T.R.N.C.

NEAR EAST UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

SWITCHABLE-HYDROPHILICITY SOLVENT LIQUID-LIQUID MICROEXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS FROM BIOLOGICAL FLUIDS PRIOR TO HPLC-DAD DETERMINATION

MALEK HASSAN

ANALYTICAL CHEMISTRY

MASTER OF SCIENCE THESIS

NICOSIA

2019

T.R.N.C.

NEAR EAST UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

SWITCHABLE-HYDROPHILICITY SOLVENT LIQUID-LIQUID MICROEXTRACTION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS FROM BIOLOGICAL FLUIDS PRIOR TO HPLC-DAD DETERMINATION

MALEK HASSAN

ANALYTICAL CHEMISTRY
MASTER OF SCIENCE THESIS

SUPERVISOR
ASSIST. PROF. DR. USAMA ALSHANA

NICOSIA
2019

Insert Approval Page here

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name **:** MALEK HASSAN

Signature **:**

Date **:** 15 May 2019

ACKNOWLEDGEMENTS

I want to start by thanking my supervisor Assist. Prof. Dr. Usama ALSHANA for his help and remarkable notes during my MSc. studies. It was a pleasure to study with such a great scientist, mentor and guide in both life and science. I will never forget our discussions in the laboratory, which sculpted my scientific personality. My extraordinary gratitude goes to him.

Moreover, I would like to thank my lecturers Prof. Dr. Mustafa SOYLAK, Prof. Dr. Jalal HANAEE, Assoc. Prof. Dr. Hayati ÇELİK and Assist. Prof. Dr. Banu KEŞANLI for their valuable courses and advice, which were very helpful in literature review and writing this thesis. Thank you for your time, efforts and continuous support.

Special thanks go to Prof. Dr. O. Yavuz ATAMAN for accepting to be the Chair of the Jury despite his crowded schedule and to all Jury members, Prof. Dr. Mehmet ÖZSÖZ, Prof. Dr. İhsan ÇALIŞ, and Assist. Prof. Dr. Banu KEŞANLI for their valuable time, contributions and comments.

Lastly, thanks to my colleagues at the Department of Analytical Chemistry at NEU; it was great to share the laboratory experience with you and wish you all the best.

To my parents and family, I owe it all to you; without you I would have never been able to write this thesis; thank you.

ABSTRACT

Hassan, M. Switchable-Hydrophilicity Solvent Liquid-Liquid Microextraction of Non-Steroidal Anti-Inflammatory Drugs from Biological Fluids Prior to HPLC-DAD Determination.
Near East University, Institute of Health Sciences, Analytical Chemistry Program, Master of Science Thesis, Nicosia, 2019.

Switchable-hydrophilicity solvent liquid-liquid microextraction was used prior to high-performance liquid chromatography with a diode-array detector (HPLC-DAD) for the determination of four non-steroidal anti-inflammatory drugs (i.e., ketoprofen, etodolac, flurbiprofen, and ibuprofen) in human urine, saliva, and milk. Optimum extraction conditions were as follows: 500 µL of switched-on *N*,*N*-dimethylcyclohexylamine as the extraction solvent, 9.5 mL of the aqueous phase, 500 µL of 20 M sodium hydroxide as a switching-off trigger, and 30 s extraction time. A portion of the final extract was directly injected into HPLC. Under optimized extraction and chromatographic conditions, limits of detection ranged between 0.04 and 0.18 µg mL^{-1} in all matrices analyzed. Excellent linearity with coefficients of determination (R^2) ranging between 0.9955 and 0.9998 and percent relative standard deviations (%RSD) of 0.9-7.7% were obtained. The proposed method was efficiently used for the extraction of the four analytes from the biological fluids with percent relative recoveries (%RR) ranging between 96 and 109%.

Keywords: Biological fluids, Liquid-liquid microextraction, Non-steroidal anti-inflammatory drugs, Switchable-hydrophilicity solvent.

ÖZET

Hassan, M. Non-Steroidal Anti-İnflamatuar İlaçların Biyolojik Sıvılardan Değiştirilebilir Hidrofilik Çözücülü-Sıvı-Sıvı Mikroekstraksiyonu ve HPLC-DAD ile Tayini.

Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Analitik Kimya Programı, Yüksek Lisans Tezi, Lefkoşa, 2019.

Değiştirilebilir hidrofilik çözücülü sıvı-sıvı mikroekstraksiyonu ve yüksek performanslı sıvı kromatografi-diyot dizisi dedektör (HPLC-DAD) ile non-steroidal anti-inflamatuar ilaçların (ketoprofen, etodolac, flurbiprofen ve ibuprofen) biyolojik sıvılarda (tükürük, idrar ve süt) tayin edilmiştir. Optimum ekstraksiyon koşulları aşağıdaki gibi bulunmuştur: 500 µL *N,N*- dimetilsiklohekzilamin (ekstraksiyon çözücü), 9.5 mL sulu faz hacmi, 500 µL, 20 M sodyum hidroksit (faz ayırıcı), ve 30 saniye ekstraksiyon süresi. Elde edilen ekstrakt HPLC'ye doğrudan enjeksiyon için uygun olarak değerlendirilmiştir. Optimum ekstraksiyon ve kromatografik koşullarda, teşhis limitleri analiz edilen matrislerde 0.04 ile 0.18 µg mL^{-1} arasında hesaplanmıştır. Kalibrasyon grafikleri, 0.9955 ile 0.9998 arasında değişen tamamlayıcılık katsayıları (R^2) ile iyi bir doğrusallık göstermiştir. Göreceli standart sapmalar (%RSD) 0.9-7.7% arasında elde edilmiştir. Önerilen yöntem, biyolojik sıvılardan dört analitin ekstraksiyonu için 96 ile 109% arasında değişen nispi geri kazanım yüzdeleri ile (%RR) verimli bir şekilde kullanılmıştır.

Anahtar Kelimeler: Biyolojik sıvılar, Değiştirilebilir hidrofiliklik çözücü, Non-steroidal anti-enflamatuar ilaçlar, Sıvı-sıvı mikroekstraksiyonu.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Definition
AAP	American Academy of Pediatrics
AAS	Atomic absorption spectrometry
ACN	Acetonitrile
AFS	Atomic fluorescence spectrometry
AKI	Acute kidney injury
BE	Back-extraction
C-18	Octadecyl
CE	Capillary electrophoresis
COX	Cyclooxygenase
CPE	Cloud-point extraction
DAD	Diode-array detector
DI	Deionized
DI-SPME	Direct immersion solid-phase microextraction
DLLME	Dispersive liquid-liquid microextraction
DMCA	*N*,*N*-dimethylcyclohexylamine
ET	Etodolac
ETD	Evaporation-to-dryness
FAAS	Flame-atomic absorption spectrometry
FASS	Field-amplified sample stacking
FBP	Flurbiprofen
FDA	Food and drug administration
GAC	Green Analytical Chemistry
GC	Gas chromatography
GFAAS	Graphite furnace-atomic absorption spectrometry
HBP/GO-HF-SLPME	Hyperbranched polyglycerol/graphene oxide nanocomposite

Abbreviation	Definition
HG	Hydride generation
HS-SPME	Headspace solid-phase microextraction
HF-LPME	Hollow fiber-based liquid-phase microextraction
HPLC	High-performance liquid chromatography
IBU	Ibuprofen
IL	Ionic liquid
KET	Ketoprofen
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LLME	Liquid-liquid microextraction
MeOH	Methanol
MEPS	Microextraction by packed sorbent
MIP	Molecularly imprinted polymer
MP	Mobile phase
MS	Mass spectrometry
MSPE	Magnetic solid-phase extraction
NP	Normal phase
NSAID	Non-steroidal anti-inflammatory drugs
ODS	Octadecyl group-bonded silica gel
RP	Reversed-phase
SDME	Single-drop microextraction
SFE	Supercritical fluid extraction
SFOD-ME	Solidification of floating organic droplet microextraction
SHS	Switchable-hydrophilicity solvents
SHS-LLE	Switchable-hydrophilicity solvent liquid-liquid extraction
SHS-LLME	Switchable-hydrophilicity solvent liquid-liquid microextraction
SOE	Salting-out extraction
SP	Stationary phase

Abbreviation	Definition
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SQT	Slotted quartz tube
S-UA-LLME-SFO	Salting-out ultrasound-assisted liquid-liquid microextraction based on solidification of a floating organic droplet
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UA-Dμ-SPE	Ultrasound-assisted dispersive micro solid-phase extraction
USP-STF	United States Preventive Task Force

CHAPTER 1

INTRODUCTION

1.1 Non-steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of pharmaceutically active compounds having anti-inflammatory, analgesic and antipyretic properties. The common mechanism of action of NSAIDs is based on inhibition, mostly not chemically related, of the metabolism of arachidonic acid by inhibiting cyclooxygenase (COX) enzymes [1]. For many years, NSAIDs have been among the most world-widely consumed medicines due to their multiple activities. The use of NSAIDs is increasing contentiously; over 22 million prescriptions include those which are written every year in the UK, and over 70 million in the US [2]. However, the real consumption of these drugs is further higher, since they are also sold over the counter.

1.1.1 Usage of NSAIDs

NSAIDs are used as a drug for humans and in veterinary. They help in the management of three common symptoms, i.e., fever, inflammation and pain. The analgesic property of these drugs expands its use to control a variety of conditions, including headaches and lower backache as the most common conditions controlled by NSAIDs, in addition to arthritis, cold or flu, period pains, injuries of joint, bone, sprains, or strains, muscle or joint complaints and toothache [3].

According to their inhibition selectivity, NSAIDs can be classified into two main classes: COX non-selective inhibitors and COX-2 selective inhibitors, and according to their chemical structure in ten categories [4] as shown in **Figure 1.1**

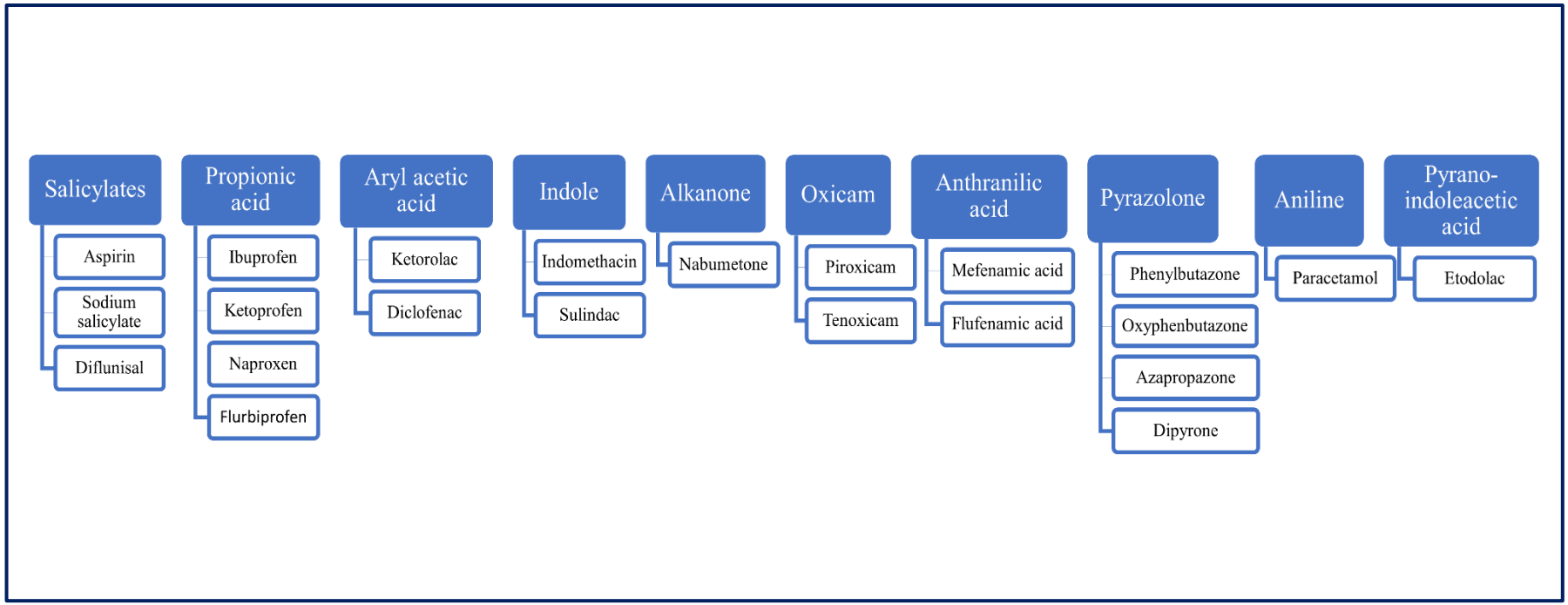


Figure 1.1 Classification of NSAIDs according to their chemical structures.

Recent studies support the use of NSAIDs in low doses, particularly aspirin, for the prevention of several types of cancer. According to the United States Preventive Task Force (USP-STF), the use of NSAIDs in low dose is recommended for individuals at the age of 50-59 having a risk of cardiovascular disease and colorectal cancer. This recommendation was based on reports of reduction of the risk associated with the use of aspirin [5]. The antineoplastic effect of aspirin is also effective in gastric and esophageal cancer, in addition to other types including breast, lung and prostate cancer. Furthermore, other studies indicated that it may improve survival rates by reducing the risk of metastasis [6, 7]. However, the results are generally conflicting and sparse [8].

Although the exact mechanisms are not clear yet [9], several NSAIDs are experimentally approved to have antineoplastic effects throughout *in vivo* and *in vitro* evidence [10, 11]. The most susceptible histological tumors to the antineoplastic activity of NSAIDs are adenocarcinomas, which comprise the majority of ovarian as well as endometrial cancer [12, 13].

In addition to the previously mentioned indications of NSAIDs, epidemiological studies have shown that long-term use of NSAIDs reduces the risk of developing Alzheimer's disease and delays its onset [14].

1.1.2 Side Effects of NSAIDs

Despite the extensive usage and different indications of NSAIDs, it is well known that they have a wide range of side effects, among them gastrointestinal side effects are the most common. Others include skin rashes, hepatitis, nephropathies, in addition to interactions with other drugs such as antihypertensive or antihyperglycemic agents [2].

Gastrointestinal side effects of NSAIDs range from mild to severe dyspeptic symptoms, development of duodenal or gastric ulceration, perforation or hemorrhage, as well as other events, which may lead to hospitalization or even death [2]. Endoscopic studies have shown a

prevalence rate of 14–25% of duodenal and gastric ulcers in NSAID users. The relative risk for developing a serious gastrointestinal complication in patients on NSAIDs has been calculated in a large meta-analysis as 2.74 and 3.09 for upper gastrointestinal, 5.93 for perforation and 7.62 for ulcer-related death. Nevertheless, Non-aspirin NSAIDs have been linked to increasing the risk of dose-dependent cardiovascular events [15, 16].

Acute kidney injury (AKI) is another serious adverse effect of NSAIDs, especially in pediatrics. AKI has an incidence of up to 30% of intensively cared [17]. In most of the cases of AKI, the causative agent is mainly a prescribed drug in more than 25% [18]. Besides NSAIDs, chemotherapeutics and antibiotics are the main causes of drug-induced AKI [19]. Even more, kidney and liver tumors were reported in animal studies on rats and mice exposed to some NSAIDs [20, 21].

The half-life of ibuprofen is prolonged in neonates, and more particularly in preterm infants, and it is excreted into human milk in minimal amounts. The clearance of paracetamol is less in neonates compared to older infants. Despite their possible excretion in mother milk, ibuprofen and paracetamol are not contraindicated for nursing mothers [22].

According to the American Academy of Pediatrics (AAP), the use of flurbiprofen, celecoxib and naproxen are compatible with breastfeeding, since the excretion is less than 1%. However, avoiding their usage if the infants have a ductal-dependent cardiac lesion is prudent. Furthermore, the long-term use, particularly of naproxen, is not recommended based on case reports of gastrointestinal tract bleeding and emesis due to the potential closure of the ductus arteriosus in neonates. Oral and injectable forms of ketorolac are entirely contraindicated in nursing mothers [23].

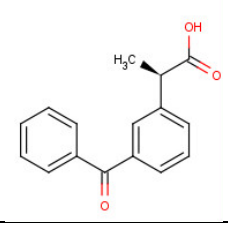
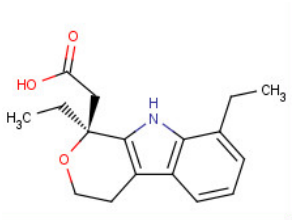
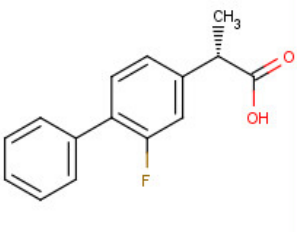
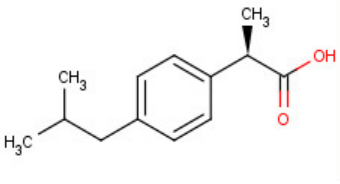
Food and Drug Administration (FDA) discourages the use of other NSAIDs in case of nursing due to limited published data and due to other various reasons. Diflunisal has a long half-life and adverse events, which may be severe, as cataracts or even fatality. Even more, the

concentrations of meloxicam in the milk of lactating animals was found to be higher than their plasma concentrations [23].

Veterinary use of NSAIDs, particularly in food-producing animals, may cause a long-term exposure of NSAIDs and their metabolites residues by consumers due to the possible entrance of these active residues into the food chain [24].

The chemical structures and physical properties of the four model NSAIDs used in this study [i.e., ketoprofen (KET), etodolac (ET), flurbiprofen (FBP) and ibuprofen (IBU)] are listed in **Table 1.1.**

Table 1.1 Chemical structures and physical properties of the studied NSAIDs.

Analyte	Chemical Structure	$logP$	pK_a	$M_r\ (g\ mol^{-1})$
Ketoprofen		3.61	3.88	254.3
Etodolac		3.44	4.73	287.4
Flurbiprofen		3.94	4.42	244.3
Ibuprofen		3.84	4.85	206.3

1.2 Sample Pretreatment

Sample pretreatment or preparation is how the sample is treated before its analysis by the analytical instrument. This process may include extraction, pH adjustment, filtration, derivatization, in addition to any other clean-up or preconcentration procedures, necessary to isolate the analytes from a complex matrix and to enrich their concentration level [25].

Analytical techniques have undergone a great advancement and improvement during the last decades as a result of the improvement in technology and industry, which led to the

advancement of most analytical techniques including chromatography, electrochemistry, spectroscopy and microscopy. Despite this sample preparation is still needed with most of the analytical techniques to minimize matrix interferences and preconcentrate the analytes as much as possible. Otherwise, the method's selectivity and sensitivity will be affected. Proper extraction of the analytes from the sample is the best choice as the sample preparation since it can significantly remove interferences and enrich the analytes which in turn would improve both selectivity and sensitivity [26].

Since it is a combination of several steps, sample preparation is the milestone of analytical method development [27], and in addition to the previously mentioned points, there is an agreement among analytical chemists that the sample preparation step itself, rather than the analytical instrumentation, is the "bottleneck" in the determination of trace and ultra-trace analytes [28]. The ideal sample preparation technique should be selective for the analytes, reproducible, and should result into a good clean-up of the sample and preconcentration of the analytes [29].

Green Analytical Chemistry (GAC) is a concept that was firstly proposed in 1999 [30] and was simultaneously adopted by Anastas, the pioneer of green chemistry, as well. Since that time, the concept was intended to either remove or minimize the environmental impact that could be caused by analytical methodologies. Among the twelve principles of green chemistry, (**Figure 1.2)** [31] six of them are related to GAC, which should be implemented to all steps of analytical methods [32]. These principles are shown in **Figure 1.3**.

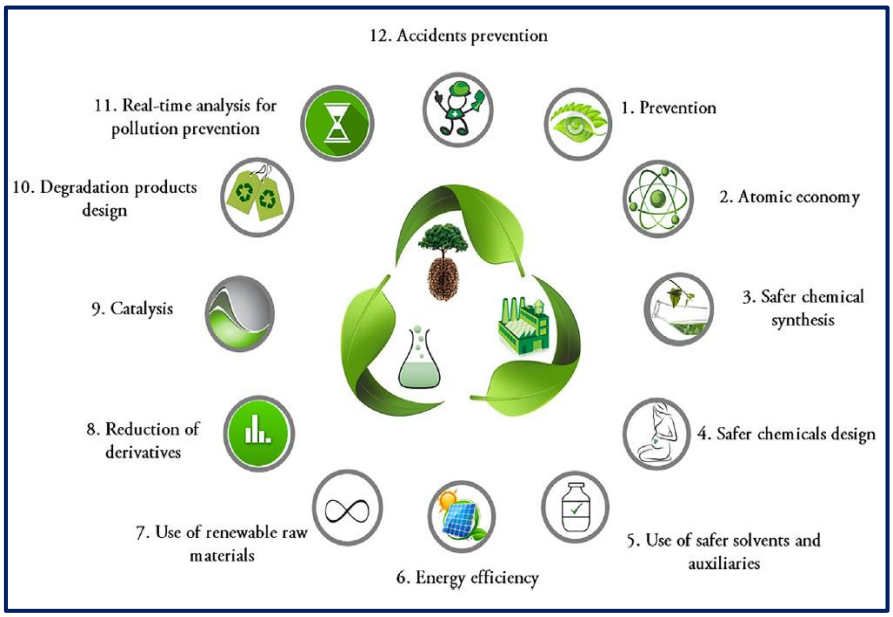


Figure 1.2 Principles of green chemistry proposed by Anastas and Warner [33].

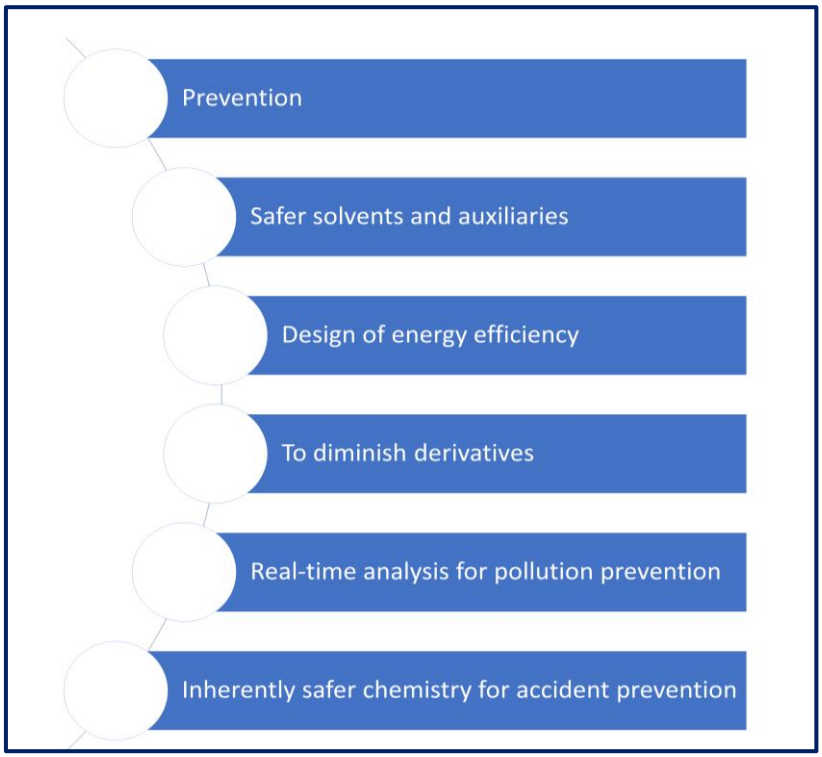


Figure 1.3 GAC principles.

It is important to mention that several innovative advancements in sample preparation since 1970 were the main milestones of GAC, which would not be achievable without these advancements. The summary of these milestones is shown in **Figure 1.4**. Different method characteristics should be focused on to assign the greenness of the analytical method, including the type, volume and nature of the solvents used, safety of operation, energy consumption, required time and waste production [32].

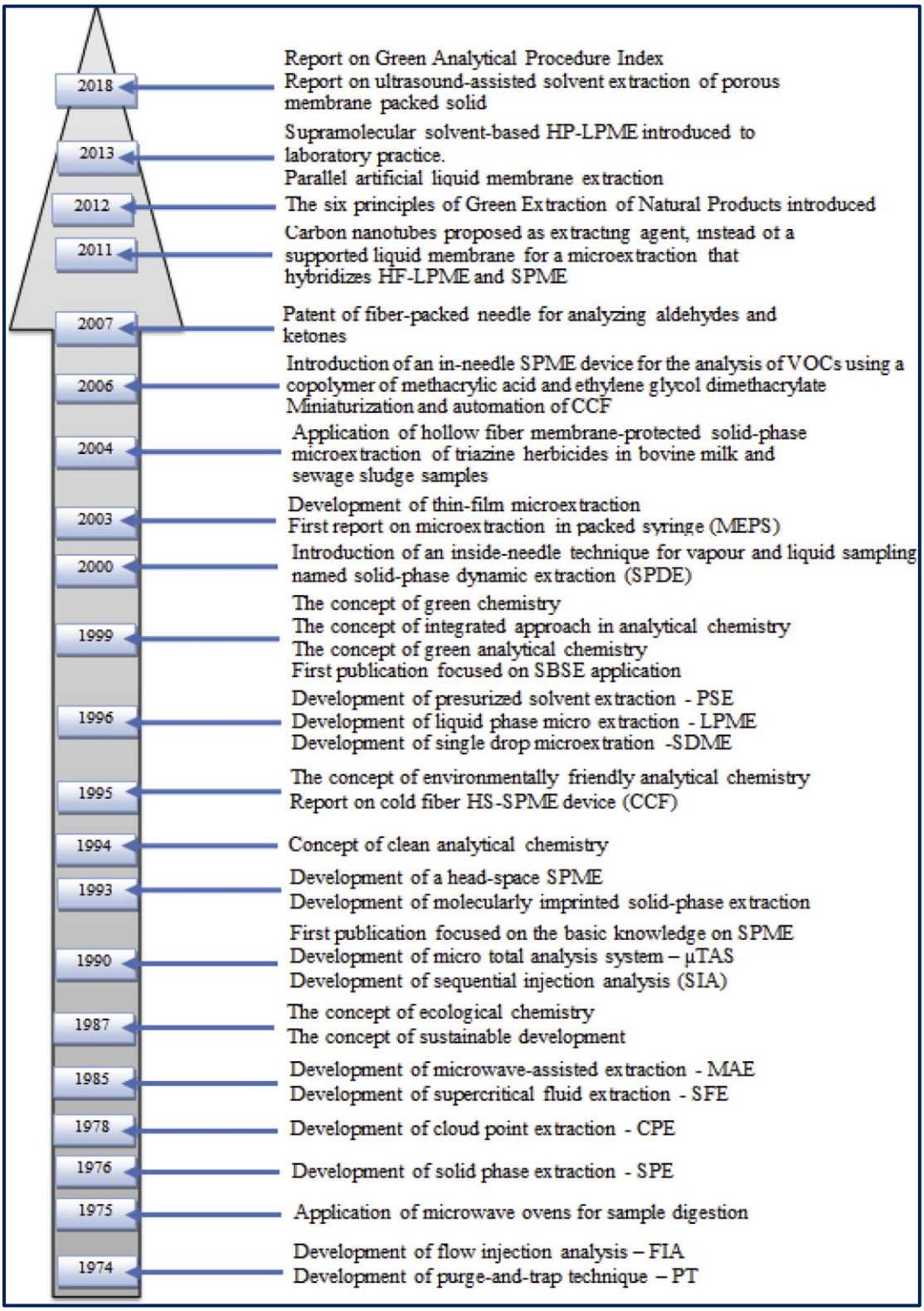


Figure 1.4 Milestones of GAC [34].

The current trend in sample preparation is shifting towards environmental friendliness, miniaturization, simplicity, automation, and cost-effectiveness. Microextraction techniques, in general, are classified as environmentally friendly, due to the significant reduction of organic solvents, and minimal waste generation. Even more, microextraction techniques have several advantages over conventional extraction techniques, which include:

1- Simplicity and ease of operation.
2- Miniaturization.
3- Low cost in general.
4- Applicability to a variety of analytes and samples.

Recently, combining microextraction techniques with other sample preparation steps such as another extraction step or sample pretreatment method are shown as a unique approach to improve the clean-up, which results in enhanced quality of the analysis [35].

1.3 Extraction Techniques

Liquid-liquid extraction (LLE), also known as partitioning or solvent extraction, is probably the oldest extraction technique. In LLE, the separation depends mainly on the different solubility of the analytes in the organic and aqueous phase. The main drawback of this method is the massive consumption of toxic organic solvents, low selectivity, limited preconcentration factors and long equilibration time.

Franz von Soxhlet invented Soxhlet extraction in 1879, who used a large sample size up to 30 g, with continuous introduction of the extraction solvent into the sample by evaporation and condensation of the former. This method is matrix-independent and does not require filtration. However, the extraction time can range between 6 to 24 h, which is among the longest extraction procedures. Moreover, the volume of the organic solvents usually used in this technique is quite large, which may be up to 500 mL [36].

