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Development and validation of a stability indicating capillary electrophoresis method for the determination of metformin hydrochloride in tablets

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ABSTRACT

A simple and a stability indicating capillary electrophoresis method was developed and validated for the analysis of metformin hydrochloride in tablet formulation. The method employed 40 mM citrate buffer at pH 6.7 as a background electrolyte with an applied voltage of 15 kV (positive polarity). The capillary used was of 68.5 cm length (60 cm to detector) and the detection wavelength was 230 nm. The method was validated in accordance with the ICH requirements, which involved accuracy, precision, linearity, selectivity and both limit of detection and limit of quantitation. The current method was linear over the concentration range of 0.2–2.0 mg/ml of metformin hydrochloride. The method was precise as reflected by inter-day and intra-day relative standard deviation (RSD) of less than 2%. The limit of detection and limit of quantitation were 2.0 μg/ml and 8.0 μg/ml, respectively. The stability indicating capability of the method was established by enforced degradation studies combined with peak purity assessment using photodiode array detection.

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1. Introduction

Metformin hydrochloride (MF), 1,1-dimethylbiguanide hydrochloride (Fig. 1) [1] is a biguanide hypoglycaemic agent commonly used for the treatment of type II diabetes [2]. Although MF was developed decades a go it remains wildly used for the treatment of diabetes either alone or in combination with other drugs. From the analytical point of view several methods for its determination in dosage form and biological fluids have been reported [3–11]. A method for determination of MFHCl in dried blood spots has also been reported using HPLC with UV detection [12]. HPLC methods have been reported for the determination of the drug in its tablet dosage form [13,14]. Near infrared spectroscopic technique has also been employed for the determination of MFHCl in dosage form [15].

However, none of the reported methods for the determination of MFHCl was shown to be a stability indicating. Therefore, it was necessary to develop a stability indicating method, for this important compound, that can be employed in stability studies and routine quality control of its preparations. Due to the high polarity of the drug, it has no sufficient retention on RP-HPLC columns and consequently efficient separation from potential degradation products might not be anticipated [16]. Many of the reported methods for the analysis of MFHCl employed a normal phase mode or a cyano column to enhance retention of the compound [4,5,9,11,17]. Recently, a normal phase silica column was employed to separate MFHCl from one of its degradation products [18]. Some of the other reported methods have employed the more common reversed phase columns (C18), but these were successful, only because nearly all of them employed MS detectors, where retention and separation are not a must [3,6,10].

In efforts to enhance retention and subsequent separation efficiency of MFHCl, ion pairing agents such as camphor sulfonic acid have been employed, but only minor improvement in retention was obtained [8,14]. Other approaches that have been reported in attempts to improve retention included derivatization with p-nitrobenzoyl chloride [19].

A capillary electrophoresis method was reported for the analysis of MFHCl in tablets. The method focused on the detection limit (sensitivity) of the method but it was not systematically developed to have a proven stability indicating power [20]. In this study we report a capillary electrophoresis method systematically optimized and validated in light of the current ICH guidelines [21] with a proven stability indicating capabilities.

2. Experimental

MF was obtained from ACDIMA Bio-centre (Amman, Jordan) and was certified to have a purity of 99.8%. Citric acid, boric

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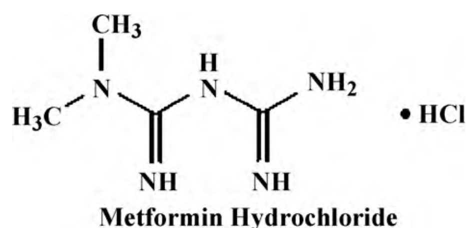

Fig. 1. Structure of metformine HCl.

acid and sodium borate were purchased from Sigma (Sigma, St. Louis, USA). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Commercial metformin tablets: Glucophage® (Merck sante, France) with the batch number 104326, was obtained from pharmacy shops in the Jordanian market.

Agilent 1000 CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector was employed. CE chemstation® software was used for instrument control, data acquisition and data analysis. Fused silica capillaries (50 μm I.D.) were obtained from Composite Metal (UK) and cut to an effective length of 60 cm (total length 68.5 cm). The pH values of the running buffer solutions were adjusted using Mettler Toledo pH meter to within ±0.02. The electrolyte solution was prepared and filtered daily.

Samples were injected hydro-dynamically for 10 s at 10 mbar for all experiments. The finally recommended conditions employed a voltage of 15 kV (positive polarity) with a background electrolyte (BGE) consisted of a citrate buffer (40 mM) with the pH value adjusted to 6.7.

