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Simultaneous Determination of Metformin and Pioglitazone in Presence of Metformin Impurity by Different Spectrophotometric and TLC – Densitometric Methods

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Received: May 21, 2018; Accepted: June 21, 2018; Published: June 22, 2018

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Abstract

New simple accurate methods were developed and validated for simultaneous determination of Metformin hydrochloride and Pioglitazone hydrochloride in presence of Metformin impurity Melamine , both in bulk powder and in pharmaceutical preparation using spectrophotometric methods and thin layer chromatography . Method A used zero order spectrophotometric technique for determination of Pioglitazone at 268nm and isoabsorbtive point spectrophotometric technique to determine Metformine at 255 nm in presence of the other drugs and used double divisor technique to determine Metformine in presence of the other drugs at 254 nm. Method B uses TLC densitometric technique for separation and simultaneous determination of the three drugs using toluene / methanol / acetic acid (5:5:0.5) as a developing system and 240nm as a scanning wavelength.

This method were validated and shown to demonstrate good accuracy and precision according to ICH guidelines.

Keywords: Metformin hydrochloride; Pioglitazone hydrochloride; Melamine; Double Divisor and TLC Densitometry;

Introduction

Metformin HCl (MET) is N; N dimethyl imido dicarbonimidic diamide hydrochloride .It is an antidiabitic drug from the biguanide class used in the management of type 2 diabetes. The major action of Metformin is increasing glucose transport across the cell membrane in skeletal muscles [1].

Pioglitazone HCl (PIO) is (±)-5-[p-[2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2, 4-thiazolidinedione hydrochloride [2]. It is an anti-diabetic drug from the thiazolidinedione hydrochloride class of drugs [3] MET and PIO combination is impartment for patients suffering from type 2 diabetes who require treatment with more than one anti-hyperglycemic drug to achieve optimal glycemic control [2].

Melamine (MEL) is 1, 3, 5-Triazine-2, 4, 6-triamine; and it is a potential impurity of Metformin. A variety of toxic effects from Melamine, including nephrolithiasis, chronic kidney inflammation and bladder carcinoma.

The literature review show that MET was determined in its bulk powder by liquid chromatography (LC) [4-10] and spectroscopy [11, 12] and capillary electrophoresis [13-15], Pioglitazone was determined by liquid chromatography (LC) [16-19], and capillary electrophoresis [20]. The binary mixture of MET and PIO was determined by some spectrophotometric methods [21-23] and determined by reversed phase liquid chromatography [2, 24-26].

The aim of this work is to develop and validate new analytical methods able to determine the tertiary mixture Metformin, Pioglitazone and Melamine in bulk powder and pharmaceutical dosage form without interference or prior separation.

Experimental

Instruments

Double beam UV_Visible spectrophotometer (SHIMADZU, Japan) .model uv-1601 PC with quartz cell of 1cm path length connected to IBM compatible computer. The software is uv - pc personal spectroscopy version 3.7.

TLC scanner densitometer (Camag, Muttenz, Switzerland). The following requirements were taken into consideration: slit dimensions, 5x0.2 mm; scanning speed, 20 mm/s; spraying rate, 10/mL; data resolution: 100 mm/step. Pre-coated silica gel aluminum plates (20x10 cm; 60 F254) were obtained from Fluka, Sigma-Aldrich Chemie Gmbh, and Germany. The sample applicator for TLC was a Linomat IV with a 100mL syringe (Camag, Muttenz, Switzerland).

Pure standards

Standard MET and PIO with claimed purity of 99.88%and 99.98% respectively according to manufacturer certificate were kindly supplied by Sigma Pharmaceuticals Industries (El Monofeya, Egypt).

Melamine was purchased from sigma Aldrich chemie (Germany) with certified purity of 99.7%.

Pharmaceutical dosage forms

Bioglita plus tablets batch no. [15675] were manufactured

by Amoun pharmaceutical Co.SAE (Cairo _ Egypt). Labeled to contain 850 mg of Metformin HCl and 15mg of Pioglitazone HCl .

Solvents and reagents

All reagents and chemicals used were of analytical grade and were used without further purification; they included:

(i) Methanol analar (Central Drug House Ltd., India)

(ii) Toluene and glacial acetic acid (Al-Nasr Pharmaceutical chemicals company, Abu Zaabal cairo – Egypt).

