Chloramphenicol Primary Calibrant
UME CRM 1301

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ABBREVIATIONS

ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
CAP	Chloramphenicol
CCQM	Consultative Committee for the Amount of Substance
c-KFT	Coulometric KFT
CMC	Calibration and Measurement Capabilities
CRM	Certified reference material
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography Diode Array Detection
HR ICP-MS	High Resolution Inductively Coupled Plasma Mass Spectrometry
HS-GC	Head Space Gas Chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
k	Coverage factor
KF	Karl Fisher
MB	Mass Balance
LGC	Dedicated Metrology Institute of England (Laboratory of the Government Chemist)
MSDS	Material Data Safety Sheet
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
NIST	National Institute of Science and Technology -USA
NMIA	National Metrology Institute of Australia
NMR	Nuclear Magnetic Resonance
qNMR	Quantitative Nuclear Magnetic Resonance
RASSF	Rapid Alert System for Food and Feed
s	Standard deviation
s_{bb}	Between-unit standard deviation;
SI	International System of Units
s_{meas}	Standard deviation of measurement data;
s_{wb}	Within-unit standard deviation
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index
T	Temperature
t	Time
TG	Thermal Gravimetry
t_{crit}	Critical t-value for a t-test, for df degrees of freedom
TRaNS	Abbreviation for the Turkish name of the software developed for Random Stratified Sample Selection

TÜBİTAK	Turkish Scientific and Technical Research Council
u	standard uncertainty
U	expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability;
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity;
u_c	combined standard uncertainty;
u_{char}	Standard uncertainty of the material characterisation;
u_{CRM}	Combined standard uncertainty of the certified value;
U_{CRM}	Expanded uncertainty of the certified value;
UME	National Metrology Institute of Turkey
u_{lts}	Standard uncertainty of the long-term stability; appropriate
u_{meas}	Measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution;
US EPA	United States Environmental Protection Agency
u_{sts}	Standard uncertainty of the short-term stability
$\bar{X}$	Arithmetic mean

ABSTRACT

CAP, a broad spectrum antibiotic that prevents protein synthesis in the bacteria, is widely used in veterinary applications for preventive actions and animal cultivation [1,2]. Illegal or improper use results in CAP residues in animal based food materials and it enters the food chain. The potential toxic effects vary from allergenic to carcinogenic [3]. Additionally by suppressing the natural fermentation it decreases the quality and taste of the food. The use of CAP is monitored by National Residue Monitoring scheme in respect to Regulation No. 5996[4] In Turkey and in respect to EU Directive 96/23/EC [6] in European Union.

This certification report covers the production and certification of chloramphenicol primary calibrant CRM - UME CRM 1301 with respect to ISO Guide 34:2009 [7]'. The details on production and analysis, data of homogeneity assessment, stability and characterization studies, statistical evaluation and conclusions have been presented and the uncertainties have been calculated in respect to ISO Guide 98-3, Uncertainty Measurement-Part 3: Guide to the Expression of Uncertainty in Measurement-GUM [8].

Certified value is presented in the table below:

Material	**Parameter**	**Mass Fraction*** [%]	**Uncertainty**** [%]
Chloramphenicol	Purity	99.58	0.15

* Certified value and uncertainty are the unweighted statistical mean qNMR and mass balance method results. Result is traceable to International System of Units (SI).

** The uncertainty of the certified value is the standard uncertainty of the measurement multiplied by the coverage factor *k=2*, which corresponds to 95 % confidence level.

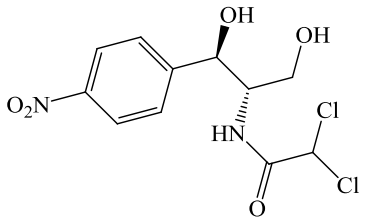
INTRODUCTION

Food and food products obtained from animals treated with CAP is banned to be served as food until the residues of this antibiotic is completely removed. This may take several months. In the Rapid Alert System for Food and Feed-RASFF entries, there are several products from different origin banned from import or export as a result of CAP residues [11]. Presence of CAP in food is regulated by legislations; therefore detection of CAP even at very low levels is extremely important. The maximum allowed amount of CAP in animal based food is reported as 0.3 µg/kg[12].

