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Chromatographic, Polarographic and Ion-Selective Electrodes Methods for Chemical Analysis of Groundwater Samples in Hydrogeological Studies

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1. Introduction

The chemical and physical characterization of groundwater and surface water is very important to understand the hydrological and geological dynamic that enriched the water in ions and organic compounds. During the water infiltration and movement into the rocks, the water is subject to numerous interactions between the aqueous and the solid phases through physical, chemical and microbial processes such as dissolution, precipitation, oxidation, reduction, complexation, ad-and desorption, filtration, gas exchange, evaporation, biological metabolism, isotopic redistribution and anthropogenic influences [1]. Groundwater in solution may have a high quantity of inorganic and organic compounds. Its contents are the combined result of the composition of surface water when entering the unsaturated zone of the soil and reactions with minerals in the rock that may modify the water composition. As a result, groundwater contains dissolved solids and gases (CO_2, O_2, H_2S) according to its initial composition, type of the rock, the partial pressure of the gas phase, pH and oxidation potential of the solution. The major ions that can be found in water are chloride, sulphate, bicarbonate, carbonate, sodium, potassium, calcium and magnesium against many others in reduced concentrations (<10 mgL^{-1}) such as iron, manganese, fluoride, nitrate, nitrite, cadmium, lead, chromium, strontium, arsenic and boron. Apart from these natural processes, the water also suffers from contamination by human activities. Solutes, such as heavy metals and organic solvents, are chemically introduced in the water systems mostly in the unsaturated zone. When water is in contact with pore gases contaminants there may be transference between the liquid and the gas states. This is an important way of volatile compounds to migrate from the subsurface. After dissolved in

water, these compounds can persist for a long time as a separate liquid phase which has prejudicial effects for the human life and the ecosystems. One of the aims of the water analysis is to obtain better knowledge concerning the water quality, residence times in the aquifer, age, recharge areas, flow paths, and also a potential or prohibitive use due to human pollution problems.

A high number of analytical analysis with several traditional techniques are no longer adequate for this purpose and the development of more green analytical techniques that can measure different ions and organic compounds with the same technique are more suitable. Over the last few decades there has been an increase growth of equipments capable of measuring very low concentrations and also analytical procedures that could concentrate the compounds and increase the signal detected, allowed the hydrogeologist to get more information about the chemical characterization of the groundwater. Equipments, such as chromatography, sensors and microdevices (e.g. microelectrodes), has undergone extraordinary developments. Most of these new analytical instruments have a lower limit to the range in which the results can be quantified and below that range where a compound can be detected but not quantified or as not detected. The quantification limits can be helpful tool for the decision to select the analytical method and equipment for the determination of a specific parameter for the hydrogeological study.

The chromatographic methods applied for the determination ions and organic compounds can be more appropriated in some cases, however the electrochemical techniques such as polarography and voltametry and the ion-selective methods can also be an alternative. The chromatographic methods can be applied to measure ion concentrations such as Cl^-, SO_4^{2-}, NO_3^-, F^-, PO_4^{3-}, Ca^{2+}, Na^+, K^+ and Mg^{2+} (ion chromatography) and to measure organic compounds using liquid chromatography (HPLC) with UV detectors (e.g. photodiode array detector (DAD)) and/or coupled with mass spectrometry such as water soluble pesticides and pharmaceuticals or gas chromatography (GC) to measure volatile organic compounds such as polycyclic musk fragrances. The polarographic and voltametric electrochemical techniques can be an option to measure the concentration of organic compounds (e.g pharmaceuticals) as well as in the same way the ion-selective electrodes for some specific ions in water matrices. Na^+, K^+ , Ca^{2+} , Mg^{2+} can be measured by ion-selective electrodes and in some cases this techniques is more advantageous than the chromatographic.

Pharmaceutical active compounds (PhAC), persistent personal care products (PPCPs) and pesticides are commonly occurring as micropollutants with a potentially significant environmental impact. The growing use of pharmaceuticals is becoming a new environmental problem, as both via human and animal urinary or fecal excretion and pharmaceutical manufacturing discharges, increasing concentrations of pharmaceuticals reach sewage treatment plants (STPs). Due to this extensive use, high concentrations of drugs are found in sewage, depending on their half-lives and metabolism. STP is therefore often ineffective in removing these substances, so that varying concentrations of them can be found in surface and groundwater.

The impact in the environment and public health arises not only from wastewater effluents discharged in aquatic media [2, 3], but also from sludge application in agriculture, since they can desorbs and contaminate the groundwater [4]. PhAC and PPCPs are becoming increasingly recognised as important micropollutants to be monitored in different matrices, groundwater, surface water drinking water and wastewater. The aquifer recharge with treated wastewater can represent an important point source of ions and organic compounds to natural aquatic systems. Due to the fact that analytical approaches normally used to quantify the abundance of these compounds are labour intensive and require various specific procedures, more simplified analytical methods need to be employed for the quantification of pharmaceutically active compounds (PhACs) and polycyclic musks in liquid and solid samples. Many studies have been carried out in different countries and geographical locations [5], the occurrence of PhAC and PPCPs in wastewater and environmental samples is highly dependent on the local diseases, treatment habits and market profiles, thus, the pollution profile and can vary significantly between different countries [5]. PhACs include many families such as antidepressives, anticonvulsants, non-steroidal anti-inflammatory drugs (NSAID), steroidal anti-inflammatory drug (SAID), drugs for asthma and allergic diseases, antihypertensives, β-blockers, lipid regulators, antibiotics, and estrogens [6]. Due to the high diversity of compounds displaying a wide variance of chemical structures, many previous studies have elected to perform a combination of analytical methods targeting specific families of compounds [7, 8]. While this strategy can be advantageous with respect to the analysis of each target group, the time-consuming and labour-intensive nature of the analytical procedures makes a high number of different methodologies undesirable when the goal of the study is to make an overall assessment of PPCPs present in environmental samples. Most of the PhACs can be analysed through High Performance Liquid Chromatography coupled with mass spectrometry HPLC-DAD-MS with the MS working with electro spray ionization in positive (ESI+) or negative (ESI-) mode with the same set of conditions after solid-phase extraction (SPE) using different adsorbent materials according to the neutral or acidic proprieties of the compounds. The musks are non-polar and volatile organic compounds and can be analysed by GC-MS after solid-phase microextraction (SPME) with different extraction fibres [9].

The polarographic and voltametric methods have been widely used for the analysis of organic compounds in samples of natural origin. However, the voltametric methods have not been widely explored for the analysis of many PhAC. The voltametric technique most used for PhAC is the direct current polarography (DCP) and differential pulse polarography (DPP) methods for the analysis of PhAC in water samples [10, 11]. The use of glassy carbon electrode has been suggested for linear sweep and cyclic voltammetric studies for some PhAC such as nifedipine [11]. Adsorptiv cathodic stripping polarographic determination of trace PhAC has been reported with high sensitivity. The detection limit obtained by these methods can be found lower or comparable to other known methods as well as the linearity range obtained. Precision of the method developed implies very low values of relative mean deviation, standard deviation and coefficient of variation. Recovery experiments showed that these methods can be used for quantitative analysis and errors of ±0.2% can be expected. The studies have shown that the polarographic and voltametric methods are

simple, reproducible and accurate and can be used to determine many PhAC in the groundwater. Despite the sophisticated instrumentation of analytical tools, complete noninvasive measurements are still not possible in most cases. More often, one or more pretreatment steps are necessary; whose goal is enrichment, clean-up, and signal enhancement during a process of sample preparation [12].

2. Groundwater monitoring plan: Sampling procedure and frequency

The groundwater monitoring plan is determined according to the needs to implement a water quality monitoring program as part of their Source Water Protection Plan. The decision is based, in part, on the high susceptibility of the aquifer and past detections of groundwater contamination and also on the characteristics of the aquifer (confined or unconfined and the soil characteristics (e.g. sand and gravel)). The thin veneer of soil at the ground surface is not a significant confining layer and cannot serve as a barrier to contaminant movement between the ground surface and the aquifer. In addition to the identified aquifer vulnerability, the groundwater contamination by volatile organic compounds (VOC's) can be measured in the source water supply from an unconfirmed spill. Although the detections will show the maximum contaminate levels, and their presence demonstrates the risk of contamination is real. The design of sampling and analysis plan include the top management priorities for developing control strategies in the source water protection plan, such as agricultural chemicals and chemicals associated with auto repair/body shops. Other concerns include underground storage tanks, potential spills along transportation routes, and surface water sources and source of water assessment included in the list of priority contaminants (Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy). In the groundwater monitoring plan, some groups of the compounds that can be included are:

1. Volatile Organic Compounds (VOCs); a group of potential pollutants that includes many solvents, musk fragrances which are the organic chemical constituents that are most commonly found in groundwater effected by domestic, commercial or industrial operations.
2. Synthetic Organic Compounds (SOCs); particularly those constituents which include the most common herbicides and pesticides and pharmaceutical active compounds found in ground water from human activities such as agriculture and domestic activities.
3. Inorganic Compounds: this will include those chemicals found in ground water most associated with agricultural land use or deicing of roadways. Nitrate and chlorine are the primary contaminants of concern.

Prior to purging and sampling a monitoring well during each monitoring event, the depth to water in the well is measured from a reference point at the top of well casing using an electric ullage tape. Using this measurement to calculate the volume of water in the well, three well volumes will be extracted by bailing. After purging, a sample will be collected for field measurement of the selected indicator parameters; pH, specific conductance, redox

potential and water temperature. Results of these measurements will be recorded on the appropriate field sampling form. Next, water samples will be obtained using the dedicated bailer for the required VOC, SOC and inorganic parameters. Upon collection, these samples will be properly labeled and stored in an iced cooler for shipment to the contract laboratory. Finally, one additional sample will be collected from the well and a second field measurement of the indicator parameters will be made. A very large volume of water sample should be collected (e.g 5 L) to analyse organic compounds and it should be collected to plastic (PET) bottles and preserved at 4°C in an isothermal bag during transportation to the laboratory. The purpose of purging – removing water continuously from the well – is to ensure that the water sampled is fresh groundwater and not stagnant casing water, which may differ significantly in quality. The use of bottom loading PVC bailers as the type of sampling equipment to purge their monitoring wells and draw samples is commonly used in the groundwater sampling. This technique is the least expensive to implement and works well with shallow monitoring wells. The purchase of a dedicated bailer for each monitoring well, will eliminating the need for decontamination between sampling events. To insure the removal of casing water and for consistency between sampling events, the technician collecting the water samples will remove three well volumes prior to sample collection. This is a conservative and accepted protocol for groundwater sampling in prolific aquifers. This suggests representative water samples from the monitoring well could be obtained just after one well volume is removed.

The sampling frequency can be defined according to the previous experience of monitoring a specific aquifer. Assuming the results for the first year groundwater monitoring, and near the spring, confirms water quality results in a well within anticipated and acceptable levels, subsequent years monitoring events can be defined as semi-annually. Preferably, sampling of monitoring wells will occur in the spring and fall of each year to better define the water quality of the aquifer at periods of high and low water levels. The collected groundwater samples will be analyzed for the same set of constituents measured during the first year of monitoring.

