J Fluoresc (2015) 25:1695–1709
DOI 10.1007/s10895-015-1656-4

ORIGINAL ARTICLE

Determination of Some Non-sedating Antihistamines via Their Native Fluorescence and Derivation of Some Quantitative Fluorescence Intensity - Structure Relationships

Michael E. El-Kommos[1] · Samia M. El-Gizawy[1] · Noha N. Atia[1] · Noha M. Hosny[1]

Received: 9 July 2015 / Accepted: 9 September 2015 / Published online: 6 October 2015

Abstract A validated simple, novel, and rapid spectrofluorimetric method was developed for the determination of some non-sedating antihistamines (NSAs); namely cetirizine (CTZ), ebastine (EBS), fexofenadine (FXD), and loratadine (LOR). The method is based on measuring the native fluorescence of the cited drugs after protonation in acidic media and studying their quantitative fluorescence intensity – structure relationships. There was a linear relationship between the relative fluorescence intensity and the concentration of the investigated drug. Under the optimal conditions, the linear ranges of calibration curves for the determination of the studied NSAs were 0.10–2.0, 0.20–6.0, and 0.02–1.0 μg/mL for (CTZ, FXD), (EBS), and (LOR); respectively. The factors affecting the protonation of the studied drugs were carefully studied and optimized. The method was validated according to ICH guidelines. The suggested method is applicable for the determination of the four investigated drugs in bulk and pharmaceutical dosage forms with excellent recoveries (97.67–103.80 %). Quantitative relationships were found between the relative fluorescence intensities of the protonated drugs and their physicochemical parameters namely: the pKa, log *P*, connectivity indexes (χ^{v}) and their squares. Regression equations (76) were obtained and not previously reported. Six of these equations were highly significant and used for the prediction of RFI of the studied NSAs.

Keywords Cetirizine · Ebastine · Fexofenadine · Loratadine · Native fluorescence · Quantitative fluorescence intensity – structure relationships

✉ Noha M. Hosny
phar_nmh2011@yahoo.com

[1] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

Introduction

In 1937, the first H1 antihistamine (thymo-ethyl-diethylamine) was synthesized. However, because of its weak activity and high toxicity, this compound was not used [1]. Clinically useful H1 antihistamines such as phenbenzamine, pyrilamine, and diphenhydramine were introduced in the 1940s [2]. Currently, H1 antihistamines constitute the second most commonly used class of medications after antibiotics [3]. H1 antihistamines are traditionally classified into six groups, based on their chemical structure to alkylamines, monoethanolamines, ethylenediamines, phenothiazines, piperazines and piperidines. This classification is, however, of limited clinical relevance, and currently H1 antihistamines are classified as first, second and third generation [4]. The older first generation antihistamines are associated with troublesome sedative and antimuscarinic effects, and are often termed as 'sedating antihistamines'. The newer generations of antihistamines, which are essentially devoid of these effects, are correspondingly termed as 'non-sedating antihistamines' (NSAs) [5].

NSAs are of potential value in the management of allergic rhinitis in which they relieve nasal and conjunctival itching, sneezing and rhinorrhoea. They are also useful in the treatment of acute and chronic urticaria [1]. NSAs down regulate the allergic inflammation directly by interfering with the histamine action at H1-receptors on sensory neurons and small blood vessels. They also decrease the antigen presentation, expression of pro-inflammatory cytokines and cell adhesion molecules, and chemotaxis. In a concentration-dependent manner, they inhibit mast cell activation and histamine release [5].

The investigated second generation non-sedating antihistamines are cetirizine (CTZ), Ebastine (EBS), loratadine (LOR) that are much more selective for peripheral H1 receptors. Fexofenadine (FXD) is the carboxylic acid metabolite of terfenadine, is one of third generation drugs. The chemical structures of the investigated drugs are given in (Fig. 1).

Literature survey reveals several methods for quantitative determination of CTZ, EBS, FXD, and LOR. Among these methods are liquid chromatography (HPLC) [6–11], gas chromatography (GC) [12], electrochemical [13–17], and spectrophotometric methods (direct UV-, derivative-spectrophotometry, formation of ion-pair complexes, and charge transfer) [18–22].

Few spectrofluorimetric methods [19, 21, 23–26] were applied for the determination of the studied drugs. We tried to introduce a simple and sensitive spectrofluorimetric method suitable and applicable for the analysis of the studied drugs in quality control processes. The simplicity and sensitivity of the proposed method are superior to other reported spectrofluorimetric methods for the investigated drugs. In the previously reported methods, procedures were complicated and time consuming (evaporation to dryness and heating in a boiling water bath for 35 min were required) [23]. In our method, the limit of detection (LOD) and limit of quantification (LOQ), which are indicative of the sensitivity of the method are lower than those of other reported methods [19, 27]. The use of the proposed method expands the applicability to bulk drugs, dosage forms and biological fluids. Unlike GC and HPLC procedures, the spectrofluorimeter is relatively simple to handle and affordable. Our interest to develop a new method for the assay of some NSAs is motivated due to their importance and high consumption rate all over the world for treating various symptoms of allergy [1] caused by the remarkable air pollution.

In the present study, it was observed that the studied drugs (CTZ, EBS, FXD, and LOR) have a native fluorescence after protonation in acidic medium. The linear ranges of calibration curves for the determination of the studied NSAs were 0.10–2.0, 0.20–6.0, and 0.02–1.0μ g/mL for (CTZ, FXD), (EBS), and (LOR); respectively. On the other hand, some quantitative

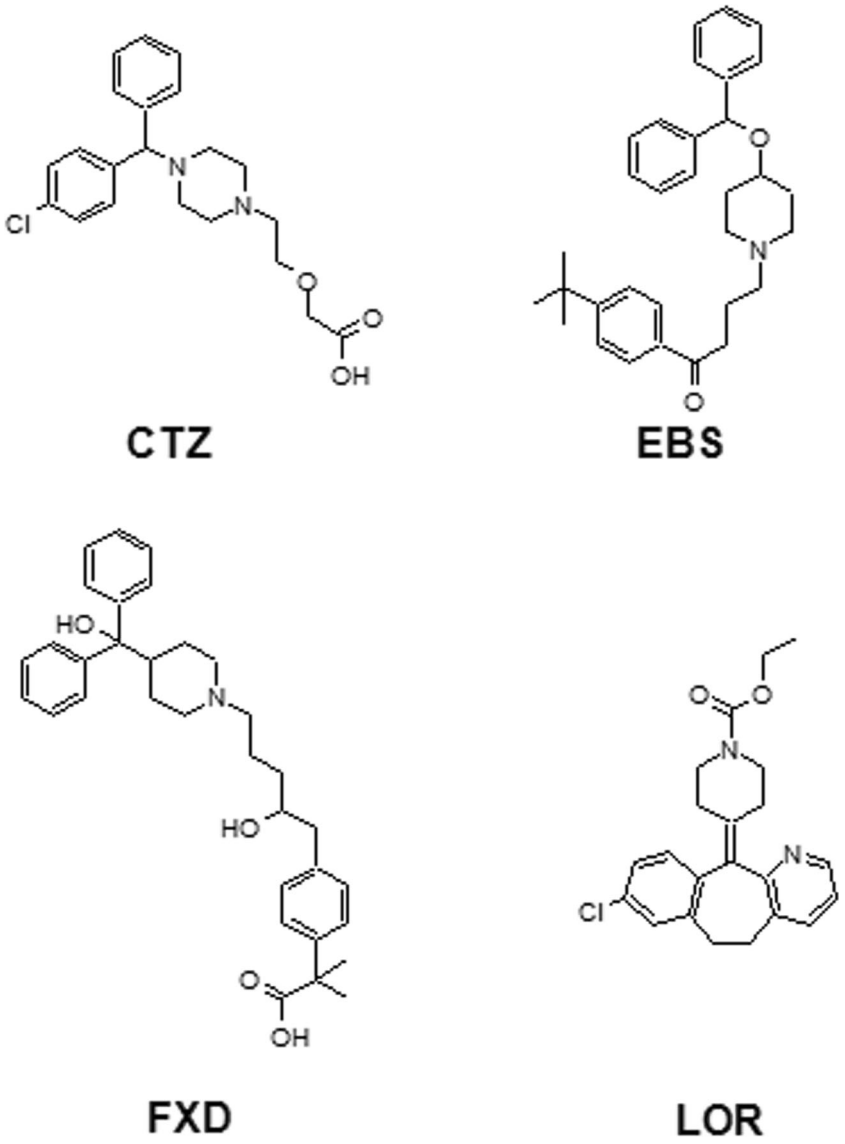


Fig. 1 Chemical structures of the investigated drugs

fluorescence intensity - structure relationships were derived. These relationships were found between the relative fluorescence intensities of the protonated drugs and their physicochemical parameters namely: the pKa, log *P,* connectivity indexes (χ^v) and their squares. Seventy six regression equations were obtained and not previously reported. Six of these correlation equations were highly significant and used for the prediction of RFI of the studied NSAs.

