An Innovative Spectrophotometric Method for the Estimation of H₁ Receptor Antagonists in Pharmaceutical Formulations

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Patel *et al.*: Estimation of H1 Receptor Antagonist

An innovative, sensitive, simple and rapid method has been developed for estimation of selective H1 receptor antagonist. The method is based on bromination of drug. The proposed spectrophotometric method was developed using potassium bromate-potassium bromide mixture in acidic condition followed by the reaction of surplus bromine with methyl orange dye and measure the absorbance at 506 nm. Beer's law was obeyed in the concentration range of 0.2-1.2 μg/ml, 0.3-1.7 μg/ml and 0.03-0.18 μg/ml for olopatadine hydrochloride, fexofenadine hydrochloride and azelastine hydrochloride respectively. The method was optimized for concentration of hydrochloride, ml of hydrochloride, ml of potassium bromate-potassium bromide mixture, ml of dye and reaction time. Developed method has been validated in accordance to International Council on Harmonisation guideline Q2R1. Statistical analysis proved that the method is accurate, sensitive, selective, precise and reproducible. The simple procedure with high accuracy, wide linearity range and sensitivity imply that the demonstrated method to be appropriate for routine estimation and quality control assay of pharmaceutical formulations.

Key words: Olopatadine hydrochloride, fexofenadine hydrochloride, azelastine hydrochloride, optimization, validation, potassium bromate-potassium bromide mixture

Olopatadine hydrochloride (HCl) (OLO), 2-[(11Z)-11-[3-(dimethylamino)propylidene]-6H-benzo[c][1] benzoxepin-2-yl]acetic acid hydrochloride[1] (fig. 1) is a white crystalline powder, almost odourless soluble in double distilled water, methanol and chloroform. It is commonly used as an anti-allergic agent. OLO is official in Indian Pharmacopoeia[2], United state pharmacopeia[3]. Fexofenadine HCl (FEX), 2-[4-[1-hydroxy-4-[4-[hydroxy(diphenyl)methyl] piperidin-1-yl] butyl] phenyl]-2-methylpropanoic acid hydrochloride[4] (fig. 2) is a white crystalline powder, almost odourless soluble in double distilled water, methanol and chloroform. It is commonly used as an anti-allergic agent. FEX is official in Indian Pharmacopoeia[5], United state pharmacopeia[6], British Pharmacopoeia[7] and European Pharmacopoeia[8]. Azelastine HCl (AZE), 4-[(4-chlorophenyl)methyl]-2-(1-methylazepan-4-yl)phthalazin-1-one hydrochloride[9] (fig. 3) is a white crystalline powder, almost odourless soluble in double distilled water, methanol and chloroform. It is commonly used as an anti-allergic agent. AZE is official in Indian Pharmacopoeia[10], British Pharmacopoeia[11] and European Pharmacopoeia[12].

OLO, FEX and AZE are selective histamine H1 antagonists and mast cell stabilizers that work by attenuating inflammatory and allergic reactions used for the treatment of seasonal and perennial allergic rhinitis and urticaria. Literature survey reveals that many analytical methods are reported for determination of mentioned H₁ receptor antagonist such as spectrophotometry[13-16], voltammetry[17-19], chromatography[20-22], high performance liquid chromatography[23-28], high performance thin layer chromatography[29], Ultra-performance liquid chromatography (UPLC)[30], Liquid chromatography–
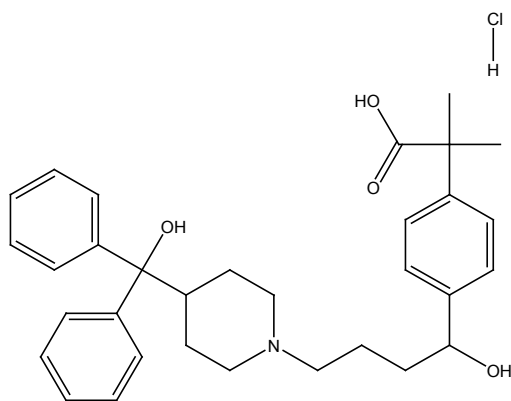
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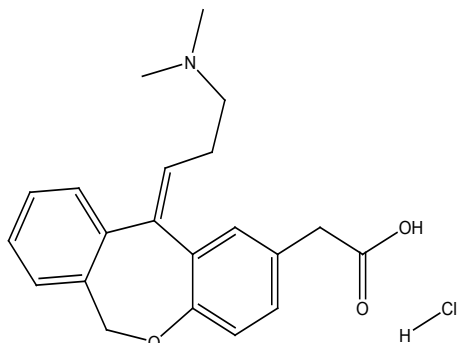
Fig. 1: Olopatadine HCl

