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Development of a new certified reference material of diosgenin using mass balance approach and Coulometric titration method

Ningbo Gong [a], Baoxi Zhang [a], Fan Hu [a], Hui Du [a], Guanhua Du [b,*], Zhaolin Gao [c], Yang Lu [a,c,*]

[a] Beijing Key Laboratory of Polymorphic Drugs, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[b] Beijing City Key Laboratory of Drug Target Identification and Drug Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China
[c] Key laboratory of Polymorph Pharmaceutical of Shandong Province, Tengzhou 277500, China

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ABSTRACT

Certified reference materials (CRMs) can be used as a valuable tool to validate the trueness of measurement methods and to establish metrological traceability of analytical results. Diosgenin has been selected as a candidate reference material. Characterization of the material relied on two different methods, mass balance method and Coulometric titration method (CT). The certified value of diosgenin CRM is 99.80% with an expanded uncertainty of 0.37% ($k = 2$). The new CRM of diosgenin can be used to validate analytical methods, improve the accuracy of measurement data and control the quality of diosgenin in relevant pharmaceutical formulations.

1. Introduction

Diosgenin (PubChem CID:99474) is a precursor of steroid hormones such as pregnenolone, progesterone, cortisone (Fig. 1), which can be found in several plant species including *Dioscorea* species, fenugreek, and *Costus speciosus* [1]. It is also an active pharmaceutical ingredient (API) in the Dioscoreae Nipponicae Rhizoma, a commonly used traditional Chinese medicine (TCM) and health food [2]. A recent study shows that diosgenin has a variety of biological activities including cancer chemopreventive and therapeutic effects [3–6], inhibiting inflammation [7–9], antioxidant [10–12], among others.

There is a dearth of analytical methods of diosgenin as a chemical and biological marker for quality control of this important medicinal material. Only a few analytical methods like HPLC [13,14] and TLC [15] have been reported till date. Coulometric titration method [16–18] has the advantages of minimal sample intake, rapid measurement, high accuracy, good reproducibility, and no corresponding reference standard requirement. This method opens a new window of opportunity for diosgenin purity assays and related production quality control.

A reference material is a material or substance one or more of whose property values are sufficiently homogenous, stable, and well established to be used for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [19]. The certification process of a candidate CRM depends on the preparation of the sample, the analytical determination and material characteristics, since the metrological traceability of the values of properties will be recorded on one certificate.

In this paper, a new CRM of diosgenin (GBW09515) was developed in compliance with ISO Guide 34 and 35 on reference materials [20,21], including procedures for sample preparation [22], homogeneity study, stability study, value assignment [23–26] and uncertainty evaluation [27–29]. The purity of diosgenin candidate CRM was determined by two independent analytical methods, including mass balance method and Coulometric titration method. The uncertainty evaluation is performed carefully with respect to mass balance method and Coulometric titration method, and then the strategy for purity determination of pure substance was discussed in detail. In China, There is a specialized agency called the NATIONAL Administrative Committee. The duties and rights were to administrate the reference materials (RMs) in China and have the proper authority to approve the reference materials. The certified reference material (CRM) means reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement

* Corresponding authors at: Beijing Key Laboratory of Polymorphic Drugs, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China (Y. Lu). Tel./fax: +86 10 63165212.
E-mail addresses: dugh@imm.ac.cn (G. Du), luy@imm.ac.cn (Y. Lu).

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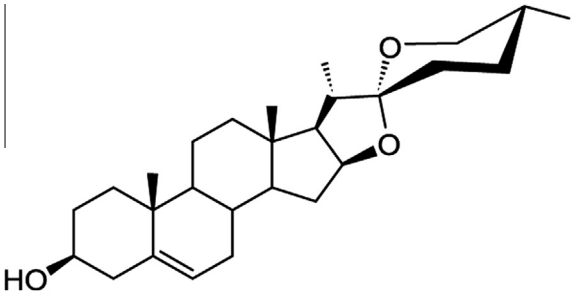

Fig. 1. Chemical structure of diosgenin.

of metrological traceability. The new CRM of diosgenin can be used to validate analytical methods, improve the accuracy of measurement data and control the quality of diosgenin in relevant pharmaceutical formulations.

2. Experiment

2.1. Materials

Diosgenin raw material with a purity of 98.5 percent was obtained from the Chengdou Mansite Botanical Development Co., Ltd (Chengdou, China). The reagents used to prepare mobile phases for the HPLC analyses were all HPLC grade, and were obtained from Thermo Fisher Scientific (USA). All other reagents are analytical grade.

