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New Matrix Compounds for the Detection of Carboxyl-Containing Nonsteroidal Anti-Inflammatory Drugs by MALDI Mass Spectrometry

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Abstract—The possibility of employing the method of mass spectrometry with matrix-assisted laser desorption/ionization (MALDI) for the rapid detection of carboxyl-containing nonsteroidal anti-inflammatory drugs has been studied. Comparison of the results obtained using the previously described and newly proposed as matrix compounds 4-(dimethylamino)benzaldehyde and *N,N*-dimethyl-*p*-phenylenediamine, showed that the latter provide the registration of mass spectra containing intense peaks of deprotonated molecules of all drugs used in the work. Comparison of the time required for their detection by various mass spectrometric methods has shown that MALDI mass spectrometry provides the highest performance in streaming analysis.

Keywords: nonsteroidal anti-inflammatory drugs, carboxyl-containing compounds, quality control, authenticity, detection, deprotonating matrices, MALDI mass spectrometry, negative ions

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that are widely used in clinical practice for rheumatic, neurological, traumatological diseases, as well as in cardiology as antiplatelet therapy. More than 30 million people worldwide take NSAIDs every day, including about 20% of patients receiving treatment in hospitals [1].

The group of NSAIDs includes drugs of various chemical structures with a different ratio of analgesic and anti-inflammatory effects. According to their chemical structure, NSAIDs have pronounced acidic or basic properties to varying degrees, depending on the presence of functional groups. The main drugs containing acidic and other functional groups include derivatives of salicylic, acetic, propionic acids, derivatives of pyrazolone and oxicams [1].

Today's Russian market for NSAIDs is predominantly monocomponent, with carboxyl-containing drugs such as diclofenac, meloxicam, ibuprofen, ketoprofen, and

ketorolac taking the leading position. Thanks to the import substitution campaign, the number of offers of domestically produced drugs has increased since 2016, however, market saturation is achieved through the release of not original, but already existing drugs [2]. In this regard, the problem of quality control of manufactured drugs is urgent, including control of the authenticity of drugs and the content of impurities in them. As the main methods for confirming the authenticity of NSAIDs, according to State Pharmacopoeia of the Russian Federation XIV ed. (GF RF XIV),¹ UV and IR spectroscopy are used. UV spectroscopy is considered

¹ General Monograph 2.1.0145.18. *Nimesulide*; General Monograph 2.1.0138.18. *Naproxen*; General Monograph 2.1.0107.18. *Ketoprofen*; General Monograph 2.1.0100.18. *Ibuprofen*; General Monograph 2.1.0022.15. *Ketorolac*; General Monograph 1.0094.18. *Diclofenac*. *The State Pharmacopoeia of the Russian Federation*, XIV ed., vol. 3, Moscow, 2018.

the most accessible method of analysis; however, it has a low information content, which reduces the reliability of the results obtained. The information content of the IR spectroscopy method is higher due to the possibility of obtaining a larger number of characteristic signals, absorption bands, which increases the level of identification reliability and allows for express streaming analysis. At the same time, the use of this method for confirming the authenticity of substances requires a laborious procedure for sample preparation of finished dosage forms (obtaining a pressed tablet with KBr) and their analysis. The identification of analytes is hampered by the presence of organic compounds (carbohydrates, polyalkylene glycols, derivatives of carboxylic acids, etc.), as well as by the presence of a shell that does not contain active substances.

Alternative methods of analysis can be various chromatographic methods, including thin layer (TLC),² gas (GC), and high performance liquid chromatography (HPLC) [3]. In the latter two cases, a variety of detector types are employed (flame ionization and katharometer for GC, ultraviolet spectrophotometric³ and light scattering for HPLC). It should be noted that by no means all of the methods mentioned are included in the articles regulating the quality control of NSAIDs by the GF RF XIV. TLC, the identification capabilities of which, in most cases, rely on the determination of the analyte retention factor and the fixation of the color change of the spot during specific derivatization, is the least informative in the mentioned list of analysis methods [4]. In order to reliably identify the separated components, it is advisable to use a combination of TLC with mass spectrometric detection (MS) [5]. However, this combination has not yet found wide application in the analysis of drugs, although there are works that describe its effective use of the. In particular, this method was used to determine NSAIDs in environmental [6] and in biological objects [7]. The main disadvantages of utilizing the method are the relative complexity of sample preparation and the selection of separation conditions [8].

