Full Automatic Clean-up Robot for Dioxin/PCB Analysis

Tohru Matsumura[1], Yuko Masuzaki[1], Atsushi Takahashi[1], Atsuko Koizumi[1],
Hiromitsu Okuyama[2], Yasuharu Kawada[2], Teruaki Higashiguchi[2]

[1]Environmental Risk Research Center, Institute of General Science for Environment,
METOCEAN Environment Inc., 1334-5 Riemon, Ohigawa, Shida, Shizuoka, 421-0212,
JAPAN
[2]Moritex Corporation, Yokohama Factory 1-3-3 Azamino-minami, Aoba, Yokohama, 225-0012, Japan

Introduction

Dioxin analysis requires several steps of clean-up procedures by combination of several column chromatography (e.g. silica gel column chromatography, carbon column chromatography) and sulfuric acid treatment. Full Automatic Clean-up Robot for Dioxin and PCB were developed.

Hardware

Robot is constituted by two apparatus. One is "sulfuric acid treatment unit" and another one is "Column Chromatography Clean-up Unit". Two apparatus can operate alone and/or combination.

Robot is constituted by syringe, pump, concentration flask, heater, stirrer, atomizer needle, needle cleaning port, column chromatograph, et al. These parts are assembled in X-Y dimensional arm (several piece of robot are shown in *Photo-1, 2 and 3)*.

To protect from line contamination, contentious on-line flow method was not employed in this system.

This robot treats six samples in parallel.

What jobs does robot do?

Put sample (hexane solution about 50mL) into the first stage of Sulfuric Acid Treatment Unit.

(A) Sulfuric Acid Treatment Unit

Put conc. H_2SO_4 into sample (hexane solution) → stirring → standing → remove H_2SO_4 by syringe → put H_2O into sample by syringe → stirring by stirring rod → standing → remove H_2O by syringe → dehydration by Na_2SO_4 → concentration by vacuum pressure and heating (0.5mL) → go To Column Chromatography Clean-up Unit (XY-arm of Column Chromatography Clean-up Unit bring samples from final stage of Sulfuric Acid Treatment Unit)

(B) Column Chromatography Clean-up Unit

Put sample (from) into multi-layer silica gel column → elution by hexane (max 250mL) → concentration (0.5mL) → put the sample into carbon column → elution by hexane (max 50 mL) → elution by (1:3) methylene chloride/hexane (max 50mL) → column reversion → elution by toluene (max 100mL) → concentration by vacuum pressure and heating for 3 fraction (50uL)

Sequence

Actions of robot are controlled by "FLOW". "FLOW" is combination of "SEQUENCE". A "SEQUENCE" has several "PROTOCOL". An image of "FLOW", "SEQUENCE" and "PROTOCOL" is shown (*Fig.-1*). An example of "SEQUENCE" and "PROTOCOL" for carbon column clean-up procedure is shown in *Table-1*.

Table-1. An example of "SEQUENCE" and "PROTOCOL" for carbon column clean-up procedure.

SEQUENCE	Carbon Column Fractionation by 3 different solvents
PROTOCOL	1. waiting after sample loading **[time (sec)]**
	2. elution by hexane **[volume (mL), flow rate (mL/min)]**
	3. waiting time after hexan elution **[time (sec)]**
	4. air blow after hexan elution **[volume (mL), flow rate (mL/min)]**
	5. elution by methylene chloride/hexane **[volume (mL), flow rate (mL/min)]**
	6. waiting time after methylene chloride/hexane elution **[time (sec)]**
	7. air blow after methylene chloride/hexane elution **[volume (mL), flow rate (mL/min)]**
	8. colume reversion **[on, off]**
	9. elution by toluene **[volume (mL), flow rate (mL/min)]**
	10. waiting time after toluene elution **[time (sec)]**
	11. air blow after toluene elution **[volume (mL), flow rate (mL/min)]**
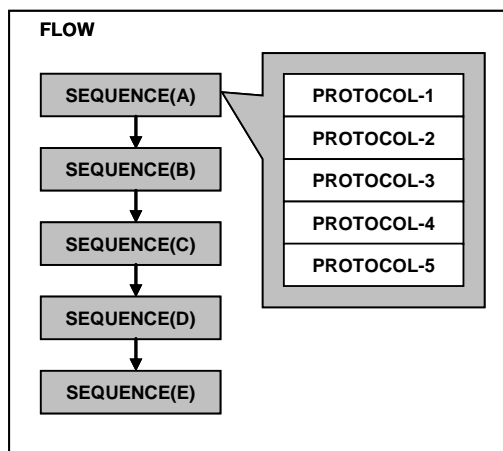
Advantage

Robot analysis has advantages compared with manual operations regarding precision/accuracy, reproducibility, cost and QA/QC. Robot writes all parameters and operation record to PC. As an example of auto recoding, a part of log file is shown in *Table-2*.

Fig.-1 An image of "FLOW", "SEQUENCE" and "PROTOCOL"

Table-2. An example of Auto Recoding (log file).

22:39:30 : **SEQUENCE NAME : Prime (Auto-Preparation before Start)**
        injection Syringe Cleaning 5(times)
        injection Syringe Plunger Stroke 50(%)
        injection Syringe Plunger Speed 22
        atomizer Needle Cleaning 3(times)
        atomizer Needle Plunger Stroke 50(%)
        atomizer Needle Plunger Speed 20

22:46:10 : **SEQUENCE NAME : Multilayer silica gel column pre-cleaning method**
        line-cleaning hexane volume 10(ml)
        line-cleaning hexane flow rate 10(ml/min)
        hexane flow rate 2.5(ml/min)
        hexane volume 200(ml)

22:46:10 multilayer silica gel column pre-cleaning (by hexane)

00:10:06 : **SEQUENCE NAME : multilayer silica gel column (1/2)**
        sample needle excess suction volume : 300(ul)
        atomizer volume : 1000(ul)
        waiting time after atomization : 20(sec)
        repeat : 3(times)
        injection Method : continuous mode

00:12:38 heater flask (A) suction volume 800

00:17:37 **SEQUENCE NAME : multilayer silica gel column (2/2)**
        hexane flow rate 2.5(ml/min)
        line cleaning hexane volume 0(ml)
        hexane volume 200(ml)
        air flow rate (after hexane elution) 10(ml/min)
        air volume (after hexane elution) 10(ml)

00:17:37 multilayer silica gel column (hexane elution)

01:39:30 **SEQUENCE NAME : Concentration**
        concentrator number 1
        concentrator temperature 50.0(DC)
        concentration limit time 240(min)
        concentrator temperature (after concentration) 25.0(DC)
        waiting time after concentration 60(sec)

01:39:37 – concentrator temperature 50.0(DC)
03:48:18 – concentration : concentrator No. 4 Finish : 128(min)
04:01:49 -- concentration : concentrator No. 6 Finish : 142(min)
04:04:00 -- concentration : concentrator No. 5 Finish : 144(min)
04:56:00 -- concentration : concentrator No. 1 Finish : 146(min)
05:01:02 -- concentration : concentrator No. 2 Finish : 151(min)
05:08:43 -- concentration : concentrator No. 3 Finish : 159(min)

Photo.-1 Concentrator for sample (hexane solution) after H_2SO_4 treatment

Photo.-2 Carbon column (Reversible)

Photo.-3

Concentrator for carbon column (fraction 3, toluene)