

Title: Stability of a 1.0 mg/ml aqueous pholcodine solution for allergy skin testing

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Accepted Article

We read with great interest the article of Bruschi et al. [1] where the authors report two patients with primary allergic reaction to pholcodine who were subsequently found by skin and serological testing to have evidence of sensitization to a neuromuscular blocking agent (NMBA). We fully agree with Bruschi et al. about the necessity to perform a more complete study addressing the link between pholcodine and NMBA anaphylaxis. However we have a better opinion about the interest of pholcodine skin testing, with less fear about non-specific histamine release as it is performed by allergologists with diluted syrups (corresponding to 0,1 to 0,01 mg/ml of pholcodine) according to our experience and to the published data [2-3]. We therefore decided to assess the stability of a pholcodine (pharmaceutical grade) 1 mg/ml solution over a 14-day period so as to be able to propose to allergologists a solution ready to dilute for standardized skin testing.

The European Pharmacopoeia (EP) monograph of pholcodine mentions the following synthesis or degradation impurities: morphine (synthesis and degradation, impurity A), codeine (derived from morphine, impurity B), and impurity D resulting from a double substitution by morpholinoethyl radical (figure 1) [4]. Codeine and morphine are known to be responsible for non-specific histamine release. Other detectable impurities are oxidation derivatives (N-oxide, N'-oxide, and N,N'-oxide).

Preparation was performed by our pharmacy preparation unit. Five hundred milligrams of pholcodine monohydrate powder (batch 22042A004, PCAS, Longjumeau, France) were dissolved in 100 ml of sterile NaCl 0.9% (Aguettant, France) and completed to 500 ml, filtered with 0.22 µm cellulose acetate filter and divided into 5 ml fractions in series of 14 glass vials. Seven of the vials were kept at 4–8°C, and the other seven were left at room temperature (20–25°C); all protected from light. All analyses were performed for two samples on days 0, 1, 2, 3, 6 and 14.

As recommended by the EP, liquid chromatography was chosen as the primary analysis technique. HPLC was carried out with Elite LaChrom® chromatographic system (VWR, Neuilly sur Marne, France) composed of a quaternary pump, a diode array detector, and an injector, coupled to EZChrom Elite™ Software Chromatography Data System. The column was a Luna® Phenomenex (Le Pecq, France) composed of 3 µm spherical particles of end-capped phenylhexylsilylated silica gel (column: 7.5 cm length, 4.6 mm diameter, 10 nm pore diameter, 400 m²/g surface). The working temperature was 35°C. The mobile phase was daily prepared

by adding 75 mL of acetonitrile to 50 mL of tetrahydrofuran for chromatography, completed to 1000 mL with 0.02 M phosphate buffer solution and adjusted to pH 7.90 $\pm$ 0.05 with 0.2 M sodium hydroxide; the pH should not exceed 8.0. Limit of quantification (signal/noise=10) for impurities A, B and D was close to 0.004%. Coefficients of variation were 1.1% for pholcodine, 27% for impurity A, 9.5% for impurity B, and 13.8% for impurity D.

We secondarily performed gas chromatography coupled to mass spectrometry (GC-MS) to allow the detection of very small amounts of impurities A, B and D. Single quadrupole mass spectrometry apparatus (DSQ II, Thermo Fischer Scientific, Villebon-sur-Yvette, France) was coupled with Focus GC system equipped with a DB-5MS non-polar capillary Agilent J&W GC column (Agilent Technologies, Massy, France) of 30 m length, 0.25 mm diameter and 0.25 μ m film thickness. Commercial stock solutions of pholcodine, codeine, morphine, and dextromoramide were supplied by Lipomed (Lipomed, Arlesheim, Switzerland).

Sterility tests were performed according to the EP [5].

We found that pholcodine concentration did not significantly vary between day 0 and day 14, whatever the storage temperature. It remained between 97.5% and 100.6%, which is within the interval required for a finished product (95.0–105.0%). Chromatograms established with a 0.05% limit of exclusion showed that impurities A, B, and D remained below the accepted threshold (max. 0.2%). Amounts of impurities for the pholcodine in solution after 14 days were almost identical to those of the control pholcodine active substance used to prepare the solution, with impurities A (0.05% vs 0.06% for the powder) and D (0.08% vs 0.09%), and absence of codeine. No N-oxides were detected and no unspecified impurity was over 0.10%. Finally, the total amount of impurities was close to that of the bulk pholcodine (0.30% vs 0.25% respectively), and far below the maximal specification (0.7%). All vials were tested for sterility and remained uncontaminated.

