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A Review On "Green Chromatography" – Reduce, Reuse, Recycle-an Art to Begin

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ABSTRACT

Green Chemistry covers analysis of sample containing organic compounds in different composition of matrices by using gas chromatography or liquid chromatography method with the use of less toxic solvents. It is important that the methods used for analysis of analyte in a sample have negligible harmful environmental impact. Green Chromatography mainly focus on making the methods greener during analysis at all the steps, starting from collection of sample, to its preparation and then its separation and finally determination of analyte. The present review includes the goals to achieve green chromatography using environmentally benign solvents and reagents, most recent contribution in the development of greener sample preparation since it has effect on whole analytical methodology, chromatographic separation techniques**,** advantages of Green Chromatography, Green micro extraction techniques (solid phase micro extraction, still bar sorptive, liquid phase micro extraction) is included with special attention. The approach in making chromatographic separations greener differs depending on the type of chromatography which includes solvent less extraction technique- aiming to eliminate or reduce the amount of organic solvents consumed without loss in chromatographic performance. In gas chromatography it is advisable not to use helium as the carrier gas because it is a non-renewable resource. GC separations using low thermal mass technology can be considered greener as energy consumption is minimized by this technology. In liquid chromatography the focus will be on the reduction of solvent consumption and replacement of toxic and environmentally hazardous organic solvents with more benign alternatives.

Keywords: Green Chromatography, Gas Chromatography, Liquid Chromatography, Solvent less Extraction, Green Analytical Chemistry.

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INTRODUCTION

Green chemistry is a vast concept have the scope for sustainable development widely used for the development of analytical methods with less environmental impact and minimum analyst occupational exposure. Advancement includes development of micro extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) techniques. Research which include separation techniques like HPLC or GC should be greener, so focus should be on the elimination of harmful solvents used in liquid chromatography (LC) and the reduction of detection time.

Green analytical chemistry is defined as clean, benign, hygienic or environmentally friendly analytical chemistry. Green analytical chemistry includes analysis of organic compound by applying quality assurance/quality control (QA/QC) and minimizing any harmful environmental impacts resulting from the analysis. Thinking of chromatographic procedures which is meeting the standards of green analytical chemistry principles, green chromatography comes to the mind first. Chromatographic procedures starts from starting from collection of sample, to its preparation and then its separation and finally determination of analyte. Green Chromatography are concerned with all the stages of analysis, but gives more attention at the steps with the greatest environmental impact that is sample preparation and the chromatographic separation all these stages are considered to be the most environmentally unfriendly and therefore have a lot of potential for "greening."

Green chromatography has 12 principles 2 and was developed to minimize the environmental impact of chemical synthesis, regents, solvents etc. Comparing chemical synthesis methods with other method, the amount of pollutant waste products generated during chromatographic analysis appears small. However, it has been noticed that one liquid chromatograph can generate one litre of liquid waste daily.

No. Principle

Explanation

Examples of principles in green analytical chemistry

1. Prevention It is better to prevent waste than to recycle or clean waste afterwards Application of direct determination methodologies, solvent less extraction techniques, miniaturization
2. Atomic economy Synthetic method is designed to increase the use of all materials required in the process to convert into the final product

3. Less hazardous chemical synthesis Synthetic method is designed to use and generate substances that minimize toxicity to human health and the environment On-line analytical waste detoxification
4. Designing safer chemicals Design chemical products to accomplish their desired function while minimizing their toxicity
5. Safer solvents and auxiliaries Minimize the use of supplementary substances wherever possible; make them completely harmless when used Toxic solvents substitution with less toxic ones, Solventless extraction techniques; direct analysis method
6. Energy Efficiency Design Energy requirements for chemical processes is reduced and synthetic methods at ambient temperature and pressure must be conducted if possible Application in microwave, ultrasound or pressure-assisted extraction. All these efforts is to minimize energy consumption
7. Renewable raw materials uses Use of renewable raw materials whenever required
8. Reduce derivatives Avoid unnecessary Derivatisation, as it requires additional reagents and it generates waste Derivatization should be avoided wherever possible
9. Catalysis Catalytic reagents are super than stoichiometric reagents –
10. Design for degradation Design such chemical products so they break down into less by-products that do not persist in the environment –
11. Real-time analysis for pollution prevention Develop such analytical methodologies which can allow real-time analysis, in-process observation and control prior to the formation of hazardous substances Develop such procedures that avail to obtain analytical results with short (preferably no) time delay
12. Inherently safer chemistry for accident prevention Substances and the type of a substance which are used in a chemical process should have power to minimize the potential for chemical accidents, including releases, explosions, and fires Application of no solvent techniques to prevent time period for monitoring ,miniaturization, and occupational exposure

Table 1:Green Chromatography Principles.

No.	Principle	Explanation	Examples of principles in green analytical chemistry
1	Prevention	It is better to prevent waste than to recycle or clean waste afterwards	Application of direct determination methodologies, solventless extraction techniques, miniaturization
2	Atomic economy	Synthetic method is designed to increase the use of all materials required in the process to convert into the final product	–
3	Less hazardous chemical synthesis	Synthetic method is designed to use and generate substances that minimize toxicity to human health and the environment	On-line analytical waste detoxification
4	Designing safer chemicals	Design chemical products to accomplish their desired function while minimizing their toxicity	–
5	Safer solvents and auxiliaries	Minimize the use of supplementary substances wherever possible; make them completely harmless when used	Toxic solvents substitution with less toxic ones, Solventless extraction techniques; direct analysis method
6	Energy Efficiency Design	Energy requirements for chemical processes is reduced and synthetic methods at ambient temperature and pressure must be conducted if possible	Application in microwave, ultrasound or pressure-assisted extraction. All these efforts is to minimize energy consumption
7	Renewable raw materials uses	Use of renewable raw materials whenever required	–
8	Reduce derivatives	Avoid unnecessary Derivatisation, as it requires additional reagents and it generates waste	Derivatisation should be avoided wherever possible
9	Catalysis	Catalytic reagents are super than stoichiometric reagents	–
10	Design for degradation	Design such chemical products so they break down into less by-products that do not persist in the environment	–
11	Real-time analysis for pollution prevention	Develop such analytical methodologies which can allow real-time analysis, in-process observation and control prior to the formation of hazardous substances	Develop such procedures that avail to obtain analytical results with short (preferably no) time delay
12	Inherently safer chemistry for accident prevention	Substances and the type of a substance which are used in a chemical process should have power to minimize the potential for chemical accidents, including releases, explosions, and fires	Application of no solvent techniques to prevent time period for monitoring ,miniaturization, and occupational exposure

