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Development and validation of liquid chromatography tandem mass spectrometry methods for the determination of gentamicin, lincomycin, and spectinomycin in the presence of their impurities in pharmaceutical formulations

K. Vučićević-Prčetić[a,*], R. Cservenák[b], N. Radulović[c,*]

[a] DSP Chromatography, Bul. Zorana Đinđića 166, Belgrade, Serbia
[b] FM Pharm, Šantićeva 92, Subotica, Serbia
[c] Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia

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ABSTRACT

Liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods for the determination of gentamicin, lincomycin and spectinomycin in the presence of their impurities were developed and tested. Chromatographic separations were achieved using gradient elution on a C18 column. All components were ionized by positive-ion electrospray and detected by multi reaction monitoring (MRM) with an LC-tandem mass spectrometer. Calibration curves were linear with correlation coefficients better than 0.99. The developed method for the determination of gentamicin provides complete base line separation of components C1, C1a, C2, C2a and C2b mentioned in the European and British Pharmacopoeias. The second developed method makes possible a simultaneous analysis of the active compounds of both lincomycin and spectinomycin. Additionally, all impurities defined in the pharmacopoeias for all three active components were determined and their identities confirmed. The methods were tested in routine quality control analysis.

1. Introduction

Aminoglycosides represent broad spectrum antibiotics which exert bactericidal activity against some Gram-positive as well as Gram-negative bacteria. Gentamicin, spectinomycin and lincomycin are among the most commonly used aminoglycosides effective in both human and veterinary applications [1,2]. Pharmaceutical formulations containing these compounds as active substances are repeatedly tested for composition as well as for the presence of impurities (Figs. 1–3). Pharmacopoeias are defining a number of HPLC, GC and microbiological methods for their determination. Gentamicin is an aminoglycoside complex mainly consisting of gentamicin C1, C1a, C2, C2a and the minor component C2b. Routinely, in the pharmaceutical industry, due to the multi component nature of gentamicin, only the relative percentage of its major constituents is measured. For the analysis of gentamicin and spectinomycin composition, Ph. Eur. defines a reversed phase LC method with electrochemical detection after postcolumn derivatization. According to the same source, lincomycin is tested for the presence of lincomycin B as the main impurity. Up to now, microbiological assays [British and European Pharmacopoeias], immunoassays, and ELISA methods neither provided the means of quantifying individual components of gentamicin, nor of impurities present in the pharmaceutical dosage forms.

Analysis of aminoglycosides is challenging due to a lack of any significant chromophore or fluorophore in these molecules. Numerous analytical methods have been used to quantify aminoglycosides, such as TLC [3], LC with spectroscopic and fluorescence detection [4–8], electrochemical detection [9–13], with evaporative light scattering detection [14–16] and also capillary electrophoresis (CE) [17,18]. Earlier LC methods and CE methods necessitated a precolumn or postcolumn derivatization (e.g. *o*-phthalaldehyde (OPA)/mercaptoacetic acid (MAA) or dansyl chloride) step to enable either UV or fluorescence detection. Although these modes of detection are quite sensitive, the obligatory derivatization step is a time-consuming process and needs well-controlled experimental conditions to produce repeatable results. If we take all this into account, mass spectrometry seems to be the technique of choice for aminoglycosides' detection in respect to very high sensitivity and positive identification, and with no derivatization steps required [18–31]. In this study, to meet this demand, reliable LC/MS/MS methods for the determination of active compounds of the antibiotics (gentamicin, lincomycin and spectinomycin) and the inherent impurities were developed and

* Corresponding authors.
E-mail addresses: katarina.vucicevic@gmail.com (K. Vučićević-Prčetić), nikoradulovic@yahoo.com (N. Radulović).

