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A trade off between separation, detection and sustainability in liquid chromatographic fingerprinting☆

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ABSTRACT

It is now recognized that analytical chemistry must also be a target for green principles, in particular chromatographic methods which typically use relatively large volumes of hazardous organic solvents. More generally, high performance liquid chromatography (HPLC) is employed routinely for quality control of complex mixtures in various industries. Acetonitrile and methanol are the most commonly used organic solvents in HPLC, but they generate an impact on the environment and can have a negative effect on the health of analysts. Ethanol offers an exciting alternative as a less toxic, biodegradable solvent for HPLC. In this work we demonstrate that replacement of acetonitrile with ethanol as the organic modifier for HPLC can be achieved without significantly compromising analytical performance. This general approach is demonstrated through the specific example analysis of a complex plant extract. A benchmark method employing acetonitrile for the analysis of *Bidens pilosa* extract was statistically optimized using the *Green Chromatographic Fingerprinting Response* (GCFR) which includes factors relating to separation performance and environmental parameters. Methods employing ethanol at 30 and 80 °C were developed and compared with the reference method regarding their performance of separation (GCFR) as well as by a new metric, *Comprehensive Metric to Compare Liquid Chromatography Methods* (CM). The fingerprint with ethanol at 80 °C was similar to or better than that with MeCN according to GCFR and CM. This demonstrates that temperature may be used to replace harmful solvents with greener ones in HPLC, including for solvents with significantly different physiochemical properties and without loss in separation performance. This work offers a general approach for the chromatographic analysis of complex samples without compromising green analytical chemistry principles.

1. Introduction

Green Chemistry arises from the understanding that the cumulative activity of humans led to contamination of water, air, soil and food by agrochemicals, industrial waste and toxic organic solvents, among others, and that keeping current practices should not be sustainable [1,2]. While analytical chemistry may be used to determine the 'greenness' of a chemical product or technology, since first described by Namiesnik in 1999 [3] it is now recognized that analytical chemistry must also be a target for green principles, in particular chromatographic methods which typically use relatively large volumes of hazardous organic solvents [4]. The most comprehensive definition of green analytical chemistry (GAC) is currently that of L.H. Lawrence being: "the use of analytical chemistry techniques and methodologies that reduce or eliminate solvents, reagents, preservatives and other chemicals that are hazardous to human health or the environment and that may also enable faster and more energy-efficient analyses without compromising performance criteria" [5]. The latter prerequisite is often the bottleneck in translating existing methods to green methods. Very recently, Gałuszka et al. have provided an excellent framework for green analytical chemistry, adapting the 12 principles of Green Chemistry [1,2] to analytical chemistry [6]. These so called 12 principles of green analytical chemistry can help the analyst to identify which points of an analytical process could be improved from the sustainability point of view [6]. Principles number 11 and 12, which state that toxic solvents should be eliminated or replaced and that the safety of the operator should be increased in

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particular identify issues which have been almost entirely ignored by analysts to date, despite them being the people who stand most to benefit [7]. Principle number 12 might be refined by including the safety of people surrounding the operator, including those with insufficient training or expertise to be aware of the potential risks [7].

Solvent (replacement, reduction or elimination) represents a substantial challenge in Green Chemistry being maybe the most active area in this field [2]. That is because solvents are closely related with mass waste in processes and syntheses, environmental pollution, workers health and safety [2]. High performance liquid chromatography (HPLC), as the name suggests, employs solvents in the composition of the mobile phases (MP), thus should be a principal target for green analytical chemistry [5,8,9]. HPLC is the most frequently employed analytical technique in chemical research and it is used routinely for quality control in various industries [8,10]. It is believed that HPLC analyses generate approximately 34 million litres of solvent waste per year worldwide [8,10]. This becomes more relevant if one considers that the most used organic solvent in such analyses is acetonitrile (MeCN), since it presents very propitious physicochemical properties for separation and detection in HPLC such as water miscibility, low boiling temperature, low viscosity of mixtures with water, ability to solvate many analytes and UV cutoff wavelength at 190 nm [8,11]. Nevertheless, it is toxic to mammals (the oral and dermal lethal dose, 50% – LD_{50} – are 2460 and 2000 mg/kg in rats and rabbits, respectively) has a half-life in water of 2–20 days and exhibits acute and chronic toxicity to aquatic life [9,11,12]. Such characteristics classify it as an undesirable solvent from an environmental point of view even on the analytical scale [9,13–15]. Despite these issues, replacement of MeCN with more benign solvent alternatives in HPLC is not a trivial task. Solvent selection rules for HPLC have developed largely empirically over more than 60 years to date and at the same time, column developments have focused on stationary phases compatible with the most commonly used solvents. Even during the recent global financial crisis when there were critical shortages of MeCN worldwide, it remained preferable for the majority of analysts to source MeCN at whatever the cost than to actively pursue replacement with a different solvent. Replacement of MeCN is arguably the most challenging goal in greening liquid chromatography [5,11,13]. The second most popular solvent in HPLC is methanol (MeOH), which also presents favourable properties, but it is readily absorbed by inhalation and dermal exposure and metabolized by humans to formaldehyde and formic acid, which are toxic and cumulative [14,16]. The wastes containing any of the both mentioned organic solvents need to be disposed as a chemical waste [5,6,11,13,17]. This picture is also observed in natural products chemistry, where the generation of harmful solvent waste from their analysis is then a contributor to environmental damage that can spoil the maintenance of natural products sources such as plants, fungi and algae. The case of *Bidens pilosa*, a medicinal plant widely used in folk medicine in many countries and listed by the Brazilian government as one of the 71 species identified for phytotherapeutic product development [18] illustrates this: about 70% of the HPLC methods developed during *B. pilosa* chemical investigations employed MeCN as the organic solvent, whereas 30% used MeOH [19–30] both of which are harmful to the environment.

