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Automated high-performance liquid chromatographic and size-exclusion chromatographic sample preparation by means of a robotic workstation

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ABSTRACT

A robotic workstation was used for the automation of standard and sample preparation for three applications: the size-exclusion chromatographic analysis of residual toluene 2,4-diisocyanate and 4,4'-diphenylmethane diisocyanate in polyurethane adhesives; the high-performance liquid chromatographic analysis of the formaldehyde, glutaraldehyde and glyoxal content of aqueous surfactant formulations; and the high-performance liquid chromatographic analysis of the tetradecanedioic acid and methylmyristate content of a reaction matrix. Sample weighing, solvent addition, mixing, standard dilutions, filtrations and solid-phase extractions were performed automatically by the workstation. The standard and sample solutions were injected on-line via the built-in injection valves into the high-performance liquid chromatographic or size-exclusion chromatographic systems.

INTRODUCTION

Today's instrumentation for high-performance liquid chromatography (HPLC) and size-exclusion chromatography (SEC) is fully automated and computer-controlled. This allows for time-efficient, unattended operation. In contrast to this, the preparation of standards and samples is very often the most tedious and time-consuming part of an analytical procedure and usually has to be done manually. In addition to time efficiency considerations, being able to avoid the manual handling of potentially toxic compounds can be a critical requirement. The use of robots for the automation of such manual procedures is common practice in today's technical environments [1,2]. The approach taken here was to use an easy-to-handle and compact robotic workstation [3] instead of a complex robot to automate simple routine procedures in liquid chromatography (LC) such as sample weighing, standard dilution, filtration or solid-phase extraction, including on-line sample injection into the chromatographic systems. A built-in balance allows for the weight tracking of all precision-relevant steps of a procedure. The workstation is designed for dedicated use as a sample preparation and injection device for liquid chromatography. Compact dimensions allow for an installation side by side with a chromatographic system on a laboratory bench or in a conventional fume hood.

EXPERIMENTAL

A Zymark "BenchMate" workstation was used, equipped with a solid-phase extraction and filtration unit and two built-in Rheodyne LC injectors with 20-μl injector loops. For filtration, disposable 30-mm PTFE filters (0.45-μm) were used. For solid-phase extraction Waters Sep-Pak Vak C_{18} cartridges were used. The 5-ml "airpush" syringe of the BenchMate was replaced by a 2-ml syringe for applications where methylene chloride had to be used to handle the high vapour pressure of this solvent. In this configuration, one of the six reagent lines had to be used for an additional airpush step at certain positions of the program to empty the sol-

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vent lines completely.

Waters 510 pumps, a Waters 680 controller, a Waters 490 four-wavelength UV detector and Waters 510 and 401 refractive index detectors were used in combination with a Nelson 6900 chromatography data system. All organic solvents were HPLC grade; water was purified with a Millipore Milli-Q system. The chromatography conditions were developed in-house. Detailed conditions are given in the figure captions.

The BenchMate procedures were written by selecting certain "preprogrammed" routines ("steps") from the BenchMate software and adding the numbers for volumes, weights, densities, etc, according to the specific application.

BenchMate procedure: 1:4 standard dilution for the SEC analysis of residual monomeric isocyanates in polyurethane adhesives

Step 1: add 2 ml of toluene 2,4-diisocyanate (TDI)–4,4'-diphenylmethane diisocyante (MDI) stock
Step 2: dilute (density: 1.3255 g/ml) 1:4 with methylene chloride making a total of 3 ml
Step 3: vortex mix for 10 s at speed 1
Step 4: wash syringe with 2 ml of air
Step 5: pre-wet filter with 0.3 ml of sample; filter 3.2 ml into next tube
Step 6: rinse filter holder with 2 ml of methylene chloride
Step 7: wash syringe with 2 ml of methylene chloride
Step 8: wash syringe with 2 ml of air
Step 9: wash LC injector with 0.7 ml of sample
Step 10: inject sample on LC 1; elute for 20 min
Step 11: end

Only the dilution ratio had to be changed to obtain the other standard concentrations. The procedures for the standard dilution for the other applications were written according to the same principle.

