

Application of videodensitometric and classical densitometric thin-layer chromatography for quantification of oxcarbazepine in pure and pharmaceutical preparations

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Abstract: The aim of the investigation was to elaborate a simple, rapid and accurate thin-layer chromatography (TLC) methods with densitometric and videoscanning detection for the determination of oxcarbazepine in pharmaceutical preparations. Analysis was performed on silica gel 60 F₂₅₄ plates in horizontal chambers with ethyl acetate-chloroform-methanol (75:20:5, v/v) as mobile phase. Densitometric assay was performed at 220 nm and videoscanning quantitation at 254 nm. The active substance was extracted from tablets with methanol. Calibration plots were constructed in the range 0.2 - 4.0 µg/spot and were correlated with good correlation coefficients ($r_{220}=0.9990$; $r_{254}=0.9919$). The methods were validated for precision and recovery. The results obtained by both techniques were compared.

Key words: thin-layer chromatography (TLC), densitometry, videodensitometry, oxcarbazepine, tablets

INTRODUCTION

Oxcarbazepine (10,11-dihydro-10-oxo-5H-dibenz[b,f]azepine-5-carboxamide) is a new antiepileptic drug used in mono- and adjunctive therapy in patients with simple or complex partial or generalised seizures. It is blocker of pre- and postsynaptic voltage-dependent sodium channels in the central nervous system [1, 2]. After oral administration, oxcarbazepine is rapidly metabolised to its pharmacologically active 10-monohydroxy metabolite (10,11-dihydro-10-hydroxy-5H-dibenz[b,f]azepine-5-carboxamide) [3].

Literature data on the determination of oxcarbazepine in pharmaceuticals describe only the application of HPLC [4], spectrophotometric [5] and Square Wave Adsorptive Stripping Voltammetry [6] methods. There is no data in the literature on the application of TLC for the analysis of this drug in pharmaceutical dosage forms and in biological material. Oxcarbazepine is frequently used in the therapy of epilepsy. Therefore, rapid and simple analytical methods are continually required for the determination its concentration in different preparations. This paper describes the application of new densitometric and videodensitometric methods for quantitative analysis of oxcarbazepine in tablets. The results obtained by the both techniques were compared.

MATERIALS AND METHODS

Chemicals. Oxcarbazepine pure substance was purchased from Sigma (St. Louis, MO, USA). Apydan tablets containing 600 mg oxcarbazepine per tablet and Trileptal tablets

containing 150 mg oxcarbazepine per tablet were obtained commercially. Methanol, ethyl acetate (Merck, Darmstadt, Germany) and chloroform (POCh, Gliwice, Poland) were of analytical reagent grade.

Standard solution. Stock standard solution of oxcarbazepine (2.0 mg/mL) was prepared by dissolving 20.0 mg pure substance in 10.0 mL methanol. Standard solution at a concentration of 0.2 mg/mL was obtained by diluting stock solution 1:10. The solutions were stored in a refrigerator at the temperature of 4°C and were stable for at least 6 weeks.

Tablet samples. Extraction of the active substance from tablets was performed with methanol. The average masses of 20 Trileptal and 20 Apydan tablets were determined. The tablets were ground up and amounts of about 0.01 g were transferred to 25-mL volumetric flasks containing approximately 15 mL methanol. The mixtures were shaken mechanically for 15 min, diluted to volume with methanol, and filtered. The resulting solutions were used for chromatographic analysis. Six microliter of each solution were applied to TLC plates, developed, dried, and scanned. The peak areas were recorded. The procedure was repeated 7 times, individually weighing the tablet powder each time.

Chromatographic procedure. Chromatography was performed on 20 cm × 10 cm silica gel 60 F₂₅₄ TLC plates (Merck, Darmstadt, Germany). Varying volumes of standard solution (1.0-20.0 µL, corresponding to 0.2-4.0 µg oxcarbazepine per spot) and 7 tablet solutions (6.0 µL, corresponding 1.6 µg) were applied to the plates by means of a 25-µL microsyringe - accuracy 0.5 µL (Hamilton, Switzerland). Chromatograms were developed to a distance of 9 cm in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) with ethyl acetate-chloroform-methanol 75:20:5 (v/v), as the mobile phase. After

development the plates were dried at room temperature. The chromatograms obtained were analyzed densitometrically by means of a Desaga (Heidelberg, Germany) CD 60 densitometer controlled by Desaga ProQuant software. The chromatograms were scanned at $\lambda=220$ nm with slit dimensions of 0.2 mm \times 4.0 mm.

