State Key Laboratory of Chemical Resource Engineering[1], Beijing University of Chemical Technology; Yangtze River Pharmaceutical Group, Beijing Haiyan Pharmaceutical Co., Ltd[2]., Beijing, China

An isolated method and assignment for critical impurities in semi-synthetic process of arbekacin sulfate

Z. H. Chen[1], H. Zhou[1], S. Wang[1], G. Li[1], J. Zhang[1] F. Wang[2], C. Li[1,*], R. Z. Qiao[1,*]

Received December 24, 2018, accepted March 8, 2019

**Corresponding authors: Prof. Dr. Renzhong Qiao, Prof. Dr. Chao Li, State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, P. R. China*
qiao_group@163.com; lichao@mail.buct.edu.cn

Pharmazie 74: 352-356 (2019) *doi: 10.1691/ph.2019.8242*

Four potential process related impurities were detected during the impurity profiling study of a semi-synthetic aminoglycoside antibiotic, arbekacin. The current preparation process from 3',4'-didehydro-dibekacin easily generates the specific impurities with similar structures to arbekacin that makes hard to separate and identify the residues. HPLC-ELSD and column chromatography loading weakly acidic cation exchange resin were used for the detection and isolation of these process impurities. Based on the synthesis and spectral data (ESI-MS/MS, ^{1}H NMR, ^{13}C NMR and 2D-NMR), the structures of these impurities were characterized as dibekacin, 3-N-γ-aminohydroxybutyric (AHB)-dibekacin, 3"-N-AHB-dibekacin and 1,3-N,N-di-AHB-dibekacin. The characterization of these impurities is discussed in detail and our current efforts may help to develop a general strategy for isolation and identification of aminoglycoside products.

1. Introduction

Arbekacin (ABK), which is often called hebekacin, is a class of compounds known as aminoglycoside antibiotics. Besides the activity against *Pseudomonas aeruginosa* and *S. aureus*, ABK shows anti-bacterial activity against methicillin-resistant *S. aureus* (Deguchi et al. 1990; Watanabe et al. 1987; Matsuhahsi et al. 1988). However, ABK monitoring should be mandatory for ensuring optimal therapeutic efficacy with minimal incidence of side effects. Arbekacin was firstly synthesized from dibekacin (DBK) with the introduction of γ-aminohydroxybutyric (AHB) in the N-1 position of dibekacin reported by Kondo et al. (1973). Our laboratory has designed a new synthetic route of ABK, as shown in Fig. 1 (Qiao et al. 2017). This method effectively improves the yield of the product and reduces the formation of by-products. An impurity profiling study toward this route was not reported to date. Therefore, the impurities of this route were researched. To the best of our knowledge, even though antibiotics are not subject to the ICH (International Conference on Harmonisation) guidelines which prescribe that more than 0.1 % of impurity should be identified, elucidating the structures of related impurities has been considered as an essential factor to ensure drug safety and quality (Kondo 1994; Kobayashi et al. 1995). Thus, it is necessary to establish a general solution for isolation, sensitive detection and identification of these impurities.

