Stable-isotope dilution analysis of 5-chloromethylfurfural (CMF)
a transient contaminant absent from liquorice

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introduction

Manufacture of liquorice involves heating/thickening of molasses to which aqueous extracts from the roots of Glycyrrhiza glabra and other flavoring ingredients have been added. A variety referred to as «salmiak» (salty liquorice) is prepared by adding up to 7% ammonium chloride during production. It is well known that heating of carbohydrates in the presence of amines produces a vast variety of condensation products that are usually considered in the context of caramelization, e.g., substituted pyrazines, pyrimidines, imidazoles or furanes. Some of these compounds have been recognized as toxic contaminants.

5-Chloromethylfurfural (CMF) appears as a rarely documented contaminant in foods with a focus on protein hydrolysates [2]. Animal studies with rodents showed an increased skin cancer activity and a strong liver carcinogenic impact of CMF [3]. The aim of this study was to elucidate whether CMF occurs as a contaminant in salty liquorice, as a result of condensation reactions in the presence of ammonium chloride.

Additionally CMF has a notable role in biomass-derived energy production. CMF can be readily obtained by the action of concentrated hydrochloric acid on common polysaccharides like starch or cellulose. For biofuel production CMF is subsequently converted into suitable ethers by alcoholysis of the chloromethyl-substituent. This reaction is reported to occur within seconds giving a first hint towards the susceptibility of CMF to nucleophilic displacement reactions ([1], see also below).
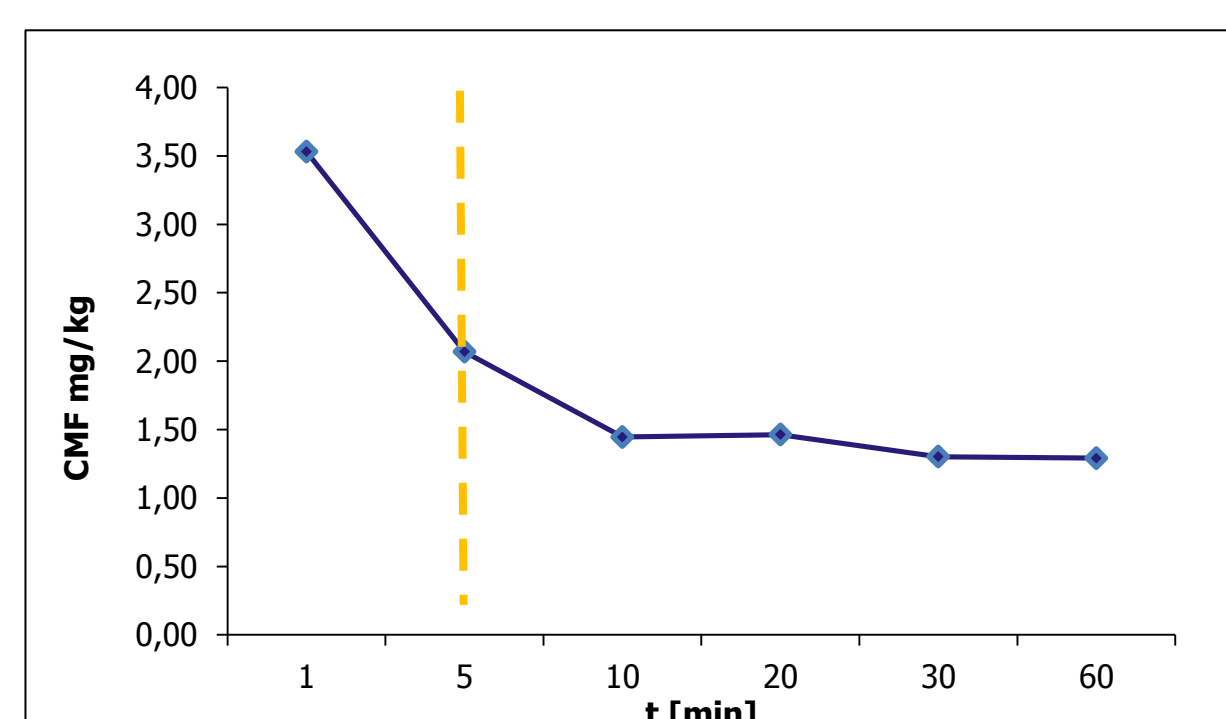
sample preparation

