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HPTLC method to study skin permeation of acylovir

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Abstract

A new simple, rapid and selective high performance thin layer chromatography (HPTLC) method is developed for the quantitation of acyclovir during in vitro skin permeation studies. Separation of guinea pig skin proteins and acyclovir was achieved by employing a mobile phase consisting of chloroform–methanol–ammonia (15:9:4, v/v/v) on precoated silica gel 60F254 aluminum plates. Densitometnic analysis was carried out at 255 nm. The limit of detection and quantification were 30 and 50 ng, respectively. The calibration curve was linear in the range of 10–20 μg/ml ($r = 0.9965$). The relative standard deviation for a sample of concentration 100 μg/ml were 1.15 and 2.85 for system and method precision, respectively. Intraday and interday variation studies gave an average 0.763 and 0.463% relative standard deviation for the three levels tested. Average recoveries of 101.8 and 100.1% were recorded for two marketed preparations studied. The method was employed to optirmize topical liposomal gel formulation of acyclovir on basis of maximum skin permeation.

Keywords: Acyclovir; High-performance thin layer chromatography (HPTLC); Skin permeation

1. Introduction

Acyclovir (9-[(2-Rydroxyethoxy)methyllguanine) is an antiviral drug used extensively against skin infections caused by herpes virus types I and II. It is reported in United States Pharmacopoeia [1], British Pharmacopoeia [2] and European Pharmacopoeia [3]. A number of analytical methods viz. Fluorimetry [4], high performance thin layer chromatography (HPLC) [5–8], potentiometric titration [9], electrophoresis [10], radioimmunoassay techniques [11] etc. have been reported. However, these methods are expensive. Monitoring of skin permeation of a drug demands a highly selective analytical technique like HPTLC. This paper describes the development and standardization of an HPTLC technique for the rapid estimation of acyclovir in skin permeation samples to study the skin penetration of acyclovir from gel formulation through guinea pig skin in vitro. The proposed method is also useful for routine analysis of prepared formulations as well as marketed preparations.

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2. Experimental

2.1. Materials

Silica gel $60F_{254}$ HPTLC plates 10×10 cm^2) were from E. Merk, Germany). Standard acyclovir was procured from Cipla Ltd, India. Acyclovir liposomal gel was developed at our laboratory. Acylcovir cream and ointment were procured from the market.

Chloroform, methanol, strong ammonia solution (25% v/v) were of analytical grade.

2.2. Instrumentation

A Camag HPTLC system (Switzerland) comprising of Camag TLC Linomat sample applicator, a 100-µl Hamilton syringe, a development chamber consisting of a Camag twin trough glass chamber and a Camag TLC scannerII attached to Perkin Elmer LCI-100 integrator were used.

2.3. Calibration points

A series of standard curves were prepared over a concentration range of 10–200 µg/ml from a stock solution of acyclovir (1 mg/ml). Solutions prepared were spotted on HPTLC plate as individual bands. the plate was developed maintaining chromatographic conditions mentoined below. Peak area (AUC) was plotted against the concentration to obtain calibration curve.

2.4. Chromatographic conditions

The conditions were as follows. Test plate: HPTLC precoated plate, silica gel $60F_{254}$, aluminum (E. Merck, Germany). Format: 10×10 cm^2, thickness: 200 µm. Spotting volume: 5µl. Band width: 3 mm. Separation technique: Ascending. Development chamber: Camaf twin trough glass chamber $10 \times 12 \times 5$ cm^3. Mobile phase: chloroform:ammonia (15:9:4, v/v/v). Time for chamber presaturation: 75 min. Relative humidity (%): 55 ± 5. Temperature: 23 ± 3°C. Migration distance: 70 mm. Migration time: 16 min. Detection wavelenght: 255 nm. Mode: absorbamce. Span: 10. Slit dimensions: 4×4 mm^2.

2.5. Method validation [12]

The method was validated for the following parameters.

2.5.1. Selectivity

A 1 cm^2one piece of freshly excised hairless defatted abdominal skin of guinea pig was cut into pieces, placed in a 10 ml volumetric flask containing distilled water and subjected to a temperature of $37 \pm$ °C for 24 h intermittently subjecting to ultrasonication to ensure maximum extraction of skin proteins. Skin proteins were similarly extracted into acvclovir solution (100 µg/ml). The two samples were analyzed separately by HPTLC as described above to study the extent of separation of skin proteins from drug and to find recovery of drug from skin permeation sample.

2.5.2. Limit of detection and quantifiacation

The limit of detection was determined by spotting six blank samples (distilled vater), developing and scanning plate as described earlier, then calculating mean peak areas and SD of the data to estimate noise of the instrument. Limit of detection then corresponds to the amount of drug which has the same area as mean $\pm$ 3 SD.

The amount of drug, the area of which equals mean $\pm$ 10 SD gave an estimate of the limit of quantification.

2.5.3. Precision

2.5.3.1. System precision. System precision was determined by spotting 5 µl of a 100 µg/ml solution of drug on the same plaaate to give six spots of the same amount (500 ng) and determining the relative SD for the data.

