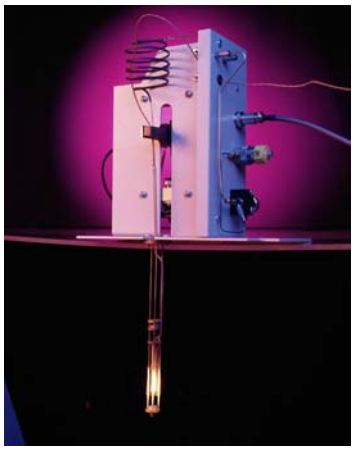
Development of Comprehensive Two-Dimensional Gas Chromatography Technology for Wine Research



Cryogenic modulator device developed at RMIT



GC×GC-TOFMS system in the RMIT Laboratory

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **RMI 01/01**

Principal Investigator: **Professor Philip Marriott**

Research Organisation: **RMIT University**

Date: **5 May 2004**

Title of Project:
Development of Comprehensive Two-Dimensional Gas Chromatography Technology for Wine Research

Author Details:
Professor Philip Marriott
Professor of Separation Science
School of Applied Science
RMIT University

5 May 2004

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Abstract:

The aim of this project was to evaluate the new technology of comprehensive two-dimensional gas chromatography (GC×GC) for a broad range of application possibilities in the wine analysis area. The technique provides enhanced separation power, and also increased sensitivity for volatile chemical analysis, both of which are relevant for the analysis of aroma components of wine. Gas chromatography is therefore widely used for this type of analysis, but often, insufficient resolution of compounds makes conclusive results difficult to achieve. The model studies that were conducted using GC×GC included general essential oil analysis for technique evaluation, then solid phase microextraction (SPME) of resveratrol from wine, and chiral analysis of selected small aroma compounds from wine.
Based on these studies, further research will be carried out with AWRI on specific projects.

Executive summary:

The GWRDC funding permitted a number of targeted applications to be conducted, which explored the capabilities of GC×GC to offer benefits to the analysis of volatile compounds in wine matrices. The RMIT University group has pioneered the development of GC×GC, primarily through its innovative program that developed a cryogenic modulation system, which is the heart of the technique. In order to demonstrate its broad utility, important strategic applications have been undertaken that offer unique solutions to chemical analysis problems. Often these problems arise from collaborators with whom we work, in order to provide direct experience with those who require high quality separations analysis.
The RMIT laboratory has also recently installed a GC×GC-TOFMS system, that provides even further identification capability for separated chemicals. In anticipation of using this equipment, the present study sought to show through a number of initial analyses that important and wide-ranging studies would be possible for wine characterization and analysis of target analytes for a variety of purposes.

This project funding was therefore modest, and directed towards for pilot applications. It was to be used for some consumables purchases, a visit to the AWRI, and to partially support attendance at a meeting by the PhD student who worked on this study, in lieu of providing a minor scholarship. All other equipment requirements were covered by the available equipment in the RMIT laboratory.

The project outcomes were:

- Demonstration of the use of GC×GC for general essential oil analysis
- The use of GC×GC combined with solid phase microextraction for analysis of resveratrol in wine. This required *in situ* chemical derivatisation for the extracted resveratrol, on the SPME device.
- The ability for GC×GC with chiral columns to resolve enantiomers and geometrical isomers of various flavour compounds using headspace and liquid injection methods.

Based on the understanding that has been achieved, and the publications produced, along with the new instrumental capabilities that we have, there is an interest in now working more closely with organisations such as the AWRI to develop more substantial projects that employ GC×GC in its full capacity for advanced chemical analysis tasks.

Background:

Over the past 3 years, the Chromatography and Molecular Separations Group of Professor Philip Marriott, Department of Applied Chemistry, RMIT University has developed and patented a new mechanism for conducting capillary gas chromatography. It uses a modulating cryotrap for very effectively and rapidly zone compressing and pulsing compounds from one column to another. This cryotraping concept has not previously been described in column GC. It can be most powerfully used when located at or near the joining of two capillary columns; the first column being of regular dimensions, and the second being short and narrow bore, so that very fast elutions are achieved on this column. If the two columns are of different stationary phase, it is then possible for compounds that are not resolved on the first column, to be separated on the second. This can be achieved over the total sample analysis, but in this case, it is necessary to modulate the pulses at a faster rate

than the peak elution width. This generates a series of successive pulses for each component. It also allows the chromatogram to be presented as a two-dimensional separation space. In this space, each compound is now represented as a discrete point of contour. In total, the number of peaks than can theoretically be separated is the product of the peak capacity on the first column, multiplied by that on the second column. This could be for example, 400 x 10 = 4000 peaks. No single column can ever reach this separation performance. Concomitant with this separation, we achieve normally about a 20-fold increase in sensitivity, since very narrow peaks are generated at the end of the second column. We now have established a range of applications that demonstrate this power, and those in the essential oil area are most relevant to the proposed wine component study here.

New Technology – new Opportunities

As an indication of the new possibilities that are offered, it is appropriate to highlight selected studies that demonstrate the new information which can be derived.

