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Liquid chromatographic methods for chloral hydrate determination

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Abstract

Liquid chromatographic methods, based on reversed-phase (RP) and anion-exchange mechanisms, have been developed for chloral hydrate determination. Both methods are preceeded by derivatization of chloral hydrate. For RP separations, different reagents [namely dansylhydrazine and *o*-(4-nitrobenzyl)hydroxylamine] have been studied, but the best results have been achieved using 1,2-benzenedithiol with UV detection at 220 nm. The anion-exchange method is based on derivatization with NaOH to form sodium formate that is then analyzed by anion-exchange, with suppressed conductivity detection. Derivatization conditions were optimized in order to reach the best yield of reaction. The optimization of the procedure allowed to determine chloral hydrate with detection limits as low as 0.2 μg/l with good linearity and reproducibility. The anion-exchange method was also applied for chloral hydrate determination in a drinking water sample. A preconcentration procedure has also been studied.

Keywords: Water analysis; Derivatization, LC; Chloral hydrate; Formate; Nitrobenzylhydroxylamine; Dansylhydrazine; Benzenedithiol

1. Introduction

Chloral hydrate is the stable product of hydration of trichloro acetaldehyde, which is poorly stable in aqueous solutions. First synthesized in 1832, it has very pungent odour and bitter taste and it is commonly added illicitly to alcoholic beverages, increasing their potency [1].

At therapeutic doses, chloral hydrate is an excellent hypnotic, while toxic doses induce severe respiratory depression and very low blood pressure. In drinking waters, chloral hydrate is present as chlorination disinfection by-product during water treatment. Following the drinking water regulations and health advisories, chloral hydrate has been included in the possible human carcinogen group. According to US Environmental Protection Agency (EPA) regulations, up to 1999, the maximum contaminant level and the maximum contaminant level goal for chloral hydrate are 60 and 40 μg/l, respectively [2].

According to EPA indications, chloral hydrate in drinking waters is analyzed after liquid–liquid extraction by gas chromatography with electron-capture detection. Most of the analytical methods existing for chloral hydrate determination are based on gas chromatography [3,4] and electrochemistry. A combination between a polarographic procedure with an amplification reaction for determination of chloral hydrate has been presented by Sulaiman and Amin [5]. This method, based on redox reactions with iodine to form iodide and then iodate allowed the determination of $6 \cdot 10^{-6}$ *M* chloral hydrate by indirect measurement.

Current literature does not provide LC methods for determination of chloral hydrate. Nevertheless, chloral hydrate has been evidenced with LC by Husain et

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al. [6] during the monitoring of trichloroacetic acid process.

In the last few years, our research group focused particular attention on drinking water contaminants, and expecially on the development of LC methods for the determination of by-products [7] and pollutants [8] coming from disinfection processes during water treatments. As a prosecution of drinking water monitoring, the aim of this work was to study LC (reversed-phase and anion-exchange based) methods for chloral hydrate determination. The methods are based on pre-column derivatization reactions. The best results have been reached through the formation of formate, when NaOH is added to a chloral hydrate solution, and through the analysis by anion-exchange chromatography. To lower the detection limits, a preconcentration step has been coupled. The method developed has been applied to the chloral hydrate determination in a drinking water sample, verifying the suitability of the technique optimized through this work.

2. Experimental

2.1. Chromatographic system

The chromatographic system used for reversed-phase measurements was a Varian (Walnut Creek, CA, USA) Model 9010 pump equipped with a Rheodyne injector with a 100 μl sample loop and a Model 332 UV–Vis variable-wavelength detector (Kontron Instruments, Milan, Italy). The chromatograms were recorded by an Axxiom Chromatography (Calabasas, CA, USA) Model 727 data station. The column used were LiChrospher 100 RP-18 end-capped 10 μm (250×4 mm I.D.), and LiChrocart RP-18 as guard column, 5 μm (4×4 mm I.D.) all by Merck (Darmstadt, Germany). Dead volume, 2.2 ml, was evaluated by the negative peak due to water.

