Regular Article

ANALYTICAL AND BIOANALYTICAL CHEMISTRY RESEARCH

Published by the Iranian Chemical Society

Anal. Bioanal. Chem. Res., Vol. 4, No. 1, 41-51, June 2017.

Selective Spectrophotometric Determination of Metformin Hydrochloride in Pharmaceuticals and Urine Using Two Nitrophenols as Chromogenic Agents

K. Basavaiah[a,*] and N. Rajendraprasad[b]

[a]*Department of Chemistry, University of Mysore, Manasagangothri, Mysuru-570 006, Karnataka, India*

[b]*PG Department of Chemistry, JSS College of Arts, Commerce & Science, B N Road, Mysuru-570 025, Karnataka, India*

(Received 16 October 2016, Accepted 27 November 2016)

Metformin hydrochloride (MFH) is an oral anti-diabetic drug of biguanide class. Two selective spectrophotometric methods were presented for the determination of MFH in pharmaceuticals. The methods were based on the measurement of yellow-coloured charge-transfer complexes formed between MFH and two poly-nitrophenols, namely, 2,4-dinitrophenol (DNP method, at 405 nm) and picric acid (PA method; at 410 nm) in dichloromethane medium. The variables which affect the complex formation were studied and optimized. Beer's law was obeyed over the concentration ranges: 2.4-48.0 and 3.2-64.0 μg ml^{-1} with molar absorptivity values of 3.24×10^3 and 2.30×10^3 l mol^{-1} cm^{-1}, with DNP method and PA method, respectively. The limits of detection (LOD) and quantification (LOQ) were calculated to be 0.13 and 0.40 μg ml^{-1} for DNP method and 0.19 and 0.59 μg ml^{-1} for PA method. Methods were validated for accuracy, precision, robustness, ruggedness and selectivity. The proposed methods were applied to the determination of MFH in tablets. The accuracy and precision of the methods were found excellent. Accuracy of the methods was ascertained by recovery test *via* standard-addition procedure. The methods were applied to spiked human urine sample without detectable interference from endogenous substances.

Keywords: Metformin hydrochloride, Nitrophenols, Spectrophotometry, Charge-transfer complex, Pharmaceuticals, Spiked human urine

INTRODUCTION

Metformin hydrochloride (MFH), chemically known as 1,1-dimethylbiguanide monohydrochloride (Fig. 1), is an oral anti-diabetic drug of biguanide class [1]. It is an antihyperglycemic agent, which improves glucose tolerance in patients with type 2 diabetes mellitus [2] by lowering both basal and postprandial plasma glucose. Since MFH continues to be one of the mostly used drugs in the management of type-2 diabetes, many methods were developed for its determination in pharmaceuticals and biological materials, including body fluids.

MFH has an official monograph each in European Pharmacopeia [3] and United Sates Pharmacopoeia [4]. Both describe titrimetry for the assay of drug in pharmaceuticals; in the former, drug solution in anhydrous formic acid-acetonitrile mixtures is titrated with perchloric acid with potentiometric end-point detection, whereas the latter involves the titration of drug in anhydrous formic acid-acetic acid medium with perchloric acid, determining the endpoint visually with crystal violet indicator.

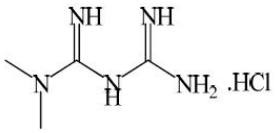


Fig. 1. Structure of MFH.

Different analytical techniques available in the literature for the determination of MFH in bulk drug and tablets include: UV-spectrophotometry [5-8], spectrofluorimetry [9], nuclear magnetic resonance spectrometry [10], atomic

*Corresponding author. E-mail: kanakapurabasavaiah@gmail.com

absorption spectrometry [11], potentiometry [12-15], conductometry [16,17], high-performance liquid chromatography [18-27], gas chromatography [28] and capillary electrophoresis [29].

Although many analytical methods were developed for determination of MFH in pharmaceuticals, most of the methods, especially chromatographic methods [18-28], lag sensitivity, inherent simplicity, cost-effectiveness *etc.* Besides, these methods were not validated properly for necessary parameters essential in most of the quality control and assessment protocols and procedures.

