Extractables Guide

Single-use Filling Bags

Shanghai LePure Biotech Co., Ltd

Catalogue

1. Introductions

The pharmaceutical single use system produced by Shanghai LePure is widely used in the bio-pharmaceutical process. Its main application fields include the research, development and production of antibody, vaccine and cell therapy products. At present, pharmaceutical single use systems are widely used in upstream, downstream and final filling processes. Therefore, end users must fully understand and verify their interactions with bio-pharmaceutical solutions and final drug products. In order to ensure the product quality, the pharmaceutical enterprises shall conduct comprehensive analysis and testing in the early stage of process development and within the scope of process monitoring and quality control, so as to prove the purity, efficacy and safety of drugs. Product safety assessment shall be carried out in the process of design and development of the single use system. These validation studies involve complete quantitative, identification and toxicological assessment of the leachables, which are the substance remained in the drug solution due to the interaction between the drug solution and the pharmaceutical single use system. Leachables are a subset of the extractables that can be extracted from pharmaceutical single use systems. Usually, the solvents and conditions for extractables testing are more severe than that for leachables. The purpose of this Guide is to provide the worst-case data on extractables to support the validation studies conducted by process developers and toxicologists.

The safety problem of disposable components, which is the most common problem in the pharmaceutical single use system, has always been the most concerned problem of many pharmaceutical enterprises, especially for the safety of extractables. For this kind of disposable components, LePure has referenced the technical data of foreign raw material suppliers, the technical guidelines for compatibility studies published by domestic CDE

(including Technical Guidelines for Compatibility Studies of Chemical Injection and Pharmaceutical Glass Packaging Container (Tentative), Technical Guidelines for Compatibility Studies of Chemical Injection and Plastic Packaging Material (Tentative), and Technical Guidelines for Compatibility Studies of Chemical Injection and Elastomeric Seals (Tentative) in China; and the Application and Technical Guidelines for Single Use System (Tentative) published by the China Center for Food and Drug International Exchange in November 2017), the technical guidelines formulated by relevant SUS organizations, and USP <665> and USP <1665>and developed a reasonable test scheme for the dissolved substances in single use system.

The potential dissolved substances in the pharmaceutical single use system produced may come from surfactants, lubricants and additives in the process of plastic processing, or from the shedding of raw materials of material structure and oligomer monomer. This study report summarizes the information on extractables from disposable components in which includes elements and organic compounds (nonvolatile compounds, semi-volatile and volatile compounds, small-molecule volatiles) in three kinds of simulated solvents, mainly referring to USP<665>, BPOG guide for pharmaceutical single use system. Elements were detected by inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-optical emission spectrometry (ICP-OES), non-volatile compounds were detected by high performance liquid chromatography-mass spectrometry/mass spectrometry(LC-MS/MS) and high performance liquid chromatography(HPLC), semi-volatile and volatile compounds were detected by gas chromatography-mass spectrometry (GC-MS), and small-molecule volatiles were detected by headspace gas chromatography-mass spectrometry (HS-GC-MS).

2. Purpose and Methodology of Extractables Testing

According to the guidelines of USP < 665 > and USP < 1665 >, the extractables test scheme was formulated respectively, and the extractables were tested according to this scheme. Before the test, LePure has confirmed the analysis and evaluation threshold (AET). According to the guidelines of Product Quality Research Institute (PQRI), AET was determined as a threshold. When the concentration of a compound exceeds the threshold, the compound should be identified, quantified and reported. In addition, the toxicity for this compound needs to be evaluated. AET is obtained through conversion according to the appropriate safety assessment threshold (SCT) or toxicological concern threshold (TTC), taking into consideration the dosage of the product. When the concentration of a compound is lower than the threshold, it can be considered that the toxicity of the compound is very low and will not be harmful to human. As LePure single use systems may be exposed to a variety of drugs and chemical reagents, and the maximum daily dosage of drugs cannot be confirmed at this stage, based on the minimum detection limit of the instrument, and according to the guide of BPOG, the report limit of 0.1μg/mL was defined as AET for organic compounds, and the report limit of 20ng/mL was set as AET for inorganic substances for this study. Converted to the concentration for surface area, AET for organic compounds was 0.016μg/cm^2, and AET for inorganic substances was 0.003μg/cm^2.

