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Spectrophotometric methods for simultaneous estimation of Flupentixol Dihydrochloride and Melitracen Hydrochloride in combined tablet dosage form

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ABSTRACT

Simple, precise and economical spectrophotometric methods have been developed for the simultaneous estimation of Flupentixol dihydrochloride and Melitracen hydrochloride in combined tablet dosage form. The first method is based on the use of simultaneous equation, the second method is based on the simultaneous equation using AUC of the two drugs, the third method is based on the use of absorbance ratio method and the fourth one is based on first order derivative method. Both the drugs obey the Beer's law in the concentration ranges employed for these methods. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Flupentixol dihydrochloride and Melitracen hydrochloride in combined tablet dosage forms.

Key words: Flupentixol dihydrochloride, Melitracen hydrochloride, Derivative Spectroscopy, Area under Curve (AUC), simultaneous equation, absorbance ratio method.

INTRODUCTION

Chemically, Flupentixol dihydrochloride (FPD) is (Z)-4-[3-[2-(Trifluoromethyl)-9H-thioxanthen-9-ylidene] propyl]-1-piperazin ethanol dihydrochloride [1] (Figure 1). It is very soluble in water, soluble in alcohol and practically insoluble in methylene chloride [2]. FPD is not official in IP and USP but official in BP. On detailed literature survey, it was found that Flupentixol can be estimated by liquid chromatographic methods individually or in combination with other drugs [3-10].

Chemically, Melitracen hydrochloride (MTH) is 3-(10,10-dimethylantracene-9-ylidene)-N,N-dimethylpropan-1-amine hydrochloride [11] (Figure 2). It is a tricyclic antidepressant. Melitracen, a bipolar thymoleptic with activating properties in low dose, was usually co-administered with Flupentixol in order to decrease the side effects. The combination has none serious side effects due to low drug dosage (10 mg Melitracen and 0.5 mg Flupentixol per tablet) [12]. Flupentixol acts by blocking the Dopamine (a neurotransmitter) receptors in the brain cells. Excess amount of dopamine receptors normally act to modify behavior and over-stimulation resulting in psychotic illness. Flupentixol blocks these receptors to control psychotic illness. Thus it is neuroleptic with anxiolytic and antidepressant properties. Melitracen acts by decreasing reuptake of norepinephrine and serotonin at the synapse resulting in high concentration of these neurotransmitters at the post-synaptic end. Thus it is antidepressant. MTH is not official in any pharmacopoeia. On detailed literature survey, it was found that MTH can be estimated by spectrophotometry [13] and by liquid chromatographic methods [14-17] individually or in combination with other drugs.

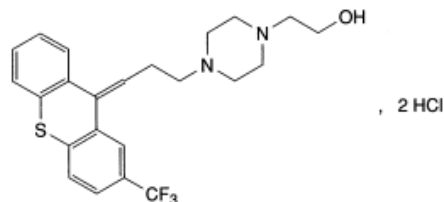


Figure 1: Chemical structure of Flupentixol Dihydrochloride

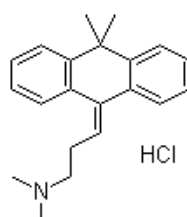


Figure 2: Chemical structure of Melitracen hydrochloride

Two spectrophotometric methods [18-19] and one liquid chromatographic method [20] is reported so far for the simultaneous estimation of these drugs in combined dosage form. Therefore, it was thought worthwhile to develop simultaneous spectrophotometric methods for the estimation of FPD and MTH from their pharmaceutical formulations.

EXPERIMENTAL SECTION

Chemicals and reagents

FPD and MTH working standards were obtained from Centaur Pharmaceutical PVT. Ltd.,(Mumbai, India). A commercial multicomponent tablet formulation was purchased from the local market. Hydrochloric acid (0.1N) of analytical grade solution was prepared in double distilled water.

Instrument

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 1.0 cm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 0.5 nm; derivative mode: ¹D (first order derivative, dA/dλ); band width (Δλ): 5.0 nm; spectral slit width: 1nm. All weights were taken on electronic balance (Denver, Germany).