In order to overcome the coherent drawbacks of LLE, another extraction technique was introduced in 1976 which proposed the use on a solid phase rather than a liquid phase for extracting the analytes. Solid-phase extraction (SPE) was able to decrease the volume of the organic solvents significantly as compared to LLE. Furthermore, it gave better extraction selectivity. However, the procedure itself was still time-consuming and although the organic solvents consumption was less than those used in LLE, analytical chemists were still not satisfied with that large volume and tried to decrease it even more. Another concern about SPE was the use of disposable cartridges, which were neither biodegradable nor environmentally friendly.

These drawbacks, mainly the massive consumption of toxic organic solvents as well as the long and time-consuming procedures, resulted into low sample throughput and non-green methods, which affected not only the environment and living microorganisms but also the researchers themselves [37]. This motivated analytical chemists to introduce contemporary extraction techniques, which would overcome these drawbacks and solve the problems of conventional extraction techniques.

Later on, cloud-point extraction (CPE) and supercritical fluid extraction (SFE) techniques were introduced, which were much more environmentally friendly. However, these two techniques were still time-consuming, especially SFE, which usually takes up to 1 h per sample. Moreover, it needs expensive special apparatus and a limited amount of the sample can be analyzed [36].

In 1987, scientists started to be more concerned about the environment and the toxicity of organic solvent on the eco-system. Thus, the concept of ecological chemistry was introduced, which led to an evolution in separation science. Three years later, the micro total analysis system was developed.

The first microextraction to evolve was termed as “solid-phase microextraction (SPME)”. Introduction of SPME by Pawliszyn and co-workers in 1990 [38] opened the door to a new era of extraction techniques and reduced the extraction volume from milliliter or even liter volumes

into microliters. SPME could solve many problems of conventional extraction techniques, which include:

1- Reduction of organic solvent consumption.
2- Miniaturization.
3- Automation.
4- Enhancement of analytes preconcentration.
5- Short extraction time.

SPME was applied for the first time to water samples in 1992 [39]. This technique provided numerous advantages, such as removing many tedious steps that were necessary for conventional methods, significantly high preconcentration factors leading to higher sensitivity and minimized analytes loss. These advantages made SPME to be widely accepted and gave it a unique reputation among analytical chemists. On the contrary, direct immersion SPME (DI-SPME) suffered from critical drawbacks, the most important being the physical instability of the fibers used, stripping and breaking of the coating, which dramatically affected the lifetime of the fibers [40]. Then, headspace SPME (HS-SPME) was introduced to enhance the lifetime as compared to DI-SPME, but the cost was still another main drawback of the technique [41].

Liquid-liquid microextraction (LLME), which emerged in the mid-to-late 1990s and was considered as an alternative to SPME [42, 43], is a miniaturized form of LLE, where the volume of the extractant is limited to smaller volume in microliters. Since LLME techniques have several advantages over SPME, they are considered to be more favorable among researchers. These advantages are:

1- Faster phase separation.
2- Easier to modify.
3- Greater extraction capability.
4- Less capital-cost, due to the lower consumption of solvents.
5- More environmentally benign.

LLME techniques can be categorized into two main categories, two- and three-phase LLME. In two-phase LLME, the extractant is in direct contact with the sample solution, which enhances the extractability but reduces the clean-up and selectivity. On the other hand, in the three-phase LLME, a third solvent, which is immiscible with the sample solution and extractant, is involved, that could increase the selectivity significantly by enhancing the clean-up efficiency [44].

Single-drop microextraction (SDME) was the first LLME to be introduced and offered an extreme reduction of the volume of organic solvent [45]. The principle of SDME is based on partitioning the analytes between the sample solution and one droplet of the extraction solvent hanged by a syringe needle. The drop is either directly immersed into the sample solution or in the headspace mode, where the latter is limited to volatile analytes only [42, 43]. Despite, ease of automation, simplicity and other advantages of this method, it suffers from droplet instability and hence low reproducibility [44].

Dispersive liquid-liquid microextraction (DLLME) was introduced by Assadi et al. in 2006 [26]. The principle of DLLME relies on the use of a third party (i.e., a disperser solvent) that is miscible with both the sample solution and the extractant. This leads to the formation of tiny droplets (emulsion) of the extractant inside the sample solution as shown in **Figure 1.5**. The emulsion formation largely increases the contact surface area between the sample solution and the extractant significantly leading to higher extraction efficiency. Hence, noticeable high preconcentration factors are obtained with this technique [44]. Besides, the equilibrium state can be achieved much faster due to the same reason, resulting in a short extraction time [46].

DLLME has gained a particular interest among researchers due to numerous advantages such as:

1- Simplicity.
2- Ease of operation.
3- Rapidness.
4- Cost effectiveness.
5- High recovery.
6- High enrichment factors.
7- Environmental benignity [26, 47].

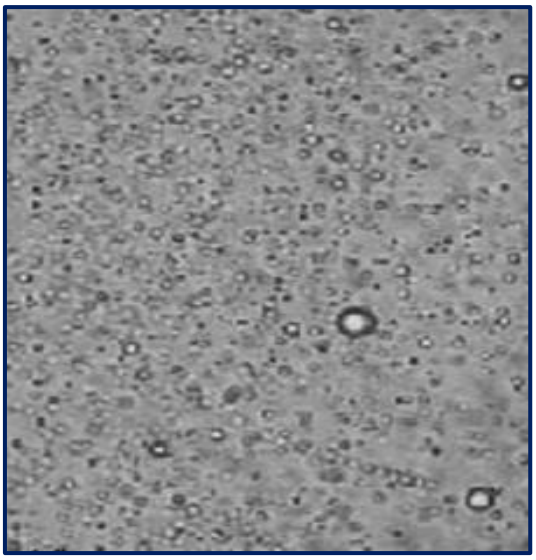


Figure 1.5 Optical microscopic photography of dispersed tetrachloroethylene in the aqueous sample [26].

Despite its several advantages, DLLME needs a centrifugation step to break down the emulsion and recover the extraction solvent, which is the most time-consuming step and is considered as an obstacle in the way of automation as well as for *in-situ* analysis. Effort has been made to overcome this limitation. Among the solutions for this problem was the use of an in-line filter to separate the organic solvent from the aqueous phase [48], or the use of hollow fiber [49, 50], among others.

Other limitations of DLLME are that it may provide low clean-up, especially with complicated matrices [44], and the use of toxic extraction solvents, which are generally halogenated hydrocarbons such as chloroform, carbon tetrachloride, chlorobenzene and tetrachloroethylene. These solvents are also incompatible with most of the reversed-phase-HPLC mobile phases. Due to this particular reason, combining DLLME with HPLC needs a further sample preparation step, mainly solvent reconstitution through evaporation-to-dryness (ETD) [46] or back-extraction (BE) [24, 51]. The general procedure of DLLME with heavy solvents is shown in **Figure 1.6**.

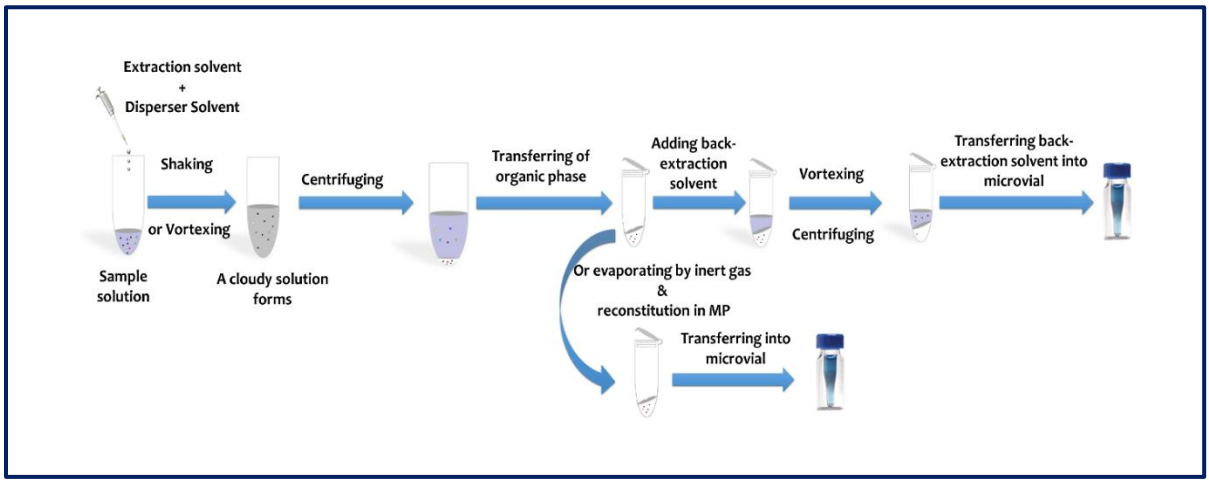


Figure 1.6 General procedure of DLLME.

Less toxic organic solvents proposed for DLLME to replace the heavy halogenated ones are low-density solvents such as 1-undecanol, 1-dodecanol, 2-dodecanol, and n-dodecanol or ionic liquids (ILs). ILs are a group of ionic organic salts with a melting point below 100 ˚C, which keeps them in the liquid form at room temperature [52]. They are known as “green solvents” that can replace conventional toxic organic solvents. Whilst several studies applied ILs in DLLME [53-57]; the main disadvantage of these solvents is their high cost due to laborious and complicated synthesis.

Low-density solvents are a good alternative for the dense chlorinated ones. However, since their densities are lower than that of water, they float on the surface after conducting DLLME, which makes their collection problematic. Several work has been conducted to overcome this limitation. Among the first attempts was the injection of deionized (DI) water to increase the level of the extractant before its collection into a capillary part in special compartment [58]. Saleh et al. [59] introduced another set, which contained a centrifuge-vial that had a conical top attached to capillary. Another study used a squeezable sample vial to direct the extractant into the capillary [60]. However, all of these methods required special tools, unlike the solidification of floating organic droplet microextraction (SFOD-ME), which was developed in 2007 [61]. SFOD-ME is simpler compared to other methods discussed previously [62]. In SFOD-ME, a low-density extraction solvent with a melting point close to room temperature is used. In such case, the floating organic drop can easily be solidified for easy collection [44].

As can be noticed, the recent trend in separation science is to modify microextraction to result in efficient, economical, miniaturized and green techniques that can overcome the limitations and disadvantages of the conventional ones.

1.4 Switchable-Hydrophilicity Solvents-Based Liquid-Liquid Microextraction

Switchable-hydrophilicity solvents (SHSs) were first introduced by Jessop et al. in 2005, and were described as “smart solvents” [63]. SHSs can be defined as a group of solvents that are immiscible with water in one form and are completely miscible in the other form. These solvents can, therefore, be switched between these two forms by changing some physicochemical properties of the system [64].

SHSs can be considered as a form of ILs but are much cheaper. The simplicity of preparing these solvents and their low cost gained them particular interest among researchers in different fields. The switching mechanism between the two forms can take place at ambient temperature and pressure by direct addition or removal of carbon dioxide, CO_2 (**Figure 1.7**). CO_2 can lead to protonation of the SHS, e.g., a tertiary amine, through to an acid-base reaction of hydrated

CO_2 or carbonic acid in the carbonated water and SHS, resulting in the hydrophilic bicarbonate salt of the SHS according to **Equation 1.1** [64]. Synthesis of SHS by purging CO_2 is shown in **Figure 1.7**.

$$NR_3 + H_2O + CO_2 \rightleftharpoons NR_3H^+ + HCO_3^- \qquad \textbf{Equation 1.1}$$

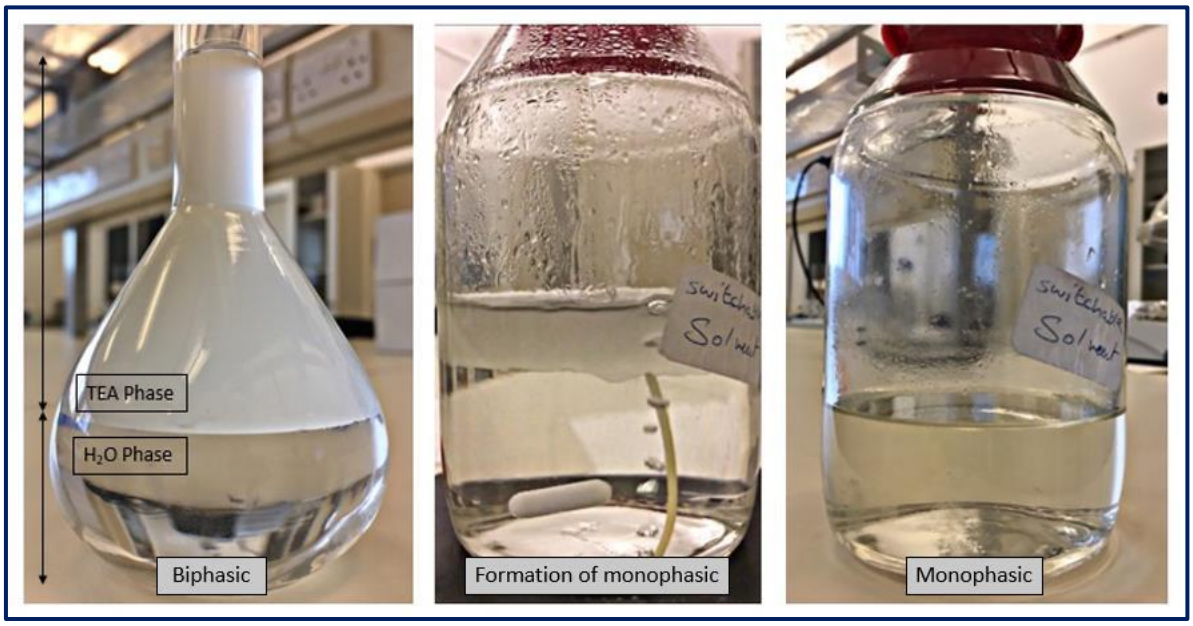


Figure 1.7 Synthesis of SHS by purging CO_2.

The first SHS reported by Jessop et al. was 1,8-diazabicyclo-[5,4,0]-undec-7-ene [63]. Then, others like amidines as well as tertiary and secondary amines have been identified as SHSs [63-66]. A list of solvents studied for their use as SHSs by Jessop et al. and their physical properties [64] are shown in **Table 1.2** and **Table 1.3**.

Monophasic SHS systems are solvents which are completely miscible with water in their "switched-off" unprotonated form (i.e., before introducing CO_2 into the system). On the contrary, if the solvent is still immiscible even after introducing CO_2 into the system, it is referred to it as a biphasic. SHSs are immiscible with water in their "switched-off" or

unprotonated from (i.e., before introducing CO_2). However, upon purging CO_2 into the mixture, they are "switched-on" to their protonated form, which is completely miscible with water.

Some guanidines are immiscible with water and can be switched on successfully, but the process is irreversible (i.e., they cannot be switched off to their unprotonated form), mainly due to their high basicity as compared to others.

It was observed that switchable amines have $logP$ values ranging between 1.2 and 2.5, otherwise, the amines will be too hydrophilic or hydrophobic and will form monophasic or biphasic, respectively. In addition, they have pK_a values higher than 9.5; amines with less pK_a do not react with carbonated water sufficiently due to insufficient basicity, preventing the switching process.

It is worth mentioning that although some amines fulfill these two criteria, they are not switchable, meaning that these criteria are necessary but not sufficient requirements for the switchable behavior. Furthermore, *N*,*N*-dimethylbenzylamine has a pK_a of 9.03 and could form a SHS, and to the best of our knowledge it, is the only exception.

Table 1.2 Tertiary amines solvents tested for their ability to use as SHS.

Behavior	Solvent	Ratio of compound to water (v: v)	$logP$	pK_a
Monophasic	Triethanolamine	1:1	-1.51	7.85
Monophasic	*N,N,N′,N′*-Tetramethylethylenediamine	1:1	0.21	9.20
Monophasic	*N*-Ethylmorpholine	1:1	0.30	7.70
Monophasic	*N*,*N*-Dimethylaminoethanol	1:1	−0.44	9.31
Monophasic	*N*,*N*-Dimethylaminopropanol	1:1	−0.08	9.76
Monophasic	*N*,*N*-Diethylaminoethanol	1:1	0.41	9.87
Monophasic	*N*,*N*-Diethylglycine methyl ester	1:1	0.76	7.75
Monophasic	*N*,*N*-Diethylaminopropanol	1:1	0.77	10.39
Monophasic	5-(Diethylamino)pentan-2-one	1:1	1.21	10.10
Monophasic	Ethyl 3-(diethylamino)propanoate	1:1	1.40	9.35
Switchable	Triethylamine	1:1	1.47	10.70
Switchable	*N*,*N*-Dimethylbutylamine	1:1	1.60	10.00
Switchable	*N*-Ethylpiperidine	1:1	1.75	10.50
Switchable	*N*-Methyldipropylamine	1:1	1.96	10.40
Switchable	*N*,*N*-Dimethylcyclohexylamine	1:1	2.04	10.50
Switchable	*N*-Butylpyrrolidine	1:1	2.15	10.40
Switchable	*N*,*N*-Diethylbutylamine	1:1	2.37	10.50
Switchable	*N*,*N*-Dimethylhexylamine	1:1	2.51	10.20
Switchable	*N*,*N*-Dimethylbenzylamine	5:1	1.86	9.03
Switchable	5-(Dipropylamino)pentan-2-one	2:1	2.15	10.15
Switchable	Diisopropylaminoethanol	1:1	1.16	10.14
Switchable	4,4-Diethoxy-*N*,*N*-dimethylbutanamine	1:1	1.48	9.83
Switchable	Ethyl 4-(diethylamino)butanoate	1:1	1.82	10.15
Switchable	*N*,*N*-Dimethylphenethylamine	1:1	2.18	9.51
Switchable	Dibutylaminoethanol	1:1	2.20	9.67
Biphasic	*N*,*N*-Dimethylaniline	1:1	2.11	5.10
Biphasic	*N*,*N*-Diisopropylethylamine	1:1	2.28	11.00
Biphasic	Tripropylamine	1:1	2.83	10.70
Biphasic	*N″*-Hexyl-*N,N,N′,N′*-tetrabutylguanidine	2:1	7.91	13.60
Biphasic	Trioctylamine	1:1	9.45	10.90
Biphasic	Propyl 3-(diethylamino)propanoate	1:1	1.85	9.45
Biphasic	*N*,*N*-Dibutylaminopropanol	1:1	2.56	10.50
Biphasic	Ethyl 3-(dipropylamino)propanoate	1:1	2.72	9.29
Biphasic	*N*,*N*-Dibutylaminobutanol	1:1	2.93	10.70

Table 1.3 Secondary amines, amidines, and guanidines solvents tested for their ability to be used as SHS.

Behavior	Solvent	Ratio of compound to water (v: v)	$logP$	pK_a
Monophasic	Diethylamine	1:1	0.71	10.92
Monophasic	Ethyl 3-(tert-butylamino)propanoate	1:1	1.38	10.09
Monophasic	tert-Butylethylamine	1:1	1.42	11.35
Monophasic	Diisopropylamine	1:1	1.46	11.07
Monophasic	*N,N,N′,N′*-Tetramethylguanidine	2:1	0.30	13.60
Monophasic	1,8-Diazabicycloundec-7-ene	2:1	1.73	12.00
Monophasic	*N*-Hexyl-*N′,N′*-dimethylacetamidine	2:1	2.94	12.00
Switchable	*N,N,N′*-Tripropylbutanamidine	2:1	4.20	12.00
Switchable	*N,N,N′*-Tributylpentanamidine	2:1	5.99	12.00
Switchable	Butyl 3-(isopropylamino)propanoate	1:1	1.90	9.77
Switchable	Propyl 3-(sec-butylamino)propanoate	2:1	1.95	9.80
Switchable	Ethyl 3-(sec-butylamino)propanoate	1:1	1.53	9.73
Switchable	Dipropylamine	1:1	1.64	11.05
Switchable	*N*-Propyl-sec-butylamine	1:1	2.03	11.05
Switchable	Di-sec-butylamine	1:1	2.43	11.02
Irreversible	*N″*-Hexyl-*N,N,N′,N′*-tetramethylguanidine	2:1	2.82	13.60
Irreversible	*N″*-Butyl-*N,N,N′,N′*-tetraethylguanidine	2:1	3.52	13.60
Irreversible	*N″*-Hexyl-*N,N,N′,N′*-tetraethylguanidine	2:1	4.43	13.60
Precipitates	tert-Butylisopropylamine	1:1	1.84	11.39
Precipitates	Ethyl 3-(isobutylamino)propanoate	1:1	1.46	9.45
Precipitates	Ethyl 4-(tert-butylamino)butanoate	1:1	1.75	10.77
Precipitates	Dibutylamine	1:1	2.61	11.28
Precipitates	Dihexylamine	1:1	4.46	11.02

Secondary amines have a different reactivity pathway, faster than the bicarbonate salt formation, which allows them to react with CO_2 directly and form ammonium carbamate salts (**Equation 1.2**), resulting in faster CO_2 uptake. Accordingly, less time is needed, i.e., less than 10 min, for switching secondary amines as compared to tertiary amines and amidines, the time for which ranges between 20 and 120 min. However, it requires higher energy to remove the CO_2 from ammonium carbamate than ammonium bicarbonate [64, 67].

$$2R_2NH + CO_2 \rightleftharpoons R_2NH_2^+ + R_2NCOO^- \qquad \textbf{Equation 1.2}$$

It was observed that some secondary amines precipitated during the switching on, as confirmed by X-ray crystallography [64], which is due to low solubility of their salts in water, limiting their use as SHS.

Despite the sparse data in the literature about biodegradation of amines, it is thought that secondary amines are more biodegradable than tertiary ones, with some exceptions [68]. (e.g., *N*,*N*-dimethylcyclohexylamine) (IUCLID Dataset for Cyclohexyldimethylamine, European Commission – European Chemicals Bureau, 2000).

Due to their several fascinating advantages besides the complete miscibility with water providing infinite surface area with aqueous solutions, the synthesis procedure itself is neither expensive nor complicated as compared to ILs. Phase separation can be instantaneous if a proper method is used (i.e., addition of a strong base), and nonetheless, the extraction system is not complicated and does not need a tertiary solvent as compared with other microextraction techniques (e.g., DLLME). SHSs were applied in the field of extraction just as soon as they have been introduced. Furthermore, no special tool or apparatus is needed for switchable-hydrophilicity solvents-based extraction (SHS-LLE) or microextraction (SHS-LLME), unlike other techniques (e.g., HF-LPME).

The first studies using SHSs as an extractant for a large scale (SHS-LLE) [65, 66, 69-71], and later on for microextraction purpose (SHS-LLME), started to gain popularity. However, the studies using SHSs as an extractant for microextraction is still growing and not yet routinely used [72]; further exploration in this field is still required.

The extraction takes place just at the phase separation step. Different methods for removing CO_2 or phase separation were examined in the literature as shown in **Figure 1.8**. Among them, the addition of a strong base such as sodium hydroxide at high concentrations seems to be the

most efficient for phase separation. Other physical and chemical methods are tedious, time-consuming and may cause serious analyte loss [72].

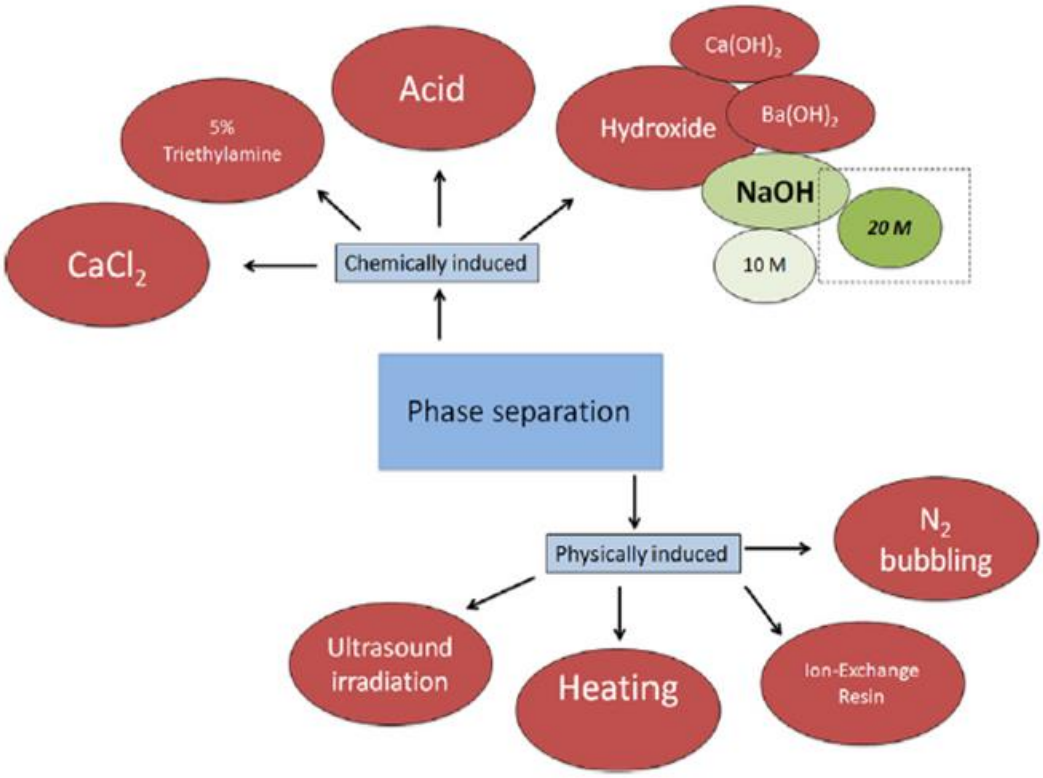


Figure 1.8 Phase separation methods [72].

1.5 High-Performance Liquid Chromatography

Chromatography is a separation method invented by Mikhail Tswett at the beginning of the 20th century. This powerful separation method finds a variety of applications in all branches of science, which has grown explosively during the last half of 20th century, mainly due to the urgent need for a powerful method that can separate complex mixtures. Numerous types of chromatography were then introduced to the field.

High-performance liquid chromatography (HPLC) gained an exceptional reputation. Martin and Synge introduced the idea of liquid chromatography, which later on led to the invention of HPLC, and they won a noble prize in chemistry in 1952 for their studies.

However, the main progress of liquid chromatography started to be noticeable in the 1960s, when scientists found that the separation may be enhanced by decreasing the inner diameter or size of the packing materials. The separation time was extremely long and separation took place at atmospheric pressure. To be able to solve this problem, scientists started to increase the flow rate of the mobile phase by merely increasing the pressure, and by doing so, they shortened the analysis time and could also increase the resolution of separation as well [73].

Chromatographic methods can be classified into two main groups based on the physical contact between the mobile phase (MP) and stationary phases (SP). Column chromatography can be further categorized into three main groups according to the mobile phase used, i.e., gas, liquid and supercritical, as shown in **Figure 1.9**.

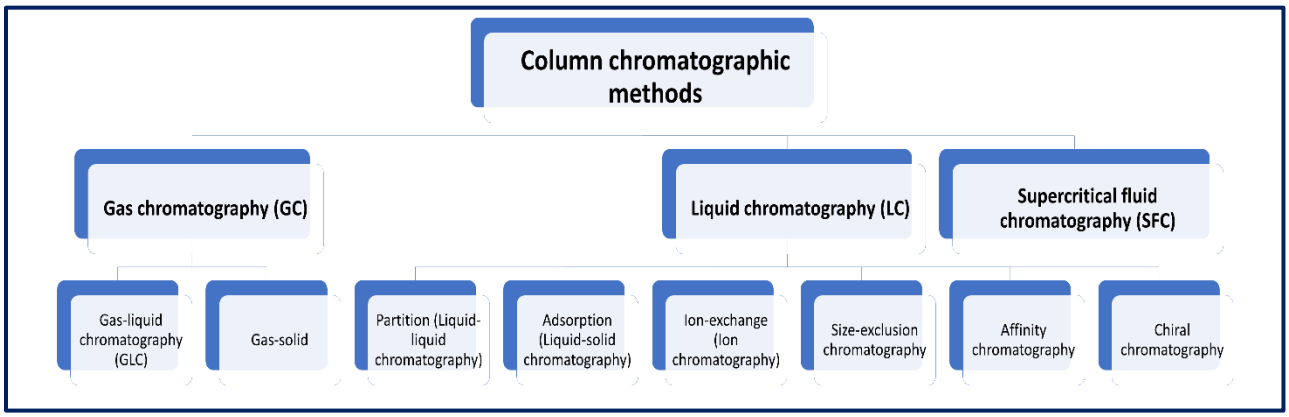


Figure 1.9 Classification of column chromatographic methods.

Liquid chromatography is considered as the most widely used analytical separation technique. The reasons behind this reputation is its applicability to a variety of analytes covering polar and nonpolar molecules, as well as inorgani, and organic ones such as amino acids, nucleic

acids, proteins, and many other macromolecules. The main advantages of liquid chromatography are listed in **Figure 1.10**.

