Journal of Chromatography A, 1456 (2016) 77–88

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Substitution of carcinogenic solvent dichloromethane for the extraction of volatile compounds in a fat-free model food system

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ARTICLE INFO

*Article history:*
Received 6 May 2016
Received in revised form 3 June 2016
Accepted 3 June 2016
Available online 16 June 2016

*Keywords:*
Volatile compounds
Substitution of CMR
Azeotrope
Ethyl acetate
Cyclopentane
Amylose complexation

ABSTRACT

Dichloromethane is known as a very efficient solvent, but, as other halogenated solvents, is recognized as a hazardous product (CMR substance). The objective of the present work is to propose substitution solvent for the extraction of volatile compounds. The most important physico-chemical parameters in the choice of an appropriate extraction solvent of volatile compounds are reviewed. Various solvents are selected on this basis and on their hazard characteristics. The selected solvents, safer than dichloromethane, are compared using the extraction efficiency of volatile compounds from a model food product able to interact with volatile compounds. Volatile compounds with different hydrophobicity are used. High extraction yields were positively correlated with high boiling points and high Log Kow values of volatile compounds. Mixtures of solvents such as azeotrope propan-2-one/cyclopentane, azeotrope ethyl acetate/ethanol, and mixture ethyl acetate/ethanol (3:1, v/v) gave higher extraction yields than those obtained with dichloromethane.



## 1. Introduction

Dichloromethane (CAS Number: 75-09-2 or methylene chloride, DCM) is a colorless, halogenated aliphatic hydrocarbon compound with an ether-like or mild sweet odor. It was extensively used in the pharmaceutical industry, in paint stripping, aerosols, adhesives and other applications such as extraction solvent for some food components. In research labs, it is known as a good solvent and used as such.

Dichloromethane, as other halogenated solvents, is recognized as a hazardous product (mainly hazard statements keys H351, suspected of causing cancer, and H373, may cause damage to organs through prolonged or repeated exposure). In terms of human toxicology, both acute toxicity and chronic toxicity must be taken into account. The acute toxicity of dichloromethane is low. The most important acute toxic effect is on the central nervous system and elevated carboxyhaemoglobin levels, and these effects are reversible, although fatalities have been reported on a number of occasions. Considering the chronic toxicity, the typical effects of high exposure to solvents are often of a neurobehavioral and cardiotoxicological nature. In addition to inhalation, dichloromethane can also be absorbed through the skin and this should be taken into account [1]. Dichloromethane was classified as a dangerous chemical with restricted uses in industry. Moreover, dichloromethane was recently moved from classification 2B (possibly carcinogenic to humans) to classification 2A meaning "probably carcinogenic to humans" [2].

In Europe, the REACH regulation foresees an authorization system aiming to ensure that the risks from substances of very high concern are properly controlled, and those substances are progressively replaced by suitable alternative substances or technologies where these are economically and technically viable [3]. For example, in the extraction of essential oils, dichloromethane was reported to be replaced by supercritical fluid ($CO_2$), diethoxymethane (CAS number: 462-95-3), dipropoxymethane (CAS number: 505-84-0), or dioxolane (CAS number: 646-06-0) [4].

In research laboratories, solvent extractions are necessary and even if amounts of solvents used are low as compared to what is used in heavy chemical industries, safety and environmental aspects must also be taken into account. The growing demand for alternative or "green" solvents that exists in industry is thus gaining research labs. To follow the principles of green chemistry, i.e., to reduce or to eliminate hazardous substances, researchers tried to found alternative solvents. Additionally, a green extraction should also reduce energy consumption, reduce unit operations and produce non denatured extract without contaminants. [5]. It is thus necessary to score how green a solvent is to compare alternative solvents. Capello et al. [6] combined the EHS (Environmental,

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http://dx.doi.org/10.1016/j.chroma.2016.06.015
0021-9673/

Health and Safety) properties and the LCA (Life Cycle Assessment) data of some solvents to compare them. They showed that ethanol for example combined a very low EHS score with a low energy demand. More recently, Tobiszewski et al. [7] compared solvents using physico-chemical data and health/hazard/environment data. They reported that the more environmentally safe solvents were among polar ones, like water, ethanol, acetone or ethyl acetate. Tobiszewski and Namieśnik [8] proposed a method using CHEM-1 model to score solvents considering their hazard. The total hazard value was 4.1 for ethanol and 39.4 for dichloromethane. Prat et al. [9] also reported a selection guide based on three scores concerning respectively safety, health, and environment. For example, ethanol was scored 4/3/3 (safety/health/environment) and dichloromethane was scored 1/7/7.

