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Article

Rapid Monitoring and Determination of Class 1 Residual Solvents in Pharmaceuticals Using Dispersive Liquid–Liquid Microextraction and Gas Chromatography–Mass Spectrometry

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A simple and rapid dispersive liquid–liquid microextraction (DLLME) method coupled with gas chromatography–mass spectrometry (GC-MS) for monitoring and determination of class 1 residual solvents, benzene (Bz), carbon tetrachloride (CT), 1,2-dichloroethane (1,2-DCE), 1,1-dichloroethene (1,1-DCE), 1,1,1-trichloroethane (1,1,1-TCE), in pharmaceuticals was developed and evaluated. The parameters affecting the extraction efficiency of analytes such as type and volume of extraction solvent, type and volume of dispersive solvent and ionic strength were investigated and optimized. 1-Octanol and methanol proved to be the most suitable extraction and dispersive solvents, respectively. The method showed linearity for 1,1-DCE, 1,1,1-TCE, CT, Bz and 1,2-DCE in the ranges of 0.001–80, 0.005–80, 0.002–80, 0.0001–40 and 0.001–80 μg/mL, respectively. The relative recoveries were in the range of 84–92, 87–98, 83–94, 89–98 and 87–96% for 1,1-DCE, 1,1,1-TCE, CT, Bz and 1,2-DCE, respectively. The obtained results showed that the proposed method can be used to monitor and determine class 1 residual solvents in pharmaceuticals.

Introduction

Residual solvents in pharmaceuticals are defined as organic volatile chemicals (OVCs) that are used or produced in the manufacture of drug substances and excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield or determine characteristics such as crystal form, purity and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. However, in such products the content of solvents should be evaluated and quantified. Therefore, testing should be performed on drug substances and excipients for residual solvents when production or purification processes are known to result in the presence of such solvents. Drug product should also be tested if a solvent is used during its manufacture.

Residual solvents are separated into three classes based on risk assessment or their potential toxicity level (1). Class 1 solvents should be avoided in all pharmaceutical manufacturing because they have known human carcinogens or they have strongly suspected carcinogens and/or environmental hazards. Table I lists some physical properties of class 1 residual solvents and their acceptance concentration limits.

Gas chromatography (GC) with the appropriate detector is the most popular solvent analysis method for qualitative and quantitative determination of OVCs in pharmaceutical products. In general, the techniques that used to screen and analyze residual solvents in pharmaceuticals can be classified into two categories: direct injection (DI) method (2–6) and methods that require the advanced sample preparation such as headspace analysis (7–9), solid-phase microextraction (SPME) (10–12) and liquid-phase microextraction (LPME) (13–15). The DI method is applied when the active pharmaceutical ingredient (API) is soluble in high boiling organic solvents, and other sample components evaporate in relatively low temperature. Because of lack of these features in many drugs, the DI method cannot be used always. However, it has the big disadvantage that non-volatile components, such as the APIs or the excipients, are also injected, and that leads to injector and column contamination. Due to the existing problems in the DI method, in the advanced sample preparation methods, the VOCs were extracted from the matrix before GC analysis. On the other hand, it should be regarded that concentrations of some VOCs, specifically class 1 residual solvents, in pharmaceuticals are at part per million (ppm) levels. The main advantage of these methods is their ability to combine sampling, extraction, clean-up and pre-concentration of analytes altogether. Therefore, the development of sample preparation methods with this ability is necessary. The comparison of these methods (headspace, SPME and LPME) shows that LPME is inexpensive than others but suffers from limited enrichment factor (EF) and long extraction time.

Assadi and coworkers (16) developed a novel microextraction technique, termed dispersive liquid–liquid microextraction (DLLME), as an extraction and pre-concentration method in 2006. In this method, the extraction is performed by an interaction between the sample and a cloud of fine extractant drops after the injection of an appropriate mixture of extraction and disperser solvents into an aqueous sample. After the formation of a cloudy solution, the surface area between the extracting solvent and the aqueous sample increases, causing a quick equilibrium state. Therefore, the extraction time becomes very short. In fact, simplicity, low cost, rapidity and high EF are the remarkable advantages of DLLME (17). Recently, a new method was developed by using the DLLME followed by capillary GC with flame ionization detector (FID) for quantitative determination of residual solvents in pharmaceuticals (18). The gas chromatography–mass spectrometry (GC-MS) technique is very powerful for qualitative and quantitative analysis compared to GC-FID. However, the power of this technique lies its ability to record mass spectra for analytes. These data can be used to determine the identity as well as the quantity of unknown components. On the other hand, the high-sensitive MS detector provides the ability to detect low concentrations of the analytes. However, there are not many methods for determination of class 1 residual

