Asian Journal of Chemistry Vol. 20, No. 2 (2008), 1130-1136

Simultaneous Estimation of Chlorhexidine Gluconate, Metronidazole, Lignocaine Hydrochloride and Triamcinolone Acetonide in Combined Dosage Form by RP-HPLC

K.S. SYED ALI ABTHEEN, R. MAHESWARI, P. SHANMUGASUNDARAM and M. VIJEYAANANDHI*

Department of Pharmaceutical Analysis, Vel's College of Pharmacy
Chennai-600 117, India
E-mail: mvaanandhi@yahoo.co.in

A simple, selective, rapid and precise reverse phase HPLC method has been developed for the simultaneous estimation of chlorhexidine gluconate, metronidazole, lignocaine hydrochloride and triamcinolone acetonide from gel dosage form. The method was carried out on Phenomenex Luna C_{18} (250 × 4.6 mm, 5 μ) column with a mobile phase consisting of sodium dihydrogen phosphate (pH 3.0 ± 0.05) buffer and acetonitrile programmed for gradient elution at a flow rate of 1 mL/min. The detecting wavelength is 230 nm using Photo diode array (PDA) detector. The retention times of chlorhexidine gluconate, metronidazole benzoate, lignocaine hydrochloride and triamcinolone acetonide were 13.50, 17.98, 10.52 and 16.65, respectively. The proposed method was also validated and it was found to be selective, accurate and precise.

Key Words: RP-HPLC, Chlorhexidine gluconate, Metronidazole, Lignocaine hydrochloride, Triamcinolone acetonide.

INTRODUCTION

Chlorhexidine gluconate[1,2] (CHG) is an antimicrobial agent. Chemically, it is designated as 1,1'-hexamethylene-*bis*-[5-(*p*-chlorophenyl) biguanide] di-D-gluconate. Chemically, metronidazole[3-5] (MZ) is 2-methyl-5-nitro-1H-imidazole-1-ethanol indicated for the topical treatment of inflammatory lesions. Lignocaine hydrochloride[6,7] (LGH) is chemically designated as acetamide, 2-(diethylamino)-N-(2,6-dimethylphenyl)-, monohydrochloride, used as local anesthetic. Triamcinolone acetonide[8-11] (TCA) is chemically pregna-1,4-diene-3,20-dione,9-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)*bis*(oxy)]-, (11β,16α)-, indicated for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses. Several analytical methods like HPLC,

spectroscopy, HPTLC are reported for the estimation of these drugs individually and in combination with other drugs. There is no analytical method reported for the simultaneous estimation of these drugs in combined dosage forms. The present work describes a simple, precise and accurate RP-HPLC[12,13] method for the simultaneous estimation of CHG, MZ, LGH and TCA in combined dosage form.

EXPERIMENTAL

Working standards: Chlorhexidine gluconate (CHG); metronidazole benzoate (MZB); lignocaine hydrochloride (LGH); triamcinolone acetonide (TCA). Most of the chemicals *viz.*, sodium dihydrogen phosphate, triethyl amine, phosphoric acid and acetonitrile are of HPLC grade.

Instrumentation: HPLC Agilent with Phenomenex Luna C_{18}, 250 × 4.6 mm, 5 µm column.

Chromatographic conditions: Column : C_{18} column, 250 × 4.6 mm, 5 µ, L1 pack; Flow rate : 1.0 mL/min; Detection wavelength : 230 nm; Injection volume : 20 µL; Column temperature : 30 °C.

Buffer preparation: 15.8 g of sodium dihydrogen phosphate and 5 mL of triethylamine were dissolved in 1000 mL water, pH adjusted to 3.0 ± 0.05 with orthophosphoric acid.

Diluent: Buffer-acetonitrile in the ratio of 50:50.

Gradient program[13]:

Time (min)	Buffer (%)	Acetonitrile (%)
0	90	10
10	50	50
20	50	50
22	90	10
30	90	10

Preparation of standard stock: Accurately about 500 mg of chlorhexidine gluconate, 200 mg of lignocaine hydrochloride and 100 mg of metronidazole benzoate were weighed and transferred into a clean, dry 100 mL volumetric flask and 20 mL of acetonitrile is added, dissolved and made up to volume with diluent.

Preparation of standard: Accurately about 10 mg of triamcinolone acetonide is weighed and transferred into a dry 100 mL volumetric flask and 10 mL of standard stock is added and made up to volume with diluent.

Sample preparation: 1 g of the sample is weighed into a 100 mL volumetric flask. 20 mL of diluent is added and sonicated for 15 min and made up to volume with diluent. The solution is filtered through 0.45 µ membrane.

Estimation: Separately injected both the standard (5 injections) and sample preparations (2 injections) into the chromatograph and the major responses are recorded. The percentage relative standard deviation of five standard injections should not be more than 2.0 %.

