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Assay of famotidine in API and dosage forms by UV direct and UV derivative spectrophotometric methods

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ABSTRACT

Simple and sensitive UV absorption spectrophotometric method (Method-A) and UV first and second derivative spectrophotometric methods (Method-B and Method-C) have been developed for the estimation of Famotidine (FMD) in pure and its pharmaceutical dosage forms. The wavelength of maximum absorbance was found to be 287.0nm from the absorption spectrum of FMD in methanol. First and second derivative spectra were obtained and from the first derivative spectrum it is found that a valley at 272.2nm has maximum amplitude and therefore validation in Method-B was carried out by measuring the amplitudes at this wavelength. Second derivative spectrum has the maximum amplitude in negative valley at 287.0nm hence the Method-C was validated by measuring amplititudes at 287.0nm. Precision was presented as standard deviation and percent of relative standard deviation of six replicate measurements and found to be within the limits. Accuracy (the mean percent of recovery of triplicate measurements) at three different concentrations i.e. 50%, 100% and 150% of target concentration was calculated and found to be within the range 98.2%-99.8%. A study of proportionality between concentration of the drug and response of the instrument was carried out and found to be linear within the range of concentrations 4-12µg/mL of FMD. Slope, intercept and correlation coefficient were calculated form the linear regression analysis and the correlation coefficient was found to be 0.9999. The developed methods were found to be precise, accurate, stable and robust therefore readily adapted for routine quality control of FMD by ordinary laboratories. The developed methods were effective for estimation of FMD in bulk and pharmaceutical preparations without any interference of other constitute in tablets of different brand names.

Keywords: Famotidine, Derivative spectrophotometer, Amplitude, Pharmaceutical preparations, Assay.

INTRODUCTION

Famotidine (FMD), a histamine-2 blocker which is used to treat and prevent ulcers in the stomach and intestines. It also treats conditions in which the stomach produces too much acid, such as Zollinger-Ellison syndrome. It effectively heals duodenal and gastric ulcers and prevents recurrence. So it is a very essential to calculate percentage of FMD within the respected formulation. Chemical name of FMD is 3-[({2-[(diaminomethylidene) amino]-1,3-thiazol-4-yl}methyl)sulfanyl]-N'-sulfamoylpropanimidamide having empirical formula and molecular weight is $C_8H_{15}N_7O_2S_3$ and 337.445grams respectively . It is a white to pale yellow crystalline compound that is freely soluble in glacial acetic acid. FMD is available as pepcid tablet for oral administration containing either 20mg or 40mg of FMD. The chemical structure of the drug is given in Fig.1.

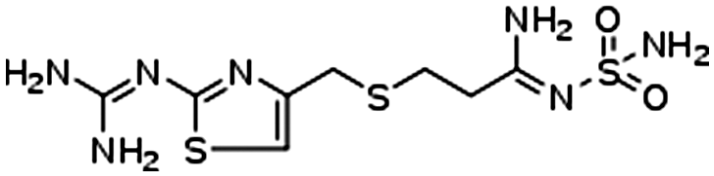

Fig.1: Chemical structure of Famotidine (FMD)

Several techniques have been adopted for the determination of Famotidine (FMD), A variety of procedures that render the UV determination of FMD have been published. A variety of UV procedures were reported for the determination of FMD as single compounds or in mixtures. Bhavik [1] developed a UV Spectrophotometric method for simultaneous estimation of FMD with other drugs in bulk and formulated tablet dosage form. Khadiga [2] reported a stability indicating UV method for the determination of FMD in combination with other drugs. Different authors [3-11] applied some colour developing reagents to estimate FMD by developing visible spectrophotometric methods. Some derivative spectrophotometric methods [12-14] were also reported to estimate the drug in combination with several drugs. Some liquid chromatographic methods [15-23] were present in the literature for the determination of FMD mostly in different biological forms, potential impurities and in combination with other drugs in dosage forms. One HPTLC method [24] was also reported to estimate the drug in bulk and formulations. Literature survey reveals that several high performance liquid chromatographic was reported in literature for the determination of FMD in different forms of biological fluids and in tablet dosage forms. One RP-UPLC method [25] was present for the simultaneous estimation of ibuprofen and FMD in pharmaceutical dosage form. Spectrophotometric simultaneous determination of Famotidine and Domperidone in combined tablet dosage form by ratio derivative and area under curve method [26], RP-HPLC and UV-derivative spectrophotometry technique [27] for the simultaneous estimation of ibuprofen and famotidine in pharmaceutical dosage form were also reported. Since derivative spectrophotometry [28-29] has its own advantages, so the author has chosen this technique to estimate the drug in pure and formulations and made some attempts to use zero, first and second derivative studies for the determination of the selected drug FMD. Therefore it seems necessary to develop a simple and fast identification method for determination of Famotidine (FMD). But UV and UV derivative Spectrophotometric methods were more sensitive than other methods, so the author has made some attempts in this direction and succeeded.