3. Information of Component and Instruments

3.1 Information of Component

Table 1 Information of Component

Name	Liquid Contact Material	Art. No.
Single-use filling bags	ULDPE	KD003-LEP-286K
Needle	Stainless steel	LE177577SU

Note: This guide is applicable to other components constructed from the same materials.

3.2 Information of Instruments

Table 2 Information of Instruments

Name	Model/Specification	Manufacturer
Gas Chromatography-Mass Spectrometry	Trace 1300/ISQ7000	Thermo Fisher
Gas Chromatography-Mass Spectrometry	7890B/5977B	Agilent
Headspace Gas Chromatography-Mass Spectrometry	TRIPLUS 300 /Trace 1310/ISQ7000	Thermo Fisher
High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry	1290/G 6470A	Agilent
High Performance Liquid Chromatography	Ultimate 3000	Thermo Fisher
Inductively Coupled Plasma-Mass Spectrometry	7800	Agilent

4. USP < 665 > Compliance

4.1 Overview of Extractables Protocol

USP<665> guide was referred to determine the extraction solution, extraction method and test instrument used in extractable study. An overview of extractables study process is provided in Table 3 and Table 4.

Table 3 Single-use Filling Bags Extractable Study Process

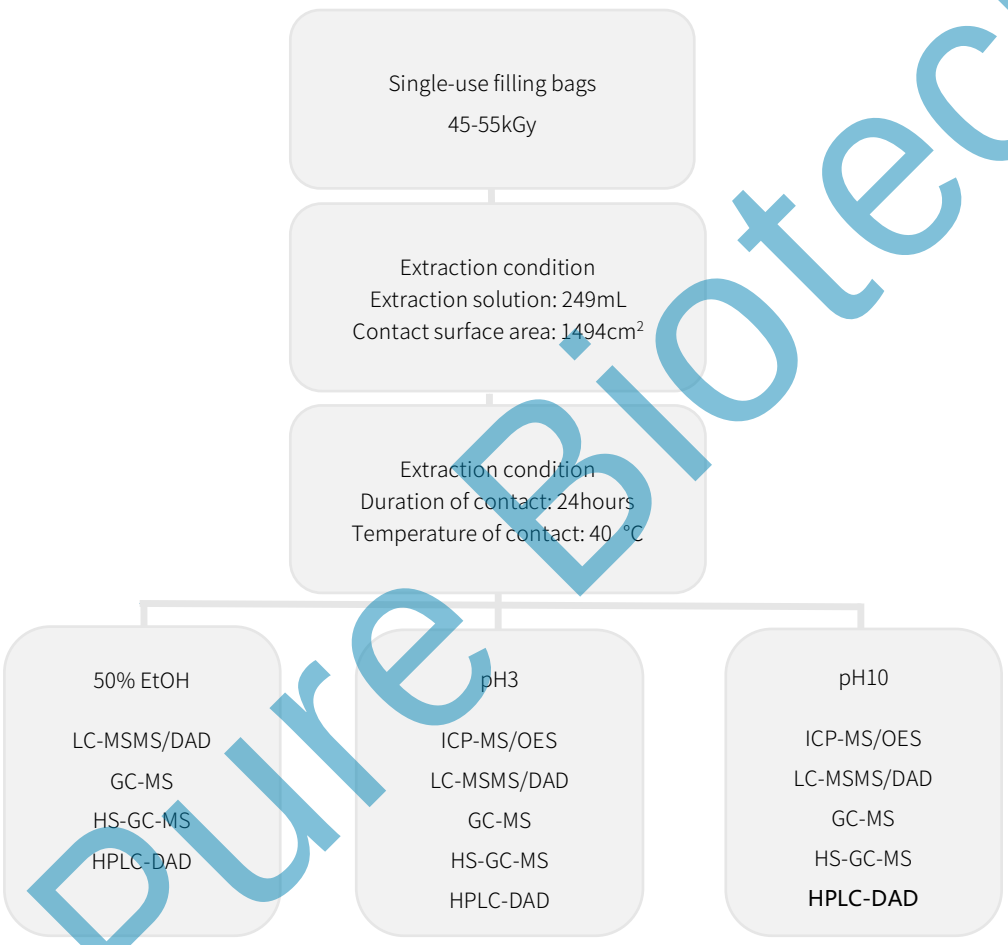
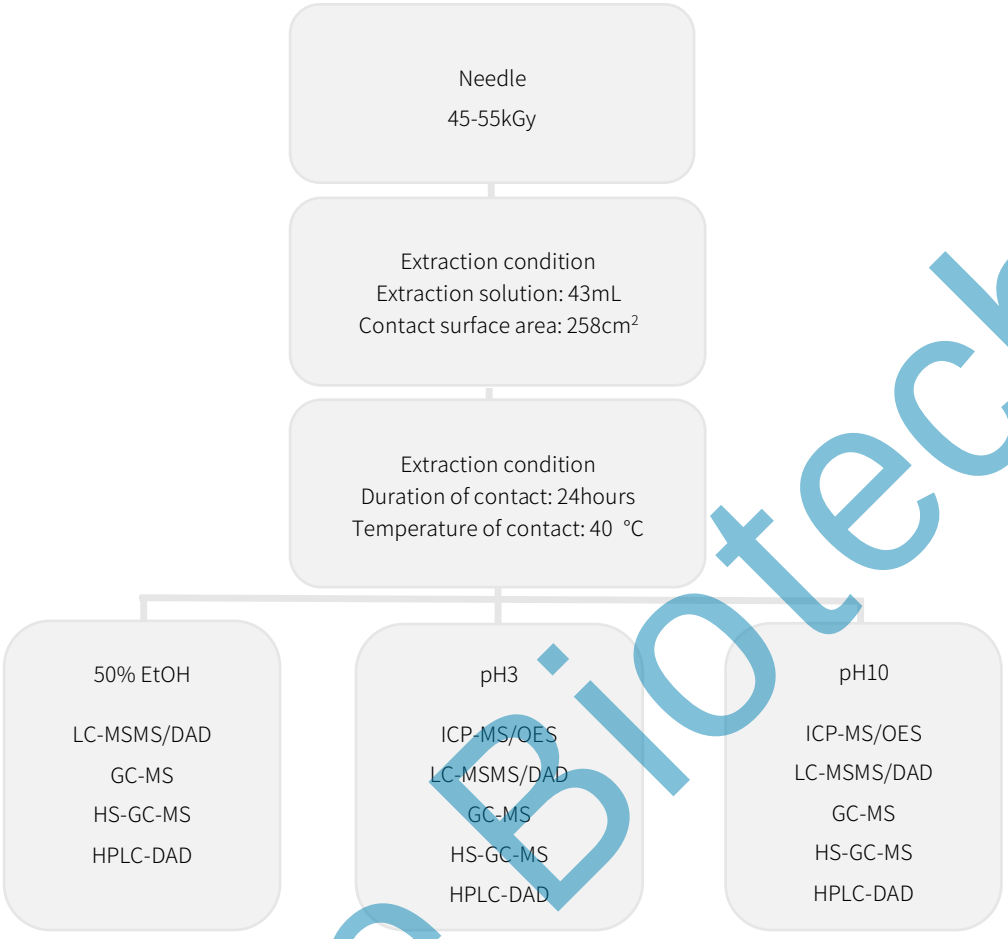


