GOVERNMENT DEGREE COLLEGE, HAYATHNAGAR

Jignaasa Student Study Project Report
On

SIMPLE, PRECISE AND LOW-COST QUANTITATIVE DETERMINATION OF PRESCRIPTION DRUGS BY CONDUCTOMETRY

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DECLARATION

We hereby declare that the study project entitled "SIMPLE, PRECISE AND LOW-COST QUANTITATIVE DETERMINATION OF PRESCRIPTION DRUGS BY CONDUCTOMETRY" *is submitted by us is original project work and it has been carried out under the supervision and guidance of Dr. B. Rajitha, Asst. Prof. of Chemistry, GDC, Hayathnagar.*

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Place: Hayathnagar

Date: 20-11-2019

Acknowledgements

We would like to express our sincere thanks to Dr. B. Rajitha, Asst. Prof. of Chemistry for her vital support, guidance and encouragement, without which, this project would not have come forth. We would also express our thanks to Sri. D. Venkateshwar Rao, Asst. Prof. of Chemistry and Dr. P. Upendra, Asst. Prof. of Chemistry for helpful discussions for making this project and we take this opportunity to thank Sri. Dr. J. Rajaram, Principal of our college for vital support and encouragement.

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CERTIFICATE

This is to certify that the study project titled "SIMPLE, PRECISE AND LOW-COST QUANTITATIVE DETERMINATION OF PRESCRIPTION DRUGS BY CONDUCTOMETRY" submitted is a bonafide project done by

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ABSTRACT

Simple, accurate and low cost conductometric methods for the quantification of prescription drugs in pure form and pharmaceutical formulations using Silver Nitrate as titrant has been developed and few drugs viz., Ciprofloxacin HCl (CIP), Ranitidine HCl (RTD) and Cetrizine di HCl (CTZ) has been studied by this method. The method is based on the formation of ion association complex between the ions present in cited drugs and titrant. Quantitative precipitation titrations are carried out by using conductometry where the solution conductance varies with addition of titrant before and after the equivalence point, so that end point can be detected by drawing the two intersecting lines. Calibration curves were constructed by using this end point. These calibration curves were used to validate the methods in terms of Limit of detection, Limit of quantification, Precision and Accuracy as per ICH guidelines. Factors affecting the conductance *viz.,* concentration of $AgNO_3$, temperature and time of reaction are optimized. The effect of excipients has also been studied and found no effect. Excellent **% Recovery values** and **Recovery standard deviation** (RSD) values ***less than 2*** indicates that these developed methods are accurate, precise and can be applied for the quantification of pure drugs and prescription drugs in the pharmaceutical industries.

Key words: Quantification, Conductometry, $AgNO_3$ solution, Drugs.

1. Aims and Objectives: To develop simple, accurate, sensitive and low-cost quantifying techniques by using conductometry for the estimation of drugs in pure form and pharmaceutical formulations (prescription drugs) using Silver Nitrate as titrant. Validation of the developed methods as per ICH guidelines and find suitability of the methods for the industries.

2. INTRODUCTION:

2.1 DRUGS: DEFINITION AND CLASSIFICATION

The French term '*drogue'* (dry herb) is the origin of the word 'drug'. No authority defined the 'drug' completely and comprehensively but it is defined in different ways by different authorities.

According to WHO a drug is any substance or product that is intended to be used to modify physiological systems or pathological states for the benefit of recipient [1] or a drug is any chemical compound that may be used on or administered to humans to help diagnose, treat, cure, mitigate or prevent disease or other abnormal conditions.

It may also be defined as a substance used in the prevention, cure or alleviation of disease or pain or as an aid in some diagnostic procedures.

Drugs can be classified in different ways on the basic of their origin, chemical structure and therapeutic action [2].

Each classification system will have its advantages and limitations and its usefulness will depend on the purpose.

2.1.1 Classification of drugs based on their origin:

i) Drug from natural origin: Herbal or plant or mineral origin, some drug substances are of marine origin.
ii) Drug from chemical as well as natural origin: Derived from partial herbal and partial chemical synthesis Chemical, example steroidal drugs

iii) Drug derived from chemical syntesis
iv) Drug derived from animal origin: For example, hormones, and enzymes.
v) Drug derived from microbial origin: Antibiotics
vi) Drug derived by biotechnology genetic-engineering, hybridoma technique
vii) Drug derived from radioactive substances.

3.1.2. Classification of drugs based on their chemical structure

The drugs are classified into several groups such as alcohols, amines, glycosides, sulphones etc., based on the functional groups available in the drug molecule.

