SIMPLE AND SENSITIVE METHOD FOR DETERMINATION OF POLYHEXANIDE IN MULTIPURPOSE SOLUTION

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RESUMEN: MÉTODO SENCILLO Y SENSIBLE PARA LA DETERMINACIÓN DE POLIHEXANIDE EN SOLUCIÓN MULTIUSO.

Un método invertido sencillo de fase HPLC ha sido desarrollado para la determinación de clorhidrato biguanudo de poli-hexametileno en la solución multiuso para las lentillas hidrófilas. La fase móvil fue acetonitrile 1%(v/v) acetato amónico de 20 mmol/L en el agua como 16: 84 (V/V) en una tasa de flujo de 1 ml/min. El clorhidrato biguanudo de poli-hexametileno fue detectado por la absorción de UV a 235 nm. El pH fue mantenido a 4,0 utilizando el ácido acético glacial. El método de Yiping et al ha sido modificado ligeramente según la necesidad. La cantidad detectada es 2 μG/ml y por eso fue llevado a cabo utilizando el método de pre concentración bajo vacio. Desde el cromatograma, fue observado que un pico claro apareció al tiempo de retención 5,883 min para el clorhidrato biguanudo de poli-hexametileno. La recuperación de droga fue encontrada a 99,38% y el método fue sencillo, rápido y propio para la concentración de la droga en la solución multiuso para las lentillas hidrófilas y para el llevar a cabo la estabilidad según la directriz de ICH para valorar la estabilidad de clorhidrato biguanudo de poli-hexametileno en la solución multiuso.

PALABRAS CLAVE: Líquido estándar de lágrima (LEL). Clorhidrato de Polihexanide (CHPH). Área bajo curva (ABC)

SUMMARY:

A simple reversed phase HPLC method has been developed for the determination of polyhexamethylene biguanide hydrochloride in multipurpose solution for hydrophilic contact lenses. The mobile phase was acetonitrile 1% (v/v) ammonium acetate 20 mM in water as 16: 84 (v/v) at a flow rate of 1 ml / min. Polyhexamethylene biguanide hydrochloride was detected by UV absorption at 235 nm. The pH was kept at 4.0 using glacial acetic acid. The method of Yiping et al has been slightly modified as needed. The quantity detected was 2 μg/ml so it was carried out using a preconcentration method under vaccum. From the chromatogram, it was observed that a distinct peak appeared at retention time 5.883 min for polyhexamethylene biguanide

hydrochloride. The recovery of drug was found to be 99.38% and the method was simple, rapid and suitable for the assay of drug in multipurpose solution for hydrophilic contact lenses and for carrying out stability as per ICH guidelines to assess the stability of polyhexamethylene biguanide hydrochloride in multipurpose solution.

KEY WORDS: Standard tear fluid (STF). Polyhexanide hydrochloride (PHNB). Area under curve (AUC)

INTRODUCTION

Polyhexamethylene biguanide hydrochloride (PHMB.HCL) has been utilized as a new antimicrobial providing reliable preservation in multipurpose solution (MPS) for hydrophilic contact lenses[1,2]. It is a polymeric compound with low skin irritancy, low eye toxicity, fast speed of kill, stable and effective over a wide pH range. It is available as 20% aqueous solution (w/v) with a molecular structure as shown in figure 1.

Fig. 1: Molecular structure of PHMB.HCl.
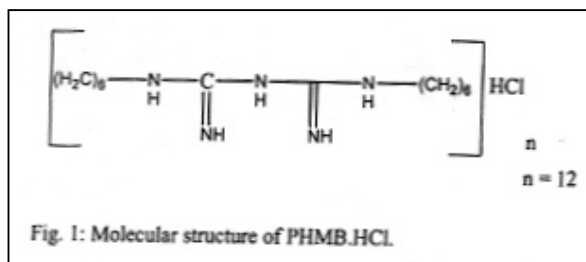
The composition of MPS is given in table 1. The MPS is found to be effective against bacteria, fungi, protozoa and viruses even at very low concentration[3,4].

Table 1: Composition of multipurpose solution (MPS)

Ingredient	Concentration
Polyhexamethylene biguanide hydrochloride	0.0002%
Boric acid	0.20%
Sodium tetraborate	0.02%
Sodium chloride	0.80%
Distilled water q.s to	100ml

In order to supervise the quality of MPS containing PHMB.HCl, it is necessary to develop the method to assay the sample of drug accurately. Reversed phase high performance liquid chromatography (RP-HPLC) with photodiode array detector (PDA) is the most frequently used method for routine determination. However, to the best of our knowledge, no RP-HPLC method for the analysis of PHMB.HCl in MPS for hydrophilic contact lenses has been reported except that of Yiping et al.[5] who has tried to establish a simple and rapid HPLC method for the routine analysis of PHMB in compound chemical disinfection.