Low cost, ease of use and maintenance, simplicity, speed, and reliability of the analytical method make visible spectrophotometry very attractive for the determination of pharmaceutical products. A perusal of the literature revealed the availability of some visible spectrophotometric methods based on different reaction schemes. A direct method, based on charge-transfer (CT) complexation reaction of MFH with iodine in acetonitrile medium, has been described [5]. Beer's law is obeyed over 1.66-72.86 µg ml^{-1} range. Based on the same reaction and employing iodine as the CT complexing agent, determination of the drug in 2-12 µg ml^{-1} concentration range has been reported by El-Bardicy *et al.* [6], in which the absorbance of the complex was measured at 295 nm. The drug was determined by Hassan *et al.* [7] through complex formation with Cu(II) in basic medium; the complex was dissolved in cyclohexylamine and measured at 540 nm. Ninhydrin is reported to form a violet colored complex with the drug, based on which a method was developed by Mubeen and Noor [30] by measuring the absorbance at 570 nm. Very recently [31], a method based on the reaction of MFH with ninhydrin and molybdate mixture yielding Ruhemann's purple product with λ_{max} at 570 nm, has been reported by Vandana *et al.* The reaction proceeds quantitatively when the reaction mixture is heated at 90 ± 1 °C for 10 min. Beer's law is obeyed over 10-30 µg ml^{-1} concentration range. The method was applied to bulk drug and tablet dosage form. Pignard [32] has reported a method based on the reaction of drug with NaOCl in the presence of NaOH and $ZnSO_4$. Methods based on the reaction of MFH with diacetyl and 1-naphthol in alkaline ethanediol [33] and bromothymol blue in phosphate buffer [34] applicable for urine, have also been described.

Dinitrophenol (DNP) and picric acid (PA) are strong electron acceptors and are known to form charge-transfer complexes with a variety of electron donors such as anthracene [35], some aniline derivatives [36] and also with some amines [37-39]. Mulliken suggested that the formation of molecular complexes from two aromatic molecules can arise from the transfer of an electron from a π-molecular orbital of a Lewis base to vacant π-molecular orbital of a Lewis acid, with resonance between this dative structure stabilizing the complex [40]. It was also noted that the possibility of complex formation through the donation of an electron from non-bonding molecular orbital in a Lewis base to a vacant π-orbital of an acceptor (n-π) [41] with resonance stabilization of the combination. Based on this chemistry, DNP and PA have been widely used as CT complexing agents for the assay of many pharmaceutical substances [42-47].

Despite their widespread use in pharmaceutical analysis [42-45], DNP and PA have not been applied for the spectrophotometric determination of MFH. This paper describes two spectrophotometric methods for the determination of MFH and its dosage form based on coloured charge transfer complex formed on reaction with 2,4-dinitrophenol (DNP method) and picric acid (PA method). A discussion of reaction pathway is also presented. The methods are rapid, extraction-free and do not involve heating/cooling steps. These methods are superior to all existing visible spectrophotometric methods in terms of sensitivity, linear dynamic range, selectivity and experimental conditions.

EXPERIMENTAL

Apparatus

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) equipped with 1-cm matched quartz cells was used for absorbance measurements.

Reagents and Materials

All reagents were of analytical reagent grade and spectroscopic grade organic solvents were used throughout the investigation. Pharmaceutical grade metformin hydrochloride (MFH), which is reported to be 99.9% pure, was received from Sanofi Aventis, Mumbai, India. Two

brands of tablets containing MEB, Glyciphage 500 mg (Franco Indian Pharmaceuticals Pvt. Ltd., Mumbai, India) and Cetapin XR 500 mg (Sanofi Aventis, Mumbai, India) used in the investigation were purchased from local commercial sources. Urine sample was collected from a 35 year old male human volunteer and used in the analysis.

2,4-Dinitrophenol (DNP, 0.05% w/v) and picric acid (PA, 0.1% w/v) solutions were prepared by dissolving the pure compounds (both from S.D. Fine Chem Ltd., Mumbai, India) in dichloromethane. A 10 % sodium hydroxide solution was prepared by dissolving 10 g of pure NaOH (Merck Ltd., Mumbai, India) in 100 ml of doubly distilled water.

Standard Metformin Base (MEB) Solution

An amount of MFH (51.2 mg) equivalent to 40 mg of base was accurately weighed and dissolved in about 20 ml of water in a 125 ml separating funnel. The solution was made alkaline with 15 ml of 10% NaOH solution and shaken successively for 2 min with 2 × 30 ml portions of dichloromethane, each extract was washed with 15 ml of water in another separating funnel. The washed extracts were pooled and passed through 5 g of anhydrous sodium sulphate. After collecting in a 100 ml volumetric flask, the volume was completed to mark with dichloromethane to provide a standard solution equivalent to 400 μg ml^{-1} MEB. Suitable aliquots were then diluted with dichloromethane to get working concentrations of 60 and 80 μg ml^{-1} MEB for use in DNP method and PA method, respectively.

Procedure for Bulk Drug: Preparation of Calibration Graph

DNP method. Different aliquots (0.2, 0.5, 1.0. . . . . .4.0 ml) of standard MEB solution (60 μg ml^{-1}) were accurately transferred into a series of 5 ml calibration flasks using a micro burette. One ml of 0.05% DNP solution was added to each flask and diluted to volume with dichloromethane. The content was mixed well and the absorbance was measured at 405 nm against the reagent blank.

