

## RESEARCH ARTICLE

Radka Vrancheva<sup>1</sup>  
Ivan Ivanov<sup>2,3</sup>  
Andrey Marchev<sup>2</sup>  
Atanas Pavlov<sup>2,3</sup>

## Qualitative and quantitative determination of protopine in *Fumaria spp.* by TLC-densitometry method

### Authors' addresses:

<sup>1</sup>Department of Analytical Chemistry, University of Food Technologies, 4000 Plovdiv, Bulgaria.

<sup>2</sup>Laboratory of Applied Biotechnologies, Institute of Microbiology "Stephan Angeloff", Bulgarian Academy of Sciences, 4000 Plovdiv, Bulgaria.

<sup>3</sup>Department of Organic Chemistry and Microbiology, University of Food Technologies, 4000 Plovdiv, Bulgaria.

### Correspondence:

Ivan G. Ivanov

Laboratory of Applied Biotechnologies, Institute of Microbiology "Stephan Angeloff", Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria.  
Tel.: +359 32 642430  
e-mail: ivanov\_ivan.1979@yahoo.com

### Article info:

Received: 25 October 2012

Accepted: 21 December 2012

### ABSTRACT

A rapid and accurate TLC-densitometry method for qualitative and quantitative determination of protopine has been developed. The best separation was achieved using a mobile phase chloroform – ethyl acetate – methanol – ammonium hydroxide (80:80:40:0.05, v/v/v/v). The results obtained by this method (CV% 3.4) corresponded well with those obtained by using a HPLC method. The reliability of the proposed method was proved through reproducibility test with alkaloid extracts from *Fumaria spp*

**Key words:** TLC, protopine, HPLC, *Fumaria officinalis* L., *Fumaria rostellata* Knaf.

## Introduction

Plants of the genus *Fumaria* have been used in the traditional medicine as anti-hypertensive, diuretics, hepatoprotectants and laxatives (to treat gastrointestinal disorders), as well as in the treatment of some skin diseases (rashes or conjunctivitis) (Suau et al., 2002a, 2002b). The biological activity of *Fumaria spp.* is mostly associated with the presence of isoquinoline alkaloids. The most important isoquinoline alkaloid is protopine (7-methyl-6,8,9,16-tetrahydrobis[1,3]benzodioxolo [4,5-c:5',6'-g]azecin-15(7H)-one). This alkaloid possess strong hepatoprotective activity (Rathi et al., 2008), inhibits histamine H1 receptors and platelet aggregation (Saeed et al., 1997), inhibits serotonin transporter and noradrenaline transporter and has an antidepressant effect (Xu et al., 2006), as well as antimicrobial, antiviral (Orhana et al., 2007) and anti-

inflammatory activities (Saeed et al., 1997). Several techniques have been used for its determination in plant extracts, including TLC (Tanahashi & Zenk, 1985; Hadjiakhoondi et al. 1999; Waksmundzka-Hajnos & Petruczynik, 2008), GC-MS (Suau et al., 2002a, 2002b; Maiza-Benabdesselam et al., 2007a, 2007b), HPLC (Soušek et al., 1999). But until now in the scientific literature has not been described TLC-densitometry method for quantitative determination of protopine from crude alkaloid extracts from *Fumaria spp.* TLC-densitometry is a suitable method for screening programs of huge number of samples selection of high-producing plant individuals because it is simple, faster and cheaper than HPLC and has appropriate sensitivity and reliability (Berkov & Pavlov, 2004).

This study describes a rapid TLC method for quantitative and qualitative determination of isoquinoline alkaloid protopine in total alkaloid extracts from different *Fumaria* species.

## RESEARCH ARTICLE

## Materials and Methods

### Chemicals and plant material

Protopine ( $\leq 95\%$ ) was obtained from Extrasynthese (France).

Aerial parts of *Fumaria officinalis* L. were collected from Sozopol and those of *Fumaria rostellata* Knaf. from Bucino village, near to Blagoevgrad, both in May 2012. Each sample was a mixture of material from several plants.

