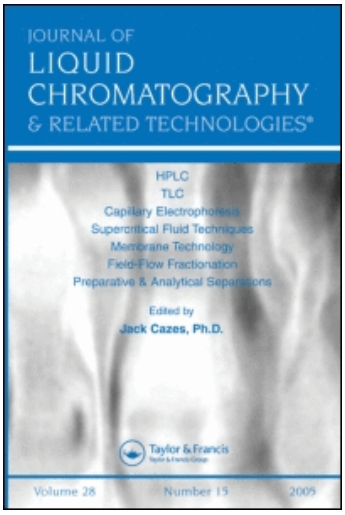

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Evaluation of Visualizing Reagents for Estradiol on Thin Layer by Densitometric Method

A. Pyka[a]; W. Klimczok[a]; D. Gurak[a]
[a] Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, Sosnowiec, Poland

To cite this Article Pyka, A. , Klimczok, W. and Gurak, D.(2008) 'Evaluation of Visualizing Reagents for Estradiol on Thin Layer by Densitometric Method', Journal of Liquid Chromatography & Related Technologies, 31: 4, 555 — 566
To link to this Article: DOI: 10.1080/10826070701812780
URL: http://dx.doi.org/10.1080/10826070701812780

Journal of Liquid Chromatography & Related Technologies®, 31: 555–566, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701812780

Evaluation of Visualizing Reagents for Estradiol on Thin Layer by Densitometric Method

A. Pyka, W. Klimczok, and D. Gurak

Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, Sosnowiec, Poland

Abstract: Five dyes as new visualizing reagents, namely gentian violet, methylene violet, methylene blue, malachite green, and Janus blue, have been used to detect estradiol on neutral aluminum oxide $60F_{254}$ and on neutral aluminum oxide $150F_{254}$. Barton's reagent, rhodamine B, and sulphuric acid were used as the comparative visualizing reagents. Limit of detection (detectability), detection index, modified broadening index, modified contrast index, and linearity range were determined for estradiol following use of these visualizing reagents. It was stated, that the proposed modified contrast index is the objective parameter describing the applied visualizing reagents. The influence of a solid support on obtained visualizing effects was found. The angles (α) between the tangents at the inflection points to the curves of the densitometric peaks are more compact on neutral aluminum oxide $60F_{254}$ than on neutral aluminum oxide $150F_{254}$. This observation indicates that the utility of any particular visualizing reagent depends on the type of chromatographic support used. For the quantitative research of estradiol investigated, relatively good properties had sulphuric acid (VI), Barton's reagent, gentian violet, and methylene violet.

Keywords: NP-TLC, Estradiol, New visualizing reagents, Dyes, Densitometry, Detectability, Modified broadening index, Detection index, Modified contrast index

Correspondence: A. Pyka, Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, 4 Jagiellońska Street, PL-41-200, Sosnowiec, Poland. E-mail: apyka@slam.katowice.pl

INTRODUCTION

The separated substances on thin-layer can be detected by the following methods: physical (individual color of substance or fluorescence of substance in UV light); chemical (colored reactions of separated substances with visualizing reagents); physicochemical (e.g., the application of isotopes as visualizing reagent); biological (the application of biodetectors). The visualizing reagents have the special significance to detect separated compounds on thin-layers. In view of the detection mechanism of the compound, the visualizing reagents can be sorted as follows: conservative reagents, which do not destroy separated substances; and destructive reagents, which destroy or change the structure of separated substances.[1–3]

Currently, the most important fields of application of thin-layer chromatography are pharmacy (30%), biochemistry, forensic chemistry, and clinical chemistry (25%), environmental (15%), food analysis and cosmetology (10%), inorganic substances (5%), and other fields (15%). Currently, the most important field of application of thin-layer chromatography is the pharmaceutical field. The number of publications in the field of pharmacy steadily increases.[1,3] This results from the fact that contemporary thin-layer chromatography is a full instrumented and automated technique.

Therefore, the research of new visualizing reagents of the various groups of organic compounds was the aim of our numerous earlier papers.[4–12] Estradiol has definite pharmacological and biological properties and occurs in many pharmaceutical preparations.[13,14] We decided to examine a series of useful new visualizing reagents for their ability to detect estradiol on thin layers, because thin layer chromatography (TLC) is a basic method for studying this drug.