BenchMate procedure: sample preparation for the SEC analysis of residual monomeric isocyanates in polyurethane adhesives

Step 1: add 5 ml of methylene chloride
Step 2: wash syringe with 2 ml of air
Step 3: vortex mix for 600 s at speed 3
Step 4: pre-wet with 0.5 ml of sample and filter 4.5 ml into next tube
Step 5: rinse filter holder with 2 ml of methylene chloride
Step 6: wash syringe with 2 ml of methylene chloride
Step 7: wash syringe with 2 ml of air
Step 8: wash LC injector with 0.7 ml of sample
Step 9: inject sample on LC 1 with run time of 20 min
Step 10: end

The sample weighing is performed automatically before the BenchMate starts with step 1 of the program.

BenchMate procedure: sample preparation for the HPLC analysis of aldehydes in an aqueous surfactant formulation

Step 1: add 5 ml of 0.0167 *M* orthophosphoric acid
Step 2: vortex mix for 60 s at speed 2
Step 3: pre-wet filter with 0.5 ml of sample; filter 4 ml into next tube
Step 4: rinse filter holder with 2 ml of 0.0167 *M* orthophosphoric acid
Step 5: wash LC injector with 0.5 ml of sample
Step 6: inject sample on LC 2 with run time of 20 min
Step 7: end

The sample weighing is performed automatically before the BenchMate starts with step 1 of the program.

BenchMate procedure: sample preparation for the HPLC analysis of dicarboxylic acids in a reaction matrix

Step 1: add 1 ml of 0.2 *M* sodium hydroxide
Step 2: condition column with 2 ml of methanol
Step 3: condition column with 5 ml of water
Step 4: vortex mix for 10 s at speed 3
Step 5: load 0.6 ml of sample onto column
Step 6: rinse column with 2 ml of 1 *M* hydrochloric acid
Step 7: rinse column with 6 ml of water
Step 8: collect 7.5-ml fraction into next tube using isopropanol–acetonitrile–water–acetic acid (40:40:20:0.1, v/v)
Step 9: vortex mix for 10 s at speed 2
Step 10: pre-wet with 0.3 ml of sample; filter 3 ml into next tube
Step 11: rinse filter holder with 2 ml isopropanol–acetonitrile–water–acetic acid
Step 12: inject sample on LC 1 with run time of 15 min
Step 13: inject sample on LC 2 with run time of 15 min
Step 14: end

The sample weighing is performed automatically before the BenchMate starts with step 1 of the program.

RESULTS AND DISCUSSION

Automated standard and sample preparation for the SEC analysis of residual monomeric isocyanates in polyurethane adhesives

Residual TDI and MDI in polyurethanes can be analyzed by using a SEC technique. The standard and sample preparation for this method consisted of the following two general steps which had to be automated: (i) perform a standard dilution series, filter and inject standards to create an external standard calibration graph; (ii) weigh adhesive samples, add methylene chloride, mix to dissolve, filter and inject for analysis.

A manually prepared standard stock solution is used for the dilution series. Note the airpush steps that were performed with the reagent syringe ("Wash syringe with 2 ml of air", see Experimental section). All the standards were run in triplicate using the BenchMate to perform the dilutions and LC injections. The reproducibility (relative standard deviations for the peak areas of each triplicate were 1.2–3.5%) and linearity of the calibration graph (correlation factor 0.9998) were excellent. The weight-tracking results for these dilutions showed that the experimental weight ratios for the different dilutions were within ± 0.5% of the programmed (theoretical) values.

