Journal of Pharmaceutical and Biomedical Analysis 112 (2015) 126–138

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Solid phase extraction in tandem with GC/MS for the determination of semi-volatile organic substances extracted from pharmaceutical packaging/delivery systems via aqueous solvent systems

Steven A. Zdravkovic*

Extractables/Leachables and GC/MS Services, Pharmaceutical Product Development, Inc., 8551 Research Way, Suite 90, Middleton, WI 53562, USA

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 9 April 2015
Accepted 13 April 2015
Available online 28 April 2015

Keywords:
Solid phase extraction
Controlled extraction study
Extractables
Polysorbate 80
GC/MS

ABSTRACT

An extractable survey is one of several studies performed on a pharmaceutical storage/delivery system as part of the process of demonstrating that the system is suitable for its intended use. In this paper, a solid phase extraction method for the preparation of aqueous extracts generated during an extractable survey is presented. The method offers a convenient means to isolate semi-volatile organic extractable compounds from aqueous extraction solvents for analysis by gas chromatography/mass spectrometry. Following the solid phase extraction procedure, derivatization is performed to convert problematic functionalities (such as amines and acids) into appropriate chromatographically friendly derivatives. Demonstration of method performance is achieved in three ways using a set of 31 commonly observed extractable substances as model compounds. First, a breakthrough experiment was performed with a 2 solvent system consisting of water and 10/90 isopropanol/water over a range of 6 mL to 100 mL. Results from this experiment show only caprolactam possessed a significant level of breakthrough in either solvent over the range of volumes evaluated. Second, a formal accuracy/precision study was conducted using a three solvent system consisting of water, 10/90 isopropanol/water and 1% polysorbate 80. This experiment demonstrates the quantitative ability of the method at levels ranging from 20 ng/mL to 50 μg/mL. Recovery values of 70% to 130% of the theoretical concentration, with relative standard deviation values of less than 15% for replicate preparations, are obtained for a majority of the compounds evaluated. Finally, a case study involving the extraction of an intravenous drug delivery bag with multiple aqueous solvent systems further demonstrates the viability of solid phase extraction for use in an extractables survey.

1. Introduction

In order to meet regulatory requirements [1–3] a pharmaceutical product's packaging/delivery system is required to have its suitability for use demonstrated as part of the drug development process. An important part of this demonstration involves the evaluation of substances unintentionally introduced by the packaging into the drug product during storage and/or use. A compound that has migrated from the package into the product in this manner is known as a leachable. Because leachables are impurities, they have the potential to affect the safety, efficacy, potency, and identity of the final product. As part of the work performed to control leachables in the product, an extractables survey is commonly carried out on the materials used in the packaging/delivery system. This survey determines compounds that may be extracted from the material using a range of solvents and exposure conditions. Compounds extracted from packaging components via a matrix and/or exposure condition other than what would be encountered for the product under typical conditions of use are referred to as extractables. Knowledge of the extractable profile of a packaging system allows for the evaluation of potential leachables. Additionally, the extractable profile can be utilized for an assessment of the materials composition for selection/qualification purposes [4,5]. Over the past 10 years, information on the scope, design, and execution of extractables surveys, as well as other relevant information related to characterization of the container closure system, has been published [6–10].

Aside from the non-specific analysis of an extract's properties including, for example, total organic carbon, pH, and UV absorbance, chromatographic instrumentation (in concert with an appropriate detector) is the preferred platform for the specific qualitative/quantitative analysis of organic extractable substances. Typically, gas chromatography is employed for the analysis of volatile and semi-volatile organic compounds, while liquid

* Tel.: +1 608 203 4364; fax: +1 608 827 8813.
E-mail address: steve.zdravkovic@ppdi.com

http://dx.doi.org/10.1016/j.jpba.2015.04.031
0731-7085/

chromatography is used for non-volatile and thermally labile organic compounds.

In order to analyze samples via chromatographic instrumentation, sample pre-treatment is often required. Issues necessitating pre-treatment include enrichment of analyte concentration to allow for the detection of compounds present at trace levels, or the removal of substances that may interfere with the analysis. Another commonly encountered issue requiring pre-treatment of the sample is a lack of compatibility between the sample matrix and the chromatographic platform. For liquid chromatography, extracts composed exclusively of organic solvent can be problematic due to the potential for severe peak distortion. Conversely, aqueous samples are typically problematic for gas chromatography due to back-flash in the inlet, peak distortion, and reactivity with certain column stationary phases. Incompatibility with liquid chromatography can be overcome with relatively simple evaporation procedures followed by reconstitution in an appropriate solvent. Alternatively, a method has been demonstrated for the analysis of extractable compounds from organic matrices directly by reverse phase liquid chromatography [11] thereby eliminating the need to perform sample pretreatment. Analysis of aqueous matrices directly by gas chromatography is a greater challenge. Direct injection of these matrices onto the instrument is problematic or impossible, while the properties of both water and the analytes it may contain make an evaporation/solvent exchange procedure prohibitively difficult. Thus, other approaches are required.

The most common techniques for the preparation of aqueous samples for analysis by gas chromatography in wide use today are liquid/liquid extraction (LLE), static or dynamic headspace extraction, solid or liquid micro-extraction, and solid phase extraction (SPE). After considering the advantages/disadvantages of each technique, it was hypothesized that a sample preparation method using SPE would provide an optimal approach for the analysis of aqueous samples generated during an extractable survey for analysis by gas chromatography/mass spectrometry (GC/MS). A review of the current literature was performed to determine if this has been explored previously. Although this review did yield a significant amount of information on the use of SPE for the analysis of a wide range of compounds from aqueous matrices, a minimal amount of information on its use for extractable surveys was found. A detailed and informative work published by Pouche et al. [12] describes an SPE method developed and validated for the isolation and subsequent analysis of several common packaging additives by liquid chromatography/mass spectrometry. The information presented in this work is valuable for the demonstration of SPE's applicability to several polymeric additives. Nonetheless, the focus was on the analysis of targeted non-volatile hydrophobic substances only. The use of SPE for the isolation of several model extractable compounds for nuclear magnetic resonance (NMR) analysis has been presented by Norwood et al. [13]. In this work, SPE is used as an isolation and solvent exchange technique for NMR. Other articles found in the literature involving SPE and pertaining to the analysis of extractable/leachable substances from packaging [14–16] focused on targeted analysis of a small number of extractable or leachable compounds in specific matrices. Despite the contributions these works provide, uncertainty exists around the use of SPE, or other techniques, for the preparation of aqueous extraction samples generated as part of an extractable survey for analysis by GC/MS.

Therefore, the goal of this work is to develop an SPE methodology specifically tailored for the recovery of packaging related compounds in aqueous matrices followed by analysis via GC/MS. In addition to development of the SPE and GC/MS procedures, derivatization of the sample is evaluated in order to ensure compounds with problematic functionalities, such as carboxylic acids and primary/secondary amines, are not missed. A set of 31 model compounds representing a wide variety of chemical species commonly encountered in extraction studies was used to develop the method as well as demonstrate its performance. Additionally, a case study utilizing the method for the preparation and analysis of aqueous extraction solvents from an intravenous drug delivery bag is presented.

2. Materials and methods

2.1. Reagents and standards

Trace analysis grade ethyl acetate was purchased from Burdick and Jackson (Morristown, NJ). Methanol was chromatographic grade and obtained from Sigma-Aldrich (St. Louis, MO) or EMD chemicals (Darmstadt, Germany). Water was produced through an in-house deionization system and had a resistivity of 18.2 MΩ cm.

Reference standards for model compounds were obtained from Sigma-Aldrich or Tokyo Chemicals America (Portland, OR). 7,9-Di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione was synthesized in house due to a lack of commercially available material. All reference standard used in this study were at least 97% pure.

N,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane catalyst (BSTFA/TMCS), *N*-methylbis(trifluoroacetamide) (MBTFA), and Trifluoroacetic Anhydride (TFAA) derivatization reagents were obtained from Sigma-Aldrich and were derivatization grade or better.

2.2. Derivatization procedure

A portion of each test solution was derivatized prior to analysis. BSTFA/TMCS was used for the test solutions adjusted to pH 2 while MBTFA was used for test solutions adjusted to pH 9. A single procedure was used for derivatization with both reagents. First, 0.5 mL of test solution and 0.2 mL of derivatization reagent were combined in a 2 mL glass auto-sampler vial. Next, each vial was sealed with a Teflon lined cap and shook by hand for about 5 s to mix. Finally, the vials were incubated in an 80 °C oven for 60 min.

2.3. GC/MS method

An Agilent (Santa Clara, CA) 7890A gas chromatograph in tandem with an Agilent 5975C single quadrupole mass spectrometer was utilized as the GC/MS system for this study. Separation was achieved on an Agilent HP-35 (35% Phenyl/65% Polydimethylsiloxane) narrow bore 30 m × 0.25 mm column with a 0.25 μm film thickness. Method settings for applicable parameters on the gas chromatograph and mass spectrometer can be found in Table 1.

