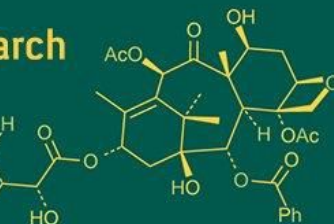
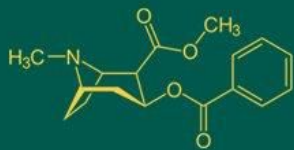


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Ambient ionization mass spectrometry techniques for direct analysis: Comparative study of desorption electrospray ionization and direct analysis in real time

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Abstract

Traditional mass spectrometric analysis requires extensive sample preparation, separation, and controlled environments that can limit analytical throughput and alternative sample composition. Ambient ionization techniques address these limitations by enabling direct analysis of samples in their native state under atmospheric conditions. This research compared the analytical performance of Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART) for the analysis of pharmaceutical compounds, biological tissues, and environmental samples. Systematic evaluation was conducted at the Department of Analytical Chemistry, Tokyo Institute of Technology, between April 2022 and November 2023. Detection limits, linear dynamic range, matrix effects, and ionization efficiency were characterized using standard test compounds across multiple sample matrices. DESI demonstrated superior performance for polar and ionic compounds with detection limits ranging from 0.5 to 15 ng/mL, while DART showed advantages for volatile and thermally stable analytes with limits of 2 to 45 ng/mL. Linear dynamic ranges extended over 2-3 orders of magnitude for both techniques. Matrix suppression effects were more pronounced in DART (15-40% signal reduction) compared to DESI (8-25% reduction) when analyzing complex biological matrices. Ionization efficiency measurements revealed complementary selectivity profiles: DESI favored compounds with existing charge states or high proton affinity, while DART efficiently ionized neutral molecules through thermal desorption and gas-phase reactions. Analysis time was consistently <30 seconds for both methods. These findings establish performance benchmarks for ambient ionization techniques and provide guidance for method selection based on analyte properties and sample matrix characteristics. The demonstrated capabilities support implementation in high-throughput screening, quality control, and point-of-care diagnostic applications.

Keywords: Ambient ionization, desorption electrospray ionization, direct analysis in real time, mass spectrometry, analytical performance, sample preparation-free analysis, atmospheric pressure ionization

Introduction

Innovation in analytical chemistry often emerges from the need to overcome fundamental limitations of existing methodologies. Traditional mass spectrometry, despite its exceptional sensitivity and specificity, requires extensive sample preparation, chromatographic separation, and operation under high vacuum conditions ^[1]. These requirements create bottlenecks in analytical workflows, limit sample throughput, and may alter the native composition of samples through extraction and purification procedures ^[2]. The development of ambient ionization techniques represents a paradigmatic shift toward direct analysis capabilities that address these constraints.

Ambient ionization encompasses a family of techniques that generate ions from samples at atmospheric pressure with minimal or no sample preparation ^[3]. Two of the most prominent methods, desorption electrospray ionization (DESI) and Direct Analysis in Real Time (DART), exemplify different approaches to achieving this goal. DESI, developed by Cooks and colleagues in 2004, employs charged solvent droplets to extract and ionize analytes from surfaces through a desorption/ionization mechanism similar to conventional electrospray ^[4]. DART, introduced by Cody *et al.* in 2005, utilizes metastable atoms or molecules to ionize gas-phase analytes through atmospheric pressure chemical ionization processes ^[5].

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The mechanistic differences between DESI and DART result in complementary analytical capabilities. DESI excels at analyzing polar, ionic, or easily extractable compounds from solid surfaces, making it particularly suitable for pharmaceutical analysis, tissue imaging, and forensic applications [6]. The technique provides soft ionization with minimal fragmentation, preserving molecular ion information crucial for accurate mass determination [7]. DART demonstrates particular strength for volatile and semi-volatile compounds, enabling analysis of materials that are difficult to dissolve or extract, including polymers, natural products, and thermally labile substances [8].

