Introduction

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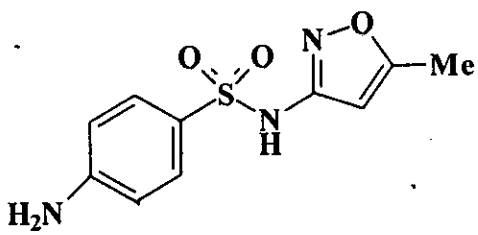
No doubt that, it is critically vital to determine the purity and concentration of any therapeutic drug in high accuracy and precision. Since most of them are toxic in high concentration, detection of the dose is very essential. One of the most important procedures is the spectrophotometric method. In this study we represent a spectrophotometric method for two sulfur containing antibiotics sulfamethoxazole and the aminoglycoside, lincomycin hydrochloride, in addition to antispasmodic drug, mebeverine hydrochloride. The applied methods are characterized by their simplicity, selectivity and high sensitivity. In this chapter, short notes are given about the physical and chemical characters, besides, mode of action and use. A historical survey on some previous works concerned with the drugs under investigation is shown briefly.

1. Literature survey for the determination of sulfamethoxazole:-



***Definition*[1]:**

Sulfamethoxazole is 4-amino-N-(5-methyl-3-isoxazolyl) benzene sulfonamide. It is one of more than 3000 synthesized sulfonamides. It contains not less than 99 % and not more than 101 % of $C_{10}H_{11}N_3O_3S$ calculated with reference to the dried substance. It has a molecular weight of 253.31.

***Characters*[1]:**

Sulfamethoxazole is an almost white crystalline powder, practically insoluble in water, freely soluble in acetone, soluble in warmed alcohol and slightly soluble in ether. It dissolves in dilute solution of NaOH. It has the melting point of 167 °C.

***Action and use*[2]:**

It has antimicrobial activity (bacteriostatic and bactericidal activity). Its mode of action depends on blocking dihydropetroate synthetase enzyme, inhibiting the formation of folic acid, an important precursor to the synthesis of nucleic acids, and hence preventing the synthesis of nucleic acids.

Several procedures have been developed for the determination of sulfamethoxazole in both pure and dosage forms. Some of them are described in brief:

Lin et al[3] provided a basic principal and experimental technique of non relative component reference multiplier derivative spectrophotometry for the determination of sulfamethoxazole (I) and trimethoprime (II). The procedure overcomes the problem of overlapping in derivative spectrophotometry and the quantitative analysis of lower content component in mixture can be done without separation. This method was investigated to assay the contents of (II) and used to assay the zero crossing derivative spectrophotometry for the assay of (I) in pharmaceutical preparations. The average recoveries of (II) and (I) were 102.5±1.63 % (cv) and 100.3±0.99 % (cv), respectively. The results showed that it can not only effectively remove the interference from each other, but also give a high sensitivity and accuracy.

Mohamed et al[4] developed a simple spectrophotometric method for the determination of 15 sulfonamides in bulk and dosage forms. The methods were based on the interaction of p-benzoquinone with sulfonamides in 0.1 M HCl. The resulting chromophore was measured at 500 nm. The effect of different variables on colour development were established. Beer's law was obeyed in concentration range of 10-50 µg/mL. Results for the analysis of different sulfonamide tablets and ophthalmic solutions marketed locally were in good agreement with that of a reference method.

Issa et al[5] developed a spectrophotometric method for micro-determination of sulfamethoxazole and trimethoprime drugs. The proposed method was based on charge transfer (CT) complex formation of the drug

with alizarin or quinalizarin in alkaline medium then measuring the developed absorbance at its maximum. The optimum conditions for maximum absorbance results were studied. Beer's law was obeyed in the ranges 10-130 and 10-190 μg/mL for sulfamethoxazole and trimethoprime respectively. For more accurate results, Ringbom optimum concentration ranges were 20-120 and 10-170 μg/mL, respectively. The molar absorbitivity and Sandell sensitivity were also calculated. The proposed method was as accurate as the USP method and is simpler than the official method. Applications of the suggested method to representative pharmaceutical sulfa drugs were presented and the validity of the method was assessed by applying the standard addition technique.