MATERIALS AND METHODS

2.1 Instrumentation

An UV-Visible spectrophotometer (UV-3000) with 1cm matched quartz cells was used for the spectral and absorbance measurements. Semi micro balance (CPA225D) was used for weighing purpose.

2.2 Method development

All the chemicals, reagents and solvents used in the present investigation were analytical grade. Distilled water used for the analysis was prepared by double distillation in the laboratory. A gift sample of FMD was provided by Dr.Reddy's Laboratory Hyderabad and different dosage forms were obtained from the local pharmacy. Working standard solution of FMD was prepared by taking about 10 mg of FMD standard accurately into a 100 mL volumetric flask, about 70 mL of methanol was added, sonicate to dissolve completely and made volume up to the mark with the same solvent. Further 20 mL of the above solution was diluted to 100mL with methanol. A series of working standard solutions of different concentrations (4-12 µg/mL) were prepared from the diluted solution of concentration 20 µg/mL.

The absorption spectrum of the working standard solution of concentration 6µg/mL was scanned in the range of wavelength 200-400nm and then first and second derivative spectra were recorded. The absorption spectrum was found to be sharp having wavelength of maximum absorbance at 287.0nm (Method-A). First derivative spectrum (Fig.3) has one positive valley at 272.2 nm and a negative valley (having maximum amplititudes) at a wavelength of 305.1nm. The proposed Method-B was validated by measuring amplititudes at this wavelength. The second derivative spectrum (Fig.4) was found to have two positive peaks at 255.2 and 313.1nm, and a negative valley at 287.0nm. The maximum displacements at above three wavelengths were measured and found that the amplititudes at 287.0nm was maximum; hence the Method-C was validated for second derivative spectrophotometry at 287.0nm.The zero crossing points in first and second derivative spectra were found to be at 287.0nm and 272.2 &305.1nm respectively.

2.3 Method Validation

Linearity and Range: Into five 10mL calibrated tubes, different aliquots (2-6mL; 20µg/mL) of standard FMD solution was taken and diluted up to the mark with methanol, shaken well and then kept aside for 10min. The absorption spectra and derivative spectra for each of the concentration were recorded over the wavelength range 200-400nm against a reagent blank under similar conditions (Fig.5-Fig.7). Linearity plots were drawn taking absorbance or amplititudes on x-axis and concentration on y-axis and were shown in Fig.8-Fig.10. The mean values of correlation coefficient, slope and intercept were evaluated by the least square regression method and were shown in Table 1.

Calibration plot: In Method-A, a linear straight line was drawn by taking absorbance values on y-axis and concentration on x-axis (Fig.8). In case of Method-B and Method-C, maximum D^1 and D^2 amplititudes were plotted against concentration of the drug (Fig.9-Fig.10). Linear least squares regression analysis was applied in three cases and slope intercept and correlation coefficient parameters were calculated and were presented in Table-1.

Precision: Precision (repeatability) of each proposed methods was calculated from the absorbance values or maximum amplititudes of five replicates of a fixed amount of FMD in total solution in D^0, D^1 and D^2 respectively. The standard deviation and percent relative standard deviation were calculated for the proposed methods and presented in Table-2.

