DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(15), 1789-1797 (1995)

PHARMACEUTICAL ANALYSIS OF β-METHYLDIGOXIN IN DOSAGE FORMS USING RP-HPLC

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method has been developed for the assay of β–methyldigoxin in Dimekor® tablets (0.1 mg) and ampules (0.2 mg/2 mL). Quantitation of cardiac glycoside in mentioned dosage forms was carried out by the incorporation of phenacetin as an internal standard. A Varian HPLC configured with a Partisil P10 ODS1 column was used for the separation and quantitation of β-methyldigoxin in pharmaceutical preparations. The mobile phase was acetonitrile-water 38 : 62 v/v with flow rate 1.6 ml/min and UV detection was set at 220 nm. The range of linearity extended from 0.01 to 0.11 mg/mL. For the quantitative analysis of β-methyldigoxin in tablets the recovery was 100.16 % and for ampules 99.50 %. The excipients did not interfere with the determination of the analysed substance. The proposed method is precise and sensitive for the examination of examine the content uniformity of tablets and is in a good agreement with PH.JUG.IV (1). A spectrofluorimetric method was used for the dissolution test by the method described in USP XXII (2).

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INTRODUCTION

β-Methyldigoxin, *Medigoxin*, 3β,12β,14β–trihydroxy-5β-card-20(22)-enolide-3-(4'''-O-methyltridigitoxoside), is produced from digoxin by selective methylation of the hydroxyl group on the C-4 atom of the terminal digitoxose.

β-Methyldigoxin is a pharmaceutically important drug for the treatment of congestive heart failure, atrial fibrillation and atrial flutter. It has the same general properties and use as digoxin, but its onset of action is more rapid. β-Methyldigoxin is therefore a better and more desirable cardiotropic agent than digoxin. The margin between the therapeutic and toxic doses of cardiac glycosides is narrow. These drugs are therefore used in low doses and quantitation in dosage forms is critical.

Numerous of papers describe various methods for the determination of digoxin as a cardiac glycoside, but there are not enough for β-methyldigoxin. Only the Japanese Pharmacopea XII (3) prescribes β-methyldigoxin in pharmaceutical dosage forms. Homogenity of β-methyldigoxin tablets was determined by a fluorimetric method using ascorbic acid, hydrogen-peroxide and ethanol (4). A micro high-performance liquid chromatographic method has been developed for the assay of β-methyldigoxin and digoxin tablets (5). In biological materials Rietbrock (6) and Hinderling (7) reported their assay using thin-layer chromatography. Radioimmunoassay combined with HPLC was developed by Malini (8). HPLC-fluorescence polarization immunoassay has been done for the determination of β-methyldigoxin and its metabolites in plasma and urine (9). The concentrations of β-methyldigoxin in serum of patients treated with this cardiotropic agent were measured by polarized immunofluorescence (10,11).

MATERIALS AND METHODS

Chemical and Reagents

All chemicals and reagents were of analytical grade. β-Methyldigoxin was kindly donated by "Boehringer"-Mannheim. The analytical grade purity was checked UV and IR spectrophotometric identification, thin-layer chromatography and specific rotation. The Dimekor® tablets and ampules were obtained from ICN Galenika, Belgrade. Phenacetin was used as a USP reference standard. For HPLC investigation Methanol - "Reanal", Hungary and Acetonitrile - "Farmitalia", Carlo Erba, were used. For the dissolution test and spectrofluorimetric determination, hydrogen-peroxide and concentrated hydrochloric acid, "Zorka"-Šabac were used. Water used for the analysis was high quality, distilled.

Instruments

The HPLC system consisted of a pump (model Solvent Delivery System Varian 9010), a variable wavelength detector model Varian 9065 Polychrom

"diode array" and an integrator (model Softwere Varian Star). A Partisil P10 ODS1 250x4.6 mm column with 10 μm particles size was used. Samples were injected using a Rheodyne Model 7125 valve injector equipped with a 50 μL sample loop.

