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Short communication

Composite glycerol/graphite/aromatic acid matrices for thin-layer chromatography/matrix-assisted laser desorption/ionization mass spectrometry of heterocyclic compounds

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ABSTRACT

New composite matrices have been suggested for the analysis of mixtures of different synthetic organic compounds (N-containing heterocycles and erectile dysfunction drugs) by thin layer chromatography/matrix-assisted laser desorption ionization time-of-flight mass spectrometry (TLC/MALDI-TOF). Different mixtures of classical MALDI matrices and graphite particles dispersed in glycerol were used for the registration of MALDI mass spectra directly from TLC plates after analytes separation. In most of cases, the mass spectra possessed [M+H]+ ions; however, for some analytes only [M+Na]+ and [M+K]+ ions were observed. These ions have been used to generate visualized TLC chromatograms. The described approach increases the desorption/ionization efficiencies of analytes separated by TLC, prevent spot blurring, simplifies and decrease time for sample preparation.

1. Introduction

Thin-layer chromatography (TLC) plays an important role in qualitative and semi-quantitative analysis of wide range of compounds [1]. The main advantages of TLC are its versatility, cheapness and rapidity. However, this method possesses some disadvantages related to the identification of separated components: usually identification is carried out by low informative chemical and optical methods or using R_f (retardation factor) values.

On the other hand, "soft" ionization mass spectrometry methods became very useful in the last decades in the analysis of a variety of organic compounds. Among them electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the most popular [2,3]. The last one seemed to be a suitable method for the detection of analytes separated by TLC due to the possibility of their desorption/ionization directly from a TLC plate [4]. The main problem of this ionization method, which is based on

laser irradiation of co-crystallyzed matrix substances and analytes [5], is a low sensitivity. This may be due to a low concentration of an analyte on the adsorbent surface and low penetration of laser beam into TLC adsorbent. Another problem is that the application of MALDI matrices solutions on the developed TLC plates blurs analytes spots. A number of approaches, based mainly on the use of different solid particles (graphite, Co, TiO_2 and others [6,7]) has been suggested to prevent such bluring. Among them graphite is of interest in TLC/MALDI due to its high tendency of laser energy absorption. At the same time some polar liquids (glycerol, ethylene glycol, diethanolamine and other) have been tested to increase the analytes 'concentration' on a sorbent surface without spots blurring. For example, graphite particles suspensions in diethanolamine and glycerol were used in the analysis of peptides, oligosaccharides and proteins [8,9]. Crecelius and colleagues have presented very impressive results, using a suspension of graphite ($\sim 2\,\mu m$) in ethylene glycol for the analysis of some antibiotics by TLC/MALDI [10]. The aim of our work was further developing of such approaches to increase the efficiency of desorption/ionization of heterocyclic compounds and reproducibility of their TLC/MALDI mass spectra.

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R¹:3-OCH3, **4,5**-OH; **R²:3**-Ph,
4-o-methylphenyl, **5**-Me; **R³:3,4**-allyl,**5**-H

1   **2**

6   **R¹7**-Me, **8**-H   **R¹9**-Cl, **10**-H; **R² 9**-H,**10**-Me

R¹ 11-H, **12**-NH₂;
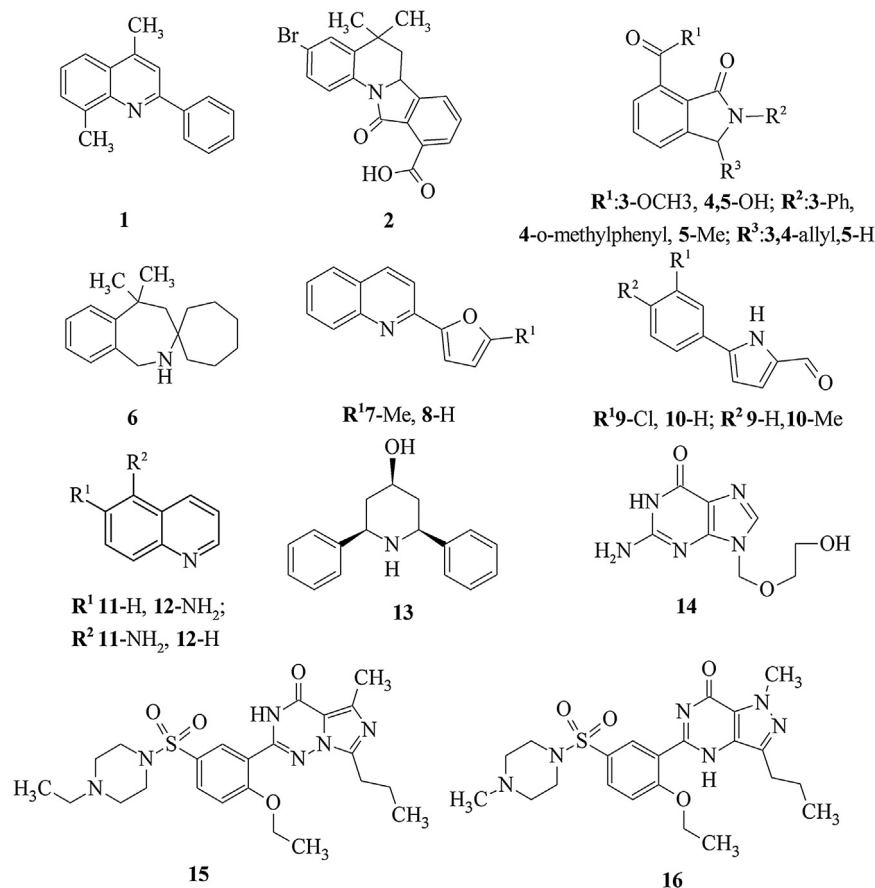**R² 11**-NH₂, **12**-H   **13**   **14**