2.2. Instruments and conditions

The HPLC analysis of the samples was conducted using an Agilent 1200 HPLC system (Agilent Technologies, Inc, USA) equipped with an Agilent Eclipse XDB-C18 (250 × 4.6 mm, 5 μm) column, and a diode array UV detector. The mobile phase was composed of acetonitrile and methanol at a ratio of 40:60, the inject concentration of samples was 1 mg mL^{-1} and the detection wavelength was 210 nm. The moisture content of the substances was determined using a Mettler-Toledo DL 31 Karl Fisher titrator. The ash was determined using SX 2.5-10 a resistance furnace, (Shanghai Shuli Instrument Co., Ltd, China), A XS105 balance from Mettlor Toledo was used for weighing samples. The volatile impurity was determined using an Agilent 7890A GC system (Agilent Technologies, Inc, USA), An DM-624 column was used for GC separation, the inject concentration of samples was 10 mg mL^{-1}. The CT analysis of the samples was conducted using a coulometer (Chinese Academy of Medical Sciences, China), The electrolyte composition of 4 mol L^{-1} hydrochloric acid, 1 mol L^{-1} potassium bromide and glacial acetic acid in a 3:3:2 ratio, and the inject concentration of samples was 1.6 mg mL^{-1}.

2.3. Preparation of CRMs

The 150 g raw material was dissolved in 9 L acetone at 60 °C, after stirring for 30 min, the solution was filtered and crystallized under ambient conditions for 12 h, then the crystals were filtered and rinsed several times with acetone and dying over 24 h under 0.01 mbar pressure at a constant temperature of 40 °C. This process repeated twice to acquire the candidate of the diosgenin CRMs. The crystals were then ground into powder, sieved through 150 μm and 74 μm filters, respectively. The resulting 74 μm to 150 μm fraction was homogenized in a rotating mixer prior to filling. The material was used to fill dark glass bottles (ca. 50 mg each), and then the bottles were fusion sealed to prevent air from leaking.

2.4. CT method validation

In a Coulometric titration the titrant is generated electrochemically by constant current from a proper electrolyte. Coulometric titrations are in many ways similar to volumetric titrations: the concentration of the titrant is equivalent to the generating current, and the volume of the titrant is equivalent to the generating time. In coulometric methods, the quantity of electrical charge required to convert a sample of an analyte quantitatively to a different oxidation state is measured.

A Pt-plate (2 × 1 cm^2) was used as the working electrode and a Pt-stick as the indicator electrode, with the electrode reaction, $2Br^- = Br_2 + 2e^-$. About 8 ml electrolytic solutions were used inside the anode compartment, and the same electrolytic solution was added into the cathode compartment to the same high as anode compartment. The generator current was set to 0.9985 mA, and the endpoint was determined using a current up to 20 μA.

The purity of diosgenin was calculated according to the following formula:

$$P_{CT} = \frac{W}{W_{sample}} \times 100\% = \left(\frac{Q_1 \times M}{n \times F} \Big/ \frac{mV_1}{V_2}\right) \times 100\% = \left(\frac{i \times t \times M}{n \times F} \Big/ \frac{mV_1}{V_2}\right) \times 100\% \quad (1)$$

where, Q_1 and m are the past power and mass of the sample, respectively. M is the mole mass of the diosgenin, n is the number of transferring electrons, and F is the Faraday's constant. V_1 and V_2 are the injection volume and the solution volumes, respectively. i is the instrument current, and t is the titration time.

2.4.1. Optimization condition

Prior to the application of the proposed method, the optimal values of the parameters causing variability of the measurements were selected. Thus, content of electrolytic solution, the concentration of electrolytic solution were optimized. Eight different conditions have been investigated. HPLC method was used to evaluate the Coulometric titration reaction. The best component and proportion of electrolyte was 4 mol L^{-1} hydrochloric acid/ 1 mol L^{-1} potassium bromide/glacial acetic acid (3:3:2, v: v: v). From the HPLC patterns, the main content diosgenin has reacted completely, and the impurities were not interfere the titration results.

2.4.2. Calibration curve

Six concentrations of standard solutions were prepared by dissolving appropriate amounts of high purity diosgenin in methanol to achieve a series of concentrations ranging from 1.242 mg mL^{-1} to 2.034 mg mL^{-1}. 250 μL of each concentration of solution was transferred by a microscale injector.

2.4.3. Precision test

The precision of the method was determined by evaluating inter- and intra-day injections of standard solutions. Six injections were performed for each day within three consecutive days.

2.4.4. Repeatability

The repeatability was confirmed with six independent analytical sample solutions prepared from the same sample batch, and variations were expressed by relative standard deviations (RSDs).

2.5. HPLC method validation

For HPLC method, the following parameters were evaluated: selectivity/specificity, linearity, limit of detection (LOD) and quantification (LOQ), intra- and inter-assay precision. For the selectivity/specificity, eight different elution conditions have been