Quantitative determination of residual 1,4-dioxane in three-dimensional printed bone scaffold

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Summary Background/Objective: A novel porous scaffold poly (lactide-co-glycolide) and tricalcium phosphate (PLGA/TCP) was developed by three-dimensional printing technology for bone defect repair. As a Class 2 solvent with less severe toxicity, content of residual 1,4-dioxane in this newly developed scaffold should be rigorously controlled when it is translated to clinical use. In this study, a headspace gas chromatography-mass spectrometric (HS-GC-MS) method and related testing protocol were developed for quantitative determination of 1,4-dioxane in the PLGA/TCP composite scaffolds.

Methods: Matrix effect analysis was used to optimise the pretreatment method of the scaffolds. Then, the procedure for testing 1,4-dioxane using HS-GC-MS was set up. The accuracy, precision, and robustness of this newly developed quantitative method were also validated before quantification of 1,4-dioxane in the scaffolds with different drying procedures.

Results: Dimethyl formamide (DMF) was the optimal solvent for dissolving scaffolds for GC-MS with proper sensitivity and without matrix effect. Then, the optimised procedure was determined as: the scaffolds were dissolved in DMF and kept at 90°C for 40 minutes, separated on a HP-5MS column, and detected by mass spectrometry. Recovery experiments
Quantitative determination of residual 1,4-dioxane

Introduction

Steroid-associated osteonecrosis (SAON) is one of the most difficult diseases to treat as it largely occurs in large joints leading to articular surface collapse and expensive joint replacement [1–3]. The prognosis of joint replacement in SAON patients is poor due to osteolysis and impaired osteogenesis [4,5]. Accordingly, research and development of osteopromotive scaffolds ready for implantation, which would be capable of activating host cells to differentiate into angiogenic and osteogenic lineage, would be desirable [6,7].

Low-temperature deposition manufacturing (LDM) is a unique rapid prototype technology [8,9] that provides accurate point-to-point control of liquid moulding materials to form scaffolds with a gradient pore structure [10,11]. In some studies, series of porous poly(lactic-co-glycolic acid) and tricalcium phosphate (PLGA/TCP) scaffold were fabricated using the LDM technique [12]. The PLGA/TCP scaffolds exhibited osteoconductive activity and could also be used as a drug carrier [11,13–20].

In the process of scaffold fabrication, 1,4-dioxane was optimised as a necessary solvent used for dissolving PLGA due to its low melting point, liposolubility and highly volatile nature. However, with two oxygen atoms, 1,4-dioxane has been shown to be carcinogenic to animals and humans [21–24]. Furthermore, 1,4-dioxane may also be toxic to liver, lungs, kidneys, and the central nervous system [25]. 1,4-dioxane was defined as a Class 2 solvent associated with less severe toxicity, which should be limited to below 380 ppm according to international regulations, such as Chinese Pharmacopoeia [26], International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [27], and United States Pharmacopoeia [28]. Therefore, it is mandatory to test residual 1,4-dioxane for PLGA/TCP scaffolds and this testing is also necessary for registration and quality control of novel scaffolds.

To date, established testing methods for 1,4-dioxane have been used for quantification analysis in liquid or liquid-like samples, such as drinking water, waste water, vaccines, and cosmetics. These samples were usually processed with double distilled water [29,30]. For scaffolds, the pretreatment is a necessary step to select a proper solvent before analysis.

It was reported that 1,4-dioxane was determined reliably in water by various techniques including direct aqueous injection, purge and trap gas chromatography–mass spectrometric (GC-MS), and GC-MS analysis of continuous liquid–liquid extraction extracts [30]. Conventional purge and trap GC-MS is strictly limited by poor purge efficiency of 1,4-dioxane whose detection limit is about 100 times higher than those efficiently purged volatile organic compounds [30]. Headspace GC-MS (HS-GC-MS) was used commonly in the determination of poorly purified organic solvents in cosmetics [31] as it could reach the target compound to eliminate the background interference in special boiling point of volatile organic compounds. Therefore, HS-GC-MS was chosen as an analysis method for scaffolds to simplify the pretreatment process and acquire high quality chromatogram or spectrum.

