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Spectrofluorimetric Method for the Determination of Alfuzosin and Flavoxate Hydrochlorides in Pharmaceuticals and Biological Fluids.

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Summary: A simple and sensitive spectrofluorimetric method was developed to determine alfuzosin-HCl and flavoxate-HCl. Maximum fluorescence intensity was achieved in pure water at 388 nm and 375 nm using λex 244 nm and 240 nm for alfuzosin-HCl and flavoxate-HCl, respectively. The optimum experimental parameters such as solvent, micelle-enhancement and pH were evaluated. Good correlations were obtained between the fluorescence intensity and concentration in the ranges of 2.5-30 ng/ml for alfuzosin and 1-6 µg/ml for flavoxate-HCl. The suggested method was successfully applied to estimate the two drugs in their tablets with average recoveries of 99.2±1.01 and 99.8±0.70%, respectively. These results were found to agree with those of reference methods. The method also retained its accuracy and precision when applied to determine alfuzosin-HCl in spiked serum or urine as judged by an average recovery of 95.4±0.87 or 100.1±0.92%, respectively. Furthermore, the method was validated according to the International Cofference on Harmonization.

Introduction

Alfuzosin-HCl (AFZ); N-[3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)-methyl-amino]propyl]oxolane-2-carboxamide hydrochloride, is alpha-1-adrenoreceptor blocker[1,2]. Flavoxate-HCl (FVX); 2-piperidinoethyl-3-methyl-4-oxo-2-phenyl-4-chromene-8-carboxylate hydrochloride, is anti-cholinergic[3,4]. Both are used for the treatment of lower urinary tract disorders associated with benign prostatic hyperplasia[1-3]. AFZ and its formulations are still non official. The reported methods revealed that the drug was determined by HPLC[5-9], spectrophoto-metric[10-12] and voltammetric[13] methods. FVX is officially assayed in BP 2001[4] by non-aqueous titration. Several techniques were used for its determination

including HPLC[14-16], GC[17,18], TLC[18] electrophoresis(19), spectroscopy[20] and volammetry[21]. Up to date nothing have been reported concerning direct spectrofluorimetric determination of both drugs. Hence the author has made an attempt to develop a new simple and rapid spectrofluorimetric method to determine AFZ and FVX in their formulations and/or biological fluids.

Experimental

Apparatus

Shimadzu RF 1501 spectrophotofluorimeter (Japan) equipped with Xenon discharge lamp and 1 cm quartz cell at low and high sensitivity with ex and em band width 10 nm, Jasco V 630-PC spectrophotometer (Japan) and Mitrohm Titranto 809 Tiamo automatic potentiometric titrator connected to Mitrohm combined-glass electrode and 804 Ti stand with stirrer (Switzerland) were used.

Materials and reagents

Double distilled was used throughout the procedure**.** Pure AFZ, 99.8±0.53% was kindly supplied by Amriya for Pharmaceutical Industries, Alexandria, Egypt. Pure FVX, 100.2±095%, was kindly supplied by Medical Union Pharmaceuticals, Ismalia, Egypt. Xatral SR tablets contain 5 mg AFZ per tablet, BN 117901[B] (Amriya for Pharmaceutical Industries, Alexandria, Egypt-under licence of Laboratories Sythelabo Group, Le Plessis Robinson, France) and Genurine^{R} tablets contain 200 mg FVX per tablet, BN 070278 (Medical Union Pharmaceuticals, Abu-Sultan, Ismalia, Egypt-under licence of Recordati, Milano, Italy) were purchased from the local market.

Standard solutions

Standard AFZ solution; 0.1mg/ml was prepared in water found to be stable for 3 days at room temp, from which a 0.01mg/ml working solution was prepared by diluting with water. Working standard FVX solution; 0.2 mg/ml in water was prepared and found to be stable for 2 days at room temp and protected from light.

