INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

SIMULTANEOUS ESTIMATION OF THIOCOLCHICOSIDE AND FLUPIRTINE MALEATE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A new simple accurate and economical reverse phase high performance liquid chromatographic method was developed for the determination of Thiocolchicoside and Flupirtine Maleate in bulk and tablet dosage form. The separation was eluted on a C_{18} column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of mixed phosphate buffer 6.5 and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1.0ml/min. The detection was made at 255 nm. The retention times were 1.96min for Malic acid, 2.52min for Thiocolchicoside and 4.97min for Flupirtine. Calibration curve was linear over the concentration range of 4-24µg/ml for Thiocolchicoside and 50to300 µg/ml for Flupirtine. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

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Key Words: Thiocolchicoside, Flupirtine Maleate, ICH guidelines

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INTRODUCTION

Analytical chemistry is a branch of chemistry that Ideals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts[1-4]. Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained". Pharmaceutical analysis plays a major role today, and it can be various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc. Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a

faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation (1-4).

Aim of present work is to develop a new simple accurate and economical reverse phase high performance liquid chromatographic method was developed for the determination of thiocolchicoside and flupirtine maleate in bulk and tablet dosage form and validated in terms of ICH guildlines**.** Hence, on the basis of literature survey it was thought to develop a precise, accurate, simple and reliable, less time consuming and less cost effective method for the estimation of Thiocolchicoside and Flupirtine

MATERIALS AND METHODS

Selection of Detector Wave Length (5-7)

An UV spectrum of 10µg /ml Thiocolchicoside and 100mcg/ml Flupirtine in diluents (methanol) in was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength of 255 nm was selected. At this wavelength Thiocolchicoside and Flupirtine standard showed good absorbance.

Prepartion of standard and sample solution prepartion

Standard stock solution preparation Weigh and transfer 8 mg of Thiocolchicoside 50 mg Flupirtine Maleateworking standard into 50 mL volumetric flask, add 30 ml diluent and sonicate to dissolve and dilute to volume with diluent. (Stock solution). Transfer 10 ml of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Preparation Previously grinded powder equivalent to 1 tablet weight in 50 mL volumetric flask add 30 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filtrate the solution through 0.45µ filter. Further dilute 10 ml with 100 ml with diluents.

Procedure Inject 10 µL of blank solution, placebo solution, three times of Standard solution, Disregard peaks due to blank and placebo.

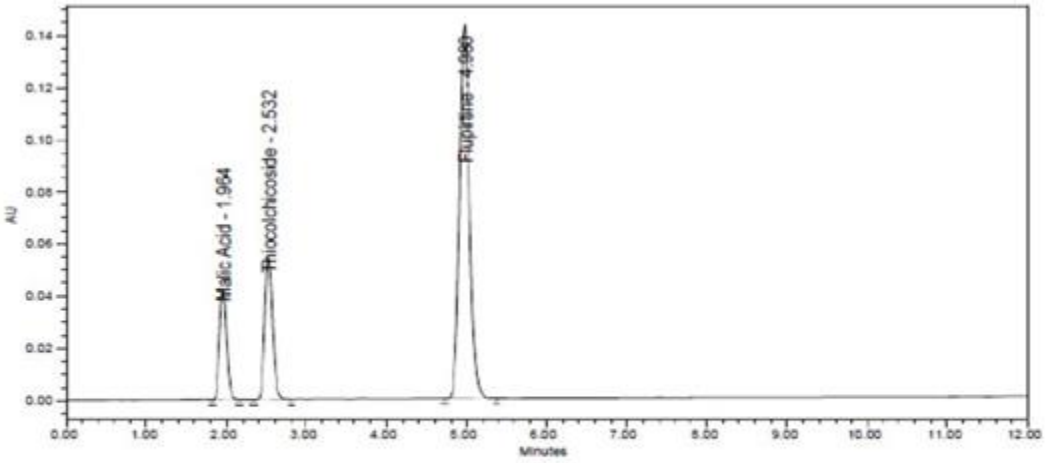
The standard solution was prepared by using Thiocolchicoside and Flupirtine for tablets working standard as per test method and injected six times into the HPLC system. The system suitability parameters, accuracy and precision were evaluated.