The purpose of this investigation was to develop a selective, rapid, yet simple and robust method for the determination of 1,4-dioxane in PLGA/TCP porous scaffolds by HS-GC-MS. The procedure included pretreatment of the scaffolds, sample enrichment by headspace apparatus, separation from other components by GC, and identification by MS. The method was validated and applied to optimise the drying process of scaffolds during fabrication that could also be generalised as standard and/or guideline for wide applications.

Experimental

Standards and reagents

1,4-dioxane (99.9%, GC grade) and dimethyl sulphoxide (GC grade) were obtained from Aladdin (Shanghai, China).
Dimethyl formamide (DMF; HPLC grade) was obtained from J&K Scientific Corporation (Beijing, China).

**Raw material of scaffolds**

PLGA: The ratio of lactic acid to glycolic acid was 75:25. Molecular weight was 250,000 Da and provided by Shandong Institute of Medical Instruments (Shandong, China).

TCP: Low temperature β-tricalcium phosphate. Range of particle size was 0.1–5 μm. It was obtained by chemical precipitation and purchased from Shanghai Bio-lu Biomaterials Co., Ltd. (Shanghai, China).

**Fabrication and surface morphologies of PLGA/TCP scaffolds**

Porous scaffolds PLGA/TCP were made by three-dimensional (3D) printing based on LDM technique (Figure 1A). Before the samples were fabricated, PLGA was dissolved in 1,4-dioxane at a ratio of 1:5 (weight/volume). TCP was mixed with the solution at a ratio of 1:4 (weight/weight) (TCP:PLGA). Homogenous slurries were controlled to be deposited into specific positions according to the model predesigned by computer animated design. The fabrication temperature in the refrigerator was −30°C. Subsequently, the scaffolds were lyophilised to remove the solvent during fabrication. Matrix blank scaffolds were fabricated followed by the process of sample, except that PLGA was dissolved in dimethylsulphoxide instead of in 1,4-dioxane. The surface morphologies of the prepared scaffold sections were observed by scanning electron microscopy (SEM, JEOL JSM-6390, Tokyo, Japan) at 15 kV and 5.0 m.

One scaffold weighed 2 g and was lyophilised at −50°C, 50 Pa for 2 days; then, it was cut into five 0.3 g of scaffolds separately, and dried at room temperature for 0 days, 3 days, 6 days, 7 days, and 9 days in vacuum drying oven with the pressure of 50 Pa for selecting the best freeze-drying process.

**Preparation of standard solutions**

Standard solutions of DMF were used for the calibration curve: 0.4 mL, 0.2 mL, 0.1 mL, and 0.05 mL of standard stock solution (1000 ppm) were transferred to five 10 mL volumetric flasks with DMF, separately, diluted with DMF to volume and mixed. Subsequently, 1 mL of 10 ppm of standard solution was transferred to a 10 mL volumetric flask, diluted with DMF to volume, and then mixed to obtain 1 ppm standard solution.

Standard solutions of Na₂CO₃ solution used for calibration curve: 2.5 mL, 1.25 mL, 0.625 mL, 0.3125 mL, and 0.128 mL of standard stock solution (400 ppm) were transferred to five 10 mL volumetric flasks with Na₂CO₃, separately, diluted with Na₂CO₃ solution (0.3 g/mL) to volume, and mixed.

Standard solutions including matrix blank scaffolds: 0.3 g of matrix blank scaffolds, which were cut into pieces of 0.2 × 0.2 × 0.2 cm³ were transferred to a headspace vial, and five parallels were prepared. Then, 4 mL of five different concentrations of standard solutions of DMF or Na₂CO₃ solution used for the calibration curve were transferred into five headspace vials one by one. All of the standard solutions described above (4 mL) were transferred to each vial and the vials were closed immediately. After heating the vial for 40 minutes at 90°C,
the HS-GC-MS of the gas in the sample vial was performed for optimisation of solutions.

Quantitation

A 0.3 g sample was weighed accurately and cut into pieces of 0.2 × 0.2 × 0.2 cm³ in a headspace vial. DMF (4 mL) was transferred to this vial, and the vial was closed immediately. After heating the vial for 40 minutes at 90°C, the HS-GC-MS of the gas in the sample vial was performed in the selective ion (masses 88) mode. The sample was quantified by the external standard method. Calibration curves were determined at the beginning of sample set in 1 day. Blanks and check standards were run for each sample set. Overall, blanks and check standards comprised a minimum of 30% of the total samples analysed during any given analysis sequence to ensure the stability of the method.