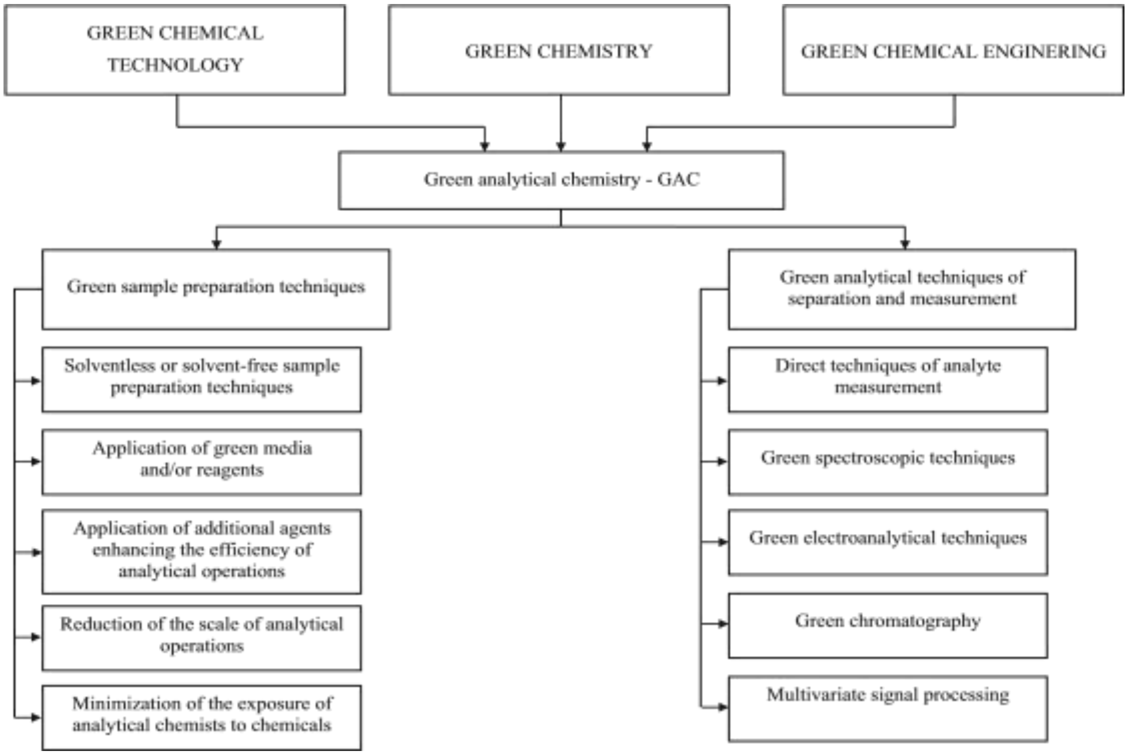

Figure 1: Principles of Green analytical chemistry which is applied to sample preparation and the final determination step.

Organic compound analysis in a sample containing different composition of matrices is usually performed using chromatographic techniques. Chromatographic techniques are used in routine research work, food and environmental analysis and in medical industry. A single analysis of liquid chromatography can generate 1-1.5 L of liquid waste daily which has maximum impact on environment 3. Hence it is the responsibility of industrial chemists to minimize pollution which is generated by their activities and to obtain reliable analytical results within a short time, with a little or no negative impact on environment. Green analytical chemistry is economical, as it promotes consumption of reagents, this creates opportunities of saving reagent for analytical laboratories and decreases the need for purchase of large amount of reagents/solvents 4.

Green chromatographic methodologies without sample preparation:

An important aspects of green Chromatographic methodologies is the either presence or absence of the sample preparation step, which is often the most polluting step in the entire chromatographic analysis procedure 5. Occupational exposure to toxic chemicals may affect the health of the analyst during sample preparation. From the point of green analytical chemistry, it is greatly advantageous to use direct chromatographic methodologies (i.e. methodologies without sample preparation) whenever possible. The main limitation of such methodologies is that it is only applicable to samples with relatively clean/pure matrices 6, as otherwise, the chromatographic columns might quickly deteriorate due to deposition of components present in sample that do not elute from the column. Water, petroleum, and spirits fractions are examples of matrices that can usually be injected into chromatographic columns without sample pre-treatment to remove the deposited components present in the sample.

Compare to liquid chromatography, gas chromatographic methods are usually more readily adaptable to the elimination of the sample preparation step. The work on direct gas chromatographic methods which was proposed by Grob and Grob was focused on the development of on-column injection 7. A combination of on-column injection with electron capture detection will allow chromatographic detection of halogenated compounds in water samples by direct aqueous injection 8, whereas a combination of direct aqueous injection with flame ionization detection 9 or mass spectrometric detection 10 will allow direct detection of hydrocarbons in water samples. The latter two detectors have problems in handling relatively large volumes of water passing through them.

The goals of green analytical chemistry is achieved by applying direct chromatographic methodologies in several ways. The consumption of materials mostly used during sample preparation (including organic solvents, fibres, sorbents, cartridges etc.) is avoided. The overall time for the analysis (starting from sample collection to getting the final results) is potentially greatly shortened. The elimination of sample preparation step allows placing the chromatograph at-line or on-line, which may further reduce the analysis time. This is especially important during process analytics, where near-real time results are very crucial.

