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Article

Capillary Electrophoresis Assay Method for Metoprolol and Hydrochlorothiazide in their Combined Dosage Form with Multivariate Optimization

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The current manuscript reports the first capillary electrophoresis method for the separation and quantification of metoprolol (MET) and hydrochlorothiazide (HCT) in their combined dosage form. MET and HCT were detected at 240 and 214 nm, respectively, using a photodiode array detector. The univariate approach was used for optimizing voltage, injection time and capillary temperature. The factorial design with response surface plots, as a multivariate approach, was used to study the effect of buffer concentration and pH on resolution, peak area and migration time. The optimum conditions were 50 mmol/L phosphate at pH 9.5, injection time 10.0 s, voltage 25 kV and capillary temperature 25°C. The method was linear in the range of 2.5–250 μg/mL for both drugs with correlation coefficients above 0.9997. Additionally, acceptable recovery of the contents of MET and HCT in their formulations (96.0–100.3%) with acceptable precision (1.38–2.60 %) were achieved. Moreover, the limits of detection of MET and HCT were 0.02 and 0.01 μg/mL, respectively, which were suitable for pharmaceutical analysis.

Introduction

The preparation of new combinations of medicines, as well as the requirements of modern industrial-scale pharmaceutical analysis, requires researchers to develop new and efficient methods for multi-quantification with separation procedures. High-performance liquid chromatography (HPLC) is a dominant separation technique, especially in pharmaceutical analysis. However, HPLC does not always fulfil all of the requirements of modern industrial-scale pharmaceutical analysis with respect to reagent consumption, analysis time, instrumentation cost and instrumentation simplicity. Capillary electrophoresis (CE), as a microfluidic-based technology, is simpler, faster, less expensive, consumes less reagent and is more efficient in separation than HPLC (1, 2). Hence, the extensive utilization of CE to generate complementary and alternative methods of pharmaceutical analysis is desirable.

To improve the therapy of cardiovascular diseases, many medicinal substances are used in combined dosage forms, as in the case of metoprolol (MET) and hydrochlorothiazide (HCT) (3–5). MET is a β-1 selective adrenoceptor antagonist. It is widely used for the treatment of mild to moderate hypertension and angina pectoris (6). MET is chemically known as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide (Figure 1A). HCT is one of the oldest thiazide diuretics used to treat hypertension (7). HCT is chemically known as 2-propanol, 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-,(±)-,butanedioate (Figure 1B).

The British Pharmacopoeia (8) and the United States Pharmacopeia (9) have addressed assay methods for MET and HCT in their single formulation. However, an official method for their simultaneous quantification in combined dosage forms has not yet been developed. Moreover, despite the widespread use of the combination of MET and HCT, few validated assay methods have been proposed using some analytical techniques. Regarding this issue, two reversed-phase HPLC methods with ultraviolet (UV) detection have been developed (10, 11). Another HPLC method with mass spectrometric detection has also been developed (12). Moreover, derivative spectrophotometric methods for simultaneous quantification of MET and HCT in pharmaceutical preparations have also been reported (13–15).

On the other hand, many CE methods have been reported for the assay of MET or HCT with other drugs. Among those methods, CE and micellar electrokinetic capillary chromatographic (MEKC) (16) have been applied for the separation and quantification of HCT with six angiotensin-II-receptor antagonists (ARAII). In the CE method, a simple electrolyte of 60 mmol/L phosphate at pH 2.5 was adopted, while the MEKC method applied 55 mmol/L phosphate buffer at pH 6.5 with 15 mmol/L sodium dodecyl sulfate (SDS). The authors reported that the CE and MEKC methods were suitable for the qualitative and quantitative determination of combined HCT–ARAIIs in pharmaceutical formulations. In another CE method (17), MET, atenolol and esmolol were separated and quantified with electrochemiluminescence detection. In that method, poly-β-cyclodextrin was added to the running buffer of 20 mmol/L phosphate at pH 10.0 to improve the separation. This method was applied to quantitatively determine MET in its pharmaceutical preparation and to monitor its pharmacokinetics in the human body. Another study proposed the coupling of CE with a capacitively coupled contactless conductivity detector for the determination of a single analysis of MET, chlorpheniramine, clomiphene, catharanthine and vinorelbine and their counter-ions (18). Dual-opposite end injection was used to hydrodynamically introduce the analytes at each end of the capillary (18). In another study, CE with laser-induced fluorescence detection was used for the assay of MET in rabbit plasma (19). In addition, CE and capillary electrochromatography