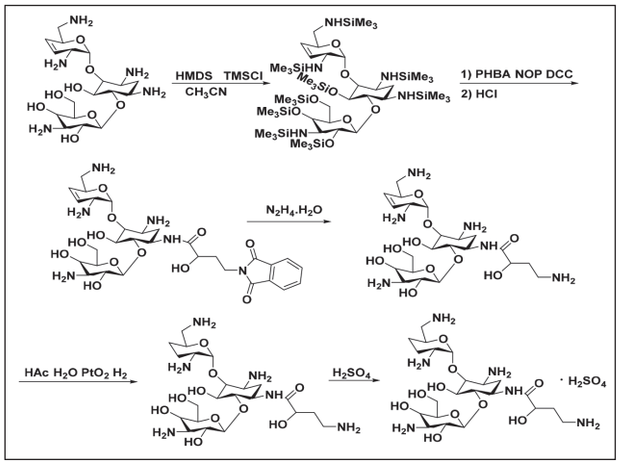

Fig. 1: Synthetic route to arbekacin sulfate

Many methods were developed to isolate and analyze aminoglycoside antibiotics and their related products (Stead 2000; Megoulas and Kouppairs 2004). The isolation of related impurities from aminoglycoside bulk drugs is a challenge due to the high polarity and strong basicity of aminoglycosides. Column chromatography with weakly acidic cation exchange resin, carboxymethyl (CM) sephadex and silica gel as main tools are used to isolate aminoglycosides (Clarot et al. 2004). An evaporative light-scattering detector (ELSD) is chosen as an universal detector to analyze nonvolatile samples (Vogel et al. 2001; Wang et al. 2006). It is a tough assignment that these substances and ABK were isolated and identified, since these related products with similar structures and polarity were derived from the same synthetic route. ESI-MS^{n} spectrometry combined with 2D nuclear magnetic resonance (2D-NMR) can provide more detailed structural information, and realize the identification of these related impurities.

The main impurities of the original synthesis pathway were the unreacted raw material dibekacin and trace amounts of unknown impurities. In our synthetic route, arbekacin and four related impurities (Fig. 2) were detected and isolated by HPLC-ELSD and weakly acidic cation exchange resin (CD-180), respectively. Further, ESI-MS/MS and 2D-NMR were employed to assign and identify the structures of these substances. This work can evaluate the composition of arbekacin sulfate bulk drug from different sources and efficiently control the content of impurities. This impurity profiling study will also be of immense importance for understanding the source of potential impurities during the synthesis of ABK.

2. Investigations, results and discussion

2.1. Optimization of HPLC-ELSD method

A SB-C18 column (250 mm × 4.6 mm ID; 5 μm) with acidic mobile phase consisting of organic solvent and trifluoroacetic acid was used to detect arbekacin and related impurities.

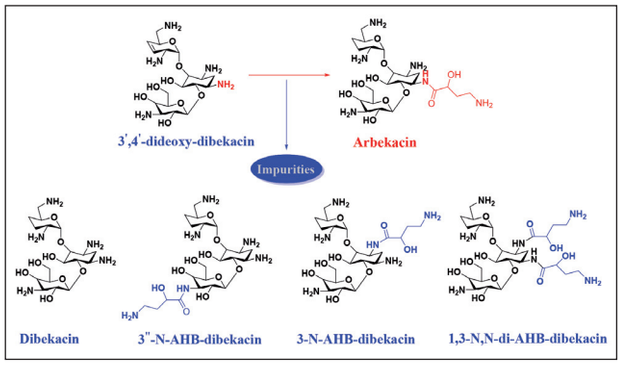

Fig. 2: Structures of arbekacin and related impurities

Various concentrations (0.5, 1, 2 M) of trifluoroacetic acid were tested to show the effect on peak shape, resolution and retention time. The results show that as the concentrations increased, the retention time of arbekacin increased and the chromatograms showed to have sharp symmetrical peaks and good separation. When trifluoroacetic acid was 0.2 M, arbekacin and related impurities were effectively separated.

Organic solvents (methanol, acetonitrile, and acetone) were tested to show the influence on peak shape, resolution and retention time. The mobile phase with the presence of methanol was able to completely separate ABK and related impurities. Therefore, methanol was selected as organic solvent.

The flow rate of mobile phase has a great influence on resolution and response. By adjusting to flow rate (1, 0.8, 0.6 ml min^{-1}), ABK and related impurities were completely separated when the flow rate was 0.8 ml min^{-1}.

2.2. Detection of impurities by HPLC-ELSD

Arbekacin sulfate sample was analyzed by the HPLC-ELSD method. Related impurities were detected. The analysis revealed the presence of four peaks marked 1 at relative retention time (RRT) of 5.495 min, 2 at RRT of 7.180 min, 3 at RRT of 9.587 min and 4 at RRT of 10.300 min in the chromatogram (Fig. 3).

