Application of two charge transfer complex formation reactions for selective determination of metformin hydrochloride in pharmaceuticals and urine

Nagaraju Rajendraprasad1* and Kanakapura Basavaiah2

Abstract

Background: Metformin hydrochloride (MFH) is a biguanide class anti-diabetic drug used to treat type-2 diabetes mellitus. Its reaction with two charge-transfer complexing agents, p-chloranilic acid (PCA) and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) in acetonitrile medium to yield coloured products measurable at wavelengths of maxima 530.0 and 460.0 nm, respectively, was conveniently used to develop two spectrophotometric methods for analyses of bulk sample and tablets.

Results: The effect of solvent, reagent concentration and reaction time to form charge-transfer (CT) complexes was meticulously studied and optimized. Under optimised conditions, the absorbance at the respective wavelength of maximum versus concentration of MFH was in linear correlation for the range from 8.0 to 320.0 μg mL−1 in PCA and DDQ methods, respectively, and correspondingly, the values of molar absorptivity of 0.733 × 103 and 0.257 × 104 L mol−1 cm−1 and Sandell sensitivity of 0.3620 and 0.0644 μg cm−2. The quantification (QL) and detection (DL) limits were 2.67 and 0.88 μg mL−1 for PCA method, and 0.33 and 0.11 μg mL−1 for DDQ method.

Conclusion: The new methods were emerged as repeatable and reproducible, with replicate measurements for intra- and inter-day variations as showed by obtained RSD values of < 2%. Within a day and between day relative errors were ≤ 2.18%. Methods were also validated for robustness, ruggedness and selectivity and agreeing results were produced. The methods were used to analyse MFH-containing tablets very accurately and precisely as reflected by the mean recovery value close to 100% and lower RSD values, respectively. Analysis of spiked human urine yielded excellent mean recoveries, indicating the absence of interference from endogenous substances.

Keywords: Metformin hydrochloride, Determination, Spectrophotometry, C-T complex, Pharmaceuticals

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Background

Metformin hydrochloride (MFH), has the IUPAC name of 1,1-dimethylbiguanide monohydrochloride (Fig. 1), is an anti-diabetic drug belonging to biguanide class [1]. MFH is the anti-hyperglycemic compound and used to treat type 2 diabetic patients [2]. The drug lowers the glucose level of plasma.

MFH is official in European (EP) [3] and the United States Pharmacopeia (USP) [4]. Both describe titrimetry to determine MFH in pharmaceuticals; in the former, drug solution in anhydrous formic acid-acetonitrile mixture is titrated with perchloric acid with potentiometric end-point detection and the latter involves the titration of MFH in anhydrous mixture of formic and acetic acids versus perchloric acid, determining the endpoint visually, with crystal violet indicator.

Different analytical techniques have been employed by different workers for MFH determination in real pharmaceutical samples and they are liquid chromatography (LC) [5–15], high performance-thin layer chromatography (HPTLC) [16–19], gas chromatography (GC) [20], capillary electrophoresis (CE) [21], uv-spectrophotometry [22–28], spectrofluorimetry [25], potentiometry [29–32] and conductometry [33]. MFH was determined simultaneously along with other therapeutic drugs in pharmaceuticals by ultra-violet spectrometry [34], LC [35–37] and CE [38]. Fourier transform infra-red spectrometric technique has also been utilised to determine chemical stability of the drug MFH [15] in pharmaceutical samples. These methods, no doubt, are selective and sensitive, but lack the simplicity and ease of operation, which are desirable for rapid routine analytical application. Visible spectrophotometry meets these criteria, but has been sparsely used for quantifying MFH.

