Journal of Chromatography, 257 (1983) 140–145
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,466

Note

Minor component tablet analysis by high-performance thin-layer chromatography

M.-L. CHENG and C. F. POOLE*
Department of Chemistry, Wayne State University, Detroit, MI 48202 (U.S.A.)
(First received October 6th, 1982; revised manuscript received October 27th, 1982)

Thin-layer chromatography (TLC) is routinely used for the qualitative and semi-quantitative analysis of minor impurities in pharmaceutical products. For this application the simplicity, speed and high sample throughput of TLC are desirable features. With modern scanning densitometry the accuracy and precision of the TLC analysis is adequate for quality control and stability testing applications. Recent advances in the practice of TLC, generally known as high-performance thin-layer chromatography (HPTLC), have increased the acceptance of HPTLC as a powerful quantitative separating tool[1–3]. In this report we wish to demonstrate that HPTLC is a suitable method for quality control applications. By way of example, the determination of three expected contaminants at concentrations below the 1% (w/w) level in tablets of metoprolol tartrate will be described. Metoprolol is a widely used selective beta-adrenoceptor blocking drug used to manage hypertension and other cardiovascular diseases. Its structure, and those of the three expected contaminants, are given in Fig. 1.

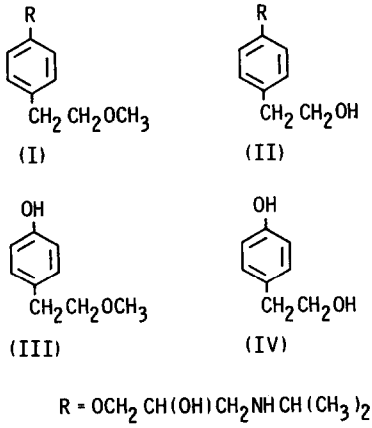

Fig. 1. Structures of metoprolol (I) and its expected contaminants 1-(isopropylamino)-3-[4-(2-hydroxyethyl)phenoxy]-2-propanol (II), 4-(2-methoxyethyl)phenol (III) and 4-(2-hydroxyethyl)phenol (IV).

0021-9673/83/0000–0000/$03.00

EXPERIMENTAL

Metoprolol tartrate, 1-(isopropylamino)-3-[4-(2-hydroxyethyl)phenoxy]-2-propanol (II) [H105/22], 4-(2-methoxyethyl)phenol (III) [H93/12], 4-(2-hydroxyethyl)phenol (IV) [H98/12] and metoprolol tartrate in tablet and injectable solution form were gifts from Hässle (Mölndal, Sweden).

The tablet samples were prepared by pulverizing five tablets in a mortar and pestle. The weight equivalent to two tablets (≈ 1.0 g) was transferred to a stoppered centrifuge tube and 5.0 ml of methanol added. The tubes were placed in an ultrasonic bath for 4.0 min and then centrifuged. Each sample was then spotted in duplicate as a 200-nl and 1.0-μl sample volume.

Chromatography was performed on 5 × 10 or 10 × 10 cm KC_{18} reversed-phase HPTLC plates (Whatman, Clifton, NJ, U.S.A.). The plates were precleaned by a single development in hexane, dried with prepurified nitrogen and redeveloped in the same direction with methanol.

Solutions were applied to the plate using a 200-nl Pt-Ir micropipette (Camag, Willmington, NC, U.S.A.) attached to an EVA-Chrom applicator (W & W Electronic Instruments, Basle, Switzerland) in volumes of 200, 400 or 1000 nl by spotting at the

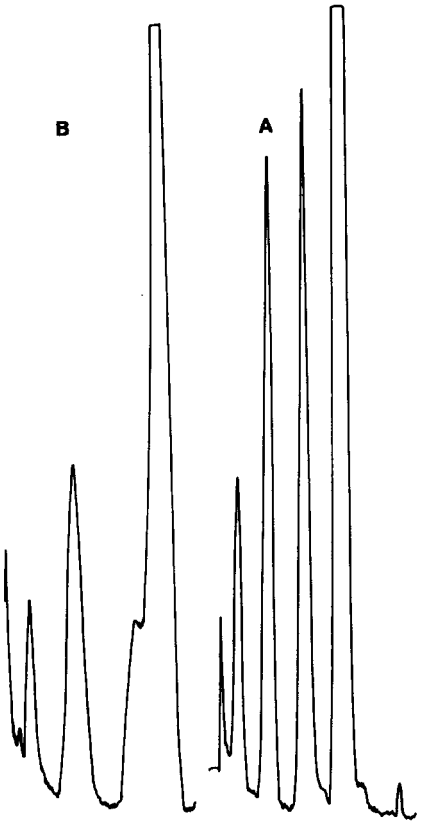

Fig. 2. Separation of a standard mixture by reversed-phase HPTLC. A, Methanol–solvent A (3:7); B, methanol–water (3:7). Solvent A = water–ammonia (9:1).

same position one, two or five times. All samples were dissolved in methanol and the spots applied in a line 0.5 cm apart and 0.5 cm from the lower edge of the plate.

