

ORIGINAL ARTICLE

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



Dezhi Yang, Fengfeng Wang, Li Zhang, Ningbo Gong, Yang Lv\*

Beijing Key Laboratory of Polymorphic Drugs, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Received 12 January 2015; received in revised form 4 March 2015; accepted 14 March 2015

## KEY WORDS

Differential scanning calorimetry;  
Mass balance;  
Coulometric titrimetry;  
Certified reference material;  
Uncertainty;  
Ferulic acid

**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.

© 2015 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*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

\*Corresponding author. Tel./fax: +86 10 63165212.

E-mail address: [luy@imm.ac.cn](mailto:luy@imm.ac.cn) (Yang Lv).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

<http://dx.doi.org/10.1016/j.apsb.2015.03.009>

2211-3835 © 2015 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 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<sup>1</sup> and prevent the decrease in Akt phosphorylation of Bad induced by focal cerebral ischemic injury<sup>2</sup>. It has also been shown to possess antiatherogenic<sup>3</sup>, antidepressant<sup>4</sup> and antioxidant<sup>5</sup> 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<sup>6</sup>. 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.<sup>7</sup>, 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*<sup>8</sup>, 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*<sup>9</sup>, 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)*<sup>10</sup>.

## 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<sup>e</sup> 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<sup>11–13</sup>. 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

$$x_{\text{si}} = \frac{\Delta H_{\text{f}} F (T_0 - T_{\text{f}})}{RT_0^2} = \frac{QMF\Delta T}{mRT_0^2} \quad (1)$$

where  $x_{\text{si}}$  is the content of solid impurities in the sample,  $\Delta H_{\text{f}}$  is the molar enthalpy of fusion of the main component,  $F$  is the melted fraction,  $\Delta T = T_0 - T_{\text{f}}$  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,  $x_{\text{vi}}$ , is calculated using

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

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

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

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  $\mu\text{L}$  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  $x_{\text{vi}}$  (water, solvent residues, etc.), organic impurities  $x_{\text{oi}}$ , inorganic impurities (sulphated ash  $x_{\text{sa}}$  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

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

The values of  $x_{oi}$  and  $x_{sa}$  are in turn calculated using Eqs. (5) and (6) respectively:

$$x_{oi} = \frac{\sum_{i=1}^n A_{oi}f_{oi}}{A_{mc}f_{mc} + \sum_{i=1}^n A_{oi}f_{oi}} \quad (5)$$

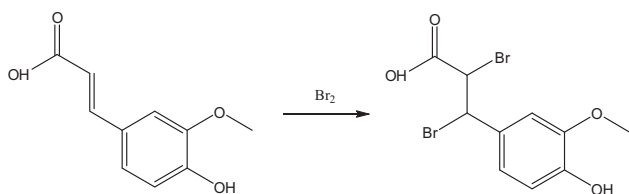
$$x_{sa}\% = \frac{m_3}{m_2} \times 100 \quad (6)$$

where  $A_{oi}$  and  $A_{mc}$  are the chromatographic peak areas of the organic impurities and main component respectively,  $f_i$  and  $f_{mc}$  are the corresponding correction factors,  $m_2$  is the initial mass of sample and  $m_3$  is the mass of sulfated ash. This method is accurate and recommended by the World Health Organization (WHO)<sup>14</sup>, European Pharmacopoeia<sup>15</sup>, International Pharmacopoeia<sup>16</sup> and the National Metrology Institute organized by the Bureau International des Poids et Mesures<sup>17</sup>.

In this study, chromatography was carried out on an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm) using a mobile phase composed of aqueous 0.5% acetic acid:acetonitrile 85:15 (v/v) delivered at 1 mL/min. The injection volume was 10 μL, column temperature 30 °C and detection wavelength 320 nm. Volatile impurities and inorganic impurities were determined as indicated above.