Green sample preparation techniques as a "gold solution"

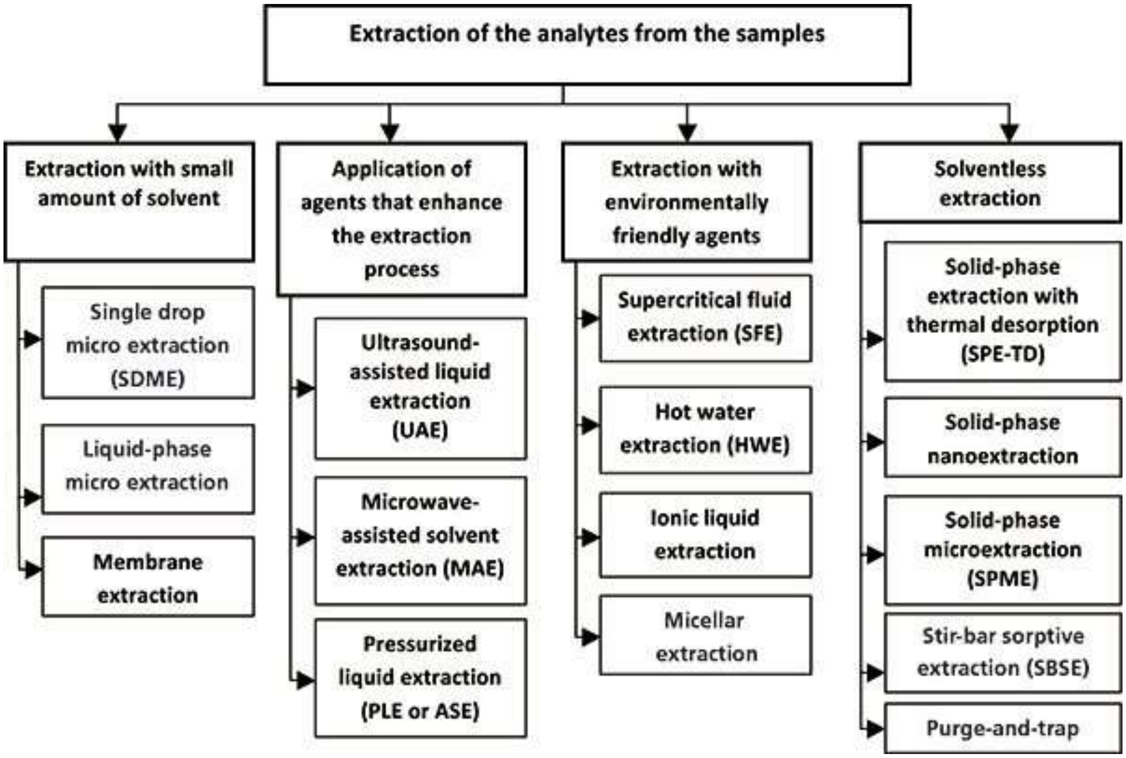

Figure 2: Gold Solution preparation techniques.

In recent years, more emphasis is given on new solutions preparation and its applications that improve the "greenness" of sample preparation techniques: 11

DLLME is the technique that has been intensively worked. DLLME uses small amount of nonpolar solvent (usually below 100 µL) and approximately 1 mL of less toxic dispersive solvent. DLLME is based on the procedures that have been proposed to determine UV filters. DLLME extraction can be done with ultrasounds that eliminate or reduce the need for dispersive solvent 12. Dispersive solvent increases the solubility of the extraction solvent. Without the use of dispersive solvent, extraction solvent volume is reduced, because small part of it is dissolved in water; and despite of low initial solvent volume used it is easy to recover large amount of extraction solvent volume which further is easy to handle 13. For example,

(1). Ultrasound-assisted extraction of chlorobenzene 13 µL can be applied to determine siloxanes in water samples. (2) In LC analysis, ionic liquids are considered as green solvents and can be used as extraction agents in DLLME. (3) Antibiotics, polycyclic aromatic hydrocarbons (PAHs), or oestrogens in water samples and pesticides in honey are used after sample is dissolved in water 14.

The alternative solution for DLLME is solidification of floating organic drop (SFO) 15. The solvents used for extraction in SFO are higher alcohols such as nonanol, decanol, or undecanol. After extraction process, the sample is subjected to a lower temperature, hence the solvent solidifies and can be easily separated from the sample matrix. These technique can be used in sample preparation before analysis of GC and LC. These technique is suitable for the extraction of compounds with log kow in the range 3–7. For example, 16

(1) Using 10 µL of 1-undecanol and 100 µL of acetonitrile as dispersive solvent, Triazines in water samples can be determined. (2) Also determination of amphetamines in urine samples and determination of pesticides in vegetables and fruits after extraction with acetone can be done by these procedure. (3) Dong et al. has extracted phytocides from soil by using water–methanol mixture and was evaporated to dryness before the residue was redissolved in methanol. Before using high performance liquid chromatography (HPLC) analysis, the samples were subjected to ionic liquid-based ultrasound-assisted DLLME. A derivatization step can easily get combined with DLLME and these derivatizing agent easily hydrolyzes in the water –sample. (4) Casado et al. has performed acetylation with simultaneous extraction of benzotriazoles on a mixture of microvolumes of acetic anhydride, acetonitrile, and toluene. (5) Farajzadeh et al. applied butylchloroformate as a derivatizing agent in DLLME for determining amantadine, which is pharmaceutically prescribed for influenza treatment, in human plasma samples.

Another current trends is solid-phase microextraction (SPME) 17. It includes automation, in situ sampling, development of in vivo applications, and the development of new coatings (non-bonded, bonded, or cross-linked polymers). Traditionally, Polydimethylsiloxane (PDMS) is been used as SPME fibre coating, but fibres like polyacrylate or polyglycol polymers are now available for the extraction of polar analytes 18. Traditionally fused-silica is used as the support for coating, but recently metal-based fibres have been developed for increased durability and improved mechanical properties. Increasing the thickness of the film during coating will increase fibre capacity and thereby increases the sensitivity, however, which can lead to increase in the extraction time required to obtain equilibration. Ionic liquids have recently been used as fibre coatings, which has opened up all the possibility to design and modify coatings to obtain greater selectivity towards analytes. In 2016, Feng et al. has isolated alcohols by applying a polymerized ionic liquid 1-vinyl-3-octylimidazolium bromide as a coating in SPME from water (even methanol) and produced good recoveries. In 2016, Ho et al. has used 1-butyl-3-methylimidazolium hexafluorophosphate-coated SPME fibre to extract chlorophenols from water 19. Applications of SPME includes the determination of bioactive compounds or contaminants directly in tissues of living organisms, or in their habitat, as demonstrated by Bessonneau et al. in corals. In vivo sampling of solid phase microextraction is enhanced to govern emerging filth in fish and evaporative halogenated organic compounds in trees, bugs and worms, higher animals, and even humans.