PA method. Aliquots (0.2, 0.5, 1.0. . . . . .4.0 ml) of a standard MEB (80 μg ml^{-1}) solution were accurately transferred into a series of 5 ml calibration flasks. To each flask, 1 ml of 0.1% PA solution was added and the content was made up to volume with dichloromethane. The content was mixed well and the absorbance measured at 410 nm against the reagent blank.

Standard graph in each method was prepared by plotting the absorbance *vs*. drug concentration, and the concentration of the unknown was computed from the respective regression equation derived using the Beer's law data.

Procedure for Tablets

Ten tablets were weighed and pulverized. An amount of powder equivalent to 40 mg metformin base was accurately weighed and dissolved in about 20 ml of water in a 125 ml separating funnel by shaking for 20 min. The metformin base solution was prepared as described under "standard MEB solution". The solution was diluted with dichloromethane to get working concentrations of 60 and 80 μg ml^{-1} MEB and analysed as described above.

Procedure for Placebo Blank and Synthetic Mixture

A placebo blank containing starch (40 mg), acacia (35 mg), sodium citrate (35 mg), hydroxyl cellulose (35 mg), magnesium stearate (35 mg), talc (40 mg) and sodium alginate (35 mg) was prepared by homogeneous mixing of all the components. About 20 mg of placebo blank was taken and its extract was prepared as described under "procedure for tablets" and analysed using the procedures of each method by taking 2 ml. To 20 mg of placebo blank, 51.2 mg of MFH was added, mixed for uniform composition and quantitatively transferred into a 125 ml separating funnel. The metformin base solution was prepared as described under "procedure for tablets". This solution, after appropriate dilution, was subjected to analysis using the procedures described earlier.

Procedure for Spiked Human Urine Sample

Urine (5 ml) was spiked with 12.8 mg of pure MFH and diluted to 10 ml with 10% NaOH, quantitatively transferred into a 125-ml separating funnel. After mixing well, the base solution was then prepared as detailed under "preparation of standard metformin base solution". Suitable aliquots of 100 μg ml^{-1} base solution were subjected to analysis.

RESULTS AND DISCUSSION

Absorption Spectra

The reaction of DNP or PA as Lewis acid with MEB as Lewis base results in the formation of an intense yellow colored product. The absorption spectra of the yellow colored products were recorded for 360-500 nm range against the corresponding blanks. The resulted yellow colored CT complexes showed maximum absorbance at 405 and 410 nm for MEB-DNP and MEB-PA, respectively (Fig. 2).

Reaction Pathway

The chemistry involved in the proposed methods is the formation of charge-transfer complex by DNP or PA acting as π-acceptor (Lewis acid) with MEB, acting as n-donor (Lewis base). DNP and PA react with such donor molecules to form charge-transfer and proton transfer complexes [43, 44,46,48,49]. When an amine is combined with a polynitrophenol, a type of force field produces an acid-base interaction and the other, an electron donor-acceptor interaction. The former interaction leads to the formation of true phenolate by proton-transfer, while the latter to a true molecular compound by charge-transfer [50]. Based on this, the mechanism in the present context can be discussed in terms of transfer of electronic charge from the primary aliphatic amine of MEB, an electron-rich molecule (a Lewis-base donor), to the ring of DNP or PA, an electron-deficient molecule as a Lewis-acid acceptor and at the same time the proton of the hydroxyl group of DNP or PA gets transferred to the primary amine of MEB (Scheme 1). The reason for the color produced lies in the formation of complexes between the pairs of molecules MEB-DNP and MEB-PA, and this complex formation leads to the production of two new molecular orbitals and, consequently, to a new electronic transition [51]. The possible reaction pathways are illustrated in Scheme 1.

Method Development

Many experimental variables, which were found to affect the color intensity and stability of the resulting complexes, were optimized to achieve maximum sensitivity and adherence to Beer's law.

Effect of Reagent Concentration

The optimum concentration of the reagent required to achieve maximum sensitivity of the developed color species in each method was ascertained by adding different amounts of DNP or PA to a fixed concentration of MEB. The results showed that 1.0 ml of 0.05% DNP or 0.1% PA solution was optimum for the production of maximum and reproducible color intensity (Fig. 3).

Effect of Solvent

In order to select a suitable solvent as reaction medium, the reagent solutions were prepared separately in different solvents, such as 1,4-dioxane, chloroform, acetonitrile, acetone, *t*-butanol, 2-propanol and dichloromethane, and the reaction with MEB was followed. Dichloromethane was best suited for the preparation of reagent solutions. Similarly, effect of diluting solvent was studied and the results showed that the ideal diluting solvent to achieve maximum sensitivity and stability of the colored species was dichloromethane in both methods.

Effect of Reaction Time and Stability of the CT Complexes

The optimum reaction time was determined by measuring the absorbance of the complex formed upon the addition of reagent solution to MEB solution at room temperature. The reaction in both methods was instantaneous. The absorbance of the resulting CT complexes remained stable for at least 15 h, thereafter.