For the investigation of the dissolution, an Erweka dissolution tester type DT6 and a Perkin Elmer 3000 Spectrofluorimeter with 1 cm glass cells, were used.

Chromatographic Conditions

The separations and detections were performed with a 50 μL sample loop. Flow rate was 1.6 mL/min. at 30°C. Detection wavelength was 220 nm. The mobile phase was prepared by mixing acetonitrile and water in ratios 38:62 v/v after filtering through the 0.45 μm Millipore filter and degassing with helium.

Internal Standard Solution

The internal standard solution was prepared by dissolving phenacetin in mobile phase (c = 0.032 mg/mL).

Standard Solutions (II)

Stock solution (I) was prepared with β-methyldigoxin in mobile phase (c = 0.04 mg/mL). HPLC standard solution (II) was prepared by adding internal standard solution in stock solution (I) for a final concentration of β-methyldigoxin 0.04 mg/mL and 0.032 mg/mL of phenacetin as internal standard solution. Solutions I and II were filtered through the 0.45 μm Millipore filter.

Sample Preparations

A sample solution of tablets was prepared by accurately weighing and pulverizing 20 tablets. After treating in ultrasonic bath, adding the internal standard solution, dissolving by mobile phase and filtering through the Millipore filter, the concentrations were as in HPLC standard solution (II). The sample solution of ampules was prepared by transferring the contents to 25 mL volumetric flask, adding the internal standard solution, diluting by mobile phase, ultrasonicating and filtering through the Millipore filter (the concentration of that sample solution corresponded to II).

Chromatographic Procedure

The concentration range for the quantitation was defined by the calibration curve method. Ten standard solutions of β-methyldigoxin from the concentration of 0.01 mg/mL to 0.11 mg/mL were prepared and analysed by the mentioned HPLC conditions. The calibration curve was constructed using the average peak areas from three chromatograms.

RP-HPLC method was used for the separation of the analysed β-methyldigoxin from Dimekor® tablets and ampules using internal standard method. The recovery values for analysed content of Dimekor® tablets and ampules were calculated.

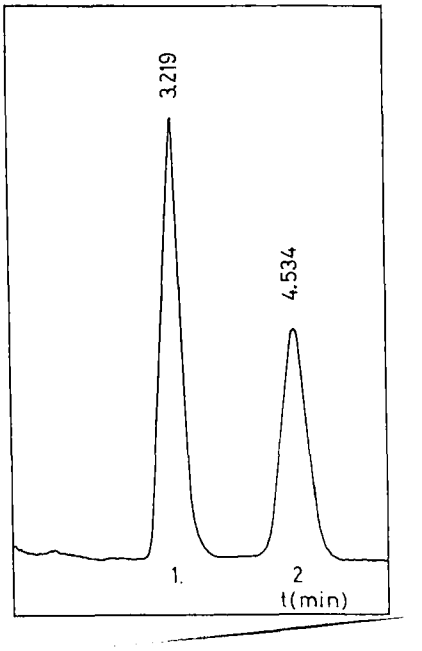

Fig. 1. Chromatogram of phenacetin (1) and β-methyldigoxin (2); mobile phase: acetonitrile-water 38:62 v/v, flow rate 1.6 mL/min., UV monitor at 220nm and sample volume 50 μL

The content uniformity was defined by separately analyzing the contents of ten tablets.

Dissolution Test

The USP dissolution test was used for the investigation of dissolution of β-methyldigoxin in Dimekor® tablets. Six tablets were dissolved in 500 mL dissolution medium for 60 minutes at 37°C in a special dissolution tester. To 1.0 mL of this solution was added: 1.0 mL of ascorbic acid (2 mg per mL of methanol), 5.0 mL of hydrochloric acid and 1.0 mL of hydrogen peroxide (2.0 mL 30 percent hydrogen peroxide was diluted to 100 mL with methanol and just prior to use, 2.0 mL of this solution was diluted to 100 mL with methanol). The fluorescence was measured after 2 hours. The fluorimetric conditions were excitation wavelength at 350 nm and emission at 485 nm. The blank solution was the mixture of ascorbic acid solution, hydrochloric acid and hydrogen peroxide in the same ratio as it had been added to the sample. A calibration curve was constructed using the fluorescence intensity versus standard concentrations in the concentration range from 0.01 to 1.00 μg/mL.

