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Simultaneous Nitrogen, Sulfur, and Mass Spectrometric Analysis after Multi-Column Switching of Complex Whiskey Flavour Extracts

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KEYWORDS

PTV-Injection, multi column switching, cryotrapping, capillary GC-MS, simultaneous mass selective/sulfur chemiluminescence/nitrogen detection, flavour compounds

SUMMARY

Interesting odour active fractions of great complexity can be obtained from whiskies by high efficiency fractional distillation using the combined azeotropic behaviour of the compounds of interest and the dominant ethanol and water matrix components.

One of these fractions in particular contains many sulfur and nitrogen compounds and the identification and corresponding organoleptic contribution of these compounds is important. The complexity of the sample presents substantial difficulties for single column mass spectrometric investigation and therefore two-dimensional cutting is the preferred approach.

The present work extends this scheme to include further additional cutting after the second column to achieve simultaneous nitrogen, sulfur, and ms investigation of the heart cuts. The procedure uses a computer-controlled user-friendly multidimensional GC system, incorporating Graphpack technology for leak-free connections with precise cutting, and represents a significant saving in total analysis time.

INTRODUCTION

Distilled spirits represent very complex mixtures of aroma compounds in an ethanol-water matrix [1]. The relative flavour contribution of these secondary aroma congeners can vary by orders of magnitude and it is quite often the case that compounds present at trace levels can exert a far greater influence on the perceived sensory characteristics than other compounds present at much higher levels. The situation is further complicated by the general problem of locating trace compounds in the presence of less interesting major components. Therefore special chromatographic techniques and detectors are required to locate and identify such low threshold trace species, with special emphasis on nitrogen and sulfur compounds.

Once an extract or concentrate of the required compounds is prepared a suitable chromatographic system must be employed to allow separation and resolution of the species of interest. By far the most effective approach for this purpose is two-dimensional capillary gas chromatography, and initial use of the specific detectors to locate the compounds of interest in the pre column chromatogram for subsequent cutting and transfer to the main column [2]. Multidimensional chromatography must be user friendly for acceptance as a routine technique outside the realms of research institutes and the Gerstel apparatus used in this work represents the commercial evolution of such a system. All cutting and transfer operations are achieved without pulsing and in automated user friendly operation. Use of micro coupling pieces incorporating Graphpack ferrules allow both pre- and main column chromatography indistinguishable from injection to a single column.

In the present work this scheme is extended to include a cross piece after the main column so that the chromatographed cut is further distributed simultaneously to a mass selective detector and specific nitrogen and sulfur detectors. This cross piece offers micro coupling design advantages similar to the column switching device and produces three simultaneous channels of information for each initial injection and subsequent cut. This approach allows a complex extract to be quickly investigated and profiled without having to disconnect and reconnect the main column to the various detectors. Grob [3] has pointed out difficulties when using the alternative press-fit connectors and T-pieces when used with recently introduced fused silica columns. Reliable leak-free connections were difficult to achieve and the problem seemed to lie in lack of bonding at the connector/T-piece conical seat. Leak-free connections are a prerequisite for any connector or distributor and use of a purpose designed cross piece with Graphpack ferrule technology offers the best technical solution for this purpose.

EXPERIMENTAL

Instrumentation. Figure 1 represents a compilation of the various components used to configure the system employed for this work. The apparatus consists of a temperature programmable cold injection system (CIS-3, Gerstel GmbH, Mülheim an der Ruhr, Germany), a multi column switching system (MCS A, Gerstel GmbH, Mülheim an der Ruhr, Germany), two HP 5890 GC ovens (Hewlett-Packard, Avondale, USA), connected by a cryotrap interface (CTS-1, Gerstel GmbH, Mülheim an der Ruhr, Germany). The second oven is equipped with a mass selective detector (HP 5971 A, Hewlett-Packard, Avondale, USA), a chemiluminescence sulfur detector (Sievers Instruments, Colorado, USA), and a nitrogen thermionic detector (TID-2, Detector Engineering and Technology, USA). Both the sulfur and nitrogen detectors use existing FID bases of the GC and are relatively easy to move between the two ovens for monitoring either the pre-column chromatogram, or main column chromatogram after transfer of a cut.