The proposed method provides a rapid and sensitive procedure for the analysis of the studied drugs in pure and in pharmaceutical formulations. The developed method provides an inexpensive tool for the determination of the studied drugs in quality control laboratories especially in developing countries where the cost is the main concern. In addition, the prediction of RFI of the studied NSAs was achieved by using highly significant regression equations.

Experimental

Apparatus

(i). Spectrofluorimetric measurements were carried out using a Shimadzu RF-5301 PC spectrofluorimeter (Tokyo, Japan) with 1 cm quartz cells. The slit width of both excitation and emission monochromators was set at 10 nm.
(ii). Ultrasonic cleaner (Cole-parmer, Chicago, USA).
(iii). Sartorious handy balance- H51 (Hannover, Germany).

Materials and Reagents

(i). CTZ. HCl was kindly supplied by Egyptian International Pharmaceutical Industries Company E.I.P.I.Co. (10^{th} of Ramadan city, Egypt).
(ii). EBS was kindly supplied by Global Napi Pharmaceutical Company (Giza, Egypt).
(iii). FXD. HCl and LOR were kindly supplied by Amoun Pharmaceutical Company (Obour, Egypt).
(iv). Acetic acid 96 %, sulphuric acid, and perchloric acid (El-Nasr Pharmaceutical Chemicals Co., Egypt). 0.5 N of each acid solution was prepared in double distilled water.
(v). Methanol (Fisher Scientific U.K. limited, United Kingdom).
(vi) All other chemicals and reagents used were of analytical grade.

Pharmaceutical Formulations

All pharmaceutical formulations were obtained from the Egyptian market as follows:

(i). Epirizine tablets (labeled to contain 10 mg CTZ. HCl per tablet);
(ii). Evastine tablets (labeled to contain 10 mg EBS per tablet);
(iii). Allerfen tablets (labeled to contain 60 mg FXD. HCl per tablet);
(iv). Mosedine tablets (labeled to contain 10 mg LOR per tablet).

Preparation of Standard Solutions

The stock standard solutions were prepared to contain 1.0 mg/mL by dissolving 50.0 mg of each drug in 25 mL methanol, and then the volumes were completed to 50 mL with the same solvent.

The working solutions were prepared by appropriate dilution of the stock solutions with methanol.

General Procedure

One milliliter of the working standard or sample solution of CTZ, EBS, FXD, or LOR was transferred into 10-mL calibrated flask. Also, 4.0 mL of 0.5 N acid solution ($HClO_4$ for CTZ and FXD, H_2SO_4 for EBS and CH_3COOH for LOR) was added. Then, the mixture was diluted to the mark with distilled water. The relative fluorescence intensity of the resulting solutions was measured at specific $\lambda_{ex.}$ / $\lambda_{em.}$ mentioned in Table 1 against a reagent blank prepared in the same manner (addition of 1 mL of methanol instead of working standard or sample solution).

Construction of Calibration Curves

Transfer different aliquots of standard solutions equivalent to 1.0–20, 2.0–60, and 0.20–10 μg of (CTZ, FXD), (EBS), and (LOR); respectively, into 10-mL calibrated flasks. Then, the assay was conducted as under general procedure. The relative fluorescence intensity was plotted versus the final concentration of the drug (μg/ml) to get the calibration graphs; alternatively, the corresponding regression equations were derived.

Procedure for Tablets

Accurately weighed powder samples obtained from 30 tablets, equivalent to 25.0 mg of each drug, were suspended in methanol, and then the volumes were completed to the mark with the same solvent in 25-mL calibrated flasks, filtered. The first portion of the filtrate was rejected then; the assay was completed as under general procedure.

Table 1 Spectral characteristics for the developed spectrofluorimetric method

Parameter	CTZ	EBS	FXD	LOR
$\lambda_{ex.}$ / $\lambda_{em.}$ (nm)	235/294	225/290	230/290	275/462
Linearity range (μg/mL)	0.10–2.0	0.20–6.0	0.10–2.0	0.020–1.0
Correlation coefficient (r)±SD[a]	$0.9999\pm7.96\times10^{-5}$	$0.9999\pm7.79\times10^{-5}$	$0.9999\pm2.40\times10^{-5}$	$0.9999\pm2.88\times10^{-5}$
R^2±SD[a]	$0.9998\pm2.06\times10^{-4}$	$0.9997\pm1.55\times10^{-4}$	$0.9999\pm4.82\times10^{-5}$	$0.9999\pm5.77\times10^{-5}$
Intercept (a)±SD[a]	−4.818±1.475	5.844±0.4533	−16.99±1.878	4.754±1.500
Slope (b)±SD[a]	148.96±13.45	23.274±0.04159	234.88±3.882	541.68±12.34
LOD[b] (μg/mL)	0.0327	0.0643	0.0264	0.00914
LOQ[c] (μg/mL)	0.0990	0.1948	0.07995	0.0277

[a] Average of six replicates

[b] Limit of detection

[c] Limit of quantitation

Method Validation

Typical analytical performance characteristics for the validation of procedures according to ICH Q2 guidelines were described in this study, including linearity, limit of detection, limit of quantification, precision, accuracy, and robustness [28].

Linearity

Linearity was determined either by plotting fluorescence intensity versus concentration or from linear regression equation RFI = $bC + a$, where RFI is the relative fluorescence intensity of the protonated drug, b is the slope, a is the intercept, and C is the concentration of the drug in μ g/mL.

Limits of Detection (LOD) and Quantification (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of y-intercept of the regression equation and S is the slope of the calibration curve.

Accuracy

To determine the accuracy of the proposed method, three levels of drug concentrations (low, medium, and high) were prepared from the stock solutions and analyzed (n =6).

Precision

Intra-day precision of the proposed method was tested by replicate analysis of three separate solutions of the working standard of CTZ, EBS, FXD, and LOR at three different concentration levels: low, middle, and high; (0.5, 1.0, and 2.0 μ g/mL) for (CTZ, EBS, and FXD) and (0.2, 0.5, and 1.0 μ g/mL) for LOR. This study was repeated for three consecutive days to determine the inter-day precision (n =6).

Recovery

The recovery of the proposed method was determined using the standard addition technique, by adding a

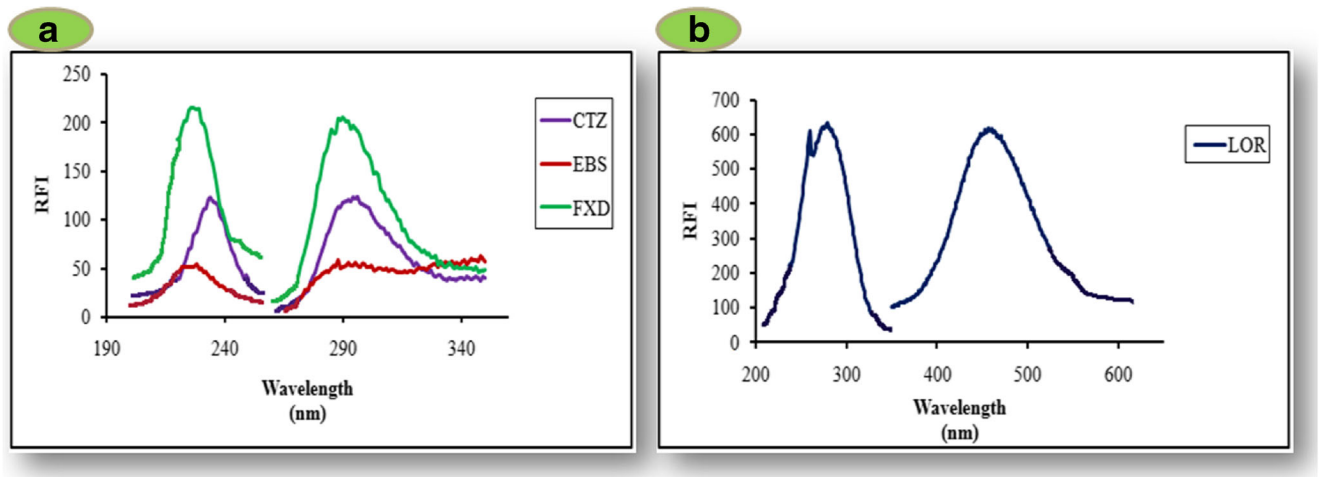


Fig. 2 Fluorescence spectra of **a-** CTZ, EBS, FXD, and **b-** LOR in acid medium (Final drug concentration=1.0 μg/mL)