In videodensitometric analysis the chromatograms were analyzed at $\lambda=254$ nm by use of a Mitsubishi CCD camera controlled by Desaga ProViDoc videodocumentation VD system. Quantitative assay was performed using Desaga ProResult software. Calibration curves were constructed by plotting the peak area against the amount of the drug spotted. The amount of the substance analyzed in each tablet was calculated by use of appropriate regression equations.

RESULTS AND DISCUSSION

The mobile phase ethyl acetate-chloroform-methanol (75:20:5 v/v) was selected as optimal for obtaining well-shaped, symmetrical single spots of oxcarbazepine. The horizontal technique and a migration distance of 9 cm were chosen as the best for chromatogram development. The R_F value of the drug was 0.46 ± 0.006 (mean \pm SD; $n=40$). The wavelength 220 nm was selected for densitometric evaluation because at this wavelength there was a maximum of the absorption spectrum of oxcarbazepine. Videodensitometric detection was performed at $\lambda=254$ nm.

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 densitometric assay the LD and QL for oxcarbazepine were found to be 0.02 and 0.05 μg per spot. In the videodensitometric procedure they were 0.1 and 0.2 μg per spot, respectively.

Calibration was carried out using 7 points. For each point, 5 measurements were made to improve the precision of the analytical procedure. The data were averaged and calibration curves calculated. The plot of the peak area versus concentration of oxcarbazepine was found to be linear in the range 0.2-4.0 μg per spot. The calibration curves were represented by the following linear regression equations:

$$y_{\text{Dens}} = 599.60x + 76.36 \quad (r = 0.9990)$$

and

$$y_{\text{VideoDens}} = 77.34x + 86.42 \quad (r = 0.9919).$$

The densitogram and chromatogram recorded for the standard solutions are presented in Figures 1 and 3.

The intra-day and inter-day precision of the method were estimated by performing 5 determinations of small (0.2 μg per spot), medium (1.6 μg per spot), and large (4.0 μg per spot) amounts of oxcarbazepine. The results obtained are listed in Table 1.

The densitometric and videodensitometric methods were successfully applied for the determination of oxcarbazepine in Trileptal and Apydan tablets. A typical chromatogram is illustrated in Figure 4. A single spot at $R_F = 0.45$ was observed in the chromatogram obtained from the drug sample extracted from the tablets. There was no interference from the excipients present in the formulations. Results of the analysis of oxcarbazepine in the pharmaceutical products were evaluated statistically; the results are shown in Table 2. Densitograms and chromatograms obtained during analysis

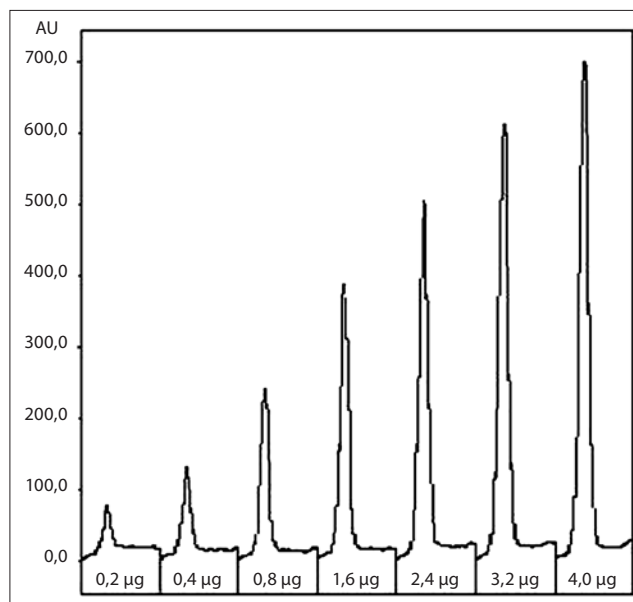


Figure 1 Densitogram recorded for standard solutions of oxcarbazepine in calibration range 0.2-4.0 μg per spot.

