New halogen-specific detector applied to the analysis of chlorinated fatty acids

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Abstract

A new halogen-specific detection method (XSD) was tested for determination of chlorinated fatty acids in marine biota. In XSD, an increased emission of ions and electrons is caused by the high-temperature combustion of halogen-containing compounds. The detection limit of methyl dichlorooctadecanoate and the selectivity at a reactor temperature of 900°C match those of electrolytic conductivity detection (ELCD). The relative standard deviation is less than 11% for ≥ 0.2 ng methyl dichlorooctadecanoate. An XSD chromatogram of a complex sample, chlorinated fatty acid methyl esters liberated from fish lipids, agreed with a previously obtained ELCD chromatogram. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chlorine in chlorinated fatty acids constitutes a substantial part of the extractable organically bound chlorine (EOCl) in marine biota [1–5], and can account for up to 90% of EOCl in fish. Chlorine bleached pulp production has long been a major

anthropogenic source of chlorinated fatty acids [6], but chlorinated fatty acids may also be formed naturally, both from biogenic and anthropogenic precursors (reviewed by Mu et al. [7]). Only a few studies of physiological effects of chlorinated fatty acids are at hand, but a number of them show that chlorinated fatty acids can affect reproduction related processes [8–10] and that they have relatively high toxicity to fish [6].

Fatty acid methyl esters (FAMEs) containing chlorine have been determined successfully by gas chromatography (GC) using Hall electrolytic conductivity detection (ELCD) [2,4,11]. Electron-capture detection (ECD) and the ELCD are the most

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commonly used methods for detecting halogenated compounds in complex sample matrices. ECD is not suitable for detection of chlorinated FAMEs, because the ECD limit of pure methyl dichlorooctadecanoate is about 0.5 ng and negative peaks are obtained from unchlorinated FAMEs in the samples, presumably resulting from a high background in the ECD system [2,12]. The GC–ELCD method has a detection limit of 0.25 ng of methyl dichlorooctadecanoate and no negative peaks are obtained from unchlorinated compounds [2].

Recently, a new halogen-specific detection method (XSD) has been introduced for the determination of halogenated compounds such as pesticides and poly-chlorinated biphenyls (PCBs) [13,14]. XSD is a thermionic method, based mainly on halogen-in-duced thermal electron emission [13]. According to a technical report [13] from the manufacturer (OI Analytical, College Station, TX, USA), XSD has a higher selectivity than ECD (expressed as chlorine/hydrocarbon ratio) in the analysis of halogenated compounds, but a lower selectivity than ELCD. The "oxidation to chlorine"-based XSD selectivity is 10^4 at a temperature of 1000°C [13], while the "reduction to HCl"-based ELCD selectivity is 10^6 [15,16]. The sensitivities, however, for chlorinated pesticides [α-benzene hexachloride (α-BHC), β-BHC, hepta-chlor, aldrin, p,p'-DDE, endrin and endosulfan] of XSD and ELCD were similar, 1 pg Cl/s [13]. The manufacturer claims that XSD has advantages over ECD and ELCD as a halogen detection method. No radioactive source is needed as in ECD. In comparison to ELCD, no solvent and pump are needed and air or oxygen can be used instead of hydrogen, making operation safer. The stabilisation time is 15–30 min, while ELCD or the ECD requires about 1 day to stabilise. The stability of XSD is better than that of ELCD and it is cheaper to maintain.

Arguments, such as these, called for an investigation. The main purpose of this work was to study if XSD can be used as an alternative detection method for the analysis of methyl esters of chlori-nated fatty acids and to obtain some information on the function of the XSD system. The detection limit of methyl dichlorooctadecanoate and the selectivity, in terms of the response ratio between chlorinated and unchlorinated FAMEs, were studied and com-pared to those previously obtained for ELCD.

