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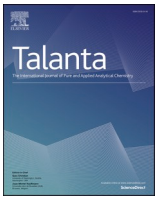

# Compatibility of cloud point extraction with gas chromatography: Matrix effects of Triton X-100 on GC-MS and GC-MS/MS analysis of organochlorine and organophosphorus pesticides

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ABSTRACT

Cloud point extraction is an environmentally benign and simple separation/concentration procedure that can be regarded as an alternative to classical liquid-liquid extraction. In the current work, it was studied the compatibility of cloud point extraction followed by back-extraction in low volume of organic solvent with gas chromatography-mass spectrometry (GC-MS and GC-MS/MS). Triton X-100 was preferred than Triton X-114 as a surfactant to produce the clouding phenomenon and hexane or isooctane was found to be appropriate organic solvents which can be used at the back-extraction step. It was observed that *ca.* 0.09 % w/w Triton X-100 was co-extracted in the organic phase (hexane or isooctane) so further study was carried out to find out its effect on the GC-MS (GC-MS/MS) measurement when liquid samples are injected without any pre-cleaning to remove the surfactant. The chromatographic separation and the mass detection were not deteriorated by the concomitant Triton X-100 for analysis of several Organochlorine and Organophosphorus pesticides (alpha-HCH, beta-HCH, gamma-HCH, Pentachlorobenzene, Hexachlorobenzene, Chlorpyrifos, Chlorpyrifos-methyl, Aldrin, Endrin, Dieldrin, alpha-Endosulfan, Heptachlor, Heptachlor-*endo*-epoxide-A, o,p-DDD, p,p-DDD, o,p-DDE, p,p-DDE, o,p-DDT and p,p-DDT). The stability of the GC system when introducing surfactant was assessed as acceptable (typically the peak area RSD% for 20 consecutive injections were below 5 %). Under the developed vaporization conditions using PTV or PSS injectors it can be deduced that Triton X-100 is deposited on the inner surface of the liner. This effect is beneficial since the resulting surfactant layer makes a surface which facilitates the pesticides transfer to the GC column. As a consequence, for some analytes, a substantial enhancement (up to 2.3 times) in the sensitivity was observed when the matrix-matched medium (0.09 % w/w Triton X-100 in organic solvent) is used compared to calibration in solely hexane or isooctane. Meanwhile, the measurement precision in the presence of Triton X-100 remains unchanged. The GC-MS/MS analysis was alternatively accomplished by the use of glass or metal liner and it was found that the glass one should be preferable. Finally, it can be concluded that cloud point extraction with Triton X-100 can be combined with GC-MS or GC-MS/MS analysis by applying liquid injection of the target analytes transferred in organic solvents such as hexane or isooctane. We have established a positive effect of Triton X-100 on the instrumental performance which is on opposite to the generally accepted concern of the negative influence of the surfactants on the gas chromatographic analysis.

## 1. Introduction

The persistent organic pollutants (POPs) are usually liposoluble organic compounds that are resistant to environmental degradation which are prone to accumulate in soils, waters, and sediments [1]. Moreover, the relatively volatile POPs could be naturally transferred at large distance in the atmosphere so they can be deposited thousands of kilometers away of their original source, especially in areas with hot climate. It is also well known that POPs can be accumulated in the adipose tissue of land-living and aquatic animals [2,3]. Humans can be exposed to POPs mainly through the food chain, the water consumption as well as inhalation from the surrounding air. If the POPs enter the human body, even at low levels, they can cause increased cancer risk, reproductive disorders, alterations of the immune system, neurotoxicity,

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**Table 1**
Precursor ions, selected scan time ranges, Quantitative and Qualitative transitions for the target analytes with corresponding collision energies (eV) used in GC-MS/MS analysis.

| Compound name | *Scan time* range, min | Precursor ion, *m/z* | Quant transition (Product ion, *m/z*) | Collision energy, eV | Qual transition (Product ion, *m/z*) | Collision energy, eV |
|---|---|---|---|---|---|---|
| Pentachlorobenzene | 2.5–4.0 | 249.9 | 214.9 | 25 | 142 | 40 |
| Hexachlorobenzene | 4.0–4.8 | 283.9 | 213.9 | 35 | 248.8 | 25 |
| alpha-HCH | 4.0–4.8 | 181 | 109 | 30 | 145 | 15 |
| beta-HCH | 4.8–6.0 | 181 | 109 | 30 | 145 | 15 |
| gamma-HCH | 4.8–6.0 | 181 | 109 | 30 | 145 | 15 |
| Chlorpyrifos-methyl | 6.0–7.5 | 286 | 270.9 | 20 | 93 | 25 |
| Chlorpyrifos | 7.5–9.6 | 196.9 | 168.9 | 15 | 107 | 40 |
| alpha-Endosulfan | 9.6–11.0 | 240.9 | 136 | 40 | 205.9 | 15 |
| Aldrin | 7.5–9.6 | 262.9 | 192.9 | 40 | 190.9 | 40 |
| Dieldrin | 11.0–13.4 | 262.9 | 192.9 | 40 | 190.9 | 35 |
| Endrin | 11.0–13.4 | 262.9 | 193 | 35 | 190.9 | 35 |
| Heptachlor | 6.0–7.5 | 271.9 | 236.8 | 25 | 116.9 | 40 |
| Heptachlor-*endo*-epoxide-A | 7.5–9.6 | 183 | 118.9 | 30 | 154.9 | 15 |
| o,p-DDE | 9.6–11.0 | 246 | 176.1 | 40 | 211 | 20 |
| p,p-DDE | 11.0–13.4 | 246 | 176.1 | 40 | 175.1 | 40 |
| o,p-DDD | 11.0–13.4 | 235 | 165.1 | 30 | 199.1 | 15 |
| p,p-DDD | 13.4–17.0 | 235 | 165.1 | 25 | 199.1 | 20 |
| o,p-DDT | 13.4–17.0 | 235 | 165.1 | 30 | 199.1 | 20 |
| p,p-DDT | 13.4–17.0 | 235 | 165.1 | 30 | 199.1 | 20 |

endocrine disruption and genotoxicity [4]. The worldwide growing concern about POPs resulted in the Stockholm Convention (2001) at which it was accepted an international agreement to restrict or eliminate the production and the use of POPs [5]. Organochlorine pesticides (OCPs) are one of the common types of POPs that are intentionally manufactured, and widely employed in the past, but their usage has been restricted and decreased in recent decades due to their environmental persistence and neurotoxicity [6]. Organophosphorus pesticides (OPPs) are the most extensively used pesticides in the world because they are less persistent than other chlorinated pesticides [7]. Another important issue is the fact that very often the metabolites of the OCPs or the OPPs are also persistent and harmful to living beings [8].