A more rapid approach to confirming the authenticity of pharmaceutical substances and drugs is to utilize mass spectrometry with ionization at atmospheric pressure

or ambient ionization without the use of additional chromatographic separation. However, the use of most of the methods of ionization at atmospheric pressure (electrospray ionization, photoionization, etc.) for this purpose is complicated by pronounced matrix effects [9]. More convenient methods of ambient ionization (direct analysis in real time, desorption ionization by electrospray, etc.), which practically do not require preliminary sample preparation [10]. However, their widespread use in analytical practice is hindered by the rather low prevalence.

Another promising method for rapid confirmation of the authenticity of pharmaceutical substances is matrix-assisted laser desorption/ionization mass spectrometry (MALDI). Originally intended for the analysis of high molecular weight compounds, now this method is increasingly employed for the detection of small molecules, including biologically active substances [14]. Its great advantage consists in the possibility of using automated data collection algorithms when laser irradiating targets with a large number of analytes deposited on them (for example, standard targets for Bruker MALDI mass spectra allow 384 samples to be applied to them simultaneously).

One of the main obstacles to the widespread use of this method is the need to select matrix compounds for the analysis of specific classes of analytes in order to ensure the best sensitivity and sometimes selectivity of the method [12, 13]. The largest number of works in the field of studying organic compounds by MALDI mass spectrometry was carried out using the mode of registration of positively charged ions. Such ions are the most stable and, under MALDI conditions, are formed as a result of protonation or cationization of analyte molecules. In some cases, approaches are used that allow the formation of a positively charged fragment in a molecule in advance [14]. However, high sensitivity can also be achieved when registering negatively charged ions. As recent studies have shown, such ions can be easily generated in the case of acids and compounds with high acidity, if matrices with a high affinity for the proton are used for this purpose and, therefore, providing the proton elimination from the analyte molecule [15].

To date, only a few such deprotonating matrix compounds have been described. For example, it was proposed to use 9-aminoacridine [16] and 3-aminoquino-

² General Monograph 2.1.0107.18. *Ketoprofen. The State Pharmacopoeia of the Russian Federation*, XIV ed., vol. 3, Moscow, 2018.

³ General Monograph 2.1.0022.15. *Ketorolac. The State Pharmacopoeia of the Russian Federation*, XIV ed., vol. 3, Moscow, 2018.

line [17] for the detection of carboxylic acids, some phenols and acids.

The purpose of this work is to study the possibility of using some of the previously described and assess the potential of the new matrices we propose for the detection of carboxyl-containing nonsteroidal anti-inflammatory drugs by MALDI mass spectrometry.

EXPERIMENTAL

The characteristics of matrix compounds used in the work are listed in Table 1. The solvents utilized were methanol, ethanol, acetone, tetrahydrofuran (THF), acetonitrile (all of the above substances were produced by Reakhim, chemically pure grade), as well as acetonitrile (FizLabPribor, Russia, HPLC grade) for HPLC analysis. *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride (Sigma-Aldrich, cat. no. T6381 USA, derivatization grade, CAS 25561-30-2) was used.

Ibuprofen (**1**) was provided by the Center for Shared Use (scientific and educational center) of the Peoples' Friendship University of Russia in the form of a pharmaceutical substance. For the analysis, its solution in THF was prepared with a concentration of 10 mg mL⁻¹. Diclofenac (**2**), ketorolac (**3**), etodolac (**4**), dexketoprofen (**5**), indomethacin (**6**), ketoprofen (**7**), naproxen (**8**), tiaprofenic acid (**9**), nimesulide (**10**) were extracted from drugs in tablet form, purchased in the pharmacy store (Table 2).

Solutions of active substances **2–10** were prepared as follows: drugs purchased in the pharmacy store (Table 2) were ground in an agate mortar to powder; the powder was placed in 2 mL silanized glass vials, 1 mL of the corresponding solvent (Table 2) was added and kept in a shaker at room temperature for 60 min.

10 μL of each of the analyte solutions were mixed with 30 μL of a solution of the template compound in THF (30 mg mL⁻¹) and applied to an MTP 384 ground steel target plate (Bruker Daltonics Inc.). To record mass spectra from a nanostructured surface, solutions of analytes without adding a matrix were applied to an MSP 96 NALDI target (Bruker Daltonics Inc.).