GC/MS analysis

HS-GC tandem MS was used to identify and quantify 1,4-dioxane. An Agilent (California, USA) 7890B GC was used for all separations. The GC was equipped with a 30m × 0.25 mm × 0.25 μm HP-5 MS column (California, USA). The GC was coupled to an Agilent 7697A headspace for injection and an Agilent 5977 mass spectrometer (MS) operated in electric ionisation mode while using the selective ion monitoring mode. Optimised parameters of GC-MS are as followed in Table 1.

Method validation

Specificity

Specificity described the ability of an analytical method to separate the analysts of interest accurately from other components expected to be present in samples. The guiding principle 9101 [26] entitled “The validation guidelines of drug quality standard analytical method” provides identification requirements and criteria for specificity testing. Blank, sample, and 100 ppm of standard solution were prepared following the preparation protocol and detected.

Accuracy

According to Chinese Pharmacopoeia 2015 Edition guideline 9101 [26], accuracy can be evaluated with spiking recovery experiments. Matrix blank can be used to evaluate recovery as described in the guideline. In our sample preparation process, 0.3 g scaffold was dissolved with 4 mL DMF. According to Chinese Pharmacopoeia [26], International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [27], and United States Pharmacopoeia [28], 1,4-dioxane in the products should be no more than 380 ppm. We convert the concentration of 1,4-dioxane in 0.3 g scaffold (i.e. 380 ppm) into the concentration in 4 mL DMF according to the following formula: 380 ppm × weight of the scaffold (0.3 g)/the weight of DMF = 380 ppm × 0.3g/(volume × density) of DMF = 380 ppm × 0.3g/(4 mL × 0.9487 g/L) = 30 ppm. This means the concentration of 1,4-dioxane in 0.3 g scaffold in 4 mL DMF should be less than 30 ppm. So, 20 ppm, 25 ppm, and 30 ppm of 1,4-dioxane standard solutions were prepared with matrix blank scaffolds in DMF; triplicate samples of each concentration were prepared. The recovery was calculated as follows: (actual concentration/theoretical concentration) × 100%.

Precision

The method precision can be determined using standard preparations [29]. Method repeatability expresses the precision of the analytical method including system precision and sample preparation under the described operating conditions. Two standards of 25 ppm were processed by two different research assistants in 2 days for determination of both intra- and inter-day precision using the same method. Every sample was analyzed six times. Relative standard deviation (RSD%) was used for the evaluation of precision.

Limit of detection and limit of quantitation

The limit of detection (LOD) is the lowest amount that can be detected, but not necessarily quantified. According to the guideline [26], it is defined as the concentration required to give a signal to noise ratio (S/N) of 3:1. The limit of quantification (LOQ) is the lowest amount that can be quantified accurately, defined as the concentration required to give a signal to noise ratio (S/N) of 10:1. The noise ratio was calculated automatically by a Mass Selective Detector (MSD) chemstation of Agilent after the target peak was integrated. A serial sample of low concentrations (100 ppb, 10 ppb, 1 ppb, 0.1 ppb) were detected and analysed.

Linearity

Series concentrations of standards of 1,4-dioxane of 1–40 ppm were prepared as per the preparation protocol described earlier. Calibration curves were constructed for each analyst, plotting (peak area of quantifier ion) versus

| Table 1 Optimised parameters of HS-GC-MS for determining 1,4-dioxane in novel porous scaffolds. |
|---------------------------------|----------------|---------------------|
| **Content** | **Item** | **Parameter** |
| Instrument | HS | Agilent 7697A |
| | GC | Agilent 7890 |
| | MS | Agilent 5977A |
| Parameters of Headspace | Temperature | 90°C |
| Parameters of GC | Time | 40 min |
| | Column | HP-5MS |
| | Process of Column | 40°C (5 min) |
| | Temperature | 320°C (5 min) |
| | Process of post run |  |
| | Gas mode | Velocity |
| | Velocity | 36.262 cm/sec |
| | Inlet mode | split |
| | Split ratio | 50:1 |
| Parameters of MS | Temperature of source | 230°C |
| | Temperature of inlet | 220°C |
| | Quantifier ion | 88 |
| | Quantifier ion | 58 |
| | Scan mode | SIM |

GC = gas chromatography; HS = head space; MS = mass spectrometry; SIM = selected ion monitoring.
concentration (ppm). Slope, intercept, and correlation coefficient were calculated using linear regression analysis.