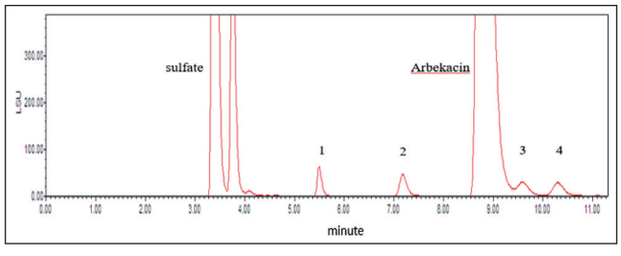

Fig. 3: HPLC-ELSD spectrum of arbekacin sulfate

2.3. Isolation of four substances by column chromatography

Arbekacin sulfate bulk drug was isolated by column chromatography. Compounds **1**-**4** were successively obtained when the concentrations of ammonia were 0.1 M, 0.2 M, 0.4 M, 0.5 M, respectively. Each substance was detected by TLC and then used as sample for structural analysis by ESI-MS/MS and NMR.

2.4. Structure elucidation of related products by ESI-MS/MS

It is a challenging task to identify the structure of these impurities only by MS, especially compounds **2** and **3** with the same mass-to-charge ratio to ABK. ESI-MS/MS was used to further analyse these substances. The data of ESI-MS/MS and proposal structures are shown in Table 1.

The related impurities and ABK follow the similar fragmentation pathway, thus, it is necessary to understand the fragment ions of ABK. The three rings in ABK were labeled as A, B and C for easy assignment. In the ESI-MS/MS spectrum of ABK with protonated molecule at m/z 553, the probable fragmentation pathway is shown in Fig. 4.

Compound **1** with protonated ion at m/z 452 was identical to dibekacin. The protonated ion at m/z 163 further indicates that there is no AHB side chain in ring B of compound **1**. (Fig. 5) It is reasonable to propose **1** as dibekacin because it is the hydrogenated product of 3', 4'-didehydro-dibekacin which was used as starting material.

Compound **2** with the $[M+H]^+$ ion is at m/z 553, which suggested that AHB substituted as a side chain on dibekacin. It produced abundant ion at m/z 425 by loss of ring A (-128 Da). Similarly, the loss of ring C (-161 Da) yield ions at m/z 392. All the other fragment ions of compound **2** were also identical with those of ABK (Fig. 6). These result indicated that AHB side chain was probably substituted to ring B like arbekacin and the structure of compound **2** was proposed to be 3-N-AHB-dibekacin.

Compound **3** with the protonated ion at m/z 553, produced the abundant ion at m/z 425 was identical to compound **2**. This suggested that the AHB side chain substituted on ring B or ring C. Ion at m/z 291 by loss of ring C (-262 Da), compared with the loss of ring C (-161 Da) in ABK, the extra 100 m/z implied the presence of an AHB side chain in ring C of 3. Ions at m/z 263 and 163 were produced by glycosidical cleavage between ring B and ring C by loss of 162 Da and 262 Da. Based on these fragmentation, we can assume that AHB side chain was substituted in ring C. The probable structure of 3 is 3''-N-AHB-dibekacin (Fig. 7).

The mass spectrum of related impurity **4** displayed $[M+H]^+$ ion at m/z 654. Compared with the protonated ABK at m/z 553, the extra mass-to-charge ratio indicated that two amino groups may be substituted by AHB side chain. Ion at m/z 526 by loss of ring A (-128Da) indicated that these two AHB side chains substituted on ring B and ring C. Loss of ring C (-161 Da) yield the ion at m/z 493, which implied there were two AHB side chains on ring B. As a consequence, the structure of compound **4** was confirmed as 1, 3-N,N-di-AHB-dibekacin (Fig. 8).

2.5. Structure elucidation of impurity 2 and impurity 3 by NMR

It is difficult to gain the subtle distinction and well-defined assignment of compounds **2** and **3** in the ABK synthetic process from the ESI-MS/MS fragmentation pathway. Subsequently, NMR experiments including ^{1}H NMR, ^{13}C NMR and HMBC spectra (details on request from the authors) were carried out to obtain detailed information of these products. In order to compare the results of these products with ABK, the NMR data of ABK are shown in Table 2 and the results of compounds **2** and **3** are shown in Table 3.

