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Development HPLC technique for determining Oxymetazoline and Isoxspurine in pharmaceutical formulations

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Abstract

A fast, high-performance liquid chromatography method was established to estimate Oxymetazoline hydrochloride and Isoxsuprine hydrochloride in pharmaceutical preparations HPLC. It consists of an Azorbax-C8 (250mm ×4.6 mm, 5µm) column with a mobile phase composed of methanol:acetonitrile: buffer (0.01 M) potassium dihydrogen phosphate with triethylamine (pH 5.5) (50: 10:40, V/V/V) eluted at 1.5 ml/min flow rate. The detection was done using a photodiode array (PDA) detector. The proposed method was validated in linearity, precision, and accuracy. The retention times of IsoxsuprineHCl and OxymetazolineHCl are 3.6 and 6.2 ±0.4 min, respectively. For both drugs, the linear range and detection limits were 1-250 µg/ml and 0.5 µg/ml, respectively. The proposed method was successfully applied to determine ISX and OXY in pharmaceutical preparations.

Key words: Oxymetazoline Hydrochloride, HPLC, Isoxsuprine Hydrochloride

1. Introduction

Isoxsuprine hydrochloride is a vasodilator that stimulates beta-adrenergic receptors. It causes blood vessels and uterine smooth muscle relaxation, and its vasodilatory effect is more pronounced in arteries supplying power than those supplying skin [1]. USP XXI [2] uses UV spectrophotometry to measure aqueous solutions of ISX around 269 and 300 nm, while the British Pharmacopoeia [3] recommends using $HClO_4$ and 1-naphtholbenzene as indicators for non-aqueous visual titration.

Oxymetazoline hydrochloride is an imidazoline derivative. This non-selective adrenergic drug used in eye and nose drops, acts on adrenergic receptors, causing severe vasospasm and increased blood pressure [4]. Oxymetazoline overdose does not cause serious toxicity, except for minor side effects in the central nervous and/or cardiovascular system caused by systemic absorption of the oxymetazoline drug into the nasal mucosa [5].

Various analytical methods have been used to determine isoxoprene hydrochloride, including high performance liquid chromatography[6-8], fluorescence[9], flow injection[10,11], spectrophotometry [12-17], gas chromatography[18] and for the determination of oxymetazoline hydrochloride, including HPLC[19-22], spectrophotometry[23-29], flow injection chemistry Luminescence[30], Ion Selective Electrode[31- 36] and Fluorescence Spectrophotometry[37] methods.

The goal of this study was to create a sensitive, fast, and repeatable HPLC technique for detecting Oxymetazoline hydrochloride and Oxymetazoline hydrochloride in pharmaceutical formulations.

2. Experimental

2.1. Apparatuses

- HPLC, short for High-Performance Liquid Chromatography (Agilent, USA). A quaternary pump from 1200 series, a photodiode array detector, An autosampler injector from Agilent, and a vacuum degasser come from the United States. HPLC present in Sohag Clinical Toxicology Lab– Sohag University Hospitals.

2.2. Materials and solvents:

- Isoxsuprinehydrochloride (99.9% m/m) was purchased from sigma and pharmaceutical grade

-Oxymetazoline hydrochloride (99.9 percent m/m) was acquired from the Iraqi state enterprise for Drug Industries, and Medical Appliances, SDI.

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- triethylamine liquid (TEA) purchased 99% from Merck, Germany.

-Methanol and acetonitrile (ACN) (all are HPLC grade) were purchased from Sigma-Aldrich (German)

-Potassium dihydrogen phosphate powder and Orthophosphoric acid liquid were purchased from Sigma- Aldrich Company (Germany)

-sodium hydroxide powder was purchased from an Egyptian Company

-Deionized water is obtained from human power one water deionizer, Sohag clinical toxicology laboratory.

Prepared all solutions with analytical grade chemicals and deionized water.