### 2.3.3. CT

CT is an important method of electrochemical analysis based on Faraday's law of electrolysis<sup>18</sup>. Here the relationship between reactive substances and consumption of electricity is described by



**Figure 1** The addition reaction between ferulic acid and bromine in the coulometric titration.

$$W = \frac{QM}{nF} = \frac{i \times t \times M}{n \times F} \quad (7)$$

where  $W$  is the weight (g) of reacting substance,  $Q$  is the electricity through the electrode (the product of current  $i$  and time  $t$ ),  $M$  is the molar mass of the reactive substance,  $F$  is the Faraday constant and  $n$  is the number of transferring electrons. Therefore, the purity of the sample can be calculated using

$$\begin{aligned} \text{Purity}\% &= \frac{W_{\text{cal}}}{W_{\text{real}}} \times 100 = \left( \frac{Q \times M}{n \times F} / C \times V_1 \right) \times 100 \\ &= \left( \frac{i \times t \times M}{n \times F} / \frac{m_4}{V_2} \times V_1 \right) \times 100 \end{aligned} \quad (8)$$

where  $W_{\text{cal}}$  is the amount (g) of reactive substance,  $W_{\text{real}}$  is the amount (g) of sample,  $C$  is the concentration (g/μL) of the reactive substance (equal to its mass  $m_4$  divided by the volume ( $V_2$  μL) of the sample solution) and  $V_1$  is the injection volume (μL) of the sample.

Purity determination of ferulic acid by CT is based on the reaction between its carbon-carbon double bond and bromine produced from the KBr electrolyte. The reaction shown in Fig. 1 involves the transfer of 2 electrons. In this study, the working and indicator electrodes were platinum and the electrolyte solution was KBr (4 mol/L) and HCl (8 mol/L) in a 1:1 ratio. The generator current was 0.9985 mA and calibration was performed using a solution of arsenious acid. The end point of the titration was indicated by the increase in current.

### 2.4. Measurement uncertainty

#### 2.4.1. DSC

According to the GUM and Eq. (3), the combined standard uncertainty of purity determined by DSC can be calculated using

$$u_{\text{DSC}}^2(\text{purity}) = \left( \frac{\partial f}{\partial x_{si}} u_c(x_{si}) \right)^2 + \left( \frac{\partial f}{\partial x_{vi}} u_c(x_{vi}) \right)^2 \quad (9)$$

$$u_{\text{DSC}}^2(\text{purity}) \approx u_c^2(x_{si}) + u_c^2(x_{vi}) \quad (10)$$

where  $u_c(x_{si})$  and  $u_c(x_{vi})$  are calculated using Eqs. (11) and (12) respectively:

$$\left( \frac{u_c(x_{si})}{x_{si}} \right)^2 = \left( \frac{u_c(Q)}{Q} \right)^2 + \left( \frac{u_c(M)}{M} \right)^2 + \left( \frac{u_c(F)}{F} \right)^2 + \left( \frac{u_c(\Delta T)}{\Delta T} \right)^2$$

**Table 1** Purity of ferulic acid CRM determined by DSC, MB method, CT and levels of volatile and inorganic impurities.

Measurement	Purity			Impurity	
	DSC (%)	MB (%)	CT (%)	Loss on drying (%)	Sulphated ash (%)
1	99.94	99.97	99.88	0.16	0.06
2	99.91	99.97	99.83	0.12	0.04
3	99.98	99.98	99.77	0.16	0.06
4	99.97	99.97	99.82	0.14	0.04
5	99.96	99.91	99.77	0.08	0.06
6	99.92	99.97	99.83	0.18	0.04
7	99.91	99.96	99.77	0.14	0.06
8	99.95	99.92	99.82	0.11	0.04
9	99.94	99.96	99.83	0.15	0.04
10	99.96	99.95	99.82	0.20	0.04
Mean	99.94	99.96	99.88	0.13	0.04
SD	0.000246	0.000232	0.000347	0.000343	0.000103

