THE DETECTION AND QUANTIFICATION OF ETHYL GLUCURONIDE IN PLACENTAL TISSUE AND PLACENTAL PERFUSATE BY HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Background
Ethyl glucuronide (EtG) is arising as a promising biomarker of heavy prenatal alcohol exposure, however its transfer across the human placenta is still unclear and is currently being investigated using the *ex vivo* placental perfusion model. This model allows for sampling from placental tissue and placental perfusate, which is a surrogate to plasma.

Objective
To develop a method for detecting and quantifying EtG in placental perfusate and tissue using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS).

Methods
A method was optimized by manipulation of the following components to attain the highest peak counts for the quantifying ions of EtG and its deuterated internal standard on the mass spectrum: cartridges used for solid phase extraction, injection method, derivatizing agent, pre-injection parameters, SPME fiber, GC ramp speed, and GC column flow.

Results
The final method utilized involved solid phase extraction of standards via UCT CleanScreen Cartridges, derivatization with heptafluorobutyric acid, and introduction into the GC via HS-SPME with adsorption to a polydimethylsiloxane fiber. The method has improved sensitivity over other methods that quantify EtG in blood using GC-MS, with detection limits of 1.6 ng/mL and 13.7 ng/g for placental perfusate and tissue, respectively. The method was applied to samples collected from the fetal reservoir during the *ex vivo* placental perfusion model and EtG was detected in the fetal circulation after 20 minutes of perfusion, indicating transfer of EtG.

Conclusions
The present method is sensitive and can be used to quantify EtG transfer during *ex vivo* placental perfusion experiments.

Key Words: *Ethyl glucuronide; gas chromatography; headspace solid-phase microextraction; biomarker; placenta; ex vivo placental perfusion model*

Ethyl glucuronide (EtG) is a minor, non-oxidative metabolite of ethanol formed by the glucuronidation of ethanol via UGT1A1 and UGT2B7 isozymes.[1] The pharmacokinetics of EtG have been determined in healthy adults who consumed a controlled dose of alcohol[2,3] and in chronic alcoholics.[4] Importantly, compared to ethanol, EtG has longer detection times in blood

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(24 hours)[4] and urine (5 days)[5], indicating its use as a marker of alcohol consumption over extended time periods.

The use of long-term biomarkers of alcohol consumption is of particular importance when screening newborns for Fetal Alcohol Spectrum Disorder (FASD), an umbrella term that covers the physiological, developmental, and behavioural outcomes in children associated with maternal consumption of alcohol *in utero*. Since maternal self-report of alcohol consumption during pregnancy is often unreliable[6], objective alcohol biomarkers have proven useful in screening for prenatal alcohol exposure.

Reports of elevated EtG in meconium, the infant's first stool[7-10], have implicated EtG as a potentially sensitive and specific biomarker of prenatal alcohol exposure, with a positive cut-off of 2 nmol/g meconium. However, EtG has also been detected in placental tissue and fetal remains[11], suggesting that EtG's presence in meconium may be due to both fetal metabolism of ethanol and transfer of maternal EtG across the placenta. These contributions need to be determined as they may influence the validity of the present EtG cut-off in meconium.

In this respect, the *ex vivo* placental perfusion model is an effective method for determining the placental transfer of xenobiotics.[12] Samples of placental tissue and placental perfusate - which is considered a plasma surrogate and avoids the need for excessive blood collection - are frequently taken during perfusions to determine the extent of placental transfer of and fetal exposure to various xenobiotics. In the current study, we explain the development of a method for EtG detection and quantification in placental tissue and perfusate by coupling headspace solid phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC-MS) after cleanup by solid phase extraction. Previous studies have used GC-MS to quantify EtG in blood samples[13-15], however the additional HS-SPME step presented in this study provides the added advantage over direct injection of increased sensitivity due to additional cleanup and accumulation of EtG on the SPME fiber.[16] This method can be used to measure EtG in placental perfusate and tissue samples collected from subsequent placental perfusions in an attempt to elucidate EtG disposition across the maternal-placental-fetal unit.

