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DART mass spectrometry and its applications in chemical analysis

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Abstract. Published data on the fundamental and applied aspects of DART mass spectrometry are surveyed. The DART ionization principles and the key parameters affecting the analytical characteristics of the method and the mass spectra of the determined compounds are considered. The advantages and drawbacks of DART mass spectrometry are discussed and the existing and prospective applications are outlined. The bibliography includes 120 references.

I. Introduction

A distinctive feature of direct analysis in real time (DART) mass spectrometry compared to conventional mass spectrometry is ionization of low-molecular-mass compounds present on the surfaces of solids or liquids in a gas stream without sample preparation or chromatographic separation of components of the sample.†

A DART source was patented in 2005 [1] and the first DART-MS study [2] was also reported in that year. However, a prototype of the commercially available DART source was used in the US Army since 2002.[3] It is believed [4] that the invention of the DART source is the most significant event in mass spectrometry since the invention of the electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) techniques. Presently, DART-MS studies are rapidly progressing and the number of publications on the subject and corresponding analytical applications increases continuously.

DART mass spectrometry allows one to carry out a fast non-contact analysis of various samples. Most often, solid samples are introduced into the ionization region with a pincer while liquid ones are introduced with a glass or polymeric rod. Evaporation/desorption of analytes from the surface of samples in a helium or nitrogen stream is accompanied by their ionization involving metastable atoms or molecules (or reagent ions). Only the low-molecular-mass components are ionized; this allows one to avoid the impact of the high-molecular-mass matrix components of the analysis. It is of interest not only to identify analytes, but also to quantify them.

To date, there are no reviews solely devoted to DART-MS and its analytical applications. A review [5] is restricted to qualitative determination and quantification of pharmaceuticals in different. It leaves the physical foundations of DART-MS and the parameters affecting analytical characteristics of the method as well as the mass spectra of compounds out of consideration.

II. The DART ionization principles

The design of a DART source is shown in Fig. 1. A gas stream passes through the discharge region where ions, electrons and metastable (excited) molecules or atoms are produced.

Opinions are different as to the nature of the discharge in the DART source. Earlier,[2, 6] Cody and Laramee (inventors of DART-MS) used the term 'electrical discharge'

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† Here one deals with a word play, namely, the abbreviation DART and a 'dart' (missile); the latter characterizes the rapidity and high accuracy of the method. From this point on, we will use the abbreviation DART-MS for DART mass spectrometry.

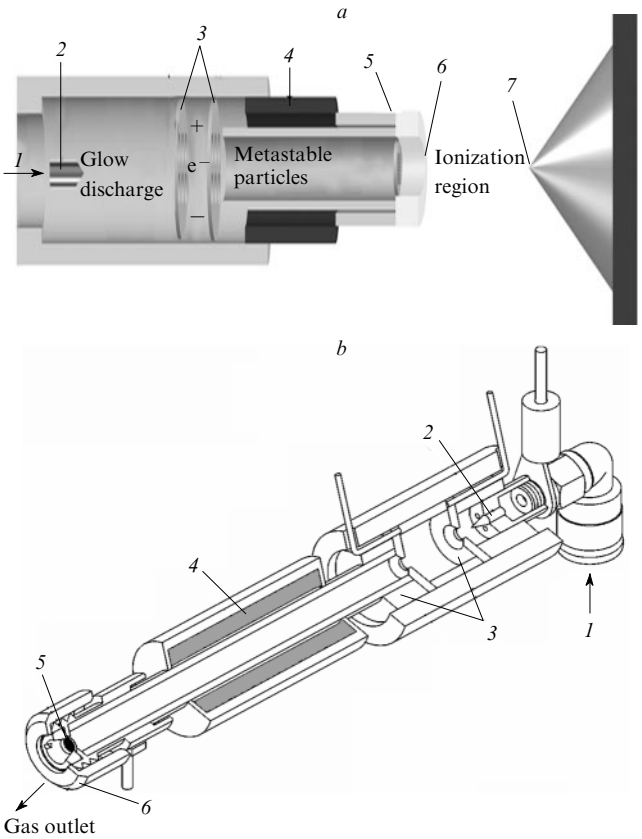


Figure 1. Schematic (*a*) and detailed view (*b*) of a DART source.[2] Gas inlet (*1*), needle electrode (*2*), disk electrodes (*3*), gas heater (*4*), grid electrode (*5*), insulator cap (*6*) and gas outlet (*7*).

without specifying its nature. Hieftje and co-workers[7] stated that a corona discharge is used in the DART source. This was also confirmed by classification of discharges depending on the potential difference applied to electrodes, namely,[8] tens of volts correspond to the arc discharge, hundreds of volts correspond to the glow discharge and a few kilovolts correspond to the corona discharge. A thorough investigation of the properties of the discharge in the DART source involving a study of reagent ions, responses to a model compound (ferrocene) as well as thermography of the stream of the inert gas used for desorption and ionization suggested that one deals with a corona-to-glow transition discharge. However, Cody emphasized in his recent study[9] on the ionization mechanism in the DART source that DART operates with a glow discharge rather than a corona discharge.

As the gas stream passes through the DART source, ions, electrons and excited atoms or molecules are produced in the discharge chamber. Then, most charged species are removed at electrodes,[6] *i.e.*, a stream of metastable gas particles leaves the source.‡ In addition, the gas is heated to provide thermal desorption of analyte molecules. The gas stream leaving the DART source is directed immediately towards the sample placed in the gap between the DART source exit and the mass spectrometer inlet. Most DART-MS experiments are carried out with helium (purity 99.999%). The possibility of using neon and nitrogen was also discussed,[2,6,9] but no such studies have been reported so far. As to argon, it was reported that metastable Ar atoms rapidly lose their energies in humid atmospheric air as a result of a reaction involving homolytic bond cleavage in the water molecule without corresponding formation of ions;[6] therefore, argon is inappropriate for DART-MS experiments. However, in 2010, the first argon DART mass-spectrometric study was reported.[10] Selective ionization of melamine was achieved using reagent ions produced upon ionization of acetylacetone and pyridine vapours in the absence of water clusters.

In most studies, the DART ionization conditions proposed by Cody and Laramee[2] were used. Namely, the potential at the gas-discharge needle of the DART source ranges from 1 to 5 kV, the first perforated disk electrode is grounded and the potentials at the second perforated electrode and at the grid electrode are about ±100 and ±250 V, respectively (the 'plus' and 'minus' sign corresponds to the positive-ion and negative-ion mode, respectively). The gas flow rate is usually at least 0.55 litre min^{-1} and the gas temperature varies from ambient temperature to 350 °C. The width of the gap between the DART source exit and the skimmer input orifice or a vacuum interface of a mass spectrometer usually varies from 5 to 25 mm.[2,6,9]

The ionization mechanisms of analytes in DART-MS are complex and poorly studied. Most studies on the subject were carried out with helium. It is assumed that a number of complex processes occur concurrently depending on the proton affinity and the ionization potential of analyte, on the analyte concentration in the sample, on the polarity and nature of the gas used and on the presence of auxiliary reactants.[2] Some authors make an analogy between the DART ionization mechanisms and the mechanisms of atmospheric pressure photo- and chemical ionization.[9,11]

When using a nitrogen or neon gas stream, production of cations begins with the Penning ionization[6,12] involving transfer of the energy of a 'long-lived' excited-state metastable atom or molecule (N*) to the analyte molecule (M) resulting in a molecular radical ion ($M^{+\bullet}$) and an electron

$$N^* + M \longrightarrow M^{+\bullet} + N + e^-.$$

This process dominates if the ionization energy of the molecule M is lower than the internal energy of the species N*.

The energy of an excited (triplet) state of a helium atom is 19.8 eV. The ionization energies of atmospheric gases and organic molecules are usually lower than 19.8 eV. When using helium, the following reaction dominates:[6,9]

$$He(2^3S) + n\,H_2O \longrightarrow [(H_2O)_{n-1} + H]^+ + OH^- + He(1^1S).$$

The reaction is characterized by a cross-section of about 10 nm^2 and is highly efficient.[6,13] Therefore, it was assumed[6] that DART ionization is not affected by humidity, although no detailed explanation and relevant experimental data were reported. However, this is not the case.

If the proton affinity of an analyte molecule is higher than that of a water cluster, transfer of a proton occurs:

$$[(H_2O)_n + H]^+ + M \longrightarrow [M+H]^+ + n\,H_2O;$$

‡ The mechanisms of processes occurring in the DART source are complex and still unclear. Some researchers believe that not all ions are removed at the source electrodes and therefore the stream leaving the source contains metastable species and ions.

also, if the proton affinity difference is large, fragmentation of ions is possible.

The influence of experimental conditions on production of alkane molecular ions was studied.[9] Shortening of the distance between the DART source exit and the mass spectrometer sampling orifice to 5 mm and an increase in the grid electrode potential from 250 to 650 V caused changes in the mass spectrum of the background, *viz*., the appearance of strong signals of $O_2^{+\bullet}$ radical cations. Under the conditions of production of $O_2^{+\bullet}$ species, the ionization mechanism changed. Namely, signals of $M^{+\bullet}$ and other odd-electron species, *e.g*., $[M-CO]^{+\bullet}$ appeared in the mass spectra of nonpolar analytes, whereas signals of protonated molecules and other even-electron species are mainly observed in the mass spectra. Presumably, the mechanism of production of odd-electron ions involved a charge exchange between $O_2^{+\bullet}$ and the analyte molecule provided that the ionization energy of the analyte was lower than the ionization energy of $O_2^{+\bullet}$ (12.07 eV):

$$He^* + O_2 \longrightarrow O_2^{+\bullet} + e^- + He,$$

$$O_2^{+\bullet} + M \longrightarrow M^{+\bullet} + O_2,$$

$$O_2^{+\bullet} + M \longrightarrow [\text{fragment ion}]^+ + O_2 + R^{\bullet},$$

$R^{\bullet}$ is an organic radical or a molecular fragment.

Production of ions was affected by the arrangement of sample introduction, *viz*., the shorter the distance between samples and the DART source exit the larger the number of molecular ions formed. The mass spectra of alkanes exhibited signals of $[M-H]^+$, $[M+O-H]^+$, $[M+O-3H]^+$, $[M+2O-H]^+$, $[M+2O-3H]^+$, $[M+3O-H]^+$, as well as $[C_nH_{2n+1}]^+$, and $[C_nH_{2n-1}]^+$ ions.

It was suggested [14] that the process begins with thermal desorption of analyte from the solid or liquid state to the gaseous state

$$M(\text{solid}) \longrightarrow M(\text{gas}),$$

and then is followed by the interaction of the analyte molecule with water cluster ions

$$[(H_2O)_n + H]^+(\text{gas}) + M(\text{gas}) \longrightarrow$$
$$\longrightarrow [M(H_2O)_{n-1} + H]^+(\text{gas}) + H_2O(\text{gas}) \longrightarrow$$
$$\longrightarrow [M + H]^+(\text{gas}) + n\,H_2O(\text{gas}).$$

In addition, it is believed that compounds characterized by low partial vapour pressure can also interact with water cluster ions in the solid state

$$[(H_2O)_n + H]^+(\text{gas}) + M(\text{solid}) \longrightarrow$$
$$\longrightarrow [M(H_2O)_{n-1} + H]^+(\text{gas}) + H_2O(\text{gas}) \longrightarrow$$
$$\longrightarrow [M+H]^+(\text{gas}) + n\,H_2O(\text{gas}).$$

This provides an explanation for the ionization of salts (*e.g*., *N*,*N*-diisopropylaminoethyl methylphosphonothioic acid) in the zwitterionic form.[2]

In revising the ionization mechanism, a so-called transient microenvironment concept was proposed for the positive-ion mode.[15] This concept takes into account the thermodynamic properties of analytes and the matrix. It is believed that the ionization mechanism involves nine gas-phase reactions (Table 1).

The mass spectra of some organic solvents including proton acceptors, benzene derivatives, alkanes and chlorinated methane derivatives were studied.[15] The mass spectra of proton acceptors mainly exhibited signals of $[Solv+H]^+$ and $[2\,Solv+H]^+$ ions; for some compounds, signals of $[2\,Solv+H-H_2O]^+$ ions with relative intensities of 15% – 16% were also observed. Most signals observed in the mass spectra of benzene derivatives belonged to $Solv^{+\bullet}$ and $[Solv+H]^+$ ions. In the case of fluorinated benzene derivatives, signals of $[Solv-F+OH]^+$, $[Solv-F+H_2O]^+$ and $[2\,Solv-2\,F+H_2O]^+$ cations were also detected. The mass spectra of alkanes showed peaks of $Solv^{+\bullet}$ and $[C_nH_{2n+1}]^+$ ions and dehydrogenation products $[Solv-n\,H]^+$ (n = 1, 2, 3). The spectra of chlorinated methane derivatives showed no signals of $M^{+\bullet}$ radical cations or $[M+H]^+$ ions; only peaks of fragment ions of different compositions were observed. In particular, the mass spectra of $CHCl_3$ containing pentene, cyclohexene or ethanol (15×10^{-4}% – 0.75%) exhibited signals of the following ions: $[C_nH_{2n+1}]^+$ (n = 3 – 6), $[CHCl_2]^+$, $[CCl_3]^+$, $[C_6H_{11}Cl]^+$ and even $[(C_2H_5OH)_n+H]^+$ (n = 1, 2) depending on the composition of the additive. As a proof for the mechanism proposed, an experimental study of solutions of ten compounds belonging to different classes showed that the presence and intensities of the signals of the $M^{+\bullet}$ and $[M+H]^+$ ions depended on the solvent used and on the analyte concentrations in the samples. The efficiency of this concept was also confirmed for solids with different content of impurities. It was concluded [15] that methanol, toluene, hexane and chloroform are the best solvents for DART ionization and that tetrahydrofuran, ethyl acetate, *N*,*N*-dimethylformamide and dimethylsulfoxide should not be used.

