Grainger J, Trout G, Goebel C

Evaluation of ionic liquid GC columns

Australian Sport Drug Testing Laboratory, National Measurement Institute, Sydney, Australia

Abstract

The measurement of the isotopic ratio of carbon in urinary steroids such as testosterone, which can be produced naturally, remains a difficult analytical problem. For the method to work the steroids which are to have their isotope ratios measured must be completely separated from all other compounds present when they elute from the gas chromatograph (GC). Unfortunately none of the GC columns currently in use permit such resolution without extensive prior clean-up of the urine sample using multiple solid phase extraction (SPE) columns or high performance liquid chromatography (HPLC).

A new range of capillary GC columns have been released that are based on ionic liquids that combine high polarity with high upper temperature limits. Two of these columns were evaluated with current GC columns of medium and low polarity to observe the effects on separation of a number of acetylated steroid standards and extracted urine samples.

It was observed that the ionic liquid columns provide more efficient separation of acetylated steroids than any column currently in routine use with GC-C-IRMS. The resolution is such that prior HPLC purification is unnecessary for the analysis of the high concentration analytes Et_Ac, A_Ac, and 11-oxo-Et_Ac in most samples. However the low concentration androstanediols would require further purification or there is potential for using a heart cutting 2DGC method using a low polarity column in the first dimension and a highly polar column in the second dimension.

Introduction

In gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) compounds analysed are combusted to CO_2 and require peaks to consist entirely of a single compound. Peak purity is currently achieved by SPE, acetylation, HPLC purification, and analysis by GC-C-IRMS using a Agilent DB17MS column. New GC columns available use ionic liquids which are more polar than currently used silicon based liquid phases, but have similar upper temperature limits.

To evaluate the ionic liquid column for its separation behaviour in 1DGC-MS, a series of acetylated steroid standards and extracted urine samples were compared to the separations achieved on a low polarity Ultra 2 column and an intermediate polarity DB17MS column.

Experimental

Urine samples were extracted following the screening protocol for GC-C-IRMS currently in force in ASDTL. In summary, 5 mL urine was extracted with Agilent/Varian BondElut C18 SPE cartridges (500 mg, 3 mL). The cartridges were conditioned with 4.5 mL methanol and 4.5 mL water, samples loaded, washed with water (4.5 mL) and 25% methanol (4.5 mL) then eluted with 100%methanol (2 x 3 mL). After evaporating to dryness, the extract was reconstituted in 1 mL phosphate buffer (pH 7), 50µL β-glucuronidase enzyme added then incubated for 2 hours at 37°C. 500 µL of 20% pH 9 carbonate buffer was added and the sample extracted with 2 x 4 mL of TBME. The extracts were evaporated to dryness and 50 µL acetic anhydride and 150 µL pyridine added. Samples were heated at 60°C for 2 hours, evaporated to dryness and reconstituted with 50 µL cyclohexane.

 ISBN 978-3-86884-039-1

Sample extracts were analysed on an Agilent 6890 Series GC System and Agilent 5973 Series Mass Spectrometer Detector in full scan mode for the acetylated steroid standards shown in Table 1. The four columns evaluated in this study were Agilent Ultra 2 (17 m x 0.2 mm x 0.11 µm – low polarity), Agilent DB17MS (30 m × 0.25 mm × 0.25 µm – medium polarity), Supelco SLB-IL59 and SLB-IL60 (25 m × 0.25 mm × 0.25 µm –high polarity).

Compound Name	Abbreviation
5α-androstanol acetate (IS for IRMS)	5α-ol_Ac
Etiocholanolone acetate	Et_Ac
Androsterone acetate	A_Ac
Pregnanediol (5β) di-acetate	PD_Ac2
5α-androstanediol di-acetate	ααβ_Ac2
5β-androstanediol di-acetate	βαβ_Ac2
11-oxo-etiocholanolone acetate	11-oxo-Et_Ac

Table 1. List of acetylated steroid standards used in the study.

Results and Discussion

A typical separation of six acetylated steroid standards on the four different columns is shown in Figure 1. The separation of the βαβ- and ααβ-diol di-acetates using the high polarity columns was more than twice that achieved on the DB17MS column. Given the separations achieved it was decided to determine whether it would be possible to analyse urine extracts directly for the acetylated steroids without HPLC purification.

