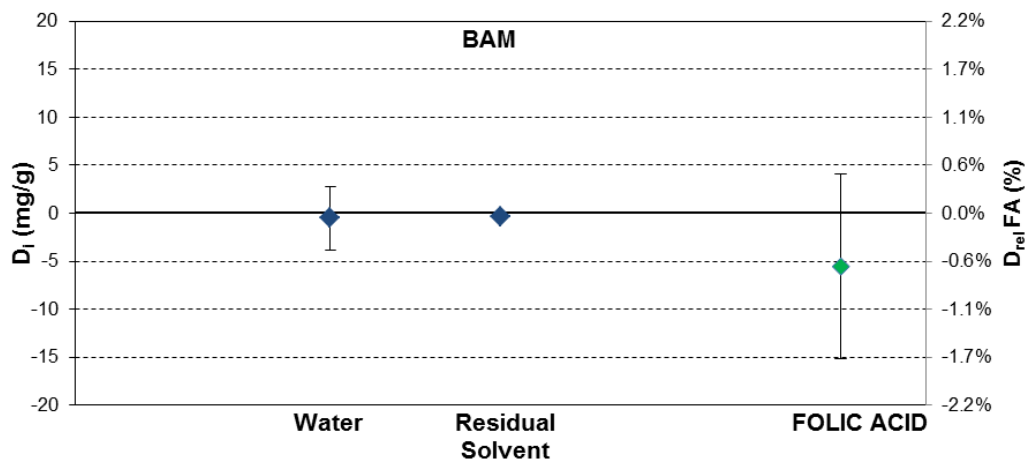


BAM

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	NMR, LC-DAD (retention time and UV-spectrum against commercial standard)
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	qNMR
Result for Folic Acid content (mg/g)	900.95, u = 0.36
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	LC-DAD
Result for total related structure impurities (mg/g)	Not determined, not all impurities quantified
Method(s) used for water content assignment	Coulometric KF titration with oven transfer
Result for water content (mg/g)	77.5, u = 0.2
Method(s) used for residual solvent assignment	Headspace GC-MS, - GC-FID, SPME-MS
Result for residual solvent (mg/g)	0, u = 0.01
Method(s) used for non-volatiles assignment	Not determined
Result for total non-volatiles (mg/g)	-

DoE plot against KCRVs

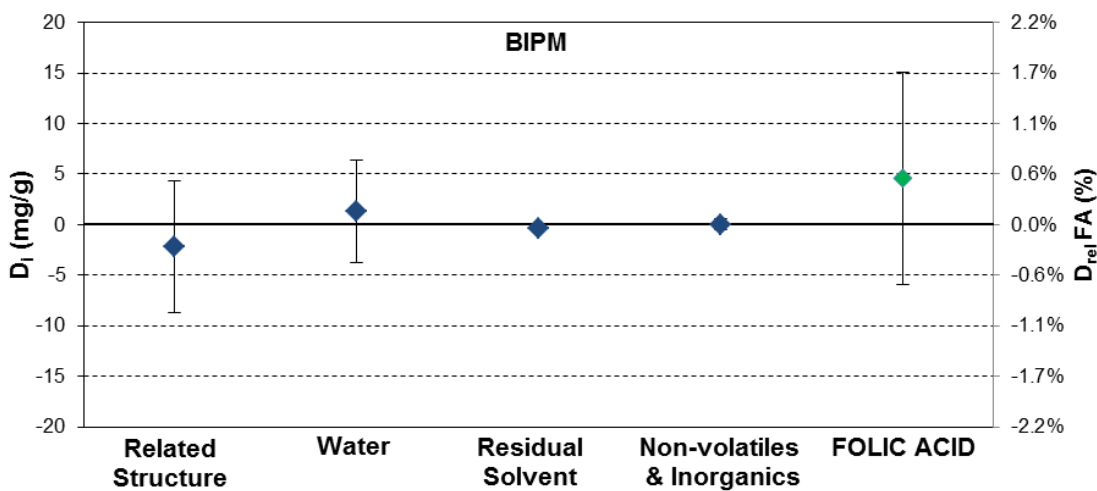


BIPM

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	LC-MS/MS, NMR
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	911.1, $u = 2.15$
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) for related structure impurity assignment	HPLC-MS/MS, HPLC-CAD
Result for related structure impurity (mg/g)	9.16, $u = 0.89$
Method(s) used for water content assignment	Coulometric Karl Fischer titration with oven transfer, TGA
Result for water content (mg/g)	79.3, $u = 1.9$
Method(s) used for residual solvent assignment	Direct GC-MS, hsGC-FID, NMR
Result for residual solvent (mg/g)	0.0, $u = + 0.15, -0$
Method(s) used for non-volatiles assignment	ICP-MS, TGA, Elemental analysis
Result for total non-volatiles (mg/g)	0.4, $u = 0.2$

