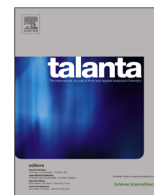
Talanta 128 (2014) 51–57

Contents lists available at ScienceDirect

Talanta

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



A novel liquid chromatography-tandem mass spectrometry method for determination of menadione in human plasma after derivatization with 3-mercaptopropionic acid

Ruijuan Liu, Mengmeng Wang, Li Ding*

Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 12 February 2014
Received in revised form
8 April 2014
Accepted 9 April 2014
Available online 30 April 2014

Keywords:
Liquid chromatography-tandem mass spectrometry
Menadione
Derivatization
3-mercaptopropionic acid
Michael addition
pharmacokinetic

ABSTRACT

Menadione (VK_3), an essential fat-soluble naphthoquinone, takes very important physiological and pathological roles, but its detection and quantification is challenging. Herein, a new method was developed for quantification of VK_3 in human plasma by liquid chromatography-tandem mass spectrometry (LC–MS/MS) after derivatization with 3-mercaptopropionic acid via Michael addition reaction. The derivative had been identified by the mass spectra and the derivatization conditions were optimized by considering different parameters. The method was demonstrated with high sensitivity and a low limit of quantification of 0.03 ng mL^{-1} for VK_3, which is about 33-fold better than that for the direct analysis of the underivatized compound. The method also had good precision and reproducibility. It was applied in the determination of basal VK_3 in human plasma and a clinical pharmacokinetic study of menadiol sodium diphosphate. Furthermore, the method for the quantification of VK_3 using LC–MS/MS was reported in this paper for the first time, and it will provide an important strategy for the further research on VK_3 and menadione analogs.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Vitamin K (VK) is a family of structurally similar and fat-soluble vitamins. All forms of VK share a common naphthoquinone ring, but differ in the position-3 side chain. There are three major types of VK, including phylloquinone (VK_1), menaquinones (VK_2), and menadione (VK_3) (see Fig. 1). VK_1 and VK_2 are the natural compounds. The most common form of VK_2 in humans is menaquinone-4 (MK-4) [1]. Although VK_3 (2-methyl-1,4-naphthoquinone) is considered as a synthetic analog, Davidson et al. [2–4] found that dietary VK_1 can be cleaved to form VK_3 by bacteria in the intestine. It is also an intermediate metabolite in the conversion of VK_1 to MK-4 [5], and plays an important role in blood coagulation as a cofactor for the synthesis of clotting factors in the liver and in bone mineralization [4,6].

Recently, the interest in VK_3 has grown because of its antitumor activity against various human cancer cells [7,8] and other actions [9–11]. It has shown antiproliferative effects against various kinds of cancer cells, including pancreatic [12], hepatic [13], oral cavity [14], breast [15], leukemia [16] and several glioma cell lines [17]. Both *in vivo* and *in vitro* studies showed a synergistic effect when VK_3 was combined with conventional chemotherapeutic agents, such as 5-fluorouracil, mitomycin C, doxorubicin, bleomycin, cisplatin, dacarbazine and so on [3,18]. The development of the VK_3 prodrugs as antihemorrhagic and anticancer agents is promising, and attracts more and more interest of scientists. To evaluate the safety and efficacy of those developing new drugs or prodrugs of VK_3, it is very important to obtain the plasma concentration data of VK_3 converted by those drugs. In addition, an increasing body of work indicates that VK deficiency may be associated with osteoporosis and possibly with hepatocarcinoma and atherosclerosis [9]. The efficacy of VK in the prevention or treatment of these diseases deserves further studies. Therefore, to detect the low endogenous concentrations and elucidate the effects and molecular mechanisms of vitamin Ks in human, it is essential to establish accurate, sensitive, and selective methods for the quantification of vitamin Ks in human plasma. There are many methods reported in the literature for determination of VK_1 [19] and VK_2 [20] in plasma, but there is no highly sensitive and effective method or LC–MS/MS method reported for the determination of VK_3 in human plasma. The reasons mainly include two aspects. On the one hand, there is no ionizable functional group in its chemical structure, so that the mass spectrometric response of it is very poor, and it is relatively difficult to establish a sensitive and robust LC–MS/MS method for its unchanged form. On the other hand, VK_3 is highly unstable in plasma, its plasma concentration level is very low, and meanwhile it decreases rapidly in plasma [21,22].

