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ARTICLES

Combination of Planar Chromatography with Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry for the Analysis of Biologically Active Compounds

R. S. Borisov, D. I. Zhilyaev, C. A. Esparsa Sandoval, N. Yu. Polovkov, and V. G. Zaikin

Institute of Petrochemical Synthesis, Russian Academy of Sciences, Leninskii pr. 29, Moscow, 119991 Russia

e-mail: borisov@ips.ac.ru

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Abstract—MALDI mass spectra of 33 pharmacological substances recorded from standard metal targets using four different matrices (HABA, DHB, IAA and AT) have been obtained for the first time. They revealed only peaks for ions $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$ with different intensities. It has been shown that MALDI spectra of the compounds can be obtained as a result of the desorption/ionization of analytes from spots on thin-layer chromatogram (TLC) (silica stationary phase) in the presence of the same tested matrices. It has been found that graphite can be efficiently used as a matrix in combination of TLC with MALDI mass spectrometry, as it eliminates the danger of the broadening of spots on TLC plates in the treatment with matrix solution and reduces the time of sample preparation.

Keywords: MALDI mass spectrometry, combination of TLC with MALDI, pharmaceutical substances, graphite as a matrix

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INTRODUCTION

Planar (thin-layer) chromatography (**TLC**) occupies an important place in the qualitative and semiquantitative analysis of complex natural, pharmaceutical, medico-biological, and chemical samples. The main advantages of TLC are the rapidity of analysis, versatility, and the low cost of equipment. However, this method also possesses disadvantages, in particular, the identification of the separated components is usually performed by different optical and chemical methods and by R_f values (response factors), which rarely are informative. A much more efficient method of the determination of structures or the identification of analytes in these cases can be the use of mass spectrometry with desorption ionization, in particular, with matrix-assisted laser desorption/ionization (**MALDI**). The advantage of this approach is the possibility of analyte conversion into an ionized state directly from a TLC plate. The application of this methodology to the analysis of drug substances is of special interest, as it ensures the rapid evaluation of the purity of drugs, distinguishing stereoisomers, and the detection of fake drugs.

With understanding of the prospects of a combination of thin-layer chromatography with MALDI mass spectrometry, some scientists started to study complex mixtures using this combined method approximately 15 years ago. However, there are very few works of such kind, particularly dealing with the low-molecular-weight drug substances.

The first approaches to such combination were based on the separation of mixtures by thin-layer chromatography and followed by the study of the chromatographic spots by MALDI mass spectrometry (off-line mode). For example, in one of the earlier works [1], the products of a series of organic reactions preliminarily separated by TLC, as well as components of carbohydrates were identified by molecular masses using the data of the recorded MALDI mass spectra. In the present work, reaction mixtures were first separated by TLC (adsorbent silica 60 F_{254}), the corresponding spots with substances on the adsorbent were scraped from the plate, extracted with acetonitrile or diethyl ether, and the extracts were applied onto a target and analyzed by MALDI mass spectrometry.

In a relatively recent work [2], a similar approach was used for the identification of quaternary protoberberine alkaloids related to the isoquinoline class and possessing bactericidal, fungicidal, insecticidal, and antiviral activity. The work was performed using a mixture of berberine and palmatine as an example, and the studied spots together with a part of a plate were cut out and fixed on a target using an adhesive glue for recording mass spectra. The substances were extracted from the remained portion of the chromatogram with

ethanol and quantitatively analyzed by electrospray ionization mass spectrometry.

More interesting seems to be an approach, in which a developed chromatographic plate is used in a MALDI mass spectrometer instead of a target and mass spectra are measured for each chromatographic spot (on-line mode). In one of the first works of such type [3], this possibility was demonstrated on an example of the study of impurities in tetracycline. In a relatively recent work, it was shown on an example of the study of trace amounts of psychotropic medications of amphetamine series, ketamine, chlorpromazine, and morphine that TLC/MALDI mass spectrometry ensures the determination of the listed compounds at the level 0.05–5 ng [4].

Along with the development of a combined TLC/MALDI mass spectrometric method for the qualitative analysis of mixtures of low-molecular compounds, works on the implementation of this methodology into the quantitative determination of components have been started. For example, in [5], for the quantitative determination of components of drug piroxicam, an internal standard tenoxicam was used, which in certain concentration was added to the mobile phase in the TLC analysis. However, differences in the cocrystallization and the efficiency of ionization of the analyte and the internal standard in the case when their structures are strongly different is a substantial limitation of the method of quantitative analysis using an internal standard. Probably the most reliable and accurate method of quantitative analysis can be based on the application of internal standards labeled by stable isotopes. This approach was used, for example, by Nicola et al. [6], who employed the D_3 analogue of cocaine as an internal standard for the quantitative determination of cocaine. For the study equal portions of solutions of cocaine and D_3-cocaine in methanol were mixed and the obtained mixture was applied on a TLC plate and chromatography was performed. After drying the chromatogram, the spot of interest was extracted with an aqueous solution of a mixture of methanol with acetic acid, and the extract was applied onto a MALDI target. Quantitative determination was performed by the ratio of peak intensities in the mass spectrum corresponding to the analyzed substance and the internal standard.