System suitability was evaluated for each analytical set to ensure the integrity of the data being produced. The following parameters were evaluated:

- Chromatographic non-interference: Verified using diluent or derivatized diluent. Any peaks which could potentially interfere with a peak of interest were noted. If an interference was present the data was evaluated using an extracted ion to obtain a pure response.
- Instrument Precision: Evaluated for the method evaluation experiments using a mixture of the model compounds injected multiple times in each sequence. Acetophenone-d_5 was used for evaluation of precision in the case study experiment. Unless otherwise justified, the %RSD for precision was required to be less than or equal to 20 for all experiments performed in this study.
- Instrument sensitivity: Evaluated for the case study experiment only. A 100 ng/mL solution of Acetophenone-d_5 was injected. The USP signal to noise ratio was required to be ≥10.

Table 1
GC/MS instrument parameters.

Instrument	
Type	Gas chromatograph
Model	Agilent 7890A
Column	
Stationary phase	35% Diphenyl/65% dimethyl polysiloxane
Column dimensions	30 m × 0.25 mm, 0.25 μm film
Flow rate	1.0 mL/min
Carrier gas	Helium
Mode	Constant flow
Inlet	
Type	Split/splitless
Mode	Splitless
Inlet liner	2 mm splitless Gooseneck, no Wool
Temperature	280 °C
Purge on time	0.5 min
Purge flow	60 mL/min
Oven	
Initial temperature	40 °C
Initial hold time	5 min
Ramp	10 °C/min
Final temperature	300 °C
Final hold time	4 min
Run time	35 min
Detector	
Type	Single quadrupole mass spectrometer
Transfer line temperature	300 °C
Mode	Scan
Scan range	29–700 AMU
Solvent delay	4 min (Neat sample/standard) 7 min (Derivatized sample/standard)
Injector	
Type	Automatic liquid sampler
Injection volume	0.4 μL

2.4. Solid phase extraction method

Four SPE stationary phases, each representing a different sorbent chemistry, were obtained for evaluation. A styrene/divinylbenzene (SDVB) column was obtained from Agilent Technologies sold under the trade name Bond Elut Plexa. It consisted of 500 mg stationary phase in a 6 mL polymeric SPE tube. A hydrophilic/lipophilic balanced (HLB) column was obtained from Sigma-Aldrich sold under the trade name Supel-Select HLB. It consisted of 200 mg of stationary phase in a 6 mL polymeric tube. An ENVI-CARB SPE column was obtained from Sigma-Aldrich and represents a graphitized carbon stationary phase. It was held in a reversible polymeric tube and contained 400 mg of stationary phase. A Supelclean ENVI-18 column was obtained from Sigma-Aldrich. It contained 500 mg of C_{18} stationary phase held in a 6 mL glass tube.

SPE columns were attached to a Supelco visiprep vacuum manifold using disposable PTFE valve liners. As necessary, 25 mL PTFE reservoirs were attached to accommodate large sample volumes. Prior to loading sample, each column was conditioned with, in succession, approximately 5 mL ethyl acetate, approximately 5 mL of methanol, and approximately 5 mL of 10/90 methanol/water. Once conditioned, a portion of the solution to be tested was added to the column. A small volume of concentrated spike standard, or acetophenone-d_5 internal standard, was then added. The remaining sample solution was added to the column to achieve the desired total volume to be loaded.

Two columns were prepared for all test solutions. The test solution in the first column was adjusted to a pH of ≤2 using 1 N HCl. The test solution in the second column was adjusted to a pH of ≥9 using 1 N NaOH. After mixing, the pH of each solution was verified via a pH test strip. Following pH adjustment, test solutions were pulled through the stationary phase at a rate of about 5 mL/min and discarded. Next, a 2 mL aliquot of 10/90 methanol/water was added as a rinse, after which maximum vacuum was applied to the columns to dry. Depending on the number and/or type of column being prepared, the drying time required was at least 30 min and no more than 90 min.

Two analyte elution procedures were employed in this work. The first, used with the column evaluation studies, utilized 2.2 mL of ethyl acetate for the C_{18}, HLB and graphitized carbon columns. A volume of 2.5 mL of ethyl acetate was applied to the SDVB columns. This difference is attributable to the increased volume of solvent absorbed by the dried stationary phase of the SDVB columns, which was determined experimentally to be 0.8 mL. For comparison, the C18 columns absorbed 0.5 mL of eluent. The volume of eluent used for each stationary phase was chosen to allow room for a quantitative transfer of eluent into a 2 mL volumetric flask. After bringing the flask to volume, the eluent was transferred into a glass culture tube containing a small amount of anhydrous sodium sulfate to dry the eluate. The second elution procedure was used with the SDVB columns in the breakthrough, accuracy/precision, and case study experiments. A volume of 2.8 mL of ethyl acetate eluent was applied to the columns and collected directly into a glass tube containing a small amount of anhydrous sodium sulfate. Mixing of the solution was performed for both elution procedures to verify the sodium sulfate was not saturated. At least 5 min was allowed to complete drying. One-half milliliter of this solution was transferred into a GC auto-sampler vial for derivatization. The remaining solution was vialed for direct analysis.

2.5. Case study

The test article for the case study was a plastic IV bag that was retrieved from a commercially available intravenous (IV) product. The specific commercial product was 0.9% Sodium Chloride Injection, USP, packaged in a 50 mL Viaflex bag (Baxter Healthcare, Deerfield, IL, product code 2B1301, lot number P3061951). These product units were obtained from VWR and were within their labeled expiry.

Extraction of the IV bag was performed utilizing four solvents:

- 80/20 Water/Isopropanol: Prepared volumetrically by combining 8 parts water and 2 parts isopropanol.
- 1% Polysorbate 80: Prepared gravimetrically by combining 5 g of polysorbate 80 and 495 g of water.
- 50 mM pH 2.5 Phosphate Buffer: Dissolved 2.75 g of sodium phosphate monobasic dihydrate and 0.84 g of phosphoric acid in approximately 400 mL of water. Brought the total volume to 500 mL in a volumetric flask. Verified that the pH was 2.5 ± 0.1.
- 50 mM pH 9.5 Ammonium Buffer: Dissolved 1.24 g of ammonium acetate in approximately 400 mL of water. Added 1.1 mL of ammonium hydroxide (30% NH_3) to the solution. Brought the total volume to 500 mL in a volumetric flask. Verified that the pH was 9.5 ± 0.1.

The saline solution contained in the bags was removed and replaced with 50 mL of extraction solvent via a glass syringe. Extraction was facilitated by placing the IV bags in a 70 °C oven for two days. After incubation, the contents of each bag was removed and transferred to a flask. In addition to the extraction samples, controls for each solvent were prepared by adding solvent into a flask and exposing to the same extraction conditions as the samples.

Each extract, along with the appropriate control, was prepared for analysis with the SPE procedure. The pH 2.5 buffer, pH 9.5 buffer, and 1% polysorbate 80 extracts were loaded directly onto the SPE column. The 80/20 water/isopropanol extract was

diluted by a factor of 2 with water prior to loading to minimize analyte breakthrough. Following the SPE procedure, the samples were derivatized followed by analysis with the GC/MS method.

Acetophenone-d_5 was present as both an external standard as well as an internal standard spiked into the samples. The response of the Acetophenone-d_5 internal standard was used for quantitation.

Identification of extractables detected in the samples was performed using NIST library search software. The classification of identification level is similar to that outlined in the PQRI OINDP recommendation [7], with the exception that elemental composition/molecular weight confirmation was not performed.

3. Results and discussion

3.1. Selection of model compounds

The selection of model compounds used for development and evaluation of the method is based on three criteria. First, analytes of interest to the method are "semi-volatile" organic compounds. Second, some hydrophilic character is desired to be consistent with compounds expected to be extracted by aqueous solvents. Finally, compounds selected represent those commonly observed in extracts of materials used in pharmaceutical product packaging and/or delivery configurations. Table 2 lists the model compounds selected for the study based on these criteria.

In order to evaluate the method's capabilities beyond what may be minimally necessary to fulfill its purpose, model compounds whose physical properties are outside the intended scope were included. Toluene, which is typically analyzed by headspace gas chromatography on a volatiles column, is included to evaluate recovery of a hydrophobic, volatile substance. Compounds such as dioctyl phthalate or stearic acid, which have been demonstrated [11] to be readily analyzed by LC/MS, are included to evaluate very hydrophobic, low volatility compounds.

3.2. Derivatization procedure development

Compounds containing amine, carboxylic acid, and/or hydroxyl functional groups often prove problematic for analysis by gas chromatography. Although there are other functionalities that are difficult to chromatograph in their natural state, these are the most commonly observed in extractables analysis based on reported extractable profiles for various materials [6,17] as well as the author's experience.

In order to obtain adequate chromatography, conversion of troublesome functionalities to more volatile and less polar analogs is a common practice. Based on published literature, [18–25], *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane catalyst, N-methylbis(trifluoroacetamide) (MBTFA), and trifluoroacetic anhydride (TFAA) were selected as potential reagents to fulfill this purpose in this work. BSTFA is a silylation reagent which is noted to have applicability for the derivatization of a wide range of functionalities in a single procedure, an attractive benefit for the scope of this work. However, the silylation of amines is noted to be difficult [23]. MBTFA and TFAA are acylation reagents capable of converting several functional groups, but are especially reactive toward amines. TFAA is reported to be the most volatile and reactive of the fluorinated anhydrides but has the disadvantage of producing acidic byproducts that must be removed prior to analysis. MBTFA is less reactive but produces inert byproducts that do not impact the performance of the chromatographic separation [21,23].