Fig. 1 shows a typical chromatogram for a polyurethane adhesive sample. Reproducibility studies, where all samples were prepared and analyzed in triplicate using the BenchMate, showed relative standard deviations (R.S.D.s) ranging from 1% to 17% for residual MDI and TDI, depending on the level of residual isocyanate (0.01–5%, w/w) in the polyurethane sample.

Automated standard and sample preparation for the HPLC analysis of aldehydes in an aqueous surfactant formulation

An HPLC technique was used to analyze glyoxal, formaldehyde and glutaraldehyde in aqueous surfactant formulations. The manual procedure consisted of the following two steps which again had to be automated: (i) perform a standard dilution series using a standard stock solution, filter and inject standards to create the external standard calibration graph; (ii) weigh samples, add 0.0167 *M* orthophosphoric acid, mix to dissolve, filter and inject for analysis.

Because an aqueous system was used for the sample preparation and chromatography, no vapour pressure problems were experienced, and the standard 5-ml airpush syringe was used without problems. It was expected that during the sample preparation the solutions might foam because of the

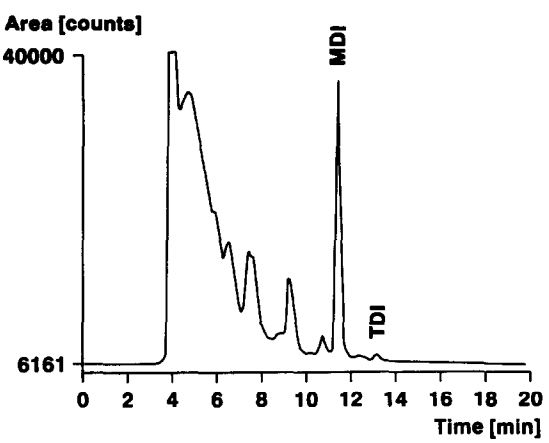

Fig. 1. Typical chromatogram of the residual MDI and TDI analysis in a polyurethane adhesive sample. Chromatographic conditions: 3 × PLGel 50 A column (5 μm particle size, 300 × 7.6 mm I.D.); methylene chloride at 1.5 ml/min; 20 μl injection volume; UV detection at 240 nm for TDI and at 280 nm for MDI.

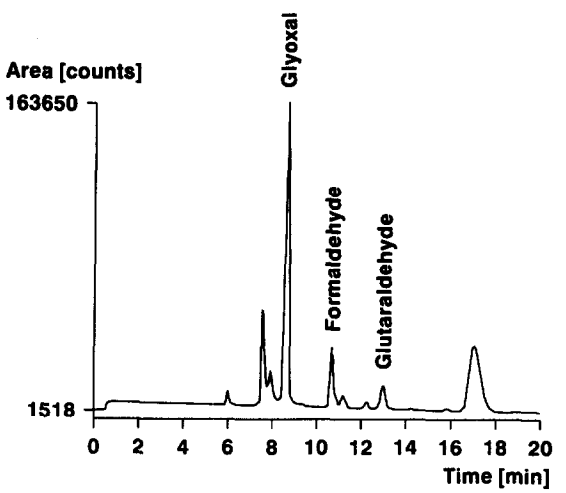

Fig. 2. Typical chromatogram of the aldehydes in an aqueous surfactant formulation. Chromatographic conditions: Shodex KC-811 column (5 μm particle size, 300 × 8 mm I.D.) with C-811 P guard column; 0.12% (v/v) orthophosphoric acid at 1 ml/min; 20 μl injection volume; refractive index detection.

TABLE I

COMPARISON OF THE ALDEHYDE ANALYSIS RESULTS FOR THE AUTOMATED *VERSUS* THE MANUAL PROCEDURE

Sample No.	Glyoxal		Formaldehyde		Glutaraldehyde	
	Automated	Manual	Automated	Manual	Automated	Manual
1	8.8	8.8	–	–	4.1	4.5
2	11.8	12.0	10.8	11.1	3.6	3.8
3	8.8	8.8	–	–	4.2	4.5
4	4.3	3.8	1.4	1.4	0.7	0.8

presence of surfactants in the aqueous formulation. No such problems were experienced.

