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RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed Formulation: Development and Validation

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARS designed the study, performed the statistical analysis and wrote the protocol. Authors PDM and MSD performed the analyses of the study and managed the literature searches. All authors read the final manuscript. Author VJK approved the final draft of manuscript.

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ABSTRACT

The reversed-phase high performance liquid chromatography (RP-HPLC) and high performance thin layer chromatography (HPTLC) methods for simultaneous estimation of Metfomin Hydrochloride (MET) and Vildagliptin (VLD) in bulk and their marketed

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combined dosage form were developed.
For RP-HPLC, separation was carried out using HiQsil C18HS (4.6mmø×250mm) analytical column and detection was carried out using variable wavelength detector. The mobile phase was composed of phosphate buffer (pH adjusted to 6 using 3M KOH): methanol: acetonitrile in the ratio of 50:30:20 v/v/v. Flow rate was kept at 0.8ml/min. The drugs- MET and VLD were retained at 3.7 minutes and 4.8 minutes respectively.
The HPTLC method was developed using Camag HPTLC system. Silica Gel 60GF254 precoated TLC plates were used as stationary phase. The mobile phase was ammonium acetate in methanol (1% w/v): Toluene; (10:0.5). The detection of spots was carried out densitometrically at 214 nm in absorbance mode. The R_f values for MET and VLD were found to be 0.44 and 0.55 respectively.
Performance characteristics of both of these RP-HPLC and HPTLC methods for simultaneous estimation of MET and VLD in bulk and their marketed combined dosage form were statistically validated as per the recommendations of ICH guidelines of analytical method validation.
The RP-HPLC method was found to be linear across concentration range of 10-60µg/mL for MET and VLD respectively. For RP-HPLC the LOD values for MET and VLD were 1.09µg/ml and 1.70µg/ml respectively and LOQ values for MET and VLD were 3.32µg/ml and 5.15µg/ml respectively.
The HPTLC method was found to be linear with across the range 1000-5000ng/spot and 500-2000ng/spot for MET and VLD respectively. For HPTLC the LOD values for MET and VLD were 17.22ng/spot and 34.60ng/spot respectively and LOQ values for MET and VLD were 52.20ng/spot and 104.85ng/spot respectively.
Both of these RP-HPLC and HPTLC methods were found to be simple, specific, linear, accurate, precise and robust, hence any of these methods can be conveniently adopted for routine analysis of the formulations containing MET and VLD, for their simultaneous estimation.

Keywords: RP-HPLC; HPTLC; metformin hydrochloride (MET); vildagliptin (VLD); marketed dosage form; validation.

1. INTRODUCTION

Metformin Hydrochloride (MET) chemically known as Imidodicarbinimidic (N, N-dimethyl-monohydrochloride) is a biguanide antihyperglycemic agent widely used for management for type II diabetes [1]. For chemical structure refer Fig. 1.

Vildagliptin (VLD) is an oral anti-hyperglycemic drug which is highly selective dipeptidyl peptidase-4(DPP-4) inhibitor. For chemical structure refer Fig. 2. VLD prolongs the action of hormone incretin that stimulates postprandial insulin secretion via direct action on pancreatic β-cells and suppress glucagon secretion by the α-cells [1]. For management for type II diabetes, patients are prescribed with the tablets each containing MET (500mg) and VLD (50mg).

Literature survey revealed that few methods are reported for simultaneous estimation of MET and VLD such as UV-visible spectroscopic method [2], RP-HPLC method [3], UPLC-MS/MS [4] but high performance thin layer chromatography (HPTLC) was not found to be reported. HPTLC becoming popular due to its advantages of low operating cost, high sample throughput and minimal sample preparation. Although, RP-HPLC method for simultaneous

estimation of MET and VLD has been reported [5], the proposed experimental work was aimed to develop and validate more economical, sensitive RP-HPLC as well as HPTLC methods for simultaneous estimation of MET and VLD from bulk and formulation.