3. Groundwater sampling and field analysis

In the sampling process of surface or groundwater, it is important to define the purpose of the collecting program, number of samples to be tested, which physical parameters and chemical constituents will be analyzed, as well as where the samples will be collected. It is difficult to obtain samples that accurately reflect the composition of groundwater in the aquifer conditions because the pressure and oxygen concentration change considerably during the sampling process. As a result, the temperature, Eh and pH of the water can change too. Atmospheric oxygen oxidizes components, which are commonly found in anoxic groundwater. Also the degassing of CO_2 will increase the pH causing carbonate precipitation ($CaCO_3$) and concomitant loss of alkalinity.

Most of this type of problems can be overcome by carefully sampling and measuring some parameters in the field such as pH, SC, Eh, and temperature, and a pressurized sample for alkalinity determination. Alkalinity can be rapidly quantified in field by the titration

method using a burette or a field alkalinity kit. In some cases, a down-hole determination of temperature, pH, specific conductance, and redox may be needed in conjunction with down-hole sample collection. Hence, any pressure-dependent reaction that will affect the water will result in different values for samples collected in situ and at the well head.

In addition, conservation of samples is typically done to ensure that they retain their physical and chemical characteristics. In the field, it is important to collect samples in clean sample bottles (500 mL high-density polyethylene bottles with polypropylene screw caps), preserve them by cooling, freezing, or acidification immediately after collecting and then storing in a chilled vacuum container in a dark place before transportation to a laboratory for analysis. It is good field practice to clean the sampling device prior to use providing that no residue remains. For that, bottles and devices should be rinsed with a sample of water being sampled to prevent any contamination. After use, acetone and distilled water can be used to rinse thoroughly.

Frequently, nitric acid is added for metals preservation, since it prevents adsorption or precipitation of cations. At the same time, the acidification limits bacterial growth and, as an oxidant, converts ferrous iron to ferric iron and precipitation as $FeOOH$. Before being analyzed, the samples can usually be preserved for all inorganic compounds during 28 days, at 4°C.

4. Analytical methods for inorganic compounds

The analytical measurement can be either qualitative or quantitative and a very large variety of instruments and techniques can be used for different types of analysis, depending on the cost, information, accuracy, and precision acquired. In most laboratories today, ion chromatography (IC) has replaced older methods of ion analysis because it offers superior sensitivity, accuracy, and dynamic range. Also, it is environment-friendly, extremely fast and versatile. Therefore, this method is particularly advantageous in the analysis of low concentrations as such in high-purity water. IC can also be used for detection and quantification of different ion species in a wide variety of water samples. However, older techniques that do not provide such high results are still in use.

With the purpose of proceeding to the efficient chemical characterization of water and to turn possible the comparison among results, laboratories, hidrogeologists and others have developed sampling protocols. For environmental analysis and determination of inorganic ions in drinking water the EPA (United States Environmental Protection Agency) publishes laboratory analytical methods (most of these methods - 120.1, 130.1, 150.2, 200.7, 206.5, 218.6, 300.1, 365.4, etc. - are published as regulations in the Code of Federal Regulations (CFR) at title *Part 136* and *40 Parts 401-503*), and specifies the type of sample that is needed, the type of sampling container to be used, the method by which the sample container is cleaned and prepared, whether or not the sample is filtered, the type of preservative that is to be added to the sample in the field, and the maximum time that the sample can be held prior to analysis in the laboratory [13].

4.1. Ion chromatographic method

Chromatography is a wide range of physic-chemical separation processes in which the components to be separated are distributed between a stationary and a mobile phase. The name for each of the various types of chromatography depends on the state of aggregation of these two phases. The introduction of high pressure in the separation system and the hardware with software for calculation of the peaks, gas and liquid chromatography have developed into one of the most comprehensive and important methods of modern instrumental analysis. Many ions (anions or cations) in the test sample are separated and quantified quickly and with high precision by an ion chromatographic system containing a guard column, a separator column, with or without suppressor device, and are measured using a conductivity detector. In the technique with chemical suppression, the background conductivity is suppressed both chemically and electronically. In contrast, the direct chromatographic technique employs eluents with salts of organic acids in low concentration on ion exchangers of very low capacity to achieve relatively low background conductivity, which can be suppressed directly by electronic means. This method is applicable to the determination of bromide, chloride, fluoride, nitrate-N, nitrite-N, orthophosphate, sulphate, calcium, potassium, sodium, magnesium and ammonium, in water.

4.2. Potentiometric titration method

The water analysis is not completely done if the carbonate and bicarbonate ions are not determined. Using the alkalinity concept, which is the capability of water to neutralize acids when the presence of calcium and magnesium carbonate ions in it is very high, it is possible to quantify the carbonate and bicarbonate ions. The total alkalinity is the contribution due to all bicarbonates, carbonates, and hydroxides present in the water; and it can be determined by potentiometric titration of an unfiltered sample (100 mL) with a standard solution of strong acid (HCl 0.1 molL^{-1}) and phenolphthalein (from pH 8 to pH 10) or methyl orange (from pH 3.1 to pH 4.4) as indicator. The result is expressed as meqL^{-1} of HCO$_3^-$ and CO$_3^-$, i.e., the volume equivalent of acid added to the water until it changes colour. This method is applicable to all types of water in the range 0.5 - 500 mgL^{-1} alkalinity as CaCO$_3$. The upper range can be extended by dilution of the original sample [14].

4.3. Ion-selective electrode method

Ion-selective electrode methods are regularly used to determine many parameters of water in field and laboratory due to their versatile sensors. They are applicable in many situations for the determination of pH, electrical conductivity (EC), hardness, calcium, sodium, potassium, magnesium and others. The electrodes coupled to a multi-parameter analyzer are designed for the detection and quantify of physical and chemical parameters with calibration for any range of values. For example the potassium ion selective electrode consists of an inert fluorocarbon body with a detachable PVC membrane unit, on the end of which is glued the ion selective membrane. The electrical potential of an ion selective electrode is a function of the activity of certain ions in an aqueous solution. This potential

can only be measured against a reference electrode, such as a saturated calomel electrode, placed in the same solution. The electrode should be used in the pH range 4-9.

The problem associated with results obtained with ion-selective methods is the uncertainty caused by instable equilibrium depending on the ionic-strength of the solution. When the electrode is placed in a water sample, the response time may go from 1 to 10 minutes or even much more, and the equilibrium point varies in conformity to the type of electrode and the parameter in measure. In the daily life of a laboratory, the main difficulty is associated with inaccurate results related to the sodium electrode because of interfering ions in the sample. Also, the response time increases considerably because of that. Another issue is the short lifetime of the membrane which, in good performance, does not extend to more than a year if it works every day. In order to produce acceptable measurements, it is highly important that the electrode chosen is in conformity with the sample characteristics.

5. Analytical methods for organic compounds

5.1. Sample preparation for chromatographic and electrochemical methods

The selection and application of the most appropriated analytical technique is related with the proprieties of the organic compound to be identified and quantified in the different samples. Among the proprieties, some of the most important are the acid-base characteristic of the compound, the polarity (polar or non-polar compounds) and the adsorption capacity measured by the octanol-water partition coefficient ($logK_{ow}$) reported for many of the organic compounds. Values of $logK_{ow}$ < 2.5 correspond to low adsorption potential, 2.5 > $logK_{ow}$ > 4.5, to media adsorption potential and $logK_{ow}$ > 4.5 to high adsorption potential [15]. The adsorption not only depends of the hydrophobicity but also from the electrostatic forces and pKa of the compound [16]. There is a linear relationship between the $logK_{ow}$ and the pKa of the most of the organic compounds [17]. Organic compound with high adsorption potential are mostly present adsorbed in the solid matrices and need a previous extraction procedure to a liquid phase before the analysis by chromatographic and electrochemical methods. The polar compounds present in water samples can be analysed taking to account the acidic or basic proprieties and most adequate adsorption media for clean-up and pre-concentration technique. The low concentration (ngL^{-1} or pgL^{-1}) mostly frequent of the organic compounds in water samples justifies the previous concentration step by the use of solid-phase extraction (SPE) methods. The non-polar compounds present also in low concentration in water samples need also a previous clean-up and pre-concentration technique by the use of solid-phase micro extraction (SPME) before analysis by chromatographic methods.

5.1.1. Solid phase extraction (SPE)

Solid-phase extraction (SPE) is the most used clean-up technique for pre-concentration of water (surface, groundwater) and wastewater samples prior to analysis of the organic compounds. The samples should be previously filtered by 0.45 µm glass fibre membranes (GF 6, <1 µm, diameter 47mm from Wathman, England) and stored at -20°C before analysis

by SPE. Different sorbents can be used for clean-up the samples: Oasis HLB (hydrophilic lipophilic balance) cartridges and reverse phase Sep-Pak C18 cartridges, which assures good recovery of compounds in a wide range of polarities. For most of the organic compounds, Oasis HLB and reverse phase (RP C18) as SPE cartridges are the most used in literature due to the polar nature of the compounds and the acidic and neutral characteristics e.g PhAC [18,19]. The selection of the SPE media is directly related with the proprieties of the compound (e.g. acidic or neutral characteristics. RP C18 is most appropriate for the neutral and Oasis HLB general it can be more appropriate for the acidic compounds.

SPE was used for the extraction and clean-up of the liquid wastewater samples. OASIS HLB cartridges (60 mg, 30 μm, Waters, Eschborn, Germany) is used for the acidic organic compounds (e.g acidic PhACs) and RP-C$_{18ec}$ cartridges (500 mg, 50 μm, Waters, Milfort, U.S.) is used for the neutral organic compounds (e.g. neutral PhACs). Each cartridge was previously conditioned with 1 mL methanol followed by 1 mL of Milli-Q water, then dried in a N$_2$-stream. For the acidic PhACs, 200 mL of filtered water and 10 µl of an internal standard were passed through the OASIS HLB cartridges at pH 2-3. For the neutral, 500 mL of filtered water and 50 µl of internal standard (e.g. meclofenamic acid) is passed through the RP-C$_{18ec}$ cartridges at pH (7-7.5). Samples passed through the SPE cartridges at a flow rate of 20 mL min^{-1} and vacuum pressure of -5 psi, then the cartridges is eluted four times with 1 mL of methanol. The methanol extracts are evaporated to 1 mL by a gentle nitrogen stream. Then, 50 µL of extract are injected into the LC-MS.

For the extraction of the organic compounds (e.g PhAC) adsorbed to the soil, sludges and solid samples, the procedure consists of ultrasonic solvent extraction (USE) using solvents (e.g. methanol/acetone) or pressurized liquid extraction (PLE) using 100% methanol. After this extraction step, non-selective, an additional clean-up can be performed with SPE [19]. The method most commonly used for extraction from solid phase is the ultrasonic solvent extraction (USE) prior to SPE. In this method, the solid sample is centrifuged for 5 min at 10 000 rpm. 2 g of the centrifuged solid sample is for extraction of the organic compounds adsorbed. The concentrated sample is mixed with 4 mL methanol in an ultrasonic bath for 5 min. The slurry is then centrifuged for 1 min at 10 000 rpm. The supernatant is collected in a separate vial and 2 mL of methanol is again added to the solid sample. Centrifugation and supernatant collection is then repeated. To ensure the extraction is complete, 2 mL of acetone is then added to the solid sample and the same procedure (i.e. ultrasonic bath, centrifugation, supernatant collection) is repeated. Then, the 4 extracts (2x2 mL of methanol and 2x2 mL of acetone) are combined and evaporated to a volume of ca. 1 mL. The concentrated extract is diluted in 150 mL of Milli-Q water prior to SPE.

