

LEPURE

Extractables Guideline

Le-Flex[®] TPE Tubing

Catalogue

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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 is 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 TPE tubing. 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 ultra performance liquid chromatography-mass spectrometry/mass spectrometry (UPLC-MSMS) and ultra 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>/<1665> and BPOG, 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 can not 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 0.1 µg/mL was defined as AET for organic compounds, and the report limit of 20 ng/mL was set as AET for inorganic substances for this study. Converted to the concentration for surface area, AET for organic compounds was 0.016 µg/cm², and AET for inorganic substances was 0.003 µg/cm².

3. Information of Component and Instruments

3.1 Information of Component

Table 1 Information of Le-Flex®TPE Tubing

Name	Material	Art. No.
Le-Flex® TPE Tubing	TPE	RKT04

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

3.2 Information of Instruments

Table 2 Information of Instruments

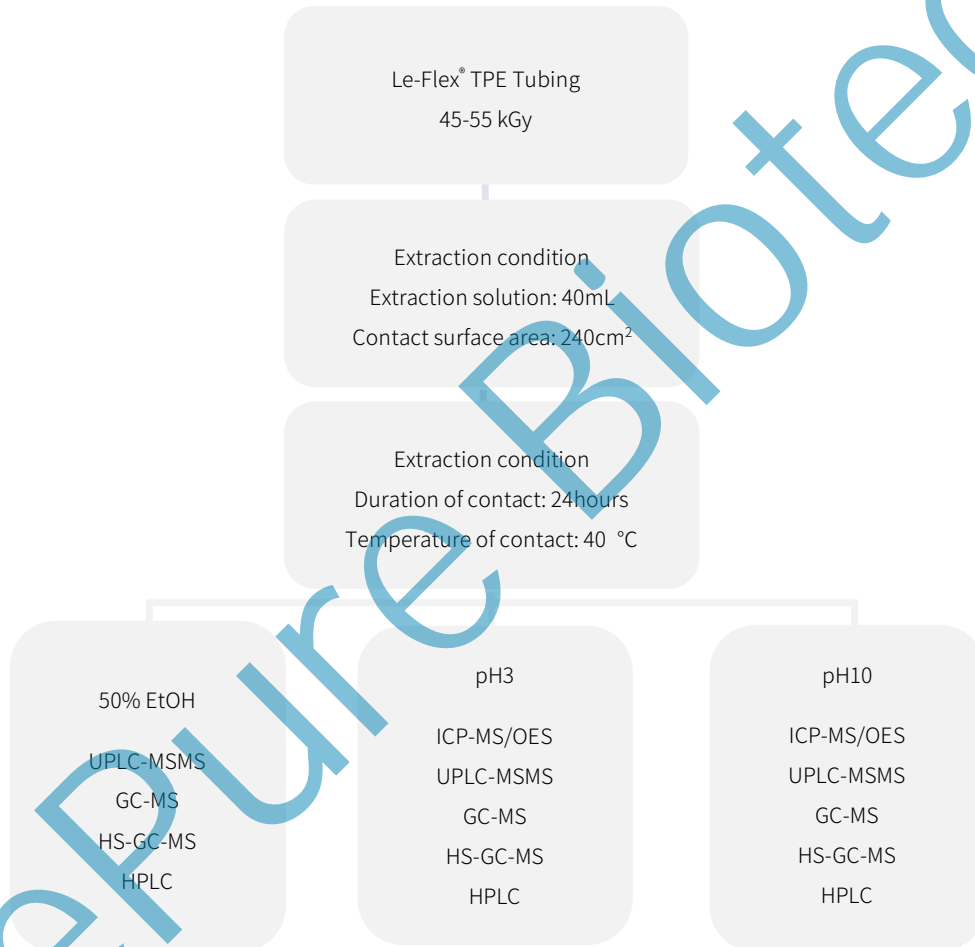
Name	Model / Specification	Manufacturer
Gas Chromatography-Mass Spectrometry	Trace 1310 / ISQ7000	Thermo Fisher
Headspace Gas Chromatography-Mass Spectrometry	TriPlus 300 HS / Trace 1310 / ISQ7000	Thermo Fisher
Ultra Performance Liquid Chromatography-Mass Spectrometry	1290 / G6470A	Agilent
Ultra Performance Liquid Chromatography-Mass Spectrometry	1290 Infinity II / G6470B	Agilent
Inductively Coupled Plasma-Mass Spectrometry	7800	Agilent
Inductively Coupled Plasma-Optical Emission Spectrometry	5110	Agilent
Ultra Performance Liquid Chromatography	Ultimate 3000	Thermo Fisher
Gas Chromatography-Mass Spectrometry	7890B / 5977B	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 the extractable study. An overview of extractables study process is provided in Table 3.

