

Evaluation of a diltiazem hydrochloride gel for the treatment of anal fissure

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ABSTRACT

This paper describes the production and evaluation of a 2% diltiazem HCl gel, as an alternative to traditional approaches such as surgery, in the treatment of anal fissure. The gel itself consists of hydroxypropylmethylcellulose in water. It was evaluated for chemical, physical and microbiological stabilities before clinical evaluation. Prior to evaluation, the method for the quantification of the drug and products of degradation, obtained under stressful conditions imposed on drug solutions, was developed and validated according to the guidelines by the International Conference on Harmonisation. Once the method was validated, and the gel produced, a stability test was carried out for six months. The gel proved to be stable at room temperature and moisture conditions without showing any product degradation, the drug content remained above 95% from the expected content, no microbiological growth was observed and the physical properties remained stable. Clinical trials, followed by continuous use in the clinic, have shown the potential use of the gel in daily clinical practice. The gel proved to be effective in the treatment of anal fissure and its prescription became part of the hospital's routine as an alternative treatment to surgery.

KEYWORDS

Anal fissure, clinical evaluation, diltiazem HCl gel, method validation

INTRODUCTION

Anal fissure, an acute or chronic disease, is a common problem which affects both male and female patients [1]. The illness is particularly distressing in patients with chronic constipation, diarrhoea and rectal inflammation. The clinical diagnosis is simple and can be carried out by physical examination and evaluation of the symptoms, namely pain, anal bleeding and local irritation. The presence of a fissure is responsible for a large percentage (20%) of symptomatic haemorrhoids [2]. The principal physiopathological factor for the appearance and chronicity of the fissure is high tonicity and blood pressure in the internal anal sphincter combined with relative ischaemia in the posterior midline

anal canal [2], which delays cicatrisation. Patients with an anal fissure have an abnormally high resting anal sphincter pressure that, if decreased, promotes the healing of the fissure [3]. However, without treatment the success of cicatrisation in healing the fissure is below 65%.

Treatment of anal fissure can be achieved with a diet rich in fibre, ingestion of liquids and the use of topical analgesics. When this is not successful, surgical or chemical sphincterotomy must be considered. Although surgical sphincterotomy is simple, presents low morbidity, rapid relief of pain, high success rates (97–98%) and low recurrences, it also has some major drawbacks such as high cost, flatulence and faecal incontinence (0.5–4.9%). Another approach in treatment of the disease requires anal dilation, which is a non-standardised procedure requiring anaesthetic. However, this technique can damage the sphincter (65%) and promote faecal incontinence (12.5%). A more recent approach uses botulinum toxin injected either in or out of the sphincter. Although only a few studies have been carried out, the success rate (75–95%) together with a low morbidity rate is promising. However, the exact location for the injection remains controversial, with patients showing faecal incontinence and a high rate of recurrence.

Alternative approaches to the treatment of anal fissure use drugs that diminish the anal pressure at rest, with no damage to the anal sphincter, thus promoting patient compliance. Those used most often are nitrates,

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e.g. glyceryl trinitrate (GTN) and calcium channel blockers, e.g. nifedipine and diltiazem, administered orally or topically. Glyceryl trinitrate, given to patients as sublingual tablets, creams or gels can successfully treat anal fissures but its side effects, namely headaches, affect patient compliance, as reflected by its low clinical success (between 25–50%) [4]. Nifedipine, an alternative to GTN, has been given to patients as a sublingual tablet with promising results. Decreases in both the resting anal sphincter pressure as well as a number of important side effects, such as cardiac palpitations, have been observed [3].

Diltiazem, in its hydrochloride salt form, has been given to patients as a tablet (60 mg) or a gel (2%) [2-7]. Diltiazem produces a dose dependent reduction of anal pressure [3], with improved results (clinical outcome and patient compliance) for the gel, namely a lower incidence of cardiac palpitations and headaches [6]. Furthermore, use of the gel avoids surgery (decreasing patient's discomfort, stays in hospital and the probability of complications) and decreases the number of recurring headaches. The optimal concentration for the drug has been established at 2% (w/w). Concentrations below 1% were not efficacious, whereas concentrations above 2% did not show improved effects [3]. Topical gels containing a 2% drug concentration have a maximum effect (28%) on reducing the anal pressure for 3 to 5 hours [3, 8]. Some clinicians have prescribed diltiazem HCl gel (2%), applied twice a day, for eight weeks, with good results [4], whereas others prefer three times a day application for a few weeks. In both cases remission of the fissure was observed without recurrence, side effects and equal [7], if not higher success [9] than observed for the nitrates, after three months' treatment. Local application improves therapeutic compliance and effect compared to oral administration, although a rash may be observed. As a consequence diltiazem gel is the first choice in the treatment of anal fissure.

