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GC/MS Application Note

Automated Direct Derivatization and GC/MS Analysis

A Robust Method for Comprehensive Metabolomic Profiling of Dried Blood Spots, Serum, and Plasma

Analytical fields

LIFE SCIENCE CLINICAL

Automated Direct Derivatization and GC/MS Analysis

A Robust Method for Comprehensive Metabolomic Profiling of Dried Blood Spots, Serum, and Plasma

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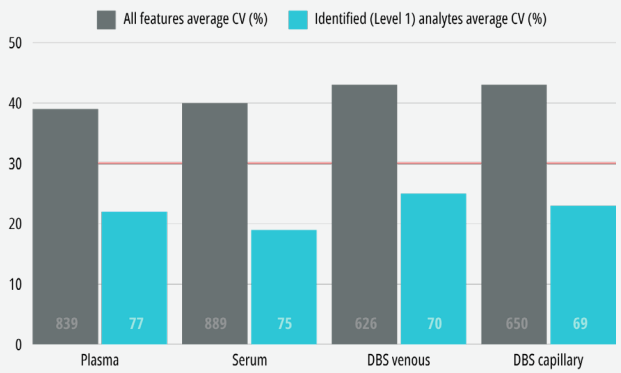
Abstract

Dried blood spots (DBS) offer a minimally invasive and efficient alternative to traditional blood collection methods for metabolomic profiling. This application note presents a fully automated, in-vial, two-step derivatization method (methoximation followed by trimethylsilylation) for gas chromatography/mass spectrometry (GC/MS) analysis of DBS, serum, and plasma sample. The workflow enables rapid analysis with a 31-minute sample-to-sample runtime. Untargeted analysis generates over 600 features per sample and over 70 analytes are identified to the RECETOX Metabolome HR-[EI+]-MS library. Routinely measured metaboites show quantitative reproducibility (CV < 30%). Untargeted analysis using MS-DIAL measured over 600 features per sample, with more than 70 metabolites confidently identified against an in-house library. This automated, high-throughput workflow significantly streamlines metabolomic analysis of various blood sample types, facilitating faster turnaround times and broader applications in research and clinical settings.

Direct and rapid methoxymation in the vial, followed by the silylation of metabolites in various blood matrices (Dried Blood Spots - DBS, Plasma, and Serum)

- Analyze approximately 40 samples within 24 hours, identifying over 70 metabolites
- Achieve quantitative reproducibility for routinely measured metabolites with CVs under 30%.



Download the workflow file

Read the full protocol online

Requires a Thermo Scientific™ TriPlus™ RSH (or **comparable**) robotic platform equipped with incubator/agitator, cooled drawer, and robotic tool change (RTC) station equipped with liquid handling tools.

Introduction

Metabolomics, the comprehensive study of small molecules (metabolites) within biological systems, offers invaluable insights into physiological processes, disease states, and therapeutic responses [1], [2], [3]. Due to its rich metabolite content and accessibility, blood is a frequent target for metabolomic profiling. Notably, dried blood spots (DBS) offer a minimally invasive alternative to traditional blood collection methods, involving the collection of a small blood volume on filter paper, which is then dried and stored [4], [5].

DBS offer numerous advantages, including simplified sample collection, reduced storage and shipping costs, and increased metabolite stability [6], [7]. Furthermore, DBS can be derived from both venous and capillary blood, each offering distinct advantages depending on the research context. Capillary blood sampling is typically performed, but the drying of venous blood on cards can be valuable for storage in resource-limited settings.

Gas chromatography/mass spectrometry (GC/MS) is a widely utilized analytical platform in metabolomics, renowned for its high sensitivity, reproducibility, and extensive metabolite coverage. However, many metabolites are not inherently volatile or thermally stable enough for direct GC/MS analysis. Chemical derivatization is a common strategy to modify these metabolites, enhancing their volatility and thermal stability for successful analysis.

