CHAPTER I

I. Introduction

I.1. General Introduction

I.1.1. Investigation of the studied drug

I.I. Dextromethorphan Hydrobromide

Dextromethorphan hydrobromide (DXM) was an antitussive drug that is found in many over-the-counter cold remedies [(1)] and cough syrups. In considerably higher dosage, DXM was a powerful psychedelic drug (Specifically, it is a dissociatives drug). The dissociatives are a major subclass of the psychedelic drugs. It's not physically addictive, but may be psychologically addictive Dextromethorphan was first patented with U.S. Patent

In 1958 the FDA approved the use of dextromethorphan as an anti-tussive, in response to the rampant recreational use of, and addition to, the new government regulated codeine that is present in cough medicines at the time. The perceived advantages of dextromethorphan in comparison to codeine were the lack of physical addiction and the absence of a sedative like effect from a normal dosage[(1)]. Two years after its approval, dextromethorphan is finally marketed in the United States as Romilar, a dextromethorphan-only pill, which touted itself as the safe cough medicine alternative to the heavily abused codeine. While it did not take recreational codeine users long to unlock the abuse potential of dextromethorphan, it took thirteen years from Romilar's initial debut for

it to be withdrawn from the shelves. The early 90s trend of DXM use followed closely to the 80s due to the War on Drugs, and the lack of information about dextromethorphan. The Internet began to take shape around the world at this time allowing many to communicate with a defined topic at hand. Much of the early dissemination of knowledge on DXM is conducted on Usenet, calling attention to an obscure recreational drug. As the availability of access to the Internet became more common, dedicated websites with more accurate (and more scientific) information concerning DXM appeared, and were easily found by many company. This flow of information has only increased with time. The growth of the Internet and the ease of spreading information also led to deaths from DXM coming to light. There are now websites largely focusing on documenting the circumstances of these deaths.

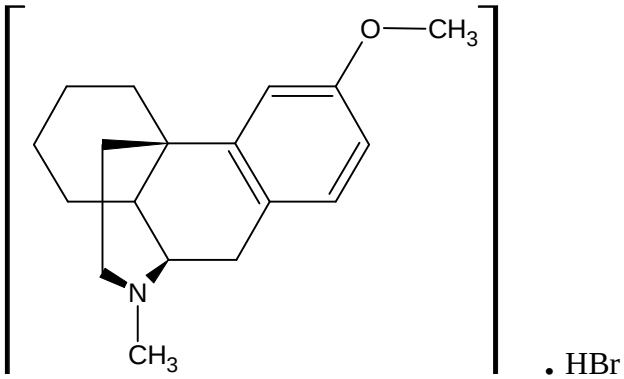
The advantages of dextromethorphan over codeine are the absence of constipation and physical dependence[(2)] it is also less sedative, and is little to no psychological effect in the doses used medically (typically no more than 30 mg, or slightly more, spread over several hours; 10-15 mg is a common dose in cough syrups). It is safe to assume that addiction is impossible, or phenomenally improbable, for use as recommended; the medical dose of dextromethorphan likely has less psychological effect than the alcohol in mouthwash, and definitely less than the caffeine in some headache remedies. Addiction has not been reported with a first plateau dose recreational dose of dextromethorphan, which extends to a 2.5 mg/kg dose (up to ten times maximal recommended dose for a 200 pound adult). A somewhat overweight adult man might have to take 15 pills to reach the top of that range.

The FDA approved dose of dextromethorphan is 30mg. In significantly higher doses of 150 mg to 2 mg, dextromethorphan is

recreationally used as a psychedelic drug that can cause dissociation and dreamlike mental effects, as well as visual and aural hallucinations that can last eight hours or longer in sufficiently high dosage, and can even include "out of body experiences" at very high doses, though this is uncommon; some use high doses for attempts at spirituality or self knowledge. Some users also report an increased ability to understand abstract concepts.

Chemical Structure.

Dextromethorphan hydrobromide (DXM)



. HBr

Empirical Formula : $C_{18}H_{25}NO_2$.HBr

Chemical Name : Morphinan,3-methoxy – 17 – methyl – (9 α - 13 α - 14 α) -,hydrobromide.

Molecular Weight : 352.32 g mol^{-1}

Appearance : White Crystalline powder With little or no odor.

Melting Range : 109.50 – 112.50 ^{0}C.

Solubility : Freely soluble in water, ethyl and methyl alcohol.

Packaging and storage : Preserve in tight containers.

Uses : as antitussive (cough suppressant).

I.I. Literature review

I.I.1.Spectrophotometric methods

Hybrid linear analysis (HLA), as a recent factor-based multivariate calibration technique, is applied for the spectrophotometer determination of ternary mixtures of pseudoephedrine hydrochloride (PSH), dextromethorphan hydrobromide (DXM), and sodium benzoate (BNZ)[(3)]. The utilized HLA is assisted by a wavelength selection procedure which is based on the calculation of the net analyte signal (NAS) regression plot in any considered wavelengths window for each test sample, in addition to a moving window strategy for searching the region with maximum linearity of NAS regression plot (minimum error indicator (EI)). HLA is applied because it is simpler to adapt to the NAS regression plot methodology, also used less factors than partial least squares (PLS). An orthogonal array design was applied for formation of calibration and prediction sets in the concentration ranges 0-7500 $mmoll^{-1}$for PSU, 0-300 $mmoll^{-1}$ for DXM, and 0-1400 $mmoll^{-1}$ for BNZ. The method had the ability to select wavelength regions that minimize the effect of non-linearity of the spectral data, in addition to that of non-modeled interferences.

The use of multiwavelength and derivative spectroscopy for the simultaneous determination of pseudoephedrine hydrochloride(PSH), dex tromethorphan hydro bromide(DXM) and chlorpheniramine maleate(CH M) is described[(4)].Volume of syrup equivalent to 30 mg (PSH), 10 mg (DXM) and 2mg (CHM), is extracted with 2x20 ml ether. Aqueous layer is retained, shaken with 5 ml 1M-NaOH, then extracted with 4x20 ml $CHCl_3$. Organic layers are combined, evaporated to dryness, and the

residue reconstituted in 0.1NHCl to give final concentrations of 150 gml^{-1} (PSH), 50 gml^{-1} (DXM) and 10 gml^{-1} (CHM). For the multi wave length approach, sample solutions are scanned from 220-350 nm and the data compared to that for mixed standards; absorption maxima (epsilon not stated) are 257 nm, 265 nm and 278 nm for (PSH), (CHM) and (DXM), respectively. For derivative spectroscopy, first derivative order is most suitable,absorbance's are measured at the optimum wavelengths of 256.4, 264 and 279.4 nm, and analyte concentration determined by simultaneous equation (equations provided). Beer's law is obeyed from 2-300 gml^{-1} (PSH), 0-100 gml^{-1} (DXM), and 1-20 gml^{-1} (CHM). RSD (n=5) ranged from 0.2-1.9% for the two methods, and recoveries from 98-102.2%. Results tabulated .

Two methods were used for the determination of dextromethorphan hydro bromide[(5)]. In the first method, a suitable portion of the formulation is spiked with 5 mg dextromethorphan hydrobromide (DXM) and diluted to 25 ml with 0.2 M NaOH. The mixture is repeatedly extracted with chloroform and the combined extracts are evaporated to dryness and reconstituted in 0.1MHCl (100 ml). Chlorpheniramine maleate was determined by measuring the absorbance of the sample solution at 310 nm. Determination of chlorpheniramine maleate and (DXM) was effected from the absorbance's at 278 and 245 nm and using these data to solve two derived simultaneous equations. In the second method, a suitable portion of the formulation is diluted to 25 ml with 2M NaOH and extracted repeatedly with chloroform. The extract is evaporated to dryness and reconstituted in methanol/acetonitrile/phosphate buffer of pH 5.8 (5:1:4) (mobile phase). After addition of caffeine (2 mg) as internal standard, the mixture is diluted to 10 ml with the mobile phase and analyzed by HPLC on a 5 μg Nucleosil-CN column (20 cm x 4 mm)

operated with the mobile phase described above (0.8 $mlmin^{-1}$) and detection at 278 nm. The recoveries for the three analytes are reported for each method and compared. Both methods gave good reproducible results.

