Determination of chlorinated fatty acids using SPE, XSD and GC/MS with particular regard to cultured human cells.

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Abstract

Chlorinated fatty acids (ClFAs) account for a considerable portion of the extractable, organically bound chlorine (EOCl) in aquatic animals. Because of analytical difficulties little is as of yet known about their behaviour in organisms. Some of the analytical problems were solved by using a recently introduced halogen specific detector (XSD) for gas chromatography. It was shown that the XSD is a good alternative to the previously used electrolytic conductivity detector to detect ClFA methyl esters (ClFAMEs). Furthermore, the XSD was found to be very easy to maintain and stable in the analysis of ClFAMEs. The XSD operates in an oxidative pyrolysis mode and the sample compounds are converted into their oxidation products. When halogen-containing compounds enter the hot detector, the detector current will increase.

The incorporation and metabolism of dichlorooctadecanoic acid in a cell-lines of human cells were studied. The acid was incorporated and degraded to dichlorohexadecanoic acid and dichlorotetradecanoic acid by β-oxidation. ClFAs were found both in the neutral lipid and in the phospholipid fractions of the cultured cells. No shorter ClFAs than dichlorotetradecanoic acid were detected indicating that no further metabolism occured. Dichlorotetradecanoic acid was released from the cells into the culture medium to a higher extent than were the other ClFAs.

An isolation method for ClFAMEs was developed in order to separate the metabolite dichlorotetradecanoic acid from vastly dominating common unchlorinated fatty acids. By using solid-phase extraction on aminopropyl silica > 1 µg of a ClFAMEs can be isolated and detected from 1 g of lipid with only 1% of the dominating unchlorinated FAMEs in the fraction containing the ClFAMEs.

Structure elucidation of ClFAs was done with gas chromatography/mass spectrometry using picolinyl esters of the ClFAs and electron ionization. The picolinyl esters made it possible to identify 5,6-dichlorotetradecanoic acid as a metabolite of 9,10-dichloroctadecanoic acid, which further supports that Cl_2FAs are to an extent degraded by β-oxidation. Methyl esters or pyrrolidides of ClFAs are not suitable for full characterisation of Cl_2FAs, such as localisation of the chlorine atoms.

Keywords: mass spectrometry, halogen sensitive detector, organohalogens, triacylglycerols, dichlorostearic acid, dichloromyristic acid, metabolism, membrane

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