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ORIGINAL ARTICLE

Development of a new ferulic acid certified reference material for use in clinical chemistry and pharmaceutical analysis

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Abstract This study compares the results of three certified methods, namely differential scanning calorimetry (DSC), the mass balance (MB) method and coulometric titrimetry (CT), in the purity assessment of ferulic acid certified reference material (CRM). Purity and expanded uncertainty as determined by the three methods were respectively 99.81%, 0.16%; 99.79%, 0.16%; and 99.81%, 0.26% with, in all cases, a coverage factor (k) of 2 (P=95%). The purity results are consistent indicating that the combination of DSC, the MB method and CT provides a confident assessment of the purity of suitable CRMs like ferulic acid.

Abbreviations: ASTM, American Society for Testing and Materials; CRM, certified reference material; CT, coulometric titrimetry; DAD, diode-array detector; DSC, differential scanning calorimetry; EDQM, European Directorate for Quality Medicine; GUM, Guide to the Expression of Uncertainty in Measurement; ISO, International Organization for Standardization; MB, mass balance; RM, reference material; SI, International System of Units; WHO, World Health Organization

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1. Introduction

Ferulic acid (4-hydroxy-3-methoxycinnamic acid), a phenolic compound present in several plants, is an important pharmaceutically active agent in the treatment of leukopenia and in providing protection against cardiovascular and cerebrovascular disease. In animal studies, ferulic acid was found to attenuate the decrease in parvalbumin expression[1] and prevent the decrease in Akt phosphorylation of Bad induced by focal cerebral ischemic injury[2]. It has also been shown to possess antiatherogenic[3], antidepressant[4] and antioxidant[5] properties.

A reference material (RM) is a material sufficiently homogeneous and stable with respect to one or more specified properties that have been established as fit for its intended use in a measurement process. A certified reference material (CRM) is an RM characterized by a metrologically valid procedure for one or more specified properties accompanied by a certificate that provides the value of the specified property, the associated uncertainty and a statement of metrological traceability[6]. Whilst it is generally agreed that CRMs are crucial to the development of assays required for clinical chemistry and pharmaceutical analysis, the number of commercially available CRMs is very limited. In fact, an analysis by Nogueira et al.[7], revealed that only a few CRMs are available for purchase in the USA and Japan. In China, many research institutions are engaged in the development of CRMs, but compared with the number of marketed drugs and related medical products, the number of CRMs remains very low.

In drug quality control, CRMs are important to ensure the purity of drugs and provide confidence in their efficacy and safety. This in turn requires reliable analytical methods that are not only accurate but also ensure the traceability of the purity values of CRMs. The International Organization for Standardization (ISO) Guideline 34, *General requirements for the competence of reference material producers*[8], recommends that the appropriate approach to drug characterization should be selected based on the type of CRM and its intended use, the competence of the laboratory involved and the quality of methods employed. In addition, ISO Guideline 35, *Reference materials–General and statistical principles for certification*[9], recommends that a laboratory employs two or more independent methods when assessing the purity of a particular CRM.

In the present study, three techniques based on different principles namely differential scanning calorimetry (DSC), the mass balance (MB) method based on high pressure liquid chromatography (HPLC) and coulometric titrimetry (CT) were compared in evaluating the purity of ferulic acid CRM for the first time. The uncertainty evaluation of the three methods was carefully performed according to the ISO *Guide to the Expression of Uncertainty in Measurement* (*GUM*)[10].

2. Material and methods

2.1. Materials

Ferulic acid CRM (GBW 09518) was obtained from the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. Indium (GBW (E) 130182) and arsenious acid solution (GBW 08666) were obtained from the National Institute of Metrology, China.

2.2. Instrumentation

DSC was performed using a Mettler Toledo DSC1/700 equipped with an autosampler. The general performance of the instrument, including heat flow, temperature and enthalpy, was calibrated monthly using indium and the programmed *In Check* method stored in STAR^e software according to the instruction manual. HPLC was performed on an Agilent 1260 system with a diode-array detector (DAD). Weight loss on drying and sulfated ash were determined using a Yiheng vacuum drying oven and muffle furnace, respectively. CT was conducted using a coulometric titrator produced by Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. A Mettler Toledo XS-105 analytical balance was also used. All instruments were subjected to mandatory annual calibration by the National Institute of Metrology to ensure that all measurements could be traced to the International System of Units (SI) and that the uncertainty in every measurement was in a traceability chain.

2.3. Methods

2.3.1. DSC

DSC can be performed rapidly with high precision and does not require a previously characterized CRM[11–13]. Its application to purity assessment is based on the heat of fusion of the sample and on the melting point of the main component which is reduced in the presence of impurities. In a eutectic system, the correlation between melting point depression and the degree of impurity is described by the van't Hoff equation in

$$x_{si} = \frac{\Delta H_f F(T_0 - T_f)}{RT_0^2} = \frac{QMF\Delta T}{mRT_0^2} \quad (1)$$

where x_{si} is the content of solid impurities in the sample, ΔH_f is the molar enthalpy of fusion of the main component, F is the melted fraction, $\Delta T = T_0 - T_f$ is the depression of the melting point, Q is the heat of fusion of the sample, m is its mass, R is the gas constant and M the molar mass of the main component. Generally, samples contain a few volatile impurities the content of which, x_{vi}, is calculated using

$$x_{vi}\% = \frac{m_0 - m_1}{m_0} \times 100 \quad (2)$$

where m_0 is the initial mass of sample and m_1 is the mass of sample determined at constant weight after drying. The actual purity is then calculated using

$$\text{Purity}\% = (1 - x_{si})(1 - x_{vi}) \times 100 \quad (3)$$

DSC was performed under a constant atmosphere of high-purity nitrogen at a flow rate of 50 mL/min and heating rate of 0.5 K/min. The instrument was cooled using a refrigerated cooling system (Huber TC45, Germany). Approximately 3 mg of sample was weighed to within 0.01 mg using a Mettler 40 μL aluminum crucible, hermetically sealed with an appropriate aluminum lid and crimped. An empty crucible and lid of the same type were used as reference.

2.3.2. The MB method

In assessing the purity of a CRM, the total of volatile impurities x_{vi} (water, solvent residues, *etc.*), organic impurities x_{oi}, inorganic impurities (sulphated ash x_{sa} determined as loss on ignition to constant weight) and main component should equal 100%. Accordingly, purity is determined by subtracting total impurities from 100% as expressed

$$\text{Purity}\% = (1 - x_{oi})(1 - x_{vi} - x_{sa}) \times 100 \quad (4)$$