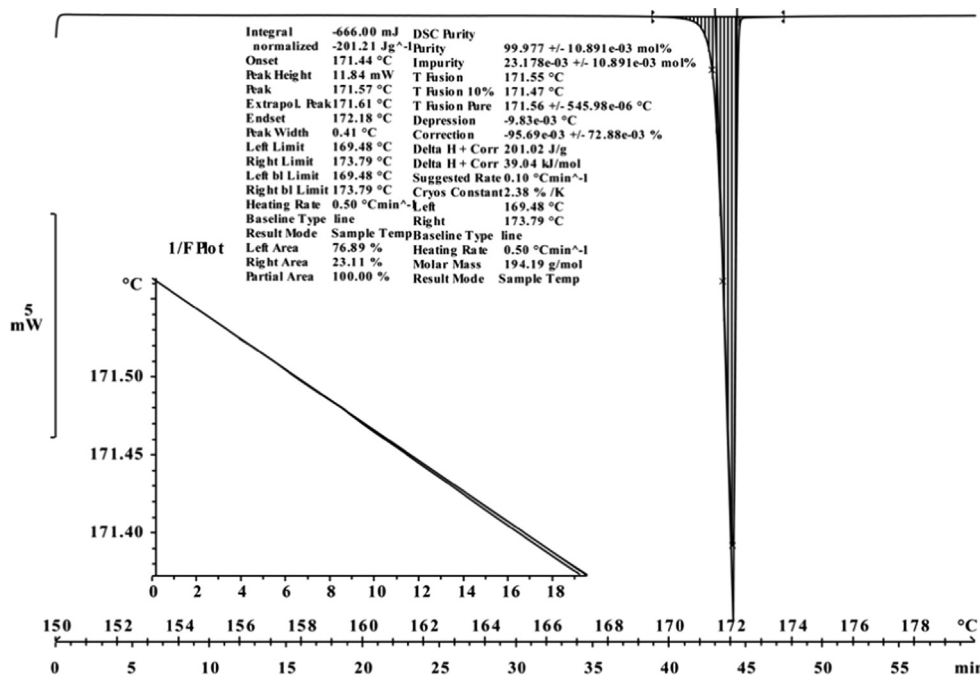


Figure 2 The DSC curve of ferulic acid CRM.

$$+ \left( \frac{u_c(m)}{m} \right)^2 + \left( \frac{u_c(T_0)}{T_0} \right)^2 + \left( \frac{u_c(f_1)}{f_1} \right)^2 \quad (11)$$

$$u_c(x_{vi}) = \sqrt{\left( \frac{\partial(x_{vi})}{\partial m_0} \right)^2 \cdot (u_{m0})^2 + \left( \frac{\partial(x_{vi})}{\partial m_1} \right)^2 \cdot (u_{m1})^2}$$

$$= \sqrt{\left( \frac{m_1}{m_0^2} \right)^2 \cdot (u_{m0})^2 + \left( -\frac{1}{m_0} \right)^2 \cdot (u_{m1})^2} \quad (12)$$

Here  $f_1$  is the correction factor which mainly includes the linear regression and integration performed by the software for purity analysis.

#### 2.4.2. The MB method

On HPLC-MS analysis, it was found that the amounts of impurities in the sample were too low to be identified. Therefore it was assumed that  $f_{oi} = f_{inc}$  and Eq. (5) becomes

$$x_{oi} = \frac{\sum_{i=1}^n A_{oi}}{A_{mc} + \sum_{i=1}^n A_{oi}} = \frac{\sum_{i=1}^n A_{oi}}{A_{tot}} \quad (13)$$

where  $A_{tot}$  is the chromatographic peak area of all components archived from the Agilent workstation software.