A direct charge-transfer (C-T) complex formation reaction of MFH with iodine in acetonitrile medium has been reported [22]. The linear correlation exists between absorbance and concentration of the range 1.66–72.86 μg mL⁻¹ MFH. A 2–12 μg mL⁻¹ MFH quantification is possible in the method of iodine-drug C-T complex measurement at 295 nm, as reported by El-Bardicy et al. [24]. A complexation reaction between MFH and Cu(II) in cyclohexylamine medium to yield purple coloured product (λmax 540 nm) was found described [25]. The MFH-ninhydrin complex, measurable at 570 nm, is reported by Mubeen and Noor [39]. Vandana et al. [40] described the assay procedure of reaction between MFH and ninhydrin-merbromine mixture to form Ruhemann’s purple product with 570 nm as wavelength of maximum. Quantitative reaction was found to occur for 10 min at 90 ± 1 °C. In this ninhydrin-merbromine mixture method, MFH can be determined over 10–30 μg mL⁻¹ concentration range. Pignard [41] has reported a method for MFH with NaOCl using NaOH and ZnSO₄ to obtain and measure yellow coloured product. Methods were also developed to determine MFH with triacetyl and 1-naphthol in alkaline ethanol [42] and bromothymol-blue in phosphate buffer [43], and these are applicable exclusively for urine samples.

The stable molecular system formed in solution between an electron-donating molecule having sufficiently low ionization energy, and an electron accepting molecule having high electron affinity and these molecules are C-T complexes. Principally, these complexes possess new and intense absorption bands in either uv or visible region of the electromagnetic spectrum. Absorption bands of this type are known as C-T bands since they involve electronic transition from an orbital on the donor atom to the vacant orbital on the acceptor. C-T complexes are formed due to transfer of an electron from a π–molecular orbital of donor (Lewis base) to a vacant π–molecular orbital of the acceptor (Lewis acid), i.e. π–π electronic transitions [44]. These complexes are of particular interest, and they were widely applicable for assay of many therapeutics by spectrophotometry [45–50].

In spite of wide utility of C-T complexation reactions in analysis of therapeutic agents [45–50], MFH, according to literature survey, has not been determined based on these versatile reactions. Taking into account the basic nature of metformin, it was made to get reacted with PCA and DDQ (as π–acceptors) in acetonitrile medium to yield coloured products. Thus, two highly feasible and simple quantitation procedures with spectrophotometry were proposed herein for MFH. The coloured radical anions such as MFH-PCA (measurable at 530.0 nm) and MFH-DDQ (measurable at 460.0 nm) complexes are of greater interest in this report to quantify MFH. MFH was able to be determined more selectively and satisfactorily in human urine sample spiked by drug by these methods.

Methods

Instrumentation

The absorbances were recorded with digital Systronics manufactured spectrophotometer (Ahmedabad, Gujarat, India) and quartz cells (path length: 1 cm).

Analytical grade reagents and spectroscopic grade organic solvents were used through the investigation. The pure drug of pharmaceutical grade metformin hydrochloride
(MFH, 99.9% pure) was procured from Sanofi Aventis, Mumbai, India, was utilised in the analysis. Cetapin XR and Glyciphage tablets (500 mg MFH/tablet), manufactured and supplied by Franco Pharmaceuticals and Sanofi Aventis of India, respectively, were obtained from local stores and used for analyses.

Preparation of stock solutions
Solutions of 0.2% (w/v) each of \( p \)-chloranilic acid (PCA) and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) were prepared freshly using standard compounds (both from S.D. Fine Chem. Ltd., Mumbai) in 1,4-dioxan (Merck, India). A 10% (w/v) solution of NaOH was prepared in doubly-deionised water.

Preparation of standard metformin base (MEB) solution from metformin hydrochloride (MFH)
An amount (128 mg) of standard MFH corresponding to 100 mg of its base (MEB) was dissolved in 20 mL of water. After adding a 15 mL of 10% NaOH solution, the content was extracted for 2 min with 2 × 30 mL portions of dichloromethane. Each extract was washed thoroughly with 15 mL of deionised water in another separating funnel. The washed and pooled extracts were then passed through 5 g of anhydrous Na\(_2\)SO\(_4\) and collected to 100 mL beaker. The solvent was then evaporated, and the residue dissolved in and diluted to 100 mL in a volumetric flask to get 1000 \( \mu \text{g mL}^{-1} \) MEB in acetonitrile. Suitable aliquots were then diluted to get 400 and finally to 80 \( \mu \text{g mL}^{-1} \) solutions in equivalent to MEB and analysed by PCA and DDQ methods, respectively.

