Journal of Chromatography A, 1271 (2013) 207–216

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

# Development of a chiral micellar electrokinetic chromatography–tandem mass spectrometry assay for simultaneous analysis of warfarin and hydroxywarfarin metabolites: Application to the analysis of patients serum samples

Xiaochun Wang[a], Jingguo Hou[a], Michael Jann[b], Yuen Yi Hon[c], Shahab A. Shamsi[a,*]

[a] Center of Diagnostics and Therapeutics, Georgia State University, 50 Decatur Street, Atlanta, GA 30303, USA
[b] Mercer University, Southern School of Pharmacy, Atlanta, GA 30341, USA
[c] National Institutes of Health, USA

ARTICLE INFO

*Article history:*
Received 4 September 2012
Received in revised form
14 November 2012
Accepted 19 November 2012
Available online 26 November 2012

*Keywords:*
Warfarin/hydroxylated-warfarin metabolites
Polymeric surfactant
Optical/positional isomers
MEKC–MS versus CEC–MS
SIM versus MRM
CYP mediated warfarin metabolism

ABSTRACT

The enantioseparation of warfarin (WAR) along with the five positional and optical isomers is challenging because of the difficulty to simultaneously separate and quantitate these chiral compounds. Currently, no effective chiral CE–MS methods exist for the simultaneous enantioseparation of WAR and all its hydroxylated metabolites in a single run. Polymeric surfactants (aka. molecular micelles) are particularly compatible with micellar electrokinetic chromatography–mass spectrometry (MEKC–MS) because they have a wider elution window for enantioseparation and do not interfere with the MS detection of chiral drugs. Using polysodium *N*-undecenoyl-L,L-leucylvalinate (poly-L,L-SULV) as a chiral pseudophase in MEKC–MS baseline separation of WAR, its five metabolites along with the internal standard was obtained in 45 min. This is in comparison to 100 min required for separation of the same mixture with packed column CEC–MS using a vancomycin chiral stationary phase. Serum samples were extracted with mixed-mode anion-exchange (MAX) cartridge with recoveries of greater than 85.2% for all WAR and hydroxywarfarin (OH-WAR) metabolites. Utilizing the tandem MS and multiple reaction monitoring mode, the MEKC–MS/MS method was used to simultaneously generate calibration curves over a concentration range from 2 to 5000 ng/mL for *R*- and *S*-warfarin, 5 to 1000 ng/mL for *R*- and *S*-6-, 7-, 8- and 10-OH-WAR and 10 to 1000 ng/mL for *R* and *S*-4′-OH-WAR. For the first time, the limits of detection and quantitation for most WAR metabolites by MEKC–MS/MS were found to be at levels of 2 and 5 ng/mL, respectively. The method was successfully applied for the first time to analyze WAR and its metabolites in plasma samples of 55 patients undergoing WAR therapy, demonstrating the potential of chiral MEKC–MS/MS method to accurately quantitate with high sensitivity.

© 2012 Elsevier B.V. All rights reserved.

## 1. Introduction

Warfarin (WAR), the most commonly prescribed anticoagulant, is clinically administered as a racemic mixture of *R*- and *S*-WAR to treat and prevent thromboembolism [1–3]. Despite its extensive use, WAR therapy is complicated by a narrow therapeutic range and wide inter-individual response to treatment [4]. The *S*-WAR is 2–5 times more potent anticoagulant, and is metabolized much quicker (∼1.5 times faster) than *R*-WAR [5]. Therefore, different *R*- and *S*- form of WAR are differentially metabolized by stereoselective metabolism. The time-consuming and laborious procedure in collecting urine samples can be eliminated by directly determining WAR enantiomers in the human plasma. In addition, to understand the mechanisms for relationships between WAR therapy and patient response, the investigation of the metabolic processes in serum samples is necessary and critical.

WAR metabolism is further characterized by a process termed as regioselective metabolism catalyzed by several important cytochrome (CYP) P450 members (Fig. 1). For example, the more potent *S*-WAR exclusively catalyzed by CYP2C9 to form 7-OH-WAR although 6-OH-WAR and 4-OH-WAR are also formed [6]. In contrast, *R*-WAR is metabolized to all five OH-WAR meta-bolites but 10-OH-WAR and 6-OH-WAR are the most abundant metabolites [7,8]. Reported drug interaction [9] and genetic studies [10–12] indicate that many of these *P450s* enzymes may play a clinical role in patient response to WAR therapy. Therefore, better understanding of the contributions of the CYP enzymes toward total WAR metabolism is warranted.

There has been significant interest in developing sensitive methods for monitoring WAR and its metabolites in serum samples.

* Corresponding author. Tel.: +1 404 413 5512; fax: +1 404 413 5505.
*E-mail addresses:* sshamsi@gsu.edu, chesas@langate.gsu.edu (S.A. Shamsi).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.11.046

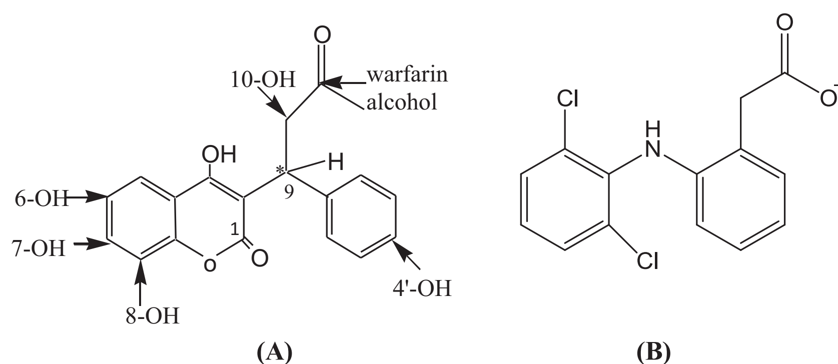


**Fig. 1.** Structures of (A) WAR and its sites of hydroxylation (indicated by arrow) to generate monohydroxylated WAR metabolites by cytochrome P450s, (B) diclofenac (DFC) sodium (used as internal standard). The asterisk indicates stereogenic center.

