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Validated Spectrofluorimetric Method for The Determination of Oxymetazoline Hydrochloride via Derivatization with 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)

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ABSTRACT

Objectives: An accurate and precise spectrofluorimetric method was developed and validated for the determination of non-selective adrenergic drug named; oxymetazoline hydrochloride (OMZ) in its pure form and nasal drops. **Method:** Based on nucleophilic substitution reaction of target drug with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) to form a highly fluorescent fluorophore with emission measured at 543 nm after excitation at 400 nm. **Results:** Under optimum conditions, the proposed method obeys Beer's law in the range of (1-12 µg mL^{-1}) and the reaction mechanism was presented. **Conclusion:** The method was validated according to ICH guidelines for accuracy, precision and was successfully applied for the determination of the drug in its pure form and nasal drops. The obtained results were statistically compared with those of the reported method and found to be in good agreement.

Keywords: oxymetazoline hydrochloride, spectrofluorimetric method, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole, NBD-Cl, derivatization

INTRODUCTION

Oxymetazoline hydrochloride chemically known as: 3-[(4,5-Dihydro-1*H*-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethylphenol hydrochloride, with molecular weight 296.84 g/mol (1**)** (See **Figure 1**). It is non-selective adrenergic drug acting as a nasal decongestant (2).

Different analytical techniques were applied for quantitative estimation of oxymetazoline hydrochloride including spectrophotometric methods (3-8), Chemiluminescence (9), TLC- densitometric (10), HPLC methods (10-15) and electrochemical (potentiometric) method (16).

4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole **Figure 2** also known as 7-chloro-4-nitrobenzo-furazan (NBD-Cl) is a stable non-fluorescent pale-yellow solid (17). It has been used as derivatizing reagent for devolvement of both spectrophotometric and spectrofluorimetric methods for determination of many amines (17-20).

The aim of this work is to develop and validate a spectrofluorimetric method for the determination of oxymetazoline hydrochloride in its pure form and its pharmaceutical nasal drops via derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl).

EXPERIMENTAL

Instruments

Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp, holographic grating excitation and emission monochromators for all measurements. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were done at medium sensitivity.

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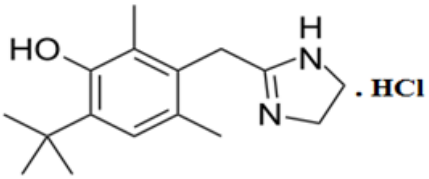

Figure 1. Chemical structure of oxymetazoline hydrochloride

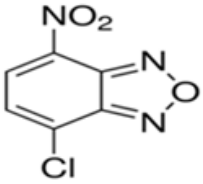

Figure 2. Chemical structure of 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl).

Chemicals and Reagents

- NBD-Cl (99%) (Sigma Chemical Co., St. Louis, USA), freshly prepared as 0.1% methanolic solution and protected from light.
- Hydrochloric acid (El-Nasr Co., Egypt), prepared as 1M aqueous solution
- Sodium bicarbonate (El-Nasr Co., Egypt), prepared as 0.2M aqueous solution
- Methanol, ethanol, acetonitrile, acetone and propanol (sigma-Aldrich, USA), all of HPLC grade
- Water used throughout the procedures was freshly double distilled.

Pure Standard

Pure oxymetazoline hydrochloride powder (99.30%) (B.NO.120M000059) was kindly supplied by Pharaonia Pharmaceuticals Company. New Borg El-Arab – Alexandria – Egypt.

Pharmaceutical Preparation

Oxymet® 0.05% 5ml drops: labeled to contain 0.05g of oxymetazoline hydrochloride per 100 ml, the product of Pharaonia Pharmaceuticals Company. New Borg El-Arab – Alexandria – Egypt. (B.NO.5557002), purchased from local market.

Standard Solutions

Stock standard solution (1mg mL^{-1}) was prepared by dissolving 100 mg of oxymetazoline hydrochloride in 50 mL water, and the volume was then completed to 100 mL with water.

Working solution (100µg mL^{-1}) was prepared by accurate transferring 10 mL of oxymetazoline hydrochloride from its stock standard solution into 100 mL volumetric flask, then the volume was completed to the mark with water.