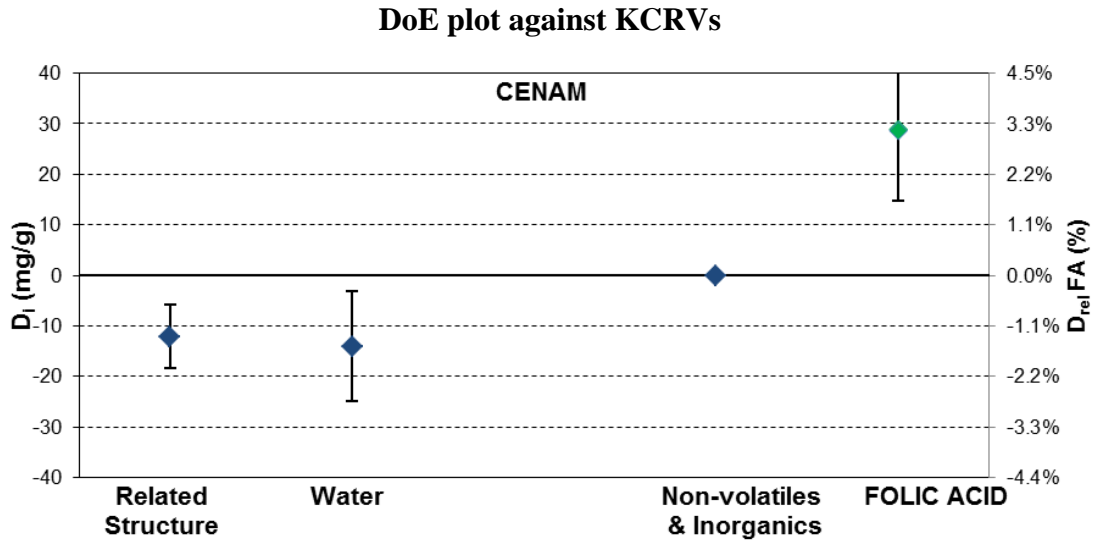
DoE plot against KCRVs



CENAM

Core Competency Claims

Not received

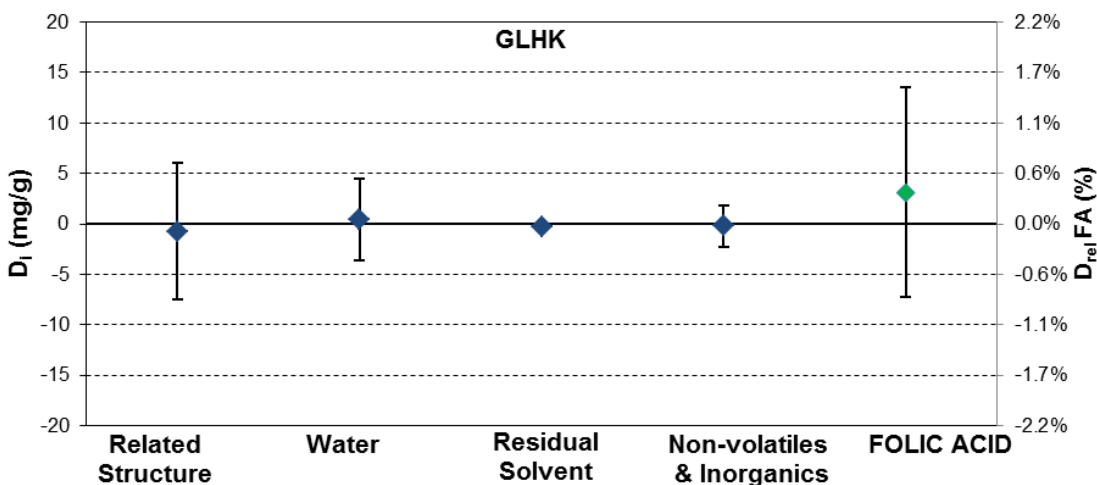


GLHK

Core Competency Claims

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	IR, MS and chromatographic equivalence
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	909.6, $u = 1.99$
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	LC-MS, LC-MS/MS and HPLC-UV
Result for related structure impurity (mg/g)	11.87, $u = 1.26$
Method(s) used for water content assignment	Coulometric Karl Fischer
Result for water content (mg/g)	78.41, $u = 1.16$
Method(s) used for residual solvent assignment	hsGC-MS
Result for residual solvent (mg/g)	< 0.1
Method(s) used for non-volatiles assignment	ICP-MS, Ion chromatography
Result for total non-volatiles (mg/g)	0.17 $u_{+} = 1$

DoE plot against KCRVs



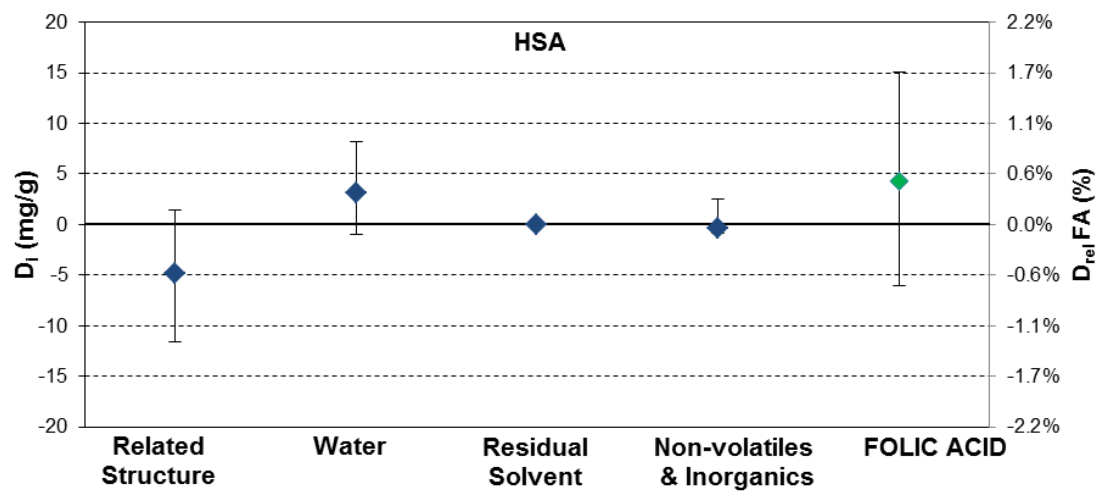
Appendix A to CCQM-K55.d Final Report version 12.01.2018