The mechanisms of production of anions under DART ionization conditions are less studied than for cations. It is assumed [2, 16] that electrons formed as a result of Penning

Table 1. Gas-phase reactions as stages of the DART ionization mechanism.[15]

No.	Reaction equation	Reaction conditions
1	$He^* + H_2O \rightarrow$ $\rightarrow He + H_2O^{+\bullet} + e^-$	$E_m(He) > E_i(H_2O)$
2	$H_2O^{+\bullet} + (H_2O)_m \rightarrow$ $\rightarrow HO^{\bullet} + [(H_2O)_m + H]^+$	$A[(H_2O)_m] > A(HO^{\bullet})$
3	$He^* + Solv \rightarrow$ $\rightarrow He + Solv^{+\bullet} + e^-$	$E_m(He) > E_i(Solv)$
4	$Solv^{+\bullet} + Solv_n \rightarrow$ $\rightarrow [Solv-H]^{\bullet} + [Solv_n+H]^+$	$A(Solv_n) > A([Solv-H]^{\bullet})$
5	$[(H_2O)_m + H]^+ + Solv_n \rightarrow$ $\rightarrow (H_2O)_m + [Solv_n + H]^+$	$A(Solv_n) > A[(H_2O)_m]$
6	$[Solv_n + H]^+ + M \rightarrow$ $\rightarrow Solv_n + [M + H]^+$	$A(M) > A(Solv_n) >$ $> A[Solv-H]^{\bullet})$
7	$Solv^{+\bullet} + M \rightarrow$ $\rightarrow [Solv-H]^{\bullet} + [M + H]^+$	$A(M) > A([Solv-H]^{\bullet}) >$ $> A(Solv_n)$
8	$Solv^{+\bullet} + M \rightarrow Solv + M^{+\bullet}$	$A([Solv-H]^{\bullet}) > A(Solv_n)$ and $E_i(Solv) > E_i(M)$
9	$[(H_2O)_m + H]^+ + M \rightarrow$ $\rightarrow (H_2O)_m + [M + H]^+$	occurs provided that the contributions of competing reactions are small

Note. Notations are as follows: $E_m(He)$ is the energy of helium atom in a metastable state (19.8 eV); m = 1, 2 or 3, n = 1 or 2; E_i is the ionization energy and A is the proton affinity, Solv stands for the matrix (solvent) molecule.

ionization rapidly slow down through collisions with gas molecules at atmospheric pressure

$$e^- \text{ (fast)} + \text{gas} \longrightarrow e^- \text{ (slow)}.$$

Then, slow electrons are trapped by atmospheric oxygen

$$e^- \text{ (slow)} + O_2 \longrightarrow O_2^{-\bullet}$$

to give radical ions $O_2^{-\bullet}$, which react with analyte molecules to produce anions. It was stated [6] (without reporting relevant experimental data) that the negative-ion mass spectra almost do not change when using nitrogen, neon or helium, but the sensitivity of detection increased in the order $N_2 < Ne < He$.

The negative-ion mode is used more rarely than the positive-ion mode. The mechanism of negative-ion production was studied [16] taking 42 model compounds (fullerenes, perfluorohydrocarbons, explosives, phenols, polyfluorobiphenyls and carboxylic acids) as examples. Based on experimental data, it was concluded that the mechanism of DART ionization in the negative-ion mode is similar to the atmospheric pressure photoionization mechanism and involves electron capture

$$M + e^- \longrightarrow M^{-\bullet},$$

dissociative electron capture

$$M + e^- \longrightarrow [M-X]^- + X^{\bullet},$$

proton transfer

$$M + Y \longrightarrow [M-H]^- + [H+Y]^+,$$

and anion (halogen) attachment

$$M + A^- \longrightarrow [M + A]^-.$$

Therefore, the mass spectra may have rather complex patterns depending on the class of compounds and their interpretation may be ambiguous. For instance, the mass spectra of aliphatic perfluorohydrocarbons exhibit signals of $M^{-\bullet}$ and $[M-F]^-$ ions, the mass spectra of nitro compounds show signals of $M^{-\bullet}$, $[M-H]^-$ and $[M-NO]^-$, the spectra of phenols and their derivatives display signals of $[M-H]^-$ anions, the spectra of toluene and anisole show signals of $[M-H+O]^-$, $[M-CH_3+O]^-$ and $[M-OCH_3+O]^-$ ions and the mass spectra of THF and 1,4-dioxane exhibit signals of $[M-H+O]^-$ and $[M-H+O_2]^-$ ions.[16]

III. Parameters affecting the DART mass spectra

The DART mass spectra are relatively simple.[2,6] The positive-ion spectra showed signals of M^+ and $[M+H]^+$ while negative-ion spectra showed signals of $[M-H]^-$. More recently, data refuting this information were reported. At present, it is clear that the DART mass spectra may be either simple or complex depending on the class of compounds studied and experimental conditions.

Factors affecting the mass spectra can be divided into two groups:

— factors due to specific features of samples (chemical compositions and molecular structures of compounds to be determined, as well as the chemical composition of the matrix); and

— factors due to experimental conditions (chemical composition and velocity of the gas stream, the temperature and the voltage at electrodes in the DART source and at the mass spectrometer inlet, and specific features of the experimental design).

In the text below we will consider how these factors influence the mass spectra. Specific features of the experimental design are also considered in this Section because their effect is closely related to other parameters of the system (temperature, velocity of the gas stream and the interface configuration of the mass spectrometer).

1. The nature of analytes

The dependence of the pattern of the DART mass spectra on the nature of analytes has been little studied and no surveys summarizing data for different classes of compounds have been reported as yet. Table 2 presents information on the regularities of ionization of some classes of chemical compounds.§

The data listed in Table 2 should be treated with criticism because there are no references and information is incomplete (*e.g.*, no data on the signals of $[M+OH]^+$ ions in the mass spectra of phosphorus-containing chemical warfare agents [17] are given). In particular, the types of mass spectrometers used are unknown. DART ionization can be practically implemented using an AccuTOF mass spectrometer (JEOL, Japan). A system of skimmers near the mass spectrometer inlet provides a necessary resolution (Fig. 2 *a*). For non-JEOL mass spectrometers, an atmospheric/vacuum interface [*e.g.*, Vapur interface (IonSense, USA)] should be used. Otherwise, operation of the mass spectrometer is impossible owing to violation of pressure balance. Such an interface uses a ceramic tube rather than a skimmer cone with a small-diameter orifice (as in the AccuTOF instrument) for gas supply.¶ The design of the interface is shown in Fig. 2 *b*.

An increase in the analyte concentration in the sample facilitates production of dimeric ions.[9,19] The response factors of analytes in DART-MS depend strongly on their nature. For instance, the sensitivity of determination of 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-4-indenone (cashmeran) is 100 times higher than that of γ-lacton-4-hydroxydecanoic acid (decal) and ethyl 2-methylpentanoate (manzanate).[20] The data of Table 3 taken from Ref. 21 characterize the DART mass spectra, but no experimental results supporting this information were reported.

Positive-ion DART mass spectra of alcohols have much more complex patterns than corresponding mass spectra of other classes of compounds.[22,23] The mass spectra of 16 alcohols recorded using a helium stream exhibited an unexpectedly large number of signals, which can be explained by oxidation of the alcohols proceeding by several

§ Initially, Table 2 was compiled by R Cody and posted on the DART-MS Discussion Board at http://groups.google.com/group/dart-mass-spectrometer-users.

¶ The Vapur interface was also developed for AccuTOF mass spectrometers. However, the advantages of its use in DART-MS have not been reported as yet.

Table 2. Signals detected in DART mass spectra of different classes of compounds.

Class of compounds	Positive ions	Negative ions	Note
Alcohols	$[M+H]^+$, $[2M+H]^+$, $[M+H-H_2O]^+$ polyatomic alcohols require derivatization	$[M-H]^-$ for phenols	dehydrogenation occurs frequently, derivatization is required for most alcohols (C_4 and longer-chain ones)
Alkanes	$M^{+\bullet}$, $[M-H]^+$, alkyl fragments, ions formed as a result of oxidation involving NO^+		only radical ions $M^{+\bullet}$ are formed provided that no Vapur interface is available and water vapour is not removed
Alkenes	usually $[M+H]^+$, some fragments		
Amines, bases	$[M+H]^+$		
Amino acids	$[M+H]^+$, some fragment ions		derivatization with dimethylaminoethanol is appropriate in some cases
Aromatics	$M^{+\bullet}$, $[M-H]^+$; production of $[M-H]^+$ is possible for some alkylaromatic compounds		
Carbonyl-containing compounds	$[M+H]^+$, $[M+NH_4]^+$		mostly $[M+NH_4]^+$ ions when using the Vapur interface
Carboxylic acids	$[M+H-H_2O]^+$; $[M+H]^+$, $[M+NH_4]^+$ in the presence of ammonia	$[M-H]^-$	
Carbohydrates	$[M+H]^+$ and dehydration products for mono- and disaccharides; larger molecules require derivatization		*in situ* derivatization with tetramethylammonium hydroxide is efficient; this results in an adduct with tetramethylammonium. If a compound contains a nitrogen atom, $[M+H]^+$ ions are detected
Ethers	$[M+H]^+$, $[M+NH_4]^+$		mostly $[M+NH_4]^+$ ions when using the Vapur interface
Explosives (hexogen, octogen, nitroglycerine, nitroglycol, penthrite, tetryl)		$[M+Cl]^-$, $[M+CH_3CO_2]^-$, $[M+NO_3]^-$	with CH_2Cl_2 as reagent gas
Halogenated (*e.g.*, chlorinated) hydrocarbons alkanes	–	Cl^-, Cl_2^-, $[M-Cl]^-$, *etc.*	
Metals	$M^{+\bullet}$ and metal oxides		some metals forming volatile derivatives (*e.g.*, acetylacetonates) can be detected. At very high cone voltages, element ions or oxide ions, or hydroxide fragments are formed
Nitroaromatics including explosives (trinitrotoluene, dinitrotoluene)	$M^{+\bullet}$, $[M+H]^+$	$M^{-\bullet}$, $[M-H]^-$	the limits of detection are lower in the negative-ion mode
Organometallic compounds	$M^{+\bullet}$, $[M+H]^+$	$M^{-\bullet}$, $[M-H]^-$	ligand abstraction producing a singly charged positive or negative ion may occur depending on the nature of analyte
Peptides	$[M+H]^+$, fragment ions	$[M-H]^-$, fragments	by and large, the method is inefficient except for the smallest peptides
Peroxides	$[M+NH_4]^+$		
Proteins	not detected, the mass spectrum may exhibit signals of amino acids		by and large, the method is inapplicable
Salts	cation	anion	depending on particular compound

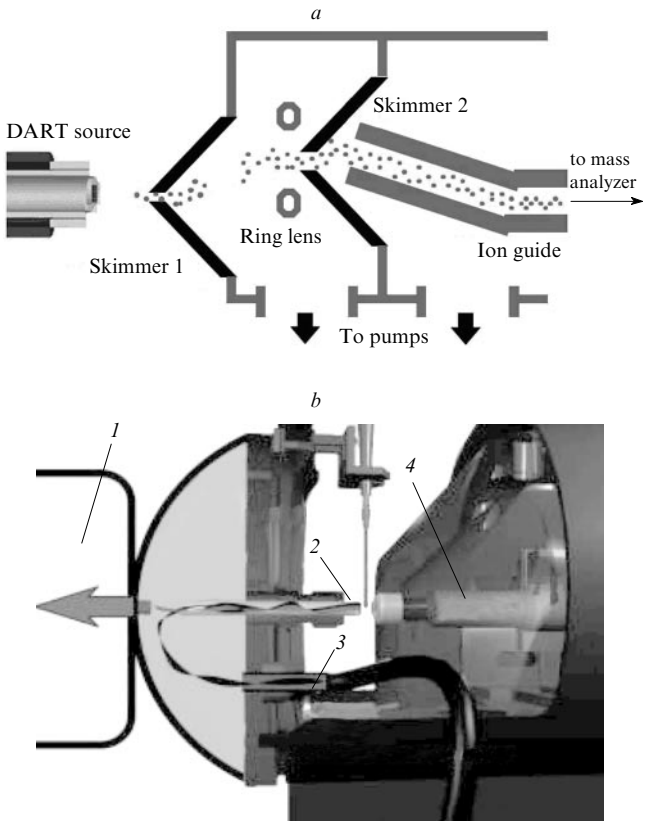


Figure 2. Schematics of a built-in vacuum interface for an AccuTOF mass spectrometer (*a*) and a Vapur interface for this and other models of mass spectrometers (*b*).
The scheme shown in Fig. 2*a* is reproduced from the AccuTOF Mass Spectrometer User Manual and Fig. 2*b* is taken from Ref. 18. Mass spectrometer (*1*), entrance tube (*2*), vacuum port (*3*) and the DART source (*4*).