The plates were developed in a short-bed continuous development chamber (Regis, Morton Grove, IL, U.S.A.). The development time was 10 min in plate position 4. The mobile phase was solvent A–methanol (3:7). Solvent A was 10% ammonia (sp.gr. = 0.88) in water. Sample retention measured as R_F values; metoprolol 0.40, II 0.57, III 0.73 and IV 0.87.

In situ scanning of the HPTLC plates was performed with a Shimadzu CS-910 scanning densitometer (Shimadzu, Columbia, MD, U.S.A.). All measurements were made in the reflectance mode, dual wavelength–single beam operation, sample wavelength λ = 280 nm and reference λ = 300 nm, slit width 0.5 mm, slit height 4.0 mm and scan rate 24 mm min^{-1}. Peak area measurements were obtained using a Spectra-Physics minigrator (Spectra-Physics, Santa Clara, CA, U.S.A.).

RESULTS AND DISCUSSION

Although many solvent systems will provide a separation of the four component standard mixture by HPTLC, the choice of conditions useful for the separation of compounds II–IV in the presence of a much larger concentration of compound I are more restrictive. First of all, compound I will overload the sample capacity of the plate leading to spot broadening in excess of that attributable to diffusional broadening. Optimum conditions require the maximum peak to peak separation of compound I from neighboring components to allow maximum sample loading. A second problem is the severe tailing of the spots observed in many solvent systems. The polar functional groups interact very strongly with the plate surface resulting in poor peak shape. The mobile phase must contain a tailing inhibitor to eliminate this effect

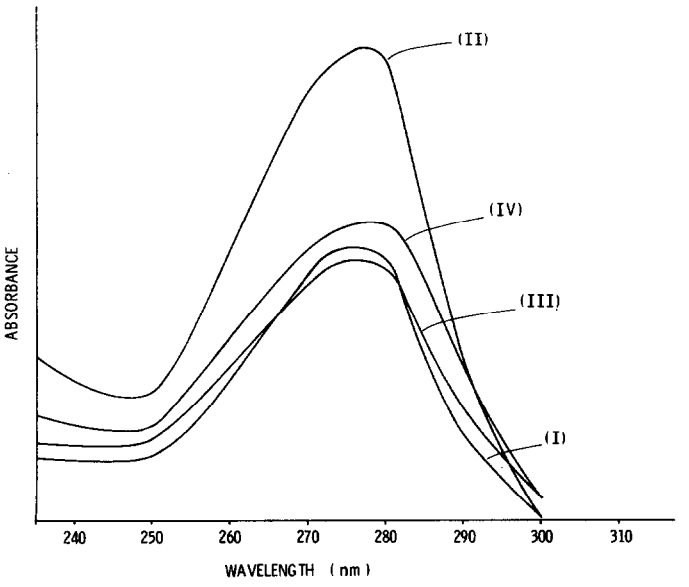

Fig. 3. UV spectra of metoprolol and its expected contaminants measured *in situ*.

without diminishing the selectivity of the solvent system. Using pH buffers or organic acids was not succesful for this purpose[4]. A basic tailing inhibitor is required; ammonia in fairly high concentration works best and is compatible with a reversed-phase separation system. The beneficial effect of including ammonia in the mobile phase can be seen from Fig. 2. The solvent system of Fig. 2A contains the optimum concentration of ammonia, while Fig. 2B is the same solvent system with an equal volume of water substituted for ammonia. The separation is incomplete in Fig. 2B and the peak widths larger due to tailing. Methanol was found to be the optimum organic component of the reversed-phase solvent system. Acetonitrile, tetrahydrofuran, dioxan, ethanol and isopropanol were less selective solvents.

The minor components are present in low concentration in the sample demanding high sensitivity for their detection. This is a function of the operating conditions of the densitometer, the absorbance properties of the sample components and the minimization of background interferences. All compounds contain a similar UV-chromophore and can be conveniently measured at λ = 280 nm (Fig. 3). Single wavelength detection provides an unstable baseline at high sensitivity, Fig. 4A. This drifting baseline results from the uneven distribution of background contaminants

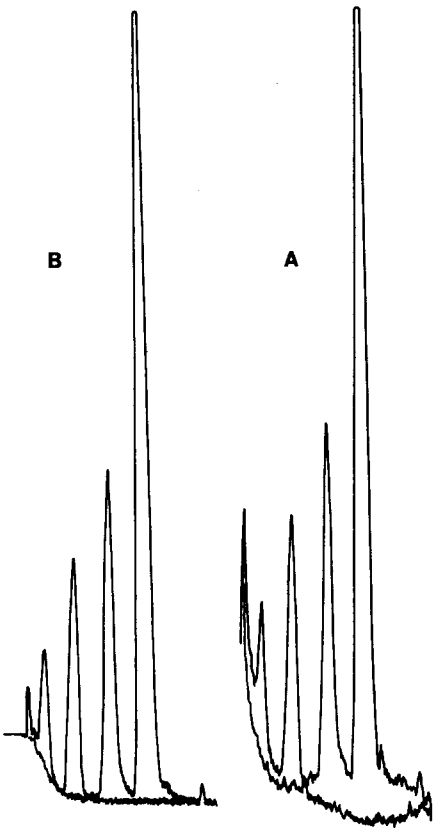

Fig. 4. Use of background correction in scanning densitometry. A, Double beam–single wavelength mode; B, dual wavelength–single beam mode.

