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## Microgravimetric method for the determination of metformin in pharmaceutical preparations and in the bulk drug

Matthieu Tubino<sup>1</sup>, Luís Francisco Bianchessi<sup>1</sup>, Marta M. D. C. Vila<sup>2</sup>

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**Abstract:** This article reports a microgravimetric method for the quantitative determination of metformin in bulk and in pharmaceutical preparations. The analysis is simple and relatively rapid. A complete analysis can be performed in only an hour and thirty minutes due to the drying procedure of the precipitate. However a sample can be treated every each five minutes. The method is based on the reaction of nickel (II) with metformin that in basic medium forms an orange precipitate. The reaction is performed in a plastic syringe. The filtration was done in a glass tube containing a sintered glass disk or in Millipore® syringe filters, with posterior weighing after drying. The analytical results were statistically compared with that obtained with a HPLC and with the titrimetric method suggested by the United States and by the Japanese pharmacopoeias.

**Keywords:** metformin; microgravimetry; nickel complex; analysis; syringe filters

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## INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous syndrome characterized by endocrine-metabolic abnormalities that modify the metabolic homeostasis in humans [1]. This pathology has been in continuous increasing and among the chronic non transmissible diseases it is one of the more relevant [2].

Metformin (1,1-dimethylbiguanidine) is an hypoglycemic agent employed in type 2 diabetes mellitus (DM2)[3]. It is usually found in the hydrochloride in 500 mg and 850 mg tablets.

Metformin chloridate is a white crystalline solid. Its molecular formula is  $C_4H_{11}N_5 \cdot HCl$  (Fig. 1) and the molecular mass 165.63 Da. Is freely soluble in water but insoluble in acetone, ether and chloroform [4,5].

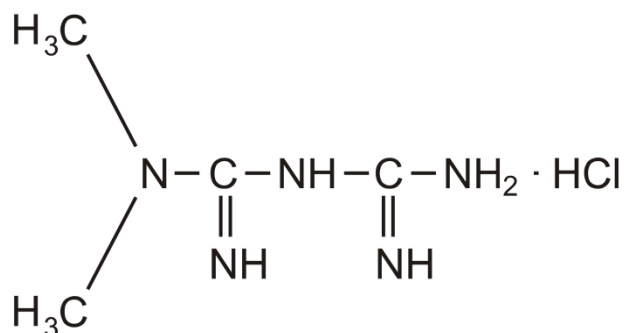


Figure 1

Some analytical procedures are reported in the literature for the determination of metformin[6,7].

An official analytical method of metformin chloridate indicated in various pharmacopoeias is the potentiometric titration in non aqueous solution[8-11]. Conductimetric titrations are proposed by Abou-Dan *et al.*[12] Catalayud *et al.* [13]. and Sartori *et al.* [14].

Spectrophotometric methods for the determination in bulk drug and in pharmaceutical preparations have been also reported. In the ultraviolet region methods some are proposed: British Pharmacopoeia [10], Annapurna *et al.* [15], Ashour and Kabbani[16]. In the visible region a method was related by Hassan *et al.* [17].

This last method was based of the colored complex formed between metformin (MF) and copper (II) with posterior dissolution in an organic solvent.

Several liquid chromatographic methods can be found in the literature [8-10,18-23].

Chromatographic HPLC procedures present good selectivity due to the separation in columns but present an environmental disadvantage that is the relatively high volume of organic solvents used. Despite this fact, it can be satisfactorily used for the simultaneous analysis of metformin and other drugs present in the same preparations [18, 23-33].

Recently Tubino *et al.* related an analytical quantitative method for the determination of metformin in pharmaceutical preparations and in the bulk drug using diffuse ultraviolet-visible reflectance [34].

Despite the reporting of a number of methods for the analysis of metformin, a deficiency is observed with respect to absolute methods, *i.e.*, that do not require calibration curves. Only three volumetric [12-14] are reported and no one for gravimetric was found.

Despite their absolute analytical characteristic classical gravimetric methods are usually very slow. However, the developing of the weighing techniques that resulted in better and faster to use balances, relatively fast gravimetric procedures can be developed [35].

The aim of the present work was to develop a reliable, simple and relatively fast method for the determination of metformin in bulk drugs and in pharmaceutical preparations.

## EXPERIMENTAL DETAILS

### Apparatus

HPLC: Shimadzu Prominence diode array SPP-M20; Micro-Bondapac column C18, length 300 mm, I.D. 3.0 mm, particle size 10  $\mu\text{m}$ . Injection volume 20  $\mu\text{L}$ .

pHmeter: Analyser, model pH 300, with a glass combined Ag/AgCl electrode; internal solution KCl 3.0 mol  $\text{L}^{-1}$ .

