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Short Communication

Assay of iodochlorhydroxyquin in cream and ointment formulations by high-performance liquid chromatography

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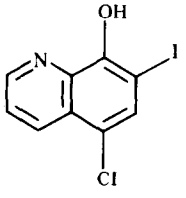
Introduction

Iodochlorhydroxyquin (5-chloro-7-iodo-8-hydroxy-quinoline) (I) has anti-microbial activity and is used in topical formulations in the treatment of skin disorders, either alone or in combination with a corticosteroid. A robust, stability-indicating method was required for the routine determination of (I) in cream and ointment preparations. GLC [1–3] was not considered because of the requirement for derivatisation before determination whereas HPLC [4, 5] methods exist without that need. Trial applications of HPLC to underivatised (I), however, resulted in methodology lacking the required reliability and the problem was ascribed to interaction between the hydroxyl groups of (I) and the various HPLC reversed-phase column packings examined. Differences in



(I)

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chromatography between different types of reversed-phase were displayed, although none of those tried was considered to be acceptable for the assay. Column packings examined were Spherisorb ODS2, Hypersil ODS, Nucleosil C18, Lichrosorb RP8 and Zorbax TMS; mobile phases were methanol–water mixtures with the addition of small amounts of acid (formic, acetic or phosphoric) or base (triethylamine or ammonia). The peak shapes for (I) exhibited varying degrees of tailing or no peak was obtained.

Acetylation of the hydroxyl group of (I) and the use of silica column HPLC is the basis of another published method [6] which gave superior chromatography to that previously tried, although in the authors' hands it still did not yield a sufficiently robust method for routine use.

More recently (I) and some analogues have been determined as their nickel complexes, but without further modification, by a reversed-phase HPLC method [7].

However, because of chromatographic problems previously encountered with underivatised (I) (and seen during development of the nickel complexation method [7]) the former derivatisation approach [6] was favoured. A less polar amino-bonded column was substituted for the silica previously used, the mobile phase was simplified and the derivatisation procedure modified. The resultant procedure satisfied initial criteria; it gave reproducible linearity of response and accuracy and was suitable for stability monitoring. The procedure is readily automated and comprises only solution stages without filtration.

Experimental

Apparatus

The chromatographic system consisted of a Constametric II pump (Laboratory Data Control, Stone, Staffs, UK), an ISS-100 autosampler (Perkin–Elmer, Beaconsfield, Bucks, UK), an LC3 variable wavelength detector (Pye Unicam, Cambridge, UK) set at 244 nm and 1.28 a.u.f.s. and a column oven or column block heater to maintain the analytical column at 35°C. Injections of 20 μl were made into the system and chromatograms were assessed by manual peak-height measurements or, alternatively, by data system. Separations were performed using a 125 × 5 mm i.d. column, slurry packed with Zorbax NH_2 phase (Du Pont, Stevenage, Herts, UK); a 300 × 5 mm i.d. precolumn packed with Porasil (Waters, Harrow, Middlesex, UK) was used between the pump and autosampler to protect the analytical column. The mobile phase flow-rate was 1.0 ml min^{-1}.

Chemicals and reagents

1-Chlorobutane was HPLC grade (Fisons, Loughborough, Leicestershire, UK), tetrahydrofuran was stabilised Laboratory Reagent grade or HPLC grade and all other solvents were Laboratory Reagent grade. Triphenylamine was at least 98% pure (Aldrich, Gillingham, Dorset, UK). The mobile phase was 1-chlorobutane–tetrahydrofuran–glacial acetic acid–methanol (97.4:2.0:0.5:0.1, v/v). The acetylating mixture was freshly prepared (daily) pyridine–acetic anhydride (1:1, v/v). An internal standard, used to aid quantitation, was added prior to derivatisation. The internal standard solution was an approximately 5 mg ml^{-1} solution of triphenylamine in tetrahydrofuran. (The appearance of extra small, but non-interfering, peaks sometimes seen in the final chromatogram may be minimised or avoided by the use of HPLC grade tetrahydrofuran and high quality pyridine and acetic anhydride.)