2.1.3 Classification of drugs based on their therapeutic action:

The drugs classified into several groups depending on their therapeutic action.

i) Cardiovascular drugs
ii) Musculo-skeletal disorders (Muscle relaxants)
iii) Antibiotics
iv) Drugs acting on Central nervous system
v) Drugs on alimentary system
vi) Drugs for genito-urinary tract
vii) Drugs for respiratory system and anti-allergics

2.2 SCOPE OF THE PROJECT:

- Pharmaceutical analysis is an important subject for the quality control of chemicals, bulk drugs and pharmaceuticals.
- This is due to the fact that bulk drugs and pharmaceuticals of maximum purity are essential for the safeguard of the health of human beings.

2.3 : PHARMACEUTICAL ANALYSIS

- Pharmaceutical Analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals.
- The term "Pharmaceutical analysis" is otherwise called quantitative pharmaceutical chemistry.
- Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the finished dosage forms.
- The most common pharmaceutical analysis is the quantitative measurement of the active pharmaceutical ingredient and related compounds in the pharmaceutical product.
- The purity and stability of the drugs are checked with the help of various physical, chemical and instrumental technologies.
- These techniques have been developed/ modified day by day with the aim to increase the selectivity, sensitivity and accuracy of the method.
- The new techniques have replaced the old ones in the various pharmacopoeias.

Thorough survey of literature reveals that a large number of techniques available for pharmaceutical analysis. The methods include chromatographic, spectroscopic and electro chemical methods.

Among all these methods conductometric determination is found simple, precise and accurate as well as cost effective.

2.4. Methods of Pharmaceutical analysis:

Literature survey revealed that almost all physical and chemical methods have been used for Pharmaceutical analysis. Some important methods are: [3-10]

i. Titrimetry
ii. Potentiometry
iii. Conductometry

iv. Other electro-analytical techniques
v. Fluorimetry
vi. Infra-red spectroscopy
vii. NMR spectrometry
viii. Mass spectrometry
ix. Chromatography
   a) Thin layer chromatography
   b) High performance thin-layer chromatography
   c) Gas chromatography
   d) High performance liquid chromatograph
x. Spectrophotometry
   a) Colorimetry
   b) Direct Spectrophotometry
   c) Oxidative kinetic spectrophotometry
   d) Charge transfer complexation
   e) Extractive spectrophotometry

2.5.: Overview on the validation of a method for quantification:

Parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. An attempt at harmonization was made for pharmaceutical applications through the International Conference on Harmonization ICH [11,12] where representatives from the industry and regulatory agencies from the United States, Europe and Japan defined parameters, requirements and, to some extent, methodology for analytical methods validation. The parameters, as defined by the ICH and by other organizations and authors, are summarized and are described in brief in the following paragraphs.

Possible analytical parameters for method validation

- Specificity
- Selectivity
- Precision
- Accuracy
- Linearity
- Range
- Limit of detection
- Limit of quantification
- Sandell's sensitivity
- Ruggedness

3 EXPERIMENTAL

3.1 Instrumentation:

Conductance for the study has been measured by using Digital Conductometer., model No: 9009, with K_{cell} =1.02 of Digisun electronics.

3.2 Preparation of standard stock solutions:

a) Drug solution:

For the analysis of drugs by Conductometric titrations, standard stock solutions (1mg/1mL) were prepared by dissolving 100mg of each drug in separate 100mL volumetric flasks with double distilled water. All the stock solutions were further diluted with the same solvent to attain working concentrations.

b) **Silver nitrate solution:**

A stock solution of 0.05M was prepared in a 100 mL standard flask by dissolving 50 mg of AgNO3 in double distilled water and further diluted to 0.005M to attain working concentration.

4. RESEARCH METHODOLOGY

4.1. About the method:

Conductometer computes the ionic solutions conductance by measuring the mobile ions conductance. In the process of conductometric titrations if a fast moving ion is replaced by a slow moving ion then the solution conductance decreases, if a slow moving ion is replaced by a fast moving ion then the solution conductance increases. At the end point abnormal changes take place in the conductance data during the titration, which leads to intersection of two straight lines in the graph (conductivity vs titrant volume) and this intersection point gives the end point. Acid-Base titrations are familiar examples for the titration method. Generally in titrations titrant neutralizes the reactant and gives the precipitated product, so the conductance varies slightly with each addition of measured titrant to the fixed volume of reactant depending upon the nature of reactant and titrant and end point can be determined from the two straight lines intersecting point by plotting a graph. This forms the basis for quantification of drugs and pharmaceuticals by this method [13-16].