RESULTS AND DISCUSSION

An UV spectrum of 10µg /ml Thiocolchicoside and 100mcg/ml Flupirtine in diluents (methanol) in was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength of 255 nm was selected. At this wavelength Thiocolchicoside and Flupirtine standard showed good absorbance. The standard solution was prepared by using Thiocolchicoside and Flupirtine for tablets working standard as per test method and injected six times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits (Table-1 and fig-1).

Table-1 Results of System Suitability

S.No	System Suitability Parameter	Observed value		Acceptance criteria
		THIOCOLCHICOSIDE	FLUPIRTINE	
1	The % RSD of peak areas	0.13	0.04	NMT 2.0
2	The Tailing factor for peak in standard solution	1.14	1.11	NMT 2.0
3	Theoretical plates	3387	7618	NLT2000
4	Resolution	--	11.83	NLT 2.0



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Malic Acid	1.964	248913	41638	13.46		1.12	2457
2	Thiocolchicoside	2.532	355354	53745	19.21	3.35	1.14	3387
3	Flupirtine	4.980	1245462	143290	67.33	11.83	1.11	7618

Fig-1 Chromatogram for System Suitability

The standard solution was prepared by using Thiocolchicoside and Flupirtine for tablets working standard as per test method and injected six times into the HPLC system.The RSD for peak areas from six replicate injections of Thiocolchicoside and Flupirtine was found to be 0.17% (Fig-2).

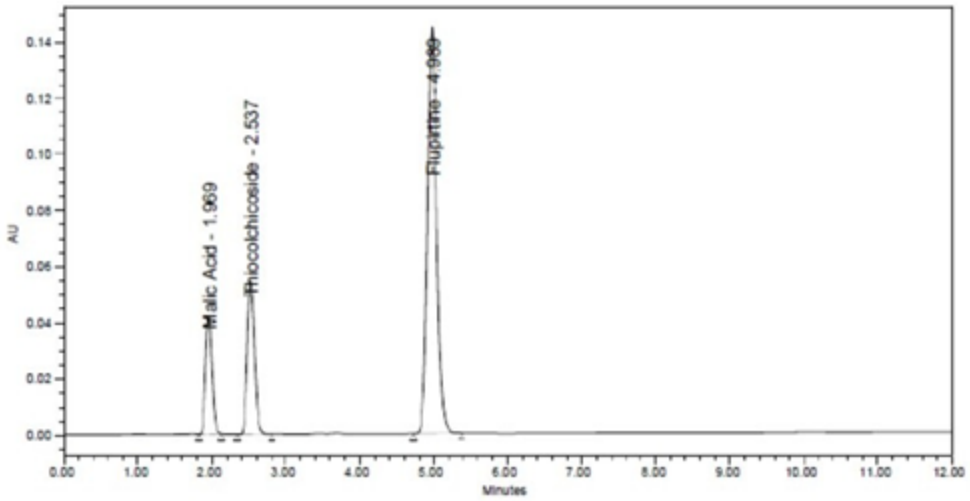


Fig-2 Chromatogram for System Precision

The precision of test procedure was evaluated for Thiocolchicoside and Flupirtine by performing the assay as per the test method. The % relative standard deviation of Thiocolchicoside and Flupirtine was found to be within the limits. The % RSD of of Thiocolchicoside and Flupirtine from the six sample preparations should be NMT 2.0% (Table-2).