Another rapidly developing technique is Stir-bar sorbent extraction (SBSE), where the majority of fibre coating use is PDMS 20. Recently, polar coating is another alternative coating technique which have been introduced and intensively investigated for the extraction of analytes having a vast range of polarities including polyacrylate and polyglycol coatings. For the extraction of chlorophenols and chloroanizoles from wine samples, ethylene glycol with silicone is the copolymer used which was found to be more effective than a polyacrylate coating 21. This copolymer is used because it allows for simultaneous extraction and preconcentration of both polar phenols and nonpolar chloroanisoles. Methacrylic acid and Divinyl benzene copolymer is another coating technique for the extraction of polar compounds from water samples. In comparison, the SBSE coating gives much better recoveries i.e. (around 100% for analysed compounds) for polar pharmaceuticals than commercial PDMS coating-based stir bar (less than 20%). As a passive sampling the still Bar is also used for the in situ extraction of nitro organic pesticides. Getting such an approach seems to be a promising technique for the collection of integrated samples. The sample preparation steps and in situ composition are both straightforward. The most appropriate selection of stir bar coating can result in sampling of polar analytes, which usually are not collected by traditional passive samplers. Stir bars can also be coated with chiral imprinted polymers or molecularly imprinted polymers (MIPs) for extraction of certain groups of analytes. The time required to perform assay is relatively long; however, the limit of detection (LOD) obtained is very low. For example, it takes three hours of SBSE extraction followed by thermal desorption, then includes two-dimensional GC separation and time-of-flight mass spectrometry (TOF-MS) for determination of organochlorine pesticides at pg/L concentration 22.

Green aspects in gas chromatography.

Gas chromatography (GC) is a technique used for the analysis of semi-volatile and volatile compounds. The choice of carrier gas should be taken into consideration when trying to make GC analysis greener. The most commonly used carrier gas in GC is Helium (He), because of its favourable chromatographic properties (high optimum linear velocity) while being non-flammable, non-toxic, safe and inert to handle. It is also a non-renewable resource, and the world’s reserves of this precious gas Helium are about to get extinct, a shortage that is likely to have far-reaching repercussions. Other carrier gas which can be used in GC is Nitrogen (N2)23. However, the optimal linear velocity of Nitrogen (N2) is low compared to helium or hydrogen, which translates into long analysis times. In addition, in the van Deemter curve the steepness of the nitrogen means a small changes in the average linear velocity (uopt) result in large changes in efficiency 24. These two factors makes the nitrogen least desirable carrier gas for GC. The best alternative carrier gas in

GC seems to be Hydrogen. The van Deemter curve of Hydrogen is very flat, and Hydrogen's uopt is the highest among the three common carrier gases compared here hence separations can be conducted at higher-than-optimal flow rates with little loss in efficiency. Consequently, the result will be shortest analysis times accomplished with hydrogen as the carrier gas. Also, hydrogen is considered as the best carrier gas with high efficiency for samples containing compounds that elute over a wide temperature range when constant pressure is used. The next approach to make GC "greener" is to reduce analysis time which will lead to increase in a sample throughput. This can be achieved by using columns having shorter length than conventional and with smaller internal diameter (i.d) without sacrificing separation and resolution. Further, increase in the uopt of the carrier gas with such columns, will contributes to the reduction of the analysis time. This is commonly referred to as fast capillary GC. The limitations of using narrow diameter columns is their reduced sample capacity, which frequently leads to column overloading 25.

There are 2 aspects in Gas Chromatography: Comprehensive two-dimensional gas chromatography (GC × GC) and Low thermal mass technology which is discussed below: 26

Low thermal mass technology

After column selectivity, temperature programming is the second important tool which has to be controlled in gas chromatography because gas chromatography works on high temperature principle. Temperature-programmed gas chromatography (TPGC) is a commonly used term used for increasing column temperature during GC analysis 27. Advantage of TPGC includes separation for solutes based on their boiling points range, improves peak symmetry for solutes along with high retention factors, and improves detection limits. The innovative work of TPGC is that it allows the removal of heavier and unwanted sample components from the column that could otherwise block the integrity of the chromatographic system.

Low thermal mass technology was introduced in 2001, with the aim to achieve ultrafast temperature programming and an unrivalled cool down time with a consumption of power approximately 1% of conventional gas chromatography. Fig.4 depicts low thermal mass column module diagram 28. LTMGC plays important role in green techniques as it overcomes the limitation of resistive heating of a GC column- and brings about two main changes: reduction of power consumption by approximately a factor of 200, and increased speed of column heating (up to 1800˚C/min; rates depend on column mass, column configuration and column void time), which can potentially reduce the analysis time. Column heating at 1800˚C/min, shows satisfactory separation at less than 0.3 min. An example of the application of LTM in the analysis of alkanes is presented in Figure. 5. Comparison of cooling rates of four LTMGC modules with a conventional

GC oven equipped with a vent deflector depicts that the thermal mass of the modules, LTM GC was 3–10 times faster when cooling down from 300˚C to 30˚C compared to conventional GC. Fig. 3 depicts Advantages of LTM, fast heating and cooling rates allow high sample throughput, resulting in high efficiency temperature programming 29. And the limitation of LTM is the host oven has to be kept constant at elevated temperature to prevent cold spots, which might have a negative impact on the capillary tubing used for interfacing the host.