Table 3. Signals detected in DART mass spectra.[21]

Negative ions	Possible analytes	Positive ions	Possible analytes
$M^{-\bullet}$	ionic compounds, some electrophilic compounds	M^+, $M^{+\bullet}$	ionic compounds, some organic compounds with low ionization potentials
Proton abstraction products $[M-H]^-$	acids, nitroaromatics	proton transfer products	bases, alkenes, low-molecular-mass alcohols, ethers, ketones, aldehydes
		$[M+H]^+$	compounds prone to H/D exchange
Adducts $[M+X]^-$	unstable nitro compounds, some halogenated hydrocarbons	other adducts $[M+Z]^+$	polar compounds, ethers, ketones, acids, peroxides

Note. X and Z are organic fragments of different structure.

mechanisms upon ionization. The proposed mechanisms involve the loss of a few hydrogen molecules, the addition of an oxygen atom, the loss of water molecules, a methylene unit or a hydrogen atom. Exceptions were 3-quinuclidinol and 2-diisopropylaminoethanol whose mass spectra exhibited strong signals of $[M+H]^+$ cations. The molecules of these alcohols contain nitrogen atoms, thus seemingly increasing the proton affinity and, as a consequence, stability of the $[M+H]^+$ cation. To simplify the DART mass spectra and to improve the sensitivity of determination by a factor of 20, it was proposed to convert the alcohols to corresponding carbamates[22] whose mass spectra exhibit strong signals of $[M+H]^+$ or $[M+NH_4]^+$ ions. Since a single-step reaction took about 16 h, it was proposed[23] to accelerate the process by activating the reactants (phenyl isocyanates) by adding various substituents.

From the aforesaid it follows that the DART mass spectra of samples of unknown chemical compositions obtained under conventional experimental conditions (ionization in a helium stream, positive-ion mode) are difficult to interpret. The mass-spectrometric techniques involving DART ionization are more appropriate for identification of specified components than for the determination of the chemical compositions of unknown components of complex mixtures. In the negative-ion mode, one should also expect complex patterns of mass spectra taking into account the possibility for several types of reactions to occur. Procedures for obtaining simple DART mass spectra of different classes of compounds, which show only signals of molecular ions or $[M+H]^+$ ions, as was reported in the early communications (see, *e.g.*, Ref. 6), have not been elaborated so far.

2. The nature of the matrix

The effects of matrices (in particular, organic solvents) used to dissolve analytes on the DART mass spectra were studied.[15] The transient microenvironment concept outlined above does hold for such matrices. In considering this concept one should take into account the fact that the matrix, in particular, a highly volatile organic solvent, acts as the source of reagent gas for DART ionization. The highest sensitivity of determination of the test compound warfarin was attained for ethyl acetate solutions.[24] The intensity of the signal of protonated molecule was solvent-dependent, *viz*., the strongest signals were observed from analytes dissolved in ethyl acetate, while the weakest signals (about 7 times weaker) were observed in dimethyl sulfoxide solutions. Other solvents were *N*,*N*-dimethylformamide, tetrahydrofuran, acetonitrile and methanol. These results were also confirmed in a study[25] whose authors recommended solutions in ethyl acetate for DART-MS. To date, there are no commonly accepted conditions for the introduction of samples into the ionization region; therefore, the choice of optimum solvents for DART-MS is still a moot question.

It was reported that there is a correlation between the DART and ESI signal responses of many compounds.[26] It is believed that high sensitivity is due to the use of high-purity volatile solvents as matrices. In some cases, it was sufficient to introduce water or acetonitrile into animal plasma samples in order to make signals more intense. The sensitivity of determination of verapamil in solution was 20 times higher than in plasma. However, the reproducibility of the signal of plasma samples (coefficient of variation was 5.86% based on 8 injections at a verapamil concentration of

1 μg ml^{-1} each) was better than that of neat solutions [coefficient of variation in a study of a verapamil solution of the same concentration in water–acetonitrile (1 : 1) mixture was 21.7%]. This was explained as follows: the higher the viscosity the weaker the effect of gravity on the shape of the sample drop during its evaporation. In addition, the calibration curves for the analytes in animal plasma and brain were almost identical. The volatile solvent facilitated thermal desorption of analyte from the surface of the sample;[27] in this case, the signal intensities depended on the extent of dilution of the model solutions. When an autosampler was used, the reproducibility of signals increased with an increase in the boiling point of the solvent. No changes in the sensitivity with T_b of the solvent were reported.

The matrix effects in biomaterials were evaluated.[18] The signals of analytes in biological media were in the range from 5% to 70% of the signal in the model solution and depended on the nature of the matrix and the analyte. Dilution of plasma with water to 1 : 1 ratio caused no changes in the relative intensity of the analyte signal; this was explained by a mutual compensation of the effect of the decrease in the analyte concentration and weakening of the matrix effects. The addition of formic acid (0.1%) favoured some increase in the signal intensity.

Since the signal responses in DART-MS depend on the composition of the matrix, this method is of limited use in the analysis of samples containing different macrocomponents. It was shown that the slopes of the calibration curves constructed for solutions in neat methanol and in methanol containing 10% of diethylamine differed by a factor of nearly two.[19] The addition of an internal standard led to better reproducibility of the results obtained, but quantification of the samples with different solvents was impossible.

The material of the probe used to introduce a sample into the ionization region affects the signal response.[26] Carboxylic compounds interacted with the surface of a glass capillary coated with the sample to be introduced into the ionization region. Taking cholic acid as an example, it was shown that the signal intensity can be increased by a few orders of magnitude using polyimide-coated capillaries.

3. Chemical composition of reagent gas in the DART source and the environment

Additional materials to Ref. 2, which are available on the Internet at http://pubs.acs.org/doi/suppl/10.1021/ac050162j/suppl_file/ac050162jsi20050223_101942.pdf, include, in particular, the DART mass spectra of toluene obtained using helium and nitrogen as reagent gases. The most intense signals in the mas spectra correspond to $[M+H]^+$ ions in the former and to $M^{+\bullet}$ ions in the latter case. Comparative experiments using these gases in the DART source were reported.[28] The helium flow rate was varied from 0.55 to 2 litre min^{-1}, while the nitrogen flow rate was 7 litre min^{-1} because lower flow rates did not produce a stable baseline signal in the DART chronograms. Other experimental conditions were identical. The helium-induced mass spectra of permethrin and deltamethrin exhibited numerous intense signals of fragment ions; the proportion of such signals increased with the gas temperature. In the nitrogen-induced mass spectra, the intensities of the fragment-ion and $[M+H]^+$ ion peaks were minimum, but intense signals of $[M+NH_4]^+$ ions were observed. The lower extent of fragmentation in the case of nitrogen was explained by lower thermal conductivity and lower energy of metastable nitrogen molecules compared to the energy of metastable helium atoms.

Analyses of As-, Fe-, Hg-, Pb-, Se- and Sn-containing organometallic compounds showed[29] that the patterns of the mass spectra depended significantly on the reagent gas used. Owing to high toxicity of the analytes, the samples were introduced with a fume extraction system over the solution. When triethylarsine was analyzed using helium, mainly $[M+H]^+$ ions and $[M+OH]^+$ adducts formed. Analysis using nitrogen revealed signals of other ions, presumably, triethylarsine cluster ions. Analysis of other compounds containing the elements listed above led to production of various fragment ions or cluster ions depending on the gas used. Based on the published data,[29] it is difficult to establish the regularities and to establish which ions are mainly produced in helium or nitrogen when analyzing organometallic compounds. Nitrogen may serve a cheap alternative to helium in many types of experiments. However, the need for application of high electric fields to excite the glow discharge, a more thorough control of gas temperature as well as the possibility of oxidation of the analytes upon ionization makes nitrogen of limited use. It can be used only in the determination of some specified compounds.[9]

The patterns of mass spectra and the origin of ions can usually be explained by various processes in the DART ionization region. However, the authors of some studies report the signals of $[M+NH_4]^+$ ions which are produced following a still unclear mechanism. Possible reasons for the formation of the $[M+NH_4]^+$ ions are as follows:

— placing an NH_4OH solution into the ionization region;[2,27,30–35]

— use of nitrogen instead of helium;[28]

— presence of ammonia-containing impurities in the sample;[36]

— presence of ammonia in the atmosphere surrounding the instrument;[37,38] the chemical composition of ions was confirmed[38] using an orbital ion trap as mass analyzer;

— ammonia excretion by human organism;[3,22,39]

— formation of ammonium ions as a result of decomposition of an amino-containing reagent (aminoacrylic acid) in the course of a chemical reaction.[40,41]

Some authors report the $[M+NH_4]^+$ ions with no information how they were produced.[23,42–44] It was shown that NH_4OH as reagent allows one to improve the sensitivity of determination. For instance, making use of NH_4OH followed by detection of the $[M+NH_4]^+$ ions provides a higher sensitivity of determination of compounds containing hydroxyl or carboxyl groups[26] and allows the sensitivity of determination of triacylglycerols to be increased by an order of magnitude.[27] Production of $[M+NH_4]^+$ ions is typical of carbonyl-containing compounds (acids, esters, ketones, peroxides).[9]

The $[M+Cl]^-$ and $[M+CF_3COO]^-$ anions are produced in the presence of dichloromethane or 0.1% trifluoroacetic acid solution placed very close to the ionization region.[2] In particular, the $[M+Cl]^-$ ions can form in the determination of nitro-containing explosives if dichloromethane is used.[45]

A first argon DART-MS study was reported.[10] The energy of an excited argon atom is 11.55 eV (*cf.* 12.6 eV for the ionization energy of water); therefore, no Penning ionization occurs. This feature was used for selective deter-

mination of melamine in powdered milk. There was the need for developing a procedure for selective ionization of melamine ($[M+H]^+$ $m/z_{theor} = 127.0732$) in the presence of 5-hydroxymethylfurfurol ($[M+H]^+$ $m/z_{theor} = 127.0395$). The sources of reagent gases were acetylacetone and pyridine, both having low ionization potentials. Acetylacetone was supplied with a syringe pump and an open vial of pyridine was placed near the ionization region. Penning ionization of acetylacetone was followed by proton transfer from the $[M+H]^+$ ion (M is acetylacetone) to the pyridine molecule. In turn, proton was transferred from the $[M'+H]^+$ ions (M′ is pyridine) to the melamine molecule. 5-Hydroxymethylfurfural was not ionized under the conditions chosen; this improved the selectivity of the method.

4. The temperature and the gas flow rate in the DART source

The temperature inside the DART source (T_1) differs from the actual temperature in the ionization region (T_2), *i.e.*, in the gap between the DART source and the input orifice of the mass spectrometer, because gas released in the environment is cooled. Experiments[42,46] gave the following relations between these temperatures:†

T_1/°C	200	450	175	250	325
T_2/°C	130	310	167	230	280

The actual temperature experienced by the sample depends not only on the temperature inside the DART source, but also on the flow rate of the gas stream passing through the source and on the sample arrangement in the ionization region. At a helium flow rate of 2 litre min^{-1}, the temperature in the ionization region was maintained at almost a constant value throughout the time interval studied (120 s after switching on helium stream). At higher flow rates (4–6 litre min^{-1}), the temperature decreased by 20 °C and more during the first 15 s and then lowered more slowly (by about 10 °C after 100 s).[46]

The effect of temperature in the DART source on the pattern of mass spectra was studied with a few samples.[24] Raising the temperature to 350 °C did not cause degradation of the analytes. The signal of the $[M+H]^+$ ions was the most intense, the signal intensities of fragment ions being at most 10%–15% of the signal intensity of the protonated molecule. Ionization of the samples may lead to oxidation of analytes; at the same time, the signal intensities of oxidation products were $\leqslant$10% of the signal intensity of the $[M+H]^+$ ions.

