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Simultaneous Estimation of Gatifloxacin and Ambroxol HCl in tablet formulation by HPTLC Method

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Abstract

A simple, specific, accurate and precise high performance thin layer chromatographic method for the analysis of gatifloxacin and ambroxol Hcl in combined tablet dosage forms is reported in this paper. The method uses aluminium plates coated with silica gel 60 F_{254} as stationary phase and n-butanol-water-methanol-ammonia 4:0.5:0.5:1(v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 310 nm. The two drugs were satisfactorily resolved with R_F values 0.33 and 0.89 for gatifloxacin and ambroxol Hcl respectively. The respective calibration plots were linear over the ranges 20-60 µg/ml and 2.5-12.5µg/ml per band. Intra-day variation as RSD (%) was 0.21 for gatifloxacin and 0.24 for ambroxol Hcl. Interday variation, as RSD (%) 0.04 for gatifloxacin and 0.4 for ambroxol Hcl. The method, which was validated in accordance with ICH guidelines, can be used for analysis and is a rapid and cost-effective quality-control tool for routine simultaneous analysis of gatifloxacin and ambroxol Hcl in combined dosage forms.

Key words: Gatifloxacin, Ambroxol HCl, HPTLC, densitometry.
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INTRODUCTION

Gatifloxacin is a synthetic broad spectrum 8-methoxy fluoro quinolone antibacterial agent. Chemically gatifloxacin is (±)-1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7 (-3methyl-1-piperazinyl) 4-oxo-3-quinoline carboxylic acid sesquinhydrine. Gatifloxacin which is widely used in chronic bronchitis, pneumonia, acute sinusitis and urinary tract infections. Ambroxol hydrochloride, chemically 4([(2-amino, 3, 5-dibromophenyl)-methyl] amino)-cyclohexyl or N-[(trans-p-hydroxy cyclo hexyl)-(2-amino-3, 5-dibromo benzyl)-amino] cyclo hexanol hydrochloride is an active metabolite disorders associated with viscid or excessive mucus and

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expectorant. Tablet is available commercially as Lancer-D. On detailed literature survey, it was found that these drugs have been estimated individually and in combinations by various methods [1-15]. The present works depicts simple, precise and accurate HPTLC method for simultaneous estimation of gatifloxacin and ambroxol HCl in tablet formulation.

MATERIALS AND METHODS

Experimental
Reagents and Chemicals
Methanol (AR grade) was obtained from Merck Specialties (Mumbai, India). n-butanol and ammonia (all AR-grade) were pro-cured from Sisco Research Laboratories (Mumbai, India). Gatifloxacin and Ambroxol Hcl reference standards were provided as gifts by A TO Z Labs, (Chennai, India), labeled to contain gatifloxacin 400mg and ambroxol Hcl 75mg, were obtained locally. Gatifloxacin (10mg) and ambroxol Hcl (10mg) were weighed separately and dissolved in 100ml of mobile phase to furnish solutions of concentration 100 μg mL^{-1} for each drug.

Chromatography
Chromatography was performed on 20cm X 10cm aluminium plates coated with 250-μm layers of silica gel 60 F$_{254}$ (E-Merck). Before use the plates were pre washed with methanol and activated at 110 ^{0}C for 5min. samples were applied as 6-mm, 5mm apart, by means of a camag linomat 4 sample applicator with 100-μL sample syringe.Linear ascending development, with n-butanol:water:methanol:ammonia 4:0.5:0.5:1(%, v/v) as mobile phase, was performed in a 20cm X10 cm Camag twin - trough glass chamber previously saturated with mobile phase vapour for 30mins. The development distance was 85mm and the develop time approximately 15mins. After development the plates were dried in current of air by means of a hair drier. Densiometric scanning at 310nm was performed with a camag scanner 3 operated by cats software version 4.05. The source of radiation used was a deuterium lamp emitting a continues a UV spectrum between to 200 and 400nm the slit dimensions were 5mm X 0.45mm and the scanning speed was 20mm s^{-1}.

Preparation of Calibration Plots
Stock solutions of the drugs in the range of 20 - 60 μg/ml of Gatifloxacin and 2.5 - 12.5 μg/ml of Ambroxol HCl were applied to an HPTLC plate. The plate was developed and scanned under the conditions described above. Each amount was analyzed three times and peak areas were recorded. Calibration plots of peak area against the respective amount of drug were established separately for gatifloxacin and ambroxol Hcl.

Procedure for Analysis of Tablet Formulation
Twenty tablets were weighed accurately and powdered. Powder equivalent to 400mg of Gatifloxacin and 75mg of Ambroxol HCl was taken and transfer to a 100ml volumetric flask containing approximately 25ml of mobile phase. The mixture was ultra-sonicated for 15mins then diluted to volume with mobile phase. The solution was filtered through a Whatman No.42 filter paper and appropriate amount of filtrate of gatifloxacin and ambroxol Hcl was applied to HPTLC plate. After chromatogram development the peak areas of the bands were measured at 310 nm and the amount of each drug in each tablet was determined from the respective calibration plot. The analysis procedure was repeated three times for the homogenous powder

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sample. The densitogram obtained from a sample solution of gatifloxacin and ambroxol Hcl is shown in Fig. 1.