A common trend in analytical chemistry is the development of methodologies for green analysis with inherent minimization and limited application of environmentally hazardous compounds [9,10]. An example of such a procedure for separation and/or pre-concentration is the cloud point extraction (CPE), also known as micelle-mediated extraction. This procedure can be regarded as an alternative to the classical liquid-liquid extraction in which the organic solvents are replaced by a small amount of non-flammable, non-volatile and non-toxic surfactants [11–13]. In CPE the target analytes are extracted in a small volume of surfactant-rich phase which further is subjected to instrumental analysis. CPE was successfully used for the extraction and pre-concentration of pesticides using a variety of surfactants before their determination by HPLC [14–20]. However, the analysis of samples with high content of surfactants by gas chromatography is problematic and quite limited. Actually, the direct introduction of the viscous surfactant-rich phase into a GC system in general is not possible. It is supposed that a high content of surfactant can (i) clog or block the GC column, (ii) adsorb onto the stationary phase and partially change its polarity, and/or (iii) elute as a series of peaks from the column over time, overlapping with the peak(s) of the target analytes [11,21]. Several approaches have been proposed to overcome these limitations aiming the combination of cloud point extraction with GC analysis i.e. separation and recovering the target analyte(s) from the surfactant-rich phase using liquid chromatography mini-column (silica gel and Florisil, cation exchange columns) [22–24]; using microwave agitation or sonication to back-extract analytes from the surfactant-rich phase into a water-immiscible solvent optionally with additional cleaning step before injection [25–28]; centrifugation [29]; applying analyte derivatization prior to introduction into the GC [30].

The present study is focused on the systematic investigation of the effect of surfactant (Triton X-100) as a matrix component in samples obtained after cloud point extraction of Organochlorine and Organophosphorus pesticides on the following GC-MS or GC-MS/MS analysis. To the best of our knowledge such fundamental investigation was not previously have been done. The scope of our study is also extended to assess the effect of Triton X-100 when different types of GC liners are used.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The studied pesticides were purchased as solid substances with a purity higher than 95 %. Alpha-HCH, beta-HCH, gamma-HCH, Pentachlorobenzene, Hexachlorobenzene, Chlorpyrifos, Chlorpyrifos-methyl, Aldrin, Endrin, Dieldrin, alpha-Endosulfan, Heptachlor, Heptachlor-*endo*-epoxide-A, o,p-DDD, p,p-DDD, o,p-DDE, p,p-DDE, o,p-DDT, p,p-DDT were supplied from Dr. Ehrenrshtorfer GmbH (Germany) and for each analyte a stock solution was prepared in acetonitrile at a concentration of 1000 μg $ml^{-1}$ (further dilution was carried out in hexane). Dichlorvos, Ethoprophos, Disulfoton, Methyl-parathion, Fenchlorphos and Prothiofos were purchased from Supelco (USA), and for each analyte, a stock solution was prepared in hexane: acetone (9:1, v/v) at a concentration of 1000 μg $ml^{-1}$ (further dilution was carried out in acetone). All stock solutions were stored in the dark at 4 °C. The chemical structures of the target analytes are shown in Table S1. Acetonitrile LC-MS grade was obtained from Honeywell (USA), HPLC grade acetone was obtained from Merck (Germany), hexane, isooctane, Triton X-114, and Triton X-100 were purchased from Sigma–Aldrich (Germany). Ultrapure water (18 MΩ) was obtained from a Chorus 1 Complete Ultrapure Water System (ELGA, PURELAB®, United Kingdom).

### 2.2. Equipment and working conditions

#### 2.2.1. UV-VIS

UV/VIS spectrophotometer ONDA UV 30 Scan (Giorgio-Bormac, Italy) was used for the assessment of the solubility of Triton X-114 and Triton X-100 in different organic solvents. For quantification, the absorption peak of the surfactants at 276 nm was registered against the corresponding organic solvent as a blank.

**Table 2**
Selected ion monitoring (SIM) ions used in GC-MS analysis in selected scan time ranges.

| *Compound name* | Scan time range, min | *Ion 1, m/z* | *Ion 2, m/z* | *Ion 3, m/z* |
|---|---|---|---|---|
| Dichlorvos | 9.25–10.00 | 109 | 79 | 185 |
| Ethoprophos | 15.25–15.85 | 158 | 97 | 126 |
| Disulfoton | 17.75–18.25 | 88 | 60 | 97 |
| Methyl-parathion | 18.75–19.30 | 104 | 125 | 263 |
| Fenchlorphos | 19.30–19.80 | 285 | 79 | 125 |
| Chlorpyrifos | 20.00–20.50 | 97 | 197 | 314 |
| Prothiofos | 22.00–22.60 | 113 | 309 | 267 |

### 2.2.2. GC-MS/MS and GC-MS

In the current study, two GC systems were used.

2.2.2.1. GC-MS/MS TSQ 9000 (Thermo Fisher Scientific, USA) with EI at 70 eV, equipped with a triple QMF mass detector and PTV injector. The system was operated by Excalibur software 4.1. An injection volume of 1 μl was injected using an autosampler AI1300, equipped with a 10 μl glass syringe. The PTV injector was used in Split mode (split ratio 5:1), starting with 65 °C initial inlet temperature followed by gradient heating at 14.5 °C $sec^{-1}$ to 260 °C. GC column TG-MS (15 m × 0.25 mm, 0.25 μm film thickness, Thermo Fisher Scientific, USA) was used for chromatographic separation. The oven temperature program was as follows 120 °C – held for 1 min; increased at the rate of 40 °C $min^{-1}$ to 155 °C, rating 4 °C $min^{-1}$ to 187 °C, rating 1 °C $min^{-1}$ to 194 °C and rating 12 °C $min^{-1}$ to a final temperature of 260 °C and held for 5 min. The solvent delay time and total analysis time were 2.5 min and 28 min, respectively. Helium (purity 99.9999 %) at a flow rate of 1.2 ml $min^{-1}$ was used as a carrier gas. The transfer line and Ion source temperatures were set at 250 °C and 230 °C, respectively.