4.2 About the drug:

a) List of drugs studied using Silver Nitrate as titrant:

1. Cetrizine di HCl (CTZ)

2. Ciprofloxacin HCl (LFX)

3. Rantidine HCl (RTD)

1. Cetrizine dihydrochloride

CTZ is a selective second generation anti histamine drug and chemically "(*RS*)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy] acetic acid dihydrochloride." – IUPAC(fig.12), CTZ causes less side effects when compared it with other first generation antihistamines due to its less ability to pass through the blood-brain barrier and became a

widely prescribed drug to treat allergic disorders like allergic rhinitis, chronic utricariaz etc.

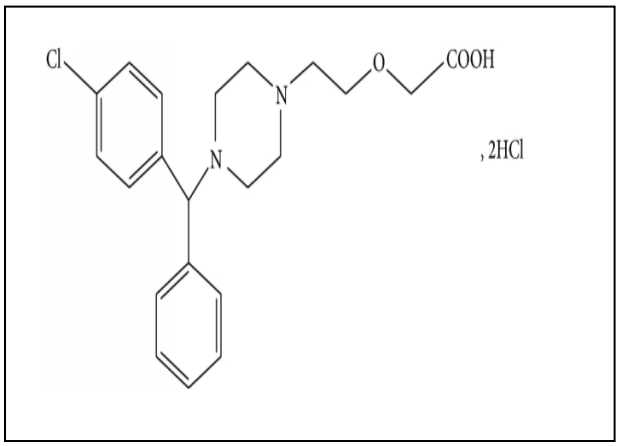

Fig 1. Structure of Cetrizine Dihydrochloride

Literature analysis confirmed that CTZ has been quantified by most of the available analytical methods in pharmaceutical preparations such as Spectrophotometry[17], HPLC[17], Spectrofluorimetry [18], Titrimetry[19], LC/MS[20] due to its significance and also acknowledged that no reports have been cited on this method of our interest for the quantification of CTZ.

2. Ciprofloxacin hydrochloride (CIP):

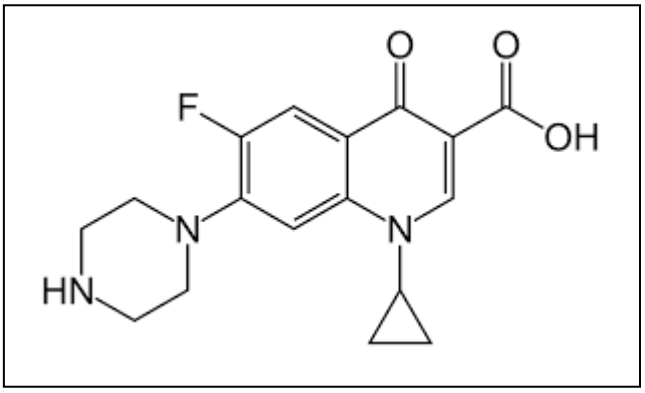

Fig 2. Structure of Ciprofloxacin HCl

Ciprofloxacin (CIP) is a second generation synthetic broad spectrum antimicrobial drug of a fluoroquinolone class. Chemically, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid [fig: 2]. The bactericidal action of CIP is a consequence from restricting the DNA gyrase and topoisomerases enzymes which are necessary for bacterial DNA recombination, transcription repair and replication [1,2]. Clinical studies has been disclosed that CIP is active against most isolates of the several bacteria both in vitro and clinical infections[21].

Due to its physiological significance various analytical methods were reported like Spectrophotometry[22], RP- HPLC [23], HPTLC[24], Spectrofluorimetry[25] and Titrimetry[26] to determine it both in dosage forms and in biological fluids. However, literature analysis disclosed that for this drug so far no article have been cited by this method of our concern.

1. **Rantidine HCl:**

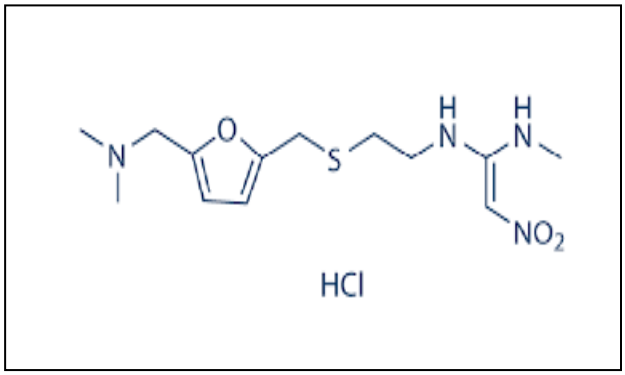

Fig 3. Structure of Ranitidine HCl

Chemically it is N, N, dimethyl-5-[2-(1-methylamine-2-nitrovinyl)ethylthiomethyl] furfurylamine hydrochloride. RTD inhibits the secretion of gastric acid, generally used as an antacid drug and also for the treatment of peptic ulcers, night heart burn due to its capacity to act as an antagonist of histamine for the H_2-receptor. [27-28]

In order to prove the amount of drug present in pharmaceutical preparations, various methods have been reported in the literature such as Spectrophotometric[29], RP-HPLC[30], HPTLC[31], Spectrofluorimetry[32] and FT-IR[33].

