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Short communication

Hyphenated ultra high-performance liquid chromatography–Nano Quantity Analyte Detector technique for determination of compounds with low UV absorption

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ABSTRACT

A novel universal aerosol-based detector Nano Quantity Analyte Detector – NQAD™, connected with an ultra-performance liquid chromatography system is described. The detector was employed for detection of selected antibiotic compounds – macrolides (oleandomycin, erythromycin, troleandomycin, clarithromycin and roxithromycin) that are hard to detect using classical UV detectors due to the lack of chromophores. The determined lowest detection limits under isocratic conditions for these compounds ranged from 3.0 to 5.4 μg/mL. The suitability of the detector connected with ultra high-performance liquid chromatography in the gradient mode was tested on a more complex mixture containing 12 antibiotics. The detector exhibited full compatibility under both the elution modes when UHPLC separations were achieved in relatively short run times.

1. Introduction

UV detection is often considered to be one of the most widespread detection techniques combined with liquid chromatography. However, the universality of UV detection is limited because it requires the presence of a chromophore in the analyte molecule. UV detectors are not sensitive enough for a great many analytes especially those with a lack of π electrons. This is especially important when using ultra high-performance chromatography (UHPLC) where low injection volumes are usually employed (up to 5 μL) and where the volume of the UV detector flow cells can be decreased. Therefore, a novel hyphenated method is introduced in this paper that combines UHPLC with a novel commercially available aerosol-based detector (Nano Quantity Analyte Detector – NQAD™).

UHPLC is a modern separation technique providing considerable high-throughput analysis compared with HPLC. Hardware adjustments allow UHPLC systems to work at extreme pressures, up to 100 MPa [1]. This is necessary for operation with sub-2 micron particles in the chromatographic columns for UHPLC applications and cannot be achieved using conventional liquid chromatographic systems [2]. UHPLC generally yields significantly higher separation efficiencies and shorter run times compared to ordinary HPLC columns. According to the Van Deemter equation, when the particle size of the chromatographic sorbent is decreased, the efficiency of the separation process increases and the efficiency does not diminish at higher flow rates or linear velocities [3].

NQAD™ is a novel aerosol-based detector for HPLC that is also termed in the literature as the condensation nucleation light-scattering detector (CNLSD) [4]. NQAD™ uses condensation nucleation technology. The principle of the technique is based on nebulization and evaporation of the mobile phase at elevated temperature and consequent analyte condensation with supersaturated auxiliary water vapor. This leads to creation of relatively large droplets that are later detected using scattered light with a laser-photodetector system set-up at perpendicular arrangement. This increase in particle size tremendously increases the light-scattering signal and dramatically increases the sensitivity in comparison to ELSD [5]. Only particles above a critical size can act as condensation nucleation sites that increase the signal–noise ratio due to discrimination of small droplets from the mobile phase. The producer states that the detector allows measuring of compounds in low nanogram on column ranges and the NQAD™ dynamic range should span from below 1 ng to over 10 μg on the column [6]. However, even lower LODs were published with a laboratory built CNLSD for various compounds [4,7]. The technique is also suitable for compounds that lack π electrons and that are thus hard to detect using UV methods. Such a universal detection technique should have extensive applications in liquid chromatography including UHPLC as it

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was already published with the laboratory built CNLSD connected to several other separation techniques including HPLC [4,7,8], capillary electrophoresis [9] and capillary electrochromatography [10].

Some representatives of macrocyclic antibiotics, macrolides, can cause difficulties in UV detection. These compounds represent a large group of very similar, naturally occurring antibiotics produced mostly by *Streptomyces* sp. They consist of a macrocyclic lactone ring to which typically two sugars, one of which is an amino sugar, are attached. They are amongst medicines that have recently been widely used to treat a broad spectrum of infectious diseases. It is assumed they can also appear in the environment through misuse [11]. This implies the necessity of suitable, reliable and high-throughput methods for their determination.

Up to the present time, in addition to GC [12], mostly HPLC with various detectors has been used for macrolide analysis. Several methods with UV detection were developed in the past [13]; however, many macrolides lack π-electrons and are thus hard to detect in the UV range even at low wavelengths. Therefore, various techniques have been investigated for macrolide detection. Fluorescence was used by e.g. Pakinaz and Khashaba [14]; however, sample pre-treatment is required for the detection. Electrochemical detection [15–18] may offer a much better choice because of the presence of electro-active groups in their molecules and is undoubtedly the most frequently used technique today. Most recently, mass spectrometry, the most universal detector by far, has also been applied to macrolide detection [11,19,20]. All the above techniques for analysis of macrolides were reviewed by e.g. Marzo and Dal Bo [21], Kanfer et al. [22] and Danaher et al. [23].

This work was concerned with testing a novel hyphenated UHPLC-NQAD™ technique employing the first commercially available detector using condensation nucleation light-scattering principle for detection of five selected macrolides (Mixture I) and 12 other antibiotics with various structures and chromatographic properties (Mixture II) and to compare this method with UV detection.