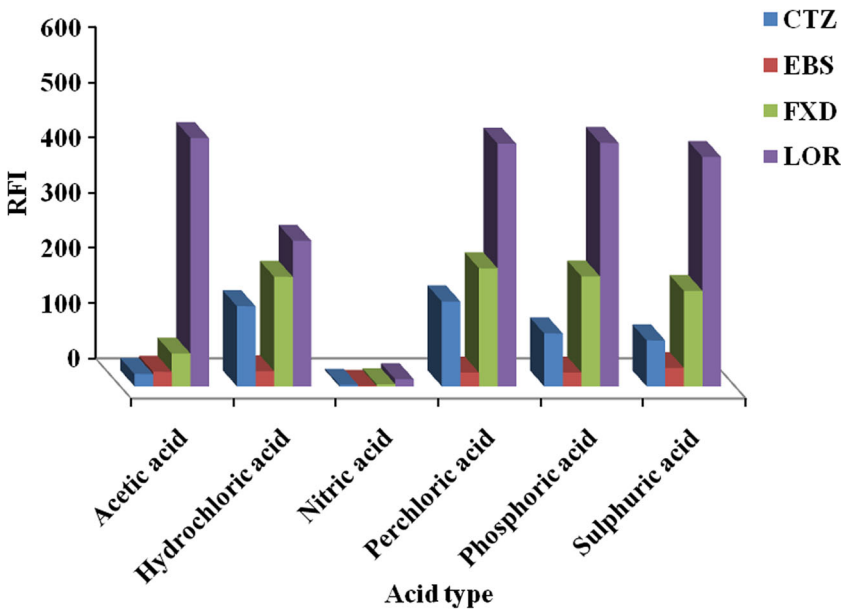


Fig. 3 Effect of acid type on the fluorescence intensity of the studied drugs (Final drug concentration=1.0 μg/mL)

known amount of standard at three different concentration levels to the pre-analyzed sample.

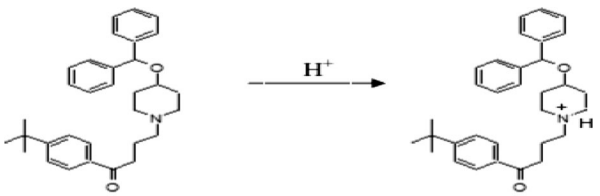
Robustness

The robustness of the proposed method was determined by studying the effect of minor changes on the RFI of the protonated drug: the concentration and volume of the acid on the method performance.

Results and Discussion

Fluorescence Spectra

The four studied drugs protonated in acidic medium. The fluorescence intensity of the protonated drugs was measured (Fig. 2). The magnitude of the increase of fluorescence intensity of the drug (RFI) was proportional to the concentration of the drug. The emission and excitation wavelengths were shown in Table 1.



Protonation of EBS

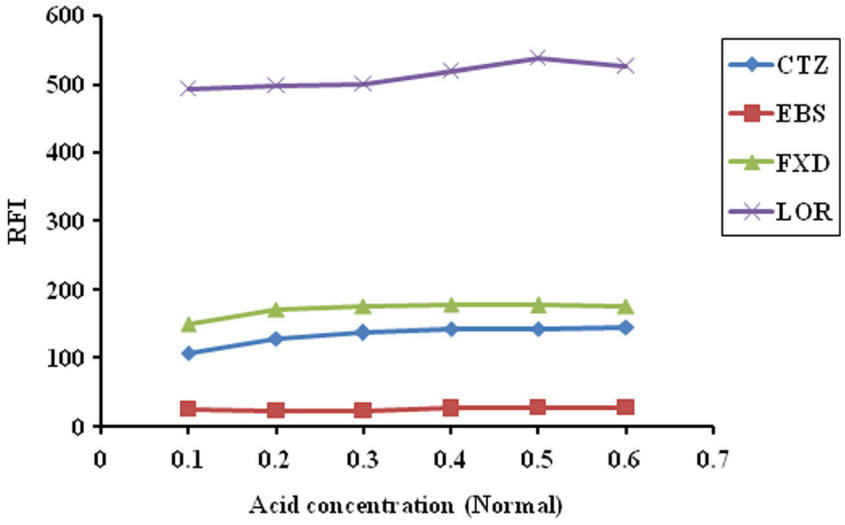


Fig. 4 Effect of acid concentration on the fluorescence intensity of the studied drugs (Final drug concentration= 1.0 μg/mL)

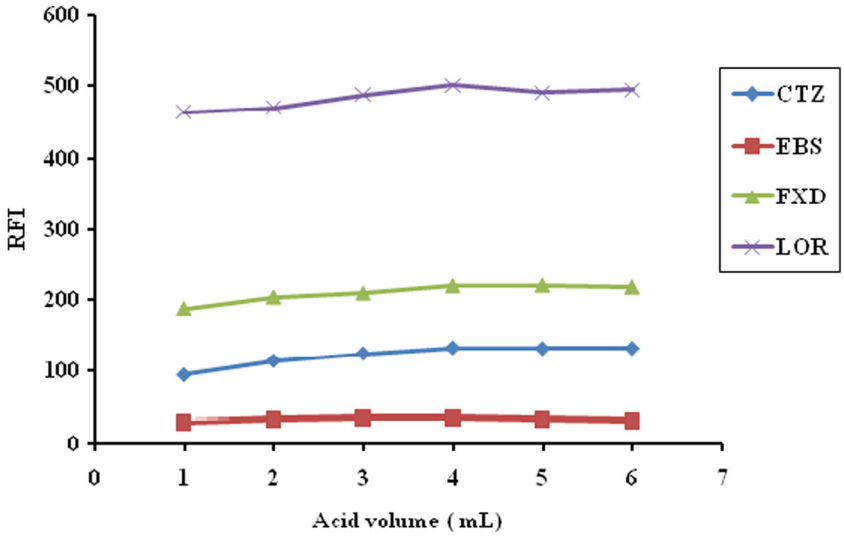


Fig. 5 Effect of acid volume on the fluorescence intensity of the studied drugs (Final drug concentration=1.0 μg/mL)

Optimum Reaction Conditions

To optimize the assay parameters, the effect of acid type, concentration, and volume, also the effect of diluting solvent were studied.

Effect of Type, Concentration, and Volume of Acid

The influence of acid on the native fluorescence of the studied drugs was investigated. The procedure was carried out using different acids such as CH_3COOH, HCl, HNO_3, H_3PO_4, $HClO_4$, and H_2SO_4. The highest fluorescence intensity of the studied drugs was observed with $HClO_4$, H_2SO_4 and CH_3COOH for (CTZ and FXD), EBS and LOR; respectively (Fig. 3). Also, different volumes (1.0–6.0 mL) and various concentrations of the selected acids (0.1–0.6 N) were added to the studied drugs. It was found that RFI of the studied drugs reached the maximum using 4 mL of 0.5 N acid solutions for all drugs (Figs. 4 and 5).

Effect of Solvent

Ethanol, methanol, propan-2-ol, dichloromethane, and distilled water were studied as diluting solvents. The highest fluorescence intensities were observed with distilled water (Fig. 6).

Validation of the Developed Method

Linearity, Detection and Quantitation Limits

Under the described experimental conditions, standard calibration curves for CTZ, EBS, FXD, and LOR were constructed by plotting the relative fluorescence intensity against the drug concentration (Fig. 7). Linear regression equation, limit of detection, and limit of quantitation for each drug are presented also in Table 1. The correlation coefficients were 0.9999 indicating good linearity.