The evaluation of the selectivity of the method was performed by using two approaches 1) Full scan mode in the mass range 20–700 amu, split ratio 5:1, and dwell time 0.2 sec. 2) SRM mode with experimentally optimized selected time intervals for each target analyte, listed in Table 1, was used to obtain more data points per peak, better reproducibility, and a higher signal-to-noise ratio. SRM mode was also used for the assessment of the chromatographic system stability. For this purpose, one precursor ion and two transitions for each target analyte were chosen as shown in Table 1. One of the transitions was used for quantitative determination (Quant transition) and the other for qualitative identification (Qual transition). The study was performed using a glass liner (PTV Liner with Three Baffles, 1 mm ID, 2.75 mm OD, 120 mm Length, Thermo Fisher Scientific, USA) or a metal liner (PTV Siltek Metal Liner, 2 mm ID, 2.75 mm OD, 120 mm Length, Thermo Fisher Scientific, USA).

2.2.2.2. Alternative measurements were carried out by GC-MS Shimadzu 2010 S E (Shimadzu Corporation, Japan) at 70 eV, equipped with a single quadrupole mass filter (QMF) and PSS injector. The GC-MS system was operated by Lab Solution software. Injection volume of 2 μl was injected by autosampler using a 10 μl glass syringe and high-pressure injection at 56.9 kPa. The PSS injector was used in Split mode, split ratio 5:1, at 280 °C temperature. GC column TG-5MS (30 m × 0.25 mm, 0.25 μm film thickness; Thermo Fisher Scientific, USA) was used. The column oven temperature program was set up as follows 80 °C – held for 2 min; increased at the rate of 8 °C $min^{-1}$ to 280 °C, rating 50 °C $min^{-1}$ to a final temperature of 350 °C. The solvent delay time and total analysis time were 5 min and 28.4 min, respectively. Helium (purity 99.9999 %) at a flow rate of 0.9 ml $min^{-1}$ was used as a carrier gas. The transfer line and Ion source temperatures were set at 250 °C and 220 °C, respectively. The study was performed using a glass liner (Split, Focus Liner, 5 mm OD, 3.4 mm ID, 95 mm Length, Trajan SGE, Australia).

The GC-MS analysis was performed in SIM mode. For each analyte were selected three target ions, listed in Table 2. Two reference ions were used as confirmation of the analyte and one target ion was selected for quantitative analysis.

### 2.3. Model of cloud point extraction procedure

The performed cloud point extraction procedure was developed in our laboratory including the following steps (Fig. 1): a 10 ml 2 % w/w aqueous solution of surfactant (Triton X-100 or Triton X-114) was prepared in a 12 ml glass conical test tube, capped with a plastic cap. The

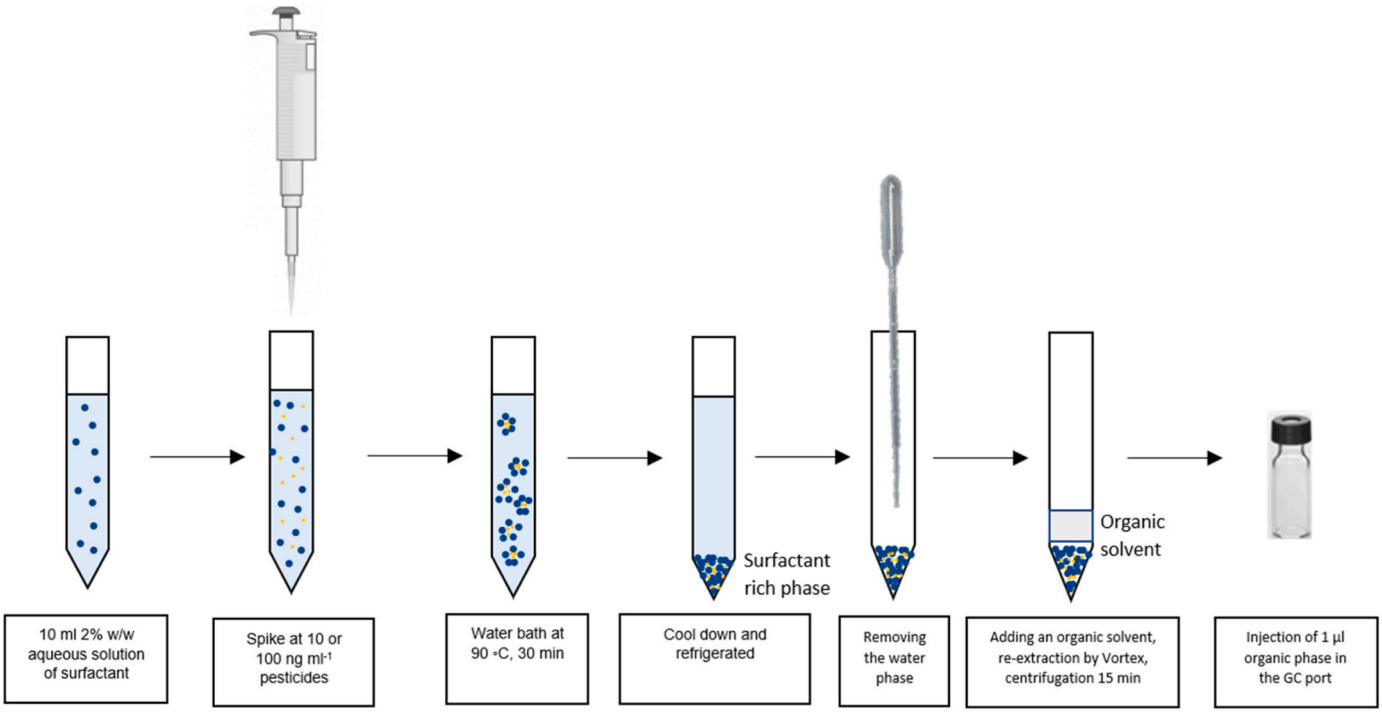


**Fig. 1.** Cloud point extraction procedure followed by Vortex assisted back extraction in organic solvent.

**Table 3**
Solubility of Triton X-100 and Triton X-114 in organic solvents in % (w/w) (n = 6).

| Solvent | Triton X-100, % | SD, % | Triton X-114, % | SD, % |
|---|---|---|---|---|
| Hexane | 0.089 | 0.003 | 0.150 | 0.005 |
| Isooctane | 0.085 | 0.003 | 0.145 | 0.004 |
| Cyclohexane | 0.466 | 0.018 | 0.781 | 0.020 |
| Ethyl acetate | 1.43 | 0.25 | 1.58 | 0.31 |