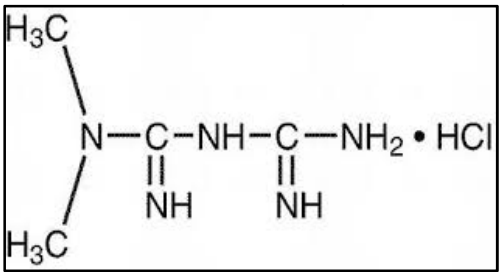

Fig. 1. Chemical structure of metformin hydrochloride

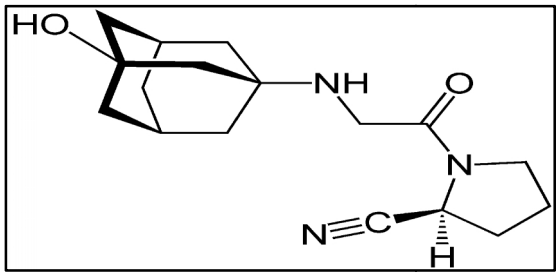

Fig. 2. Chemical structure of vildagliptin

2. MATERIALS AND REAGENTS

Working standards of pharmaceutical grade MET, VLD were obtained as generous gifts from Glenmark generics, Mumbai. Methanol, acetonitrile were purchased from S.D. Fine Chemicals, Mumbai. Instrument details and specifications for both HPLC and HPTLC methods are given in Table 1.

2.1 Instrument

Table 1. Instruments and specifications

Instrument	HPLC	HPTLC
Make and model	Agilent 1200 Series HPLC system, Agilent , U.S.	Camag, switzerland
Specification	Quaternary Gradient	TLC scanner 5
Sampling mode	Autosampler	Manual with Linomat applicator
Detection	Variable wavelength detector	UV
Software	EZChrom	winCATS(ver.1.4.1)

3. EXPERIMENTAL [6-18]

3.1 Analytical Method Development (AMD): (Refer Table 2)

Table 2. Experimental procedures of HPLC and HPTLC method development

Sr. no.	System/ method	RP-HPLC	HPTLC
3.1.1	Preparation of Standard and working solution	100 mg VLD and MET each were accurately weighed and transferred into 100 ml volumetric flask separately and volume was made upto 100 ml with distilled water. Working solution was prepared from	Standard stock solutions of VLD and MET were prepared separately by dissolving 100 mg of drug in 100 ml methanol to obtain concentration 1000µg/ml (1000ppm).

Sr. no.	System/ method	RP-HPLC	HPTLC
		standard solution. 1ml from each of stock solutions were pipetted out and transferred to 10ml volumetric flask and volume was made upto the mark with mobile phase.	
3.1.2	Preparation of Sample Solution for simultaneous estimation from marketed tablet formulation	Twenty tablets were accurately weighed and crushed into a fine powder. The weight of powder equivalent to 500 mg of MET and 50 mg of VLD was transferred into 100 ml volumetric flask. To this solution distilled water was added, mixture was sonicated to dissolve the drug and then volume was made up to the mark with distilled water. The solution was filtered through 0.45 µm filter paper and filtrate was appropriately diluted to get desired concentration of MET (500 µg/ml) and VLD (50 µg/ml).	Twenty tablets were accurately weighed and crushed into a fine powder. The weight of powder equivalent to 500 mg of MET and 50 mg of VLD was transferred into 100 ml volumetric flask. To this solution methanol was added , mixture was sonicated to dissolve the drug and then volume was made up to the mark with methanol. The solution was filtered through 0.45 µm filter paper and filtrate was appropriately diluted to get desired concentration of MET (500 µg/ml) and VLD (50 µg/ml).
3.1.3	Selection of detection wavelength	UV absorption spectra for 10 ppm solution of each MET, VLD individually and their mixture were generated by scanning over range of 200-400 nm using UV Visible spectrophotometer	10 ppm solution of each MET, VLD individually and their mixture were scanned densitometrically over the range of 200-400 nm using TLC scanner of Camag HPTLC.
3.1.4	Optimisation of chromatographic conditions	Many preliminary trials were carried out for selection and optimisation of: • Stationary phase • Mobile phase • Flow rate • Injection volume • Column temperature	Many preliminary trials were carried out for selection and optimisation of : • Mobile phase • Sample application rate • Injection volume • Saturation time • Band length

3.2 Analytical Method Validation

Performance characteristics of analytical HPLC and HPTLC methods were statistically validated as per the recommendations of ICH guidelines for analytical method validation [6]. Validation parameters and procedures followed for their determination are tabulated in Table 3.

