Indirect Atomic Absorption Spectrometric Determination of Clindamycin Hydrochloride & Moxifloxacin Hydrochloride in Pure and in Pharmaceutical Formulations through Ion-Associate Formation with Phosphotungstate

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Abstract—A new simple, selective, accurate and precise indirect atomic absorption spectrometric method has been developed for the quantitative determination of clindamycin hydrochloride (CM.HCl) and moxifloxacin hydrochloride (Mf.HCl) drugs. The method is based on the reaction of each studied drug with an accurately measured excess of phosphotungstate reagent to form ion-associate precipitate and the unreacted excess phosphotungstate was determined by atomic absorption spectrometry of tungsten at 255.1 nm. The molar concentrations of drugs, which equivalent to the consumed phosphotungstate reagent, were then indirectly determined. The optimum conditions for the precipitation reactions and for the tungsten atomic absorbance measurements have been carefully investigated. The mole ratio method and the elemental analysis of the formed ion-associate precipitates were measured. The present developed method has been successfully applied for the determination of the studied drugs in pure solutions and in their commercial pharmaceutical formulations. There were insignificant interferences from most of the common excipients used. Regression equation parameters and limits of detection and quantitation were obtained. Rectilinear calibration graphs were observed in the concentration ranges 92.28-599.82 µg/mL for CM.HCl and 87.58-569.27 µg/mL for MF.HCl with recovery percent ranged from 97.03 to 99.74 and relative standard deviations ranged from 0.78 to 3.11% which indicated a good accuracy and precision compared with the official and reported methods as revealed by F-and t-tests.

Index Terms— Atomic absorption spectrometry, Clindamycin, Moxifloxacin, Phosphotungstate.

1 INTRODUCTION

CLINDAMYCIN hydrochloride (CM.HCl) with molecular formula $C_{18}H_{33}N_2O_5S.HCl$ is a broad-spectrum antibiotic that belongs to the lincosamide group. It acts mostly as a bacteriostatic antibiotic. The most common clinical conditions in which clindamycin is used are: infections in gynecology, gingiva infections, respiratory tract, skin and soft tissue infections, intra-abdominal infections, pneumonia caused by Pneumocystis jiroveci, toxoplasmosis, malaria, babesiosis, and acne. Clindamycin is available in several pharmaceutical forms, which can be administered orally, intravenously, intramuscularly or intradermally [1]..

Moxifloxacin hydrochloride (MF.HCl) with molecular formula $C_{21}H_{24}FN_3O_4.HCl$ is antibiotic prescription medicine for the treatment of certain bacterial infections [2]. It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Moxifloxacin hydrochloride is used for treatment of sinusitis and lung infections like pneumonia and chronic bronchitis, treatment of bacterial eye infections [3]. It is used for the treatment of acute bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis, community acquired pneumonia, complicated and uncomplicated skin and skin structure infections, and complicated intra-abdominal infections [4]. The structural formulas of CM.HCl and MF.HCl are shown in Fig. 1.

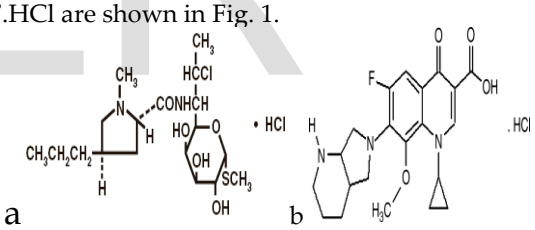

Fig. 1.The chemical structure of the investigated drugs, a. clindamycin HCl and b. moxifloxacin HCl.

Phosphotungstate anion (PT), $[PW_{24}O_{40}]^{3-}$, is a precipitating reagent has long been used in separations of the amino acids. It was used as protein precipitating to assay cholesterol and uric acid in blood and urine [5]. It also was used as an efficient and recyclable catalyst for the synthesis of polysubstituted quinolones and other phosphotungstate-incorporated metal–organic compounds [6, 7].

Several methods have been reported for the quantitative determination of clindamycin HCl and moxifloxacin HCl in pharmaceutical and biological samples, among these are high performance liquid chromatography (HPLC) [8-12] molecular spectrophotometry [13, 19] and potentiometry [20, 21]. However, most of the analytical methods employed for determination of the cited drugs are require extraction step and/or involve expensive and sophisticated experimental set up which many ordinary laboratories cannot afford.

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Atomic absorption spectrometry (AAS) occurs in the forefront of the most sensitive and widely used analytical techniques. It is a versatile, simple sensitive, selective and high speed instrument for direct determination of metal ions, but its use in indirect determination of nonmetallic anions such as drugs is limited. However, in recent decade, it has found considerable applications for the determination of various drugs [22-26].

To the best of our knowledge, indirect AAS method has not been reported yet in the literatures to the determination of CM.HCl and MF.HCl drugs based on ion-associate precipitation of the drug with phosphotungstate reagent. Thus, the present work was made to develop a validated indirect AAS procedure for the determination of these drugs. The method was performed by precipitating CM.HCl or MF.HCl drug with an excess of sodium phosphotungstate, $Na_3[PW_{24}O_{40}]$, (NaPT) as inorganic complex reagent and measuring the tungsten content of phosphotungstate (PT) ions $[PW_{24}O_{40}]^{3-}$ in the filtrate using AAS which proved to be successful for the indirect determination of these compounds in bulk powders as well as in pharmaceutical dosage forms. Comparing the proposed method with some of the published ones reveals that this method is simple, accurate, need no prior separation steps and can be applied for determination of CM.HCl and MF.HCl drugs in commercial samples with insignificant interference from commonly used excipients.

