

A NOVEL MERCURIMETRIC TITRATION ASSAY OF METFORMIN HYDROCHLORIDE USING MERCURY (II) THIOCYANATE–IRON (III) NITRATE INDICATOR SYSTEM

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ABSTRACT

A simple, rapid, reliable and novel titration procedure for the mercurimetric assay of metformin hydrochloride (MHCl) based on measurement of the chloride content of its hydrochloride salt is described. In this experiment, initially a synthetic mercury(II) thiocyanate $[\text{Hg}(\text{SCN})_2]$ indicator was prepared by titrating mercury(II) nitrate $[\text{Hg}(\text{NO}_3)_2]$ against potassium thiocyanate (KSCN) in the 1:2 stoichiometric ratio using ferric nitrate $[\text{Fe}(\text{NO}_3)_3]$. After addition of a known amount of $\text{Hg}(\text{NO}_3)_2$ to the indicator, the solution was further titrated against the aqueous solution of MHCl until the original red color reappeared. The concentration of chloride present in the volume (of MHCl) utilized for the reaction was found to be reacting in the 2:1 stoichiometric ratio with the $\text{Hg}(\text{NO}_3)_2$ taken in the second titration. The statistical treatment of the experimental data obtained by determining MHCl in the concentration range 0.8281 to 8.281 g/lit, indicates that the procedure is precise and accurate. The procedure was further applied for the analysis of MHCl in tablet formulations. The average recovery and accuracy were found to be in agreement with claimed by the manufacturer. The common excipients used in tablet formulations did not interfere in the determination of MHCl by this procedure as observed by the recovery experiment using standard addition method. The chemistry involved in titration, the action of an indicator at the end point and the factors that affecting the reaction stoichiometry are discussed.

Keywords: Chloride assay, Mercurimetric titration, Mercury (II) nitrate, Mercury(II) thiocyanate-iron(III) nitrate indicator system, Metformin hydrochloride, Metformin hydrochloride tablets.

INTRODUCTION

Argentometric¹⁻³ and mercurimetric^{4,5} determination of chloride in the hydrochloride salt of the drug are the common practice to find the concentration of the drugs. The essence of the present study of the determination of MHCl is also based on the quantitation of the chloride in the drug hydrochloride. The traditional spectrophotometric methods⁶⁻⁹ for determination of chloride are based

on replacement of the thiocyanate ions in an undissociated $\text{Hg}(\text{SCN})_2$ and measurement of the absorbance of the red iron(III)-thiocyanate complex. The ability of iron(III) to form thiocyanate complexes of different composition¹⁰, therefore these methods⁶⁻⁹ reflect non-linearity in the Beer's law at higher concentration of the chloride.

The well known and commonly used titrimetric methods for determination of chloride includes

Mohr method¹¹ in which the neutral solution of chloride is directly titrated against silver nitrate (AgNO_3) using potassium chromate indicator. The anions like phosphate, arsenate, chromate, sulfide, and oxalates are showing the interference in the determination of chloride in neutral solution¹⁰. So, in Volhard procedure¹¹ initially, the precipitation reaction of chloride with known and excess of AgNO_3 is carried out in dilute nitric acid and the residual AgNO_3 is estimated by back titration using thiocyanate solution. The co-precipitation of thiocyanate ions over silver chloride (AgCl) precipitate is the major problem associated Volhard's method, which can be little bit eliminated by coagulating the AgCl precipitate with the nitrobenzene. Fajans method¹⁰⁻¹¹ of the determination of chloride using AgNO_3 utilizes fluorescein and dichlorofluorescein as the adsorption indicator for determination of chloride in neutral and in acidic medium respectively. The extent of the adsorption of the indicator on the surface of the AgCl precipitate is related to the experimental conditions. In turbidimetric titration¹²⁻¹³ a solution of AgNO_3 was added into chloride solution and turbidance of the AgCl suspension is measured for determination of chloride. The refractive index difference and particle dimension of the AgCl are changing continuously during the course of titration; as a result, it does not produce an appropriate end point in the turbidimetric titration curve.

