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TLC-DENSITOMETRIC METHOD FOR QUALITATIVE ANALYSIS OF BETAMETHASONE AND ITS RELATED COMPOUNDS IN PHARMACAUTICAL PREPARATIONS

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Abstract: A new simple and rapid TLC-densitometric procedure for the separation and identification of betamethasone and its related substances, betamethasone-17,21-dipropionate, betamethasone-17-valerate, betamethasone-21-valerate and also betamethasone disodium phosphate was developed. One of the chromatographic systems proposed in this study, which has been satisfactory applied in separation of four pairs of examined compounds was silica gel $60F_{254}$ (E. Merck, Art. 1.05554) and a mixture containing chloroform-methanol-acetic acid (99.5%) in volume composition 28 : 5 : 0.5. Densitometric measurements were done using densitometer TLC Scanner 3 at 246 nm. The proposed method was checked in terms of its specificity for the determination of betamethasone-17,21-dipropionate and betamethasone disodium phosphate in commercially available products containing both compounds, separately, as active ingredients. The results showed that the method is suitable for qualitative analysis of betamethasone derivatives in simple and combined pharmaceuticals in various dosage forms e.g., lotion and injection solution. It also can be applied in quality control of pharmaceutical formulations of betamethasone and its related compounds in form of salts and esters.

Keywords: betamethasone, glucocorticoids, TLC-densitometry, NP-TLC, RP-TLC

Betamethasone (B) and its derivatives, betamethasone dipropionate, betamethasone disodium phosphate and also betamethasone valerate are synthetic glucocorticoids given orally in form of tablets, parenterally, by inhalation, by local injection, or administrated topically in the different disorders in which corticosteroids are indicated (1). B is a corticosteroid with anti-inflammatory and immunosuppressive properties, used especially to treat conditions such as arthritis, hormone and immune system disorders, allergic reactions, certain skin and eye conditions, breathing problems, and certain cancers. It is applied as a topical cream, ointment, lotion gel and tablets. As was reported by Pastuszka and Kaszuba in excellent review paper, the therapeutic effect of betamethasone derivatives depends mainly on potency of the drug, type of vehicle, application and also on the range of genetic factors describing individual sensitivity (2). It is well known that in order to achieve better therapeutic effect of glucocorticosteroids including betamethasone derivatives, they are often combined with other bioactive substances e.g., salicylic acid as keratolytic and antiseptic agent. Combination drugs containing betamethasone dipropionate and salicylic acid in form of ointment or liquid are widely used in dermatology in the therapy of skin diseases e.g., in plaque psoriaris, atopic dermatitis, seborrhoeic dermatitis, because it is well tolerated medication by patients and provides better penetration of betamethasone dipropionate into the skin. Moreover, B is available in a number of pharmaceutical preparations in combination with other steroids such as dexamethasone acetate, flumethasone pivalate or with antibacterial agents like, for instance, antibiotics (gentamicin) (2-8). Additionally, a mixture of B with its related compounds e.g., with betamethasone dipropionate, betamethasone disodium phosphate or with betamethasone valerate is present in various pharmaceutical formulations.

Official method recommended by United States Pharmacopoeia (USP) and by Polish Pharmacopoeia (FP) for the identification and assay

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of B and its derivatives combined with other agents including antibiotics and preservatives in pharmaceutical dosage form is high performance liquid chromatography (HPLC) (9, 10). Numerous research papers reported applicability of this method in combination with UV, UV-DAD and MS/MS detectors in quality and quantity control of some dermatological products containing betamethasone derivatives and some cosmetics (11-15). Past study showed other analytical methods: spectrophotometry and voltammetry in analysis of betamethasone in biological samples (16-20). As was reported in above-mentioned papers (11-15), HPLC method is highly efficient for the determination of B but rather expensive and time consuming, because requires repeated solvent extraction of multicomponent pharmaceutical preparations containing B and its related compounds.

Therefore, it was tried to develop a simple and inexpensive TLC-densitometric method for separation and identification of B and some derivatives: betamethasone-17,21-dipropionate (BP), betamethasone-17-valerate (BV17), betamethasone-21-valerate (BV21) and also betamethasone disodium phosphate (BPh) in mixture. Additionally, the optimum chromatographic conditions, which were found for the separation of all examined compounds, were used for the separation and next identification of both, betamethasone-17,21-dipropionate and betamethasone disodium phosphate in their pharmaceutical formulations. According to our knowledge, until today there is only one TLC-densitometric procedure which was successfully applied in the quantification of betamethasone-17,21-dipropionate in combined pharmaceutical preparation (in the presence of salicylic acid and nipagin in a form of lotion) (21). Identification and quantification of betamethasone disodium phosphate in pharmaceutical dosage form by TLC-densitometry has not been yet officially published in research papers and also in both pharmacopoeias (9, 10).

Thus, the objective of this work was to develop a rapid, simple TLC-densitometric method for the separation and identification of the selected betamethasone derivatives including BPh and BP in mixture. The results of this study might be an indicator for further investigations concerning the quantitative analysis of examined betamethasone compounds.