2 MATERIALS AND METHODS

2.1 Apparatus

Atomic absorption spectrometric measurements were carried out on an atomic absorption spectrometer (NOVA 300, Germany) with nitrous oxide-acetylene flame and a tungsten hollow-cathode lamp under the following operation conditions: wavelength 255.1 nm, slit-width 0.2 nm, relative noise 1.0 and lamp current 20 mA.

The pH values of solutions were measured using a digital pH-meter (Jenway, 3505,UK). All Fig.s were carried out on Toshiba computer using Microsoft excel 2010.

2.2 Materials and Reagents

All chemicals used were of analytical reagent grade. Pure samples of CM.HCl and MF.HCl were generously supplied by some Yemeni pharmaceutical companies. Double distilled water was used throughout. Sodium phosphotungstate (NaPT) was obtained from British Drug Houses (BDH), while sodium tungstate was Aldrich product. Pharmaceutical preparations assayed were purchased from Yemeni local markets.

The following available commercial pharmaceutical preparations were analyzed:

Clinacin®, capsules (Shiba Pharma. Co. Ltd, Yemen), labeled to contain 150 mg CM.HCl per capsule.

Clinaderm®, solution, (Philadelphia Pharmaceuticals Co., Amman, Jordan) labeled to contain 10 mg clindamycin HCl per mL.

Moxival®, tablet (Global Pharma. Sana'a, Yemen) labeled to contain 400 mg moxifloxacin HCl per tablet.

Zimoxaxacin®, tablet (Zin Laboratories Ltd, Nagpur, Maharashtra State, India) labeled to contain 400 mg moxifloxacin HCl per tablet.

2.3 Preparation of Stock Solutions

Using 100-mL calibrated flasks, standard solutions containing 0.005 $mol.L^{-1}$ of each investigated drug were prepared by dissolving the accurately weighed 0.2307 g of pure clindamycin hydrochloride, (CM.HCl), in double distilled water or by dissolving 0.2190 g of pure moxifloxacin hydrochloride, (MF.HCl), in methanol.

Stock standard solution of tungsten (10,000 μg/mL) was prepared by dissolving an accurate mass 1.795 g of sodium tungstate dihydrate, $Na_2WO_4.2H_2O$, in about 20 mL of double distilled water. 10 mL of 10% (w/v) NaOH solution was added, diluted to 100 mL and the solution was stored in a polyethylene bottle till it was used for the preparation of the standard calibration curve.

A $3.0x10^{-3}$ $mol.L^{-1}$ sodium phosphotungstate (NaPT), $Na_3PW_{12}O_{40}$, solution containing (6619 μg/mL of tungsten) was prepared by dissolving an accurate mass 0.8839 g of NaPT into a 100-mL volumetric flask and the solution was stored in a plastic bottle.

More dilute working solutions were prepared daily by appropriate dilutions.

For placebo solution, synthetic mixture containing common excipients was prepared. Starch, glucose, sodium citrate and magnesium stearate were homogeneous mixed and 20 mg of the resulting mixture was transferred into 100-mL calibrated flask. Then, 20 mg of pure CM.HCl or MF.HCl drug was added and the solution was diluted to the mark with double distilled water.

Solutions for sodium hydroxide, (NaOH), hydrochloric acid, (HCl), and sodium chloride, (NaCl), were prepared and used for adjusting pH and ionic strength of the medium.

2.4 Formation and Characterization of Ion-Associate Precipitates

The base of the proposed method is the precipitation of ion-associate formed from the reaction of each studied drug with

PT reagent. Clindamycin-phosphotungstate (CM-PT) and moxifloxacin-phosphotungstate (MF-PT) ion-associates have been formed by mixing solutions containing 0.0010 M of NaPT reagent with the requisite amount of CM.HCl or MF.HCl drug. The resulting precipitates were left in contact with their mother liquor to assure complete coagulations. The precipitates were filtered, washed with double distilled water and dried at the room temperature. Portions of the obtained precipitates were subjected to CHN elemental analysis (table 1).

2.4.1 Stoichiometric Ratio

To establish the mole ratio between CM or MF drug and the PT reagent, mole ratio method of continuous variations of equimolar solutions was employed [27, 28]. The drug and the reagent were mixed in various proportions. Different amounts (1.0-6.0 mL) aliquots of 0.01 mol.L^{-1} CM.HCl or MF.HCl were added to 1.0 mL of 0.01 mol.L^{-1} NaPT standard solution in which the total volume of drug and reagent was maintained at 25 mL, (Fig.2). Each resulting mixture was shaken, left to stand, filtered and then the atomic absorbance was measured. The atomic absorbances obtained were plotted against the volumes of each drug.