Preparation of standard stock solutions

FPD and MTH standard solutions.—Accurately 10 mg each of standard FPD and MTH were weighed and transferred to two separate 100 ml volumetric flasks and dissolved in 0.1N HCl and further diluted with the 0.1N HCl solvent to obtain standard solutions of FPD and MTH having final concentrations of 100 μgml⁻¹ each.

Method I: Simultaneous Equation Method

Series dilutions of the standard stock solutions were made separately by pipetting out 0.1, 0.5, 1.0, 2.0, 4.0, 5.0 and 6.0 ml of standard stock solutions into separate 10 ml volumetric flasks and diluting to volume with 0.1N HCl to produce the concentrations ranging from 1.0-50.0 μg ml⁻¹ FPD and 1.0-60.0 μg ml⁻¹ for MTH respectively. The above solutions were scanned over the range of 400 nm to 200 nm against blank. The λ_{max} was found to be at 229.5 nm for FPD and 258.5 nm for MTH, respectively. Figure 3 represents the overlain spectra of FPD and MTH. The absorbencies of the standard solutions were measured at 229.5 nm and 258.5 nm and calibration curves were plotted by taking concentration on x-axis and absorbance at 229.5 nm or 258.5 nm on Y-axis (Graph 1 and Graph 2) and the regression analysis of calibration curves and absorptivity values of both these drugs are presented in Table 1 and Table 2.

Two simultaneous equations [21] (in two variables C₁ and C₂) were formed using these absorptivity values.

$$A_1 = (0.061154) C_1 + (0.046968) C_2 \quad (1)$$

$$A_2 = (0.022978) C_1 + (0.043642) C_2 \quad (2)$$

Where, C₁ and C₂ are the concentrations of FPD and MTH measured in μgml⁻¹, in the sample solutions. A₁ and A₂ are the absorbance of mixture, at selected wavelengths of 229.5 nm and 258.5 nm respectively.

By applying the Cramer's rule [22] to equations 1 and 2, the concentration C_{FPD} and C_{MTH}, can be obtained as follows

$$C_{\text{FPD}} = \frac{A_2(0.046968) - A_1(0.043642)}{-0.00159}$$

And

$$C_{\text{MTH}} = \frac{A_1(0.022978) - A_2(0.061154)}{-0.00159}$$

Method II: Area Under Curve Method

In the simultaneous equation using AUC method, the area under curves of the recorded spectrums were measured at the selected wavelength ranges, 224 to 234 nm (for FPD) and 253.5 to 263.5 nm (for MTH) and calibration curves were plotted by taking concentration on x-axis and AUC at 224 to 234 nm or 253.5 to 263.5 nm on Y-axis (Graph 1 and Graph 2) and the regression analysis of calibration curves and absorptivity values (X) of both these drugs are presented in Table 1 and Table 2. The 'X' values were determined as, X = Area under curve of component (from 224 to 234 nm or 253.5 to 263.5 nm)/concentration of the component in μgml^{-1} . A set of two simultaneous equations framed using these 'X' values as follows,

$$A_1 = 0.58526C_1 + 0.48068C_2 \text{ - (at } \lambda \text{ 224.0-234.0 nm) -- (1)}$$

$$A_2 = 0.23219C_1 + 0.43202C_2 \text{ - (at } \lambda \text{ 253.5-263.5 nm) -- (2)}$$

Where, C_1 and C_2 are the concentrations of FPD and MTH measured in μgml^{-1} , in the sample solutions. A_1 and A_2 are the area under curve of sample solutions at the wavelength range, 224 to 234 nm and 253.5 to 263.5 nm, respectively

By applying the Cramer's rule²² to equations 1 and 2, the concentration C_{FPD} and C_{MTH} , can be obtained as follows

$$C_{\text{FPD}} = \frac{A_2(0.48068) - A_1(0.43202)}{-0.14123}$$

And

$$C_{\text{MTH}} = \frac{A_1(0.23219) - A_2(0.58526)}{-0.14123}$$

Method III: Absorbance Ratio Method (Q-Analysis)