model solution was spiked with pesticides at a final concentration of 10 ng $ml^{-1}$ (for GC-MS/MS analysis) or 100 ng $ml^{-1}$ (for GC-MS analysis). The glass tube was placed in a water bath at 90 °C (above the cloud point temperature of 2 % w/w Triton X-100 which is 64 °C) for 30 min which results in the formation of two phases: surfactant-rich phase on the bottom and upper water phase. The tube was left at room temperature (24 ± 2 °C) to cool down for 10 min and placed for 30 min in a refrigerator at 4 °C to increase the viscosity of the surfactant-rich phase. The upper water phase was removed by Pasteur Pipettes. Then 2 ml of water was added to the remaining surfactant-rich phase (*ca.* 1 ml) in order to decrease its viscosity. Further, 2 ml of non-miscible organic solvent (one of hexane, isooctane, cyclohexane, or ethyl acetate) was added and back-extraction assisted by Vortex agitation was proceeded for 10 min. The solution was centrifuged for 15 min at 2500 rpm and placed in a freezer at −22 °C to facilitate the separation of the two phases. The upper organic phase was pipetted and submitted to measurement by i) UV-VIS (all extraction systems), ii) GC-MS (Triton X-100/isooctane) or iii) GC-MS/MS (Triton X-100/hexane).

## 3. Results and discussions

### 3.1. Quantification of co-extracted triton X-100 and triton X-114

Cloud point extraction can be applied to extract pesticides from a water medium [22,26,27,30]. Usually, as a result of this pre-concentration procedure, a surfactant-rich phase is obtained in which the target analytes are collected but some amount of the initial water solution is also included. Hence the straightforward combination of cloud point extraction with GC-MS or GC-MS/MS analysis based on liquid sample introduction into the injector system is not possible due to the direct injection of high contents of surfactant and water into the GC. A possible option to overcome this obstacle is to further transfer the target analytes from the surfactant-rich phase into a small volume of easily volatile organic solvent compatible with the GC instrumentation. However, at the last extraction step, it will be beneficial the amount of co-extracted surfactant to be reduced as much as possible aiming to protect the GC liner and/or column from any deterioration. For this reason, a study focused on the co-extraction of the most common surfactants used in cloud point extraction (Triton X-100 and Triton X-114) into solvents with different properties (hexane, isooctane, cyclohexane, and ethyl acetate) was carried out using the procedure described in section 2.3.

The quantitative analysis of the co-extracted Triton X-100 or Triton X-114 in the studied solvents was assessed by registering the UV-VIS spectra of the organic phases which had been added to the surfactant-rich phase. In each organic solvent (hexane, isooctane, cyclohexane and ethyl acetate) both of the surfactants have the same absorption maximum ($\lambda_{max}$ = 276 nm). Fig. S1 represents a UV-VIS spectrum of 0.05 % w/w standard solution of Triton X-100 in hexane. The concentration of Triton X-100 (or Triton X-114) was determined by absorbtion measurements at 276 nm (6 replicates) in each organic medium.

The achieved results as weight percentages (Table 3) show that the equilibrium content (solubility) of Triton X-100 in each organic solvent is significantly lower than the corresponding concentration of Triton X-114. Meanwhile, the level of Triton X-100 in hexane and isooctane is statistically identical and the lowest obtainable. For this reason, the following experiments were set up to simulate cloud point extraction using Triton X-100 with further transfer of the target analytes in hexane or isooctane. Hence the matrix effects arising in the GC-MS or GC-MS/MS analysis were assessed by matrix-matched media, which is expected to be obtained after cloud point extraction, containing 0.09 % (w/w) Triton X-100 dissolved in hexane or isooctane. It is worth mentioning that the aforementioned equilibrium concentration of Triton X-100 migrating from the surfactant-rich phase to hexane or isooctane in the back-extraction step should remain constant regardless of the initial concentration of the surfactant used in the first step of extraction.

### 3.2. Evaluation of the selectivity of the GC - MS/MS method

Hexane and a matrix-matched medium (0.09 % w/w Triton X-100 in hexane) were subjected to measurements by GC-MS/MS using the instrumental parameters prescribed in section 2.2.2.1 (1). The total ion current (TIC) chromatograms in Full scan mode (mass range 20–700 amu adjusted to the molecular weight of Triton X-100) were registered and compared (Fig. S2). It was found that both chromatograms were identical which leads to the fact that no peaks due to Triton X-100 are evident when matrix-matched medium is injected. Possible reasons for

**Table 4**
Retention times and signal stability of the target analytes when matrix-matched samples were injected in GC-MS/MS with glass or metal liner.

| Compound name | Glass liner | | | Metal liner | | | |
|---|---|---|---|---|---|---|---|
| | Rt, min | Rt SD[a], min | Area, RSD%[a] | Rt, min | Rt SD[a], min | Area, RSD%[a] | Area, RSD%[b] |
| Pentachlorobenzene | 3.03 | 0.005 | 1.60 | 3.04 | 0.003 | 6.15 | 2.31 |
| alpha-HCH | 4.49 | 0.005 | 1.23 | 4.53 | 0.002 | 7.06 | 1.94 |
| Hexachlorobenzene | 4.61 | 0.005 | 2.39 | 4.63 | 0.003 | 5.99 | 3.17 |
| beta & gamma-HCH | 5.18 | 0.004 | 1.25 | 5.22 | 0.004 | 6.21 | 1.84 |
| Chlorpyrifos-methyl | 6.82 | 0.004 | 3.43 | 6.87 | 0.002 | 7.37 | 3.02 |
| Heptachlor | 6.90 | 0.003 | 3.83 | 6.94 | 0.003 | 6.64 | 3.76 |
| Aldrin | 7.87 | 0.004 | 2.08 | 7.91 | 0.004 | 6.47 | 3.55 |
| Chlorpyrifos | 8.33 | 0.002 | 2.66 | 8.39 | 0.005 | 6.87 | 1.56 |
| Heptachlor-*endo*-epoxide-A | 9.35 | 0.002 | 3.43 | 9.41 | 0.001 | 8.43 | 3.77 |
| o,p-DDE | 10.34 | 0.002 | 1.83 | 10.41 | 0.003 | 7.49 | 1.63 |
| alpha-Endosulfan | 10.39 | 0.003 | 1.93 | 10.45 | 0.004 | 8.11 | 2.43 |
| Dieldrin | 11.43 | 0.003 | 4.58 | 11.51 | 0.005 | 6.79 | 5.69 |
| Endrin | 12.34 | 0.006 | 3.74 | 12.42 | 0.007 | 6.01 | 3.99 |
| p,p-DDE | 11.67 | 0.001 | 2.00 | 11.76 | 0.005 | 6.52 | 2.41 |
| o,p-DDD | 12.01 | 0.003 | 2.14 | 12.12 | 0.006 | 6.80 | 2.50 |
| p,p-DDD & o,p-DDT | 13.74 | 0.002 | 2.46 | 13.88 | 0.005 | 7.81 | 2.06 |
| p,p-DDT | 15.88 | 0.008 | 4.47 | 16.02 | 0.008 | 9.02 | 3.68 |

