Halogen selective detection techniques and their use in the determination of chlorinated fatty acids

Literature review

by

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Summary

The electron capture detector (ECD), the electrolytic conductivity detector (ELCD), the halogen selective detector (XSD), the atomic emission detector (AED) and the mass spectrometer (MS) are very useful tools for the analysis of individual halogenated organic compounds after chromatographic separation. The ECD is superior to the other detectors concerning analysis of polyhalogenated aromatic organic compounds, but the XSD and the ELCD have selectivities superior to that of the ECD, expressed as the Cl/HC ratio. The ELCD and the XSD are also very useful, and superior to the ECD, in the analysis of chlorinated fatty acids, compounds that may account for up to 90% of extractable organically bound chlorine (EOCl) in marine biota. The major benefit with the AED is that it can confirm the content of the individual halogens. The AED sensitivity, with regard to chlorine, is comparable to that of the ELCD and XSD. MS is useful for identification of compounds and by using selective ion monitoring (SIM) the mass spectrometer can also be used as a halogen selective detector and low detection limits can be reached.

A colourimetric technique, potentiometric titration, a volumetric method, neutron activation analysis (NAA) and X-ray fluorescence do not give any information about the structure of individual halogenated compounds but are very useful tools for the selective determination of the total halogen content of a sample, as adsorbable organically bound halogens (AOX), total organically bound halogens (TOX) and extractable organically bound halogens (EOX). The colourimetric technique, potentiometric titration and the volumetric method are based on the determination of halides formed by combustion of a halogen containing compound. The NAA and X-ray fluorescence analysis methods have the advantage that the sample can be analysed without being destroyed.

Contents

1. Introduction

The considerable amount and the complexity of chlorinated organic compounds in nature and the fact that the anthropogenic ones often are suspected of having long-lasting toxic properties in the environmental make essential detection methods for this kind of compounds. Each compound may occur only in small amounts, therefore trace determination is important. Different detection methods can be selected depending on what kind of information concerning the chlorinated organic compounds are of interest. The focus of this review is halogen selective detection of individual organohalogen substances. To determine individual substances, the sample components must be separated. Common methods for separation of substances in complex matrices are gas chromatography (GC) and liquid chromatography (LC). Here, mainly halogen selective detectors that can be connected to a GC will be considered. Detection techniques that can be used for this purpose are ECD, ELCD, XSD, AED, and MS. The detection of organohalogen substances such as chlorinated fatty acids will be discussed in particular. The major part of extractable organically bound chlorine (EOCl) in marine biota is formed by chlorinated fatty acids. In fish lipids they may account for up to 90% of EOCl [1]. Concepts such as TOX, AOX and EOX will also be discussed, including halogen element selective detection methods used for these kind of measurements.

2. Detection of individual organochlorine substances.

2.1 Electron capture detection (ECD)

One of the most common detectors used for organochlorine compounds is the electron capture detector (ECD) [2-5]. The ECD is very sensitive to molecules that contain electronegative functional groups such as halogens, peroxides, ketenes, anhydrides, quinones, and nitro compounds. It is insensitive to amines, alcohols, and hydrocarbons [6-9]. The sensitivity for halogenated compounds increases nearly exponentially with the number of halogenated atoms and it also depends on how the halogenated compounds are bound in the molecule [2,10]. The ECD is the most sensitive detector for polyhalogenated aromatic compounds. Detection limits of a few fg are possible for polyhalogenated pesticides [11], but the ECD is not very selective compared to halogen selective detectors such as the XSD and the ELCD. ECD can only be used in pesticide analysis when the extract is very clean [2]. Extracts not pure enough result in chromatograms with high noise levels. Compounds with low response in the ECD, generate peaks with relative high intensity in the ECD chromatogram, if they occur in high concentration in the extract. This results in a high noise level and decreased detection limits.

The ECD utilises the ability of some compound to undergo electron attachment, which can be either dissociative or non-dissociative [12,13]. The effluent from the column is passed through the ionisation chamber containing a radioactive source (Ti^3H, Sc^3H, or ^{63}Ni), which emits ß-particles (Figure 1). Electrons from the emitter ionise the carrier gas molecules (often nitrogen). This is a rapid process, <0.1 µs and it produce a plasma of positive ions, radicals, and thermal electrons, which migrate to the anode. A small constant current, the standing current, is generated. Electron-absorbing compounds in the carrier gas stream react with the thermal electrons to produce negative ions or neutral molecules, which are swept from the cell. The net result is a reduction in the number of electrons found at steady state, equalling a

drop in the standing current. The maximum current is found in the absence of capturing compounds and a peak is therefore a detector current "valley". The response is non-linear unless the detector is pulsed [9,14]. In such a case the ECD voltage is applied as a sequence of narrow pulses with a duration and an amplitude which is sufficient to collect the very mobile electrons but not the heavier, slower negative ions. An electron concentration is built up inside the cell during the interval between pulses. When the pulse is applied, the concentration of electrons is essentially reduced to zero. During most of the time no field is applied, which enables the free electrons to reach thermal equilibrium with the gas molecules [14,15].

