

Determination of 2,2-Dimethylbutyrate (HQK-1001) in Human Plasma and Urine by LC-MS/MS

Weimin Wang¹, Ping Guo¹, Futian Han¹, Jing Ke¹, Harry Zhao¹, Zhongping (John) Lin¹, Patrick Bobbitt²
¹ Frontage Laboratories, Inc., Malvern, PA; ² HemaQuest Pharmaceutical Inc., Seattle WA

Overview

A robust LC-MS/MS method was developed and validated for the quantification of HQK-1001 in human plasma and human urine using LC-MS/MS with solid phase extraction plus chemical derivitization.

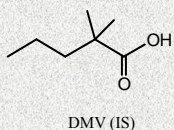
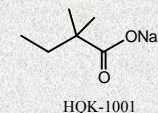
Assay Summary

- Sample extraction by solid phase extraction and chemical derivitization.
- Calibration curve range (HQK-1001): 0.1 to 50 µg/mL
- Sample volume: 100 µL
- Matrix: Human Plasma (K₃-EDTA) and Human Urine
- Method: ESI(+)-RP-HPLC-MS/MS

Introduction

Analysis of short-chain fatty acids in biological samples has proven to be challenging to bioanalytical chemists and yet it is increasingly demanded in both drug discovery PK screening and clinical sample analysis. However, HQK-1001 has a low response in either positive or negative ionization mode of LC-MS/MS. A derivitization method can increase the responses of the HQK-1001 in MS/MS detection as well as improve the separation of the short-chain fatty acid from endogenous interferences. This study presents a method that combined the solid phase extraction, which served as an effective approach to clear-up biological samples, with derivitization to improve the efficiency and reproducibility of quantitative accuracy

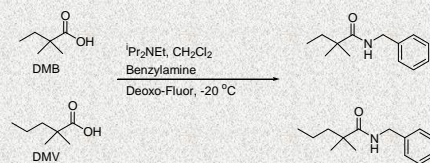
Figure 1. Chemical Structures of HQK-1001 and its Analogue 2,2-Dimethylvaleric Acid (DMV) as the Internal Standard (IS)



Methods

Extraction Procedure

Aliquots of human plasma/urine were fortified with the internal standard (DMV) and loaded onto an Oasis HLB SPE cartridge. After appropriate treatment, the samples were eluted with acetonitrile. The collected solution was subjected to chemical derivitization with Deoxo-Fluor as the agent. The carboxyl group was converted into the corresponding amide. After being dried down under N₂, the samples were reconstituted with ACN/ H₂O (25/75) and injected into the LC-MS/MS system.



Chromatographic Conditions

Column: Unison UK-C18 (30X2.0 mm, 3 micron)
 Mobile Phase A: 0.1 % formic acid in H₂O
 Mobile Phase B: 0.1 % formic acid in MeOH/H₂O(98/2)
 Injection Volume: 5 µL
 Needle Wash Solvent: Mobile Phase B
 Elution: Gradient
 Run Time: 4.6 minutes

MS/MS Detection

Mass Spectrometer: Sciex API 4000
 Ionization: Positive Ion Electrospray
 Mode: MRM

Table 1. Monitored Transitions

Compound	Q1 Mass m/z	Q3 Mass m/z
HQK-1001	206.2	71.1
DMV (IS)	220.2	85.1

Results

Linearity

- The validated concentration range: 0.1 to 50 µg/mL for HQK-001
- Correlation coefficient: r² ≥ 0.990
- LLOQ: Signal/Noise at least 20
- Specificity: Six lots of blank human serum were screened and interference was within the acceptance criteria 20% of LLOQ

Table 3. Results – Intrarun Precision and Accuracy for QC Samples in Human Plasma

Sample	%CV	%Nominal	N
LLOQ	14.4	104.0	6
QC Low	5.9	107.3	6
QC Mid	1.7	102.7	6
QC High	5.6	92.5	6

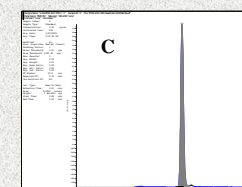
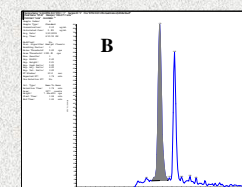
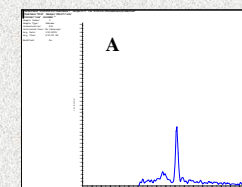
Table 4. Results - Interrun Precision and Accuracy for QC Samples in Human Urine

	Concentration (µg/mL)	0.3			19			38		
		n	Mean	%CV	n	Mean	%CV	n	Mean	%CV
Intrarun-1	n	6	6	6	6	6	6	6	6	6
	Mean	0.320	20.3	37.2						
	%CV	5.5	4.2	2.3						
	%Nominal	106.7	107.1	98.0						
Intrarun-2	n	6	6	6	6	6	6	6	6	6
	Mean	0.284	20.2	36.9						
	%CV	8.7	6.4	6.6						
	%Nominal	94.7	106.5	97.1						
Intrarun-3	n	6	6	6	6	6	6	6	6	6
	Mean	0.298	17.9	35.3						
	%CV	10.1	6.4	4.3						
	%Nominal	99.2	94.0	93.0						
Interrun	n	18	18	18	18	18	18	18	18	18
	Mean	0.301	19.5	36.5						
	%CV	9.2	8.1	5.0						
	%Nominal	100.2	102.5	96.0						

Results – Stability

- 3 Freeze/Thaw Cycles in human urine
- Bench-top stability in human urine (at room temperature): at least 6 hours
- Processed sample stability (at room temperature): at least 93 hours in human plasma and at least 45 hours in human urine

Figure 3. Chromatograms for Blank Plasma (A), LLOQ (0.1 µg/mL) (B) and IS (C)



Results from Sample Assay

- More than 2000 samples have been analyzed in the lab with good results in support of a few clinical studies.
- Incurred Sample Stability (ISR) has been evaluated using 20 samples from a clinical study, the results met the acceptance criteria.

Conclusions

A simple high throughput LC-MS/MS method for determination of HQK-1001, a short-chain fatty acid in human plasma and human urine has been developed and validated. The method was robust, sensitive and reliable. The validated high throughput method has been successfully used in support of a few clinical studies.