The Islamic University Journal (Series of Natural Studies and Engineering)
Vol.15, No. 1, pp 13 -22 , **2007**, ISSN 1726-6807, http// www.iugaza.edu.ps/ara/research

Formulation and stability evaluation of 1% w/v oral solution of Bromhexine hydrochloride for veterinary use.

Abdel Naser Zaid, PhD
Faculty of Pharmacy, An-Najah National University,
Nablus, Palestine
E-mail: dal_naser@yahoo.com

Abstract: Purpose: The aim of this study is to develop bromhexine hydrochloride 1 %w/v oral solution for veterinary use and to evaluate its stability. Methods: Solutions of Bromhexine hydrochloride (1%w/v) were prepared by dissolving bromhexine hydrochloride in benzyl alcohol at 50 °C then alcohol 96 % v/v; Tween 80 and purified water were added. The obtained solution was filled in amber glass bottles, and the solution was stored at 25 °C/60 % relative humidity (RH) and at 40 °C /75% RH. The strengths of bromhexine hydrochloride were determined by High performance liquid chromatographic assay at 0, 2, 4, 6, 8, 10, 12, 16, 20 and 24 months. The concentrations of the drug were directly related to the peak area. pH, odor, color and crystal formation was also monitored. Results: The degradation of bromhexine hydrochloride 1% w/v oral solution was faster at 40 °C/75% RH than at 25 °C /60% RH. No significant differences were found between the initial and final pH value for the solution at the studied conditions. No detectable changes in color, odor or precipitations were observed for the solutions stored at the upper conditions. Conclusions: Bromhexine hydrochloride 1% w/v oral solution could be formulated and remains stable for at least 2 years when is stored at 25°C /60% RH and for 16 months when stored at 40 °C /75% RH.

الملخص: هدف البحث: هدف هذه الدراسة هو ابتكار محلول فموي بتركيز 1% من مادة بروموهكسين هايدروكلورايدو ذلك للاستخدام البيطري، بالإضافة إلى تقييم ثباتية هذا المحلول المائي. أسلوب البحث: تم تحضير محلول من البروموهكسين هيدروكلورايد و ذلك بإذابته في الكحول البنزيلي على درجة حرارة 50 مئوية، و من ثم تم إضافة الكحول، التوين 80، و الماء المنقى على التوالي. تمت تعبئة المحلول الذي تم الحصول عليه في علب زجاجية داكنة حيث تم تخزين بعضها على درجة حرارة 25مئوية و 60% رطوبة نسبية، و البعض الآخر تم تخزينه على درجة حرارة 40 مئوية و 75% رطوبة نسبية. تم تقدير تراكيز البروموهكسين هيدروكلورايد بواسطة الفحص الكروماتوغرافي السائلي ذو القدرات العالية و ذلك في الأشهر0، 2، 4، 6، 8، 10، 12 ،16، 20و 24، تم حساب تركيز الدواء عن طريق مقارنة المساحة تحت المنحنى كما تم فحص كل من pH، الرائحة، اللون و الترسبات . النتائج: التفكك في مادة البروموهكسين هيدروكلورايد في المحلول الفموي 1 % كان أسرع في العينات المخزنة على درجة حرارة 40 مئوية و 75% رطوبة نسبية

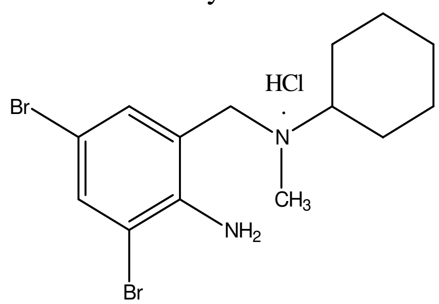
مقارنة مع ذلك المخزن على درجة حرارة 25 مئوية و 60% رطوبة نسبية، كما أنه لا توجد فروق في pH بين بداية الدراسة و نهايتها. لا يوجد أي تغيير في لون و رائحة المحلول، كما أنه لا توجد ترسبات في العينات المخزنة تحت الظروف المذكورة طيلة فترة الدراسة.
الاستنتاج: يمكن تحضير المحلول الفموي برومهكسين هايدروكلورايد 1% و إبقاؤه ثابتا لمدة لا تقل عن سنتين عند تخزينه على درجة حرارة 25 مئوية و 60% رطوبة نسبية، و لمدة لا تقل عن 16 شهرا" عند تخزينه على درجة حرارة 40 مئوية و 75% رطوبة نسبية.