For ion chromatographic measurements, the apparatus consisted of a model QIC ion chromatograph (Dionex, CA, USA) equipped with a single piston DQP pump. The volume of the sample loop was 200 μl. The analytical columns included a guard column IonPac AG11 (50×4 mm I.D.) and a separator column IonPac AS11 (250×4 mm I.D.) from Dionex. The resin of the columns is composed of 13 μm polystyrene–divinylbenzene substrate agglomerated with a completely aminated anion-exchange latex. The ion-exchange capacity is approximately 45 μeq/column. Eluent solutions contained NaOH at proper concentration. Detection was achieved by a conductivity detector. Eluent conductivity has been suppressed by an Anion MicroMembrane Suppressor (AMMS-II). The regenerant solution was a 25 m*M* sulfuric acid solution. A chromatographic data system (AI-450, Dionex) was used for data collection and processing. Chromatograms were recorded at room temperature. Retention times represent the average values of at least three injections. Dead volume, 1.8 ml, was evaluated by the negative peak due to water.

Eluent flow rate was set, in both cases, at 1 ml/min. For sample pretreatment, OnGuard-H (Dionex) disposable cartridges, containing a cation-exchange sulfonic acid resin in the hydrogen form, have been used.

2.2. Reagents and solutions

Chloral hydrate, *o*-(4-nitrobenzyl)hydroxylamine hydrochloride, acetonitrile were from Fluka (Buchs, Switzerland), 1,2-benzenedithiol, sodium hydroxide, sodium hydrogencarbonate, sulphuric acid, dodecyl sulfate sodium salt, dansylhydrazine were Merck products, formic acid, sodium carbonate anhydrous and sodium tetraborate were from Aldrich (Milwaukee, WI, USA), while ammonium chloride was from Carlo Erba (Milan, Italy).

All aqueous solutions were prepared by high purity water obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Eluents were filtered through 0.22-μm filters before use.

3. Results and discussion

Preliminary work has been done analyzing chloral hydrate by RPLC, using a mobile phase containing 15 m*M* aqueous NH_4Cl–CH_3CN (80:20). At these conditions, chloral hydrate eluted at 5.1 min. The calibration plot obtained for chloral hydrate solutions of 1.7, 3.3, 6.6, 8.3 and 16.5 mg/l had a correlation coefficient $r^2=0.997$. Due to its low absorbance,

detection limit, evaluated at 201 nm, was 1.7 mg/l (0.01 m*M*).

3.1. Derivatization reactions

3.1.1. Reversed phase ion interaction chromatography

In order to lower the detection limit for chloral hydrate determinations, some derivatization reactions have been studied. Liquid chromatographic analysis of aldehydes can be performed through their derivatization and spectrophotometric detection with compounds containing hydrazine groups [9,10]. The derivatization of chloral hydrate with dansylhydrazine and *o*-(4-nitrobenzyl)hydroxylamine performed at controlled temperature conditions and at different pH values did not evidence the formation of a chromatogarphic peak in addition to that of the excess of reagent, thus confirming that the carbonylic group of trichloroacetaldehyde in water solution is completely hydrated and not available for formation of hydrazones.

A reaction between –SH and C=O groups has been exploited further. A 2.42 m*M* 1,2-benzenedithiol solution has been reacted for 1 h at 70°C with an equimolar solution of chloral hydrate. Reaction product obtained and absorption spectra of blank and derivatized solutions are shown in Fig. 1. Solutions of blank and derivatized chloral hydrate have been acidified at pH 3 in order to protonate the sulfur atoms of the adduct formed and then analyzed by reversed-phase ion interaction chromatography with a mobile phase containing acetonitrile–water (40:60, v/v) and 9 m*M* NaCl, 6 m*M* sodium dodecyl sulfate, 19 m*M* HCOOH, pH 3 (Fig. 2). It has been noted that the peak area of derivatized chloral hydrate remains stable with time, while 1,2-benzenedithiol reduces its peak area.

The derivatization reaction does not occur at lower temperature and it is sensitive to concentrations of chloral hydrate; in fact, concentrations of chloral hydrate of 20 mg/l do not react neither with an equimolar solution nor with a ten fold excess of 1,2-benzenedithiol. Since this concentration is relatively high for trace analysis purposes, the system has not further been studied.