EXPERIMENTAL

Thin Layer Chromatography

TLC was performed on 10 cm × 20 cm aluminium plates precoated with 0.20 mm layers of neutral aluminum oxide $60F_{254}$ (E. Merck, #1.05550) and neutral aluminum oxide $150F_{254}$ (E. Merck, #1.05551). The plates were prewashed with methanol-chloroform (1:1, v/v) and dried for 24 h at room temperature (18 ± 1°C). The plates were then activated at 120°C for 30 min. Standard solutions of estradiol (Sigma-Aldrich) containing 25.00, 20.00, 16.00, 12.80, 10.24, 8.19, 6.55, 5.24, 4.19, 3.36, 2.68, 2.15, 1.72, 1.37, 1.10, 0.88, 0.53, 0.32, and 0.10 mg were prepared in 5 mL ethanol. The solutions of the estradiol (5 μL) were spotted manually using a microcapillary (Camag, Switzerland) on the chromatographic plates. The mobile phase (50 mL) was placed in a classical chamber (Camag, Switzerland) and the chamber was saturated with the mobile phase for 30 min. Plates with estradiol were

developed with a mixture of toluene and ethyl acetate (1:1, *v*/*v*) as mobile phase at room temperature (18 ± 1°C). The development distance was 7 cm.

Visualizing Reagents Investigated

New Visualizing Reagents

Gentian violet (Fluka, Switzerland), methylene violet (Michrom, England), methylene blue (POCh, Poland), malachite green (POCh, Poland), and Janus blue (Michrom, England) were used as 50 mg/100 mL solutions in distillated water.

Comparative Visualizing Reagents

Barton's reagent, rhodamine B, and sulphuric acid (VI) were used as the comparative visualizing reagents.[3]

Barton's Reagent[3]

Potassium hexacyanoferrate (1 g) (POCh, Poland) was dissolved in 100 mL water (solution I) and 2 g iron (III) chloride hexahydrate (POCh, Poland) was dissolved in 100 mL water (solution II). Immediately before use, 100 mL water, 8 mL solution I, 2 mL solution II, and 1 mL 32% hydrochloric acid (POCh, Poland) in a measuring cylinder were mixed and made up to 100 mL with methanol.

Rhodamine B Reagent[3,15]

Rhodamine B (POCh, Poland) reagent was used as 50 mg/100 mL solutions in distillated water.

Sulphuric Acid[3]

A solution of sulphuric acid (VI) in methanol (1:19, v/v) was also applied for detection of estradiol.

The dried plates were dipped in particular visualizing reagent solutions for 5 sec. The plate dipping in a solution of sulphuric acid (VI) was then heated to 120°C for 20 min. The remaining plates, after dipping in solution of visualizing reagents, were dried for 24 h at room temperature (18 ± 1°C).

Spectrodensitometric Analysis

The spectrum was recorded using Camag Scanner TLC 3. The radiation sources were a deuterium and wolfram lamps emitting a spectrum between 190 and 800 nm. The start wavelength was 200 nm and end wavelength was

700 nm. The slit dimensions were 12.00 × 0.90 mm, Macro; the optimized optical system was resolution; the scanning speed was 20 nm s^{-1}; the data resolution was 1 nm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order.

Densitometric Analysis

Densitometric scanning was then performed at the respective wavelength (Table 1) with a Camag Scanner TLC 3 operated in the absorbance mode and controlled by winCATS 1.4.1 software. The radiation sources were a deuterium or wolfram lamp emitting a spectrum between 190 and 800 nm. The slit dimensions were 12.00 × 0.90 mm, Macro; the optimized optical system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 μm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order. Each track was scanned three times and baseline correction (lowest slope) was used.

Modified Broadening Index, Detection Index, and Modified Contrast Index

Modified Broadening Index

The broadening index was modified and was calculated as

$$I_{broad} = \frac{25}{p_2} \times 1000\left(\frac{\mu g}{AU}\right) \quad (1)$$

Table 1. Absorption maximum of estradiol on neutral aluminum oxide $60F_{254}$ and neutral aluminum oxide $150F_{254}$

	λ_{max} (nm) of estradiol on neutral aluminum oxide	
Detection way	$60F_{254}$	$150F_{254}$
Without using visualizing reagent	205	207
Rhodamine B	228	283
Barton's reagent	233	229
Sulphuric acid	278	532
Gentian violet	226	598
Methylene violet	227	204
Methylene blue	681	683
Malachit green	645	653
Janus blue	205	205

where 25 μg of the analyzed estradiol in 5 μL of solution was applied to the chromatographic plate, and p_2 is the spot area [AU] of 25 μg of analyzed estradiol after the plate has been dipped in reagent solution.

