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Note

Rapid high-performance liquid chromatographic determination of pramoxine hydrochloride in topical cream and suppositories

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Pramoxine hydrochloride is the active ingredient of numerous pharmaceutical preparations. Quantitation of pramoxine hydrochloride in a pharmaceutical preparation is complicated by substantial interferences from the high lipoid excipients. A direct analysis of an aerosol cream by gas–liquid chromatography[1] (GLC) resulted in a lengthy sample preparation and 30 min chromatographic separations. Tuesley *et al.*[2] reported a non-aqueous titration of a similar preparation without interferences. Others reported non-aqueous titrimetry[3], spectrophotometry[3], and high-performance liquid chromatography (HPLC)[1], all of which require removing the excipients prior to quantitation. The non-aqueous titrimetric and spectrophotometric techniques are non-specific and the GLC method is time consuming. This paper presents a rapid and stability-indicating HPLC technique to determine pramoxine hydrochloride in cream and suppositories.

EXPERIMENTAL

Reagents

Acetonitrile was distilled in glass UV grade from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Methanol and 2-propanol were HPLC grade from Fisher Scientific (Fair Lawn, NJ, U.S.A.). A pramoxine hydrochloride USP reference standard (United States Pharmacopeia, Washington, DC, U.S.A.) was used as the analytical standard. Dibutyl phthalate (Aldrich, Milwaukee, WI, U.S.A.) was used as the internal standard. Phosphoric acid and potassium phosphate dibasic anhydrous were AR grade from J. T. Baker (Phillipsburg, NJ, U.S.A.). A 0.2 *M* potassium phosphate (dibasic) pH 7.5 buffer was prepared by dissolving 3.5 g of potassium phosphate dibasic anhydrous in 100 ml of distilled water. The pH of the solution was adjusted to 7.5 with 50% aqueous phosphoric acid.

Internal standard preparation

A 2-ml portion of dibutyl phthalate was diluted with methanol to 50 ml. A 10-ml portion of the resulting solution was further diluted with methanol to 100 ml.

Standard preparation

Into a 25-ml volumeric flask, a 45-mg portion of pramoxine hydrochloride

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USP reference standard was accurately weighed. The pramoxine hydrochloride was dissolved and diluted with methanol to volume. A working standard solution was prepared by diluting 10.0 ml of standard preparation and 5.0 ml of internal standard preparation with methanol to 100 ml.

Sample preparation

A portion of a sample equivalent to approximately 18 mg of pramoxine hydrochloride was weighed accurately into a 250-ml erlenmeyer flask. A volume of 15 ml of 2-propanol and 40 ml of methanol were added to the flask. The sample was warmed over a steam bath to dissolve the cream or melt the suppository, and then 40 ml of methanol and 5.0 ml of internal standard were added. The solution was cooled in a cold water bath (10°C or lower) for about 5 min. A portion of the resulting mixture was filtered through a 0.4 micrometer polycarbonate membrane (Nuclepore, Pleasanton, CA, U.S.A.) prior to injection into the chromatograph.

Apparatus

The HPLC system consisted of a Model 870 chromatographic pump (DuPont Instruments, Wilmington, DE, U.S.A.), a Rheodyne 7125 injection valve with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.), a Model 450 variable wavelength detector (Waters Assoc., Milford, MA, U.S.A.) and an Autolab System I electronic integrator (Spectra-Physics, Santa Clara, CA, U.S.A.). Chromatographic separations were performed on a Waters Assoc. μBondapak C_{18} column (30 cm × 3.9 mm I.D.) and chromatograms were recorded on a Model 385 Linear recorder (Linear Instruments, Irvine, CA, U.S.A.). A 3 cm × 4.6 mm I.D. C_{18} guard column (Brownlee Labs., Santa Clara, CA, U.S.A.) was placed between the injector and the analytical column, and a 15 cm × 4 mm I.D. guard column packed with 0.032–0.063 mm silica gel (ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.) was installed between the chromatographic pump and the injector.