### Extraction of alkaloids

Intact plants were dried at room temperature and powdered. Samples of 150 mg of drug were moistened with ethanol (3x5ml) in an ultrasonic bath for 15 min. The combined extracts were concentrated under vacuum and dissolved in 2x2 mL of 3% sulfuric acid. The neutral compounds were removed by extraction (three times) with diethyl ether. The alkaloids were fractionated after basification of the extracts with 1 mL of 25% ammonia and extraction with chloroform (3x3 mL). The chloroform extracts were then dried over anhydrous sodium sulfate and evaporated to dryness.

### TLC-analysis

A standard solution containing 1 mg/mL of protopine was prepared in ethanol. Aliquots of the stock solution containing 5, 10, 15, 20, 25 and 30  $\mu\text{g}$  of standard were spotted together with 15  $\mu\text{L}$  of the samples of unknown alkaloid concentration onto silica gel aluminium plates (ALUGRAM SIL G, 20x20), (Macherey-Nagel, Germany) using different solvent systems: **1**) Ethyl acetate:methanol:ammonium hydroxide (170:20:10, v/v/v) (Hadjiakhoondi et al., 1999); **2**) Petroleum ether:diethyl ether:methanol (100:100:6, v/v/v) (Chelombiko et al., 1971); **3**) Chloroform:methanol:ammonium hydroxide (190:10:1, v/v/v) (Tanahashi & Zenk, 1985); **4**) Chloroform:ethyl acetate:methanol (80:80:40, v/v/v) (Nino et al., 2006); **5**) Chloroform:ethyl acetate:methanol:ammonium hydroxide (80:80:40:0.05, v/v/v/v). The alkaloids were visualized by triplicate spraying with Dragendorff reagent. Ten minutes after the final spraying, the plates were scanned at an optical resolution of 600 dpi using an HP Scanjet® 2200c. For the quantification of protopine was used QuantiScan® (version 2.1 Biosoft, Cambridge, UK) image analysis software. The protopine content of each sample of unknown alkaloid concentration was calculated from the peak areas of the densitogram by comparison with standard analyzed on the same plate.

### HPLC analysis

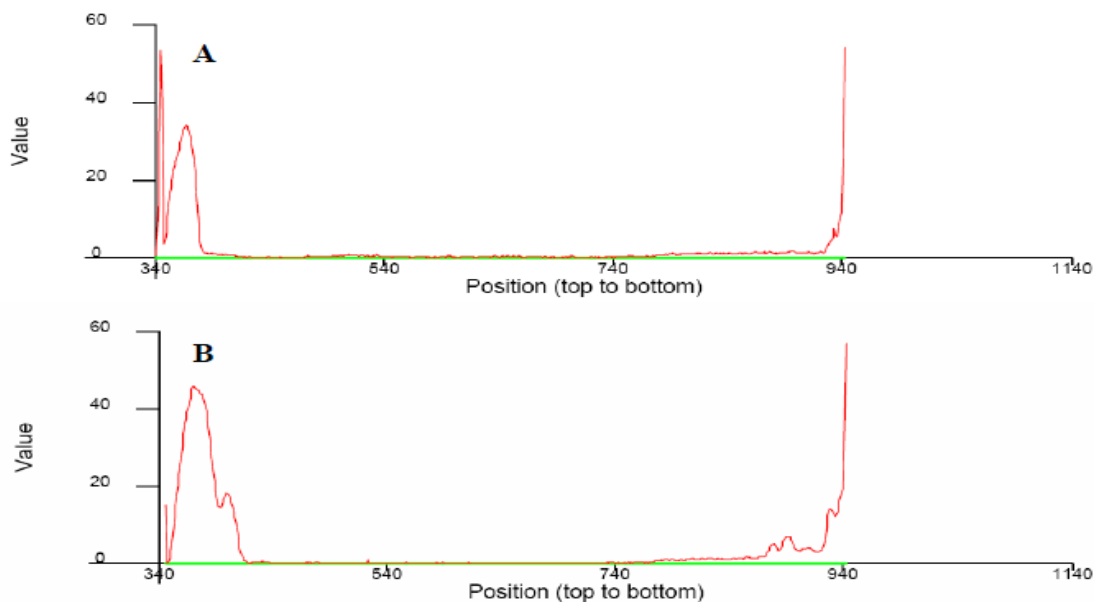
Protopine concentration was defined using Waters 1525 Binary Pump HPLC systems (Waters, Milford, MA, USA), Waters 2484 dual  $\lambda$  Absorbance Detector (Waters, Milford, MA, USA), equipped with Supelco Discovery HS C<sub>18</sub> column (5  $\mu\text{m}$ , 25 cm  $\times$  4.6 mm) and Breeze 3.30 software. Solvents for the preparation of the mobile phases were: **I**) 10.1 mL of triethylamine added to 1 L redistilled water, adjusted with H<sub>3</sub>PO<sub>4</sub> to pH 2.5 and **II**) acetonitrile. The mobile phases used were: **A**: 80% of **I** and 20% of **II** (v/v), and **B**: 40% of **I** and 60% of **II** (v/v). The mobile phase program was: 0–2 min 100% **A**, 2–15 min 77% **A** and 23% **B**, 15–30 min 60% **A** and 40% **B** (Soušek et al., 1999). UV detector was set at 290 nm and the volume of injection was 20  $\mu\text{L}$ . For analyses, protopine standard and the alkaloid fractions were dissolved in 1 mL 1 N HCl in ethanol and were injected into the HPLC system. All measurements were performed at 26°C and the mobile phase flowrate was 1.0 mL/min. Each determination was repeated five times.

