The use of flash chromatography for purification geranylo-β-glycoside synthesized in the Koenigs-Knorr reaction

DZIADAS MARIUSZ[1], Beata Liberek[2], Henryk H. Jeleń[1]

[1]*Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Poznań, Poland;*
[2] *Sugar Chemistry Chair, Faculty of Chemistry, Gdańsk University, Gdańsk, Poland*
Corresponding email: *mariusz.dziadas@up.poznan.pl*

Glycosides occur widely in plant material and include a substantial reservoir of aromatic compounds that are immobilized as a non-volatile precursors. For the analysis of these compounds, especially to monitor hydrolysis processes internal standards are required, that would mimic the behavior of analytes of interest. Due to the complex nature of food as a matrix, quantification of flavor compounds released from glycosides after hydrolysis or quantification of glycosides require an appropriate internal standard and for SIDA (Stable Isotope Dilution Analysis) isotopomers are used. The greatest limitation in using such standards is their limited availability. The synthesis of geranylo-β-D- glucopyranoside was performed to check the pathway to synthesize isotopomers for SIDA analysis in future.

The paper presents a synthesis of geraniol-beta-glucoside in the presence of Ag2O (63% yield) by the Koenigs-Knorr from tetra-*O*-acetyl-β-D-glucopyranobromide. A rapid method for purification of a reaction product using flash chromatography on a manually packed column filled with 100g of SiO2 60 (40-63um, Merck) in one hour was developed. Flash chromatography method was developed based on data obtained from TLC analysis, using a mobile phase composition that was selected in TLC in order to achieve appropriate rates of RF, for each of the two purification steps (after the synthesis of RF=0.38 with toluene:ethyl acetate 5:1 and after deacetylation (MeONa) RF=0.65 chloroform:methanol 4:1). In order to identify reaction product analysis was carried out using ^{1}H and ^{13}C NMR geranylo-β-D-glucose - H1 - δ 4.27 (d) $J_{1,2}$ 8.0 Hz, H2 - δ 3.17 (t) $J_{2,3}$ 8.4 Hz, H3 - δ 3.30 (t) $J_{3,4}$ 8.8 Hz, H4 - δ3.28 (t) $J_{4,5}$ 8.8 Hz, H5 - δ 3.22 (ddd) 2xH6 - δ 3.86 (dd) δ 3.66 (dd) $J_{6,6}$ 12.0 Hz $J_{6,5}$ 5.6 Hz $J_{6',5}$ 2.0 Hz, 1-CH_2 - δ 4.34 (dd) δ 4.24 (dd) J_{gem} 11.6Hz J 6.4 Hz, 2-CH - δ 5.36 (t) J 6.4; 6.8 Hz, 3-CCH_3 - δ 1.68 (s), 4-CH_2 - δ 2.04 (t) J 7.6 Hz, 5-CH_2 - δ 2.11 (m) J 6.4; 6.8 Hz, 6-CH - δ 5.01 (t) J 6.4; 6.8 Hz, 7-CCH_3 - δ 1.60 (s), 8-CH_3 - δ 1.67 (s).

In order to verify the conformation of beta-glycoside and the ability to be used as the internal standard enzymatic hydrolysis using a complex

enzyme preparation of AR-2000, which contain a specific beta-glucosidase activity in combination with SPME.
This work is a preliminary step to carry out the specific synthesis of glycoside with labeled aglycone. This will provide internal standards for direct analysis of glycosides by GC/MS after derivatization or an indirect analysis after hydrolysis of glycoside and determination of the volatile aglycone using appropriate analytical technique (SPE, SPME, GC/MS) to quantify them.

Determination of melamine in fresh milk with Single hollow fiber SLM extraction based on ion pair mechanism combined with HPLC

LEIHONG GAO[1], Jan Åke Jönsson[2]

[1] Department of Science, Hebei University of Science and Technology, Yuhua East Road NO. 70, 050018, Shijiazhuang, China.
[2] Division of Analytical Chemistry, Lund University, P.O.Box 124, 221 00, Lund, Sweden.
glh272003@yahoo.com, Jan_Ake.Jonsson@organic.lu.se

In this work, a novel analytical method based on single hollow fiber SLM extraction and HPLC was developed for the rapid analysis of melamine in fresh milk. The conditions of the extraction were investigated and optimized. As a result, a supported liquid membrane containing 6-undecanone and di-2-ethylhexyl phosphoric acid was used. The extractions were made from 25 mL aqueous donor phase (milk sample) with pH 5.0 to a more acidic acceptor phase (36 µL 1 M HCl) and the mass transfer was driven by the proton gradient between these phases [1,2]. For 5 mg/L spiked milk sample and 60 min extraction time, an enrichment factor of 21(extraction efficiency 6%) was obtained. Spike recovery of this method was 98%. It was found that the proposed method provided linear range from 0.2 to 50 mg/L (r^2 = 0.9998), low detection limit (LOD) of 0.003 mg/L and LOQ of 0.005 mg/L. The obtained results demonstrated that hollow fiber SLM extraction combined with HPLC is a simple and rapid method for the analysis of melamine in milk.