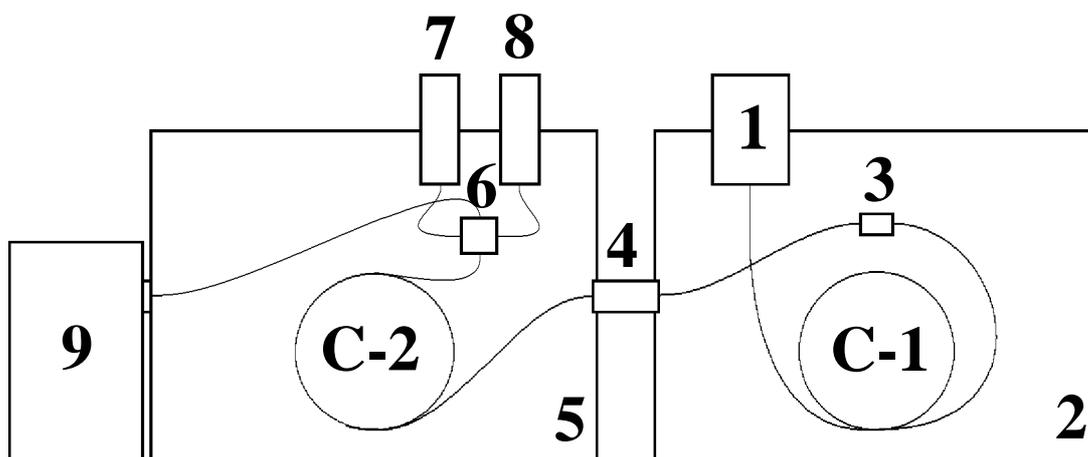


Figure 1. Schematic diagram of the applied system which consists of a temperature programmable cold injection system with a septumless sampling head (1), a GC (2) configured with a monitor FID, column switching device (3) and pneumatics, connected via a heated transfer line incorporating a cryotrap (4) to a second GC (5) which has a micro cross-piece (6) installed after the main column with short column segments to nitrogen (7), sulfur (8) and msd (9) detectors.

The Sievers chemiluminescence detector offers significant advantages for trace sulfur compound analysis in complex mixtures. The detection mechanism is based on FID flame conversion of organic sulfur to sulfur monoxide followed by monitoring the chemiluminescent reaction of the sulfur monoxide with ozone. This two-step requirement makes the detector both particularly specific and immune from quenching due to co-eluting compounds. A recent study [4] emphasised this latter advantage in a comparative evaluation of the SCD with an atomic emission detector (AED).

Analysis conditions.

Columns:

Pre-column in GC 1	15 m Carbowax 20M		
	$d_i = 0,25 \text{ mm}$	$d_f = 0,25 \mu\text{m}$	
Main column in GC 2	60 m 5% Diphenylpolysiloxane		
	$d_i = 0,25 \text{ mm}$	$d_f = 0,25 \mu\text{m}$	
Column segments to selective detectors	5 m 5% Diphenylpolysiloxane		
	$d_i = 0,25 \text{ mm}$	$d_f = 0,25 \mu\text{m}$	
Column segment to MSD	15 m 5% Diphenylpolysiloxane		
	$d_i = 0,25 \text{ mm}$	$d_f = 0,25 \mu\text{m}$	

Pneumatics:

Carriergas	He	$p_i = 240 \text{ kPa}$	split x:30
Control flow		$p_c = 175 \text{ kPa}$	10 ml/min
FID	H ₂ , 30 ml/min	Air, 300 ml/min	N ₂ , 30 ml/min
FID / SCD	H ₂ , 300 ml/min	Air, 200 ml/min	N ₂ , 10 ml/min
TID-2	H ₂ , 5 ml/min	Air, 60 ml/min	