Table.1. Comparison of various methods used for the quantitative analysis of drugs (AMX, FBS, ITP and MBV)

1. Cetrizine di Hcl					
Method	**UV-Visible**	**RP-HPLC/HPLC**	**TLC/HPTLC**	**Fluorimetry**	**Titrimetry**
Range (µg/mL, except	8-90	5-40	40-2000	0.04-0.4	0.000005-0.1 M
Accuracy %RSD	0.95	0.44	100.19		1.100
Precision %Recovery	1.05	0.96	99.70	99.49	100.04
LOD (µg/mL,exceptTLC)	1.10	0.25	3.94	0.0027	0.000005 M
LOQ (µg/mL,exceptTLC)	3.40	0.76	13.14	0.0083	
2.Ciprofloxacin HCl					
Range (µg/mL, except	5-50	0.39 $x10^3$-50 $x10^3$	80-280 ng/spot	20-1000 ng/mL	
Accuracy %RSD	0.442	4.2	0.76	2.12	3.7
Precision %Recovery	99.42	98.7	99.61	98.5	98.4
LOD (µg/mL,exceptTLC)	0.127	0.11 $x10^3$	60 ng/spot	5 ng/mL	-
LOQ (µg/mL,exceptTLC)	0.718	0.35 $x10^3$	80 ng/spot	18 g/mL	-
3.Ranitine HCl					
Range (µg/mL, except	0.3-3.2	3-150	2-20 µg/spot	40-1200 ng/mL	-
Accuracy %RSD	0.78	0.610	0.80-0.91		-
Precision %Recovery	99.67	100.33	100.09	98.97	-
LOD (µg/mL,exceptTLC)	0.08	1.17	0.549	0.04	-
LOQ (µg/mL,exceptTLC)	0.24	3.55	1.664	0.12	-

Conducting experiment in our college lab

PROCEDURE:

4.3. Assay of pure drug sample:

Aliquots of 1-10 mL standard drug solution (each mL solution has 1mg of pure drug) i.e. 1-10mg of pure drug solution were transferred into a 50 mL standard flask and filled the flask with double distilled water upto the mark. Each solution conductivity was measured by transferring it into a beaker, immersing the conductivity cell in it and titrating against 0.005M Silver Nitrate for CTZ, CIP and RTD drug solutions by taking the titrant in a burette. For each addition of titrant to the solution conductivity was measured after stirring 2 minutes and corrected each value with the below mentioned equation for dilution effects, assuming that the conductivity is a linear function of dilution.

$$\Omega\text{-}1 \text{ correct} = \Omega\text{-}1 \text{ obs } [V1+V2/V1]$$

Where Ω-1 correct = corrected electrolytic conductivity

Ω-1 obs = observed electrolytic conductivity

V_1= initial volume of the solution

V_2= volume of reagent added

A graph was constructed between corrected conductivity Vs added titrant volume and the end point was ascertained graphically at the intersection of two lines.

The amount of drug used for this study was calculated by using this equation:

Amount of drug= V.M.R / N

Where V= volume of titrant added

M=molecular weight of the drug

R=molar concentration of the titrant

N=number of moles of titrant consumed by one mole of drug.

5.4. Optimization of the quantification parameters:

a) Effect of solvent:

Conductometric titrations were carried out by using the different solvents like acetone, ethanol, methanol and double distilled water to dissolve the drug and reagent in order to assess the suitable solvent for these experiments.

Preliminary experimental values revealed that double distilled water was the most suitable solvent which showed higher conductance and sharp end point.

b)Effect of reagent concentration:

In order to fix the optimum concentrations of the $AgNO_3$

i). Different concentrations ($1x10^{-3}$, $5x10^{-3}$ and $1x10^{-2}$) $AgNO_3$ solutions were used to titrate against fixed amount of investigated drug solution.

