

*Review Article*

**Green Bioanalytical Chemistry: A Review**

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*Received 26 Feb. 2016; received in revised form 06 April 2016; accepted 06 April 2016*

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**ABSTRACT**

Green Chemistry is the utilisation of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products. Green chemistry is about Waste minimisation at source, Use of catalysts in place of reagents, Using non-toxic reagents, Use of renewable resources, Improved atom efficiency, Use of solvent free or recyclable environmentally benign solvent systems. The introduction of the dimension of green chemistry into the assessment of analytical methods should be a natural development trend in chemistry and should coincide with its general policy. Some of the principles of green chemistry, such as, prevention of waste generation; safer solvents and auxiliaries; design for energy efficiency; safer chemistry to minimize the potential of chemical accidents; development of instrumental methods are directly related to analytical chemistry. Investigation of GAC methodologies encompasses a number of strategies to minimize or to eliminate the use of toxic substances and the generation of wastes. The main focus has been the development of new routes to minimize the amounts of side products and to replace toxic solvents. Recent trends in green bioanalytical chemistry involves various strategies as development of chiral stationary phases for HPLC separation; advances in analytical chemistry using the unique properties of ionic liquids, chemical sensors and biosensors, bio analysis based on nanoporous materials, materials-based approaches to minimizing solvent usage in analytical sample preparation, microplasmas for analytical applications of lab-on-a-chip, applications of nanomaterials in enantio separation and related techniques, recent trends in counter-current chromatography.

**KEYWORDS**

Green bioanalytical chemistry, Green Chemistry, HPLC, Nanomaterials; Waste management.

## **1. INTRODUCTION**

The introduction of the dimension of green chemistry into the assessment of analytical methods should be a natural development trend in chemistry and should coincide with its general policy.

Some of the principles of green chemistry, such as, prevention of waste generation; safer solvents and auxiliaries; design for energy efficiency; safer chemistry to minimize the potential of chemical accidents; development of instrumental methods are directly related to analytical chemistry.<sup>[1]</sup>

### *1.2. History*

The term green chemistry was first used in 1991 by P. T. Anastas in a special program launched by the US Environmental Protection Agency (EPA) to implement sustainable development in chemistry and chemical technology by industry, academia and government. In 1995 the annual US Presidential Green Chemistry Challenge was announced. Similar awards were soon established in European countries. In 1996 the Working Party on Green Chemistry was created, acting within the framework of International Union of Applied and Pure Chemistry. One year later, the Green Chemistry Institute (GCI) was formed with chapters in 20 countries to facilitate contact between governmental agencies and industrial corporations with universities and research institutes to design and implement new technologies.

The first conference highlighting green chemistry was held in Washington in 1997. Since that time other similar scientific conferences have soon held on a regular basis.

The first books and journals on the subject of green chemistry were introduced in the 1990s, including the *Journal of Clean Processes and Products* (Springer-Verlag) and *Green Chemistry*, sponsored by the Royal Society of Chemistry. Other journals, such as *Environmental Science and Technology* and the *Journal of Chemical Education*, have devoted sections to green chemistry. The actual information also may be found on the Internet.

### *1.3. The Idea of Green Chemistry*

The concept of green chemistry has appeared in the United States as a common research program resulting from interdisciplinary cooperation of university teams, independent research groups, industry, scientific societies and governmental agencies, which each have their own programs devoted to decreasing pollution. Green chemistry incorporates a new approach to the synthesis, processing and application of chemical substances in such a manner as to reduce threats to health and the environment. This new approach is also known as:<sup>[2]</sup>

- Environmentally benign chemistry
- Clean chemistry
- Atom economy
- Benign-by-design chemistry

### *1.4. Analytical Chemistry*

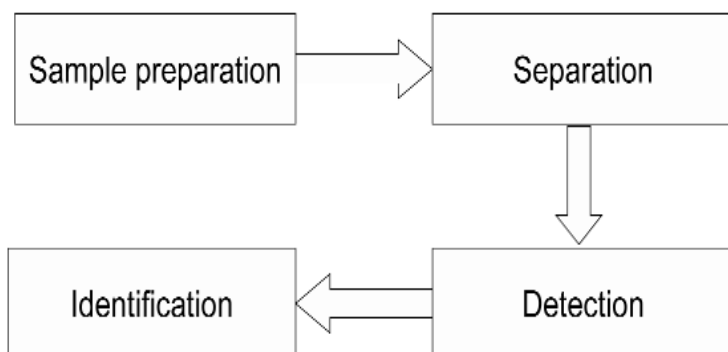
Chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. Qualitative analysis gives an indication of the

identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components. The separation of components is often performed prior to analysis.<sup>[3]</sup>

- Analytical chemistry seeks ever improved means of measuring the chemical composition of natural and artificial materials
- The techniques of this science are used to identify the substances which may be present in a material and determine the exact amounts of the identified substances
- Qualitative: provides information about the identity of an atomic, molecular or bio molecular species
- Quantitative: provides numerical information as to the relative amounts of species

### *1.5. Analytical System*

Every system of chemical analysis can be divided into several steps to which different approaches can be applied (Fig.No.1). Analysis starts with treatment of the sample and its preparation for further separation into components. The components have to be detected in a way that allows the quantification of the separated components and gives necessary characteristics and possibly unique data for the identification of a substance.<sup>[1]</sup>



**Fig.No.1:** The general scheme of an analytical system

### *1.5. Green Chemistry*

#### *1.5.1. Definition*

Green Chemistry is the utilisation of a set of principles that reduces or eliminates the use or generation of hazardous substances in the design, manufacture and application of chemical products.