Prepared solutions

Standard stock solutions (1mg/ml)

An amount of 0.025 gm of each drug were accurately weighed into 3 separated 25ml volumetric flask ,then the volume in each flask was made up to the mark with methanol

Standard working solution (100µg/ml)

A volume of 2.5 ml of each stock solution of drugs were diluted to 25ml with methanol in 3 separated 25ml volumetric flasks.

Laboratory prepared mixtures

Mixtures containing different ratios of MET, PIO and MEL were prepared using their standard working solutions in methanol.

Methodology

Linearity and construction of calibration curve

Spectrophotometric method

Different aliquots of PIO , MET and MEL were transferred from working solution (100µg/ml) to three separate series of 10ml volumetric flask and the volume was completed to the mark by methanol to obtained concentration ranges (3µg/ml -25µg/ml) , (10µg/ml - 50µg/ml) and (10 µg/ml– 33 µg/ml) from PIO , MET and MEL respectively . The spectrum of each concentration was recorded against methanol as blank. The spectra were observed for selecting of the suitable wavelength for zero order, double divisor and isoabsorptive point.

HPTLC - densitometric method

Into 3 different groups of 10ml volumetric flasks aliquots of MET, PIO and MEL were accurately transferred from their working solution, the volume was then made up with methanol. A 10µl aliquots of each solution was spotted as band of 5mm width on TLC plates to obtain concentration ranges of (3 - 20µg) , (3 - 12µg) and (0.5 - 5µg) PIO , MET and MEL respectively .

The peak area were recorded using a scanning wavelength of 240 nm and calibration curves were constructed by plotting the integrated peak area versus the concentration in µg / band for each compound and the regression equations were computed .

Analysis of laboratory prepared mixtures

Accurate aliquots were transferred from MET , PIO and MEL working solutions into a series of 10ml volumetric flasks completed to volume with methanol and mixed well to obtain tertiary mixtures of different ratios then the absorption spectra of each solution was recorded and the same procedures mentioned under calibration curves were applied in order to determine MET , PIO and MEL in the laboratory prepared mixtures by both spectrophotometric techniques and TLC densitometric method.

Application to pharmaceutical formulation

Bioglita plus tablet are labeled to contain 850 mg MET and 15mg PIO per tablet. The contents of 10 tablets were powdered. An amount of the powder equivalent to 100 mg of MET and PIO were separately transferred into 100ml volumetric flask , 50ml of methanol was added to each of them and sonicated for 30min , cooled and then the volume was completed to mark to obtained 1000 µg /ml of MET and PIO stock solution and then the solution was filtered . Appropriate dilution of the prepared solutions were made to prepare working solutions containing 100 µg / ml of each of PIO and MET and the procedures under construction of calibration curves were followed for both spectrophotometric techniques and TLC densitometric method.

Result and discussion

Spectrophotometric methods

Spectrophotometry is a widely used analytical technique because of its simplicity, low cost and its time consuming

Determination of PIO by zero order

PIO can be determined by zero order spectrophotometry at 268 nm where MET and MEL show no absorbance (Figure 2).

The absorbance spectra of pure PIO solutions of different concentrations (3 _ 25 µg / ml) are recorded against methanol as blank (Figure 3).

The calibration curve was constructed relating the absorbance of PIO at 268 nm to concentration (µg / ml) and the regression equation was computed showing a linear relationship (Figure 4).

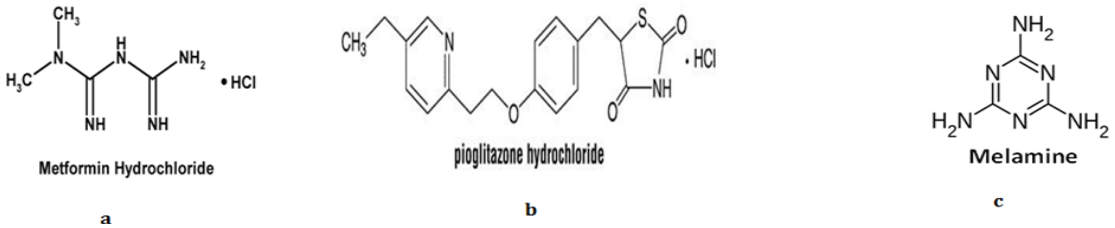

Figure1: Chemical structure of (a) Metformin hydrochloride, [b] Pioglitazone hydrochloride and (c) Melamine

