Short Chain Fatty Acid Analysis in Human Plasma

To support microbiome research, Pine Lake Laboratories has developed an assay for short chain fatty acids (acetic, butyric, and propionic acid) in human plasma. These targeted metabolites can be used as an indicator of microbiome activity in research subjects and as biomarkers to evaluate the effect of various pharmaceutical drug treatments during pre-clinical and clinical trials. The method involves extracting the short chain fatty acids form plasma and then analysis by direct injection GC-MS. The LOQ for all three short chain fatty acids is 1.00 ug/mL.This white paper will describe the method and highlights the performance of the method.

1. Method Description

A. Standard and QC Preparation

Standards and QCs are prepared by spiking known levels of each short chain fatty acid into plasma. The endogenous level of each short chain acid in the plasma used to prepare the standards and QCs had previously been determined by standard addition. The final concentration is then the total of the amount added and the endogenous level.

B. Sample Preparation

The following steps are taken to prepare the plasma samples for analysis:

1. Pipette 0.4 mL of each standard, QC, blank and sample into centrifuge tubes
2. Spike each tube with internal internal standards (acetic acid-d_3, butyric acid-d_3 and propionic acid-d_3).
3. Add 50 uL acetotrile, vortex and centrifuge.
4. Transfer supernatant to glass GC vial for analysis.

C. Instrumental Analysis

Samples are analyzed by GC-MS using a Nukol capillary column (15 m x 0.23 mm, 0.25 µm). Details available upon request.

2. Results

A. Linearity and Range

For all three short chain fatty acids, the range of the method was 1.00 to 100 µg/mL with R^2 > 0.995. Representative calibration curves can be found in **Figures 1-3**.

Figure 1 Representative calibration curve for Acetic Acid (1.00 – 100 µg/mL)

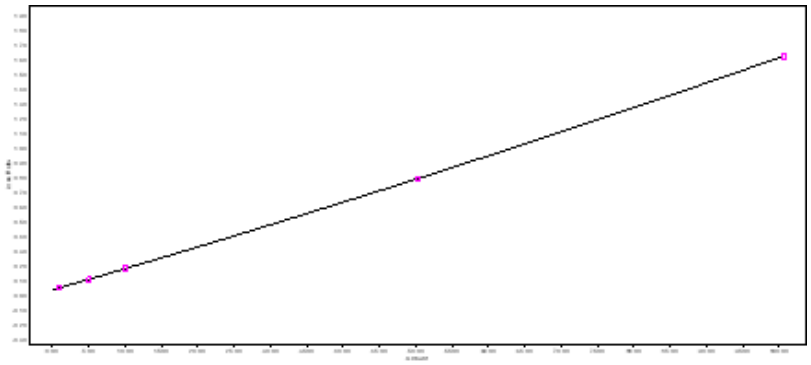


Figure 2 Representative calibration curve for Butyric Acid (1.00 – 100 µg/mL)

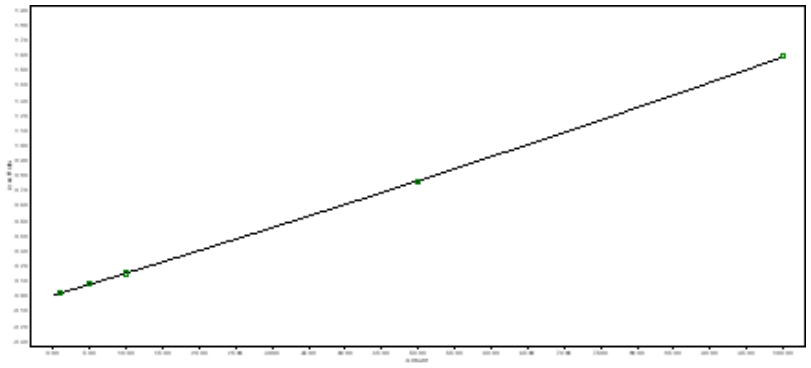
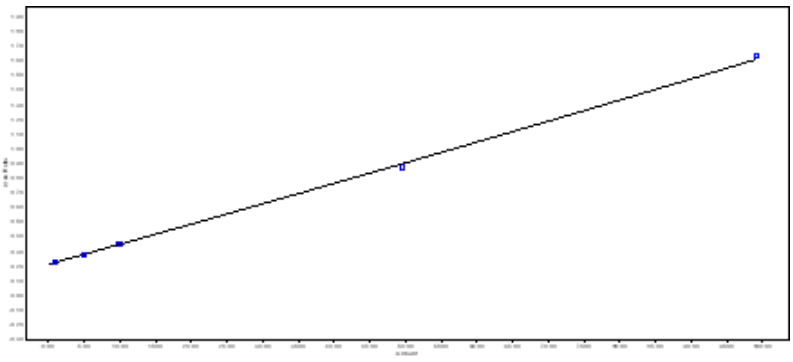