HSA

Core Competency Claims

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	<ul style="list-style-type: none"> (1) NMR (2) Reference standards of folic acid from different sources (NIM China, Sigma Aldrich, Schircks Laboratories, and Dr Ehrenstorfer) and HPLC-DAD to match retention time
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	<ul style="list-style-type: none"> (1) HPLC-DAD for identification and quantification of related structure impurities using relative peak area approach (2) LC-MS/MS for identification of related structure impurities (3) GC-MS for identification of residual organic solvent (4) GC-FID for quantification of residual organic solvent (5) Karl Fischer coulometry for quantification of water (6) TGA for quantification of non-volatiles/inorganics (7) ICP-OES for quantification of non-volatiles/inorganics
Result for Folic Acid content (mg/g)	910.8, $u = 2.4$
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	<ul style="list-style-type: none"> (1) HPLC-DAD for identification and quantification of related structure impurities using relative peak area approach (2) LC-MS/MS for identification of related structure impurities
Result for related structure impurity (mg/g)	7.71, $u = 0.314$
Method(s) used for water content assignment	Karl Fischer coulometry
Result for water content (mg/g)	81.08, $u = 1.94$
Method(s) used for residual solvent assignment	<ul style="list-style-type: none"> (1) GC-MS for identification of residual organic solvent (2) GC-FID for quantification of residual organic solvent
Result for residual solvent (mg/g)	0.374, $u = 0.153$
Method(s) used for non-volatiles assignment and	<ul style="list-style-type: none"> (1) TGA for quantification of non-volatiles/inorganics (2) ICP-OES for quantification of non-volatiles/inorganics
Result for total non-volatiles (mg/g)	0 (< 2), $u = +1.44, -0$

DoE plot against KCRVs for HSA

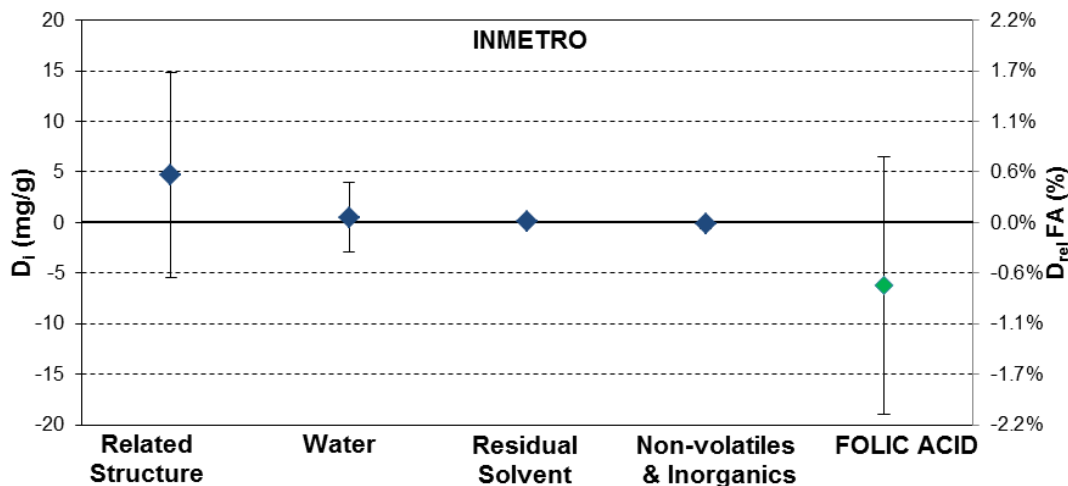


INMETRO

Core Competency Claims

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	MS (from LC-MS/MS) and NMR spectra according to folic acid structure; UV spectrum and LC retention time according to a commercial reagent of folic acid
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	qNMR combined with mass balance Mass balance considered related structure substances, water, residual solvent (acetone) and inorganics
Result for Folic Acid content (mg/g)	900.3 ± 8.4 (k=2)
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	HPLC-PDA: N-(4-aminobenzoyl)-L-glutamic acid (impurity A) and 6-formylpterine were determined by external calibration; unidentified impurities were calculated by area normalization
Result for related structure impurity (mg/g)	17.3 ± 7.9 (k=2)
Method(s) used for water content assignment	Karl Fischer direct sampling coulometric titration
Result for water content (mg/g)	78.5 ± 1.1 (k=2)
Method(s) used for residual solvent assignment	HS-GC-MS
Result for residual solvent (mg/g)	0.467 ± 0.025 (k=2)
Method(s) used for non-volatiles assignment and	ICP: sodium and silicon by quantitative ICP-OES method; minor inorganics were determined by semi-quantitative ICP-MS method
Result for total non-volatiles (mg/g)	0.211 ± 0.034 (k=2)

DoE plot against KCRVs

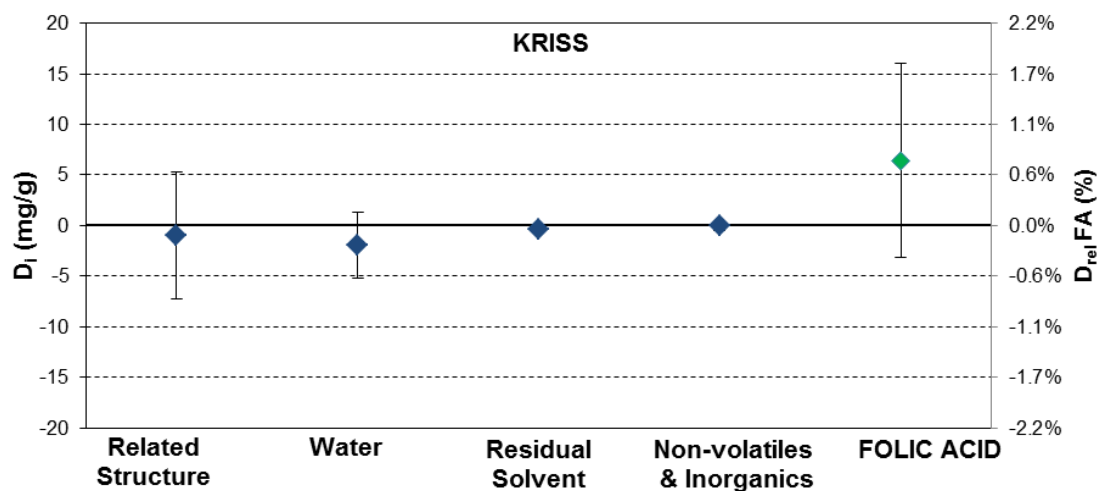


KRISS

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	LC-MS, LC-UV, NMR
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	912.90 ± 0.66 mg/g (with 95 % of confidence level , k=2.45)
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) used for related structure impurity	LC-UV
Result for related structure impurity (mg/g)	11.60 ± 0.40 mg/g (with 95 % of confidence level , k=1.97)
Method(s) used for water content assignment	Coulometric Karl Fischer titration with oven-drying
Result for water content (mg/g)	76.06 ± 0.67 mg/g (with 95 % of confidence level , k =3.18)
Method(s) used for residual solvent assignment	Headspace GC-MS
Result for residual solvent (mg/g)	0.00 ± 0.18 mg/g (with 95 % of confidence level , k=2.12)
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	0.33 ± 0.34 mg/g (with 95 % of confidence level , k =3.18)

