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A new, effective and robust Method to couple Gas Chromatography and Isotope Ratio Mass Spectrometry

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1 Abstract

A new method to couple gas chromatography (GC) to isotope ratio mass spectrometry (IRMS) is presented. The crucial step in all types of GC/IRMS interfaces is removal of the solvent. While conventional interfaces feature this process after the GC column, it is performed before any solvent enters the column in the new interface. This vastly simplifies the device and numerous connections can be abandoned. As a consequence chromatographic resolution as well as maintenance effort is improved dramatically.

2 Introduction

The possibility to perform compound specific isotope analysis (CSIA) by coupling gas chromatography to isotope ratio mass spectrometry (GC/IRMS) on principle is known since 1976 [10]. This development represents a major breakthrough in the field of stable isotope analysis, especially regarding the measurement of the bioelements at natural isotopic abundances. It has facilitated enormous progress in scientific disciplines as diverse as geology, ecology, biochemistry, and the forensic sciences, to name but some. In doping control for instance, it now provides the possibility to successfully discriminate physiological steroid production from the illicit administration of the corresponding synthetic hormone [3, 2, 1, 11, 12, 5].

The crucial process in GC/IRMS is the online conversion of organic compounds into simple gases suitable for analysis in a gas isotope ratio mass spectrometer. In case of carbon and nitrogen stable isotope analysis this is virtually always performed by quantitative oxidation of the analytes into CO_2 and NO_x, where nitrous oxides subsequently are converted to N_2. Therefore the technique is often referred to as GC/C/IRMS, the ’C’ standing for ’combustion’. Before combustion may be performed, the complete removal of any solvent is required. Otherwise the excess of the solvent rapidly would destroy the combustion reactors,

which possess restricted reservoirs of metal oxides as oxidizing agents. Although the release of oxygen is not required here the same holds true on principle for isotope analyses of D/H and $^{18}O/^{16}O$ by thermal conversion (GC/TC/IRMS), a recently introduced technique [8]. Solvent residues will be pyrolysed and then will plug up the reaction tube.

The necessity of proper solvent removal affects the technique, because numerous valves, tees and capillary connections are employed for this purpose. The emerging dead volumes may largely affect the chromatographic separation. An excellent resolution however is badly required for valid isotope ratio measurements because peak overlap may induce dramatic systematic error.[6, 4] Moreover the critical parts reside inside the GC cabinet where they suffer severe thermal stress. Therefore GC/IRMS systems are prone to leakage, which in turn renders maintenance extraordinary tedious and time consuming.

We therefore designed a GC/IRMS system which achieves complete removal of the solvent before the chromatographic separation process is started. The key to this is employment of a cooled injection system which is operated in solvent vent mode. Due to residual pressure in the injection port however small amounts of solvent still enter the GC column and hence the reactor. Therefore solvent removal is supported by an auxiliary pump which generates a rough vacuum in the split line.

Due to this design, nearly all the critical parts mentioned before could be removed from the system while complete solvent removal still is guaranteed.

3 Experimental

A CIS 4 cooled injection system (Gerstel, Mülheim, Germany) was mounted into an Agilent GC model 6890 (Agilent Techn., Palo Alto, CA, USA). The GC was coupled to Delta plus XP gas isotope ratio mass spectrometer (Thermo, Bremen, Germany) by a GC combustion interface CI III (Thermo). The latter was modified as depicted in figure 1.