15   **16**

Fig. 1. Structures of studied compounds.

2. Experimental

2.1. Analytes, solvents and materials

In the present work we analyzed different mixtures of nitrogen-containing heterocyclic compounds (1–14) synthesized and characterized as described in [11] (Fig. 1). Vardenafil and silde-

nafil standards (15,16) were commercially acquired from EDQM (France).

Traditional organic matrices for MALDI such as 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), 3β-indoleacrylic acid (IAA), dithranol (AT) and glycerol were purchased from Fluka (Austria), Sigma (China), Aldrich Chemical Co. (Belgium) and Fluka (Austria) respectively. Graphite

Table 1
Retention factors, type of cationization, absolute intensities (I, arb.u.) and signal/noise ratios (S/N) for most abundant peaks in MALDI mass spectra of compounds 1–16 recorded using stainless steel target and IAA matrix (SST), TLC plate and IAA matrix (TLC-IAA), TLC plate and composite glycerol/graphite/IAA matrix (TLC-C).

Comp.	Rf	SST			TLC-C			TLC-IAA		
		Ion, m/z	I	S/N	Ion, m/z	I	S/N	Ion, m/z	I	S/N
1	0.89[a]	[M+H]⁺,234	$2.7*10^5$	1061	[M+H]⁺,234	$7.5*10^3$	39	[M+H]⁺,234	$9.1*10^4$	403
2	0.82[b]	[M+Na]⁺,408	$2.9*10^4$	175	[M+Na]⁺,408	$1.1*10^4$	158	[M+Na]⁺,408	$6.1*10^4$	206
3	0.12[a]	[M+H]⁺,308	$1.5*10^5$	585	[M+Na]⁺,330	$1.7*10^4$	163	[M+Na]⁺,330	$9.1*10^4$	386
4	0.83[b]	[M+H]⁺,308	$1.4*10^5$	576	[M+H]⁺,308	$2.7*10^3$	53	[M+H]⁺,308	$8.4*10^4$	401
5	0.68[b]	[M+H]⁺,192	$6.8*10^5$	1890	[M+Na]⁺,214	$3.5*10^3$	60	[M+Na]⁺,214	$3.5*10^5$	629
6	0.20[b]	[M+H]⁺,244	$5.3*10^5$	1794	–[d]	–	–	[M+H]⁺,244	$2.6*10^5$	370
7	0.67[a]	[M+H]⁺,210	$1.4*10^5$	608	[M+H]⁺,210	$1.7*10^3$	65	[M+H]⁺,210	$1.6*10^4$	217
8	0.64[a]	[M+H]⁺,196	$4.5*10^5$	1412	[M+H]⁺,196	$1.3*10^3$	74	[M+H]⁺,196	$1.1*10^4$	174
9	0.34[a]	[M+H]⁺,206	$5.3*10^4$	203	[M+H]⁺,206	875	18	[M+H]⁺,206	$3.8*10^4$	163
10	0.33[a]	[M+H]⁺,186	$2.4*10^5$	668	[M+H]⁺,186	$1.1*10^4$	91	[M+H]⁺,186	$7.6*10^4$	365
11	0.58[b]	[M+H]⁺,145	$3.3*10^5$	1169	[M+Na]⁺,168	$1.9*10^4$	89	[M+Na]⁺,168	$1.1*10^5$	482
12	0.53[b]	[M+H]⁺,145	$1.2*10^5$	421	[M+Na]⁺,168	$2.7*10^4$	78	[M+Na]⁺,168	$5.3*10^4$	102
13	0.19[a]	[M+H]⁺,254	$3.5*10^5$	709	[M+H]⁺,254	$1.6*10^3$	21	[M+H]⁺,254	$7.3*10^3$	34
14	0.10[b]	[M+H]⁺,226	$4.1*10^5$	1011	–[d]	–	–	[M+H]⁺,226	$2.2*10^4$	240
15	0.69[c]	[M+H]⁺,489	$2.3*10^5$	1507	[M+H]⁺,489	$1.1*10^4$	87	[M+H]⁺,489	$1.3*10^5$	821
16	0.69[c]	[M+H]⁺,475	$1.9*10^5$	1056	[M+H]⁺,475	$4.1*10^3$	67	[M+H]⁺,475	$4.5*10^4$	328

[a] Solvent A.
[b] Solvent B.
[c] Solvent C.
[d] No corresponding peaks were reagistered.