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Simultaneous estimation of vildagliptin and metformin hydrochloride by using RP-HPLC in bulk and pharmaceutical dosage form

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Abstract

The estimation of Metformin hydrochloride and Vildagliptin by simultaneous method using RP-HPLC method was performed by using Chromosil ODS C₁₈ column having 250 x 4.6mm 5 μ , and mobile phase containing 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40% v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively. The all validation parameters were accepted within the limits as per ICH guidelines. The specificity was performed under stress conditions like acid, base, peroxide, light and heat and observed the degradation of drugs. It was found to be within the acceptance limit. Hence it was concluded that the developed method was found to be simple, precise, accurate and rapid method for the routine analysis of Vildagliptin and Metformin hydrochloride API and pharmaceutical dosage form in laboratories.

Keywords: metformin hydrochloride, vildagliptin, RP-HPLC method

Introduction

Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption-desorption steps on the stationary phase [1-3].

Types of HPLC techniques [4-8]

The types of chromatography based on following parameters include

Based on modes of chromatography

- Reversed phase chromatography
- Normal phase chromatograph

Based on principle of separation

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Based on elution technique

- Isocratic separation
- Gradient separation

Based on the scale of operation

- Analytical HPLC
- Preparative HPLC

High performance liquid chromatography [9-16]

High Performance Liquid Chromatography (HPLC) is one of the chromatography techniques, It is most widely used analytical method. HPLC utilizes a liquid mobile phase to separate the components of a mixture. These components (or analytes) are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure.

In the column, the mixture is resolved into its individual components. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases.

2. Materials and equipments used

Metformin, Vildagliptin, Di Potassium Hydrogen Phosphate, Methanol, Chromosil C₁₈ column, HPLC.

3. Results and Discussions

Table 1: Optimized Chromatographic conditions for galvusmet

Mobile phase	Di Potassium Hydrogen Phosphate:Methanol 60:40(v/v)
Detector	Photo Diode Array (PDA)
Pump mode	Isocratic
Ph	9.2
Diluents	Mobile phase
Column	Chromosil C ₁₈ column (250mm X 4.6 mm, 5μ)
Column Temp	35°C
Wavelength	258nm
Injection Volume	10 μl
Flow rate	0.5 ml/min
Run time	7 minutes
Retention Time	1.43min for Metformin and 5.32min for Vildagliptin

Optimized method

The optimization was performed by using the column Chromosil ODS C18 column 250 x 4.6mm 5μ, mobile phase 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40%v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively.

Table 2: System suitability parameters of Galvusmet tablet

S. No.	Parameter	Vildagliptin	Metformin
1.	Retention Time	5.337	1.433
2.	Tailing	1.181	1.721
3.	Theoretical Plates	7594	3621
4.	%RSD(n=5)	0.2	0.4
5.	Similarity Factor	0.9816	0.9796

Table 3: Assay for vildagliptin and metformin hydrochloride tablet

S. No.	Name of the drugs	Mean sample peak area n=6	Standard peak area	Labelled amount (Mg/Tab)	Amount found (Mg)	% Assay (95-105%)
1	Vildagliptin	2085678	2010811	50mg/ tablet	48.9mg	97.8
2	Metformin	2893648	2924916	500mg/ tablet	512mg	102.4