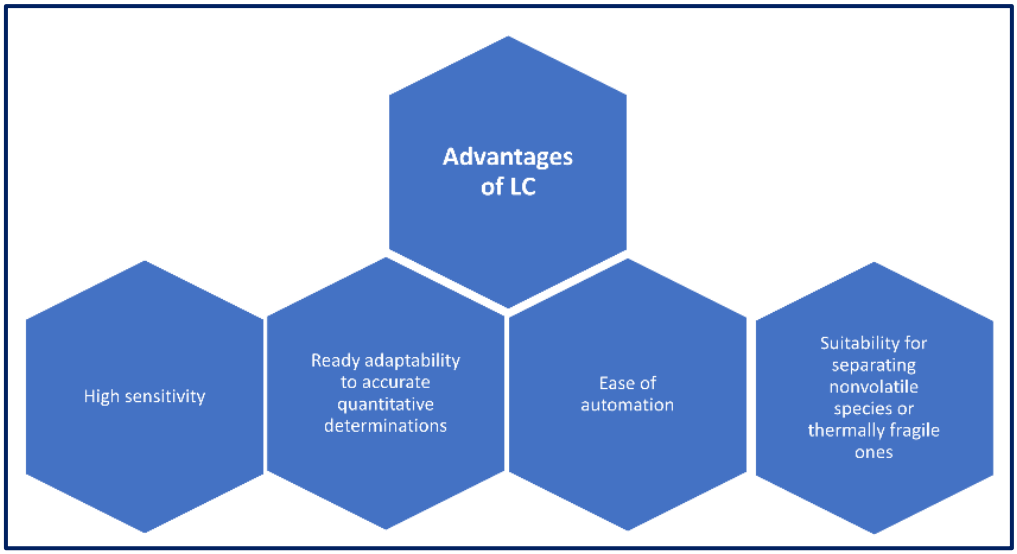


Figure 1.10 Advantages of LC.

Among others, partition liquid chromatography is the most commonly used one, which has two different modes depending on both mobile and stationary phases. The first is a normal phase (NP), which uses nonpolar mobile phases (e.g., n-hexane, ethyl acetate, etc.) and polar stationary phase (e.g., silica gel, alumina, etc.) and is mainly used for polar analytes. On the contrary, in the reversed-phase (RP) mode, the mobile phase is relatively polar (e.g., water, ACN, MeOH, THF) and the stationary phase is made of nonpolar particles such as octadecyl (C-18) group-bonded silica gel (ODS). The latter is more favorable since the solvents used are much less toxic as compared to the NP mode. Furthermore, the majority of analytes having low polarity would show more interactions with the stationary phase in the RP as compared to NP. Choosing the mode depends mainly on the suitability of the analytes under investigation as illustrated in **Figure 1.11**.

In chromatography, the separation takes place due to the distribution of the analytes between the mobile and the stationary phases. Both phases should be carefully chosen in order to provide

a rational equilibrium of the analytes between the two phases for a good separation to be achieved. Distribution of the analytes among the two phases can be calculated from the distribution coefficient (K) as shown in **Equation 1.3**.

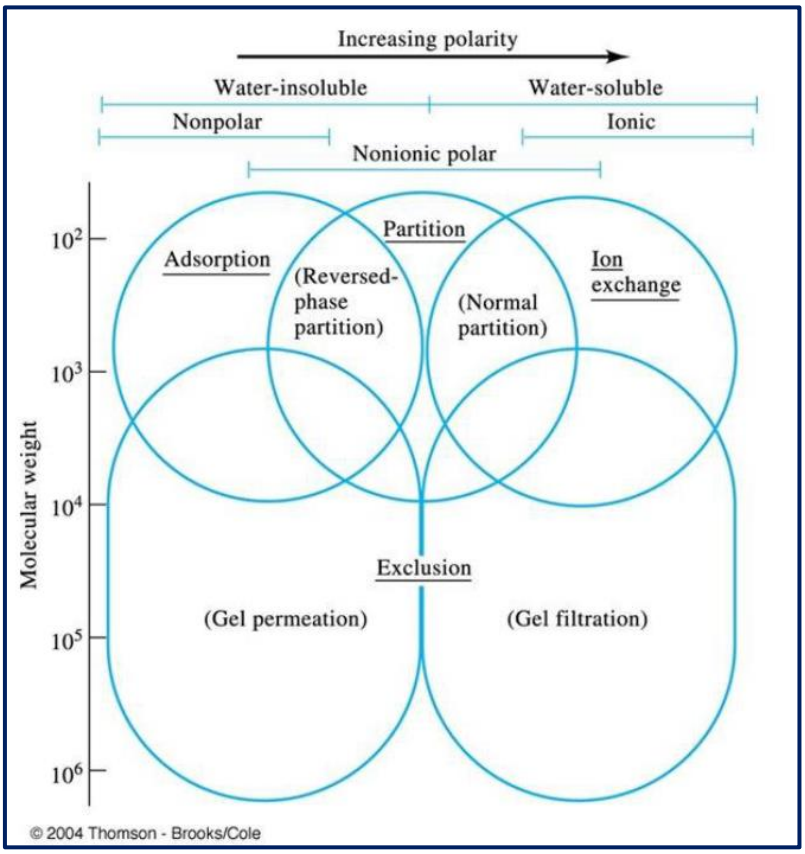


Figure 1.11 Modes for LC.

$$K = \frac{Concentration\ of\ the\ analytes\ in\ the\ stationary\ phase}{concentration\ of\ the\ analytes\ in\ the\ mobile\ phase} \qquad \textbf{Equation 1.3}$$

Choosing the suitable mode depends upon the polarity of the analytes, molecular weight, and degree of ionization. The logarithm of partition coefficient P (i.e., $logP_{O/W}$) is an important parameter on which the chromatographer can depend to estimate the polarity of the analytes. This parameter can be defined as the ratio between the analyte concentration in two immiscible phases (i.e., octanol and water), which is calculated as follows:

$$logP_{O/W} = log\frac{[analyte\ in\ n-octanol]}{[analyte\ in\ water]} \qquad \textbf{Equation 1.4}$$

As noticed in **Equation 1.4**, polar analytes are expected to have low $logP_{O/W}$ value since its concentration in water would be larger than its concentration in n-octanol, and vice versa.

The degree of ionization can be calculated using graphs of percentage microspecies distribution versus pH. MarvinSketch is among the useful programs available for quick plotting of such graphs. In addition, several physicochemical properties can be predicted using this program.

1.5.1 HPLC Instrumentation

HPLC has seven main components are shown in **Figure 1.12**, namely: mobile phase reservoir, pump, injection loop, column, detector, data acquisition and waste collection bottle. In addition to the main parts of HPLC, extra accessories can be combined to the instrument to enhance the performance, such as quaternary pump, degasser, autosampler, column thermostatic jacket or oven or fraction collector, etc.

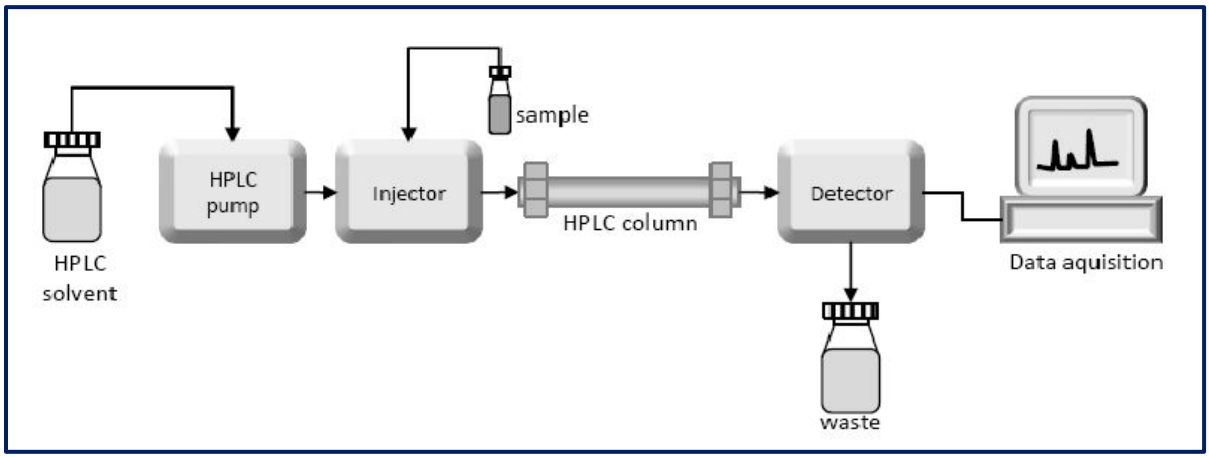


Figure 1.12 HPLC instrumentation.

1.5.2 Elution Modes in HPLC

Elution in HPLC can be either isocratic or gradient. The first is delivering a constant composition of the mobile phase during the analysis. Whereas, in the gradient elution, the composition of the mobile phase can be varied during the analysis.

The isocratic elution is simpler and more common than the gradient one since it does not require a quaternary pump. Also, factors affecting the separation in the isocratic mode can be better understood. However, isocratic elution may suffer from the common "general elution problem" (i.e., a long time gap between analytes having different polarities) which prolongs the analysis time needed in the isocratic mode significantly. Gradient elution can solve this kind of problem with a proper resolution by varying the mobile phase composition during the analysis. Another superiority of gradient elution is that it can separate structurally similar analytes with a higher resolution which are difficult to achieve with the isocratic mode.

When gradient elution is applicable, the preliminary gradient scan can provide a piece of valuable information and the chromatographer can analyze the peaks by some calculations (**Equation 1.5**) in order to decide whether isocratic elution can be possible or not, besides, the composition needed for isocratic elution can be estimated.

$$\Delta t_g = t_f - t_i \qquad \textbf{Equation 1.5}$$

where, Δt_g is the difference in the retention time of the final (i.e., t_f) and initial peak (i.e., t_i).

After running the gradient scan and calculating Δt_g, the final decision can be made depending on estimations related to t_g (i.e., total gradient time) as given in **Figure 1.13**. However, if the Δt_g value is very small then gradient elution may be applied to enhance the resolution. If

isocratic elution is possible, then, the suitable composition of the mobile phase can be estimated by dividing Δt_g by 2, and the composition corresponding to that retention time can be adopted.

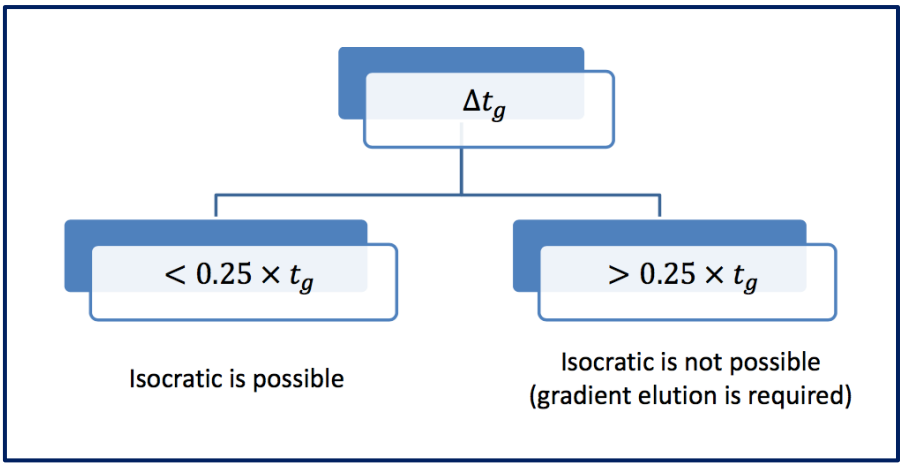


Figure 1.13: Deciding on the elution mode.

1.5.3 Optimization of HPLC Conditions

In HPLC optimization, the systematic approach is always preferred over the "Random walk" (i.e., changing the HPLC conditions randomly or uncoordinatedly), because the first can provide the analyst with better understanding of the effect of separation conditions on the separation within a shorter time. It is possible to obtain a good separation with the "Random walk". However, understanding the interactions and correlations between different parameters might be infeasible, resulting into a higher number of experiments as compared to the systematic approach.

1.5.4 Factors Affecting Resolution

Resolution (R_s) is a well-known term in chromatography that describes the degree of separation between neighboring bands or peaks. There are three factors affecting the resolution, i.e., retention (or capacity), number of theoretical plates (efficiency) and selectivity.

1. Retention (or capacity) factor (k') can be obtained from the chromatogram using **Equation 1.6**.

$$k' = \frac{t_R - t_M}{t_M} \qquad \textbf{Equation 1.6}$$

where, t_R is the retention time of the analyte and t_M is the dead time (i.e., the retention time of an unretained species in the column).

Improving (k') can be carried out by changing the mobile phase composition, the column temperature or the mobile phase pH either by adding pH modifier (e.g., acetic acid, trifluoroacetic acid, etc.) or a buffer (e.g., acetate, citrate, phosphate, etc.).

2. Number of theoretical plates (efficiency) (N) can be obtained from the chromatogram using **Equation 1.7**.

$$N = 16\left(\frac{t_R}{W}\right)^2 \qquad \textbf{Equation 1.7}$$

where, t_R is the retention time of the analyte and W is its peak width.

The efficiency can be enhanced via increasing the column length, internal diameter, or decreasing particle size or by changing the flow rate.

3. Selectivity factor ($\propto$) can be obtained from the chromatogram using **Equation 1.8**.

$$\propto = \frac{k'_B}{k'_A} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \qquad \textbf{Equation 1.8}$$

where, $(t_R)_A$ and $(t_R)_B$ are the retention times of the first and second analyte, respectively, in the critical pair and t_M is the dead time.

Changing the column type or the mobile phase identity can improve the selectivity. Unlike N and k', $\propto$ describes a critical peak pair. These factors can be combined to improve R_s, as given in **Equation 1.9**.

$$R_s = \frac{\sqrt{N_{av}}}{4} \times \frac{k'_{av}}{k'_{av} + 1} \times \frac{\propto - 1}{\propto} \qquad \textbf{Equation 1.9}$$

As can be noticed from **Figure 1.14,** R_s is so dependent on $\propto$, since any small change in the later can significantly improve R_s. Improving N can also improve R_s but less significantly. On the other hand, increasing k' up to ca. 10 can improve R_s, beyond which it would have less effect on resolution.

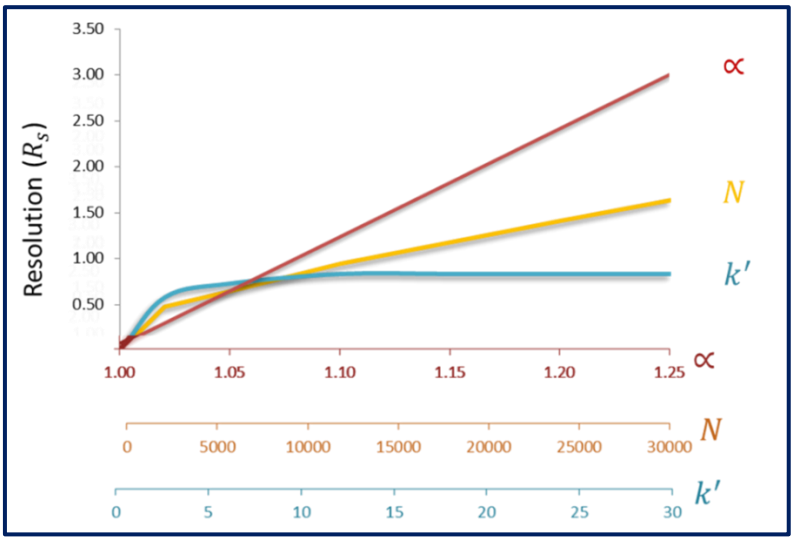


Figure 1.14: Effect of k', $\propto$ and N on R_s.

In order to have a reasonable control of the different parameters and to minimize the number of optimization experiments, a good understanding of the various factors affecting the

separation is required. These parameters include the type of column packing, particle size, column dimensions, column temperature, flow rate, composition and identity of the mobile phase, pH of the mobile phase, type and concentration of the mobile phase modifier, etc. In the systematic approach for optimizing HPLC conditions, the optimization should be dependent on how much change in resolution is needed, which in turn can be done by evaluating the chromatogram from preliminary experiments [74]. However, it is crucial to choose the most suitable HPLC methodology in the first place. After that, the chromatographer can decide which parameter to optimize according to the need for changing $\propto$, k' or N.

A systematic approach toward separation in RP-HPLC is summarized in **Figure 1.15**. First, an initial injection is done and the chromatogram is evaluated. For example, if the R_s of the critical pair in the chromatogram is poor and k'_{av} is outside the optimum range (i.e., of $2 \leq k'_{av} \leq 10$), then improving k'_{av} should be the first choice, since $\propto$ may change the chromatogram completely and N will not improve it enough to fit into the required range. On the contrary, if R_s is marginal and k'_{av} is already within the range, the best solution would be to improve N.

Increasing $\propto$ can improve R_s significantly as mentioned previously. However, it may change the selectivity (i.e., peak order). Mostly, the case where improving $\propto$ is desirable is when k'_{av} is already within the optimum range but R_s of the two adjacent peaks yet is much less than baseline resolution (i.e., 1.5). In such a case, trying to improve N would require a very long separation time to achieve a sufficient resolution.

Although increasing $\propto$ can provide the shortest possible separation times, it often involves much effort if the change is enormous. Since predicting the right conditions to improve $\propto$ is complicated and may bring the optimization back to the preliminary step, it is better to start with optimizing k'_{av}.

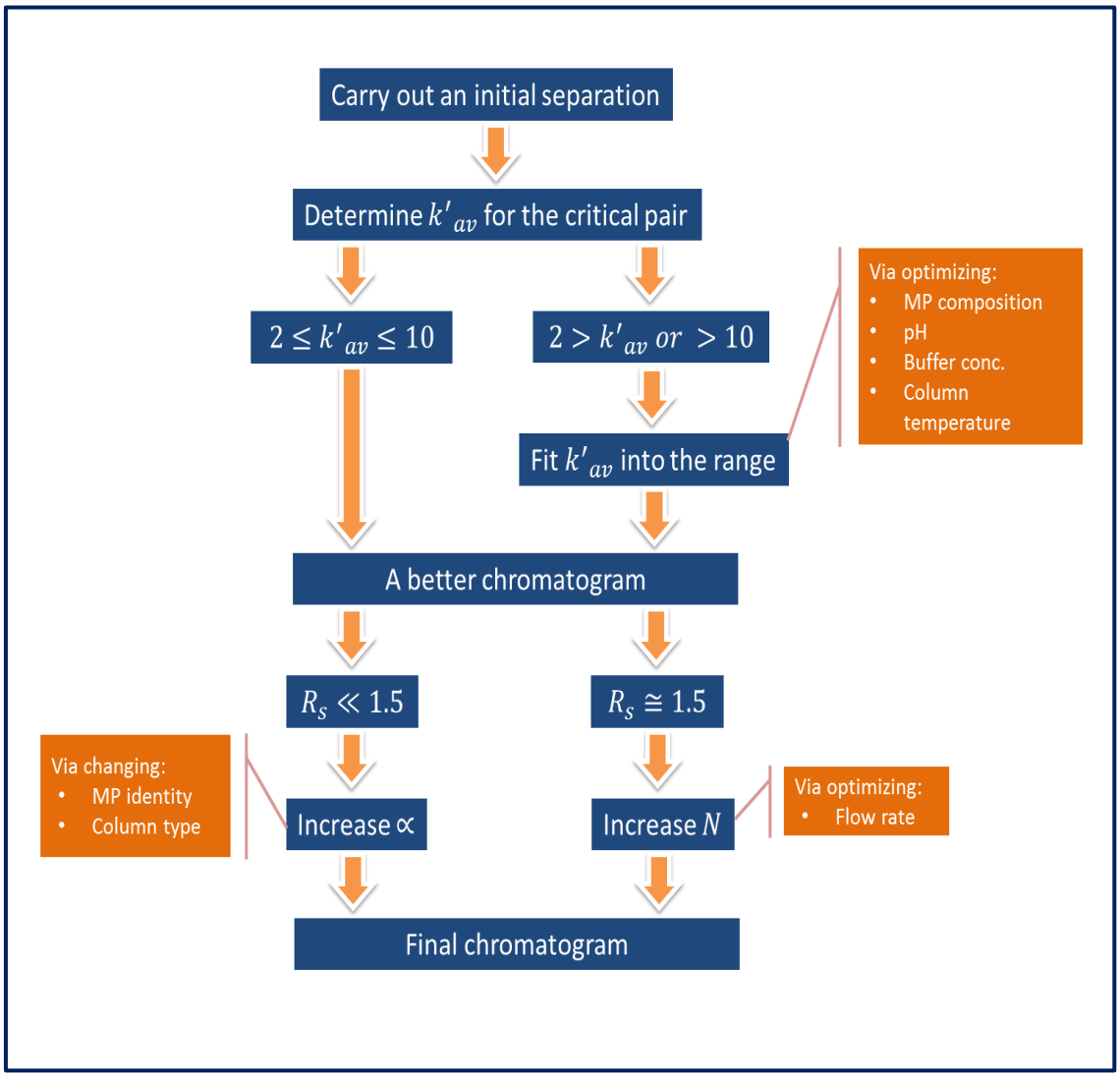


Figure 1.15: A systematic approach to HPLC optimization [74].

1.6 Literature review

1.6.1 SHS-LLME

Although SHSs were introduced by Jessop et al. in 2005 [63], its first use in the microextraction context was done in 2014 [72]. In this study, benz[a]anthracene was extracted from water samples before its determination using fluorescence spectrophotometry. Since then, it started to grasp the attention of researchers working in this field. The rapid increase of publications where SHS-LLME was used is shown in **Figure 1.16,** reaching approximately 37 publications in about five years.

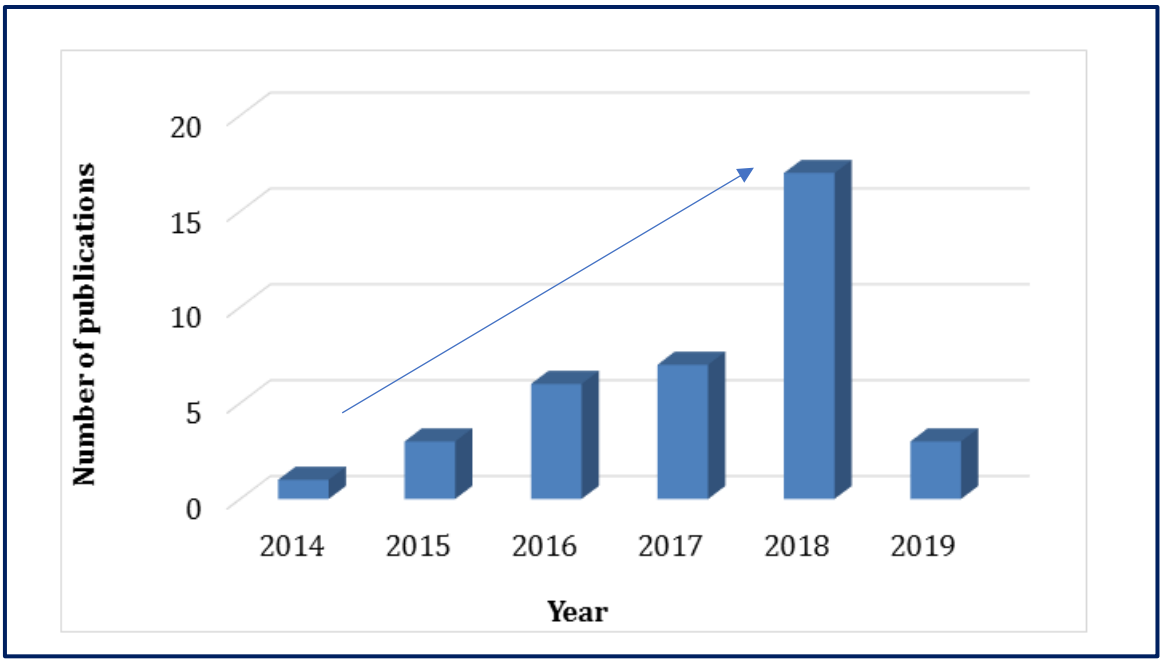


Figure 1.16 Number of publications using SHS-LLME (Web of Science, May 2019).

Although the first publication used SHS-LLME for studying molecular analyte, the technique was later applied for cadmium [75] and copper [76]. Just a few months later, the number of publications in both molecular (**Table 1.4**) and atomic (**Table 1.5**) fields is almost equal nowadays (**Figure 1.17**), which shows the applicability of this technique to a variety of atomic and molecular analytes.

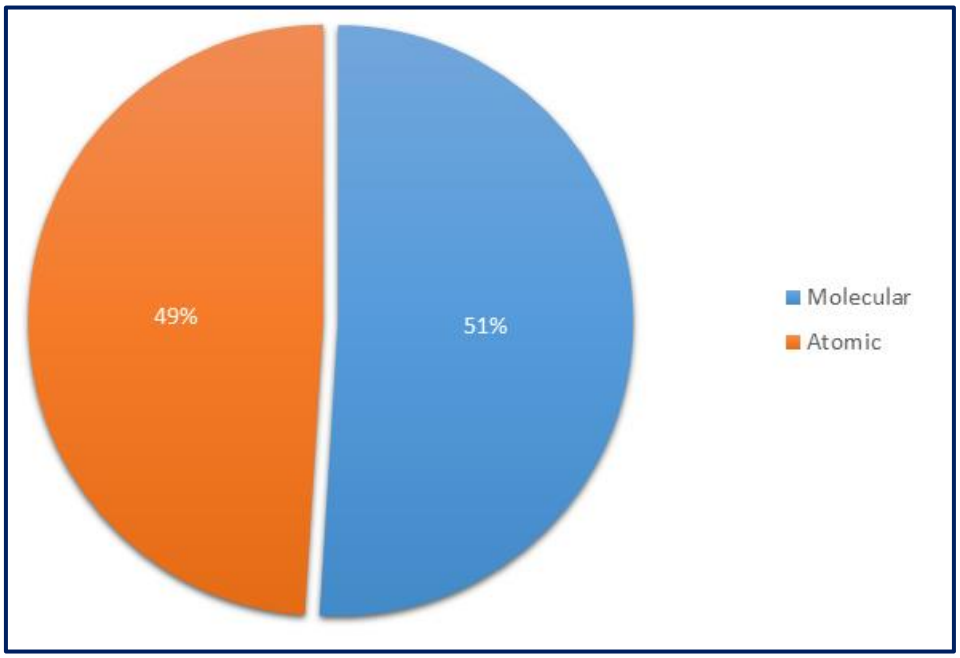


Figure 1.17 Type of analytes studied using SHS-LLME (Web of Science, May 2019).

Another point related to SHS-LLME, which drew the attention of researchers was the possibility to automate this method. The first attempt to automate SHS-LLME was done in 2015. This was done using a syringe and peristaltic pumps prior to HPLC for the determination of ofloxacin in human urine samples [77].

During the last five years, researchers used SHS-LLME for studying environmental, biological and food samples as shown in **Figure 1.18**. Some studies used SHS-LLME for studying pharmaceuticals [78, 79], in addition to only one publication studying the applicability of this method to plant samples. In this study, SHS-LLME was used to determine protoberberine alkaloids in *Rhizoma coptidis* samples [80].

As mentioned earlier, the first application of SHS-LLME was done using fluorescence spectrometry [72], and it was the only one using this technique. Other two studies used SHS-LLME prior to UV-Vis to determine uranium [81] and mercury [82] in environmental samples.

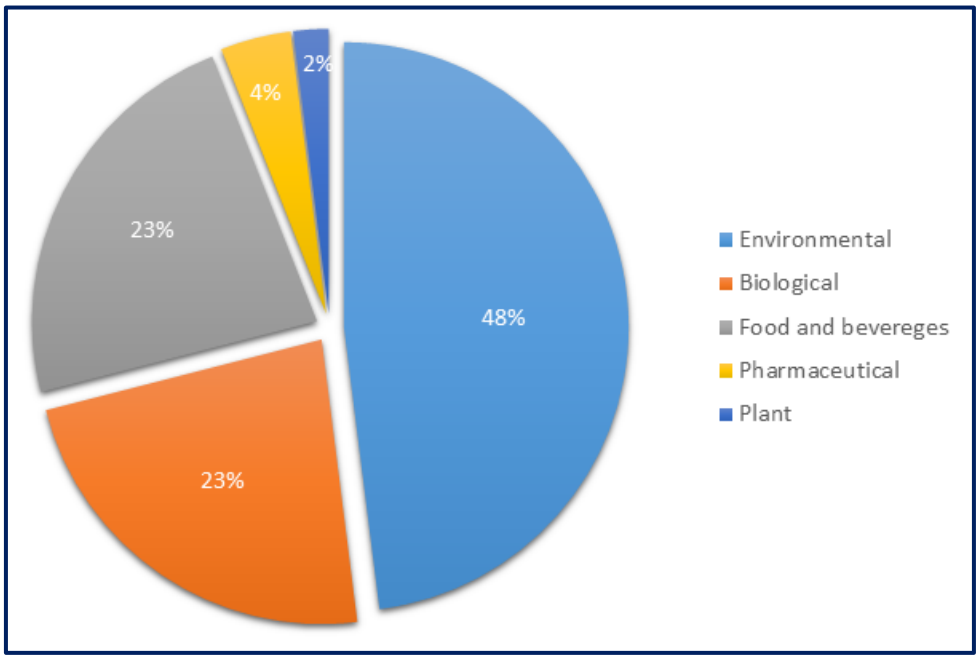


Figure 1.18 Type of samples studied using SHS-LLME (Web of Science, May 2019).