The most important problems arising in the combination of TLC with MALDI mass spectrometry are the selection of a matrix and of the most suitable method for mixing it with the analyte in the spot. Indeed, when a matrix solution is applied onto a plate, the broadening of the spot can occur. In a result, the cocrystallization of analyte with the matrix in such a broadened spot can be extremely nonuniform, which can result in substantial time consumption for the search for a region on the plate with satisfactory mass-spectrometric characteristics. One of solutions for this problem was proposed in [7] and consists in the treatment of the TLC plate with a solvent followed by its pressing to the target with a preliminarily applied matrix layer. As a result, the simultaneous extraction of analytes and their application onto the matrix occurs. A more optimum is the approach in which the whole TLC plate is coated with a matrix layer [3].

The published papers on the application of a combination of TLC with MALDI mass spectrometry to the analysis of pharmaceuticals are of occasional character. Therefore, we started a systematic study in this field trying to develop a general methodology. A wide range of drug substances with different structures and different functional groups were taken as test objects. In the present paper, we will discuss a study of the possibility to recording MALDI mass spectra of these substances directly from TLC plates, testing of the effect of various adsorbents for TLC on the results obtained, and the selection of the most suitable matrices and solvents. The next stage will include the development of a TLC method for the separation and analysis of these substances and the development of methods for their chemical modification (derivatization) to determine elements of structure and the nature of functional groups by MALDI mass spectrometry.

It should be noted that a combination of TLC with mass spectrometry based on other desorption ionization methods is also being developed. In particular, works on the application of DART (direct analysis in real time) mass spectrometry combined with TLC are performed [8].

EXPERIMENTAL

Used substances, solvents, and materials. Pharmaceutical substances bearing various functional groups (Aldrich Chemical Co., Belgium) were used in the work (Table 1).

2-(4-Hydroxyphenylazo)-benzoic acid (**HABA**) (99.8%, Fluka, Austria), 2,5-dihydroxybenzoic acid (**DHB**) (99.5%, Aldrich Chemical Co, Belgium), 3β-indoleacrylic acid (**IAA**) (99.0%, Sigma, China), and 1,8,9-anthracenetriol (**AT**) (99.0%, Fluka, Austria) were used as matrices.

Tetrahydrofuran (**THF**) was used to dissolve pharmaceutical substances and matrices.

For the registration of MALDI mass spectra, a steel target MTP ground steel (Bruker, Germany) containing 384 cells for the application of analytes with the matrix was used.

For the registration of MALDI mass spectra from TLC plates, commercially available plates with a fixed silica layer (Macherey-Nagel ALUGRAM Xtra Sil G unmodified silica 60) and an alumina layer (Macherey-Nagel, Alox-25) were tested.

A graphite matrix was obtained using a soft pencil.

Equipment. MALDI mass spectra were obtained by desorption/ionization from metal targets and TLC plates on a Bruker autoflex speed mass spectrometer equipped with a solid body UV-laser with $\lambda = 355$ nm

Table 1. Drug substances studied in this work

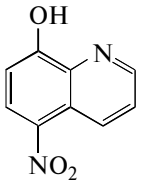
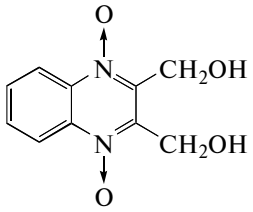
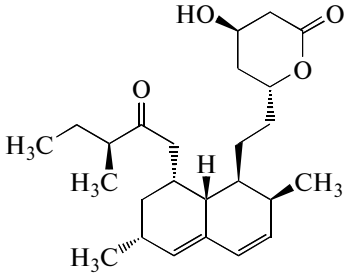
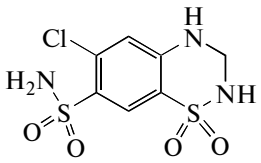
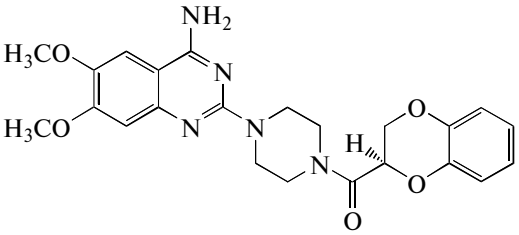
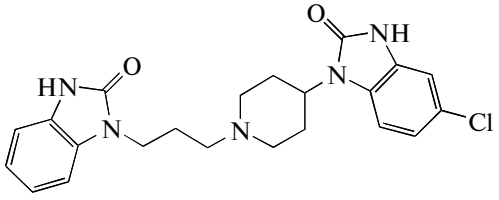
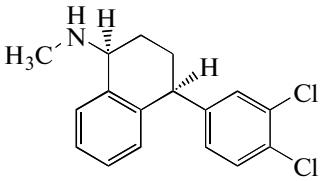
No. of compound	Chemical (brand) name	Structure	Empirical formula, molecular mass, Da
		Hydroxyl compounds	
1	5-nitro-8-quinolinol (nitroxoline)		$C_9H_6N_2O_3$ 190
2	1,4-di-*N*-oxide of 2,3-bis-(hydroxymethyl)-quinoxaline (dioxidine)		$C_{10}H_{10}N_2O_4$ 222
3	8-(2-[4-hydroxy-6-oxooxan-2-yl]ethyl)-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2-methylbutanoate (lovastatin /mecavor)		$C_{24}H_{36}O_5$ 104
		Compounds with amino group	
4	6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide, 1,1-dioxide (hypothiazide)		$C_7H_8ClN_3O_4S_2$ 297
5	1-(4-amino-6,7-dimethoxy-2-quinazolin-yl)-4-[(2,3-dihydro-1,4-bezodioxin-2-yl)carbonyl]piperazine (doxazosin)		$C_{23}H_{25}N_5O_5$ 451
6	6-chloro-3-[1-[3-(2-oxo-3*H*-benzimidazol-1-yl)-propyl]piperidin-4-yl]-1*H*-benzimidazol-2-one (domperidone)		$C_{22}H_{24}ClN_5O_2$ 425
7	4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine (sertraline)		$C_{17}H_{17}Cl_2N$ 305