Variations in reaction time, derivatization reagent volume, and temperature were evaluated for each derivatization reagent. A mixture of the model compounds listed in Table 2 was prepared at 10 μg/mL in ethyl acetate for this purpose. Compounds in the model compound mixture which undergo derivatization, and the derivative produced, are included in the analytical description column of this table.

Results for evaluation of BSTFA/TMCS as a derivatization reagent show silylation was completed for the carboxylic acid and hydroxyl group functionalities at room temperature with a reagent to sample volume ratio of 0.1 mL/0.5 mL. Aniline, caprolactam, and dibenzylamine showed only partial derivatization, even for a reagent to sample volume ratio of up to 0.4 mL/0.5 mL held at 80 °C for 1 h. Consequently, an additional experiment was performed using a more aggressive procedure similar to what was outlined in other works [22–24] to ascertain if complete derivatization of these compounds could be achieved. This experiment involved a 1:1 ratio of sample and dimethylformamide (a known catalyst of the reaction) and BSTFA/TMCS reagent. Following derivatization at 70 °C for 1 h, the solution was brought to 1 mL with dichloromethane. Results for this procedure showed a significant negative chromatographic impact from the reagents, and effectively eliminated the first 12 min of the chromatogram from producing useable data. Peak distortion was observed for analytes of interest, likely due to the large volume of reagent causing partitioning in the inlet or a solvent effect on-column. As a result of the poor chromatography, it could not be determined if complete derivatization for caprolactam, aniline, and dibenzylamine was achieved. This procedure was not studied further, as results for the more mild conditions achieved complete derivatization of the problematic functionalities, excluding the amines, and produced acceptable chromatography. No significant chromatographic interferences, as illustrated in Fig. 1, from about 7 min until the end of the separation were observed.

Although ambient conditions and a low 0.1 mL/0.5 mL ratio worked well for the BSTFA/TMCS reagent, incomplete derivatization was occasionally noted for less reactive functionalities. As a means to overcome this, and increase the robustness of the procedure, a ratio of 0.2 mL/0.5 mL with incubation at 80 °C for 60 min was employed with success for the remainder of the study.

Results for the evaluation of TFAA showed complete derivatization of the amines and several of the hydroxyl group containing compounds was achieved without incubation. Although TFAA performed well, the trifluoroacetic acid byproduct generated damaged the column. Incorporation of an extraction procedure using 1 N sodium hydroxide failed to neutralize/remove the acid byproduct and also impacted the completeness of the derivatization.

Results for MBTFA showed derivatization of caprolactam, aniline, and a limited group of the hydroxyl containing compounds is achieved with incubation. The derivatized form of dibenzylamine was identified, but consistent responses were not obtained suggesting the reaction is not complete. No acid by products were produced by this reagent and a relatively clean chromatographic profile is obtained, as illustrated in Fig. 1.

MBTFA was ultimately selected as a complementary reagent for the method, mainly to perform derivatization of amines which are not adequately covered with the BSTFA/TMCS reagent. As with BSTFA, a ratio of 0.2 mL/0.5 mL incubated at 80 °C for 60 min was found to be successful.

3.3. Gas chromatography/mass spectrometry method development

Initial evaluation of the chromatographic separation was performed on a HP-5MS (5% phenyl/95% polydimethylsiloxane) column with a 20 m length, 0.18 mm diameter, and a 0.18 μm film thickness. Separation of a 10 μg/mL mix of the model compounds was found to be less than adequate and contained several partially co-eluting peaks. A switch to a more polar HP-35 (35% phenyl/65%

Table 2
Information for the model compound set..

Chemical name (colloquial name or abbreviation)	Identifier number	CAS number	Molecular weight	Boiling point (°C)	Log $P_{o/w}$ [1]	Analytical description	Approximate retention time (min)	Quantitation limit (ng/mL)
2-Oxohexamethyleneimine (caprolactam)	1	105-60-2	113.2	136	0.66	pH 9, TFA Derivative	15.7	90
Cyclohexanone	2	108-94-1	98.1	155	0.81	Neutral	9.3	67
Phenylamine (aniline)	3	62-53-3	93.1	184	0.90	pH 9, TFA Derivative	13.3	135
2-(2-Butoxyethoxy)ethyl acetate (2BEA)	4	124-17-4	204.3	245	1.30	pH 2	16.7	176
1,4-Diacetylbenzene	5	1009-61-6	162.2	263	1.34	Neutral	18.9	162
Hydroxybenzene (phenol)	6	108-95-2	94.1	182	1.46	pH 2, TMS Derivative	10.8	128
Phenyl methyl ketone (acetophenone)	7	98-86-2	120.2	202	1.58	Neutral	12.8	52
2-benzofuran-1,3-dione (phthalic anhydride)	8	85-44-9	148.1	295	1.60	pH 2	17.5	90
Hexanoic acid	9	142-62-1	116.2	202	1.92	pH 2, TMS Derivative	11.2	66
1,3-Benzothiazole (benzothiazole)	10	95-16-9	135.2	228	2.01	Neutral	15.6	187
Methyl hexyl ketone (2-octanone)	11	111-13-7	128.2	173	2.37	Neutral	10.4	125
1-Hydroxycyclylhexyl Phenyl Ketone (irgacure 184)	12	947-19-3	204.3	322	2.44	Neutral	21.4	747
2-Ethylhexanoic acid	13	149-57-5	144.2	228	2.64	pH 2, TMS Derivative	12.5	37
Dibenzylamine	14	103-49-1	197.3	270	2.67	pH 2	21.6	591
Metthyl-2 benzoylbenzoate (M2BB)	15	606-28-0	240.3	351	2.70	pH 2	24.6	144
Methylbenzene (toluene)	16	108-88-3	92.1	111	2.73	Neutral	4.5	84
2-Ethylhexan-1-ol (2-ethylhexanol)	17	104-76-7	130.2	186	2.73	pH 2, TMS Derivative	11.0	74
2-Phenylphenol	18	90-43-7	170.2	286	3.09	Neutral, TMS Derivative	19.5	38
Diphenylmethanone (benzophenone)	19	119-61-9	182.2	305	3.18	Neutral	20.9	96
(4,4′-(Propane-2,2-diyl)diphenol) Bisphenol A	20	80-05-7	228.3	220	3.32	pH 2, TMS Derivative	25.4	138
7,9-Di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (7,9-DTBD)	21	82304-66-3	276.4	NA	3.55	pH 2	23.4	450
Ethyl 4-ethoxybenzoate (E4EB)	22	5432-17-7	194.2	275	3.60	pH 2	19.1	125
2-Fluorobiphenyl	23	321-60-8	172.2	248	3.96	Neutral	17.1	22
Anthracene	24	120-12-7	178.2	340	4.45	Neutral	22.7	81
Di(*n*-butyl) phthalate (dibutyl phthalate)	25	84-74-2	278.3	340	4.50	pH 2	23.9	123
Decane	26	124-18-5	142.3	174	5.01	Neutral	8.8	92
2,6-Bis(1,1-dimethylethyl)-4-methylphenol (BHT)	27	128-37-0	220.4	265	5.10	Neutral	18.2	59
2-(5-Chloro-2-benzotriazolyl)-6-*tert*-butyl-*p*-cresol (Tinuvin 326)	28	3896-11-5	315.8	450	5.55	Neutral	29.1	466
Octamethylcyclotetrasiloxane (OMCTS)	29	556-67-2	296.6	176	6.74	pH 2	8.1	78
Di(2-ethylhexyl) phthalate (dioctyl phthalate)	30	117-81-7	390.6	384	7.60	pH 2	28.5	351
Octadecanoic Acid (stearic acid)	31	57-11-4	284.5	383	8.23	pH 2, TMS Derivative	24.7	545

[1] Log $P_{O/W}$ values were obtained using EPIWEB 4.1 Software.

polydimethylsiloxane) column was made. Separation of the test mix on this column was vastly improved. The only co-elution observed was phenol/2-ethyl-1-hexanol in the direct analysis and phenol/hexanoic acid in the BSTFA derivatized standard. Fig. 2 displays the chromatograms obtained for the non-derivatized working standard on both the HP-5MS and HP-35 columns to illustrate the full separation. Separation of the BSTFA/TMCS and MBTFA derivatized model compound mix on the HP-35 column is displayed in Fig. 3. The HP-35 column was selected based on the improvement in separation it provided. Although this column worked well for the model compound mix, and may be better suited for more polar extractables expected to be encountered in aqueous extraction solvents, the columns performance beyond this set of compounds cannot be inferred.