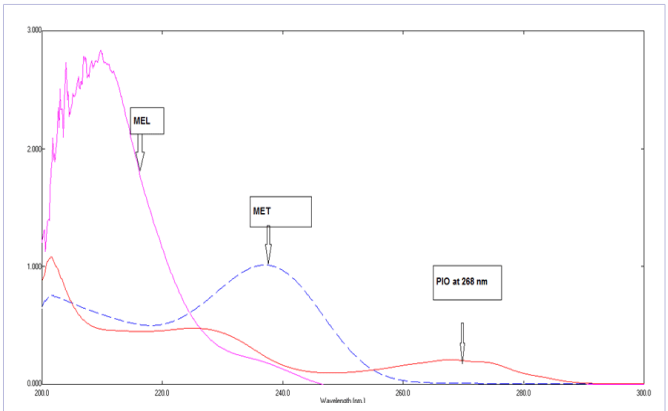

Figure 2: Absorption spectra of Metformin 10µg (solid line) , Pioglitazone 10 µg (dashed line) and Melamine (dotted line) 10 µg

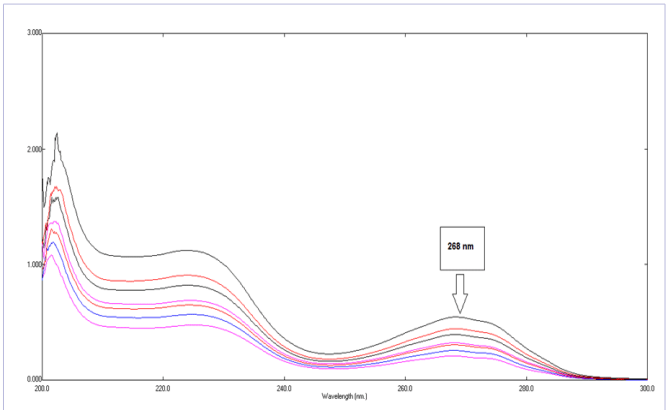

Figure 3: The UV absorbance spectra of different concentration (3 – 25 µg/ml) of PIO

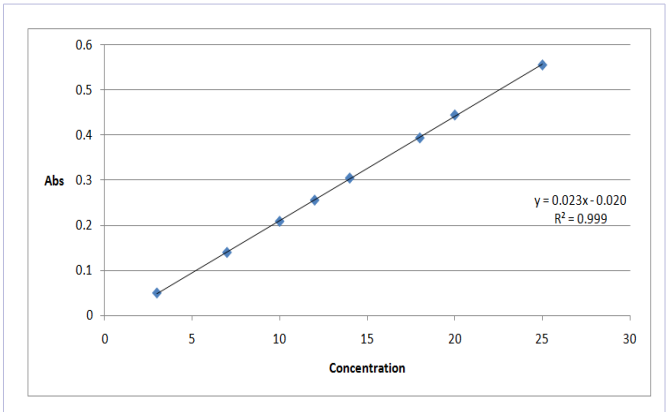

Figure 4: Calibration curves relating the absorbance of PIO at 268 nm to the concentration (µg / ml)

Double divisor method ratio spectra for determination of Metformin 254nm

Absorption spectra of pure MET ,different ternary mixture and dosage form solutions were recorded and divided by the standard spectrum of PIO / MEL binary mixture containing 10µg/ml of each and the second derivative of the spectra was obtained (using 4 as delta lamda and 10 as scaling factor) (Figure 5).

