1. Homogeneity studies

1.1 Homogeneity studies of genistin candidate CRM

Table 1

The results of homogeneity

Random No.	Purity (%)		
	1	2	3
20	99.67	99.67	99.71
46	99.66	99.66	99.67
49	99.65	99.69	99.66
104	99.65	99.68	99.66
128	99.68	99.67	99.69
186	99.65	99.64	99.68
316	99.67	99.67	99.68
342	99.70	99.66	99.68
377	99.65	99.67	99.68
400	99.68	99.69	99.70
444	99.68	99.66	99.68
453	99.66	99.68	99.69
461	99.65	99.66	99.65
465	99.67	99.66	99.66
487	99.68	99.65	99.67

Table 2

The results of ANOVA

	SS	df	MS	F	P-value	F crit
Between	4.39×10^{-7}	14	3.14×10^{-8}	1.47	0.18	2.04
Within	6.40×10^{-7}	30	2.13×10^{-8}			
Total	1.08×10^{-6}	44				

$$u_h = \sqrt{\left(MS_{between} - MS_{within}\right)/n} = \sqrt{\left(3.14 \times 10^{-8} - 2.13 \times 10^{-8}\right)/3} = 5.78 \times 10^{-5}$$

1.2 Homogeneity studies of genistein CRM

Table 3

The results of homogeneity

Random No.	Purity（%）		
	1	2	3
27	99.28	99.31	99.33
41	99.30	99.31	99.30
59	99.32	99.30	99.32
128	99.30	99.32	99.30
151	99.33	99.31	99.31
189	99.31	99.32	99.32
209	99.30	99.30	99.32
210	99.31	99.31	99.31
228	99.32	99.29	99.30
262	99.33	99.33	99.31
343	99.31	99.29	99.30
345	99.29	99.31	99.31
395	99.29	99.30	99.30
473	99.30	99.32	99.30
495	99.32	99.31	99.32

Table 4

The results of ANOVA

	SS	df	MS	F	P-value	F crit
Between	2.25×10^{-7}	14	1.61×10^{-8}	1.19	0.33	2.04
Within	4.07×10^{-7}	30	1.36×10^{-8}			
Total	6.32×10^{-7}	44				

$$u_h = \sqrt{(MS_{between} - MS_{within})/n} = \sqrt{(1.61 \times 10^{-8} - 1.36 \times 10^{-8})/3} = 2.91 \times 10^{-5}$$

2. Stability studies

2.1 Short-term stability of genistin candidate CRM

Table 5

The results of short-term stability

Conditions	Time (day)	Purity (%)				S
		1	2	3	Mean	
high temperature (60 °C)	0	99.64	99.67	99.73	99.68	0.000458
	7	99.71	99.69	99.72	99.71	0.000153
	14	99.67	99.67	99.66	99.67	0.000058
high humidity (90 % RH)	0	99.68	99.68	99.66	99.67	0.000115
	7	99.71	99.69	99.68	99.69	0.000153
	14	99.66	99.69	99.66	99.67	0.000173
high illumination (4500 lx)	0	99.67	99.65	99.72	99.68	0.000361
	7	99.67	99.67	99.69	99.68	0.000115
	14	99.66	99.72	99.69	99.69	0.000300

Table 6

The results of t-test

t value	Time (day)		t_{crist}
	7	14	
high temperature	0.956	0.500	
high humidity	1.809	0.277	2.776
high illumination	0.152	0.369	

Table 7

The results of uncertainty evaluation of short-term stability

Conditions	Linear equation	S	u_{sts}(Conditions)	u_{sts}
high temperature	$y = -9.52 \times 10^{-6}x + 9.97 \times 10^{-1}$	3.13×10^{-4}	4.42×10^{-4}	
high humidity	$y = -2.38 \times 10^{-6}x + 9.97 \times 10^{-1}$	3.80×10^{-4}	5.38×10^{-4}	8.98×10^{-4}
high illumination	$y = 7.14 \times 10^{-6}x + 9.97 \times 10^{-1}$	4.00×10^{-4}	5.66×10^{-4}	