After evaluation, an oven program consisting of a 40 °C initial temperature, 10 °C/min thermal ramp, and 300 °C final hold temperature was found to work well for the separation. A splitless injection with a 0.4 μL injection volume was found to produce acceptable chromatography and instrument precision. Other parameters, such as inlet temperature and flow rate, were set based

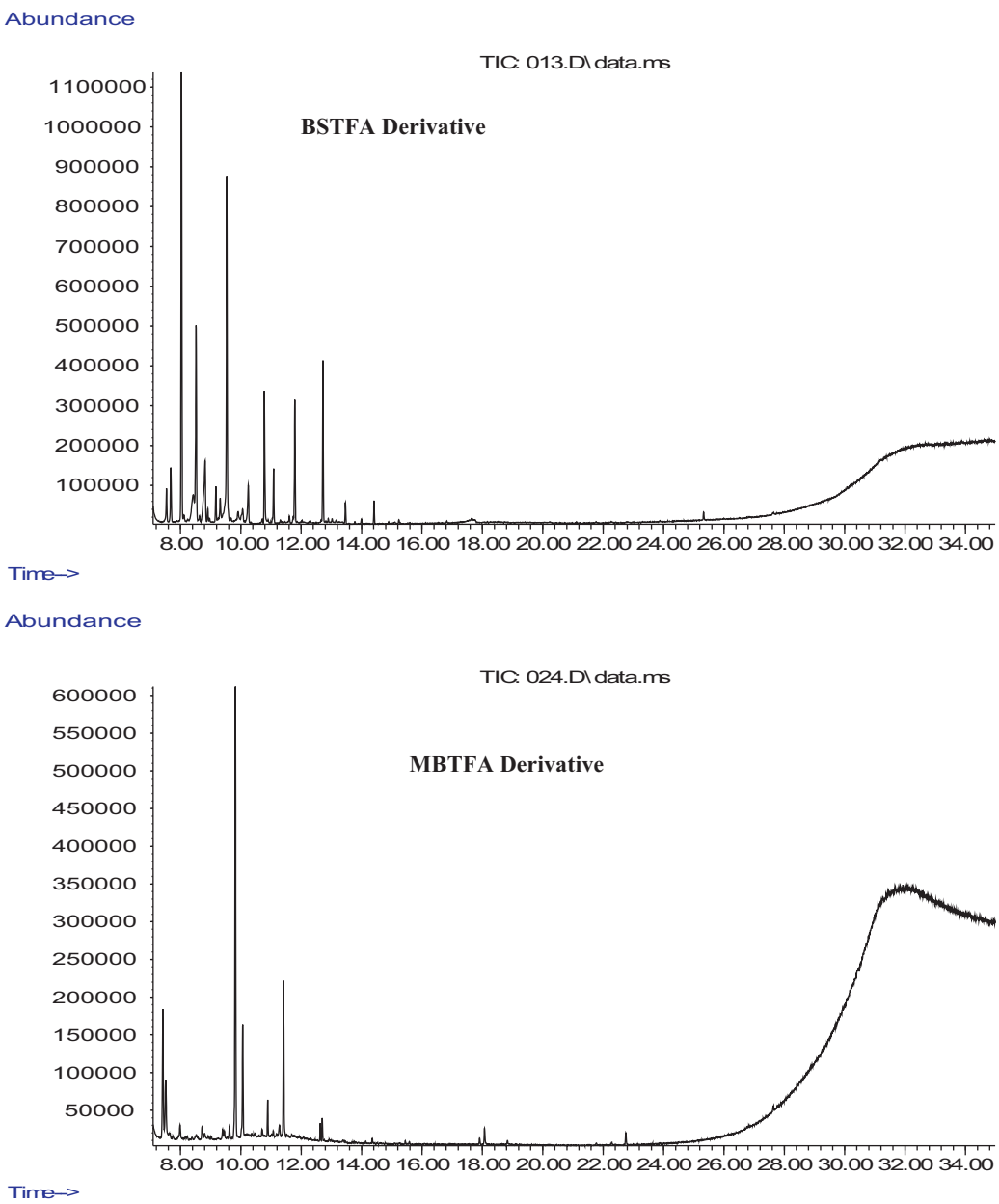

Fig. 1. Total ion chromatograms for BSTFA/TMCS and MBTFA derivatized ethyl acetate diluent. Several peaks attributable to derivatization artifacts are present for both reagents prior to about 13 min. The number and intensity of these peaks is not so significant as to preclude the detection and identification of extractables detected in this region.

on typical values for the column dimension used and analytes evaluated. An in-depth optimization study of these parameters was not performed.

Method sensitivity was evaluated by determining the quantification limit of each model compound in the test mix. Standard solutions were prepared at 0.1 μg/mL, 0.5 μg/mL, 1 μg/mL, and 10 μg/mL. Each standard level was analyzed directly as well as after BSTFA/TMCS and MBTFA derivatization. Data obtained for the lowest concentration producing a response near a signal to noise ratio of 10 was used to estimate the USP signal to noise ratio for each compound.

Calculation of the USP signal to noise ratio is defined in Eq. (1) with H being the height of the peak response and h representing the height of the baseline noise determined over a range at least 5 times the width of the peak. Once the signal to noise ratio was calculated, the concentration corresponding to a USP signal to noise ratio of 10 was determined by Eq. (2). Quantification limit concentration values obtained for each model compound are listed in Table 1.

$$\text{USP S/N ratio} = \frac{2H}{h} \tag{1}$$

$$\text{Concentration equal to a USP S/N ratio of 10} = \frac{\text{Standard concentration(ng/mL)}}{\text{USP signal to noise ratio of peak}} \times 10 \tag{2}$$

3.4. Solid phase extraction method development

The most critical variable in the solid phase extraction method is the mode used and subsequent stationary phase selected. As the focus of this work is the recovery of compounds from aqueous matrices, chromatographic modes, and the associated stationary

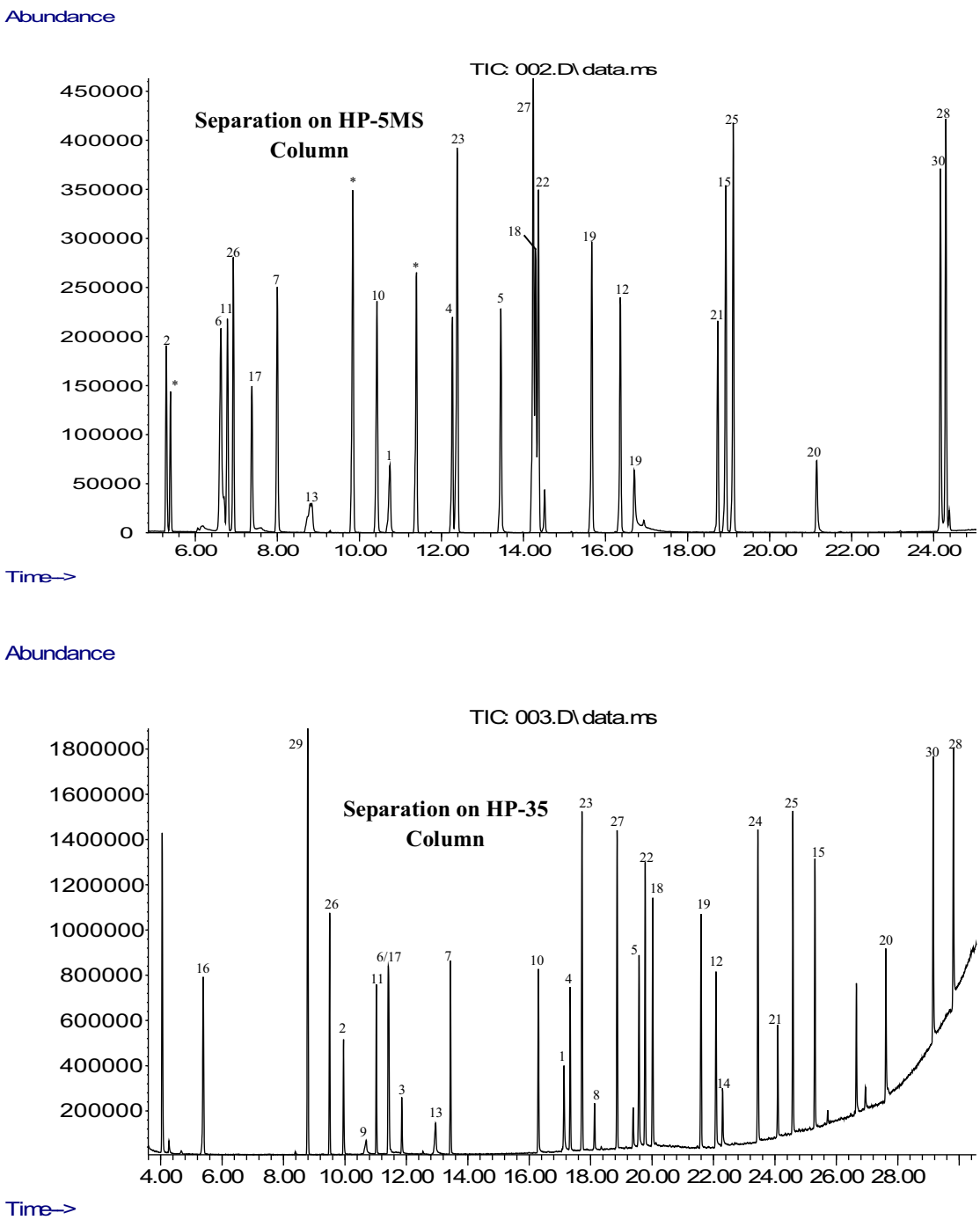

Fig. 2. Comparison of the model compound mix separation on HP-5MS and HP-35 columns. See Table 2 for numerical identifiers for each compound. Peaks marked with an * correspond to hexanal, naphthalene, and tridecane, respective to their elution times, which were included in the test mix used for initial evaluation of separation on the HP-5MS column but were not included for the remainder of the study. Phthalic anhydride, anthracene, and OMCTS were not present in this mix. Several peaks are co-eluting, or partially co-eluting, on the HP-5 column. Baseline separation of all but 1 peak pair is achieved with the HP-35 column. Retention is also improved, most notably for toluene (Peak 16, not observed on the HP-5 column), cyclohexanone (peak 2), and phenol (peak 6) 6.