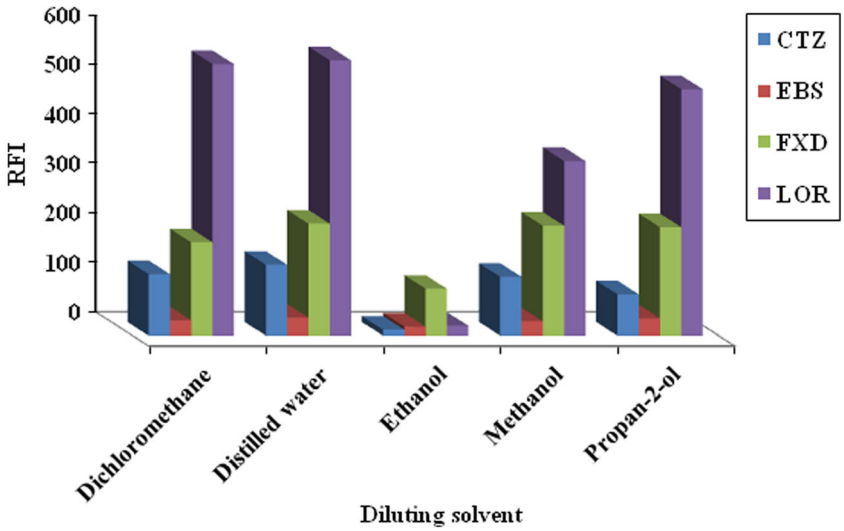


Fig. 6 Effect of diluting solvent on the fluorescence intensity of the studied drugs (Final drug concentration=1.0 μg/mL)

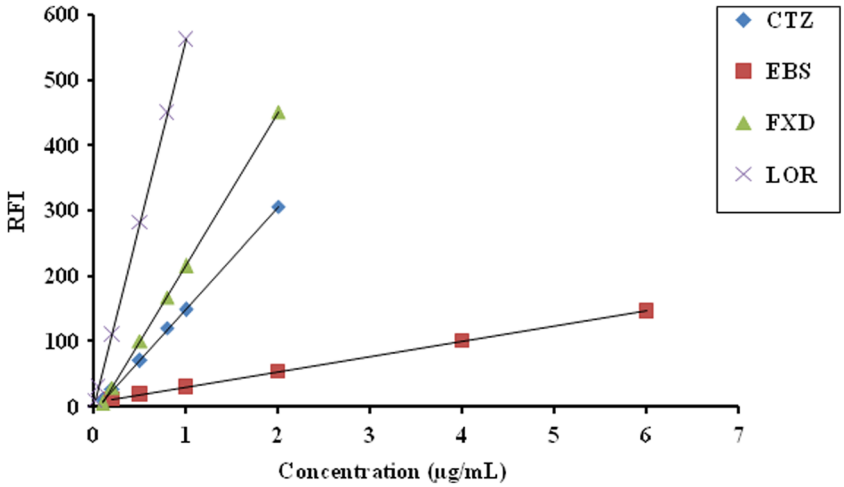


Fig. 7 Calibration curves of relative fluorescence intensities of the studied non-sedating antihistamines

Precision and Accuracy

RSD% values for CTZ, EBS, FXD, and LOR for intra-day and inter-day precisions were ranged from 0.22 to 2.28 indicating good repeatability. Accuracy was determined by comparing measured concentrations of CTZ, EBS, FXD, and LOR with the actual values and expressed as percentage in Tables 2 and 3. The accuracy of the developed method for the studied drugs ranged from 98.03 to 101.72 % indicating good accuracy of the developed method. The obtained accuracy and precision were satisfactory for quality control measurements.

Robustness

The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters without changes in quantitation. For the determination of the method's robustness, two factors were selected from the analytical procedure to be examined in the robustness testing; concentration and volume of the acid.

Results are shown in Table 4. It was found that none of these variables had a significant effect on the determination of investigated drugs. This provides an indication of the reliability of the proposed method during the normal usage, so the developed spectrofluorimetric method is considered robust.

Application of the Developed Method to Pharmaceutical Preparations

Different commercial dosage forms of the studied drugs were successfully analyzed by the developed method, and results were compared with those obtained by reported methods [23, 26] as shown in Table 5. It was observed that there was no significant difference between results obtained by the developed method and the reported methods as indicated by *t* - and *F* -tests. Additionally, recovery experiments were carried out for the studied drugs in their respective pharmaceutical

Table 2 Accuracy of the proposed spectrofluorimetric method for analysis of some Non-Sedating Antihistamines at three concentration levels

Drug	CTZ			EBS			FXD			LOR		
Conc. (µg/mL) Exp. No.	0.50	1.0	2.0	0.50	1.0	2.0	0.50	1.0	2.0	0.20	0.50	1.0
1	100.09	100.49	101.96	100.18	102.44	98.71	98.73	97.28	100.30	97.94	100.62	99.15
2	99.54	100.41	101.42	96.92	102.70	100.76	99.95	98.32	100.75	98.40	101.58	101.91
3	99.71	100.19	102.80	95.91	100.31	101.81	101.87	98.51	100.57	97.35	100.61	101.88
4	99.57	99.39	101.45	100.89	98.71	101.96	101.57	98.98	99.80	101.91	100.99	98.08
5	99.62	99.53	102.07	101.01	100.39	98.12	101.72	98.03	101.35	99.19	99.52	103.78
6	98.53	100.85	100.60	97.99	101.01	99.02	97.89	97.05	102.48	101.32	99.02	101.56
Mean ± SD*	99.51± 0.52	100.14± 0.57	101.72± 0.74	98.81± 2.18	100.92± 1.48	100.06± 1.66	100.29± 1.70	98.03± 0.74	100.87± 0.94	99.35± 1.86	100.39± 0.95	101.06± 2.08
C.V**	0.52	0.57	0.73	2.20	1.47	1.66	1.70	0.75	0.93	1.87	0.94	2.06

*Average of six replicates

**Coefficient of variation

Table 3 Intra-day and Inter-day precision of the proposed spectrofluorometric method for some NSAs

Authentic drug	Concentration added* (μg/mL)	Found* (μg/mL)	Intra-day precision		Inter-day precision	
			% Recovery±SD**	RSD %	% Recovery±SD**	RSD %
CTZ	0.50	0.4988	99.83±1.14	1.14	99.70±0.22	0.22
	1.0	1.011	100.84±0.41	0.41	101.26±0.82	0.81
	2.0	2.019	99.97±0.44	0.44	101.94±0.56	0.55
EBS	0.50	0.5001	101.43±1.88	1.85	98.60±2.19	2.22
	1.0	1.504	99.98±1.20	1.20	100.78±1.36	1.35
	2.0	2.004	101.71±1.09	1.07	98.65±1.31	1.32
FXD	0.50	0.4911	97.98±0.44	0.45	98.45±0.93	0.94
	1.0	0.9836	98.22±0.63	0.64	98.49±0.22	0.23
	2.0	2.020	100.55±0.57	0.57	101.46±0.85	0.83
LOR	0.20	0.2007	97.76±0.50	0.51	102.95±1.39	1.35
	0.50	0.4962	100.15±0.84	0.84	98.32±0.61	0.62
	1.0	1.001	99.98±2.28	2.28	100.12±1.41	1.41

* Average of twelve replicates

**Average of six replicates

formulations by standard addition method. The results in Table 6 indicate that the developed method is convenient for all investigated drugs with good recoveries, and there is no interference from either the co-administered drugs or frequently encountered excipients. The proposed method is sensitive, accurate, and precise. It is suitable for the determination of the studied drugs in their dosage forms in quality control laboratories.

Derivation Quantitative Fluorescence Intensity - Structure Relationships

It is well known that the relative fluorescence intensity is dependent on the molecular structure and linking pattern or bonding scheme of the atoms in the fluorescent species. The conversion of structural formula into numerical values or indexes, which encode structural information, will be extremely helpful. Thus, a numerical description of a molecule derived from knowledge of the molecular structure itself can be developed.

There are two general aspects of structure which can be identified and expressed in the form of numerical parameters:

***a- The topology of the molecule*:** information about the identities of atoms and their electronic properties and connections.

***b- The molecular topography*:** various three-dimensional aspects e.g., size, shape, branching, volume and surface area of the molecule.