The determination of the detection limits of compound **1** by MALDI mass spectrometry was carried out by sequential two-fold dilution of the analyte solution

(10 mg mL⁻¹) with subsequent registration of its mass spectrum using the corresponding matrix compound. When determining the detection limits of analytes, the minimum signal-to-noise ratio was taken as 3.

To estimate the analysis time required for the detection of analytes by GC/MS, 10 μL of *N,O*-bis-(trimethylsilyl)trifluoroacetamide was added to 100 μL of a solution of compound **1** in acetonitrile (2 mg mL⁻¹) and kept on a shaker for 5 min at 70°C. After silylation, 1 μL of the derivatized extract was injected into the GC injector.

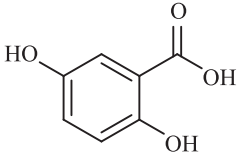
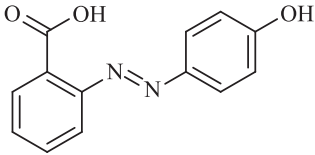
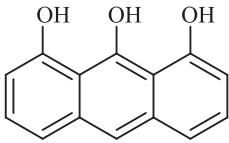
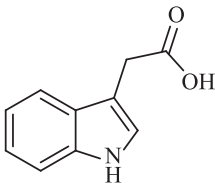
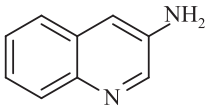
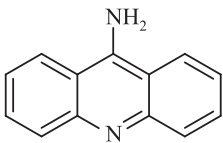
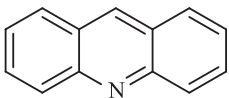
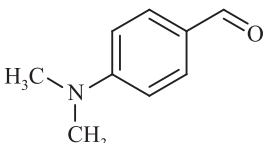
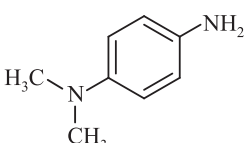
To estimate the analysis time required for the detection of analytes by HPLC/MS, a solution of compound **1** in acetonitrile (1 mg mL⁻¹) was filtered by a liquid chromatograph autosampler without using a chromatographic column.

MALDI mass spectra were recorded on a Bruker autoflex speed mass spectrometer (Bruker Daltonics Inc.) equipped with a solid-state UV laser with $\lambda = 355$ nm, in the mode of recording positive and negative ions with a reflectron. The maximum laser energy is 8 kJ m⁻². Electrospray ionization (ESI) mass spectra were recorded on a Shimadzu LCMS-8040 mass spectrometer (Shimadzu) equipped with a triple quadrupole mass analyzer in the negative ion recording mode. GC/MS analysis was carried out using a Thermo Focus DSQ II chromatomass spectrometer (Varian VF-5ms capillary column, 15 m long, 0.25 mm inner diameter, stationary phase layer thickness 0.25 μm, carrier gas helium). Operating mode: injector temperature 250°C, initial temperature of the chromatograph thermostat 45°C, then 1 min isotherm, heating at a rate of 15 deg min⁻¹ to 250°C. Operating mode of the mass spectrometer: ionization energy by electrons 70 eV, ion source temperature 250°C, scanning in the range of 10–800 Da at a speed of 1.5 scan s⁻¹, the resolution is unity over the entire mass range.

RESULTS AND DISCUSSION

As already noted, one of the main disadvantages of the MALDI mass spectrometry method is the need for empirical selection of matrix compounds. In this study, aimed at developing a method for the analysis of NSAIDs, we studied the possibility of using both traditional matrices (DHB, HABA, AT, IAA, AQ, AA) (Table 1) and the matrix compounds that we first tested (AC, DMABA, DMAPA). Since most NSAIDs are

Table 1. Matrix compounds used in the work

Structure	Name	Abbreviation
	2,5-Dihydroxybenzoic acid	DHB
	2-(4-Hydroxyphenylazo) benzoic acid	HABA
	1,8,9-Anthracetriol	AT
	3-Indole acrylic acid	IAA
	3-Aminoquinoline	AQ
	9-Aminoacridine	AA
	Acridine	AC
	4-(Dimethylamino)benzaldehyde	DMABA
	<i>N,N</i> -Dimethyl- <i>p</i> -phenylenediamine	DMAPA

low-molecular-weight compounds, products of their ionization appear in low mass range, in which intense peaks of products of ionization and fragmentation of matrix compounds are observed. Therefore, in

addition to MALDI, a method was investigated for analysis by surface-assisted laser desorption/ionization mass spectrometry using commercially available nanostructured surface targets (NALDI).