Calculation: For three drugs (chlorhexidine gluconate, lignocaine HCl and triamcinolone acetonide) the assay values are calculated using the formula:

$$\frac{\text{Avg. sample area} \times \text{standard weight} \times \text{standard dilution}}{\text{Avg. standard area} \times \text{sample weight} \times \text{sample dilution}} \times \text{Standard purity}$$

For metronidazole*:

$$\frac{\text{Avg. sample area} \times \text{standard weight} \times \text{standard dilution} \times A}{\text{Avg. standard area} \times \text{sample weight} \times \text{sample dilution} \times B} \times \text{Standard purity}$$

where A = molecular weight of metronidazole; B = molecular weight of metronidazole benzoate. *The values obtained are for metronidazole benzoate, the assay values are calculated in terms of metronidazole using the above formula.

The results are determined for the assay; the percentage relative standard deviations are calculated for each drug's assay value and are tabulated in Table-1.

Validation[13,14]

System suitability: The standard solution is prepared at working concentration and analyzed as per the method. The major suitability parameters studied are resolution, tailing factor and number of theoretical plates. The results are tabulated in Table-2.

Linearity and range: The linearity of the HPLC method was demonstrated for standard solutions equivalent to 50 to 150 % of the working strength. Six standard solutions at concentrations within the mentioned range were prepared and analyzed as per the method.

The linearity was demonstrated for chlorhexidine gluconate solutions in concentration ranging from 0.05 to 0.15 mg/mL, for metronidazole benzoate solutions from 0.005 to 0.015 mg/mL, for lignocaine hydrochloride solutions from 0.10 to 0.30 mg/mL and for triamcinolone acetonide solutions from 0.005 to 0.015 mg/mL.

The line of best fit is drawn by plotting concentration *vs.* peak area response and from the calibration curve the regression value is determined for each drug. The results are tabulated in Table-3.

System precision: The standard solution is prepared at working concentration and analyzed in replicate as per the method. The results obtained by repeating the estimation procedure were observed to be good.

Precision of the method is expressed in terms of percentage relative standard deviation of the data obtained, here in this case the peak area obtained. The results are tabulated in Table-4.

Method precision: The sample solution is prepared at working concentration and analyzed in replicate as per the method. Precision of the method is expressed in terms of percentage relative standard deviation of the data obtained, here in this case the percentage label claim obtained. The results are tabulated in Table-5.

Accuracy: The accuracy of the method is determined by analyzing three solutions containing chlorhexidine gluconate, metronidazole, lignocaine hydrochloride and triamcinolone acetonide and at *ca.* 50, 100 and 150 % of the working strengths spiked with placebo. The percentage recovery results obtained are listed in Table-6.

RESULTS AND DISCUSSION

The mobile phase composed of sodium dihydrogen phosphate (pH 3.0 ± 0.05) buffer and acetonitrile programmed for gradient elution offered maximum resolution for the drugs at the wavelength of about 230 nm at which all the drugs had maximum absorption.

The assay values are calculated in terms of percentage label claim and were found to be 93.39 for chlorhexidine gluconate, 102.96 for metronidazole, 106.27 for lignocaine hydrochloride and 99.33 for triamcinolone acetonide with percentage relative standard deviations of 1.03, 0.84, 0.93 and 1.16, respectively (Table-1).

TABLE-1
QUANTITATIVE ESTIMATION

Sample	Label claim (mg)	Amount present (mg)	Label claim (%)*	RSD (%)
CHG	1.0	0.933	93.39	1.03
MZB	1.0	1.030	102.96	0.84
LGH	2.0	2.090	106.27	0.93
TCA	0.1	0.099	99.33	1.16

*Each value is mean of three values.

System suitability parameters like resolution, tailing factor and number of theoretical plates were studied, having values for resolution not less than 2.0, for tailing factor not more than 2.0 and for number of theoretical plates not less than 2000 and thus found to be suitable (Table-2).

TABLE-2
SYSTEM SUITABILITY

Sample	Resolution	Tailing factor	No. of theoretical plates
CHG	8.55	2.00	13240
MZB	3.18	1.20	24641
LGH	-	1.72	32686
TCA	7.50	1.22	31058

The linearity studies were performed with standard solution prepared at serial concentrations. There exists linearity in the concentration range of 0.05 to 0.15 mg/mL for chlorhexidine gluconate, 0.005 to 0.015 mg/mL for metronidazole, 0.10 to 0.30 mg/mL for lignocaine hydrochloride and 0.005 to 0.015 mg/mL for triamcinolone acetonide. The values for coefficient of regression were found to be not less than 0.9990 (Table-3).

TABLE-3
LINEARITY AND RANGE

Sample	Linearity Range (mg/mL)	Regression coefficient
CHG	0.050 to 0.150	0.9992
MZB	0.005 to 0.015	0.9990
LGH	0.100 to 0.300	0.9994
TCA	0.005 to 0.015	0.9994

System precision (Table-4) and method precision (Table-5) are performed with five replicate solution of standard and sample (in working concentration), respectively. The percentage relative standard deviations values obtained with these studies were found to be not more than 2 %.