phases, potentially suitable for this purpose were considered. Chromatographic modes noted to be most applicable to the analysis of organic compounds from aqueous matrices are ion exchange and reverse phase. Ion exchange chromatography is applicable for the recovery of polar ionic compounds from aqueous matrices, which is an attractive characteristic. However, since this approach requires optimization for the specific class of analyte being analyzed, may not work well for neutral compounds, and typically involves aqueous or more polar eluents, it was not investigated. Reverse phase chromatography, by principal, retains non-polar substances in polar mobile phases, typically via hydrophobic carbon based stationary phases such as C_{18} or C_8. While these may be the most popular reverse phase sorbents, they are certainly not the only options available. In fact, a review of the literature [26–31] showed styrene/divinylbenzene (SDVB) polymers, hydrophilic/lyphophilic balanced (HLB) styrene polymers, and graphitized carbon sorbents have been successfully used for the recovery of polar compounds from aqueous matrices. Despite their applicability to polar compounds, these stationary phases still operate predominantly by a reverse phase mechanism and thus are expected to recover more

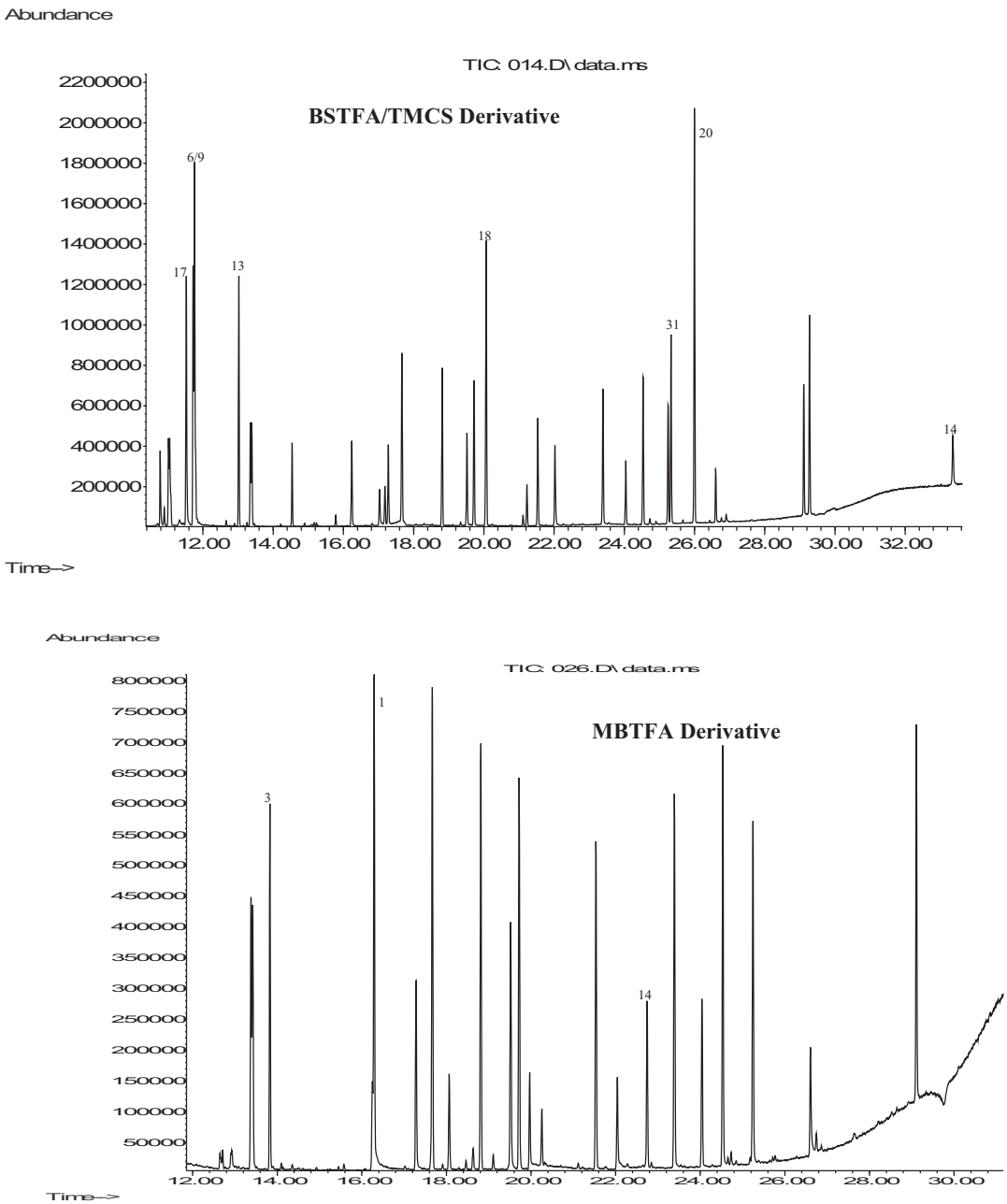

Fig. 3. Total ion chromatogram of BSTFA/TMCS and MBTFA derivatives of the 10 μg/mL model compound mix. See Table 2 for numerical identifiers for each compound. Only compounds which formed derivatives are identified. The chromatography for the derivatized model compound mixtures illustrates the significant improved in peak shape and response as compared to the non-derivatized chromatography. For example, compounds such as aniline (peak 3) and 2-ethylhexanoic acid (peak 13) are present in the derivatized chromatography with responses several times higher than the non-derivatized results. Stearic Acid (peak 31) is detected in the derivatized results but was not observed in the non-derivatized chromatography.

hydrophobic compounds as well. Based on this information, these phases were selected for further evaluation.

Each stationary phase was evaluated by loading and recovering the model compounds listed in Table 2. Results for this evaluation, in the form of the percent of each analyte recovered relative to an external standard response, are displayed in Table 3. With the exception of the graphitized carbon phase, most compounds showed adequate recoveries, defined as being within 70% to 130% of the theoretical concentration. The graphitized carbons stationary phase showed no recovery for all but nine of the model compounds indicating it is not suitable for the purposes of this work. Overall, the SDVB phase produced acceptable analyte recovery for the most model compounds and none were found to have recovery values of less than 50%. Based on these results, the SDVB phase was determined to be at least comparable, if not modestly better, than the other two stationary phases evaluated. Accordingly, it was selected as the stationary phase for use in the method.

In order to eliminate the need to bring the eluent to an exact volume prior to analysis, and to improve reproducibility, the use of an internal standard was incorporated into the method. 2-fluorobiphenyl was included in the initial test mix to determine its suitability for this purpose. Its selection as a potential internal standard is justified by the fact that it is not a polymeric additive, elastomeric additive, or related substance, and has been previously evaluated [32,33] for this purpose. Recovery experiments performed during the development of the method showed

Table 3
Model compound recovery results for the stationary phase screening experiment.

Compound	Mean (n = 2) percent recovered (20 μg loaded on column)			
	HLB	C_{18}	SDVB	Graphitized Carbon
Caprolactam	36	42	80	42
Cyclohexanone	101	95	101	109
Aniline	53	45	58	0
2BEA	100	98	109	103
1,4-Diacetylbenzene	95	95	109	0
Phenol	86	87	111	36
Acetophenone	100	96	103	0
Phthalic anhydride	18[1]	73	82[1]	0
Hexanoic acid	97	96	115	0
Benzothiazole	99	97	101	0
2-Octanone	100	99	97	88
Irgacure 184	98	91	103	0
2-Ethylhexanoic Acid	97	96	111	18
Dibenzylamine	27	90	76[1]	0
M2BB	97	98	101	0
Toluene	37	0	62	0
2-Ethylhexanol	99	98	115	78
2-Phenylphenol	77	98	109	0
Benzophenone	85	95	89	0
Bisphenol A	3[1]	99	111	0
7,9-DTBD	100	99	102	0
E4EB	85	97	96	0
2-Fluorobiphenyl	82	95	90	0
Anthracene	81	79	61[1]	0
Dibutyl phthalate	79	93	83	0
Decane	65	0	77	34
BHT	89	87	94	0
Tinuvin 326	85	88	85	0
OMCTS	30	73	58	60
Dioctyl phthalate	86	93	94	0
Stearic acid	76[1]	91	123	0

[1] Percent difference from mean was ≥15.