5.1.2. Solid phase micro extraction (SPME)

The most used technique for the determination of the non-polar (e.g polycyclic musk fragrances (PMF)) in water, wastewater, soil and sludge samples of the WWTP is the headspace solid-phase micro extraction (SPME), followed by GC-MS analysis [19]. SPME is also a pre-concentration and clean-up technique previous to analyze by GC-MS. Due to their elevated lipophilicity (logK_{ow} = 5.90-6.35), most of non-polar organic compounds are,

therefore, sorbed onto soil or sludge and suspended matter. In literature, analytical methods are reported for analyzing polycyclic musk fragrances (PMF) in soil, sediments and sludge using soxhlet or pressurized liquid extraction (PLE) with dichloromethane, silica gel, alumina columns and gel permeation chromatography (GPC) as clean-up methodology previous to GC-MS analysis [18]. In all the cases, several clean-up steps must be applied to the extracts before chromatographic analysis. The SPME is a solventless technique that simplifies the long and tedious processes of sample preparation and analyte extraction in a single step. The SPME technique is a very sensitive technique that can be applied to adsorb the volatile and non-polar compounds released from the aqueous or solid phase to the headspace completely isolated where a fiber of an adsorbable material or can be immerged in the liquid sample to extract selectively the target compounds [20]. The fibers can be of polyacrilate (PA), polydimethylsiloxane (PDMS), divinylbenbenzene (DVB), PDMS/DVB and carboxen-PDMS (CAR-PDMS) and carbowax-DVB (CW-DVB), carboxen-PDMS-DVB (PDMS-DVB-CAR) and they are selected according to the characteristics of the compound that need to be extracted. The head space technique is more used than the immerged fiber in the liquid phase due to the matrix characteristic of some samples that are inappropriate for the submerged fiber.

The extraction of non-polar organic compounds (e.g. musks) from the water, wastewater, soil, sediment and sludge samples was carried out by solid phase micro extraction (SPME) with fibres previously described. The fibres are pre-conditioned prior to use for 30 min at 250 °C. 2 g of sample is added to a vial with 0.5 g NaCl and 10 μL of an internal standard. The fibre was exposed to the sample headspace in a sealed vial with a Teflon lid for 15 min at 90°C. The fibre was thermally desorbed and analysed by GC-MS.

Sludge is a very complex sample and the extraction of the organic pollutants from the matrix usually implies solvent extraction of the dried soil or sludge samples assisted by accelerated solvent extraction, sonication, microwave heating, solid phase extraction (SPE), simple agitation or solid phase micro extraction (SPME). The determination of non-polar organic compounds in solid samples samples by SPME with different fibers can be influenced by the extraction temperature, fiber coating, agitation, pH and salting out on the efficiency of the extraction. An extraction temperature of 100 °C and sampling the headspace over the stirred sludge sample using PDMS/DVB as fiber coating lead to best effective extraction of the musks in general. The method proposed is very simple and yields high sensitivity, good linearity and repeatability for all the analytes with limits of detection at the ngg^{-1} level. The total analysis time, including extraction and GC analysis, in only 40 min, and no manipulation of the sample is required. The GC-MS with MS in electronic impact (EI) in positive mode analytical technique is the most appropriate for the identification and quantification of the polycyclic musk fragrances (PMF) [9].

5.1.3. Chromatographic analysis of organic compounds

The liquid chromatography coupled with mass spectrometry (LC-MS), liquid chromatography tandem mass spectrometry (LC-MS/MS) and liquid chromatography with diode array detector and coupled with mass spectrometry (LC-DAD-MS) with MS in

electrospray (ESI) or atmospheric pressure chemical ionization (APCI) in positive or negative mode are the analytical techniques most adequate for the identification and quantification of organic compounds (e.g PhACs, pesticides, herbicides) in water and wastewater since most of the compounds present neutral and acidic polar characteristics [18]. The GC-MS, LC-DAD-MS and LC-MS/MS techniques are important techniques used for most of organic compounds and their metabolite or reaction products identification and quantification [21, 22]. Due to their selectivity and sensitivity, they are particular important and powerful methods for metabolite or by-product identification of many reactions in the environment (such as biodegradation, photo-oxidation, chemical oxidation and others). Even when a definitive assignment of chemical structures is not possible and, therefore, only tentative degradation pathways can be proposed, GC-MS is so far the most frequently used tool of analysis for identifying transformation products [23]. Winckler used GC-MS for study the ibuprofen metabolites generated by biodegradation processes. Two important advantages of GC-MS methods are the large amount of structural information they yield by the full scan mass spectra obtained under electronic impact (EI) ionization and the possibility of using commercial libraries, making identification of unknowns feasible. However GC-MS has important drawbacks because of its scan capability for analyzing the very polar, less volatile compounds typically generated by these photo-processes [24]. Because of this limitation derivatization techniques should be considered for protection of the polar group by the chemical reaction for a specific period and temperature conditions to get a non-polar derivatized compound that is more compatible with the GC-MS analysis. Many compounds can be used as derivatized reagents to give this protection to the molecule (e.g. MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide), BSTFA (*N,O*-bis(trimethylsilyl)-trifluoroacetamide), TMS (Trimethylsulfonium hydroxide solution) and MTBSTFA (*N-tert*-Butyldimethylsilyl-*N*-methyl)trifluoroacetamide) depending on the chemical structure of the original compound to derivatize. The analysis of degradation products is a highly challenging task. First, the chemical structure of intermediates is unknown, although it can be assumed that primary degradation products are structurally related to the parent pharmaceuticals. Second, standard material for structure elucidation is seldom available. Third, degradation products are present at low concentrations. Therefore, advanced and extended identification methodologies are needed for full structural elucidation of organic compounds (e.g pharmaceutical) degradation products and other new organic compounds that could be found in surface and groundwater as result of chemical and biological transformations. Samples containing the organic compound are typically separated by LC or GC, and either directly injected or pre-concentrated by SPE, lyophilization, evaporation, solvent extraction (e.g. liquid–liquid extraction), or SPME. Many chromatographic techniques can be applied for product isolation prior to nuclear magnetic resonance (NMR). During GC or LC separation, degradation product retention times may provide the first source of identification information. One major point of attention during GC analysis is the thermal stability of pharmaceutical degradation products. High GC-inlet temperatures can decompose thermal labile compounds. They may be used to estimate the polarity and volatility of the degradation products and can be compared, if available, with the standards. For a more accurate identification, degradation product spectral data have to be collected by

use of dedicated detection instruments such as UV spectroscopy or mass spectrometry (MS) [22]. When standard compounds are available, LC-UV or GC-MS spectral data of the unknown degradation products are compared with those of standard compounds. GC-MS also allows spectra comparison with extended databases (e.g., from NIST or Wiley). However, in the majority of cases, standards or databases are not available and data on molecular weight, elemental composition, and chemical structure have to be collected by GC-MS, LC-MS, high resolution (HR)-MS, or multidimensional MS (MS^n). Analysis of the parent compound molecule and analogous products as well as isotope labeling strengthens identification. Next to these hyphenated techniques, direct UV photodetection, direct infusion (DI)-MS, and nuclear magnetic resonance (NMR) analysis may provide supplementary data to complement the chromatographic separation.

The MS analysis of the sample is frequently one of the most used techniques for degradation product of target organic compounds identification and also for the biotransformation products of organic compounds studies without analysis of standards, parent molecules, or analogous products. This is possible since the structure of the parent molecule is also known. Another possibility can be as suggested by Doll and Frimmel, in [25] where they made clear distinction between unequivocally identified organic compound (e.g pharmaceutical) degradation products, based on comparison of LC retention time and UV spectrum with standards, and tentatively identified degradation products, based on LC-MS fragmentation analysis with comparison with standards [25]. The proposed techniques can be applied for a wide range of degradation products, i.e., biodegradation and photodegradation products from pharmaceuticals, as well as degradation products from other micro pollutants such as musks. Although standard compounds and GC-MS spectra may be readily obtained for transformation products resulting from AOP treatment or biotransformation metabolites of widely variable PhAC chemical structures and musks studied, these resources are frequently unavailable commercially to confirm the chemical structures for many other organic compounds and some of this standards need to be synthesized in laboratory. Chromatographic separation by GC or LC is an indispensable part of the analytical procedure when multi residue analysis is the focus. The choice between both chromatographic techniques is especially based on the polarity and thermal stability of the target compound.

Another option to confirm the product and metabolite chemical structures is the use of combining the information of LC-DAD-MS and LC-MS/MS. The presence of diclofenac has been reported in natural waters and in wastewater treatment plant effluents as a consequence of its incomplete elimination with conventional wastewater treatment [26, 27]. Direct photolysis can produce photo transformation products that are commonly analyzed by GC-MS, LC-MS and LC-MS/MS techniques combined in order to get confidence in the chemical structures produced during the process. LC-MS and LC-MS/MS allow the separation of semi-polar and polar degradation products without extensive derivatization. Moreover, aqueous samples can be directly injected when concentrations of intermediates are high compared to the instrument detection limit. The retention time of the degradation products can provide information on degradation product polarity. This is a tool for product identification in addition to stronger identification methods.

The mass spectrometry (MS) is considered a principal tool for identifying new products of oxidation of organic compounds, especially since it enables an efficient analysis of trace amounts of analytes in complex organic mixtures [22]. Such complicated environmental or biological samples require separation of components prior to mass spectrometric analysis, which justifies the use of hyphenated techniques such as GC-MS or LC-MS. The single-stage quadrupole (Q) and an ion trap (IT) both hyphenated to a GC, and a quadrupole-time-of-flight (QTOF) MS coupled to an LC can also be used. In the Q mass analyzer, the ions generated in the source undergo electron impact (EI) fragmentation, which results in complex, ambiguous spectral data and hence in non-selectivity that is its main disadvantage [22]. In contrast, the IT mass detector has the unique ability to isolate and to accumulate ions. By iterating ion trapping and scanning, it allows the generation of collision-induced dissociation (CID) spectra of the parent and fragment ions (and their fragment ions), thus increasing the level of confidence in assigning a particular structure [22]. Alternatively, the hybrid QTOF, in which the final resolving mass filter of a triple Q is replaced by a TOF analyzer, not only allows MS^2 operation but also has the necessary accuracy and resolution to give exact-mass measurements [22,28]. Together with MS methods, both chromatographic techniques complement each other to account for a wide range of polarity, acidic-basic characteristics and different functional groups formed during UV degradation or other oxidation techniques. LC-MS methods can also be used for the identification of metabolites produced by organisms like diclofenac in fish bile with electrospray ionization quadrupole-time-of-light mass analyser (QTOF) [28]. The combined use of both GC-MS and LC-MS analysis for detection of organic compounds such as pharmaceutical degradation products targets two different purposes: (a) increasing the range of detectable degradation products or (b) confirmation of suggested degradation products. A large range of degradation products is detected used GC-MS for detection of non-polar degradation products and LC-MS for semi-polar and polar degradation products during advanced oxidation of diclofenac and dipyrone [29].

