7.6 AccuTOF-DART Mass Spectrometry

7.6.1 Introduction

7.6.1.1

Direct Analysis in Real Time (DART) is an atmospheric pressure ionization technique that can analyze solids, liquids and gases by placing the test material into a heated gas flowing through the sampling area. Ionization occurs from the surface of the sampling medium. This ion source is coupled with an accurate mass time-of-flight mass spectrometer (AccuTOF).

7.6.1.2

While ionization can be done in both positive and negative mode, the large majority of chemical materials give usable spectra in positive ion mode. Ionization in positive ion mode is accomplished by charging a heated helium gas stream, forming metastable helium ions which react with ambient water vapor, producing hydronium ions which subsequently react with the sample molecules to induce ionization. The mechanism of positive and negative ion production with the DART is discussed by Cody, et. al. (See Reference 7.6.3.1)

7.6.1.3

In general, DART ionization produces spectra with a characteristic peak of the protonated or deprotonated molecular ion. These ions are measured at their exact mass in the AccuTOF mass spectrometer. Elemental composition calculations, based on empirical formulas, can be performed on these ions to determine whether they fall within a specified range, usually measured in millimass units (mmu), of a known compound. Full identification by the protonated or deprotonated molecular ion only is not possible if other isomers exist.

7.6.2 General Screening and Negative Ion Methods

7.6.2.1 Instrumentation, Instrument Parameters and Materials

7.6.2.1.1

The DART ion source is coupled to a JEOL AccuTOF™ mass spectrometer (JMS-100LC) and operated in either positive-ion or negative-ion mode. This system is controlled by "Mass Center" software.

7.6.2.1.2

Method parameters are listed in Table 1 below. Parameters including Mass range, DART Temperature and Ionization mode may change to best suit the analytes in question. Ionization mode and ionization temperature should be clearly delineated in the title of the data file (example LS11-3211_DartPos250deg)

Table 1: Method Parameters for DART_POS_SWITCH_01 (General Screening Method - Positive Mode) and for DART_NEG_SWITCH_01 (Negative Ion method - negative ion mode)

Parameter	Setting	Parameter	Setting
IonGuidePeak Voltage	600V	MassRange	60 - 600 Daltons (Da)
Orifice1 Voltage	Variable (20V, 30V, 60V, 90V)	Helium Flow Rate	2.5 L/min.
Spectrum Recording Interval	Every 0.25 seconds	Gas Heater Temperature	250 ºC
Orifice2 Voltage	Approximately 5V	Discharge Electrode Needle	4000V
Ring Lens Voltage	Variable Approximately 3-6V	Electrode1	7.60V
Orifice1 Temperature	80 ºC	Electrode2	250V

7.6.2.1.3
Internal mass calibration is accomplished using a dilute solution of polyethylene glycol (PEG) 600 (Emerald Biosystems, Bainbridge Island, WA) in methanol.

7.6.2.1.4
Separate tune files need not be established where the only difference is in the orifice1 voltage.

7.6.2.1.5
Blanks of the glass melting point tube utilized for the sample (liquids) or empty forceps (solids) should be run prior to the sample within the run. If any contaminant is present that interferes with the analyte peaks of interest, the blank and sample shall be re-run.

7.6.2.2 Procedure

7.6.2.2.1
Although samples may be run in any chemical state, it is recommended that powders, tablets and capsules be dissolved in a suitable solvent (e.g., methanol, methylene chloride, ammonia saturated chloroform). If the sample does not ionize suitably in a solvent, solid samples may be introduced.

7.6.2.2.2 I
n general, liquid samples are run by dipping the closed end of the glass melting point tubes into the sample solution and then immediately inserting the tube into the DART gas stream for several seconds. Replicate samplings (2-3) are recommended within the data file to more fully represent spectra of the analytes of interest. Let the melting point tube cool briefly between samplings in order to achieve better consistency when sampling volatile solvents. Other sampling methods (e.g., solids, plant materials, gases, dried liquids) may be also be run by holding with forceps and inserting directly into the DART gas stream.

7.6.2.2.3
Bring DART and AccuTOF out of standby mode, load an appropriate DART tune file and set the AccuTOF to "Operate".

7.6.2.2.4
PEG 600 calibrant solution shall be run within each data file. Replicate samplings (2-3) are recommended for proper internal mass calibration.