Such an aqueous solution of 1.0 mg/ml pholcodine is stable during a 14-day period, with no significant amount of codeine or morphine that could generate non-specific histamine release. It could be suitable for performing dilutions in standardized cutaneous or *in vitro* tests enabling exploration of pholcodine sensitization in patients with suspected pholcodine allergy, but also in patients with NMBA anaphylaxis.

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- Accepted Article
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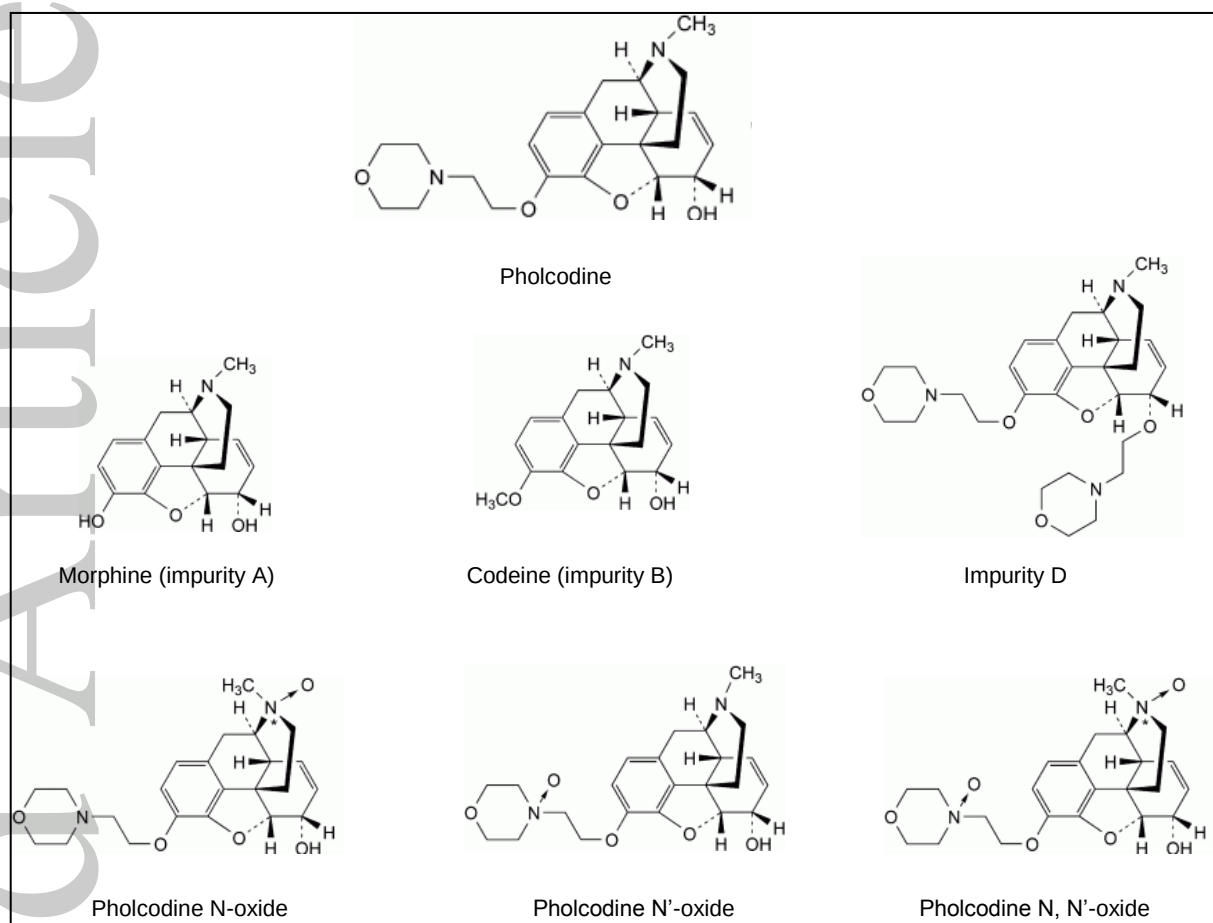


Figure 1. Chemical structures of pholcodine and its synthesis or degradation impurities.