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APPLICATION OF DEEP EUTECTIC SOLVENTS FOR THE DETERMINATION OF RESIDUAL SOLVENTS IN PHARMACEUTICALS USING HEADSPACE GAS CHROMATOGRAPHY

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LIST OF ABBREVIATIONS

API – Active pharmaceutical ingredient
CA – Citric acid
ChCl – Choline chloride
DES – Deep eutectic solvent
DEG – Diethyl glycol
DSC – Differential calorimetry scanning
DMF – Dimethylformamide
DTA – Differential thermal analysis
EU – European Union
ECD – Electron capture detector
FTIR - Fourier-transform infrared spectroscopy
FID – Flame ionization detector
GC – Gas chromatography
GLC – Gas-liquid chromatography
GSC – Gas-solid chromatography
GMP – Good manufacturing practices
Glu – Glucose
HS-GC – Headspace gas chromatography
HS – Headspace
HBD – Hydrogen bond donor
HBA – Hydrogen bond acceptor
IR – Infrared
IL – Ionic liquids
ICH - The International Council for Harmonization
MS – Mass spectrometry
ORS – Organic residual solvents
PTV – Programmed temperature vaporizing
PEG – Polyethylene glycol
PID – Photoionization detector
ppm – Parts per million
PDE – Permitted daily dose
TGA – Thermogravimetric analysis
TCD – Thermal conductivity detector
TEG – Triethylene glycol
US – The United States
USP – The United States pharmacopeia
HS-SME – Headspace-solvent microextraction
SPME – Solid-phase microextraction

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INTRODUCTION

Organic solvents are commonly present and used in the pharmaceutical industry, and they can be used in many manufacturing steps and processes such as extraction and purification. Therefore, drying techniques are utilized to remove these undesirable organic solvents after the manufacturing process. However, trace amounts can be unavoidable in the final products even after drying and processing. These trace amounts in the final products are commonly known as organic residual solvents, and they can potentially have harmful and toxic effects.

Consequently, the pharmaceutical industry maintains strict control over organic residual solvents, with pharmacopoeias establishing acceptable limits for their presence in the final products. Therefore, analysis methods are utilized by manufacturers to determine ORSs in final products in order to comply with pharmacopoeias. Gas chromatography is considered the optimal choice and most suitable technique of analysis, due to its capability to separate, identify and detect volatile substances with low detection limits. However, selecting a proper sample matrix and calibration media is a crucial step in this technique.

Deep eutectic solvents were selected to be investigated as suitable sample matrix for residual solvents determination using HS-GC. This selection is due to their exceptional properties, biodegradability, ease of synthesis and non-toxic nature.

The **aim of the research work** is to investigate the application of deep eutectic solvents as a sample matrix and calibration media for the determination of organic residual solvents in pharmaceuticals using HS-GC technique. In order to achieve this goal, few objectives were set:

- To synthesise deep eutectic solvents using microwave assisted heating, and study their thermal stability using HS-GC.
- To investigate the performance of DESs as a matrix media for different ORSs determination using HS-GC method.
- To select an appropriate DES for optimisation of the HS-GC method.
- To apply the developed optimised method in common pharmaceutical products determination of ORSs.

1. DEEP EUTECTIC SOLVENTS

1.1 Definition, classification, synthesis.

Over the last decades, the societal expectations for the pharmaceutical industry to rely more on sustainable materials and reagents has grown significantly in order to reduce the high consumption of materials, and eliminate the production of hazardous wastes. Consequently, this has led chemists and pharmaceutical manufacturers to increase the efforts towards designing greener and more sustainable chemical processes and materials to cope up with this growing trend. The pharmaceutical industry is one of the largest users of organic solvents, and that accounts for 75-80% of the waste that is associated with synthesis of APIs. In addition to that, solvents are routinely used for cleaning reaction vessels, reaction media, purifications and separations [1]. With the growing interest in green chemistry, a new class of solvents has emerged as a promising alternative to conventional organic solvents due to their sustainable preparation methods using highly accessible and natural sources.

Abbott et al. investigated different mixtures of amides and quaternary ammonium salts to form low melting point eutectics which show many advantages such as sustainability, biodegradability with the possibility to produce these mixtures from readily available sources [2]. In 2003, Abbott et al. was the first to use the term “deep eutectic solvents (DES)” to describe the resulting mixtures they investigated, and the term eutectic came from a Greek origin that means easy (or lowest) melting [3] which reflects the interesting property of the final mixtures to have lower melting point than their pure components. This property was attributed to the complexation between a hydrogen bond donors and hydrogen bond acceptors to form hydrogen bonds at a well-defined stoichiometric proportions [2].

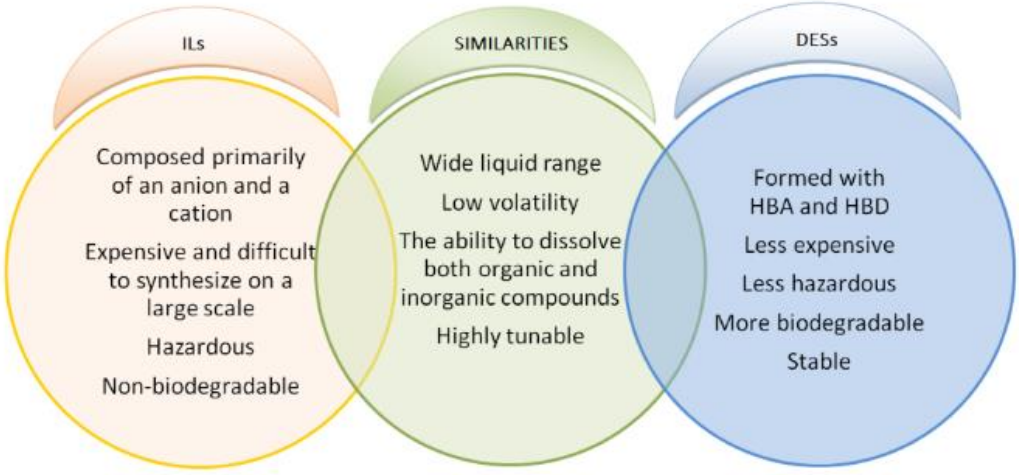

Fig. 1. Similarities and differences of ILs and DESs [4].

In 2014, Abbott et al. presented a review in which they defined DES as systems formed from a eutectic mixture of Lewis or Brønsted acids and bases which can contain a variety of anionic and/or cationic species [5]. But due to the limited published work dedicated to

understanding and blotting a clear definition of DES in the last century, this term has been interchangeable and misused as a subclass of ionic liquids [6]. Płotka-Wasylka et al. introduced a review with emphasis on the differences and similarities between DESs and ILs. The differences between DESs and ILs can be attributed to two basic ways, the nature of the starting materials and the formation method of the final complex [4] which are summed in **Fig. 1**.

Deep eutectic solvents can be classified into 5 types depending on the complexing agent used (**Table 1**), and they can presented using the general formula (1) below:

$$Cat^{+}X^{-}zY \qquad (1)$$

Where Cat^{+} is any ammonium, phosphonium or sulfonium cations, and X^{-} is a Lewis bast which is generally a halide anion, Y is either a Lewis or Brønsted acid, and z refers to the number of Y molecules that interact with the anion.

Table 1. general formula and classification of DESs.

Type	General formula	Terms
Type I	$Cat^{+}X^{-}zMCl_{x}$	M = Zn, Sn, Fe, Al, Ga, In
Type II	$Cat^{+}X^{-}zMCl_{x} \cdot yH_2O$	M = Cr, Co, Cu, Ni, Fe
Type III	$Cat^{+}X^{-}$zRZ	Z = $CONH_2$, COOH, OH
Type IV	$MHalx + RZ = [MClx{-}1\,] + \cdot\, RZ \cdot [MClx{+}1\,]$	M = Al, Zn; Z = $CONH_2$, OH
Type V	HBA + HBD	HBA is non-ionic

Type I is an analogous type to the well-studied metal halide/imidazolium salt systems, and this type of DESs is formed using metal halides such as $ZmCl_2$, LiCl, AgCl combined with imidazolium salts. Non-hydrated metal halides exhibit low melting point, and therefore few hydrogen bond doners are available to form this type of DESs [7].

Type II eutectics are identical to type I but they are formed using hydrated metal halides and choline chloride, this type of DESs are more feasible to use in large industrial scale due to their relatively low cost and their inherent air and moisture insensitivity.

Type III eutectics are formed using hydrogen bond acceptors (e.g. choline chloride, proline) and hydrogen bond donors (e.g., alcohol, carboxylic acids, amides, saccharides) as presented in **Fig 2**. This type of eutectics is easy to prepare, relatively has a low cost and can be tailored and designed to be applied in many applications due to the wide range of hydrogen bond donors that have been studied and available today. This type of eutectics is the most popular and widely used nowadays due to their versatility and adaptability to be applied to many applications including extraction of glycerol from biodiesel [8], cellulose derivative synthesis [9].

In 2007, Abbott et al. have investigated metal-Containing anions and cations which are formed using disproportionation processes [10] which can be formed between metal hydrates (or metal halide hydrate) and simple alcohols or amides, and those eutectics were termed as type IV DESs.

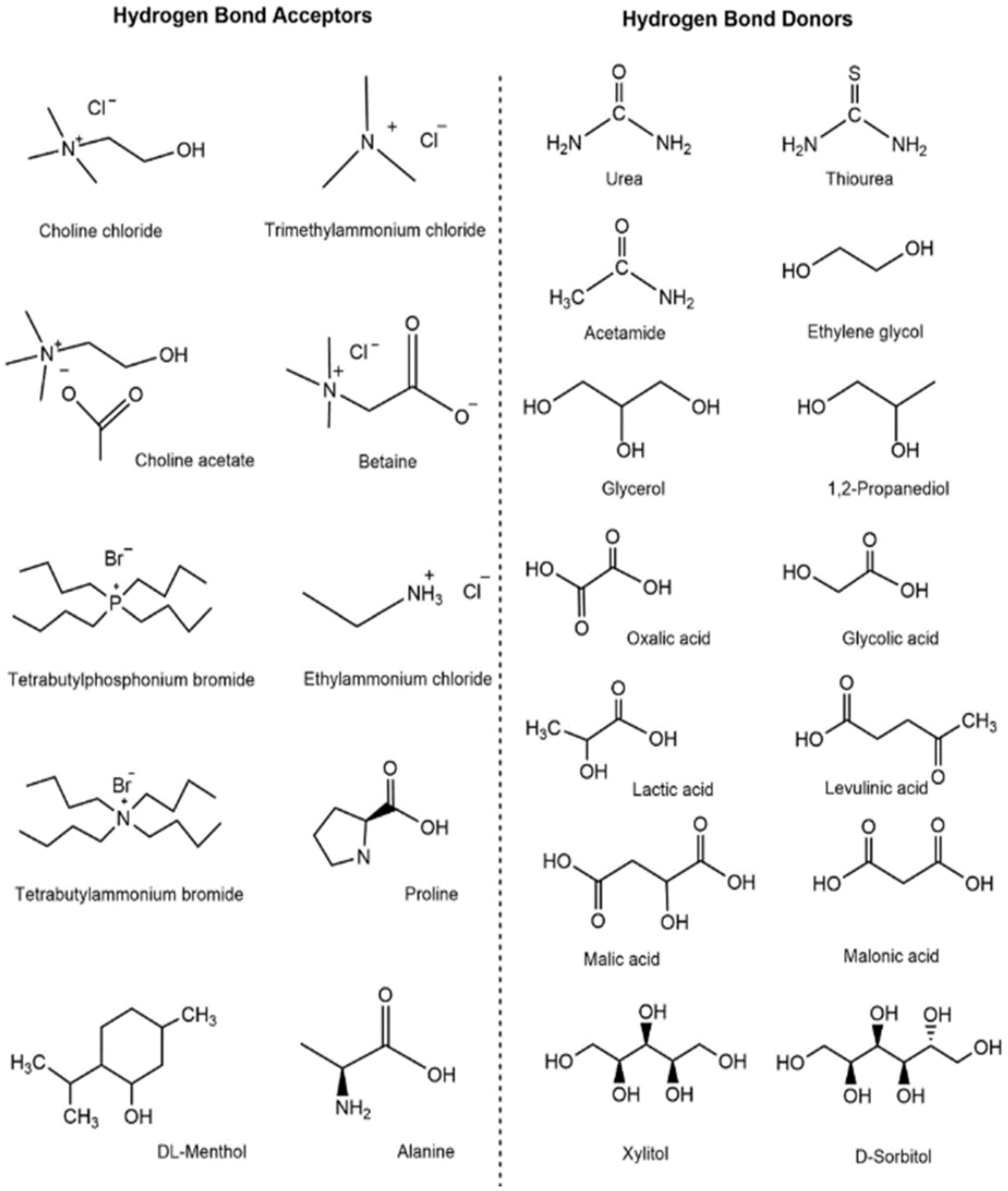

Fig 2. Commonly used HBA and HBD for DESs preparation.