A calibration curve relating the peak amplitude of MET at 254 nm to the concentration in µg / ml was constructed and the regression equation was computed showing good linearity in the concentration range of (10 – 45 µg/ ml) (Figure 6 and 7)

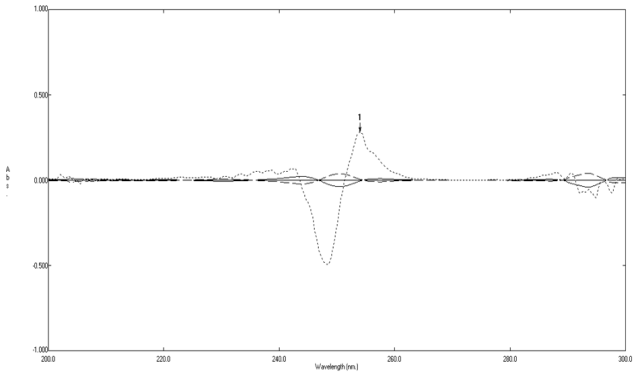

Figure 5: The double divisor second derivative spectra of MET , MEL and PIO using (PIO and MEL 10 µg) as divisor

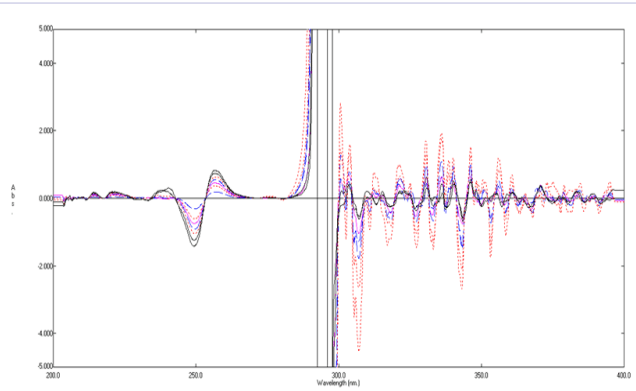

Figure 6: Double divisor second derivative spectra of pure MET (10 – 45 µg /ml) using PIO /MEL (10 µg /ml) as divisor

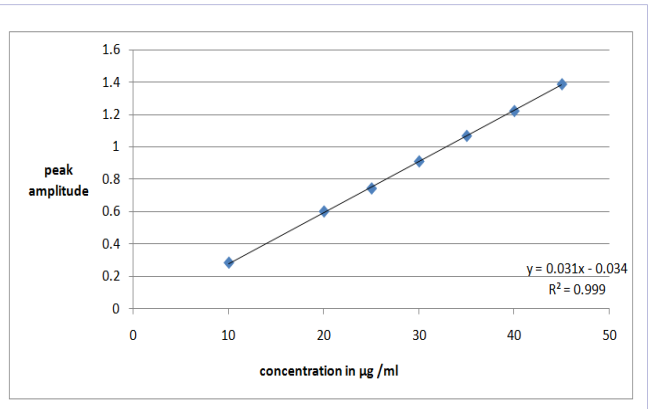

Figure 7: Calibration curve relating peak amplitude of double divisor second derivative spectra of 4 MET to its concentration in µg /ml .

Isoabsorptive point spectrophotometric method for determination of MET in presence of PIO and MEL

Determination of Metformin at 255nm (isoabsorptive point) using methanol as blank

In the isoabsorptive point technique, the concentration of PIO was determined by measuring the absorbance at 268 nm. Then calculate the total mixture concentration by measuring the absorbance at 255 nm. To determine the absorbance of MET we subtract absorbance of the calculated concentration of PIO from the total absorbance of mixture at 255 nm. A calibration curve was constructed relating the absorbance of PIO at 268 nm to concentration (µg / ml) and the regression equation was computed showing a linear relationship (Figure 9).

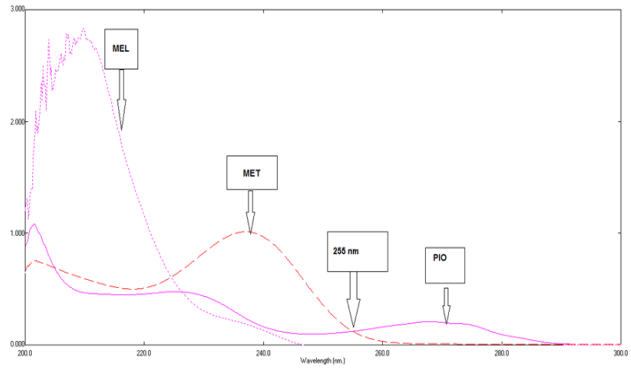

Figure 8: MET 10 µg (dash line) , PIO 10 µg (solid line) and MEL 10 µg (dot line)