Dextromethorphan hydrobromide was described by two different methods[6], viz. second-order derivative spectrophotometer (2DS) and multi wavelength spectrophotometer(MWS), for the determination of dextromethorphan hydro bromide (DXM) and pseudoephedrine hydrochloride (PSH) in liquid dosage forms are presented. Portions (5 ml) of a commercial syrup sample containing (DXM) (5 or 7.5 mg) and (PSH) (30 or 15 mg) are extracted with 2 x 10 ml diethyl ether. The ethereal layers are discarded, the aqueous layer is made alkaline with 5 ml 1MNaOH and extracted with 3 x 20 ml and 10 ml portions $CHCl_3$. The combined organic extracts are dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue is dissolved in 0.1MHCl and the volume is made up to 100 ml. For 2DS, the absorbance is measured at 285.9 and 293 nm for (DXM) and 271.6 nm for PSH. For MWS, the absorbance was measured at 278, 263, 257 and 251 nm, along with those of mixed standard solutions. The absorbance were processed by matrix equations (given) and correlated to determine the concentrations of DXM and PSH. Beer's law was obeyed from 5-20 and 60-240 μg/ml, respectively, for DXM and PSH. Recoveries of DXM and PSH were 98.40-101.90 and 98.83-102.13 and 99.70-101.60 and 99.13-101.77 %, respectively, for 2DS and MWS methods. For 2 commercial form- ulations, values of DXM and PSH were in good agreement with labelled values by both 2DS and MWS methods.

Determination of dextromethorphanHBr and bromhexineHCl in tablets by fist derivative spectrophotometry was described [(7)]. Powdered tablets, containing about 15mg bromhexine HCl (BR), were transferred into a 50 ml volumetric flask and dispersed in 30 ml methanol for 5 min by ultrasonic vibration. The suspension is diluted to30 ml volume with methanol and filtered; 25 ml is transferred to a 50 ml volumetric flask and adjusted to volume with phosphate buffer solution (pH 6). A 3ml aliquot is diluted to 25 ml with 50% methanol in phosphate buffer. UV determination by first-derivative spectroscopy of (BR) and dextromethorphan HBr (DXM) is carried out using zero-crossing and peak-to-base measurement at 234 and 324 nm, respectively. The method is linear in the range 6-30 and 12-60 μg/ml for BR and DXM with correlation coefficients of 0.9999 for both. The limit of detection was 0.103 μgml^{-1} for BR and 0.033 μgml^{-1} for DXM. Details of a HPLC method developed as the reference method are given. Results obtained by first-derivative spectrophotometer agree well with those obtained by this HPLC method.

A densitometry used for determination of dextromethorphan hydro bromide and doxylaminesuccinate in syrups and its validation[(8)]. dextromethorphan hydrobromide and doxylaminesuccinate syrups (3ml) are vortex mixed with 3ml 25% ammonia for 10 min, ultraso- nicted for 10 min and extracted with 4 ml ammonia and 2 x 2.5 ml hexane. The hexane extracts are diluted to 10 ml, a 8 ml portion is evaporated to dryness and the residue to dissolved in 1 ml hexane. The resulting solution is spotted onto silica gel 60 F254 plates and the plates are developed in methanol/25% ammonia (14:1) to 8 cm. The analyte spots are identified by scanning in absorbance-reflectance mode at 200-400 nm and quantified by measuring the absorbance at 223nm.Calibration graphs were linear for 2.4-12.2 μg /spot of dextromethorphan and 1.5-7.4 μg /

spot of doxylamine with detection limits of 0.13 μg / spot and 0.14 μg/spot, respectively. Recoveries are 99.61-101.21 % and 98.56-102.1% for dextromethorphan and doxylamine, respectively.

Second-derivative spectrophotometric used for determination of mixtures of phenyl propanolamine hydrochloride and dextromethorphan hydrobromide in some pharmaceutical preparations[(9)]. A portion of the contents of capsules or a portion of powdered lozenges is mixed with methanol , and the second-derivative absorption spectra of the filtered solution. are recorded from 220 to 320 nm. Peak amplitudes are measured between the max. at 260 nm and its minimum at 257 nm for phenylpropanolamine hydrochloride (PP) and corresponding measurements are made at 291.5 and 287.5 nm for dextromethorphan hydrobromide (DXM).Calibration graphs are rectilinear from 20-350 $\mu g ml^{-1}$ and 16-200$\mu g ml^{-1}$ for PP and DXM, respectively, and recoveries from simulated capsules and lozenges are 98.0 ± 2.4% and 99.9 ±2.0%, respectively.

Second-derivative photodiode-array spectroscopy method was used for determination of pseudoephedrine hydrochloride, chlorphenir-amine maleate and dextromethorphan hydrobromide in tablet[(10)]. A chew able paediatric tablet containing the cited drugs Pseudoeph hydrochloride (PSH), chlorpheniraminemaleate (CHM), and dextromethorphanhydrobromide (DXM) is grounded and dissolved in 100 ml of 0.1M acetate buffer of pH 5.0. The solution. Is set aside for one hour at room temperature, then the second-derivative spectrum of a filtered aliquot is recorded from 240-300 nm. Results are calculated from the absorbance's at 240, 250, 260, 270, 280, 290 and 300 nm. Some interference is caused by excipients; therefore, a placebo solution. is treated as a fourth standard. Sensitivities are 7.5, 1.0 and 5.0 $\mu g\ ml^{-1}$ for PSH, CHM and

DXM, respectively. Results of analysis of the tablet and of a sustained-release formulation agreed well with those obtained by HPLC.

I.I.2. Chromatography methods

HPLC method with fluorometric is used for simultaneous determination of dextromethorphanhydrobromide (DXM) and its three metabolites (dextrorphan (DX),3-methoxymorphinan (MM),3-hydroxymorphinan (HM)) in human[(11)]. Extraction protocol using an n-heptane/ethyl acetate (1: 1) solvent mixture that achieved recoveries of 70-90% with an insignificant interference from the plasma matrix. The analysis was performed on a phenyl column with isocraticelution, a mobile phase composed of 20 % methanol, 30 % acetonitrile, and 50 % KH_2PO_4 buffer (10 mM, with adding 0.02% of TEA; adjusted with phosphoric acid to pH 3.5), and a run time of only 15 min. Linear calibration curves were constructed in the concentration range of (1-200) M for DXM and its three metabolites. The lower limit of quantitation in human plasma was 1.0 M for each compound.

Liquid chromatography (LC) as anew rapid and sensitive method has been developed and validated for thesimultaneous determination of phenylephrine hydrochloride, paracetamol, and chlorpheniramine maleate and dextromethorphan hydrobromide in pharmaceutical preparations [(12)]. The separation was achieved on a C18 column using a gradientmobile phase of acetonitrile-sodium perchlorate (pH 3, 0.01 M) at a flow rateof 1.4 mL min^{-1}. Detection was at 204 nm. Pseudoephedrine hydrochloride was selected as internal standard. The recovery of the drugs ranged from 97.8 to100.9%. Central composite design was used during validation to calculate method robustness and the percentage of sodium perchlorate,

temperature and flow rate were investigated as factors. The method was found to be applicable for the determination of the four compounds in sugar-coated tablets.