2.4.2 Effect of Some Experimental Variables on Precipitation Reaction

To select the optimum conditions, the effects of some experimental variables on the solubility of each ion-associate precipitate were examined by measuring the atomic absorbance of the dissociated PT ions. For this purpose, AAS of tungsten was measured in three series of 25-mL calibrated flasks having solutions with different values of pH, ionic strength or temperature. In order to study the effect of pH, 12 calibrated flasks of buffer solutions covering the acid to alkaline range were prepared and to each flask, excess amount of ion-associate precipitate was added. The mixtures were shaken, left to stand, filtered and the atomic absorbance of tungsten in PT ions was measured (Fig 3). The same way was followed to investigate the effect of ionic strength, where NaCl was used to prepare solutions containing different ionic strengths (Fig. 4). The effect of temperature (Fig. 5) was investigated by heating the ion-associate precipitates in a water bath and the atomic absorbance was measured at different temperatures.

2.4.3 Solubility of Ion-Associate Precipitates

The solid ion-associate was added in excess to a solution of the optimum pH and ionic strength. The solution was shaken and left to stand for several days to attain equilibrium, then the saturated solution was filtered into a dry beaker (rejecting the first few mL of the filtrate) and the amount of tungsten complex ion released was measured by standard additions method, in which four equal portions of the filtrate were taken. To each portion, a certain amount of tungsten standard solution was added and the atomic absorbance was measured to evaluate the concentration of the tungsten complex ions and hence the solubility and the solubility product constant (K_{sp}) of each ion associate were calculated.

2.5 General Procedure

For analysis of CM.HCl and MF.HCl drugs, different aliquots (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 6.5 mL) of each standard drug solution (0.005 M) were quantitatively transferred into a series of 25-mL calibrated flasks, to get ($2.0x10^{-4}$–$1.3x10^{-3}$ M). To each flask, 4.0 mL of 0.003 mol.L^{-1} PT solution was added, and made up to the mark with the optimum pH and ionic strength solution. The solutions were shaken well, left to stand for 20 minutes and then the mixture was filtered through Whatman no 42 filter paper. The filtrate solutions were aspirated into a nitrous oxide/acetylene flame and the atomic absorbance signals were measured at 255.1 nm, against reagent blank solution prepared in the same way without the drug. The unreacted PT ion concentration was determined from the relevant calibration curve. The PT consumed in ion-associate formation, which equivalent to the drug, was calculated by the subtraction, and thus the concentration of the drug was indirectly determined.

2.6 Preparation of Pharmaceutical Samples

Certain amounts of tablets, (from each dosage form), were weighed, crushed in a clean agate mortar, powdered and triturated well. For capsules, the contents of 10 capsules were emptied as completely as possible, accurately weighed and mixed. Accurately weighed amount of fine powder containing 230.7 mg of CM.HCl or 219.0 mg of MF.HCl was dissolved in sufficient double distilled water with continuous shaking, then the content was filtered and the filtrate was transferred into 100-mL volumetric flask and diluted to the mark to obtain standard solutions of $5x10^{-3}$ mol.L^{-1} of each drug. For analysis of solution, certain volumes of pharmaceutical liquids were pipetted into 100-ml calibrated flasks and completed to the marks by double distilled water. Aliquots of these pharmaceutical solutions were quantitatively transferred into 25-mL calibrated flasks and then the general procedure was followed. Blank samples, containing all of the reagents in the same volumes, were prepared and analysed by the procedure.

3 RESULTS AND DISCUSSION

3.1 Reaction and Stoichiometric Ratio

Both (CM.HCl) and (CM.HCl) reacted with NaPT and gave coagulated precipitates which form the basis of the indirect determination of the studied drugs.

The molar ratio of each formed ion-associate was experimentally determined according to the procedure previously described. Fig. 2 depict the variation of atomic absorbance with the addition of varying volumes of (1.0×10^{-2} mol.L^{-1}) CM.HCl

or MF.HCl to a fixed volume (1.0 mL) of (1.0×10^{-2} mol.L^{-1}) NaPT solution in which the concentration of PT was kept constant and that of the drug was varied.

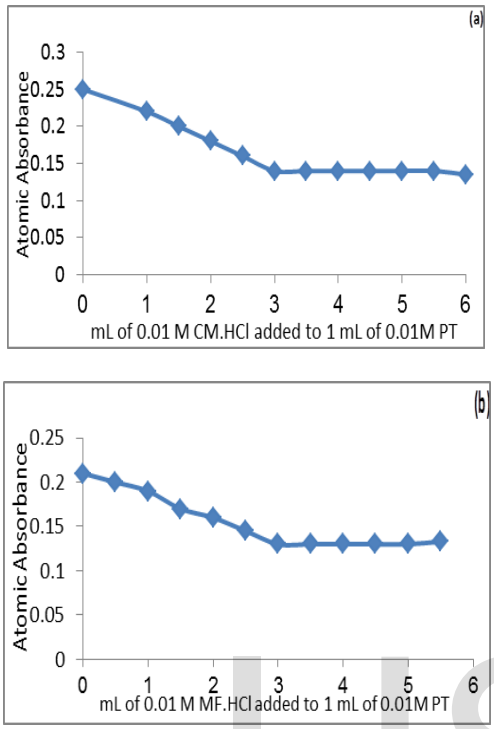

Fig..2. Mole ratio for the reaction of CM.HCl (a) and MF.HCl (b) with phosphotungstate

The results of molar ratio and CHN analysis (Table 1) showed that one mole of $[PW_{24}O_{40}]^{3-}$ anion react with 3 moles of CM or MF cation to form one mole of ion-associate.

