Quantification of Short Chain Fatty Acids (acetate, butyrate, propionate) in human blood with ion exclusion chromatography

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ABSTRACT

Short Chain Fatty Acids (SCFAs), i.e. acetate, propionate and butyrate, are mainly produced by bacterial fermentation of undigested carbohydrates in the human colon. Most important are omega-3, omega-6 and unsaturated fatty acids as being important for a healthy lifestyle. SCFAs are fundamental for proper intestinal flora and they can help to prevent type 2 diabetes. SCFAs such as acetate and propionate show promise as candidates to increase satiety-enhancing properties of food. Here we describe a simple method for determining organic acids in human blood.

1. Introduction

Short Chain Fatty Acids (SCFAs), i.e. acetate, propionate and butyrate, are mainly produced by bacterial fermentation of undigested carbohydrates in the human colon [1]. Colorectal cancer is one of the so-called 'westernized diseases' and the second leading cause of cancer death worldwide [2]. Omega-3, omega-6 and unsaturated fatty acids are important for a healthy lifestyle. SCFAs may certainly contribute to a healthy lifestyle, in particular in respect of intestinal flora, and it is possible that they are key factors in the prevention of type 2 diabetes [3]. In addition, SCFAs may have a role in appetite regulation and energy homeostasis [4]. Therefore, SCFAs such as acetate and propionate are discussed as health-promoting compounds that increase satiety-enhancing properties of food. Nevertheless, the diagnostic measurement of SCFAs is diagnostic challenging. Currently, most of the investigations measuring SCFAs so far have been only performed in stool. There is convincing data showing that fecal SCFAs is a good biomarker of the gut microbiota ecosystem and dynamics of SCFAs in the human body [5].

However, analysis of SCFAs from stool samples is elaborating and still challenging [6]. Moreover, most protocols for the analysis of SCFAs rely on gas chromatography-mass spectrometry (GC-MS), which of course is a very powerful and reliable but also requires sophisticated equipment [7]. Therefore, a simple and reliable method to analyse SCFA in human blood would be diagnostic helpful. We here present a simple method for determination of SCFAs in human blood samples by ion chromatography.

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2. Materials and methods

2.1. Sample storage and chemicals

All samples were stored at −40 °C and thawed at room temperature prior to analysis. A SCFA standard stock of 5 mg/l were obtained from Metrohm (Filderstadt, Germany). Standards for acetate (#51791), butyrate (#08089), propionate (#51716) at concentrations of 1000 mg/l were obtained from Sigma-Aldrich (Taufkirchen, Germany). Chemicals for eluent A (0.5 mM $HClO_4$) and regeneration solution B (50 mmol LiCl/l) were obtained from Methrom and prepared in deionized water.

2.2. Sample preparation

200 μl of serum was diluted in 800 μl of deionized water in an Eppendorf tube. The samples were then vortexed for 2 min (VWRVX-2500 Multi-tube vortex mixer, VWR International, Darmstadt, Germany) followed by centrifugation (5 min, $3041 \times g$) in a Rotana 460 Eppendorf centrifuge (Eppendorf, Hamburg, Germany). Subsequently, the supernatants were filtered through a 0.2 μm Chromafil$^{(R)}$ Xtra Ca-20/25 filter (Machery-Nagel, Düren, Germany). Subsequently, 20 μl of the filtrate were injected into the ion chromatography autosampler using the following setup: Column: Metrosep Organic Acids 250/7.8 (Metrohm); Pre-column: Metrosep Organic Acids Guard 4.6 (Metrohm); Prefilter: Metrosep Organic Acids Guard/3.5 (Metrohm); column flow 0.5 ml/min. The ion chromatography system was coupled with a conductivity detector and an In-line Degasser suppressor module.

3. Results

The results of the validation for acetate, butyrate and propionate are depicted in Fig. 1, showing that all parameters are linear over the entire measurement range. The elution chromatogram showed spurious peaks for all parameters, which however did not cause any analytical problems.