This paper reports the development, production, quality control, stability (physical, chemical and microbiological) and clinical evaluations of a 2% diltiazem hydrochloride gel for the treatment of anal fissure. Furthermore, the work exemplifies the use of a drug in the preparation of a medicine for a small number of patients in hospital according to international and national guidelines. Likewise, other pathologies and patients may benefit from the example reported here.

MATERIALS

Diltiazem hydrochloride and its analytical standard were supplied by Laboratórios Delta, SA (Portugal). The hydroxypropylmethylcellulose was Methocel E4M (Colorcon, UK),

propilenoglycol supplied by VPereira, SA (Portugal) and sterile water (B. Braun, Portugal). The gel was packaged in aluminium tubes (VPereira, SA, Portugal). Potassium phosphate (analytical grade), triethylamine, methanol and Whatman 42 filters, all supplied by Merck (Germany) were used for the preparation of the chromatographic mobile phase.

METHODS

Preparation of the gel

The formulation (see Table 1) and the preparation of the gel (for 100 g) have been previously described [10]. Briefly, both the diltiazem hydrochloride (2 g) and the propilenoglycol (10 g) were mixed thoroughly (Kenwood Chef Mixer, UK) for 10 minutes and then hydroxypropylmethylcellulose (2 g) was added to the mixture and mixed for another 5 minutes. Once the mixture was produced, hot water (85 g; 70°C) was added slowly up to the quantity required to produce the gel (100 g). The system was left stirring for 12 hours to allow complete dispersion of the cellulose derivative. After production of the gel it was left unstirred for 3 hours under vacuum to release the air incorporated, prior to packaging in aluminium tubes (50 g per tube).

Evaluation of the gel

The gel was evaluated for organoleptic characteristics (colour, feeling to touch), viscosity (Brookfield DV-II viscosimeter, Brookfield, USA), total number of viable microorganisms [11], assay of the drug, and detection of the degradation of drug moieties by high pressure liquid chromatography (HPLC).

Assay of diltiazem hydrochloride

The assay of the drug was carried out by HPLC according to a technique described previously [12]. Briefly, 100 mg of gel was dispersed in 50 mL of methanol and submitted to ultrasound for 5 minutes, prior to filtration. Then 10 µL of the solution was injected in a liquid chromatographer (Merck-Hitachi, Germany) fitted with a column LiChroCART 125-4 and LiChrospher 100 RP-8 (5 µm). The mobile phase was made of a mixture of acetonitrile-phosphate (6.9 g of KH_2PO_4 in 1,000 mL water, adjusted to pH 3 with HCl 0.1 M prior to the addition

Table 1: Composition of the gel (100 g)

Components	Quantity (g)
Diltiazem HCl	2
Propilenoglycol	10
Hydroxypropylmethylcellulose	3
Water	85

of 0.5 mL of triethylamine) in equal parts. The solution was filtered through 0.22 μm filters (MSI-micro separations). The flow was set to 1 mL/minute and the assay was carried out at room temperature. Detection of the drug was by ultraviolet spectrophotometry ($\lambda = 240 \text{ nm}$) and the value compared to those observed in a calibration curve produced from solutions with different concentrations of the drug (analytical standard). The method was validated for specificity (gel with drug versus gel without drug, i.e. placebo), linearity (seven solutions with increasing concentrations of drug), precision (10 replicates of the gel were considered), accuracy (triplicates of gels with 50%, 100% and 150% of drug), and limit of detection and quantification (by continuous dilution of the gel). The limit of quantification was considered as three times greater than the limit of detection [13, 14].

In order to detect possible products of degradation after production of the gel, water solutions of the drug (5 mg/mL) were submitted to magnesium permanganate 3 M (for one week), acidic medium (HCl, 5 N) and alkaline medium (NaOH, 5 N), heat (100°C, 5 minutes), light (direct sun exposure, for one week) to promote degradation of the drug. The solutions (10 μL) were then analysed by HPLC (with a diode array) to detect any degradation products which may have formed. No products of degradation are described in the European Pharmacopoeia [15], thus this procedure was used to provide further information on the development of the gel.