Procedure

Linearity

Aliquots of AFZ working solution in water, 0.01 mg/mL equivalent to 0.25-3.0 µg of the drug were transferred to a series of 100-mL volumetric flasks and completed to volume with water. The fluorescence intensity of each solution was measured at λem 388 nm using λex 244 nm at low sensitivity. In another set of 100-mL volumetric flasks, accurate volumes of working FVX solution, 0.1 mg/mL corresponding to 0.1-0.6 mg FVX were diluted to the mark with water. Their fluorescence intensities were measured at 375 nm after excitation at 240 nm at high sensitivity. Calibration curves were constructed relating each drug concentrations to fluorescence intensities and the corresponding regression equations were computed.

Application to pharmaceutical formulations

Ten Xatral SR or Genurin^{R} tablets were weighed and finely ground. A weighed amount of the fine well mixed powder equivalent to one mg AFZ or 20 mg FVX was transferred to a 100-mL beaker containing 60ml water and shaked by sonication for 10 min. Then filtered into a 100-mL volumetric flask and completed to volume with water to obtain aqueous solution labeled to contain 0.01 mg/mL AFZ or 0.2 mg/mL FVX. Each solution was analysed by spectrofluorimetry as detailed under linearity and drug concentration was calculated from the proper regression equation.

Application to biological fluids

A. Bovine serum- A standard AFZ solution 0.1 mg/mL was prepared in methanol. In standard eppendorf tubes 0.02-0.12 mL of the methanolic solution were added to 0.5 mL serum and the volume was completed to one mL with methanol. The tubes were vortex for 5min, then centrifuged for 20 min at 5000 rpm. 0.25 mL of the protein-free supernatant from each tube was transferred to a 100-mL volumetric flask, diluted to volume with water and analysed exactly as described

under linearity. Blank values were computed by treating drug-free serum in the same manner.

B. Human urine- one mL urine was spiked with 10 mg AFZ and diluted to 100 mL with water in a volumetric flask. Then 5 ml of this solution was further diluted with water to 50 mL to obtain a working solution labeled to contain 0.01 AFZ analysed fluorimetrically as detailed under linearity. Blank used was similarly treated AFZ-free urine.

Results and discussion

AFZ and FVX were predicted to be efficient fluorophores due to their highly conjugated and rigid molecular structures. The influence of rigidity has been involved to account for the increasing in fluorescence of organic molecules that enhanced by electron donating substituents as it increase the likelihood for radiative deactivation. This has been fulfilled in AFZ by the fusion of the benzene ring with pyrimidine ring in its quinazolinyl moiety and by the substitution with amino and methoxy groups, Scheme 1. Also for FVX the rigid and planar structure obtained from the fusion of benzene and pyran rings in its flavone nucleus together with the further conjugation with a phenyl ring has been expected to lower the rate of non-radiative relaxation to the point where fluorescence has point to occur[2-6,22], Scheme 1.

Reveiwing the literature, no direct fluorimetric methods were reported for both drugs except fluorescence-detection of AFZ in HPLC[5,6].

Figs 1and 2 showed that AFZ exhibited λem at 388 nm using λex 244 nm and FVX has λem at 375 nm after excitation at 240 nm.

It was noteworthy to mention that the proposed fluorimetric procedure can not be used for stability-indication of both drugs. Their degradation products were prepared and separated by refluxing AFZ with 2 N NaOH for 2 h[9] and FVX with conc. HCl for 2h[3].

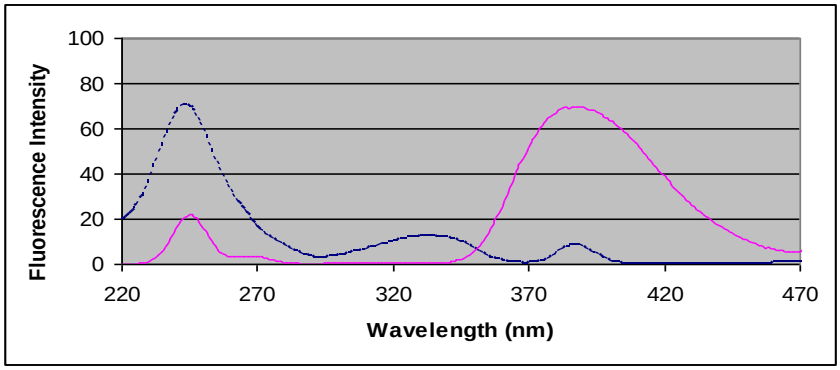


Fig. 1. Excitation and emission spectra of 20 ng/ml Alfuzosin-HCl in water (λex 244nm , λem 388nm).

