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STABILITY-INDICATING ASSAY FOR ORPHENADRINE HYDROCHLORIDE BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid method based on reversed-phase high-performance liquid chromatography (HPLC) is described for the separation and quantitation of orphenadrine hydrochloride and its major degradation product *o*-methylbenzhydrol. The method has been applied to the assay of orphenadrine hydrochloride and *o*-methylbenzhydrol in the commercially available tablet and injectable formulations and in a hospital-formulated syrup. The method is stability-indicating and has been used for preliminary studies of the degradation of orphenadrine [1% (m/v)] in aqueous solutions stored at various pH values at elevated temperature. The assay results obtained for the dosage forms and the preliminary stability study confirm the HPLC method as being suitable both for routine quality control and for stability studies on orphenadrine hydrochloride preparations.

INTRODUCTION

Orphenadrine hydrochloride is indicated in the treatment of all forms of Parkinsonism and is also widely used in psychiatric medicine for the prevention and control of extrapyramidal side effects induced by major tranquillisers, such as the phenothiazines. Orphenadrine hydrochloride is commercially available in tablet and injection form. Some psychiatric patients are, however, unwilling or unable to take tablets, so that it becomes necessary to formulate an oral liquid preparation of the drug for clinical use.

Although several studies on the metabolic fate of orphenadrine hydrochloride in rat[1], monkey[2] and man[3] have been reported, no detailed information on analytical methods for orphenadrine hydrochloride has been published. Bioanalytical methods based on thin-layer chromatography[3], non-aqueous titration[4], UV-spectroscopy[5],

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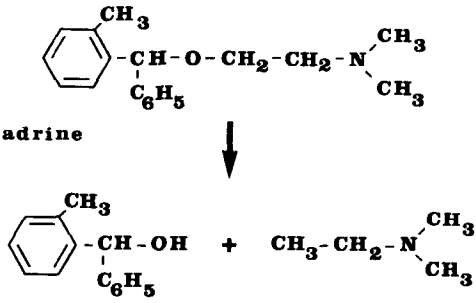

Orphenadrine

o-methylbenzhydrol

Scheme 1.

gas-liquid chromatography[6] and fluorimetry[7] have been described. For the analysis of dosage forms, a stability-indicating method is required to discriminate between the parent compound, orphenadrine hydrochloride, and its major degradation product, *o*-methylbenzhydrol (Scheme 1). A stability-indicating method is essential for the development of a new pharmaceutical formulation, such as that required in the present case for routine, large-scale use in the hospital service.

The present work reports the development and evaluation of a simple, precise and sensitive stability-indicating assay by reversed-phase high-performance liquid chromatography (HPLC) for the rapid analysis of orphenadrine hydrochloride and *o*-methylbenzhydrol in various dosage forms. The application of this method to a pilot study on the stability of orphenadrine hydrochloride in aqueous solutions at elevated temperature is also described.

EXPERIMENTAL

Reagents and materials

HPLC-grade acetonitrile (Rathburn Chemicals, Walkerburn, U.K.) was used as received. Potassium dihydrogen phosphate was AnalaR grade (BDH, Poole, U.K.) and sodium lauryl sulphate was of a grade specifically purified for biochemical work (Fisons, Loughborough, U.K.). All eluent mixtures in glass-distilled water were filtered through a Millipore® 0.45-μm HF filter using an all-glass apparatus, before degassing for 10 min in an ultrasonic bath under reduced pressure.

Orphenadrine hydrochloride powder was supplied by A. H. Cox (Barnstaple, U.K.) and the major degradation product, *o*-methylbenzhydrol, was kindly provided by Riker Laboratories (Loughborough, U.K.); both compounds were used as received. Orphenadrine hydrochloride (Disipal) tablets and injection were obtained from Brocades (Weybridge, Surrey, U.K.). The glacial acetic acid and perchloric acid were of a grade for non-aqueous titration (BDH).

Equipment

The liquid chromatograph was assembled in the laboratory from commercially available units, comprising a constant-flow pump (Gilson Model 302, Gilson, Villiers-le-Bel, France) and a variable-wavelength UV detector with an 8-μl flow cell

(CE212, Cecil Instruments, Cambridge, U.K.), operated at 220 nm. A 100 × 5 mm I.D. stainless-steel column (Shandon-Southern Instruments, Cheshire, U.K.) was slurry-packed with a microparticulate bonded reversed-phase packing (5-μm ODS Hypersil, Shandon-Southern Instruments) by the upward displacement technique. Sample introduction was by SGE 20-μl microsyringe (Scientific Glass Engineering, Melbourne, Australia) and loop valve injector (Rheodyne, CA, U.S.A.). The chromatograph was flushed clean at the end of each day with methanol–water (60:40, v/v) to prevent salt deposition in the system. Using the same eluent, the column performance was checked regularly with a test mixture of phenol and *p*-cresol, the average number of theoretical plates (*N*) being over 40,000 plates/m. The pH meter used was a Corning-EEL Model 113 (Corning Medical, Essex, U.K.).