Analysis of animal plasma samples showed[26] that the temperature inside the DART source influences the sensitivity of determination. High temperatures favour desolvation and transition of analytes to the gas phase; however, too high temperatures caused the samples to dry and char immediately. As a result, the analyte molecules entered the gas phase more slowly. The optimum temperature inside the source was 300 °C. In addition, the intensity of the analyte signal increased with an increase in the flow rate of helium passing through the DART source (data for two compounds). However, high flow rates broke the pressure balance in the mass spectrometer and it automatically shut down; also, sample droplets were blown into the mass spectrometer. Therefore, a limiting flow rate of 3.5 litre min^{-1} was recommended. Other parameters (width of the gap between the DART source and the orifice of the mass spectrometer, the speed of motion of the sample, voltage at electrodes) had little effect on signal intensities, but particular ranges of these parameters studied by Zhao *et al.*[26] were not reported.

The range of temperatures inside the DART source from 150 to 200 °C was reported as optimum in another study.[47] At higher temperatures (above 250 °C), thermal desorption of analytes (silylated metabolites) was too fast while the data acquisition rate of the scanning mass spectrometer was not high enough and thus the signals were less intense. Raising the temperature led to partial decomposition of analytes. When using an AccuTOF mass spectrometer (nearly 375 μm inlet orifice), the optimum helium flow rate was 2.5–3 litre min^{-1}. The total number of signals detected increased with an increase in the helium flow rate, but at flow rates higher than 3 litre min^{-1} the orifice of the mass spectrometer contaminated and the reproducibility decreased owing to increased turbulence of the gas stream. Contrary to this, coupling the DART source with the hybrid quadrupole-time-of-flight mass spectrometer through a Vapur interface was optimum at a minimum flow rate (0.55 litre min^{-1}). Analogously, the temperature range 150–250 °C in the DART source was optimum in the determination of substituted benzenes in environmental water.[48] A wider range of optimum temperatures (150–350 °C) was indicated for the analysis of stabilizers in polypropylene samples;[32] here, the operating temperature was 250 °C.

A study[49] on the effect of the temperature in the DART source on the signal intensities of Voriconazole samples revealed no bell-shaped signal intensity-*vs*.-temperature dependence. The DART source was used in combination with a quadrupole mass spectrometer and the temperature interval studied was from 50 to 500 °C. Analytes were introduced into the ionization region in the form of solutions (concentration 1 mg ml^{-1}). No temperature dependence of the signal intensity of the $[M+H]^+$ ion and some characteristic fragment ions of Voriconazole was observed, although it was concluded that the results are best reproduced in the temperature range 200–350 °C. Raising the temperature revealed a trend towards an increase in the relative intensities of the signals of fragment ions (relative to the signal of the protonated molecule). It was concluded that control of the gas temperature plays a significant role in the control of fragmentation.

An optimum temperature of 425 °C was chosen for DART quantitative analysis of biological fluids.[18] It is believed that ionization at lower temperatures is inefficient, while at higher temperatures, analytes decompose. High-temperature ionization was recommended[50] for analytes with molecular masses higher than 600.

Analysis of organometallic compounds[29] involved optimization of the gas flow rate and temperature for particular gas (nitrogen or helium). The DART source was coupled with a linear ion trap. The optimum flow rates were 2.5–3.5 litre min^{-1} for nitrogen and 6.5 litre min^{-1} (and more) for helium. The temperature influenced the intensities of signals of different analytes in different manner, namely, they both increased and decreased in the temperature range

† The data in the first two columns (from the left) were taken from Ref. 42. Other data were taken from Ref. 46. Relations between the temperatures reported in those studies are different because the authors used different types of mass spectrometers and the distances from the DART source exit to the temperature measurement points were also different.

studied (25 – 350 °C). No heating of the gas was required for analysis of highly volatile compounds. It was concluded that the main goal of heating is to ensure thermal desorption of analytes from the surface of the sample for further ionization/dissociation of the gas-phase clusters. The effect of temperature on the mass spectra obtained using helium or nitrogen was studied.[28] Signals of $[M + NH_4]^+$ ions were detected in both cases, but their origin remains unclear. When using helium, raising the temperature from 100 to 150 °C led to a more intense signal of the $[M + H]^+$ ion and to weakening of the signal of the $[M + NH_4]^+$ ion; on further heating, the signal of the $[M + H]^+$ ion became less intense owing to fragmentation. Contrary to this, experiments with nitrogen gas in the temperature range from 100 to 250 °C revealed a continuous increase in the $[M + NH_4]^+$ signal intensity. Depending on the position of the sample with respect to the orifice of the mass spectrometer, the signals of the $[M + H]^+$ ions became more or less intense with an increase in the helium flow rate.

Temperatures below 200 °C were recommended for analysis of olive oil and determination of triacylglycerols in it[27] because at high temperatures the signal intensites of $[M + NH_4]^+$ ions abruptly decrease (ammonia vapour was used as reagent). Signals of fragment ions became more intense in the temperature range studied (100 – 450 °C).

Based on the gas dynamics calculations, simulation of temperature gradients and experiments with an in-house built DART source, it was shown[14] that the intensity of a signal depends on position of the sample in the ionization region (often, this was not reported, especially in the early publications on DART-MS). Placing the sample in an optimum manner in the ionization region allows the sensitivity of determination to be increased by an order of magnitude.[51]

Pioneering publications on the improvement of selectivity by varying the temperature in the DART source have been reported in 2009 – 2010. A study[52] of the chemical compositions of eucalypt leaves and small branches revealed the possibility of detecting different classes of compounds depending on their boiling points (at 50, 100, 200 and 300 °C). For instance, methanol, ethanol, acetone, terpenes and sesquiterpenes were detected below 100 °C; a feature of the mass spectra was the presence of signals of $[2M + H]^+$ ions whose intensity decreased as the helium temperature increased. At 200 °C, terpenes with higher boiling points were detected. Above 300 °C, signals of pyrolysis products of plant fibres, *viz*., aldehydes, ketones and substituted furans and benzenes appeared in the spectra.

The design of DART sources allows one to create temperature gradients, but the first publication on DART temperature programming was reported only in 2010.[33] Thermal separation of three components with the same integer m/z ratios, namely, 158.057 ($[M + NH_4]^+$ ion of 5-nitro-1*H*-pyridin-2-one), 158.075 ($[M + NH_4]^+$ ion of *O*-isopropyl methylphosphonofluoridate, sarin) and 158.191 ($[M + H]^+$ ion of *N*,*N*-dimethyloctyl-1-amine) was carried out using ammonia vapours. Temperature programming from 75 to 400 °C at a rate of 1.5 °C s^{-1} caused separation of the components with time due to the differences in the boiling points. Probably, this approach can also be used for DART-MS analysis of more complex mixtures.

5. Voltage at electrodes of the DART source and at the inlet cone of mass spectrometer

The possibility of fragmentation of ions in the DART source with an increase in the voltage at the inlet cone of a mass spectrometer was first reported[2] without discussing experimental data. More recently, the possibility of controlling fragmentation by varying the inlet cone voltage was confirmed in the literature. For instance, an inlet cone voltage of 20 V was used[53] to detect $[M + H]^+$ ions while initiation of collisionally induced dissociation of analytes required a voltage of 40 and 60 V. When detecting, *e.g*., melamine, signals of not only $[M + H]^+$ ions, but also of the $[M + H - CH_2N_2]^+$ and $[M + H - H_2O]^+$ ions and some other fragment ions were observed.

As the cone voltage increased, the extent of fragmentation of drug molecules also increased.[54] Interestingly, in some cases an increase in the voltage to 90 V allowed one to distinguish between different isomers based on the character of fragmentation. The model analyte solutions in methanol had a relatively high concentration of 1 mg ml^{-1}. However, these results contradict the results obtained[55] in the analysis of 25 samples of cocaine, *viz*., isomers of impurities are indistinguishable even at high cone voltages.

Additional information on analytes can be obtained by varying the inlet cone voltage.[39] Three inlet cone voltages (15, 40 and 70 V) caused a minimum, moderate and intense collisionally induced dissociation, respectively. At 40 V, production of dimeric ions $[2M + H]^+$ and $[2M + NH_4]^+$ was minimum. The pattern of the mass spectra changed depending on the voltage. Not only signals of $[M + H]^+$ ions, but also those of $[M - H_2O + H]^+$, $[M - CO_2 + H]^+$, $[M + O + H]^+$ and $[M - CO_2 + H]^+$ ions appeared in the spectrum. Analogously, fragmentation of nucleotides and nucleosides was studied[56] at an inlet cone potential increased to 80 V; the potential difference at the skimmer was 65 V, being appropriate for fragmentation of non-covalently bound adducts.

Nevertheless, it was reported[36] that conventional operation requires an inlet cone voltage of 60 V while higher voltages are needed when fragmentation ‡ is desired.

The maximum (in absolute value) voltage at the inlet cone of the mass spectrometer reported to date is −240 V (negative-ion mode).[21,57] From the available data it follows that, using voltages from −140 to −240 V, one can detect the $^{79}Br^-$ and $^{81}Br^-$ isotopes with high accuracy in the absence of overlapping peaks because all organic ions whose peaks could overlap with the peaks of the bromide ions undergo fragmentation at such voltages. Fragmentation of ions was also facilitated by using a high cone voltage which was increased from 20 to 150 V.[27]

A study of organometallic compounds showed that the signal intensities depend on the voltage at the gas-discharge electrode (needle electrode) of the DART source.[29] The signal intensities increased with the voltage and a value of 4000 V was chosen to be optimum. The intensities of

‡ Fragmentation due to increased cone voltages is called (i) in-source collision induced dissociation (in-source CID)[27,39] or (ii) in-source collision activated dissociation (in-source CAD),[53] which has the same sense as in-source CID.[27,39] However, the term cone-induced dissociation (CID)[25] seems to be more correct in the case of DART mass spectrometry.

analyte signals depended on the voltage at electrodes of the DART source, which contradicts the published data,[26] according to which no significant influence of the voltage at electrodes of the DART source on the signal intensity (the voltage range studied was not reported) was observed. As the voltage at the disk electrode (it serves for removal of ions produced in the source after discharge) changed from −300 to 0 V, the signals of analytes were almost the same, but as this voltage changed from 0 to 100 V, the signal intensities decreased abruptly. Further increase in the voltage had no effect on the signal intensity. At nonzero voltage at the grid electrode needed to minimize ion–ion recombination at the DART source exit, the signals of almost all analytes weakened.

An analysis of publications concerning optimization of DART experiments and fragmentation of compounds belonging to different classes showed that universal conditions providing high sensitivity and unambiguous identification of mass spectra have not been established so far. In our opinion, for efficient optimization of experimental conditions, it is necessary to study in detail the mass spectrum of the background and to choose the conditions for obtaining the most intense signals of water cluster ions because effective production of $[M+H]^+$ ions from analytes in turn requires a high content of corresponding reagent ions. We failed to find publications concerning this approach to the optimization of experimental conditions. On the contrary, the authors of most publications dealt with $m/z \geqslant 50$; this made impossible monitoring of the intensity of the signal of the $(H_2O)_2H^+$ cluster ion which plays a key role in the transfer of a proton to the analyte molecule.[8]

IV. Fields of application of DART mass spectrometry

1. Qualitative analysis

Most often, qualitative analysis by DART-MS involves identification of components of various mixtures based on the m/z ratios; therefore, the possibility of identification of organic compounds depends strongly on the mass spectrometer used.

The first design of the DART source allowed it to be used only with the AccuTOF time-of-flight mass spectrometer. Most studies published so far also have been carried out using this combination. Modern DART sources are compatible with the Agilent and Bruker (Germany) and ThermoFinnigan, Applied Biosystems and Waters (USA) mass spectrometers. A combination of the DART source with an AccuTOF or some other high-resolution mass spectrometer allows one to identify analytes based on the their molecular masses and empirical formulas.

An AccuTOF mass spectrometer has a specified resolution of 6000–7000; the distance between two neighbouring peaks in the mass spectrum at which they are separated at half maximum corresponds to ~0.01 Da at $m/z = 100$ and ~0.1 Da at $m/z = 1000$. The average mass accuracy in the determination of 16 alcohols was ±0.00004 Da.[22] The high accuracy was explained by thorough maintenance of the instrument and by control of experimental conditions. However, in other studies the mass accuracy was usually no better than the specified value (5 ppm using an internal standard for calibration), *i.e.*, 0.0005 Da at $m/z = 100$ and 0.005 Da at $m/z = 1000$.

In the text below we will consider publications concerning quantitative analysis by DART-MS. The publications are grouped based on the type of analyte.

a. Analysis of pharmaceuticals and medicinal substances

Rapid detection of counterfeit pharmaceuticals by DART-MS was for the first time demonstrated taking a study on artesunate-containing antimalarial products as an example.[34] The $[M-H]^-$ or $[M+NH_4]^+$ ions were detected (using ammonium hydroxide; otherwise, only fragment ions produced). The mass spectra of some counterfeit drugs exhibited the $[M+H]^+$ and $[M+NH_4]^+$ ions of artemisinin, as well as $[2M+H]^+$ and $[2M+NH_4]^+$ ions and fragment ions.

