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Stability indicating reversed-phase ion-pairing liquid chromatographic determination of vertilmicin sulfate as bulk drug and in injections

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Abstract

A simple reversed-phase high performance liquid chromatographic method was developed for the analysis of vertilmicin sulfate, a novel aminoglycoside (AG). UV detection was used to determine vertilmicin sulfate and its related compounds in drug substance and products without sample derivatization. The method was used to determine the content of vertilmicin and its related compounds and test the stability of vertilmicin sulfate as drug substance and in injections, which was required for registration of new drug.
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Keywords: Vertilmicin sulfate; Aminoglycoside; Liquid chromatography; Stability

1. Introduction

Vertilmicin sulfate (Fig. 1) belongs to a class of compounds known as aminoglycoside (AG) antibiotics. It is a novel drug that was found in the synthesis of netilmicin and is under registration in China. Aminoglycosides inhibit the protein synthesis of microorganisms, resulting in a rapid, concentration-dependent bactericidal action. Aminoglycosides are particularly active against aerobic gram-negative bacilli and are used to treat tuberculosis, particularly in cases of suspected multiple-drug resistance. However, these drugs can give rise to adverse reactions, including ototoxicity and nephrotoxicity, which is almost always reversible when treatment is discontinued [1]. In addition, vertilmicin was proved to have less toxicity than netilmicin.

As a new drug, vertilmicin sulfate is under registration in China. So it is required to develop a suitable method to determine the content of vertilmicin sulfate and its related compounds produced during synthesis, purification and storage. And it is necessary to test the stability of vertilmicin sulfate in new drug substance and products.

The structure of vertilmicin sulfate, shown in Fig. 1, indicates that vertilmicin has three primary amines, two second amines, three OH groups and one double-bond. Like many aminoglycosides, vertilmicin sulfate lacks a suitable chromophore, which is necessary for UV detection. For this reason, the analysis of aminoglycosides is usually performed using pre-column [2–8], post-column derivatization [9] methods or other detections, such as evaporative light scattering detection [10], pulsed electrochemical detection [11–13] or mass spectrometry [12,14]. But sample derivatization can increase the complexity of an HPLC method by requiring additional system components, such as an extra reagent pump, a reaction coil and a mixing. Other disadvantages of sample derivatization include the possible creation of degradation products and the introduction of impurities as a result of the derivatization procedure. And other detections like mass spectrometry are not widely used, while direct UV detection is more broadly applied to quality control because of its convenience and generalization.

TLC has also been used as a qualitative method for routine quality and stability testing of aminoglycosides for lack of UV absorption, as is described in the British Pharmacopoeia

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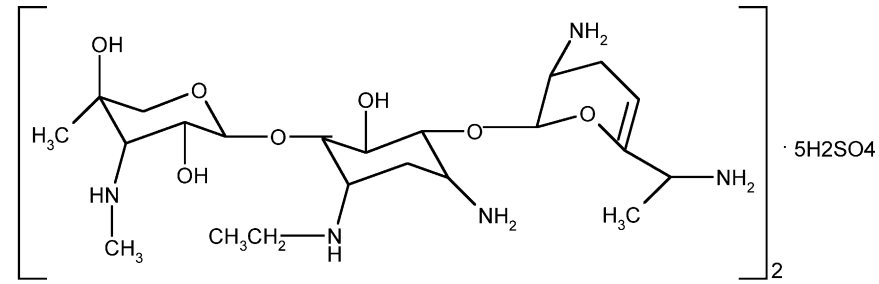

Structure of Vertilmicin Sulfate

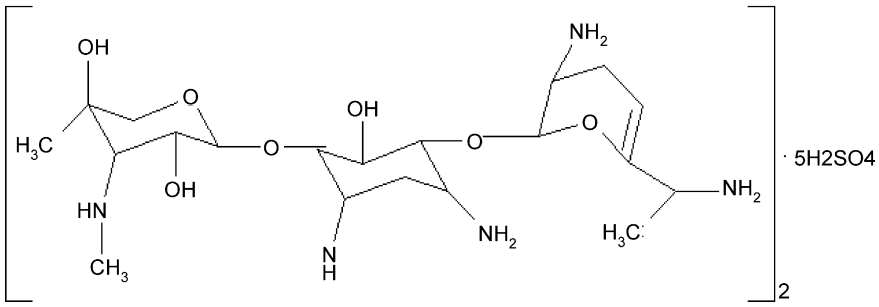

Fig. 1. Structure of vertilmicin sulfate and impurity A.

for the quality control of kanamycin and neomycin [15]. But due to low sensitivity, TLC with color reaction was difficult to determine related compounds precisely at trace level. Only one method was reported to determine vertilmicin in plasma [16], which needed a long-time sample preparation and a complex derivatization.

