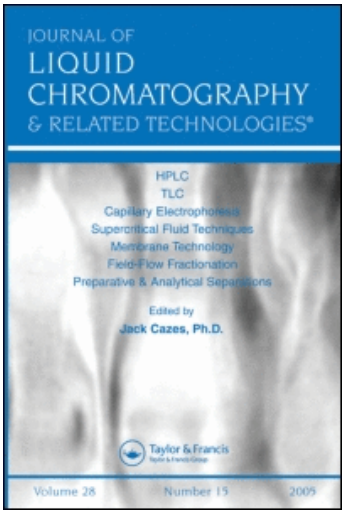

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DENSITOMETRIC AND VIDEODENSITOMETRIC DETERMINATION OF NADOLOL AND PINDOLOL IN TABLETS BY QUANTITATIVE HPTLC

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ABSTRACT

New, simple, precise, and rapid high performance thin layer chromatography (HPTLC) methods have been developed for the determination of nadolol (N) and pindolol (P) in tablets. The stationary phase was silica gel $60F_{254}$ and the mobile phase was ethyl acetate-methanol-glacial acetic acid ($49 + 49 + 2$, v/v). Detection and quantification were done densitometrically at 270 nm and by videodensitometric scanning at 254 nm. In a densitometric procedure, the linearity range was 0.2–1.2 μg/10 μL for N and P. In a videodensitometric assay, the linearity ranges were 2.0–12.0 and 0.2–1.2 μg/10 μL for N and P, respectively. Precision was validated by replicate analyses of standard solutions, and accuracy by analysis of fortified samples. In

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densitometric procedure, the precision (RSD) obtained for the standard solutions ranged from 1.14 to 2.80 and from 0.74 to 1.85% for N and P, respectively. For the videodensitometric assay, the RSD values ranged from 0.68 to 2.36 and from 0.79 to 3.20% for N and P, respectively. The results obtained by both techniques were compared.

Key Words: Nadolol; Pindolol; Tablets; Quantitative HPTLC

INTRODUCTION

Nadolol (5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3, 4-tetrahydro-2,3-naphthalenediol) and pindolol (1-(1H-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol) are nonselective β-adrenergic antagonists, which have widespread and important uses in the management of cardiac arrhythmia, angina pectoris, and hypertension. Pindolol also exhibits a membrane-stabilizing action on myocardial muscle fibrils and intrinsic sympathomimetic activity.[1] Thin layer chromatography was used for identification of nadolol and pindolol in raw material or pharmaceuticals[2–9] and in biological material.[10–12] The TLC assays of their enantiomers were also described using chiral ion interaction agents, chemical derivatization, or chiral stationary phases.[13–16] The quantitative TLC procedures for determination of nadolol and pindolol in plasma and urine were elaborated by densitometric evaluation of the intrinsic fluorescence.[17,18] No quantitative HPTLC methods for determination of these drugs in pharmaceutical preparations were found in the literature. We described new, simple, precise, and rapid procedures for the quality control of nadolol and pindolol formulations. Quantitation was achieved by conventional optical scanning and by videodensitometry, and the results obtained by the both techniques were compared.

EXPERIMENTAL

Reagents and Chemicals

Nadolol and pindolol USP substances, Apo-Nadol® 80 mg and Apo-Pindol® 10 mg USP tablets were obtained from Apotex Inc. (Canada). Ethyl acetate, methanol LiChrosolv®, and glacial acetic acid (pro analysis grade) were purchased from E. Merck (Germany).

Instrumentation and Layers

A Camag (Switzerland) HPTLC system equipped with an automatic TLC Sampler III, TLC Scanner 3, and CATS V 4.05 software was used. Videodensitometry was performed with a Desaga (Germany) VD 40 Video system comprising the Cab UVIS, in conjunction with a high-resolution Mitsubishi color-video CCD camera (mode CP 700D) with a horizontal resolution of 430 TV lines and a standard sensitivity of 1 lx. A Desaga videodocumentation system ProViDoc, version 3.02 and a Desaga program for quantitative analysis ProResult, version 3.00.139 were applied. The horizontal Teflon DS II chambers with mobile phase distributors from Chromdes (Lublin, Poland) were used. TLC was performed on 10×20 cm HPTLC plates coated with 0.25 mm layers of silica gel $60F_{254}$ from E. Merck (Germany). A single bath number was used throughout the calibration and validation procedures.