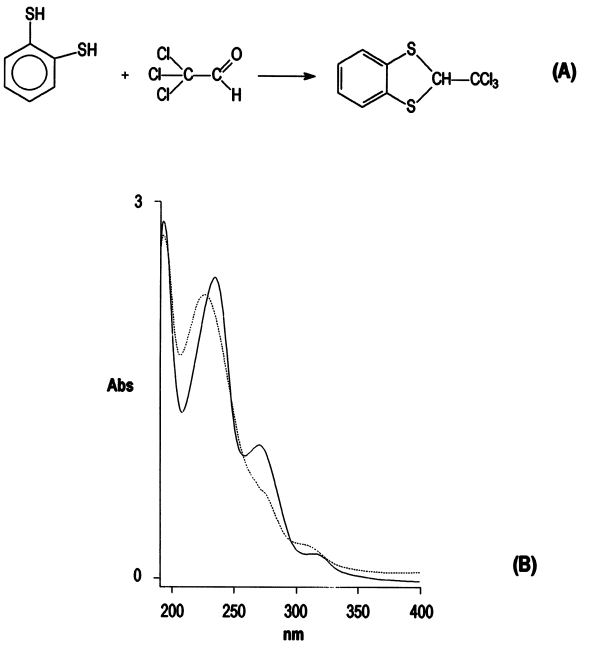

Fig. 1. (A) Derivatization of trichloroacetaldehyde with 1,2-benzenedithiol. (B) Absorption spectra (reference water) for 0.14 m*M* 1,2-benzenedithiol (solid line) and 20 mg/l chloral hydrate derivatized (dotted line). Reaction conditions: 2.42 m*M* 1,2-benzenedithiol and 400 mg/l chloral hydrate. 1 h at 70°C.

3.2. Anion-exchange chromatography

The derivatization of chloral hydrate and the subsequent IC determination has been performed exploiting an alkaline hydrolysis [11] reaction. A NaOH solution was used. This reaction, shown in Fig. 3, forms sodium formate and chloroform. Formate has been then detected by suppressed anion-exchange chromatography. Reaction has been performed in 5 m*M* NaOH at different concentration of chloral hydrate in the range: 2–5 mg/l. A blank was also prepared. Blank and chloral hydrate solutions have been reacted for 1 h at 65°C. In order to reduce the NaOH contribution to the detection, an OnGuard-H cartridge for the neutralization of blank and chloral hydrate solutions has been used. Acidification through cartridges avoids introduction of further ionic species that could interfere in the chromatogram. Solutions have been then injected and eluted on an IonPac AS11 by 2.5 m*M* NaOH. The chromatogram evidenced the formation of two peaks at

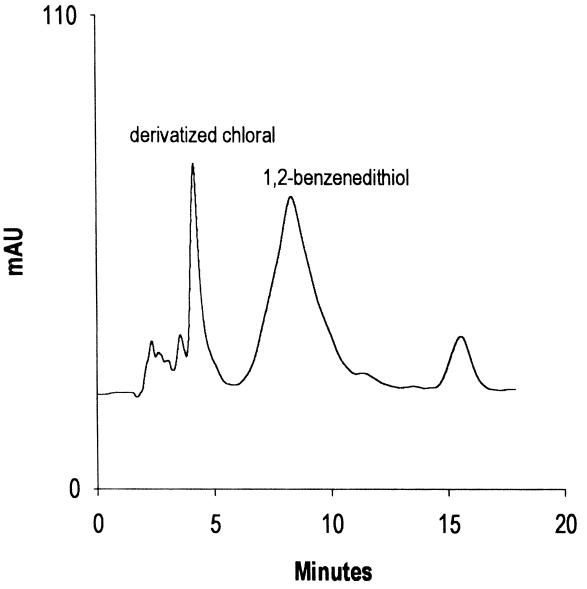

Fig. 2. RP ion interaction separation of derivatized chloral hydrate. Reaction conditions: 1,2-benzenedithiol and chloral hydrate 1 h at 70°C. Column: LiChrospher 100 RP-18 endcapped. Eluent: CH_3CN–water (40:60 v/v), 9 m*M* NaCl, 6 m*M* sodium dodecyl sulfate, 19 m*M* HCOOH, aqueous pH 3. Sample loop: 100 μl. Detection: UV 220 nm. Sample: 0.24 m*M* chloral hydrate in 0.22 m*M* 1,2-benzenedithiol.

1.8 and 2.4 min. By addition of known amounts of sodium formate in the reacted solution, the first peak has been attributed to $HCOO^-$ ion. The second peak has been attributed to chloride ions due to dichlorocarbene formed by chloroform in presence of OH^- medium. Derivatized solution is stable also after 1 week after reaction and does not need particular storing conditions.