Type V eutectics are a new generation of hydrophobic solvents, which were investigated in 2015 for the first time as extractants [11]. This type of DESs is formed between a hydrogen bond donor and a hydrogen bond acceptor, (e.g. menthol and thymol at 1:1 ratio [12], decanoic acid and menthol at 1:1 ratio [13]). This novel class of hydrophobic DESs have shown improved and unique properties, with interesting applications in academia and industry [14]. Some of the commonly used HBD and HBA to prepare DESs are presented in **Fig 2**.

Deep eutectic solvents are versatile mixtures which can be synthesized by relatively simple and economical process at various ratios. Many methods of DESs synthesis have been investigated including:

1. *Heating method*, in this method the mixture of the HBA and HBA is heated to a certain temperature and stirred until a homogenous liquid is obtained. And this method is considered to be the most popular method and have been widely used to prepare different mixtures of DESs [15], however some high temperatures may cause degradation of the eutectic mixture.
2. *Grinding method* is simply based on continuous grinding of the components until a homogenous mixture is obtained which was also investigated by Florindo et al. using different ratios of cholinium chloride and carboxylic acids [15].
3. *Freeze-drying method* by dissolving HBD and HBA in water, mixing, freezing, followed by freeze-drying to obtain the DES. This method have been investigated and proven to be a suitable approach to prepare DESs [16].
4. *Evaporation method* which consists of dissolving the components in water, followed by evaporation at 50 °C was reported by Dai et al. [17].
5. *Ultrasound-assisted method* has also been recently reported by Santana et al. as a relatively faster and efficient method of DESs preparation [18].

Preparation of DESs depends mainly on hydrogen bond network formation between HBD and HBA and in addition to that, intermolecular interactions between components including Van der Waals and electrostatic forces are also present [19]. For instant, Aziz et al. prepared a mixture of choline chloride and urea at a molar ratio of 1:2 where the mixture was heated at 80 °C and mixed until clear liquid appeared [20] as presented in **Fig. 3** below.

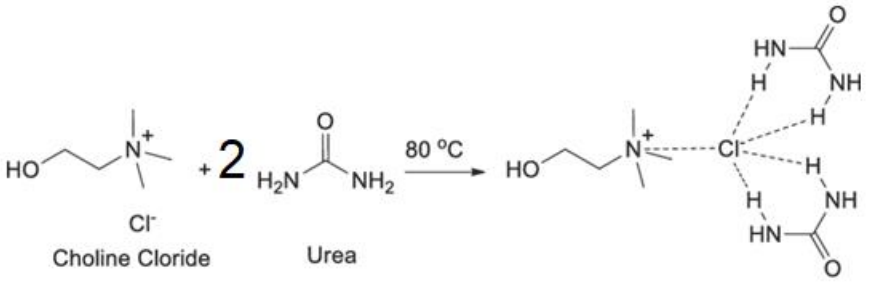

Fig. 3. An example of deep eutectic solvent preparation.

1.2 Properties

Deep eutectic solvents have unique properties which made them a promising target by researchers to investigate and study. DESs can be chemically tailored for specific applications, due to the wide variety of compounds that can be used as precursors for DESs formation [21]. However, the main physicochemical properties of DESs are their melting temperature, density, viscosity, ionic conductivity, surface tension and polarity.

Freezing temperature is an essential factor to consider when designing an analytical methods and DESs systems that can be effectively used in an application. However, deep eutectic solvents are characterized by having a lower freezing point than the precursors used to make them, some examples are shown in **Table 2**.

Table 2. Freezing Point Temperatures of a Selection of DESs.

HBA	mp/°C	HBD	mp/°C	HBA: HBD (molar ratio)	DES Tf/°C	reference
Choline chloride	303	urea	134	1:2	12	[2]
Choline chloride	303	1-methyl urea	93	1:2	29	[2]
benzyltriphenyl phosphonium chloride	345−347	ethylene glycol	−12.9	-	47.91	[22]
$ZnCl_2$	293	urea	134	-	9	[10]
Choline chloride	303	citric acid	149	1:1	69	[2]

Deep eutectic solvents are mixtures of more than one component and those systems can be represented using a solid-liquid phase diagram called the *phase behavior* diagram. This diagram shows the melting temperature in a function of the mixture composition, considering a binary system of compounds A and B, the eutectic point the melting temperature at which both melting curves of the two compounds meet (**Fig. 4**).

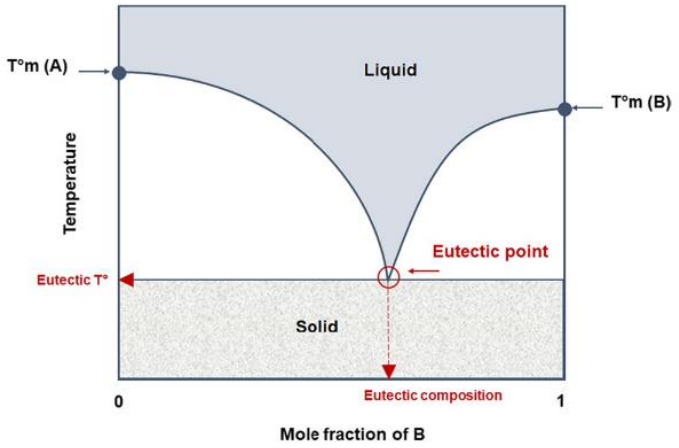

Fig. 4. Phase diagram of a binary mixture, representing the eutectic point of the system.

Most DESs have a freezing point that ranges between -69 and 149 °C, and mostly present a freezing point lower than 150 °C [23]. However, freezing point of DESs is affected by the choice of HBD, the nature of the organic salt and it's anion [2], as well as the molar ratio between HBD and HBA [24]. Moreover, it was shown that the method of preparation can also influence the freezing point value [9]. On the other hand, there is no correlation between the freezing point of starting materials and the final freezing point of the DESs formed [25], [23].

Density is considered to be one of the most important parameter to describe physical properties of liquids, and it depends on the nature of the starting materials that are used to form the DESs. Most reported densities of DESs are ranging between 1.0 and 1.3 $g.cm^{-3}$ at 25 °C which is higher than water [21]. On the other hand, densities lower than water were also reported of hydrophobic DESs [26]. The differences between densities of different DESs can be described by the Hole theory [27], which assumes that DESs to be composed of holes of approximate dimension and size, and those holes affect the properties of DESs [7]. During DESs preparation, when HBD were added to HBD, formation of hydrogen bonds takes place which reduces the free available space between molecules, and consequently increases the density of DESs. The density of DESs is also affected by molar ratio of HBD and HBA, and this feature can be used to manipulate the final density of resultant DES as reported in [28], [29]. In addition to that, the density of DESs decreases with temperature increase, and this relationship can be described in terms of isobaric thermal expansion coefficients, which reflects the available free volume of DESs. These coefficients are used to express the compressible behavior of DESs [7], and they are obtained from experimental densities at different temperatures of DESs:

$$\alpha P = -\rho^{-1}(\partial\rho/\partial T)_P \qquad (2)$$

Equation (2) states that the coefficient of thermal expansion (α) is equal to the negative reciprocal of the density (ρ) multiplied by the partial derivative of density with respect to temperature (T) at constant pressure (P). This equation provides a quantitative relationship between the density and temperature of a material while keeping the pressure constant.

Consequently, a linear decrease in DESs density was observed with an increase in temperature, which is ascribed to the availability of more free spaces between the HBA and HBD of DESs and hence the increase in the holes size in the eutectics.

Viscosity is an important physical property of DESs and it has been reported and investigated extensively in literature. Most of the reported viscosities of DES are relatively high at room temperature (η >100 mPa.), due to the rich hydrogen bond network between DESs components. However, a broad range of viscosities have been reported, including sugar-based DESs which present extremely high viscosities (12,730 mPa·s for 1:1 choline chloride: sorbitol at 30 °C and 34,400 mPa·s for 1:1 choline chloride: glucose at 50 °C) as well as even higher viscosities were recorded (85,000 mPa·s for 1:2 choline chloride: zinc chloride at 25 °C) [23]. On the other hand, very low viscosity DESs were reported for hydrophobic DESs based on DL-menthol (7.61 mPa·s at 25 °C for 1:3 DL-menthol: octanoic acid) [30]. The viscosity of DESs is affected by many factors including the nature of starting components [10], molar ratio [31], temperature [2] as well as water content [32].

Ionic conductivity is mainly affected and controlled by viscosity, most DESs show poor ionic conductivity at room temperature. However, temperature increase results in a decrease in viscosity and therefore an increase in conductivity [23], [33]. In addition to that, this property is also affected by the nature of precursor HBD and HBA [25] as well as the water content [32].

Surface tension is an essential property of DESs which depends on the intensity of intermolecular forces forming the liquid, namely the hydrogen bonds. However, liquids with

higher viscosity show higher surface tension, which consequently linearly decrease with temperature increase [34].

Polarity is a key property to consider when designing a method, due to the fact that it reflects the solvation capability of the DESs and therefore determines the suitability of the DES for a specific application. When designing an analytical method, the analyte's polarity needs to be matched with the DES's polarity to promote an efficient solvation of the analyte.

Refractive index is another key property of DESs which should be tuned to fit some specific applications. In a recent investigation in 2022, Chen et al. conducted a systemically study on the refractive index of DESs, and it was shown that DESs present relatively high values between 1.4 and 1.5 compared to water which has a value of 1.334 [35].

*Thermal stability. S*tudying the thermal stability of eutectic systems is an important step to prevent hydrogen bond network from dissociation, and hence decomposition of the DES with the temperature increase. Consequently, forming materials decompose due to the increased temperature following the hydrogen bond dissociation [36]. Chemat et al. investigated the thermal decomposition temperature of a ternary DES systems of choline chloride, urea and L-arginine with different ratios and under different temperatures (**Fig. 5**). Obtained data showed that with the addition of L-arginine, the thermal stability of the ternary systems increased, and this observation might be attributed to the intermolecular interactions and coordinating nature of the ions [37], which is speculated to aid in preventing the "escape" of molecules from the hydrogen bond network [38] which leads to the decomposition of deep eutectic system.

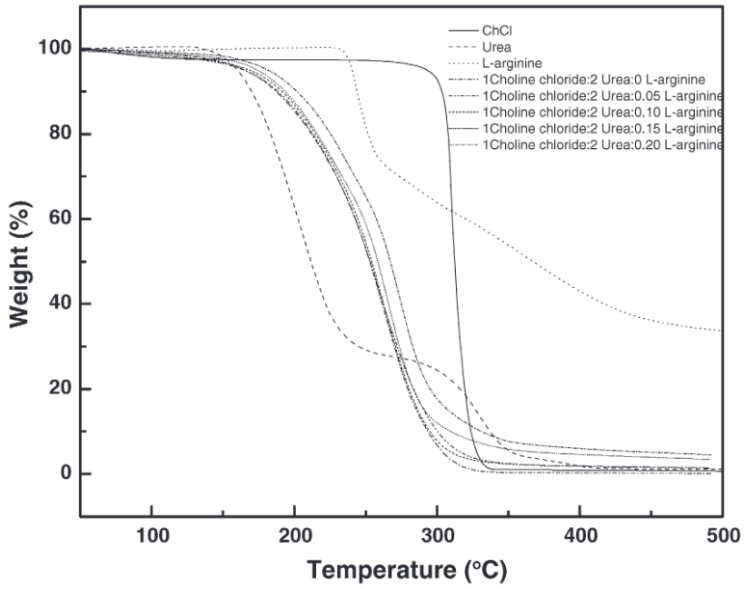

Fig. 5. Thermal decomposition temperatures of pure components and DESs with different ratios. [37]

1.3 Water effect on deep eutectic solvents properties

Water presence in deep eutectic systems can be unavoidable due to the hygroscopic nature of many DESs, and therefore studying its effect on eutectic systems' properties is of a great importance. Dihydrogen monoxide is one of few natural compounds that represents a hydrogen bond donor and acceptor, and consequently, it has a strong interaction with the hygroscopic components of the DESs hydrogen bond network.

Water can be considered as an impurity in many DESs, although some DESs are made of the hydrated form of metal halides. Water can be used as a cosolvent in many DESs, and it can be used to modify many essential physicochemical properties of DESs such as viscosity, conductivity, density as well as freezing point. In addition to that, water content which can be involved in the hydrogen bonding within DESs can be investigated and revealed using nuclear magnetic resonance (NMR) [16], and by Fourier transform infrared spectroscopy [39].

