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Review

Green chromatography

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ABSTRACT

Analysis of organic compounds in samples characterized by different composition of the matrix is very important in many areas. A vast majority of organic compound determinations are performed using gas or liquid chromatographic methods. It is thus very important that these methods have negligible environmental impact. Chromatographic techniques have the potential to be greener at all steps of the analysis, from sample collection and preparation to separation and final determination. The paper summarizes the approaches used to accomplish the goals of green chromatography. While complete elimination of sample preparation would be an ideal approach, it is not always practical. Solventless extraction techniques offer a very good alternative. Where solvents must be used, the focus should be on the minimization of their consumption. The approaches used to make chromatographic separations greener differ depending on the type of chromatography. In gas chromatography it is advisable to move away from using helium as the carrier gas because it is a non-renewable resource. GC separations using low thermal mass technology can be greener because of energy savings offered by this technology. In liquid chromatography the focus should be on the reduction of solvent consumption and replacement of toxic and environmentally hazardous solvents with more benign alternatives. Multidimensional separation techniques have the potential to make the analysis greener in both GC and LC. The environmental impact of the method is often determined by the location of the instrument with respect to the sample collection point.
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1. Introduction

"Green chemistry", "clean chemistry", "benign chemistry", etc., are all terms used to describe approaches that minimize the use of feedstock, consumption of reagents and energy, as well as generation of wastes in the chemical industry. Another goal of such practices is the elimination of hazardous substances by substituting them with more benign ones. The concept of green chemistry was introduced by Anastas with the 12 principles of green chemistry (Table 1) [1]. The principles became guidelines for chemists who design new chemicals, materials and processes. Green chemistry is a concept falling into the scope of sustainable development.

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Table 1
Green chemistry principles and their implementation in green analytical chemistry.

No.	Principle	Explanation	Examples of implementation in green analytical chemistry
1	Prevention	It is better to prevent waste than to treat or clean waste afterwards	Application of solventless extraction techniques, application of direct determination methodologies, miniaturization
2	Atom economy	Design synthetic methods to maximize the incorporation of all materials used in the process into the final product	–
3	Less hazardous chemical syntheses	Design synthetic methods 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 auxiliary substances wherever possible; make them innocuous when used	Substitution of toxic solvents with less toxic ones; solventless extraction techniques; direct analysis
6	Design for energy efficiency	Minimize the energy requirements of chemical processes and conduct synthetic methods at ambient temperature and pressure if possible	Application of microwave, ultrasound or pressure-assisted extraction to minimize energy consumption (much shorter extraction time)
7	Use of renewable feed stocks	Use renewable raw materials or feedstock whenever practicable	–
8	Reduce derivatives	Minimize or avoid unnecessary derivatization if possible, as it requires additional reagents and generates waste	Derivatization should be avoided when possible
9	Catalysis	Catalytic reagents are superior to stoichiometric reagents	–
10	Design for degradation	Design chemical products so they break down into innocuous products that do not persist in the environment	–
11	Real-time analysis for pollution prevention	Develop analytical methodologies needed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances	Development of procedures that allow obtaining analytical results with short (preferably no) time delay
12	Inherently safer chemistry for accident prevention	Choose substances and the form of a substance used in a chemical process to minimize the potential for chemical accidents, including releases, explosions, and fires	Application of solventless techniques to prevent occupational exposure, real time monitoring, miniaturization

Green analytical chemistry is a branch of green chemistry concerned with different aspects of chemical analysis. Fig. 1 illustrates how green analytical chemistry can be applied to sample preparation and the final determination step. While most of the twelve green chemistry principles apply to all areas of chemistry, some of them apply directly to analytical chemistry, e.g. the need for real-time monitoring for pollution prevention.

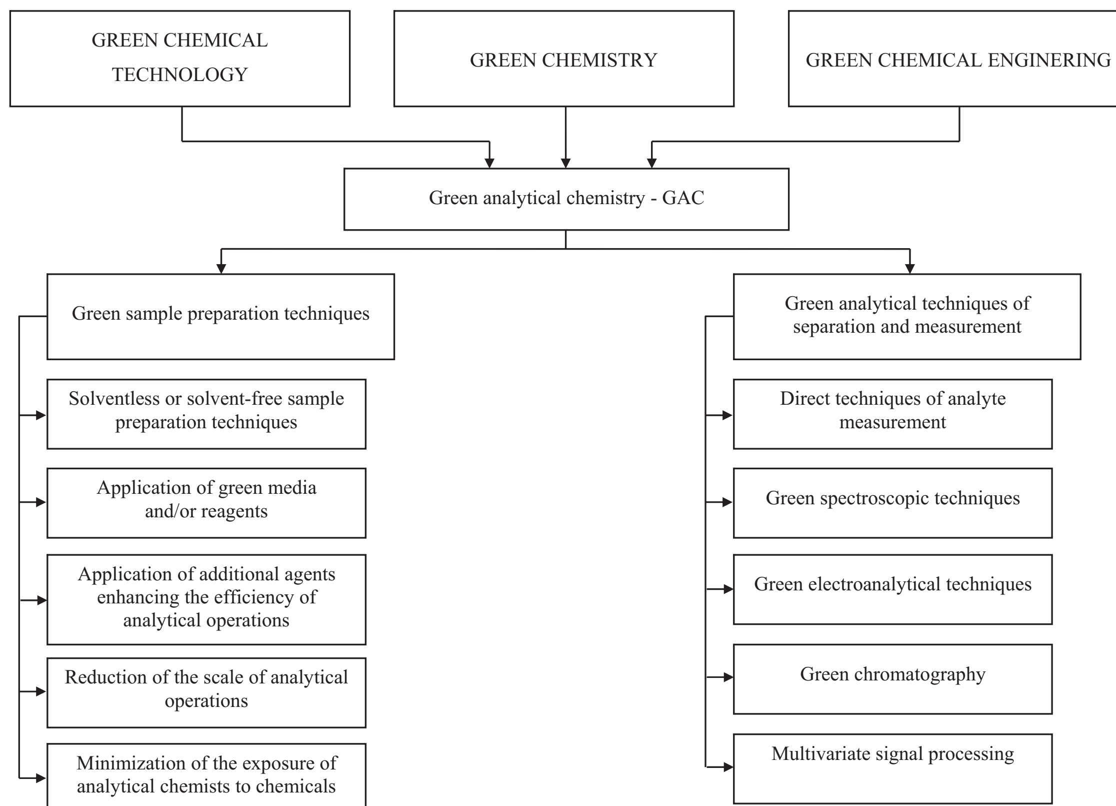

Fig. 1. Green analytical chemistry principles applied to sample preparation and the final determination step.

The interest in green analytical chemistry is growing. Books dedicated to this topic have been published, including "Green Analytical Chemistry" [2], "Challenges in Green Analytical Chemistry" [3] and "Handbook of Green Analytical Chemistry" [4]. Special issues devoted to green analytical chemistry were also published in journals including Trends in Analytical Chemistry (2010) [5], and more recently Analytical and Bioanalytical Chemistry [6] and Bioanalysis [7–12].

Determination of organic compounds in various matrices is usually performed using chromatographic techniques. Chromatographic procedures are used in research, as well as routine medical, industrial, food and environmental analysis. This translates into a very large number of chromatographic determinations performed each and every day around the world. A single liquid chromatograph can potentially generate 1–1.5 L of liquid waste daily [13]. Environmental impact of solvents consumed during chromatographic determinations should not be neglected. Just as industrial chemists' responsibility is to minimize pollution generated by their activities, analytical chemists' responsibility is to obtain reliable analytical results within a short time, with little or preferably no negative environmental impact. Fortunately, economy is usually on the side of green analytical chemistry, as it promotes protocols that consume no or just small amounts of reagents. This creates opportunities for savings for analytical laboratories, as they do not need to purchase large amounts of reagents and/or solvents. Similarly to green spectroscopy [14] or green electrochemical methods, there is place for green chromatography within the framework of green analytical chemistry [13,15].

The aim of this paper is to present the ways in which chromatography can become greener (see Fig. 2). Different stages of chromatographic procedures are taken into consideration. Liquid chromatography and gas chromatography are compared from the point of view of green analytical chemistry. In addition, various options to reduce the environmental impact of sample preparation prior to chromatographic analysis are presented. The role of miniaturization in sample preparation and chromatographic separations is also stressed.

2. Chromatographic methodologies without sample preparation

An important green aspect of chromatographic methodologies is the presence or absence of the sample preparation step, which is often the most polluting in the entire chromatographic analysis procedure [16]. Occupational exposure to toxic chemicals during sample preparation may affect the health of the analyst. From the perspective of green analytical chemistry, it is highly advantageous to use direct chromatographic methodologies (i.e. methodologies not requiring sample preparation) whenever possible. The main limitation of such methodologies is that they are only applicable to samples with relatively clean matrices [17], as otherwise the chromatographic columns might quickly deteriorate due to deposition of sample components that do not elute from the column. Water, spirits and petroleum fractions are examples of matrices that can usually be injected into chromatographic columns without sample pretreatment.

Gas chromatographic methods are usually more readily adaptable to the elimination of the sample preparation step than liquid chromatographic ones. The early work on direct gas chromatographic methods was focused on the development of on-column injection, proposed by Grob and Grob [18]. A combination of on-column injection with electron capture detection allowed chromatographic determination of halogenated compounds in water samples by direct aqueous injection [19], whereas a combination of flame ionization detection [20] or mass spectrometric detection [21] with direct aqueous injection allowed direct determination of hydrocarbons in water samples. The latter two detectors have problems handling the relatively large volumes of water passing through them in this method, though.

Application of direct chromatographic methodologies meets the goals of green analytical chemistry in several ways. The consumption of materials that would have been used during sample preparation (including organic solvents, sorbents, cartridges, fibers, etc.) is avoided. The total time of the analysis (from sample collection to obtaining the results) is potentially greatly shortened. The lack of sample preparation step allows placing the chromatograph at-line or on-line, which may further reduce the analysis time. This is especially important in process analytics, where near-real time results are crucial. Direct chromatographic methodologies thus meet the 11th principle of green chemistry that pushes analytical chemistry toward minimizing environmental impact of chemical processes through real-time monitoring.

3. Green sample preparation

Sample preparation is considered a crucial part of analytical protocols based on chromatographic separation, identification and quantitative determination of a wide spectrum of analytes, especially in samples characterized by complex composition of the matrix. There are different ways leading to "greening" of sample preparation. Representative examples include:

- elimination (or at least reduction) of the amounts of solvents and reagents used in the analysis,
- miniaturization of instruments and reduction of the scale of analytical operations,
- integration of various operations and automation/robotization of sample preparation,
- proper sealing of all vessels used during sample preparation,
- solvent recovery and reuse,
- application of green media (e.g. ionic liquids, supercritical fluids or superheated water),
- application of factors enhancing the effectiveness of sample preparation (e.g. elevated temperature and/or pressure, microwave and ultrasonic energy).