The mercurimetric determination of the chloride involves the direct titration against $\text{Hg}(\text{NO}_3)_2$ using diphenylcarbazone (DPC) indicator¹⁴. Although DPC is a suitable indicator for this titration but the mixture of DPC with bromothymol blue gives better end point, which was demonstrated by Clarke¹⁵ and applied for the determination of chloride in plant materials¹⁶. But the detection of the exact end point in this titration is a difficult task without precise adjustment of the pH (3.2–3.3). Finally, all these titrimetric methods mentioned above are associated with some kind of difficulty in determination of chloride. However, titrimetry is the most common, convenient and favorite tool in all analytical laboratories due to its inherent reproducibility, accuracy and low costing absolute method of analysis.

Metformin is used to lower blood glucose in patients with non-insulin dependent diabetes mellitus¹⁷. It is orally administered drug in the form of its hydrochloride salt (Fig.1) having the molecular formula ($\text{C}_4\text{H}_{11}\text{N}_5\text{HCl}$) and the molecular weight 165.62 g/mol.

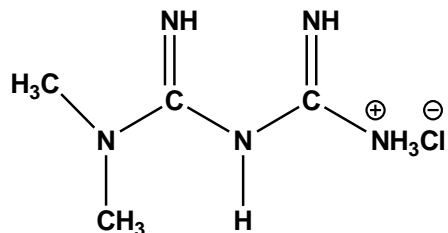


Figure 1: Chemical structure of the metformin hydrochloride

Several methods have been proposed in the literature for quantification of MHCl including titration with perchloric acid¹⁸, conductometric determination using copper sulfate¹⁹ and silver nitrate²⁰, a potentiometric assay with PVC membrane sensors²¹⁻²², and photofluorimetric²³ evaluation by using chrysenequinone and 1-naphthol. In addition, high performance liquid chromatography²⁴, reversed phase-liquid chromatography²⁵, liquid chromatography-mass spectrometry²⁶, tandem mass spectrometry²⁷⁻²⁸, gas chromatography²⁹, capillary electrophoresis³⁰ and spectrophotometric³¹⁻³² analysis. Most of these methods are time-consuming and required an expensive instrumentation.

In the previous study, we have utilized the $\text{Hg}(\text{NO}_3)_2$ reagent and $\text{Hg}(\text{SCN})_2\text{-Fe}(\text{NO}_3)_3$ indicator system for the titrimetric determination of ranitidine hydrochloride³³ as well as for determination of the chloride concentration in the water of green coconuts³⁴. However, there is no report on the titrimetric assay of the MHCl using $\text{Hg}(\text{NO}_3)_2$ reagent and $\text{Hg}(\text{SCN})_2\text{-Fe}(\text{NO}_3)_3$ indicator system. So, in the present study, an attempt is made to report the original approach that allows a simple and rapid titrimetric assay of MHCl. In this procedure, initially a synthetic $\text{Hg}(\text{SCN})_2$ indicator was prepared by titrating $\text{Hg}(\text{NO}_3)_2$ against KSCN up to the red color end point using $\text{Fe}(\text{NO}_3)_3$. Then a known amount of the $\text{Hg}(\text{NO}_3)_2$ was added to the indicator solution and further titrated against an aqueous solution of MHCl

until the original red color reappeared. The concentration of MHCl present in the volume (of MHCl solution) utilized for titration was found to be reacting in the 2:1 stoichiometric ratio with the known amount $\text{Hg}(\text{NO}_3)_2$ taken in the second step of the titration.

Experimental

Chemicals and Reagents

All the chemicals used were of the analytical grade. Double distilled water was used to prepare all the solutions. A standard 0.05M of KSCN, MHCl and sodium chloride (NaCl) solutions were prepared in water. A 0.05M $\text{Hg}(\text{NO}_3)_2$ solution was prepared in 0.5M nitric acid. A 0.2M of $\text{Fe}(\text{NO}_3)_3$ solution was prepared in 3.0M perchloric acid (HClO_4). The molarity of $\text{Hg}(\text{NO}_3)_2$ was adjusted exactly equal to 0.05M by standardizing against standard 0.05M KSCN using $\text{Fe}(\text{NO}_3)_3$ indicator. The metformin-containing tablets, *Metfor*, *Glycomet*, *Diabetrol*, *Reclimate*, *Gemer 1* and *Actizide-M* were purchased from commercial sources and subjected to analysis.

