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Short Communication

Quantitative reproducibility study with automated microcolumn liquid chromatography

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ABSTRACT

Studies were conducted to evaluate the quantitative reproducibility and long-term system stability for a reversed-phase microcolumn liquid chromatographic system. The relative standard deviation found was 0.38% for a major component and 0.7–1.6% for minor impurities using external standard calibration and automated sample injection. System longevity was studied over an eight-month operating period using repetitive injections of synthetic samples in an automated fashion. Excellent column stability was observed with minimal increase in operating pressure or observable loss in resolution. The results obtained suggest that microcolumn liquid chromatography is well-suited for routine applications.

INTRODUCTION

Miniaturization of a packed column liquid chromatography (LC) system using fused-silica columns was initially investigated almost a decade ago [1–5] with the recognized benefits of reduced consumption of mobile and stationary phases, increased mass sensitivity with concentration sensitive detectors, high separation efficiencies and posibility of new detection techniques [6,7]. More recently, the low volumetric dispersion given by such microcolumns has been exploited by interfacing in a multidimensional approach with capillary gas chromatography [8–11], conventional liquid chromatography [12,13], and capillary supercritical fluid chromatography [14]. Although studies in column preparation and system use have continued, the use of microcolumn LC has not yet found widespread routine utilization, mainly due to perceived difficulties of operation, instrument availability, and to the relative lack of published information concerning quantitative reproducibility and long-term system stability, which are of great importance to analysts involved in the solution of day-to-day problems. Recent reviews have summarized the practical details, theory and applications of microcolumn LC [15,16]. In order for the benefits of the technique to be fully exploited and for the technique to be more widely practiced, a microcolumn LC system should yield quantitative results which are equivalent to those obtained using conventional systems. Unfortunately, few quantitative studies using microcolumn LC have been reported [17].

In this paper, studies conducted in the evaluation of an automated microcolumn LC (Micro LC) system in terms of quantitative reproducibility and long-term system stability are described.

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EXPERIMENTAL

The system used consisted of an Isco LC 500 (Isco, Lincoln, NE, USA) syringe pump operated at constant flow-rate, which was connected to a Model A2N14W injection valve (Valco Instruments, Houston, TX, USA) equipped with a 100-nl internal rotor. The injection valve was either operated manually, or was automated using an air-driven Digital Valve Interface (Valco). In either case, the valve was allowed to remain in the inject position for two seconds prior to returning the load position.

An ABI 757 variable-wavelength detector (Applied Biosystems, Santa Clara, CA, USA) with a longitudinal flow cell [18] of 8-mm path length and 70-μm diameter (LC Packings, San Francisco, CA, USA) was operated at 214 and 254 nm.

Columns were made from fused silica (Polymicro Technologies, Phoenix, AZ, USA), 15 cm × 250 μm I.D. equipped with a porous ceramic bed support [19] and were packed at 400 atm with Spherisorb ODS, 5-μm particle diameter (Phase Separations, Haupagge, NJ, USA) as a slurry (5:1; in acetonitrile. The eluent used was acetonitrile–0.05 *M* KH_2PO_4 (30:70) at a flow-rate of 4.5 μl/min. An NEC portable 30386SX computer (NEC Information Systems, Boxborough, MA, USA) was used to control pump filling, sample introduction, collect raw data, integrate the chromatogram and store and plot the results, utilizing user-written software.

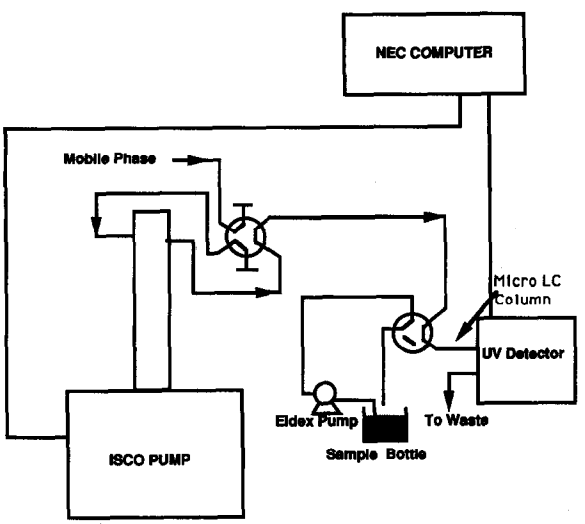

Fig. 1. Diagram of the automated microcolumn LC system.

