Comprehensive two-dimensional

supercritical fluid and
gas chromatography

(SFCxGC)

By

Andre Venter

Submitted in partial fulfillment of the requirements for the degree

Doctor of Philosophy

In the

Faculty of natural and agricultural sciences

University of Pretoria

February 2003
Comprehensive two-dimensional supercritical fluid and gas chromatography (SFCxGC)

Submitted by

Andre Venter

In partial fulfillment of the requirements for the degree

Doctor of Philosophy

Supervisor: Prof. E.R. Rohwer

Department of Chemistry

Faculty of natural and agricultural sciences

University of Pretoria

Summary

A novel chromatographic method was devised that makes use of the superb group separation power of normal phase supercritical fluid chromatography (SFC) combined with a fast second separation by a resistively heated gas chromatograph (GC). The SFC was operated isothermally with stopped flow to provide the time required for the GC analysis. The GC analysis had a typical cycle time of 1 minute. During this time the GC column was independently heated at a rate of 450°C/min to 250°C and actively cooled down again to -50°C before the next GC injection takes place. This was achieved with an in-house designed, resistively heated, temperature programmable gas chromatograph. Various temperature measurement circuits were also evaluated. An interface was developed that allows transfer between the SFC and the GC in such a way that the entire eluent from the first separation is analyzed by the second separator. Chromatographic resolution was not lost during the transfer process from the first to the second separation stages. The interface also allows for the exchange of the carrier gas used in the second gas chromatographic separation to provide for the maximum separation speed. In the first separation, a silica gel packed column and the novel application of a silica gel porous layer open tubular capillary column was used for SFC group separation. The SFCxGC_{np} was applied to petrochemical samples and essential oils and the results were compared to that obtained with a commercially available GCxGC system.
# Chapter 1
## Introduction
1.1 Project history ................................................................. 1
1.2 Background ............................................................................ 2
   1.2.1 Chromatography in a nutshell .............................................. 2
   1.2.2 History ............................................................................ 2
   1.2.3 Modern Chromatography .................................................... 3
   1.2.4 Multidimensional Chromatography ....................................... 4
   1.2.5 Comprehensive multidimensional chromatography .............. 5
1.3 Comprehensive supercritical fluid and gas chromatography ......... 7
   1.3.1 Comments on existing SFCxGC attempts ............................. 7
   1.3.2 Advantages of SFCxGC .................................................... 8
1.4 Approach ............................................................................. 8
1.5 Presentation and arrangement ................................................ 9

# Chapter 2
## Fundamental principles of comprehensive multidimensional chromatography
2.1 Multidimensional techniques ................................................ 11
2.2 Multidimensional chromatography .......................................... 11
2.3 Comprehensive Multidimensional Chromatography ................ 12
2.4 Orthogonality ....................................................................... 12
2.5 Resolution in two-dimensional chromatograms ....................... 13
2.6 Peak capacity of comprehensive multidimensional systems ........ 16
2.7 Sample dimensionality and Ordered Chromatograms .......... 20
2.8 Historical overview of multidimensional instrumentation ............. 23
   2.8.1 Thin layer chromatography .............................................. 23
   2.8.2 Planar column chromatography ......................................... 23
   2.8.3 Electrophoretic techniques .............................................. 24
   2.8.4 High performance liquid chromatography. HPLC x HPLC ....... 24
   2.8.5 Gas chromatography GC x GC .......................................... 25
      2.8.5.1 Modulators ............................................................ 25
Chapter 3

Fast Gas Chromatography: Theoretical considerations

3.1 Introduction ........................................................................................................ 29
3.2 Optimization of resolution for fast gas chromatography ................................. 31
3.3 Optimization of separation speed ...................................................................... 35
    3.3.1 The influence of capacity factor ................................................................. 36
    3.3.2 The influence of selectivity ......................................................................... 37
    3.3.3 Influence of carrier gas flow rate and pressure drop .................................. 37
    3.3.4 The influence of column radius .................................................................. 37
    3.3.5 The influence of diffusion coefficients ....................................................... 38
    3.3.6 The relative contributions of column diameter v/s carrier gas identity ...... 39
3.4 Temperature programmed analysis .................................................................. 40
    3.4.1 Heating rates ............................................................................................. 40
    3.4.2 Normalized heating rates ......................................................................... 41
    3.4.3 Default Optimum Heating Rate .................................................................. 41
3.5 Achieving fast heating rates .............................................................................. 42
    3.5.1 Resistive heating ......................................................................................... 42
    3.5.2 Methods of making columns electrically conductive ................................. 43
    3.5.3 Methods of sensing temperature ............................................................... 44
    3.5.3.1 Resistance measurements ....................................................................... 45
    3.5.3.2 Resistance measurement with superimposed AC signal ....................... 46
    3.5.3.3 Separate sensing wire ............................................................................ 46
    3.5.3.4 Infrared temperature sensing ................................................................. 46
    3.5.4 Temperature Control ................................................................................ 46
    3.5.5 The Control variable ................................................................................. 46
3.6 Chapter conclusion ............................................................................................ 50
Chapter 4
Fast gas chromatography: Design, construction and evaluation