DESs supramolecular network change. Water addition is a relatively simple way of tailoring DESs properties to enhance their performance and properties, if they are added in adequate amounts. On the other hand, DESs structures and properties are defined and formed by intermolecular interactions, namely hydrogen bonding, Coulomb forces as well as dispersion forces, and an excess addition of water can result in a disturbance if this order and results in total decomposition of the DES [4].

Density. Water content effect on density was investigated by Florindo et al. [15] on choline chloride based DES, where the solvents were left in contact with air for a month and the content of the retained water was shown to range from 14 to 20% with. Obtained data showed that while going from 20 to 80 °C, the density of water saturated samples was lower than the density of dried samples, with a only a 5% difference. Same effect was reported by shah et al. when they investigated the effect of adding up to 65 wt.% of water to choline chloride: urea DES at 30 °C [40]. Consequently, the presence of water in eutectic systems slightly affects the density of the DESs. On the other hand, Dai et al. reported that the density of glucose: choline: water DES decreased linearly with water content increase [32].

Viscosity and conductivity. Unlike density, viscosity and conductivity are highly sensitive to water presence and content in DESs. Agieienko et al. investigated the effect of water content on viscosity and density of choline chloride: urea systems, it was shown that density was decreased by 0.14% with around 0.008 mass fraction of water, while viscosity was decreased by 22% with a 0.005 mass fraction of water [40]. In addition to that, Due et al. [41] reported a decrease in viscosity (**Fig. 6**) by more than 13 times from 1080 mPa·s to 81 mPa·s at 298 K, and an improve in conductivity (**Fig. 7**) by one order of magnitude from 0.5 mS/cm to 4.9 mS/cm at 313 K, using choline chloride: urea DES systems with a water content range between 0 (dry) to 6 wt% (hydrated).

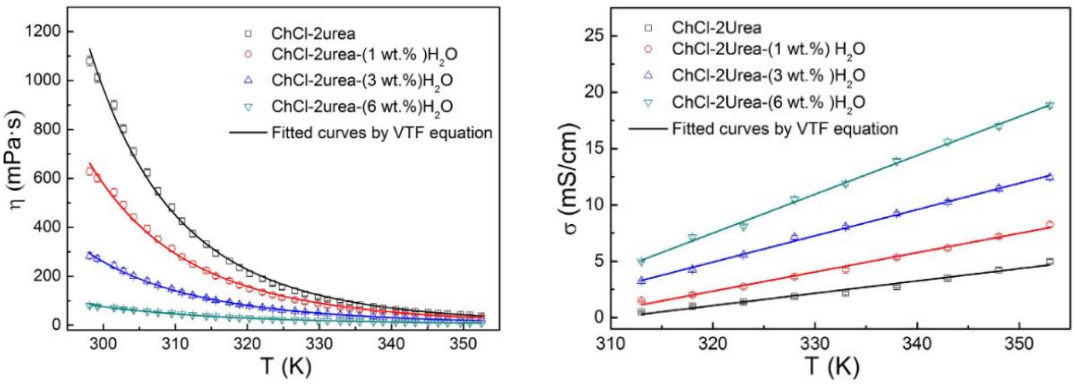

Fig. 6. Viscosity (on the left) and conductivity (on the right) of choline chloride: urea DESs with different water wt% as a function of temperature.

Polarity is directly connected with solubilization capability of the DESs and it is reflected in the tailoring process of the DES to a specific application, and water addition can have a significant effect on the DES polarity. Polarity of DESs can be evaluated using solvatochromatic dyes and the estimation of its visible absorption maximum (λmax). Gabriele et al. investigated the water addition effect on the polarity of different DESs using Nile Red positive solvatochromatic dye; which shows a visible absorption maximum (λmax) shift towards a longer wavelength (lower energy) when dissolved in an increasingly polar media [42]. They reported an increase in polarity after the addition of 25 wt% to different DESs systems, namely DEG (diethylene glycol), TEG (triethylene glycol) and PEG (polyethylene glycol) 200 (**Fig. 8**).

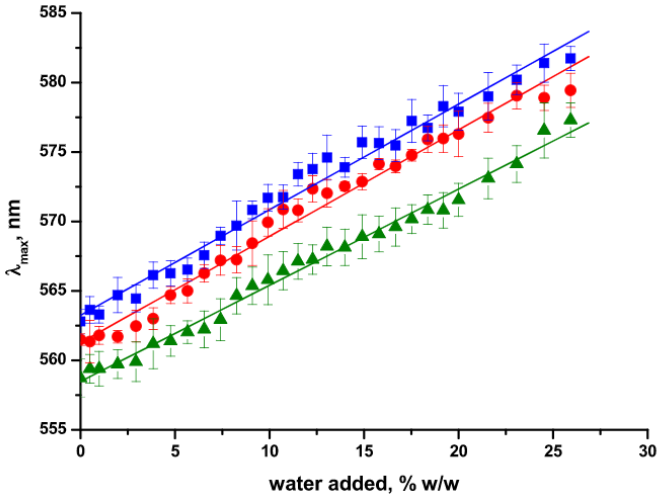

Fig. 8 The effect of water addition percent on maximum absorbance (λmax) of Nile red in the DESs at room temperature. ChCl/DEG (blue), ChCl/TEG (red), ChCl/PEG 200 (green) [42].

Freezing point. As highlighted before, water addition can disturb the hydrogen bond network structure which is the main matrix of the deep eutectic solvent system, and excess

addition can reach a point where water becomes the solvent, and the components are solvated in water [42]. Smith et al. investigated water addition effect on freezing point using ternary systems of urea/ChCl/H_2O with various ratios [43], reported data showed a depression in the freezing point with more water addition until a minimum was reached when 6 equivalent of water (0.67 mol fraction) has been added (**Fig. 9**). On the other hand, an increase in freezing point was observed with a fraction of water higher than 0.67 (X_{water}). Moreover, similar data trend was reported by Meng et al. where they studied the impact of water addition on melting temperature of urea/ChCl DESs [44].

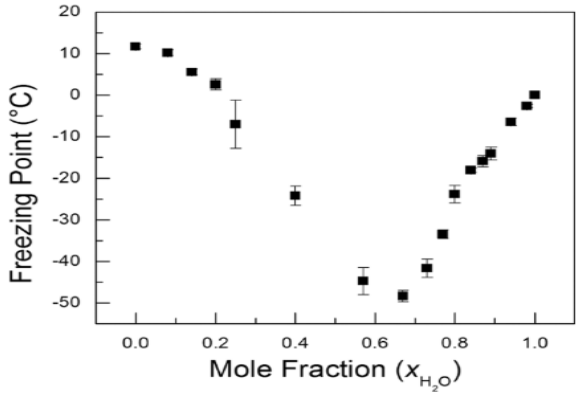

Fig. 9. Freezing point of 2:1 urea/ChCl DES as a function of the mole fraction of water added.

1.4 Applications of deep eutectic solvents in analytical chemistry field

From the analytical chemistry perspective, deep eutectic solvents are viewed as an attractive alternative to conventional organic solvents, which are toxic, and highly volatile. Deep eutectic solvents ae designer media which can be tailored and utilized to many applications including:

- *chromatography,* DESs can be used as a mobile phase or mobile phase additive [45], it can also be utilized as a stationary phase [46], or a surface modifier of materials used in chromatography [47], [48]. DESs were also utilized to determine bioactive terpenoids [49] using headspace-solvent microextraction (HS-SME) coupled with GC.
- *Microextraction* applications of target analytes from complex media, followed by quantification using analytical instruments, including liquid-liquid extraction [50], [51].
- DESs can also be used as an *eluent* following dispersive solid-phase extraction procedure [52], [53], and it was successfully employed in determining target analytes using an environmentally friendly solvents.

Many other different applications have been investigated to utilize DESs unique properties and advantages that they offer over the conventional solvents, such as: their potential in nanotechnology, and their applications in organic reactions including lipase-catalyzed reactions, synthesis of polymers and related materials, as well as their use as organic synthesis media [54].

2. ORGANIC SOLVENTS IN PHARMACEUTICAL INDUSTRY

2.1 Introduction

Solvents are defined as substances that are capable of dissolving or dispersing other substances (solutes) to form a solutions, which are usually in liquid form, and they are accounted for between 80 and 90% of the utilized masses in chemical operations [55]. Consequently, solvents play a crucial role in the pharmaceutical industry, including:

1. Extraction: solvents are used to extract active ingredients from complex matrixes such as plants, animals and microorganisms. For example, ethanol, hexane and acetone mixtures are used to extract lycopene from raw tomatoes [56].
2. Purification: solvents are used to purify active ingredients and final products of impurities like salts, and other organic substances. For example, alcohols are used to purify products from proteins by precipitation [57].
3. Formulation: solvents are extensively used as a media of formulation to produce dosage forms in pharmaceutical industry, such as tablets, capsules as well as injections [58].
4. Crystallization: various solvents can be used to form crystals of active ingredients, to improve the purity and stability of final products [59].
5. Cleaning: solvents are extensively used in cleaning procedures of laboratory and manufacturing equipment [60].

In pharmaceutical industry, organic solvents are commonly used due to the many advantages they offer, and therefore, they are constantly present in production processes. As a consequence, the pharmaceutical industry is considered to be the largest consumer of organic solvents per amount of final product [61], examples are shown in **Table 3**.

Table 3. Examples of the most commonly used organic solvents in pharmaceutical industry and some of their properties [62].

Common organic solvents	Properties
Ethanol	Colorless, flammable solvent that is widely used in pharmaceutical industry due to its high solvability of many APIs, and it can also be used as a disinfectant.
Acetone	Clear colorless solvent which is commonly used in manufacturing of APIs.
Methanol	Colorless flammable substance which can be used as a solvent and reactant in chemical synthesis.
Dichloromethane	Colorless, volatile solvent which can be used in extraction and purification of natural products.
Ethyl acetate	Colorless, flammable solvent which is commonly for purification of natural products, such as plant extracts and essential oils.

Due to the fact that many organic solvents are being used and utilized for their advantages in the pharmaceutical industry, solvent selection is considered to be a crucial step in drug development and manufacturing, because they can have a big influence on so many aspects of the manufacturing process such as cost, safety, efficacy as well as quality of the final product [63]. Besides, many organic solvents are volatile and have low boiling point, and can form vapor in room temperature, which present many health and environmental risks, consequently, many big pharmaceutical manufacturers developed their own solvent selection guides [63] to ensure proper handling and minimize any potential risks. Moreover, organic solvents have a lipophilic nature, and hence they can rapidly cross body membranes, and can cause acute and chronic adverse effects [64], and they can cross blood brain barrier which can cause many neurotoxic adverse effects [65], which can result in chronic neurological dysfunctions.

With that being said, organic solvents can exhibit negative effects if they are inhaled, ingested, or come into contact with skin or if they are improperly handled, in addition, they can cause many environmental risks when released into the environment by manufacturers [58], [66]. Therefore, organic solvents use is strictly regulated, and many green chemists are studying and developing innovative alternatives and methods to minimize organic solvents use, and their hazardous wastes, as well as, their presence in final pharmaceutical products [58].

2.2 Removal of Organic Solvents

Drug manufacturing process can be complicated and involves multiple steps (**Fig. 10**), which need to be carefully monitored and regulated. However, each step of the drug production can potentially get contaminated by the organic solvents that are being used, and therefore, drying steps are involved to removed undesirable organic solvents. Many drying techniques are commonly used in pharmaceutical manufacturing including:

1. Freeze-drying, in which the sample is frozen, and sublimation process takes place under reduced pressure to remove undesirable solvents [67].
2. Fluidized Bed Dryer [68], in this technique the powder is suspended in an upward stream of heated air, which aid in the reduction of moisture content of the powder.
3. Spray drying (**Fig. 11**), in which the solvent or powder is pumped through a nozzle to create a slurry, which faces a counter flow of heated gas [69]
4. Rotary Drying using an industrial scale equipment which bring the powder in contact with heated gas to reduce solvent, and moisture content [70].
5. Microwave-Vacuum Drying which offers several advantages to heat sensitive substances, it combines the advantage of quick solvent removal as well as the lowering of boiling temperature, due to pressure reduction [71].

Choosing the suitable technique is highly dependent on the type of solvents involved, the source and ways of contamination [61], the physical and chemical properties of the solvent, cost and time needed for the solvent removal process, as well as safety consideration where the removal process should take place under vacuum conditions. However, trace amounts of organic solvents can remain in the final products after the drying process, and those remaining entities are referred to as residual solvents [72].