Table 1: LC/ESI-MS and proposed chemical structures of impurities in arbekacin

Substance	t_R (min)	$[M+H]^+$(m/z)	Fragment ions (m/z)	Proposed chemical structure
1	5.495	452	324, 291, 163, 129	Dibekacin
2	7.180	553	425, 392, 264, 162	3-N-AHB-dibekacin
Arbekacin	8.791	553	425, 392, 264, 162	1-N-AHB-dibekacin
3	9.587	553	425, 291, 263, 163	3″-N-AHB-dibekacin
4	10.300	654	526, 493, 365,162	1,3-N,N-di-AHB-dibekacin

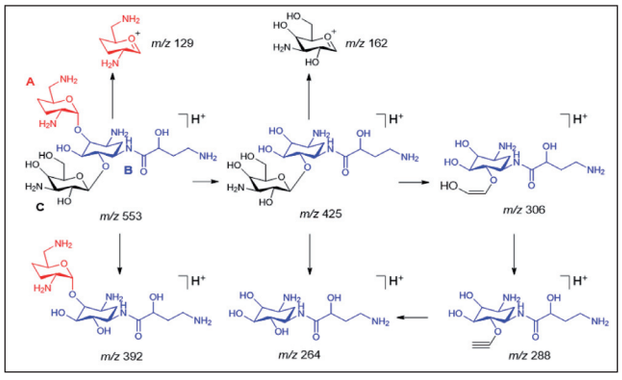

Fig. 4: Probable fragmentation pathway of arbekacin

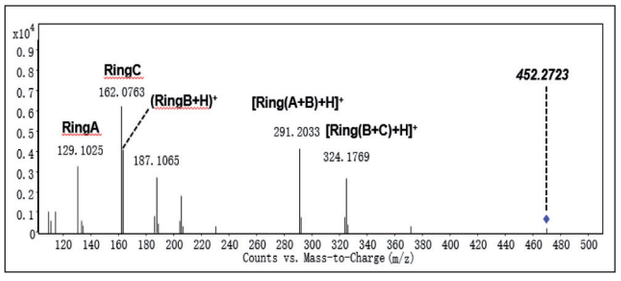

Fig. 5: ESI–MS/MS spectrum acquired from $[M+H]^+$ ions of **1** at m/z 452

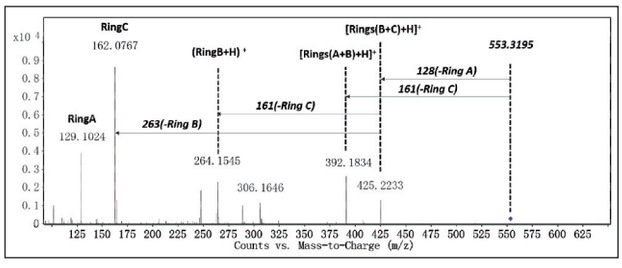

Fig. 6: ESI–MS/MS spectrum acquired from $[M+H]^+$ ions of **2** at m/z 553

As to compound **2** (Table 3), the signal of AHB side chain [δC 175.2 (C-1'''), 69.5 (C-2'''), 31.4 (C-3'''), 36.2 (C-4''')] was observed. Compared with the ^{1}H NMR data of ABK, a downfield chemical shift of 0.69 ppm occurred at H-1 position and an upfield chemical shift of 0.53 ppm occurred at H-3. In the ^{13}C NMR spectrum, downfield of 1.4 ppm at position of C-3 and upfield of 1.4 ppm at C-1 compared to those of ABK. ^{1}H and ^{13}C NMR data all suggested that an AHB moiety was substituted on amino group of C-3, not C-1 like ABK. The result was further proved by HMBC spectrum. The HMBC spectrum of compound **2** showed that C-1'''was coupled with H-3, H-2'''and H-3'''. Hence, the chemical structure of **2** was verified as 3-N-AHB-dibekacin.