In this paper, HPLC was firstly used to determine the content of vertilmicin sulfate and its related compounds for testing the stability of new drug substance and its two injections.

2. Experimental

2.1. Chemicals and reagents

Vertilmicin sulfate standard, drug substance (batch numbers: 20020114, 20020220, 20020318) and its two injections (100 mg vertilmicin/2 ml (20020701, 20020702, 20020703) and 100 mg vertilmicin/100 ml (20020711, 20020712, 20020713)) with different additives were offered by Zhejiang Conler Pharmaceutical Co. Ltd. (Wenzhou, China). Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). Sodium heptanesulfonate was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Phosphoric acid and triethylamine (analytic reagent grade) were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). The water used was deionized.

2.2. HPLC conditions

The chromatographic system (Agilent HP1100 series HPLC instrument, America) consisted of an Agilent G1311A QuatPump fitted with a G1322A Degasser and a manual sample injector equipped with a 100 μl sample loop. The detector used was an Agilent G1314A VWD. The separation was performed on an Agilent XDB-C_8 (particle size 5 μm, 150 mm × 4.6 mm ID) column. The mobile phase consisted of aqueous buffer (containing 20 mM sodium heptanesulfonate and 30 mM triethylamine, phosphoric acid was added to adjust pH to 2.5)–acetonitrile (75:25, v/v). And the proportion was changed from 75:25 to 74:26 while 2 ml injection was analyzed for related compounds. The mobile phase was filtered through a 0.45 μm pore size membrane filter prior to mixing and ultrasonically degassed after mixing. Chromatography was performed at 30 °C with a flow rate of 1 ml min^{-1}. The analytes were detected at 201 nm.

2.3. Storage of sample for stability [17]

2.3.1. Influence factor testing

Vertilmicin sulfate substance and two injections (one batch respectively) had been stored under condition of high temperature (60 ± 2 °C), high humidity (RH 95 ± 5%) and strong light (4500 ± 500 lx) for 10 days respectively (RH means relative humidity).

2.3.2. Accelerated testing

Vertilmicin sulfate substance and two injections (three batches respectively) had been stored under condition of 40 ± 2 °C/RH 75 ± 5% for 6 months.

2.3.3. Long-term testing

Vertilmicin sulfate substance and two injections (three batches respectively) had been stored under condition of 25 ± 2 °C/RH 60 ± 5% for 12 months.

2.4. Preparation of solutions

An amount of 500 mg of vertilmicin sulfate standard (refined from substance) was accurately weighed and dissolved in 50 ml of water. Then this stock solution was diluted 10 times with aqueous buffer (containing 20 mM sodium heptanesulfonate and 30 mM triethylamine, phosphoric acid was added to adjust pH to 2.5) to obtain a working standard solution of 1 mg ml^{-1}.

2.5. Preparation of sample solutions

An amount of 50 mg of vertilmicin sulfate substances, accurately weighed, was dissolved in 50 ml aqueous buffer to prepare a sample solution of 1 mg ml^{-1}.

As for 2 and 100 ml-injection, sample solutions of 0.9 mg ml^{-1} were finally obtained by diluting with aqueous buffer.

2.6. Injection of solutions

An amount of 10 μl of these sample solutions was injected to determine the content of vertilmicin sulfate and 50 μl of these sample solutions was injected to determine the content of related compounds. The levels of related compounds were all calculated using the response factor of vertilmicin.