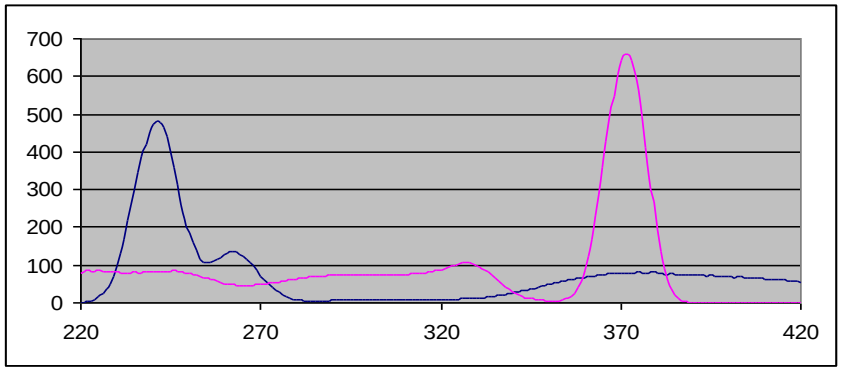
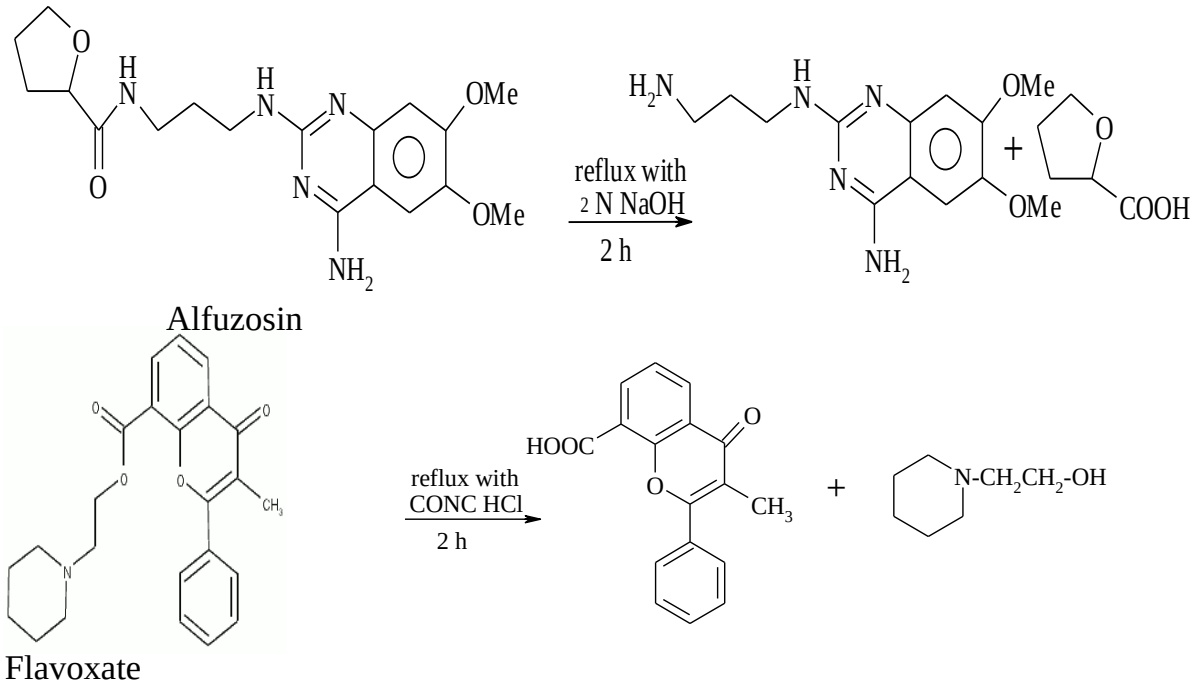


Fig.2. Excitation and emission spectra of 6µg/ml Flavoxate-HCl in water (λex 240nm , λem 375nm).