Table 3. Analytical method validation: parameters and their determination

<table>
<tr><th>Parameter</th><th>Method/procedure followed</th></tr>
<tr><td>Specificity</td><td>As per ICH, Specificity should be carried out to ensure identity of an analyte. To determine specificity chromatograms/ densitograms were obtained for blank, MET, VLD individually and their mixture.</td></tr>
<tr><td>Accuracy</td><td>Accuracy was established across the specified range of analytical procedure by adding known added quantities of analyte to the synthetic mixture of drug product components and to the combined dosage form. As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentrations levels in triplicate. (e.g., 3 concentrations/ 3 replicates each). Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of 10ppm solution of synthetic mixture of MET and VLD Recovery studies were also performed on tablets containing MET and VLD.</td></tr>
<tr><td>Precision</td><td>Precision was carried out at two levels.
Repeatability: Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/ 3 replicates each)
Intermediate Precision: Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure. Intraday and interday precision studies were performed by taking 9 determinations of 3 concentrations/3 replicates each, at 3 times in a same day and on 3 different days, respectively.
Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.</td></tr>
<tr><td>Detection limit and quantification limit</td><td>Detection limit (DL) or limit of detection (LOD) and quantification limit (QL) or limit of quantitation (LOQ) is determined based on the standard deviation of the response and the slope of calibration curve.
DL (LOD): $LOD = \frac{3.3\,\sigma}{S}$ QL (LOQ): $LOQ = \frac{10\,\sigma}{S}$
σ = Standard deviation of response estimated based on the calibration curve.
S = Slope of the calibration curve.</td></tr>
<tr><td>Linearity</td><td>A linear relationship was evaluated across the range of 10 to 60 mg for both drugs namely MET and VLD.
As per ICH, for the establishment of linearity, a minimum of 5 concentrations are recommended.
Linearity is reported by the value of the correlation coefficient, y-intercept, and slope of the regression line along with a plot of the data.</td></tr>
<tr><td>Robustness</td><td>Robustness was evaluated for proving the reliability of an analytical method with respect to deliberate variations in method parameters.
To establish robustness of analytical method following factors were studied.
HPLC:
• Stationary phase
• Mobile phase
• Flow rate
• Injection volume
• Column temperature
HPTLC:
• Mobile phase
• Sample application rate
• Injection volume
• Saturation time
• Band length</td></tr>
</table>

4. RESULTS AND DISCUSSION

Results of experimental work of RP-HPLC method and HPTLC method are discussed in two separate sections 4.1 and 4.2 respectively.

4.1 RP-HPLC Method Development and Validation

4.1.1 RP- HPLC method development

4.1.1.1 Selection of wavelength

UV absorption spectra for 10 ppm solution of each MET, VLD individually and their mixture were overlaid and 220 nm was selected as a detection wavelength for simultaneous chromatographic determination of MET and VLD.

4.1.1.2 Optimization of chromatographic conditions

According to the literature survey for RP-HPLC, it was observed that both the drugs MET and VLD were well retained over C18 column respectively. Thus, in order to get optimum resolution simultaneously C18 column was selected. Many preliminary trials were carried out for selection of mobile phase, some are tabulated in Table 4.

Table 4. Optimization trials for mobile phase composition

Mobile phase components	Compositions
Methanol: water	(60:40),(80:20)
ACN: water	(50:50),(70:30)
100mM ammonium acetate buffer(pH 5):ACN	(50:50)
20mM ammonium acetate buffer(pH 5.5):ACN	(30:70)
20 mM phosphate Buffer (pH 4):Methanol :ACN	(50:40:10)

Different flow rate in the range of 0.5 to 1.5 ml/min and different injection volumes in the range of 20 µl to 100 µl were tried. Optimized mobile phase selected was composed of 50 mM Phosphate Buffer (pH 6): Methanol: Acetonitrile (50:30:20).

Optimized chromatographic conditions are tabulated in Table 5.