Development of HPLC procedure

The reversed-phase HPLC method was developed by systematic optimisation of the detection wavelength and of each eluent parameter in turn: type and concentration of organic modifier, eluent pH, type and concentration of pairing ion, and eluent flow-rate, as discussed below.

Protocol for quantitation

Quantitation was performed using peak heights measured to the baseline extrapolated from the leading edge of the peak. The structurally similar compound, diphenhydramine hydrochloride, was selected as the internal standard. The internal standard was incorporated in standards and tests by quantitative dilution at concentrations of 600 μg/ml and 50 μg/ml for orphenadrine hydrochloride and *o*-methylbenzhydrol assays, respectively, to give peak heights comparable to those anticipated for the analytes. The single-point bracketting technique was employed for all routine quantitative measurements. In this procedure, a group of test samples, preceded and followed by a standard mixture of comparable concentration, is assayed with respect to the average standard peak-height ratio.

Analysis of dosage forms

The dosage forms investigated included the commercially available Disipal tablets (50 mg), Disipal injection (20 mg/ml) and a hospital-formulated orphenadrine hydrochloride syrup (1%, m/v).

The orphenadrine hydrochloride content of eight samples of the injection and of the syrup was determined by HPLC as follows: a 2.0 ml-sample of injection (equivalent to 40 mg orphenadrine hydrochloride) was combined with 10.0 ml of an aqueous solution of 3.0 mg/ml diphenhydramine hydrochloride in a 50.0-ml volumetric flask and made up to volume with distilled water to give an internal standard concentration of 600 μg/ml. An analogous procedure was used for the syrup, except that the 4.0 ml sample was added last, using the reverse dilution technique to overcome problems of sample viscosity.

A sample of crushed orphenadrine hydrochloride tablets (equivalent to 70 mg orphenadrine hydrochloride) was combined with 10.0 ml of an aqueous solution of 6 mg/ml diphenhydramine hydrochloride in a 100.0 ml volumetric flask to give an internal standard concentration of 600 μg/ml, and then made up to volume with acetonitrile–water (50:50, v/v) mixture. The flask was placed in a sonic bath for 30

min and the extract filtered. Two 15-μl injections of each solution were assayed and the orphenadrine hydrochloride content of each dosage form calculated.

The *o*-methylbenzhydrol content of six samples of the injection, the syrup and tablets was also determined. A 2.0-ml sample of the injection was combined with 5.0 ml of an aqueous solution of 250 μg/ml diphenhydramine in a 25.0 ml volumetric flask and made up to volume with distilled water to give an internal standard concentration of 50 μg/ml. Again an analogous procedure was used for the syrup, except that the 10.0 ml sample was added by the reverse dilution technique. Each orphenadrine hydrochloride tablet (50 mg) was sonicated for 30 min in a 25.0 ml volumetric flask with 5.0 ml of an aqueous solution of 250 μg/ml diphenhydramine hydrochloride and then made up to volume with acetonitrile–water (50:50, v/v) mixture. Two 15-μl injections of each solution were assayed for their *o*-methylbenzhydrol content.

In the British Pharmacopoeial monograph for orphenadrine hydrochloride tablets[8], an official assay method is prescribed for orphenadrine hydrochloride and this was therefore selected as a referee method for the HPLC assay of the tablets. Samples of crushed Disipal tablets (equivalent to 70 mg orphenadrine hydrochloride) were assayed independently by the BP 1980 non-aqueous titration method[9]. Orphenadrine hydrochloride in the powdered tablet sample was dissolved as completely as possible in 5 ml of distilled water and 5 ml of 2 *M* hydrochloric acid. Then the orphenadrine hydrochloride was extracted with four 15-ml quantities of chloroform. The combined extracts were filtered and 10 ml of a solution of mercuric acetate (4%, m/v) in glacial acetic acid added before titrating the orphenadrine potentiometrically against 0.02 *M* perchloric acid.

