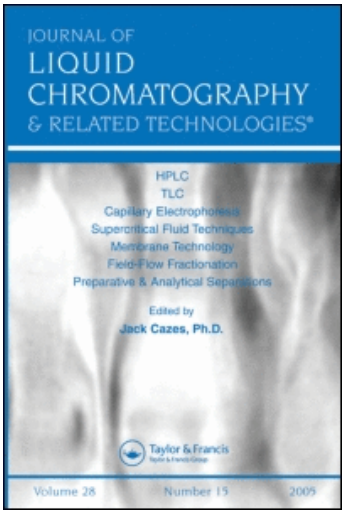

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF ARBEKACIN SULFATE INJECTION AND ITS RELATED SUBSTANCES BY HPLC USING EVAPORATIVE LIGHT SCATTERING DETECTION

Jian Wang[a]; Xiaojun Hu[b]
[a] Zhejiang Institute for Food and Drug Control, Hangzhou, P.R. China [b] Medical Information Center, School of Medicine, Zhejiang University, Hangzhou, P.R. China

Online publication date: 08 February 2010

To cite this Article Wang, Jian and Hu, Xiaojun(2010) 'DETERMINATION OF ARBEKACIN SULFATE INJECTION AND ITS RELATED SUBSTANCES BY HPLC USING EVAPORATIVE LIGHT SCATTERING DETECTION', Journal of Liquid Chromatography & Related Technologies, 33: 4, 441 — 451
To link to this Article: DOI: 10.1080/10826070903574170
URL: http://dx.doi.org/10.1080/10826070903574170

Journal of Liquid Chromatography & Related Technologies, 33:441–451, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903574170

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF ARBEKACIN SULFATE INJECTION AND ITS RELATED SUBSTANCES BY HPLC USING EVAPORATIVE LIGHT SCATTERING DETECTION

Jian Wang[1] and Xiaojun Hu[2]

[1]*Zhejiang Institute for Food and Drug Control, Hangzhou, P.R. China*
[2]*Medical Information Center, School of Medicine, Zhejiang University, Hangzhou, P.R. China*

□ *A new and simple HPLC-ELSD method for the determination of arbekacin sulfate injection and its related substances was developed. The column was Agilent SB-C_{18} (250 × 4.6 mm, 5μm). The mobile phase was 200 mM trifluoroacetic acid – acetonitrile (91:9) flow rate was 0.8 mL min^{-1}. The detector used was an Dikma SEDEX 75 ELSD detector. The drift tube temperature was 50°C. The pressure of nebulizing gas was 3.5 bar. Good separation of arbekacin from the main related substances could be achieved. The standard curve was rectilinear in the range of 500 ~ 2500 μg mL^{-1} (r = 0.9990). The average recovery of arbekacin is 100.8% (R.S.D = 1.5%, n = 9). The average recovery of dibekacin is 104.7% (R.S.D = 2.5%, n = 9). The limit of detection of arbekacin was 4.5 μg mL^{-1}. The limit of detection of dibekacin was 5.0 μg mL^{-1}. The method is simple and rapid, and the results are accurate and reproducible.*

Keywords arbekacin sulfate, arbekacin sulfate injection, dibekacin, HPLC-ELSD

INTRODUCTION

Arbekacin sulfate belongs to a class of compounds known as aminoglycoside antibiotics. Post column derivatization method is used for the determination of related substances, and microbiological assays of antibiotics is used for the assay in Japanese Pharmacopoeia.[1] Like many aminoglycosides, arbekacin sulfate lacks a suitable chromophore, which is necessary for UV detection. For this reason, the analysis of arbekacin sulfate is performed using precolumn or postcolumn derivatization methods.[1–3] Such methods, which need sample treatment, make more complex the HPLC system (reaction coil, extra pump, etc.) and were very time consuming. In fact, several drawbacks could be listed against a sample derivatization

Correspondence: Xiaojun Hu, Medical Information Center, School of Medicine, Zhejiang University, Hangzhou 310058, P.R. China. E-mail: xjhu@zjuem.zju.edu.cn

process: introduction of non controlled impurities, degradation products, and the most important, impurities of the analyte lacking the specific functional group required for derivatization could not be detected.