In quantitative assay of two components by Q- analysis method, absorbances were measured at the isobestic wavelength and maximum absorption of one of the two components. From the overlain spectra of FPD and MTH shown in Figure 3, absorbances were measured at the selected wavelengths i.e 239 nm (isobestic wavelength) and 258.5 nm (wavelength of maximum absorption of MTH). The concentration of each component can be calculated by mathematical treatment of mentioned equation.

$$\text{Concentration of FPD} = \frac{Q_O - Q_N}{Q_T - Q_N} \times \frac{A}{\epsilon_1}$$

$$\text{Concentration of MTH} = \frac{Q_O - Q_T}{Q_N - Q_T} \times \frac{A}{\epsilon_2}$$

Where, A = Absorbance of sample solution at isobestic point

ϵ_1 and ϵ_2 = Absorptivity of FPD and MTH at isobestic wavelength.

Q_T = Absorptivity of FPD at 258.5 nm/ Absorptivity of FPD at 239 nm.

Q_N = Absorptivity of MTH at 258.5 nm / Absorptivity of MTH at 239 nm.

Q_O = Absorbance of sample solution at 258.5 nm / Absorbance of sample solution at 239 nm

Method IV: First-Order Derivative Spectroscopy

The spectrums obtained in Method I was derivatised to obtain first derivative spectrum. The two spectra were overlain as shown in Figure 6. It appeared that FPD showed zero crossing at 229 nm while MTH showed zero crossing at 244.5 nm. At the zero crossing point of FPD (229 nm), MTH showed a substantial $dA/d\lambda$, whereas at the zero crossing point of MTH (244.5 nm), FPD showed a substantial $dA/d\lambda$. Hence the wavelengths 229 nm and 244.5 nm were selected as analytical wavelengths for determination of MTH and FPD, respectively. These two wavelengths can be employed for the estimation of MTH and FPD without any interference from the other drugs in their combined formulation. Calibration curves were plotted by taking $dA/d\lambda$ on Y-axis and concentrations on X-axis (Graph-1 and Graph-2). The regression analysis of calibration curves are presented in Table 1 and Table 2.

Preparation of tablet sample solution

Twenty tablets each containing Flupentixol dihydrochloride INN equivalent to 0.5 mg Flupentixol and Melitracen hydrochloride INN equivalent to 10 mg Melitracen were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 0.5 mg of Flupentixol dihydrochloride and 10 mg Melitracen hydrochloride was transferred to a 100 ml volumetric flask and 50 ml 0.1N HCl was added. After ultrasonic vibration for 30 min, the mixture was diluted to volume with 0.1N HCl and filtered through Whatman filter paper # 41. Appropriate aliquots were subjected to the above methods and the amounts of FPD and MTH were determined. Percent labeled claim and Standard Deviation (S.D) was calculated and the results are presented in Table 3.

Validation of methods

Linearity: For all the methods, 6-point (1-50 μgml^{-1} FPD and 1-60 μgml^{-1} MTH) calibration curves were prepared on 3 different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method.

Precision: The intraday and interday precisions of the proposed spectrophotometric methods were determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of FPD (5, 10, and 20 μgml^{-1}) and MTH (5, 10, and 20 μgml^{-1}) and the results are reported in terms relative standard deviation (RSD; Table 4).

Accuracy: This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding known amounts of FPD and MTH reference materials to a prequantified sample solution. Aliquots of sample solutions containing FPD and MTH at 1 μgml^{-1} and 20 μgml^{-1} respectively were transferred to three 10 ml volumetric flasks containing, respectively, 0.8, 1, and 1.2 μgml^{-1} FPD and 16, 20 and 24 μgml^{-1} MTH reference solution. The contents were mixed and diluted to volume in order to obtain final concentrations of 1.8, 2 and 2.2 μgml^{-1} FPD and 36, 40 and 44 μgml^{-1} MTH, respectively. The recoveries were verified by estimation of drugs in triplicate preparations at each specified concentration level.