2.1. Method

New capillaries were flushed for 1 h with 1 M sodium hydroxide. Between injections, the capillary was conditioned with 0.1 M sodium hydroxide (1 min) followed by the background electrolyte (BGE) for 2 min. The BGE were prepared by dissolving the correct amount of boric acid or citric acid in 800 ml of double distilled water, titrating with 1 M NaOH, to the required value of pH and completing the volume to 1 l. BGE so prepared were filtered through 0.45 μm membrane filters.

2.2. Preparation of standard solutions and samples

Stock solutions of MFHCl (10 mg/ml) were prepared by dissolving 200 mg of MFHCl with distilled water in 20 ml volumetric flask and completing the volume properly. Separate aliquots (0.5, 1, 2, 2.5, 4 and 5 ml) of stock solution were transformed to a 25 ml volumetric flask and diluted with distilled water to make concentrations: 0.2, 0.4, 0.8, 1, 1.6 and 2 mg/ml, respectively.

Tablets containing 500 mg of MFHCl were accurately weighed and crushed to fine powder. An appropriate amount of the crushed powder equivalent to 100 mg was transferred into 100 ml volumetric flask, diluted to volume with water and sonicated for 10 min, obtaining a final concentration of 1.0 mg/ml of MFHCl. The solution was filtered through 0.45 μm membrane filters and an appropriate portion was placed in a CE vial.

2.2.1. Linear range, accuracy, precision and sensitivity

The peak area for MFHCl was plotted against concentration to construct calibration curves (6 concentration points). The method of least square was employed to examine linearity of the curve. Five curves were constructed and the average values for intercepts, slopes and correlation coefficients were reported. Both intra-day and inter-day precisions were determined employing solutions prepared from Glucophage® tablets as described in Section 2.3. The final filtrate was properly diluted to contain lowest, intermediate and highest concentrations (0.2, 1.0 and 2.0 mg/ml) on the calibration curve. Six separate solutions were prepared at each concentration level and electropherograms obtained within the same day to assess the intra-day, and over a period of 3 days (1–2 injections/day) to assess the inter-day precision. Detection limit and quantification limit were taken as the concentrations at which the peak responses were 3 and 15 times the average noise level, respectively.

2.2.2. Selectivity and forced degradation studies

Selectivity and forced degradation studies were assessed according to guidance of ICH by subjecting a sample of standard MFHCl solution to forced degradation using neutral (distilled water), acidic (0.1 M HCl), basic (0.1 M NaOH), oxidative (10% H_2O_2) and photolytic media (exposure for UV light at 254 nm for 24 h). Neutral, acidic, basic and oxidative solutions were refluxed at 80 °C for 6 h. The peak purity of MFHCl in the obtained electropherograms was assessed with the aid of the photodiode array detector. Potential interference from tablet additives was assessed by subjecting commercially available tablets to analysis and subsequent proof of peak purity. In addition some of the commonly used tablet additives such as starch, lactose, magnesium stearate, sucrose and carboxymethyl cellulose were examined for potential interference with MFHCl peak. 200 mg of each additive were subjected to extraction, as described in Section 2.3, and injected onto capillary under the same conditions.

2.3. Application of the optimized method to commercial samples (Glucophage®)

Commercially available tablets containing 500 mg MFHCl/tablet (Glucophage®) were subjected to analysis using the proposed CE method. Briefly, a quantity of the powdered tablets containing 0.1 g of MFHCl was shaken for15 min with 70 ml of water then diluted to 100 ml with water. The solution was filtered using normal filter paper disregarding the first 20 ml. 10 ml of the filtrate was diluted to 50 ml using the running electrophoresis buffer (citrate). The solution was then filtered through 0.45 μm membrane filter and injected into the CE system.

3. Results and discussion

From preliminary degradation studies of MFHCl, the compound appeared very stable but degraded mostly in NaOH. Because degradation in NaOH produced largest number of new peaks, the sample degraded in NaOH was employed for further optimization of the method. Through method development, the most critical separation was observed between MFHCl and one of the degradation products in the NaOH-degraded sample (relative retention time = 0.91). Therefore, the degree of resolution between MFHCl and this degradation product was considered through method development. The first step in method development was to choose a suitable type and pH of the background electrolyte. The two buffer systems, citrate and borate, were examined in order to cover a pH range 4.5–10. All attempts with pH values 8.2–9.7 using borate buffer at different concentrations (15–60 mM) failed to produce acceptable peak shape. The peak of MFHCl was distorted with severe tailing. The use of citrate buffer, on the other hand, at a concentration of 40 mM and pH value of 4.5–6.7 (at a temperature of 25 °C, 9 kV) resulted in improvement of peak shape of MFHCl. The migration time for MFHCl was shown to increase as the pH decreased (within the range 4.5–6.7) with accompanying peak broadening. The optimum pH value was taken as pH 6.7 as it provided the highest efficiency. A potential explanation for the

obtained efficiency at pH 6.7 could be that, at pH 6.7 the internal wall of the capillary is sufficiently ionized (unlike at pH 4.5, pka for silanol groups is ~7.7 [22]) to allow reasonable EOF which minimizes diffusion and associated peak broadening. On the other hand at the pH value of 6.7, the internal surface of the capillary is not sufficiently charged (as is the case at pH 8–10) thus decreasing potential binding of MFHCl to the internal wall.

