ELSEVIER

Journal of Chromatography A, 692 (1995) 1–9

JOURNAL OF CHROMATOGRAPHY A

Automated procedure for determination of trace amounts of aldehydes in drinking water

Ran Wu*, Landy B. White

Thermo Separation Products, P.O. Box 5116, Fremont, CA 94537, USA

Abstract

According to method 8315 of the US Environmental Protection Agency, the analysis of aldehydes in drinking water includes manual derivatization, solid-phase extraction and HPLC separation. The procedure is time consuming and labor intensive. A new column-switching technique has been developed to automate the formaldehyde analysis with a standard HPLC system. This method replaces the sample loop on the injection valve of the autosampler with a precolumn where sample cleanup and preconcentration occur. Acetonitrile was used to dissolve the derivatizing reagent and as a system flush to reduce the blank contamination. The method is evaluated for reproducibilities, linearities, spike recoveries and minimum detection limits for two aldehydes.

1. Introduction

Formaldehyde is a natural oxidation product of many organic compounds [1]. It is also formed by incomplete combustion of many organic substances and is widely present in the atmosphere [2]. The major sources of formaldehyde found in drinking water are from the discharge of industrial wastes and oxidative water treatment processes [3]. Formaldehyde is a potential mutagen and carcinogen in laboratory animal tests [4,5]. Because of its adverse effect on human health, routine testing for formaldehyde and acetaldehyde in drinking water is required by the US Environmental Protection Agency (EPA) in some areas [6].

The current EPA method 8315 for the determination of aldehydes in drinking water involves derivatization with 2,4-dinitrophenylhydrazine (DNPH), manual solid-phase extraction and preconcentration, followed by HPLC separation [7–10]. The procedure is tedious, labor intensive and time consuming. To automate the procedure would normally require an expensive and complex robotic system. Presented here is a newly developed automated method utilizing a personal computer (PC)-controlled sample processing workstation that performs the derivatization and accomplishes the sample cleanup and preconcentration with a conventional HPLC system. The only modification of this HPLC system is the replacement of the sample loop with a precolumn on the injection valve of the autosampler. Through the customized PC-controlled sample processing workstation, the sample was derivatized by adding DNPH and mixing in the autosampler. Then the derivatized sample was metered into the precolumn (typically 500 to 1500 μl) and washed using an on-board prep syringe drive. Later, the sample was on-line eluted to the analytical column for HPLC separation. The common high blank response prob-

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00660-1

lem for formaldehyde analysis [3,11] was improved by dissolving DNPH in acetonitrile instead of ethanol [9], and a system flush with acetonitrile at the end of each injection.

Compared to the traditional on-line column-switching technique which requires an extra washing pump and an external switching valve, this column-switching technique requires a rather simple HPLC system. The automated method is simple, efficient and the amount of waste generated by this method is reduced significantly.

2. Experimental

2.1. Equipment

A TSP SpectraSYSTEM liquid chromatograph (Thermo Separation Products, Fremont, CA, USA) consisting of a P4000 pump coupled with SCM400 solvent conditioning module, an AS3000 autosampler with sample preparation option, a UV2000 detector, PC1000 software and a PC-controlled sample processing workstation loaded with HyperAccess modem software (Hilgraeve, Monroe, MI, USA) was used.

Mega Bond Elut C_{18} cartridges (2 g) and the Vac-Elut vacuum manifold from Varian Sample Prep Products (Varian, Harbor City, CA, USA) were used for manual solid-phase extraction.

2.2. Chemicals

DNPH (70%, w/w, purity) and acetaldehyde (99% purity) were obtained from Aldrich (Milwaukee, WI, USA). Formaldehyde (37% formalin solution) was purchased from Sigma (St. Louis, MO, USA). DNPH-derivatized formaldehyde and DNPH-derivatized acetaldehyde standard mixture (1000 mg/l each in methanol) was obtained from Accustandard (New Haven, CT, USA). Glacial acetic acid and HPLC-grade acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ, USA). Sodium hydroxide pellets were purchased from Mallinckrodt (Paris, KY, USA).