The spectrums were recorded in the UV range and then analyzed. The results are reported in terms of % recovery (Table 5).

Specificity: Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific.

Robustness: The robustness of the proposed methods was tested by changing parameters such as wavelength range and slit width. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

Ruggedness: Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot in different laboratories by different analyst using similar operational and environmental conditions; data is presented in Table 6.

RESULTS AND DISCUSSION

FPD is very soluble in water but MTH is sparingly soluble in water, hence 0.1N HCl was selected as solvent for this study in which both the drugs were soluble and stable throughout the study. Figure 3 shows overlaid zero-order spectra of FPD and MTH at $10 \mu\text{gml}^{-1}$ and the spectra showed a λ_{max} of 229.5 nm and 258.5 nm for FPD and MTH, respectively. Also, each of which absorbs at the λ_{max} of the other hence, simultaneous equation method, simultaneous equation using AUC method, absorbance ratio method and first-order derivative methods were used to estimate FPD and MTH in presence of each other. In Method II (Figure 4 and 5), study was carried out at two wavelength ranges i.e 224-234 nm/219-239 nm and 253.5-263.5 nm/248.5-268.5 nm, but good linearity range was obtained at the wavelength range of 224-234 nm and 253.5-263.5 nm.

Figure 6 shows the overlaid first-derivative spectra of FPD and MTH at $10.0 \mu\text{g/ml}$ and the spectra showed a ZCP (zero cross point) of FPD (229 nm) where MTH could be analyzed and ZCP of MTH (244.5 nm) where FPD could be analyzed.

It was also observed that with the increase in FPD and MTH concentration, the responses are increased (Graph 1 and 2). The responses for FPD and MTH were found to be linear in the concentration range of $1-50 \mu\text{gml}^{-1}$ FPD and $1-60 \mu\text{gml}^{-1}$ MTH for all the methods.

The assay results for FPD and MTH in its pharmaceutical dosage forms (Table 3) obtained by using the different spectrophotometric methods were showed that there was no significant difference in the content of FPD and MTH determined by the different spectrophotometric methods. Hence all the methods can be used for the estimation of the drugs in their combined pharmaceutical formulations.

The recoveries of FPD and MTH (Table 5) were found to be in the acceptable range. Excipients used in the formulation did not interfere with response of either of the drugs at their respective analytical wavelengths. Also, no significant change in response of FPD and MTH was observed by changing parameters such as wavelength range and slit width. The intra-day and inter-day

precision values (%RSD) were calculated (Table 4) and results were found to be in the acceptable range for FPD and MTH. Ruggedness of proposed methods were determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (Table 6). Hence, the methods are precise, specific, accurate, ruggedness and robust for estimation of FPD and MTH.

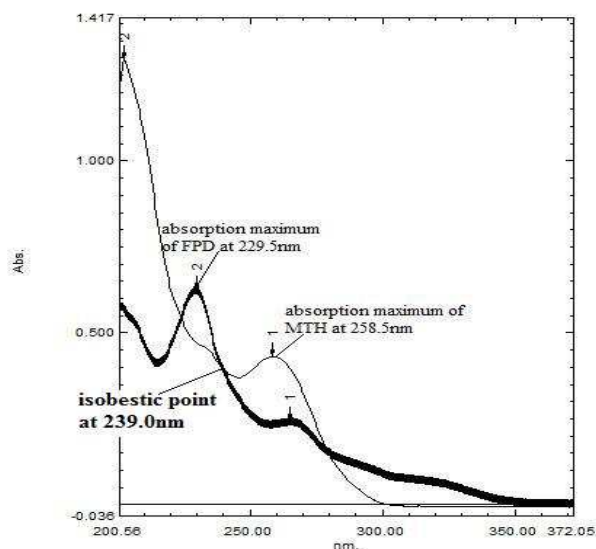


Figure 3: Overlain zero-order absorption spectra of FPD ($10 \mu\text{gml}^{-1}$) and MTH ($10 \mu\text{gml}^{-1}$)

