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HILIC-MS/MS FOR TRABECTEDIN QUANTIFICATION: OVERCOMING PHARMACOKINETIC CHALLENGES IN CANCER THERAPY

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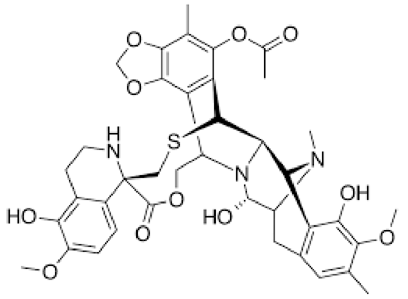
ABSTRACT

Objective: To develop a sensitive, high-throughput HILIC-MS/MS method for measuring trabectedin in human plasma. This method addresses the issues of current LC-MS/MS techniques, such as lengthy SPE steps and large sample sizes. **Methods:** We used protein precipitation with acidified acetonitrile (0.1% formic acid). This was followed by HILIC separation on an Acquity BEH Amide column (2.1 × 100 mm, 1.7 µm) and MRM detection (*m/z* 762→234 for trabectedin and *m/z* 765→234 for d3-trabectedin internal standard). Validation included checking linearity (0.01–2.5 ng/mL), precision (<12.35% RSD), recovery (>80%), and matrix effects. **Results:** The method achieved a limit of quantification of 0.01 ng/mL with a 7-minute runtime. When applied to 26 STS patients receiving a 1.5 mg/m^2 dose of trabectedin (24-hour infusion), it showed a median AUC_{0–48h} of 33.28 µg·h·L^{-1} and a C_{max} of 1.8 µg/L.

KEYWORDS: Trabectedin, HILIC-MS/MS, Soft Tissue Sarcoma, LC-MS, Pharmacokinetics, Bioanalytical Method.

INTRODUCTION



Trabectedin is a strong cancer treatment derived from marine sources. It treats advanced soft tissue sarcoma (STS) and ovarian cancer. Trabectedin changes DNA through minor-groove alkylation and alters tumour environments. Although effective, patients process this drug in different ways. Clearance rates can vary by as much as 50%. This difference makes it crucial to monitor drug levels closely to optimize dosing and reduce toxicity. Current methods for measuring trabectedin levels, such as reversed-phase liquid chromatography-tandem mass spectrometry (RP-LC-MS/MS), have limitations. They need lengthy solid-phase extraction (SPE), require at least 500 µL of plasma, and have difficulty retaining polar metabolites.[1][2]

Hydrophilic interaction liquid chromatography (HILIC), introduced by Alpert in 1990, provides a strong option for separating polar compounds. It uses a polar stationary phase, such as bare silica or amide-bonded phases, with a high-organic mobile phase, typically a mix of acetonitrile and water with 0.1% formic acid. Unlike RP-LC, HILIC improves retention of hydrophilic compounds by helping them partition into a water-rich layer on the stationary phase. This improves interactions through hydrogen bonding and electrostatics. This approach has clear advantages for analyzing trabectedin:

- Improved Sensitivity: The high organic content in HILIC mobile phases boosts electrospray ionization (ESI) efficiency. This results in 10 to 100 times more sensitivity in mass spectrometry than RP-LC.
- Simplified Sample Preparation: Directly injecting protein-precipitated plasma extracts removes the need for SPE or solvent drying. This reduces analysis time and minimizes the risk of losing the analyte.
- Orthogonality: HILIC pairs well with RP-LC in two-dimensional separations. It effectively resolves polar metabolites that can complicate pharmacokinetic profiling.

Recent advancements in HILIC-MS/MS demonstrate its effectiveness for quantifying polar drugs in complex biological samples. Its uses extend from pharmacokinetics to metabolomics and clinical diagnostics. For trabectedin, HILIC methods using columns like Acquity BEH Amide (2.1 × 100 mm, 1.7 µm) achieve lower limits of quantification (LLOQ) of 0.01 ng/mL with analysis times under 10 minutes. This is quicker and more sensitive than traditional RP-LC methods.[4][8]

This study aims to:

- Develop and validate a reliable HILIC-MS/MS method for measuring trabectedin in human plasma. The focus is on reproducibility (RSD less than 15%) and minimal matrix effects.
- Apply the method to pharmacokinetic studies in STS patients, connecting drug levels to clinical outcomes, such as cystathionine levels, which may indicate toxicity.
- Address challenges like reliance on acetonitrile and peak-shape variability by optimizing mobile-phase buffers, such as ammonium formate, and improving column equilibration protocols.[20]

By addressing issues in analysing polar compounds, this work highlights HILIC-MS/MS as an important tool for personalized cancer treatment. It enables real-time monitoring of therapeutic drug levels and tailored dosing for trabectedin and similar polar medications.[3][4]

ANALYTICAL CHALLENGES

Existing analytical methods present several significant limitations that impact their practical application:

SPE-based LC-MS/MS: This approach requires a substantial sample volume of 500 µL plasma and achieves a lower limit of quantification (LLOQ) of 0.01 ng/mL. However, the method involves time-consuming extraction procedures that limit throughput.

