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Journal of Chromatography B, 795 (2003) 363–370
JOURNAL OF CHROMATOGRAPHY B
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Short communication

Automated method for determination of glutardialdehyde residues in flexible endoscopes after disinfection

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Received 20 March 2003; received in revised form 10 July 2003; accepted 15 July 2003

Abstract

Glutardialdehyde (GDA) is the most commonly used disinfectant for flexible endoscopes. After inappropriate rinsing of endoscopes residual GDA in the narrow endoscope channels may lead to toxic effects in patients. Common methods for determination of aldehydes in water involve derivatization with 2,4-dinitrophenylhydrazine (DNPH), liquid–liquid or solid-phase extraction and HPLC determination. Since derivatization and extraction is both time-consuming and labor-intensive only a small number of samples can be measured. Thus, we developed a fully automated method which includes a conventional HPLC system, a programmable autosampler, and UV detection. After GDA derivatization using DNPH the samples remain in the aqueous phase and no preconcentration of the analyte is necessary. The samples are automatically derivatized through the autosampler. While derivatization in one sample takes place the previous sample is injected and measured by HPLC. Our method is well suited for screening residual GDA in endoscopes as it is both time- and labor-saving.

Keywords: Endoscopes; Glutardialdehyde

1. Introduction

Since its introduction in the early sixties, glutardialdehyde (GDA) is increasingly used for disinfection and sterilization of heat-sensitive instruments and materials that may be damaged by other physical and chemical methods. Due to its excellent sporocidal activity, GDA has been classified as chemosterilizing agent. Its antimicrobial activities, mechanisms, and usage has been well reviewed [1,2].

GDA is the most commonly used disinfectant for flexible endoscopes [3–6]. The expanding use of endoscopes in diagnosis and surgical treatment of patients require safe and effective methods of cleaning and disinfection. However, due to the narrow-lumened channels of flexible endoscopes adequate cleaning and disinfection is difficult. As a final step, it is recommended that the narrow channels should be rinsed properly to ensure removal of all traces of GDA. Otherwise, inadvertant contact between the patient

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doi:10.1016/S1570-0232(03)00567-1

and residual GDA may lead to toxic effects, such as GDA-induced necrosis, colitis, and proctitis [7–11]. According to the symptoms observed, other studies also assume toxic effects of GDA; however, residual GDA concentrations were not determined in these studies.

Low to high residual GDA concentrations were found in endoscope channels and in rinsing water. Lynch et al. [12] measured GDA concentrations of up to 0.1% in the rinsing water, Rozen et al. [13] determined 0.2% of GDA equivalents in rinsing water and in endoscope channels. Farina et al. [14] observed that GDA levels were higher and more variable after manual disinfection (<0.2–159.5 mg/l) than after automatic disinfection (<0.2–6.3 mg/l).

These studies show that monitoring the residual GDA concentration is desirable in order to reduce the risk of toxic effects in patients during endoscopy. There are a large number of nonspecific methods for the determination of aldehydes, e.g. photometric and fluorimetric methods. However, possible contaminants such as organic residues occurring in the rinsing water may interfere with these methods and lead to inaccurate results. Specific methods use HPLC or GC separation and detection after derivatization of aldehydes [15–20]. Among a large number of derivatization reactions, the 2,4-dinitrophenylhydrazine (DNPH) method has gained outstanding importance. Aldehydes are derivatized with DNPH in acidic media, yielding their corresponding hydrazone derivatives. After an appropriate reaction time liquid–liquid or solid-phase extraction of the hydrazone derivatives is performed, followed by HPLC determination [21]. These methods are labor-intensive and time-consuming.

In addition, only small volumes should be used to rinse the processed endoscopes in order to avoid residual GDA within the channels from being highly diluted. Depending on the internal diameter of the narrow channels and the length of the endososcopes, sample volumes of not more than 5–15 ml are available for investigation.

Therefore, a method which allows close monitoring and sensitive determination of residual GDA in small sample volumes is required. In this paper, we describe an automated procedure based on derivatization with DNPH and separation through HPLC. Sample derivatization is performed automatically by means of a programmable autosampler. Manual sample pretreatment involving preconcentration with liquid–liquid or solid-phase extraction is not necessary.