As CMF appears susceptible to aqueous hydrolysis additional stabilization measures must be taken during sample preparation. Therefore saturated sodium chloride was used to dissolve the liquorice specimens within a maximum of five minutes (see also below). Subsequent liquid-liquid extraction with dichloromethane provided a yellow solution that was filtered through silica for further purification. Final solvent evaporation provided samples suitable for GC/MS analysis.
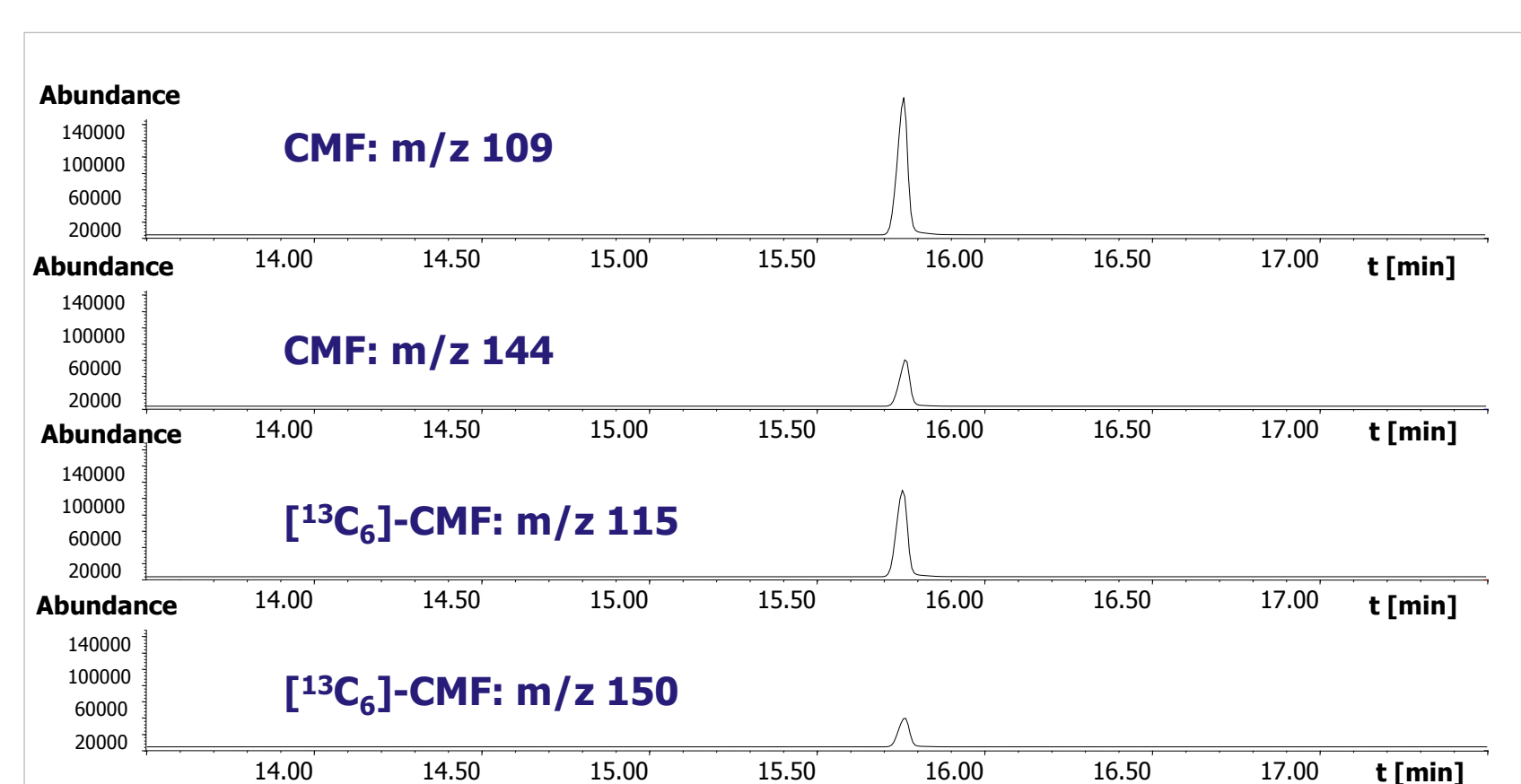
CMF hydrolysis & stabilization

Stabilization of chloroalkyl-substituted analytes against hydrolysis is often achieved by addition of sodium chloride. To investigate if sodium chloride addition also stabilizes CMF in aqueous environments stability experiments were carried out. Therefore CMF was incubated with saturated sodium chloride at room temperature and conversion to HMF was followed by GC/MS for one hour.