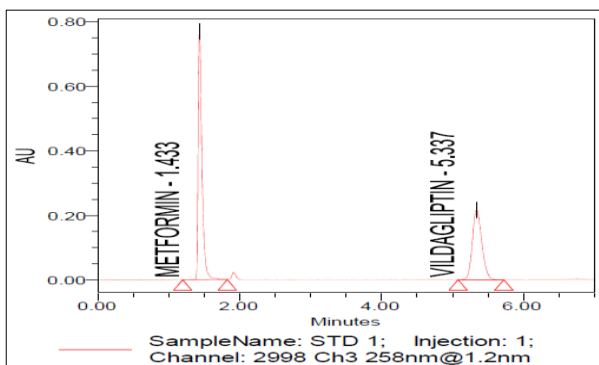


Fig 1: Typical chromatogram of mixed standard

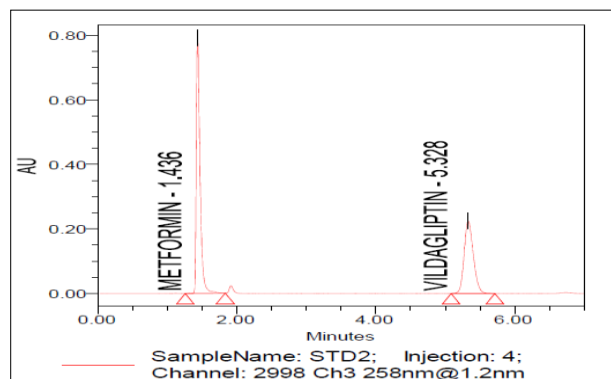


Fig 3: Typical chromatogram of standard for specificity

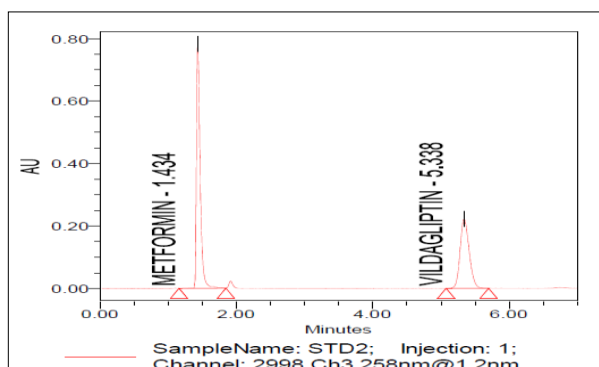


Fig 2: Typical chromatogram of sample

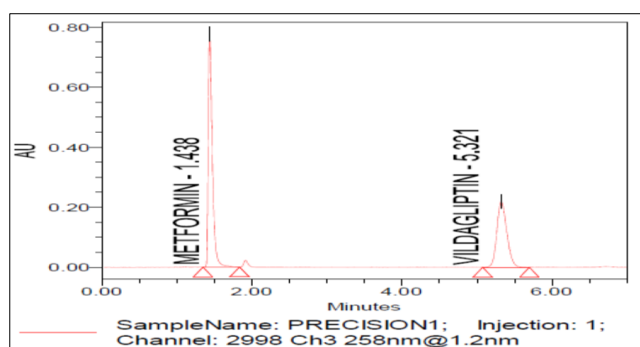


Fig 4: Typical chromatogram of Precision

Table 4: Precision of metformin

S.no.	Sample	Inj	Name	RT	Area	USP tailing	USP plate count	S/N
1	PRECISION1	1	METFORMIN	1.438	2961791	1.731	3618	1567.542
2	PRECISION2	1	METFORMIN	1.440	2995569	1.728	3576	1814.325
3	PRECISION3	1	METFORMIN	1.439	2995425	1.739	3587	1685.032
4	PRECISION4	1	METFORMIN	1.441	2999213	1.730	3588	1657.370
5	PRECISION5	1	METFORMIN	1.441	2972181	1.694	3579	1953.594
6	PRECISION6	1	METFORMIN	1.441	2989081	1.705	3615	1472.850
Mean				1.44	2985543	1.721	3593	1691.785
Std. Dev					15102			
%RSD					0.5			

Table 5: Precision of Vildagliptin

S. No.	Sample	Inj	Name	RT	Area	USP resolution	USP tailing	USP plate count	S/N
1	Precision1	1	Vildagliptin	5.321	2014022	22.451	1.189	7718	442.282
2	Precision2	1	Vildagliptin	5.321	2032453	22.405	1.186	7649	510.228
3	Precision3	1	Vildagliptin	5.314	2026948	22.280	1.193	7522	471.588
4	Precision4	1	Vildagliptin	5.313	2034497	22.391	1.185	7650	464.966
5	Precision5	1	Vildagliptin	5.312	2059452	22.460	1.187	7821	556.683
6	Precision6	1	Vildagliptin	5.307	2053236	22.534	1.191	7837	419.727
Mean				5.314	2036768	22.420	1.188	7699	477.579
Std. Dev					16872				
%RSD					0.8				