Detection Index[15,16]

The detection index is defined as:

$$I_{det} = \frac{m_1}{p_1}\left(\frac{\mu g}{AU}\right) \tag{2}$$

where m_1 is the smallest quantity of estradiol detected [μg] with the visualizing reagent (limit of detection), and p_1 is the spot area of the substance [AU] at the limit of detection of the estradiol.

Modified Contrast Index

The modified contrast index was calculated as

$$I_{Contr(modif)} = \frac{h}{\alpha}\left(\frac{AU}{o}\right) \tag{3}$$

whre h is the height of denstitometric band [AU] of 25 μg of analyzed estradiol, and α is the angle [°] between the tangents at the inflection points to the curves of the densitometric band of estradiol.

The modified broadening indices, detection indices, and modified contrast index were calculated by use of the Eqs. (1), (2), and (3), respectively.

RESULTS AND DISCUSSION

Estradiol was detected on plates precoated with neutral aluminum oxide $60F_{254}$ and neutral aluminum oxide $150F_{254}$ The neutral aluminum oxides 60 and 150 have the pore diameter equal to 6 nm and 15 nm, respectively. The neutral aluminum oxide 60 has the specific surface areas in the range $180 \div 200\ m^2/g$. However, neutral aluminum oxide 150 has the specific surface area equal to $70\ m^2/g$.[17] Five new visualizing reagents (known as dyes), namely gentian violet, methylene violet, methylene blue, malachite green, and Janus blue were used to analyze the estradiol. However, rhodamine B, Barton's reagent and sulphuric acid were used as comparative visualizing reagents for detection of estradiol. Estradiol was also densitometrically detected without the use of visualizing reagent. The absorption maximum of estradiol without the use of visualizing reagents and after detection by the use of particular visualizing reagents is presented in Table 1. The densitometric analyses were performed at respective wavelengths, which are mentioned in Table 1. Without the use of visualizing

reagents, and by means of all visualizing reagents, it was possible to detect the estradiol investigated in the amount of 25 μg. The colors of chromatographic spots and background colors, without using a visualizing reagent and after estradiol detection with visualizing reagents on neutral aluminum oxide $60F_{254}$ and neutral aluminum oxide $150F_{254}$, are presented in Table 2. All obtained chromatographic spots of estradiol, after application of particular visualizing reagents, were durable and visible for over 24 hours.

The modified broadening index was proposed in this work. A good visualizing reagent has a relatively large numerical value of modified broadening index for a particular substance detected (small spot area, which refers to 25 μg of substance detected). The modified broadening indices for the estradiol investigated are presented in Table 3. The R_F values of the estradiol investigated on neutral aluminium oxide $60F_{254}$ and neutral aluminium oxide $150F_{254}$ are equal to 0.48 and 0.52, respectively. These reagents can be used to identify estradiol analyzed by TLC, based on R_F values, and on the different colors of the chromatographic spots. The detection indices of estradiol are also presented in Table 3. The detection index indicates the ratio of the minimal number of micrograms of the estradiol to the area of the chromatographic spot in AU. The area of the spot was assessed by the densitometric method.

The limits of detection of the estradiol investigated with visualizing reagents tested, linearity range, and densitometric band characteristic of 25 μg estradiol on neutral aluminum oxide $60F_{254}$ and neutral aluminum

Table 2. Color of chromatographic spots and background colors after estradiol detection with particular visualizing reagents on neutral aluminum oxide $60F_{254}$ and neutral aluminum oxide $150F_{254}$

	Neutral aluminum oxide $60F_{254}$ (E. Merck, #1.05550)		Neutral aluminum oxide $150F_{254}$ (E. Merck, #1.05551)	
Detection way	Color of estradiol spot	Background color	Color of estradiol spot	Background color
Without using visualizing reagent	lack of colored spot in visible light	white	lack of colored spot in visible light	white
Rhodamine B	dark pink	pink	dark pink	pink
Barton's reagent	dark blue/green	willow-green	dark blue/green	willow-green
Sulphuric acid	light orange	light beige	light pink	light beige
Gentian violet	violet/blue	violet	dark violet	violet
Methylene violet	grey/blue	light grey	grey/blue	light grey
Methylene blue	blue	dark blue	light blue	blue
Malachite green	green	light green	green	dark blue
Janus blue	blue	navy blue	blue	dark blue