More recently, the same research group has reported a number of studies on DART-MS analysis of antimalarial pharmaceuticals[58–62] and a small review on the methods of identification of counterfeit grigs.[63] In 2009, a new source combining DART ionization and desorption electrospray ionization (DESI) was proposed.[64] In 2010, a mixed-type source in which DART ionization was integrated with IR laser ablation metastable-induced chemical ionization (LAMICI) appeared.[65] The latter source was used to obtain the mass spectra of pharmaceuticals and algal tissues. The resolution was determined by the crater size produced under the action of a laser pulse rather than by the diameter of the metastable gas jet. The limit of detection was 15–30 pg. Other publications concerning DART-MS analysis of pharmaceuticals and food additives are also available.[2, 66–71]

The most vantage and fastest is analysis of samples in the form of pharmaceutical tablets. In this case, the DART mass spectra often exhibit only the characteristic signals of the active pharmaceutical ingredients (API) because the matrix is non-volatile. As an example, Fig. 3 presents the DART mass spectrum of Mexidol (API is ethylmethylhydroxypyridine). A strong signal of the protonated API molecule in the DART spectrum provides an instant confirmation of the presence of the desired component in the tablet.[69]

The results of analysis of the API of different pharmaceuticals obtained by DART-MS and HPLC-mass spectrometry (HPLC-MS) were compared.[24] For most analytes, the ESI mass spectra showed signals corresponding to $[M+H]^+$ ions; these signals were also observed under DART ionization conditions, but their relative intensities

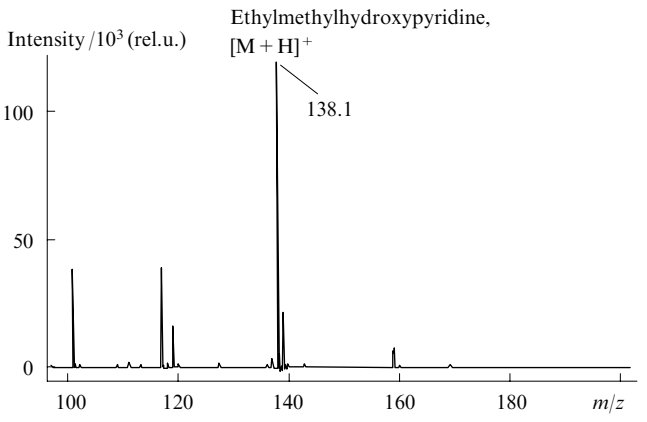


Figure 3. DART mass spectrum of Mexidol tablet.

were one or two orders of magnitude lower than in the former case.

DART-MS can be used in different stages of preclinical pharmaceutical development as a tool for [19]

— identification of reaction products that cannot be detected by HPLC-MS;

— evaluation of the extent of reaction completion in the course of chemical synthesis;

— express analysis of preparative-scale HPLC fractions;

— photodegradation studies of the API in encapsulated pharmaceuticals;

— semiquantitative determination of API in tablets.

DART mass spectrometry has found an application in forensic science as a tool for confirmation of the chemical composition of drugs.[54] The limits of detection of analytes in solutions were about $10-30$ ng ml^{-1}. This method allows one to detect a larger number of impurities compared to GC-MS. However, an analysis of 25 cocaine samples using a DART source coupled with a time-of-flight mass spectrometer showed [55] that the final results of analysis depend on human factor and that for many samples reliable results can be obtained after multiple repetition of the analysis.

A drawback of DART-MS, which makes it of limited use in quality control of pharmaceticals, is costly and cumbersome equipment. At the same time, a mobile less-than-45-kg mass spectrometer equipped with a DART source and suitable for quality control of pharmaceticals was reported.[43]

b. Analysis of products of organic synthesis

A number of studies on characterization of products of organic synthesis by DART-MS are available.[19, 24, 50, 72] DART-MS analysis allow one to characterize poorly soluble compounds whose identification by conventional methods used in organic synthesis is difficult. In particular, DART-MS was used to confirm the chemical composition of insoluble polycyclic aromatic hydrocarbons.[73] Sampling of analyte microparticles was done using a capillary with a closed tip. To perform DART-MS analyses of powders,[19, 25, 54, 72] the samples were preliminarily dissolved in a small volume of solvent or placed in an envelope made of filter paper and then introduced in the ionization region. Recently,[74] DART-MS was applied to analyze powdered samples mixed with magnetic particles taking analysis of mustard powder as an example.

A number of studies is devoted to the fragmentation mechanisms of various compounds in the DART source.[40, 41, 56, 75] For instance, the fragmentation mechanisms of nucleotides and nucleosides in the positive- and negative-ion modes were investigated.[56]

c. Analysis of biomaterials

DART-MS can found application in any field of analysis related to detection of low-molecular-mass components. In the case of bioanalysis, such a field is proteomics for which DART-MS is of limited use because it enables ionization of low-molecular-mass compounds (usually, with molecular masses of at most 1000 Da) only. The applicability of DART mass spectrometry for peptide analysis was suggested earlier;[2] however, no experimental studies on the subject have been reported as yet. Recently, applications of DART-MS to protein studies were reported,[76–78] but the method was used for the determination of only low-molecular-mass compounds, *e.g.*, products of enzymatic reactions.

DART-MS was employed for the determination of taxoid compounds in the callus of *Taxus wallichiana* [30] and for chemical profiling [§] of roots and identification of tropane alkaloids in them.[79] DART-MS is suitable for the identification of (i) methyl esters of fatty acids in cell bacteria [80] and (ii) components in plant samples including wheat,[25] elderberry,[31] eucalypt [52] and bamboo.[81] The structure of cannabioid indole in plant drug raw material illegally traded in Japan was established in a joint DART-MS/GC-MS/HPLC-MS/NMR study.[82] The cultivars of *Piper betle* Lynn. can be differentiated using the DART mass spectra obtained in the analysis of *Piper betle* leaves.[83]

Application of DART-MS for analysis of living organisms gives quite interesting results.[84] The chemical composition of cuticular hydrocarbons (pheromones) from *Drosophila melanogaster* was determined. The technique was non-lethal and nondestructive and involved carefully controlled depressions of the abdomen with a small steel probe followed by introducing the probe into the ionization region. Experiments revealed male and female differences in the chemical composition of pheromones. Also, the compositions of pheromones taken from virgin female flies and after some time intervals upon successful copulation were also different.

Of particular interest is DART-MS analysis of biological fluids and tissues, because drug discovery and the search for biomarkers of various diseases involve analysis of a large number of same-type samples, most often, by HPLC-MS. Here, the most expensive stages include sample preparation and chromatographic separation of analyzed mixtures. Analysis by DART-MS makes it possible to identify amino acids and caffeine in saliva,[2] to determine pharmaceuticals and their metabolites in blood, urea and tissues from different organs,[18, 26] metabolomic profiling [46, 47] and detection of ovary cancer biomarkers in the analysis of derivatized components of serum.[85]

d. Food analysis

γ-Hydroxybutyrate can be determined by DART-MS in alcoholic drinks at a level of 10^{-3}% (Ref. 2) and in salt on the rim of a drinking glass at a level of 100 ng. A method of screening for γ-hydroxybutyric acid in drinks in the negative-ion mode with a detection limit of 0.05 mg ml^{-1} was developed.[86]

DART-MS offers some advantages over GC-MS in the analysis of soft drinks.[87] Antimicrobial additives, synthetic sweetening agents, acidity regulators and saccharides were identified based on the signals corresponding to $[M+H]^+$ or $[M-H]^-$ ions without sample preparation and chromatographic separation.

DART-MS was used to identify melamine in contaminated pet food.[53] An unusual four-step method of identification involves not only the determination and comparison of exact m/z values, but also an analysis of the fragmentation data obtained at increased inlet cone voltages and the data on H/D exchange. The reliability of identification can be improved using the isotope ratios characteristic of compounds of specified chemical compositions.[31]

§ In this case, measurements of mass spectra were used as fingerprints for further studies.

The determination of strobilurin fungicides and pesticides in wheat grains was reported.[25,57] Milled grains to be analyzed were placed in an envelope made of filter paper at $\leqslant 200$ °C. Quantitative analysis was done for ethyl acetate extracts of milled grains at 300 °C.

DART-MS can be used for quality control of olive oils.[27] The mass spectra exhibited signals corresponding to $[M+NH_4]^+$, $[M+H-RCOOH]^+$, $[M+H-RCOO]^+$, $[M+H-RCO]^+$, $[RCO]^+$ and $[RCO-H_2O]^+$ ions. For correct and reproducible operation of the DART autosampler, it was proposed to dilute the samples of olive oils with an organic solvent to a 1 : 1 ratio.

DART-MS was applied to determine the components of garlic and some other alliums and possible mechanisms of reactions involving them.[40,41,75] Signals of some intermediates whose formation was postulated earlier based on the results of calculations were observed for the first time in the DART mass spectra. The $[M+H]^+$, $[M+NH_4]^+$ and $[2M+H]^+$ ions were detected.

DART-MS coupled with Fourier Transform ion cyclotron resonance mass spectrometry (resolution of such a mass spectrometer is 60 000 – 70 000) offers some additional advantages.[35] That study concerns the determination of sugars in the flesh of a red grapefruit using the data for the $[M+NH_4]^+$, $[2M+H]^+$, $[2M+NH_4]^+$ and $[M+NH_4-H_2O]^+$ ions (a container with an NH_4OH solution was placed near the ionization region). Capsaicin can be identified in habanero pepper juice using signals corresponding to the $[M+H]^+$, $[2M+H]^+$ and $[M+O+H]^+$ ions detected in the mass spectra. High relative intensity of signals of dimeric ions was explained by ion – ion and ion – molecule interactions in the hexapole mass spectrometer.

e. Analysis of everyday objects

DART-MS can be used for the determination of phthalates in toys made of PVC.[42] Signals corresponding to $[M+H]^+$, $[M+NH_4]^+$, $[2M+H]^+$ and $[2M+NH_4]^+$ ions were detected in the spectra of phthalate solutions in toluene (concentrations more than 100 μg ml^{-1}). The limits of detection in solid samples were evaluated using plates made of PVC containing phthalates (0% – 25%). The limit of detection was less than 0.1% for the $[M+H]^+$ ion and less than 5% for the $[M+NH_4]^+$ ion. This contradicts the data,[26,27] according to which the sensitivity of determination for the $[M+NH_4]^+$ ion is much higher than for the $[M+H]^+$ ion.

DART mass spectrometry was employed to identify additives (plasticizers, amtioxidants, dyes, stabilizing agents) in food packaging.[51] In a study of the content of additives (plasticizers) in PVC gaskets for lids of glass jars [38] the limits of detection of thirteen different additives using signals corresponding to $[M+H]^+$ and $[M+NH_4]^+$ ions were 0.1% – 5%, but for three more additives no signals were observed even at contents ranging from 5% to 40%. The limits of detection of the same additives in toluene extracts were at least 1%. The possibility of rapid identification of 21 stabilizers in polypropylene samples [32] and of the chemical composition of decomposition products of these stabilizers at $T > 190$ °C was shown. The content of stabilizers in the model samples was about 2 mg g^{-1}.

The possibility of establishing document authenticity by comparing the DART mass spectra characteristic of different types of ink was studied.[88]

f. Analysis of environmental objects

DART-MS in the negative-ion mode can be used for the determination of bromine-containing inhibitors of combustion in various environmental objects [21] including indoor dust as well as the identification of thiabendazole pesticide in plant leaves.[57] A DART-MS/Fourier Transform ion cyclotron resonance mass spectrometry study was used to identify some components of crude oil.[35]

g. Analysis of chemical warfare agents and explosives

The possibility of identification of nitro compounds was mentioned,[2] but experimental mass spectra were reported only for nitroglycerine detected on the surface of a necktie (the NO_2^-, NO_3^-, $C_3H_5O_3^-$ and $[M+Cl]^-$ ions were detected) and some explosives containing at a level of 3×10^{-4} % in water from a muddy pond. The use of DART-MS for the detection of inorganic explosives (ammonium nitrate, sodium azide and sodium perchlorate) are available. Chemical warfare agents can be identified and the mass spectrum of *O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothiolate (VX agent) is reported, which exhibits a signal of $[M+H]^+$ ion (signal-to-noise ratio > 100 000 for less than 1 μg of the substance). Even salts, *e.g.*, *N*,*N*-diisopropylaminoethyl methyl methylphosphonothioic acid (a zwitterionic derivative of VX) can be detected and analyzed by DART-MS. The mass spectrum of this salt was also reported in Ref. 2.

Feasibility of DART-MS for detection of chemical warfare agents on 20 different surfaces was reported.[3] The spectra exhibit signals of peroxide and tetrazine compounds. The appearance of intense signals corresponding to $[M+NH_4]^+$ ions was explained by human factors. The sensitivity of determination was on the average 500 pg per 25 mm^2 of surface area and depended on the skimmer voltage.