2.3. Preparation of standard solutions, spiked samples and reagent solutions

The working standard was made by diluting 10 μl of the DNPH-formaldehyde and DNPH-acetaldehyde standard mixture to 1 ml with deionized water (10 mg/l). Serial dilutions were made from the working standard to different concentrations for the calibration curves.

A manually spiked sample was made by spiking formaldehyde and acetaldehyde solutions into 100 ml of deionized water in a Nalgene bottle for determination of the method linearity, reproducibility and spike recovery.

To make 500 mg/l of DNPH derivatizing reagent, 7.15 mg of DNPH (70%, w/w) was dissolved in 10 ml of acetonitrile. The 5 *M* sodium acetate buffer was prepared by adjusting 28.5 ml of glacial acetic acid to pH 5 with 5 *M* sodium hydroxide, then diluting the solution to 100 ml with deionized water.

2.4. Experimental conditions

A 1.5 cm × 3.2 mm RP-18 Newguard column (7 μm particle size, silica-based C_{18} guard column) was used for the precolumn (Applied Biosystems, San Jose, CA, USA) on the injection valve. The samples in the sample vials were preserved at 4°C in the autosampler before the analyses. The separation was carried out at room temperature on a 15 cm × 4.6 mm Keystone Deltabond AK column (5 μm silica particles bonded with a polymeric stationary phase) equipped with an integral guard column (Keystone Scientific, Bellefonte, PA, USA). The mobile phase was isocratic acetonitrile–water (35:65) at a flow-rate of 1 ml/min. Detection was by UV absorption at 360 nm.

2.5. Experimental procedures

Sample preparation

In accordance with EPA method 8315, 4 ml of acetate buffer were manually pipetted into 100 ml of the spiked sample, then the sample was adjusted to pH 5 with sodium hydroxide. A 1-ml

volume of this sample was transferred into a sample vial and placed in the autosampler. Through the RS232 communication with the autosampler, the customized sample processing workstation manipulated the autosampler's syringe movements, injection valve, flush valve and solvent valve. Fig. 1 shows the autosampler configuration for sample preparation.

A 60-μl volume of DNPH was added into the sample vial by the autosampler's sample syringe. Then the sample vial was transferred to the built-in heater/mixer to vortex mix the sample for 10 min at room temperature for the derivatization reaction. Using the column-switching technique (described next), 500 μl of the derivatized sample were loaded on the precolumn for sample cleanup and preconcentration, followed by on-line HPLC separation.

Column-switching mechanism

Fig. 2 illustrates the column-switching mechanism which consists of the following steps:

Step 1: The precolumn was preconditioned with water. Then the pump flow was stopped, and the injection valve was moved to "inject" position. The prep syringe drew a large volume of sample into the solvent holding loop (see Fig. 2a).

Step 2: The injection valve was moved to "load" position, and the prep syringe pushed the sample onto the precolumn for trace enrichment (see Fig. 2b).

Step 3: The pump flow was restarted, as the prep syringe flushed 5 ml of water through the precolumn to perform sample cleanup (see Fig. 2c).

Step 4: The injection valve was moved to "inject" position, and the mobile phase back flushed the sample out of the precolumn and completed the injection onto the analytical column for HPLC separation (see Fig. 2d).

Step 5: While the sample was analyzed on the HPLC system, the prep syringe rinsed and conditioned the precolumn with water and started to process the next sample (see Fig. 2e).

Another set of the manually spiked samples was processed according to the original EPA method 8315 as a control group to compare to the automated method. The procedures included manual derivatization, C_{18} solid-phase extraction and conventional HPLC separation with 20-μl injection on the sample loop.