DoE plot against KCRVs

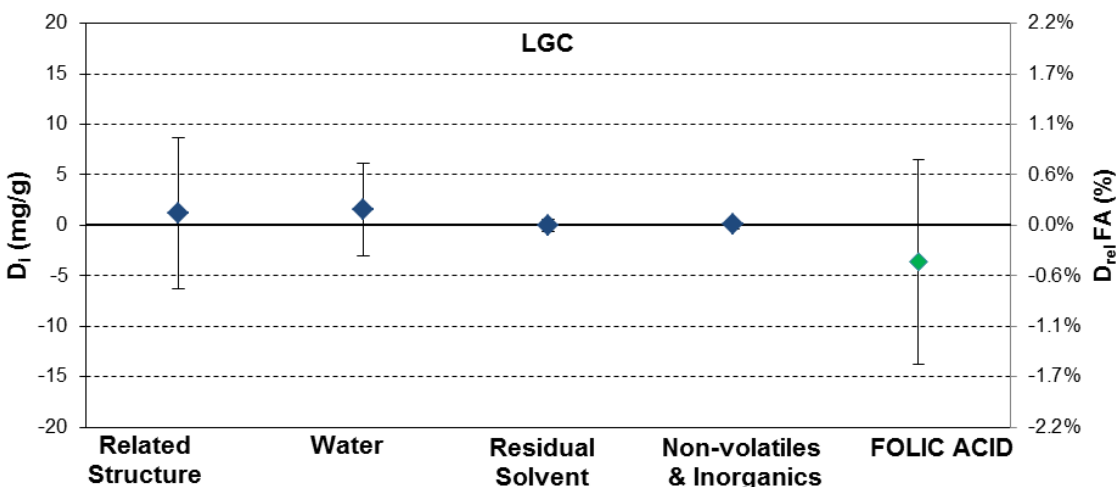


LGC

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	NMR, HPLC-UV-MS/MS
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Combination of mass balance approach (indirect) and Q-NMR approach (direct)
Result for Folic Acid content (mg/g)	902.9, $u = 1.6$
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) used for related structure impurity assignment	UPLC-DAD, Q-NMR, HPLC-UV-MS/MS
Result for related structure impurity (mg/g)	7.12, $u = 2$
Method(s) used for water content assignment	Coulometric Karl Fischer titration with oven transfer, TGA
Result for water content (mg/g)	79.52, $u = 1.61$
Method(s) used for residual solvent assignment	Headspace GC-MS, NMR
Result for residual solvent (mg/g)	0.373, $u = 0.274$
Method(s) used for non-volatiles assignment and	ICP-MS, TGA
Result for total non-volatiles (mg/g)	0.429, $u = 0.107$

DoE plot against KCRVs

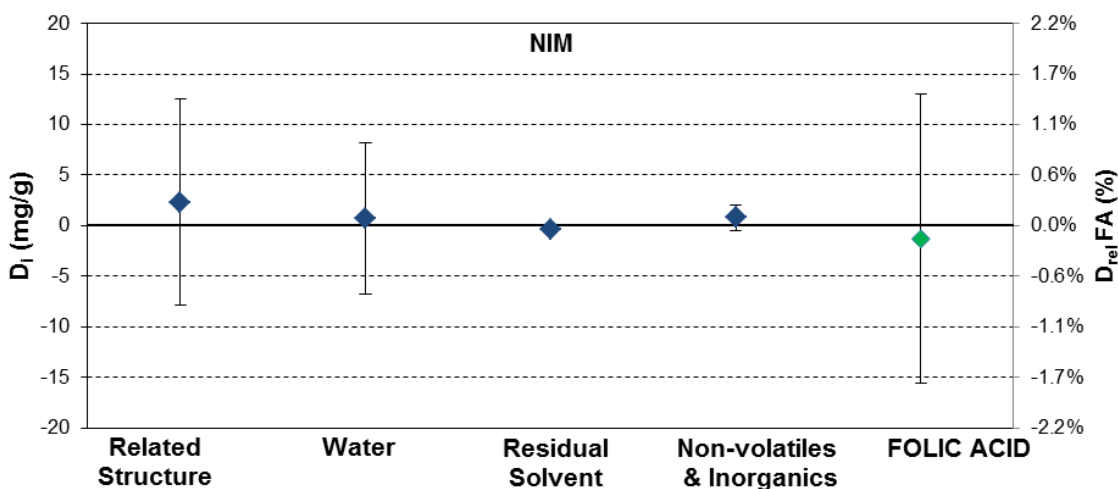


NIM

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	LC-MS/MS
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	LC-DAD, KFT, ICP-MS, Head-Space GC, etc.
Result for Folic Acid content (mg/g)	905.21, $u = 5.2$, $U = 10.54$
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) for related structure impurity assignment	LC-DAD, LC-MS/MS
Result for related structure impurity (mg/g)	14.91 $u = 4.01$, $U = 8.02$
Method(s) used for water content assignment	KFT, Element Analysis
Result for water content (mg/g)	78.70 $u = 3.37$, $U = 6.73$
Method(s) used for residual solvent assignment	Head-Space GC
Result for residual solvent (mg/g)	< 0.01
Method(s) used for non-volatiles assignment	ICP-MS, TGA, IC
Result for total non-volatiles (mg/g)	1.18 $u = 0.59$, $U = 1.19$

