Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 674–681

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Ultra high performance liquid chromatography tandem mass spectrometry analysis of quorum-sensing molecules of *Candida albicans*

Petr Greguš[a], Hana Vlčková[a], Vladimír Buchta[b], Jan Kestřanek[c], Lucie Křivčíková[b], Lucie Nováková[a,*]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Department of Clinical Microbiology, Faculty of Medicine in Hradec Králové, Charles University, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic
[c] Department of Obstetrics and Gynecology, Faculty of Medicine in Hradec Králové, Charles University, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic

ARTICLE INFO

Article history:
Received 7 April 2010
Received in revised form 28 May 2010
Accepted 28 May 2010
Available online 8 June 2010

Keywords:
Quorum sensing
Farnesol
Tyrosol
UHPLC–MS/MS
Bio-analytical method

ABSTRACT

Candida albicans is generally one of the most commonly isolated fungal pathogen from human body. It is a frequent cause of nosocomial infections, bloodstream infections, urinary infections and mucosal infections of oral cavity and vagina *C. albicans* can grow as hyphae, pseudohyphae, or budding yeast. Morphological conversion of a yeast form to pseudohyphal or hyphal one is often characterized by the change of commensal status to an invasive form. Farnesol and tyrosol can participate in these transformation processes as quorum sensing molecules together with some physical–chemical factors.

A new analytical method for identification and quantification of biologically active substances farnesol and tyrosol using ultra high performance liquid chromatography (UHPLC) in connection with tandem mass spectrometry was developed. The analytes were separated on Acquity BEH C18 analytical column using binary mobile phase consisting of acetonitrile and formic acid 0.075% (75:25) at flow-rate 0.20 ml/min. SRM (selected reaction monitoring) mode was applied in order to ensure sufficient selectivity and sensitivity using the first most intensive transition as a quantitative (121 > 77 and 205 > 121) and second one for the confirmation purposes (121 > 93 and 205 > 109). The method was validated in terms of linearity (>0.9994), precision (0.5–3.8% RSD), accuracy (78.9–106.0%), LOD (limit of detection) and LOQ (limit of quantitation). The method can serve as an analytical tool for the detection and determination of quorum-sensing molecules in biological samples.

1. Introduction

The polymorphic fungus *Candida albicans* is one of the most important yeast in medicine. It is a member of the indigenous microbiota of mucosa and skin in humans and animals and is thought to be acquired during passage through the birth canal. *C. albicans* has been recently used as a model for studying the basic biology of fungi as well. For fungi exhibiting yeast–mycelium dimorphism the dependence of cell morphology on initial cell density has been termed an inoculum size effect. The inoculum size effect in the dimorphic yeast results from the production of quorum-sensing molecules (QSMs). The QSMs indentified in *C. albicans* are farnesol, farnesic acid, and tyrosol [1–6].

QSMs are extracellular chemical signals, and are produced continuously in response to increasing density of microbial population to coordinate action of the cells. Their production is usually not dependent on the type of carbon source or nitrogen source or on the chemical nature of the growth medium. In general, these signals can regulate some important virulent, morphological, and physiological properties through activation of proper genes. Majority of studies concern QSM of bacteria [7,8], but there is a growing number of reports about QSM in fungi, especially yeasts. The main two QSMs in *Candida* under study are farnesol and tyrosol, their structures are shown in Fig. 1. While farnesol blocks the yeast-to-mycelial dimophic transition of *C. albicans*, tyrosol supports the development of filamentous form of this yeast [5,6,9].

Originally, analytical methods for the determination of farnesol and tyrosol were developed separately. Farnesol was first discovered to be QSM of *C. albicans* [1], while tyrosol function in the QS was described later [2]. It would be however highly convenient to develop one analytical method being able of simultaneous determination of farnesol and tyrosol, because such method would give direct information about the concentrations of both QSM independently of sample origin and thus enable better identification of cell morphology and description of invasive stadium. This could be helpful in diagnostic and treatment approaches, e.g. in vaginal candidosis.

Farnesol is a volatile molecule of terpenoid structure, therefore GC–MS (gas chromatography with mass spectrometry detection) was often a method of choice for its determination [1,10,11]. Both

* Corresponding author. Tel.: +420 495067381; fax: +420 495067164.
E-mail address: nol@email.cz (L. Nováková).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.05.029

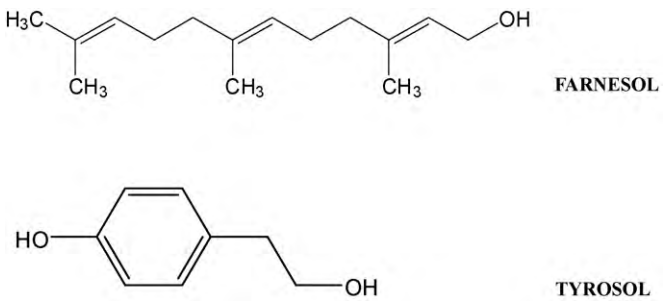

Fig. 1. Chemical structures of farnesol and tyrosol.

