Forensic Science International 231 (2013) 195–198

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Ethyl glucuronide identified in commercial hair tonics

Torsten Arndt *, Stefanie Schröfel, Karsten Stemmerich

Bioscientia Institut für Medizinische Diagnostik GmbH, Konrad-Adenauer-Straße 17, D-55218 Ingelheim, Germany

ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form 29 April 2013
Accepted 2 May 2013
Available online 14 June 2013

Keywords:
Alcohol
Ethyl glucuronide
Hair testing
Hair tonic
LC–MS/MS

ABSTRACT

Background: Ethyl glucuronide (EtG) in hair is considered as a specific marker of ethanol consumption. Prompted by a report of positive EtG hair testings due to hair treatment with an EtG containing hair lotion, commercially available herbal hair tonics from supermarkets, drug-stores, and health food stores were analyzed for the presence of EtG and ethyl sulfate (EtS).
Methods: LC–MS/MS (QTRAP 5500 mass spectrometer) was done in multiple reaction monitoring (MRM), enhanced product ion (EPI) and MS^3 mode. The lower limit of quantitation was 0.05 mg/L for EtG and the cut-off for the detection of EtS 0.01 mg/L.
Results: Altogether 11 hair tonics from 8 manufacturers were tested, with 1 product in 3 different lots. EtG ranged between 0.07 and 1.06 mg/L (7 products from 4 manufacturers) and was almost identical in the 3 lots of 1 product (1.01–1.06 mg/L). EtS was found in 3 out of the 11 hair tonics.
Conclusions: EtG is quite frequently present in commercially available herbal hair tonics. Using EtG in hair as a marker of alcohol (ab)use, one has to consider external sources of EtG and has to assess the use of hair care products, esp. if the patient denies any ethanol intake. Whether EtS is a more reliable alcohol (ab)use marker, as sometimes discussed, should be critically assessed against the background of its broad use in large amounts in industrial chemistry.

1. Introduction

Ethyl glucuronide (EtG) is a minor hepatic metabolite of ethanol and is used as a marker of ethanol consumption [1,2]. Recently, Sporkert et al. [3] reported about positive EtG hair testings caused by hair treatment with an EtG containing herbal hair lotion. The present study aimed to assess whether this has been a single observation for that specific hair lotion or whether EtG appears more frequently in commercial hair care products.

2. Materials and methods

2.1. Reagents

EtG and EtS were from Lipomed (Bad Säckingen, Germany). An EtG-D5 and EtS-D5 internal standard solution was from Recipe (Munich, Germany). Rotisolv® Ultra LC–MS Water and acetonitrile were from Carl Roth GmbH (Karlsruhe, Germany) and formic acid approx. 98% for mass spectrometry was from Fluka Sigma–Aldrich (Germany). The DRI® ethyl alcohol testkit was delivered by Thermo Scientific (Passau, Germany).

2.2. Ethanol measurement

The ethanol content in the hair tonics was measured by a NADH method on an Olympus AU680 analyzer (Beckman-Coulter). This method was validated for the determination of ethanol in serum and urine but not for ethanol in hair tonics. The analysis results are thus given as approximate ethanol concentrations (Table 1).

2.3. LC–MS/MS

LC–MS/MS for EtG and EtS was done with a modified version of a commercially available testkit (ClinMass® Ethyl Glucuronide, Recipe, Munich, Germany). We modified this testkit to achieve utmost sensitivity and esp. to allow the inclusion of enhanced product ion scan and MS^3 experiments for an utmost reliable identification of EtG in hair tonics.

2.4. Instrumentation

MRM, product ion and MS^3 experiments were done with a 1260 Infinity LC (Agilent, Waldbronn, Germany) coupled to a MistraSwitch column oven (Maylab Analytical Instruments, Vienna, Austria) and a QTRAP 5500 mass spectrometer (AB Sciex, Darmstadt, Germany). A PAL HTC-xt autosampler (CTC Analytics, Zwingen, Switzerland) was used as autosampler.

2.5. Sample preparation

To 10 μL of standards or hair tonic samples were added 100 μL internal standard solution (EtG-D5 and EtS-D5) and 1 mL Rotisolv® Ultra LC–MS water.