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Table I
Class 1 Residual Solvents, Acceptance Limit, Solubility in Water and Their Boiling Points

Solvent	Acceptance limit (μg/mL)	Solubility in water (g/L) at 20°C	Boiling point (°C)
1,1-DCE	8	2.5	31.2–31.7
1,1,1-TCE	1,500	1.3	74
CT	4	0.81	76.7
Bz	2	1.8	80.1
1,2-DCE	5	8.7	83.5–84.1

solvents in the literature (9, 18, 19). Therefore, developing an inexpensive and fast analytical method for monitoring and determination of class 1 residual solvents in pharmaceuticals is essential. To the best of our knowledge, this study is the first report describing determination of class 1 residual solvents in pharmaceuticals by using DLLME and GC-MS analysis.

This study presents a new and rapid method for monitoring and determination of class 1 residual solvents in pharmaceuticals by DLLME with gas chromatography–mass spectrometry. Analytical parameters of the DLLME-GC-MS method were compared with previously reported methods.

Experimental

Chemicals

Chemicals, such as benzene (Bz), carbon tetrachloride (CT), 1,2-dichloroethane (1,2-DCE), 1,1-dichloroethene (1,1-DCE), 1,1,1-trichloroethane (1,1,1-TCE), chlorobenzene (CB), methanol, 1-octanol, acetonitrile, acetone, ethanol and sodium carbonate were purchased from Merck and Fluka chemical companies. Aqueous solutions were prepared with deionized water from a Milli-Q system (Millipore, USA). Zidovudine, lamivudine, acyclovir and ribavirin were obtained from Hetero (Hyderabad, India). The structures of these drugs are shown in Supplementary Material, Figure S1.

Instrumentation

The injection was performed using a 1-μL Hamilton GC syringe. The GC-MS system (Agilent Technologies 6890N GC system and inert MSD model 5975C with Triple-Axis Detector, USA) equipped with a HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) was used. Helium was used as the carrier gas at the constant flow rate of 1.0 mL/min. The split ratio was adjusted at 1/50. The oven temperature was initially set at 40°C for 12 min, then it was raised to 180°C at a rate of 20°C/min and left constant for 1 min. The injector temperature was kept at 250°C. Transfer line was set at 300°C and quadrupole mass spectrometer was scanned over the 50–250 m/z range.

Standard solutions preparation

A primary stock solution (1000 μg/mL) of the five organic solvents (CT, 1,2-DCE, 1,1,1-TCE, 1,1-DCE and Bz) was prepared by dissolving appropriate volumes of these organic solvents in methanol. Internal standard solution (1,000 μg/mL) was prepared by dissolving the CB in methanol. Working standard solutions were prepared by proper serial dilution in deionized water. Aqueous standard solutions were prepared by mixing 10 mL of working standard solutions with 0.1 mL of internal standard solution and 0.3 g sodium carbonate.

Internal standard calibration can be used to compensate for variation in analyte recovery and absolute peak areas due to matrix effects and GC injection variability. Prior to the extraction, a known quantity of a known additional analyte (0.1 mL of CB) was added to each sample and standard solution.

Pharmaceutical samples preparation

Aqueous sample solutions were prepared by mixing 100 mg of drug substance, 10 mL of deionized water, 0.1 mL of internal standard solution and 0.3 g of sodium carbonate. Sample solutions were subjected to the optimized DLLME method.

DLLME procedure

Under the finally optimized conditions, 10.0 mL of the sample or standard solution, 0.3 g of sodium carbonate and 0.1 mL of internal standard solution were transferred to a 15.0-mL screw cap glass tube with conical bottom. Then, a mixture of 10 μL of methanol (dispersive solvent) and 60 μL of 1-octanol (extraction solvent) was injected into the sample solution using a syringe, and the mixture was gently shaken manually for several seconds. A cloudy solution consisting of very fine droplets of 1-octanol dispersed into the sample solution was formed, and the six organic solvents (CT, 1,2-DCE, 1,1,1-TCE, 1,1-DCE, Bz and CB as internal standard) were extracted into the fine droplets. After centrifuging for 3.0 min at 6,000 rpm, the extraction solvent was floated on the surface of solution. After removing the lower phase, the extraction solvent was removed with a 10-μL syringe and transferred into the microtube. Finally, 0.5 μL of this solution was injected onto the GC-MS system for analysis.

