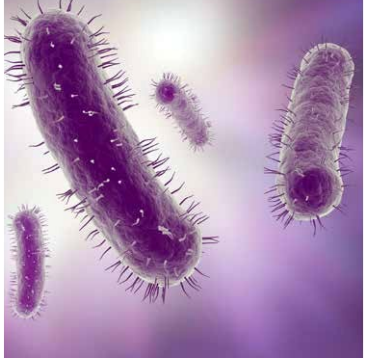
Application Note
Antibiotics



The most reliable LC-ECD applications for Antibiotics analysis

Aminoglycosides
Amikacin
Framycetin Sulphate
Gentamicin Sulphate
Kanamycin Sulphate
Lincomycin
Neomycin
Spectinomycin
Tobramycin
Streptomycin

Macrolide antibiotics
Azithromycin
Azaerythromycin
Clarithromycin
Erythromycin
Roxithromycin

Streptomycin Sulfate According to USP

- **Meets all requirements of U.S. Pharmacopeia 38-NF33, 2015**
- **Dedicated ALEXYS analyzer for Antibiotics**
- **Flow cell with Au working electrode and stainless steel AUX**
- **Reproducible and robust**

Summary

The Streptomycin sulphate analysis was evaluated using the exact method and conditions described in the official 2015 USP monograph [5]. In this application note, typical results obtained with an ALEXYS® LC-EC system are reported, demonstrating its performance for the assay of Streptomycin sulphate in commercial pharmaceutical preparations.

Introduction

Streptomycin (figure 1) was the first aminoglycoside antibiotic described in 1944 by Waksman *et al* [1] and is produced by microbial fermentation of the actinobacterium *Streptomyces griseus*. It was shown to inhibit the growth of aerobic gram-positive and gram-negative bacteria as well as the tubercle bacilli, and was in fact the first effective treatment for tuberculosis. Besides its common use for clinical treatment in humans it is also utilized as veterinary drugs and crop-protection agent. Like other aminoglycosides, streptomycin is potentially oto- and nephrotoxic. Streptomycin and its derivatives can be analyzed using ion-pair reversed phase liquid chromatography (RP-LC) in combination with UV detection at 195 nm or 205 nm [2,3], but this method has limitations. It requires high concentrations of the compounds to be detected by UV absorbance due to the absence of a good chromophore. Pulsed Amperometric Detection (PAD) is a better choice. Streptomycin and its impurities have a molecular structure which contain functional groups that can be oxidized and detected by PAD with superior selectivity and sensitivity [4]. High Pressure Anion Exchange followed by PAD-mode detection (HPAEC-PAD) is the method prescribed by the U.S. Pharmacopeia (USP) to assay streptomycin sulfate [5].

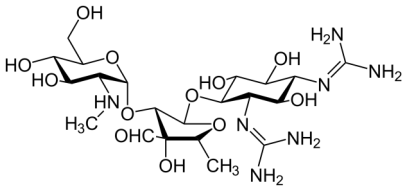


Figure 1. Structural formula of streptomycin

This application note presents typical results obtained with the ALEXYS® analyzer for Antibiotics, demonstrating its performance for the assay of streptomycin sulphate in commercial pharmaceutical preparations.

Method

The analysis was performed using an ALEXYS analyzer (figure 5). This system contains the P6.1L isocratic pump with integrated dual channel degasser and a solvent switch, enabling step-gradient separation. For detection, the system contains a DECADE Elite electrochemical detector. A summary of the LC-ECD conditions are given in table 1.