Fig. 2: Fexofenadine HCl
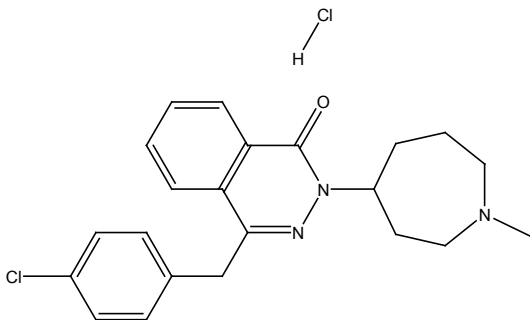
Fig. 3: Azelastine HCl

mass spectrometry (LC–MS)[31] and stability indicating method[32] for have been employed. The objective of this study is to develop a relatively economic, valid, accurate, precise and sensitive colorimetric method for the estimation of various H_1 receptor antagonists in the pure form and pharmaceutical dosage form. Since most of the reported methods have been found to be less sensitive and complicated, there is a true demand to develop a sensitive method for the estimation of same. Thus, the present investigation aims to develop a sensitive and cost-effective method for the estimation of various H1 receptor antagonists in the pharmaceutical dosage form using the spectrophotometric technique. The proposed method has the advantages of great sensitivity and simplicity along with good accuracy and precision. The method is applied successfully for the

estimation of various H1 receptor antagonists like OLO, FEX and AZE in the respective pharmaceutical dosage forms without the interference of excipients. The color developed was stable for a long period of time; hence, this method can be extended for the routine assay of OLO, FEX and AZE in the respective pharmaceutical formulations. The method was validated as compliance with International Conference on Harmonization (ICH) guidelines[33].

MATERIALS AND METHODS

OLO of pharmaceutical grade was kindly supplied as gift sample by USV Pvt. Ltd., Mumbai, India and were certified to contain 99.65 % (w/w), on dried basis. The nasal spray containing 665 µg/spray OLO was procured from Walgreens, market of USA which is used for analysis of pharmaceutical formulation. FEX of pharmaceutical grade was kindly supplied as gift sample by Camper healthcare, Ganpat Vidyanagar, Mehsana, India and were certified to contain 99.75 % (w/w), on dried basis. The FEX tablets contain 60 mg/tablet FEX was procured from Walgreens, market of USA which is used for analysis of pharmaceutical formulation. AZE of pharmaceutical grade was kindly supplied as gift sample by Sun pharma Pvt. Ltd. Vadodara, Gujarat, India and were certified to contain 99.89 % (w/w), on dried basis. The nasal sprays were procured from Janata super market, Mehsana, Gujarat, India containing 0.1 % w/v of AZE. Analytical reagent (AR) graded potassium bromate, potassium bromide, methyl orange dye, concentrated HCl and double distilled water used were purchased from Finar Chemicals Pvt. Ltd. The spectrophotometric analysis was performed using a double beam ultraviolet (UV)-visible spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells. In addition, analytical balance (CP224S, Sartorius, Germany), ultrasonic cleaner (Frontline FS 4, Mumbai, India), volumetric flasks, beakers and pipettes of borosilicate glass were used in the study.

Preparation of stock solution:

Stock solutions were prepared by weighing 10 mg of OLO, FEX and AZE respectively. The weighed drugs were transferred to the separate 100 ml volumetric flask for OLO, FEX and AZE respectively and label them appropriately. Volumes were made up to the mark with double distilled water to obtain a solution containing

100 µg/ml. The solutions was further diluted with the same solvent to obtain final concentration of 10 µg/ml for OLO and FEX where 1 µg/ml for AZE.

Preparation of reagents:

Preparation of HCl solution xM: HCl solution (0.1, 0.2, 0.3, 0.4 and 0.5 M), prepared by diluting the appropriate volume (xM HCl prepared by transferring 85x ml of concentrated HCl to 1000 ml) of concentrated acid with double distilled water.