In order to get the best yield of reaction, derivatization has been performed at different experimental conditions. If reaction is performed at ambient temperature, its yield is only 32%, while if chloral hydrate standard solution is injected as such without NaOH derivatization, the yield is the 27% of that performed in NaOH at controlled temperature.

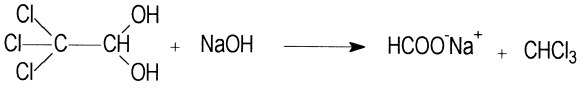

Fig. 3. Derivatization reaction of chloral hydrate with sodium hydroxide.

In order to increase peak response, the effect of NaOH concentration has been studied at different chloral hydrate concentrations (2, 4, 5, 10 mg/l), as shown in Fig. 4. A concentration of 10 m*M* NaOH has been chosen as optimal for chloral hydrate derivatization. In Fig. 5 the derivatization of 200, 400, 800 μg/l chloral hydrate is shown at the conditions optimized. As shown in Fig. 5A, the chromatogram of blank provides a signal that interfere with the peak of formate. Correlation coefficients (r^2) for formate and chloride peaks were respectively 0.999 and 0.998. Relative standard deviation calculated on three different derivatization reactions was 6.1%.

In order to improve the separation conditions and to avoid the interference for chloral hydrate determination, experiments on tetraborate eluents have been performed. Fig. 6 shows the dependence of chloral hydrate retention time as a function of tetraborate concentration (2.5–10 m*M*) in the eluent. As expected, at the lowest eluent concentration chloral hydrate is sufficiently separated from the void volume; good reproducibility and linearity (r^2= 0.9998 for 0.6–1.0 mg/l chloral hydrate) are also

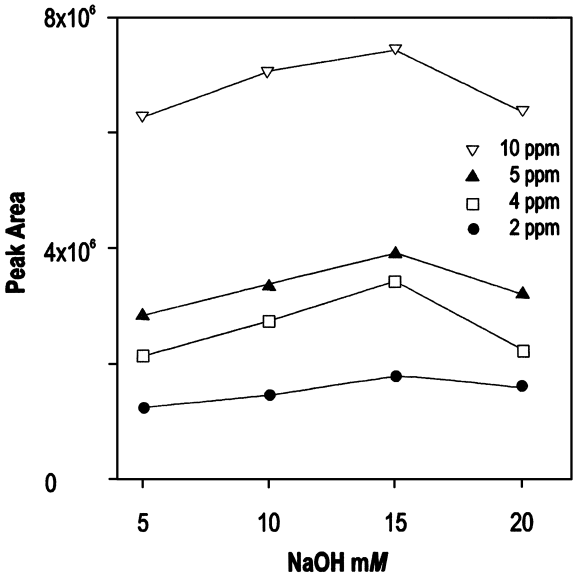

Fig. 4. Effect of NaOH concentration on the yield of reaction chloral hydrate→sodium formate. Reaction conditions: NaOH and chloral hydrate concentrations as shown, 1 h at 65°C. Column: IonPac AS11. Eluent: $NaHCO_3+Na_2CO_3$=2.0 m*M*, $NaHCO_3$:Na_2CO_3=3:1.

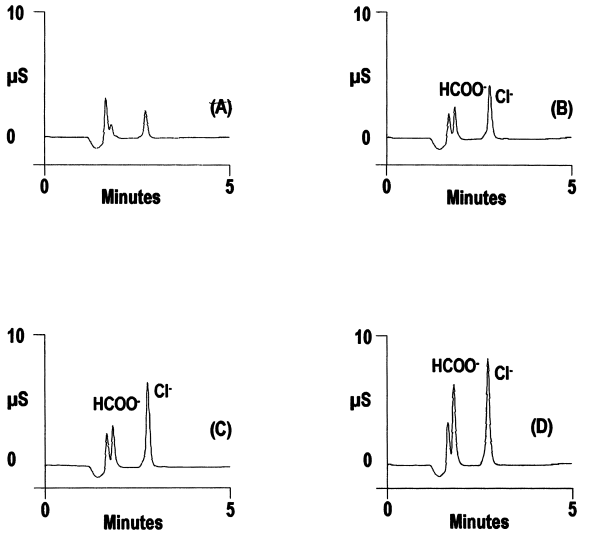

Fig. 5. Anion-exchange separation of derivatized chloral hydrate. Reaction conditions: 10 m*M* NaOH and chloral hydrate as shown, 1 h at 65°C. Column: IonPac AS11. Eluent: 2.5 m*M* NaOH. Sample loop: 200 µl. Detection: suppressed conductivity. (A) blank. (B) 200 µg/l chloral hydrate. (C) 400 µg/l chloral hydrate. (D) 800 µg/l chloral hydrate.

