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Research Article

Simultaneous Estimation of UV Spectroscopy and Method Development and Validation for Clofibrate and Tolbutamide by RP-HPLC

Mukku Sonia*, Gope Edward Raju, Kandregula Uma Maheswari, Doonaboyina Raghava, Kavala Nageswara Rao

Department of Pharmaceutical Analysis, K.G.R.L College of Pharmacy, Bhimavaram-534201, Andhra Pradesh, India.

Abstract
High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tolbutamide and Clofibrate was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tolbutamide and Clofibrate were found to be from 100-500 µg/ml of Tolbutamide and 1-5µg/ml of Clofibrate. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Tolbutamide and Clofibrate. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.
Keywords: Phosphate buffer, Inertsil C_{18} column, Tolbutamide and Clofibrate, RP-HPLC.

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Corresponding author
Mukku Sonia,
Department of Pharmaceutical Analysis,
K.G.R.L College of Pharmacy,
Bhimavaram-534201, A.P, India

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1. Introduction

Clofibrate is an antilipidemic agent similar to gemfibrozil. It acts to lower elevated serum lipids by reducing the very low-density lipoprotein fraction (Sf 20-400) rich in triglycerides. Serum cholesterol may be decreased, particularly in those patients whose cholesterol elevation is due to the presence of IDL as a result of Type III hyperparathyroidism. Tolbutamide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It is structurally similar to acetohexamide, chlorpropamide and tolazamide and belongs to the sulfonylurea class of insulin

secretagogues, which act by stimulating β cells of the pancreas to release insulin.

2. Methodology

Instrumentation:

Instruments used in HPLC Alliance waters and model no.2695 separation module. UV spectrophotometer software LANBINDIA UV 3000. PH meter model no.AD 1020. Weighing machine model no. Afcoset ER-200A. Borosil pippettes, burettes and beakers.

Materials and reagents:

Clofibrate and Tolbutamide were gifted by the cipla laboratories. Water, methanol and acetonitrile for HPLC are supplied merck and potassium phosphate was supplied by finger chemical pvt ltd.

Method development:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 30: 70 v/v respectively. UV spectrum of 10 µg / ml Clofibrate and Tolbutamide in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 260. At this wavelength both the drugs show good absorbance. The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5 m) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

Chromatographic conditions:

The cinematographic conditions were succesfully developed for the separation of clofibrate and tolbutamide by using Waters HPLC with auto sampler and PAD. Inertsil ODS (4.6 x 150mm, 5µm) column, flow rate was 1ml per min. Detection wave length was 260 nm. Mobile phase ratio was 30% buffer and 70% methanol buffer (6.8 grams of potassium dihydrogen ortho phosphate in1000 ml water pH adjusted with ortho phaosparic acid.).

3. Results and Discussion

Table 1: Results of method precession for Tolbutamide

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 2: Results of method precession for Clofibrate

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

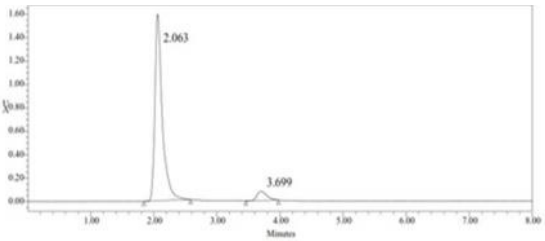

Figure no 1: chromatogram for standard injection

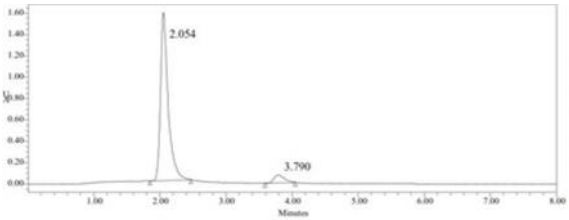

Figure .2 chromatogram for sample injection

Table 3: Results of Intermediate precision for Tolbutamide

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 4: Results of Intermediate precision for Clofibrate

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Table 5: accuracy (recovery) data for Tolbutamide

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	99.84%

Table 6: accuracy (recovery) data for Clofibrate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	100.51%

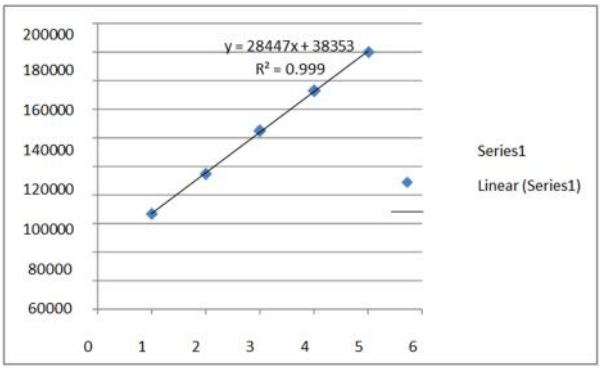

Figure 3: calibration graph for Tolbutamide

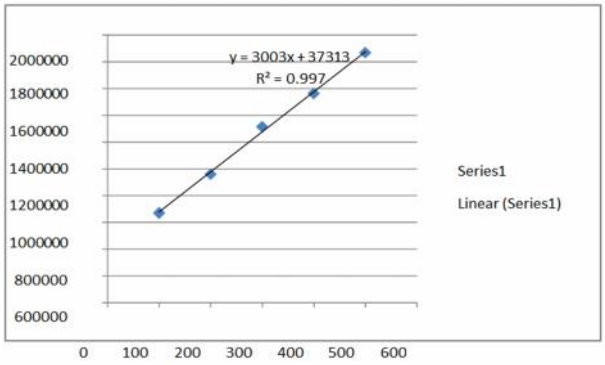

Figure 4: calibration graph for Clofibrate

Table 7 : Analytical performance parameters of Tolbutamide and Clofibrate

Parameters	**Tolbutamide**	**Clofibrate**
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 8: Results of LOD

Drug name	**Baseline noise (µV)**	**Signal obtained (µV)**	**S/N ratio**
Tolbutamide	52	152	2.9
Clofibrate	52	156	3

Table 9: Results of LOQ

Drug name	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
Tolbutamide	52	522	10.03
Clofibrate	52	524	10.1

Table 10: Flow Rate (ml/min) data for Tolbutamide

S. No	**Flow Rate(ml/min)**	**System Suitability Results**	
		USP Plate Count	**USP Tailing**
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 11: flow rate (ml/min) data for Clofibrate

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Tolbutamide and Clofibrate was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Tolbutamide and Clofibrate were found to be from 100-500 g/ml of Tolbutamide and 1-5 g/ml of Clofibrate. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Tolbutamide and Clofibrate. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision and Vincristine in API and Pharmaceutical dosage form.

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