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Influence of Modifiers, Extractants, and Trappers on Lipid Composition with Liquids in Standard State Extraction, Supercritical Fluid Extraction and Trapping by Supercritical Fluid Extraction, Part II

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Abstract

Modifiers have a broad array of influences on extraction with liquids in standard state, supercritical fluid extraction (SFE), trapping by SFE and supercritical fluid chromatography (SFC). They can significantly change the qualitative and quantitative results. Quantitative and qualitative results can be influenced by different extractants and modifiers in different ways as it was shown by Brondz *et al.* at 2007 in "The real nature of the indole alkaloids in *Cortinarius infractus*: Evaluation of artifact formation through solvent extraction method development", *J. Chromatography A*, 1148, 1-7. The choice of correct extractant, modifier, and trapper to the bulk mobile phase for supercritical fluids (SFs) or for liquids in subcritical or in the liquids in standard state is a challenge in any extraction procedure. This is the second paper in a sequence that describes the influence of extractants and modifiers on the performance of SFs and results of extraction with liquids in standard state and SFE. Here, attention is given to possible mistakes in qualitative and quantitative results by poor understanding of the influence of extractants, modifiers, and trappers on extraction and trapping process by a careless choice of extractant, modifier, and trapper for extraction with liquids in standard state and SFE. The SF chosen for discussion in the paper is CO_2. However, similar effects can be observed with use of other SFs and fluids in subcritical and standard states. In this paper, the discussion of lipids, fatty and carboxylic acids have been chosen as target analytes for extraction, trapping and analysis. Some examples from extraction with liquids in the standard state and trapping in the supercritical state (collection) have been furnished with the wrong extractant, modifier, or trap-

per which is presented for illustration of inappropriate choice of extractants, modifiers, and trappers.

Keywords

Modifiers, Extractants, Trappers, Supercritical Fluid Chromatography, Supercritical Fluid Extraction, Extraction with Liquids in Standard State, Lipids, Fatty Acids, Carboxylic Acids, Extraction of Fatty and Carboxylic Acids

1. Introduction

Correct extraction of a mixture of natural, industrial, or synthetic products as lipids at the preparation stage for analysis or for other use is a significant challenge. Lipids are a very heterogeneous class of substances, especially lipids from natural samples [1] [2]. Classification of substances that belong to lipids is not uniform [3] [4] [5]. However, lipids can be mainly divided into those that can be saponified and those that cannot be saponified by their ability to react with bases and water. Saponification is the base hydrolysis of an ester in the presence of water. The ester is attacked by hydroxide anion. By this, the chain of reactions is initiated (**Figure 1**).

The reaction of saponification is reversible. The reverse reaction is esterification and proceeds in a water-free environment in the presence of alcohol. It has been catalyzed by +H, cations of different metals, and nonmetallic specimens [5] (**Figure 2**).

2. Extraction of Lipids and Fatty Acids

Fatty acids (FAs) are one of the families in a broad array of substances called lipids [1] [2] [3] [4]. FAs are substances that, in addition to carboxylic function, can contain some other functions [1] [2] [3] [4] [5]. Different approaches to extraction of lipids, free fatty acids (FFAs), and carboxylic acids have been described in [6] [7] [8]. Several extraction methods for lipids were recommended by Folch *et al.* [6]

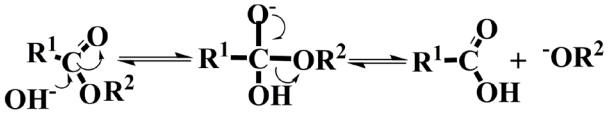

Figure 1. Mechanism of base hydrolysis (saponification).

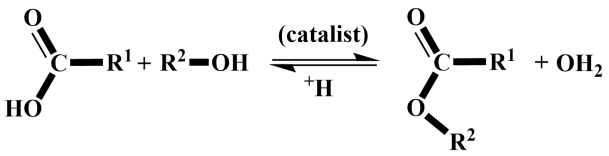

Figure 2. The esterification reaction catalyzed by +H.

and Bligh and Dyer [8]. FFAs and carboxylic acids are a heterogeneous family with some restrictions toward use of extractants, modifiers and trappers; the methods of analysis were discussed in [5] and verification of results correctness in [9] [10]. Seldom in natural samples do FAs present as a single class with carboxylic function only; more usually, natural samples present a broad mixture of very different classes of lipids and FAs [1] [2] [3] [4] [5] [11] [12] [13] [14]. The methods such as those described in [6] [8], where alcohol has been used, are not appropriate for extraction of FFAs, especially with additional functions [9]. Bulk alcohol as solvent or solvent modified with alcohol [9] [10], or alcohol trapper can lead to artifacts (McDaniel, L.H. and Taylor, L.T. (1999) Esterification of Decanoic Acid during Supercritical Fluid Extraction Employing either Methanol-Modified Carbon Dioxide or a Methanol Trap. *Journal of Chromatography A*, 858, (2), 201-207).