#### *1.5.2. Green Chemistry is about*

- Waste Minimisation at Source
- Use of Catalysts in place of Reagents
- Using Non-Toxic Reagents

- Use of Renewable Resources
- Improved Atom Efficiency
- Use of Solvent Free or Recyclable Environmentally Benign Solvent systems

### *1.5.3. Why Do We Need Green Chemistry?*

- Chemistry is undeniably a very prominent part of our daily lives.
- Chemical developments also bring new environmental problems and harmful unexpected side effects, which result in the need for ‘greener’ chemical products.
- A famous example is the pesticide DDT.
- Green chemistry looks at pollution prevention on the molecular scale and is an extremely important area of Chemistry due to the importance of Chemistry in our world today and the implications it can show on our environment.
- The Green Chemistry program supports the invention of more environmentally friendly chemical processes which reduce or even eliminate the generation of hazardous substances.
- This program works very closely with the twelve principles of Green Chemistry.<sup>[4]</sup>

### *1.6. Green Analytical Chemistry*

- The relationship between green chemistry and analytical chemistry can be treated in two ways.
- Analytical chemistry is a subject to control and justify green chemistry. This is where analytical chemistry is an efficient tool for conformation of the green result of a chemical product or technology.
- On the other hand, chemical analysis methods need solvents, reagents, and energy, and they generate waste.
- The principles of green chemistry, suggested by Anastas and Warner <sup>[2]</sup>, are directly related to analytical chemistry as well, the most important of them being,
  - Prevention of waste generation;
  - Safer solvents and auxiliaries;
  - Design for energy efficiency; and
  - Safer chemistry to minimize the potential of chemical accidents.

#### *1.6.1. The Principles of Green Chemistry*

Green chemistry is commonly presented as a set of twelve principles proposed by Anastas and Warner <sup>[2]</sup>. The principles comprise instructions for professional chemists to implement new chemical compounds, new synthesis and new technological processes.

The first principle describes the basic idea of green chemistry — protecting the environment from pollution. Remaining principles are focused on such issues as atom economy, toxicity, solvent and other media using consumption of energy, application of raw materials from

renewable sources and degradation of chemical products to simple, nontoxic substances that are friendly for the environment.

### **1. Prevention**

It is better to prevent waste than to treat or clean up waste after it has been created.

### **2. Atom Economy**

Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

### **3. Less Hazardous Chemical Syntheses**

Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

### **4. Designing Safer Chemicals**

Chemical products should be designed to effect their desired function while minimizing toxicity.

### **5. Safer Solvents and Auxiliaries**

The use of auxiliary substances (e.g. solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

### **6. Design for Energy Efficiency**

Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

### **7. Use of Renewable Feed stocks**

A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

### **8. Reduce Derivatives**

Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

### **9. Catalysis**

Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

### **10. Design for Degradation**

Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

### **11. Real-time analysis for Pollution Prevention**

Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances

### **12. Inherently Safer Chemistry for Accident Prevention**

Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

The selected examples for implementing the 12 principles in laboratory and industry are presented in Table No.1.

**Table No.1:** Examples of Implementation of Green Chemistry Principles into Practice.

Sr. No.	PRINCIPLE	EXAMPLES
1	Preventions	Use of solvent less sample preparation techniques <sup>[5]</sup>
2	Atom economy	Hydrogenation of carboxylic acid to aldehyde using solid catalysts
3	Less hazardous chemical Synthesis	Adipic acid synthesis by oxidation of cyclohexine using hydrogen peroxide <sup>[6]</sup>
4	Designing safer chemicals	New , less hazardous pesticide (e.g. Spinosad ) <sup>[7]</sup>
5	Safer solvents and auxiliaries	Supercritical fluid extraction , synthesis in ionic liquids <sup>[8]</sup>
6	Design for energy efficiency	Polyolefines-polymer alternatives to PWC (polymerization may be carried with lower energy consumption <sup>[9]</sup>
7	Use of renewable feed stocks	Production of surfactants <sup>[10]</sup>
8	Reduce derivatives	On-fibre derivatization vs. derivatization in solution in sample preparation <sup>[11]</sup>
9	Catalysis	Efficient Au(III)-catalysed synthesis of b-enaminones from 1,3-dicarbonyl compounds and amines <sup>[12]</sup>
10	Design for degradation	Synthesis of biodegradable polymer <sup>[13]</sup>
11	Real-time analysis for pollution prevention	Use of in-line analyser for waste water monitoring
12	Inherently safer chemistry for accident prevention	Di-Me carbonate(DMC) is an environmentally friendly substitute for Di-Me sulphate and Me halides in methylation reactions <sup>[14]</sup>

### *1.7. Application of the Principles of Green Chemistry*

#### *1.7.1. In Analytical Chemistry*

Analytical chemistry is considered to be a small-scale activity, but this is not always true in the case of controlling and monitoring laboratories whose number of runs performed is high. This makes an analytical laboratory comparable with the fine chemicals or pharmaceutical industry. The use of instrumental methods instead of wet chemistry, automation, and minimization is a new trend in analytical chemistry, making this branch of chemistry more sustainable.

In this study, widespread separation methods are considered and an attempt is made to characterize them against the above-mentioned principles. Special attention is given to capillary electrophoresis (CE), which provides a very good opportunity to improve analytical chemistry by replacing many chromatographic methods that consume large volumes of solvents. The choice of different solvents and micronization in analytical chemistry is also discussed. <sup>[1]</sup>

*1.7.1.1. Separation Methods in Analytical Chemistry*

The increasing demand for faster, more cost-effective, and environmentally friendlier analytical methods is a major incentive to improve the classical procedures used for sample treatment in environmental analysis. In most classical procedures, the use of rapid and powerful instrumental techniques for the final separation and detection of analytes contrasts with the time-consuming and usually manual methods used for sample preparation, which slows down the total analytical process. The efforts made in this field in the past 10 years have led to the adaptation of the existing methods and development of new techniques to save time and chemicals, and improve overall performance. In these approaches, miniaturization has been a key factor in designing integrated analytical systems to provide higher sample throughput and/or unattended operation. The list of possible environmentally friendly sample-preparation techniques is long and includes the following more or less self-explanatory types:

**a) SOLVENT MICRO-EXTRACTION**

- 1) In-vial liquid–liquid extraction (in-vial LLE)
- 2) Single-drop micro-extraction (SDME)
- 3) Liquid-phase micro-extraction (LPME)
- 4) Liquid–liquid–liquid micro-extraction (LLLME)

**b) SORPTION MICRO-EXTRACTION AND LIQUID DESORPTION**

- 1) Solid-phase extraction (SPE)
- 2) In-tube solid-phase micro-extraction (in-tube SPME)
- 3) Fibber-in-tube solid-phase extraction (fibber-in-tube SPE)
- 4) Single short column (SSC)