2-fluorobiphenyl had recovery values in the 70–90% range. Ideally, an internal standard should be recovered both precisely and accurately (i.e. as close to 100% as possible) to prevent bias of the other results. For this reason, 2-fluorobiphenyl was not selected as an internal standard. Instead, acetophenone was chosen for this purpose. Justifications for selection of acetophenone as an internal standard include precise and accurate recovery via the SPE procedure, a neutral nature that does not derivatize, and commercial availability in high purities from multiple sources. The use of acetophenone directly as an internal standard is not advisable, as it is a known extractable compound and thus would obscure any response of this substance if it were present in an extract sample. Instead, acetophenone-(phenyl-d_5) was used. Use of a deuterium analog allows for differentiation between the internal standard and any intrinsic acetophenone while featuring the benefits of acetophenone's chromatographic properties.

3.5. Evaluation of analyte breakthrough during sample loading

As a consequence of the dosing regiments and/or fill volumes associated with some pharmaceutical products, sub part-per-million analytical thresholds are often needed to assess product safety. In order to adequately detect extractable substances at these levels a certain degree of analyte enrichment is required. In principal, SPE offers a means to achieve this degree of enrichment through its ability to load an indefinite amount of sample onto the stationary phase. However, in reality, large sample volumes can result in desorption of retained analytes from the stationary phase during the loading process. This phenomenon is referred to as analyte breakthrough. While hydrophobic analytes retain strongly on a reverse phase sorbent and have minimal breakthrough concern, more hydrophilic compounds, as well as hydrophobic compounds in certain matrices (for example, aqueous containing an organic modifier), may have capacity factors less than the total volume applied. In these situations problematic levels of analyte breakthrough would be encountered. Due to the need for the method to be able to analyze both hydrophobic and relatively hydrophilic extractables, and provide for high levels of enrichment, an assessment of breakthrough is important and was performed.

The first breakthrough experiment utilized water as solvent. Water is selected to represent an extraction sample consisting of pure water, a common extraction solvent. It also represents aqueous solvents containing polar modifiers such as sugars, salts, or other compounds not expected to desorb analytes from the stationary phase with a propensity greater than water. The second experiment evaluated a solution of 10/90 (v/v) isopropanol/water. This mixture represents a water/alcohol extraction solvent used as is or diluted down from a higher organic ratio. The presence of organic may also represent a product formulation which contains surfactants that have a solubilizing effect for more hydrophobic substances.

To evaluate breakthrough, model compounds were first loaded onto the stationary phase by spiking into 2 mL of water. Although this may not be representative of a large volume containing a homogeneous distribution of analytes, it ensures each sample is comparable. Solvent volumes of 0 mL (control), 6 mL, 12 mL, 25 mL, 50 mL, and 100 mL were evaluated by passing the solvent over the pre-loaded stationary phase. For each volume, two columns were prepared to allow for adjustment to pH ≥ 9.5 and pH ≤ 2.5. The extent of breakthrough was assessed by calculating the percent of each analyte recovered relative to the theoretical mass loaded. The percent recovered for the control, mean percent recovered for volumes showing minimal breakthrough, and the percent relative standard deviation (%RSD) of the percent recovered for these volumes were determined. These results can be found in Table 4.

Results for water showed no significant change in percent recovered over the range of volumes tested for all compounds with the exception of caprolactam and stearic acid. Stearic acid was retained

Table 4
Breakthrough experiment results.

Compound	Water					10% Isopropanol/90% water		
	Percent recovered in control (0 mL)	Maximum volume with minimal breakthrough observed (mL)	Mean percent recovered for volumes showing minimal breakthrough[1]	%RSD of percent recovered values[1]	Percent recovered in control (0 mL)	Maximum volume with minimal breakthrough observed (mL)	Mean percent recovered for volumes showing minimal breakthrough[1]	%RSD of percent recovered values[1]
Caprolactam	66	6	66	NA	80	0	NA	NA
Cyclohexanone	107	100	111	7	109	6	108	1
Aniline	67	100	88	12	85	12	83	7
2BEA	104	100	103	3	116	100	105	6
1,4-Diacetylbenzene	82	100	88	3	82	100	82	2
Phenol	94	100	105	6	106	25	108	3
Acetophenone	90	100	102	7	101	100	100	1
Phthalic anhydride	100	100	71	13	116	100	112	7
Hexanoic acid	76	100	89	4	99	50	96	3
Benzothiazole	83	100	93	4	110	100	95	10
2-Octanone	105	100	104	5	102	100	104	5
Irgacure 184	94	100	98	2	101	100	93	8
2-Ethylhexanoic Acid	102	100	101	3	91	100	93	4
Dibenzylamine	80	100	63	21	130	100	108	14
M2BB	87	100	100	5	106	100	100	9
Toluene	86	100	80	7	53	100	60	6
2-Ethylhexanol	94	100	100	7	96	100	99	7
2-Phenylphenol	97	100	100	4	99	100	91	13
Benzophenone	84	100	88	2	99	100	90	9
Bisphenol A	65	100	56	6	76	100	61	9
7,9-DTBD	99	100	106	8	106	100	105	10
E4EB	86	100	92	4	91	100	89	9
2-Fluorobiphenyl	87	100	81	6	88	100	90	4
Anthracene	70	100	68	3	53	100	56	12
Dibutyl phthalate	82	100	90	6	86	100	86	6
Decane	90	100	81	8	89	100	83	5
BHT	98	100	92	5	98	50	84	8
Tinuvin 326	66	100	48	9	97	6	93	6
OMCTS	86	100	71	8	61	100	70	6
Dioctyl phthalate	80	100	92	7	104	50	93	10
Stearic acid	101	25	81	11	55	12	42	11

[1] Mean percent recovered and standard deviation values are based on results showing minimal breakthrough, up to 5 for those showing no significant breakthorugh for any volume evaluated.

well up to 25 mL, which would still allow an appreciable degree of enrichment to be obtained. Caprolactam, the most hydrophilic compound in the model compound mix, showed obvious breakthrough occurred at volumes greater than 6 mL.

A greater incidence of analyte breakthrough was observed for the 10/90 isopropanol/water solvent evaluation. These results are consistent with the presence of isopropanol in the solution, which is expected to decrease the capacity factors of all analytes evaluated. Despite this, only caprolactam, cyclohexanone, and Tinuvin 326 had breakthrough evident at volumes less than 12 mL.

3.6. Demonstration of accuracy and precision

As a consequence of the wide range of sample matrices that may be encountered in a given extractables survey, a comprehensive determination of accuracy and precision is not possible. Instead, water, 1% Polysorbate 80, and 10/90 isopropanol/water were chosen to demonstrate method performance. As a corollary to this, it is important to note that although accuracy/precision is evaluated, the intent is not to "validate" the method. These results are reported to demonstrate method capability and provide confidence in its ability to perform its function. It may be neither necessary, nor appropriate, to perform method validation at the extractable survey stage of a pharmaceutical packaging/delivery systems characterization. The development and validation of methods for the analysis of targeted compounds is typically performed in separate studies following the extractable survey.

Analyte concentrations and solution volumes chosen for the accuracy/precision experiment are a reflection of the expected extraction characteristics of water, polysorbate 80 and isopropanol/water solvents. Water is a weak extraction solvent as, by nature, it is polar; most extractable type compounds are typically hydrophobic and thus have marginal solubility in water at best. Therefore, the accuracy/precision experiment performed for the water matrix involved a 100 mL volume spiked at 20 ng/mL. Polysorbate 80 is a surfactant commonly used in parenteral products to stabilize aqueous formulations. As a surfactant, polysorbate 80 increases the solubility of hydrophobic compounds in aqueous solution, which has been reported [34] to increase the migration of container related contaminants into a drug product. Consequently, it is often desired to have an extraction solvent or drug placebo containing polysorbate 80 to evaluate these characteristics. For the purpose of this work, a volume of 4 mL 1% polysorbate 80 spiked at 5 μg/mL was evaluated. Extraction solvents composed of some proportion of organic solvent and water are commonly included in an extractables survey as a means of exaggerating the extraction of the material. To simulate this, an evaluation of a 50/50 isopropanol/water spiked at a concentration of 50 μg/mL was performed.

Water and 1% polysorbate 80 were spiked in-column and then loaded directly onto the stationary phase. Isopropanol/water was spiked and then diluted to an organic concentration of 10% to minimize analyte breakthrough. As pure solvents, water and isopropanol/water produced clean chromatographic profiles. Polysorbate 80 is a relatively complex substance and produced

Table 5
Accuracy/precision experiment results.