Table 6: Assay of precision

S. No.	Sample weight	Sample area of vildagliptin	Sample area of metformin	%Assay of vildagliptin	%Assay of metformin
1	1246	2014022	2961791	98	99
2	1246	2032453	2995569	99	100
3	1246	2026948	2995425	99	100
4	1246	2034497	2999213	99	100
5	1246	2059452	2972181	100	99
6	1246	2053236	2989081	100	100
Std. deviation		16872	15102	0.82	0.51
% RSD		0.8	0.5	0.83	0.51

Table 7: Data of Intraday precision

S. No.	Sample Weight	Vildagliptin Area	Metformin Area	%Assay of Vildagliptin	%Assay of Metformin
1	1246	2094158	2953216	102	99
2	1246	2034087	2997291	99	100
3	1246	2024507	2971913	99	99
4	1246	2026048	2973758	99	100
5	1246	2026917	2986560	99	100
6	1246	2020185	2983724	99	100
Mean		2037650	2977744	99	100
Std.Dev		28047	15146	1.37	0.51
%RSD		1.4	1.1	1.36	0.51

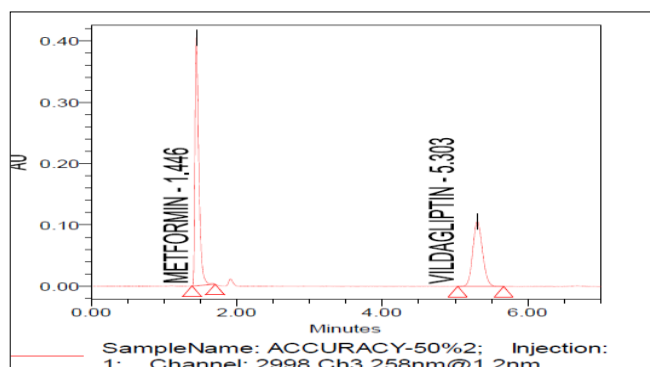


Fig 5: Typical chromatogram of accuracy 2 50%

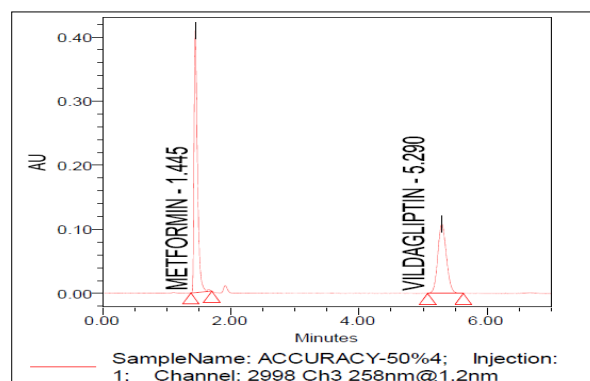


Fig 6: Typical chromatogram of accuracy 4 50%

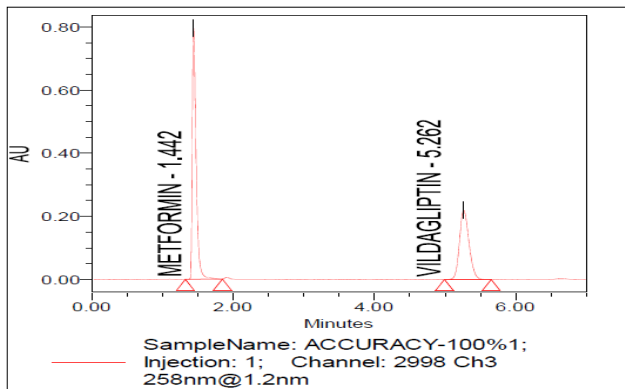