2. Experimental

2.1. Chemicals

2.1.1. Aldehyde solution

Formaldehyde (37% formalin solution) and GDA (50% aqueous solution) were obtained from Merck (Germany). Acetaldehyde was purchased from Acros (Belgium). A stock solution containing formaldehyde, acetaldehyde, and GDA was prepared by dissolving 100–130 mg/l of each aldehyde in deionized water. This stock solution was diluted 1:500 for testing the aldehyde stability. Serial dilution were made from the stock solution to different concentrations for the calibration curves.

2.1.2. Derivatization solutions

DNPH was obtained from Merck (Germany). A DNPH solution is prepared by dissolving 1 g of DNPH in 100 ml acetonitrile. This solution may be stored.

The derivatization solution (DerSol) for the automated derivatization of samples in the autosampler is prepared by adding 1 ml of H_3PO_4 (42%) to 10 ml of DNPH solution. This solution is transferred to a larger autosampler vial in the autosampler rack. One milliliter of the samples are pipetted into HPLC vials. For derivatization, according to the autosampler program, 10 μl of the derivatization solution (DerSol) are added to this sample volume of 1 ml.

If derivatization is combined with an automatically 1:10 dilution of the samples a diluted derivatization solution (DerSol-Dil) is used. This solution is prepared by adding 1 ml of the DNPH solution and 100 μl H_3PO_4 (42%) into 100 ml distilled water. For every sample one HPLC vial is filled with 1.3 ml of this derivatization solution, a second vial is filled with the sample. According to the autosampler program 144 μl of the respective sample is transferred into this volume, thus yielding a 1:10 dilution in combination with derivatization.

Manual derivatization is performed by adding 1 ml DNPH solution and 50 μl H_3PO_4 (42%) to a 100 ml standard solution of GDA. If derivatization is performed directly in the HPLC vials corresponding volumes are used.

2.2. Apparatus

HPLC measurements were performed using a Shimadzu HPLC consisting of a degassing unit (DG-1210), two pumps (LC-10AD), a mixing chamber, a SIL-10A programmable autosampler with sample preparation options, a column oven (CTO-10AS) and a photodiode-array detector (SPD-M10A). The column oven was set at 25 °C. Absorbance was measured at 360 nm. Equipment and data acquisition were controlled with the Class-VP software in combination with the system controller (SCL-10A).

For separation of the aldehydes, a Nucleosil 100-5 C18 Nautilus, 125 mm × 2 mm column (Macherey-Nagel, Düren, Germany) was used in combination with a guard column (30 mm × 3 mm) consisting of the same material. The eluent flow rate was set to 0.25 ml/min. A 50 μl sample loop was used for injection. The separation was carried out with a binary gradient, consisting of (1) a 8 mmol aqueous Na_2HPO_4 solution adjusted to pH 8 with H_3PO_4 and (2) acetonitrile. The gradient starts at 75% aqueous Na_2HPO_4 solution and changes to 100% acetonitrile within 15 min. This composition is held for 10 min. Subsequently, the initial eluent composition is reached within 5 min and the column is reequilibrated for 20 min.

3. Results

Our initial intention was to manually derivatize the samples directly in the HPLC sample vials since only small volumes of just a few milliliters are available. Following an appropriate reaction time the samples should be measured by HPLC without previous preconcentration. The preconcentration step commonly involves extraction and redissolution in an organic solvent like acetonitrile. Without preconcentration the hydrazones formed remain in the aqueous phase. The formation of hydrazone derivatives is time-dependent. Cotsaris and Nicholson [22] determined an optimal reaction time for the aldehydes and DNPH of about 30–60 min. Subsequent decomposition of some of the hydrazones may occur in aqueous solutions.

