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MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CARDIAC GLYCOSIDES IN β-METHYLDIGOXIN AND DIGOXIN TABLETS

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SUMMARY

A micro high-performance liquid chromatographic (micro-HPLC) method has been developed for the assay of β-methyldigoxin and digoxin tablets. Quantitation of cardiac glycosides in tablets was carried out by the incorporation of dexamethasone as an internal standard. The procedure consisted of disintegration of tablets, extraction with acetone–ethanol (9:1) and injection for micro-HPLC on an ODS micro column, using acetonitrile–water (28:72) for β-methyldigoxin tablets and methanol–water (1:1) for digoxin tablets; the effluent was monitored by UV detection at 220 nm. The average values of the contents in β-methyldigoxin and digoxin tablets were 99.6 and 100.2% of the labelled amounts, respectively. The proposed method is sufficiently precise and sensitive to examine the content uniformity of tablets.

INTRODUCTION

β-Methyldigoxin and digoxin are pharmaceutically important drugs for the treatment of congestive heart failure and atrial fibrillation. β-Methyldigoxin is produced from digoxin by selective methylation of the hydroxyl group on the C-4 atom of the terminal digitoxose. These cardiac glycosides are used in low doses and there is a narrow margin between the therapeutic and toxic doses. Therefore, it is extremely important to evaluate the exact amounts of cardiac glycosides present in pharmaceutical dosage forms.

Previously published methods of determining digoxin in tablets have utilized colorimetric[1], fluorometric[2], thin-layer chromatographic[3] and gas–liquid chromatographic[4] techniques, but they did not always yield accurate quantitation of intact digoxin in tablets. High-performance liquid chromatography (HPLC) has been shown to be an effective method for the determination of digitalis glycosides in pharmaceutical preparations. Yoshino *et al.*[5] have reported the use of a MicroPak NH_2 column to separate β-methyldigoxin and its related steroids, but satisfactory resolution has not been obtained. The usefulness of a silica gel normal-phase system in the HPLC analysis of digoxin tablets has also been described[6]. The HPLC method employing a reversed-phase column has been widely used for the analysis of digoxin in tablets[7–11].

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On the other hand, Ishii *et al.*[12] have developed micro-HPLC for measuring micro-scale quantities. Previously, we reported the separation and quantitation of digitalis glycosides in *Digitalis purpurea* leaves using micro-HPLC[13,14]. The present paper describes the determination of β-methyldigoxin and digoxin tablets by means of micro-HPLC on a reversed-phase column with UV detection (220 nm) based on the butenolide ring.

EXPERIMENTAL

Instruments

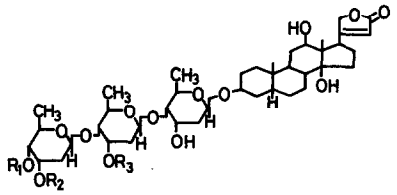
The apparatus was a Famillic-100 micro high-performance liquid chromatograph (Japan Spectroscopic, Tokyo, Japan) equipped with an Uvidec-100 UV spectrophotometer monitoring the absorbance at 220 nm. The micro flow-through cell consisted of a quartz tube with a volume of 0.3 μl. The micro column (95 mm × 0.5 mm I.D.) was a PTFE tube packed with SC-01 (Japan Spectroscopic). This was a reversed-phase column containing 5-μm porous silica particles linked covalently with octadecylsilyl (ODS) groups. The separations were performed under ambient conditions.

Materials

β-Methyldigoxin, dimethyldigoxin 1 and 2 were kindly donated by Yamanouchi (Tokyo, Japan). Digoxin was obtained from Aldrich (Milwaukee, WI, U.S.A.) and dexamethasone from Sigma (St. Louis, MO, U.S.A.). The structures of these cardiac steroids are given in Fig. 1. Digoxigenin, digoxigenin monodigitoxoside and bisdigitoxoside were prepared by hydrolysis of digoxin according to the procedure of Haack *et al.*[15]. All of these materials were checked for homogeneity by thin-layer chromatography (TLC), and solvents were purified by redistillation prior to use.

