DEVELOPMENT STUDIES OF THE CANDIDATE CERTIFIED REFERENCE MATERIAL OF SODIUM DICLOFENAC

Gisele E. B. Silva[1], Suzane M. Queiroz[1], Raquel Nogueira[1], Valnei S. Cunha[1], Eliane C. P. Rego[1], Werickson F. C. Rocha[1], Gabriela F. Moreira[2], Janaína M. Rodrigues[1]

[1] National Institute of Metrology, Standardization and Industrial Quality (Inmetro), Chemical Metrology Division (Dquim), Xerém/RJ, Brazil, dquim@inmetro.gov.br

[2] National Institute of Metrology, Standardization and Industrial Quality (Inmetro), Materials Metrology Division (Dimat), Xerém/RJ, Brazil

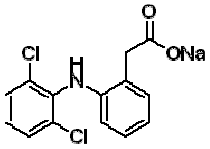
Abstract: This paper describes the development studies of a new sodium diclofenac certified reference material (CRM), which can ensure metrological traceability of measurements results to the International System of Units (SI) and can be used for quality control, validation of analytical methods, and calibration of reference materials of this active pharmaceutical ingredient (API).

Key words: sodium diclofenac, certified reference material (CRM), active pharmaceutical ingredients (API), high performance liquid chromatography (HPLC).

1. INTRODUCTION

The National Institute of Metrology, Standardization and Industrial Quality (Inmetro) started to develop certified reference materials (CRM) of active pharmaceutical ingredients (API) in 2008. After having produced the CRMs of captopril [1] and metronidazole [2], studies are being conducted to certify the candidate CRM of sodium diclofenac (Fig. 1), which is a largely used non-steroidal anti-inflammatory agent.

Fig. 1: Sodium diclofenac structural formula



CRMs of APIs are highly pure substances, which are fully characterized concerning their impurity profile and also evaluated regarding homogeneity between batch units and stability under transport and storage conditions (short- and long-term stability studies). They are accompanied by a certificate, which declares the API mass fraction and its measurement uncertainty.

These CRMs can ensure metrological traceability of measurement results to the International System of Units (SI), a requisite of laboratories accredited by ABNT NBR ISO/IEC 17025:2005 standard [3], and can be used in the quality control of pharmaceutical raw materials and finished products, validation of analytical methods, as well as calibration of non-certified reference materials.

The development of pharmaceutical CRMs is a pioneer project in Brazil, since the Inmetro's captopril CRM is the first Brazilian CRM of an API. Additionally, the project is also relevant in the international scenario, since only a few API CRMs are offered in the market, e.g., four CRMs from the United States Pharmacopeia (USP).

2. MATERIALS AND METHODS

The certification of the Inmetro first batch of sodium diclofenac candidate CRM is based on the requirements of the ISO Guides 34:2009 [4] and 35:2006 [5] and comprises the following steps:

2.1 Analysis of the material according to the Brazilian Pharmacopeia IV [6] and United States Pharmacopeia 34 [7] to verify its adequacy for use as candidate CRM.

2.2 Batch production, carried out as follows: amber glass flasks were filled with 525 mg sodium diclofenac (nominal mass: 500 mg, excess: 5 %) under controlled temperature and humidity conditions, and then were closed with rubber stoppers and aluminum seals (Fig. 2).

Fig. 2: Sodium diclofenac candidate CRM

2.3 HPLC-DAD method validation [8, 9], followed by determination of organic impurities (characterization step 1). The CRM was dried at 105 °C/3 h before the test. A high performance liquid chromatography (HPLC) system (Shimadzu) consisting of a LC-020AT quaternary pump, a DGU on-line degasser, a SPD-20A photodiode array detector, and a CBM-20 interface was employed.

A method based on the United States Pharmacopeia 34 (USP 34) [7] was used under the following experimental conditions: HPLC reversed-phase column C18, 250 x 4.6 mm i.d., 5 µm Luna (Phenomenex); mobile-phase methanol-potassium dihydrogen phosphate buffer 0.005 M pH 2.5 (70:30, V/V), flow-rate 1.0 mL/min, detection wavelength 254 nm, injection volume 20 µL. Solutions containing 750 and 7.5 µg g^{-1} of sodium diclofenac (previously dried) were prepared in methanol-water (70:30, V/V) in triplicate from each candidate CRM flask, and each solution was injected three times into the HPLC system. This test was repeated on three different days.