Intermediate Precision: To evaluate intermediate precision (reproducibility) measurements were performed on different days under the same experimental conditions. In the present study intermediate precision of each proposed method was ascertained from the absorbance values and amplititudes obtained for five replicates of a fixed amount of FMD in total solution on two different days. The standard deviation and percent relative standard deviation were calculated in each case and presented in Table-3.

Accuracy: Accuracy, concordance between the measured value and the true or most probable value was determined at three different amounts (50%, 100%, and 150%) of FMD within the Beer's law limits were taken, measurements were made thrice in each concentration. Standard deviation and percent of relative standard deviation were calculated for three replicate measurements at three concentrations. The results were recorded in Table 4(a)-Table 4(c).

Robustness: Robustness of a method is a study of the effect of small variation of the experimental conditions on reproducibility of the measurements. In the present investigation a study of robustness was carried out by making a small change in wavelength (±2) of measurements. The results of robustness of the D^0, D^1 and D^2 spectroscopy were represented in Table-5.

Limit of detection (LOD) and limit of quantization (LOQ): The LOD and LOQ of the proposed methods were calculated by using standard deviation of the intercept (σ) and slope (s) of the calibration curve. These were calculated by using the formulae LOD=3σ/s and LOD=10σ/s and are presented in Table-6.

2.4 Assay of pharmaceutical formulations

The average weight of five tablets of FMD was accurately calculated and these tablets were grinded well into a uniform powder. Test solution of 6µg/mL was prepared as explained in preparation of working standard solution by taking an amount of the tablet powder equivalent to10 mg of FMD. Three different concentration solutions at 50%, 100% and 150% of target concentration were also prepared in similar manner. Pepcid tablets of 20mg and 40mg were analyzed by the validated method by measuring absorbance and amplititude of working standard solution and sample solution. The amount of drug present was evaluated in terms of percent of recovery of six replicates and the results were presented in Table-7.

RESULTS AND DISCUSSION

The wavelength of maximum absorbance was found to 287nm. The first derivative spectrum crossed zero point at 287.0nm leaving one positive peak at 272.2 nm and a negative peak at 305.1nm. The second derivative spectrum was found to have two positive peaks at 255.2 and 313.1nm, and a negative valley at 287.0nm. The maximum displacements at above three wavelengths were measured and found that the amplititudes at 287.0nm was maximum; hence the method was validated for second derivative spectrophotometry at 287.0nm.The zero crossing points in second derivative spectra were found to be at 287.0nm and 272.2 &305.1nm respectively. The developed method was found to be linear within the range of concentration 4-12µg/mL in direct, first derivative and second derivative methods. Low values of standard deviation and percent of relative standard deviation (% RSD) indicate that the developed method was precise.

The mean percent of recovery and percent of relative standard deviation were evaluated at 50%, 100% and 150% concentration levels. The mean percent of recovery and percent of relative standard deviation were found to be 98.8% (0.234), 99.8% (0.98), 99.8% (1.94). High percent of recovery values support for good accuracy of the method. Limit of detection (LOD) and limit of quantitation (LOQ) values were found to be 0.31&0.842, 0.026&0.034 and 0.019&0.029 in zero order, first and second derivative studies respectively, these values indicate that the developed method was sensitive. The method has been proved robust at ±2nm Wavelength variation. Pharmaceutical formulations were analysed and percent of recovery was calculated and found to be present between 99.40-101.88.

Table-1: Linearity of the proposed Method

S. No.	Concentration µg/mL	Absorbance	D^1Amplitude*	D^2Amplitude*
1	4.0	0.259	0.009	1.57E-3
2	6.0	0.379	0.015	2.38 E-3
3	8.0	0.515	0.019	3.20 E-3
4	10.0	0.650	0.024	4.00 E-3
5	12.0	0.814	0.029	4.75 E-3
Slope		0.067	0.0024	0.0004
Intercept		0.0104	9.0E.-5	4.0E-6
Correlation Coefficient		0.9999	0.9993	0.9999

**D^1 and D^2 are first order and second order derivative spectra*

Table-2: Precision for the developed method

S.No	Concentration µg/mL	Zero Order	First Order	Second Order
Average*		0.3794	0.0150	2.388 E-3
Standard Deviation*	6.0	1.673E-3	2.2E-4	1.79E-05
%RSD*		0.4410	1.4434	0.7491

