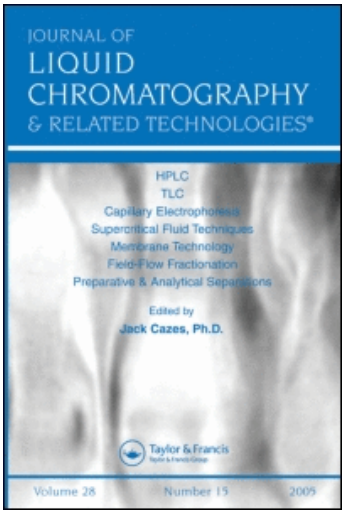

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DENSITOMETRIC DETERMINATION OF URSODEOXYCHOLIC ACID IN PHARMACEUTICAL FORMULATIONS IN FORM OF TABLETS AND CAPSULES

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□ *In this work a very simple and rapid TLC densitometric method used to quantitative analysis of ursodeoxycholic acid in commercial drugs was presented. Ursodeoxycholic acid (UDCA) was effective extracted from pharmaceutical formulations in form of tablets and capsules by means of methanol. Chromatography was performed on glass plates precoated with silica gel 60 F_{254} (E. Merck, Art. 1.05715) with the use of mixture:* n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 as a mobile phase. Under these optimum conditions the R_F value for ursodeoxycholic acid is equal to 0.48. After visualization of spots with the use of 10% H_2SO_4 and next heating them in temp. 120° C, the chromatograms were quantitative scanned by densitometer at maximum wavelength $\lambda = 360$ nm. On the basis of obtained results it was stated, that elaborated method can be used in routine quantity control of ursodeoxycholic acid in selected pharmaceutical formulations.*

Keywords bile acids, densitometry, TLC, ursodeoxycholic acid

INTRODUCTION

Ursodeoxycholic acid is one of the bile acids which exist in human and animal bile. Moreover, it has been used as pharmacological active substance in treatment of gallstone cholestasis in form of tablets and capsules of pharmaceutical preparations.[1] Scientific literature describes a lot of examples about ursodeoxycholic acid application in medicine.[2] Determination of ursodeoxycholic acid level in biological fluids is widely presented in many papers. Although different analytical methods used for bile acid analysis, the chromatographic techniques such as GC and HPLC with fluorescence or UV-detection of bile acids after their prior derivatization are usually

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applied.[3,4] There are a view reports only about qualitative and quantitative analysis of bile acids like chenodeoxycholic and ursodeoxycholic acid in commercial drugs. The methods of bile acids determination in pharmaceutical preparations such as: HPLC and HPTLC with fluorescence detection of bile acids and also capillary electroforesis allow for analysis of pharmacological active bile acids like: ursodeoxycholic acid (UDCA), dehydrocholic acid (DHCA), deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) in respective drugs.[5–7]

The aim of this work was to find the optimum chromatographic conditions for quantitative determination of ursodeoxycholic acid in selected pharmaceutical formulations with the use of TLC densitometric method. TLC and HPTLC with UV-densitometric detection were used for analysis of many pharmaceutical products like: fusidic acid, piperine, oxcarbazepine, levetiracetam, rivastigmine.[8–11] Densitometric technique is very useful in pharmaceutical analyses because is very quick and easy to work.

EXPERIMENTAL

Materials and Chemicals

Standard Substance

Ursodeoxycholic acid (UDCA), No. U5127 (99%, Sigma-Aldrich) was used as standard. A stock solution of ursodeoxycholic acid (50 mg/mL) was prepared by dissolving respective amount of standard substance in methanol (POCh, Gliwice, Poland).

Pharmaceutical Formulations

Ursodeoxycholic acid (UDCA) in form of tablets and capsules in quantity 250 mg in both drugs was examined. The two pharmaceutical preparations of ursodeoxycholic acid were produced commercially.

Solvents

The following components of the mobile phase: *n*-hexane (Merck, Germany), ethyl acetate (POCh, Gliwice, Poland), acetic acid 99.5% (POCh, Gliwice, Poland), were used for TLC analysis. Sulfuric acid, 95% (Chempur, Piekary Śląskie, Poland) was used to prepare a visualizing reagent. All chemicals were analytical grade.

Apparatus

Densitometer: Desaga (Germany), Model CD 60 equipped with Windows–compatible ProQuant software was used.

Thin – Layer Chromatography

Stationary Phase

Chromatographic analysis was performed on the glass chromatographic plates precoated with silica gel 60 F_{254}(E. Merck, Art. 1.05715) 10 cm × 20 cm. The plates were activated at 120°C for 30 minutes before using in all cases.