Most of the studies used atomic absorption spectrometry (AAS), as shown in **Figure 1.19**, which were as follows: Copper in environmental sample using flame-atomic absorption spectrometry (FAAS) [76], lead and cadmium in water, tea and human hair samples using graphite-furnace atomic absorption spectrometry (GFAAS) [83], cadmium in water, vegetable, fruit and cigarette samples using FAAS [75], palladium in water samples using GFAAS [84], vanadium in water and food samples using GFAAS [85], cobalt in tobacco and food samples using FAAS [86], nickel in tobacco and food samples using FAAS [87], silver and cobalt in bovine milk, orange juice, vitamin B12 (methylcobalamin) pill and tap water using FAAS [79], cadmium, nickel, lead and cobalt in water, urine and tea infusion samples using FAAS [88], cobalt in egg yolk and vitamin B12 pill using slotted quartz tube (SQT-FAAS) [78], arsenic in water samples using HG-AAS [89], cadmium in environmental samples using SQT-FAAS [90], palladium in water samples using SQT-FAAS [91], palladium in automotive catalytic converters, roadside dust and river water using FAAS [92], and cadmium in baby food samples using FAAS [93]. In addition, one study applied hydride generation atomic fluorescence spectrometry (HG-AFS) to determine arsenic and selenium in environmental

water and liver samples [94]. A summary of analytical instruments applied after SHS-LLME is given in **Figure 1.19.**

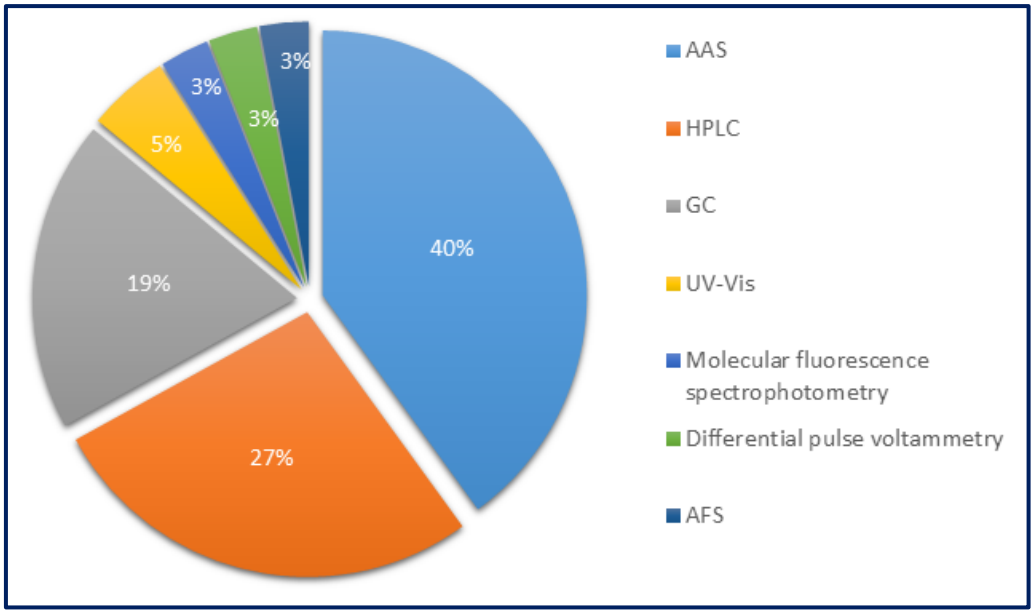


Figure 1.19 Type of instruments used with SHS-LLME (Web of Science, May 2019).

Chromatography is another technique which had been combined with SHS-LLME in the literature, particularly, gas chromatography (GC) and HPLC. In general, SHS-LLME-GC does not require any further pretreatment after the extraction step, such as solvent reconstitution throughout evaporation-to-dryness (ETD), which is due to volatility of SHSs [95-98]. However, in some studies, ETD was applied for other reasons such as derivatization of the analyte [99] or when the solvent was incompatible with the detector used in GC [100, 101].

On the other hand, SHS-LLME-HPLC needed an extra step before injecting the extract into the instrument, which was due to the low solubility of the SHS in the switched-off form in the mobile phase. In cases where the mobile phase contained more than 90% of organic solvent, the extract could be injected directly into the system in its switched-off form without any further treatment [102-104]. However, the majority of chromatographic methods needed solvent reconstitution as the typical pretreatment method to overcome the miscibility problem

with the mobile phase [80, 105, 106]. Another solution was to dissolve or dilute the extract in a mixture of acid and/or organic solvent before injecting the extract into HPLC [77, 107, 108] or through back-extraction the analytes into an aqueous phase [109].

The majority of studies used tertiary amines as SHS, as shown in **Figure 1.20**, besides secondary amines, fatty acids, and amides. The main reason behind the widespread use of these solvents was the proper physical properties, low cost, applicability and stability of these solvents after being switched on.

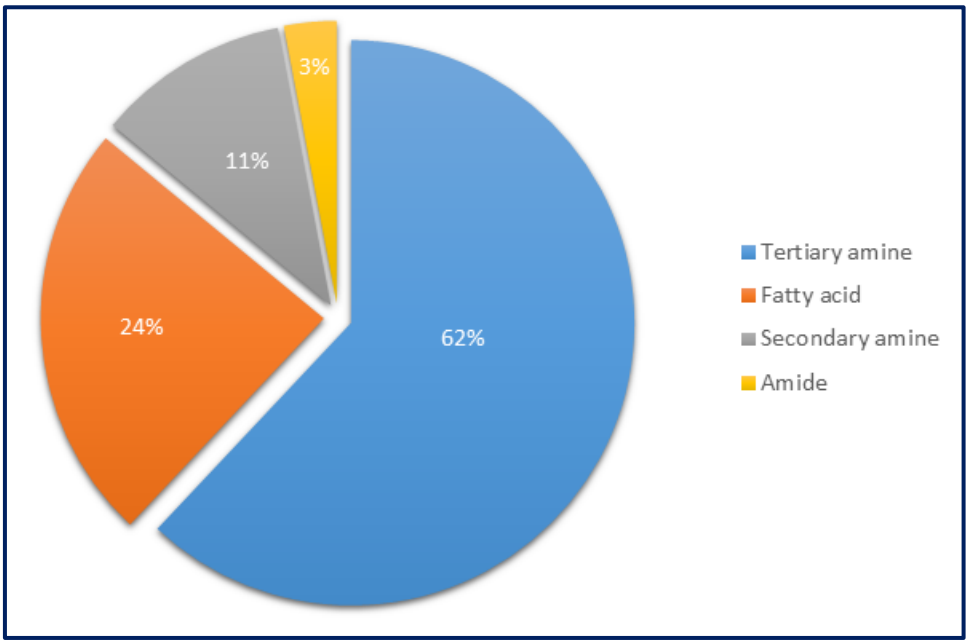


Figure 1.20 Type of SHS used in literature (Web of Science, May 2019).

Table 1.4 Summary of SHS-LLME methods for molecular analytes (Web of Science, May 2019).

Analyte	Sample	SHS/ Volume (µL)	Instrument	Ref.
Nitrazepam	Aqueous	*N*,*N*-Dipropylamine, 100	Differential pulse voltammetry	[110]
Benz[a]anthracene	Water	DMCA, 375	Fluorescence spectrophotometer	[72]
Methadone, tramadol	Human urine	Dipropylamine, 400	GC-FID	[96]
4-n-Nonylphenol	Municipal wastewater	*N*,*N*-Dimethylbenzylamine, 1000	GC-ID^4-MS	[95]
Methamphetamine	Human urine	Dipropylamine, 100	GC-MS	[99]
Endocrine disruptors, pesticides, hormones.	Water	*N*,*N*-Dimethylbenzylamine, 750	GC-MS	[97]
Quaternary ammonium herbicide, paraquat	Human urine, plasma, river water, apple juice	TEA, 375	GC-MS	[100]
Fluoxetine, estrone, pesticides, endocrine disruptors	Wastewater	*N*,*N*-Dimethylbenzylamine, 500	GC-MS	[98]
11 Drugs	Human urine	DMCA, 166	GC-MS	[101]
Chloramphenicol	Water	DMCA, 333	HPLC-DAD	[108]
Protoberberine alkaloids	*Rhizoma coptidis*	TEA, 350	HPLC-DAD	[111]
Ofloxacin	Human urine	Hexanoic acid, 50	HPLC-FLD	[77]
Ofloxacin	Chicken meat	Dichloromethane and acrylic acid, 600	HPLC-FLD	[106]
Fluoroquinolones	Shrimp	Nonanoic acid, 4	HPLC-FLD	[109]
Steroid hormones	Water	Nonanoic acid, 100	HPLC-UV	[107]
Sudan dyes	Solid food	Hexanoic acid, 300	HPLC-UV	[104]
Sudan dyes	Spices	Hexanoic acid,130	HPLC-UV	[102]
Paraquat	Biological, river water	TEA, 250	HPLC-UV	[103]
Bisphenols	Beverages	DMCA, 391	HPLC-UV	[105]

Table 1.5 Summary of SHS-LLME methods for atomic analytes (Web of Science, May 2019).

Analyte	**Sample**	**SHS/ Volume (µL)**	**Instrument**	**Ref.**
Cadmium	Water, vegetable, fruit, cigarette	TEA, 375	FAAS	[75]
Cobalt	Tobacco, food	*N*,*N*-Dimethyl-n-octylamine, 200	FAAS	[86]
Nickel	Tobacco, food	1-Ethylpiperidine, 400	FAAS	[87]
Silver and cobalt	Bovine milk, orange juice, vitamin B12 pill, tap water	Hexanoic acid, 300	FAAS	[79]
Cadmium, nickel, lead, cobalt	Water, urine and tea infusion	TEA, 450	FAAS	[88]
Palladium	Automotive catalytic converters, roadside dust, river water	DMCA, 300	FAAS	[92]
Cadmium	Baby food	TEA, 250	FAAS	[93]
Copper	Environmental	TEA, 500	FAAS	[76]
Lead and cadmium	Water, tea, human hair	TEA, 1000	GFAAS	[83]
Palladium	Water	TEA, 376	GFAAS	[84]
Vanadium	Water, food	Decanoic acid, 112,	GFAAS	[85]
Arsenic	Water	Diethylenetriamine, 1400	HG-AAS	[89]
Arsenic, selenium	Environmental water, liver	Sodium nonanoate, 5.4 (mg)	HG-AFS	[94]
Cobalt	Egg yolk and vitamin B12 pill	*N*,*N*-Dimethylbenzylamide, 500	SQT-FAAS	[78]
Cadmium	Environmental	*N*,*N*-Dimethylbenzylamine, 500	SQT-FAAS	[90]
Palladium	Water samples	*N*,*N*-Dimethylbenzylamine, 250	SQT-FAAS	[91]
Uranium	Environmental	TEA, 500	UV-Vis	[81]
Mercury	Environmental	DMCA, 500	UV-Vis	[82]

1.6.2 NSAIDs

Due to several side effects of NSAIDs, possible over-dose consumption, high stability in eco-system and possible accumulation in the environment, analytical chemists proposed numerous analytical methods to monitor NSAIDs and their metabolites in environmental, biological and food samples.

NSAIDs were extracted from several matrices using a variety of extraction techniques. These methods include LLE [112, 113], SPME using polymer monolithic column based on deep eutectic solvents which was connected directly to HPLC to determine NSAIDs in aqueous samples [114], HF-LPME to determine some NSAIDs and their metabolites during wastewater treatment [115], hyperbranched polyglycerol/graphene oxide nanocomposite (HBP/GO−HF-SLPME) to determine NSAIDs in hair and wastewater samples [116].

DLLME was combined with field-amplified sample stacking in capillary electrophoresis (FASS-CE) for the determination of NSAIDs in milk and dairy products [24], IL-DLLME was used to determine NSAIDs in tap and river water samples [117], as well as ultrasound-assisted emulsification microextraction after *in situ* derivatization of NSAIDs was used prior to GC-mass spectrometry (MS) analysis [118].

In a study, two extraction methods were combined [i.e., ultrasound-assisted dispersive micro-solid-phase extraction (UA-Dμ-SPE) and salting-out ultrasound-assisted liquid-liquid microextraction based on solidification of a floating organic droplet (S-UA-LLME-SFO)] to determine NSAIDs in wastewater, human urine and plasma samples [119].

Microextraction by packed sorbents (MEPS) was also applied to extract NSAIDs from human urine [120], besides, selective extraction with molecularly imprinted polymers (MIPS) [121], and magnetic solid-phase extraction (MSPE) [122].

1.7 Aim of This Study

This study aimed at expanding the applicability of switchable-hydrophilicity solvent liquid-liquid microextraction (SHS-LLME) throughout direct injection into the HPLC column, which eliminates centrifugation, back-extraction (BE) or evaporation-to-dryness (ETD). The proposed method was applied for the extraction and determination of four NSAIDs [i.e., ketoprofen (KET), etodolac (ET), flurbiprofen (FBP) and ibuprofen (IBU)] in biological fluids (i.e., milk, saliva and urine). Relevant experimental parameters affecting the method efficiency were systematically studied and optimized, which included the type and volume of the extraction solvent, volume of phase separator, volume of the aqueous phase, extraction and centrifugation time as well as type of sample introduction.

To the best of our knowledge, this is the first report on the application of SHS-LLME prior to HPLC for the determination of NSAIDs in biological fluids. Other novelties include the first attempt to combine salting-out extraction (SOE) with SHS-LLME in order to minimize the complexity of the biological samples and the introduction of the extract resulting from SHS-LLME directly into HPLC by online switching on.

CHAPTER 2

EXPERIMENTAL

2.1 Instrumentation

The experiments were performed using an Agilent Technologies 1200 series HPLC system (USA) equipped with a degasser, a quaternary pump, an autosampler, a column thermal jacket and a diode-array detector (DAD). ChemStation (Rev. B.03.01, Agilent Technologies, USA) was used for evaluating chromatograms. Separation of the analytes was carried out using a reversed-phase column (i.e., Grom-Sil 80 Octyl-4 FE, 4.6 mm ID x 250 mm, 3 μm, Alltech Grom, Germany), a mobile phase consisting of ACN:1.0% TFA (in aqueous solution), 40:60 (%, v/v) at pH* 1.4, a flow rate of 0.8 mL min^{-1}, a separation temperature of 40 °C and an injection volume of 20 μL. The DAD detector was operated at a wavelength of 230 nm during optimization of separation and extraction conditions for monitoring the analytes. However, for quantitation, the maximum wavelength (λ_{max}) of each analyte was used (i.e., KET 256 nm, ET 224 nm, FBP 246, and IBU 224 nm).

2.2 Reagents and Solutions

KET ($logP$ 3.61, pK_a 3.88) and ET ($logP$ 3.44, pK_a 4.73) were kindly provided by Nobel İlaç (Istanbul, Turkey), FBP ($logP$ 3.94, pK_a 4.42) and IBU ($logP$ 3.84, pK_a 4.85) were obtained from Abdi İbrahim İlaç Sanayi ve Tic. A.Ş. (Istanbul, Turkey). HPLC-grade acetonitrile (ACN) was acquired from VWR (France), *N*,*N*-dimethylcyclohexylamine (DMCA, $logP$ 1.99, pK_a 10.2) and sodium hydroxide were from Sigma-Aldrich (Germany), triethylamine (TEA, $logP$ 1.26, pK_a 10.2) was from Sigma-Aldrich (Belgium), sodium chloride was from Merck (Denmark), phosphoric acid was from Merck (Switzerland), and trifluoroacetic acid (TFA) was from Merck (France). All reagents were at least of analytical grade unless otherwise stated. Deionized (DI) water (18.2 MΩ-cm), obtained using Purelab Ultra Analytic (ELGA LabWater,

UK), was used for all aqueous solutions. $logP$ and pK_a values were calculated using MarvinSketch (Version 5.3.8, ChemAxon, USA).

2.3 Apparatus

Isolab digital ultrasonic bath (Germany) was used for ultrasonication and degassing of solvents. Centrifugation was performed with Hettich Eba 20 centrifuge (Germany), while vortexing was performed on a Heidolph Reax top Vortex. Eppendorf micropipette (Sigma-Aldrich, USA) and tips were used for sample collection and transfer, Borucam glass vacuum filtration set, Whatman (Germany) regenerated cellulose membrane filters (0.2 μm), and Chromfil (China) sterile nylon syringe filters (0.22 μm) were used for filtering the solvents and sample solutions. A Blomberg refrigerator was used for sample preservation. All pH measurements were performed using pH meter (Mettler Toledo, SevenEasy, Switzerland) with a pH electrode (InLab Micro Pro-ISM, Mettler Toledo, Switzerland).

2.4 NSAIDs Standard Solutions

Individual stock solutions of the four NSAIDs were prepared at a concentration of 1000 mg L^{-1} in ACN and stored at -15 °C until use. At each working session, mixed standard working solutions were prepared from the stock solutions by appropriate dilution with DI water. Filtration of all solutions and samples was carried out before use via vacuum filtration.

2.5 Synthesis of SHSs

Three SHSs were prepared using TEA, DMCA and a mixture of both at a 1:1 (v/v) ratio. Equal volumes of DI water and the tertiary amine were transferred into a screw-capped glass bottle, and then CO_2 was gently purged into the immiscible mixture through a capillary Teflon tube passing from the cap, which had another hole to prevent pressure build-up in the bottle. Purging of the gas was stopped when a transparent one-phase solvent was obtained, which was stored at 4 °C and used on need. These solvents were stable as one phase for at least four months.

2.6 Sample Collection and Pretreatment

Human milk samples (around 50 mL) were collected from a 30-year old healthy volunteer, who had breastfed for twelve months. Saliva and urine samples were obtained from a 25-year old healthy male volunteer. The volunteers were instructed not to use any NSAID for at least one week and to discard the first few milliliters of the milk and urine before collection. The volunteer was asked to wash his mouth with DI water three times before collecting the saliva. All samples were analyzed on the same day as they were collected.

Ethical approval was obtained from Near East University Ethical Committee (Project Number: YDU/2019/67-771) to evaluate the applicability of the proposed method, and then the volunteers were asked to use the drugs and samples were collected for genuine samples studies as described above.

2.7 Salting-Out Extraction (SOE)

The samples were transferred into a 15-mL screw-cap conical centrifuge graduated polypropylene test tube, spiked with prescribed concentrations of the NSAIDs and were vortexed for one minute before they were allowed to rest for 15 min in a dark place for equilibration to take place. Next, the mixture was centrifuged for 15 min at 6000 rpm at ambient temperature, and then 2.0 mL of the clear transparent solution were transferred into another test tube. Then, 100 µL of phosphoric acid were added, and the mixture was vortexed for 1 min, followed by the addition of 4.0 mL of ACN and 1.0 mL of saturated sodium chloride. Upon vortexing (for 1 min) and centrifugation (for 3 min at 6000 rpm), the upper layer of ACN (c. 3.5 mL) was collected.

2.8 SHS-LLME

A portion of the ACN resulting from the SOE (i.e., 3.0 mL) was transferred into a test tube, 500 µL of the switched-on DMCA were added, and the volume was completed to 13.0 mL with

DI water. The addition of 500 µL of 20 M sodium hydroxide immediately switched off the SHS, resulting into the formation of a cloudy solution. Vortexing the mixture for 30 s enhanced the extraction of the analytes into the SHS. Within about 1 min, phase separation occurred, resulting into a supernatant layer of the switched-off DMCA without the need for a centrifugation step. A representative portion of the switched-off DMCA was directly injected into HPLC without any further pretreatment.

2.9 Sample Introduction into HPLC

A portion of 100 µL of the amine layer resulting from SHS-LLME was collected using an HPLC syringe and 20 µL injected directly into HPLC after transferring it into a microvial without any further pretreatment except while studying the BE and ETD. A schematic presentation of the general SHS-LLME procedure with direct injection into HPLC is given in **Figure 2.1.**

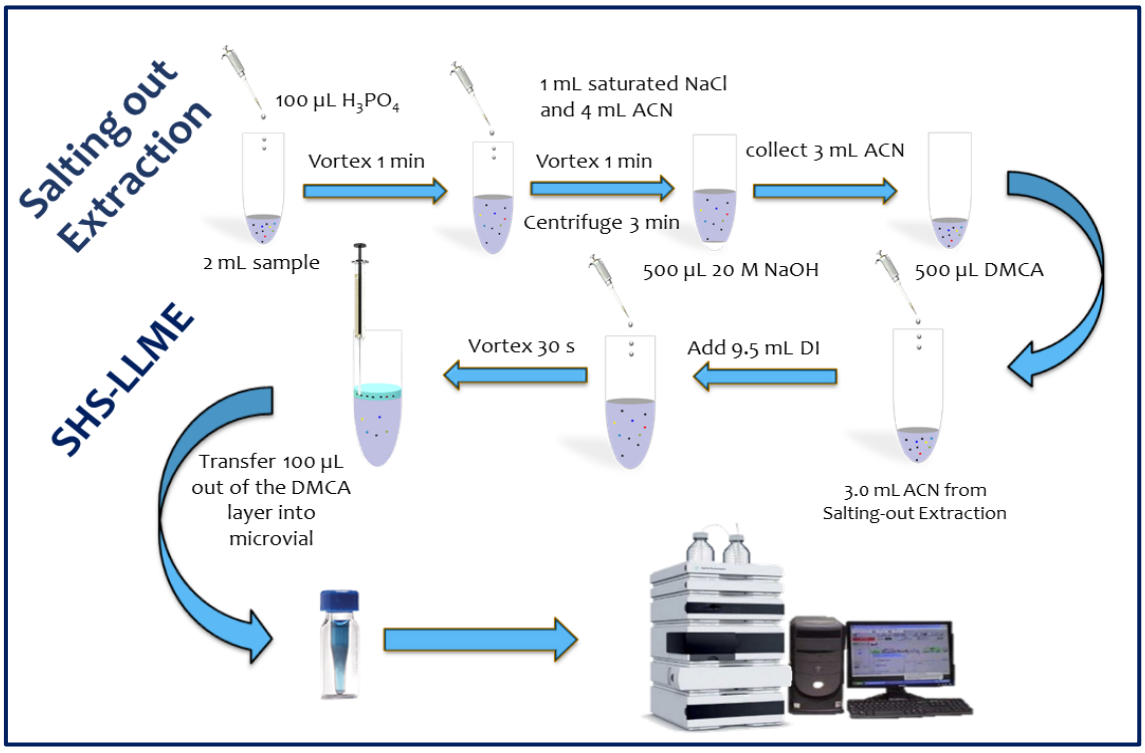


Figure 2.1 General SHS-LLME procedure.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Optimization of HPLC Conditions

HPLC parameters were optimized in a previous work at our laboratory [123], the optimum conditions are summarized in table **Table 3.1**.

Table 3.1 Optimum HPLC conditions.

Column	Grom-Sil 80 Octyl-4 FE, 4.6 mm ID x 250 mm, 3 μm
Flow rate	0.8 mL min^{-1}
Temperature	40 °C
Detector/ Wavelength (for monitoring all analytes)	DAD. 230 nm (BW 4). Reference 360 nm (100 BW)
Injection volume	20 μL
Mobile phase	ACN:1.0% TFA, 40:60 (%, v/v)
pH*	1.4

3.2 Sample Pretreatment

The complexity of biological fluids and the presence of the analytes at trace or even ultra-trace concentrations necessitates the use of an efficient sample clean-up and preconcentration method prior to the determination to minimize matrix effect, increase selectivity, prevent damage to the instrument and to increase sensitivity. Also, the removal of significant interferences from the sample would result in proper control of the composition of the final extract, which improves reproducibility and robustness of the method. Furthermore, it is highly desirable to have a single extraction procedure that would be applied to a wide variety of samples (e.g., urine, saliva, milk) with minimum, if any, modifications due to the matrix-to-

matrix difference (e.g., ionic strength or fat content). In this study, SHS-LLME combined with a simple SOE step is proposed to fulfill these requirements as mentioned earlier.

SOE with ACN is a homogeneous liquid-liquid extraction method that involves a simple one-step solvent extraction of analytes from biological samples (i.e., urine, saliva and milk) followed by salting-out the water-miscible organic solvent by adding an inorganic salt like sodium chloride. The use of ACN to induce protein precipitation is a simple and effective approach routinely employed in clinical and biomedical laboratories for sample clean-up [124]. Meanwhile, the addition of inorganic salt for salting-out may both greatly enhance the extraction efficiency of the analytes from the aqueous solution and further simplify the matrix. SOE has the advantages of high extraction rate, good reproducibility and simple operation process [125].

In any method development process, the preliminary experiments are of high importance. In order to minimize the time and effort during optimization, physicochemical properties of the analytes should be taken into consideration. The first physical property of the studied NSAIDs to be checked was the $logP$ value (**Table 1.1**), which ranged between 3.44 and 3.94. These values indicate that the studied analytes have intermediate to low polarity; they were taken into consideration while choosing the extraction solvents.

Although polarity has a high impact on choosing the solvents and preliminary conditions, it is not enough alone since NSAIDs are weak acids. Accordingly, ionization and pK_a values should also be considered. The microspecies distribution of the analytes was calculated using MarvinSketch application to be able to build the preliminary experiment method. The microspecies distribution of the analytes versus pH are shown in **Figure 3.1**, **Figure 3.2**, **Figure 3.3** and **Figure 3.4**.

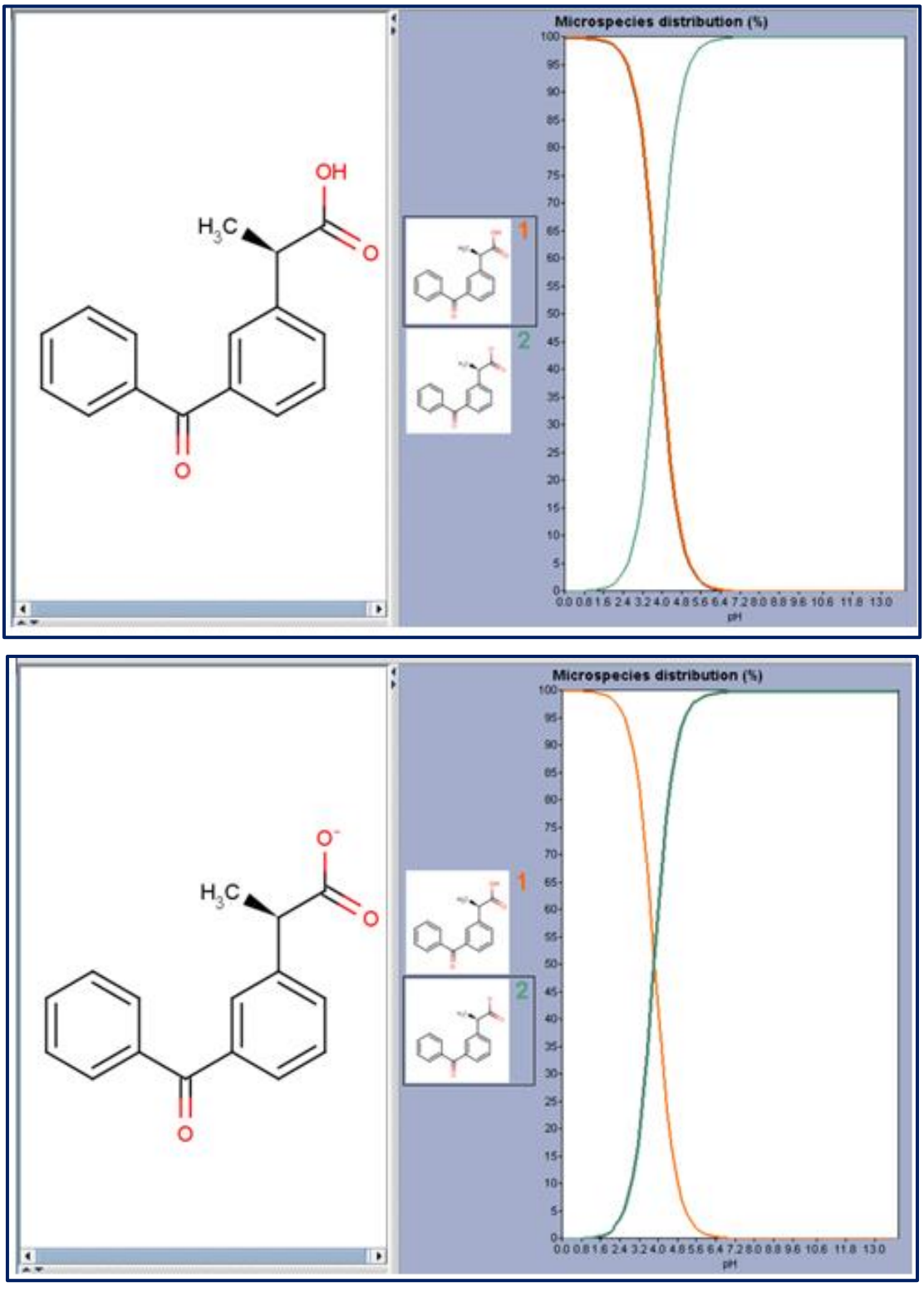


Figure 3.1 Microspecies distribution of KET.