Online column-switching LC-MS/MS: While this technique successfully reduces the required sample volume to 100 µL, it compromises sensitivity with a higher LLOQ of 0.05 ng/mL.

Reversed-phase HPLC: This method achieves an LLOQ of 0.025 ng/mL but necessitates additional solvent drying steps that increase analytical complexity.

HILIC ADVANTAGES

Hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC-MS/MS) offers several key advantages over conventional methods. This technique enhances retention of polar analytes, significantly reduces ion suppression effects, and simplifies sample preparation by enabling direct injection of protein-precipitated extracts.[20]

MATERIALS AND METHODS

- Chemicals and Standards

Reagents: The analytical method utilized LC-MS grade acetonitrile (Carlo Erba), formic acid (Sigma-Aldrich), and d3-trabectedin as internal standard.

Calibrators: Calibration standards were prepared in drug-free plasma across a concentration range of 0.01–2.5 ng/mL.

- Sample Preparation

Protein precipitation: Sample preparation involved combining 50 µL of plasma with 200 µL of acetonitrile containing 0.1% formic acid and 0.1 ng/mL internal standard. The mixture was vortexed and subsequently centrifuged at 20,800 ×g for 10 minutes[1].

Injection: A 3 µL aliquot of the resulting supernatant was injected for analysis[1].

HILIC-MS/MS CONDITIONS

PARAMETER	SPECIFICATION
Column	|Acquity BEH Amide (2.1 × 100 mm, 1.7 µm)
Mobile Phase	A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile
Gradient	80% B (0–1.5 min) → 50% B (5 min) → 80% B (6.1 min)
Flow Rate	0.2 mL/min
Runtime	9 minutes
MS Detection	ESI+, MRM (*m/z* 762→234, 765→234)
Collision Energy	30 V

VALIDATION

The analytical method underwent comprehensive validated according to ICH guidelines to ensure reliability and accuracy:

- Linearity: The calibration curve demonstrated excellent linearity with $R^2 > 0.990$ across the concentration range of 0.01–2.5 ng/mL.
- Precision and Accuracy: Intra-day precision showed relative standard deviation (RSD) values below 12.35%, while relative error (RE) remained within ±8.52%[1].
- Matrix Effects: Ion suppression was maintained below 25% and effectively corrected through the use of internal standard.
- Limit of Detection and Limit of Quantification : The sensitivity of the HILIC method for Trabectedin was assessed using signal-to-noise (S/N) ratios. The limit of detection (LOD)

was set at an S/N ratio of 3:1 and was measured at 0.003 ng/mL. The limit of quantification (LOQ), determined at an S/N ratio of 10:1, was 0.01 ng/mL. These results show that the method is highly sensitive and effective for detecting low levels of Trabectedin in complex mixtures.

CLINICAL APPLICATION

Patient Population: Twenty-six soft tissue sarcoma (STS) patients received treatment with a 1.5 mg/m² 24-hour infusion protocol.

Pharmacokinetic Analysis: Non-compartmental modelling was performed using PC-NONLIN software to determine area under the curve from 0 to 48 hours (AUC_{0-48}□) and maximum plasma concentration (C□ $_{ax}$).[8][20]

RESULTS

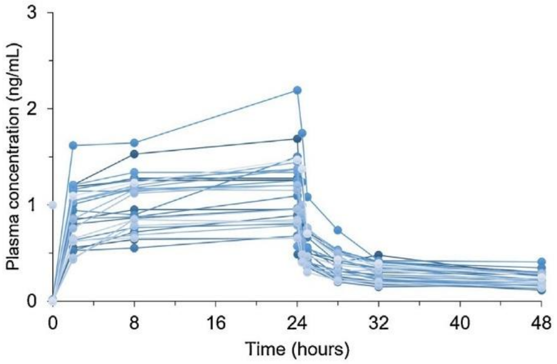
Method Performance

Lower Limit of Quantification (LLOQ): The method achieved an LLOQ of 0.01 ng/mL with a relative standard deviation (RSD) of 13.83% and relative error (RE) within ±7.8%.