Table 1. (Contd.)

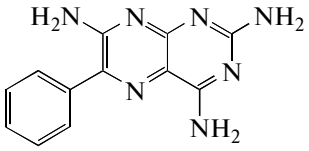
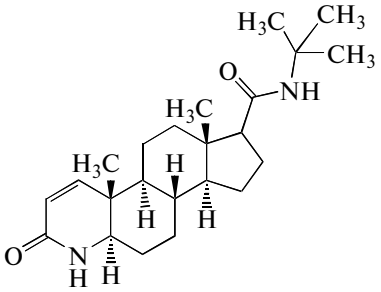
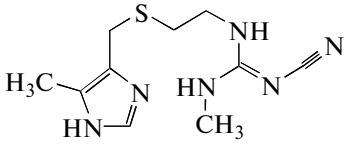
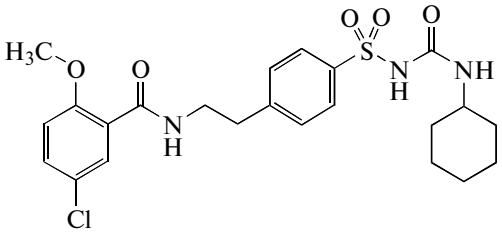
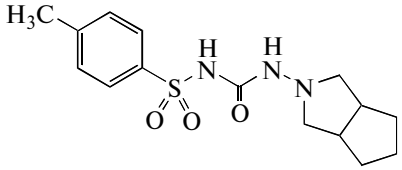
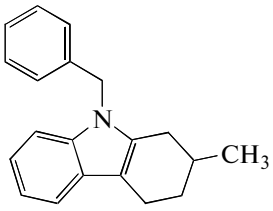
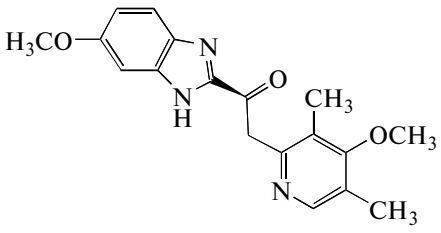
No. of compound	Chemical (brand) name	Structure	Empirical formula, molecular mass, Da
8	6-phenyl-2,4,7-pteridinetriamine (triamterene)		$C_{12}H_{11}N_7$ 253
9	*N*-*tert*-butyl-3-oxo-4-aza-5α-androst-1ene-17β-carboxamide (finasteride)		$C_{23}H_{35}N_2O_2$ 372
10	*N*-cyano-*N*'-methyl-*N*''-(2[[(5-methyl-1*H*-imidazol-4-yl)methyl]-thio]ethyl)guanidine (cimetidine)		$C_{10}H_{16}N_6S$ 252
11	5-chloro-*N*-(4-[*N*-[cyclohexylcarbonyl)sulfonyl]phenethyl)-2-methoxybenzamide (glibenclamide)		$C_{23}H_{28}ClN_3O_5S$ 493
12	*N*-[[(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-yl)-amino]carbonyl]-4-methylbenzenesulfonamide (gliclazide)		$C_{15}H_{21}N_3O_3S$ 323
13	9-benzyl-2-methyl-2,3,4,9-tetrahydro-1*H*-bcarboline (diazoline)		$C_{19}H_{20}N_2$ 276
14	6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1*H*-benzimidazole (omeprazole)		$C_{17}H_{19}N_3O_3S$ 345

Table 1. (Contd.)