The terms "solventless" or "solvent-free sample preparation" are used frequently. A large number of review papers available in the literature focused on different aspects of sample preparation for chromatographic analysis. Table 2 lists examples of the most important green sample preparation techniques [13,22–27].

4. Green aspects of gas chromatography

Gas chromatography (GC) is a technique of choice for the analysis of semi-volatile and volatile compounds. Implementation of the principles of green chemistry into gas chromatography can be performed in many ways. Minimizing the amount or eliminating the solvent during sample preparation prior to final chromatographic analysis is highly recommended as discussed above. The choice of carrier gas may also be considered when trying to make GC analysis greener. Helium (He) is the most commonly used carrier gas in GC because of its favorable chromatographic properties (high optimum linear velocity) while being non-toxic, non-flammable, inert and safe to handle. However, He is also a non-renewable resource, and the world's reserves of this precious gas are about to run out, a shortage that is likely to have far-reaching repercussions. Nitrogen (N_2) can also be used as a carrier gas in GC. However, its optimal linear velocity (u_{opt}) is low compared to helium or hydrogen, which translates into long analysis times. In addition, the steepness of the

Table 2
Examples of green sample preparation techniques.

Technique	Principle	Applications/analytes	Examples of matrices	Green aspects	Ref.
Miniaturized extraction techniques					
Solid phase extraction techniques					
Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction (QuEChERS)	Uses a small amount of organic solvent. Employs a novel, quick dispersive solid phase extraction cleanup Two main steps: • Solvent extraction (intensively shaken) • Dispersive SPE (dSPE)	Multiresidue, multiclass pesticides Antibiotics Drugs	Fruits Vegetables Soil	Low consumption of solvents and other materials	[27–30]
Solid-Phase Microextraction (SPME)	Based on partitioning of analytes between the extraction phase and the sample matrix • A fiber coated with a polymeric stationary phase is placed in the sample (direct mode – DI-SPME) or its headspace (HS-SPME) • Analytes partition into the stationary phase until equilibrium is reached • Desorption of the concentrated analytes into an analytical instrument	Aroma, pharmaceutical, forensic samples BTEX, diesters, pesticides, PCBs, benzaldehyde, acethophenone, tridecane, monohalogenated benzene, phenolic compounds, aromatic amines, arylamines, formaldehyde, PAHs, triazines, chlorinated hydrocarbons, MTBE, odorants, malodorous sulfur compounds, organophosphate triesters, dodecane, isocyanates	Gaseous phase Water Sediment Food Sand Clay Fish Industrial effluents	• Eliminates the need for solvents • Combines sampling, analyte isolation and enrichment into one step	[22,31]
Microextraction by Packed Sorbent (MEPS)	• Sample collection by a sorbent (a bed or a coating of the inner surface of a chromatographic column) • Cleanup to remove interfering compounds • Analyte extraction with a suitable solvent • Sample introduction to a chromatographic injector • Sorbent bed regeneration – washing with solvent and a washing solution (50 μL) • Sorbent types: C2, C8, C18, C8-SCX, SAX, SCX, PS-DUB, MIP, HILIC	Drugs and their metabolites: Local anesthetic drugs Anticancer drugs roscovitine, olomoucine, busulphan, cyclophosphamide Neurotransmitters dopamine and serotonin Methadone, cocaine and cocaine metabolites	Water Urine Plasma Blood	• Significantly reduces the solvent volume (10–50 μL) and the sample volume Short extraction time (about 1 min), low energy consumption	[32,33]
Stir-bar Sorptive Extraction (SBSE)	Sorption of analytes onto a thick film polymer (e.g. PDMS) coated on a magnetic stir bar. Can be used in direct or headspace modes	Water, air, food pollution, biological and biomedical fields Organic compounds: VOCs, PAHs, PCBs	Water Air	Solventless sample preparation when thermal desorption is applied for analyte desorption	[34–43]
Solid Phase Nanoextraction (SPNE)	Based on strong affinity of analytes toward gold nanoparticles • Aqueous samples (500 μL) are mixed with colloidal gold solution • Quantitative binding of analytes to the surface of gold nanoparticles • Centrifugation to recover the nanoparticles	PAHs	Water	Reduced organic solvent consumption	[44]
Liquid extraction techniques					
Single Drop Microextraction (Liquid–Liquid Microextraction) (SDME or LLME)	• A microdrop of solvent is suspended from the tip of a conventional microsyringe or PTFE rod • Analytes diffuse into the droplet • The microdrop is retracted into the syringe, then injected into a chromatographic injector Two modes of SDME: – Direct immersion (DI-SDME) – the acceptor organic phase is in direct contact with the aqueous sample, – Headspace SDME (HS-SDME) – the acceptor phase is in the gaseous phase above the sample	Hypericins (in deproteinated plasma, urine) PAHs, VOCs including alcohols, chlorobenzenes, phenols, amines, organotins, trihalomethanes, BTEX Semi-volatile organic compounds (SVOCs)	Water Air	Very small volume of reagents and solvents (1–2 μL)	[45–47,23,48]
Liquid-Phase Microextraction (LPME)	• In dynamic LPME, a microsyringe is used as a microseparating funnel for the extraction of target analytes. Analyte-enriched microdrop is injected into a chromatograph • In two-phase hollow fiber (HF) LPME mode, organic solvent is immobilized inside a porous polypropylene capillary tube and placed in contact with the water sample. Analytes partition into the solvent through the pores of the capillary • In three-phase HF-LPME mode, analytes cross the organic solvent embedded in the pores of a semi-permeable membrane and concentrate in a third phase inside the lumen of the capillary after modification of their solubility (e.g. through pH change)	Drug/pharmaceuticals analysis: amphetamines, antidepressants, amino alcohols, anabolic steroids, basic drugs, cocaine, methamphetamine, Environmental analysis: drugs, pesticides, aromatic amines, BTEX, phenols, fatty acids, fungicides, pesticides, haloacetic acids, organic pollutants, food and beverage analysis: carbaryl, ochratoxin A, pesticides Peptide analysis: peptides Miscellaneous: octanol–water partitioning	Blood Urine Human breast milk Hair Drinking, river and sewage water Saliva Serum Soil	• Negligible consumption of organic solvent, high potential to pre-concentrate target analytes • Reduced generation of wastes and relatively low sample consumption	[49]

Ionic Liquid Dispersive Liquid–Liquid Microextraction (IL-DLLME)	• Three phase system is applied: sample/extraction solvent/disperser solvent miscible with both • Ionic liquid is used in DLLME instead of a volatile organic solvent as the extraction solvent • Extremely large area of the contact of the solvent and aqueous phase is established (rapid transfer of analytes from the aqueous phase to the extraction phase)	PAHs, organophosphorus pesticides, chlorobenzenes, chlorophenols, phenols, trihalomethanes, methomyl phthalate esters, anilines, polybrominateddiphenyl ethers	Water	Eliminates the use of highly toxic chlorinated solvents (ionic liquids are considered green solvents)	[50–52,49]
Membrane extraction					
Supported Liquid Membrane Extraction (SLME)	• Porous PTFE membrane impregnated with organic solvent is applied • Analytes are transferred through the membrane due to concentration gradient aq (donor)/org (membrane)/org (acceptor) phase combination is used	Pesticides, amines, metal ions, drugs, chlorophenols	Water	Reduction in the volume of solvent used	[53–56]
Microporous Membrane Liquid–Liquid Extraction (MMLLE)	• Similar to SLME • Involves a microporous membrane; aq (donor)/org (membrane)/org (acceptor) phase combination is used	Pesticides, herbicides 4-(dimethylamino) benzoate	Water Urine Red wines	Reduced consumption of reagents	[54]
Membrane Extraction with a Sorbent Interface (MESI)	• A non-porous polymeric membrane for analyte extraction from a gas or liquid phase is used • Analytes are removed from the inner membrane surface by a stream of gas and transferred to a sorbent where they undergo preconcentration • The sorbent trap is periodically thermally desorbed and the concentrated analytes are injected to a chromatographic system	BTEX, aliphatic and aromatic organics, alcohols	Water Air	• Solventless technique • Semi-continuous analysis	[57]
Membrane Assisted Solvent Extraction (MASE)	• A three-phase system (aqueous phase/non-porous polymeric membrane/organic phase) • Organic solvent receptor phase	PCBs PAHs Phenols	Water Beverages	Reduced consumption of reagents	[58]
Microdialysis sampling	• A probe equipped with a semipermeable membrane is placed in the sample • A solution of ionic composition similar to the surrounding fluid is pumped through the probe by a syringe pump, leading to analyte concentration gradient between the perfusate and the surrounding medium • Analytes diffuse into the probe due to the concentration gradient • The solution coming from the probe (dialysate) is then delivered to an appropriate analytical system	Metals Pesticides Proteins Aniline, 2-chloroanaline Sugars Inorganic anions	Body fluids of living organisms Water Soil Food Sludge	• Small sample volume • Reduced consumption of reagents	[59,60]
Membrane-Separated Liquid Extraction (MSLE)	• A porous membrane is applied • Analytes are transferred through the membrane due to concentration gradient; aq (donor)/org (membrane)/aq (acceptor) phase combination is used	Polar compounds (organic acids) Ionic compounds (metal ions)	Aqueous samples	Low solvent consumption	[61]
Thin-Film Microextraction (TFME)	• Thin poly(dimethylsiloxane) membrane is used • Principle the same as in SPME (a membrane is used instead of a fiber)	Hydrophobic semivolatile compounds, PAHs Polar phenolic compounds	Water Seawater	Solventless	[62]
Alternative solvents					
Ionic liquids (ILs)	• Dissolve organic and inorganic compounds • Non-volatile, thermally-stable (up to ∼300 °C) • Used as alternatives to traditional organic solvents	• Supported liquid membrane extraction • Chiral ILs applied in chromatographic SCE separation of chiral mixtures • Application in microextraction √ SDME √ LPME √ Dispersive liquid–liquid microextraction (DLLME) √ Coatings of SPME fibers	Solid or liquid samples	• Green solvents • Do not emit harmful vapors	[63,64]

Table 2 (*Continued*)