These results indicated that $AgNO_3$ solutions lower or higher than 0.005M concentrations were not suitable to perform titrations with these drugs of our interest as above or below of these concentrations of the reagents the conductance readings were unstable, required more time to obtain constant conductance readings and identification of end point was very poor. Hence 0.005M of $AgNO_3$ were chosen as optimum concentrations in order to get the highly stable conductance values with 2 minutes of mixing after each addition of titran and also to minimize the dilution effect on the conductance readings throughout the titration.

c) Effect of Temparature:

These titrations were carried out at room temperature. Conductance readings were increased throughout the titration with rise of temperature but no change at the end point value for a fixed amount of drug solution.

d) Linearity:

In order to assess whether the proposed method performing any fixed or proportional preference, a simple linear regression of the $AgNO_3$ volumes against observed drug concentration were calculated. Slopes of the regression lines were applied with student's t-test values at 95% confidence level and revealed that it didn't differ considerably from the ideal value of unity. The observed standard deviation (SD) values are acceptable at least for the studied concentrations.

e) Accuracy and Precision:

To evaluate the precision each experiment was repeated 3 times and accuracy is measured in terms of % recovery and % RSD. Excellent percent recovery and RSD being less than 2 for each drug exhibits accuracy and precision of the developed methods (Table 3).

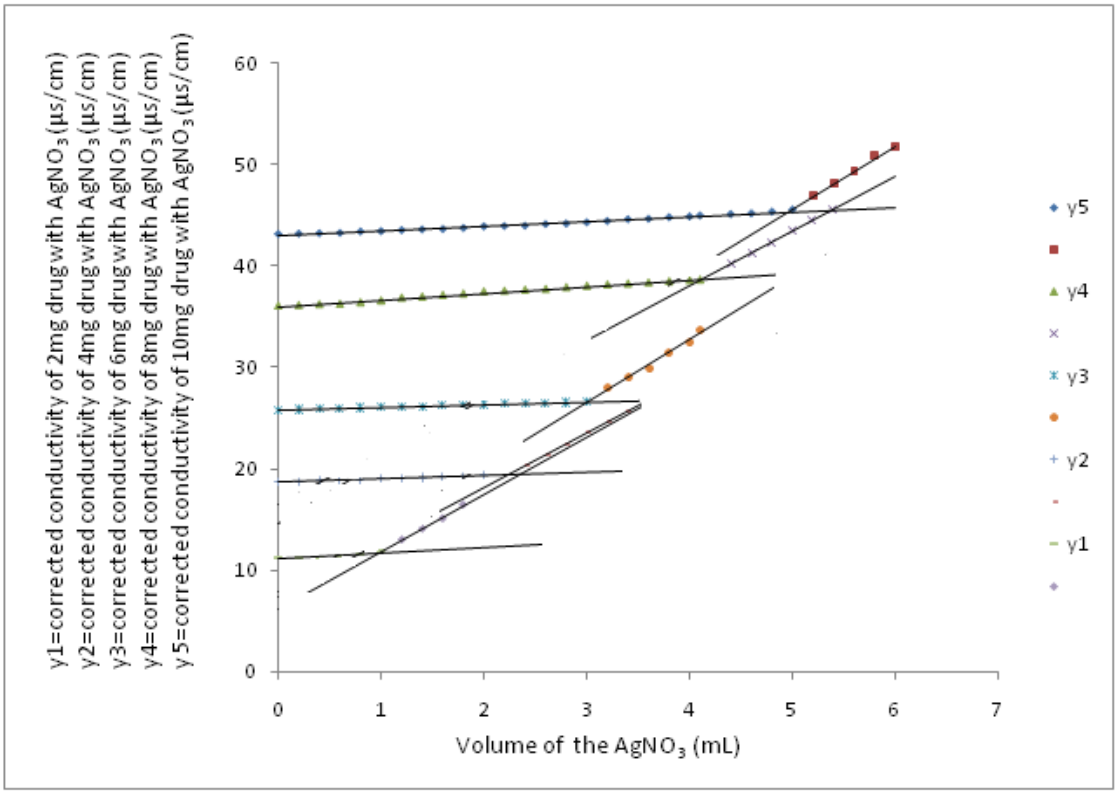

Fig 4 : Conductometric titration curves of 2mg, 4mg, 6mg, 8mg and 10 mg of Cetrizine di HCl by using Silver Nitrate as titrant