Compound	Number of preparations[1]	100 mL of water spiked at 20 ng/mL		4 mL of 1% TWEEN 80 spiked at 5 μg/mL		2 mL of 50% isopropanol/50% water spiked at 50 μg/mL	
		Mean percent recovered	% RSD	Mean percent recovered	% RSD	Mean Percent Recovered	% RSD
Caprolactam	6	13	31	43	15	13	4
Cyclohexanone	12	87	15	91	3	96	7
Aniline	6	79	7	96	4	98	1
2BEA	6	92	11	102	2	100	3
1,4-Diacetylbenzene	12	107	5	102	1	100	4
Phenol	6	99	10	91	18	102	1
Acetophenone	12	103	2	100	1	100	1
Phthalic anhydride	6	180	35	110	11	113	4
Hexanoic acid	6	108	2	104	1	99	1
Benzothiazole	12	104	3	108	6	99	1
2-Octanone	12	107	10	94	3	94	1
Irgacure 184	12	106	9	115	5	89	17
2-Ethylhexanoic acid	6	98	2	99	2	101	1
Dibenzylamine	6	21	114	187	9	109	12
M2BB	6	107	4	100	2	99	8
Toluene	12	98	3	53	15	54	20
2-Ethylhexanol	6	188	8	103	1	110	2
2-Phenylphenol	6	100	1	93	7	100	2
Benzophenone	12	105	3	103	3	89	4
Bisphenol A	6	104	3	82	24	101	4
7,9-DTBD	6	111	9	136	4	99	11
E4EB	6	99	2	99	1	87	6
2-Fluorobiphenyl	12	73	3	99	1	81	2
Anthracene	12	80	19	94	11	72	11
Dibutyl phthalate	6	86	9	101	3	79	7
Decane	12	122	3	63	7	80	7
BHT	12	59	12	88	7	85	3
Tinuvin 326	12	59	16	103	20	66	13
OMCTS	6	12	24	54	10	59	15
Dioctyl phthalate	6	72	9	85	7	72	9
Stearic acid	6	76	15	NA[2]	84	4	

[1] Compounds that recovered well in both low and high pH preparations had results for both reported. Compounds that were affected by pH, or required derivatization, had recovery values reported for that condition only.
[2] Due to a large amount of stearic acid intrinsically present in the matrix, accurate quantitation is not possible.

several responses determined to be intrinsic to it. These responses did not have a significant impact on the specificity of the separation in either the directly analyzed or derivatized samples. The only exception was a relatively significant amount of stearic acid which made quantitation of this compound in the test mix not possible.

Table 5 displays the percent of each analyte recovered as well as the %RSD of the recovery values obtained in this experiment. Caprolactam proved to be the most difficult compound to recover and was found to have poor recovery values in all three solvents. Problematic recovery for this target is suspected to be a function of its hydrophilic nature, although compounds with similar log *P* values, such as cyclohexanone and aniline, were found to recover well. It is expected that the recovery of caprolactam will improve as the solvent volume is decreased and concentration is increased based on results from the initial column evaluation and breakthrough experiment results. Dibenzylamine was also found to recover inconsistently across the solvents and concentrations evaluated. This may be a result of the underivatized amine functionality in the molecule impacting its chromatographic response. It is noted that while dibenzylamine was found to convert to both TMS and TFA derivatives, the completeness of derivatization was inconsistent and thus its underivatized form had to be evaluated.

In some cases, less than ideal recovery values were observed for the other compounds evaluated. Despite these results, the overall results of the accuracy/precision study showed quantitative (recovery within 70% to 130% of the theoretical concentration) and precise (%RSD ≤15%) recovery for the majority of target analytes are achieved for all three solvents evaluated. For those that were not recovered within the desired range it is important to note that they were still recovered to some degree, generally >50% of the theoretical concentration.

3.7. Case study

Analysis of aqueous extracts from a single IV bag configuration was performed to illustrate the use of the method for genuine samples generated as part of an extractables survey. This packaging configuration was selected as the subject of this evaluation for several reasons. First, IV bags are a common and well recognized means for the storage and delivery of therapeutic solutions. Thus, they are frequently the subject of extractable surveys for parenteral products employing them as a storage/delivery system. Second, IV bags often have relatively large fill volumes (≥50 mL) administered in a short time (<24 h) resulting in large volumes administered on a daily basis. This combination may cause analytical difficulties due to the need for the analytical threshold to be set at the part-per-billion level in order to ensure compounds present are adequately characterized. The use of SPE, as proposed in this work, offers a means to overcome this challenge. Finally, IV bags are readily available from generic suppliers making them easily obtainable for this work.

In this study, the IV bag was extracted with multiple solvents at 70 °C for a duration of 2 days. Two aqueous buffers, one at pH 2.5 and one at pH 9.5, a 20/80 mixture of isopropanol and water, and a 1% polysorbate 80 solution were selected as extraction solvents. This range of solvents is consistent with those that may be included in a typical extractable survey to evaluate the impact pH, organic content, and formulation components have on the extractable profile. An extraction temperature of 70 °C and an extraction duration of 2 days were selected to represent a beyond worst case exposure scenario for a parenteral product that contacts the IV bag for a relatively short period (≤1 week) at low exposure temperatures (≤20 °C).

Table 6
Case study results.

Extractable identification	Retention time (min)	Identification level	Extractable mass (μg/IV bag)		1% Polysorbate 80	80/20 Water/ isopropanol extract
			pH 2.5 Extract	pH 9.5 Extract		
Cyclohexanone	9.2	Confirmed	8.0	6.9	22.7	2.8
2-Ethylhexanol, TMS	11.2	Confirmed	ND	ND	40.5	ND
Aniline, TFA	13.2	Confirmed	3.2	ND	ND	ND
Nonanoic Acid, TMS	15.4	Confident	ND	4.8	ND	ND
Pyrrole 2-carboxylate, TMS	15.7	Confident	ND	0.6	ND	ND
Decanoic acid, TMS	16.6	Confident	ND	3.6	ND	ND
Phthalic Anhydride	17.5	Confirmed	ND	3.7	ND	ND
Adipic acid, TMS	17.7	Confident	ND	1.8	ND	ND
Butylated hydroxytoluene	18.5	Confirmed	ND	ND	40.0	ND
Dodecanoic Acid, TMS	18.9	Confident	ND	31.7	110	2.2
Vanillin, TMS	19.1	Tentative	ND	ND	ND	0.9
Benzoic acid, 2-ethylhexyl ester	20.9	Confident	ND	ND	16.0	ND
Tetradecanoic acid, TMS	21.0	Tentative	ND	8.9	ND	0.3
Dibenzylamine	21.6	Confirmed	5.3	ND	ND	ND
Hexadecanoic acid, TMS	22.9	Confident	ND	5.3	406	ND
Mono(2-ethylhexyl) phthalate, TMS	24.8	Confident	ND	39.3	ND	ND
Octadecanoic Acid, TMS	24.9	Confident	ND	ND	736	ND
Unspecified Phthalate	25.3	Tentative	ND	ND	5.8	ND
Di(Octyl) phthalate isomer	27.7	Confident	ND	ND	48.8	ND
Di(Octyl) phthalate isomer	28.3	Confident	ND	ND	404	ND
Di(2-ethylhexyl) Phthalate	28.4	Confirmed	0.2	12.2	60756	8.4

ND = not detected.

The extractable profile obtained from the analysis of the extraction samples via the solid phase extraction-GC/MS method is presented in Table 6. As a means to access the completeness of the extractable profile generated by the SPE-GC/MS method a comparison to the available literature [6,22,35,17] on the subject of material composition/extractable profiles of PVC is made. Although the exact formulation of the material analyzed in this study vs. that reported in the literature are likely not identical, correlations can still be made as these materials require certain additives to fulfill their functions; thus, any obvious omissions from the results could indicate a non-representative profile was obtained.

In order to achieve the desired characteristics of the PVC material a significant amount (≥20% w/w) of a plasticizer is added to the PVC base resin. Given the high mass of this substance in the material it is logical to expect it to contribute to the extractable profile of the material. Indeed, a review of the extractable profile obtained shows the presence of di(2-ethylhexyl)phthalate, the most common plasticizer historically used to plasticize PVC. The relatively low mass of this substance quantified in the aqueous buffers and isopropanol/water extraction solvents is consistent with its hydrophobic nature. The fact that polysorbate 80 produced the largest extractable mass is consistent with the extraction properties of this solvent reported in the literature [36]. In addition to the plasticizer, several compounds which are related to it are observed in the results. Most notably, phthalic anhydride, which is actually a reaction product of phthalic acid formed in the GC inlet, benzoic acid 2-ethylhexyl ester, and mono(2-ethylhexyl) phthalate are identified. These extractables are formed by loss of one, or both, of the 2-ethylhexyl side chains of this molecule.

Other prominent additives in PVC material which may produce extractables include secondary plasticizers, acid scavengers, heat stabilizers, and lubricant/slip agents. The series of "fatty" carboxylic acids observed in the extractable profile is likely attributable to degradation products of the metal stearate salt heat stabilizers/acid scavengers. No extractables were observed that could be correlated to a lubricant or secondary plasticizer. Although this discrepancy may seem significant, it is most likely a function of the physical properties of these compounds. For example, epoxidized vegetable oils are typically used as a secondary plasticizer while erucamide, or a similar long-chain amide, is used as the lubricant/slip agent. Both of these additives are very hydrophobic and are not volatile. Thus, they would not be expected to produce an appreciable response by GC analysis and may not extract well into the aqueous solvent systems explored in this work.

In addition to extractables related to major additives of PVC, several other compounds which could not be correlated are observed. Some can be reconciled based on knowledge of their function. For example, BHT is an antioxidant while cyclohexanone is a bonding solvent used in the construction of the bags. Others, while not necessarily well characterized, were detected and thus are constituents of this materials extractable profile.