Results

Extraction solvent and its volume

The correct choice of extraction solvent is crucial for optimizing the DLLME procedure. The extraction solvent should show low solubility in water, high affinity for the analyte and good chromatographic behavior. There were two reasons for choosing 1-octanol as extracting solvent: (i) immiscibility in water and (ii) high boiling point that leads to elute after then analytes and no overlapping takes place (13).

The effect of extraction solvent volume was studied by rapidly injecting the mixture of 10 μL of methanol with different volumes of 1-octanol (60–200 μL). The results in Figure 1 show that increasing the volume of 1-octanol results in decrease in the extraction efficiency of analytes. The reason for this behavior can be attributed to an increase in the volume of organic phase that led to a decrease in the EF.

Dispersive solvent and its volume

In the DLLME method, type of dispersive solvent and its volume can be affected by the extraction efficiency of analytes. In this study, methanol, acetonitrile, acetone and ethanol were examined as dispersive solvents. As can be seen from Figure 2,

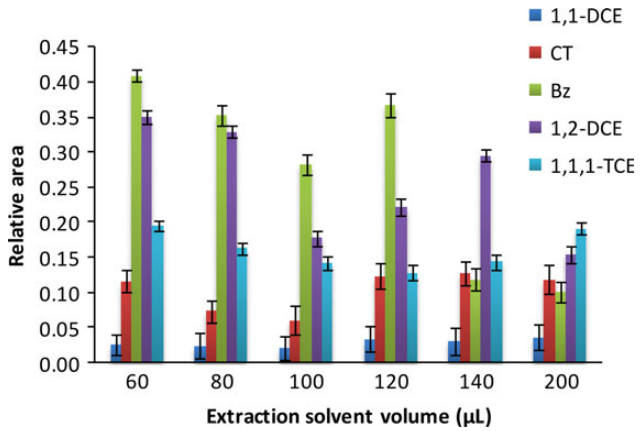

Figure 1. Effect of extraction solvent volume on the extraction efficiency of analytes. Extraction conditions: extraction solvent, 1-octanol; sodium carbonate concentration, 3% w/v; dispersive solvent, methanol; dispersive solvent volume, 10 μL. From left to right; 1,1-DCE, CT, Bz, 1,2-DCE and 1,1,1-TCE.

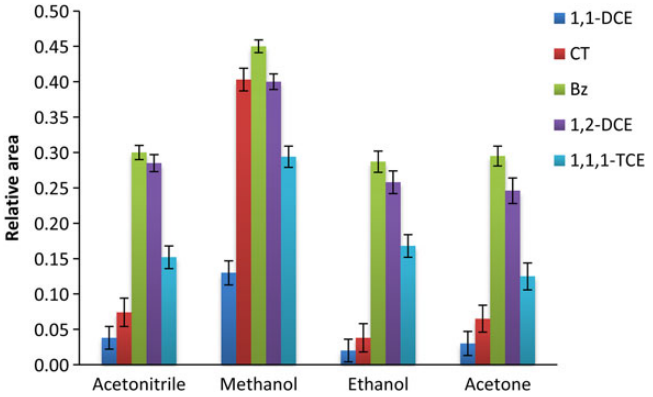

Figure 2. Effect of dispersive solvent on the extraction efficiency of analytes. Extraction conditions: extraction solvent, 1-octanol; sodium carbonate concentration, 3% w/v; extraction solvent volume, 60 μL; dispersive solvent volume, 10 μL. From left to right; 1,1-DCE, CT, Bz, 1,2-DCE and 1,1,1-TCE.

methanol has the highest extraction efficiency for all analytes. Therefore, methanol was chosen as dispersive solvent.

Different volumes of methanol as dispersive solvent were investigated in the range 10–200 μL. As shown in Figure 3, by increasing methanol volume, the extraction efficiency of analytes decreases. Addition of methanol led to increase the solubility of 1-octanol and decrease the organic phase volume. Then, reduction of extraction efficiency occurred. Therefore, 10 μL of methanol was selected as the optimum dispersive solvent volume for all subsequent experiments.