Mobile Phase

A mixture of *n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 (v/v/v) was used as a mobile phase for quantitative analysis of ursodeoxycholic acid in investigated pharmaceutical preparations.

Preparation of Calibration Plot

The following methanolic solutions of ursodeoxycholic acid in concentrations: 50, 40, 30, 20, 10 and 5 mg/mL were used to prepare a calibration plot. The respective standard solutions in quantities of 3 µl each were spotted on the glass chromatographic plates (10 cm × 20 cm, Art. 1.05715) with the use of micropipettes (5 µL, Camag, Switzerland), equivalent to: 0.15; 0.12; 0.09; 0.06; 0.03 and 0.015 mg UDCA/spot, respectively. The chromatograms were developed at room temperature in a 20 cm × 20 cm horizontal chamber (Camag, Switzerland) using *n*-hexane-ethyl acetate–acetic acid in volume composition 22:22:5. This mobile phase allows for obtaining the optimum R_F values of ursodeoxycholic acid. The development distance was 14 cm. Mobile phases of 50 mL were used in all cases. Next the plates were dried at room temperature 18 ± 1°C using a fume cupboard. The spots were visualized by spraying them using 10% water solution of sulfuric acids and then heating the plates at 120°C for 20 minutes. After visualization, the chromatograms of bile acids were scanned with the use of densitometer Desaga (Germany) at the optimum for ursodeoxycholic acid wavelength $\lambda = 360$ nm. This wavelength was experimentally found on the basis of peak area measurements for ursodeoxycholic acid standard solution recorded in the range 200–360 nm. The results of spot area [AU] for standard solution of UDCA were used to prepare a calibration plot. Each analysis was repeated six times.

Extraction of Ursodeoxycholic Acid from Pharmaceutical Preparations

For quantitative analysis of pharmaceutical formulations, a single tablet or capsule of drugs containing ursodeoxycholic acid (in quantity of 250 mg) was pulverized with pestle in a porcelain mortar. Next the ursodeoxycholic acid was extracted from the powders with 7 mL of methanol for 10 minutes. The extraction of ursodeoxycholic acid was complete. After

10 minutes the mixture was centrifuged. The supernatant was used as test solution in quantitative analysis. The extraction was performed six times.

Assay of Ursodeoxycholic Acid in Tablets and Capsules

The applied chromatographic plates 10 cm × 20 cm (E. Merck, Art. 1.05715) were activated at 120°C for 30 minutes before using. After activation, the extracts of respective drugs and standard solution of ursodeoxycholic acid in concentration 0.0214 g/mL (equivalent to the content of ursodeoxycholic acid in both examined extracts) in quantities of 3 µl each were spotted on the glass chromatographic plates with the use of micropipettes (5 µL, Camag, Switzerland). The chromatograms were developed at room temperature in a 20 cm × 20 cm horizontal chamber (Camag, Switzerland), using *n*-hexane-ethyl acetate–acetic acid in volume composition 22:22:5. The development distance was 14 cm. Mobile phases of 50 mL were used in all cases. Next the plates were dried at room temperature 18 ± 1°C using a fume cupboard. The spots were visualized by spraying them using 10% water solution of sulfuric acids and then heating the plates at 120°C for 20 minutes. The chromatograms of standard solution and extracts of ursodeoxycholic acid were scanned with the use of densitometer Desaga (Germany) at the maximum for ursodeoxycholic acid and its extracts wavelength $\lambda = 360$ nm. The results of spot area [AU] obtained for extracts were used to calculate of ursodeoxycholic acid content in examined drugs by means previously prepared calibration curve. Each analysis was repeated six times.

Validation of the Method

The TLC method with densitometric detection was validated for specificity, linearity, precision, accuracy, linearity, LOD (limit of detection) and LOQ (limit of quantitation). The values of respective statistical parameters for elaborated method are given in Tables 1 and 2.

RESULTS AND DISCUSSION

In presented work, the methanolic standard solution of ursodeoxycholic acid and its methanolic extracts of respective pharmaceutical formulations containing UDCA as pharmacological active substance were examined.

The first step in our investigations was to optimize the chromatographic conditions, which allowed to quantitative determination of UDCA with the use of TLC densitometric technique such as: kind of stationary phase, mobile phase composition and maximum wavelength for UV-spectrum of UDCA. To find the maximum wavelength in UV for UDCA and its extracts, the UV-spectrum was made in the range 200–360 nm.