General procedures

Procedure for bulk drug

Construction of calibration line PCA method
A clean micro-buret was filled with 400.0 \( \mu \text{g mL}^{-1} \) MEB solution and transferred 0.1, 0.25, 0.5, 1.0, ....... 4.0 mL into an array of 5 mL calibrated flasks. The solvent volume in each flask was increased to 4.0 mL with acetonitrile. 1.0 mL of PCA was added and contents were mixed and kept standby for 10 min. Then, the absorbance was recorded in reference to the reagent blank employing 530.0 nm as absorption maximum.

DDQ method
Different aliquots of 80.0 \( \mu \text{g mL}^{-1} \) MEB solution so as to cover 1.60 to 64.0 \( \mu \text{g mL}^{-1} \) concentrations were accurately transferred to a sequence of 5 mL calibrated flasks, the volume was raised to 4 mL with the aid of acetonitrile, 1 mL of DDQ solution added and after mixing, the contents were let stand ahead for 2 min. Each solution was used and recorded the absorbance at 460.0 nm versus the reagent blank.

The calibration lines of MEB concentration in \( \mu \text{g mL}^{-1} \) against absorbance in each method were constructed and used to calculate the MEB concentration in \( \mu \text{g mL}^{-1} \) in unknowns. Otherwise, the respective regression equation derived was also used to avail the concentrations in unknowns.

Procedure for tablets
Finely grounded tablet powder contained with 100 mg MEB was dissolved in about 20 mL of water and poured into a 125 mL separating funnel. The procedure of
conversion of MFH to MEB was followed. The 400.0 and 80.0 μg mL⁻¹ solutions in respect of MEB were obtained by dilution and correspondingly used for assay by PCA and DDQ methods. Aliquots of appropriate volumes were befit for assay by the procedures as described under construction of calibration line.

Procedure for selectivity by analyses of placebo and synthetic mixture

A homogeneous powder of 40 mg each of acacia, hydroxyl cellulose, magnesium sodium alginate, stearate, starch, sodium citrate and talc was composed as placebo. Accurately 20 mg of placebo was used and extracted prepared by following procedure described for tablets. A 2-mL aliquot of placebo extract was used for analysis following the procedures described in PCA and DDQ methods separately.

A synthetically prepared mixture of placebo and drug in the form of powder was constituted by mixing 128 mg MFH and 100 mg placebo. Metformin base (MEB) solution in acetonitrile was prepared using the procedure for preparation of placebo or tablet extract and was used for analyses by PCA and DDQ procedures. The percentage recovery of MEB was then calculated.

Procedure for spiked human urine sample

Urine (5 mL) was spiked with 128 mg of pure MFH, diluted to 10 mL with 10% NaOH and carefully taken in the separating funnel. Then, the procedure to prepare basic form of MFH was intact followed. After evaporation, the residue was constituted in acetonitrile and the resulting solution was diluted to get 400 and 80 μg mL⁻¹ with acetonitrile, and used in PCA method or DDQ

Table 1 Results showing the effect of solvents on the absorbance of C-T complexes when 160 and 32 μg mL⁻¹ MEB used in the reaction in PCA and DDQ methods, respectively

| Solvent      | PCA method | DDQ method |
|--------------|------------|------------|
|              | λmax, nm   | Absorbance of blank at λmax | Absorbance of product of MEB and PCA at λmax | λmax, nm | Absorbance of blank at λmax | Absorbance of product of MEB and DDQ at λmax |
| Acetonitrile | 530.0      | 0.013      | 0.496      | 460.0 | 0.006      | 0.520      |
| Chloroform   | 525.0      | 0.014      | 0.232      | 450.0 | 0.018      | 0.315      |
| Methanol     | 520.0      | 0.019      | 0.331      | 450.0 | 0.024      | 0.321      |
| Ethanol      | 520.0      | 0.014      | 0.292      | 450.0 | 0.011      | 0.268      |
| Acetone      | 520.0      | 0.014      | 0.232      | 445.0 | 0.017      | 0.260      |
| 1,4-Dioxane  | 520.0      | 0.018      | 0.124      | 450.0 | 0.019      | 0.160      |
| Dichloromethane | 525.0     | 0.023      | 0.212      | 450.0 | 0.022      | 0.302      |
| Dimethylformamide | 525.0     | 0.028      | 0.224      | 450.0 | 0.024      | 0.319      |

*Wavelength maximum of C-T complexes in respective solvent*
method, respectively. The drug contents recovered (in %MEB) were calculated for each case.