Sample preparation

One β-methyldigoxin 0.1-mg tablet was placed in 20-ml test-tube to which were added 0.3 ml of distilled water. After ultrasonication for 2 min in an ultrasonic cleaning bath, the suspension was extracted with 5 ml of acetone–ethanol (9:1) containing dexamethasone (0.03895 mg) as an internal standard. The mixture was further ultrasonicated for 5 min and centrifuged at 1400 *g* for 5 min. The supernatant was



Digoxin : $R_1=R_2=R_3=H$
β-Methyldigoxin : $R_1=CH_3$, $R_2=R_3=H$
Dimethyldigoxin 1 : $R_1=R_3=CH_3$, $R_2=H$
Dimethyldigoxin 2 : $R_1=R_2=CH_3$, $R_3=H$

Fig. 1. Structures of digoxin and its methylated compounds.

transferred to a test-tube and evaporated *in vacuo*. The residue obtained was redissolved in 0.1 ml of methanol and analyzed by micro-HPLC.

The composite assay of β-methyldigoxin tablets was performed by accurately weighing an amount of the powder from ten ground tablets corresponding to 0.1 mg of β-methyldigoxin and following the procedure described above.

For the assay of a digoxin 0.25-mg tablet, one tablet was extracted with 5 ml of acetone–ethanol (9:1) containing dexamethasone (0.11826 mg) and the sample was prepared in the same manner as for the β-methyldigoxin tablet.

Chromatographic procedure

A 0.2-μl volume of each sample was injected into the liquid chromatograph and the flow-rate was adjusted to 8 μl/min. The mobile phase for each separation is listed with each chromatogram. β-Methyldigoxin and digoxin in tablets were determined by the internal standard method. Calibration curves were constructed using the average peak areas from three chromatograms.

Recovery test for β-methyldigoxin

β-Methyldigoxin (0.04235 mg) and an internal standard (0.03895 mg) were added to each β-methyldigoxin tablet, and the mixtures were then extracted with acetone–ethanol (9:1). The sample preparation and chromatographic procedure were carried out in the manner described above.

RESULTS AND DISCUSSION

An initial study was directed towards the chromatographic separation of β-methyldigoxin and related compounds. Fig. 2 depicts the chromatogram obtained

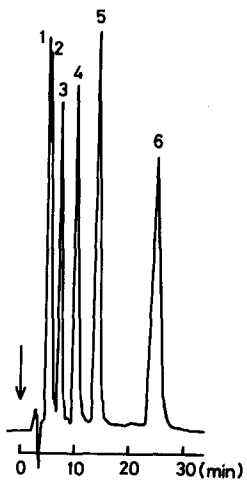


Fig. 2. Separation of a mixture of β-methyldigoxin, digoxin, their decomposition products and an internal standard. Peaks: 1 = digoxigenin; 2 = digoxigenin monodigitoxoside; 3 = digoxigenin bisdigitoxoside; 4 = digoxin; 5 = dexamethasone; 6 = β-methyldigoxin. Conditions: Jasco SC-01 column (95 mm × 0.5 mm I.D.); mobile phase, acetonitrile–water (28:72); flow-rate, 8 μl/min; UV monitor at 220 nm; sample volume, 0.2 μl.

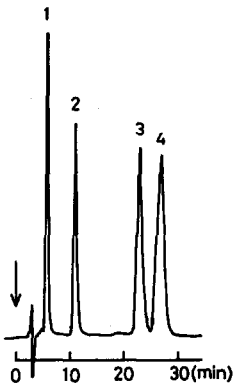


Fig. 3. Separation of a mixture of digoxin and its methylated compounds. Peaks: 1 = digoxin; 2 = β-methyldigoxin; 3 = dimethyldigoxin 1; 4 = dimethyldigoxin 2. Conditions: mobile phase, acetonitrile–water (33:67); other conditions as in Fig. 2.