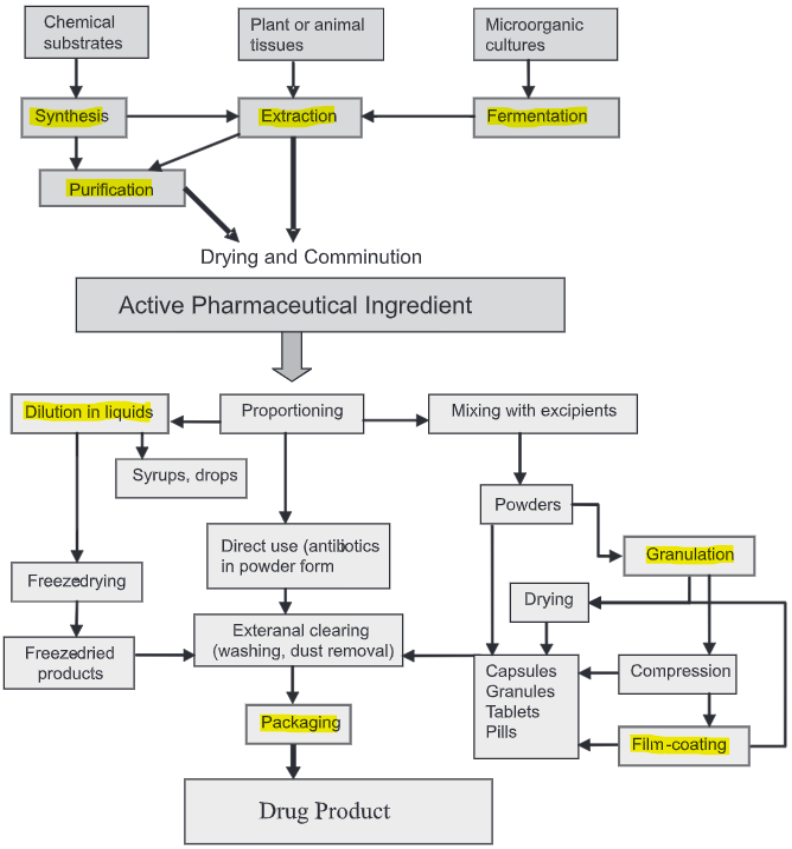

Fig. 10. An illustration of general selected manufacturing process in pharmaceutical industry, the highlighted boxes represent the steps which can be potentially contaminated by organic solvents [61].

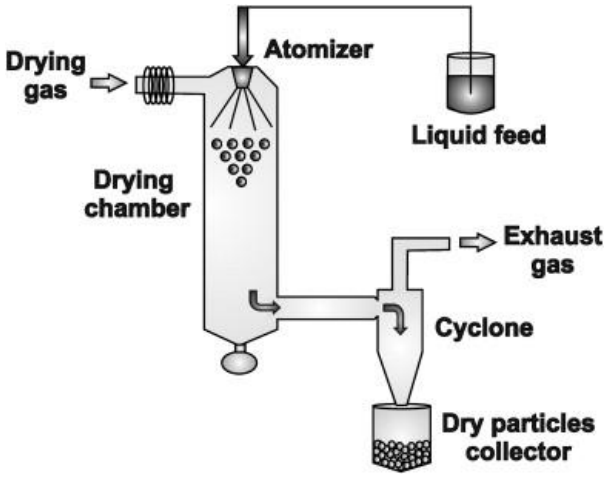

Fig. 11. An illustration of spray drying process in pharmaceutical industry [69].

2.3 Residual Solvents in Pharmaceutical Industry

Impurities control is a critical issue in the process of drug manufacturing, and they need to be tightly controlled and identified. Impurities are defined as the unwanted chemicals that remain with the active pharmaceutical ingredients, or develop during formulation or during storage of the final product, due to the degradation of either the API or additives [73]. Moreover, the International Conference on Harmonization (ICH) has published guidelines to control and evaluate impurities in new drug products [74], substances [75], as well as residual solvents [76].

Residual solvents are organic volatile chemicals that are used during the manufacturing process or gets generated during the production, and they are difficult to be removed completely, and those residues are should be controlled and lowered to meet the safety data [73]. According to ICH guideline Q3C solvents are divided into four classes [76] as following:

- Class 1 (**Table 4**) which includes solvents that have been identified or highly suspected to cause adverse effects on human health including carcinogenicity, genotoxicity, or developmental toxicity.
- Class 2 (**Table 5**) solvents are recommended to be limited due do their potential to cause non-genotoxic carcinogenesis or irreversible toxicity, such as neurotoxicity or teratogenicity, they also are a potential cause for other significant reversible toxicities. In ICH guides, limits for this group is listed in two different ways, namely by part per million (ppm) concentration as well as permitted daily exposure (PDE).
- Class 3 (**Table 6**) solvents have a PDE of 50 mg (0.5%) or less per day, higher concentration might also be accepted if the manufacturer proves that the amount is realistic and complies with the manufacturing capabilities and good manufacturing practices (GMP).
- Class 4 solvents have limited toxicological data, and if manufacturers are using them, they need to provide a proper justification of the residual content in their products [76].

Table 4. Class 1 solvents and their limits (ppm), and concerns for limiting [76].

Solvent	Concentration Limit (ppm)	Concerns
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 5. Class 2 solvents and their limits in term of their permitted daily exposure (mg/day) as well as concentration limit (ppm) [76].

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6	600
1,2-Dimethoxyethane	1	100
N,N-Dimethylacetamide	10.9	1090
N,N-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	20	2000
2-Methoxyethanol	0.5	50
Methylbutylketone	0.5	50
Methylcyclohexane	11.8	1180
N-methylpyrrolidone	48.4	4840
Nitromethane	0.5	50
Pyridine	2	200
Sulfolane	1.6	160
Tetralin	1	100
Toluene	8.9	890
1,1,2-Trichloroethylene	0.8	80
Xylene	21.7	2170

Table 6. Class 3 solvents which are limited by GMP and other quality requirements [76].

Class 3 solvents		
Acetic acid	Tert-Butylmethyl ether	Ethanol
Heptane	Dimethyl sulfoxide	Ethyl acetate
Acetone	Isopropyl acetate	Ethyl ether
Isobutyl Acetate	Methyl acetate	Ethyl formate
Anisole	3-Methyl-1butanol	Formic acid
1-Butanol	Methyl ethyl ketone	Pentane
2-Butanol	2-methyl-1-propanol	1-Pentanol
2-propanol	Propyl acetate	Triethylamine
Butyl acetate	2-methyltetrahydrofuran	1-Propranol

2.4 Analytical Methods for Organic Residual Solvents Determination

Organic residual solvents can be unavoidable in many industrial pharmaceutical manufacturing processes, even after processing, such as the synthesis of APIs and formulation, therefore they need to be strictly regulated and monitored [61]. As a result, the ICH Harmonized Guidelines, which have been set by EU, Japan and US under ICH topic Q3C, to regulate the analysis methods which are used to detect residual solvents as well as their acceptable limits [72]. Eventually, USP has adopted the ICH Q3C guidelines set, and in addition to that, USP revised the different limits, and methods for determination of residual solvents.

Loss of weight was the first analytical method to be added to pharmacopoeias, and this method can be performed under vacuum or normal pressure, furthermore, this method is considered to be a simple non demanding method. However, loss of weight method exhibits many disadvantages, such as lack of specificity, high limit of detection (About 0.1%), as well as the relative high amount of sample needed for analysis (1 to 2 grams), and it's sensitivity to atmospheric humidity [72]. Thermogravimetric analysis was also employed as a non-specific method to detect residual solvents in different samples [77], in which, the mass of the sample is measured as a function of temperature or time while being subjected to a controlled temperature program in a controlled atmosphere. TGA method is a successor to the loss of weight method and it showed an improved sensitivity (Limit of detection about 100 ppm), and it requires less sample volume (5-20 mg) [72]. In addition to the previously mentioned methods, DTA and DSC have been also been used for residual solvents determination [77], [78].

The loss of weight method and it's successors lack specificity, and the general content of residual solvents can be obtained when using more than one solvent in the production procedure, and therefore, their use is limited nowadays [72]. Infrared spectroscopy and Fourier transformation infrared spectroscopy have been employed to determinate residual solvents in different samples, but they showed many disadvantages including interference between matrix and analytes bands, as well as their low sensitivity (limit of detection above 100 ppm) [72]. None of the above methods can meet the sensitivity requirements in ICH guidelines and pharmacopoeias, and consequently, they have limited, and rather rare applications and use in the pharmaceutical industry.

As a consequence, gas chromatography technique is considered to be the most efficient analytical method for the determination of residual solvents in the pharmaceutical industry, due to the nature of the organic solvents that are being used in the industry, which have relatively low boiling points and a generally thermal stable nature [72], [78].

3. ORGANIC RESIDUAL SOLVENTS ANALYSIS BY GAS CHROMATOGRAPHY

3.1 Introduction

Among the different testing methods of residual solvents, GC is considered the optimal choice and most suitable method of analysis, due to its capability to separate, identify and detect volatile substances with low detection limits [78]. Over the years, GC methods have dominated the field of residual solvents analysis in the pharmaceutical industry, due to the volatile nature of the most organic solvents that are being utilized and used in the field [72]. Consequently, GC methods were adopted for residual solvents analysis in pharmacopoeias where they were explained, described and presented in general chapters and different individual monographs.

Modern technology advancement in the analytical chemistry field have led to the development of appropriate systems which are designed to analyze specific groups of substances and samples, and consequently led to developing efficient systems with shorter time of analysis and lower limit of detection [72]. However, the pharmaceutical industry present a wide variety of sample types that need to be analyzed and regulated, therefore the main concern in developing an appropriate method of detection is sample introduction (injection) to the GC system. The choice of injection technique is determined by the type of sample, type of analyte, their quantity and available laboratory tools.

3.2 Gas Chromatography Sample Introduction Methods

Gas chromatography techniques can be divided according to sample injection method into three main categories, direct injection, HS-GC, and SPME. Moreover, the selection of an appropriate sample injection method plays a vital role in obtaining accurate and reliable results, as each method has its own advantages and limitations. Therefore, it is essential to consider the factors which play a major role in choosing the appropriate method, such as sample type, analysis time, available equipment.

3.2.1 Direct Injection

Direct injection is commonly used and preferred in the pharmaceutical industry, due to its simplicity and reliability, where the sample is directly introduced to the GC system using syringes or an autosampler. The sample is delivered via the injection port which is designed to introduce the sample into the GC column, and this process is followed by vaporization of the sample, followed by elution using a carrier gas (Helium, nitrogen, etc.). Direct injection technique is usually preferred for residual solvents analysis of drug substances, but when it comes to more complex samples such as tablets or syrups, more extensive sample preparation techniques needs to be employed [72]. Sample preparation techniques, such as extraction and pre-concentration, help overcome obstacles like limited solubility or difficulty in vaporizing the matrix, enabling analysis of complex samples [78].

Direct injection have different modes including:

- *Split injection* is used for complex samples where carrier gas flow is split between a "split vent" and the capillary column. The sensitivity of the analysis is reduced in this mode, due to the loss of the more volatile substances in the sample. Whereas, in *splitless injection* mode sensitivity is increased, as most of the injected sample is transferred to the analysis column.
- *On-column injection*, where the sample gets injected into the pre-column followed by solvent venting, then the analytes get transferred onto the analytical column to perform separation.
- Programmed temperature vaporizing (PTV) injection is considered to be time consuming, due to having many parameters that needs to be optimized.

3.2.2 Headspace

In the pharmaceutical industry, headspace technique is commonly used for residual solvent analysis [78] due to its simplicity and time-saving benefits. This method relies on the indirect introduction of the analytes into the gas chromatography system; by extracting the volatile and semi volatile compounds from the sample vial's headspace region with direct contact with the sample as presented in **Fig. 12** [77]. Headspace-sampling techniques are typically categorized as dynamic headspace analysis and static headspace analysis. Dynamic headspace analysis offers the benefit of a low detection limit, while static headspace analysis is known for its ease of use and automation.

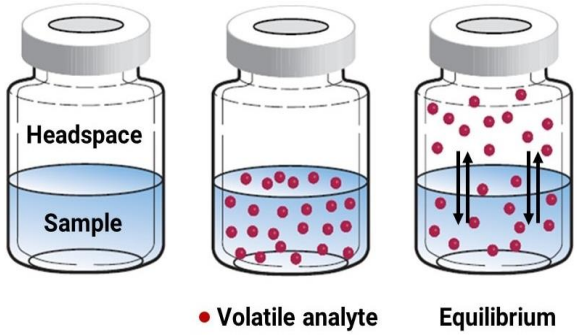

Fig. 12. Sealed headspace vial used for sample introduction into GC system.

In dynamic HS-GC system, a continuous flow of inert gas is swept over the surface of the sample matrix, which results in the volatile residual solvents being transferred into the a trap (see **Fig. 13A**) where they are accumulated before the introduction to the analysis column [79]. This method is particularly useful when analyzing samples that contain a very low concentration of residual solvents, consequently low detection limits have been reported in picogram-per-milliliter range [80].