### Reagents

All the employed reagents were of analytical grade, excepting the metformin hydrochloride that was a pharmaceutical grade 98.5%, certified product. The pharmaceutical preparations were purchased in drugstores of the region.

## Solutions

*Standard solution of metformin hydrochloride:* prepared in calibrated 50 mL volumetric flask; about 7.5 grams (weighed to 0.1 mg) of the drug (98.5 % certified metformin hydrochloride) was dissolved in Milli Q Pure class water.

*Nickel chloride 1 mol L<sup>-1</sup> solution:* 11.9 g of NiCl<sub>2</sub>·6H<sub>2</sub>O were dissolved with Milli Q Pure grade water in a 50 mL volumetric flask.

*NaOH 2 mol L<sup>-1</sup> solution:* 4.0 g of NaOH were dissolved in Milli Q Pure grade water in a 50 mL volumetric flask.

*HClO<sub>4</sub> 0.1 mol L<sup>-1</sup> in glacial acetic acid* [11]: 8.5 mL of perchloric acid were dissolved in 500 mL of glacial acetic acid; 21 mL of acetic anhydride were added; the volume was completed to 1000 mL with glacial acetic acid. The solution was standardized with potassium biphthalate.

*Standard (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub> pH 5.00 buffer solution:* 5.0 g of the salt were dissolved in 500 mL of pure water. The pH was adjusted with NaOH 0.2 mol L<sup>-1</sup>, prepared by dilution from that of 2.0 mol L<sup>-1</sup>, to pH = 5.00 ± 0.05.

*Mobile phase, HPLC:* 500 mL of methanol and 500 mL of the (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub> buffer [28].

*Standard solutions for the calibration curve, HPLC:* 507.6 mg of the drug (containing 500.0 mg of metformin hydrochloride) was exactly weighed and transferred to a 100.0 mL volumetric flask and dissolved with 50.0 mL of methanol. The volume was completed with the pH 5.00 buffer solution. From this 5000 ppm solution 8.00 mL were taken and transferred to a 200.0 mL volumetric balloon. The volume was completed with the mobile phase above described. From this 200.0 ppm stock solution 120.0, 130.0, 140.0, 150.0, 160.0, and 170.0 ppm concentrations were obtained through adequate dilutions.

## Samples treatment

### *Proposed method*

20 tablets were accurately weighed in an analytical balance to 0.1 mg. From the total mass the mean weight of a tablet was calculated. An aliquot of about 1.0 g (about 800 mg of metformin

hydrochloride) with accuracy of 0.1 mg was weighed into an assay tube. 2.0 mL of water were added following stirring to dissolve the drug and centrifugation during 5 minutes at 4500 rpm to separate no soluble excipients. The supernatant was carefully transferred to a 10.0 mL volumetric flask. 2.0 mL more were added to the residue into the tube and the process was repeated. Three extraction were performed and always the supernatant was introduced into the 10.0 mL balloon. The volume was completed to the mark with water.

### *HPLC method* [28]

20 tablets were accurately weighed in an analytical balance to 0.1 mg and carefully crushed in a mortar.

An aliquot of the pharmaceutical preparation, containing *c.a.* 500.0 mg of metformin hydrochloride was exactly weighed and transferred to a 100.0 mL volumetric flask and dissolved with 50.0 mL of methanol. The volume was completed with the pH 5.00 buffer solution.

From this 5000 ppm solution 2.00 mL were taken and transferred to a 50.0 mL volumetric balloon. The volume was completed with the mobile phase above described. From this 200 ppm solution a 140 ppm solution was prepared by dilution, *i.e.*, 3.50 mL were diluted to 5.00 mL with water.

### *2.4.3. Sample treatment* [8]

20 tablets were carefully weighed to 0.1 mg and crushed in a mortar. An aliquot containing about 60 of metformin was weighed to 0.1 mg, transferred to a 150 mL beaker and solubilized with 50 mL of acetic anhydride and 4 mL of formic acid. Titration was point-to-point performed with a standard solution of perchloric acid 0.1 mol L<sup>-1</sup>.