Keywords: Formulation, Stability, Bromhexine Hydrochloride, Veterinary,

1. Introduction:

Bromhexine hydrochloride (Figure 1) is a mucolytic expectorant which exhibits its action by increasing bronchial secretions and reducing their viscosity. In addition, it produces in an increase in immunoglobulin levels in airway secretions. This agent was recently recommended as a new therapy for pathological states, such as alcoholic chronic pancreatitis where there is an increased viscosity of the pancreatic juice (1). It may be administered and has been used as ancillary therapy in the management of bronchopneumonia in horses, calves and pigs, as well as for the treatment of amniotic fluid aspiration in newborn calves and piglets (2). In an uncontrolled study, bromhexine has been reported to increase mucociliary clearance in patients with chronic bronchitis (3). Furthermore bromhexine hydrochloride may be administered in combination with antimicrobial agents in the treatment of respiratory infections, due to its capacity to disrupt the mucopolysaccharides of bronchial secretion and as results in enhancing the bronchial penetration of antimicrobial drugs (4). Martin et al (5) found that bromhexine hydrochloride increases oxytetracycline concentration within the secreted mucus, causing the reverse of mucospissic activity of oxytetracycline in vivo. Also patients given bromhexine hydrochloride and amoxicillin combinations had favorable clinical responses at the end of the course of treatment. The infection was completely resolved for 46% among the drug bromhexine hydrochloride and amoxicillin combinations and 34 % of patients on drug amoxicillin alone (6). Another investigation of bromhexine and amoxicillin combinations showed no side effects due to bromhexine, and all in all it may be recommended in the treatment of acute respiratory infections (7). In addition, bioavailability of the potent macrolide erythromycin antibiotic was increased after its administration as combined with bromhexine hydrochloride (8). In veterinary clinical studies, Escoula L, et al (9) found that injecting of bromhexine hydrochloride (Quentan®) with spiramycin resulted in an increase in spiramycin concentration in bovine nasal secretions. This potentiation explains the growing interest in administering combinations of bromhexine hydrochloride and antibiotics in the treatment of infectious diseases of the respiratory tract. Other dosage forms available for human use include tablets, aerosol, elixir and injectable solutions [10]. In the veterinary field bromhexine hydrochloride is formulated as powder in strength of 1%w/w for reconstitution of oral suspension, 0.5% w/v oral solution and 0.3 %w/v for intramuscular administration [11-12]. Since bromhexine hydrochloride is slightly soluble in alcohol and very poorly soluble in water therefore its formulation as solution was very difficult especially when strengths higher than 0.3 % w/v were required. Therefore, the development of bromhexine hydrochloride 1%w/v oral solution presents a challenge to improve the bioavailability of bromhexine hydrochloride in comparison with the solid dosage forms and make the mixing of the drug with antibiotics for respiratory infections for animal uses an easier task. Subsequently bromhexine hydrochloride 1% w/v oral solution (for veterinary use) is important from the commercial point of view, since this will result in reducing of the production cost in the pharmaceutical industry. In fact the same weight of bromhexine hydrochloride available in one liter of 0.5% w/v solution (commercially available) is also present in one half liter when 1 % w/v bromhexine hydrochloride solution is prepared. In the commercial sense, this means a reduction in size of the packing material to half the size of the solution available in the market and as result, easier transportation of this packing specifically in Palestine when considering the large number of checkpoints which represents true obstacles to traveling and transportation of the goods. For all of the above mentioned reasons, many attempts were carried out in order to produce a liquid formulation which contains bromhexine hydrochloride in a concentration of 1% w/v [12-13].The investigated formulation must demonstrate stability for long periods of time in order to make its production and commercialization at an industrial level an easy task.

