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ORIGINAL PAPER

Stability–Indicating Spectrofluorimetric Methods for the Determination of Metolazone and Xipamide in Their Tablets. Application to Content Uniformity Testing

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Abstract A highly sensitive, simple and rapid stability-indicating spectrofluorimetric method was developed for the determination of metolazone (MET) and xipamide (XPM) in their tablets. The proposed method is based on the measurement of the native fluorescence of MET in methanol at 437 nm after excitation at 238 nm and XPM in alkaline methanolic solution at 400 nm after excitation at 255 nm. The fluorescence–concentration plots were rectilinear over the range of 2.0– 20.0 ng/mL for MET and 0.2– 2.0 μg/mL for XPM, with lower detection limits (LOD) of 0.35 ng/mL and 0.02 μg/mL and a lower quantification limit (LOQ) of 1.05 ng/mL and 0.07 μg/mL for MET and XPM, respectively. The method was successfully applied to the analysis of MET and XPM in their commercial tablets and the results were in good agreement with those obtained using the official and comparison methods, respectively. Furthermore, content uniformity testing of the studied pharmaceutical tablets was also conducted. The application of the proposed method was extended to stability studies of MET and XPM after exposure to different forced degradation conditions, such as acidic, alkaline, oxidative and photolytic degradation conditions, according to ICH Guidelines. Moreover, the method was utilized to investigate the kinetics of the alkaline, acidic and photolytic degradation of MET. The apparent first-order rate constants and half-life times were calculated. Proposals for the degradation pathways for both MET and XPM were postulated.

Keywords Metolazone · Xipamide · Spectrofluorimetry · Stability-indicating · Tablets · Content uniformity

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Introduction

Metolazone (Fig. 1a; 7-Chloro-1,2,3,4-tetrahydro-2-methyl-3-(2-methylphenyl)-4-oxo-6-quinazolinesulfonamide) and xipamide (Fig. 1b; 4-chloro-2',6'-dimethyl-5-sulfamoylsalicylanilide) [1] are diuretics with similar actions and uses to those of the thiazide diuretics. They are orally administered for treatment of edema associated with heart failure and for management of hypertension [2].

MET is official in the United State Pharmacopoeia (USP) [3], the British Pharmacopoeia (BP) [4], and the European Pharmacopoeia (EP) [5].

Literature survey revealed that, several analytical methods were reported for the determination of MET in raw material and pharmaceutical formulations including; spectrophotometry [3, 6–10], HPTLC [11, 12], and liquid chromatography [3, 13–15]. Meanwhile, some analytical methods have been reported for the determination of MET in biological matrices including; fluorimetry [16], HPLC [17–22], liquid chromatography- tandem mass spectrometry (LC-MS) [23–26].

Regarding XPM, it is not official in any of the pharmacopoeias. However, various analytical methods have been published for its determination either per se or in pharmaceutical preparations viz spectrophotometry [27–34], spectrofluorimetry [29], TLC-densitometry [28, 32, 35], HPLC [35–44], and electrochemical analysis [45].

Spectrofluorimetry has been widely applied for the analysis of several compounds of pharmaceutical interest owing to its highly sensitive, selective, easily operated and economical technique.

To the best of our knowledge, up till now only one fluorimetric method [16] has been published concerning the determination of MET in urine with 50 times less sensitivity than the proposed method. However, HPLC methods with fluorimetric detection

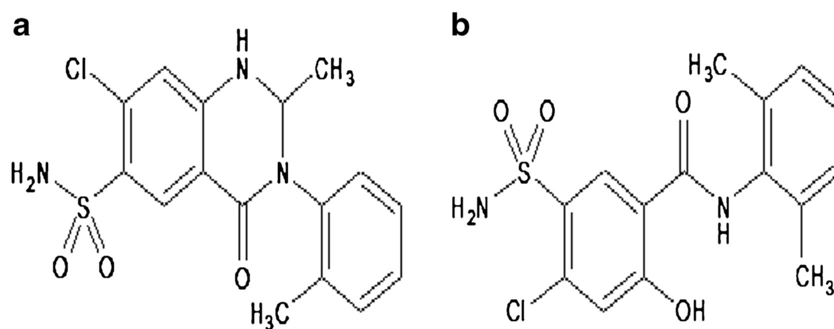


Fig. 1 Structural formulae of: **a**: Metolazone **b**: Xipamide

have been reported [18–21] for MET. For XPM, a fluorescence quenching spectrofluorimetric method [29] have been reported; based on ternary complex formation with eosin and lead (II) in the presence of methylcellulose as surfactant.

None of the reported methods was described as a stability-indicating method for the determination of MET or XPM or introduced a degradation kinetic study of MET.

The current study was aimed to develop and validate a simple, rapid and sensitive spectrofluorimetric methodology for the determination of MET and XPM utilizing the native fluorescence of MET in methanol and of XPM in alkaline methanolic solution. The present study is simpler and more time saving, with no need for derivatization reaction compared with the reported spectrofluorimetric method for the analysis of XPM [29]. The proposed method was fully validated according to ICH guidelines [46], and successfully applied for the determination of the studied drugs in their tablets. Furthermore, the proposed method was extended to establish the inherent stability of both drugs under different stress conditions such as alkaline, acidic, oxidative and photolytic conditions.

Experimental

Apparatus

A RF-1501 Shimadzu spectrofluorophotometer (Japan) with a xenon lamp was used with the excitation and emission slits set at 5 mm. A 1-cm quartz cell was used for all measurements. A Consort NV P-901 pH –Meter (Belgium) was used for pH measurements. Ultrasonic bath, model SS 101 H 230, USA was used for dissolving materials and reagents.

Camag UV-Lamp (S/N 29000), dual wavelength (254/336), 2 X8W(Muttenz, Switzerland) was used in the photo stability study.

Materials and Reagents

All chemicals and pharmaceuticals used were of Analytical Reagent grade and Pharmaceutical grade, and the solvents were of HPLC grade, and distilled water was used throughout the work.