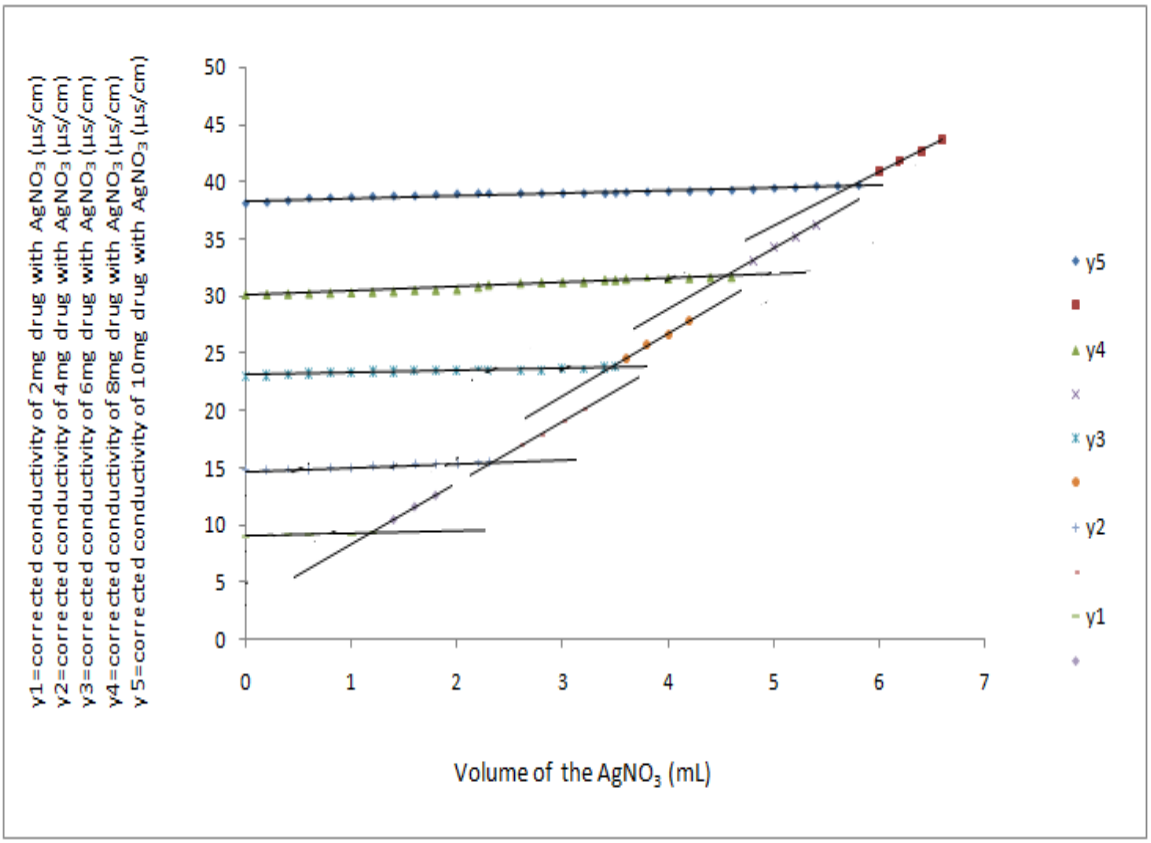

Fig 5: Conductometric titration curves of 2mg, 4mg, 6mg, 8mg and 10 mg of Ciprofloxacin HCl by using Silver Nitrate as titrant