** Statistical analysis applied on five replicates of measurements*

Table-3: Study of Intermediate Precision of the proposed method

Statistical parameter	Zero order	First order	Second order
Average*	0.3806	0.0150	2.394 E-3
Standard Deviation*	2.074E-3	1.64E-4	2.07E-05
%RSD*	0.5448	1.0939	0.8662

** Statistical analysis applied on five replicates of measurements*

Table-4(a) Accuracy of the developed method (Zero derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.91	98.2%	
100%	10.0	9.88	98.8%	98.8%
150%	15.0	14.9	99.3%	

Table-4(b) Accuracy of the developed method (First derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.99	99.8%	
100%	10.0	9.98	99.8%	99.8%
150%	15.0	14.97	99.8%	

Table-4(c) Accuracy of the developed method (Second derivative)

%Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5.0	4.99	99.8%	
100%	10.0	9.98	99.8%	99.8%
150%	15.0	14.97	99.8%	

Table-5 Robustness of the proposed method

wavelength	Absorbance(Zero)	Amplitude(First)	Amplitude(second)
285	0.295	0.008	0.0031
287	0.306	0.010	0.0030
289	0.298	0.009	0.0030

Table-6 LOD and LOQ of FMD

Parameter	Zero Derivative	First Derivative	Second Derivative
LOD	0.310	0.026	0.019
LOQ	0.842	0.034	0.029

Table-7 Assay of pharmaceutical formulations

S.No.		Formulation	Labeled Amount	Amount Found*	SD	%Recovery	% RSD
1	D^0	Pepcid Tablet	20 mg	19.94	0.571	99.70	0.5727
2		Pepcid Tablet	40mg	40.57	0.792	101.43	0.7809
1	D^1	Pepcid Tablet	20 mg	20.08	0.376	100.40	0.3745
2		Pepcid Tablet	40mg	39.76	1.024	99.40	1.0302
1	D^2	Pepcid Tablet	20 mg	19.89	0.972	99.45	0.9774
2		Pepcid Tablet	40mg	40.75	1.068	101.88	1.0483

Average of six determinations, SD=standard deviation, RSD=relative standard deviation, D0, D1andD2 were represent zero, first and second order derivatives

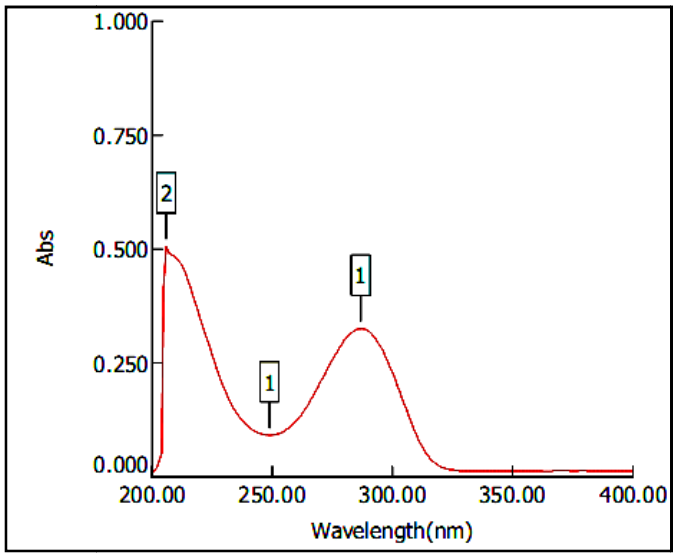

Fig. 2: Absorption spectrum of FMD (6 µg/mL)

