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Short Communication

Simultaneous determination of yohimbine hydrochloride, strychnine nitrate and methyltestosterone by ion-pair high-performance liquid chromatography

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ABSTRACT

A reversed-phase ion-pair chromatographic method was developed for the simultaneous determination of yohimbine hydrochloride (YOH), strychnine nitrate (SN) and methyltestosterone (MT) in a pharmaceutical preparation (tablet). YOH, SN and MT were extracted and separated within 15 min on an octadecylsilano-silica gel column (15 cm × 4.6 mm I.D., 5 μm particle size). The mobile phase was a mixture of $5 \cdot 10^{-3}$ *M* sodium octanesulphonate solution (pH 5.5) and methanol (40:60, v/v). The ultraviolet absorption detector was set at 254 nm, and noscapine was used as an internal standard. The recoveries of YOH, SN and MT from a known sample were 101.9 and 101.8 and 99.4%, respectively. By this method, these components in commercial tablets could be simultaneously determined without any interference. The method is sufficiently sensitive, reproducible and accurate.

INTRODUCTION

Yohimbine hydrochloride (YOH) is an indole alkaloid isolated from the bark of *Corynante yohimbe*. Because it is a vasodilator, this alkaloid has been used alone or in combination with strychnine nitrate (SN) or methyltestosterone (MT) as an aphrodisiac. In recent years, analyses of samples containing YOH alone and in combination with either SN of MT have been carried out by high-performance liquid chromatography (HPLC) [1–6].

No report has dealt with the analysis of a mixture of YOH, SN and MT. In the present study, which attempted the simultaneous determination of YOH, SN and MT in a pharmaceutical tablet preparation, ion-pair HPLC with a column packed with octadecylsilane (ODS)-gel was used. A simple and reproducible procedure was established. This is described in the following section.

EXPERIMENTAL

Chemicals and reagents

YOH was the product of Tokyo-Kasei Kogyo (Tokyo, Japan). SN and noscapine were purchased from Sanko Seiyaku (Tokyo, Japan). MT was purchased from Wako Junyaku Kogyo (Osaka, Japan). YOH, SN, MT were used as standard substances. Noscapine was used as an internal standard for HPLC. The other chemical reagents and solvents were of analytical grade.

Conditions of chromatography

The HPLC system used consisted of 880 PU-In-

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telligent pumps, an 875 UV variable-wavelength detector, a 7125 Rheodyne injector, an RC 150 recorder, an 860-CO column oven and a column packed with Fine Pak SIL-C_{18}S (15 cm × 4.6 mm I.D., 5 μm particle size). All these parts were manufactured by Nippon Bunko Kogyo (Tokyo, Japan). The elution was carried out with an ultrasonicated mobile phase, a mixture of methanol and $5 \cdot 10^{-3}$ *M* sodium octanesulphonate solution (pH 5.5, 60:40, v/v), at a flow-rate 1.5 ml/mm and with a column temperature of 45°C. The eluate was monitored at 254 nm.

Preparation of standard solution

YOH, SN and MT were separately dissolved in the 60:40 (v/v) mixture of methanol and water, and standard solutions of 30, 200 and 600 μg/ml were prepared. Noscapine was dissolved in the same solvent as described above, and two internal standard solutions containing it at (1) 5000 μg/ml and (2) 100 μg/ml were prepared.