Technique	Principle	Applications/analytes	Examples of matrices	Green aspects	Ref.
Supercritical Fluid Extraction (SFE)	• A substance in supercritical state is applied as the extracting agent (temperature and pressure of the substance above the critical values) • The most common supercritical fluids include carbon dioxide, nitrous oxide, ethane, propane, n-pentane, ammonia and sulfur hexafluoride	Petroleum hydrocarbons, PAHs, PCBs	Medical plants, food, seeds, fruits, leaves; flowers, rhizomes, soil, drugs, sediments, water, animal tissues	Minimization or complete elimination of organic solvents	[65,66]
Subcritical Water Extraction, or Hot Water Extraction, or Pressurized Water Extraction (SWE, HWE or PWE)	• Superheated water used as the extracting solvent • Water below the supercritical state (T_c = 374 °C, P_c = 218 atm) can perform very selective extractions of polar (at lower temperatures), moderately polar, and nonpolar (at higher temperatures) organic pollutants	Semi-volatile and non-volatile compounds: PCBs, PAHs, pesticides, monoterpenes, triazines explosives, isoflavones, lignans, saponins, anthocyanins, dioxins	Soil Waste sludge	• Elimination of organic solvents • Most of the fluid phase is recyclable	[67]
Assisted extraction					
Microwave-Assisted Extraction (MAE)	• Microwave energy used to enhance the extraction process • Sample can be heated, homogenized, digested • Two modes: pressurized MAE or atmospheric MAE	PAHs, PCBs, organochlorine pesticides, and alkanes	Blood, food, environmental, biological, industrial samples	• Reduction of solvent and sample consumption • Saving time and energy	[24]
Ultrasonic Extraction (USE)	• Ultrasonic energy is used, therefore acoustic cavitation takes place • Bubbles of gas are formed, then implode, resulting in local increases in pressure and temperature • Two types of ultrasonic devices: ultrasonic probes or baths	Metals	Solid samples	• Reduced solvent consumption • Saving time and energy	[16]
Pressurized Liquid Extraction, or Pressurized Fluid Extraction (PLE or PFE)	• Extraction at high pressure and temperature above the atmospheric pressure boiling point • Elevated temperature increases analyte solubility and diffusion rate, and decreases viscosity and surface tension of the solvent • Elevated pressure improves the penetrability of the extractant into the pores of the matrix	Organic compounds	Food Biological samples	• Reduced solvent consumption • Possible application of environmentally-friendly solvents (e.g. ethanol, methanol) • Savings in time and energy	[16]

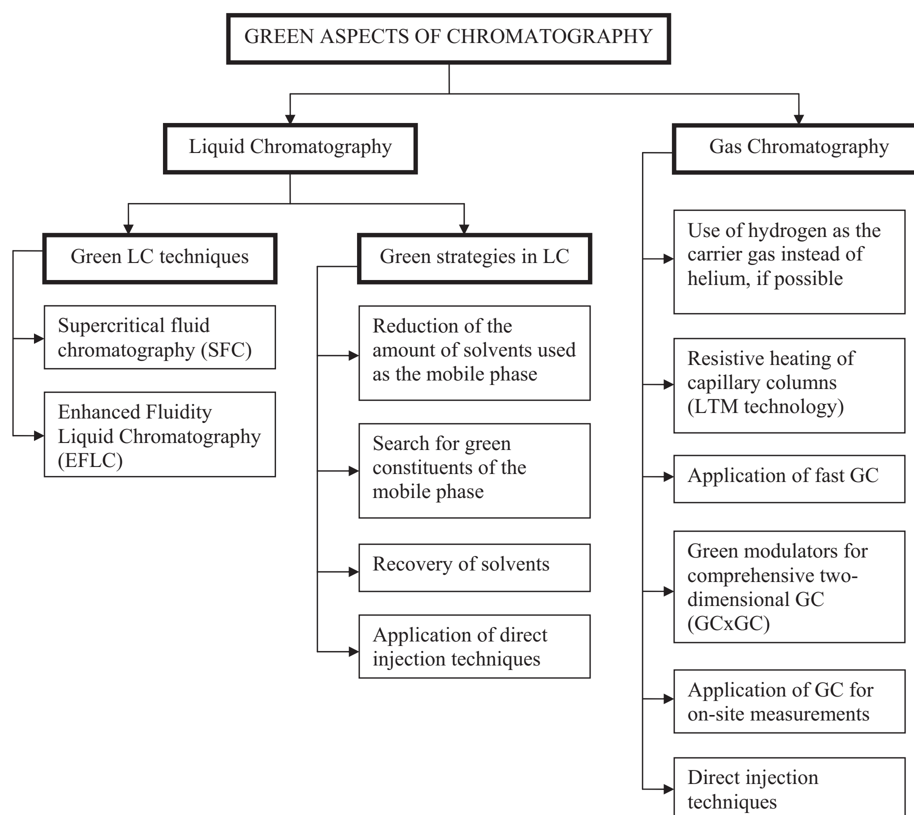

Fig. 2. Different approaches to greening of chromatographic methodologies.

nitrogen van Deemter curve means that small changes in the average linear velocity result in large changes in efficiency. These two factors make nitrogen the least desirable carrier gas for GC.

Hydrogen seems to be the best alternative as a carrier gas in GC. Hydrogen's u_{opt} is the highest of the three common carrier gases compared here, and the van Deemter curve is very flat, hence separations can be conducted at higher-than-optimal flow rates with little loss in efficiency. Consequently, the shortest analysis times can be accomplished with hydrogen as the carrier gas. Also, the wide range over which high efficiency is obtained makes hydrogen the best carrier gas for samples containing compounds that elute over a wide temperature range when constant pressure is used. Methods where He is used as the carrier gas can be translated into methods using hydrogen without sacrificing analysis speed, resolution, and sensitivity. Furthermore, hydrogen gas generators are available, which overcomes concerns about safety of hydrogen gas cylinders.

The next approach to make GC "greener" is the reduction of the analysis time leading to increased sample throughput. This may be achieved by using columns shorter than conventional and with smaller internal diameter (i.d.) without sacrificing separation and resolution. Furthermore, u_{opt} of the carrier gas is increased with such columns, which contributes to further reduction of the analysis time. This solution is commonly referred to as fast capillary GC. The downside of using narrow diameter columns is their reduced sample capacity, which frequently leads to column overloading.

4.1. Low thermal mass technology

In gas chromatography, temperature programming is often considered the second most important parameter to control after column selectivity [68]. The use of increasing column temperature during GC analysis is referred to as temperature-programmed gas chromatography (TPGC). TPGC brings many advantages including better separation for solutes with a wide boiling points range, improved peak symmetry for solutes with high retention factors, and improved detection limits [69]. Moreover, TPGC allows the removal of unwanted heavier sample components from the column that could otherwise compromise the integrity of the chromatographic system.

To achieve ultrafast temperature programming and an unprecedented cool down time with a power consumption of approximately 1% of conventional gas chromatography, low thermal mass technology was introduced in 2001 [70,71]. Fig. 3 presents low thermal mass column module diagram. LTMGC is considered a green technique because resistive heating of a GC column brings about two main advantages: reduction of power consumption by approximately a factor of 200, and increased speed of column heating (up to 1800 °C/min; achievable rates depend on column mass, configuration and column void times), which can potentially reduce the analysis time. An example of the application of LTM in the analysis of alkanes is presented in Fig. 4. It has been shown that even at 1800 °C/min, separation was satisfactory and was achieved in less than 0.3 min.

Luong et al. [68] presented a comparison of cooling rates of four LTMGC modules to a conventional GC oven equipped with a vent deflector. Depending on 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. Fast heating and cooling rates allow high sample throughput, resulting in high efficiency temperature programming. Other advantages of the LTM technology are presented in Fig. 5.

Although the LTM technology has many advantages, it is not without its drawbacks. First, the host oven has to be kept constantly at elevated temperature to prevent cold spots, which might have a negative impact on the capillary tubing used for interfacing the host

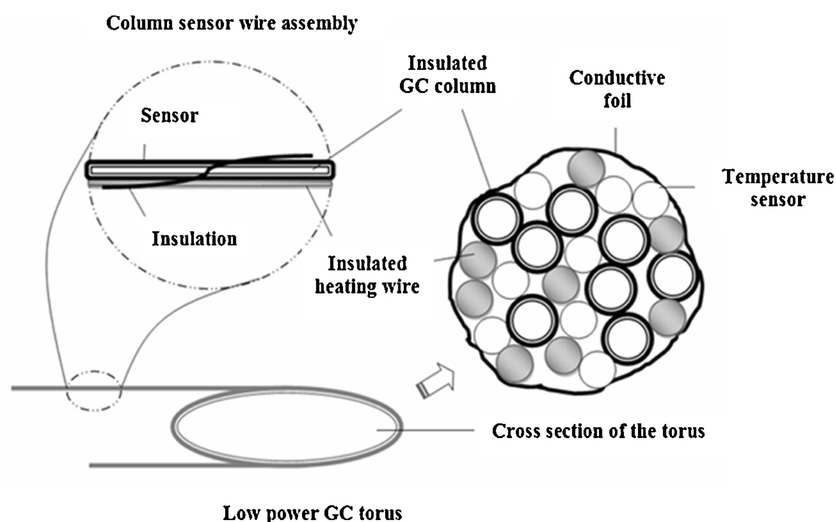

Fig. 3. LTM column module diagram (Patents: US 6,209,386; US 6,217,829; US 6,490,852; US 6,682,699).

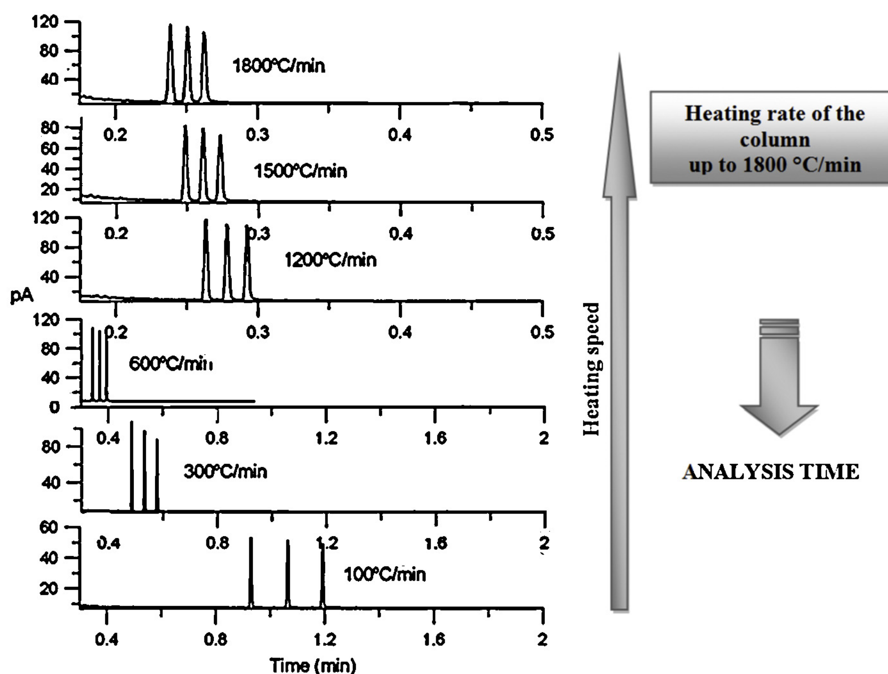

Fig. 4. Separation of C14–C16 n-alkanes on a 2 m × 0.1 mm × 0.12 μm (5%-phenyl)-methylpolysiloxane capillary column for a range of temperature programming rates from 100 °C/min to 1800 °C/min [68].