A partial least-squares multivariate calibration, was used for the determination of dextromethorphan hydrobromide (DXM), pseudoephedrine hydrochloride (PSE) and triprolidine hydrochloride (TRP) in syrup samples [(13)]. The method allows the simultaneous quantification of the analytes that the closely overlapping spectral bands are not efficiently solved. The calibration set consisting of 27 samples with 60, 80, 100 mg ml^{-1} of DXM, 180, 240, 300 mg mL^{-1} of PSE and 8, 10, 12 mg ml^{-1} of TRP was used for calibration matrix. The absorbances were recorded between 230 and 320 nm every 5 nm.

A simple and reliable reversed-phase high performance liquid chromatographic method (HPLC) for the routine analysis of pseudoephedrine hydrochloride and dextromethorphanhydrobromide in composite pseud- oephedrine hydrochloride dry suspension has been established[(14)]. HPLC determination for the drug is performed in a Lichrospher C_6H_6 column and detected at 220 nm. Acetonitrile-H_2O-H_3PO_4 (50:50:0.1, v/v, pH 2.5, containing 1.0 gl^{-1} sodium dodecyl sulfate) is used as the mobile phase and the flow rate is 1.2 $mlmin^{-1}$. The method is proved to be linear in the ranges of 1.03-206 mgl^{-1} and 5-200 mgl^{-1} for dextromethorphan hydro-bromide and pseudoephedrine hydrochloride, respectively. The relative standard deviations of intra-assay (n=7) and inter-assay (n=5) are 1.0 % and 1.5 %for pseudoephedrine hydrochloride analysis and 1.8% and 2.2 % for dextromethorphan hydrobromide analysis.The recoveries of pseudo- ephedrine hydrochloride and dextromethorphan hydrobromide are 95.7 % -98.7 % and 100.0 %-101.8 %, respectively. The method has

been successfully applied to the simultaneous determination of pseudoephedrine hydrochloride and dextromethorphan hydrobromide

HPLC method with fluorimetric was used for determination of dextromethorphan, and its main metabolites in human plasma is developed and validated[(15)]. The method involved a simple and rapid protein precipitation protocol, using a mixture of $ZnSO_4$ and methanol. The analysis was performed on a 3 min, C18 Tracer Excel 15 cm x 0.4 cm i.d. column by gradient elution in which mobile phase, consisted of potassium dihydrogen phosphate buffer (pH=3),0.01M)/methanol tetrahydrofuran 68.5:31:0.5), and mobile phase (B) consisted of methanol / tetrahydrofuran (93.25:6.75). Linear calibration curves are obtained in the range of 10-500 $ngml^{-1}$ for dextromethorphan, dextrorphan and hydroxymorphinan. The limit of quantization (LOQ) was 10 $ngml^{-1}$ for each compound.The maximum within and between days precisions are 7.4 and 7.8 %, respectively. The accuracies at four different concentration levels ranged from 88.2 to 111.5 %. The recoveries are between 88.0 and 108.6%. The assay method is successfully applied to determine dextromethorphan metabolic ratio after an oral dose of 30 mg of dextromethorphan hydrobromide.

A rapid and accurate HPLC method is described[(16)] for the simultaneous determination of acetaminophen, dextromethorphen hydro bromide and pseudoephedrine hydrochloride in a new cold formulation. Chromatographic separation of the three pharmaceuticals was performed on a Hypersil CN column (150 x 5.0 mm, 5 mum) with a mobile phase mixture of an ion-pairing solution, methanol and acetonitrile (25:57:18), at a flow rate of 1.0 $mlmin^{-1}$, with detection at 220 nm. Separation is complete in less than 10 min. The method are validated for linearity,

accuracy, precision, limit of quantization and robustness. Linearity, accuracy, and precision are found to be acceptable over the ranges of 2.06 similar to 20.6 $mgml^{-1}$ for acetaminophen, 0.202 similar to 2.02 $mgml^{-1}$ for pseudoephedrine hydrochloride and 0.042 similar to 1.06 $mgml^{-1}$ for dextromethorphen hydro bromide. .

HPLC method was used for the quantitative determination of paracetamol, dextromehorphnhyrobroide, pseudoephedrine hydrochloride ,and diphenhydramine hydrochloride in dispersed tablets[(17)]. The chromatographic conditions are as follows. Diamonsil-C18 (200 mm x 4.6mm, 5.0 mum). The mobile phase consisted of a mixture of acetonitrile-methanol 0.05 mol/l potassium dihydrogen phosphate (5:1:25) for paracetamol and UV detection at 245 nm. The mobile phase consisted of a mixture of sodium format buffer (pH 3.3) acetonitrile-methanol-sodium lauryl sulfate (32:28:5:0.06,v/v/v/w) for dextromethorphanhydro bromide pseudoephedrine hydrochloride and diphenhy draminehydrochloride and UV detection at 256 nm. Results The calibration curves are linear in the range of 2.06-20.60 $\mu gml^{-1}$for paracetamol (r=0.9997),0.11-1.06 $mgml^{-1}$ for dextromethorphan hydro bromide (r=0.9994),0.20-2.02 $mgml^{-1}$ for pseud- oephedrine hydro chloride (r=0.9995),0.10-1.01 $mgml^{-1}$ for diphenhyd ramine hydrochloride (r= 0.0.9997) . The average recoveries are 100.2%, 99.28%, 100.6% and 100.7%, respectively. Conclusion The method is accurate and reliable for quality control of this compound preparation.

High-performance liquid chromatography method was used for quantative determination of dextromethorphan hydrobromide in Plasma[(18)] . Plasma (1 ml) is agitated with 0.25 ml saturated Na_2CO_3 and 2 ml n-hexane containing 0.1% triethylamine for 20 min then centrifuged

for 5 min. The organic phase is separated and evaporated; the residue is dissolved in water and diluted with mobile phase to 200 µg of solution. Dextromethorphan hydrobromide (DXM) in 50 µg DXM of the solution is determined by HPLC on a 4 µg Novapak C8 column (15 cm x 3.9 mm i.d.), with aqueous 50% acetonitrile containing 0.5% glacial acetic acid and the pH adjstent to 4.3 as mobile phase at 1 ml/min and fluorimetric detection at 308 nm (excitation at 280 nm). The calibration graph for DXM is linear, with detection limit of 0.2 $ngml^{-1}$. The recovery is 93.7% with RSD of 3.53%. There is no interference.

HPLC method is used for determination of guaiphenesin , pseu-doephedrin hydrochloride and dextromethrphan hydrobromide in syrup[(19)].**A** sample (2 ml) is shaken with 0.9 g NaCl, 4 drops of an ammonia reagent and 10-15 ml methanolic 92% $CHCl_3$ (thrice), and the combined organic extracts is washed with 5 ml saturated NaCl solution. The organic layer is dried over anhydrous Na_2SO_4 and then evaporated; the residue is dissolved in 5ml of 50% methanol. Guaiphenesin (GPH), pseudoephedrinehydro- chloride (PSH) and dextromethorphan hydrobromide (DXM) in 10µGPH of the solution areseparated and determined byHPLC on a 5µ Spherisorb C_8 column (25cm x4.6 mm i.d.), with methanolic 2.5 mM-hexane sulfonic acid/H_2O(containing2% triethylamine/$_3$M-H_3PO_4 (1400:1000:68) as mobile phase (1 ml/min) and detection at 257 nm. The calibration graphs for GPH, PSH and DXM are linear from 2-10, 0.3-1.5 and 0.15-0.65µg, respectively. Recoveries were 99.7-100.8%.