Even though the consumption of solvents per run can be reduced due to recent improvements in LC and related technologies (e.g. state-of-art columns are often shorter, narrower and packed with smaller particles, thus allowing a reduction in solvent consumption without loss of efficiency) [8,9,31,32], the daily contact of analysts with harmful solvents such as MeCN and MeOH could potentially cause negative impact on their health [4,6,15,16]. Furthermore, the high backpressure generated by small particle packed columns often requires ultra high-pressure liquid chromatography (UHPLC) systems, which are expensive and not available in most laboratories [33]. From a holistic perspective, the environmental cost of replacing operational HPLC systems with newer systems that simply operate at higher pressures does also not support the general principles of Green Chemistry.

Acetone might be a greener alternative to the replacement of MeCN in reversed phase high performance liquid chromatography (RP-HPLC) [12,34]. However, as its UV cut-off extends out to 330 nm, it limits its use with ultraviolet absorbance based detectors [35]. Ethanol (EtOH), which can be obtained by fermentation processes from renewable sources, appears as a biodegradable, non-toxic solvent option for HPLC-UV analyses [4,5,8,13,36]. As described by Welch et al. the use of EtOH also offers the possibility of analysis without generation of toxic waste, and the use of instrumentation outside traditional laboratory boundaries including in sensitive environments or developing areas where chemical waste disposal is not yet well established or its price is prohibitively expensive [8]. The widespread availability of EtOH, which is also produced from renewable sources in so called *organic crops* in Brazil, also supports Green Chemistry concepts [1]. EtOH presents various advantageous physical properties for LC in general (low volatility, miscibility with water and UV cutoff wavelength at 210 nm), but the high viscosity of EtOH/water solutions has been identified as a significant technical limitation [5,8,36]. Some strategies can be adopted to overcome the problem of high backpressure across the column. The simple reduction of the mobile phase (MP) flow rate to respect the pressure limit of a conventional HPLC system was successfully applied for the replacement of MeOH with EtOH for the development of chromatographic fingerprints of medicinal plants [7]. Another option is to heat the water–ethanol MP to decrease its viscosity [5,37]. As the thermodynamics and kinetics of the LC separation are a function of temperature [38], this option can be strategically explored when a harmful solvent is intended to be replaced with a greener option which belongs to a different group of selectivity. That is, the use of high temperature may not only decrease MP viscosity, but also improve selectivity and efficiency of the chromatographic process [37,39] employing the greener solvent. Such replacement at elevated temperature might lead to a greener chromatographic method [5,8,9].

Thus, the selection of the organic solvent in LC should not be done only as function of separation and detection parameters, but as a trade off between them and those related to sustainability.

Based on these considerations, the comparative performance of MeCN and EtOH for the establishment of HPLC-UV fingerprints of an extract of *B. pilosa* was investigated keeping in mind the principles of Green Chemistry/Green Analytical Chemistry [1–3,5,6,9,32]. Thus, automated, multivariate and multianalyte resources saving experimental designs were adopted to globally optimize chromatographic methods for both solvents from a heuristic perspective. For that a metric for a more comprehensive comparison of LC analyses was developed here. The approach adopted in this work was kept as generic as possible in order to demonstrate that the same approach can be applied as described to other samples and related techniques.

2. Materials and methods

2.1. Chemicals and reagents

The MeCN (Honeywell Burdick & Jackson, USA) and absolute EtOH (Scharlau, Spain) used were HPLC grade. The anhydrous acetic acid (AcOH) (Merck, Germany) was AR (ACS) grade.