**c) THERMAL DESORPTION**

- 1) Solid-phase micro-extraction (SPME)
- 2) Stir-bar-sorptive extraction (SBSE)

**d) MATRIX SOLID-PHASE DISPERSION**

- 1) Matrix solid-phase dispersion (MSPD)

**e) ENHANCED FLUID/SOLVENT EXTRACTION**

- 1) Supercritical-fluid extraction (SFE)
- 2) Pressurized-liquid extraction (PLE)
- 3) Subcritical-water extraction (SWE)
- 4) Microwave-assisted extraction (MAE)
- 5) Sonication-assisted solvent extraction (SASE)

**f) THERMAL DESORPTION FROM SOLIDS**

- 1) Direct thermal desorption (DTD)
- 2)

As regards liquid samples, it is probably true to say that SPE (in all of its modes and formats) is the preparation method of first choice, especially when more polar analytes are involved. An online (and automated) solid-phase extraction–liquid chromatography (SPE-HPLC) is a fully mature approach and is, moreover, the technique that can easily be miniaturized. As for most of the other sample-preparation techniques, many of these were introduced only very recently, and

novel approaches keep emerging. SPME and SBSE are primarily useful for liquid samples. Although a wider range of analytes can be covered with the latter technique, less expensive automation and easy handling seem to be advantages over the former approach.

### *1.7.2. Electrophoresis*

The “green” principles of analytical chemistry can be most easily achieved in electrically driven separation methods due to the low consumption of solvent and sample. A typical volume of the separation capillary in capillary zone electrophoresis (CZE) is about 5 $\mu$ L, and such volume is also required for eluent. Although the low consumption of the eluent has been frequently pointed out as an advantage of CZE over high-pressure liquid chromatography (HPLC), this has never been discussed in the context of green chemistry.

The comparison of these two methods (Table No. 2) indicates directions in the development of separation methods—decrease of sample size, low consumption of solvent, higher selectivity, faster analysis time, mechanically simpler instrument. The situation is changing very fast in the CZE where growing number of new methods and instrumental solutions are published in journals and presented at conferences. During this time, CZE is overcoming the problems of instability of separation and lack of sensitivity of detectors, and more laboratories are starting to consider the capillary electrophoresis (CE) as a standard procedure for the separation of complex samples.

### *1.7.3. Micronization in Separation Methods*

Micronization is an important approach to minimize the waste generated and is essential for analysis when the amount of sample available is very small (less than microliters). This is also influenced by combinatorial chemistry, which has stimulated the search for the alternative separation approaches. The key to a rapid and efficient synthesis is not only the parallel arrangement of reactions, but simple workup procedures so as to circumvent time-consuming and laborious purification steps.

The similar reasoning applies even more to microfluidics in the case of which the amount of eluent consumed is even lower than in CZE. Miniaturized total analysis systems were first proposed as a novel concept for chemical sensing in 1990, developing the field of microfluidics and leading to the vision of lab-on-a-chip. TAS integrates all steps required in chemical analysis :- sampling, pre-processing, and measurement into a single device via miniaturization, resulting in an improved selectivity and detection limit compared to conventional sensors. Also, the dramatic downscaling and integration of chemical assays hold a considerable promise for a faster and simpler onsite monitoring of priority pollutants and make these analytical microsystems particularly attractive as “green analytical chemistry” screening tools.

### *1.7.4. Alternative Solvents*

Most methods of sample treatment and further separation into components use solvents, and this is the main source of organic waste. The search for an alternative solvent is an important step in making an analysis “greener” and environmentally friendlier. It appears that alternative solvents like supercritical fluids and ionic liquids (ILs) have one extra dimension which makes them even



more attractive for researchers—tunability. The ability to fine-tune the properties of the solvent medium will allow this to be selected to replace specific solvents in a variety of different processes or create new methods for processing (analysing) samples.

#### *1.7.4.1. Supercritical fluids*

The most popular substances used in supercritical fluid processing: water ( $T_k = 374.1\text{ }^\circ\text{C}$ ;  $p_k = 218.3\text{ atm}$ ) and carbon dioxide ( $T_k = 31.1\text{ }^\circ\text{C}$ ;  $p_k = 72.8\text{ atm}$ ). The supercritical CO<sub>2</sub> is nontoxic, easy to purify, and relatively inert.

Using supercritical fluids instead of organic solvents for extraction is becoming more popular for most liquid–solid extractions, especially when the supercritical CO<sub>2</sub> is used as a solvent. There are already some official U.S. EPA methods where the supercritical CO<sub>2</sub> is replacing organic solvents in sample preparation to extract target compounds. This replacement saves analysis time and minimizes the consumption of organic solvent. An additional green aspect of using gases in supercritical conditions allows saving of energy because of the absence of distillation and solvent evaporation.

An easy tuning of the solubility and other parameters of solvent by temperature and/or pressure is possible. In this way, the use of solvents in supercritical conditions means expanding the overall spectrum of solubility, polarity, and volatility properties of solvents and mobile phases. The same solvent can be used in different applications and procedures.

Despite all that is positive, the methods using supercritical fluids are not widespread, and there is a lack of standard procedures in controlling laboratories. This may be explained by the fact that methods are technically complicated and there is a need for a careful and precise control of processing (extraction) parameters because the solubility in the supercritical fluid is very sensitive to the changes of pressure and temperature. Also, collection of the analyte after the extraction is a critical step for analysis performance, because of possible losses of analyte. Different systems have been developed, among them liquid trap, solid-phase trap, cold trap, etc. . All this means that the process of extraction using supercritical fluids is not fully studied, and there is a lot of room for further development to establish reliable analysis methods based on supercritical fluids. One possible direction is online coupling of the extraction unit and the separation unit with suitable detector where possible losses of analytes when transferring from one instrument to another are minimized.

Using supercritical fluids in chromatography, it is possible to fill in the gap between gas and liquid, providing a continuum of mobile-phase properties and widening possibilities of one instrument.

#### *1.7.4.2. Ionic liquids*

ILs are salts with a melting point close or below room temperature. They form liquids composed of ions. This gives these materials, when used as solvents, the potential to behave very differently from conventional molecular liquids. Their physical properties are very promising for green chemistry applications: they are non-volatile liquids and good solvents for many organic and inorganic materials.