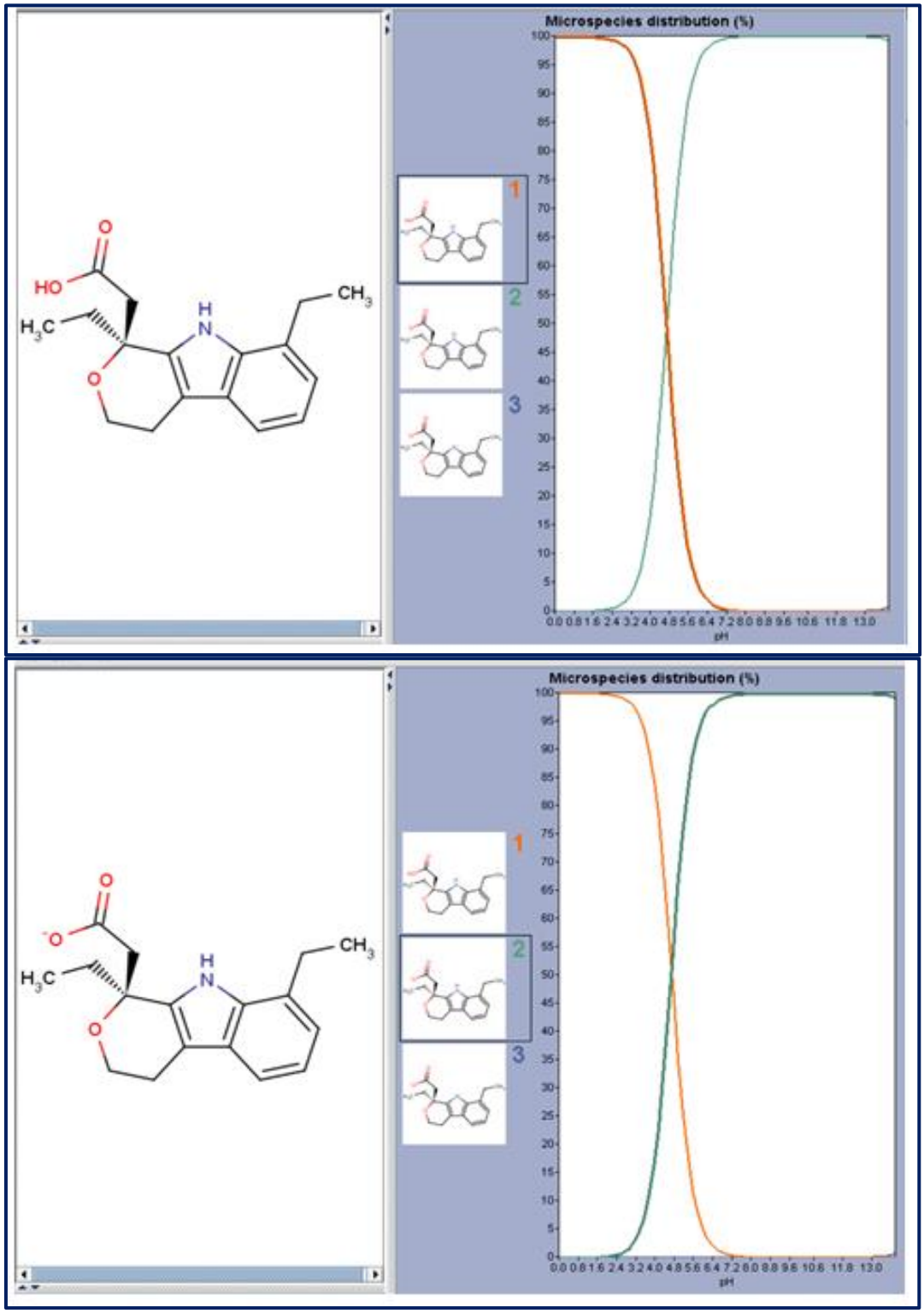


Figure 3.2 Microspecies distribution of ET.

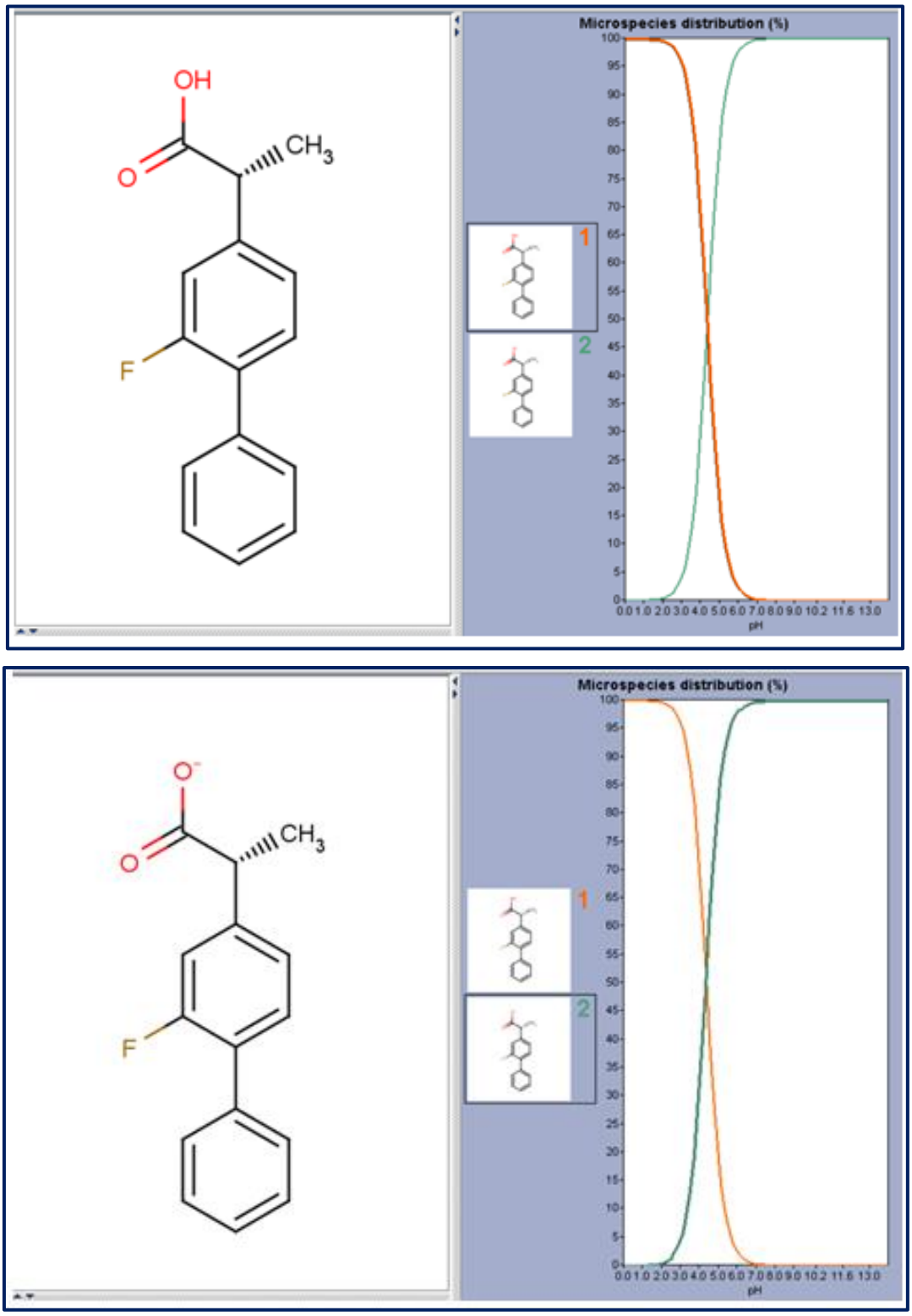


Figure 3.3 Microspecies distribution of FBP.

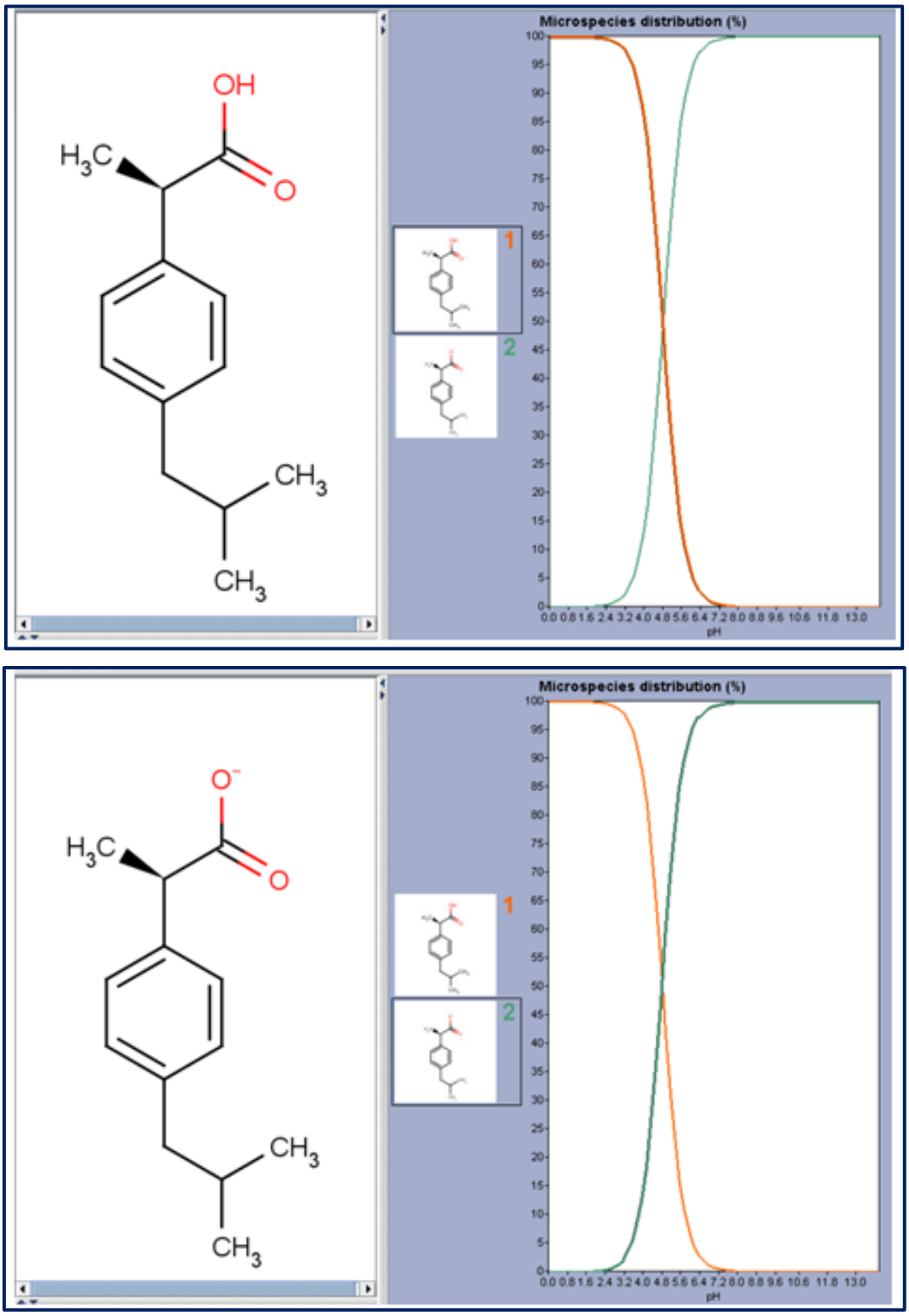


Figure 3.4 Microspecies distribution of IBU.

As noticed from the microspecies distribution (**Figure 3.1**, **Figure 3.2**, **Figure 3.3** and **Figure 3.4**) of the four NSAIDs, all of them may be present either in the neutral or ionized form, except for ET, which has a third form but with a negligible amount (< 1%). The first is generally dominant at pH below 4, whereas the ionized form starts to be dominant at higher pH. Accordingly, if the solutions containing these NSAIDs acidified, it would make the neutral form dominant for a better interaction with the reversed-phase stationary phase resulting in better resolution. The information revealed by the microspecies were also used in the SOE. Through acidifying the sample solution with 100 µL phosphoric acid, the neutral form was dominant, which increased the affinity of the analytes toward ACN. In addition, deproteinization of the samples was enhanced.

The high fat, proteins and lipids content in biological fluids, particularly milk samples, can be lowered noticeably by simply centrifuging the sample before subjecting it to SOE, as shown in **Figure 3.5**. Hence, centrifugation for 15 min at 6000 rpm was applied before SOE.

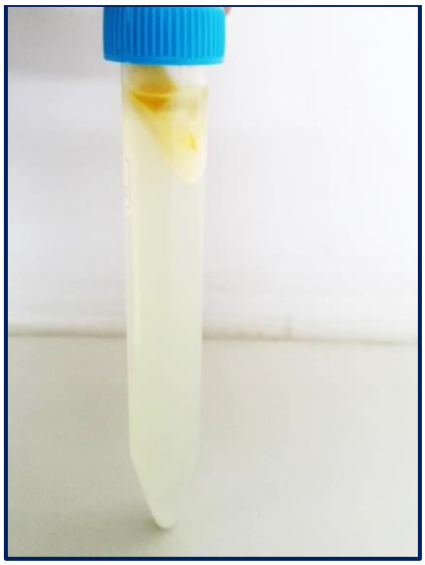


Figure 3.5 Milk sample after centrifugation at 6000 rpm for 15 min.

3.3 Salting-Out Extraction (SOE)

SOE can fulfill GAC aspects, more than most other sample pretreatment techniques that either use larger amounts of organic solvents, have longer operation times or need expensive and special apparatus (**Table 3.2**). Using SOE is superior from a GAC perspective due to the following reasons:

1. Elimination of tedious steps.
2. Lower energy consumption.
3. Less consumption of organic solvents.
4. High safety.
5. Low possibility of contamination, decomposition or analyte loss.
6. Eco-friendliness.
7. Low-cost.

Table 3.2 Comparison of greenness issues of common sample preparation techniques.

Extraction method	**Greenness**				**Need for special equipment**	**Ref.**
	Time	**Energy**	**Safety**	**Solvent**		
Soxhlet extraction	6-24 h	High consumption	Exposure risk to organic vapors	150-500 mL of organic solvents	Yes	[32]
Microwave-assisted extraction	10-30 min	Moderate consumption	Potential explosion risks with closed vessels	10-40 mL of organic solvents	Yes	
Supercritical-fluid extraction	10-60 min	Moderate consumption	Unsafe (high pressure and temperature)	2-5 mL (solid trap); Supercritical CO_2 is used as an extraction solvent	Yes	
Accelerated solvent extraction	10-20 min	Moderate consumption	Unsafe (high pressure and temperature)	10-40 mL of organic solvents	Yes	
Ultrasound-assisted extraction	<1 h	Moderate consumption	Safe; extractions performed at atmospheric pressure and room temperature	Medium volume	Yes	
SOE	<5 min	Low consumption	Safe; extractions performed at atmospheric pressure and room temperature	4 mL of organic solvent	No	This study

In addition, SOE shows high defattening and deproteinization effect, unlike other some sample pretreatment techniques as can be seen from **Figure 3.6**. Among other solvents, ACN is the most commonly used solvent in SOE for this purpose [126, 127].

Using a centrifugation step before SOE, the SOE step itself, and using ACN results into a significant sample clean-up of biological samples. In the past, this could be achievable by using tedious conventional extraction methods that needed special apparatus and/or toxic non-polar solvents such as n-hexane.

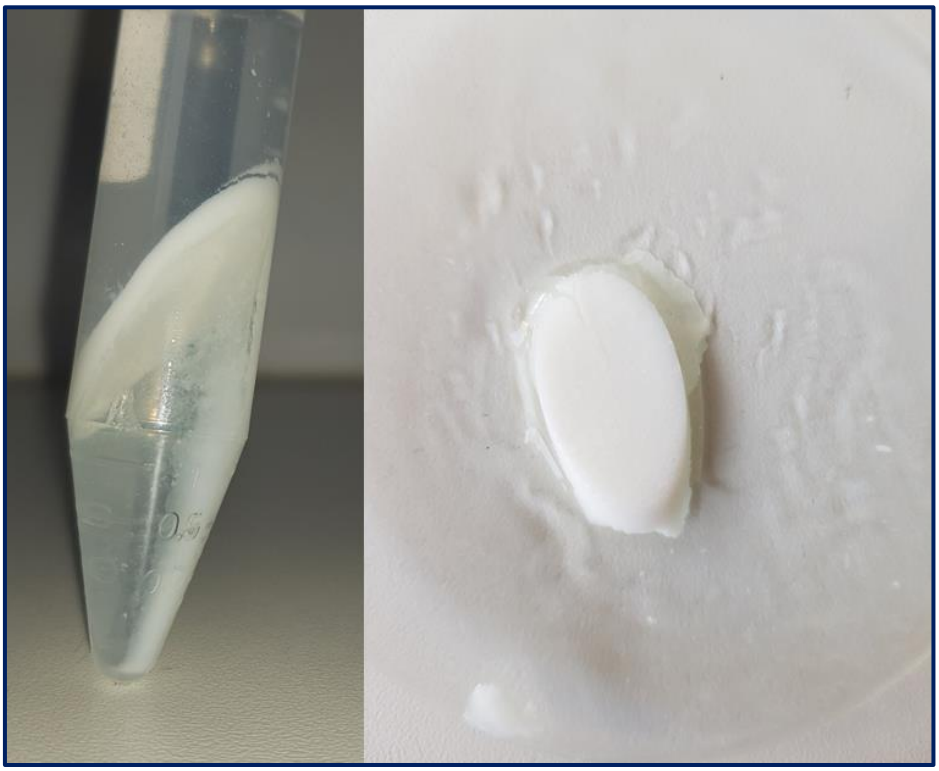


Figure 3.6 Clean-up of milk sample with SOE.

In addition to all above-mentioned advantages of using SOE before SHS-LLME, it is worth mentioning that SHS-LLME would be difficult to perform with biological samples if the SOE step is eliminated, since the organic extraction solvent would be saturated with fats, lipids, and proteins coming from the sample, as shown **Figure 3.7**. Even more, combining SOE with SHS-LLME follows the current trend in bioanalysis that using two extraction steps, where the first

is a simple technique intended mainly for clean-up, while the second is a microextraction technique for preconcentration of the analyte(s) and further clean-up.

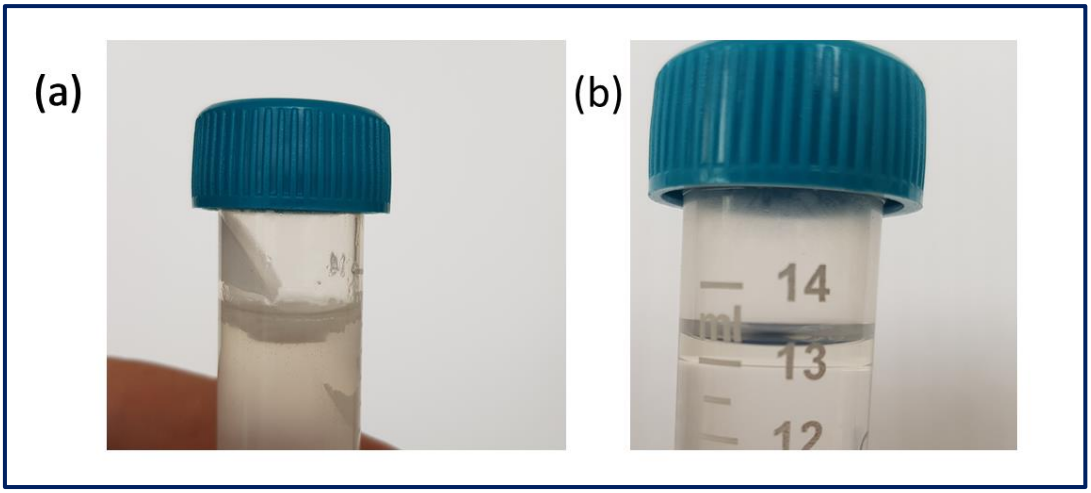


Figure 3.7 Milk sample after (a) SHS-LLME, (b) SOE-SHS-LLME.

In conclusion, the centrifugation step can enhance the sample clean-up, acidifying the sample solution before starting with SOE would increase the recovery of the analytes. Furthermore, the use of SOE before SHS-LLME is necessary to have a "cleaner" extract that can be injected into HPLC.

3.4 Optimization of SHS-LLME Parameters

The most influential parameters on SHS-LLME, which included the type and volume of the extraction solvent, volume of sodium hydroxide as a switching-off trigger, volume of the aqueous phase, addition of n-hexane, extraction time, centrifugation time and type of sample introduction into the instrument were all studied in detail and optimized. Peak areas were used to evaluate the impact of each parameter on the extraction efficiency of the method using the one-factor-at-a-time approach. Optimization of SHS-LLME conditions was performed using spiked human milk samples.

3.4.1 Optimization of the Type and Volume of Extraction Solvent

The selection of an appropriate SHS as the extraction solvent should consider specific properties such as: (a) the solvent should have extractive tendency towards the target analytes, (b) it must have two forms (hydrophilic and hydrophobic) that are interconvertible by the addition or removal of CO_2 from the system, and (c) as hydrophobic solvents, they should be immiscible with water in their neutral unprotonated form (switched-off) and, when CO_2 is used as a switching trigger, they should be converted into their water-miscible protonated forms (switched-on) to establish a stable two-phase system [128]. Tertiary amines, including TEA and DMCA, are ideal solvents for SHS-LLME and are the most commonly used ones for this purpose [103, 105].

In order to evaluate the effect of the type of SHS, TEA, DMCA, and a mixture of both at a 1:1 (v/v) ratio were studied. It was observed that DMCA gave the highest peak area, in average, for the four analytes (**Figure 3.8**), which might be due to higher hydrophobicity of DMCA as compared to TEA as revealed by their $logP$ values (**2.2**).

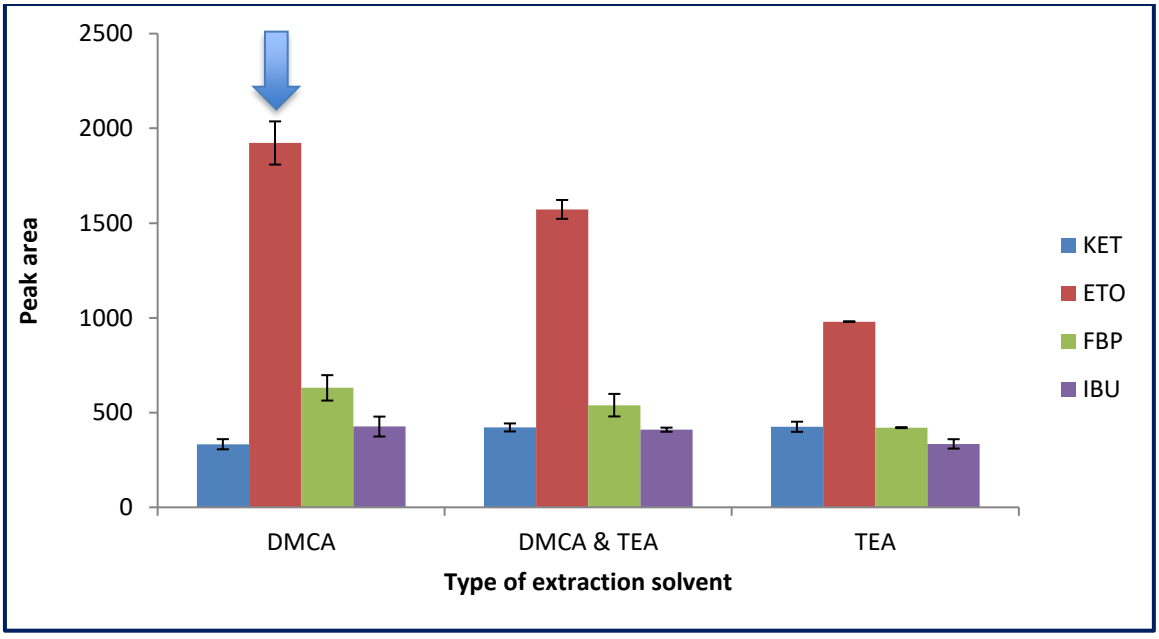


Figure 3.8 Effect of the type of SHS used as extraction solvent in SHS-LLME.

The volume of the extraction solvent can directly affect the volume of the resulting SHS phase and analyte recovery. Different volumes of the switched-on DMCA (i.e., 200, 250, 500, 750, 1000 and 1250 µL) were studied. As shown in **Figure 3.9**, 500 µL was found to be optimum. Beyond this volume, peak areas generally decreased due to the increase of the supernatant volume.

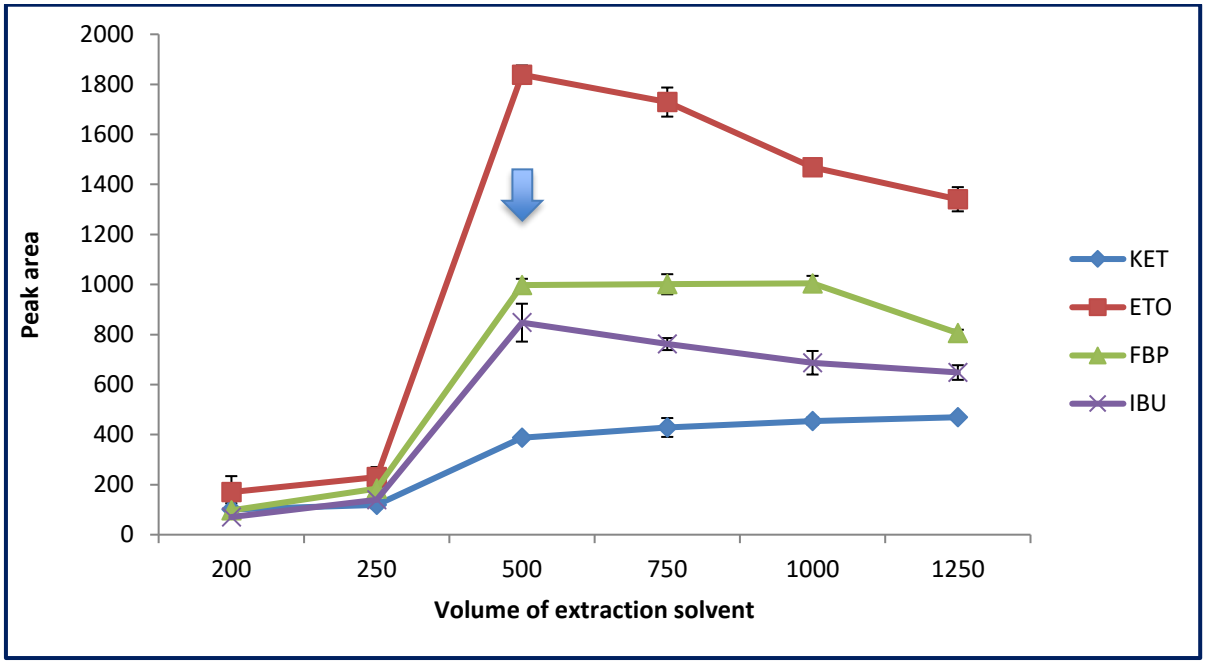


Figure 3.9 Effect of the volume of extraction solvent in SHS-LLME.

3.4.2 Optimizing the Volume of Sodium Hydroxide as a Switching-Off Trigger

The addition of high concentration of sodium hydroxide (e.g., 20 M) to the homogeneous extraction system is a crucial step in SHS-LLME, which was necessary to induce phase separation through switching off the tertiary amine via removal of CO_2. Another important aspect is that such a high concentration would increase the ionic strength in the donor phase significantly minimizing the effect of ionic matrix-to-matrix difference. Different volumes of 20 M sodium hydroxide ranging from 300 to 700 µL were tested. As shown in **Figure 3.10**,

there was a slight increase in peak area up to 500 µL, beyond which it remained constant except for ET. Hence this volume was considered optimum for subsequent experiments.

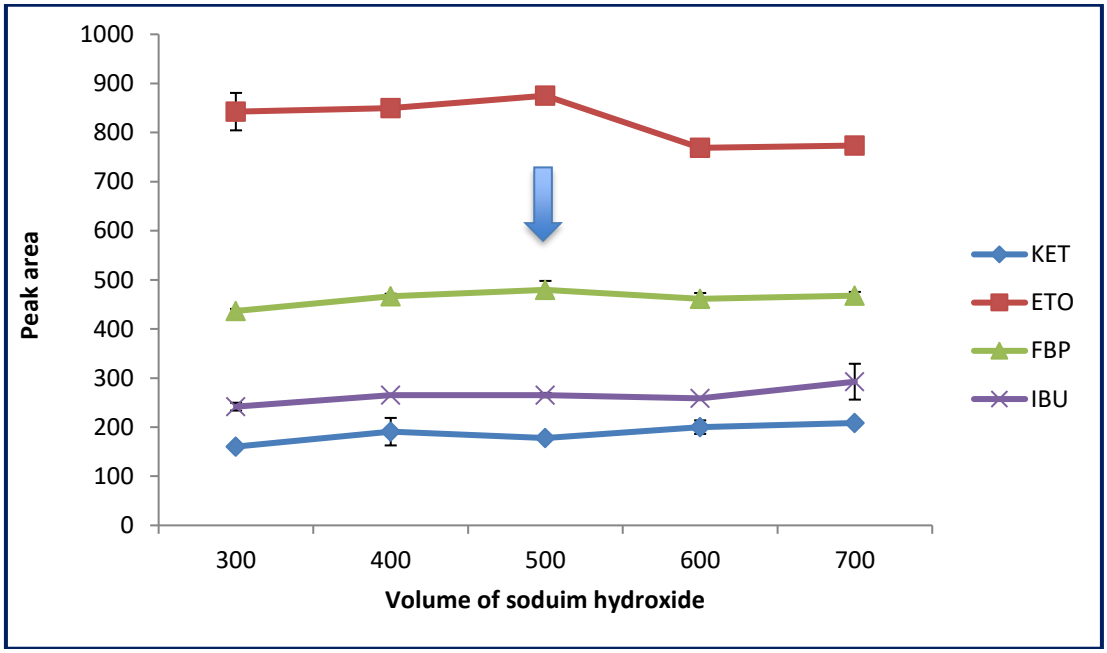


Figure 3.10 Effect of the volume of sodium hydroxide used as switching-off trigger in SHS-LLME.

