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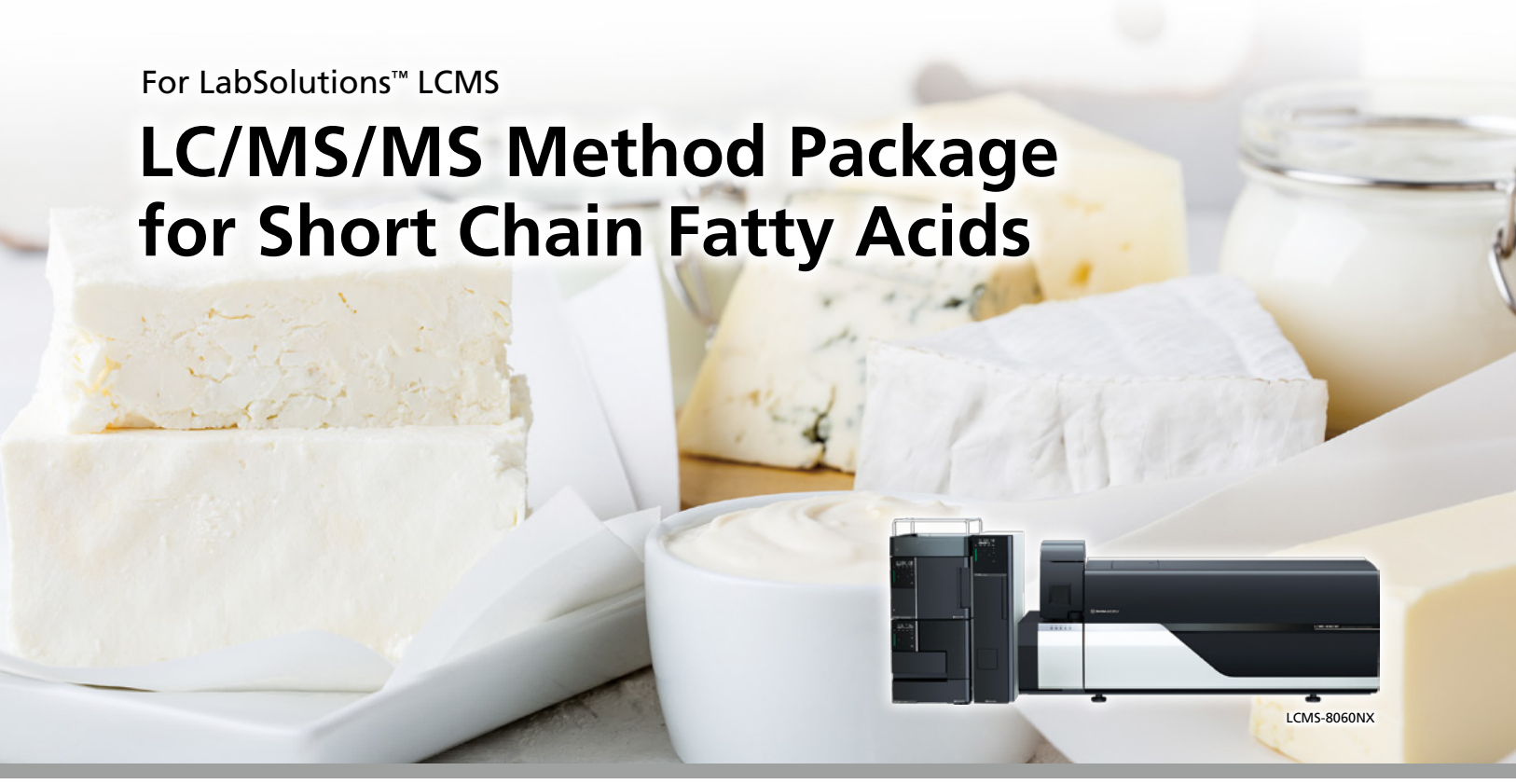
For LabSolutions™ LCMS

LC/MS/MS Method Package for Short Chain Fatty Acids



Short-Chain Fatty Acids and Organic Acids Targeted for Analysis

Of the short-chain fatty acids produced by intestinal bacteria, acetic acid, propionic acid, and butyric acid are well known, and it has been reported that there are some connections between them and lifestyle-related diseases such as obesity and diabetes. Generally speaking, short-chain fatty acids are highly volatile and highly hydrophilic. This makes it difficult to perform LCMS analysis using a conventional reversed phase system. For that reason, this method package targets short-chain fatty acids (C2 to C5) that have been derivatized using 3-nitrophenylhydrazine (3-NPH). After setting MRM transitions, it can be used for the simultaneous analysis of 22 components, including organic acids related to the central metabolic pathways.