-We also made Isoxsuprine stock solution with a concentration of 1000µg/ml by dissolving 0.01g Isoxsuprine hydrochloride in 10 ml of deionized water

.- In addition, we prepared a stock solution of oxymetazoline with a concentration of1000µg/ml by dissolving 0.01 g of Oxymetazoline hydrochloride in 10 ml of deionized water,

-Working mix standard of (Iso + Oxy) solutions was prepared by diluting the stock solution for Iso and Oxy to a final concentration of 500 in deionized water.

-Phosphate [6,18] buffer preparation with triethylamine: 1.36 gm of potassium dihydrogen phosphate (KH2PO4) dissolved in 800 ml deionized water, then added 1ml triethylamine. The pH was adjusted by adding sodium hydroxide (1M) and ortho-phosphoric acid (1M) and measuring with a pH meter until the proper pH values were attained. Then the volume was filled to 1liter with deionized water.

2.3. Instrumentation and chromatographic conditions

The proposed method was performed using HPLC (Agilent, USA). At 25°C, the analytical column was kept. The analytes were eluted from the column using a mobile phase that was pumped at 1.5 ml/min. Methanol, acetonitrile, and buffer (0.01M) potassium dihydrogen phosphate with triethylamine (pH 5.5) were used to make the mobile phase (50:10:40, v/v/v).The column was equilibrated for two minutes. According to the absorption spectra for drugs, as shown in Figures 1&2, the detection was done at 220 nm.

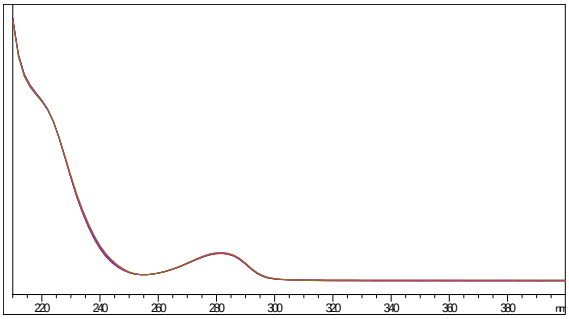

Figure (1): Absorption spectra of ISO (10 µg/ml)

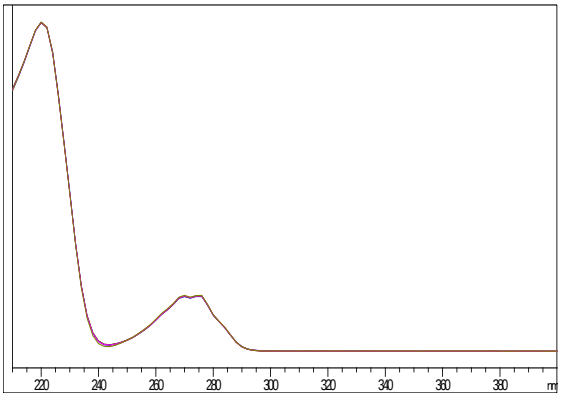

Figure (2): Absorption spectrum of OXY (10 µg/ml)

2.4. **Solutions of pharmaceutical forms**

• Twenty commercial ISO (Isoxuprine® Cairo industry, Egypt) tablets were precisely weighed and finely pulverized. A quantity of the powder corresponding to 10 mg of ISO was precisely measured and dissolved in D.W, stirring well into a 100 mL volumetric flask. After filtering, the solution was utilized as a stock solution (200 g/mL). Distilled water was used to make other pharmaceutical tablet solutions.

•Oxymet nasal drops 0.05%, (200 µg/ml): Provided from the Cairo company for drug industries, Cairo-Egypt. We combined two containers of the medication (each containing 10 mL of 0.05 percent OMZ), and 2 mL of the resultant solution was diluted with D.W to 50 mL in a volumetric flask to prepare a concentration of 200 µg/mL OXY concentration.