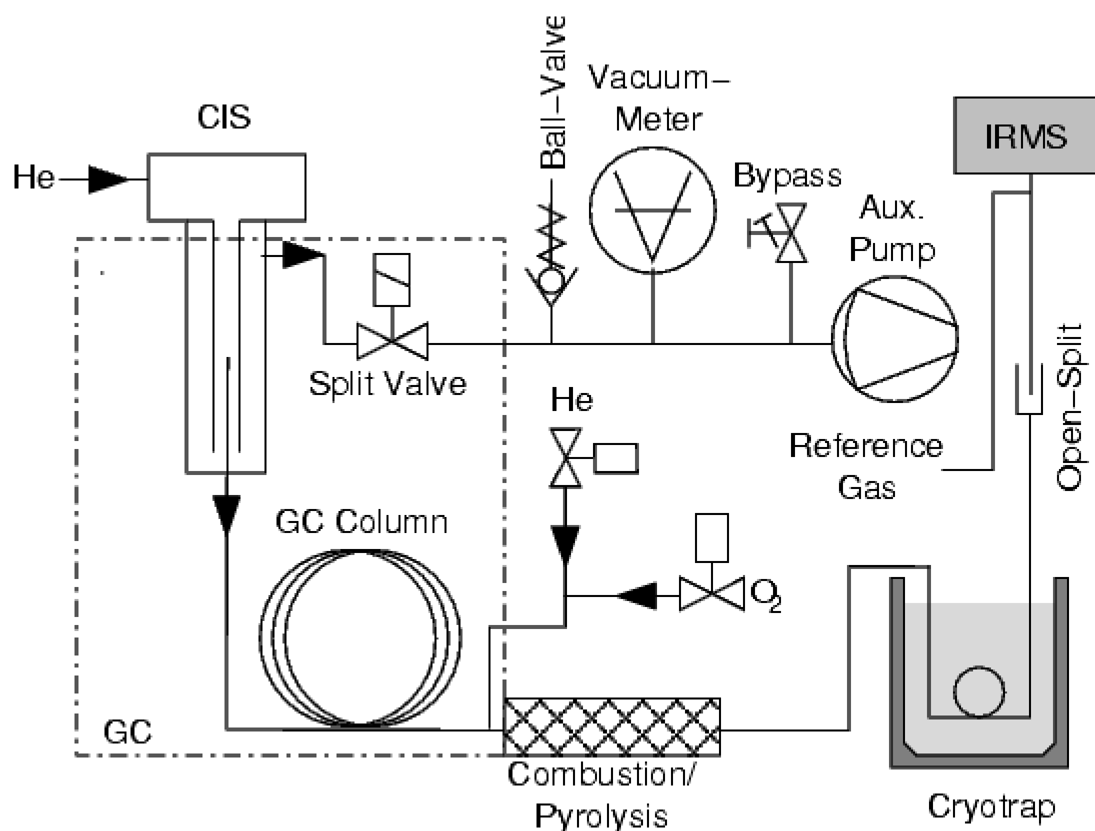

Figure 1: Schematic drawing of the new GC/IRMS interface

The capillaries, unions, and tees required for the backflush system were removed. The Nafion(R) watertrap was repleced by a Dewar vessel containing a dry ice/acetone mixture (ca. -70°C). The reactor itself now is connected to the GC column by a post column reaction tee (Valco Instruments, Houston, TX, USA). This tee serves to recharge the reactor with oxygen. Moreover a permanent helium flow of roughly 0.3 mL/min is added here. This purges the connection and hence any dead volume is virtually removed. Another important advantage is emergence of a self sealing effect which avoids the necessity of application of excessive torque in order to manage a leak tight connection. This always bears the risk of breaking the reaction tube.

The split line of the GC now is connected to an all purpose laboratory pump. The latter is operated during the solvent vent interval and is started *via* a relay. A rough vacuum of *ca.* -0.3 bar is generated which can be adjusted by a controllable bypass. For safety reasons a ball valve is incorporated into the split line. This guarantees outflow of the carrier gas under any circumstances.

4 Results & Discussion

The removal of the solvent was complete, even when large volume injections were performed. At least seven *mu*L acetone could be injected without emergence of a solvent peak. However a signal of *ca.* 250 mV height and of upto 60 s width could be observed during early intervals. Its intensity showed some variation with the vaccum in the split line and with the the injected volumes. Most likely this is due to impurities in the solvent. Usage of freshly distilled solvents caused disappearance of this signal. Anyway the intensity of this ghost signal is comparable to that of moderately concentrated analytes an does not affect the interface design with any restrictions.