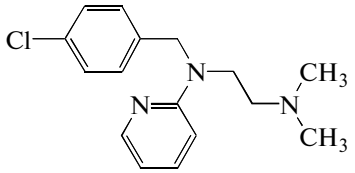
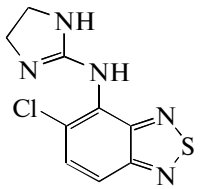
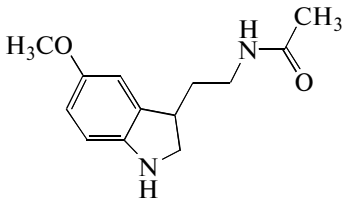
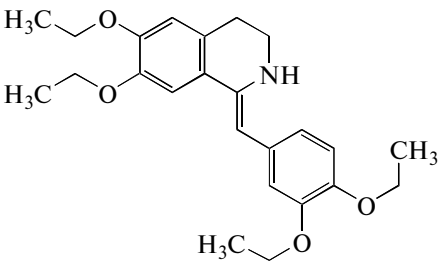
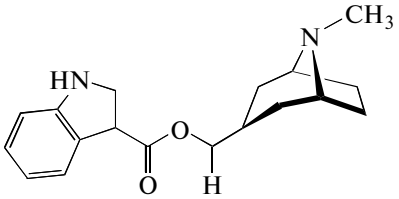
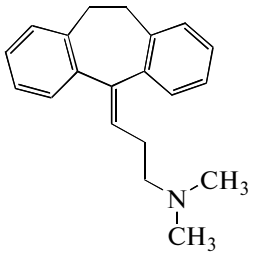
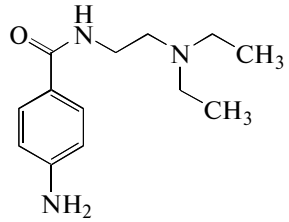
No. of compound	Chemical (brand) name	Structure	Empirical formula, molecular mass, Da
15	*N*,*N*-dimethyl-*N*-4-chlorophenyl)-*N*-(2-pyridyl)ethylenediamine (suprastin)		$C_{16}H_{20}ClN_3$ 289
16	5-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-benzo[*c*][1,2,5]thiadiazol-4-amine (tizanidine)		$C_9H_8ClN_5S$ 253
17	*N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]ethanamide (melaton)		$C_3H_{16}N_2O_2$ 232
18	1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline (drotaverine, No-Spa)		$C_{24}H_{31}NO_4$ 397
19	(1a*H*, 5a*H*)-8-methyl-8-azabicyclo[3,2,1]octan-3a-yl ether of 1*H*-indole-3-carboxylic acid (tropisetron)		$C_{17}H_{20}N_2O_2$ 284
20	5-(3-dimethylaminopropylidene)-10,11-dihydrodibenzocycloheptene (amitriptyline)		$C_{20}H_{23}N$ 277
21	4-amino-*N*-[2-(diethylaminoethyl]benzamide (acetylprocainamide)		$C_{13}H_{21}N_3O$ 235

Table 1. (Contd.)

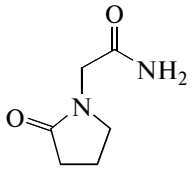
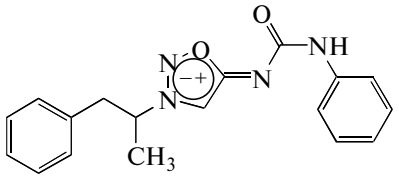
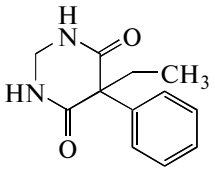
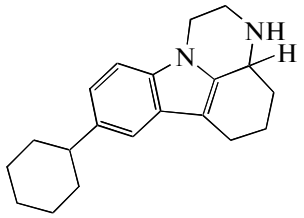
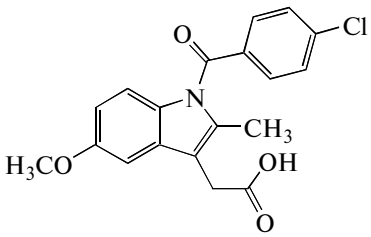
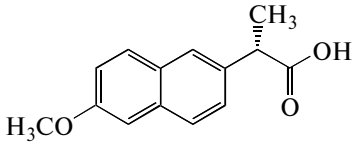
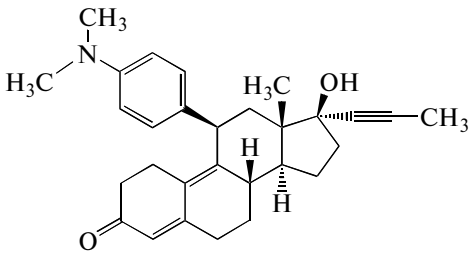
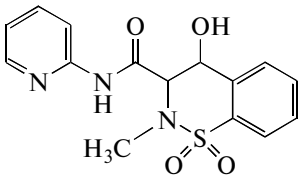
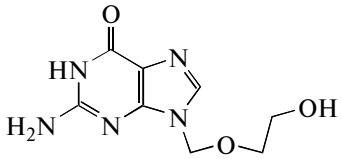
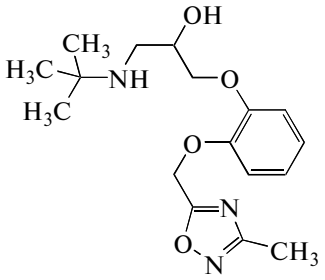
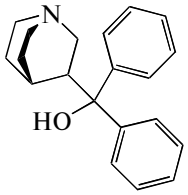
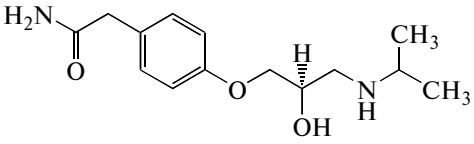
No. of compound	Chemical (brand) name	Structure	Empirical formula, molecular mass, Da
22	2-oxo-1-pyrrolidine-acetamide (piracetam)		$C_6H_{10}N_2O_2$ 142
23	5-(phenylcarbomoylimino)-3-(1-phenylpropan-2-yl)-5*H*-1,2,3-oxodiazol-3-ium-2-ide (sydnocarb)		$C_{18}H_{18}N_4O_2$ 322
24	5-ethyl-5-phenylhexahydropyrimidine-4,6-dione (haxamidine)		$C_{12}H_{14}N_2O_2$ 218
25	2,3,3a,4,5,6-hexahydro-8-cyclohexyl-1*H*-pyrazino(3,2,1-*j*,*k*)carbazole (tetrindole)		$C_{20}H_{27}ClN_2$ 330
Substances with –COOH group			
26	2-(1-[(4-chlorophenyl)-carbonyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetic acid (indomethacin)		$C_{19}H_{16}ClNO_4$ 357
27	6-methoxy-a-methyl-2-naphthaleneacetic acid (naproxen)		$C_{14}H_{14}O_3$ 230
Substances with various functional groups			
28	17β-hydroxy-11β-(4-dimethylaminophenyl)-17α(1-propynyl)estra-4,9-dien-3-one (mifepristone)		$C_{29}H_{35}NO_2$ 429