3. Results and discussion

3.1. Validation

The ability of the chromatographic system to separate vertilmicin from its possible impurities was investigated. For the related compound test, samples stored under relevant stress conditions (light, heat, acid/base hydrolysis and oxidation) were analyzed to demonstrate the specificity of the method. The results of chromatograms for the determination of vertilmicin sulfate and its related compounds were shown in Figs. 2–6. The retention time of vertilmicin was about 10 min (15 min while assaying 2 ml injection for related compounds). The linear equation was obtained as follows: y (peak areas) = 2602.8x (concentrations) − 172.2 (r = 0.9998, n = 6, 0.25–5 mg ml^{-1}); y = 17159x + 18.86 (r = 0.9963, n = 4, 0.01–0.04 mg ml^{-1}). The results of the precision and recovery of the HPLC method are presented in Tables 1 and 2. The precision of the method for vertilmicin sulfate were all less than 2% R.S.D. for both within-day and between-day assays. The relative recoveries of vertilmicin sulfate were all around 100%, which were obtained from determining the content of vertilmicin sulfate in some injection blank samples spiked with vertilmicin sulfate. For assay of related compounds, the limit of detection of vertilmicin was 1 μg ml^{-1} (the injection volume was 50 μl, S/N = 3) and the limit of quantification is 3 μg ml^{-1} (S/N = 10). One related compound was repeatedly found above an apparent level and we purified it and conducted to characterize its structure, which was called impurity A (the structure is shown in Fig. 1).

3.2. Results of determination of vertilmicin sulfate and its related compounds in drug substance

The results of the stability of drug substance in different conditions are shown in Table 3. The data shows that ver-

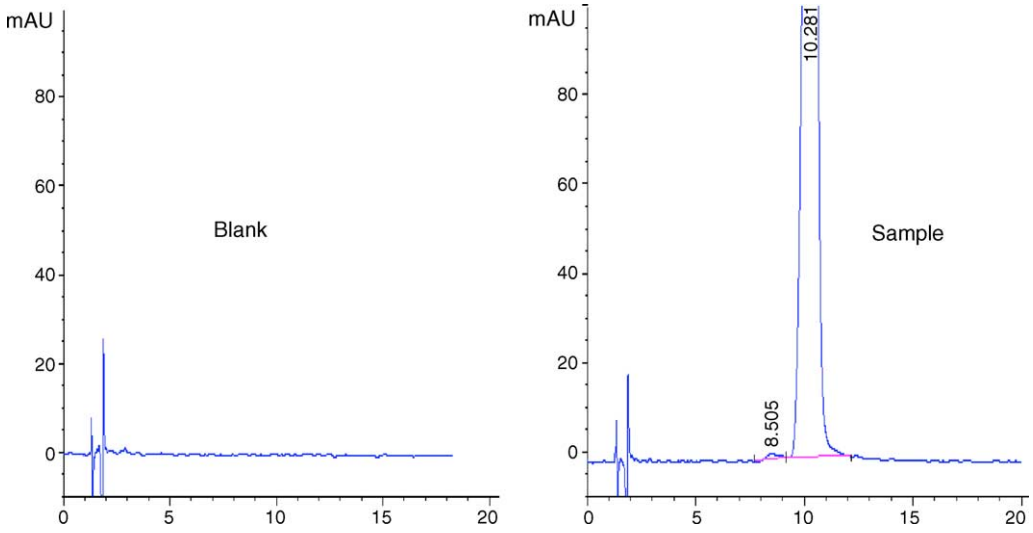

Fig. 2. Chromatogram of drug substance determining for related compounds (Agilent XDB-C_8 column, the mobile phase is aqueous buffer (containing 20 mM sodium heptanesulfonate and 30 mM triethylamine, phosphoric acid was added to adjust pH to 2.5)–acetonitrile (75:25, v/v)).