Simultaneous determination of chlorpheniramine maleate(I), dextromthorphan hydrobromide and phenylpropanol amine hydrochl-

oride from cough syrup by HPLC[20]. Cough syrup (5 ml) is weighed, diluted to 10 ml with H_2O, then 1.0 ml mixed with 2 ml internal standard (1.0 $mgml^{-1}$ dicyclohexyl phthalate) and diluted to 10 ml as previous. A 100 µg l portion of the solution is analyzed on a 10µg Bondapak C18 column (30 cm x3.9 mm), operated with 0.01M - Na_2HPO_4 / acetonitrile / methanol / triethylamine (20:55: 25:0.1) adjusted to pH 7.5 with H_3PO_4 as mobile phase (1 ml/min) and detection at 261 nm. All four compounds are separated within 15 min. Calibration graphs are linear from 5-80 $µgml^{-1}$ chlorpheniramine maleate (CHM), 20-220 $µgml^{-1}$ dextromethorphan hydro bromide (DXM), and 75-250 $µgml^{-1}$ phenylpropanolamine hydro- chloride(PPH).Limit of detec- tion and limit of quantization are, respectively:1.2 and 3.9 $µgml^{-1}$ (CHM) 5.0 and 16.8 $µgml^{-1}$ (DXM), 6.9 and 23.1 $µgml^{-1}$ (PPH). RSD ranged from 1.64-1.98% for standards (n = 5) and 1.1-1.7% for syrup samples (n = 7). Recoveries (n = 3)are 101.11-101.54%.

Quantification of dextromethorphan hydrobromid and clemastine fumarate in pharmaceutical by HPLC with ultraviolet absorotion densit- ometry[21]. Clemastine fumarate (CF) in tablets, and Dextromethorphan hydrobromide (DXM) in solid and liquid gelcaps are analyzed on silica gel 60 F254 GLP HPTLC plates (20 cm x 10 cm for DXM analysis and 10 cm x 10 cm for CF analysis), cleaned with dichloromethane/ methanol (1:1) and ethyl acetate/methanol/concentrated NH_3 (17:1:2) for DXM and dichloromethane/methanol/concentrated NH_3 (90:10:1) for CF as mobile phase and detection at 225 nm for DXM and 216 nm for CF. Amounts of DXM in taplets and gel caps ranged from 100 to 114% of the label values with RSD from 1.2 to 1.9% and recoveries of 99.4%. For CF tablets heaving are 99-103% of the label values with RSD of 2.2% and recoveries of 97.9%.

Gas chromatography method used for determination of dextromethorphan hydrobromide, ephedrine hydrochloride and chlorpheniramine from cough syrup[22].Its (10g) is mixed with 1.0 ml aqueous brom hexane hydrochloride (5.0 mg/ml; internal standard) and 20 ml 1M-NaOH. After shaking for 2.0 min, the mixture are allowed to stand for 5 min then extracted by shaking with $CHCl_3$ (3 x 20 ml) for 5 min. The combined extracts are evaporated to dryness and the residue reconstituted in 2 ml $CHCl_3$.A 2µg portion of the resulting solution is analyzed by GC on a SS column of 5% SE 30 on Chromos orb W HP (80-100 mesh) temperature programmed from 135 ^{0}C (held for 1 min) to 250 ^{0}C (held for 5 min) at 80 ^{0}C/min and operated with N2 as carrier gas at 30 ml/min and FID. Calibration graphs are linear for 0.5-5.0 mg/ml dextromethorphan hydrobromide (DXM) and ephedrine hydrochloride (EP) and 0.25-2.5 mg/ml chlorpheniramine maleate (CHM). Detection limits and quantification limits were 0.3 and 1.0 mg/ml for both DXM and EP, and 0.15 and 0.6 mg/ml for CHM, respectively. Recoveries are 100.78%, 98.25% and 98.07% for DXM , EP and CHM , respectively. RSD (n = 5) were 1.72-2.2%.

Guaifenesin, pseudoephedrine hydrochloride and dextromethorphan hydro bromide were determined in cough syrup by HPLC method[23]. C18 column (25cm x4.6 mm i.d) with methanol / H_2O/ ammonium format buffer (45:54:1) as mobile phase(1 ml/min ;1.5 ml/min after 9 in 1 ml/ min after 19 min) and detection at 257 nm. Calibration graphs are linear, with the exception of guaifenesin, from 60-108 mg/ml and 30-54 mg/ml for pseudoephedrine hydrochloride and dextromethorphan hydro bromide, respectively.RSD are 7.87%, 0.803% and 0.45% and ecoveries are 100.126%, 99.89% and 99.13% for guaifenesin, pseudoephedrine

hydrochloride and dextromethorphan hydro bromide, respectively. The merits of the method are briefly discussed.

High-performance Thin-layer chromatography method was used for determination of pseudoephedrine sulphate, azatadine maleate and dextromethorphan hydro bromide[(24)]. Ground tablets are dissolved in methanol, filtered and the filtrate made up to 25 ml with methanol. A 4 ml portion is added to 1 ml of 4 mg/ml naproxen (internal standard), made up to 10 ml with methanol and 5µg applied to a silica gel 60 F254 HPTLC plate with CH_2Cl_2/ acetone/ methanol/triethylamine (70:40:5:2) as mobile phase. The plates are densi- tometrically scanned at 257 nm and the calibration graph is linear from 0.05-54µg. RSD for pseudoephe-drine sulphate, azatadine maleate and dextromethorphan hydro bromide are 1.25, 1.2 and 1.4%, respectively. Recoveries are 98-102% .

Determination dextromethorphan hydrobromide in bulk form and dosage formulations by HPLC[(25)]. The dextromethorphan hydro bromideis analyzed on a 10 µg -Bondapak C18 column (30 cmx3.9mm i.d.) with acetonitrile/ 40 m acetate buffer of pH 4.3 (3:1) as mobile phase (1.5 ml/min) and detection at 278 nm. The method is applied to the analysis of powdered tablets, syrup or drops, which are extracted and diluted with mobile phase. Liberally (2 mg/ml in mobile phase) is added as internal standard before HPLC. The recovery of (DXM) from tablets is 100.9 ± 0.29% (n=4) and the RSD is 0.41% (n = 6).

Ion-pair liquid chromatographymethod was used for determintion of dextromethorphan hydrobromide in sachet[(26)]. The contents of a sachet of the cited cold medication are dissolved in H_2O and, after sonication for

15 min. the solution is filtered. A 50 µg portion of the filtrate is analyzed on a Shandon Hypersil Phenyl-2 (5 µg) end-capped column (25 cm x 4.6 mm i.d.) with a mobile phase (2ml/min) of aqueous 5% acetonitrile containing 50ml monosodium phosphate ,125 mlM-tetrabutylammonium hydrogen sulfate and 1ml pentane sulfuric acid and adjusted to pH 2.5 with 1M-NaOH; detection is at 210 and 290 nm. Recoveries are close to 100%, calibration graphs are linear and the minimum amounts of active drug substances that could be quantified are 0.1% of the 100% label claim with detection at 210 nm, and 1% with detection at 290 nm. The minimum detection limits are ~10 ng, and the RSD (n = 20) obtained by two analysts on different days are 0.4-2%

I.I.3 Electro – analytical methods

Determintion dextromethorphan hydrobromide, diphenhydramine hydrochloride and phenylephrine hydrochloride in syrups can be achieved by capillary electrophoresis[27]. The separation of basic nitrogenous compounds commonly used as active ingredients in cold medicine formulations by micellar electro kinetic capillary chromatography and capillary zone electrophoresis with direct absorptiometric detection is investigated. The type and composition of the background electrolyte (BGE) are investigated with respect to separation selectivity and BGE stability. BGE of 10 ml M sodium dihydrogenphosphate/ sodiumtetraborate buffer containing 10ml M-SDS and 10% acetonitrile, pH 9.0 is found to be optimal. Dextromethorphan hydrobromide, diphenhydramine hydroch- loride and phenylephrine hydrochloride are baseline-separated in less than 11 min, giving separation efficiencies of up to 494 000 theoretical plates, reproducibility of corrected peaks areas below 3% relative standard deviation and

concentration detection limits from 2.5 to 5.5 mg/ml. Detection is performed at 196 and 214 nm .