Table 3 Extractable Study Process



4.2 Results of Extractables Testing

The elemental, non-volatile, semi-volatile and volatile extracts in Le-Flex® TPE Tubing were detected. In addition to, 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.

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4.2.1 Results of Elemental Impurities

Table 4 Results of Elemental Impurities

Element	ICH Q3d Class	Conc. ($\mu\text{g}/\text{cm}^2$)	
		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
W	N/A	<LOR	<LOR

Element	ICH Q3d Class	Conc. ($\mu\text{g}/\text{cm}^2$)	
		pH3	pH10
Mg	N/A	0.004	0.004
Al	N/A	<LOR	0.004
Ti	N/A	<LOR	<LOR
Mn	N/A	<LOR	<LOR
Fe	N/A	<LOR	<LOR
Zn	N/A	<LOR	<LOR
Si	N/A	0.015	0.050
Sr	N/A	<LOR	<LOR
Be	N/A	<LOR	<LOR
In	N/A	<LOR	<LOR

Note: "LOR" means limit of report, and the concentration of LOR is $0.003 \mu\text{g}/\text{cm}^2$.

4.2.2 Results of Organic Compounds

Table 5 Results of Organic Compounds

Model Solvent	Analytical Method	Compound Name	CAS	Conc. ($\mu\text{g}/\text{cm}^2$)
50%EtOH	UPLC-MSMS	Caprolactam	105-60-2	0.032
	UPLC-MSMS	N-Cyclohexylformamide	766-93-8	0.062
	UPLC-MSMS	N, N-Dimethylcyclohexylamine	98-94-2	0.062
	UPLC-MSMS	Octanoic acid	124-07-2	0.080
	UPLC-MSMS	Lauric acid	143-07-7	0.025
	UPLC-MSMS	Myristic acid	57677-52-8	0.013
	UPLC-MSMS	Palmitic acid	57-10-3	0.15
	UPLC-MSMS	Stearic acid	57-11-4	0.031
	UPLC-MSMS	Unknown	N/A	0.027
	GC-MS	Erucamide	112-84-5	0.13
	GC-MS	1-Dodecene	112-41-4	0.019
	GC-MS	Undecanal	112-44-7	0.027
	GC-MS	2,4,6-Trimethylbenzaldehyde	487-68-3	0.017
	GC-MS	1-Tetradecanol	112-72-1	0.021
	GC-MS	Undecenoic acid	112-38-9	0.020
	GC-MS	Methyl oleate	112-62-9	0.072
	GC-MS	4-Propylbenzoic acid	2438-05-3	0.020
	pH3	UPLC-MSMS	N-Cyclohexylformamide	766-93-8
UPLC-MSMS		N, N-Dimethylcyclohexylamine	98-94-2	0.011
GC-MS		Cyclohexanone	108-94-1	0.018
GC-MS		Hexanoic acid	142-62-1	0.020
pH10	UPLC-MSMS	N-Cyclohexylformamide	766-93-8	0.021
	UPLC-MSMS	N, N-Dimethylcyclohexylamine	98-94-2	0.021
	UPLC-MSMS	Myristic acid (1-13C)	57677-52-8	0.011
	UPLC-MSMS	2-Hexadecenoic Acid	629-56-1	0.038
	UPLC-MSMS	Palmitic acid	57-10-3	0.016
	GC-MS	Cyclohexanone	108-94-1	0.034

Note: "LOR" means limit of report. The concentration of LOR for UPLC-MSMS is $0.008 \mu\text{g}/\text{cm}^2$, the concentration of LOR for GC-MS and HS-GC-MS is $0.016 \mu\text{g}/\text{cm}^2$.