Each HPLC run requires 50 min. As sample processing progresses, the time that elapses until measurement of the current sample by HPLC increases. During this time interval, the hydrazone derivatives may decompose and thus lower the detection signal. Therefore, stability of the hydrazones in aqueous solution was monitored. In addition to GDA, formaldehyde and acetaldehyde were also measured and the results were compared. After manual sample derivatization the stability of the derivatives was determined by repeated injection of the aqueous mixture. The corresponding aldehyde concentrations ranged between 2.0 and 2.5 mg/l. The diamonds in Fig. 1a and b show the time course of formaldehyde and GDA hydrazone concentrations. The formaldehyde hydrazone concentration remained stable over 17 h and yielded a reproducible signal with 3% standard deviation. Similar results were obtained for acetaldehyde (data not shown). The acetaldehyde hydrazone derivative did not decompose over the 17 h period, with peak areas displaying 4% standard deviations.

In contrast to formaldehyde and acetaldehyde hydrazones, the GDA hydrazone decomposed by approximately 40% over 17 h. Due to this instability it is impossible to derivatize all samples simultaneously and keep them in the aqueous sample matrix until measurement by HPLC. Rather, after adding DNPH and H_3PO_4, a constant and optimal reaction time for every sample is a prerequisite for GDA derivatization. The GDA hydrazone must then either be extracted from the aqueous phase and redissolved in an organic solvent (e.g. acetonitrile) or directly be measured with HPLC.

Hence, we modified our strategy and decided that all steps be carried out by means of a programmable autosampler. The autosampler was programed to perform both sample derivatization and HPLC measurement automatically. This method presents the advantage that sample pretreatment becomes superfluous. The only requirement is to transfer a defined sample or derivatization solution volume (DerSol-Dil) into the HPLC vials; this volume depends on the autosampler program selected.

Autosampler programs are frequently used when derivatization time is short. Reagents are automatically added to the sample vial, and after a short reaction time