MATERIALS AND METHODS

Materials

PHMB. HCl was obtained from Avecia Biocides, Manchester, U.K. Acetonitrile and water HPLC grade were from S.D. chemicals, Mumbai, India. Ammonium acetate, glacial acetic acid were of analytical grade and were used as received.

HPLC instrumentation and chromatographic conditions

HPLC was performed with a Shimadzu class VP series manual injector fitted with a 20 μl loop, a model SPD- 10 UV detector, a model LC- ATVP pump operated in an isocratic mode and a 4.6 mm i.d sign 50 mm Shim- pack CLC- ODS C18 column, particle size 5 μm. The mobile phase was 16:84 (v/v) of acetonitrile -1% (v/v) and ammonium acetate 20 mM in water and it was pumped out at 1 ml / min. The mobile phase was filtered through a 0.45 μm membrane filter and ultrasonified before use. The UV absorption spectrum of PHMB.HCl indicated the presence of an analytically useful absorption band with a maximum at 235 nm as shown in figure 2.

Fig. 2: UV spectrum of PHMB.HCl
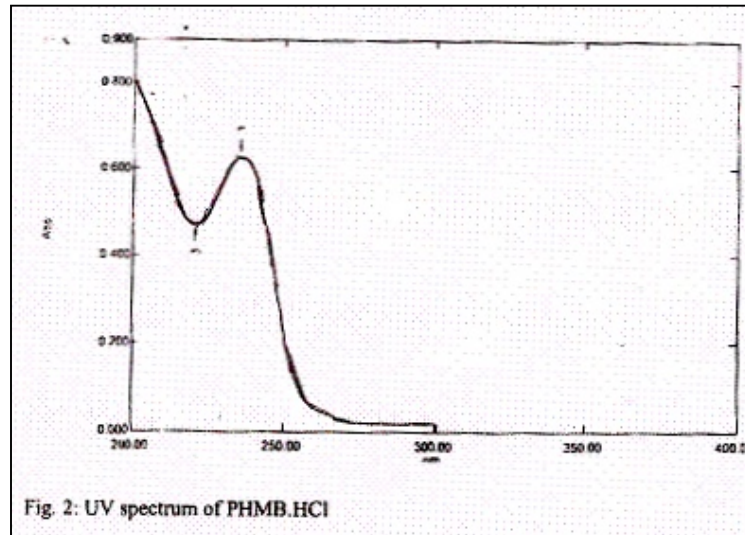
The 20 μl samples of MPS which was pre-concentrated under vacuum was injected and chromatogram was obtained. The temperature was maintained at 30 °C. Under this condition, PHMB.HCl was eluted at 5.883 min as shown in figure 3. Interferences from other ingredients of MPS were not observed.

Fig. 3: Chromatogram of PHMB.HCl (retention time 5.883 min)
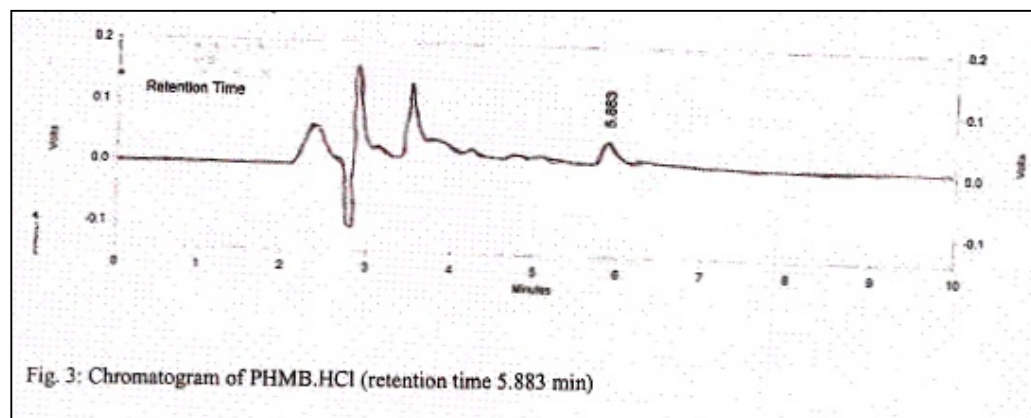
Preparation of Standard Curve

From the drug sample (20% w/v), 1.0 ml was pipetted and transformed into 100 ml capacity volumetric flask and volume was made up to 100 ml with filtered standard tear fluid (STF) of pH 7.4. The composition of STF was as mentioned in table 2.