3.4.3 Optimization of the Volume of the Aqueous Phase

Addition of water to ACN resulting from the SOE step (**Section 2.7**) was necessary for triggering off the SHS to take place since sodium hydroxide (20 M) would be immiscible with ACN containing the switched-on SHS. However, the volume of water in the donor solution would also affect the extraction efficiency from several points of view. It would change the polarity of the donor solution, the donor-to-acceptor ratio, and solubility of the switched-off SHS. Furthermore, due to its high concentration, sodium hydroxide would have a salting-out effect on the analytes, the switched-off SHS and ACN, which is eventually affected by the volume of water.

The overall effect of adding different volumes of water on extraction efficiency was investigated within the range of 2.5 to 10.5 mL. As can be seen from **Figure 3.11**, peak areas generally increased up to 9.5 mL, after which they decreased. Consequently, 9.5 mL was taken as the optimum volume for water in the donor phase.

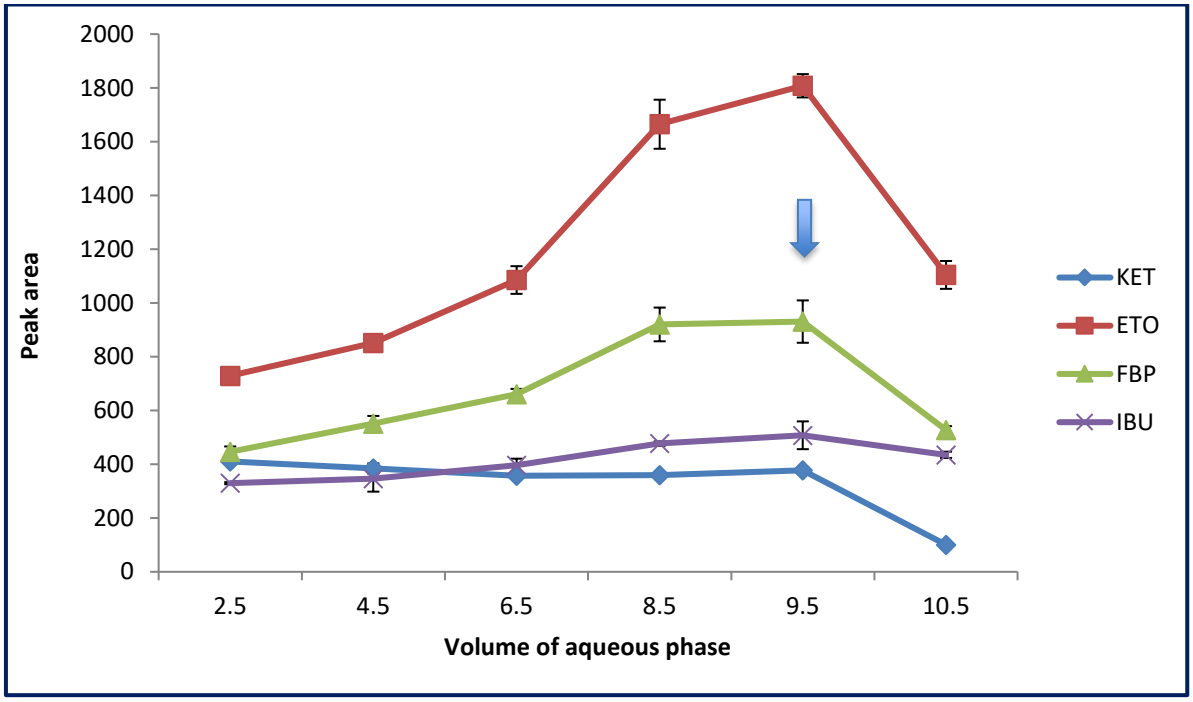


Figure 3.11 Effect of the aqueous phase in the SHS-LLME.

3.4.4 Effect of Addition of n-Hexane

The low polarity of conventional organic solvents used in DLLME, when applied for the extraction of analytes from biological samples, generally necessitates the use of *n*-hexane as a defattener solvent for a better sample clean-up, which decreases the greenness of the method and complicates the procedure.

Up to this point, *n*-hexane was added for the same purpose. In this experiment, the effect of removing *n*-hexane from the procedure was examined. It was found that neither peak areas

(**Figure 3.12**) nor the chromatograms were affected. Based on these results, the experiments were continued without the addition of *n*-hexane in later experiments.

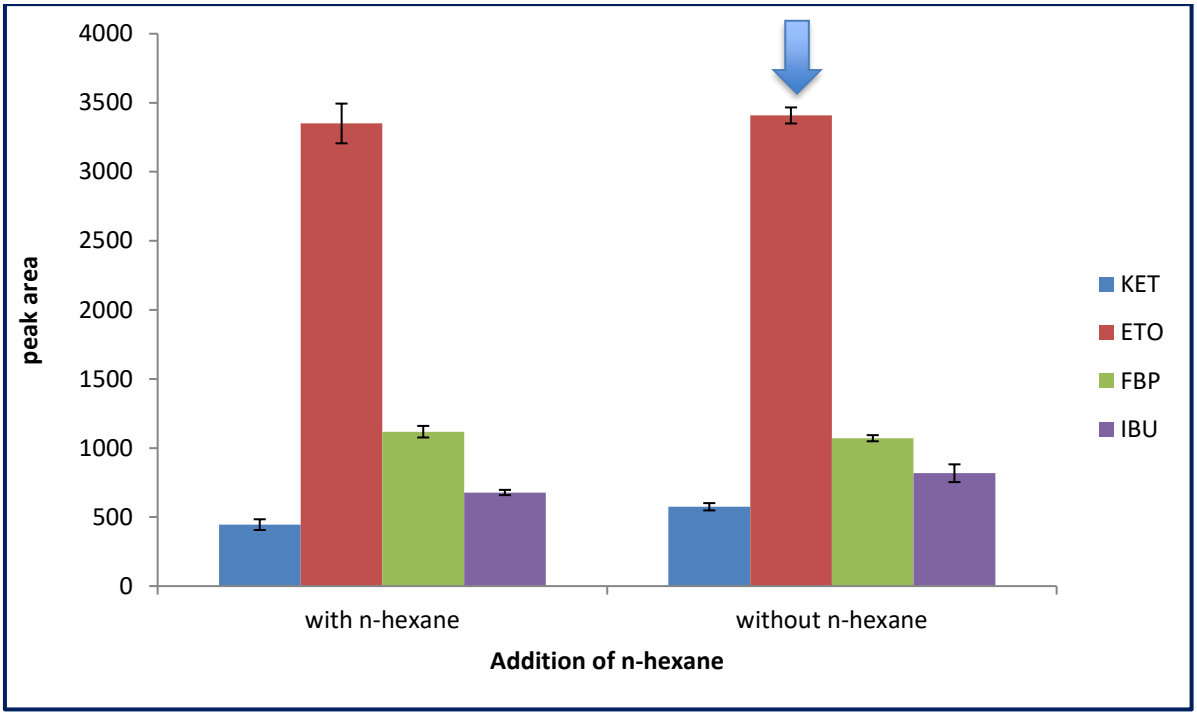


Figure 3.12 Effect of addition of n-hexane prior to SHS-LLME.

The high clean-up efficiency obtained in this method was linked to (i) the use of ACN deproteinization through SOE, in addition to SOE itself (ii) the use of 20 M sodium hydroxide, and (iii) the higher polarity of the SHSs used in this study. This significant sample clean-up can be considered as one of the main advantages of the proposed method and further contributes to its greenness.

3.4.5 Effect of Extraction Time

In SHS-LLME, extraction time (or period) can be defined as the time interval between the addition of sodium hydroxide and phase separation, which corresponds to the vortex time in this study. Vortex assistance can enhance extractability of the analytes by increasing the

probability of collision between sodium hydroxide and the switched-on SHS as well as between the switched-off SHS and the analytes.

The effect of extraction time was examined in the range of 0–60 s. The results, shown in **Figure 3.13**, indicated that extraction efficiency increased up to 30 s, beyond which it remained constant. In SHS-LLME, expectedly, equilibrium should be reached very rapidly since it is a homogeneous extraction, whereby the surface area of contact between the extraction solvent and the analytes is infinitely large. Considering these results, 30 s was considered optimum for extraction time.

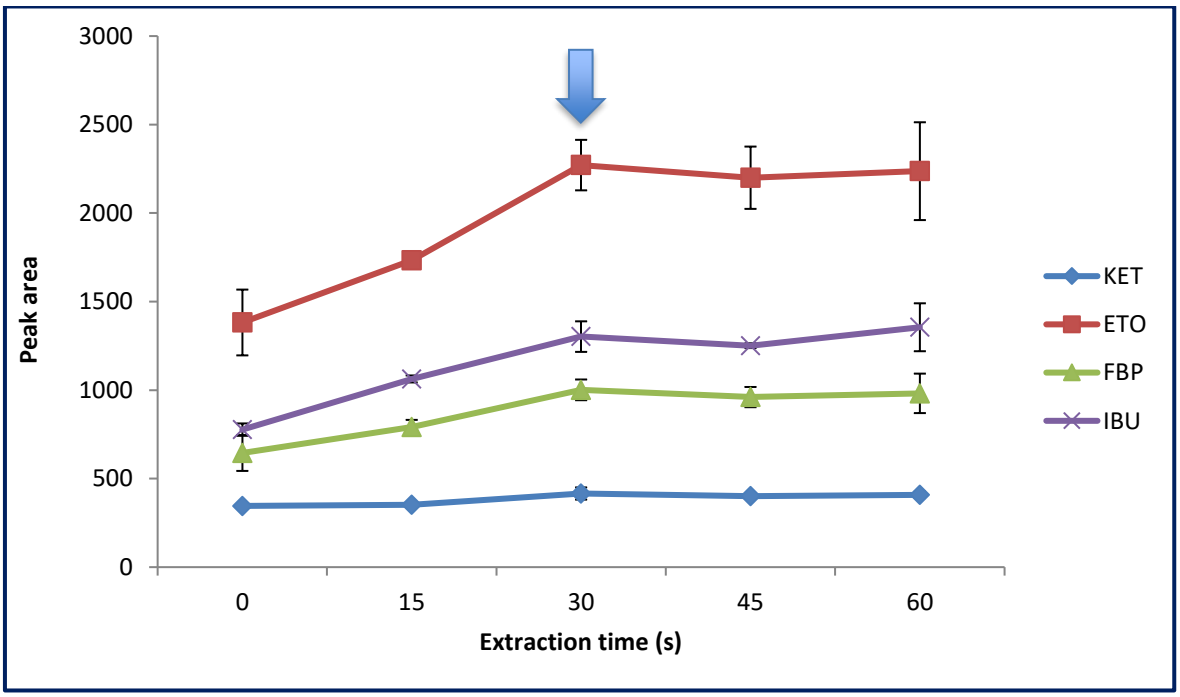


Figure 3.13 Effect of extraction time in SHS-LLME.

3.4.6 Effect of Centrifugation Time

Many LLME techniques (e.g., DLLME) require centrifugation to separate the donor phase from the acceptor phase, especially when further sample preparation is needed like BE or ETD.

This centrifugation step was always considered as one of the main limitations of those methods due to more prolonged procedures and difficulty in automation and *in-situ* analysis.

In this experiment, optimization of the centrifugation time was studied using direct injection of the acceptor phase as a sample introduction technique. Therefore, it was not necessary to collect all of the acceptor phase. Moreover, peak areas were not affected by the centrifugation time even when the centrifugation step was eliminated entirely (**Figure 3.14**). Accordingly, the rest of the optimization studies and quantitation experiments were performed without centrifugation for direct injection. It was only used when BE and ETD were investigated in the sake of comparison with direct injection.

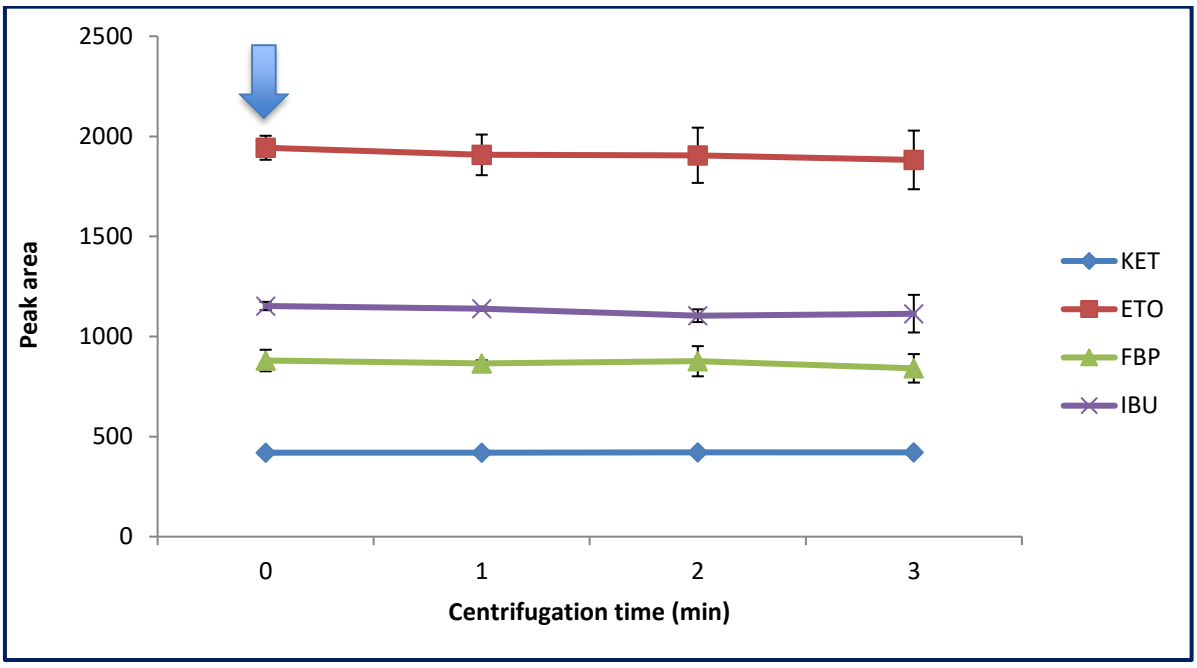


Figure 3.14 Effect of centrifugation time on SHS-LLME.

3.5 Type of Sample Introduction

Sample introduction into HPLC is a critical step in the analysis because different variables should be taken into consideration; one of the most important being the miscibility of the final

extract with the mobile phase. When the final extract is immiscible with the mobile phase, BE or ETD are generally applied. However, these two steps are time-consuming, tedious, cost-ineffective and may result in analyte loss. Therefore, it is highly desirable that the final extract be miscible with the mobile phase for direct injection to be applied.

The presence of TFA in the mobile phase [i.e., 40:60 (a:b, v/v) at pH* 1.4] immediately switched on the SHS (pK_a values are given in **2.2**) to its ionized form upon injection, resulting in complete miscibility of the SHS and reduced the retention time significantly (close to the solvent front), which prevented any peak overlap with the analytes and gave a "clean" baseline. It is noteworthy that DMCA and TEA absorbed up to 240 nm.

BE and ETD were also conducted for a comparison purpose. Even though BE was fast, low recoveries were obtained due to the low solubility of the analytes in the BE solution (i.e., 60 µL of 1.0 M NaOH). ETD not only resulted in low recoveries due to loss of analytes but was also tedious and time-consuming (ca. 200 µL of DMCA evaporated in approximately 40 min). After ETD, the residue was reconstituted into 200 µL of the mobile phase. Therefore, the superiority of direct injection over the other two methods is that direct injection provides the highest possible recovery without any further pretreatment step. Also, unlike for the other two methods, there is no need to collect all of the upper acceptor phase, which is a tedious step and requires centrifugation. A representative volume was enough for direct injection. Extraction recoveries (shown in terms of peak areas) obtained with the three methods are shown in **Figure 3.15**.

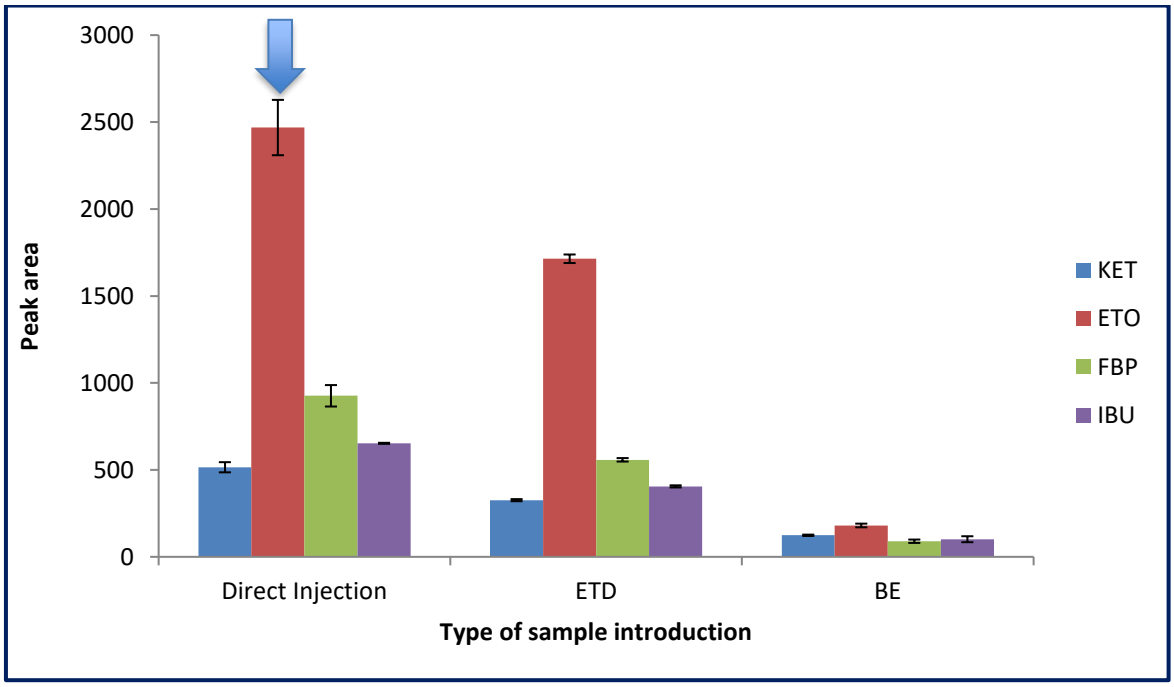


Figure 3.15 Effect of the type of sample introduction.

Miscibility of the two SHSs used in this study (i.e., DMCA and TEA) with different mobile phases containing ACN or MeOH with and without 1.0% TFA was investigated using a 1:1 (v/v) ratio in a test tube. Centrifugation was also performed to confirm miscibility. As shown in **Figure 3.16**, miscibility of the SHSs increased with increasing the organic solvent in the mobile phase. Addition of TFA to the mobile phase expanded the range of miscibility due to switching-on of the SHS. TEA was found to be miscible over a wider range of mobile phase compositions than DMCA. ACN and MeOH showed similar ranges of miscibility with both SHSs.

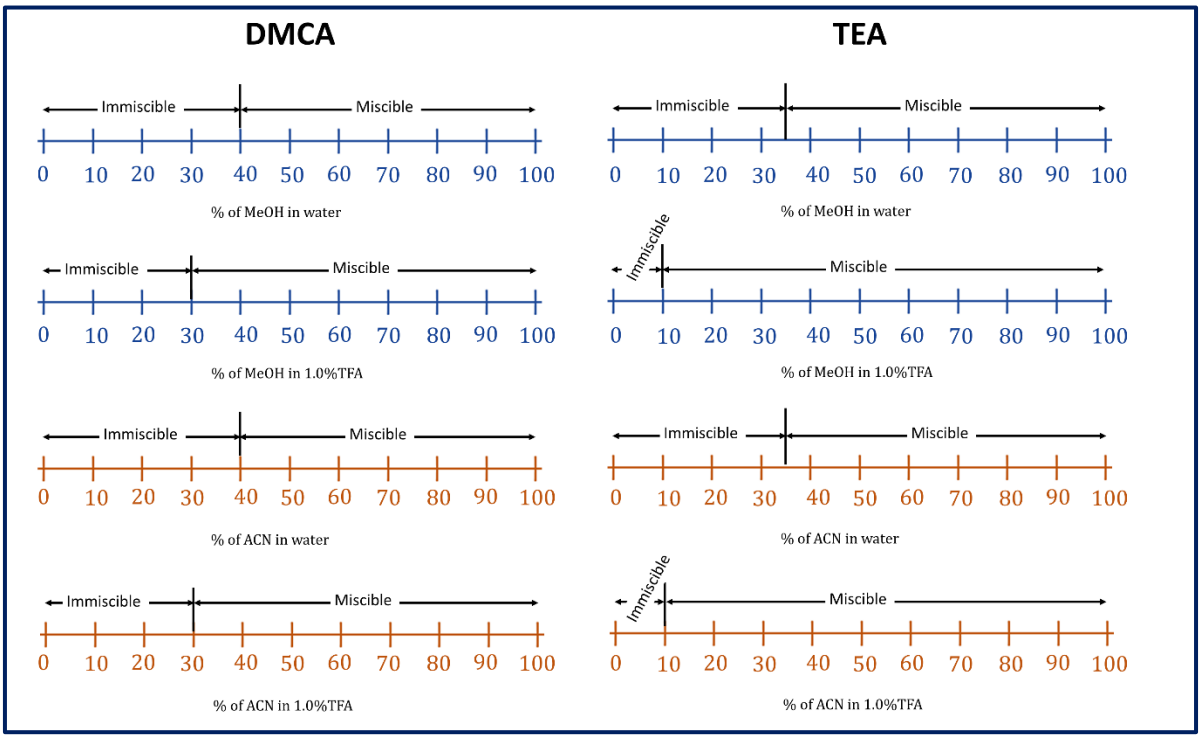


Figure 3.16 Miscibility of SHSs used in this study with different mobile phases. The ratio of SHS to the mobile phase was 1:1 (v/v).

3.6 Optimum SHS-LLME Conditions

The optimum SHS-LLME parameters in this study are summarized in **Table 3.3**.

.

Table 3.3: Optimum SHS-LLME conditions.

Extraction solvent	DMCA
Volume of extraction solvent	500 µL
Volume of sodium hydroxide	500 µL
Volume of the aqueous phase	9.5 mL
Extraction time	30 s

3.7 Analytical Performance

In order to evaluate the performance of the proposed method, matrix-matched calibrations were constructed using drug-free saliva (**Figure 3.17**), milk (**Figure 3.18**) and urine (**Figure 3.19**) samples and spiking the samples at concentration levels of 1.0, 2.0, 3.0, 4.0 and 5.0 μg mL^{-1} for each analyte, with three parallel replicates at each level. Analytical performance parameters including regression equations, coefficients of determination (R^2), limits of detection (LOD), limits of quantitation (LOQ), linear dynamic ranges (LDR), and percentage relative standard deviations (%RSD) are listed in **Table 3.4**.

Table 3.4 Figures of merit of SHS-LLME-HPLC.

Matrix	NSAID	Regression Equation[a]	R^2	LOD[b] (µg mL^{-1})	LOQ[c] (µg mL^{-1})	LDR[d] (µg mL^{-1})	%RSD[e]	
							Intraday	Interday
Saliva	KET	$y = 69.05(\pm 0.524)x - 2.91(\pm 1.59)$	0.9994	0.06	0.22	0.22-100	1.3	2.1
	ET	$y = 245.23(\pm 3.94)x - 16.39(\pm 11.94)$	0.9974	0.14	0.46	0.46-100	3.3	6.5
	FBP	$y = 144.74(\pm 0.70)x - 3.76(\pm 2.12)$	0.9998	0.04	0.14	0.14-100	0.9	1.5
	IBU	$y = 67.41(\pm 1.14)x + 6.17(\pm 3.46)$	0.9971	0.14	0.48	0.48-100	4.2	7.7
Urine	KET	$y = 81.34(\pm 0.37)x + 2.06(\pm 1.12)$	0.9998	0.04	0.13	0.13-100	0.9	1.4
	ET	$y = 318.80(\pm 4.75)x + 32.81(\pm 14.39)$	0.9978	0.13	0.42	0.42-100	3.4	6.3
	FBP	$y = 171.98(2.47)x + 17.43(7.49)$	0.9979	0.12	0.41	0.41-100	2.5	3.5
	IBU	$y = 68.62(1.47)x + 7.17(4.44)$	0.9955	0.18	0.61	0.61-100	3.7	7.3
Milk	KET	$y = 76.63(\pm 0.60)x + 4.27(\pm 1.80)$	0.9994	0.07	0.22	0.22-100	1.7	3.7
	ET	$y = 311.29(\pm 4.23)x + 24.35(\pm 12.81)$	0.9982	0.12	0.39	0.39-100	2.8	5.9
	FBP	$y = 167.62(\pm 2.41)x + 9.78(\pm 7.31)$	0.9979	0.12	0.41	0.41-100	2.4	4.2
	IBU	$y = 64.37(\pm 1.33)x + 9.17(\pm 4.02)$	0.9958	0.18	0.59	0.59-100	3.4	5.4

[a] $Peak\ area\ =\ slope(\pm SD) \times\ [NSAID\ concentration\ (\mu g\ mL^{-1})] +\ intercept(\pm SD)$.

[b] Limit of detection.

[c] Limit of quantitation.

[d] Linear dynamic range.

[e] Percentage relative standard deviation ($n = 3$).

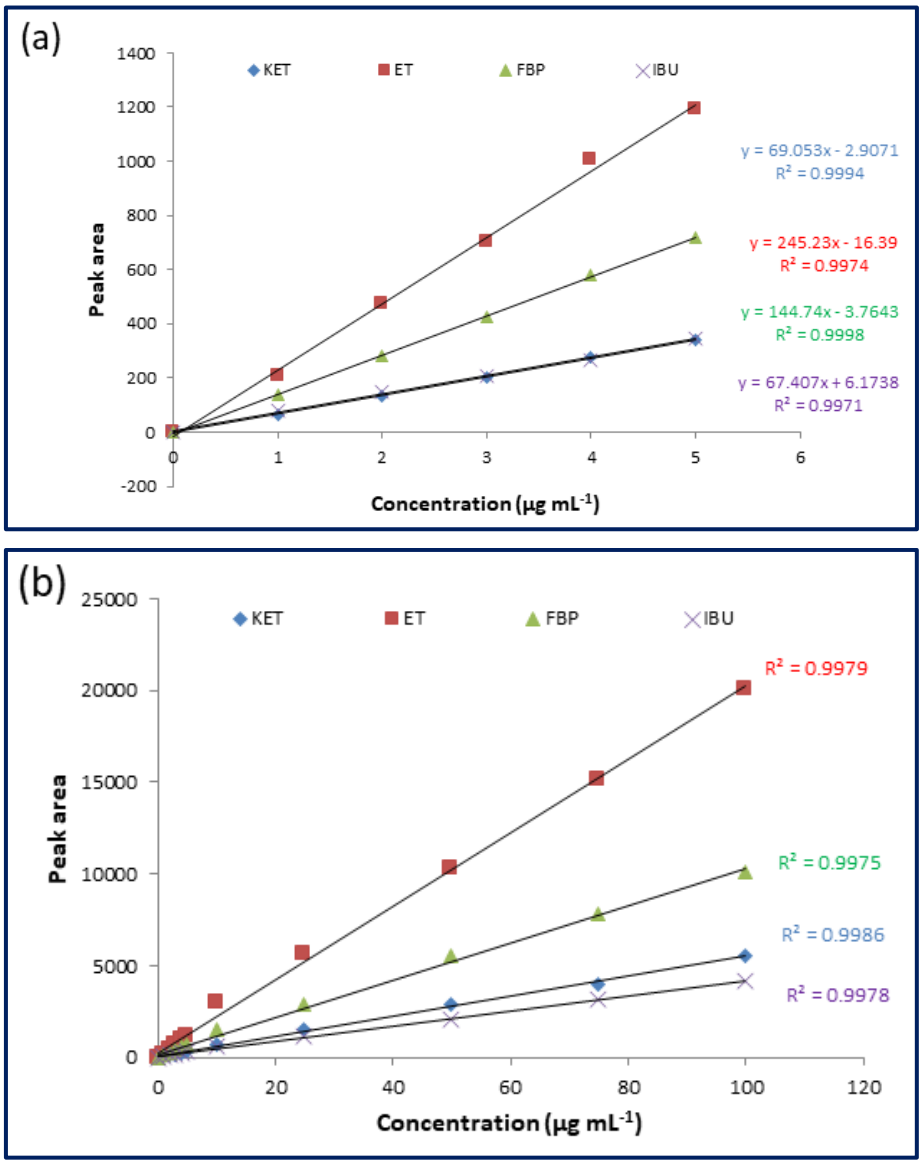


Figure 3.17 (a) Calibration curves of NSAIDs in saliva (b) LDR.