METHODS

Materials

Stock ethyl glucuronide and penta-deuterated ethyl glucuronide (EtG-d_5) internal standard solutions were purchased from Cerilliant (Round Rock, TX). Columns used for solid phase extraction were UCT Clean Screen Extraction Columns (200mg/3mL/50pkg; Chromatographic Specialties Inc., Brockville, ON), Aminopropyl NH_2 Columns (Sopachem, Eke, Belgium), and OASIS MAX columns (Waters Corporation, Milford, MA). An Optima L-80 XP Ultracentrifuge (Beckman Coulter) was used for blended tissue centrifugation. Splitless liners (2mm x 5 x 95) were purchased from Chromatographic Specialties Inc and SPME 100 μm polydimethylsiloxane red and black fibers were purchased from Supelco Analytical (Bellefonte, PA). Stock grade methanol, formic acid, and heptafluorobutyric and pentafluoropropionic acid derivatizing agents were purchased from Sigma-Aldrich (St. Louis, MO).

Perfusate

Maternal and fetal perfusate used in the Motherisk laboratory for the *ex vivo* placental perfusion experiment consists of 10.9 g/L M199 tissue culture medium (Sigma Aldrich, St. Louis, MO), dextran (maternal, 7.5 g/L; fetal, 30.0 g/L), glucose (maternal, 2.77 mM), antipyrine (maternal, 1 mM), heparin (2000 U/I), and kanamycin (100 mg/L). Perfusate solutions were made monthly and stored at 4°C.

Preparation of Stock Solutions and Standards

Working stock solutions of EtG were prepared by making 1:10 serial dilutions from 100 μg/mL stock EtG in methanol (stock solutions were 10 μg/mL, 1 μg/mL, 100 ng/mL, and 10 ng/mL).

These stock solutions covered the range of concentrations used for standards, validation, and calculation of limit of detection (LOD) and limit of quantification (LOQ). Working stock solution of 1 μg/mL EtG-d_5 was prepared by diluting 100 μg/mL stock EtG-d_5 in methanol. All stock solutions were stored at -20°C as per the manufacturer's recommendations (Cerilliant,

Round Rock, TX). For calibration standards, 1 mL standards were prepared with blank perfusate, 50 ng/mL EtG-d_5, varying concentrations of EtG ranging from 0-500 ng/mL, and 50 μL formic acid. For tissue standards, blank 1.00 ± 0.01 g tissue samples were suspended in 3 mL deionized water with 50 ng/g EtG-d_5, varying concentrations of EtG ranging from 0-500 ng/g, and 150 μL formic acid. Blank standards, containing neither EtG nor EtG-d_5, were also prepared for each batch as a control for equipment functionality.

Sample Collection

Perfusate and tissue samples were collected during the perfusion of a human term placenta with EtG by means of the *ex vivo* placental perfusion model that has been described in detail previously[17] and adapted in our Motherisk laboratory.[18,19] Briefly, term placentae were obtained from scheduled elective Caesarian sections at the obstetrics ward at St. Michael's Hospital in Toronto, Ontario. Research ethics board approval was obtained from the hospital and mothers gave written consent prior to delivery. Dual circulation was established by means of maternal and fetal perfusate in place of blood (see *Perfusate* section for ingredients), roller pumps to establish blood flow, and flasks that act as maternal and fetal "reservoirs" (i.e. systemic circulations).

After assessing placental integrity and viability, 1 μg/mL EtG was added to the maternal perfusate, and fetal samples were collected at 0, 10, 20, 30, and 60 minutes to quantify maternal-fetal transfer. After 60 minutes of perfusion, the experiment was terminated and both the perfused cotyledon and an adjacent unperfused cotyledon were isolated for determination of EtG concentration in tissue. Perfusate samples and the 2 cotyledons were stored at -20°C until analysis.

Sample Preparation

Thawed fetal samples collected from the placental perfusion were prepared in a similar way to calibration standards. Solutions were prepared in labeled Eppendorf tubes by combining 950 μL sample and 50 μL (1 μg/mL) EtG-d_5. After vortexing, 50 μL formic acid was added to each tube in preparation for solid phase extraction. One sample from the thawed adjacent unperfused lobule and 3 samples from the thawed perfused lobule were prepared by weighing 1.00 ±0.01 g tissue, transferring samples to labeled plastic tubes, suspending samples in 3 mL deionized water, and adding 50 μL (1 μg/mL) EtG-d_5 to each sample. Formic acid (150 μL) was added to each tube and samples were vortexed thoroughly. Samples were blended on ice for three 30 second intervals each using a POLYTRON PT 10-35 laboratory homogenizer (Kinematica Inc., Littau/Lucerne, Switzerland) on level 7. Samples were transferred to centrifuge tubes and spun using an Optima L-80 XP Ultracentrifuge (Beckman Coulter, Brea, CA) with a Ti 50.2 rotor for 30 minutes at 28,700g and 4°C. The supernatant was collected for subsequent solid phase extraction.