Stability study

A preliminary study on the stability of orphenadrine hydrochloride in aqueous solutions was carried out at various pH values. Aqueous solutions of orphenadrine hydrochloride (1%, m/v) were prepared in 0.2 *M* citric acid–sodium hydroxide buffers at pH 2, 3, 4, 5, 6 and 7. Each solution was assayed in duplicate immediately to establish the initial orphenadrine hydrochloride concentration. Individual flasks were stored in water baths at either 25 or 70°C, samples being withdrawn daily. Each sample was cooled, 4.0 ml added to 10.0 ml of an aqueous 3 mg/ml diphenhydramine hydrochloride solution in a 50.0-ml volumetric flask and made up to volume with distilled water. A 15-μl aliquot was subjected to HPLC analysis in duplicate.

RESULTS AND DISCUSSION

Choice of packing material

Previous work of Bergh and De Vries[10] using a bonded reversed-phase C_{18}-column permitted the separation of diphenhydramine hydrochloride and some of its metabolites, viz. N-desmethyldiphenhydramine, benzhydrol, N,N-didesmethyldiphenhydramine, benzophenone and 2-methyldiphenylmethoxy acetic acid. Since orphenadrine hydrochloride is closely related in chemical structure to diphenhydramine hydrochloride, an ODS-packing material was selected. In this laboratory 5-μm ODS-Hypersil has been shown to give good quantitative performance and was therefore selected as the packing material for the present work.

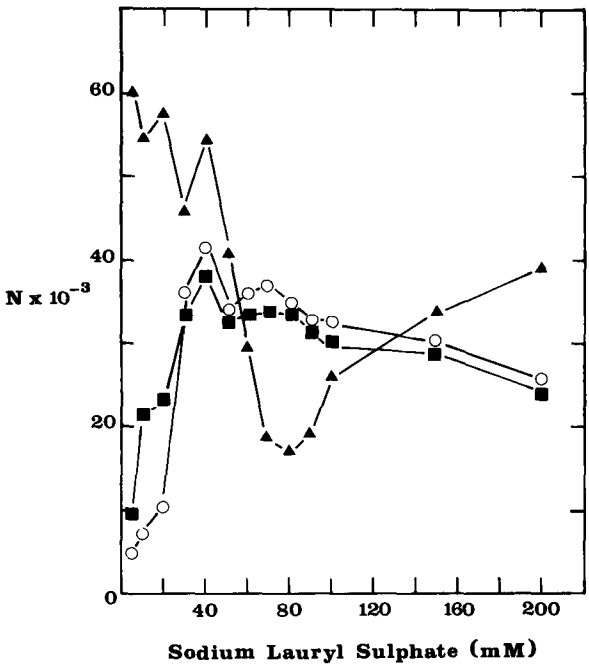

Fig. 1. Relationship of the number of theoretical plates per metre (N) to sodium lauryl sulphate concentration, for orphenadrine hydrochloride (■), diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

Mobile phase optimisation

Preliminary study. Since operation at the alkaline pH necessary to suppress ionisation of orphenadrine (pK_a = 9.0) would be inadvisable due to degradation of the packing material above pH 9, it was decided to examine a reversed-phase ion-pair system. Sodium lauryl sulphate (SLS) was selected as a potential pairing ion. With an initial eluent comprising methanol–aqueous 50 m*M* potassium dihydrogen phosphate pH 7 (60:40, v/v) containing 10 m*M* SLS, a split peak for orphenadrine hydrochloride was observed. When methanol was replaced by acetonitrile as the organic modifier, the peak-splitting effect was eliminated. With the eluent acetonitrile–aqueous 50 m*M* potassium dihydrogen phosphate pH 7 (50:50, v/v) containing 10 m*M* SLS, a 15-μl injection of aqueous orphenadrine hydrochloride (100 μg/ml) yielded a single peak with marked tailing, indicating the need to optimise the sodium lauryl sulphate concentration and/or the pH. The pH of the eluent [acetonitrile–50 m*M* buffer (50:50, v/v), containing 10 m*M* SLS] was initially adjusted by drop-wise addition of 2 *M* sodium hydroxide or 0.25 *M* sulphuric acid, giving values in the range of pH 3 to 7 to permit a rapid assessment of the pH-effect before more detailed examination. The highest chromatographic efficiency for orphenadrine hydrochloride in terms of the number of theoretical plates per metre (N) was observed at about pH 4.0, as discussed below.