The mass fraction of each organic impurity in the samples (w_{org}), expressed in g/100 g, was determined by peak area ratio, according to eq. 1:

$$w_{org} = \frac{A_{imp\_750}\, x\, 100}{(A_{diclo\_7,5}\, x\, DF) + \sum A_{imp}} \quad (eq. 1)$$

where A_{imp_750} is the peak area of each organic impurity in the 750 µg g^{-1} solution, $A_{diclo\_7,5}$ is the sodium diclofenac peak area in the 7.5 µg g^{-1} solution, *DF* is the dilution factor (750 to 7.5 µg g^{-1}), and ΣA_{imp} is the sum of organic impurities' peak areas in the 750 µg g^{-1} solution.

The combined standard uncertainty of the organic impurities mass fraction (u_{org}) was determined according to eq. 2:

$$u_{org} = \sqrt{\sum_{i=1}^{N} (\partial_{org} / \partial_{xi})^2\, u_{xi}^2} \quad (eq. 2)$$

where $\partial_{org}/\partial_{xi}$ is the sensitivity coefficient or partial differential of the mass fraction of organic impurities with respect to each uncertainty component x_i, and u_{xi} is the standard uncertainty of each input estimate x_i.

2.4 Determination of volatiles (characterization step 2) by i) loss on drying test [7], ii) headspace GC-FID [7], and iii) Karl Fischer coulometric titration using a Karl Fischer coulometer (831 model, Metrohm AG) equipped with generator electrode without diaphragm, current generator electrode (400 mA), and platinum indicator electrode (10 µA), connected to an oven sample processor (774 model, Metrohm). The experimental conditions were: oven temperature, 105 °C; end-point voltage, 50 mV; extraction time, 300 s; nitrogen-flow, 100 mL min^{-1}.

The estimation of the combined standard uncertainty of the volatiles mass fraction (u_{vol}) was based on the repeatability of results and on the analytical balance error (eq. 3).

$$u_{vol} = x \sqrt{(SD/\sqrt{n}\, x)^2 + (U/k\, m)^2} \quad (eq. 3)$$

where *SD* is the standard deviation, *n* is the number of measurements, *x* is the mean of results, *U* is the expanded uncertainty of the analytical balance, *k* is the coverage factor (*k* = 2 for 95 % confidence level), and *m* is the average weighed mass.

2.5 Determination of inorganic impurities mass fraction (characterization step 3). Considering that sodium is present in the sodium diclofenac structure (Fig. 1), the residue on ignition test (600 ºC), which is largely used to estimate inorganic impurities in pharmaceuticals, could not be applied; therefore, the ICP-MS analysis will be performed. The combined standard uncertainty of the inorganic impurities' mass fraction (u_{inog}) can be estimated by eq. 3.

3.6 Homogeneity study [4, 5]. For this test, 17 CRM flasks selected at random were analyzed, without prior drying. The HPLC method was based on the Brazilian Pharmacopeia IV [6] and consisted of the following experimental conditions: HPLC reversed-phase column C8, 15 x 4,6 mm i.d., 5 µm Luna (Phenomenex); mobile-phase methanol-potassium dihydrogen phosphate buffer 0.005 M pH 2.5 (70:30, V/V), flow-rate 1.0 mL/min, detection wavelength 254 nm, injection volume 50 µL.

The uncertainty due to between-bottle (in)homogeneity (u_{bb}) was calculated using eq. 4:

$$u_{bb} = \sqrt{(MS_{between} - MS_{within})/n} \quad (eq. 4)$$

where $MS_{between}$ is the mean square between groups, MS_{within} the mean square within groups, and *n* the number of replicates.

2.7 Short- and long-term stability studies [4, 5] were carried out without prior drying of the candidate CRM. The HPLC method was the same used for the homogeneity test.

For the short-term stability study, the classical design was used. 10 candidate CRM flasks selected at random were exposed simultaneously to 50 °C. On pre-determined days (1, 3, 14, 17, 21, 24, 42, 44, 55, and 57), one flask was taken out of the oven and individually analyzed (solutions prepared in triplicate, three injections into the HPLC system). Three other CRM flasks, kept at the reference temperature of 25 °C and analyzed in the same way, were used as controls (t = 0).

