Comparison between a home-made LC-MS/MS method in measuring hair EtG and a commercial methodology: results and implications.

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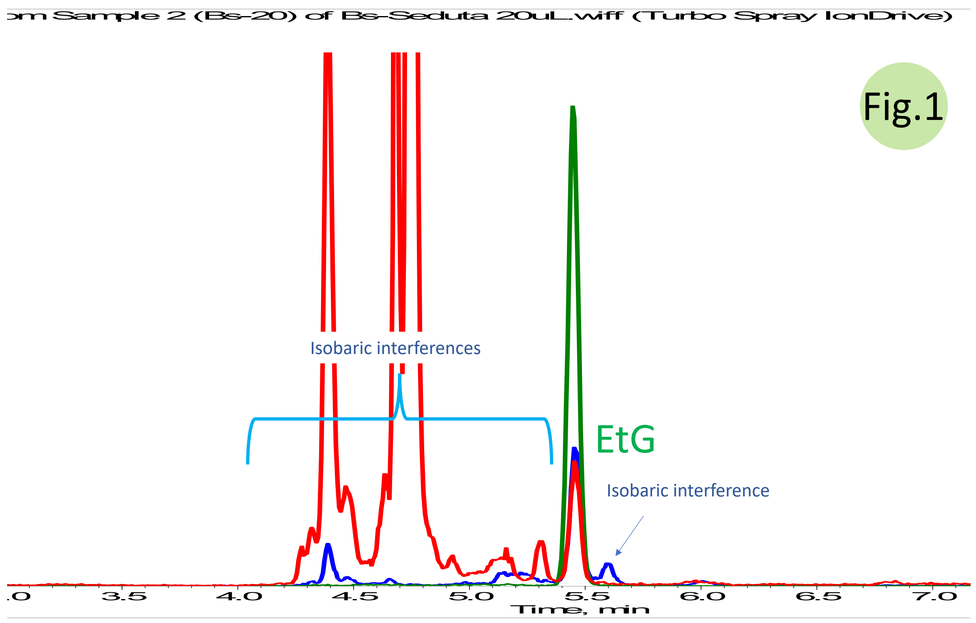
Introduction

Ethyl-glucuronide (EtG) in hair is one of the alcohol consumption markers. Several methods for measuring it by LC-MS/MS have been published. Nonetheless, due to the chemical properties of ETG, there are some concerns when running its quantification in real samples, some related to the extraction procedure and some to isobaric interferences. For addressing these issues, a comparison has been conducted in measuring some patient samples with both a home-made developed methodology and a commercially available method, the latter mainly centered on a different LC column.

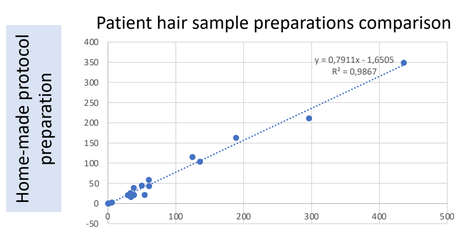
Fig.1



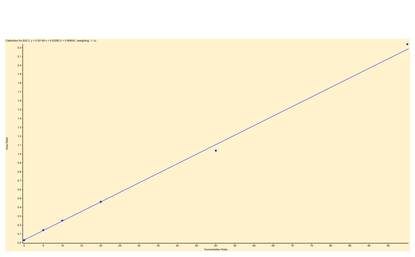
Method

For 20 anonymized patient hair samples, extraction has been conducted according the commercial BSN kit (p.n. EUM 04200), and through a home-made protocol involving quite similar steps (CH_2Cl_2 + MetOH cleaning, followed by an overnight extraction). See Fig.1.
In the home-made approach a RP-column bathed by a H_2O/ACN gradient and an Agilent 8040 LC-MS/MS is used for measurements. Commercial kit is centered on a proprietary column (with different chromatographic capacity and selectivity) and measurements have been conducted on a Sciex 6500 LC-MS/MS. See Fig.2.
In order to exclude any bias originated by the sample preparation, additional experiments have been run on extracts from 38 anonymized patient hair samples read by both the home-made and the Kit instrumental settings (Fig.3).