## Methods

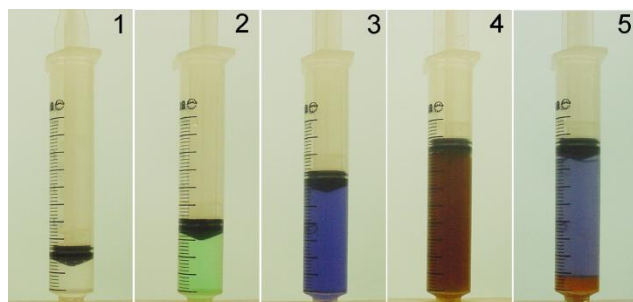
### *Proposed microgravimetric method*

The proposed method was based on the reaction of metformin with nickel(II) in aqueous medium forming an insoluble complex [12]. The reaction was performed inside a plastic 10 mL syringe. The 2.00 mL aliquot of the sample was first measured with a pipette, placed in a 5 mL beaker and then carefully

sucked with the syringe as can be seen in Figure 2. In sequence the other solutions are successively placed in this same beaker and sucked into the same syringe. The solutions were always introduced in the following order: 2.00 mL sample; 1.5 mL nickel(II)  $1.0 \text{ mol L}^{-1}$ ; 3.0 mL  $\text{NH}_3$  concentrated ( $17 \text{ mol L}^{-1}$ ); 2.0 mL NaOH  $2.0 \text{ mol L}^{-1}$ . The precipitate is quantitatively formed in about 3 minutes (Fig. 3).



**Figure 2**

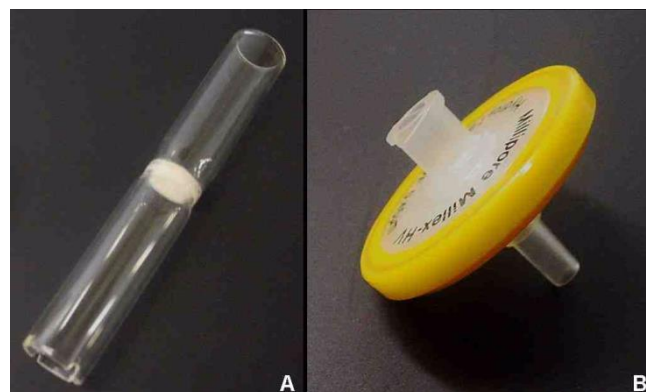


**Figure 3**

The precipitate was separated using a tube with a sintered G3 glass filter [35] (Fig. 4A) or a syringe filter (Millipore, Millex HV,  $0.45 \mu\text{m}$  pore, 33 mm diameter) (figure 4B) previously weighed.

In the case of the glass tube vacuum pump was used to filtrate the solid but a centrifuge can be used [35].

The solid in the filter was carefully washed with about 2.5 mL of concentrated  $\text{NH}_3$  following drying in an oven at  $120^\circ\text{C}$  for one hour. From the obtained weight of metformin as precipitate its quantity in the pharmaceutical preparation was calculated.



**Figure 4**

#### *Potentiometric method [8,9]*

The method recommended by the American and Japanese pharmacopoeias was used [8,9], using perchloric acid solution as titrant.

#### *HPLC method [28]*

This procedure is similar to that recommended by USP [8], British [10] and Japanese [9] pharmacopoeias. Buffer  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  pH=5.00 and methanol 50:50 v/v as mobile phase was used at  $1 \text{ mL min}^{-1}$ . The column ( $300 \times 3.0 \text{ mm}$ ) was a Micro-Bondapak C18 Waters,  $10 \mu\text{m}$  diameter particle size; temperature  $40^\circ\text{C}$ ,  $\lambda_{\text{max}}$  225 nm and injection volume  $20 \mu\text{L}$ .

#### *Statistical treatment*

For statistical comparison of the data obtained with the proposed method *versus* the reference methods the *t* test of Student and the Snedecor **F** test were used [36].

## **RESULTS AND DISCUSSION**

The orange color precipitate of nickel-metformin complex is easily formed but the solution must be strictly mixed in the indicated order: metformin, nickel, ammonium hydroxide, sodium hydroxide. Mainly in the case of sodium hydroxide solution that is added in order to increase the pH, if it is mixed before the ammonium hydroxide, nickel hydroxide precipitates and the formation of the desired complex does not occur.

It is important to pay attention to the molar excess of nickel, with respect to metformin (MF), in

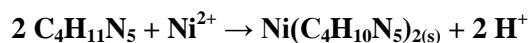
order to guarantee the quantitative formation of the complex.

The solution of metformin presents no color, unless an organic colorant is present. The addition of the green solution of Ni(II) turns all solution in this color. The addition of the ammonium hydroxide causes change to a blue color as the result of the  $\text{Ni}(\text{NH}_3)_6^{2+}$  hexamine complex. With the posterior sodium hydroxide solution an orange species is formed in solution, precipitating soon after as  $\text{Ni}(\text{MF})_2$ . The sodium hydroxide solution must be slowly and in three portions so the precipitate will be gradually formed.