HPLC mobile phase: 0.01 *M* potassium phosphate (dibasic) pH 7.5 buffer–

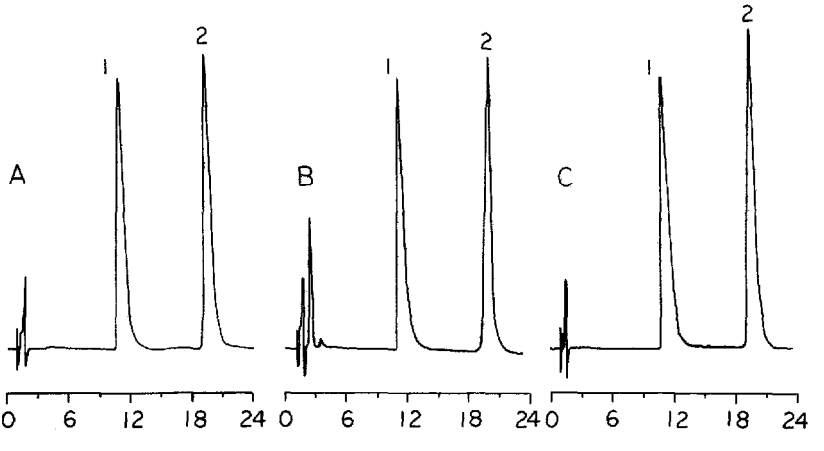

Fig. 1. Typical HPLC chromatograms of pramoxine hydrochloride containing internal standard. (A) working standard; (B) cream sample preparation; (C) suppository sample preparation. Conditions as stated in text. Time scale in minutes. Peaks: 1 = pramoxine hydrochloride; 2 = internal standard.

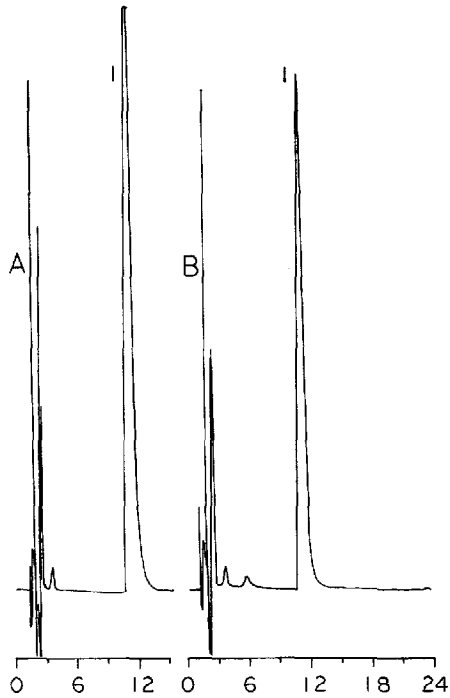

Fig. 2. HPLC chromatograms of cream. (A) Before UV degradation; (B) after UV degradation. Detector sensitivity: 0.8 a.u.f.s. Conditions as stated in text. Time scale in minutes. Peak 1 = pramoxine hydrochloride.

acetonitrile (10:11) with a flow-rate of 2.0 ml/min was used for this investigation. Detector: 224 nm at 1.6 a.u.f.s.

RESULTS AND DISCUSSION

The procedure described herein is a rapid and simplified accurate method for the determination of pramoxine hydrochloride in pharmaceutical preparations. Since an internal standard is used, the quantitation of the final volume of the sample preparation is not needed. A complete removal of the excipients before chromatography is also unnecessary because the soluble excipients left in the sample preparation do not interfere with the assay. Typical chromatograms of the working standard and sample preparations of cream and suppositories are shown in Fig. 1.