EXPERIMENTAL

Chemicals and reference standards

The reference standards of betamethasone (CAS No. 378-44-9), betamethasone-17,21-dipropionate (CAS No. 5593-20-4), betamethasone-17-valerate (CAS No. 2152-44-5), betamethasone-21-valerate (CAS No. 2240-28-0) and also betamethasone disodium phosphate (CAS No. 151-73-5) were from commercial source - Sigma-Aldrich (St. Louis, MO, USA). The following mobile phase components: methanol, chloroform, acetone, n-hexane, ethyl acetate, acetic acid (99.5%) for liquid chromatography were purchased from POCh (Gliwice, Poland). Distilled water was obtained from Department of Analytical Chemistry (Medical University of Silesia, Sosnowiec, Poland). Standard solutions of five examined compounds at concentration of 5 mg/mL each were prepared in ethanol (96%, pure for analysis) from POCh (Gliwice, Poland). The reference standard mixtures: containing five examined compounds (M5) in quantity of 5 mg each in 1 mL: B, BP, BV17, BV-21 and also BPh were prepared. Other standard mixtures are consisted of B, BPh, and salicylic acid (M3) mixed at the same concentration like in the case of M5. The last variant of standard mixture was prepared by mixing both solutions of B and BPh at concentration of 5 mg/mL each (M2). Each solution (5 µL) was spotted on the chromatographic plates.

Pharmaceutical preparations

The commercially available pharmaceutical preparations of the combination: BPh + salicylic acid (0.5 mg + 20 mg) per mL in form of lotion and also marketed injection solution consisting of 5.3 mg of BPh per mL in form of an ampoule (5 mL) were used. A sample of lotion (1 mL) diluted with 200 µL of ethanol (96%) and also the commercial injection solution were spotted on TLC plates in quantity of 5 µL together with standards.

Materials

In preliminary study, in order to find the optimum chromatographic conditions for the complete separation of five examined compounds, different aluminum chromatographic plates (20 × 20 cm) were applied such as the chromatographic plates for NP-TLC precoated with with 0.20 mm layers of silica gel $60F_{254}$ without concentrating zone (E. Merck, Darmstadt, Germany, Art. 1.05554), with concentrating zone (E. Merck, Darmstadt, Germany, Art. 1.05583), and also the chromatographic plates used for RP-TLC analysis, silica gel 60 RP-$18F_{254}$ (E. Merck, Darmstadt, Germany, Art. 1.05559).

Apparatus

TLC Densitometer: Camag (Muttenz, Switzerland) equipped with TLC Scanner 3 con-

trolled by WinCATS 1.4.2 software. Deuterium lamp was as a source of radiation emitting continuous UV spectrum between 190 and 450 nm.

The 5 µL Camag micropipettes (Muttenz, Switzerland) were used to apply the solutions on the plates.

Chromatographic chamber: twin-trough chamber for 20 × 10 cm plates (Art. 0.222.5221, Camag, Muttenz, Switzerland).

Chromatography

A thin-layer chromatography was performed on aluminum plates (Art. 1.05554, Art. 1.05559, Art. 1.05583), which were cut from original plates into 10 × 10 cm before use. Next, the plates were activated at 120°C for 30 min. Micropipette (5 µL) was used for samples application. The plates were developed at room temperature (20°C) in a twin-trough chromatographic chamber with the use of several mobile phase systems:

- chloroform-acetone in volume composition 7 : 1;
- *n*-hexane-acetone-acetic acid (99.5%) in the volume compositions: 38.5 : 11 : 0.5, 38.5 : 11 : 0.7, 38.5 : 11 : 2, 38.5 : 11 : 1.2 and 38.5 : 11 : 4;
- *n*-hexane-ethyl acetate-acetic acid (99.5%) in volume composition 35 : 10 : 5;
- chloroform-ethyl acetate in volume composition 33.5 : 16.7;
- acetonitrile-water in volume compositions: 80 : 20, 65 : 35 and 50 : 50;
- chloroform-methanol-water in the volume compositions: 45 : 11 : 1, 38 : 11 : 1, 38 : 11 : 2, 30 : 11 : 1, 38 : 11 : 0, 38 : 9 : 1, 38 : 9 : 2 and 38 : 5 : 1;
- chloroform-methanol-acetic acid (99.5%) in volume composition 28 : 5 : 0.5.