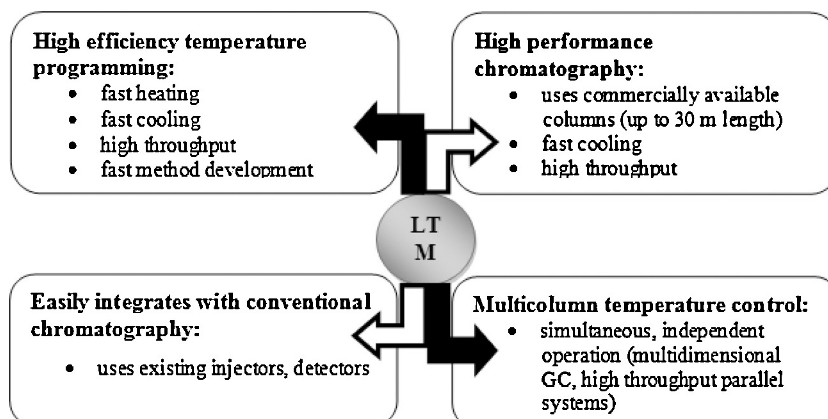

Fig. 5. Advantages of low thermal mass technology [68,70,71].

GC with the LTM module [68]. Constant heating of the oven negates to some extent the energy savings brought about by direct resistive heating. Moreover, if LTMGC is used with a splitless injector or with a cool-on column injection system, an uncoated yet deactivated transfer line should be used to connect the injector with the column module [68]. If the host oven is at a higher temperature than the LTMGC module, thermal conditioning of the LTMGC prior to analytical work is required. This is 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 [68].

Another technology based on direct resistive heating of nickel-clad fused silica GC columns has been introduced by VICI [72]. This direct resistive heating method can achieve heating rates as high as 800 °C/min, as well as sub-one minute cooling times from 360 °C to 40 °C for 5-m columns, while peak power consumption is around 70 W [72]. The resistively heated nickel-clad fused silica columns are suitable for fast GC analysis and for portable instruments because of rapid heating and cooling, low power consumption, small size and high reliability. These advantages make this technology “green”.

4.2. Comprehensive two-dimensional gas chromatography (GC × GC)

Comprehensive two-dimensional gas chromatography (GC × GC) is the most powerful separation technique for volatile and semi-volatile analytes, especially when combined with time-of-flight mass spectrometry (TOFMS). This technique has been known for over 20 years, as it was introduced in 1991 [73]. The following features distinguish a comprehensive two-dimensional gas chromatography system from conventional one-dimensional GC:

- *Two different columns*: the first (typically 30–60 m long) is usually non-polar, while the second (~0.5–2 m) is polar/semi-polar; however, reverse configurations and other column combination can also be used.
- *Modulator*: placed between the two chromatographic columns, prevents the loss of separation achieved in the first column and enables two-dimensional separation.
- *Detectors*: very narrow peaks eluting from the second column (~50–400 ms) require high-speed data acquisition systems (sampling at least 50–100 Hz to obtain 10–20 points per peak) [74].

GC × GC allows better separation of sample components in complex matrices than conventional 1D-GC, while requiring the same (or only slightly longer) time for separation, with comparable reagents consumption and the same sample volume. In principle, this makes GC × GC greener compared to one-dimensional gas chromatography. The great separation power of GC × GC–TOFMS allows the determination of target analytes in complex matrices with little to no sample preparation, which results in significant time and reagent savings. An example of this is shown in Fig. 6. The matrix components in this figure are represented by the continuous band in the lower part of the plot [75]. Without extensive sample preparation, chlorfenvinphos could not be separated from the other components of the carrot extract using 1D-GC–TOFMS, and could not be recognized based on the mass spectrum. On the other hand, separation and identification of this compound was possible using GC × GC–TOFMS, and its mass spectrum corresponded closely to the library spectrum.

The modulator is the most important component of a GC × GC system. The purpose of this device is to continuously collect small portions of the eluate from the first column and inject them into the second column at regular intervals [76]. Development of efficient modulators that make it possible to collect and introduce the analytes to the second column in a fast and repeatable manner has been and still remains the main challenge for the technology. The history of the development of GC × GC modulators has been recently recounted in two review articles [74,77]. One of the most popular approaches to modulation these days is based on analyte trapping in the stationary phase of the GC column [78]. The analytes are trapped in the stationary phase at low temperature. At the end of each modulation period the capillary is rapidly heated to release the analytes in the form of a narrow band into the second column. This process is known as thermal modulation, and is the most popular approach these days. Thermal modulation is usually carried out using cold and hot gas streams to alternately cool and heat segments of the capillary column [79,80]. The main disadvantages of this approach include somewhat complex design and the need for cryogenic agents (liquid CO_2 or N_2), which are cumbersome to handle and expensive to use. The consumption of liquid cryogens compromises the benefits of GC × GC as a green separation technique, hence greener modulation methods are being developed.

Differential flow modulators are an alternative to thermal modulators. In these modulators the effluent from the first column is collected in a sampling loop (or loops). Before the loop becomes 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 these modulators is somewhat simpler than that of thermal modulators, and they require no cryogenic agents, making them more economical and greener. However, they do suffer from some limitations. The maximum length of the modulation period is determined by the volume of the loop and is usually limited to ~2 s. Band compression is accomplished by pressurization of the sampling loop content, which is not as efficient as thermal focusing. As a consequence, the sensitivity of GC × GC with differential flow modulation is usually worse than with thermal modulators. Finally, the high carrier gas flow rates in the second dimension preclude direct coupling of the second column to mass spectrometers. Splitting of the effluent can be used, but leads to further reduction of the sensitivity of the method.

A new type of thermal modulator introduced recently can be a viable alternative to cryogenic modulators. The device is known as consumable-free modulator (CFM). It uses ambient air flow for cooling [81]. The design of the CFM is schematically illustrated in Fig. 7. This type of modulator eliminates the consumption of cryogenic agents, which makes it green. It allows modulation of compounds in the volatility range from C5 n-alkane to C40. It has no moving parts and does not require any consumables, which makes it ideal for in situ analyses, another important aspect of green chemistry.

The stop-flow modulation technique reported in the literature [82] provides the ability to reduce the consumption of cryogenic agents and at the same time eliminates the dependence of the analysis time in the second dimension on the modulation duration. In this modulator the carrier gas flow in the first column can be stopped while continuously supplying the gas to the second column. Separation of the components in the second dimension can therefore be carried out for a time longer than the modulation period. This technique allows the use of optimized conditions in both columns, leading to more efficient separation.

5. Green liquid chromatography

Liquid chromatography is generally considered less green than gas chromatography, as it requires solvents for the separation. On the flip side, this offers more possibilities for “greening”. In theory,

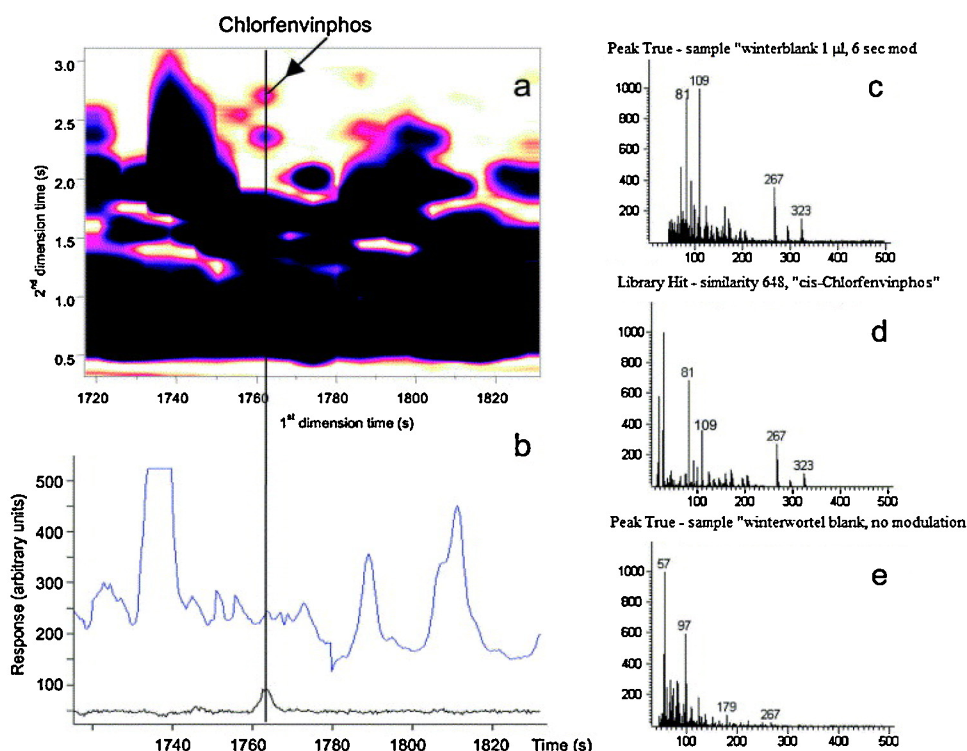

Fig. 6. Comparison of chromatograms of carrot extract obtained by GC × GC–TOFMS (a) and 1D-GC–TOFMS (b). Mass spectrum obtained after GC × GC separation (c), library spectrum (d) and mass spectrum obtained after 1D-GC separation (e) [75].

one continuously operated analytical liquid chromatograph equipped with a conventional LC column (15–25 cm in length, 4.6 mm i.d., packed with 5 µm 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. Although this volume of waste is small compared to the amount of sewage and waste generated by a typical large industrial company, some big companies use hundreds of liquid chromatographs in their research laboratories and in

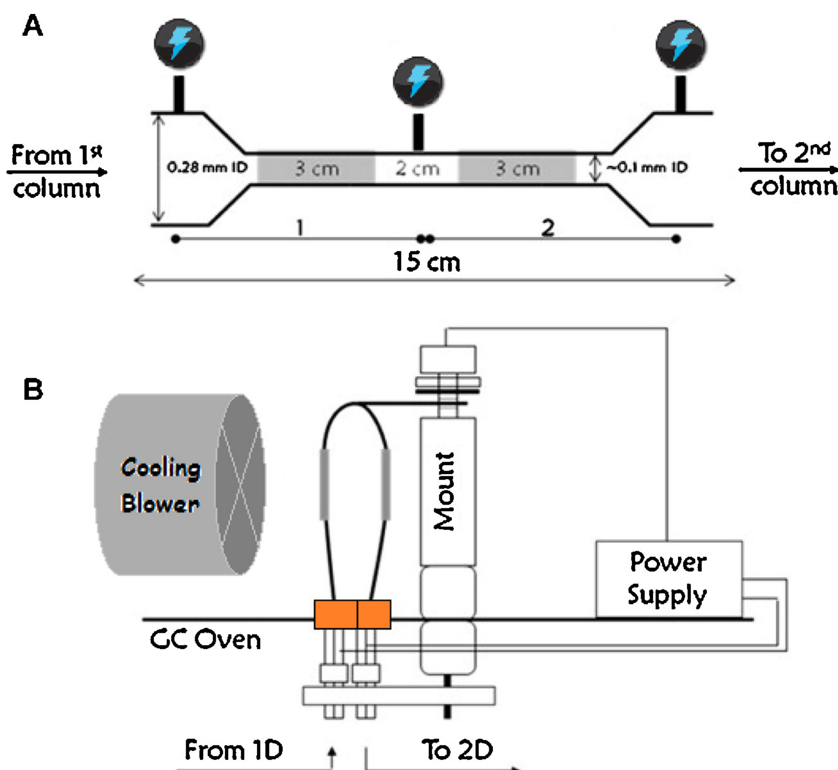

Fig. 7. CFM modulator. A – narrowed capillary with two trapping zones (stationary phase trapping areas marked using gray color); resistive heating through three contacts provides two-stage modulation. B – the modulator placed on a chromatographic oven; cooling of the trapping capillary accomplished by a blower [76].