Evaporative light scattering detection (ELSD) is described as a universal detection mode suitable for non-adsorbing analytes.[4–7] The chromatographic mobile phase is nebulized with an inert gas and evaporated in a drift tube. The response does not depend on the solute optical properties, any compound less volatile than the mobile phase could be detected. The detector response is now well described and shows a double logarithmic relationship between the signal and the analyte concentration. Such response allows all molecules of the sample to give a proportional signal (same sensitivity). This principle is in good agreement with the search of impurities in pharmaceutical products.

This principle aim of this work was to develop a rapid and simple chromatographic method, which allows a direct sample introduction without any derivatization. HPLC-ELSD method was first used to determine the content of arbekacin sulfate injection and its related substances. The method development uses a novel low pH stable reversed phase silica column and a simple mobile phase that was designed not to contain any non-volatile reagents. This permits the use of evaporative light scattering detection that enables the detection of arbekacin sulfate injection and its related substances.

EXPERIMENTAL

Chemicals and Reagents

Arbekacin sulfate reference substance, dibekacin reference substance, injections (batch numbers: ARI_7-6201, ARI_7-6202, ARI_7-6203, ARI-6201, ARI-6202, ARI-6203) were offered by Reyon Pharmaceutical Co. Ltd. (Chungbuk, Korea); trifluoroacetic acid and acetonitrile were chromatographic grade.

Apparatus

An Agilent 1100 series liquid chromatography (LC) system equipped with a binary pump was connected to an Agilent G1313A autosampler. Chromatographic separation was carried out at room temperature using an Agilent SB-C_{18} analytical column (250×4.6 mm, 5 μm). The mobile phase consisted of 200 mM trifluoroacetic acid-acetonitrile (91:9). Detector used was an Dikma SEDEX 75 ELSD detector. The drift tube temperature was 50°. The pressure of nebulizing gas was 3.5 bar. The flow rate was 0.8 mL min^{-1}.

Sample Preparation

Arbekacin sulfate injections were simply diluted with water to obtain a concentration level within the working range, concentrations of arbekacin solution used for the assay and related substances, were 1.0 and 5.0 mg mL^{-1}, respectively.

Quantitation

An external standard calibration curve with three calibration points ranging from 80 to 120% of the analytical assay concentration (1.0 mg mL^{-1}) was used for the arbekacin assay. A separate external standard calibration curve was used for the assay of low level related substances, with three calibration points ranging from 1.0 to 2.0% of the sample concentration (5.0 mg mL^{-1}). Calculations were based on peak areas.

RESULTS AND DISCUSSION

Method Development

Selection of the Mobile Phase

It is difficult to retain the AGs in the reversed phase mode even with purely aqueous eluents. Therefore, most chromatographic methods were based on some forms of ion-pair modes or basic mobile phases. In order to permit the use of evaporative light scattering detection, the mobile phases can not contain any non-volatile reagents. Perfluoranated carboxylic acids, such as heptafluorobutyric acid,[5,6] were reported for use as ion-pair reagents to facilitate the retention of AGs in the reversed phase mode. An Agilent SB-C_{18} (250 × 4.6 mm, 5 μm) column with acidic mobile phase consisting of trifluoroacetic acid and organic solvent was tested to separate arbekacin from its impurities, and the HPLC chromatograms showed to have sharp symmetrical peaks and good separation.

The mobile phases consisting of various concentrations of trifluoroacetic acid (50, 100, 200 mM) were tested to investigate the influence on peak shape, resolution, and retention time. The results showed that as the concentrations increased, the retention time of arbekacin increased and the chromatograms showed to have sharp symmetrical peaks and good separation. If the concentration of trifluoroacetic acid was lower than 200 mM, the arbekacin could not be completely separated from dibekacin (a main impurity in arbekacin). However, high concentration (200 mM) with pH below 2 would do harm to the chromatographic column. Therefore, 200 mM trifluoroacetic acid was selected as the ion-pair reagent.

The mobile phases with the presence of methanol, acetonitrile, and acetone had some influence on the resolution and peak shape; the mobile

TABLE 1 Influence of Flow Rate on Resolution and Peak Shape

Flow Rate (mL min^{-1})	Plate of Arbekacin	Resolution Between Arbekacin and Dibekacin
1.0	6242	3.13
0.8	7337	3.50
0.6	8029	3.90

phase with the presence of acetonitrile was the best to separate dibekacin from other impurities. Therefore, acetonitrile was selected as the organic solvent. The mobile phase consisting of 200 mM trifluoroacetic acid – acetonitrile (91:9) was optimal.