TABLE-4
SYSTEM PRECISION

S. No.	Peak areas			
	CHG	MZ	LGH	TCA
1	3483817	6163437	2406523	324598
2	3459713	6157824	2402824	325475
3	3456397	6152163	2402066	325749
4	3456397	6152553	2402066	325888
5	3457807	6149647	2403283	325944
Average	3463434	6155125	2403683	325531
RSD (%)	0.33	0.09	0.07	0.17

TABLE-5
METHOD PRECISION

S. No.	Label claim (%)			
	CHG	MZ	LGH	TCA
1	93.85	103.01	106.77	100.31
2	94.14	102.87	106.75	99.69
3	92.37	102.94	107.20	100.00
4	92.31	102.89	106.78	98.75
5	92.30	102.96	106.78	99.37
Average	92.99	102.93	106.85	99.62
% R.S.D	0.99	0.05	0.18	0.60

The accuracy of the method was assessed by spiking the drugs at the levels of 50, 100 and 150 % with placebo and calculating the percentage recovery. The values for percentage recovery were found to be 98.49 for chlorhexidine gluconate, 99.48 for metronidazole, 98.56 for lignocaine hydrochloride and 98.08 for triamcinolone acetonide. Thus the values for percentage were found to be within the standard acceptable limit of 98.0 to 102.0 % (Table-6).

TABLE-6
ACCURACY

Sample	Level (%)	Theoretical conc. (mg/mL)	Measured conc. (mg/mL)	Recovery* (%)
CHG	50	0.0501	0.0493	98.49
	100	0.1002	0.0986	
	150	0.1500	0.1480	
MZ	50	0.0501	0.0498	99.48
	100	0.1003	0.0998	
	150	0.1504	0.1497	
LGH	50	0.1003	0.0992	98.56
	100	0.2007	0.1973	
	150	0.3010	0.2964	
TCA	50	0.0052	0.0051	98.08
	100	0.0104	0.0102	
	150	0.0156	0.0153	

*Each value is mean of three values.

Thus the developed method was found to be accurate, precise and specific for the estimation of chlorhexidine gluconate, metronidazole, lignocaine hydrochloride and triamcinolone acetonide. The method can thus be adopted for the assay of chlorhexidine gluconate, metronidazole, lignocaine hydrochloride and triamcinolone acetonide in gel formulation.

ACKNOWLEDGEMENT

The authors are thankful to Fourrts India Laboratories, Kelambakkam, for providing the necessary facilities.

REFERENCES

1. L. Havlíková, L. Matysová, L. Nováková, R. Hájková and P. Solich, *J. Pharm. Biomed. Anal.*, **43**, 1169 (2007).
2. Y. Zhu,Y. Yang and Q. Qi, *Wei Sheng Yan Jiu*, **32**, 51 (2003).
3. A. Mishal and D. Sober, *J. Pharm. Biomed. Anal.*, **39**, 819 (2005).
4. D.K. Bempong, R.G. Manning, T. Mirza and L. Bhattacharyya, *J. Pharm. Biomed. Anal.*, **38**, 776 (2005).
5. M.S. Ali, R.S. Chaudhary and M.A. Takieddin, *Drug Develop. Ind. Pharm.*, **25**, 1143 (1999).
6. W.F. El-Hawary, *J. Pharm. Biomed. Anal.*, **27**, 97 (2002).
7. P.V. Devarajan, M.H. Adani and A.S. Gandhi, *J. Pharm. Biomed. Anal.*, **22**, 685 (2000).
8. S. Sudsakorn, L. Kaplan and D.A. Williams, *J. Pharm. Biomed. Anal.*, **40**, 1273 (2006).
9. S. Yasser, El-Saharty, N.Y. Hassan and F.H. Metwally, *J. Pharm. Biomed. Anal.*, **28**, 569 (2002).
10. S.A. Döppenschmitt, B. Scheidel, F. Harrison and J.P. Surmann, *J. Chromatogr. B: Biomed. Sci. Appl.*, **682**, 79 (1996).
11. L. Matysova, R. Hajkova, J. Sicha and P. Solich, *Anal. Bio-anal. Chem.*, **376**, 440 (2003).
12. P.D. Sethi, HPLC: Quantitative Analysis Pharmaceutical Formulations, CBS Publishers and Distributors, New Delhi (India), pp. 101-137 (2001).
13. R. Snyder, J. Kirkland and L. Glajch, Practical HPLC method development, A Wiley International Publication, edn. 2, pp. 100-109, 303-313, 686-698 (1997).
14. Validation of Analytical Procedures/Methodology, ICH hormonised Triplicate Guidelines, pp. 1-8 (1996).

(*Received*: 27 January 2007; *Accepted*: 1 October 2007) AJC-5947