As can be seen, CMF hydrolysis is not prevented by saturated sodium chloride, but sufficient quantities remain extractable within minutes. Also CMF and [$^{13}C_6$]-CMF are equally susceptible to hydrolysis and hence this loss will be completely compensated from an analytical point of view. A five minute extraction time was finally selected as a compromise between sample dissolution and analyte hydrolysis.

synthesis & analytical characterization

CMF, * = $^{12/13}C$

CMF, as well as a [$^{13}C_6$]-labelled isotopomer, were obtained in a one-step procedure starting from either [$^{12}C_6$]- or [$^{13}C_6$]-fructose, where repeated dehydration and concomitant chlorination are achieved in a heated solution of lithium chloride in concentrated hydrochloric acid [4,5].

Both compounds were recovered from the very dark reaction mixture by extraction with dichloromethane and subsequent flash chromatography as colorless oils ([$^{12}C_6$]: 85%, [$^{13}C_6$]: 88%). ^{1}H-NMR (DMSO-d_6) δ 4.91 (s, 2H), 6.81 (d, 1H, J = 3.45 Hz), 7.49 (d, 1H, 3.75 Hz), 9.59 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 37.0, 112.8, 152.7, 156.2, 178.6); GC–MS: >99%.

Mass spectrometric analysis of CMF and elucidation of the fragmentation pathway was much facilitated by analysis of the synthesized [$^{13}C_6$]-isotopomer. Thus, comparison of the chlorine patterns, e.g., 144/146 or 87/91, and the subsequent loss of carbons as observed by the mass difference between the ^{12}C- and ^{13}C-isotopomer, e.g., 109/115 (six carbons) to 81/86 (five carbons) revealed the fragmentation pathway as shown below.