2.6. Chromatography

The main settings were: autosampler: 15 °C, column: Recipe ClinMass EtG 100 × 2 mm at 40 °C, injection volume: 20 μL, eluent: 0.01% (v/v) formic acid (A) and acetonitrile (B), gradient: 0–3.8 min 0.3 mL/min 10% B, 3.8–4.0 min increase of flow rate to 0.6 mL/min, 4.0–6.0 min 0.6 mL/min 10% B, 6.0–6.5 min increase to 90% B, 6.5–10.0 min 0.6 mL/min 90% B, 10.0–10.5 min decrease to 10% B, 10.5–13.0 min

Abbreviations: EtG, ethyl glucuronide; EtS, ethyl sulfate.

* Corresponding author. Tel.: +49 6132 781 349; fax: +49 6132 781 428.
E-mail address: torsten.arndt@bioscientia.de (T. Arndt).

0379-0738/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2013.05.010

Table 1
Ethyl glucuronide (EtG) and ethyl sulfate (EtS) in herbal hair tonics as determined by LC–MS/MS (lower limit of calibration 0.05 mg/L for EtG and lower limit of detection 0.01 mg/L for EtS) and approximate ethanol content determined by a NADH method.

Hair tonic[a]	EtG [mg/L]	EtS	EtOH [%] (v/v)
1, lot 1	1.04	Negative	21
1, lot 2	1.01	Negative	22
1, lot 3	1.06	Negative	22
2	0.17	Negative	39
3	0.07	Negative	32
4	0.09	Positive	25
5	Negative	Positive	29
6	Negative	Positive	0.3
7	Negative	Negative	11
8	Negative	Negative	38
9	Negative	Negative	31
Sporkert et al. [3] [b]	2.7 mg/L	No data	44

[a] To avoid misuse of the analysis data by patients and accused, product names and manufacturers can be obtained from the corresponding author.
[b] A herbal hair lotion.

0.6 mL/min 10% B, 13.0–13.5 min decrease of flow rate to 0.3 mL/min, 13.5–15.0 min 0.3 mL/min 10% B.

2.7. Mass spectrometry

General settings for the MRM, enhanced product ion scan, and MS^3 experiments were: ESI negative, curtain gas (CUR) 50 psi, ion spray (IS) −3500 V, temperature (TEM) 600 °C, nebulizer gas (GS 1) 50 psi zero air, turbo gas (GS 2) 50 psi nitrogen.

2.8. MRM experiments

Ethyl glucuronide 221 → 75 (collision energy (CE) −20 V, declustering potential (DP) −40 V), 221 → 85 (CE −22 V, DP −30 V), ethyl glucuronide-D5 226 → 85 (CE −22 V, DP −25 V), ethyl sulfate 125 → 97 (CE −22 V, DP −25 V), 125 → 80 (CE −40 V, DP −35 V) and ethyl sulfate-D5 130 → 98 (CE −24 V, DP −20 V), dwell time 50 ms per MRM.

2.9. Enhanced product ion scan (EPI) experiments

A time period 1 from 0 to 4.5 min was used for MRM and for ion trap experiments: MRM experiments were done for ethyl glucuronide and ethyl glucuronide-D5, using a dwell time of 35 ms per MRM. Ion trap experiments were done to obtain the enhanced product ion spectrum for ethyl glucuronide with the following settings: Q1 precursor ion 221, Q2 collision energy −15 V, collisionally activated dissociation gas (CAD) set at medium, declustering potential −85 V, Q3 used in the linear ion trap (LIT) mode, fixed fill time 200 ms, scan range 50 to 230 *m/z*, Q0 trapping, scan rate 10,000 Da/s. A time period 2 from 4.5 to 15 min was used only for MRM experiments for ethyl sulfate and ethyl sulfate-D5, using a dwell time of 100 ms per MRM.