Method Validation [16]

To study intra-day variation, three mixed standard solutions containing gatifloxacin and ambroxol Hcl were prepared and applied to the plates. All solutions were analysed on the same day to record any intra-day variation in the results. To study inter-day variation, analysis of three mixed standard solutions of same concentration was performed on three different days. To confirm the specificity of the method gatifloxacin and ambroxol Hcl were applied to an HPTLC plate and developed and scanned as described above.

Recovery Studies

To check the accuracy of the method, recovery studies were conducted after addition of standard drug solution at three different levels to pre-analyzed sample solution.

RESULTS AND DISCUSSION

Method Development

Different mobile phase containing different proportions of toluene, methanol, acetone, n-butanol, water, ammonia, ethyl acetate were examined (data not shown). Finally n-butanol-water-methanol-ammonia 4:0.5:0.5:1(v/v) was selected as mobile phase because it resulted in acceptable resolution between the bands with R_F values of 0.33 for gatifloxacin and 0.89 for ambroxol Hcl. The optimum chamber saturation time with mobile phase vapour was 10 min.

Validation

Standard calibration plots were linear over the range 20-60 µg/ml and 2.5-12.5 µg/ml for 2.5-12.5 µg/ml for gatifloxacin and ambroxol Hcl respectively, correlation coefficients were 0.9998 and 0.999 respectively. Intra-day variation, as RSD (%), was 0.21 for gatifloxacin and 0.24 for ambroxol Hcl respectively. Interday variation, as RSD (%) 0.04 for gatifloxacin and 0.4 for ambroxol Hcl respectively. Excipients present in the formulation did not interfere with the peaks of gatifloxacin and ambroxol Hcl. The spectra acquired for gatifloxacin and ambroxol Hcl extracted from tablet were also compared with those acquired from gatifloxacin and ambroxol Hcl standards, correlation was good, indicating the method was specific. The validation data are summarized given in table-I.

The study the accuracy and precision of the method, recovery was determined at three levels-50, 100 and 150%. Results from recovery studies are reported in table-II.

The method was also evaluated by assay of commercially available tablets containing gatifloxacin and ambroxol Hcl. Five replicate analyses were performed on accurately weighed amounts of the tablets. The assay (%) was 100.48 for gatifloxacin and 99.48 for ambroxol Hcl with standard deviation of 0.87 and 0.18 receptively.

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Table –I: Validation Data

Method characteristic	Gatifloxacin	Ambroxol Hcl
Linearity range (µg/ml)	20-60	2.5-12.5
Correlation coefficient (r)	0.9998	0.999
Accuracy (%, n=3)	100.28	99.25
Inter-day Precision (n=3)	0.21	0.24
Intra-day Precision (n=3)	0.08	0.4
Specificity	Specific	Specific

Table- 2: Results of recovery studies

Drug	Label claim (mg/tablet)	Amount added (%)	Amount recovered(mg)	Recovery (%)
Gatifloxacin	400	50	450.12	100.03
		100	500.08	100.01
		150	550.89	100.16
Ambroxol Hcl	75	50	124.79	99.83
		100	174.13	99.5
		150	224.62	99.81
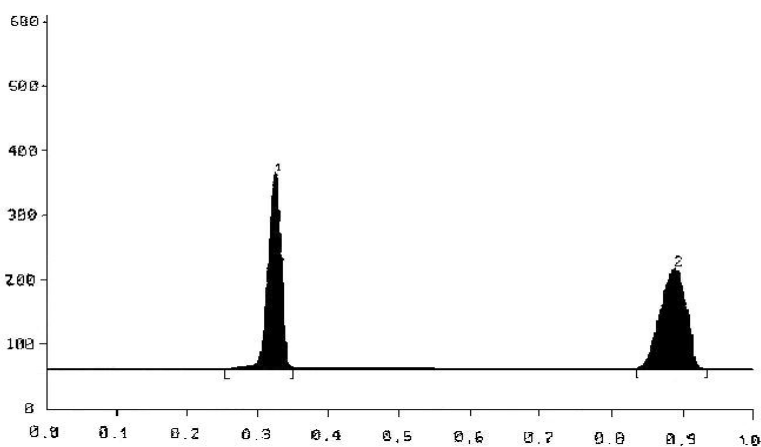
Figure-1: Densitogram Of Sample
(1) Gatifloxacin (2) Ambroxol Hcl

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CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today HPTLC is rapidly become a routine analytical technique due to its advantages of low operating costs high sample throughput and the need for minimum sample preparation.

This validated HPTLC method proved to be simple, fast, accurate and precise and can thus be used for routine analysis of gatifloxacin and ambroxol Hcl in combined tablet dosage forms.

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