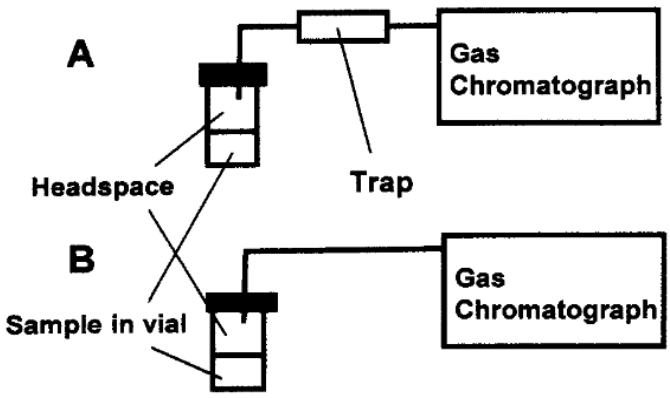

Fig. 13. Dynamic vs. static headspace sampling technique. (A) Dynamic headspace uses a trap to concentrate and collect volatile residual solvents before introduction to the column. (B) static headspace technique in which a volume of the headspace gas phase is introduced directly to column [78].

Whereas in static HS-GC system, a small volume of the sample is placed in a sealed headspace vial and heated to a specific temperature to promote the transfer of analytes into the headspace gas phase (**Fig. 13B**), a sampled volume of the headspace gas phase is then introduced into the GC system for separation and analysis [78]. This technique is usually preferred when the pharmaceutical samples are readily soluble in solvents, such as water or dimethyl sulfoxide [78]. Detection limits of a nanogram-per-milliliter for residual solvents in pharmaceutical products were reported for static HS-GC [80].

3.2.3 Solid-Phase Microextraction

The pharmaceutical industry often uses solid-phase microextraction as a sample preparation technique in gas chromatography to detect residual solvents due to its ability to achieve low limits of detection [78]. In this technique, an extraction phase is dispersed on a solid support, and exposed to the sample for a period of time to allow partition of the analytes between the extraction phase and sample matrix, and desorption of the extracted analytes are then introduced to the GC system [81]. SPME is generally classified into 2 modes according to the position of the extraction phase (**Fig. 14**), direct SPME and headspace SPME.

In residual solvent analysis, headspace SPME is the preferred mode of analysis where the analytes are transported, and extracted from the headspace (air barrier) before reaching the SPME extracting phase (as shown in **fig. 20**). This approach can serve to protect the fiber coating from being damaged by the non-volatile and high molecular mass components of the sample matrix [82]. Additionally, headspace SPME was shown to be able to extract a wide range of organic compounds [83], and it offers execution simplicity and low cost of hardware too [78].

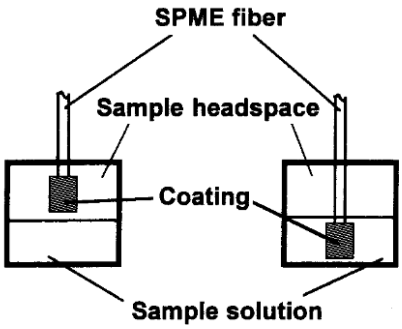

Fig. 14 Modes of Solid solid-phase microextraction sampling technique. (A) Headspace SPME, where the fiber coating is exposed to the headspace phase above the sample. (B) Direct SPME, where the fiber coating is immersed in the sample matrix [78].

3.3 Separation Step in Gas Chromatography

The separation step of analytes is performed using different capillary and wide-bore columns, this process is achieved by carrying the mixture of analytes using a mobile phase (carrier gas) such as nitrogen or hydrogen through the column which is packed by the stationary phase. Two types of columns are employed and used in gas chromatography systems, which includes capillary (narrow-bore) and packed open tubular (wide-bore) columns (see **Fig. 15**) [84].

Columns

Packed columns	Capillary columns
Stationary phase is coated directly in the column	Stationary phase is coated with the inner wall of the column
Applicable for both GSC and GLC	Applicable only for GLC
Liquid phase is adsorbed onto the surface of the beads in a thin layer or onto the solid inert packing	Liquid stationary phase is immobilized on the capillary tubing walls

Fig. 15. Types of columns used in Gas chromatography (GSC is a short for gas-solid chromatography, GLC is a short for gas-liquid chromatography).

Capillary columns are typically preferred over packed columns, with an estimation that over 80% of gas chromatography applications are using capillary columns, due to its fast and efficient separation [84]. Capillary columns are long tubes which can be made out of glass, metal, or quartz. Capillary columns are hollow structures, with a diameter which ranges between 50 to 500 micrometer, and they offer a wide variety of stationary phases which can coat the

inside of the column [72]. In residual solvents analysis, the most popularly used stationary phases are listed in **Table 7**.

Table 7. Popular column phases used for residual solvents determination.

Stationary phase	United States Pharmacopoeia phase name	Additional comments
6%-Cyanopropylphenyl-94%-dimethyl polysiloxane	Equivalent to USP phase G43	Recommended by pharmacopoeias, low/mid polarity
6% Cyanopropyl-phenylmethylpolysiloxane	Equivalent to USP phase G43	Recommended by pharmacopoeias, low/mid polarity
Polyethylene glycol (PEG)	Close equivalent to USP phase G16	Recommended by pharmacopoeias, high polarity
5% Phenylmethylpolysiloxane	Equivalent to USP Phase G27	Non-polar
Polydimethylsiloxane	Equivalent to USP Phase G2	Non-polar
50% Trifluoropropylmethylpolysilox-ane	Close equivalent to USP Phase G6	Mid/high polar

3.4 Gas Chromatography Detectors for Organic Residual Solvent Analysis

When a suitable detector is used, gas chromatography systems can separate, identify and quantify substances with low detection limits. Generally, wide selection of detectors can be employed in GC systems, and they could be classified into universal detectors [85] and selective detectors [86], examples of which given in **Table 8**. In residual solvents analysis, flame-ionization detectors and mass spectrometer detectors are the most appropriate choices. If the analytes are known or suspected, the FID is preferred, where it detects almost all carbon containing molecules, except for small molecular compounds such as carbon monoxide [87], which consequently makes it ideal for organic residual solvent detection. Moreover, flame-ionization detectors are commonly deployed in GC systems for residual solvents analysis due to their low detection limits, general reliability as well as its wide linear dynamic range [88].

On the other hand, mass spectrometer detectors are the preferred choice in residual solvents analysis, when analytes are unknown and an extra level of identification is needed [72]. MS detectors are highly sensitive and selective detectors (see **Table 8**) which work by ionizing the molecules in the sample, followed by separation based on mass-to-charge-ratio. Consequently, MS detectors provide both qualitative and quantitative data about the analytes [86], which can be either deployed in scan mode as a universal detector or in ion monitoring mode as a selective detector.

Table 8. Commonly used detectors in gas chromatography and their characteristics [88].

Detector	Type	Approximate detection limit	Selectivity	Dynamic linear range
Flame ionization detector	Universal (organic/carbon compound)	2×10^{-12}g/s	None, universal	Greater than 10^7
Electron capture detector	Selective (halogens and other electron withdrawing groups)	Highly variable as low as 5×10^{-15}g	None	10^4
Photoionization detector	Universal	2×10^{-13}g/s	Based on ionization energy	Greater than 10^7
Thermal conductivity detector	Universal	4×10^{-10}g/ml	None	Greater than 10^7
Mass spectrometry	Universal or selective	Variable, as low as 25 femtogram	Variable, can be used for selective ions	10^5

4. EXPERIMENTAL PART

4.1 Materials and Reagents

The reagents which were used in the experiments include HBDs, HBAs, ten common organic solvents in the pharmaceutical industry, and 3 different pharmaceutical products for the testing of the developed method.

Methanol ≥ 99.8 % Merck, Ethanol 96% Vilniaus degtinė, 1-Butanol ≥ 99.7% Sigma-Aldrich, 1-Propanol ≥ 99.7% Sigma-Aldrich, Acetone ≥ 99.9 % Merck, Chloroform ≥99.5% Sigma-Aldrich, Carbon tetrachloride ≥ 99 Sigma-Aldrich, Chlorobenzene ≥ 99.8% Sigma-Aldrich, Cumene ≥ 98 Sigma-Aldrich, Acetonitrile ≥ 99.5% WVR, DMF ≥ 99 Sigma-Aldrich, D-Glucose ≥ 99% Alfa Aesar, Distilled water.

Selected commonly used pharmaceutical products from local pharmacies were used to test the method, ACTIFED® 60mg/2.5mg Tablets (pseudoephedrine hydrochloride/Triprolidine hydrochloride) (McNeil). Flavamed® 30mg tablets (ambroxol hydrochloride) (BERLIN-CHEMIE MENARINI). Gripex® 500/30/15mg tablets (paracetamol/pseudoephedrine hydrochloride/ dextromethorphan hydrobromide) (US Pharmacia).

4.2 Equipment and Conditions

Different DESs were synthesized according to the conditions listed in **Table 9** using microwave reactor Monowave 450 (Anton Paar) with the exception of ChCl: Urea, which was prepared by conventional heating using a laboratory oven set at the specified temperature (80 ^{o}C). A ternary DES system (ChCl: CA: Glu) was synthesized using choline chloride, citric acid monohydrate, and glucose, with different water contents (15% and 20%).

Table 9. DES synthesis conditions and methods.

DES	HBA	HBD 1	HBD 2	Molar ratio	Water additive %	Temp. (^{o}C)	Time (seconds)
ChCl: Urea	ChCl	Urea	-	1:2	0	80	600*
ChCl: CA	ChCl	CA	Glu	1:1:1	15	80	90
ChCl: CA: Glu	ChCl	CA	Glu	1:1:1	20	80	90
ChCl: CA	ChCl	CA	-	1:1	15	80	60
ChCl: CA	ChCl	CA	-	1:1	20	80	60
ChCl: Glu	ChCl	Glu	-	1:1	15	80	30
ChCl: Glu	ChCl	Glu	-	1:1	20	80	30

HBA – hydrogen bond acceptor, HBD – hydrogen bond donors, ChCl – Choline chloride Glu – glucose, CA – citric acid monohydrate.
* Casual heating was performed using a temperature of 80 ^{o}C due to stability issues when using microwave reactor for ChCl: Urea sample.

Headspace gas chromatography experiments were performed on a PerkinElmer Clarus 580 series gas chromatograph (PerkinElmer, USA) equipped with a flame ionization detector

(temperature 250°C, hydrogen flow 40 ml min–1, air flow 400 ml min–1, auxiliary gas (helium) flow 30 ml min–1). The GC system was equipped with the ZB-WAX plus capillary column (30 m long, 0.32 mm internal diameter and stationary phase layer thickness 1 μm) (Phenomenex, USA). Headspace extraction and sample introduction was performed on a PerkinElmer Headspace Sampler Turbomatrix 16 (PerkinElmer, USA) equipped with a balanced pressure system. Twenty milliliter headspace vials were used in all experiments. A headspace vial was positioned in the HS autosampler and equilibrated at selected temperature. The needle temperature and the transition line temperature were by 10°C higher than the headspace vial equilibration temperature. The settings of the headspace sampler were 1 min for pressurization and 0.05 min for injection. The GC oven temperature was programmed as follows: 35°C for 5 min from 35 to 85°C at 10°C min^{-1} and from 85°C to 200°C at 25°C min^{-1}. The total analysis time was 14.6 min.

4.3 Detailed Experimental Description

4.3.1 Deep Eutectic Solvents Synthesis

Quantities needed for synthesizing 10 g of each DES was calculated using the following equation (3):

$$n_1 \cdot M_1 + n_2 \cdot M_2 = \rightarrow f = \frac{n1 \cdot M1 + n2 \cdot M2}{m} \quad (3)$$

where n_1 – the amount of substance of component 1; n_2 – the amount of substance of component; M_1 – the molar mass of first component ; M_2 – the molar mass of second component; m – total mass of DES; f – dilution factor.

Water content was calculated as a fraction of the total required volume of the DES, other required masses were calculated using f, by multiplying the molar mass of the component by the required amount of component in gram, and finally divide it by f.

Table 10. Quantities used to prepare 10 g of each DES with different water content.

Molar ratio	HBA	HBA Mass (g)	HBD 1	HBD 1 mass (g)	HBD 2	HBD 2 mass (g)	Water (g)
1:2	ChCl	5.37	Urea	4.63	-	-	0
1:1:1	ChCl	2.32	CA	3.18	glucose	3.00	1.50
1:1:1	ChCl	2.18	CA	3.00	glucose	2.82	2.00
1:1	ChCl	3.42	CA	4.92	-	-	1.50
1:1	ChCl	3.23	CA	4.63	-	-	2.00
1:1	ChCl	3.71	glucose	4.79	-	-	1.50
1:1	ChCl	3.49	glucose	4.51	-	-	2.00

The calculated quantities were weighted and placed in a microwave reactor vial equipped with a magnetic bar stirrer. The vials were then placed in the microwave reactor (except for ChCl: Urea) to perform the microwave assisted synthesis of the DES using the conditions in **Table 9**. The programming of the reactor simply consisted of 3 steps: firstly, *heating* to reach the desired temperature (**Table 9**), followed by *holding* for a period of time with stirring, and finally *cooling* the sample down to 50^{o} C.

