Development of Certified Reference Material of methamphetamine hydrochloride

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Abstract. This work aimed to present the production of certified reference material (CRM) of methamphetamine hydrochloride, as an important tool to ensure the quality of forensic results. Methamphetamine is a synthetic drug derived from amphetamine, which potentially stimulates the central nervous system, and its prolonged use can cause excessive anxiety, euphoria, bipolar disorder, psychosis, among other health damages. To produce this batch of CRM, homogeneity and transport stability studies were carried out by HPLC-PDA using the results of chromatographic area corrected by the mass fraction of the sample in the analyzed solution. Evaluation of stability under storage conditions and characterization of the material were performed by ^{1}H qNMR, a primary measurement procedure. The CRM showed neither considerable heterogeneity nor tendency to instability under transport conditions (temperature of 50 ºC up to 21 days) and storage conditions (20-25 °C). The certified purity value of methamphetamine hydrochloride was (999 ± 12) mg/g, equivalent to a mass fraction of (99.9 ± 1.2) g/100g (k = 2).

1. Introduction

Methamphetamine is a synthetic drug derived from the amphetamine molecule. Amphetamine compounds are central nervous system stimulants, causing increased concentrations of the neurotransmitters dopamine, serotonin, and norepinephrine in the brain[1,2]. This induces greater alertness, increased excitement, euphoria, excessive anxiety, and personality disorders[3]. Although therapeutically some of these amphetamine compounds can be used to treat narcolepsy, attention deficit hyperactivity disorder, and obesity, its abusive use causes irreversible damage[4]. Methamphetamine hydrochloride (N-methyl-1-phenylpropan-2-amine; hydrochloride, MA.HCl, Figure 1) is an unpredictable and lethal drug, more potent than amphetamine, due to the additional methyl moiety that makes it more lipophilic and, therefore, facilitates the crossing of the blood-brain barrier[5]. Methamphetamine on the illegal market is also known as ice, crank, meth, glass, speed, and crystal. Hydrochloride is the most common form due to the solubility of this compound in water and acids, can be used orally, injected, intravenously, and intranasally [6,7]. The United Nation Office on Drugs and Crime(UNODC) estimates that there are 27 million users of amphetamine-type stimulants, being the third largest group of users, after cannabis and opioids[8].

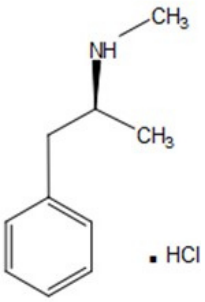

Figure 1. Chemical structure of methamphetamine hydrochloride.

Identification of MA.HCl is extremely important in several types of crimes. Judicial processes resulting from seizure and repression operations in general require that the results of forensic analysis are reliable in order to avoid questioning due to possible analytical weaknesses.
The use of CRM by laboratories is a tool proposed by the ISO/IEC 17025 standard[9] to guarantee the quality of routine analysis, as well as to establish metrological traceability in calibration.CRMs are high purity substances, stable, homogeneous, and fully characterized regarding their composition, which are accompanied by a certificate or similar type of document declaring the specifiedvalue of a given property, and the associated measurement uncertainty[10]. CRMs are capable of ensuringmetrological traceability of measurement results to the international system of units (SI), which means the accuracy and comparability of results over time and space[9-12]. By definition, the metrological traceability is the property of a measurement result in which the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty,which means that traceability and uncertainty are intimately linked[12-14]. Laboratories accredited underISO/IEC 17025 are required to prove the metrological traceability of their measurement results[9].The present work was aimed at developing a MRC of MA.HCl following the requirements from ISO 17034 standard[11], as a tool to improve the robustness of the reports of forensic chemistry laboratories, as wellas a strategy for the implementation of public security actions.