One of the advantages of ILs is thermal robustness. This means that the wide thermal operating range (typically,  $-40$  to  $200$  °C) is possible that enables a wide range of kinetic control for reactions that proceed in ILs.

The most popular ILs are 1-alkyl-3-methyl imidazolium salts and 1-alkyl pyridinium salts with a multiple selection of anions. The search for applications is intensifying in each area of analytical chemistry—electrochemistry, chromatography, and electrophoresis, even mass spectrometry. The number of publications on ILs is increasing almost exponentially. The basis for this activity is an easy preparation of salts with different ion constituents. This ability might best be described as the “chemical tunability” of ILs—a class of solvents with members possessing similar physical properties but different chemical behaviour. ILs can be applied not only in the existing methods whose sensitivity and selectivity of analysis need to be improved, but their different behaviour and properties can offer original solutions in chemical analysis as well.

ILs have good solvating properties, which together with a large range of spectral transparency make them suitable solvents for spectroscopic measurements of a wide range of species including organic, inorganic, and organometallic compounds. Notably, a variety of transition-metal complexes, which are unstable in other media, may be studied in room-temperature ILs. The use of room-temperature ILs as solvents for UV, visible, and IR spectroscopy for highly charged complex ions with high or low-oxidation states like  $[MX_n]_y^-$  complexes (M = transition metal; X = Cl, Br) circumvents the problems of solvation and solvolysis and permits reliable solution spectra to be recorded for these species. Spectroscopic measurements of solvatochromic and fluorescent probe molecules in room-temperature ILs provide insights into solvent intermolecular interactions, although interpretation of the different and generally no correlated “polarity” scales is sometimes ambiguous.

It is demonstrated that task-specific ILs have advantages over common solvents used as separation media in an LLE process achieving high efficiencies and selectivities of separation. The development of simple systems for cleaning of fuels is of high practical importance. The extraction of sulphur and nitrogen compounds from gasoline and diesel oil by ILs indicates that such a process could be an alternative to common hydrodesulphurization for deep desulfurization down to values of 10 ppm S or even lower.

### *1.8. Greener Sample Pretreatments*

The public concern over protecting the environment has induced chemists to look for new sample-preparation techniques that could reduce the adverse environmental impact of organic solvents. <sup>[15]</sup>

Table No.3 summarizes the characteristics of the main sample-treatment methods developed to avoid or to reduce use of organic solvents, as selected from papers identified in the literature as describing clean, green or environmentally friendly methods.

Microwave-assisted extraction (MAE) has been applied to the extraction of organic compounds from very different types of matrix. It employs less organic solvent and a shorter extraction time than traditional extraction methods.

MAE has been proposed for GAC extraction of atrazine, simazine and prometryne from synthetic-soil samples, using water and some organic solvents. <sup>[16]</sup> Triazines could be efficiently

extracted with 30-ml water as they provide a cheap, safe, environmentally-friendly alternative to organic solvents.

MAE can be used together with a micellar system to extract organic compounds from soils. This method was used for the analysis of phenols in soils and provided a viable, greener alternative by replacing organics with surfactants.<sup>[17]</sup>

*1.8.1. The main advantages of MAE are:*

- i) Short extraction time;
- ii) Reduction in the amount of sample required;
- iii) High sample throughput;
- iv) Reduced cost; and,
- v) Great safety, since it does not require the use of hazardous materials and can be contained in closed reactors.

SFE also offers an attractive alternative to overcome the unfavourable effect of non-polar organic solvents employed in extracting non-polar compounds.

The main advantages of SFE are:

- i) It can achieve high concentrations;
- ii) It is quantitative;
- iii) It is fast;
- iv) It is simple; and,
- v) It is selective.

SFE is also an environmentally-friendly analytical methodology that can be automated easily and completely. SFE has been employed in extraction of pesticide residues from plants<sup>[18]</sup> and fruits.<sup>[19]</sup>

In the same way, accelerated solvent extraction (ASE), also known as pressurized solvent extraction (PSE), pressurized fluid extraction (PFE), pressurized liquid extraction (PLE) and solvolytic extraction, is a solid-liquid extraction process performed at high temperatures (50–200 C) and high pressures (10–15 MPa). ASE is a form of PSE similar to SFE, although, in ASE, the extraction is carried out under pressure to maintain the solvent in its liquid state at high temperature, but always below its critical condition. Although the solvent used in ASE is usually organic, pressurized hot water can also be used. Nowadays, ASE is considered a potentially, attractive, alternative technique for extracting organic compounds from environmental or biological matrices<sup>[20]</sup> and for food applications<sup>[21]</sup>, its main advantages over traditional extraction methods being dramatic decreases in the amount of solvent used and the extraction time.

In SPME, sorbent-coated silica fibres are used to extract analytes from aqueous or gaseous samples. After extraction, the fibres can be desorbed by using small amounts of organic solvents or transferring thermally the fiber directly into the injection port of a GC. Based on the first approach, seven phenols were extracted from water using oxidized multi-walled carbon nanotubes for extraction and 70 IL of acetonitrile : water (70:30) for elution<sup>[22]</sup>. However, SPME

with thermal desorption has been used for the analysis of snow samples by GC <sup>[23]</sup>, avoiding completely the use of organic solvents.

SBSE is not as popular a technique as SPME, but it has been used as a green alternative for extracting pesticide residues in sugarcane<sup>[24]</sup>.

LPME is essentially miniaturised liquid–liquid extraction, in which the analyte moves between the bulk aqueous phase and a very small volume of organic solvent. Recent developments use a single droplet of solvent, suspended at the tip of a needle and exposed to the sample solution (SDME), and they have been employed for extracting aniline derivatives from water samples in a two-step procedure<sup>[25]</sup>.

By using membrane-based devices, different ideas have been developed to extract and pre-concentrate different types of analytes, avoiding or reducing the amount of organic solvents.

In LLLME, a thin film of organic solvent is immobilized in the pores of a polypropylene hollow fiber placed on the exterior of the hollow fiber carrying the donor aqueous phase. The pH of sample is adjusted to neutralize the target compound and the internal channel of the fiber acts as the acceptor aqueous phase, with a pH adjusted to ionize the target compounds. With stirring, neutral compounds in the donor phase are extracted into the organic film on the fiber and then back extracted into the acceptor phase inside the fiber. This technique has been used in extracting herbicides from milk<sup>[26]</sup> and aniline derivatives from water samples<sup>[25]</sup>.