Perfusate and tissue samples were extracted through UCT Clean Screen Extraction Columns (200 mg/3 mL, United Chem, Bristol, PA) via a vacuum manifold. The protocol for extraction was as follows: (1) Condition cartridges with 1 mL 1 % formic acid solution; (2) Add 1 mL sample and pull through slowly, leaving vacuum on 5 kPa for 5 minutes; (3) Add 1 mL water and pull through slowly, leaving vacuum on 10 kPa for 15 minutes; (4) Change collection vials and elute with 2 mL 2% formic acid in methanol solution, pulling though slowly.

Eluted standards and samples were transferred to SPME vials and dried with N_2 gas on a 35°C hot plate. Each vial was then derivatized with 40 μL heptafluorobutyric anhydride (HFBA) and heated at 80°C for 15 minutes. Finally, samples were dried briefly with N_2 gas and loaded onto the GC tray for injection.

GC-MS Instrumentation and Conditions

All samples were analyzed using a Shimadzu QP2010 Plus GC-MS coupled to an AOC-5000 Autosampler (Shimadzu, Columbia, MD, USA).

The GC-MS was operating in negative chemical ionization mode and samples were analyzed using Shimadzu GCMSsolution version 2.51 software. After drying off excess HFBA, samples were further derivatized using a CTC-agitator with the following parameters: pre-incubation at 2 minutes with 1 minute of agitation

and 15 second stop intervals, fiber extraction for 10 minutes with 1 minute of agitation and 15 second stop intervals, 5 minutes desorption, agitator speed 250 rpm and agitator temperature 90°C.

Samples were passed through a splitless liner and separated using a DB-1HT column (10 μm thickness, 15 m length, 0.25 mm diameter; Agilent, Mississauga, ON) with helium as the carrier gas. The GC oven temperature ramp was: 70°C, hold for 2 minutes, increase to 280°C at a rate of 12°C/minute. The injection temperature was 260°C and the column flow was 1.2 mL/minute. Ion source and interface temperatures were both 250°C. Detector voltage was 0.7 kV above the calibrated baseline. The MS was operating in SCAN acquisition mode and was programmed to analyze an ion window of 200-425 (m/z).

RESULTS

Method Optimization

Several conditions were optimized to maximize peak chromatographic areas counts. Solid phase extraction was carried out using protocols for OASIS MAX,[20] Aminopropyl NH_2[21] and UCT Clean Screen cartridges.[22] Derivatization with both heptafluorobutyric anhydride (HFBA) and pentafluoropropionic anhydride (PFPA) was evaluated. Additionally, 3 methods for introducing sample into the GCMS were compared: direct injection, headspace injection, and injection after HS-SPME. For the latter, injection using 100 μm polydimethylsiloxane red and black fibers were compared. Different GC temperature ramp speeds and GC column flow speeds were compared as well. The results of the optimization experiments are summarized in Table 1. The final protocol presented for each parameter yielded the best results and this was used for analysis of perfusate and tissue samples from the perfusion experiment.

Calibration Curve, Limits of Detection and Quantification

Perfusate calibration curves were prepared by spiking blank perfusate (1 mL total volume) with 1, 10, 50, 100, 250, and 500 ng/mL EtG and 50 ng/mL EtG-d_5. Tissue calibration curves were prepared by spiking 1.00 ± 0.01 g tissue suspended in 3 mL deionized water with 5, 10, 50, 100, 250, and 500 ng/g EtG and 50 ng/g EtG-d_5. These concentrations were expected to cover the range seen in perfusate and tissue samples collected after perfusion with 1 μg/mL EtG, a concentration that was chosen based on blood EtG levels detected in healthy adults who consumed a moderate dose (1 mg/kg) ethanol.[23] Standard curves were generated by calculating the EtG:EtG-d_5 ratio of peak area count for each sample and plotting it as a function of EtG concentration. Regression lines were generated and regression analysis was performed. Calibration curves were linear within these ranges from perfusate and tissue, with a $R^2 > 0.99$. Three calibration curves were generated for each matrix, and average slope, y-intercept, and R^2 values for perfusate and tissue are presented in Table 2.