Accurate chemical measurements, require utilization of CRMs for proving the traceability and quality of measurement. There is no primary calibrant CRM present for residual CAP analysis. This is the basic motivation in the production and certification of CAP CRM. This report presents certification of CAP CRM.

The structure and some of the chemical properties of CAP is presented in Table 1.

Table 1. The structure and some of the chemical properties of CAP

	
CAS No	56-75-7
Molecular Weight	323.13 g/mol
Compound Name	Chloramphenicol
Chemical Formula	$C_{11}H_{12}Cl_2N_2O_5$
IUPAC Name	2.2-dichloro-N-[(1S,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide

PARTICIPANTS

All the stages of CRM production and certification have been performed at TÜBİTAK UME Chemistry Group Laboratories.

MATERIAL PROCESSING

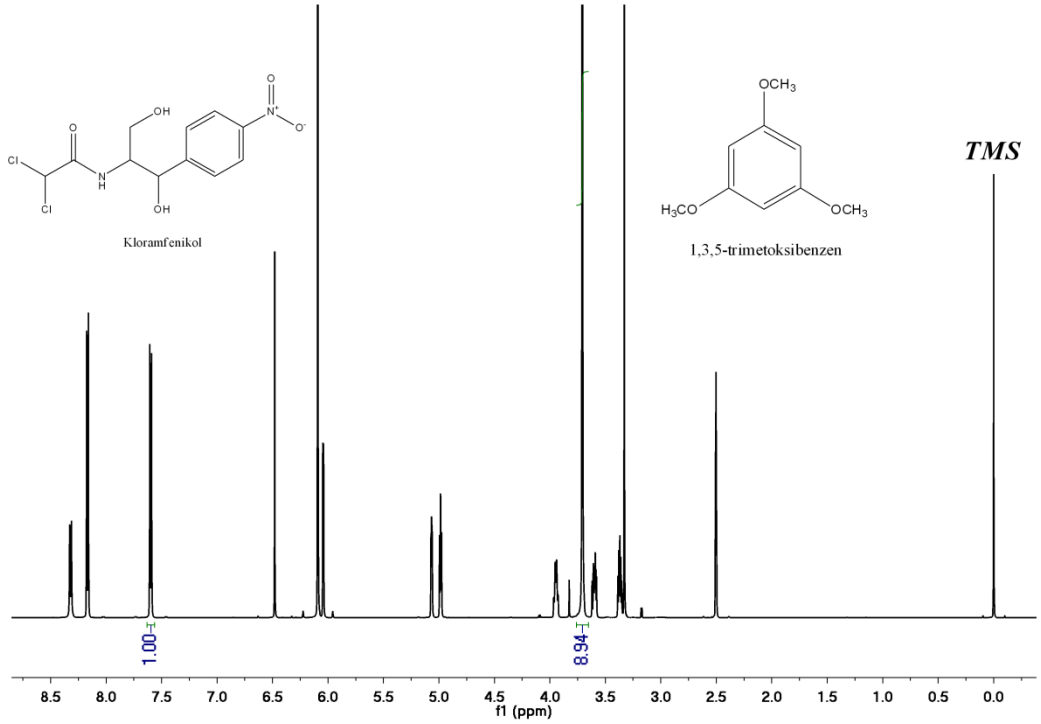
Chloramphenicol, is purchased from Acros Organics (Belgium).The reported purity of the product is 98%, which is based on HPLC analysis.

UME CRM 1301, was first homogenized for four hours in the 3D mixer (WAB-Willy A. Bachofen AG Maschinenfabrik, Turbula, Switzerland), then 500 bottles were manually filled into amber vials with aluminium crimp caps, in glove box (MBraun, LABmaster, Almanya) under argon atmosphere. Each unit has 100 mg CAP. The batch has been labelled. After the selection samples for different stages of CRM production, the remaining units have been stored in dark at (25 ± 3) °C under controlled conditions.