Metolazone was kindly provided by Pharmaceutical Div., Pennwalt Corp., Rochester, N.Y. The purity percentage of MET was 100.45 %. The purity was established by applying the USP method [3].

Xipamide was kindly provided by Egyptian INT. Pharmaceutical Industries CO. (EIPICO), Egypt.

Sodium dodecyl sulphate (SDS; 95 %) and cetyl trimethyl ammonium bromide (CTAB; 99 %) were purchased from Winlab (UK).

Acetonitrile was obtained from Sigma- Aldrich (Germany) and Methanol was purchased from Tedia (USA).

Hydroxy propyl β-cyclodextrin (HP-β-CD) was obtained from Merck (Germany).

Tween- 80, methyl cellulose, acetone, ethanol, acetic acid 96 %, sodium acetate trihydrate, boric acid, sodium hydroxide, 30.0 %hydrogen peroxide, sulfuric acid; were all obtained from El-Nasr Pharmaceutical Chemical Co. (ADWIC; Egypt).

Acetate buffer (0.2 M, pH 3.6–5.0) and borate buffer (0.2 M, pH (6.0 – 10.0) solutions were freshly prepared. SDS, CTAB, methylcellulose, HP-β-CD and Tween-80 were prepared as 0.25 % w/v aqueous solutions.

Metenix tablets; batch # 099536, labeled to contain 5.0 mg metolazone per tablet (Sanofi-aventis S.A.E, Egypt) were purchased from commercial sources in the local pharmacy. Xipamid HEXAL® tablets; batch # CR1503, labeled to contain 10.0 mg xipamide per tablet (HEXAL AG industriestraBe, Holzkirchen, Germany) were obtained as a gift from a friend in Germany.

Standard Solutions

Stock solutions of 400.0 μg/mL of MET and XPM were prepared by dissolving 20 mg of each in 50 mL methanol with the aid of an ultrasonic bath. Working standard solutions were prepared by appropriate dilution of the stock solutions with methanol. Solutions of MET were protected from light with aluminium foil. All solutions were stored in the refrigerator and found to be stable for at least 10 days without alteration.

General Procedures

Procedures for Calibration Graphs

Aliquot volumes of the standard solutions covering the working concentration range were transferred into a series of 10 mL volumetric flasks so that the final concentrations were in the range of 2.0–20.0 ng/mL for MET and 0.2– 2.0 μg/mL for XPM. 2.0 mL of 0.4 M NaOH was added to XPM flasks and mixed, and then all the solutions were completed to the mark with methanol and vigorous mixed. The fluorescence intensities were measured at 437 nm after excitation at 238 nm for MET and at 400 nm after excitation at 255 nm for XPM. The relative fluorescence intensities were plotted against the final concentrations of the drugs. Alternatively, the corresponding regression equations were derived.

Procedures for Tablets

Accurately weighted quantities of the mixed contents of 10 powdered tablets equivalent to 5.0 mg MET or 10 mg XPM were transferred into a 25 mL volumetric flask and about 20 mL of methanol were added. The contents of the flask were sonicated for 30 min, completed to the volume with the same solvent and filtered. The filtrate was further diluted with methanol to get working standard solutions to be assayed by subjecting to the general procedure as described under "Procedures for calibration graphs". The nominal contents were calculated either from previously plotted calibration graphs or using the corresponding regression equations.

Procedures for Content Uniformity Testing

The same procedure applied for the analysis of MET and XPM in tablets was followed using one tablet as a sample. Ten different metenix or xipamid hexal® tablets were analyzed and the uniformity of their contents was tested by applying the official USP [3] Guidelines.

Procedures for Stability Studies

Procedure for Alkaline and Acidic Degradation

- For MET

Aliquots of appropriate methanolic MET standard solution (equivalent to 5.0 μg of the drug) were transferred into a series of small tubes; 5 mL of either 0.2 M NaOH or 0.1 M HCl were added. The solutions were heated in boiling water bath for different time intervals (10–70 min). At the specified time, the contents of each tube were cooled, neutralized to pH 7.0 and the solutions were then transferred into a series of 25.0 mL volumetric flasks. The volumes were completed with distilled water. 1.0 mL of each solution was then transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs" was performed. The drug concentration was derived either from the previously plotted calibration graph, or using the regression equation.

- For XPM

Aliquots of appropriate methanolic XPM standard solution (equivalent to 250.0 μg of the drug) were transferred into a series of small tubes; 5.0 mL aliquots of 2.0 M NaOH or 2.0 M HCl were added. The solutions were heated in a boiling water bath for 2.0 hrs. At the specified time, the contents of each tube were cooled, neutralized to pH 7.0 and the solutions were then transferred into a series of 25.0 mL volumetric flasks. The volumes were completed with water. 1.0 mL of each solution was then transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs". was performed. The drug concentration was derived either from the previously plotted calibration graph, or according to the regression equation.

Procedure for Oxidative Degradation

Aliquots of appropriate methanolic MET or XPM standard solutions (equivalent to 5.0 μg of MET or 250.0 μg of XPM) were transferred into a series of 25 mL volumetric flasks; 5 mL of either H_2O_2 solution (2–30 %, w/v) were added. The volumes were completed with distilled water. 1.0 mL of each solution was then transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs" were performed. The drug concentration was derived either from the previously plotted calibration graph, or according to the regression equation.

Procedure for Photolytic Degradation

Under UV-light

- For MET

  Aliquots of appropriate methanolic MET standard solution (equivalent to 5.0 μg of the drug) were transferred into a series of 25.0 mL volumetric flasks and completed to the volume with either methanol, water or methanol:water mixture (50:50, v/v). The volumetric flasks were exposed to UV-lamp at a wavelength of 366 nm at a distance of 15.0 cm placed in a wooden cabinet for different time intervals (0.5–4.0 hr). At the specified time, 1.0 mL of each solution was transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs" was performed.