Fig 7: Typical chromatogram of accuracy 1 100%

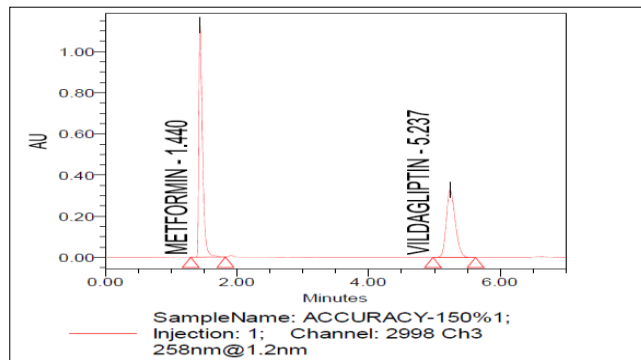


Fig 9: Typical chromatogram of accuracy 150%

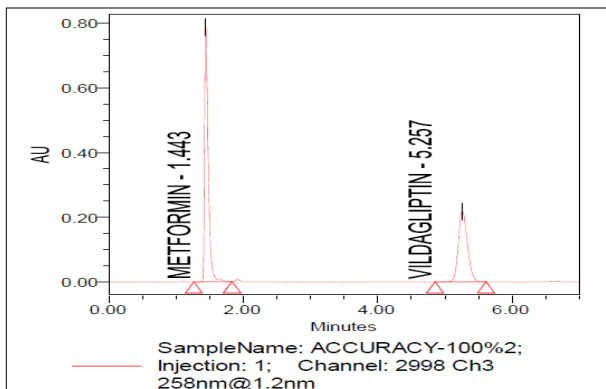


Fig 8: Typical chromatogram of ACCURACY 2 100%

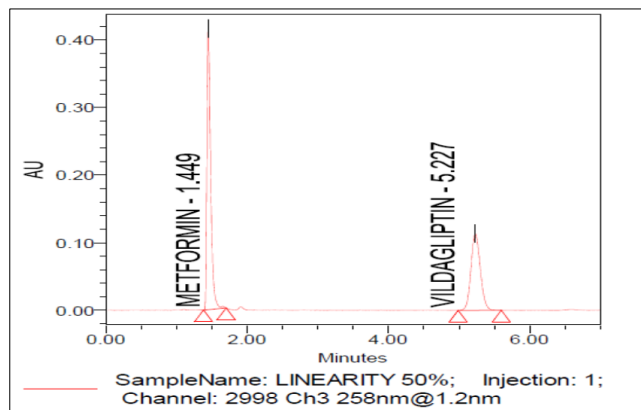


Fig 10: Typical chromatogram of linearity

Table 8: Accuracy of vildagliptin

Spiked level	Sample weight	Sample Area	µg/ml added	µg/ml found	%Recovery	%Mean
50%	621.00	997834	199.358	194.62	98	
50%	622.00	992244	199.679	193.53	97	
50%	615.00	987707	197.432	192.64	98	97
50%	619.00	996135	198.716	194.29	98	
50%	623.00	991543	200.000	193.39	97	
50%	620.00	996664	199.037	194.39	98	
100%	1246.00	2041682	400.000	398.21	100	
100%	1245.00	2034527	399.679	396.81	99	100
100%	1246.00	2064285	400.000	402.62	101	
150%	1869.00	3096685	600.000	603.97	101	
150%	1868.00	3098866	599.679	604.40	101	
150%	1869.00	3094341	600.000	603.52	101	101
150%	1867.00	3099493	599.358	604.52	101	
150%	1869.00	3073826	600.000	599.52	100	
150%	1869.00	3094182	600.000	603.49	101	

