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Research

Newer RP-HPLC Method Development And Validation For The Simultaneous Estimation Of Diloxanide Furoate, Tinidazole In Dosage Form

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Diloxanide and Tinidazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (25:75% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240 nm. The retention time of the Tinidazole and Diloxanide was 2.256, 5.427 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Tinidazole and 25-125mg/ml of Diloxanide. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Diloxanide, Tinidazole, RP-HPLC, validation.

INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that

is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

MATERIALS AND METHODS

Diloxanide Furoate from Sura labs, Tinidazole from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Tinidazole and Diloxanide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15 ml of Tinidazole and 0.75 ml of Diloxanide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 40ºC
Column : Phenomenex Gemini C18 (4.6×150mm, 5µ)
Mobile phase : Methanol: Water (25:75% v/v)
Flow rate : 1ml/min
Wavelength : 240 nm
Injection volume : 10 µl
Run time : 10 min

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 250ml (25%) of Methanol, 750ml of Water (75%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Water (25:75% v/v)
Column : Phenomenex Gemini C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 240 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 10 minutes

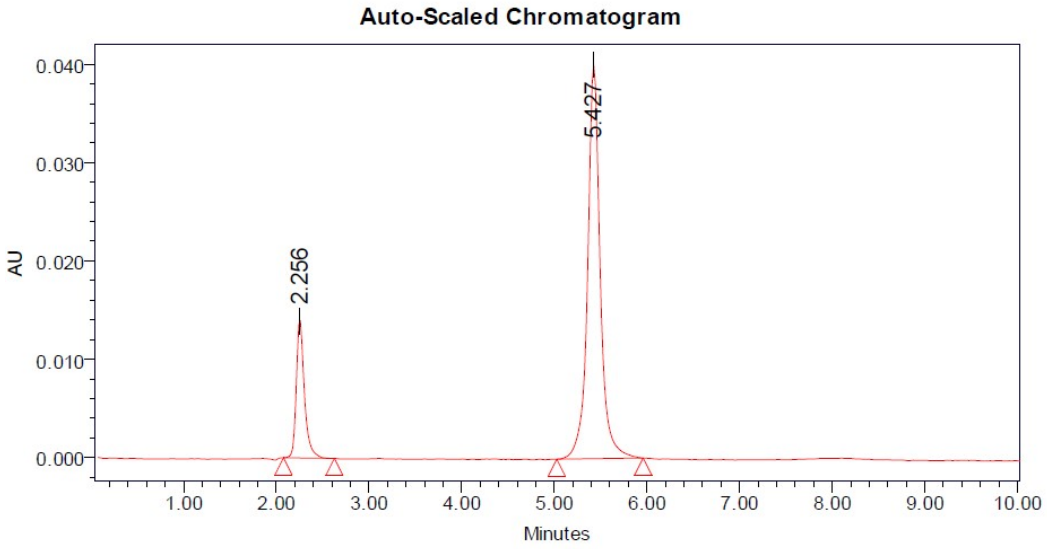

Fig 1: Optimized Chromatogram

Table 1: Peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tinidazole	2.256	84994	13905		1.32	5535
2	Diloxanide	5.427	377906	39948	16.27	1.03	9101

From the above chromatogram it was observed that the Tinidazole and Diloxanide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

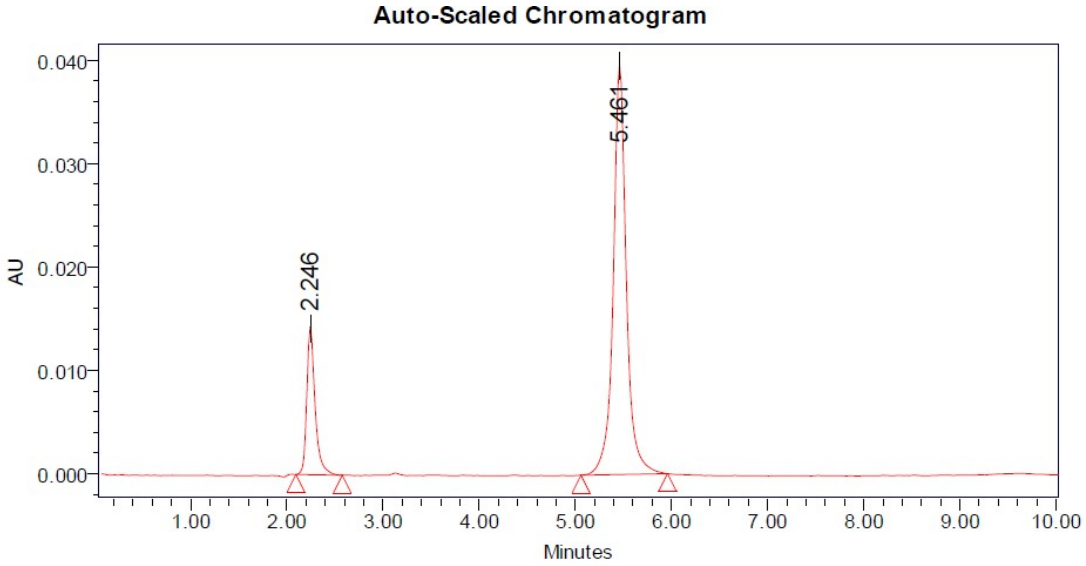

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tinidazole	2.246	86052	33061		1.32	5506
2	Diloxanide	5.461	364678	39373	16.42	1.02	9146