Nickel-63 is a better energy source than tritium and can be used up to 400 °C, but the sensitivity of the nickel detector is about five times less than that of a tritium unit. Another advantage of using nickel-63 is that the detector is much less sensitive to contamination [14].

Residual oxygen and water must be removed form the carrier and make-up gases to avoid excessive noise [11].

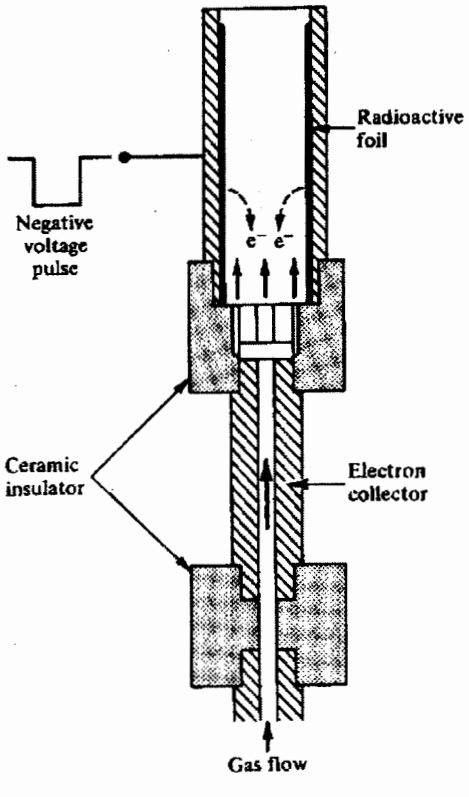

Figure 1. Electron capture detector [14].

2.2 Electrolytic conductivity detector (ELCD)

When used for gas chromatography the ELCD is sensitive to compounds containing halogen, sulphur, and nitrogen [16,17]. For analysis of halogenated organic compounds the ELCD is relatively sensitive and more selective than the ECD [2]. The ELCD has the advantage that the extracts do not need to be purified as far as when using ECD [16]. The US Environmental Protection Agency has recommended the ELCD for detection of chlorinated pesticides, PCBs and other halogenated compounds. The detection limit is as low as about 50 pg of chlorine [18]. ELCD has also been used with success for the analysis of methyl esters of chlorinated fatty acids [18-20]. The ELCD is more suitable than the ECD for analysis of chlorinated fatty acid methyl esters [18], because methyl esters of unchlorinated fatty acids generate negative peaks when monitored by ECD [10]. ELCD can also be used in liquid chromatography [22,23].

As a GC detecor the ELCD can be operated in an oxidative mode or a reductive mode. When the organic compounds are combusted in oxygen or air (the oxidative mode), HCl, SO_2, SO_3, CO_2 and N_2 are formed from compounds containing chlorine, sulphur, carbon, and nitrogen (the formation of NO_2 is negligible). The combustion products are dissolved in a polar solvent, before entering the conductivity detector. The conductivity of the solvent changes as the combustion products are dissolved. The sensitivity for CO_2 is very low because of the inability for CO_2 to absorb rapidly in the polar solvent. The sensitivity of nitrogen containing compounds is also low because N_2 changes the solvent conductivity very little [16,17]. If hydrogen is used as combustion gas (reductive mode), HCl, H_2S, CH_4, and NH_3 are the products. The response from hydrogen sulphide is very low, compared to HCl, because its dissociation constant is too low. CH_4 gives no response either. For the analysis of halogenated compounds the detector is operated in the reductive mode and a slightly acidic electrolyte, n-propanol, is used as solvent. The n-propanol is made acidic by an ion exchange column. The conductivity of this solvent is to a very low degree effected by H_2, NH_3 and CH_4. The detector selectivity for chlorinated hydrocarbons, relative to hydrocarbons is approximately 10^5 in the oxidative mode and 10^6 in the reductive mode [17].

The major components of an ELCD are a reactor, in which the compounds are combusted, and a circulating-solvent system, where the formed reaction products are dissolved, and the change of the solvent conductivity is measured. A block diagram of the ELCD system is shown in Figure 2. From the GC column the sample and carrier gas enter the reactor to be combusted. The reactor consists of a nickel tube, which acts as a catalyst, and it is heated to some 850 °C (Figure 3). The reaction products formed in the reactor are directed through the gas inlet into the conductivity cell assembly (Figure 4), where the combustion products easily can be adsorb in the solvent, resulting in a changed solvent conductivity. The gas passes through the centre of the cell while the solvent, clinging to the walls, is introduced into the conductivity measuring cell. The gas and the solvent are mixed again after the conductivity cell; the gas/solvent mixture is directed to the solvent reservoir, were the gas is vented. The electrolytic conductivity of a solution is directly proportional to the specific conductance of the solution, which has the advantage that the response is directly proportional to the chlorine content in the extract [17]. Analyte ions are removed from the solvent by an ion exchange column [21].