2. Principle of the XSD system

Except for the pneumatics and the detector con-troller (controlling the reactor temperature and pro-cessing the detector signal), the XSD system consists of three major parts [13]: a jet inlet assembly in the detector base, a reactor assembly, and a probe assembly. Through the jet inlet, a mixture of com-bustion gas (air) and the GC column effluent enters the reactor tube 1, which is surrounded by the temperature regulated reactor core (Fig. 1). The core also serves as an auxiliary anode, which is said to increase the current collection efficiency [13]. The combustion gas also purges the outside of the jet. The probe assembly consists of an anodic platinum coil, 2 (in the application note [13] erroneously named "cathode"), wrapped around and in contact with an alkali glass ceramic rod, 3, and a cathodic platinum bead, 4, at the end of the rod. A potential, measured to 27 V, is applied over the anode and
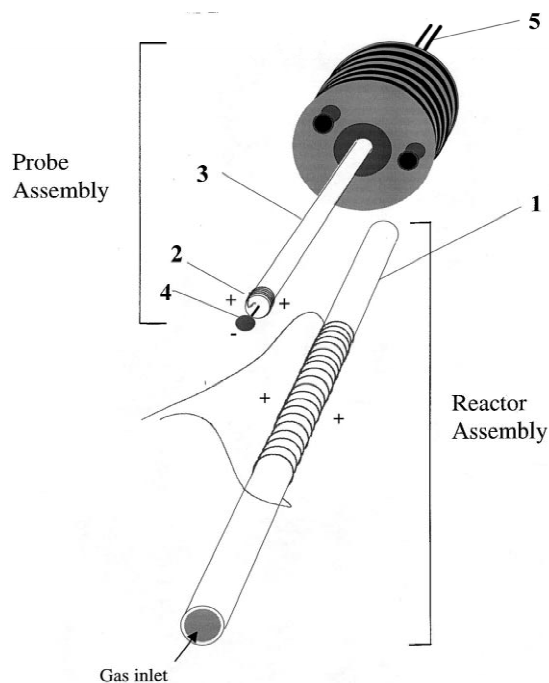

Fig. 1. A simplified, schematic drawing of the XSD system with reactor core and probe assembly consisting of: (1) reactor tube, surrounded by the temperature regulated reactor core, (2) anodic platinum coil, (3) alkali glass ceramic rod, (4) cathodic platinum bead and (5) connector to the detector controller.

cathode and a background current is obtained, probably caused by thermal electrons emitted from the cathode at high temperature. The current is transferred via connector, 5, to the detector controller and quantified by an external data handling device.

The mechanism for the XSD response to halogenated compounds is not commonly known [13]. The exact identification of oxidation products formed in the XSD system has not been made. In principle, there are three thermionic mechanisms: positive surface ionisation, negative surface ionisation, and thermal electron emission [13,21]. In contrast to the ELCD system (when used for halogen-selective detection), the XSD reactor operates in an oxidative pyrolysis mode and the sample compounds are converted into their oxidation products. When halogen-containing compounds enter the detector, the detector current increases. According to the report [13], in addition to hydrogen halide, free halogen atoms are formed during high-temperature combustion of halogen-containing compounds. The free halogen atoms exist in the gas phase in equilibrium with their non-dissociated dimers, and this equilibrium is driven towards the free halogen atoms as the temperature increases. The ''halogens'' are adsorbed onto the alkali activated cathode and desorbed as either a neutral halogen atom, a negatively charged ion, or as an alkali halide molecule [13,17]. This can be explained by a homolytic cleavage of molecules generating free halogen atoms or a heterolytic cleavage generating negatively charged halogen-containing fragments. Negative halogen ions are formed on a cathodic surface when the work function of the cathode is lower than the electron affinity of a neutral halogen atom. The platinum cathode can be activated by alkali which will decrease the work function of the cathode from that of platinum to a value close to that of the alkali element (i.e., from 5.65 eV to between 2 and 3 eV [18,19]). The work function of the alkali activated platinum cathode is now lower than the electron affinity of chlorine (3.6 eV [18,19]) and an electron transfer to the chlorine can occur. The energy then released per electron transfer is supposed to generate a local temperature increase followed by a larger flow of ''thermal'' electrons [13].

The transfer of alkali from the ceramic rod to the cathode (the activation of the cathode) is not explained. In the works by Rice [20] and Roberts [21] on vapour detectors for tracing the presence of certain impurities in gases, halogen-containing compounds were explained to remove alkali from the surface of the ceramic rod close to the anode, which was considered to be an alkali ion emitter. Rice [20] claimed that as soon as the halogen content is consumed, a new layer of alkali is built up on the surface by diffusion from the bulk of the ceramic rod and the current returns to its original value. However, it is also possible that the concentration of alkali at the surface of the ceramic rod is reduced due to evaporation of an alkali halide.

The detector response depends on the reactor temperature, which can be set at 800, 900, 1000 and 1100°C. The response also depends on the time a sample compound spends in the detector, which is governed by the flow (10–40 ml/min) of the combustion gas, being either air or oxygen. At a higher flow, the compound will remain for too short a time in the detector and the response will decrease, due to incomplete combustion. At very low flows (<10 ml/min), the peak width increases [13].