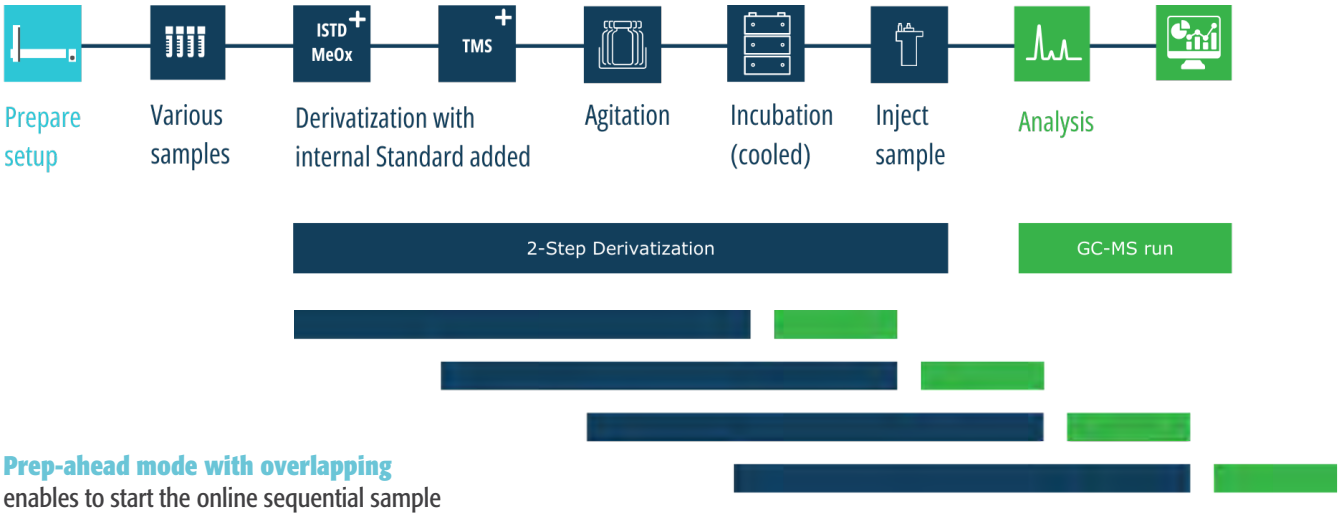
A prevalent two-step derivatization approach involves methoximation (MeOx), targeting carbonyl groups, followed by trimethylsilylation (TMS), reacting with various functional groups containing active hydrogens [8]. This derivatization not only improves volatility and thermal stability but also reduces peak tailing and enhances the separation and detection of metabolites in GC/MS.

Traditionally, derivatization has been performed in batches, offering efficiency but potentially introducing variability if samples are not treated uniformly [9]. The method presented in this application note is based on a previously published, fully automated workflow for GC/MS-based metabolomics (Jbebli et al. 2025). This workflow employs a fully automated, in-vial derivatization approach using a Thermo Scientific TriPlus RSH autosampler, combining the advantages of sequential processing (consistency) with the speed and efficiency of automation.

The automated process utilizes an x,y,z robot to perform derivatization steps directly within sample vials, eliminating manual transfer and minimizing errors or contamination. The robot's programmable, precise sequence ensures consistent reaction times and conditions for each sample, achieving a sample-to-sample runtime of 31 minutes. To further enhance throughput for large sample series, the workflow incorporates a "prep-ahead" mode with overlapping sample preparation, allowing the robot to initiate derivatization for the next sample while the current one undergoes GC/MS analysis. This strategy maximizes efficiency without sacrificing accuracy or precision.

In this application note, we detail this automated, in-vial derivatization method for GC/MS analysis of DBS (both venous and capillary), serum, and plasma samples, focusing on its application to untargeted metabolomic analysis.

We assess the workflow's performance by calculating the coefficient of variation (CV%) for metabolites identified through untargeted analysis (comparison to the RECETOX library), demonstrating the robustness and applicability of this method for a wide range of metabolomic studies.



Prep-ahead mode with overlapping enables to start the online sequential sample preparation and measurement ahead of time for more efficiency and throughput.

Results

Untargeted Data Processing using MS-DIAL

Non-targeted data processing was performed in MS-DIAL (version 4.9.221218). MS-DIAL parameters for feature alignment used a retention time tolerance of 0.05 min in combination with an EI spectral similarity of 75%. For analyte identification, deconvoluted features were matched to the RECETOX Metabolome HR-[EI+]-MS library using an alkane retention index tolerance of 40 units and m/z tolerance of 0.003. Level 1 identification represents confirmed structures based on matching to authentic reference standards within the library. Features with CVs below 30% were considered reproducibly measured, a commonly accepted threshold in metabolomics.

Table 1 summarizes the features annotated in each sample type and the analytes of interest identified in blood samples (Level 1). Using a threshold of 30% CV, 449, 450, 264, and 329 features are reproducibly measured in serum, plasma, DBS venous, and DBS capillary, respectively.

The variation in six replicates was determined by calculating the CV% for each feature. The results are plotted in a violin plot (Figure 1) to visualize the distribution of variation in each sample. A dashed horizontal line at 30% aids in quickly assessing the CV values and indicates the threshold for reproducible measurement.