Composition of the CT Complexes and Conditional Stability Constants

The composition of the CT complex was established by Job's method of continuous variations [52] using equi-molar concentrations of the drug (base form) and reagents (4.48×10^{-4} M in DNP method and 2.16×10^{-4} M in PA method). Five solutions containing MEB and the reagent (DNP or PA) in various molar ratios, with a total volume of 5 ml, were prepared. The absorbance was subsequently measured at 405 nm in DNP method and 410 nm in PA method. A plot of absorbance *vs.* mole-ratio (Fig. 4) gave a maximum at a molar ratio of $X_{max} = 0.5$ which indicated the formation of 1:1 CT complex between MEB and reagent (DNP or PA). Because of the steric hindrance to nitrogen attached to

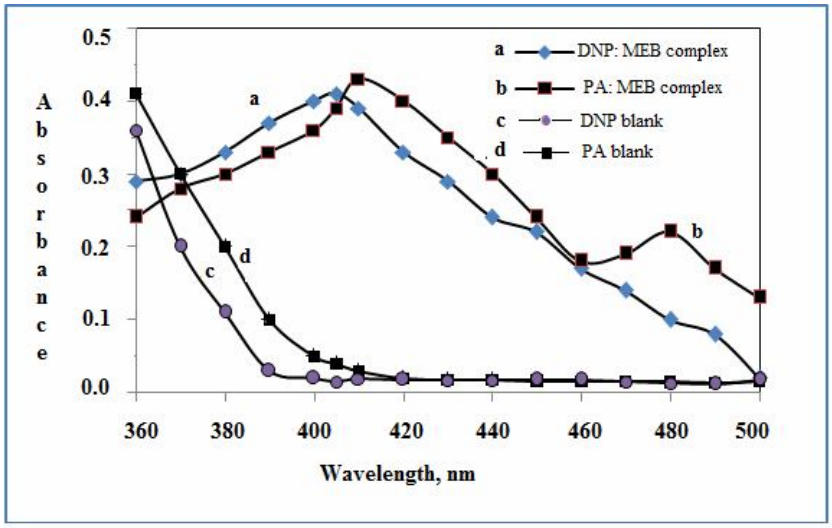
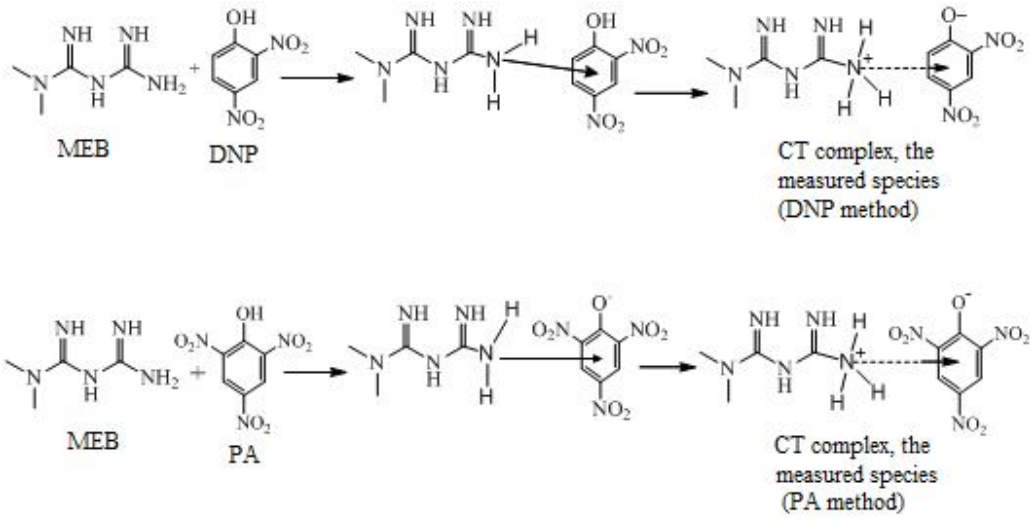


Fig. 2. Absorption spectra of charge-transfer complexes: a. 2 ml of 60 µg ml^{-1} MEB+1 ml of 0.05% DNP+2 ml of dichloromethane; b. 2 ml of 80 µg ml^{-1} MEB + 1 ml of 0.1% PA+ 2 ml of dichloromethane c.1 ml of 0.05% DNP+ 4 ml of dichloromethane & d. 1 ml of 0.1% PA+ 4 ml of dichloromethane.



Scheme 1. The probable reaction pathways of formation of MEB: DNP and MEB: PA CT complexes

tertiary carbon, only the nitrogen attached to primary carbon is believed to be involved in CT complex formation as shown in Scheme 1.