from the separation of β-methyldigoxin, digoxin and their decomposition products such as digoxigenin, digoxigenin monodigitoxoside and bisdigitoxoside. For the selection of an internal standard, a variety of compounds were investigated and dexamethasone, which can be separated satisfactorily from these substances, was found to be the most suitable. Micro-HPLC was performed on an ODS bonded silica column (SC-01) using acetonitrile–water (28:72) as the mobile phase at a flow-rate of 8 μl/min. A detection wavelength of 220 nm was used, account being taken of the α,β-unsaturated lactone ring attached at C-17 of the steroid nucleus. The separation is sufficiently good and reproducible to permit quantitative work. The PTFE column size employed was much smaller than those in conventional HPLC. Also, the possibility of contamination by digoxin and dimethyldigoxins is present in the manufacture of β-methyldigoxin. Therefore, the chromatographic behaviour of these glycosides was investigated. The separation of digoxin, β-methyldigoxin, dimethyldigoxin 1 and 2 was achieved when acetonitrile–water (33:67) was used as the eluent, as shown in Fig. 3.

On the basis of these data, the determination of β-methyldigoxin in tablets was then undertaken. A β-methyldigoxin tablet was disintegrated by ultrasonication and extracted with acetone–ethanol (9:1) containing an internal standard. Fig. 4 illustrates a typical chromatogram of the extract of a β-methyldigoxin 0.1-mg tablet. The excipients present were ascertained not to interfere with the peaks due to β-methyldigoxin and dexamethasone. No peaks corresponding to the retention time of the internal standard were detected in a chromatogram of the extract in the absence of an internal standard. There was no contamination by dimethyldigoxins in the chromatogram using a mobile phase of acetonitrile–water (33:67). A linear calibration graph was obtained by plotting the peak area ratios of β-methyldigoxin to an internal standard against the amount of β-methyldigoxin (mg): $y = 18.56\,x + 0.02473$, with a correlation coefficient of 0.9996. The range of linearity extends from 0.04 to 0.16 mg. The data for the composite tablet assay are given in Table I. The mean value for the percentage of the label claim was 100.3% with a standard deviation of 0.88%. The accuracy of the assay method was examined by adding an amount of β-meth-

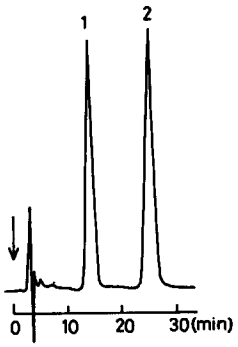


Fig. 4. Chromatogram of the extract of a β-methyldigoxin tablet with an internal standard. Peaks: 1 = dexamethasone; 2 = β-methyldigoxin. Conditions as in Fig. 2.

yldigoxin standard to the tablet sample. Recoveries for the eight samples ranged from 99.5 to 100.1% with a mean of 99.7% and a standard deviation of 0.23%. From the investigation of the tablet recovery *versus* the number of extraction times, the extraction procedure proposed was ascertained to be sufficient for a rapid leaching of β-methyldigoxin from the tablet matrix. These results confirmed the validity of the micro-HPLC procedure and its applicability to the quantitation of β-methyldigoxin tablets.

The determination of digoxin tablets was also undertaken. Fig. 5 shows the chromatogram of digoxin, its degradation products (digoxigenin, its mono- and bis-digitoxosides) and dexamethasone as an internal standard. These compounds were separated into five peaks using methanol–water (1:1) as the eluent. It is interesting that the elution order of digoxin and dexamethasone was reversed when acetonitrile–water was used instead of methanol–water as the mobile phase. The

TABLE I

RESULTS OF THE COMPOSITE ASSAY OF β-METHYLDIGOXIN 0.1-MG TABLETS BY THE MICRO-HPLC METHOD

Sample number	*Weight of tablet powder (mg)*	*Amount found (mg)*	*Amount calculated in terms of one tablet* (mg)*	*Percent of label claim*
1	118.651	0.10050	0.10028	100.28
2	120.424	0.10152	0.09981	99.81
3	114.166	0.09570	0.09924	99.24
4	118.496	0.10001	0.09992	99.92
5	113.402	0.09527	0.09946	99.46
6	120.134	0.10265	0.10116	101.16
7	133.180	0.11354	0.10093	100.93
8	132.008	0.11343	0.10173	101.73
Mean ± S.D.				100.3 ± 0.88

* The average weight of one tablet was 118.393 mg (n = 15).