Table 1. Overview of measured features and identified metabolites across samples.
*Analytes are annotated by comparison with RECETOX Metabolome HR-[EI+]-MS library.

Sample	Features number	All features average CV (%)	Identified analytes (Level 1*)	Identified analytes average CV (%)
Plasma	889	40	77	**22**
Serum	839	39	75	**19**
DBS venous	626	43	70	**25**
DBS capillary	650	43	69	**23**

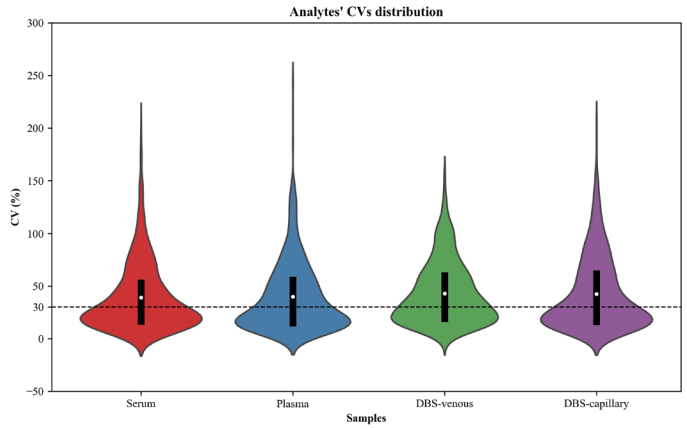


Figure 1. Violin plots of the coefficients of variation (CV) for peak area of features detected following GC-Orbitrap MS metabolomics analysis of various blood samples. Serum, plasma, DBS capillary, and DBS venous samples were analyzed in six replicates. A greater proportion of features are reproducibly measured, i.e., below the 30% CV threshold (dashed line), in liquid blood samples. The median is indicated by a white dot and the interquartile range (IQR) by a black box.

Summary

Metabolomics, the study of small molecules within biological systems, provides crucial insights into health and disease. Dried blood spots (DBS) offer a compelling alternative to traditional blood collection methods for metabolomic profiling.

This application note demonstrates a streamlined workflow for high-throughput gas chromatography/mass spectrometry (GC/MS) analysis of DBS, serum, and plasma samples, leveraging a fully automated, in-vial derivatization process. This automation reduces variability, minimizes manual error, and enhances consistency. The method routinely identifies over 70 metabolites in untargeted analyses using MS-DIAL software and the RECETOX Metabolome HR-[EI+]-MS library, providing a comprehensive overview of the metabolite profile and enabling discovery of novel biomarkers or metabolic pathways.

The coefficient of variation (CV%) across replicates was calculated to assess variability, with 449, 450, 264, and 329 features showing CVs below 30% in serum, plasma, DBS venous, and DBS capillary, respectively, indicating good reproducibility. Importantly, the automated workflow enabled the analysis of approximately 40 samples within 24 hours, showcasing its high-throughput capability.

The inherent versatility of the robotic platform allows for seamless integration of additional sample preparation steps. For instance, a Dilutor Tool can be incorporated for generating dilution series, or a centrifuge can be added to perform more complex extractions.

The combination of high throughput, robustness, automation, and inherent versatility positions this method as a valuable tool for large-scale metabolomic studies, biomarker discovery, and routine clinical diagnostics.

Materials and Methods

This application note utilizes the materials and methods described in the previously published protocol by Jbebli et al. 2025, available here.

The workflow described can also be adapted for the analysis of other sample types, as detailed in the following published SOPs.

Biomarker Analytical Laboratories. (2021). SOP for metabolite profiling of seminal plasma via GC Orbitrap. Zenodo https://doi.org/10.5281/zenodo.5734331

Biomarker Analytical Laboratories. (2022). SOP for metabolite profiling of urine via GC-MS. Zenodo https://doi.org/10.5281/zenodo.7462217

Biological Materials

- Dried capillary blood spots (DBS-capillary): Collected via finger prick on Whatman 903 cards, dried overnight, and stored at -80 °C with desiccant.
- Dried venous blood spots (DBS-venous): 100 µl of venous blood spotted onto Whatman 903 cards, dried overnight, and stored at -80 °C with desiccant.
- Pooled human serum
- Pooled human plasma

(Details on the origin and processing of serum and plasma are available in the original protocol.)