3. Results and discussion

3-1.Chromatographic condition optimization

Several factors, including acceptable detector wavelength, mobile phase composition, pH, and flow rate, were investigated to establish the optimal condition that provides the optimum separation and peak shape for both pharmaceuticals (figure3). The choice of the UV spectra showed the highest peak height for analytes at 220 nm. The mobile phase ratio between organic solvents and aqueous was changed using various methanol or acetonitrile:phosphate buffer combinations (30:70, 35:65, 40:60, 45:55, 50:50, and 60:40). With a sample injection volume of 100L, the pH of the mobile phase ranged between 2.0 and 7.0. Equilibrating the HPLC system with the first mobile phase composition, followed by five injections of the same standard, was used to assess system appropriateness. Agilent ChemStation Software determined system appropriateness characteristics such as resolution (Rs) and tailing factor (T) for the HPLC system. Rs and T are advised to be 2 and 2, respectively. General standards verified the approach to fulfill USFDA recommendations. We evaluated the

method´s specificity, sensitivity, linearity, accuracy, precision and recovery to test its integrity [32].

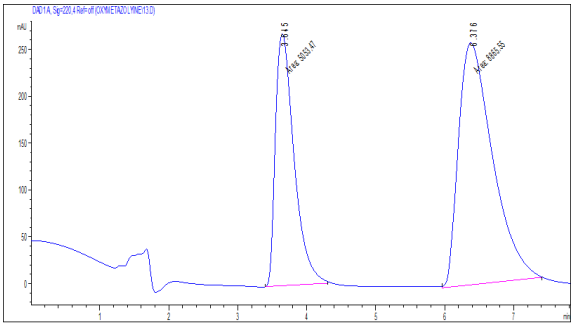

Figure (3): Typical chromatogram of ISO (50µg/mL) and OXY (15 mg/ml) under the optimum chromatographic conditions at ambient temperature.

3.2. Analytical performance

Working calibrators (1, 10, 50, 100, 200, and 250 µg/ml) for Iso and Oxy were made in deionized water. Calibration curves (n = 6) were constructed over the concentration range of 1 – 250 µg/ml for each drug. The normalized peak area of Oxy and Iso was measured and plotted against the theoretical concentration of the spiked standards. The correlation coefficients, slopes, intercepts, linear range, detection, and quantification limitations in Table (1) were determined using least-square linear regression analysis. At each calibration level, the mean accuracy was tested using back-calculated concentrations. The quantitative accuracy ought to be within 20% of the objective. Negative quality control samples were analyzed after each linearity sample to determine any carry-over.

- Six distinct blank specimens (no analyte) were used for evaluating specificity. We achieved this specificity by looking for any signal to assess the manner of co-eluting chromatographic peaks that might interfere with analyte identification. A blank samples (no analyte added) were evaluated with each batch to identify peaks that could be interfere with analyte detection.
- Each analyte detection limit (LOD) and quantification (LOQ) were calculated to determine the method's sensitivity. The lowest concentration at which the analyte signal-to-noise ratio was three was designated as LOD. An adequate retention period and suitable chromatography (peak shape and resolution) were used to determine peak height.

The lowest concentration at which the analyte signal-to-noise ratio was ten was defined as LOQ.

- The linearity of the method was assessed using the regression line. A coefficient of determination is used to represent it (r2). We used a minimum r2 of 0.99 to establish linearity.

Table 1: Statistical treatment analytical values for calibration curves

Parameter	Value OXY	Value ISO
Regression equation	y = 103.6x + 148.4	y = 48.76x + 106.2
Correlation coefficient, r	0.9989	0.9989
Linear range (µg/mL)	1-250	1-250
Limit of detection, LOD (µg/mL)	0.50	0.50

The accuracy and precision for each analyte (Oxy and Iso) were calculated in three repetitions at LQC, MQC, and HQC (5,75,and 220 g/ml). The accuracy was determined by comparing the mean computed Oxy and Iso concentrations in validation samples to the target amounts. It is given as a percentage bias. Precision was obtained by dividing the standard deviation by the computed mean concentration and represented in terms of percent relative standard deviation (RSD %). See table (2) .