The standard dilution programs were very similar to the adhesive application. Again, triplicate standard dilutions and calibration runs resulted in excellent linearity (correlation factor 0.9999) and reproducibility with R.S.D.s ranging from 0.3 to 2.9% for the peak-area counts of each triplicate. The weight-tracking results for these dilutions showed that the experimental weight ratios for the different dilutions were within ± 0.3% of the programmed (theoretical) values.

Fig. 2 shows an example of a typical chromatogram of the aldehydes in an aqueous surfactant formulation. A comparison of manually generated aldehyde analysis results with the results from the BenchMate shows excellent agreement (Table I). Reproducibility studies, where all samples were prepared and analyzed in triplicate, showed that the R.S.D.s ranged from 0.2 to 16.3% depending on the aldehyde level.

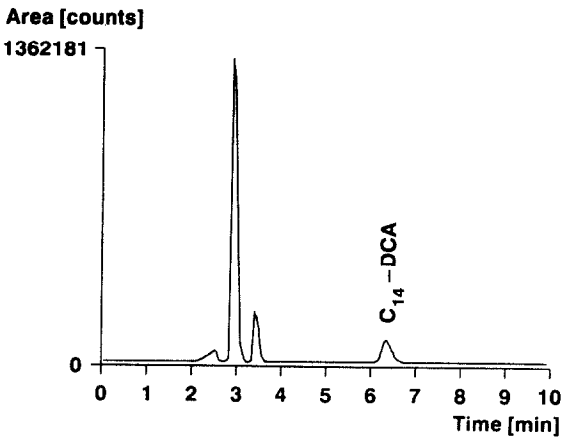

Fig. 3. Typical chromatogram of tetradecanedioic acid (C_{14}-DCA) from a reaction matrix. Chromatographic conditions: LiChrosorb C_{18} column (10 μm particle size, 250 × 4.6 mm I.D.; Phenomenex); isopropanol–acetonitrile–water–acetic acid, 40:40:20:0.1 (v/v), for tetradecanedioic acid; 45:45:10:0.1 (v/v) for methylmeristate (chromatogram not shown) at 1 ml/min; 20 μl injection, volume: refractive index detection.

Automated standard and sample preparation for the HPLC analysis of dicarboxylic acids in a reaction matrix

α, ω-Dicarboxylic acids, in this case tetradecanedioic acid, can be analyzed by reversed-phase HPLC [4]. The manual procedure consisted of the following two steps which had to be automated: (i) a standard dilution series, filtration of the standard solutions and injection into the HPLC system; (ii) sample weighing, addition of 0.2 *M* sodium hydroxide, solid-phase extraction, filtration and injection into the HPLC system for analysis.

Via the BenchMate's two built-in injection valves, the sample was injected into two independent isocratic HPLC systems. Elution times of 10 min allowed for a fast analysis of the two components of interest, the reaction product tetradecanedioic acid and methylmyristate, which is the starting material for this reaction. Fig. 3 shows a typical chromatogram of the tetradecanedioic acid portion in a sample. Good analytical precision was found with a correlation factor of 0.9972 for the linearity of the calibration graphs, R.S.D.s of ± 0.5% for the standard dilution series, and R.S.D.s of 1.7–4.0% for the dicarboxylic acid content of the samples.

CONCLUSIONS

The results of the three applications discussed here demonstrate that a robotic workstation can provide nearly complete automation of many of the most tedious and time-consuming sample preparation procedures for liquid chromatography. Utilizing the BenchMate robotic workstation proved to be straightforward. The system's weight-tracking feature provided excellent reliability and precision. In principle, this workstation could be used for routine gas chromatography sample preparation and even simple derivatization procedures. The only drawback is its current inability to handle autosampler vials. This feature would be the most desirable option for such a robotic workstation in a chromatography laboratory.

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