The choice of ceramic rod material is important for having a sensitive and long lasting detector, because the halogen mediated removal of alkali from the surface of the ceramic rod eventually results in a lack of alkali and the ceramic rod must be replaced by a new one.

Different alkali metal salts such as iodides of lithium, sodium, potassium, rubidium and caesium and also silicates of sodium and potassium have been shown suitable. A material that seems to give excellent sensitivity and very long constant life time is synthetic Leucite (K_3O–Al_2O_3–SiO_2=1:1:4) [21]. Neither the operator's manual [17] nor the application note [13] mention which alkali material is used in the ceramic rod of the Model 5360 XSD system.

3. Experimental

3.1. Studies of chlorinated fatty acids

The purity of the chemicals used, *cis*-9-octadecenoic acid (99%; Sigma, St. Louis, MO, USA), borontrifluoride (20% solution in methanol; Merck,

Hohenbrunn, Germany), cyclohexane (>99.5%; Riedel-de Haën, Seelze, Germany), chloroform (99.8%; J.T. Baker, Deventer, The Netherlands), and acetone (>99.5%; J.T. Baker), was controlled in blank procedures. Water was taken from a Milli-Q water purification system (Millipore, Molsheim, France). The water was extracted with cyclohexane before use. All glassware was rinsed with acetone (>99.5%) and heated at 250°C for 15 h before use. 9,10-*threo*-Dichlorooctadecanoic acid was prepared from *cis*-9-octadecenoic acid, which was dissolved in chloroform and exposed to chlorine for half an hour in darkness and cooled by dry ice [22]. The acid was esterified with 20% borontrifluoride in methanol [23]. The purity of methyl dichlorooctadecanoate was studied by GC with electron ionisation mass spectrometry [22] and found to be 85% (based on the total ion chromatogram). The methyl dichlorooctadecanoate was used for calibrating the GC–XSD response.

A Shimadzu gas chromatograph, Model 17 A, was equipped with a BPX5 (5% phenyl–polysil-phenylenesiloxane, 25 m×0.22 mm I.D., film thickness 0.25 µm) fused-silica capillary column (Polynom, Stockholm, Sweden). A manual wide bore injection technique was used. Helium (99.997%; Air Liquide, Malmö, Sweden) was used as carrier gas. The injector temperature was 280°C and the carrier gas flow was 1 ml/min. The column temperature program was 90°C (held for 2 min) to 160°C at 20°C/min and then to 300°C at 10°C/min (held for 5 min). An XSD system (OI Analytical) Model 5360, was connected to the column. Medical air (Air Liquide) was used as combustion gas.

3.2. Tests with methyl dichlorooctadecanoate

Solutions of methyl dichlorooctadecanoate in the concentration range of 0.2 to 80 µg/ml were used for optimising the XSD conditions. The relative standard deviation (RSD) was calculated from five manual injections of 1 µl of every standard solution. Different reactor temperatures (900°C, 1000°C and 1100°C) and different flows of medical air (8–32 ml/min) were selected. For measuring the selectivity, in terms of the methyl dichlorooctadecanoate/ methyl octadecenoate response ratio, a sample containing 0.8 µg/ml of methyl dichlorooctadecanoate

and 5 mg/ml of methyl octadecenoate (ratio $1:6\cdot10^3$) was prepared and 1 µl of the sample was injected. To study whether the response of methyl dichloro-octadecanoate changed if a mixture of unchlorinated FAMEs were present, a sample containing FAMEs liberated from cow milk was used. The concentration of methyl dichlorooctadecanoate was 80 µg/g of milk FAMEs. To confirm that no signal was generated from unchlorinated compounds in high concentrations, FAMEs liberated from lipids of cow milk and cultured mammalian cells [24], respectively, were injected in amounts of 10 µg and 28 µg, respectively.

3.3. Analysis of a fish lipid sample

Transesterification of fish lipids to FAMEs and enrichment of the chlorinated FAMEs have been described earlier [2,4]. The final samples were stored in cyclohexane at −20°C. Chlorinated FAMEs enriched from an earlier well-examined eel sample from Idefjord [22] were studied by GC–XSD. The column temperature program was 90°C (held for 2 min) to 280°C at 4°C/min (held for 10 min). The XSD chromatogram (17 µg FAMEs injected) was compared to an earlier recorded ELCD chromatogram [4,11,22].

