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Authors: Silvia Gosiewska, Burhan Ozalp, Olaf Schouten, Rob van der Hoeven, Peter Lankhorst, Adriana Carvalho de Souza

Affiliation & sponsor of the project: DSM Biotechnology Center, Alexander Flemminglaan 1, 2613 AX, Delft, The Netherlands

Presenting author: Scientist (PhD)

**Fast and robust analytical workflow for isolation and identification of unknown impurities during product development**

During the chemical or fermentative process, often unexpected byproducts are being formed. The knowledge of the structure of these compounds leads to understanding how these components could be formed and give an opportunity to prevent their formation and/or increase the yield of the desired product. The identification of the unknown byproducts in the mixture of main product and known byproducts is usually very difficult as the new impurities can be present on very low level and can differ structurally only slightly from the other compounds present in the sample.

Fast and practical workflow has been developed for the identification of the chemical structure of impurities. The workflow is based on isolation of impurities using a semi preparative HPLC and followed by high resolution mass spectrometry and high field NMR analyses for structure elucidation.

Semi-preparative HPLC system using 10 to 20 mm i.d. columns can be used for the isolation of the unknowns in amount as low as 0.1% (1g/L). The amount of isolated material, few milligrams or less of the desired component, is sufficient for the 2D-NMR identification with high field NMR equipment.

The workflow has been applied successfully for structure elucidation of several unknown impurities in various biotech products e.g. fermentative production of steviol glycosides.