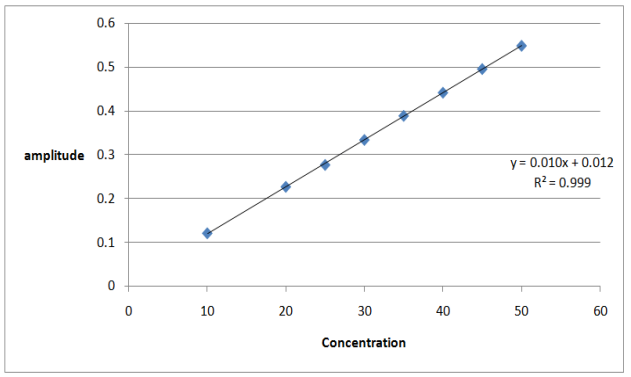

Figure 9: Calibration curve relating the peak amplitude of MET at 255 nm to the concentration

Tlc - densitometric method

The TLC densitometric technique was successfully applied for the determination of MET, PIO and MEL in pure form, mixtures and dosage form. This method offers a simple way to quantify directly on TLC plate by measuring the optical density of the separated bands, in order to obtain optimum separation among the studied drugs. Different trials have been carried out to reach the optimum developing system ,scanning wavelength , band dimension and slit dimension [27].

Different developing systems with different ratios have been tested such as toluene: methanol, chloroform: methanol and toluene: methanol: acetic acid

The best results concerning chromatographic separation, peak symmetry and linearity were obtained upon using the system (toluene – methanol - acetic (5:5:0.5) by volume). The obtained Rf values were 0.8, 0.2 and 0.5 for PIO, MET and MEL, respectively (Figure 10 and 11).

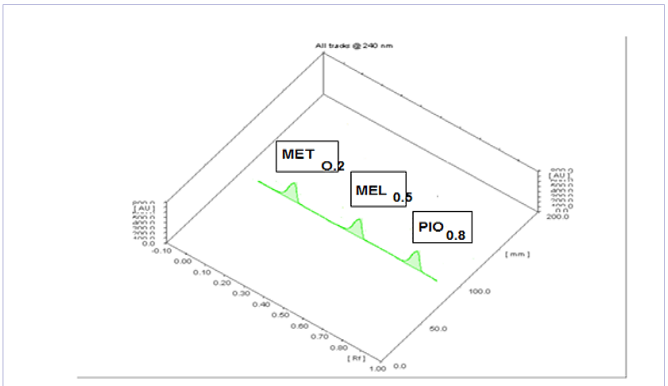

Figure 10: TLC densitogram of mixture of MET , PIO and MEL.

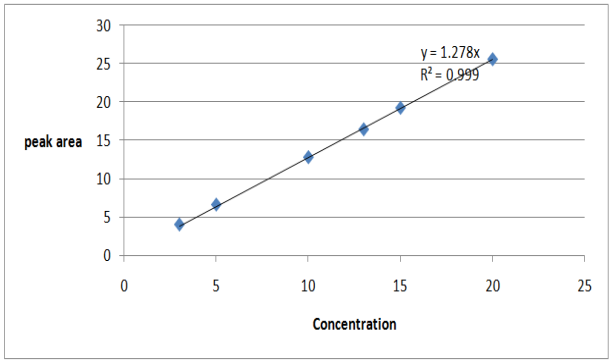

Figure 11: Calibration curve relating the peak area of TLC peaks of PIO to its concentration in µg / band .

Different scanning wavelength such as 210nm .225nm and 240nm were tried but 240nm was the best scanning wavelength that showed high sensitivity with minimum noise for all the drugs.