For the long-term stability study, CRM samples are being kept at the recommended storage conditions (25 ºC). The first analysis was performed at the beginning of the studies, the second after a 10-week storage period. Along the year of certification studies, two other analyses will be carried out. After conclusion of the long-term stability studies, a monitoring program of the CRM batch will be started.

Eq. 5 is used to estimate the uncertainty due to stability (u_{stab}), namely u_{sts} (short-term stability) and u_{lts} (long-term stability).

$$u_{stab} = s_{(b1)}\, t \quad (eq. 5)$$

where $s_{(b1)}$ is uncertainty of the slope (standard error in b_1, regression analysis), and *t* is the time (transport / storage).

2.8 Determination of the sodium diclofenac mass fraction by mass balance [10]. The API mass fraction (w_{API}), expressed in g/100 g, was determined by mass balance (eq. 6):

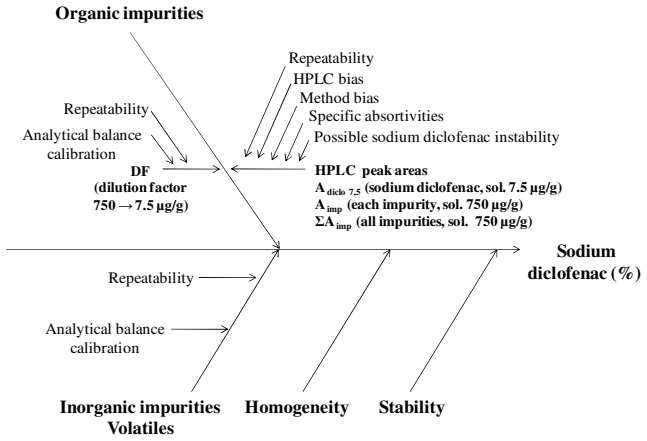
$$w_{API} = 100 - \Sigma w_{org,i} - \Sigma w_{inorg,i} - \Sigma w_{vol,i} \quad (eq. 6)$$

Where $\sum w_{org,i}$, $\sum w_{inorg,I}$, and $\sum w_{vol,i}$ are the respective sums of mass fractions of organic, inorganic, and volatile impurities, expressed in g/100 g.

2.9 Estimation of the CRM combined standard uncertainty (u_{CRM}) [11, 12]:

The uncertainty sources of the mass fraction of sodium diclofenac CRM can be seen in Fig. 3.

Fig. 3. Cause-and-effect diagram showing the uncertainty sources in the certification of the sodium diclofenac CRM



The combined standard uncertainty of the CRM (u_{CRM}) can be calculated according to eq. 7 [11, 12]. This equation is derived from the law of propagation of uncertainties, which consists of "the square root of the total variance obtained by combining all the uncertainty components" [12].

$$u_{\mathrm{CRM}} = \sqrt{{u_{\mathrm{org}}}^2 + {u_{\mathrm{inorg}}}^2 + {u_{\mathrm{vol}}}^2 + {u_{\mathrm{bb}}}^2 + {u_{\mathrm{sts}}}^2 + {u_{\mathrm{lts}}}^2} \quad (eq.7)$$

For the sodium diclofenac candidate CRM, eq. 7 can be reduced to eq. 8, since the CRM will be dried by the customer prior to use. Therefore, u_{vol} can be considered as zero. If the u_{sts} value obtained is smaller than u_{lts}, it will also be disregarded for u_{CRM} calculation.

$$u_{\mathrm{CRM}} = \sqrt{{u_{\mathrm{org}}}^2 + {u_{\mathrm{inorg}}}^2 + {u_{\mathrm{bb}}}^2 + {u_{\mathrm{sts}}}^2 + {u_{\mathrm{lts}}}^2} \quad (eq.8)$$

The expanded uncertainty (U), which is "the interval within which the value of the measurand is believed to lie with a higher level of confidence" [12] is estimated by eq. 9.

$$U = u_{\mathrm{CRM}}\, k \quad (eq.9)$$

2.10 Confirmation of the sodium diclofenac mass fraction by using a primary method or another secondary method.

3. RESULTS AND DISCUSSION

The results of the studies carried out with the sodium diclofenac candidate CRM are detailed below.