Table 1. (Contd.)

No. of compound	Chemical (brand) name	Structure	Empirical formula, molecular mass, Da
29	8-[hydroxy(pyridin-2-ylamino)methylidene-9-methyl-10,10-dioxo-10λ^6-thia-9-azabicyclo[4.4.0]deca-1,3,5-trien-7-one (piroxicam)		$C_{15}H_{13}N_3O_4S$ 331
30	2-amino-9-(2-hydroxyethoxy)methyl)-1*H*-purin-6(9*H*)-one (acyclovir)		$C_8H_{11}N_5O_3$ 225
31	5-[2]-*tert*-butylamino-2-hydroxypropoxy[phenoxymethyl]-3-methyl-1,2,4-oxydiazole (proxodolol)		$C_{17}H_{25}N_3O_4 \cdot HCl$ 371
32	α,α-diphenyl-3-quinuclidinemethanol (fencarol)		$C_{20}H_{23}NO$ 293
33	4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]benzeneacetamide (atenolol)		$C_{14}H_{22}N_2O_3$ 266

and a reflectron. The spectra were recorded in the positive ion monitoring mode. The maximum energy of the laser was 8 kJ/m^2.

For the visualization of spots in thin-layer chromatograms, illumination with an UV lamp in a CAMAG UV cabinet (Switzerland) was used.

Sample preparation. To obtain MALDI mass spectra from targets, the solutions of analytes (2 mg/mL) and matrices (30 mg/mL) were mixed in THF in the ratio 1 : 2 (v/v) in a separate vial, 1 μL of a mixture was applied onto a steel target and dried under a drying fan.

Prior to recording mass spectra from TLC plates, solutions of analytes in THF (2 mg/mL) were applied onto a TLC plate. After the evaporation of the solvent, the solution of a matrix (mg/mL) was sprayed on a plate, and the plates were kept in a vacuum desiccator for an hour.

When graphite matrix was used, the plate with the applied analyte after drying was placed in an UV cabinet, the contours of visualized spots were outlined with a graphite pencil, and graphite grid was applied over the whole spot.