2. Experimental

Chemicals and sample preparation
All solvents were HPLC grade (Tedia, Rio de Janeiro, Brazil), maleic acid CRM 8792.0001 (certified purity: (999.9 ± 1.7) mg/g) was from the Brazilian National Institute of Metrology, Quality and Technology (Inmetro, Duque de Caxias, RJ, Brazil); deuterium dioxide (D_2O) from Cambridge Isotope Laboratories (Andover, USA), and water type I by Millipore Sigma, Burlington, MA).The MA.HCl samples used for certification were provided by Federal Police with judicial authorization,through a technical cooperation agreement between both parties. Samples were grinded, homogenized and bottled in amber glass vials with rubber stopper and aluminum seal containing approximately 50 mg.

Instrumentation
HPLC-PDA. ACQUITY UPLC–PDA System (model Xevo TQ; Waters) was used for the homogeneity and transport stability studies, and preliminar determination of purity. Liquid chromatographic separation was performed using an Acquity UPLC BEH C18 column (1.7 µm × 2.1 × 50 mm; Waters, Milford, MA, USA), with a flow rate of 0.3 mL/min, and an injection volume of 10 µL at 27.5º C column temperature, and wavelength detector (λ = 210). Mobile phase A consisted of water 0.1% (v/v) with trifluoroacetic acid (TFA), mobile phase B was acetonitrile (can) with 0.1 % (v/v) TFA. Gradient mode elution (0-1 min, 10% B, 1-6 min, 10-40% B, 6-10 min, 40-80% B, 10-11,01 min, 80-10% B, and 11,01-12 min, 10% B).

Karl Fischer coulometric titration. The water content was determined using a Karl Fischer coulometer

(852 model, Metrohm AG, Bleiche West, Switzerland) equipped with a generator electrode without a diaphragm, a current generator electrode (400 mA) and a platinum indicator electrode (10 µA). The results were processed with Tiamo 2.4 software, 2006 version (Metrohm).

NMR. Data acquisition was performed in a nuclear magnetic resonance (NMR) spectrometer system Avance III, 500 MHz (Bruker Daltonics, MA, USA), equipped with a Prodigy cryoprobe (CPP TCI 500S1 H&F-C/N-D-05 Z), operating at 11.7 T and 298 K. For this measurement, it was used a zg pulse sequence, 90° pulse was calibrated for each acquisition centered at 4.4 ppm, acquisition time was 3.28 s, with 64 k points, 16 transients, relaxation delay of 57 s, maintaining free induction decay (FID) resolution at 0.31 Hz/pt. The ^{1}H NMR spectrum was referenced with the D_2O solvent signal at 4.8 ppm. Data were processed using TopSpin 3.6.5 software from Bruker. Acquisition replicates (n=3) of each tube were performed randomly. The spectra were processed with MestreNova 14.1.1 program, Mestrelab Research SL (Santiago de Compostela, Spain). Peak multiplicities were designated by the following abbreviations: s, singlet; d, doublet; t, triplet; dd, double doublet, and m, multiplet.

Sample preparation and measurement uncertainty
Sample solutions were gravimetrically prepared using calibrated analytical balances Sartorius modelo MSA 2.7S (Goettingen, Alemanha), Mettler Toledo XS 205 (Greifensee, Suíça), and Mettler Toledo modelo XS 1003 S (Greifensee, Suíça), with a resolution of 0.0001 mg, 0.01 mg, and 0.001 g. The measurement uncertainties were calculated using the propagation uncertainty approach as described in the ISO guide for the expression of measurement uncertainty[12].

Homogeneity study
Between-unit homogeneity was studied with 3 true replicates analyses of 10 units selected in a random stratified sampling scheme. Within-unit homogeneity was evaluated in 6 true replicates from a single unit. From each flask, 300 µg mL^{-1} solutions were prepared in triplicate, and each solution was injected three times into the HPLC system in a single injection sequence, as previously described. To prevent block effect or trend along the sequence, the first injection block (groups) was analyzed in ascending filling order, the second in random order, and the third in descending order. Results were evaluated through the area of MA.HCl (analyte) corrected by the mass fraction of the solution, determined according to equation 1 (Table 1). This result does not have an absolute meaning, as it does not represent the concentration or mass fraction of MA.HCl in the sample. However, the variation of this relative parameter in the samples is a measure of the homogeneity of the material.