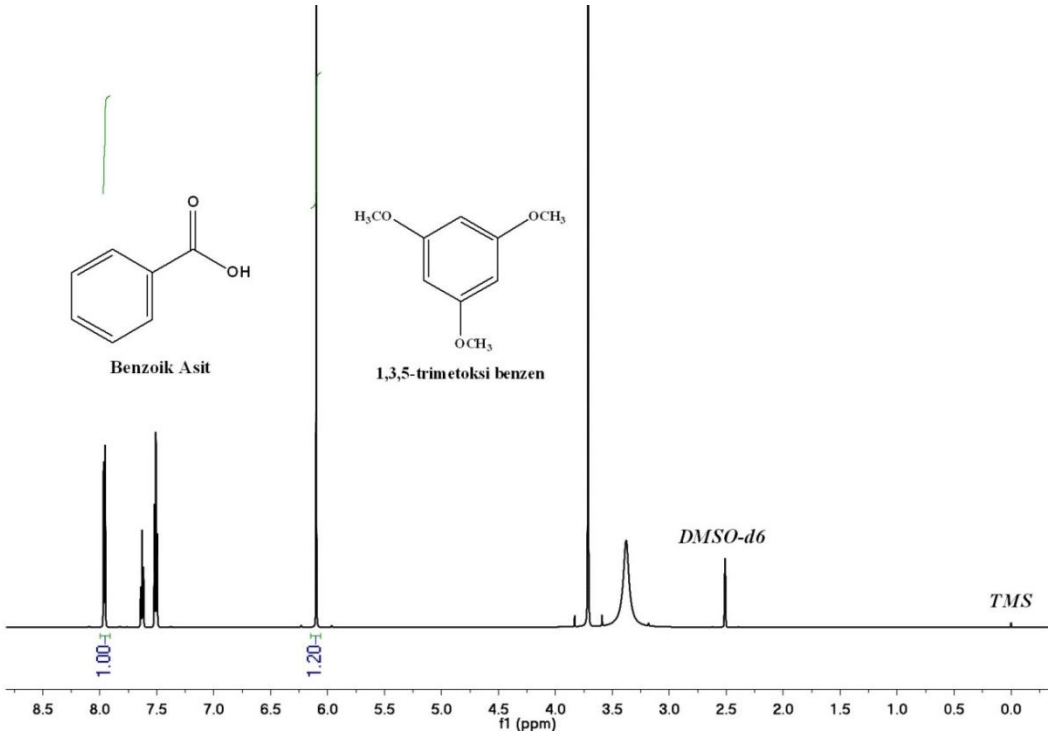
HOMOGENEITY

In order to quantify the between unit heterogeneity to certify the purity, 10 units of UME CRM 1301 have been chosen by utilizing TRaNS. TRaNS has an algorithm to separate the whole batch into groups with equal number of units and then choose one unit from each group randomly. The number of units has been determined by taking the cubic root the total number of units.

The units separated for this study has been analysed under repeatability conditions. An in-house validated qNMR purity assessment method has been utilized at this stage. Measurements have been performed by 600 MHz NMR instrument, in two parallel with 6 instrument repetitions. A sample spectrum and the spectrums of CRMs utilized in quantification have been presented below (Figure 1).



(a)



(b)

Figure 1. Sample ^{1}H-NMR spectrums a) Chloramphenicol and 1,3,5-trimetoxyibenzene, (b) benzoic acid and 1,3,5-trimetoxybenzene

A random sequence has been followed for the measurement in order to differentiate between the variance resulting from filling and analytical sequence. In other words the analytical sequence is different from the filling order. Data and the technical details about qNMR technique have been presented in Table 2 and Table 16, respectively. The values obtained show normal distribution.

Data has been subjected to regression analysis. Statistical significance on the slope has been tested with t-test in 95 % confidence level. There is no statistical significance arising from filling or analysis sequence.

Table 2. Measurement results for homogeneity assessment.

Unit No	**Purity** [%]	
	Result 1	**Result 2**
20	99.23	99.48
168	99.62	99.65
181	99.75	99.72
201	99.25	99.81
274	99.77	99.69
297	99.59	99.81
308	99.25	99.87
399	99.58	99.40
419	99.51	99.58
431	99.52	99.50

Data set has been subjected to Grubbs' test in 95 % confidence level for outliers. No outliers have been detected. Within unit heterogeneity has been analysed by ANOVA. Results have been presented in Table 3.

