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Analysis of metalaxyl racemate using high performance liquid chromatography coupled with four kinds of detectors

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ABSTRACT

Chiral stationary phase-high performance liquid chromatography coupled with various detectors has been one of most commonly used methods for analysis and separation of chiral compounds over the past decades. Various detectors exhibit different characteristics in qualitative and quantitative studies under different chromatographic conditions. Herein, a comparative evaluation of HPLC coupled with ultraviolet, optical rotation, refractive index, and evaporative light scattering detectors has been conducted for qualitative and quantitative analyses of metalaxyl racemate. Effects of separation conditions on the peak area ratio between two enantiomers, including sample concentration, column temperature, mobile phase composition, as well as flow rate, have been investigated in detail. In addition, the limits of detection, the limits of quantitation, quantitative range and precision for these two enantiomers by using four detectors have been also studied. As indicated, the chromatographic separation conditions have been slight effects on ultraviolet and refractive index detections and the peak area ratio between two enantiomers remains almost unchanged, but the evaporative light scattering detection has been significantly affected by the above-mentioned chromatographic conditions and the corresponding peak area ratios varied greatly. Moreover, the limits of detection, the limits of quantitation, and the quantitative ranges of two enantiomers with UV detection were remarkably lower by 1–2 magnitudes than the others.

1. Introduction

Chirality is ubiquitous in life sciences, pharmaceuticals, fine chemicals, and materials, etc. [1–5]. Enantiomers may exhibit different physiological, pharmacological, and toxic activities in the chiral environment, although having same physical and chemical properties. Only one or several enantiomers in many chiral pharmaceuticals exhibit pharmacologically active, and the presence of enantiomeric impurities could lead to potentially serious consequences. Thus, separation, quantitative determination, in-process quality control, and pharmacological studies of chiral compounds have been one of hotspots in research of novel pharmaceuticals.

In recent years, chiral stationary phase-high performance liquid chromatography (HPLC) coupled with various detectors, e.g. ultraviolet (UV) detector, optical rotation (OR) detector, refractive index (RI) detector and evaporative light scattering (ELS) detector, has been one of the most commonly used methods in chiral separation studies [1,2,6–15]. Due to high sensitivity, high stablity, and low cost, UV detector has been widely applied in detection of analytes with typical chromophoric groups, and showed some potential drawbacks, such as unsuitablity for analytes without typical chromophores and limit of solvent [16–19]. As being a universal detector, evaporative light scattering detector is suitable for analytes with less volatility than mobile phase [17,20–25]. Most of analytes with/without typical chromophores are able to be detected through ELS detector. For refractive index detector, sample is detected on the basis of difference between its refraction and mobile phase's refraction [26–29], and it has some potential shortages, such as low sensitivity, susceptibility to disturbance from eluents, baseline instability, and gradient incompatibility. Moreover, optical rotation detector as a kind of selective detector is only suitable to detection of optically active substances and non-optically active compunds couldn't interfere with determintion of enantiomers [30–33].

Chromatographic conditions have some potential impacts on sensitivity and precision of these detectors and peak area normalization method can't be directly used for quantitation of enantiomers under some conditions. Toussaint group reported a comparative study on determination of chiral non-aromatic alco-

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hols by HPLC coupled with four different detectors [33]. The peak area ratio between enantiomers by using the ELS detector was remarkably different with the others. Wipf and his co-worker reported HPLC determinations of enantiomeric ratios through three detectors, namely, UV, ELS, and electrical aerosol detector [29]. ELS detector didn't reflect real enantiomeric purity under different elution conditions in comparison with the others, and might be complemented through adopting a suitable calibration protocol. HPLC coupled with ELS detector was applied in detection and determination of a mixture of enantiomers, and the effects of some factors on integration of chromatographic peaks, such as molecular weight and volatility of analytes and peak shape, have been discussed in detail [34,35], which indicated that non-linear response for ELS detector has been the main factor leading to deviation.

Metalaxyl is an acetanilide-type fungicide and contains an asymmetrically substituted C atom, thereby having two enantiomers (Fig. 1). It shows high activity against fungi of the order Peronosporales and good effectiveness against diseases caused by oomycetes, and has been widely employed in the agriculture field from 1977, for seed treatment, banded or broadcast soil application, and foliar spray [36,37]. Usually, plant treatment is performed with the racemic mixture. However, some results indicated that the *R*-metalaxyl has been the primary active ingredient relative to the *S*-enantiomer and the anti-fungicidal activity mostly originated from the *R*-enantiomer [37]. In addition, metalaxyl is photolytically and hydrolytically stable with about a half-time of 400 days under natural sunlight, and 7–118 days at 20 °C from pH 5 to pH 9 in hydrolysis [37–39]. Thus, its long-term accumulation in soils and groundwater could cause a potentially serious consequence to the environment and public health [40,41].

