



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

## Simultaneous determination of chloramphenicol and hydrocortisone acetate in cream using TLC densitometry method

\*Nia Kristiningrum, Mia Rakhmawati

Research and Development Centre in Laboratory of Chemical Pharmacy, University of Jember, East Java, Indonesia

### ABSTRACT

A rapid and reproducible TLC method was developed for the determination of hydrocortisone acetate and chloramphenicol in cream. The analytes were dissolved with methanol and chromatographed on silica Gel GF 254 TLC plate using chloroform:ethyl acetate in the ratio of 1:1.5 (v/v) as mobile phase. Spots at R<sub>f</sub> 0.29 and R<sub>f</sub> 0.59 were recognized as chloramphenicol and hydrocortisone acetate, respectively. Quantitative analysis was done through densitometric measurement at wavelength 265 nm. Method was found linear over the concentration range of 300-900 ng/spot with the correlation coefficient of 0.999 and 0.998 for hydrocortisone acetate and chloramphenicol, respectively. Specificity showed calculation of purity and identity more than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) of the method were 23.84 and 71.51 ng/spot for hydrocortisone acetate, 21.06 and 63.18 ng/spot for chloramphenicol. The precision of this method was less than 2.8% whereas the means of the recovery data were 100.40±0.579% for hydrocortisone acetate and 100.24±1.20% for chloramphenicol. The proposed method has been applied to the determination of hydrocortisone acetate and chloramphenicol in commercial cream formulations and the recovery of label claim were 99.23±0.66% (chloramphenicol) and 99.25±0.41% (hydrocortisone acetate) for brand A and 100.32±0.87% (chloramphenicol) and 100.53±0.78% (hydrocortisone acetate) for brand B. The developed method was successfully used for the assay of hydrocortisone acetate and chloramphenicol. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

**Key Words:** Validation, chromatography, analysis, linearity, reproducible, accuracy.

### INTRODUCTION

Hydrocortisone acetate (Figure 1-A), is a corticosteroid that is able to cope with low potential itching and reduce inflammation caused by dermatitis. Hydrocortisone can prevent or suppress the onset of symptoms of radiation-induced inflammation, infection, chemicals, and allergen (Gunawan *et al.*, 2007). Chloramphenicol (Figure 1-B), an antibiotic, possesses broad spectrum antibacterial activity and is used for the treatment Gram positive and Gram negative bacterial infections. The combination of hydrocortisone acetate and chloramphenicol are used for dermatitis and anti-infection. Many analytical method including spectrophotometric (Blanco *et al.*, 1999) and TLC (Bhawani *et al.*, 2010) have been reported for the determination of hydro-

cortisone acetate. TLC-densitometry also reported for the estimation of chloramphenicol and prednisolone acetate in their individual and combined pharmaceutical formulations (Musharraf *et al.*, 2012). However, no TLC densitometry method is available for quantitative determination of hydrocortisone acetate and chloramphenicol simultaneously in its pharmaceutical dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise TLC-densitometry method for determination of hydrocortisone acetate and chloramphenicol simultaneously.

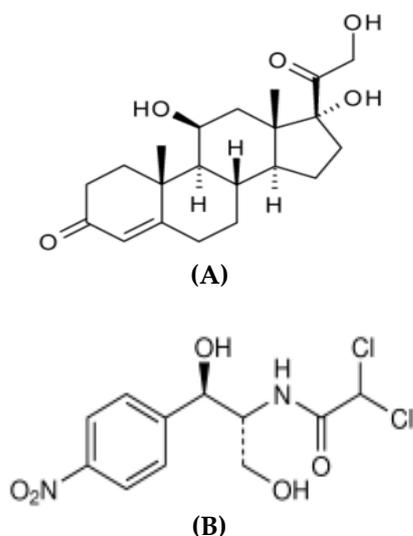
### EXPERIMENTAL

#### Material and Reagents

Chloramphenicol working standard (Wuhan Grand Pharmaceutical Group Co., Ltd.), Hydrocortisone acetate (Tianjin Tianyao Pharmaceuticals Co., Ltd.), Methanol, chloroform (Merck) and ethyl acetate

