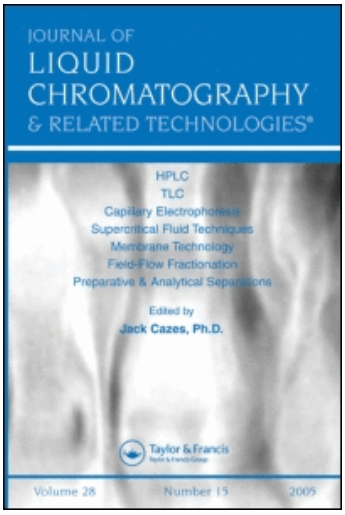


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# NEW VISUALIZING REAGENTS FOR SELECTED PHENOLIC DRUGS INVESTIGATED BY THIN LAYER CHROMATOGRAPHY

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# NEW VISUALIZING REAGENTS FOR SELECTED PHENOLIC DRUGS INVESTIGATED BY THIN LAYER CHROMATOGRAPHY

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## ABSTRACT

Thirteen new visualizing reagents have been used to detect 13 phenolic drugs following thin layer chromatography on silica gel layers. Limits of detection (detectability), detectability index, and broadening index were determined for these drugs following use of these visualizing reagents. Aniline blue and brilliant green were the best and most universal visualizing reagents for the phenolic drugs investigated. Densitograms of selected phenolic drugs after spraying with aniline blue and brilliant green are presented.

## INTRODUCTION

Many phenol derivatives have definite pharmacologic and biological properties.[1–5] The drugs that require special attention are: bamethane, salicylanilide, thymol, eugenol, niclosamide, methyldopa, and norepinephrine.

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There are good analytical and physicochemical reasons for describing new visualizing reagents; these reasons, and the most important reagents and techniques for different types of organic compounds, have been described elsewhere.[6–9]

We decided to examine a series of useful visualizing reagents for their ability to detect certain compounds on thin layers, because thin layer chromatography (TLC) is a basic method for studying these drugs.

## EXPERIMENTAL

### Thin Layer Chromatography

TLC was performed on $20 \times 20$ cm glass plates precoated with 0.25 mm layers of silica gel 60 (E. Merck, # 1.05721). The plates were activated at 120°C for 30 min. Standard solutions containing different concentrations of phenolic drugs (Sigma) were prepared in methanol (for bamethane- **BM**), ethanol (for ethamivan- **EM**, hexachlorophene- **HP**, salicylanilide- **SA**, pyrocatechine- **PC**, thymol- **TM**, pentazocine- **PZ**, phloroglucinol- **PG**, eugenol- **EG**), acetone (for niclosamide- **NS**), a mixture of ethanol – water (4 : 1, v/v; for terbutaline- **TB**), a mixture of methanol – water (3 : 2, v/v; for methyldopa- **MD**), and a mixture of methanol – formic acid (8.5 : 1.5, v/v; for norepinephrine- **NP**). Microsyringes (1 and 100 μL; Hamilton) were used to spot the solutions on the plates. The particular drugs were spotted separately on a plate. Plates with methyldopa, norepinephrine, terbutaline, bamethane, and ethamivane were developed with a mixture of glacial acetic acid – *n*-butanol – water (1 : 4 : 1, v/v) as mobile phase. Plates with phloroglucinol, pentazocine, hexachlorophene, pyrocatechine, niclosamide, salicylanilide, and thymol were developed with a mixture of chloroform – methanol (9 : 1, v/v). The plate with eugenol was developed with benzene as mobile phase.

### Visualizing Reagents Investigated

Alkaline blue (**A**), aniline blue (**B**), neutral red (**C**), and brilliant green (**D**) were used as 50 mg/100 mL solutions in water. Bromophenol blue (**E**), bromothymol blue (**F**), brilliant cresyl blue (**G**), thymol blue (**H**), phenol red (**I**), bromocresol green (**J**), and helasol green (**K**), were used as 50 mg/100 mL solutions in 2% aqueous sodium hydroxide solution. Bromophenol blue (**E**) solution was prepared directly before use.

Additionally, brilliant cresyl blue (**L**) and bromocresol green (**M**) were used as 50 mg/100 mL solutions in water. The chromatograms were sprayed with 2% aqueous solutions of $CuSO_4$, and then aniline blue (**B**) or brilliant green (**D**).