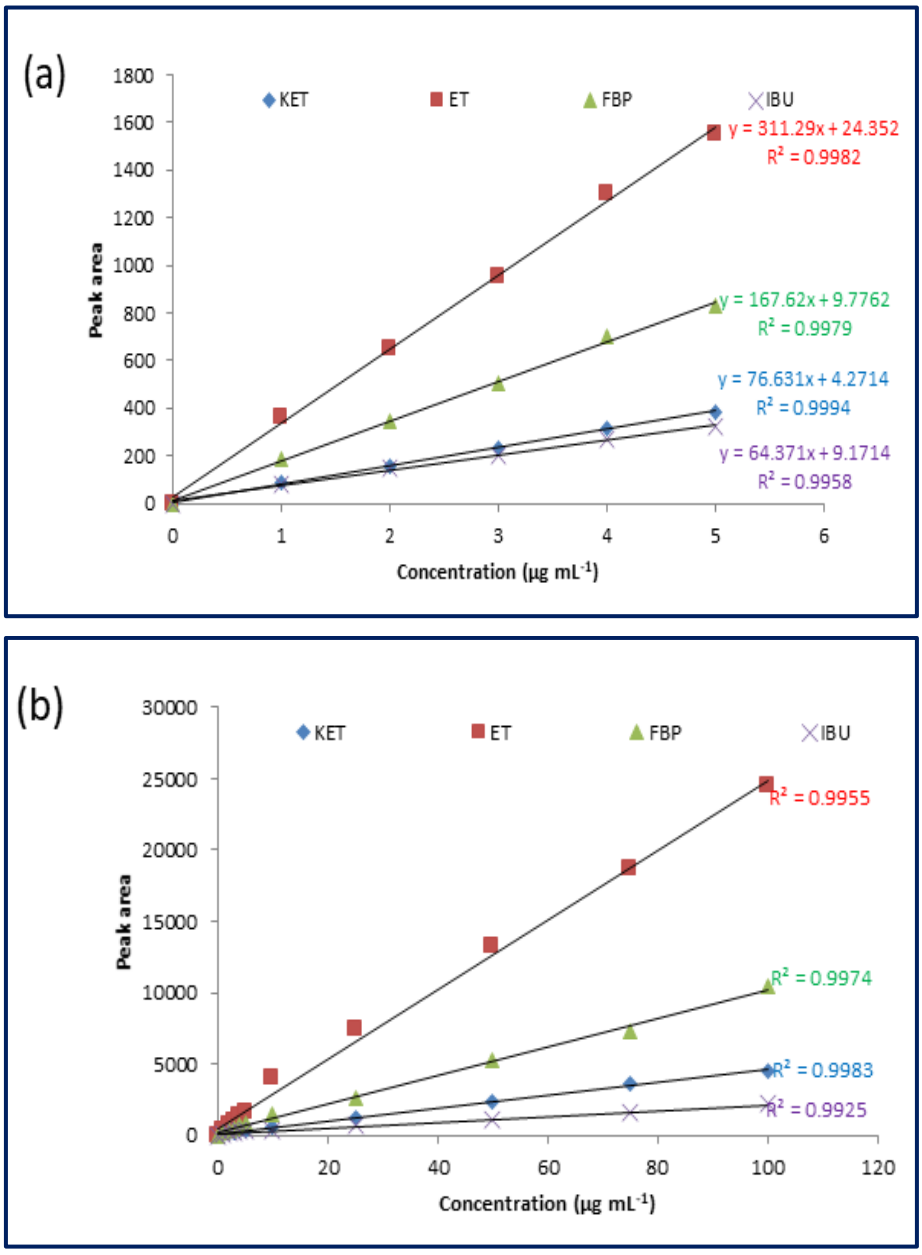


Figure 3.18 (a) Calibration curves of NSAIDs in milk (b) LDR.

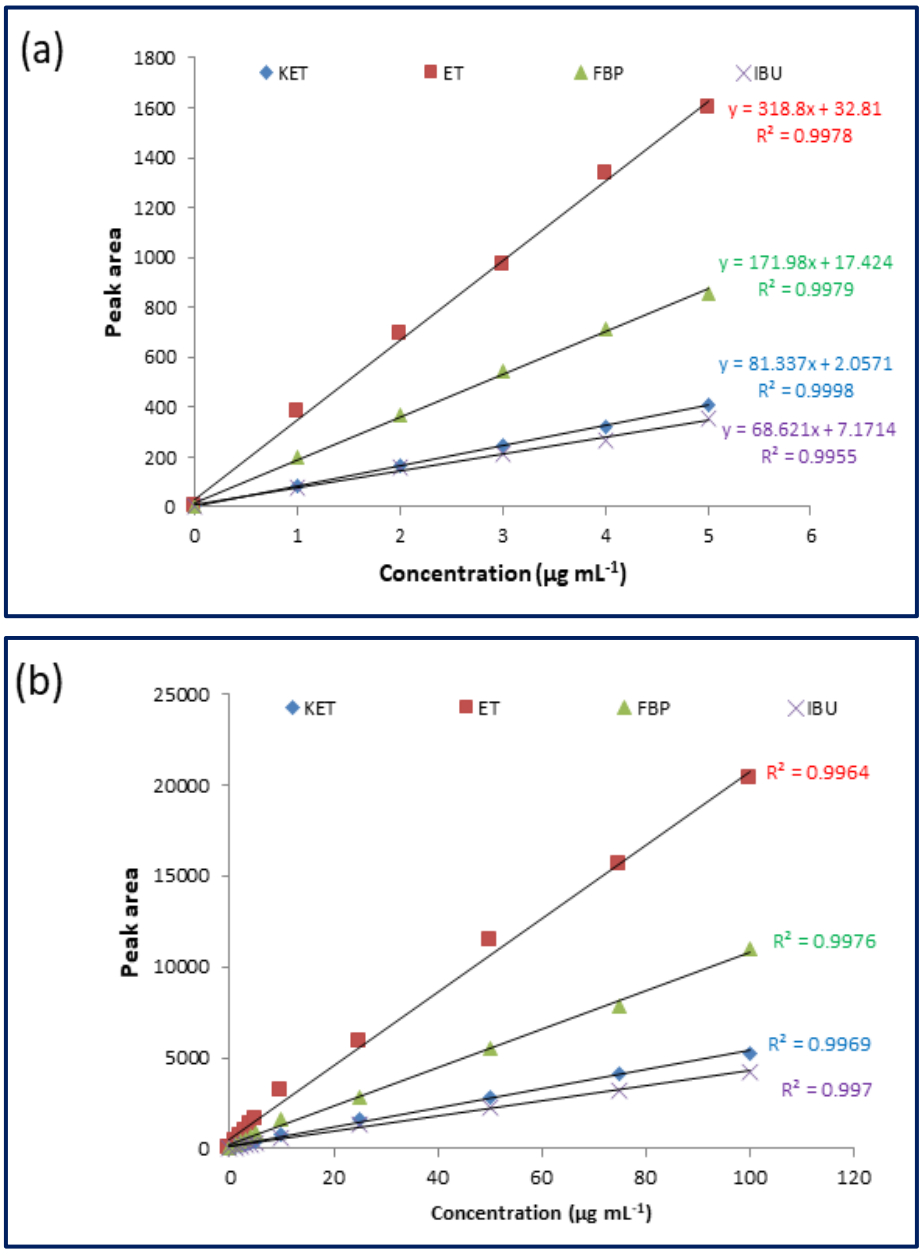


Figure 3.19 (a) Calibration curves of NSAIDs in Urine (b) LDR.

The response was linear over the concentration range from their corresponding LOQ to 100 µg mL^{-1} for all analytes, with R^2 ranging between 0.9955 and 0.9998. LODs (calculated based on $3S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the regression equation) ranged between 0.04 and 0.18 µg mL^{-1} and LOQs (calculated based on $10S_b/m$)

ranged between 0.13 and 0.61 µg mL^{-1}. Reproducibility of the proposed method was evaluated in terms of intra-day and inter-day precision, by repeating the calibration graphs for each NSAID in the same day and within three consecutive days, respectively. The results, expressed as the average %RSD of peak areas, were in the range of 0.9-4.2 and 1.4-7.7% for intra-day and inter-day, respectively, despite the complexity of the studied matrices.

3.8 Matrix Effect and Recovery Studies

In order to evaluate the applicability of the proposed SOE-SHS-LLME-HPLC method and to investigate the presence of any potential matrix effect, recovery studies were performed by spiking milk, saliva, and urine at three concentration levels. Percentage relative recoveries (%RR) obtained for spiked samples are listed in **Table 3.5**. Accordingly, %RR values obtained from matrix-matched calibrations were in the range of 95.7-109.2%.

Table 3.5 Percentage relative recoveries of NSAIDs from biological fluids.

NSAID	Added (µg mL^{-1})	Found (µg mL^{-1})			%RR[a]		
		Milk	Saliva	Urine	Milk	Saliva	Urine
KET	-	<LOD	<LOD	<LOD	-	-	-
	2.00	2.02	1.98	2.01	100.9	98.3	101.2
	3.00	2.99	2.93	2.95	99.5	98.1	100.6
	4.00	4.05	4.11	4.06	101.2	101.5	99.0
ET	-	<LOD	<LOD	<LOD	-	-	-
	2.00	2.01	2.00	2.06	100.4	99.5	103.2
	3.00	2.97	2.91	2.86	99.0	98.0	98.3
	4.00	4.11	4.27	4.36	102.7	104.0	102.2
FBP	-	<LOD	<LOD	<LOD	-	-	-
	2.00	2.01	1.98	2.03	100.4	98.7	102.5
	3.00	2.97	2.95	3.01	98.9	99.4	102.1
	4.00	4.14	4.18	4.25	103.4	101.1	101.6
IBU	-	<LOD	<LOD	<LOD	-	-	-
	2.00	2.13	2.22	2.43	106.5	104.5	109.2
	3.00	3.03	2.99	2.99	101.1	98.5	99.9
	4.00	4.10	3.97	3.80	102.5	96.8	95.7

Matrix effect was studied by calculating the p-value of the calibrations in the different biological fluids using one-way ANOVA and it was found that it is significant at $p < 0.01$, which indicated no matrix effect between these three matrices. Typical chromatograms of unspiked and spiked samples are shown in **Figure 3.20**, **Figure 3.21** and **Figure 3.22**. Absence of interfering peaks at the retention times of the analytes indicated good selectivity of the proposed method.

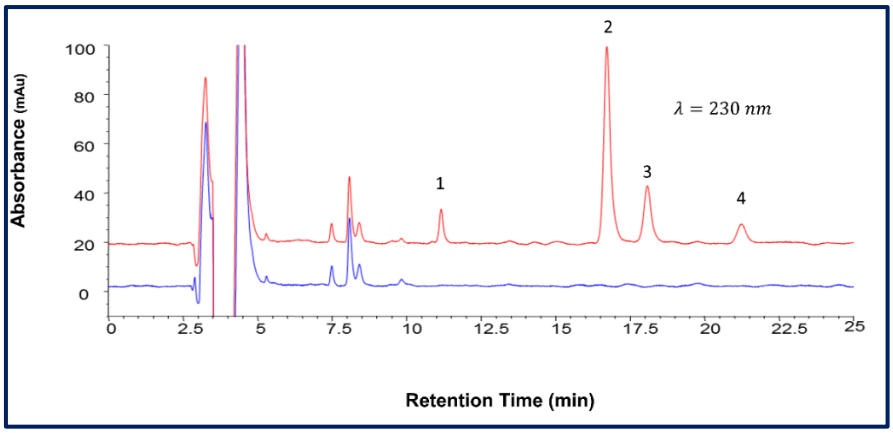


Figure 3.20 Representative chromatograms of mother milk samples extracted and analyzed under optimum SHS-LLME-HPLC conditions. (Top chromatogram: sample spiked at 5.0 µg mL^{-1} of each analyte; bottom: unspiked sample).

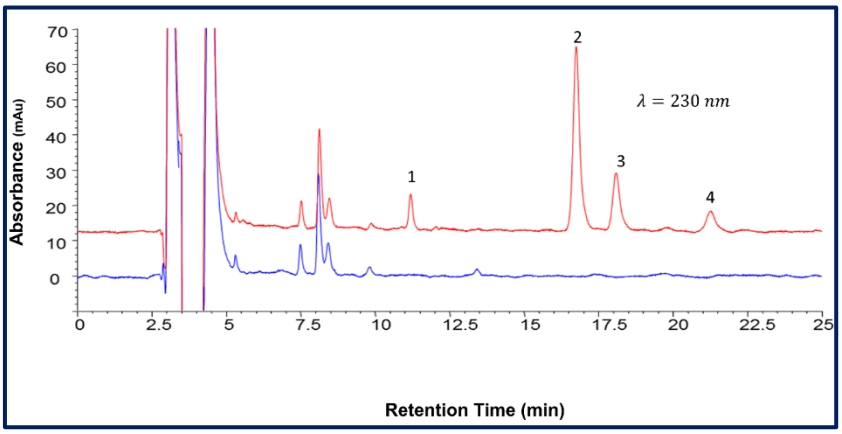


Figure 3.21 Representative chromatograms of saliva samples extracted and analyzed under optimum SHS-LLME-HPLC conditions. (Top chromatogram: sample spiked at 5.0 µg mL^{-1} of each analyte; bottom: unspiked sample).

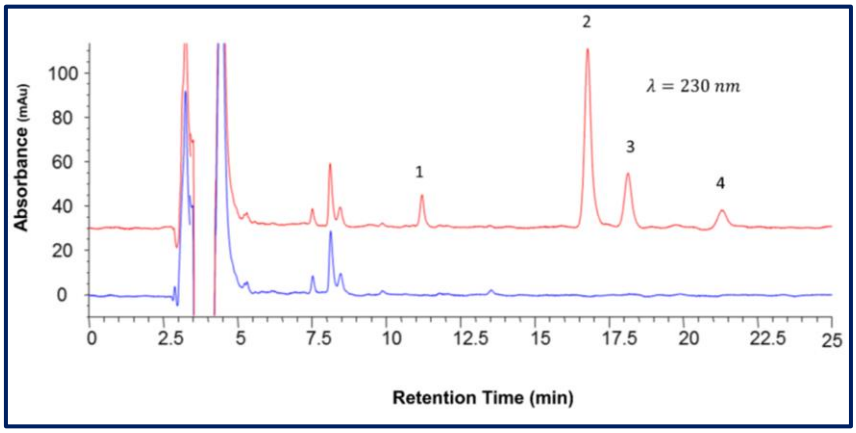


Figure 3.22 Representative chromatograms of urine samples extracted and analyzed under optimum SHS-LLME-HPLC conditions. (Top chromatogram: sample spiked at 5.0 µg mL^{-1} of each analyte; bottom: unspiked sample).

3.9 Application to Genuine Samples

For the milk samples, the volunteer was asked to take a tablet containing 200 mg of FBP, and the sample was collected after two hours. From the chromatogram shown in **Figure 3.23**, the excreted concentration was calculated as 0.16 µg mL^{-1}.

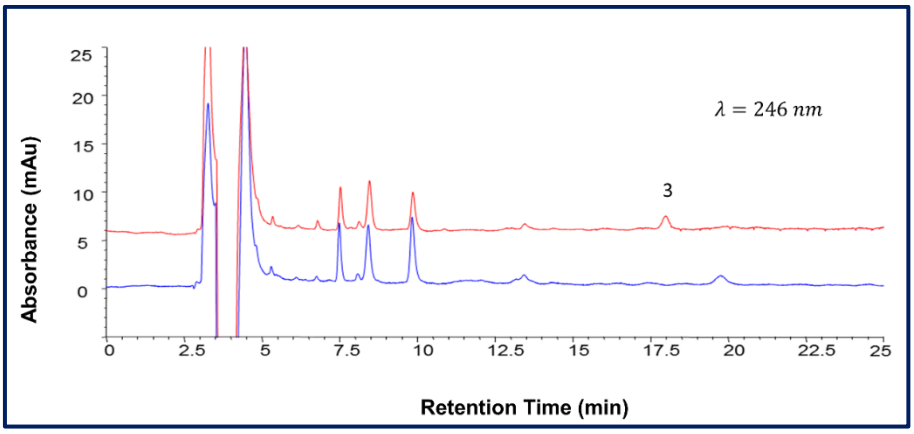


Figure 3.23 Top: genuine mother milk sample containing FBP, bottom: blank (drug-free) sample.

For the saliva and urine, the volunteer was asked to take a tablet containing 400 mg of ET and to collect the samples after two hours. The concentration of ET in the urine was found as 31.37 µg mL^{-1} (**Figure 3.24**), whereas, its concentration in saliva was below LOD (**Figure 3.25**).

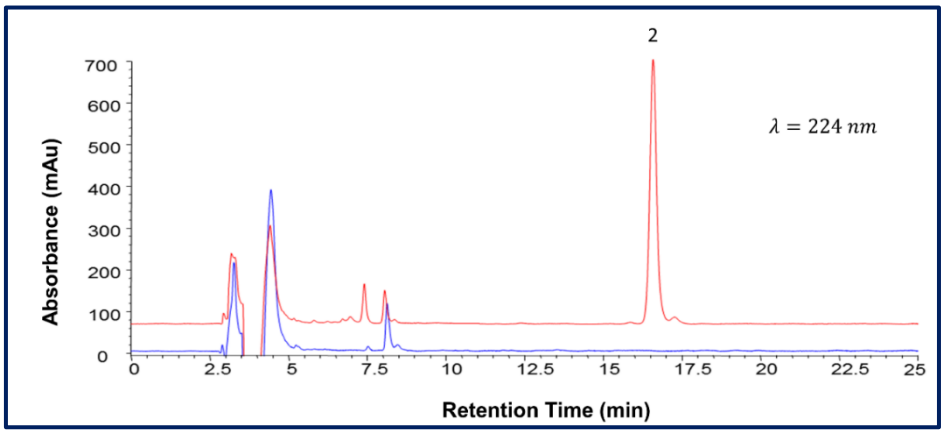


Figure 3.24 Top: genuine urine sample containing ET, bottom: blank (drug-free) sample.

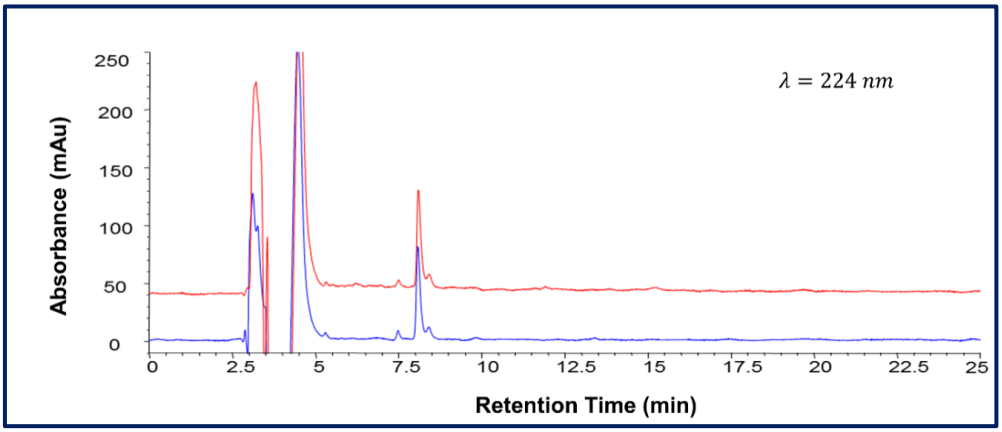


Figure 3.25 Top: genuine saliva sample containing ET, bottom: blank (drug-free) sample.

3.10 Comparison with Other Methods

The proposed SHS-LLME-HPLC method for the selected NSAIDs was compared with other reported methods considering aspects such as extraction time, the volume of organic solvents, and sensitivity expressed in terms of LOD. In comparison with other methods, the main advantages of the proposed method are the elimination of centrifugation, BE or ETD, as well as significant reduction of time and amount of organic extraction solvents consumed.

As shown in **Table 3.6**, the extraction time in this study was only 0.5 min, which was due to complete miscibility of the switched-on form of the extraction solvent with the sample solution. Hollow-fiber liquid phase microextraction (HF-LPME) [115, 129] required a much longer time for equilibrium to be established. Liquid-liquid extraction (LLE) [112] required large amounts of organic solvents (i.e., 8000 µL) and a further step for preconcentrating the analytes through ETD (i.e., 1.5 h). Although single-drop microextraction (SDME) [130] used a small volume, this technique suffers from low robustness due to the ease of dislodgment of the suspended organic droplet during the extraction process. Both tandem air-agitated liquid-liquid microextraction (TAALLME) [131] and DLLME [24] used chlorinated solvents and required centrifugation in addition to BE or ETD steps. Mass spectrometry (MS) detectors are inherently more sensitive than UV/DAD, but they are much more expensive and are not affordable by many analytical laboratories.

Table 3.6 Comparison of SHS-LLME-HPLC with other reported methods for extraction and determination of NSAIDs.

Sample	**Extraction method/ Technique[a]**	**Extraction time (min)**	**Volume of organic solvents (µL)**	**LOD[b] (µg mL^{-1})**	**Ref.**
Wastewater	HF-LPME-LC-MS	300	-	7.1-89.3 (ng mL^{-1})	[115]
Human urine	HF-LPME-HPLC-UV	45	-	5-15 (ng mL^{-1})	[129]
Bovine milk	LLE-LC-MS/MS	10	8000	0.10-62.96 (µg kg^{-1})	[112]
Human urine	SDME-CE-DAD	10	300	1.0-2.5	[132]
Wastewater and human plasma	TAALLME-HPLC-UV	9	99.5	0.1-0.3 (ng mL^{-1})	[131]
Bovine milk and dairy products	DLLME-FASS-CE-DAD	2	2150	3.0-13.1 (µg kg^{-1})	[24]
Saliva, milk, and urine	SHS-LLME-HPLC-DAD	0.5	250	0.04-0.18	This study

[a] Hollow-fiber liquid phase microextraction (HF-LPME), Liquid-liquid extraction (LLE), Single-drop microextraction (SDME), Tandem air-agitated liquid-liquid microextraction (TAALLME), Dispersive liquid-liquid microextraction (DLLME).

[b] Limit of detection.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

In this study, a method based on salting-out extraction (SOE) combined with switchable-hydrophilicity solvent liquid-liquid microextraction (SHS-LLME) is proposed prior to reversed-phase HPLC for the extraction and determination of four non-steroidal anti-inflammatory drugs (NSAIDs) in human milk, saliva, and urine.

Miscibility of the final extract with the mobile phase facilitated direct injection into the instrument, which could eliminate the need for centrifugation and any further treatment after the SHS-LLME. As a result, the extraction time of SHS-LLME was as short as 0.5 min.

This method offered numerous advantages over conventional sample preparation techniques, which include environmental friendliness due to the use and generation of smaller volumes of nontoxic and biodegradable organic solvents, simplicity, cost-effectiveness, short extraction time, and ease of operation.

Despite the complexity of the matrices studied, the method showed high relative recoveries, reproducibility and selectivity, which indicates significant cleaning up of the sample.

The method was applied to a genuine sample containing NSAIDs, and the concentrations of excreted drugs were higher than LOQ, except for the saliva (below LOD), which gives the method a high potential to be used in routine analysis or for future studies.

Different steps enhanced the clean-up process in this method, combining centrifugation before SOE, using ACN in the SOE procedure and the SOE itself cleaned up the samples efficiently, which was proved by the negligible matrix effect.

A rational combination of SHS-LLME and HPLC was introduced by this method, by adding acid modifier to the mobile phase in order to online switching on of the amine layer; as a result of this a lower UV cut-off of these amines was obtained. In addition, higher miscibility with mobile phase and fewer interactions between the amine and the stationary phase is achieved, which facilitate an early elution of the SHS (i.e., close to the solvent front). Moreover, the direct injection into HPLC after SHS-LLME was achievable, which provides superiority over other existing sample introduction techniques.

The results proved that SOE-SHS-LLME could be of interest for the determination of NSAIDs in biological fluids. The extraction time of SHS-LLME was short (30 s), and the steps were not as complicated as in the other techniques. Moreover, superior analytical performance over other commonly applied methods, such as the ability to directly inject the final extract into HPLC gives SHS-LLME a high potential to be applied to other analytes with HPLC or other instruments.

REFERENCES

[1] P. Jedziniak, T. Szprengier-Juszkiewicz, K. Pietruk, E. Sledzinska, J. Zmudzki, Determination of non-steroidal anti-inflammatory drugs and their metabolites in milk by liquid chromatography-tandem mass spectrometry, Analytical and Bioanalytical Chemistry 403(10) (2012) 2955-2963.

[2] R.I. Russell, Non-steroidal anti-inflammatory drugs and gastrointestinal damage - problems and solutions, Postgraduate Medical Journal 77(904) (2001) 82-88.

[3] G.A. Green, Understanding NSAIDs: From aspirin to COX-2, Clinical Cornerstone 3(5) (2001) 50-59.

[4] J.C. Frölich, A classification of NSAIDs according to the relative inhibition of cyclooxygenase isoenzymes, Trends in Pharmacological Sciences 18(1) (1997) 30-34.

[5] S. Friis, A.H. Riis, R. Erichsen, J.A. Baron, H.T. Sorensen, Low-Dose Aspirin or Nonsteroidal Anti-inflammatory Drug Use and Colorectal Cancer Risk A Population-Based, Case-Control Study, Annals of Internal Medicine 163(5) (2015) 347-U70.

[6] J. Chubak, E.P. Whitlock, S.B. Williams, A. Kamineni, B.U. Burda, D.S.M. Buist, M.L. Anderson, Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the US Preventive Services Task Force, Annals of Internal Medicine 164(12) (2016) 814-U80.

[7] A.T. Chan, S. Ogino, C.S. Fuchs, Aspirin Use and Survival After Diagnosis of Colorectal Cancer, Jama-Journal of the American Medical Association 302(6) (2009) 649-659.

[8] F. Verdoodt, S.K. Kjaer, S. Friis, Influence of aspirin and non-aspirin NSAID use on ovarian and endometrial cancer: Summary of epidemiologic evidence of cancer risk and prognosis, Maturitas 100 (2017) 1-7.

[9] M.J. Thun, E.J. Jacobs, C. Patrono, The role of aspirin in cancer prevention, Nature Reviews Clinical Oncology 9(5) (2012) 259-267.

[10] S.M. Fischer, E.T. Hawk, R.A. Lubet, Coxibs and Other Nonsteroidal Anti-Inflammatory Drugs in Animal Models of Cancer Chemoprevention, Cancer Prevention Research 4(11) (2011) 1728-1735.

[11] M.J. Thun, S.J. Henley, C. Patrono, Nonsteroidal anti-inflammatory drugs as anticancer agents: Mechanistic, pharmacologic, and clinical issues, Jnci-Journal of the National Cancer Institute 94(4) (2002) 252-266.
[12] P.M. Rothwell, M. Wilson, J.F. Price, J.F.F. Belch, T.W. Meade, Z. Mehta, Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials, The Lancet 379(9826) (2012) 1591-1601.
[13] P.M. Rothwell, F.G.R. Fowkes, J.F.F. Belch, H. Ogawa, C.P. Warlow, T.W. Meade, Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials, The Lancet 377(9759) (2011) 31-41.
[14] K.P. Townsend, D. Pratico, Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs, Faseb Journal 19(12) (2005) 1592-1601.
[15] E.M. Antman, J.S. Bennett, A. Daugherty, C. Furberg, H. Roberts, K.A. Taubert, Use of nonsteroidal Antiinflammatory drugs an update for clinicians - A scientific statement from the American Heart Association, Circulation 115(12) (2007) 1634-1642.
[16] S. Trelle, S. Reichenbach, S. Wandel, P. Hildebrand, B. Tschannen, P.M. Villiger, M. Egger, P. Juni, Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis, Bmj-British Medical Journal 342 (2011).
[17] L.N. Faught, M.J.E. Greff, M.J. Rieder, G. Koren, Drug-induced acute kidney injury in children, British Journal of Clinical Pharmacology 80(4) (2015) 901-909.
[18] M.L. Bentley, H.L. Corwin, J. Dasta, Drug-induced acute kidney injury in the critically ill adult: Recognition and prevention strategies, Critical Care Medicine 38 (2010) S169-S174.
[19] L. Oni, D.B. Hawcutt, M.A. Turner, M.W. Beresford, S. McWilliam, C. Barton, B.K. Park, P. Murray, B. Wilm, I. Copple, R. Floyd, M. Peak, A. Sharma, D.J. Antoine, Optimising the use of medicines to reduce acute kidney injury in children and babies, Pharmacology & Therapeutics 174 (2017) 55-62.
[20] G. Dowling, P. Gallo, E. Malone, L. Regan, Rapid confirmatory analysis of non-steroidal anti-inflammatory drugs in bovine milk by rapid resolution liquid chromatography tandem mass spectrometry, Journal of Chromatography A 1216(46) (2009) 8117-8131.
[21] T. Hu, T. Peng, X.J. Li, D.D. Chen, H.H. Dai, X.J. Deng, Z.F. Yue, G.M. Wang, J.Z. Shen, X. Xia, S.Y. Ding, Y.N. Zhou, A.L. Zhu, H.Y. Jiang, Simultaneous determination of thirty non-

steroidal anti-inflammatory drug residues in swine muscle by ultra-high-performance liquid chromatography with tandem mass spectrometry, Journal of Chromatography A 1219 (2012) 104-113.