Preparation of potassium bromate-potassium bromide (KBr-KBrO$_3$) mixture: Accurately weighed 1.67 g KBrO3 and 5.95 g KBr and dissolved in 100 ml distilled double distilled water[34,35] then transferred 10 ml of above solution to 100 ml volumetric flask and diluted up to mark with double distilled water.

Preparation of methyl orange dye: Dissolve 0.01 % methyl orange in double distilled water.

General procedure:

Accurately transferred 1 ml of OLO, FEX and AZE stock solutions to 10 ml separate volumetric flasks then added 2 ml 0.1 M HCl and added 2 ml KBr-KBrO$_3$ reagent in all 10 ml volumetric flasks, allowed the reaction mixtures to bromination of drugs for 30 min, lastly excess bromine reacted with added 2 ml methyl orange dye[35] and formed pink colour in acidic condition.

Optimization of experimental variables:

The effect of acid concentration on the measured species was investigated by following the general procedure. The effect of 2 ml of HCl of different concentrations (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 M) was studied by measuring the absorbance of the coloured product using a fixed concentration of drugs 1.0 µg/ml (fig. 4), simultaneously the effect of ml of optimized concentration of HCl (0.5-3.0 ml) was investigated by same procedure (fig. 5), it is clear that the absorbance of the coloured product remained constant with 2.0-3.0 ml of 0.2-0.5 M HCl, 2.0-3.0 ml of 0.2-0.5 M HCl and 1.5-3.0 ml of 0.1-0.5 M HCl for OLO, FEX and AZE respectively. Therefore, 2.0 ml of 0.2 M HCl for OLO and FEX, 1.5 ml of 1.0 M HCl for AZE was selected for method.

The effect of ml of KBr-KBrO$_3$ mixture and ml of methyl orange dye were optimized, too by following general procedure. The effect of different ml of KBr-KBrO$_3$ mixture (1.0-6.0 ml, fig. 6) and methyl orange dye (0.5-3.0 ml, fig. 7) were observed by measuring the absorbance of coloured products using same concentration of drugs, it is clear that the coloured products gave highest absorbance with 3.0, 2.0 and 4.0 ml of KBr-KBrO$_3$ mixture and 2.0, 2.0 and 2.5 ml of methyl orange dye for OLO, FEX and AZE respectively.

The effect of time on the reaction between drugs and KBr-KBrO$_3$ mixture in the presence of HCl was studied by keeping all other reaction conditions unchanged. The absorbance of the coloured products were measured at different time intervals (5.0-78.0 min, fig. 8) and the result showed that the reaction was completed after 40, 55 and 30 min and remained stable for at least 22-24 h, 5-6 h and 28-30 h for OLO, FEX and AZE respectively (fig. 9).

Calibration curve:

Appropriate aliquots (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) stock solution of OLO were transferred to 10 ml volumetric flasks, then added 2.0 ml of 0.2 M HCl followed by 3.0 ml of KBr-KBrO$_3$ mixture to each flask, keep all the flasks for 40 min at least to complete bromination of drug, lastly added 2.0 ml of methyl orange dye and diluted up to mark with double distilled water to obtain final concentrations 0.2-1.2 µg/ml.
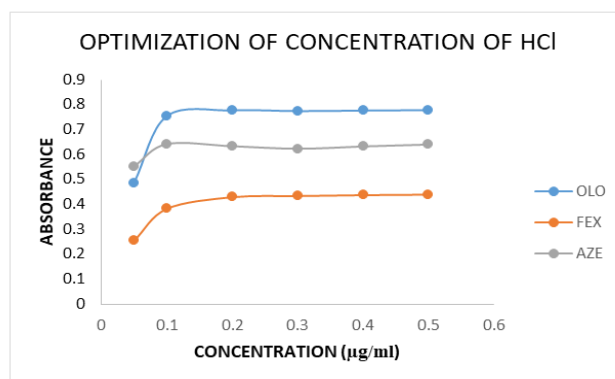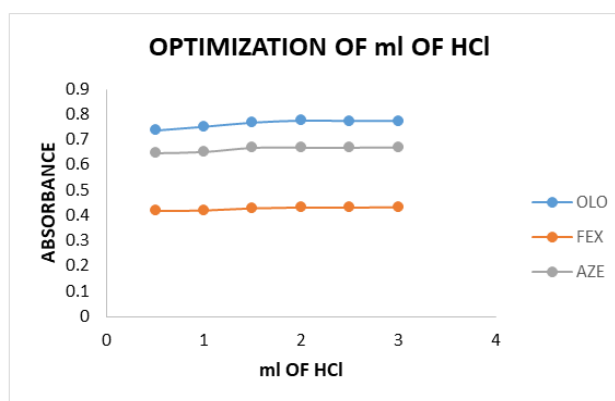