According to the GUM and Eq. (4), the combined standard uncertainty of purity determined by the MB method  $u_{MB}$  can be calculated using

$$u_{MB}^2(\text{purity}) = \left( \frac{\partial f}{\partial x_{oi}} u_c(x_{oi}) \right)^2 + \left( \frac{\partial f}{\partial x_{vi}} u_c(x_{vi}) \right)^2 + \left( \frac{\partial f}{\partial x_{sa}} u_c(x_{sa}) \right)^2 \quad (14)$$

$$u_{MB}^2(\text{purity}) \approx u_c^2(x_{oi}) + u_c^2(x_{vi}) + u_c^2(x_{sa}) \quad (15)$$

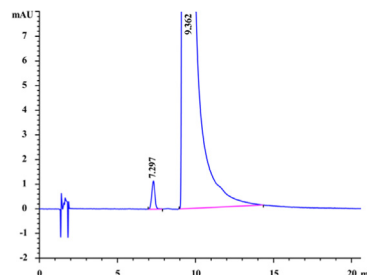


Figure 3 The HPLC-DAD chromatogram of ferulic acid CRM.

where  $u_c(x_{oi})$  and  $u_c(x_{sa})$  are in turn determined using Eq. (16) and (17):

$$\left( \frac{u_c(x_{oi})}{x_{oi}} \right)^2 = \left( \frac{u_c(A_{oi})}{A_{oi}} \right)^2 + \left( \frac{u_c(A_{tot})}{A_{tot}} \right)^2 + \left( \frac{u_c(f_2)}{f_2} \right)^2 \quad (16)$$

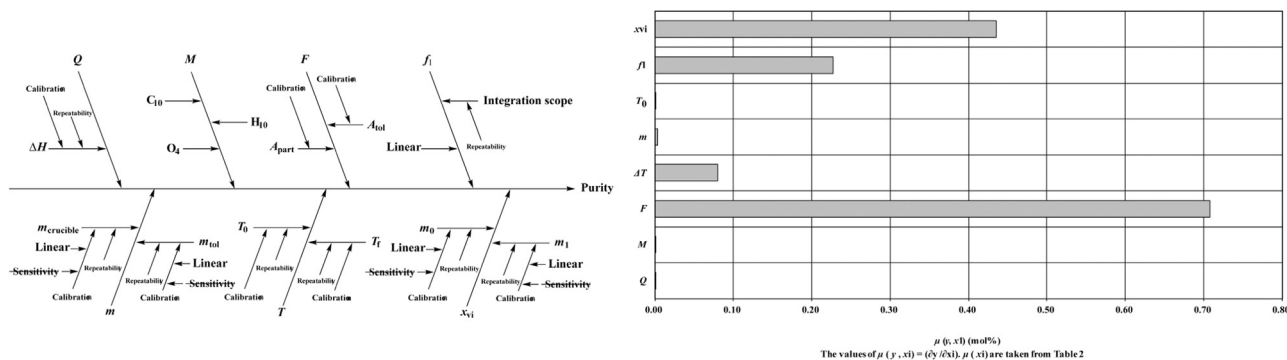
$$u_c(x_{sa}) = \sqrt{\left( \frac{\partial(x_{sa})}{\partial m_2} \right)^2 \cdot (u_{m2})^2 + \left( \frac{\partial(x_{sa})}{\partial m_3} \right)^2 \cdot (u_{m3})^2}$$

$$= \sqrt{\left( \frac{1}{m_2} \right)^2 \cdot (u_{m2})^2 + \left( \frac{m_3}{m_2^2} \right)^2 \cdot (u_{m3})^2} \quad (17)$$

Here  $f_2$  is the correction factor which mainly includes the integration performed by the software for purity analysis.

#### 2.4.3. CT

According to the GUM and Eq. (8) and given that  $n$  and  $F$  are constants in Eq. (8), the combined standard uncertainty of purity



**Figure 4** Possible sources and contributions to uncertainty in the purity determination of ferulic acid CRM by DSC.

**Table 2** Quantification of the relevant uncertainties in the determination of the purity of ferulic acid CRM by DSC, MB method and CT.

