HPLC determination of metronidazole and chlorhexidine acetate in Taizou lotion

LI Xiao-yan(*Nanning Institute for Drug Control Nanning Gwangxi* 530001)

ABSTRACT: OBJECTIVE To establish the method on determination of metronidazole and chlorhexidine acetate in Taizou lotion by HPLC. **METHOD** An HPLC method was established by using a C_{18} column (250 mm × 4.6 mm , 10μm) as analytical column , methanol-acetonitrile-triethylamine buffer solution (0.05 mol·mL^{-1} ,adjusted pH to 2.5 with H_3PO_4) (30∶ 30∶ 40 ,v/v) as mobile phase , prednisone acetate as an internal standard . The flow rate was 1.0 mL·min^{-1} , and detection wavelength was at 254 nm. **RESULTS** The linearity was obtained over the range of 32 ~ 128μg·mL^{-1} for metronidazole (r = 0.99996) and 20 ~ 80μg·mL^{-1} for chlorhexidine acetate (r = 0.99997) , respectively . The average recovery of method was 100.4 % with RSD 0.19 % for metronidazole and 99.94 % with RSD 0.20 % for chlorhexidine acetate , respectively. The intra-day and inter-day RSD for metronidazole were 0.12 % (n = 6) and 0.32 % (n = 5) , that for chlorhexidine acetate were 0.18 % (n = 6) and 0.35 % (n = 5) , respectively. **CONCLUSIONS** The method is simple , rapid , accurate and can be used for the quality control of the preparation.

KEY WORDS: metronidazole chlorhexidine acetate Taizou lotion HPLC
中图分类号 : R917.101 ; R984　　文献标识码 : B　　文章编号 :1007-7693(2003)03-0210-03

高效液相色谱法测定泰唑洗液中甲硝唑和醋酸氯己啶的含量

李小燕(南宁市药品检验所,广西　南宁 530001)

摘要:目的　建立泰唑洗液中甲硝唑和醋酸氯己啶含量的 HPLC 测定方法。方法　采用 C_{18} 柱(250 mm × 4.6 mm , 10μm) , 流动相为甲醇-乙腈-三乙胺缓冲液(0.05 mol·mL^{-1} ,用磷酸调节 pH 至 2.5) (30∶ 30∶ 40 ,v/v) ,内标物为醋酸泼尼松。流速为 1.0 mL·min^{-1} , 检测波长为 254 nm。结果　线性范围 : 甲硝唑 32 ~ 128μg·mL^{-1} (r = 0.99996) ,醋酸氯己啶 20 ~ 80μg·mL^{-1} (r = 0.99997) 。精密度 : 日内及日间 RSD 为 : 甲硝唑 0.12 % (n = 6) 和 0.32 % (n = 5) ; 醋酸氯己啶 0.18 % (n = 6) 和 0.35 % (n = 5) 。平均回收率甲硝唑为 100.4 %(RSD = 0.19 % n = 5) ,醋酸氯己啶为 99.94 % (RSD = 0.20 % , n = 5) 。结论　本法简便 , 快速 , 准确 , 可用于该制剂的质量控制。

关键词 : 甲硝唑 ; 醋酸氯己啶 ; 泰唑洗液 ; 高效液相色谱法

1　Introduction

Taizou lotion is a hospital preparation that composed of metronidazole and chlorhexidine acetate. It possessed anti-inflammatory and antibiotic functions . It can be used effectively in the treatment of vaginitis and external genitalia inflammation that caused by anaerobes , fungus , gonococcus and other causative agents. Different methods are described in the literature for analysis of solution of chlorhexidine acetate and metronidazole by double wavelength spectrophotometry[1] , dual-wavelength factor-multiratio method[2] and linear programming spectrophotometry[3] . In this paper , a reversed-phase HPLC method for the determination of metronidazole and chlorhexidine acetate in Taizou lotion was reported .

2　Experimental

2.1　Materials and Apparatus

Metronidazole RS , chlorhexidine acetate RS and prednisone acetate RS were obtained from National Institute for the Control of Pharmaceutical & Biological Products. Methanol and acetonitrile were of HPLC grade. Water was deionized and freshly distilled. Triethylamine and phosphoric acid were of AR grade . Radix notoginseng perfume was commercial products. Taizou lotion were obtained from Nanning Maternity and Infant Health Hospital.

The apparatus used was Shimadzu LC-10ATvp equipped with a Shimadzu SPD-10Avp UV detector , and a model 7725i sample injector (Rheodyme , cotati , CA , USA) equipped with a 20μL loop. Chromatographic data were processed by a computer of a Weima chromatographic workstation (Shenzhen shenruan electronic co. , Shenzhen China) .