Effect of ionic strength

The salt addition to the sample may influence the efficiency of extraction in the DLLME process. In order to examine the effect of the ionic strength of samples on the extraction efficiency of analytes, a series of experiments were performed using the aqueous standard solutions containing different percentages of sodium carbonate (0–7%, w/v). As shown in Figure 4, the presence of the salt considerably enhanced the extraction efficiency of the analytes, reaching a maximum at 3.0% w/v of sodium

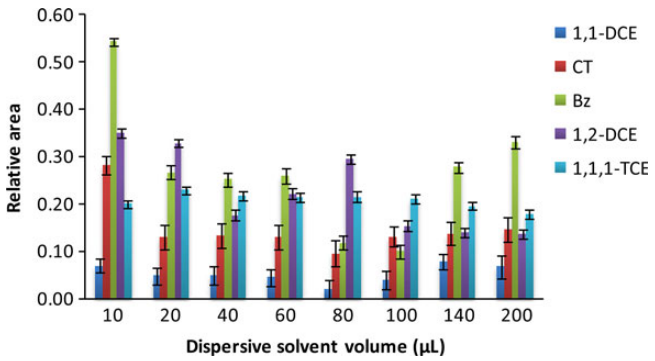

Figure 3. Effect of dispersive solvent volume on the extraction efficiency of analytes. Extraction conditions: extraction solvent, 1-octanol; sodium carbonate concentration, 3% w/v; dispersive solvent, methanol; extraction solvent volume, 60 μL. From left to right; 1,1-DCE, CT, Bz, 1,2-DCE and 1,1,1-TCE.

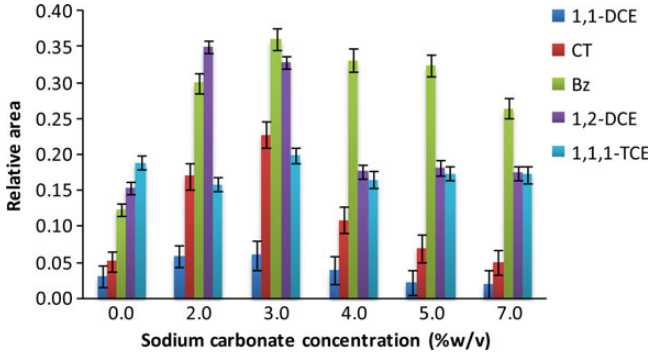

Figure 4. Effect of ionic strength on the extraction efficiency of analytes. Extraction conditions: extraction solvent, 1-octanol; extraction solvent volume, 60 μL; dispersive solvent, methanol; dispersive solvent volume, 10 μL. From left to right; 1,1-DCE, CT, Bz, 1,2-DCE and 1,1,1-TCE.

carbonate. It is observed that at higher sodium carbonate concentrations, extraction efficiency of analytes decreased. This behavior can be attributed to the increase of sample viscosity. Consequently, transfer of analytes from the sample matrix to organic phase becomes difficult (20). Therefore, 3.0% w/v of salt was selected as the optimum concentration for all subsequent experiments.

Method validation

Linearity of the DLLME-GC-MS method was evaluated by extracting and injecting standard solutions of mixtures of 1,1-DCE, 1,1,1-TCE, CT, Bz and 1,2-DCE under the optimized conditions. R^2 values of calibration curves were ≥0.997 for the five analytes that approved the linearity of the proposed method. The limits of quantification (LOQs) based on the signal-to-noise ratio (S/N) of 10 were 1.0, 5.0, 2.0, 0.1 and 1.0 μg/L for 1,1-DCE, 1,1,1-TCE, CT, Bz and 1,2-DCE, respectively. Other analytical parameters for the DLLME-GC-MS method are summarized in Table II.

The accuracy of the method was investigated by determining the relative recovery of analytes spiked in several APIs such as zidovudine, lamivudine, acyclovir and ribavirin. Spiked concentration levels (25, 50 and 100% of acceptance limit) were selected according to the acceptance limits of these solvents in pharmaceuticals except for 1,1,1-TCE. Supplementary Material, Figure S2