Microbiological evaluation

Microbiological evaluation of the gel was carried out according to the European Pharmacopoeia [11] for liquid preparations for oral and rectal use (counting of total viable microorganisms). A challenge test was also carried out to ascertain the preservation ability of the gel [11].

Protocol for the evaluation of stability

The stability of the gel was evaluated according to guidelines from the International Conference on Harmonisation (ICH) regarding the stability of medicines [13, 14]. Three batches of the gel were produced at different times (same formulation and processing conditions) and stored for 180 days in aluminium tubes protected from light, at $22 \pm 3^\circ\text{C}$ in an environmental chamber (Heraeus, Germany). At different time intervals (0, 7, 16, 30, 60, 90 and 180 days) samples were collected and analysed. The tests and the techniques were the same as for the finished product. The gel was considered stable when the assay of the drug provided a result above 95%, the other properties remained stable and no degradation products were observed in the chromatogram.

Clinical evaluation of the gel

Patients suffering from acute and chronic anal fissure pathology from the colonproctology outpatient clinic were selected and followed up for one year. The criteria for selection were the presence of symptoms for more than two months, no response to other therapies and demographic characteristics, e.g. gender, age, lifestyle. Twenty-five patients, of which eight were male and 17 female, with a positive diagnosis for anal fissure, were enrolled in the study. Patients were advised to apply 2 cm of diltiazem gel twice a day (in the morning and in the evening), 1 cm inside the anus and 1 cm outside, for eight weeks. The outcomes were evaluated based on the clinical records of the patients: diagnosis, clinical response and overall evolution of their condition. The endpoints of the study were the absence of symptomatology and fissure epithelialisation at the eighth week. If the endpoints were not achieved the application of the diltiazem gel was continued for another eight weeks. These outcomes were compared with the date and the amounts of the gel dispensed. Patients were elected for surgery if the fissure persisted at the 16th week. The study was approved by the hospital's ethical committee.

RESULTS

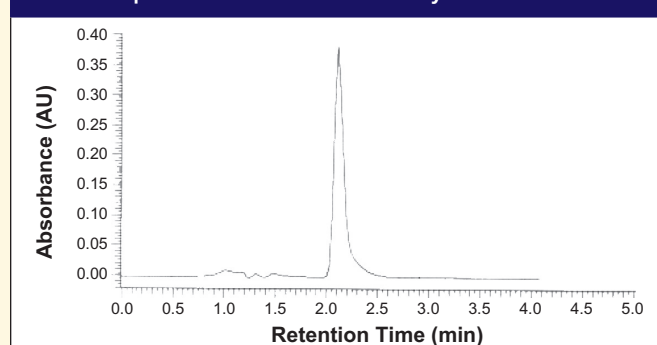
Validation of the drug assaying method

For proper characterisation of the gel it was necessary to validate the analytical method used to quantify the drug in both the finished product and throughout the gel's life before its expiry date.

The method was validated for specificity, linearity, accuracy, precision and limits of detection and quantification. Figure 1 shows a chromatogram of the drug in solution. The results for the validation of the technique were as follows:

- The specificity of the method was evaluated when both gels, with and without diltiazem HCl, were assayed.

Figure 1: Typical chromatogram observed for the quantification of diltiazem hydrochloride



In the absence of the drug no peak in the chromatogram due to the drug was observed.

- The linearity of the method was observed when several solutions with different concentrations of the drug were prepared. A linear relationship between the signal produced by the HPLC detector and the concentration of the drug was observed ($R^2 = 0.9991$).
- The precision was evaluated through the analysis of replicates and showed an average of 95.6% of drug recovered and a relative standard deviation (RSD) of 0.41%. The low RSD proves the precision of the method.
- The method was considered accurate when the results observed for expected amounts (50%, 100%, and 150%) showed only a small bias (-1.13 for 50%, -2.44 for 100% and -1.59 for 150%).