Herein, a comparative evaluation of HPLC coupled with UV, RI, OR and ELS detectors has been conducted for qualitative and quantitative determinations of metalaxyl enantiomers. Effects of chromatographic conditions on the peak area ratio, such as concentration, column temperature, mobile phase composition, and flow rate, have been investigated in detail.

2. Experimental

2.1. Chemicals and materials

Racemic metalaxyl (purity > 98%) was bought from Wuhan Fengzhulin Chemexpress Co., Ltd (Wuhan, China). Optically pure *S*-(+)-metalaxyl and *R*-(−)-metalaxyl were obtained through preparative separation of metalaxyl racemate by simulated moving bed chromatography [42]. Ethanol and *n*-hexane were of HPLC-grade and purchased from Lab Science, Inc (Houston, Texas, US). Nitrogen with purity higher than 99.999% was used as the evaporation gas. EnantioPak OD column (150 mm × 4.6 mm, 5 μm) was friendly provided by Guangzhou Research & Creativity Biotechnology Co., Ltd (Guangzhou, China).

2.2. Instruments

Chromatographic analysis experiments have been performed with a 1200 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, a quaternary pump, an auto injector with a 100 μL sample loop, a column oven, a multiple wavelength UV detector, a RI detector, and the Agilent Chemstation software. Polarimetry of chiral compounds has been carried out by using IBZ Chiralyser-MP optical rotation detector (IBZ Messtechnik GMBH, Hannover, Germany). In addition, an Alltech Model 3300 evaporative light scattering detector (Grace Davison Discovery Sciences, Deerfield, IL, USA) was used in this study. These four detectors were individually connected to the same HPLC system. Minor difference between retention times of two enantiomers was present in the obtained chromatograms because of different flow cells and pipelines of these four detectors.

2.3. Chromatography

Solutions of racemic metalaxyl, *S*-(+)-metalaxyl, and *R*-(−)-metalaxyl were prepared through dissolving the corresponding samples and diluting with the mobile phase, respectively. Mixture of *n*-hexane/ethanol was freshly prepared and degassed in an ultrasonic bath before use.

Enantioseparation of metalaxyl was achieved on the EnantioPak OD column by normal-phase HPLC at 25 °C, without otherwise specified. Sample solutions (20 μL) were injected twice and the flow rate was 1.0 mL min^{-1}. The UV detection wavelength of metalaxyl was set to 220 nm. The optical unit's temperature for refractive index detector was 35 °C. For evaporative light scattering detector, the flow rate of nitrogen was set at 1.5 L min^{-1}, the temperature of the drift tube was 35 °C, and the gain was set at 1. Optical rotation detector was used for identification of the enantiomers. The average and the range of optical rotation detector were set at off and 4.0 m deg, respectively.

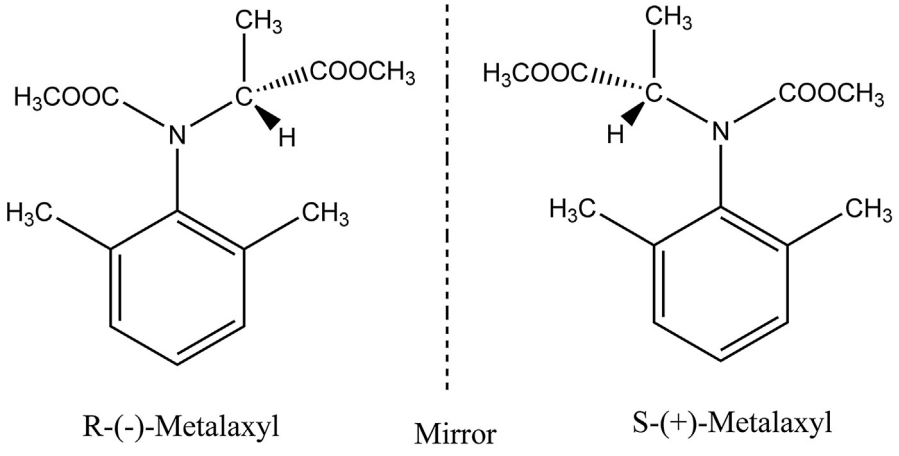

Fig. 1. Molecular structures of two metalaxyl enantiomers.