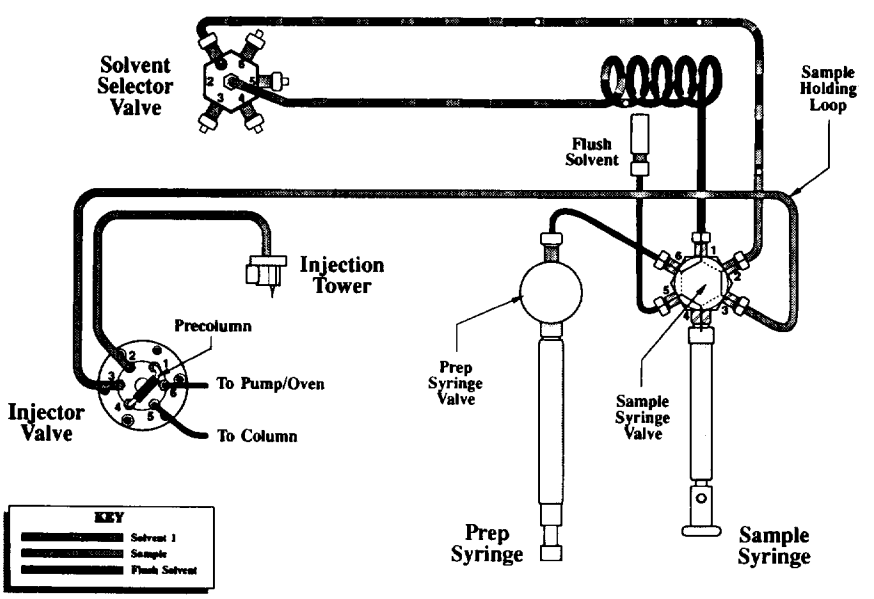

Fig. 1. Autosampler configuration for customized column-switching mechanism.