Short-stability study
The short-term stability study was performed at 50 °C (simulated transport temperature) for 28 days in an isochronous design. Stability was evaluated from 10 units selected by the stratified random approach. Every 7 days, 2 units were removed and kept at reference temperature (20-25 °C) for later analysis. At the end of the 28 days, the 8 flasks corresponding to 7, 14, 21 and 28 days of study were added to two others, which remained at reference temperature. All CRM flasks were analyzed on the same day (300 µg mL^{-1} solution of MA.HCl) by HPLC-PDA. The evaluation of the results was based on the $A_{corrected}$ in an analogous way to the one presented for the study of homogeneity equation 1 (Table 1).

Long-stability study
Long-term stability was evaluated under storage conditions (20-25 °C), during 6 months with monthly analysis (0, 1, 3, 5, and 6 months), by the classic design. The analysis was performed by ^{1}H quantitative nuclear magnetic resonance (^{1}H qNMR), using maleic acid MRC (999.9 ± 1.7) mg/g (k = 2), as an internal standard (IS). For this analysis, approximately 10.0 mg of sample and 9.4 mg of IS were weighed and dissolved in 1 mL of D_2O by vortexing. At each time point, two flasks were analyzed in 3 true replicates. The evaluation of the stability of MA.HCl under storage conditions was performed using linear regression.

Candidate CRM Characterization
The identification of related substances was previously determined by HPLC-PDA. For this, two columns were evaluated for MA.HCl selectivity: Acquity UPLC BEH C8 and C18 (100 mm x 2.1 mm

i.d., 1.7 µm), Waters (Milford, USA), applying the chromatographic method previously described. The water content was determined with coulometric Karl Fischer titration using 6 replicates of 0.03 g (total of 6 flasks) by direct addition of samples into the titration vessel. Characterization was performed by ^{1}H qNMR, a primary measurement procedure, using maleic acid MRC as an IS. The mass fraction of MA.HCl (w_A) was determined by qNMR using equation 2 (Table 1).

Estimation of uncertainties

The CRM property value was the mass fraction determined with ^{1}H qNMR and its uncertainty (u_{CRM}) was obtained by combining the uncertainties of the characterization study itself, homogeneity, short-term stability, and long-term stability. The uncertainties were combined in a relative way since the uncertainties of the homogeneity and the short-term stability were obtained using $A_{corrected}$ and not the mass fraction as used for characterization and long-term stability studies. The uncertainty estimations were performed according to the ISO GUM[15], a supplement for Monte Carlo Simulation[16], and the Eurachem/Citac Guide[13] (equations in Table 1).

The uncertainty due to between-bottle (in)homogeneity (u_{bb}) was calculated with equations (3) and (4) (Table 1) [17]. The short- and long-term stability uncertainties (u_{sts} and u_{lts}, respectively) were estimated using equation (5) (Table 1)[17]. The combined standard uncertainty of the CRM property value (u_{CRM}) was estimated with equation (6) (Table 1), which corresponds to the law of propagation of uncertainties. Both stability uncertainties (u_{sts} and u_{lts}) were considered for calculating u_{CRM}. The expanded uncertainty of the certified property value (U_{CRM}) was calculated with equation (7) (Table 1), using a coverage factor (k) of 2, for an uncertainty level of 95%. The combined standard uncertainty of the MA.HCl mass fraction ($u(w_A)$) determined with ^{1}H NMR was estimated by equation (8) (Table 1) according to GUM[15].

