Journal of Chromatography, 200 (1980) 261–266
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,121

Note

Semi-automated high-performance liquid chromatographic method for the determination of diacetolol hydrochloride in medicated animal diets

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(Received July 8th, 1980)

Diacetolol (I), reported[1] to be the major metabolite of acebutolol (II) in man, has been found[2] to be an effective β-adrenergic receptor antagonist for the treatment of cardiac arrythmias.

As part of a programme of toxicological work concerned with both acebutolol hydrochloride and diacetolol hydrochloride, a long-term feeding study of diacetolol hydrochloride in mice is being undertaken.

In order to be able to determine the concentration and uniformity of concentration of diacetolol hydrochloride in batches of the medicated diets and to determine the stability of the drug substance in the medicated diets, it was necessary to develop suitable analytical procedures.

Previous work in our laboratories had shown that high-performance liquid chromatography (HPLC) was readily applicable in the development of both quality control and stability-indicating procedures for acebutolol hydrochloride, and this method was adapted for use with diacetolol hydrochloride.

EXPERIMENTAL

Apparatus

Chromatography was carried out using a Model M6000A solvent delivery system (Waters Assoc., Northwich, Great Britain) or a Model 750/03 high-pressure pump (Applied Chromatography Systems, Luton, Great Britain) pumping at 2.0 ml/min; an automatic injector Model 725, fitted with a 10-μl loop (Micromeritics, via Coulter Electronics, Harpenden, Great Britain); a 25 cm × 4.6 mm I.D. stainless-steel column, packed in our laboratories with Partisil-5, using zero-dead-volume end fittings (fittings and packing from Whatman, Maidstone, Great Britain); a Model CE 2012 reference channel, variable wavelength UV monitor (Cecil Instruments, Cambridge, Great Britain) or a Spectromonitor III (Laboratory Data Control, Stone, Great Britain) operating at a wavelength of 230 nm; and a Servoscribe 1s potentiometric recorder (Smiths Industries, London, Great Britain) operating at a chart speed of 300 mm/h with a full-scale sensitivity of 10 mV.

Reagents

Acetonitrile, methanol anhydrous and ammonia solution (specific gravity

0021-9673/80/0000–0000/$02.25

0.88) of Laboratory Chemical grade (May & Baker, Dagenham, Great Britain) were used without purification. Distilled water was further treated using a Milli-Q water purification system [Millipore (U.K.), London, Great Britain] before use.

The mobile phase employed was acetonitrile–methanol–0.1 *M* aqueous ammonia (3:1:1, v/v).

Diacetolol hydrochloride, DL-1-(2-acetyl-4-acetamidophenoxy)-2-hydroxy-3-isopropylaminopropane (I) hydrochloride (M&B 16942A); acebutolol hydrochloride. DL-1-(2-acetyl-4-*n*-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane (II) hydrochloride; DL-1-(2-acetyl-4-aminophenoxy)-2-hydroxy-3-isopropylaminopropane (III); DL-1-(2-acetyl-4-acetaminophenoxy)-2,3-dihydroxypropane (IV); 2-acetyl-4-acetamidophenol (V) and 2-acetyl-4-aminophenol (VI) were prepared at May & Baker. The structures of these compounds are shown in Fig. 1.

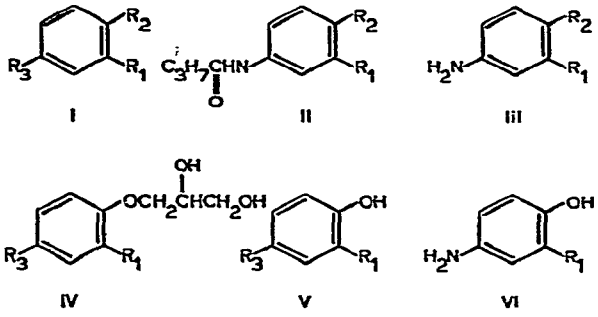

Fig. 1. Diacetolol hydrochloride and its major impurities/decomposition products.

Preparation of sample solution

A small plug of glass wool is inserted into a glass chromatography column (25 cm × 2.5 cm I.D., fitted with 2-mm bore tap) and an accurately weighed portion of the diet (10 g) weighed into the column. A graduated flask (100 ml capacity) is placed under the column, methanol is poured into the column and the flow-rate adjusted to *ca.* 20 ml/min. The first 100 ml of eluate is collected in the graduated flask. This solution is diluted as necessary with methanol to yield a final solution containing *ca.* 50 μg of diacetolol hydrochloride per millilitre.