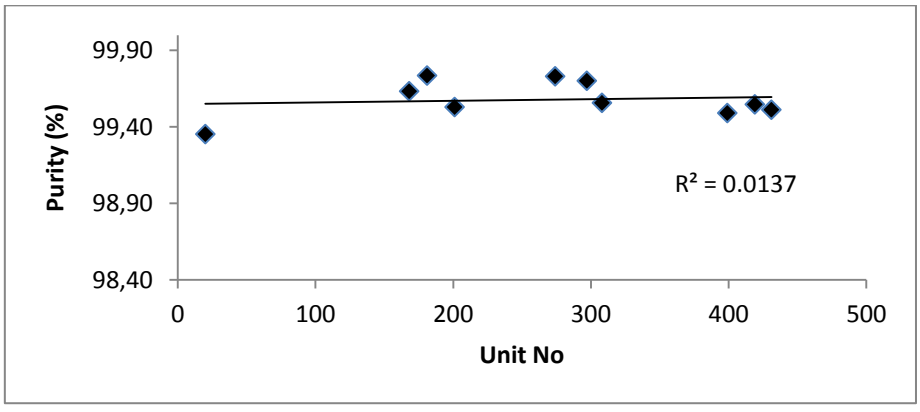


Figure 2. Measurement results for homogeneity assessment.

Table 3. One-tailed ANOVA results for homogeneity data.

SUMMARY			
Groups	**Replicates**	**Average** [%]	**Variance**
20	2	99.35	0.03
168	2	99.63	0.00
181	2	99.74	0.00
201	2	99.53	0.15
274	2	99.73	0.00
297	2	99.70	0.02
308	2	99.56	0.19
399	2	99.49	0.02
419	2	99.55	0.00
431	2	99.51	0.00

ANOVA					
Source of Variation	***SS***	***df***	***MS***	***F***	F_{crit}
Between Groups	0.27	9	0.03	0.70	3.02
Within Groups	0.42	10	0.04		

As $MS_{between}$ (0.03) < MS_{within} (0.04), the random variation between (s_{bb}) units cannot be estimated. The maximum hidden, between bottle heterogeneity (u^*_{bb},) was evaluated as follows [13]:

$$u^*_{bb} = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{N(n-1)}}$$

where u^*_{bb},, is 0.10 %.

ANOVA results also confirm the absence of statistical significance ($F = 0.70 > F_{crit} = 3.02$).

High purity materials are expected to be homogeneous. However, any contamination arising from material processing should be checked and quantified. We can conclude that the units are homogeneous for a minimum sample intake of 10 mg, which is the minimum amount used in NMR analysis.

STABILITY

Two different stability tests have been conducted to simulate transportation conditions (short term stability) and storage conditions (long term stability) in order to test the effects of environmental conditions. The first study takes four weeks and second takes eight months for testing the time and temperature effects on the stability. Certain number of samples has been kept for specific time periods and under certain temperatures and at the end transferred to the reference temperature (-20 °C), where no instability is expected (isochronous setup). All of the samples have been analysed at the same time after the last units transferred to the reference temperature, under repeatability conditions in random order. All of the samples have been analysed twice. Experimental details are the same as in homogeneity assessment and are presented in Annex II.

Short Term Stability Results:

26 units kept at 4 °C, 25 °C and 45 °C separately for four weeks for short term stability study. (Table 4). For each test period and time 4 units were transferred to reference temperature until the end of whole test period (isochronous setup)[14].

Data (Table 5) were evaluated individually for each storage temperature (Table 6). The obtained data were evaluated individually for each temperature. The results were screened for outliers using the Grubbs test. No outliers detected.

Furthermore, the data were evaluated against storage time and regression lines of mass fraction versus time. The slopes of the regression lines were tested for statistical significance. For all elements, the slopes of the regression lines were not significantly different from zero (on 95 % confidence level) at all temperature values selected (Table 7, Figure 3).

