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Description and validation of coupling high performance liquid chromatography with resonance Rayleigh scattering in aminoglycosides determination

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ABSTRACT

In view of the fact that many substances generally exhibit very little ultraviolet absorbance and the absence of native fluorescence, a new strategy with simple instrumentation and excellent analytical performance combining high performance liquid chromatography (HPLC) with resonance Rayleigh scattering (RRS) was developed. It was validated for the quantification of aminoglycosides (AGs). This fact was also carefully calculated by quantum chemistry. However, the sensitivity was probably limited by the volume of flow-through cell. Therefore, the result calls for a suitable one to ensure optimal RRS signal. Interestingly, when serum or urine samples of analytes were analyzed by this method, they were all well resolved without any interference, which would hold a new perspective to be applied in the determination of substances in biological matrix.

1. Introduction

Since the early 1990s, RRS as one type of Rayleigh scattering (RS) -based techniques has received much attention [1,2]. It is a special elastic scattering produced when the wavelength of resonance light scattering is located in or close to the molecular absorbed band [3,4]. The meaning of RRS was well apprehended by Lu et al. [5]. Although the assay method of RRS is similar with localized surface plasmon resonance (LSPR), RRS differs from LSPR which is one of the signature optical properties of noble metal nanoparticles. LSPR arises when the incident photon frequency is resonant with the collective oscillation of the conduction electrons in the metal nanoparticles [6–8]. Due to its high sensitivity, simplicity and low cost, RRS has been successfully coupled with other analytical methods to implement simultaneously separation [9–13]. Nevertheless, few studies have focused on the improvement of instrumentation about HPLC–RRS association. Throwing off the immovable RRS technique, a novel on-line chromatographic strategy with resonance light scattering detection was proposed.

Aminoglycosides (AGs) are a group of highly potent and wide spectrum antibiotics that are used to treat certain bacterial infections [14]. These antibiotics participate in a large variety of biologically relevant molecular recognition processes involving both RNA and protein receptors [15,16]. Based on the affinity of these synthetic aminoglycosides for RNA, it is reasonable to believe that AGs can potentially act as regulators of gene expression [17]. They are also shown to stabilize the hybrid DNA·RNA duplex [18,19]. Unfortunately, a high plasma level of some AGs may cause serious adverse ototoxicity and nephrotoxicity. Thus, monitoring of AGs levels frequently and carefully in both pharmaceuticals and biological fluids is in great demand.

Various microbiological [20,21], electrochemical [22–25], spectrophotometric [26,27] and chromatographic methods [28,29] for the determination of AGs in different matrices have been reviewed. Among these methods, chromatographic methods appear to be the prevailing technique because of their simple operation. Nevertheless, in view of the fact that AGs generally exhibit very little ultraviolet (UV) absorbance or fluorescence absence, derivatization techniques are usually required in chromatographic determinations [30,31]. However, these methods sometimes take considerable time and derivatives are lower stability. Numerous efforts have been spent or being investigated to seek non-derivatization methods for the analysis of AGs, such as mass spectrometric detection [32–34], pulsed electrochemical detection [35,36] and evaporative light scattering detection [37]. However, many major or minor detectable ingredients in pharmaceutical intermediate and final products give rise to a lot of serious interferences to the detection of AGs. Interestingly, we found a novel strategy without interference in analytes determination.

Herein, a novel HPLC–RRS system (Fig. 1) was shown here and validated in the determination of AGs. Both theoretical and experimental analysis had been proved that AGs reacted with Congo red (CR) to form binary compounds simultaneously which lends

Abbreviations: RRS, resonance Rayleigh scattering; HPLC–RRS, the method coupling HPLC with RRS; AGs, aminoglycosides; CR, Congo red; AMK, amikacin; NTL, netilmicin; ETM, etimicin; RSD, the relative standard deviations.

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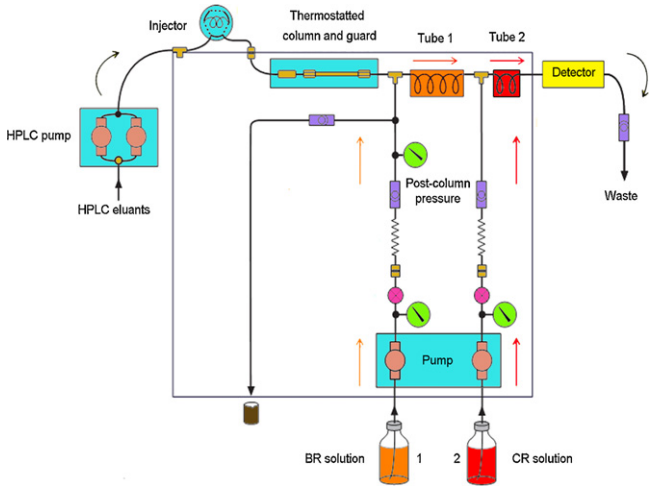

Fig. 1. Schematic diagram of HPLC–RRS system.