2.10. MS^3 experiments

A time period 1 from 0 to 4.5 min was used for the MRM experiments for ethyl glucuronide and ethyl glucuronide-D5 with a dwell time of 35 ms per MRM. Q1 was used for separation of the 1st precursor ion of *m/z* 221. Q2 was used as a collision cell with a collision energy of −15 V, CAD set at high, declustering potential −85 V. Q3 was used for separation of the 2nd precursor ion of *m/z* 203 (corresponding to a loss of H_2O between Q1 and Q3, 221 → 203) and as linear ion trap with a fixed fill time of 200 ms, excitation time 20 ms, excitation energy 45 mV, scan range *m/z* 50–170. Time period 2 (4.5–15 min) was used only for MRM for ethyl sulfate and ethyl sulfate-D5 (dwell time 100 ms per MRM).

2.11. Calibration

A six point calibration was used for quantification of EtG (0.05–2.00 mg/L) and qualitative analysis of EtS (0.01–0.40 mg/L). An EtG in urine analysis method validated according to [4] was used in this study. EtG concentrations measured for the native hair tonic samples and for 1:10 dilutions showed an excellent agreement (data not shown), proofing that the EtG in urine LC–MS/MS method was well suited for the EtG analysis in the hair tonic matrices.

3. Results and discussion

An initial test series with hair cosmetics used by the stuff of our laboratory already yielded 1 hit regarding the presence of EtG [5]. This was a hair tonic based on alcoholic plant extracts, like in the case of a hair lotion described by Sporkert et al. [3]. Pure synthetic hair care products (shampoos, gels, sprays, etc.) were tested EtG negative (data not shown). Thus, the present study focused on herbal hair tonics.

Altogether, 11 hair tonics from supermarkets, drug-stores, and health food stores were tested and EtG concentrations between 0.07 and 1.06 mg/L were obtained (Table 1). Three different lots of 1 product showed an almost identical EtG concentration between 1.01 and 1.06 mg/L (Table 1).

EtG identification was done by LC–MS/MS (Fig. 1), enhanced product ion (EPI) spectra (Fig. 2), or MS^3 spectra (Fig. 3). Figs. 1–3 show the corresponding ion chromatograms and mass spectra for

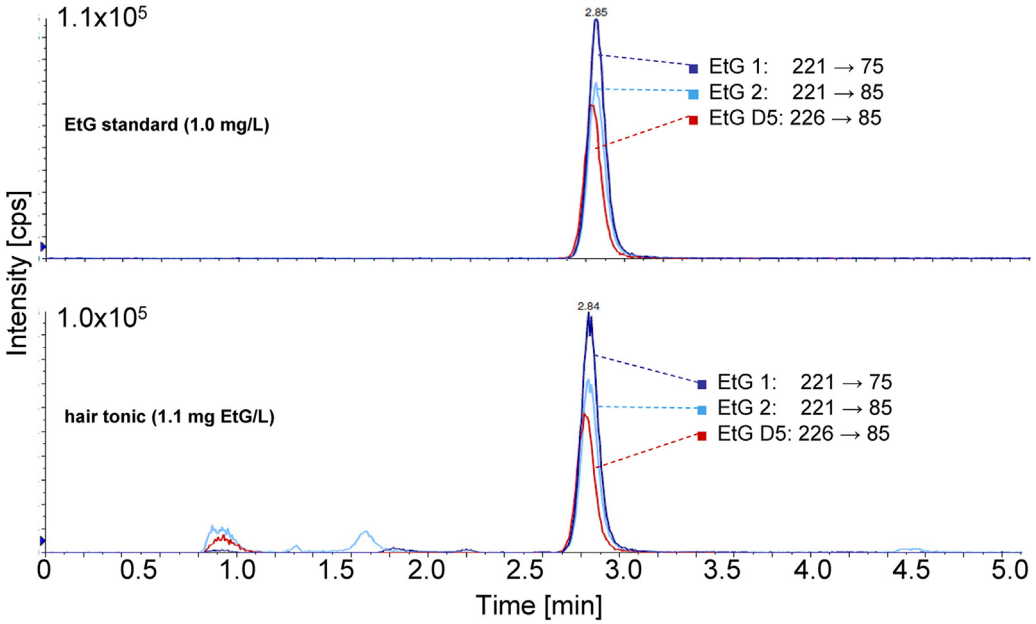

Fig. 1. LC–MS/MS ion chromatogram of an aqueous ethyl glucuronide standard (1.00 mg/L) and of a commercially available hair tonic (EtG content approx. 1.10 mg/L). EtG-D5 = internal standard.