Table 5. Optimized chromatographic conditions

Stationary phase	BDS HYPERSIL C18 (4.6mmΦ×250mm)
Mobile phase	50 mM phosphate buffer (pH 6): Methanol: Acetonitrile (50:30:20)
Flow rate	0.8ml/min
Detection wavelength	220nm
Injection volume	50 µl

Chromatogram obtained using these optimised chromatographic conditions both drugs- MET and VLD were well resolved and retained at 3.7 minutes and 4.8minutes respectively, representative chromatogram is shown in Fig. 3.

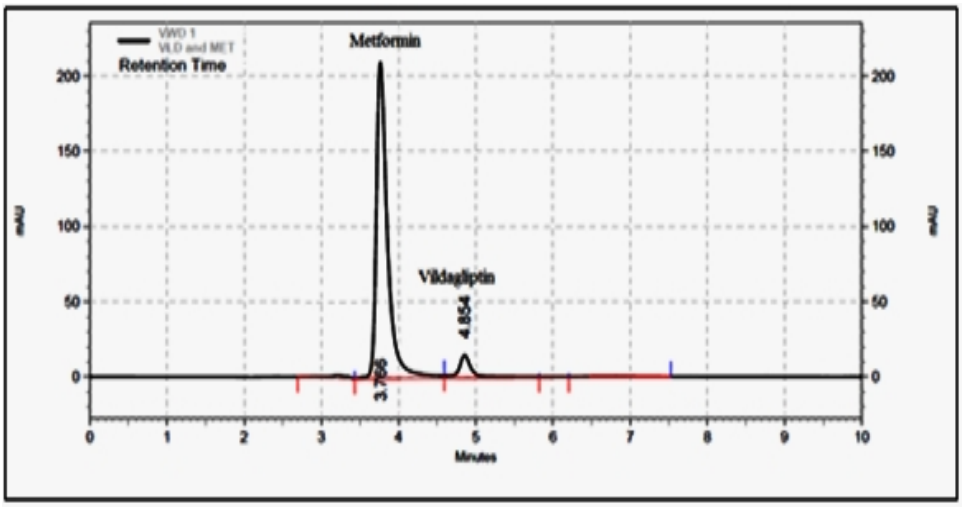

Fig. 3. Representative chromatogram of MET and VLD

4.1.2 RP- HPLC method validation

4.1.2.1 Specificity

Separate chromatograms were obtained for blank, MET, VLD individually and their mixture. The overlay of chromatograms of blank, MET, VLD individually and their mixture is shown in Fig. 4. The overlaid chromatogram indicated no interfering peak or baseline noise at the respective retention times of MET and VLD. Thus, it ensures the identity of both analytes under study and hence proves the specificity of a method.

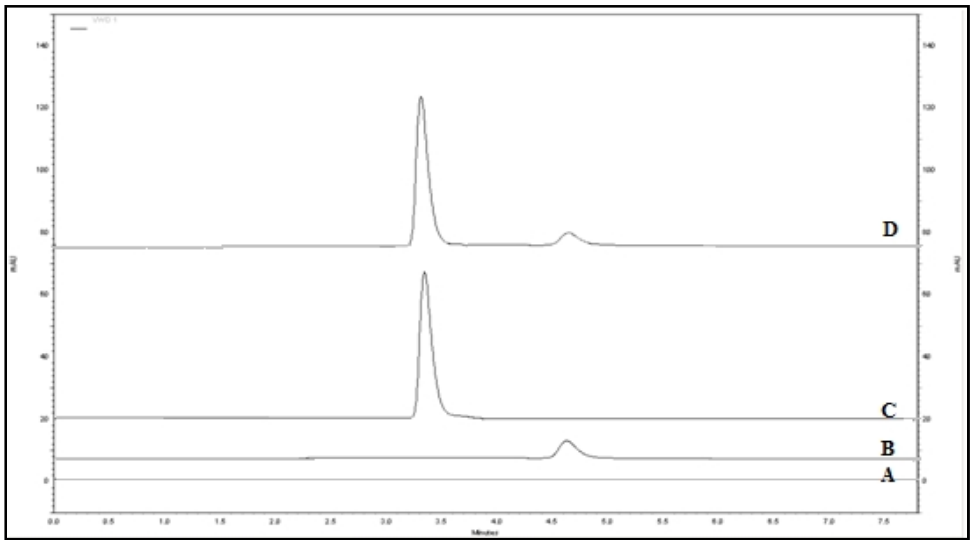

Fig. 4. An overlay of chromatograms of blank, MET, VLD individually and their mixture
[A: Blank run, B: VLD, C: MET, D: Mixture of MET and VLD.]