Table II
Figures of Merit for the DLLME-GC-MS Method

Analyte	Repeatability	Accuracy	Sensitivity		Linearity			
	RSD[a] (%)	Recovery[b] (%)	LOD[c] (μg/L)	LOQ[d] (μg/L)	LR[e] (μg/L)	r^2	Slope	Intercept
1,1-DCE	3.2	87.8	0.3	1.0	1.0–80,000	0.9987	0.0017	0.0028
1,1,1-TCE	3.5	92.5	1.5	5.0	5.0–80,000	0.9993	0.0079	0.0196
CT	3.8	88.2	0.5	2.0	2.0–80,000	0.9994	0.0072	0.017
Bz	3.0	92.8	0.04	0.1	0.1–40,000	0.9991	0.065	0.374
1,2-DCE	3.4	91.8	0.3	1.0	1.0–80,000	0.9976	0.0144	0.0506

Concentration of working standard solutions: 1,1-DCE and 1,2-DCE: 0.001, 0.01, 0.1, 1.0, 10, 30, 60 and 80 μg/mL; 1,1,1-TCE: 0.005, 0.01, 0.1, 1.0, 10, 30, 60 and 80 μg/mL; CT: 0.002, 0.01, 0.1, 1.0, 10, 30, 60 and 80 μg/mL and Bz: 0.0001, 0.001, 0.01, 0.1, 1.0, 10, 20 and 40 μg/mL.
[a]Relative standard deviation for $n = 6$.
[b]Mean relative recovery ($n = 3$).
[c]Limit of detection (S/N = 3).
[d]LOQ (S/N = 10).
[e]Linearity range.

Table III
Accuracy Data for Spiked Analytes in Several APIs

API	1,1-DCE		1,1,1-TCE		CT		Bz		1,2-DCE	
	Added concentration (μg/mL)	Recovery (%) ± SD[a]	Added concentration (μg/mL)	Recovery (%) ± SD	Added concentration (μg/mL)	Recovery (%) ± SD	Added concentration (μg/mL)	Recovery (%) ± SD	Added concentration (μg/mL)	Recovery (%) ± SD
Zidovudine	2	84 ± 5	10	91 ± 3	1	87 ± 5	0.5	92 ± 3	1.25	89 ± 4
	4	86 ± 4	40	95 ± 4	2	86 ± 6	1	96 ± 2	2.5	91 ± 2
	8	89 ± 2	80	94 ± 3	4	84 ± 4	2	96 ± 2	5	93 ± 2
Lamivudine	2	88 ± 6	10	90 ± 5	1	92 ± 3	0.5	89 ± 4	1.25	87 ± 3
	4	87 ± 4	40	92 ± 2	2	89 ± 5	1	93 ± 3	2.5	96 ± 4
	8	92 ± 3	80	93 ± 2	4	94 ± 5	2	92 ± 3	5	95 ± 1
Acyclovir	2	91 ± 4	10	98 ± 6	1	83 ± 5	0.5	90 ± 4	1.25	88 ± 3
	4	85 ± 3	40	96 ± 5	2	88 ± 4	1	95 ± 1	2.5	92 ± 4
	8	89 ± 2	80	91 ± 2	4	87 ± 2	2	98 ± 2	5	90 ± 2
Ribavirin	2	86 ± 7	10	88 ± 4	1	88 ± 3	0.5	90 ± 3	1.25	90 ± 5
	4	89 ± 4	40	87 ± 3	2	87 ± 4	1	92 ± 1	2.5	95 ± 2
	8	88 ± 4	80	95 ± 1	4	93 ± 1	2	91 ± 2	5	96 ± 2

[a]Mean relative recovery ± standard deviation ($n = 3$).

shows the chromatograms of unspiked and spiked zidovudine after extraction. It is clear from the unspiked chromatogram of zidovudine (Supplementary Material, Figure S2A) that there were no interfering compounds at analyte retention times. Table III lists the obtained relative recoveries from the analysis of spiked samples. As can be seen, relative recoveries were in the range of 84–92, 87–98, 83–94, 89–98 and 87–96% for 1,1-DCE, 1,1,1-TCE, CT, Bz and 1,2-DCE, respectively.

Figure 5 shows the chromatograms of blank and analytes that extracted under optimized conditions. As it was observed from the chromatograms, there were no interfering peaks in blank at retention times corresponding to analyte peaks.