Resolution and response are strongly dependant on flow rate and the increase of flow rate resulted in poor resolution. The experimental results showed that flow rate was selected to be 0.8 mL min^{-1} in order to separate completely arbekacin from dibakacin (at greater flow rate arbekacin may overlap with impurities, while at smaller flow rate, peak broadening and asymmetry would increase, resulting in a decrease of ELSD response). The results were shown in Table 1.

Peak shape and resolution are strongly dependant on sample concentration, and the increase of sample concentration resulted in poor peak shape and resolution. It was satisfactory, that for the assay of arbekacin and related substances, the concentrations were 1.0 and 5.0 mg mL^{-1}, respectively.

Optimization of ELSD Conditions

In this experiment, a Dikma Technologies SEDEX 75 evaporative light scattering detector was used. The drift tube temperature was tested at 40, 45, and 50°C to study the influence on ELSD response and resolution. The results showed that there was higher ELSD response at lower temperature, but there was better separation of arbekacin from dibakacin at higher temperature. The drift tube temperature at 50°C was satisfactory. The results are shown in Table 2.

The pressure of the nebulizing gas (2.5, 3.5, 4.0 bar) was tested to study the influence on ELSD response and signal-to-noise, and the results showed

TABLE 2 Influence of Drift Tube Temperature on Resolution and Peak Shape

Drift Tube Temperature (°C)	Plate of Arbekacin	Resolution Between Arbekacin and Dibekacin
50	6432	3.30
45	6242	3.13
40	6153	2.92

that 3.5 bar was satisfactory. The gain, ranging from 4 to 6, was tested and the results showed that ELSD response increased with a higher gain value but resulted in an increase of baseline noise. Therefore, the gain was set at 4 and 6 for the assay of arbekacin and related substances, respectively.

Method Validation

A preliminary method validation was performed to determine if the HPLC system was acceptable with respect to the specificity, linearity of response, precision, accuracy, and to determine the limit of detection.

Specificity

The ability of the chromatographic system to resolve arbekacin sulfate from its possible impurities was investigated. Dibekacin and excipient were examined in order to assure that they do not interfere (peak overlapping) with arbekacin. For the related substances test, samples were stored under relevant stress conditions (light, heat, acid/base hydrolysis, and oxidation, respectively). Samples showed light and heat stability, while degradation compounds were produced under acid/base hydrolysis and oxidation conditions. The experiment showed that the arbekacin injection under acid/base hydrolysis produced some dibekacin. Arbekacin could be completely separated from dibekacin, excipient, and degradants. The chromatograms for the determination of arbekacin sulfate injection and its related substances were shown in Figures 1–7.

Acid degradation: The arbekacin sulfate injection of 1.0 mL was introduced into a test tube, 0.5 mL of 1 M HCl was added and heated for 30 min at 100°C. After 30 min, the drug treated with 1 M HCl was neutralized with 1 M NaOH and diluted with water to 10 mL. The solution was injected into the HPLC system.

Basic degradation: The arbekacin sulfate injection of 1.0 mL was introduced into a test tube, 0.5 mL of 1 M NaOH was added and heated for 30 min at 100°C. After 30 min, the drug treated with 1 M NaOH was neutralized with 1 M HCl and diluted with water to 10 mL. The solution was injected into the HPLC system.

Oxidative degradation: The arbekacin sulfate injection of 1.0 mL was introduced into a test tube, 0.5 mL of 30% H_2O_2 was added and heated for 30 min at 100°C. After 30 min, the drug treated with 30% H_2O_2 was diluted with water to 10 mL. The solution was injected into the HPLC system.

Heat degradation: Of arbekacin sulfate injection, 1.0 mL was introduced into a test tube and heated for 30 min at 100°C. After 30 min, the