The alkaline hydrolysate of AFZ was neutralized with 5 N HCl, checked for complete degradation by TLC and IR [9], then analysed by the proposed fluorimetric method. It was found to interfere seriously with λem at 388 nm of the intact drug. However, the main degradate of FVX, 3-methylflavone-8-carboxylic acid, precipitated from hot conc. HCl was filtered after dilution and checked for its purity by FTIR using KBr disc and ^{1}H NMR in deuterated DMSO[3]. Although, it was sparingly soluble in water, yet the small amout soluble had significant reading at 375 nm, preventing the selective determination of FVX. This may be ascribed to the presence of the same fluorophoric moieties in each pure drug and its degradate as obvious in the following scheme:



Scheme 1: The proposed degradation pathway of alfuzosin-HCl and flavoxate-HCl.

Optimization of experimental parameters

To obtain maximum sensitivity and adequate selectivity, factors affecting the native fluorescence of AFZ and FVX at 388 and 375 nm were studied after excitation at 244 and 240 nm, repectively.

Both drugs are water-soluble, but it was desirable to study the variation of drugs fluorescence with changes in the dielectric constant of the medium. So water and different organic solvents were tried as solvent, maximum fluorescence was achieved in water and dramatically decreased in methanol ethanol and acetonitrile. In acid media as 0.1N H_2SO_4 or 0.1N HCl, the fluorescence intensity of AFZ decreased in both acids but that of FVX diminished only in H_2SO_4 and in HCl it was nearly the same as in water. Thus water was recommended as solvent throughout this work, which adds to the benefits of the method.

The pH was found to be of no effect on the fluorescence intensity of both drugs using acetate buffers (pH 3-6) and phosphate buffers (pH 3-7). Again

micelle-enhanced fluorescence was studied by adding one ml (containing 100µg/mL) of each sensitizer solution; ß-cyclodextrin, gelatin and sodium dodecyl sulfate (SDS) to the aqueous drug solution. The results indicated that the fluorescence intensity of AFZ was nearly constant in pure water and in presence of ß-cyclodextrin or gelatin but decreased in presence of SDS. However, that of FVX was highly decreased in presence of the three sensitizers therefore, non of them was used in this work.

As a result, the determination of AFZ and FVX was performed by measuring their native fluorescence intensities in pure water at 388 nm and 375 nm after excitation at 244 and 240 nm, respectively.

Validation

The validity of the method was checked according to the International Conference on Harmonization and USP[23] by testing for the linearity, accuracy, precision and specificity.

Linearity- Regression analysis of Beer's plots at the specified wavelengths in a concentration range of 2.5-30 ng/mL AFZ and 1-6 µg/mL gave good correlation, r^2 = 0.9997 and 0.9991, respectively. The regression parameters calculated from the calibration graphs along with the SD of the slope, intercept and residual together with the lower limit of detection, LOD were resumed in Table 1.

Precision- It was evaluated by the assay repeatability of three concentrations of AFZ (2.5, 15 and 30 ng/mL) and FVX (1, 3 and 6 µg/mL) in triplicates in each day of three successive days. Good precision was obtained as prevealed by the intraday RSD% that ranged between 0.30-0.89 and the interday ones that were 0.80-1.21 for both drugs (Table 1) which proved the ruggedeness of the proposed method.

Accuracy- It was checked by 9 determinations of each standard drug. A mean percent of 100.1± 0.54 for AFZ and 99.7 ± 0.83 for FVX was obtained, Table 1. It was also checked by the recovery of added standard AFZ or FVX to a known conc. of commercial tablets. As prevealed in Table 2, good recoveries of 101.1±0.94 and

98.9±1.24% of added standard were obtained for the two mentioned drugs, respectively

Table (1): Characteristic parameters of the regression equations and assay validation results of the proposed spectroflurimetric method.