The chamber was previously saturated with vapors of 50 mL of mobile phase for 30 min. In all experiments the migration distance was 80 mm. The distance between the tracks was 15 mm. After development, the plates were dried for 20 h at 20°C in a fume cupboard. Densitometric and spectrodensitometric analyses were carried out by Camag TLC Scanner 3 and controlled by WinCATS 1.4.2 software. To find the maximum absorbance of five examined steroids and also salicylic acid, the densitometric measurements were performed at multiwavelengths from $\lambda = 200$ to 400 nm, at the wavelength interval of 25 nm at each step. The optimum wavelength for all investigated steroids was $\lambda = 246$ nm. The identity of the salicylic acid spots were determined by scanning in absorbance mode at $\lambda = 300$ nm. The chromatographic bands were measured by spectrodensitometric analysis under the following conditions: the slit dimensions were 10.00 × 0.40 mm, Macro; the optimal optical system was light; the scanning speed was 20 mm/s and 20 nm/s; the data resolution was 100 µm/step and 1 nm/step, respectively, for densitometric and spectrodensitometric analysis. Each analysis was repeated three times.

The mean value of obtained R_F results and also average value of band width (w) of each examined compound were used to describe the efficacy of the separation and identification of betamethasone derivatives by the use of proposed TLC-densitometric method.

Calculating of separation factors

In order to estimate the results of the separation of four examined pairs of betamethasone derivatives: BPh/B, B/BV17, BV17/BV21 and also BV21/BP (on the basis of obtained densitograms), the following separation factors for adjacent peaks were calculated: ΔR_F, α and also R_F^{α} according to the equations [1- 3]:

$$\Delta R_{F(1,2)} = R_{F1} - R_{F2} \quad [1]$$

$$R^{\alpha}_{F(1,2)} = \frac{R_{F1}}{R_{F2}} \quad [2]$$

where: R_{F1} and R_{F2} are the values of the two adjacent peaks, and $R_{F1} > R_{F2}$.

$$\alpha = \frac{\frac{1}{R_{F1}} - 1}{\frac{1}{R_{F2}} - 1} \quad [3]$$

where: R_{F1} and R_{F2} are the values of the two adjacent peaks, and $R_{F1} < R_{F2}$.

Specificity (selectivity) of the method

According to current guidelines of analytical methods applied in quality and quantity control of pharmaceuticals such as ICH guidelines (22) and also on the basis of other guides published by Ferenczi-Fodor et al. (23, 24), by Nagy-Turák (25) and by Kobyłka and Komsta (26), each analytical method including TLC requires evaluation of specificity which is a part of validation procedure. As was reported in the above presented guidelines, it must be proven that the developed method can separate a substance determined from the impurities, excipients and/or degradation products (related compounds).

In this work, the specificity of the proposed method in order to use it in qualitative analysis of BP in the presence of salicylic acid in commercial lotion and also BPh in another marketed product was checked on the basis of the results obtained for

the optimum chromatographic conditions, which enabled complete separation of the examined compounds from other excipients present in both pharmaceuticals such as their degradation products and related substances (e.g., B).

To confirm the specificity of the developed TLC-densitometric method, the comparison of obtained chromatographic bands from standard solution and also coming from respective pharmaceutical was made.

Moreover, to estimate the effect of resolution of examined betamethasone and its related substances, the separation factor (R_S) for all adjacent bands (on densitogram) coming from investigated compounds was calculated as (27):

$$R_S = \frac{2d}{w_{b1} + w_{b2}} \quad [4]$$

where: d = a distance between the centers of two adjacent chromatographic bands; w_{b1} and w_{b2} = bands width at the base line.

RESULTS AND DISCUSSION

The purpose of work reported herein was to develop a simple and rapid TLC-densitometric method for the separation and identification of B and its related compounds such as BP, BV17, BV21 and also BPh in mixture. The developed method was applied in further steps of this study for the qualitative analysis of some of the investigated steroid