Ion-pair reagent. The effect of ion-pair reagent concentration was then examined in the range 5–200 m*M*, using the same eluent adjusted to pH 4.0. The relation-

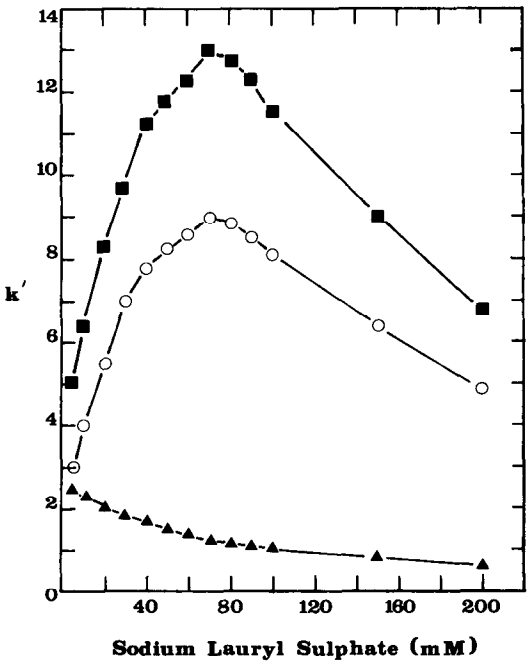

Fig. 2. Relationship of the phase capacity ratio (k') to sodium lauryl sulphate concentration, for orphenadrine hydrochloride (■), diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

ship of N and of the phase capacity ratio (k') to SLS concentration is illustrated in Figs. 1 and 2 for orphenadrine hydrochloride, the internal standard diphenhydramine hydrochloride and the degradation product *o*-methylbenzhydrol. Optimum column efficiency and complete resolution of all three components was observed at 40 mM SLS. The degradation product appeared to be particularly sensitive to SLS concentration, even though it would not be expected to form ion pairs. The retention behaviour of the two ionised solutes followed the classical model, with a maximum k' value at 80 mM SLS (Fig. 2), illustrating the possibility for fine-tuning ion-pair separations of this kind.

Organic modifier. The concentration of acetonitrile was varied from 40 to 70% (v/v), keeping other eluent parameters constant [aqueous 50 mM potassium hydrogen phosphate (pH 4.0), with a total eluent concentration of 40 mM SLS]. The optimum column efficiency for all three compounds corresponded to a composition of 50% (v/v) acetonitrile, as shown in Fig. 3. As would be expected for a reversed-phase system, the k' values of the three compounds decreased with increased acetonitrile concentration (Fig. 4). Excellent resolution and analysis were observed at an acetonitrile concentration of 50% (v/v), which was therefore selected for further work.

Eluent pH. Finally, the effect of eluent pH was re-examined. The relationship of both N and k' to eluent pH for orphenadrine hydrochloride, diphenhydramine hydrochloride and *o*-methylbenzhydrol is illustrated in Figs. 5 and 6, which confirmed that for all three compounds the highest chromatographic efficiency corresponded to pH 4.0. Although the k' values were relatively insensitive to pH, the

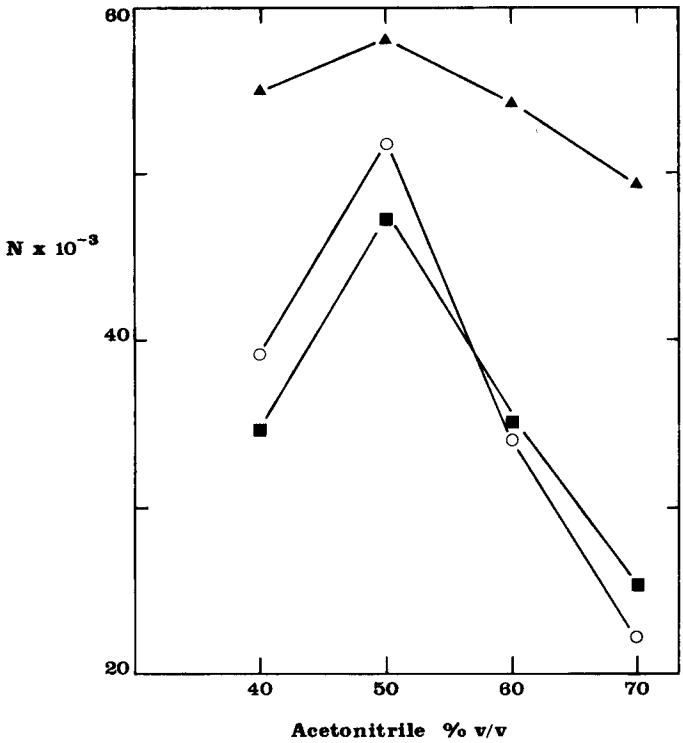

Fig. 3. Relationship of N (plates/m) to acetonitrile concentration, for orphenadrine hydrochloride (■), diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

