AUTOMATED CARBON-COLUMN SYSTEM FOR CLEANUP OF POLYHALOGENATED DIBENZO-P-DIOXIN AND DIBENZOFURAN EXTRACTS

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ABSTRACT

A fully automated carbon-column LC system is described which provides for unattended processing of batches of up to ten (10) individual extracts from dioxin/furan sample preparation methods. The microprocessor-based system is constructed with commercially available hardware modules commonly found in environmental analysis laboratories, and performs automatic injection, elution and fraction-collection with minimal operator interaction.

INTRODUCTION

Carbon-column cleanup measures have seen widespread use in laboratories utilizing current analytical protocols for the determination of polyhalogenated dibenzo-p-dioxin and polyhalogenated dibenzofuran in environmental sample matrices. Current techniques are both tedious and time-consuming in the manual interaction demanded for their execution. Certain safety hazards exist, as well, in the handling of the concentrated extracts isolated from such samples.

Subsequent to confirming the utility of pump-fed LC systems for carbon column applications (vs. gravity-fed techniques) through comparison studies on extracts processed under common PCDD/PCDF methods, the investigators considered the potential for automating the entire process. Expected benefits were to be: 1) dramatically decreased operator involvement and a resulting increase in laboratory efficiency and, 2) increased safety through elimination of extended sample handling. The central theme for design and construction was the economical application of hardware common to labs involved in such work.

DESIGN and CONSTRUCTION

Overall Concept

The system is designed to perform in the same manner as a typical HPLC apparatus, through the use of an autosampler, injection valve, solvent pump, fraction collector and control system. The following additional components have been added to accommodate the carbon-column chromatographic scenario:

1) A stream-switching valve ahead of the solvent pump to provide selective delivery of the various solvents.
2) A six-port valve ahead of the separation column to provide switchable backflushing.
3) A ten-port, multi-column valve for the selection of a virgin column for each extract.

Overall control of the various components of the system is achieved through the use of an inexpensive, compact (6" x 4") microcontroller. This 6502-based microcomputer offers 4K of RAM, a real-time clock, 32 user-addressable input/output ports, and has a ROM-based, abbreviated BASIC interpreter built in. This controller proved to be an ideal choice, in that it allows the entire system to be run without the dedication of a large, expensive personal computer and the required additional I/O board and control program. User-specified control programs may be either typed-in prior to operation or burned into EPROM chips for permanent storage. The controller operates on 9VDC from a typical plug-in transformer. The EPROM plug will accept a variety of popular chips.

Autosampler

A Micromeritics 728 Autosampler (Micromeritics Inst. Corp., 5680 Goshen Springs Road, Norcross, GA 30093 USA) was chosen for automatic delivery of extract to the system. The autosampler delivers a user-determined amount of a known volume of total extract to the injection valve's fixed-volume sample loop. The autosampler cycle specifics are programmed on the front panel, with cycle initiation performed by the external microcontroller. Line rinses are achieved through "sampling" of solvent in alternate vials on the autosampler carousel.

Solvent Pump

Any HPLC solvent pump is suitable for this system. A pressure-sensor is recommended for automatic shutdown under clogged column or blocked tubing conditions.

Solvent Switching Valve

A Valco six-port rotary stream-switching valve (low pressure, teflon fittings) is utilized (VICI, PO Box 55603, Houston, TX 77255 USA) along with a Valco electric multi-position actuator. Control is applied externally from microcontroller. Solvent switching times are specified in the control program. Solvent inlets are applied to sequential ports of the valve in the order of their use to minimize switching steps during the elution processes.

Injection Valve

A Valco six-port switching valve (VICI) with 2-position electric actuator is used, with switching control coming from the external microcontroller. This valve holds a fixed-volume, interchangeable sample loop for regulated, quantitative delivery to the separation column.

Column-Selection Valve

A Valco 10-port CST-type column-selection valve with electric multi-position actuator is used to provide a separate column for each extract. External control is applied by the microcontroller. Spliced directly into the backflush valve, this valve sequentially switches each column into the sample path.

Backflush Valve

A Valco six-port switching valve (VICI) with 2 position actuator, controlled by the external microcontroller is used to direct the sample/solvent flow forward or backward on the separation column.

Fraction Collector

An ISCO Retriever II multi-fraction collector and Model 3100 Fraction Programmer are employed for the collection of the various fractions (ISCO, P.O. Box 5347, Lincoln, NB 68505 USA). The collection sequence is keyed into the programmer, with subsequent external triggering of the collection cycle by the microcontroller. Void volumes are programmed to a waste vessel, with fractions of interest delineated through the input of time windows.

Microcontroller

A Basicon MC1-i microcontroller (Basicon, Inc. 11895 N.W. Cornell Rd., Portland, Oregon 97229 USA) provides all control functions for the system, most of which are in the form of timed contact closures. This single-board unit utilizes a condensed, ROM-based BASIC programming language to control a 24-port peripheral interface port, providing TTL-level high/low signals at were developed to supplement the low sink/source nature of these port lines.

SYSTEM OPERATION

On initial use, the microcontroller is programmed by typing in the BASIC code for the desired sequence. Commonly used sequences may be transferred to the EPROM for non-volatile storage.

Freshly packed columns are inserted on the column-selection valve and conditioned with solvent either through manual triggering of the flow path or brief program control. Samples are loaded onto the autosampler carousel, and the wash cycle is keyed in on the front panel. After keying the desired time-windows into the fraction controller, the central microcontroller is started, sending all valves to the proper positions for loop-loading. Sample extract is loaded onto the loop, with subsequent switching for injection onto the column. The solvent selector performs the appropriate routings in tandem with the backflush valve, while the fraction collector distributes the various fractions into separate pyrex tubes. Simultaneously, the autosampler performs line-rinsing and indexes to the next sample. Subsequent to collection, the column-selector indexes to the next fresh column for the following injection while the injection valve returns to the loop-load position. An optional UV detector upstream from the fraction collector may be used to monitor effluents, potentially precluding the often expensive analysis of samples which are too concentrated or contaminated.

DISCUSSION

The described system reduces operator involvement by approximately 80% in comparison with manual, gravity-feed techniques. In addition, at least four physical sample-handling steps are removed from the procedure. Proper rinse timings and assembly of valve fittings prove to result in negligible system carry-over. The automatic, sequential solvent-selection and backflush-switching tasks provide a more reliable system in relation to potential human error in execution of the relatively high number of manipulations demanded in the manual procedure.

The use of an autosampler in this system--i.e., a device which does not quantitatively transfer all of the extract to the chromatography setup--imposes the requirement of starting the procedure with a **known volume** of extract, with subsequent consideration of the fraction of this total represented by the injection-loop volume.

Suggestions involving automated column-heating during the backflush mode will be investigated as potential enhancements to the overall performance of this sort of column cleanup system.