2.5.3.2. Method precision. The method precision was studied similarly as in case of system precision for the same concentration (500 ng) but at six different times, developing and scanning each plate separately. The relative standard deviation for the data was determined.

2.5.4. Ruggedness

A solution of concentration 80 μg/ml was prepared and analyzed on dav 0 and after 24, 48 and 72 h. Each time 5 μl of the solution was spotted each time. Data was treated for relative SD deviation to assess ruggedness of the method.

2.5.5. Interday atid intraday variation studies

To ensure ruggedness of the method, elaborate inter and intraday variation studies were carried out at three levels (50, 400 and 1000ng). The concentrations chosen fell in the linearity range including the highest and tile lowest points of the calibration curve. Samples were analyzed in tniplicates each morning, and evening for three days. Data was treated for relative standard deviation.

2.5.6. Assay

2.5.6.1. Extraction procedure from gels. Approximately 100mg of gel was weighed and transferred to a 10 ml volumetric flask. Extraction of drug was carried out in distilled water bv ultrasonication for 30 min. The volume was made to 10 and 2 ml of this stock dispersion were diluted to 10 ml with distilled water to give a final concentration of 100 μg/ml of acvclovir. The sampie was then filtered through G-3 sintered glass filter to separate the precipitated gellant polymer and analyzed subsequently.

Placebo gels were analyzed similarly to study potential Interference. Excipients used in case of placebo formulation 1 were EpIkuron 200® SHg (a phospholipid), cholesterol, vitamin E acetate, Carbopol-940 and triethanolamine. Placebo formulation 2 contains Proliposome 3080 S® (a phospholipid), cholesterol, vitamin E acetate, Carbopol-940 and triethanolamine. A conventional placebo gel containing only Carbopol-940 and Triethano amine was also prepared and analyzed.

2.5.7. Extraction procedure from marketed formulations

Marketed formulation-1 was cream based while formulation-2 was a water washable ointment. Extraction of the drug from the two marketed formulations was carried out as described for gel. The stock solution was diluted similarly to give a final concentration of 100 μg/ml of acyclovir. The resulting turbid dispersion was centrifuged at 5000 rpm for 30 min, where the insoluble ingredients accumulated as a thin layer at the surface of the liquid. The clear aqueous layer was carefully siphoned off and analyzed. The amount of acyclovir was calculated as % w/w of drug in dosage form from linear regression analysis and expressed as average of three readings.

2.5.8. Recovety studies (accuracy)

To determine accuracy of the proposed method, recovery studies from both marketed formulations were also carried out at four levels (100, 150, 175 and 200%) by standard addition method. Samples were analyzed in triplicate.

2.5.9. Skin permeation studies [13]

Skin permeation studies were carried out in Keshary Chein type diffusion cells maintained at 37 ± 1°C with receiver compartment containing 10ml phosphate buffer pH 7.4 with 0.02% w/v sodium azide to prevent microbial growth. Freshly excised hairless defatted abdominal skin of guinea pig was mounted with derinis facing the receiver cell. Then 200–250 mg gel was applied on an area of one square cm. inside a Teflon ring placed on the epidermal side. Drug concentration in the receiver cell was analyzed at the end of 24 h by the method described above.

The amount of drug penetration was calculated as amount of drug in mg penetrating per square cm of skin per day from gel containing 10 mg drug. The skin permeation of drug from liposomal gels was compared against topical marketed preparations as well as gel without phospholipid (conventional gel).

3. Results and discussion

3.1. Optimization of chromatogram

Various systems were tried in order to obtain a compact spot of acyclovir. it was observed that pH had an important role in determinling the migration of the drug in the mobile phase. A

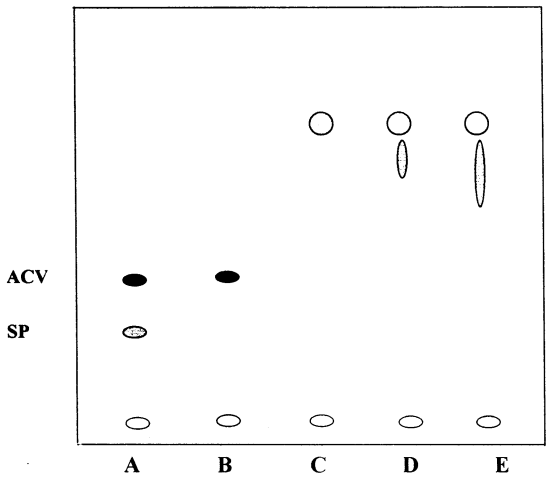

Fig. 1. Schematic representation of separation of acyclovir from skin proteins on TLC plate (after development). A, drug + skin proteins; B, pure drug (ACV); C, placebo of conventional gel; D, placebo formulation 1; E, placebo formulation 2. Acyclovir (ACV) ($R_f = 0.3 \pm 0.03$; skin proteins (SP), R_f = ranging from 0 to 0.2; conventional gel (C), $R_f = 0.7 \pm 0.05$; placebo formulation 1 (D), R_f ranging from 0.6 to 0.7; placebo formulation 2 (E), R_f ranging from 0.5 to 0.7.

mobile phase with a pH of 8.5 was found to be optimum. The suitable mobile phase was found to be chloroform–methanol–ammonia (15:9:4, v/v/v) to obtain a compact spot of drug. The proposed method gave a dense and compact spot of acyclovir with an R_f value of 0.3 ± 0.03 ($n = 6$) while skin proteins of guinea pig skin were diffused between R_f, of 0–0.2 (Fig. 1). The variation in Rf of skin proteins can be attributed to biological variation. An average of 99.97% of drug could be recovered from sample containing skin proteins.