Results
Spectral characteristics
The reaction between MEB and PCA resulted an intense reddish violet complex with analytical wavelength of maximum ($\lambda_{\text{max}}$) at 530.0 nm (Fig. 2). The PCA radical anion was assumed to be formed there. In resembling to the reaction of MEB with PCA, the free base of MFH, MEB also reacted with DDQ to produce an intense orange-red colored complex with highly intense band at 460.0 nm. Two other bands (at 510.0 and 570.0 nm) (Fig. 2) were also seen in the spectra, and the choice of 460.0 nm was made because of sensitivity of the reaction product and low blank absorbance.

Method development
Optimization of reaction conditions
Optimum conditions were established by measuring absorbance of either MEB-PCA or MEB-DDQ C-T complexes, by varying parameters separately and individually at a time while others maintained intact or unvaried.

Effect of volume of PCA and DDQ
Figure 3 illustrates the impact of PCA and DDQ concentrations on their respective complexes with MEB. MEB was allowed to react with either 0.5–3 mL of 0.2% PCA or 0.5–
3 mL of 0.2% DDQ. Maximum and minimum absorbance values were obtained for sample and blank, respectively, when 1 mL of PCA or DDQ used in PCA or DDQ method.

Effect of dissolution or reaction solvent
MEB was reacted with either PCA or DDQ in acetonitrile, chloroform, ethanol, methanol, acetone, 1,4-dioxane, dichloromethane and dimethylformamide. Maximum sample absorbance and minimum blank absorbance were observed in acetonitrile. The respective results, come across during the fixing up of solvent are presented in Table 1 below, indicated that acetonitrile as a suitable solvent.

Effect of time and stability of C-T complexes
The times required to form a stable C-T complexes were fixed by measuring absorption of the respective complex formed upon adding the PCA or DDQ solution to MEB solution at different intervals of time. The color formation was complete in 10 min in PCA, and 2 min in DDQ methods were stable for 2.5 and 3 h of time in PCA and DDQ methods, respectively.

Investigation of stoichiometry of CT complexes
Principle of Job’s continuous variations method [51] was invaded to evaluate the reaction stoichiometry between MEB and either PCA or DDQ. In this experiment, equimolar solutions of drug and reagent were mixed out of 5.0 mL total volume. The plots of absorbance at wavelength of maximum versus mole ratio of MEB in each method were prepared (Fig. 4). The maximum absorbance of both MEB-PCA and MEB-DDQ C-T complexes at mole ratio of 0.5 indicated that the drug in base form (MEB) and reagent (PCA or DDQ) followed a 1:1 reaction stoichiometry.

Method validation
The optimised experimental conditions have been invaded and the final procedures for PCA and DDQ...
methods have been described. The validity of both the analytical methods has been assessed for linearity, accuracy, precision, robustness and ruggedness on par with the ICH guidelines [52, 53]. The details and consequent assessment results from validation are presented in sections below.

**Evaluation of linearity with calibration and sensitivity parameters**

A linear relationship was originated between absorbance at \( \lambda \text{max} \) and concentration of MEB (Fig. 5) in the ranges of concentrations mentioned below (Table 2). The method of least squares was followed and the slopes (m), intercepts (b) and correlation coefficients \( \beta \) in PCA and DDQ methods were calculated and are also reported (Table 2). The calibration or linear range, molar absorption coefficient, Sandell’s sensitivity and ranges of PCA and DDQ methods are also tabulated (Table 2). The detection (DL) and quantitation limits (QL) have been calculated using the slope of the calibration curve and the standard deviation of the blank absorbance readings of respective method as per the ICH Guidelines [53]. The formulae used to calculate DL and QL are given below:

\[
DL = \frac{3.3 \times SD}{m} \quad \text{and} \quad QL = \frac{10 \times SD}{m}
\]

where SD is the standard deviation of absorbance of set of blanks and m is the slope of the calibration curve of respective method. The values of DL and QL for PCA and DDQ methods have been reported in Table 2.