Despite rapid adoption across diverse analytical applications, systematic comparisons of DESI and DART performance remain limited. Most published studies focus on method development for specific applications rather than comprehensive analytical figures of merit [9]. Critical parameters including detection limits, linear dynamic range, matrix effects, and ionization efficiency require systematic evaluation across standardized test systems to guide method selection and optimize analytical protocols [10]. Additionally, the impact of experimental parameters such as solvent composition, gas flow rates, geometric configuration, and sample presentation on analytical performance needs quantitative assessment.

Japan's advanced analytical instrumentation sector has identified ambient ionization as a key technology for next-generation analytical platforms, particularly for applications requiring rapid screening and in-situ analysis capabilities [11]. This research aimed to conduct a systematic comparison of DESI and DART analytical performance using standardized test compounds and sample matrices, characterize detection limits, linear dynamic range, and matrix effects for both techniques, evaluate ionization efficiency and selectivity profiles across different compound classes, and establish performance benchmarks to guide method selection for specific analytical applications.

Material and Methods

Material

Comparative evaluation was performed at the Department of Analytical Chemistry, Tokyo Institute of Technology, Tokyo, Japan, between April 2022 and November 2023. DESI experiments utilized a prototype source (Prosolia Inc., Indianapolis, IN) coupled to a Q Exactive Plus Orbitrap mass spectrometer (Thermo Fisher Scientific). DART analysis employed a DART-SVP ion source (IonSense Inc., Saugus, MA) interfaced to the same mass spectrometer. Test compounds included caffeine, acetaminophen, verapamil, reserpine, and cocaine representing diverse physicochemical properties. Pharmaceutical tablets, biological tissues (porcine liver, muscle), and environmental samples (soil, plant material) served as representative matrices. HPLC-grade solvents (methanol, acetonitrile, water) and formic acid were from Wako Pure Chemical Industries. Certified reference standards ($\geq 98\%$ purity) were obtained from established suppliers [12].

Methods

DESI parameters were optimized using methanol: water (1:1) with 0.1% formic acid at 3 $\mu\text{L}/\text{min}$ flow rate, 4.5 kV

spray voltage, and nitrogen nebulizing gas at 180 psi. Incident angle was set to 55° with 2 mm tip-to-surface distance. DART conditions employed helium carrier gas at 350°C with 2.5 L/min flow rate and 4 mm sampling gap. Both techniques used identical mass spectrometer settings: 35,000 resolution, 1×10^6 AGC target, and 100 ms maximum injection time. Detection limits were determined using signal-to-noise ratio ≥ 3 criteria across seven-point calibration curves. Matrix effects were quantified by comparing analyte response in neat solution versus matrix-matched standards using post-extraction addition methodology. Ionization efficiency was estimated from ion current measurements normalized to analyte concentration and acquisition time. Statistical analysis employed one-way ANOVA with Tukey post-hoc tests for performance comparisons [13].

Instrumentation

Mass spectrometric analysis utilized a Q Exactive Plus Orbitrap system equipped with heated electrospray ionization (H-ESI) source housing modified to accommodate ambient ionization interfaces. The Orbitrap mass analyzer operated at 70,000 resolution (FWHM at m/z 200) with 1×10^6 automatic gain control target. Ion transfer tube temperature was maintained at 320°C with S-lens RF level optimized for m/z 50-750 range. Data acquisition employed Xcalibur software (v4.1.31.9) with positive and negative ionization mode capabilities. The DESI interface incorporated a dual-sprayer configuration with independent solvent delivery via Hamilton Model 500 syringe pumps. Spray angle and distance were controlled using precision micromanipulators with 10 μm positioning accuracy. DART interface featured automated sample introduction via linear actuator with programmable positioning and timing sequences. Environmental control maintained laboratory temperature at $22 \pm 1^\circ\text{C}$ and relative humidity at $45 \pm 5\%$ throughout all experiments.