DoE plot against KCRVs

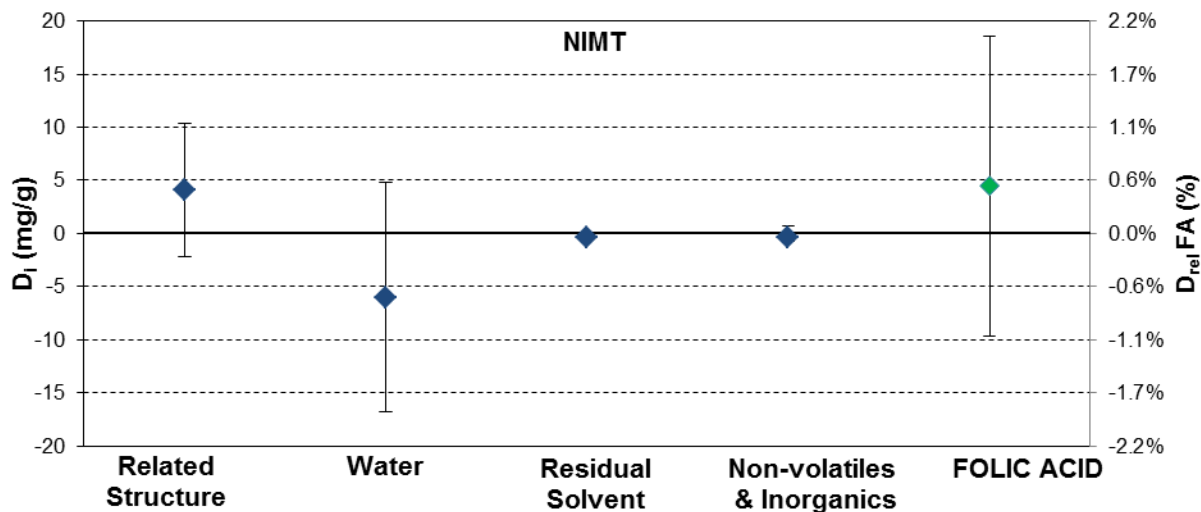


NIMT

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	LC-MS/MS HPLC-PDA by comparing with authentic folic acid and N-(4-aminobenzoyl)-L-Glutamic acid (Folic Acid Imp A) (traceability provided by in-house purity assessment)
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	911.47, $u = 5.18$
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) used for related structure impurity assignment	HPLC-PDA
Result for related structure impurity (mg/g)	16.69, $u = 0.17$
Method(s) used for water content assignment	Karl Fischer titration
Result for water content (mg/g)	71.83, $u = 5.15$
Method(s) used for residual solvent assignment	hsGC-FID
Result for residual solvent (mg/g)	0 (not detected)
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	0 (< 1)

DoE plot against KCRVs

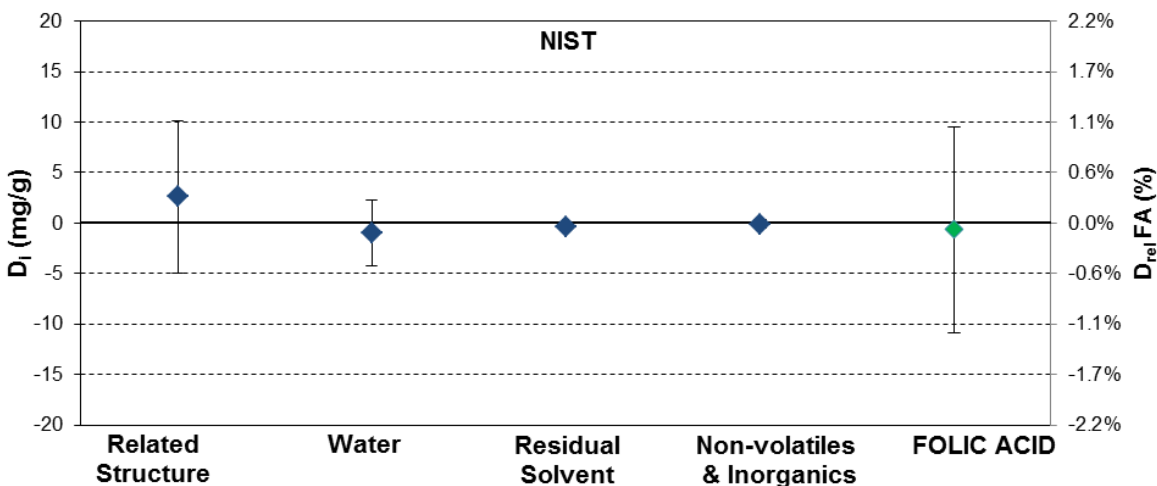


NIST

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	NMR (HSQC, COSY), LC-MS
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	qNMR and mass balance
Result for Folic Acid content (mg/g)	905.9 (-3.1 / +3.5)
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) for related structure impurity assignment	LC-UV, LC-MS
Result for related structure impurity (mg/g)	15.2 (-3.8 / +4.4)
Method(s) used for water content assignment	Karl Fischer titration
Result for water content (mg/g)	77.0 ± 0.35
Method(s) used for residual solvent assignment	head-space GC
Result for residual solvent (mg/g)	< 0.01
Method(s) used for non-volatiles assignment	ICP-MS, Ion chromatography
Result for total non-volatiles (mg/g)	0.27 ± 0.10