stable-isotope dilution analysis

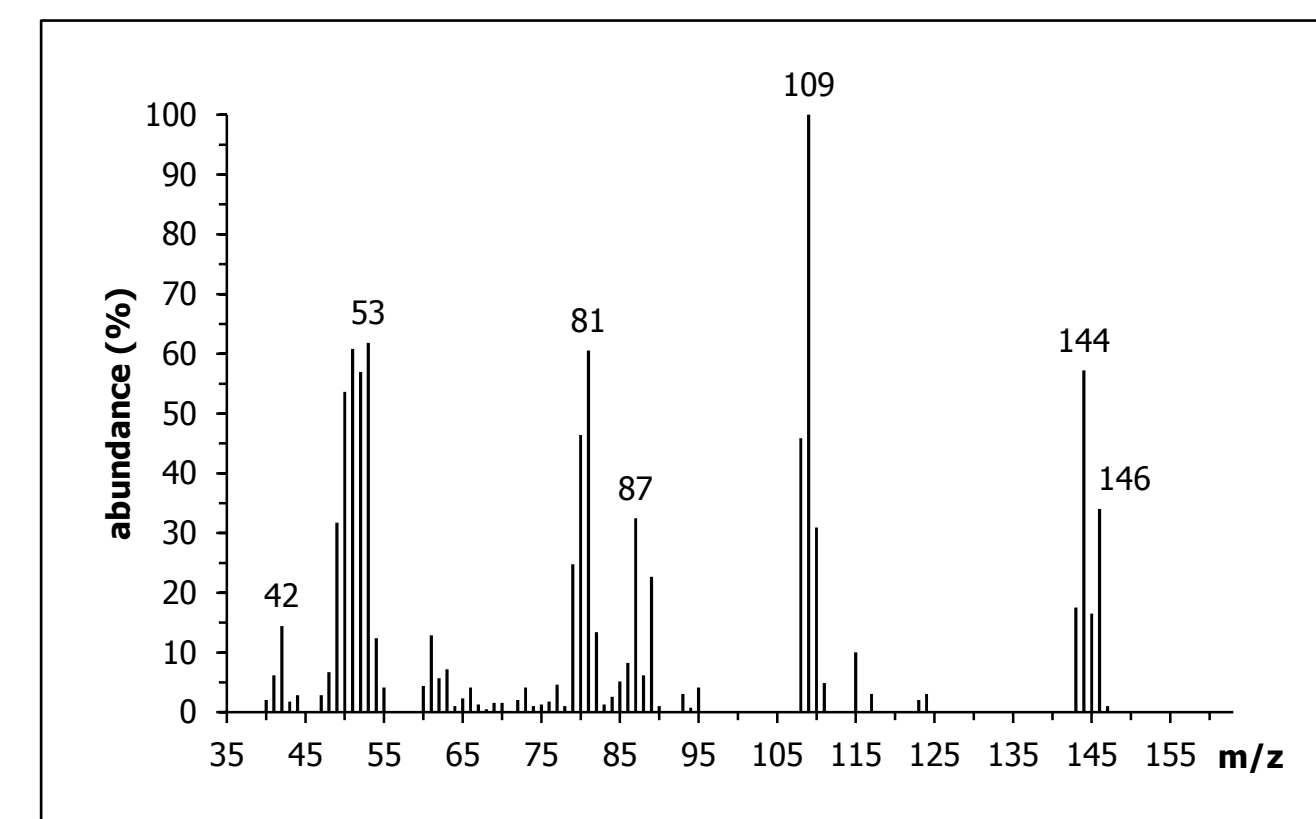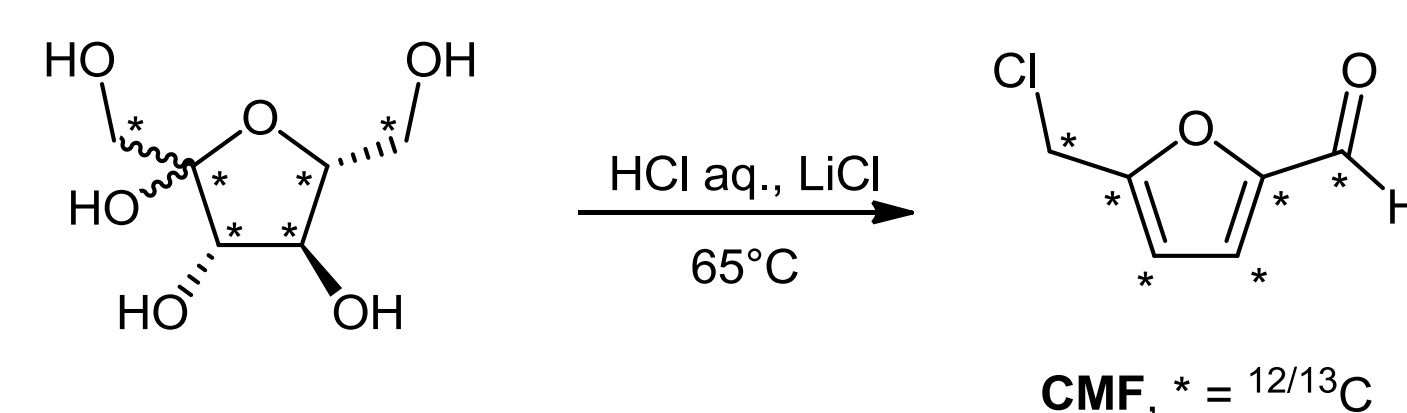| column	Restek Rxi 5MS 30 m × 0.25 mm × 0.25 µm
temperature	40°C (5 min) 6°C/min to 150°C (5 min)
carrier gas	15 psi, He
Inj. Vol.	2 µL
EI (70 eV)	CMF: m/z 109, 144 [$^{13}C_6$]-CMF: m/z 115, 150