Mass technology

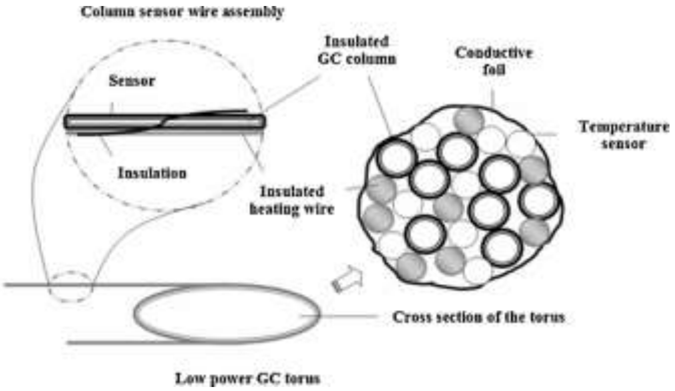

Figure 3: Advantages of low thermal

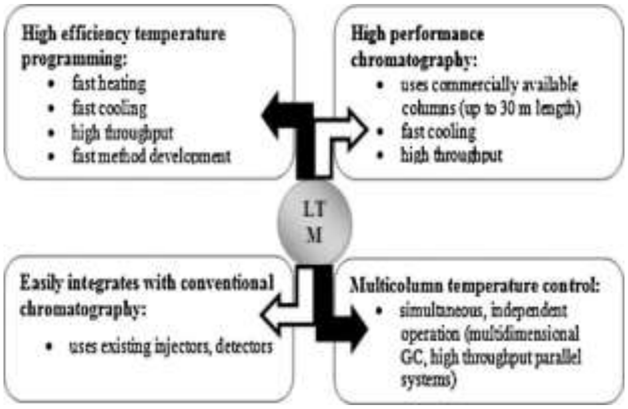

Figure 4: LTM column module diagram

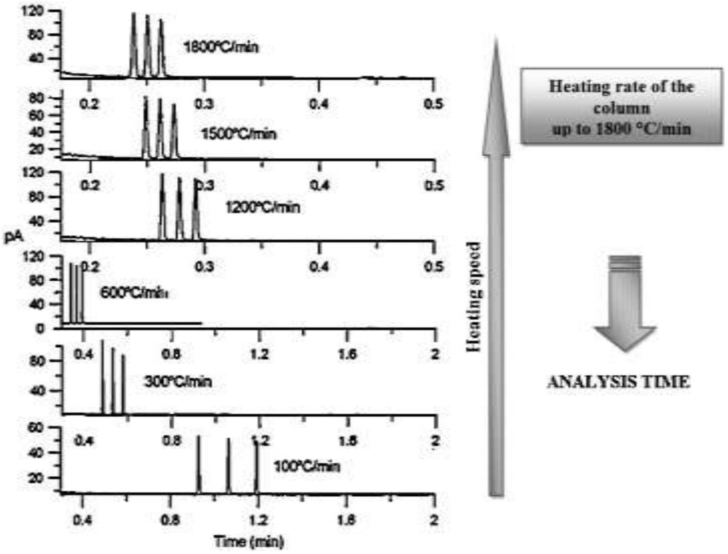

Figure 5: Separation of C14–C16 n-alkanes on a 2 m × 0.1 mm × 0.12 m (5%-phenyl)- methyl polysiloxane capillary column for a range of temperature programming rates from 100 ◦C/min to 1800 ◦C/min

GC with the LTM module:

Constant heating of the oven brings down the energy savings to some extent that brings about by direct resistive heating. Moreover, when LTMGC is used with a Splitless injector or with a cool-on column injection system 30, connect the injector with the column module by using an uncoated yet deactivated transfer line. Sometimes host oven is at a higher temperature than the LTMGC module, because impurities coming from the carrier gas or impurities such as septum bleed or stationary phase decomposition products can be accumulated in the LTM module, hence during such situation thermal conditioning of the LTMGC is required.

Two-dimensional gas chromatography (GC × GC)

Comprehensive two-dimensional gas chromatography (GC × GC) is the separation technique for semi-volatile analyte and volatile substance, especially when it is combined with time of-flight mass spectrometry (TOFMS). The distinguishing features of comprehensive two-dimensional gas chromatography system from conventional one-dimensional GC is as follows:31

- Difference in Columns: The first is usually non-polar sized (30–60 m long), while the second is polar/semi-polar sized (~0.5–2 m).
- Modulator: It is kept between the two chromatographic columns, cautiously prevent the loss of separation in the first column and gives two-dimensional separation.

- Detectors: It shows very narrow peaks eluting from the second column sized (~50–400 m) hence, require high-speed data acquisition systems (sampling at least 50–100 Hz to obtain 10–20 points per peak).

Two-dimensional GC × GC allows good separation of components in sample containing complex matrices than conventional 1D-GC. It requires only the same sample volume, same (or only slightly longer) time for separation and comparable reagents. The high resolution power of GC × GC–TOFMS allows the determination of analytes of interest in complex matrices with little or no sample preparation, which results in significant time and reagent savings hence achieve the goal of green chromatography. An example of this is shown in Fig.6. In this figure the matrix component represented by the continuous band in the lower part of the plot-Chlorfenvinphos cannot be separated from the other components of the carrot extract using 1D-GC–TOFMS, and cannot be detected based on the mass spectrum, but separation and identification of this compound was possible using two-dimensional GC × GC–TOFMS, and its mass spectrum corresponded closely to the library spectrum 32.

Another most important component of a GC × GC system is the modulator. The purpose of modulator is to collect small portions of elute from the first column and inject them into the second column at regular intervals. These days the approach of modulation is based on trapping of analyte in the stationary phase of the GC column. The analytes are trapped in the stationary phase at low temperature. After completion of each modulation period the capillary is rapidly heated to release the analytes in the form of a narrow band and transferred into the second column. This process is known as thermal modulation and it is usually carried out using cold and hot gas streams to cool and heat the segments of the capillary column alternatively 33. The main disadvantage of thermal modulation includes complexity of structure and requires cryogenic agents (liquid CO2 or N2), which are difficult to handle and costly to use. Hence modification in thermal modulation is required which can be obtained by consuming liquid cryogens that brings benefits to GC × GC and creates a green separation technique.

To overcome the limitation of thermal modulators alternative modulator is developed which is Differential flow modulators 34. Working of these modulators includes effluent from the first column is collected in a sampling loop, and before the loop gets overfilled, its content is flushed at a very high flow rate to the second column by an auxiliary stream of carrier gas. The design of differential flow modulators is simple, no cryogenic agents is required, making it more economical and greener 35. Limitation of these modulator is, length of the modulation period is based on the volume of the loop and is usually limited to ~2 s. Band compression is achieved by pressurization of the sampling loop content, which is not as efficient as thermal focusing. So overall sensitivity of GC × GC with differential flow modulation is usually worse than with thermal modulators.