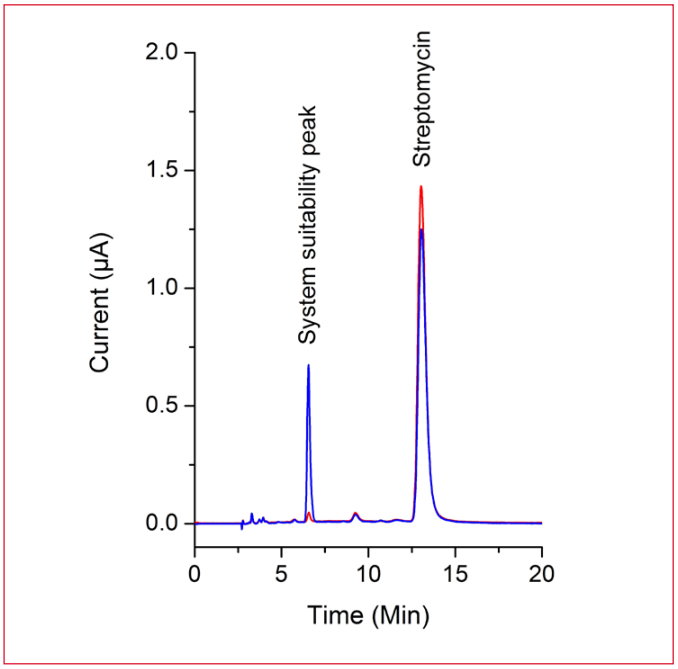


Figure 2. Chromatogram overlay of a system suitability solution (blue), and standard (red), both containing 30 µg/mL Streptomycin RS in water. Only the system suitability solution was heated for 1 hour at 75 °C. The main degradation peak is designated 'system suitability peak' in the chromatogram.

Table 1

Conditions	
HPLC*	ALEXYS Antibiotics base system - Isocratic (incl. the DECADE Elite electrochemical detector) + flow cell
Column	CarboPac™ PA1, 50 x 4 mm + 250 x 4 mm All columns: Thermo Scientific™ Dionex™
Mobile phase A	70 mM sodium hydroxide (separation)
Mobile phase B	200 sodium hydroxide (for column clean-up)
Flow rate	0.5 mL/min
Injection	20 µL
Temperature	30 °C
Flow cell**	SenCell™ with Au WE, salt bridge RE, AST 2
PAD-mode (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	about 0.3 µA
ADF	0.5 Hz
Range	2 µA/V

*) Note that the presented data are obtained with an older version of the ALEXYS LC system than shown in fig 5. **) Original work done with the 3 mm Au VT-03 sb, 50 µm spacer

Separation

The USP (38-NF33) method for streptomycin sulfate is based on isocratic separation using an anion exchange column and alkaline mobile phase (pH = 12.8) followed by PAD. One of the thermal degradation products of streptomycin (induced by heating the standard solution for 1 hour at 75 °C) is used to check the system suitability of the assay.

The monograph prescribes the use of an anion-exchange column for the separation of streptomycin with the following details: size 250 x 4 mm as polystyrene/divinylbenzene substrate agglomerated with quaternary amine functionalized latex beads, about 9 µm to 11 µm in diameter (USP column packing L46). The separation is based on isocratic elution followed by a step-gradient (table 2). A column clean-up/ regeneration step after isocratic elution is necessary to remove late eluting (thermal) degradation products present in the streptomycin products and standards. These degradation products may result from storage or chemical degradation during manufacture. Especially the heat-treated system suitability standard exhibits a large response from a late-eluting impurity. Without the step-gradient column clean-up step, this impurity elutes after 160 minutes.

Table 2

Step-gradient program

Time (min)	*Mobile phase*	*Description*
0 - 22	70 mM NaOH	Isocratic elution & detection
22 - 40	200 mM NaOH	Column clean-up/regeneration
40 - 85	70 mM NaOH	Equilibration at starting conditions

Mobile phase preparation

To minimize the introduction of carbonate ions in the mobile phase, the eluents were carefully prepared manually using a 50% w/w carbonate-free NaOH solution (commercially available). The diluent was DI water (resistivity >18 MΩcm) which was sonicated and sparged with Helium 5.0 prior to use. The mobile phase should be prepared in plastic bottles instead of glass. The mobile phases were blanketed with Helium 5.0 (0.5 bar overpressure) during the analysis, to prevent the introduction of CO_2 over time.

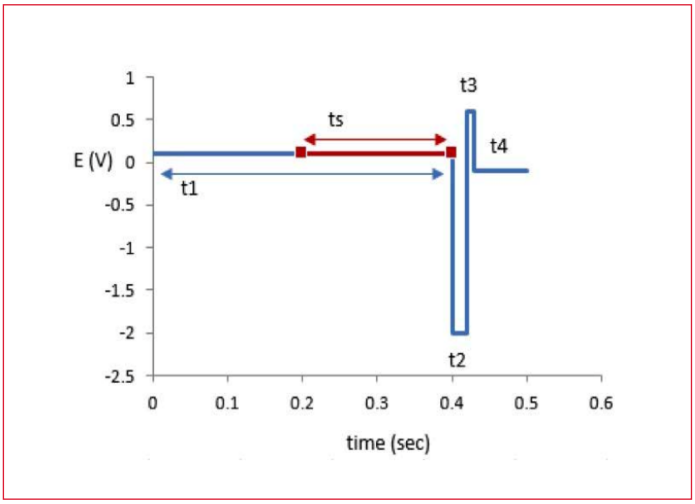


Figure 3: 4-step PAD potential waveform as applied for the detection of streptomycin according the USP monograph.

