Journal of Pharmaceutical and Biomedical Analysis 112 (2015) 155–168

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Exploring the possibilities of capacitively coupled contactless conductivity detection in combination with liquid chromatography for the analysis of polar compounds using aminoglycosides as test case☆

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ARTICLE INFO

Article history:
Received 24 September 2014
Received in revised form 1 December 2014
Accepted 5 December 2014
Available online 17 December 2014

Keywords:
C^4D
LC
Aminoglycosides
Ion-pairing reagents
Explorative study

ABSTRACT

The analysis of highly polar (often charged) compounds which lack a strong UV absorbing chromophore is really challenging. Despite the numerous analytical methods published, the demand for a simple, robust and cheap technique for their analysis still persists. Here, reversed phase (RP) liquid chromatography (LC) with capacitively coupled contactless conductivity detection (C^4D) was explored for the first time as a possible method for separation and detection of various aminoglycoside (AMG) antibiotics which were taken as typical test compounds: tobramycin (TOB), spectinomycin, streptomycin, amikacin, kanamycin A and kanamycin B. C^4D was performed using a commercially available as well as a laboratory made cell. As ion-pairing reagents (IPR) four perfluorinated carboxylic acids were used: pentafluoropropionic acid, heptafluorobutyric acid, nonafluoropentanoic acid (NFPA) and pentadecafluorooctanoic acid (PDFOA). 0.125 mM NFPA-acetonitrile (ACN) (90:10) or 0.125 mM PDFOA-ACN (70:30) as mobile phases were suitable to detect TOB with reasonable retention times. However, NFPA was preferred for practical reasons. Its applicable concentration range in the mobile phase was strongly restricted by loss of chromatographic performance at lower levels and excessive background conductivity at higher levels. Overall repeatability and robustness of the method were rather poor which was explained by the relatively low IPR levels. Selectivity between the tested AMGs was mainly influenced by the number of protonated amino groups per molecule making it impossible to separate compounds of equal net charges. Problems encountered with gradient elution, hydrophilic interaction liquid chromatography (HILIC) and separation at high pH without IPRs are also discussed.

1. Introduction

The separation and detection of compounds with a highly polar character lacking a UV absorbing chromophore in their structure, has always been an obstacle as the most commonly used analytical technique, reversed phase (RP) liquid chromatography (LC) with ultraviolet detection (UV), cannot be applied as such. Taking aminoglycosides (AMGs) as example (Fig. 1), separation techniques like paper chromatography [1], thin layer chromatography [2], ion-exchange chromatography [3], gas–liquid chromatography after trimethylsilylation [4], ion-pair (IP) LC [5,6] and capillary electrophoresis (CE) [7] have been reported. Among these, IP-LC has been used extensively as the use of ion-pairing reagents (IPR) helped to retain otherwise highly polar, charged AMG molecules on the RP stationary phase. To allow their detection by UV or fluorescence detection methods, colorimetric reaction with ninhydrin [8], pre-column or post-column derivatization with reagents having a UV chromophore or a fluorophore have been described [9–12]. However, these methods are rather cumbersome. Derivatization procedures are not preferred due to the constraints of being tedious and time consuming, giving problems with quantitation because of variability in reaction completeness and possible toxicity of some derivatizing agents.

Direct detection techniques are always preferable for the sake of simplicity and correct quantitation. Among the direct detection techniques, pulsed electrochemical detection (PED) has been used

☆ Selected papers presented at 10th International Symposium on Drug Analysis, Liege, Belgium. June 22-25, 2014.

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http://dx.doi.org/10.1016/j.jpba.2014.12.015
0731-7085/