The proposed precipitation reactions suggest that the drugs and the reagent are first dissociated into their corresponding ions and the drug-reagent ion-associate precipitates are thereafter formed by electrostatic interaction between CM or MF drug cation and PT reagent anion.

The net reactions occurring are as follows:

Three moles of CM.HCl or MF.HCl reacted with one mole of sodium phosphotungstate to form sodium hydrochloride and clindamycin–phosphotungstate or moxifloxacin–phosphotungstate

$3CM.HCl$ or $3MF.HCl$ drug + NaPT reagent → (CM-PT) or

MF-PT ion-associate +3NaCl

$$3C_{18}H_{33}N_2O_5S.HCl_{(aq)} + Na_3PW_{24}O_{40(aq)} \rightarrow (C_{18}H_{33}N_2O_5S.H)_3{-}$$

$$PW_{24}O_{40(s)} + 3NaCl_{(aq)}$$

$$3C_{21}H_{24}FN_3O_4.HCl_{(aq)} + Na_3PW_{24}O_{40(aq)} \rightarrow (C_{21}H_{24}FN_3O_4.H)_3{-}$$

$$PW_{24}O_{40\,(s)} + NaCl_{(aq)}$$

The solubility values of 6.44×10^{-5} M, 6.60×10^{-5} M and the solubility product constant values (K_{sp}) of 4.65×10^{-16}, 5.13×10^{-16} were obtained for CM-PT and MF-PT ion-associate, respectively, indicating relatively good stability complexes.

Studying the actual composition of these formed ion-associate precipitates was performed using CHN analysis. The %C, %H and %N calculated and those measured for each ion associate are listed in Table 1, which also affirmed that CM and MF form 3:1 (drug-reagent) ion-associate with PT.

TABLE 1
ELEMENTAL ANALYSIS RESULTS OF ION-ASSOCIATE PRECIPITATES COMPOSITION.

	Ion-Associate Precipitate			
	CM-PT		MF-PT	
Element	Measured%	Calculated%	Measured %	Calculated%
C	15. 64	15. 57	18.23	18.48
H	2, 51	2.47	1.82	1.85
N	2.06	2.02	3.06	3.08

3.2 Optimization of Reaction Conditions

In order to establish the best conditions for the formation of the ion-associate precipitates and for the atomic absorbance measurements, the procedure was optimized by an invariable method (keeping all variables constant except one). After comparing the atomic absorbance at different values of pH, ionic strength and temperature, it was found that acid media has a solubilizing effect on the ion-associate precipitates leading to dissociate PT and subsequently high atomic absorbance of tungsten. The optimum pH, for both ion-associates, was found to be at near neutral and low alkali media. Respecting to the ionic strength effect, 0.58 mol.L^{-1} and 0.71mol.L^{-1} of NaCl concentrations were found to be the high optimum values for the least solubility of the CM-PT and MF-PT ion-associate, respectively. Higher salt concentrations showed relatively solubilizing effect. Regarding the temperature effect, the temperature range of about (20-60 °C) was found to be negligible effect. Higher temperature showed relative solubilizing effect on the ion-associate precipitates producing higher results for the atomic absorption measurements.

The optimum conditions are those in which the ion-associate precipitate exhibited the lowest solubility. This means that the formed ion-associates were more stable and least soluble at pH=6.5-9.1 (Fig. 3), lower than 0.58 mol.L^{-1} ionic strength (Fig. 4) and there was no significant effect of temperature within the range of 20-60 °C (Fig. 5).

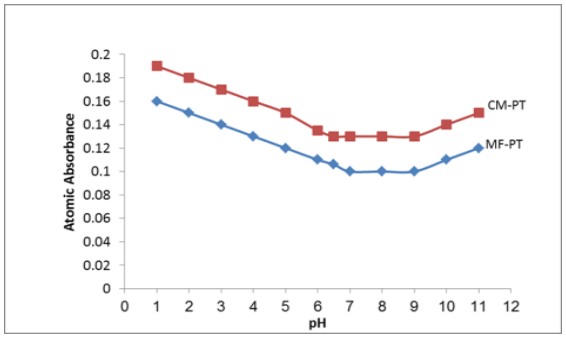

Fig.3. Effect of pH on the solubility of the precipitated ion-associates.

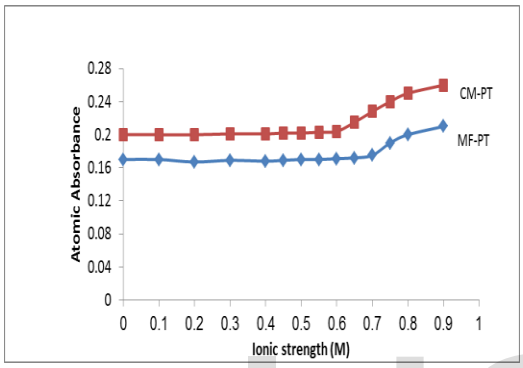

Fig. 4. Effect of molarity of NaCl on the solubility of the precipitated ion-associates.

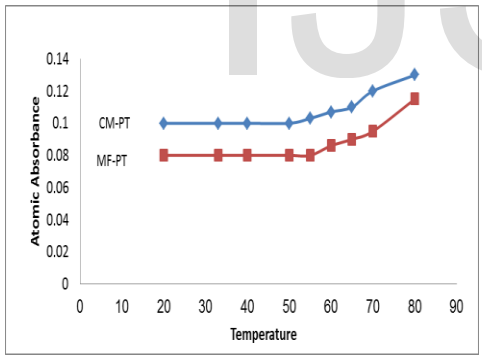

Fig. 5. Effect of temperature on the solubility of the formed ion-associates.