Table 9: Accuracy of metformin

Spiked level	Sample weight	Sample Area	µg/ml added	µg/ml found	%Recovery	%Mean
50%	621.00	1504436	1993.579	2014.35	101	
50%	622.00	1508720	1996.709	2020.09	101	
50%	615.00	1508932	1974.318	2020.37	102	101
50%	619.00	1500663	1987.159	2009.30	101	
50%	623.00	1503577	2000.000	2013.20	101	
50%	620.00	1503188	1990.369	2012.68	101	
100%	1246.00	2998202	4000.000	4014.42	100	
100%	1245.00	2980187	3996.709	3990.30	100	100
100%	1246.00	2989292	4000.000	4002.49	100	
150%	1869.00	4426663	6000.000	5927.04	99	
150%	1868.00	4414119	5996.790	5910.25	99	
150%	1869.00	4430292	6000.000	5931.90	99	99
150%	1867.00	4425319	5993.579	5925.24	99	
150%	1869.00	4434462	6000.000	5937.49	99	
150%	1869.00	4454596	6000.000	5966.44	99	

Table 10: Recovery of galvusmet

S. No.	Recovery	Vildagliptin	Metformin
1.	50%	97	101
2.	100%	100	100
3.	150%	101	99

Table 11: Linearity data of Metformin

S.no.	Sample	Inj	Name	RT	Area	USP Tailing	USP Plate count	S/N
1	Linearity 50%	1	Metformin	1.449	1486807	1.601	3864	769.974
2	Linearity 75%	1	Metformin	1.446	2223941	1.625	3753	1254.816
3	Linearity 100%	1	Metformin	1.445	2963845	1.671	3780	1827.286
4	Linearity 125%	1	Metformin	1.446	3709470	1.764	3508	1829.243
5	Linearity 150%	1	Metformin	1.443	4446840	1.775	3416	2319.757

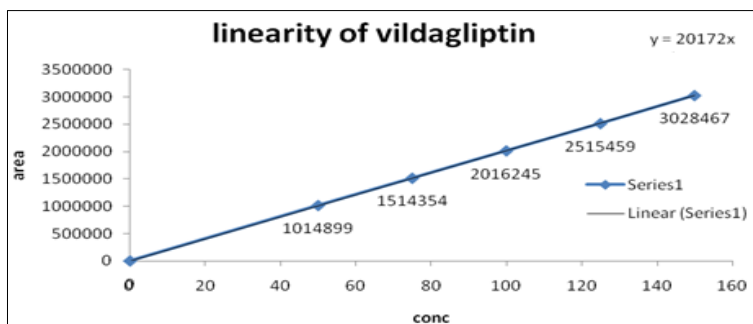


Fig 11: Linearity of Vildagliptin

Table 12: Linearity data of vildagliptin

S. No.	Sample	Inj	Name	RT	Area	USP Resolution	USP Tailing	USP Plate count	S/N
1	Linearity 50%	1	Vildagliptin	5.227	1014899	22.203	1.102	7599	209.492
2	Linearity 75%	1	Vildagliptin	5.212	1514354	22.418	1.095	7873	353.292
3	Linearity 100%	1	Vildagliptin	5.197	2016245	22.154	1.155	7643	512.435
4	Linearity 125%	1	Vildagliptin	5.192	2515459	21.390	1.188	7130	524.074
5	Linearity 150%	1	Vildagliptin	5.179	3028467	21.404	1.242	7178	683.893