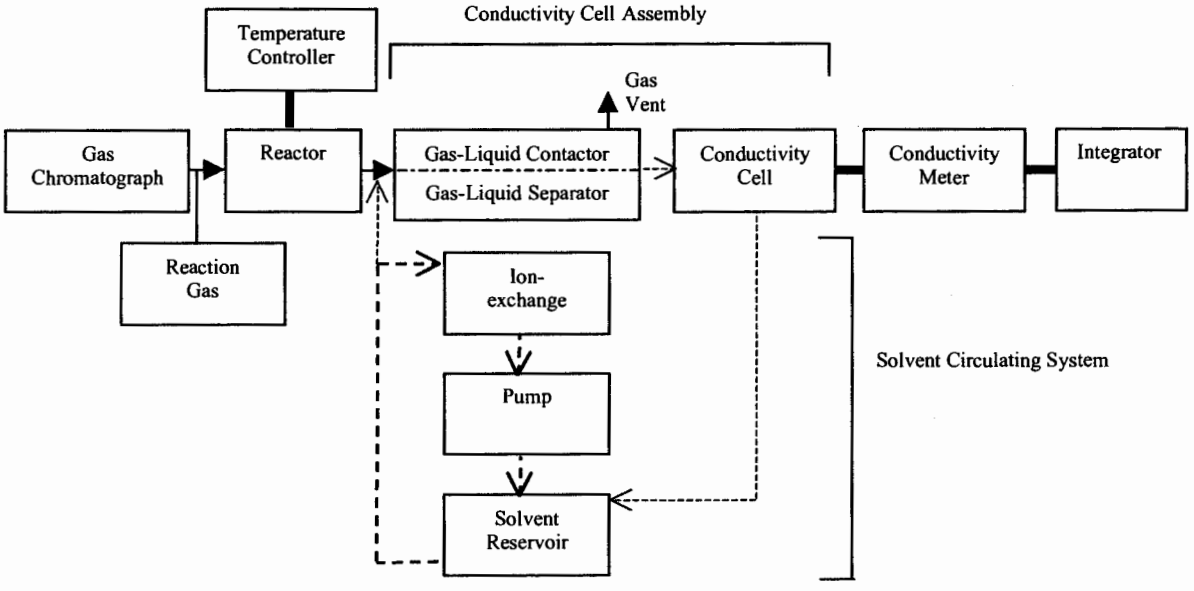

Figure 2. Block diagram of the electrolytic conductivity detector system [21,24]. Dotted lines indicate the solvent flow.

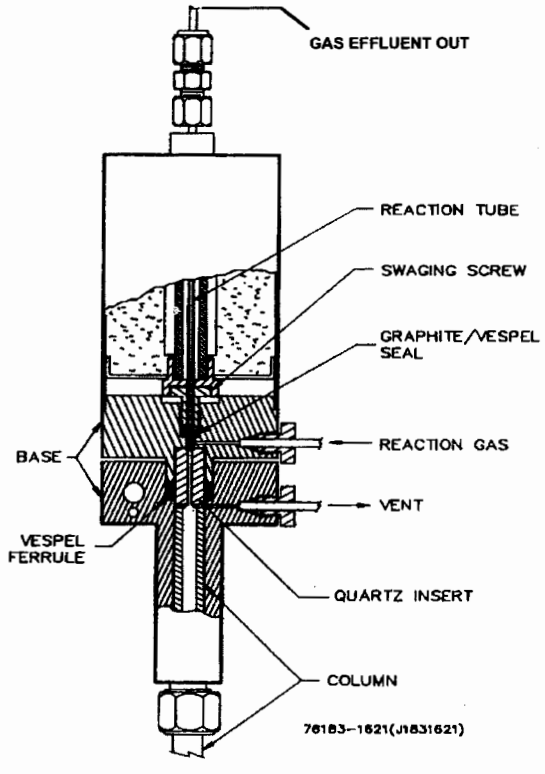

Figure 3. The reactor in an electrolytic conductivity detector, being connected to the conductivity cell assembly via the "gas effluent out" [21].