- For XPM

Aliquots of appropriate methanolic XPM standard solution (equivalent to 125.0 μg of the drug) were transferred into a series of 25.0 mL volumetric flasks and completed to volume with either methanol, water or methanol: water mixture (50:50, v/v). The volumetric flasks were exposed to UV-lamp at a wavelength of 254 nm at a distance of 15.0 cm placed in a wooden cabinet for different time intervals (1.0–24.0 hr). 1.0 mL of each solution was transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs" was performed.

Under Sunlight

Aliquots of appropriate methanolic MET or XPM standard solutions (equivalent to 2.5 μg of MET or 125.0 μg of XPM) were transferred into a series of 25 mL volumetric flasks and completed to volume with methanol. The volumetric flasks were exposed to sunlight for different time intervals (0.5–48.0 hr). 1.0 mL of each solution was transferred into 10.0 mL volumetric flask and the "Procedures for calibration graphs" was performed.

Results and Discussion

MET exhibits an intense native fluorescence in methanolic solution at 437 nm after excitation at 238 nm (Fig. 2a). It is clear that, MET exhibits three excitation wavelengths of 238, 268 and 340 nm. A wavelength of 238 nm was selected as the optimum excitation one since it yields the sharpest emission spectrum with the most sensitivity.

XPM was found to exhibit a native fluorescence in alkaline methanolic solution at 400 nm after excitation at 255 nm (Fig. 2b). XPM exhibits two excitation wavelengths of 238 and 255 nm. A wavelength of 255 nm was selected as the optimum excitation wavelength

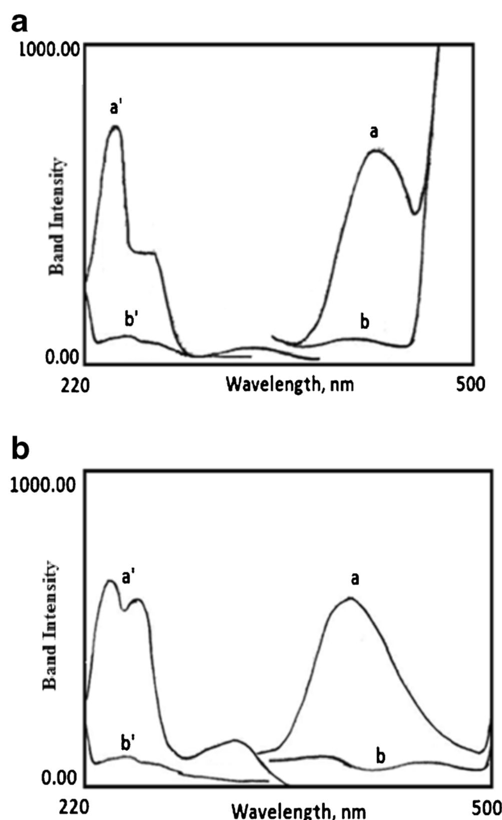


Fig. 2 Fluorescence spectra of: (**a**) a': Excitation and a: Emission spectra of (20.0 ng/mL) of MET in methanol. b': Excitation and b: Emission spectra of methanol (blank). **b** a': Excitation and a: Emission spectra of (2.0 μg/mL) of alkaline methanolic solution of XPM (2.0 mL of 0.4 M NaOH/10 mL methanol). b': Excitation and b: Emission spectra of alkaline methanol (2.0 mL of 0.4 M NaOH/10 mL methanol) (blank)

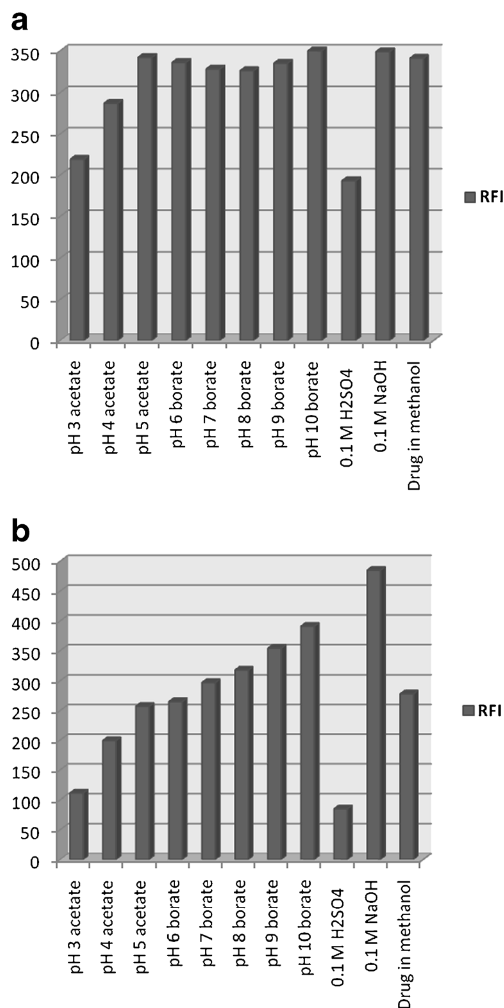


Fig. 3 Effect of pH (0.2 M acetate buffer and 0.2 M Borate buffer; 1.0 mL of each), (0.1 M H_2SO_4, 0.1 M NaOH; 1 mL of each) on RFI of: (**a**) MET (10 ng/mL) (**b**) XPM (2.0 μg/mL)

since it gave emission spectrum with best reproducibility and linearity and nearly the same sensitivity. Practically, when trying to make calibration of XPM using 238 nm as the excitation wavelength, it was found that the results were not reproducible and the calibration plot was not rectilinear over the used concentration range. This was due to high blank reading (0.4 M NaOH/methanol) at 400 nm by excitation at 238 nm. Being phenolic compound, its intrinsic fluorescence intensity was enhanced (240 %) in the presence of sodium hydroxide solution due to phenolate formation.

As a consequence, we aimed to utilize these emission bands of MET and XPM, in order to explore a new methodology for the analysis of MET and XPM in tablets. Different experimental parameters affecting the fluorescence intensities of MET and XPM were carefully studied and optimized. Such factors were changed individually, where others kept constant. Furthermore, the developed methods were applied to establish the inherent stability of MET and XPM under different stress conditions. In addition, content uniformity testing of MET and XPM tablets was performed.