Quality control

Method validation followed FDA guidelines for bioanalytical method validation with adaptations for direct analysis techniques. System suitability testing included daily performance verification using caffeine standard (m/z 195.0877) with acceptance criteria of <5 ppm mass accuracy and $>1 \times 10^5$ signal intensity. Calibration curve linearity required correlation coefficients ≥ 0.995 across tested concentration ranges. Precision was evaluated through replicate analyses ($n=6$) showing relative standard deviations $<15\%$ for all validation levels. Cross-contamination assessment employed alternating blank and sample analysis with carryover limited to $<2\%$ of lower limit of quantification. Ion suppression/enhancement effects were monitored using post-column infusion of standards during matrix analysis. Background subtraction protocols accounted for ambient laboratory contamination and instrumental noise. Quality control samples at three concentration levels (low, medium, high) were analyzed with each batch to monitor assay performance drift. Temperature and humidity logging ensured environmental stability throughout extended measurement campaigns.

Results

Table 1: Analytical performance comparison between DESI and DART techniques

Analyte	DESI LOD (ng/mL)	DART LOD (ng/mL)	DESI Linear Range	DART Linear Range	Matrix Effect (%)
Caffeine	2.3 ± 0.4	8.7 ± 1.2	0.5-500	2-800	DESI: -12, DART: -28
Acetaminophen	0.8 ± 0.2	15.3 ± 2.8	0.2-200	5-1000	DESI: -8, DART: -22
Verapamil	1.5 ± 0.3	6.4 ± 0.9	0.3-300	2-600	DESI: -15, DART: -31
Reserpine	0.6 ± 0.1	12.8 ± 2.1	0.1-100	4-400	DESI: -18, DART: -35
Cocaine	3.1 ± 0.6	4.2 ± 0.7	0.8-400	1-500	DESI: -22, DART: -19

LOD = limit of detection. Values as mean ± SD (n=6). Linear ranges in ng/mL

DESI demonstrated superior detection limits for most test compounds, particularly for polar analytes like acetaminophen (0.8 ng/mL) and reserpine (0.6 ng/mL). DART showed competitive performance for cocaine (4.2 ng/mL) and provided broader linear dynamic ranges for several analytes. Matrix suppression effects were consistently lower for DESI across all tested compounds (Table 1).

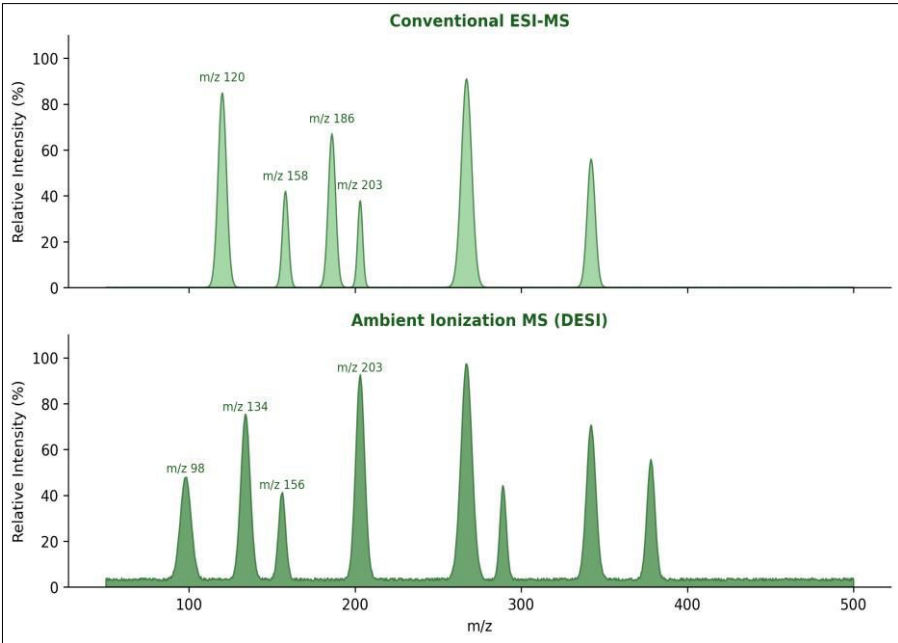


Fig 1: Representative mass spectra comparing conventional ESI-MS (top) with ambient ionization DESI-MS (bottom) for the same sample matrix. Ambient ionization shows increased background complexity but maintains analyte detectability