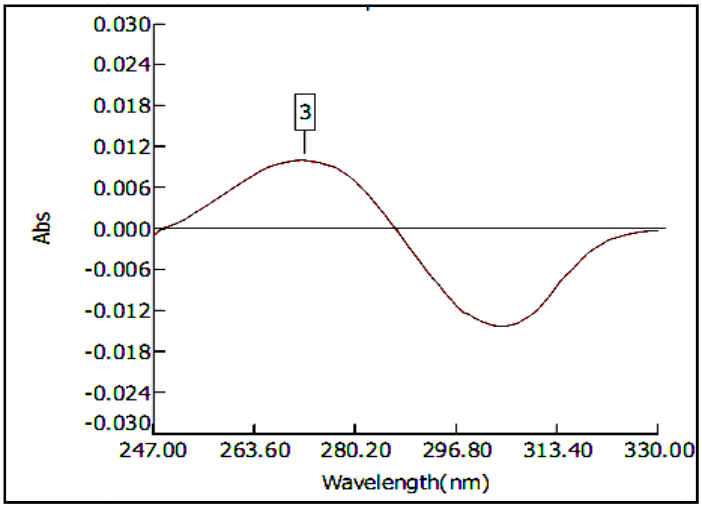

Fig. 3: First derivative spectrum of FMD (6 µg/mL)

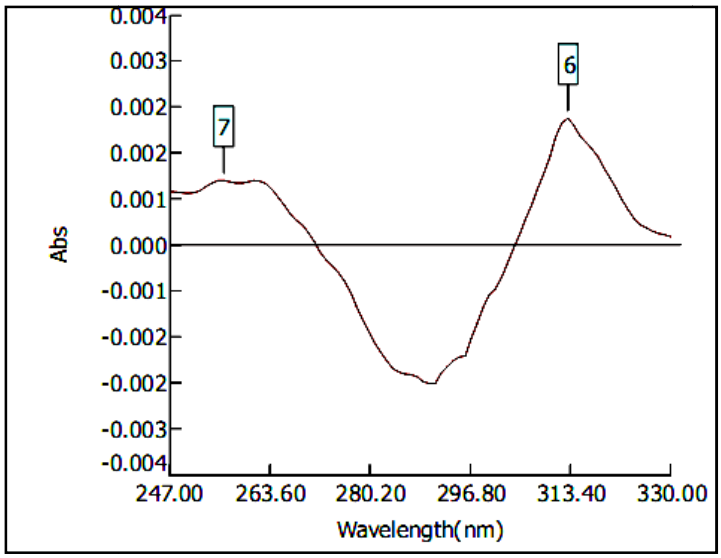

Fig: 4: UV second derivative spectrum of FMD (6µg/mL)

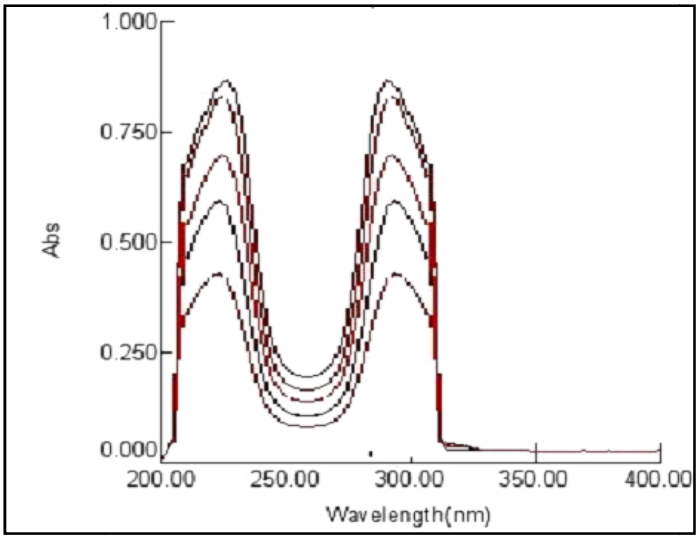

Fig. 5: Absorption spectra of FMD (4-12µg/mL)