Optimization of Experimental Conditions

Effect of pH

The influence of pH on the fluorescence of MET and XPM was studied using different types of buffers covering the whole pH range, such as 0.2 M acetate buffer over the pH range 3.0–5.0 and 0.2 M borate buffer over the pH range 6.0–10.0, in addition to 0.1 M H_2SO_4 and 0.1 M NaOH (Fig. 3: a & b). For both drugs, the use of buffer did not enhance the RFI over the entire pH range studied. It was noticed that bellow pH 5.0, the RFI of MET and XPM was decrease. Above pH 5.0 and up to pH 10.0, the RFI of MET did not change, while that of XPM increase with increasing the pH. 0.1 M H_2SO_4 was found to decrease the RFI of both drugs, while

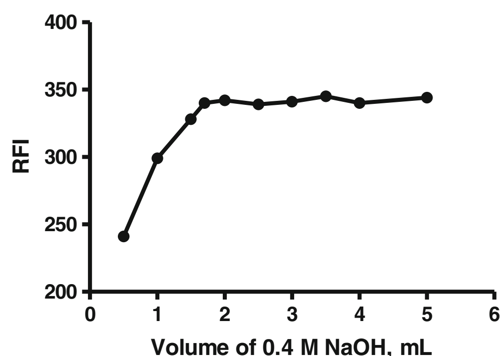


Fig. 4 Effect of volume of 0.4 M NaOH on RFI of XPM (1.0 μg/mL)

Table 1 Effect of different diluting solvents on the RFI of the studied drugs

Diluting solvent	RFI	
	MET (10 ng/mL)	XPM (1.0 μg/mL)
Methanol	339	341
H_2O	348	335
Ethanol	256	366
Acetonitrile	197	253
Acetone	0	0

0.1 M NaOH caused an enhancement of the fluorescence intensity of XPM compared with its methanolic solution. This enhancement of the RFI of XPM with the addition of 0.1 M NaOH was increased with increasing the concentration of NaOH. Thus, 0.4 M NaOH was conveniently used.

It was found that maximum RFI of MET was achieved in methanolic solution without the addition of any buffer, while that of XPM needed the addition of NaOH.

Effect of the Volume of NaOH

The influence of the volume of NaOH on the RFI of XPM was studied using increasing volumes of 0.4 M NaOH. It was found that increasing volumes of 0.4 M NaOH solution resulted in a corresponding increase in RFI up to 2.0 mL, after which no further increase in RFI was attained. Therefore, 2 mL 0.4 M NaOH solution

Table 2 Analytical performance data for the determination of the studied drugs by the proposed methods

Parameter	MET	XPM
Linearity range	2.0 – 20.0 (ng/mL)	0.2 –2.0 (μg/mL)
Intercept (a)	7.03	16.08
Slope (b)	32.90	321.64
Correlation coefficient (r)	0.9999	0.9998
S.D. of residuals ($S_{y/x}$)	4.06	2.62
S.D. of intercept (S_a)	3.46	2.23
S.D. of slope (S_b)	0.26	1.68
S.D.	0.84	0.61
% RSD [a]	0.84	0.61
% Error [b]	0.34	0.25
LOD[c]	0.35 (ng/mL)	0.02 (μg/mL)
LOQ[d]	1.05 (ng/mL)	0.07 (μg/mL)

[a] Percentage relative standard deviation

[b] Percentage relative error

[c] Limit of detection

[d] Limit of quantitation

was chosen as the optimal volume for maximum fluorescence intensity of XPM (Fig. 4).

Effect of Diluting Solvent

The effect of different diluting solvents on the RFI of MET and XPM was investigated using water, ethanol, methanol, acetonitrile, and acetone. It was found that methanol was the best solvent for dilution, as it gave the highest RFI and the lowest blank reading with reproducible results (Table. 1). Water gave nearly the same results as methanol but with reduced reproducibility. Ethanol caused a very slight increase in the RFI of XPM and decrease in the RFI of MET. Using acetonitrile resulted in decrease of the RFI of MET and XPM due to physical interaction between the drug and solvent molecules leading to transfer of energy from the drug to the solvent molecules via collision and hence decrease fluorescence intensity. On the other hand, acetone greatly quenched the fluorescence of both drugs and that was due to intersystem crossing process.

Effect of Time

The effect of time on the RFI of the two drugs was also studied. It was found that the fluorescence intensity remained stable for more than 3 hr in the dark.

Validation of the Method

Linearity and Range

Assessment of linearity of the assay method was performed by replicate analysis of six sets for each drug (standard calibration plots). The relative fluorescence intensity vs. concentration plots were linear over the range 2.0–20.0 ng/mL for MET and 0.2– 2.0 μg/mL for XPM. Linear regression analysis of the data gave the following equations:

$$RFI = 7.03 + 32.90\,C \qquad (r = 0.9999) \quad \text{for MET}$$

$$RFI = 16.08 + 321.64\,C \qquad (r = 0.9999) \quad \text{for XPM}$$

Table 3 Assay results for the determination of the studied drugs in pure form by the proposed and comparison methods

Compound	Proposed method			Official method [3]		
MET	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (μg/mL)	Amount found (μg/mL)	% Found
	2.0	2.020	101.01	20.0	20.326	101.63
	6.0	6.016	100.28	40.0	39.956	99.89
	10.0	10.058	100.59	60.0	60.570	100.95
	14.0	13.827	98.76	80.0	79.456	99.32
	18.0	17.929	99.61			
	20.0	20.148	100.74			
Mean			100.17			100.45
± S.D.			0.84			1.04
t-test			0.48			(2.306)*
F-test			1.53			(5.409)*
				Comparison method [43]		
XPM	**Amount taken (μg/mL)**	**Amount found (μg/mL)**	**% Found**	**Amount taken (μg/mL)**	**Amount found (μg/mL)**	**% Found**
	0.2	0.202	100.90	10.0	10.136	101.36
	0.6	0.597	99.45	16.0	15.785	98.65
	1.0	1.001	100.09	20.0	19.996	99.98
	1.4	1.405	100.36	26.0	26.083	100.32
	1.8	1.787	99.30			
	2.0	2.008	100.41			
Mean			100.09			100.08
± S.D.			0.61			1.12
t-test			0.014			(2.306)*
F-test			3.35			(5.409)*

N.B. Each result is the average of three separate determinations

*The figures between parentheses are the tabulated t and F values at P=0.05 [47]

Where: RFI is the relative fluorescence intensity, C is the concentration of the drug in ng/mL for MET and in μg/mL for XPM and r is the correlation coefficient.

