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Development of high performance liquid chromatography methods with charged aerosol detection for the determination of lincomycin, spectinomycin and its impurities in pharmaceutical products

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ABSTRACT

Novel and simple liquid chromatography methods with charged aerosol detection (LC–CAD) for simultaneous quantitation of lincomycin and spectinomycin and its related substances have been developed and tested. This type of analysis is complicated due to the different chromatographic behavior of these two agents and the lack of chromophores in spectinomycin complex. CAD seems to be a promising alternative to overcome these difficulties. It shows a consistent inter-analyte response, independent of chemical structure of an analyte. It also enables the direct quantification of related substances for which no reference standards were available, with good accuracy and precision. Chromatographic separations were achieved using a C18 Hypersil® Gold column, with mobile phases consisting of water, acetonitrile and trifluoroacetic acid. All impurities were identified using time-of-flight mass spectrometry with electrospray ionization. The developed methods have been successfully used in the routine quality control analysis of pharmaceutical preparations.

1. Introduction

Spectinomycin produced by *Streptomyces spectabilis* belongs to aminoglycoside antibiotics and lincomycin produced by *Streptomyces lincolnensis* is a lincosamide antibiotic. Pharmaceuticals being a combination of these two antibiotics (in a 2:1 ratio) are widely used in veterinary medicine for the treatment of bacterial gastrointestinal and respiratory infections [1,2]. In human medicine, spectinomycin is used principally against *Neisseria gonorrhoeae* [3]. Due to their natural synthesis, both of them remain a mixture of similar compounds. Spectinomycin is the principal component of its complex. In solution, it undergoes a ring opening and closure of the hemiketal function. This results in an equilibrium mixture of four possible anomers, which may, or may not, co-elute in a chromatographic analysis. The chemical structures of both mentioned antibiotic and their related substances are presented in Figs. 1 and 2.

Few chromatographic methods combined with electrochemical detection (ED) [4] and mass spectrometry (MS) [5,6] have been reported in literature for the determination of lincomycin. The European Pharmacopoeia (Ph. Eur.) defines a HPLC-UV method for lincomycin assay and related substances test [7]. This method is rather simple to apply, it uses a very popular UV detector and a C8 column and produces repeatable results, so it may be used in a routine analysis successfully.

For spectinomycin, the current Ph. Eur. monographs define a reversed phase HPLC–ED method after post-column derivatization [8,9], whereas the United States Pharmacopoeia (US pharmacopoeia) prescribes gas chromatography with flame ionization detection (GC–FID) [10,11] for the assay determination, without defining the related substances test. Pharmacopoeial methods are hardly reproducible, so other methods have been developed. Some are performed using pre-column or post-column derivatization procedures [12–14], which are typically cumbersome, difficult to reproduce and may result in introduction of non-controlled impurities. Therefore, non-derivatizing HPLC methods with ED [15–17], MS [18,19] and evaporative light scattering detection (ELSD) [20–22] have been developed.

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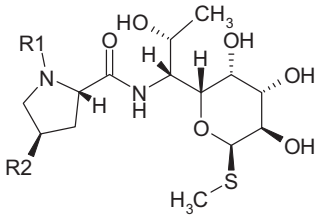
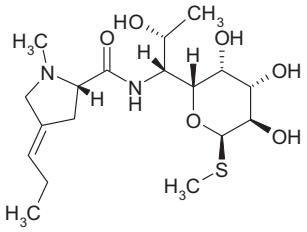
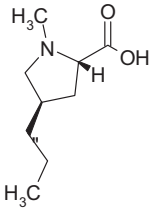


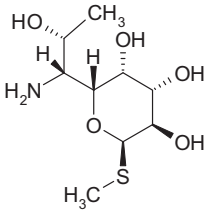
Compound	R1	R2
Lincomycin	CH_3	C_3H_7
Lincomycin B	CH_3	C_2H_5
Impurity A (α-amide epimer)	CH_3	C_3H_7
Impurity C	H	C_3H_7
Impurity D (7-*epi*-lincomycin)	CH_3	C_3H_7



Impurity B
(propylidene analogues)



Impurity E
(4-propyl hygric acid)

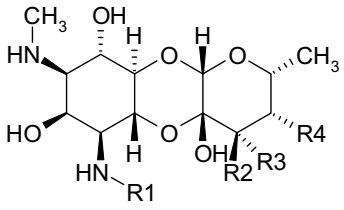


Impurity F
(methyl-1-thiolincosaminide)

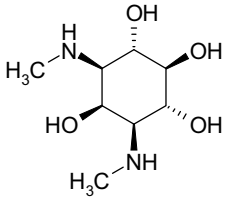
Fig. 1. Chemical structures of lincomycin and its related substances.

As far as we are aware, there are only two chromatographic methods with pulsed amperometric detector (PAD) [23] or MS [24] describing the simultaneous determination of both compounds in pharmaceuticals in one run. This type of analysis is complicated due to the difference in the chromatographic behavior of these two agents.

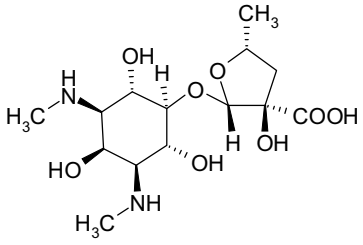
Corona Charged Aerosol Detection (CAD) seems to be a promising alternative to overcome the difficulties mentioned above. It shows consistent inter-analyte response, independent of chemical structures of an analyte, under identical mobile phase and CAD conditions. Its features have been described previously [25] and an extensive overview of CAD's applications has been reported by



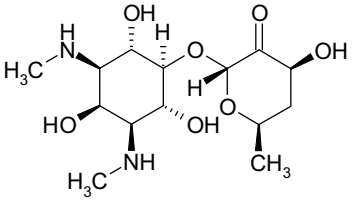
Compound	R1	R2	R3	R4
Spectinomycin	CH_3	R2+R3=O		H
(4*R*-dihydrospectinomycin)	CH_3	OH	H	H
Impurity C (4*S*-dihydrospectinomycin)	CH_3	H	OH	H
Impurity D (dihydroxyspectinomycin)	CH_3	H	OH	OH
Impurity E (*N*-desmethylspectinomycin)	H	R2+R3=O		H
Impurity G (tetrahydroxyspectinomycin)	CH_3	R2+R3=O		OH



Impurity A
(Actinamine)



Impurity B
(Actinospectinoic acid)



Impurity F
(Triol spectinomycin)

Fig. 2. Chemical structures of spectinomycin and its related substances.