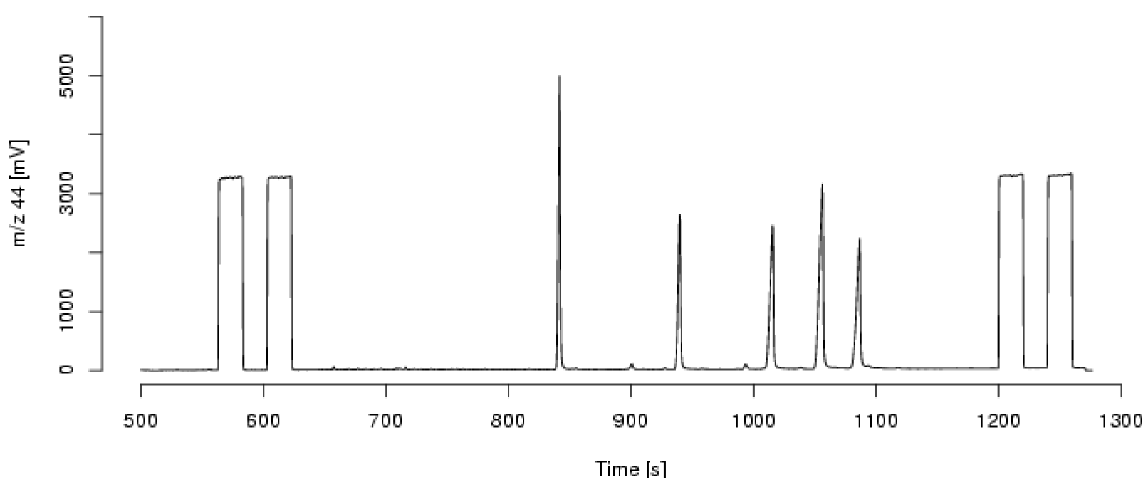

Figure 2: GC/C/IRMS analysis of a standard mixture five different undervatized steroids. In the order of elution: 3α-androstanol, etiocholanolone, 11-ketoetiocholanolone, 5βpregnane-3α,20α-diol, 11-hydroxyandrosterone.

Figure 2 shows the result of a GC/C/IRMS analysis of an underivatized steroid standard mixture. The peaks appear sharp, narrow, and symmetrical. The compounds contain up to three oxygens. Obviously even relatively polar steroids can be analysed on the new interface without the requirement of derivatisation. This is considered a big advantage, because three major problems induced by derivatisation can be circumvented. 1) Degradation of the reactor (silicon or fluorine in the derivatisation moiety), 2) adulteration of the original isotope ratios of the analytes, and 3) bias due to isotope effects during the derivatisation reaction.

A more detailed view of the analysis is presented in figure 3. It also shows the comparison to a measurement of the same standard mixture on an unmodified interface.