Another membrane-based approach is called supported- liquid-membrane extraction (SLME) or membrane- assisted solvent extraction (MASE). In this case, separation occurs when compounds are transported to a greater extent than others from a donor phase through the membrane into an acceptor phase. For non-porous membranes, the efficiency of the transport of compounds depends to a large extent on the partition coefficient between the different parts of the extraction system, so good selectivity can be achieved by choosing appropriate membrane material and organic acceptor phases. This technique has been used for green extraction of pesticide residues from juice <sup>[24]</sup> using only 800 l of organic solvent.

#### *1.8.2. CPE offers advantages:*

- i) It is inexpensive;
- ii) Good concentration efficiency;
- iii) Low environmental toxicity; and,
- iv) Safety.

Surfactants can dissolve organic compounds entrapped in the micellar phase. Complete separation of the micellar phase and the original water phase is achieved as a function of temperature and the presence of some salts. The use of surfactants to increase the solubility of organic compounds in water has been successfully combined with SPE to extract pesticides from vegetables.<sup>[27]</sup>

Beyond sample-preparation methods previously commented upon, derivatization of surface molecules is a valuable tool in making sample treatment greener. Molecular imprinting has become a powerful method for preparing robust materials that can recognize specific chemical

species. However, applications as real alternatives or complements to biomolecules are limited to some extent by inherent shortcomings (e.g., non-specificity and low affinity of binding sites). However, chelating solid phases can be made by immobilizing chelating agents on appropriate supports. This methodology has been successfully applied to extracting trace-metal ions from water samples<sup>[28]</sup>

### *1.9. Green Analytical Methodologies*

Investigation of GAC methodologies encompasses a number of strategies to minimize or to eliminate the use of toxic substances and the generation of wastes. The main focus has been the development of new routes to minimize the amounts of side products and to replace toxic solvents.

#### *1.9.1. Screening Methodologies*

It is clear that one of the aims of the GAC is to reduce the number of samples to be analysed by classical, non environmentally friendly methodologies and also to reduce the waste generated as a result. However, this reduction in the number of samples for analysis should be done in a safe, controlled way. It can be achieved by using the so-called “screening methods” that involve procedures to indicate whether target analytes are present above or below a threshold but also comprise those that provide fast acquisition of semi-quantitative data about all components of a sample.

In general, screening methods tend to be qualitative, involving little or no sample treatment, and the response is used for immediate decision-making, with confirmation requiring a conventional alternative. Put plainly, a screening method is a simple measurement that provides a “yes/no” response, avoiding the need to process a large number of samples so as to limit complex sample treatments of conventional techniques to those samples with positive responses.

It is important to note the great efforts made by the scientific community in this direction in recent years. In this respect, it is interesting to mention immunoassays (IAs), which were first developed for monitoring insulin in blood in 1960 and are now usually employed in clinical chemistry to determine hormones, drugs and viruses in biological samples. Enzyme-linked immune sorbent assay (ELISA) is the most common IA technique employed. The main advantage of those biology-based analytical-screening techniques is the complete replacement of organic solvents by aqueous media and the consequent reduction of toxic wastes.

As well as so-called rapid colorimetric tests employed to determine the presence of some inorganic compounds, we should mention the different analytical techniques commonly employed for rapid analysis of elemental composition of samples,

- X-ray fluorescence, which provides excellent qualitative or semi-quantitative data without any pre-treatment inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS), which are the most sensitive, selective techniques for multi-elemental determination of several components in the same sample

- Mass spectrometry (MS), which offers good advantages for selective determination of compounds and sensitive determination of organic pollutants, providing fast sequential information on multiple compounds with high level of sensitivity and,
- Ion-mobility spectrometry (IMS), which is usually used for screening explosives at airports and detection of compounds from pyrolysis, detecting chemicals for the military industry, including warfare agents, and monitoring stack-gas emissions in industry.

### *1.9.2. Replacement of Toxic Reagents*

The use of flow-based procedures has contributed to achieving greener analytical methods, by automation and miniaturization, but also by replacing toxic reagents by non-contaminating reagents.

Guava leaf extract has been used as an alternative natural reagent for the FI determination of Fe without the need for further purification.

The use of SPE in combination with FIA systems has been proposed as a way of replacing toxic reagents. A time-based, multi-syringe FI (MSFI) approach was developed for automating disk-based sorbent extraction of nitro-substituted phenol isomers followed by on-line simultaneous determination of individual species by diode-array spectrophotometry. The method involved on-line enrichment of target analytes and removal of potentially-interfering matrix components. The nitro phenol isomers were eluted with an alkaline solution and the UV-vis spectra were recorded. Deconvolution of strongly-overlapping spectra was done using multivariate regression models.

A GAC procedure was developed for nitrate determination in natural waters based on direct spectrophotometric measurements, using an FIA system with an anion-exchange column. The proposed method employed only one reagent (HClO<sub>4</sub>) and avoided interferences of humic acid, NO<sub>2</sub>, PO<sub>3</sub>\_4, Cl, SO<sub>2</sub>\_4 and Fe<sup>3+</sup>. The same principle was applied in the SIA determination of phosphate in urine. The interferences of Ca, due to the crystallisation of calcium phosphate, were avoided using a cation-exchange resin.

### *1.9.3. Minimization of Wastes*

The substitution of all toxic reagents employed in chemical analysis is not easy, so reduction of the amounts employed should be also considered. In this sense, multi commutation has the advantages of minimizing both reagent consumption and waste generation. In this approach, micro-volumes of samples and reagents are sequentially inserted into the reaction coil of a single line manifold, providing a simple system, suitable mixing conditions, and easy optimization of the sample/reagent ratio, and avoiding excessive use of reagents. Multi commuted flow systems can be designed with solenoid micro-pumps that can reproduce the micro-volumes of solutions dispensed, thus down scaling the methods automated.

### *1.9.4. Recovery of Reagents*

The recovery of reagents provides a satisfactory way to cut down on the side effects of analytical methods, as it is an important step towards achieving zero emissions in research.