Preparation of standard solution

A standard solution is prepared by dissolving *ca.* 0.1 g (accurately weighed) of diacetolol hydrochloride in 100 ml of methanol and subsequently diluting a 5-ml aliquot of this solution to 100 ml with methanol.

Injection sequence

Portions of the diluted sample and standard solutions are placed in clean vials, provided for use with the automatic injector, so that each sample solution is injected immediately after, and prior to, the standard solution.

RESULTS AND DISCUSSION

The chromatographic conditions chosen involve the use of a silica column in weak ion-exchange mode rather than the classical adsorption mode[3]. This technique has been applied in our laboratories to a wide range of separations involving basic drugs with an alkylamine side-chain.

The main advantages of this technique are that:

(i) Only the basic components of a mixture are significantly retained on the column whilst non-basic materials, including most formulation excipients, elute at the solvent front, reducing the possibility of column degeneration from species irreversibly adsorbing on the packing[4] and simplifying the separation.

(ii) Such components are much more efficiently chromatographed using this mode of separation than using reversed-phase C_{18}-bonded phases[5]. It is well known[4,6] that ammonia solutions cause gradual dissolution of the silica column packing; however, under normal conditions of use the advantages gained by increased column efficiency more than outweigh the disadvantage of a shortened column life.

The chromatographic conditions were developed to give optimum separation of diacetolol (I; retention time 11 min) from the most likely manufacturing impurity (II; retention time 9 min) and the amide decomposition product of diacetolol (III; retention time 13 min) and to permit levels of II and III to be detected in diacetolol down to 0.1% (w/w) (see Fig. 2).

Inspection of the structural formula of diacetolol indicated that compounds IV, V and VI are also possible decomposition products. Using the chromatographic conditions described above, it was found that all these compounds eluted at the solvent front (see Fig. 2).

In order to determine the likely decomposition pathway(s), samples of diacetolol were degraded under various conditions. Treatment with 0.1 *M* hydrochloric acid under forcing conditions yielded the amine product (III). Treatment with 0.1 *M* sodium hydroxide solution under forcing conditions yielded a more complex mixture. In addition to III, two additional peaks were discernible in the chromatogram, one with a retention time of 5.5 min and the other at the solvent front. The compound corresponding to the peak with a retention time of 5.5 min is considered to have been formed by an important decomposition pathway and has been isolated by semi-preparative HPLC, submitted for structural analysis and identified as DL-1-hydroxy-2-(2-acetyl-4-acetamidophenoxy)-3-isopropylaminopropane (VII).

Thus the chromatographic conditions chosen were capable of resolving at least six manufacturing impurities/decomposition products from diacetolol; however, this evidence alone does not imply that the method is stability-indicating. It was considered necessary to check that there were no decomposition products with the same retention time as diacetolol. Therefore, a solution of diacetolol partially decomposed in 0.1 *M* sodium hydroxide solution was chromatographed using the conditions outlined above and the eluant collected corresponding to the peak in the chro-