3.1 / 3.2 Analysis of the material and production of the candidate CRM batch

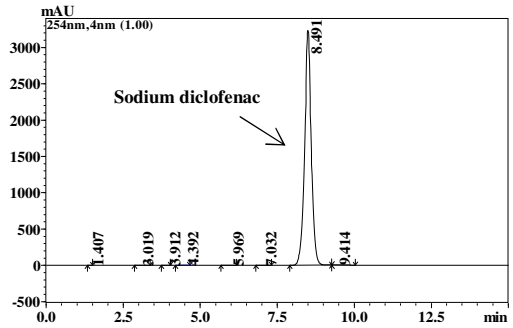
After initial pharmacopeial analyses of the sodium diclofenac, the material was considered appropriate to become a candidate CRM and was filled into glass flasks was previously described. The weight control of the filled flasks resulted in average weight 524.15 mg (n = 10), *RSD* 1.48 %, largest weight deviation from the average 2.6 %.

3.3 Characterization step 1: Determination of organic impurities by HPLC-DAD

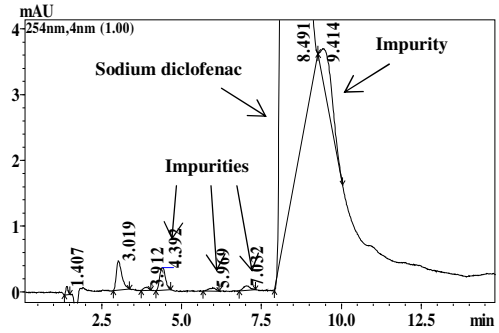
The related substances test described in the pharmacopeial monographs are able to detect residual impurities from the organic synthesis and degradation products. The HPLC method from the Brazilian Pharmacopeia IV (Fig. 4 a-c) was evaluated for this purpose. However, the USP 34 method (Fig. 5 a-c) revealed to be more appropriate for the quantification of organic impurities, since it uses a larger column (250 mm length) and results in more efficient separations.

Fig. 4. Determination of organic impurities by HPLC method from the Brazilian Pharmacopeia IV

(a) sample concentration: 750 µg/g



(b) sample concentration: 750 µg/g (enlarged view)



(c) sample concentration: 7.5 µg/g

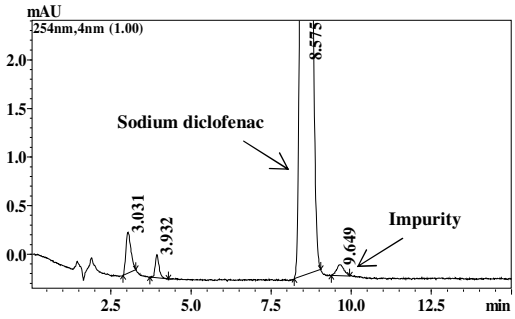
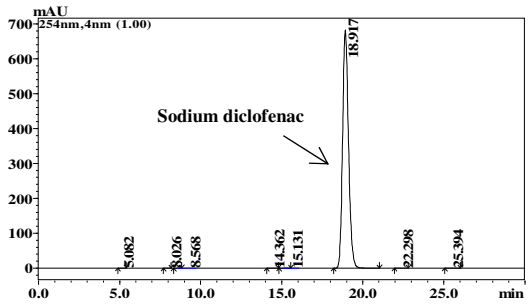
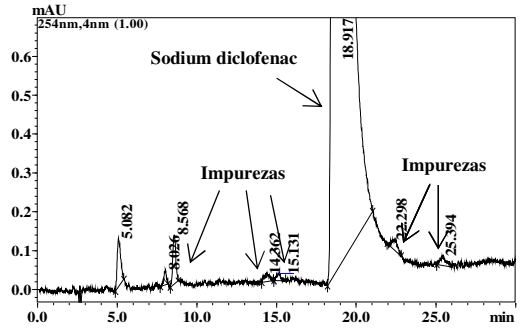


Fig. 5. Determination of organic impurities by HPLC method from USP 34

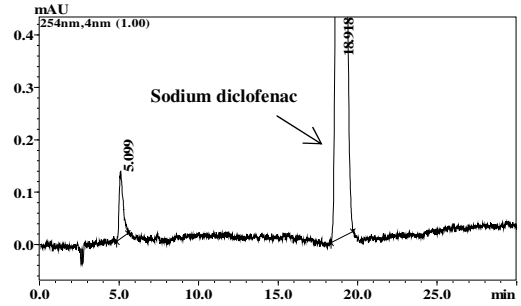
(a) sample concentration: 750 µg/g



(b) sample concentration: 750 µg/g (enlarged view)



(c) sample concentration: 7.5 µg/g



The results of the related substances test can be seen in Table 1. For certification purposes, the peak area ratio calculation was used (eq. 1). The results complied with the limit concentrations for diclofenac related compound A, individual, and total impurities, respectively [7]: 0.2 g/100 g, 0.2 g/100 g, and 0.5 g/100 g.