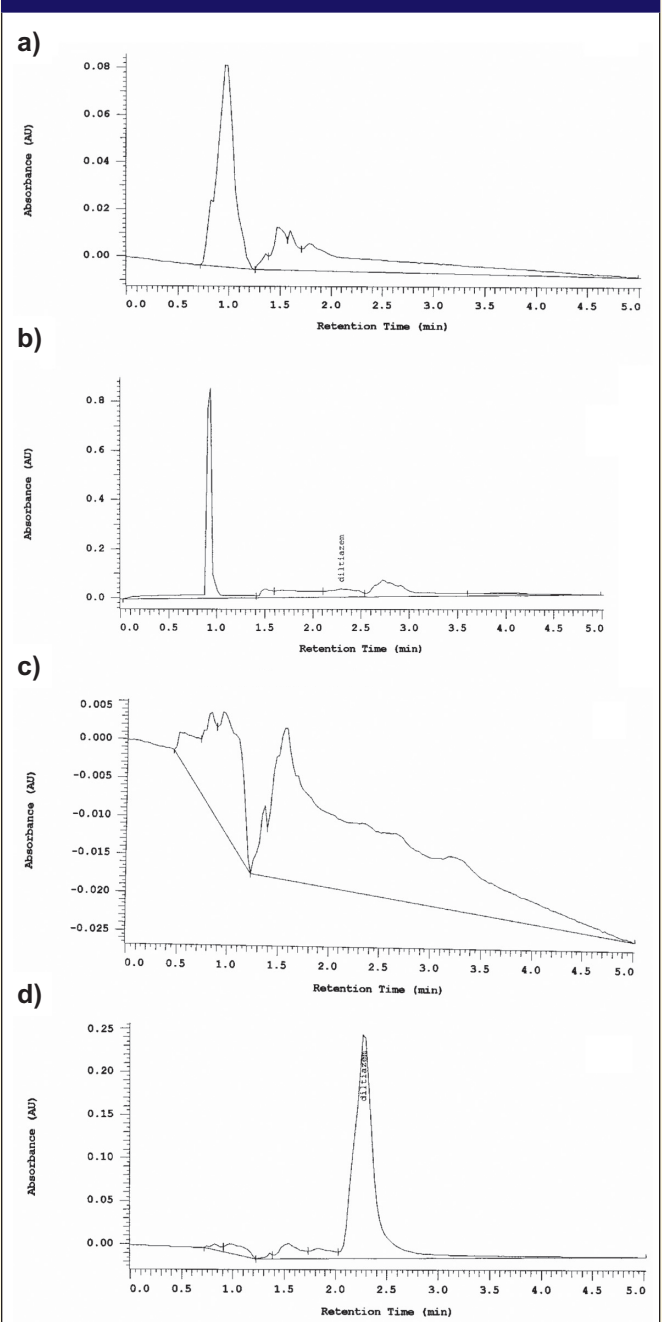
Stability of the gel

Preliminary results have also shown that no preservative was required and patients were pleased with the organoleptic characteristics of the gel. Both physicians and patients identified the proposed formulation as the most suitable for the intended application, particularly with concerns in relation to the percentage of HPMC in which the amount of 2% diltiazem HCl was deemed adequate. Consequently, the formulation was adopted and its processability and stability considered.

The stability of the preparation was evaluated for both the drug and the gel. Different drug solutions were placed under stressful conditions and the chromatograms evaluated in a chromatographer connected to a diode array. The results presented in Figure 2 summarise the modifications observed in the chromatograms when the quantification of the drug was carried out at $\lambda = 240$ nm. The chromatograms clearly show that the shapes of the peaks are completely different from the one observed in Figure 1; thus it was possible to continue the evaluation of the drug at that wavelength, following the analytical procedure.

The results show (see Table 2) that the viscosity decreased slightly during the six months of storage, whereas the amount of drug assayed remained high throughout (see Table 3). Although a small decrease in the percentage of the drug quantified was observed, the amount remained well above 95%, i.e. within $100 \pm 5\%$ (accepted specification for finished products, see Table 3). The initial microbiological evaluation of the gel after one month of production showed no growth of microorganisms even when a challenge test was carried out. These results were confirmed when the stability was monitored for the lifetime of the gel (see Table 4), where the absence of microorganisms

Figure 2: Chromatograms produced when drug solutions were exposed to a) acidic medium, b) alkaline medium, c) oxidative medium and d) heat conditions



was obvious. Microbiological control was carried out by counting the total viable microorganisms and *E. coli* [11]. The accepted number for oral preparations is 10^3 for microorganisms and 10^2 for yeasts per gram or millilitre of preparation. From the results presented in Table 4 one can observe that the gel remained stable for the period of analysis.