The ^{13}C and ^{1}H NMR spectra of compound **3** were similar to that of **2** and ABK. It also has extra AHB side chain signals. In ^{13}C spectrum, downfiled of 1.4 ppm at position C-3'' compared to that of arbekacin and downfield of 1 ppm compared to that of **2**, suggesting that an AHB side chain was substituted on C-3''. The presence of extra AHB moiety signals [δH 4.30(1H, dd, J = 3.1, 7.7 Hz, H-2'''), 2.10 (1H, m, H-3'''), 1.95 (1H, m, H-3'''), 3.18 (1H, m, H-4''')], coupled with an upfield shift of 0.65 ppm at H-3'' (ring C) in the ^{1}H NMR spectrum, supported the deduction on the basis of the ^{13}C NMR spectrum. HMBC spectrum also showed that C-1''' was coupled with H-3'', H-2''', H-3''', which further proved the suggestion.

All ^{1}H, ^{13}C and HMBC NMR signals of compounds **2** and **3** are shown in Table 3. All of the structure deductions agreed with the results of HPLC and ESI-MS/MS.

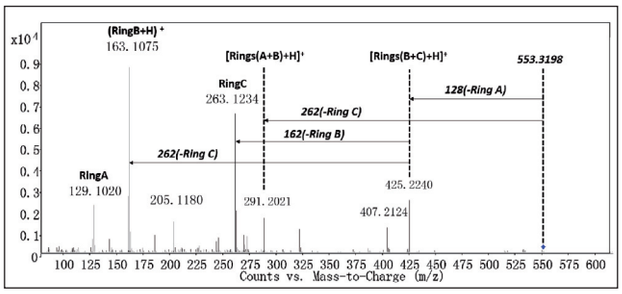

Fig. 7: ESI–MS/MS spectra acquired from $[M+H]^+$ ions of **3** at m/z 553

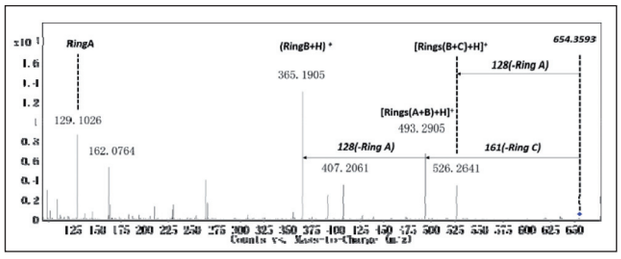

Fig. 8: ESI–MS/MS spectra acquired from $[M+H]^+$ ions of **4** at m/z 654

The structure of related impurities elucidated by NMR and ESI-MS/MS, no substitution in amino group of ring A took place. The reason was that 2' amino group of ring A has large steric hindrance and side chain did not react with the 2' amino group. The 6' amino group had large solvation and hydrogen-bonding so that the active ester was not easy to react with the 6' amino group in a polar solvent.

In this work, we explored a general evaluation of arbekacin sulfate and four related impurities derived from the same synthetic route. Column chromatography loading weakly acidic cation exchange resin and HPLC-ELSD were used to isolate and analyse the title products, respectively. High resolution ESI-MS provided precise mass-to-charge ratio for these compounds, and subsequent ESI-MS/MS analysis offered clear fragmentation pathway that suggesting possible structures of similar compounds. 2D-NMR assays gave the growing evidence for distinguishing arbekacin and related products, especially to different positions of AHB side chain that identified by HMBC spectra.

The isolation and analysis of arbekacin and related products can provide important information for product synthesis and ensuring drug safety and quality. The present work may have the potential to provide a general isolated and analytical strategy for aminoglycoside derivatives and, in particular, be applied to the research and production of semi-synthetic antibiotic drugs.

3. Experimental

3.1. Reagents and chemicals

The sample of arbekacin sulfate bulk drug was synthesized in our laboratory according to JP 6167224B2. HPLC grade methanol was purchased from J&K Scientific Ltd. (Beijing, China). Other reagents were of analytical grade reagent and purchased from Beijing Chemical Works (Beijing, China). Weakly acidic cation exchange resin (CD-180) was purchased from Huazhen Sci. & Tech. CO., Ltd. (Shanghai, China).