Fig. 4: Optimization of concentrations of HCl

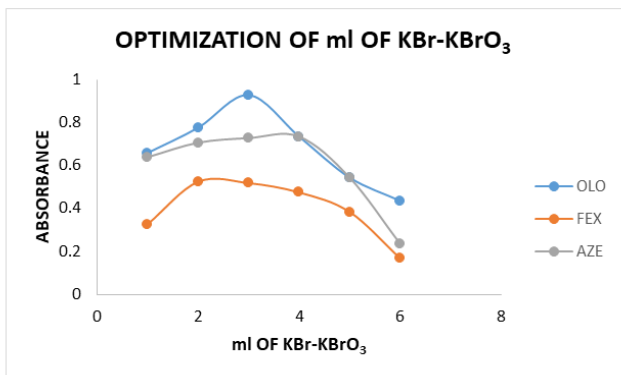**Fig. 5: Optimization of ml of HCl**

OPTIMIZATION OF ml OF KBr-KBrO₃

Fig. 6: Optimization of ml of KBr-KBrO₃

OPTIMIZATION OF ml OF DYE
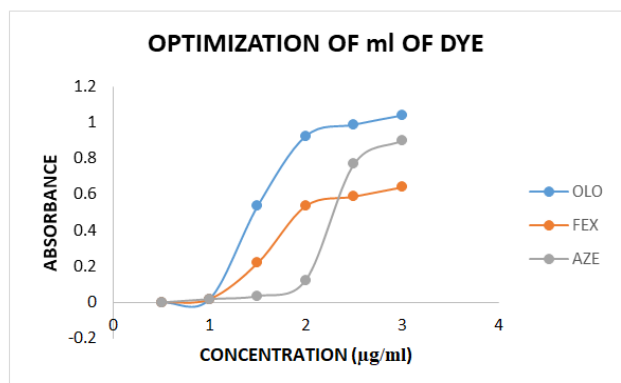
Fig. 7: Optimization of ml of methyl orange dye

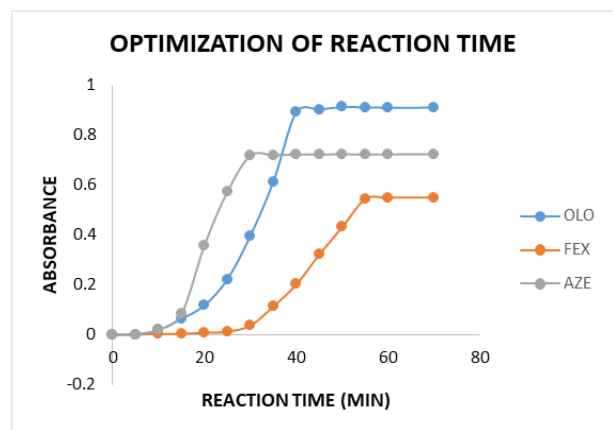**OPTIMIZATION OF REACTION TIME**

Fig. 8: Optimization of reaction time

Appropriate aliquots (0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5 and 1.7 ml) stock solution of FEX were transferred to 10 ml volumetric flasks, then added 2.0 ml of 0.2 M HCl followed by 2.0 ml of KBr-KBrO₃ mixture to each flask, keep the all flasks for 55 min at least to complete bromination of drug, lastly added 2.0 ml of methyl orange dye and diluted up to mark with double distilled water to obtain final concentrations 0.3-1.7 μg/ml. Appropriate aliquots (0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 ml) stock solution of AZE were transferred to 10 ml volumetric flasks, then added 1.5 ml of 0.1 M HCl followed by 4.0 ml of KBr-KBrO₃

mixture to each flask, keep the all flasks for 30 min at least to complete bromination of drug, lastly added 2.5 ml of methyl orange dye and diluted up to mark with double distilled water to obtain final concentrations 0.03-0.18 μg/ml. The solutions were mixed well and scanned in the visible range (400-800 nm, fig. 10), absorbances were recorded at wavelength of maximum absorbance (λ_{max}) (506 nm). Calibration curves were constructed (fig. 11-fig. 13) by plotting absorbances versus concentrations of drugs and regression equations was computed.