Table 1. Equations used in the certification studies of the MA.HCl candidate MRC

Equations		Variables
$A_{corrected} = A_{analyte}/FM_{solution}$	(1)	$A_{corrected}$ = HPLC peak area corrected by mass fraction of the MA.HCl in the analyzed solution $A_{analyte}$= MA.HCl peak area (300 µg g^{-1} solution); $FM_{solution}$ = mass fraction of MA.HCl in the solution
$w_A = \frac{I_a}{I_{is}} \times \frac{M_a}{M_{is}} \times \frac{N_{is}}{N_a} \times \frac{m_{is}}{m_a} \times w_{is}$	(2)	w_A or w_{is} = mass fractions of analyte or internal standard (g/100g) I_a or I_{is} = integrals of analyte or internal standard M_a or M_{is} = molar masses of analyte or internal standard Na or N_{is} = number of protons of analyte or internal standard signals ma or m_{is} = weighted masses of analyte or internal standard
$u_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n_r}}$	(3)	u_{bb} = uncertainty due to between bottle (in)homogeneity (g/100g) $MS_{between}$ = mean square between groups MS_{within}= mean square within groups n_r = number of replicates
$u_{bb} = \sqrt{\frac{MS_{within}}{n_r}} \times \sqrt[4]{\frac{2}{dfMS_{within}}}$	(4)	dfMS$_{within}$= degrees of freedom of mean square within groups
$u_{sts} = u_{lts} = s_{(b1)}t$	(5)	u_{sts} or u_{lts} = uncertainty due to short- or long-term stability studies, respectively s(b1) = slope uncertainty (g/100g day^{-1}or g/100 g week^{-1}) t = time (days, weeks)

$u_{CRM} = \sqrt{u^2_{C(WA)} + u^2_{bb} + u^2_{sts} + u^2_{lts}}$	(6)	u_{CRM} = combined standard uncertainty of certified property value (g/100g)
$U_{CRM} = u_{CRM}\, k$	(7)	U_{CRM} = expanded uncertainty of certified property value (g/100g)
$u_c(wA) = \sqrt{u^2_{(\frac{I_a}{I_{is}})} + u^2_{(Ma)} + u^2_{(mis)} + u^2_{(ma)} + u^2_{(Wis)}}$	(8)	$u_{C(w_A)}$ = combined standard uncertainty of certified property value w_A determined by ^{1}H qNMR (g/100g)

3. Results and Discussion

Homogeneity study

The homogeneity results showed no trend across filling order or chromatographic injection sequence. In the homogeneity testing, the one-way analysis of variance for the 11 flasks analyzed in triplicate resulted in a mean square between flasks ($MS_{between}$) of 2006.4794 and a mean square within flasks (MS_{within}) of 3608.3478. Using equations (3) and (4) (Table 1).

The MS_{within} was greater than the $MS_{between}$, however, as the purity of the candidate CRM is very high (the batch presented only 0.01 mg/g of impurities), it is not expected that the difference between the replicates was caused by heterogeneity inside the flask, but rather by variations in the weighing of the samples. In addition, the repeatability of the HPLC-PDA method was high with a relative standard deviation (RSD) from 0.14 to 0.29%, so any minor differences in chromatographic area results would be noticed. Thus, the value of MS_{within} was not considered for calculating the u_{CRM}. The uncertainty related to the homogeneity for CRM of MA.HCl was 2006.479, equivalent to 0.27% of the mean value of the area ratio results (753027.496).

Short- and long-term stability studies

The short-term stability study was carried out to evaluate the CRM stability under transport conditions (50 ºC) for 28 days by HPLC-PDA, using an isochronous design. All flasks were analyzed at the end of the study. The evaluation of the results was based on the $A_{corrected}$ as applied in the homogeneity study. The results of the short-term stability study are shown in Figure 2. Measurand tendency change was observed when the results of the area corrected were plotted in order of analysis or in order of study time. After removing the last study point (28 days), the curve slope was considered insignificant ((t_{b1} value smaller than t_{crit} (1.1436 < 1.995)), and therefore this material proved stable under transport conditions (50 ºC, 21 days). Using equation 5 (Table 1), based on the value of $s(_{b1})$ = 50.3385651 (regression analysis) and on the 21-day period, the short-term stability uncertainty (u_{sts}) was 1057.110, equivalent to 0.14% of the mean value of the area ratio results (758471.543).