PROCEDURE

Construction of Calibration Curves

Different aliquots equivalent to (10 – 120 µg) from working standard solution of oxymetazoline hydrochloride were accurately measured and transferred into a test tube set to prepare different concentration covering the linearity range (1-12 µg mL^{-1}), then 1mL (0.1% NBD-Cl) was added followed by 1.5 mL of (0.2M) $NaHCO_3$. The reaction mixtures were allowed to proceed in thermostatically controlled water bath at 60 °C for 30 minutes, and then cooled to room temperature. After cooling, the reaction mixture was acidified by adding 1mL of 1M HCl, and completed to volume with methanol. The relative fluorescence intensity was measured at λem.= 543 nm after excitation at λex.= 400 nm.

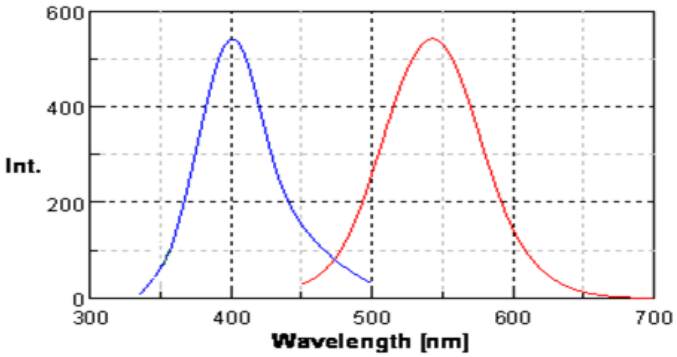

Figure 3. Excitation and emission spectra of the reaction product of oxymetazoline hydrochloride (7 µg mL-1) with 0.1% NBD-Cl

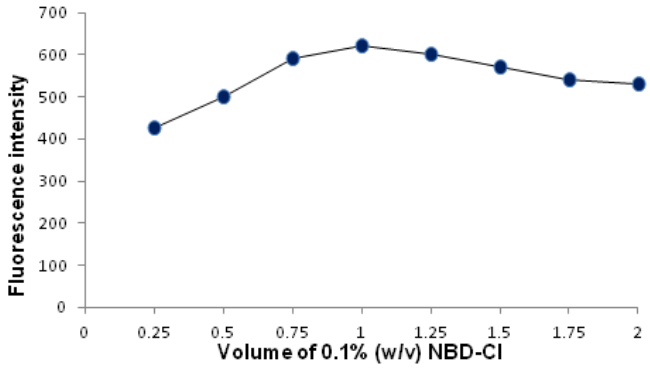

Figure 4. Effect of volume of 0.1% NBD-Cl on the fluorescence intensity of reaction product at λem 54 nm

Application to Pharmaceutical Preparation

Contents of 5 **Oxymet**® drops were mixed well. A volume (4 ml) equivalent to 2 mg of oxymetazoline hydrochloride was transferred into 20-ml volumetric flask and completed to volume with distilled water to obtain a solution labeled to contain 100µgml^{-1} of oxymetazoline hydrochloride.

The solution was analyzed using the procedure described previously and oxymetazoline hydrochloride concentration obtained from the corresponding regression equation.

RESULTS AND DISCUSSION

Oxymetazoline hydrochloride doesn't has a native fluorescence, so its derivatization with fluorigenic reagent was necessary for spectrofluorimetric determination. 4-chloro-7-nitrobenzo-2-oxa-1,3- diazole (NBD-Cl) an electroactive halide reagent, which was considered as a likely target for good nucleophiles, thus upon reaction of oxymetazoline hydrochloride with (NBD-Cl), a dark yellow-colored fluorescent derivative was formed, which exhibited maximum fluorescence intensity at (λem.= 543 nm) after its excitation at wavelength (λex.= 400nm). The excitation and emission spectra for the reaction product of oxymetazoline hydrochloride with (NBD-Cl) was shown in **Figure 3.**

Optimization of Experimental Conditions

Different experimental parameters affecting the fluorescence intensity were studied and optimized.

Effect of (NBD-Cl) volume

The influence of NBD-Cl volume was studied using different volumes of 0.1% (w/v) NBD-Cl solution ranging from (0.25-2 mL), it was found that 1mL of 0.1% (w/v) NBD-Cl produce the highest FI as shown in **Figure 4.**