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To study the effect of the reagent concentration, different amount of 0.003 M NaPT reagent were added to different concentrations of CM.HCl or MF.HCl drug and the atomic absorbances were measured to get the volume of the reagent that gave the optimal absorption. Hence, 4.0 mL of standard 0.003 M NaPT per 25 mL of the reaction mixture was employed as the excess amount of reagent solution for quantitative precipitation of the studied drugs.

3.3 Calibration Curve of AAS

A standard calibration curve was constructed by plotting the atomic absorbance of tungsten at 255.1 nm versus the tungsten concentration (μg/mL) and linear equation for the standard curve was calculated by linear regression which was used to estimate the PT concentration and subsequently the concentration of the drugs. Spectral data and analytical conditions for the AAS measurements are listed in Table 2

TABLE 2
SPECTRAL DATA AND ANALYTICAL CONDITIONS FOR THE AAS MEASUREMENTS OF TUNGSTEN

Expression (Unit)	Quantity
Wavelength, λ (nm)	255.1
slit-width (nm)	0.2
lamp current (mA)	20
pH	6.5-9.1
Ionic strength (mol.L^{-1})	0.58 , 0.71
Temperature (°C)	20-60
*Range concentration of W (μg/mL)	100-1000
**Regression equation	A = 0.0003c + 0.0847

Calculated in the basis of tungsten molar weight

** The absorbance-concentration curve of the tungsten*

A is the atomic absorbance and c is the concentration in (μg/mL)

3.4 Indirect AAS Determination of Drugs in Pure Samples

As described in experimental section, CM.HCl and MF.HCl drugs have been indirectly determined depending on the relation between their concentrations and the atomic absorption measurements obtained for the unreacted PT reagent. The obtained stoichiometric results demonstrated that three moles of CM.HCl or MF.HCl drug reacted with one mole of PT reagent. The standard PT reagent was added in excess to that required to react with each drug and the excess reagent in filtrate was aspirated through the nitrous oxide-acetylene flame to measure the atomic absorbance of tungsten of unreacted PT and then the concentration of PT reacted, which corresponded to the concentration of the investigated drug was calculated as the total PT added minus the PT measured by AAS. Finally, the molar masses (461.4 and 437.9 g.mol^{-1}) of CM.HCl and MF.HCl drugs respectively were used to calculate the concentration of each drug as μg/mL.

Molar concentration of the drug = The total PT reagent added

– The unreacted PT measured by AAS.

μg/mL of the drug = (Molar concentration)(molecular mass)(1000)

TABLE 3
QUANTITATIVE DETERMINATION OF CM.HCL AND MF.HCL IN PURE SAMPLES

Studied drugs	Taken (μg/mL)	[a] Found±SD (μg/mL)	[b] RSD	[c] Er (%)	Recovery (%)
CM.HCl	92.28	91.37 ±1.59	1.72	0.99	99.01
	184.56	182.98±2.53	1.37	0.86	99.14
	276.84	274.76±3.96	1.43	0.75	99.25
	369.12	366.24±3.47	0.94	0.78	99.22
	461.40	460.20±3.97	0.86	0.26	99.74
	553.68	548.53±8.19	1.48	0.93	99.07
	599.82	593.28±9.66	1.61	1.09	98.91
MF.HCl	87.58	86.74±1.47	1.68	0.96	99.04
	175.16	173.97±2.05	1.17	0.68	99.32
	262.74	261.08±2.44	0.93	0.83	99.37
	350.32	348.67±2.73	0.78	0.47	99.53
	437.90	435.10 ±3.90	0.89	0.64	99.36
	525.48	519.86 ±6.73	1.28	1.07	98.93
	569.27	562.67±8.65	1.52	1.16	98.84

[a] *Mean±standard deviation estimated from five replicate determinations.*

[b] *RSD Percentage relative standard deviation.*

[c] *Er Percentage relative error.*

In pure samples, both drugs were precisely and accurately determined over the linear concentration ranges 92.28-599.82 μg/mL for CM.HCl and 87.58-569.27 μg/mL for MF.HCl with relative errors (Er%) in the range of 0.26-1.09% for CM.HCl and 0.47-1.16%, for MF.HCl. The relative standard deviations (RSD) were in ranges of 0.86-1.72 and 0.78-1.68 for CM.HCl and MF.HCl respectively, (Table 3).

The assay results obtained from the proposed method were statistically compared with those obtained from the official [2] and reported methods [12] using t- and F- tests.

3.5 Method Validation

The validation assays for the proposed method was performed with regard to accuracy, precision, linearity, detection limit and quantification limit.

3.5.1 Accuracy and Precision

The accuracy of the proposed method was checked by performing relative error and percentage recovery experiments at seven concentration levels covering wide range of each drug in the pure samples. The values of percentage recovery and relative error, shown in Table 3, indicate the good accuracy. In order to the precision of the method, the repeatability of the results was assessed through analysis of the studied drugs in pure solutions and in pharmaceutical preparations by calculating standard deviation (SD) of five replicate determinations. The low values of relative standard deviation (RSD) reflect the precision of the method (Table 3).