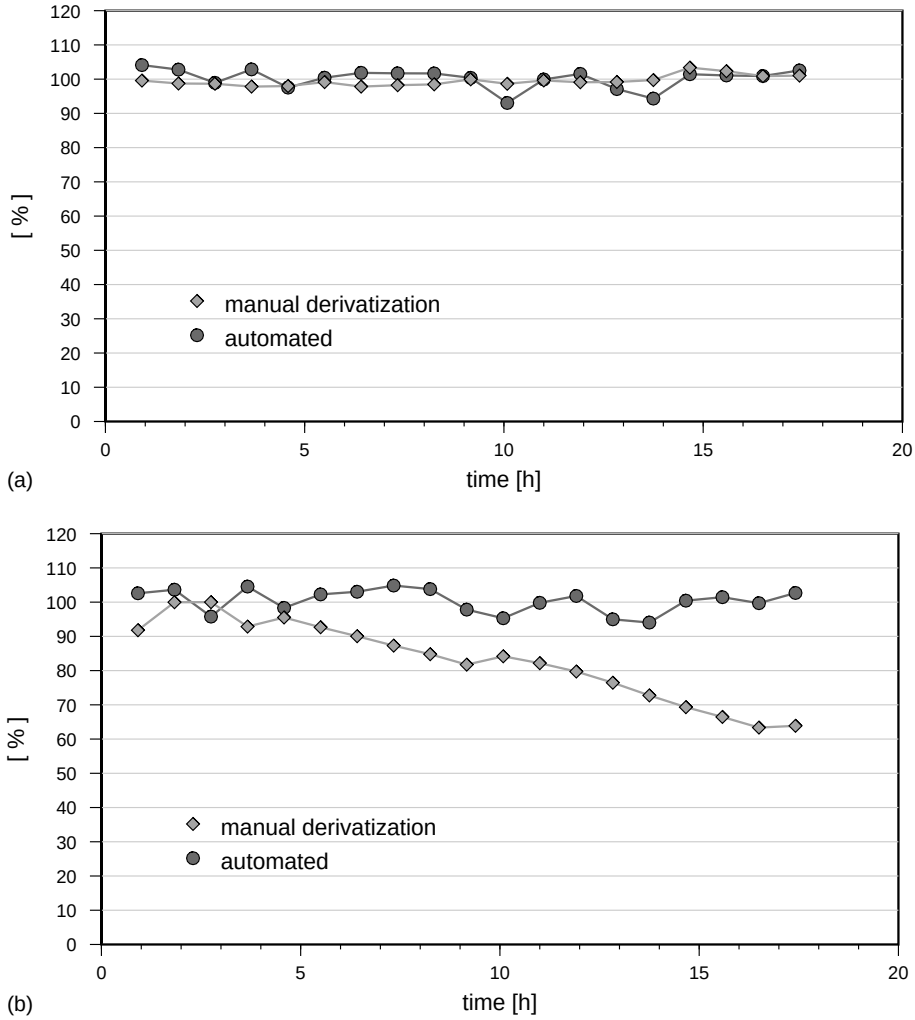

Fig. 1. Reproducibility of formaldehyde (a) and GDA (b) measurements after manual derivatization (diamonds) and automated derivatization (circles) in aqueous solution.

the sample is injected into the HPLC system. In the case of aldehyde derivatization an appropriate reaction time lasts 30–60 min, which is the time needed for one HPLC run. Therefore, derivatization of one sample and measurement of a previously derivatized sample can be combined.

A suitable autosampler program can be divided into two parts. The first part contains the instructions for derivatization of a sample by adding the derivatization solution DerSol. In view of the long reaction time, derivatization is performed in the vial following the vial with the sample which is to be injected next. A volume of 10 μl of the reagent is added to a sample volume of 1 ml. To ensure an entire and reproducible derivatization, the sample is then mixed three times by rapidly drawing and releasing 350 μl of the mixture.

The second part of the program instructs the autosampler to inject the preceeding sample. While this sample is measured with HPLC the derivatization can take place in the treated sample. After finishing the HPLC run the derivatized sample becomes the sample which is to be injected next and the process starts again. This procedure provides a constant derivatization time for every sample which is composed of the

run time for HPLC including equilibration time and the time for processing the autosampler program.

Fig. 1a and b show the time course for formaldehyde and GDA using the automated derivatization program. No difference between manual and automated derivatization was noted for formaldehyde. Both methods showed constant peak signals with about 3% deviation. Acetaldehyde (data not shown) yielded similar results with about 4% standard deviation. Hence, both methods can be used to measure these aldehydes. Conversely, an obvious difference between manual derivatization (varying reaction time) and automated derivatization (constant reaction time) was observed for GDA regarding stability and reproducibility. Over 17 h, only the automated procedure revealed constant peak areas for GDA with 4% standard deviation.

Linearity of the method was studied using concentrations ranging from 10 μg/l to 3 mg/l for each aldehyde. Seven concentrations were prepared by diluting the stock solution. Each concentration was measured five times. Within the measured concentration range adequate linearity was observed for formaldehyde and acetaldehyde (formaldehyde: $y = 195580 + 3071417x$, $r = 0.999$; acetaldehyde: $y = 180603 + 4349081x$, $r = 0.999$; y in arbitrary units). Linearity was not evaluated for higher concentrations. The regression line for formaldehyde is shown in Fig. 2a. GDA also produces adequate linearity reaching a