Table 4 Needle Extractable Study Process



4.2 Results of Extractables Testing

The elemental, non-volatile, semi-volatile and volatile extracts in Single-use filling bags were detected. In addition, we focused on the antioxidants and their degradation products, fatty acids, phthalate plasticizers, polycyclic aromatic hydrocarbons, lubricants, siloxanes, vulcanizing agents, nitrosamines and other additives during the experiment and data analysis.

4.2.1 Results of Elemental Impurities

Table 5 Results of Elemental Impurities for Single-use Filling Bags

Element	ICH Q3d Class	Conc. (μg/cm²)	
		pH3	pH10
Cd	Class 1	<LOR	<LOR
Pb	Class 1	<LOR	<LOR
As	Class 1	<LOR	<LOR
Hg	Class 1	<LOR	<LOR
Co	Class 2A	<LOR	<LOR
V	Class 2A	<LOR	<LOR
Ni	Class 2A	<LOR	<LOR
Tl	Class 2B	<LOR	<LOR
Au	Class 2B	<LOR	<LOR
Pd	Class 2B	<LOR	<LOR
Ir	Class 2B	<LOR	<LOR
Os	Class 2B	<LOR	<LOR
Rh	Class 2B	<LOR	<LOR
Ru	Class 2B	<LOR	<LOR
Se	Class 2B	<LOR	<LOR
Ag	Class 2B	<LOR	<LOR
Pt	Class 2B	<LOR	<LOR
Li	Class 3	<LOR	<LOR
Sb	Class 3	<LOR	<LOR
Ba	Class 3	<LOR	<LOR
Mo	Class 3	<LOR	<LOR
Cu	Class 3	<LOR	<LOR
Sn	Class 3	<LOR	<LOR
Cr	Class 3	<LOR	<LOR
B	N/A	<LOR	<LOR
Na	N/A	<LOR	<LOR