**Accuracy, repeatability and intermediate variation**

The assay procedures were repeated seven times within the day and five times on different days to evaluate repeatability and intermediate variation, respectively. The accuracies have also been reported in the mean time for proposed PCA and DDQ methods. Solutions of different concentrations of MFH equivalent to MEB were assayed at three levels. The results, expressed as %RSD, were ≤ 1.13 in intra-day and ≤ 1.75 (Table 3) in inter-day variations indicating high precision with respect to repeatability and less deviations in the results of assay in the study of intermediate variations of both the methods. Accuracy, expressed as %RE, between the mean/average concentrations and true or taken concentrations of MEB. The %RE values in Table 3 were calculated for the studied concentrations of MEB, were ≤ 2.18% demonstrated the high accuracies of presented PCA and DDQ methods.

**Evaluation of robustness and ruggedness**

Small incremental changes were made in the volume of reagent and time. The effects of the changes were studied on the absorption of complex systems for study of robustness. In ruggedness study, assays were done by three analysts, and also by a single analyst performing analysis on three different instruments in the same laboratory. The intermediate precision values, expressed as %RSD, were from 0.47 to 2.98 indicating acceptable robustness and ruggedness. Table 4 below is dedicated to highlight the overall results of these studies.

**Selectivity**

After subjecting the placebo extract for analysis the resembling results with reagent blank confirmed inactive role of substances in placebo. The selectivity was also ensured by analysing synthetic mixture extract. The analysis yielded a percentage recovery values of MFH from 97.89 to 101.70 with a standard deviation below 2%.

### Results of percent recovery of MFH in the analysis of spiked human urine

| Method     | Spiked concentration, \( \mu g \text{ mL}^{-1} \) | Found ± SD | % Recovery ± RSD |
|------------|-----------------------------------------------|------------|------------------|
| PCA method | 200.00                                        | 197.43 ± 1.09 | 98.72 ± 0.72     |
| DDQ method | 40.00                                         | 39.15 ± 0.92  | 97.87 ± 1.05     |

*Average of five determinations; SD is standard deviation
demonstrated the non-interference of additives in a synthetic mixture in MFH quantification.

Application to tablets
Cetapin XR 500 mg (product X) and Glyciphage 500 mg (Product Y) tablets were subjected to analysis by general quantification procedures. The mean recoveries were calculated and compared with official method [3] statistically. The results of statistical tests as revealed by the Student’s t and variance ratio F values [52] were in very good agreement. The mean recovery values of MFH from PCA, DDQ and official methods with standard deviation, t and F values are indicated below in Table 5.

Accuracy by recovery study
The accuracy and selectivity were further confirmed by performing a recovery test. To a fixed quantity of the pre-analyzed tablet extract, pure MFH solutions were spiked at three levels of different concentrations and total MFH was determined by following proposed PCA and DDQ methods. Three replicates from each level were assayed; the recoveries of MFH (pure) were calculated and compiled in Table 6. These results reveal that commonly added adjuvants did not show interference. Further, the results also outlined the selectivity of analytical methods with accurate determination.

Application to spiked human urine
The proposed analytical procedures were employed to determine MFH in extract of human urine spiked by pure drug so that the usefulness of them for physiotherapeutic administration of drug to be made possible. The obtained results of spiked human urine analysis are presented below in Table 7.