The improvement of analytical methods confirms that for the majority of the organic trace contaminants, microbial degradation does not lead to mineralization but rather to the formation of a multitude of transformation products. In order to evaluate whether an organic contaminant was transformed to non-toxic products or even mineralized, it is important to know the transformation pathways. Modern hybrid mass spectrometry systems provide the accurate masses of the new products and deliver information of mass fragments which can be used to identify the chemical structure. However, with the exception of very simple reactions (e.g. hydrolysis of amides and esters) the MS spectra are often not sufficient to obtain and confirm the chemical structures of the transformation products [24]. In general, there are a couple of structural modifications which lead to products with the same accurate masses and similar mass fragments of the parent compound. Without the knowledge of chemical/microbial reactions and/or measurements with alternative methods, the suggested product chemical structure could be incorrect. One possible solution for structural confirmation of the transformation products is nuclear magnetic resonance spectroscopy (NMR). However, a drawback of NMR is the elevated quantity needed of a relatively pure isolated standard, not very easy to achieve for the low concentrations.

6. Electrochemical analysis of organic compounds

The pharmaceutical, pesticides and flame retardant are considered emerging organic compounds, some of them are considered xenobiotic compounds. Analytical measurement procedures of these organic compounds can not only fallow LC-MS and GC-MS techniques but also electrochemical analytical techniques. The scope of organic compounds analysis includes the analytical investigation of bulk materials, the intermediates, and degradation products of substances that can be expected to find in the environment resulting from the different urban and rural sources and promote environmental impact in soil, surface and groundwater and consequently in human and health. The presence of these compounds in groundwater and surface water can enter in the urban water cycle and affect the drinking water systems and agriculture when irrigated with contaminated water with the organic compounds. The growing use of pharmaceuticals is becoming a new environmental problem, as both via human and animal urinary or fecal excretion and pharmaceutical manufacturing discharges, increasing concentrations of pharmaceuticals reach sewage treatment plants (STPs). Due to this extensive use, high concentrations of drugs are found in sewage, depending on their half-lives and metabolism. STPs are often ineffective in removing these substances, so that varying concentrations of them can be found in surface and groundwater. In recent years, increasing attention has been paid to the determination of pharmaceuticals, pesticides and flame retardants in water samples. Until now, many analytical methods reported in the literature have been carried out by gas and high-performance liquid chromatography, usually in combination with mass spectrometry (GC–MS, LC–MS), capillary electrophoresis mass spectrometry and high-performance liquid chromatography-photochemically induced fluorimetry (LCPIF). Unfortunately, all these reliable methods are very expensive, and it would be better to use different analytical methods, which do not require expensive instrumentation and which therefore could be used even in less highly developed areas. It is necessary that analytical methods and results comply with the following requirements: 1) the analytical techniques used provide reliable results with a fast turnaround time; 2) the obtained results provided will remain consistent throughout the development cycle of the substances; and if possible, 3) the techniques are transferable to laboratories doing more repetitive testing.

Electrochemistry has always provided analytical techniques characterized by instrumental simplicity, moderate cost and portability. Electroanalytical techniques can easily be adopted to solve many problems of organic compounds with a high degree of accuracy, precision, sensitivity and selectivity, often in spectacularly reproducible. First examples of the organic compound (e.g. pharmaceutical) analysis using by polarographic methods were described in the 1930s and 1940s. Most of the pharmaceutical active compounds (PhAC) were found to be as an electrochemically active. Modern electrochemical methods are now sensitive, selective, rapid and easy techniques applicable to analysis in the pharmaceutical fields, and indeed in most areas of analytical chemistry. They are probably the most versatile of all trace PhAC analysis. Electroanalytical methods are also widely used in specific studies and monitoring of industrial materials, biological and environment samples. The electroanalytical techniques at varying levels of sensitivity are required to solve analytical

problems. This kind of assays require high specificity, low detection and determination limits and capable of determining drugs and their metabolites with nanogram (ng) or picogram (pg) level simultaneously. Voltammetric techniques have been extremely useful in measuring drinking water, wastewater, groundwater, surface water, metabolites and urinary excretion of drugs following low doses, especially when coupled with chromatographic methods. In many cases, modern electroanalytical techniques like square wave voltammetry (SWV) can be available alternative to more frequently used spectrometric or separation methods. The volumetric instrument involves a cell with three electrodes immersed in a solution containing the analyte and also an excess of nonreactive electrolyte called supporting electrolyte. One of the three electrode is the working electrode (e.g. microelectrode (ME) of vitreous carbon (VC), or mercury electrodes such as dropping mercury electrode (DME), static mercury electrode (SME) and hanging mercury drop electrode (HMDE)), whose potential varied linearly with time, the other electrode is the reference electrode (commonly a saturated calomel, or a silver/silver chloride electrode (Ag/AgCl/KCl(sat.))) and the third electrode is the counter electrode, which is often a coil of platinum wire or a pool of mercury that simply serves to conduct electricity from the signal source through the solution to the electrode).

6.1. Electrode preparation

The fundamental process in electrochemical reactions is the transfer of electrons between the electrode surface and molecules in the interfacial region (either in solution or immobilized at the electrode surface). The kinetics of this heterogeneous process can be significantly affected by the microstructure and roughness of the electrode surface, the blocking of active sites on the electrode surface by adsorbed materials, and the nature of the functional groups (e.g., oxides) present on the surface. Therefore, there has been considerable effort devoted to finding methods that remove adsorbed species from the electrode and produce an electrode surface that generates reproducible results. Some of these methods have also resulted in the activation of the electrode surface (as judged by an increase in the rate of electron transfer). These methods include mechanical polishing, heat pretreatment, and electrochemical pretreatment. The most common method for surface preparation is mechanical polishing. The protocol used for polishing depends on the application for which the electrode is being used and the state of the electrode surface. There are a variety of different materials available (e.g., diamond, alumina, silicon carbide), with different particle sizes suspended in solution (BAS supplies 0.05 μm alumina polish and 1, 3, 6, and 15 μm diamond polishes). The pad used for polishing also depends on the material being used for polishing - Texmet pads are used with alumina polish, and nylon pads should be used with diamond polish.

Working electrodes supplied by BAS have first been lapped to produce a flat surface, and have then been extensively polished to a smooth, mirror-like finish at the factory. Therefore, they typically only require repolishing with 0.05 μm or 1 μm diamond polish by the user in between experiments. Materials that have a rougher surface (e.g., electrodes which have been scratched) must first be polished using a larger-particle polish in order to remove the surface defects. After the defects have been removed, the polishing should continue with

successively smaller-particle-size polish (e.g., 15 μm, then 6 μm, then 3 μm, and then 1 μm). Once polishing has been completed (this can require from 30 s to several minutes, depending upon the state of the electrode), the electrode surface must be rinsed thoroughly with an appropriate solvent to remove all traces of the polishing material (since its presence can affect the electron transfer kinetics).Alumina polishes should be rinsed with distilled water and diamond polishes with methanol or ethanol. The rinsing solution should be sprayed directly onto the electrode surface. After the surface has been rinsed, electrodes polished with alumina should also be sonicated in distilled water for a few minutes to ensure complete removal of the alumina particles. If more than one type of polish is used, then the electrode surface should be thoroughly rinsed between the different polishes. The effect of any surface pretreatment can be determined by its effect on the rate of electron transfer. This can be judged qualitatively by examining the separation of the peak potentials in a cyclic voltammogram of a molecule whose electron transfer kinetics are known to be sensitive to the state of the surface; a more quantitative determination can be made by calculating the value of ks from this peak potential separation. For example, ks for potassium ferricyanide at glassy carbon surface following a simple polishing protocol was found to lie in the range 0.01 - 0.001 cm s^{-1} (this should be compared with the values measured for ks for a platinum electrode, which are at least one order of magnitude larger) [30]. The strong dependence of the electron transfer kinetics of ferricyanide on the state of the electrode surface means that there can be significant variations in the peak potential separation after each polishing. Polishing alters the microstructure, roughness, and functional groups of the electrode surface in addition to removing adsorbed species [30, 31]. For example, the electrode surface can be contaminated by the agglomerating agents required to keep the alumina particles suspended in solution and by the components of the polishing pad. The presence of these species can have a deleterious effect on the electron transfer kinetics by blocking the active sites for the electron transfer reaction. Polishing is often used in combination with another pretreatment (e.g., heat or electrochemical). For many other systems, the simple polishing described above is adequate (for example, when using non-aqueous electrolytes, since blocking of active sites by adsorbed species is less common in such electrolytes than in aqueous solutions). Another method for preparation of the electrode surface that is becoming more widely used is electrochemical pretreatment (ECP), particularly for electrodes which cannot readily be polished (e.g., carbon fiber cylinder electrodes). ECP consists of applying conditioning potentials to the electrode surface before the experiment. As for polishing, this has the effect of removing adsorbed species and altering the microstructure, roughness, and functional groups of the electrode surface. The precise ECP protocol depends upon the application and varies considerably. The potential waveforms typically are held at, or cycle to, a large positive or negative potential, either using steps or sweeps (constant potential, potential scan, triangular wave and square wave. Although the development of the preconditioning protocols has been largely empirical, the pretreated electrode surface has been characterized in order to elucidate the reasons for the activation of the electrode surface [31]. For glassy carbon electrodes, in addition to the removal of adsorbed species, the preconditioning potential leads to the formation of an oxygen-rich layer on the carbon surface. This layer contains

oxides as well as other oxygen-containing functional groups which may catalyze electron transfer reactions (the composition of the functional groups in this layer is sensitive to the pretreatment conditions and depends on the solution pH as well as the potentials used for the pretreatment) [31]. The oxide layer can also adsorb and/or exchange ions from the solution, which leads to improved detection limits. However, electrochemical pretreatment of electrodes increases the background current of the electrode relative to that of a polished electrode, which may be disadvantageous for some applications.

6.2. Pre-treatment and enrichment of surface and groundwater water samples for voltametry analysis

A solid-phase extraction (SPE) can also be used for pre-concentration of surface water samples for electrochemical analysis. 2L volume samples were adjusted to pH 3 and 7 with concentrated hydrochloric acid and sodium hydroxide, respectively and then passed through an SPE cartridge (conditioned with acetone, methanol and water) using a vacuum system [32]. After the cartridges had been left to dry for 30 min, the drugs were eluted with 5mL of methanol. The choice of methanol as extracting agent was suggested by its strong eluent ability and its inactivity on the electrodes used. Extracts were diluted with 5mL of 0.2M $NaClO_4$ before electrochemical analysis. In some cases 1mL of the extract obtained at pH 7 was then diluted with 9mL of 0.1M NaOH before analysis [32].