Table 3
Information on the types of LC according to the column internal diameter [2].

Type of LC	Column diameter (mm)	Flow rate (μL/min)	Characteristics
Conventional	2.1 < i.d. ≤ 5.0	300–10,000	The most commonly used columns Large selection of commercially available stationary phases
Narrow-bore	2.1 ≤ i.d. ≤ 3.0	200–1000	Have several combined characteristics of microbore and standard-bore columns Narrow-bore LC can be easily implemented on conventional equipment with little modification Advantages: reduction in the stationary phase amount, lower solvent consumption and higher mass sensitivity Lower volumetric flow rates cause less damage to pumps, thus extending their life spans and reducing mechanical trouble
Microbore-LC	1.0 ≤ i.d. < 2.0	50–400	Low flow rate and narrow tubing allow reducing the consumption of organic solvents Ideal for the analysis of samples of limited availability
Capillary-LC	0.1 ≤ i.d. ≤ 1.0	0.4–200	Allows for the reduction of organic waste production Capillary flow is generated by a flow rate controller The capillary is very flexible and easily bent
Nano-LC	0.025 ≤ i.d. ≤ 0.1	25×10^{-6}–4000×10^{-6}	Nano-flow is generated by a flow rate controller Nearly a solvent-free system A suitable detector fornano-LC is MS coupled with a nano-spray source Fused-silica capillary, polyether ether ketone (PEEK) and polymer – sheated fused silica (PEEK-sil) tubing is used The dead volume of the tubing is significant due to the very low flow rate To decrease the sample loading time, high flow rate (10–30 μL/min) is used More difficult to handle and operate compared to conventional LC

process control, resulting in thousands of liters of toxic waste produced every day. Consequently, reduction in the amount of solvents used for LC separations is highly desirable.

A good strategy to minimize the consumption of organic solvents, and consequently the production of organic waste, is to modify the relevant column-related parameters [83]. Reduction of solvent consumption can be accomplished by reducing the mobile phase flow rate, which is possible when the internal diameter (i.d.) of the column is reduced. To obtain comparable separations, when the column i.d. is reduced. To obtain comparable separations, when the column i.d. is reduced, the flow rate has to be scaled down by the square of the column diameter Eq. (1) [83].

$$F_{\text{down-scaled}} = F_{\text{conventional}} \left(\frac{\text{i.d.}_{\text{down-scaled}}}{\text{i.d.}_{\text{conventional}}} \right)^2 \quad (1)$$

Reduction of the column i.d. is often accompanied by an increase in analytical sensitivity (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 [83]. On the other hand, extracolumn effects affect the efficiency much more with small diameter columns, which might lead to significant loss in resolution. Columns with internal diameters down to ~2 mm can be handled with conventional LC systems; further miniaturization of chromatographic columns has green character only if micro- and nano-liter pumps are used. The use of capillary-LC and nano-LC may bring other advantages, such as high efficiency and high sensitivity, especially when only small sample volumes are available for the analysis. Information on the types of LC according to the internal column diameter is presented in Table 3. Many applications of capillary-LC and nano-LC have been reported. Table 4 gives examples of such applications, including the parameters of the columns used, composition of the mobile phases and type of detection.

Reduction of the solvent consumption may be also achieved by increasing the chromatographic productivity, which can be done by reducing the particle size and shortening the column length. The same number of plates can be obtained using 5 μm particles (column length of 15 cm) and 2 μm particles; however, in the latter case a column length of 5 cm is sufficient. Reduction in the column length shortens the analysis time, increasing the throughput [83].

Particle size reduction in HPLC typically cannot be implemented without instrumental modifications resulting in reduced extra-column dispersion as well as higher mobile phase delivery pressure, as column backpressure increases significantly when smaller diameter particles are used. Recently, many ultra-high pressure (UHPLC) instruments have become commercially available. The main advantage of UHPLC methods is shorter analysis time, which, together with the reduction of column length and diameter, makes the separation greener. The performance of conventional and UHPLC systems is compared in Fig. 8, where the speed of analysis for different particle sizes is compared for a 400 and a 1200 bar instrument. The *x*- and *y*-scales present log N (plate number) and $\log(H/u_0)$ (height equivalent to theoretical plate over optimum velocity), the latter being related to the analysis time. For high throughput separations requiring 10,000 plates, the analysis times are 10 min for 5 μm particles at 400 bar, 2.5 min for 1.8 μm particles at 400 bar and 1.6 min for 1.8 μm particles at 1200 bar, which means that the analysis can be ~6 times faster when using 1.8 μm particles at 1200 bar compared to 5 μm particles at 400 bar. For 25,000 and 100,000 plates, the reduction of the analysis time by a factor of ~5 and ~3.5, respectively, can be accomplished.

Another parameter that can be used as a powerful variable in liquid chromatography is temperature. It is well know that temperature of the column affects selectivity, efficiency and detectability. Because it is easier to change temperature during the method development stage compared to other parameters such as mobile phase composition or buffer pH, elevated temperature has become a popular parameter used to make HPLC greener. Temperature changes may be very useful in selectivity tuning, especially for polar and ionizable compounds. The benefits of using elevated temperature in LC are summarized in Fig. 9.

Elevated temperature in LC cannot be applied without instrumental adaptations, of which the most important are [109]:

- The column must be supplied with a thermostat.
- The mobile phase must be preheated before it enters the column and with most detectors cooled after it leaves the column (this guarantees that the detector signal will not be influenced by fluctuations in the eluent's temperature).
- A stable stationary phase must be used.

Although high-temperature LC brings many benefits, it also has limitations. For example, high-temperature HPLC cannot be used for the analysis of complex mixtures when analytes are thermally unstable. In addition, temperature stability of the stationary phase

Table 4
Applications of microbore, capillary and nano-columns including parameters of the column used, composition of the mobile phase and type of detection.

Application	Analytes	Matrix	Stationary phase	Mobile phase	Technique used	Mobile phase volume per analysis	Ref.
Microbore column							
Drug analysis	OXZ, MHD	Rat brain	C18; 15 cm × 1.0 mm; 5 μm particles	A: water/ACN/FA (98:2:0.1, v/v) B: water/ACN/FA (20:80:0.1, v/v)	LC–MS/MS	1 mL	[84]
	Ofloxacin	Corneal precipitate	C18; 15 cm × 1.0 mm; 5 μm particles	Water/ACN/FA (90:10:0.1, v/v)	LC–MS/MS	1 mL	[85]
	R-ofloxacin S-ofloxacin	Sewage	C18; 10 cm × 2.1 mm; 1.7 μm particles	MeOH/water/FA (80:20:0.1, v/v)	LC–MS/MS	3.3 mL	[86]
Protein, peptide analysis	Proteins, peptides	Phloem sap of Perilla and lupine plants	C18; 15 cm × 1.0 mm; 5 μm particles	ACN/water, TFAA	HPLC–MALDI-TOF-MS	–	[87]
	Proteins, peptides	Standard solution	C8; 15 cm × 1 mm	A: ACN/TFAA B: ACN/water/TFAA	LC–MALDI-MS LC–MALDI-MS/MS	3.6 mL	[88]
Toxin analysis	MC-LR	Scum from a hepatotoxic Oscillatoriaagardhii bloom	C18; 10 cm × 1 mm	A: 5% ACN:5% MeOH:0.5% FA B: 49% ACN:49% MeOH:0.5% FA	LC–MS	0.5 mL	[89]
	MC-LR, MC-RR, MC-YR	Water	C18; 10 cm × 1 mm; 3.5 μm particles	ACN/FA 10–30% (20 min) – 30–40% (30 min) – 40–70% (5 min) – 70% (5 min)	HPLC–MS	0.9 mL	[90]
			C18; 10 cm × 2.1 mm; 3.5 μm particles			12 mL	
Hormone analysis	Insulin	Urine	C18; 5 cm × 1 mm; 3.5 μm particles	A: 0.1% CH_3COOH:0.01% TFAA B: ACN:0.1% CH_3COOH:0.01% TFAA	LC–MS/MS	2.66 mL	[91]
	TRH	Rat brain	C18; 15 cm × 1 mm	2% 1-propanol, 20% acetonitrile, 0.1% TFAA in water	HPLC–MS	1 mL	[92]
Inorganic analysis	As(III), As(V), MMA, DMA, Se(IV), Se (VI)	Water	15 cm × 1 mm; 3 μm particles	Water with NH_4NO_3 and $NH_4H_2PO_4$	HPLC–ICP-MS	0.4 mL	[93]
Neuro-transmitter analysis	Acetylcholine, choline	Rat brain	15 cm × 1 mm; 5 μm particles	50 mM PB at pH 6.5, containing 1 mM S-1-O, 2 mM TMAB, 0.5 mM $Na_2EDTA \cdot 2H_2O$ and 0.5% (v/v) ProClin 150 in water	IC	1.35 mL	[94]
	Substance P, CGRP, dynorphin A	Rat spinal cord	C8; 10 cm × 1 mm; 5 μm particles	ACN/water/FA at a ratio of 0:100	HPLC–ESI/MS/MS	2.4 mL	[95]
Capillary column							
Drug analysis	Paclitaxel, docetaxel	Cancer cells	C18; 15 cm × 0.5 mm, 3.5 μm particles	A: ACN/water (10:90, v/v) containing 2 mM AM with FA B: ACN/water (90:10, v/v) containing 2 mM AM	LC–MS/MS	0.17 mL	[96]
	Ximelagatran and its metabolites	Pig liver	C4; C18; 15 cm × 0.2 mm; 0.5 μm particles (homemade)	A: 10 mM AM and 4.5 mM AA B: 10 mM AM, 4.5 mM AA and 95% ACN (v/v)	LC–MS/MS	0.1 mL	[97]
Environmental analysis	Chlorophyll a, chlorophyll b	Water	C18; 15 cm × 0.5 mm; 5 μm particles	EtOH/water (95:5, v/v)	LC–UV–DAD	0.4 mL	[98]
Nucleotide analysis	dAMP, dGMP, dCMP, dTMP	Standard solution	C18; 15 cm × 0.3 mm; 3 μm particles	20 mM CH_3COONH_4/MeOH (95:5, v/v)	HPLC–ICP–MS	30 μL	[99]
Hormone analysis	TRH	Rat brain	C18; 8 cm × 0.2 mm; 5 μm particles	0.1% TFA, 3% 1-propanol, 20% acetonitrile	HPLC–MS	4.8 μL	[92]
Flavonoid analysis	Almond flavonoids	Standard solution	C18; 15 cm × 0.3 mm; 3 μm particles	A: water:MeOH:FA (92.5:7.5:0.1, v/v/v) B: CAN:FA (100:0.1, v/v)	HPLC–DAD–MS HPLC–DAD–MS/MS	0.12 mL	[100]
Pesticide analysis	Chloro-phenoxy acid herbicides and their esters	Apple juice	C18; 15 cm × 0.3 mm; 3 μm particles	Water:MeOH:0.8% H_3PO_4	LC–UV	0.44 mL	[101]
Nano column							
Biosample analysis	Penicillin antibiotics	Liver, kidney	C18; 10 cm × 0.1 mm; 3 μm particles	CAN/water/AM with FA (90:10:0.1, v/v/v)	LC–UV LC–MS	9 μL	[102]