Table 2: Viscosity of the gel throughout the study

Viscosity (cP × 10 ³)							
Batch number	Day						
	0	7	16	30	60	90	180
1	25.6	25.3	24.3	24.0	24.1	22.4	21.4
2	21.6	21.3	19.7	19.8	19.5	20.8	20.3
3	23.1	22.8	22.5	21.2	21.0	22.2	20.8

Clinical evaluation

In the study 21 patients (84%) did not present any recurrence of the illness, whereas three (12%) presented with some symptoms. One patient stopped treatment (4%) for reasons not related to the illness or the treatment itself. None of the patients in the study noted symptoms such as those found during treatment with nitrates, e.g. headaches.

DISCUSSION

The development of the formulation has shown that delivery of diltiazem hydrochloride to patients was possible with hydroxypropylmethylcellulose, water and propilenoglycol. The gel formed showed adequate physical properties for the topical application of the drug. This was possible by involving physicians and patients at the early stages of development. One can assume that either the drug, or the excipient or the dosage form itself, is not a proper medium for the development of microorganisms, although a large amount of water is present.

Table 3: Recuperation of diltiazem hydrochloride from the three batches throughout the study

Diltiazem hydrochloride (%; average ± standard deviation)							
Batch number	Day						
	0	7	16	30	60	90	180
1	99.2 ± 0.6	98.4 ± 0.8	99.2 ± 1.5	99.5 ± 4.6	98.9 ± 1.0	98.4 ± 1.4	96.6 ± 0.4
2	101.2 ± 2.0	99.8 ± 0.3	100.3 ± 0.4	99.3 ± 1.8	100.2 ± 1.7	100.6 ± 1.7	98.8 ± 0.4
3	101.3 ± 1.5	98.0 ± 0.2	100.9 ± 2.9	98.9 ± 2.0	99.3 ± 1.9	99.5 ± 2.6	99.4 ± 0.5

Table 4: Results of the microbiological control over the three batches of gel for the period of analysis (n = 3)

Number of colonies forming units per millilitre (cfu/mL)							
Type of microorganism	Day						
	0	7	16	30	60	90	180
Bacteria	0	0	0	0	0	0	0
Yeast	0	0	0.3	0	9.0	0.7	0
<i>E. coli</i>	absent	absent	absent	absent	absent	absent	absent

Although the European Pharmacopoeia identifies the impurities of the drug it does not describe the products of degradation, therefore they had to be generated to allow the comparison with possible products of degradation of the drug in the gel upon storage. Fortunately none were observed and thus one can accept the stability of the preparation for the six months of the study.

The simplicity of the gel's formulation was required to promote an easy and flexible production of the gel at the hospital's facilities; for example, lack of space, human resources and time had to be considered. At present, 14 batches of gel have been prepared and dispensed to patients with significant clinical relevance, namely through high patient compliance. As a consequence this protocol has been shared with other hospitals and the number of patients benefiting from the gel is continuously increasing.

CONCLUSION

This work has proved it is possible to prepare a 2% diltiazem hydrochloride gel in the hospital for outpatients. It was prepared in the easiest way to facilitate its preparation and dispensing. The gel has proved to be stable under stress conditions since the stability tests have not shown drug degradation products throughout the gel's lifetime. The test was carried out for 180 days and due to the stability of the gel it can be assumed that the diltiazem HCl gel (2%), stored at room temperature (22 ± 0.3°C) was stable chemically, physically and microbiologically for the period of the study (180 days), therefore the expiring date can be six months after production. This period fits the

routine of the hospital because this is a reasonable period of time to predict and evaluate the number of patients enrolled for treatment, i.e. the patients entering the treatment, the ongoing patients, and the ones leaving the treatment.

The preparation of the gel and the quantification of the active moiety have been proved to be adequate according to the validation of the method carried out to quantify the drug and its products of degradation.

Although the number of patients considered in the study was small and the period of follow up was short, the study strongly suggests that the application of the gel can

replace the surgical procedures in the treatment of chronic anal fissure, without showing side effects, avoiding a stay in the hospital which has benefits for both patients and the national health service.

Finally, this work has shown that it is possible to prepare medicines for a small number of patients according to

international and national guidelines for quality and can be extended to other drugs and pathologies.

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