Atmospheric organic analysis has been conducted for many years. Our study of Melbourne urban atmospheric samples, showed that many of these prior studies were probably erroneous in their conclusions, having failed to measure many of the relevant compound classes. An estimate was that our new method allowed up to 40% more organic constituents to be measured in the samples. This analytical study was published in Nature (2000), confirming the significance of the observations. Subsequent to this, we have applied the new technique to exhaust gas analysis, using the sampling method of SPME.

Recently we have had much success in chiral GC analysis, both in single column and comprehensive two-dimensional gas chromatography modes. We have shown that it is possible to measure the chiral ratios of target compounds in complex mixtures, such as essential oils. We have also applied our methods to the measurement of target compounds in perfumes, which is not too dissimilar to the analysis requirements of fragrance compounds in natural samples such as wines.

With the successful pilot study completed as part of the initial pilot study with the AWRI we are now able to undertake more substantial studies of importance to the wine analysis area.

The initial project aims were:

1. To evaluate a broad range of application possibilities for the new high resolution, sensitive comprehensive two-dimensional gas chromatography method in the wine analysis area. This remains a long-term goal as we explore the use of GCxGC in this area.
2. To develop specific applications to demonstrate the capabilities and further identify projects that will benefit from the procedures that can be introduced..

Project Aims and Performance targets:

The project aims were open-ended. Because they involved the demonstration of the new technology, which would then identify further opportunities, the major aim was to select a number of typical studies that were of interest to collaborators (e.g.AWRI) and to demonstrate the results that could be achieved. The interest has been sufficiently positive to lead to a number of new applications, such as the analysis of wine lactone in a real wine matrix, using chiral phase columns to resolve the isomers, and GC×GC to separate these from wine matrix components. This study is currently underway. Performance targets for the RMIT group, whilst not set directly for this project, remain the publication of original research results. This was achieved in the present project.

Method:

The general approach to chemical analysis by using GC methods was followed. Sampling by using SPME was also conducted. These were not part of the research goals, which was to evaluate GC×GC, but are integral parts of the method.

The development of a GC×GC method involves:

Selecting the appropriate injection / introduction procedure

Selection of the proper column set that should maximize the 'orthogonality' of the separation. We use a low polarity / high polarity column set for essential oil-type analyses.

Select the optimum conditions for the analysis – temperature program, modulation rate, data acquisition rate, length of second dimension column. Adjust according to initial results.

Normally, it is necessary to try to compare results with one dimension analysis, or with GCMS, but this is usually futile because the separation achieved by these methods are inadequate. Now, we use the GC×GC-TOFMS system for confirmation of separated peaks.

Each study had a different requirement according to the introduction and sampling method, and the types of columns to be used.

Results/Discussion:

(i). General essential oil analysis – analysis of ginger volatiles
This showed the general principles of SPME headspace sampling for a variety of different ginger samples, and the reproducibility of the position of peaks in 2D separation space – an important parameter for routine analysis. The ability to analyse for very small components in the presence of major components that would otherwise interfere was apparent.

(ii) trans-resveratrol analysis
SPME sampling using a polar fibre was used for solution-sampling of resveratrol in wine. The fibre was then dried in a nitrogen flow to remove water traces, then derivatised in the headspace of a derivatising fluid, to allow the volatile derivative of resveratrol to be made. Injection into the GC then permitted analysis of this in wine and spiked wine samples.

(iii) Small flavour compounds in wine.
2- and 3-methyl isomers of butanol, butyric acid, and the acetate and ester were sampled, and separated on a chiral first dimension column, by using GC×GC. These volatile compounds required a thicker phase trapping column to be used with the cryotrapping step, but very good modulation peak shapes were achieved. The ability to separate these components from matrix interferences that occur in single column GC was shown. Both spiked wine and authentic wine samples were analysed.

Outcome/Conclusion:

Project outputs

These were successful in that GC×GC was demonstrated to be a suitable technique for wine aroma and flavour analysis. The selected studies proved that GC×GC is able to offer much better sensitivity and separation power for these applications. For the student working on the project, the published papers met the requirements of a research degree. In this respect, the intended performance targets were achieved.

The only outstanding study required fro this area is o use the new TFOMS system for mass spectrometry detection of separated compounds, and in particular for isotopically enriched samples. This is the next phase of the collaborative study with AWRI.

Future opportunities
These research results should now be used as a springboard for further evaluation of the trace and complex chemical flavour compounds in wine and grape. Wherever GC is needed for a specific analysis task, where complex samples are to be analysed, there will be an obvious opportunity for GC×GC to provide superior analytical data. The system developed in RMIT is simple and effective, and not costly. Improved data handling systems are still required. There are a number of new studies that are needed, as outlined below.

The project essentially was designed to demonstrate the proof of concept for use of GC×GC for wine analysis. The benefits to users will be when the system is tested in applications that can deliver specific analysis objectives, such as chiral analysis for enantiomeric compounds that allow wine quality to be evaluated, or the study of complex pyrazines in grape berries, or any other similar study.