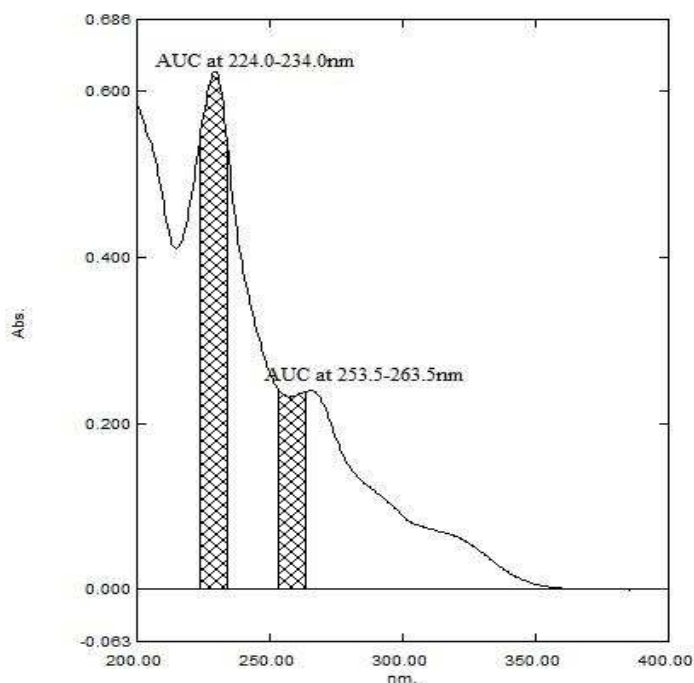


Figure 4: Absorption spectra of FPD ($10 \mu\text{gml}^{-1}$) in 0.1N HCl showing AUC at selected wavelengths

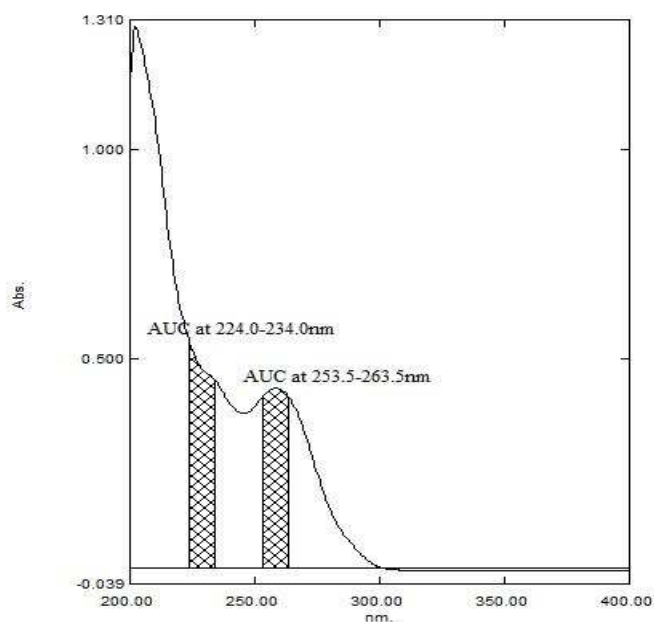


Figure 5: Absorption spectra of MTH (10 µgml⁻¹) in 0.1N HCl showing AUC at selected wavelengths