Table-2 Results of Repeatability

INJECTION	**THIOCOLCHICOSIDE**		**FLUPIRTINE**	
	RT	**Area**	**RT**	**AREA**
1	2.532	355354	4.98	1245462
2	2.531	356953	4.977	1247928

3	2.532	355022	4.984	1247749
4	2.535	356062	4.983	1244905
5	2.537	355990	4.985	1245435
6	2.532	356238	4.981	1245043
MEAN	2.533166667	355936.5	4.982	1246087
STD.DEV	0.002316607	680.94104	0.002944	1375.08647
%RSD	0.09	0.19	0.06	0.11

The precision of test procedure was evaluated for Thiocolchicoside and Flupirtine by performing the assay as per the test method. The % relative standard deviation of Thiocolchicoside and Flupirtine was found to be within the limits. The % RSD of % Assay of Thiocolchicoside and Flupirtine from the six sample preparations should be NMT 2.0% (Fig-3).

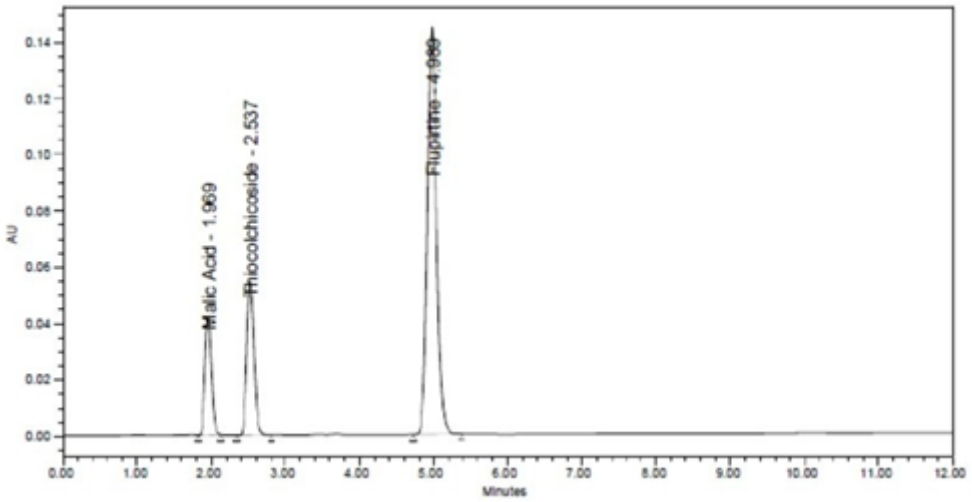


Fig-3 Chromatogram for Reproducibility

It was established that the linearity of test method is from 25% to 150% for Thiocolchicoside and Flupirtine of the target assay concentration. The Correlation Coefficient should be not less than 0.997 (Table-3).

Table-3 Results of Linearity

S.NO	**SPIKE**	**STANDARD**		**AREA**	
		THIOCOLCHIC	**FLUPIRTINE**	**THIOCOL**	**FLUPIRTI**
1	25%	4	50	94473	328490
2	50%	8	100	187789	651770
3	75%	12	150	280856	973266
4	100%	16	200	356504	1239013
5	125%	20	250	449825	1564373
6	150%	24	300	555324	1932640
Correlation coefficient				0.999161	0.999965
Slope (m)				21985	6118
Intercept (c)				10064	33680

To determine the accuracy of the test method samples were prepared by spiking Thiocolchicoside and Flupirtine API with the equivalent amount of placebo at 50%, 100%, and 150% of the target concentration. Three samples were prepared in triplicate. The average % recovery of Thiocolchicoside and Flupirtine was found to be within the limits.

CONCLUSION

Present study describe about A new simple accurate and economical reverse phase high performance liquid chromatographic method was developed for the determination of Thiocolchicoside and Flupirtine Maleate in bulk and tablet dosage form. The separation was eluted on a C_{18} column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of mixed phosphate buffer 6.5 and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1.0ml/min. The detection was made at 255 nm. The retention times were 1.96min for Malic acid, 2.52min for Thiocolchicoside and 4.97min for Flupirtine. Calibration curve was linear over the concentration range of 4-24 µg/ml for Thiocolchicoside and 50 to 300 µg/ml for Flupirtine. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

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