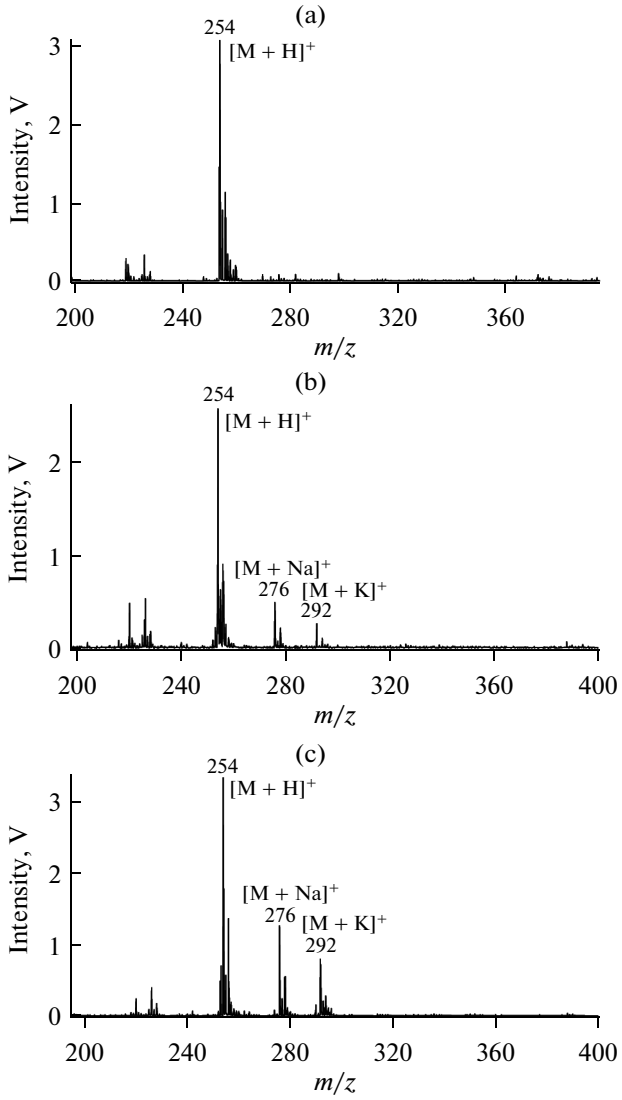


Fig. 1. MALDI mass spectra of tizanidine (**16**) recorded using a metal target and matrices (a) DHB; (b) TLC plates and DHB matrix; (c) TLC plates and graphite as a matrix.

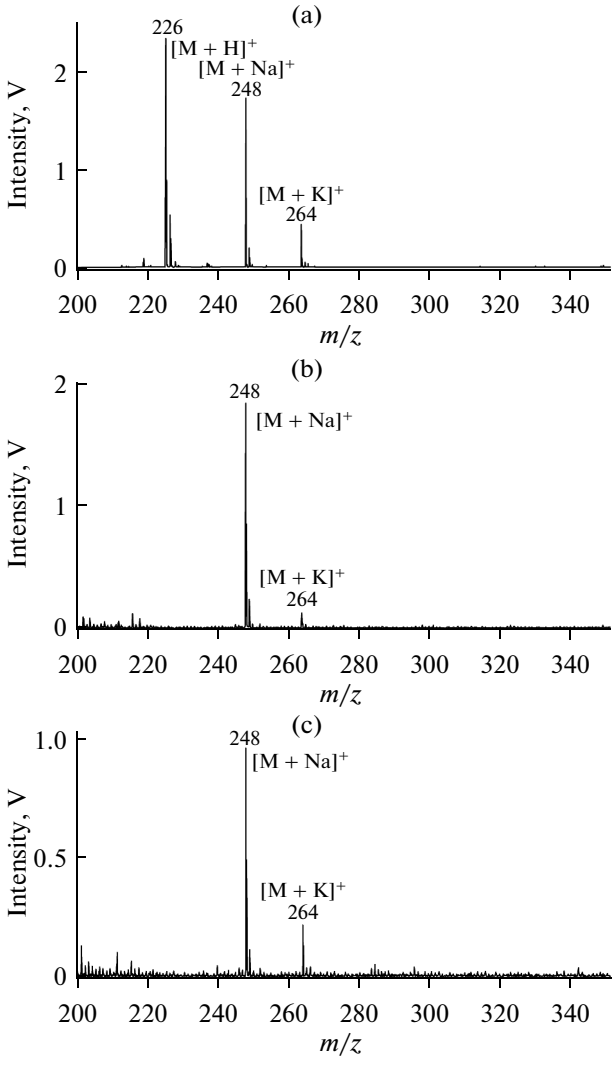


Fig. 2. MALDI mass spectra of acyclovir (**30**) recorded using a metal target and matrices (a) AT; (b) TLC plates and AT matrix; (c) TLC plates and graphite as a matrix.

RESULTS AND DISCUSSION

Possibilities of using a combination of TLC with MALDI mass spectrometry for the investigation and determination of drug substances were studied on an example of a big series of compounds bearing various functional groups: hydroxyl, carboxyl, and amino groups, as well as several groups of different nature. The structures and names of all compounds are presented in Table 1.

At the first stage of the work, we studied the ability of all selected compounds to undergo desorption/ionization in the presence of a matrix directly from standard metal targets used in MALDI mass spectrometry. Four matrices were tested: HABA, DHB, IAA, and AT. It was found that the main products of desorption/ionization recorded in MALDI mass spectra were the following ions: $[M + H]^+$ (protonated molecule) and adducts with sodium and potassium cations $[M + Na]^+$ and $[M + K]^+$ (Figs. 1a, 2a). Ions of the first type formed by the protonation of molecules because of the presence of a carboxyl or a weak acidic hydroxyl group in the matrix, as well as because of the potential presence of a substance in the form of a salt with an organic or an inorganic acid (most often with HCl). Ion adducts with sodium and potassium cations formed as a result of interaction with these ions washed from the glassware by the solvent used. The relationship of peak intensities of these three types of ions strongly depends on the nature of analyte, matrix used, and the presence of acid in the sample (for example, in the salt composition). The data of the recorded mass spectra are presented in the summarized Table 2 (peak intensity with the maximum height