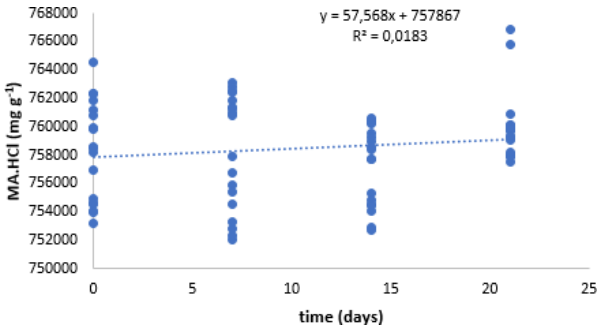

Figure 2. Results for the short-term stability study of MA.HCl candidate CRM.

The long-term stability study (storage conditions at 20 - 25 ºC) for 6 months was evaluated using classical design. The samples were individually analyzed by qNMR on pre-determined periods (0, 1, 3, 5, and 6months). Linear regression of data showed that the slope of the curve was not significantly different from zero, ((t_{b1} value smaller than t_{crit} (0.7561 < 2.0032)), therefore this material proved stable

at 20 - 25 °C for 6 months. Uncertainty was determined by the product of the time interval between characterization and the expiry date of the certificate. Figure 2 shows the results of the long-term stability study of the MA.HCl candidate CRM. The long-term stability uncertainty (u_{lts}) was estimated as 5.1 mg g^{-1} which is equivalent to 0.51% according to equation 5 (Table 1), considering s($_{b1}$) as 0.0053097 (regression analysis) and "t" as the storage shelf-life of 967 days (approximately 3 years).

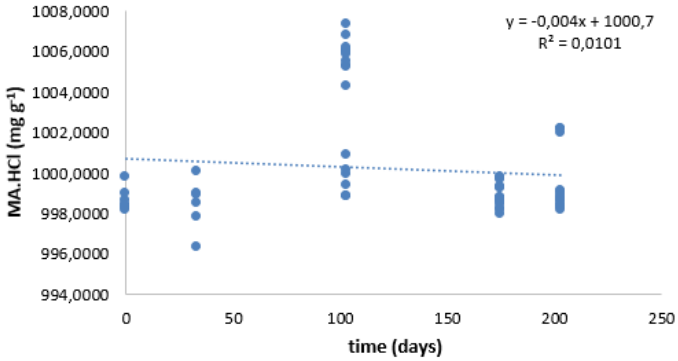

Figure 3. Results for the long-term stability study of MA.HCl candidate CRM.

Characterization

The related substances were evaluated by HPLC-PDA and water content by coulometric Karl Fischer titration. Characterization was performed by ^{1}H qNMR, a potential primary method of measurement, using maleic acid MRC as an IS.

The HPLC-PDA analyses showed a low-intensity peak detected close to the mais component peak (1000 µg mL^{-1} solution of MA.HCl), Figures 4a and 4b. However, the impurity signal intensity was very close to the chromatogram noise, in the order of 0.01 mg/g, a value lower than the material target uncertainty (u_{CMR}) of 1.5 mg/g. Both columns C8 and C18 presented good method repeatability, with approximately 0.2% RSD (n=10), Figures 4c and 4d, respectively.

The water mass fraction determined by Karl Fischer coulometric titration was 0.40 mg/g of MA.HCl, with a standard uncertainty (u_{water}) of 0.35 mg/g for k = 2.