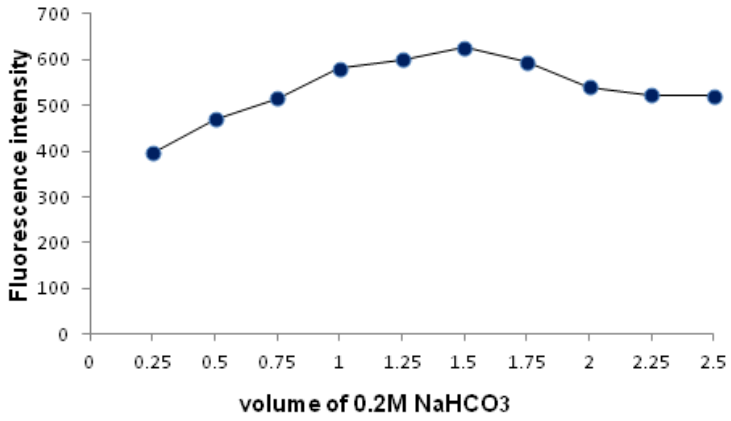

Figure 5. Effect of volume of 0.2 M NaHCO3 on the fluorescence intensity of reaction product at λem 543 nm

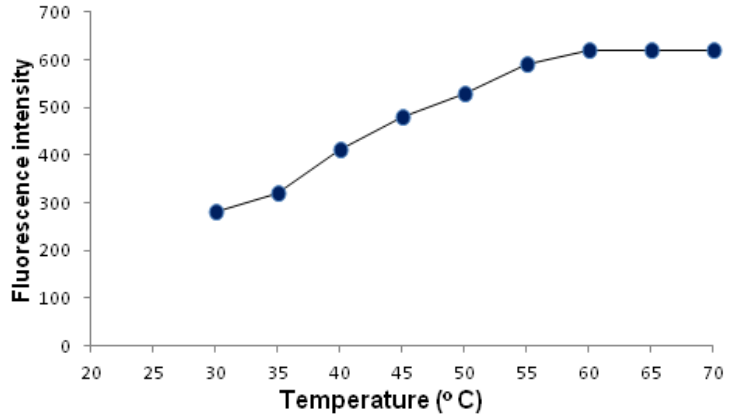

Figure 6. Effect of temperature (°C) on the fluorescence intensity of reaction product at λem 543 nm

Effect of ($NaHCO_3$) volume

The reaction of oxymetazoline hydrochloride with NBD-Cl should be carried out in alkaline medium (pH ~8.3) in order to generate the nucleophile from oxymetazoline hydrochloride. The influence of $NaHCO_3$ volume was studied using different volumes of 0.2 M $NaHCO_3$ solution ranging from (0.25-2.5 mL), it was found that 1.5 mL of (0.2 M) $NaHCO_3$ produces the highest FI as shown in **Figure 5**.

Effect of temperature

The influence of temperature the reaction was carried out at different temperatures (30-70 ^{0}C), it was found that the reaction was dependent on the temperature and the FI increased as the temperature increased and the maximum FI was obtained at 60 ^{0}C as shown in **Figure 6**.

Effect of heating time

In order to determine the time required for completion of the reaction, the reaction was carried out at different reaction time interval (5-40 min.). The results indicated that the optimum time was 30 min as shown in **Figure 7**.

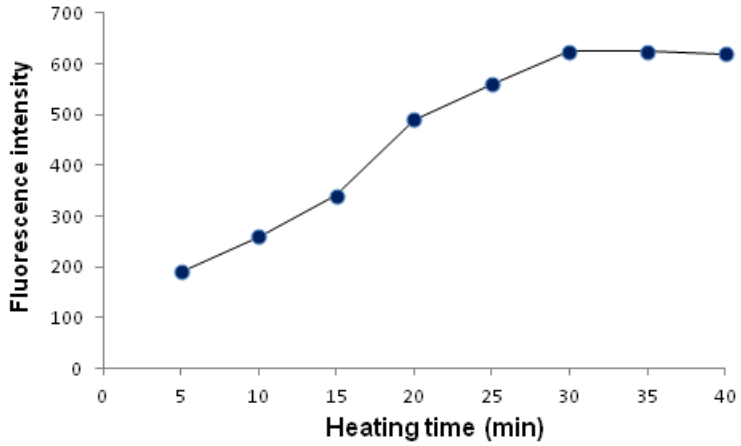

Figure 7. Effect of heating time at 60 °C on the fluorescence intensity of reaction product at λem 543nm