## Results and Discussion

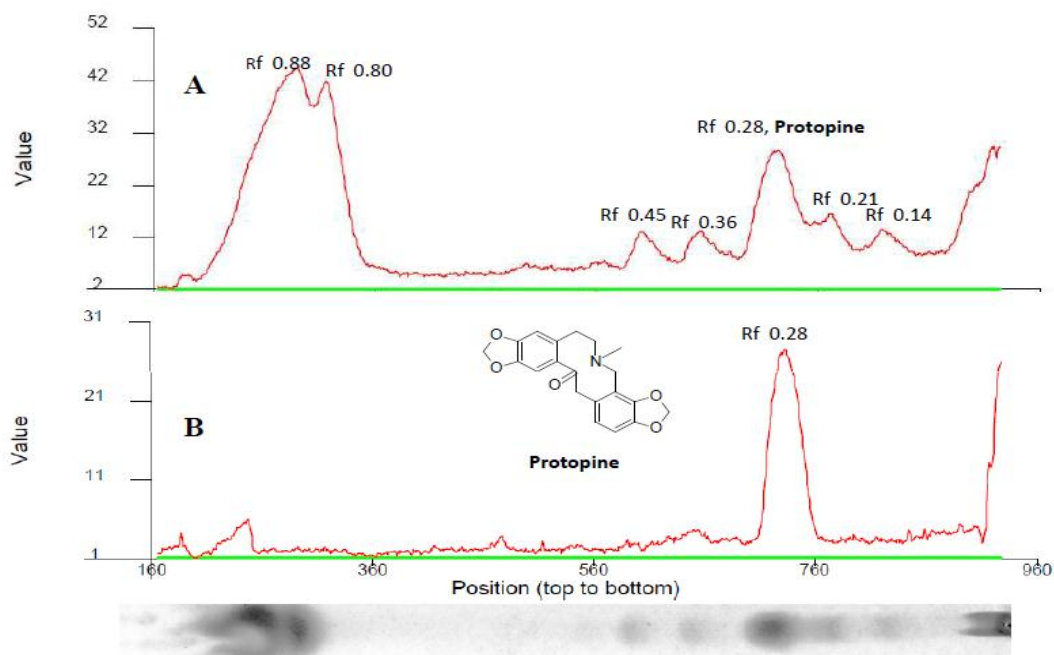
The aim of this work was to establish a TLC method for fast screening and selection of protopine-rich plants of the genus *Fumaria*. In our study we used a different mobile system. When were used the mobile phases **1** or **3**, there was no sufficient separation and all alkaloids were in one spot at the top of the densitograms (Figure 1). When were used the solvent systems **2** or **4**, the alkaloids were at the start line of the TLC-plate. The best results were obtained using following solvent system: chloroform:ethyl acetate:methanol:ammonium hydroxide (80:80:40:0.05, v/v/v/v), (Figure 2). In this case, 7 different alkaloids were separated with R<sub>f</sub> values of 0.14, 0.21, 0.28, 0.36, 0.45, 0.80, 0.88 (Figure 2). To improve the spot shapes and separation of isoquinoline alkaloids we basified mobile phase by adding an ammonium hydroxide in small concentration, because most of the them are strong bases. Priority of the developed TLC method was to perform separation of protopine from the mixtures of other accompanying isoquinoline alkaloids from *Fumaria* spp.