The conditional stability constants ($\log K_f$) values of MEB-DNP and MEB-PA complexes were calculated using the formula:

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M (n)^n}$$

where A and Am are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the mole concentration of

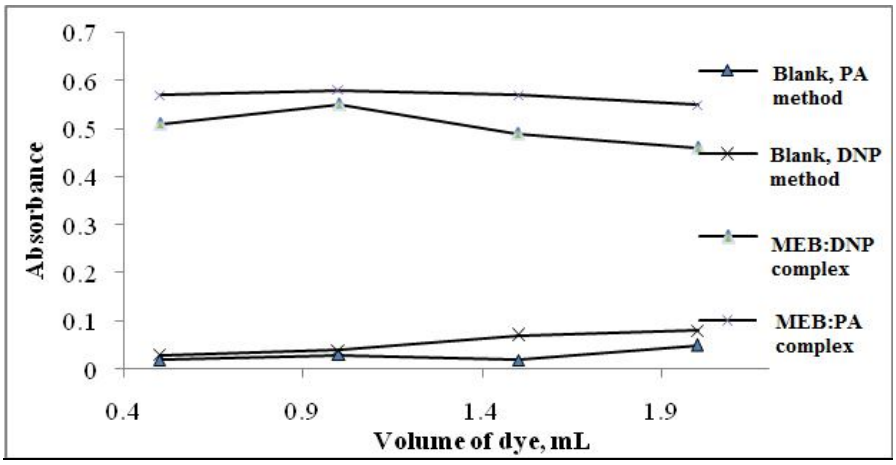


Fig. 3. Effect of reagent concentration on the color development. DNP method (Blank): 0.05% DNP; MEB-DNP complex: 25 µg ml^{-1} MEB. PA method (Blank): 0.1% PA; MEB-PA complex: 40 µg ml^{-1} MEB.

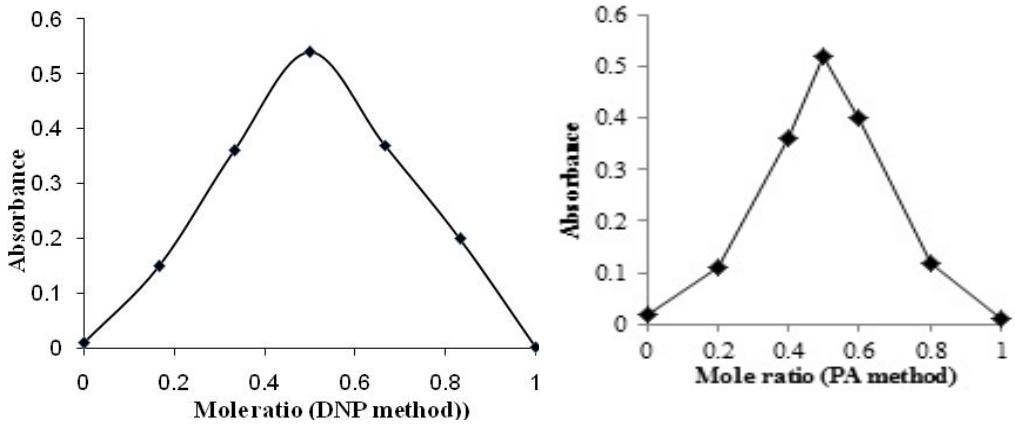


Fig. 4. Job's plots to establish composition of CT complexes DNP Method: 4.48×10^{-4} M each of MEB & DNP PA Method: 2.16×10^{-4} M each of MEB & PA.

drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drug. The $\log K_f$ values were found to be 7.42 and 6.92 for DNP method and PA method, respectively.

Method Validation

The proposed methods were validated for linearity, sensitivity, selectivity, accuracy, precision, robustness, ruggedness and recovery according to the current *ICH* guidelines [53].

Linearity and Sensitivity

Under the optimized experimental conditions, the standard calibration curves were constructed by plotting absorbance *vs.* MEB concentration. The linear regression equations were obtained by the method of least squares. Beer's law range, molar absorptivity, Sandell's sensitivity, correlation coefficient, standard deviation of intercept (S_b), standard deviation of slope (S_m), limits of detection (LOD) and quantification (LOQ) for both methods are summarized in Table 1.

Table 1. Sensitivity and Regression Parameters

Parameter	DNP Method	PA Method
λ_{max} (nm)	405	410
Color stability (h)	15	15
Linear range (µg ml^{-1})	2.4-48	3.2-64
Molar absorptivity (ε) (M^{-1} cm^{-1})	3.24×10^3	2.30×10^3
Sandell sensitivity (µg cm^{-2})[a]	0.0511	0.0720
Limit of detection (LOD) (µg ml^{-1})	0.13	0.19
Limit of quantification (LOQ) (µg ml^{-1})	0.40	0.59
Regression equation, ($Y^b = b + mx$)		
Intercept (b)	0.0137	0.0096
Slope (m)	0.0202	0.0142
Standard deviation of m (S_m)	2.13×10^{-3}	1.61×10^{-3}
Standard deviation of b (S_b)	7.2×10^{-4}	1.5×10^{-4}
Regression coefficient (r)	0.9994	0.9993

[a]Limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. [b]Y = b + mx, where Y the absorbance, X concentration in µg ml^{-1}, b intercept, m slope.

