

Development of HPTLC–UV Absorption Densitometry Method for the Analysis of Alprazolam and Sertraline in Combination and Its Application in the Evaluation of Marketed Preparations

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Abstract

A new simple, sensitive, and reproducible high-performance thin-layer chromatography method for the estimation of alprazolam and sertraline in combination is developed using silica gel plates with fluorescent indicators. The system is equipped with an automated sample applicator, and the detection was performed at 254 nm by using UV absorption densitometry. The mobile phase consists of carbon tetrachloride, methanol, acetone, and ammonia in the ratio 12:3:5:0.1. The retention factor values for alprazolam and sertraline are found to be 0.52 and 0.70, respectively. The limit of detection of alprazolam and sertraline in the mixture of given proportion is observed to be 0.05 µg/mL and 2.5 µg/mL and the limit of quantitation is 0.2 µg/mL and 10 µg/mL, respectively. The method has shown good linearity in the range of 0.2 µg/mL to 0.65 µg/mL for alprazolam ($R^2 > 0.9953$) and 10 µg/mL to 32.5 µg/mL for sertraline ($R^2 > 0.9942$). The intra- and inter-assay ($n = 5$) variations in the linear range are less than 4% for alprazolam and 6% for sertraline. Three pharmaceutical products containing this combination are analyzed to test the applicability of the new method. The percentage of alprazolam and sertraline in the tablets studied range from 97.7% to 102.82% and 96.5% to 99.9%, respectively.

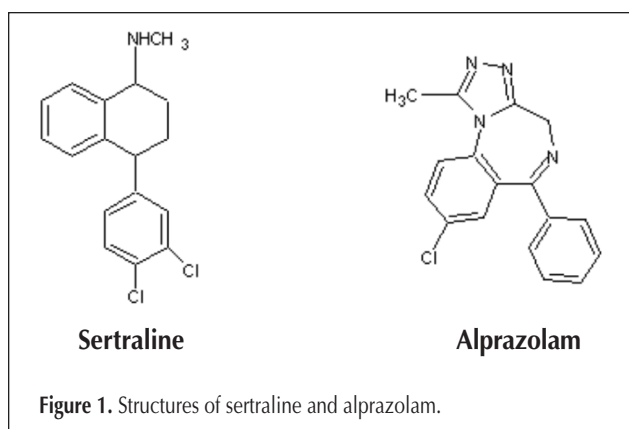
Introduction

Sertraline [1S,4S-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthalenamine] (Figure 1) is a potent and selective inhibitor of neuronal serotonin uptake indicated in the treatment of major depressive episodes (1). It has only very weak effects on neuronal uptake of norepinephrine and dopamine. At clinically effective doses, sertraline also inhibits the uptake of serotonin by human blood platelets.

Alprazolam [8-chloro-1-methyl-6-phenyl-4H-s-triazolo(4,3,α)(1,4) benzodiazepine] (Figure 1) is a benzodiazepine class tranquilizer used in the relief of anxiety symptoms (2), and it is the treatment of panic disorder and anxiety associated with depression. It enhances the action of human body's γ -amino butyric acid, a primary inhibitory neurotransmitter found in the brain and spinal cord.

Coadministration of benzodiazepines along with selective serotonin reuptake inhibitors is common in the treatment of panic disorders. Goddard et al. (3) reported that rapid stabilization of panic symptoms can be safely achieved using a combination of clonazepam and sertraline. Fisekovic et al. (4) reported the beneficiary effect of sertraline in combination with alprazolam in the treatment of panic disorders.

Previously, high-performance liquid chromatography (HPLC) methods were reported for determination of sertraline and alprazolam individually. A reversed-phase HPLC method for the simultaneous estimation of sertraline and alprazolam in combined capsule dosage form was reported (5). To date, no high-performance thin-layer chromatography (HPTLC) method was available in the literature for determination of



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alprazolam and sertraline. This paper reports a simple, sensitive, and reproducible HPTLC method for the simultaneous determination of sertraline and alprazolam and its applicability in the estimation in three marketed tablets. The developed method can be used to estimate these drugs in quality control during and after manufacturing of the combined dosage forms where large number of samples can be handled simultaneously. The present method is more sensitive than the reported HPLC method.