Table 4 (*Continued*)

Application	Analytes	Matrix	Stationary phase	Mobile phase	Technique used	Mobile phase volume per analysis	Ref.
Drug analysis		Pharmaceuticals					
Food analysis		Milk					
Biosample analysis	4-ABP	Human bladder cells and rat bladder tissue	C18; 0.073 mm × 0.043 mm; 5 μm particles	MeOH/water/AA (70:30:0.1, v/v/v)	LC–MS	20 μL	[103]
Enantiomeric separation	Nonsteroidal anti-inflammatory drugs	Standard solution	C18; 0.1, 0.05 mm i.d., packed with different RP particles, different lengths of column	CAN:water (30:70, v/v), 30 mM of TM-β-CD	LC–UV–vis	10 μL	[104]
	Nonsteroidal anti-inflammatory drugs	Standard solution	Silica coated with cellulose tris(3-chloro-4-methylphenylcarba-mate); 25 cm × 0.100 mm i.d., 5 μm particles	MeOH/ACN; AF	HPLC–UV–vis	4 μL	[105]
Flavonoid analysis	Flavanones and flavanone glycosides	Standard solution	Silica coated with phenylcarbamate-propyl-β-CD stationary phase; 22 cm × 0.1 mm; 5 μm particles	MeOH/water/TEAA	LC–UV–vis	12 μL	[106]
Food analysis	Phenolic compounds	Olive oil	C18; 0.075 mm × 0.01 mm; 3 μm particles	Water/ACN/AA	LC-ESI-TOF-MS	28.8 μL	[107]
Lipids analysis	Phospholipids (PC, PE, PI, PS, PL)	Mouse liver and brain	C18; 15 cm × 0.075 mm	A: ACN/water (50:50, v/v) B: isopropanol/ACN (90:10, v/v)	LC–MS/MS	12 μL	[108]

AA, acetic acid; 4-ABP, 4-aminobiphenyl; ACN, acetonitrile; AF, ammonium formate; AM, ammonium acetate; dAMP, deoxyadenosine 5′-monophosphate; dGMP, deoxyguanosine 5′-monophosphate; dCMP, deoxycytidine 5′-monophosphate; dTMP, thymidine 5′-monophosphate; DMA, dimethylarsinic acid; FA, formic acid; OXZ, oxcarbazepine; MC, microcystin; MeOH, methanol; MHD, 10,11-dihydro-10-hydroxycarbamazepine; MMA, monomethylarsenic acid; PB, phosphate buffer; S-1-O, sodium 1-octanesulphonate; TEAA, % triethylammonium acetate; TMAB, tetramethylammonium bromide; TFAA, trifluoroacetic acid; TM-β-CD, heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin; TRH, thyrotropin-releasing hormone.

has to be taken into consideration. When silica-based columns are used under RP conditions, the temperature should not exceed 60 °C in most cases, especially in acidic or basic buffered eluents. Because of these limitations, the properties of the analytes of interest and the stationary phases must be carefully considered before using elevated temperature LC [109].

The next strategy for greening liquid chromatography is the search for "green" components of the mobile phase. Typical mobile phases used in RPLC include acetonitrile/water and methanol/water mixtures. Although both acetonitrile and methanol are toxic, the latter has lower disposal costs, therefore it should be selected over acetonitrile whenever possible. According to the guide on "green" mobile phases, water, acetone, methanol, and ethanol can be treated as environmentally friendly LC phase. Ethanol is a particularly desirable solvent for green liquid chromatography, as it has properties similar to acetonitrile and methanol, but is less volatile, less toxic and has lower disposal costs. However, ethanol is not without its drawbacks, including high viscosity of ethanol/water mixtures and the restrictions in trading. Acetone is yet another alternative. It has good solubilizing properties and is perfectly miscible with other solvents such as water. However, it is not widely utilized as a mobile phase in LC because it is a strong UV absorber in the region up to 340 nm, which makes it impractical when UV detection is used [93]. Moreover, acetone is highly volatile and therefore difficult to pump.

Green liquid chromatography can sometimes benefit from the use of monolithic stationary phases. Their macroporous structure induces low pressure drops and allows the use of high mobile phase flow rates, which leads to shorter analysis times. However, unless the column diameter is reduced, this does not bring about solvent savings. On the other hand, these columns offer the possibility of using mobile phases of higher viscosity, for example ethanol/water mixtures.

Replacement of organic solvents with substances under supercritical conditions is the next approach to make liquid chromatography "greener". Slight changes in temperature and pressure around the critical point of supercritical fluids (SFs) result in important changes in physical properties such as density, which can be used to tune the solubility and other parameters of the solvent. Using solvents under supercritical conditions expands their spectrum of solubility and volatility. Consequently, supercritical fluids (especially carbon dioxide) can be an excellent choice as chromatographic mobile phases.

Supercritical fluids are considered green mobile phases because of their limited environmental impact, low disposal costs, reduced consumption of toxic solvents and additives, lack of toxicity (in most cases), residue-free removal of the solvent from the extract and the raffinate, and the ability to recover the solvent almost completely. The reduction in the use of organic solvents results in cost, health and safety benefits, as well as faster and cleaner sample recovery during experimental procedures. Supercritical fluids offer high solubililizing power, high mass transfer capability and high selectivity, therefore they find applications in separation techniques increasingly often.

Supercritical carbon dioxide reclaimed from the atmosphere is the most common SF used as a mobile phase in chromatography. The main advantages of supercritical CO_2 (very low viscosity, high diffusivity, good solubilizing power) result in fast and efficient separations, often better than what can be achieved in HPLC. Because carbon dioxide is a non-polar eluent, organic solvents such as methanol must be added to increase its polarity, but usually at less than 30%.

The use of fluids other than CO_2 has also been reported in the literature; however, these fluids do not have any distinct advantages. They often have high critical temperature and may be flammable (especially organic solvents). Inorganic supercritical fluids such as

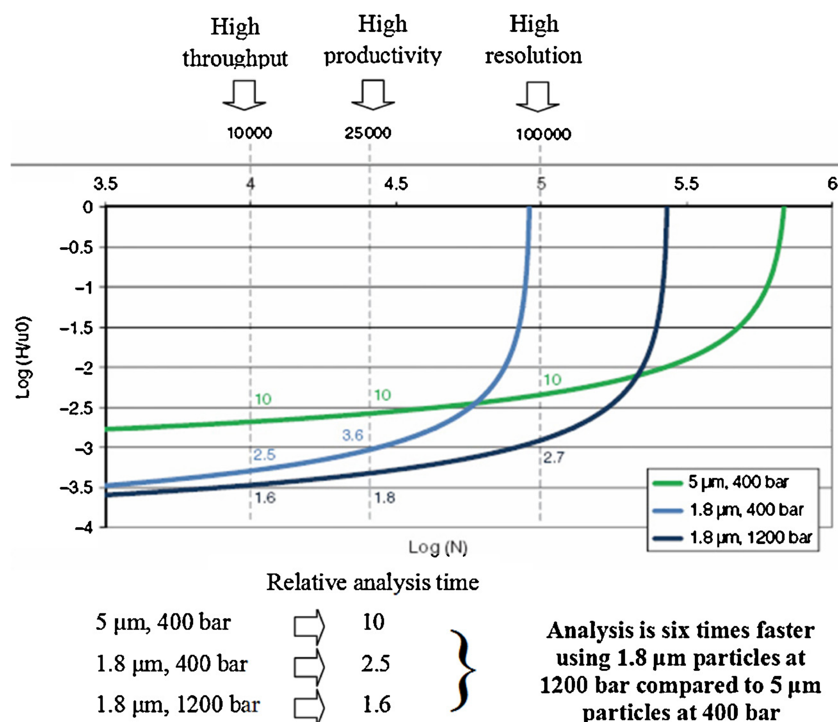

Fig. 8. Kinetic plots for different particles on a 400 and 1200 bar instrument [83].

ammonia or nitrous oxide also have significant drawbacks, such as neurotoxicity or strong oxidizing character in the latter case.

Supercritical fluid chromatography (SFC) is used increasingly often for analytical, semi-preparative and preparative scale purification of chiral compounds, including production of enantiomers. The use of SFC for chiral separations has been one of its most successful applications due to the high diffusivity and low viscosity of the supercritical mobile phase. SFC is replacing HPLC in many pharmaceutical applications, not only for purification, but also as a standard screening and method development tool for chiral compounds. The main advantages of SFC compared to HPLC include higher efficiency, faster method development, faster column equilibration, flexibility in solvent selection and reduced generation of toxic and hazardous wastes.

A comparative study of enantiomeric separation of several antiulcer drugs such as omeprazole, lansoprazole, rabeprazole and pantoprazoleusing SFC and HPLC on Chiralpak AD column was presented by Toribio et al. [110]. Chiral separation by HPLC was only achieved for omeprazole and pantoprazole, while SFC allowed enantiomeric separation of all the compounds studied. Moreover, even when the separation was possible by HPLC, the peaks were broadened. In SFC, resolution between critical pairs was in most cases higher than 2, and the analysis time was less than 10 min. HPLC provided lower selectivity and resolution, and the retention times were longer. Another disadvantage of HPLC was higher consumption of organic solvents. Moreover, due to problems with miscibility of hexane in the normal-phase mode, only ethanol and 2-propanol could be used as polar modifiers, while in SFC a wider spectrum of modifier choices was possible.

Although both SFC and LC in general can provide good enantioselectivity, SFC provides noticeable advantages in terms of the mobile phase flow rate, resolution, analysis time and consumption of organic solvents. Phinney [111] compared the chiral separation of metroprolol on a Chiralcel OD chiral stationary phase by SFC and LC (Fig. 10). Complete resolution of the enantiomers was accomplished with both techniques; however, the mobile phase flow rate in SFC was four times the rate used for LC, which allowed the analytes to be separated in less than one third of the time required for LC separation (SFC: 6 min; LC: 22 min).