- *Resolution between two drugs must be not less than 2, Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Assay (Standard)

Table 3: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Tinidazole	2.256	84994	13905		1.32	3535
2	Diloxanide	5.427	377906	39948	16.27	1.03	9101
3	Tinidazole	2.249	86394	14163		1.38	3701
4	Diloxanide	5.430	376779	39935	16.13	1.05	9360
5	Tinidazole	2.248	85870	14082		1.40	3684
6	Diloxanide	5.443	375760	39609	16.19	1.05	9228

Assay (Sample)

Table 4: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Tinidazole	2.247	86092	36065		1.36	9506	1
2	Diloxanide	5.452	376779	37984	16.42	1.38	9511	1
3	Tinidazole	2.246	86052	33061		1.32	9487	2
4	Diloxanide	5.461	364678	39373	16.42	1.04	9146	2
5	Tinidazole	2.243	84182	39537		1.03	9228	3
6	Diloxanide	5.466	385423	39457	16.48	1.02	9247	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times = \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Tinidazole and Diloxanide in pharmaceutical dosage form was found to be 99.4 %.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY
Tinidazole

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	51080
66.6	10	92208
100	15	139140
133.3	20	180998
166.6	25	223920

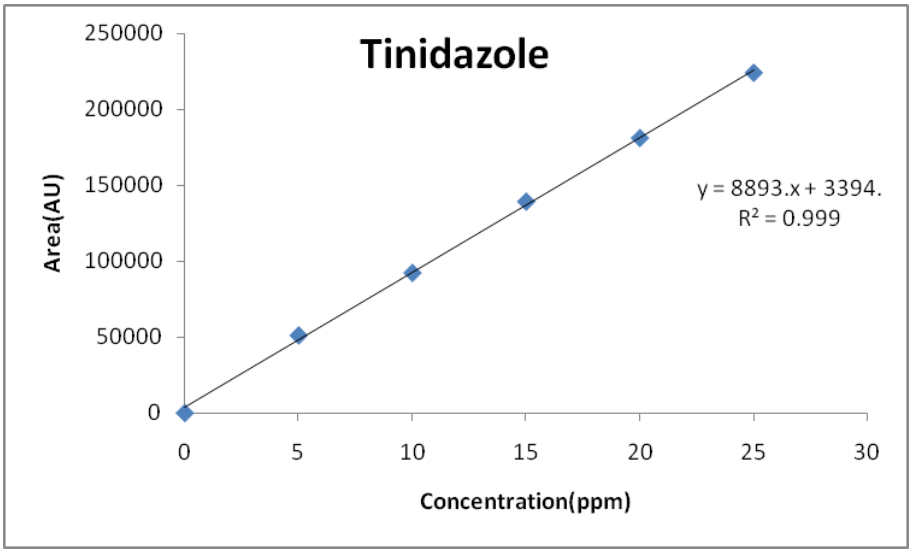

Fig 3: Calibration graph for Tinidazole

Diloxanide

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	25	224573
66	50	441895
100	75	635379
133	100	842226
166	125	1041381

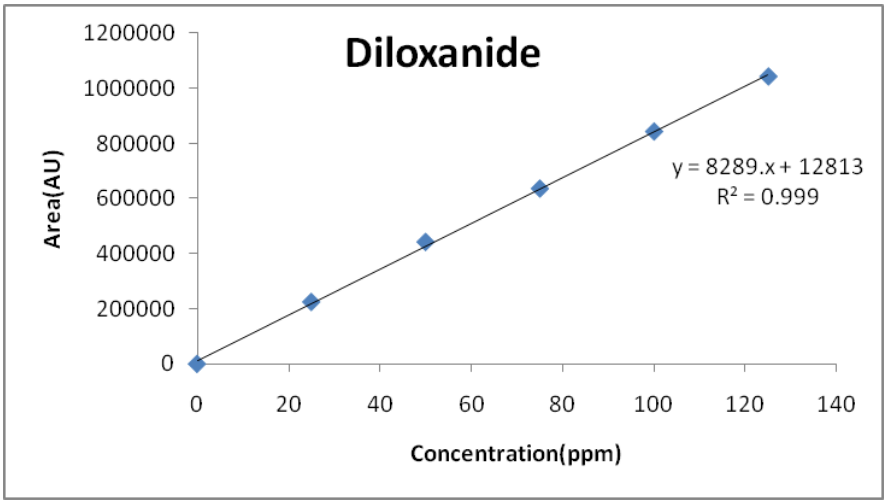

Fig 4: calibration graph for Diloxanide

REPEATABILITY

Table 5: Results of repeatability for Tinidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tinidazole	2.269	85148	13802	3405.7	1.4
2	Tinidazole	2.255	85369	13826	3338.4	1.4
3	Tinidazole	2.252	85451	13797	3474.5	1.4
4	Tinidazole	2.267	85812	13858	3422.2	1.4
5	Tinidazole	2.260	87007	14018	3326.6	1.3
Mean		2.264	87210	13986	3416.4	1.4
Std. Dev			85999.6			
% RSD			887.5			
			1.0			