Feng et al[6] proposed a spectrophotometric method for the analysis of compound sulfamethoxazole tablets by a charge transfer reaction where powdered tablets, equivalent to 0.1 g of sulfamethoxazole (I), were dissolved in dilute HCl and the solution was filtered and diluted to 500 mL with H_2O. A portion (1.0 mL) was treated with 2.0 mL of ethanolic 0.15 M p-benzo-quinone, 1.0 mL of 0.5 M HCl, 0.5 mL of ethanol and H_2O to 5.0 mL then heated at 60 °C for 1 hour. The absorbance was measured at 500 nm. To determine trimethoprime (II), 0.1 g of the powdered sample was dissolved in warm $CHCl_3$ then the solution was filtered and diluted to 50 mL. 0.5 mL portion of the solution was treated with 2.0 mL of ethanolic 7.35 mM chloranilic acid and $CHCl_3$ to 5 mL, and after 20 minutes, at room temperature, the absorbance was measured at 530 nm. Beer's law was obeyed for 4-55 μg/mL of (I) and 20-210 μg/mL of (II). Recoveries were >99 %. There was no interference. The results were compared with those obtained by the method of the Chinese Pharmacopoeia.

Charge transfer reaction of sulfonamide drugs with p-benzoquinone was studied by ***Zhou et al***[7] where the methods of ***Iskandar et al***[8] were modified for the determination of sulfa drugs. For sulfadiazine (I) and sulfamethoxazole (II), powdered tablets containing 100 mg of I or II were dissolved in dilute HCl, the solution was filtered, and The filtrate was diluted to 100 mL with H_2O. 0.5 mL portion of the resulting solution was mixed with 2.5 mL of ethanolic 0.15 M p-benzoquinone solution, 1.0 mL of 0.5 M HCl and H_2O to 5 mL. The solution was heated to 60-65 °C for 1 h and the absorbance was measured at 500 nm. Beer's law was obeyed up to 54 and 55 μg/mL of I and II, respectively. The corresponding recoveries were 103.2 and 101 %. Results agreed with those obtained by literature methods.

Ceric (IV) oxidative spectrophotometric method for the determination of sulfamethoxazole (I) in the presence of trimethoprime (II) in tablets was illustrated by ***Husain et al***[9]. Portions of standard sulfamethoxazole (I) (1.0 mg/mL) solution in 0.5 M H_2SO_4 were mixed with sufficient 0.5 M H_2SO_4 to make the solution 1 M overall. A 7.5 X excess of 0.5 % Ce (IV) solution in 0.5 M H_2SO_4 was added and the solution was diluted to 10 mL with H_2O. The solution was equilibrated for 2 minutes with 10 mL $CHCl_3$ then the organic layer was removed, dried over anhydrous Na_2SO_4 and its absorbance was measured at 460 nm against reagent blank. Beer's law was obeyed from 0.075-0.35 mg/mL of (I). Trimethoprime (II), which also gave an orange-red colour in the presence of Ce (IV), did not interfere because the color was not stable or extractable into $CHCl_3$. The method was applied to the analysis of (I) in powders and tablets also containing (II). Recoveries were

98.93-100.15 % with RSD of 0.1-2.65 %. Results agreed well with those obtained by the British Pharmacopoeia method.

Analysis of various very slightly water soluble drugs in micellar medium was studied by ***Issopoulos***[10]. Potentiometric and visual determination of sulfamethoxazole in pure form. Sulfamethoxazole (I) (100-1000 mg) was dissolved in aqueous 0.05 M cetyltrimethyl ammonium bromide surfactant and diluted to 100 mL with the same solvent. A portion of the solution containing 10-30 or 150-500 mg of (I) was titrated potentiometrically with 2.5 mM or 0.05 M NaOH, either manually using the first and second derivatives of the titration curve or automatically at pH 7.51 or 8.42 for the two (I) concentrations, respectively. For visual titration, 10 drops of phenol red or phenolphthalein were added to the solution (I) before titration. The drug was determined in the range 0.053-0.5 mM. The RSD for 13-125 mg (I) were 0.31-1.36 % (n = 5). The results agreed with those obtained by the official method.