Table 4 Robustness of the developed spectrofluorometric method

Drug	% Recovery*±SD			
	CTZ	EBS	FXD	LOR
No variation**	100.18±0.67	101.66±0.99	99.98±1.01	102.67±0.73
Acid conc.				
0.4 normal	97.17±1.54	99.89±1.37	99.37±0.47	100.72±1.92
0.6 normal	100.67±0.88	101.85±2.14	99.80±1.04	101.35±1.87
Acid volume				
3.0 mL	99.97±0.45	98.50±1.71	99.37±0.47	100.72±1.92
5.0 mL	98.89±1.27	101.83±0.54	99.80±1.04	101.35±1.87

* Average of three replicates

** No variation in the assay condition of the proposed method. The optimized condition was 4.0 mL of 0.5 N acid (Acetic acid for LOR, Perchloric acid for CTZ and FXD, and Sulphuric acid for EBS). Final drug conc. = 1.0 μg/ml

Table 5 Determination of investigated Non-Sedating Antihistamines in their dosage forms

Authentic drug	Dosage form	% Recovery[a]±SD		t- value[b]	F- value[b]
		Proposed method	Reported method		
CTZ	Epirizine® tablets	100.73±1.73	100.33±0.78*	0.520	4.92
EBS	Evastine® tablets	99.86±2.20	98.84±1.18*	0.997	3.48
FXD	Allerfen® tablets	101.06±1.47	100.33±1.01*	1.010	2.12
LOR	Mosedine® tablets	100.14±0.92	100.08±1.34**	0.079	2.12

[a] Average of six determination±standard deviation

[b] Theoretical values at 95 % confidence limit; t=2.228, F=5.053

* Reference [26]

** Reference [23]

In the present work, the topology of the molecule was expressed using several electronic parameters namely: the pKa (amino group) and log *P* of the studied drug. These electronic parameters were obtained from literature [29–35] and included as the electronic contribution variables.

The molecular topography was expressed using the molecular connectivity (χ^v) adapted by Kier and Hall [36] as a descriptive title for the general method leading to indexes derived from the molecular structure. The structural formula of the compound is written as a molecular skeleton without hydrogens. Each carbon atom is designated by a cardinal number, which is a count of the number of adjacent carbon atoms. This count of adjacent or formally bonded carbon atoms is called the delta value (δ). The molecular skeleton is then dissected into all constituent bonds, each designated by the two carbons, i and j, forming the bond. A value for each bond is computed from the equation $(\delta_i\,\delta_j)^{-0.5}$. The molecular connectivity index is the simple sum of the computed bind values over the entire molecule according to the equation [36]:

$$^{1}\chi^{v} = \Sigma\left(\delta_i\,\delta_j\right)^{-0.5}$$

Where the prefix 1 indicates that the index is for a one bond dissection of the molecule.

To calculate the second order molecular connectivity index ($^{2}\chi^{v}$), a term for each two bond fragment (three contiguous

Table 6 Assay of tablets by standard addition method

Authentic drug	Dosage form	Declared amount (mg/tab.)	Working conc. (μg/mL)	Authentic drug added (μg/mL)	Found of added (μg/mL)	% Recovery of added ± SD*	C.V
CTZ				0.25	0.256	102.44 ± 2.32	2.26
	Epirizine® tablets	10.0	0.25	0.75	0.778	103.80 ± 0.85	0.82
				1.75	1.777	101.54 ± 1.88	1.85
EBS				0.25	0.258	103.11 ± 0.74	0.72
	Evastine® tablets	10.0	0.25	0.75	0.732	97.67 ± 0.80	0.82
				1.75	1.721	98.33 ± 0.92	0.94
FXD				0.25	0.252	100.68 ± 2.13	2.12
	Allerfen® tablets	60.0	0.25	0.75	0.763	101.70 ± 1.33	1.31
				1.75	1.743	99.62 ± 1.78	1.79
LOR				0.20	0.193	96.27 ± 0.51	0.53
	Mosedine® tablets	10.0	0.30	0.50	0.515	103.08 ± 1.08	1.05
				0.70	0.691	98.72 ± 0.72	0.73

* Average of six replicates

Table 7 Some physicochemical parameters of the studied drugs

Drug	pKa*	Log *P***	$^{1}\chi^{v}$	$^{2}\chi^{v}$	$^{3}\chi^{v}$	$^{4}\chi^{v}$	$^{1}\chi^{2}$	$^{2}\chi^{2}$	$^{3}\chi^{2}$	$^{4}\chi^{2}$
CTZ	8.00	1.70	2.17	1.17	0.53	0.25	4.71	1.37	0.28	0.0625
EBS	10.32	2.74	5.70	5.08	3.62	3.42	32.49	25.81	13.1	11.7
FXD	9.53	2.81	6.49	5.35	4.50	3.10	42.12	28.62	20.25	9.61
LOR	5.00	5.20	1.63	0.70	0.31	0.15	2.66	0.49	0.096	0.0225

*References of pKa values are [29–32]

** References of Log *P* values are [33–35]

- Molecular connectivity indexes and their squares were calculated

atoms i, j and k) is computed using the following general equation:

$$^{2}\chi^{v} = \Sigma\left(\delta_i \delta_j \delta_k\right)^{-0.5}$$

On the other hand, the general equations are used to calculate the third and fourth order molecular connectivity indexes are $^{3}\chi^{v}=\Sigma\ (\delta_i\ \delta_j\ \delta_k\ \delta_l)^{-0.5}$ and $^{4}\chi^{v}=\Sigma\ (\delta_i\ \delta_j\ \delta_k\ \delta_l\ \delta_m)^{-0.5}$; respectively.

To include the effect of unsaturation in the calculated connectivity indexes, a double bond would be counted twice when calculating the d value for adjacent atoms e.g.,

$$^{2}C = {}^{3}C - {}^{3}C = {}^{2}C$$

This procedure takes explicit account of the valence and the hybrid state of each carbon atom. The modified delta value

Table 8 Regression analysis of relative fluorescence intensities of the studied Non-Sedating Antihistamines versus molecular connectivity indexes

Parameter	Type of equation	Equation No.	a	b	c	d	r	R^2	F	SE
$^{1}\chi^{v}$	**Linear**	**1**	451.1	−52.50			0.5912	0.3496	1.075	221.6
	Log.	**2**	499.0	−216.2			0.6662	0.4438	1.596	204.9
	Exp.	**3**	437.9	−0.2600			0.6536	0.4272	1.492	207.9
	Power	**4**	557.1	−1.072			0.7523	0.5659	2.607	181.0
	Polynomial (degree 2)	**5**	1635	−869.3	101.2		0.9706	0.9420	32.50	66.15
	Polynomial (degree 3)	**6**	**2890**	**−2029**	**403.4**	**−23.75**	**0.9999**	**0.9999**	**427,802**	**0.5941**
$^{2}\chi^{v}$	**Linear**	**7**	406.5	−55.76			0.6168	0.3803	1.227	216.3
	Log.	**8**	354.4	−153.3			0.7083	0.5018	2.014	193.9
	Exp.	**9**	369.5	−0.2900			0.6632	0.4399	1.571	205.6
	Power	**10**	273.9	−0.7750			0.7967	0.6347	3.475	166.1
	Polynomial (degree 2)	**11**	1295	−1199	186.6		0.9995	0.9990	2053	8.572
	Polynomial (degree 3)	**12**	**1332**	**−1268**	**219.6**	**−4.033**	**0.9999**	**0.9999**	**72,108**	**1.447**
$^{3}\chi^{v}$	**Linear**	**13**	362.6	−56.96			0.5418	0.2936	0.8311	230.9
	Log.	**14**	262.2	−110.3			0.6659	0.4434	1.5961	204.9
	Exp.	**15**	281.1	−0.27833			0.5895	0.3475	1.0653	221.9
	Power	**16**	171.5	−0.5517			0.7483	0.5600	2.545	182.2
	Polynomial (degree 2)	**17**	561.2	−533.5	102.9		0.8118	0.6590	3.864	160.4
	Polynomial (degree 3)	**18**	**1285**	**−2688**	**1066**	**−116.0**	**0.9999**	**0.9999**	**62,597**	**1.553**
$^{4}\chi^{v}$	**Linear**	**19**	370.2	−78.13			0.6172	0.3809	1.230	216.2
	Log.	**20**	213.8	−92.05			0.6744	0.4548	1.668	202.9
	Exp.	**21**	325.6	−0.445			0.6190	0.3832	1.242	215.8
	Power	**22**	133.5	−0.49			0.7347	0.5398	2.346	186.4
	Polynomial (degree 2)	**23**	347.0	40.81	−34.15		0.6204	0.3849	1.252	215.5
	Polynomial (degree 3)	**24**	**1297**	**−5362**	**3151**	**−494.6**	**0.9999**	**0.9999**	**882,626**	**0.4136**

a is the intercept. b, c, and d are the first, second and third slopes; respectively