#### \*Corresponding Author:

Nia Kristiningrum, Lecturer  
Department of Chemistry, Faculty of Pharmacy  
Jember University, Kalimantan street I No 2  
Jember, Indonesia 68121  
E-mail: [neeya\\_k@yahoo.co.uk](mailto:neeya_k@yahoo.co.uk)  
Contact No.: 08123074417



**Figure 1: Structures of hydrocortisone acetate (A) and chloramphenicol (B).**

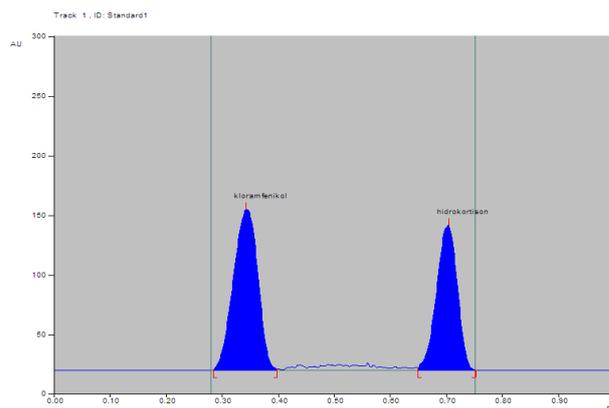
(Riedel-de Haën). Commercial cream containing hydrocortisone acetate and chloramphenicol.

#### Preparation of standard solutions and pharmaceutical samples

Stock standard solution was always freshly prepared by dissolving 25 mg of each Hydrocortisone acetate and Chloramphenicol in 25ml methanol, individually and in combination. Working standard solutions were prepared by dilution of stock solution with methanol to get solutions in concentration range of 150-450 ppm. For sample preparation, cream was weighed so that contain equivalent of 2,5 mg Hydrocortisone acetate and 2 mg chloramphenicol. The cream was dissolved in 25ml volumetric flask, and approximately 15 ml of methanol was added. The mixture was ultrasonic and diluted to 25ml.

#### TLC Method and Chromatographic Condition

Planar chromatography was performed by spotting the sample on precoated TLC silica gel GF 254 (20 x 10 cm) using 2.0 $\mu$ l glass capillaries. A Camag Twin Through Chamber containing a mixture of chloroform and ethyl acetate (1:1.5) was saturated. The spots move to a distance of 9 cm. Densitometric scanning was performed on Camag TLC Scanner 3 in the absorbance mode at 265 nm for all measurements. The slit dimension was kept at 6.00 mm x 0.30 mm and a scanning speed of 20 mm/s was employed. Chloramphenicol was detected at Rf 0.29 and



**Figure 2: Densitogram of standard chloramphenicol (Rf: 0.29) and hydrocortisone acetate (Rf : 0.59), Chloroform : ethyl acetate (1.0: 1.5, v/v).**

Hydrocortisone acetate was detected at 0.59. Quantitative evaluation was performed via peak areas by WinCat<sup>®</sup> software (version 1.4.1.8154).

#### Method Validation

The developed method was validated with the following parameters.

##### Specificity

The Specificity of this method was determined by analyzing standard and sample. Specificity was showed by purity and identity test that determined by scanning at 200nm-400nm. Calculations for identity checks ( $rS,S$  and  $rS,A$  where S is spectrum standard and A is spectrum sample and purity checks ( $rS,M$  and  $rM,E$  where S = start, M = center; and E = end of spectrum).

##### Linearity

The evaluation of the calibration curve's linearity was done based on spots of the standard solutions prepared in methanol at the concentrations 150, 200, 250, 300, 350, 400 and 450 ppm. Peak area was recorded for each concentration and a calibration curve was obtained by plotting peak area vs concentration.