Preparation of sample solution

At least twenty tablets were weighed accurately and finely powdered. A portion of the powder, corresponding to about ten tablets, was weighed accurately, 40 ml of 10% sodium hydroxide solution were added, and the mixture was shaken for 10 min on a mixer. After centrifugation (3000 rpm, 10 min, 1500 *g*), the residue was collected and the supernatant liquid decanted [containing L-methionine (ME), thianine hydrochloride (VB_1), riboflavin (VB_2) and folic acid (FA)] as thoroughly as possible. Two aliquots of 20 ml of 10% sodium hydroxide solution were then added to the residue, and the mixture was shaken for 10 min on a mixer, centrifuged again, and the supernatant liquid decanted as thoroughly as possible. The residue was extracted with four 50-ml portions of chloroform. The chloroform extracts were combined, and the chloroform was evaporated on a water bath. A 10-ml volume of methanol was added just when the odour of chloroform was no longer apparent, and the solution was evaporated to dryness. An accurately measured aliquot of 25 ml of methanol–water mixture (60:40, v/v) was added to the residue, and this solution was filtered through a disposable filter unit and used to prepare sample solutions. The determination was performed using the same method (HPLC).

Quantitation

According to the formulation of pulspin tablet (Ishihara Pharmaceutical Industry, Chiba, Japan), the portions of YOH, SN, and MT corresponding to their weight in ten tablets were accurately weighed. These portions were combined in a mortar, thoroughly mixed until a uniform powder was obtained, and dissolved in the mixture of methanol and water (60:40, v/v). This solution was diluted with the mixture accurately to 25 ml, filtered through a disposable filter unit (0.45 μm, Chromatodisk 13A, for aqueous systems, Kurabo), and used to prepare sample solutions.

Sample solution A (for the determination of YOH and MT). A 5-ml portion of the sample solution and 2 ml of the internal standard solution 1 were accurately combined, and the mixture was diluted accurately to 20 ml with the mixture of methanol and water (60:40, v/v). A 5-μl portion of this solution was analysed for YOH and MT by HPLC.

Sample solution B (for the determination of SN). A 5-ml portion of the sample solution and 0.1 ml of the internal standard solution 2 were accurately combined, and the mixture was diluted accurately to 20 ml with the mixture of methanol and water (60:40, v/v). A 20-μl portion of this solution was analysed for SN by HPLC. Based on the peak-height ratio of the substance to be determined to the internal standard, the concentrations of YOH, SN and MT were calculated by a conventional procedure with the relevant calibration curves.

RESULTS AND DISCUSSION

Choice of system

An ODS-silica gel column was used for the ion-pair HPLC. Mobile phases varying in composition were examined in order to achieve the complete separation of YOH, SN and MT in a compound preparation. The best separation was obtained with the mixture of methanol and $5 \cdot 10^{-3}$ *M* sodium octanesulphonate solution (60:40, v/v). The effects of column temperature were studied at 40–50°C, the column temperature being maintained by means of a column oven. The best separation within a reasonable retention time (t_R) was obtained at 45°C.

Fig. 1 shows chromatograms obtained under the conditions as described above.