Analysis of marketed formulation:

Accurately transferred 0.1 ml OLO nasal spray solution to 100 ml volumetric flask diluted up to the mark with double distilled water. Dissolve 1 FEX tablet powder to 100 ml volumetric flask, sonicate for 30 min to completely dissolve FEX and filter properly and diluted up to mark with double distilled water, dilute the above solution to obtain final concentration 10 μg/ml. Accurately transferred 0.5 ml AZE nasal spray solution to 100 ml volumetric flask diluted up to mark with double distilled water. Transferred 1 ml of sample solutions
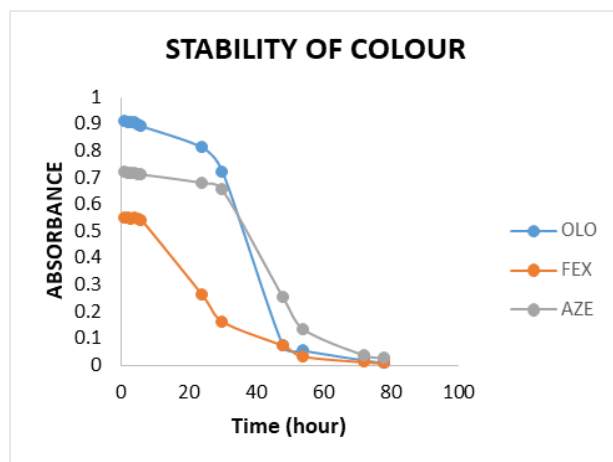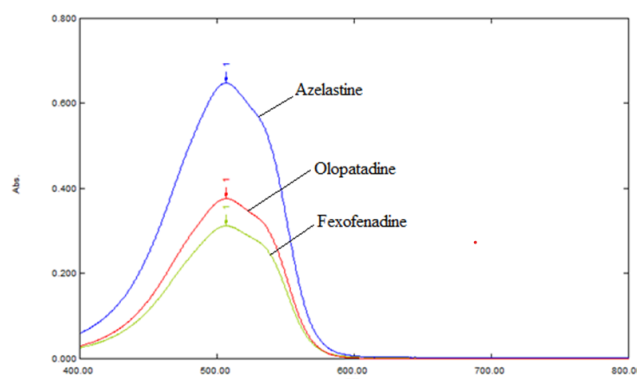
STABILITY OF COLOUR

Fig. 9: Stability of colour

Fig. 10: Representative absorbance spectra of OLO (0.4 μg/ml), FEX (0.7 μg/ml) and AZE (0.09 μg/ml) at 506 nm
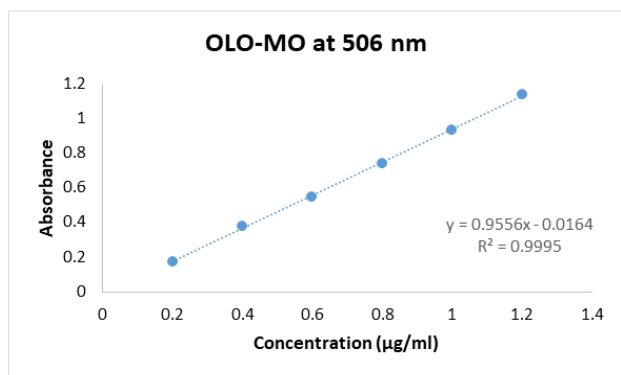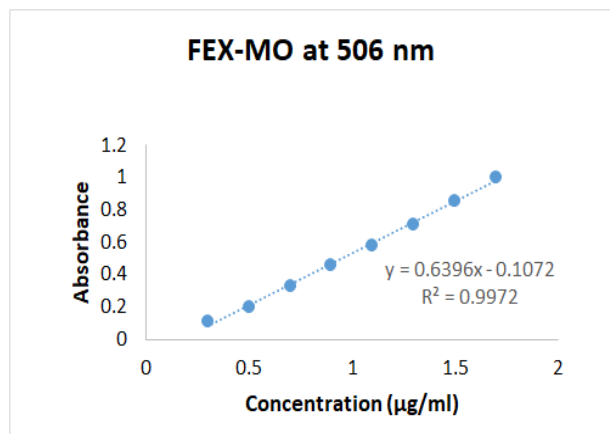
Fig. 11: Calibration curve of OLO