HPTLC determination of sulfamethoxazole (I) and trimethprime (II) (CO-trimoxazole) from pharmaceutical formulations was illustrated by ***Lalla et al***[11]. Silica gel 60F 254 *HPTLC* plates (20x10 cm^2), with $CHCl_3$/ methanol (9:1) as mobile phase were used to determine I and II in tablets. The tablets were crushed and prepared for analysis according to the Indian Pharmacopoeia procedure and standard solutions of the two drugs were prepared in methanol. The plates were densitometrically scanned at 254 nm. R_F values for I and II were 0.5 and 0.2, respectively. Detection and quantification limits were 35 and 50 ng for I, 15 and 25 ng for II, respectively.

Calibration graphs for I and II were linear from 250-1050 ng and 50-250 ng, respectively.

Simultaneous spectrophotometric determination of sulfamethoxazole (I) and trimethoprime (II) in drugs was provided by ***Hassouna***[12]. Crushed tablets (100 mg) were dissolved in methanol and the solution was diluted to 100 mL. Syrup dosage forms were diluted with methanol. Prepared samples were analyzed by spectrophotometry, with absorption measurements from 200-350 nm at 2 nm intervals. The spectral data were subjected to classical least square (CLS) using the computer program of CASAP or ORIGIN. Using the first derivative method, I and II were determined at 288 and 240 nm, respectively, the calibration graphs were linear from 4-20 μg/mL for both drugs and the mean recoveries of I and II were 108.64 % and 103.47 % respectively, with RSD of 7.50 % and 7.26 % respectively. Using CLS, the mean recoveries for both drugs were 100 % with RSD of 0.85 %.

Determination of ingredients in compound Xaiokexin tablets with statistical stimulation spectrophotometry was studied by ***Du et al***[13]. Sample equivalent to 30 mg sulfamethoxazole (I), obtained from 10 finely ground tablets, was dissolved in anhydrous ethanol to 250 mL and filtered. 2.0 mL portion of the filtrate was taken and diluted to 50 mL with more ethanol. The absorbance values at wavelengths (212, 214, 218, 230, 248, 250, 258, 262, 268, 288 and 290 nm) of the diluted solution were measured. The data were fed to a computer for calculation with use of a modified simplex method. The stepwise regression procedure was similarly applied. The recoveries were 94.8-110.3 % with RSD of 0.91-3.9 %.

Charge transfer reaction of tetrachlorobenzoquinone with sulfa drugs was illustrated by ***Zhou et al***[14]. Portions of a standard solution of sulfdiazine (I) were mixed with 2.0 mL of 3 M tetrachlorobenzoquinone, 5 mL 0.1 M NaB_4O_7 buffer of pH 9, then the mixture was diluted to 10 mL with H_2O. The solution was heated at 50 °C for 2 hours, cooled to room temperature and the absorbance was measured at 356 nm against reagent blank. Two other sulfa drugs, sulfmethazine (II) and sulfamethoxazole (III) were also analyzed at the same time. The calibration graphs for I, II, and III were linear from 2-20, 2-52 and 1.8-33 mg/L, respectively. The method was applied to the analysis of I and III in tablets and compound Sinomin tablets, with recoveries of 93.5-102.3 %. Results were comparable with those published in the literature.

Simultaneous determination of sulfamethoxazole (I) and trimethoprime (II) by spectrophotometry was studied by ***Zhou et al***[15]. A sample of a mixture of I and II was dissolved in H_2O and 0.1 M HCl was added to 50 mL. The absorbance values of the solution were measured at 240 and 260 nm where I and II were determined using calibration graphs. Beer's law was obeyed up to 12 mg/L for both I and II. The recoveries were 99.7-100.1 % with RSD of 2.3-2.6 %. The method was employed to the analysis of compound Sinomin tablets and injection solutions.

Multiple factor method and its application in spectrophotometric assay of multicomponent system was studied by ***Zhou et al***[16]. The principle of the cited method was described mathematically. 1.0 mL portion of synthetic sample solution of sulfamethoxazole (I) and trimethoprime (II) was diluted to 50 mL with 0.1 M HCl. The absorbance was scanned over 210-

290 nm at intervals of 2 nm against reagent blank. Beer's law was obeyed up to 12 μg/mL of (I) and (II). Results showed mean recoveries and RSD (n = 9) of 99.79 % and 0.45 % for (I) & 100.67 % and 0.95 % for (II), respectively. The method has been used to determine (I) and (II) in tablets and injection solutions. Results agreed with those obtained by the method of Chinese Pharmacopoeia.