Standard procedure

About 30 mg of (I) of defined potency was accurately weighed into a 100-ml volumetric flask, dissolved in and diluted to volume with tetrahydrofuran and mixed well. Into a suitable vial were transferred 2.0 ml of solution, 2.0 ml of internal standard solution and 3.0 ml of acetylating mixture. The vial was closed using a cap without a metal liner and the contents were mixed well and heated in a water-bath at 60°C for 25–30 min. The solution was cooled to room temperature and then 1 ml was transferred to another vial and evaporated to dryness under a fume hood using a stream of dry nitrogen until the smell of pyridine or acetic anhydride was absent. The residue was dissolved in 5 ml of 1-chlorobutane and mixed well.

Sample procedure

About 1 g of cream or ointment [for formulations containing 3% m/m of (I)] was accurately weighed into a 100-ml volumetric flask, about 30 ml of tetrahydrofuran was added and the mixture placed in an ultrasonic bath until complete solution was effected. The solution was diluted to volume with tetrahydrofuran, mixed well and then treated as for the standard solution.

Chromatography

Duplicate 20 μl injections of each standard and sample solutions were made and averaged data was used to calculate results. Peak heights for (I) and internal standard were measured and the peak-height ratios (I)/internal standard calculated. Alternatively a data system measuring peak heights or areas was set to perform the same function. Sample assays were calculated in the usual manner.

Results and Discussion

A typical chromatogram is shown in Fig. 1. Linearity of response was evaluated using four standard solutions over the range 50–200% of expected concentration and shown to be acceptable by a correlation coefficient (r) of greater than 0.999.

Recoveries of (I) from ointment and cream blanks, spiked at 80, 100 and 120% of theory, were in the range 97–103% with mean recoveries of 101.1% for the cream and 99.7% for the ointment. No interfering peaks were seen in formulation blanks or from sample solvent.

The reproducibility of the method was evaluated by three different analysts (in two different laboratories) who each assayed three weights of the same ointment and cream batches using different apparatus on different occasions. Ointment assays displayed a relative standard deviation (RSD) of 2.0% and cream assays a RSD of 2.8%. Mean assays were 2.94% and 2.95% for ointment and cream, respectively, compared with a label claim of 3.00%.

Final assay solutions were shown to be stable for at least four days when stored in the dark, although exposure in a light cabinet decomposed the acetate derivative of (I). An accelerated degradation study was performed, subjecting (I) to heat (105°C), light (in a light cabinet) and 1 M sodium hydroxide or 1 M hydrochloric acid for one day. The resultant samples (after neutralisation for the last two challenges) were assayed by the above procedure. Only the alkali treatment gave significant breakdown although no extra peaks were seen. The method has, however, detected light degradation of (I)

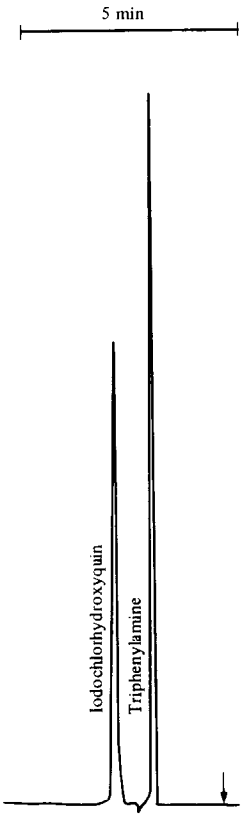

Figure 1

Table 1
Assay data for iodochlorhydroxyquin* (% m/m) on cream and ointment batches (label claim 3.00% m/m) stored at room temperature

	Creams								Ointments	
	1	2	3	4	5	6	7	8	1	2
Initial	2.95	—	(2.96)	2.88	(2.91)	2.76	2.89	—	2.92	2.91
6 months	—	—	(3.03)	2.86	(2.88)	2.88	2.83	—	2.84	2.94
12 months	2.98	3.09	(2.81)	—	—	2.78	—	3.09	—	—
18 months	—	—	(3.08)	—	—	—	—	—	—	—
30 months	—	—	—	—	2.83	—	—	—	—	—
36 months	—	—	2.88	—	—	—	—	—	—	—

*Values given in brackets were obtained by a non-stability indicating, colorimetric assay.

acetate as several extra peaks eluting between the two desired peaks on the chromatogram and is suitable for stability monitoring.

The procedure was also applied to cream and ointment samples stored at room temperature. The results compare favourably with the label claim of 3.00% (m/m) and are given in Table 1.

It was found necessary to allow a 20-min analysis time for ointments to allow elution of formulation excipient(s). The procedure has been shown to be precise, accurate and sufficiently rugged for routine use, although currently only limited data for different batches have been accumulated.

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