In the recent years, SFC popularity has increased not only because of its greener character, but also because of wider availability of commercial instrumentation, including accessories that can covert a conventional HPLC system to one that is SFC-capable. SFC columns are similar to those used in NP-HPLC; however, SFC-specific stationary phases such as 2-ethyl pyridine have also been developed, providing improved selectivity. SFC is traditionally regarded as a separation technique for non-polar substances, but it has been also applied to anionic, cationic and chiral compounds, as well as to drugs and proteins.

Enhanced fluidity (EF) liquid mixtures used as mobile phases for the separation of moderately polar to polar compounds in LC are another “green” approach. EF liquid mixtures are polar liquids such as alcohol, to which high proportions of soluble gases such as CO_2 have been added. These mixtures share many of the positive attributes of both SFs and liquids [112]. As with supercritical fluids, the polarity of EF liquids can be varied by changing the pressure. Moreover, EF liquid mixtures require low pressures to maintain a single phase [112]. By adding high proportions of carbon dioxide to methanol or ethanol with a glassy carbon stationary phase, chromatographic advantages including lower pressure drops, increased optimum linear velocity, improved efficiency, and decreased analysis time were obtained without sacrificing the mobile phase strength [113]. Because of their low viscosity, EF liquid mixtures enable the use of long capillary columns (1 m or more) to produce highly efficient separations [112]. The knowledge of the properties of EF liquids is important to correctly choose operating parameters for a given separation, therefore phase diagram information is required. For some mixtures this information can be obtained from the literature, but for many others experimental determination is required. Enhanced fluidity liquid chromatography (EFLC) has been utilized in reversed- and normal-phase LC, as well as for size exclusion separations. It also has found application in chiral separations [114]. EFLC was found to be more efficient and selective than SFC or conventional HPLC.

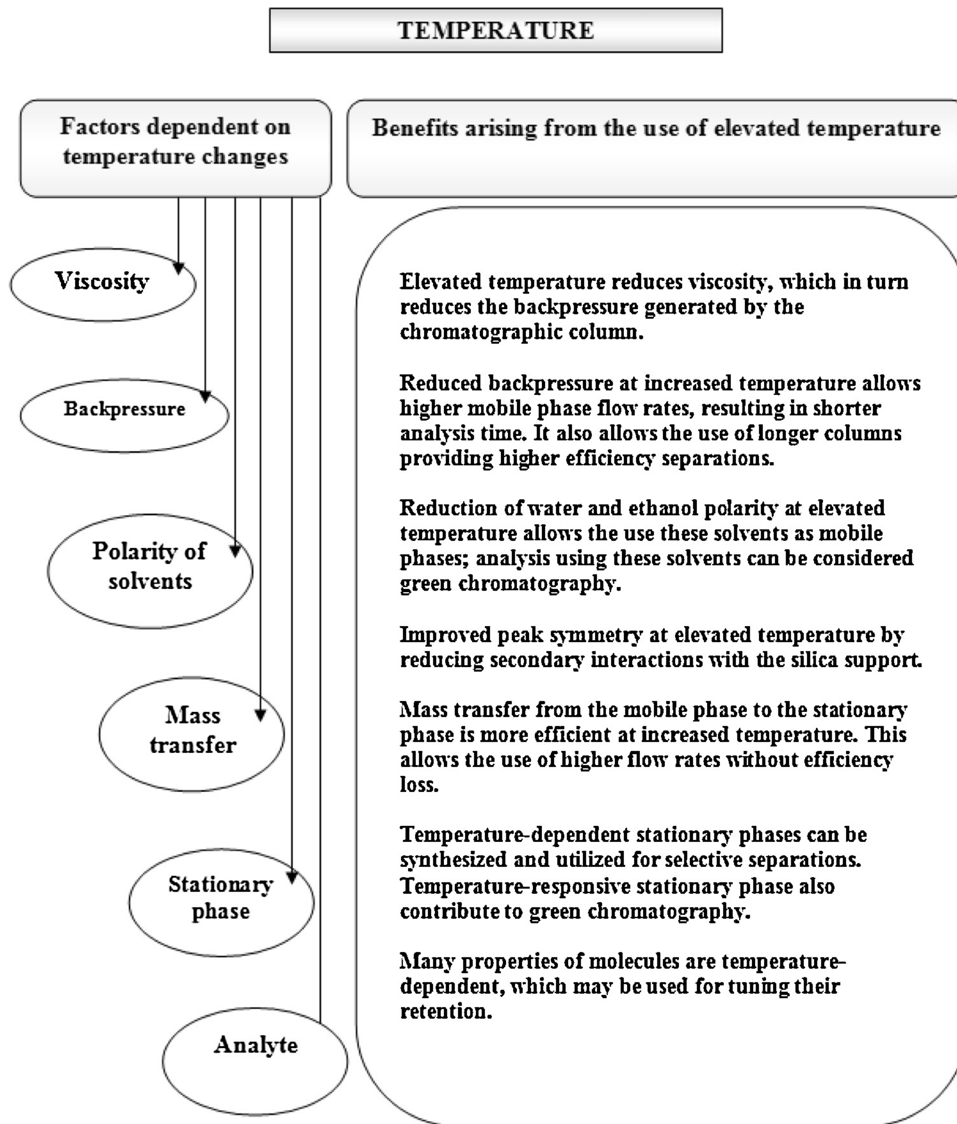

Fig. 9. Factors dependent on temperature changes and the benefits arising from the use of elevated temperature in HPLC.

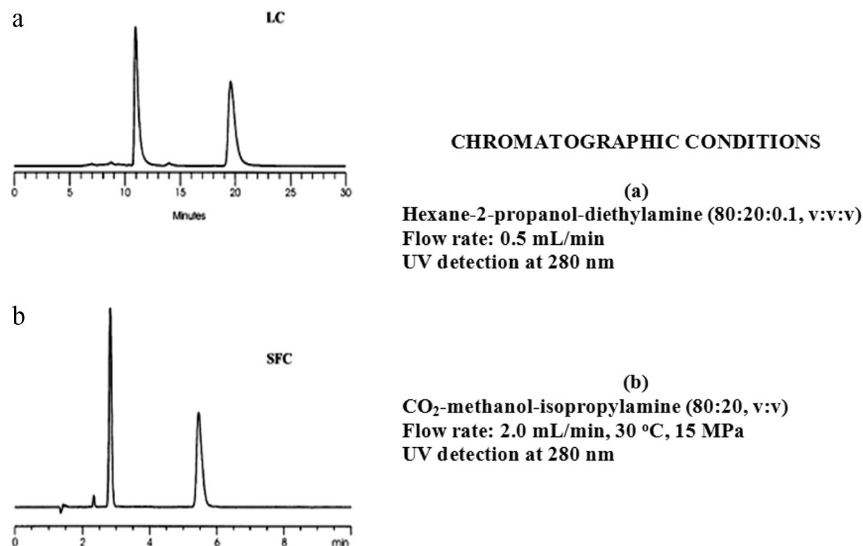

Fig. 10. Comparison of LC and SFC techniques for the separation of metoprolol on a Chiralcel OD CSP by SFC and LC. From [111] with permission.

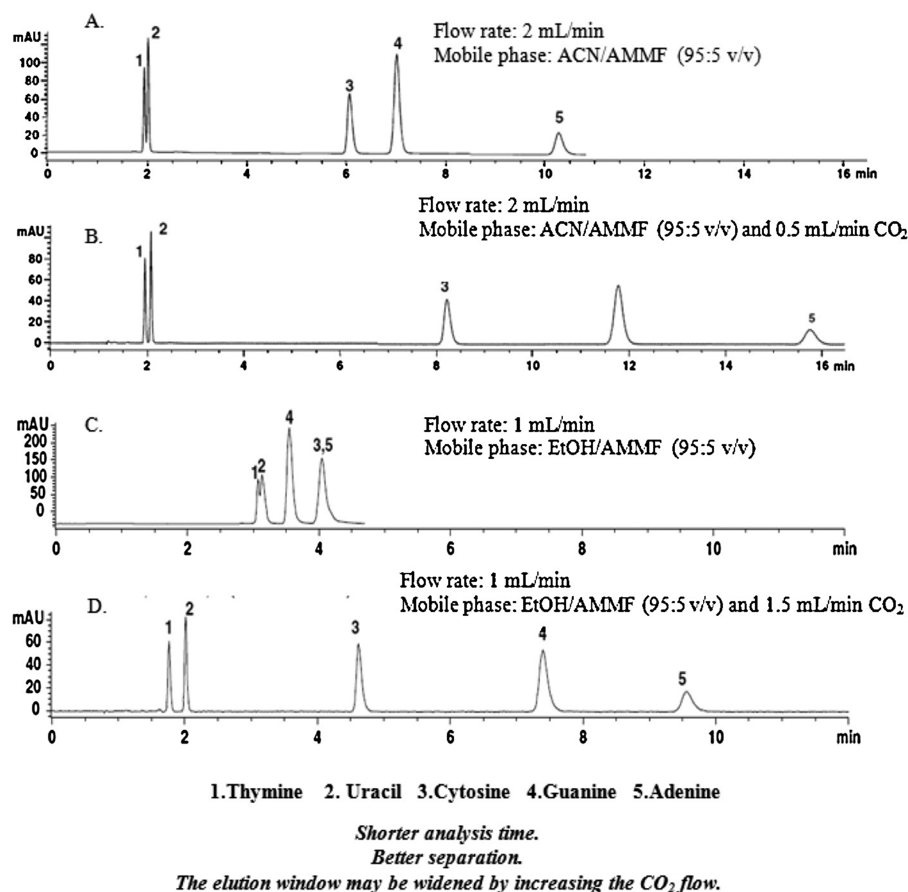

Fig. 11. Separation of nucleobases by HILIC using acetonitrile/water and ethanol/water (A and C) and enhanced-fluidity mobile phases (B and D). From [117] with permission.

Recently, EF liquid mixtures were used in HILIC mode. HILIC is a term introduced by Alpert [115] to describe a variant of NP-LC, in which polar stationary phases are used in combination with aqueous/organic mobile phases to separate analytes based on differences in hydrophilicity. Compared with hydrophobic solvents, hydrophilic solvents have lower volatility and are commonly used in reverse-phase liquid chromatography (RP-LC). They include acetone, acetonitrile, ethanol, methanol and tetrahydrofuran. In HILIC, separation is achieved by partitioning between a water-enriched layer on the surface of a polar stationary phase, and a mobile phase that contains a high percentage of organic solvent [115]. Although methanol and acetonitrile are by far the most commonly used solvents, the more eco-friendly ethanol and water mixtures can successfully replace those solvents [116,117]. In that case, HILIC can be treated as a green mode of LC, especially when ammonium acetate together with ammonia (in the case of alkaline mobile phases) or formic acid with acetic acid (in the case of acidic mobile phases) are selected as the additives [117]. These additives are more eco-friendly than phosphate and borate buffers. The features of HILIC using enhanced-fluidity mobile phases and ethanol instead of acetonitrile are illustrated by the analysis of nucleobases shown in Fig. 11. It has been shown that the addition of CO_2 to a mobile phase composed of ethanol/buffer allows replacing acetonitrile/buffer mobile phases in HILIC separations.