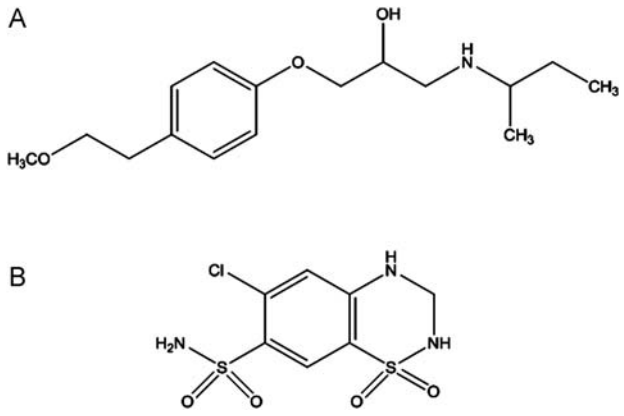

Figure 1. Chemical structures of MET (A); HCT (B).

(CEC) have been used with UV detection for the assay of HCT and losartan in tablets (20). The CE separation was conducted using an electrolyte of 100 mmol/L borate at pH 9 with trimethyl-β-cyclodextrins (20). Furthermore, another CE method with UV detection was developed for atenolol and chlorthalidone in pharmaceutical formulations (21). The running buffer was 20 mmol/L phosphate at pH 9.0 (21). Amiloride, in addition to the latter two drugs, was also assayed by CE with UV detection using a running buffer of 25 mmol/L phosphate at pH 9.0 (22).

Despite all the CE methods described previously, no CE method has yet been reported for the assay of both MET and HCT in their combined dosage form. Therefore, the current study propose the development of a new CE method for the separation and simultaneous quantification of MET and HCT in their binary mixture.

Experimental

Instrumentation

A P/ACE MDQ CE system with a photo-diode array detector from Beckman (Fullerton, CA) was used in the current study. The 32 Karat Software Version 7.0 was used for data handling. A 50.2-cm long × 50 μm i.d. fused-silica capillary was used for separation. The capillary was housed in a cartridge with a detector window of 100 × 800 μm (10 cm to the detector, short end injection). For sample loading, hydrodynamic injection mode was applied. Before each run, the capillary was sequentially washed by 0.1 mol/L sodium hydroxide for 1.5 min, distilled water for 1.5 min and the separation electrolyte for 2.0 min.

Chemicals, reagents and samples

Deionized water was used throughout the experiments. MET and HCT, which were supplied by Sigma-Aldrich (Taufkirchen, Germany), were obtained from Drug Control Centre (Riyadh, Saudi Arabia). HPLC-grade methanol and analytical-grade hydrochloric acid, phosphoric acid, sodium hydroxide and sodium phosphate were supplied by Merck (Darmstadt, Germany). Lopressor HCT tablets including different contents of MET and HCT were prepared by Novartis Pharmaceuticals Corporation (East Hanover, NY).

Preparation of standard solutions

Stock standard solutions of MET and HCT were prepared using an appropriate amount of each drug. The drugs were dissolved by few drops of methanol and then completed with 10% acetonitrile to yield a final concentration of 1.0 mg/mL. The stock solutions of both drugs were stored at 4°C and equilibrated at room temperature before use. Working mixed standard solutions were daily prepared by dilution in the running buffer in the appropriate way.

Preparation of pharmaceutical samples

Twenty tablets were powdered. Amounts equivalent to 50 mg MET and 25 mg HCT were weighed and dissolved by methanol in a 100-mL volumetric flask. The solution was filtered through a 0.45-mm PTFE membrane filter. The filtrate was diluted with the running buffer to yield different concentrations of MET and HCT. Three placebo samples including different concentrations of excipients (cellulose compounds, colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch, glycolate, stearic acid and sucrose) were also prepared.