Table 2. Drugs and solvents used in the work and in the course of sample preparation

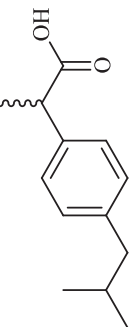
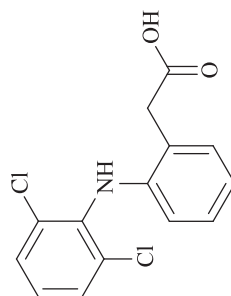
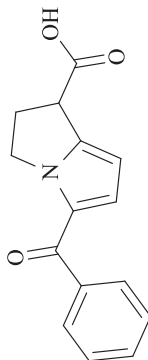
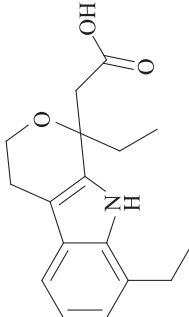
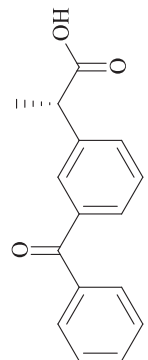
Active substance	Structure	M_w	Tradename	Description of the drug	Manufacturer of the drug	Solvent
Ibuprofen		206	Ibuprofen	Pharmaceutical substance	Hebei Chisure Biotechnology Co., Ltd (China)	Acetone
Diclofenac		296	Diclofenac	Enteric-film coated tablets, 25 mg of active substance per tablet	JSC "Sintez" (Russia)	Ethanol
Ketorolac		255	Ketanov®	Film coated tablets, 10 mg of active substance per tablet	S.K. Therapy S.A. (Romania)	Methanol
Etodolac		287	Nobedolac®	Film coated tablets, 400 mg of active substance per tablet	Nobel Ilach Sanayi ve Tijaret A.Sh. (Turkey)	Acetonitrile
Dexketoprofen		254	Dexalgin® 25	Film coated tablets, 25 mg of active substance per tablet	Berlin-Chemie AG/ Menarini Pharma HmbX (Germany/Italy)	Ethanol

Table 2. (Contd.)

Active substance	Structure	M_w	Tradename	Description of the drug	Manufacturer of the drug	Solvent
Indomethacin		357	Indomethacin Sopharma	Enteric-film coated tablets, 25 mg of active substance per tablet	Sopharma AD (Bulgaria)	Tetrahydrofuran
Ketoprofen		254	Ketoprofen Organica	Film coated tablets, 100 mg of active substance per tablet	JSC "Organika" (Russia)	Acetone
Naproxen		230	Nalgezin®	Film coated tablets, 275 mg of active substance per tablet	Krka, d. d., Novo mesto (Slovenia)	Ethanol
Tiaprofenic acid		260	Surgam®	Film coated tablets, 300 mg of active substance per tablet	Sanofi-Aventis (France)	Ethanol
Nimesulide		308	Nimesulide	Tablets, 100 mg of active substance per tablet	LLC "Atoll" (Russia)	Acetone

Table 3. Results of using various matrix compounds for recording the mass spectra of negative ions of the studied compounds^a

Compound	Matrix compounds								
	2,5-dihydroxy-benzoic acid	2-(4-hydroxy-phenyl-azo)benzoic acid	1,8,9-anthracene triol	3-indole acrylic acid	3-amino-quinoline	9-amino-acridine	acridine	4-(dimethyl-amino)-benz-aldehyde	<i>N,N</i> -dimethyl- <i>p</i> -phenylene-diamine
Ibuprofen	–	–	–	–	++	+	+	+++	++
Diclofenac	–	–	–	–	+	–	–	++	++
Ketorolac	–	+	–	+	+	–	–	++	+
Etodolac	–	+	+	–	++	–	–	+++	+++
Dexketoprofen	–	–	–	–	+	–	–	+++	++
Indomethacin	–	–	–	–	++	+	–	+++	++
Ketoprofen	–	–	–	–	+	+	–	+++	+++
Naproxen	–	–	–	–	+	–	–	+++	++
Tiaprofenic acid	–	–	+	–	++	+	–	+++	++
Nimesulide	–	–	+	–	++	++	+	+++	+++

^a “–” No peak of the deprotonated molecule in the mass spectrum. The peak of the deprotonated molecule is present in the mass spectrum, but “+” its intensity is less than the intensities of other ion peaks in the mass spectrum; “++” its intensity is comparable to the intensities of other ion peaks in the mass spectrum; and “+++” its intensity is significantly higher than the intensities of other ion peaks in the mass spectrum.