Element	ICH Q3d Class	Conc. (μg/cm²)	
		pH3	pH10
W	N/A	＜LOR	＜LOR
Mg	N/A	＜LOR	＜LOR
Al	N/A	＜LOR	＜LOR
Ca	N/A	＜LOR	＜LOR
Ti	N/A	＜LOR	＜LOR
Mn	N/A	＜LOR	＜LOR
Fe	N/A	＜LOR	＜LOR
Zn	N/A	＜LOR	＜LOR
K	N/A	＜LOR	＜LOR
Si	N/A	0.362	0.306

Note: “LOR” means limit of report, and the concentration of LOR is 0.003μg/cm².

Table 6 Results of Elemental Impurities for Needle

Element	ICH Q3d Class	Conc. (μg/cm²)	
		pH3	pH10
Cd	Class 1	＜LOR	＜LOR
Pb	Class 1	＜LOR	＜LOR
As	Class 1	＜LOR	＜LOR
Hg	Class 1	＜LOR	＜LOR
Co	Class 2A	＜LOR	＜LOR
V	Class 2A	＜LOR	＜LOR
Ni	Class 2A	＜LOR	＜LOR
Tl	Class 2B	＜LOR	＜LOR
Au	Class 2B	＜LOR	＜LOR
Pd	Class 2B	＜LOR	＜LOR
Ir	Class 2B	＜LOR	＜LOR
Os	Class 2B	＜LOR	＜LOR
Rh	Class 2B	＜LOR	＜LOR
Ru	Class 2B	＜LOR	＜LOR
Se	Class 2B	＜LOR	＜LOR
Ag	Class 2B	＜LOR	＜LOR
Pt	Class 2B	＜LOR	＜LOR
Li	Class 3	＜LOR	＜LOR
Sb	Class 3	＜LOR	＜LOR
Ba	Class 3	＜LOR	＜LOR
Mo	Class 3	＜LOR	＜LOR
Cu	Class 3	＜LOR	＜LOR
Sn	Class 3	＜LOR	＜LOR
Cr	Class 3	＜LOR	＜LOR
B	N/A	＜LOR	＜LOR
Na	N/A	＜LOR	＜LOR
W	N/A	＜LOR	＜LOR
Mg	N/A	＜LOR	＜LOR
Al	N/A	＜LOR	＜LOR
Ca	N/A	＜LOR	＜LOR

Element	ICH Q3d Class	Conc. (μg/cm²)	
		pH3	pH10
Ti	N/A	＜LOR	＜LOR
Mn	N/A	＜LOR	＜LOR
Fe	N/A	0.100	0.009
Zn	N/A	0.005	＜LOR
K	N/A	＜LOR	＜LOR
Si	N/A	＜LOR	＜LOR

Note: “LOR” means limit of report, and the concentration of LOR is 0.003μg/cm²

4.2.2 Results of Organic Compounds

Table 7 Results of Organic Compounds for Single-use Filling Bag

Model Solvent	Analytical Method	Compound Name	CAS	Conc. (μg/cm²)
50% EtOH	LC-MS/MS	Palmitic acid	57-10-3	0.020
	LC-MS/MS	Stearic acid	57-11-4	0.068
	GC-MS	Hexanoic acid	142-62-1	0.26
pH3	HS-GC-MS	Trimethylsilanol	1066-40-6	0.062
pH10	HS-GC-MS	Trimethylsilanol	1066-40-6	0.026

Note: "LOR" means limit of report. The concentration of LOR for LC-MS/MS is 0.008μg/cm², the concentration of LOR for GC-MS and HS-GC-MS is 0.016μg/cm².

