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Spectrophotometric microdetermination of anti-diabetic drug metformin HCl in pharmaceutical formulation and biological fluids

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ABSTRACT

Two simple, sensitive and accurate spectrophotometric methods have been proposed for the determination of metformin (Mf) pharmaceutical formulations and biological fluids. The methods are based on the measurement of absorbances of tris (*o*-phenanthroline) iron (II) [FPL, method A] and tris (bipyridyl) iron (II) [FBL, method B] complexes at 510 and at 520 nm, respectively. Reaction conditions have been optimized to obtain coloured complexes of higher sensitivity and longer stability. The absorbances were found to increase linearly with increase in concentrations of Mf which were corroborated by correlation coefficient values. The complexes obeyed Beer's law over the concentration range of 0.2–6.00.02-6.0μg ml^{-1} for Mf. The proposed methods were successfully applied to the determination of metformin HCl in bulk drugs, pharmaceutical formulations and biological fluids (obtained from diabetic patients undergoing treatment with metformin, HCl) without interference by the common co-formulated substances. Statistical comparison of the results with the reference method showed good concurrence and indicated no significant difference in accuracy and precision.

KEYWORDS

Metformin HCl (Mf);
Spectrophotometry;
Redox reactioms;
Tris (*o*-phenanthroline) and tris (bipyridyl) iron (II) complexe;
Pharmaceutical analysis;
Biological fluids.

INTRODUCTION

Diabetes and its abnormalities are a major health problem in the modern society. It is characterized by disruption of insulin production, leading to high blood glucose concentration and other complications such as neuropathy, renal dysfunction and cardiopathy[1]. The two categories of diabetes mellitus are type I (insulin-dependent) and type II (non-insulin dependent). Type II diabetes is a progressive and complex disease that is difficult to manage effectively in the long-term. Metformin (Mf), N, N-dimethylimidodicarbonimidic diamide (Scheme 1) is an orally administered antihyperglycemic drug used to treat type II diabetes. It helps diabetics to control the amount of glucose (sugar) to a normal level and maintains this level in their blood. Metformin can be used alone or in conjunction with other medications, sulfonylureas, alpha-glucosidade inhibitors, or insulin. Although metformin's exact mechanism of action is not completely understood, it decreases the intestinal absorption of dietary carbohydrates, inhibits hepatic glucose production and gluconeogenesis or, perhaps most importantly, increases the sensitivity of muscle cells to insulin by enhancing peripheral glucose uptake and utilization[2].

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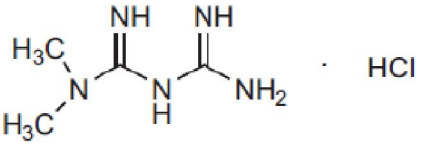

Metformin hydrochloride (Mf)

Drug quality control is a branch of analytical chemistry that has a wide impact on public health, and so development of reliable quick and accurate methods for the active ingredient determination is very important. A variety of separation techniques have been proposed for assay determination of metformin. These include gas chromatography[3-5] capillary electrophoresis[6] conductometry[7] or voltammetry[8]. Nevertheless, most of these methods involve several manipulation steps before the final result of the analysis, have poor selectivity or require expensive apparatus.

Spectrophotometric methods are the most commonly used techniques and continue to enjoy wide popularity. The common availability of the instrumentation, the simplicity of procedures, speed, precision and accuracy of the technique still make spectrophotometric methods attractive. Also, spectrophotometric methods of analysis are more economic and simpler, compared to other methods. Spectrophotometric determination of metformin is based on reaction with NaOCl followed by NaOH and ZnSO4 to give a yellow color[9]. Spectrophotometric determination of metformin in biological fluids has been also suggested by reactions of metformin with bromothymol blue in phosphate buffer[10] or formation of Cu-Mf complex with an absorption maximum at 540nm[11]. In addition spectrophotometric determination of Mf via charge-transfer complex with iodine was also suggested[12].

Redox reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds[13-18]. In such reactions, the drug substance was oxidized by a suitable oxidizing agent; such as Fe (III), Ce (IV) or potassium permanganate, where the produced lower oxidation state cation was determined spectrophotometrically with a suitable chelating agent. None of these reagents have been previously used for the spectrophotmetric analysis of Mf.

The present study was dedicated to investigate the application of Fe (III)–tris (*o*-phenanthroline (FPL) or Fe (III)-tris (bipyridyl) (FBL) systems for the spectrophotometric determination of Mf in their pharmaceutical dosage forms and biological fluids (urine or plasma). The proposed methods can be applied for quality control analysis, where modern and expensive apparatus such as GLC, HPLC are not available.