Method	Parameter
DSC	$u_c(Q)/Q$ $8.60 \times 10^{-4}$
	$u_c(M)/M$ $2.41 \times 10^{-5}$
	$u_c(F)/F$ $7.08 \times 10^{-1}$
	$u_c(\Delta T)/\Delta T$ $8.04 \times 10^{-2}$
	$u_c(m)/m$ $2.49 \times 10^{-2}$
	$u_c(T_0)/T_0$ $8.76 \times 10^{-4}$
	$u_c(f_1)/f_1$ $2.27 \times 10^{-1}$
	$u_c(x_{vi})/x_{vi}$ $4.35 \times 10^{-1}$
MB method	$u_c(A_{oi})/A_{oi}$ $1.68 \times 10^{-1}$
	$u_c(A_{tot})/A_{tot}$ $4.03 \times 10^{-3}$
	$u_c(f_2)/f_2$ $1.83 \times 10^{-1}$
	$u_c(x_{vi})/x_{vi}$ $4.35 \times 10^{-1}$
	$u_c(x_{sa})/x_{sa}$ 1.15
CT	$u_c(i)/i$ $5.28 \times 10^{-4}$
	$u_c(t)/t$ $2.77 \times 10^{-4}$
	$u_c(M)/M$ $2.41 \times 10^{-5}$
	$u_c(m_4)/m_4$ $5.52 \times 10^{-4}$
	$u_c(V_1)/V_1$ $5.10 \times 10^{-4}$
	$u_c(V_2)/V_2$ $3.27 \times 10^{-4}$
	$u_c(f_3)/f_3$ $2.40 \times 10^{-6}$

determined by CT is given by

$$\left(\frac{u_{CT}(\text{purity})}{\text{Purity}}\right)^2 \approx \left(\frac{u_c(i)}{i}\right)^2 + \left(\frac{u_c(t)}{t}\right)^2 + \left(\frac{u_c(M)}{M}\right)^2 + \left(\frac{u_c(m_4)}{m_4}\right)^2 + \left(\frac{u_c(V_1)}{V_1}\right)^2 + \left(\frac{u_c(V_2)}{V_2}\right)^2 + \left(\frac{u_c(f_3)}{f_3}\right)^2 \quad (18)$$

where  $f_3$  is the correction factor which mainly includes the uncertainty in the current calibration of the coulometric titrator used in this study.

2.4.4. Expanded measurement uncertainty

When the measurement uncertainty is evenly distributed and the confidence interval is 0.95, the coverage factor  $k$  of the extended measurement uncertainty  $U$  is 2. Therefore, in the present study, the expanded measurement uncertainty  $U$  of these methods was calculated using

$$U_{\text{method}} = u_{\text{method}}(\text{purity}) \times 2 \quad (19)$$

3. Results

The purities and impurities determined using the different methods are listed in Table 1. Data from each method were analyzed using Gravel booth inspection and no outliers were found. The results obtained by DSC, the MB method and CT were not significantly different based on Students  $t$ -tests.

3.1. DSC

The heat flow temperature curve and plot of  $T_f$  versus  $1/F$  for DSC of ferulic acid CRM are shown in Fig. 2. As indicated by Eq. (1), data for the latter fits well to a straight line. The purity of the sample determined by DSC according to Eq. (3) was 99.81%. The combined standard uncertainty calculated using Eqs. (9)–(12) was 0.08% giving a  $U$  value of 0.16% ( $k=2, P=95\%$ ).

3.2. The MB method

A typical HPLC chromatogram of ferulic acid CRM is shown in Fig. 3. The main peak at 9.362 min indicates ferulic acid and the peak at 7.297 min is clearly due to an impurity. The purity of the sample determined using the MB method according to Eq. (4) was 99.79%. The combined standard uncertainty calculated using Eqs. (14)–(17) was 0.08% giving a  $U$  value of 0.16% ( $k=2, P=95\%$ ).

3.3. CT

The purity of the sample determined by CT according to Eq. (8) was 99.81%. The combined standard uncertainty calculated according to Eq. (18) was 0.13% giving a  $U$  value of 0.26% ( $k=2, P=95\%$ ).

4. Discussion

4.1. DSC

Purity determination by DSC depends on the heating rate of the instrument which according to the American Society for Testing and Materials (ASTM) E 928-08<sup>19</sup> should be in the range 0.3–0.7 K/min. Using different heating rates in this range indicated that heating rate has no significant effect on purity determination and accordingly the heating rate was set to 0.5 K/min.