Plates 20 cm × 20 cm were sprayed with 10 mL of these reagents. Plates were evaluated 5 min after spraying (variant 1). They were then heated at 100°C for 15 min and re-examined (variant 2).

## Broadening Index and Detectability Index

Broadening Index[10]

The broadening index is defined as

$$I_{broad} = \frac{100}{P_2} \times 100 \qquad \left[\frac{\mu g}{mm^2}\right] \tag{1}$$

where 100 μg of the analyzed substance in 10 μL of solution was applied to the chromatographic plate, and $p_2$ is the spot area of 100 μg of analyzed substance after the plate has been sprayed with visualizing reagents and heated at 120°C for 30 min.

Detectability Index[11]

The detectability index is defined as:

$$I_{det} = \frac{m_1}{P_1} \qquad \left[\frac{\mu g}{mm^2}\right] \tag{2}$$

where $m_1$ is the smallest quantity of substances detected [μg] with the visualizing reagent (limit of detection), and $p_1$ is the spot area of the substance [$mm^2$] at the limit of detection of the substance.

The broadening indexes and detectability indexes were calculated by use of the Eq. (1) and (2).

## Densitometric Analysis

Densitometric measurement was performed in the reflectance mode with a Shimadzu CS-9001 PC scanning densitometer coupled with a 486IBM-compatible PC. Plates were scanned in zigzag mode over the sample zones.

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## RESULTS AND DISCUSSION

Eleven visualizing reagents applied as 13 visualizing systems (known as alkacimetric and redoximetric indicators), were used to analyze the drugs. By means of these visualizing reagents: alkaline blue (**A**), aniline blue (**B**), bromophenol blue (**E**), bromothymol blue (**F**), alkaline solution of brilliant cresyl blue (**G**), bromocresol green (**J**), helasol green (**H**), aqueous solution of brilliant cresyl blue (**L**), it was possible to detect all of the drugs investigated in the amount of 100 μg. By use of the remaining visualizing systems (**C, D, I, K, M**), it was also possible to detect 100 μg of the drugs investigated, except: methyldopa, terbutaline, norepinephrine, ethamivan. These could not be detected by means of neutral red (**C**); ethamivan, which could not be detected by means of helasol green (**D**), thymol blue (**H**), and aqueous solution of bromocresol green (**M**); bamethane and salicylanilide could not be detected with phenol red.(**I**)

A good visualizing reagent has a relatively large numerical value of broadening index for a particular substance detected (small spot area, which refers to 100 μg of substance detected).

The broadening indexes for the drugs investigated, along with the best visualizing reagents, are presented in Table 1. The $R_F$ values of the drugs investigated are also given.

The limits of detection of the phenol drugs investigated with the visualizing reagents tested, directly after spraying (variant 1), or after 30 min heating at 120°C (variant 2), as well as the detection indexes, are presented in Table 2. The results of our research shows that the visualizing effect depends on the chemical structure of the visualizing reagent, as well as the structure of the substance detected. The limits of detection of the drugs investigated shows that, only in some cases, is the detection better after the plates were heated. Levels of detection of the phenolic drugs investigated were in the following ranges: for pyrocatechine 0.3–5.0 μg; for pentazocine 0.5–100 μg; for norepinephryne 0.6–100 μg; for niclosamide 0.8–50 μg; for salicylanilide 0.8–100 μg; for methyldopa 1.2–4.8 μg; for terbutaline 2.0–30 μg; for thymol 2.0–100 μg; for hexachlorophene 3.0–25 μg; for phloroglucinol 3.0–30 μg; for bamethane 10.0–100 μg; for eugenol 5.0–10 μg; and for ethamivan 40.0–100 μg. The most sensitive detections were obtained for: pyrocatechine with alkaline blue (**A**), brilliant green (**D**), helasol green (**K**) (300 ng); pentazocine with aniline blue (**B**) (50 ng); norepinephrine with bromothymol blue (**F**) (600 ng); niclosamide and salicylanilide with brilliant green (**D**) (800 ng); methyldopa with aqueous solution of bromocresol green (**M**) (1.2 μg).