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 can not 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 6). An appropriate limit value can be chosen based on treatment cycle and treatment route to conduct safety assessment.

Table 6 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 compounds and elements, there is no high-risk compounds or elements detected for Le-Flex®TPE Tubing. 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 7 Instrument Method for Elements Impurity by ICP-OES

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

Table 8 Instrument Method for Elements Impurity by ICP-MS

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

Appendix 2: Study of organic compounds

Table 9 Scan Method for Semi-Volatile Compounds by GC-MS
(Polarity Column)

Column	TG-WAXMS (30 m*0.25 mm*0.25 μ m)		
Injection Volume	1 μ L		
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 1.0 mL/min		
Acquisition Type	Full Scan 30-550; SIM+SCAN		
Temperature Program			
No.	Rate (°C/min)	Temp (°C)	Hold Time (min)
1	/	40	5
2	10	230	6

Table 10 Scan Method for Semi-Volatile Compounds by GC-MS
(Non-polarity Column)

Column	HP-5MS UI (30 m*0.25 mm*0.25 μ m)		
Injection Volume	1 μ L		
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 1.2 mL/min		
Acquisition Type	Full Scan 35-550; SIM+SCAN		
Temperature Program			
No.	Rate (°C/min)	Temp (°C)	Hold Time (min)
1	/	60	5
2	20	220	1
3	10	300	8

Table 11 Scan Method for Volatile Compounds by HS-GC-MS

Headspace Condition	80 °C 30 min		
Column	TG-624 SILMS (60 m*0.25 mm*1.4 µm)		
Injection Volume	1000 µL		
Carrier Gas	He		
Split Flow	5 mL/min		
Temperature	Injection Temp: 250 °C; MS Source Temp: 230 °C; Transferline Temp: 230 °C		
Flow Control Mode	Constant press, press is 124.11 kPa		
Acquisition Type	Full Scan: 35-300; SIM+SCAN		
Temperature Program			
No.	Rate (°C/min)	Temp (°C)	Hold Time (min)
1	/	40	2
2	8	90	4
3	6	200	15

Table 12 Instrument Method for Anti Oxidant 2246, 2,3,4,5,6-Pentachlorophenol, N,N'-Diphenylthiourea by UPLC-MSMS

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

Table 13 Instrument Method for Fatty Acid by UPLC-MSMS

Mass Condition			
Scan Type	SIM	Polarity	Negative
Gas Temp	300 °C	Gas Flow	7 L/min
Nozzle Voltage	500 V	Sheath Gas Temp	300 °C
Sheath Gas Flow	11 L/min	Capillary	4000 V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1 mm*100 mm*1.8 µm)	Flow Rate	0.3 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid+5 mmol/L Ammonium acetate aqueous 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 14 Instrument Method for BPA by UPLC-MSMS

Mass Condition			
Scan Type	SIM	Polarity	Negative
Gas Temp	300 °C	Gas Flow	7 L/min
Nozzle Voltage	500 V	Sheath Gas Temp	300 °C
Sheath Gas Flow	11 L/min	Capillary	4000 V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1 mm*100 mm*1.8 µm)	Flow Rate	0.3 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid+5 mmol/L Ammonium acetate aqueous solution B: Methanol		
Time	3 min		

Table 15 Instrument Method for Melamine by UPLC-MSMS

Mass Condition			
Scan Type	SIM	Polarity	Negative
Gas Temp	300 °C	Gas Flow	7 L/min
Nozzle Voltage	500 V	Sheath Gas Temp	300 °C
Sheath Gas Flow	11 L/min	Capillary	4000 V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1 mm*100 mm*1.8 µm)	Flow Rate	0.3 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid+5 mmol/L Ammonium acetate aqueous solution B: Methanol		
Time	3 min		

Table 16 Instrument Method for Bis(2,4-di-tert-butylphenyl) phosphate by UPLC-MSMS