Short-Chain Fatty Acids
Acetic acid
Propionic acid
Butyric acid
Isobutyric acid
Valeric acid
Isovaleric acid

Organic Acids
2-oxobutyric acid
2-hydroxyglutaric acid
α-ketoglutaric acid
β-hydroxybutyric acid
Isocitric acid
Oxaloacetic acid
Citric acid
Glyoxylic acid
Glycolic acid
Succinic acid
Lactic acid
Pyruvic acid
Fumaric acid
Maleic acid
Malonic acid
Malic acid

MRM Transition Settings

When making MRM transition settings, selectivity can be enhanced by specifying characteristic product ions for 3-nitrophenylhydrazine derivatives. Note that some organic acids have a carbonyl group derived from ketones, so 3-nitrophenylhydrazine derivatives that reacted with carboxylic acid and the carbonyl group are targeted for MRM transition.

Pretreatment Protocol Including Derivatization

The entire protocol necessary for pretreatment, including the derivatization step using 3-nitrophenylhydrazine, is contained in the Instruction Manual. By following these procedures, an analyst should be able to perform the entire process, from derivatization to data acquisition and data analysis, immediately after the package is introduced.

For LabSolutions LCMS Software

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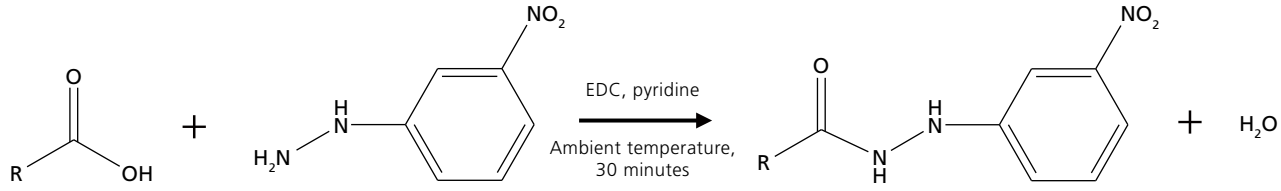
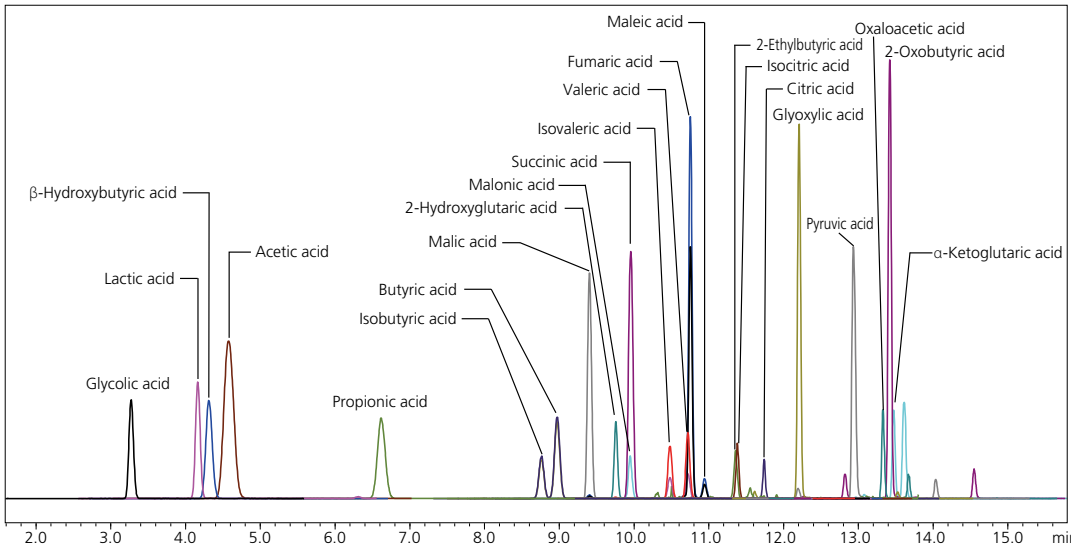
For derivatization using 3-nitrophenylhydrazine, carbodiimide is used as a condensing agent, and reaction proceeds under mild conditions. Moreover, in order to efficiently carry out reactions with both short-chain fatty acids and organic acids, this method package includes derivatization using 3-NPH instead of the more commonly used 2-NPH. Also, 3-NPH derivatizations after reactions are stable, and quantitative analysis of the short-chain fatty acids or organic acids in the sample can be performed through reaction with an appropriate amount of derivatization reagent. Additionally, as reactions take place in water solutions using this derivatization reagent, the sample will not be dried and volatile components will not be lost.



Derivatization Reaction with 3-nitrophenylhydrazine (3-NPH)

The following example shows an analysis using the LCMS-8060 of a standard mixed solution of short-chain fatty acids/organic acids. An MRM chromatogram of short-chain fatty acids/organic acids derivatized using 3-NPH is shown. As can be seen, sufficient separations of the components, such as the structural isomers butyric acid, isobutyric acid, fumaric acid, maleic acid, citric acid, and isocitric acid, were obtained. This method package includes MRM transitions that were formulated beforehand in order to achieve optimization for 3-NPH derivatizations for short-chain fatty acids/organic acids. As a result, short-chain fatty acids (C2 to C5) and organic acids representative of central metabolic pathways, which are difficult to analyze with LCMS using a conventional reversed phase system, can be analyzed simultaneously.



Remarks and Precautions

LabSolutions LCMS Ver. 5.99SP2 or later is required.

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