GC/MS-chromatogram (Agilent 7890A/5975C) of a spiked liquorice sample (CMF and [$^{13}C_6$]-CMF: 1 mg/kg, measurement conditions are given in the table).
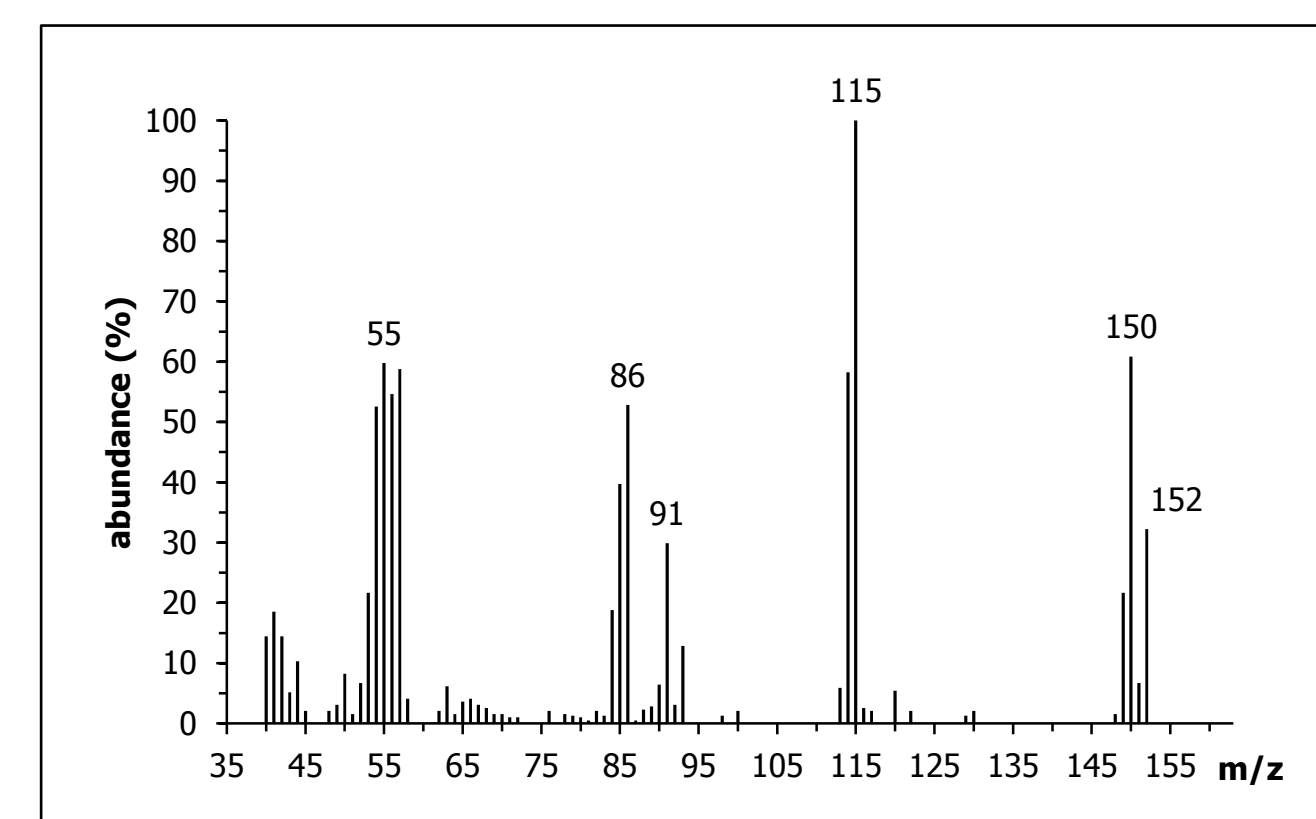
The presented method was validated following internationally accepted standards [6]. For this purpose analyte-free liquorice was spiked with CMF/[$^{13}C_6$]-CMF and subsequently processed according to the established procedure. The described method is linear over a suitable range (5-50 µg/kg) and sensitive enough to reach toxicologically relevant levels. (LOD = 10 µg/kg, LOQ = 50 µg/kg). As [$^{13}C_6$]-CMF was applied as a stable labeled internal standard a more than satisfactory recovery (92-103%) was achieved. The method was therefore judged suitable for CMF analysis in liquorice.