Results and Discussion

Preliminary investigation

Spectrum scans for MET and HCT were performed individually in a range of 190–400 nm. The λ_{max} for MET and HCT were found to be 240 and 214 nm, respectively, which were set as the optimum. Additionally, the stability of MET and HCT was examined in stock solutions. The results showed that no significant degradation occurred when solutions were stored at 4°C for 72 h.

Method optimization

The proposed CE method was optimized to obtain acceptable resolution, large peak area and less analysis time. To this end, some electrophoretic parameters, instrumental and chemical, were considered. Both univariate and multivariate approaches were adopted for optimization processes. For the latter approach, it is advisable to keep parameters as small as possible (23–31). Hence, conditions presumed to be interacting with each other were optimized by the multivariate approach. Accordingly, the factorial design and response surface plots, as multivariate methods, were used for optimizing buffer concentration and pH (32–34). The univariate approach was used for optimizing instrumental conditions, including voltage, injection time and capillary temperature.

Univariate optimization

To optimize the instrumental conditions, the most common range of voltage used in CE, i.e. 5–30 kV, was examined in the current study (1). High voltage reduces analysis time while low voltage enhances separation. In the current study, 25 kV was set for further optimization because better separation was achieved at a short time. In addition, 1.0–20.0 s, as the most common range of injection time used in CE, was examined at a pressure of 0.5 psi (1). A long injection time improves signals

but causes some loss in resolution and peak symmetry. In the current study, a better result was obtained at an injection time of 10.0 s. Furthermore, the practicable range of capillary temperature, namely 5–30°C, was applied (1). No significant effect from capillary temperature on analysis time and resolution was recorded. Thus, 25°C, as the normal capillary temperature, was set.

Multivariate optimization

To optimize chemical conditions, the 3^2 full-factorial design was adopted for optimizing buffer concentration and pH. The base 3 stands for the minimum, medium and maximum levels of experimental conditions. The power 2 stands for the number of experimental conditions. Based on previous studies (16, 17, 21, 22), a wide range of pH, up to 9.5, was adopted in phosphate buffer. Hence, the levels of buffer concentrations of 25, 37 and 50 mmol/L at pH levels of 4.6, 7.0 and 9.5 were applied. Accordingly, nine experiments were conducted. Because acceptable resolution was obtained in all conditions, the responses of peak height and migration time were considered for setting the optimum conditions. Following the practice of the response surface method (23–31), the obtained data were interpolated using the values −1, 0 and +1 instead of the minimum, medium and maximum levels of the experimental conditions. Then, the response surface plots were constructed (Figures 2–5).

Figures 2 and 3 show that the effect of pH on the peak area of MET and HCT at high buffer concentration is more than that at low buffer concentration. In contrast, the effect of buffer concentration on the peak area of MET at high pH is more than that at low pH (Figure 2) and vice versa for HCT (Figure 3). Fortunately, the largest peak areas of both drugs were obtained at high buffer concentration and high pH values. However, buffer concentration recorded an insignificant effect on migration time at high pH values and vice versa at low pH values, especially for MET (Figures 4 and 5). In general, the shortest migration time of both drugs was obtained at high pH levels. In conclusion, 50 mmol/L phosphate at pH 9.5 was set as the optimum values. The optimum pH of phosphate buffer that was adopted in the current study is comparable with that of previous studies (21, 22), which adopted pH ranges of 9.0–10.0 in phosphate buffer.