Assay Procedure

Step-I: Preparation of indicator

A 5.0ml of 0.05M of $\text{Hg}(\text{NO}_3)_2$ was transferred into a 250-ml titration flask containing 15 ml of distilled water. In this solution, 10 ml of 0.2M of $\text{Fe}(\text{NO}_3)_3$ in 3.0M HClO_4 was added. The content in the flask was titrated against 0.05M KSCN placed in a 10-ml micro-burette. This process was performed by intermittent till a faint red color developed. This termed here as the first end point of the titration. The burette reading (V_{KSCN}) was recorded. The exact molarity [$M_{\text{Hg}(\text{NO}_3)_2}$] of $\text{Hg}(\text{NO}_3)_2$ was calculated using following Eq. (1):

$$M_{\text{Hg}(\text{NO}_3)_2} = \frac{(M_{\text{KSCN}} \times V_{\text{KSCN}})}{(V_{\text{Hg}(\text{NO}_3)_2} \times N)} \quad \dots \text{Eq. (1)}$$

In Eq. (1), M_{KSCN} is the molarity of KSCN, $V_{\text{Hg}(\text{NO}_3)_2}$ is the volume of $\text{Hg}(\text{NO}_3)_2$ taken for titration, $N(=2)$ is the number of moles of KSCN reacting with one mole of $\text{Hg}(\text{NO}_3)_2$. At the end point, this solution consists little amount of red colored monothiocynatoiron(III) nitrate, $[\text{Fe}(\text{SCN})](\text{NO}_3)_2$ and $\text{Hg}(\text{SCN})_2$ and $\text{Fe}(\text{NO}_3)_3$. This solution was used as an indicator in the second step of the titration.

Step-II: Determination of MHCl

After preparation of the indicator solution, exactly 5.0ml of standard 0.05M $\text{Hg}(\text{NO}_3)_2$ was added to the flask. This addition disappears the red color of the solution. The content in the titration flask was titrated against 0.05M MHCl solution slowly from a 10-ml micro burette until the original red color reappeared. This termed here as the second end point of the titration. At this end point, the burette reading (V_{MHCl}) of the MHCl solution was recorded. The molarity (M_{MHCl}) of the MHCl solution was calculated as shown below:

$$M_{\text{MHCl}} = \frac{(M_{\text{Hg}(\text{NO}_3)_2} \times V_{\text{Hg}(\text{NO}_3)_2} \times N)}{V_{\text{MHCl}}} \quad \dots \text{Eq. (2)}$$

In Eq. (2), $V_{\text{Hg}(\text{NO}_3)_2}$ is the volume of $\text{Hg}(\text{NO}_3)_2$ was taken in the second step of titration.

The strength of the MHCl in g/lit (*viz.* mg/ml) was calculated by multiplying molarity (M_{MHCl}) with the molecular weight (165.62 g/mol) of the MHCl. The amount of MHCl (C_{MHCl}) in 100 ml solution was calculated by using following Eq. (3).

$$C_{\text{MHCl}} = M_{\text{MHCl}} \times \text{MW} \times 100 \quad \dots \text{Eq. (3)}$$

Quantification of MHCl in tablets

A single tablet containing MHCl was weighed and ground into a fine powder. An accurate amount of 0.200 to 0.400 g of MHCl powder was dissolved in water and diluted to 100 ml in the calibrated flask. The solution was shaken thoroughly and filtered using a Whatman No. 41 filter paper. This filtrate was filled in the 10-ml micro burette and assayed as per the above procedure. The molarity, strength, and amount of MHCl in 100 ml solution were calculated as mentioned above.

Assay by standard addition method

After preparation of indicator solution by step-I, exactly 5.0ml of standard 0.05M $\text{Hg}(\text{NO}_3)_2$ was added in titration flask. Initially, a known amount of (4.0 ml, 6.0 ml or 8.0 ml of 0.05M) of MHCl was added as a standard and then content was further titrated by adding MHCl sample solution from 10-ml micro burette till original red color was reappeared. The burette reading (V_{SAM}) of the MHCl sample solution was recorded. The molarity (M_{SAM}) of the MHCl

solution in the sample solution was calculated using following Eq.(4):

$$M_{\text{SAM}} = \frac{[(M_{\text{Hg(NO}_3)_2} \times V_{\text{Hg(NO}_3)_2} \times N) - (M_{\text{MHCl}} \times V_{\text{MHCl}})]}{V_{\text{SAM}}}$$

In Eq. (4), M_{MHCl} and V_{MHCl} are molarity and volume of 0.05M MHCl respectively which was added as MHCl standard. Furthermore, the strength and the amount of MHCl in 100 ml sample solution were calculated as described above.