Simultaneous determination of the components in multi component Zenxiao Lianhuang tablets using artificial neural network spectroscopy was studied by ***Yan et al***[17]. A sample obtained from 10 ground tablets was dissolved in anhydrous ethanol then diluted with 0.1 M HCl to 250 mL. A 10 mL portion of the filtrate was further diluted to 100 mL with the acid for absorption scanning between 200-350 nm. The obtained data were fed into a computer for processing with previously trained back-propagation artificial neural network then calculating the amounts of sulfadiazine, sulfamethoxazole and trimethoprime. The recoveries were 98.2-103 %. The effect of network parameters on the analytical results was also discussed.

Simultaneous multivariate spectrophotometric analysis of binary and ternary mixtures of sulfamethoxazole, trimethprime and phenazopyridine in tablets was illustrated by ***Ribone et al***[18]. The feasibility was evaluated from analyzing binary mixtures of sulfamethoxazole and trimethprime & ternary mixtures of sulfamethoxazole, trimethoprime and phenazopyridine hydrochloride by spectrophotometry in conjunction with multivariate partial least square calibration. Calibration models were developed with use of binary and ternary training sets of 9 and 15 samples, respectively. In the analysis of tablets, a portion of finely ground material was sonicated for 20 minutes with

70 mL of 0.04 N NaOH, the solution was filtered, the filtrate was diluted to 100 mL and the absorption spectrum was measured in the range 200-600 nm. Results were presented for various pharmaceutical preparations and synthetic binary and ternary mixtures of the cited compounds. Recoveries of analytes in synthetic mixtures were 98.3-105.8 % for binary mixtures and 100-106.2 % for ternary mixtures, whilst for pharmaceutical preparations, the recoveries were 93-118.8 and 86.4-99.9 % for binary and ternary mixtures, respectively.

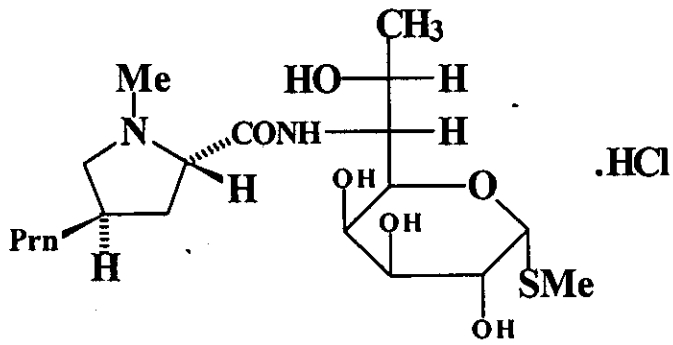
Quantitative determination of sulfamethoxazole (I) by second derivative differential pulse polarography was developed by ***Liang et al***[19]. Standard I solution, containing 50.7 mg dissolved in 50 mL 10 % HCl before adding 50 mL H_3BO_3/KOH buffer of pH 9 and H_2O to 100 mL. Second derivative differential pulse polarography was performed with measurement of the peak at -1.45 V (*vs.* Ag/AgCl). The calibration graph was linear from 0.08-0.8 mM, with detection limit of 8.6 nM. Excipients did not interfere. The method was applied to I tablets, with recovery of 100 % and RSD of 0.5 %. Results were compared with those obtained by dead stop titration.

Determination of sulfamethoxazole (I) in Sinomin tablets by micellar-enhanced spectrofluorimetry was studied by ***Luo et al***[20]. Sample equivalent to 10 mg (I), obtained from ground tablets, was dissolved in anhydrous ethanol to 100 mL. Portions of the supernatant solution were diluted with H_2O to give a 10 µg/mL solution of (I). Portions (2.0 mL) of acetic acid/sodium acetate buffer of pH 4 and 3.0 mL of 50 mg/mL cetyl trimethyl ammonium bromide were added and the solution was diluted with H_2O to 25 mL. The fluorescence intensity was measured at 346 nm (excitation at 261 nm). The calibration graph was linear from 80-800 ng/mL of (I), with detection limit of

16 ng/mL. The solution was stable for 30 minutes. Co-formulated trimethoprime did not interfere.