[22] E. Jacqz-Aigrain, R. Serreau, C. Boissinot, M. Popon, A. Sobel, J. Michel, O. Sibony, Excretion of ketoprofen and nalbuphine in human milk during treatment of maternal pain after delivery, Therapeutic Drug Monitoring 29(6) (2007) 815-818.

[23] H.C. Sachs, C. Drugs, The Transfer of Drugs and Therapeutics Into Human Breast Milk: An Update on Selected Topics, Pediatrics 132(3) (2013) E796-E809.

[24] U. Alshana, N.G. Goger, N. Ertas, Dispersive liquid-liquid microextraction combined with field-amplified sample stacking in capillary electrophoresis for the determination of non-steroidal anti-inflammatory drugs in milk and dairy products, Food Chemistry 138(2-3) (2013) 890-897.

[25] M.E.T. Padron, C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, Microextraction Techniques Coupled to Liquid Chromatography with Mass Spectrometry for the Determination of Organic Micropollutants in Environmental Water Samples, Molecules 19(7) (2014) 10320-10349.

[26] M. Rezaee, Y. Assadi, M.R.M. Hosseinia, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction, Journal of Chromatography A 1116(1-2) (2006) 1-9.

[27] M. Sajid, Porous membrane protected micro-solid-phase extraction: A review of features, advancements and applications, Analytica Chimica Acta 965 (2017) 36-53.

[28] M. Rutkowska, K. Owczarek, M. de la Guardia, J. Plotka-Wasylka, J. Namiesnik, Application of additional factors supporting the microextraction process, Trac-Trends in Analytical Chemistry 97 (2017) 104-119.

[29] M.A. Fernandez-Peralbo, M.D.L. de Castro, Preparation of urine samples prior to targeted or untargeted metabolomics mass-spectrometry analysis, Trac-Trends in Analytical Chemistry 41 (2012) 75-85.

[30] J. Namiesnik, Pro-ecological education - Chemical Faculty of the Technical University of Gdansk, Poland, Environmental Science and Pollution Research 6(4) (1999) 243-244.

[31] P.T. Anastas, Green chemistry and the role of analytical methodology development, Critical Reviews in Analytical Chemistry 29(3) (1999) 167-175.
[32] C. Bendicho, I. De La Calle, F. Pena, M. Costas, N. Cabaleiro, I. Lavilla, Ultrasound-assisted pretreatment of solid samples in the context of green analytical chemistry, Trac-Trends in Analytical Chemistry 31 (2012) 50-60.
[33] B.A. de Marco, B.S. Rechelo, E.G. Totoli, A.C. Kogawa, H.R.N. Salgado, Evolution of green chemistry and its multidimensional impacts: A review, Saudi Pharmaceutical Journal 27(1) (2019) 1-8.
[34] A. Kurowska-Susdorf, M. Zwierzdzynski, A.M. Bevanda, S. Talic, A. Ivankovic, J. Plotka-Wasylka, Green analytical chemistry: Social dimension and teaching, Trac-Trends in Analytical Chemistry 111 (2019) 185-196.
[35] J. Moreda-Pineiro, A. Moreda-Pineiro, Recent advances in combining microextraction techniques for sample pre-treatment, Trac-Trends in Analytical Chemistry 71 (2015) 265-274.
[36] S.P. Xie, M.C. Paau, C.F. Li, D. Xiao, M.M.F. Choi, Separation and preconcentration of persistent organic pollutants by cloud point extraction, Journal of Chromatography A 1217(16) (2010) 2306-2317.
[37] A. Zgola-Grzeskowiak, T. Grzeskowiak, Dispersive liquid-liquid microextraction, Trac-Trends in Analytical Chemistry 30(9) (2011) 1382-1399.
[38] C.L. Arthur, J. Pawliszyn, Solid-phase microextraction with thermal-desorption using fused-silica optical fibers, Analytical Chemistry 62(19) (1990) 2145-2148.
[39] C.L. Arthur, L.M. Killam, S. Motlagh, M. Lim, D.W. Potter, J. Pawliszyn, Analysis of substituted benzene compounds in groundwater using solid-phase microextraction, Environmental Science & Technology 26(5) (1992) 979-983.
[40] C. Nerin, J. Salafranca, M. Aznar, R. Batlle, Critical review on recent developments in solventless techniques for extraction of analytes, Analytical and Bioanalytical Chemistry 393(3) (2009) 809-833.
[41] N. Lorenzo-Parodi, W. Kaziur, N. Stojanovic, M.A. Jochmann, T.C. Schmidt, Solventless microextraction techniques for water analysis, Trac-Trends in Analytical Chemistry 113 (2019) 321-331.

[42] H.H. Liu, P.K. Dasgupta, Analytical chemistry in a drop. Solvent extraction in a microdrop, Analytical Chemistry 68(11) (1996) 1817-1821.
[43] M.A. Jeannot, F.F. Cantwell, Solvent microextraction into a single drop, Anal. Chem. 68(13) (1996) 2236-2240.
[44] Y. Yamini, M. Rezazadeh, S. Seidi, Liquid-phase microextraction - The different principles and configurations, Trac-Trends in Analytical Chemistry 112 (2019) 264-272.
[45] S.R. Liu, P.K. Dasgupta, Liquid droplet - A renewable gas sampling interface, Analytical Chemistry 67(13) (1995) 2042-2049.
[46] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid-liquid microextraction method, Journal of Chromatography A 1217(16) (2010) 2342-2357.
[47] S. Berijani, Y. Assadi, M. Anbia, M.R.M. Hosseini, E. Aghaee, Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection - Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water, Journal of Chromatography A 1123(1) (2006) 1-9.
[48] B. Ebrahimpour, Y. Yamini, A. Esrafili, Emulsification liquid phase microextraction followed by on-line phase separation coupled to high performance liquid chromatography, Analytica Chimica Acta 751 (2012) 79-85.
[49] K. Wang, N. Li, L. Lei, X. Yang, Z.B. Wang, D. Li, S. Zang, H.Q. Zhang, A.M. Yu, Z.W. Zhang, Collection and separation of extract in dispersive liquid-liquid microextraction with hollow fiber, Analytical and Bioanalytical Chemistry 408(12) (2016) 3359-3367.
[50] D. Lopes, A.N. Dias, V. Simao, E. Carasek, Determination of emerging contaminants in aqueous matrices with hollow fiber-supported dispersive liquid-liquid microextraction (HF-DLLME) and separation/detection by liquid chromatography - Diode array detection, Microchemical Journal 130 (2017) 371-376.
[51] U. Alshana, N. Ertas, N.G. Goger, Determination of parabens in human milk and other food samples by capillary electrophoresis after dispersive liquid-liquid microextraction with back-extraction, Food Chemistry 181 (2015) 1-8.
[52] H.Y. Yan, H. Wang, Recent development and applications of dispersive liquid-liquid microextraction, Journal of Chromatography A 1295 (2013) 1-15.

[53] L.M. Ravelo-Perez, J. Hernandez-Borges, M. Asensio-Ramos, M.A. Rodriguez-Delgado, Ionic liquid based dispersive liquid-liquid microextraction for the extraction of pesticides from bananas, Journal of Chromatography A 1216(43) (2009) 7336-7345.
[54] Y.C. Fan, Z.L. Hu, M.L. Chen, C.S. Tu, Y. Zhu, Ionic liquid based dispersive liquid-liquid microextraction of aromatic amines in water samples, Chinese Chemical Letters 19(8) (2008) 985-987.
[55] Y. Liu, E.C. Zhao, W.T. Zhu, H.X. Gao, Z.Q. Zhou, Determination of four heterocyclic insecticides by ionic liquid dispersive liquid-liquid microextraction in water samples, Journal of Chromatography A 1216(6) (2009) 885-891.
[56] M.H. Mallah, F. Shemirani, M.G. Maragheh, Ionic Liquids for Simultaneous Preconcentration of Some Lanthanoids Using Dispersive Liquid-Liquid Microextraction Technique in Uranium Dioxide Powder, Environmental Science & Technology 43(6) (2009) 1947-1951.
[57] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Development of an ionic liquid based dispersive liquid-liquid microextraction method for the analysis of polycyclic aromatic hydrocarbons in water samples, Journal of Chromatography A 1216(36) (2009) 6356-6364.
[58] M.A. Farajzadeh, S.E. Seyedi, M.S. Shalamzari, M. Bamorowat, Dispersive liquid-liquid microextraction using extraction solvent lighter than water, Journal of Separation Science 32(18) (2009) 3191-3200.
[59] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, Ultrasound-assisted emulsification microextraction method based on applying low density organic solvents followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples, Journal of Chromatography A 1216(39) (2009) 6673-6679.
[60] L. Guo, H.K. Lee, Low-density solvent-based solvent demulsification dispersive liquid-liquid microextraction for the fast determination of trace levels of sixteen priority polycyclic aromatic hydrocarbons in environmental water samples, Journal of Chromatography A 1218(31) (2011) 5040-5046.
[61] M.R.K. Zanjani, Y. Yamini, S. Shariati, J.A. Jonsson, A new liquid-phase microextraction method based on solidification of floating organic drop, Analytica Chimica Acta 585(2) (2007) 286-293.

[62] M.I. Leong, S.D. Huang, Dispersive liquid-liquid microextraction method based on solidification of floating organic drop for extraction of organochlorine pesticides in water samples, Journal of Chromatography A 1216(45) (2009) 7645-7650.
[63] P.G. Jessop, D.J. Heldebrant, X.W. Li, C.A. Eckert, C.L. Liotta, Green chemistry - Reversible nonpolar-to-polar solvent, Nature 436(7054) (2005) 1102-1102.
[64] J.R. Vanderveen, J. Durelle, P.G. Jessop, Design and evaluation of switchable-hydrophilicity solvents, Green Chemistry 16(3) (2014) 1187-1197.
[65] P.G. Jessop, L. Phan, A. Carrier, S. Robinson, C.J. Durr, J.R. Harjani, A solvent having switchable hydrophilicity, Green Chemistry 12(5) (2010) 809-814.
[66] P.G. Jessop, L. Kozycz, Z.G. Rahami, D. Schoenmakers, A.R. Boyd, D. Wechsler, A.M. Holland, Tertiary amine solvents having switchable hydrophilicity, Green Chemistry 13(3) (2011) 619-623.
[67] F. Bougie, M.C. Iliuta, Sterically Hindered Amine-Based Absorbents for the Removal of CO2 from Gas Streams, Journal of Chemical and Engineering Data 57(3) (2012) 635-669.
[68] I. Eide-Haugmo, H. Lepaumier, A. Einbu, K. Vernstad, E.F. da Silva, H.F. Svendsen, Chemical stability and biodegradability of new solvents for CO2 capture, 10th International Conference on Greenhouse Gas Control Technologies 4 (2011) 1631-1636.
[69] A.R. Boyd, P. Champagne, P.J. McGinn, K.M. MacDougall, J.E. Melanson, P.G. Jessop, Switchable hydrophilicity solvents for lipid extraction from microalgae for biofuel production, Bioresource Technology 118 (2012) 628-632.
[70] C. Samori, D.L. Barreiro, R. Vet, L. Pezzolesi, D.W.F. Brilman, P. Galletti, E. Tagliavini, Effective lipid extraction from algae cultures using switchable solvents, Green Chemistry 15(2) (2013) 353-356.
[71] A. Holland, D. Wechsler, A. Patel, B.M. Molloy, A.R. Boyd, P.G. Jessop, Separation of bitumen from oil sands using a switchable hydrophilicity solvent, Canadian Journal of Chemistry-Revue Canadienne De Chimie 90(10) (2012) 805-810.
[72] G. Lasarte-Aragones, R. Lucena, S. Cardenas, M. Valcarcel, Use of switchable solvents in the microextraction context, Talanta 131 (2015) 645-649.
[73] D.A. Skoog, F.J. Holler, S.R. Crouch, Principles of Instrumental Analysis, 6th Edition ed., Brooks/Cole Publishing Co.2007.

[74] U. Alshana, Separation and quantitation of some platinum group metals by RP-HPLC, MSc. Thesis, Department of Chemistry, Middle East Technical University, Ankara, November 2004.
[75] E. Yilmaz, M. Soylak, Switchable polarity solvent for liquid phase microextraction of Cd(II) as pyrrolidinedithiocarbamate chelates from environmental samples, Analytica Chimica Acta 886 (2015) 75-82.
[76] E. Yilmaz, M. Soylak, Switchable solvent-based liquid phase microextraction of copper(II): optimization and application to environmental samples, Journal of Analytical Atomic Spectrometry 30(7) (2015) 1629-1635.
[77] C. Vakh, A. Pochivalov, V. Andruch, L. Moskvin, A. Bulatov, A fully automated effervescence-assisted switchable solvent-based liquid phase microextraction procedure: Liquid chromatographic determination of ofloxacin in human urine samples, Analytica Chimica Acta 907 (2016) 54-59.
[78] Z. Tekin, S. Erarpat, A. Sahin, D.S. Chormey, S. Bakirdere, Determination of Vitamin B12 and cobalt in egg yolk using vortex assisted switchable solvent based liquid phase microextraction prior to slotted quartz tube flame atomic absorption spectrometry, Food Chemistry 286 (2019) 500-505.
[79] A.G. Moghadam, M. Rajabi, M. Hemmati, A. Asghari, Development of effervescence-assisted liquid phase microextraction based on fatty acid for determination of silver and cobalt ions using micro-sampling flame atomic absorption spectrometry, Journal of Molecular Liquids 242 (2017) 1176-1183.
[80] S. Hu, J. Xue, X. Yang, X. Chen, R.Q. Wang, X.H. Bai, Sodium dodecyl sulfate sensitized switchable solvent liquid-phase microextraction for the preconcentration of protoberberine alkaloids in Rhizoma coptidis, Journal of Separation Science 41(18) (2018).
[81] M. Soylak, M. Khan, E. Yilmaz, Switchable solvent based liquid phase microextraction of uranium in environmental samples: a green approach, Analytical Methods 8(5) (2016) 979-986.
[82] M. Khan, M. Soylak, Switchable solvent based liquid phase microextraction of mercury from environmental samples: a green aspect, Rsc Advances 6(30) (2016) 24968-24975.

[83] S. Zhang, B.B. Chen, M. He, B. Hu, Switchable solvent based liquid phase microextraction of trace lead and cadmium from environmental and biological samples prior to graphite furnace atomic absorption spectrometry detection, Microchemical Journal 139 (2018) 380-385.
[84] M. Ezoddin, K. Abdi, N. Lamei, Development of air assisted liquid phase microextraction based on switchable-hydrophilicity solvent for the determination of palladium in environmental samples, Talanta 153 (2016) 247-252.
[85] Naeemullah, M. Tuzen, A new portable switchable hydrophilicity microextraction method for determination of vanadium in microsampling micropipette tip syringe system couple with ETAAS, Talanta 194 (2019) 991-996.
[86] Z.M. Memon, E. Yilmaz, M. Soylak, Switchable solvent based green liquid phase microextraction method for cobalt in tobacco and food samples prior to flame atomic absorption spectrometric determination, Journal of Molecular Liquids 229 (2017) 459-464.
[87] M. Reclo, E. Yilmaz, M. Soylak, V. Andruch, Y. Bazel, Ligandless switchable solvent based liquid phase microextraction of nickel from food and cigarette samples prior to its micro-sampling flame atomic absorption spectrometric determination, Journal of Molecular Liquids 237 (2017) 236-241.
[88] A. Habibiyan, M. Ezoddin, N. Lamei, K. Abdi, M. Amini, M. Ghazi-khansari, Ultrasonic assisted switchable solvent based on liquid phase microextraction combined with micro sample injection flame atomic absorption spectrometry for determination of some heavy metals in water, urine and tea infusion samples, Journal of Molecular Liquids 242 (2017) 492-496.
[89] J. Ali, M. Tuzen, T.G. Kazi, Determination of Arsenic in Water Samples by Using a Green Hydrophobic-Hydrophilic Switchable Liquid-Solid Dispersive Microextraction Method, Water Air and Soil Pollution 228(1) (2017).
[90] M. Firat, S. Bodur, B. Tisli, C. Ozlu, D.S. Chormey, F. Turak, S. Bakirdere, Vortex-assisted switchable liquid-liquid microextraction for the preconcentration of cadmium in environmental samples prior to its determination with flame atomic absorption spectrometry, Environmental Monitoring and Assessment 190(7) (2018) 8.
[91] M. Firat, E.G. Bakirdere, An accurate and sensitive analytical strategy for the determination of palladium in aqueous samples: slotted quartz tube flame atomic absorption

spectrometry with switchable liquid-liquid microextraction after preconcentration using a Schiff base ligand, Environmental Monitoring and Assessment 191(3) (2019) 9.

[92] M. Reclo, E. Yilmaz, Y. Bazel, M. Soylak, Switchable solvent based liquid phase microextraction of palladium coupled with determination by flame atomic absorption spectrometry, International Journal of Environmental Analytical Chemistry 97(14-15) (2017) 1315-1327.

[93] E. Vessally, E. Ghorbani-Kalhor, R. Hosseinzadeh-Khanmiri, M. Babazadeh, A. Hosseinian, F. Omidi, M.H. Ebrahimi, Application of switchable solvent-based liquid phase microextraction for preconcentration and trace detection of cadmium ions in baby food samples, Journal of the Iranian Chemical Society 15(2) (2018) 491-498.

[94] A. Shishov, M. Wieczorek, P. Koscielniak, D. Dudek-Adamska, A. Telk, L. Moskvin, A. Bulatov, An automated continuous homogeneous microextraction for the determination of selenium and arsenic by hydride generation atomic fluorescence spectrometry, Talanta 181 (2018) 359-365.

[95] S. Erarpat, S. Bodur, D.S. Chormey, S. Bakirdere, Switchable solvent liquid-phase microextraction-gas chromatography-quadrupole isotope dilution mass spectrometry for the determination of 4-n-nonylphenol in municipal wastewater, Microchemical Journal 144 (2019) 1-5.

[96] H. Ahmar, M. Nejati-Yazdinejad, M. Najafi, K.S. Hasheminasab, Switchable Hydrophilicity Solvent-Based Homogenous Liquid-Liquid Microextraction (SHS-HLLME) Combined with GC-FID for the Quantification of Methadone and Tramadol, Chromatographia 81(7) (2018) 1063-1070.

[97] D.S. Chormey, S. Bodur, D. Baskin, M. Firat, S. Bakirdere, Accurate and sensitive determination of selected hormones, endocrine disruptors, and pesticides by gas chromatography-mass spectrometry after the multivariate optimization of switchable solvent liquid-phase microextraction, Journal of Separation Science 41(14) (2018).

[98] S. Erarpat, A. Caglak, S. Bodur, S.D. Chormey, G.O. Engin, S. Bakirdere, Simultaneous determination of fluoxetine, estrone, pesticides, and endocrine disruptors in wastewater by gas chromatography-mass spectrometry (GC-MS) following switchable solvent-liquid phase microextraction (SS-LPME), Analytical Letters 52(5) (2019) 869-878.

[99] S.K. Shahvandi, M.H. Banitaba, H. Ahmar, Development of a new pH assisted homogeneous liquid-liquid microextraction by a solvent with switchable hydrophilicity: Application for GC-MS determination of methamphetamine, Talanta 184 (2018) 103-108.
[100] N. Lamei, M. Ezoddin, N.R. Kakavandi, K. Abdi, M. Ghazi-khansari, Ultrasound-assisted switchable solvent in determination of quaternary ammonium herbicide paraquat in biological, environmental water, and apple juice samples using chemical reduction process coupled to GC-MS detection, Chromatographia 81(6) (2018) 923-930.
[101] F.M. Xu, Q. Li, W.L. Wei, L.Y. Liu, H.B. Li, Development of aliquid-liquid microextraction method based on aswitchable hydrophilicity solvent for the simultaneous determination of 11 drugs in urine by GC-MS, Chromatographia 81(12) (2018) 1695-1703.
[102] M. Hemmati, M. Rajabi, Switchable fatty acid based CO2-effervescence ameliorated emulsification microextraction prior to high performance liquid chromatography for efficient analyses of toxic azo dyes in foodstuffs, Food Chemistry 286 (2019) 185-190.
[103] N.R. Kakavandi, M. Ezoddin, K. Abdi, M. Ghazi-Khansari, M. Amini, S.J. Shahtaheri, Ion-pair switchable-hydrophilicity solvent-based homogeneous liquid-liquid microextraction for the determination of paraquat in environmental and biological samples before high-performance liquid chromatography, Journal of Separation Science 40(18) (2017) 3703-3709.
[104] A. Pochivalov, P. Davletbaeva, K. Cherkashina, A. Lezov, C. Vakh, A. Bulatov, Surfactant-mediated microextraction approach using switchable Chock for hydrophilicity solvent: HPLC-UV determination of Sudan dyes in solid food samples, Journal of Molecular Liquids 271 (2018) 807-814.
[105] X.R. Wang, M. Gao, Z.N. Zhang, H.D. Gu, T.T. Liu, N.N. Yu, X.D. Wang, H.L. Wang, Development of CO2-mediated switchable hydrophilicity solvent-based microextraction combined with HPLC-UV for the determination of bisphenols in foods and drinks, Food Analytical Methods 11(8) (2018) 2093-2104.
[106] I. Timofeeva, S. Timofeev, L. Moskvin, A. Bulatov, A dispersive liquid-liquid microextraction using a switchable polarity dispersive solvent. Automated HPLC-FLD determination of ofloxacin in chicken meat, Analytica Chimica Acta 949 (2017) 35-42.
[107] A. Shishov, I. Sviridov, I. Timofeeva, N. Chibisova, L. Moskvin, A. Bulatov, An effervescence tablet-assisted switchable solvent-based microextraction: On-site

preconcentration of steroid hormones in water samples followed by HPLC-UV determination, Journal of Molecular Liquids 247 (2017) 246-253.
[108] X. Di, X. Wang, Y.P. Liu, X.J. Guo, Solid-phase extraction coupled with switchable hydrophilicity solvent-based homogeneous liquid-liquid microextraction for chloramphenicol enrichment in environmental water samples: a novel alternative to classical extraction techniques, Analytical and Bioanalytical Chemistry 411(4) (2019) 803-812.
[109] A. Pochivalov, I. Timofeeva, C. Vakh, A. Bulatov, Switchable hydrophilicity solvent membrane-based microextraction: HPLC-FLD determination of fluoroquinolones in shrimps, Analytica Chimica Acta 976 (2017) 35-44.
[110] S. Shahraki, H. Ahmar, M. Nejati-Yazdinejad, Electrochemical determination of nitrazepam by switchable solvent based liquid-liquid microextraction combined with differential pulse voltammetry, Microchemical Journal 142 (2018) 229-235.
[111] S. Hu, J. Xue, X. Yang, X. Chen, R.Q. Wang, X.H. Bai, Sodium dodecyl sulfate sensitized switchable solvent liquid-phase microextraction for the preconcentration of protoberberine alkaloids in Rhizoma coptidis, Journal of Separation Science 41(18) (2018) 9.
[112] E. Dubreil-Cheneau, Y. Pirotais, M. Bessiral, B. Roudaut, E. Verdon, Development and validation of a confirmatory method for the determination of 12 non steroidal anti-inflammatory drugs in milk using liquid chromatography-tandem mass spectrometry, Journal of Chromatography A 1218(37) (2011) 6292-6301.
[113] W. Zheng, K.H. Yoo, A.M. Abd El-Aty, D.H. Park, J.M. Choi, S.K. Kim, Y.S. Kang, H.X. Zhang, A. Hacimuftuoglu, A.E. Bekhit, J. Wang, J.H. Shim, H.C. Shin, Quantitative determination of carbasalate calcium derived metabolites, acetylsalicylic acid and salicylic acid, in six animal foods using liquid-liquid extraction method coupled with liquid chromatography-tandem mass spectrometry, Food Chemistry 278 (2019) 744-750.
[114] R. Wang, W.Q. Li, Z.L. Chen, Solid phase microextraction with poly(deep eutectic solvent) monolithic column online coupled to HPLC for determination of non-steroidal anti-inflammatory drugs, Analytica Chimica Acta 1018 (2018) 111-118.
[115] E. Larsson, S. al-Hamimi, J.A. Jonsson, Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid

phase microextraction and liquid chromatography mass spectrometry, Science of the Total Environment 485 (2014) 300-308.
[116] Z. Rezaeifar, Z. Es'haghi, G.H. Rounaghi, M. Chamsaz, Hyperbranched polyglycerol/graphene oxide nanocomposite reinforced hollow fiber solid/liquid phase microextraction for measurement of ibuprofen and naproxen in hair and waste water samples, Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 1029 (2016) 81-87.
[117] C. Toledo-Neira, A. Alvarez-Lueje, Ionic liquids for improving the extraction of NSAIDs in water samples using dispersive liquid-liquid microextraction by high performance liquid chromatography-diode array-fluorescence detection, Talanta 134 (2015) 619-626.
[118] C.H. Lee, Y. Shin, M.W. Nam, K.M. Jeong, J. Lee, A new analytical method to determine non-steroidal anti-inflammatory drugs in surface water using in situ derivatization combined with ultrasound-assisted emulsification microextraction followed by gas chromatography mass spectrometry, Talanta 129 (2014) 552-559.
[119] S. Arghavani-Beydokhti, M. Rajabi, A. Asghari, Coupling of two centrifugeless ultrasound-assisted dispersive solid/liquid phase microextractions as a highly selective, clean, and efficient method for determination of ultra-trace amounts of non-steroidal anti-inflammatory drugs in complicated matrices, Analytica Chimica Acta 997 (2018) 67-79.
[120] S. Magiera, S. Gulmez, A. Michalik, I. Baranowska, Application of statistical experimental design to the optimisation of microextraction by packed sorbent for the analysis of nonsteroidal anti-inflammatory drugs in human urine by ultra-high pressure liquid chromatography, Journal of Chromatography A 1304 (2013) 1-9.
[121] T. Martinez-Sena, S. Armenta, M. de la Guardia, F.A. Esteve-Turrillas, Determination of non-steroidal anti-inflammatory drugs in water and urine using selective molecular imprinted polymer extraction and liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis 131 (2016) 48-53.
[122] H. Alinezha, A. Amiri, M. Tarahomi, B. Maleki, Magnetic solid-phase extraction of non-steroidal anti-inflammatory drugs from environmental water samples using polyamidoamine dendrimer functionalized with magnetite nanoparticles as a sorbent, Talanta 183 (2018) 149-157.

[123] S. Alsoul, Dispersive liquid-liquid microextraction of non-steroidal anti-inflammatory drugs in human milk and their determination by HPLC, MSc. Thesis, Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, Nicosia, TRNC, May 2018.

[124] X. Xiong, L. Zhang, L.T. Cheng, W. Mao, High-throughput salting-out assisted liquid-liquid extraction with acetonitrile for the determination of anandamide in plasma of hemodialysis patients with liquid chromatography tandem mass spectrometry, Biomedical Chromatography 29(9) (2015) 1317-1324.

[125] T.T. Li, L. Zhang, L. Tong, Q.F. Liao, High-throughput salting-out-assisted homogeneous liquid-liquid extraction with acetonitrile for determination of baicalin in rat plasma with high-performance liquid chromatography, Biomedical Chromatography 28(5) (2014) 648-653.

[126] J. Zhang, H.Q. Wu, E. Kim, T.A. El-Shourbagy, Salting-out assisted liquid/liquid extraction with acetonitrile: a new high throughput sample preparation technique for good laboratory practice bioanalysis using liquid chromatography-mass spectrometry, Biomedical Chromatography 23(4) (2009) 419-425.

[127] Y.Q. Tang, N.D. Weng, Salting-out assisted liquid-liquid extraction for bioanalysis, Bioanalysis 5(12) (2013) 1583-1598.

[128] F. Zare, M. Ghaedi, R. Jannesar, L. Tayebi, Switchable polarity solvents for preconcentration and simultaneous determination of amino acids in human plasma samples, New Journal of Chemistry 42(12) (2018) 10007-10015.

[129] Y.B. Cha, S.W. Myung, Determination of Non-Steroidal Anti-Inflammatory Drugs in Human Urine Sample using HPLC/UV and Three Phase Hollow Fiber-Liquid Phase Microextraction (HF-LPME), Bulletin of the Korean Chemical Society 34(11) (2013) 3444-3450.

[130] M.A. Jeannot, A. Przyjazny, J.M. Kokosa, Single drop microextraction-Development, applications and future trends, Journal of Chromatography A 1217(16) (2010) 2326-2336.

[131] M. Bazregar, M. Rajabi, Y. Yamini, A. Asghari, M. Hemmati, Tandem air-agitated liquid-liquid microextraction as an efficient method for determination of acidic drugs in complicated matrices, Analytica Chimica Acta 917 (2016) 44-52.

[132] A. Garcia-Vazquez, F. Borrull, M. Calull, C. Aguilar, Single-drop microextraction combined in-line with capillary electrophoresis for the determination of nonsteroidal anti-inflammatory drugs in urine samples, Electrophoresis 37(2) (2016) 274-281.