TABLE 1 The Statistical Parameters for Calibration Plot

Parameter	Ursodeoxycholic Acid (Standard)
Wavelength [nm]	360 nm
R_F	0.48 ± 0.01
LOD [mg per spot]	0.014
LOQ [mg per spot]	0.041
Linearity range [mg per spot]	0.030–0.120
Slope	167763.0 ± 10243.5
Intercept	12004.0 ± 841.59
Correlation coefficient (R)	0.9963
n	4
Confidence level	99%
Standard error (s)	687.16
F-ratio	268.22

Note: *n*-number of points used to construction the calibration plot; *F*-value of the Fisher test; LOD-limit of detection; LOQ-limit of quantitation.

Figure 1 shows the UV-spectrodensitograms of UDCA standard solution at $\lambda = 200$, 250, 300 and 360 nm performed on glass plates precoated with silica gel 60 F_{254} (E. Merck, Art. 1.05715) and developed with mixture: *n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 as a mobile phase and then visualized by the use of 10% H_2SO_4 in temp. 120°C. The maximum wavelength of UV-spectrum for UDCA and its extracts from examined drugs is at $\lambda = 360$ nm. This wavelength was used in quantitative investigations. The spectrodensitograms for methanolic extracts of pharmaceutical formulations and UDCA as standard solution at maximum wavelength $\lambda = 360$ nm presents Fig. 2.

The examples of densitograms for both extracts recorded at $\lambda = 360$ nm confirm that, there were no interferences from excipients present in tablets and capsules of commercial formulations.

TABLE 2 The Results of Quantitative Analysis of Ursodeoxycholic Acid in Extracts from Examined Pharmaceutical Formulations

Parameter	Ursodeoxycholic Acid (Tablets)	Ursodeoxycholic Acid (Capsules)
n	6	6
Wavelenght [nm]	360 nm	360 nm
R_F [cm]	0.48 ± 0.01	0.48 ± 0.02
Specificity	+	+
Accuracy	95.3%	97.2%
Precision	5.29%	6.15%
Amount of ursodeoxycholic acid found in drugs [mg/spot]	0.102 ± 0.0054	0.104 ± 0.0064

Note: *n*-the number of samples

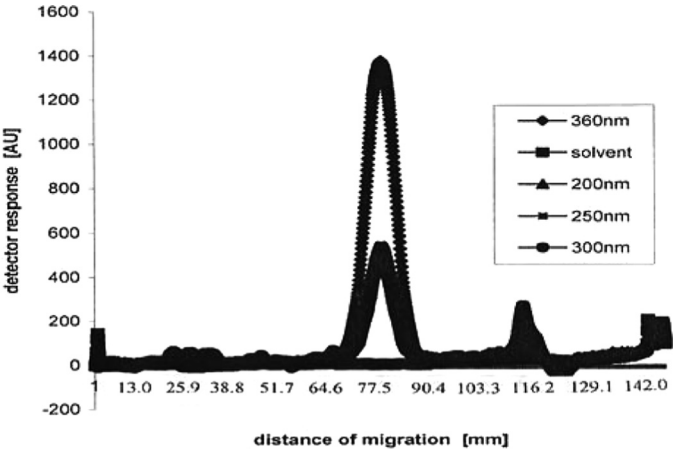

FIGURE 1 UV-densitograms obtained for ursodeoxycholic acid as standard at $\lambda = 200$, 250, 300 and 360 nm.

To describe specificity of elaborated method, the R_F value of UDCA standard solution and its extracts from pharmaceutical formulations in form of tablets and capsules and also UV-spectrum for the both examined compounds were compared. The R_F values of UDCA standard and its extracts obtained on silica gel 60 F_{254} chromatographic plates (E. Merck, Art. 1.05715) and developed with mixture: *n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 are equal to 0.48. Very good correspondence between UV-spectrum of UDCA standard and its methanolic extracts from pharmaceutical preparations was observed (Fig. 2.). This fact decides about specificity of proposed method.

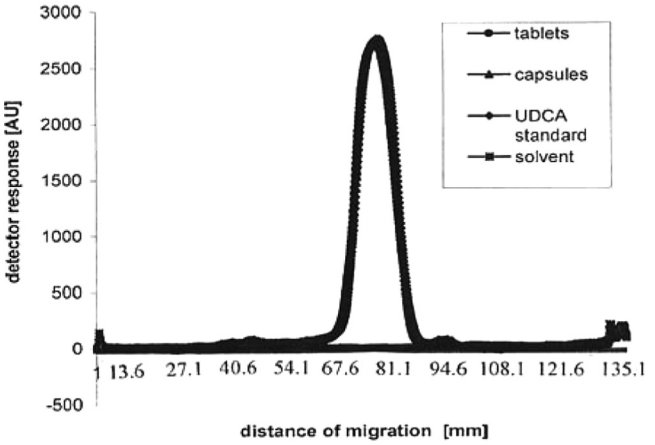

FIGURE 2 UV-densitograms obtained at $\lambda = 360$ nm from ursodeoxycholic acid as standard solution and from extracts of pharmaceutical formulations in form of tablets and capsules.