Intra- and inter-day precisions were assessed using three concentrations of standard drug solutions and RSD values were obtained within the same day to evaluate repeatability (inter-day precision) and over three consecutive days to evaluate intermediate precision (intra-day precision). The RSD calculated for intra and inter day were found to be not significantly different. The results obtained from the proposed method were also statistically compared with those obtained from the official and reported methods using student's (t-test) for accuracy and variance ratio (F-test) for precision. The calculated F-value and t-test at 95% confidence level (Table 4) were lower than the tabulated values for F- and t-test. This means that the present indirect AAS method is comparable and there are no significant differences between the proposed method and the official or reported method.

TABLE 4
STATISTICAL EVALUATION AVERAGES FOR DETERMINATION OF CM.HCL AND MF.HCL

Parameters	CM.HCl	MF.HCl
[a] Range (µg/mL)	92.28-599.82	87.58-569.27
[b] Taken-Found Regression equation	y=0.9896x+0.0513	y=0.9905x+0.06
Slope (a)	0.9896	0.9905
Intercept (b)	0.0513	0.06
Correlation Coefficient (R^2)	0.9999	0.9999
Standard deviation, SD (µg/mL)	4.77	4.00
Relative standard deviation RSD (%)	2.02	1.82
Relative error Er (%)	1.33	1.39
F-value (9.61)[C]	2.73	2.48
t-value (2.776 [C]	1.24	0.96
Detection limit DL (µg/mL)	6.12	5.50
Quantitation limit QL (µg/mL)	20.41	18.37

[a] *Calculated in the basis of the drug molar weight*

[b] *y= ax+b where y and x are the taken and found concentrations of drug, respectively*

[c] *Tabulated F-value and t-value at the 95% confidence level and for four degrees of freedom.*

3.5.2 Linearity and Range

To check the linearity, standard solution of tungsten, (100 to 1000 µg/mL), were measured and the graph between concentration and absorbance was plotted. Each measurement was performed five times to check the reproducibility. The obtained calibration graph is straight line and the correlation coefficient is found to be 0.9998 (Fig. 6).

Under the experimental conditions, standard calibration curve was applied for the determination of the studied drugs based on indirect AAS measurements. The observed atomic absorbance readings for all of the solutions were within the linear portion of the curve.

Calibration curves between µg/mL taken and µg/mL found concentrations for each drug were also constructed and the linear relationships were obtained. Taken-found linear regression equations and some other statistical treatments of the results obtained are listed in Table 4, indicating the excellent linearity with high correlation coefficients.

3.5.3 Detection Limit and Quantitation Limit

Sensitivity of the method could be determined, through the detection limit (DL) and quantification limit (QL). They were calculated using the following equations: DL=3.3×σ/s, QL=10.0×σ/s. Where σ is the standard deviation of replicate determination values and s is the slope of the calibration graph. Based on these equations and according to Internation al Conference on Harmonization (ICH) [29], the detection limits (6.12, 5.50) and the quantification limits (20.41, 18.37) were obtained for CM.HCl and MF.HCl drugs, respectively (Table 4).

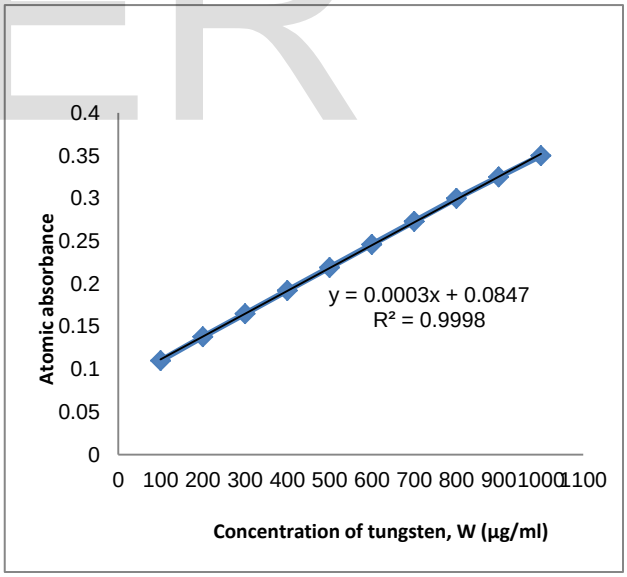

Fig. 6. Standard calibration curve for determination of tungsten by AAS.

3.5 Selectivity

Since the formation of each drug-reagent ion-associate requires the existence of positively and negatively charged species, the presence of commonly encountered additives and