| Table 8 | Details with performance characteristics of reported visible spectrophotometric methods |
|---------|-------------------------------------------------------------------------------------|
| Reagents and solvents used | Methodology employed | Linear range | Molar absorption coefficient, L mol⁻¹ cm⁻¹ | Remarks | Reference No. |
| Iodine and acetonitrile | The absorbance of yellow C-T complex of MFH with iodine measured in acetonitrile at: | 1.656–72.86 mg mL⁻¹ | a) 1.03 × 10⁴ b) 2.27 × 10⁴ c) 3.63 × 10⁴ | Lower analytical wavelengths employed where photometric errors are possible. Incomplete validation reported | [22] |
| Iodine and dichloroethane | The absorbance of yellow C-T complex of MFH with iodine measured in acetonitrile at 295 nm | 2–12 μg mL⁻¹ | – | – | [24] |
| Copper(II) sulfate and cyclohexylamine | Measurement of purple coloured product at 540 nm | 0.5–2 mg mL⁻¹ | 2.27 × 10³ | Less sensitive and larger volume of organic solvent required. | [25] |
| Ninhydrin and alkali | Measurement of MFH-ninhydrin complex at 570 nm | 8–18 μg mL⁻¹ | 57 | Less sensitive and incompletely validated. | [39] |
| Ninhydrin and ammonium molybdate mixture | A C-T complex of MFH with ninhydrin formed at 90 C measured at 570 nm | 10–30 μg mL⁻¹ | 9.49 × 10³ | Not applicable for room temperature analysis. Incompletely validated. | [40] |
| NaOCl, NaOH and ZnSO₄ | Measurement of yellow product | – | – | The reaction is not suitable for routine metformin assay, since the amount of NaOCl required has to be comparable to that of MEB and an excess of NaOCl destroys the color development. Moreover the method was developed only for urine analysis. | [41] |
| Triacetyl, 1-naphthol and alkaline ethandiol | Measurement of colored products | – | – | Applicable to urine sample. Incomplete method validation. | [42] |
| Bromothymol blue in phosphate buffer | – | – | – | – | [43] |
| p-Chloranilic acid (PCA) and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) and acetonitrile | Measurement of C-T complex of MEB with: | a) 8.0 to 320.0 μg mL⁻¹ b) 1.6 to 64.0 μg mL⁻¹ | a) 0.733 × 10⁴ b) 0.257 × 10⁴ | Methods have been completely validated. Applicable to analyse dosage forms and urine samples. | Present work |
**Discussion**

Various methods were reported by researchers for MFH determination. The articles describing the analytical procedures using chromatographic [5–20], electrophoretic [21] and spectrofluorimetric [25] techniques were described as selective methods for MFH. Unfortunately, the need for sophisticated instruments and highly skilful operator to execute the assay plan made them disadvantageous. On the other end, the reported ultra-violet spectrophotometric methods [22–28] are also suffering from limitations such as shorter analytical wavelength where the possible photometric error arises, less sensitivity, narrow linear range or additional extraction steps with organic solvents. The potentiometric methods [29–32] are usable only with the fabricated electrodes or sensors which have not been thoroughly validated and moreover with shorter life time. Requirement of larger sample size per titration is the major drawback of conductometric method [33]. The methods developed for MFH using spectrophotometry [34], liquid chromatography [35–37] and capillary electrophoresis [38] are applicable for simultaneous determination of MFH in the presence of other therapeutics. There are few visible spectrophotometric methods were also developed and published. The details, performance characteristics and limitations of reported visible spectrophotometric methods are summarised below in Table 8.

The limitations or disadvantages of reported spectrophotometric methods have been showed in the above table (Table 8), and thus, a clear footstep was taken to develop simple analytical methods with complete validation to quantify MFH in pharmaceuticals and urine.

Metformin base (MEB) as n-donor, reacts with PCA and DDQ, the π-acceptors, to form C-T complexes, which dissociates into radical anions in solvents such as acetonitrile. The e⁻ transfer process from the donor to the acceptor mostly occurs, and consequently, the intensely colored radical anions observed. The probable pathway is indicated below:

\[
\text{MEB} + \text{A} \rightarrow \text{MEB-A} \rightarrow \text{MEB}^+ + \text{A}^- \quad \text{(Radical anion)}
\]

The reaction between π-acceptors and the hydrochloride salts of amines is infeasible because of inability of N of amine in contributing e⁻ [50]. Since MFH contains HCl, it was converted to base form, i.e. MEB, extracted into dichloromethane, solvent evaporated and finally the solution in acetonitrile obtained [54]. The possible reaction schemes showing the pathways for formation of MEB-CAA and MEB-DDQ C-T complexes are outlined in Scheme 1 in analogous to previous works [47, 55–59].