Experimental

Materials

Alprazolam and sertraline were kindly gifted by Unichem Laboratories (Ahmedabad, India). Precoated HPTLC plates

were made of silica gel 60 F₂₅₄ of size 10 cm × 20 cm purchased from Merck (Mumbai, India). All the solvents were of analytical grade purchased from Merck (Mumbai, India). Tablets of three brands, Restyl Plus (Brand 1; Cipla, Mumbai, India), Trika Plus (Brand 2; Unichem, Ahmedabad, India), and Alprax Plus (Brand 3; Torrent, Ahmedabad, India) all containing alprazolam (0.5 mg) and sertraline (25 mg) per tablet, were purchased from a local pharmacy.

Preparation of standard solutions

Standard solutions

Alprazolam (100 mg) was accurately weighed into a 100-mL volumetric flask, dissolved in methanol (~20 mL), and the solution was diluted to volume with the same solvent to get a standard solution of 1 mg/mL. One milliliter of this solution was diluted 10 times in volumetric flask to prepare the working standard solution (0.1 mg/mL). Similarly, sertraline working standard solution of concentration 5.0 mg/mL was prepared in methanol.

Solutions for calibration curve

All the working standards solutions contain a mixture of alprazolam and sertraline. The alprazolam concentrations in the mixture solutions were 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, and 650 ng/mL, and the concentrations of sertraline were 1, 1.5, 2, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, and 32.5 µg/mL.

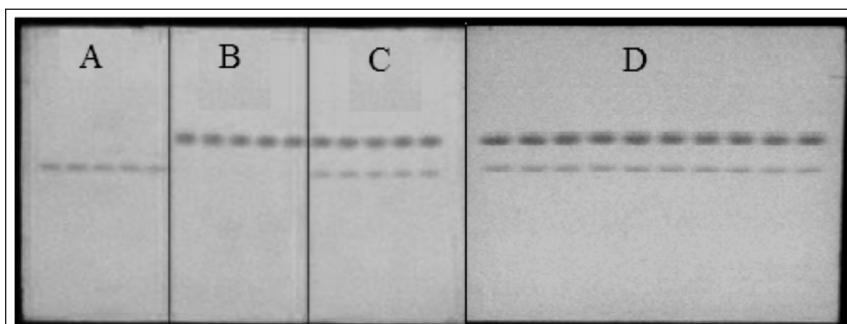


Figure 2. Bands showing alprazolam (0.3 µg/mL) (A), sertraline (15 µg/mL) (B), mixture in same concentration (C), and tablet extract (containing 0.5 µg/mL of alprazolam and 25 µg/mL of sertraline) (D).

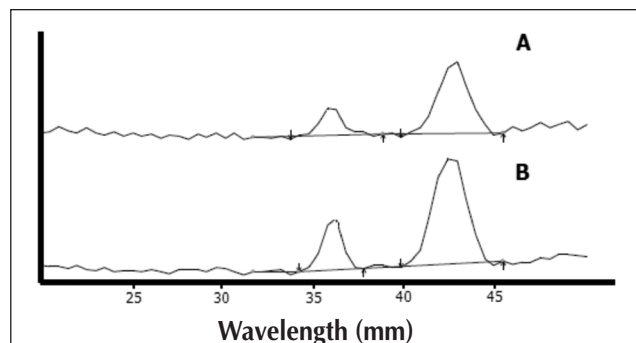


Figure 3. Densitogram showing separation of alprazolam (0.3 µg/mL) and sertraline (15 µg/mL) in standard mixture (A) and separation of alprazolam (0.5 µg/mL) and sertraline (25 µg/mL) from tablets (B).

