Stability of a Parenteral Formulation of Betamethasone and Levobupivacaine

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Abstract

Background: The therapeutic management of syndromes presenting simultaneously pain and inflammation often requires the administration of anesthetic and corticosteroid drugs by epidural administration. In this article, we studied a mixture that combines betamethasone and levobupivacaine, which demonstrates prolonged analgesic effects. To our knowledge, the stability of such a mixture in epidural solution has not been examined. **Objective:** To evaluate the chemical, physical, and microbiological stability of an extemporaneously prepared mixture. **Methods:** A solution of betamethasone acetate 1 mg/mL, betamethasone phosphate 1 mg/mL, and levobupivacaine hydrochloride 0.83 mg/mL was prepared in saline. The components were analyzed by high-performance liquid chromatography for up to 270 days of storage, protected and exposed to light, at room temperature, and stored in the refrigerator and at 45°C. In addition, sterility, organoleptic properties, and pH of the admixture were monitored. **Results:** There are no significant differences between drug concentrations obtained at room temperature and at refrigerated temperature. The accelerated conditions (45°C) demonstrated different results among the actives: betamethasone acetate and levobupivacaine hydrochloride are affected while betamethasone phosphate remains stable. The stability of the mixture does not depend on light exposure. The validity period of the different components in the mixture was estimated as 120 days for betamethasone phosphate and 163 days for levobupivacaine hydrochloride; betamethasone acetate remained unchanged during 155 days. **Conclusion:** Analgesic mixtures of betamethasone and levobupivacaine can be stored at ambient temperature in polypropylene vials for up to 120 days at the studied concentrations. These data enable the rationalization of the centralized preparation in the hospital pharmacy.

Keywords

quality assurance, stability, pain management, parenteral therapy, admixtures

In the Pain Unit of the University of Valencia General Hospital, clinical evidence of a synergistic activity between levobupivacaine and betamethasone has been obtained in recent years. Extemporaneous mixtures have been used to avoid injecting patients.¹ This practice results in unnecessary work and cost for the hospital pharmacy as the components cannot be reused. For this reason, many studies about combinations of anesthetic and analgesic or opioid drugs have been reported in the literature. The technical information of Chirocane (AbbVie Farmacéutica, SLU, levobupivacaine hydrochloride) reports data on mixtures with clonidine, morphine, fentanyl, and sufentanil, in different concentrations. Nevertheless, there is no information about this particular combination.

From an industrial point of view, this mixture has very little interest as both drugs are not protected by patent. It is also very difficult to substantiate an authorization of a combined drug.

Pain is one of the major concerns for the hospitalized population. There is a clinical need for improvement of therapy, for inpatients and outpatients.

Considering these facts, a study on stability was designed to optimize the preparation of a batch at the hospital pharmacy, while maintaining the high quality of the drug.²

Methods

Materials

Betamethasone was used as a commercially available drug (Celestone Cronodose) marketed as a 2 mL vial containing 6 mg of betamethasone sodium phosphate (BSP) and 6 mg betamethasone acetate (BA). The pH of this suspension is 7.21 ± 0.01 . Levobupivacaine hydrochloride (LBUP) is

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marketed under the name of Chirocane and presented as infusion bags containing 1.25 mg/mL, with a pH of 5.27 ± 0.01 .

Preparation of Solutions. The mixture was prepared in a laminar flow hood, grade A/B controlled work area.³ This facility guarantees sterility of the final product as the raw materials are sterile marketed drugs. The standard operational procedure used at the Pharmacy Service in the clinical routine was followed. A large scale was used to prepare enough volume to conduct the study. Fifty vials of Celestone Chronodose (100 mL) were homogenized and transferred to a 500 mL sterile flask. Chirocane was added (200 mL of 1.25 mg/mL). The contents were mixed until a homogeneous state was achieved, resulting in a clear transparent solution of pH 7.4. The actual concentrations were 1 mg/mL of BSP and BP and 0.83 mg/mL of LBUP. Using sterile polypropylene vials, 2 mL was aliquoted and sealed. Samples were placed randomly on 4 racks depending on storage conditions. Eight glass vials were prepared to test initial and final points for comparison.