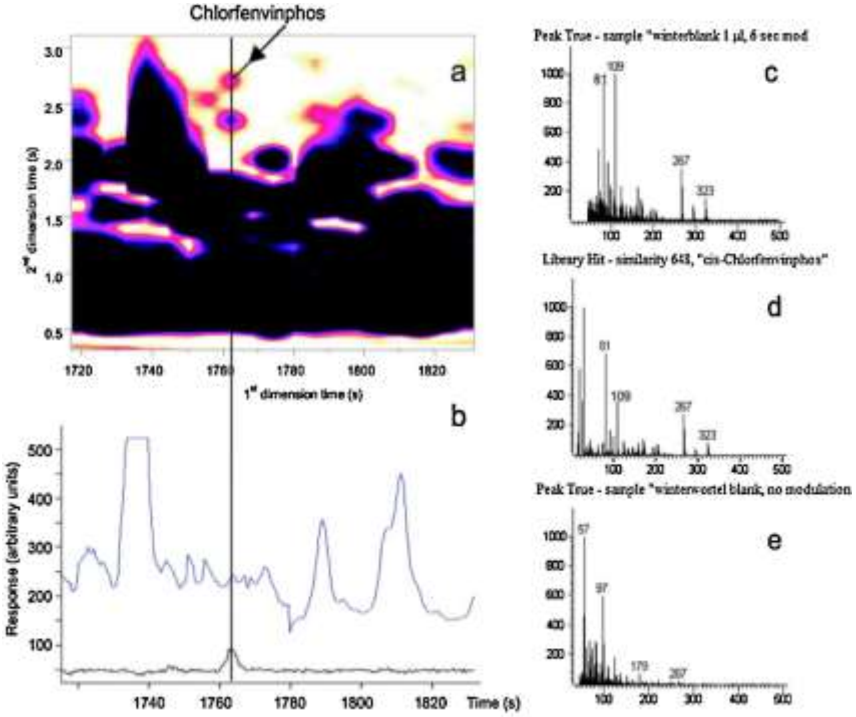

Figure 6: Chromatograms of carrot extract by GC × GC–TOFMS

(a) 1D-GC–TOFMS (b). Mass spectrum obtained after 2D-GC × GC separation

(c) Library spectrum

(d) mass spectrum obtained after 1D-GC separation

Green Aspects In Liquid Chromatography

Comparing with gas chromatography, Liquid chromatography is generally considered as less green, as it requires solvents for their separation. On the other side, this offers more possibilities for “greening”. Theoretically one uses analytical liquid chromatograph equipped with a conventional LC column (15–25 cm in length, 4.6 mm i.d., packed with 5 m particles) and operated at a flow rate of 1 mL/min produces ∼1500 mL of waste per day, meaning ∼500 L of waste per year 36. Although the volume of waste produced by this method is small compared to the amount of sewage and waste generated by a typical large industrial company. Reducing the internal diameter of the column will in return reduces solvent consumption and the mobile phase flow rate. Effective separations will be achieved when the column i.d is reduced. The flow rate has to be decreased by the square of the column diameter. Analytical sensitivity is increased by reduction of the column i.d (mainly when UV, fluorescence and electrospray ionization mass spectrometry are used as detectors) owing to the reduced dilution of the solutes in the mobile phase and the appearance of more concentrated bands at the detector. On the other hand, effects of larger diameter column affects the efficiency much more than smaller diameter columns, which might lead to significant loss in resolution. Solvent consumption can also be reduced by increasing the chromatographic productivity, which can be done shortening the column length and by reducing the particle size 37. To prepare column length of 15 cm with the same number of plates, 5 m particles can be used whereas for column length 5 cm, 2 m particles is sufficient. Reducing the column length will shortens the analysis time, in return increases the throughput. Temperature is the other parameter that can be used as a powerful variable in liquid chromatography. Temperature of the column affects selectivity, efficiency and detectability. The parameter temperature is easier to change during the method development stage compared to other parameters such as mobile phase composition, buffer or pH, hence elevated temperature has become popular parameter used in HPLC to make it greener. Change in Temperature is very useful in selectivity tuning, especially for polar and ionisable compounds. Benefits of using elevated temperature in Liquid Chromatography are summarized in Fig. 7

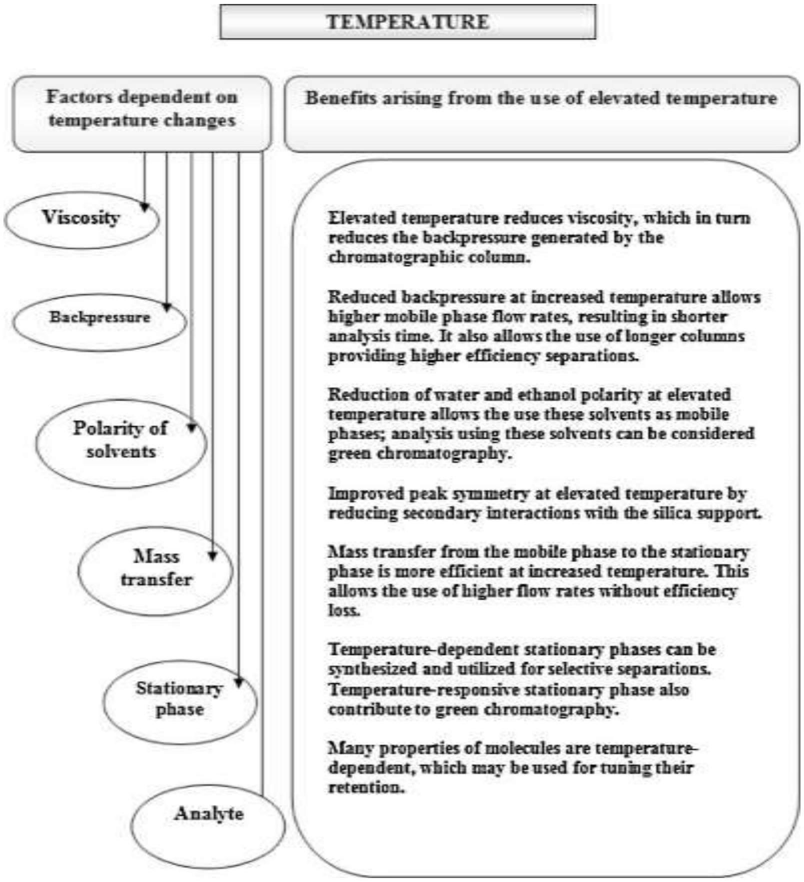

Figure 7: Factors dependent on change in temperature and the benefits arising from the use of elevated temperature in HPLC.