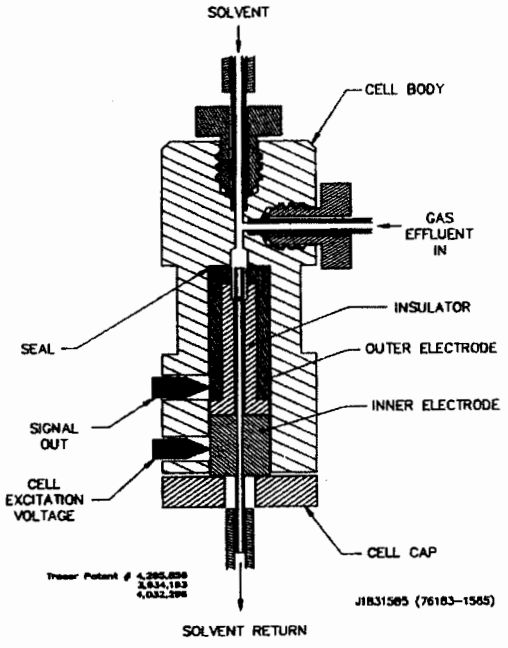

Figure 4. The conductivity cell assembly in an electrolytic conductivity detector, being connected to the reactor via the "gas effluent in" [21].

2.3 Halogen selective detector (XSD)

Recently a new halogen specific detector, XSD, has been introduced. The XSD works in many applications, for example in the determination of halogenated compounds such as chloro-pesticides and polychlorinated biphenyls, PCBs [25,26]. The XSD is comparable to the ELCD in its capacity to detect halogenated organic compounds. Studies have shown that the XSD has a higher selectivity, expressed as the chlorine/hydrocarbon ratio, than the ECD, but that the XSD selectivity is less than that of the ELCD. The XSD selectivity is 10^4 at the recommended operation temperature of 1000 °C, while the ELCD selectivity is 10^5 [25]. However, it has been shown that the selectivity halogenated fatty acid methyl ester/fatty acid methyl ester is increased to $>>10^4$ at 900 °C [27]. The XSD and the ELCD have similar sensitivities, 1 pg Cl/sec [25]. The XSD has the advantage over the ELCD as a halogen detector that no solvent is added, no pumps are needed, the stabilisation time is only one hour while the ELCD requires half a day, the stability is better, and the XSD is cheaper to maintain [25,27].

The XSD is a thermoionic device, based upon three mechanisms, positive surface ionisation, PSI, negative surface ionisation, NSI, and thermal electron emission, TEE, for generation of an electrical current [25]. The work done by Rice [28] and Roberts [29] about electrical vapour detectors for detecting the presence of certain substances or impurities in gases, laid the foundation for the XSD.

Except for the pneumatics and the detector controller, the XSD consist of three major parts: the jet inlet assembly in the detector base, the reactor assembly, and the probe assembly (Figure 5). A mixture of combustion gas (air) and the GC column effluent enters the reactor core through a jet inlet. The combustion gas flows into the reactor base through two lines: one line purges the outside of the detector jet and the other line purges the jet inlet. The probe assembly consist of an alkali glass ceramic tube with a cathode coil (wrapped around the ceramic tube) and an anode platinum bead at the end of the ceramic tube.

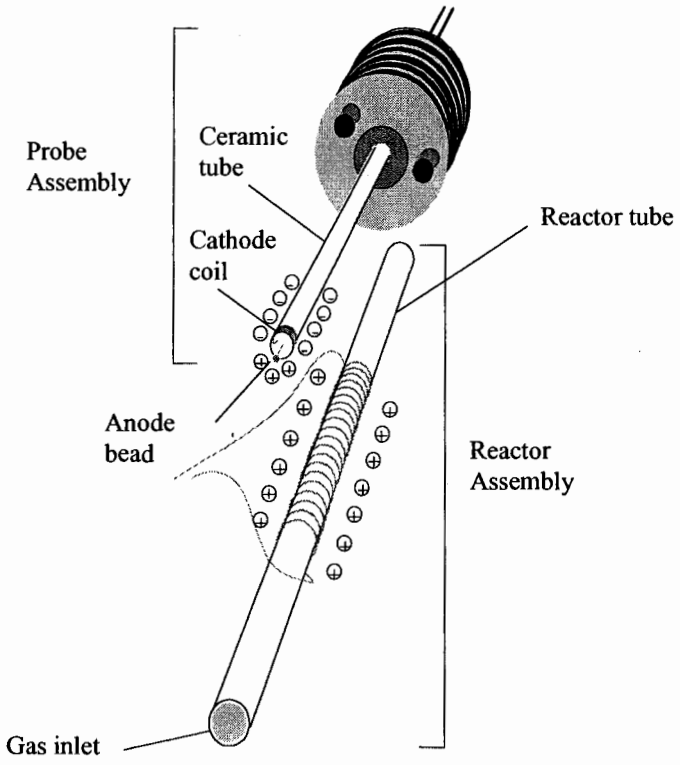

Figure 5. A simplified sketch of the XSD detector [27].