Table 8 Results of Organic Compounds for Needle

Model Solvent	Analytical Method	Compound Name	CAS	Conc. (μg/cm²)
50% EtOH	LC-MS/MS	Palmitic acid	57-10-3	0.12
	LC-MS/MS	Stearic acid	57-11-4	0.068

Note: "LOR" means limit of report. The concentration of LOR for LC-MS/MS is 0.008μg/cm², the concentration of LOR for GC-MS and HS-GC-MS is 0.016μg/cm².

5. Safety Assessment

The toxicity of extractables and leachables must be evaluated for the effects on both patients and process. Although almost any quantity of certain compounds in a drug is considered unacceptable (e.g., ICH Q3C class-1 solvents), the toxicity of extractables or leachables must be observed in the broader context of the following criteria, the actual concentration of the leachables in the final drug, the mode of administration, the dosage, the duration of treatment, the number of patients, and the risk benefit assessment.

Therefore, the toxicity is not only related to the identification and concentration of the extractables, or only related to the amount of leachables in the process fluid or pharmaceutical intermediates. The daily intake of patients can be obtained taking into consideration information of extractable concentration, model solvent volume, test component contact area, process component contact area, process batch and dosage. The daily intake of single compound can be compared with PDE value. For compounds or unknown substances whose PDE value cannot be obtained, the worst scenario can be assumed.

1) All extractables are migrating to the final product.
2) All extractables are considered as DNA reactive impurities (genotoxicant).

The purpose of determining the toxicological concern threshold (TTC) in ICH M7 is to define a common acceptable exposure level for compounds that have gone through toxicological studies (see Table 9). An appropriate limit value can be chosen based on treatment cycle and treatment route to conduct safety assessment.

Table 9 Acceptable Intakes for an Individual Impurity

Duration of treatment	⩽1 month	>1-12 months	>1-10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Depending on the toxicity categorization and concentration of each detected compound and elements, there is no high-risk compounds or elements detected for Single-use filling bags. The toxicological assessment of extractables or leachables for drug products should be performed based on the process conditions and clinical dose for patient.

Appendix 1: Study of Elements Impurity

Table 10 Instrument Method for Elements Impurity by ICP-OES

RF Power	1200W	Plasma Gas Flow Rate	12.00L/min
Pump Rate	12.00rpm	Auxiliary Gas Flow Rate	1.00L/min
Atomized Gas Flow Rate		0.70L/min	

Table 11 Instrument Method for Elements Impurity by ICP-MS

RF Power	1550W	Plasma Gas Flow Rate	15.0L/min
Peristaltic Pump Speed	0.10rps	Auxiliary Gas Flow Rate	0.90L/min
Sampling Depth	10mm	Nebulizer Gas Flow Rate	1.01L/min
Nebulizer Chamber Temperature		2°C	

Appendix 2: Study of Organic Compounds

Table 12 Scan Method for Semi-Volatile Compounds by GC-MS (Polar Column)

Column	TG-WAXMS (30m*0.25mm*0.25μm)		
Injection Volume	1μL		
Carrier Gas	He		
Split Ratio	Splitless		
Temperature	Injection Temp.: 230°C,Transferline Temp.: 230°C, MS Source Temp.: 230°C		
Flow Control Mode	Constant flow, rate is 1.0mL/min		
Acquisition Type	Full Scan 30-550; SIM		
Temperature Program			
No.	Rate (°C/min)	Temp. (°C)	Hold Time (min)
1	/	40	5
2	10	230	6

Table 13 Scan Method for Semi-Volatile Compounds by GC-MS (Non-polar Column)

Column	HP-5MS UI (30m*0.25mm*0.25μm)		
Injection Volume	1μL		
Carrier Gas	He		
Split Ratio	Splitless		
Temperature	Injection Temp.: 300°C, Transferline Temp.: 300°C, MS Source Temp.: 300°C		
Flow Control Mode	Constant flow, rate is 1.2mL/min		
Acquisition Type	Full Scan 35-1000； SIM		
Temperature Program			
No.	Rate (°C/min)	Temp. (°C)	Hold Time (min)
1	/	60	5
2	20	220	1
3	10	300	8

Table 14 Scan Method for Volatile Compounds by HS-GC-MS

Headspace Condition	80°C 30min

Column	TG-624SILMS (60m*0.25mm*1.4μm)
Injection Volume	1000μL
Carrier Gas	He
Split Flow Rate	5mL/min
Temperature	Injection Temp.: 250°C, Transferline Temp.: 230°C, MS Source Temp.: 230°C
Flow Control Mode	Constant pressure, pressure is 124.11kpa
Acquisition Type	Full Scan: 35-300; SIM