HPTLC Analysis

Volumes 10 μL of standard and samples were applied to the plates as spots, by means of a Camag autosampler described above. The standards and samples were applied to the same plate as a control for reliable quantification. HPTLC was performed with ethyl acetate-methanol-glacial acetic acid ($49 + 49 + 2$, v/v). Plates were developed at room temperature for a distance of 8 cm beyond the origin using vapor-equilibrated TLC chambers. The development time was 18–20 min, after which the plates were allowed to air dry. Densitometry of samples and standards was performed at 270 nm using a deuterium lamp and the scanner described above in reflectance/absorbance mode, with the slit dimension 4.0×0.30 mm and the scanning speed 5 mm/sec. The wavelength used was found to provide maximum absorption by measurement of *in situ* spectra of standard zones, by use of the spectral mode of the densitometer. Videodensitometry was performed at 254 nm, using a system described above, in absorption mode with a shutter speed 1/50 sec.

Procedure for Calibration

The working solutions of nadolol (N) and pindolol (P) were prepared by dissolving 0.25 g of N, 0.025 g of N, and 0.025 g of P in 25 mL of methanol. They were stored at 4°C and were stable for at least one month. Varying volumes (0.2–1.2 mL) of working solutions were taken in different 10 mL volumetric flasks and diluted to the mark with methanol. The series of concentrations covering the range 2.0–12.0 μg of N (adequate for videodensitometric assay) and

the range 0.2–1.2 μg of N and P (adequate for densitometric scanning of N and for the both assays of P) were produced. Ten μL of each of these solutions was applied on HPTLC plates. Calibration curves were constructed by plotting peak areas against the amount of the drug in μg/10 μL (per spot), and the linear relationship was evaluated by calculation of the regression line by the method of least squares. The weights of drugs in sample zones were determined automatically from their areas by extrapolation from these curves.

Procedure for Assays in Tablets

Twenty tablets of N and P were accurately weighed and the average weights were calculated (the mean weight of tablet was 0.4024 g and 0.1587 g for N and P, respectively). The tablets were pulverized and the amounts of the powders equivalent to 40 mg of N and 30 mg of P were taken in different 50 mL volumetric flasks, shaken for 30 min, and diluted to the mark with methanol. The solutions were filtered through Whatman No. 42 filter paper. Five mL of filtrates was further diluted to 50 mL with methanol. Ten μL of these solutions was applied to the HPTLC plate, developed, dried, and scanned. For videodensitometric assay of N, the first filtered solution (without dilution) was used. The contents of drugs in the sample zone were determined automatically from the calibration curves. For each tablet analysis, percent recovery was determined by comparing the theoretical contents predicted by the label declaration to the mean experimental contents of the sample zones.

RESULTS AND DISCUSSION

A mobile phase ethyl acetate-methanol-glacial acetic acid (49 + 49 + 2, v/v) was selected as optimal. The active substances were quickly eluted and sufficiently separated from the solvent front to improve the quantitative assay. The R_f values were 0.45 ± 0.006 and 0.61 ± 0.005 (mean ± SD) for N and P, respectively. The detection limit (DL) and quantification limit (QL) were determined visually by establishing the minimum levels at which the analyte could be reliably detected and quantified with acceptable accuracy and precision. In a densitometric assay, the LD and QL for nadolol were found to be 0.05 and 0.1 μg per spot. In the videodensitometric procedure they were 0.2 and 1 μg per spot, respectively. The LD and QL for pindolol were found to be 0.05 and 0.1 μg per spot, in densitometric and videodensitometric procedures. Calibration procedures were done using six points. For each point, five measurements were made to improve the precision of an analytical procedure. The data were averaged and calibration curves were calculated. In the densitometric assay, the plot of the

peak areas versus the concentration of N or P was found to be linear in the range 0.2–1.2 μg per spot (10 μL) for both drugs. The calibration curves were represented by the following linear regression equations:

$$y_N = 2160.53x + 222.68 \ (r = 0.997)$$
$$y_P = 19503.07x + 14.367 \ (r = 0.992)$$

For the videodensitometric assay, the plot of the peak areas versus the concentration of the drug was found to be linear in the range 2.0–12.0 and 0.2–1.2 μg per spot for N and P, respectively. The calibration curves were represented by the following linear regression equations:

$$y_N = 761.4x - 387.70 \ (r = 0.996)$$
$$y_P = 12758.29x + 190.2 \ (r = 0.996)$$

where y = area, x = concentration of the drug in μg per spot.