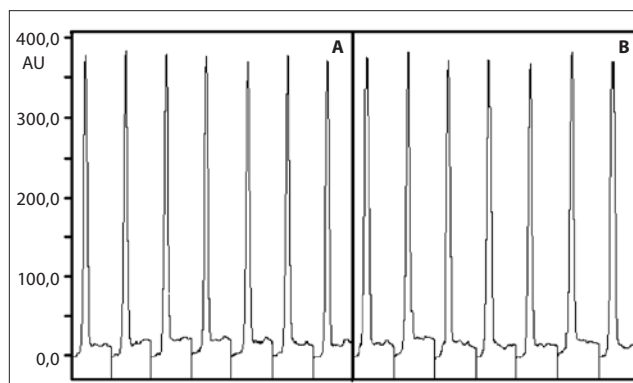


Figure 2 Densitograms obtained during analysis of pharmaceutical preparations: (A) – Trileptal tablets; (B) – Apydan tablets.

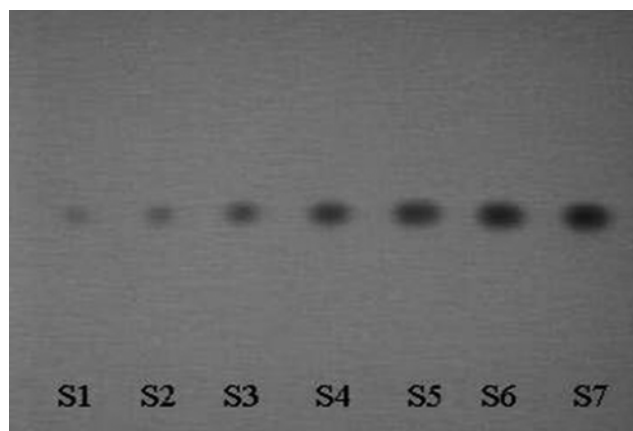


Figure 3 Chromatogram obtained from analysis of oxcarbazepine calibration solutions (S1 – S7) on a silica gel TLC plate developed with ethyl acetate-chloroform-methanol (75:20:5, v/v), as mobile phase.

of pharmaceutical formulations are shown in Figures 2 and 4.

Comparison between the densitometry and videodensitometry was performed by Snedecor's F-test (precision) and Student's t-test (accuracy). Results are shown in Table 3. There were no significant differences between the two elaborated methods.

Table 1 Intra-day and inter-day precision of the TLC-system^{a)}

Amount (µg/spot)	Intra-day precision		Inter-day precision	
	RSD (%) Densito- metry	Videodensito- metry	RSD (%) Densito- metry	Videodensito- metry
0.2	1.05	2.36	1.17	3.10
1.6	0.56	0.98	0.74	1.26
4.0	1.38	1.15	1.48	1.63

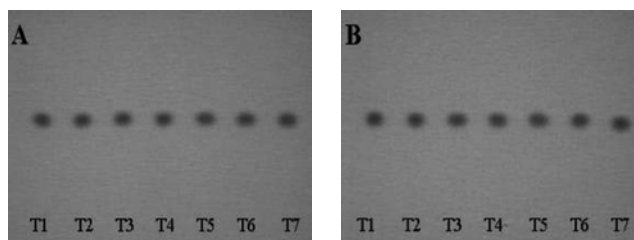
^{a)} n=5

Table 2 Statistical evaluation of results obtained from determination of oxcarbazepine in pharmaceutical preparations

	Densitometry	Videodensitometry
<i>Apydan</i>		
Amount claimed [mg]	600	600
Mean amount found [mg]	604.45	601.03
Recovery [%]	100.74	100.17
Variance	20.8954	3.6789
Standard deviation	4.5711	1.9181
Relative standard deviation [%]	0.76	0.32
95% Confidence interval	599.65-609.25	599.02-603.05
<i>Trileptal</i>		
Amount claimed [mg]	150	150
Mean amount found [mg]	150.67	150.56
Recovery [%]	100.45	100.37
Variance	1.2273	1.1521
Standard deviation	1.1078	1.0734
Relative standard deviation [%]	0.74	0.71
95% Confidence interval	149.50-151.83	149.43-151.68

Table 3 Comparison of precision and accuracy between densitometry and videodensitometry

Test	Apydan tablets	Trileptal tablets
F-test (precision)	5.679 (p = 0.079)	1.065 (p = 0.946)
T-test (accuracy)	1.689 (p = 0.137)	0.177 (p = 0.863)

**Figure 4** Chromatogram obtained from analysis of tablet samples (T1 -T7) on a silica gel TLC plate developed with ethyl acetate-chloroform-methanol (75:20:5, v/v), as mobile phase. (A) – Trileptal, (B) – Apydan.

CONCLUSION

The densitometric and videodensitometric methods described in this paper are accurate, precise, and simple to perform in routine analysis. They show adequate linearity, and no matrix effect occurred.

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