Registration of MALDI mass spectra of positively charged ions using all tested traditional matrix compounds and NALDI targets gave the expectedly unsatisfactory result: most of the mass spectra obtained did not contain signals corresponding to analytes. In cases where peaks of protonated (compound **2**) or cationized molecules (compounds **1**, **7**, **9**) were present in the mass spectra, the signal-to-noise ratio for them did not exceed 40. Obviously, polar carboxyl groups present in analyte molecules significantly reduces the probability of participation of analytes in ionization processes associated with protonation, and interaction of the carboxyl groups with metal cations leads to the formation of salts, rather than charged complexes.

On the contrary, the mass spectra of negatively charged ions in most cases contained noticeable peaks corresponding to the products of deprotonation of analyte molecules. However, for most matrices, such as DHB, HABA, AT, IAA, which usually provide the transfer of a proton to the analyte molecule, the intensity of the recorded peaks of negatively charged ions was at the noise level. A similar picture was observed for the case of matrixless NALDI, where transfer of a proton from weakly acidic silanol groups on the surface of nanowires is the main mechanism of ionization. Substantially better

results were obtained using deprotonating matrices: the intensity of the corresponding ion peaks and the signal-to-noise ratio were tens and hundreds of times higher. Comparative analysis of the data obtained showed that AQ and previously not described as matrix compounds DMABA and DMAPA are the most universal matrix compounds (Fig. 1, Table 3). It is quite obvious that such compounds have a high affinity for the proton, which ensures easy elimination of the acidic proton from the analyte molecule, therewith in the gas phase of the MALDI torch the $[\text{analyte} - \text{H}]^-/[\text{matrix} + \text{H}]^+$ equilibrium is significantly shifted towards the producing the negatively charged deprotonated molecules.

In the course of the work, examinations were carried out to establish the best matrix of the three mentioned. For compound **1**, the detection limits were determined by the serial dilution method: when using AQ as a matrix compound, it was 4×10^{-2} mg mL⁻¹, and when using DMABA and DMAPA, 1×10^{-3} and 2×10^{-3} , respectively.

It should be noted that the use of deprotonating matrices makes it possible to record MALDI mass spectra for NSAIDs that do not contain carboxyl groups. For example, the sulfamide group present in the