Reproducibility was determined by plotting one set of standards in low and high concentrations of the drugs, on five separate plates. The obtained values were compared with theoretical amounts. The assays in the standard solutions showed a sufficient precision of the HPTLC systems. In the densitometric assay, the RSD values ranged from 1.14 to 2.80 and from 0.74 to 1.85% for N and P, respectively. In the videodensitometric procedure, they ranged from 0.68 to 2.36 and from 0.79 to 3.20% for N and P, respectively. The results of these determinations are presented in Table 1. A linear regression program, provided by software, was used to produce the calibration curves relating to the standard zone weights and their optimized scan areas. The standards and the samples were

Table 1. Precision of the HPTLC Systems

Amount Declared μg/Spot		Amount Found* (Mean ± SD) μg/Spot		Relative Standard Deviation %	
N	P	N	P	N	P
Densitometry					
0.3	0.3	0.30 ± 0.009	0.30 ± 0.006	2.80	1.85
0.9	0.9	0.90 ± 0.010	0.91 ± 0.007	1.14	0.74
Videodensitometry					
3.0	0.3	3.05 ± 0.072	0.31 ± 0.010	2.36	3.20
9.0	0.9	9.02 ± 0.061	0.91 ± 0.007	0.68	0.79

*n = 5.
N = nadolol, P = pindolol.

developed on the same plate as a control for reliable quantification. The analytes weights in the sample zones were determined automatically from their areas by extrapolation from calibration curves. The nadolol and pindolol contents in commercial brands of tablets were analyzed according to the procedures described above. In the densitometric procedure, the contents were found to be 80.81 ± 0.86 and 9.93 ± 0.08 (mg per tablet, mean $\pm$ SD) for N and P, respectively. In the videodensitometric assay, they were 80.28 ± 1.22 and 9.99 ± 0.06 mg per tablet for N and P, respectively. The RSD values were 1.06 and 0.79% for N and P, respectively, in the densitometric procedure. In the videodensitometric assay, they were 1.52 and 0.58% for N and P, respectively. The results of these determinations are shown in Table 2.

The relative low RSD values indicate that the methods are sufficiently precise. The accuracy of the proposed methods was confirmed by recovery experiments from the fortified samples. Three different levels of standards (50, 100, and 150% of theoretical weight predicted by the label declaration) were added to the weighed portions of powdered tablets (adequate to the mean weight of tablets), shaken, filtered, and diluted with methanol, according to the procedure given above. Each level was repeated three times, and the percentage recoveries were calculated. Recoveries of nadolol and pindolol were 99.71–102.05 and 98.41–101.57%, respectively, for the densitometric procedure. For the videodensitometric assay, they were 97.15–101.57 and 97.37–101.14% for N and P, respectively. These results, given in Table 3, indicate that the methods are sufficiently accurate and precise, and that there is no interference from excipients present in the tablets analyzed. Comparison of nadolol and pindolol results in tablets, by densitometric and videodensitometric methods, was done using

Table 2. Assays of Apo-Nadol® 80 mg and Apo-Pindol® 10 mg Tablets

Amount Found (Mean ± SD)* mg/Tablet		Recovery (Mean)* %		Relative Standard Deviation %	
N	P	N	P	N	P
Densitometry					
80.81 ± 0.858	9.93 ± 0.078	101.01	99.25	1.06	0.79
Videodensitometry					
80.28 ± 1.219	9.99 ± 0.058	100.36	99.88	1.52	0.58

*n = 5.
N = nadolol, P = pindolol.

Table 3. Results of Recoveries of Nadolol and Pindolol from the Fortified Samples

Recovery* %		Relative Standard Deviation %	
N	P	N	P
Densitometry			
99.71–102.05	98.41–101.57	0.88	1.05
Videodensitometry			
97.15–101.57	97.37–101.14	1.38	1.11

*n = 9.
N = nadolol, P = pindolol.

F-Snedecor and t-Student tests. At P = 95% the differences in precision of the both methods were not significant.

In summary, new HPTLC assays are developed and validated for quantitation of two, widely used in therapy, β-blockers in tablets. The systems used for quantitative analysis enable reproducible, rapid, and convenient analysis of the both drugs. Our methods can achieve recoveries as a percentage of tablet label values, standard deviations for replicate analyses, and recoveries for fortified samples that are well within the range of values required for use in a pharmaceutical analytical laboratory. The results demonstrated for accuracy and precision can be successfully compared with those reported for HPLC analyses.

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