2. Experimental

2.1. Chemicals, standard solutions

The solvents used in UHPLC were of gradient grade. Acetonitrile 99.95% (ACN) Biosolve and Methanol 99.95% (Chromapur GG) were purchased from Chromservis (Czech Republic).

The standard stock solutions were prepared with methanol–water (50:50, v/v) at a concentration level of 1 mg/mL. Standard solutions with the required concentration were obtained by dilution of the stock solutions with methanol–water (50:50, v/v).

Mixture I contained the following macrolides: oleandomycin (OLE), erythromycin (ERY), troleandomycin (TRO), clarithromycin (CLA) and roxithromycin (ROX) at a concentration of 50 μg/mL.

Mixture II contained following antibiotics: metronidazole (MET), vancomycin (VAN), chloramphenicol (CHL), cycloheximide (CYC), lincomycin (LIN), griseofulvin (GRI), clindamycin (CLI), clarithromycin (CLA), roxithromycin (ROX), rapamycin (RAP), streptovitacin A (STV) and carbomycin (CAM) at a concentration of 62.5 μg/mL.

MET, VAN, CHL, CYC, LIN, GRI, CLI, ERY, CLA, ROX and RAP were obtained from Sigma–Aldrich, Germany and were of UV grade (>95%). STV, OLE, TRO and CAM were purer than 90% and were kindly provided by Prof. Jaroslav Spížek, Institute of Microbiology of the Academy of Sciences of the Czech Republic, v.v.i., Czech Republic.

2.2. UHPLC

Samples were analyzed with the Acquity UHPLC system (Waters, Milford, MA, USA) using Waters BEH C18 column (50 mm × 2.1 mm I.D., particle size 1.7 μm), column temperature, 50 °C; data sample rate, 20 Hz; filter constant, 0.5; injection volume, 5 μL; flow rate, 0.4 mL/min. Mobile phases consisted of water (A) and acetonitrile (B), both containing 0.01% or 0.04% NH_4OH for NQAD™ and UV detection, respectively. The stock solution of aqueous NH_4OH solution had a concentration of 24%.

2.2.1. Isocratic mode

Mixture I was separated under isocratic conditions A:B 55:45 (v/v). The separated compounds were detected by both the NQAD™ and UV techniques.

2.2.2. Gradient mode

Mixture II was separated under gradient conditions (min/%A) 0/5; 7/27; 12/33; 17/88; 18/100; 20/100. Each analysis was followed by a column equilibration step (2 min). The separated compounds were detected by both the NQAD™ and UV techniques.

2.3. NQAD™ detection

The Quant NQAD™ (Quant Technologies, LLC; Blaine, Minnesota, USA) was used for detection of antibiotics under

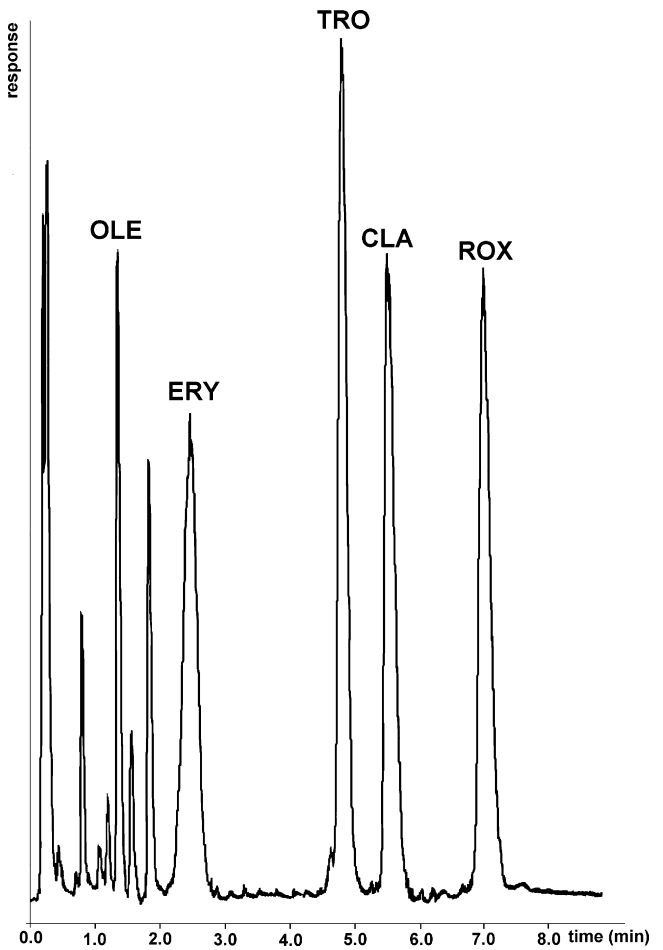

Fig. 1. Isocratic separation of Mixture I with NQAD™ detection. OLE, ERY, TRO, CLA, ROX at a concentration of 50 μg/mL, BEH C18 column (50 × 2.1 mm I.D., 1.7 μm), column temperature, 50 °C; injection volume, 5 μL; flow rate, 0.4 mL/min, water (A) and acetonitrile (B), both containing 0.01% NH_4OH (55:45, v/v), NQAD™ detection: 35 °C, gain 10×.