6.3. Ion-selective electrode method

Potentiometric methods also play a significant role in pharmaceutical, biological, clinical and environmental analysis with the introduction of new ion-selective electrodes [33]. It is still one of the most promising analytical tools capable of determining both inorganic and organic substances in pharmaceuticals. Ion selective electrodes (ISEs) belong to the oldest established chemical sensors. Their response characteristics including selectivity, kinetic process and basic thermodynamic process are comparatively well understood. ISEs are mainly membrane based devices, consisting of permeable selective ion-conducting materials, which separate the sample from the inside of the electrode. On the inside is a filling solution containing the ion of interest at a constant activity. The membrane is usually water insoluble, nonporous and mechanically stable. ISEs membranes always contain a substantial concentration of the primary ion salt, which is typically in the mM range. This practice clearly favors the occurrence of co-transport and thus brings about a less than optimal detection limit. The composition of the membrane is designed to yield a potential that is primarily due to the ion of interest via selective binding processes. In addition, the membrane backside is usually contacted with a relatively concentrated internal filling solution of a primary ion salt. These ions can be released into the sample by a zero-current ion flux according to two principal mechanisms. These are the co-transport of the primary ion with a counter-ion and the counter-transport with another ion of the same charge type. The ion transfer mechanisms not only limit the experimental lower detection level but also bias the assessment of the selectivity behavior of the ISE. Selectivity coefficients are typically determined from the responses to the primary and the interfering ions. ISEs are most

frequently employed in different variants. The interest in ISEs increased dramatically in recent years since it was shown that the detection limit of conventional ISEs can be lowered towards the picomolar (pM) concentration level, enabling potentiometric trace-level analysis. Membrane materials, possessing different ion recognition properties, have thus been developed to impart high selectivity. The Nernst equation is normally used to describe the ideal response of these cells. To overcome the disadvantages of the ISEs, (slow electrode response, poor selectivity towards other acidic or basic gases, long recovery time, and practical limitations for miniaturization), new concepts have been proposed such as the replacement of the pH glass electrode by a polymeric membrane selective to pH. Depending on the nature of the membrane material used to impart the desired selectivity, ISEs can be divided into three groups: glass, liquid and solid electrodes. Glass electrodes are responsive to univalent cations. Ion-sensitive glass membranes are used in this kind of the ISE. The most common glass electrodes are known as the pH electrode. These electrodes have been widely used for pH measurements. Other types of glass electrodes are used for cation measurements such as sodium, ammonium, and potassium. Liquid-membrane-type ISEs, based on water – immiscible liquid substances impregnated in a polymeric membrane, are widely used for direct potentiometric measurements.

This type of ISE is particularly important because it permits direct measurements of several polyvalent cations as well as certain anions. The membrane-active component can be an ion exchanger or a neutral macrocyclic compound. Ion exchanger and neutral carrier electrodes are the best known liquid-membrane-type ISEs. Most of the ISEs work has been related to the development of solid membranes that are selective primarily to anions. Solid-state ISEs based on immobilization of ion recognition sites in the conducting polymer membrane represent another area of great interest.

The solid state membrane can be made of single crystals, polycrystalline pellets, or mixed crystals such as fluoride, iodide, chloride, bromide, thiocyanide, etc. More directly related to pharmaceutical analysis is the considerable interest devoted to polymeric membranes selective to organic compounds such as drug-active compounds. Considerable work has been devoted to the development of solid membranes that are selective primarily to anions. The solid-state membrane can be made of single crystals, polycrystalline pellets or mixed crystals. Solid-state ISEs contain three major elements. The polymeric matrix, one or several mediators and the active material are constituted these three major elements. PVC is often selected as the polymeric matrix. However aminated and carbocylated-PVC, hydroxylated-PVC, cellulose acetate, silicone rubber, or polyurethane derivatives can also be used as polymeric matrix. The mediators can also be plasticizers. The active materials such as ion pair, ion exchanger or neutral carrier must exhibit high lipophilicity in order to remain in the organic membrane, even upon repeated exposure to aqueous samples. These three major elements must be fully compatible and dissolved in the same hydrophobic solvent. For high membrane sensitivity of this kind of ISE, the active material must rapidly and reversibly exchange the ion. Covalent binding of ion-recognition sites to conducting polymers should result in very durable solid-state ISEs that are easily miniaturized, e.g., electropolymerization of specifically functionalized monomers. The optimal composition of the membrane will be selected in order to provide the best characteristics such as Nernstian

slope, the lowest detection limit, the shortest response time, the widest linearity range, the highest selectivity, the best reproducibility, and the longest lifetime. Other types of solid-state ISEs are coated-wire ISEs and solid-state electrodes without an internal filling solution [33]. Coated-wire electrodes are prepared by coating an appropriate film directly onto a conductor thus eliminating the internal filling solutions. It can be prepared simply by dipping the solid substrate into a solution containing the dissolved polymer, the plasticizer and an ion-pair, and allowing the solvent evaporates. The analytical performance of solid-state ion-selective electrodes where conducting polymers are used as ion-to-electron transducer has been significantly improved. This ion-responsive membrane is commonly based on polyvinyl chloride (PVC) while the conductor can be graphite or metallic-based (Pt, Ag, Cu, Ru, Al, etc.) and can be of any convenient geometric shape (wire, disc, etc.). Coated wire electrodes can be prepared by using other polymers and modified polymers such as poly acrylic acid and modified poly vinyl-benzyl chloride, etc. These electrodes are inexpensive, simple and easy to prepare. The measuring concentration range is varied between 10^{-5} and 10^{-1} M. Despite these advantages, coated-wire ISEs may suffer from reproducibility and long-term stability problems because of the poorly defined contact and mechanism of charge transfer between the membrane coating and conducting transducer. New concepts for preparing coated wire ISEs appear to improve their analytical performance particularly with respect to stability and reproducibility. The ability to eliminate the internal electrolyte solution and decrease the detection limits compared with the traditional ISEs are significant advantages. The detection limit of such coated-wire or solid-state electrodes without an internal filling solutions is on the nanomolar (nM) level. Also, these electrodes demonstrate high stability similar to those of conventional ISEs. Possible interferences in ISEs generally originate from compounds that are structurally related to the pharmaceutically active compounds under investigation such as metabolites, intermediates of synthesis, homologues of other pharmaceutically active compounds, or drug excipients exhibiting similar pharmacological properties. The determination and screening of drugs by the method of direct potentiometry with solid-state ISEs offers a rapid and simple procedure satisfying all requirements of pharmaceutical analyses in groundwater and other organic compounds.

6.4. Voltametric and polarographic techniques

Five main potential excitation signals can be used in voltametry such as polarography linear scan voltametry (LSV), normal pulse voltametry (NPV), differential pulse polarography (DPV), square wave voltametry (SWV) and cyclic voltametry (CV) for the analysis of organic compounds in surface water, groundwater and wastewater. Square wave voltammetric (SWV) technique is among the most sensitive means, for the direct evaluation of concentrations; it can be widely used for the trace analysis, especially on pharmaceutical compounds. This method is the source of a fair amount of confusion. The problem arises from the number of waveforms employed, which are frequently described as simply square wave voltammetry. In this context it will be consider three basic groups: the Kalousek, Barker, and Osteryoung formats. Square wave voltammetric technique originates from the Kalousek commutator and Barker's square wave polarography. Kalousek constructed an

instrument with a rotating commutator which switched the potential of the dropping. Kalousek square wave technique is a lower frequency method, which measures the current only on the reverse half cycle of the square wave (SW). The Barker format is the simplest to visualize. The waveform is a direct analog to sinusoidal ac voltammetry with a symmetric square wave of frequency and amplitude riding on either a ramp or slow staircase waveform. Osteryoung format is the most common form of SW techniques [34]. This waveform differs from the other SW techniques in that the base potential increases by amplitude for each full cycle of the square wave. The current is measured at the end of each half cycle. This wave form can be applied to a stationary electrode or static mercury drop electrode. In this case the time interval is arranged to allow the drop to grow to a pre-determined size. The response consists of discrete current-potential points separated by the potential increment ΔE. Hence ΔE determines the apparent scan rate, which is a number of current-potential points within a certain potential range. The currents increase proportionally to the scan rate. Frequently, the response is distorted by electronic noise and a smoothing procedure is necessary for its correct interpretation. In this context, it is better if ΔE is as small as possible.

The advantage of SWV is that a response can be found at a high effective scan rate, thus reducing the scan time. For this reason SWV is employed more often than normal pulse voltammetry (NPV) and differential pulse voltammetry (DPV) techniques. Whereas NPV and DPV function with effective sweep rates between 1 and 10 mVs^{-1}, SWV can reach 1 Vs^{-1}. There are advantages: greater speed in analysis and lower consumption of electroactive compounds in relation to DPV, and reduced problems with blocking of the electrode surface. Also, in comparison to both linear sweep and cyclic voltammetry, it as a much broader dynamic range and lower limit of detection because of its efficient discrimation of capacitance current. Analytical determinations can be made at concentrations as low as 10 nM. SWV is 4 and 3 times higher than the DPV response, for reversible and irreversible systems, respectively. Therefore, typical SWV measurements take only 1-5 s whereas DPV requires much longer analysis times at about 2-4 min. [34]. Frequencies of 1-100 cycles per second permit the use of extremely fast potential scan rates. This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal-to-noise ratio.

The other advantage of SWV, the difference of current is larger than either forward or reverse currents, so the height of the peak is usually quite easy to read, thus increasing the accuracy. The forward current i_2, reverse current i_1, or difference current ($i = i_2 - i_1$) can be used as the response in this technique. The net current has only very small charging current contributions, and in typical experiments the total faradaic charge is much less than equivalent to a monolayer of material. That is, the system is charged very little by the perturbation. The position and shape of the net current response are remarkably insensitive to size and shape of electrode. A further advantage of the current difference output is that, when the signal lies in the oxygen reduction plateau, the response due to the reduction of oxygen is subtracted out. The sensitivity increases from the fact that he net current is larger than either the forward or reverse components. Also, the sensitivity of SWV is higher than

that of NPV and DPV. Square wave voltammetry is a powerful electrochemical technique that can be applied in both electrokinetic and quantitative determination of redox couples strongly immobilized on the electrode surface [31, 34]. In general, computer-based data acquisition may revolutionize the whole area of data collection in electrochemistry since more complex waveforms and current gathering techniques may be employed. This technique requires the power and flexibility of the mini-computer for its development and modern microprocessors for its commercial implementation. Microprocessors can be used to compensate for the practical problem of solution resistance and recently menu-selectable software has been incorporated in a stand-alone instrument which allows background subtraction and signal differentiation. The inherent speed of SWV can greatly increase sample throughput in batch and flow analytical operations. The method can be quite rapid and lends itself to the monitoring of rapid processes such as liquid chromatography. Simplex optimization to maximize peak current by varying the waveform parameters has been examined and SWV has also been used in thin lays. Because of the sensitivity and rapidity SWV is useful for drug analysis in their dosage forms and biological and water samples. The low detection and determination limits permit the analysis of trace amount of drug compound. SWV method was applied to numerous drug active compounds. In addition, SWV detection can also be used to resolve co-elution or co-migrating species for LC and CE methods. Electroanalytical applications of drugs using SWV technique can be consider into direct and stripping measurements. Some pharmaceutical compounds that are analyzed directly, i.e. without accumulation of reactant or product of the electrode reaction. The stripping methods are based either on the accumulation of amalgams and metal deposits, or on the adsorptive accumulation of pharmaceutical compounds and metal complexes.