EXPERIMENTAL

Reagents and chemicals

All the chemicals were of the analytical reagent grade and double distilled water was used throughout the experiments.

FPL and FBL were prepared[19] as follows:

FPL was prepared by mixing 0.198 g of tris (o-phenanthroline (PNL) with 2 ml of 1 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate (FAS) and diluting with distilled water to 100 ml.

FBL was prepared by mixing 0.16 g of tris (bipyridyl) in 2 ml of 1 M HCl with 0.16 g of ferric ammonium sulphate dodecahydrate (FAS) and diluting with distilled water to 100 ml.

Acetate buffer solutions of various pH values (2.5-6.0) were prepared as recommended previously[20]

Authentic samples

Authentic metformin hydrochloride ($C_4H_{11}N_5$, HCl, M.W.165.6 g mol^{-1}) sample was supplied by the CID co. Egypt. Contents of Mf were assigned to be 98.23%. Metformin HCl working solution was prepared by dissolving 0.01 g of pure Mf in 50 ml of double distilled water, completed to 100 ml with double distilled water to obtain the working standard solution of concentration 100µgml^{-1}.

CIDOPHAGE, 500 and 850 mg, were purchased from local drug stores and used as Mf samples.

Apparatus

Perkin elmer Lambada 20 double beam self recording spectrophotometer with 10 mm quartz cell, connected to PC software. HANA pH meter HI 8417 with pH sensitivity of ± 0.05 pH units.

General procedure

Aliquots of 100 µgml^{-1} Mf standard solution were transferred into a series of 10 mL calibrated flasks followed by 2.0 ml FPL (Method A) or 1.5 ml FBL

(Method B) reagent solution and 2.5 mL acetate buffer solution pH 4.00. The reaction medium was heated on a water bath at 60 ^{0}C for 15 minutes, cooled at room temperature and the volume was diluted to the mark with double-distilled water. The colored complexes formed were measured at 510 and 520 nm for methods A and B, respectively, against a reagent blank treated similarly. The absorbance values were recorded and plotted against drug concentration in μg ml.

Analytical applications

Spectrophotometric determination of Mf in dosage forms

Ten Cidophage tablets (either 500 or 850 mg) were weighed and grinded to finely divided powdered. An accurately weight of the powder contain 500 mg Mf was transferred into a 100 mL calibrated flask, and dissolved in about 50 ml of distilled water. The contents of the flask were swirled, sonicated for 10 min, completed to volume with water and filtered. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration and analyzed by the proposed and official methods[21].

Spectrophotometric determination of Mf in biological fluids

Aliquots of 1.0 ml of biological fluid (urine or plasma) were spiked with different concentration levels of Mf. The samples were treated with 0.1 ml of 70% perchloric acid diluted to 10 ml. The resulting solution was vortexing for 1.0 minute and centrifuged for 10 minutes at 13000 rpm in ambient temperature. The supernatants were transferred into test tubes and neutralized with 1.0 M NaOH solution, then analyzed according to the recommended procedures. The absolute recovery was determined from calibration graphs or regression equations. The blank was prepared by treating the drug-free urine and plasma in the same manner, each measurement was repeated for five times.

RESULTS AND DISCUTION

Ferric ion play a prominent role in the spectrophotometric determination of many pharmaceutical drugs, acting as an oxidant; where a ferric ion gets reduced to ferrous ion in extent equivalent to the drug concentration. The liberated Fe (II) can be determined using selective chelating agents such as 1,10-phenanthroline and 2,2$^-$-bipyridyl[22].

The proposed methods A and B are based on the formation of tris(o-phenanthroline) or tris (bipyridyl)-iron(II) chelate upon reduction of the corresponding Fe (III) complexes with Mf. Due to the critical role of the reaction conditions on the analysis performance, parallel studies were carried out on both proposed methods including the influence of measuring wavelength, buffer, concentration and sequence of reagent addition were tested in details to select the optimal possessing the best sensitivity and selectivity towards Mf.

Effect of reaction variables

Wavelength selection

The absorption spectra of the colored complexes in the proposed methods showed a characteristic λ_{max} values as in (Figure 1). Iron (II)-tris (bipyridyl) showed absorption maximum at 520nm while the corresponding tris (o-phenanthroline) complex absorbs at 510nm.

Effect of pH

Different buffer media; universal, phosphate, borate, and acetate buffer solutions, were examined to achieve maximum color intensity at fixed Mf concentration. Acetate buffer proved to be the most favorable one accompanied with the highest absorbance values.