Table 2. Relative peak intensities of protonated ($I_{rel, H}$) and sodium ($I_{rel, Na}$) and potassium ($I_{rel, K}$) cationized ions of analyte molecules obtained from steel targets using 1,8,9-anthracenetriol (AT), 2,5-dihydroxybenzoic acid (DHB), 2-indoleacrylic acid (IAA), and 2-(4'-hydroxyphenylazo)benzoic acid (HABA) as matrices

Compound	Matrix											
	AT			DHB			IAA			HABA		
	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$
1	0	0	0	100	83	33	0	0	0	0	0	0
2	100	8	0	100	9	0	0	0	0	100	20	0
3	0	100	14	0	100	41	0	100	6	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	100	23	3	100	16	0	100	9	4	100	4	0
6	0	0	0	100	83	3	63	100	16	100	15	4
7	100	44	0	100	0	0	100	15	19	100	0	29
8	0	100	0	0	0	0	100	25	9	100	5	15
9	100	83	6	100	72	10	13	100	10	13	100	10
10	0	100	90	0	100	29	31	100	38	25	100	33
11	0	31	100	0	100	13	0	100	13	13	80	35
12	100	45	4	100	43	8	28	100	23	100	50	9
13	100	0	0	100	0	23	100	0	0	100	0	0
14	0	60	100	0	0	0	0	0	0	100	0	0
15	100	0	0	100	0	0	100	0	0	100	0	0
16	100	0	0	100	0	0	100	0	0	100	0	0
17	100	75	8	8	1.5	0.5	73	100	27	0	100	14
18	100	0	0	100	0	0	100	0	0	0	0	0
19	100	0	0	100	0	0	100	0	0	100	4	10
20	100	0	0	100	0	0	100	0	0	100	0	4
21	100	0	0	100	0	0	100	0	0	0	0	0
22	0	0	0	0	100	19	0	0	0	0	100	17
23	100	87	3	73	87	100	100	16	0	0	0	0
24	0	0	0	0	0	0	0	0	0	50	100	17
25	0	0	0	0	0	0	0	0	0	100	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	100	0	0	100	0	0	100	0	0	100	0	0
28	0	0	0	100	21	18	56	100	25	85	2	0
29	0	0	0	0	0	0	0	0	0	100	0	0
30	100	63	33	100	13	9	45	100	31	31	100	25
31	0	0	0	0	0	0	0	0	0	100	27	0
32	100	0	0	100	0	0	100	0	0	100	0	0
33	100	0	0	100	18	7	100	12	0	100	7	3

in the spectra is taken for 100%). It should be noted that no peaks of fragment ions formed in the fragmentation of the above cations of analytes and matrices were observed in the MALDI mass spectra.

Among the studied compounds, only for compounds **4** and **26** we did not succeed to register mass spectra in any of the studied matrices. At the same time, for compounds **24** and **25** spectra were obtained only in the presence of HABA. It is interesting that, even in the presence of an AT matrix, the molecule of which does not contain carboxylic groups, sometimes $[M + H]^+$ ions were found. It is not improbable that phenolic hydroxyl groups in AT, which are of weak acidic character, can favor protonation of the analyte molecules. In some cases, the presence of an acid in a salt-like sample (for example, in chlorohydrates) can facilitate protonation.

Though in most of cases we could obtain MALDI spectra using various matrixes, in the general case, no dependence of the efficiency of ionization (which is determined by the energy of laser radiation and the yield of different ions) in the presence of the studied matrices on the structure of the studied substances or, at least, on the character of functional groups was found. At the same time, in each particular case it was necessary to select the most suitable matrix to obtain MALDI mass spectra.

The second stage of the work consisted in the study of the possibility of recording MALDI mass spectra directly from TLC plates. To evaluate the probability of the formation of background ions as a result of ionization of the chromatographic phase and to ensure the ionization of analytes, we studied the applicability of two phases, alumina and silica. It appeared that alumina was completely unsuitable as a stationary adsorption chromatographic phase in the combined use of TLC and MALDI mass spectrometry. The addition of any of the above matrices to an analyte on a TLC plate with alumina did not make possible the recording of peaks of ions with a significant signal-to-noise ratio, which is indicative of the impossibility or recording MALDI mass spectra using plates with this type of chromatographic phase.

At the same time, when silica was used as a stationary phase, peaks of cationized and protonated analyte molecules were observed in the mass spectra. Peaks of ions corresponding to ionized molecules of the chromatographic phase or to their fragments were not observed in the mass spectra (Figs. 1b, 2b). Because of this fact, TLC plates with a silica-based stationary phase, which in most cases ensures the registration of MALDI mass spectra were taken for the further studies.

An important obstacle should be mentioned. Initially the application of matrix solutions onto TLC plates was performed directly in the zone of analyte elution of determined under UV light. However, it was found that such application led to the repeated chromatography of analytes (in this case, the solvent in which the matrix was applied acted as a mobile phase), which was accompanied by spot broadening and irregular cocrystallyzation with the matrix. Therefore, to eliminate this phenomenon, we used a deposition method, in which the whole plate was coated with a uniform matrix layer. This approach ensures the recording of MALDI mass spectra for all of the studied compounds using various matrices.