Temperatures:

CIS	60°C; ↗ 280°C;	12°C/s
Oven 1	60°C; ↗ 250°C;	5°C/min
Oven 2	60°C; ↗ 220°C;	3°C/min
CTS	200°C; ↘ -150°C;	12°C/s
	↗ 200°C;	12°C/s
FID	250°C	
FID / SCD	280°C	
TID-2	350°C	
MSD	280°C	

Detectors:

Monitor detector in GC 1	FID
Main detectors in GC 2	FID / SCD (Sulfur-selective)
	TID-2 (Nitrogen-selective)
	MSD (Mass-selective)
	Scan 30-350 amu

For column switching and transfer of cuts the cryotrap is cooled with LN₂ from its normal temperature of 200°C to -150°C two minutes before the cut, followed by heating at 25°C/second to 200°C for “re-injection” of the focused cut to the second column and to the cross-piece and three detectors. All devices (both oven programs, cryotrap, msd etc.) are linked by remote start cables so that a single injection to the pre-column initiates the total program sequence. The SCD and TID-2 signals were monitored on a dual terminal Hewlett-Packard 5880A GC using external analogue inputs. By removing the SCD probe from its FID this can alternatively function as a normal FID.

RESULTS

Figures 2a, b, c represent three analyses of a complex whiskey extract run consecutively on a single column and into three separate detectors (FID, TID-2 and SCD). The traces serve to highlight the problem that mass spectrometric investigation of many of the indicated nitrogen and sulfur compounds would be very difficult in such a complex matrix.