Fig.2



Hair samples	Kit protocol	Home-made
1	124.9	115.3
2	436.2	348.8
3	135.8	103.7
4	6.2	2.7
5	38.7	21.4
6	54.5	21.3
7	0.6	<5
8	0.6	<5
9	1.4	<5
10	0.3	<5
11	189.2	163
12	61	43
13	34	16.2
14	50.1	44.7
15	37.9	20.5
16	60.5	58.7
17	38.1	38.8
18	33.5	25.8
19	29.5	21
20	296.4	211.1



Results

- Comparison of the two full deployed protocols shows a bias of 20%, attributable to different calibrators (due to the esiguity of the number of patient hair samples reproducibility tests have been hampered).
- Excluding the sample preparation variation and within a general good correlation, discrepancies have been observed for some samples. Their LC-MS/MS traces show as a column with different chromatographic capacity and selectivity can mitigate the isobaric interference impact. Different fragment ion-ratios between analyte and deuterated internal standard are evidenced by Fig.4.

Single prep samples	Kit-LC Readings	Home-made readings
1	115,3	>100
2	348,8	>100
3	2,7	3,34
4	21,4	22,23
5	21,3	25,91
6	13,6	13,18
7	0	<5
8	0	0
9	0	<5
10	0	<5
11	0	<5
12	163	163
13	16,2	23,27
14	44,7	43,52
15	20,5	28,95
16	63	120
17	3,7	<5
18	24	28,23
19	0	0
20	37,4	41,64
21	26,5	24,16
22	25,1	28,41
23	15	25,45
24	3,7	7,70
25	13,9	22,25
26	9,5	18,19
27	0	0
28	57,7	69,46
29	16,7	18,24
30	34,8	48,04
31	96,7	97,98
32	25,8	26,08
33	21	26,98
34	62,7	82,52
35	42,1	51,75
36	54,3	82,88
37	89,7	77,92
38	65,2	71,24

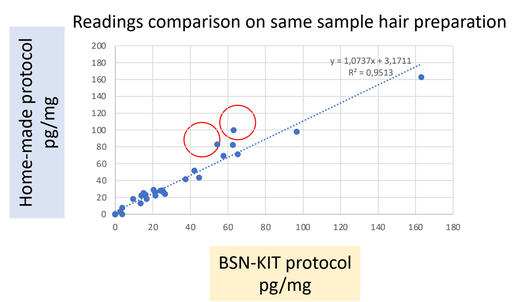


Fig.3

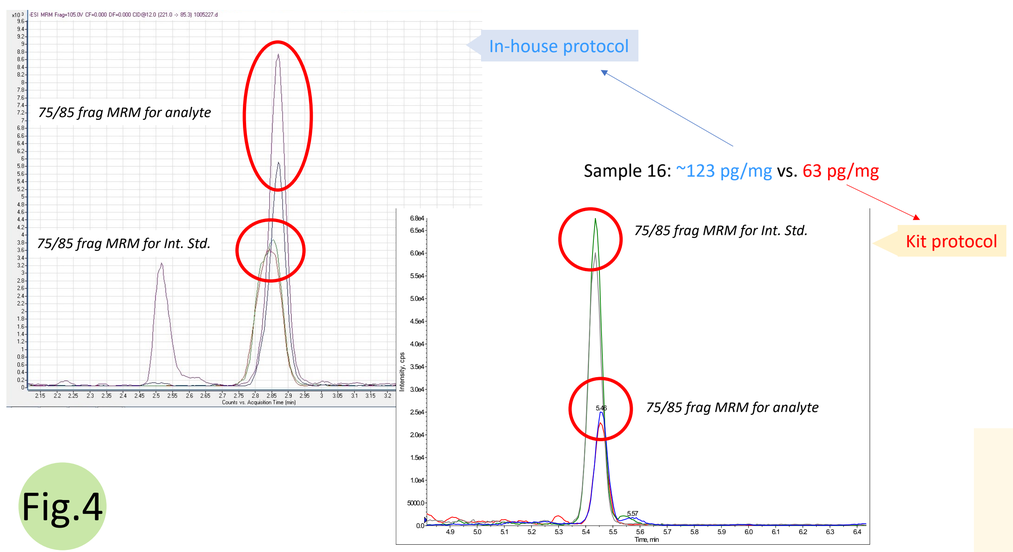


Fig.4

Conclusion

The number of patient samples analyzed allowed to verify a good correlation when the two protocols are independently conducted. Nevertheless, it can be seen as for some specific patient samples the use of more selective chromatographic columns can make the analytical result more accurate, more immune to isobaric interferences, the latter potentially hampering the EtG quantification in real samples.

Ref. **Determination of ethyl glucuronide levels in hair for the assessment of alcohol abstinence**
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Development of a Column-Switching HPLC-MS/MS Method and Clinical Application for Determination of Ethyl Glucuronide in Hair in Conjunction with AUDIT Detecting High-Risk Alcohol Consumption
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