Currently, the most common enantioselective analytical technique used for the determination of WAR and its metabolites in biological samples is high performance liquid chromatography (HPLC) based on either achiral or chiral methods and detection by UV [13–16], combination of UV/fluorescence and circular dichroism [17,18] as well as mass spectrometry (MS) [3,19–25]. Generally, multiple research groups focus on resolving *R*- and *S*-WAR [3,13–15,23], but not simultaneous enantiomeric separation of all OH-WAR enantiomers. A more recent study reported an elegant method for the analysis of WAR and OH-WAR enantiomers in plasma samples by ultra performance liquid chromatography (UPLC)–MS/MS using a combination of two columns (phenyl achiral column and chiral Astec Chirobiotic V column) connected in series with identical mobile phase [25]. However, HPLC method requires large volumes of organic solvent. In addition, relatively large injection volume requirement in HPLC is also unfavorable when the patient samples are volume-limited. For example, when extensive research is needed to understand the correlation of metabolite formation with genetic data and other separation techniques, capillary electrophoresis (CE) has a clear advantage.

The technique of CE has been extensively developed as a complementary enantioselective analytical technique to conventional HPLC in the past decade [26–31]. Electrokinetic chromatography (EKC) using low molecular mass chiral selectors (e.g., charged cyclodextrins, macrocyclic antibiotics, crown ethers, unpolymerized micelles) are capable of separating both charged and uncharged molecules simultaneously with high efficiency, selectivity and flexibility. A weak point, which still remains using the aforementioned chiral selectors for CE is UV-detection sensitivity. Coupling EKC or MEKC with ESI-MS is very attractive and advantageous. However, direct coupling of MEKC to MS is problematic using conventional surfactants forming micelles. This is mainly due to production of background ions from surfactant monomers resulting in cluttering of mass spectrum and signal suppression [32,33]. Several methods have been introduced to overcome these limitations of MEKC–MS including: (i) use of high-molecular-mass polymeric surfactants [34–36], (ii) use of the partial filling technique [37], (iii) counter-migration [38], and the use of atmospheric pressure photoionization [39,40]. Among the various approaches for chiral CE–ESI-MS, the use of high molecular mass polymeric chiral surfactants (also called molecular micelles or micelle polymers) is considered to be a simple and convenient for a variety of chiral compounds. This is because molecular micelles are covalently stabilized micellar aggregates, which remain intact in solution phase inside the capillary. In addition, they are not fragmented in the gas phase of the mass spectrometer, showing notable advantages. These include zero critical micelle concentration, very stable micellar structure, low surface activity, low volatility as well relatively narrow polydispersity and significantly less MS signal suppression. All of the aforementioned characteristics make polymeric surfactants very compatible chiral selector for on-line MEKC–ESI-MS [34–36,41–43].

In our previous study [35], MEKC–ESI-MS method for the analysis of WAR enantiomers was developed, using a polymeric surfactant, polysodium *N*-undecenoyl-L,L-leucyl-valinate (poly-L,L-SULV), as a pseudostationary phase. The method was applied to the determination of only *R*- and *S*-WAR in patient samples. Thus, the simultaneous enantio-separation of WAR and its hydroxylated metabolites by MEKC–MS remains unexplored. This article begins with the development of MEKC–MS method for simultaneous separation of WAR and all OH-WAR metabolites. Comparison of the MEKC–MS versus packed column CEC–MS as well comparison of single quadrupole and triple quadrupole mass spectrometer are provided in details for such analysis. Optimization of solid phase extraction (SPE) procedure for generation of calibration curve, improving limit of quantitation and limitation of detection (LOD) of WAR and metabolites in serum samples using MEKC–ESI-MS/MS with excellent sensitivity and specificity is illustrated. Finally, the MEKC–ESI-MS/MS study was expanded to apply the method to analysis of 55 patient samples receiving WAR therapy.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Racemic WAR, racemic 4′-, 6-, 7-, 8- and 10-OH-WAR, non-racemic diclofenac sodium (used as internal standard (I.S.)), HPLC-grade acetonitrile (ACN), methanol (MeOH), ethanol (EtOH) and isopropanol (IPA), analytical-grade ammonium acetate (as 7.5 M $NH_4OAc$ solution) and dipeptide (L,L-leucylvalinate) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Ammonium hydroxide and acetic acid were obtained from Fisher Scientific (Springfield, NJ, USA). The 3 μm vancomycin chiral stationary phase (CSP) was donated by Advanced Separation Technologies, Inc. (Whippany, NJ, USA). Water used in all experiments was triply deionized and obtained from Barnstead Nanopure II water system (Barnstead International, Dubuque, IA, USA). Oasis mixed-mode anion-exchange (MAX) cartridges (3 $cm^3$, 100 mg) were supplied by Waters (Milford, USA). The dipeptide surfactant monomer of sodium (L,L-SULV) was synthesized following the procedure described in reference [44]. The surfactant monomer was polymerized using a total dose of 20 M rad of $^{60}Co$ γ-radiation by Phoenix Memorial Laboratory (University of Michigan, Ann Arbor, MI). Blank and patient's human plasma were obtained from Mercer University Southern School of Pharmacy (Atlanta, GA, USA) and stored under −80 °C until the assay was performed.

Download English Version:

https://daneshyari.com/en/article/1201516

Download Persian Version:

https://daneshyari.com/article/1201516

Daneshyari.com