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Short Communication

High performance thin layer chromatographic determination of tolperisone hydrochloride

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1. Introduction

Tolperisone hydrochloride is used as a muscle relaxant. At present only a few assay methods are reported, using HPLC [1] and potentiometric titration [2]. There has been an increasing demand for a suitable method for the assay of tolperisone hydrochloride. This paper describes a simple high performance thin layer chromatographic method (HPTLC) for the determination of tolperisone hydrochloride in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

An HPTLC system from Camag (Switzerland) was used. It consisted of an automated TLC sampler, an automated multiple development chamber and a TLC scanner 3. A 691 pH meter (Metrohm) was also used.

2.2. Chemicals

All chemicals were of analytical reagent grade and used without further purification.

The following reagents were used: acetic acid anhydrous; acetone; chloroform; dichloromethane and perchloric acid (BDH Chemical, UK); crystal violet; ethanol; methanol; potassium hydrogen phthalate; glacial acetic acid and *n*-butanol (E. Merck Darmstadt, F.R.G.); tolperisone hydrochloride and lidocaine hydrochloride (Sigma Chemical Company, St. Louis, MO, USA).

2.3. Recommended procedure

2.3.1. Construction of calibration curve

Known amounts of standard tolperisone hydrochloride (dissolved in absolute ethanol) in the range of 0.5–5.0 μg ml^{-1} were applied on an

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HPTLC plate (10 × 20 cm) precoated with silica-gel G60 using an automated TLC sampler. The plate was developed for 15 min in an automated multiple development chamber, using methanol or methanol:acetone:butanol (50:45:5, v/v/v) as mobile phase. The peak area of each spot was determined by using a TLC scanner 3 with UV detection at 260 nm. Calibration curve was constructed by plotting the peak area against various concentrations of tolperisone hydrochloride.

2.3.2. Procedure for the assay of dosage forms

Not less than twenty tablets were accurately weighed and finely powdered. A sample amount equivalent to ca. 100 mg of tolperisone hydrochloride was accurately weighed, transferred into a 50-ml volumetric flask and diluted to the mark with absolute ethanol. Then a ca. 400 ppm solution of tolperisone hydrochloride was prepared from the above solution by dilution with absolute ethanol. The injection containing 100 mg ml^{-1} tolperisone hydrochloride and 2.5 mg ml^{-1} lidocaine hydrochloride was used. The contents of five injections were mixed together and 1 ml of the sample was taken, transferred into a 50-ml volumetric flask and made up to 50 ml with absolute ethanol. Then a ca. 400 ppm solution of tolperisone hydrochloride was prepared by dilution with absolute ethanol. Each sample solution was taken and analyzed by using the method described in Section 2.3.1.

3. Results and discussion

3.1. Optimization of experimental conditions

The thin layer chromatographic separations of tolperisone hydrochloride and lidocaine hydrochloride were carried out, using dichloromethane, methanol, dichloromethane: butanol (3:1), dichloromethane:ethanol (1:1, v/v) and methanol:acetone:butanol (50:45:5, v/v/v) as mobile phases, and silica gel G60-precoated plates (20 × 20 cm) were used. After development, the plates were dried and the spots were detected under short wavelength ultraviolet light at 254 nm with a UV viewer. The results showed that among the mobile phases tested, methanol or methanol:acetone:butanol (50:45:5, v/v/v) gave better separation of tolperisone hydrochloride and lidocaine hydrochloride as shown in Fig. 1. The R_f values of tolperisone hydrochloride and lidocaine hydrochloride using the former mobile phase were 0.61 and 0.91, respectively while those obtained by using the latter mobile phase were 0.50 and 0.86, respectively. Therefore, these two solvent systems were chosen as appropriate solvents for the development of the HPTLC method.

3.2. Limit of detection

The lower detection limit of tolperisone hydrochloride was also investigated. When methanol

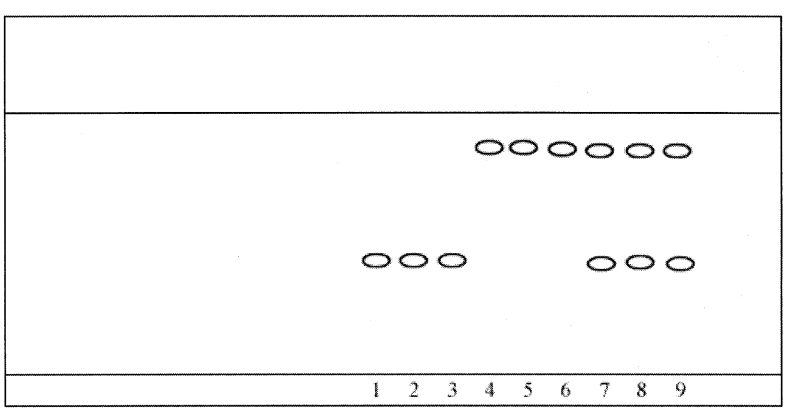

Fig. 1. Chromatogram obtained from the separation of 5 μl of standard solution containing 5 μl of tolperisone hydrochloride and lidocaine hydrochloride using methanol:acetone:butanol (50:45:5, v/v/v) as mobile phase. 1–3 = Spots of toperisone hydrochloride, 4–6 = spots of lidocaine hydrochloride, 7–9 = spots of tolperisone hydrochloride and of lidocaine hydrochloride.