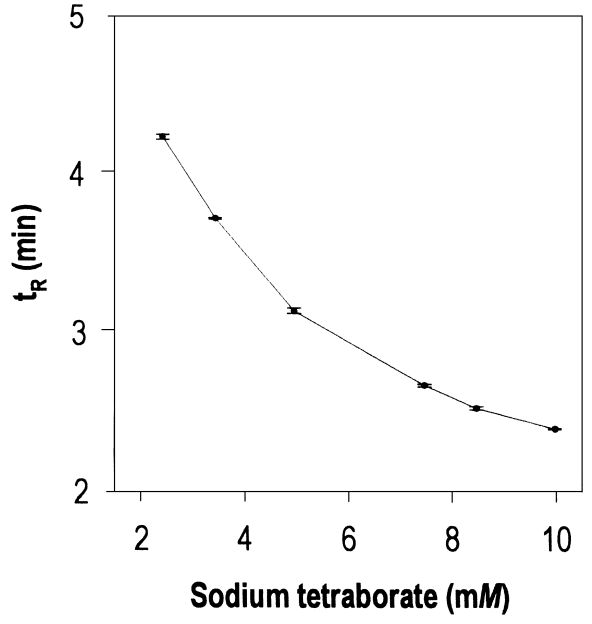

Fig. 6. Effect of sodium tetraborate concentration on the retention of formate. Reaction conditions as Fig. 4. Column: IonPac AS11. Error bars represent the standard deviations calculated from triplicate injections of three different sets of derivatization reactions.

ensured. Since tetraborate-based eluents reduce interferences in the blank, the pretreatment by the On-Guard-H cartridge has been then removed. In order to match derivatization and elution conditions in tetraborate medium, reaction of chloral hydrate has also been made with 5 m*M* tetraborate for 1 h at 65°C. Since yield of transformation of chloral hydrate in tetraborate medium was the 65±6% of that obtained by NaOH, the best conditions were chosen as 10 m*M* NaOH for derivatization and 2.5 m*M* tetraborate for separation. At these experimental conditions, the yield of transformation of chloral hydrate to formate has been determined and was found to be 74.0%. Detection limit, calculated as three times the background noise was 0.2 µg/l. A chromatogram of 0.4 µg/l chloral hydrate and its blank is shown in Fig. 7.

3.3. Analysis of drinking water

The suitability of the procedure developed has been tested for chloral hydrate determination in a drinking water sample. The sample solution has been filtered on 0.22 µm filters and divided into five aliquots. In order to take into account the concentration of formate already occurring in water, the first aliquot was injected as such without derivatization.

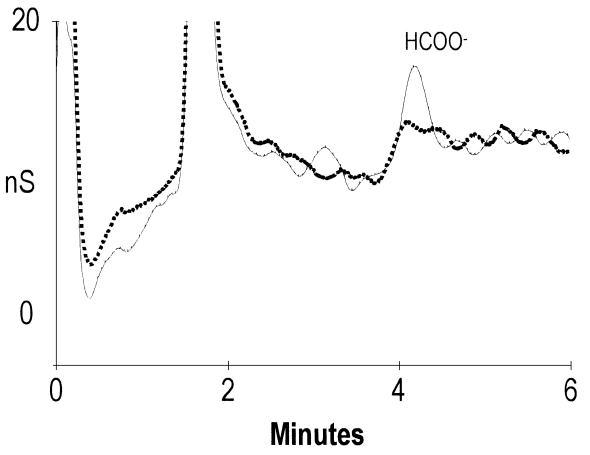

Fig. 7. Determination of trace amounts (0.4 µg/l) of chloral hydrate (solid line) and its blank (dotted line). Reaction conditions as Fig. 4. Column: IonPac AS11. Eluent: 2.5 m*M* sodium tetraborate. Sample loop: 200 µl. Detection: suppressed conductivity.

