (42)	0.07 (30)	0.07 (30)	0.08 (34)	0.03 (14)	0.01 (5.3)	0.05 (6.5)	0.03 (3.8)	0.02 (2.7)	0.01 (1.6)	0.01 (1.5)	0.01–0.08 (1.5–42)
O-, m-, p-tolualdehydes	0.06 (36)	0.03 (16)	0.01 (6.6)	0.06 (38)	0.02 (9.3)	0.01 (6.2)	0.02 (3.4)	0.02 (2.7)	0.01 (2.1)	0.01 (1.3)	0.02 (3.8)	0.01–0.06 (1.3–38)
Hexaldehyde	0.09 (42)	0.04 (20)	0.04 (19)	0.11 (53)	0.03 (14)	0.03 (13.1)	0.03 (5.1)	0.02 (2.8)	0.02 (2.4)	0.01 (1.3)	0.02 (3.3)	0.01–0.11 (1.3–42)
2,5-Dimethylbenzaldehyde	0.11 (75)	0.03 (22)	0.12 (81)	0.25 (166)	0.02 (13)	0.01 (5.3)	0.03 (6.2)	0.02 (3.2)	0.01 (2.4)	0.01 (1.6)	0.03 (5.5)	0.01–0.25 (1.3–166)
Ranges	0.04–0.13 (9.0–75)	0.03–0.09 (9.6–34)	0.03–0.12 (6.6–81)	0.03–0.25 (21–166)	0.03–0.09 (2.6–21)	0.01–0.04 (2.9–17)	0.03–0.09 (1.9–9.8)	0.02–0.05 (1.4–5.1)	0.01–0.07 (1.2–5.1)	0.01–0.06 (1.2–4.0)	0.01–0.05 (0.5–5.5)	

[a] Detection limits expressed in ppbv (volume of carbonyl compound in air volume).
[b] Detection limits expressed in pg.

CC, is comparable to previous values found by HPLC–MS [40]. These results indicate the good sensitivity of RRLC to CCs determination.

Nevertheless their expression, DLs found using RRLC indicate that application of this technique is a viable task that allows the determination of carbonyl compounds in environmental samples after derivatization to hydrazones using usual sampling and sample preparation conditions [32]. According to these results this technique allowed to obtain better DLs and improved sensitivity when compared to HPLC. Moreover, many RRLC chromatograms showed very narrow peaks and in some cases a resolution that indicated an opportunity for the improvement of the method and the determination of other hydrazones, as well.

3.5. Application

Air samples were collected during 2 h intervals (08:00–21:00) in May 21st, 2009, following the Method EPA TO-11 [32]. Hydrazones were eluted from SPE cartridges by the addition of ACN (5 mL) [32]. Part of final solutions were transferred to 2 mL vials and analyzed by RRLC using the Z-RN column (Zorbax Eclipse Plus C18; 50 mm × 2.1 mm × 1.8 μm), a mobile phase composed of MeOH:water:THF:IsoproOH (Table 4) and detection at 360 nm. Calibration curves were obtained with standards solutions containing all CCs and concentrations ranging between 2.00 and 500 $\mu g L^{-1}$. Blanks run in parallel showed no significant concentrations of CC.

Individual CC concentrations are shown in Fig. 4a and b. Formaldehyde (8.22–9.78 ppbv) predominated in all periods followed by acetaldehyde (1.77–3.99 ppbv) and propanone (1.89–3.26 ppbv). Propionaldehyde (<0.02–0.89 ppbv) and butyraldehyde (<0.04–0.93 ppbv) showed comparable concentrations (Fig. 4a). Acrolein and crotonaldehyde were not detected. Benzaldehyde (<0.01–3.04 ppbv) predominated among heaviest CCs although isovaleraldehyde (<0.01–1.39 ppbv) and hexaldehyde

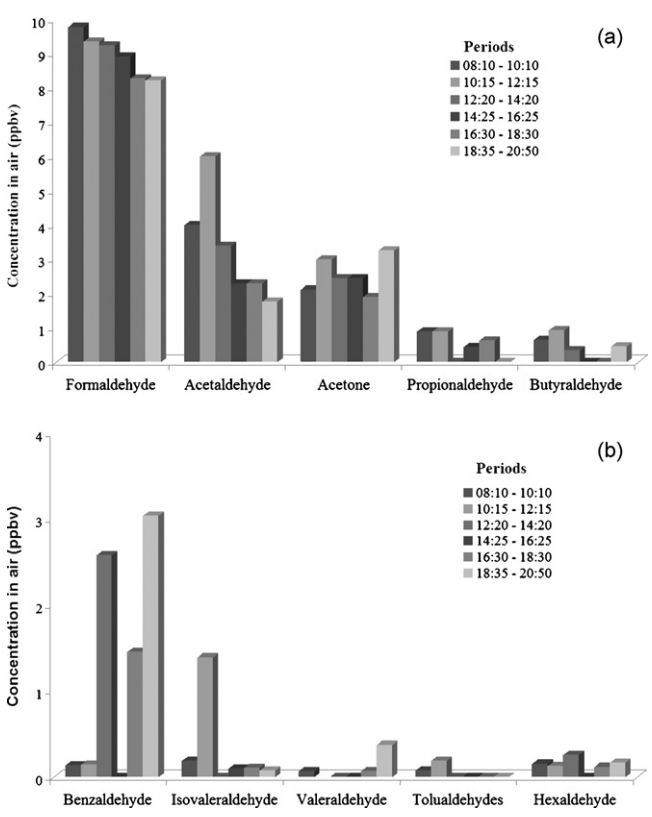

Fig. 4. Variation of individual CC concentrations in air samples (ppbv) in different periods of the studied day (a) C_1–C_4 CCs and (b) >C_5 CCs.