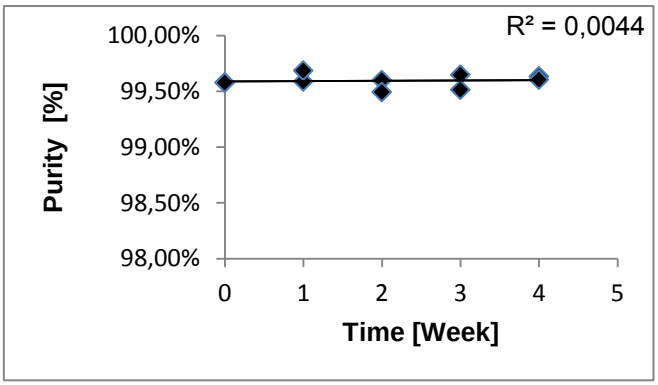
Table 4. Isochronous Setup for short term stability study

Unit Number	Temperature [°C]	Time [Week]
402	-20	0
464	-20	0
416	4	1
454	4	1
74	4	2
100	4	2
433	4	3
458	4	3
73	4	4
266	4	4
370	25	1
375	25	1
326	25	2
212	25	2
197	25	3
247	25	3
292	25	4
305	25	4
112	45	1
152	45	1
11	45	2
126	45	2
60	45	3
345	45	3
7	45	4
141	45	4

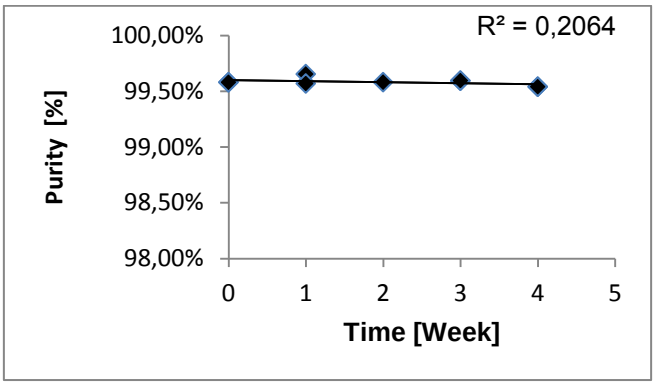
Table 5. Data for short term stability evaluation.

Time [Week]	**Purity** [%]		
	4 °C	**25 °C**	**45 °C**
0	99.58	99.58	99.58
0	99.58	99.58	99.58
1	99.59	99.65	99.49
1	99.69	99.57	99.56
2	99.60	99.58	99.50
2	99.50	99.54	99.52
3	99.65	99.59	99.61
3	99.52	99.60	99.58
4	99.64	99.54	99.58
4	99.61	99.54	99.66

a) 4 °C



b) 25 °C



c) 45 °C

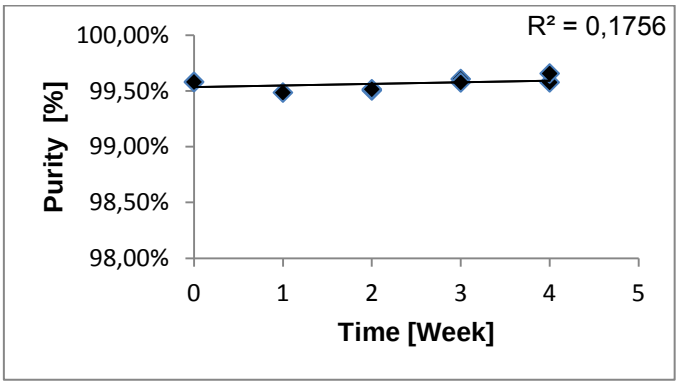


Figure 3. Regression lines for short term stability data for different temperatures: a) 4 °C, b) 25 °C and c) 45 °C.

Table 6. Single Factor ANOVA of the short-term stability measurement results.

Groups (storage temperature) [°C]	**No. Of Replicates**	**Average Mass Fraction** [%]	**Variance**		
-20	2	99.58	0		
4	8	99.60	4.22×10^{-7}		
25	7	99.60	1.49×10^{-7}		
45	7	99.55	1.48×10^{-7}		
ANOVA					
Source of Variance	***SS***	***df***	***MS***	***F***	F_{crit}
Between Groups	1.00×10^{-6}	3	3.36×10^{-7}	1.42	3.10
Within Groups	4.75×10^{-6}	20	2.37×10^{-7}		
Total	5.75×10^{-6}	23			

Table 7. Statistical evaluation of short-term stability measurement results.

Statistical Parameters	**4 °C**	**25 °C**	**45 °C**
Slope	2.57×10^{-5}	3.00×10^{-6}	3.07×10^{-5}
Slope/s_b	1.37×10^{-4}	8.34×10^{-5}	8.71×10^{-5}
Significance	-	-	-
u_{sts}	0.01%	0.01%	0.01%

Transfer of UME CRM 1301 samples at ambient temperatures (not exceeding 45 °C) does not cause a significant instability of the CRM samples.