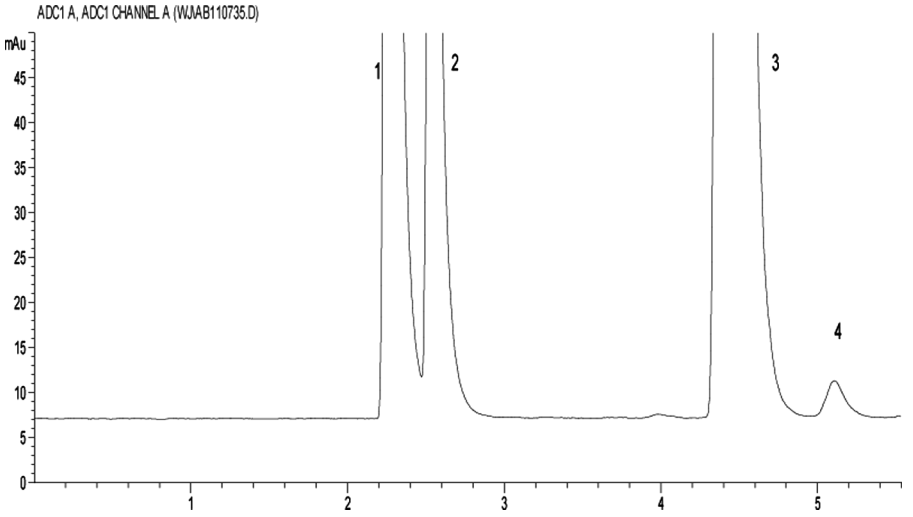

FIGURE 1 Chromatogram of arbekacin spiking with 2% dibekacin; 1. sulfate; 2. excipient; 3. arbekacin; 4. dibekacin.

drug was diluted with water to 10 mL. The solution was injected into the HPLC system.

Photolytic degradation: Of arbekacin sulfate injection, 1.0 mL was introduced into a Petri dish, which is exposed to light at 4500 Lx for 48 hrs. After 48 hrs, the drug was diluted with water to 10 mL. The solution was injected into the HPLC system.

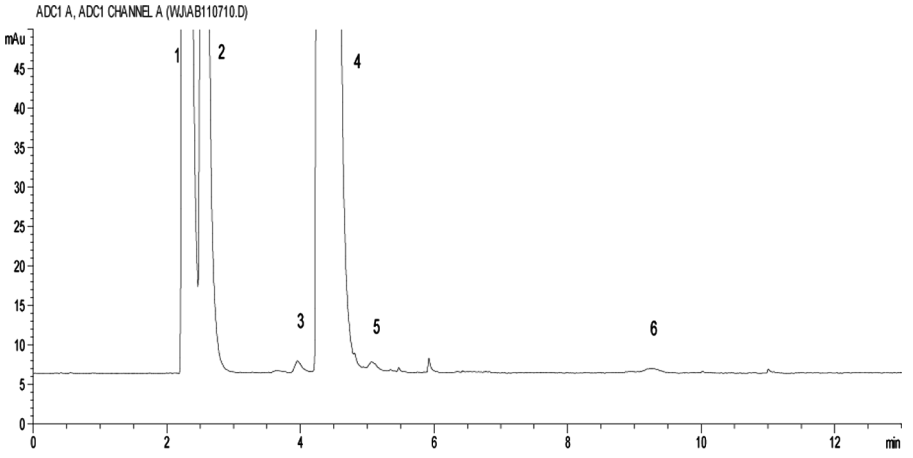

FIGURE 2 Chromatogram of related substances in arbekacin sulfate injection. 1. sulfate; 2. excipient; 4. arbekacin; 5. dibekacin; 3. and 6. other impurities.

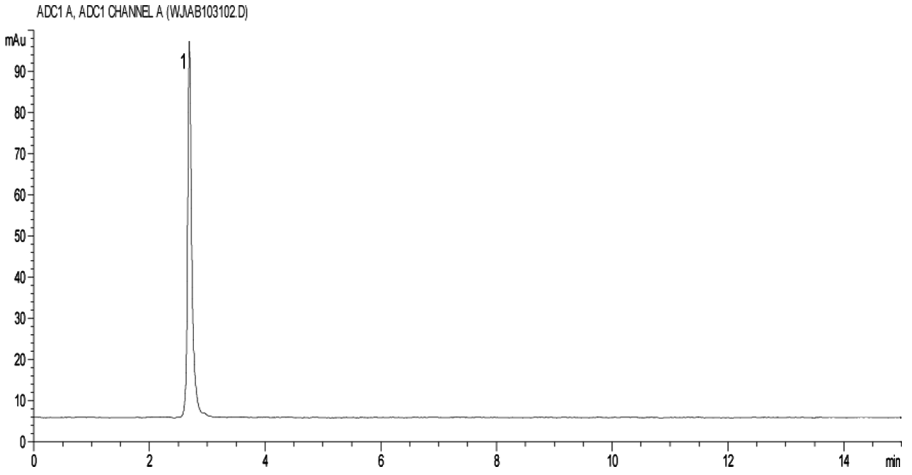

FIGURE 3 Chromatogram of excipient. 1. excipient.