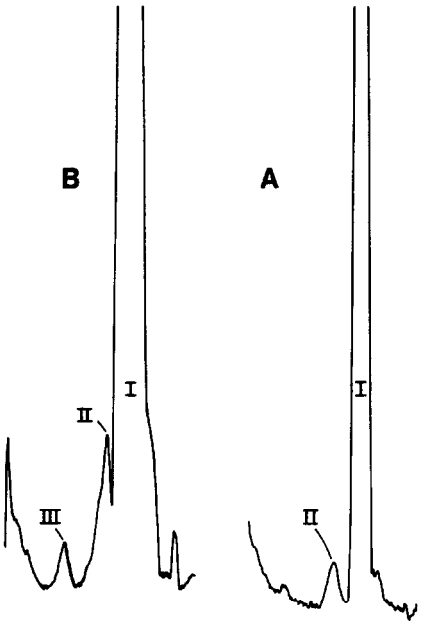

Fig. 5. Determination of compounds II and III in the methanol extractable portion of a metoprolol tablet. A, 200-nl sample size; B, 1.0-μl sample size.

adsorbed on the plate. Various methods of precleaning the plates were not effective in completely eliminating this contribution to the detector signal. It can be corrected for by operating in the dual wavelength–single beam mode. A reference wavelength, close to the measuring wavelength, at which the sample shows minimum absorption is selected for this purpose. From Fig. 3 it can be seen that λ = 300 nm is a suitable choice. In the dual wavelength–single beam mode absorbance in the reference beam is substracted from the sample beam. Provided there is little change in the background absorbance signal between λ = 280 and 300 nm a stable baseline will be obtained. This is clearly the case in Fig. 4B and reliable measurements of peak areas can be obtained. Under these conditions detection limits for compounds II–IV were established as 15.0, 5.10 and 10.20 ng, respectively.

Fig. 5 illustrates the results obtained for a tablet sample. Only compounds II and III were found in detectable concentrations, compound IV, if present, would have a concentration below 0.01 % (w/w) of the methanol extractable portion of the tablet. For this reason, the separating system described in the Experimental section was optimized to provide the best separation of compounds I, II and III with compound IV moved close to the solvent front. Fig. 5A represents a 200-nl sample extract from which compound II can be quantitatively determined. Fig. 5B is the same sample but spotted at a higher concentration of 1.0 μl. Compound III can be readily detected at this concentration but compound II is now fused with I. To determine both compounds II and III the methanol extract should be spotted at both sample volumes.

In Table I are summarized some typical results for the concentration of compounds II and III in tablets of metroprolol tartrate stored at 37°C for various periods

TABLE I

CONCENTRATION OF IMPURITIES IN METOPROLOL TABLETS*

Sample No.	*Initial data experiment started*	*Concentration (%, w/w)***	
		Compound II	*Compound III*
1	71-11-08	0.250 ± 0.001	<0.01
2	73-12-05	1.030 ± 0.018	0.039 ± 0.005
3	73-12-05	0.71 ± 0.01	0.090 ± 0.026
4	74-05-25	1.650 ± 0.004	0.054 ± 0.011
5	74-12-05	0.380 ± 0.005	<0.01

* Per cent of methanol extractable portion of tablet not total tablet weight.

** Average R.S.D.: compound II, 6%; compound III, 20%, $n = 5$. Compound III was determined at levels close to the detection limit. At this concentration the principal source of error was integrator reproducibility (13.9%) due to the low number of total counts.

of time. No correlation was found between the age of the tablets and the concentration of compounds II and III. Compounds II and III are probably by-products generated during the synthesis of metoprolol. For the tablets analyzed in Table I, compound II was present in amounts ranging from 0.25 to 1.65% (w/w), average 0.78% (w/w). Compound III was found in some samples in significantly lower amounts ranging from 0.09 to <0.01 % (w/w). The average concentration in the three samples containing compound III was 0.06% (w/w). A sample of a metoprolol injectable solution, concentration 1 mg ml^{-1}, was also analyzed and found to contain 0.33% (w/w) of compound II and undetectable amounts of compounds III and IV.

ACKNOWLEDGEMENTS

Work in the authors' laboratory is supported by AB Hässle and the Michigan Heart Association.

REFERENCES

1 A. Zlatkis and R. E. Kaiser (Editors), *High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977.
2 W. Bertsch, S. Hara, R. E. Kaiser and A. Zlatkis (Editors), *Instrumental HPTLC*, Hüthig, Heidelberg, 1980.
3 D. C. Fenimore and C. M. Davis, *Anal. Chem.*, 53 (1981) 252A.
4 L. Zhou, C. F. Poole, J. Triska and A. Zlatkis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 440.