DoE plot against KCRVs

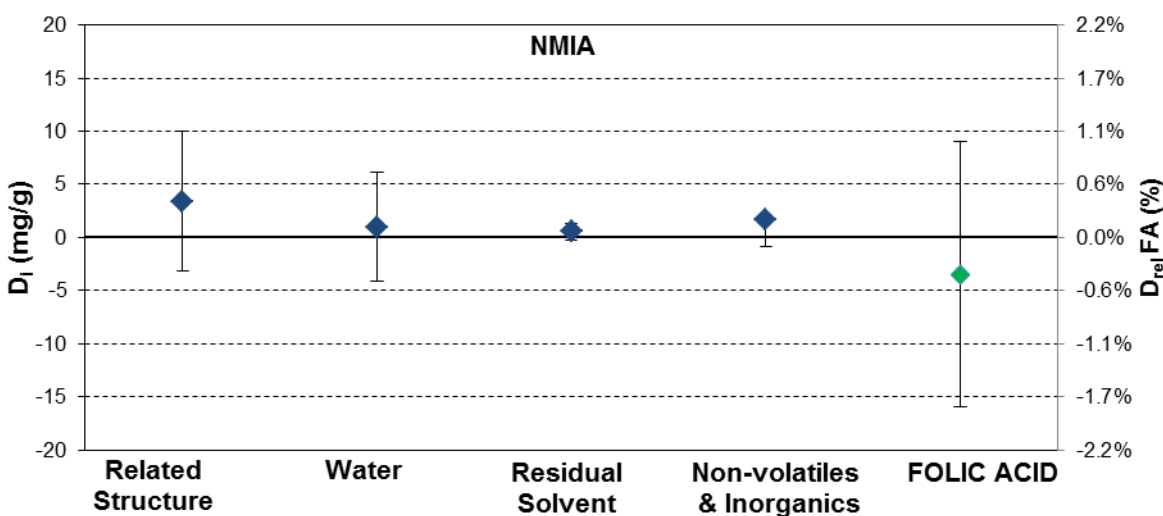


NMIA

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	HPLC-UV, ¹ H NMR, electrospray mass spectrometry.
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance and qNMR
Result for Folic Acid content (mg/g)	903, $u = 4$
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) used for related structure impurity assignment	High performance liquid chromatography with UV detection. (content)
Result for related structure impurity (mg/g)	16
Method(s) used for water content assignment	Karl-Fischer in Hydranal AK solution
Result for water content (mg/g)	79
Method(s) used for residual solvent assignment	¹ H NMR spectroscopy under qNMR conditions
Result for residual solvent (mg/g)	0.9
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	0, $u = 1.1$ (below LOD of 2 mg/g)

DoE plot against KCRVs

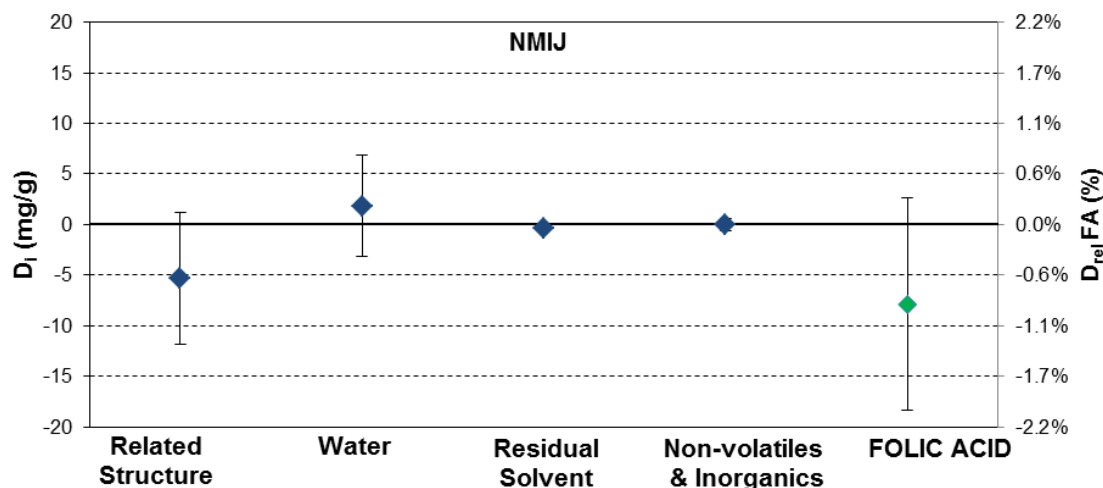


NMIJ

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	qNMR, LC-UV, LC-CAD, KFT, TGA
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	qNMR, LC-CAD
Result for Folic Acid content (mg/g)	898.6, u = 7.7
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) for related structure impurity assignment	LC-UV, LC-CAD, LC-TOFMS
Result for related structure impurity (mg/g)	7.24, u = 0.11
Method(s) used for water content assignment	Coulometric Karl Fischer titration
Result for water content (mg/g)	79.81, u = 1.35
Method(s) used for residual solvent assignment	qNMR, GC-FID
Result for residual solvent (mg/g)	0.05, u = 0.03
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	0.35, u = 0.43