Results and Discussion

The majority of drug compounds have been assayed by determining the chloride content in their hydrochloride salt using silver salts¹⁻³ and mercury salts^{4-5,33}. In the earlier study, we utilized 1:1 mercuric–thiocyanate reagent for direct determination of chloride³⁵ and mercuric-thiocyanate absorbing system for spectrophotometric determination of chloride³⁶. The proposed procedure of the assay of the MHCl is also based on the determination of chloride content in the hydrochloride salts of the drug. In an aqueous medium, MHCl ionizes by giving the protonated drug moiety and the chloride ion facilitating the mercurimetric titration of the MHCl via measurement of the chloride concentration.



Stability and reactivity of the complexes

Practically, during the formation of complex, there is a competition between two or more ligands for the metal ion in an aqueous solution. The complex formation reactions are the ligand replacement process. The ligands in the coordination sheath of the metal ion are replaced step wisely by the other ligands having strong combining capacity with the metal ion³⁷. The value of formation constant or stability constant (log K) determines the stability of the complex.

The cumulative formation constant (CFC) of the chloride³⁷⁻³⁸ complexes of mercury(II) [Hg(II)] are reported as log $K_1=6.74$, log $K_2=13.22$, log $K_3=14.07$ and log $K_4=15.07$ indicates the formation of $[\text{Hg}(\text{Cl})]^+$, $[\text{Hg}(\text{Cl})_2]$, $[\text{Hg}(\text{Cl})_3]^{-1}$ and $[\text{Hg}(\text{Cl})_4]^{-2}$ complexes respectively. The values of log K_2 , log K_3 and log K_4 of these complexes does not differs

widely; consequently, with the addition of the chloride solution into the Hg(II) solution, there is a possibility of the formation of all complexes simultaneously. Consequently, in the direct titration of chloride against Hg(II), the correct reaction stoichiometry becomes indistinguishable.

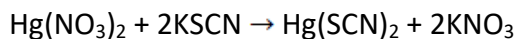
The CFC of thiocyanate³⁷⁻³⁸ complexes of the Hg(II) are reported as log $K_2=17.47$ and log $K_4=21.23$ respectively this indicates the formation of $[\text{Hg}(\text{SCN})_2]$ and $[\text{Hg}(\text{SCN})_4]^{-2}$ complexes only. Since no report³⁷⁻³⁸ on the values of log K_1 and log K_3 designates no possibility of formation of the $[\text{Hg}(\text{SCN})]^+$ and $[\text{Hg}(\text{SCN})_3]^{-1}$ complexes. In addition, the value of log K_2 and log K_4 of the thiocyanate complexes of Hg(II) differs widely. This makes practicable for determination of Hg(II) using thiocyanate titrant¹¹ up to $[\text{Hg}(\text{SCN})_2]$ reaction product by using $\text{Fe}(\text{NO}_3)_3$ indicator. The same theme of determination of Hg(II) is applied here for preparation of the indicator solution (step-I of the procedure).

The complexometric reaction study³⁷⁻³⁸ illustrates that both the chloride and thiocyanate ions form sufficiently stable complexes with Hg(II), but the reactivity of chloride towards Hg(II) is quite more; since it displaces thiocyanate ion from the $\text{Hg}(\text{SCN})_2$ and makes it feasible to determine chloride⁶⁻⁹ as well as the detection of the end point of the titration in the proposed procedure. In the present investigation, the reaction of the chloride with the Hg(II) was controlled in the 1:1 stoichiometric ratio by making the reaction competitive for the chloride and thiocyanate ions towards Hg(II) ion. Probably this is possible only by adding chloride solution into the solution of Hg(II) containing thiocyanate ions.

Chemistry of the indicator

The mercurimetric determination of the chloride concentration is the well-known examples of the complexometric titration¹⁵⁻¹⁶. At acidic pH, the reaction of chloride against Hg(II) is taking place in the 2:1 stoichiometric ratio, that forms the basis of many titrimetric methods employed in the drug analysis⁴⁻⁵ as well as the analysis of chloride in the plant materials¹⁶. The present titration of MHCl determination also involved the reaction of chloride

with a known concentration of $\text{Hg}(\text{NO}_3)_2$ using $\text{Hg}(\text{SCN})_2\text{-Fe}(\text{NO}_3)_3$ indicator system. This $\text{Hg}(\text{SCN})_2$ indicator was prepared by titrating $\text{Hg}(\text{NO}_3)_2$ against KSCN in the 1:2 stoichiometric ratio using $\text{Fe}(\text{NO}_3)_3$.



