



# Rapid Analysis of Ethyl Sulfate and Ethyl Glucuronide in Human Urine

*A novel approach to detect and quantify EtG and EtS in human urine samples using a simple sample preparation procedure with reliable direct analysis by LC-MS/MS*

*An Overview of the verified iMethod™ Application for EtG and EtS, Version 1.0 for Cliquid® Software*

## Introduction

Ethyl glucuronide (EtG) is a direct metabolite of ethanol (alcohol). The presence of EtG in urine can be used to detect the ingestion of alcohol up to 80 hours after the initial ingestion. EtG testing is not subject to adulteration, fermentation or cross reactivity. Ethyl sulfate (EtS) has also been identified as a second specific metabolite of ethanol. Measuring both EtG and EtS provides greater sensitivity and accuracy.

In our new iMethod™ Application, we present a novel analytical method that allows the accurate and reliable detection and quantification of EtG and EtS in human urine samples using a simple sample preparation procedure with direct analysis by LC-MS/MS. This procedure produces highly reliable and accurate quantitative results.

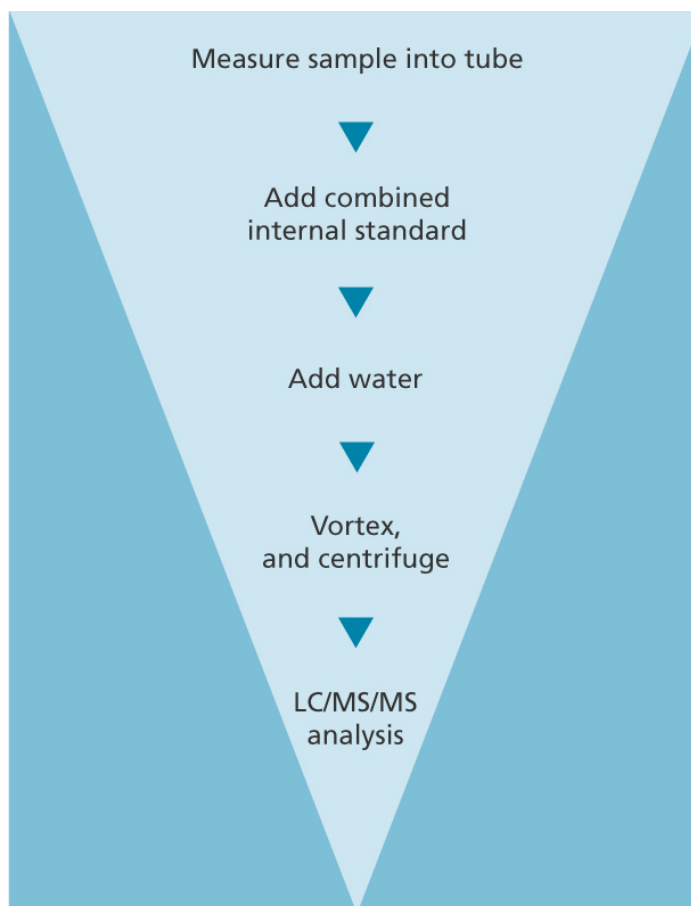
## Method Overview

The basic outline of the sample preparation procedure for this analysis is shown in Figure 1. Briefly, the urine sample has internal standards added and is then diluted with water for analysis by LC-MS/MS.

This method was optimized using the following specific supplies and equipment beyond basic lab equipment and reagents: (1) Phenomenex Synergi Hydro-RP analytical HPLC column (4 µm, 100 x 4.6 mm), (2) Shimadzu Prominence LC system, and (3) API 3200™, 3200 QTRAP®, API 4000™ or 4000 QTRAP® MS/MS system.