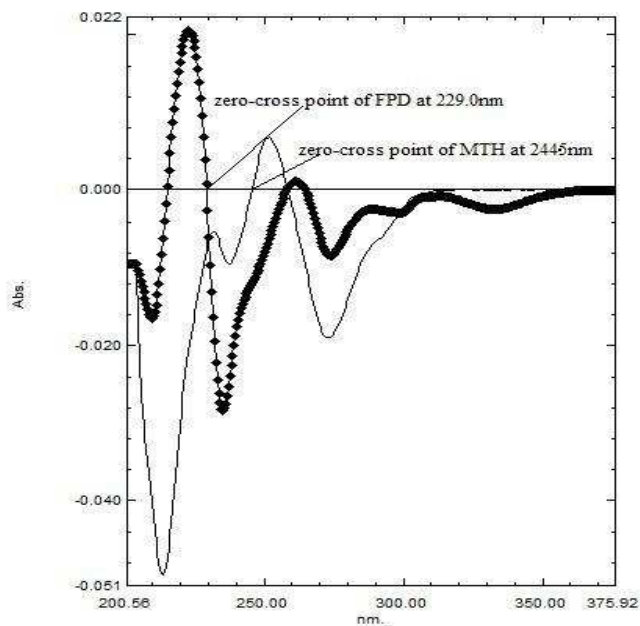
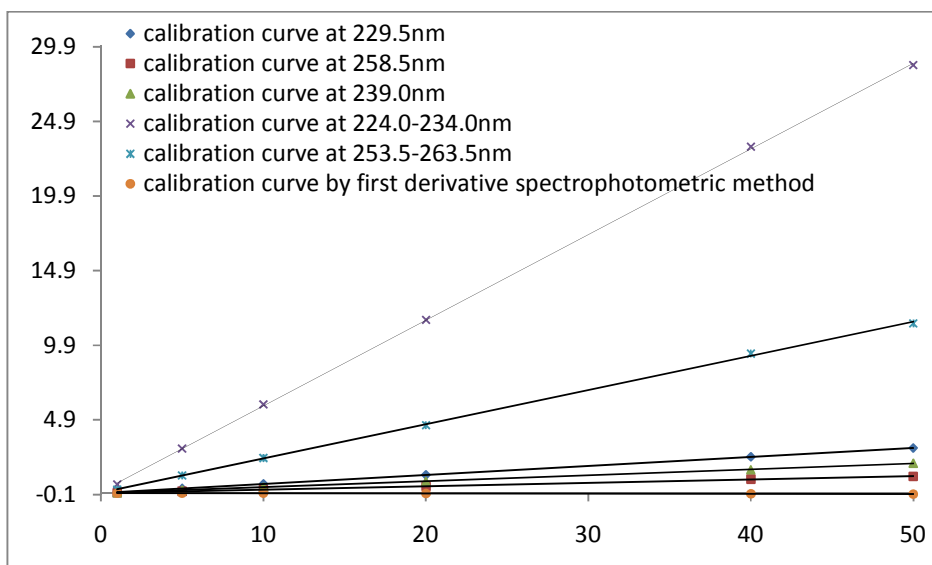
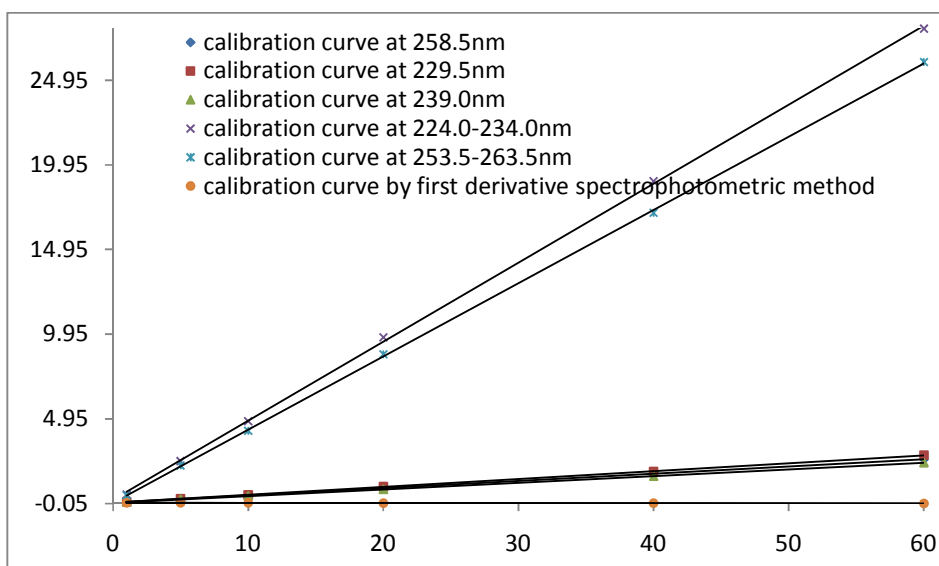


Figure 6: Overlain First-order derivative spectrum of FPD (10 µgml⁻¹) and MTH (10 µgml⁻¹).