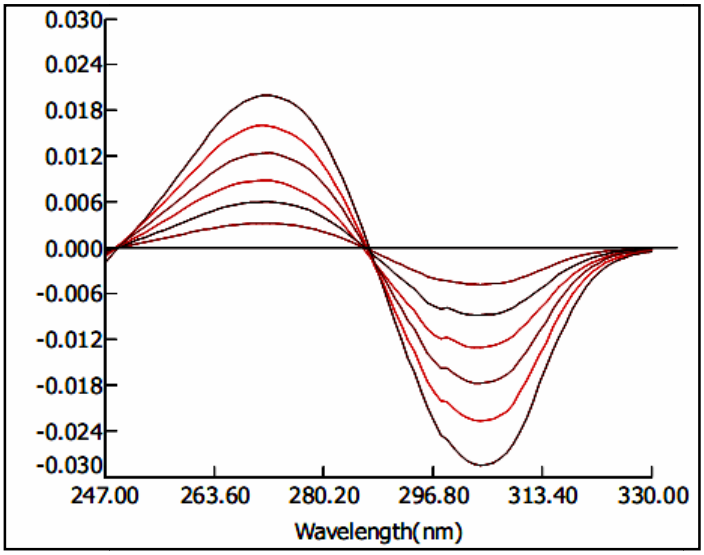

Fig. 6: First Order Derivative spectra of FMD (4-12 µg/mL)

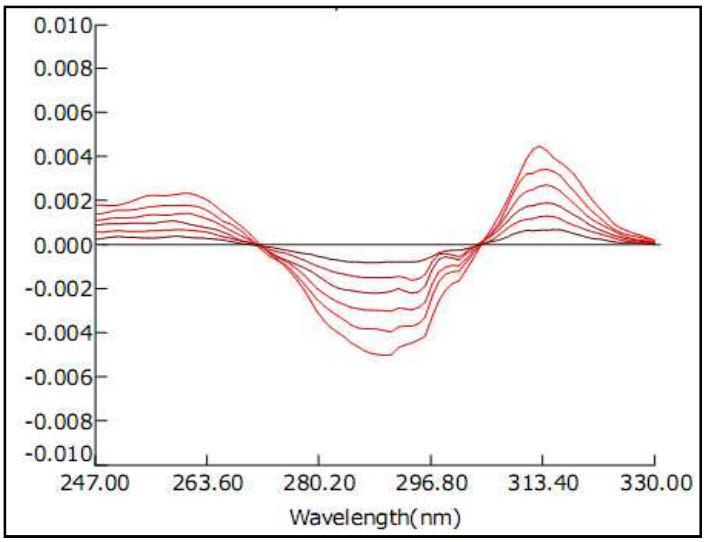

Fig. 7: Second Order Derivative spectra of FMD (4-12 µg/mL)