TABLE 5
QUANTITATIVE DETERMINATION OF CM.HCL AND MF.CL IN PHARMACEUTICAL FORMULATIONS

Analyte	Sample	Taken (μg/100mL)	[a] Found±SD (μg/100mL)	[b] RSD (%)	[c] Er (%)	Recovery (%)
CM.HCl	Clinacin® (150 mg/capsule)	92.28	90.77±2.54	2.75	1.64	98.36
		184.56	181.74±4.17	2.26	1.53	98.47
		276.84	273.52±6.40	2.31	1.20	98.80
		369.12	362.88±9.67	2.62	1.69	98.31
		461.40	450.56±11.72	2.54	2.35	97.65
	Clinaderm (10mg/mL)	92.28	90.19±2.87	3.11	2.26	97.74
		184.56	181.20±5.02	2.72	1.82	98.18
		276.84	272.88±7.06	2.55	1.43	98.57
		369.12	361.11±11.44	3.10	2.17	97.83
		461.40	450.74±13.70	2.97	2.31	97.69
MF.HCl	Moxival® (400 mg/tablet)	87.58	86.19±2.49	2.84	1.59	98.41
		175.16	172.81±4.52	2.58	1.34	98.66
		262.74	259.95±5.12	1.95	1.06	98.94
		350.32	342.26±6.52	1.86	2.30	97.70
		437.90	424.89±9.50	2.17	2.97	97.03
	Zimoxaxacin® (400 mg/tablet)	87.58	85.99±2.61	2.98	1.81	98.19
		175.16	172.30±4.90	2.80	1.63	98.37
		262.74	258.93±6.07	2.31	1.45	98.55
		350.32	342.23±8.83	2.52	2.31	97.69
		437.90	425.24±11.78	2.69	2.89	97.11

[a] *Mean±standard deviation estimated from five replicate determinations.*

[b] *Percentage relative standard deviation.*

[c] *Percentage relative error.*

excipients added to the pharmaceutical dosage forms maybe interfered with the studied drugs. Therefore before dealing with the analysis of the pharmaceutical preparations, the selectivity of the proposed procedure was experimentally investigated by the analysis of prepared standard solutions of CM.HCl or MF.HCl in the absence and in the presence of varying amounts of the commonly used excipients including (starch, glucose, sodium citrate and magnesium stearate). The results obtained indicated that there was no interference from the ingredients and fillers used in the most drug products. Thus, no extraction was needed to separate the desired compounds from the drug matrices and the proposed method is selective enough for analysis both studied drugs in their pharmaceutical dosage forms.

3.6 Application of The Method to Analysis of Pharmaceutical Forms

The proposed method was successfully applied to the determination of CM.HCl and MF.HCl in their commercial pharmaceutical tablets, capsules and solutions by using direct calibration procedure. The concentration ranges, the relative errors, Er, and the relative standard deviations, RSD, obtained for each pharmaceutical dosage form are listed in Table 5. The accuracy was assessed by investigating the errors for five concentration levels with five replicates of each concentration. The results attained by the proposed method indicated relatively wide concentration ranges and good accuracy. The values of percentage relative standard deviation of the proposed indirect AAS method were found to be not exceeding 3.11%, indicating acceptable level of the precision (Table 5). The analysis of tablets, capsules and solutions samples is also evident that the results were unaffected by the presence of excipients as shown by the satisfactory levels of accuracy obtained. Statistical analysis of the results obtained (Table 4) indicated that the proposed procedure was as accurate and precise as the reported methods.

4 CONCLUSION

This paper reported a new example of indirect atomic absorption spectrometric method application in drugs analysis. The method was developed for the determination of CM.HCl and MF.HCl drugs based on formation of an ion-associate precipitate between each drug and PT reagent. The factors affecting on the ion-associate precipitates were studied and the conditions were optimized. The molar ratio and the solubility of each formed ion-associate were determined. The proposed indirect AAS was successfully applied for determination of the investigated drugs either in their pure forms or in their corresponding pharmaceutical preparations without interference from commonly used excipients. Student's t-test and F-test indicated the absence of systematic errors and no significant differences between the proposed and official methods.

REFERENCES

[1] The International Pharmacopoeia, Clindamycin hydrochloride, Ninth Edition, 2019

[2] British Pharmacopoeia, Her Majesty Stationery Officer, London, UK, 2013.

[3] R. Singh, K.R. Ledesma, K. Chang, J. Hou, R.A. Prince, and V.H. Tam, Pharmacodynamics of Moxifloxacin Against a High Inoculum of Escherichia Coli in an in Vitro Infection Model," J. Antimicrobial Chemotherapy, *Vol.64*,, pp 556-562, 2009.

[4] R.K. Gunda, J.N. kumar, Formulation Development and Evaluation of Moxifloxacin HCl Fast Dissolving Tablets", Pharm Methods, 8(2) 160-167, 2017.

[5] R.E. Watts, "A Simplified Alkaline Phosphotungstate Assay for Uric Acid in Serum, Ann. din. Biochem. 11, 103-111, 1994.

[6] M. Dabiri, S. Bashiribod, "Phosphotungstic Acid: An Efficient, Cost-effective and Recyclable Catalyst for the Synthesis of Polysubstituted Quinolines," Molecules, 14, 1126-1133, 2009.

[7] Q. Han, L. Zhang, C. He, J. Niu, C. Duan, Metal–Organic Frameworks with Phosphotungstate Incorporated for Hydrolytic Cleavage of a DNA-Model Phosphodiester, Inorg. Chem., 51, 5118–5127, 2012.

[8] M. Mifsud, J. Vella, V. Ferrito, "A simple HPLC method for the determination of clindamycin in human plasma," J. Chem. and Pharm. Res., 6(1), 696-704, 2014.

[9] L. Rajendar, N.R. Potnuri, and N. Rao, "A stability Indicating RP-HPLC Method for the Simultaneous Estimation of Metronidazole, Clindamycin and Clotrimazole in Bulk and Their Combined Dosage Form," World J. Pharm. Sci, 3(1), 93-103, 2015.