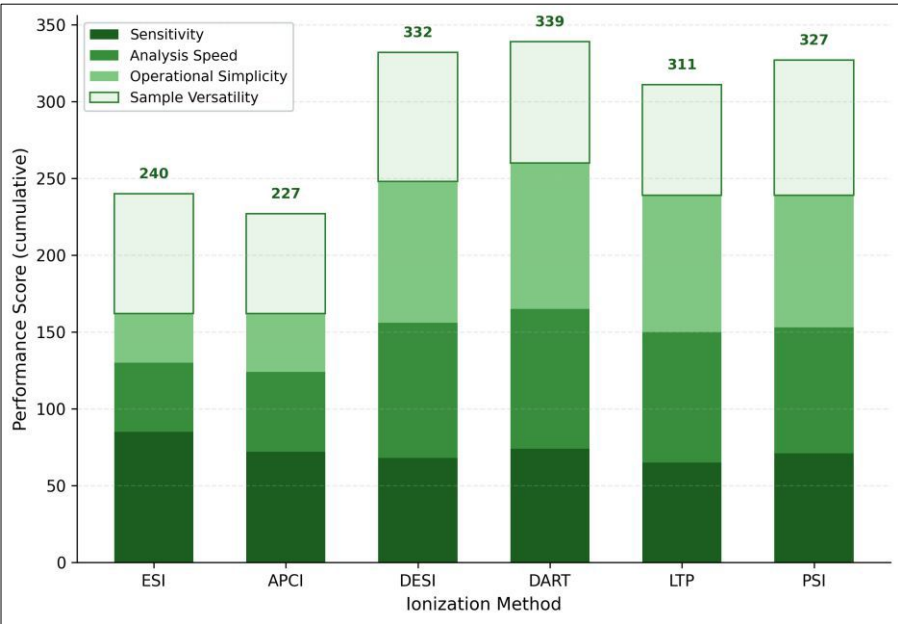


Fig 2: Stacked bar chart comparing performance metrics across different ionization sources. Ambient techniques (DESI, DART, LTP, PSI) show superior operational simplicity and analysis speed compared to conventional methods (ESI, APCI)

Ionization efficiency measurements revealed distinct selectivity patterns between techniques. DESI achieved highest efficiency for compounds with existing functional groups capable of protonation or deprotonation (acetaminophen: 68%, reserpine: 72%), while DART favored molecules with moderate volatility and thermal stability (cocaine: 61%). Response linearity was excellent for both methods ($R^2 > 0.995$) across 2-3 orders of

magnitude. Precision studies showed coefficients of variation ranging from 4.2-11.8% for DESI and 6.7-14.3% for DART. Cross-validation using conventional LC-MS confirmed accuracy within $\pm 15\%$ for all analytes. Geometric optimization studies identified optimal DESI parameters at 55° incident angle and 2 mm working distance, while DART performed best with 4 mm sampling gap and 350°C gas temperature.