5.1. Two-dimensional liquid chromatography

In general, one-dimensional (1D) chromatographic techniques are incapable of providing complete resolution of the components of very complex samples including biochemical and environmental ones, which means that numerous chromatographic runs are required to fully characterize such samples. This is not desirable from the point of view of green chemistry due to the large amounts of organic solvents and additives required leading to the generation of large amounts of waste, long analysis times and high costs [118]. The quest for a substantial increase in the number of compounds that can be separated into individual peaks in a chromatographic run (increased peak capacity) led to the development of multidimensional techniques. A multidimensional separation refers to a technique in which more than one separation mechanism is applied to the same sample [118]. The combination of different separation mechanisms helps improve resolution and increase the separation space [118].

2D-LC can be performed in two modes:

- *Heart-cutting:* selected fraction(s) from the first dimension (D1) are directed to the second dimension (D2) via a 6-port switching valve. Applicable when only a few constituents of the sample matrix require additional separation power [119].
- *Comprehensive two-dimensional liquid chromatography*: the entire effluent from D1 is directed to D2 via an 8-/10-port valve in the form of subsequent fractions. Complementary selectivity and compatibility of the stationary and mobile phases is required. Applicable when a large number of complex sample constituents require characterization [119]. The transfer of fractions between the two columns can be performed on- or off-line [120].

The advantages of comprehensive two-dimensional liquid chromatography (LC × LC) include prevention of sample contamination or sample loss, as well as the possibility of automation. On the other

Table 5
Examples of on-line and at-line chromatographic methods.

Chromatographic device	Investigated medium	Analytes	Remarks	Ref.
On-line				
GC–FID	Ambient air	Hydrocarbons	Operating in trigger mode with NMVOC analyser	[124]
GC–ECD	Drinking water	Trihalomethanes	Capillary membrane sampler, analysis time of 20 min	[125]
GC–MS	Drinking water	Trihalomethanes	Purge and trap sample preparation, analysis time of 5 min	[126]
IC–fluorescence detection	Drinking water	Haloacetic acids	Post column derivatization with nicotinamide, analysis in 1 h	[127]
GC–MS	Drinking water	Trihalomethanes	Analysis time 3 min	[128]
At-line				
GC–MS	Groundwater	Explosives	Analysis time ~1 h	[129]
GC–μFID	Water	BTEX	Analysis time 3 min, sample prep by headspace SPME	[130]
GC–TCD	Air	Gaseous components	Analysis time 6 min	[131]
GC–MS	Air	VOC	Analysis time 3 min	[132]
GC–μFID	Water	Amines	Derivatization step, SPME, analysis time 22 min	[133]
GC–FID	Wastewater	VOC	20 min analysis time, purge and trap sample preparation	[134]
GC–μFID	Air	Acetaldehyde	SPME sample preparation, 30 min analysis time	[135]
IC	Water	Major cations	No sample preparation	[136]

hand, more complex instrumentation is required, and data handling and optimization of the operating conditions become critical issues [120]. On-line LC × LC may be considered a "green" technique because a single run is sufficient to fully characterize the sample in most cases. The advantages of this approach include reduction in solvent consumption and waste generation, as well as shorter analysis time compared to off-line LC × LC. Fast separations in the second dimension can be accomplished using high temperature HPLC, which is a green approach (as explained above). Another solution to reduce the total analysis time in comprehensive 2D HPLC is to use short columns [121].

As mentioned earlier, majority of conventional 1D RPLC separations are performed using acetonitrile or methanol as the organic modifiers. The main reasons for this are the low UV cutoff of both solvents, as well as their low viscosity. In 2D LC, low UV cutoff is not required in the first dimension of the system. This allows the use of more eco-friendly solvents such as acetone or ethanol, which may be very useful in changing chromatographic selectivity to allow spreading of the chromatographic peaks across the entire 2D-separation space [122].

The next approach to make 2D LC "greener" is the use of temperature programming in the first dimension of a system using reverse phase in both dimensions. This allows reducing the amount of the organic modifier in the first dimension separation, which in turn makes focusing of the analytes injected into the second dimension column easier. Temperature programming in the second dimension might help eliminate instrument-related problems associated with excessive delay volumes and with forming reproducible organic solvent mixtures on sub-minute timescales [122].

6. Location of the chromatograph with respect to the investigated object

The traditional approach to chromatographic analysis involves collection of the sample, transport to the laboratory and final analysis (usually preceded by sample preparation). All of these activities generate negative environmental impact. The use of portable chromatographs or on-line process analysers is both greener approaches.

There are four possibilities to locate the analytical device with respect to the investigated medium [123]:

- o *Off-line*: sample is collected from the investigated medium, transported to the laboratory and analyzed after sample preparation. Negative environmental impact is related to sample transportation and preservation (a preservation agent might need to be added to the samples). There is a risk of representativity loss during prolonged time of transportation, storage and preparation.
- o *On-line*: the analytical device is located near the investigated medium. Sample is periodically automatically collected and analyzed. In case of chromatographic analysis, *on-line* sample preparation is usually applied. Sample is neither transported nor stored, hence the risk of sample representativity loss is minimized. Application of large amounts of reagents (e.g. for sample preservation) is usually avoided in this case.
- o *At-line*: the analytical device is brought to the investigated medium by the analyst. Sample is analyzed at the site of collection, usually after some minor sample preparation operations. Similarly to *on-line* mode, consumption of reagents is minimized and the time from sample collection to sample analysis is relatively short.
- o *In-line*: the measuring device is located in the investigated medium. The measurement is taken in real-time or under near real-time conditions. This mode of locating analytical device is limited to techniques that do not require sample preparation or instrument calibration. Location of a chromatograph in *in-line* mode is unlikely.

Some applications of chromatographic techniques allow the chromatograph to be located *on-line* or *at-line*, meeting the requirements of green analytical chemistry. Selected examples are presented in Table 5.

7. Miniaturization in chromatography

Miniaturization of analytical instrumentation brings about many advantages, of which space savings are probably the least important. Miniaturized separation systems typically require fewer consumables, produce less waste, and their energy consumption is vastly reduced compared to full-size laboratory systems [137,138]. They can often be carried to the sampling site, allowing on-line or at-line analysis. The speed of separation and method sensitivity can also usually be improved with miniaturized systems [139]. These characteristics make miniaturized separation systems inherently green. For example, as discussed above, reduction of the column diameter and length in LC allows lower flow rates of the mobile phase to be used, reducing overall solvent consumption compared to conventional methods. The amount of sample required for analysis with miniaturized systems is typically smaller compared to conventional analysis, which is very important in many research areas, including forensic science, biomedical science, etc. Lower flow rates reduce dilution of the analyte in the mobile phase, improving detection sensitivity when concentration-dependent detectors are used (e.g. UV–vis). Reduction of the separation column dimensions is also one of the most effective approaches when developing novel

Table 6
Classification of portable chromatographs [140].

Type	Weight (kg)	Purpose	Advantages
Compact	10–25	Mobile and stationary laboratories	Reduction of energy consumption, costs, materials and space. Similar characteristic to those of conventional chromatographs
Portable, transportable, field	5–15	On-site analysis	Rapid analysis, gas and power self-supporting
Chip-based chromatographs	0.2–3	On-site analysis, handheld	Very fast analysis, for relatively simple analytical problems, fully self-supporting, restricted analytical capabilities
Specially designed chromatographs; micro-chromatographs	<0.2	Space investigations	Automated analysis, resistant to impact and shaking

Table 7
Chip-based chromatography.

Chromatographic technique	Description, advantages	Application	Ref.
LC on-chip	Superior efficiency compared to conventional LC. Facile positioning of detection cells. Low unit cost. Shorter analysis times. Low pressure drops for a given performance. Flat flow profile significantly reduces band-broadening when compared to conventional LC methods; this is due to the fact that the mobile phase is driven by electro-osmosis [141,142]	Analysis of highly complex peptide mixtures	[143]
		Biomarker discovery	[144]
		Enantiomer analysis	[145]
		Post-translational modifications: • Glycopeptide profiling • Phosphoproteome analysis	[146,147]
		Nucleolar proteome	[148]
GC on-chip	Provide rapid analysis. Easy to operate. Low-power demands. The favored substrates for microfabricated GC columns are silicon, porous silicon, carbon nanotubes, parylene. The silicon-based fabrication technology approach has been motivated by the advantages offered by the precision at the micron scale level, component integration, miniaturization and batch production. Low GC flow rates to minimize vacuum-pumping requirements. The use of a modular approach to adapt to different environments [149]	Biomedical science	[146]
		Analysis of volatile compounds	[150]
		Alkanes analysis	[151]
		Environmental analysis Atmospheric-air analysis	[147]
CEC on-chip	Most easily executed in an open-channel format (i.e. the stationary phase is immobilized on the channel walls) The separation efficiency with microfabricated CEC columns appears to be equivalent to that of 1-μm-particle-diameter packed columns Separation can be performed faster in a microchip than in conventional methods because higher electrical field strengths (V/cm) can be applied on a microchip than in capillaries. The possibility to easily integrate other elements to the analytical process, such as filtration, concentration of a sample chemical and derivatization of the analytes before separation [152]	Analysis of polycyclic aromatic hydrocarbons	[153]
		Enantiomers analysis	[154]
		Peptides analysis	[155]

stationary phases in LC because it enables evaluation of the often limited amounts of experimentally synthesized phases [137].

Miniaturization brings about the advantage of instrument portability, which allows on-site analysis. This fulfills the 11th principle of green chemistry calling for real-time analysis for pollution prevention. Table 6 presents a classification of portable chromatographs together with a summary of their advantages.

The concept of lab-on-a-chip, first introduced in the 1990s, resulted in the race to develop chip-based separation systems. Such chromatographs, with the separation chips often as small as a coin, are easily portable, consume very little energy and chemicals, and can potentially be very cheap when mass produced. Table 7 summarizes some of the features of chip-based separation systems and present examples of their applications.

Miniaturized separation systems usually require microscale sample preparation. As discussed earlier, numerous microscale sample preparation techniques have been developed in the recent years, including SPME, MEPS, SPNE, LPME, etc. Research in this area is on-going.

8. Summary

Chromatographic techniques have the potential to be greener at all steps of the analysis, from sample collection through its preparation to separation and final determination. An ideal chromatographic method would yield analytical results without using any consumables; it would be performed in *in-line* mode, without sample preparation. Unfortunately, sample preparation prior to chromatographic analysis is required in most cases. Consequently, solventless, miniaturized sample preparation techniques should be used whenever possible to minimize solvent consumption.

As most chromatographic determinations performed are routine ones, it is extremely important to perform them with methodologies that have small environmental impact. Future progress in greening analytical chromatography is expected to be accomplished through miniaturization, shortening of the analysis time and improvements of the resolving power.

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