The most important characteristic analytical data for the separation of these SCFAs are summarized in Table 1 and data on quality control accuracy from QC samples (low, medium, and high) for each organic acid are given in Table 2.
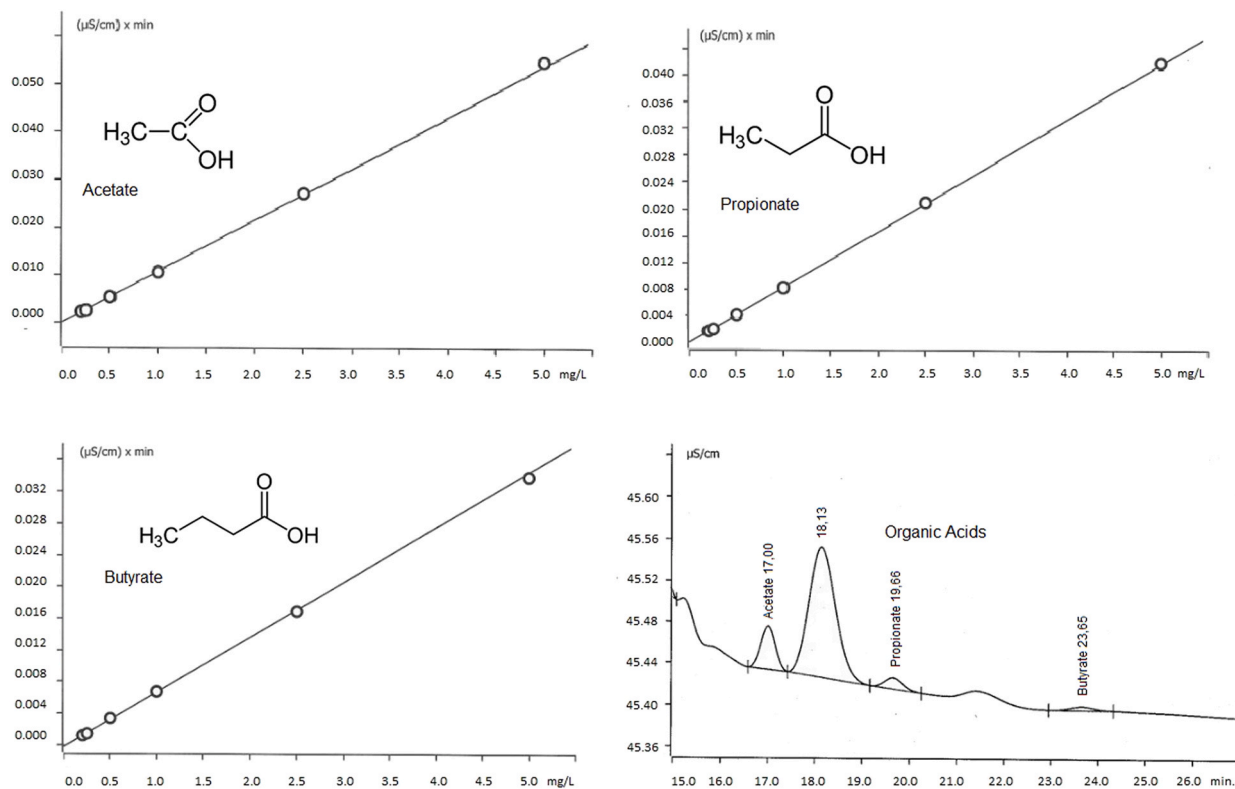

Fig. 1. Determination of the limit of quantification (LOQ) of acetate, butyrate and propionate in serum chromatogram using exclusion chromatography.

Table 1
Analytical data.

Analyte	Linearity	Correlation coefficient	LOQ	Average	SD	RSD (%)	Bias (%)
Acetate	0.2–5.0 mg/l	R: 0.999	0.2 mg/l	0.19	0.01	4.7	−2.6
Butyrate	0.2–5.0 mg/l	R: 0.999	0.2 mg/l	0.2	0.01	3.2	−2.2
Propionate	0.2–5.0 mg/l	R: 0.999	0.2 mg/l	0.2	0.01	3.2	−2.2

Abbreviations used are: LOQ, limit of quantification; RSD, relative standard deviation.

Table 2
Accuracy from Quality Control (QC) samples (n = 10).

Analyte	QC (l)	QC (m)	QC (h)	QC (l) RSD	QC (m) RSD	QC (h) RSD
Acetate	0.2 mg/l	1.3 mg/l	1.6 mg/l	5.80%	1.10%	0.70%
Butyrate	0.2 mg/l	0.4 mg/l	0.6 mg/l	5.50%	8.60%	4.20%
Propionate	0.2 mg/l	0.5 mg/l	0.8 mg/l	3.40%	4.50%	2.50%

Abbreviations used are: l, low; m, medium; h, high; RSD, relative standard deviation.

4. Discussion and conclusion

The method has a measuring range between 0.2 and 5.0 mg/l (Table 1), which could probably be extended to higher concentrations. The limits of quantification of 0.2 mg/l are sensitive enough to reliably detect these analytes in blood. The QC accuracy in all cases is <9% (Table 2), suggesting that the methodology is rather robust. The method is inexpensive and offers a reliable way to measure SCFAs, while previous methods based on mass spectrometry (e.g. GC-MS) are often expensive [8]. Furthermore, the quantification limits of the ion chromatography are comparable to other methods and are completely sufficient for SCFA determination. As derivatisation is not necessary with this method, the method further saves time and money. The linearity between 0.2 and 5.0 mg/l corresponds to the essential occurrence and fully meets all criteria. Furthermore, compared to GC methods that are often influenced by extraction yield, the proposed methodology does not require elaborating processing steps preventing loss during sample handling.

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