Laboratory wastes are not discarded directly into the environment but are treated off-line. However, this practice increases the running costs of analytical laboratories and creates a

problem through the accumulation of toxic residues. A green alternative to waste storage is therefore on-line recovery of solvents and toxic or expensive reagents.

A useful way of recovering reagents on-line is to incorporate a cat ion exchange mini-column after a flow through cell. In this way, the main reagent can be regenerated on-line and heavy-metal ions can be removed from the waste, thus reducing both reagent consumption and waste generation.

#### *1.9.5. On-Line Decontamination of Wastes*

As described above in the context of GAC, special emphasis should be placed on assessing the environmental impact of new methods in addition to traditional goals (e.g., accuracy, precision, sensitivity, and LODs). In 1994, different flow methods proposed involved an additional effort to detoxify wastes generated. Basically, on-line treatment of waste involves adding a decontamination step after analytical measurement to obtain clean waste.

In 1999, to detoxify wastes on-line, it was suggested using:

- i) Thermal degradation,
- ii) Oxidative detoxification,
- iii) Photo degradation, and,
- iv) Bio degradation,

#### *1.9.6. Reagent-Free Methodologies*

Methods based on direct measurements of untreated samples can be the best options to make analytical determinations greener.

FT-Raman spectrometry has been used as a reagent free methodology. The quantitative determination of different analytes performed directly on the sample, solid or liquid, using standard glass vials as sample cells. eliminates reagent consumption and avoids waste generation, also reducing the need for sample handling and the contact of the operator with potentially toxic substances. This technique has been used for the determination of iprodione in solid-pesticide formulations and for the analysis of sweetener formulations.

The evolution of Chemometric has supported development of solvent-free methodologies based on mathematical treatment of signals obtained by direct measurements on untreated solid or liquid samples. Partial least squares NIR (PLS-NIR) provides a direct procedure for pesticide determination in formulations. NIR spectroscopy using transmittance measurements, in combination with Chemometric, has proved to be a powerful tool for the determination of the peroxide index in edible oil using PLS calibration without the need to use solvents or derivatization reagents.

Another example of solvent-free methodology is based on photo acoustic-Fourier-transform infrared-spectroscopy (PAS-FT-IR), which was applied to determine Mancozeb in agrochemicals. The method involved direct measurement of the transmittance spectra of solid samples.

Other reagent-free methodologies proposed in the literature include photo-induced mercury cold/chemical vapour generation (PI-CVG), which uses sample matrix as a reductant. It was proposed for atomic fluorescence detection of trace mercury in wine or liquor samples. The new

method is based on the reduction of mercury by wine ethanol irradiated with UV light. The standard-addition method was used for real sample analysis to achieve the reagent-free goal.

Direct measurement of the elemental composition of samples through energy dispersion X-ray fluorescence (ED-XRF) is not called a green methodology. However, it is clear that these measurements involve no sample treatment and can be done without creating wastes. <sup>[15]</sup>

### *1.10. Recent Trends in Green Bioanalytical Chemistry*

#### *1.10.1. Development of Chiral Stationary Phases For High-Performance Liquid Chromatographic Separation*

Chromatography technology based on chiral stationary phases (CSPs) for enantio separation is widely used for resolution and preparation of biochemicals (e.g., drugs, foods, fragrances and pollutants). In this review, we focus on the development of CSPs for high-performance liquid chromatography (HPLC), including the recognition mechanism, applications and limitations of classical CSPs, newly discovered types of CSP, and also the methods for the rational design of future CSPs on the basis of computational chemistry <sup>[29]</sup>

#### *1.10.2. Advances In Analytical Chemistry Using The Unique Properties Of Ionic Liquids*

Ionic liquids (ILs) are regarded as non-molecular solvents, as they are composed entirely of cations and anions. ILs possess several excellent unique properties (e.g., low volatility, high thermal stability, specific electrochemical characteristics, easy design, tunable viscosity, and miscibility with water or organic solvents). These properties make ILs attractive candidates for various analytical applications, the number of publications on which has increased exponentially in the past decade. This article presents an overview of representative applications of ILs in advances in analytical chemistry benefited from the unique properties of ILs, including the development achieved by using ILs as extraction solvents, dissolution solvents and separation media. <sup>[30]</sup>

#### *1.10.3. Graphenes in Chemical Sensors And Biosensors*

This review covers the current state of the art of using graphenes in electrochemical and optical chemical sensors and biosensors. We first discuss the various types of graphenes, graphene oxides and the like, and also give a definition for each. This is followed by a section on the use of non-modified materials (“plain graphenes”) in mainly electrochemical and optical chemical sensors and (bio) sensors. The next section summarizes the various kinds of sensors based on composite materials containing graphenes, with sub-sections on electro-chemical, field-effect-transistor-based, fluorescent, chemiluminescent and colorimetric sensors. We show that the use of graphenes alone or in composite form can improve the performance of chemical sensors and biosensors, particularly with respect to dynamic ranges, lower limits of detection, selectivity and size of instrumentation. <sup>[31]</sup>

#### *1.10.4. Bio Analysis Based On Nanoporous Materials*



Nano porous materials possess nanometer-sized pore distribution and are widely used in bio sensing. The unique properties of Nano porous materials include large surface area, good chemical, thermal and mechanical stability, very uniform pore distribution with tunable pore size, high adsorption capacity, and an ordered porous network for free diffusion of substrates and reaction products. Usage of Nano porous materials can significantly improve the analytical performance of biosensors in biomedical diagnosis and monitoring of food and environmental quality. This article reviews some major advances in bio analysis based on Nano porous materials, including bio sensing based on zeolite, mesoporous silica, mesoporous carbon, mesoporous metal and metal oxide. These Nano porous materials have showed promising applications in electrochemical bio sensing, electro catalysis, proteomics analysis and bio recognition.<sup>[32]</sup>