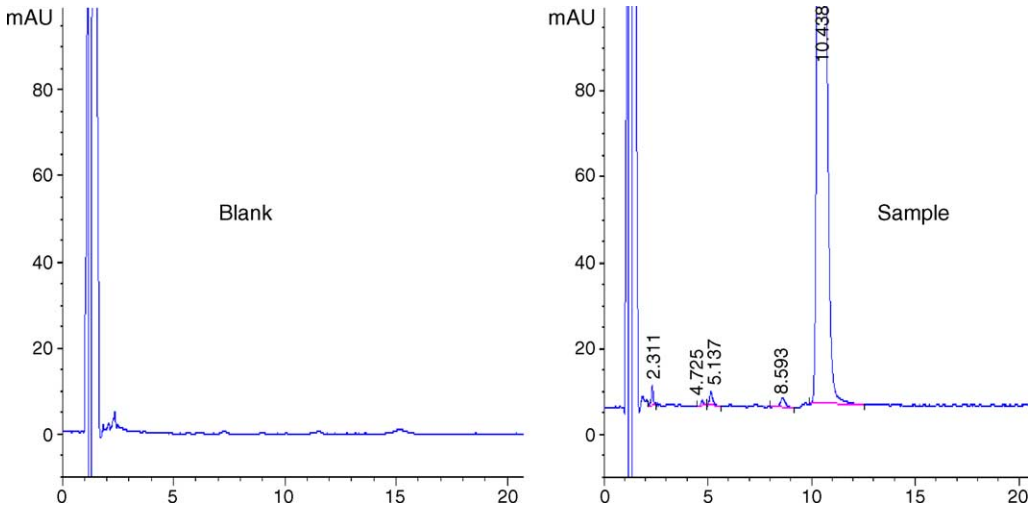

Fig. 3. Chromatogram of 100 ml injection determining for related compounds (HPLC conditions are same to Fig. 2).

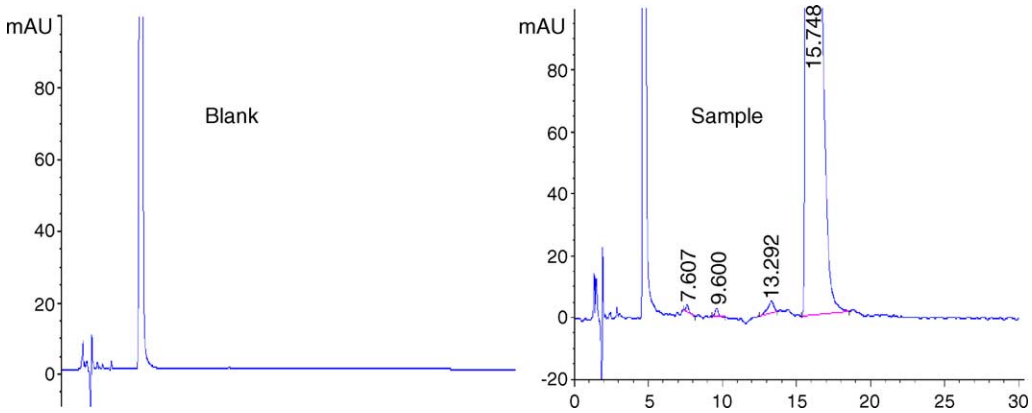

Fig. 4. Chromatogram of 2 ml injection determining for related compounds. (Agilent XDB-C_8 column, the mobile phase is aqueous buffer (containing 20 mM sodium heptanesulfonate and 30 mM triethylamine, phosphoric acid was added to adjust pH to 2.5)–acetonitrile (76:24, v/v)).

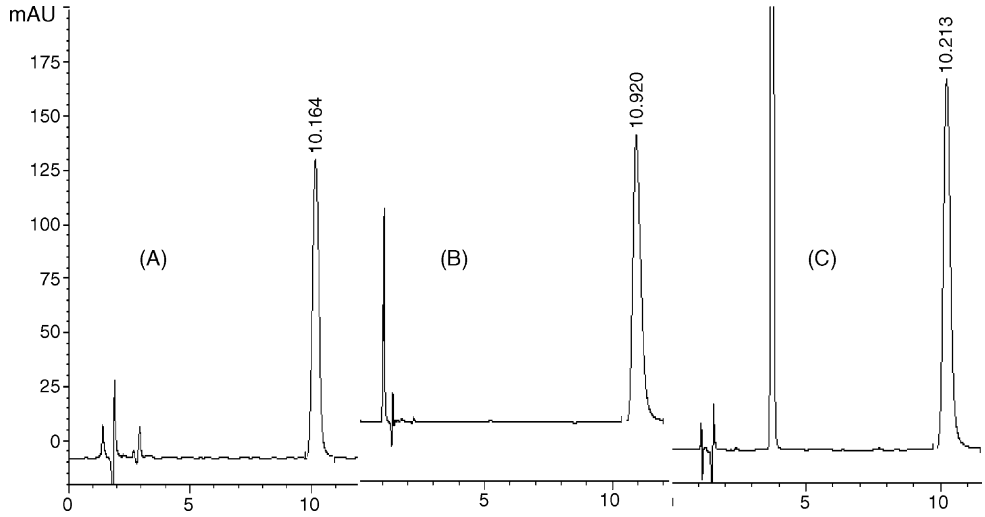

Fig. 5. Chromatograms of determination for the content of vertilmicin sulfate (A: sample of substance, B: sample of 100 ml injection, C: sample of 2 ml-injection). (HPLC conditions are same to Fig. 2).