Table 2. Results of Intra-day and Inter-day Accuracy and Precision Study

Method	MEB taken (µg ml^{-1})	Intra-day accuracy and precision (n = 7)			Inter-day accuracy and precision (n = 5)		
		MEB found (µg ml^{-1})	RE (%)	RSD (%)	MEB found (µg ml^{-1})	RE (%)	RSD (%)
DNP method	16.0	16.09	0.56	2.03	16.11	0.69	1.33
	24.0	23.72	1.17	0.96	23.67	1.34	1.06
	32.0	32.25	0.78	0.98	32.31	0.97	0.82
PA method	24.0	24.20	0.83	0.59	24.26	1.08	0.91
	36.0	35.87	0.36	1.18	36.17	0.47	0.72
	48.0	48.65	1.35	1.53	48.36	0.75	2.19

RE-relative error, RSD-relative standard deviation

Table 3. Results of Method Robustness and Ruggedness Study, Expressed as %RSD

Method	MEB taken ($\mu g\ ml^{-1}$)	Robustness	Ruggedness	
		Reagent volume[a]	Inter-analysts (n = 3)	Inter-instruments (n = 3)
DNP method	16	1.12	0.89	1.02
	24	1.22	0.62	1.07
	32	1.94	0.90	1.13
PA method	24	0.55	1.22	1.08
	36	0.39	1.46	1.22
	48	1.66	1.32	0.64

[a]The volumes of DNP (DNP method) and PA (PA method) used were: 1.0 and 1 ± 0.2 ml.

Table 4. Results of Analysis of Tablets by the Proposed Methods and Statistical Comparison of the Results with the Official Method

Tablet analyzed	Label claim, mg/tablet[a]	Found (Percent of label claim ± SD)[b]		
		Official method	DNP method	PA method
Glyciphage	500	98.67 ± 0.76	99.45 ± 1.56 t = 0.42 F = 4.21	100.3 ± 1.12 t = 2.68 F = 2.17
Cetapin XR	500	101.3 ± 1.13	100.4 ± 1.77 t = 0.96 F = 2.45	99.87 ± 1.76 t = 1.53 F = 2.43

[a]Amount in mg per tablet; [b]mean value of 5 determinations. Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77 and tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39.

Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, pure drug (MEB) solutions at three different concentration levels (within the working range) were prepared and analysed during the same day (intra-day) and on five consecutive days (inter-day). The results presented in Table 2 reveal fair accuracy and precision of the methods.

Robustness and Ruggedness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate

Table 5. Results of Accuracy Assessment by Recovery Test

Tablet analyzed	DNP method				PA method			
	MEB in tablet ($\mu g\ ml^{-1}$)	Pure MEB added ($\mu g\ ml^{-1}$)	Total found ($\mu g\ ml^{-1}$)	Pure MEB recovered (Percent ± SD)[a]	MEB in tablet ($\mu g\ ml^{-1}$)	Pure MEB added ($\mu g\ ml^{-1}$)	Total found ($\mu g\ ml^{-1}$)	Pure MEB recovered (Percent ± SD)[a]
Glyciphage	15.91	8.00	24.32	101.7 ± 1.23	24.07	12	34.84	96.58 ± 1.76
	15.91	16.00	31.46	98.59 ± 1.66	24.07	24	48.36	100.6 ± 1.12
	15.91	24.00	40.11	100.5 ± 1.08	24.07	36	62.17	103.5 ± 1.88
Cetapin XR	16.06	8.00	24.44	101.6 ± 1.34	23.97	12	36.72	102.1 ± 1.14
	16.06	16.00	31.68	98.80 ± 0.89	23.97	24	46.90	97.78 ± 1.03
	16.06	24.00	39.12	97.66 ± 1.00	23.97	36	59.17	98.66 ± 0.57

[a]Mean value of three measurements.

Table 6. Results of Determination of MEB in Spiked Human Urine

Method	Spiked concentration ($\mu g\ ml^{-1}$)	Found[a] ± SD	%Recovery ± RSD
DNP method	24.0	23.41 ± 0.74	97.54 ± 1.56
PA method	36.0	36.94 ± 0.91	102.6 ± 1.44

[a]Mean value of five determinations; RSD is relative standard deviation.

variations in experimental parameters and provides an indication of its reliability during normal usage [54]. To evaluate the method robustness, reagent volume was altered slightly and its effect was found to be negligible.