Parameters	**Alfuzosin-HCl**	**Flavoxate-HCl**
λem	388 nm	375 nm
λex	244 nm	240 nm
Calibration range	2.5-30 ng/mL	1-6 μg/mL
LOD	1 ng/mL	0.2 μg/mL
Regression parameters		
Slope Sb±SD	3.441±0.2	8.23±0.15
Intercept Sa±SD	0.208±0.42	2.68±0.64
Residual SD (Sy/x)	0.498	0.657
Correlation coeffeceint	0.9997	0.9991
Accuracy (mean±SD%)	100.1±0.54	99.7±0.83
Precision (RSD%)*		
Intraday	0.30-0.56	0.43-0.89
Interday	0.80-1.05	0.95-1.21

. * 9 determinations

Specificity- The successful determination of both drugs in Xatral SR and Genurin® tablets using the proposed fluorimetric method without interferences from excipeints and additives proved its specificity; with average recoveries of 99.2±1.01 and 99.8±0.70% were obtained. No significant differences was found between these results and those of a compendial method for AFZ[12] or the official method for FVX[4] when stasistical comparison was carried out by adopting student-t test and F-test at p 0.05, Table 2. However, the proposed method was of much higher sensitivity and simplicity.

Moreover, the attainment of this high sensitivity especially for AFZ encouraged its application to determine it in spiked bovine serum and human urine. The drug was directly analysed in urine samples, but for serum, a simple

deprotination was carried out with methanol to avoid background variations, followed by centrifugation and the clear cetrifugate containing AFZ was analysed.

Table (2): Application of the proposed spectrofluorimetric method and reference methods[4,12] to determine alfuzosin-HCl and flavoxate-HCl in their formulations.

Formulation	Proposed method	Reference method
Xatral SR tablets	98.7	
	100.3	
	97.9	
	99.1	
	100.1	
Mean±SD	99.2±1.0 %	98.6±0.76 [12]
t-value	1.009 (1.86)	
F-value	1.72 (6.4)	
Standard addition	101.9±0.94 %	
Genurin® tablets		
	98.9	
	100.7	
	99.6	
	100.2	
	99.4	
Mean±SD	99.8±0.70%	98.9±0.92[4]
t-value	1.734 (1.86)	
F-value	1.73 (6.4)	
Standard addition	98.9±1.24	

Ref (4): involved non-aqueous titration of FVX with $HClO_4$ in presence of mercuric Acetate. Ref(12) included spectrophotometric measurement of the reaction product of AFZ with ninhydrin in DMF at 575 nm. Figures in parenthes are the tabulated values.

Table 3 shows AFZ average recoveries of 95.4 ± 0.87% from serum and 100.1± 0.92% from urine samples.Unfortunately, FVX could not be analysed in biological fluids as setting the instrument on high sensitivity for its quantitation giving rise to high blank readings (no medium sensitivity in the instrument used)

Conclusion

This study presents the development and validation of a new simple spectrofluorimetric procedure to analyse alfuzosin-HCl and flavoxate-HCl in their

tablets with simple extraction using water. It prove highly suitable for the analysis of alfuzosin-HCl in biological samples; serum and urine, as it exhibits a large stockes shift and high sensitivity that avoiding potential background interferences from sample matrix. The proposed procedure has been validated according the ICH documents.It has been found to be more sensitive than most of the curretly accepted methods especially for alfuzosin-HCl and offers fast response, lower cost and environmental protection.

Table (3) : Application of the proposed spectrofluorimetric procedure to determine alfuzosin- HCl in serum and urine

Serum			**Urine**		
Added	Found	Recovery	Added	Found	Recovery
ng /ml	ng / ml	%	µg /ml	µg /ml	%
5	4.75	95	3	2.994	99.8
10	9.61	96.1	10	10.05	100.5
15	14.18	94.5	20	20.01	100
25	23.7	94.8	25	24.73	98.9
30	28.95	96.5	30	30.42	101.4
Mean±SD%		95.4±0.87			100.1±0.92

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