Figure 1: Structure of Bromhexine Hydrochloride



2. Materials and Methods

2.1 Reagents and Materials

All chemicals and reagents were USP/NF or ACS (American chemical Society) grade and were used without further purification. The Bromhexine hydrochloride powder (lot. BRX956200402, Kempex, Holland), benzyl alcohol (Lot. CHCHLT0002, Bayer Jermany) , Tartaric acid (lot. F12502, ETs Legre-Mante, Marseilles, France) Ethanol 96% v/v (Lot. K31869783, Merk, Tween 80 (Lot. 215041, Esterchem, (m) SDN.BHD, Malaysia. High performance liquid chromatography-grade (HPLC) methanol Acetonitrile, Sodium perchlorate , di-amonium hydrogen phosphate and sodium -1-hexanesulfonate were of ACS and Merck grade.

2.2. Equipment

Bromhexine hydrochloride was quantified using a liquid chromatographic system consisted of a pumb (LC-6A, Shimidazu Corporation, Duisbourg, Germany), a 100μL manual injector (Rheodyne®, Bensheim, Germany). All pH values were measured with a pH 211 microprocessor pH meter from Hanna Instruments (Woonsocket, Rhode Island). An electrical laboratory stirrer (Phipps& Bird. Inc., Richamond Virgina) for mixing and an electrical laboratory balance (Type AEy-20G Shimidazu Corp. Japan) for weighing were used. For purified and distilled water a Reverse Osmosis (R.O.) water system (model HP-300 Cuno/ water factory system / USA) was used for preparation of purified and distilled water.

2.3. Preparation of formulations

Many trials were conducted to prepare bromhexine hydrochloride solutions. These trials were initially evaluated for variations in pH, color, odor and precipitate formation. The best formulation which showed no variation in the above mentioned properties was chosen for long term stability studies (table 1). Sample preparations for stability studies were prepared according to table 1 and the subsequent procedure of preparation. All blank and active-drug solutions were prepared in duplicate and stored in 100 ml amber glass bottles. Preparations were stored at 25 °C /60% relative humidity (RH) and 40% °C/75% RH. Each solution was visually assessed for color and clarity before it was poured into the bottles and the initial pH value was recorded. Approximately 5 ml of solution from each of the bottles, which were labeled 1% solution and blank Solution, was removed for analysis and apparent pH measurement. The preparations were sampled at 0, 2, 4, 6, 8, 10, 12, 16, 20, and 24 months. All samples were tested for pH and analyzed for drug concentration.

Table 1: Formulation of 1% w/v Bromhexine Hydrochloride Oral Solution

Ingredients	% Concentration	Amounts
Bromhexine Hydrochloride	1	50 g
Benzyl alcohol	5	250 mL
Ethanol	40	2000 mL
Tween 80	4	200 mL
Tartaric acid	0.1	5 g
RO water qs	100 %	5000 mL
R.O. : Reverse Osmosis, qs : as needed	mL: milliliter	g : gram

Procedure of preparation of the formulation in table 1:

1) Dissolve Bromhexine hydrochloride in benzyl alcohol at 50°C (solution I).
2) Add alcohol 96% v/v to solution I and mix well to form solution II.
3) Add tween 80 to solution II and mix well to form solution III.
4) Dissolve Tartaric acid in 100 ml of purified water and then mix with solution III to achieve solution IV.
5) Dilute solution IV with purified water to obtain 1000 ml solution and then filter the resultant solution using 0.45 μm Millipore filter paper.