[a] For 20 measurements.
[b] For measurements starting from 11th to 20th injection.

the last could be i) the detection of some *m/z* ratios could be skipped due to the insufficient measurement time because of the selected wide mass range, ii) accepting that Triton X-100 remains in the liner due to the used temperature program of the PTV injector or iii) the amount of the surfactant passing through the chromatographic column is too low due to the used split ratio. Further study was carried out by measurement of the native matrix-matched medium and the same blank sample but spiked at 100 ng $ml^{-1}$ with the target analytes using SRM mode of detection prescribed in section 2.2.2.1 (2) (Fig. S3). The RTIC chromatograms of the blank were compared with the ones corresponding to the solution containing spiked pesticides. It was observed that at each transition of any target analyte, there were no isobaric interferences caused by Triton X-100 which presents as a matrix component. The last fact shows that even to assume that some amount of the surfactant enters the GC column its presence is not influencing the selectivity of the measurements under SRM mode. It should be noted that the SRM transitions of beta-HCH and gamma-HCH as well as the ones of p,p-DDD and o,p-DDT are identical (Table 1) and have very close retention times so both compounds were only partially resolved. For this reason, beta and gamma-HCH and respectively p,p-DDD/o,p-DDT were analyzed as a sum of their signals.

### 3.3. Stability of the chromatographic system

When combining GC analysis with a sample preparation procedure in which surfactants are used then a common approach is to clean up the obtained final solution from the long-chain amphiphilic molecules before injection. The last is recommended due to the number of potential negative effects which are expected to occur if surfactants enter the GC liner or/and column. In the current study for a proposed procedure of cloud point extraction (see section 2.3), it was studied the stability of the GC-MS/MS system when injecting the final organic phase without any purification of the co-extracted Triton X-100. The aim was to distinguish any negative effect caused by the long-term injection of the surfactant as a matrix component of the sample. For this purpose, the GC system was preconditioned by 3 injections of 0.09 % (w/w) Triton X-100 in hexane (matrix-matched blank) followed by 20 consecutive injections of the same matrix spiked with 100 ng $ml^{-1}$ of the target pesticides to assess the precision of their retention times and the peak areas measured in SRM mode of detection (see section 2.2.2.1). Two series of measurements were carried out by using a glass liner and alternatively by using a metal one. The obtained retention times (Rt), their standard deviations (Rt SD), and the relative standard deviation of the signal areas (Area, RSD%) are shown in Table 4. The data analysis showed that highly repeatable retention times were observed (all standard deviations were lower than 0.01 min). It was also found that the retention times do not shift from the ones registered by injection of standard solutions in pure hexane. The last could be explained with the assumption that not substantial amount of the surfactant enters the GC column which remains with unchanged properties.

Concerning the signal precision, it was found that its value was acceptable for the purpose of the trace pesticide analysis (when the glass liner was used for 20 consecutive replicates most of the calculated RSD% were below 2.5 % with the highest value of 4.58 %). When the analytes pass through the metal liner the calculated area RSD% for 20 successive injections were relatively higher (5.99–9.02 %) than the ones obtained by the glass liner. However, if only the second half of the spiked replicates with numbers from 11 to 20 were used for area RSD% calculations then substantial improvement in the precision was observed (Table 4). As it was mentioned above it could be assumed that Triton X-100 practically does not enter the GC column under the working conditions (see section 2.2.2.1). Hence it could be expected that the injected amount of the surfactant is vented during the split mode of introduction and/or retained on the inner liner surface. So, it can be concluded that 3 injections of a matrix-matched medium are sufficient to condition the GC system with mounted glass liner but in the case of metal one it is needed to proceed additional injections in order to achieve better precision. It seems that the utilization of metal liner will cost more time for the conditioning of the GC system but this will be needed only if the solutions with a matrix-matched medium are injected after samples in pure hexane. If only a long-term analysis of samples containing 0.09 % (w/w) Triton X-100 in hexane (corresponding to matrix-matched standards and phases obtained after cloud point extraction) is running then the prolonged preconditioning will not be necessary.

**Table 5**
Comparison of the line slopes in matrix-matched and hexane calibration for the target analytes measured by GC-MS/MS and using glass or metal liner.

| *Compound name* | *Glass liner* | | *Metal liner* | |
|---|---|---|---|---|
| | Slope ratio | SD | Slope ratio | SD |
| Pentachlorobenzene | 0.97 | 0.02 | 1.27 | 0.07 |
| Hexachlorobenzene | 0.96 | 0.03 | 1.43 | 0.08 |
| alpha-HCH | 0.96 | 0.01 | 1.26 | 0.06 |
| beta & gamma-HCH | 0.95 | 0.01 | 1.31 | 0.07 |
| Chlorpyrifos-methyl | 1.02 | 0.06 | 2.03 | 0.13 |
| Chlorpyrifos | 1.12 | 0.02 | 2.11 | 0.15 |
| alpha-Endosulfan | 0.99 | 0.02 | 1.64 | 0.12 |
| Aldrin | 0.96 | 0.02 | 1.61 | 0.11 |
| Dieldrin | 0.90 | 0.04 | 1.78 | 0.13 |
| Endrin | 1.08 | 0.03 | 1.95 | 0.31 |
| Heptachlor | 1.03 | 0.02 | 1.66 | 0.14 |
| Heptachlor-*endo*-epoxide-A | 0.97 | 0.02 | 1.65 | 0.16 |
| o,p-DDE | 0.98 | 0.02 | 1.67 | 0.11 |
| p,p-DDE | 1.14 | 0.04 | 1.86 | 0.12 |
| o,p-DDD | 1.14 | 0.03 | 1.92 | 0.14 |
| p,p-DDD & o,p-DDT | 1.12 | 0.04 | 2.30 | 0.17 |
| p,p-DDT | 1.10 | 0.03 | 1.64 | 0.21 |