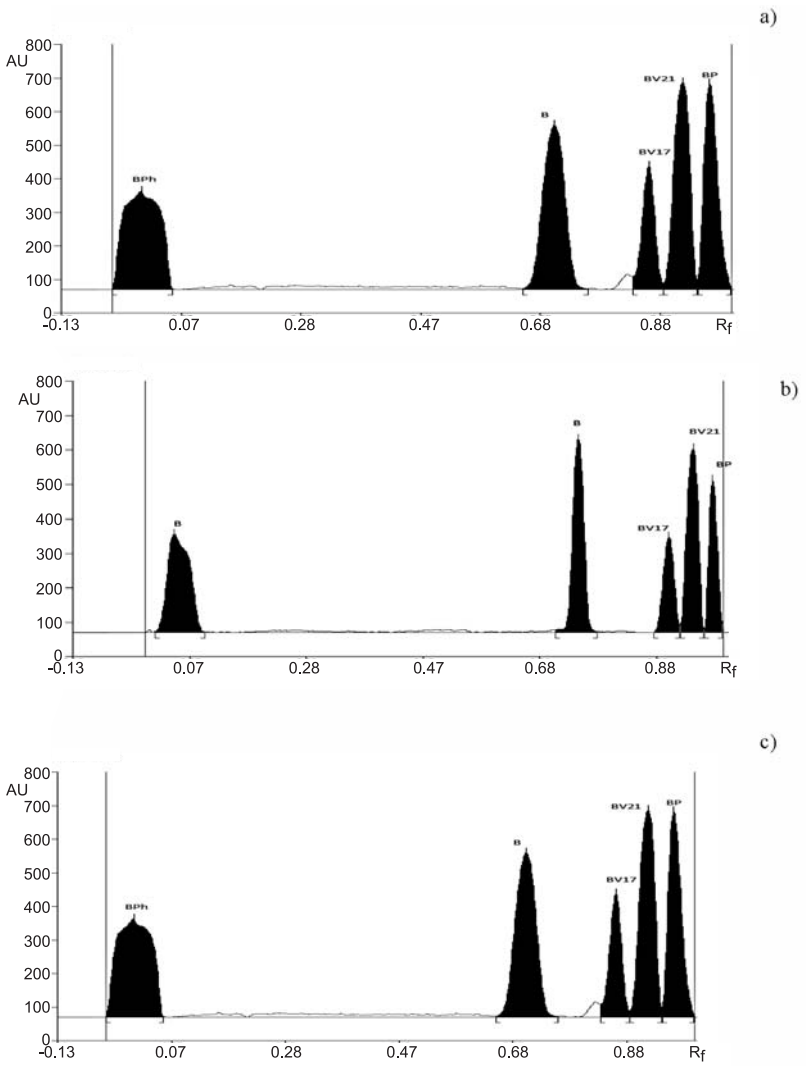

Figure 1. Densitograms registered from chromatograms of model mixture containing B, BV17, BV21, BP and BPh at 246 nm obtained on silica gel $60F_{254}$ (Art 1.05554) using the following mobile phase systems: chloroform-methanol-water 45 : 11 : 1 (v/v/v) (a), chloroform-methanol-water 38 : 11 : 2 (v/v/v) (b) and chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v) (c); for abbreviations see text

Table 1. Effect of resolution of betamethasone and its four examined derivatives obtained under selected chromatographic conditions.

Chromatographic conditions: mobile phase/chromatographic plates	Separated pairs of adjacent peaks on densitograms			
	BPh/B	B/BV17	BV17/BV21	BV21/BP
Chloroform-methanol-water 45 : 1 : 1 (v/v/v) silica gel 60F_{254} Art. 1.05554	+	+	+	+
Chloroform-methanol-water 38 : 11 : 1 (v/v/v) silica gel 60F_{254} Art. 1.05554	+	+	+	+
Chloroform-methanol-water 38 : 11 : 2 (v/v/v) silica gel 60F_{254} Art. 1.05554	+	+	+	+
Chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v) silica gel 60F_{254} Art. 1.05554	+	+	+	+
Chloroform-methanol-water 38 : 11 : 1 (v/v/v) silica gel 60 RP-18F_{254} Art. 1.05559	+	+	+	-
Chloroform-acetone 7 : 1 (v/v) silica gel 60 RP-18F_{254} Art. 1.05559	+	+	+	-
Chloroform-acetone 7 : 1 (v/v) silica gel 60F_{254} Art. 1.05554	-	+	+	+
n-Hexane-ethyl acetate-acetic acid (99.5%) 35 : 10 : 5 (v/v/v) silica gel 60 RP-18F_{254} Art. 1.05559	+	+	+	+/-
Acetonitrile-water 80 : 20 (v/v) silica gel 60F_{254} Art. 1.05554	+	+	+	-
Acetonitrile-water 80 : 20 (v/v) silica gel 60 RP-18F_{254} Art. 1.05559	+	+	+	-

Where: (+) = good separated , (-) = not separated, (+/-) = poorly separated, B = betamethasone, BV17 = betamethasone-17-valerate, BV21 = betamethasone-21-valerate, BP = Betamethasone-17,21-dipropionate, BPh = Betamethasone disodium phosphate.

compounds: BP and also BPh in pharmaceuticals containing BP in the presence of salicylic acid and BPh, respectively, as the active ingredients. As was reported above, many procedures (11-15), including HPLC were satisfactorily applied for the quality control of B derivatives in commercial products (e.g., cosmetics) and in biological samples. High-performance liquid chromatography is a very favorite analytical tool (accurate, precise) and widely used in separating the complex mixture of molecules including steroids in biological and also in pharmaceutical systems but in comparison with TLC, this technique is more expensive (price of columns) and required time consuming pre-treatment steps. Therefore, thin-layer chromatography (TLC) is still a very popular and frequently used analytical method in drug analysis because of its advantages: many samples can be analyzed quickly in one step without additional clean-up of samples and also solvents (28-31). For this reason, this technique is easy to perform and inexpensive in comparison with other chromatographic methods. As was described in the literature, a progress in TLC equipment and new developments in instrumentation of TLC e.g., densitometry, caused that modern TLC may in some cases be comparable to HPLC in terms of selectivity, precision and sensitivity (29). However, until today, both Pharmacopoeias (USP and FP) don't show the real possibilities of TLC in terms of its application in quantitative analysis of drugs including B and its derivatives (32). Pharmacopeial monographs describe only TLC semiquantitative purity test of these compounds in bulk substances and drugs (9, 10). A lack of the official pharmacopoeial TLC-densitometric procedure suitable for identification and quantification of B derivatives: BP, BV17, BV21 and also BPh in pharmaceuticals, shows that there is a need to develop a rapid and simple TLC-densitometric method for the separation and identification of these compounds in commercially available products. A special attendance was devoted to analysis of BPh in model mixture and in pharmaceutical product containing BPh.