The syrup was treated with 2 ml aqueous theophylline (1.2 mg/ml; internal standard) and electrophoresis buffer is added to 50 ml acetaminophen (paracetamol; I), pseudoephedrine hydrochloride (II), dextromethorphan hydrobromide (III), chlorpheniramine maleate (IV) and sodium benzoate (V) in the filtered solution are determined by high-performance capillary electrophoresis on a quartz column[28]. (72 cm x 50µg, effective length 52cm) operated at 30 ^{0}C, with $NaH_2PO_4/Na_2B_4O_7$ buffer of pH 8.5±0.2 as electrophoresis buffer, at voltage of 30 kV and detection at 200 nm. Separation completed in 80 min. The calibration graphs for I, II, III, IV and V are linear from 0.0124-0.112, 0.0601-0.541, 0.0206-0.185, 0.0046-0.0414 and 0.0401-0.361 g/l, respectively. The within- and between-day RSD were 0.3-2.4 and 0.8-3.3%, respectively. The recoveries were 99-101.2% .

capillary electrophoresis can be used for determination dextromethorphan hydrobromide in alkaline media[29].Compounds that are either neutral or positively charged in acidic media, e.g., etenzamide, guaiphenesin, parac- etamol and potassium guaiacolsulfonate, are determined in 50 m M-SDS in 15mM-phosphate buffer of pH 11.0 as carrier, whereas those that are negatively charged in alkaline media,e.g., chlorpheniramine maleate, dextromethorphan hydrobromide, dihydrocodeine phosphate, methyleph- edrinehydrochloride and noscapine, are determined in 50 ml phosphate buffer of pH 3.0 as carrier. Hydrostatic injection for 10 s and on-column detection at 185 nm are used. Recoveries are 92.7-109% and RSD (n = 3) are 0.5-6.3%.

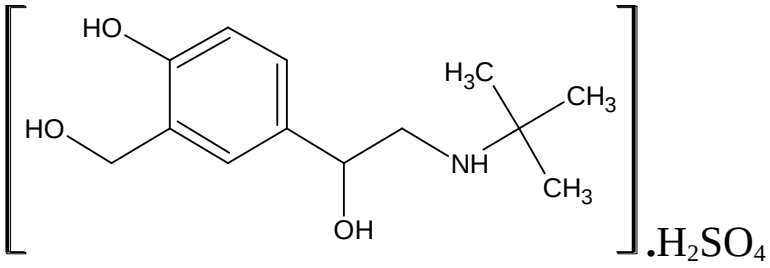
Determination of dextromethorphan hydrobromide in pharmaceutical perparations used by double membrane selective electrode based on dextro- methorphan-5-nitrobarbiturate[30]. A Pt-wire electrode is coated first with a 0.1-mm-thick membrane prepared from plasticized PVC resin containing tetrabutylammonium bromide and then with a 0.2-mm-thick layer of PVC resin containing the dextromethorphan (DXM)-5-nitrobar-biturate ion-pair complex. The electrode is used with a double-junction Ag-AgCl reference electrode for determination of (DXM).The electrode response is linear from 50 µg to10 µg, with a detection limit of 10 µg. The response is constant between pH 4 and 10 and the compositions of the membranes are optimized. At 0.1ml, the electrode potential drift is 3.4 mV day^{-1} with a coeff. of variation (n = 10) of 0.55%. Of 14 species examined, significant interferences are observed only with dextrometho-rphan hydro bromide and caffeine. The method is used to determine (EP) in pharmaceuticals; results agreed well with the certified values .

I.II. Salbutamol sulfate.

Salbutamol is used to relieve and prevent bronchospasm associated with asthma, bronchitis, emphysema and other pulmonary disorders where bronchospasm is a complicating factor. It is also indicated in the prevention of exercise-induced bronchospasm. Salbutamol is a relatively selective beta-2-adrenoreceptor against bronchial smooth. Administration by inhalation results in direct stimulation of beta-2 receptors in bronchial smooth muscle and hence bronchodilator. This is thought to be due to stimulation of adenyl cyclase by salbultamol, resulting in increased levels of cyclic AMP within cells [(31)]. These are thought to inhibit the entry of calcium ions into the cells, thus inhibiting smooth muscle contraction. High levels of cyclic AMP in mast cells also inhibit the release of histamine and SRS-A. Whilst salbutamol is well-tolerated, particularly when compared with previous therapies such as theophylline, like all medications there exists the potential for adverse drug reactions to occur especially when in high doses, or when taken orally or intravenously.

Chemical Structur:

Salbutamol Sulphate (SIS)



Empirical Formula : $(C_{13}H_{21}NO_3)_2 . H_2SO_4$

ChemicalName : 4-Hydroxy-3-hydroxy methyl - ((tert-butylamino) methyl) -benzyl alcohol

Molecular Weight : 239.3 g mol^{-1}

Appearance : White Crystalline it is odorless and almost taste less

Melting Range : 157-158 ^0C

Solubility : Freely soluble in water, ethanol, slightly soluble in ether .

Packaging and storage : Preserve in airtight containers

Uses : As a bronchodilator, especially in the treatment of asthma.

I.II. Literature review

I.II.1.Spectrophotometric methods

Four simple, rapid, accurate, precise, reliable and economical spectrophotometric methods have been proposed for simultaneous determination of salbutamol sulphate (SIS), bromhexine hydrochloride(BH) and etofylline (ET) in pure and commercial formulations without any prior separation or purification[32].They were first derivative zero crossing spectrophotometry (method 1), simultaneous equation method (method 2), derivative ratio spectra zero crossing method (method 3) and double divisor ratio spectra derivative method (method 4). The ranges for SIS, BH and ET were found to be 1-35 mg mL^{-1}, 4-40 mg mL^{-1} and 5-80 mg mL^{-1}. For methods 1 and 2, the values of limit of detection (LOD) were 0.2314 mg mL^{-1}, 0.4865 mug mL^{-1} and 0.2766 mg mL^{-1} and the values of limit of quantitation (LOQ) were 0.7712 mg mL^{-1}, 1.6217 mg mL^{-1} and 0.9221 mg mL^{-1} for SS, BH and ET, respectively.For method 3, LOD values were 0.3297 mg mL^{-1} , 0.2784 mg mL^{-1} and 0.7906 mg mL^{-1} and LOQ values were 0.9325 mg mL^{-1}, 0.9282 mg mL^{-1} and 2.6352 mg mL^{-1} for SS, BH and ET, respectively. For method 4, LOD values were 0.3161 mg mL^{-1} , 0.2495 mg mL^{-1} and 0.2064 mg mL^{-1} and LOQ values were 0.9869 mg mL^{-1}, 0.8317 mg mL^{-1} and 0.6879 mg mL^{-1} for SIS, BH and ET. The precision values were less then 2% RSD. for all four methods. The common excipients and additives did not interfere in their determinations. The results obtained by the proposed methods have been statistically compared by means of Student t-test and by the variance ratio F.

Three different methods developed for the determination of salbutamol sulfate (SIS), in pure drug form and in dosage forms, were discussed[33]. The methods were based on the oxidation-abomination

reaction of the drug by bromine generated in situ by the interaction of bromate with bromide in acid medium. In titrimetry the drug is titrated directly with bromate in the presence of a large excess of bromide and in sulfuric acid medium using methyl red as indicator. Spectrophotometric measuments based on addition of a measured excess of bromate-bromide mixture to the sample solution in sulfuric acid medium followed by the estimation of surplus bromine by reacting it with a definite amount of methyl orange dye and measuring the absorbance at 510 nm. The amount of bromate reacting corresponds to the sample content. The kinetic method depends on the linear relationship between the concentration of the drug and time for oxidation and bromination as indicated by the bleaching of the methyl orange acid color by the bromine generated in situ.Titrimetry is applicable in the 2-20 mg range. In spectrophotometeric method, Beer's law is obeyed in the 0.5-5.0 μgml^{-1} range whereas concentrations in the 5.0-25.0 μgml^{-1} range can be determined by the kinetic method. The effect of common excipients and additives in tablets is discussed. The procedures have been successfully applied to dosage forms.