2.2 Short-term stability of genistein candidate CRM

Table 8

The results of short-term stability

Conditions	Time (day)	Purity (%)				S
		1	2	3	Mean	
high temperature (60 °C)	0	99.32	99.31	99.30	99.31	0.000100
	7	99.32	99.29	99.30	99.30	0.000153
	14	99.29	99.32	99.31	99.31	0.000153
high humidity (90 % RH)	0	99.32	99.31	99.30	99.31	0.000100
	7	99.30	99.32	99.29	99.30	0.000153
	14	99.30	99.28	99.33	99.30	0.000252
high illumination (4500 lx)	0	99.28	99.29	99.32	99.30	0.000208
	7	99.30	99.29	99.31	99.30	0.000100
	14	99.29	99.33	99.31	99.31	0.000200

Table 9

The results of t-test

t value	Time (day)		t_{crist}
	7	14	
high temperature	0.632	0.316	
high humidity	0.632	0.426	2.776
high illumination	0.250	0.800	

Table 10

The results of uncertainty evaluation of short-term stability

Conditions	Linear equation	S	u_{sts}(Conditions)	u_{sts}
high temperature	$y = -2.38 \times 10^{-6}x + 9.93 \times 10^{-1}$	1.50×10^{-4}	2.12×10^{-4}	
high humidity	$y = -4.76 \times 10^{-6}x + 9.93 \times 10^{-1}$	1.56×10^{-4}	2.21×10^{-4}	3.27×10^{-4}
high illumination	$y = 9.52 \times 10^{-6}x + 9.93 \times 10^{-1}$	8.16×10^{-5}	1.15×10^{-4}	

2.3 Long-term stability of genistin candidate CRM

Table 11

The results of long-term stability

Time (month)	Purity (%)							S
	1	2	3	4	5	6	Mean	
0	99.66	99.67	99.68	99.67	99.65	99.69	99.67	0.00014
1	99.67	99.69	99.64	99.68	99.65	99.67	99.67	0.00019
2	99.65	99.66	99.71	99.66	99.68	99.65	99.67	0.00023
4	99.65	99.67	99.7	99.67	99.67	99.65	99.67	0.00018
6	99.67	99.67	99.69	99.66	99.67	99.68	99.67	0.00010
12	99.68	99.67	99.68	99.67	99.66	99.65	99.67	0.00012

Table 12

The results of uncertainty evaluation of long-term stability

Linear equation	S	u_{lts}
$y = 7.75 \times 10^{-7}x + 9.97 \times 10^{-1}$	3.82×10^{-4}	4.66×10^{-4}

2.4 Long-term stability of genistein candidate CRM

Table 13

The results of long-term stability

Time (month)	Purity (%)							S
	1	2	3	4	5	6	Mean	
0	99.30	99.31	99.32	99.33	99.32	99.31	99.32	0.00010
1	99.29	99.30	99.33	99.31	99.34	99.33	99.32	0.00020
2	99.30	99.33	99.34	99.31	99.30	99.30	99.31	0.00018
4	99.30	99.32	99.32	99.34	99.29	99.32	99.32	0.00018
6	99.30	99.28	99.32	99.32	99.30	99.32	99.31	0.00016
12	99.31	99.33	99.30	99.29	99.32	99.31	99.31	0.00014

Table 14

The results of uncertainty evaluation of long-term stability

Linear equation	S	u_{lts}
$y = -5.79 \times 10^{-6}x + 9.93 \times 10^{-1}$	1.89×10^{-4}	2.30×10^{-4}

3. Characterization Studies

3.1. Purity determination by Differential scanning calorimetry method

Table 15

The results of purities determined by DSC

No.	Purity (mol%)	
	genistin	genistein
1	99.65	99.32
2	99.68	99.31
3	99.66	99.26
4	99.71	99.30
5	99.68	99.28
6	99.66	99.31
7	99.71	99.30
8	99.70	99.27
9	99.66	99.39
10	99.69	99.30
mean	99.68	99.30
s	0.000233	0.000357