Recommendations:

There a number of future research directions that are of interest, that GC×GC can address. These are listed as research questions;
Can GC×GC be used to provide a comprehensive flavour and volatile profiling tool, such that the chemical characterization of these compounds can be revealed in a single analysis?
Can these results be used to provide a method of establishing wine type or variety, and even region?
Can statistical (chemometric) methods be used to compare and contrast wines, and maybe understand further understand 'metabonomic' issues in wine.
Are there other target analysis areas of interest (e.g. amino acid profiles) that should be now investigated?

Some of these studies may impinge on viticultural practices, that assist in following flavour generation and maturation processes in grape berries, in different climatic and environmental conditions.

Appendix 1: Communication:

The investigation of comprehensive two-dimensional gas chromatography (GC×GC) technology for application to advanced separations in wine analysis was described in two separate papers:
(i) Use of solid phase microextraction (SPME) for resveratrol analysis in wine, with on-fibre derivatisation and GC×GC.
(Y. Shao, P. Marriott, H. Hügel. Solid-Phase Microextraction/On-Fibre Derivatization with Comprehensive Two Dimensional Gas Chromatography analysis of *trans*-Resveratrol in Wine. *Chromatographia* 57(**2003**) S349-353)
(ii) Application of enantiooselective separations systems for chiral analysis of wine flavour components (2- and 3-methyl isomers of butanol, butyric acid and its esters and acetates).
(Y. Shao, P. Marriott. Separation of Positional Isomers by the Use of Coupled Shape-Selective Stationary Phase Columns. *Anal. Bioanal. Chem.,* 375 (5) (**2003**) 635-642)
The latter of these was an analytical problem that the AWRI sought a method for.

Two further publications featuring essential oils analysis that assisted our understanding of the analytical procedures developed with the support of this project were:
(iii) Solid-Phase Microextraction – Comprehensive two-dimensional gas chromatography of Ginger (*Zingiber officinale*) Volatiles.
(Y. Shao, P. Marriott, R. Shellie, H. Hugel. *Flavour Fragr. J.*, 18 (**2003**) 5-12)
(iv) Ultra-High Resoloution Capillary Gas Chromatography by Using Cryogenic Modulation.
(P. Marriott, R. Ong, R. Shellie, R. Western, Y. Shao, R. Perera, L. Xie, A. Kueh, P. Morrison. *Aust. J. Chem.,* 56 (**2003**) 187-191).
Recently, AWRI has requested our assistance in analysis of wine lactone and its deuterated analogues in a real wine matrix, in order to overcome interferences that compromise conventional analysis. Our GC×GC–TOFMS system should be critical in providing high-level analytical data.

Appendix 2: Intellectual Property:

NIL

Appendix 3: References

NIL

Appendix 4: Staff

Professor Philip Marriott (Project leader)
Ms Yajing Shao (PhD candidate; performed all the analysis)
Dr Danielle Ryan (Post Doctoral researcher; will offer future support to on-going projects)
Mr Paul Morison (Technical assistance)

All team members are at Australian Centre for Research on Separation Science (ACROSS)
RMIT University

Appendix 6: Budget reconciliation

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION
Statement of Receipts and Expenditure - **FORM B**
For the year ending 30 June 2003

Trust Fund :	RESEARCH TRUST FUND	FUNDING	$5000
Project No :	RMI 01/01	Salaries	
Grantee :	Prof Philip Marriott	Travel	800
Title of Project :	Development of comprehensive two-dimensional gas chromatography technology for wine research	Operating	4200
		Capital	
		Total Funding	5000

EXPENDITURE										
	Salaries		Travel		Operating		Capital		Total	
	$	¢	$	¢	$	¢	$	¢	$	¢
A Uncommitted (c/f 1 July)										
B Outstanding Commitments (c/f 1 July)										
C Refunds of funding										
D Cash Received From Trust Fund									5000	00
E Approved transfers (from Form C)										
F Cash available (A+B-C+D±E)										
G Expenditure									5000	00
H Outstanding Commitments (30 June)									0	
I Total funds Committed (G-H)									5000	00
J Uncommitted (30 June) (F-I)										
K Other income (Paid to Trust Funds)										

Note : *Row B should be the same as Row H from the previous year and Row A the same as Row J from the previous year.*

I hereby certify that this statement of expenditure is correct.

PHILIP J. MARRIOTT — Printed Name
13 Apr 2004 — Date

Expenditure Summary

Total Income to RMI 01/01 & Continuation of project	2001 $5,000 2002 $5,000
TOTAL	**$ 10,000**
Expenditure	2001 $417.60 2002 $5315.07 2003 $4891.04
TOTAL	**$ 10,623.71**
(balanced of expenditure over income covered by transfer from other grant sources)	
Note that surplus of 2001 grant carried forward to continuing grant in 2002	

Breakdown of Expenditure

2002 Expenditure	
Consumable materials	$4322.03
Minor equipment	$765.45
Travel to AWRI Adelaide	$227.59
TOTAL	**$5315.07**
2003 Expenditure	
Consumables	$3197.32
Travel	$1693.72
TOTAL	**$4891.04**