Indirect potentiometric titration of sulfamethoxazole (I) in the presence of trimethoprime in co-trimazole tablets using copper based mercury film electrode was studied by ***Abdul Kamal Nazer et al***[21]. Ground tablets were stirred with 0.5 M NaOH for 30 minutes. After filtration, the filtrate was adjusted to pH 8.2 with 0.1 M NaOH/ 0.1 M HNO_3 then mixed with 0.2 M triethanolamine/ nitrate buffer of pH 8.2 and 0.1 M $AgNO_3$. Unreacted $AgNO_3$ was titrated against 0.1 M NH_4SCN using a Cu-based Hg-film electrode prepared as described by ***Riyazuddin***[22], as the indicator electrode. Under optimum conditions, the method enabled determination of 1-10 mg I with RSD (n = 7) 1.32 % and recovery of 99.88 %. No interference was caused by excipients present in dosage forms. Results obtained were compared with those of the BP method.

2. Literature survey for the determination of lincomycin hydrochloride:-



Definition[1]*:*

Lincomycin Hydrochloride is the mono hydrate of methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro-α-D-galacto-octopyranoside hydrochloride. It is produced by *streptomyces lincolnensis var lincolensis*. It contains not less than 82.5 % and not more than 93 % $C_{18}H_{34}N_2O_6S$-HCl calculated with reference to the anhydrous substance.

Characters:

Lincomycin is an almost white crystalline powder, very soluble in water, slightly soluble in alcohol, sparingly soluble in acetone and practically insoluble in ether. It has a melting point of 145-147 °C. The molecular formula is $C_{18}H_{34}N_2O_6S$-HCl, H_2O. It has the molecular weight of 461.

Action and use*[2]*:

Lincomycin is an antimicrobial drug. It may be either bactericidal or bacteriostatic. It can inhibit protein synthesis in bacteria through binding to the 50S subunit of the ribosome, and the binding site is a 23S rRNA. This may interfere with formation of initiation complexes for peptide chain synthesis or may interfere with aminoacyl translocation reactions.

Many works were done for estimation of lincomycin hydrochloride in both pure and pharmaceutical formulations; some of them are briefly illustrated below:

Spectrophotometric and indirect determination of lincomycin by atomic absorption spectroscopy (*AAS*) was studied by ***El-Ries***[23]. The reaction of lincomycin with cupric ions in alkaline medium was taken as a basis for the colorimetric and indirect *AAS* determination of lincomycin hydrochloride. The AAS procedure was based on the extraction of copper-lincomycin complex at pH 11, into n-butanol. The copper content in this extract was determined by *AAS*. The response was linear up to 30 µg/mL of lincomycin. The method was accurate, sensitive and simple. The proposed methods were applied to pharmaceutical preparations with fine accuracy.

Study of adsorptive voltammetry for lincomycin hydrochloride was illustrated by ***Liu et al***[24]. A sample (0.1 g) was dissolved in H_2O and then diluted to 100 mL which was further 10 fold diluted. A portion (0.1 mL) of the solution was treated with 0.5 mL of 1.0 M NaOH and 1.0 mL of 1.2 M Na_2SO_3 and then H_2O was added to 25 mL. Adsorptive voltammetry was performed with measurement of the peak potential at -1.38 V (*vs.* the SCE).

The calibration graph was linear for 1-12 μM lincomycin hydrochloride. The RSD was 0.94-1.4 %.

Preparation of the all solid state lincomycin ion selective electrode and its applications was studied by **Xu** *et al*[25]. The cited *ISE* was made by using lincomycin (I)-silicotungstate ion pair complex as the electroactive material and the graphite rod as the electrode substrate. The response was linear for 0.1-50 μM of (I) with a slope of 58.3 mV and a detection limit of 25 μM. The optimum pH was 5-8. The electrode had a life's pan of six months and was applied to the potentiometric determination of injection solutions. Interference from chlorpheniramine maleate and tetracaine was observed.