Table I. Table Showing the Mean Percentage of Alprazolam and Sertraline in Three Marketed Tablet Brands

Tablets	Alprazolam (%)	Sertraline (%)
Brand 1	97.7	99.9
Brand 2	102.82	96.5
Brand 3	101.54	98.72

HPTLC conditions

Analysis was performed on silica gel 60F₂₅₄ plates. Sample and standard solutions were applied by using a Desaga automated sample applicator (Desaga, Heidelberg, Germany) equipped with 100-µL syringe. The settings were: band length, 6 mm; application rate, 4 s/µL; table speed (scan speed), 10 mm/s; distance between bands, 2 mm; distance from the plate edge, 1.0 cm; and distance from the bottom of the plate, 2.0 cm. The volumes applied for each analysis were 3.50 µL.

The plates were developed to a distance of 4.5 cm beyond the origin with carbon tetrachloride–methanol–acetone–ammonia in the ratio of 12:3:5:0.1 in a vapor-equilibrated Desaga chamber. The vapor equilibration time was 25 min. After development, the plates were air-dried for 5 min. The sample and standard zones were quantitated by scanning at 254 nm with Densitometer CD60 (Desaga). The linear regression calibration curve of zone weights and scan areas were plotted by using Desaga ProQuant version 1.03.200.

Method validation

The standard solutions were chromatographed for inter- and intraday assay variation ($n = 5$). The calibration curves were obtained by plotting the peak area against concentration for linearity of alprazolam and sertraline in the above concentration range. The accuracy of the method was determined using external standard addition. Known amounts of standard

drugs were added at four different levels, and each determination was carried out in triplicate. The limit of detection (LOD) and quantitation (LOQ) were obtained.

Application to the analysis of marketed tablets

Alprazolam and sertraline in three brands of marketed tablets was determined. Ten tablets of each brand were weighed and finely powdered. Accurately weighed powder equivalent to 2.5 mg of alprazolam and 125 mg of sertraline was transferred to a 25-mL volumetric flask, dissolved in methanol (~10 mL), and shaken for 15 min. The solution was then diluted to volume with the same solvent, mixed, and finally filtered through Whatmann No. 42 filter paper (Whatman, Middlesex, UK). A sample (1 mL) of the filtrate was serially diluted to get a concentration of 500 ng/mL of alprazolam and 25 µg/mL of sertraline, and this solution was used for analysis. The analysis was done in triplicate, and the amounts of alprazolam and sertraline were calculated from the calibration curve.

Results and Discussion

After the development, the HPTLC plates containing fluorescent indicator are shown as compact bands of sertraline and alprazolam on green background when viewed under 254 nm UV light (Figure 2). The retention factor values for alprazolam and sertraline were found to be 0.52 and 0.70, respectively. The densitogram of alprazolam and sertraline in combination obtained using Densitometer CD60 is shown in Figure 3. The LOD of alprazolam and sertraline in the mixture of given proportion was found to be 0.05 µg/mL and 2.5 µg/mL, and the LOQ was 0.2 µg/mL and 10 µg/mL, respectively. The method has shown good linearity in the range of 0.2 µg/mL to 0.65 µg/mL for alprazolam ($R^2 > 0.9953$) and 10 µg/mL to 32.5 µg/mL for sertraline ($R^2 > 0.9942$). The intra and inter assay ($n = 5$) variations in the linear range were less than 4%

for alprazolam and 6% for sertraline.

Three pharmaceutical tablet products containing this combination were analyzed to test the applicability of the new method. The percentage of alprazolam and sertraline in the tablets studied are shown in Table I and range from 97.7% to 102.82% and 96.5% to 99.9%, respectively.

Conclusion

The HPTLC method developed for the simultaneous determination of alprazolam and sertraline in solid dosage forms is accurate, precise, rapid, and selective. It can, therefore, be easily and conveniently used for routine quality control analysis.

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