Table 3. Modified broadening index and detection index for estradiol detected on neutral aluminum oxide $60F_{254}$ and neutral aluminum oxide $150F_{254}$

	Neutral aluminum oxide $60F_{254}$ (E. Merck, #1.05550)		Neutral aluminum oxide $150F_{254}$ (E. Merck, #1.05551)	
Detection way	Modified broadening index μg/(AU)	Detection index μg/(AU)	Modified broadening index μg/(AU)	Detection index μg/(AU)
Without using visualizing reagent	1.916	0.32/495	2.188	0.32/409
Rhodamine B	2.184	0.32/985	8.144	0.32/686
Barton's reagent	1.051	0.32/1070	0.454	0.53/1629
Sulphuric acid	0.559	0.10/850	1.028	0.10/450
Gentian violet	2.203	0.32/760	2.292	0.32/796
Methylene violet	1.835	0.32/705	2.953	0.53/490
Methylene blue	4.707	3.36/1385	5.565	2.68/1455
Malachit green	1.536	0.88/1025	1.895	0.53/4171
Janus blue	2.059	2.15/1560	2.782	0.88/935

oxide $150F_{254}$ are presented in Tables 4 and 5, respectively. The detection of estradiol becoming into a chromatographic spot, was also noted by densitometric estimation. The visualizing reagents reported in this work can be used as new detection reagents for the qualitative and quantitative determination of estradiol. The smallest quantity of estradiol (0.10 μg) was detected with sulphuric acid as the visualizing reagent on both studied chromatographic adsorbents. The estradiol can be detected in a quantity equal to 0.32 μg by the densitometric method, without the use of a visualizing reagent, and after detection with rhodamine B, Barton's reagent, gentian violet, and methylene violet on neutral aluminum oxide $60F_{254}$. However, on neutral aluminum oxide $150F_{254}$ the estradiol can be detected in a quantity equal to 0.32 μg by the densitometric method, without the use of a visualizing reagent, and after detection with rhodamine B and gentian violet.

It was stated, that all applied ways of detection permit obtaining a linear dependence between of the area of the densitometric band and the quantity of spotted estradiol. The range of linearity is different for particular applied visualizing reagents and depends on the applied chromatographic adsorbent. It was stated, that the largest range of linearity (0.53 ÷ 25.00 μg) was obtained after detection with gentian violet in the case of estradiol analyses performed on neutral aluminum oxide $60F_{254}$. The smallest quantities of estradiol (linearity range is 0.32 ÷ 6.55 μg) can be quantitatively determined with sulphuric acid in the case of estradiol analyses performed on neutral aluminum oxide $150F_{254}$. However, it was stated, that the largest range of linearity (0.88 ÷ 20.00 μg) was obtained after detection with gentian

Table 4. Characteristic of densitometric band of estradiol, limit of detection, modified contrast index, and linearity range on neutral aluminum oxide $60F_{254}$

Detection way	Densitometric band characteristic of 25 μg estradiol			Modified contrast index (AU/o)	Limit of detection (μg)	Linearity range (μg $spot^{-1}$) (r, correlation coefficient)
	Area (AU)	Height (AU)	α			
Without using visualizing reagent	13048	246	14.5°	16.966	0.32	1.10÷25.00 r = 0.9951
Rhodamine B	11447	202	21.5°	9.395	0.32	0.88÷25.00 r = 0.9979
Barton's reagent	23786	255	13°	19.615	0.32	0.53÷10.24 r = 0.9975
Sulphuric acid	44709	497	13°	38.231	0.10	0.88÷12.80 r = 0.9974
Gentian violet	11348	220	15°	14.667	0.32	0.53÷25.00 r = 0.9943
Methylene violet	13622	221	17°	13.000	0.32	0.88÷3.36 r = 0.9975
Methylene blue	5311	74	71°	1.042	3.36	5.24÷25.00 r = 0.9976
Malachit green	16277	216	24°	9.000	0.88	1.72÷20.00 r = 0.9964
Janus blue	12139	127	30°	4.233	2.15	4.19÷20.00 r = 0.9952

Table 5. Characteristic of densitometric band of estradiol, limit of detection, modified contrast index, and linearity range on neutral aluminum oxide $150F_{254}$