4.1.2.2 Linearity

Six serial dilutions of MET and VLD were prepared using a standard stock solutions. Responses were recorded as peak area. The peak areas were plotted against concentrations to obtain the calibration curve. The RP-HPLC method was found to be linear across concentration range of 10-60µg/mL for MET and VLD respectively. The values of correlation coefficient, y intercept and slope of regression line are shown in Table 6.

Table 6. Values for linearity

Parameter	RP-HPLC	
	MET	**VLD**
Linearity range	10-60µg/ml	10-60µg/ml
R^2	0.9947	0.9973
y-intercept	361238	68992
Slope	211781	14904

4.1.2.3 Limit of detection and limit of quantitation

Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slope of regression line. The calculated values of LOD and LOQ for MET and VLD are shown in Table 7.

Table 7. LOD and LOQ

Parameter	RP-HPLC	
	MET	**VLD**
LOD	1.09 µg/ml	1.70 µg/ml
LOQ	3.32 µg/ml	5.15 µg/ml

4.1.2.4 Accuracy

Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The recovery studies on bulk drugs were performed in triplicates of three concentration levels viz. 80%, 100%, 120% of 10 ppm solution of synthetic mixture of MET and VLD. The percent recovery was calculated from the data obtained, the results are tabulated in Table 8.

Table 8. Accuracy: recovery studies on bulk drugs for RP-HPLC

	Observations					Inference
Drug	**% level**	**Concentration before spiking (µg/ml)**	**Total concentration after spiking (µg/ml)**	**Amount recovered (µg/ml)**	**% recovery**	
MET	80	10	18	17.69	98.30%	Acceptable recovery hence accurate
	100	10	20	19.86	99.30%	
	120	10	22	22.24	101.15%	
VLD	80	10	18	17.22	95.70%	
	100	10	20	19.84	99.20%	
	120	10	22	22.28	101.30%	

Recovery studies were also performed on tablets containing MET and VLD. The marketed tablets of MET and VLD were triturated and sample solution was prepared which yield a concentration of MET (500 µg/ml) and VLD (50 µg/ml). To this solution known amount of MET and VLD were added at three concentration levels viz. 80%, 100%, 120%. Then these samples were diluted with mobile phase with a dilution factor of 20 and injected for HPLC analysis. % Recovery values for both analytes-MET and VLD were back calculated from response obtained for dilute solution. Results are tabulated in Table 9.

The method was found to be accurate for simultaneous estimation of MET and VLD from bulk and tablet formulation with acceptable % recovery known added amount of analyte in sample.

4.1.2.5 Precision

The results of intraday and interday precision studies are tabulated in Tables 10 and 11 respectively. Percent RSD values for both intraday and interday precision were found within acceptable limit.

4.1.2.6 Robustness

To determine robustness of analytical HPLC method changes observed in retention time and response were recorded. Method was found to be reliable and robust as method performance (retention time and response) is not much affected by deliberate variations in mobile phase composition and its pH, column temperature and flow rate. The results obtained are tabulated in Table 12.

4.2 HPTLC Method Development and Validation

4.2.1 HPTLC method development

4.2.1.1 Selection of wavelength

UV absorption spectra for 10 ppm solution of each MET, VLD individually and their mixture were overlaid Fig. 5 and 214 nm (an Isobestic wavelength) was selected as a detection wavelength for simultaneous chromatographic determination of MET and VLD.