Luo *et al*[26] provided a method for detecting and quantitating lincomycin residue in salmon tissues by ion pair reversed phase liquid chromatography (*LC*) with electrochemical detection at 0.9 V. Lincomycin was extracted from tissues by homogenizing with 0.01 M KH_2PO_4 buffer (pH 4.5) and centrifuging the mixture. Water-soluble proteins were precipitated by adding Na_2WO_4 and H_2SO_4 then removed by centrifugation. The buffer extract was then passed through a C18 solid-phase extraction cartridge. Lincomycin was eluted with 50 % acetonitrile in water, and the elute containing lincomycin was extracted with ethyl acetate. After the solvent had been evaporated, the residue was redissolved in mobile phase & analyzed by *LC*. The method had a limit of detection of 7 ng/g lincomycin for salmon muscle and 12 ng/g for salmon skin. The limit of quantitation was 17 ng/g for salmon muscle and 24 ng/g for salmon skin. Average

recoveries of lincomycin spiked at 50, 100 and 200 ng/g were ≥ 85 % for salmon muscle and ≥ 80 % for salmon skin.

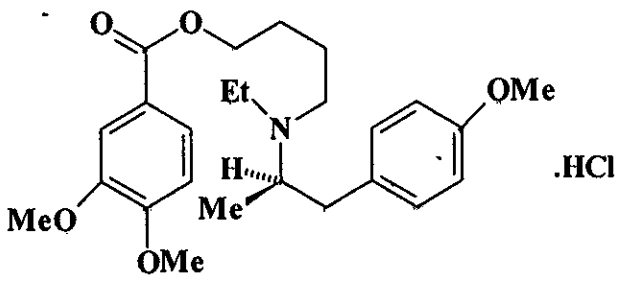
Fang et al[27] illustrated a method for the determination of lincomycin and lincomycin B in bulk drug and pharmaceutical formulations by capillary zone electrophoresis with amperometric detection. Bulk drug was diluted to about 50 µg/mL with 0.1 M NaOH. Ampoule solutions (300 mg/mL) were diluted 5000 fold with 0.1 M NaOH. Powder equivalent to one tablet (250 mg) or capsule (250 mg) was shaken with 50 mL H_2O for 10 minutes with sonication. The solution was made up to 100 mL and filtered. 1.0 mL portion of the filtrate was diluted to 50 mL with 0.1 M NaOH. The resulting solution was analyzed on a capillary (80 cm x 25 µm i.d.) with 0.1 M NaOH as the electrophoretic medium, operated at 26 kV with wall jet amperometric detection at +675 mV (*vs.* Ag/AgCl). Lincomycin and lincomycin B were separated within 15 minutes with a resolution of 2.3. Calibration graphs were linear from 10-150 µg/mL. Detection limits were about 1 µg/mL for both compounds. Recoveries were > 95 % and RSD were < 1 %. There was no interference from excipients.

Liquid chromatographic method for separation of lincomycin from its related substances was developed by ***Orwa et al***[28]. Lincomycin hydrochloride (I) or tablet, soluble powder or injections were extracted or diluted with H_2O to give an expected concentration of 2 mg/mL. Portions (20 µL) were analyzed on a column (25 cm x 4.6 mm i.d.) of base-deactivated 5 µm Supelcosil LC-ABZ C_{12-18} at 45 °C, with a mobile phase (1.0 mL/min) of 2.25 % acetonitrile, 2.72 % KH_2PO_4 adjusted to pH of 5

with 3.48 % K_2HPO_4 and 0.067 % methane sulfonic acid in H_2O and detection at 210 nm. This method gave a good separation of I, lincomycin Band 7-epilincomycin, as well as of three unidentified minor impurities. Calibration graphs were linear for 1.2-2.8 mg/mL of I, with a lower limit of determination of 0.015 % of the nominal content of the formulation and a detection limit of 0.005 %. At 2 mg/mL the RSD was 0.07 % (n = 3). The concentrations of the related substances, calculated as I, were reported for four formulations. Use of standard octadecyl columns and other base-deactivated columns gave less satisfactory separations.

Determination of lincomycin hydrochloride by flow-injection chemiluminescence analysis was developed by ***Wang et al***[29]. Portions of a standard lincomycin hydrochloride (I) solution were diluted with H_2O to 50 mL at pH 6.5. A portion of the solution was injected into the cam steam of 0.1 % H_2O_2 in a flow-injection manifold (FIM). The carrier stream was merged with reagent stream of 5 mM $NaIO_4$ in 1.1 M H_3PO_4, all at pump rate of 3 mL/min. The FIM was set up with a valve to detection cell distance of 60 mm and the chemiluminescence intensity produced by the merged stream was measured. The calibration graph for I was linear from 0.1 - 90 µg/mL, with detection limit of 36 ng/mL. No interference was observed. The method was applied to the analysis of I in injection solutions, with recoveries of 98-101 % and RSD of 1-1.1 %.