Statistical analysis [47] of the data gave high value of the correlation coefficient (r) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small value of the percentage relative standard deviation and the percentage relative error (Table. 2). These data proved the linearity of the calibration graphs.

Limits of Quantification (LOQ) and Limits of Detection (LOD)

The limits of quantitation (LOQ) were determined by establishing the lowest concentrations that can be measured according to the ICH Q2 (R1) recommendation [46] below which the calibration graph is non-linear. The limits of detection (LOD) were determined by evaluating the lowest concentrations of the analytes that can be readily detected. The results are also summarized in Table 2.

The values of LOQ and LOD were calculated according to the following equations [46]:

$$\mathrm{LOQ} = 10Sa/\mathrm{b}$$

$$\mathrm{LOD} = 3.3Sa/\mathrm{b}$$

Where Sa is the standard deviation of the intercept of the regression line and b is the slope of the calibration graph.

Accuracy and Precision

Statistical analysis [47] of the results obtained by the proposed method and official method [3] for MET or comparison method [44] for XPM using Student's t-test and variance ratio F-test showed no significant differences between the two methods regarding accuracy and precision (Table 3).

The intra-day precision was evaluated by determination of three concentrations of each drug in pure forms on three successive occasions. The inter-day precision was also evaluated through replicate analysis of three concentrations for a period of 3 successive days. The results of intra-day and inter-day precision are summarized in Table 4. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed methods.

Selectivity The selectivity of the method was investigated by observing any interference encountered from common excipients in different formulations. It was shown that these compounds did not interfere with the results of the proposed method.

Pharmaceutical Applications

The proposed method was applied to the determination of MET and XPM in tablet dosage form (Table 5). The results shown in Table 5 are in good agreement with those obtained using the official and comparison methods [3, 43]. Statistical analysis of the results obtained using Student's t-test and

Table 4 Precision data for the determination of the studied drugs by the proposed methods

Parameter		MET (ng/mL)			XPM (μg/mL)		
		4.0	8.0	16.0	0.2	0.6	1.0
Intraday	% Found	100.27	98.66	99.51	100.90	98.91	100.09
		101.53	99.79	100.85	98.60	102.37	101.40
		99.42	101.03	100.14	100.10	99.45	99.09
	Mean	100.41	99.83	100.17	99.87	100.24	99.19
	S.D.	1.06	1.19	0.67	1.17	1.86	1.16
	% RSD	1.06	1.19	0.67	1.17	1.86	1.16
	% Error	0.61	0.69	0.39	0.68	1.07	0.67
Interday	% Found	100.82	99.41	100.36	99.15	99.45	99.28
		99.11	100.62	97.84	97.57	100.11	100.09
		100.05	102.32	99.45	100.90	103.24	100.89
	Mean	99.99	100.78	99.22	99.21	100.93	100.09
	S.D.	0.86	1.46	1.28	1.67	2.03	0.81
	% RSD	0.86	1.45	1.29	1.68	2.01	0.80
	% Error	0.49	0.84	0.74	0.97	1.16	0.46

N. B. Each result is the average of three separate determinations

Table 5 Assay results for the determination of the studied drugs in their tablet dosage form by the proposed methods

Parameter	Proposed method			Official method[3]		
	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (µg/mL)	Amount found (µg/mL)	% Found
Metenix 5 mg Tablet	6.0	6.003	100.06	5.0	4.868	97.36
	10.0	9.907	99.07	10.0	10.188	101.88
	14.0	14.175	101.25	15.0	15.019	100.13
	18.0	17.914	99.52	20.0	19.924	99.62
Mean			99.98			99.75
±S.D.			0.94			1.86
t-test			0.22			(2.447)*
F-test			3.91			(9.276)*
Xipamid Hexal® 10 mg Tablet				comparison method [43]		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Found	Amount taken (µg/mL)	Amount found (µg/mL)	% Found
	0.4	0.401	100.28	10.0	9.981	99.81
	0.8	0.812	101.45	16.0	15.783	98.64
	1.2	1.181	98.43	20.0	20.413	102.07
	1.6	1.590	99.38	26.0	25.823	99.32
Mean			99.89			99.96
±S.D.			1.29			1.49
t-test			0.076			(2.447)*
F-test			1.33			(9.276)*

N.B. Each result is the average of three separate determinations

*The figures between parentheses are the tabulated t and F values at $P=0.05$ [47]

variance ratio F-test [47] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

Content Uniformity Test

Due to the high sensitivity of the proposed method and its ability to rapidly measure the fluorescence intensity of a single tablet extract with sufficient accuracy, the method is ideally suited for content uniformity testing. The steps of the test were adopted according to the USP [3] procedure. The acceptance value (AV) was calculated and it was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated excellent drug uniformity for MET and XPM as shown in Table 6.

Stability Studies and Degradation Kinetics

Degradation in Alkaline and Acidic Conditions

Being an amide, MET was found to be highly labile to alkaline and acidic hydrolysis. The degradation in 1 M NaOH or 1 M HCl at 100 °C was so fast that, almost the entire drug was degraded within 15 min. Subsequently, degradation studies were performed using either 0.2 M NaOH or 0.1 M HCl solutions. The degradation of MET in alkaline and acidic conditions at boiling temperature was found to be time dependent; it was observed that the RFI decreased gradually by increasing boiling time with either NaOH or HCl. Fig. 5 (a& b) revealed that drug concentration decreased with increasing boiling time intervals in alkaline and acidic medium. Plotting the logarithms of drug concentrations against time resulted in straight lines as shown in Fig. 6 (a, b) for alkaline and acidic degradation. The apparent first order rate constants and half-life times for both alkaline and acidic degradation were calculated (Table 7)

Alkaline and acidic degradation of MET occurred significantly at boiling temperature, where, at lower temperatures the degradation was mild. The alkaline and acidic treatments of MET are expected to cause cleavage of its amide bond resulting in decrease of the RFI intensity of the drug.