DoE plot against KCRVs

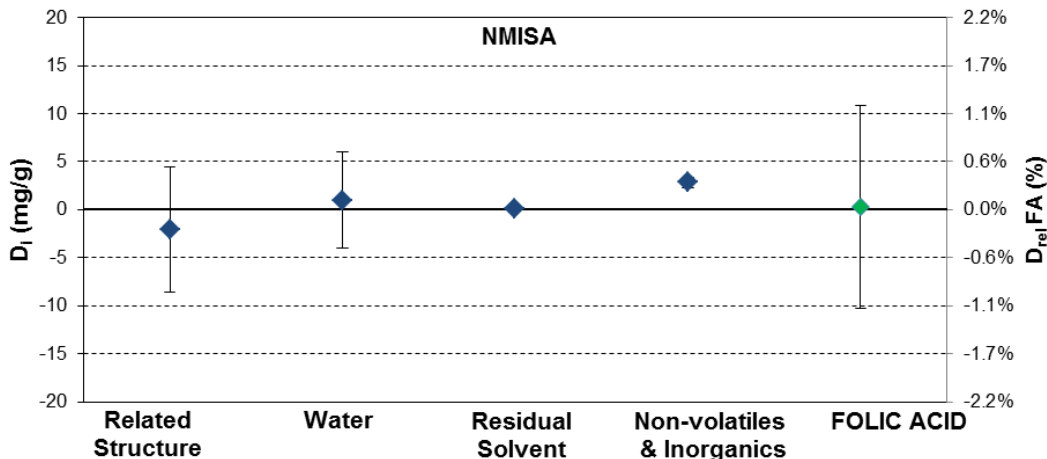


NMISA

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	Mass spectrum, UV absorption spectrum, proton NMR and retention time match against authentic reference standard of folic acid
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	906.8 ± 5.1 (k=2, 95% LOC)
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) for related structure impurity assignment	High Performance Liquid Chromatography with UV, and Mass Spectrometric detection. (HPLC-UV and HPLC-ESI-MS), Mass spectrum, UV absorption spectrum and retention time match against authentic reference standard of folic acid, external calibration and peak area%
Result for related structure impurity (mg/g)	10.5 ± 2.8 (k=2, 95% LOC)
Method(s) used for water content assignment	Karl Fischer Coulometric titration by automated oven transfer, direct insertion and thermal gravimetric analysis
Result for water content (mg/g)	79.0 ± 4.1 (k=2, 95% LOC)
Method(s) used for residual solvent assignment	Bracketing external calibration, Solid Phase Micro-Extraction (SPME) Gas Chromatography with Time-of-Flight Mass Spectrometric detection (GC-TOFMS) for residual solvent screening, Headspace GC-FID) for residual solvent
Result for residual solvent (mg/g)	0.50 ± 0.25 (k=2, 95% LOC)
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	3.2 ± 1.6 (k=2, 95% LOC)

DoE plot against KCRVs

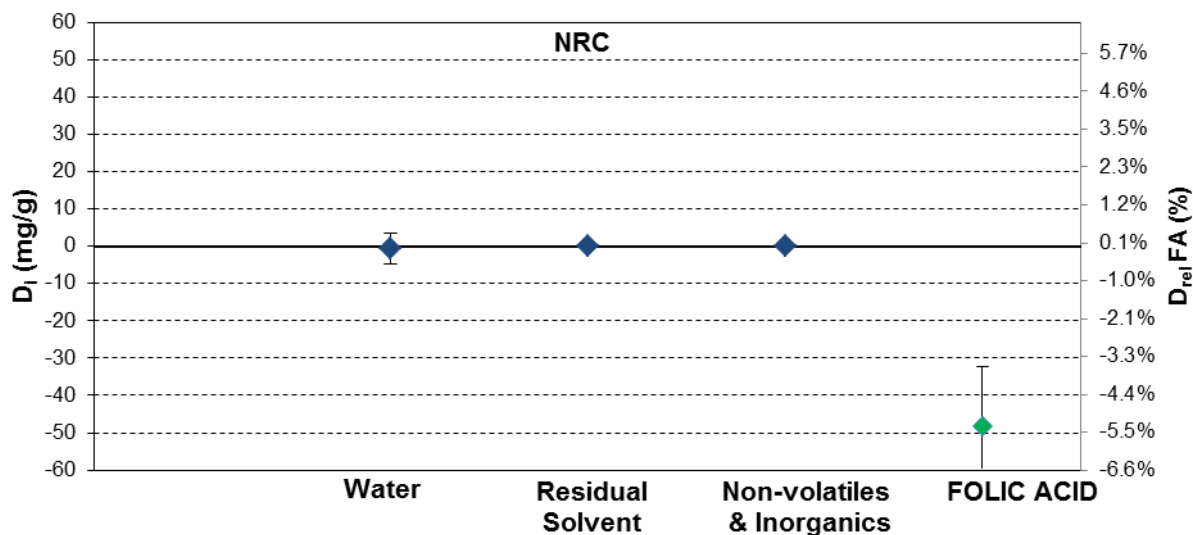


NRC

Core Competency Claims

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	¹ H, ¹³ C – NMR; MS, UV
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	¹ H – qNMR; LC-UV/MS, TGA, KF
Result for Folic Acid content (mg/g)	858.3 (6.3)
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	¹ H – qNMR; LC-UV/MS
Result for related structure impurity (mg/g)	61.4 (6.8) mean and standard uncertainty
Method(s) used for water content assignment	KF coulometric - oven transfer, cross check with TGA
Result for water content (mg/g)	77.5 (1.2) mean and standard uncertainty
Method(s) used for residual solvent assignment	¹ H – qNMR
Result for residual solvent (mg/g)	0.29 (0.03) mean and standard uncertainty
Method(s) used for non-volatiles assignment	TGA, microbalance
Result for total non-volatiles (mg/g)	0.28 (+ 0.31, -0.28) mean and standard uncertainty