##### Limit of Detection and Quantification

Standard solution were prepared at the concentration 20, 30, 40, 50 ppm, 60 and 90 ppm and 2  $\mu$ l of each of these solutions was spotted on the TLC plate. Peak area was recorded for each concentration. Limit of detection (LOD) and limit of

**Table 1: Optimum condition for analysis of hydrocortisone acetate and chloramphenicol.**

Parameters	Data
Solute	Methanol
Eluent	Chloroform : ethyl acetate = 1:1.5 (v/v)
Stationary phase	Silica gel GF 254
Wavelength	265 nm
Concentration optimum	Chloramphenicol - 250 ppm Hydrocortisone acetate - 200 ppm

**Table 3: Result of precision evaluation of hydrocortisone acetate.**

Measurement <sup>a</sup>	RSD value (%) (n=6) <sup>b</sup>
1	0.769%
2	1.749%
3	1.735%
Average RSD	1.418%

<sup>a</sup>Each measurement was performed by the same analyst and on a different plate and in different days.

<sup>b</sup>Evaluated by one analyst on one plate (repeatability).

quantification (LOQ) were determined using software validation method version 1.13.

#### Precision

The precision of this method was performed by repeatability and intermediate precision studies. Repeatability studies was performed by analyzing one concentration of the drug for six times on the same day. The intermediate precision was checked by repeating studies on three different days.

#### Accuracy

The accuracy of this method was evaluated through recovery experiments by adding three different amounts of hydrocortisone acetate and chloramphenicol standards i.e. 40, 50 and 60% of the concentration samples. Each concentration were replicated (n=3).

#### Analysis of Marketed formulations

The Samples that is contain of hydrocortisone acetate and chloramphenicol (brand A and brand B) were prepared as sample preparation method. Each of samples were replicated (n=3) and spotted on plates. The analysis was done in the same way as described earlier.

**Table 2: Result of precision evaluation of chloramphenicol.**

Measurement <sup>a</sup>	RSD value (%) (n=6) <sup>b</sup>
1	1.394%
2	1.024%
3	1.075%
Average RSD	1.164%

<sup>a</sup>Each measurement was performed by the same analyst and on a different plate and in different days.

<sup>b</sup>Evaluated by one analyst on one plate (repeatability).

**Table 4: Accuracy result of commercial creams.**

Analyte	Label claim [%] (mean ± SD)	Added [%]	Recovery
Chloramphenicol	100.65% ± 1.164	40%	100.91% ± 0.57
		50%	98.94% ± 0.55
		60%	100.87% ± 1.15
		Average recovery ± SD	100.24 % ± 1.20
Hydrocortisone acetate	100.14% ± 1.418	40%	100.24% ± 0.40
		50%	100.76% ± 0.40
		60%	100.21% ± 0.85
		Average recovery ± SD	100.40 % ± 0.57

## RESULTS AND DISCUSSION

### Optimum Condition

Table 1 shows the optimum conditions for analysis of hydrocortisone acetate and chloramphenicol simultaneously using TLC densitometry. The mobile phase of chloroform : ethyl acetate (1 : 1.5 v/v) give the efisien chromatogram. Efficiency of chromatogram was evaluated by the value of *Number of Theoretical Plate* (N), *Height Equivalent to a Theoretical Plate* (H) and *resolution* (Rs). The Rf of analytes are 0.29 for chloramphenicol and 0.59 for hydrocortisone acetate (Figure 2). Concentration optimum for hydrocortisone acetate was 200 ppm and chloramphenicol was 250 ppm.

### Method validation

From the TLC densitometry, showed that analyte spots in samples were identical with standards. Purity check of the analyte spots using winCATS software also showed that analyte spots were pure.