An Eldex Model A high-pressure metering pump (Anspec, Ann Arbor, MI, USA) equipped with a 0.5-μm in-line filter (Alltech, Milwaukee, WI, USA) was used to continuously circulate sample through the injection valve. A diagram of the Micro LC system set-up is included in Fig. 1. A program was written for the computer to allow automated refilling of the pump, which has a 50-ml capacity. The slide switch at the back of the pump was set to external, and the interfacing to the Isco pump was accomplished through 5-V TTL level signals to the 37 pin D-sub connector. The manual 3-way valve of the pump was replaced with an air actuated 6-port valve (Valco). During pump filling, the outlet port of the pump and the line to the 4-port injection valve were blocked. After the pump piston reached the bottom of its stroke, the 6-port valve was rotated and the pump was switched to the constant pressure mode in order to rapidly repressurize the system. After allowing one minute to reach the desired column pressure, the pump was switched to the constant flow mode.

The sample used for the quantitative study consisted of 2,4-dichlorophenoxyacetic acid (2,4-D) as a major component and various related compounds, *o*-chlorophenoxyacetic acid (OCPAA), *p*-chlorophenoxyacetic acid (PCPAA), 2,6-dichlorophenoxyacetic acid (2,6-D), *p*-chlorophenol (PCP), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) and 2,4-dichlorophenol (2,4-DCP) (Pfaltz & Bauer, Waterbury, CT, USA and Aldrich Chemicals, Milwaukee, WI, USA). Compounds were dissolved in acetonitrile–water (50:50). Long-term system stability was studied using a solution containing acetophenone, phenol and methyl benzoate (Aldrich).

RESULTS AND DISCUSSION

Reproducibility studies were conducted using synthetic samples containing a major component and a series of minor impurities, as this represents a typical analysis conducted in the assay of chemical products.

Due to the geometry of the valco injection valve sample loops typically used for microcolumn LC [20] and because the components of interest were not soluble in a weaker solvent than the mobile phase in order to allow peak focussing [21] tailing

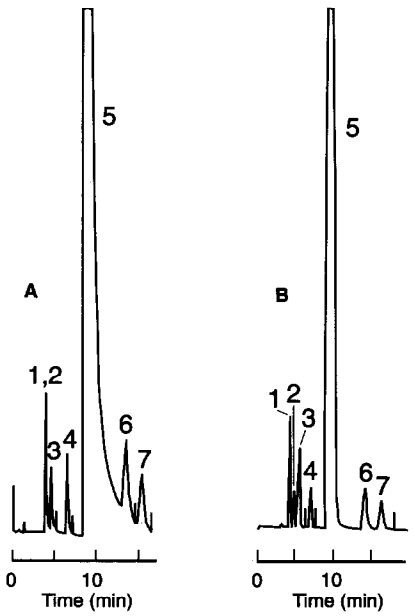

Fig. 2. Chromatograms of 2,4-D and related impurities. (A) Injection valve left in the inject position for the duration of the analysis. (B) Injection valve returned to the load position after 2 s. Column: 15 cm × 250 μm I.D. fused silica packed with Spherisorb ODS, particle diameter (d_p = 5 μm; eluent: acetonitrile–0.05 *M* KH_2PO_4 (30:70); flow: 4.5 μl/min; Injection loop size: 100 nl; detector: ABI 757, longitudinal flow cell of 8 mm path length; wavelength: 214 nm. Peaks: 1 = *o*-Chlorophenoxyacetic acid, 2 = *p*-chlorophenoxyacetic acid, 3 = 2,6-dichlorophenoxyacetic acid, 4 = *p*-chlorophenol, 5 = 2,4-dichlorophenoxyacetic acid, 6 = 2,4,6-trichlorophenoxyacetic acid and 7 = 2,4-dichlorophenol.