3. Literature survey for the determination of mebeverine hydrochloride:-



Definition[30]:

Mebeverine hydrochloride is (RS)-4-[ethyl (4-methoxy-α-methyl phen-ethyl) amino] butyl veratrate hydrochloride. It contains not less than 99% and not more than 101 % of $C_{25}H_{35}NO_5$-HCl, calculated with reference to the dried substance. It has the molecular weight of 466.

Characters:

It is an almost white crystalline powder, very soluble in water, freely soluble in 96 % ethanol and practically insoluble in ether. It has the melting point of 105-107 °C.

Action and use[30]:

It has myotropic spasmolytic properties with a strong and selective action on the smooth muscles of gastro-intestinal tract particularly of the colon.

Many methods have been developed for the estimation of mebeverine hydrochloride in pure and pharmaceutical preparations, some of them are summarized below:

Abdel-Gawad[31], developed a spectrophotometric method for the determination of trace amounts of chloroquine phosphate and mebeverine hydrochloride by ion- pair extraction with Rose Bengal (RB). The method depended on extraction of the associates formed between tertiary amines and RB. The reddish- purple ion associated complex was readily extractable in dichloro-methane and exhibited absorption maxima at 560 and 557 nm for chloroquine phosphate and mebeverine hydrochloride, respectively. Beer's law was obeyed in the concentration range 0.5-8.0 µg/mL of chloroquine phosphate and mebeverine hydrochloride. The proposed method can be applied to the determination in pharmaceutical preparations.

Hassan et al[32], provided a colourimetric method for the determination of mebeverine hydrochloride in tablets by charge transfer complexation. Powdered samples (100 mg) were mixed with 3 x 25 mL of H_2O, filtered and made up to 100 mL with H_2O. A portion (25 mL) was mixed with few drops of 10 % NaOH and extracted with 3 x 25 mL $CHCl_3$, filtered and made up to 100 mL with the extaractant. The solution was further diluted to 0.01 mg/mL (solution A) or 0.1 mg/mL (solution B). Solution A was mixed with 3.0 mL iodine, diluted to 10 mL with $CHCl_3$ and kept in the dark for 30 minutes prior to analysis (method A). Solution B was heated to 70 °C to remove the solvent then cooled and mixed with 1.0 mL tetracyanoethylene (TCNE) (method B) and 1.5 mL tetracyanoquinodimethane (TCNQ) (method C). Analysis was performed with Perkin Elmer 550 S *UV-Vis* double beam spectrophotometer.

Absorbences were measured at 292, 416 and 840 nm for methods A, B, and C, respectively. Beer's law was obeyed from 0.5-30 µg/mL, 5.0-15 µg/mL, and 5.0-25 µg/mL for methods A, B, and C, respectively, with corresponding molar absorpitivities of 1.42 x 10^5, 1.46 x 10^4 and 1.59 x 10^4 L mol^{-1} cm^{-1}. This method has been applied to the analysis of commercial tablets.

Extractive spectrophotometric determination of mebeverine using Erichrome Black T and Alizarin Red S was illustrated by ***Reddy et al***[33]. Powdered tablets equivalent to 100 mg mebeverine hydrochloride (I) were dissolved in H_2O. A portion of the solution was mixed with dilute HCl and 2.0 mL of 2 % Erichrome Black T or 2.0 mL 0.25 % Alizarin Red S then diluted to 10 mL with H_2O. The mixture was extracted for 3 minutes with 10 mL $CHCl_3$ and the absorbance of the organic phase was measured at 500 or 410 nm for determination of (I) as its complex with Erichrome Black T or Alizarin Red S, respectively. Beer's law was obeyed from 5-40 µg/ml (I) (ε = 14000 L $mol^{-1}cm^{-1}$) and 20-100 µg/mL (I) (ε = 4890 L $mol^{-1}cm^{-1}$) for the two reagents, respectively, and the corresponding RSD (n = 8) were 0.945 % and 1.14 %. Recoveries were 99.1-100.02 %. Excipients did not interfere.