DoE plot against KCRVs

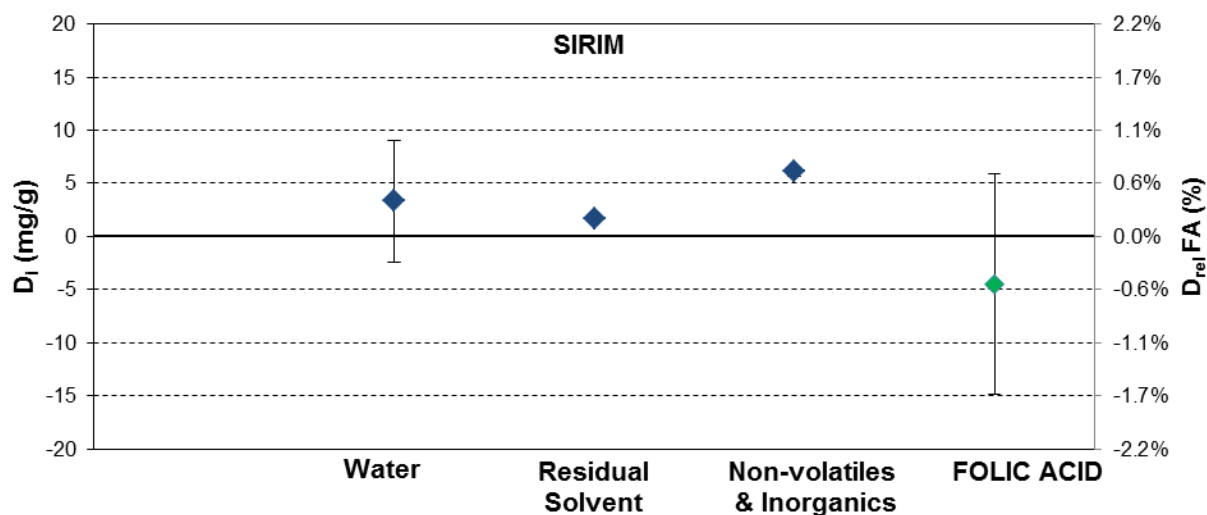


SIRIM

Core Competency Claims

• Value assignment of Primary References: Main component mass fraction and uncertainty	
Identity verification	NMR
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	qNMR
Result for Folic Acid content (mg/g)	902, U = 4
• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)	
Method(s) for related structure impurity assignment	Not determined
Result for related structure impurity (mg/g)	-
Method(s) used for water content assignment	Karl-Fischer
Result for water content (mg/g)	81.310, U = 4.736
Method(s) used for residual solvent assignment	Thermogravimetric Analyser (TGA)
Result for residual solvent (mg/g)	2.022 U = 0.149
Method(s) used for non-volatiles assignment	Thermogravimetric Analyser (TGA)
Result for total non-volatiles (mg/g)	6.460 U = 0.150

DoE plot against KCRVs

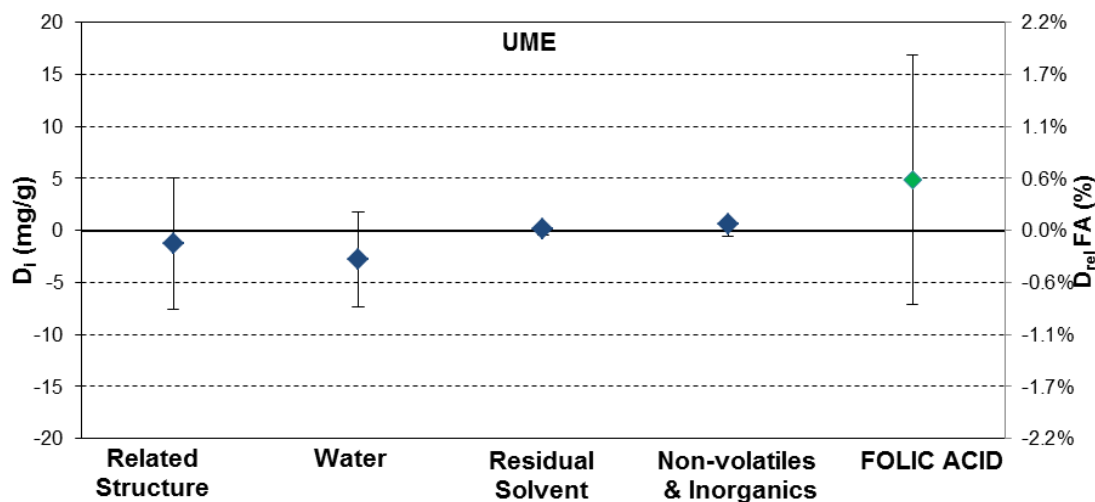


UME

Core Competency Claims

<p>• Value assignment of Primary References: Main component mass fraction and uncertainty</p>	
Identity verification	HPLC-UV, LC-MS, HRMS and NMR
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass Balance (HPLC-UV, TGA, Karl-Fischer coulometry, TD GC-MS, HS GC-MS), NMR
Result for Folic Acid content (mg/g)	911.365 (u=3.620)
<p>• Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only)</p>	
Method(s) used for related structure impurity assignment	Mass Balance (HPLC-UV, TGA, Karl-Fischer coulometry, TD GC-MS, HS GC-MS), NMR
Result for related structure impurity (mg/g)	11.310 (u=0.244)
Method(s) used for water content assignment	Coulometric Karl Fischer titration with oven transfer and thermal gravimetric analysis
Result for water content (mg/g)	75.225 (u=1.597)
Method(s) used for residual solvent assignment	HS GC-MS, TD GC-MS, qNMR
Result for residual solvent (mg/g)	< 0.5 (u=0.001)
Method(s) used for non-volatiles assignment	TGA
Result for total non-volatiles (mg/g)	Non-volatiles/ inorganics < 1 (u = 0.001) Other impurities, Grease (by qNMR) 0.6 (u=0.01)

DoE plot against KCRVs



VNIIM

Core Competency Claims

<ul style="list-style-type: none"> • Value assignment of Primary References: Main component mass fraction and uncertainty 	
Identity verification	LC-MS
Method(s) used for assignment of Folic Acid mass fraction content of CCQM-K55.d	Mass balance
Result for Folic Acid content (mg/g)	912,07 ± 1,56
<ul style="list-style-type: none"> • Value assignment of Primary References: Minor component/impurity class mass fraction and uncertainty (required for mass balance approaches only) 	
Method(s) used for related structure impurity assignment	LC/DAD (LC/MS for identification)
Result for related structure impurity (mg/g)	12,20 ± 1,51
Method(s) used for water content assignment and confirmation	Karl Fisher Titration with oven transfer
Result for water content (mg/g)	75,61 ± 0,39
Method(s) used for residual solvent assignment and confirmation	GC/MS/TD
Result for residual solvent (mg/g)	0,044 ± 0,005
Method(s) used for non-volatiles assignment and confirmation	ICP/MS/MS
Result for total non-volatiles (mg/g)	0,076 ± 0,004

DoE plot against KCRVs