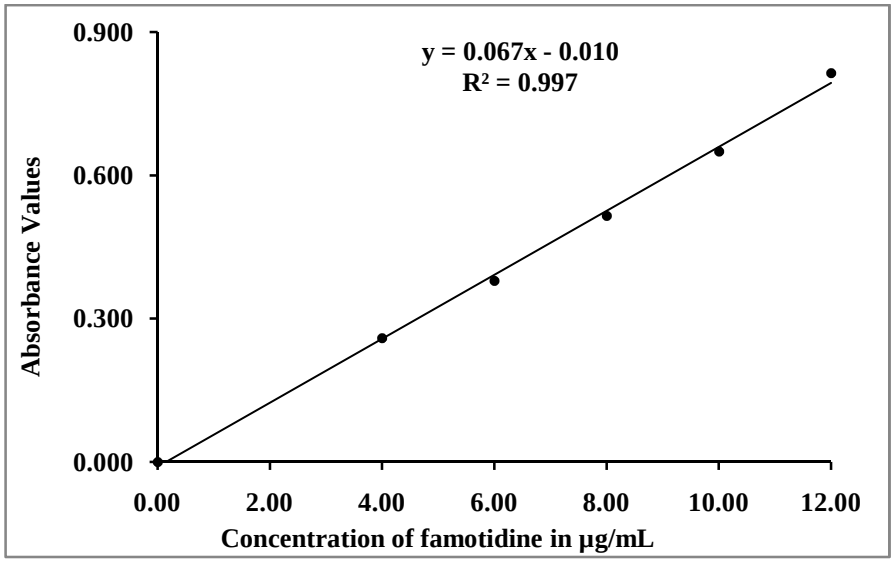

Fig. 8: Linearity plot of absorbance against concentration of FMD

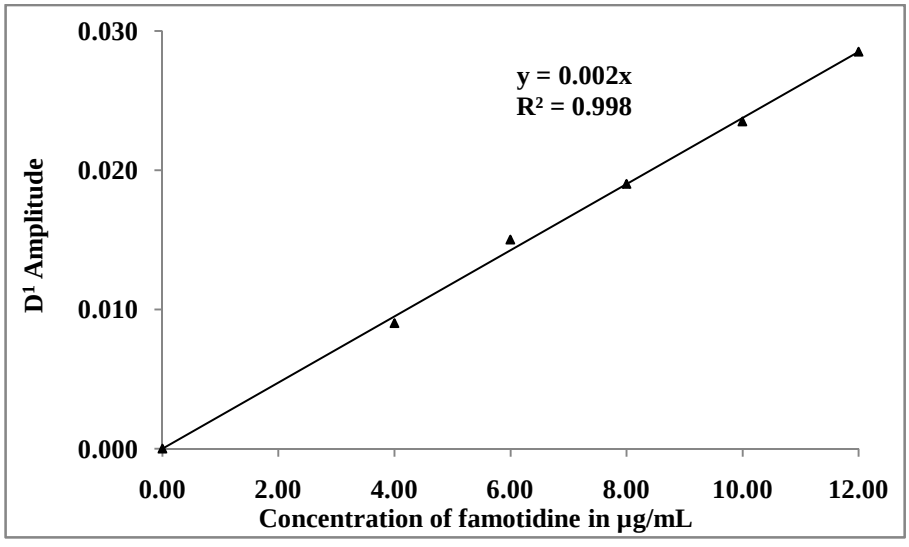

Fig. 9: Calibration plot of first derivative amplititudes against concentration of FMD

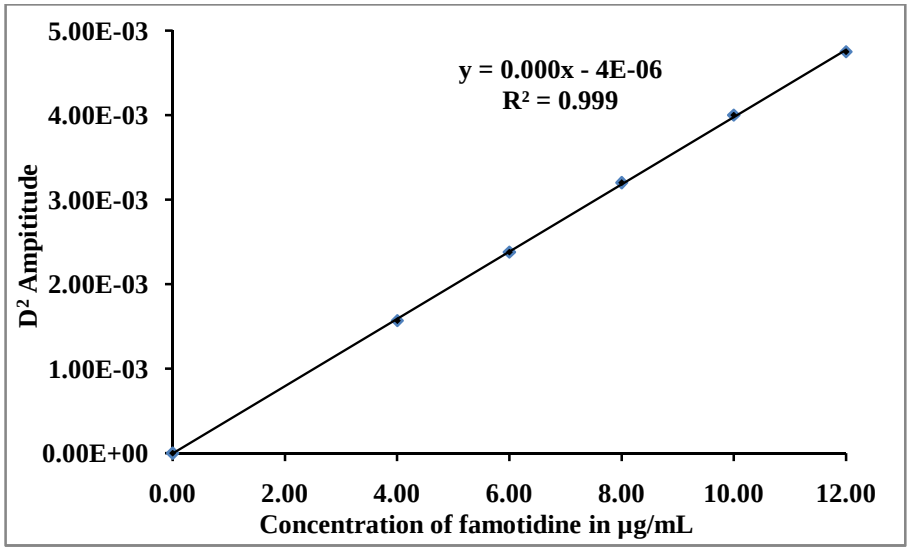

Fig. 10: A linear straight line drawn between second derivative amplititudes and concentration of FMD

CONCLUSION

The developed UV Spectrophotometric methods were effective for quantitative determination of FMD in bulk and pharmaceutical preparations without any interference of other constitute in the formulation. Tablets of different brand names were analyzed by the proposed methods and assay of the drug was calculated. The derivative spectrophotometric methods developed by the author were simple sensitive, selective, reproducible, and stable. The developed methods could be readily adapted to routine quality control of FMD by ordinary laboratories.