Figure 3 Representative calibration curve for Propionic Acid (1.00 – 100 µg/mL)



B. Accuracy and Precision

A representative lot of plasma was spiked at three separate levels with known amounts of each of the three short chain fatty acids. Results are in **Table 1**. Accuracy was acceptable for all three short chain fatty acids with average recovery within ± 15.0% of target for each level. Similarly precision was acceptable at ≤15.0% for each level.

Table 1. Recovery of SCFAs Spiked into Plasma

Level	Acetic Acid			Butyric Acid			Propionic Acid		
	ug/mL	Replicate	% Recovery	ug/mL	Replicate	% Recovery	ug/mL	Replicate	% Recovery
Low	2.00	1	95.0	2.00	1	119.5	2.00	1	111.7
		2	107.0		2	97.3		2	110.9
		3	85.1		3	90.1		3	108.1
		4	114.1		4	89.5		4	101.4
		5	90.1		5	111.1		5	106.9
		6	87.0		6	94.5		6	112.4
		Ave	**96.4**		**Ave**	**100.3**		**Ave**	**108.6**
		StD	**11.7**		**StD**	**12.2**		**StD**	**4.1**
		%RSD	**12.1**		**%RSD**	**12.2**		**%RSD**	**3.8**
Mid	10.0	1	98.8	10.0	1	107.7	10.0	1	108.7
		2	108.3		2	115.2		2	114.4
		3	119.5		3	108.0		3	117.7
		4	92.2		4	89.6		4	83.9
		5	93.7		5	86.1		5	103.5
		6	113.6		6	87.6		6	93.7
		Ave	**104.4**		**Ave**	**99.0**		**Ave**	**103.7**
		StD	**11.2**		**StD**	**12.7**		**StD**	**12.9**
		%RSD	**10.7**		**%RSD**	**12.8**		**%RSD**	**12.4**
High	50.0	1	106.8	50.0	1	119.4	50.0	1	123.5
		2	117.5		2	108.1		2	103.9
		3	115.0		3	98.5		3	97.1
		4	89.7		4	86.1		4	85.6
		5	107.8		5	93.3		5	91.2
		6	99.6		6	85.7		6	91.6
		Ave	**106.1**		**Ave**	**98.5**		**Ave**	**98.8**
		StD	**10.2**		**StD**	**13.2**		**StD**	**13.6**
		%RSD	**9.6**		**%RSD**	**13.4**		**%RSD**	**13.7**

Conclusion

This simple yet effective GC/MS analysis can be used to accurately determine the concentration of acetic, butyric, and propionic acid in plasma. The method is specific, accurate, and precise with a large quantifiable range for short chain fatty acids. Additional short chain fatty acids not included in the method evaluation reported in this white paper can also be detected and quantitated using this method.

About the Author

Kurt L. Moyer has more than 30 years of pharmaceutical and medical device development experience spanning all areas from discovery support to marketed products. His primary expertise is in the areas of bioanalysis, extractables and leachables, method development and validation, identification of impurities and metabolites, and GLP/GMP compliance. Prior to joining Pine Lake Laboratories, Dr. Moyer served as a Senior Research Investigator for Sanofi Aventis and a Research Scientist for the DuPont Pharmaceutical Company. Dr. Moyer received his Ph.D. in biochemistry from Villanova University.

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