7.6.2.2.5
In positive ion mode a methyl stearate standard shall be run within each casework data file. In negative ion mode, a stearic acid standard shall be run within each casework data file. The methyl stearate protonated molecule (299.295 Da) or the stearic acid deprotonated molecule (283.2637) can also be used for drift compensation. Caffiene or other standard can also be run in any data file as a second accuracy check.

7.6.2.2.6
After data collection has finished, the DART ion source should be switched to standby to conserve helium.

7.6.2.2.7
Averaged, background subtracted, centroided spectra are used for all data reduction. Data reduction may be done in TSSPro software.

7.6.2.2.8
Mass calibration is performed utilizing the PEG600 spectra in the 20V function. This calibration can be performed in either the JEOL or TSSpro software when data is generated.

7.6.2.2.8.1
Data files, each of which include a calibration data file, are archived. Overwriting the working calibration file is acceptable since they can be regenerated.

7.6.2.2.8.2
Internal mass calibration should be applied to all functions where data will be used.

7.6.2.2.9
If manual mass drift compensation is performed, only the 20V orifice 1 setting is used. In the TSSPro software, the mass in question is set as the reference mass, and the exact calculated value is entered. A new (one point) calibration is then performed. For this reason, whenever manual drift compensation is used, the compound used should be near the sample compound in molecular weight.

7.6.2.2.9.1

Overwriting the working calibration file is acceptable since they can be regenerated.

7.6.2.2.9.2

If used, internal drift compensation calibrations are applied to all samples and standards (and all orifice 1 voltage settings) introduced in a data file.

7.6.2.2.10

In positive ion mode, after performing PEG600 internal mass calibration, the protonated molecule (20V setting) of methyl stearate shall be within ± 5 mmu of the calculated value of 299.295 Da. In negative ion mode, after performing PEG600 internal mass calibration, the deprotonated molecule (20V setting) of stearic acid shall be within ± 5mmu of the calculated value of 283.2637 Da.

7.6.2.2.10.1

The spectrum demonstrating that the methyl stearate protonated molecules, or the stearic acid deprotonated molecules are within the above acceptance criterion shall be included in the case record.

7.6.2.2.11

Data processing : Spectra should be saved in JEOL-DX format for use by other data reduction software (e.g., TSSpro, SearchFromList, Elemental Composition Workshop). Repeat as necessary for spectra at other orifice1 settings.

7.6.2.2.12

Return AccuTOF and DART to standby mode. Do not turn the DART gas off before the temperature is below 200C.

7.6.2.3 Data Interpretation

7.6.2.3.1

Data from the 20V function is used to obtain information regarding molecular weight and should be searched against the 20V Mass library.

7.6.2.3.2

Data from functions with higher Orifice1 values result in greater fragmentation, and can be searched against libraries or databases for those voltages. Care must be taken to evaluate search results using both types of information.

7.6.2.3.3

Comparison of spectra to in-house library/database search results, when available should be included in the case record.

7.6.2.3.4

Identification for any compound by this method must include comparison with a reference standard, examined after the sample, run on the same day as the sample. The sample must be within ± 5 mmu of the standard, at all orifice 1 voltages, for identification. Indentifications must also include similar fragmentation of the sample and standard at higher voltages. Associations made between a sample and a known or control material need only meet the ± 5 mmu tolerance for a representative number of component peaks at the 20V orifice 1 setting.

7.6.3 References

7.6.3.1

Cody RB, Laramee JA, Nilles JM, Durst HD. "Direct Analysis in Real Time (DART) mass spectrometry" JEOL News 2005; 40(1): 8-12.

7.6.3.2

JEOL, Inc. Application note: LC/MS: Identification of unknowns by combining exact mass measurement with the NIST 02 Mass Spectral Database Similarity Search, www.JEOL.com/ms/accutof.html, January 2003.

7.6.3.3

Laramee, J, Cody RB, Nilles, JM and Durst HD, "Forensic Application of DART (Direct Analysis in Real Time) Mass Spectrometry)" in Forensic Analysis on the Cutting Edge, Wiley Interscience, Hoboken, NJ, 2007, pp. 175-195

7.6.3.4

Pfaff, AM and Steiner, RR, "Development and validation of AccuTOF-DART™ as a screening method for analysis of bank security device and pepper spray components", Forensic Science International, July 2010