TABLE 2 Method Sensitivity

Matrix	Perfusate	Tissue
Slope	0.0204	0.0134
Intercept	0.0596	0.0451
Mean R^2	0.9991	0.9948
LOD	1.6 ng/mL	13.7 ng/g
LOQ	4.8 ng/mL	41.6 ng/g

The limit of detection (LOD) and limit of quantification (LOQ) were determined by spiking perfusate and tissue with low concentrations of EtG (1-25 ng/mL for perfusate, 5-50 ng/g for tissue), generating regression lines and performing regression analysis. LOD and LOQ were calculated using the equations $LOD = 3\sigma/S$ and $LOQ = 10\sigma/S$, where σ represents the standard deviation of the linear regression line and S represents the slope of the line. Three low concentration calibration curves were generated for each matrix, and average LOD and LOQ values for perfusate and tissue are presented in Table 2.

TABLE 1 Summary of protocols used to optimize method of EtG extraction and quantification in placental perfusate and tissue

Category	Materials used	Method	Results	Ref
Solid Phase Extraction	Aminopropyl NH_2 Columns	• Condition with 3 mL methanol, 3 mL water, 3 mL acetonitrile • Add sample • Wash with 1 mL n-hexane • Elute with 2 mL 2% NH_3	No signal	21
	OASIS MAX Cartridges	• Condition with 2 mL methanol, then 2 mL water • Add sample • Wash with 1 mL NH_4OH, 2 mL methanol • Elute with 2 mL 2% formic acid in methanol	Poor signal, unable to integrate individual peaks	20
	UCT CleanScreen Cartridges	• Condition with 1 mL 1% formic acid • Add sample • Wash with 1 mL water • Elute with 2 mL 2% formic acid in methanol	Clear individual peaks for EtG and internal standard	22
Injection Method	Direct injection	Dry samples, derivatize for 30 minutes, reconstitute in 50 μL ethyl acetate, inject 2 μL	Background noise masked signal	20
	Headspace injection	Agitation for 20 minutes, inject 2.5 mL at 36 μL/sec at 90°C	Poor signal, unable to integrate individual peaks	25
	Headspace SPME	Dry samples, derivatize for 15 minutes, analyte adsorbs to SPME fiber before fiber injection	Highest area counts, effective peak separation	22
Derivatizing Agent	PFPA	Used 10 μL for comparison to HFBA	Poor signal for internal standard quantifying ion	27
	HFBA	Used 10 μL for comparison to PFPA	Strong EtG and internal standard peaks	22
Pre-injection Parameters	-	Derivatized samples pre-incubated in agitator for 5 minutes followed by fiber extraction for 20 minutes	Good area counts, SPME fiber swelled after each batch	25
	-	Derivatized samples dried with N_2 briefly, pre-incubated in agitator for 2 minutes followed by fiber extraction for 10 minutes	Same area counts as other method, SPME fiber lasted longer	22
SPME Fiber	Carboxen/ PDMS fiber (75μm, black)	-	Higher counts than red fiber, however fiber stripped at higher EtG concentrations	22
	PDMS fiber (100 μm, red)	-	Counts not as high as black fiber, however, fiber lasted for more runs	22
Ramp Speed	-	GC oven temperature increased from 70°C to 280°C at 15°C/minute	Peaks did not drop to baseline, difficult to integrate	-
	-	GC oven temperature increased from 70°C to 280°C at 12°C/minute	Peaks dropped to baseline	-
Column Flow	-	1.0 mL/min	Internal standard peak incorporated into nearby M199 peak	-
	-	1.2 mL/min	Effective separation of internal standard and M199 peaks	-

EtG, ethyl glucuronide; GC, gas chromatography; HFBA, heptafluorobutyric acid; M199, medium 199; PDMS, polydimethylsiloxane; PFPA, pentafluoropropionic acid; SPME, solid phase microextraction

FIG. 1 Sample chromatographs of the quantifying ion for EtG (399) and EtG-d_5 (404) after extraction of (A) 100 ng/mL EtG from perfusate; (B) 100 ng/mL EtG-d_5 from perfusate; (C) 100 ng/g EtG from tissue; (D) 100 ng/g EtG-d_5 from tissue.