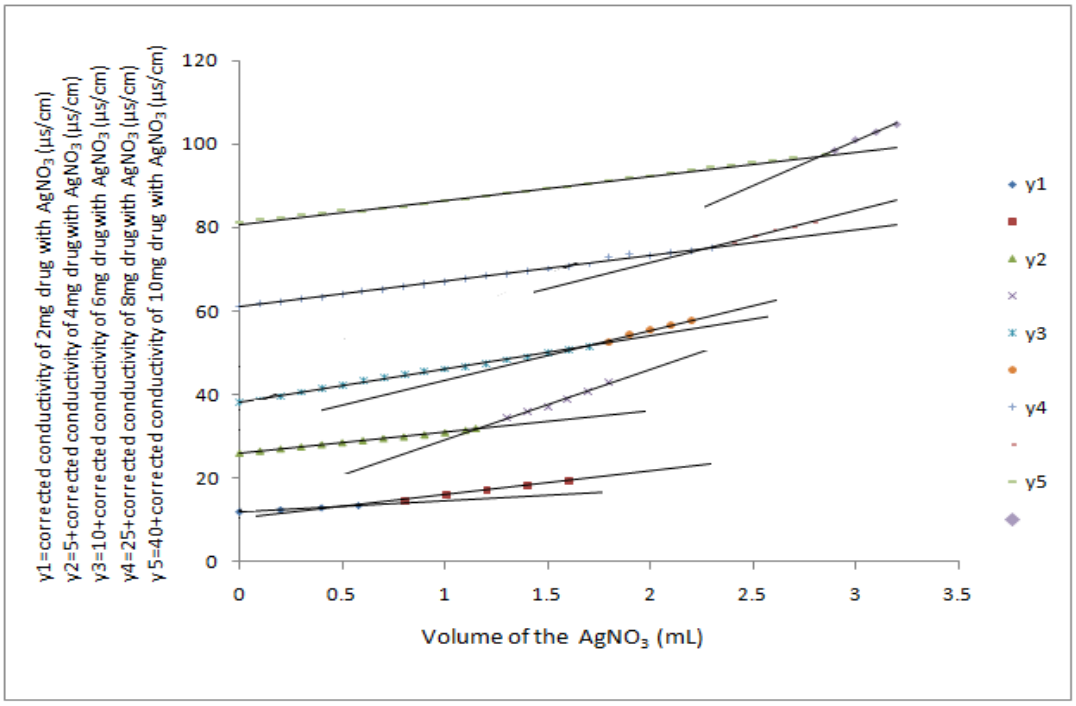

Fig 6: Conductometric titration curves of 2mg, 4mg, 6mg, 8mg and 10 mg of Ranitidine HCl by using Silver Nitrate as titrant

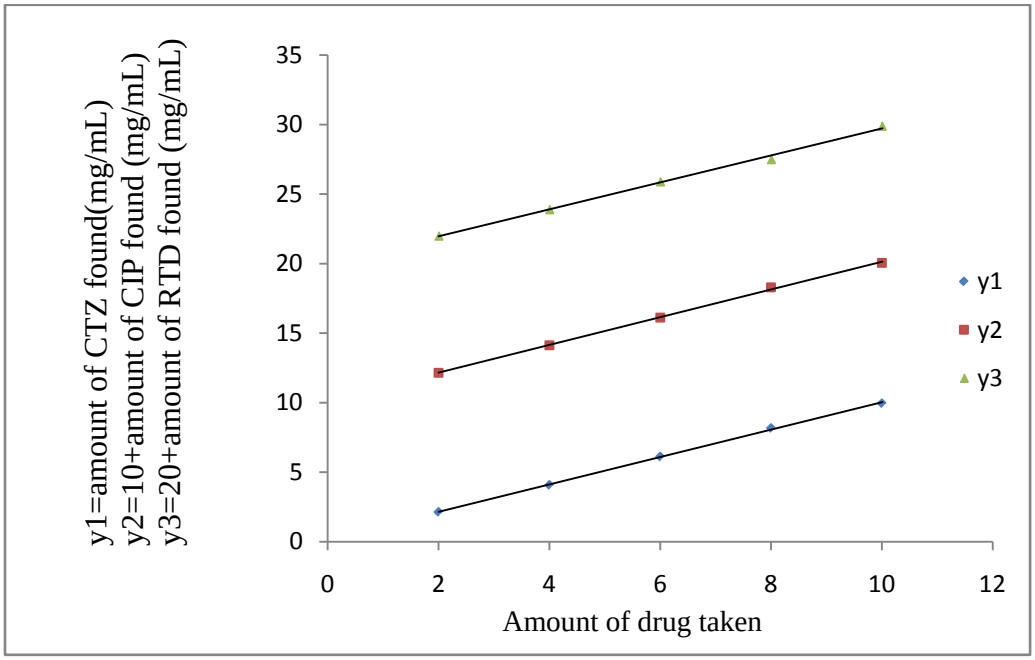

Fig 7 : Calibration curves of Cetrizine di Hydrochloride, Ciprofloxacin Hydrochloride and Ranitine Hydrochloride

4.5. Procedure for analysis of Pharmaceuticals:

For all these pharmaceutical preparations double distilled water used as a solvent and Whatman No:42 filter paper used for filtering the residue.

1. Certizine di HCl:

Five tablets of Okacet containing 10 mg each amounting about 50 mg of

Cetrizine was finely powdered. A portion of the powder equivalent to 100 mg of CTZ accurately weighed and transferred into a calibrated flask. This drug powder dissolved in double distilled water and kept aside for10 minutes. This solution was shaken well and the residue was filtered through filter paper. Same solvent was used to wash the residue and to adjust the volume up to the mark. This solution was used as a stock sample solution and further diluted to get a required concentration to complete the analysis by following the recommended procedures.

2. Ciprofloxacin

Each tablet of Cipro contains 500 mg Ciprofloxacin as active substance was weighed and crushed to a fine powder. An amount of tablet powder equivalent to 100 mg of CIP was weighed exactly and transferred into a clean 100 ml volumetric flask containing water. This solution was kept aside for few minutes, shaken well and filtered through a filter paper, washed the residue with the same solvent and adjusted the volume to the mark with water. It was used as a stock sample solution for further experiments. Working standard solutions were prepared by adding same solvent step wise to the stock sample solution.