4.3.2 Headspace Gas Chromatography Method Development

Ten different organic solvents were selected (listed in **Table 11**) from different classes according to ICH Q3C guidelines (**Table 4, 5, 6**), due to their common use in the pharmaceutical industry and their presence as residual solvents.

Table 11. Selected commonly used solvents in pharmaceutical industry with their classes according to ICH Q3C guidelines.

Selected solvents	Classes according to ICH Q3C guideline
Carbon tetrachloride	1
Chlorobenzene	2
Acetonitrile	
Methanol	
Chloroform	
1-butanol	3
1-propanol	
Acetone	
Ethanol	
Cumene	

Samples of the selected analytes were prepared separately in water, and HS-GC analysis was performed according to the conditions in chapter 4.2 to determine the retention time of the ten selected analytes.

The method was further optimized (heating time, injection time) using the selected DES as sample matrix. Heating time optimization was performed using 4 sample of DES 1 g, each sample was spiked with a fixed amount of organic solvents mixture, peak areas were plotted against 4 different times (5, 10, 15 and 20 minutes) to evaluate the adequate amount of analytes which got transferred to the gas phase. Similarly, to optimize sample injection time, 4 samples (1 g each) of the selected DES were spiked, analyzed and peak areas were plotted using 4 points (0.01, 0.03, 0.05 and 0.07 minutes).

4.3.3 Deep Eutectic Solvents Thermal Stability

The thermal stability of the 7 synthesized deep eutectic solvents were determined at 80 °C, 100 °C and 120 °C by adding 1 g of the DES to a headspace vial and running the method described in chapter 4.2 previously. The detection of new peaks at increasing temperature indicates the degradation of the synthesized DES or the presence of volatile/semi-volatile impurities, therefore the resultant chromatograms were inspected to detect the appearance of any new peaks with temperature increases.

4.3.4 Deep Eutectic Solvent Selection

1 g of each synthesized DESs were spiked with a mixture of the selected organic solvents (2 mg/g) to a fixed final concentration of 5 ppm in 7 different headspace vials, and analyzed with HS-GC method given in chapter 4.2. The obtained peak areas were normalized and evaluated to investigate the effect of varying the matrices on different ORS with regards to their recovery from the matrix of the sample.

4.3.5 Calibration Curve

The calibration curve was prepared by spiking the selected DES with a mixture of the organic solvents (2 mg/g) in dimethylformamide to a final concentration of 100 ppm, and further diluting to different concentrations (60, 30, 10, 5, 1 ppm) using DES. Peak areas of ORS were determined and plotted against the concentrations in ppm, coefficient of determination, linear equation were calculated using Microsoft Excel.

4.3.6 Optimized Method Application in Common Pharmaceuticals

Three commonly used pharmaceutical products were purchased from local market (ACTIFED®, Flavamed®, Gripex®), and they were used to apply the optimized method of ORS determination using HS-GC and DES as a sample matrix. Three samples were prepared separately for each product by grinding the tablets and diluting the sample 5 times using DES, by weighing 0.2 g of the powder and adding 0.8 g of the selected DES to dispense the powder. The samples were sealed in the HS vials, sonicated in water bath for 10 minutes, and introduced to the HS-GC sampler, GC analysis was conducted according to the selected conditions after the optimization in chapter 4.3.5 using the selected DES as a sample matrix.

4.4 Results and discussion

4.4.1 Deep Eutectic Solvents synthesis

DESs were successfully synthesized using the microwave assisted heating method (except for ChCl: Urea) and the quantities described in chapter 4.3.1 and were consequently used in the following experiments. ChCl: Urea was synthesized using conventional heating duo to its

observed degradation following the microwave assisted, this could be attributed to the oscillating effect [89] which can cause polar substance (Urea) to decompose and form by-products. On the other hand, conventional heating doesn't directly interact with the substance, and it primarily heats the sample from outside and towards the center.

4.4.2 Headspace Gas Chromatography Method Development

The separation conditions using HS-GC were optimized in order to separate all substances in short period of time. Under the conditions described in chapter 4.2 all analytes were base line separated (**Fig. 16**), and retention times of the selected ORSs were obtained (**Table 12**). Parameters and the column were selected according to the best separation results, and the pharmacopoeias recommendations.

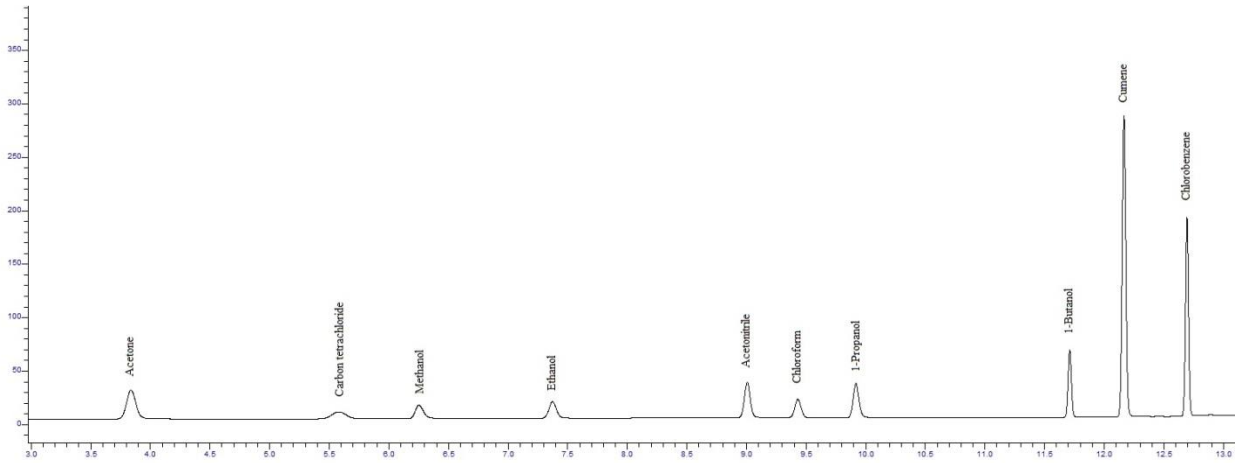

Fig. 16. Chromatogram of separated analytes after HS-GC method development with base line separation.

Table 12. Retention time of the selected organic solvents.

Organic residual solvent	Retention time (minutes)
Acetone	3.8
Carbon tetrachloride	5.6
Methanol	6.1
Ethanol	7.4
Acetonitrile	9
Chloroform	9.4
1-propanol	9.9
1-butanol	11.7
Cumene	12.15
Chlorobenzene	12.7

4.4.3 Deep Eutectic Solvents Thermal Stability

Synthesized DESs' stability was evaluated at different temperatures, to detect the presence of any peaks that might overlap with the target ORSs' peaks (Chromatograms presented in Appendix 1-3). 100 ^{o}C was selected where all the synthesized DES were stable and showed no observable peak formation. A Peak at (t = 6.2 minutes) consistently appeared in all the resulting chromatograms (**Fig. 17**). Further investigation revealed that the observed peak is attributed to methanol, which originates from being an impurity in the choline chloride which was used as a HBA in the synthesized DESs. On the other hand, the results showed no formation of other peaks up to 100 ^{o}C, which may interfere with the identification of the target ORSs.

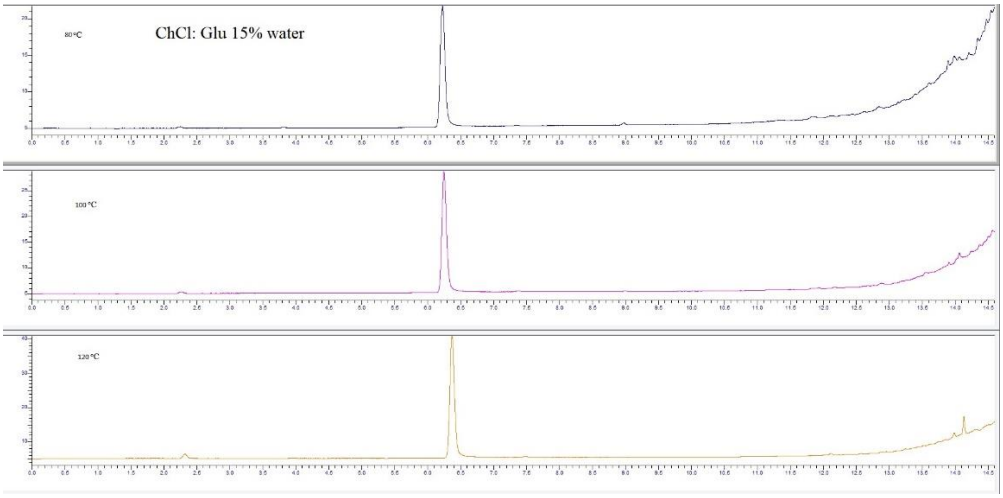

Fig. 17. Thermal stability test of DES, peak at 6.2 minutes represent methanol impurity.

4.3.4 Deep Eutectic Solvent selection

DESs were investigated for their efficiency as a calibration media for headspace GC determination of organic residual solvents. Normalized peak areas of the selected ORSs are given in **Fig. 18**. Since methanol was present as an impurity that is originating from choline chloride, therefore it was excluded from further evaluations.

	Acetone	Carbon tetrachloride	Ethanol	Acetonitrile	Chloroform	1-propanol	1-butanol	Cumene	Chlorobenzene
ChCl-CA-Glu 15% H_2O	91%	100%	100%	97%	100%	100%	100%	100%	100%
ChCl-CA-Glu 20% H_2O	66%	100%	73%	74%	91%	74%	78%	96%	93%
ChCl-CA 15% H_2O	70%	96%	77%	75%	87%	77%	77%	95%	94%
ChCl-CA 20% H_2O	51%	97%	57%	56%	82%	60%	64%	93%	89%
ChCl-Glu 15% H_2O	92%	98%	95%	94%	83%	90%	87%	95%	93%
ChCl-Glu 20% H_2O	79%	100%	82%	81%	86%	84%	89%	99%	95%
ChCl-Urea	100%	91%	96%	100%	74%	84%	76%	90%	89%

Percent Abundance (based on peak area)

Fig. 18. Normalized percent abundance of different ORS in different DESs as calibration media.

The selected organic solvents which were categorized as polar solvents (acetone, ethanol, acetonitrile, etc.) showed greater recovery dependence on the used DES, which can be attributed to the DESs composition of the rich hydrogen bond network, which can interact with the polar solvents and hence lower the transfer to the headspace. In contrast, non-polar solvents including cumene, chlorobenzene, and carbon tetrachloride did not show a significant change in response to the type of DES used, which can be attributed to their greater recovery from the DESs.

Furthermore, the impact of water content on the ORSs recovery was investigated, revealing a noteworthy correlation. Notably, an increase in water content from 15% to 20% resulted in a 10-27% reduction in the recovery of the polar solvents (Acetone, Ethanol, Acetonitrile, etc.), which could be attributed to the water addition distributive effect on the DESs' structure and hydrogen bond network (see chapter 1.3). On the other hand, non-polar solvents showed no significant change in recovery due to water content variation.

ChCl-CA-Glu 15% H_2O was selected as the best media of calibration for further experimentation, optimization and calibration curve preparation, because it was least affected by the changing of the analyte and it showed more consistent results across different ORSs.

4.3.5 Headspace Gas Chromatography Method Optimization

The conditions (i.e., injection time, heating time) of HS-GC were optimized with ChCl-CA-Glu 15% H_2O using the selected ORSs and their peak areas as an indicator. Moreover, the analysis of the data presented in **Fig. 19** which shows the effect of changing the injection time on the peak areas of different ORS, peak areas grew significantly from 0.01 to 0.05 minutes and then remained relatively unchangeable after 0.05 minutes.