Table 2: Composition of standard tear fluid (STF) at pH 7.4

Ingrdients	Concentration
Boric acid	0.2g
Sodium tetraborate	0.02g
Sodium chloride	0.80g
Distilled water q.s.to	100ml

From this solution 1.0 ml was further diluted up to 100 ml with the STF of pH 7.4 in a 100 ml volumetric flask in order to obtain a stock solution of 20 µg /ml. From this stock solution different volumes were withdrawn and diluted with STF of 7.4 for obtaining different concentration from 0.25 µg /ml to 7.5 µg /ml. The volume of each standard solution was 10 ml and all these were filtered through a membrane filter (0.45 µm). The 20 µl of each solution was injected into the HPLC column in order to obtain the chromatogram and AUC (area under the curve) was calculated. The observed values are given in table 3.

Table3 : HPLC reading of standard solution of PMHB.HCl (detection 235nm)

| Concentration (µg/ml)	AUC (Average)	Regressed (AUC)	±SD
0.25	110121	114268	0.632455532
0.50	120634	119580	0.894427191
1.00	131147	130204	0.983191563
1.50	141660	140828	0.894427191
2.00	152171	151452	0.894427191
2.50	162686	162076	0.894427191
3.00	173199	172700	0.632455532
3.50	183712	183324	0.894427191
4.00	194224	193948	0.894427191
4.50	204738	204572	0.632455532
5.00	215251	215196	0.983190011
5.50	225764	225820	0.894427191
6.00	236277	236444	0.632455532
6.50	246789	247068	0.816494089
7.00	257302	257692	0.894427191
7.50	267815	268316	0.632455532

n=6, results are the mean of six readings

Coefficient of correlation $(r^2) = 0.9994$

Equation of regressed line $y = 21248x + 108956$, where y is regressed AUC

Slope(m) = 21248, x is the concentration (µg/ml), intercept (c)= 108956.

The method was repeated for six times i.e. n=6. A standard plot PHMB. HCL was drawn by plotting concentration on X- axis and AUC on Y- axis and is shown in figure 4.

Fig. 4: Standard plot of PHMB.HCl in HPLC analysis
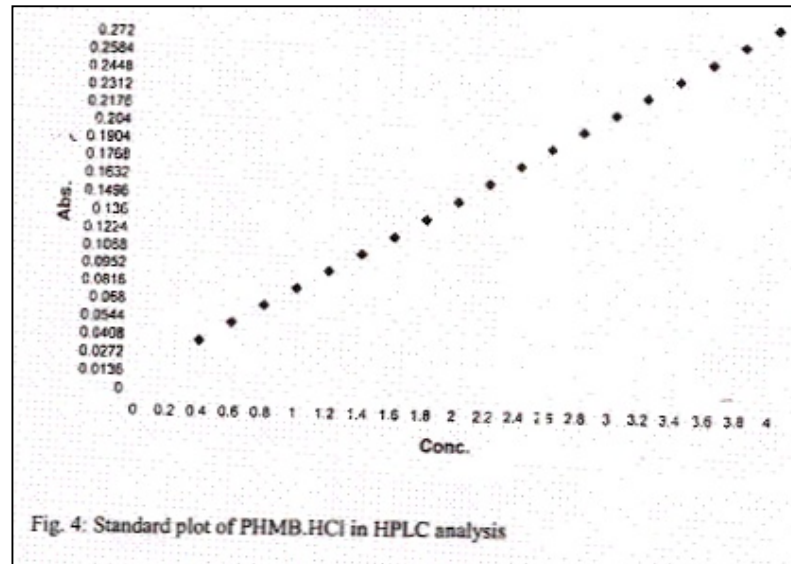
From the point observed the regressed line was drawn and the equation of this regressed line was calculated. The coefficient of correlation (r2) was also determined. The coefficient of correlation (r2) = 0.9994 and equation of regressed line y= 21248x+ 108956, where y is regressed AUC and slope (m) is 21248, x is the concentration (μg / ml) and intercept (c) is 108956.

Determination of PHMB.HCl in MPS under Stability Studies as per ICH Guidelines

Under the stability studies of the formulation, 20 μl of the sample undiluted were injected into an HPLC column, using similar conditions as in the case of the standard curve and AUC was obtained. The drug concentration obtained by extrapolation of the AUC of the sample under the standard curve and the potency of the drug in the sample was calculated.