Mass Condition			
Scan Type	SIM	Polarity	Negative
Gas Temp	300 °C	Gas Flow	7 L/min
Nozzle Voltage	500 V	Sheath Gas Temp	300 °C
Sheath Gas Flow	11 L/min	Capillary	4000 V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1 mm*100 mm*1.8 µm)	Flow Rate	0.3 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid+5 mmol/L Ammonium acetate aqueous solution B: Methanol		
Time	3 min		

Table 17 Instrument Method for other Non-Volatile Compounds
by UPLC-MSMS

Mass Condition			
Scan Type	MRM	Polarity	Positive
Gas Temp	300 °C	Gas Flow	7 L/min
Nozzle Voltage	500 V	Sheath Gas Temp	300 °C
Sheath Gas Flow	11 L/min	Capillary	4000 V
Liquid Chromatography Condition			
Column	Eclipse plus C18 (2.1 mm*100 mm*1.8 µm)	Flow Rate	0.3 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 0.1% formic acid +5 mmol/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 18 Instrument Method for Phthalate plasticizer Compounds by GC-MS

Column	HP-5MS UI (30 m*0.25 mm*0.25 μ m)		
Injection Volume	1 μ L		
Carrier Gas	He		
Split Ratio	Splitless		
Temperature	Injection Temp: 300 $^{\circ}$ C; Transferline Temp: 300 $^{\circ}$ C; MS Source Temp: 300 $^{\circ}$ C		
Flow Control Mode	Constant flow, rate is 1.2 mL/min		
Acquisition Type	Full Scan 35-550; SIM		
Temperature Program			
No.	Rate ($^{\circ}$ C/min)	Temp ($^{\circ}$ C)	Hold Time (min)
1	/	60	5
2	20	220	1
3	10	300	8

Table 20 Instrument Method for Vulcanizer by UPLC

Column	Hypersil GOLD C18(250 mm*4.6 mm*5 μ m)
Mobile Phase	Methanol-Water (90:10)
UV Wavelength	280 nm
Injection	10 μ L
Column Temp	25 $^{\circ}$ C
Flow Rate	1.0 mL/min
Run Time	15 min

Table 21 Instrument Method for PAHs Compounds by GC-MS

Column	HP-5MS UI (30 m*0.25 mm*0.25 µm)		
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 is 1.2 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 22 Instrument Method for Nitrosamines by UPLC-MSMS

Mass Condition			
Scan Type	MRM	Ionization Type	APCI+
Gas Temp	250 °C	Gas Flow	7 L/min
Sheath Gas Temp	250 °C	Sheath Gas Flow	7 L/min
Nozzle Voltage	500 V	Capillary	3000 V
Liquid Chromatography Condition			
Column	ZORBAX SB-CN 2.1 mm*100 mm, 1.8 µm-Miclon	Flow Rate	0.4 mL/min
Column Temp	40 °C	Injection Volume	5 µL
Elution Mode	Gradient Elution		
Mobile Phase	A: 5 mmol/L Ammonium acetate aqueous solution added 0.1 % formic acid B: Methanol		
Run Time (min)	Phase A	Phase B	
0	90	10	
5	5	95	
8	5	95	
8.1	90	10	
10	90	10	

Table 23 Instrument Method for 2-MBT by UPLC-MSMS

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

Reference

1. USP<665> PLASTIC MATERIALS, COMPONENTS, AND SYSTEMS USED IN THE MANUFACTURING OF PHARMACEUTICAL DRUG PRODUCTS AND BIOPHARMACEUTICAL DRUG SUBSTANCES AND PRODUCTS
2. USP<1665> CHARACTERIZATION OF PLASTIC MATERIALS, COMPONENTS, AND SYSTEMS USED IN THE MANUFACTURING OF PHARMACEUTICAL DRUG PRODUCTS AND BIOPHARMACEUTICAL DRUG SUBSTANCES AND PRODUCTS
3. BPOG Best Practices Guide for Evaluating Leachables Risk in Biopharmaceutical Single-Use Systems
4. BioPhorum best practices guide for extractables testing of single-use components
5. ICH Q3D(R1) Guideline for elemental impurities
6. ICH M7(R1) Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
7. The international pharmaceuticals guidance on application and technology of single use system

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