Method ruggedness [54] was determined by having the analysis done by three analysts and also by a single analyst performing analysis on three different instruments. The results presented in Table 3 showed no statistical differences between results obtained by different analysts and instruments suggesting that the proposed methods are rugged.

Selectivity

The recommended procedures were applied to the analysis of placebo blank and the resulting absorbance readings in both methods were same as that of the reagent blank, confirming non-interference from the placebo. The analysis of synthetic mixture solution prepared as described earlier, yielded percent recoveries of 98.61 ± 1.66 (n = 5) and 100.7 ± 0.57 (n = 5) for DNP method and PA method, respectively. The results of this study showed that the inactive ingredients did not interfere in the assay indicating the high selectivity of the proposed methods and its utility for routine determination of tablets form.

Application to Tablets

The proposed methods were successfully applied to the determination of MEB in two brands of tablets and the

results are compiled in Table 4. The results obtained were statistically compared with those obtained by the official method [3], which uses a procedure of potentiometric titration of MFH with perchloric acid in formic acid-acetonitrile medium. As can be seen from the results presented in Table 4, the calculated *t*- and *F*-values at 95% confidence level did not exceed the tabulated values for four degrees of freedom. This indicates that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision.

Accuracy by Recovery Study

The accuracy and selectivity of the proposed methods were further ascertained by performing a recovery study. Pre-analysed tablet powder was spiked with pure MFH at three concentration levels and the total was determined by the proposed methods. The results presented in Table 5 clearly indicate that the excipients present in the tablets did not interfere in the assay.

Application to Spiked Urine

The proposed methods were also applied to the determination of MFH in spiked human urine sample and the results are presented in Table 6.

CONCLUSIONS

This is the first report on the application of 2,4-dinitrophenol and picric acid as chromogenic agents for the sensitive and selective determination of metformin hydrochloride and its dosage form by spectrophotometry. It is noteworthy that the methods donot involve any drastic experimental conditions unlike the reported methods. These are the simplest spectrophotometric methods ever reported for metformin since these methods involve mixing metformin base and reagent solutions followed by absorbance measurement. This ease of performance is truly reflected in high accuracy and precision of the results for tablets, as well as for spiked human urine.

ACKNOWLEDGEMENTS

Authors thank Sanofi Aventis, Mumbai, India, for gifting metformin hydrochloride pure sample. Prof. K. Basavaiah gratefully acknowledges the financial assistance by UGC, New Delhi, India, in the form of BSR Faculty fellowship.

REFERENCES

[1] British Pharmacopoeia, Her Majesty's Stationery Office, London, UK, 2007.
[2] C.J. Bailey, M.R.C. Path, R.C. Turner, N. Engl. J. Med. 334 (1996) 574.
[3] European Pharmacopoeia, Strasbourg, Council of Europe 6.0 (2007) 2370.
[4] The United States Pharmacopoeia, XXXII Revision, the National Formulary XXVII, Rockville, USP Convention 2905 (2009).
[5] S. Ashour, R. Kabbani, Anal. Lett. 36 (2003) 361.
[6] M.G. El-Bardicy, S.Z. El-Khateeb, A.K.S. Ahmad, H.N. Assad, Spectrosc. Lett. 22 (1989) 1173.
[7] S.S.M. Hassan, W.H. Mahmoud, M.A.F. Elmosallamy, O.H.M. Othman, Anal. Chim. Acta 378 (1999) 299.
[8] P. Umapathi, J. Ayyappan, S.D. Quine, Trop. J. Pharm. Res. 11 (2012) 107.
[9] N.M. Rao, J. Bagyalarshmi, T.K. Ravi, Int. J. Pharm. Sci. Res. 1 (2010) 262.
[10] S.Z. El-Khateeb, H.N. Assad, M.G. El-Bardicy, A.S. Ahmad, Anal. Chim. Acta 208 (1988) 321.
[11] V.K. Sharma, A. Bhattacharya, Asian J. Chem. 22 (2010) 8273.
[12] M.S. Rizk, H.M. Abdel-Fattah, Y.M. Issa, E.M. Atia, Anal. Lett. 26 (1993) 415.
[13] A.F. Shoukry, M.S. Rizk, H.M. Abdel-Fattah, Y.M. Issa, E.M. Atia, J. Chem. Technol. Biotechnol. 60 (1994) 217.
[14] M.S. Rizk, H.M. Abdel-Fattah, Y.M. Issa, E.M. Atia, J. Chem. Technol. Biotechnol. 61 (1994) 415.
[15] M.S. Rizk, Electroanalysis 7 (1995) 687.
[16] J.M. Calatayud, M.C.P. Marti, P.C. Falco, Anal. Lett. 18 (1985) 1381.
[17] M. Abou-dan, S. Ashour, H. Abou-dan, Asian J. Chem. 13 (2001) 1.
[18] F. Al-Rimawi, Talanta 79 (2009) 1368.
[19] M. Kar, P. Choudhury, Indian J. Pharm. Sci. 71 (2009) 318.