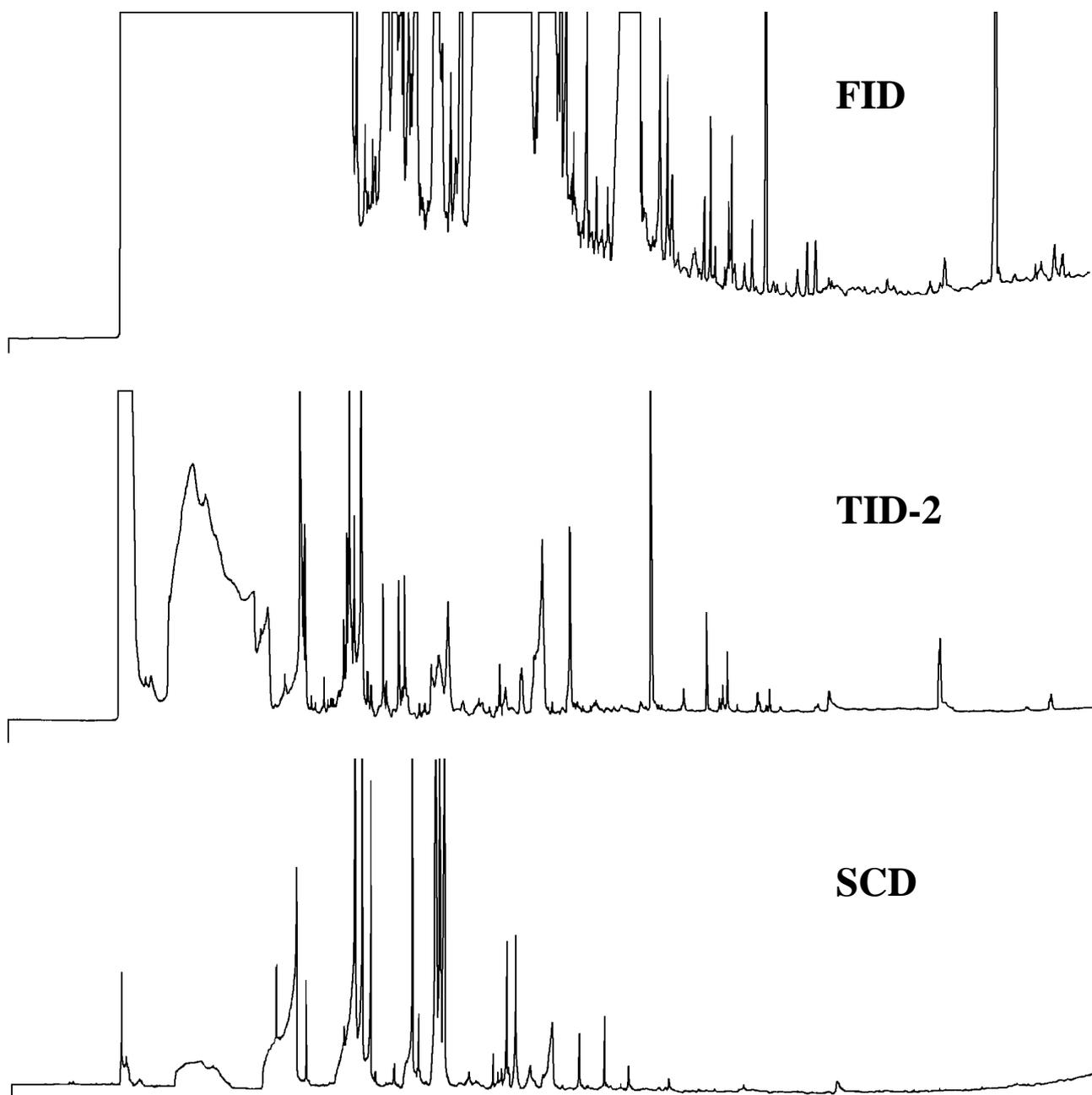


Figure 2. Single column analysis of a whiskey extract with separate FID-, Nitrogen- and Sulfur-detection.

A two-dimensional chromatographic capability is practically a mandatory requirement to achieve a solution and the present work incorporates this approach with additional subsequent simultaneous cross-piece distribution to the three detectors.

The apparatus was assembled as outlined in **Figure 1**, but for purposes of initially testing the integrity of the cross-piece the main column in the second oven was connected directly to an injector in the same oven. **Figures 3a, b, c** show the simultaneous traces from injection of a ppm level test solution consisting of two sulfur compounds, a pyrazine, and a thiazole containing both heteroatoms. The chromatography is more than acceptable and the simultaneous patterns show the utility and productivity of the technique.