Graph 1: Calibration curves of FPD



Graph 2: Calibration curves of MTH

Table 3. Assay results of FPD and MTH in pharmaceutical dosage form (Tablet) using the proposed spectrophotometric methods

Drug	Label Claim (mg/tab)	% Label Claimed \pm SD(n=5)			
		Method I	Method II	Method III	Method IV
FPD	0.5	99.76 \pm 0.48	99.65 \pm 0.21	98.97 \pm 0.45	99.24 \pm 0.27
MTH	20	100.32 \pm 0.45	99.98 \pm 0.23	100.15 \pm 0.04	99.87 \pm 0.45

Table 4: Precision studies of FPD and MTH by proposed spectrophotometric methods

method	Intraday (n=3); (RSD, %)						Interday (n=3); (RSD, %)					
	Drug Conc. taken (μgml^{-1})						Drug Conc. taken (μgml^{-1})					
	FPD			MTH			FPD			MTH		
	5	10	20	5	10	20	5	10	20	5	10	20
I	0.34	0.44	0.57	0.33	0.45	0.58	0.36	0.46	0.59	0.37	0.49	0.62
II	0.42	0.42	0.35	0.65	0.43	0.61	0.59	0.91	0.46	0.47	0.40	0.38
III	0.67	0.76	0.54	0.54	0.28	0.75	0.38	0.67	0.74	0.48	0.73	0.56
IV	0.54	0.52	0.38	0.54	0.51	0.65	0.87	0.68	0.76	0.95	0.50	0.67

Table 5: Results for Accuracy studies of FPD and MTH by proposed spectrophotometric methods

method	Accuracy (% recovery)					
	FPD			MTH		
	80% 1+0.8 μgml^{-1}	100% 1+1 μgml^{-1}	120% 1+1.2 μgml^{-1}	80% 20+16 μgml^{-1}	100% 20+20 μgml^{-1}	120% 20+24 μgml^{-1}
I	99.80	100.21	101.80	98.60	100.90	99.79
II	99.67	100.65	100.98	99.02	100.12	100.34
III	98.47	100.16	100.15	99.56	100.34	100.21
IV	98.78	99.97	99.79	99.56	100.03	100.16

Table 6: Ruggedness data of $10 \mu\text{gml}^{-1}$ FPD and $10 \mu\text{gml}^{-1}$ MTH

Drug	Analyst I, %RSD				Analyst II, %RSD			
	methodI	methodI I	methodII I	methodI V	methodI	methodI I	methodI II	methodIV
FPD	0.44	0.54	0.38	0.57	0.46	0.52	0.41	0.61
MTH	0.52	0.43	0.62	0.32	0.57	0.39	0.65	0.35

Table 1: Regression analysis of calibration Curves and Absorptivity values of FPD