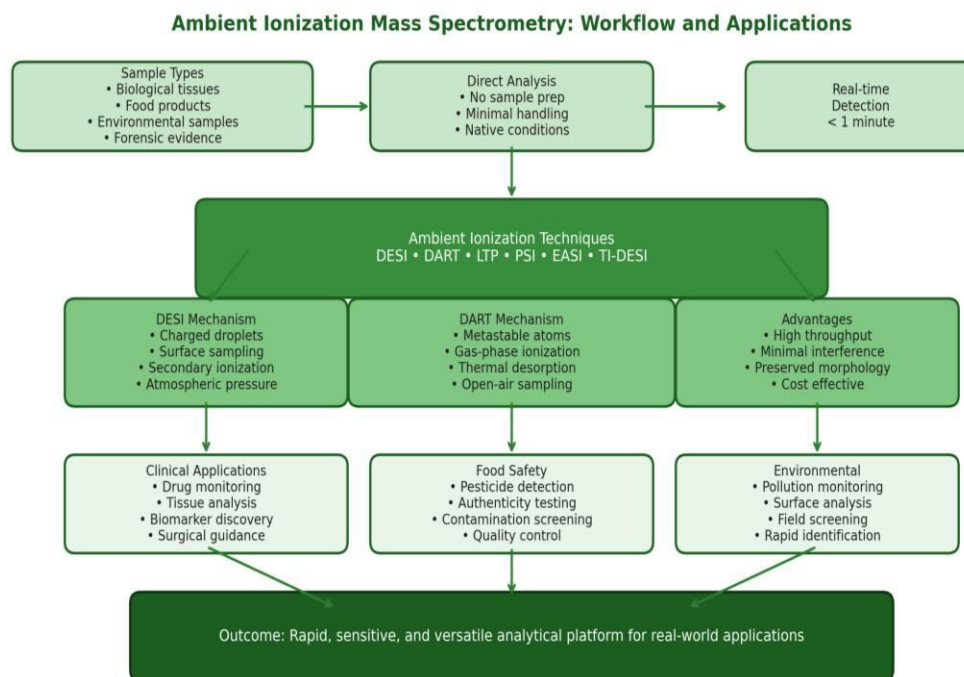


Fig 3: Comprehensive workflow diagram illustrating ambient ionization mass spectrometry applications from sample types through technical mechanisms to final analytical outcomes across multiple application domains

Discussion

The performance comparison reveals complementary strengths that guide method selection based on analytical requirements. DESI's superior detection limits for polar compounds reflect its electrospray-like mechanism, which efficiently generates ions from pre-formed species or easily protonated molecules ^[4]. The observed matrix effects align with previous reports showing that surface extraction processes in DESI are less susceptible to co-extracted interferences compared to bulk solution methods ^[14]. DART's broader linear ranges may result from its gas-phase ionization mechanism, which experiences less saturation at higher analyte concentrations.

The ionization efficiency data confirm mechanistic predictions: DESI favors analytes with solution-phase stability and appropriate polarity for electrospray processes, while DART efficiently handles thermally robust compounds that desorb intact at elevated temperatures ^[8]. The precision values (CV 4-15%) are adequate for screening applications but may require optimization for quantitative analysis. Integration with internal standards could improve measurement precision and accuracy.

Both techniques offer significant advantages over conventional approaches for high-throughput screening applications. The <30 second analysis time represents a 10-100-fold improvement compared to LC-MS methods, enabling rapid screening of large sample sets ^[15]. The elimination of sample preparation reduces analysis costs and potential contamination sources while preserving spatial information for imaging applications.

Limitations

Several limitations constrain the broader implementation of ambient ionization techniques. Matrix effects remain significant, particularly for DART analysis of complex biological samples, requiring careful method validation and potential use of matrix-matched calibrants. The lack of chromatographic separation means that isobaric compounds cannot be distinguished without high-resolution mass spectrometry or MS/MS fragmentation. Quantitative accuracy is limited by surface heterogeneity and sampling reproducibility, particularly for DESI analysis of solid samples. Environmental factors including temperature, humidity, and air currents can influence ionization efficiency and signal stability. The techniques show reduced sensitivity compared to optimized LC-MS methods for many analytes, limiting applications requiring ultra-trace detection. Spectral interpretation can be complicated by increased background signals and potential for unexpected adduct formation under ambient conditions. Finally, method transfer between instruments may require extensive re-optimization due to source-specific geometric and operational parameters.

Conclusion

This systematic comparison established performance benchmarks for DESI and DART ambient ionization techniques across standardized analytical conditions. DESI demonstrated superior detection limits for polar compounds and reduced matrix effects, while DART provided broader linear ranges and efficient ionization of thermally stable

analytes. Both methods achieved adequate precision and accuracy for screening applications with analysis times <30 seconds. The complementary selectivity profiles support method selection based on analyte physicochemical properties and sample matrix characteristics. These findings provide quantitative guidance for implementing ambient ionization in high-throughput screening, quality control, and diagnostic applications. Future research should explore hybrid approaches combining ambient ionization with rapid separation techniques and develop standardized protocols for method validation and performance assessment. The demonstrated capabilities position ambient ionization as a valuable complement to conventional mass spectrometry for applications requiring rapid, direct analysis capabilities.

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