Discussion

A new and rapid method for monitoring and determination of class 1 residual solvents in pharmaceuticals by using DLLME-GC-MS was developed. The extraction efficiency for the target analytes by DLLME is influenced by several extraction parameters, such as extraction solvent and its volume, type and volume of dispersive solvent, sample pH and ionic strength. Exception of sample pH, effect of the other parameters on the analytes (CT, 1,2-DCE, 1,1,1-TCE, 1,1-DCE and Bz) and extraction efficiency were investigated and optimized in the previous section. The sample pH plays an important role in the extraction efficiency of the ionizable organic compounds in all liquid–liquid extraction (LLE) techniques. However, in this study all analytes are neutral and have not any charge in the pH range. Nevertheless, over the 2–8 pH range, a series of experiments were performed. The obtained results showed that pH does not have a significant effect on extraction efficiency of analytes. Therefore, there was no need to investigate the pH effect in further experiments. Finally, pharmaceutical samples were analyzed with the optimized DLLME procedure.

The analytical parameters of the proposed method were compared with several reported methods in the literature (Table IV). The results show that the limits of detection (LODs) and LOQs of class 1 residual solvents were improved by using the DLLME-GC-MS. The proposed method can be surely used to monitor and determine the class 1 residual solvents in pharmaceuticals.

Conclusions

The dispersive liquid–liquid microextraction technique coupled with capillary column gas chromatography–mass spectrometry (DLLME-GS-MS) was successfully applied to monitor and

Table IV
The Results of Analytical Performance for Several Reported Methods and the Current Method

Analyte	Method	LOD (μg/mL)	LOQ (μg/mL)	RSD (%)	Recovery (%)	LR (μg/mL)	r^2	Analysis time[a] (min)	Reference
1,1-DCE	HS-GC-FID	7.4	–	7.3	–	0.15–15	0.990	35	(19)
1,1,1-TCE		18.8	–	6	–	0.15–15	0.993		
CT		45	–	6	–	0.15–15	0.998		
Bz		4.8	–	2.4	–	0.15–15	1.000		
1,2DCE		30	–	1.6	–	500–5,000	0.999		
1,1-DCE	HS-GC-MS	1.9	–	15.9	–	0.15–15	0.992	35	(19)
1,1,1-TCE		2.1	–	4.7	–	0.15–15	0.997		
CT		0.71	–	5.7	–	0.15–15	0.998		
Bz		0.7	–	6.2	–	0.15–15	0.994		
1,2-DCE		8.2	–	2.3	–	500–5,000	0.999		
1,1-DCE	HS-GC-FID	0.16	0.55	–	–	3.96–198	0.9997	43	(9)
1,1,1-TCE		0.48	1.68	–	–	0.72–360	0.9925		
CT		0.69	2.42	–	–	2.08–104	0.9970		
Bz		0.01	0.04	–	–	1.36–68	0.9999		
1,2-DCE		0.1	0.33	–	–	0.96–48	1.0000		
1,1-DCE	DLLME-GC-FID	0.003	0.009	3.8	31[b]	0.01–32	0.999	25	(18)
1,1,1-TCE		0.005	0.017	3.5	61[b]	0.01–32	0.997		
CT		0.011	0.04	3.7	71[b]	0.05–160	0.996		
Bz		0.0006	0.002	3.2	39[b]	0.002–6.400	0.996		
1,2-DCE		0.005	0.015	6.1	29[b]	0.01–32	0.999		
1,1-DCE	DLLME-GC-MS	0.0003	0.001	3.2	87.8	0.001–80	0.9987	25	CM[c]
1,1,1-TCE		0.0015	0.005	3.5	92.5	0.005–80	0.9993		
CT		0.0005	0.002	3.8	88.2	0.002–80	0.9994		
Bz		0.00004	0.0001	3.0	92.8	0.0001–40	0.9991		
1,2-DCE		0.0003	0.001	3.4	91.8	0.001–80	0.9976		

[a]Analysis time includes sample preparation and run time.
[b]Absolute recovery.
[c]Current method.