The effect of the concentration of citrate was examined in the range 10–60 mM using a temperature of 25 °C, a voltage of 9 kV and a pH value of 6.7. The migration time was found to increase slightly for MFHCl but significantly for other degradants, with increasing buffer concentrations. A compromise between efficiency and migration time (overall) was chosen at 40 mM.

The effect of temperature was studied in the range 15–30 °C. As would be expected, higher temperature values resulted in sharper peaks and lower migration times. Therefore, 30 °C was chosen as the optimum temperature value that produced reasonable efficiency and resolution. The effect of voltage was studied in the range 10–25 kV and the value of 15 kV was chosen as the optimum because it keeps reasonable separation between the critical pair of peaks in the degradation solution within a reasonable time. Thus the finally recommended conditions of the proposed CE method were 40 mM citrate buffer at pH 6.7, temperature of 30 °C at 15 kV (Fig. 2).

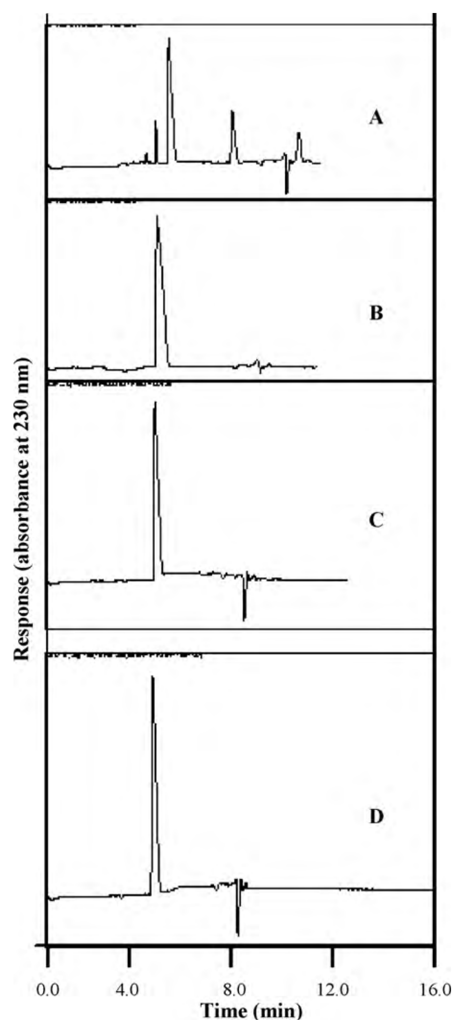

Fig. 2. Representative electropherograms at the finally recommended conditions (40 mM) citrate, pH value of 6.7 at 30 °C and a voltage of 15 kV for (A) NaOH-degraded samples of MFHCl, (B) Standard MFHCl solution, (C) HCl-degraded samples of MFHCl, and (D) H_2O_2-degraded samples of MFHCl. The main peak in all electropherograms corresponds to MFHCl and other peaks correspond to unknown degradation products.

3.1. Method validation

3.1.1. Linearity

The linearity was evaluated and established by triplicate analysis of the standard solutions of MFHCl. The obtained peak areas were plotted against the corresponding concentration to generate calibration curves. Good linearity was evident ($R^2 = 0.9997$) over the examined concentration range 0.2–2.0 mg/ml. A typical calibration equation was $y = 485.5x + 6.31$ where y is peak area and x is the concentration of the standard solution expressed in mg/ml.

3.1.2. Precision

The precision of the method, evaluated as the repeatability was studied by calculating the relative standard deviation (RSD%) of the peak area for six determinations of solutions prepared from Glucophage® tablets at lower (0.2), intermediate (1.0), and high (2.0) concentrations. All determinations were made on the same day and under the same experimental conditions. The obtained RSD values were 1.12%, 0.88% and 0.84% for the lowest, intermediate and highest concentrations, respectively, which indicate the acceptable repeatability of the method.