F = F-ratio between the variances of the observed and calculated values at 95 % probability

SE = Overall standard error of the correlation

Most Statistically significant relationships are presented in bold digits

Table 9 Regression analysis of relative fluorescence intensities of the studied Non-Sedating Antihistamines versus the squares of the molecular connectivity indexes

Parameter	Type of equation	Equation No.	a	b	c	d	r	R^2	F	SE
$^1\chi^2$	**Linear**	**25**	357.6	−5.980			0.5285	0.2793	0.7750	233.2
	Log.	**26**	499.1	−107.8			0.6661	0.4436	1.595	204.9
	Exp.	**27**	270.4	−0.02333			0.5705	0.3255	0.9652	225.6
	Power	**28**	557.4	−0.5333			0.7513	0.5644	2.591	181.3
	Polynomial (degree 2)	**29**	527.2	−50.30	1.033		0.8003	0.6404	3.562	164.8
	Polynomial (degree 3)	**30**	1223	−284.9	12.50	−0.1492	0.9988	0.9976	837.8	13.41
$^2\chi^2$	**Linear**	**31**	352.5	−8.351			0.5667	0.3212	0.9462	226.4
	Log.	**32**	354.4	−76.92			0.7085	0.5019	2.0155	193.9
	Exp.	**33**	279.2	−0.04000			0.5896	0.3476	1.0655	221.9
	Power	**34**	274.0	−0.3850			0.7958	0.6333	3.454	166.4
	Polynomial (degree 2)	**35**	465.6	−126.72	4.176		0.8151	0.6644	3.959	159.2
	Polynomial (degree 3)	**36**	800.0	−524.2	33.39	−0.5510	0.9996	0.9993	2837	7.292
$^3\chi^2$	**Linear**	**37**	321.8	−10.29			0.4570	0.2089	0.5280	244.38
	Log.	**38**	262.2	−55.35			0.6656	0.4431	1.591	205.0
	Exp.	**39**	220.7	−0.04000			0.4980	0.2480	0.6596	238.3
	Power	**40**	171.4	−0.2717			0.7471	0.5582	2.526	182.6
	Polynomial (degree 2)	**41**	360.6	−60.59	2.649		0.6909	0.4773	1.826	198.6
	Polynomial (degree 3)	**42**	771.5	−2322	282.2	−8.340	0.9999	0.9999	17,966	2.899
$^4\chi^2$	**Linear**	**43**	355.3	−22.48			0.6201	0.3845	1.249	215.6
	Log.	**44**	213.8	−46.00			0.6744	0.4548	1.668	202.9
	Exp.	**45**	307.4	−0.1283			0.6014	0.3617	1.133	219.5
	Power	**46**	133.5	−0.2417			0.7338	0.5386	2.334	186.6
	Polynomial (degree 2)	**47**	345.8	45.00	−6.135		0.6790	0.4610	1.711	201.7
	Polynomial (degree 3)	**48**	783.3	−10,367	1956	−91.90	0.9982	0.9963	545.3	16.61

a is the intercept. b, c, and d are the first, second and third slopes; respectively

F = F-ratio between the variances of the observed and calculated values at 95 % probability

SE = Overall standard error of the correlation

Table 10 Regression analysis of relative fluorescence intensities of the studied Non-Sedating Antihistamines versus pKa or log *P*

Parameter	Type of equation	Equation No.	a	b	c	d	r	R^2	F	SE
pKa	**Linear**	**49**	956.6	−87.86			0.9197	0.8458	10.97	107.9
	Log.	**50**	1571	−645.7			0.9346	0.8736	13.82	97.70
	Exp.	**51**	5134	−0.4267			0.9453	0.8936	16.81	89.60
	Power	**52**	81,954	−3.043			0.9465	0.8959	17.21	88.64
	Polynomial (degree 2)	**53**	1632	−282.1	12.84		0.9390	0.8817	14.91	94.50
	Polynomial (degree 3)	**54**	**14,572**	**−5701**	**733.0**	**−30.73**	**0.9999**	**0.9999**	**574,951**	**0.5124**
log *P*	**Linear**	**55**	−177.2	132.4			0.8745	0.7648	6.504	133.2
	Log.	**56**	−176.8	390.3			0.7974	0.6358	3.492	165.8
	Exp.	**57**	31.75	0.4983			0.9252	0.8559	11.88	104.3
	Power	**58**	35.12	1.376			0.8933	0.7979	7.899	123.5
	Polynomial (degree 2)	**59**	416.9	−255.5	54.14		0.9422	0.8877	15.80	92.09
	Polynomial (degree 3)	**60**	25,198	−28,501	9811	−1008	0.9999	0.99973	7594	4.458

a is the intercept. b, c, and d are the first, second and third slopes; respectively

F = F-ratio between the variances of the observed and calculated values at 95 % probability

SE = Overall standard error of the correlation

Most Statistically significant relationship is presented in bold digits

Table 11 Multiple linear regression analysis of relative fluorescence intensities of the studied Non-Sedating Antihistamines versus pKa or log P and connectivity indexes or their squares

RFI=a+b pKa+c (χ^vor χ^2)				RFI=a+b log*P*+c (χ^vor χ^2)					
Parameter (1)	Parameter (2)	Equation No.	a	b	c	r	R^2	F	SE
pKa	$^1\chi^v$	**61**	1194	−150.9	70.24	0.9997	0.9993	724.1	10.21
	$^2\chi^v$	**62**	1275	−153.1	70.76	0.9958	0.9915	58.49	35.77
	$^3\chi^v$	**63**	1246	−143.2	73.55	0.9998	0.9996	1260	7.738
	$^4\chi^v$	**64**	1268	−143.9	86.23	0.9825	0.9652	13.88	72.45
	$^1\chi^2$	**65**	**1238**	**−141.5**	**7.752**	**0.9999**	**0.9999**	**170,526**	**0.6654**
	$^2\chi^2$	**66**	1278	−144.9	10.45	0.9964	0.9927	68.21	33.14
	$^3\chi^2$	**67**	1209	−132.7	13.77	0.9993	0.9985	344.4	14.79
	$^4\chi^2$	**68**	1249	−137.9	22.24	0.9734	0.9474	9.007	89.11
log *P*	$^1\chi^v$	**69**	−3.350	115.3	−30.13	0.9277	0.8606	3.086	145.1
	$^2\chi^v$	**70**	−18.35	113.9	−32.92	0.9394	0.8824	3.753	133.2
	$^3\chi^v$	**71**	−60.96	118.5	−32.57	0.9232	0.8524	2.887	149.3
	$^4\chi^v$	**72**	−35.75	115.1	−50.61	0.9548	0.9116	5.155	115.5
	$^1\chi^2$	**73**	−67.21	119.2	−3.357	0.9193	0.8452	2.730	152.9
	$^2\chi^2$	**74**	−58.99	117.6	−5.107	0.9356	0.8753	3.510	137.2
	$^3\chi^2$	**75**	−99.32	122.8	−5.673	0.9078	0.8242	2.344	162.9
	$^4\chi^2$	**76**	−44.03	115.7	−15.13	0.9626	0.9266	6.306	105.3

a is the intercept. b, c, and d are the first, second and third slopes; respectively

F = F-ratio between the variances of the observed and calculated values at 95 % probability

SE = Overall standard error of the correlation

Most Statistically significant relationship is presented in bold digits

calculated according to this procedure is called the valence delta (δ^v) and the computed connectivity index is called the valence chi (χ^v).

Another progress in this field was the treatment of hetero-atoms introduced by Kier and Hall [37] where the delta value for each heteroatom is calculated according to the equation:

$$\delta^v = z^v - h$$

Where z^v is the number of valence electrons and h is the number of hydrogen atoms attached to the heteroatom. Kier and Hall also reported the δ^v values of different heteroatoms in various hybrid states.

In this work, we tried to co rrelate the experimentally measured RFI of the studied drug with the pKa (amino group) and log *P* values of the studied drugs, as well as the first, second, third and fourth order connectivity indexes and their squares for the substituent at amino group. These parameters are summarized in Table 7.

Similar correlations were previously reported for phenothiazines [38] and quinolone antibacterials [39] where highly significant relationships were obtained between molar absorptivities of certain derivatives and some physicochemical parameters of the parent compound.