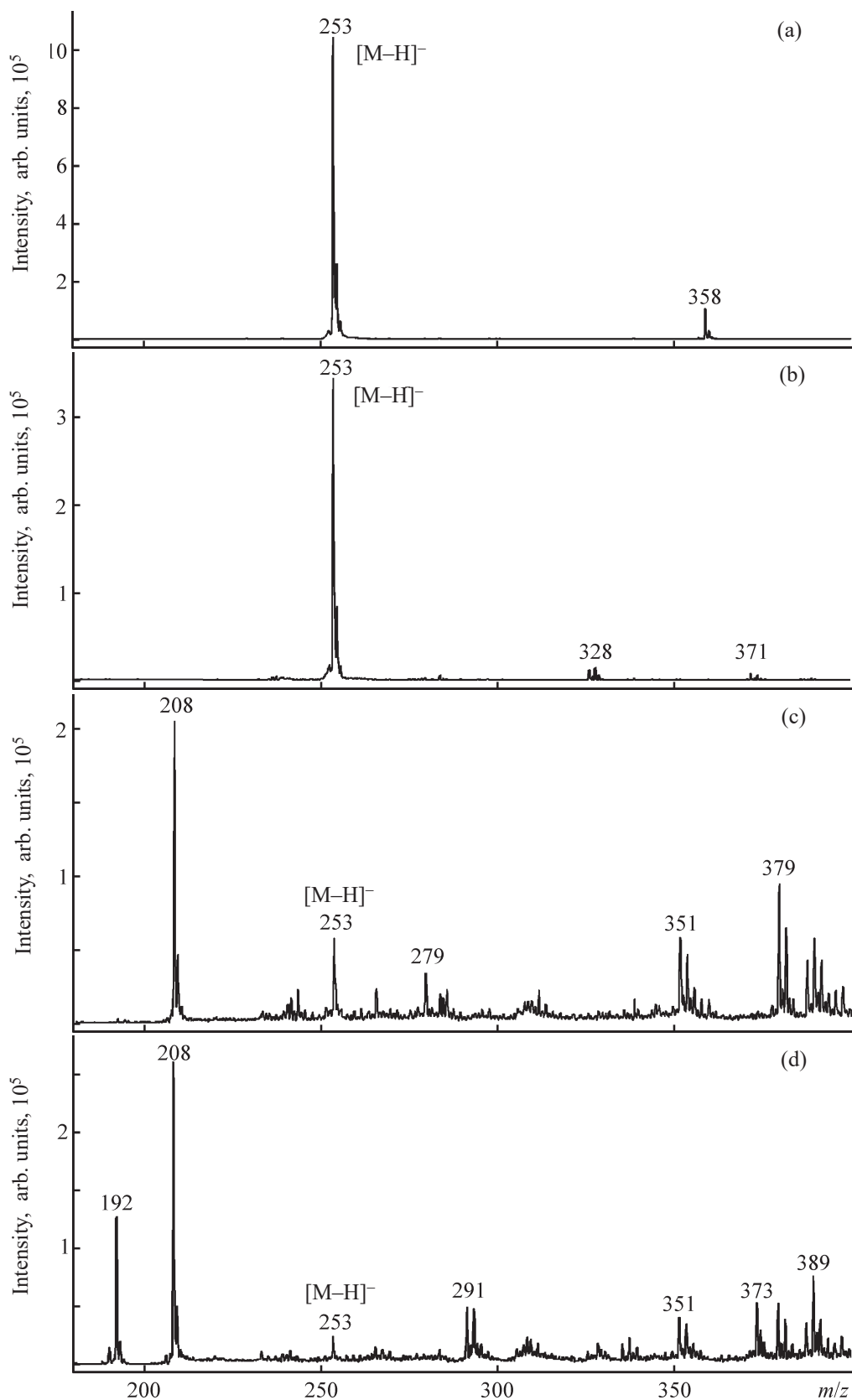


Fig. 1. MALDI mass spectra of the Ketoprofen organic active substance, obtained using matrix compounds: (a) 4-(dimethylamino)-benzaldehyde, (b) *N,N*-dimethyl-*p*-phenylenediamine, (c) 3-aminoquinoline, (d) 9-aminoacridine.

preparation of nimesulide **10** also provides the generation of deprotonated ions. In this case, as for compounds **1–9**, the mass spectrum of positively charged ions of nimesulide contained only low-intensity peaks of ions of protonated and cationized molecules.

A comparative assessment was performed of the time required for confirming the presence of an active substance by other mass spectrometric methods (GC/MS and HPLC/MS-ESI), based on the compound **1** analysis data. In the first case, besides a relatively long analysis time (14 min), an additional stage (silylation) of sample preparation was required, which increased the total analysis time of one sample to 19 min. In the second case, the analysis required significantly less time, since the mode of direct injection of the analyte solution into the ion source was used. The total analysis time of one sample, including the dissolution of the sample, its filtration, and registration of the mass spectrum, was on average 9 min, which is less than the time required to register the MALDI mass spectrum (11 min, taking into account the target transporting into the instrument). At the same time, the transition to streaming analysis of a large number of samples by MALDI mass spectrometry made it possible to significantly reduce the total analysis time to 3 min per sample due to the need for only a single transporting the target with several deposited samples into the instrument and automatic registration of the mass spectrum. At the same time, for HPLC/MS-ESI the possibility of reducing the analysis time is much less, and in the flow mode the analysis of one sample took 6 min.

CONCLUSIONS

The MALDI mass spectrometry can be used for the rapid identification of pharmaceutical substances and drugs, as well as for the qualitative determination of acid-containing nonsteroidal anti-inflammatory drugs in samples of various genesis (biological, natural). For NSAIDs containing carboxyl or sulfamide groups, it is proposed to use deprotonating matrices, which provide high sensitivity of the MALDI mass spectrometry method in the mode of recording negatively charged ions. Obviously, the proposed method allows the selective detection of acidic substances against the background of other components that do not undergo deprotonation. It was found that 4-(dimethylamino)-benzaldehyde and *N,N*-dimethyl-*p*-phenylenediamine are the most effective among the proton-eliminating

matrix compounds tested in the work. These compounds can be recommended for applying as matrices when using a combination of TLC/MALDI-MS methods.

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CONFLICT OF INTEREST

M. L. Khrushcheva is the scientific editor of the Journal of Applied Chemistry, other co-authors declare that there is no conflict of interest requiring disclosure in this article.

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