%RSD for sample should be NMT 2, The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 6: Results of method precession for Diloxanide

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Diloxanide	5.274	370076	40629	9075.5	1.1	15.4
2	Diloxanide	5.266	370126	40937	9120.4	1.1	15.6
3	Diloxanide	5.265	372484	41279	9212.4	1.1	15.3
4	Diloxanide	5.278	376524	41454	8883.0	1.1	15.3
5	Diloxanide	5.305	381812	41320	9041.5	1.1	15.3
Mean		5.319	382550	41133	8974.1	1.1	15.3
Std. Dev			375595.4				
% RSD			5620.2				
			1.5				

%RSD for sample should be NMT 2, The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 7: Results of Intermediate precision for Tinidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tinidazole	2.248	84029	13603	3519.3	1.4
2	Tinidazole	2.245	84202	13520	3372.9	1.4
3	Tinidazole	2.242	84745	13636	3411.8	1.4
4	Tinidazole	2.239	85442	13775	3323.5	1.3

5	Tinidazole	2.243	85535	13768	3433.4	1.4
6	Tinidazole	2.246	85699	13739	3336.9	1.3
Mean			84942			
Std. Dev			720.3716			
% RSD			0.8			

- *%RSD of five different sample solutions should not more than 2*

Table 8: Results of Intermediate precision for Diloxanide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Diloxanide	5.284	366831	40102	9180.2	1.1	15.8
2	Diloxanide	5.293	368856	40464	9155.6	1.1	15.5
3	Diloxanide	5.306	370174	39977	9039.6	1.0	15.5
4	Diloxanide	5.319	370603	40748	9119.3	1.1	15.8
5	Diloxanide	5.346	372578	39772	9183.9	1.1	15.6
6	Diloxanide	5.352	376550	40083	9009.1	1.1	15.9
Mean			370932				
Std. Dev			3349.09				
% RSD			0.9				

%RSD of five different sample solutions should not more than 2, The %RSD obtained is within the limit, hence the method is rugged.

Table 9: Results of Intermediate precision Day 2 for Tinidazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tinidazole	2.255	85442	40102	9180.2	1.4
2	Tinidazole	2.260	85535	40464	9155.6	1.4
3	Tinidazole	2.242	85699	39977	9039.6	1.4
4	Tinidazole	2.245	84657	40748	9119.3	1.3
5	Tinidazole	2.260	86754	39772	9183.9	1.4
6	Tinidazole	2.255	85908	40083	9009.1	1.3
Mean			85665.83			
Std. Dev			682.4683			
% RSD			0.7			

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision for Diloxanide

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Diloxanide	5.266	368856	39977	9039.6	1.0	15.5
2	Diloxanide	5.265	370174	40748	9119.3	1.1	15.8
3	Diloxanide	5.306	370603	39772	9183.9	1.1	15.6
4	Diloxanide	5.293	369542	40083	9009.1	1.1	15.9
5	Diloxanide	5.265	371265	56430	9023.8	1.2	15.1
6	Diloxanide	5.266	378531	47652	9123.1	1.0	15.3
Mean			371495.2				
Std. Dev			3546.193				
% RSD			0.9				

%RSD of five different sample solutions should not more than 2, The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

The accuracy results for Tinidazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	69862.33	7.5	7.47	99.6	98.8%
100%	135467.7	15	14.8	98.6	
150%	199976	22.5	22.1	98.2	

The accuracy results for Diloxanide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	322954	37.5	37.4	99.7	99.7%
100%	632155	75	74.7	99.6	
150%	945870.3	112.5	112.5	100	

Robustness

Table 11: Results for Robustness

Tinidazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	84994	2.256	5535	1.32
Less Flow rate of 0.9 mL/min	89987	2.505	5891	1.27
More Flow rate of 1.1 mL/min	80653	2.046	5085	1.20
Less organic phase	89987	2.505	5098	1.20
More organic phase	80654	2.046	5123	1.27

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Diloxanide

Parameter used for sample analysis	Peak Area	Retention	Theoretical plates	Tailing
Actual Flow rate of 1.0 mL/min	377906	5.427	9101	1.01
Less Flow rate of 0.9 mL/min	397680	5.599	9407	1.03
More Flow rate of 1.1 mL/min	327899	4.576	9584	0.98
Less organic phase	396750	5.599	9407	1.02
More organic phase	339025	4.576	9584	0.99

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Diloxanide and Tinidazole in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Diloxanide and Tinidazole was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water (25:75% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Diloxanide and Tinidazole in bulk drug and in Pharmaceutical dosage forms.

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