According to the technical report from OI Analytical, Texas, USA, the XSD reactor operates in an oxidative pyrolysis mode [25]. Compounds are converted into their oxidation products. Products containing halogen generates an emission of alkali ions from the anode [28,29]. According to the technical report free halogen atoms are formed during the combustion of halogen containing compounds. The free halogen atoms will adsorb on a negatively charged platinum electrode, which is activated by neutralisation of alkali ions emitted from an anodic surface. Free halogen atoms exist in the gas phase, in equilibrium with their nondissociated dimers. This equilibrium is driven towards the dissociated halogen atoms as the temperature increases. The halogen species emitted from the cathodic surface can consist of neutral atoms, negatively charged halide ions, or alkali-halide molecules [25,30,31]. A thermal electron emission on the cathode occurs due to halogen adsorption (which further decreases the work function of the platinum electrode), or by an increase of the local temperature of the cathode (due to the electron affinity of the halogen, or the heat formed by the formation of alkali halides). Positive alkali ions are created on the anodic surface when neutral alkali atoms hit the anodic surface. This occurs when the ionisation potential of the neutral atom or molecule is less than the work function of the anodic surface [25]. The emission current is created by free electrons, as well as negative and positive ions and is measured by an electrometer circuit.

2.4 Atomic emission detection (AED)

The AED is an element-specific detector. It has long been used for metal analysis and it is also widely used for both qualitative and quantitative analysis. Recent developments in plasma sources have expanded the number of applications. The AED has shown to be useful

also for screening chloro- and bromocompounds in complex mixtures The detector response is almost independent of the structure of the molecule in which the studied compound is present [2,32,33]. The Cl/C selectivity is 3500 and the detection limit was 25 pg/s in a study of chlorinated pesticides [34]. The detection limit can be improved to about one pg/s [35].

At room temperature, essentially all of the atoms of a sample of matter are in the ground state. The heat of a flame, an electric arc, a spark, or plasma excitation can bring electrons to a higher orbital. When the electrons return to their ground state, emission of photons or radiation will appear [2,32,36]. An atomic emission detector consists of three major components; the sampling device and ion source, the spectrometer and the detector, and the read-out source (Figure 6) [32,36]. The sampling device can for example be a peristaltic pump or a GC. The source, where the electrons are excited, is selected in accordance with the requirements of the desired analysis. Plasma, arc, and spark sources are more energetic atomisation sources compared to the heat of a flame, and offer benefits such as better spectra for most elements and permit lower detection limit for compounds that are highly resistant to decomposition. Arc excitation is more sensitive while spark sources are more stable for analysis of solid samples. Plasma sources are used for solution and for gas samples [32].

A plasma is a high-temperature electrical conducting gas mixture containing cations and electrons in relative high concentrations [32,37]. In a DC-arc plasma jet a DC-arc discharge is formed between electrodes in flowing-gas streams of argon or helium. The inductively coupled plasma (ICP) discharge is the most effective emission spectroscopic source today. The discharge is caused by the effect of a radio-frequency field, without electrodes, on flowing argon. The radio-frequency signal creates a magnetically induced eddy current, in the flowing argon. Ionisation of the flowing gas is initiated by a spark [32]. Microwave plasma discharge is produced with or without electrodes by microwave fields in stationary or flowing gas streams in a microwave cavity [32,33]. An AED system with microwave-induced helium plasma has been used for determination of organohalogens [33,38-40]. The eluent from the GC is introduced into microwave-energised helium plasma, with a high temperature, that is coupled to a diode-array optical emission spectrometer. All the elements in the sample are atomised to excite their characteristic atomic spectra. A spectrometer is capable of detecting emitted radiation from 170-780 nm [6,33]. The response to carbon, chlorine, bromine and iodine is measured at 496, 479, 478, and 206 nm, respectively [39]. Detectors used in emission instrument systems are based on photographic emulsions and photoelectric transducers.

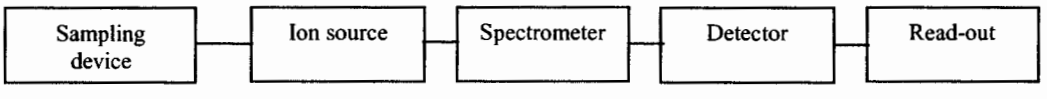

Figure 6. Block diagram of the atomic emission system.

2.5 Mass spectrometry (MS)

Mass spectrometry is one of the most applicable of all chemical analytical tools. It provides both quantitative information and information about the structural composition of inorganic and organic molecules. The mass spectrometer creates charged particles that are separated

according to their mass/charge ratio and the relative abundance of ionised species of each mass is measured. This generates a mass spectrum indicating the relative numbers of different ions. The major advantage of mass spectrometry is the very high sensitivity for many compounds compared to other analytical techniques and its ability to identifying unknown compounds [41-43].