Table 13: LOD&LOQ values of Galvusmet

S. No.	Parameter	Vildagliptin	Metformin
1.	LOD	0.0015	0.005
2.	LOQ	0.0043	0.014

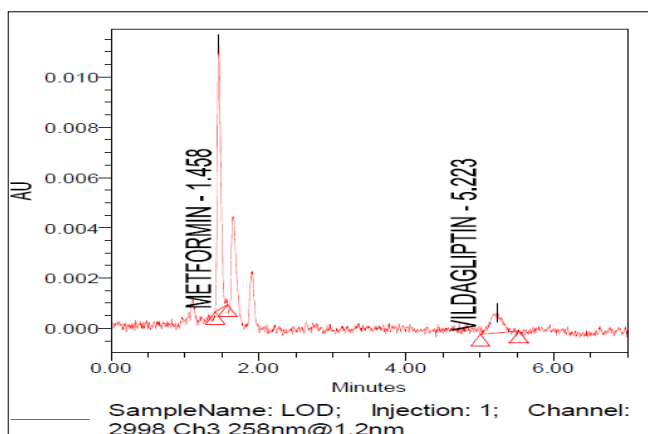


Fig 12: Typical Chromatogram of LOD

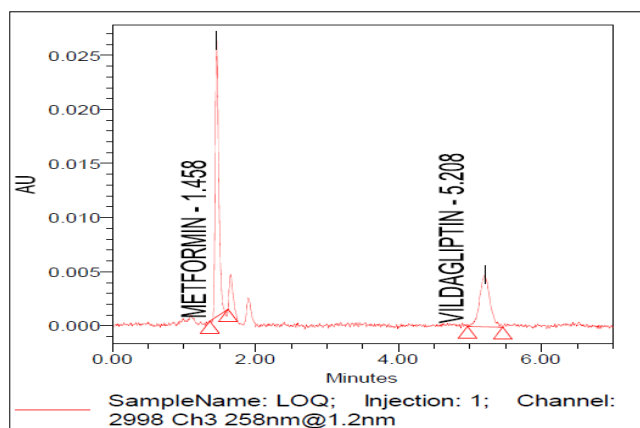


Fig 13: Typical Chromatogram of LOQ

Table 14: Comparison data of Robustness of Vildagliptin

S. No.	Analyte	Parameter	Flow 0.4ml	Flow 0.6ml	Temp 33°C	Temp 237°C
1.	Vildgliptin	Retention Time	6.392	4.367	5.236	5.160
2.	Vildgliptin	Tailing	1.203	1.164	1.186	1.152
3.	Vildgliptin	Theoretical Plates	8465	7068	7752	7958

Table 15: Comparison data of Robustness of Metformin

S. No.	Analyte	Parameter	Flow 0.4ml	Flow 0.6ml	Temp 33°C	Temp 37°C
1.	Metformin	Retention Time	1.805	1.209	1.450	1.447
2.	Metformin	Tailing	1.748	1.749	1.749	1.710
3.	Metformin	Theoretical Plates	3576	3540	3584	3615

Table 16: Forced degradation data of Galvusmet

S.no.	Sample weight	Vildagliptin Area	Metformin Area	%Assay of vildagliptin	%Assay of metformin	%Degradation of vildagliptin	%Degradation Of metformin
Heat	1246	1894179	2745878	94	94	-6	-6
Acid	1246	1563464	2054657	78	70	-22	-30
Base	1246	1616193	2014049	80	69	-20	-31
Peroxide	1246	1519582	1836252	76	63	-24	-37
Light	1246	1871891	2782466	93	95	-7	-5

4. Conclusion

The estimation of Metformin hydrochloride and Vildagliptin by simultaneous method using RPHPLC method was performed by using Chromosil ODS C₁₈ column having 250 x 4.6mm 5 μ , and mobile phase containing 0.1M Di Potassium hydrogen phosphate and Methanol at the ratio (60:40%v/v) adjust the pH:9.2 by using Ortho phosphoric acid. The flow rate was 0.5ml/min and detection was observed at 258nm. The retention time of Metformin and Vildagliptin was 1.43min and 5.32min respectively. The all validation parameters were accepted within the limits as per ICH guidelines. The specificity was performed under stress conditions like acid, base, peroxide, light and heat and observed the degradation of drugs. It was found to be within the acceptance limit. Hence it was concluded that the developed method was found to be simple, precise, accurate and rapid method for the routine analysis of Vildagliptin and Metformin hydrochloride API and pharmaceutical dosage form in laboratories.

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