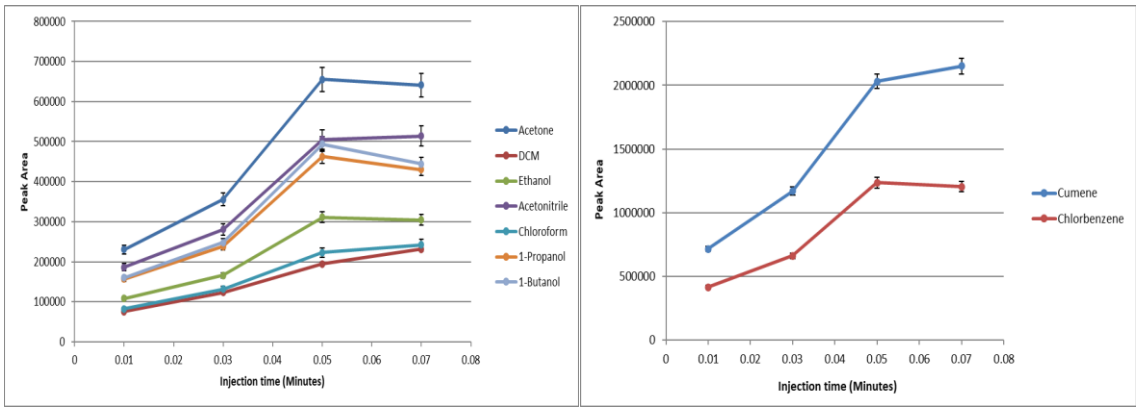

Fig. 19. HS injection time optimization, peak area vs. injection time.

Additionally, heating time was optimized and data is shown in **Fig. 20** which illustrates the effect of heating time on peak areas of different ORSs, it was shown that 15 minutes were needed for most of the selected ORSs to reach equilibrium and transfer to the headspace. Moreover, further heating time showed no significant effect on the analytes transfer to the headspace and hence equilibrium was reached, and no further heating time is required.

The following criteria were selected; injection time of 0.05 minutes, heating time of 15 minutes to reach equilibrium between headspace and DES.

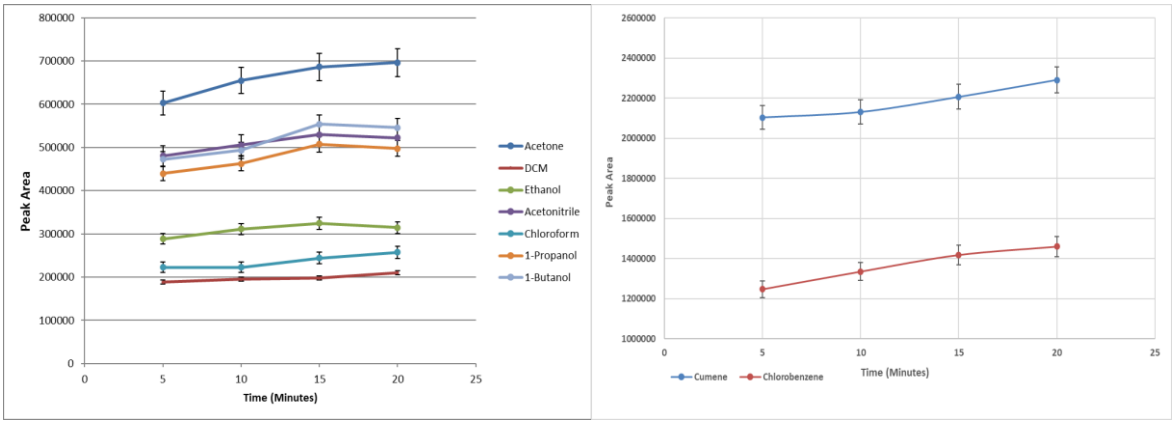

Fig. 20 HS heating time optimization, peak area change vs. heating time.

4.3.6 Calibration Curve

A six-points calibration curve was built for each analyte as presented in appendix 4. Linear equation, coefficient of determination (R^2) of the trendline of each analyte is presented in **Table 13** which were calculated using Microsoft Excel.

The obtained linear equation of each analyte is necessary to quantify the presence of organic residual solvents in the pharmaceutical product. These equations will help determine if the acquired results and concentrations adhere with the accepted limits in the pharmacopeias (**Table 4-6**).

Table 13 Calibration curves data for the selected ORSs.

ORS	Linear Equation	R^2
Acetone	y = 4861.3 x - 4423.2	0.9979
Carbon tetrachloride	y = 1749.5 x - 2708.4	0.9985
Ethanol	y = 1749.5 x - 2708.4	0.9984
Acetonitrile	y = 1749.5 x - 2708.4	0.9982
Chloroform	y = 2200 x - 2928.5	0.9991
1-propanol	y = 3546.5 x - 3154	0.9984
1-butanol	y = 3877.3 x - 2593.5	0.9985
Cumene	y = 3877.3 x - 2593.5	0.9991
Chlorobenzene	y = 3877.3 x - 2593.5	0.9989

The acquired coefficients of determination (R^2) are relatively high and range between 0.9979 to 0.9991 which, comply with the recommended coefficient of determination ($0.98 < R^2$) in the pharmaceutical industry [90], meaning that the fitted trendlines are very close to the data and are representative.

4.3.7 Optimized Method Application in Common Pharmaceuticals

Three samples of pharmaceuticals were analyzed and chromatograms were obtained for each product (presented in **Fig. 22**).

In **Fig. 22a**, peak 2 at 6.3 minutes was expected and it represents the methanol impurity in the used DES, however, peak 1 at 3.8 minutes corresponds to acetone. In **Fig. 22b** peak 2 at 6.3 minutes corresponds to the methanol impurity, and peak 1 at 3.8 minutes corresponds to acetone as an ORS in the product at a concentration lower than 5 ppm. On the other hand, Gripex sample (**Fig. 22c**) showed two major peaks at 11.5 minutes as well as at 6.3 minutes (methanol impurity in DES). Peak 2 which is observed at 11.5 minutes can't be certainly identified due to limitation of flame ionization detector and the unavailability of a reference material at that retention time.

Upon quantitative analysis of the observed peaks in **Fig 22**, peaks at 3.8 minutes, they exhibited a considerable small magnitude and they fell below the lower limit of the first point in the calibration curve (5 ppm after calculating the initial 5 times dilution of the drug sample powder). As we can see in **Fig. 21**, acetone signal intensity remains strong even at the lowest calibration point (5 ppm), so it would be feasible to calibrate acetone at even lower concentrations. However, there is no need to do so for quantifying acetone in pharmaceutical products. This is because the acceptable limit for acetone in pharmaceutical substances is very high (5000 ppm) [91]. Moreover, the developed method allows us to make measurements 1000 times below the acceptable limit.

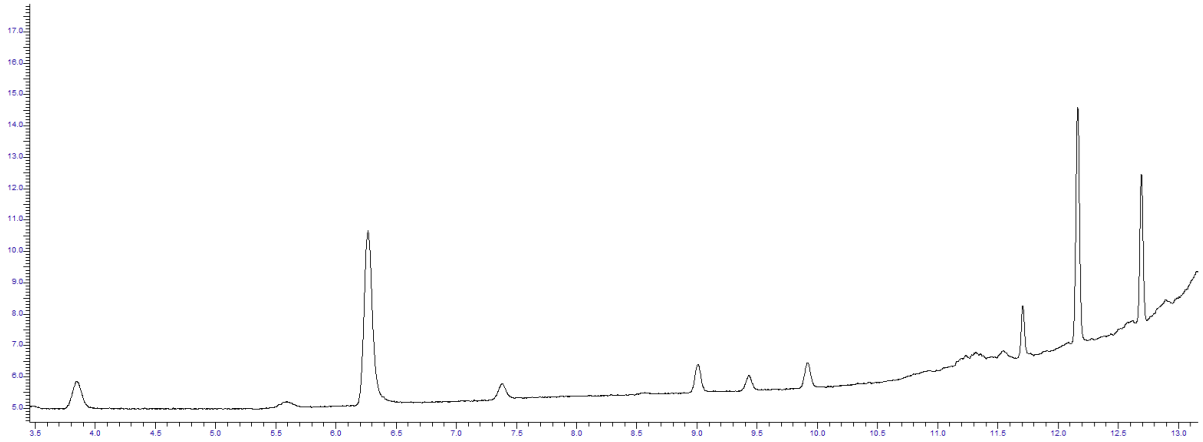

Fig. 21. Chromatogram of the selected ORSs, at a concentration of 5 ppm.

The developed method using DESs allowed us to measure 2nd and 3rd class of ORSs with a limit of detection far below the regulatory requirements set by pharmacopeias. On the other hand, to measure class 1 ORSs with proper accuracy, we need lower limits of determination which can't be achieved using the developed method. However, it should be noted that this limitation is not attributed to the use of DES as sample matrix, but to measure and quantify such low concentrations, the implementation of SPME and HS trap is necessary, as mentioned in chapter 3.2.2 and 3.2.3.

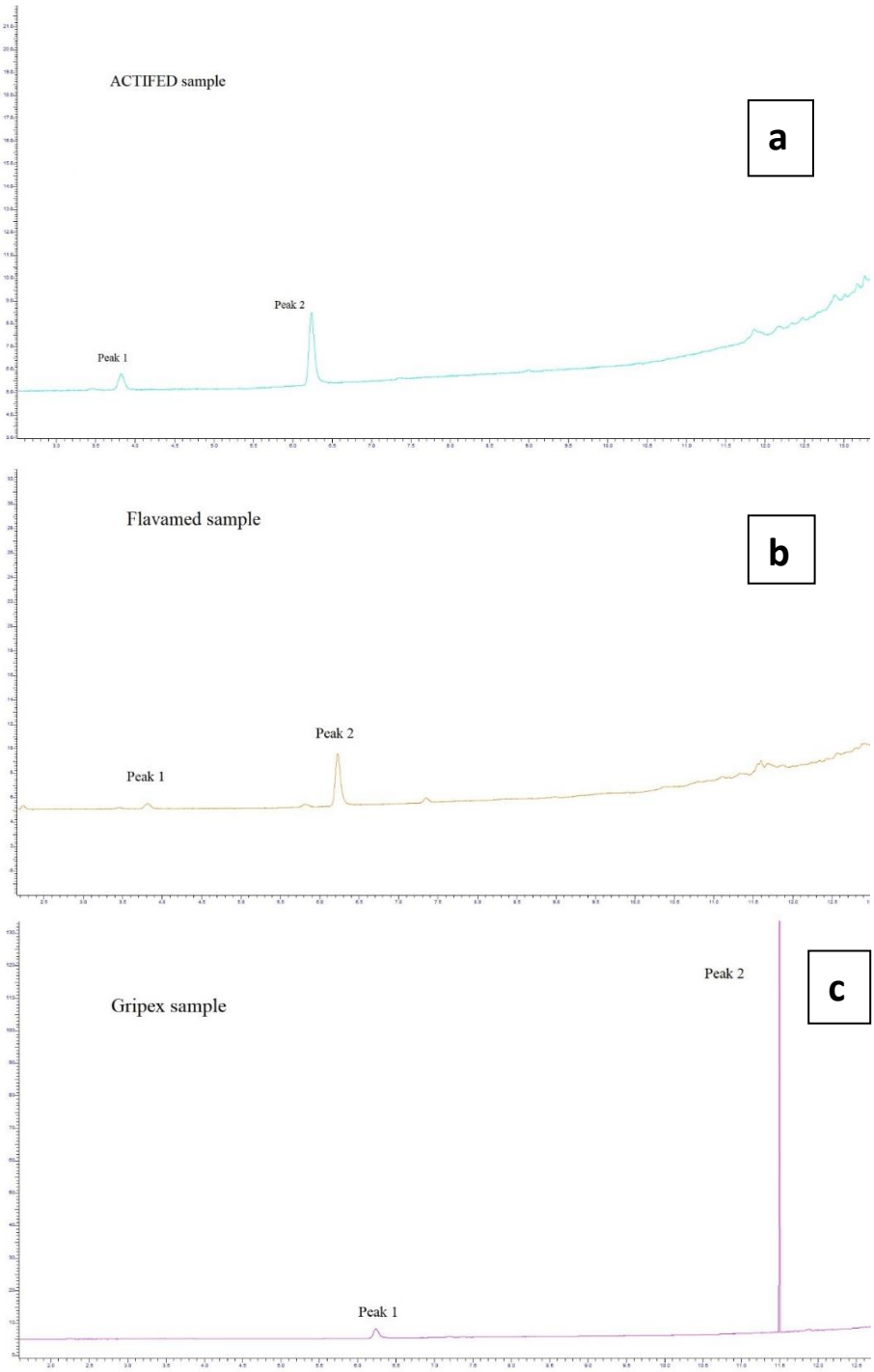

Fig. 22. Chromatograms of analyzed pharmaceutical products. ACTIFED sample chromatogram (**a**), peak 1 (3.8 minutes) and peak 2 (6.3 minutes). Flavamed sample chromatogram (**b**), peak 1 (3.8 minutes) and peak 2 (6.3 minutes). Gripex sample chromatogram (**c**), peak 1 at 6.3 minutes and peak 2 at 11.5 minutes.