Conc. (μgml^{-1})	Abs. at 229.5 nm	Absorptivity at 229.5nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	Abs. at 258.5nm	Absorptivity at 258.5nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	Abs. at 239nm	Absorptivity at 239.0nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	AUC (224-234nm)	Absorptivity at 224-234 nm	AUC (253.5-263.5 nm)	Absorptivity at 253.5-263.5nm	dA/d λ at 244.5nm
1	0.06	0.06	0.023	0.023	0.041	0.041	0.586	0.586	0.233	0.233	-0.001
5	0.313	0.0626	0.117	0.0234	0.201	0.0402	2.984	0.5968	1.185	0.237	-0.006
10	0.624	0.0624	0.232	0.0232	0.401	0.0401	5.948	0.5948	2.345	0.2345	-0.013
20	1.218	0.0609	0.451	0.02255	0.78	0.039	11.604	0.5802	4.551	0.22755	-0.025
40	2.429	0.060725	0.927	0.023175	1.569	0.039225	23.201	0.580025	9.343	0.233575	-0.049
50	3.015	0.0603	1.127	0.02254	2.001	0.04002	28.687	0.57374	11.375	0.2275	-0.062
r \pm %RSD	0.9998 \pm 0.0058		0.9996 \pm 0.0153		0.9997 \pm 0.0322		0.9999 \pm 0.0514		0.9996 \pm 0.0473		0.9997 \pm 0.0494
Slope \pm %RSD	0.0601 \pm 0.2540		0.0226 \pm 0.4425		0.0394 \pm 0.6382		0.5735 \pm 0.0786		0.2289 \pm 0.1765		-0.0012 \pm -0.9491
Intercept \pm %RSD	0.0111 \pm 0.90091		0.0026 \pm 1.4808		0.0016 \pm 1.3203		0.1172 \pm 0.2147		0.0292 \pm 1.2013		0.00007 \pm 0.8287
mean		0.061154		0.022978		0.039924		0.585261		0.232188	
SD		0.001091		0.000358		0.000723		0.00906		0.003862	
%RSD		1.784258		1.5591		1.811929		1.548088		1.663277	

Table 2: Regression analysis of calibration Curves and Absorptivity values of MTH

Conc. (μgml^{-1})	Abs. at 258.5 nm	Absorptivity at 258.5nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	Abs. at 229.5 nm	Absorptivity at 229.5nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	Abs. at 239 nm	Absorptivity at 239.0nm ($\text{ml } \mu\text{g}^{-1}\text{cm}^{-1}$)	AUC (224-234nm)	Absorptivity at 224-234nm	AUC (253.5-263.5nm)	Absorptivity at 253.5-263.5nm	dA/d λ at 229nm
1	0.043	0.043	0.046	0.046	0.041	0.041	0.482	0.482	0.429	0.429	-0.001
5	0.223	0.0446	0.24	0.048	0.201	0.0402	2.461	0.4922	2.196	0.4392	-0.004
10	0.439	0.0439	0.47	0.047	0.401	0.0401	4.813	0.4813	4.234	0.4234	-0.008
20	0.89	0.0445	0.952	0.0476	0.78	0.039	9.756	0.4878	8.773	0.43865	-0.017
40	1.738	0.04345	1.851	0.046275	1.569	0.039225	18.972	0.4743	17.127	0.428175	-0.033
60	2.544	0.0424	2.816	0.046933	2.369	0.04002	27.989	0.466483	26.023	0.433717	-0.048
r \pm %RSD	0.9991 \pm 0.0451		0.9992 \pm 0.0551		0.9995 \pm 0.0724		0.9992 \pm 0.0600		0.9992 \pm 0.0603		0.9992 \pm 0.0551
Slope \pm %RSD	0.0423 \pm 0.3608		0.0467 \pm 0.5385		0.039 \pm 1.0256		0.4655 \pm 0.2477		0.4327 \pm 0.2345		- 0.0008 \pm -1.25
Intercept \pm %RSD	0.0166 \pm 1.2048		0.0037 \pm 1.5746		0.0014 \pm 1.2461		0.1733 \pm 0.4580		0.0065 \pm 1.5385		0.0003 \pm 0.5528
mean		0.043642		0.046968		0.039924		0.480681		0.432024	
SD		0.000862		0.000759		0.000723		0.00925		0.006271	
%RSD		1.974343		1.616985		1.811929		1.924285		1.451429	

CONCLUSION

All the methods that were developed for the determination of FPD and MTH in the presence of each other are based on different analytical techniques. All the methods were validated and found to be simple, sensitive, accurate, and precise. In spite of the low content of FPD, all the methods were successfully used to estimate the amount of FPD and MTH present in tablet formulations without the need for addition of standard FPD. Comparison of the assay results obtained for FPD and MTH in tablet formulations by using these methods indicated no significant difference. Hence, all the methods can be used successfully for routine analysis of combined tablet dosage forms of FPD and MTH.

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