The best detection reagents for the drugs investigated (being phenol derivatives) were: helasol green (**D**), as well as aniline blue (**B**). Therefore, these two visualizing reagents were used for densitometric research. Bamethane,

***Table 1.*** $R_F$ Values[a] and the Best Broadening Indexes ($I_{broad}$) [$\mu g/mm^2$][a,b] with Visualizing Reagents Selected for Phenolic Drugs Investigated

| Phenolic Drug | Drug Symbol | $R_F$ | $I_{broad}$ ($\mu g/mm^2$) | Visualizing Reagent |
|---|---|---|---|---|
| Methyldopa | MD | 0.523 | 67 | brilliant cresyl blue (**G**) |
| Norepinephrine | NP | 0.560 | 99 | alkaline blue (**A**) |
| Terbutaline | TB | 0.688 | 270 | aniline blue (**B**) |
| Bamethane | BM | 0.778 | 455 | brilliant green (**D**) |
| Ethamivan | EM | 0.874 | 345 | aniline blue (**B**) |
| | | | 303 | brilliant-cresyl blue (**G**) |
| Phloroglucinol | PG | 0.264 | 152 | bromocresol green (**M**) |
| | | | 143 | neutral red (**C**) |
| | | | 139 | brilliant green (**D**) |
| Pentazocine | PZ | 0.305 | 84 | bromophenol blue (**E**) |
| Hexacholophene | HP | 0.675 | 71 | brilliant green (**D**) |
| Pyrocatechine | PC | 0.721 | 62 | bromocresol green (**M**) |
| Niclosamide | NS | 0.811 | 278 | bromothymol blue (**F**) |
| | | | 270 | helasol green (**K**) |
| | | | 250 | thymol blue (**H**) |
| Salicylanilide | SA | 0.920 | 217 | alkaline solution of bromocresol green (**J**) |
| | | | 182 | aqueous solution of bromocresol green (**M**) |
| Thymol | TM | 0.962 | 135 | bromocresol green (**M**) |
| | | | 128 | helasol green (**K**) |
| Eugenol | EG | 0.356 | 68 | bromothymol blue (**F**) |

[a]Average, $n = 5$.
[b]Evaluation after heating at 120°C for 30 min.

terbutaline, methyldopa as well as hexachlorophene, salicylanilide, and niclosamide were detected with brilliant green (**D**), and then the densitometric analysis of chromatograms was determined. Terbutaline and norepinephrine were detected with aniline blue (**B**). For hexachlorophene (10.0 μg), salicylanilide (15.0 μg), and niclosamide (5.0 μg) an optimum wavelength of incident light $\lambda = 617.9$ nm was selected. Densitograms of hexachlorophene, salicylanilide, and niclosamide, after spraying with brilliant green (**D**), are shown in Fig. 1. For the path on which bamethane (100 μg), terbutaline (100 μg), and methyldopa (50 μg) are placed and detected by means of brilliant green (**D**), $\lambda = 380.5$ nm was selected as optimum wavelength. Densitograms for these three drugs are presented in Fig. 2. The peak derived from methyldopa has an irregular shape, suggesting that methyldopa has impurities, which (using the given mobile phase) could not be completely separated. For terbutaline

***Table 2.*** Detectability [μg], and Detectability Index ($I_{detec}$) [μg/mm$^2$][a] for Selected Phenolic Drugs[b] and Visualized with Reagents[b]