Quantitative analysis of mebeverine in dosage forms by *HPLC* was studied by ***Al-Deeb et al***[34]. Mebeverine hydrochloride (I) was determined in two forms; (i) powdered tablets and (ii) a liquid formulation. Powdered tablets were dissolved in methanol and 5 mL were centrifuged for 5 minutes. 50 µL portion was mixed with 10 µL methyl paraben and diluted with mobile phase to 10 mL. The liquid (mebeverine HCl solution; 10 mL) was mixed with methanol and shaken for 5 minutes . The solution (5.0 mL) was

centrifuged for 10 minutes, then a 50 µL portion was mixed with 10 µL methyl paraben and diluted with mobile phase to 10 mL. Portions (20-25 µL) of the resulting solutions from both procedures were applied to a 10 µm Bondapak C_{18} column (30 cm x 3.9 mm i.d.) with 0.05 M ammonium acetate buffer/acetonitrile (9:11) of pH 5.2 as mobile phase and detection at 263 nm. Calibration graphs were linear from 0.5-10 µg/mL of (I), recoveries (n = 10) were from 97.9-100.8 % and the quantitation limit was 5 ng/mL. The RSD was < 2.2 %.

Spectrophotometric determination of mebeverine hydrochloride was illustrated by ***Sreedhar et al*** [35]. Crushed tablets equivalent to 100 mg mebeverine hydrochloride (I) were dissolved in H_2O. Portions ≤ 2.0 ml were mixed with (i) 3.0 mL 10 mM HCl and 0.5 mL 6.18 mM fast green (FGF), diluted to 10 mL with H_2O and extracted with 10.0 mL $CHCl_3$, followed by absorbance measurement of the organic layer at 625 nm within 20 minutes. (ii) 8.0 mL glycine hydrochloride buffer solution of pH 2 and 5.0 mL 3.2 mM bromothymol blue, diluted to 15 mL with H_2O and extracted with benzene, followed by absorbance measurement of the extract at 405 nm after five minutes equilibrium; or (iii) 2.0 mL phthalate hydrochloride buffer solution of pH 3 and 5.0 mL 0.171 M cobalt thiocyanate, diluted to 15 mL with H_2O then extracted with 10 mL benzene, followed by absorbance measurement of the extract at 625 nm after 5 minutes equilibration. Beer's law was obeyed from 2-40, 2-25, and 100-600 µg/mL for the three methods, respectively (ε = 9320, 16700 and 606 L $mol^{-1}cm^{-1}$, respectively). The RSD (n = 6) were 0.64-1.45 % and recoveries were 99.3-100.2 %. Excipient didn't interfere. Detection limits were not stated.

Application of first derivative *UV*-spectrophotometery, *TLC*-densitometry and liquid chromatography for the simultaneous determination of mebeverine hydrochloride (I) and sulpiride (II) was developed by ***EL-Walily et al***[36]. The first method depends on first-derivative ultraviolet spectrophotometry, with zero-crossing measurement method. The first derivative amplitudes at 214.2 and 221.6 nm were selected for the assay of (I) and (II), respectively. Calibration graphs followed Beer's law in the range of 10-30 and 2-8 µg/mL, and the linearity was satisfactory (r = 0.9999), for (I) and (II), respectively. The second method was based on the application of thin layer chromatographic separation of both drugs followed by the densitometric measurement of their spot areas. After separation on silica gel GF254 plates, using ethanol: diethyl ether: triethyl amine (70: 20: 10 v/v) as mobile phase, the chromatographic zones corresponding to the spots of (I) and (II) were scanned at 262 and 240 nm, respectively. The calibration function was established in the ranges of 4-12 µg for (I) and 2-8 µg for (II). The third method was an internal standard procedure based on high performance liquid chromatographic separation of the two drugs on a reversed phase Bond pack CN column. The detection was done at 243 nm using buclizine hydrochloride as internal standard. All chromatographic methods showed good linearity, precision and reproducibility. No spectral or chromatographic interference from the tablet excipients was found. The proposed methods were successfully applied to the assay of commercial tablets and content uniformity test. The procedures were rapid, simple and suitable for quality control application.