XPM was found to be insusceptible to acid degradation; after boiling with 2.0 M HCl for 2 hr no evidence of degradation of the drug was observed. The alkaline hydrolysis of XPM with 2.0 M NaOH for 2 hr at boiling temperature

Table 6 Results of content uniformity testing of MET and XPM tablets using the proposed methods

Parameter	Percentage of the label claim	
	Metenix Tablet	Xipamid Hexal® Tablet
Data	98.74	100.52
	99.64	101.70
	99.64	98.63
	97.67	99.52
	98.95	100.06
	100.51	99.83
	99.10	101.12
	101.32	97.79
	100.47	98.88
	101.14	102.16
Mean ($\overline{X}$)	99.72	100.02
± S.D.	1.15	1.39
% RSD	1.15	1.39
% Error	0.36	0.44
Acceptance value (AV) [3]	2.76	3.34
Max. allowed AV (L1) [3]	15	15

was accompanied by a slight increase in the fluorescence intensity (23 %). This could be explained by the formation of a degradation product that also exhibited a high native fluorescence. Alkaline degradation of XPM occurred significantly at boiling temperature, where, at lower temperatures no degradation was observed. Previous report [48] confirmed that XPM was stable in acidic as well as in basic media at room temperature, but when XPM is acted upon by strong alkali and elevated temperature. The alkaline treatment of XPM was expected to cause cleavage of its amide bond resulting in the formation of products of higher native fluorescence intensity.

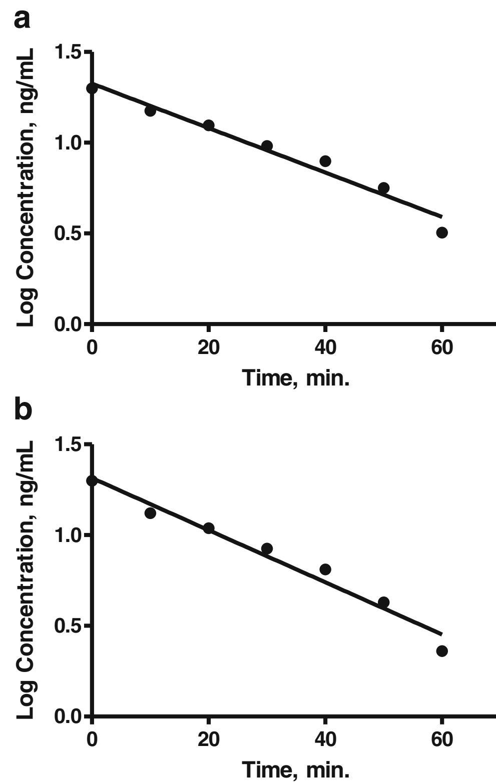


Fig. 6 Plot of different heating times vs. log MET concentration at boiling temperature. a With 0.2 M NaOH (b) With 0.1 M HCl

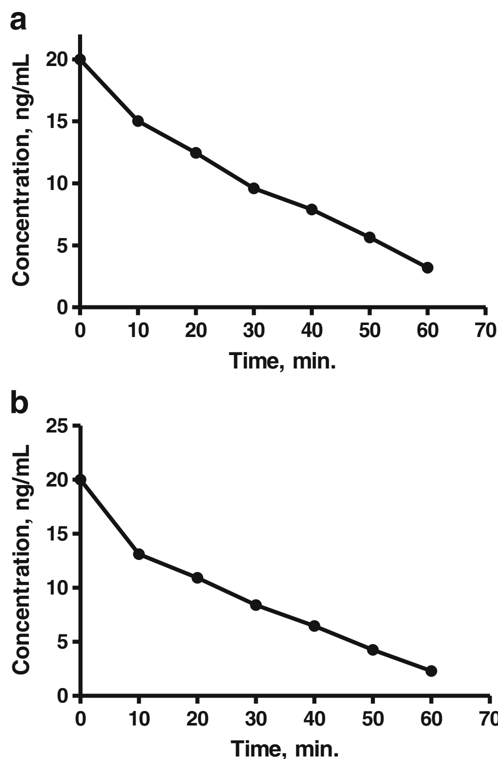


Fig. 5 Plot of different heating times vs. MET concentration at boiling temperature. a With 0.2 M NaOH. b With 0.1 M HCl

Table 7 Results of the degradation study of MET under different stress conditions on the kinetic parameters of MET (20 ng/mL) at boiling temperature

Degradation condition		Reaction rate constant (K, min^{-1})	Half life time ($t_{1/2}$, min)
Alkaline degradation (0.2 M NaOH, 100 °C)		0.0283	24.49
Acidic degradation (0.1 M HCl, 100 °C)		0.0332	20.87
Photolytic UV degradation	Methanol	0.0076	91.18
	Methanol/Water (50/50, v/v)	0.0059	117.46
	Water	0.0071	97.60