Table 1
Reproducibility and accuracy of the HPTLC assay using methanol as mobile phase

Amount of tolperisone hydrochloride added (mg)	Amount of tolperisone hydrochloride found ($n = 5$) (mg)	% Recovery	% CV	% Error
7.6	7.73	101.67	1.2	1.67
8.9	8.97	100.81	1.3	0.81
10.0	9.92	99.18	1.0	0.82
	Average	100.55	1.2	1.10

Table 2
Reproducibility and accuracy of the HPTLC assay using methanol:acetone:butanol (50:45:5, v/v/v) as mobile phase

Amount of tolperisone hydrochloride added (mg)	Amount of tolperisone hydrochloride found ($n = 5$) (mg)	% Recovery	% CV	% Error
8.1	8.06	99.49	0.9	0.51
10.1	10.05	99.46	1.0	0.54
12.5	12.52	100.20	0.8	0.20
	Average	99.71	0.9	0.41

was used as mobile phase, the limit of detection (defined as three times of the baseline noise) was calculated to be 20.12 ng spot^{-1} of tolperisone hydrochloride. When methanol:acetone:butanol (50:45:5, v/v/v) was used as mobile phase, the detection limit was 4.12 ng spot^{-1} of tolperisone hydrochloride. This indicated that the latter mobile phase exhibited five times better sensitivity than that of the former, since the latter mobile phase gave the lower blank level than that of the former with good reproducibility, leading to the higher signal to noise ratio and hence, the lower detection limit, better precision and accuracy.

3.3. Calibration graphs

The calibration graphs for tolperisone hydrochloride using methanol as mobile phase were linear over the ranges 0.02–0.10, 0.20–0.50, 0.50–5.0 and 5.0–25.0 μg ml^{-1}. Linear regression analysis gave correlation coefficients of 0.9999, 0.9987, 0.9981 and 0.9986, respectively. When methanol:acetone:butanol (50:45:5, v/v/v) was used as mobile phase, linear calibration graphs were obtained over the ranges 0.004–0.020, 0.030–0.400, 0.500–5.00 and 5.00–25.00 μg ml^{-1}. Linear regression analysis gave correlation coefficients of 0.9987, 0.9999, 0.9987 and 0.9987, respectively. Therefore all the calibration graphs showed good correlation coefficient values which indicated excellent agreement.

3.4. Precision and accuracy

The reproducibility and accuracy of the proposed method were verified by analysing the aliquots of Mydocalm injection sample solutions spiked with various amounts standard tolperisone hydrochloride. The results are shown in Tables 1 and 2.

Better precision and accuracy were obtained when methanol:acetone:butanol (50:45:5, v/v/v) was used as mobile phase, compared with those obtained by using methanol alone as mobile phase.

3.5. Application

The proposed HPTLC was applied to the analyses of two tablet formulations and one injection formulation of tolperisone hydrochloride. A comparative determinations of tolperisone hy-

Table 3
HPTLC and potentiometric assay of tolperisone hydrochloride in pharmaceutical preparations

Sample	% Of the label claim[a]	
	HPTLC	Potentiometric titration
Tolperisone hydrochloride Tablet 1	99.7	96.7
Tolperisone hydrochloride Tablet 2	100.0	97.8
Tolperisone hydrochloride Injection	99.8	97.0

[a] Average of 10 determinations.

drochloride in the same samples were also carried out by potentiometric method [2]. The results are summarized in Table 3. Excellent correlation between the two methods was obtained.

The method could also be used for the determination of tolperisone hydrochloride in the presence of lidocaine hydrochloride as shown in Fig. 1.

4. Conclusion

The present HPTLC assay method is very precise, sensitive and accurate. It offers several advantages of low cost, simple and rapid. It affords simultaneous determination of tolperisone hydrochloride and lidocaine hydrochloride. No interference from excipients or impurities is encountered. The method was also evaluated for analysing commercial formulation and comparing the results with those obtained by the official method. Satisfactory agreement between the results was obtained.

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