Detection way	Densitometric band characteristic of 25 μg estradiol			Modified contrast index (AU/o)	Limit of detection (μg)	Linearity range (μg $spot^{-1}$) (r, correlation coefficient)
	Area (AU)	Height (AU)	α			
Without using visualizing reagent	11424	192	13°	14.769	0.32	1.37 ÷ 20.00 r = 0.9972
Rhodamine B	3068	51	33°	1.545	0.32	0.53 ÷ 8.19 r = 0.9928
Barton's reagent	55006	342	33°	10.364	0.53	1.37 ÷ 10.24 r = 0.9928
Sulphuric acid	24313	309	14°	22.071	0.10	0.32 ÷ 6.55 r = 0.9976
Gentian violet	10906	127	20°	7.700	0.32	0.88 ÷ 20.00 r = 0.9879
Methylene violet	8466	154	33°	4.667	0.53	1.37 ÷ 25.00 r = 0.9983
Methylene blue	4492	62	64.5°	0.961	2.68	5.24 ÷ 25.00 r = 0.9974
Malachit green	13194	155	35.5°	4.366	0.53	1.10 ÷ 20.00 r = 0.9987
Janus blue	8986	112	30.5°	3.672	0.88	2.15 ÷ 25.00 r = 0.9974

violet in the case of estradiol analyses performed on neutral aluminum oxide $150F_{254}$. Densitometric evaluation of obtained densitometric bands of 25 μg estradiol was described by the area of densitometric band [AU], the densitometric band height [AU], and the angle (α) between the tangents at the inflection points to the curves of the densitometric band, formulated in degrees [°].

To depict the contrast index[15] the following test of estradiol was carried out: 25 μg of estradiol were dropped, in turn on the starting line, next their mobile phase was evolved and afterwards each spot was developed by means of another visualizing reagent. The contrast index represents two independent values, namely the angles (α) between the tangents at the inflection points to the curves of the densitometric band, formulated in degrees, and the densitometric band high [AU].[15] The modified contrast index was proposed in this work. The modified contrast index indicates the ratio of the height of the densitometric band [AU] of estradiol to the angle (α) between the tangents at the inflection points to the curves of the densitometric band formulated in degrees [°]. The best visualizing reagent for estradiol has the highest value of the modified contrast index. The numerical values of a contrast index and modified contrast index indicate that methylene blue has poorer detection properties for estradiol than remaining visualizing reagents on both the investigated chromatographic adsorbents. High values of the angle, a low value of densitometric band height, and a low value of modified contrast index indicate that methylene blue cannot be regarded as a suitable visualizing reagent for estradiol. The numerical values of a contrast index and modified contrast index indicate that on neutral aluminum oxide $60F_{254}$ the best detection properties for estradiol have sulphuric acid. However, from among new visualizing reagents, the best detection properties have gentian violet and metylene violet. Similar detection properties to gentian violet and methylene violet have Barton's reagent. Poor numerical values of a contrast index and modified contrast index were obtained on neutral aluminum oxide $150F_{254}$ in relation to analyses performed on neutral aluminum oxide $60F_{254}$. The numerical values of a contrast index and modified contrast index indicate that on neutral aluminum oxide $150F_{254}$ the best detection properties for estradiol have a solution of sulphuric acid prepared according to a definite procedure.

The visualizing reagents, known as dyes, proposed in this work are not universal visualizing reagents. In an earlier work, all five dyes, namely gentian violet, methylene violet, methylene blue, malachite green, and Janus blue, were tried for nicotinamide detection. It was stated that the above mentioned visualizing reagents did not give color chromatographic spots with nicotinamide.[18] The applied new visualizing reagents are non-destructive reagents. This fact has definite analytical and physicochemical significance. The obtained visualizing effects and non-destructive properties of applied visualizing reagents in relation to estradiol indicate, that progress in the range of analysis of estradiol on thin layer has taken place.

CONCLUSION

The visualizing reagents proposed in this work should provided a supplement to those used previously for the detection of estradiol. The study also provides information about the physicochemical, analytical, and pharmaceutical importance of the new visualizing reagents proposed. The influence of a solid support on obtained visualizing effects was found. The angles (α) between the tangents at the inflection points to the curves of the densitometric peaks are more compact on neutral aluminum oxide $60F_{254}$ than on neutral aluminum oxide $150F_{254}$. This observation indicates that the utility of any particular visualizing reagent depends on the type of chromatographic support used. For quantitative research of estradiol investigated, relatively good properties had sulphuric acid (VI), Barton's reagent, gentian violet, and methylene violet. It was stated, that the proposed modified contrast index is the objective parameter describing the applied visualizing reagents.