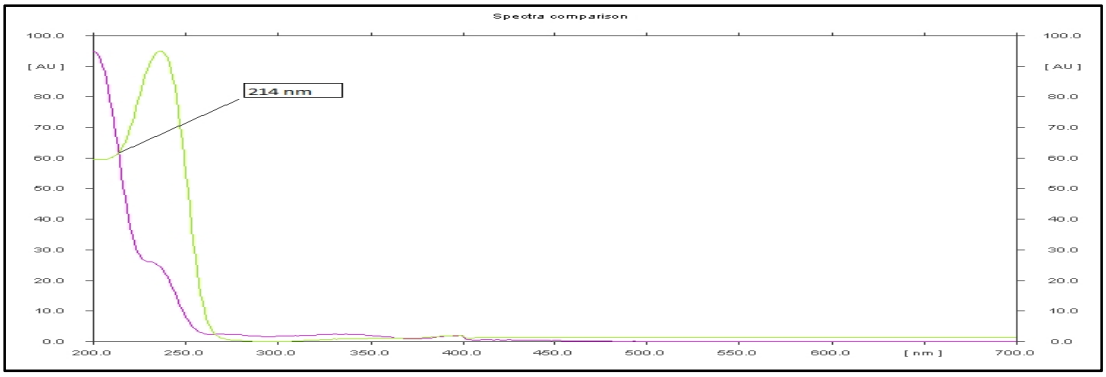

Fig. 5. An overlay of UV spectra of MET and VLD

Table 9. Accuracy: recovery studies for tablet formulation

Drug	% level	Observations and results					Inference
		Concentration before spiking (µg/ml)	Total concentration after spiking (µg/ml)	Concentration injected after dilution with mobile phase (µg/ml)	Amount recovered (µg/ml)	% recovery analyte	
MET	80	500	900	45	44.24	98.33%	Acceptable % recovery, hence accurate.
	100	500	1000	50	51.10	102.21%	
	120	500	1100	55	53.58	97.43%	
VLD	80	50	90	4.5	4.51	100.32%	
	100	50	100	5	4.96	99.32%	
	120	50	110	5.5	5.57	101.44%	

4.2.1.2 Optimization of chromatographic conditions

According to the literature survey for HPTLC, it was observed that both the drugs MET and VLD were well retained over Silica Gel 60GF_{254} precoated TLC plates. Many preliminary trials were carried out for selection of mobile phase, some are tabulated in Table 13.

Different application volumes in the range 0.1-100 µl were tried with variable saturation time. Optimized mobile phase selected was ammonium acetate in methanol (1% w/v): Toluene (10:0.5).

The plates were prewashed with methanol and activated at 60°C for 20 minutes prior to use. Optimized chromatographic conditions are tabulated in Table 14.

Densitogram obtained using these optimised chromatographic conditions both drugs- MET and VLD gave highest resolution, minimum tailing and Rf values 0.44 and 0.55 respectively.

Table 10. Intraday precision studies

Level	Observations						Inference
	MET			VLD			
	Low	Mid	High	Low	Mid	High	
Concentration (µg/ml)	20	40	50	20	40	50	Acceptable % RSD, hence precise
%RSD	1.13	0.47	0.55	1.79	1.33	0.43	

Table 11. Interday precision studies

Level	Observations						Inference
	MET			VLD			
	Low	Mid	High	Low	Mid	High	
Concentration (µg/ml)	20	40	50	20	40	50	Acceptable % RSD, hence precise
%RSD	0.64	0.38	0.35	1.85	0.72	0.31	

Table 12. Robustness:effect on retention time and response by variation in mobile phase composition and its pH, column temperature and flow rate

Method parameters and variations	Level of variations	MET		VLD	
		%RSD	Change in retention time (Minutes)	%RSD	Change in retention time (minutes)
Proportion of organic phase in mobile phase 50:30 (±2):20	-2	0.4785	0.0254	0.8547	0.1478
	+2	0.8457	0.0478	0.9874	0.2547
Flow rate (0.8±0.2)	-0.2	0.5247	0.2584	0.3987	0.4578
	+0.2	0.8947	0.4580	0.4751	0.0458
Column Temperature 29º±5º	-5º	1.1478	2.5478	2.1487	0.8457
	+5º	0.9874	1.1475	1.5874	0.4751
pH	-2	0.2354	0.0547	0.5442	0.0214
	+2	0.4178	0.0235	0.2478	0.1487