2.2　Chromatographic conditions and Chromatograms

The column used was a KF-C18 , 250 mm × 4.6mm I.D, 10μm . (Dalian Institute of Chemical Physics , the Chinese Academy of Sciences) The detection wavelength was set at 254nm . The mobile phase consisted of a mixture of methanol , acetonitrile and triethylamine buffer solution (0.05 mol• L^{-1} , adjusted pH to 2.5 with H_3PO_4) (30∶ 30∶ 40 V/ V) . This solution was filtered through a 0.45μm membrane and degassed under vacuum before using. The flow rate was 1.0 mL• min^{-1} and the whole operation was under room temperature . 20μL of the sample solution was injected into HPLC. Typical chromatograms of a reference , sample and blank (prepared according to the prescription) are illustrated in Figure 1 . The retention times of metronidazole , chlorhexidine acetate and internal standard were 3.46 , 5.54 and 8.23 minutes , respectively. The overall chromatographic run time was 10 minutes . The blank did not interfere the determination.

2.3 Methods and Results

2.3.1 Preparation of Standard and Internal standard solution

Dissolved accurately weighed quantity of metronidazole RS and chlorhexidine acetate RS separately in methanol to obtain a solution having a known concentration of about 800μg• mL^{-1} for metronidazole and about 500μg• mL^{-1} for chlohexidine acetate as standard solution . Dissolved accurately weighed quantity of prednisone acetate RS in methanol to obtain a internal solution having a known concentration of about 700μg• mL^{-1} .

2.3.2 System Suitability

The column efficiency for chlorhexidine acetate was 2900 theoretical plates . The resolution between the two closest peak pairs (metronidazole and chlorhexidine acetate) was 5.2 .

2.3.3 Linearity and Correction Factor

Separately transferred an accurately measured volume of standard solution 1.0 , 1.5 , 2.0 , 2.5 , 3.0 , 3.5 and 4.0 mL to 25 mL volumetric flasks containing 2.0 mL of internal standard solution , diluted with mobile phase to volume and mixed . Separately injected 20μL these solution , recorded the chromatograms , and observed that the relationship between the peak areas of the analytes and that of the internal standard and the analyte concentration was linear (Table 1)

2.3.4 Precision of injection

Injected 20μL of the same sample solution into the chromatograph, replicates of six , recorded the chromatograms , and measured the ratio of the analytes and internal standard areas. The results of RSD were 0.12 % for metronidazoloe , and 0.18 % for chlorhexidine acetate .

Tab 1 Calibration graphs for metronidazole and chlorhexidine acetate using prednisone acetate as internal standard

表 1 甲硝唑和醋酸氯己啶的标准曲线(以醋酸泼尼松为内标物)

Component	Quantification equation : $Y(\mu g \cdot mL^{-1}) = a + bx$ (ratio of analyte / internal standard areas)				Correction factor
	Concentration range	a	b	r	
metronidazole	32 ~ 128	146	2.65	0.99996	2.5039
chlorhexidine acetate	20 ~ 80	39.3	0.0118	0.99997	0.6992

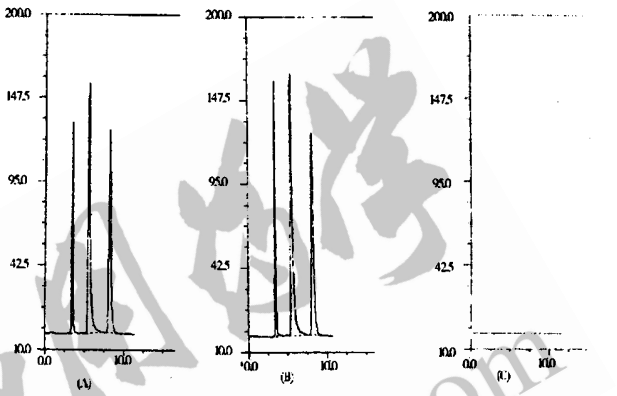

Fig 1 HPLC chromatograms of chemical reference substance (A) , sample (B) and blank (C)

图 1 对照品(A) ,样品(B) 和辅料(C) 的高效液相色谱图

1 . metronidazole ;2 . chlorhexidine acetate ;3 .internal standard

1 .甲硝唑 ;2 .醋酸氯己啶 ; 3 .内标

2.3.5 Repeated Test

Prepared 5 lots of the same batch sample solution , separately determined according to the procedure described in section "2.3.7" for sample assay . The result of average content was 99.18 % (RSD = 0.25 %) for metronidazoloe , 101.4 % (RSD = 0.32 %) for chlorhexidine acetate .