**Robustness of the method**

Robustness can be described as the ability to reproduce the (analytical) method under different circumstances. When a small change exists, a robust test is set up to evaluate the robustness of a method. Special factors changed in GC-MS detection were columns of different brands or batch numbers, different solid phase, different types of supports, flow rate of carrier gas, temperature of column, inlet, and detection [26]. In this validation, we used two different columns (HP-5ms and DB-624) to detect the same sample for evaluating the robustness. Each column detected samples in triplicate.

**Application in optimising the drying procedure**

Scaffolds dried at 37°C in 0 days, 3 days, 6 days, 7 days, and 9 days in vacuum drying oven were quantified by optimised conditions. The content of 1,4-dioxane was compared with different scaffolds processed in different drying procedures.

**Statistical analysis**

The peak areas of 1,4-dioxane in different extracts were presented as means ± standard deviation of three repeated experiments. T test was performed using SPSS 16.0 software (Chicago, IL, USA) for comparison with statistical significance at $p < 0.05$.

**Results**

**Porous property of 3D printing PLGA/TCP scaffolds**

The photo of 3D printing porous scaffold is shown in Figure 1A. From the SEM images, we found that the scaffolds had well interconnected macropore structures (Figure 1B), and numerous micropores were observed on the wall surface of the scaffold framework, with pore sizes ranging from 5 μm to 50 μm (Figures 1C–1F).

DMF was the optimal solvent for dissolving scaffolds for GC-MS.

In this investigation, two solvents (double distilled H$_2$O, DMF) and two sodium solutions (NaCl solution and Na$_2$CO$_3$ solution) were chosen as optimising solvents for sample preparation (Figure 2A). The order of the peak area of 1,4-dioxane was: double distilled Na$_2$CO$_3$–H$_2$O > NaCl–H$_2$O > H$_2$O > DMF ($p < 0.01$ vs. H$_2$O, $n = 3$) (Figure 2B). However, the area of 1,4-dioxane in each concentration of the calibration curves without matrix were twice that of those in which matrix was dissolved with Na$_2$CO$_3$–H$_2$O. These differences were not found between the calibration curves with or without matrix blank scaffolds dissolved with DMF.

**Figure 2** Optimisation of the solvent in pretreatment process. (A) Standard solution of 25 ppm dissolved with different solutions. (B) Quantified area of 1,4-dioxane in different solutions using the same head space gas chromatography mass spectrometry parameter. **$p < 0.01$ ($n = 3$), vs. ddH$_2$O. (C) Matrix effect of standards dissolved with Na$_2$CO$_3$–H$_2$O or dimethyl formamide (DMF). The concentrations of standard dissolved with Na$_2$CO$_3$–H$_2$O were 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm. The concentrations of the standard dissolved with DMF were 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 40 ppm. AB*S = abundance × second; dd = double distilled; ppm = parts per million.
(Figure 2C), indicating that the matrix effect of samples significantly existed when dissolved with \( \text{Na}_2\text{CO}_3-\text{H}_2\text{O} \). Thus, DMF was the optimal solvent for dissolving scaffolds for GC-MS.

Validation of the method

System suitability
The 70 eV electron ionization mass spectral ions from 1,4-dioxane include the molecular ion, \( m/z \) 88 ([M]+, quantifier ion) and fragment ions, \( m/z \) 58 ([M-CH\(_2\)O]+, qualifier ion). The retention time of the target mass was 3.266 min. There was 100% chromatographic resolution of the peaks (resolution, (RS) > 1.5) (Figure 3). The tailing factor of this target peak was 2.26, and the number of theoretical plates was 36,954.

Specificity
The results showed that the method was specific as there was no other peak to interfere with the peak of interest, and as for the retention time no significant difference was observed from the standard components (Figure 4).