A. Two-dimensional liquid chromatography

One-dimensional (1D) chromatographic techniques is not capable to give better resolution of components specially the complex samples containing environmental and biochemical ones, hence several chromatographic runs is required for these technique to completely characterise such samples, which is not desirable in making green chemistry due to usage of large amounts of organic solvents leading to generation of large amount of by products and waste, extending the analysis time and in return making the method costly 39.

The demand for separating number of compounds into individual peaks in a chromatographic run (increase peak capacity) leads to the development of multidimensional techniques. These technique is based on more than one separation mechanism to the same sample. Hence, combination of different separation mechanisms is applied to same sample to improve resolution and increase the separation space.

2D-LC is performed in two modes: 40

- Heart-cutting: Applicable when only some constituents of the sample matrix require additional separation power. In these method selected fraction from the first dimension (D1) are placed to the second dimension (D2) via a 6-port switching valve.
- Comprehensive two-dimensional liquid chromatography: Applicable when a large number of complex sample constituents require characterization. In these method the entire effluent from D1 is directed to D2 via an 8-/10-port valve in the form of subsequent fractions. Proper Selectivity and Compatibility of the stationary and mobile phases is required. Fractions can be transferred between the two columns on-line or off-line.

LOCATION OF THE CHROMATOGRAPH ON-LINE OR AT-LINE (STEP FOR REDUCING THE ENVIRONMENTAL IMPACT)

Traditional approach for chromatographic analysis involves collection of the sample, transport to the laboratory and final analysis (usually done before sample preparation). All these activities generate negative environmental impact. Hence, the greener approach can be obtained by use of portable chromatographs or on-line process analysers. Four possibilities to locate the analytical device with respect to the investigated medium. 41

Off-line: Sample is collected from the investigated medium, then it is transported to the laboratory and finally analysed after sample preparation. Sample transportation and preservation (a preservatives might need to be added to the samples) creates negative environmental impact. There is a risk of loss during prolonged time of transportation, storage and preparation.

On-line: The analytical device is located near the investigated medium. Sample is automatically collected periodically and analysed. On-line sample preparation is usually applied in case of chromatographic analysis because in this case sample is neither transported nor stored, hence the risk of sample representatively loss is minimized. In this case application of large amounts of reagents (e.g. for sample preservation) is usually avoided.

At-line: The analyst brings the analytical device to the investigated medium. Sample is analysed at the site of sample collection, after some minor sample preparation operations. It is similar to on-line mode, as it minimize consumption of reagents and the time from sample collection to sample analysis is relatively short.

In-line: The measuring device is located near the investigated medium. The measurement is done at real-time or under near real-time conditions. This mode of locating the analytical device is limited to that techniques that do not require instrument calibration or sample preparation. It is unlikely to locate chromatograph in an in-line mode.

ADVANTAGES OF MINIATURIZATION IN CHROMATOGRAPHY

Analytical instrumentation-miniaturization brings about many advantages, out of which space savings are probably the least important. Miniaturized separation systems typically requires fewer consumables, as they produce less waste, and their energy consumption is reduced vastly compared to full-size laboratory systems. Miniaturization can often be carried to the sampling site, allowing on-line or at-line analysis. Miniaturized systems also improves the speed of separation and method sensitivity and these characteristics makes the miniaturized separation systems inherently green. For example, as discussed above, reducing the column diameter and length of LC column will allow lower flow rates of the mobile phase, hence reducing overall solvent consumption compared to conventional methods. Compared to conventional analysis, the amount of sample required for analysis with miniaturized systems is typically smaller which is very important in many research areas, including biomedical science, forensic science etc. Lowering the flow rate reduces dilution of the analyte in the mobile phase, hence improving detection sensitivity when concentration-dependent detectors are used (e.g. UV–Vis). Reducing the separation column dimensions is also one of the most effective approaches while developing novel stationary phases in LC because it allows evaluation of the limited amounts of experimentally synthesized phases. 42

Greener Chromatography Solvent Selection Guide

Ethyl acetate: Ethanol 3:1 (v/v) solution is used in replacement for dichloromethane (DCM) in flash chromatography purifications and other associated HPLC methods. Which step towards achieving a green chromatography. 43

Compound selection:

Solvent mixtures for green chromatography are used to evaluate the purification of “drugs like” molecule that medicinal chemists regularly prepare and purify. Thin-layer chromatography (TLC) is used as an alternative green solvent systems that use dichloromethane as an alternative because it is considered to be greener. Selected compounds were classified into three families: Neutral, Basic and Acidic Compounds.44

- Neutral subsets-absence of carboxylic acid or an aliphatic amine groups. It do not require any acidic or basic additives because it cause tailing or streaking during elution.
- Basic compounds contain tertiary aliphatic amines and a basic solvent additive (NH4OH) was necessary to prevent tailing.
- Acidic compounds contain carboxylic acids functional group, and an acidic solvent additive (AcOH) both helps to prevent tailing.

Greener chromatography solvent selection evaluation method

The relative eluting strength of a test compounds was determined by TLC analysis by using mixture of solvents. All the compounds were spotted in parallel manner on a single TLC plate, and the compound was run with a specific solvent mixture and different eluents were obtained. The retention frequency (Rf) value was measured for each individual compound. Then, the individual Rf values were averaged to give an average retention frequency (Rfavg) value for the compound in that specific solvent mixture. Solvent mixtures were systematically evaluated at varying concentrations of polar eluent. 45

SUMMARY

At all steps of the analysis chromatographic techniques have the potential to be greener, starting from sample collection through its preparation to separation and final determination. Many developments and changes have been observed to make chromatography techniques more environmentally friendly. An ideal chromatographic method would be performed in the in-line mode, without sample preparation to yield analytical results without using any consumables. Unfortunately, in most cases sample preparation is required prior to chromatographic analysis. Consequently, to minimize solvent consumption Solvent less, miniaturized sample preparation techniques should be used whenever possible. As most of the chromatographic determinations performed are routine ones, it is important to perform those methodologies that have small environmental impact. Progress in future for analytical chromatography to be greener is expected to be accomplished through miniaturization, shortening of the analysis time and improvements of the resolving power. Things to be highlighted is that there are frequent efforts made to qualitatively measure and compare the chromatographic procedures in terms of possible harm to the environment. Although this is just a step towards recent development, there are still a lots of issues, such as the amount and toxicity of solvents and reagents, to be improved upon to make the chromatography "greener".