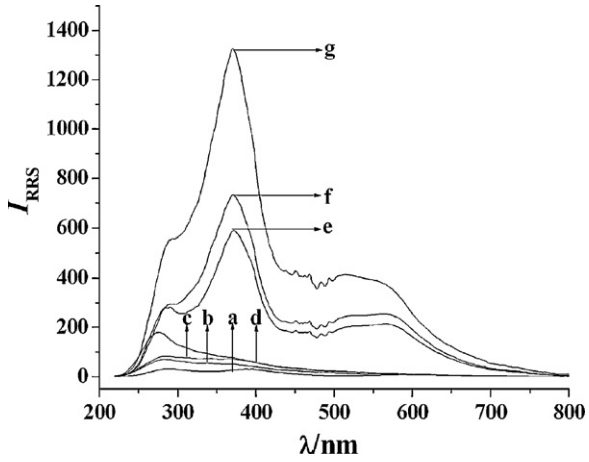

Fig. 2. The RRS spectra, (a) CR, (b) AMK, (c) ETM, (d) NTL, (e) NTL and CR, (f) ETM and CR and (g) AMK and CR.

further support for future investigation. To the best of our knowledge, this is the first report that HPLC–RRS was employed to determine three AGs and confirmed by distinct mediums. The new strategy exhibited simple instrumentation and excellent analytical performance. Meanwhile, it would hold a new perspective to be applied in substances lack of useful spectroscopic and electrochemical properties.

2. Experimental

2.1. Apparatus

An Agilent 1100 liquid chromatography (Agilent Technologies, CA, USA) consisted of G1322A online degasser, G1311A pump, G1316A column oven, G1321A fluorescence detector. A PCX5200 post-column derivatization instrument (Pickering Laboratories, Inc., USA), A Hitachi F-2500 spectrofluorophotometer (Hitachi Ltd., Tokyo, Japan) and a multimode TMSPM atomic force microscope (Veeco Instruments Inc., USA), the AFM images were analyzed using the Nanoscope Quadrex software. The surfaces of ion-association complexes were scanned by the Tapping Mode probes (Veeco model TESP7). Double distilled water was prepared by a Millipore SZ-93 system (Shanghai Yarong Biochemical Apparatus Co.).

2.2. Chemicals and reagents

Amikacin (AMK), netilmicin (NTL) and etimicin (ETM) were purchased from National Institute for the Control Pharmaceutical and Biological Products (Beijing, China). Congo red (CR, Shanghai Chemical Reagent Stocking and Providing Station). Britton–Robinson buffer solution with different pHs was prepared by mixing the mixed acid (composed of 0.04 mol L^{-1} H_3PO_4, HAC and H_3BO_3 with 0.2 mol L^{-1} NaOH) in proportion. Double distilled water was used throughout. All reagents were filtered through a 0.2 μm pore size filter membrane (Millipore, Bedford, MA, USA).

2.3. Preparation of standards

AMK, NTL and ETM were weighted and dissolved in water to prepare a stock solution of 1.0 mg mL^{-1} separately. All the solutions were stored at 0–4 °C in darkness. Standard solutions with three AGs of different concentrations were prepared by serial dilutions of the stock solutions. Acetonitrile of twice volume was added to 200 μL human serum to precipitate protein. The solution was vortex-mixed for 1 min and centrifuged for 10 min at 14,000 r min^{-1}. Clear supernatant was transferred to a clear 1.5 mL centrifuge tube. The organic phase was separated and evaporated to dryness at 40 °C in a vacuum drying oven, then reconstituted with 100 μL mobile phase. The treatment to urine was the same.

2.4. HPLC–RRS parameters

Chromatographic separation of the parent drugs was achieved on reverse phase analytical column of Synergi Hydro-RP (150 mm × 4.6 mm, 4 μm, Phenomenex, CA, USA). The flow rate was 0.4 mL min^{-1} of 0.1% trifluoroacetic acid (pH 2.2) and the temperature of the column oven was 30 °C. The injection volume was 20 μL for the determination. The RRS detection was monitored at $\lambda_{ex} = \lambda_{em} = 370$ nm. The total analysis time was not more than 25 min.

3. Results and discussion

3.1. Selection of the chromatographic parameters

The RRS spectra of the AGs and Congo red were analyzed in the buffer solution separately. After that, 40 μL AGs (10 μg mL^{-1}), 1 mL buffer solution and 1 mL CR were successively added into a 10.0 mL calibrated flask. The mixture was then diluted with water to 10.0 mL and mixed thoroughly. The RRS spectra were recorded by scanning from 200 to 700 nm on the Hitachi F-2500 spectrofluorophotometer. The RRS spectra of our target compounds were reported in Fig. 2.

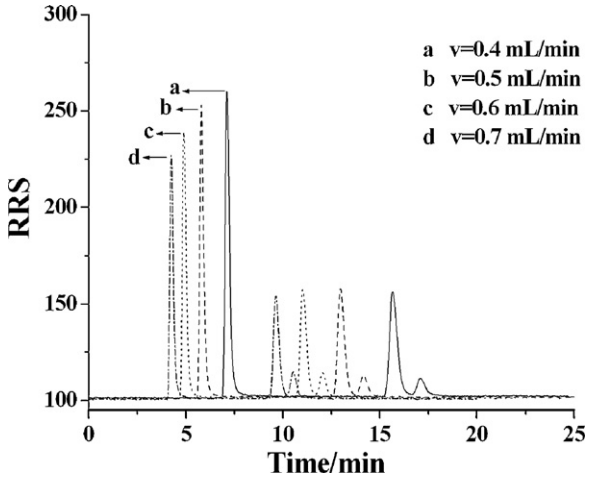

Fig. 3. Effect of flow rates of mobile phase in HPLC–RRS.