As a reference method for the quantification of protopine in this study was used HPLC. The calibration curve was linear in the concentration range 10–100  $\mu\text{g}/\text{mL}$  with correlation coefficient 0.998. Reproducibility of the proposed TLC method is presented in Table 1. Deviations for *Fumaria officinalis* L. and *Fumaria rostellata* Knaf. are 3.43 and 3.42.

## RESEARCH ARTICLE



**Figure 1.** Densitogram obtained from the alkaloid extracted from *Fumaria officinalis* L. on silica 60  $F_{254}$  with: **A** – solvent system **1** ethyl acetate:methanol:ammonium hydroxide (170:20:10, v/v/v); **B** – solvent system **3** chloroform:methanol:ammonium hydroxide (190:10:1, v/v/v).



**Figure 2.** Densitogram from chromatogram of the alkaloid extracted from *Fumaria officinalis* L (**A**) and the protopine (**B**) on silica 60  $F_{254}$  with solvent system **5** chloroform:ethyl acetate:methanol:ammonium hydroxide (80:80:40:0.05, v/v/v/v).

## RESEARCH ARTICLE

**Table 1.** Reproducibility test: determination of protopine in sample *F. officinalis* L. and *F. rostellata* Knaf. by TLC and HPLC methods.

| Number | <i>Fumaria officinalis</i> L. |       | <i>Fumaria rostellata</i> Knaf. |       |
|--------|-------------------------------|-------|---------------------------------|-------|
|        | TLC                           | HPLC  | TLC                             | HPLC  |
| 1      | 269.6*                        | 262.1 | 324.9                           | 341.6 |
| 2      | 274.6                         | 273.7 | 316.7                           | 310.8 |
| 3      | 282.6                         | 285.4 | 312.7                           | 330.7 |
| 4      | 261.2                         | 268.1 | 313.7                           | 335.0 |
| 5      | 260.7                         | 276.3 | 339.1                           | 354.3 |
| Mean   | 269.8                         | 273.1 | 321.4                           | 334.5 |
| ±SD    | 9.2                           | 8.7   | 11.0                            | 7.1   |
| CV%    | 3.4                           | 3.21  | 3.42                            | 2.13  |

Legend: \*µg protopine/g DW

These results corresponded well with those obtained by HPLC (3.21 and 2.13). The difference in qualitative determination of protopine between HPLC and TLC analysis is just  $9.7 \pm 0.1\%$  and consequently the TLC-method that has been developed was the reliability for fast screening of protopine and other isoquinoline alkaloids. The distinctions that were observed could be described to the variable conditions of spraying and to differences between the layers themselves. The spraying of the layers is of fundamental importance in obtaining reproducible results (Berkov & Pavlov, 2004).

The described TLC method is faster, cheaper and easier for the determination of protopine in comparison with HPLC. Moreover, this method can be applied not only in the laboratory, but also under field conditions for the rapid screening huge number plant individuals of wild populations of *Fumaria* spp.