Inter-day precision was assessed by performing six determinations of Glucophage® solutions, prepared as above, over a period of three days. The obtained RSD values were 1.72, 1.48 and 1.43, respectively. Generally the obtained RSD values indicate acceptable level of inter-day and intra-day precision.

3.1.3. Accuracy

According to the ICH guidance and in cases where the tablet additives (placebo) were not available [21] accuracy could be concluded once precision, linearity and specificity were established. Thus the proposed method can be concluded accurate based on that argument. However, additional proof of accuracy was obtained as follows: (1) for analysis of raw material, accuracy was assessed by three replicate determinations of three different solutions of MFHCl containing 0.8, 1.0 and 1.2 mg/ml, corresponding to 80%, 100% or 120% of the analytical concentrations, respectively. The obtained concentration values when compared to the nominal values produced accuracies of 100.51, 99.98, and 100.97 for the concentrations 80%, 100% and 120%, respectively, with an average RSD value of 0.91%. (2) For the assay of dosage forms, standard addition approach was employed. Tablets of MFHCl were assayed directly and then after adding fixed amounts of the standard drug to their powder. The difference between the results obtained for the directly analyzed samples and the spiked tablet's powder should correspond to the amount of the drug added in each case. Recovery values expressed as percentage recovery of the added drug were 100.1%, 100.5% and 99.9% for added amounts of 20, 40, and 80 mg/100 mg of the expected amount of MFHCl, respectively ($n = 5$, RSD was less than 1.6%).

Limit of detection was taken as the concentration that produced a response to noise ratio of three which was found to be 1.4 μg/ml. LOQ was decided as the concentration that produced a response to noise ratio of 10 or more and that was found to be 6.0 μg/ml. At 6.0 μg/ml an RSD value of 1.96 was obtained which ensures the suitability of this value as LOQ.

3.1.4. Selectivity

Selectivity of the method was established by subjecting samples of MFHCl standard to degradation in NaOH, HCl, H_2O_2 and UV light. The degradation solutions were subjected to electrophoresis using the proposed method. Only the sample subjected to NaOH showed significant new peaks in their electropherograms (Fig. 2). In all cases

Table 1
Results for ruggedness studies.

pH value	6.5	6.7	6.9	6.7	6.7	6.7
Citrate concentration (mM)	40	40	40	38	40	42
Resolution	3.65	3.75	3.81	3.55	3.75	3.64
Precession	1.35	1.31	1.42	1.37	1.31	1.39

the purity of MFHCl was assessed with the aid of PDA detector. Peak slicing technique with the aid of PDA detector was employed to check for peak purity. Absorption spectra were obtained at the upslope, apex and down slope of the MFHCl peaks. In all cases the three overlaid UV spectra were identical, indicating peak purity, and consequently the selectivity of the method.

The proposed method was employed for the analysis of the commercially available MFHCl tablets (Glucophage®). The obtained overlaid UV spectra for the peaks of MFHCl in the analyzed tablets were identical indicating the purity of the peak and lack of interference from the relevant formulation additives. Moreover, samples of starch, lactose sucrose, magnesium stearate and carboxymethyl cellulose when subjected to the proposed method did not show any peaks that might interfere with that of MFHCl.

3.1.5. Ruggedness

The most important parameters that are likely to be changed and consequently influence the analytical performance of the method were the concentration and the pH value of the background buffer. Other parameters such as the applied voltage and temperature were actually very well controlled by the machine. Therefore, the effect of slight changes of buffer concentrations and pH on the performance of the method was studied. The criteria assessed were the resolution of the critical pair in the NaOH-degraded MFHCl samples as well as the precision for the peak area for MFHCl. Results are summarized in Table 1. In general the obtained data suggest a reasonable robustness of the method.

3.1.6. System suitability test

The most critical separation was observed between MFHCl and one of the degradants in the NaOH-degraded sample (relative retention time = 0.91). Therefore, resolution between this degradation and MFHCl was taken as system suitability criteria. The minimum obtained value for resolution of this critical pair was 3.5 therefore a resolution value of 3.5 could be taken as the system suitability criteria. In addition, based on precision studies, the precision of the peak area of MFHCl should be less than 1.5 for triplicate injections of the standard solution (1 mg/ml).

4. Conclusion

A stability indicating assay method was successfully developed for the determination of MFHCl in tablets. The method was shown to have sufficient linearity, accuracy, precision, sensitivity, selectivity and robustness. MFHCl was shown to be highly stable with maximum degradation in alkaline solution. The proposed method was shown to effectively separate MFHCl from its major degradation products obtained under alkaline condition. The method has the additional advantages of employing aqueous system rather than the potentially toxic organic solvents, and minimum sample preparation steps.

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