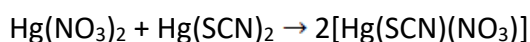
After equivalence point of the titration, a trace concentration of KSCN reacts with $\text{Fe}(\text{NO}_3)_3$ gives the red colored $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$ in the solution at the end point.



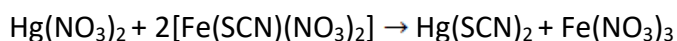
Thus, the indicator solution consists of $[\text{Hg}(\text{SCN})_2]$, $\text{Fe}(\text{NO}_3)_2$ with a little amount of $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$. All the reaction products are soluble because of the highly acidic pH (~0.80 to 0.85) created by the HClO_4 and HNO_3 which were employed in the preparation of $\text{Fe}(\text{NO}_3)_3$ and $\text{Hg}(\text{NO}_3)_2$ reagents respectively.

Chemistry of titration reaction

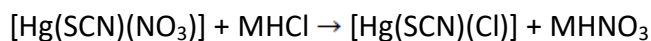
The second step of the procedure describes the mercurimetric determination of chloride or MHCl . When an indicator was prepared by using a 5.0ml of 0.05M $\text{Hg}(\text{NO}_3)_2$ and exactly same amount of $\text{Hg}(\text{NO}_3)_2$ (*viz.* 5.0 ml of 0.05M) was taken for the second step of the titration; then $\text{Hg}(\text{NO}_3)_2$ reacts with the $\text{Hg}(\text{SCN})_2$ by forming $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ as below:



Furthermore, the addition of $\text{Hg}(\text{NO}_3)_2$ also vanishes the red color of the indicator solution because of the conversion of red colored $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$ into the colorless $\text{Hg}(\text{SCN})_2$.

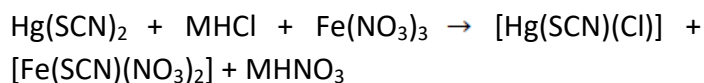


Thus, before the titration against the MHCl , the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ and $\text{Hg}(\text{SCN})_2$ with $\text{Fe}(\text{NO}_3)_3$ are the reacting species present in the titration flask. When this solution titrated against the MHCl solution, then chloride ions (of the MHCl) are reacting initially with the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ through replacing the nitrate ions.



As long as, the titrimetric reaction does not reach to 1:1 stoichiometry, the initially linked thiocyanate ions [to $\text{Hg}(\text{II})$] does not allow for the chloride ions to

increase coordination number of $\text{Hg}(\text{II})$ greater than two. Thus up to the equivalence point, both the thiocyanate and the chloride ions have equal reactivity towards $\text{Hg}(\text{II})$, which makes this reaction competitive for both the ions. After completion of the reaction in the 1:1 stoichiometric ratio, the extra chloride ions (of the MHCl) displaces the thiocyanate ions from the $\text{Hg}(\text{SCN})_2$ and it regenerates the red colored $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$ on reaction with $\text{Fe}(\text{NO}_3)_3$ at the end point of the titration.



The identical red color intensity at first and the second end point of the titrations designates equal concentration of $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$, which proves that the titration is free from the titration errors.

Stoichiometry of titration

When the exactly same amount of $\text{Hg}(\text{NO}_3)_2$ was employed in the preparation of the indicator solution (step-I of the titration) as well as for the titration of the MHCl (step-II of the titration), then a small amount of $\text{Hg}(\text{SCN})_2$ was generated in the indicator solution. The concentration of this $\text{Hg}(\text{SCN})_2$ presides over the amount of the $[\text{Fe}(\text{SCN})(\text{NO}_3)_2]$ that was formed in the first step of the titration. With reference to above discussion, the proposed procedure of the mercurimetric determination of the MHCl involves the reaction of chloride ions (of the MHCl) against the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ in the presence of $\text{Hg}(\text{SCN})_2$ and $\text{Fe}(\text{NO}_3)_3$. It was observed that one mole of the MHCl was reacting with the one mole of the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ or two moles of the MHCl were reacting with one mole of the $\text{Hg}(\text{NO}_3)_2$, that was taken in the second step of the titration.