| Visualizing Agents[b] | | Symbol of Drugs[b] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MD | NP | TB | BM | EM | PG | PZ | HP | PC | NS | SA | TM | EG |
| **A** | Detectability[c] | – | 10 | 30 | 15 | – | – | 10 | 10 | 0.3 | 20 | 20 | 30 | 5 |
| | $I_{detec}$[d] | 4/37 | 10/36 | 30/25 | 15/39 | 100/39 | 5/25 | 5/31 | 10/75 | 0.3/10 | 20/33 | 20/58 | 30/19 | 5/62 |
| **B** | Detectability[c] | – | 2 | 20 | 10 | 50 | – | 0.5 | 3 | 0.4 | 7 | 7 | 5 | 5 |
| | $I_{detec}$[d] | 4/43 | 2/25 | 10/31 | 10/25 | 50/18 | 5/27 | 0.5/68 | 3/26 | 0.3/10 | 7/15 | 7/39 | 5/62 | 5/103 |
| **C** | Detectability[c] | – | – | – | – | – | 30 | 10 | 10 | 2 | 30 | 40 | 50 | – |
| | $I_{detec}$[d] | – | – | – | 100/117 | – | 30/33 | 5/21 | 10/86 | 2/77 | 30/65 | 40/51 | 50/38 | – |
| **D** | Detectability[c] | – | 4 | 20 | – | – | 5 | 10 | 10 | 1 | 0.8 | 0.8 | 2 | 5 |
| | $I_{detec}$[d] | 4/26 | 2/22 | 20/52 | 30/9 | – | 5/20 | 7/39 | 5/61 | 0.3/17 | 0.8/18 | 0.8/16 | 2/42 | 5/58 |
| **E** | Detectability[c] | 4 | 3 | 5 | 40 | 50 | 5 | 5 | 10 | 2 | 15 | – | 30 | 10 |
| | $I_{detec}$[d] | 4/19 | 3/10 | 5/10 | 40/52 | 40/83 | 5/18 | 5/25 | 10/43 | 2/26 | 5/15 | 100/68 | 10/22 | 10/56 |
| **F** | Detectability[c] | 4.8 | 0.6 | – | – | 50 | 20 | 4 | 5 | 5 | 10 | 30 | 20 | 5 |
| | $I_{detec}$[d] | 4.8/8 | 0.6/28 | 3/11 | 40/46 | 50/41 | 20/32 | 4/59 | 5/45 | 5/21 | 10/24 | 30/34 | 20/24 | 5/37 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **G** | Detectability[c] | 4.8 | 2 | 2 | – | 100 | 2 | 2 | 5 | 5 | 7 | 10 | 100 | 5 |
| | $I_{detec}$[d] | 4.8/14 | 2/18 | 2/17 | 40/30 | 50/27 | 2/23 | 2/60 | 5/66 | 1/28 | 7/39 | 10/28 | 100/199 | 5/76 |
| **H** | Detectability[c] | 3.2 | 10 | – | – | – | 8 | 5 | 10 | 4 | 50 | 100 | 20 | 10 |
| | $I_{detec}$[d] | 3.2/7 | 10/35 | 20/26 | 25/28 | – | 8/25 | 5/116 | 5/37 | 4/64 | 10/23 | 50/23 | 20/24 | 10/35 |
| **I** | Detectability[c] | 4 | 0.5 | – | – | 100 | 10 | 100 | 30 | 0.5 | 15 | – | 100 | 5 |
| | $I_{detec}$[d] | 4/27 | 0.7/5 | 1/16 | – | 50/16 | 3/21 | 100/200 | 10/85 | 0.5/10 | 15/67 | – | 100/152 | 5/97 |
| **J** | Detectability[c] | 4 | 1 | 20 | 40 | 50 | 8 | 5 | 10 | 2 | 5 | 60 | 100 | 5 |
| | $I_{detec}$[d] | 4/42 | 1/17 | 10/48 | 40/33 | 40/32 | 8/31 | 5/13 | 10/117 | 1/24 | 5/14 | 60/39 | 100/94 | 5/83 |
| **K** | Detectability[c] | 3.2 | 1 | – | – | 50 | 8 | 100 | 20 | 0.3 | 5 | 100 | 100 | 10 |
| | $I_{detec}$[d] | 3.2/9 | 1/20 | 2/26 | 30/35 | 50/34 | 8/25 | 100/219 | 10/71 | 0.3/11 | 5/15 | 80/84 | 100/78 | 5/127 |
| **L** | Detectability[c] | – | – | – | – | 100 | 5 | 100 | 20 | 2 | 50 | 70 | 100 | 5 |
| | $I_{detec}$[d] | 1.2/15 | 100/258 | 20/47 | 50/14 | 100/56 | 5/31 | 100/258 | 10/40 | 2/42 | 20/57 | 70/35 | 100/103 | 5/62 |
| **M** | Detectability[c] | – | 2 | – | – | – | 10 | 4 | 25 | 0.7 | 15 | 25 | 30 | 5 |
| | $I_{detec}$[d] | 1.2/18 | 2/27 | 10/25 | 50/15 | – | 10/24 | 2/84 | 25/44 | 0.7/19 | 15/30 | 25/19 | 30/25 | 5/58 |

[a]Average, $n = 5$.
[b]Codes as indicated in section "Experimental".
[c]Initial evaluation.
[d]Evaluation after heating at 120°C for 30 min.