Calibration curves were plotted to related the integrated peak area versus the corresponding concentrations in the concentration range of 3-12µg/band ,3-20 µg/band and 0.5 - 5 µg/band for MET ,PIO and MEL respectively (Figure 11 , 12 and 13) . The regression equations were computed and found to be:

AMEL = 3.6831C + 0.0117	R^2 = 0.9999
AMET = 2.78C - 0.8607	R^2 = 0.9996
APIO=1.2787C	R^2 = 0.9997

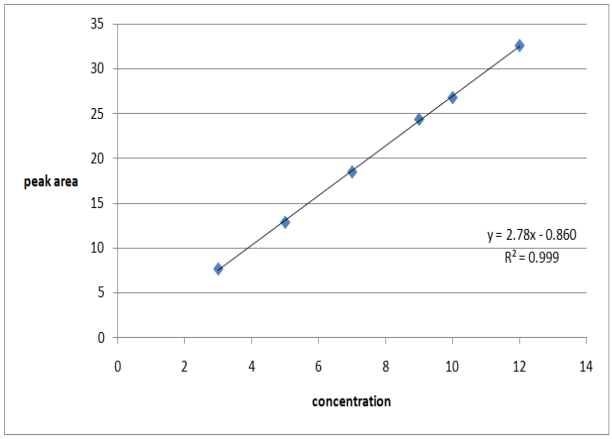

Figure 12: Calibration curve relating the peak area of TLC peaks of MET to its concentration in µg / band .

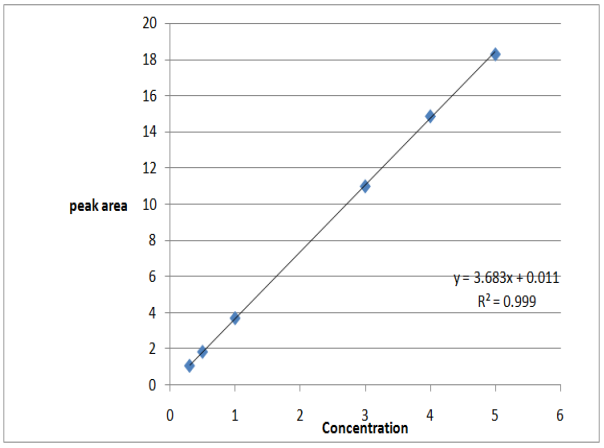

Figure 13: Calibration curve relating the peak area of TLC peaks of MEL to its concentration in µg / band .

Method validation

Validation of the methods was carried out according to ICH recommendation

Linearity and range

The calibration range of the studied drugs was established through considerations of the practical range necessary according to Beer-lamberts law to give accurate, precise and linear results. Linearity ranges are shown in table [1].

Good linearity is evident from the high values of the correlation coefficient and low values of intercept

Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations and the results are shown in (Table 1).

Precision

Repeatability

Three concentrations (5, 10, 15 µg mL-1 of MET and PIO) were analyzed three times intra-daily using the proposed methods. Good results and acceptable relative standard deviations (RSDs) were obtained (Table 1).

Table 1: Regression and analytical parameters of the proposed zero order, isospestic point, double divisor and TLC –densitometric methods for determination of Metformin, Pioglitazone and Melamine

Parameters	Zero order	Double divisor	Isospestic point	TLC-densitometric method		
	PIO	MET	MET	PIO	MET	MEL
Range	3-25µg	10-45µg	10-50µg	3-20µg	3-12µg	0.5-5µg
Slope	0.0231	0.03153	0.0107	1.2	2.7	3.68
Intercept	0.0207	-0.0341	0.0119	0.07	0.8	0.019
Correlation coefficient	0.9999	0.999903	0.9999	0.9997	0.9996	0.9999
Accuracy Mean ±SD	99.98±0.68	100.168±0.72	100.46±0.75	100.4 ± 1.09	100.24± 1.1	99.33±1.4
Precision Repeatability[A] RSD	0.689	0.72	0.756	1.08	1.1	1.45
Intermediate [B] precision RSD	0.731	0.75	0.876	1.17	1.3	1.65

[A] The intraday precision (n=3), average of three different concentrations repeated three times within one day.
[B] The interday precision (n=3), average of three different concentrations repeated three times on three

Intermediate precision

The previous procedure was repeated interdaily on 3 different days for the analysis of the chosen concentration. Good results and acceptable RSD s were obtained.

Selectivity

Selectivity of the proposed analytical method was assessed by the analysis of different synthetic laboratory mixtures contained different ratio of (MET and PIO) within their linearity ranges satisfactory results are shown in table 2.

Specificity of the proposed methods are evident from the spectrophotometric mehods and HPTLC in figure 11which also show no interference between them.