3.3. Analytical applications

We validated the new HPLC technique to determine ISO and OXY in pharmaceutical forms under optimum conditions. Two concentrations for ISO tablets, OXY nasals, and two spiked concentrations in river water samples for each drug were studied after their solutions were prepared as recommended previously. Table (3) show that the results of three replicate analyses of each piece obtained by the proposed method were in acceptable agreement with the spiking amounts. The results obtained (recoveries values) are summarized in Table 3 and compared with those of standard methods [2]. Two tests (Student's t-and F-test) [38] were used to compare the performance of the current process with standard procedures. The calculated values at a 95% confidence level did not surpass the theoretical ones, showing a considerable difference in the accuracy and precision of the examined approaches.

Table (2): precision and accuracy of method for determination of Oxy and Iso for (n = 9; 3 sets for 3 days)

Actual spiked concentration (µg/ml)	OXY				ISO			
	Measured concentration		Accuracy (Bias%)	Precision (RSD%)	Measured concentration		Accuracy (Bias%)	Precision (RSD%)
	Mean	SD			Mean	SD		
(Intra day)with in- day (n=3)								
5	4.62	0.26	-7.60%	5.63%	4.95	0.70	-1.05%	6.11%
75	79.71	5.56	6.28%	4.98%	75.48	5.16	0.63%	5.41%
220	232.80	6.68	5.82%	3.73%	228.63	6.34	3.92%	2.77%
(Inter day) between- day (n=9)								
5	4.57	0.17	-8.59%	3.72%	4.68	0.41	-6.40%	4.31%
75	77.99	3.85	3.99%	4.94%	76.58	4.35	2.11%	5.68%
220	227.70	11.09	3.50%	4.87%	226.30	0.13	2.86%	4.48%

Table 3: Applications of the proposed method in pharmaceutical samples and comparison of the proposed method with standard method using t- and F-statistical tests

Isoxsuprine hydrochloride										
Dosage form	proposed method					Official method				
	Taken conc. (µg/mL)	Found conc.(µg/mL)	Rec.%	Mean	RSD %	Taken conc. (µg/mL)	Found conc. (µg/mL)	Rec.%	Mean	RSD %
Duvaline Tablet (10 mg)	35.00	36.64	104.68	101.76	5.13	8.00	7.86	98.29	97.24	2.31
	175.00	172.98	98.84		3.11	12.00	11.54	96.19		1.45
Pure ISO				101.16					99.65	
t (4.30)[c] F (19.0)[c]	t-test =2.98 F-test =16.11									
Oxymetazoline hydrochloride										
Oxymet Nasal drops (0.05%)	35.00	31.99	91.40	96.28	4.30	1.00	0.973	97.30	99.81	1.01
	175.00	177.03	101.16		5.35	3.00	3.07	102.33		0.79
Pure OXY				101.50					102.00	0.70
t (4.30)[c] F (19.0)[c]	t-test=0.87 F-test =5.68									

[C]: theoretical values of t-test and F-test at degree of freedom $n_1=n_2=2$

4. Conclusion

This paper describes a validated HPLC method to determine ISO and OXY in pharmaceutical formulations. Showed the technique to be accurate, precise, and suitable for analyzing ISO and OXY in tablet and nasal drop formulations. The results of this method show the ISO and OXY peak responses to be precise and linear over the range of 1–250 µg/mL. The mean percent recovery of ISO and OXY was less than 101.16 and 101.50, respectively.

This method has been successfully applied to determine ISO and OXY in pharmaceutical tablets and nasal formulations. It is evident that, compared to previous published HPLC techniques, the proposed approach has distinct benefits such as high sensitivity, low organic solvent consumption (100µl), and a quick analysis time of fewer than 7 minutes. The method can be applied in quality control laboratories using a basic chromatographic apparatus to determine both medications in one chromatographic run due to its strong validation requirements.

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