chromatographic efficiency of both ionised solutes was very sensitive to pH. It is therefore necessary to control this parameter very carefully to ensure assay uniformity from one batch of eluent to another. The optimum flow-rate of the eluent giving a high column efficiency and an acceptable retention time was found to be 2.0 ml/min. The phase capacity ratios for the three compounds were unaffected by the flow-rate of the eluent over the range examined (0.5–3.0 ml/min).

Optimised composition. The eluent composition finally selected for the separation of orphenadrine hydrochloride, its degradation product *o*-methylbenzhydrol and the internal standard diphenhydramine hydrochloride, comprised acetonitrile–aqueous 50 m*M* potassium dihydrogen phosphate (50:50, v/v), containing 40 m*M* SLS and adjusted to pH 4.0, at a flow-rate of 2.0 ml/min. Under these conditions the mean k' (n = 12) for each of the three compounds at ambient temperature (*ca.* 21°C) were 11.3, 1.7 and 7.8, respectively. The chromatogram illustrated in Fig. 7 shows satisfactory resolution of the compounds with a total analysis time of about 8.5 min, as required for routine quality control of dosage forms. Orphenadrine hydrochloride was well resolved from its major degradation product *o*-methylbenzhydrol, thus enabling the assay to be used for stability studies.

Choice of detection wavelength

In acidic conditions orphenadrine hydrochloride has a characteristic aromatic

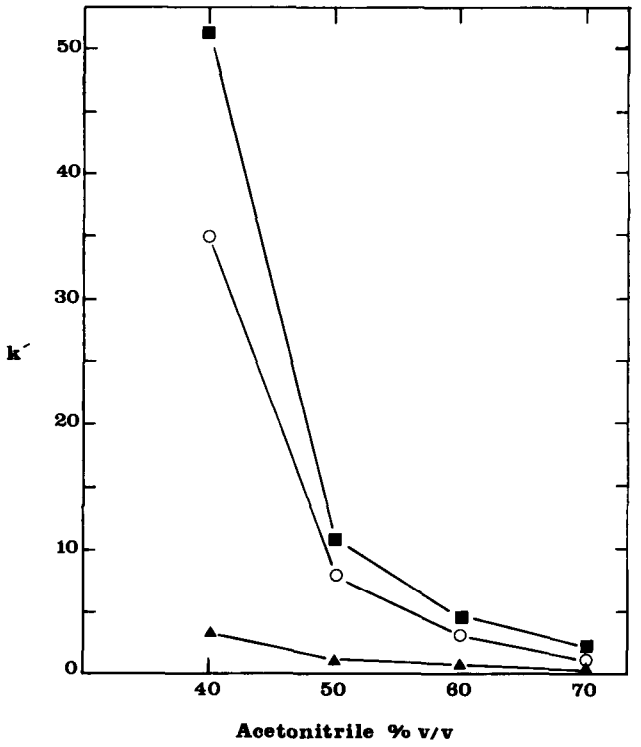

Fig. 4. Relationship of k' to acetonitrile concentration, for orphenadrine hydrochloride (■), diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

UV absorption maximum at 264 nm with low absorptivity ($A\,1^{1\%}_{cm} = 24$) and a highly absorptive peak at 220 nm, which was therefore chosen for increased detection sensitivity. The absorption spectrum of o-methylbenzhydrol is similar. At this wavelength the detection limit for orphenadrine hydrochloride (defined as the amount corresponding to a signal-to-noise ratio of 2) was found to be approximately 10 ng injected on column; for the sharper *o*-methylbenzhydrol peak, the detection limit was 1 ng on column.

Quantitative performance

Since all components were observed as sharp, well-resolved peaks, constant in shape, quantitation was performed using peak-height measurements. The peak-height ratios of replicate injections (15 μl) of an aqueous solution of orphenadrine hydrochloride (800 μg/ml) and diphenhydramine hydrochloride (600 μg/ml) were measured, when the relative standard deviation (R.S.D.) was found to be 0.14% ($n = 9$). For replicate injections of an aqueous solution of *o*-methylbenzhydrol (10 μg/ml) and diphenhydramine hydrochloride at a concentration (50 μg/ml), chosen to give comparable peak height, the R.S.D. of peak-height ratios was 0.23% ($n = 8$). These results indicate that very high precision can be obtained from the peak-height ratio measurement for quantitation of orphenadrine hydrochloride and *o*-methylbenzhydrol.