Several factors may affect the results of purity determination by DSC. Here the possible sources of uncertainty in the DSC method were carefully identified and calculated based on Guideline CG 4 of the Eurachem/Citac working group: *Quantifying uncertainty in analytical measurement*<sup>20</sup>. The relevant sources of uncertainty and their contributions to purity determination by DSC are shown in Fig. 4 and the results are shown in Tables 1 and 2.

4.2. The MB method

The MB method is commonly used to assess sample purity and is generally applied as HPLC with mass spectrometry (MS) to identify impurities. However, in this study impurities in the sample produced no signal in MS despite investigating the effect

of increasing sample concentration and chromatographic run time and the use of gradient elution and multi-wavelength detection. As a result, the correction factor for each impurity could not be determined and purity was assessed by HPLC with UV detection.

As for DSC, the possible sources of uncertainty in the MB method were identified and quantified based on Guideline CG 4 of

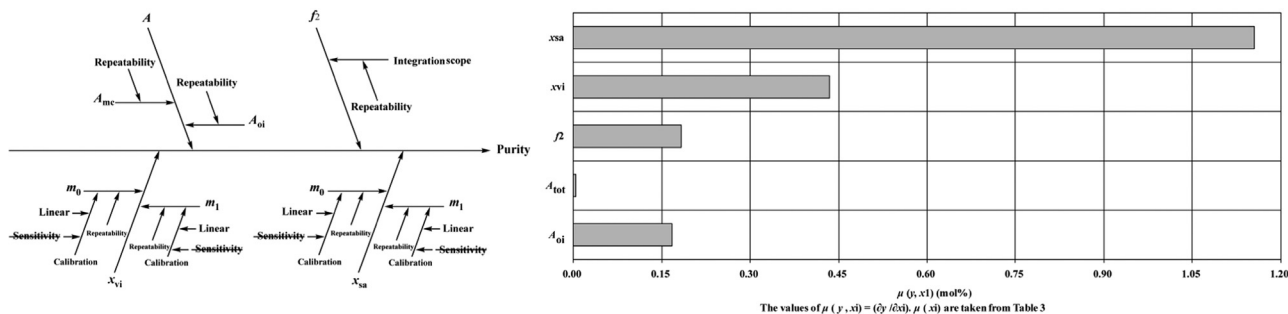


Figure 5 Possible sources and contributions to uncertainty in the purity determination of ferulic acid CRM by the MB method.