* Corresponding author. Tel./fax: +86 25 83271485.
E-mail addresses: dinglidl@hotmail.com, dinglihg@sina.com (L. Ding).

http://dx.doi.org/10.1016/j.talanta.2014.04.039
0039-9140/© 2014 Elsevier B.V. All rights reserved.

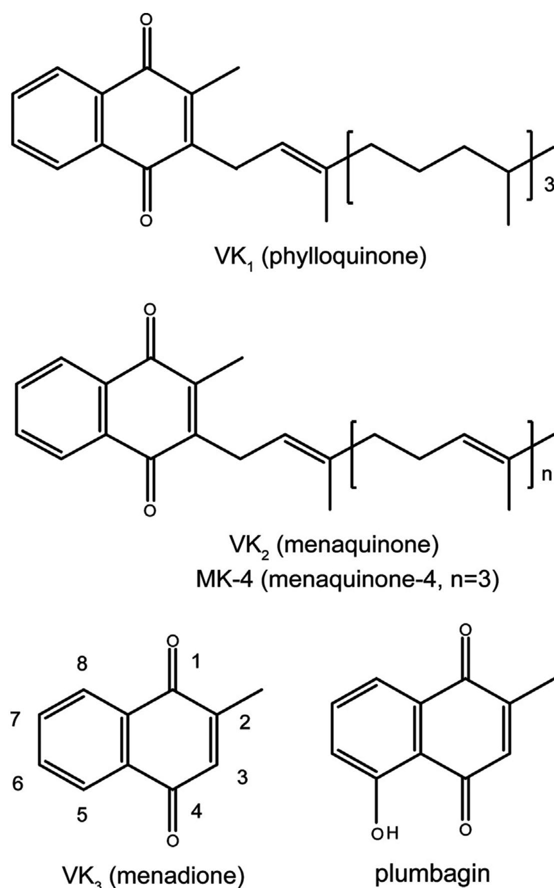


Fig. 1. Chemical structures of vitamin Ks and plumbagin. Note the *n* on vitamin K_2, which represents the number of unsaturated isoprenyl groups.

Various methods [5,6,23–27] have been reported for the determination of VK_3 in various matrices. Among the methods only a few were reported for the determination of VK_3 in plasma. Akman et al. [23] reported a differential pulse polarographic assay for VK_3 in plasma with a low limit of quantification (LLOQ) of 30 ng mL^{-1}. Hu et al. [24] reported the HPLC method for the determination of VK_3 in rabbit plasma with an LLOQ of 10 ng mL^{-1}. Both methods were not sensitive enough for determining the basal VK_3 level in plasma. Recently, Al Rajabi et al. [5] reported an HPLC method using a C_{30} column, post-column zinc reduction and fluorescence detection to measure urinary VK_3 with an LLOQ of 0.3 pmol mL^{-1}. The method offered increased sensitivity, but it only applied to the determination of VK_3 in urine not in plasma. Furthermore, VK_3 is not endogenously fluorescent and requires a post-column zinc reduction for fluorescent detection. To the best of our knowledge, although the LC–MS/MS methods for determination of vitamin K_3-glutathione conjugate in hepatocytes [28] and in liver [29] have been published over the years, no successful methods for the quantitative determination of VK_3 using LC–MS/MS are reported so far.