Table 1. Organic impurities (related substances) mass fraction of the candidate sodium diclofenac CRM

	Mass fraction (g/100 g)	u_{org} [(1)]
1	0.023448	0.003968
2	0.024456	0.004153
3	0.024007	0.004061
Average	**0.023970**	-
SD	0.000505	-
CV (%)	2.106981	-
Largest value	-	**0.004153**

[(1)] u_{org} = combined standard uncertainty of organic impurities' mass fraction

3.4 Characterization step 2: Determination of volatiles

The results obtained by the three different methods employed for determination of volatiles mass fraction (w_{vol}) are shown in Table 2. The results complied with the volatiles mass fraction limit of 0.5 g/100 g [6].

Table 2. Volatiles mass fraction of the sodium diclofenac candidate CRM

Test	Volatiles type	n [(1)]	w_{vol} (g/100 g)	u_{vol} [(2)] (g/100 g)
Loss on drying	Water and residual solvents	14	0.269403	0.012213
Karl Fischer	Water	10	0.243973	0.023132
Headspace GC-FID	Residual solvents	6	Not detected	-

[(1)] n = number of candidate CRM flasks
[(2)] u_{vol} = combined standard uncertainty of volatiles mass fraction

The loss on drying result was considered the most appropriate to indicate the volatiles mass fraction, since it corresponds to the total amount of water and residual solvents eliminated under the test conditions (105 ºC/3 h).

Since this drying procedure will be carried out by the customer prior to use, which is an usual procedure recommended for all pharmacopeial standards that are not degraded by temperature, the volatiles will be considered as zero for the mass balance calculation, and u_{vol} value will be disregarded for u_{CRM} calculation.

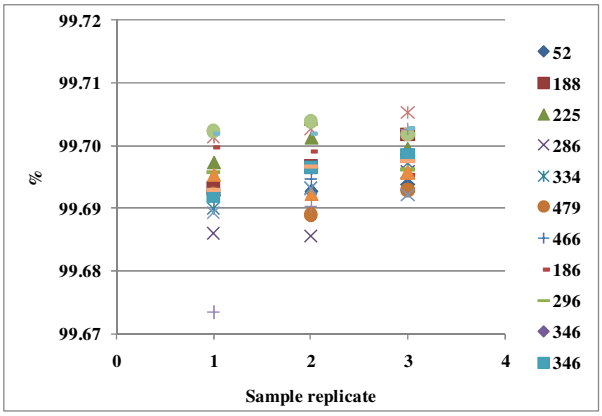
3.5 Characterization step 3: Determination of inorganic impurities

A commonly used method for determination of inorganic impurities, the residue on ignition test, could not be applied to the sodium diclofenac, since it has sodium in its structure (Fig. 1), which is not ignited at 600 ºC. According to the USP 34 monograph of sodium diclofenac, the inorganic impurities should be determined by using a limit test for heavy metals. However, this is not a quantitative methodology and cannot be used for certification purposes. Therefore, another test for quantification of inorganic impurities, especially ICP-MS, will be used.

3.6 Homogeneity testing

The between-bottle homogeneity test results are shown in Fig. 6. The uncertainty due to between-bottle (in)homogeneity (u_{bb}) was estimated as 0.003566 g/100 g (eq. 4).

