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Investigation of two-dimensional high performance liquid chromatography approaches for reversed phase resolution of warfarin and hydroxywarfarin isomers

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ABSTRACT

Several offline and online 2D HPLC methods were investigated for the reversed phase resolution of a complex mixture of closely related warfarin and hydroxywarfarin isomers. By combining reversed phase achiral/chiral HPLC separation with UV-triggered fraction collection and subsequent chiral/achiral reversed phase HPLC analysis of collected fractions, complete resolution of all 12 components of the mixture was possible. In addition, a faster method was developed from online 2D HPLC analysis where multicomponent fractions from the first dimension are simultaneously chromatographed in the second dimension.

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1. Introduction

The chromatographic resolution of complex mixtures of closely related molecules is an important area of current interest for pharmaceutical analytical chemistry [1–4]. A variety of tools for such problems have become available in recent years, ranging from improved stationary phases [5] to the use of multidimensional chromatography [6–11]. In a recent investigation into the separation of a mixture of the drug, warfarin, and related hydroxylation metabolites, excellent fast separations of all six components was obtained by either UHPLC or SFC, while complete resolution of all 12 stereoisomers present in the sample was afforded by chiral SFC analysis using a Chiralcel OD-3 column with 25 mM isobutylamine in methanol as polar modifier [12–14].

Attempts at chromatographic resolution of all 12 components by chiral HPLC analysis was not successful, although partial deconvolution of overlapping peaks by examining characteristic fragments in the ESI(+)-MS mode did allow for separation of 10 isomeric species, with two of the 6- and 8-hydroxywarfarin isomers overlapped [13]. While chiral SFC affords impressive separation of all components within 8 min, the lack of a reversed phase chiral HPLC method was disappointing, as many bioanalysis laboratories do not yet possess SFC instrumentation, and instead rely upon reversed phase LC–MS as the analytical technique of choice [3]. In this study we investigate the chromatographic separation of this complex mixture of warfarin and related hydroxylation metabolites by offline and online 2D chiral reversed phase HPLC methodologies.

2. Experimental

2.1. Instrumentation

Offline 2D experiments were performed on a HPLC system consisted of an Agilent Series 1100 G1311A binary pump, a G2260A PrepALS auto sampler, a G1322A degasser, a G1316A thermostatted column compartment, a G1315B diode array detector and a G1364A AFC automated fraction collector (Agilent Technologies, Palo Alto, CA, USA). The system was controlled by Chemstation® Rev. B.04.03 [16] software.

Core shell HPLC screening was performed on an Agilent 1100 system. The Agilent system was comprised aa modified G1312A binary pump, a modified G1367A WPALS autosampler, a G1379S degasser, a G1316A thermostatted column