#### *1.10.5. Materials-Based Approaches To Minimizing Solvent Usage In Analytical Sample Preparation*

The marriage of materials and analytical chemistry has been an important development in sample preparation. Also, miniaturized sample preparation is gaining more interest, with the advantages of much lower consumption of organic solvents, improved labour efficiency and shorter extraction times. This review describes recent developments of sorbent-based sample-preparation methods, including primarily procedures for minimizing solvent usage and focusing on preparation and applications of interesting materials as sorbents. We discuss and assess several novel materials (i.e. graphene, ionic liquids, polymeric ionic liquids, molecularly-imprinted polymers, and metal-organic frameworks) in some of the latest published works on sample preparation. We emphasize the performance of these new sorbents in the extraction of analytes from environmental, biological and food samples, and evaluate their merits and shortcomings.<sup>[33]</sup>

#### *1.10.6. Microplasmas for Analytical Applications of Lab-On-A- Chip*

The concept of “lab-on-a-chip” has expanded within recent years and has numerous potential applications in analytical chemistry. As a result, greater emphasis has been placed on research into microplasmas, which can act as sample-excitation sources for lab-on-a-chip devices. These miniaturized versions of full-sized plasma sources have become popular for optical emission spectrometry, mass spectrometry and atomic spectroscopy. Microplasmas for lab-on-a-chip can offer an element-specific or molecule-specific, label-free detection method. The development of microfabrication techniques makes it possible to integrate microplasma sources on chips within analytical instruments, reducing the operating and purchasing costs while increasing instrument portability. We review current and prospective generation, fabrication and application of microplasma chips in lab-on-a-chip research.<sup>[34]</sup>

#### *1.10.7. Applications of Nanomaterials in Enantio separation And Related Techniques*

Chirality is an important, universal phenomenon in nature. For the in-depth study of pharmacology and biology, efficient enantioselective technologies are indispensable. Nanomaterials with large surface-to-volume ratio and specific physical and chemical properties have demonstrated great potential in chiral discrimination. Many publications show that

utilization of nanomaterials could improve the selectivity, the stability and the efficiency of enantioseparation. This review summarizes the applications of various enantioselective nanomaterials, including mesoporous silica, organic polymers, metal-organic frameworks, metal nanomaterials, magnetic nanomaterials, carbon nanotubes and well-oriented chiral nanolayers. After proper preparation and modification, these functionalized nanomaterials are effective for chiral separation through their specific enantioselective adsorption, especially when they are used as stationary or pseudo-stationary phases in chiral chromatographic separation, such as thin-layer chromatography, high-performance liquid chromatography, gas chromatography and capillary electrophoresis.<sup>[35]</sup>

#### *1.10.8. Recent Trends in Counter-Current Chromatography*

Counter-current chromatography (CCC) has been widely used as a preparative chromatography technique since Ito first developed it in the late 1960s. This technique uses a support-free liquid stationary phase, which is retained by gravity or centrifugal force. The analytes can access the whole stationary phase, leading to effective separation. In the past few decades, high-speed CCC has been a versatile preparative-scale separation technique, especially in isolation of natural products. The aim of this review is to describe different aspects of recent interesting applications in CCC, including instrumentation, solvent selection and elution methods.<sup>[36]</sup>

## **2. REFERENCES**

1. Koel M, Kaljurand M (2006) Application of the principles of green chemistry in analytical chemistry. *Pure Appl. Chem.* 78:1993–2002.
2. Anastas P T, Warner J C(1998) *Green Chemistry:Theory and Practise*, Oxford University Press, Oxford. 2:30.
3. Holler F, Skoog J, Douglas A, Donald M, *et al.*( 1996) *Fundamentals of analytical chemistry*. Philadelphia, Saunders College Pub.1:1982 - 859.
4. <http://www.worldofteaching.com>
5. Namieoenik J, Wardencki W (2000) Solventless sample preparation techniques environmental analysis. *J. High Resol. Chromatogr.* 23:297.
6. Sato K, Aoki M, Noyori R A (1998) Green Route to Adipic Acid: Direct Oxidation of Cyclohexenes with 30 percent hydrogen peroxide. *Science.* 281: 1646.
7. [http:// www.epa.gov/greenchemistry](http://www.epa.gov/greenchemistry)
8. Bardley D, Dyson P, Welton T(2000) Room temperature ionic liquids. *Chem. Rev.* 9 (5):18.
9. Romano U, Garbassi F. (2000) The environmental issue, A challenge for new generation polyolefins. *Pure Appl. Chem.*72:1383.
10. Nicolas N, Benvegnu T, Plusquellec D. (2002) Surfactants from renewable resources. *Actualite Chimique.*70:11-12.
11. Stashenko EE, Puertas AM, Salgar W, Delgado W, Martinez JR, *et al.* (2000) Solid-phase microextraction with on fibre derivatization applied to the analysis of volatile carbonyl compounds. *Chromatogr. J.* 175: A.886.