chemical ionization and electron ionization were employed. These methods however were developed only for qualitative purposes to confirm a presence of farnesol molecule in various matrices including *C. albicans* strains [1], *Mycastor coypus* anal scent glands [10] or tobacco smoke [11]. Further, HPLC–UV (high performance liquid chromatography with ultra-violet detection) was used for the identification purposes using retention times [1], however in complicated matrices this approach might miss sufficient selectivity. Further, farnesol metabolites (farnesyl-glucuronide) and farnesol with other related compounds were determined using HPLC in connection with ESI-MS (electrospray ionization mass spectrometry) [12,13]. Ionization was typically performed in negative ion mode. $[M-H]^-$ served as a precursor ion for further MS/MS quantitation of farnesol or its glucuronide. According to our knowledge, only one method was developed for quantitative purposes and fully validated for the determination of farnesol in rat liver and testis [12]. ESI-MS/MS detection is highly advantageous from the selectivity and sensitivity point of view. However, the procedure reports a necessary derivatization step in order to reach sufficient sensitivity, which complicates the method and makes the application time-consuming. No fast, simple and sensitive method for the qualitative and quantitative analyses of farnesol was found in the literature. An overview of analytical methods used for the determination of farnesol and its related compounds is shown in Table 1.

Tyrosol was often determined with other phenolic compounds in virgin olive oils [14,15] or in human LDL-fraction after digestion of olive oil [16,17] or in olive mill wastewaters [18]. HPLC using UV (typically 280 nm) or MS detection was a method of choice [14–22]. MS detection is unequivocally more convenient, because it provides better sensitivity and selectivity especially in complicated matrices. Most of HPLC methods were developed and fully validated for quantitative purposes using ESI-MS/MS which provides very high selectivity in complicated matrices [14,16–18]. ESI was performed in negative ion mode using $[M-H]^-$ as a precursor ion for further SRM (selected reaction monitoring) quantitation. Typical sensitivity of such methods reached LOQ (limit of quantitation) around 1 ng/ml levels. Other methods for the determination of tyrosol employed GC–MS with EI [21] or MEKC (micellar electrokinetic chromatography) [22]. An overview of analytical methods used for the determination of tyrosol and its related compounds is shown in Table 2.

The aim of this work was to develop fast, reliable, sensitive and selective analytical method for the simultaneous determination of farnesol and tyrosol as quorum-sensing molecules of *C. albicans* using UHPLC–MS/MS method. While tyrosol was quite successfully evaluated in terms of quantity using specific ESI-MS/MS methods (although not all method provided all quantitative and validation results), mostly only qualitative analysis of farnesol was typically performed. According to our knowledge, simultaneous determination of farnesol and tyrosol has never been performed before. Analytical approaches employed various instrumentations for the analysis of the two analytes. GC was often used for the analysis of

Table 1
An overview of analytical methods used for the determination of farnesol and its relative substances.

Determined substances	Matrix sample prep.	Method/stationary phase	Mobile phase	Detection	Validation data	Analysis time	Ref.
Farnesol	*Candida albicans* Extraction by ethyl acetate	**HPLC**-RP C18 5 μm 4.6 mm × 250 mm	MeOH:H_2O (4:1)	UV 210 mm	Qualitative method	NA	[1]
Farnesol	*Candida albicans* Extraction by ethyl acetate	**GC**-DB5 column 30 m	NA	EI-MS CI-MS	Qualitative method	11 min	[1]
Farnesol and isomers Esters of farnesol	*Mycastor coypus* - anal scent glands	**GC**-ZB-FFAP Coated capillary column 30 m × 0.25 mm, 0.25 μm	Helium	EI-MS CI-MS	Qualitative method	NA	[10]
	Flash chromatography	XTI-5 coated capillary col 5 m × 0.25 mm, 0.25 μm		IR			
Farnesol Farnesylacetone Farnesyl-methylether	Tobacco smoke Extraction by hexane	**GC**-capillary column (122-5562, DB-5MS 5% phenyl 95% methyl, 15 m × 0.20 mm, 0.33 μm film)	NA	EI-MS	Qualitative method	NA	[11]
Farnesol, geranyl-geraniol	Rat liver and testis LLE	**HPLC**-Symmetry Shield C8 5 μm 2.1 mm × 150 mm IS = n-pentadecanol	ACN, 10 mM ammonium acetate, acetic acid (90:10:0.1)	ESI-MS/MS $[M-H]^-$ derivatization	rec = 108–111% LOQ = 0.15 ng/g LOD = 0.05 ng/g	8 min	[12]
Farnesyl-glucuronide	Liver microsomes Sonication	**HPLC**-Spherisorb ODS 5 μm 2.1 mm × 150 mm	A: 0.1% formic acid B: ACN + 0.1% formic acid, gradient elution	ESI-MS/MS $[M-H]^-$	LOD = 30 fmol	7 min	[13]

Abbreviations: MS, mass spectrometry; ESI, electrospray ionization; EI, electron ionization; CI, chemical ionization; ACN, acetonitrile; MeOH, methanol; IS, internal standard; rec, recovery; LLE, liquid–liquid extraction; NA, data not available.