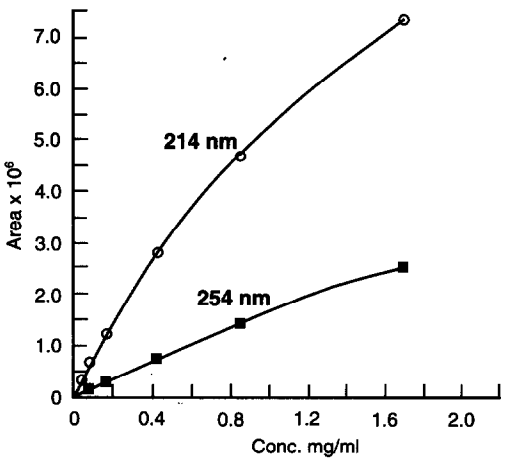

Fig. 3. Plot of response *versus* concentration obtained for 2,4-dichlorophenoxy acetic acid.

was observed in the resulting peak profiles when, during sample introduction, the valve was turned to the inject position and left there for the duration of the analysis, as illustrated in Fig. 2A. Returning the valve to the load position after a few seconds eliminated this problem as illustrated in Fig. 2B.

Detector linearity was evaluated by injection of varying concentrations of 2,4-D at 214 and 254 nm. In both cases deviations from linearity were observed at concentrations above 0.5–0.8 mg/ml (Fig. 3). Initial reproducibility experiments were conducted using a manual injection procedure (manual

TABLE I

REPRODUCIBILITY FOR THE ANALYSIS OF 2,4-DICHLOROPHENOXY ACETIC ACID USING MICROCOLUMN LIQUID CHROMATOGRAPHY (MICRO LC)

Compound	Manual injection (n = 11)			Automated injection (n = 11)		
	$\bar{x}^a$ (%)	T (%)	R.S.D. (%)	$\bar{x}^a$ (%)	T (%)	R.S.D. (%)
o-Chlorophenoxyacetic acid	1.43	0.03	2.0	2.45	0.02	0.73
p-Chlorophenoxyacetic acid	1.04	0.05	4.8	2.78	0.04	1.5
2,6-Dichlorophenoxy acetic acid	1.63	0.10	6.1	2.45	0.02	0.74
p-Chlorophenol	1.36	0.05	3.7	2.55	0.04	1.6
2,4-Dichlorophenoxyacetic acid	92.4	1.11	1.2	86.7	0.33	0.38
2,4,6-Trichlorophenoxyacetic acid	1.27	0.01	3.9	2.48	0.02	0.82
2,4-Dichlorophenol	1.52	0.05	3.3	2.28	0.03	0.40

[a] $\bar{x}$ = Mean value.

valve switching to the load position after injection), however the reproducibility obtained using this approach was considered unacceptable when compared to typical reproducibility values obtained using conventional LC systems (Table I). Automation of the injection process as described in the experimental section yielded much improved results, as illustrated in Table I. The reproducibility values [relative standard deviation (R.S.D.) of 0.38% for the major component] are comparable to results obtained using conventional LC systems.

The long-term system stability was evaluated using a solution containing acetophenone, phenol and methyl benzoate, and the system was automated to inject every 20 min. The Eldex pump was used to continuously circulate the sample solution through the 4-port injection valve. Filtering of the sample stream was considered important to insure that pressure buildup in the column due to particulate material did not occur. The system was operated continuously for eight months, using computer controlled refill of the pump cylinder. The same column was used throughout the study, and injections were performed at a rate of twenty per day during the first three weeks of the study. Thereafter, the injection rate was 3–5 per day for the remainder of the eight-month period. Retention time reproducibility over the eight-month period was 1% without any temperature control of the column or pump. Peak area response (R.S.D.) ranged from 0.7% to 1.2%. No significant pressure increase or column performance deterioration was observed.

Microcolumn LC has been shown to yield quantitative reproducibility which is considered as good as that obtained using conventional size liquid chromatographic systems. Automation of the injection process significantly improved reproducibility results. The stability and reliability observed over an eight-month operating period suggest that microcolumn LC is very suitable for routine applications.

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