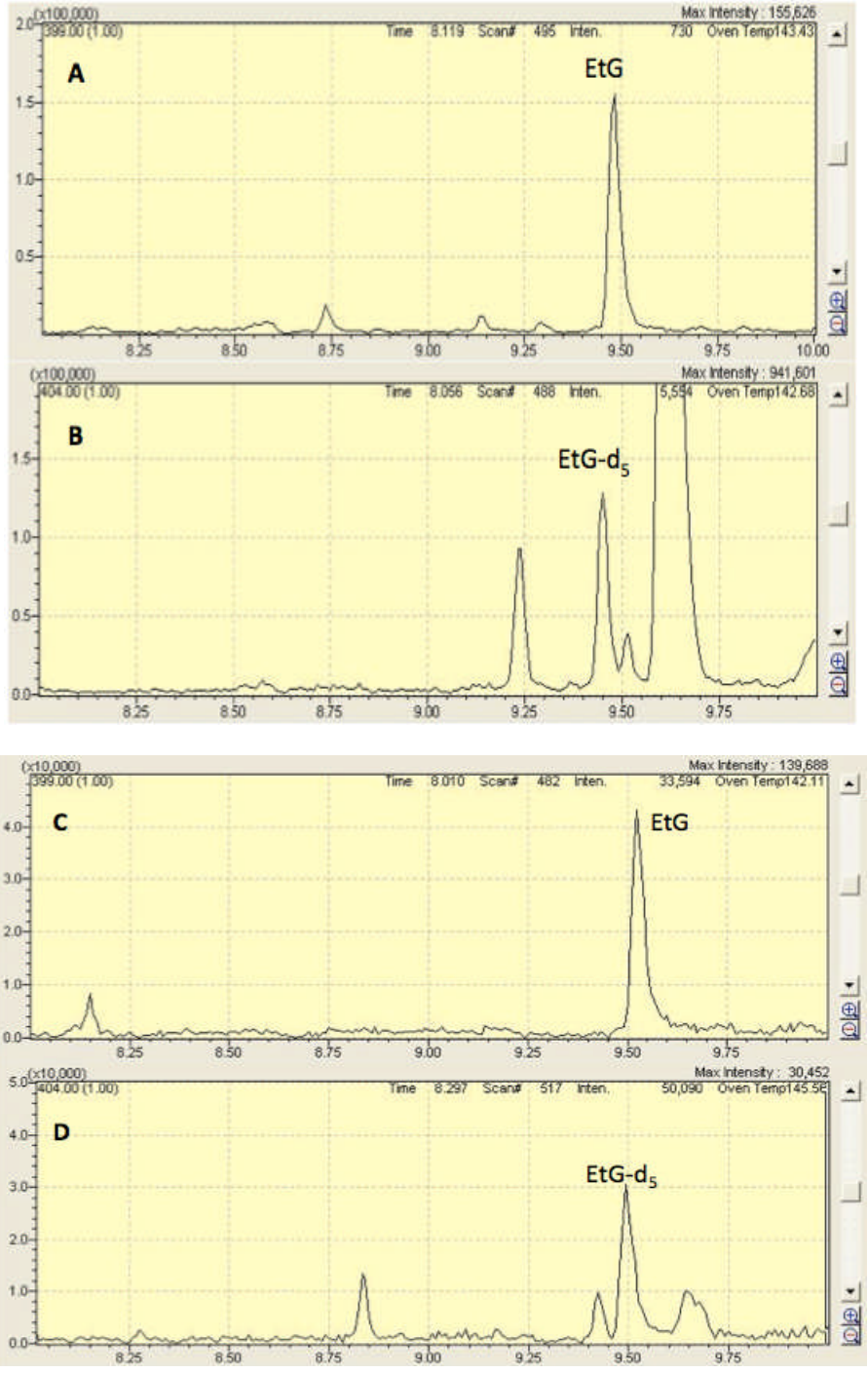

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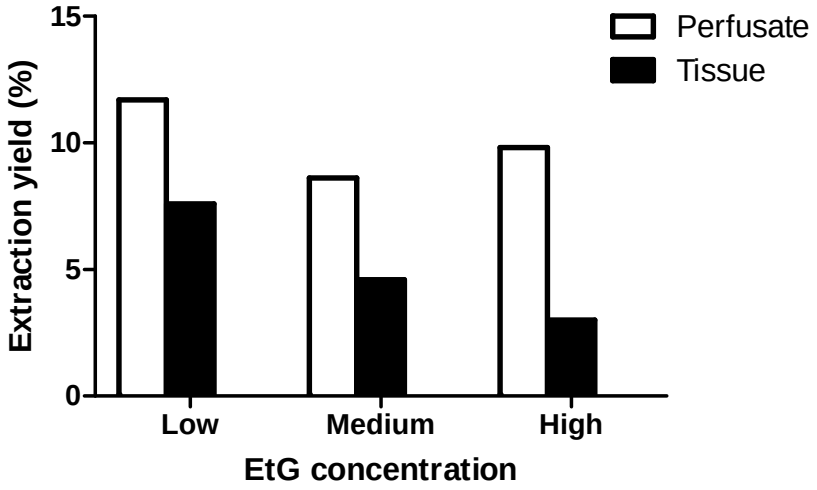
FIG. 2 Extraction yields during HS-SPME. Extraction yields were calculated by comparing the peak area of the EtG quantifying ion after HS-SPME to direct injection of the same concentration. Low = 10; medium = 100; high = 500; units = ng/mL for placental perfusate and ng/g for placental tissue.



Reproducibility and Extraction Yield

Intra- and inter-day CVs for perfusate and tissue are reported in Table 3. Extraction yields ranged from 8.6-11.7% for perfusate and 3.0-7.6% for placental tissue (Figure 2).

TABLE 3 Summary of inter- and intra-day variability for final protocol (n=3 for each).

Matrix	Inter-day CV (%)	Intra-day CV (%)
Perfusate	13.5	14.6
Tissue	28	14.1

Analysis of Perfusion Samples

This method was used to determine the presence of EtG in fetal perfusate during placental perfusion of 1 μg/mL maternal EtG and in the perfused placental tissue after the experiment. EtG concentrations in the fetal perfusate over the 60-minute perfusion are shown in Table 4. Using this method, EtG was first detectable after 10 minutes of perfusion and first quantifiable after 20 minutes. After 60 minutes, EtG levels did not appear to have reached a plateau. Perfused placental tissue contained 222.1-398.5 ng/g EtG after 60 minutes of perfusion (Table 5). EtG was not detected in a sample taken from an adjacent unperfused lobule.

TABLE 4 EtG concentration in fetal reservoir during a 1-hour perfusion experiment of 1 μg/mL EtG initially added to the maternal reservoir using the *ex vivo* placental perfusion model.