Temperature Program			
No.	Rate (°C/min)	Temp. (°C)	Hold Time (min)
1	/	40	2
2	8	90	4
3	6	200	15

Table 15 Instrument Method for Antioxidant 2246 And Pentachlorophenol by LC-MS/MS

Mass Condition			
Scan Type	MRM	Polarity	Negative
Gas Temp.	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp.	300°C
Sheath Gas Flow	11L/min	Capillary	3500V
Liquid Chromatography Condition			
Column	Eclipse plusC18 (2.1mm*100mm*1.8μm)	Flow Rate	0.3mL/min
Column Temp.	40°C	Injection Volume	5μL
Elution Mode	Isocratic Elution		
Mobile Phase	Add 0.1% formic acid-methanol (5: 95) to 5mmol/L ammonium acetate aqueous solution		

Table 16 Instrument Method for Fatty Acid by LC-MS/MS

Mass Condition			
Scan Type	SIM	Polarity	Negative
Gas Temp.	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp.	300°C
Sheath Gas Flow	11L/min	Capillary	3500V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1mm*100mm*1.8μm)	Flow Rate	0.3mL/min
Column Temp.	40°C	Injection Volume	5μL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid + 5 mmol/L ammonium acetate solution B: Methanol		
Time (min)	A (%)	B (%)	
0.00	15	85	
5.00	2	98	
9.00	2	98	
9.10	15	85	
12.00	15	85	

Table 17 Instrument Method for Other Non-volatile Compounds by LC-MS/MS

Mass Condition			
Scan Type	MRM	Polarity	Positive
Gas Temp.	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp.	300°C
Sheath Gas Flow	11L/min	Capillary	3500V
Liquid Chromatography Condition			
Column	Eclipse plusC18 (2.1mm*100mm*1.8μm)	Flow rate	0.3mL/min
Column Temp.	40°C	Injection Volume	5μL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid + 5mmol/L ammonium acetate solution B: Methanol		
Time (min)	A (%)	B (%)	
0.00	80	20	
10.00	2	98	
27.90	2	98	
28.00	80	20	
30.00	80	20	

Table 18 Scan method for other Non-Volatile Compounds by LC-MSMS/DAD

Mass Condition			
Scan Type	SCAN	Polarity	Positive/Negative
Gas Temp	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp	300°C
Sheath Gas Flow	11L/min	Capillary	Positive:3500V /Negative:3000V
Liquid Chromatography Condition			
Column	Eclipse plusC18 (2.1mm*100mm*1.8μm)	Flow Rate	0.3mL/min
Column Temp	40°C	Injection Volume	5μL
Detector	DAD	UV Wavelength	254nm
UV Wavelength Range	190nm~400nm	UV Reference Wavelength	360nm
Elution mode		Gradient Elution	
Mobile Phase	A：0.1% formic acid +5mmol/L Ammonium acetate aqueous solution；B：Methanol		
Time（min）	A（%）	B（%）	
0.00	80	20	
10.00	2	98	
27.90	2	98	
28.00	80	20	
30.00	80	20	

Appendix 3: Study of Specially Concerned Compounds

Table 19 Instrument Method for Phthalate Plasticizers and Polycyclic Aromatic Hydrocarbons by GC-MS

Column	HP-5MS UI (30m*0.25mm*0.25μm)		
Injection Volume	1μL		
Carrier Gas	He		
Split Ratio	Splitless		
Temperature	Injection Temp.: 300°C, Transferline Temp.: 300°C, MS Source Temp.: 300°C		
Flow Control Mode	Constant flow, rate is 1.2mL/min		
Acquisition Type	Full Scan 35-1000; SIM		
Temperature Program			
No.	Rate (°C/min)	Temp. (°C)	Hold Time (min)
1	/	60	5
2	20	220	1
3	10	300	8