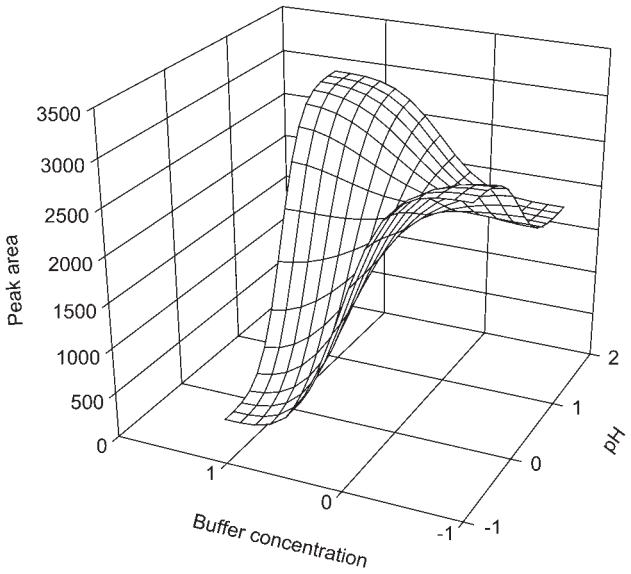

Figure 3. Response surface plot of the peak area of HCT against buffer concentrations (25, 37 and 50 mmol/L) and pH values (4.6, 7.0 and 9.5); fixed conditions: fused-silica capillary column (50.2 cm length × 50 μm i.d.), hydrodynamic injection time of 10 s at pressure of 0.5 psi, separation voltage of 25 kV, column temperature of 25°C, 50 μg/mL MET and 25 μg/mL HCT.

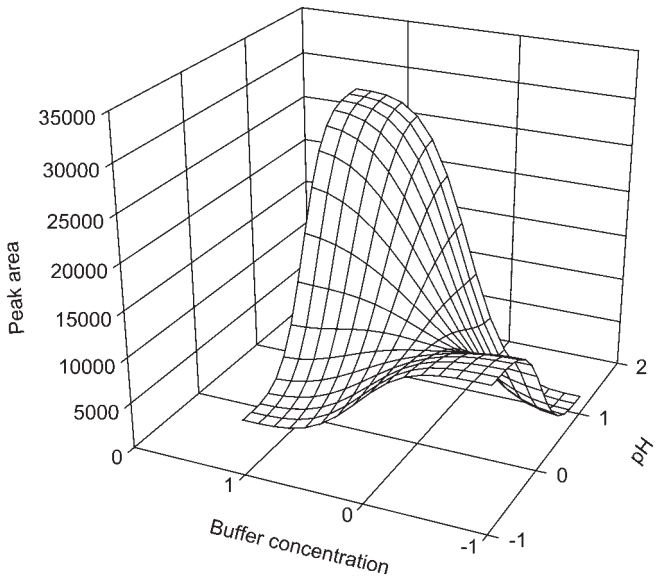

Figure 2. Response surface plot of the peak area of MET against buffer concentrations (25, 37 and 50 mmol/L) and pH values (4.6, 7.0 and 9.5); fixed conditions: fused-silica capillary column (50.2-cm length × 50 μm i.d.), hydrodynamic injection time of 10 s at pressure of 0.5 psi, separation voltage of 25 kV, column temperature of 25°C, 50 μg/mL MET and 25 μg/mL HCT.

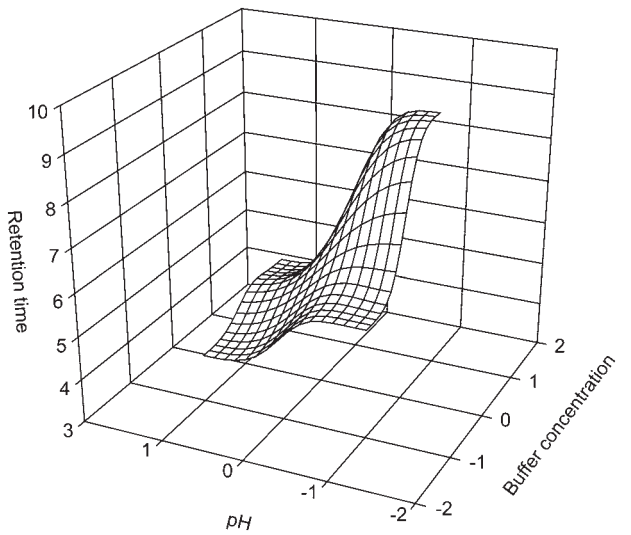

Figure 4. Response surface plot of the retention time of MET against buffer concentration (25, 37 and 50 mmol/L) and pH values (4.6, 7.0 and 9.5); fixed conditions: fused-silica capillary column (50.2 cm length × 50 μm i.d.), hydrodynamic injection time of 10 s at pressure of 0.5 psi, separation voltage of 25 kV, column temperature of 25°C, 50 μg/mL MET and 25 μg/mL HCT.