According to obligatory validation procedures of analytical methods, the aim of the first step in this study was to find the optimum chromatographic conditions (proper mobile phase and kind of chromatographic plates), which allowed to achieve satis-

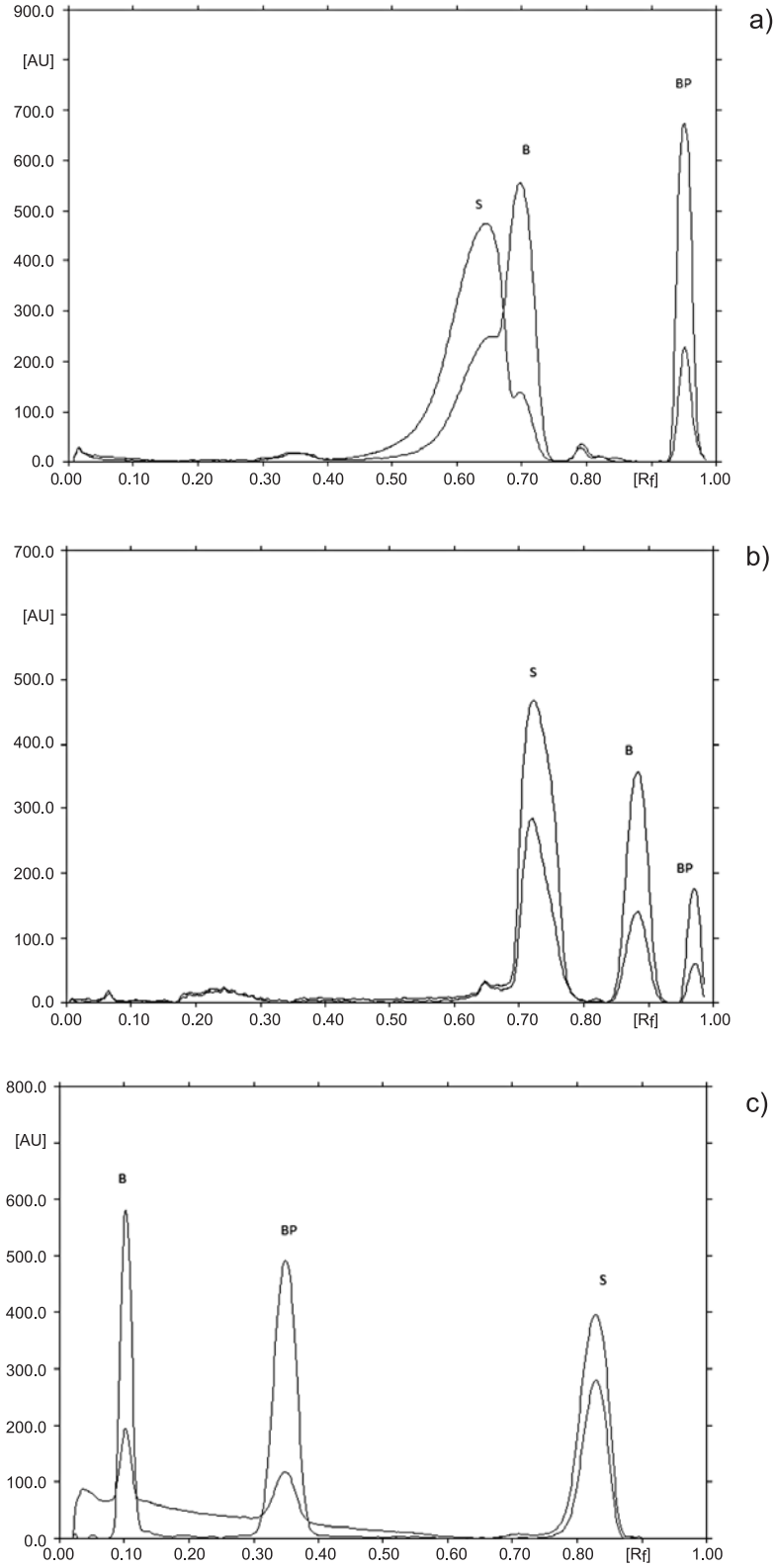

Figure 2. Densitograms registered from chromatograms of model mixture containing B, BP and S at 246 nm (for B and BP) and also at 300 nm for S obtained on silica gel $60F_{254}$ (Art 1.05554) using the following mobile phase systems: chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (a), acetonitrile-water 80 : 20 (v/v) (b), and on silica gel $60F_{254}$ Art. 1.05583 and by *n*-hexane-ethyl acetate-acetic acid (99.5%) 35 : 10 : 5 (v/v/v) (c); for abbreviations see text

factory separation of B and four its derivatives mentioned above, including BP and BPh in mixture. In this preliminary study, various chromatographic conditions (different adsorbents and mobile phases) in normal and reversed phase system (NP-TLC and RP-TLC) were tested. The influence of some of them on efficacy of separation of five compounds was summarized in Table 1. On the basis of these data, it could be observed that among several chromatographic systems used (described in Experimental part) those which give satisfactory resolution of four pairs of examined compounds namely: BPh, B, BV17, BV21 and also BP in mixture are:

- chloroform-methanol-water 45 : 11 : 1 (v/v/v);
- chloroform-methanol-water 38 : 11 : 1 (v/v/v);
- chloroform-methanol-water 38 : 11 : 2 (v/v/v);
- and also chloroform-methanol-acetic acid (99.5%) in volume composition 28 : 5 : 0.5 and silica gel $60F_{254}$ Art. 1.05554 for all of them.

By the use of these chromatographic systems good resolution between each pair of five investigated compounds: BPh/B, B/BV17, BV17/BV21 and BV21/BP was achieved. Examples of densitograms of separated mixtures containing these compounds presented in Figure 1 confirm this fact. The choice of the presented chromatographic systems from all tested as the optimum for the separation and identification of five examined betamethasone compounds were done on the basis of the results of earlier studies focused on the separation and identification of seven bile acids (free and conjugated with respective amino acids) and some steroid hormones in mixture (33-38). In previous study, it was concluded that the following separation factors: ΔR_F, R_s, α and also R^{α}_F are very useful for estimation of the separation results of each pair of adjacent peaks on the densitogram. It was found that for each such pair obtained under above-mentioned chromatographic conditions the two important separation factors were satisfactory when: $\Delta R_F \geq 0.05$ and $R_s > 1.00$. It could be concluded that, similarly like in case of another group of steroids namely bile acids, $R_F \geq 0.05$ and $R_s > 1.00$ for each pair of betamethasone compounds resulted in their good separation. Moreover, based on the obtained results (Table 1), it could be observed that NP-TLC system is better than RP-TLC for separation of five examined compounds. However, it might be noted that the biggest problem in separation of all investigated mixtures referred to separation of BV21 from BP and also BPh from B (Table 1). The mobile phase: acetonitrile-water (80 : 20, v/v) recommended by US Pharmacopoeia for HPLC analysis of betamethasone derivatives was not satisfactory for the separation of BV21 from BP. Modification of mobile phase: chloroform-methanol-water by changing the water as a component of this mobile into acetic acid (99.5%) improved the resolution of examined compounds, especially BV21 from BP. Moreover, the obtained spots were compact in comparison with those formed by the use of mobile phase containing water. The second pair BPh/B, which was difficult to separate by the use of chloro-

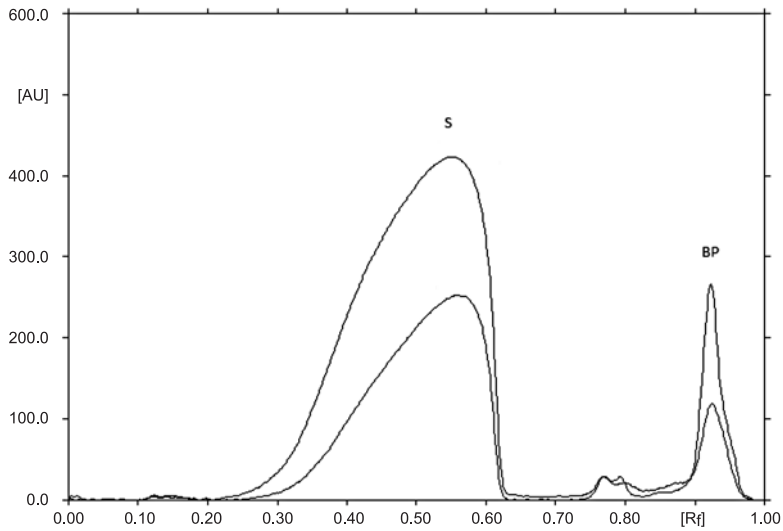

Figure 3. Densitogram obtained from commercial product containing BP and S (lotion) at 246 nm for BP and at 300 nm for S, developed on silica gel $60F_{254}$ (Art 1.05554) using mobile phase system: chloroform-methanol-acetic acid 28:5:0.5 (v/v/v); for abbreviations see text

Table 2. Separation factors of four pairs of examined compounds obtained by means of mobile phase: chloroform-methanol-acetic acid (99.5%) in volume composition: 28 : 5 : 0.5 on silica gel $60F_{254}$ (E. Merck, Art. 1.05554).