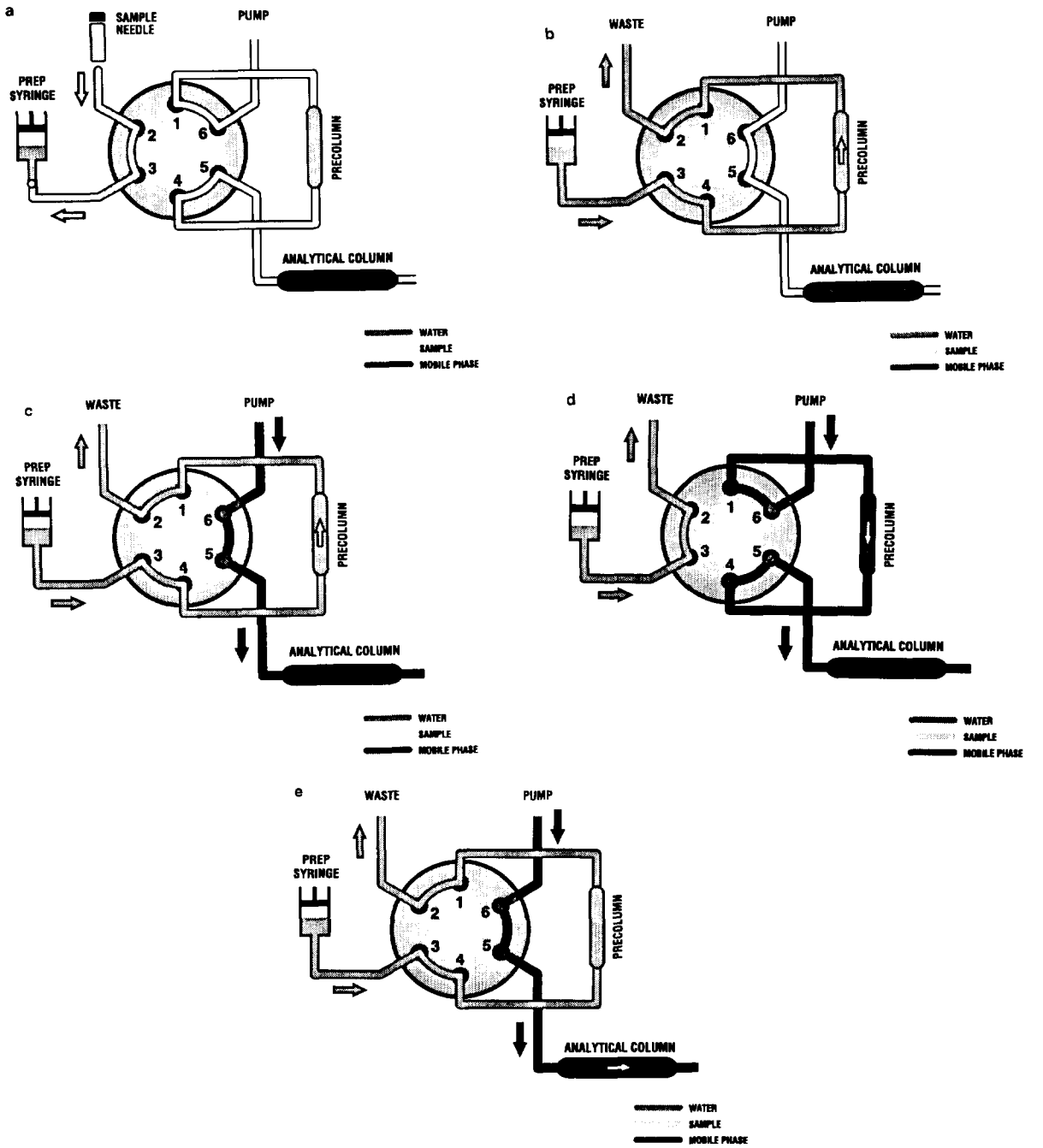

Fig. 2. Detailed illustrations of the column-switching mechanism on a conventional HPLC system. See text.

3. Results

Fig. 3 shows a typical chromatogram for the DNPH-derivatized formaldehyde and DNPH-derivatized acetaldehyde from the column-switching system. Fig. 4 shows a chromatogram of 1 μg/l for both prederivatized aldehydes using a 500-μl sample on the column-switching system.

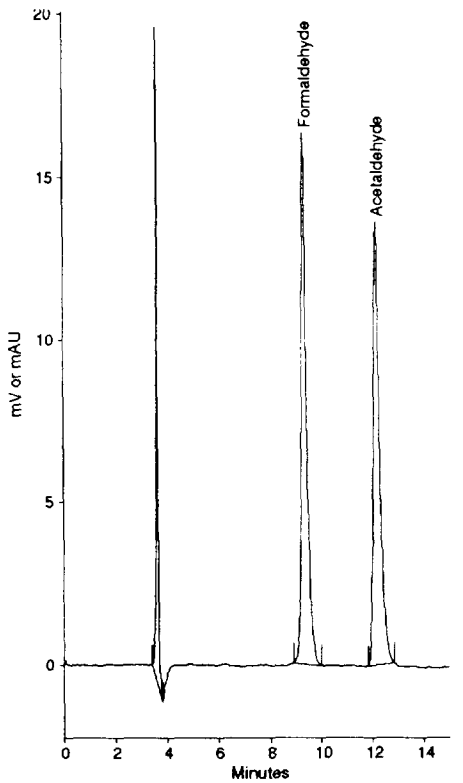

Fig. 3. Chromatogram of DNPH-derivatized formaldehyde and DNPH-derivatized acetaldehyde on the column-switching system. The injection volume was 500 μl, and the concentration was 100 μg/l in water each.

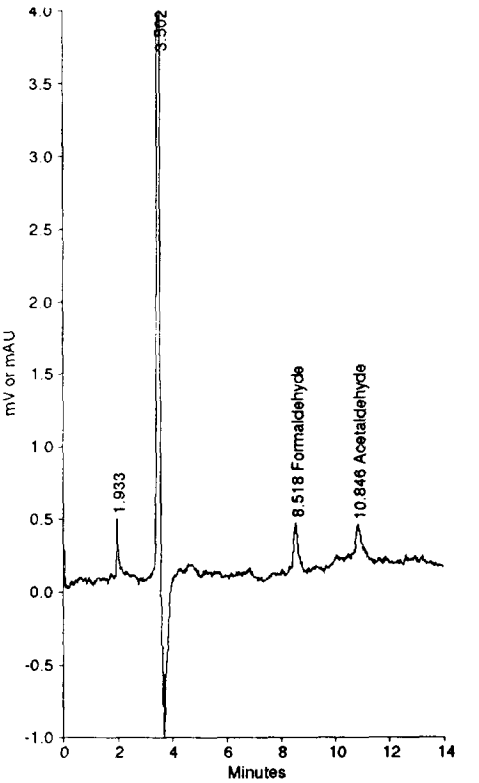

Fig. 4. DNPH-derivatized formaldehyde (1 μg/l) and 1 μg/l of DNPH-derivatized acetaldehyde on the column-switching HPLC system. The injection volume was 500 μl.