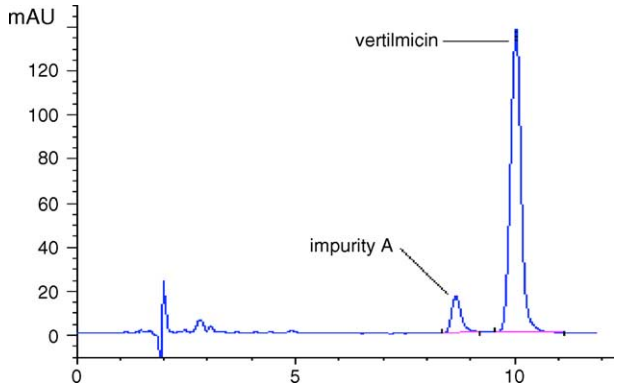

Fig. 6. Chromatogram of vertilmicin spiking with impurity A. (HPLC conditions are same to Fig. 2).

Table 1
Precision and accu racy for the analysis of vertilmicin sulfate

Concentration (mg/ml)	Within-day R.S.D. (%, $n = 3$)	Between-day R.S.D. (%, $n = 3$)
0.25	1.17	1.81
1.00	0.13	1.37
2.00	0.29	0.48

Table 2
Relative recoveries of vertilmicin sulfate in injection blank

Injection blank (ml)	Amount spiked (mg ml^{-1})	Amount found[a] (mg ml^{-1})	Relative recovery (%)
2	0.72	0.73 ± 0.01	101.38
	0.90	0.90 ± 0.003	99.87
	1.08	1.08 ± 0.01	100.25
100	0.72	0.72 ± 0.01	100.38
	0.90	0.92 ± 0.004	101.71
	1.08	1.10 ± 0.01	102.02

[a] Mean ± S.D. of quintuplicate analyses.

tilmicin sulfate substance was stable in long-term testing, accelerated testing, high temperature testing and strong light testing except for high humidity testing. The content of vertilmicin sulfate decreased greatly in high humidity testing because of the hygroscopic property of the drug. So vertilmicin sulfate substance must be stored under dry conditions.

3.3. Results of determination of vertilmicin sulfate and its related compounds in 2 ml injections

The results of the stability of 2 ml injection in different conditions are shown in Table 4. The data shows that vertilmicin sulfate 2 ml injection was stable in different storage conditions. The content changes of vertilmicin sulfate were all less than 5%.

3.4. Results of determination of vertilmicin sulfate and its related compounds in 100 ml injections

The results of the stability of 100 ml injection in different conditions are shown in Table 5. The data shows that vertilmicin sulfate 100 ml injection was stable in long-term testing, accelerated testing, high humidity testing and strong light testing except for high temperature testing. The content of vertilmicin sulfate decreased 6.72% and the content of total related compounds increase to 3.58% in high temperature testing. Therefore, it can be concluded that vertilmicin sulfate 100 ml injections are unstable in high temperature condition and must be stored in refrigerator.

3.5. Development of the chromatography

It is difficult to retain the AGs in the reversed-phase mode even with purely aqueous eluents. Therefore, most chromato-

Table 3
Results of analysis of drug substances' stability

Items	Batches	Storage time[a]	Content of vertilmicin sulfate (%, $n = 2$)	Content of total related compounds (%)
Accelerated testing	20020114	0 M	86.67 ± 0.14	0.58
		6 M	86.13 ± 0.13	1.18
	20020220	0 M	86.71 ± 0.23	0.50
		6 M	86.29 ± 0.21	1.11
	20020318	0 M	86.74 ± 0.20	0.48
		6 M	86.35 ± 0.18	0.97
Long-term testing	20020114	12 M	85.64 ± 0.15	1.45
	20020220	12 M	85.56 ± 0.12	1.31
	20020318	12 M	85.63 ± 0.11	1.23
High temperature testing	20020114	5 D	86.67 ± 0.20	0.66
	20020114	10 D	85.84 ± 0.22	1.65
High humidity testing	20020114	5 D	58.60 ± 0.22	0.69
	20020114	10 D	57.71 ± 0.17	1.28
Strong light testing	20020114	5 D	86.31 ± 0.18	0.82
	20020114	10 D	86.21 ± 0.23	0.99

[a] M, months; D, days.