Linearity of Response

It is now well known that ELSD gives a non-direct linear response. A plot of log I versus log$m$ provides a linear response as a plot of the peak area versus the sample concentration in double logarithmic coordinates.

For the assay of arbekacin sulfate, the linearity of response was determined by preparing, in duplicate, five arbekacin sulfate solutions ranging from 50 to 250% of the assay concentration (1.0 mg mL^{-1}). Each solution was analyzed using the recommended HPLC system. The regression curves

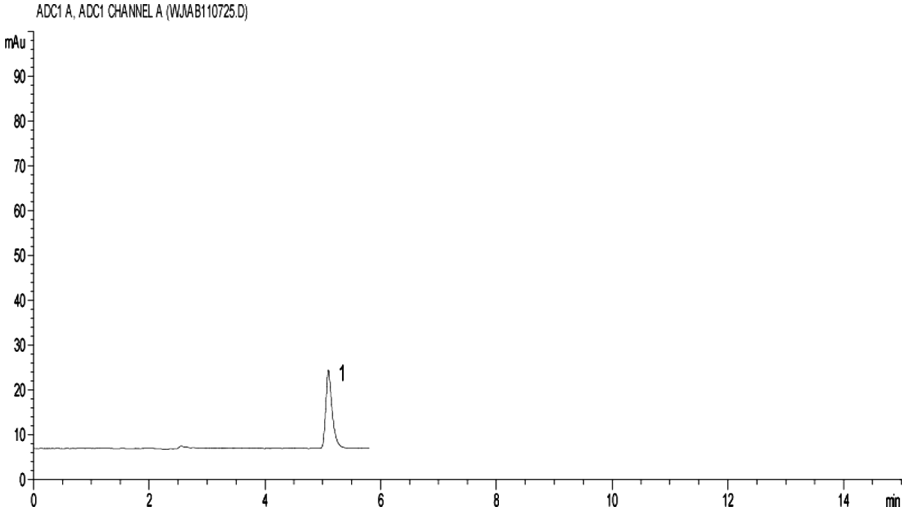

FIGURE 4 Chromatogram of reference substance in related substances. 1. dibekacin.

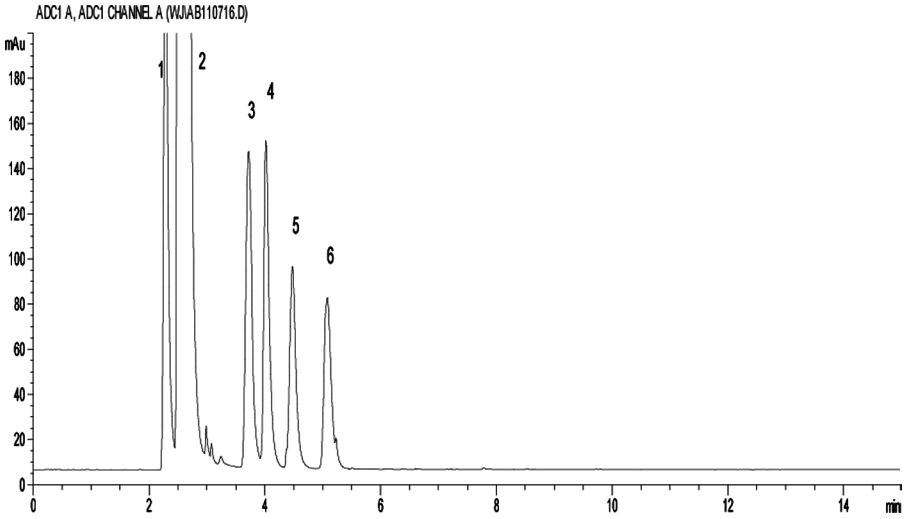

FIGURE 5 Chromatogram of arbekacin sulfate injection by acid damage.

were obtained by plotting log (concentration) versus log (peak area). The regression equation was log A = 1.229 logC − 0.880. The corresponding coefficient r was 0.9990. The result indicated good linearity.