4. Conclusions

A method utilizing SPE with derivatization followed by analysis via GC/MS was developed and evaluated for the analysis of a diverse range of organic semi-volatile type extractable substances from aqueous extraction solvents. Adequate levels of analyte recovery were demonstrated for up to 100 mL of water and isopropanol/water illustrating the potential for high enrichment factors to be provided by the method. Accuracy results for compounds spiked into water at 20 ng/mL, 1% polysorbate 80 at 5 μg/mL, and isopropanol/water at 50 μg/mL showed percent recovery values in the range of 70%–130% for 82% of the solvent/compound combinations. Precision results for this evaluation produced %RSD values of less than or equal to 15 in 88% of the solvent/compound combinations. After comparing the extractable profile obtained for PVC material generated in this study to information available in the literature it is concluded, within the limitation of such a comparison, that a representative extractable profile was obtained.

Acknowledgments

The author would like to acknowledge James Mullis and Julie Sutcliffe for proof reading and providing valuable feedback on this work.

References

[1] U.S. Department of Health and Human Services, Food and Drug Administration Guidance for Industry, Container Closure Systems for Packaging Human Drugs

and Biologics, U.S. Department of Health and Human Services, Food and Drug Administration Guidance for Industry, Rockville, MD, 1999 (May 1999).
[2] Food and Drug Administration, Code of Federal Regulations, 21CFR211.94, Control of Components and Drug Product Containers and Closures, Title 21, Volume 4, Chapter 1.
[3] European Medicines Agency, Guideline on Plastic Immediate Packaging Materials, CPMP/QWWP/4359/03. EMEA/CVMP/205/04, European Medicines Agency, 2005 (May 19 2005).
[4] C.L.M. Stults, J. Mikl, O. Whelehan, B. Morrical, W. Duffield, L.M. Nagao, A risk based approach to management of leachables utilizing statistical analysis of extractables, AAPS PharmSciTech (2014) 1–12.
[5] C.L.M. Stults, J.M. Ansell, A.J. Shaw, L.M. Nagao, Evaluation of extractables in processed and unprocessed polymer materials used for pharmaceutical applications, AAPS PharmSciTech (2014) 1–15.
[6] D. Jenke, Compatibility of Pharmaceutical Products and Contact Materials, John Wiley and Sons, New Jersey, NJ, 2009.
[7] Product Quality Research Institute, PQRI Leachables and Extractables Working Group Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, Product Quality Research Institute, Arlington, VA, 2006.
[8] D. Paskiet, D. Jenke, D. Dall, C. Houston, D.L. Norwood, I. Markovic, The product quality research institute (PQRI) leachables and extractables working group initiatives for parenteral and ophthalmic drug products, PDA J. Pharm. Sci. Technol. 67 (2013) 430–447.
[9] D.J. Ball, D.L. Norwood, C.L.M. Stults, L.M. Nagano, Extractables and Leachables Handbook: Safety Evaluation and Best Practices Applied to Inhalation Drug Products, John Wiley and Sons, New Jersey, NJ, 2012.
[10] A. Feilden, Update on Undertaking Extractable and Leachable Testing, Smithers Rapra Technology, Shawbury, Shrewsbury, Shropshire, UK, 2010.
[11] S.A. Zdravkovic, M.D. Bruss, R.F. Piccoli, D.M. Wood, A method utilizing ultra-high performance liquid chromatography with ultraviolet and mass spectrometric detection for the analysis of material extracts produced during a controlled extraction study, PDA J. Pharm. Sci. Technol. 68 (2014) 504–526.
[12] C. Pouche, F. Lafay, L. Wiest, R. Baudot, D. Leonard, C. Cren-Olive, Monitoring the extraction of additives and additive degradation products from polymer packaging into solutions by multi-residue method including solid phase extraction and ultra-high performance liquid chromatography–tandem mass spectrometry analysis, Anal. Bioanal. Chem. 406 (2014) 1493–1507.
[13] D.L. Norwood, J.O. Mullis, M. Davis, S. Pennino, T. Egert, N.C. Gonnella, Automated solid phase extraction (SPE) LC/NMR applied to the structural analysis of extractable compounds from a pharmaceutical packaging material of construction, PDA J. Pharm. Sci. Technol. 67 (2013) 267–287.
[14] D. Shen, H. Lian, T. Ding, J. Xu, C. Shen, Determination of low level ink photoinitiator residues in packaged milk by solid-phase extraction and LC–ESI/MS/MS using triple quadrupole mass analyzer, Anal. Bioanal. Chem. 395 (2009) 2359–2370.
[15] N.C. Maragou, E.N. Lampi, N.S. Thomadis, M.A. Kouparris, Determination of Bisphenol A in milk by solid phase extraction and liquid chromatography–mass spectrometry, J. Chromatogr. A 1129 (2006) 165–173.
[16] J. Li, Y. Cai, Y. Shi, S. Mou, G. Jiang, Analysis of phthalates via HPLC–UV in environmental water samples after concentration by solid-phase extraction using ionic liquid mixed hemimicelles, Talanta 74 (2008) 498–504.
[17] D. Jenke, Extractable/leachable substances from plastic materials used as pharmaceutical product containers/devices, PDA J. Pharm. Sci. Technol. 56 (2002) 332–371.
[18] D.R. Knapp, Handbook of Analytical Derivatization Reactions, first ed., John Wiley and Sons, New Jersey, NJ, 1979.
[19] R.J. Wells, Recent advances in non-silylation derivatization techniques for gas chromatography, J. Chromatogr. A 843 (1999) 1–18.
[20] A. Ahuja, Derivatization in gas chromatography, J. Pharm. Sci. 65 (1976) 163–182.
[21] Guide to Derivatization Reagents for GC, Supelco Bulletin 909A, Sigma-Aldrich, 1997, Retrieved from ⟨http://www.sigmaaldrich.com⟩.
[22] D. Jenke, T. Castner, T. Egert, et al., Extractables characterization for five materials of construction representative of packaging systems used for parenteral and ophthalmic drug products, PDA J. Pharm. Sci. Technol. 67 (2013) 448–511.
[23] H. Kataoka, Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis, J. Chromatogr. A 733 (1996) 19–34.
[24] BSTFA Instructions. Thermo Scientific, Retrieved from ⟨http://fscimage.fishersci.com/images/D00370~.pdf⟩.
[25] M.D. Hernando, M. Mezcua, M.J. Gómez, O. Malato, A. Agüera, A.R. Fernández-Alba, Comparative study of analytical methods involving gas chromatography–mass spectrometry after derivatization and gas chromatography–tandem mass spectrometry for the determination of select endocrine disrupting compounds in wastewaters, J. Chromatogr. A 1047 (2004) 129–135.
[26] N. Masqué, R.M. Marcé, F. Borrull, New polymeric and other types of sorbents for solid-phase extraction of polar organic micropollutants from environmental water, TrAC, Trends Anal. Chem. 17 (1998) 384–394.
[27] M.-C. Hennion, C. Cau-Dit-Coumes, V. Pichon, Trace analysis of polar organic pollutants in aqueous samples—tools for the rapid prediction and optimisation of the solid-phase extraction parameters, J. Chromatogr. A 823 (1998) 147–161.
[28] M.-C. Hennion, Solid phase extraction: method development, sorbents and coupling with liquid chromatography, J. Chromatogr. A 856 (1999) 3–54.
[29] C.W. Huck, G.K. Bonn, Recent developments in polymer-based sorbents for solid-phase extraction, J. Chromatogr. A 885 (2000) 51–72.
[30] M.-C. Hennion, Graphitized carbons for solid phase extraction, J. Chromatogr. A 885 (2000) 73–95.
[31] S. Weigel, R. Kallenborn, H. Huhnerfuss, Simultaneous solid phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography–mass spectrometry, J. Chromatogr. A 1023 (2004) 183–195.
[32] J.O. Mullis, A. Granger, C. Quin, D.L. Norwood, The analytical evaluation threshold (AET) concept, sensitivity and analytical uncertainty, in: Conference Proceedings, Leachables and Extractables, Smithers-Rapra, Dublin, Ireland, 2008.
[33] D. Jenke, A. Odufu, Utilization of internal standard response factors to estimate the concentration of organic compounds leached from pharmaceutical packaging systems and application of such estimated concentrations to safety assessment, J. Chromatogr. Sci. 50 (2012) 206–212.
[34] D. Jenke, A general assessment of the physiochemical factors that influence leachables accumulation in pharmaceutical drug products and related solutions, PDA J. Pharm. Sci. Technol. 65 (2011) 166–176.
[35] A. Teasdale, M. Jahn, S. Bailey, A. Feilden, G. Taylor, M.L. Corcoran, R. Malick, D. Jenke, C.L.M. Stults, L.M. Nagao, Controlled extraction studies applied to polyvinyl chloride and polyethylene materials: conclusions from the ELSIE controlled extraction study pilot program, AAPS PharmSciTech (2014) 1–11.
[36] D. Jenke, Evaluation of model solvent systems for assessing the accumulation of container extractables in drug formulations, Int. J. Pharm. 224 (2001) 51–60.