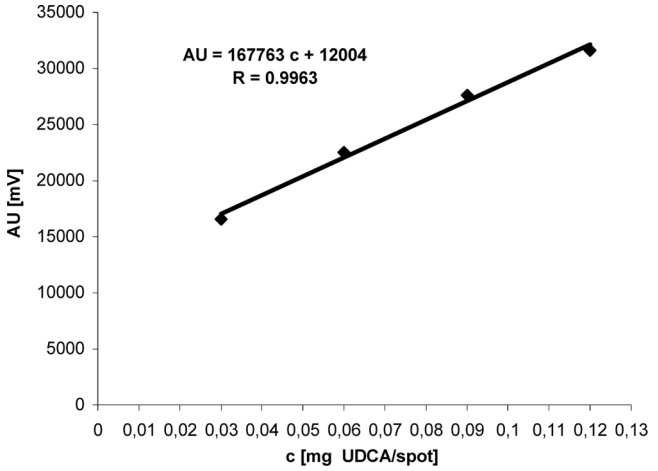

FIGURE 3 Linear calibration plot for ursodeoxycholic acid.

The next part of this work was to determine the UDCA content in investigated formulations in form of tablets and capsules using calibration plot and then validation of elaborated method. The calibration plot was set up direct peak area against the content of ursodeoxycholic acid spotted on the chromatographic plates as methanolic solutions. The equation of this curve was estimated with the use of linear regression analysis. Figure 3 shows calibration plot for ursodeoxycholic acid and its linear equation: $AU = 167763.0 \times c + 12004.0$, where: AU is the peak-area response and c is the content of ursodeoxycholic acid [mg/spot]. The linear range for this relationships was 0.030–0.120 mg/spot.

The statistical parameters of presented calibration plot such as: *R*-correlation coefficient; *s*-standard deviation; *F*-value of the Fisher test; LOD-limit of detection LOQ-limit of quantitation are listed in Table 1.

The LOD of this method is 0.014 [mg/spot] and LOQ is equal to 0.041 [mg/spot]. The values of statistical properties for calibration plot are satisfactory. R-value shows its good linearity. Presented linear calibration plot was used for determination of UDCA content in extracts of pharmaceutical preparations.

Results of quantitative analysis of ursodeoxycholic acid in both examined pharmaceutical formulations: tablets and capsules obtained with the use of UV-densitometric method (at $\lambda = 360$ nm) on glass plates (Art. 1.05715), developed with the use of mobile phase *n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 at 18°C are presented in Table 2.

From the data of UDCA content in pharmaceutical preparations obtained in six repeats it follows, that the content (mean ± standard

deviation) of ursodeoxycholic acid from one tablet is equal to 0.102 ± 0.0054 mg/spot and similarly in the case of capsule is equal to 0.104 ± 0.0064 mg/spot. The obtained results of UDCA amount in pharmaceutical formulations were comparable to the values declared by producers. This method was successfully validated according to specification recommended by GLP (Good Laboratory Practice), GMP (Good Manufacturing Practices) and also by ICH (International Conference of Harmonization) for pharmaceutical products. The precision of elaborated method is 5.29% for tablets and much worse for capsules 6.15%, respectively. Accuracy of the described method as determined by comparison with the values declared by producers was higher than 95%. The summarized validation is presented in Table 2.

CONCLUSIONS

On the basis of obtained results it was stated that, the chromatographic TLC conditions such as: glass plates precoated with silica gel 60 F_{254} (E. Merck, Art.1.05715) and mixture of *n*-hexane–ethyl acetate–acetic acid in volume composition 22:22:5 used as a mobile phase allow for quantitative analysis of ursodeoxycholic acid in selected pharmaceutical formulations by means of densitometric detection at $\lambda = 360$ nm. The successful validation of elaborated method for specificity, linearity, accuracy, precision, LOD and LOQ indicates, that TLC densitometric method can be used in routine quantitative control analysis of ursodeoxycholic acid in selected pharmaceutical preparations in form of tablets and capsules.

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