## Acknowledgement

The work was supported by the Bulgarian Science Foundation, Bulgarian Ministry of Education, Youth and Science (contract DMU 03/77-2011).

## References

- Berkov S, Pavlov, A. 2004. A rapid densitometric method for the analysis of hyoscyamine and scopolamine in Solanaceous plants and their transformed root cultures. *Phytochem. Anal.*, 15(3): 141-145.
- Chelombitko VA, Muraveva DA, El Sawi Y. 1971. Protopine and allocryptopine from *Argemone mexicana*. *Khimiya Prirodnikh Soedineni.*, 2(208): 204.
- Hadjiakhoondi A, Morteza-Semnani K, Inanloo HR, Pirali-Hamedani M, Shafiee A. 1999. Angoline and other alkaloids from the roots of *Glaucium oxylobum* Boiss. and Buhse. *DARU.*, 7(3): 31-35.
- Maiza-Benabdesselam F, Khentache S, Bougoffa K, Chibane M, Adach S, Chapeleur Y, Max H, Laurain-Mattar D. 2007a. Antioxidant activities of alkaloid extracts of two Algerian species of *Fumaria*: *Fumaria capreolata* and *Fumaria bastardii*. *Rec. Nat. Prod.*, 1(2-3): 28-35.
- Maiza-Benabdesselam F, Chibane M, Madani K, Max H, Adach S. 2007b. Determination of isoquinoline alkaloids contents in two Algerian species of *Fumaria* (*Fumaria carpeolata* and *Fumaria bastardii*). *African J. of Biothech.*, 6(21): 2487-2492.
- Nino J, Hernández JA, Correa YM, Mosqueta OM. 2006. *In vitro* inhibition of acetylcholinesterase by crude plant extracts from Colombian flora. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, 101(7): 783-785.
- Orhana I, Özçelik B, Karaoğlu T, Sener B. 2007. Antiviral and antimicrobial profiles of selected isoquinoline alkaloids from *Fumaria* and *Corydalis* species, *Zeitschrift für Naturforschung C.*, 62(1-2): 19-26.
- Rathi A, Srivastava AK, Shirwaikar A, Rawat AKS, Mehrotra S. 2008. Hepatoprotective potential of *Fumaria indica* Pugsley whole plant extracts, fractions and an isolated alkaloid protopine. *Phytomedicine*, 15: 470-477.
- Saeed SA, Gilani AH, Majoo RU, Shah BH. 1997. Anti-thrombotic and anti-inflammatory activities of protopine. *Pharmacological research*, 36(1): 1-7.
- Soušek J, Guedon D, Adam T, Bochořáková H, Táborská E, Válka I, Šimánek V. 1999. Alkaloids and organic acid content of eight *Fumaria* species. *Phytochem. Anal.*, 10: 6-11.
- Suau R, Cabezudo B, Rico R, Nájera F, López-Romero JM. 2002a. Direct determination of alkaloid contents in *Fumaria* species by GC-MS. *Phytochem. Anal.*, 13: 363-367.
- Suau R, Cabezudo B, Rico R, López-Romero JM, Nájera F. 2002b. Alkaloids from *Fumaria sepium* and *Fumaria agrarian*. *Biochem. Syst. Ecol.*, 30: 263-265.
- Tanahashi T, Zenk MH. 1985. Isoquinoline alkaloids from cell suspension culture of *Fumaria capreolata*. *Plant cell Reports.*, 4: 96-99.

**RESEARCH ARTICLE**

- Waksmundzka-Hajnos M, Petruczynek A. 2008. TLC of isoquinoline alkaloids. – In: Waksmundzka-Hajnos M, Sherma J, Kowalska T. (eds), *Thin Layer Chromatography in Phytochemistry*, CRC Press, Taylor & Francis Group, p. 641-684.
- Xu LF, Chu WJ, Qing XY, Li S, Wang XS, Qing GW, Fei J, Guo LH. 2006. Protopine inhibits serotonin transporter and noradrenaline transporter and has the antidepressant-like effect in mice models. *Neuropharmacology* 50: 934-940.