Long Term Stability Results:

CAP, the first commercialised antibiotic is known to be stable in solid form, but small amount of degradation is reported when it is exposed to heat or gamma rays for sterilization [9,10]. Our studies on short term stability, however has shown that degradation is not dramatically affecting the purity value (Figure 4). In respect to the literature and the short term stability results, long term stability has been monitored for 8 months.

Selected samples were stored at 25 °C for 8 months. At the end of each month, two (main and two replacement) samples were transferred to reference temperature (Table 8). Measurements were performed in an order different than filling order under repeatability conditions, in order to differentiate between the variance between filling and analysis. Analysis were carried out using qNMR technique with the experimental parameters reported in Annex 2. Two subsamples were prepared per sample. Two replicates were measured in qNMR and the results were presented in Table 8.

Statistical evaluation of data is presented in Table 9. Grubbs' test was applied for outliers and no outliers were detected.

Table 8. Long Term Stability Data.

Time [Months]	**Purity** [%]	**Unit No.**
0	99.76	268
0	99.60	315
1	99.52	367
1	99.65	180
2	99.61	32
2	99.59	327
3	99.65	198
3	99.52	339
4	99.56	481
4	99.59	17
5	99.61	41
5	99.64	65
6	99.59	150
6	99.45	217
7	99.56	280
7	99.58	378
8	99.54	437
8	99.68	494
22	99.74	7
22	99.66	8

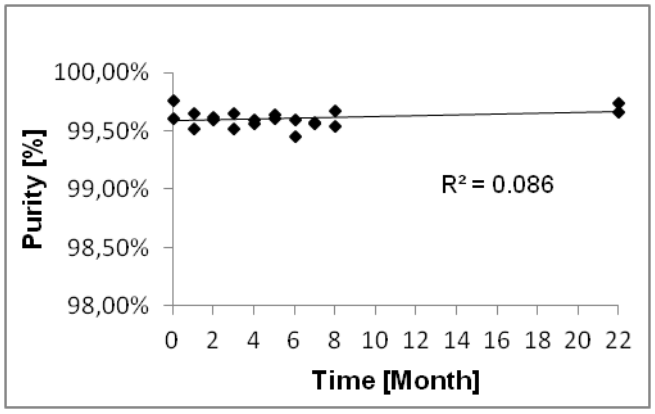


Figure 4. Regression analysis with long term stability data.

Table 9. Statistical evaluation of Long Term Stability data.

Statistical Parameters	Value*
Slope	1.79 x 10^{-5}
Slope / s_b	5.04 x 10^{-5}
Significance (95%, Confidence interval)	-
u_{lts}[3 years]	0.18 % (0.10 %)*

* Value in the parentheses is the calculated value for 22 month monitoring period.

Slope of the time vs. purity graph is not largely different from the zero, which indicate that the data is not statistically significant in 95 % confidence level.

Uncertainty component for long term stability u_{lts} for three years is calculated using the formula below [15], where RSD_{stab} is the standard deviation of 20 results, $\overline{x}$ is the average time data, and x is the predetermined shelf life.

$$u_{lts} = \frac{RSD_{stab}}{\sqrt{\sum\left(X_i - \overline{X}\right)^2}} \cdot x$$

25 °C is the recommended storage temperature for the CRM in the light of the statistical data. The stability of the product will be monitored through its recommended shelf life.

CHARACTERIZATION

Characterization of UME CRM 1301 has been performed by two different analytical techniques in respect to ISO Guied 34: qNMR is a direct method, and mass balance is an indirect method for purity assessment [10].