Using the spectrofluorimetric method the recovery value of Dimekor® tablets was calculated and content uniformity of Dimekor® tablets was determined.

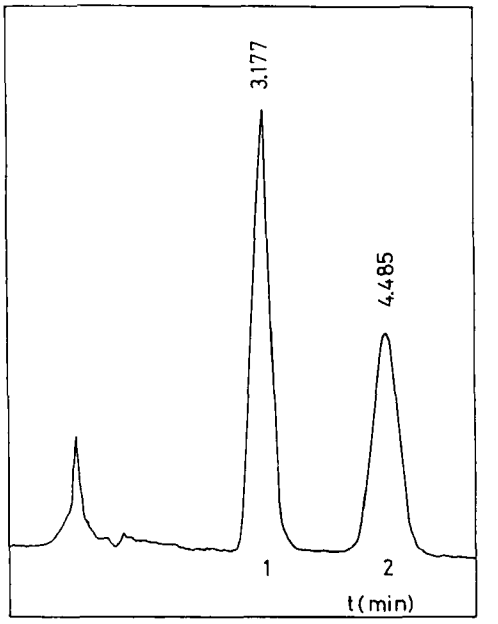

Fig.2. Chromatogram of phenacetin (1) and β-methyldigoxin (2) in Dimekor® tablets; mobile phase: acetonitrile-water 38:62 v/v, flow rate 1.6 mL/min., UV monitor at 220 nm and sample volume 50 µL

RESULTS AND DISCUSSION

For the separation and determination of β-methyldigoxin in the Dimekor® tablets and ampules, the internal standard method was chosen. For the selection of an internal standard, a variety of compounds were investigated and phenacetin, which can be separated satisfactorily from β-methyldigoxin, was found to be the most suitable one. The representative chromatogram (Fig.1.) shows that the separation of β-methyldigoxin from phenacetin is excellent with the retention time for β-methyldigoxin of 4.489 min. and for phenacetin of 3.178 min.

On the basis of these data, the determination of β-methyldigoxin in Dimekor® tablets and ampules was then undertaken. Figures 2 and 3 illustrate the typical chromatograms of the extract of a β-methyldigoxin from tablets and ampules. The present excipients did not interfere with the peaks due to β-methyldigoxin and phenacetin.

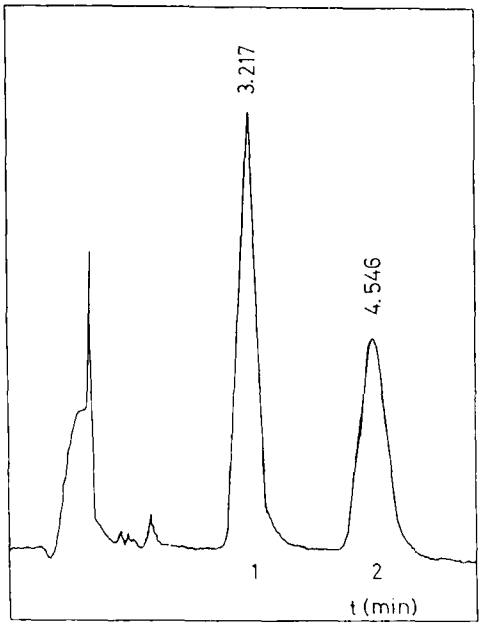

Fig.3. Chromatogram of phenacetin (1) and β-methyldigoxin (2) in Dimekor® ampules; mobile phase: acetonitrile-water 38:62 v/v, flow rate .6 mL/min., UV monitor at 220 nm and sample volume 50 μL.