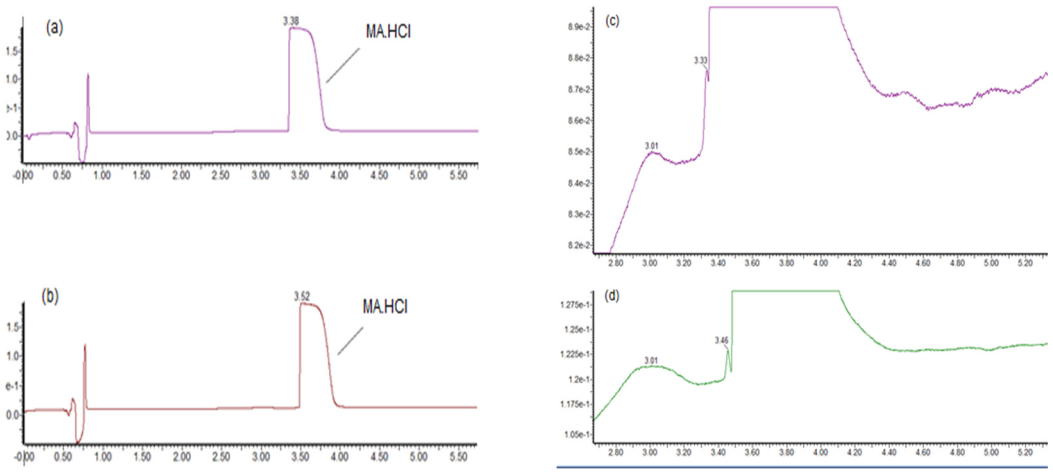

Figure 4. HPLC–PDA chromatograms of related substances test of MA.HCl candidate CRM (at 1000 µg mL^{-1}) columns C8 (a) and columns C18 (b), enlargement (c), showing the peaks of MA.HCl (t_R = 3.38 min) and unknown impurities (t_R = 3.33 min) with column C8, enlargement (d), showing the peaks of MA.HCl (t_R = 3.52 min) and unknown impurities (t_R = 3.46 min) with column C18.

The structural identification of MA.HCl was confirmed by ^{1}H NMR analysis. The ^{1}H NMR spectrum (500 MHz, D_2O) of the MA.HCl MRC candidate showed the following signals (Figure 5): δ 1.27 ppm (d, 3H); δ 2.70 ppm (s, 3H); 2.90 ppm (dd, 1H); 3.07 ppm (dd, 1H); 3.53 ppm (m, 1H); 7.44 ppm - 7.31 ppm (m, 5H).

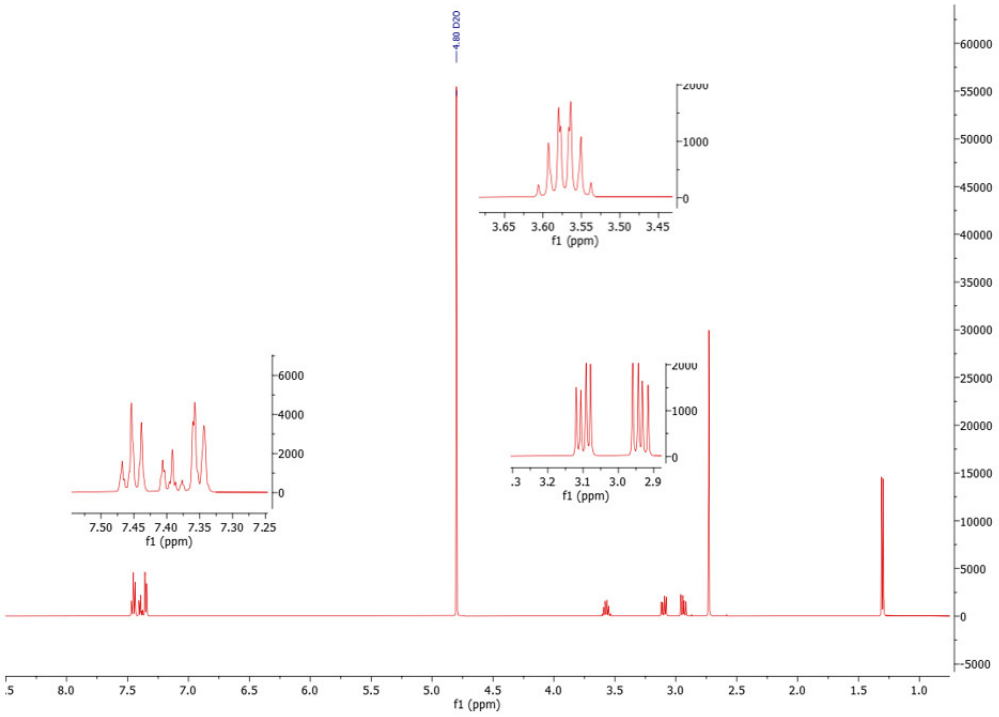

Figure 5. 500 MHz ^{1}H NMR spectrum for MA.HCl candidate CRM in D_2O.