Detection

The USP method for assaying streptomycin prescribes the use of a flow cell with a gold (Au) working electrode (WE), Ag/AgCl reference electrode (RE) and stainless steel auxiliary electrode (AE). Both the SenCell as well as FlexCell (with stainless steel inlet block) from Antec Scientific match these requirements. A 4-step potential waveform is used (as prescribed in the USP monograph) to detect streptomycin and its impurities on the Au working electrode, see Table 1 and Figure 3. This particular 4-step waveform with a pulse duration of 500 ms has been claimed to have as benefits: (1) a consistent long-term peak area response and (2) minimal electrode wear [6].

Sample preparation

Standard preparation: 15 mg of USP Streptomycin Sulfate RS was accurately weighted and dissolved in 50 mL of water in a volumetric flask (sonicated for 1 minute and mixed).
The obtained solution was subsequently 10x diluted using a 100 mL volumetric flask to obtain a final concentration of 0.03 mg/mL.

System suitability solution: 10 mL of the above mentioned standard was heated to 75 °C for 1 hour and cooled down to ambient prior to use

.

Assay preparation: 30 mg of a commercial streptomycin sulfate sample (Sigma-Aldrich, pn S6501, batch SLBD3728V) was transferred to a 100 mL volumetric flask and diluted with water to volume (sonicated for 1 minute and mixed). 10 mL was subsequently transferred to a second 100 mL volumetric flask and diluted to volume with water, resulting in a final concentration of 0.03 mg/mL. Prior to use, the water contents of the commercial Streptomycin Sulfate sample was determined as specified in the monograph (loss on drying).

Loss on drying: An accurately weighted amount (around 100 mg) of Streptomycin sample was dried under vacuum (5 mm Hg) at 60 °C for 3 hours. The dried sample was re-weighted and the loss on drying calculated. This analysis was performed in duplicate and an average weight loss of 3.4% was calculated, which was within the USP criteria (< 5.0%). The weight loss reported by the manufacturer of this specific sample in the certificate of analysis was 3.1%.

Results

System Suitability

In Figure 2 an overlay is shown of the chromatograms obtained with the USP standard solution (red curve) and the system suitability solution (blue curve). The retention times for the system suitability peak and streptomycin were 6.55 and 13.08 minutes, respectively. It is evident from figure 2 that the response of the main degradation product at 6.55 min increased significantly (more than 18 fold increase in peak height) after the heat treatment.

The USP monograph for Streptomycin Sulfate specifies a set of tests to check system suitability. The chromatograms shown in Figure 2 were used to evaluated the system suitability, and the results are listed in table 3. It is evident that all system suitability requirements are met.

Linearity, repeatability & LOD

The linearity for Streptomycin was investigated in the concentration range of 5 µg/mL – 40 µg/mL. In this concentration range the correlation coefficient for peak area was better than 0.995. The relative standard deviation (RSD) of the retention time, peak area and height were determined for 6 replicate injections of the USP Streptomycin Sulfate RS standard solution. The RSD's were 0.1%, 0.5% and 0.5%, respectively for the Streptomycin peak. The Limit of Detection

Table 3

USP system suitability paramters

Parameter	*USP criterium*	*Measured*
Relative retention time (main degradation product)	0.5	0.5
Resolution (main degradation product - streptomycin)	>3.0	11.3
Column efficiency (streptomycin)	> 1 000	3 853
Tailing factor (streptomycin)	<2.0	1.5
RSD peak area, n=6 (streptomycin)	<5%	0.7%

(LOD) for streptomycin, calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 30 segments of 0.5 min), was about 0.1 µmol/L (70 ng/mL).

Sample analysis

As an example, a commercially available sample of streptomycin sulfate was analyzed (Sigma Aldrich, S6501 Streptomycin sulfate salt; batch SLBD3728V). The sample is abbreviated as 'sample SLBD3728V' from this point onwards.

The chromatogram obtained from sample SLBD3728V is shown in Figure 4. The potency (contents) of streptomycin in a sample is calculated as:

Potency (in µg/mg) = 1000 x (C x P / WU) x (rU / rS)

C = Concentration in mg/mL of the USP Streptomycin Sulfate RS in the standard preparation.