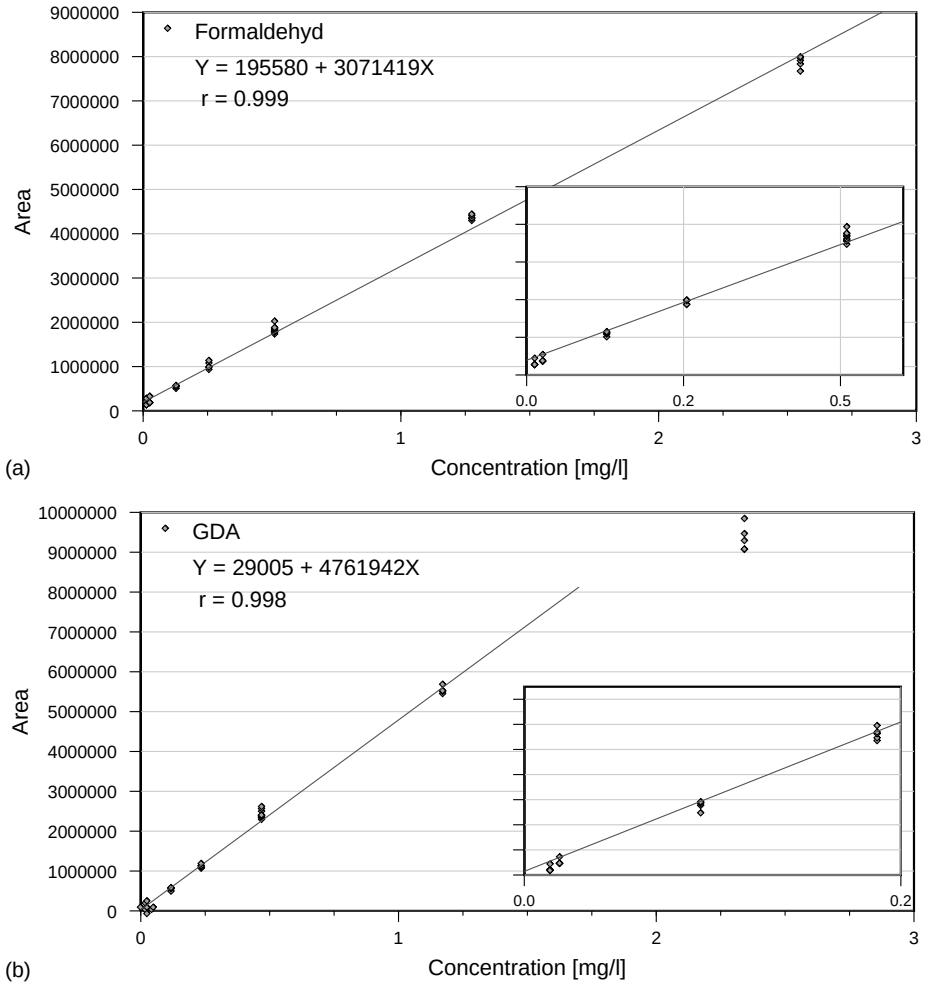

Fig. 2. Linearity and estimated regression line parameters for formaldehyde (a) and GDA (b).

maximum concentration of about 1.5 mg/l. At higher concentrations the linearity curve flattened (Fig. 2b) due to peak brodening. As a consequence, samples containing higher GDA concentrations must be diluted in order to achieve accurate results.

The detection limit was calculated as three-fold baseline noise. Using an injection volume of 50 μl a detection limit of 0.47 μg/l was found for GDA, corresponding to 24 pg of GDA injected. In view of an analytical column with an internal diameter of 2 mm, the injection volume of 50 μl appeared relatively large and could have produced broader peaks. However, since an aqueous sample was injected the solvent was weaker than the mobile phase. Thus, the solutes were concentrated at the top of the column leading to peak sharpening. Representative chromatograms with aldehyde concentrations of 200 μg/l using an injection volume of 50 μl and concentrations of 2 mg/l using a volume of 5 μl are shown in Fig. 3. When using 50 μl no significant increase in peak width was observed in comparison with 5 μl. Even injection volumes greater than 50 μl could be used which would lead to a further decrease of detection limits. In view of the large injection volume and the aqueous nature of the samples a column material was selected that tolerates even a 100% aqueous mobile phase without any organic modifiers.

In practice, not only low or medium concentrations but also concentrations above the linear section of the calibration curve may occur, e.g. high residual GDA concentrations due to incomplete final rinse of endoscopes. Hence, automated derivatization can be performed along with simultaneous dilution (e.g. by factor 10 or 100) so that manual dilution is no longer necessary, which further simplifies the method.

A 1:10 dilution is achieved by transferring a given sample volume to a given volume of the diluted derivatization solution (DerSol-Dil). For example, in our autosampler program, 144 μl of the sample is pipetted into a vial containing 1 300 μl DerSol-Dil. The DNPH and H_3PO_4 concentrations in the derivatization solution are brought to levels identical with concentrations in undiluted samples. Other dilution factors may be achieved by simply varying the relation of the sample volume and the volume of DerSol-Dil.