HPLC purity expressed as a ratio of CAP peak area to the sum of the areas from all peaks in the chromatogram. The total area for all of the components summed up and assumed to be equal to 100%.

$$P_{CAP} = P_{HPLC} - (w_{su} + w_{inorganicresidue})$$

P_{CAP} : CAP Purity

P_{HPLC} : CAP Purity calculated by HPLC

w_{SU} : Water amount

$w_{anorganikkalıntı}$: Inorganic residue amount

The UV active impurities were detected in highly concentrated CAP solution (5000 mg/L), then with optimal concentrations (ca. 200 mg/L) peak areas were normalized and purity was determined. For the analysis Thermo Finnigan Surveyor (USA) with PDA Plus Detector was utilized. The mean purity is calculated as (99.59 ± 0.16) % (k=2, norm), which is the arithmetic mean of ten different units. Detector response was assumed to be equal for all of the components.

cKFT measurements resulted a water content of (0.0881 ± 0.0016) % (mean ± SD of ten replicate). SRM 2890 was used as calibrant in cKFT analysis. Analysis were carried out by Mettler Toledo C30 coulometrik KF titrator (Switzerland)

For the analysis in TGA 2-6 mg sample was used. Thermogravimetric experiments were carried out using Exstar SII TG/DTA 7300 (Japan). The experiments were conducted at constant oxygen concentration. All of the experiments were carried out at 50 mL/min flow rate and a heating rate of 10 °C/min the temperature range of 25 °C to 750 °C, where the oxygen flow was allowed at 500 °C.

In the spectrum of TGA analysis, no weight loss was observed, which indicates that the sample has no free water molecules or residual organic solvent. However at 150 °C, besides melting of the sample, an endothermic weight loss by 0.3 % was observed. This weight loss was interpreted as, the release of crystal water by melting. There is an exothermic weight loss between (220 - 270) °C, which is the result of thermally unstable groups leaving the molecule.

For the analysis of residual organic solvents, HS-GC method was used. The amount of residual organic solvent is less than the limit of detection. Analyses were performed by GC-MS/MS Thermo Trace, TSQ Quantum XLS, (USA) which is equipped with a head space sampling apparatus. Technical details were presented in Annex II.

For the analysis of the inorganic impurity content of UME CRM 1301, after an elemental screening with ICP-MS Sodium, Magnesium, Potassium, Calcium, Iron, Vanadium and Aluminium has been detected. UME CRM 1301 samples were analysed with HR-ICP-MS for the above mentioned elements. In the analysis of these elements NIST SRM 3152a for sodium, NIST SRM 3131a for Magnesium, NIST SRM 3141a for Potassium, NIST SRM 3109a for Calcium, NIST SRM 3126a for Iron, NIST SRM 3165 for Vanadium and NIST SRM 3101a for Aluminium was utilized. Analyses were carried out in respect to US EPA 6020[19]. Results have been presented in Table 10. Total amount of inorganic content is very low therefore it is not affecting the certified value and its corresponding uncertainty.

Table 10. Inorganic residue amount

Unit No	**Amount** [µg/kg]							
	Na	Al	Ca	K	Mg	Fe	V	
57	4.18	0.24	2.28	0.52	0.26	0.11	0.002	**Total***
261	4.48	0.12	1.05	0.47	0.25	0.12	0.002	
316	4.60	0.12	0.96	0.51	0.26	0.10	0.002	
								217.03

* For the calculation of total amount the most abundant oxide salts were taken into account.

The mean purity is calculated as (99.66 ± 0.15) % (k = 2, norm) for which two different internal standards (benzoic acid and trimethoxybenzene) were utilized and measurement traceability is linked to NIST SRM 350b.

VALUE ASSIGNMENT AND UNCERTAINTY CALCULATIONS

The certified value is calculated as the mean of the results obtained from both of the methods (Table 11). Uncertainty value is the results of characterization study. The uncertainty contributions from homogeneity and stability studies were neglected. Even though the long term stability results were indicated instability for the first eight months, after twenty two months the uncertainty of characterization is valid.

Table 11. The certified value and the uncertainty for UME CRM 1301.

Parameter	**Value [%]**
Purity (MB)	99.49
Purity (qNMR)	99.67
Purity (CRM)	**99.58**
u_{MB}	0.019
u_{qNMR}	0.074
U_{CRM}	**0.15 (k = 2, norm)**

Expanded uncertainty reported by the certified value was calculated by k = 2 coverage factor which is representing 85 % confidence level. The uncertainty value reported was calculated by combining the uncertainty values evaluated by the two methods. Measurement uncertainty was calculated by combining the uncertainties of mass balance method and qNMR technique, with the formula given below.

$$U_{CRM} = k \cdot u_{char} = k \cdot \sqrt{u_{MB}^2 + u_{qNMR}^2}$$

The certified purity for UME CRM 1301 Chloramphenicol Primary Calibrant is presented below.