Identification of 15 explosives on different surfaces (glass, polyurethane foams, steel, wood, asphalt) was reported.[45] Solid model samples were prepared by drying 2 μl aliquots on Al foil, which resulted in 2 μg deposition of analytes. The surfaces were rubbed with an Al foil. All spectra exhibited signals corresponding to $[M+Cl]^-$, M^- or $[M-H]^-$ ions (with dichloromethane as reagent gas). When no dichloromethane was used, the instrument was tuned to produce $NO_2^{-\bullet}$ radical ions, signals corresponding to $[M+NO_2]^-$ and $[M+NO_3]^-$ ions were observed in the spectra of nitro compounds.

h. Identification of flavours and fragrances

The capabilities of GC-MS and DART-MS (a time-of-flight mass spectrometer was used in the latter case) were compared in the analysis of mint oil.[89] The duration of GC-MS analysis of a sample diluted with methanol to a 1 : 1000 ratio was 10 min, while a DART-MS analysis of neat oil took only 5 s; however, the mass spectrum showed no signals of protonated molecule, which made the identification of components difficult. Signals corresponding to $[M+NH_4]^+$, $[2(M-2H)+H]^+$, $[M-H_2O+H]^+$ ions, *etc.*, were detected.

Analysis of flavours and fragrances deposited on strips (to simulate a surface analysis), fabric and hair, as well as analysis of cooling agents in chewing gums by DART-MS was reported.[20] In addition to the $[M+H]^+$ ion peaks, the spectrum also exhibited peaks corresponding to $[M-2H+H]^+$ ions. An additional confirmation of the

possibility of distinguishing between aromatizing agents based on their mass spectra was reported.[43]

i. Determination of the structure and properties of nanomaterials

A good example of a study on nanomaterials is provided by analysis of self-assembled dodecanethiol monolayers on gold surface by DART-MS.[90] Although in most cases DART-MS is reported as a low-destructive technique, repeated analysis of monolayers is impossible because of structure violation.¶ Structural features of the monolayers were determined based on the m/z of molecular ions produced. Self-assembly of nanostructures from amphiphilic perylene diimide particles to nanostrips and nanofibres in various solvents was studied by DART-MS coupled with UV, fluorescence and NMR spectroscopies.[91]

j. Analysis of inorganic substances

The results of DART-MS analysis of iodine and red phosphorus have been reported.[37] The mass spectrum of an iodine sample exhibited intense signals corresponding to I_2^+, I_3^+, I_4^+, $[I+NH_3]^+$, $[I+2\,H_2O]^+$, $[I_2+H_2O]^+$. The mass spectrum of red phosphorus showed signals corresponding to $[P+O]^+$ and $[P+O_2]^+$ ions.

In a study on the identification of sulfur-containing gases evolved from drywall samples,[92] the following anions were detected: S_2^-, SO_2^-, HS_2^-, SO_3^-, S_2O^-, SO_4^-, S_4^-, *etc*. In the negative-ion mode, the background was clean.

k. Identification of components of mixtures of organic compounds

From the aforesaid it follows that DART-MS is widely used in qualitative analysis. Since the resolution of a time-of-flight mass spectrometer is not high enough and the identification of signals in the mass spectra of different classes of compounds is ambiguous, this instrument is of limited use in DART-MS for the identification of unknown components of mixtures. Usually, the ratio of the number of identified components to the total number of signals detected in the analysis of complex multicomponent systems is small. For instance, only 12%–28% of the total number of detected signals (depending on the sample, ~600–800) were identified.[77] Taking into account the variety of fragmentation pathways of compounds belonging to different classes, one can suggest that a DART source coupled with a time-of-flight mass spectrometer should first of all be used in the search for specified compounds.

Coupling of DART ionization with mass spectrometers having a higher resolution offers great prospects for identification of components of complex mixtures. A DART-MS study using an orbital ion trap was reported in 2010.[38] The m/z ratios were determined to an accuracy of about 0.0001. In addition, DART-MS can be coupled with Fourier transform ion cyclotron resonance mass spectrometry.[35] The m/z values were also determined to the fourth decimal place. Mention was made that DART-MS allows one to detect only volatile low-molecular-mass components. It was assumed that the use of additives (ammonium hydroxide) will favour extension of the set of possible analytes. Contrary to this, we believe that selective ionization using reagent gases will favour reduction of the number of possible analytes; this feature should be used for the differentiation between compounds.

¶ Thus, DART-MS in analysis of nanomaterials cannot be treated as low-destructive technique because in this case violation of the surface nanolayer is important from the standpoint of non-destructive analyses considerations (unlike analysis of more homogeneous objects for which destruction of surface monolayers on going to the interior can be ignored).

2. Quantitative analysis

The intensity of a signal depends on position of the sample in the ionization region; therefore, a problem in quantitative analysis by DART-MS consists in bad reproducibility of positioning. In the early studies, samples were placed in the ionization region manually. More recently, a number of positioning devices were developed; this was followed by a rapid increase in the number of publications concerned with quantitative analysis by DART-MS.

Carrying out quantitative analysis requires construction of calibration curves and, as a consequence, homogeneous reference samples with a specified content of analyte. There is a few studies on semiquantitative analysis of solid samples by DART-MS, but the most accurate results can be obtained in the analysis of liquid samples, because solid reference samples with exact and uniformly distributed concentrations of analytes are difficult to prepare.

a. Analysis of liquid samples

When performing quantitative analysis of liquid samples by traditionally used mass spectrometry techniques, *e.g.*, HPLC-MS, an internal standard is often added to the analyte and the calibration curves are constructed using the analyte-to-internal standard signal ratios rather than the absolute intensities of analyte signals.[93] One can assume that this allows one to compensate the errors in the sample positioning in the ionization region; therefore, internal standards were used in most publications concerning quantitative analysis by DART-MS. However, taking into account the proposed mechanisms of ionization in the DART source (see above), if the analyte and the internal standard have different boiling points, their ionization will occur asynchronously and thus introduce errors in the results of analysis. Indirect evidence for the correctness of this assumption is provided by the results of a study[33] in which thermal separation of analyte signals was attained using a temperature-programmed DART ion source. To date, studies on correlations between the results of quantitative analysis by DART-MS using internal standards and the boiling points of the analytes and standards are unavailable.

Manual sampling of liquid specimens is most often done using glass capillaries with closed tips. For better reproducibility of positioning, an AutoDART HTC PAL (Leap Technologies, USA) autosampler was developed.[94] It provides high throughput of analyses, high accuracy of positioning of each sample in the ionization region and is equipped with interchangeable DIP-It glass tips. The autosampler costs about USD 7000. The DIP-It tips are manually placed in the DIP-It holders (also relatively cheap parts). The DIP-It holders for 1 and 12 tips are available; they provide high reproducibility of positioning compared to the manual input.

There is an alternative type of a probe for liquid samples which is about four times more sensitive.[95] The sample probe was made by coiling twelve turns of 0.12 mm nickel chromium resistance wire around a syringe needle. This probe has a large surface area and can hold large-volume liquid samples. This probe was used to study the kinetics of release of the aromatizing agent from the chewing gum to

saliva. To avoid the matrix effects and the need for using internal standards, the samples diluted with 50% ethanol solution (1 : 50) were analyzed. The lower limit of detection attained with the new probe was 2 ng ml^{-1} (*cf.* about 6 ng ml^{-1} for conventional closed-tip glass capillaries used for DART-MS analyses). The authors of other publications on quantitative analysis by DART used closed-tip glass capillaries or glass DIP-It probes.

The determination of fungicides in wheat grains was carried out [25, 57] with pre-extraction with ethyl acetate (prochloraz was the internal standard) using an AutoDART HTC PAL autosampler and an AccuTOF time-of-flight mass spectrometer. The correlation coefficient of the calibration curves for five fungicides was more than 0.99 in the whole concentration range studied (6 – 1200 μg kg^{-1}, or 3 – 600 ng ml^{-1} with respect to extract). The limits of detection were 5 – 30 ng g^{-1} (or 2.5 – 15 ng ml^{-1} with respect to extract), being 3 to 6 times higher than those attained by HPLC-MS/MS. The advantages of the proposed technique include 'softer' requirements for the extract and, as a consequence, a relatively simple procedure for sample preparation.

The results of quantitative analysis by DART-MS of solutions of four (three phosphorus-containing and one sulfur-containing) chemical warfare agents in dichloromethane and in muddy water from a small natural creek have been reported.[17] The internal standards were deuterated analogues of the analytes. Presumably, samples were introduced into the ionization region manually (no autosampling devices were mentioned). The calibration curves constructed for all analytes were linear (correlation coefficient >0.99) in the concentration range 5 – 1500 ng ml^{-1} for both replicate measurements ($N = 7$) in each solution and for single-measurement procedure. The authors emphasized that analysis is extremely rapid and there is no need for the analysis of blank solutions (a conventional step in quantitative mass spectrometry) because DART-MS experiments do not involve contact between the sample and the instrument. We believe that periodic analysis of 'blank' samples is required because contamination of the entrance part of the vacuum interface by solid particles or microdroplets from the sample may produce a false signal.

Quantification of pharmaceuticals in biological fluids and tissues in preclinical and clinical studies is an important potential field of application of DART-MS. Such studies require analyses of a large array of same-type samples and are usually performed by HPLC/ESI-MS using a triple quadrupole mass spectrometer. The most expensive stages include sample preparation and chromatographic separation.[96] Each sequence of analyses includes at least 18 samples and the analysis takes a few hours to complete. The duration of a single analysis, as a parameter, is important not only from the standpoint of the cost of determination, but also from the standpoint of correctness of the results obtained. Indeed, the sensitivity of the system may vary during the operation over a period of a few hours and this cannot always be compensated by the internal standard technique.[97] Therefore, the use of DART-MS for quantification of pharmaceuticals and their metabolites in biological systems is very topical.

Quantification of pharmaceuticals in plasma by DART-MS using a triple quadrupole mass spectrometer, an AutoDart HTC PAL autosampler and a vacuum interface was reported.[26] The limits of detection of 39 pharmaceuticals in plasma varied from 0.25 to 20 ng ml^{-1}; the limit of detection for 80% of the tested compounds was about 5 ng ml^{-1}. The quantification dynamic range was evaluated for more than ten analytes. The linear range of these compounds was about 0.5 – 2000 ng ml^{-1} for DART and nearly 0.5 – 5000 ng ml^{-1} for ESI-MS. Taking four metabolites (glucuronides) as examples, it was shown that their possible decomposition in the DART source was not followed by an increase in the intensity of the signal of the daughter ion and therefore did not affect the results of analysis. The results obtained in the studies of the pharmacokinetic properties of loperamide and verapamil by HPLC-MS and DART-MS were almost identical. The advantages of DART-MS included no sample preparation and a rapid analysis of samples (54 s), while the drawbacks of the technique were a lower sensitivity and no ionization of some analytes.

Similar results were obtained in a study [18] where the determination of pharmaceuticals in biomaterials was also carried out by DART-MS using a triple quadrupole mass spectrometer. The authors reported that an AutoDart HTC PAL autosampler and the Vapur interface allowed the detection sensitivity to be improved by a factor of 10 to 100 times (presumably, compared to the detection sensitivity of a system with no Vapur interface). At the same time, without the Vapur interface, the system was unable to maintain stable vacuum within the limits of the normal operating pressure of the mass spectrometer.[18] The matrix effects were evaluated for different classes of compounds; the intensity ratios of analyte signals in untreated plasma and in the model solution were from 0.05 to 0.7. The matrix effects were also evaluated for different tissues; the most pronounced matrix effects were observed for brain tissues. In some cases, proteins were precipitated with acetonitrile to reduce the matrix effects. The limits of detection of pharmaceuticals in plasma were at a level of 1 ng ml^{-1} (and lower), which met the requirements of most bioanalytical applications. By and large, there were good correlations between the data obtained by DART-MS and HPLC/ESI-MS, but the former method was 2 to 10 times less sensitive than the latter. As a rule, no internal standards were used. The following drawbacks of DART-MS were recognized:

— due to the absence of chromatographic separation the mass spectra exhibit signals of all components of the mixture introduced into the ionization region; this complicates the interpretation of these spectra and the sensitivity to some specified analytes may reduce owing to the overlap of their signals with the matrix signals and to secondary ion-molecular reactions;

— a trend towards breakdown of some labile bonds in metabolites (*e.g.*, glucuronides) upon ionization and in the absence of the possibility to detect compounds with molecular masses higher than 1000 Da.

The results of pharmacokinetic studies of some biologically active compounds in a nettle extract were reported.[98] The pharmacokinetic curves of nicotinamide, adenine, synephrine and osthole in serum were studied. Peptides, proteins and polysaccharides were separated by ethanol precipitation followed by centrifugation. The supernatant was analyzed by DART-MS. The concentrations of analytes in serum were of the order of 100 ng ml^{-1} and more. The calibration curves were constructed using four points each corresponding to the data obtained from replicate measurements ($N = 7$). Note that neither the

Table 4. Quantification of liquids with DART-MS.