### 3.4. Analytical performance and assessment of the non-spectral matrix effect in instrumental analysis

To the best of our knowledge, no previous study has assessed the non-spectral matrix effects of Triton X-100 as a concomitant compound in samples analyzed by GC-MS or GC-MS/MS. The influence of Triton X-100 on the instrumental sensitivity was established by comparison of the slopes of the calibration curves achieved via the measurement of sets of 5 standard solutions (with concentrations up to 15 ng $ml^{-1}$) prepared in pure hexane as well as in 0.09 % (w/w) Triton X-100 in hexane, respectively (eq. (1)). Each standard level was measured in triplicate and calculations were accomplished by the weighted regression approach, using $1/c^2$ as weighting factors where *c* denotes the standard concentration [31,32]. The standard deviations of the slopes were also evaluated and the propagated standard deviation of the corresponding slope ratios were further calculated.

$$Slope\ ratio = \frac{Slope\ (Matrix\text{-}match)}{Slope(Hexane)}, \quad (1)$$

The obtained results as slope ratios when alternatively, the glass or the metal liners were used are presented in Table 5.

When the glass liner was used it was observed that the achieved sensitivity for most of the target analytes was statistically identical in both studied media – pure hexane and matrix-matched one. For several compounds (Chlorpyrifos, Endrin, p,p-DDE, o,p-DDD, p,p-DDD & o,p-DDT, p,p-DDT) it was observed little enhancement (~10 %) of the registered sensitivity and only for Dieldrin, it was detected a 10 % decrease in the slope ratio. The utilization of the metal liner brought different results – for all target analytes, the calibration slopes substantially increase in a matrix-matched medium that contained Triton X-100 compared to samples in pure hexane. The enhancement was in the range of 126–230 % (Table 5). However, it can be noticed that the standard deviations of the slope ratios when the metal liner had been used were greater than in the case of glass liner application. This is a consequence of the registered relatively high values for the corresponding standard deviations of the term *Slope (Hexane),* in eq. (1),

**Table 6**
Analytical characteristics of the GC-MS/MS measurements in hexane and a matrix-matched medium using glass liner.

| Compound name | Hexane calibration | | | | Matrix-matched calibration | | | |
|---|---|---|---|---|---|---|---|---|
| | Slope Area ml $ng^{-1}$ | $R^2$ | LOD, ng $ml^{-1}$ | LOQ, ng $ml^{-1}$ | Slope Area ml $ng^{-1}$ | $R^2$ | LOD, ng $ml^{-1}$ | LOQ, ng $ml^{-1}$ |
| Pentachlorobenzene | 4847 | 1.00 | 0.02 | 0.06 | 4685 | 1.00 | 0.05 | 0.15 |
| Hexachlorobenzene | 8055 | 0.99 | 0.04 | 0.12 | 7703 | 1.00 | 0.05 | 0.17 |
| alpha-HCH | 6177 | 1.00 | 0.03 | 0.11 | 5937 | 1.00 | 0.06 | 0.19 |
| beta & gamma-HCH | 5665 | 1.00 | 0.02 | 0.06 | 5399 | 1.00 | 0.03 | 0.10 |
| Chlorpyrifos-methyl | 1258 | 0.98 | 0.93 | 3.09 | 1288 | 0.96 | 0.26 | 0.88 |
| Chlorpyrifos | 4340 | 1.00 | 0.25 | 0.83 | 4846 | 1.00 | 0.10 | 0.33 |
| alpha-Endosulfan | 1269 | 1.00 | 0.08 | 0.25 | 1256 | 1.00 | 0.08 | 0.26 |
| Aldrin | 1413 | 1.00 | 0.08 | 0.27 | 1354 | 1.00 | 0.07 | 0.24 |
| Dieldrin | 606 | 0.98 | 0.20 | 0.68 | 548 | 0.99 | 0.11 | 0.36 |
| Endrin | 731 | 0.99 | 0.11 | 0.36 | 788 | 0.99 | 0.09 | 0.30 |
| Heptachlor | 2707 | 1.00 | 0.06 | 0.18 | 2785 | 1.00 | 0.06 | 0.20 |
| Heptachlor-*endo*-epoxide-A | 632 | 0.99 | 0.09 | 0.31 | 616 | 1.00 | 0.07 | 0.23 |
| o,p-DDE | 9260 | 0.99 | 0.12 | 0.41 | 9095 | 1.00 | 0.05 | 0.15 |
| p,p-DDE | 7620 | 0.99 | 0.10 | 0.32 | 8684 | 1.00 | 0.07 | 0.25 |
| o,p-DDD | 17,928 | 1.00 | 0.05 | 0.16 | 20,430 | 0.99 | 0.04 | 0.13 |
| p,p-DDD & o,p-DDT | 22,054 | 0.99 | 0.15 | 0.52 | 24,606 | 1.00 | 0.20 | 0.65 |
| p,p-DDT | 2924 | 1.00 | 0.08 | 0.25 | 3221 | 1.00 | 0.10 | 0.33 |

**Conditions:** 5-level calibration curves up to 15 ng $ml^{-1}$. Standards in each level are injected in triplicate.

**Table 7**
Analytical characteristics of the GC-MS/MS measurements in hexane and a matrix-matched medium using metal liner.