The hydrodynamic of the voltametry can be obtained by stirring the cell solution with a ordinary magnetic stirrer, by rotating the microelectrode in order to help the analyte flow and migration from the solution to the working electrode under the influence of an electric field, convection resulting from stirring or vibration and diffusion due to concentration differences between the film of liquid at the electrode surface and the bulk solution. All the efforts need to be made to minimize the effect of migration by introduction of an inactive supporting electrolyte. When the concentration of supporting electrolyte exceeds that of the analyte by 50- to 100- fold, the fraction of the total current carried by the analyte approaches zero and result in the rate of migration of the analyte toward the electrode of opposite charge becomes essentially independent of the applied potential.

The applied potential between the microelectrode and the reference electrode can be obtained by the application of the Nernst equation for an electrode reaction of A + ne⁻ ↔ P:

$$E_{appl} = E_A^o - \frac{0.0592}{n} log \frac{C_P^o}{C_A^o} - E_{ref} \qquad (1)$$

Where E_{appl} is the potential applied between the microelectrode and the reference electrode, C_P^o and C_A^o are the molar concentrations of P (product in the bulk solution changed by electrolysis that tends to zero with the reaction in the microelectrode) and A (analyte in the bulk solution unchanged by electrolysis), respectively, in a thin layer of solution at the

electrode surface only. It is also assumed that the electrolysis, over short periods of time, does not alter the bulk solution concentration appreciably because the electrode surface is very small.

Some application examples of the electrochemical analysis of pesticide-acaricide (amitraz) [35] and PhAC (nifedipine) [11] are now presented. Electrochemical analysis is used for the determination of amitraz [35]. Amitraz is a formamide acaricide used predominantly in the control of ectoparasites in livestock and honeybees. Amitraz hydrolysis is rapid and occurs under acidic conditions, exposure to sunlight and biodegradation by microorganisms. The main hydrolysis product of amitraz, 2,4 dimethylaniline, is recalcitrant in the environment and toxic to humans. An electrochemical method for the determination of total amitraz residues and its final breakdown product, 2,4 dimethylaniline. Cyclic voltammetry at a glassy carbon electrode showed the irreversible oxidation of amitraz and 2,4 dimethylaniline in the presence of spent cattle dip. A limit of detection in the range of 8.5×10^{-8} M for amitraz and 2×10^{-8} M for 2,4 dimethylaniline is obtained using differential pulse voltammetry. Feasibility studies in which the effect of supporting electrolyte type and pH had on electroanalysis of amitraz and its degradants, showed that pH affects current response as well as the potential at which amitraz and its degradants are oxidised. Britton-Robinson buffer is found to be the most suitable supporting electrolyte for detection of amitraz and its degradants in terms of sensitivity and reproducibility [35]. Studies performed using environmental samples showed that the sensitivity and reproducibility of amitraz and 2,4 dimethylaniline analyses in spent cattle dip is comparable to analyses of amitraz and 2,4 dimethylaniline performed in Britton Robinson buffer. In addition, the feasibility of measuring amitraz and 2,4 dimethylaniline in environmental samples is assessed and compared to amitraz and 2,4 dimethylaniline analyses in Britton-Robinson buffer. Amitraz and 2,4 dimethylaniline is readily detectable in milk and honey. The biological degradation of amitraz and subsequent formation of 2,4 dimethylaniline is readily monitored in spent cattle dip. The breakdown of amitraz to 2,4 dimethylaniline can be monitored using cyclic voltammetry.

Another application of the electrochemical methods has been developed for the trace determination of nifedipine.in a simple and rapid differential pulse polarographic [11].A well-defined single peak with Ep value of -0.51 V is obtained in 0.1M acetate buffer (pH 5.0). The linearity is valid up to 5×10^{-5} M (r =0.9995) with minimum detection limit of 3.5×10^{-8} M. Precision of the method developed is implied from the values of relative mean deviation, standard deviation and coefficient of variation, which are 2.05%, 1.1 and 3.2% respectively. Marketed formulations of nifedipine have been analyzed by calibration and standard addition methods. The studies have shown that the method is simple, reproducible and accurate and can be used in the analysis of this PhAC in water. The use of glassy carbon electrode has been suggested for linear sweep and cyclic voltammetric studies. Adsorptive cathodic stripping polarographic determination of trace nifedipine has been reported with high sensitivity. Anodic electrochemical behavior based on the oxidation of dihydropyridine ring to form pyridine derivative compound has been reported as good signal. However, the detection limit obtained by this method is found to be lower as compared to the known method. Also, linear range is found to be much wider than the reported values.

In recent years, increasing attention has been paid to the determination of pharmaceuticals, pesticides and other organic compounds in water samples. Until now, many analytical methods reported in the literature have been carried out by gas and high-performance liquid chromatography, usually in combination with mass spectrometry (GC–MS, LC–MS). Unfortunately, all these reliable methods are very expensive, and it would be better to use different analytical methods, which do not require expensive instrumentation and which therefore could be used even in less highly developed areas. The electrochemical methods applied to monitoring of pharmaceuticals, pesticides and other organic compounds can be another on determination in surface waters and groundwater as a result of incomplete removal of some of these persistent compounds in sewage and in the wastewater treatment plants.

6.5. Parameters affecting the voltametry

6.5.1. Effect of the ionic strength

Voltammetric measurements in solutions of very low ionic strength, including those without deliberately added supporting electrolyte, became possible by using microelectrodes, electrodes with at least one essential dimension in the range of micrometers or less [36]. Voltammetric measurements without supporting electrolyte departed significantly from the traditional way such measurements were performed. 'Traditional' voltammetry required an excess of electro-inactive ions to make the solution sufficiently conductive, to make a compact double layer, and to suppress migration of electro-active species. Electrode processes at microelectrodes are usually associated with very low currents in the range of nano- (nA) or picoamperes (pA). Consequently, it might seem straightforward that even in solutions of very high resistance, the ohmic IR drop is very low. However, there is one specific phenomenon which contributes to lowering of the ohmic drop. This is an increase in ionic strength in the depletion layer while the electrode reaction of an uncharged substrate advances. The ionic strength increases as a result of the formation of the charged products accompanied by drawing of appropriate amounts of counter ions from the bulk solution. This allows voltammetric measurements in solutions of low conductivity, e.g. solutions without added supporting electrolyte or simply solutions of low support ratio, which is the ratio of the concentration of supporting electrolyte to the concentration of the reactant. It should be mentioned here that it is possible to have both a relatively high conductivity and a low support ratio; this is the case with a very high concentration of electro-active species. For neutral reactants, the use of regular-size electrodes, with sizes in the range of mm, is practically not possible in solutions of low ionic strength. To correct the effect of the ionic strength during the voltammetric analysis, different buffered medium are used with acetate, phosphate, citrate, borate at different pH from 3 to 9 depending on the acidic or basic proprieties of the organic compound being analyzed [34, 37].

6.5.2. Dissolved oxygen effect

Dissolved oxygen is easily reduced at the microelectrode. An aqueous solution saturated with air exhibits two waves attributed to the first reduction of O_2 to H_2O_2 and a second

reduction of H_2O_2 to H_2O corresponding to two waves of identical height. The presence of oxygen often interferes with the accurate determination of the other species. The oxygen removal is ordinary the first step in amperometric procedures. The deaeration is obtained by injection of an inert gas (*sparging*) for several minutes. Gaseous nitrogen (N_2) or another inert gas should also be introduced in the cell solution in order to reduce the oxygen effect response in the working electrode and minimize the effect of the oxygen in the voltammogram [37].

6.5.3. pH effect

The half-wave potentials for organic compounds are markedly pH dependent. pH variation may result in a change in reaction product. The electrode process consumes or produces hydrogen ions that will change the pH of the solution at the electrode surface unless the solution is buffered. These changes affect the reduction potential of the reaction and cause drown-out, poorly defined waves. When the electrode is affected by pH changes, nonlinearity in the diffusion current/concentration relationship will encountered. In organic polarography, good buffering is vital for the generation of reproducible half-wave potentials and diffusion currents. The polarograms of the PhAC (e.g nifedipine) were recorded in different buffer systems. Single symmetrical peak was obtained in Britton-Robinson buffer from pH 3.0 to 10.0, acetate buffer from pH 4.0 to 6.0, borate buffer from pH 7.5 to 11.0, Mcllavaine buffer from pH 3.5 to 7.0 and Clark-Lubs buffer from pH 5.0 to 10.0. Acetate buffer at pH 5.0 was selected for all studies as a single sharp peak with high degree of reproducibility was obtained under these conditions. The effect of maxima suppressor was studied using Triton-X- 100, gelatin and bromophenol blue. In the absence of maxima suppressor, the peak was highly unsymmetrical. Addition of gelatin (0.1%, 0.5 ml), bromophenol blue (0.1%, 0.5 ml) or Triton-X-100 (0.1%, 0.5 ml) improves the symmetry of the peak. With addition of 0.5 ml of 0.1% Triton-X-100, a narrow, symmetrical peak was obtained. With every 0.5 ml addition of Triton-X-100 there was about 8% decrease in the diffusion current. Hence, 0.5 ml of 0.1% Triton-X-100 was selected as the optimum concentration for further studies [37].

6.5.4. Functional groups effect

The reactive functional groups of the organic compounds can be expected to produce one or more polarographic waves. The carbonyl groups including aldehydes, ketones and quinones produce polarographic waves. In general, aldehydes are reduced at lower potencials than ketones. The conjugation of carbonyl double bond also results also in lower half-wave potentials. Certain carboxylic acids are reduced polarographically, although simple aliphatic and aromatic monocarboxylic acid is not. Dicarboxylic acid such as fumaric, maleic or phtalic acid in which the carboxylic group is conjugated with one another give characteristic polarograms. Most peroxides and epoxides yield polarograms. Nitro, nitroso, amino oxide azo groups are generally reduced at the dropping electrode. Most organic halogens groups produce a polarographic wave which results from replacement of the

halogen group with an atom of hydrogen. Carbon/carbon double bond is reduced when it is conjugated with another double bond, an aromatic ring or an unsaturated group. Hydroquinone and mercaptans produce anodic waves. Most of these functional groups can be detected by the use of dropping mercury electrode (DME) [37, 38].

6.5.5. Solvents effects

The solubility frequently dictates the use of solvents or pure water for organic polarography. Aqueous mixtures containing different amounts of such miscible solvents such as glycols, dioxane, acetonitrile, alcohols, acetic acid can be employed. The supporting electrolytes such as lithium or tetra alkyl ammonium salts can also be used.