Sample Stability

The Stability studies were carried out according to the ICH guidelines and for this purpose MPS which were developed on pilot scale were kept at 40°C and 75% RH in humidity chamber for 6 months. The samples were withdrawn at intervals of 0, 1, 2, 4 and 6 months and analyzed for drug content by the HPLC method of analysis as mentioned before. The results are given in table 4.

Table 4: Mean drug content values of stability studies at 40 ± 0.5°C & 75% RH for MPS with PHMB.HCl.

Time (days)	Mean drug content (μg /ml)	Percent drug remaining	Log % drug remaining	Slope	Degradation rate costant K (days⁻¹)
0	2.062	100	2.000		
30	2.049	99.37	1.9972		
60	2.028	98.35	1.9928	14.66×10^{-5}	33.76×10^{-5}
120	2.019	97.91	1.9908		
180	2.000	96.99	1.9867		

The results of the stability studies carried out as per ICH guidelines were compared with those of the sample at 0 day. The data converted into log percent drug remaining and it was plotted against time in days as shown in figure 5.
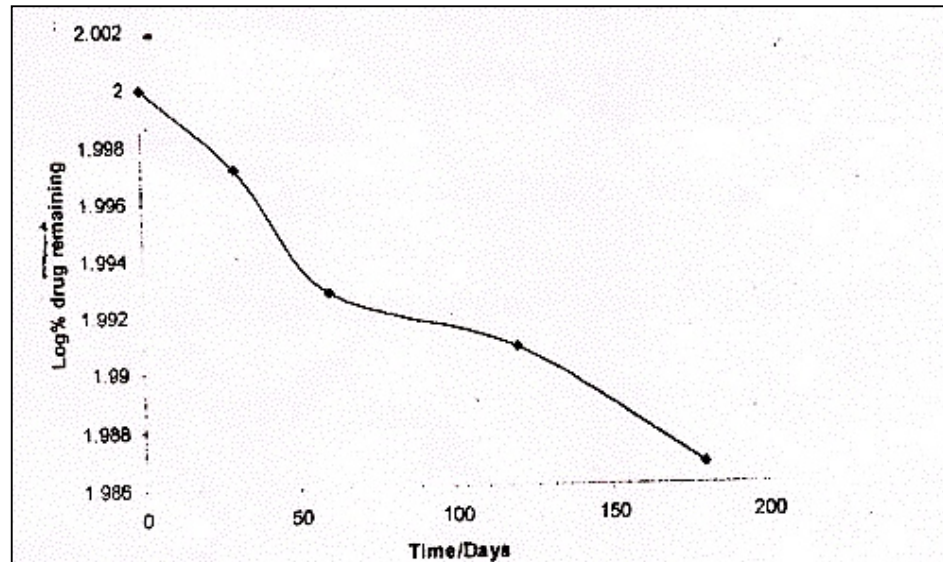
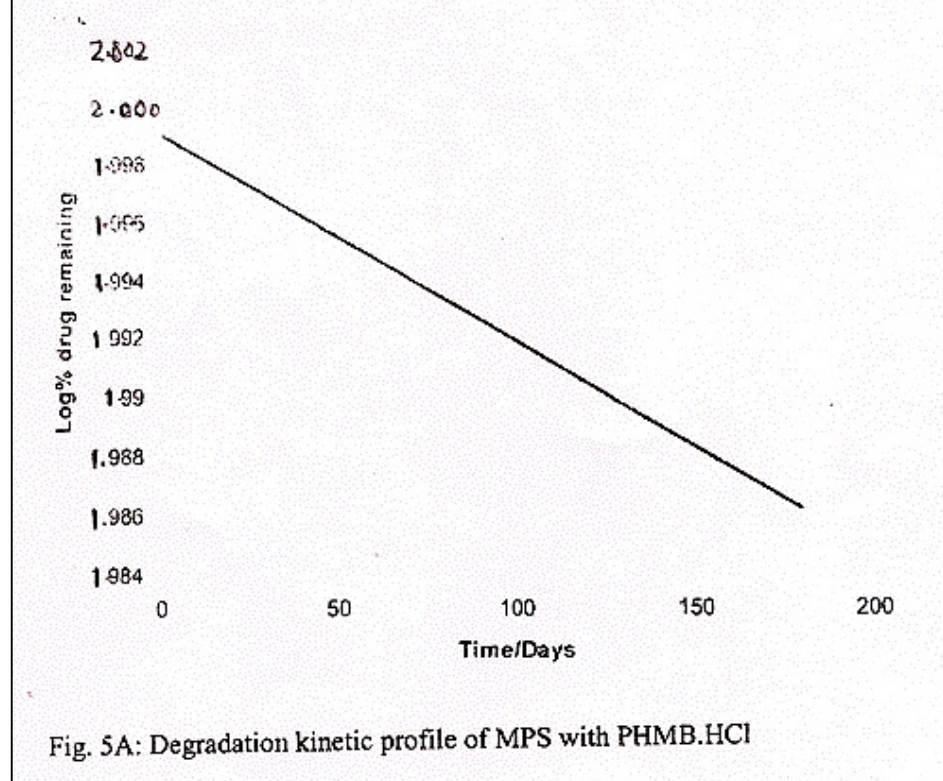Fig. 5: Degradation kinetic profile of MPS with PHMB.HCl (ICH guidelines)