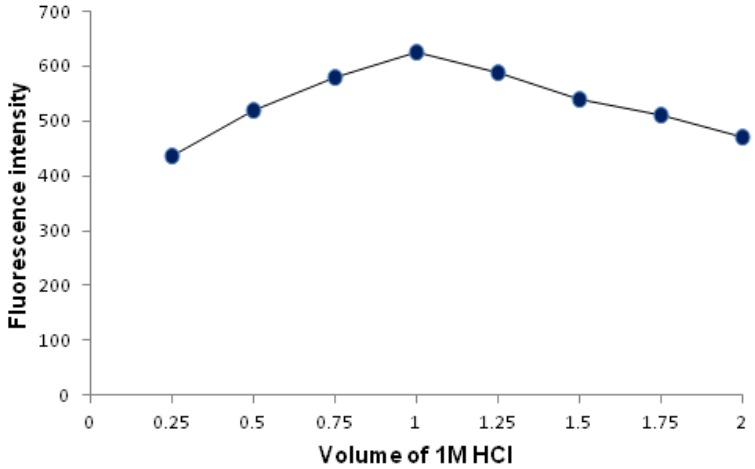

Figure 8. Effect of volume of 1M HCl on the fluorescence intensity of reaction product at λem 543 nm

Effect of HCl volume

Addition of HCl (17) to the reaction mixture before the measurement of the FI was necessary for remarkably decreasing the background fluorescence (duo to the hydrolysis product of NBD-Cl to the corresponding hydroxyl derivative namely, 7-hydroxy-4 nitrobenzoxadiazole (NBD-OH) (21). The fluorescence of NBD-OH was found to be quenched in strong acidic medium (pH ≤ 1), where the reaction product was not affected, the reaction was carried out using different volumes of 1M HCl ranging from (0.25-2mL). The optimum volume of HCl required for acidification was found to be 1 mL of 1M HCl as shown in **Figure 8**.

Effect of diluting solvent

In order to select the most appropriate solvent for diluting the reaction solution, different solvents involve: water, methanol, ethanol, acetonitrile, acetone and propanol were studied. The highest FI was obtained upon using methanol as shown in **Figure 9**.

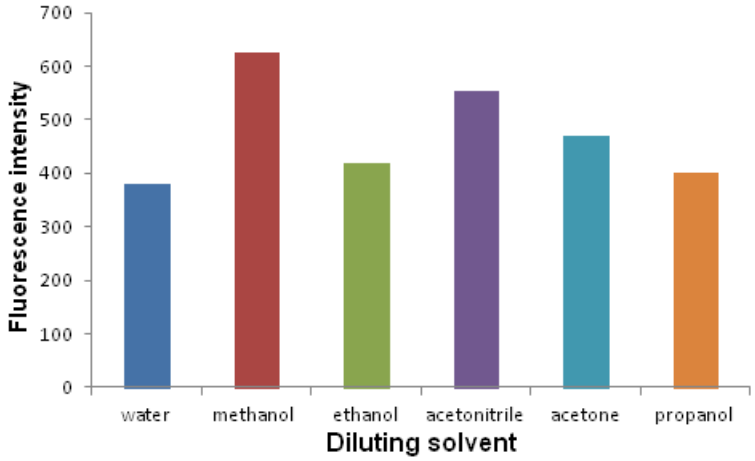

Figure 9. Effect of diluting solvent on the fluorescence intensity of reaction product at λem 543 nm

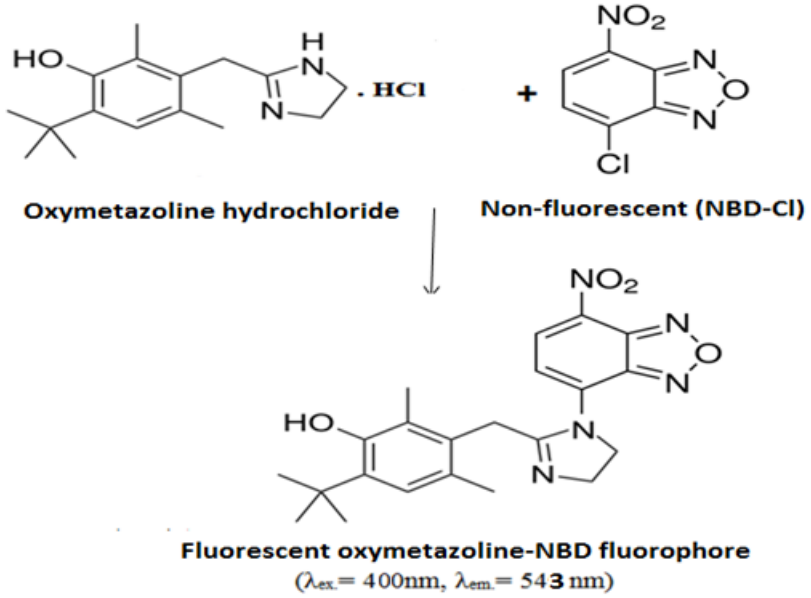

Figure 10. The Proposed reaction pathway between oxymetazoline hydrochloride and NBDCl

Mechanism of the reaction

NBD-Cl is an electroactive halide reagent, which was considered as a likely target for good nucleophiles, oxymetazoline has free terminal secondary amino group considered as good nucleophile, react with NBD-Cl through nucleophilic substitution forming highly fluorescent dark yellow fluorophore, the suggested reaction pathway between oxymetazoline hydrochloride and NBD-Cl was shown in **Figure 10**.