The method is thus simple, rapid and separates skin proteins from drug adequately to allow for accurate quantification of drug during skin permeation studies.

3.2. Calibration plots

The peak area versus drug concentration was plotted to construct a standard curve of ACV. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation $r = 0.9965 \pm 0.00033$; slope = 1250.913 ± 45.63 and intercept = 100213.4 ± 3764.1 ($n = 6$) over the concentration range studied. The RSD for within day and day-to-day analysis was found to be less than 2% in all these cases.

3.3. Validation

The precision of the method was expressed in terms of relative standard deviation. Excellent method precision and system precision was evident with a low RSD of 2.85 and 1.15%, respectively. Table 1 shows a low RSD of 0.539% between peak area values obtained for the same drug solution at 0, 24, 48 and 72 h proving the ruggedness of the method. This indicates that acyclovir is stable during the extraction procedure as well as during analysis. The results of intra-day and inter-day variation analysis are shown in Table 2. The ruggedness of the method was further proved by the low average RSD of 0.763 and 0.462% obtained for peak areas for intra day and inter day variation studies, respectively.

The limit of detection was found to be 30 ng where the drug could be detected without any noise, The limit of quantitation was 50 ng, The results for assay and recovery studies presented in Table 3 indicate that the developed method can be used to quantify acyclovir in pharmaceutical dosage forms (gel, cream and ointment).The extraction procedure is very simple with no interfer-

Table 1
Ruggedness ($n = 3$)

Amount of drug (ng)	Time (h)	Peak area values	Mean ± SD	% RSD
400	0	678009.55	675038.16 ± 3641.66	0.539
	24	669831.95		
	48	675332.72		
	72	676978.40		

Table 2
Interday and intraday variation ($n = 3$)

Experimnetal level	Theoretical	Experimental content (ng)	% RSD
Level 1			
Within day	50	50.35	0.69
Interday	50	50.24	0.48
Level 2			
Within day	400	404.08	1.02
Interday	400	401.26	0.32
Level 3			
Within day	1000	1005.8	0.58
Interday	1000	1005.9	0.59

ence during quantification from excipients present which was confirmed by analyzing placebo gels similarly. Excipients of conventional gel showed an R_f of 0.7. In placebo formulation 1 and 2 different exciplents were used. The R_f of excipients in case of placebo formulation 1 ranged from 0.6–0.7 and those of placebo formulation 2 ranged from 0.5–0.7. Thus adequate separation of excipients from drug was achieved eliminating excipient interference during analysis. Method showed excellent recovery from marketed creams, the average recovery being 101.8 and 100.1%, respectively, proving the accuracy of the method as well as the suitability of the extraction procedure. High percent of recovery shows that there is no interference from excipients used in marketed formulations.

Table 4
In vitro skin permeation data od acyclovir from different formulations ($n = 6$)

Formulation	Skin permeation (μg/cm^2 per day) mean ± SD
Liposomal gel	1431.54 ± 36.25
Liposomal gel	2174.91 ± 14.67
Conventional gel	105.21 ± 8.99
Marketed	156.677.65
Marketed	261.78 ± 12.315

3.4. Skin permeation studies

The method was employed in selecting the optimum formulation on basis of maximum permeation of acyclovir through guinea pig skin. The results are as shown in Table 4. No interference during analysis due to skin proteins was encountered. Skin proteins being more polar in nature had a lower R_f value ranging from 0 to 0.2.

4. Conclusion

The proposed HPTLC method of analysis is simple, rapid and selective. Statistical data analysis proves that the method is reproducible and

Table 3
Assay recovery analysis ($n = 3$)

Formulation	Label claim, % w/w	Label claim, %	Observed, % (mean ± SD)	% Recovery
Marketed 1[a]	5	100	102.98 ± 1.79	102.98
		150	150.51 ± 1.64	100.34
		175	177.88 ± 1.54	101.64
		200	199.98 ± 2.50	99.99
Marketed 2[b]	5	100	101.16 ± 0.29	101.16
		150	154.41 ± 1.22	102.94
		175	171.27 ± 1.14	97.86
		200	197.58 ± 2.25	98.79
Liposomal gel	5	100	101.54 ± 0.44	101.54
Conventional gel	5	100	102.40 ± 0.65	102.40

[a] Average recovery = 101.22%.
[b] Average recovery = 100.19%.

precise. The method was thus found to be economical and can be employed for routine analysis of the drug in pharmaceutical formulations as well as to monitor skin permeation of drug.

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