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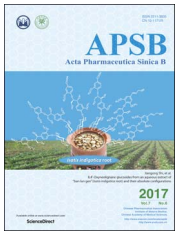

ORIGINAL ARTICLE

Rapid and sensitive liquid chromatography–tandem mass spectrometric method for the quantitative determination of potentially harmful substance 5,5′-oxydimethylenebis (2-furfural) in traditional Chinese medicine injections

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KEY WORDS

5,5′-Oxydimethylenebis (2-furfural);
LC–MS/MS;
Quantitative analytical method;
Traditional Chinese medicine injection;
Quality control

Abstract With the rapid development and wide application of traditional Chinese medicine injection (TCMI), a number of adverse events of some TCMIs have incessantly been reported and have drawn broad attention in recent years. Establishing effective and practical analytical methods for safety evaluation and quality control of TCMI can help to improve the safety of TCMIs in clinical applications. In this study, a sensitive and rapid high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method has been developed and validated for the quantitative determination of potentially harmful substance 5,5′-oxydimethylenebis (2-furfural, OMBF) in TCMI samples. Chromatographic separation was performed on a C18 reversed-phase column (150 mm × 2.1 mm, 5 μm) by gradient elution, using methanol–water containing 0.1% formic acid as mobile phase at the flow rate of 0.3 mL/min. MS/MS detection was performed on a triple quadrupole mass spectrometer with positive electrospray ionization in the multiple reaction-monitoring mode. The method was sensitive with a limit of quantification of 0.3 ng/mL and linear over the range of 0.3–30 ng/mL (r=0.9998). Intra- and inter-day precision for analyte was <9.52% RSD with recoveries in the range 88.0–109.67% at three concentration levels. The validated method was successfully applied to quantitatively determine the

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compound OMBF in TCMIs and glucose injections. Our study indicates that this method is simple, sensitive, practicable and reliable, and could be applied for safety evaluation and quality control of TCMIs and glucose injections.

1. Introduction

As a new dosage form of traditional Chinese medicine (TCM), traditional Chinese medicine injection (TCMI) is considered to be a great achievement of modernization of TCM. TCMIs have been extensively used in China to treat a variety of diseases, including bacterial and viral infections, musculoskeletal disorders, cancer, cardiovascular and cere-brovascular dysfunction.[1–3] However, many serious adverse drug reactions (ADRs) of TCMI in some patients, including anaphylactic shock and fatal anaphylaxis, have been reported in recent years.[4–6] Because TCMI is a complex concoction made from extracts derived from a single herb or a group of herbs in a composite formula, and chemical ingredients in herb medicine, which vary greatly with the geographical origin of the species, time of harvest, cultivation practice, methods of processing, and storage condition, further contribute to the complexity and instability of TCMI.[7–11] Moreover, the quality control of TCMI is still unresolved for its complex composition. Particularly, current methods for monitoring the potentially harmful components in TCMI produced in the procedure of preparation, transportation and storage, are inadequate. Thus, it is imperative and urgent to develop practicable and reliable analytical methods for the purposes of improving the safety and quality of TCMI.

5-Hydroxymethyl furfural (5-HMF, $C_6H_6O_3$, Fig. 1A), a common product of the Maillard reaction, is generated by acid-catalysed thermal dehydration from fructose, saccharose and to a lesser degree from glucose.[12–14] Thus, it can be easily found in many processed sugar- or starch-rich foods and heat-sterilised glucose/fructose solutions for pharmaceutical preparations.[15–17] Excessive 5-HMF can cause skin irritation, damage to striated muscles,[18] liver cancer,[19] or induce aberrant crypt foci in the colon.[20] Therefore, the content of 5-HMF in dextrose injection was strictly limited in *Chinese Pharmacopoeia* and *United States Pharmacopoeia*.[21,22] In our previous study, we have found that there were significant differences in the content of 5-HMF in TCMI samples produced by different manufacturers or even different batches from the same manufacturer[23].

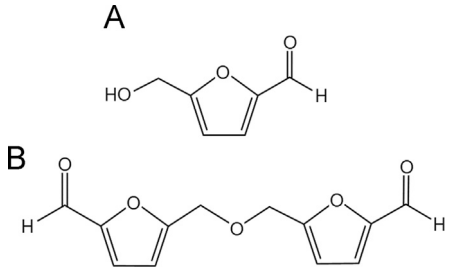

Figure 1 The chemical structures of (A) 5-hydroxymethyl furfural and (B) 5,5′-oxydimethylenebis (2-furfural).

Due to the unstable character of 5-HMF, it is readily hydrolyzes to levulinic acid and formic acid under acidic aqueous conditions.[24,25] In addition, 5-HMF can also participate in hydrogenation, esterification and polymerization reactions.[26] 5,5′-Oxydimethylenebis (2-furfural, OMBF, $C_{12}H_{10}O_5$, Fig. 1B), a coloured polymer of 5-HMF, is a by-product of the Maillard reaction,[27] and is generally produced from acid-catalyzed dehydration reaction of 5-HMF.[28,29] We occasionally found this compound in TCMI samples through imitating the high-temperature/high-pressure sterilization procedures of glucose injection production. Our further study revealed that OMBF has immunosensitizing potential by acting as a neo-antigen or neo-epitope to elicit a mixed type-1 and type-2 immune response, and exposure to OMBF may represent a safety concern for humans.[30] However, no studies have been reported on screening this potentially harmful substance in TCMIs and glucose injections. We herein developed a rapid, simple and sensitive HPLC–MS/MS method for quantitative determination of OMBF in TCMIs and glucose injections. The developed method in this study could rapidly and sensitively determine OMBF with short analysis time, low limits of detection and quantification, and could also contribute to improve the safety and quality of TCMI and glucose injections.

2. Material and methods

2.1. Chemicals and reagents

OMBF was prepared using the procedure outlined by Larousse et al.[31] A purity of >98% was detected by HPLC. Formic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol was purchased from Merck (Muskegon, MI, USA). Pure water was obtained from the Wahaha Group Co., Ltd. (Hangzhou, China). Other chemicals were of analytical grade.

TCMI samples (Shuxuening Injection, Qingkailing Injection, Chaihu Injection, Huangqi Injection, Xuesaitong Injection, Shuanghuanglian Injection, Shenmai Injection, Mailuoning Injection, Zhiyinhuang Injection, Tianmasu Injection, Gegensu Injection, Chuanhuning Injection, Guanxinning Injection, Dengzhanxixin Injection, Shengmai Injection, Ciwujia Injection, Chuanxinlian Injection, Danxiangguanxin Injection, Xiyanping Injection) and glucose injection samples produced by different Chinese pharmaceutical factories were purchased at local pharmacies.

2.2. Sample preparation

Accurately weighed OMBF was dissolved in methanol to prepare a 0.335 mg/mL stock solution. Standard working solutions of OMBF at concentrations of 0.3, 0.6, 1.2, 2.4, 5, 15 and 30 ng/mL were prepared by serial dilutions of the stock solution with methanol. TCMI samples and glucose injection samples were