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REFERENCES

[1] Kesur BR, Salunkhe DRVR, Chandrakant S, Magdum DR, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 271, 4(4).
[2] Khadiga. Kelani M, Aziz AM, Hegazy MA, Laila Abdel Fattah, *Analytical letters*", **2002**, 35(6), 1055, 35(6).
[3] Nafisur Rahman, Mohammad Kashif. *Il Farmaco*, **2003**, 1045, 58(10).
[4] Kamath BV, Shivram K, Saroj Vangani , *Analytical Letters*, **1992**, 2239, 25(12).
[5] Ayad MM, Abdalla Shalaby, Abdellatef HE, Hosny MM, *Analytical and Bioanalytical Chemistry*, **2003**, 710, 376(5).
[6] Nafisur Rahman, Mohammad Kashif, *Analytical Sciences*, **2003**, 907, 19(6).
[7] Amin S, Shama SA, Ahmed IS and Gouda EA, *Analytical Letters*, **2002**, 1851, 35(11).
[8] Mohammed Ibrahim Walash, Mohie Khalid Sharaf-El-Din, Mohamed El-Sayed Metwally and Mostafa Reda Shabana, *Journal of the Chinese Chemical Society*, **2005**, 71, 52.
[9] Kelani KM, Aziz AM, Hegazy MA, Laila Abdel Fattah, *Spectroscopy Letters: An International Journal for Rapid Communication*,**2002**, 543, 35(4).
[10] Al-Ghannam, Sheikha, Belal, Fathalla, *Journal of AOAC International*, **2002**, 1003, 85(5).
[11] Kanakapura Basavaiah, Okram Zenita, *Quim. Nova*, **2011**, 735, 34(5).
[12] Sahu R, Preeti Nagar, Bhattacharya S, Deepti Jain, *Indian Journal of Pharmaceutical Sciences*, **2006**, 503, 68(4).
[13] Deep Patel P, Shah RR, Patel AP, Tank PK, *An international journal of pharmaceutical sciences*, **2012**, 2506, 4.
[14] Shah DA, Suthar DJ, Nagda CD, Chhalotiya UK, Bhatt KK, *Arabian journal of chemistry*, **2012**,1,5.
[15] Zarghi, Shafaati A, Foroutan SM, Khoddam A, *Journal of Pharmaceutical and Biomedical Analysis*,**2005**,677, 39.
[16] Helali N, Darghouth F, Monser L, *Chromatographia*, **2004**, 455, 60(7-8).
[17] Diane AI, Ashiru, Rajesh Patel, Basit AW, *Journal of Chromatography B*, **2007**, 235860(2).
[18] Abdel-Moety EM, Khattab FI, Kelani KM, AbouAl-Alamein AM, II *Farmaco*, **2003**, 931, 57(11).
[19] Nawel Helali, Lotfi Monser, *Journal of Separation Science*, **2008**, 276, 31(2).

[20] Dragica, Stafilov Trajce, *Journal of the Serbian Chemical Society*, **2003**, 883, 68(11).
[21] Eva Anzenbacherova, Karolina Filipova, Milan Nobilis, Pavel Anzenbacher, *Journal of Separation Science*, **2003**, 722, 26(8).
[22] Khatal Laxman D, Kamble Asmita Y, Mahadik Mahadeo V, Dhaneshwar R, Sunil, *Journal of AOAC International*, **2010**, 765, 93(3).
[23] Paraskevas D, Tzanavaras, *Central European Journal of Chemistry*, **2011**, 446, 9(3).
[24] Pawar SM, Patil BS, Patil RY, *International Journal od Advances in Pharmaceutical Science*, **2010**, 54, 1(1).
[25] Yarram Ramakoti Reddy, Kakumani Kishore Kumar, Reddy MRP, K Mukkanti K," *Pharmaceutical Methods*, **2012**, 57, 3(2).
[26] Dipali D, Tajane, Sacchidanand R. et al., *Der Pharmacia Sinica*, **2011**, 60, 2 (3).
[27] Rajani Sekhar V, Padmanabha Reddy Y, Ramalingam P, Harihara Theja D, , *Der Pharmacia Sinica*, **2013**, 160, 4(2).
[28] Trinath. M, Saurabh K. Banerjee, Hari Hara Teja. Dand C. G. Bonde , *Der Pharmacia Sinica*, **2010**, 1 (1): 36
[29] Vivekkumar K. Redasani, Amit R. Kothawade, Bhushan J. Mali, Sanjay J. Surana, *Der Chemica Sinica*, **2011**, 298, 2 (4).