Analyte	Sample	Linear dynamic range	Internal standard	Conditions	Ref.
γ-Hydroxy-butyrate	urea	to 800 μg ml^{-1}	γ-hydroxybutyrate-d_6 (50 μg ml^{-1})	autosampler not mentioned	see [a]
39 Pharma-ceuticals	plasma	0.5 – 2000 ng ml^{-1}	no internal standard was used for most analytes	autoDart HTC PAL autosampler and Vapur interface to improve sensitivity	26
Novel pharma-ceuticals	biomaterials (plasma, tissue homogenates)	minimum concen-tration 0.1 ng ml^{-1}, linear dynamic range is 3 – 4 orders of magnitude	using an isotopically labeled standard or without the use of an internal standard (accuracy and reproducibility were comparable)	the same	18
Nicotinamide, adenine, synephrine and osthole	serum	of the order of a few ng ml^{-1}	internal standard not mentioned	autosampler not mentioned	98
Promazine	urea	1 – 500 μg ml^{-1}	chloropromazine, 50 μg ml^{-1}	the same	6
Chemical warfare agents (4 compounds)	solutions in dichloromethane	5 – 1500 ng ml^{-1}	isotopically labelled standards	autosampler was not used. 7 sample introductions for each concentration	17
Strobilurin fungicides	crops and their extracts	12 – 1200 ng ml^{-1}	proxhloraz (250 ng ml^{-1})	autoDart HTC PAL autosampler	25

[a] http://www.jeolusa.com/RESOURCES/AnalyticalInstruments/DocumentsDownloads/tabid/337/Default.aspx?EntryId = 44

internal standard nor an autosampler were specified. A 3 μl sample was deposited on a borosilicate capillary which was placed in a helium plasma jet and held there 'until the entire sample was consumed'. The sample introduction procedure was only briefly outlined; the general idea of quantification by DART-MS of a fixed volume of the sample ignoring its positioning during a few seconds deserves more thorough studies. The pharmacokinetic properties of flavonoids in an elderberry extract enriched with phenolic acids, polyphenolic compounds and other flavonoids were studied.[99] Slow-dissolve lozenges containing this extract were administered to patients and then the concentrations of the extracts in blood were studied. As in Ref. 98, the preparation of serum samples also involved precipitation with ethanol and centrifugation. A capillary coated with 10 μl of extract was introduced into the ionization region and held there until complete disappearance of the signal of the sample. Demethoxycurcumin and methyl linoleate were used as internal standards. The maximum concentrations of cyanidin in serum determined by DART-MS and HPLC-MS were 3.1 ± 1.0 and 5.4 ± 2.8 nmol litre^{-1}, respectively. It was concluded that the DART-MS and HPLC-MS data are comparable; however, the pharmacokinetic curves obtained by these methods for the same samples are significantly different.

The results of DART-MS quantification of various liquids are presented in Table 4.

When considering these data, one is getting the impression that quantification by DART-MS using internal standards (most often, isotopically labelled ones) is possible in the concentration range from a few ng ml^{-1} to several μg ml^{-1}. However, data that contradict this conclusion are also available. For instance, it was shown [51] that, despite the use of an AutoDart-97 CTC PAL autosampler, the signal was nonlinear and the content of additives (plasticizers, dyes, *etc.*) in the model solutions could not be determined. The correlation coefficient for the determination of bis(2-ethylhexyl) ester of benzene-1,2-dicarboxylic acid in the concentration range 0.1 – 100 μg ml^{-1} was less than 0.45. No signal of the analyte was detected at concentrations lower than 0.1 μg ml^{-1}. Experiments were done on a triple quadrupole mass spectrometer, the DART source was mounted using the interface for atmospheric pressure ionization sources. A direct analysis of curcumin in drinks [100] was characterized by a correlation coefficient of 0.9823 for the calibration curves in the concentration range 5 – 100 μg ml^{-1} (model solutions of curcumin in acetone); this indicates high reproducibility of analyses. However, the results obtained by DART-MS and by HPLC-UV differ by 44%, *i.e.*, the results of DART-MS analysis were incorrect for quantification of curcumin. Presumably, this can be due to the matrix effects.

b. Analysis of solid samples

The feasibility of semiquantitative [19] analysis of API in pharmaceuticals was shown taking 100-mg tablets containing 0.25, 2.5 and 25 mg of an API (not specified) as examples. Tablets were positioned in the ionization region with tweezers. For each API dose, replicate measurements ($N = 5$) were analyzed, and the average height of the API peak detected in the single ion monitoring mode and corresponding to the protonated molecule was used to construct a calibration curve with a correlation coefficient of 0.9998; this indicates good reproducibility. Although no experimental data were reported, it was concluded that DART-MS can be used for express analysis of API in

pharmaceutical tablets provided a rather high percentage of the API. Probably, the correctness of quantitative analysis was low.

An automated sample holder (IonSense, USA) for analysis of pharmaceutical tablets and, probably, for better reproducibility of their positioning in the ionization region is commercially available; however, no studies on its application have been reported so far.

The calibration curves for analysis of curcumin in curry powder [100] were constructed using reference samples (curcumin-adsorbed silica gel) prepared by dissolving curcumin in methanol, adding silica gel, concentration *in vacuo* and mixing with silica gel containing no curcumin. The correlation coefficient of the calibration curve was 0.9093 in the concentration range $(5-100)\times10^{-4}$%. The accuracy of the measurements of the curcumin content in drinks and curry powder was evaluated by comparing the DART-MS and HPLC-UV data. The results of determination of curry powder differed by 10%. Probably, the matrix effects were minimum when using the reference samples; however, this is to be revised.

The possibility of semiquantitative analysis of aromatic strips with deposited flavours and fragrances using a DART source coupled with a triple quadrupole mass spectrometer was reported.[20] The relative standard deviations of peak heights varied from (6–15)% to 40%. At best, the limit of detection was 100 pg. Analytes (aliquots of 10 μl) were deposited on strips. It was concluded that semiquantitative analysis requires standartization of the shape of the samples.

A custom-designed sample holder was fabricated to position insecticide-treated bednets for malaria prevention in the ionization region. The bednet was fixed between the DART source exit and the mass spectrometer inlet, exposing a 20 mm^2 bednet area to the gas stream. Semiquantitative determination of insecticides in bednets by DART is possible.[28] The correlation coefficient was $\geqslant 0.983$; the limit of detection of deltamethrin was 0.5 mg m^2 for both helium and nitrogen used in the DART source. No data on lower levels of deltamethrin were reported in that study.

An autosampler was home built to pull cotton swabs with analytes in the ionization region. The analytes were transferred to the swabs from corresponding surfaces by rubbing.[39, 101–104] This device can be used for semiquantitative determination of chemical compounds deposited on different surfaces.

From the aforesaid it follows that the potential of DART-MS for quantitative analysis of solid samples has been studied poorer than for analysis of liquids. The limits of detection of analytes in solid samples, expressed in percent, are a few orders of magnitude higher than for liquid samples. We believe this can be explained by the fact that in DART-MS analysis of liquids, ionization of analytes is accompanied by evaporation of the solvent (in fact, the sample is concentrated). In the case of solid samples, ionization of the sample produces no changes in its volume and mainly occurs from the surface; the most part of the analyte distributed in some way inside the sample is not transferred to the detection system. Therefore, a comparison of the limits of detection for the solid and liquid samples seems to be incorrect.

V. Coupling of DART mass spectrometry with sample preparation and chromatography

Initially, the main advantages of DART-MS included no sample preparation and chromatographic separation. However, recently, studies on the extension of the capabilities of determination of chemical compounds by coupling DART-MS with sample preparation and different kinds of chromatography have been reported.

1. Coupling with sample preparation

To carry out rapid screening of urea for drugs, it was proposed to use coupling of microextraction by packed sorbent with DART-MS.[105, 106] In direct inlet systems, the intensities of urea signals are not high enough. The aim of sample preparation is to remove impurities, to transform analytes to more appropriate forms (presumably, ammonium derivatives because elution occurred in the presence of ammonium hydroxide) and pre-concentration to improve the detection sensitivity. Extraction was performed with a microsyringe (volume 100–250 μl). A microcartridge filled with the sorbent (1–2 mg) was placed between the barrel and the needle. The volume of the sample passing through the microcartridge was to 250 μl while the volume of organic wipe was 20 to 50 μl. The limits of detection of ecgonine methyl ester, benzoylecgonine, cocaine and cocaethylene in urea were 4–24 ng ml^{-1}. Extraction of a single sample took at most 2 min; a total of 5 μl of preconcentrated sample was placed in the gap between the DART source and the orifice of the mass spectrometer. Quantitative analyses were performed using internal standards (deuterated analogues of the analytes). The lower limits of detection were 65–95 ng ml^{-1}. A comparison with other methods gave the following results.

Method	I	II	III	IV
c /ng ml^{-1}	65–95	10–500	~10	~100–260

Here I stands for the combination of the DART source with microextraction by packed sorbent, II stands for GC-MS, III stands for HPLC-MS; IV stands for HPLC-UV; and c is the lower limit of detection.

The sensitivity of DART mass spectrometry is not high enough for the determination of UV filters (benzophenone, 2-ethylhexyl *p*-(*N*,*N*-dimethylamino)benzoate, 4-*tert*-butyl-4′-methoxydibenzoylmethane, homomenthyl salicylate, 2-ethylhexyl salicylate, octocrylene and 4-methylbenzylidenecamphor) in environmental water, therefore, it was proposed to combine DART-MS with stir bar sorptive extraction.[48] A 2-cm long stir bar coated with a 0.5-mm polydimethylsiloxane layer was used. Analytes were concentrated from liquid samples on the surface of the bar and then the bar was placed in the DART ionization region to record the mass spectra. This enabled semiquantitative determination, *viz*., the correlation coefficients were higher than 0.959, the reproducibility varied from 5% to 30%, the limits of detection were less than 40 ng $litre^{-1}$ and the volume of the sample extracted was 250 ml. A comparison of the results of determination obtained by DART ionization and by GC-MS with thermal desorption showed that in the former case, the limits of detection were 1 or 2 orders of

magnitude higher than in the latter case depending on the nature of the analyte. However, DART-MS provided high throughput of analyses and the detection of analytes at a level of tens of nanogrammes per litre, which approximately corresponded to the concentration of analytes typically found in surface water affected by swimming activities. It was suggested to use this approach for pre-screening and selection of a limited set of samples which can then be analyzed by more sensitive methods, *e.g.*, thermal desorption GC-MS.

2. Coupling with high-performance liquid chromatography

The first study on coupling of HPLC to DART-MS has been reported in 2010.[107] Such a combination was practically implemented using a simple interface device for transfer of the HPLC effluent from the chromatograph to the ionization region. The method HPLC/DART-MS offers some advantages over traditional HPLC-MS. It also differs from atmospheric pressure chemical ionization in that under the DART conditions the discharge region and the ionization region are spatially separated. It is important that HPLC/DART-MS allows one to use conventional salt buffers (including phosphate ones) for HPLC; this considerably extends the scope of the method. It was established that under the DART ionization conditions the mass spectrometer is a mass rather than concentration detector. The optimum parameters for the operation in the negative-ion mode are as follows: eluent flow rate 1 ml min^{-1}, helium temperature 300 – 350 °C and grid electrode voltage −75 eV. The analytical characteristics of the method have been studied. For instance, the limits of detection of parabenes in the negative-ion mode were 20 – 55 ng ml^{-1}, the linear dynamic range was 200 – 10 000 ng ml^{-1}; for pyrazine derivatives, the higher detection sensitivity was attained in the positive-ion mode, the limits of detection were 0.1 – 0.3 ng ml^{-1}; the correlation coefficient for the concentration range studied (1 – 50 ng ml^{-1}) was more than 0.993.

3. Coupling to gas chromatography

The possibility of coupling DART-MS to gas chromatography was reported recently.[9] To this end, the capillary column was extended by 0.5 m outside of the gas chromatograph and placed in a copper tube heated to 250 °C. Approximately 0.5 cm of the column was extended beyond the end of the copper tube directly in the DART ionization region. The capabilities of such a combination were tested using a model mixture of hydrocarbons. Depending on the distance between the DART source exit and the orifice of the mass spectrometer, one of two different ionization mechanisms dominated and the mass spectra resembled the electronic ionization (at a distance of nearly 5 mm, molecular ions were the most abundant) or chemical ionization (at a distance of about 15 mm, protonated molecules dominated) mass spectra. In the latter case, the detection sensitivity was nearly tenfold lower. The advantages of GC/DART-MS analysis include the possibility of using higher carrier gas flow rates and another ionization mechanism, which allows one to detect compounds that are difficult to analyze by conventional GC/EI-MS technique (*e.g.*, corrosive compounds).