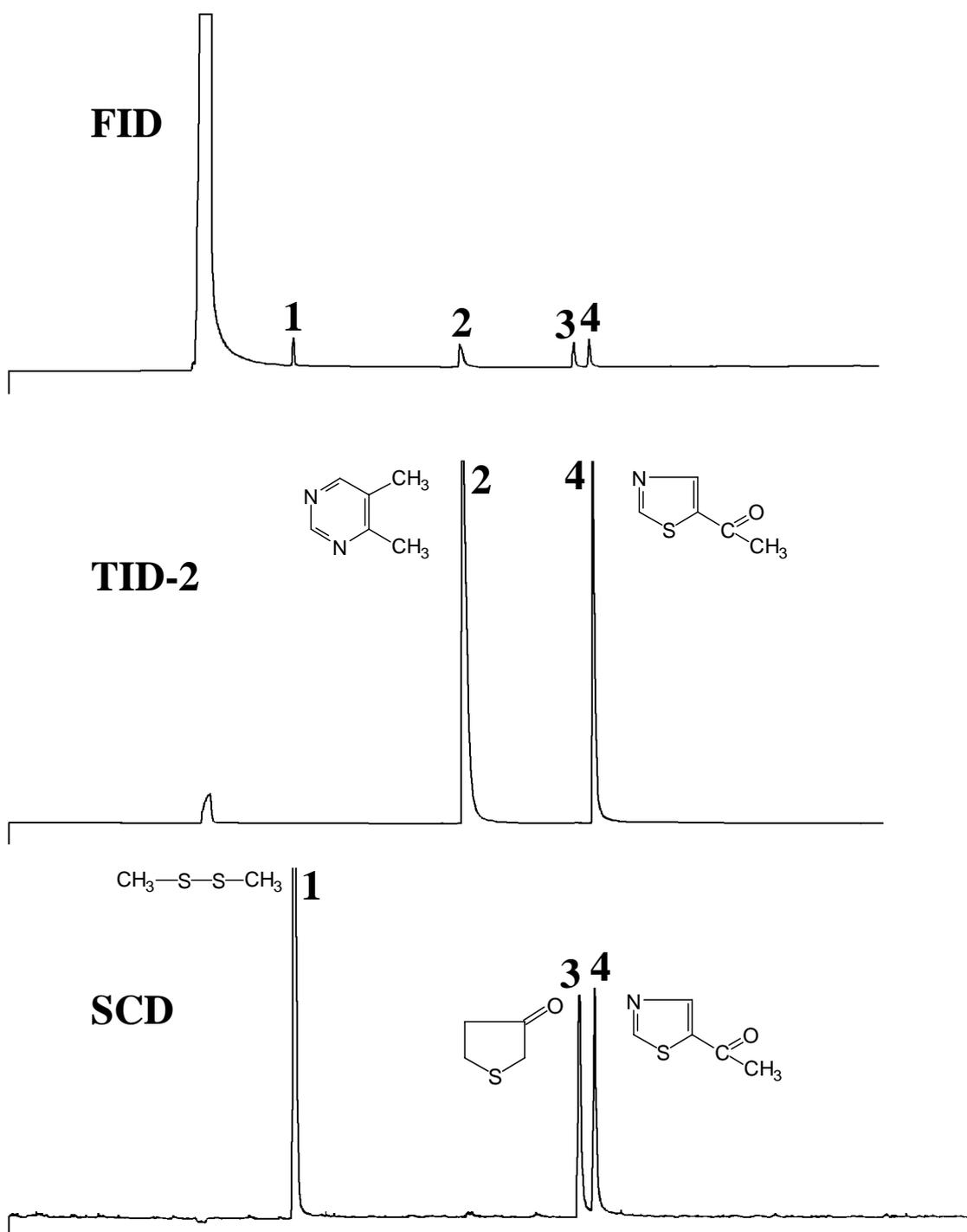


Figure 3. Single column analysis of a test-mix with simultaneous FID-, Nitrogen- and Sulfur-detection.

The main column is then connected to the pre-column to establish the full configuration (**Figure 1**), and **Figures 4a, b, c** and **d** show a selected cut from the pre-column, which is cryofocused and passed to the main column for simultaneous ms, sulfur and nitrogen detection. The resolution gain indicated by the TIC trace guarantees the homogeneity of the vast majority of these peaks, and the required sulfur and nitrogen species can be easily located in the TIC trace from their respective specific traces.

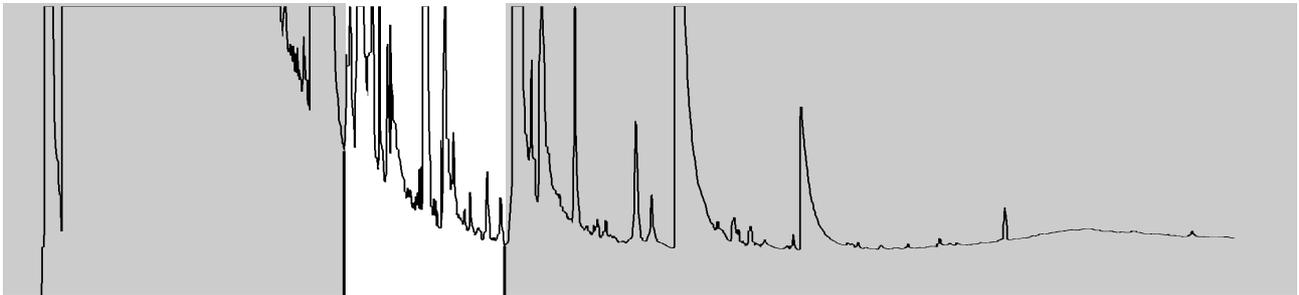


Figure 4a. Pre-column chromatogram.