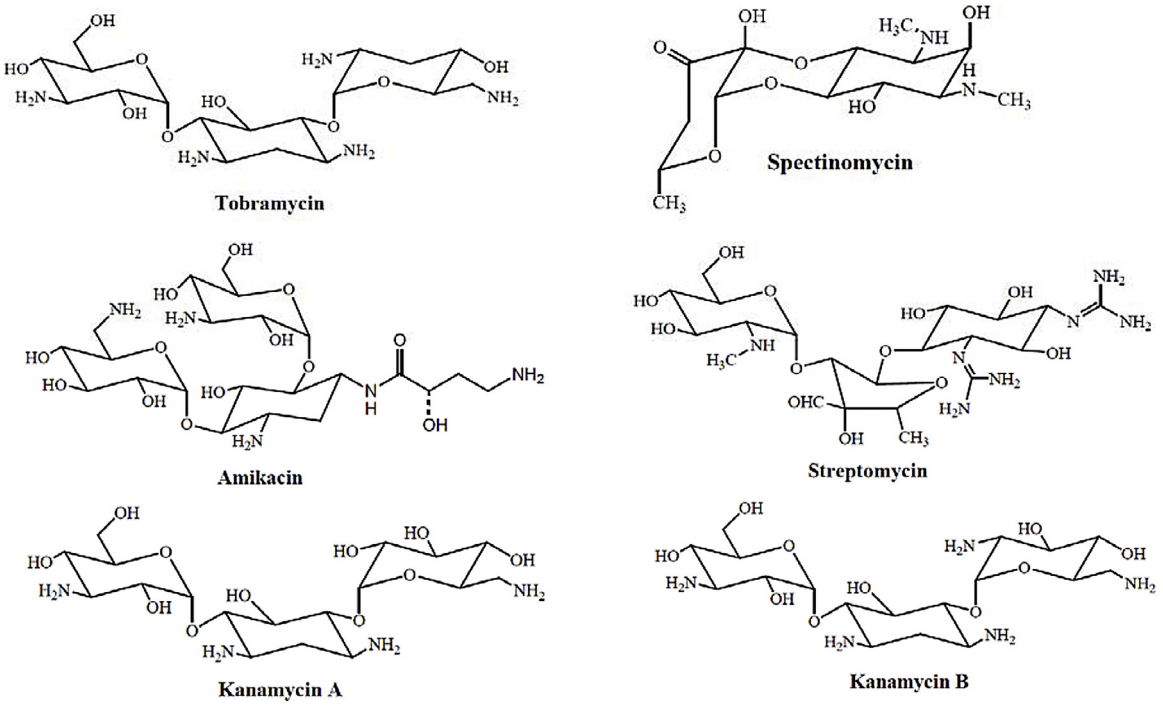

Fig. 1. Structure of some aminoglycosides.

extensively for AMG analysis. Over the years, PED has gained popularity and a large number of methods using LC-PED for the analysis of AMGs in bulk drugs, pharmaceutical dosage forms and biological fluids have been reported in literature [5,6]. Monographs of several AMGs in the European Pharmacopeia (Ph. Eur.) prescribe LC-PED for determination of related substances and assay [13].

Nonetheless, PED has been unable to acquire unopposed acceptance as direct detection technique for analytes that lack a UV absorbing chromophore. This is due to various reasons: (1) To be detected by PED the analytes should have an oxidizable group. (2) During detection, the analytes are oxidized and the reaction products adsorb on the surface of the working electrode which causes fouling of the electrode. Though a cleaning step to remove the adsorbed analytes is incorporated in different PED waveforms, it is not always sufficient and after some time mechanical cleaning is required. After cleaning the electrodes, a relatively long equilibration time is needed. (3) Some experience of the analyst in working with the apparatus and maintaining it is required to obtain repeatable quantitative results with PED. These aspects of PED have drawn severe criticism which has encouraged the evaluation of other detectors to detect molecules without chromophores as potential replacements of PED.

A group of such alternative detectors comprises aerosol based detectors, such as the evaporative light scattering detector (ELSD) [14] and charged aerosol detector (CAD) [15]. However, they require volatile mobile phase constituents, thus limiting the choice of reagents and often also the selectivity of the LC method. Furthermore, they are known to give a non-linear area response to the analyte quantity and a log-log transformation of these data is required to obtain a linear relationship. Also, ELSD was found to have a lower sensitivity for tobramycin (TOB) than PED [16]. Due to the above mentioned disadvantages, aerosol based detectors were not considered as plausible alternatives of PED.

LC combined with mass spectrometry (MS) has also been used for the analysis of AMGs. MS is a highly sensitive and informative technique with identification capabilities, but it is rather expensive to be used for routine analysis [17–21]. Refractive index detection [15] has also been used, but poor sensitivity and reproducibility, incompatibility with gradient elution and sensitivity to changes in the ambient temperature have limited its use as detection technique.

It is clear from the above discussion that an alternative detection technique for these compounds is highly desirable. Capacitively coupled contactless conductivity detection (C^4D) is another technique that can be used for the analysis of molecules without a chromophore. The detector was developed and first introduced by Zemann et al. and Fracassi da Silva and do Lago [22,23]. A number of detailed studies describing the fundamental principles of C^4D are available in literature [24–29]. The advantages of the detector are its relatively simple structure, low price, lack of direct contact between liquid and electrode surface and the possibility of direct coupling with other detectors due to its non-destructive nature. The majority of C^4D applications can be found in the coupling with CE [27,30,31]. However, some articles combine C^4D with LC using conventional as well as capillary columns [32–35]. In general, the LC-C^4D methods use strongly diluted mobile phases of simple composition, since one of the prerequisites of detectability in conductivity detection is a sufficiently low background conductivity.

The suitability of this detector for the analysis of AMGs in combination with CE has been demonstrated by Law et al. [36] and El-Attug et al. [37–39]. Even though there have been numerous publications on assay and impurity profiling using CE, LC still persists as the leading separation technique, presumably because of its better repeatability, robustness and widespread use. Therefore, it was decided to explore the suitability of LC-C^4D. In this paper, detection of AMG antibiotics by C^4D coupled to LC is described for the first time. Strong limitations of the method are demonstrated and pitfalls encountered during method development are discussed.