A linear calibration curve was obtained by plotting the peak area ratios of β-methyldigoxin to an internal standard against the amount of β-methyldigoxin: $y = 2.493x + 0.4145$ with a correlation coefficient of 0.9996. The range of linearity extended from 0.01 to 0.11 mg/mL.

For the quantitation of β-methyldigoxin in tablets and ampules the following equation was used:

$$c_s = c_i \frac{A_s}{A_i} F$$

where is: c_S - sample concentration,
c_i - internal standard concentration
A_S - sample peak area
A_i - internal standard peak area
F - detector response factor for analysed substance.

Table 1.

RP-HPLC determination of β-methyldigoxin in Dimekor® tablets and spectrofluorimetric results of dissolution test

Nominal content (mg/tab.)	HPLC Found (%)	HPLC Content uniformity (%)	Dissolution test Found (%)
0.1	101.11	90.02	96.7
0.1	99.80	94.43	93.3
0.1	97.59	102.93	95.0
0.1	99.67	107.29	95.0
0.1	99.12	99.32	96.7
0.1	100.66	103.13	111.7
0.1	99.74	110.95	105.0
0.1	101.93	107.90	120.0
0.1	101.34	96.42	116.7
0.1	100.62	101.08	120.0
< >	100.16	101.35	105.0
S	1.14	6.62	11.1
CV	1.14	6.53	10.6

< > - mean average (%)
S - standard deviation (%)
CV - coeffficient of variation (%)

Detector response factor F for β-methyldigoxin was determined for the standard solution (II) under the same HPLC conditions and calculated from the equation:

$$F = \frac{c_1 A_2}{c_2 A_1}$$

where is: c_1 - phenacetin concentration

c_2 - β–methyldigoxin concentration

A_1 - phenacetin peak area

A_2 - β-methyldigoxin peak area

Quantitative assay of β-methyldigoxin in Dimekor® tablets and ampules was performed and the results obtained (Table I and II) were in a good agreement with the manufacturers nominal content (standard deviation for tablets is S = 0.00123 mg/tab. and for ampules S = 0.00311 mg/amp.). Recoveries for ten samples ranged from 97.59 to 101.93 % with a mean 100.16 % and a standard deviation of 1.14 % .

Table 2.

RP-HPLC determination of β-methyldigoxin in Dimekor® ampules

Nominal content (mg/2mL)	Found (%)
0.2	98.13
0.2	101.87
0.2	100.06
0.2	101.39
0.2	99.71
0.2	101.55
< >	100.45
S	1.56
CV	1.55

The results obtained for the content uniformity determination are given in Table I and are in agreement with the PH.YUG.IV.

The results confirmed the validity of the RP-HPLC procedure and its applicability to the quantitation of β-methyldigoxin tablets and ampules.

Dissolution test of Dimekor® tablets was carried out after the method described in USP XXII. Spectrofluorimetric method was applied for the assaing of β-methyldigoxin in Dimekor® tablets (Table II) by measuring the fluorescence intensity of analysed samples. The contents of β-methyldigoxin in Dimekor® tablets after the dissolution test were within 93.3-120 % with the mean value of 105.01 % which is in agreement with the prescription of content digression. For the quantification of β–methyldigoxin in the dissolution test of Dimekor® tablets, the calibration concentration range from 0.04 to 0.24 μg/mL, was used. The recovery of the single-tablet content uniformity of β-methyldigoxin was 95.50% .

A linear spectrofluorimetric calibration curve was obtained for the concentration range from 0.01 to 1.00 μg/mL with the regresion function y = 504x - 0.51. That makes this sensitive spectrofluorimetric method suitable for the determination of β-methyldigoxin in a wide concentration range.

CONCLUSIONS

In conclusion, the authors propose the RP-HPLC assay method as accurate, precise and sensitive enough for the determination of β-methyldigoxin in pharmaceutical dosage forms.

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