Nineteen samples were extracted and analysed using the ionic liquid columns. For the method to work, it would require that each compound elutes as a pure peak with no interference from adjacent peaks. Peak purity was assessed by comparing the ratio of the area of the TIC to the sum of the areas of the three major ions for each of the analytes in both the standards and the samples (Figure 2).

The results showed that this was possible in all samples for A_Ac, Et_Ac, and in all but five samples for 11-oxo-Et_Ac. The PD_Ac2 peak was of adequate purity in approximately half the samples. However interferences were observed in most samples for the βαβ-diol_Ac2 and in virtually all samples for the ααβ-diol_Ac2 due to their low concentrations.

Given the separations achieved on the high polarity columns, there is potential for their use in the second dimension of a 2DGC-C-IRMS system. 2DGC-C-IRMS has been used by Brailsford *et al* (2012) in the analysis of a number of steroid compounds, but not for the analysis of androstane diols. In theory a heart-cut 2DGC-C-IRMS method using a low polarity column in the first dimension, and sending heart cuts to a high polarity column (second dimension) should separate the diols and the Et_Ac and A_Ac effectively from interferences such that their carbon isotope ratios could be measured.

 ISBN 978-3-86884-039-1

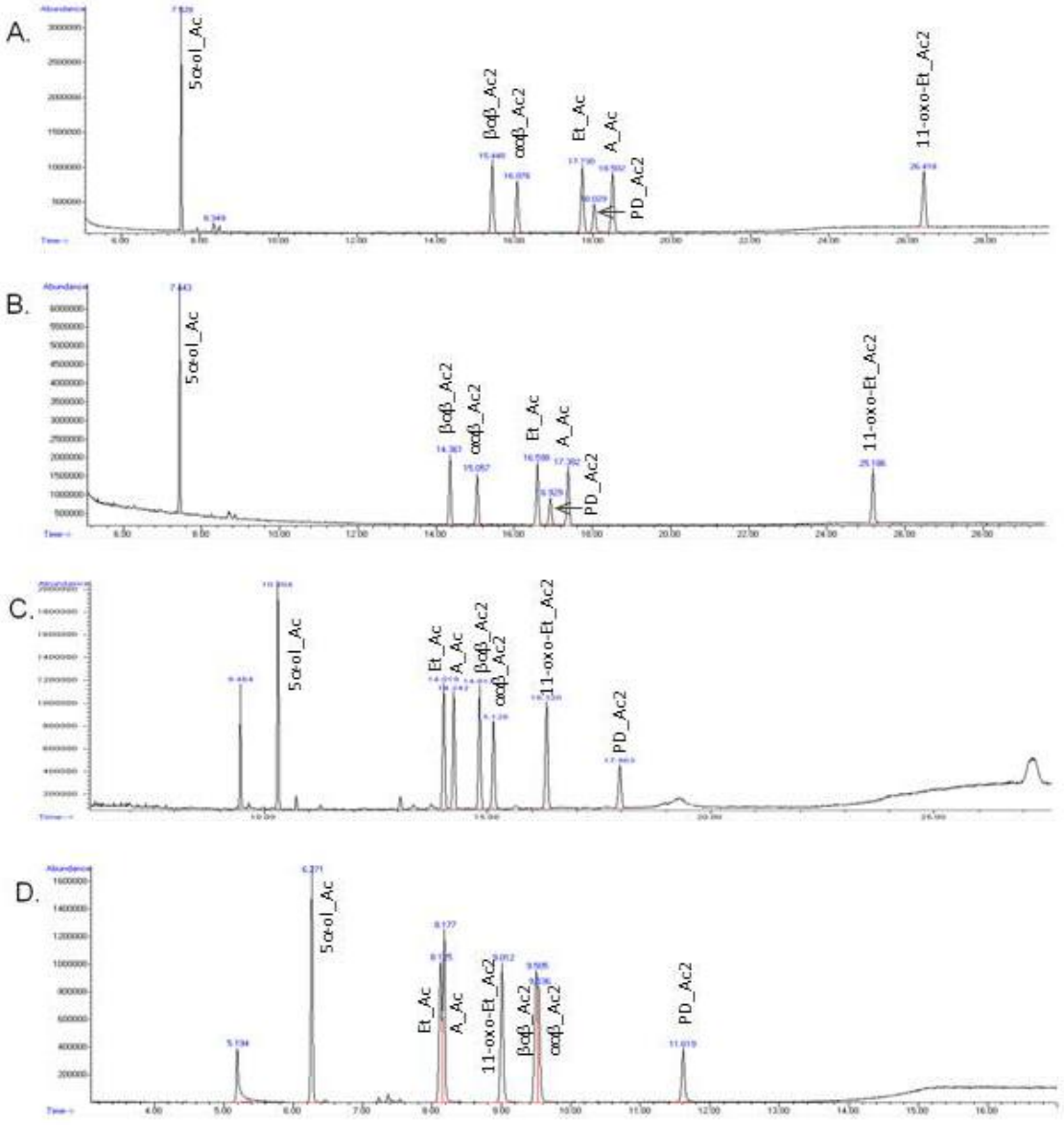

Figure 1. Separation of acetylated steroid standards using A) SLB-IL59 column; B) SLB-IL60 column; C) DB17MS column; and D) Agilent Ultra 2 column.

 ISBN 978-3-86884-039-1

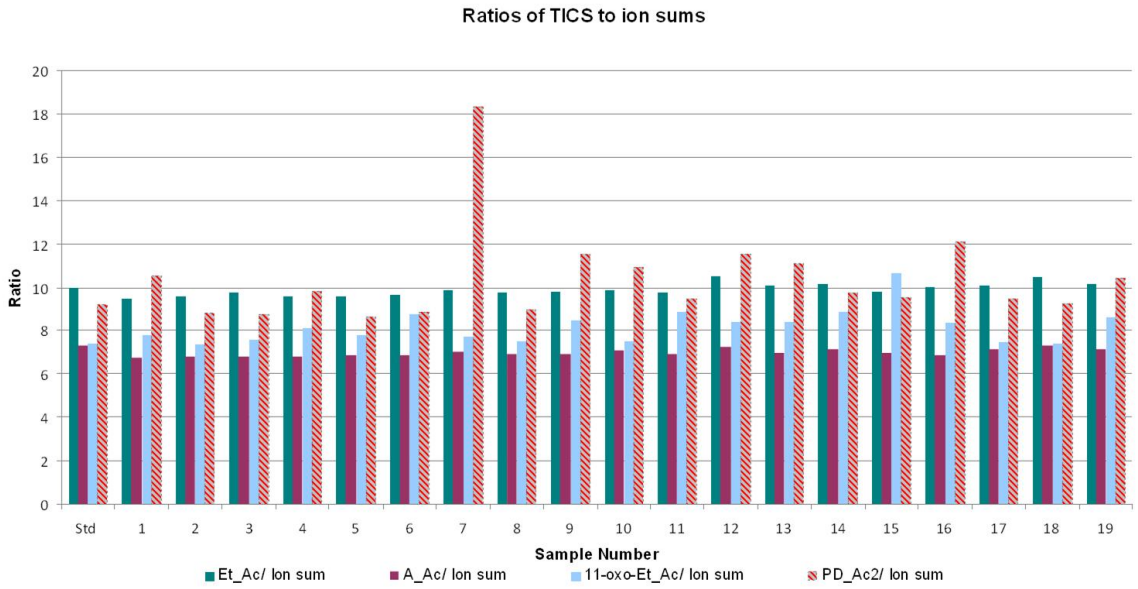

Figure 2. Peak purity of samples and standard for Et_Ac, A_Ac, 11-oxo-Et_Ac and PD_Ac.

Conclusions

The new ionic liquid columns SLB-IL59 and SLB-IL60, provide more efficient separation of acetylated steroids than any column currently in routine use with GC-C-IRMS. The resolution is such that prior HPLC purification is unnecessary for the analysis of the high concentration analytes Et_Ac, A_Ac, and 11-oxo-Et_Ac in most urine samples. With the low concentration diols a heart cutting 2DGC method using an Agilent Ultra 2 column in the first dimension and an SLB-IL59/60 column in the second dimension should be capable of producing pure peaks for GC-C-IRMS analysis.

References

[1] Brailsford A D, Gavrilović I, Ansell R J, Cowen D A, Kicman A T. (2012) Two-dimensional gas chromatography with heart-cutting for isotope ratio mass spectrometry analysis of steroids in doping control. *Drug Test. Analysis* **4,** 962-969.

Acknowledgements

This project was supported by the Australian Government through the Sport Anti-Doping Program.