The detection limits for both aldehydes on the conventional HPLC system, using a 20-μl injection were about 25 μg/l (see Fig. 5), based on a S/N ratio of 3. The detection limits for both aldehydes on the column-switching system therefore have improved significantly over the conventional HPLC system because of the ability to load a large volume of sample on the precolumn. The sample loading volumes on the precolumn were linear to the signal responses. Fig. 6 shows the linear relations for different loading volumes (between 200 and 1000 μl) and the corresponding signal responses at 2 mg/l level for both aldehydes. Tables 1 and 2 summarize the performance of the automated column-switching method for formaldehyde analysis in comparison with the original EPA method 8315. The injection reproducibilities on the column-switching system; using prederivatized standards; were calculated based on seven consecutive 500-μl samplings from seven individual vials at the concentrations between 0.1 and 1 mg/l. The R.S.D. for the conventional HPLC system for both aldehydes was calculated from seven consecutive injections of 20 μl from the same vial at the concentrations between 0.1 and 1 mg/l. Therefore, the injection reproducibilities for the column-switching system (0.8–2.9%) were comparable with the conventional HPLC system (0.4–4.2%). The automated method was defined as automatically adding 60 μl of DNPH into 1 ml of manually spiked samples, vortex mixing the sample in the heater/mixer, followed by column-switching technique and on-line HPLC separation. Based on the same statistical calculation described above, the automated method reproducibilities were 1.3–1.9% versus 0.6–1.3% for

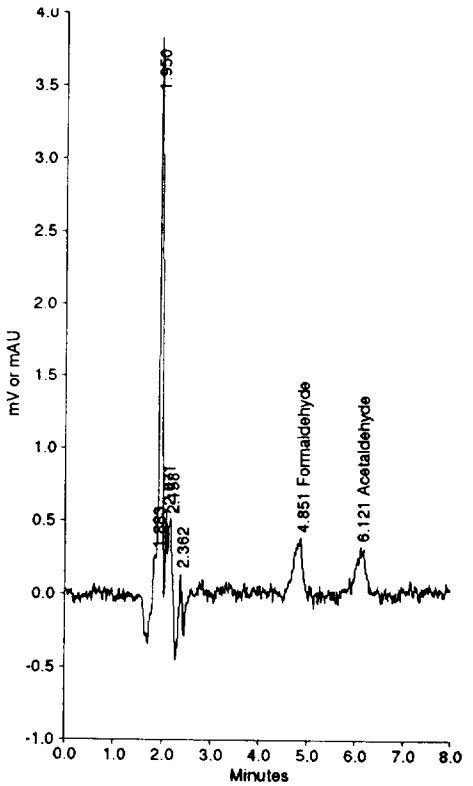

Fig. 5. DNPH-derivatized formaldehyde (25 μg/l) and 25 μg/l of DNPH-derivatized acetaldehyde on the conventional HPLC system. The injection volume was 20 μl.

the manual EPA method. The study of the efficiency of vortex mixing for formaldehyde analysis indicates the derivatization reaches a plateau in 10–20 min. The spike recoveries were calculated according to the calibration curve generated from the commercially prederivatized aldehyde standard. The data were obtained from spiking 0.1 to 0.5 mg/l of formaldehyde and acetaldehyde standard solutions into two batches of deionized water samples. In comparing the spike recoveries of the automated method (90–105%) to 86% recovery reported by the EPA for organic free water, the performance of the automated method was very comparable.