Accuracy, precision, robustness, and linearity
The accuracy, precision, robustness, and linearity of the method are summarised in Table 2. Recovery experiments gave 97.9—100.7% recovery for 1,4-dioxane (Table S1). The repeatability of the method was expressed as % RSD of five continuous standards, and the %RSD of peak area was 0.6%. %RSD of retention time and peak area that described intraday precision were 0.01% and 0.58%, respectively (n = 6). Interday precision was calculated to describe intermediate precision of retention time and peak area, which were 0.02% and 0.62% (n = 12), respectively (Table S2). The linear range for 1,4-dioxane was determined as 1—40 ppm. The calibration curves were linear for all the standard components with correlation coefficient ≥ 0.9999. LOD and LQD for 1,4-dioxane were 1 ppb and 5 ppb, respectively. The results showed that the resolution, tailing factor, and the number of theoretical plates when the column was changed to DB-624 met the system suitability requirements. %RSD of six samples was 1.6% between Hp-5ms and DB-624 (Table S3), implying that the area of target peak had little changes. All of the results met the requirement of the guideline 9101 described in the Chinese Pharmacopoeia 2015 Edition [26] (Table 2).

Figure 3  Chromatography and spectrum for the determination of 1,4-dioxane. (A) Extract ion gas chromatography mass spectrometry (GC-MS) of 1,4-dioxane of sample. (B) Extract ion GC-MS of 1,4-dioxane of standard. (C) Mass spectrum of 1,4-dioxane of sample. (D) Mass spectrum of 1,4-dioxane of standard.
Application in the translation of PLGA/TCP porous scaffolds

The established method has been used to monitor the drying process and control the quality of the scaffolds. The result showed that 1,4-dioxane in the scaffolds lyophilised (−50°C, 50 Pa) for 2 days and then dried in vacuum (25°C, 50 Pa) for 7 days were below 380 ppm. Extension of freeze drying time to 9 days did not reduce the content of 1,4-dioxane (Figure 5). According to the data above, the

Table 2  Validation of the method.

| Items                        | Tested value | Accepted range [26] |
|------------------------------|--------------|---------------------|
| Linearity                    |              |                     |
| R²                           | 0.99999      | /                   |
| Range (ppm)                  | 1–40         | /                   |
| Accuracy (Recovery)          |              |                     |
| 20 ppm                       | 97.9% ± 3.76 | 90–108%             |
| 25 ppm                       | 100.7% ± 2.41|                     |
| 30 ppm                       | 98.9% ± 1.18 |                     |
| Precision (RSD)              |              |                     |
| Repeatability (n = 6)        | RT           | 0.01% < 3%          |
| Area                         | 0.58%        |                     |
| Intermediate precision (n = 12) | RT        | 0.02% < 6%          |
| Area                         | 0.62%        |                     |
| Robustness (RSD)             |              |                     |
| 1.60%                        | /            |                     |

RSD = relative standard deviation; RT = retention time.

Figure 4  The method shows good specificity. (A) There is no peak in the selected ion monitoring (SIM) chromatography of blank. The same retention time (min) of 1,4-dioxane in the SIM chromatography of sample (B) and SIM chromatography of 25 ppm standard (C) without other peaks to interfere.

Figure 5  The method shows good specificity. (A) There is no peak in the selected ion monitoring (SIM) chromatography of blank. The same retention time (min) of 1,4-dioxane in the SIM chromatography of sample (B) and SIM chromatography of 25 ppm standard (C) without other peaks to interfere.
scaffolds should be freeze-dried for 2 days and then dried in vacuum more than 7 days to control the content of 1,4-dioxane to satisfy the safety limit.

**Discussion**

Three-dimensional printing porous scaffolds are promising regenerative strategies for bone defect repair in orthopaedics [6,7]. As a newly developed medical product, safety issues are considered as the most important ones. As a Class 2 solvent with less severe toxicity, content of residual 1,4-dioxane in the novel 3D printing PLGA/TCP scaffolds should be rigorously controlled [26]. We first developed an HS-GC-MS method for testing 1,4-dioxane in PLGA/TCP porous scaffolds. This method utilised a reproducible and highly recovery sample preparation process, a more efficient separation technology, and a specific single-ion monitoring mass detection to quantify 1,4-dioxane in PLGA/TCP porous scaffolds.