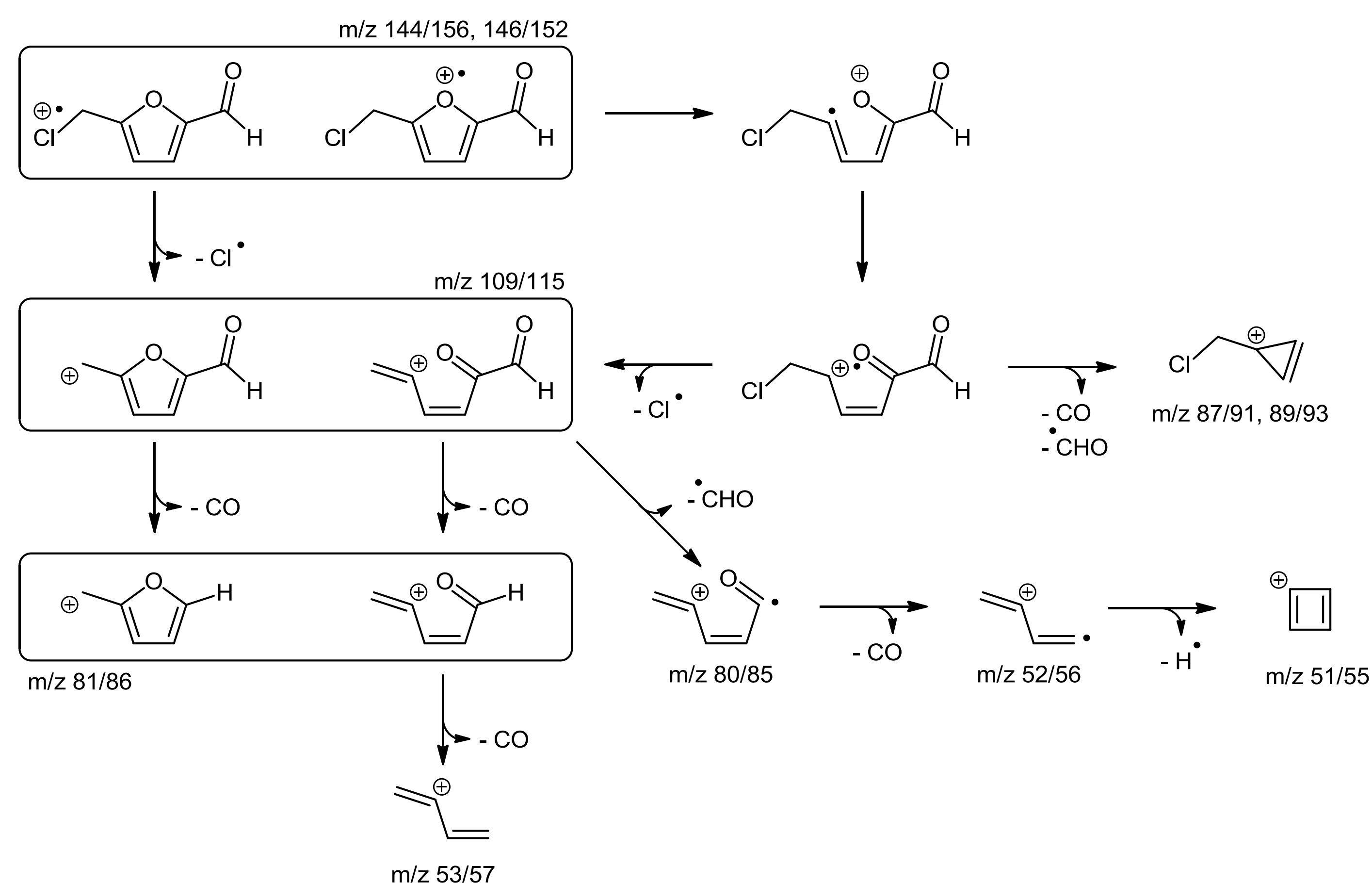
liquorice samples

The objective of this study was to investigate whether CMF occurs as an endogenous food born toxicant in liquorice flavored with ammonium chloride. Therefore 15 commercially available liquorice products with varying levels of added ammonium chloride were analyzed using the presented method. None of the samples contained measurable amounts of CMF (LOD = 10 µg/kg).

As no toxic effects of CMF are expected at concentrations below this limit and under normal dietary exposure, CMF is not a relevant food born toxicant in ammonium chloride flavored liquorice.

references

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