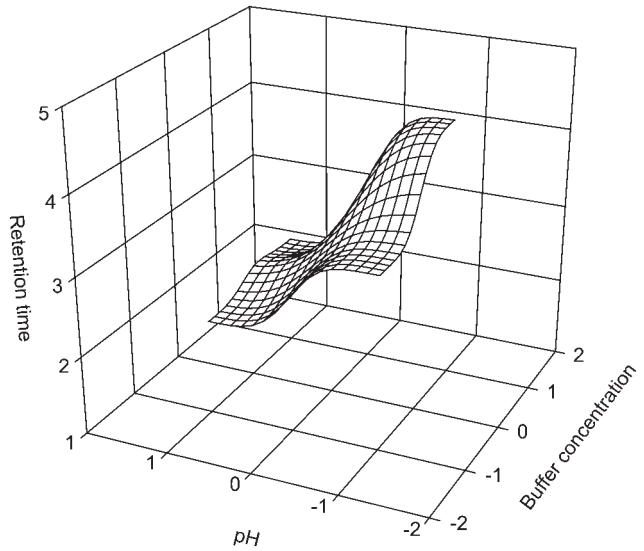

Figure 5. Response surface plot of the retention time of HCT against buffer concentration and pH values (4.6, 7.0 and 9.5); fixed conditions: fused-silica capillary column (50.2 cm length × 50 μm i.d.), hydrodynamic injection time of 10 s at pressure of 0.5 psi, separation voltage of 25 kV, column temperature of 25°C, 50 μg/mL MET and 25 μg/mL HCT.

Table I
Method Validation Results

Analytical feature	MET	HCT
Retention time (min)	2.3	3.3
Peak symmetry	1.12	1.00
Number of theoretical plates	13,258	12,130
Weighed regression of Calibration equation	PA = 0.0023C − 2.1429*	PA = 0.0018C − 1.9853
Correlation coefficient	0.9997	0.9999
Linear range (μg/mL)	2.5–250	2.5–250
Recovery (%)	98.7–100.3	96.0–99.0
Intra-day precision (RSD%, $n = 7$)	1.42	1.38
Inter-day precision (RSD%, $n = 5$)	2.60	2.09
LOD (μg/mL)	0.02	0.01
LOQ (μg/mL)	0.07	0.04

*PA: peak area; C: concentration (μg/mL).

Method validation

The proposed method was validated according to the guidelines of the primary regulatory agencies, namely the International Conference on Harmonization (ICH) Technical Requirements for Registration of Pharmaceuticals for Human Use (35) and the International Union of Pure and Applied Chemistry (IUPAC) (36). The results are introduced in Table I.

Separation efficiency

Resolution (R), peak symmetry (PS) and the number of theoretical plates (N) were calculated using Equations (1–3), respectively (28, 29), in which t_R is a migration time; w_b is the peak width at the base of a peak; A_f is the area of front peak; At is the area of back peak; $w_{0.1}$ is the peak width at the 10% of peak height, A and B are the widths of an asymmetrical peak from t_R to the left and right sides, respectively. MET and HCT were eluted in a relatively short time (9.0 min) with acceptable resolution and numbers of theoretical plates. Borate buffer at pH 9.5 was also examined in the current study. That and this condition also achieved sufficient separation with a resolution of 17.0. However, borate buffer recorded a longer analysis time (6.0 min) with migration times of 3.6 min for MET and 5.7 min for HCT.

$$R = \frac{2(t_{R2} - t_{R1})}{w_{b1} + w_{b2}} \quad (1)$$

$$PS = \frac{A_f}{A_t} \quad (2)$$

$$N \approx \frac{41.7(t_r/w_{0.1})^2}{A/B + 1.25} \quad (3)$$

Linearity

For calibration, a long series of mixed standard solutions including MET and HCT in different concentrations was examined under the optimized conditions. As shown in Table I, due to the optimization of peak areas, wide ranges of linearity for both drugs were obtained with a good correlation, with coefficients of ≥ 0.9997. Previous CE methods (16) recorded narrower ranges of linearity for HCT than our CE method. The previous methods reported a range of linearity for HCT of 40–200 μg/mL when it was separated from irbesartan and a range of linearity of 30–150 μg/mL when it was separated from losartan.