REFERENCES

1. KoelM., KaljurandM. Green Analytical Chemistry, Royal Society of Chemistry, Cambridge, 2010; 6(12): 542-245.
2. Anastas P. T., WarnerJ. C. Green Chemistry: Theory and Practice. Oxford University Press, New York, 1998; 9(137): 319-324.
3. Welch C. J., Wu N., Biba M., Hartman R., Brkovic T., Gong X., Helmy R., Schafer W., Cuff J., Pirzada Z. Chromatographic Techniques, 2010; 1(29): 667-720.
4. De la Guardia M., Garrigues S. Challenges in Green Analytical Chemistry, Royal Society of Chemistry, 2011; 5(127):34-53.
5. TobiszewskiM., MechlinskaA., ZygmuntB., Namiesnik J. Trends Anal. Chemistry, 2009; 6(28): 734-789.
6. TobiszewskiM., Namiesnik J. Trends Anal. Chem, 2012; 12(35): 67-72.
7. GrobK., Grob JrK. Chromatogr.A, Green Analytical Chemistry, Royal Society of Chemistry, 2000; 32(78): 311-345.
8. GrobK. ChromatographyJ, 2011; 3(84): 781-820.
9. Kubinec R., Adamuscin J., Jurdakova H., Foltin M., Ostrovsky I., Kraus A., Sojak L. Flame Ionisation Chromatograph A, 2014; 12(34): 90-110.
10. Aeppli C., Berg M., Hofstetter T. B., Kipfer R., Schwarzenbach R. P. Mass Spectrometry, Chromatography A, 2008; 11(32):116.
11. Guardia M., Garrigues S. John Wiley & Sons, Handbook of Green Analytical Chemistry, 5th ed., Europiean Publication, 2012; 2(34):1202-1300.
12. De la GuardiaM., Trends Anal. 2010; 4(29):577-590.
13. De la GuardiaM., Sampling Techniques. 2012; 5(56):625-670.
14. Li.F, Ploch.S. Gold Solution preparation Techniques, 2012; 2(12):1259-1260.
15. Bojko B., Pawliszyn J. Bioanalysis, 2012; 2(16): 1263-1726.
16. De la GuardiaM., Garrigues S. Analytical Chemistry, 2013; 2(34):1267-1345.
17. Kaljurand M., Koel M. Green Chemistry, 2012; 4(12):1271-1323.

18. WandZ. Chromatography Techniques, 2012; 4(20):1285-1452.
19. Ghosh.C. Bioanalysis, 2012; 7(34):1377-1456.
20. Bendicho C., Lavilla I., Pena-Pereira F., Romero V. AnalJ, Analytical Spectrophotometry, 2012; 9(45):1831-1980.
21. WangJ. Chemistry, 2014; 7(35):811-930.
22. OuyangG., Pawliszyn J. TOF-MS , 2006; 7(90):1059-1064.
23. Pena-PereiraF, LavillaI., Bendicho C. Gas Chromatography, 2010; 7(29):617-620.
24. Armenta S., Garrigues S., de la Guardia M. Van Demeter curve in Gas Chromatography, 2008; 12(27): 497-560.
25. Jakubowska N., Polkowska Z., Namiesnik J. Gas Chromatography Techniques, 2005; 2(35): 217-230.
26. Aufartová J., Mahugo-Santana C., Sosa-Ferrera Z., Santana-Rodríguez J. J., Nováková V., Solich P. Two Dimensional Green Chromatography, 2011; 12(70): 33-45.
27. Pawliszyn J. Temperature-programmed gas chromatography, 2010; 4(12):45-78.
28. Anastassiades M., Lehotay S. J., Stajnbaher D., Schenck F. J. Low Thermal mass column module 2003; 4(45): 412-456.
29. Mastovská K, Lehotay S, Anastassiades M, Analytical Chemistry, 2010; 8(77):8129.
30. Plössl F., Giera M., Bracher F. Types of Column Injection System, Chromatography A , 2006; 5(19):16.
31. Risticevic S., Niri V. H., Vuckovic D., Pawliszyn J. One Dimensional Chromatography, 2009; 3(45):393-450.
32. El-BeqqaliA, KussakA., Abdel-RehimM. GC × GC–TOFMS, Chromatography A, 2006; 4(112):234.
33. Abdel-RehimM. Capillary Column Chromatography, 2009; 8(23):234-244.
34. Kawaguchi M, Ito R, Saito K., Nakazawa H, Pharm J. Differential flow modulators, Biomed. Anal.2006; 5(12):100.
35. Baltussen E., Sandra P., David F., Cramers C. Differential flow modulators, Microcolumn J, 2010; 12(56):737.
36. OchiaiN., SasamotoK, KandaH. Liquid Chromatography, Global Anal. Solutions, 2008; 3(12): 34
37. DavidF., Sandra.P. Liquid Chromatography, J. Chromatogr. A, 2012; 4(12):1124
38. Tienpont B., David F., Benijts T., Sandra P. Liquid Chromatography, PharmJ. Biomed. Anal. 2009; 4(56):43.

39. Grodkreutz S. R., Swenson M. M., Secor L. B. Stoll D.R, Liquid Chromatography, Chromatography.A, 20012; 5(1228):31
40. Grivel C., Dehunex A., Heinisch S. Modes of 2D-LC, Chromatography. J, 2008; 8(13):10
41. Brown M. A, Emmert G. L. Location of Chromatography, Analytical Chemistry. 2015;2(15):58
42. Slentz B. E., Penner N. A., Regnier F.E, Miniaturization of Analytical System, 2015; 7(15):13
43. Yashine Y.I, Yashine Y. L. Flash Chromatography, 2013; 2(10):26
44. Chang C. C., Her G. R. Guide for Solvent selection, 2012; 3(5):40
45. Saito Y. TLC Analysis. 2014; 2(13): 22