In order to show that this method is stability-indicating, a portion of the cream was placed in a beaker and set under a 435-W ultraviolet (UV) lamp (Hanovia Chemical and Manufacturing Co., Newark, NJ, U.S.A.) for about 2 h. The degraded cream was then taken through the assay procedure. Fig. 2 shows the chromatograms of the cream before and after degradation. The cream was about 24% degraded. In treating a suppository similarly to the cream, the suppository was about 71% degraded after 3 h of UV irradiation. Fig. 3 shows the chromatograms of suppositories before and after degradation. In both cases, the degradation products are seen eluting before the

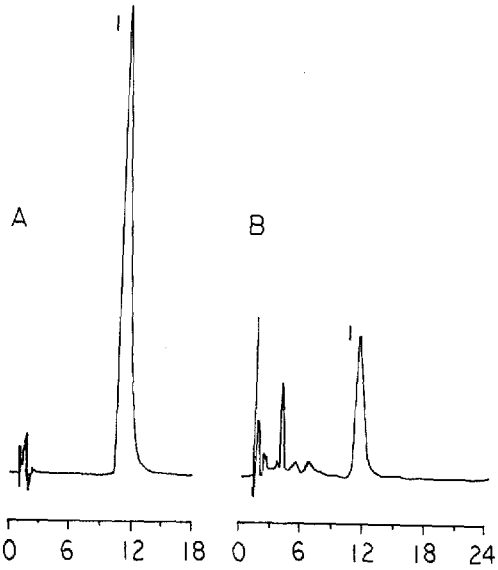

Fig. 3. HPLC chromatograms of suppository. (A) Before UV degradation; (B) after UV degradation. Detector sensitivity: 1.28 a.u.f.s. Conditions as stated in text. Time scale in minutes. Peak 1 = pramoxine hydrochloride.

drug and internal standard and do not interfere with the assay. The method is therefore a stability-indicating method.

Light stability of pramoxine hydrochloride in the alcoholic solution used in this method was also investigated. A working standard solution of pramoxine hydrochloride in a volumetric flask was placed on a bench top and exposed to normal laboratory light for 3 days. It was assayed against an identical preparation stored in darkness. No degradation was detected in either sample.

Method validation

A linear relationship between the peak area ratio and concentration was obtained from 0.036 to 0.36 mg/ml of pramoxine hydrochloride solutions.

Standard addition–recovery studies were performed at the 50, 100 and 150% potency levels of pramoxine hydrochloride in the formulations. Recoveries ranged from 99.5 to 100.3% and 99.2 to 100.7% for the cream and suppositories, respectively. The results are shown in Tables I and II.

TABLE I

RECOVERY OF PRAMOXINE HYDROCHLORIDE FROM CREAM

Potency level (%)	*Amount added (mg)*	*Amount recovered (mg)*	*Recovery (%)*
50	9.17	9.12	99.5
100	18.34	18.4	100.3
150	27.51	27.4	99.6

TABLE II

RECOVERY OF PRAMOXINE HYDROCHLORIDE FROM SUPPOSITORIES

Potency level (%)	*Amount added (mg)*	*Amount recovered (mg)*	*Recovery (%)*
50	9.09	9.02	99.2
100	18.18	18.3	100.7
150	27.28	27.3	100.1

TABLE III

PRECISION DATA FOR THE HPLC ANALYSIS OF PRAMOXINE HYDROCHLORIDE IN CREAM

Run	*Analyst*	*Day*	*Pramoxine · HCl (%)*
1	1	1	1.03
2	1	1	1.01
3	1	2	1.02
4	1	2	1.03
5	2	3	1.03
6	2	3	1.03
		Mean	1.03
	Standard deviation		±0.01
	Relative standard deviation		±0.8

TABLE IV

PRECISION DATA FOR THE HPLC ANALYSIS OF PRAMOXINE HYDROCHLORIDE IN SUPPOSITORIES

Run	*Analyst*	*Day*	*Pramoxine · HCl (%)*
1	1	1	0.964
2	1	1	0.985
3	1	2	0.979
4	1	2	0.984
5	2	3	0.994
6	2	3	0.998
		Mean	0.984
	Standard deviation		±0.012
	Relative standard deviation		±1.2

Precision data were generated from the HPLC analysis of typical lots of cream and suppository formulations by two analysts over a three day period and these data are summarized in Tables III and IV.

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