Time (minutes)	Concentration (ng/mL)
0	ND
10	<LOQ
20	19.6
30	36.5
60	92.8

TABLE 5 EtG concentration in placental tissue after 1-hour perfusion experiment of 1 μg/mL EtG initially added to the maternal reservoir using the *ex vivo* placental perfusion model.

Sample	Concentration (ng/g)
1	398.5
2	222.1
3	287.8
Average	302.8
SD	89.2

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DISCUSSION

This study is the first to use HS-SPME coupled with GC-MS to measure EtG in both placental tissue and fetal perfusate. Detecting EtG in these matrices has a variety of uses. Tissue can be used in forensic cases when access to blood or urine is limited. Additionally, since EtG does not appear to bind to erythrocytes[24] and since there are no reports of EtG binding to plasma proteins, analysis of EtG in perfusate is likely similar to analysis in plasma or even whole blood. Most importantly, these matrices are used for measurement of EtG transfer across the human placenta using the *ex vivo* placental perfusion model. Based on preliminary data reported in this study from a perfused lobule and fetal samples collected during 1 hour of perfusion, EtG appears to cross the placenta and bind to placental tissue. This finding would indicate that EtG previously detected in meconium is likely of both maternal and fetal origin. Further analysis of EtG transfer using a greater sample size, longer perfusion time, and more controlled perfusion conditions is needed to support the data from this study.