Table 20 Instrument method for PAHs compounds by GC-MS

Column	HP-5MS UI（30m*0.25mm*0.25μm）		
Injection Volume	1 μL		
Carrier Gas	He		
Split Ratio	Splitless		
Temperature	Injection Temp：300°C；Transferline Temp：300°C；MS Source Temp：230°C; MS Quad Temp: 150°C		
Flow Control Mode	Constant flow, rate is1.2 mL/min		
Acquisition Type	Full Scan 35-550；SIM		
Temperature Program			
No.	Rate（°C/min）	Temp（°C）	Hold time（min）
1	/	60	5
2	20	220	1
3	10	300	8

Table 21 Instrument Method for Vulcanizing Agent by HPLC

Column	Hypersil GOLD C18 (250mm*4.6mm*5μm)
Mobile Phase	MeOH:Water (90:10)
UV Wavelength	280nm
Injection Volume	10μL
Column Temp.	25°C
Flow Rate	1.0mL/min
Run Time	15min

Table 22 Instrument method for 2-MBT by LC-MS/MS

Mass Condition			
Scan Type	MRM	Polarity	Positive
Gas Temp	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp	300°C
Sheath Gas Flow	11L/min	Capillary	3500V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1mm*100mm*1.8μm)	Flow Rate	0.2mL/min
Column Temp	30°C	Injection Volume	5μL
Elution mode		Gradient Elution	
Mobile Phase	5mmol/L Ammonium acetate aqueous solution added 0.1% formic acid-methanol（5：95）		
Run Time	4min		

Table 23 Instrument Method for Nitrosamines by LC-MS/MS

Mass Condition			
Scan Type	MRM	Polarity	Positive
Gas Temp.	300°C	Gas Flow	7L/min
Nozzle Voltage	500V	Sheath Gas Temp.	300°C
Sheath Gas Flow	11L/min	Capillary	3500V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1mm*100mm*1.8μm)	Flow Rate	0.3mL/min
Column Temp.	30°C	Injection Volume	5μL
Elution Mode	Isocratic Elution		
Mobile Phase	Add 0.1% formic acid-methanol (10: 90) to 5mmol/L ammonium acetate aqueous solution		
Time (min)	A (%)	B (%)	
0.00	30	70	
5.00	2	98	
6.00	2	98	
6.10	30	70	
8.00	30	70	

Appendix 3:

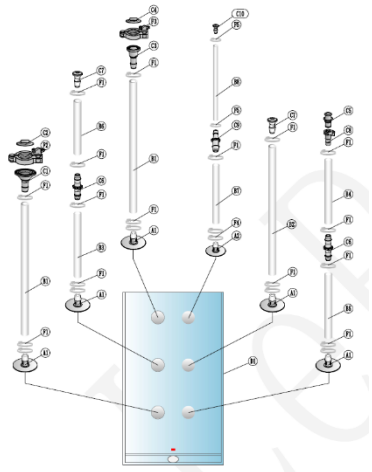


Fig 1： Drawing of Single-use Filling Bags （LeKrius®）

Serial No.	Description	Material
D1	3D Single-use bag	ULDPE
A1	Port	PE
A2	Port	PE
B1	Silicone tube (Liveo Pharma 50)	Silicone
B2	Silicone tube (Liveo Pharma 65)	Silicone
B3	Silicone tube (Liveo Pharma 65)	Silicone
B4	Silicone tube (Liveo Pharma 50)	Silicone
B5	Silicone tube (Liveo APT)	Silicone
B6	Silicone tube (Liveo Pharma 50)	Silicone
B7	Silicone tube (Liveo Pharma 50)	Silicone
B8	Silicone tube (Syntegon)	Silicone
C1	Chuck interface	PP
C2	Closing plate	PE
C3	Chuck interface	PP
C4	Closing plate	PP
C5	MPX quick-connection male cap	PC
C6	Two-way equal-diameter connector	PP
C7	Plug	PP
C8	MPX quick-connection female connector	PSF
C9	Two-way connector	PP
C10	No. 4 bottom-magnetic stirring impeller	PP

Reference

1. USP<665> Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products
2. USP<1665> Characterization and Qualification of Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products
3. BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components Used in Biopharmaceutical Manufacturing
4. BioPhorum Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems Used in Biopharmaceutical Manufacturing
5. ICH Q3D(R1) Guidelines for Elemental Impurities
6. ICH M7(R1) Evaluate and Control DNA Reactive (Mutagenic) Impurities in Drugs to Limit Potential Carcinogenic Risk
7. Application and Technical Guide of Disposable Use System.

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