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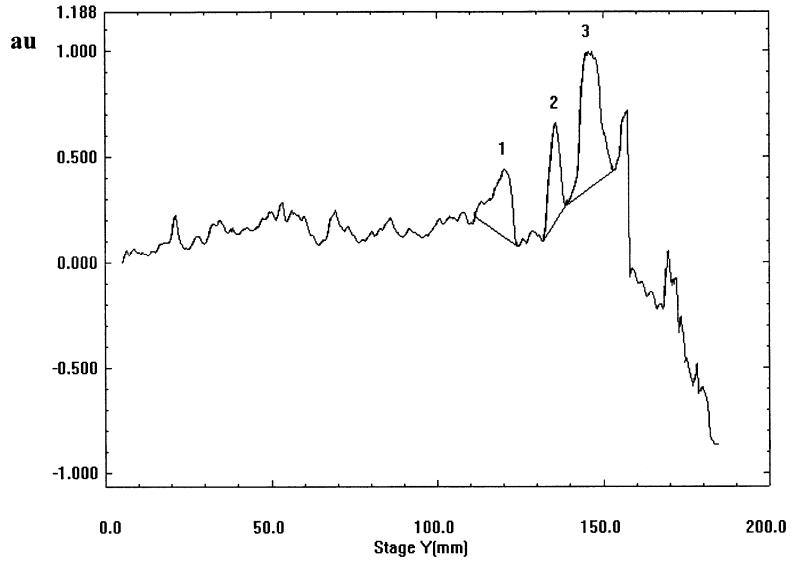


***Figure 1.*** Densitogram of hexachlorophene (1), niclosamide (2), and salicylanilide (3) after spraying with brilliant green (**D**).

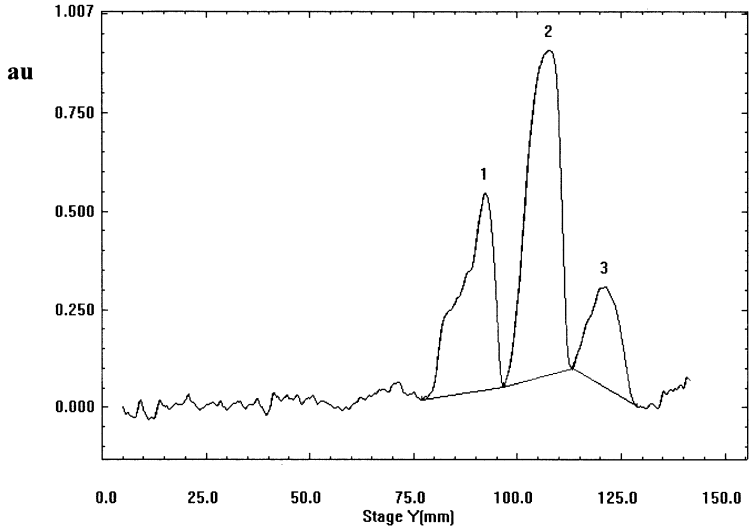


***Figure 2.*** Densitogram of methyldopa (1), terbutaline (2), and bamethane (3) after spraying with brilliant green (**D**).

(15.0 μg) and norepinephrine (5.0 μg), detected by means of aniline blue (**B**), an optimum wavelength for densitometric analysis, $\lambda = 371$ nm, was chosen. Densitograms obtained for terbutaline and norepinephrine are presented in Fig. 3. The densitograms shows that both aniline blue (**B**) and brilliant green (**D**) have good properties as visualizing reagents in relation to the substances detected. However, brilliant green (**D**), because of its properties, causes heterogeneity of adsorbent surface observed as noise. This effect is not observed for aniline blue (**B**).

