

Antibiotic Analysis by HPLC-ECD According USP/EP Monographs

Analyzing aminoglycoside and macrolide antibiotics in bulk products and pharmaceutical formulations.

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With the increasing demand in product quality and safety assurance for antibiotics, sensitive analytical methods are required. HPLC with electrochemical detection (HPLC-ECD) is the best analytical platform that fulfils all the criteria including sensitive impurity profiling, and the detection of by-products at low cost of operation and ownership (unlike LC/MS).

To guarantee ease of use and reliable results, Antec Scientific has developed a dedicated Antibiotics Analyzer that can be used for any type of aminoglycoside or macrolide antibiotics analysis.

ECD-PAD: the ideal detection

Both categories of antibiotics (aminoglycosides and macrolides), as well as most relevant impurities and by-products, contain one or more carbohydrate moieties on each molecule. This makes the use of pulsed amperometric detection (PAD) the ideal analytical technique, not only for the analysis of the antibiotic itself but in particular for the sensitive analysis of impurities and by-products. The preceding separation can be based either on reversed phase HPLC with post column addition of sodium hydroxide or the use of high performance anion exchange chromatography (HPAEC).

Two types of electrochemical flow cells are available: The FlexCell with easy



Figure 2. ALEXYS Antibiotics Analyzer for aminoglycosides and macrolides antibiotics analysis, based on RP-HPLC or HPAEC with PAD (3 or 4 steps) detection. Inserts: (A) FlexCell (routine use) and (B) SenCell (in case of EP/USP requirement). Sensitivity FlexCell 100 nmol/L, SenCell 10 nmol/L.

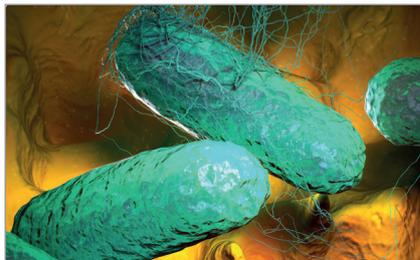


Figure 1. Salmonella typhi, a Gram-negative bacterium causing typhoid fever, which is treated with the macrolide antibiotic azithromycin.

exchangeable gold (Au) working electrode for routine use and the SenCell with integrated Au electrode and a stainless-steel auxiliary electrode, which is a requirement in some EP/USP monographs.

Example: netilmicin sulfate analysis according EP 8.1

Netilmicin is a semi-synthetic aminoglycoside antibiotic synthesized by alkylation of sisomicin (1-N-ethyl derivative). It is an effective antibiotic used against a wide range of gram-positive and gram-negative bacteria. Netilmicin is available as injectable and ophthalmic pharmaceutical preparations.

During the synthesis of netilmicin, by-products are formed at low concentrations such as 2'-N-ethyl and 6'-N-ethyl derivatives of sisomicin (alkylation products) and 1-N-ethylgaramine (hydrolysis product). UV detection is not suitable for the detection of low levels of netilmicin and its impurities because they have a weak UV chromophore. However, pulsed amperometric detection (PAD) can be successfully utilized as described in the European Pharmacopoeia 8.1 (2014). A chromatogram recorded under the required conditions is shown in Figure 3. The separation of netilmicin

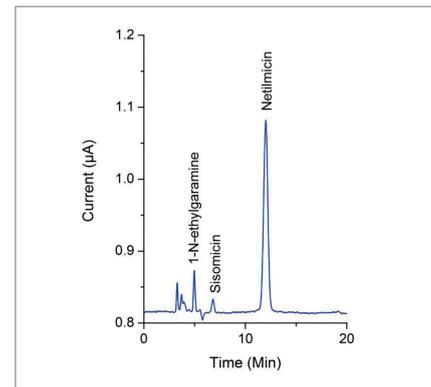


Figure 3. 20 µL injection of a standard consisting of 10 µg/mL netilmicin sulfate CRS (CRS - Chemical Reference Substances), 10 µg/ml sisomicin sulfate CRS and 8.2 µg/ml 1-N-ethylgaramine sulfate CRS in mobile phase. ALEXYS Antibiotics Analyzer.

and its major impurities, i.e., sisomicin and 1-N-ethylgaramine, are based on RP-HPLC followed by post column addition of NaOH solution upfront PAD detection, and it meets all the system suitability criteria of the EP method.

Conclusions

With the ALEXYS Antibiotics Analyzer the EP criteria for composition and impurity analysis in commercial netilmicin formulations are easily fulfilled. Comprehensive, scientific product applications are available for aminoglycosides such as: amikacin, framycetin, gentamicin, kanamycin, lincomycin, neomycin, spectinomycin, streptomycin, tobramycin to name a few. In addition, macrolide antibiotics such as azithromycin, clarithromycin, erythromycin, and roxithromycin can also be analyzed by the ALEXYS Antibiotics Analyzer.