2.4. Preparation of blank and standard solutions

Blank solutions were prepared in the same manner as described in table 1, but without the use of bromhexine hydrochloride. Blank solutions were labeled as blank solutions. Standard solutions were prepared using bromhexine hydrochloride of the same batch, which was used to prepare the formulation to yield the desired range of standard curve preparations and controls. The mobile phase solution was used as the diluting solvent, to get the desired concentrations ranged from 10μg/mL to 100μg/ml.

2.5. Analytical Method

To establish the bromhexine hydrochloride content in solution blank, standard and samples were analyzed using the HPLC method. The formulation for the 1%w/v bromhexine hydrochloride solution is shown in table 1.

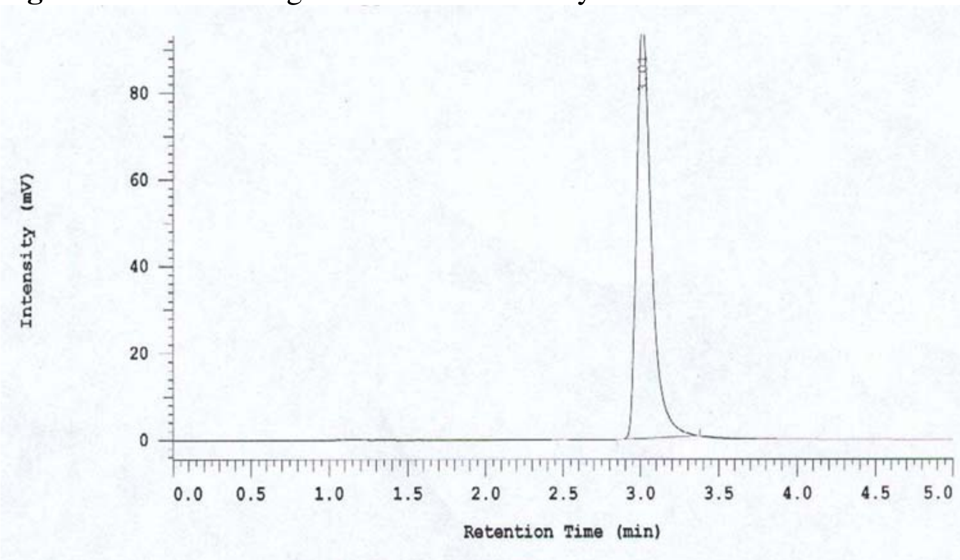
2.5.1. Chromatographic conditions

A LiCrospher® 60 RP-8, 5 μm , 4x 250mm column (Merck, Darmstadt, Germany) was used as the stationary phase. The flow rate of 1 mL/minute was used throughout the run. The ultraviolet variable-wavelength detector

(SPD-6A, Shimidazu, Duisberg, Germany) was set at 225 nm. The mobile phase was prepared by dissolving 6 g of sodium perchlorate, 2 g of diammonium hydrogen phosphate and 1,8g of sodium -1- hexanesulfonate in 400 ml of distilled water, add acetonitrile up to 1000 mL, shake well and adjust the pH to 3±0.2 using 1N phosphoric acid. The mobile phase was filtered through an HPLC- certified 0.45 -μm FP vercel membrane filter (Gelman Sciences, Ann Arbor, Michigan) with the help of a Millipore filter holder (part 4, Millipore Filter Corporation, Bedford, Massachusetts) and degassed using nitrogen gas. The mobile phase was used as the diluting solvent for the preparation of standard and sample solution. The flow rate was 1 mL/minute, the ultraviolet variable wave length detector was set at 225 nm, the injection volume was 80 μL and the temperature was ambient. The run time was 6 minute. The 1% bromhexine hydrochloride solution was diluted 10 times to yield the same final concentration as 0.1 % bromhexine hydrochloride solution. Drug concentrations were determined using a liquid chromatograph.