| Compound name | Hexane calibration | | | | Matrix-matched calibration | | | |
|---|---|---|---|---|---|---|---|---|
| | Slope Area ml $ng^{-1}$ | $R^2$ | LOD, ng $ml^{-1}$ | LOQ, ng $ml^{-1}$ | Slope Area ml $ng^{-1}$ | $R^2$ | LOD, ng $ml^{-1}$ | LOQ, ng $ml^{-1}$ |
| Pentachlorobenzene | 2826 | 0.96 | 0.08 | 0.26 | 3578 | 0.98 | 0.05 | 0.18 |
| Hexachlorobenzene | 4470 | 0.96 | 0.08 | 0.28 | 6385 | 0.99 | 0.04 | 0.15 |
| alpha-HCH | 3064 | 0.99 | 0.19 | 0.64 | 3857 | 0.98 | 0.05 | 0.17 |
| beta & gamma-HCH | 5066 | 0.98 | 0.05 | 0.18 | 6615 | 0.98 | 0.06 | 0.20 |
| Chlorpyrifos-methyl | 621 | 0.95 | 0.35 | 1.18 | 1261 | 0.98 | 0.20 | 0.68 |
| Chlorpyrifos | 1826 | 0.96 | 0.24 | 1.79 | 3856 | 0.98 | 0.06 | 0.19 |
| alpha-Endosulfan | 603 | 0.94 | 0.10 | 0.34 | 991 | 0.99 | 0.19 | 0.62 |
| Aldrin | 719 | 0.97 | 0.85 | 2.82 | 1154 | 0.98 | 0.21 | 0.71 |
| Dieldrin | 298 | 0.98 | 1.57 | 5.23 | 529 | 0.98 | 1.62 | 5.39 |
| Endrin | 422 | 0.87 | 2.81 | 9.37 | 825 | 0.98 | 1.22 | 4.08 |
| Heptachlor | 1456 | 0.85 | 0.10 | 0.35 | 2414 | 0.96 | 0.08 | 0.28 |
| Heptachlor-*endo*-epoxide-A | 269 | 0.96 | 0.23 | 0.77 | 444 | 0.97 | 0.42 | 1.41 |
| o,p-DDE | 4391 | 0.95 | 0.11 | 0.32 | 7340 | 0.99 | 0.04 | 0.14 |
| p,p-DDE | 3811 | 0.95 | 0.09 | 0.31 | 7098 | 0.99 | 0.17 | 0.58 |
| o,p-DDD | 7829 | 0.95 | 0.20 | 0.66 | 15,035 | 0.99 | 0.04 | 0.14 |
| p,p-DDD & o,p-DDT | 8990 | 0.95 | 0.60 | 1.99 | 20,635 | 0.98 | 0.06 | 0.19 |
| p,p-DDT | 1316 | 0.91 | 2.04 | 6.82 | 2156 | 0.98 | 0.12 | 0.41 |

**Conditions:** 5-level calibration curve in the range of 0–15 ng $ml^{-1}$. Standards in each level are injected in triplicate.

when the analytes pass through the metal liner (the lowest values for the coefficients of determination were observed for calibration in pure hexane and usage of metal liner – Tables 6 and 7). The last can be explained by the retention or/and interaction of the pesticides occurring on the bare metal surface of the liner. So, it could be assumed that the observed increase in the analyte signals in the presence of Triton X-100 is due to processes localized in the liner. We suppose that at the vaporization step (under the conditions depicted in section 2.2.2.1.), Triton X-100 is deposited on the inner surface of the liner and the surfactant starts to have a preserving effect against pesticide retention or/and interaction, especially in the case when analytes with relatively high boiling points are passing through the metal liner.

Tables 6 and 7 summarize the analytical characteristics derived from the accomplished weighed regression analysis when varying the applied liner and the media of the calibration solutions. It is noticeable that the obtained values for the coefficient of determination ($R^2$) do not deteriorate in the presence of 0.09 % Triton X-100 which was valid for both used liners. The presented LODs and LOQs were calculated based on the 3s and 10s criteria, respectively. The values of the standard deviations of the regression line intercepts were assumed as representatives for the standard deviation of the blank signal [33,34]. From Table 6 it is evident that the performance characteristics of the glass liner GC-MS/MS system are not influenced substantially by the presence of Triton X-100 as a matrix component. In the case of metal liner GC-MS/MS analysis (Table 7), it was found that for the number of analytes the LODs (LOQs) decreased by more than two times i.e. for Hexachlorobenzene, alpha-HCH, Chlorpyrifos-methyl, Chlorpyrifos, Aldrin, Endrin, o,p-DDE, o,p-DDD, p,p-DDD & o,p-DDT, p,p-DDT. For the rest of the analytes, the performance characteristics do not differ substantially when switching the sample medium. In pure hexane as well as in a matrix-matched medium the obtained sensitivities by the glass liner were higher than the ones achieved by the metal liner. For some analytes (Pentachlorobenzene, beta & gamma-HCH, alpha-Endosulfan, Aldrin, Dieldrin, Endrin, Heptachlor-*endo*-epoxide-A, p,p-DDE, p,p-DDT) it also can be seen lower LODs (LOQs) when the glass liner GC-MS/MS was applied compared to the application of metal liner. For this reason, it can be concluded that the use of glass liners should be preferable.

### 3.5. Comparative measurement by GC-MS

Further study of the effect of a matrix-matched medium (0.09 % w/w Triton X-100 in isooctane) was carried out with a new set of seven target

**Table 8**
Analytical characteristics of the GC-MS measurements in isooctane and a matrix-matched medium using glass liner.

| Compound name | | Calibration in isooctane | | Matrix match calibration | | | |
|---|---|---|---|---|---|---|---|
| | Rt, min | Slope Area ml $ng^{-1}$ | $R^2$ | Slope Area ml $ng^{-1}$ | $R^2$ | *Slope ratio* | SD |
| Dichlorvos | 9.42 | 157 | 1.00 | 197 | 1.00 | 1.26 | 0.04 |
| Ethoprophos | 15.53 | 56 | 1.00 | 73 | 1.00 | 1.30 | 0.01 |
| Disulfoton | 17.91 | 167 | 1.00 | 216 | 1.00 | 1.29 | 0.01 |
| Methyl parathion | 19.07 | 29 | 1.00 | 45 | 1.00 | 1.57 | 0.03 |
| Fenchlorphos | 19.39 | 73 | 1.00 | 99 | 1.00 | 1.36 | 0.01 |
| Chlorpyrifos | 20.22 | 57 | 1.00 | 76 | 1.00 | 1.34 | 0.03 |
| Prothiofos | 22.31 | 42 | 1.00 | 64 | 1.00 | 1.53 | 0.02 |

**Conditions:** 5-level calibration curve in the range of 0–1000 ng $ml^{-1}$.

analytes (Table 2) with conventional GC-MS system (Shimadzu 2010 SE) under the instrumental parameters pointed in section 2.2.2.2. Isooctane was used as an alternative organic solvent to hexane. Since the solubility of Triton X-100 in isooctane and hexane is practically identical (Table 3) a matrix-matched medium simulating the final solution after cloud point extraction again was adjusted to contain 0.09 % w/w of the surfactant dissolved in isooctane. It was found that the retention times of all the seven analytes were identical in solely isooctane and matrix-match medium. Table 8 gives the calculated slope ratios (eq. (1)) and their propagated standard deviations derived from the calibration lines fitted on measurements of standards in pure isooctane as well as in a matrix-matched medium (5 standards with concentration up to 1000 ng $ml^{-1}$). The regression models were built-up by the weighted regression approach, using $1/c^2$ as weighting factors. It is evident that there is an enhancement of the sensitivities in matrix-matched calibration in the range of 126–157 %. LODs (LOQs) were also calculated in the same way as mentioned in section *3.4* and it was found that the performance characteristics of the glass liner GC-MS system are not influenced substantially by the presence of Triton X-100 as a matrix component. The discussed results further prove that cloud point extraction with Triton X-100 can be combined with GC-MS or GC-MS/MS analysis by applying liquid injection of the target analytes transferred in organic solvents such as hexane or isooctane.