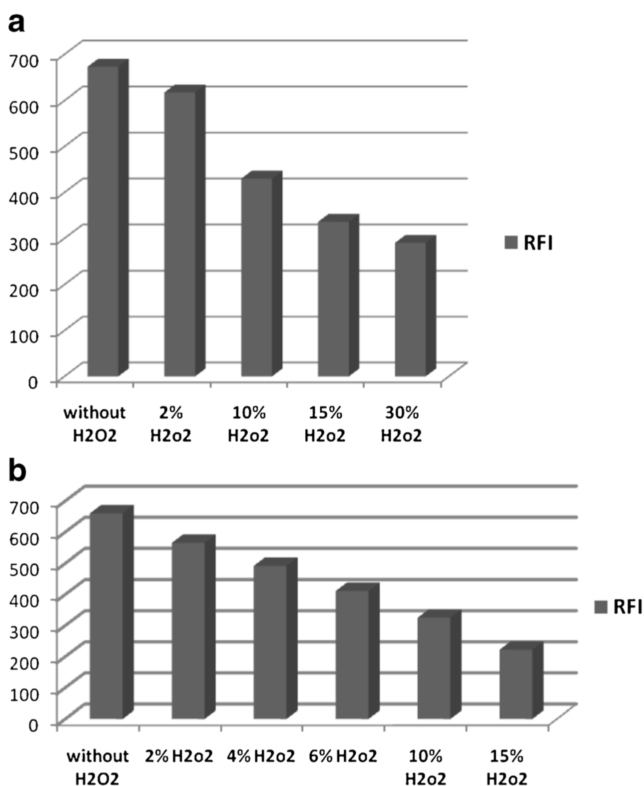


Fig. 7 Effect of the %concentration of H_2O_2 (5 mL) at room temperature on RFI of : **a** MET (20.0 ng/mL) (**b**) XPM (2.0 μg/mL)

Oxidative Degradation

Considerable degradation of MET and XPM was observed under oxidative conditions. It was found that treating the drugs with H_2O_2 solution at room temperature, according to the procedure described above, resulted in immediate degradation of MET and XPM. The amounts of degraded drugs were dependent on the concentration of H_2O_2 added (Fig. 7: a&b); 49 % degradation of MET and 66 % of XPM were observed upon using 15.0 % H_2O_2 solution. The oxidative degradation of MET is proposed to proceed via oxidation of the nitrogen atom yielding the corresponding N-Oxide derivative of MET leading to decrease in the RFI of MET. Oxidation of tertiary amino group (like in MET) into the corresponding oxide was previously reported in the literature [49, 50]. The oxidative degradation pathway of XPM was expected to be through the oxidation of the phenolic hydroxyl group with the formation of quinonoid structure resulting in decrease of the RFI intensity of the drug as in the case of ethamsylate drug previously reported [51].

Photolytic Degradation

The effect of UV-light on the stability of MET was studied by exposing the drug solutions in different solvents (methanol, water, methanol/water mixture, 50/50, v/v) to the UV-light at 254 and 366 nm. No considerable degradation of MET upon exposure to the UV-light at 254 nm was observed in any of the three solutions, even after 48 hr, 31 % degradation only occurred. However, by exposing different MET solutions to UV-light at 366 nm, the degradation rate was greatly enhanced. It was found that a significant decrease in the RFI of MET was observed upon exposure of the drug to UV-light at 366 nm for 4 hrs. (80 % degradation). Appreciable

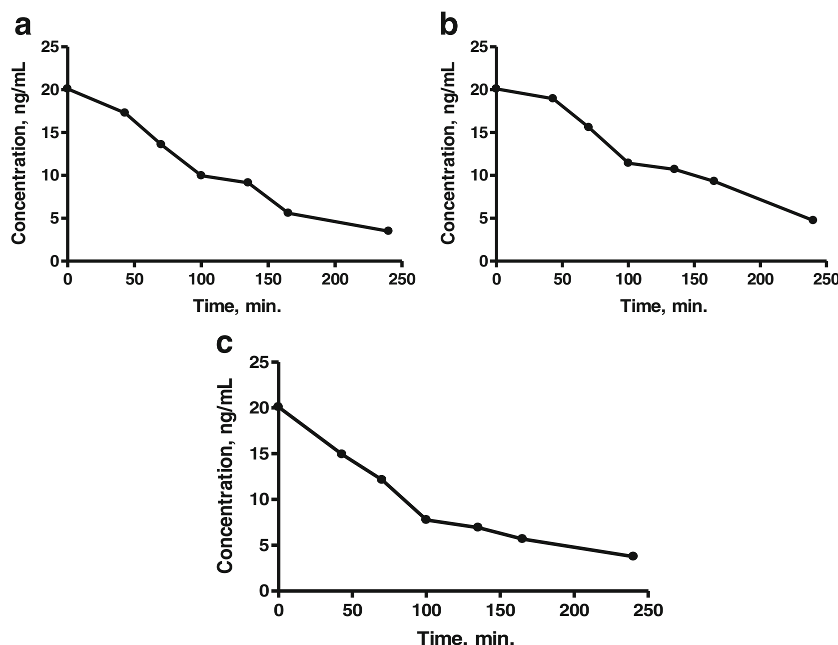


Fig. 8 Plot of different exposing times to UV lamp (366 nm) vs. MET concentration in: (**a**) Methanolic solution. (**b**) Methanol/water mixture (50/50). (**c**) Aqueous solution