Table 16

The results of uncertainty evaluation of DSC method

	$u_c(Q)/Q$	$u_c(M)/M$	$u_c(F)/F$	$u_c(\Delta T)/\Delta T$	$u_c(m)/m$	$u_c(T_0)/T_0$	$u_c(f_1)/f_1$	$u_P(DSC)$
genistin	1.01×10^{-3}	1.61×10^{-5}	8.16×10^{-2}	8.98×10^{-3}	1.70×10^{-2}	5.29×10^{-4}	4.55×10^{-2}	3.05×10^{-4}
genistein	8.54×10^{-4}	2.59×10^{-5}	3.68×10^{-2}	1.48×10^{-2}	2.72×10^{-2}	4.96×10^{-4}	2.91×10^{-2}	3.91×10^{-4}

3. 2. Purity determination by Coulometric titrimetry method

Table 17

The results of purities determined by CT

No.	Purity (%)	
	genistin	genistein
1	99.68	99.31
2	99.61	99.37
3	99.58	99.30
4	99.83	99.20
5	99.64	99.42
6	99.72	99.33
7	99.71	99.33
8	99.70	99.30
9	99.64	99.32
10	99.69	99.42
mean	99.68	99.33
s	0.000679	0.000642

Table 18

The results of uncertainty evaluation of CT method

	$u_c(i)/i$	$u_c(t)/t$	$u_c(M)/M$	$u_c(m)/m$	$u_c(V_1)/V_1$	$u_c(V_2)/V_2$	$u_c(f_2)/f_2$	$u_P(CT)$
genistin	4.60×10^{-4}	4.59×10^{-4}	1.61×10^{-5}	5.68×10^{-4}	5.10×10^{-4}	8.16×10^{-5}	2.40×10^{-6}	1.15×10^{-3}
genistein	1.42×10^{-3}	3.47×10^{-4}	2.59×10^{-5}	1.37×10^{-3}	5.10×10^{-4}	1.63×10^{-4}	2.40×10^{-6}	2.06×10^{-3}

4. Value assignment

Table 19

The results of value assignment

	Method	Purity	n	S	t	t_{crist}	df	$u_p(Method)$	u_p
genistin	DSC	99.68	10	0.000233	0.00	2.18	12	3.05×10^{-4}	1.10×10^{-3}
	CT	99.68	10	0.000679				1.00×10^{-3}	
genistein	DSC	99.30	10	0.000357	1.29	2.13	15	3.91×10^{-4}	2.12×10^{-3}
	CT	99.33	10	0.000642				2.06×10^{-3}	

5. u_{CRM} and U_{CRM} estimation

Table 20

The results of u_{CRM} and U_{CRM} estimation

	Purity (%)	u_h	u_{sts}	u_{lts}	u_p	u_{CRM}	U_{CRM}
genistin	99.68	5.78×10^{-5}	8.98×10^{-4}	4.66×10^{-4}	1.10×10^{-3}	1.45×10^{-3}	2.91×10^{-3}
genistein	99.32	2.91×10^{-5}	3.27×10^{-4}	2.30×10^{-4}	2.12×10^{-3}	2.17×10^{-3}	4.35×10^{-3}

6. Results of CRM analysis

Table 21

The results of CRM analysis

	Purity (%)	U_{CRM} (%)	k	P
genistin	99.7	0.3	2	0.95
genistein	99.3	0.5		

7. Cross-checked result

Table 22

The results of cross-checked method

	MB Purity (%)	Water (%)	Sulphate ashes (%)	Residual ssolvents	HPLC Purity (%)
genistin	99.66	0.12	0. 20	0.17	99.95
genistein	99.32	0.19	0. 12	0.23	99.86