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Short Communication

Determination of pralidoxime chloride in pharmaceutical dosage forms by isotachophoresis*

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Introduction

Pralidoxime chloride (PAM-2Cl), is a reactivator of organophosphate-inhibited cholinesterase. PAM-2Cl alone or in combination with atropine has therapeutic value as an antidote to poisoning by organophosphate agents or drugs acting as cholinesterase inhibitors. Existing methods commonly used for the analysis of PAM-2Cl are HPLC [1, 2], TLC [3], spectrofluorimetry [4], spectrophotometry [5, 6], polarography [7] and non-aqueous titration [8].

Because of the cation nature of the pyridinium oximes, capillary isotachophoresis (ITP) can be considered as a convenient analytical method for their determination. Accordingly in this study it was sought to develop a simple and low cost ITP method for the determination of PAM-2Cl in dosage forms.

Experimental

Materials

Pralidoxime chloride, (2-[(hydroxyimino)-methyl]1-methylpyridinium chloride) (purity 99.5%) was synthesized at the Laboratories of Bosnalijek, Sarajevo. Pralidoxime tablets, each containing 500 mg PAM-2Cl; pralidoxime injection containing 1 g PAM-2Cl; pralidoxime autoinjector ampoules containing 450 mg PAM-2Cl and 2 mg atropine sulphate were obtained from Bosnalijek, Sarajevo. All other chemicals were of analytical grade purity (Merck). Redistilled water was used throughout.

Calibration

The stock solution of 5 mg ml^{-1} PAM-2Cl was prepared in water. The dilutions of the stock solutions were made so that the injection of 1 μl into the ITP analyser corresponded to amounts of 1–5 μg of PAM-2Cl.

Sample preparation

Samples of PAM-2Cl injections and PAM-2Cl autoinjector ampoules were diluted with redistilled water to 2.6 mg ml^{-1} and 2.4 mg ml^{-1}, respectively.

Disintegrated tablets containing 500 mg of PAM-2Cl were dissolved in 200 ml water. The solution was mixed for 10 min, filtered and injected.

Isotachophoretic conditions

ITP analyses were carried out on an LKB (Bromma, Sweden) model 2210 tachophor equipped with a 630 mm PTFE capillary. The leading electrolyte contained potassium ions (10 mM), with acetate as the counter ion (pH 4.7) and the terminating electrolyte was β-alanine (10 mM). Triton (2 g l^{-1}) was used as an additive in the leading electrolyte. Usually runs were started at a current of 250 μA which was gradually reduced to 50 μA shortly before

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Table 1
Results of calibration

Amount of PAM-2Cl injected (μg)	Length* (mm)	RSD (%)
0.625	3.0	2.7
1.25	5.5	2.5
2.50	10.5	3.4
3.75	16.5	2.0
5.00	22.0	1.7

*Average of five replicates; regression equation $y = 4.36x + 0.039$, $Sxy = 0.308$; coefficient of correlation $r = 0.994$.

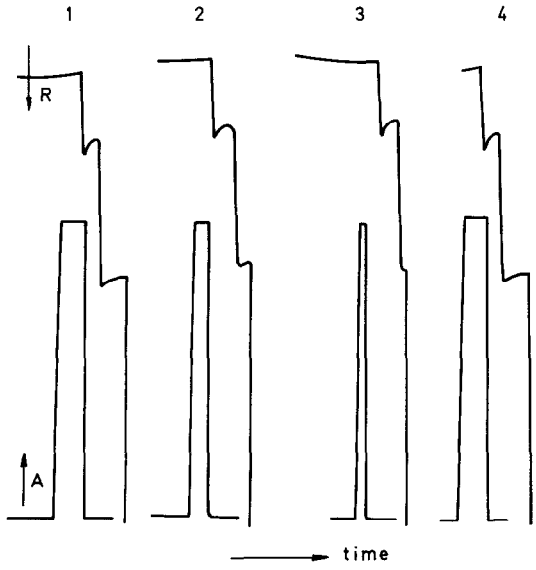

Figure 1
Isotachopherogram of PAM-2Cl standards: 2.5 μg (1), 1.25 μg (2), and 0.625 μg (3) and analysis of PAM-2Cl in autoinjector ampoules: 2.4 μg (4); the conductivity signal (R) is shown together with the UV signal (A) at 254 nm.

Table 2
Assay of PAM-2Cl in dosage forms

PAM-2Cl dosage forms	Nominal amount (μg)	Amount found* (μg)	RSD (%)
Tablets	2.50	2.45	1.30
Injections	2.66	2.60	1.73
Autoinjector ampoules	2.40	2.45	2.60

*Mean ($n = 7$).

the cation was due to be detected. Analyses were carried out at a temperature of 20°C and took 15 min. The volumes injected were 1 μl. UV absorption was used for detection and quantification.

Results and Discussion

For the determination of PAM-2Cl, a calibration graph was used. The calibration plot (zone length vs the sample amount) was found to be linear up to 5 μg. The calibration data are given in Table 1. Values of relative standard deviation (1.7–3.4%) confirmed the reliability of the method. At a chart speed of 10 mm min^{-1} the limit of detection was 0.150 μg. Isotachopherograms of standards and the analysis of PAM-2Cl in autoinjector ampoules are shown in Fig. 1. Although the PAM-2Cl autoinjector ampoules contained PAM-2Cl and atropine sulphate, the amount of the latter injected could not be detected. In addition, it was demonstrated that, since the method allows separation of PAM-2Cl and atropine sulphate, even higher concentrations of atropine did not interfere with PAM-2Cl determination. No interference was found from tablet excipients.

Results of the determination of PAM-2Cl in dosage forms are shown in Table 2. Recoveries were (97.8–102.1%) in relation to the labelled claim; the relative standard deviation obtained (1.3–2.6%) confirmed the reproducibility of the method.

In summary, by allowing the rapid and accurate determination of PAM-2Cl, the isotachophoretic method was found to be suitable for the routine analysis of PAM-2Cl in dosage forms.

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