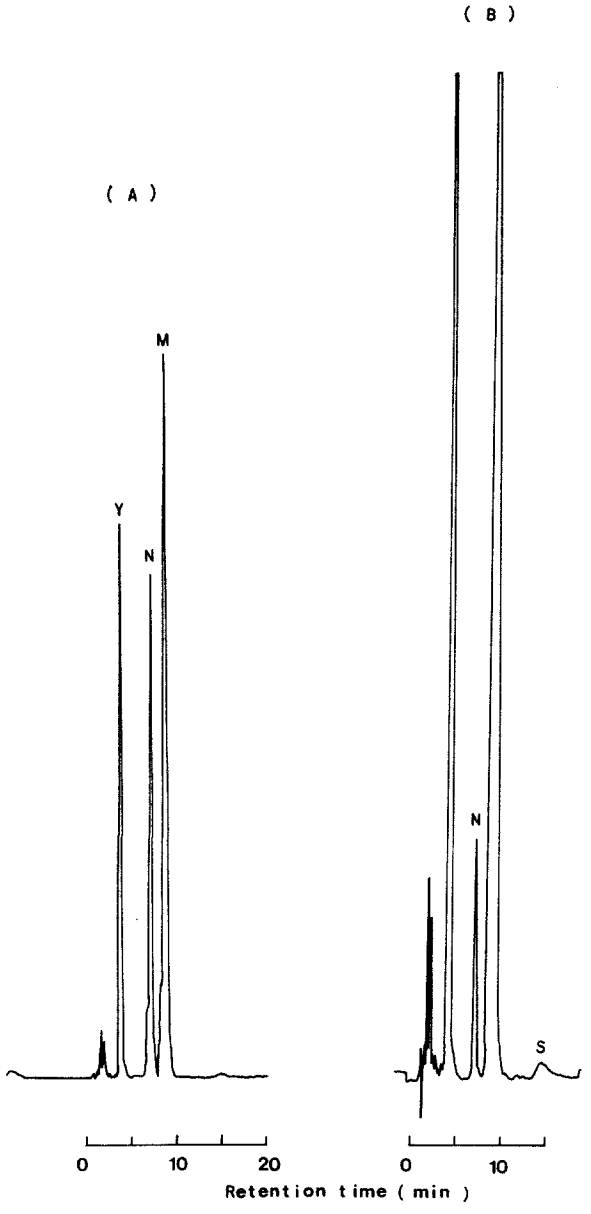

Fig. 1. Chromatograms of (A) yohimbine hydrochloride and methyltestosterone and (B) strychine nitrate in commercial tablets. Peaks: Y = yohimbine hydrochloride; N = noscapine; M = methyltestosterone; S = strychnine nitrate. Retention time: Y = 4 min; N = 7.3 min; M = 9.3 min; S = 15 min.

Calibration

Calibration curves were linear between 60 and 300 μg/ml for YOH ($y = 0.945x + 0.058$, $r = 0.999$), between 5 and 30 μg/ml for SN ($y = 12.408x - 0.034$, $r = 0.999$) and between 20 and 100 μg/ml for MT ($y = 3.608x - 0.009$, $r = 0.999$). Detection limits at a signal-to-noise ratio of 3 were 10 μg/ml for YOH, 1.5 μg/ml for SN and 2.86 μg/ml for MT.

TABLE I

RECOVERIES OF YOHIMBINE HYDROCHLORIDE, STRYCHNINE NITRATE AND METHYLTESTOSTERONE FROM A MIXTURE OF STANDARDS

Compound	Added (mg)	Recovery (%) ($n = 8$)	C.V. (%) ($n = 8$)
YOH	3.00	101.9	1.48
SN	0.30	101.8	1.06
MT	1.50	99.4	0.94

Recovery

Using the formulation of a commercially available compound preparation, a sample was prepared by combining known amounts of these three drugs and was analysed to assess their recovery. As shown in Table I, more than 99% of added YOH, SN and MT could be recovered. The coefficients of variation (C.V.) for the recovery tests were less than 1.5%.

Application

A chromatogram of commercial tablet extract is shown in Fig. 1A and B. The determination of YOH, SN and MT in commercial tablets is shown in Table II. Other ingredients, such as ME, VB_1, VB_2 and FA, did not interfere with the analysis.

TABLE II

DETERMINATION OF YOHIMBINE HYDROCHLORIDE, STRYCHNINE NITRATE AND METHYL TESTOSTERONE IN COMMERCIAL TABLETS

Tablets: according to the label, one tablet contains 3 mg of yohimbine hydrochloride, 1.5 mg of methyltestosterone, 0.3 mg of strychnine nitrate, 100 mg of L-methionine, 3 mg of thiamine hydrochloride, 3 mg of riboflavin and 0.1 mg of folic acid.

Compound	Label claim (mg per tablet)	Amount found (mean ± S.D., $n = 5$) (mg per tablet)
YOH	3.00	3.10 ± 0.17
SN	0.30	0.31 ± 0.02
MT	1.50	1.54 ± 0.08

In brief, the ion-pair HPLC method described is an excellent method which affords a simple simultaneous determination of YOH, SN and MT. The results were satisfactorily reproducible.

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