The purity of the IS in mg/g (999.9 ± 1.7) was included in equation 3 (Table 1) so that the final result would be in this unit. The signals used for quantitative analysis were 6.4 ppm for maleic acid, and 2.88 ppm (ddd, 2H + s, 3H) and 7.39 ppm (m, 5H) for MA.HCl. The quantification of MA.HCl was performed by the average of these two signals.

To estimate the measurement uncertainty ($u(w_a)$), the classic approach of the GUM[12] was used, considering all sources of uncertainty of the input quantities of the measurand according to equation 2 (Table 1). The sources of uncertainty are plotted in an Ishikawa diagram as depicted in Figure 6. The results of the mass fraction and measurement uncertainty are shown in Table 2.

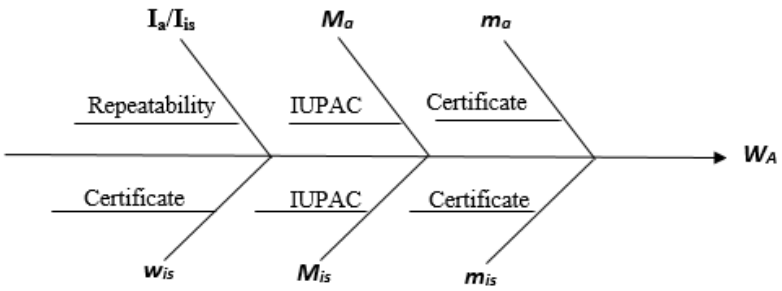

Figure 6. Ishikawa diagram illustrating the uncertainty sources affecting MA.HCl mass fraction.

Table 2. Uncertainty combination data obtained for the characterization of MA.HCl by ^{1}H qNMR

Signals	w_A(MA.HCl) (mg/g)	$u_{(Wa)}$ (mg/g)	$u_{c(Wa)}$ (mg/g)
7.4 ppm	998.60	0.8728	0.9170
2.88 ppm	998.98	0.9170	
Average	998.79		

The mass fraction of the MA.HCl determined with ^{1}H qNMR was (998.8 ± 1.8) mg/g, for k = 2. The HPLC-PDA analysis results and the water content were compatible with the purity by qNMR.

CRM certified value and estimation of uncertainties

The certified value is the one with the highest confidence in their accuracy and for which all known or potential sources of error were researched and considered. The characterization value of the MA.HCl CRM was determined by ^{1}H qNMR.
In order to calculate the expanded uncertainty (U_{CRM}), contributions from standard uncertainty due to homogeneity, short-term stability, long-term stability, and characterization were evaluated. For this, the combined standard uncertainty (u_{CRM}) and expanded uncertainty (U_{CRM}) for the certified reference material was calculated according to equations (6) and (7) (Table 1), using the uncertainty of the MA.HCl mass fraction determined with ^{1}H qNMR ($uc_{(wa)}$), equation (8). The calculated combined standard uncertainty (u_{CRM}) was 6.0 mg/g.
The certified value with its expanded uncertainty (U_{CRM}) for a confidence level of approximately 95 % and a coverage factor k = 2 was: MA.HCl mass fraction: (999 ± 12) mg/g or (99.9 ± 1.2) g/ 100g.

4. Conclusions

The approach for evaluating the results of homogeneity and short-term stability studies proposed in this work by measuring the corrected chromatographic area, obtained by the ratio between the analyte response and the mass fraction of the material in the solution, was satisfactory.
The first batch of the CRM of MA.HCl produced by Inmetro was finalized and complied with all the requirements. The certified value with its expanded uncertainty (U_{CRM}) for a confidence level of approximately 95 % andcoverage factor k = 2, was (99.9 ± 1.2) g/100g. This material will be an important tool for forensic laboratories to ensure the metrological traceability of measurement and the quality of the forensic results.

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