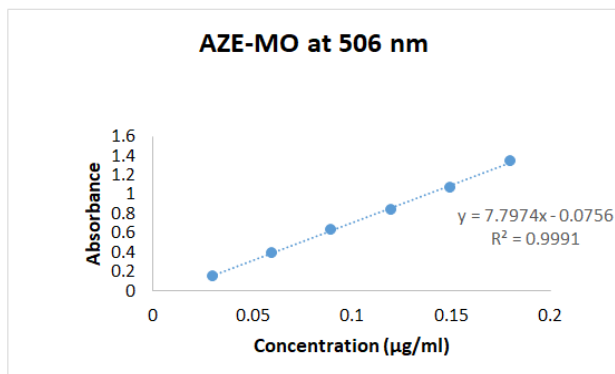**Fig. 12: Calibration curve of FEX**

Fig. 13: Calibration curve of AZE

to 10 ml separate volumetric flask and followed the optimized procedure to obtain coloured product. Absorbance of obtained solution was measured at 506 nm and the concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from Beer's law.

Method validation:

The proposed method was validated as per the ICH guideline Q2R1, for the parameters like accuracy, linearity, precision, detection limit and quantitation limit. The linearity of the method was performed with the concentrations 0.2-1.2 µg/ml, 0.3-1.7 µg/ml and

0.03-0.18 µg/ml of OLO, FEX and AZE respectively. Calibration curves were constructed by plotting absorbances versus concentrations of drug and regression equations was computed (Table 1).

The method precision (repeatability of the instrument was checked by repeated scanning (n=6) and measuring the absorbance of solution of OLO (0.6 µg/ml), FEX (1 µg/ml) and AZE (0.06 µg/ml) without changing the parameter of the method. Also, the intra-day and inter-day precision of the proposed method was evaluated by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 w for 3 different concentrations of sample solutions of OLO (0.4, 0.6 and 0.8 µg/ml), FEX (0.6, 0.8 and 1.0 µg/ml) and AZE (0.06, 0.09 and 0.12 µg/ml). The results were reported in terms of percentage relative standard deviation (% RSD).

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated using following formulae: LOD=3.3 (SD)/S and LOQ=10 (SD)/S, where SD=standard deviation of response and S=average of the slope of the regression line.

The accuracy of the method was determined by calculating recoveries by the standard addition method[13]. Known amounts of standard solution of OLO and FEX were added at 50 %, 100 % and 150 % levels to pre quantified sample solutions of drugs. Where, AZE was added at the known amount of 80 %, 100 % and 120 % levels to pre quantified sample solutions of drugs.

RESULTS AND DISCUSSION

Bromate-bromide mixture in acid medium shows as an equivalent solution of bromine and has been widely used for the assay of several organic and bio active pharmaceutical compounds. The proposed method describes the *in situ* generation of bromine by the action of the hydrochloric acid on $KBr-KBrO_3$ mixture. In the present method varying concentrations of drug solutions were reacted with a fixed and known excess amount of generated bromine in hydrochloride acid medium and after a predetermined time, the un-reacted bromine is determined by treating with a known fixed amount of methyl orange and measuring the absorbance at 506 nm (fig. 8). A linear relation has been found between absorbance and concentration of drugs which formed the basis for quantification of the drug.

The calibration curve was found to be linear over the range of 0.2-1.2 µg/ml, 0.3-1.7 µg/ml and

TABLE 1: REGRESSION DATA FOR CALIBRATION CURVE

Parameters	OLO	FEX	AZE
Wavelength (nm)	506 nm	506 nm	506 nm
Beer's law limit (µg/ml)	0.2-1.2	0.3-1.7	0.03-0.18
Regression equation			
y=mx+c	y=0.9553x+0.0165	y=0.6396x-0.1072	y=7.7974x-0.0756
Slope (m)	0.9553	0.6396	7.7974
Intercept (c)	0.0165	0.1072	0.0756
Correlation coefficient (r^2)	0.9995	0.9972	0.9991