Separation factor	Pairs of adjacent peaks on densitograms obtained from standard mixtures (M2, M3 and M5) of examined compounds						
	BPh/B	B/BV17	BV17/BV21	BV21/BP	S/B	B/BP	S/BP
ΔR_F	0.69	0.14	0.06	0.05	0.05	0.25	0.30
α	119.50	2.28	1.80	2.36	1.27	10.00	10.80
R^{α}_F	35.50	1.20	1.07	1.05	1.08	1.35	1.46
R_S	8.23	2.78	1.25	1.11	0.70	3.21	7.12

form-acetone in volume composition 7 : 1 and silica gel $60F_{254}$ Art. 1.05554 could be successfully separated by the use of other mobile phase systems presented in Table 1, for example, containing a mixture chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v). Using these chromatographic conditions, the following R_F values for examined compounds were determined: $R_{F(BPh)} = 0.02 \pm 0.01$, $R_{F(B)} = 0.71 \pm 0.02$, $R_{F(BV17)} = 0.85 \pm 0.01$, $R_{F(BV21)} = 0.91 \pm 0.01$ and $R_{F(BP)} = 0.96 \pm 0.01$. The values of resolution factors (ΔR_F, R_s, α and also R^{α}_F) obtained for each examined pair, and also well separated and symmetric peaks of BPh, B, BV17, BV21, BP observed on densitogram (Fig. 1c) caused that the following chromatographic conditions: chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v) as a mobile phase and silica gel $60F_{254}$ (Art. 1.05554) were selected as the best (optimum) for the further steps regarding the qualitative analysis of pharmaceuticals containing: BP and BPh in form of lotion and injection solution, respectively.

TLC-densitometric analysis of pharmaceuticals containing BP and BPh, separately was evaluated with regard to obligatory analytical procedures designed for quality control of pharmaceutical preparations in term of its specificity and selectivity to determine BP and BPh as active ingredients in their commercial products in the presence of other components, like, for example, salicylic acid (in the case of BP) and including the substances related to both BP and BPh such as B (their degradation product). Densitogram registered from chromatograms of model mixture containing B, BP and salicylic acid (S) at 246 nm (for B and BP) and also at 300 nm for S obtained on silica gel $60F_{254}$ (Art 1.05554) using the mobile phase system: chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v) (Fig. 2a) indicates that according to previous suggestion this solvent mixture and silica gel $60F_{254}$ (Art. 1.05554) are optimum for the separation of BP (active ingredient) from its related compound B (potential degradation product). Moreover, this conclusion confirmed the values of ΔR_F, R_s, α and also R^{α}_F obtained for this separated pair B/BP from model mixture M3 (B + BP + S) presented in Table 2. Good resolution is observed between S and BP. Unfortunately, worse results of separation could be observed on the basis of both: densitogram and value of calculated separation factor R_s ($R_s < 1$) for S/B. To sum this part of study, it was suggested that the developed TLC-densitometric method will be also specific (allows to separate BP from B and S) by use of the same chromatographic plates (Art. 10.05554) and acetonitrile-water 80 : 20 (v/v) as mobile phase (Fig. 2b). Another optimum conditions are silica gel $60F_{254}$ plates (Art. 1.05583) developed by n-hexane-ethyl acetate-acetic acid (99.5%): 35 : 10 : 5 (v/v/v) (Fig. 2c). Under these conditions, it was possible to obtain satisfactory results of separation, comparable to those presented by Wulandari and Indrayanto by means of silica gel and ethanol (96%)-toluene-chloroform-glacial acid in volume composition: 6.0 : 20 : 14 : 0.5 (21). Thus, it could be said that the TLC procedure described in this paper could be an alternative to that presented by these authors. Additionally, applicability of developed chromatographic conditions are confirmed in Figure 3 obtained from commercially available preparation of BP and S.

The third step of this study included the application of developed TLC-densitometric procedure using silica gel $60F_{254}$ (Art. 1.05554) and mobile phase system: chloroform-methanol-acetic acid (99.5%) in volume composition: 28 : 5 : 0.5 to qualitative analysis of the second compound belonging to betamethasone group: BPh. According to our knowledge, there is no official TLC-densitometric procedure in pharmacopoeias and published in research papers. Therefore, the objective of this study was also to find the best conditions, which allowed to separate BPh from its related substance e.g., B and next, to apply them to verify the presence