In addition to the matrices tested at the first stage of the work (AT, DHB, IAA, and HABA), we also used graphite. Recently we showed [9] that graphite provides a quite efficient desorption/ionization of analytes from a TLC adsorbent. In this case, a spot in the TLC containing the adsorbed analyte was visualized under UV light and its contours were outlined by a soft graphite pencil. Additionally a graphite grid was applied onto the spot. A comparison of signal-to-noise ratios and laser energies necessary for obtaining a stable analytical signal demonstrated that the use of graphite as a matrix requires somewhat higher laser energies and the corresponding signal-to-noise ratio in most of cases decreased (Figs. 1c, 2c).

The analysis of the obtained mass spectra showed (Table 3) that, in the case of all tested matrices, peaks of ions associated with the ionization of analyte molecules through the attachment of a proton or a sodium or a potassium cation were recorded. When graphite was used, we could expect that only adducts with sodium and potassium cations formed, but not the $[M + H]^+$ ions, because there was no source of hydrogen in the system. In many cases this was the case. However, peaks of protonated molecules $[M + H]^+$ were also often detected in the spectra. Probably they formed because of the presence of an acid in the drug. Although it was possible that a molecule of a carboxyl-bearing analyte itself could be a donor of proton.

It should be emphasized that the use of graphite allowed us to substantially reduce the time of sample preparation. As it was mentioned above, an additional argument for the application of this matrix is the possibility of the prevention of the broadening of the analyte spot in its contact with the matrix solution. It should be noted that the results obtained using graphite as a matrix are comparable with the results obtained using organic matrices. Thus, the application of graphite in many cases can be more preferable, because it excludes problems arising in experiments with matrix solutions and is characterized by a simple sample preparation.

CONCLUSIONS

For the first time we obtained MALDI spectra of 33 drug substances, which were recorded using standard metal targets and four different matrices (HABA, DHB, IAA, and AT) and are characterized only by peaks of $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$ ions with different ratios of intensities. It was shown for the first time that MALDI mass spectra of these sub-

Table 3. Relative peak intensities of protonated ($I_{rel, H}$) and sodium ($I_{rel, Na}$) and potassium ($I_{rel, K}$) cationized ions of analyte molecules obtained from TLC plates using 1,8,9-anthracenetriol (AT), 2,5-dihydroxybenzoic acid (DHB), 2-indoleacrylioc acid (IAA), and 2-(4'-hydroxyphenylazo)benzoic acid (HABA) and graphite as matrices

Compound	Matrix														
	AT			DHB			IAA			HABA			graphite		
	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$	$I_{rel, H}$	$I_{rel, Na}$	$I_{rel, K}$
1	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0
2	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	100	0	0	0	0	0	0	0	0	0	0	100	82
4	0	0	0	0	0	0	0	0	0	0	0	0	100	16	29
5	100	2	0	100	5	0	100	50	15	100	0	0	100	29	16
6	0	0	0	0	100	0	100	30	73	0	96	100	26	100	86
7	100	9	11	0	0	0	0	0	0	0	0	0	100	0	0
8	100	11.4	0	55	100	0	100	11	0	0	0	0	0	100	69
9	100	0	0	33	100	29	0	0	0	0	0	0	19	100	18
10	100	0	0	0	100	50	24	24	100	0	0	0	0	100	22
11	0	0	0	0	0	0	0	0	100	0	13	100	0	73	100
12	47	100	27	0	100	25	0	100	54	0	0	0	0	7	100
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	100	36
15	0	0	0	0	0	0	35	100	0	0	0	0	100	0	0
16	100	84	36	100	18	11	100	72	0	100	31	0	100	32	25
17	0	0	0	0	100	27	11	100	34	0	44	100	0	100	50
18	100	0	0	80	100	31	100	11	7	0	0	0	100	40	21
19	0	0	0	0	0	0	0	0	0	0	0	0	100	11	23
20	0	0	0	0	0	0	0	0	0	95	73	100	100	35	37
21	0	0	0	0	0	0	0	0	0	0	0	0	51	100	73
22	0	32	100	0	0	0	0	0	100	0	0	0	0	67	100
23	0	0	0	0	0	0	0	0	0	0	0	0	100	23	5
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	100	37	28	100	44	0	50	100	0	100	22
27	0	0	0	0	100	36	0	100	33	36	60	100	0	100	82
28	100	89	0	0	100	0	74	56	100	0	0	0	0	100	0
29	100	0	0	0	100	46	23	0	100	0	11	100	0	0	0
30	100	59	0	50	100	0	0	100	9	0	0	0	4	100	20
31	100	0	0	0	0	0	100	0	0	0	60	100	0	0	0
32	100	11	0	0	100	30	8	100	57	0	50	100	0	83	100
33	100	6	1	30	100	14	0	0	0	0	0	0	0	0	0

stances can be obtained as a result of desorption/ionization from a TLC spot (silica stationary phase) in the presence of the same matrices. It was found that, for a combination of TLC with MALDI mass spectrometry, it is sufficient to use graphite as a matrix, which eliminates the risk of spot broadening in TLC in treatment with a matrix solution and reduces the time of sample preparation.

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