Table 12 Prediction of relative fluorescence intensities of the studied Non-Sedating Antihistamines

Drug	Relative fluorescence intensities							Error (%)					
	Calculated						Observed						
	6	12	18	24	54	65		6	12	18	24	54	65
CTZ	143.14	143.18	142.8	145.71	145.32	142.74	143.05	0.0629	0.0909	0.0698	1.859	1.587	0.217
EBS	30.3	31.87	27.15	29.48	32.84	29.86	29.41	3.026	8.36	7.684	0.238	11.66	1.462
FXD	217.77	219.26	213.49	221.28	219.58	216.28	216.61	0.536	1.223	1.440	2.156	1.371	0.152
LOR	551.13	550.97	550.83	561.93	552.45	551.29	551.10	0.00544	0.0236	0.0490	1.965	0.245	0.0345

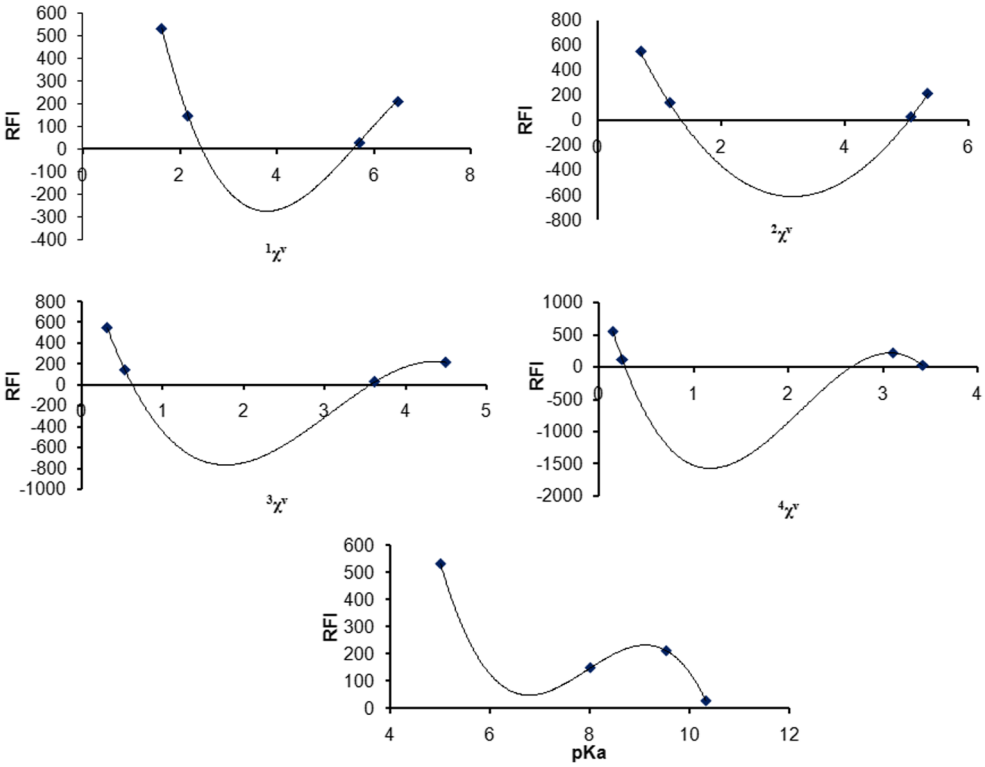


Fig. 8 Polynomial regression curves (degree 3) of equations # (6, 12, 18, 24 and 54)

Computation

Intel CORE i3 PC equipped with Microsoft Excel 2007® data analysis tool pack.

Mathematical and Statistical Treatment of Data

a- The correlation between the experimentally measured RFI of the studied drugs obtained in the previously "Results and Discussion" section and the corresponding drug concentrations was carried out using linear regression model to obtain the intercept (a), the slope (b), the correlation coefficient (r) and the determination coefficient (r^2).

b- The correlation between the experimentally measured RFI of the studied drugs and the studied parameters obtained from literature [29–35], was carried out using simple or multiple regression analysis models in the Microsoft Excel 2007® data analysis tool pack.

Results and Discussion

The first, second, third and fourth valence connectivity indexes and their squares for R group linked on amino group of the investigated drugs were calculated according to the general rules of Kier and Hall [36] mentioned under "Derivation Quantitative Fluorescence Intensity - Structure Relationships" section. In addition, pKa and log *P* values were obtained from references [29–35]. All types of possible correlations were calculated including;

a- Linear curve fit. $Y = a + bX$
b- Logarithmic curve fit. $Y = a + b \ln X$
c- Exponentialc curve fit. $Y = a e^{bX}$
d- Power curve fit. $Y = a X^b$
e- Polynomial (degree 2). $Y = a + bX + cX^2$
f- Polynomial (degree 3). $Y = a + bX + cX^2 + dX^3$

Where Y=RFI, and X=pKa, log *P*, $^1\chi^v$, $^2\chi^v$, $^3\chi^v$, $^4\chi^v$, or their squares.

On the other hand, multiple linear regression analysis was performed including several parameters representing the structural features that differ from one drug to another. All curves were fitted by the method of least squares. Regression equations obtained were evaluated to estimate the best fit by calculating their correlation coefficient (r), standard error (SE), and the significance of correlation by the F- test. The relations obtained are presented in Tables 8, 9, 10 and 11.

It is evident from the obtained 76 relations (These equations are given serial number from 1 to 76):

i. Regarding the type of regression equation, the best curve fit, as estimated by correlation coefficient, standard error

and significance of correlation by F- test, is generally the polynomial regression of degree 3 (Tables 8, 9 and 10). The numbers of these equations are #6, 12, 18, 24, and 54.

ii. pKa and log *P* of the studied drugs were used with connectivity indexes or their squares for multiple linear regression analysis. Excellent correlation of RFI versus pKa and $^{1}\chi^{2}$ was obtained for all the studied drugs according to the general equation (65). The regression model obtained in equation #65 demonstrated that an increase in the $^{1}\chi^{2}$ value with a decrease in the pKa value resulted in an increase in the measured RFI.

Equation	Equation No.
$RFI = -23.75\ (^{1}\chi^{v})^{3} + 403.4\ (^{1}\chi^{v})^{2} - 2029\ ^{1}\chi^{v} + 2890$	6
$RFI = -4.033\ (^{2}\chi^{v})^{3} + 219.6\ (^{2}\chi^{v})^{2} - 1268\ ^{2}\chi^{v} + 1332$	12
$RFI = -116.0\ (^{3}\chi^{v})^{3} + 1066\ (^{3}\chi^{v})^{2} - 2688\ ^{3}\chi^{v} + 1285$	18
$RFI = -494.6\ (^{4}\chi^{v})^{3} + 3151\ (^{4}\chi^{v})^{2} - 5362\ ^{4}\chi^{v} + 1297$	24
$RFI = -30.73\ (pKa)^{3} + 733.0\ (pKa)^{2} - 5701\ pKa + 14572$	54
$RFI = 1238 - 141.5\ pKa + 7.752\ ^{1}\chi^{2}$	65

iii. These six equations (# 6, 12, 18, 24, 54 and 65) were exploited for the calculation of RFI of NSAs as shown in Table 12. The close concordance of the predicted with the experimentally measured relative fluorescence intensities is quite evident. Figure 8 shows polynomial regression curves (degree 3) of equations # (6, 12, 18, 24 and 54).

Conclusion

The developed spectrofluorimetric method for the determination of some non-sedating antihistamines (cetirizine, ebastine, fexofenadine, and loratadine) via their native fluorescence is novel, simple, rapid, and selective, sensitive, precise, accurate, and convenient. Hence, the proposed method should be useful for routine quality control purposes. On the other hand, some quantitative fluorescence intensity - structure relationships were derived. These relationships were found between the relative fluorescence intensities of the protonated drugs and their physicochemical parameters namely; the pKa, Log *P,* connectivity indexes (χ^{v}) and their squares. Seventy six regression equations were obtained and not previously reported. Six of these correlation equations were highly significant and used for the prediction of RFI of the studied NSAs.

Disclosure All the authors of the paper do not have a direct financial relation with the commercial identity mentioned in the paper.

Conflict of Interest All the authors declare that there is no conflict of interests in their submitted paper.