ACKNOWLEDGMENT

This research was financed by the Ministry of Science and Higher Education by resources reserved for science in the years 2005–2008 as research project No. T09A 155 29.

REFERENCES

1. Jork, H.; Funk, W.; Fischer, W.; Wimmer, H. *Dünnschicht-Chromatographie, Reagenzien und Nachweismethoden, Physicalische und Chemische Nachweismethoden: Grundlagen, Reagenzien I*; VCH: Weinheim, Germany, 1989.
2. Sherma, J., Fried, B., Eds.; *Handbook of Thin-Layer Chromatography: Revised and Expanded*, 3rd edn.; Marcel Dekker, Inc.: New York, 2003.
3. Jork, H.; Funk, W.; Fischer, W.; Wimmer, H. *Thin-Layer Chromatography: Reagents and Detection Methods, Physical and Chemical Detection Methods: Activation Reactions, Reagents Sequences, Reagents II*; VCH: Weinheim, Germany, 1994; Vol. 1b.
4. Pyka, A.; Sliwiok, J. Application of thymol blue and bromothymol blue to the detection of the alcohols in TLC. J. Planar Chromatogr. Mod. TLC **1992**, *5*, 282–283.
5. Wardas, W.; Pyka, A. New visualizing agents for fatty vitamins in TLC. Chem. Anal. (Warsaw) **1995**, *40*, 67–72.
6. Wardas, W.; Pyka, A. Visualizing agents for cholesterol derivatives in TLC. J. Planar Chromatogr. Mod. TLC **1998**, *11*, 70–73.
7. Wardas, W.; Pyka, A. Visualizing agents for esters of higher fatty acids in TLC. J. Planar Chromatogr. Mod. TLC **2001**, *14*, 8–15.
8. Pyka, A.; Gurak, D.; Bober, K. New visualizing reagents for selected phenolic drugs investigated by thin layer chromatography. J. Liq. Chromatogr. & Rel. Technol. **2002**, *25*, 1483–1495.

9. Pyka, A.; Gurak, D.; Bober, K.; Niestrój, A. Application of new visualizing agents for selected essential oil components in TLC. Chem. Anal. (Warsaw) **2002**, *47*, 691–699.
10. Pyka, A.; Bober, K. Visualizing agents for short chain fatty acids in TLC. J. Planar Chromatogr. Mod. TLC **2005**, *18*, 141–146.
11. Pyka, A. Phenolic drugs, new visualizing reagents for detection in TLC. In *Encyclopedia of Chromatography*, 2nd edn.; Cazes, J., Ed.; Taylor & Francis, Inc., 2005; 1267–1273.
12. Pyka, A. Phenolic drugs: new visualizing reagents for detection in TLC. In *Encyclopedia of Chromatography*; Cazes, J., Ed.; Taylor & Francis, Inc., 2007; 1–9.
13. Makarevich, A.E. Disorders of sex hormone status in patients with chronic obstructive pulmonary disease. Wiad. Lek. **2003**, *56* (3/4), 140–146.
14. Crha, I.; Hruba, D.; Fiala, J.; Ventruba, P.; Zakova, J.; Petrenko, M. The outcome of infertility treatment by in-vitro fertilisation in smoking and non-smoking women. Cent. Eur. J. Public. Health **2001**, *9* (2), 64–68.
15. Sliwiok, J. The Application of fuchsine dyes in the detection of higher fatty acids by thin-layer chromatography. Microchem. J. **1968**, *13* (1), 108–110.
16. Gregorowicz, Z.; Sliwiok, J. Indexes for estimation of developing reagents in thin-layer chromatography. Microchem. J. **1970**, *15* (1), 60–63.
17. Rabel, F.M. Sorbents and Precoated Layers in Thin-Layer Chromatography. In *Handbook of Thin-Layer Chromatography: Revised and Expanded*, 3rd edn.; Sherma, J., Fried, B., eds. Marcel Dekker, Inc.: New York, 2003; 99–133.
18. Klimczok, W.; Pyka, A.; Gurak, D. Application of densitometry and spectrodensitometry for the evaluation of the new visualizing reagents for selected drugs, The *XXXI* Symposium "Chromatographic Methods of Investigating the Organic Compounds", Katowice-Szczyrk, June 4th–6th, 2007, P-14, p. 61.

Received July 20, 2007
Accepted September 10, 2007
Manuscript 6182