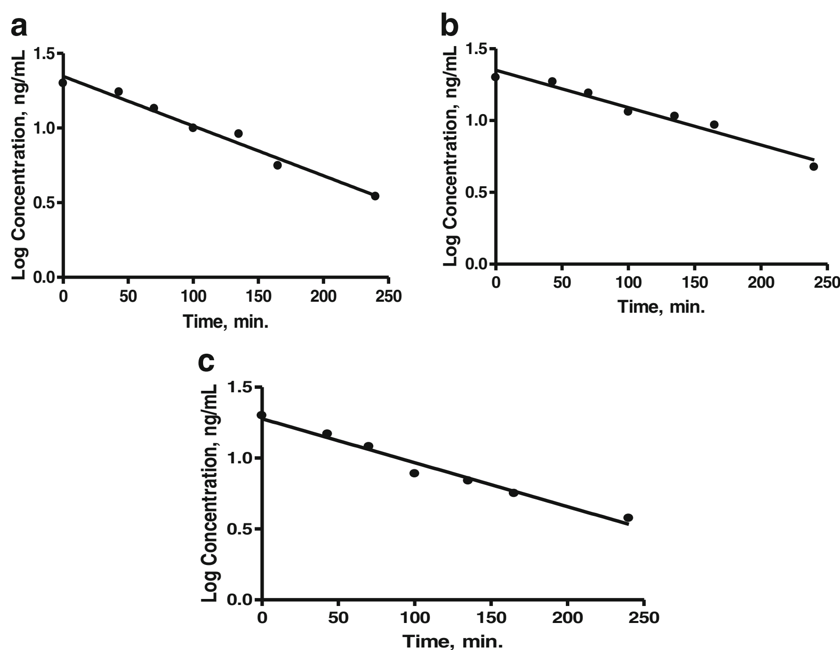
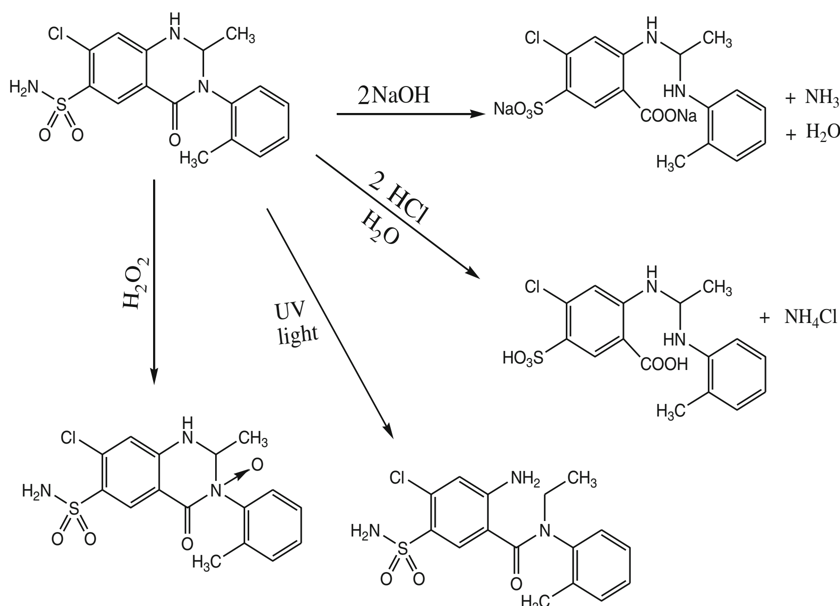


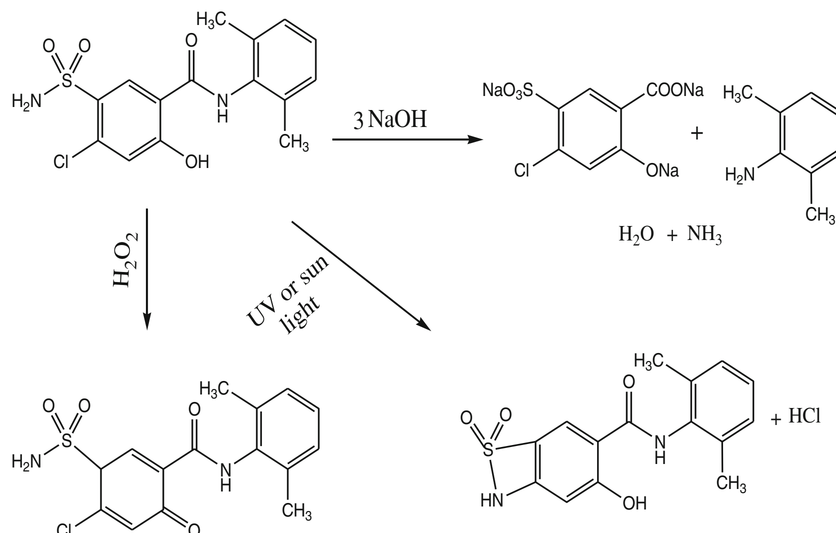
Fig. 9 Plot of different exposing times to UV lamp (366 nm) vs. Log MET concentration in: (**a**) Methanolic solution. (**b**) Methanol/water mixture (50/50, v/v). (**c**) Aqueous solution

degradation of MET was carried out in methanol, methanol/water mixture and in water when irradiated for the same period but with slower rate with respect to methanol/water mixture. Figure (8: a&b&c) revealed that drug concentration decreased with increasing exposing time intervals. Plotting the logarithms of drug concentrations against time resulted in straight lines as shown in Fig. (9: a& b&c). The apparent first order rate constants and half-life times for photolytic degradation in different solvents were calculated (Table 7). A proposal for the degradation pathway of MET has been postulated as shown in Scheme 1. It is expected that, the photolytic degradation of MET proceeds through photohydrolysis. This proposal is based on previous report concerned with the photodegradation of furosemide which has similar structure to MET [52].

In case of XPM, a marked increase in the RFI was observed upon exposure of the drug solution in different solvents (methanol, water, methanol/water mixture, 50/50, v/v) to UV-light at 254 nm for 6 hr (116 % increase) and at 366 nm for 40 min (197 % increase). This increase was more significant in water and methanol/water mixture rather than in methanol. The increase of RFI of XPM under UV was attributed to photo-cyclization which is one of the expected mechanisms of photodegradation [53].



Scheme 1 Proposed pathway of alkaline, acidic, oxidative and UV degradation of MET



Scheme 2 Proposed pathway of alkaline, oxidative and UV/sun light degradation of XPM

Methanolic solution of MET was stable to sun-light even after exposure for 48 hrs. On the other hand, methanolic solution of XPM was stable to sun-light for about one hour; after which RFI was increased gradually with time (80 % increase in RFI after 30 hr).

Degradation pathways for both MET and XPM were demonstrated in Scheme 1, 2, respectively.

Conclusion

A simple and sensitive spectrofluorimetric method was developed for the determination of MET and XPM. The proposed method is rapid, less time–consuming and does not require the elaborate treatment associated with chromatographic methods; moreover, it is sensitive, with no need for derivatization reactions compared with the reported spectrofluorimetric methods for the determination of XPM. By virtue of its simplicity and rapidity, the proposed method could be applied to the analysis of the two drugs in their tablet dosage forms. Furthermore, content uniformity testing of the studied tablets was also conducted. Additionally, the proposed method has been adapted for stability studies of the two drugs as a novel stability–indicating method.

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