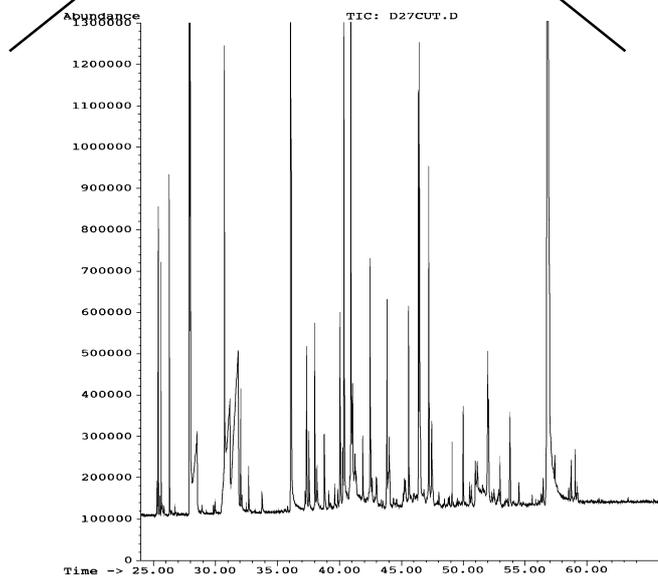


Figure 4b. Main column chromatogram, TIC.

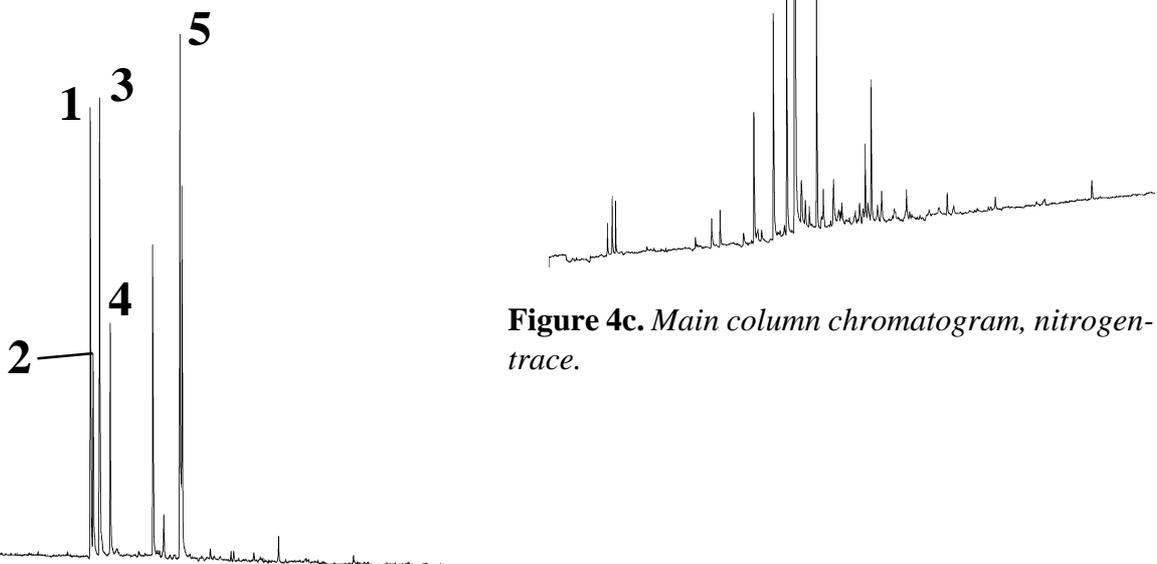


Figure 4c. Main column chromatogram, nitrogen-trace.

Figure 4d. Main column chromatogram, sulfur-trace.

The integrity of the cross-piece, and indeed the total system, can be further seen in **Figures 5a** and **b**. Here the TIC and FID runs are compared using the same cut as for **Figure 4**. The configuration now is cross-piece to MSD, NPD and FID, i.e. the SCD probe is removed to allow this detector to revert to normal FID function. The similarity of the traces indicates a very high degree of confidence in the system design and performance.