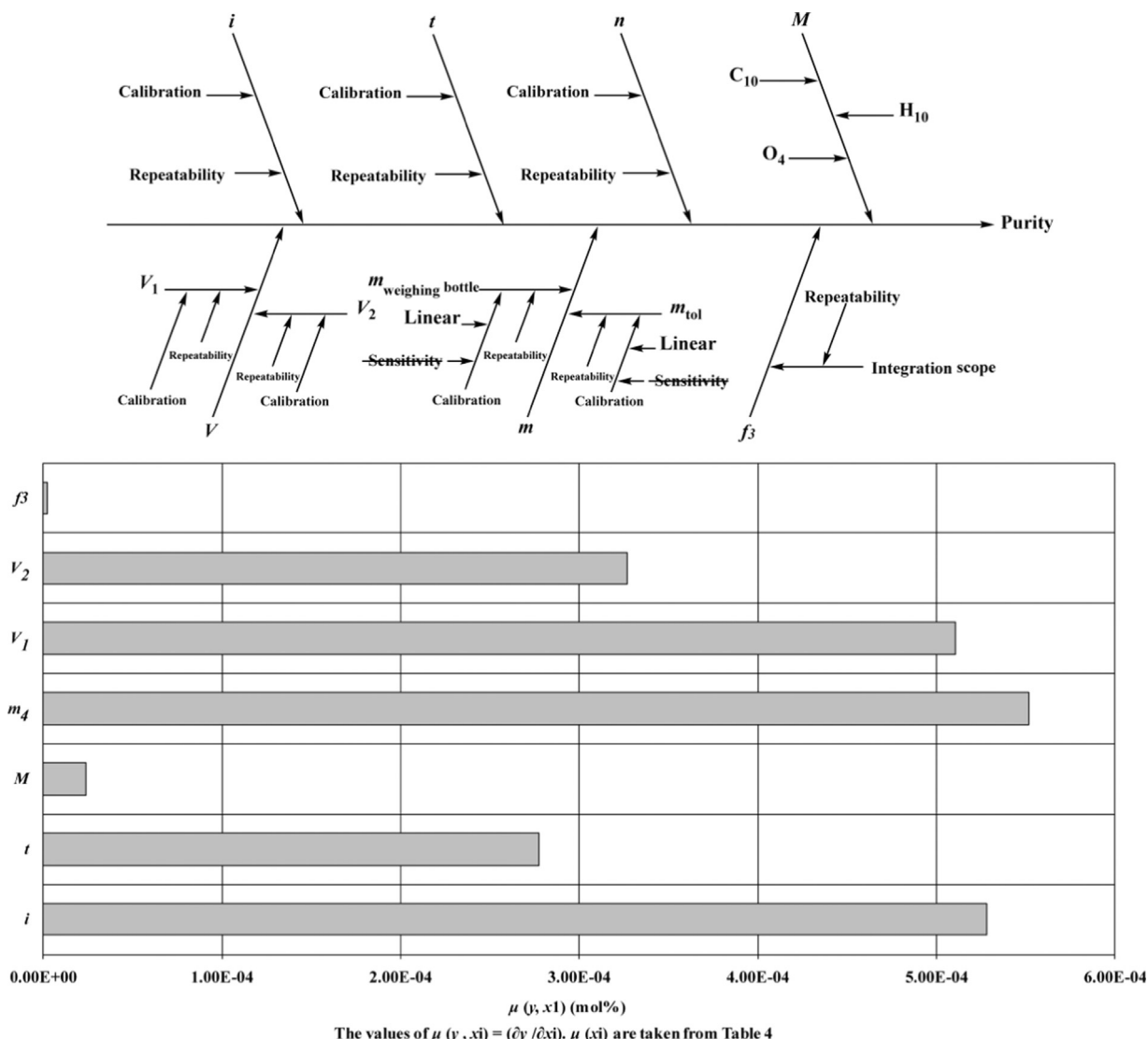


Figure 6 Possible sources and contributions to uncertainty in the purity determination of ferulic acid CRM by CT.



the Eurachem/Citac working group<sup>20</sup>. The sources of uncertainty and their contributions to purity determination are shown in Fig. 5 and the results in Tables 1 and 2.

#### 4.3. CT

The limitation of this method is that organic impurities containing unsaturation (double bonds, activated benzene rings) can also undergo addition reactions with bromine and bias the results. However, a previous study showed that the destruction experiment does not produce any degradation products in the sample.

The possible sources of uncertainty in the CT method were carefully identified and are shown in Fig. 6. The corresponding uncertainties were calculated and are given in Tables 1 and 2.

## 5. Conclusions

In this study, three analytical techniques based on different principles were used to determine the purity of ferulic acid CRM for the first time. Consistent results were obtained providing confidence in the purity assessment. CT and DSC have the advantages of practicality, speed and reproducibility and do not require a previously characterized reference standard. However, they have a narrow working range which limits their applicability for purity determination. They therefore act as complementary techniques to the MB method for substances that react with bromine (CT) or for detecting impurities which can form a eutectic mixture with the principal component (DSC).

Ferulic acid CRM has been approved and assigned as a primary reference material by the General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China. The CRM number is GBW 09518, and its purity is 99.8% with an expanded uncertainty of 0.5% ( $k=2$ ,  $P=0.95$ ).

## Acknowledgments

The work was financially supported by the Ministry of Science and Technology of the People's Republic of China (No. 2007FY130100) and Major Scientific and Technological Special Project for "Significant New Drugs Creation" of China during the 12th Five-Year Plan Period (No. 2012ZX09301002-001-013).