3.2. Apparatus

3.2.1. HPLC

HPLC was performed on a Waters series 2695 liquid chromatography system (Waters Technologies, USA), equipped with a bibasic pump, autosampler and Evaporative Light Scattering Detector (ELSD 2424, Waters Technologies, USA). Data were acquired using Waters Empower 3 software. A SB-C18 column (250 mm × 4.6 mm ID; 5 µm) size was employed. Methanol was used as phase A, trifluoroacetic acid (0.2 M) was chosen as phase B and the volume ratio of methanol to aqueous trifuoroacetic acid was 4:96. The flow rate was set at 0.8 mL min^{-1}, and injection volume was 30 µL. The postrun time was 12 min. Column oven temperature was 30 °C. The temperature of drift tube was 60 °C. The carrier gas pressure was 40 psi.

Table 2: ^{1}H NMR, ^{13}C NMR and HMBC data of arbekacin

No.	Arbekacin sulfate δH(J/Hz)	δC	HMBC(C-H)
1	4.04(1H, m)	48.9	H-2’’’, H-6, H-2, H-3
2Heq	1.76(1H, m)	30.8	H-4, H-3, H-1
2Hax	2.12(1H, m),		
3	3.47(1H, dd, 3.2, 5.8)	48.8	H-4, H-2, H-1
4	3.99(1H, t ,3.2)	78.0	H-1’, H-5, H-3,H-2
5	3.83(1H, dd, 9.2, 18.5)	74.9	H-6, H-4
6	3.94(1H, m)	80.0	H-1’’, H-5, H-1
1’	5.75(1H, d, 3.4)	94.5	H-4, H-2’, H-3’
2’	3.58(1H, t, 10.1)	48.7	H-4’, H-3’, H-1’
3’	2.09(1H, dd, 4.1, 7.8)	20.7	H-5’, H-4’, H-2’, H-1’
4’	1.86(1H, m)	25.6	H-6’, H-5’, H-3’, H-2’
	1.50(1H, m)		
5’	4.11(1H, m)	65.7	H-6’, H-4’, H-3’
6’	3.17(1H, m, 3.1, 13.4)	42.7	H-5’, H-4’
	3.00(1H,dd, 8.4, 13.4)		
1’’	5.10(1H, d, 3.7)	97.9	H-3’’, H-2’’
2’’	3.75(1H,dd, 4.9, 13.7)	68.1	H-4’’, H-3’’, H-1’’
3’’	3.44(1H, m)	55.1	H-5’’, H-4’’, H-2’’, H-1’’
4’’	3.73(1H, m)	65.8	H-6’’, H-5’’, H-3’’, H-2’’
5’’	4.04(1H, dd, 4.4, 10.0)	72.0	H-6’’, H-4’’, H-3’’
6’’	3.76(1H, m)	69.8	H-5’’, H-4’’
	3.69(1H, dd, 3.8, 10.9)		
1’’’		175.4	H-1, H-2’’’, H-3’’’
2’’’	4.21(1H, dd, 3.8, 9.3)	69.5	H-1, H-3’’’, H-4’’’
3’’’	2.11(1H, m)	31.1	H-2’’’, H-4’’’
	1.94(1H, dd, 4.0, 9.3)		
4’’’	3.10(1H, t, 7.24)	36.9	H-2’’’, H-3’’’

d, doublet; t, triplet; m, mutiplet; dd, doublet of doublet; J, 1H-1H coupling constants.

3.2.2. ESI-MS/MS Instrumentation

ESI-MS/MS analysis was performed using an Xevo G2 Qtof mass spectrometer equipped with an electrospray ionization (ESI) source and ion trap mass analyzer. All of the data were processed using Qualitative Analysis, version B.06.00.
MS was in positive-ion electrospray mode. Nitrogen was used as both sheath and auxiliary gas. Other MS Parameter were set as follows: capillary voltage = 3,500 V, fragment voltage = 125 V, skimmer voltage= 60 V, drying gas temperature = 350°C and collision energy = 25 V. The mass range was kept at m/z 0–800.