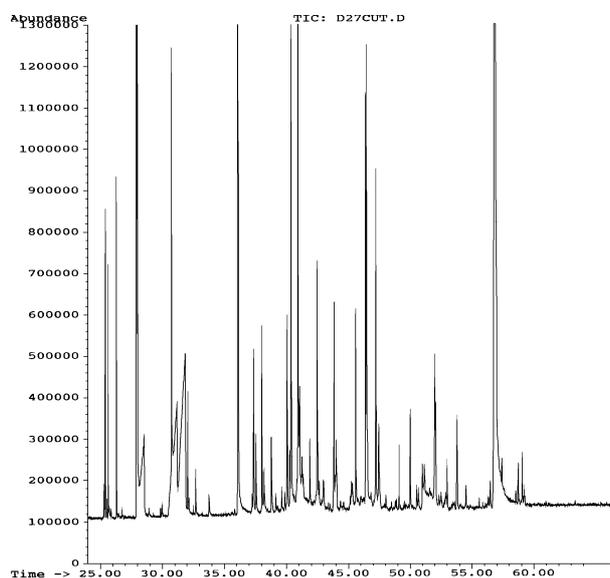


Figure 5a. Main column chromatogram, TIC.

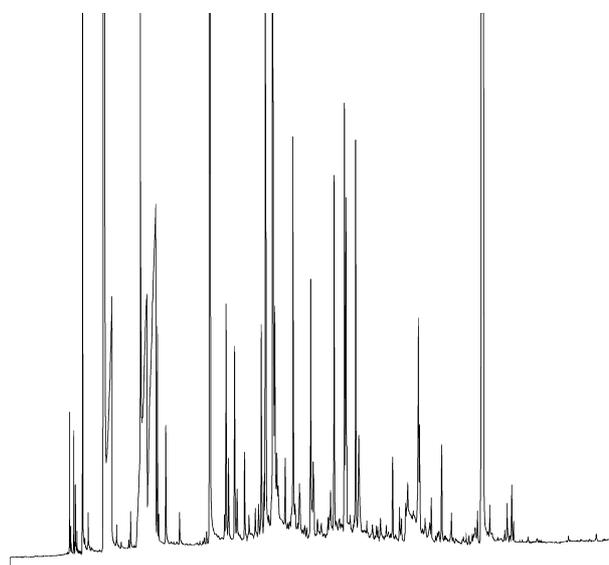


Figure 5b. Main column chromatogram, FID.

Figure 6 identifies examples of some sulfur and nitrogen compounds found by this approach, and their ms elution pattern can be easily matched to the specific sulfur and nitrogen traces. It is clear that injection of a complex sample ten to fifteen times with successive cuts from the pre-column trace would allow rapid MS location of unknown sulfur and nitrogen compounds in the sample.

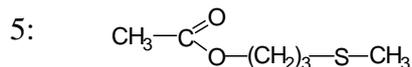
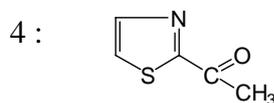
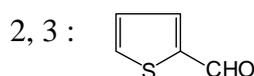
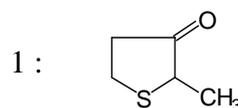
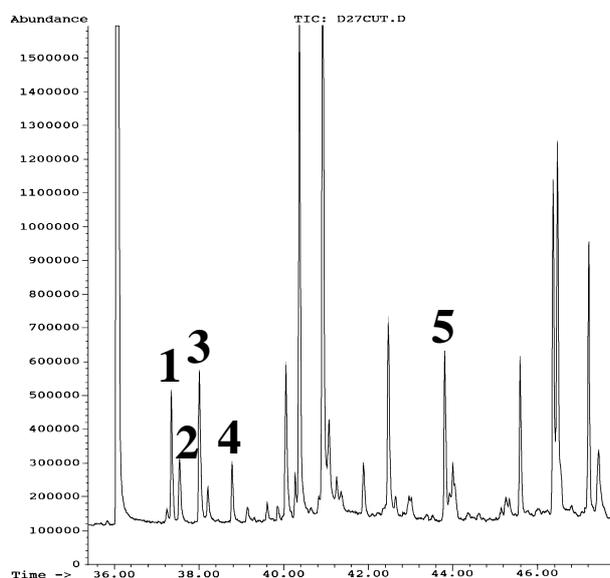