3. Rantidine HCl:

For the analysis of commercial formulations, Two tablets of **RANTIDINE (150 mg)** were taken and grounded to a fine powder. A small portion of the tablet powder equivalent to 100mg was weighed accurately and dissolved it by transferring into a 100 mL calibrated flask which contains solvent. This solution was stirred well and filtered through a filter paper. Same solvent was used to wash the residue and to adjust the volume to the mark. It was used as a stock sample

solution and aliquot of this stock solution further diluted by adding the same solvent to get the working standard concentrations within the Beer's Law limit to complete the assay by following the above mentioned procedure.

Table 2 : Analytical and regression parameters for the determination of the drugs by Conductometric titration with $AgNO_3$.

Parameter	CTZ	CIP	RTD
Concentration of drug (mg mL^{-1})	1-10	1-10	1-10
Sandell's sensitivity (mgcm-2)	0.001006	0.000999	0.0005
LOD (mg mL^{-1})	0.126767	0.235443	0.004662
LOQ (mg mL^{-1})	0.384143	0.713464	0.014128
Slope, b	0.994	1.001	2.002
Intercept, a	0.048	0.009	0.017
Correlation co-efficient R	0.9989	0.999	0.9985
Regression equation Y=a+bx	0.994x+0.048	1.001x+0.009	2.002x+0.017
Standard deviation of intercepts (Sa)	0.038184	0.071418	0.002828
Standard deviation of slopes (Sb)	0.007778	0.008485	0.007071

Table 3 : Recovery studies to evaluate the accuracy and precision of the methods on pure drug samples by conductometric titration with $AgNO_3$

Drugs	Amount taken mg/mL	Amount found mg/mL	Error (%)	Recovery (%)	RSD (%)	Proposed method mean±SD
CTZ	2 4 6 8	2.06 3.95 6.08 8.03	1.50 1.25 1.33 0.37	98.50 98.75 98.67 99.62	0.5710	98.88±0.504
CIP	3 5 7 9	3.02 4.99 7.06 9.13	0.67 0.20 0.86 1.44	99.33 99.80 99.14 98.55	0.519591	99.21±0.515
RTD	2 4 6 8	2.01 3.95 5.98 8.03	0.50 1.25 0.33 0.37	99.50 99.67 99.67 99.62	0.079138	99.61±0.079

Table 4 : Application of proposed methods for the analysis of drugs in pharmaceutical preparations by conductometric titration with $AgNO_3$

Drug	Amount taken ($mgmL^{-1}$)	Amount found ($mg\ mL^{-1}$)	Error (%)	Recovery (%)	RSD (%)	Proposed method Mean±SD
CTZ	2.5 4.5 6.5 8.5	2.47 4.56 6.58 7.95	1.20 0.89 0.31 0.59	98.80 99.11 99.69 99.41	0.388	99.25 ±0.384
CIP	3.5 5.5 7.5 9.5	3.51 4.95 7.53 8.96	0.28 0.91 0.4 0.42	99.71 99.09 99.6 99.58	0.279	99.496 ±0.2765
RTD	3.5 5.5 7.5 9.5	3.48 5.56 7.55 9.44	0.57 0.72 0.67 0.63	99.42 99.27 99.33 99.36	0.0656	99.35 ±0.0652

6. RESULTS AND DISCUSSION:

Calibration curves revealed that quantitative precipitation titrations can be carried out by using conductometry where the solution conductance varies with addition of titrant before and after the equivalence point, so that end point can be detected by drawing the two intersecting lines. On using Silver Nitrate as titrant for the estimation of the studied drugs Silver Chloride was precipitated upto neutralization and these conductance readings led to a straight line during the first division of the titration curve. Conductance readings for the excess of titrants added after neutralization of the drug led to the second division of the titration curve. Equivalent points have been obtained for each drug by determining the intersecting point of titration curves and calibration graphs constructed between amount of drug taken and amount of drug found with r^2 =0.999(approx) showing high accuracy and precision of the method. To assess the validity of the proposed methods, these methods were applied on drugs in its pure form and pharmaceutical preparations and statistical analysis of the data obtained revealed that these developed methods are accurate, precise and can be applied over a concentration range of 1-10mg for all the studied drugs.

7. CONCLUSION:

Above methods are simple, sensitive, rapid, accurate and cost-effective which rely on the use of simple techniques and chemicals but provide reasonably good results on par with the expensive and highly sophisticated techniques like HPLC. Thus these methods can be used as alternatives for the quantification of bulk drugs and pharmaceuticals.

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