The reaction stoichiometry *viz.* the number (N) of moles of MHCl reacting with one mole of $\text{Hg}(\text{NO}_3)_2$ was first studied by determining the MHCl in the range 0.8281 to 8.281 g/lit (0.005M to 0.05M) using the standard MHCl solutions. The value of N was calculated by using following Eq. (5):

$$N = \frac{(M_{\text{MHCl}} \times V_{\text{MHCl}})}{(M_{\text{Hg}(\text{NO}_3)_2} \times V_{\text{Hg}(\text{NO}_3)_2})} \quad \dots \text{Eq. (5)}$$

In Eq. (5), M_{MHCl} and V_{MHCl} are the molarity and the burette reading of the MHCl solution. The value of N found to be correct only when the concentration of the $\text{Hg}(\text{NO}_3)_2$ employed in the second titration was smaller or equal to that was taken for preparation of indicator solution (table 1 and 2). At this experimental condition along with the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$, the $\text{Hg}(\text{SCN})_2$ was present in the in the titration flask for detection of the end point of the titration. Consequently in presence of the $\text{Hg}(\text{SCN})_2$ the reaction stoichiometry was found to be correct. When an indicator was prepared by using a 5.0ml of 0.05M $\text{Hg}(\text{NO}_3)_2$ and the lower amount (1.0, 2.0, 3.0 or 4.0ml) of $\text{Hg}(\text{NO}_3)_2$ was employed for the titration (step-II), at this situation excess amount of $\text{Hg}(\text{SCN})_2$ in the indicator solution gives the sharper end point of the titration.

When larger amount (more than 5.0ml) of $\text{Hg}(\text{NO}_3)_2$ was used in the second step of the titration, then the formation of $\text{Hg}(\text{SCN})_2$ is not possible. At this condition along with the $[\text{Hg}(\text{SCN})(\text{NO}_3)]$ and the free $\text{Hg}(\text{II})$ ions (not lined with thiocyanate) were present in the titration flask. These free $\text{Hg}(\text{II})$ ions are reacting with chloride by giving chloro-complexes of coordination number greater than two. Consequently, the value of N was observed to be increased (table 1 and 2). Under this condition, the development of the red color at the end point of the titration can be predicted because of the following reaction:

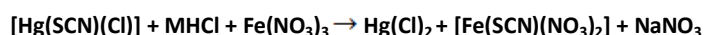


Table 1: Results obtained in determination of reaction stoichiometry by varying the concentration of the $\text{Hg}(\text{NO}_3)_2$ for the titration

Composition of the indicator			Volume of 0.05M $\text{Hg}(\text{NO}_3)_2$ taken for titration (ml)	Volume of 0.05M MHCl required for titration (ml)	[MHCl: $\text{Hg}(\text{NO}_3)_2$] Stoichiometry found
Amount of 0.2 M $\text{Fe}(\text{NO}_3)_3$ (ml)	Amount of 0.05M $\text{Hg}(\text{NO}_3)_2$ (ml)	Amount of 0.05M KSCN (ml)			
10.0	5.0	10.0	2.0	4.0	2.000:1
10.0	5.0	10.0	4.0	8.0	2.000:1
10.0	5.0	10.0	5.0	10.0	2.000:1
10.0	5.0	10.0	6.0	12.05	2.008:1
10.0	5.0	10.0	8.0	16.09	2.012:1
10.0	5.0	10.0	10.0	20.20	2.010:1

Table 2: Results obtained in determination of reaction stoichiometry by varying the concentration of the $\text{Hg}(\text{SCN})_2$ in the indicator

Composition of the indicator			Volume of 0.05M $\text{Hg}(\text{NO}_3)_2$ taken for titration (ml)	Volume of 0.05M MHCl required for titration (ml)	[MHCl: $\text{Hg}(\text{NO}_3)_2$] Stoichiometry found
Amount of 0.2 M $\text{Fe}(\text{NO}_3)_3$ (ml)	Amount of 0.05M $\text{Hg}(\text{NO}_3)_2$ (ml)	Amount of 0.05M KSCN (ml)			
10.0	1.0	2.0	5.0	10.4	2.08:1
10.0	2.0	4.0	5.0	10.3	2.06:1
10.0	3.0	6.0	5.0	10.2	2.04:1
10.0	4.0	8.0	5.0	10.1	2.02:1
10.0	5.0	10.0	5.0	10.0	2.00:1
10.0	6.0	12.0	5.0	10.0	2.00:1

