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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TINIDAZOLE SODIUM AND DILOXANIDE FUROATE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and selective LC method is described for the determination of Tinidazole and Diloxanide furoate in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of Mixed Phosphate buffer (KH2PO4+K2HPO4) pH 6.5 and 70 volumes of Acetonitrile with detection of 270 nm. Linearity was observed in the range 36-84 µg /ml for Tinidazole (r^2 =0.9987) and 30-70µg /ml for Diloxanide furoate (r^2 =0.9977) for drugs estimated by the proposed methods was in good agreement with the label claim.The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Tinidazole, Diloxanide furoate, tablet dosage forms

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.Tinidazole is an anti-parasitic drug used against protozoan infections. It is widely known throughout Europe and the developing world as a treatment for a variety of amoebic and parasitic infections. It was developed in 1972.Tinidazole is a prodrug and antiprotozoal agent. The nitro group of tinidazole is reduced

in *Trichomonas* by a ferredoxin-mediated electron transport system. The free nitro radical generated as a result of this reduction is believed to be responsible for the antiprotozoal activity. It is suggested that the toxic free radicals covalently bind to DNA, causing DNA damage and leading to cell death. The mechanism by which tinidazole exhibits activity against *Giardia* and *Entamoeba* species is not known, though it is probably similar. Diloxanide furoate is an anti-protozoal drug used in the treatment of Entamoeba histolytica and some other protozoal infections. Although it is not currently approved for use in the United States, it was approved by a CDC study in the treatment of 4,371 cases of Entamoeba histolytica from 1977 to 1990.Diloxanide furoate is a luminal amebicide used in the treatment of *Amebiasis*.[33] It is considered the luminal agent of choice for amebiasis. The drug was discovered by *The Boots Company Plc* in 1956 and introduced as *Furamide*. The *Furamide* brand is now owned by Abbott Laboratories. It is not available in the US. In India it is available as *Amicline* by *Franco-Indian*(1-5).Aim is to develop new RP HPLC method for the simultaneous estimation of Tinidazole and Diloxanide furoate in pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Tinidazole (6, 7)

10 mg of Tinidazolewas weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Diloxanide furoate

10mg of Diloxanidefuroatewas weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 60 mg of Tinidazoleand 50 mg of Diloxanide furoate in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Tinidazoleand 50 µg/ml of Diloxanide furoate is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 60 mg of Tinidazoleand 50 mg of Diloxanide furoate in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 60 µg/ml of Tinidazole and 50 µg/ml of Diloxanide furoate is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet containsDiloxanide furoate-500mg tinidazole-600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of diloxanide furoateand tinidazole(µg/ml) were prepared by dissolving weight equivalent to 500 mg of diloxanide Furoateand600 mg of Tinidazoleand dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 50µg/ml of diloxanide Furoateand 60µg/ml of Tinidazolewas made by adding 1 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The

absorption curve shows characteristic absorption maxima at 305 nm for tinidazole, 257nm for Diloxanidefuroateand270nm for the combination.

The amount of tinidazoleand diloxanidefuroatepresent in the taken dosage form was found to be 99.49% and 98.70% respectively (Table-1).

Table -1 Assay Results

Tinidazole			**Diloxanide furoate**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	8685.497	8703.275	8751.280	8795.754
Injection-2	8653.021	8795.754	8815.057	8757.343
Injection-3	8718.749	8764.092	8771.274	8811.240
Injection-4	8840.648	8739.320	8943.783	8756.063
Injection-5	8742.316	8765.937	8863.844	8756.731
Average Area	8728.046	8753.676	8829.048	8775.426
Tablet average weight	1200		1200	
Standard weight	60		50	
Sample weight	120		120	
Label amount	600		500	
std. purity	99.2		99.3	
Amount found in mg	**596.95**		**493.48**	
Assay(%purity)	**99.49**		**98.70**	

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of tinidazole and diloxanide furoate is 0.9987 and 0.9977 (Fig-1 and 2). The relationship between the concentration of tinidazole and diloxanide furoate and area of tinidazole and diloxanide furoate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

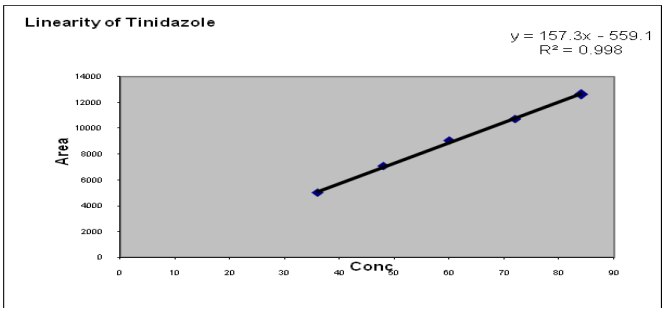

Fig-1 Linearity graph of tinidazole

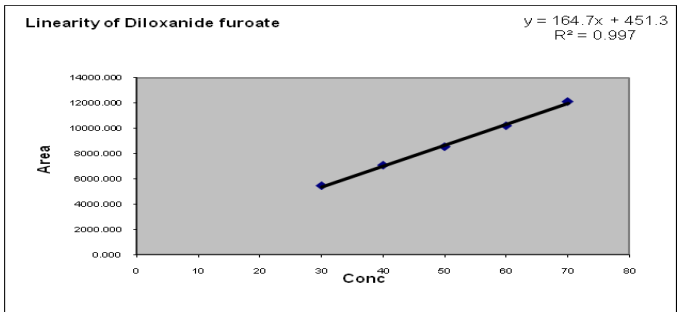

Fig-2 Linearity graph of diloxanide furoate

The percentage mean recovery of tinidazole and diloxanide furoate is 99.58% and 99.98% respectively. The LOQ for this method was found to be 1.21µg/ml & area 189.87 for tinidazole and 0.96 µg/ml & area 158.30 for diloxanide furoate.From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-2).

Table -2 Results for Ruggedness

TINIDAZOLE	**%Assay**	**DILOXANIDE FUROATE**	**%Assay**
Analyst 01	100.96	**Analyst 01**	102.00
Analyst 02	99.30	**Analyst 02**	99.11
%RSD (Area)	0.72	**%RSD (Area)**	1.13
%RSD(Rt)	0.08	**%RSD(Rt)**	0.09

CONCLUSION

From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation of Tinidazole and Diloxanidefuroate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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