Table 13. Optimized chromatographic conditions

Mobile phase components	Compositions
Ethyl acetate: toluene: methanol	(7:2:2)
Butanol: GAA: water	(6:2:2)
Acetone: methanol: toluene: glacial acetic acid	(4:3:2:1)
Ammonium acetate	(1%w/v)
Ammonium acetate: toluene	(1%w/v:1)

Table 14. Optimized chromatographic conditions

Mobile phase	Ammonium acetate in methanol (1% w/v): toluene (10:0.5)
Sample application volume	10µl
Detection wavelength	214nm
Saturation time	30 minutes

4.2.2 HPTLC method validation

4.2.2.1 Specificity

Separate densitograms were obtained for blank, MET, VLD individually and their mixture to ensure the identity of both analytes under study namely MET and VLD. The labelled overlay densitogram of blank, MET, VLD individually and their mixture is shown in Fig. 6.

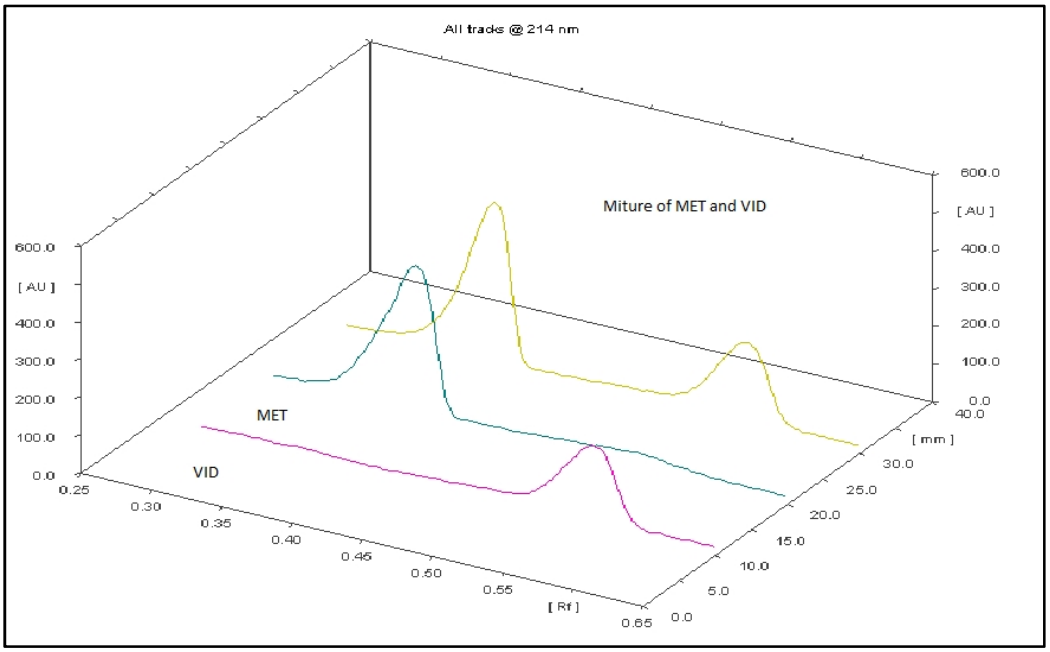

Fig. 6. An overlay of densitograms of MET, VLD individually and their mixture

4.2.2.2 Linearity

Six serial dilutions of MET and VLD were prepared using a standard stock solutions. Responses were recorded as peak area. The peak areas were plotted against

concentrations to obtain the calibration curve. The values of correlation coefficient, y intercept and slope of regression line are shown in Table 15.

4.2.2.3 Limit of detection and limit of quantitation

Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slope of regression line. The calculated values of LOD and LOQ for MET and VLD are shown in Table 16.

4.2.2.4 Accuracy

The accuracy of the method was determined by calculating recoveries of MET and VLD by the standard addition method. The analyzed samples were spiked with extra concentration levels 80%, 100%, 120% of 10 ppm solutions and the mixtures were reanalyzed by the proposed method. Recovery analyses were repeated three times for each level of all samples. Results are tabulated in Table 17.