## Method data and results

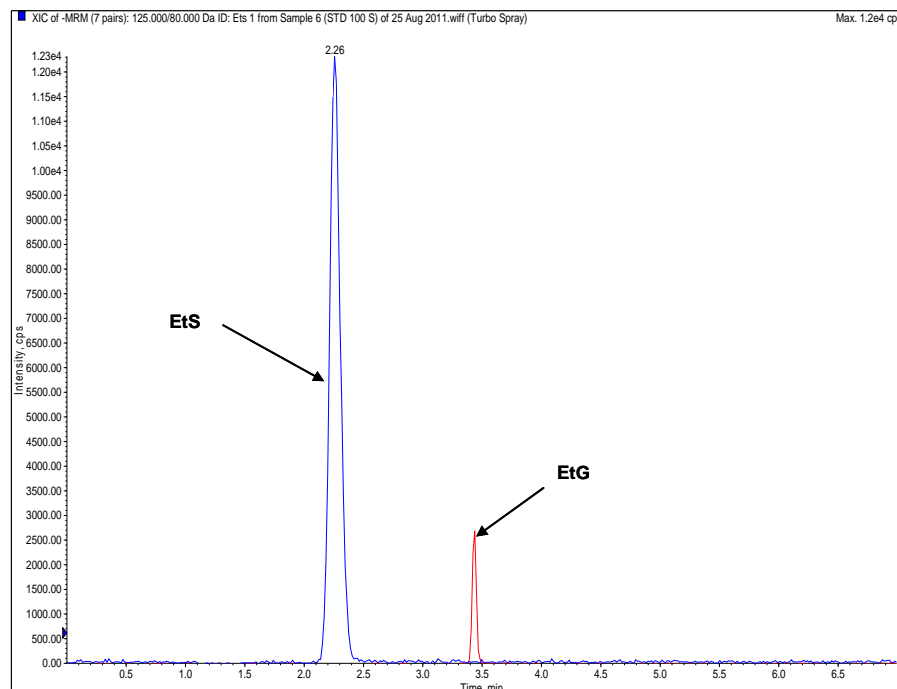
The method performance was verified in multiple labs using fortified control samples. A sample chromatogram of the EtG and EtS MRMs and the accuracy and precision results obtained in the method verification are shown in Figure 2 and Table 1.



**Figure 1. Sample preparation overview for analysis of EtG and EtS in human urine samples.**

Results show that both EtG and EtS can be accurately quantified in this procedure, with accuracies ranging from 99.2 to 104% for both EtG and EtS.

These results show that this method is accurate, reliable, and robust for the quantification of EtG and EtS in human urine.

**Figure 2. Sample chromatogram of MRMs of EtG and EtS detected at 100 ng/mL in a human urine sample.****Table 1. Outline of results obtained for this iMethod™ Application.**

	% CV	% Accuracy, 30 ng/mL	% CV	% Accuracy, 250 ng/mL	% CV	% Accuracy, 3000 ng/mL	Calibration range (ng/mL)	Correlation Coefficient
EtG using 3200 QTRAP®	4.78	100	3.65	103	2.28	104	10 to 4000	0.9997
EtS using 3200 QTRAP®	2.11	104	1.27	104	0.970	101	10 to 4000	1.0000
EtG using 4000 Q TRAP®	5.80	101	4.84	99.9	3.90	101	10 to 4000	0.9990
EtS using 4000 Q TRAP®	4.82	101	4.36	101	3.71	99.2	10 to 4000	0.9993

## System requirements

	Description/Part No.
HPLC column	Phenomenex Synergi Hydro-RP analytical HPLC column 4 um, 100 x 4.6 mm) (P/N 00D-4375-E0)
HPLC system	Shimadzu Prominence LC system
Mass spectrometer	AB SCIEX API 3200™, 3200 QTRAP®, API 4000™ or 4000 QTRAP® systems
iMethod™ Application Product Information	iMethod™ Application for EtS and EtG Version 1.0 for Cliquid Software Part number 5017191

Additional details on system, reagent, and equipment requirements for this iMethod™ Application, as well as more technical details can be provided by contacting customer support at [support@absciex.com](mailto:support@absciex.com).

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