Accuracy and precision of the experiment

In the aqueous solution, MHCl and NaCl ionize in a similar way. Therefore, the results obtained in the analysis of MHCl were simultaneously matched by analyzing standard NaCl. The five replicate measurements of the

determination of chloride and MHCl were carried out by using 0.005M, 0.01M, 0.025M and 0.05M standard NaCl as well as MHCl solutions respectively. The accuracy of the procedure in the determination of chloride and MHCl are presented in the form of relative errors. Similarly, the precision of the procedure in the determination of chloride and MHCl are expressed in terms of relative standard deviations. The results presented in Table-3 and Table 4, indicate that the proposed procedure is accurate and reproducible for determination of chloride and MHCl. As reaction stoichiometry was observed same in determination of chloride (in NaCl) as well as MHCl indicates that the protonated drug moiety, MH^+ had no effect on the complex formation reaction and the color intensity at the end point.

Table 3: Results obtained in the determination of the accuracy and precision of the procedure in determination of chloride in NaCl

Volume of 0.05M $Hg(NO_3)_2$ taken for titration (ml)	Expected amount chloride require for reaction (mg)	Strength/ molarity of chloride found* (M)	Amount of chloride found* (mg)	Absolute error (mg)	Relative error (%)	Deviation	Standard deviation
1.0ml	3.55	0.0500	3.551	0.004	0.10	0.001	0.166
2.0ml	7.10	0.0500	7.094	-0.031	-0.43	0.001	0.354
3.0ml	10.65	0.0500	10.643	-0.034	-0.32	0.001	0.274
4.0ml	14.20	0.0500	14.207	0.036	0.26	-0.001	0.300
5.0ml	17.75	0.0500	17.757	0.036	0.20	-0.001	0.361
				Average	-0.19 %		0.291

*Average value of the five determinations

Table 4: Results obtained in the determination of the accuracy and precision of the procedure in the determination of MHCl

Volume of 0.05M $Hg(NO_3)_2$ taken for titration (ml)	Expected amount MHCl require for reaction (mg)	Strength/ molarity of MHCl found* (M)	Amount of MHCl in found* (mg)	Absolute error (mg)	Relative error (%)	Deviation	Standard deviation
1.0ml	16.562	0.0500	16.563	0.007	0.04	0.000	0.057
2.0ml	33.124	0.0500	33.110	-0.068	-0.20	0.000	0.092
3.0ml	49.686	0.0500	49.720	0.172	0.35	-0.001	0.063
4.0ml	66.248	0.0500	66.183	-0.325	-0.49	-0.001	0.059
5.0ml	82.8105	0.0500	82.762	-0.245	-0.30	-0.001	0.059
				Average	-0.60		0.066

*Average value of the five determinations

Application of the procedure

The MHCl in aqueous solution ionizes to give the protonated drug moiety and the chloride ion. The liberated chloride is being reacted in the 2:1 stoichiometric ratio with the $Hg(NO_3)_2$ or in the 1:1 stoichiometric ratio with the $[Hg(SCN)(NO_3)]$. Therefore, the procedure was found applicable for the quantitative determination of the MHCl through determination of the chloride content in its hydrochloride salts. The results of this analysis are present in table 5. The percent recovery in the analysis of the MHCl drug was found in the range 99.59 to 101.11 %, so the procedure is accurate.

Standard addition method

The accuracy of the proposed procedure (employed for the determination of MHCl) was further ascertained by using standard addition method. In this study, before the titration against sample solution of the MHCl, the known amount of pure MHCl was first added in the titration flask and the solution was further titrated against the sample solution of MHCl. The results of this analysis are presented in table 6. In the analysis of drug by the standard addition method, the percent recovery of the drug was also found nearly equal to 100% (table 6). Therefore, neither the end-point of the titration nor the molarity of sample MHCl was affected by the protonated drug moiety and the common tablet excipients.