(99.58 ± 0.15) % (k = 2, norm)

TRACEABILITY

Assigned value is the statistical average of two different methods. First was mass balance approach composed of HPLC-UV, KF and ICP-MS and the second was qNMR. Both of the methods have completely different sample preparation procedures. Therefore, independence of the assigned value from the sample preparation part can be concluded.

HPLC-UV, LC-MS/MS, KF, ICP-MS and qNMR methodologies were used for analyte separation and quantification and defining the measurand. When possible a CRM is applied in all of the methodologies. Particularly HPLC method, when it is used for purity determination is known to be self sufficient and does not require a CRM.

Traceability of UME CRM 1301 is supported also by CCQM-K81 (Chloramphenicol as residue in pig muscle)[20] and CMC entries; G3KI-4300 and G3KI-4400 high purity chemicals, organic compounds [21].

UME CRM 1301 is traceable to SI.

INSTRUCTIONS FOR USE

Storage conditions

The material should be stored at a temperature of (25 ± 3) °C. However TUBITAK UME cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of open samples.

Safety precautions

The usual laboratory safety measures apply. MSDS should be investigated before use.

Intended Use

This material is intended to be used for method performance control and validation purposes. For assessing the method performance, the measured values of the CRMs are compared with the certified values [22]. The procedure can be described briefly as:

- Calculate the absolute difference between mean measured value and the certified value (Δ_m).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}):

$$u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2}$$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ) using a coverage factor of two (k = 2), corresponding to a confidence interval of approximately 95%.

If $\Delta_m \leq U_\Delta$ then there is no significant difference between the measurement result and the certified value, at a confidence level of about 95%.

ACKNOWLEDGEMENTS

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REVISION HISTORY

Date	Remarks
05.12.2014	First issue.

ANNEXES

ANNEX I:Technical Details Applied in Mass Balance Approach

Table 12. HPLC-DAD parameters

Column	C18 Synergi MAX-RP 150 x 4.6 mm, 5 µm
Mobile Phase	40:60 Methanol:water (isocratic)
Temperature	25 °C
Flow Rate	1 mL/min
Wavelength	273 nm

Table 13. Head Space Automatic Sampling Apparatus Parameters

Sample volume	2 mL
Incubation Time	10 min
Vial Oven Temperature	100 °C
Transfer Temperature	90 °C
Injector Temperature	90 °C

Table 14. GC Method Parameters

Column	HT-5 column (15 m x 0.25 mm, ø 0.10 µm film thickness)
Injection Mode and Volume	Split, 0.35 mL
Injection Temperature	250 °C
Split Ratio	1:20
Carrier Gas Flow Rate	He, 1 mL/min
GC oven Programme	40 °C 1 min, heating at 10 °C/min up to 240°C, hold for 2 min

Table 15. HR ICP-MS Analytical Parameters

Parameter	Value
Power	1105 W
Pump speed	15 rpm
Argon plasma gas	16 L/ min
Argon auxiliary gas	0.8 L/ min
Argon nebulizer gas	1.075 L/min
Nebuliser	Seaspray
Spray chamber	Quartz, Cyclonic

ANNEX II Technical Details for qNMR Analysis

qNMR experiments were performed for ^{1}H and $^{13}C\{1H\}$ in deutorated dimetiyl sulphoxide, DMSO-d6, at 599.77 MHz and 150.83 MHz, where 5 mm multinuclei liquid probe was utilized in connection to 600 MHz NMR spectrometers. 99.9 % deutorated atom purity DMSO-d6 (Merck, Germany, S5566287), with 0.1 % TMS has been used without further purification. ^{1}H and $^{13}C\{1H\}$ NMR experiments has been conducted at 7.4 µs and 13.8 µs 90º signal width. NMR parameters were presented in table 16.

Table 16. NMR parameters

Parameter	**Value**
Pulse angle	90º
Receiver gain	Autogain
Number of Scans (nt)	32
Relaxation Delay (d1)	40 s
Acquisition time (at)	3.4 s
Transform size (time domain)	64 K
Spectral Width (sw)	9615.4 Hz
Line broadening (lb)	0.3 Hz
Temperature	25 °C