Quantification of Ethylglucuronide (ETG), a marker for chronic excessive alcohol consumption, in hair by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

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Overview

A rapid, robust, sensitive and specific LC-MS/MS assay has been developed for the quantification of ethylglucuronide (ETG) in hair.

Introduction

Ethanol (alcohol) is converted to acetaldehyde and subsequently to acetic acid by liver enzymes (Figure 1). The traditional approach to assess the extent of alcohol consumption is through blood and urine analysis but unless the blood or urine samples are stored in appropriate specimen containers, with preservatives such as fluoride oxalate, ethanol can be produced as an artifact of bacterial fermentation post collection[1]. This can result in elevated and hence inaccurate ethanol concentrations in these samples which can potentially effect the interpretation of any results obtained.

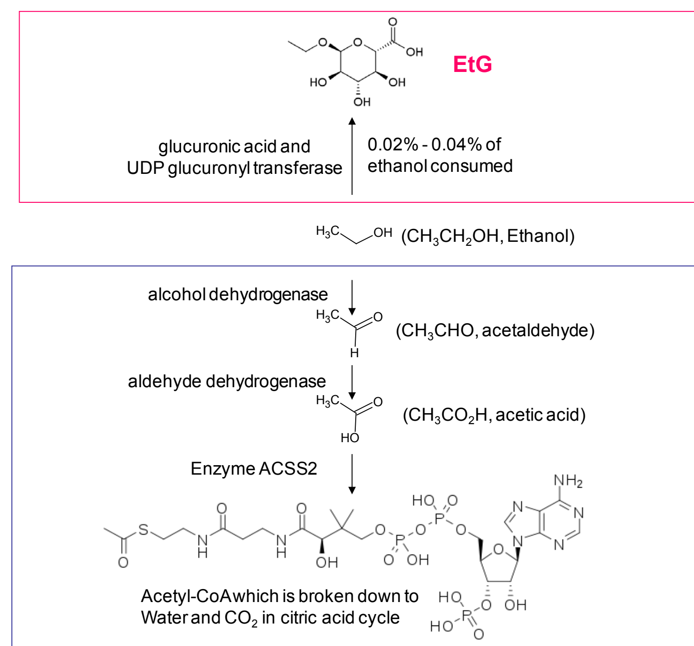

Figure1. Metabolism of ethanol in the liver

An advantage of the use of ETG in hair is that it is not subject to the effect of bacteria and also offers the opportunity to extend the window of detection (up to 6 months) compared to hours-days in blood and urine[2]. As ethanol metabolism is independent of dose, blood and urine concentrations can vary widely between individuals, however with hair analysis the use of a 30 pg/mg cut off, as proposed by the Society of Hair Testing (SoHT)[3], can be applied as standard. Methods to detect ETG in blood and urine have previously been reported[4,5] however ethanol concentrations are more commonly measured and accepted internationally as an assessment of alcohol consumption. Below, we present a method to detect ETG in hair using LC/MS/MS analysis which has been developed in accordance with ISO17025 standards. The method uses a solid phase extraction step for sample clean-up similar to previously reported methods[6] and has been developed leveraging the sensitivity achievable on the QTRAP® 5500 LC/MS/MS system (Figure 2). For the detection of ETG, LC-MS/MS has the advantage over Gas chromatography Mass Spectrometry (GC/MS) in that it detects the native ETG species which does not have to be derivatized first [e.g. into a trimethylsilyl (TMS) derivative[7]] prior to analysis.

Experimental

Sample Preparation

Hair samples were incubated in water and extracted using an anion exchange SPE cartridge and then reconstituted. During the extraction the samples were spiked with ETG-D5 which acted as the internal standard.

LC-MS/MS Methods

Figure 2. QTRAP® 5500

The final reconstituted extracts were run over a 7 minute gradient using HILIC chromatography on an Agilent HILIC+ 3.5 µM 100 x 2.1 mm column, at 40 ºC and the gradient conditions shown in Figure 3 using an Agilent 1290 HPLC System. Mobile phase A was Acetonitrile containing formic acid and B was water containing formic acid and ammonium formate.

Step	Total Time (mins)	Flow rate µl/min	A %	B %
0	0	400	95	5
1	2	400	95	5
2	2.1	1100	60	40
3	3.3	1100	60	40
4	3.4	1500	95	5
5	7	1500	95	5

Figure 3. HPLC gradient conditions for ETG analysis

The LC-MS/MS method was performed on an AB SCIEX QTRAP® 5500 system (Figure 2) equipped with Turbo V™ source and ESI probe set in negative mode at an ionspray voltage of -4500 V. The MS conditions used for the experiment are shown in Figure 4.