**Table 5: Results of analysis of hydrocortisone acetate and chloramphenicol in pharmaceutical formulation.**

Formulation	%Recovery ± SD of chloramphenicol	%Recovery ± SD of hydrocortisone acetate
Brand A	99.23% ± 0.66	99.25% ± 0.41
Brand B	100.32% ± 0.87	100.53% ± 0.78

**Table 6: Validation parameters for standard hydrocortisone acetate and chloramphenicol.**

Parameter	Hydrocortisone acetate	Chloramphenicol
Specificity	Purity test $\geq 0.99$ Identity test $\geq 0.99$	Purity test $\geq 0.99$ Identity test $\geq 0.99$
Linearity	$r = 0.999$ $V_{xo} = 1.423\%$	$r = 0.998$ $V_{xo} = 2.361\%$
Sensitivity	LOD=23.84ng/spot LOQ=71.51ng/spot	LOD=21.06ng/spot LOQ=63.18ng/spot
Precision	Average RSD= 1.418%	Average RSD= 1.164%
Accuracy	Average recovery $\pm$ SD = 100.% $\pm$ 0.57%	Average recovery $\pm$ SD = 100.24 $\pm$ 1.2%

The values of  $r_{S,M}$  and  $r_{M,E}$  were  $> 0.999$ , demonstrating that proposed TLC method is highly specific. Linearity of Hydrocortisone acetate gave the equation  $Y = 669.882 + 5.889X$  with correlation coefficient ( $r^2$ ) value of 0.999. Linearity of Chloramphenicol gave the equation  $Y = 864.717 + 6.774X$  correlation coefficient ( $r^2$ ) value of 0.998. The LOD and LOQ (Limit of Detection and Quantification) were found to be 11.918 ppm and 35.754 ppm for hydrocortisone acetate, 10.530 ppm and 31.590 ppm for chloramphenicol. All the values of the repeatability and intermediate precision evaluation were less than 2.8% (table 2 and 3). The three measurements were performed within one laboratory by same analyst on different plates and in different days. The accuracy of the proposed method were 100.40 $\pm$ 0.57% for hydrocortisone acetate and 100.24 $\pm$ 1.20% for chloramphenicol (table 4). The summary of data validation parameters is listed in table 6.

### Analysis of Marketed formulations

The result of the analysis of marketed formulations brand A and brand B showed that there was no interference from the excipients. The result given in table 5 indicate that the concentrations of hydrocortisone acetate and chloramphenicol in cream is within the requirements (USP) 90-110% for hydrocortisone acetate and 90-130% for chloramphenicol.

### CONCLUSION

A new TLC method has been developed for the identification and quantification of hydrocortisone acetate and chloramphenicol in cream simultaneously. The method was found to be simple, rapid, specific, sensitive, precise and accurate for estimation and can be conveniently employed for the routine quality control analysis hydrocortisone acetate and chloramphenicol in cream.

### REFERENCES

- Bhawani, S.A., Sulaiman, O., Hashim, R., Mohamad Ibrahim, M.N. (2010) Thin Layer Chromatographic Analysis of Steroids : A Review. *Tropical Journal of Pharmaceutical Research*, 9 (3): 301-312. [\[DOI\]](#)
- Blanco, M., Coello, J., Iturriaga, H., MasPOCH, S., Villegas, N. (1999) Kinetic Spectrophotometric Determination of Hydrocortisone Acetate in a Pharmaceutical Preparation by Use of Partial Least – Squares Regression, *Analyst*, 124 : 911-915. [\[DOI\]](#)
- Gunawan, S.G., Setiabudy, R., Nafrialdi dan Elysabeth. (2007) *Farmakologi dan Terapi Edisi 5*. Jakarta: FKUI.
- Musharraf, S.G., Fatima, U., Sultana, R. (2012) Stress Degradation Studies and Development of Stability – Indicating TLC – densitometry Method for Determination of Prednisolone Acetate and Chloramphenicol in Their Individual and Combined Pharmaceutical Formulations, *Chemistry Central Journal*, 6 (7) : 2-9.
- The United State Pharmacopoeia 25-National Formulary 20. Asia Edition. Rockville: United State Pharmacopoeial Convention.