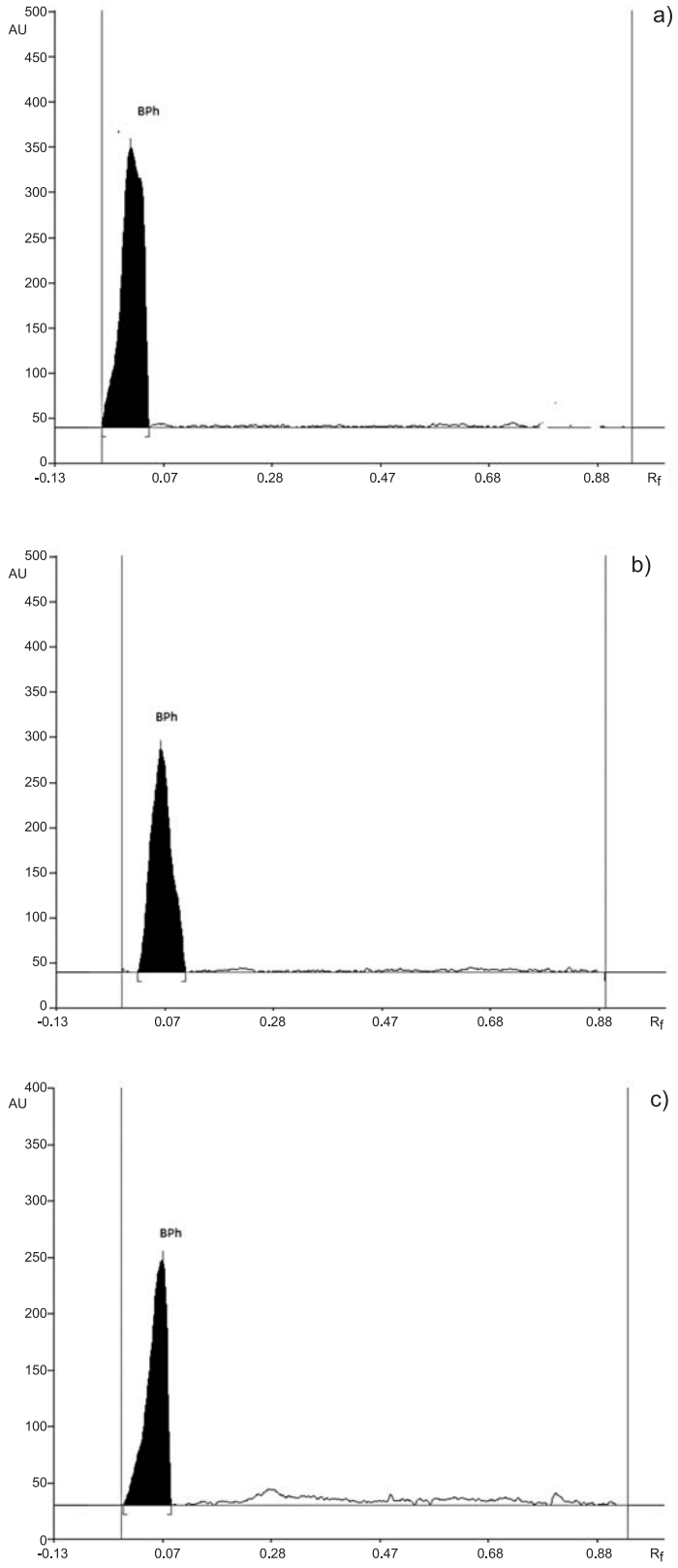

Figure 4. Densitograms from chromatograms of injection solution containing BPh, registered at 246 nm and obtained on silica gel $60F_{254}$ (Art 1.05554) using the following mobile phase systems: chloroform-methanol-acetic acid 28 : 5 : 0.5 (v/v/v) (a), chloroform-methanol-water 45 : 11 : 1 (v/v/v) (b) and by chloroform-methanol-water 38 : 11 : 2 (v/v/v) (c); for abbreviation see text

of BPh in commercially available product (injection solution). The separations factors obtained for this pair (BPh/B) by means of mobile phase system mentioned above, presented in Table 2, and also densitograms obtained from injection solution containing BPh (Fig. 4) confirm that the developed TLC procedure is suitable for qualitative analysis of BPh in commercial product. It allowed to separate BPh from its related substance e.g., B. Moreover, there are no additional peaks from excipients on densitogram obtained from the examined commercial product by use the optimum mobile phase (Fig. 4a). Next, densitograms presented in Figure 4, obtained on silica gel $60F_{254}$ (Art 1.05554) using the following mobile phase systems: chloroform-methanol-water 45 : 11 : 1 (v/v/v) (Fig. 4b) and also chloroform-methanol-water 38 : 11 : 2 (v/v/v) (Fig. 4c) indicate that besides the mixture of chloroform-methanol-acetic acid (99.5%) 28 : 5 : 0.5 (v/v/v) the two solvent mixtures give also good results of qualitative analysis of BPh in pharmaceuticals and could be applied alternatively to that one.

The study showed that the proposed TLC-densitometric procedure could be successfully applied for the determination of B and its related compounds such as BP, BV17, BV21 and also BPh in model mixture and in their selected pharmaceuticals e.g., lotion containing BP as active ingredient and also injection solution of BPh. Our study will be continued in terms of full validation of developed TLC-densitometric method in order to applied it in quantification of examined betamethasone derivatives in pharmaceuticals.

CONCLUSIONS

The results of this work show that a very simple and rapid TLC-method combined with densitometry for the separation and identification of B and its related substances: BP, BV17, BV21 and BPh was developed. The main advantage of this method is short time of analysis and possibility of analysis of five examined compounds in one step without additional clean-up of sample and solvents. It was stated that the proposed method is specific and acceptable for the detection of BP and BPh in commercially available products containing both compounds as active ingredients.

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