4.1 Introduction ........................................................................................................51
4.2 Instrumentation ...............................................................................................52
  4.2.1 Column and electrical connections ...........................................................52
  4.2.2 Current Control .......................................................................................54
  4.2.3 Temperature sensing ...............................................................................55
    4.2.3.1 Philips circuit ..................................................................................56
    4.2.3.2 Current mirror circuit .......................................................................57
    4.2.3.3 The Resistance Measurement Circuit ..............................................58
    4.2.3.4 Thermocouple Measurements .........................................................59
    4.2.3.5 Fixed current ramp .........................................................................61
  4.2.4 Data collection .........................................................................................61
  4.2.5 Reproducibility .......................................................................................61
  4.2.6 Optimization of rate ...............................................................................62
4.3 Results and discussion ....................................................................................62
  4.3.1 General comments ..................................................................................62
  4.3.2 Additional heating of injector and detector legs ......................................64
  4.3.3 The Philips circuit ..................................................................................65
  4.3.4 The current mirror circuit .......................................................................68
  4.3.5 Resistance of heating element by Ohms' law ..........................................68
  4.3.6 Thermocouples ......................................................................................71
    4.3.6.1 Thermocouple basics ......................................................................71
    4.3.6.2 Very small thermocouples ...............................................................72
    4.3.6.3 Peak profiles in comparison to a stirred air bath GC .......................72
    4.3.6.4 Thermocouple placement ...............................................................73
    4.3.6.5 Stability problems ..........................................................................75
  4.3.7 Fixed function without feedback .............................................................75
  4.3.8 Retention time reproducibility .................................................................75
4.4. Conclusions ...................................................................................................81
Chapter 5
SFC: Theoretical considerations

5.1 Introduction ..........................................................................................................83
5.2 Supercritical fluids defined ..................................................................................84
5.3 Supercritical fluids as mobile phase in chromatography ......................................85
  5.3.1 Diffusion coefficients ....................................................................................86
  5.3.2 Viscosity .........................................................................................................86
  5.3.3 Solvation ........................................................................................................88
5.4 Parameters affecting retention in SFC .................................................................89
  5.4.1 Density ...........................................................................................................90
  5.4.2 Pressure ..........................................................................................................91
  5.4.3 Temperature ...................................................................................................92
5.5 Stationary phases used with SFC .........................................................................94
5.6 Using phase ratio to reduce retention of oxygenates ............................................94
5.7 Conclusions ..........................................................................................................97

Chapter 6
SFC: Demonstration of group separation

6.1 Introduction ..........................................................................................................98
6.2 Experimental .........................................................................................................99
  6.2.1 Instrumentation for packed column PAH group separation .......................99
  6.2.2 Instrumentation for PLOT column separation .............................................100
6.3 Results and discussion .......................................................................................101
  6.3.1 Demonstration of the group separation of petrochemical samples using a silica gel packed column .........................................................101
  6.3.2 Investigations into the group separation achieved with the PLOT column 102
    6.3.2.1 PLOT column oxygenate elution pattern .............................................102
    6.3.2.2 The influence of temperature on group resolution ................................103
    6.3.2.3 The influence of pressure on group resolution .......................................104
    6.3.2.4 Comments on flow rates and runtimes ...............................................105
    6.3.2.5 Applications of the Silica gel PLOT column ......................................107
6.4 Conclusions ........................................................................................................109
Chapter 7
The Modulator: Background and literature survey

7.1 Introduction........................................................................................................110
7.2. The modulator...................................................................................................110
7.3 Stationary Phase Focusing..............................................................................114
7.4 The sweeping arm thermal modulator .........................................................115
7.5 The Cryogenic Modulator..............................................................................116
7.6 Diaphragm Valve Modulator..........................................................................117
7.7 Non-mechanical modulators ..........................................................................117
  7.7.1 Thermal modulation with hot and cold gas jets.......................................117
  7.7.2 Resistive multi-segmented thermal-gradient modulator ......................118
7.8 Conclusion .......................................................................................................118