The high sensitivity of this analytical method allowed for accurate determination of EtG transfer from maternal to fetal circulation in the *ex vivo* perfusion model. Other studies that have analyzed EtG in blood via GCMS achieved higher LODs, ranging from 37-100 ng/mL.[13-15] Comparatively, the LOD and LOQ for perfusate in this study were approximately 2 ng/mL and 5 ng/mL. Several factors might contribute to the comparatively lower limits seen in this study compared to others. Firstly, placental perfusate is a "cleaner" matrix than whole blood and does not require initial protein denaturing and centrifugation to remove cellular fractions. Secondly, these previous methods introduced sample into the GC via direct injection, whereas in this study samples were introduced via SPME. This latter method of sample injection allows for introduction of a concentrated amount of analyte into the GC[16] compared to only 1-2 μL of a reconstituted solution of analyte, as seen with direct injection.[25] Lastly, the higher sensitivity in this study may be due to the derivatizing agent and protocol utilized. Samples in this study were derivatized with both PFPA and HFBA to determine that HFBA produced higher area counts at anticipated peaks, and samples were derivatized at an optimal temperature for 30 minutes. The low LOQ established for this method was essential for accurately measuring the initial appearance of EtG in the fetal perfusate over the first hour, where concentrations ranged from 0-100 ng/mL. Analytical sensitivity allowed for the quantification of EtG in the fetal circulation within 20 minutes, which gives some insight on potential tissue binding and saturation during the initial 20 minutes.

As demonstrated in Table 1, the validation of this method was lengthy, as many changes were required to obtain a final method that was specific, accurate, and sensitive enough to detect EtG in placental perfusate and tissue. The two major limitations to the validation of this method were the higher degree of inter- and intra-day variability, and the low extraction yield. Inter- and intra-day variability was 8-17% and 5-15% for perfusate and 17-38% and 14-35% for tissue. Variability in tissue samples was greater than in perfusate samples likely due to the inherent compositional complexity and heterogeneity of tissue. SPME itself is a source of variability and several sources of imprecision have been proposed.[26] Heterogeneity among tissue samples will lead to different degrees of adsorption to the SPME fiber and competition with EtG for adsorption sites. Fiber use can also lead to variability, as there can be carryover of background substances to subsequent samples. A more detailed optimization of fiber adsorption/desorption times and temperatures could reduce this carryover.

Extraction yield was calculated for this experiment as the ratio of detector count for analyte extracted from either perfusate or tissue to detector count for pure un-extracted analyte. For the latter, EtG was added directly from the stock solution to the vial, evaporated, derivatized, and injected. The experimental recoveries for perfusate and tissue at varying EtG concentrations were 9-12% and 3-8%, respectively, indicating that overall recovery was rather poor. For tissue, one source of low EtG yield was likely analyte loss during homogenation and centrifugation. For perfusate, where samples were immediately extracted after preparation, sources of EtG loss could be due to the use of only 1 mL sample prior

to solid phase extraction and to interference of one or more of the ingredients in the M199 media with the SPE cartridges. While recovery was low for this study, the detection method was still sensitive enough to accurately measure all maternal and fetal samples.

In summary, the present method for analyzing placental perfusate and placental tissue offers added sensitivity over previous methods analyzing EtG in blood via GC-MS. This increased sensitivity is necessary for determining the specific timing and extent of placental transfer of EtG by means of the *ex vivo* placental perfusion model, which is an objective currently under investigation at our laboratory. The transplacental disposition of EtG will help elucidate the origin of EtG detected in neonatal meconium and may guide future EtG cut-off values when screening for prenatal alcohol exposure.