3.2.3. NMR Instrumentation and methods

The ^{1}H NMR, ^{13}C NMR, and 2D NMR (COSY, HSQC, HMBC) were recorded at 600 MHz on a Bruker AVANCE NMR spectrometer. The samples were dissolved in dimethyl sulfoxide-d_6 or deuterium oxide at a concentration of 60 mg/mL. The raw data were processed using Bruker topsin 2.0 software. The ^{1}H chemical shift values were reported on the δ scale in ppm relative to TMS (δ = 0.00 ppm) and in the ^{13}C NMR the chemical shift values were reported relative to DMSO-*d6* (δ = 39.50 ppm).

3.3. Sample preparation for HPLC analysis

According to our synthetic route, we synthesized the crude arbekacin sulfate for HPLC analysis and separation. The crude Arbekacin sulfate was diluted with water to obtain a final assay concentration of 200 μg mL^{-1}. The final sample solutions were filtered through 0.22 μm membrane filter for HPLC analysis.

Acknowledgements: The work was supported by the National Natural Science Foundation of China (21672021, 21572018, 21372024 and 21232005) and by the State Key Labor-atory of Natural and Biomimetic Drugs.

Conflicts of interest: None reported.

References

Clarot I, Chaimbault P, Hasdenteufel F, Netter P, Nicolas A (2004) Determination of gentamicin sulfate and related compounds by high-performance liquid chromatography with evaporative light scattering detection. J Chromatogr A 1031: 281-287.

Deguchi K, Yokota N, Koguchi M, Nakane Y, Fukushima Y, Fukuyama S, Nishimura Y (1990) Measurement of mics on 16 antimicrobial agents for freshly isolated methicillin-resistant Staphylococcus aureus strains. Jpn J Antibiot 43: 623-635.

Kobayashi Y, Uchida H, Kawakami Y (1995) Arbekacin. Int J Antimicrob Agents 5: 227-230.

Kondo S (1994) Development of arbekacin and synthesis of new derivatives stable to enzymatic modifications by methicillin-resistant Staphylococcus aureus. Jpn J Antibiot 47: 561-574.

Kondo S, Iinuma K, Yamamoto H, Maeda K and Umezawa H (1973) Syntheses of 1-n-{(s)-4-amino-2-hydroxybutyryl}-kanamycin b and -3’, 4’-dideoxykanamycin b active against kanamycin-resistant bacteria. J Antibiotics 26: 412-415.

Matsuhashi Y, Yamasato H (1988) The enzymatic mechanisms of resistant to aminoglycoside antibiotics in methicillin-cephem-resistant Staphylococcus aureus. Jpn J Antibiot 41: 523-529.

Megoulas NC, Kouppairs MA (2004) Enhancement of evaporative light scattering detection in high-performance liquid chromatographic determination of neomycin based on highly volatile mobile phase, high-molecular-mass ion-pairing reagents and controlled peak shape. J Chromatogr A 1057: 125-131.

Qiao RZ, Zhang J, Zhou H, Li G, Cai Y, Guo LL (2017) Japan Patent 6,167,224B2.

Stead DA (2000) Current methodologies for the analysis of aminoglycosides. J Chromatogr B 747: 69-93.

Table 3: ^{1}H NMR, ^{13}C NMR and HMBC data of impurities (2, 3) of arbekacin sulfate