Fig. 6: Homogeneity test results for the sodium diclofenac candidate CRM

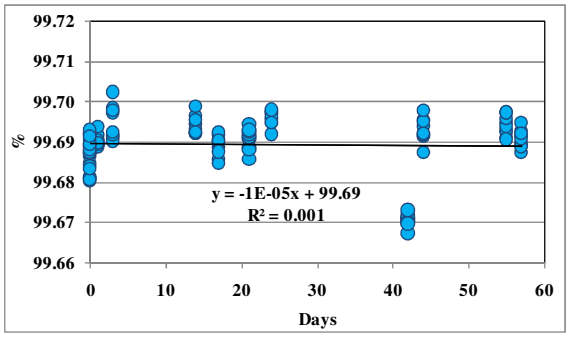


3.7 Short-term stability studies

The short-term stability studies were carried out according to the classical approach, which means that all flasks were exposed to 50 ºC at the same time, and then taken from the oven and analyzed individually on pre-determined days. This type of experimental design is reported to result in larger uncertainty values, since it is performed under reproducibility conditions [4, 5]. Even if the isochronous design (one sample introduced on the oven at a time, and then all samples analyzed together), which occurs under repeatability conditions, could result in smaller uncertainties, the classical design was still preferred. Another reason for that is the long HPLC analysis time, which makes difficult to perform analyses of a large number of samples in a single set.

The short-term stability study results can be seen in Fig. 7. The regression analysis indicated a *p*-value larger than 0.05 (0.87), demonstrating that the curve slope was insignificant. Therefore, there was no indicative of CRM degradation along the study period of 57 days at 50 ºC. The uncertainty due to the short-term stability (u_{sts}) was estimated as 0.001804 % (eq. 5).

Fig. 7. Short-term stability study results for the sodium diclofenac candidate CRM



3.8 Long-term stability studies

The long-term stability studies are in progress and will proceed until one year is completed. After this period, a monitoring program will be started. Along this initial 10-week study period, there was no evidence of MRC degradation.

3.9 Determination of sodium diclofenac mass fraction by mass balance and estimation of the CRM combined standard uncertainty (u_{CRM})

The results for the sodium diclofenac MRC obtained until the moment are summarized in table 3. The sodium diclofenac mass fraction of the candidate CRM will be calculated by mass balance according to eq. 6, while the CRM combined standard uncertainty (u_{CRM}) and expanded uncertainty (U_{CRM}) will be estimated according to eq. 8 and 9.

Table 3. Preliminary results for the sodium diclofenac candidate CRM

	w_{API} (g/100 g)	u_c [(1)] (g/100 g)
Organic impurities	0.023970	0.004153
Inorganic impurities	[(2)]	[(2)]
Volatiles	0.269403 [(3)]	0.012213 [(3)]
Short-term stability		0.001804
Long-term stability		[(2)]
Homogeneity		0.003566

(1) Combined standard uncertainty.
(2) Studies in progress.
(3) The mass fraction of volatiles will not be included either in the mass balance or in the u_{CRM} calculation, considering that the CRM will to be dried prior to use.

3.10 Confirmation of the sodium diclofenac mass fraction

According to the ISO Guides 34:2009 and 35:2006 [4, 5], the certification of reference materials shall be carried out by a primary method, by two independent secondary methods, or by interlaboratory studies.

The mass balance approach is based on three different methods, namely HPLC-DAD for determination of organic impurities, loss on drying for volatiles, and ICP-MS for inorganics impurities. The HPLC-DAD and the ICP-MS are secondary methods, while the loss on drying is a primary method, since it is based on gravimetry. Therefore the mass balance results shall be cross-checked by another independent secondary method, or by a primary method.

The analysis of sodium diclofenac by Differential Scanning Calorimetry (DSC) unfortunately was not possible, due to the analyte decomposition at the temperatures required for the test.

Another option is the use of quantitative Nuclear Magnetic Ressonance (qNMR), a candidate primary method, which is based on the comparison of integrals of ^{1}H signals of the analyte with ^{1}H signals of a certified reference material like benzoic acid. This test will be carried out.

4. CONCLUSIONS

The certification studies of the sodium diclofenac CRM will proceed, in order to offer the third Brazilian CRM of API on the market. Until now, all the obtained results were satisfactory.

The development of the sodium diclofenac CRM makes part of a pioneer project of Inmetro to develop CRMs of active pharmaceutical ingredients, in order to guarantee metrological traceability of measurement results to the SI. The studies are developed in the Organic Analysis

Laboratory (Labor) of Chemical Metrology Division (Dquim) of Inmetro (Fig. 9).

Fig. 9. The pharmaceuticals laboratory, part of the Organic Analysis Laboratory (Labor) of Inmetro

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