The acidic hydrochloride of MFH was eliminated out by extraction and obtained the drug in basic form (MEB). The basic nitrogenous group of MEB was utilised to react with PCA and DDQ to develop two new visible spectrophotometric methods based on the measurement of absorbance of 1:1 in stoichiometric MEB-PCA and MEB-DDQ C-T complexes at 530.0 and 460.0 nm, respectively. The reaction between MEB and reagents took place in 10 and 2 min to form intense MEB-PCA and MEB-DDQ C-T complexes, respectively, and respective complexes were stable for 2.5 and 3 h. Acetonitrile was showed greater selectivity for complexes’
stability over other solvents such as methanol, ethanol, acetone, 1,4-dioxane, chloroform, dichloromethane, dichloroethane and dimethylformamide. The validation of methods was performed according to ICH Guidelines [52, 53]. The calibration curves were linear over the concentration ranges of 8.0–320.0 and 1.6–64 μg mL⁻¹ MEB in PCA and DDQ methods, respectively, with molar absorptivity values of 7.33 × 10⁴ and 2.57 × 10⁴ L mol⁻¹ cm⁻¹. The regression coefficient values close to unity indicated the closed correlation between concentration of MEB and absorbance at respective analytical λmax in both the methods. The values of Sandell sensitivity, DL and QL of both the methods revealed the acceptable sensitivity. The values of RSD of 0.54 to 1.75% and RE of 0.62 to 2.18% declared the excellent precision and accuracies of proposed methods to the assay of MFH in the form of MEB. The methods were also complied for robust nature, as per the results expressed as %RSD obtained from deliberate variations of reagents and reaction time. Besides the results in %RSD from study of intermediate variation also revealed the excellent ruggedness of the methods. The statistical tests between the results of proposed (PCA and DDQ methods) and official methods yielded a satisfactory outcome of non-significant differences between performances of proposed and official methods with respect to accuracy and precision. The procedures were checked for selectivity by applying to the analysis of placebo and synthetic mixture. The inactive role of compounds in placebo and synthetic mixture was observed. The absorbance of placebo extract was same as blank and the recovery of MEB from synthetic mixture was close to 100%. The assessment of selectivity of PCA and DDQ methods respectively. The values very close to 100% declared greater selectivity of PCA and DDQ methods. The methods’ applicability to determine MFH in the form of MEB was also proved by spiked human urine analysis and in which the percent recovery of MEB was 97.87 to 98.72.

Conclusion
This is the first article utilising substituted p-benzoquinones to rapidly and reliably quantify metformin hydrochloride (MFH) in original/pure sample and tablets by spectrophotometry. The hallmarks of the presented methods are simplicity and ease of performance without the need for pH adjustment, heating, extraction and/or multireaction step as found in methods of already reported articles. The methods involve simple mixing of the reactants and absorbance measurement almost immediately. The advantageous factors of proposed methods are wide linear dynamic ranges, acceptable accuracies, precision, robustness and ruggedness. The selectivity studies produced confident results, and they permitted the MFH determination without the need for time consuming sample preparation steps. However, the methods require the drug to be present in its base form, which is obtained by an extraction step.

Abbreviations
MFH: Metformin hydrochloride; PCA: p-Chloranilic acid; DDQ: 2,3-Dichloro-5,6-dicyano-p-benzoquinone; DL: Detection limit; QL: Quantitation limit; RSD: Relative standard deviation; RE: Relative error; CT: Charge transfer; MEB: Metformin base; CAA: p-Chloranilic acid

Acknowledgements
Authors thank Sanofi Aventis, Mumbai, India, for gifting metformin hydrochloride pure sample. The first author is grateful to the JSS Mahavidyapeetha, Mysuru, and the Principal of JSS College of Arts, Commerce and Science, B N Road, Mysuru, India, for providing the facilities to pursue this work.

Authors’ contributions
NR and KB contributed equally to the work and in the overall work of manuscript preparation. Both authors read and approved the manuscript for further processing.

Funding
The work is not funded by any of the government or non-government bodies. The work is self-financed one.

Availability of data and materials
Data and material are available upon request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no conflict of interest.

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Received: 15 May 2020 Accepted: 17 August 2020
Published online: 28 September 2020

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