6.5.6. Electro deposition step effect

Only a fraction of the analyte is deposited during the electrodeposition step. The quantitative results depend not only upon control of electrode potential but also upon factors as electrode size, length of deposition and stirring rate for both the sample and standard solutions employed for calibration. Microelectrodes for stripping methods are formed from a variety of materials including mercury, gold, silver, platinum and carbon in various forms. The most popular electrode is the hanging mercury drop electrode (HMDE) which consists of a single drop of mercury in contact with platinum wire [37].

7. Chromatografic and electrochemical method validation

7.1. Chromatographic method validation

7.1.1. Calibration curves, limits of detection (LOD) and quantification (LOQ)

Standards are obtained using the stock solutions diluted in methanol. Six-point calibration curve are normally used for each compound, ranging from 5 to 200 ng L^{-1}, depending on the expected concentration range in the surface and groundwater samples. The regression coefficient of the resulting calibration curves should be >0.95 for all compounds. Ten blank samples are analysed by LC-MS (with methanol) and GC-MS (with *n*-hexane) to determine the lowest signal/noise ratio of each analyte. Limits of detection (LOD) for the analytes are calculated by the formula 3xSD/*m*, where SD is the standard deviation of the lowest signal/noise ratio of the analyte and *m* is the slope of the calibration curve. Limits of quantification (LOQ) are calculated as 10xSD/*m*.

Low LOD and LOQ are obtained for the non-polar organic compounds such as musks for Ternes and Salgado in [9, 19], when analysed by GC-MS as well as the recoveries were very high as presented in Table 1 for Smyth and Salgado in [9, 39].

The LOD and LOQ and recoveries obtained water samples of acidic and neutral PhAC, antibiotics and estrogens are presented in Table 2 for the compounds detected in this study. The results showed that the analytical procedure for PhAC enables the detection of

a substantial number of pharmaceuticals with LOD and LOQ comparable with many studies and laboratories using the LC-MS(ESI+) for the detection of this organic compounds.

Compound	LOD μM	LOQ μM	Recoveries %	Reference
Cashmeran	4.85E-09 1.02E-7	1.94E-08	83±3	[9] [39]
Celestolide	8.2E-09 6.56E-8	2.46E-08	85±4	[9] [9]
Galaxolide	3.88E-09 4.26E-8	1.55E-08	94±2 82	[9] [19] [39]
Phantolide	4.10E-09 6.97E-8	1.64E-08	97±2	[9] [39]
Tonalide	3.88E-09 3.26E-8	7.75E-09	82±3 78	[9] [19] [c]
Traseolide	7.75E-09 5.04E-9	2.33E-08	85±4	[9] [39]

[9] Salgado et al., 2010; [19] Ternes et al. (2005); recoveries obtained with groundwater; [39] Smyth et al. (2008)

Table 1. LOD and LOQ obtained by GC-MS(EI+) for Musk (non-polar organic compounds)

The estrogens are the main exception, where tandem MS should be used in order to detect these compounds to a concentration that is relevant to assess their potential environmental impact [8]. However, some other compounds, namely carbamazepine, showed lower LOD and LOQ with SPE (RP-C_{18}) followed by LC-DAD-MS(ESI+) when compared to results obtained with SPE (RP-C_{18}) and GC-MS [7]. The LOQ for carbamazepine was similar to that found through LC-MS/MS [8]. In Sacher, three separate analytical methods were used for antibiotics, antibiotics were analysed together with the other acidic compounds [7]. While Sacher [7] found a lower LOD and LOQ for amoxicillin, their recovery was lower than Salgado [9] (36% vs. 83%).

7.1.2. Determination of recoveries in chromatography

For the determination of the percentage of recovery of organic compounds in water samples, the samples are spiked with analytes dissolved in a stock solution (each at 1 mg mL^{-1} methanol). The water samples are spiked with 100 μg L^{-1} of analyte and internal standard (e.g. meclofenamic acid). After spiking, the samples are stirred for homogenisation and to enable a sufficient contact of analytes and standards with the matrix. Relative recoveries are determined in relation to a MilliQ water standard solution, also spiked with 100 μg L^{-1} of analyte and internal standard. Recoveries of the analytes in the individual clean-up steps are determined by SPE for acidic and neutral organic compounds in water

matrices as well as MilliQ water, and analysed by LC-DAD-MS. The relative recoveries are calculated from the analyte areas in the influent matrix, subtracting the analyte area quantified in the original unspiked matrix, divided by the area of the MilliQ standard sample, Table 1 and 2.

Compound	LOD μM	LOQ μM	Recoveries %	Ref	Compound	LOD μM	LOQ μM	Recoveries %	Ref
Acidic PhACs					**Neutral PhACs**				
Captopril	2.3E-08	6.91E-08	66±10	[9]	Atenolol	1.13E-08 9.02E-9	3.76E-08 3.08E-8 1.88E-7	>94±5 67 98	[9] [7] [8]
Clofibric acid	6.17E-08 2.19E-8	2.06E-07 7.45E-7 2.07E-6	>98±1 103 82	[9] [7] [8]	Caffeine	1.39E-07	4.69E-07	>82±1	[9]
Diclofenac	2.2E-08 2.74E-8	7.55E-08 9.15E-7 1.58E-6	>79±5 70 89	[9] [7] [8]	Carbamazepine	8.47E-09 4.07E-8	2.97E-08 1.36E-7 4.24E-8	>75±1 74 92	[9] [7] [8]
Enalapril	2.13E-08	7.45E-08	>88±3	[9]	Clorazepate	4.16E-08	1.39E-07	-	[9]
Flurbiprofen	7.38E-08	2.38E-07	>65±1	[9]	Dimethylaminophenazone	1.26E-07 1.86E-8	4.11E-07 6.06E-8 4.33E-7	>83±3 66 93	[9] [7] [8]
Furosemide	5.75E-08	1.91E-07	66±10	[9]	Domperidone	7.04E-09	2.11E-08	-	[9]
Ibuprofen	6.8E-08 1.70E-8	2.23E-07 5.87E-7 2.44E-6	>89±9 110 81	[9] [7] [8]	Etofenamate	5.42E-08	1.82E-07	-	[9]
Indomethacin	1.58E-08 1.22E-8	5.18E-08 4.08E-7 1.13E-6	>89±9 114 90	[9] [7] [8]	Fluoxetine	4.93E-08	1.65E-07	-	[9]
Ketoprofen	8.27E-08 1.89E-8	2.72E-07 6.34E-7 1.98E-6	>96±5 104 94	[9] [7] [8]	Fluticasone	5.62E-08	1.91E-07	>60±6	[9]
Naproxen	7.83E-08 1.66E-8	2.57E-07 5.70E-7 2.18E-6	>86±2 105 91	[9] [7] [8]	Hydroxazine	4.02E-08	1.34E-07	>87±1	[9]
Antibiotics					Indapamide	1.64E-08	4.93E-08	73±1	[9]
Amoxicillin	3.56E-08 1.26E-8	1.18E-07 4.14E-7	>83±4 36	[9] [7]	Nimesulide	4.55E-08	1.49E-07	>86±1	[9]
Ampicillin	8.6E-09	3.15E-08	>67±1	[9]	Paroxetine	7.2E-08	2.37E-07	>82±6	[9]
Estrogens					Ramipril	2.16E-08	7.44E-08	>86±12	[9]
17-α-ethynylestradiol	7.09E-08	2.33E-07	- 76	[9] [8]	Salbutamol	4.6E-08 1.09E-8	1.51E-07 3.81E-8 2.10E-6	>94±1 66 61	[9] [7] [8]
Estrone	6.67E-08	2.22E-07	104±12 82	[a] [8]	Tramadol	6.67E-08	2.23E-07	>86±2	[9]
β-estradiol	1.47E-08	4.41E-08	- 76	[a] [8]					

[9] Salgado et al., 2010; [7] Sacher et al. (2001); recoveries obtained with surface water; [8] Ternes (2001); recoveries obtained with WWTP effluent.

Table 2. LOD and LOQ obtained by LC-MS(ESI+) for PhAC polar (organic compounds)

7.2. Electrochemical method validation: Voltametry

7.2.1. Calibration curves, limits of detection (LOD) and quantification (LOQ)

A calibration curve is obtained by taking different known concentrations of the organic compound in a supporting electrolyte under specific experimental conditions and recording the polarograms and plotting di_P vs. organic compound concentration curve. The wave height/peak height of the polarogram was found to be proportional to the organic compound concentration.

Compound	Electrode type	LOD/LOQ μM	Method	Ref	Compound	Electrode type	LOD/LOQ μM	Method	Ref
Acidic PhACs					**Neutral PhACs**				
Captopril	SMDE HMDE	2.9E-3 2.3E-3	-	[34] [34]	Atenolol	NGITO GCE	0.13 0.16E+3	DPV DPV	[33] [33]
Diclofenac	ion-selective	4.0E-3	Potencio-metry	[33]	Caffeine	DE	-	CV	[33]
Ketoprofen	SMDE	3.9E-7	-	[34]	Nifedipine	GCE	1.1-4.3E-5	CV	[33]
Naproxen	Pt DE	1.04 1.3E-4	CV, LSV DPV	[33] [33]	Nimesulide	CGE	5.0E-8	DPV	[33]
Antibiotics					Propranolol	CPE	2.0E-7	DVP	[33]
Tetracycline	Gold	0.96	CV	[33]	Paroxetine	SMDE	6.2E-5	-	[34]
Azythromycin	CPE GCE	6.2E-7 9.24E-7	SWV DPV	[33]; [34] [33]	Fluoxetine	GCE	1.0	-	[34]
Estrogens					Hydroxyzine	GCE	3.0E-6	-	[34]
17-α-ethynylestradiol	CPE HDME	3.0E-8 5.9E-7	DPV -	[33] [34]	Indapamide	CPE	5.0E-6	DPV/SWV	[33]
Estrone	-	-	-	-	Ramipril	HMDE	1.2	-	[34]
β-estradiol	GCE Gold	4.0E-5 6.6E-8	DPV -	[33] [34]	Salbutamol	GCE	2.5E-7	Amp. Det	[34]
Pesticides					Sertraline	HMDE	1.98E-4	-	[34]
Atrazine	HMDE	0.37	-	[34]	Tramadol	GCE	2.2	-	[34]
Ametryn	Gold	1.08E-7	SWV	[40]					

Abbreviations: CPE: carbon paste electrode; GCE: glassy carbon electrode; HMDE: hanging mercury drop electrode; NGITO: nano-gold particles modified indium tin oxide; SMDE: static mercury drop electrode; DE: Dropping electrode; Amp Det: Amperometric detection. [33] Uslu et al., 2007; [34] Dogan-Topal et al., 2010;[40] Tavares et al., 2005

Table 3. LOD, LOQ and electrochemical method applied to the determination of different organic compounds

The calibration curve of the PhAC (e.g. nifedipine) is obtained by the diffusion current increased linearly with increase in the drop time of 0.4 to 2.0 s. At drop times above 2 s the increase in diffusion current is not linear. With increase in pulse amplitude from 20 to 100 mV, the diffusion current showed a linear calibration plot is obtained from 5.0×10^{-7} M to 5.0×10^{-5} M of nifedipine which gives an equation of regression line with coefficient of

correlation 0.9997 indicating high degree of current-concentration linearity in this range. The Ep value is obtained is in the range of -0.47 to -0.60 V, which is attributed to the reduction of nitro group [11]. The lowest determinable limit of nifedipine is found to be 3.5×10^{-8} M. The electrochemical behaviour of acidic and neutral PhACs is studied by cyclic voltammetry and pulse voltammetric techniques on mercury, carbon nanotube paste, carbon paste and gold electrodes. The best results, in terms of sensitivity, linearity range and detection limits, are obtained by differential pulse voltammetry (DPV) for ofloxacin (LOD 5.2 μM), differential pulse polarography (DPP) for clofibric acid (LOD 4.7μM) and normal pulse voltammetry (NPV) for diclofenac (LOD 0.8 μM) and propranolol (LOD 0.5μM). This is obtained when an enrichment step of approximately two orders of magnitude is performed by a solid-phase extraction procedure (SPE) in order to concentrate the samples and tested on spiked river water samples [33, 34].