P = Designated streptomycin contents in µg per mg USP Streptomycin Sulfate RS.

Wu = Weight, in mg, of the streptomycin sample taken to prepare the assay preparation.

rU = Streptomycin peak area obtain from the chromatogram of the assay preparation.

rS = Streptomycin peak area obtain from the chromatogram of the standard preparation.

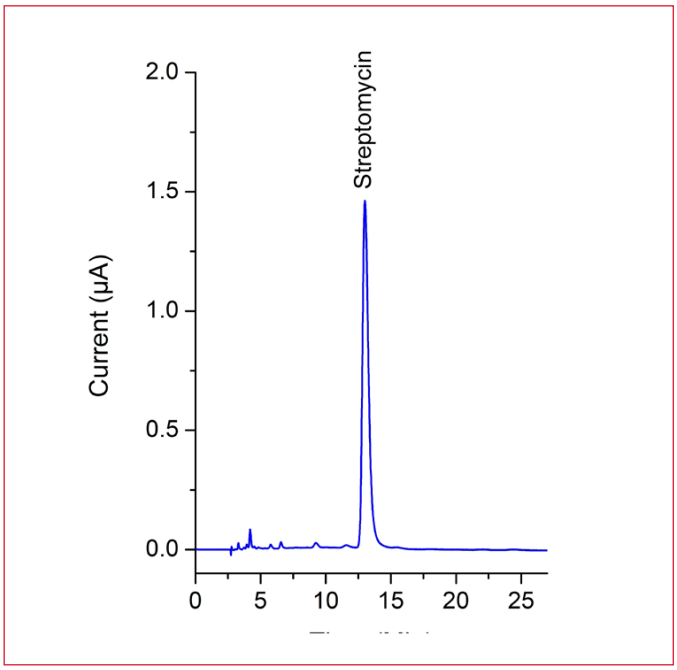


Figure 4: Analysis of Streptomycin sample SLBD3728V, 30 µg/mL in water

Table 4

Assay

Sample	*USP criteria*	*Measured*
SLBD3728V	650 - 850 µg/mg	717 µg/mg

The result of the sample potency assay is given in Table 4 together with the USP criteria. The contents of streptomycin in sample SLBD3728V was found to be within the specified limits of the USP monograph.

References

1. S.A. Waksman, Streptomycin: Background, Isolation, Properties and Utilization, Science, 118 (1953), 259 266
2. T.J. Whall, Determination of Streptomycin sulfate and Dihydrostreptomycin Sulfate by High-Performance Liquid Chromatography, J. Chromatogr., 219 (1981), 89-100
3. E. Adams, M. Rafiee, E. Roets, J. Hoogmartens, Liquid Chromatographic Analysis of Streptomycin Sulfate, J. Pharm. Biomed. Anal., 24 (2000), 219–226
4. W.R. LaCourse, Pulsed Electrochemical Detection in High Performance Liquid Chromatography, John Wiley & Sons, New York, 1ed, 1997
5. Streptomycin Sulfate, The United States Pharmacopeia 38th ed., National Formulary 33th, United States Pharmacopeial Convention, Rockville MD, (2015), 5360
6. R.D. Rocklin, A.P. Clarke, M. Weitzhandler, Improved longterm reproducibility for pulsed amperometric detection of carbohydrates via a new quadruple-potential waveform, Anal. Chem, 70, (1998), 1496 1501

Conclusion

The dedicated ALEXYS analyzer (Antibiotics base system -isocratic + flow cell) offers a tailored solution to assay streptomycin sulfate using the official method of the USP.

Figure 5: The ALEXYS analyzer for Streptomycin, consisting of the ALEXYS Antibiotics base system - Isocratic, and dedicated flow cell and bottles. The base system consists of a P6.1L pump with integrated Solvent Switch Valve (SSV) capable of running step gradients, an AS6.1L autosampler, an ET 210 Eluent tray for helium blanketing, and the DECADE Elite electrochemical detector. The system is delivered with DataApex™ Clarity™ Chromatography Data System (CDS) software.

Ordering information

Detector only	
176.0035B	DECADE Elite SCC electrochemical detector
116.4121	SenCell 2 mm Au sb
Recommended ALEXYS configuration	
180.0058W	ALEXYS Antibiotics base system - Isocratic
116.4121	SenCell 2 mm Au sb
184.0205	PPCO bottle assembly, 2L, Helium 2x

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333