[10] B.M. Prakash, and J.S. Nehal, "Novel Stability- Indicating RP-HPLC Method for the Simultaneous Estimation of Clindamycin Phosphate and Adapalene along with Preservatives in Topical Gel Formulations," Sci. Pharm., 82, 799–813, 2014.

[11] S. Ashour, "New, Simple and Validated RP-HPLC Method for Quality Control of Moxifloxacin," SOJ Pharmacy and Pharmaceutical Sciences 3,3, 1-6, 2016.

[12] M.V. Priya, P. Madhavan and P. Kumar, "A validated RP- HPLC Method for the Analysis of Moxifloxacin Hydrochloride in Pharmaceutical Dosage Forms and Stability Studies," J. Chem. Pharma. Res., 7(5), 836-841, 2015.

[13] A.I. Mohamed, A.M. Abd-Motagaly, O.A. Ahmed, S. Amin and A.I.M. Ali, "Investigation of Drug–Polymer Compatibility Using Chemometric-Assisted UV-Spectrophotometry," Pharmaceutics, 9, 7, 1-13, 2017.

[14] M. Dedic, E. Becic, B. Imamovic, and N. Ziga, "Determination of Clindamycin Hydrochloride Content in 1% Clindamycin Lotion," Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 50, 49-54, 2018.

[15] S. Ashour, "Rapid Spectrophotometric Methods for the Determination of Moxifloxacin and Sildenafil in Pharmaceutical Preparations Based on Reaction with 4-Aminoantipyrine," Acta Scientific Pharmaceutical Sciences, 4,4, 02-10, 2020.

[16] K.A.M. Attia, M.W.I. Nassar, M.A. Hasan, "A Comparative Study of Different Approaches for Stability- Indicating Determination of Moxifloxacin Hydrochloride in Presence of Its Acidic-Induced Degradation Product," Int. J. Pharm. & Pharm. Res., 6 (4), 255-280, 2016.

[17] S. Ashour, R. Bayram. "Development and Validation of Sensitive Kinetic Spectrophotometric Method for the Determination of Moxifloxacin Antibiotic in Pure and Commercial Tablets," Spectrochimica Acta A., 140, 216-222, 2015.

[18] R.M.W. Kazan, H. Seddik, M. Aboudane, "Development and Validation of Spectrophotometric Method for Determination of Moxifloxacin HCL in Bulk and Pharmaceutical Formulations," International Journal of Academic Scientific Research, Volume 5, Issue 2, 14-24, 2017.

[19] S. Nayak, U. Srinivasa, K.S. Kamath, and A.R. Shabaraya, "UV Spectroscopic Analysis of Moxifloxacin HCl in Simulated Tear Fluid. Int. J. Universal. Pharmacy. Bio. Sci.4(5), 11-15, 2015.

[20] Q. Zhou, N. Long, L. Liu L, H. Zhai and M. Zhu, "Electrochemical Determination of Moxifloxacin Hydrochloride Based on Molecularly Imprinted Polymer Modified Carbon Paste Electrode," Int. J. Electrochem. Sci., 10, 5069-5076, 2015.

[21] M.S. Rizk, H.A. Merey, S.M.Tawakkol and M.N. Sweilam, "New Potentiometric Determination of Clindamycin Hydrochloride in Pharmaceuticals," Research and Review Electrochemistry (RREC), 4(5), 161-167, 2013.

[22] M.M. Baraka, M.E. El-Sadek, L.M. Abdel-Aziz, S.S. El-bermawi", Spectrophotometric Determination of Omeprazole Sodium and Ranitidine Hydrochloride Using 2,4-Dinitrofluorobenzene and 4-chloro-7-nitrobenzen-2-oxa-1,3-diazole Accompanied With a Kinetic Study," American J. Curr. Anal. Chem. 1,1-23, 2014.

[23] P. Venkatehswarlu, B. Gouthami, "An Overview of Recent Applications of Atomic Absorption Spectroscopy in Determination of Inorganic Impurities in Drugs and, Plants and its Extracts", International Journal of Basic and Applied Sciences, 1,1, 19-27, 2015.

[24] S. Dadfarnia, A.M. Hajishabani, H.F. Rad, "Indirect Determination of Sulfadiazine by Cloud Point Extraction/Flow Injection-Flame Atomic Absorption Spectrometry", J. Chinese Chem. Soc,. 58, 503-508, 2011.

[25] M.K. Hammood, "An Indirect Atomic Absorption Spectrophotometric Determination of Cefotaxime in Pharmaceutical Formulations by Using Rhodium(II) as a Mediating Metal," National J. Chem. 41, 27-37, 2011.

[26] L. Bruno, F.L. Vitalib, E.S. Chaves, "Exploring the Versatility of an Atomic Absorption Spectrometer: Application to Direct Molecular Determination of Caffeine and Propranolol", J. Braz. Chem. Soc. 27, 4, 794-798, 2016.

[27] P. Jop, Method for finding the formula of a complex. Ann Chim. 9, 113-203, 1928.

[28] J. Rose, Advanced Physico-chemical Experimental. Pittman, London, 67, 1964.

[29] Validation of analytical procedures: text and methodology, in International Conference on Harmonization (ICH), Q2 (R1), IFPMA, Geneva, Switzerland, November, 2005.