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RP HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND FURAZOLIDONE PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of Metronidazole and Furazolidone in tablet dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile with detection of 266nm. Linearity was observed in the range 20-60 µg/ml for Metronidazole and 10-30 µg/ml for Furazolidone (r^2 =0.999) for the amount of 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.

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KEY WORDS: Metronidazole, Furazolidone, tablet dosage forms

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INTRODUCTION

Chromatography equipment look rather intimidating to anyone who has not handled them before, but on a closer look and as you get familiar with the equipment you realize that behind the network of wires, complex plumbing and circuitry is a simple machine with only a few major parts. Different combinations of these parts namely pumps, detectors and injectors yield an infinite number of configurations based on the application. Just like an understanding of human anatomy makes you conscious of the vital role of each and every body organ towards your well being and vitality. Similarly you need to have a good understanding of the parts of your HPLC system to generate data of highest reliability. A conceptual understanding of the function of each component will add to your comfort level with your HPLC system. You will ensure long time usage with high reliance on output data. The present module is intended to serve this very purpose and in simple terms you will appreciate the role of each part and its contribution to overall system efficiency. HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatalised, thermally unstable and have high molecular weights. The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively

retained on the basis of physico-chemical interactions between the analyte molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components. We now introduce you to the significance and role of each component part of the HPLC system.

A nitroimidazole used to treat amebiasis; vaginitis; trichomonas infections; giardiasis; anaerobic bacteria; and treponemal infections. It has also been proposed as a radiation sensitizer for hypoxic cells. Metronidazole is a prodrug. Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death. A nitrofuran derivative with antiprotozoal and antibacterial activity. Furazolidone binds bacterial DNA which leads to the gradual inhibition of monoamine oxidase. Furazolidone and its related free radical products are believed to bind DNA and induce cross-links. Bacterial DNA is particularly susceptible to this drug leading to high levels of mutations (transitions and transversions) in the bacterial chromosome (1-4).

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase.Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task (5).

Hence aim is to develop new RP HPLC method for the simultaneous estimation of Metronidazole and Furazolidone pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax) (6-8)

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 metronidazole

10 mg of Metronidazole was 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 furazolidone

10mg of Furazolidone was 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 10mg of metronidazole and 10 mg of furazolidone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 200 µg/ml of Metronidazole and100 µg/ml of furazolidone is prepared by pipette out 2ml from metronidazole and 1.0ml from furazolidone and make up the volume 10ml with mobile phase. This solution is used for recording chromatogram

Preparation of samples for Assay

10 tablets (each tablet contains furazolidone-100mg metronidazole-200 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of furazolidone and metronidazole were prepared by dissolving weight equivalent to 100 mg of furazolidone and 200mg of metronidazole and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-

micron syringe filter and Sonicated for 5 min and dilute to 10ml with 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 resulting spectra are shown in the fig-1.

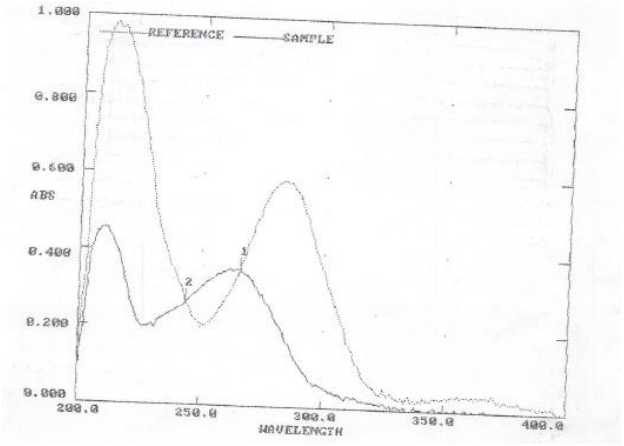

Fig-1UV-VIS spectrum of Metronidazole and Furazolidone and the isosbestic point was 266nm

The amount of metronidazole and furazolidone present in the taken dosage form was found to be 99.97% and101.52 % respectively (Fig-2).

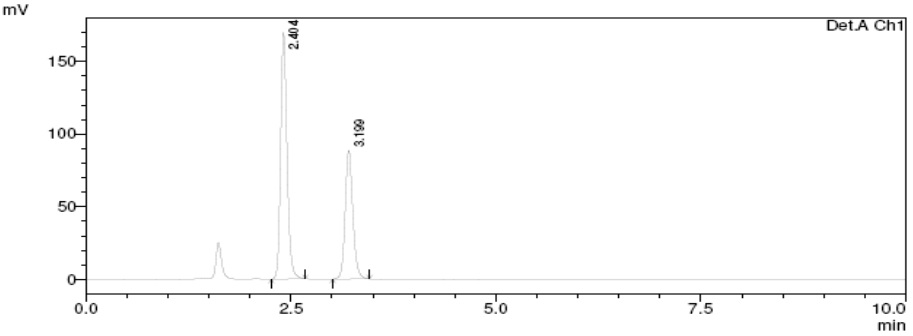

Fig-2 Chromatogram of Assay

The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-1 and 2).