Creating the necessary gaseous ion fragments from the sample in the mass spectrometer can be done in different ways. Electron ionisation (EI) is the most commonly used ionisation technique. EI is based on that electrons that have an energy of 70 eV bombard the sample molecules. Some of the molecules are positively ionised by loss of electrons. These molecular ions normally undergoes fragmentation. The detection of the resulting ionised fragments generates a mass spectrum. EI mass spectra usually contain information about the structure of a compound. To ascertain the molecular weight of a compound softer ionisation techniques such as chemical ionisation (CI) can be used. This technique is based on that a reagent gas is ionised by EI ionisation in the source, and the reagent ion react with the sample molecule and molecular ion or a molecular adduct ion is formed [41-43]. Chemical ionisation can be performed in the positive (PICI) or the negative (NICI) mode. In NICI negative ions are formed by resonance capture of a thermalized electron, dissociative capture of a low energy electron, and ion-molecular reactions that occur between ions in the ion source and neutrals [44]. The formation of ions by resonance capture of an electron is strongly dependent on the electron affinity of the analyte. The dissociative capture ion forming reactions is strongly dependent upon the energy spectrum of the electrons in the ion source, which depends on the energy of the primary ion beam, the nature of the gases in the ion source, the pressure, and the electric field in the source [44]. The selectivity of NICIMS is dependent upon the construction of the ion source and mass analyser, and the reagent gas mixture. Using a reagent gas mixture of hydrocarbon with methylene chloride and/or oxygen have found to be powerful for analysis of alkylating agents such as aliphatic polyhalides, phosphate esters, and carbamates. The selectivity of the gas mixture is high, neutral lipids are virtually transparent [44]. NICI have shown to be useful for the determination of organochlorine pesticides [44-45]. In general even-electron negative ions contain less energy than the positive ions and thus produce fewer fragments. Therefore, a collision-induced dissociation tandem mass spectrometry (CID MS/MS) is suitable for analysing negative fragments [43].

Negative ion mass spectra of organohalogen compounds may show peaks at m/z 35 and 37, m/z 79 and 81, and m/z 127 and 129, ions which correspond to chlorine, bromine and iodine, respectively, and their isotopes. The ratio between the ions at m/z 35 and 37 for chlorine is 10:3, which is the mass ratio of the naturally occurring chlorine isotopes [45]. The ratio between the naturally occurring bromine isotope ions at m/z 79 and 81 is 1:1 [46].

The relatively high natural abundance of the heavier isotopes at m/z 37, 81 and 129 results in clusters which are easy to recognise in fragment ions and molecular adduct ions of both positive and negative charge. The number of chlorine/bromine atoms in the molecule can be determined from the isotopic clusters in the mass spectrum [46-48]. By searching in a full-scan mass spectrum, by computer analysis, for the certain halogen clusters patterns, in which ions appear with certain mass ratios, mass spectrometry can be used as halogen selective detector. This technique have been used for analysing compounds containing one to ten chlorine atoms [48].

Milder ionisation techniques, such as CI, used for analysis of chlorine containing compounds is particularly suited for detecting chlorine adducts [44]. NICI is suggested to be a

suitable technique for analysis of organochlorine pesticides because this technique generates ions with strong intensity at m/z 35 and 70 [45]. NICI is also used for analysis of polybrominated diphenyl ethers (brominated flame retardants) at the Institute for Applied Environmental Research (ITM), Stockholm University. They take advantage of the fact that some aromatic organobromine compounds generate mass spectra that are totally dominating by the bromide ions m/z 79 and 81, caused by electron capture ionisation. The possibility for the bromine radical to capture an electron is higher than the possibility for the molecule to capture it [49].

Another method useful for detecting target compounds with known spectral characteristics, with a maximum sensitivity, is selected ion monitoring (SIM). In SIM analysis characteristic ions from target compound is selected. A full-scan mass spectrum is obtained by repeatedly scanning over a specified mass range every time unit [41]. A SIM mass spectrum is obtained by repeatedly scanning over a few specified selected ions every time unit. This results in longer dwell-time at each m/z value and a higher response for the selected ions. For analysis of halogen-containing compounds, typical fragments such as m/z 35 and 37 for chlorine isotopes, and m/z 79 and 81 for bromine isotopes, can be selected. For analysis of chlorine or bromine containing compounds by CI, also characteristic adduct ions can be selected. SIM can also be used for quantification [43].

Isotope dilution mass spectrometry (IDMS) in negative ionisation mode can be used for analysis of halogens, except for fluorine [50]. It can be used for geochronology and in nuclear technology analysis [43]. It has been used in determination of halogens in rock [51]. This technique is also a method for certification of standard reference material [43,50]. The theory of this method is based on the relative abundance of isotopes and their probability of occurrence. An exact known quantity of labelled internal standard is added, the sample and the added isotopes is equilibrated. When the labelled internal standard is added, the peak corresponding to the characteristic peak is moved to a different position in the spectrum, according to the number and nature of the atoms that were used in the labelling. The ratio between these two signal intensities is used to measure their relative proportion and the exact quality of the substance can be determined [48,50]

ICP-MS is also a technique that can be used for analysis of halogen. This technique has been used for analysis of halogen species of humic substances [52] and for determination of halogens in geological and biological samples [53].