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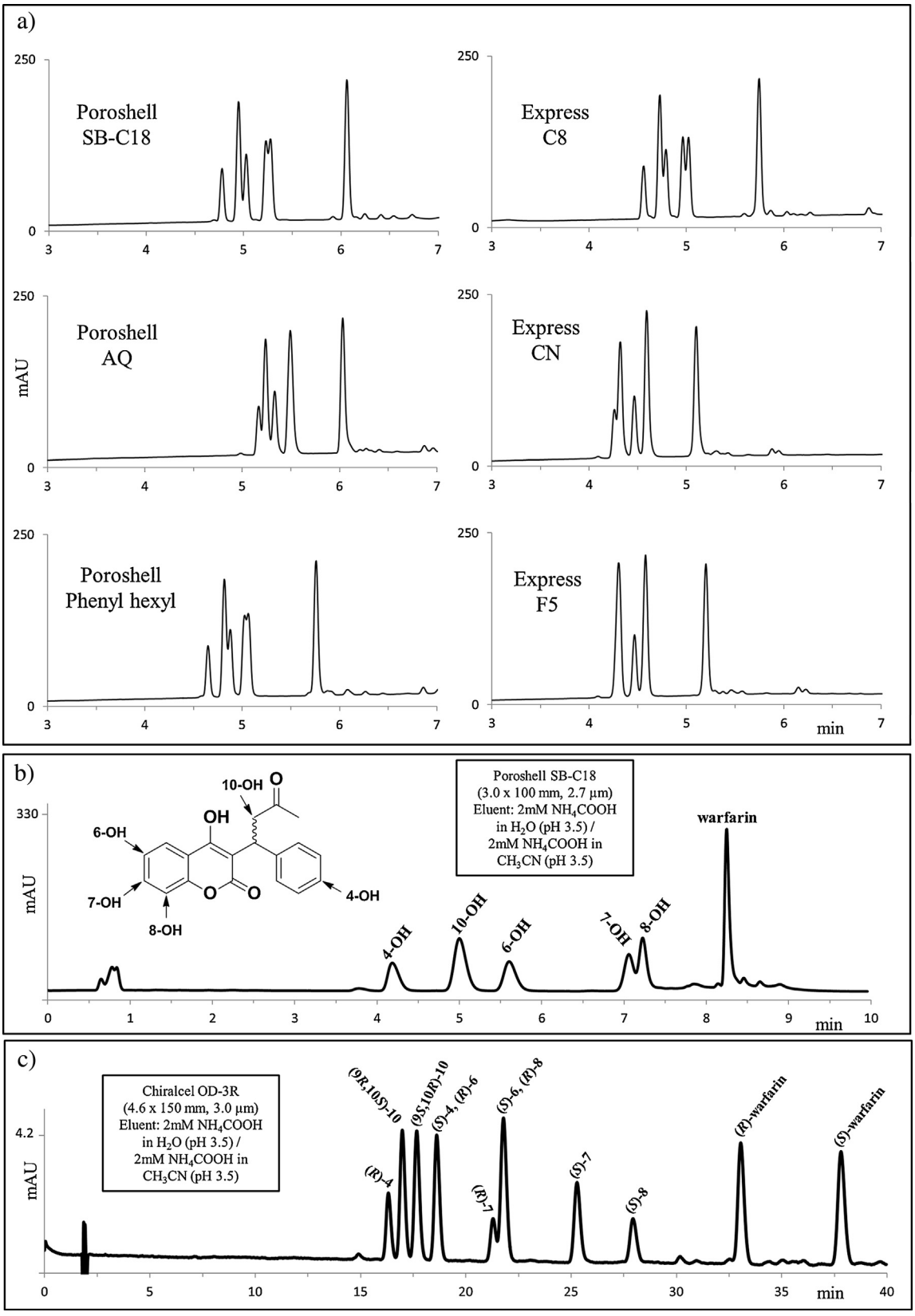

Fig. 1. Chromatographic analysis of warfarin and hydroxywarfarins mixture. (a) Reversed phase core shell HPLC screening. Six columns: 3.0 mm × 100 mm, 2.7 μm Poroshell (SB-C18, AQ, phenyl hexyl) and Ascentis Express (C8, CN, F5). Temperature: 40 °C, UV detection: 210 nm. Sample: 1 μL injection of warfarin and hydroxywarfarins mixture in CH_3OH. Flow rate: 0.75 mL/min. Eluents: A: 2 mM NH_4COOH in H_2O (pH 3.5), B: 2 mM NH_4COOH in 90/10 CH_3CN/H_2O (pH 3.5). Gradient: from 10 to 95% B in 8 min, hold at 95% for 2 min. (b) Representation of warfarin and hydroxywarfarin structures and optimized reversed phase core shell HPLC method. Temperature: 40 °C, UV detection: 210 nm. Injection volume: 10 μL. Flow rate: 0.75 mL/min. Gradient: 32% B for 3 min then ramp at 90% in 2 min, go to back to initial conditions in 0.1 min. (c) Optimized enantioseparation of warfarin and hydroxywarfarins by a reversed phase chiral HPLC method: column: OD-3R (4.6 mm × 150 mm, 3 μm); temperature: 25 °C. UV detection: 280 nm. Injection volume: 5 μL. Flow rate: 1.0 mL/min. Gradient: from 25 to 50% B in 40 min.

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