[20] S.I. Bhoir, I.C. Bhoir, M. Sundaresan, Indian J. Pharm. Sci. 65 (2003) 650.
[21] A.E. Gindy, M.W. Nassar, N.M.E. Abasawy, K.A.S. Attia, M.A. Shabrawi, J. AOAC Int. 93 (2010) 1821.
[22] K. Devendra, S. Saurabh, R. Rachumallu, K.D. Rakesh, World J. Pharm. Pharm. Sci. 3 (2014) 745.
[23] P. Gomes, J. Sippel, A. Jablonski, M. Steppe, J. Pharm. Biomed. Anal. 36 (2004) 909.
[24] J. Radhakrishna, J. Satyanarayana, A. Satyanarayana, J. Pharm. Biomed. Anal. 29 (2002) 873.
[25] M. Vasudevan, J. Ravi, S. Ravisankar, B. Suresh, J. Pharm. Biomed. Anal. 25 (2001) 77.
[26] D.H. Khanolkar, V.M. Shindhe, Indian Drugs 36 (1999) 739.
[27] M.S. Arayne, N. Sultana, M.H. Zuberi, Pakistan J. Pham. Sci. 19 (2006) 231.
[28] R.T. Sane, V.J. Banavalikar, V.R. Bhate, V.G. Nayak, Indian Drugs 26 (1989) 647.
[29] I.I. Handom, A.K.J. Bani, A.M. Abushoffa, J. Pharm. Biomed. Anal. 53 (2010) 1254.
[30] G. Mubben, K. Noor, Indian J. Pharm. Sci. 71 (2009) 100.
[31] P.P. Vandana, J.D. Subhash, H.K. Suvarna, T.K. Sudarshan, O.K. Pramod, J. Indian Chem. Soc. 91 (2014) 171.
[32] P. Pignard, Ann. Biol. Clin. 20 (1962) 325.
[33] G. Siest, F. Ross, J.J. Gabou, Bull. Soc. Pharm. 58 (1963) 29.
[34] E.R. Garret. J. Tsau, J. Pharm. Sci. 16 (1972) 1404.
[35] A. Bhal, B.S. Bhal, Text Book of Advanced Organic Chemistry, 1st ed., 1977, p. 1069.
[36] N. Singh, A. Ahmad, Canadian J. Anal. Sci. Spectrum. 54 (2009) 292.
[37] G. Saito, Y. Matsungo, Bull. Chem. Soc. Japan 44 (1971) 3328.
[38] Y. Matsungo, G. Saito, Bull. Chem. Soc. Japan 45 (1972) 1972.
[39] G. Saito, Y. Matsungo, Bull. Chem. Soc. Japan 46 (1973) 714.
[40] R.S. Mulliken, J. American Chem. Soc. 72 (1950) 600.
[41] R.S. Mulliken, J. American Chem. Soc. 74 (1952) 811.
[42] K.N. Prashanth, K. Basavaiah, Chem. Sci. J. 3 (2012) 1, Article ID CSJ-71.
[43] M.S. Raghu, K. Basavaiah, J. Assoc. Arab Univ. Basic Appl. Sci. 12 (2012) 33.
[44] C.S. Xuan, Z.Y. Wang, J.L. Sing, Anal. Lett. 31 (1998) 1185.
[45] W.F. El-Hawary, N.M. Issa, A. Talat, Int. J. Biomed. Sci. 3 (2007) 50.
[46] S. Sadeghi, M. Shamshipur, Anal. Lett. 31 (1998) 2691.
[47] E. Regulska, M. Terasiewiez, H. Prizanonska-Tarasiewiez, J. Pharm. Biomed. Anal. 27 (2002) 335.
[48] F.A. El-Yazbi, A.A. Gazy, H.M. Mahgoub, M.A. El-Sayed, R.M. Youssef, J. Pharm. Biomed. Anal. 31 (2003) 1027.
[49] M.E. Mahrous, Anal. Lett. 25 (1992) 269.
[50] Y.M. Issa, A.S. Amin, Anal. Lett. 26 (1993) 2397.
[51] W. Kemp, Organic Spectroscopy, 3rd ed., Replika Press, India, 2006, p. 274.
[52] A.S. Douglas, M.W. Donald, Principles of Instrumental Analysis, New York, Holt, Renehart, Winston, str., 1971, p. 104.
[53] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Complementary Guideline on Methodology, London, 2005.
[54] ICH Harmonised Tripartite Guideline prepared within the Third International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), Text on Validation of Analytical Procedures, 1994.