The formaldehyde linearity on the column-switching system was obtained by running the prederivatized formaldehyde standard between the concentrations of 0.05 and 1 mg/l. The five points on the calibration curve represented the average of seven peak area measurements at each concentration. All the peak area measurements had R.S.D.s of less than 3%. The standard error for each point was less than 2%. When peak area measurements were linearly correlated with concentrations using the equation peak area = concentration · 6 995 347 + 108 551, the correlation coefficient was 0.9991. For manually spiked formaldehyde samples,

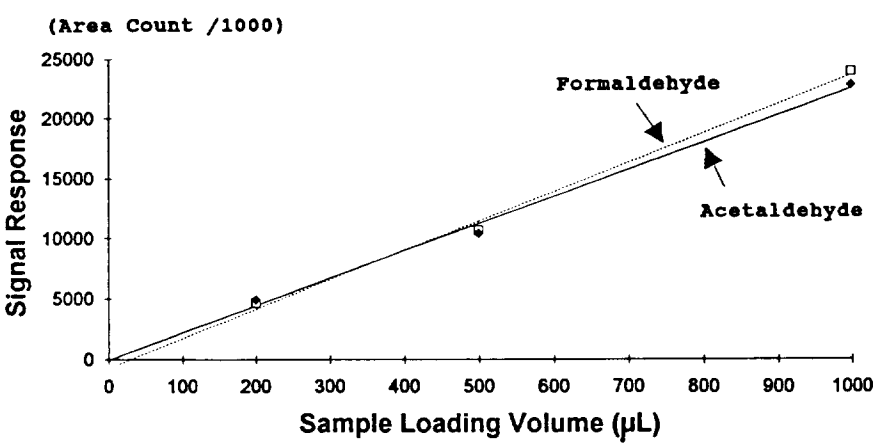

Fig. 6. Linear plots of the sample loading volume on the precolumn versus signal response for formaldehyde and acetaldehyde at a concentration of 2 mg/l level. The correlation coefficient for formaldehyde (□, dotted line) was 0.9975. The correlation coefficient for acetaldehyde (◆, solid line) was 0.9971.

Table 1
System performance

	Column-switching HPLC system	Conventional HPLC system
Formaldehyde		
Reproducibility (R.S.D., %)	0.8–2.9	0.5–4.2
Detection limit (μg/l)	< 1	< 25
Acetaldehyde		
Reproducibility (R.S.D., %)	0.9–2.6	0.4–4.2
Detection limit (μg/l)	< 1	< 25

A comparison between column-switching and conventional HPLC systems using the commercially derivatized formaldehyde and acetaldehyde standards. The experimental conditions were described in the *Column-switching mechanism* section under Experimental.

using the equation peak area = concentration · 6 595 335 + 393 258, the correlation coefficient was 0.9999 based on the same statistical calculation described above.

The common problem of a high blank response for formaldehyde analysis can be improved by substituting acetonitrile for ethanol (on EPA method 8315) to dissolve DNPH, in addition to flushing the system with acetonitrile at the end of each analysis. Using these procedures, the formaldehyde readings in blank samples were reduced by 10 to 25 μg/l. The blank acetaldehyde reading was minimized to zero.

Drinking water samples were collected from six different local areas and analyzed with the previously described automated method. All but one of the formaldehyde concentrations found were below the limit of detection of the EPA method (see Table 3). No problems were encountered with the sample cleanup as stated.

4. Discussion

The current EPA method 8315 for the determination of aldehydes in drinking water requires manual derivatization, solid-phase extraction and HPLC separation. This study shows that the entire procedure can be fully automated with a conventional HPLC system.

The traditional on-line column-switching sys-

Table 2
Method performance

	Fully automated method	Manual extraction and HPLC separation
Formaldehyde		
Reproducibility (R.S.D., %)	1.3–1.4	0.6–0.9
Spike recovery (%)	90–105	86
Acetaldehyde		
Reproducibility (R.S.D., %)	1.4–1.9	1.0–1.3
Spike recovery (%)	90–104	93–98

A comparison between the fully automated method (automated derivatization, column switching and on-line HPLC separation) and the EPA manual extraction method using the manually spiked water samples. The experimental conditions were described in the *Experimental procedures* section under Experimental.

Table 3
Real sample analyses

Local area	Formaldehyde (μg/l)[a]	Acetaldehyde (μg/l)
1	11.0	ND
2	8.9	ND
3	5.7	ND
4	7.4	ND
5	6.6	ND
6	6.9	ND

The analysis results of drinking water samples collected from six local areas. The sample preparation procedures and column-switching technique were described in the *Experimental procedures* section under Experimental. ND = Not detected.