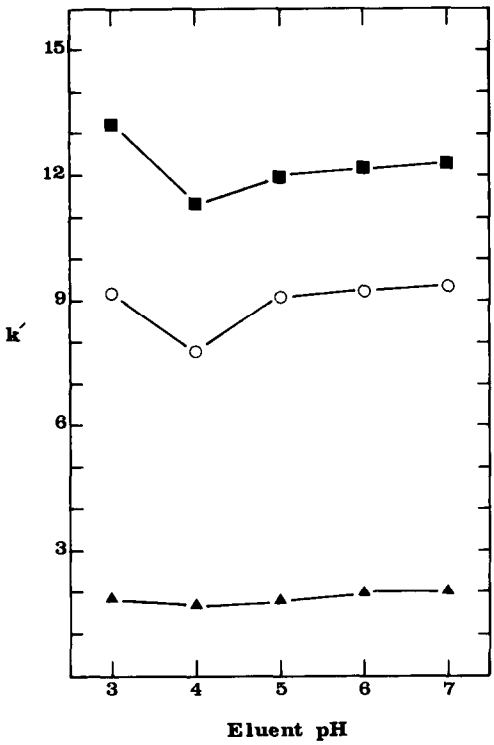

Fig. 5. Relationship of N (plates/m) to pH, for orphenadrine hydrochloride (■); diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

Analytical curves of peak-height ratios (with respect to internal standard at the appropriate concentration) against analyte concentration for orphenadrine hydrochloride (0–800 μg/ml) and for *o*-methylbenzhydrol (0–50 μg/ml) were rectilinear and passed through or close to the origin. The corresponding regression equations were: $y = 0.012x + 0.015$ ($n = 12$) at 0.5 a.u.f.s. for orphenadrine hydrochloride; and $y = 0.264x + 0.0006$ ($n = 10$) at 0.05 a.u.f.s. for *o*-methylbenzhydrol. At the zone of highest relative precision near the top of each calibration curve, the confidence limits ($p = 0.95$) were, respectively, 800 ± 2.8 μg/ml for orphenadrine hydrochloride and 50 ± 0.5 μg/ml for *o*-methylbenzhydrol.

The regression analysis data for the calibration curves of orphenadrine hydrochloride and *o*-methylbenzhydrol indicate that the quantitative performance of the assay was good. The linearity of calibration and low intercept observed, both for orphenadrine hydrochloride and for *o*-methylbenzhydrol, permitted the single-point bracketting technique to be employed for all routine quantitative measurements.

Analysis of dosage forms

The British Pharmacopoeial (BP) assay for orphenadrine hydrochloride tablets was employed as a referee method for the HPLC assay of Disipal tablets, as discussed above. The recovery of orphenadrine hydrochloride as a percentage of the theoretical content according to the initial weight of the sample was 99.5% (R.S.D., 0.38%; n

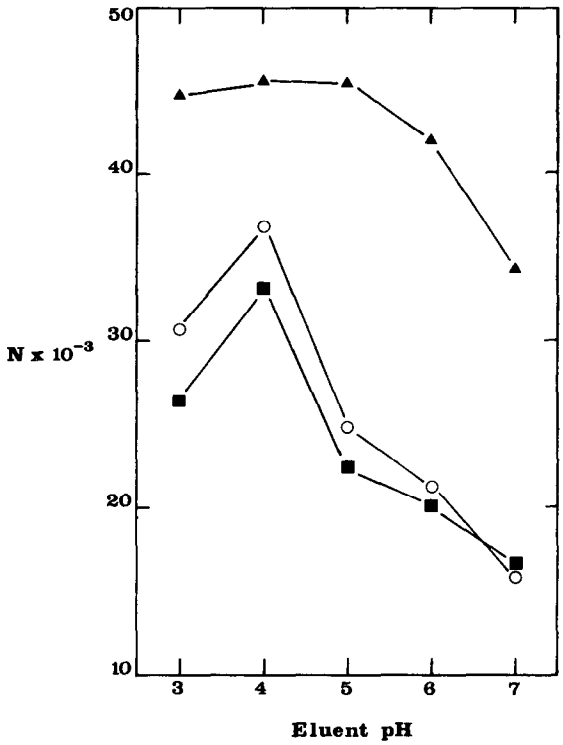

Fig. 6. Relationship of k' to pH, for orphenadrine hydrochloride (■), diphenhydramine hydrochloride (○) and *o*-methylbenzhydrol (▲); chromatographic conditions as in text.