MS is a very important detector also in liquid chromatography (HPLC) and capillary electrophoresis (CE).

2.6 Determination of chlorinated fatty acids

Chlorinated fatty acid methyl esters (FAMEs) have been successfully determined by GC using the Hall electrolytic conductivity detector (Hall detector, or ELCD) [18-20]. The GC/ELCD method has a detection limit of 250 pg of methyl dichlorooctadecanoate [18]. The ECD is not suitable for the detection of chlorinated fatty acid methyl esters, because the detection limit of methyl dichlorooctadecanoate by ECD is about 500 pg and negative peaks are occasionally encountered in the ECD chromatograms of FAMEs [18], making interpretation of chromatograms difficult. Unchlorinated FAMEs are less electron capturing and are therefore detected as negative peaks, presumably resulting from a high background created in the ECD [10]. The XSD has also been shown to be a suitable detector in the

analysis of chlorinated fatty acids. It can be compared to the ELCD and the detection limit is 0.2 ng of methyl dichlorooctadecanoate [27].

AED have also been used in studies of halogenated fatty acids [35,54]. The benefit of this detector is that it can confirm the content of chlorine, bromine and iodine in the molecules.

MS have been an important technique for the identification of chlorinated fatty acids [24,55]. Different ionisation techniques, such as EI, CI and fast atom bombardment (FAB) have been used. CI and FAB are useful tools for obtaining molecular weight information. FAB has been used in the identification of chlorohydroxy fatty acids [56]. PICI has been used in identification chlorooctadecanoic acids [57-60]. NICI has also been used in identification of chlorooctadecanoic acids, but instead of analysing the fatty acids as methyl esters (normally used for GC analysis of fatty acids), they were analysed as pentafluorobenzyl esters [57]. EI have been used for obtaining information about the structure of chlorinated fatty acid methyl esters, but the molecular ion is essentially missing in the EI mass spectrum, because of the loss of chlorine atoms. The most abundant fragments of dichlorinated fatty acid methyl esters are dechlorinated, but monochlorinated fragments, with very low abundance, could also be observed [57].

High performance liquid chromatography with MS (HPLC/MS) using plasma spray ionisation (PSI) have been used to analyse chlorinated triacylglycerols [57] and HPLC/MS using electrospray ionisation (ESI) have been used to analyse chlorinated fatty acids in organs of rat [61]. By monitoring certain isotope ions different chlorinated fatty acids were identified.

Most MS techniques used for the detection of chlorinated fatty have been SIM, in the CI-mode [58-61] and in the EI-mode [58,59], where only known ions were monitored, and unknown chlorinated fatty acids remained undetected.

3. Quantitative determination of the total halogen content in organic material

The total content of organohalogens can be measured in various ways. Organohalogens with high molecular weights (> 1000 g/mol) in water and soil samples can be measured by adsorbing them onto activated carbon particles, adsorbable organically bound halogen; (AOX) [62]. The determination of AOX is based on the measurement of hydrogen halides formed during an incineration of the organic matter on the adsorbed carbon particles. The combustion occurs in an oxygen atmosphere at high temperatures, 800-1000 °C [62-64]. The formed hydrogen halides are absorbed in a polar reagent. The hydrogen halides dissociate in the polar reagent and the free halides can be determined by a spectrofotometric, coulometric, potentiometric, or by a volumetric method. AOX are commonly used for water and soil analyses [62].

The fat-soluble part of organohalogens with molecular weights in the range of approximately 100-3000 g/mol is measured by extraction with an organic solvent, extractable organically bound halogen (EOX) [62]. The determination of EOX can also be made by measuring hydrogen halide formed during incineration [62]. It can also be determined without combustion of the sample, by neutron activation analysis (NAA, see Section 3.1) or X-ray fluorescence [62,65,66; Section 3.2]. EOX usually stands for a few percent of AOX in water and sediments.

Total organohalogens (TOX) is a sum parameter and it is determined by combustion of the whole collect sample. The formed hydrogen halides can be measured by the same way as for AOX and EOX [63]. Hydrogen halides are formed from both organically bound halogens and inorganic halides during combustion. For determining TOX the inorganic halides are removed by washing the sample with acidic nitric solution. If the inorganic halides are not removed the total amount of halogens TX is determined.