Temperature of storage and light exposure were tested. Four batches were analyzed:

- A: Room temperature, light exposure
- B: Room temperature, protected from light
- C: In the oven at 45°C, protected from light
- D: Refrigerated (2–4°C), protected from light

The sampling was conducted at the start of the study and then at 3, 7, 15, 30, 70, 90, 180, and 270 days after mixture preparation.

Physical Stability

Three parameters were assessed for physical stability: color, particulate matter, and pH. Visual control was used for color and appearance (Ph. Eur. Method 2.2.2)⁴ and visible particles (Ph. Eur. Method 2.9.20)⁴ in the vial. The pH was recorded by Crison micro-pH electrode 5208.

Chemical Stability

Chemical stability was monitored by quantitative analysis of each of the active ingredients.

Optimization of the chromatographic conditions for simultaneous analysis was not possible with the available facilities. BSP and LBUP were analyzed together and BA was quantified in a different analysis.

Analysis

High-performance liquid chromatography (HPLC) methods used were as in the USP⁵ for BSP. A PerkinElmer HPLC

system (PerkinElmer Inc, Shelton, CT) employing a binary pump, micro-vacuum degasser, autosampler, and ultraviolet detector. Agilent Technologies Chemstation Version 4.0 Software was used for the analysis.

For BSP and LBUP, separation was achieved on a reversed-phase column (Ultrabase RP-C18 100 × 4.6 mm, 2.5 μm) with an isocratic mobile phase containing buffer monobasic potassium phosphate (0.01 M; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and methanol (4:6, v/v; J.T. Baker, Deventer, Holland). The mobile phase flow rate was 1.0 mL/min, with an injection volume of 50 μL. The detection was carried out with a diode array detector at 254 and 210 nm for BSP and LBUP, respectively.

BA analysis used the same system, with the mobile phase adjusted to 3:7, v/v. The detection was carried out at 254 nm.

The HPLC methods were validated according to the international guideline with regard to specificity, range of linearity, precision, and accuracy.⁶

Microbiological Stability

Microbiological stability was assessed at the end of the study by a general bacteriology test, planted on blood agar and incubated for 48 hours at 30°C. Fungi test was carried out on liquid Sabouraud chloramphenicol agar at 37°C for 3 days and growth monitored.

Data Analysis

Differences among concentrations of every drug were established by ANOVA, followed by Tukey test when applicable, using SSPS 19.0.

The zero-order kinetic model was fitted to the remaining concentration of every component versus time by least squares linear regression (Microsoft Excel 2013). Goodness of fits were established by significance of the regression (*F* test).⁷ The lower limit of the confidence interval of 95% was used to estimate the expiry date as the time at which 90% of the active was expected. This value was considered as the stability cutoff parameter in the absence of physical and microbiological changes.

Results and Discussion

The control parameters of stability are different depending on the active ingredient and the pharmaceutical form concerned. They are generally grouped into studies of chemical, physical, and microbiological stability,⁸ even though some properties are interrelated. The design of the study is organized in these 3 parts.

Chemical stability was checked by quantification of the active ingredients. Chemical degradation is sometimes accompanied by physical changes such as discoloration,

Table 1. Validation Parameters Obtained.

Parameters	BSP	BA	LBUP
Linearity (r^2)	0.9999	0.9990	0.9999
Interday precision (CV %)	≤ 2.8	≤ 2.49	≤ 4.30
Accuracy (RE %)	≤ 7.25	≤ 19.1	≤ 2.78
LOD (mg/mL)	0.007	0.022	0.001
LOQ (mg/mL)	0.014	0.058	0.003
Range (mg/mL) ^a	0.225-1.25	0.225-1.25	0.20-1.00

Abbreviations: BSP, betamethasone sodium phosphate; BA, betamethasone acetate; LBUP, levobupivacaine hydrochloride.