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REFERENCES

1. Foti RS, Fischer MB. Assessment of UDP-glucuronosyltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs. Forensic Sci Int 2005;153:109-116.
2. Halter CC, Dresen S, Auwaerter V, Wurst FM, Weinmann W. Kinetics in serum and urine excretion of ethyl sulfate and ethyl glucuronide after medium dose ethanol intake. Int J Legal Med 2008;122:123-128.
3. Hoiseth G, Bernard JP, Karinen R, et al. A pharmacokinetic study of ethyl glucuronide in blood and urine: applications to forensic toxicology. Forensic Sci Int 2007;172:119-124.
4. Hoiseth G, Morini L, Polettini A, Christophersen A, Morland J. Blood kinetics of ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. Forensic Sci Int 2009;188:52-26.
5. Borucki K, Schreiner R, Dierkes J, et al. Detection of recent ethanol intake with new markers: comparison of fatty acid ethyl esters in serum and of ethyl glucuronide and the ratio of 5-hydroxytryptophol to 5-hydroxyindole acetic acid in urine. Alcohol Clin Exp Res 2005;29:781-787.
6. Gareri J, Lynn H, Handley M, Rao C, Koren G. Prevalence of fetal ethanol exposure in a regional population-based sample by meconium analysis of fatty acid ethyl esters. Ther Drug Monit 2008;30:239-245.
7. Backdash A, Burger P, Goecke TW, et al. Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium from newborns for detection of alcohol abuse in a maternal health evaluation study. Anal Bioanal Chem 2010;396:2469-2477.
8. Morini L, Groppi A, Marchei E, et al. Population baseline of meconium ethyl glucuronide and ethyl sulfate concentrations in newborns of nondrinking women in 2 Mediterranean cohorts. Ther Drug Monit 2010;32:359-363.
9. Pichini S, Marchei E, Magnarelli F, et al. Assessment of prenatal exposure to ethanol by meconium analysis: results of an Italian multicenter study. Alcohol Clin Exp Res 2012;36:417-424.
10. Tarcomnicu I, van Nuijs AL, Aerts K, et al. Ethyl glucuronide determination in meconium and hair by hydrophilic interaction liquid chromatography-tandem mass spectrometry. Forensic Sci Int 2010;196:121-127.
11. Morini L, Falcon M, Pichini S, et al. Ethyl-glucuronide and ethyl-sulfate in placental and fetal tissues by liquid chromatography coupled with tandem mass spectrometry. Anal Biochem 2011;418:30-36.
12. Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther 2011;90:67-76.
13. Janda I, Alt A. Improvement of ethyl glucuronide determination in human urine and serum samples by solid-phase extraction. J Chromatogr B Biomed Sci Appl 2001;758:229-234.
14. Schmitt G, Aderjan R, Keller T, Wu M. Ethyl glucuronide: an unusual ethanol metabolite in humans. Synthesis, analytical data, and determination in serum and urine. J Anal Toxicol 1995;19:91-94.

15. Shen BH, Yan H, Zhao H, Zhuo XY. [Determination of ethyl glucuronide in blood by GC-MS/MS]. Fa Yi Xue Za Zhi 2009;25:431-433.
16. Pragst F. Application of solid-phase microextraction in analytical toxicology. Anal Bioanal Chem 2007;388:1393-1414.
17. Miller RK, Wier PJ, Maulik D, di Sant'Agnese PA. Human placenta in vitro: characterization during 12 h of dual perfusion. Contrib Gynecol Obstet 1985;13:77-84.
18. Derewlany LO, Leeder JS, Kumar R, et al. The transport of digoxin across the perfused human placental lobule. J Pharmacol Exp Ther 1991;256:1107-1011.
19. Pollex EK, Anger G, Hutson J, Koren G, Piquette-Miller M. Breast cancer resistance protein (BCRP)-mediated glyburide transport: effect of the C421A/Q141K BCRP-single-nucleotide polymorphism. Drug Metab Dispos 2010;38:740-744.
20. Kerekes I, Yegles M, Grimm U, Wennig R. Ethyl glucuronide determination: head hair versus non-head hair. Alcohol Alcohol 2009;44:62-66.
21. Yegles M, Labarthe A, Auwarter V, et al. Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers. Forensic Sci Int 2004;145:167-173.
22. Agius R, Nadulski T, Kahl HG, et al. Validation of a headspace solid-phase microextraction-GC-MS/MS for the determination of ethyl glucuronide in hair according to forensic guidelines. Forensic Sci Int 2010;196:3-9.
23. Hoiseth G, Yttredal B, Karinen R, et al. Ethyl glucuronide concentrations in oral fluid, blood, and urine after volunteers drank 0.5 and 1.0 g/kg doses of ethanol. J Anal Toxicol 2010;34:319-324.
24. Hoiseth G, Morini L, Polettini A, et al. Serum/whole blood concentration ratio for ethylglucuronide and ethyl sulfate. J Anal Toxicol 2009;33:208-211.
25. Aleksa K, Walasek P, Fulga N, et al. Simultaneous detection of seventeen drugs of abuse and metabolite in hair using solid phase micro extraction (SPME) with GC/MS. Forensic Sci Int 2012;218:31-36.
26. Pawliszyn J, Pawliszyn B, Pawliszyn M. Solid phase microextraction (SPME). Chem Educator 1997;2:1-7.
27. Jurado C, Soriano T, Gimenez MP, Menendez M. Diagnosis of chronic alcohol consumption. Hair analysis for ethyl-glucuronide. Forensic Sci Int 2004;145:161-166.