CONCLUSIONS

1. Six DESs were successfully synthesized using microwave assisted heating and one DES (ChCl: Urea) was synthesized using conventional heating. It was demonstrated that such DES cannot be made by microwaves because of stability. Thermal stability of the synthesized DESs was assessed using HS-GC, showing that up to 100 ºC all DES are stable.
2. The DESs were investigated for their efficiency as a calibration media. Polar ORSs showed greater recovery dependence on the composition of the used DES, while non-polar ORSs did not show a significant change in response to the type of DES used. Additionally, Water content increase (15 to 20%) was shown to reduce the recovery of polar ORSs by 10-27%.
3. Choline chloride: Citric Acid: Glucose (1:1:1) with 15% water content was shown to be the most efficient sample matrix for the residual solvents determination, HS-GC conditions were optimized using this DES, and were set to 0.05 minutes injection time and 15 minutes heating time.
4. A 6 points calibration curve was built for each ORSs and 3 different common pharmaceutical products were evaluated for the presence of ORSs using the optimized method. The method was shown to be able to detect class 2 and 3 ORSs at limits way lower than the acceptable limits by pharmacopeias. However, for class 1 ORSs, lower limits of determination are needed, which can't be achieved using HS-GC only. In such cases, SPME or trap HS-GC are necessary.

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SUMMARY

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Application of Deep Eutectic Solvents for the Determination of Residual Solvents in Pharmaceuticals Using Headspace Gas Chromatography

Organic residual solvents can be unavoidable in pharmaceutical industry, even after drying, due to the organic solvents presence in many manufacturing steps. These ORSs can present many harmful effects which need to be avoided. Consequently, the presence of ORSs in pharmaceuticals is being strictly regulated by pharmacopeias to control and avoid these harmful effects using sensitive and accurate analytical methods. Headspace gas chromatography HS-GC is considered the optimal choice among the different analytical methods due to the volatile nature of the ORS, moreover, the selection of an appropriate sample matrix is a crucial step for this method.

Deep eutectic solvents (DESs) have received a great deal of attention in many industries, due to their promising advantages and properties. In this work, different DESs were synthesized using microwave-assisted heating (except for ChCl: Urea 1:2) and investigated for their potential as a sample matrix for ORSs determination using HS-GC technique by investigating the resulting peak areas.

Upon investigation, polar ORSs showed greater recovery dependence on the composition of the used DES, while non-polar ORSs did not show a significant change in response to the type of DES used. Consequently, among the different selected DESs, choline chloride: citric acid: glucose (1:1:1) with 15% water content showed the best results and highest ORSs recovery using HS-GC. Injection and heating time were optimized using the selected DES to an injection time of 0.05 minutes and a heating time of 15 minutes. A calibration curve was built for each ORS using the optimized conditions of HS-GC and the DESs as a sample matrix for common pharmaceuticals.

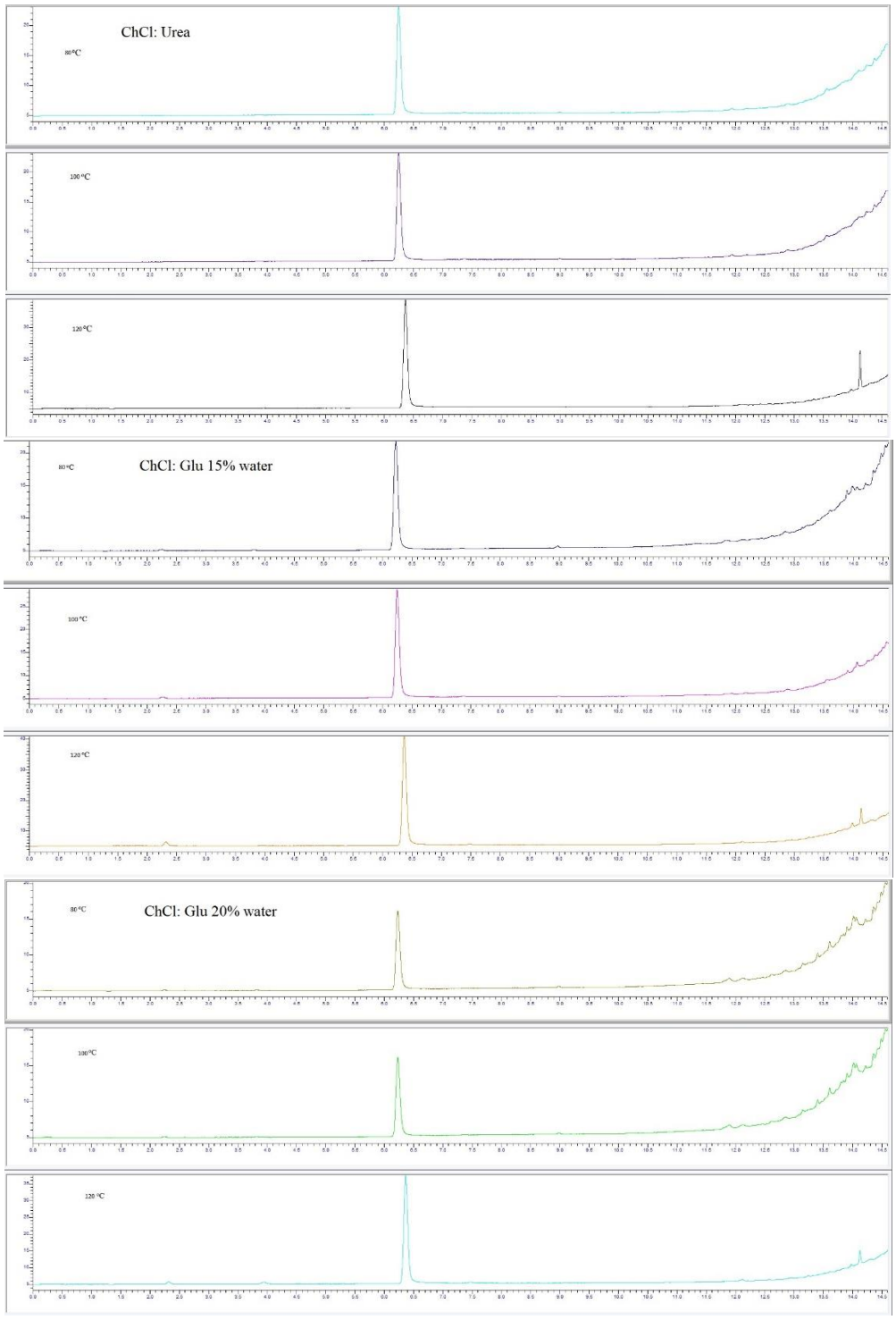
The developed method was shown to be able to detect class 2 and 3 residual solvents at limits way lower than the acceptable limits by pharmacopeias. However, for class 1 residual solvents, lower limits of determination are needed, which can’t be achieved using HS-GC only. In such cases, it should be noted that this limitation is not attributed to the use of DESs as sample matrix, the use of SPME or trap HS-GC is necessary. More investigation into their possible applications and effect of water addition as well as possible mixtures is needed to utilize their full potential as an effective green analytical method.

APPENDICES

1. Appendix 1. Thermal stability experiment chromatograms A
2. Appendix 2. Thermal stability experiment chromatograms B
3. Appendix 3. Thermal stability experiment chromatograms C
4. Appendix 4. Calibration curves of ORSs

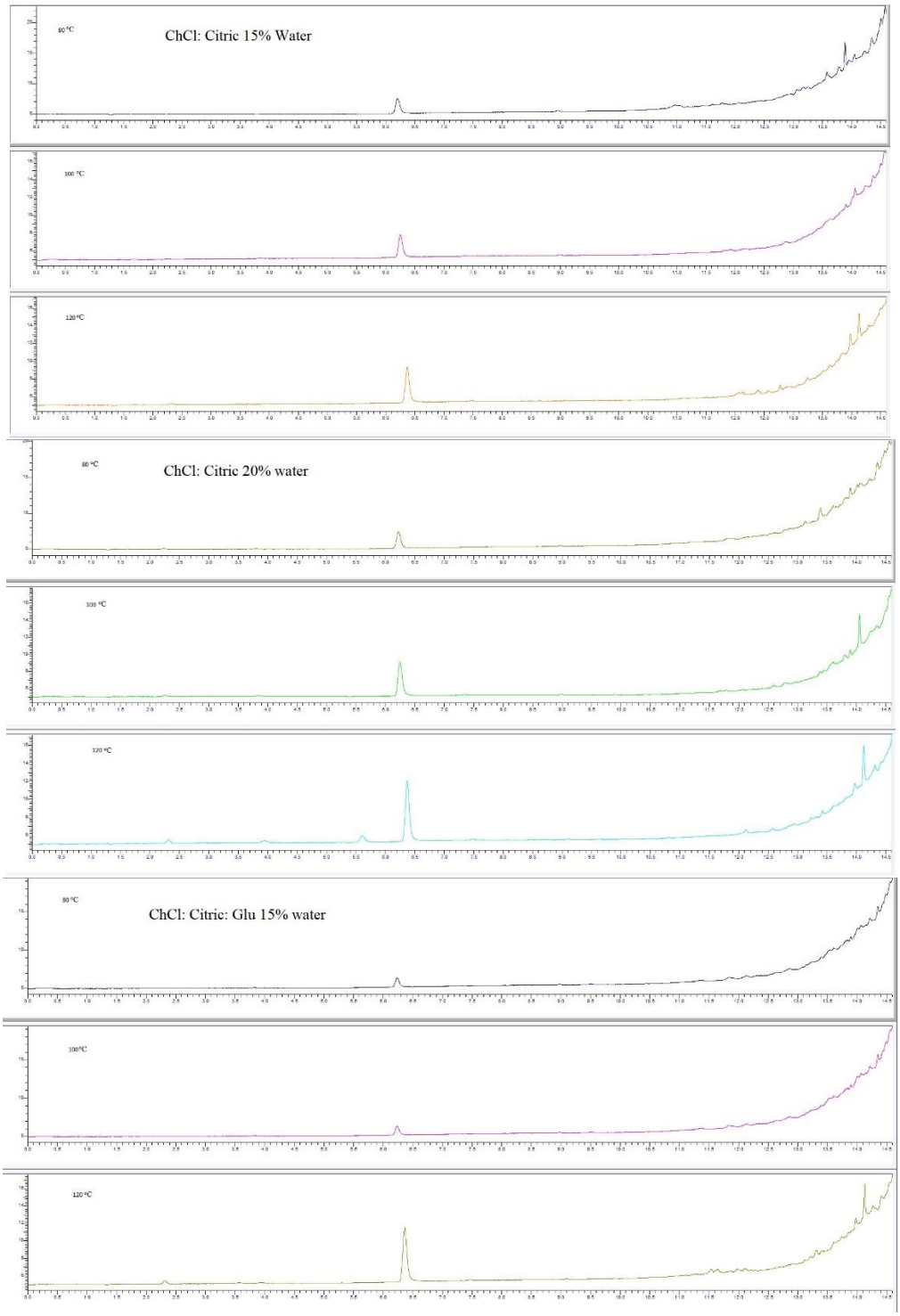
APPENDIX 1

Thermal stability experiment chromatograms A



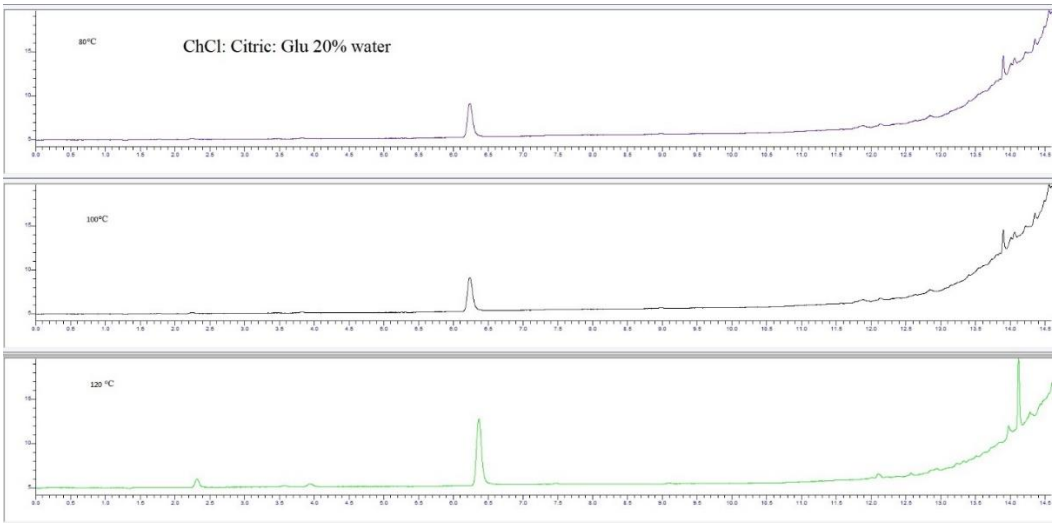
APPENDIX 2

Thermal stability experiment chromatograms B



APPENDIX 3

Thermal stability experiment chromatograms C



APPENDIX 4

Calibration curves of ORSs