^aThe concentration ranges from 25% to 125% of the nominal concentration in the mixture (ie, 1.0 mg/mL of BA and BPS; and 0.83 mg/mL for LBUP).

odor, turbidity, or precipitation. Physical changes are not necessarily related to chemical degradation. We selected turbidity as an indication of the presence of particles and pH. This latter is important as a change in the acid-base environment of the drug is a factor of instability. Stability can also relate to the release of products such as plasticizers of PVC bags and adsorption of the ingredients of the solution in the container surface. This was taken into account for the final packaging used. Parenteral solutions must be sterile before the expiry date. Microbiological instability studies were also applied.⁸

Considering standard conditions for our climate zone ($14.8 \pm 0.1^\circ\text{C}$, 52%), the study can estimate the validity period of the preparation.

Chemical Stability

The validation studies revealed that the methods are highly specific for the studied drugs.⁹ Table 1 illustrate these results.

The profile of concentration versus time for the different actives studied are presented in Figure 1. Every graph presents 4 plots corresponding to the different conditions assayed.

It can be seen that the different salts of betamethasone exhibit different sensitivity with respect to temperature in this media. BSP is stable along 9 months, regardless of temperature. On the contrary, the acetate salt exhibits a decrease of 11% at 70 days at 45°C . This feature has not been pointed out before as most of the research related to the stability of this drug is devoted to topical formulations.¹⁰ Our finding is important in a hospital setting, as this should be taken into account for the preparation of mixtures. The interest of the corticosteroid by itself and in combination of other drugs can be demonstrated on the basis of recent articles on HPLC methods that allow routine analysis.¹¹

Considering LBUP, a high dependency on temperature is also observed at 45°C : 2 days of exposure results in a 10% decrease, pointing a very low stability that is also confirmed at other conditions. This drug has been studied in different admixtures, and its use is widespread for different anesthetic and analgesic purposes. Surprisingly, the combination with betamethasone has not been checked before.^{12,13}

Comparison of batches A and B does not demonstrate differences for any of the drugs assayed. It can be concluded that the influence of light exposure in degradation is negligible for this preparation. The ANOVA and Tukey tests do not show statistical differences in concentration. No differences were detected when comparing the concentration of BSP, BA, and LBUP at the initial and end result regardless of the packing (glass vs polypropylene, data not shown).

No changes occurred during the study period in color (the content of the vials were kept transparent) or concerning crystallization of nuclei (not detected). Regarding pH, only a slight, nonsignificant decrease from 7.4 to 6.8 could be seen after 6 months at high temperature (45°C).

The microbiological study found that the mixture meets the necessary sterile conditions after 6 months.

The European Pharmacopeia states that commercially available drugs must contain 100 ($\pm 10\%$) of the active ingredient dose of the label strength. This is the case for levobupivacaine hydrochloride (Pharm Eur. Monograph 0541), betamethasone acetate (Pharm Eur. Monograph 0975), and betamethasone phosphate (Pharm Eur. Monograph 0810). Therefore, the expiry date is estimated as the time after preparation at which drug concentration decreases to 90% of the declared concentration.

This value is calculated as the lower limit of the 95% confidence interval of the zero-order regression line of the concentration profile. They are presented in Figure 2. The figure shows the period at which 90% of the declared concentration differs with the considered drug. The least stable is BSP at 120 days, followed by LBUP at 163 days. BA remains unchanged during 155 days. To maintain the quality of the mixture, the less stable fixes the maximum period it can be used.

Conclusions

This study suggests that epidural formulation mixtures of betamethasone and levobupivacaine are chemically, microbiologically, and physically stable for up to 4 months at ambient temperature in sealed polypropylene vials. The studied concentration is the standard for the combination of

Figure 1. Percentage profile versus time of (A) BSP, (B) BA, and (C) LBUP. Long dash lines represent the linear regression.

Figure 2. Graphical determination of the validity period for every compound present in the mixture: (A) BSP, (B) BA, and (C) LBUP. Long dash lines represent the 95% confidence interval of the regression.

these drugs; it is used as such or after dilution with saline to individualize the rate of perfusion. This study enables the centralized preparation after reconstitution in the hospital pharmacy.

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Declaration of Conflicting Interests

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