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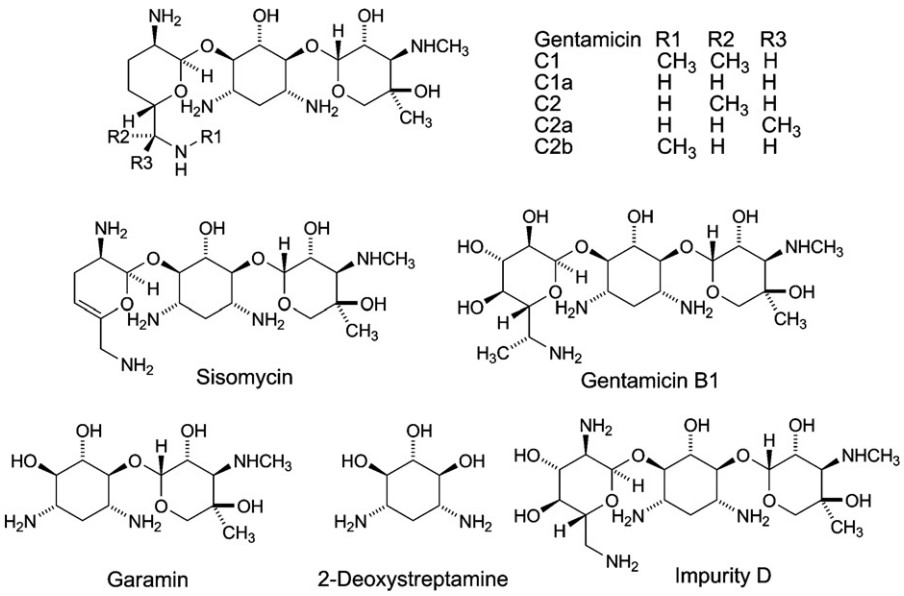

Fig. 1. Structures of gentamicin active compounds and its impurities.

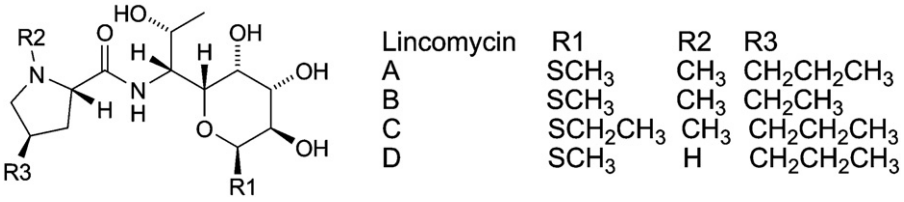

Fig. 2. Structures of lincomycin and its impurities.

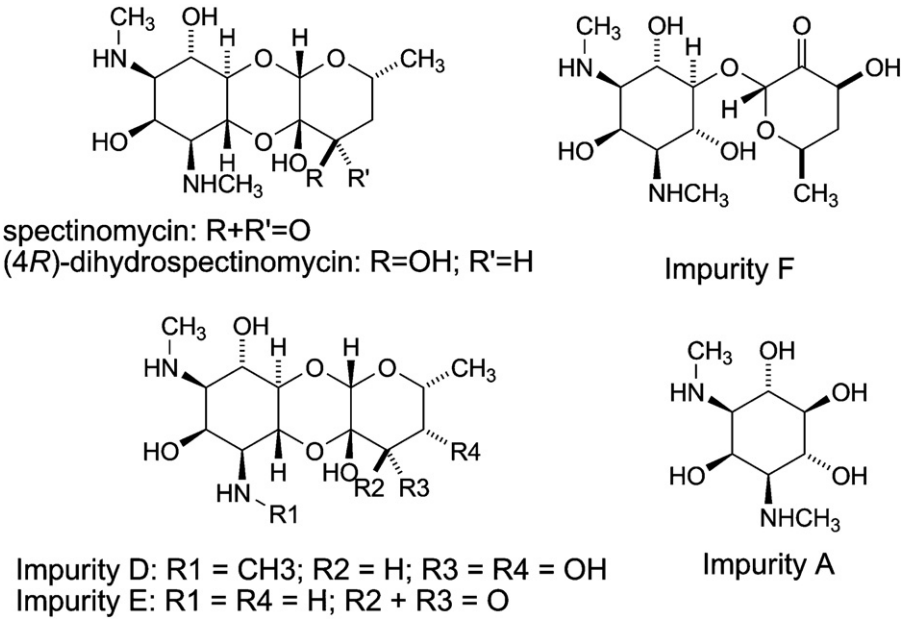

Fig. 3. Structures of spectinomycin and its impurities.

tested. The developed (two) methods were validated for assay of the active compounds and determination of their impurities with quantification expressed as percentage of the active compound. The methods were tested through the analysis of commercially available pharmaceutical dosage forms that contained either one or two antibiotics.

2. Experimental

2.1. Instrumentation

LC was performed using an Agilent Technologies HPLC system 1200 series (Waldbronn, Germany) equipped with a quaternary