Pulse techniques such as DPV, DPP and NPV gave the best results with all drugs in terms of sensitivity, linearity range and detection limits. These techniques is then applied to the determination of the same drugs in spiked river water samples, after a preliminary enrichment step of two orders of magnitude based on solid-phase extraction [32].

The main advantages of the method based on SPE/pulse voltammetry are that it can be applied directly to analysis of surface waters without any separation or derivatization of samples, and it is simple, rapid and inexpensive. Unfortunately, the method cannot be applied to analysis of real environmental samples, because its sensitivity does not allow determination of the drugs at their actual concentrations in surface waters (10^{-7} g/L), even after the enrichment step. Nevertheless, as the concentrations of drugs most frequently found in surface waters have been increasing at a dramatic rate – from 10^{-9} to 10^{-7} g/L in the last 20 years – it will be possible, in principle, to use this SPE/pulse voltammetric method as a good alternative to high-cost and time-consuming chromatographic methods.

7.2.2. Recovery percentages

To determine the percentage recovery, a fixed quantity of organic compound sample solution is taken and it is added three different (5, 10 and 15 μg) levels of working standard of the organic compound in MilliQ water and water sample. At each level the polarograms is recorded seven times and the amount of the organic compound is computed using the formula:

$$Percentage\ recovery = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \qquad (2)$$

Where N is the number of observations, X is the amount of the organic compound added and Y is the amount of the organic compound obtained. The same procedure is adopted for the water samples of organic compound at two different initial concentrations [11].

7.3. Electrochemical method validation: Selective electrodes and ion chromatography

7.3.1. Errors in quantitative analysis: Accuracy and precision

There is a correct value for any measurement, which, nevertheless, remains largely unknown. But, for the purpose of comparison, measurements made by an established method or by an accredited institution are accepted as the true value. All results are subjected to some degree of uncertainty. The difference between the result from the experimental measurement and the true result is called error. The error can be absolute or relative. It is absolute when the numerical difference from the true value is known and it is relative if the error is expressed as a percentage of the measured value.

Errors can occur during water analysis. Depending on the basis of their origin, errors can be of various types: gross, systematic (determinate) and random (indeterminate). Gross error occurs when a measurement is invalidated by a major event, such as the failure of equipment. On the other hand, systemic errors occur when the same experiment is repeated several times and the individual measurements cluster around a mean value. The deviation from the true value is a measure of systematic error and it is often estimated as the deviation of the mean from the true value. They have a Gaussian distribution and equal probability of being above or below the mean. It can be corrected or fixed if the true value of a measurement is known minimizing the difference between the mean of results and the true value or when comparing the same quantity using different analytical methods. Finally, the random errors are associated with the inaccuracies of the worker manipulating an experimental procedure.

Some of the sources of systematic and random errors include improper sampling techniques and handling of sample, mistakes by operators, and inadequate knowledge of a particular experimental procedure, incorrect use, calibration and faulty instruments, erroneous preparation of solutions, and use of contaminated glassware and reagents. Other sources of random error are long experimental techniques, especially when there are fluctuations in the conditions at the various stages of an experiment. Also, the low ionic strength in groundwater when measured with electrode may result in errors since the liquid junction potential across the porous ceramic plug of the calomel electrode varies with the composition to be measured [14].

All measurements have some degree of uncertainty and the error cannot be eliminated completely, although its magnitude and nature can be estimated or reduced with improved techniques. Uncertainty can be estimated with different degrees of precision by experimental measurements and statistical analyses. It is also important to quantify the closeness of an experimental result to the true, generally designated of accuracy. Precision of results is always affected by random error; it can be expressed as the standard deviation of results from their mean obtained from a set of replicates. The mean is the arithmetic average of a set of replicate results.

Almost all analytical process, including sample preparation and analysis, require calibration against standard solutions of known concentrations covering the concentration range expected in the sample. Calibration provides a basis for comparison with a test solution to enable the concentration or other parameter of the test solution to be determined with a high degree of certainty. In water analysis it is common to calibrate equipments using graphical methods and linear regression of internal or external calibration process. The detection limit and the precision of measurements affect the calibration results mainly when the measure is done using the selective electrode. The oscillation during the measurement and the uncertainty of the results is high in this methodology.

7.3.2. Accuracy of chemical analysis by electroneutratily

The concept of accuracy, precision and reliability can be applied to assess the laboratory results. The ability to report what is in the sample is the laboratory accuracy and the ability to reproduce results is the precision. The hydrogeologist can check on the overall procedure of laboratory submitting duplicate samples from the same source or with a known concentration for analysis or requesting the samples to be analyzed in different laboratories. The inter laboratories comparison of results may lead to the detection the problems. To note is that, at low concentrations, there may be huge variations in results of duplicate analysis of water given that the sensitivity of the method is insufficient. Sometimes the incompatibility of different elements found together in a sample can be a warning that something went wrong [41].

The electroneutrality (EN) characteristic of the water is often used as a quick and dirty check on the completeness of the analysis and on the accuracy of the laboratory. The electroneutrality is expressed by equation (3) so that the sum of the charges of all cations must equal the sum of the charges of all the anions present in water solution (principle of electrical neutrality). If the EN is less than 5%, the analysis was correctly done, in terms of ionic balance. When the EN is higher than 5%, the results are not acceptable; either the analysis is erroneous or one or more significant ions were omitted from the analysis. If the difference is greater than 10%, a big error occurred during the process due to reduced concentrations in very pure water (total dissolved solids < 50 mgL^{-1}). In most laboratories, values up to 2% are inevitable. When the EN value is more than 5%, the sampling and analytical procedures should be examined. However, EN is only applicable for major elements since for minor elements it is highly difficult to estimate. For this reason the sum only considers cations Na^+, K^+, Mg^{2+}, and Ca^{2+}, and anions Cl^-, HCO_3^-, SO_4^{2-} and NO_3^- although sometimes other ions such as NH_4^+, Fe^{2+}, H^+, Al^{3+} contribute significantly for the electroneutrality.

$$Electro\ neutrality\ (EN)\% = \frac{(\sum cations - \sum anions)}{(\frac{1}{2}\sum cations + \sum anions)} x100 \tag{3}$$

At the end of the water analysis, the results are examined to assess the consistency of the methodologies used. Table 4 shows the EN applied to data at low and high mineralized water (from 28 μScm^{-1} to 5630 μScm^{-1}) with proposes to evaluate the error in the results.

Sample	(1) EC μScm^{-1}	(2) HCO_3^- $meqL^{-1}$	(3) SO_4^{--} $meqL^{-1}$	(3) Cl^- $meqL^{-1}$	(3) NO_3^- $meqL^{-1}$	Σ anions	(4) Ca^{++} $meqL^{-1}$	(4) Mg^{++} $meqL^{-1}$	(4) Na^+ $meqL^{-1}$	(4) K^+ $meqL^{-1}$	Σ cations	EN %
1	28.5	0.15	0.07	0.19	0.01	0.42	0.20	0.05	0.18	0.00	0.43	2.4
2	33.4	0.15	0.05	0.20	0.01	0.42	0.16	0.00	0.24	0.03	0.43	2.3
3	39.7	0.17	0.09	0.17	0.01	0.44	0.23	0.03	0.18	0.01	0.45	2.2
4	40.0	0.24	0.05	0.26	0.04	0.60	0.23	0.04	0.32	0.02	0.61	1.7
5	69.2	0.15	0.07	0.32	0.20	0.75	0.28	0.09	0.32	0.04	0.73	2.7
6	112.4	0.50	0.26	2.05	0.39	3.20	0.87	0.73	1.63	0.03	3.26	1.9
7	325 457	3.19 3.90	0.61 0.51	0.85 0.57	0.10 0.01	4.75 4.99	2.62* 3.79	0.16	3.17* 0.94	0.52* 0.46	6.31 5.35	28.2 5.8
8	743	6.27	1.52	1.98	0.13	9.9	3.69*		6.13*	0.06*	9.88	0.2
9	997 1080	8.82 5.82	1.89 2.71	3.03 3.38	0.08 0.02	13.82 11.93	3.99* 7.94	0.99	11.6* 3.29	0.12* 0.08	15.71 12.3	12.8 3.1
10	1452	7.45	4.57	4.44	0.71	17.17	5.10*		18.96*	0.15*	24.21	34.0
11	3080	1.85	24.1	12.83	0.03	38.81	7.45*		70.4*	0.49*	78.34	67.5
12	5630	9.26	17.99	14.09	4.35	45.69	8.85*		76.09*	1.45*	86.39	61.6

* Measured with selective electrodes: Consort models 3315B, 3031B, 3041B and 3011B, (1) Measured with multiparameter analyser, Consort, model C833 and electrode S211B; (2) Determined by potentiometric titration method; (3) Quantified using a ionic chromatographic, Dionex, DX-120, columns IonPac AS14 e AG14, eluent 3.5 mM Na_2CO_3/1.0 mM $NaHCO_3$, with chemical suppression and method 300.0 A by EPA; (4) Quantified using a ionic chromatographic, Metrohm, 761 Compact IC, METROSEP Cation 1-2 (6.1010.000), eluent 4 mM $C_4H_6O_6$/1mM $C_7H_5NO_4$, without chemical suppression and method indicated by Metrohm, application bulletin n° 257/1, for the determination of alkaline metals.

Table 4. Electroneutrality (EN) analysis for different groundwater samples

8. Conclusions

The chromatographic techniques for the analysis of inorganic and organic compounds are more expensive but for most of the cases the limits of detection and quantification are lower when compared with the electrochemical methods such as voltammetry or ion selective electrodes. This justifies the use in most of reported results in literature obtained by chromatographic methods when compared with the electrochemical methods. However, in some specific cases and using the best voltammetric technique, it is possible to achieve comparable results of detection for estrogens (e.g. 17α-ethynylestradiol and β-estradiol), antibiotics (e.g azithromycin), pesticides (e.g. ametryn) and PhAC (e.g. propranolol).

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