For the assay of related substances (low level linearity), five arbekacin sulfate solutions and five dibekacin solutions were prepared with concentrations ranging from 0.5 to 2.5% of the sample assay concentration (5.0 mg mL^{-1}). The solutions were injected into the HPLC system. The

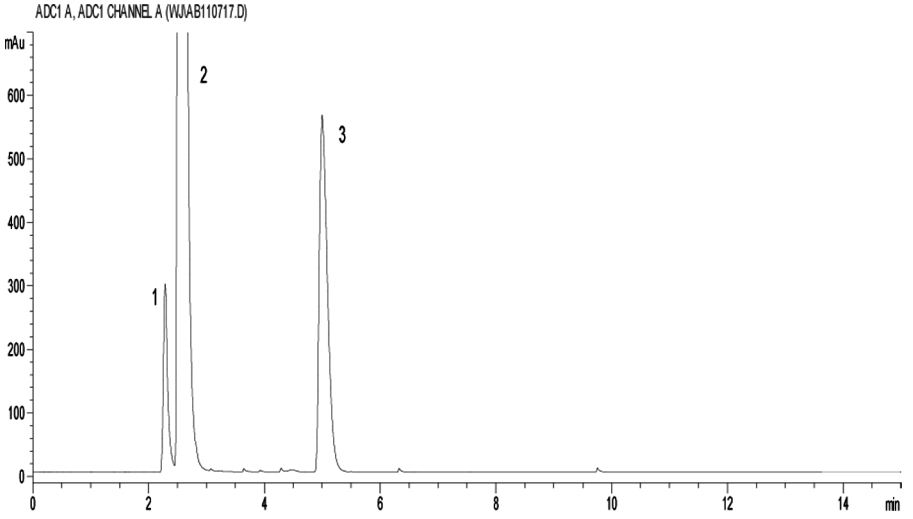

FIGURE 6 Chromatogram of arbekacin sulfate injection by base damage.

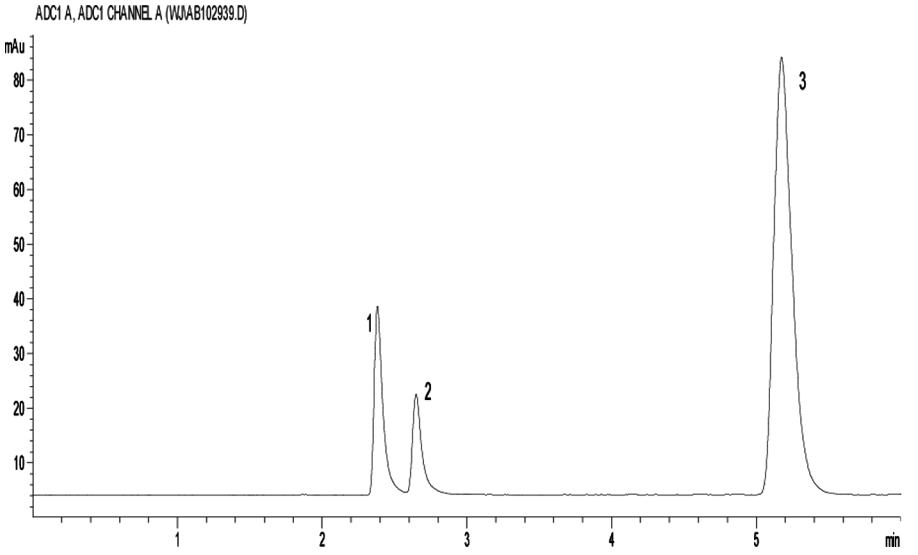

FIGURE 7 Chromatogram of arbekacin sulfate injection assay. 1. sulfate; 2, excipient; 3, arbekacin.

regression curves were obtained by plotting log (concentration) versus log (peak area). The regression equation of arbekacin sulfate was log A = 1.159 logC−0.145. The corresponding coefficient r was 0.9985. The regression equation of dibekacin was log A = 1.187 logC−0.206. The corresponding coefficient r was 0.9970. The result indicated good linearity.

Accuracy of the Assay and Related Substances

For the assay of arbekacin sulfate, the accuracy of the new method was evaluated by performing a recovery experiment, which was obtained from determining the content of arbekacin sulfate in some injection blank samples spiked with arbekacin sulfate. The average recovery was 100.8% (R.S.D = 1.5%, n = 9) and revealed sufficient accuracy. The result of the recovery for the assay of arbekacin sulfate is shown in Table 3.