Some of the visualizing reagents reported in this work can be used as new detection reagents for the qualitative determination of phenolic drugs. The colors of chromatographic spots of phenolic drugs investigated, obtained with selected reagents (including the best reagents – aniline blue (**B**) and brilliant green (**D**)) are presented in Table 3. These reagents can be used to identify compounds analyzed by TLC, based on $R_F$ values, and on the different colors of the chromatographic spots.



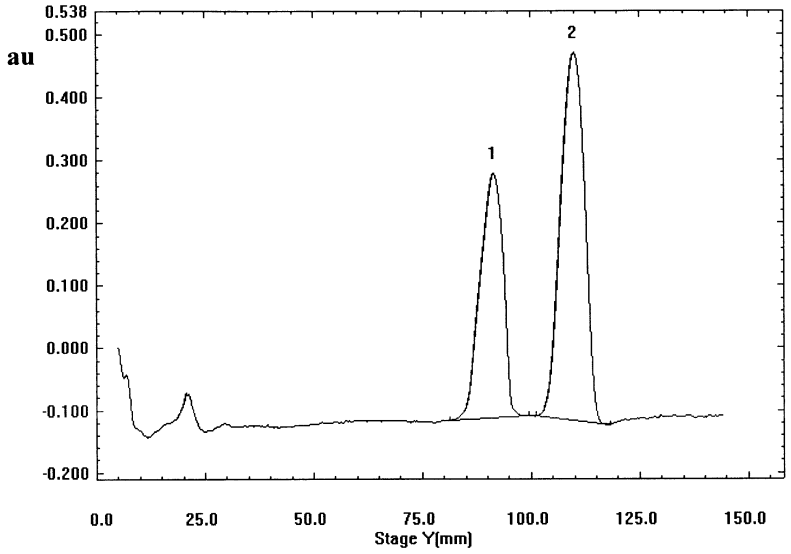


***Figure 3.*** Densitogram of norepinephrine (1) and bamethane (2) after spraying with aniline blue (**B**).

***Table 3.*** Description of the Chromatographic Spots with Selected Visualizing Reagents; Based on the Detection of 100 μg of the Phenolic Drugs

| Symbol of Drugs[a] | Visualizing Agents[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **B2**[b] | D2 | **E1**[c] | **F2** | **G2** | H2 | **J1** | **M2** |
| **MD** | light yellow | light yellow | brown | brown | brown | brown/ black | brown | light brown |
| **NP** | yellow/ brown | orange/ red/yellow | brown/ black | yellow brown | light brown | light brown | light orange/;red | brown |
| **TB** | yellow green | green | light green | green | light brown | yellow | white/ gray | light yellow |
| **BM** | light blue | white | light blue | light pink | light pink | light blue | white/ blue | white/ blue |
| **EM** | white/ blue | – | celadon | white/ blue | light yellow | – | light yellow | – |
| **PG** | yellow | yellow green | orange/ red | light yellow | light brown | gray | orange | orange |
| **PZ** | white | celadon | light yellow | light violet | white/ gray | white/ yellow | white/ blue | light violet |
| **HP** | light blue | green | white/ violet | blue | white/ blue | beige | blue | light yellow |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PC** | light brown | light orange/red | dark brown | light brown | brown | brown | brown/ black | brown | |
| **NS** | light blue | green | orange/ red/ brown | light brown | light yellow | light yellow | light brown | light yellow | |
| **SA** | white/ blue | green | – | light blue | light gray | light beige | light blue | white | |
| **TM** | blue | yellow green | light violet | blue | white blue | pink blue | white/ gray | white/ blue with white border | |
| **EG** | light blue | green | light pink | light yellow | light yellow | light brown | brown | light blue with white border | |
| Background | light blue | light green | dark violet | light blue | light blue | beige | light gray | light celadon | |

[a]Codes as indicated in section "Experimental."
[b]"2"-Evaluation after heating at 120°C for 30 min.
[c]"1"-Initial evaluation.

## CONCLUSION

The visualizing reagents proposed in this work, should provide a supplement to those used previously for the detection of phenolic drugs. The study also provides information about the physicochemical, analytical, and pharmaceutical importance of the new visualizing reagents proposed. Particular applications will have these visualizing reagents, with substances present in mixtures analyzed, give diversified colors of chromatographic spots. For quantitative research of phenolic drugs investigated, relatively good properties had aniline blue and brilliant green.

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