References

1. Mahdy MA, Webster RN (2011) Histamine and antihistamines. Anaesth Intensive Care Med 12(7):326–8
2. Thurmond RL, Gelfand EW, Dunford PJ (2008) The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov 7:41
3. Golightly LK, Greos LS (2005) Second-generation antihistamines: actions and efficacy in the management of allergic disorders. Drugs 65:341
4. Sweetman SC (2009) Martindale "The Complete Drug Reference", 36th edn. Pharmaceutical Press, London, pp 561–584
5. Simons F, Estelle R, Simons KJ (2011) Histamine and H1-antihistamines: celebrating a century of progress. J Allergy Clin Immunol 128(6):1139–1150
6. Trivedi RK, Patel MC, Jadhav SB (2011) A rapid, stability indicating RP-UPLC method for simultaneous determination of ambroxol hydrochloride, cetirizine hydrochloride and antimicrobial preservatives in liquid pharmaceutical formulation. Sci Pharm 79(3):525–543
7. Hadad GM, Emara S, Mahmoud WM (2009) Development and validation of a stability-indicating RP-HPLC method for the determination of paracetamol with dantrolene or/and cetirizine and pseudoephedrine in two pharmaceutical dosage forms. Talanta 79(5): 1360–1367
8. Konieczna L, Plenis A, Olędzka I, Kowalski P, Bączek T (2010) Rapid RP-LC method with fluorescence detection for analysis of fexofenadine in human plasma. Chromatographia 71(11-12):1081–1086
9. Plenis A, Konieczn L, Olędzka I, Kowalski P (2010) Rapid analysis of loratadine in human serum by high-performance liquid chromatography with fluorescence detection. Acta Chromatogr 22(1):69–79
10. Ibrahim F, Sharaf El-Din MK, Eid MI, Wahba ME (2011) Validated stability indicating liquid chromatographic determination of ebastine in pharmaceuticals after pre column derivatization: application to tablets and content uniformity testing. Chem Cent J 5:24
11. Maher HM, Sultan MA, Olah IV (2011) Development of validated stability-indicating chromatographic method for the determination of fexofenadine hydrochloride and its related impurities in pharmaceutical tablets. Chem Cent J 5(1):76
12. Johnson R, Christensen J, Lin CC (1994) Sensitive gas-liquid chromatographic method for the determination of loratadine and its major active metabolite, descarboethoxyloratadine, in human plasma using a nitrogen-phosphorus detector. J Chromatogr B Biomed Appl 657(1):125–131
13. Patil RH, Hegde RN, Nandibewoor ST (2011) Electro-oxidation and determination of antihistamine drug, cetirizine dihydrochloride at glassy carbon electrode modified with multi-walled carbon nanotubes. Colloids Surf B: Biointerfaces 83(1):133–138
14. Rachidi M, Digua K, Hubert P, Faouzi MA, Cherrah Y, Bouklouze A (2006) Analytical validation of potentiometric method for cetrizonium ion. Anal Lett 39(8):1699–1708
15. Aleksi MM, Radulovi VI, Kapetanovi VP, Savi VM (2010) The possibility of simultaneous voltammetric determination of desloratadine and 3-hydroxydesloratadine. Acta Chim Slov 57: 686–692
16. Ghoneim MM, Mabrouk MM, Hassanein AM, Tawfik A (2001) Polarographic behaviour of loratadine and its direct determination in pharmaceutical formulation and human plasma by cathodic adsorptive stripping voltammetry. J Pharm Biomed Anal 25(5–6): 933–939
17. Güngör SD (2004) Electrooxidation of cetirizine dihydrochloride with a glassy carbon electrode. Pharmazie 59(12):929–933

18. Mahgoub H, Gazy AA, El-Yazbi FA, El-Sayed MA, Youssef RM (2003) Spectrophotometric determination of binary mixtures of pseudoephedrine with some histamine H1-receptor antagonists using derivative ratio spectrum method. J Pharm Biomed Anal 31(4):801–809
19. Sharaf El-Din MK, Ibrahim FA, Eid MI, Wahba MEK (2011) First and second derivative synchronous fluorescence and spectrophotometric spectroscopy for the simultaneous determination of fexofenadine hydrochloride in presence of its degradation products: application to stability studies. Acta Chim Slov 58:278–287
20. El-Kousy N, Bebawy LI (1999) Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods. J Pharm Biomed Anal 20(4):671–679
21. Gazy AA, Mahgoub H, El-Yazbi FA, El-Sayed MA, Youssef RM (2002) Determination of some histamine H1-receptor antagonists in dosage forms. J Pharm Biomed Anal 30(3):859–867
22. Sevgi TU (2010) Extractive spectrophotometric determination of cetirizine dihydrochloride in pure and pharmaceutical preparations. J Food Drug Anal 18(6):440–446
23. Walash MI, Belal F, El-Enany N, Eid M, El-Shaheny RN (2011) Stability-indicating micelle-enhanced spectrofluorimetric method for determination of loratadine and desloratadine in dosage forms. Luminescence 26(6):670–679
24. Alothman ZA, Bukhari N, Haider S, Wabaidur SM, Alwarthan AA (2010) Spectrofluorimetric determination of fexofenadine hydrochloride in pharmaceutical preparation using silver nanoparticles. Arab J Chem 3(4):251–255
25. Ibrahim F, El-Din MK, Eid MI, Wahba ME (2011) Validated stability-indicating spectrofluorimetric methods for the determination of ebastine in pharmaceutical preparations. Chem Cent J 5(1): 11
26. Sharaf El-Din MK, Ibrahim F, Eid MI, Wahba ME (2012) Validated spectroflurimetric determination of some H1 receptor antagonist drugs in pharmaceutical preparations through charge transfer complexation. J Fluoresc 22(1):175–191
27. Abd El-Hay SS, Colyer CL, Hassan WS, Shalaby A (2011) Spectrofluorimetric determination of etodolac, moxepril HCl and fexofenadine HCl using europium sensitized fluorescence in bulk and pharmaceutical preparations. J Fluoresc. doi:10.1007/s10895-011-0954-8
28. (2005) ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology, Q2 (R1) Geneva, http://www.ich.org
29. Pagliara A, Morin D, Testa B, Urien S, Carrupt PA, Tillement JP, Jolliet P, Rihoux JP, Morin C (1998) Molecular properties and pharmacokinetic behavior of cetirizine, a zwitterionic H1-receptor antagonist. J Med Chem 41:853–863
30. Segarra V, López M, Ryder H, Palacios MJ, Roberts DJ (1999) Computer-assisted comparison of the structural and electronic dispositions of ebastine and terfenadine. Drug Saf 21(Suppl 1):45–61
31. Olsén L, Ingvast-Larsson C, Larsson P, Broström H, Bondesson U, Sundqvist M, Tjälve H (2006) Fexofenadine in horses: pharmacokinetics, pharmacodynamics and effect of ivermectin pretreatment. J Vet Pharmacol Ther 29(2):129–135
32. Ramulu G, Kumar YR, Vyas K, Suryanarayana MV, Mukkanti K (2011) A new validated liquid chromatographic method for the determination of loratadine and its impurities. Sci Pharm 79(2): 277–291
33. Hansch C, Leo A, Hoekman D (1995) Exploring QSAR - hydrophobic, electronic, and steric constants, DC: American Chemical Society, Washington 172, 176
34. Abou-Gharbia M, Moyer JA, Nielsen ST, Webb M, Pate U (1995) New antihistamines: substituted piperazine and piperidine derivatives as novel H1-antagonists. J Med Chem 38: 4026–4032
35. Moffat AC, Osselton MD, Widdop B (2004) Clarke's analysis of drugs and poisons. Pharmaceutical press, London
36. Kier LB, Hall LH (1986) Molecular connectivity in structure-activity analysis. Wiley, New York, pp 10–22
37. Kier LB, Hall LH (1976) Molecular connectivity VII: specific treatment of heteroatoms. J Pharm Sci 65:1806–1809
38. El-Kommos ME, Youssef AF (1987) Colorimetric determination of phenothiazine drugs. 4- Correlation between molar absorptivity and F, R and χ parameters of ring substituents. Bull Pharm Sci, Assiut Univ 10(2):21–33
39. El-Kommos ME, Saleh GA, El-Gizawy SM, Abou-Elwafa MA (2006) Quantitative fluorescence intensity - structure relationships of certain quinolone – metal chelates. Bull Pharm Sci, Assiut Univ 29(2):289–299