For the assay of related substances, the accuracy of the new method was evaluated by performing a recovery experiment, which was obtained from determining the content of dibekacin in some arbekacin sulfate injections

TABLE 3 The Recoveries of Arbekacin Sulfate for the Assay of Arbekacin Sulfate

	1	2	3	4	5	6	7	8	9
Amount spiked (mg)	7.81	7.81	7.81	9.95	9.95	9.95	11.64	11.64	11.64
Amount found (mg)	7.77	7.74	7.74	10.17	10.21	10.28	11.65	11.76	11.70
Relative recovery (%)	99.44	99.16	99.14	102.24	102.58	103.28	100.05	101.00	100.50

TABLE 4 The Recoveries of Dibekacin for the Assay of Related Substances

	1	2	3	4	5	6	7	8	9
Amount spiked (mg)	8.06	8.06	8.06	10.07	10.07	10.07	12.08	12.08	12.08
Amount found (mg)	8.73	8.42	8.58	9.95	10.37	10.52	12.88	12.68	12.65
Relative recovery (%)	108.28	104.50	106.43	98.85	103.00	104.48	106.63	104.97	104.71

spiked with dibekacin. The average recovery was 104.7% (R.S.D = 2.5%, n = 9) and revealed sufficient accuracy. The result of the recovery for the assay of related substances is shown in Table 4.

Precision of the Assay

Six replicate sample solutions at 100% of the test concentration (1.0 mg mL^{-1}) were prepared and then assayed for arbekacin using the recommended HPLC system and sample preparation. The relative standard deviation (R.S.D) value was 1.3% (n = 6). The result was satisfactory.

Limit of Detection (LOD)

Its determination could be made by the calculation of the signal-to-noise ratio. A ratio of 3 was selected and successive dilutions of the test solution gave a LOD relative to the arbekacin peak of 0.1% (m/m). The limit of detection of arbekacin was 4.5 μg mL^{-1}. The limit of detection of dibekacin was 5.0 μg mL^{-1}. The limit was in good agreement with that required by the FDA for the assay of related substances.

Analysis of Arbekacin Sulfate Injection

Six batches of arbekacin sulfate injections were analyzed using the recommended HPLC system and sample preparation. The results of

TABLE 5 The Results of Assay and Related Substances Determination for Arbekacin Sulfate Injection (n = 2)

Batches	Content of Arbekacin (%)	Content of Dibekacin (%)	Content of Total Impurity Except Dibekacin (%)
ARI_7-6201 T	103.4	0.45	0.60
ARI_7-6202 T	102.3	0.53	0.68
ARI_7-6203 T	101.2	0.43	0.69
ARI-6201 T	101.3	0.48	0.65
ARI-6202 T	101.5	0.53	0.60
ARI-6203 T	101.9	0.50	0.69

determination of assay and related substances for arbekacin injections are shown in Table 5.

CONCLUSION

The described HPLC-ELSD method provides a rapid and simple analysis for arbekacin sulfate injections and its related substances without derivatization. The method is accurate and reproducible.

ACKNOWLEDGMENTS

This paper is supported by the Zhejiang province project of analysis and test (2009F70027).

REFERENCES

1. Society of Japanese Pharmacopoeia. The Japanese Pharmacopoeia fifteenth edition. 2006, 311–313.
2. Stead, D.A. Current methodologies for the analysis of aminoglycosides. J. Chromatogr. B **2000**, *74*, 69–93.
3. Edder, P.; Cominoli, A.; Corvi, C. Determination of streptomycin residue in food by solid phase extraction and liquid chromatography with post-column derivatization and fluorometric detection. J. Chromatogr. A **1999**, *830*, 345–351.
4. Vogel, R.; Defillipo, K.; Reif, V. Determination of isepamicin sulfate and related compounds by high performance liquid chromatography using evaporative light scattering detection. J. Pharm. Biomed. Anal. **2001**, *24*, 405–412.
5. Megoulas, N.C.; Koupparis, M.A. 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 **2004**, *1057*, 125–131.
6. Clarot, I.; Chaimbault, P.; Hasdenteufel, F.; Netter, P.; Nicolas, A. Determination of gentamicin sulfate and related substances by high-performance liquid chromatography with evaporative light scattering detection. J. Chromatogr. A **2004**, *1031*, 281–288.
7. Jian, W.; Xiaojun, H. Determination of spectinomycin hydrochloride and its related substances by HPLC-ELSD and HPLC-MS^n. J. Chromatogr. B **2006**, *834*, 178–182.