Chapter 8
The modulator: Design, construction and characterization

8.1 Introduction.......................................................................................................119
8.2 Suggested modulator designs ........................................................................120
  8.2.1 Two-stage continuous modulator with pressure modulation ..............121
  8.2.2 Stopped flow pressure modulation .........................................................122
8.3 Experimental....................................................................................................123
  8.3.1 Hardware design ......................................................................................123
  8.3.2 Demonstration of the interface .................................................................124
8.4 Results and Discussion ...................................................................................126
  8.4.1 Number of modulation stages .................................................................126
  8.4.2 Run time of the modulated 1st dimension chromatogram ..................126
  8.4.3 The influence of modulation on SFC flow rates .....................................127
  8.4.4 Advantages to the stopped flow interface .............................................128
  8.4.5 Influence of stopped-flow modulation on the SFC chromatogram ......129
  8.4.6 Modulation programming ..........................................................130
8.5 Conclusions....................................................................................................130
Chapter 9
Demonstration of the comprehensive two-dimensional SFCxGC

9.1 Introduction .................................................................131
9.2 Experimental..............................................................133
  9.2.1 The supercritical fluid chromatograph .........................133
  9.2.2 The modulator .........................................................133
  9.2.3 Resistively heated gas chromatograph .........................134
  9.2.4 Description of the operation of the SFCxGC .................136
    9.2.4.1 Control before a run ..........................................136
    9.2.4.2 Control during a run ..........................................137
    9.2.4.3 Data collection and handling ..............................137
9.3 Results and Discussion ................................................138
  9.3.1 Chromatograms obtained with the packed column ..........138
    9.3.1.1 Analysis of a standard mixture ............................138
    9.3.1.2 Analysis of a commercial petrol sample ................141
    9.3.1.3 Analysis of a diesel sample .................................143
  9.3.2 Chromatograms obtained with the PLOT column .............146
    9.3.2.1 Analysis of a standard mixture of oxygenates ..........146
    9.3.2.2 Analysis of an unleaded petrol sample ..................149
    9.3.2.3 Analysis of a diesel sample (Natref LCO) ...............149
    9.3.2.4 Analysis of a lemon essential oil .........................153
9.4 Conclusions ..............................................................153

Chapter 10
Conclusions

10.1 Group separation with SFC as a first separation dimension ..154
10.2 Fast resistive heating for boiling point distribution in SFCxGC ...........................................155
10.3 The stopped flow pressure drop modulation interface ........157
10.4 Advantages to SFCxGC ...............................................157
10.5 SFCxGC Applications .................................................158
List of LabVIEW Programs

(Requires LabVIEW 5.1 or higher)

1. Fast GC Phillips circuit
   a. Simple control.llb
      • Run simple control.vi
   b. Temperature calibration.llb
      • Run temperature calibration.vi

2. Fast GC Resistive heating circuit
   a. Simple control.llb
      • Run simple control.vi
   b. Temperature calibration.llb
      • Run temperature calibration.vi

3. Fast GC with thermocouple
   a. Fast GC.llb
      • Run fast GC.vi
   b. Simple control.llb
      • Run simple control.vi

4. SFCxGC
   a. SFCxGC.llb
      • Run SFCxGC1.vi

5. Data compilation
   • Run columns-to-matrix.vi
Acknowledgements

I would like to express deep gratitude to my supervisor, Professor Egmont Rohwer. Through your wise guidance, encouragement and support, I grew not only as a scientist, but also as a person.

Thank you, Mr. Tony Hasset for your continuous helpfulness, encouragement and patience. Without you, research in our labs would be much harder.

I would like to thank Mr. Gerrie Roos and Dr. Fanie vd Walt for your valuable contributions in explaining electronics and designing circuits.

Thank you, Fabian Muelburger for your help with the data compilation program.

Thank you, Peter Makgwane for watching over the machine and all your help.

And thank you, to all my colleagues, Dr. Collion Chen, Dr. Erla Harden, Alexander Whaley, Maria Fernandes-Whaley, Werner Welthagen, Emmanuel Zellelow who contributed in more ways than I can mention.

Thank you, Eugene Smit for editing the text.

This research was made possible thanks to grants from The Foundation for Research Development, financial support from SASOL and from Scientific Development and Integration (Pty) Ltd together with the THRIPP fund. I would like to express special thanks to SDI for providing a supportive work environment that allowed me to finish this thesis.