[a] The EPA reported method detection limit for formaldehyde is 7.2 μg/l. These values include reagent blank concentration of approximately 2 μg/l formaldehyde.

tem requires an extra pump and an external switching valve in addition to the conventional HPLC system. The column-switching mechanism described here, however, replaces the sample loop with a precolumn and completes the same procedure without additional HPLC equipment. The data show the performance of the automated column-switching system for formaldehyde analysis is either comparable to or better than the original EPA method.

One of the most significant advantages of this automated method is its efficiency compared to the original EPA method. The sample preparation feature of the autosampler enables the automation of the derivatization reaction by the prep syringe and the heater/mixer. The prep syringe adds the derivatizing reagent to the sample vial. The heater/mixer vortex mixes the sample to ensure a homogeneous reaction. The column-switching technique replaces the manual solid-phase extraction for sample cleanup and trace enrichment, followed by on-line HPLC separation. The overall sample preparation and analysis time for each sample is less than 30 min and can be performed simultaneously with the previous analysis. Furthermore, the organic solvent wastes generated by this automated method have been reduced to less than 10 ml for each sample.

The ubiquitous occurrence of formaldehyde in the environment makes the detection of low levels of formaldehyde almost impossible. One way to avoid the high blank response problem is to rigorously purify each chemical used in the method so that chemicals are aldehyde-free. However, the EPA specifies the use of ethanol to dissolve DNPH. The EPA reported detection limit for acetaldehyde was 171 μg/l which included 130 μg/l acetaldehyde reading from blank samples. The high background reading of acetaldehyde in the EPA method results from the oxidation of ethanol. Using acetonitrile as a substitute for ethanol to dissolve DNPH will avoid this source of acetaldehyde contamination. Another advantage of using acetonitrile is that DNPH is more soluble in acetonitrile than in ethanol. To reduce formaldehyde contamination, a system flush with acetonitrile will eliminate the majority of the formaldehyde residues remaining in the system, thus minimizing the system background reading for the next injection.

This is a simple and reliable method for automating the aldehydes analysis in drinking water. Although the method was developed in the laboratory in a clean and controlled environment, expanding the methodology to other sample matrices will require studies of the lifespan and selectivity of the precolumn. Obviously, more complicated sample matrices will have a more pronounced effect upon the precolumn selectivity and useful life. Through the sample processing workstation, the automated derivatization capability of the autosampler and the column-switching technique described in this paper are potentially applicable to automate many other HPLC analyses in the future.

Acknowledgements

The authors appreciate the valuable suggestions from Vance Nau and Fran Lai, and also acknowledge support from Lenore Kelly and Marty Hartigan.

References

[1] *Investigation of Selected Potential Environmental Contaminants: Formaldehyde*, US Environmental Protection Agency, Office of Toxic Substances, Washington, DC, 1976.

[2] J. de Andrade and R. Tanner, *Atmos. Environ., Part A*, 26A (1992) 819.

[3] E. Cotsaris and B. Nicholson, *Analyst (Cambridge, U.K.)*, 118 (1993) 265

[4] B. Tomkins, J. McMahon and W. Caldwell, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 835.

[5] R.C. Craftstorm, A.J. Jr. Fornace, H. Autrup, J.F. Lechner and C.C. Harris, *Science*, 220 (1983) 216.

[6] *Preliminary Assessment of Suspected Carcinogens in Drinking Water*, US Environmental Protection Agency, Office of Toxic Substances, Washington, DC, 1985.

[7] P. Whittle and P. Rennie, *Analyst (London)*, 113 (1988) 665.

[8] M. Bicking, W. Cooke, F. Kawahara and J. Longbottom, *J. Chromatogr.*, 455 (1988) 310

[9] *EPA Method 8315; Fed. Reg.*, Vol. 1, Section B, Revision 2 (1990) (CFR260.11 and CFR270.6 for SW846).

[10] G. Chiavari, G. Torsi and A. Asmundsdottir, *Ann. Chim. (Rome)*, 82 (1992) 349.

[11] X. Zhou and K. Mopper, *Environ. Sci. Technol.*, 24 (1990) 1482.