= 6). The recovery of orphenadrine hydrochloride in tablets by the HPLC method was 100.4% with respect to the BP method. There was no statistically significant difference by Student's *t*-test between either method for the orphenadrine hydrochloride content of the Disipal tablets.

The method was then used to determine the orphenadrine hydrochloride content of the commercially available Disipal injection and a hospital-formulated syrup. Data for the orphenadrine hydrochloride content, presented in Table I, are calculated

TABLE I

ASSAY DATA FOR ORPHENADRINE HYDROCHLORIDE IN DOSAGE FORMS

Sample	*Nominal content*	*n*	$\bar{x}$ *(mg)*	*Recovery* (%)*	*R.S.D. (%)*
Disipal tablets batch No. 2153	50 mg	6	49.9	99.9	0.39
Disipal injection batch No. 82130-3019	40 mg in 2 ml	8	39.9	99.8	0.16
Hospital-formulated syrup	50 mg in 5 ml	8	49.9	99.8	0.22

* Relative to nominal label strength.

TABLE II

ASSAY DATA FOR DEGRADATION PRODUCT *o*-METHYLBENZHYDROL IN DOSAGE FORMS

Sample	*o-Methylbenzhydrol content*	*n*	*R.S.D. (%)*	*% (m/m) of the mean content of orphenadrine hydrochloride (Table I)*
Disipal tablets batch No. 2153	16.9 μg/tablet	6	1.5	0.034
Disipal injection batch No. 82130-3019	45.6 μg/2 ml	6	0.35	0.114
Hospital-formulated syrup	30.15 μg/5 ml	6	3.7	0.060

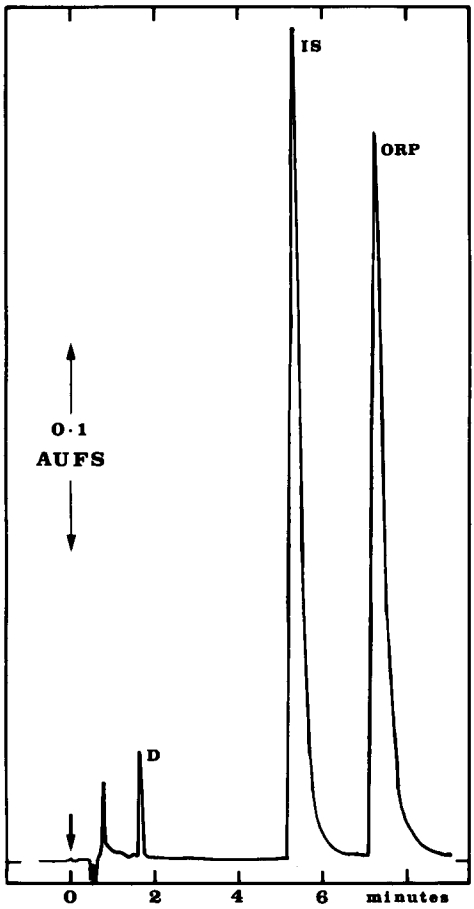

Fig. 7. Chromatogram showing the separation of orphenadrine hydrochloride (ORP), internal standard diphenhydramine hydrochloride (IS) and degradation product *o*-methylbenzhydrol (D). Chromatographic conditions as in text.