Considering analytical performance, the choice of an alternative solvent must also take into account the whole analytical procedure. Sample preparation step is crucial to ensure good analytical performance; and it is also the most polluting step of the whole analytical procedure [10]. Researchers working on volatile compounds extraction were used to make solvent extractions using dichloromethane because of its intermediate polarity and its high volatility, and some of them tentatively replaced dichloromethane by other solvents but most of the researchers preferred to use solvent-less alternative extraction methods, such as SPME [11]. Few authors [12–15] reported the use of "green" solvents for extraction in food products. In particular, Filly et al. [16] evaluated the performance of eight alternative solvents among which ethyl acetate (CAS number: 141-78-6) and dimethylcarbonate (CAS number: 616-38-6). These seemed to be promising alternative solvents for *n*-hexane to extract very hydrophobic compounds such as limonene (log Kow = 4.38, estimated from EPI suite) and carvone (log Kow = 2.71, estimated from EPI suite). Another way to green extraction procedure is also to reduce the amount of solvent used, to reduce degradation of extracted compounds, and to reduce energy consumption. For example, the use of ultrasound-assisted extraction permits reducing energy consumption and carbon emission [17].

The objectives of the present work are

- to give a summary of the most important physico-chemical characteristics to select an appropriate solvent for the extraction and analysis of volatile compounds,
- to select alternative solvents on the basis of the physico-chemical characteristics, and on their health/safety/environment characteristics
- and to compare the extraction efficiency of the selected solvents for volatile compounds extraction from a fat-free model food product based on polysaccharides able to interact with volatile compounds. Volatile compounds with a large range of hydrophobicity are considered to assess the robustness of the extraction. The extraction method used is an ultra-sound assisted one.

Finally, safe solvents are proposed for the extraction of volatile compounds.

## 2. Selection of extraction solvents

Different properties are required for a solvent to be used in the extraction of volatile compounds from food products, for analysis by gas chromatography.

- Immiscibility with water: the extracting solvent must be immiscible with the solution to be extracted. As food products are generally water-based solutions or dispersions, the extracting solvent must be an organic solvent. The more polar the organic solvent, the more it is miscible (soluble) with water.
- The volatile compounds to be extracted, which are present in a food product, should also be soluble in the extracting solvent. As volatile compounds are mainly hydrophobic compounds, hydrophobic solvents are required. From this point of view, dichloromethane has a very particular structure: it is polar (dipolar moment: 1.14 D), but it is also a good solvent for hydrophobic molecules (log Kow = 1.25); it is hard to find a substitution solvent with such properties.
- The extracting solvent should be sufficiently volatile so that it can be removed easily from the extract by distillation. High vapor pressure is preferable. This point is crucial if the extract has to be concentrated by solvent evaporation. Vapor pressure is positively correlated with the enthalpy of vaporization, which is linked with the energy to remove the solvent.
- The purity of the solvent is important, all the most as the extract has to be concentrated.
- It is usually desirable if the solvent is non-toxic and not flammable. Unfortunately, few solvents are known to meet both criteria. . .Some solvents are not toxic but flammable (e.g., diethyl ether, hazard key H224 extremely flammable liquid and vapor). Some are not flammable but toxic (e.g., dichloromethane, chloroform, classified CMR2, hazard key H351: suspected of causing cancer). Some solvents are both toxic and flammable (e.g., benzene, hazard keys H225: highly flammable liquid and vapor, H340: may cause genetic defects, H350: may cause cancer) [18].

To sum up, the following parameters will be of interest to select the solvent:

- Low boiling point: it is the temperature at which the vapor pressure of the liquid equals the pressure surrounding the liquid and the liquid changes into a vapor. As the obtained extract will be analyzed by GC, solvents with low boiling points will elute rapidly at the beginning of gas chromatography and will not hide the peak of volatile compounds on the chromatogram. Low boiling points allow sample concentration by solvent evaporation at low temperature, i.e., with less thermo-denaturation of the sample. This parameter is correlated to vapor pressure [19].
- High vapor pressure: vapor pressure or equilibrium vapor pressure is defined as the pressure exerted by a vapor in thermodynamic equilibrium with its condensed phases (solid or liquid) at a given temperature in a closed system. The equilibrium vapor pressure is an indication of a liquid's evaporation rate. It relates to the tendency of particles to escape from the liquid (or a solid). A substance with a high vapor pressure at normal temperatures is often referred to as volatile. As a general trend, vapor pressures of liquids at ambient temperatures increase with decreasing boiling points. Volatility is directly related to a substance's vapor pressure. At a given temperature, a substance with higher vapor pressure vaporizes more readily than a substance with a lower vapor pressure. Solvents with high vapor pressure could be easily removed to concentrate the extract.
- High log Kow: the partition coefficient "P" is a ratio of concentrations of un-ionized compound between octan-1-ol and water. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log Kow. The log Kow value of a compound is used as a measure of hydrophobicity. The octanol-water partition coefficient (log Kow) is probably the single most important parameter influencing solubility [20].
- Low solubility in water: if a solvent is soluble in water, it will be impossible to de-mix the two phases and to separate the extract.
- Low dipole moment "μ": polar molecules interact through dipole–dipole intermolecular forces and hydrogen bonds. Molecular polarity is dependent on the difference in electronegativity between atoms in a compound and the asymmetry of the compound's structure. Polarity underlies a number of physical