## References

1. Sung JH, Kim MO, Koh PO. Ferulic acid attenuates the focal cerebral ischemic injury-induced decrease in parvalbumin expression. *Neurosci Lett* 2012;**516**:146–50.
2. Koh PO. Ferulic acid prevents the cerebral ischemic injury-induced decrease of Akt and Bad phosphorylation. *Neurosci Lett* 2012;**507**:156–60.
3. Kwon EY, Do GM, Cho YY, Park YB, Jeon SM, Choi MS. Anti-atherogenic property of ferulic acid in apolipoprotein E-deficient mice fed Western diet: comparison with clofibrate. *Food Chem Toxicol* 2010;**48**:2298–303.
4. Zeni AL, Zomkowski AD, Maraschin M, Rodrigues AL, Tasca CI. Ferulic acid exerts antidepressant-like effects in the tail suspension test in mice: evidence for the involvement of the serotonergic system. *Eur J Pharmacol* 2012;**679**:68–74.
5. Kanski J, Aksenova M, Stoyanova A, Butterfield DA. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems *in vitro*: structure-activity studies. *J Nutr Biochem* 2002;**13**:273–81.
6. International Organization for Standardization (ISO). In: *Guideline 30/Amd.1: terms and definitions used in connection with reference materials*. Geneva, CH: ISO; 2008 amd. 1.
7. Nogueira R, Garrido BC, Borges RM, Silva GEB, Queiroz SM, Cunha VS. Development of a new sodium diclofenac certified reference material using the mass balance approach and <sup>1</sup>H qNMR to determine the certified property value. *Eur J Pharm Sci* 2013;**48**:502–13.
8. International Organization for Standardization (ISO). *Guideline 34: general requirements for the competence of reference material producers*. Geneva, CH: ISO; 2009.
9. International Organization for Standardization (ISO). *Guideline 35: reference materials – general and statistical principles for certification*. Geneva, CH: ISO; 2006.
10. International Organization for Standardization (ISO). *Guide to expression of uncertainty in measurement (GUM)*. Geneva, CH: ISO; 1995.
11. Höhne GWH, Hemminger WF, Flammersheim HJ. *Differential scanning calorimetry*. 2nd ed. Heidelberg: Springer; 2003.
12. Gao JM, Ding LX, Hu CQ. A comparative uncertainty study of the purity assessment of chemical reference substances using differential scanning calorimetry (DSC) and mass balance method. *Thermochim Acta* 2011;**525**:1–8.
13. Lim OB, Oliver EA. The purity determination of three compendial steroids by differential scanning calorimetry. *Purity Determinations by Thermal Methods*. ASTM, Philadelphia; 1984.
14. World Health Organization (WHO). *General guidelines for the establishment, maintenance and distribution of chemical reference substance*. WHO Technical Reports Series No 885, Part A, 5. Geneva: WHO; 1999.
15. European Directorate for Quality Medicine (EDQM). *Establishment of reference standards, European pharmacopoeia*. 6th ed. Strasbourg: EDQM; 2008.
16. World Health Organization (WHO). *Evaluation of chemical reference substance, supplementary information, Part A, 3.3, international pharmacopoeia*. 3rd ed. Geneva: WHO; 2003.
17. Ma K, Wang H, Zhao M, Xing J. Purity determination and uncertainty evaluation of theophylline by mass balance method, high performance liquid chromatography and differential scanning calorimetry. *Anal Chim Acta* 2009;**650**:227–33.
18. Gong NB, Liu SC, Xu W, Shi YQ, Du GH, Lu Y. Purity assessment of luteolin reference material candidates by coulometric titration method. *Anal Methods* 2013;**5**:784–8.
19. ASTM. *Standard E 928-08: test method for purity by differential scanning calorimetry*. Pennsylvania: ASTM International; 2008.
20. Eurachem/Citac Working Group. *Guideline CG 4: quantifying uncertainty in analytical measurement*. 2nd ed. London: Eurachem/Citac Working Group; 2000.