12. Acardi A, Bianchi G, Giuseppe S, Marinelli F, *et al* (2003) Gold catalysis in the reaction of 1,3-dicarbonyls with nucleophiles. *Green Chemistry*. 5 (1): 64.
13. Scott G (2000) Green polymers. *Polym. Degrad. Stab.* 68 (1): A.886.
14. Tundo P, Selva M, Memoli S (2000) Dimethylcarbonate as a green reagent(Green Chemical Syntheses and Processes). *ACS Symp. Ser.* 87.
15. Armenta S, Garrigues S, De la Guardia M (2008) *Green Analytical Chemistry*. TrAC. 27.
16. Xiong G, Tang B, He X, Zhao M, Zhang Z, Zhang Z, *et al.* (1999) Comparison of microwave-assisted extraction of triazines from soils using water and organic solvents as the extractants. *Talanta*. 48:333.
17. Ferrera Z, Santana RJ, Santana MC (2005) Comparison of microwave-assisted extraction of phenols from soils using water and organic solvents as the extractants. *Anal. Bioanal. Chem.* 382: 125.
18. Zuina VG, Yariwake JH, Bicchi C (2003) Supercritical fluid extraction. *Chromatogr.J.*;125: A 985.
19. Pearce KL, Trenerry VC, Were S. (1997) Supercritical fluid extraction of pesticide residues from strawberries. *J Agric. Food Chem.* 45: 153.
20. Beck J, Totsche KU, Knabner K (2008) A rapid and efficient determination of natural estrogens in soils by pressurised liquid extraction and gas chromatography-mass spectrometry. *Chemosphere*. 71: 954.
21. Andre MK, Pedro and Marcia MC (2005) Nondestructive Determination of Solids and Carotenoids in Tomato Products by Near-Infrared Spectroscopy and Multivariate Calibration. *Anal. Chem.* 77: 2505-2511.
22. Liu X, Ji Y, Zhang Y, Zhang H, Liu M, *et al.* (2007) Oxidized multiwalled carbon nanotubes as a novel solid-phase microextraction fiber for determination of phenols in aqueous samples. *Chromatogr. J.* 10: A 1165.
23. Kos G, Ariya P (2007) Determination of a wide range of volatile and semivolatile organic compounds in snow by use of solid-phase micro-extraction (SPME). *Anal. Bioanal. Chem.* 57: 385.
24. Gomes ZV, Schellin M, Montero L, Yariwake JH, Augusto F, Popp P, *et al.* (2006) Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction as enrichment techniques for the determination of pesticide and benzopyrene residues in Brazilian sugarcane juice. *Chromatogr. J.* 180: A 1114.
25. Sarafraz-YA. (2006) Comparison of Hollow Fiber and Single-Drop Liquid-Phase Microextraction Techniques for HPLC Determination of Aniline Derivatives in Water. *Chromatographia*. 63: 563.
26. Zhu L, Ee KH, Zhao L, Lee HK (2002) Analysis of phenoxy herbicides in bovine milk by means of liquid-liquid-liquid microextraction with a hollow-fiber membrane. *Chromatogr. J.* 335: A 963.
27. Zhu HZ, Cui YM, Zheng XW, Han HR, Yang MM, *et al.* (2007) Determination of trace trichlorfon by high performance liquid chromatography with UV detection based on its catalytic effect on sodium perborate oxidizing benzidine. *Anal. Chim. Acta.* 166: 584.

28. Sharma RK (2001) Design, synthesis, and application of chelating polymers for separation and determination of trace and toxic metal ions A green analytical method. *Pure Appl. Chem.* 73: 181.
29. Tang M, Zhang J, Zhuang S, Liu W, *et al.* (2012) Development of chiral stationary phases for high-performance liquid chromatographic separation. *TrAC.* 39: 180–194.
30. Zhi-qiang T, Jing-fu L, Pang L (2012) Advances in analytical chemistry using the unique properties of ionic liquids. *TrAC.* 39: 218–227.
31. Kochmann S, Hirsch T, Wolfbeis OS (2012) Graphenes in chemical sensors and biosensors. *TrAC.* 39: 87–113.
32. Dai Z, Ju H (2012) Bioanalysis based on nanoporous materials. *TrAC.* 39: 149–162.
33. Huang Z, Lee HK (2012) Materials-based approaches to minimizing solvent usage in analytical sample preparation. *TrAC .* 39: 228–244.
34. Luo D, Duan Y (2012) Microplasmas for analytical applications of lab-on-a-chip. *TrAC .* 39:254–266.
35. Chang C, Wang X, Bai Y, Liu H, *et al.* (2012) Applications of nanomaterials in enantioseparation and related techniques. *TrAC .* 39:195–206.
36. Hu R, Pan Y. (2012) Recent trends in counter-current chromatography. *TrAC.* 40:15–27.
37. Table 2: Comparison of Some Parameters of Liquid Chromatography and Capillary electrophoresis.

**Table 2:** Comparison of Some Parameters of Liquid Chromatography and Capillary electrophoresis.

	<b>Liquid chromatography (HPLC)</b>	<b>Capillary electrophoresis (CZE)</b>
<b>Injected volume</b>	1-100uL	0.001-0.1uL
<b>Flow rate of liquid phase</b>	1-10mL/min	0.000001-0.0001mL/min
<b>Flow profile</b>	Parabolic	Plug
<b>Number of peaks separated</b>	20-30	20-100
<b>Analysis time</b>	10-60min	1-20min
<b>Separation efficiency</b>	>10000 plates	>100000 plates
<b>Separation technique</b>	High pressure with complicated pumping system	Electrical field with stable high voltage source, no back pressure
<b>Solvents</b>	Different solvents for different columns	Different solvents in the same column
<b>Level of development</b>	Mature technique	Young, developing fast

**Table 3:** Some Green Alternatives, Obtained from Papers in Which One of The Objectives was to Make Analytical Methods Greener.

<b>Extraction method</b>	<b>Analyte</b>	<b>Matrix</b>	<b>Solvent</b>	<b>Amount solvent per sample</b>	<b>Ref.</b>
<b>MAE</b>	Triazines	Soils	Water	30mL	[16]
	Phenols	Soil	POLO: water (5:95)	8mL	[17]
<b>SFE</b>	Pesticide residues	Plants	CO2//n-hexane	1mL	[18]
	Pesticide residues	Strawberries	CO2//acetone	10mL	[19]
<b>ASE</b>	Estrogens	Soils	Acetone	n.c	[20]
	Caretenoids	Food	Methanol / ethyl acetate/light petroleum	n.c	[21]
<b>SPME</b>	Phenols VOC	Water	Acetonitrile: Water(70:30)	70uL	[22]
		Snow	Thermal desorption	-	[23]
<b>SBSE</b>	Pesticide residues	Juice	Thermal desorption	-	[24]
<b>SDME</b>	Aniline derivatives	Water	Extract. Benzyl alcohol-ethyl acetate, 80:20	Extrc:150uL	[25]
			Retroextrc:HCL (pH2)	Retroextrc:1uL	
<b>LLLME</b>	Phenoxy herbicides	Bovine milk	DS:sample+ HCL (0.5M) OS:1-octanol AS:0.1M NaOH	AS:7uL	[26]
<b>LLLME</b>	Aniline derivatives	Water	DS: sample+ NaOH(pH13) OS:benzyl alcohol-ethyl acetate, 80:20	AS:3uL	[25]

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			AS:HCL (ph2)		
<b>MASE</b>	Pesticide residues	Juice	DS:sample+ NaCL (saturated) AS: cyclohexane	AS:800uL	[24]
<b>Micelle mediated extraction</b>	Trichlorfon	Cabbage	SDS 0.01M	200mL	[27]
<b>Medications of surfaces</b>	Cu	Water	HCL0.1N	10mL	[28]

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