A simple spectrophotometric method for the determination of salbutamol sulfate and pyrantel pamoate is described[(34)] . The absorbance of the blue colored species produced when the drugs react with the Folin-Ciocalteu reagent in alkaline medium is measured. The method is applicable to the concentration range of: 1.0-12.0 $\mu gml^{-1}$and 2.5-25 μgml^{-1} for salbutamol sulfate and pyrantel pamoate, respectively. The apparent molar absorptivities and Sand ell sensitivities of the colored species are found to be 2.33 x 14 $lmol^{-1}.cm^2$ and 24.68 ngcm-^{2} and 1.45 x14 $lmol^{-1}$ cm and 42.56 ng/cm^2 for salbutamol sulfate and pyrantel pamoate, respectively. Statistical data treatment is proved the accuracy and

precision of the method to be sufficient. Excipients added to the studied pharmaceuticals did not introduce any interferences. The procedure described is successfully applied for the determination of the bulk drugs and their pharmaceutical formulations.

Three spectrophotometicr methods were used for estimation of salbutamol and theophylline from tablets[35]. Crushed tablets equivalent to 50 mg of theophylline (THP) were mixed with 8 mg pure salbutamol sulfate (SIS) and the mixture is dissolved in dis water and diluted to 100 ml with 0.05 M-NaOH. The solution is filtered, then 2.4 ml of the filtrate is diluted to 50 ml with 0.05M-NaOH. Absorbance measurements was made at 244.6 and 274.6 nm, THP and SBS are determined by solving simultaneous equations. Alternatively, second-derivative absorbance spectra are obtained and measurements are made at 283.2 and 314 nm for the direct determination of THP and SBS , respectively. In a third method, the difference between the absorbance of the prepared sample at 244.6 and 283.2 nm is used to determine THP, and the difference between the absorbance's at 274.6 and 314 nm is used to determine SIS ; the wavelengths are selected on the basis of minimal interference from the component not being determined at each wavelength. Beer's law is obeyed for THP up to 20, 40 and 40 μgml^{-1} for the three methods, respectively. The corresponding figures for SIS are 20, 30 and 20 μgml^{-1}. Recoveries are quantitative for both analytes by all three methods .

Spectrophotometric methods were used for estimation of salbutamol using chlorinated quinines in tablets was described[36]. Powdered tablets equivalent to 10 mg salbutamol are heated in a boiling water bath for 20 min with ~12 ml DMF and 0.5 g activated charcoal. The solution is cooled, diluted to 25 ml with DMF and filtered. After discarding the first

few ml, the filtrate is analyzed by two methods. Method (i): a portion of the filtrate is mixed with 0.7 ml of 1% chloranil in acetone or 2 ml of 1% dichlone in acetone, the solution is heated at 60 ^{0}C for 10 and 30 min, respectively, the volume is adjusted to 5 ml with DMF and the absorbance is measured at 550 and 490 nm, respectively. For method (ii) the filtrate is mixed with 0.4 ml 1% chloranil in acetone or 0.5 ml 1% dichlone in acetone and 0.9 or 0.5 ml 10% acetaldehyde in propan-2-ol, respectively. The solutions are heated at 60 ^{0}C for 10 min, diluted to 5 ml with propan-2-ol and the absorbance is measured at 660 nm and 570 nm, respectively. Beer's law is obeyed for 100-300 mg/l of salbutamol using chloranil and 50-200 mg/l using dichlone by method (i) and 20-70 mgl^{-1} and 30-80 mgl^{-1}, respectively, by method (ii). .

A colorimetric method is developed for the determination of the four phonetic drugs, etilefrine hydrochloride(ET), ritodrine hydrochloride (RI), isoxsuprine hydrochloride(IS) and salbutamol sulfate (SIS)[(37)]. Powdered tablets, equivalent to 20 mg of each drug, are dissolved in 50ml methanol and a 4-8 ml portion of the solution is removed and evaporated to dryness on a boiling water bath. The residue is treated with 2 ml (3 ml for ET and RI) HNO_3/ H_2SO_4 (1:1) for 20 min. (10 min for ET and RI) at 80 ^{0}C (100 ^{0}C for IS). On cooling, the solution is diluted to 50 ml and a 2 ml portion is withdrawn, mixed with 5.0 ml acetone and 5 ml 2% NaOH and diluted to 25 ml with H_2O. The absorbance of the diluted solution is measured at 375, 386, 384 and 386 nm, for ET, RI, IS and SIS respectively, a reagent blank. Beer's law is obeyed from 4.8-16 µgml^{-1} of each drug and the detection limits are 0.454, 0.414, 0.452 and 0.383 µgml^{-1} for ET, RI, IS and SA, respectively. The method is applied to the analysis of various pharmaceutical preparations and the results are in agreement with those obtained by official methods.

I.II.2.Fluorimetric methods

A flow-injection method is proposed for the determination of salbutamol[38]. The method involves the condensation of salbutamol with 4-aminoantipyrine in the presence of hexacyanoferrate (HXF) in alkaline medium, producing a colored quinoneimide that is detected absorptiometrically at 500 nm. The values of four variables (two reactor lengths and two reagent concentrations) are optimized by means of the sequential simplex method and their influence studied in univariant way. The method is validated and compared with the HPLC method established in the United States Pharmacopoeia (USP). Linearity is demonstrated in the range 0-74.1 mgl^{-1} of salbutamol sulfate (r2 = 0.9999). Commercial samples of pharmaceuticals containing salbutamol sulfate (tablets and oral solutions) are analyzed and the results obtained with the proposed method agree with the USP method in less than 1.6%, with precision similar to the HPLC method (1-2% RSD). The sampling frequency is 75 samples/hour.

The determination of salbutamol with Folin-Ciocalteau reagent (FC) using a flow injection analysis technique (FIA) with spectrophotometer detection at 750 nm is described[39]. The lab-made FIA system consisted of a peristaltic pump Gilson Mini pulse 3 equipped with Tygon tubes, double 6-port external Vici Valco sample injector and S 2000/SAD500 fiber optic spectrophotometer. It was controlled by a PC with use of originally compiled Lab VIEW -- supported software containing the mathematical library with various statistical functions for off-line data evaluation. Concentration, volume of reagents and flow rate are optimized by a simplex method. The proposed system is used for the direct determination of salbutamol sulfate in the tablets and the human

urine without preliminary pre-treatment of the sample. The negative effect of interfering substances (excipients of the tablets and matrix of the urine) is overcome by a solid phase extraction (SPE), when salbutamol is adsorbed on the solid phase in the micro column, which is integrated directly into the flow system. Pre-treatment of the sample takes place directly in the flowing stream. The sample throughput without carryover of on-line SPE is 60-80 samples per hour. With the SPE column (Baker--carboxylic acid), salbutamol is determined in the linear range from 1.0 to 15 µg ml^{-1} (RSD = 1.2%), with detection limit (3sigma) 0.1 µgml^{-1} and a frequency of 40-60 samples per hour in the water solutions. The salbutamol is determined in the linear range from 2 to 20 µg ml^{-1} (RS = 1.7 %), with detection limit (3sigma) 1 µg ml^{-1} and a frequency of 30 samples per hour in the sample of the human urine.