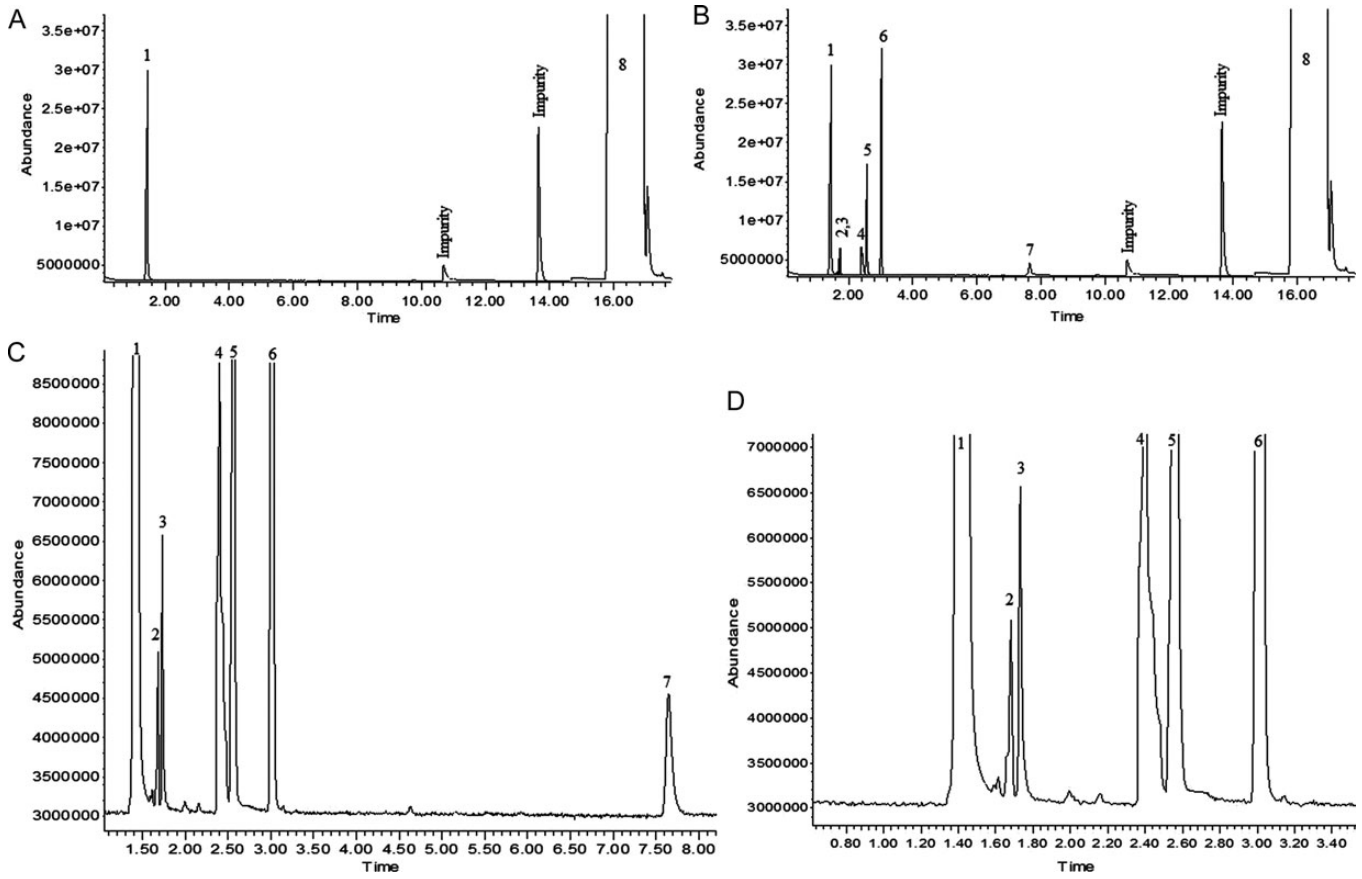

Figure 5. Total ion chromatograms (TIC) of blank extract (A), and standard solution mixture after DLLME under optimized conditions (B). Concentrations of all analytes and internal standard were 10 μg/mL. Expanded TIC for better presentation of smaller peaks (C) and (D). Peaks identification: (1) methanol (dispersive solvent, 1.44 min), (2) 1,1-DCE (1.63 min), (3) CT (1.76 min), (4) 1,1,1-TCE (2.40 min), (5) 1,2-DCE (2.56 min), (6) Bz (3.02 min), (7) CB (internal standard, 7.65 min) and (8) 1-octanol (extraction solvent, 16.02 min).

determine class 1 residual solvents in pharmaceuticals. Compared with DI, headspace, SPME and LPME methods, DLLME has the advantages of simplicity, rapidity, low cost, high recovery and EF. The quantitative data including, linearity, repeatability, accuracy, LOD and LOQ demonstrated DLLME-GS-MS as a suitable method for trace level determination of class 1 residual solvents in pharmaceuticals.

Supplementary Material

Supplementary materials are available at *Journal of Chromatographic Science* (http://chromsci.oxfordjournals.org).

Acknowledgments

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