2.3.6 Recovery

Separately piped 2.00 , 2.25 , 2.50 , 2.75 and 3.00 mL of the standard solution into 25 mL of volumetric flasks containing 2.0 mL of internal standard , added radix notoginseng perfume to about quantity of the prescription , diluted with mobile phase to volume , and mixed .(the concentrations of these solution were equal to 80 % ~ 120 % of concentration of the sample solution) . Determined and calculated according to the procedure described in section "2.3.7" for sample assay . The recovery of metronidazoloe and chlorhexidine acetate were shown in Table 2 .

Tab 2 Recovery results (n = 5)

表 2 回收率测定结果(n = 5)

metronidazole				Chlorhexidine Acetate			
added (mg)	determined (mg)	Average Recovery (%)	RSD (%)	added (mg)	determined (mg)	Average Recovery (%)	RSD (%)
1 .600	1 .607	100 .4	0 .18	1 .000	1 .002	100 .2	0 .22
1 .800	1 .804	100 .2	0 .13	1 .125	1 .123	99 .82	0 .23
2 .000	2 .006	100 .3	0 .15	1 .250	1 .248	99 .84	0 .20
2 .200	2 .216	100 .7	0 .20	1 .375	1 .376	100 .1	0 .21
2 .400	2 .410	100 .4	0 .21	1 .500	1 .496	99 .73	0 .19

2 .3 .7 Sample Assay

Transferred an accurately measured volume of Taizou lotion , equivalent to about 40 mg of metronidazole , to a 100 mL volumetric flask , diluted with water , and mixed . Piped 5 mL of this solution into a 25 mL volumetric flask containing 2 .0 mL of internal standard , diluted with mobile phase to volume , and mixed , for sample solution . Injected 20μL of this solution into the chromatograph , recorded the chromatograms , analytes quantification were carried out using the internal standard method according to the correction factor in section "2 .3 .3" . The results were shown in Table 3 .

Tab 3 Results of sample determination (labeled amount %, n =5)

表 3 样品测定结果 (标示量 %, n=5)

Lot No	Metronidazole	Chlorhexidine acetate
1	102 .3	94 .08
2	99 .54	96 .83
3	99 .06	94 .18
4	99 .18	101 .4

2 .3 .8 Stability test

Determined the same sample solution (Lot No .4) , in replicates of six , in different times of a day ,and determined the solution on five different days . Calculated the contents and RSD. The intra-day were 99 .18 % and 0 .12 % for metronidazole , 101 .4 % and 0 .18 % for Chlorhexidine acetate (n = 6) . The inter day were 99 .06 % and 0 .32 % for metronidazole , and 101 .6 % and 0 .35 % for Chlorhexidine acetate (n = 5) . The results showed that the sample solution were stabile at least in 5 days

3 Discussion

3 .1 Selection of an appropriate pH value

The selection of an appropriate pH of mobile phase is very important because it can affect the separation mode . It has been reported that low pH (<2) can result in corrosion of the interior wall of the column while high pH(>8) result in dissolution of silica gel . In this paper , we found that retention time and type of peak of chlorhexidine acetate was markedly changed by modifying the pH of the buffer solution , the lower pH value , the better the type of peak . Thus the best separation efficiency can be achieved with a pH of 2 .5 .

3 .2 Selection of internal standard

Analytes quantification was carried out using the internal standard method , employing prednisone acetate . It was observed that the type of peaks and retention times of trimethoprim (3 .13 minutes) , sodium benzoate (5 .08 minutes) , diazepam (14 .01 minutes) , cortisone acetate (9 .72 minutes) and prednisone acetate (8 .92 minutes) . At last , prednisone acetate was chosen as internal standard .

3 .3 Selection of an detection wavelength

Separately scanned the solution of metronidazole , chlorhexidine acetate and prednisone acetate from 200 nm to 400 nm , the three analytes had maximum absorption at 228 nm , 259 nm and 240 nm , respectively . In this study the detection wavelength was chosen to be 254 nm . At this wavelength , the good sensitivity can be achieved .

4 Conclusion

Because of its simplicity , good recovery and precision , the HPLC method can be successfully used in routine quality control analysiss for Taizou Lotion .

References

[1] Chen Guo pin . Quantitative determination of hibitane and metronidazole in taixiao gargle by double wavelength spectrophotometry[J] . Chin Hosp Pharm J . 1994 ,14 (9) : 416 .

[2] Wu Lin , Zhou Wei mei . Determination of chlorhexidine acetate and metronidazole in Taizuo gargle by dual-wavelength factor-multiratio method[J] . Chin Hosp Pharm J . 1997 ,17(4) : 168 .

[3] Li Xiao yan . Simultaneous determination of chlorhexidine acetate and metronidazole in Taizuo lotion by linear-programming spectrophotometry[J] . Chin Hosp Pharm J . 2001 ,21(9) :50 .

收稿日期 :2002-01-28