Robustness

The recommended TLC-Densitometric method was found to remain unchanged with small changes in method parameters e.g.: changing acetic acid ratio in the developing system ± 0.02 mL, changing saturation time ± 5 min and changing the scanning wavelength ± 1 nm. Which assessed the robustness of the validated method

System suitability testing parameters

When system suitability testing was done, we obtained acceptable results and the peaks information was given in the resolution (Rs) and selectivity factors (α) values were above 1 and 1.5, respectively, which ensured good separation of each component from the other (Table 4).

Table 2: Determination of Metformin, Pioglitazone and Melamine in laboratory prepared mixtures by the spectrophotometric method

Drugs	pioglitazone		Metformin			
Method	Zero order		double divisor		Isospestic point	
Mix .no.	Taken	Recovery	Taken	Recovery	Taken	Recovery
1	20	101.8	25	102.1079	20	99.14
2	15	100.3	30	101.8095	25	100.1
3	10	99.8	50	101.2127	30	99.8
4	12	101.6	40	98.7381	40	100.13
5	18	101.4	45	99.05467	45	99.8
6	14	101.94	20	98.5873	50	99.6
Mean ±RSD	101.4±0.83		100.2517±1.63034		99.9±0.5	

Table 3: It shows the results of determination of Metformin hydrochloride and Pioglitazone hydrochloride in its pharmaceutical formulations by the spectrophotometric method, proposed TLC - densitometric methods and application of standard addition technique.

Pharmaceutical preparation	TLC			SPECTROPHOTOMETRIC METHODS								
		ZERO ORDER			ISOSPESTIC			DOUBLE DIVISOR				
Bioglita plus	Taken (µg/ band)	Found (%±SD)	Standard addition technique (mean±SD)	TAKEN (µg/ml)	FOUND (%±SD)	STANDARD ADDITION TECNIQUE (mean±SD)	TAKEN (µg/ml)	FOUND (%±SD)	STANDARD ADDITION TECNIQUE (mean+ SD)	TAKEN (µg/ml)	FOUND (%±SD)	STANDARD ADDITION TECNIQUE (mean±SD)
Met	5	100.2 ±0.54	100.14±1.09				30	90.4 ±.49	98.7±.4	15	95.89±1.6	102.45±1.4
Pio	7	99.8 ± 0.34	101.3 ± 1.19	10	99.8±0.86	100.9±0.73						

Table 4: System suitability testing parameters of TLC densitometric method

Parameters	PIO	MET	MEL
Selectivity factors	1.44		2.09
Resolution	3.4		2.4
Capacity factors	0.25	4	1
Symmetry factors	0.8	0.59	0.77

Table 5: It shows the statistical comparison of the results obtained by the proposed methods and the established method

Items	HPTLC			Spectrophotometric methods		Reported HPLC method [c] [26]	
	MET	PIO	MEL	MET	PIO	MET	PIO
Mean	100.1	101.3	99.8	100.46	99.98	101.1667	100.7167
SD	1.2	2.2	1.2	0.75	0.68	1.47196	1.738294
N	6	6	6	6	6	6	6
Variance	1.2	0.5	1.7	0.5	0.1	2.166667	3.021667
Student T test [a]	0.5	0.3	0.6	2.6	0.01		
F – value [b]	0.3	0.9	0.16	0.2	0.7		

[a] figures in parentheses represent the corresponding tabulated values of T at P=0.05
[B] figures in parentheses represent the corresponding tabulated values of F at P=0.05
[C] HPLC method : on a Hypersil ODS-C18 column with 5 µm particle size using the mobile phase acetonitrile-water-acetic acid (75 + 25 + 0.3), adjusted to pH 5.5 with liquor ammonia, at a flow rate of 0.5 mL/min [26]

Conclusion

The developed methods have advantages over the published methods in being more simple, rapid, cost effective and data processing steps are not time consuming. Spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations allowing rapid determination at relatively low cost. The advantages of TLC-densitometric method is its ability to determine the studied drugs using one and the same developing system and scanning wavelength, several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost . The developed methods can be easily adopted for routine quality control analysis of MET and PIO.

The advantages of this spectrophotometric methods are reducing time and cost. Also the proposed TLC –densitometric method has the advantage of being more sensitive than other developed method.

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