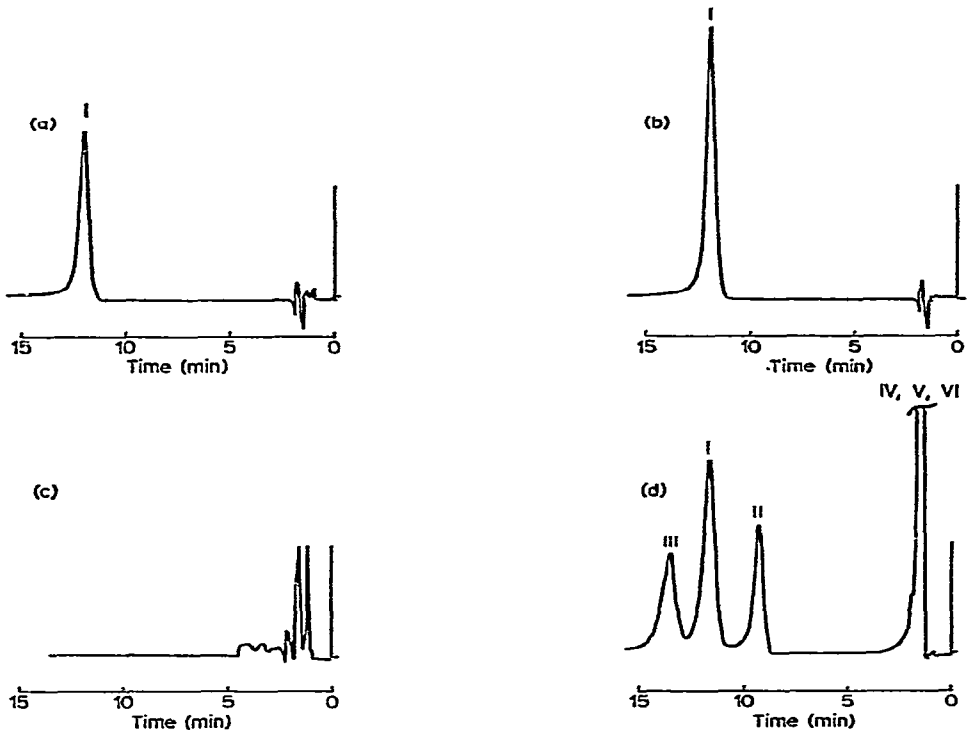

Fig. 2. Typical chromatograms of (a) sample, (b) standard, (c) unmedicated diet, and (d) standard impurities/decomposition products. For peak identification numbers, see Fig. 1.

matogram with the same retention time as diacetolol. The eluent was rechromatographed on a reversed-phase C_{18}-column (S5 ODS, Phase Separations, Queensferry, Great Britain). Only one peak was discernible in this chromatogram, strongly indicating that all the degradation products were resolved from diacetolol on the silica column.

The calibration curve for diacetolol hydrochloride was shown to pass through the origin and to be linear, for concentrations up to 1250 μg/ml.

The procedure was applied by two independent operators to three laboratory-prepared mixtures of diacetolol hydrochloride and one batch of rodent diet. The recoveries obtained by the two operators (Table I) were all within the range 95–106%.

In our laboratories the procedure has been successfully applied to over 200 samples of medicated diets and premixes containing between *ca.* 500 and 300,000 ppm of diacetolol hydrochloride using many batches of rodent diet and two batches of diacetolol hydrochloride. Determinations were undertaken by several operators using different high-performance liquid chromatographs.

Unmedicated diets run simultaneously with medicated diets have shown no trace of components with similar retention times to diacetolol or to the major impurities/decomposition products of the drug substance, except for one case when 50 ppm (estimated as and expressed in terms of diacetolol) of a component with the same retention time as diacetolol was found in a 10-g unmedicated sample extracted in metha-

TABLE I

RECOVERIES ON LABORATORY-PREPARED MIXTURES OF DIACETOLOL HYDROCHLORIDE AND RODENT DIET

Diacetolol hydrochloride content of diet (ppm)	*Recovery (%)*	
	Operator 1	*Operator 2*
500	101.2, 102.8	105.6, 104.0
20,000	100.4, 100.2	95.6, 98.1
60,000	99.5, 100.8	101.0, 99.8

nol and chromatographed undiluted. This peak was considered to have been caused by carry-over from a previous injection.

As developed, the procedure allows the extractions to be performed during the working day with the automatic injection of samples during overnight running of the HPLC, the extract solution having been found to be stable over a 24-h period.

More recently, the system was further automated by using a Hewlett-Packard 3353 Laboratory Automation System. A BASIC language computer program, developed in our laboratories, is used to control the automatic injection sequence, to store processed data from each injection on a tape cartridge and to compare the data from each sample with those from the standard injected immediately prior to and after that sample. On completion of chromatography, a summary report is issued listing the relevant information for each sample (*e.g.* concentrations of main components and impurities/decomposition products).

Recovery experiments undertaken using this system were compared with the previous quantitation method, *i.e.* measurement of peak height by rule. The results are in good agreement (Table II).

TABLE II

COMPARISON OF RECOVERIES BY PEAK HEIGHT (MANUAL MEASUREMENT) AND PEAK AREA (ELECTRONIC MEASUREMENT) FOR LABORATORY-PREPARED DIETS

Diacetolol hydrochloride content of diet (ppm)	*Recovery (%) using*	
	Peak height	*Peak area*
10,000	100.8, 99.5	101.2, 99.5
20,000	97.6, 97.9	98.2, 98.0
40,000	96.0, 99.0	96.7, 98.8
60,000	98.9, 99.6	98.9, 99.3

ACKNOWLEDGEMENTS

The authors thank Messrs. R. C. Fedynec and A. Plewa and Mrs. J. M. Chilver for technical assistance during the preparation of this paper, and the staff of Physical Chemistry Department, May & Baker, for technical discussion concerning the identification of the structure of the alkaline rearrangement product, (VII).

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