Other three aliquots have been spiked with 30–80 μg/l chloral hydrate while the last one was left as such as the blank. The four aliquots have been reacted with 10 m*M* NaOH for 1 h at 65°C and injected. In the drinking water matrix, r^2 for formate peak was 0.9995 ($n=4$).

3.4. Preconcentration of derivatized chloral hydrate

Although the very sensitive detection limits achieved, a procedure enabling chloral hydrate preconcentration has been studied. Among the different resins tested (reversed-phase and anion-exchange based materials), the IonPac AG11 guard column has been chosen as preconcentration substrate. Solutions of 50 ml of chloral hydrate of different concentrations (40–120 μg/l), derivatized as previously described and the blank, have been 1:10 diluted, and loaded into the guard column in order to evaluate the recovery yield. Species have then been eluted by 5 ml of 2.5 m*M* NaOH (preconcentration ratio=10). The eluate has been then injected in the chromatographic system and eluted in the separation column with 2.5 m*M* NaOH. A preconcentration recovery of 62±5%, calculated for the formate peak, has been obtained. An additional set of experiments has been performed in order to achieve lower detection limits and evaluating the chromatographic behaviour with sodium tetraborate eluent. Therefore, following the previous procedure, 50 ml of 5 μg/l chloral hydrate, derivatized, and diluted 1:10 have been preconcentrated in the AG11 column, and eluted with 5 ml of 2.5 m*M* NaOH. Fig. 8 shows the chromatograms obtained for the preconcentration of 0.5 μg/l chloral hydrate and for its blank.

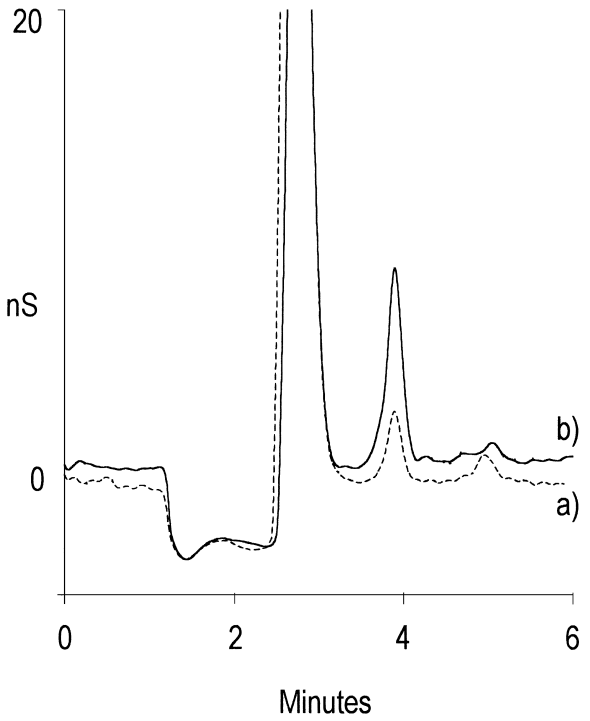

Fig. 8. Preconcentration of 0.5 μg/l chloral hydrate. Reaction and chromatographic conditions as Fig. 7. Preconcentrator: IonPac AG11. Preconcentration factor: 10. Sample: (a) blank, (b) 0.5 μg/l chloral hydrate.

4. Conclusions

Two liquid chromatographic methods, based on reversed-phase (RP) and anion-exchange mechanisms, have been studied for chloral hydrate determination. At equimolar concentrations and at controlled temperature, 1,2-benzenedithiol can be used for determination of relatively high chloral hydrate concentration by RP (>20 mg/l) at $\lambda=220$ nm. The IC method is based on derivatization of chloral hydrate with NaOH to form sodium formate that is then analyzed by anion-exchange chromatography, with suppressed conductivity detection. Derivatization conditions were optimized in order to achieve the best yield of reaction. The method developed enables the determination of chloral hydrate at concentration levels (0.2 μg/l) well below the legislation limits (40–60 μg/l) with good linearity and reproducibility. A preconcentration procedure, based on anion-exchange enrichment of formate, has also been tested. The IC method developed was applied to chloral hydrate determination in a drinking water sample.

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