	2			3		
	δH(J/Hz)	δC	HMBC(C-H)	δH(J/Hz)	δC	HMBC(C-H)
1	3.35(1H, m)	50.3	H-6, H-2, H-3,	3.48(1H, m)	49.9	H-6, H-2, H-3,
2-Heq	1.78(1H, m)	30.6	H-4, H-3, H-1	1.91(1-H, m)	28.6	H-4, H-3, H-1
2-Hax	1.81(1H, m)			2.40(1-H, dd, 4.3, 8.4)		
3	4.00(1H, m)	47.4	H-2”’, H-4, H-2, H-1	3.36(1H, m)	48.7	H-4, H-2, H-1
4	3.78(1H, d ,3.5)	77.0	H-1’, H-5, H-3, H-2	3.92(1H, m)	77.3	H-1’, H-5, H-3,H-2
5	3.83(1H, dd, 2.6, 5.8)	75.3	H-6, H-4	3.80(1H, t, 9.0)	74.4	H-6, H-4
6	3.61(1H, d, 9.7)	84.6	H-1”, H-5, H-1	3.74(1H, dd, 3.8, 5.8)	84.0	H-1”, H-5, H-1
1’	5.60(1H, d, 3.2)	94.2	H-4, H-2’, H-3’	5.76(1H, d, 3.5)	94.5	H-4, H-2’, H-3’
2’	3.31(1H, m)	48.8	H-4’, H-3’, H-1’	3.47(1H, m)	48.7	H-4’, H-3’, H-1’
3’	1.61(1H, dd, 3.5, 12.6)	20.9	H-5’, H-4’, H-2’, H-1’	1.94(2H, m)	20.6	H-5’, H-4’, H-2’, H-1’
	1.82(1H,m)					
4’	1.42(1H, dd, 3.4, 13.6)	25.7	H-6’, H-5’, H-3’, H-2’	1.50(1H, dd, 3.0, 11.8)	25.6	H-6’, H-5’, H-3’, H-2’
	1.66(1H, m)			1.86(1H, m)		
5’	3.83(1H, m)	65.1	H-6’, H-4’, H-3’	4.13(1H, m)	65.9	H-6’, H-4’, H-3’
6’	2.90(1H, dd, 8.2, 13.4)	42.7	H-5’, H-4’	3.17(1H, dd, 3.1, 13.4)	42.7	H-5’, H-4’
	3.11(1H, dd, 3.1, 13.4)			3.07(1H, dd, 7.3, 13.6)		
1”	5.02(1H, d, 3.4)	100.3	H-3”, H-2”	5.04(1H, d, 3.7)	101.0	H-3”, H-2”
2”	3.78(1H, dd, 4.2, 12.4)	68.8	H-4”, H-3”, H-1”	3.75(1H, dd, 4.6, 13.9)	69.6	H-4”, H-3”, H-1”
3”	3.30(1H, m)	54.8	H-5”, H-4”, H-2”, H-1”	4.09(1H, m)	53.7	H-1”’,H-5”, H-4”, H-2”, H-1”
4”	3.57(1H, m)	66.1	H-6”, H-5”, H-3”, H-2”	3.50(1H, m)	66.9	H-6”, H-5”, H-3”, H-2”
5”	3.75(1H, t, 3.8)	72.5	H-6”, H-4”, H-3”	3.90(1H, m)	73.3	H-6”, H-4”, H-3”
6”	3.75(1H, m)	59.9	H-5”, H-4”	3.76(1H, m)	60.4	H-5”, H-4”
	3.67(1H, dd, 4.4, 7.8)			3.69(1H, dd, 5.1, 12.5)		
1”’		175.2	H-3, H-2”’, H-3”’		176.8	H-3”’, H-2”’, H-3”’
2”’	4.19(1H, dd, 4.1, 7.9)	69.5	H-3”’, H-4”’	4.30(1H, dd, 4.1, 7.7)	69.7	H-3”’, H-4”’
3”’	1.92(1H, m)	31.4	H-2”’, H-4”’	1.95(1H, m)	30.9	H-2”’, H-4”’
	2.08 (1H, m)			2.10(1H, m)		
4”’	3.12(2H, m)	36.2	H-2”’, H-3”’	3.18(2H, m)	36.5	H-2”’, H-3”’

d, doublet; t, triplet; m, mutiplet; dd, doublet of doublet; J, ^{1}H-^{1}H coupling constants.

Vogel R, Defillipo K, Reif V (2001) Determination of isepamicin sulfate and related compounds by high performance liquid chromatography using evaporative light scattering detection. J Pharm Biomed Anal 24: 405-412.

Wang J, Hu X, Tu Y, Ni K (2006) Determination of spectinomycin hydrochloride and its related substances by HPLC–ELSD and HPLC–MS^{n}. J Chromatogr B 834: 178-182.

Watanabe T, Goi H, Hara T, Sugano T, Tanaka Y, Kazuno Y, Matsuhashi Y, Yamamoto H, Yokota T (1987) Antibacterial activities of arbekacin, a new aminoglycoside antibiotic, against methicillin-cephem-resistant staphylococcus aureus. Jpn J Antibiot 40: 349-365.