To evaluate the method under practice conditions we determined GDA recovery rates using Teflon tubes. The latter were chosen as substitutes for endoscope channels which are frequently made of Teflon. The tubes had a length of 1 m and an internal diameter

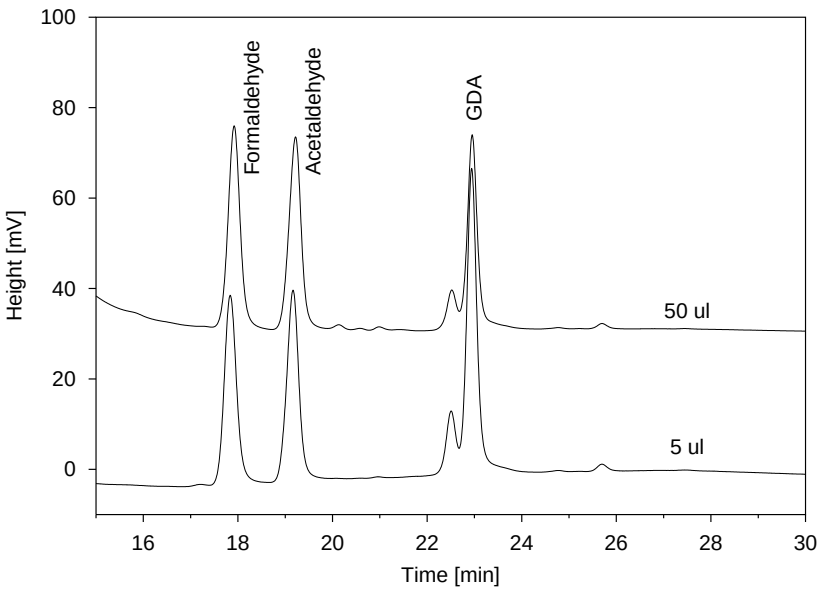

Fig. 3. Chromatograms of aldehyde samples with concentrations of approximately 2 and 0.2 mg/l and injection volumes of 5 and 50 μl, respectively (the peak in front of GDA is glyoxal, a contaminant in the GDA standard).

of 2 mm corresponding to a volume of about 3 ml. Six Teflon tubes were filled over a period of 1 h with a GDA solution containing 2–3 g/l GDA, which is a concentration commonly used in commercial disinfectants. The tubes were then rinsed with 7 ml of water, resulting in a total volume of 10ml. The GDA concentrations in disinfectants are high enough to precipitate GDA hydrazones formed through direct derivatization. Therefore, we diluted the aqueous samples 1:1000. Residual GDA concentrations in endoscopes after final rinsing would obviously be measured directly without any dilution. The diluted samples were then automatically derivatized and measured by means of HPLC.

GDA recovery rates for the six tubes were 99, 95, 94, 93, 89, and 82%. The mean value was 92% and the standard deviation 6%. Since screening samples for residual GDA in endoscopes would be measured without manual dilution, even a smaller variation in recovery rates can be expected.

4. Conclusions

Due to the toxic effects resulting from disinfectant residues contained in endoscope channels, the European Standard for Washer-Disinfectors for endoscopes [23,24] requires appropriate detection methods for the determination of such process residuals. The sampling and analytical methods shall be capable of determining the presence of process chemical at concentrations below that specified as potentially harmful.

Traditional methods for the determination of aldehydes in water requires manual derivatization, solid-phase extraction, and HPLC detection [21,25]. Since these methods are labor-intensive and time-consuming, only a small number of endoscopes can be tested at a time. Our study shows that determination of residual GDA concentrations can be fully automated by means of conventional HPLC equipment and a programmable autosampler. Since no additional equipement is necessary the method can be rapidly established in laboratories. GDA concentrations in the low microgram range per liter up to 2 mg/l can be measured without pretreatment of samples. Higher concentrations can also be measured automatically through derivatization in combination with an automated sample dilution. This time- and effort-saving method is well suited for screening for GDA residues in endoscopes.

Acknowledgements

We wish to express our gratitude to Heike Neumann for her valuable technical assistance during preparation, performance, and evaluation of the experiments. We also wish to thank Bode Chemie, Hamburg, Germany for providing financial support for this study.

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