Each of the solutions was injected six times. The relative standard deviation for replicate injections of each of the sample or standard was found to be less than 1%. The retention time of bromhexine hydrochloride was 3.1 (± 0.053) minutes. For the study, aliquot stability samples of bromhexine hydrochloride oral solution (1%) was diluted to yield the desired final concentration (20μg/mL) for analysis. A bromhexine hydrochloride standard solution of 20 μg/mL was assayed periodically as a control (see Figure 2 for the representative chromatogram.). The peak-area response from injections of standard bromhexine hydrochloride solutions with concentration of 10, 20, 40, 60, 80 and 100 μg/mL was used for the standard curves produced by linear regression of peak areas against bromhexine hydrochloride concentrations. The standard curve was linear with r^2 values of 0.9995 to 1 over the working range of bromhexine hydrochloride concentrations. The intra and inter-day coefficients of variation were less than 1%.

Figure 2: A Chromatogram of Bromhexine hydrochloride Standard Solution



3 Results and Discussion

3.1. Assay

The assay method used for this study was developed and validated by Tantishaiyakul V. et al. (14), who found it precise and accurate, with intra-day being 0.1 to 1% and the inter-day variability being 1,6%; the percent standard deviation (SD) was 0,9, based on five readings. The concentration of the drug was directly proportional to the area under the curve (range tested from 10 to 100 µg/mL) demonstrating a linearity of the method. The volume of the injected samples must remain constant to ensure the linearity between the concentration of the drug and peak areas. Thus the method was shown to be stability indicating after studies of bromhexine hydrochloride under stressful chemical and physical conditions. The degradation products obtained under both acidic and basic conditions did not interfere with the peak of bromhexine hydrochloride. Thus the method can be considered specific for bromhexine hydrochloride.

3.2. Chemical Stability Results

The behavior of bromhexine hydrochloride solutions 1% stored in amber glass bottles at 25 °C/60% RH and 40 °C/75% RH is summarized in table 2. As expected the rate of degradation of bromhexine at 40 °C/ 75 RH was faster than bromhexine hydrochloride at 25 °C but still stable within the limit of two years and the solution could be recommended within this time limit. Benzyl alcohol (15), alcohol 96%v/v and tween 80 system offers a good co-solvent for bromhexine hydrochloride. This co-solvent system is chemically compatible with bromhexine hydrochloride. Further examination of the data shows that on month 24 bromhexine hydrochloride concentration was 96,301 %, when the solution was stored at 25 °C/60% RH, while only 90,01 % of bromhexine hydrochloride was recover after 16 months of storage at 40 ° /75% RH.

Table 2: Concentration of Bromhexine Hydrochloride in the Solution with Respect to Time.

	Percent drug remaining	
Time (months)	25 °C	40 °C
0	100	100
2	99.884	98.6
4	99.504	96.901
6	99.205	95.410
8	99.000	94.303
10	98.537	93.198
12	98.023	92.30
16	97.641	90.01
20	96.816	a
24	96.301	a
R^2	0.9931	0.990
Standard error	± 0.11069	± 0.223
Significance	< 0.03	< 0.03
R^2 : R square	a: analysis not performed at this interval	

3.3. Apparent pH and physical appearance

No significant difference was found between initial and final pH values (6.3-6.5) for all tested samples which were stored at 25 °C/ 60% RH and at 40 °C / 75% RH. Concerning the appearance of the solution, no detectable change in odor, color or precipitate was observed at the end of the study for the solutions stored at 25 °C/ 60% RH and at 40 °C / 75% RH. Visually, no microbial growth was detected for any of the samples.

4. Conclusions

As evident from the preceding discussion, the bromhexine hydrochloride 1% solution prepared and stored at 25 °C / 60% RH was stable for more than 2 years and only for 16 months if stored at 40 °C / 75% RH. This suggest that our formulation is suitable for preparing bromhexine hydrochloride at high concentration, which offer a decrease in the solution packing material, an issue which results in money saving in production and less problem in transportation and less cost to consumers. In the future further study should be recommended to examine the stability of this formulation at 25 °C and 60% RH more than two years to evaluate the complete stability of the formulation under these conditions. Also a bioequivalency study should be carried out between the 1% bromhexine hydrochloride powder for oral solution and 1 % bromhexine hydrochloride oral solution to evaluate the expected increase in the absorption due to the liquid dosage form in order to decrease the daily dose of this drug an issue which lead to a further economic benefit and. In the future this formulation may be the fundamental step in order to develop this formulation strength for human use.