### 3.6. Analyte recoveries

The optimization of the factors influencing on the cloud point extraction of the pesticides from water medium was not a primary objective of the current study. However, the procedure described in section 2.3. is based on our preliminary study of several parameters influencing both steps - cloud point extraction and back-extraction. The migration of all target analytes from the water medium to the surfactant-rich phase was not influenced by the pH in the range 5–9. It was also found that increasing the initial concentration of Triton-X 100 up to 2 % w/w resulted in an increased amount of all extracted pesticides, while the subsequent rise in surfactant levels did not show any further significant improvement in the extraction efficiency. For some target analytes, the dependence of the registered signals as a function of the initial Triton X-100 concentration is shown on Fig. S4. Concerning the back-extraction in hexane it was found that vortex agitation is preferable than ultrasound one. Treating the model solutions above 10 min by vortex resulted in no improvement of the obtained recoveries. Meanwhile, to the isolated surfactant-rich phase (about 1 ml) it was necessary to add at least 2 ml of water to lower the viscosity, which facilitates further mixing with hexane in the back-extraction step. The CPE procedure was tested for extraction of the studied analytes from model water solutions at pH = 7. The obtained recoveries when hexane or isooctane was used at the back-extraction step are given in Table 9. It can be seen that for the most of the pesticides are achieved relatively high recoveries with acceptable precision. In Table 9 it can also be found alternative recoveries from other separation/concentration procedures. It can be concluded that the achieved recoveries in the current work are

**Table 9**
Analyte recoveries (%) after CPE and back-extraction in an organic solvent.

| | Compound name | Current study | | Previously reported studies | | | |
|---|---|---|---|---|---|---|---|
| | | Recovery, % | SD | Ref. [35] | Ref. [36] | Ref. [37] | Ref. [38] |
| VABE[a] in hexane | Pentachlorobenzene | 76 | 6 | | | | |
| | Hexachlorobenzene | 83 | 5 | | | | |
| | alpha-HCH | 72 | 4 | | | | 96 |
| | beta & gamma-HCH | 66 | 5 | 89/81 | | | 81/99 |
| | Chlorpyrifos methyl | 74 | 5 | | | | |
| | Chlorpyrifos | 92 | 5 | | | | |
| | alpha-Endosulfan | 88 | 6 | 80 | | | 78 |
| | Aldrin | 88 | 4 | 37 | | | 86 |
| | Dieldrin | 93 | 8 | 83 | | | 64 |
| | Endrin | 89 | 7 | 88 | | | 88 |
| | Heptachlor | 87 | 3 | 43 | | | 94 |
| | Heptachlor-*endo*-epoxide-A | 84 | 5 | 76 | | | |
| | o,p-DDE | 89 | 6 | | | | 86 |
| | p,p-DDE | 102 | 7 | 80 | | | |
| | o,p-DDD | 87 | 7 | | | | 83 |
| | p,p-DDD & o,p-DDT | 90 | 8 | 91 | | | 85/73 |
| | p,p-DDT | 105 | 12 | 87 | | | 75 |
| VABE[a] in isooctane | Dichlorvos | 5 | 1 | | 72 | | |
| | Ethoprophos | 54 | 3 | | 80 | | |
| | Methyl parathion | 35 | 3 | | 44 | 45 | |
| | Fenchlorphos | 73 | 5 | | | | |
| | Chlorpyrifos | 85 | 5 | | 86 | | |
| | Prothiofos | 83 | 7 | | | | |

**Conditions: 10 ng ml-1 (GC-MS/MS detection) or 100 ng ml-1 (GC-MS detection) of pesticides extracted from water medium at pH=7 (n = 3)**
Ref. [35] Solid-phase extraction, according to EPA method 8081 B/3535.
Ref. [36] Liquid-liquid extraction, according to EPA method 8141 B/3510.
Ref. [37] Liquid phase micro-extraction in 1-undecanol.
Ref. [38] Liquid-liquid extraction in hexane.
[a] VABE – Vortex Assisted Back Extraction.

consistent with the ones obtained from other authors as well as with those stated as achievable by solid phase extraction according to EPA method 8081 B [35] or liquid-liquid extraction proposed in EPA method 8141 B [36].

## 4. Conclusions

Cloud point extraction with Triton X-100 followed by back-extraction with hexane or isooctane can be successfully combined with GC-MS or GC-MS/MS detection of liquid sample injections. The co-extracted surfactant, which is injected into the gas chromatographic system, is at a relatively low level (0.09 %) and does not deteriorate the instrumental measurements in SRM mode as well as the long-term stability for several Organochlorine and Organophosphorus pesticides. The SRM scan mode allows for selective determination of the target analytes without the presence of isobaric interference from the matrix. Moreover, probably due to the deposition of Triton X-100 on the inner liner surface the surfactant acts as an analyte protectant resulting in enhanced sensitivity. The last effect is more obvious for pesticides with relatively high boiling points passing through a metal liner. The observed increase is due to the detergent's assumed deactivation of the liner. However, despite the highly pronounced effect when using the metal liner, the best performance characteristics are obtained when glass liner is used. The metal liner is needed to be conditioned longer than the glass liner to achieve consistent precision. A significant figure of merit for the studied combination CPE-back extraction-GC-MS (or GC-MS/MS) analysis is the possibility to be accomplished a matrix-matched calibration using hexane or isooctane spiked with 0.09 % w/w Triton X-100.

## Declaration of generative AI and AI assisted technologies in the writing process

During the preparation of this work the authors do not used Generative AI and AI assisted technologies in the writing process**.**

## CRediT authorship contribution statement

**Asya D. Hristozova:** Methodology, Validation, Investigation, Writing - original draft, Visualization. **Kiril K. Simitchiev:** Conceptualization, Formal analysis, Writing - review & editing. **Veselin J. Kmetov:** Project administration, Funding acquisition. **Erwin Rosenberg:** Resources, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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