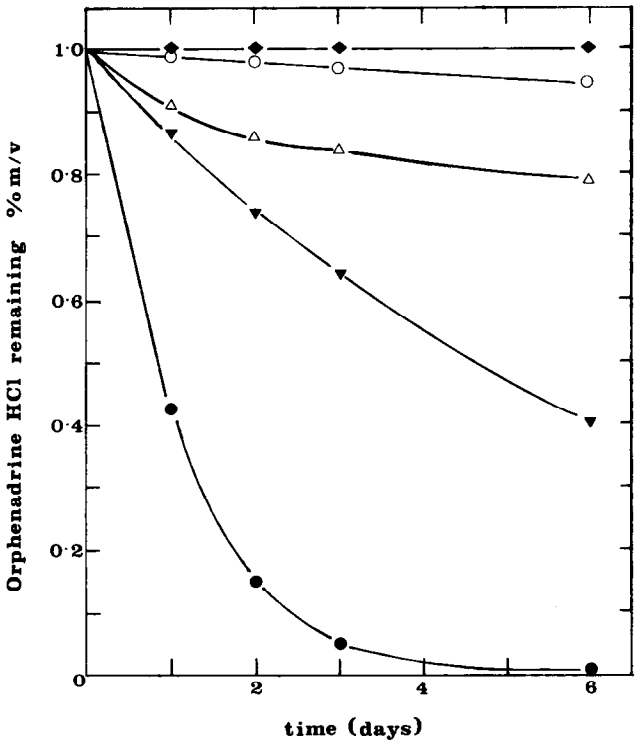

Fig. 8. Concentration (%, m/v) of orphenadrine hydrochloride remaining with respect to time, at a storage temperature of 70°C. Aqueous orphenadrine hydrochloride solutions: pH 2 (●), pH 3 (▼), pH 4 (○), pH 5 (◆) and pH 7 (△).

as a percentage of the nominal content together with the R.S.D. for the three dosage forms.

The concentration of the major degradation product *o*-methylbenzhydrol was also determined for each of the dosage forms. The results for the three dosage forms are represented in Table II as the *o*-methylbenzhydrol content per "unit dose", and as a percentage (m/m) of the mean content found for orphenadrine hydrochloride (Table I). The results in Table I indicate that the HPLC assay is both accurate and precise. The precision and sensitivity of the assay for *o*-methylbenzhydrol (Table II) is such that nanogram quantities of the degradation product can be detected in the dosage forms examined. This approach could be used to assess the change in concentration of the parent compound in cases where very low levels of degradation have occurred.

Pilot stability study

The concentration of orphenadrine hydrochloride remaining as a function of time at each pH, at a storage temperature of 70°C, is illustrated in Fig. 8. This data can be expressed as a linear function of time if pseudo-first-order kinetics are assumed, as shown in Fig. 9, where the log (concentration remaining) is plotted against time. After 6 days' storage at 25°C, less than 3% degradation was found in the control

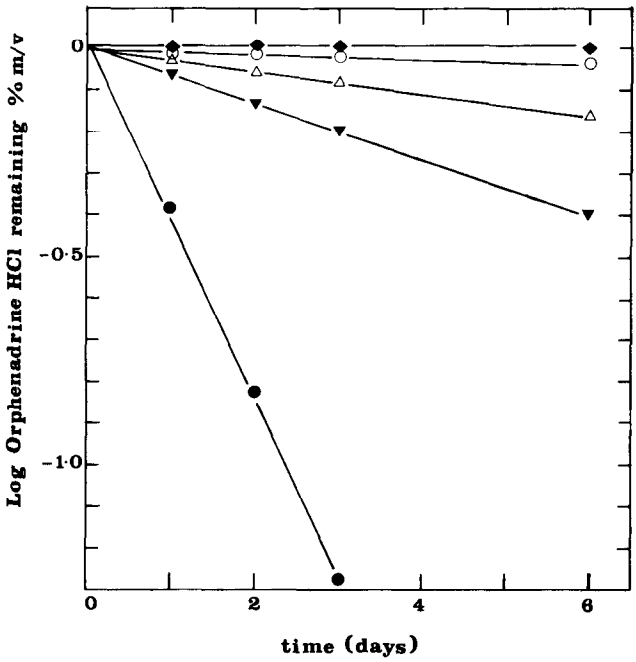

Fig. 9. Log [orphenadrine hydrochloride concentration (%, m/v) remaining] with respect to time at a storage temperature of 70°C. Aqueous orphenadrine hydrochloride solutions: pH 2 (●), pH 3 (▼), pH 4 (○), pH 5 (◆) and pH 7 (△).

solutions at the same values of pH. As illustrated in Figs. 8 and 9, there is marked degradation of orphenadrine hydrochloride at pH 2 and 3. Aqueous solutions, therefore, appear to be most stable at about pH 5.

CONCLUSIONS

The HPLC method described permits the rapid and precise quantitation of orphenadrine hydrochloride and its major degradation product *o*-methylbenzhydrol in pharmaceutical dosage forms. The assay procedure developed has been shown to be stability-indicating and has been used for a stability study on orphenadrine hydrochloride in aqueous solution at various pH values. This pilot study indicates that an oral liquid orphenadrine hydrochloride preparation should be formulated at about pH 5 for optimum stability.

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