Acknowledgement:

We wish to thank Al-Wafa Medical & Veterinary Supplies Co. for the financial support of this research.

References

[1] Tscimoto T, Takano M., Tsurano T., Hoppo K., Matsumura Y. Kuriyama S., Fukuitt, - 2004. Mediatinal pancreatic pseudocyt caused by obstruction of the pancreatic duct was eliminated by bromhexine hydrochloride. Intern Med., 43 (11): 1034-8.

[2] The Merk Veterinary Manual – 1991. Mucolytic expectorant, 7th ed., Rahway; N.J., U.S.A. Merck & Co., Inc.: p. 1404.

[3] Thomoson ML, Pavia P, Greggy I, Stark VE. -1974. The effect of bromhexine on mucociliary clearance from the human lungs in chronic bronchitis. Scand J Respir Dis. (Suppl), 90: 75-79.

[4] Bergogne, berezin, Berthelot G., Kafe HP., Doournovo P.- 1985. Influence of a fluidifying agent (bromhexine) on the penetration of antibiotics into respiratory secretions. Int. J Clin Pharmacol Res. 5 (3):341-4.

[5] Martin GP, Loveday BE, Mariot C.- 1993. Bromhexine plus oxytetracycline: the effect of combined administration upon the

rheological properties of mucus from the mini-pig., J Pharm Pharmacol. Feb; 45 (2): 126-30.

[6] Roa CC Jr, Dantes RB.- 1993. Clinical effectivness of combination of bromhexine and amoxicillin in lower respiratory tract infection. A randomized controlled trial. Arzneimittelforschung. Mar; 45 (3): 267-72.

[7] Taskar VS, Sharma RR, Goswami R, John PJ, Mahashur AA.- 1992. Effect of bromhexine on sputam amoxicillin levels in lower respiratory infections. Arzneimittelforschung. Mar; 86 (2): 157-60.

[8] Bergone-Berzin E, Pierre J, Dournovo P.- 1982. Effect of a secretolytic (bromhexine) on the penetration of erythromycin into bronchial secretions. Fortschr Med. Jun; 100 (3): 1169-71.

[9] Escoula L, Larrieu G, Camguilhem R.- 1981. Enhancement of spiramycin concentration by bromhexine in the bovine nasal secretions. Ann Tech Vet. 12 (3): 317-20.

[10] Repertorio Farmaceutico Italiano, Bisolvon®, 8th ed., Mi, Italy, Cedof s.p.a., p. A-257, 1998.

[11] L'Informatore Farmaceutico di Veterinaria e Zootecnia, Quentan®, 13th ed., Mi, Italy, Massons s.p.a., p. 415, 2002.

[12] http://www.Boehringer-ingelheim.com. uk/products/documents/BisolvonpowderDatasheet-2005.pdf.

[13] Schubert Ma, Muller-Goymann CC.- 2001. Solubilization of bromhexine hydrochloride in aqueous lecithin dispersios. Physicochemical characterization of interactions between drug and carrier. pharmazie. Sep; 56 (9): 237-43.

[14] Tantishaiyakul V, Poeaknapo C, Sribun P, Sirisuppanon K.- 1998. Simultaneous determination of dextromethorphan HBr and bromhexine HCl in tablets by first –derivative spectrophotometry. J Pharm Biomed Anal. Jun; 17 (2): 237-43.

[15] Karabit MS, Juneskans OT, Lundgren P. Studies on the evaluation of preservatives efficacy 11: the determination of antimicrobial characteristics of benzyl alcohol. J Clin Hosp Pharm, 11: 281-289, 1986.