TABLE 2: ACCURACY DATA

Drug	Level	Amount present (µg/ml)	Amount added (µg/ml)	% Mean recovery±*SD
	I	0.3694	0.2	98.37±1.42
OLO	II	0.3694	0.4	99.12±0.73
	III	0.3694	0.6	98.99 ±1.00
	Level	Amount present (mg/tablet)	Amount added (mg)	% Mean recovery±*SD
	I	59.56	30.00	97.64±1.43
FEX	II	59.56	60.00	98.94±1.56
	III	59.56	90.00	96.68±0.64
	Level	Amount present (µg/ml)	Amount added (µg/ml)	% Mean recovery±*SD
	I	0.05	0.04	101.6±1.00
AZE	II	0.05	0.05	102.4±1.48
	III	0.05	0.06	102.6±0.87

*SD: Standard Deviation

TABLE 3: SUMMARY OF VALIDATION PARAMETERS FOR AN INNOVATIVE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF H_1 RECEPTOR ANTAGONIST IN PHARMACEUTICAL FORMULATIONS

Parameters	OLO	FEX	AZE
Wavelength (nm)	506 nm	506 nm	506 nm
Beer's law limit (µg/ml)	0.2-1.2	0.3-1.7	0.03-0.18
Regression equation			
y=mx+c	y=0.9553x+0.0165	y=0.6396x-0.1072	y=7.7974x-0.0756
Slope (m)	0.9553	0.6396	7.7974
Intercept (c)	0.0195	0.1072	0.0756
Correlation coefficient (r^2)	0.9995	0.9972	0.9991
Method precision Repeatability (n=6, % *RSD)	0.25	0.31	0.3
Interday precision (n=3, % *RSD)	1.32-1.79	1.14-1.88	0.43-1.70
Intraday precision (n=3, % *RSD)	0.57-1.30	0.73-1.05	0.66-1.32
LOD (µg/ml)	0.01	0.02	0.001
LOQ (µg/ml)	0.04	0.07	0.004
% Recovery±*SD (n=3)	99.33±0.96	98.42±0.70	97.84±0.90
Assay±*SD (n=3)	98.24±1.36	97.71±1.30	102.2±0.54

*SD: Standard Deviation; *RSD: Relative Standard Deviation

0.03-0.18 µg/ml for OLO, FEX and AZE respectively. The data of regression analysis of the calibration curves is shown in Table 1. The proposed method was successfully applied to the determination of pharmaceutical dosage forms of respective drugs. The results were comparable with the corresponding labelled amounts. The developed method was also found to be linear. The results of repeatability data was found to be 0.25, 0.31 and 0.30 RSD values for OLO, FEX and AZE respectively, low value of RSD indicates that proposed method is repeatable. The RSD values for inter-day precision were 1.32-1.79, 1.14-1.88 and 0.43-1.70, while for intra-day precision were 0.57-1.30, 0.73-1.05 and 0.66-1.22 for OLO, FEX and AZE respectively. The RSD values of intermediate precision less than 2 indicates the propose method is reproducible.

TABLE 4: ASSAY RESULTS FOR NASAL SPRAY FORMULATION

Sample No.	OLO		FEX		AZE	
	Label claim (µg/spray)	% Label claim (%)	Label claim (mg/tablet)	% Label claim (%)	Label claim (% w/v)	% Label claim (%)
1	665	98.76	60	99.27	0.1	97.54
2	665	97.84	60	98.09	0.1	97.82
3	665	98.28	60	98.56	0.1	98.41
4	665	98.57	60	95.51	0.1	96.08
5	665	99.07	60	97.05	0.1	98.95
6	665	98.00	60	97.78	0.1	98.21
Mean		98.42	Mean	97.71	Mean	97.84
*SD		0.465	*SD	1.30	*SD	0.902

*SD: Standard Deviation

The LOD value was found to be 0.01, 0.02. 0.0015 µg/ml of OLO, FEX and AZE respectively, while LOQ value was 0.04, 0.07, 0.0045 µg/ml of OLO, FEX and AZE respectively. The results of recovery studies (Table 2) were 98.83±0.40, 98.68±0.63 and 102.6 ±0.87 % of OLO, FEX and AZE respectively. Findings of all validated parameters are summarized in Table 3. The proposed study, spectrophotometric method was developed for the estimation of H1 receptor antagonists and validated as per ICH guidelines.

Statistical analysis proved that method was accurate, precise and repeatable. The developed method was found to be simple, sensitive and inexpensive for analysis. The method was successfully used for determination of drug in a pharmaceutical formulation. Assay (Table 4) results for nasal spray formulation of OLO using proposed method showed 98.42±0.47 %, tablet of FEX contains 97.71±0.71 and nasal spray formulation of AZE contains 97.84±0.90.

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