Table 15. Values for linearity

Parameter	HPTLC	
	MET	**VLD**
Range	1000-5000 ng/spot	500-2000 ng/spot
R^2	0.999	0.991
y-intercept	633.7	866.4
Slope	80.67	54.46

Table 16. LOD and LOQ

Parameter	HPTLC	
	MET	**VLD**
LOD	17.22 ng/spot	34.60 ng/spot
LOQ	52.20 ng/spot	104.85ng/spot

Table 17. Accuracy:recovery studies on bulk drugs for HPTLC

Drug	Observations					Inference
	% Level	**Concentration before spiking (ng/spot)**	**Total concentration after spiking (ng/spot)**	**Amount recovered (ng/spot)**	**% recovery**	
MET	80	100	180	172.0	95.5%	Acceptable recovery hence accurate
	100	100	200	197.8	98.9%	
	120	100	220	219.0	99.5%	
VLD	80	100	180	169.5	94.1%	
	100	100	200	194.4	97.2%	
	120	100	220	220.58	100.2%	

To check the recovery of the drug at different levels in the formulations by optimized method, the marketed tablets containing MET (500mg) and VLD (50mg) were triturated, equivalent amount of powder blend was weighed and sample solution was prepared to yield a concentration of MET (500 µg/ml) and VLD (50 µg/ml). To this solution known amount of MET and VLD were added at three concentration levels viz. 80%, 100%, 120%. The results of recovery studies for tablet formulation are tabulated in Table 18.

Table 18. Accuracy:recovery studies for tablet formulation

Drug	Amount of drug in formulation (mg/tablet)	Concentration of drug solution (µg/ml)	Amount of standard Added (%)	Concentration of drug solution after spiking (µg/ml)	Total amount of drug taken (ng/spot)	Total amount of drug found (ng/spot)	Total percentage of drug found (%)
MET	500	500	80	900	9000	8845.2	98.28
			100	1000	10000	10334.0	103.34
			120	1100	11000	10750.3	97.73
VLD	50	50	80	90	900	897.39	99.71
			100	100	1000	1027.8	102.78
			120	110	1100	1094.39	99.49

4.2.2.5 Precision

Intraday and interday precision studies were performed by taking 9 determinations of 3 concentration levels (low, mid, high) /3 replicates each, at 3 times in a same day and on 3 different days, respectively. The results of intraday and interday precision studies are tabulated in Tables 19 and 20 respectively. Percent RSD values for both intraday and interday precision were found within acceptable limit.

4.2.2.6 Robustness

To determine robustness of analytical HPTLC method changes observed in the concentration of the mobile phase and saturation time. Effect of this change on both the Rf values and peak areas were evaluated by calculating the relative standard deviations (%RSD) for each. The results obtained are tabulated in Table 21.

Table 19. Intraday precision studies

Level	Observations						Inference
	MET			VLD			
	Low	Mid	High	Low	Mid	High	
Concentration applied (ng/spot)	2000	4000	5000	500	1000	2000	Acceptable % RSD, hence precise
S.D.	38.5	37.74	37.75	52.61	66.56	54.68	
%RSD	1.675	0.964	0.602	2.05	1.75	1.12	

Table 20. Interday precision studies

Level	Observations						Inference
	MET			VLD			
	Low	Mid	High	Low	Mid	High	
Concentration applied (ng/spot)	2000	4000	5000	500	1000	2000	Acceptable % RSD, hence precise
S.D.	39.84	16.44	19.86	22.05	53.58	10.71	
%RSD	1.772	0.427	0.319	0.819	1.37	0.22	

Table 21. Robustness:effect on retardation factor and response by variation in mobile phase and saturation time

Method parameters and variations	MET	VLD
	%RSD for retardation factor(R_f)	%RSD for retardation factor(R_f)
Concentration of mobile phase	0.547	0.417.
Saturation time	0.047	0.324

5. CONCLUSION

The developed RP-HPLC and HPTLC methods have been statistically validated following the recommendations of ICH guidelines and both methods were found to be specific, accurate, precise and robust. Validation studies indicated that the proposed method is suitable for the simultaneous estimation of MET and VLD in bulk and in pharmaceutical

formulation. Any of these methods can be conveniently adopted for routine analysis of the formulations containing MET and VLD.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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