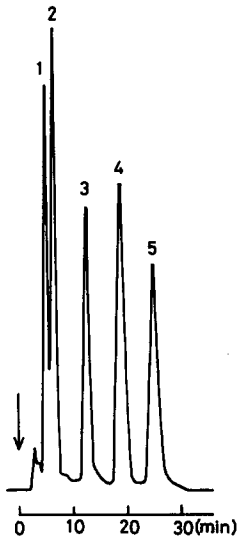


Fig. 5. Separation of a mixture of digoxin, its decomposition products and an internal standard. Peaks: 1 = digoxigenin; 2 = digoxigenin monodigitoxoside; 3 = digoxigenin bisdigitoxoside; 4 = dexamethasone; 5 = digoxin. Conditions: mobile phase, methanol–water (1:1); other conditions as in Fig. 2.

extraction procedure for digoxin tablets was similar to that described for β-methyldigoxin tablets. A representative chromatogram of the extract of a digoxin 0.25-mg tablet after addition of dexamethasone is shown in Fig. 6. The calibration graph of the peak area ratio of digoxin to dexamethasone against the weight of digoxin (mg) was linear over the range 0.1–0.4 mg, and the regression equation was $y = 6.164\,x - 0.02236$ with a correlation coefficient of 0.9997. The micro-HPLC method was satisfactory for the determination of digoxin in tablets.

This assay was applied to the study of single-tablet content uniformity of β-

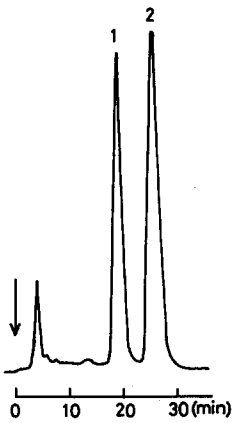


Fig. 6. Chromatogram of the extract of a digoxin tablet with an internal standard. Peaks: 1 = dexamethasone; 2 = digoxin. Conditions as in Fig. 5.

TABLE II

RESULTS OF SINGLE-TABLET ASSAYS OF β-METHYLDIGOXIN AND DIGOXIN TABLETS BY THE MICRO-HPLC METHOD

Tablet number	*Percent of label claim*		*Tablet number*	*Percent of label claim*	
	*β-Methyldigoxin**	*Digoxin***		*β-Methyldigoxin**	*Digoxin***
1	98.5	100.1	11	100.1	100.5
2	97.9	101.6	12	99.6	100.1
3	99.4	99.5	13	99.7	101.8
4	101.2	100.5	14	101.6	98.5
5	99.6	100.0	15	101.2	99.9
6	97.2	100.8	16	98.8	100.3
7	98.3	99.6	17	97.0	100.0
8	99.3	100.3	18	101.7	101.1
9	99.6	100.4	19	99.2	99.4
10	100.0	99.6	20	101.4	100.2
			Mean ± S.D.	99.6 ± 1.4	100.2 ± 0.76

* 0.1-mg tablet.
** 0.25-mg tablet.

methyldigoxin 0.1-mg tablets and digoxin 0.25-mg tablets. The assay results obtained from 20 determinations of randomly selected tablets are collected in Table II. The data indicate that the contents were within 97.0–101.7% (99.6 ± 1.4%, mean ± S.D.) of the label claim for β-methyldigoxin tablets and within 98.5–101.8% (100.2 ± 0.76%) for digoxin tablets. These values show excellent agreement with the manufacturers nominal content.

In conclusion, the micro-HPLC assay method described has been demonstrated to be accurate, precise and sensitive enough for the determination of β-methyldigoxin and digoxin in individual tablets. The use of dexamethasone as an internal standard enables the quantitative analysis of these cardiac glycosides. Micro-HPLC is a convenient and inexpensive method in comparison with conventional HPLC, because of the simplicity of micro column packing and the use of small amounts of the packing material and eluent. Consequently, this method is suitable for the routine quality assurance of pharmaceutical formulations. It is hoped that it can be adopted for the determination of cardiac steroids in other dosage forms.

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