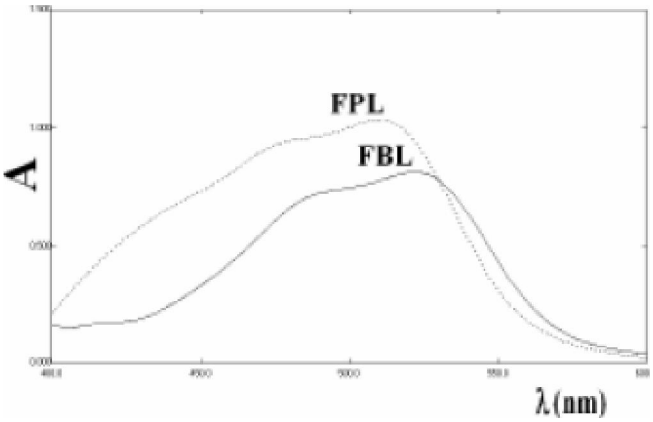

Figure 1 : Absorption spectra of FPL and FBL complexes in presence of 4.0 ppm Mf.

pH adjustment is necessary, especially in acidic medium, because the reaction was affected by change of pH in the range of (3.0–8.0) and the optimum pH value was 4.00 (Figure 2a). Moreover, 2.5 mL buffer solution was sufficient for method A and method B, for com-

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plete color development (Figure 2b).

Effect of the reagent concentration

The addition of 2 ml of FPL or 1.5 ml of FBL reagent was sufficient to obtain the maximum and reproducible absorbance for the studied complexes. Smaller amounts gave incomplete complex formation while larger concentration had no effect on complex formation, although the absorbance increased slightly due to the background of the reagent used.

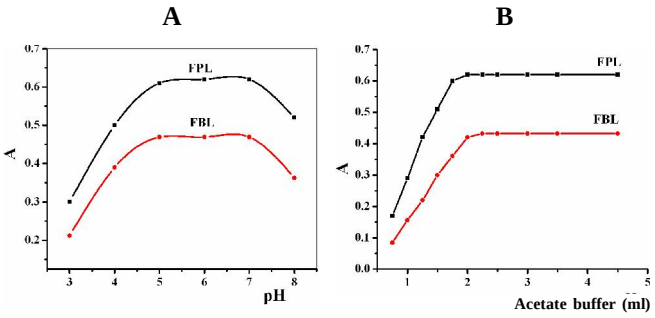

Figure 2 : Effect of pH and ml added of acetate buffer on the absorbance of the FPL and FBL complexes in presence of 2.0 ppmMf.

Effect of reaction temperature and heating time

The effect of temperature and heating time on the development of the colored complexes were studied. The reaction of Mf with both reagents slowly proceeds at room temperature and accelerated by heating. Maximum absorbance was obtained after heating on a water bath at 60 ^{0}C for about 15 minutes with both methods and further heating caused no appreciable change in the color. Moreover, the formed colored complexes were stable for more than 24h.

Sequence of addition

The optimum sequence was defined by following to color intensity and maximum absorbance on changing the sequences of addition of drug, reagent and buffer. The best condition was "drug – reagent – buffer" for the highest absorbance and stability.

Analytical data

Adherence to Beer's law was studied by measuring the absorbance values of solutions varying in drug concentrations. The analytical parameters such as molar absorptivity, Sandell's sensitivity, detection limit, slope, intercept, correlation coefficients were described (TABLE 1). The calibrations graphs are described by the regression equation: Y= a+bX (where Y= absorbance, a= intercept, b= slope and X= concentration in μgml^{-1}) obtained by the method of least squares.

Accuracy and precision

The accuracy and precision of the methods (within-assay and between assays) were determined at the Mf concentrations cited in TABLE 2. The within-assay precision was assessed by analyzing five replicates of each sample as a batch in a single assay run, and the between-assays precision was assessed by analyzing the same sample, as triplicate, in two separate assay runs. The relative standard deviations (RSD) were less than 0.95% (TABLE 2). This level of precision was adequate for the quality control analysis of Mf.

TABLE 1 : Characteristics of the metformin calibration graph for the developed FPL and FBL methods

Parameter	Units	Method	
		FPL	FBL
λ_{max}	(nm)	510	520
Beer's Range	($\mu g\ mL^{-1}$)	0.2–6.0	0.2–6.0
Ringbom range	($\mu g\ mL^{-1}$)	0.3-6.0	0.3-6.0
Detection limits	($\mu g\ mL^{-1}$)	0.08	0.1
Quantification limits	($\mu g\ mL^{-1}$)	0.17	0.2
Molar absorpitivity		4.38×10^4	3.38×10^4
Sandell sensitivity	($\mu g\ cm^{-2}$)	0.0247	0.01976
Regression equation		(Y=a+bX)*	
Slope (b)		0.24951	0.20687
S.D. of slope (S_b)		2.00×10^{-3}	1.1×10^{-3}
Intercept (a)		8.50×10^{-3}	1.95×10^{-3}
S.D. of intercept (S_a)		5.80×10^{-4}	3.00×10^{-4}
Correlation coefficient		0.9998	0.9961
Calculated t-values (2.57)**		1.07	0.76
Calculated F-test (5.05)*		2.47	2.03