Sample Preparation

- Dried Blood Spots (DBS): 3.3 mm punches were taken from DBS cards and placed directly into glass amber vials with 0.2 ml integrated inserts, sealed with magnetic caps.
- Liquid Blood (Serum, Plasma): Samples were homogenized by gentle shaking. 3 µl of liquid blood was dispensed into autosampler vials with 200 µl inserts, sealed with magnetic caps.
- Blanks: For DBS, blanks consisted of 3.3 mm filter paper punches in vials with inserts. For liquid blood samples, empty vials with caps were used.

(Further details on sample preparation, including the specific vials, caps, and inserts used, are provided in the original protocol.)

Automated Sample Preparation

Automated sample preparation was performed on a Thermo Scientific™ TriPlus™ RSH autosampler equipped with an incubator/agitator, cooled drawer, and automatic tool change (ATC) station (comparable to PAL System RTC - Robotic Tool Change).

The automated method was created using the Sampling Workflow Editor (SWE) and is fully detailed in the original protocol. The SWE is comparable to the PAL Method Composer (PMC).

Briefly, the internal standard mix is added to each vial with the methoximation solution and incubated. Then, the MSTFA working solution is added to each vial, followed by further incubation. After cooling, the derivatized samples are ready for injection.

The sequence included details about the injection list, instrumental method, sample position, and injection volume.

GC/HRMS Analysis

Following automated derivatization, 1 µl of the derivatized sample was injected into the GC/HRMS system in splitless mode. The GC/MS parameters, including column, carrier gas, oven temperature program, ionization settings, and data acquisition parameters, were as previously published (see SOPs) and are detailed in the original protocol. Data acquisition was performed using either Thermo Scientific Xcalibur or Chromeleon software.

(For a comprehensive list of reagents, equipment, and detailed GC-HRMS parameters, please refer to the original protocol.)

References

[1] A. V. Aderemi, A. O. Ayeleso, O. O. Oyedapo, and E. Mukwevho, "Metabolomics: A scoping review of its role as a tool for disease biomarker discovery in selected non-communicable diseases," Metabolites, vol. 11, no. 7, 2021, doi: 10.3390/metabo11070418.

[2] I. S. Grønningsæter, H. K. Fredly, B. T. Gjertsen, K. J. Hatfield, and Ø. Bruserud, "Systemic Metabolomic Profiling of Acute Myeloid Leukemia Patients before and During Disease-Stabilizing Treatment Based on All-Trans Retinoic Acid, Valproic Acid, and Low-Dose Chemotherapy," Cells, vol. 8, no. 10, p. 1229, Oct. 2019, doi: 10.3390/cells8101229.

[3] J. M. Wilkins and E. Trushina, "Application of metabolomics in Alzheimer's disease," Front Neurol, vol. 8, no. JAN, pp. 1–20, 2018, doi: 10.3389/fneur.2017.00719.

[4] J. Déglon et al., "Automated system for on-line desorption of dried blood spots applied to LC/MS/MS pharmacokinetic study of flurbiprofen and its metabolite," J Pharm Biomed Anal, vol. 54, no. 2, pp. 359–367, Jan. 2011, doi: 10.1016/j.jpba.2010.08.032.

[5] R. V. Oliveira, J. Henion, and E. Wickremsinhe, "Fully-automated approach for online dried blood spot extraction and bioanalysis by two-dimensional-liquid chromatography coupled with high-resolution quadrupole time-of-flight mass spectrometry," Anal Chem, vol. 86, no. 2, pp. 1246–1253, Jan. 2014, doi: 10.1021/ac403672u.

[6] K. Li, J. C. Naviaux, J. M. Monk, L. Wang, and R. K. Naviaux, "Improved Dried Blood Spot-Based Metabolomics: A Targeted, Broad-Spectrum, Single-Injection Method," Metabolites 2020, Vol. 10, Page 82, vol. 10, no. 3, p. 82, Feb. 2020, doi: 10.3390/METABO10030082.

[7] N. H. Tobin et al., "Comparison of dried blood spot and plasma sampling for untargeted metabolomics," Metabolomics, vol. 17, no. 7, pp. 1–9, Jul. 2021, doi: 10.1007/S11306-021-01813-3/METRICS.

[8] O. Fiehn, "Metabolomics by gas chromatography-mass spectrometry: Combined targeted and untargeted profiling," Curr Protoc Mol Biol, vol. 2016, Apr. 2016, doi: 10.1002/0471142727.mb3004s114.

[9] H. Miyagawa and T. Bamba, "Comparison of sequential derivatization with concurrent methods for GC/MS-based metabolomics," J Biosci Bioeng, vol. 127, no. 2, pp. 160–168, Feb. 2019, doi: 10.1016/j.jbiosc.2018.07.015.

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