The hydrogen halides, formed during combustion can be measured by a colourimeter, microcoulometric titration, and potentiometric or by a volumetric method. The colourimetric method is based on that the hydrogen halides are absorbed in reagent and the formed product is measured at an appropriate wavelength in a spectrophotometer [67]. Microcoulometric titration is based on that a constant current is passed through a cell and the substances to be determined reacts with a reagent that can be generated electrolytically [68]. The current changes rapidly when ions from the reagent appear in the solution that happens at the end point, when all substance is consumed. The time taken for the reaction to go to completion is used for measuring the amount of the substance that was present from the beginning. For determination of chloride a chloride meter, a constant current coulometry titration cell, is used [68]. This instrument generates silver ions electrolytically from a silver anode. The silver ions are removed from the solution as silver chloride. When all chloride ions are consumed the silver ions will appear in the solution and the current will rise. Potentiometric titration is based on that an ionselective electrode (ISE) detects rapid changes in activity that occurs at the equivalence point of the titration [68,69]. The ISE detector can be a solid state electrode, which consist of a solid insoluble crystalline material with specificity for a particular ion. The membrane permits movement of ions in the crystal, and the ions which disrupt the structure in the crystal the least are the most mobile. These usually have the smallest charge and diameter. Only ions that are similar to the internal mobile ions can gain access to the membrane from the outside. Equilibrium is established between the mobile ions in the crystal and similar ions in the solution that is measured. The electrode can not be used for measuring concentration of Cl^- below 10^{-5} M, because the chloride concentration in the equilibrium between AgCl and Cl^- will be in the region of 10^{-5} M. Performing the measurement at a lower temperature, 5 °C, however, the detection limit can be lowered to 10^{-6} M [69]. The solubility of AgCl decreases when the temperature is decreased. For measuring chloride, bromide and iodide a membrane containing $AgCl/Ag_2S$, $AgBr/Ag_2S$ and AgI/Ag_2S, respectively are used [68]. A surface reaction between the sample halide ions and the membrane silver ion alter the activity of the structure in the crystal, resulting in a potential difference across the membrane. The time taken for the cell potential to reach 1 mV from the final equilibrium is the response time.

A liquid-membrane electrode consisting of an ion-selective materiel dissolved in a nonpolar organic solvent can also be used to determine chloride [68].

A volumetric method used for determine the chlorine content in a solution is based on that chloride ion is reacted with silver ions. Iodine and starch indicator are added to the sample, which is finally titrated with potassium iodide until end point. The consumed volume of potassium iodide solution is determined [70]. Detection limits of 2 ppm Cl^- in lipids have been obtain by this method [70].

3.1 Neutron activation analysis (NAA)

NAA is an element selective method and it is very useful for determine the total content of halogens in a sample. NAA can be used for quantitative analysis of most elements.
The sample is irradiated with neutrons to create radionuclides. The energy from of the spectral peaks is used for identify the elements and the intensity of radiation is used for the quantification. Gamma-ray spectrometry is commonly used for measuring the induced radioactivity in the sample. The probability of a nuclear reaction is dependent on the nature of the target nuclide and the energy of the bombarding neutron. The production of radioactive atoms, N*, is equal to the difference between the rate of formation and the rate of decay. The neutrons most commonly used in NAA are thermal-energy neutrons (0,04 eV) produced by nuclear-fission reactors [71-72]. The sensitivity for chlorine is 0.01 µg, irradiated for 1 h or less in a thermal flux of 1.8×10^{12} neutrons/cm^2/s [73].

3.2 X-ray fluorescence

This is the most widely used x-ray technique for quantitative analysis, but it can also be used for qualitative analysis [74-75]. X-rays are generated by bombarding the sample with high-energy particles such as electrons (10-100 keV), protons (100 keV-5 MeV) or with X-ray photons. When an atom is bombarded an electron is ejected from one of the inner shells of the atom. An electron from a higher energy shell fills this vacancy. A new vacancy is created and this is filled by an electron from a higher shell, each new vacancy is filled until the excited atom returns to its ground state. Each electron transition results in the emission of a characteristic x-ray spectral line. Only certain electronic transitions are possible. The energies or wavelengths of the X-ray spectral lines are used to identify the elements and the intensity of radiation is used for the quantification. In X-ray fluorescence the remitted X-radiation, called the characteristic radiation from the analyte elements, is measured. Bombardment by protons or other particles that also can lead to the emission of fluorescent X-rays is called particle induced X-ray emission, PIXE and it is very useful for very thin samples because protons do not penetrate deeply into matter [75].

4. Conclusions

- The ECD is the most sensitive detector for the analysis of polyhalogenated aromatic compounds, but it is not suitable for the determination of chlorinated fatty acids.
- ELCD and XSD are very sensitive detectors, suitable for the analysis of chlorinated fatty acids and for analysis of organohalogens in complex samples. The XSD is easier to maintain and safer to operate compared to the ELCD.
- AED can confirm the content of individual halogens and the sensitivity, concerning chlorine, can be compared to that of the ELCD and XSD.
- MS is very useful for identification of unknown compounds and it has very low detection limits for certain compounds. It can be used as a halogen specific detector.
- Colourimeter, potentiometric titration and volumetric method are useful tools for determining the total content of halogen as AOX and EOX, in a sample.
- NAA and X-fluorescence are also useful techniques for AOX and EOX determination, and the samples do not have to be combusted before analysis.

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