Fig. 5A: Degradation kinetic profile of MPS with PHMB.HCl

The slope of the line was determined, and from this, the degradation rate constant 'K' in days was calculated.

RESULTS AND DISCUSSION.

An HPLC method of analysis of PHMB. HCl was carried using the method of Yiping et al after slight modification. Under the method, UV detection at 235 nm was performed. The UV spectrophotometric determination was carried out in isotonic STF of

pH 7.4. For this purpose, the drug solution was scanned in UV range and PHMB.HCl exhibited γ max at 235 nm. The drug was detected accurately with recovery of 99.38%. The standard curve under HPLC analysis between AUC and concentration obeyed Lambert's Beer law between the concentration of 7.5 µg/ml and a high value for the coefficient of correlation was observed. This

method of analysis was found to be simple, rapid and accurate for the determination of PHMB.HCl. The sample was preconcentrated by vaccum before subjecting it to HPLC studies.

Stability studies were carried out as per ICH guidelines in order to see the stability of drug in the MPS. Under the ICH methodology, sufficient number of containers of the product were kept at 40 ± 0.5 °C and RH 75% in a humidity chamber for six months. The samples were withdrawn at different time intervals and analyzed for the drug (PHMB.HCl) by the HPLC method of analysis. The log percent of drug remaining was plotted against time in days and from the curve, the slope was determined. By using the slope, the degradation rate constant was determined and it was found to be 33.76×10?5 days?1. The amount of drug degraded was less than 5 % of the total. Hence an arbitrary shelf life of two years can be assigned to the product as mentioned by ICH guidelines.

CONCLUSIONS

The HPLC method with slight modification of Yiping et al was found to be precise, accurate and suitable. The method was found to be suitable to fix an adequate shelf life for the product.

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This study determines the stability of a multipurpose solution (MPS) used as antimicrobial and for preservation of hydrophilic contact lenses by detecting the stability of its main active compound: polyhexamethylene biguanide hydrochloride. This new antimicrobial is said to present advantages such as low eye toxicity, stability over a wide pH range and fast kill speed.

The authors describe a method for quantitation of the active compound found in this MPS by means of HPLC improved from a previously described method by Yiping et al. The improvements achieved a much lower limit of detection for the method. Stability analyses for the MPS at certain storage conditions were performed up to six months proving the methodology valid for determination of the stability of the compound of interest with adequate accuracy and following the ICH guidelines.

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The HPLC technique used for the determination of drug stability is an essential part of pharmaceutical formulation development. In this article, the authors provides a thorough description of high-performance liquid chromatography (HPLC) for the determination of PHMB.HCL in the multipurpose solution. PHMB.HCL is an antiseptic belong to the biguanide group, which are cationic substances well known for their effective action against microbial infection.

Nowadays, there are few methods for the study of PHMB.HCL. The main reason is the complexity of the sample composition,

that it is always present as mixture of oligomers. Furthermore, the lack of aromatic groups makes that detection via UV-absorption is difficult. The authors in this article development a HPLC method, in terms of throughput, accuracy and cost-effectiveness that it covers essential aspects of enhance efficient separation, such as mobile phase preparation and optimization of HPLC analysis. Analysis of the results showed satisfactory precision ranging. Thus, the detection limits were of 2 μg/mL. It is also demonstrated that the high sensitivity allows the method to be used for development as prophylactic or therapeutic drugs against various eye infections.

Furthermore, this article summarises the stability studies of PHMB.HCL in the multipurpose solution. Results presented show that the amount of drug degraded was less than 5% of the total. These data present new information about of a simple method for the determination of PHMB in eye drops as well as its possible application in pharmacokinetic studies in ophthalmology, since the technique can produce reliable analytical results quickly and relatively cheaply.