Table 5: Results obtained in the quantitation determination of the MHCl in the tablet formulations by the proposed procedure

Drug Formulation	Weight of the tablet (mg)	Sample weight taken for analysis (mg)	Molarity of the MHCl found (M)	Amount of MHCl found in the sample (mg)	Amount of the MHCl found in tablet (mg)	Recovery of MHCl found in analysis (%)
Metfor (500 mg MHCl)	542	406	0.02260	374.30	499.68	99.93
	544	270	0.01515	250.91	505.53	101.11
	538	266	0.01504	249.05	503.83	100.77
Glycomet (250 mg MHCl)	272	181	0.01013	167.77	252.12	100.85
	267	267	0.01508	249.75	249.75	99.90
	268	178	0.01011	167.44	252.11	100.84
Diabetrol (500 mg MHCl)+ Glibenclamide	551	370	0.02031	336.37	500.92	100.18
	555	352	0.01923	318.48	502.16	100.43
	559	274	0.01482	245.45	500.75	100.15
Glucoford (500 mg MHCl)+ Glibenclamide	578	399	0.02084	345.15	499.99	99.99
	584	356	0.01852	306.72	503.16	100.63
	588	256	0.01315	217.79	500.23	100.05
Gemer 1 (500 mg MHCl)+ Glimepiride	568	380	0.02021	334.58	500.12	100.02
	564	338	0.01818	301.09	502.41	100.48
	568	266	0.01418	234.92	501.63	100.33
Glynase-MF (500 mg MHCl)+ Glipizide	558	358	0.01951	323.12	503.64	100.73
	559	292	0.01571	260.13	497.98	99.59
	552	270	0.01481	245.36	501.62	100.32

Table 6: Results obtained in the quantitation determination of the MHCl in the tablet formulations by proposed procedure using standard addition method

Drug Formulation	Volume of the 0.05M MHCl was added as standard (ml)	Weight of the tablet (mg)	Sample weight taken for analysis (mg)	Molarity of the MHCl found (M)	Amount of MHCl found in the sample (mg)	Amount of the MHCl found in the tablet (mg)	Recovery of MHCl found in analysis (%)
Metfor (500 mg MHCl)	4.0	542	406	0.02273	376.45	502.55	100.51
	6.0	544	270	0.01504	249.92	501.87	100.37
	8.0	538	266	0.01493	247.19	499.96	99.99
Glycomet (250 mg MHCl)	4.0	272	181	0.01014	167.85	252.25	100.90
	6.0	267	267	0.01503	249.75	249.05	99.62
	8.0	268	178	0.01010	167.29	251.87	100.74
Diabetrol (500 mg MHCl)+ Glibenclamide	4.0	551	370	0.02027	335.72	499.94	99.98
	6.0	555	352	0.01923	318.48	502.16	100.43
	8.0	559	274	0.01493	247.27	504.46	100.89

Glucoford (500 mg MHCl)+ Glibenclamide	4.0	578	399	0.02098	347.47	503.35	100.67
	6.0	584	356	0.01835	303.89	498.52	99.71
	8.0	588	256	0.01334	220.94	507.46	101.49
Gemer 1 (500 mg MHCl)+ Glimepiride	4.0	568	380	0.02027	335.71	501.80	100.36
	6.0	564	338	0.01802	298.45	498.00	99.60
	8.0	568	266	0.01428	236.60	505.22	101.04
Glynase-MF (500 mg MHCl)+ Glipizide	4.0	558	358	0.01948	322.63	502.88	100.57
	6.0	559	292	0.01575	260.85	499.37	99.87
	8.0	552	270	0.01493	247.27	505.53	101.11

Conclusion

In conclusion, the proposed procedure of determination of the MHCl is found to be quite simple, inexpensive and reliable in comparison to the above-mentioned methods requiring the sophisticated instrumentation. The results obtained in the analysis of the MHCl are found to be in agreement with those obtained in determination chloride in standard NaCl solutions; this demonstrates that neither the protonated drug moiety nor the tablet excipients interfere in the determination of MHCl by this procedure. The percent recovery values indicate the procedure is excellent for the mercurimetric determination MHCl in acidic solution without precise adjustment of the PH. With the proposed procedure the determination of MHCl is possible in the homogenous solution, consequently, it is free from the titration errors those are commonly encountered by co-precipitation in the argentometric assay of MHCl. Titrimetry is the absolute method of analysis consequently the proposed procedure is suitable for routine analysis of MHCl in pure form and in tablet formulations for controlling the quality of the product. Furthermore, given procedure may apply for determination of other drugs (existing in their hydrochloride form) as well as for assay of chloride concentration in different aqueous samples.

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