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were calculated. LOD was obtained as the concentration of a solute resulting in a peak height three times the baseline noise level for a pharmaceutical sample. LOQ was calculated as 10 times the baseline noise level for a pharmaceutical sample. As shown in Table I, the obtained LOD and LOQ of both drugs were suitable for pharmaceutical analysis. Additionally, the LOQ of the current CE method is better than that of a previous CE method (18), which was 0.12 μg/mL. The previous CE method applied a dual-opposite end injection and contactless conductivity detection.

Precision

The intra-day precision was evaluated by analyzing three solutions obtained from tablet formulations. Three concentrations of each of MET and HCT were examined. Each sample was analyzed seven times per day. The inter-day precision was evaluated by analyzing the same samples over five consecutive days. The relative standard deviation (RSD) values were calculated. In general, acceptable precision was obtained in the range of 1.38–2.60%.

Recovery and application

The CE method was applied to different doses of MET and HCT in their tablet formulations. For example, a typical electropherogram obtained from a sample was depicted in Figure 6.

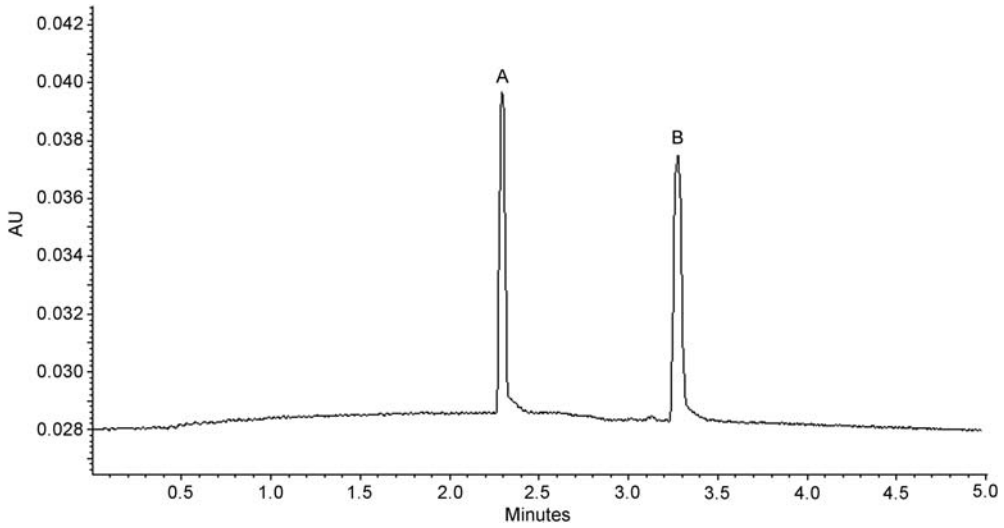

Figure 6. Typical electropherograms obtained from Lopressor HCT tablets including 50 mg/mL MET and 25 mg/mL HCT, obtained under electrophoretic conditions of 50 mmol/L phosphate at pH 9.5, fused-silica capillary column (50.2 cm length × 50 μm i.d.), hydrodynamic injection time of 10 s at pressure of 0.5 psi, separation voltage of 25 kV and column temperature of 25°C: MET (A); HCT (B).

Table II
Application of CE Method to Lopressor HCT Tablets

Content (mg)		Recovery (%)	
MET	HCT	MET	HCT
50	25	98.3	99.0
100	25	100.3	97.8
100	50	98.7	96.0

Placebo samples were also subjected to CE analysis. No peaks were recorded, indicating good selectivity of the method. To apply the proposed CE method, the same samples were analyzed in parallel using a previously validated HPLC method (10). The results were applied as reference values for the recovery calculation (Table II). In general, suitable recovery to pharmaceutical analysis was achieved using our CE method for both drugs in their formulations (96.0–100.3%). The recovery of HCT using the current method is also comparable to that of a previous CE method (16), which was approximately 96.0%.

Conclusion

For the first time, a CE technique was utilized to adopt a new method for the separation and quantification of MET and HCT in their combined dosage form. The adopted CE method is simple, because a simple electrolyte was used with no additives. The method is also rapid, because sufficient separation was obtained in a short time. In addition, the CE method recorded good recovery with acceptable precision. Therefore, the method is suitable to be applied for routine analysis in laboratories of pharmaceutical industry for the purpose of quality control.

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