*** *Y = a + bX*, where *Y* is the absorbance of the colored complex and *C* is the concentration of Mf in µg.**

**** Values in parentheses are the theoretical values for t- and *F*-value at 95% confidence limits and five degrees of freedom**

Interference studies

In pharmaceutical analysis, it is important to test the selectivity of the method towards the excipients added to the pharmaceutical preparations, such as glucose, starch, talc, lactose, sucrose. The proposed spec-

TABLE 2 : Accuracy and precision of the proposed methods for analysis of Mf (n=5)

Method	Taken (µg mL^{-1})	Found (µg mL^{-1})			
		Within-assays		Between-assays	
		Found	RDS	Found	RDS
FPL	1.0	0.92	0.82	0.94	1.20
	3.0	2.87	0.68	3.0	0.75
	4.5	4.48	0.80	4.62	0.62
	6.0	9.89	1.10	9.11	1.15
FBL	1.0	1.02	0.45	1.09	0.56
	3.0	3.10	0.65	2.89	0.88
	4.5	4.08	0.45	4.35	0.56
	6.0	6.13	0.62	5.85	0.44

trophotometric methods have the advantages that the measurements are performed in the visible region, away from the UV-absorbing interfering substances that might be co-extracted from Mf containing dosage forms. Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation (sodium lauryl sulfate, magnesium stearate, starch sodium glycolate, lactose spray dried, carboxymethylcellulose PA 102, talc, titanium dioxide, microcrystalline cellulose, hydroxypropylcellulose and pregelanitizated starch), their is no interference indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Analytical applications

The obtained satisfactory validation results made the proposed methods suitable for the routine quality control analysis of Mf and its dosage forms pharmaceutical formulations (CIDOPHAGE 500 and 850 mg tablets). The results obtained by the proposed methods were statistically compared with those obtained from the official pharmacopoeia method. The obtained mean values of the labeled amounts ranged from 99.90 ± 0.70, and 99.40 ±0.5%, using A, and B methods, respectively as recorded in TABLE 3. In the t- and *F*-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level[23]. This indicated similar precision and accuracy in the analysis of Mf in its formulations. It is evident from these results that all the proposed methods are applicable to the analysis of Mf in its tablets with comparable analytical performance.

The high sensitivity attained by the proposed methods allows the determination of Mf, in biological fluids (urine and plasma samples). The method was used to determine the amount of Mf in a healthy male 12 h or 24h after an intake of one tablet of Mf, which contains 500 or 850 mg Mf. Mf was detected and the results

TABLE 3 : Determination of Mf in tablets pharmaceutical preparations and biological fluids by the proposed and official method

Sample	Recovery		
	FPL	FBL	Official method
CIDOPHAGE (500mg)	Recovery 100.2±1.1 Variance 0.71 F* 2.82 t* 1.93	Recovery 99.4±1.2 Variance 0.59 F 2.53 t 0.55	Recovery 99.6±1.3
CIDOPHAGE (850mg)	Recovery 99.8±0.71 Variance 0.55 F=1.74 t=0.73	Recovery 99.7±1.2 Variance 0.49 F 2.03 t 0.92	Recovery 101.0±1.0
Urine sample	Recovery 97.4±1.0 Variance 0.44 F 3.13 t 0.88	Recovery 99.6±0.7 Variance 1.05 F 3.05 t 1.22	Recovery 100..0±1.4
Plasma sample	Recovery 100.2±1.1 Variance 1.02 F 2.76 t 1.57	Recovery 100.2±1.1 Variance 0.71 F 2.41 t 1.38	Recovery 99.4±0.9

were tabulated in (TABLE 3).

CONCLUSION

The redox reaction of metformin hydrochloride (Mf) using Fe^{3+} has been investigated. The formation of Fe^{2+}-phen and Fe^{2+}-bipy complexes were utilized in the development of simple, accurate, highly sensitive, low reagent consumption, no interference with good precision and accuracy spectrophotometric methods A and B for the analysis of Mf in pure form as well as in dosage and biological fluids. With these methods, one can do the analysis at low cost without losing accuracy. The

proposed methods can be used as alternative methods to the official ones for the routine determination of capsules. This encourages their successful use in routine analysis of Mf in quality control laboratories and they involve very simple procedures.

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