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Organic acid derivatization protocol for mass spectrometry analysis

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Manon Gilson¹, Guillaume Bayon-Vicente¹, Simone Krings¹, Ruddy Wattiez¹, Baptiste Leroy¹

¹UMons



Manon Gilson

UMons

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We use this protocol and it's working

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Abstract

This protocol outlines the derivatization and the detection of organic acids using mass spectrometry analysis. This technique is highly sensitive and allow the detection of low-concentration samples.

Materials

Prepare the following solutions in 66% MS-grade acetonitrile:

- 200 mM 3-NPH HCl in ACN 66%
- 50 mM EDC in ACN 66%
- 7% pyridine in ACN 66%
- 5% formic acid (in MS-grade water)

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- 1 Prepare the following solutions in 66% MS-grade acetonitrile (ACN):
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 - 50 mM EDC in ACN 66%
 - 7% pyridine in ACN 66%
 - 5% formic acid (in MS-grade water)
- 2 Dilute the samples if necessary (This technique is highly sensitive and allows the analysis of samples with low concentration).
 - Caution: MOPS (3-(*N*-morpholino)propanesulfonic acid) saturates 3-NPH, preventing proper derivatization of organic acids. A dilution is necessary to achieve a maximum MOPS concentration of 1 mM
- 3 Prepare the reaction mixture:
 - Pipette 50 μ L of the sample.
 - Add 25 μ L of 200 mM 3-NPH HCl.
 - Add 25 μ L of 50 mM EDC.
 - Add 25 μ L of 7% pyridine solution.
- 4 Incubate the samples at 30°C for 5 hours at 500 rpm.
- 5 Add 125 μ L of 5% formic acid to stop the reaction