Different from the liquid or liquid-like sample [29,30], 1,4-dioxane should be extracted from the solid scaffold at the first step. Four solutions were used; as a result, the detected amount of 1,4-dioxane was exactly inversely related to its solubility in different solution, and Na$_2$CO$_3$ solution showed the highest sensitivity due to the salting-out effect [32]. However, as the first method to test the gas from a solid porous structure, we further investigated the matrix effects of the different solutions. There were two different extraction systems, one in Na$_2$CO$_3$ solution, retaining the porous structure of the scaffolds, and the other in DMF solution without any porous structures. In DMF solution, the scaffolds were dissolved into powders, and 1,4-dioxane was distributed in solution phase and gas phase. The detected amount was determined by its solubility in DMF, so the detected amount of 1,4-dioxane were the same in DMF with or without matrix blank scaffolds. In Na$_2$CO$_3$ solution with the matrix blank scaffold, 1,4-dioxane was distributed in three phases: scaffold, solution, and gas, and the detected amount was determined not only the solubility in Na$_2$CO$_3$ but also the attachment in the porous scaffolds. Thus, the detected amount of 1,4-dioxane in Na$_2$CO$_3$ solution only was more than that in the Na$_2$CO$_3$ solution with matrix blank scaffolds (Figure 2C). The fabricated porous scaffolds had both regular macropores, with the size among 300 $\mu$m to 500 $\mu$m, as well as irregular micropores with size from 5 $\mu$m to 50 $\mu$m pores (Figure 1B–1F) [11,13], which might be the cause of matrix effects. Thus, we selected DMF to extract 1,4-dioxane from the scaffolds as the pretreatment process of the GC-MS methods. The following method’s validation results further confirmed the feasibility of this procedure.

This method was validated with good accuracy and reproducibility and met the methodological requirements of the guideline 9101 described in the Chinese Pharmacopoeia 2015 edition. It is one of the indispensable files in medical device approval and registration. We used this established method to monitor the drying process of scaffolds to guarantee the residual 1,4-dioxane less than 380 ppm, according to Chinese Pharmacopoeia [26]. Our results showed that 1,4-dioxane in the scaffolds lyophilised ($-50^\circ$C, 50 Pa) for 2 days and then dried in vacuum ($25^\circ$C, 50 Pa) for 7 days were below 380 ppm (Figure 4). After examining the three-phase diagram of 1,4-dioxane, we found that the conditions of $-50^\circ$C and 50 Pa for lyophilising are not efficient for removing 1,4-dioxane (Figure S1) because in this environment 1,4-dioxane prefers solid rather than gas; therefore, we will further optimise the drying process by lowering the pressure or increasing the temperature to improve the freeze-dry efficiency. Our newly established quantitative method will also be used to verify this hypothesis.
In fact, when we used this established method to test 1,4-dioxane in the PLGA raw material from different suppliers, we detected chloroform in some batches (Figure S2). However, if we want to accurately quantify the chloroform or dichloromethane, the pretreatment (extract) process is commonly used, while the parameters of GC-MS should be specifically tuned for each target solvent to meet the requirements of method validation.

Conclusion

In this investigation, the HS-GC-MS method was firstly developed for testing 1,4-dioxane in PLGA/TCP porous scaffolds. This method was validated to meet the requirements in the Chinese Pharmacopoeia 2015 Edition, which provided residual 1,4-dioxane test methods in the PLGA/TCP scaffolds for CFDA registration. First, we resolved the method of pretreatment of the solid scaffold to eliminate matrix effect in the development process. The analysis method had been used to optimise the process of drying of scaffolds to satisfy the product requirement. In addition, it is an important tool for quality control of the composite scaffolds.

Translational significance

Three-dimensional printing porous scaffolds are promising regenerative strategies for bone defect repair in orthopaedics [6,7]. As a newly developed medical product, safety issues are considered as the most important ones. As a Class 2 solvent with less severe toxicity, content of residual 1,4-dioxane in the novel 3D printing PLGA/TCP scaffolds should be rigorously controlled according to international regulations, such as Chinese Pharmacopoeia [26], International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [27] and United States Pharmacopoeia [28]. In this work, we firstly developed an HS-GC-MS method for testing 1,4-dioxane in PLGA/TCP porous scaffolds. It is an important file in medical device approval and registration. It has been applied as an enterprise standard to optimise the drying process of scaffolds and monitor the quality of scaffolds in the industrialisation process.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jot.2017.06.004.

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