	Q1 Mass (amu)	Q3 Mass (amu)	CE (V)
ETG	220.9	85	-22
	220.9	75	-20
ETG - D 5	226	85	-22
	226	75	-20

Figure 4. MS/MS conditions used for ETG analysis

Results and Discussion

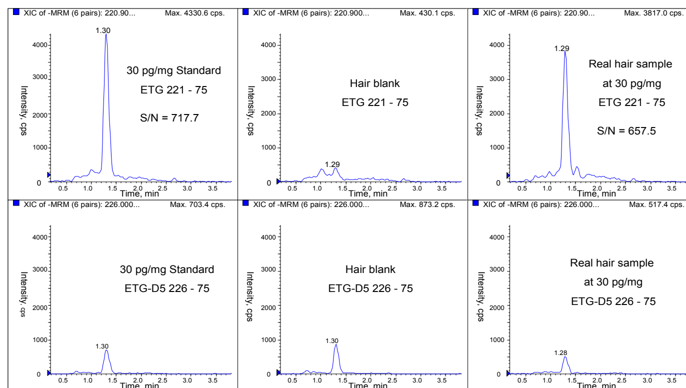

Figure 5. Extracted ion chromatograms of hair samples. The top row represents ETG traces and the bottom row the corresponded ETG-D5 internal standard chromatograms.

As can be seen in Figure 5, ETG elutes early in the HPLC run so the latter high flow part of the gradient is present to clean and re-equilibrate the HPLC column prior to the next injection. From the signal to noise shown in Figure 5 it can be seen that ETG can be easily detected at low pg/mg concentrations in hair. When a calibration line of spiked hair extracts is analyzed the response is also shown to be linear over the range tested (Figure 6).

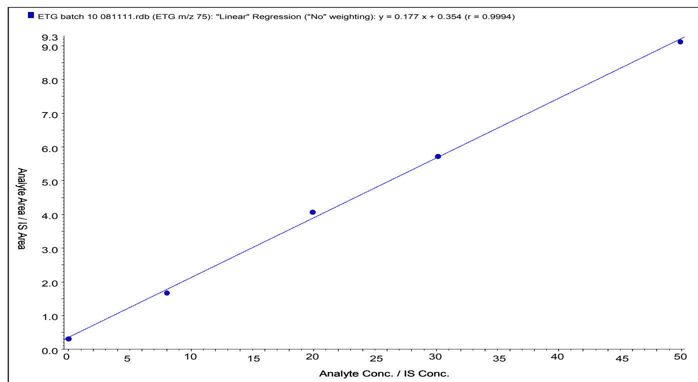

Figure 6. Calibration line for extracts of hair spiked at different levels 8 - 50 pg/mg. The calibration line shown is generated using the MRM transition 221 ->75

In this method several transitions for ETG were detected but it was found that product ions of 75 and 85 seemed to be optimal for the samples run so far. Additional transitions for the internal standard were also acquired but again the 226-75 transition was the strongest with the least amount of matrix interference and was therefore used for quantification.

Summary

From the results presented, it can be seen that the use of the very sensitive QTRAP® 5500 LC/MS/MS system has allowed for detection of ETG in the pg/mg range and therefore an assessment of alcohol consumption from extracts of hair. The results show that the responses observed are linear over the range tested.

Moving forward there are further plans to see if the use of selective techniques like the use of Differential Mobility Separation (DMS) SelexION™ technology which is available for QTRAP® 5500 LC/MS/MS system will remove more of the observed matrix effects. There are also plans to expand this approach to the analysis of drugs of abuse in hair so that long term drug abuse can be monitored.

Acknowledgements

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References

1. Saady, J. J., Poklis, A. and Dalton, H. P. (1993). Journal of Forensic Sciences 38, 1467–1471.
2. Dahl, H., Stephanson, N., Beck, O. and Helander, A. (2002). Journal of Analytical Toxicology 26, 201–204.
3. Society of Hair Testing Recommendations. "Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2011", www.soht.org.
4. Weinmann, W., Schaefer, P., Thierauf, A., Schreiber, A. and Wurst, F. M. (2004). Journal of the American Society for Mass Spectrometry 15, 188–193.
5. Høiseth G , Bernard JP , Karinen R , Johnsen L , Helander A , Christophersen AS , Mørland (2007). J . Forensic Sci Int. 172(2-3), 119-24.
6. Ines Janda, Wolfgang Weinmann, Thorsten Kuehnle, Martina Lahode and Andreas Alt (2002) Forensic Science International Volume 128, Issues 1-2, 14, Pages 59-65.
7. Andreas Alt, Ines Janda, Stephan Seidl and Friedrich-Martin Wurst (2000). Alcohol and Alcoholism, 35 (3) 313 - 314.

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