Table-1 Results for system suitability of metronidazole

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.404	924225	25888	1.27
2	2.407	916710	25783	1.26
3	2.406	922097	25770	1.24
4	2.406	921097	25775	1.27
5	2.412	919838	25857	1.24

6	2.409	919810	25875	1.26
Mean	2.407	920629.5	-	-
SD	0.003	2528.3	-	-
%RSD	0.12	0.27	-	-

Table-2 Results for system suitability of furazolidone

Injection	RT	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.199	577339	33742	1.19	4.75
2	3.204	572957	33657	1.18	4.72
3	3.201	570728	33684	1.19	4.71
4	3.201	571728	33752	1.17	4.74
5	3.208	569261	33684	1.18	4.75
6	3.205	570393	33725	1.20	4.77
Mean	3.203	572067.7	-	-	-
SD	0.003	2869.5	-	-	-
%RSD	0.10	0.50	-	-	-

A graph was plotted for metronidazole and furazolidone against the concentrations of the solutions and the peak areas. The correlation coefficient R^2 was determined and was found to be 0.999 for metronidazole(Fig-3) and 0.999 for furazolidone(Fig-4).

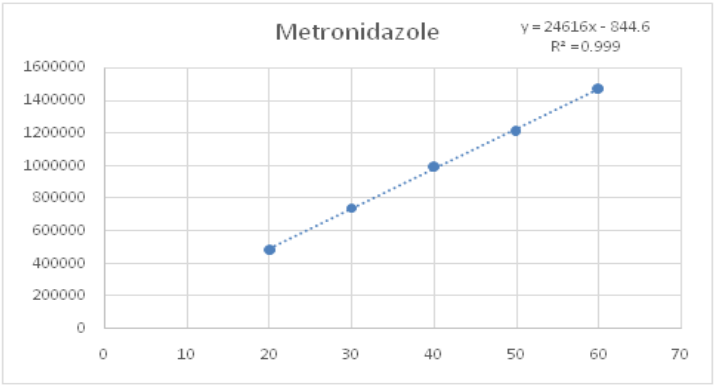

Fig-3 Graph for Linearity data of metronidazole

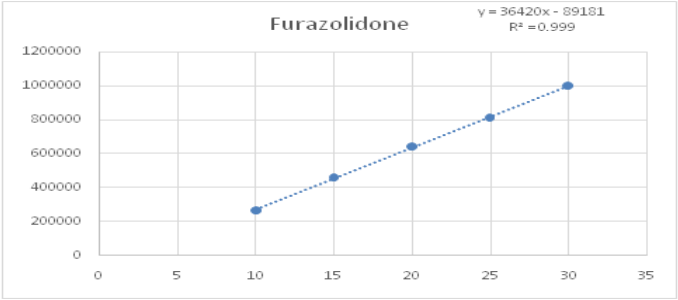

Fig-4 Graph for Linearity data of furazolidone

The % recovery of metronidazole and Furazolidone should lie between 98% and 102%. The % mean recovery of metronidazole and furazolidone was foundbetween 98.0 to 102.0. The tailing factor was found to be within the limits on small variation of flow rate and wavelength. The %RSD between two analysts assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Metronidazole and Furazolidone 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.

REFERENCES

1. Skoog DA, West DM, Holler FJ. *Introduction of analytical chemistry*. Sounder college of publishing, Harcourt Brace college publishers, 1996, 1-5.

2. Sharma B K. *Instrumental method of chemical analysis* Meerut, 1999, 175-203.

3. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. *Journal of Pharmaceutical Technology*, 5, 2003, 110-114.

4. Willard H, et al. *Instrumental methods of analysis*" 7th edition CBS publisher and distributors, New Delhi, 1991, 436-439.

5. J. Kavitha, C.H. Krishna Kishore, K.S. Lakshmi Estimation of metronidazole, furazolidone and loperamide in veterinary formulation by RP-HPLC*International Journal of Pharma and Bio Sciences* 5(1):P187-P198

6. Murali D and Rambabu C**.** Simultaneous Determination of Metronidazole and Furazolidone in Combined Tablet Dosage Form: Development and Validation of a Stability Indicating HPLC Method - *Anal Chem Ind J*, 2019, 16(14).

7. Code Q2B. *Validation of Analytical Procedures; Methodology*. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996, 1-8. 14.

8. ICH Q2A. *Validation of analytical methods, definitions and terminology*", ICH Harmonized tripartite guideline, 1999