3.4. Studies of XSD

A used reactor was dismantled with the purpose to study the composition of the detector components. The reactor core, ceramic rod, and jet inlet were cut to pieces. The elemental composition of the pieces was analysed by a JEOL, JSM-840-A scanning electron microscope, using the software Link ISIS.

The chemical equilibria in the XSD system on combustion of a chlorine-containing sample, were simulated by using HSC Chemistry for Windows, chemical reaction and equilibrium software with extensive thermochemical database, Version 3.0 (Outokumpo Research, Pori, Finland). A temperature interval of 500–1500°C was studied and a pressure of 1.0 bar was assumed. The following raw materials were selected: Cl_2 (g), H_2O (g), N_2 (g) and O_2 (g) in amounts of 5 µmol, 100 µmol, 80 mol and 20 mol, respectively. Reactions where the ions H^+ and OH^- were present were also studied.

4. Results and discussion

4.1. Detection of methyl dichlorooctadecanoate

The highest response of methyl dichlorooctadecanoate (in the range 0.2–80 ng) was obtained at 1100°C at an air flow of 10–20 ml/min. As the detector temperature was decreased from 1100°C to 1000 and 900°C, respectively, the response of both 1 and 5 ng of methyl dichlorooctadecanoate was reduced by 50–55 and 80–85%. The reduced response at low detector temperatures has been explained by a reduced concentration of free chlorine atoms in the gas phase [Cl (g)] due to formation of the non-dissociated chlorine dimer [Cl$_2$ (g)] [13]. However, the results of the theoretical study of the chemical equilibria obtained for combustion of a chlorine-containing sample (Fig. 2), shows that the formation of Cl$_2$ (g) is negligible and the concentration of Cl (g) only decreases by 20 and 40% as the reactor temperature is reduced from 1100 to 1000 and 900°C, respectively. These data do not tally with the reduced response of methyl dichlorooctadecanoate. However, the apparent advantage of performing the analyses at 1100°C, cannot be utilised because the selectivity was reduced at the higher temperatures. The highest selectivity, expressed as the response ratio between methyl dichlorooctadecanoate (0.8 ng) and methyl octadecenoate (5 μg) was obtained at 900°C. The selectivity was about $3 \cdot 10^3$ at

1100°C and $1 \cdot 10^4$ at 1000°C (the same value as determined for chlorine/hydrocarbon response ratio [13]). At 900°C, no signal was obtained from 5 μg of methyl octadecenoate, which means that the XSD selectivity, at 900°C, is much higher than $1 \cdot 10^4$, thus being comparable with the chlorine/hydrocarbon response ratio of ELCD.

A constant response was obtained at an air flow between 10 and 20 ml/min. However, as the flow of the combustion gas was increased from 20 to 25 and 32 ml/min, the response of methyl dichlorooctadecanoate (1, 5, and 50 ng) was reduced by 30 and 50%, respectively. The decreased response at an increased flow agrees with the information from the manufacturer [13], who claims that the short retention time in the detector, results in an incomplete combustion. Another explanation might be dilution of the Cl (g), which results in a decreased probability of the Cl (g) to reach the cathode. At a flow less than 10 ml/min the response was very low. The manufacturer's explanation [13] that the combustion is incomplete at a flow less than 10 ml/min, seems to be unreasonable, because the amount of oxygen (present during the elution of a chromatographic peak) was calculated to be enough to combust up to 10 μg methyl dichlorooctadecanoate. At an air flow between 10 and 20 ml/min and a reactor temperature at 900°C, the detection limit of methyl dichlorooctadecanoate was 0.2 ng (two times the noise), which is similar to the detection limit in GC–ELCD [2]. The detector response was linear in the range of 0.2–8 ng of methyl dichlorooctadecanoate (Fig. 3a), but when larger amounts of methyl dichlorooctadecanoate were injected the response became non-linear (Fig. 3b). According to the technical report [13], the detector can become non-linear when injecting high concentrations of chlorinated compounds due to the formation of Cl$_2$ (g) in the gas phase. However, the chemical equilibria in the XSD (Fig. 2) suggest that the concentration of the Cl$_2$ (g) is negligible and will not affect the response. An alternative explanation to the non-linear phenomenon is possibly an insufficient access to alkali atom-activated surfaces.