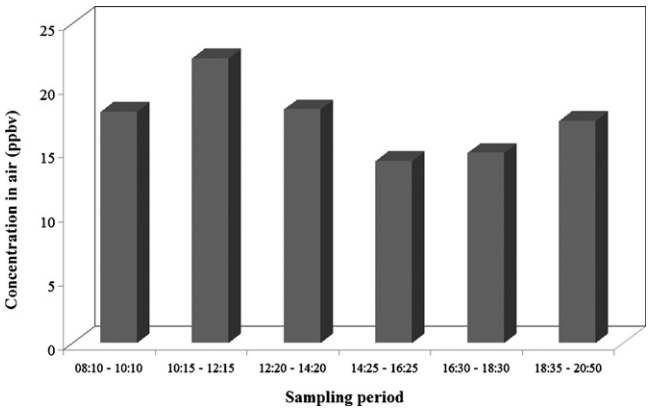

Fig. 5. Variation of total CC concentrations in air samples (ppbv) in different periods of studied day.

(<0.01–0.26 ppbv) were also found in most samples. Other heavier CCs (valeraldehyde and tolualdehye) were found in low concentrations in some samples while dimethylbenzaldehyde (0.22 ppbv) was oserved only in the sample collected between 10:15 and 12:15 (Fig. 4b). As far as we are concerned, some CCs are being reported here for the first time in the atmosphere of the Metropolitan Area of Rio de Janeiro City and this is the first study of CCs in the atmosphere of Niterói City, RJ, Brazil.

Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde and valeraldehyde showed lower concentrations (10–33%) than those recently reported to an area of high automotive traffic of Rio de Janeiro City [36]. A comparison of data obtained in this study and those found in studies carried out in other countries [15–17] indicates that concentrations of CCs are lower than those found in industrial areas of Korea [15]. Concentrations of formaldehyde found in Santiago, Chile [16] (3.9 ± 1.4 ppbv) were lower than these found in this study. Butyraldehyde (3.3 ± 3.4 ppbv) that showed comparable concentrations with acetaldehyde (3.0 ± 0.9 ppbv) and propanone (2.5 ± 1.0 ppbv) in Santiago [16] was found in relatively low concentrations in Niterói. When compared to our data, low concentrations of formaldehyde were also found in Hong Kong [17], although acetaldehyde concentrations were higher than those found in Niterói City. The predominance of formaldehyde towards acetaldehyde in Brazilian areas has been attributed to the disseminated use of compressed natural gas as an automotive fuel [36].

Total CC concentration varied between 14.20 and 20.25 ppbv (Fig. 5) with a maximum corresponding to the period between 10:15 and 12:15 h. A subsequent decrease with a minimum corresponding to the period between 14:25 and 16:25 h and a posterior increase of total CC concentrations were also observed. This fluctuation is typical of total atmospheric CC concentrations because it is very influenced by automotive traffic. These results were lower than those found in Rio de Janeiro City [36], indicating that Niterói is a less polluted area according to this criterion.

4. Conclusions

The present study showed that a number of conditions can be optimized using different solvent compositions (binary, ternary and quaternary mixtures of acetonitrile, methanol, tetrahydrofuran, isopropanol and water) to separate a mixture of 15 hydrazones of carbonyl compounds by RRLC. Most of these conditions show several advantages when compared to those found using a specific HPLC column (S-HW) that is designed for this separation. Incomplete or lack of separation of tolualdehyde hydrazones represented a several drawback in almost all chromatographic conditions.

Gradients of MeOH:water:THF:IsoproOH and water:THF:IsoproOH run using RRLC and a Zorbax Eclipse Plus C18 (50 mm × 2.1 mm × 1.8 μm) column provided the best analytical conditions, considering parameters such as analysis time, peak symmetry, ACN and mobile phase consumption rates and resolutions of propanone, acrolein and propionaldehyde and of the tolualdehyde isomers. Our results showed that RRLC can be applied in CCs determination in air without the need of any change in the usual EPA TO-11 sampling conditions.

The application of the developed method to air samples collected in Central Area of Niterói, RJ, Brazil allowed the quantification of 12 individual CC and of tolualdehyde isomers as a group. CC concentrations varied between values below their individual detection limits (0.01 and 0.09 ppbv) to 9.78 ppbv. Formaldehyde (8.22–9.78 ppbv) and acetaldehyde (1.77–3.99 ppbv) predominated among CCs. Total CCs varied between 14.20 and 20.25 ppbv with a maximum corresponding to the period between 10:15 and 12:15 h. These results are lower than those found in Rio de Janeiro City, indicating a less polluted area when this criterion is considered. As far as we know this is the first report of CC in medium Brazilian Cities.

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