In recent decades, LC–MS/MS has been proven to be an extremely sensitive and specific technique for biomedical analysis. However, some chemical substances are difficult to detect by the wide-spread LC–MS/MS method due to the lack of ionizable functional group. Introducing ionizable group, by a pre-column derivatization method, into the structure of these poorly ionizable drugs is a useful strategy to improve both detection specificity and sensitivity by mass spectrometry [30–32].

Menadiol sodium diphosphate (MSD) is a prodrug of VK_3. It is converted to its active form VK_3 *in vivo*. The drug is currently undergoing phase II clinical trials in China as an antihemorrhagic agent. In this study, we developed the sensitive LC–MS/MS method to determine VK_3 in human plasma using a simple derivatization reaction of VK_3 with 3-mercaptopropionic acid. The method was successfully applied to the clinical pharmacokinetic study of MSD, and the plasma concentration-time profiles of VK_3 in human after oral administration of MSD were acquired for the first time. The proposed method also provides a new strategy for the detection of menadione analogs.

2. Experimental

2.1. Optimization of the derivatiazation procedures

Derivatization efficiencies were evaluated at various reaction temperatures, time, media, amounts of derivatization reagent and pH levels. The optimum derivatization conditions of VK_3 with MPA were determined by the amounts of the derivatized VK_3.

2.2. Preparation of calibration standard and quality control samples

The primary stock solution of 1.0 mg mL^{-1} VK_3 was prepared by dissolving accurately weighed amounts of the reference substance in methanol. Working solutions of VK_3 were prepared at concentration levels of 1.5, 5.0, 15, 50, 150, 500, 1000, 1750 and 2500 ng mL^{-1} by serial dilution of the primary stock solution with methanol. The working solution of the internal standard (IS, 200 ng mL^{-1}) was prepared by diluting a 1.0 mg mL^{-1} plumbagin stock solution with methanol. All solutions were kept at −20 °C in dark and brought to room temperature before use. The derivatizing reagent was freshly prepared by diluting the 3-mercaptopropionic acid (MPA, 1.22 g mL^{-1}, 20 °C) to the concentration of 122 μg mL^{-1} with methanol.

We prepared VK_3-free blank plasma by exposing plasma from healthy subjects to light for eight hours[33]. The treated plasma contained no detectable VK_3. The calibration standards of VK_3 were prepared by spiking 20 μL of the corresponding working solutions mentioned above into 1.0 mL treated blank plasma to yield the concentrations of 0.03, 0.1, 0.3, 1.0, 3.0, 10, 20, 35 and 50 ng mL^{-1} for VK_3. The quality control (QC) samples were prepared using a different stock solution of VK_3 to obtain the plasma concentrations of 0.08, 0.2, 2.0 and 40 ng mL^{-1}.

All operations were performed under red lamps to avoid any losses due to light sensitivity of VK_3 and plumbagin.

2.3. Sample preparation

2.3.1. Extraction procedure

After being drawn from volunteers, the blood samples were collected into heparinized tubes in ice-water bath, and centrifuged immediately at 4 °C to separate the plasma. An aliquot of 1.0 mL plasma sample was transferred to a glass tube in the ice water bath and 20 μL of the IS working solution (200 ng mL^{-1}) was added and vortex-mixed for 10 s. Then 5 mL ethyl acetate was added to the mixture, vortex-mixed for 3 min, and centrifuged for 8 min at 1700 g. The supernatant was separated and evaporated to dryness under a gentle stream of nitrogen in a water bath of 35 °C.

2.3.2. Derivatization procedure

The residue of the extracted plasma sample was reconstituted with 100 μL MPA methanol solution (122 μg mL^{-1}) and 100 μL methanol-water (1:1, v/v). After being vortex-mixed for 3 min, the mixture was reacted at 70 °C for 2 h, and then centrifuged for 5 min at 17000 g. An aliquot of 5 μL supernatant was then used for the LC–MS/MS analysis.

Download English Version:

https://daneshyari.com/en/article/7679483

Download Persian Version:

https://daneshyari.com/article/7679483

Daneshyari.com