The XSD response of methyl dichlorooctadecanoate was the same even if unchlorinated FAMEs from cow milk were present and 10 μg of this complex matrix itself did not generate any signal.
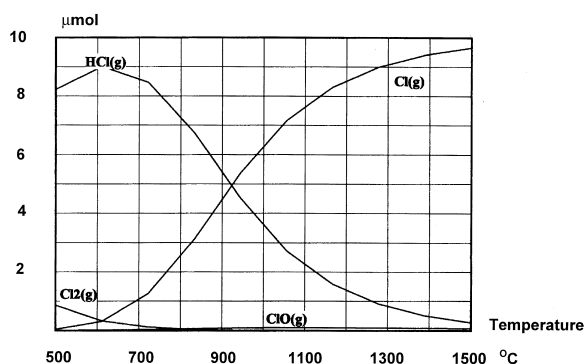

Fig. 2. Chemical equilibria on combustion of a chlorine-containing sample simulated with HSC Chemistry for Windows, Outokumpo Research, Finland. Chlorine-containing compounds having the highest concentrations at equilibrium are HCl (g), Cl (g), Cl$_2$ (g) and ClO (g). For details, see Experimental.
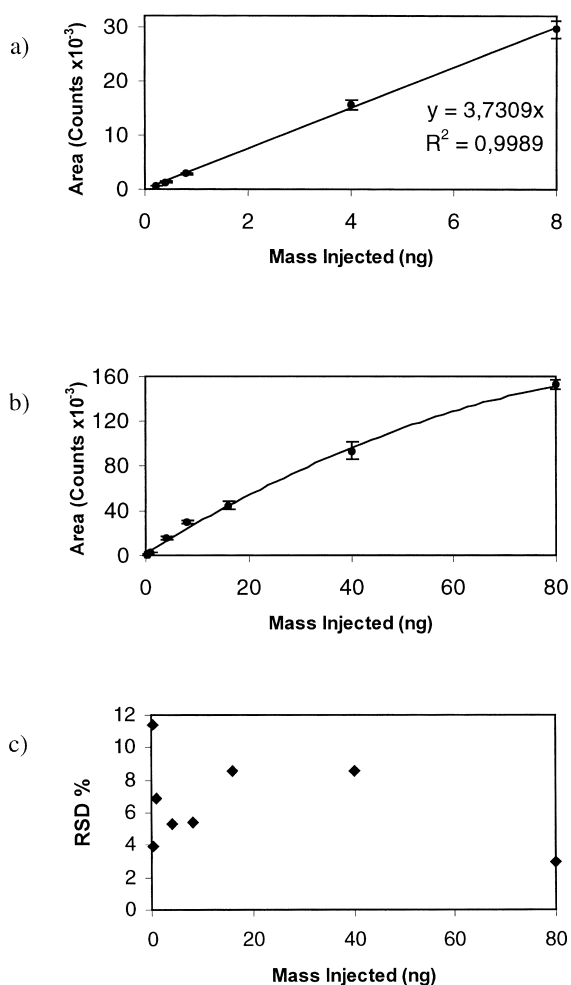
Fig. 3. Dose–response curve for XSD on injecting (a) 0.2–8 ng and (b) 0.2–80 ng of methyl dichlorooctadecanoate. (c) Relative standard deviation for 0.2–80 ng. The XSD temperature was 900°C and the reaction gas flow was 13.5 ml/min.

Neither was any signal obtained when injecting 28 μg of the mammalian cell FAMEs.

The XSD response had an RSD between 3 and 11% for the different injected amounts (Fig. 3c), which was considered acceptable when regarding the manual wide bore injection technique used. The stabilisation time for the XSD system varied between 1 and 2 h and the response to chlorinated FAMEs was almost the same (<5% difference) even if it lasted a few months between injections, which suggests that the system is stable.

4.2. Eel FAMEs

A number of chlorinated fatty acids have earlier been identified in lipids of eel from the Idefjord between Sweden and Norway [22] and these compounds were now also detected by XSD. By comparing an earlier obtained eel ELCD chromatogram and an XSD chromatogram of this study, equal patterns (Fig. 4) were found and the relative intensities of the peaks were similar in the chromatograms. The seemingly smaller signal-to-noise ratio in the XSD chromatogram (900°C) than in the ELCD chromatogram presented might be an effect of less material being injected into the GC–XSD system. The authors [22] did not mention the amount injected to the GC–ELCD system since that study concerned identification of the halogenated FAMEs. Additionally, as mentioned above, the XSD detection limit matches that of ELCD.
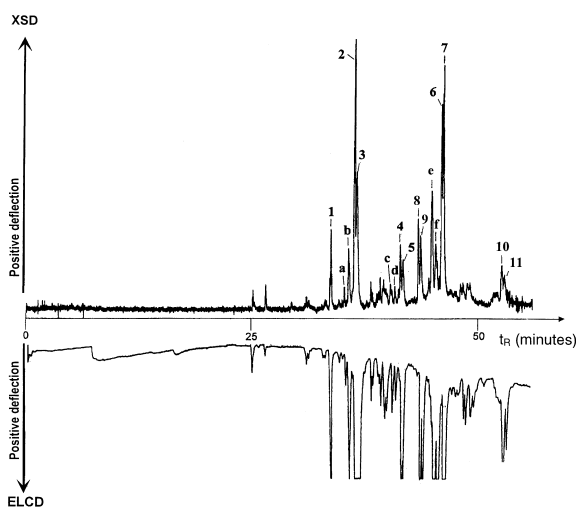