Potentiometer detection of salbutamol sulfate using electrodes[(40)]. containing its ion-exchangers with phosphotungstic acid, phosphomolyb-dic acid or a mixture of both is applied in flow-injection systems in the Nernstian range of the response of the electrodes. The selectivity of the electrodes towards different cat ions is studied in comparison with that obtained under batch conditions. The method is subsequently used to determine the drug in its pharmaceutical preparations.

I.II.3 Chromatography methods

A HPLC and a HPCE method for the separation of two enantiomers of salbutamol sulfate was presented[(41)].The separation of HPLC is performed on a Chiral-AGP column of 100 mm x 4.0 mm. 10 $mmoll^{-1}$ Ammonium format is used as mobile phase. The separation of HPCE is performed on afused silica capillary (50 mum x 67 cm, 50cm effective length). 2% beta-Cyclodextrin sulfated sodium salt in 20 $mmoll^{-1}$ sodium

dihydr- ogen phosphate (pH3.5) is used as running buffer. Sample is injected hydrodynamically into the capillary. The applied voltage is -20kV.The detection is set at 226 nm in both methods. The two enantiomers of salbutamol sulfate could be separated by HPLC and HPLC. The resolute- ions of HPLC and HPCE are 1.4 and 14.0 respectively. The linearity range of HPCE is 5–250 μgml^{-1},the detection limit of HPCE is 2.0μgml^{-1}. The method of HPCE is better than HPLC for the separation of salbutamol sulfate, and easy and reliable.

A fast, reliable and specific method for the screening, confirmation, determination and quantization of salbutamol by HPLC is developed and validated[42].Thedescribed procedure includes asingle robust chiral HPLC determination employing a Teicoplanin stationary phase. The method is evaluated for specificity, linearity, precision and accuracy. Under the chromatographic conditions of the method, known impurities are separated from the active principle.

HPLC method for determination of salbutamol in aerosol inhalers[43]. Sample (50 mg) is sonicated with 10 ml phosphate buffer of pH 7 and 75ml 15% methanol and the mixture is filtered. Portions (10µg) of filtrate were analyzed for sodium cromoglycate (SCG) and salbutamol sulfate (SIS) by HPLC on a 5 µg Alltima C18 column (15 cm x4.6 mm I'd.),with 20 ml ammonium acetate (pH adjusted to 6 with glacial acetic acid) / methanol (10:3) as mobile phase at 1 ml/min and detection at 227 nm. The calibration graphs for SCG and SBS are linear from 0.1-0.5 and 0.0125-0.1 mg/ml, respectively. The average recoveries of SCG and SBS are 99.12and 99.98%,with corresponding RSD of1.1and 1.0%, respe-ctively.

High-performance Thin-layer chromatography method was used for determination of salbutamol[44] .The analytes (10ml) in methanolic solution at 200-400mgl^{-1} were spotted onto percolated silica gel 60F254 plates, as 8mm bands, with methan /$CHCl_3$/triethylamine(5.5/4.5/0.05)as mobile phase. The resulting chromatograms are evaluated at 276 nm with a scanning densitometer. A linear calibration is obtained between 20-580 mg/l. Assays of salbutamol sulfate and bromhexine hydrochloride gave 1.98 mg/5 ml (RSD = 1.4%) and 3.97 mg5 ml^{-1} (RSD = 1.1%) for one brand and 1.99 mg/tablet (RSD =1.62%) and 7.98 mg/tablet (RSD=1.41) for a second brand respectively. Recoveries are >98.8% .

I.II.4 Electro – analytical methods

A simple and efficient method for electrochemical analysis is used for determination of SIS using polymer film coated modified electrode[45]. A glassy carbon electrode (GCE) is successfully modified with electropolymerized film of aminosulfonic acid (ASA) in pH 6.8 phosphate buffer solution (PBS). Cyclic voltammetry (CV) were used to study the electrochemical properties of the polymer film and the appropriate condition for electropolymerization process. The voltammetric behavior of SIS at the PASA GC CMEs has been investigated, the results suggest that the PASA GC CMEs have good effect of electrocatalytic oxidation action to SIS, also propose the mechanism toward SIS. The flow-injection irreversible biamperometry analysis method was studied under the applied potential difference of 0.0 V to determinate SIS. In 0.1 mol l^{-1} (pH 6.80) phosphate buffer solution, a sensitive and irreversible oxidation peak was obtained at the PASA GC CMEs. Under the optimum conditions, SIS can be determined from the range 2.0 x 10^{-6} to 1.0 x 10^{-3} mol l^{-1} with the sampling frequency of 100 samples per hour. The detection limit for SIS was 6.5 x 10^{-7} mol l^{-1} and

the RSD for 20 replicate determinations of 4.0 x 10-5 mol l^{-1} SBS is 1.85%. The method is simple, with high selective rapid and sensitive. The method is applied to the determination of SIS in the drug with satisfactory results. Moreover, the physiologically common interferents (i.e., sucrose, lactose, citric acid, and citrate) negligibly affected the response of SBS. The PASA GC CMEs film-coated electrode exhibited a stable and sensitive response to salbutamol sulfat in the presence of electrocatalysis oxidation.

Conductmetric method for determination of salubtamol sulfate in their pharmaceutical formulations was described[(46)]. Tablet or syrups containing one of the bronchodilators are extracted or diluted with water, the conductivity cell is immersed in solution and the solutions are titrated with 10mlM-phosphotungstic acid or phosphom- olybdic acid. Readings are taken at 1-2 min after additions and corrected for dilution. Recoveries from standards of 17.3-103.8 mg of salbutamol sulfate, 7.79-62.31 mg of reporter hydrochloride and 13.08-78.48 mg of pipazethate hydrochloride with both titrants are in the range 99.54-100.54%, with RSD (n= 5) of 0.16-0.52%. Similar values for the nominal contents are obtained with commercial formulations.

An internal solid contact salbutamol ion-selective sensor is fabricated[(47)] based on a conducting poly aniline film immobilized on a glassy carbon electrode surface and a plasticized poly vinyl chloride (PVC) sensing membrane containing an ion-pair complex of salbutamol (SIS) with tetraphenylborate. Effects of various conditions for the electro polymerization of aniline, including the conducting support, aniline concentration, solution pH and polymer film thickness are investigated. The sensor showed a Nernstian slope of 60.4 mV/decade (25^0C) in the

SIS concentration range of 1.0 $x10^{-1}$ to $4.0x10^{-5}$M, low detection limit (1.2×10^{-5} M), and fast response time (15 ~ 30 s). This sensor is successfully applied to the determination of SIS in the pharmaceutical preparation which showed 99.1-100.6% recovery.

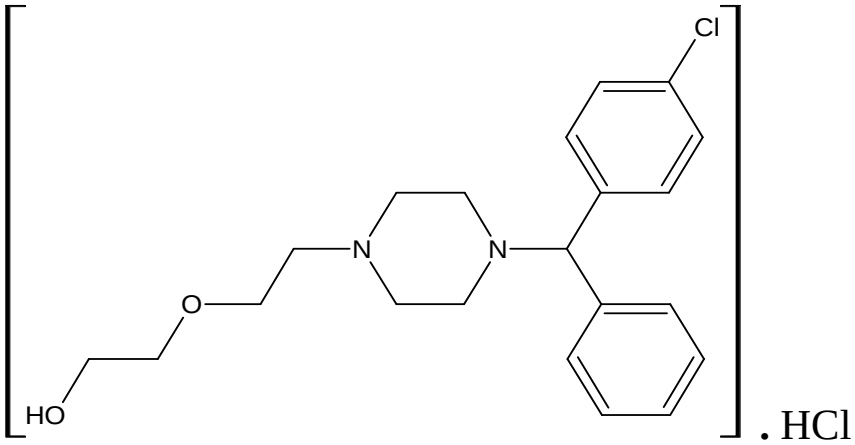
I.III. Hydroxyzine Hydrochloride

Hydroxyzine hydrochloride is an antihistamine with ant cholinergic (drying) and sedative properties that is used to treat allergic reactions. Histamine is released by the body during several types of allergic reactions and to a lesser extent during some viral infections, such as the common cold. When histamine binds to its receptors on cells, it causes changes within the cells that lead to sneezing, itching, and increased mucus production. Antihistamines compete with histamine for cell receptors, however, when they bind to the receptors they do not stimulate the cells. In addition, they prevent histamine from binding and stimulating the cells. After ingection, the molecule of hydroxyzine is changed slightly, and this changed hydroxyzine that also binds to cells.(This changed hydroxyzine, called an active metabolite--is cetirzine (Zyrtec), which is now an FDA-approved drug.) Though both hydroxyzine and cetirizine act as antihistamines, hydroxyzine causes sedation as a side effect, and cetirizine does not[48,49]. Hydroxyzine is originally approved by the FDA in 1957.