3. Extraction of FA with Liquid in the Standard State: Artifacts Appear as the Result of Alcohols as the Extractant

FFAs with additional functions are very sensitive to extraction by alcohols [9] [10] or trapping (collection) with alcohols by reacting with an extractant, trapper, or modifier present in the extractant or trapper (collector) by producing esters. The appearance of artifacts was clearly demonstrated in [9] [10] (**Figure 3** and **Figure 4**).

The investigations [9] were conducted in our laboratory after receiving the conflicting results [11] with published by Steglich *et al.* [15]. Steglich *et al.* have published [15] in the prestigious journal *Tetrahedron Letters* and claimed discovery of a new alkaloid—*infractine* in the mushroom *C. infractus* (**Figure 5**). Infractine in IUPAC nomenclature is methyl 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate.

Our investigations into the chemotaxonomy of the *Cortinarius* family [10] [11] [16] [17] [18] revealed the absence of infractine in the samples analyzed by HPLC-MS, SFC-MS, and SFC with multianalysis methods by using mass spectrometry-, ultraviolet- and corona-charged aerosol detection (CCAD) [10] [11] [16] [17] [18]. Instead of infractine, we found 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoic acid (**Figure 3** and **Figure 6**).

The presence of 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoic acid was shown in *C. infractus* [9] [10] by GC-MS, SFC-MS, and SFC with multianalysis methods, and the absence of infractine in *C. infractus*, without any doubt, has been supported in [9] [10] [16] [17] [18].

4. The Appearance of Artifacts during Supercritical Fluid Extraction (SFE) Employing Either Methanol-Modified CO_2 or Other Alcohols, or Methanol as a Trapping Collector

SFE is a very popular and economical way to obtain natural substances from vegetable, animal, and marine products [19] [20] [21] [22]. Fragrant and volatile

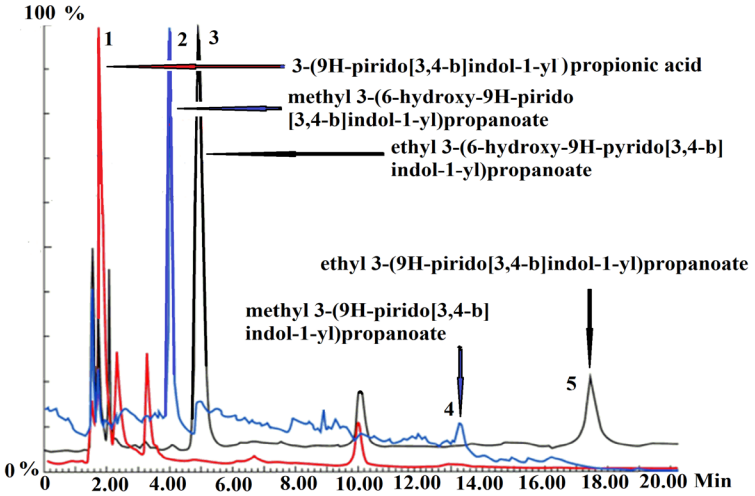

Figure 3. Total ion current (TIC) recorded by HPLC-MS analysis of *Cortinarius infractus*. It is an overlay of a red line, which is the TIC for a water extract of *C. infractus*; the blue line is the TIC for a MeOH extract of *C. infractus*; and the black line is the TIC for an EtOH extract of *C. infractus*. Chromatography and mass-spectrometric conditions are given in [9]. The structures of substances under the peaks: 1 is 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoic acid, 2 is methyl 3-(6-hydroxy-9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate, 3 is ethyl 3-(6-hydroxy-9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate, 4 is methyl 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate, and 5 is ethyl 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoate [9].