4. Coupling to thin-layer chromatography

Coupling of thin-layer chromatography (TLC) to DART-MS is of particular interest for analysis of liquid samples with complex chemical compositions (*e.g.*, various extracts, tinctures, drinks, biological fluids).† The advantages of planar chromatography and DART mass spectrometry over HPLC include the possibility of detection of all components in the sample including those retained in the starting zone of the chromatogram, no limitations in the choice of eluent and no 'memory effects'.[108, 109]

The first publication on coupling of DART-MS to high-performance TLC (HPTLC) was reported in 2006.[110] This type of analysis was used to determine isopropylthioxanthone in milk, yoghurt and fat and thus to confirm the data obtained by HPTLC with fluorescence detection (HPTLC-FLD) and HPTLC/ESI-MS. Coupling of HPTLC to DART-MS involved no pre-extraction of analytes from the TLC plate for subsequent mass spectrometric analysis. A spot whose position on the HPTLC plate was preliminarily determined by densitometric scanning, was marked with a pencil. The zones of interest on the plate were cut and analyzed by DART-MS. Isopropylthioxanthone containing in the spot was identified at a content of 3.2 ng (and more).

The possibility of coupling HPTLC to DART-MS was also demonstrated for rapid determination of acrylamide in drinking water.[111] HPTLC/DART-MS was used to confirm the data obtained by HPTLC-FLD and HPTLC/ESI-MS. It was also shown [112] that HPTLC/DART-MS is suitable for quantitative analysis of isopropylthioxanthone and caffeine solutions. With no internal standard under manual positioning of the HPTLC plate the correlation coefficient of the calibration curve was 0.8202 (*cf.* a value of 0.9892 obtained using an isotopically labelled internal standard). The signal-to-noise ratio for the signal from a spot containing about 1 ng of isopropylthioxanthone was 84. Additives to mineral oils can be identified taking organozinc compounds as analytes.[113] The mass spectrum exhibited no signals corresponding to $[M+H]^+$ ions, but relatively intense signals corresponding to fragment ions of zinc-free analyte molecules were observed. HPTLC/DART-MS was used to confirm the data obtained by other analytical methods.

TLC/DART-MS can be used for monitoring the extent of completion of organic reactions in real time. Some compounds with molecular masses above 600 were detected at 500 °C (temperature in the DART source).[50] In these cases, TLC separation was performed on glass-backed TLC plates because the plastic plates will melt at temperatures above 250 °C. In addition, TLC/DART-MS is suitable for the separation and identification of curcumin, demethoxycurcumin and bisdemethoxycurcumin in turmeric extracts.[100]

Coupling of HPTLC to DART-MS provides a spatial resolution better than 1 mm and can be used to distingush between two bands separated by a distance of 3 mm. The spatial resolution of DART-MS requires further studies, especially in connection with the fact that a new model for the DART ion source was developed, which enables better positioning of samples and allows one to scan the whole TLC plates (without cutting them). Earlier, before the new model of the ion source, the gas stream was directed into the mass spectrometer, *i.e.*, horizontally. This imposed limitations on analysis of TLC plates because the sample was

† Most studies on the subject were carried out under the supervision of Prof. G Morlock (Stuttgart, Germany).

positioned in the ionization region in such a manner that the gas stream passed across its lateral surface and the sample did not block gas supply to the mass spectrometer. The new model allows one to vary the tilt angle of the DART source relative to the input orifice of the mass spectrometer within 45°. No studies of analytical characteristics and new possibilities provided by this configuration of the DART source were reported as of the end 2010. Of particular interest, in our opinion, is the possibility of analyzing TLC plates. A 3D scanner for studies of wide surfaces was proposed along with this modifcation of the DART source. It will enable coupling of DART-MS to planar chromatography and the use of DART-MS to obtain 2D mass spectrometric images of the surface distruibution of low-molecular-mass compounds. This approach is attractive for mass spectrometric analysis of histologic slides, which at present is most often performed by MALDI mass spectrometry[114] which is of limited use for the analysis of low-molecular-mass compounds due to the overlap of the signals of analytes with the signals of the matrix. In this connection one should determine the spatial resolution under DART ionization conditions and make efforts at optimizing it.

VI. Conclusion

Since DART mass spectrometry has emerged quite recently, its methodology is far from being clearly formulated and many potential fields of application have not been studied as yet. An attractive feature of the method is the absence of sample preparation and chromatographic separation. This considerably shortens the duration of experiments and makes the analysis much cheaper. However, the available information on qualitative and especially quantitative analysis is still scarce and difficult to systematize. These data were obtained for different types of compounds in different matrices. The effect of particular parameters of the ion source and the mass spectrometer on the analytical characteristics has not been studied for a representative set of compounds and matrices. It is unclear how quantitative measurements should be carried out, *viz*., is it possible to operate without positioning devices and to use internal standards not labelled with stable isotopes. Methods of preparation of solid reference samples and quantitative parameters of their analysis have not be studied systematically. Specific features of the coupling of DART-MS to different types of mass spectrometers and the analytical potential of such combinations are still to be studied. Reactive DART-MS requires further development, especially for analysis of nonvolatile substances. In this case, the experience accumulated in ESI mass spectrometric investigations may appear to be useful.[115–117] Progress in the methodology of qualitative and quantitative analysis by DART-MS can be achieved only based on complex investigations using a representative set including moderate-volatile and nonvolatile organic compounds using different matrices and substrates for liquid samples.

The dependences of detection sensitivity on the type of analyte, on the nature of the sample, on the procedure for sample introduction and on reagent gases are to be elucidated. For instance, published data on reagent gases are reduced to ammonia vapours for production of $[M + NH_4]^+$ ions and dichloromethane for production of $[M + Cl]^-$ ions. Also, $[M + CF_3COO]^-$ ions may appear when using trifluoroacetic acid. Regularities of the appearance of signals corresponding to $[M + NH_4]^+$ ions in DART mass spectra require further investigations because, according to published data, these ions are also often observed in the absence of ammonia vapours in the ionization region. In particular, signals corresponding to these ions in the DART mass spectrum were observed[118] even for poly(ethylne glycol) which is most widely used for calibration in the coupling of DART ionization to high-resolution mass spectrometry. As an example, Fig. 4 presents the mass spectrum with a series of intense signals corresponding to $[H(OCH_2CH_2)_nOH + NH_4]^+$. It seems of great promise to establish the conditions under which the number of signals in a DART mass spectrum will be equal to the number of components in the system under study. This will considerably enhance the potential of DART-MS in the analysis of complex mixtures of unknown compounds.

The possibility of improving the detection sensitivity due to an increase in the surface area of the tip used for sampling of liquid analytes has been poorly studied. Improvement of detection sensitivity upon a change in the tip material (polyimide-coated glass) was reported in a sole study.[26]

The possibility of sorptive extraction of analytes from liquid solutions followed by placing the sorbent in the DART ionization region should also be studied thoroughly. There is only one publication[48] on the subject in which analytes were concentrated using a magnetic stir bar coated with polydimethylsiloxane.

Potentially, coupling of DART-MS to TLC provides a unique chance for using the ultrahigh selectivity of mass spectrometry in direct analysis from plates in gas stream (online) without extracting agents that make the spots diffuse and violate the chemical compositions of the spots due to the presence of impurities. Potentially, in this case one can also detect those components of the samples that do not move with the mobile phase (this is an advantage of TLC/DART-MS over the HPLC-MS technique). However, to date the capabilities of TLC/DART-MS have been studied fragmentarily using a few systems and analytes. One can assume that a drawback of TLC/DART ionization consists in the motion of analytes into the interior of the stationary phase on the TLC slide. In this connection, coupling of TLC to DART-MS offline (with pre-extraction of spots from the TLC slide using a CAMAG extractor) still can offer some advantages. It is important to compare the advantages and drawbacks of the coupling of TLC to mass spectrometry with online and offline DART ionization.

DART-MS analysis of dried spots of biological fluids (especially, urea and saliva which is important for non-invasive diagnosis) on filter paper or on some other support seems to be an attractive field of application. This approach was proposed in 2009 for the analysis of blood spots on paper using other methods of ionization.[119, 120] Application of this approach in clinical diagnosis will simplify the storage, transportation and analysis of biological fluids.

As to other potential fields of application, no studies have been reported so far in the following areas which seem to be particularly promising for further progress of DART-MS:

— rapid screening of various dosage forms including not only identification, but also quantification of API;
— analysis of bee products;
— screening of biological fluids for doping agents;
— identification of drugs in hair and nails;

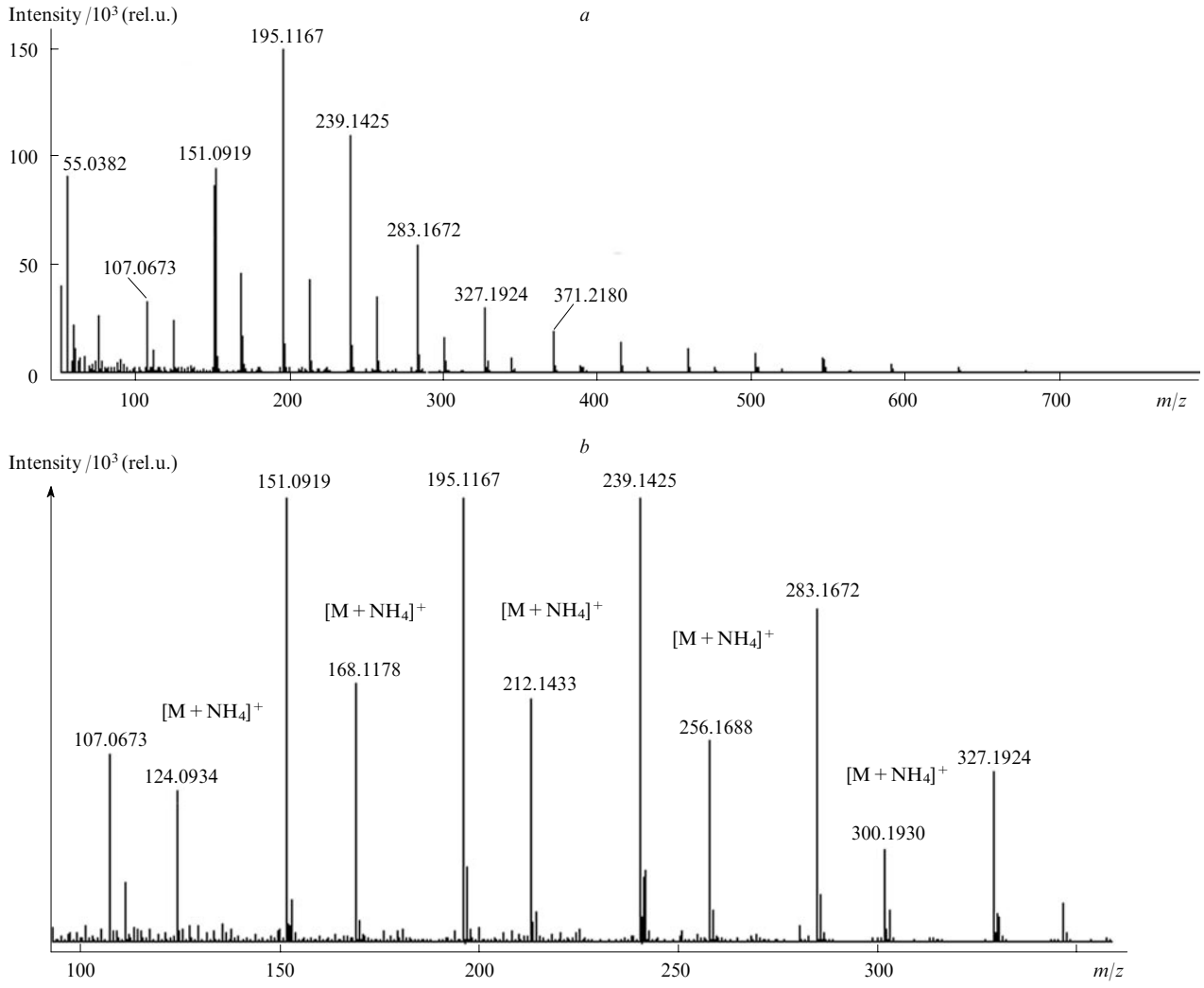


Figure 4. Mass spectrum of poly(ethylne glycol) (*a*) and its magnified fragment (*b*).
Shown are strong signals of detected characteristic ions $[M + 18]^+$, where $M = H(OCH_2CH_2)_nOH$.

— coupling of different kinds of TLC and DART-MS for analysis of complex multicomponent mixtures.

Extension of capabilities and the evolution of DART mass spectrometry require systematic investigations of its analytical potential and the development of a methodology including the research and optimization of instrumental parameters that affect the analytical characteristics of the method, elaboration of approaches to improvement of these characteristics, as well as expansion of DART-MS/TLC studies. Probably, in the nearest future one should expect an increase in the number of publications concerning the scope and limitations of DART mass spectrometry and on novel fields of application of this method

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