Hydroxyzinehydrochloride[50] is maximal effect about 30 to 60 minutes after it is taken. Its effects last for 4 to 6 hours. Therefore, it is often prescribed to be taken every 4 to 6 hours as needed for relief of allergy-related symptoms. When used to combat insomnia, it is prescribed to be taken 30 to 60 minutes before bedtime as needed. Patients over the age of 60 years are especially sensitive to the sedating effects of hydroxyzine, and the dose should be reduced. Hydroxyzine can be taken with or without food.

Chemical Structure

Hydroxyzine Hydrochlorid (HXZ)



Empirical Formula : $C_{21}H_{27}Cl\ N\ _2O_2HCL$

Chemical Name : 1-(p-chlorobenzhydryl)4-(2-(2-hydroxyethoxy)- ethyl) piperazine –one monohydrochloride.

Molecular Weight : 447.8 g mol^{-1}

Appearance : White Crystalline powder or it is odor less .

Melting Range : 192–193 ^{0}C.

Solubility : Freely soluble in water, alcohol and methyl alcohol .

Packaging and storage : Preserve in tight containers.

Uses : antihistamine (especially for purities) .

I.III. Literature review

I.III.1 spectrophotometric methods.

Spectrophotometer method for the determination of hydroxyzine hydrochloride by the formation of hydroxyzinium tetraiodocadmiate (HXZ)[51].by reaction with cadmium nitrate and the determination of the excess Cd by EDTA titration. An aqueous solution of hydroxyzine hydrochloride (containing 5.1to51mg of the pure substance) is mixed with 20.0 ml of 6.35mM-$CdNO_3$, 5 ml of 2M-H_2SO_4 and 20ml of 10% KI. The white ppt of solution is filtered off and washed. Cadmium is determined in the filtrate and washings by titration with 20 ml EDTA using Eriochrome Black T indicator.

I.III.2. Chromatography methods

A rapid, simple and specific liquid chromatography-electrospray ionization mass spectrometry method has been developed and validated for the determination of hydroxyzine hydrochloride in human plasma[52]. Samples were separated using a Thermo Hypersil-HyPURITYC18 reversed-phase column (150 mm x 2.1 mm i.d., 5 mum). The mobile phase consisted of 50 mM ammonium acetate (pH 4.0)-methanol – acetonitrile (45 : 36 : 19, v/v). Hydroxyzine and its internal standard were measured by electrospray ion source in positive selective ion monitoring mode. The method was validated with a linear range of 1.56-200.0 g mL^{-1} and the lowest limit of quantification was 1.56 g mL^{-1} for hydroxyzine hydrochloride (r_2 = 0.9991). The extraction efficiencies were about 70 % and recoveries of the method were in the range of 93.5-104.4 %. The intra-day relative standard deviation (RSD) was less than 8.0 % and inter-day RSD was within 7.4%. QC samples were stable when kept at

ambient temperature for 12 h at -20 ^{0}C for 30 days and after four freeze-thaw cycles. The method has been successfully applied to the evaluation of pharmacokinetics and bioequivalence of two hydroxyzine hydrochloride formulations in 12 healthy Chinese volunteers after an oral dose of 25 mg.

An HPLC method for the simultaneous determination of ephedrine hydrochloride, theophylline, papaverine hydrochloride and hydroxyzine hydrochloride in anti-asthmatic tablets is described[(53)]. Tablets are pulverized and extracted by solvent [acetonitrile/H_2O (1:4) adjusted to pH 2 with o-phosphoric acid] with sonication followed by filtration. Analysis of 10 µg is performed on a Nucleosil 5 µg C18 column (15cmx 4.6 mm i.d.) connected to a dual channel variable programmable wavelength detector. Two solvents are used as mobile phase: acetonitrile/H_2O (1:19) adjusted to pH 2.4 with o-phosphoric acid (A) and acetonitrile/H_2O (2:3) also adjusted to pH 2.4 (B). The composition of mobile phase is changed from 2%B, 98%A to 100% B, 0%A, according to a gradient programmer (graphically presented) at 1.5 ml/min and detection at 220 and 240 nm (programmer details given). Linearities, recoveries and RSD details are tabulated. The method is reliable, reproducible and easy to perform.

Hydroxyzine is determined in injection solution[(54)] by HPLC on Partisil PAC-10 cyanoamine with 10 ml N-K_2HPO_4 of pH 7-acetonitrile (4:1) as mobile phase, tetracine hydrochloride as internal standard, and detection at 232 nm. Recovery is 100.1%, coeff. of variation are 0.25 to 1.2% and the limit of detection is ~22 ng. The separation took less than 4 min.

A voltammetric procedure was used for determination hydroxyzine hydrochloride[(55)]. electrochemical study of hydroxyzine at a glassy

carbon electrode was carried out in the Britton-Robinson universal buffer of pH 2-11. Hydroxyzin was oxidized in a single two-electron irreversible process controlled mainly by adsorption. A simple, sensitive and time-saving square-wave adsorptive anodic stripping voltammetric procedure has been developed for determination of hydroxyzine in its commercial tablets and human serum without prior extraction. The optimized procedural conditions were: frequency = 120 Hz, scan increment =10 mV, pulse-amplitude = 25 mV, accumulation potential = -0.3 V, accumulation time = 90-300 s and a Britton-Robinson universal buffer of pH 4 as a supporting electrolyte. Mean recoveries of 100.5 ± 0.71 and 98.6 ± 1.12 % (n = 5) were achieved for assay of hydroxyzine in AtaraxR 10 and 25 mg dosage forms, respectively. Limit of detection of 1.5 x 10^{-8} mol L^{-1} (5.624 ng mL^{-1}) and limit of quantitation of 5.0 x 10^{-8} mol L-1 (18.746 ng mL^{-1}) were achieved in human serum with a mean recovery of 98.4 ± 1.22%, without prior extraction of the drug. Moreover, the described procedure was applied for evaluating the pharmacokinetic parameters of hydroxyzine in plasma of two healthy volunteers after administration of a single oral dose (AtaraxR--25 mg).

The conductmetric titration of hydroxyzine hydrochloride (I) with ammonium molybdate is studied in aqueous medium[(56)]. Measurements were made below the critial micelle concentration of I (i.e, 41.7mlM at 298 K) by adding 0.1 ml portions of the titrant with stirring (2 min) and standing (2 min), in order to attain equilibrium before the next addition. The determination is repeated five times and mean concentration. values are calculated. The calibration graph is rectilinear from 0.4-1.6 mg ml^{-I}, and the coeff. of variation ranged from 0.1-0.3 %. The method provided a fast and accurate means for determining hydroxyzine hydrochloride in pharmaceutical preparations.