Figure 6. Identification of some sulfur and nitrogen containing compounds.

The cryofocusing function can be effective to ensure good peak shape for all transferred compounds eluting from the second column. It also becomes more necessary in the case of more volatile compounds or as the temperature difference between a transfer and the second oven starting temperature decreases. **Figures 7a** and **b** show a cut with and without cryofocusing and the earlier peak deformation without cryofocusing is obvious.

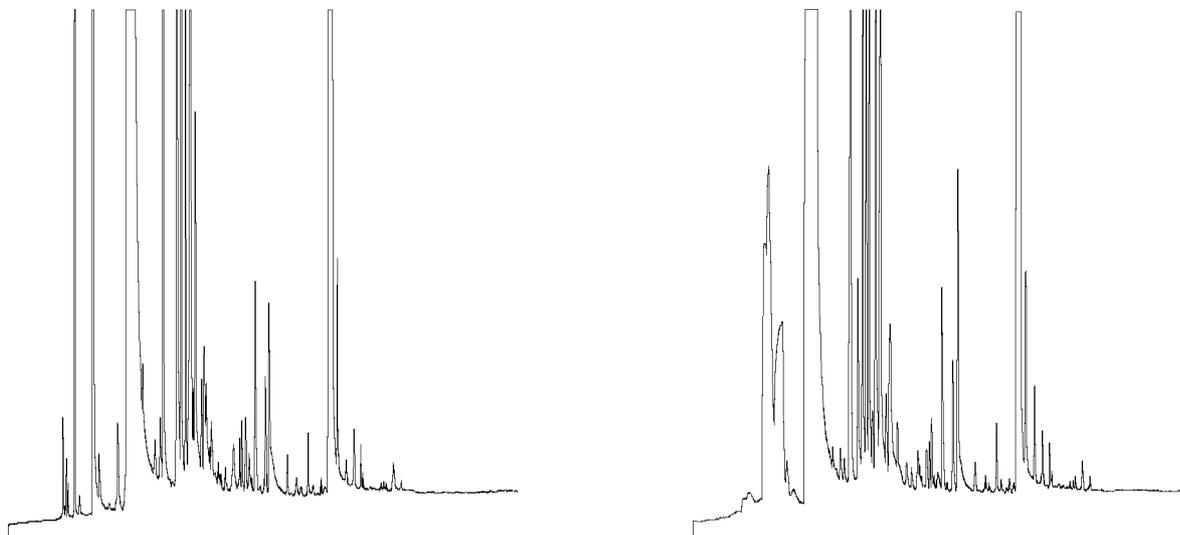


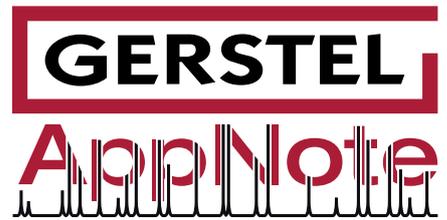
Figure 7a. *Main column chromatogram, with cryofocusing.* **Figure 7b.** *Main column chromatogram, without cryofocusing.*

CONCLUSIONS

Advances in gas chromatographic simplification of complex samples can be obtained by carefully integrating available equipment and detectors to produce powerful, user-friendly productive systems. Of primary importance for the success of this approach is the quality and superior design of the coupling pieces, hardware, and software, to achieve this integration. The Gerstel system used in this work represents such a successful approach to complex alcoholic beverage flavour analysis.

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