Table 4
Results of analysis of 2 ml injections' stability

Items	Batches	Storage time	Content of vertilmicin sulfate (%, $n = 2$)	Content of total related compounds (%)
Accelerated testing	20020701	0 M	100.86 ± 0.23	1.01
		6 M	97.74 ± 0.24	1.88
	20020702	0 M	100.28 ± 0.28	1.28
		6 M	97.40 ± 0.32	2.23
	20020703	0 M	100.39 ± 0.25	1.39
		6 M	97.00 ± 0.19	2.34
Long term testing	20020701	12 M	99.56 ± 0.21	2.19
	20020702	12 M	99.12 ± 0.24	2.40
	20020703	12 M	99.83 ± 0.28	2.41
High temperature testing	20020701	5 D	99.85 ± 0.26	1.51
	20020701	10 D	98.40 ± 0.28	2.89
High humidity testing	20020701	5 D	100.46 ± 0.18	1.58
	20020701	10 D	98.79 ± 0.27	1.76
Strong light testing	20020701	5 D	100.81 ± 0.23	1.80
	20020701	10 D	98.86 ± 0.25	2.69

Table 5
Results of analysis of 100 ml injections' stability

Items	Batches	Storage time	Content of vertilmicin sulfate (%) ($n = 2$)	Content of total related compounds (%)
Accelerated testing	20020711	0 M	102.78 ± 0.22	1.13
		6 M	98.99 ± 0.25	1.73
	20020712	0 M	103.39 ± 0.23	1.11
		6 M	99.29 ± 0.29	1.72
	20020713	0 M	104.34 ± 0.30	0.95
		6 M	99.45 ± 0.19	1.77
Long term testing	20020711	12 M	99.31 ± 0.26	2.18
	20020712	12 M	99.70 ± 0.26	1.98
	20020713	12 M	100.82 ± 0.26	1.85
High temperature testing	20020713	5 D	101.43 ± 0.19	1.74
	20020713	10 D	97.62 ± 0.29	3.58
High humidity testing	20020713	5 D	99.53 ± 0.23	0.90
	20020713	10 D	98.82 ± 0.27	1.75
Strong light testing	20020713	5 D	104.13 ± 0.32	0.98
	20020713	10 D	102.41 ± 0.24	1.01

graphic methods were based on some forms of ion chromatography [6,9,10,13,14], typically ion-exchange modes or ion-pair modes, or used special columns [12]. Sodium alkyl sulfonate [9,10,13], heptafluorobutyric acid [6,14] were reported many times for use as ion-pairing agents to facilitate the retention of AGs in the reversed-phase mode. But organic acid cannot be used at low UV wavelengh like 201 nm.

Different columns, such as C_{18} column, C_8 column, CN column, Silica column and NH_2 column were tested to separate vertilmicin and its impurities with or without adding ion-pairing solvent. The influence of different columns and other conditions, such as pH, temperature, buffer, and organic phase were evaluated using plates, resolution and symmetry factor, and conditions concerned above were proved to have the best separation and sharp peak.

4. Conclusions

The described HPLC method provides a rapid and simple assay of vertilmicin sulfate and its related compounds without derivatization. This method can be used for determination of vertilmicin sulfate and its related compounds in drug substance and injections for quality control and stability testing. Vertilmicin sulfate substance and injections were found to be stable during 6 months accelerated testing and 12 months long-term testing. But the drug substance was hygroscopic and 100 ml injection was unstable in high temperature condition. The drug substance must be stored under dry conditions and 100 ml injection must be stored refrigerated. The stability testing would be further performed to obtain the expiration date of drug substance and injections.

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