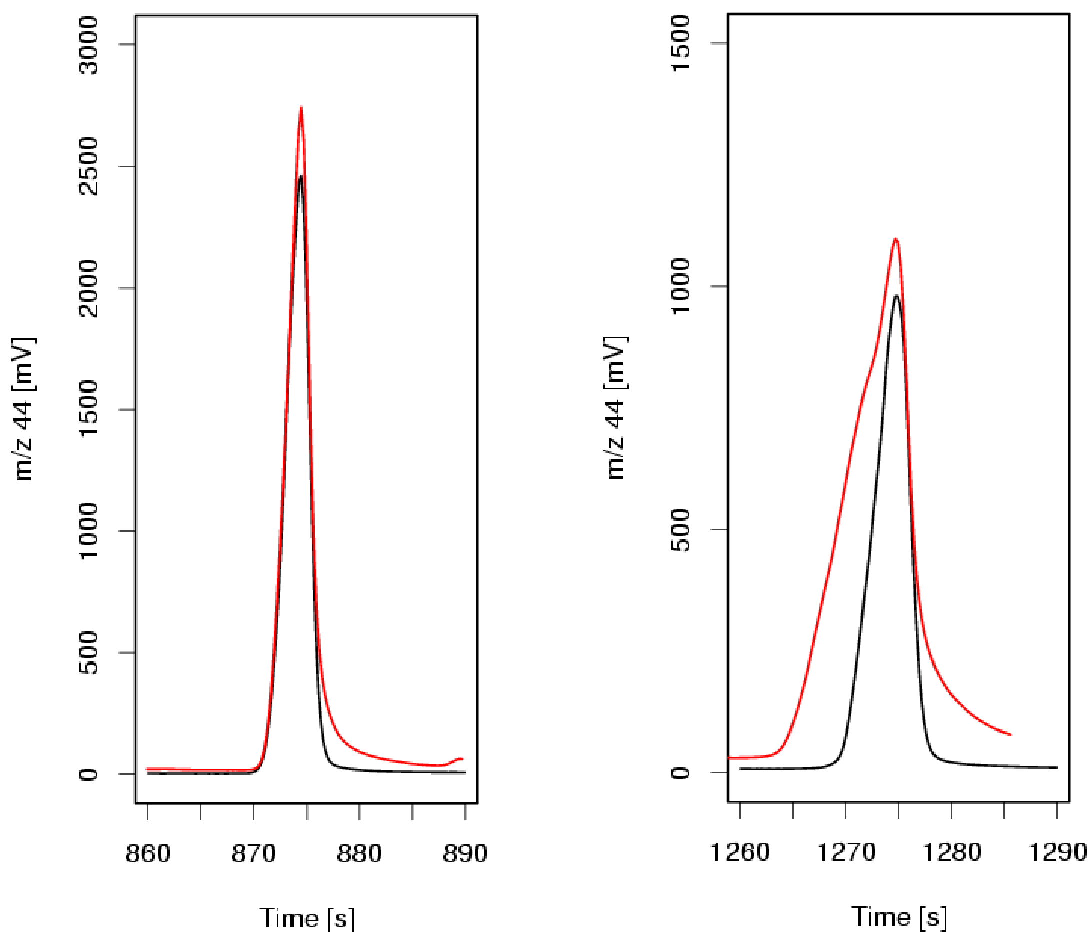

Figure 3: Comparison of the signals obtained by measurement on a conventional and on the new GC/C/IRMS system. The retention times are normalised to identical values. Only the earliest (androstanol, left panel) and the latest eluting compound (11-hydroxyandrosterone, right panel) are shown.

It becomes clear immediately that the advantages of the new interface are especially pronounced in respect to high boiling compounds. Small but significant improvements can be observed for androstanol. The analysis of 11-hydroxyandrosterone exhibits excessive tailing on the conventional interface and this is virtually absent on our new interface. Also fronting is largely reduced by the new design. However, some fronting still remains. We attribute this to overload of the column rather than to the properties of the interface. Obviously the conventional interface mostly blurs fronting by the general broadening of the peak and hence column overload never became an issue.

The necessity to modify existing GC/IRMS interfaces in order to improve chromatography

and maintenance was previously recognised. Goodman [7] developed an interface which took advantage from droppping the backflush design. However, a rotary valve inside the GC was employed to vent the solvent. Hence mechanical parts still were exposed to thermal stress and the solvent still was allowed to pass the column. The Goodman-interface moreover was characterized by a fused silica capillary which was fed through the combustion furnace and which housed the copper wires required for oxidation. Rogers and Trout [9] adopted this idea and suggested an interface which still was operated in backflush mode. The development of these interfaces obviously has been discontinued, most likely because of the very short lifetime of fused silica capillaries at temperatures required for quantitative combustion.

The maintenance of our new interface is much easier than that of any conventional one and does not suffer from the recently mentioned restrictions present in the alternative designs. The numerous connections in conventional interfaces usually become leaky sooner or later. The mounting of new columns and the exchange of expendable items (especially glass pressfits) is delicate and time consuming and it always bears the risk of causing some damage. With exception of the connection to the reactor all these critical parts could be removed where the remaining union is much easier to handle than the conventional one.

Recapitulating we feel our new GC/IRMS interface is foremost characterised by the following advantages.

1. Largely improved chromatography.
2. Improved sensitivity.
3. Possibility of large volume injections.
4. Omission of derviatisation, at least with respect to the test compounds.
5. Reduced maintenance efforts and hence larger sample throughput.

Possible problems can be identified in the analysis of low boiling compounds. Effective trapping in the injection port may be difficult under these conditions. Use of adsorbents may represent a way out. However, for doping control currently only steroids are compounds of significant interest so that this does not represent a significant restriction. It is also not possible to seperate the GC from the reactor anymore due to the loss of the backflush option. Contamination possibly present in the sample in high amounts therefore bears the possibility to rapidly degrade the reactor. However, we feel this is relatively unlikely because usually sample preparation for GC/IRMS is very specific.

We feel that adaptation of our design to any existing GC/IRMS system should be possible

without major problems. This bears the potential to facilitate much more effective testing in doping analysis.

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