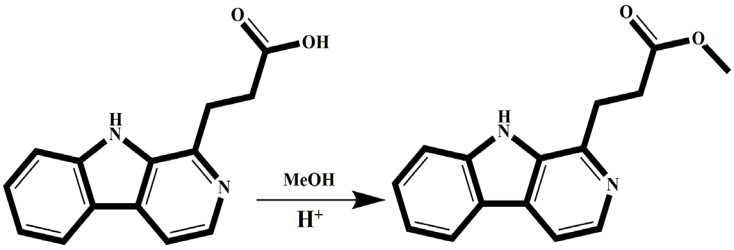

Figure 4. Esterification of 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoic acid from natural sources *C. infractus* by extraction with methanol, and appearance of artifact in the elaboration of Steglich *et al.* [15].

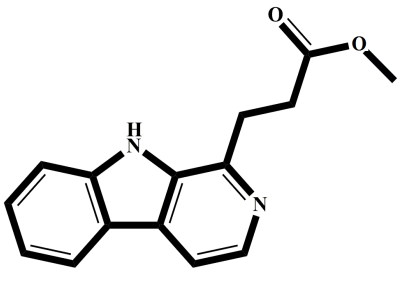

Figure 5. Structure of infractine proposed by Steglich *et al.* in [15].

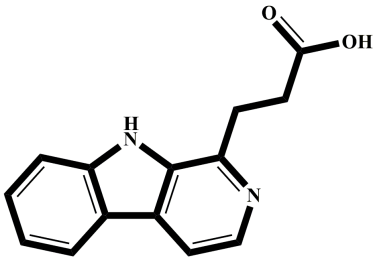

Figure 6. Structure of 3-(9*H*-pyrido[3,4-*b*]indol-1-yl)propanoic acid [9].

compounds for the food, perfumery, and pharmaceutical industries have been extracted and collected using SFE with modifiers or with bulk SC-CO_2 [23]-[32]. Comparison between traditional hydrodistillation (HD) of volatile oils and modern extraction with SFs has been presented in [33] [34] [35]. All these samples can contain FFAs.

FFA often has been collected with methanol or another alcohol as a trapper [36]. However, under SFE or collection (trapping) in the presence of alcohols, esterification of FFAs [37] or transesterification of fatty acid esters (FAEs) can happen. The use of a nonalcoholic collector such as hexane (as well as other nonalcoholic collectors) has been used to avoid esterification of FFAs or transesterification of fatty acid esters FAEs [38].

Transesterification of FAEs in supercritical alcohols is a well-known phenomenon. The kinetics of transesterification of FAE from waste vegetable oil (WVO) in supercritical methanol (SCM) and supercritical ethanol (SCE) were studied in [39] [40]. By applying elevated temperature, 99% transesterification of FAEs in supercritical alcohols was achieved. Transesterification of vegetable oils in an alcohol environment in subcritical fluid (SbCF) and SCFs was described in [41]. The esterification of decanoic acid during SFE by CO_2 modified with methanol was studied in [37].

5. Discussion

The results described in [37] bear strong witness to the possibility of esterification of FFAs and to this artifact's creation by using alcohols in SFC and SFE procedures, especially under collection of fractions by alcoholic containing traps. The need to be careful in the choice of extractants [9], modifiers, and trappers [37] in extraction or collecting samples containing FFAs is obvious. Significant esterification of FFA occurred during the trapping step in the presence of methanol [37], even without elevation of temperature or catalysis. Both the presence of +H catalyst and elevation of temperature can increase the reaction rate [37]. Methanol without the presence of external artificial +H catalyst and elevation of temperature may esterify some sensitive FFAs, as was shown in [9]. The wrong choice of extractant [15], modifier, or trapping substance [37] can lead to mistakes.

In recent years, a new development in trapping techniques has appeared, name-

ly the use of a solid-phase trap [42]. Solid-phase trapping is extensively used for polychlorinated polyaromatic hydrocarbons [43] and petroleum hydrocarbons [44]. Studies of solid-phase extraction, trapping of volatile oils have also been published [45]; however, to my knowledge, there is no description of the use of solid-phase trapping in SFE of lipids, including FFAs and the results. It would be interesting to investigate the influence on the composition of FFAs by trapping material in SFE solid-phase trapping.

6. Conclusion

The nonalcoholic extractant [9] or collector should be used to avoid esterification of FFAs or transesterification of fatty acid esters FAEs [38].

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