Stability of fluorescent fluorophore

The effect of time on the stability of the Fluorescent oxymetazoline-NBD fluorophore was studied by measuring the FI at different time intervals. It was found that the FI values remain constant for at least 24 hours at room temperature.

The optimum variables affecting the reaction of oxymetazoline hydrochloride with NBD-Cl were summarized in **Table 1**.

Table 1. Optimization of variables affecting the reaction of oxymetazoline hydrochloride with NBD-Cl

Variable	Studied range	Optimum
Excitation wavelength (nm)	350 – 520	400
Emission wavelength (nm)	490 – 600	543
(0.1 %, w/v) NBD-Cl	0.25-2 mL	1 mL
0.2 M NaHCO3	0.25-2.5 mL	1.5 mL
Temperature (°C)	30 – 70	60
Heating time (min)	5-40	30
1M HCl	0.25-2 mL	1mL
Diluting solvent	Water, methanol, ethanol, propanol, acetone, acetonitrile.	Methanol
Stability of oxymetazoline-NBD fluorophore	1- 24 hr.	24 hr. at room temp.

Table 2. Assay validation sheet of the proposed method

Parameters		Spectrofluorimetric method
λex. & λem.		400 & 543 (nm)
Linearity range (µg ml^{-1})		1 – 12
LOD (µg ml^{-1})		0.178
LOQ (µg ml^{-1})		0.541
Regression Equation		F* = 76.50 C** + 20.85
Coefficient of determination (r^2)		0.9997
Accuracy (mean ± SD)		99.97± 0.673
Precision	Intra-day	0.629
	Inter-day	0.718

F is the fluorescence intensity.*
*C** is concentration in g ml^{-1}*

Method validation (22)

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines in terms of linearity, range, LOD, LOQ, accuracy and precision.

Linearity and range

The method obeys the Beer's law in the range of 1-12 µg mL^{-1}, **Table 2,** illustrated the regression parameters of the calibration curve and determination coefficient of the drug analyzed.

Limits of detection and quantitation

LOD was found to be 0.178µg mL^{-1}, while LOQ was found to be 0.541µg mL^{-1}, as shown in **Table 2.**

Accuracy and precision

Accuracy of the proposed procedure (R%) was found to be 99.97. Intra-day precision (repeatability day precision) was found to be 0.629, while inter-day precision (intermediate precision) was found to be 0.718, **Table 2**. Good %R confirms excellent accuracy.

The validity of the proposed procedure is further assessed by applying the standard addition technique showing mean recovery of added ± SD of 99.22 ± 0.455 %. Results are presented in **Table 3**.

Table 3. Recovery study of oxymetazoline hydrochloride in Oxymet® nasal drops by the proposed spectrofluorimetric method by adopting standard addition technique

Pharmaceutical Taken (μgmL^{-1})	Pure added (μgmL^{-1})	Pure found (μgmL^{-1})	Recovery %
4	2	1.97	98.79
	4	3.96	99.06
	6	5.94	99.16
	8	7.98	99.86
Mean ± SD%			**99.22± 0.455**

Table 4. Statistical comparison for the results obtained by the proposed method and the reported method for the analysis of oxymetazoline hydrochloride in Oxymet ® nasal drops

Parameters	Proposed method	Reported method[4]
n*	5	5
$\bar{X}$	99.98	99.95
SD	0.241	0.542
RSD%	0.241	0.542
t**	0.087 (**2.3**06)	——
F**	5.020 (6.**3**88)	——

** No. of experiments.*

*** The values in the parenthesis are tabulated values of t and F at p= 0.05 level of significance.*

Statistical analysis

Statistical comparison between results obtained by applying the proposed procedure and those obtained by applying the reported method (4) showed less calculated t and F values than the tabulated ones revealing no significant difference in accuracy and precision, as shown in **Table 4**.

CONCLUSION

This work introduced an accurate spectrofluorimetric method for the determination of oxymetazoline hydrochloride in its pure form and nasal drops based on nucleophilic substitution reaction with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) to form a highly fluorescent yellow fluorophore. The proposed method is suitable for the routine analysis of oxymetazoline hydrochloride in quality control laboratories.

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