As the ammonia complexes with Ni(II), forming the hexamine complex a soluble species, the precipitation of nickel as hydroxide when sodium hydroxide is added is avoided and the excess of this cation can be easily removed by washing the precipitate with the concentrated ammonium hydroxide.

The remainder ammonia in the precipitate can be easily eliminated by heating in an oven at 120 °C.

The reaction of formation of the  $\text{Ni}(\text{MF})_2$  complex, which precipitates as an orange color solid, can be summarized by the following equation which clearly shows the necessity of a base to shift reaction to the right:



A glass tube containing a G3 porosity sintered glass disc<sup>35</sup> was used to filter the precipitate or alternatively a Millipore syringe filter (Millipore, Millex HV, 0.45 µm pore, 33 mm diameter). The same samples were analyzed using the titrimetric potentiometric procedure recommended by pharmacopeias [8,9] and by a HPLC method [28]. This last procedure was preferred as it uses external standard. All the essays were done in triplicate.

**Table 1.** Comparison of the determination of metformin hydrochloride in pharmaceutical preparations using the proposed method, HPLC[28] and that of potentiometric titration according pharmacopoeias[8,9]. Values are in milligrams per tablet ± SD.

Sample	Label	Grav. <sup>t</sup>	Grav. <sup>f</sup>	HPLC	Official
I <sup>a</sup>	850	841.3±1.7	841.7±7.5	870.1±15.1	852.2±5.5
	RSD (%)	0.2	0.9	1.7	0.6
II <sup>b</sup>	500	490.2±2.4	492.9±9.0	511.5±2.9	499.9±4.5
	RSD (%)	0.5	1.8	0.6	0.9

<sup>a</sup> The tablet also contains microcrystalline cellulose, crospovidone, colloidal silicon dioxide, magnesium stearate, polyvinylpyrrolidone, croscarmellose sodium, sodium starch glycolate.

<sup>b</sup> The tablet also contains microcrystalline cellulose, crospovidone, colloidal silicon dioxide, magnesium stearate, povidone, hypromellose, polyethylene glycol.

Grav.<sup>t</sup> = proposed gravimetric; filtration using glass tubes with sintered disc (n=3).

Grav.<sup>f</sup> = proposed gravimetric; filtration using syringe filters (n=3).

HPLC = High pressure liquid chromatography (n=3).

Official = Potentiometric titration (n=3).



The results shown in table 1 were statistically analyzed using the paired Student's  $t$  test and the

Snedecor F test [36] (Table 2).

**Table 2.** Comparison of the analytical results using the Student's paired  $t$  test and the Snedecor F test [36].

Methods compared	$t_{\text{calc}}$		$F_{\text{calc}}$	
	I	II	I	II
Grav <sup>t</sup> vs. Grav <sup>f</sup>	0.1	0.4	19.5	14.1
Grav <sup>t</sup> vs. Official (Pot.)	2.7	2.7	10.5	3.5
Grav <sup>f</sup> vs. Official (Pot.)	1.6	1.0	1.9	4.0
Grav <sup>t</sup> vs. HPLC	2.7	8.0	78.9	1.5
Grav <sup>f</sup> vs. HPLC	2.4	2.8	4.1	9.6
HPLC vs. Official (Pot.)	1.6	3.1	7.5	2.4

$v = (n_A + n_B - 2) = 4$ ; at the 95 % confidence level.  $t_{\text{tab}}=2.8$  and  $F_{\text{tab}}=19.0$ ; at the 99% confidence level  $t_{\text{tab}}=4.604$  and  $F_{\text{tab}}=99.0$

Grav.<sup>t</sup> = proposed gravimetric; filtration using glass tubes with sintered disc.

Grav.<sup>f</sup> = proposed gravimetric; filtration using syringe filters.

HPLC = High pressure liquid chromatography.

Official method: potentiometric titration.

It can be observed that the calculated  $t$  and  $F$  values are smaller than those tabulated, when the proposed gravimetric procedures are compared with the potentiometric official method, indicating equivalent results and precision. In the HPLC cases two small differences are observed in  $t$  values and one in  $F$  values when comparison is done with the gravimetric method where a glass tube with sintered disc is used and with the official titrimetric procedure. However, if the 99% confidence level is considered all results become in agreement.

## CONCLUSION

The proposed method presents some interesting characteristics. It is easy and relatively fast to be

performed; it is very reliable and inexpensive; a calibration plot is unnecessary because it is an absolute method; it is a green procedure as it does not use organic solvents or toxic substances (excepting small quantities of ammonia) and those used are in small quantities. Therefore the proposed method can be suggested for the analysis of metformin in the bulk drug and in pharmaceutical preparations.

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