Fig. 4. XSD gas chromatogram of chlorinated FAMEs liberated from an earlier well-examined eel [4,11,22]. By comparison with a similarly obtained ELCD gas chromatogram, the XSD peaks are tentatively assigned the following identities: saturated compounds: (1) methyl dichlorotridecanoate, diastereomeric (*erythro-* and *threo-*) forms of (2, 3) methyl dichlorotetradecanoate, (4, 5) methyl dichlorohexadecanoate, (6, 7) methyl dichlorooctadecanoate, (8, 9) isomers of methyl tetrachlorotetradecanoate and (10, 11) isomers of methyl tetrachlorooctadecanoate. Unsaturated compounds: (a, b) isomers of methyl dichlorotetradecenoate, (c, d) isomers of methyl dichlorohexadecenoate, and (e, f) isomers of methyl dichlorooctadecenoate. The XSD reactor temperature was 900°C and the reaction gas flow was 13.5 ml/min.

4.3. Composition of the detector components

X-Ray analysis in combination with scanning electron microscopy (SEM) showed that the reactor tube contained aluminium, probably in the form of aluminium oxide, which is a common ceramic material used for high temperature applications. No alkali metal was found in the reactor tube. The ceramic rod contained aluminium, silica and potassium. This agrees with the synthetic Leucite material, which was suggested as a suitable ceramic material [21]. Platinum and zirconium were also found in the ceramic rod, probably representing a contamination from the anode and the cathode. The jet inlet was found to consist of stainless steel. Silica was also found in the jet inlet, possibly originating from a glass lining in the tube. It is possibly advantageous to use potassium instead of sodium in the ceramic rod, because potassium has a lower work function, 2.30 eV, than sodium, 2.75 eV [18,19]. The difference between the work function of the potassium activated platinum and the electron affinity of chlorine will then be larger, thus probably making the detector more sensitive.

4.4. Chemical equilibria in the detector on combustion of a sample

The theoretical study of chemical equilibria showed that, under the selected conditions, the concentration of HCl (g) is highest at about 610°C (Fig. 2) and decreases by 90% as the temperature is increased from 610 to 1300°C. Cl (g) is the major component at temperatures exceeding 930°C. The concentration of Cl_2 (g) is low in the temperature interval studied and decreases as the temperature increases because the equilibrium between Cl (g) and Cl_2 is driven towards Cl (g) at high temperatures. At a temperature of 900°C (the selected temperature for XSD analysis), the concentration of HCl (g) is slightly higher than that of Cl (g). Other chlorine-containing compounds such as ClO (g), HClO (g), NOCl (g) are also formed (Fig. 2), but at 900°C the concentrations of these compounds contribute to less than one percent of the total halogen constituents. When also accounting for the ions H^+ and OH^-, the proportion between the different constituents was almost the same at equilibrium.

According to the producer's information [13], the Cl (g) generates the increased current. The larger proportion of Cl (g) in the higher temperature range also tallies with the increased sensitivity found at reactor temperatures of 1000 and 1100°C. However, for obtaining a better understanding of the intriguing function of XSD, further studies concerning how the response is influenced by changing temperatures and air flow must be performed for different kinds of halogenated compounds.

5. Conclusions

(1) Chlorinated fatty acid methyl esters can be selectively determined by XSD. (2) The XSD detection limit for methyl dichlorooctadecanoate matches that of ELCD, 0.2 ng. (3) The selectivity, in terms of the chlorinated/unchlorinated FAMEs ratio, matches that of ELCD, $>>10^4$. (4) The XSD system has an RSD between 3 and 11%, when injecting 0.2–80 ng of methyl dichlorooctadecanoate.

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