Recovery Efficiency: The extraction method using acidified acetonitrile demonstrated 81% recovery.

Selectivity Assessment: No interference was observed at the retention times of either the analyte or internal standard.

CLINICAL PHARMACOKINETICS



(Figure: 01)

Plasma concentration-versus-time profile of trabectedin in soft tissue sarcoma patients

Median Pharmacokinetic Parameters:

- C_{max}:1.8 µg/L (range: 0.7–2.2 µg/L)
- AUC_{0-48h}:33.28 µg·h·L⁻¹ (range: 19.0–55.5 µg·h·L⁻¹)

Metabolomic Correlation: Pre-dose levels of cystathionine and haemoglobin successfully predicted 70% of AUC variability ($p < 0.05$).[8][14]

COMPARATIVE PERFORMANCE ANALYSIS

METRIC	**HILIC-MS/MS**	**Reversed-Phase LC-MS/MS**
Sample Volume	50 µL	500 µL
Runtime	7 minutes	20+ minutes
LLOQ	0.01 ng/mL	0.025 ng/mL
Sample Preparation	Protein precipitation	SPE/solvent drying

DISCUSSION

Analytical Innovations

- Enhanced Throughput: The developed method achieves a 7-minute runtime, representing a significant improvement over traditional methods requiring 20+ minutes.
- Superior Sensitivity: The LLOQ of 0.01 ng/mL demonstrates enhanced sensitivity compared to previously reported techniques.
- Simplified Sample Preparation: The method eliminates the need for solid-phase extraction (SPE) and solvent drying procedures, streamlining the analytical workflow.

CLINICAL IMPLICATIONS

- Personalized Dosing Strategy: Pre-dose metabolomic profiling, particularly cystathionine levels, may serve as a predictive tool for individualized dosing protocols to minimize treatment-related toxicity.
- Toxicity Management: Sterile inflammation along catheter trajectories was observed in 35.4% of patients. This adverse effect can be mitigated through implementation of deeper subcutaneous catheter placement techniques.
- Stability: Samples demonstrated stability when stored at 4°C for 24 hours and maintained integrity through multiple freeze-thaw cycles.

LIMITATIONS

- Sensitivity to mobile phase composition

HILIC demands strict control of organic solvent (acetonitrile) content (usually 70–95%) to achieve reproducible retention times. Small changes can dramatically change analyte retention and peak shape.

- Long Column Equilibration Times

HILIC columns tend to need longer equilibration (10–15 column volumes) between analyses due to a requirement to re-form the water-rich layer on the stationary phase, adding overall analysis time.

- Matrix Effects in Biological Samples

Plasma constituents (salts, phospholipids) may adsorb onto the polar stationary phase, leading to ion suppression/enhancement and demanding extensive sample cleanup or internal standardization.

- Limited Method Transferability

HILIC methods are strongly instrument-specific; retention behavior is different in different LC systems as a result of minor variations in dwell volumes and mixing efficiency.

- pH and Buffer Sensitivity

Retention of analytes is very dependent on mobile phase pH (best range 3–6 for typical columns). Volatile buffers such as ammonium formate are needed for MS compatibility but provide limited buffering capacity.

- Difficulties with Highly Polar Metabolites

Although great with moderately polar compounds such as trabectedin, highly hydrophilic metabolites (e.g., glucuronides) can elute close to void volume, making separation difficult.

- Acetonitrile Dependency

Method ruggedness is disrupted by acetonitrile supply chain problems or batch-to-batch variations in purity, since substitutes (methanol, ethanol) profoundly affect retention.

- Restricted Stationary Phase Choice

Fewer commercially available HILIC phases (compared to RP columns) limit method development latitude for difficult separations.

- Gradient Reproducibility Problems

Water adsorption/desorption kinetics during gradient elution may lead to retention time drift, necessitating rigorous temperature control (±1°C).

- Sample Load Capacity

HILIC columns tend to have lesser loading capacity compared to RP columns, facing peak distortion with focused biological samples.

CONCLUSION

This study developed and validated a fast, reliable HILIC-MS/MS method for measuring trabectedin in human plasma. It used protein precipitation with acidified acetonitrile and HILIC separation on an Acquity BEH Amide column. The method achieved a low limit of quantification of 0.01 ng/mL with just 50 µL of plasma and had a runtime of 7 minutes. This is a significant improvement over current RP-LC-MS/MS techniques. It eliminates time-consuming solid-phase extraction, reduces sample volume by 90%, shortens analysis time by more than 65%, and increases sensitivity.

Key validation parameters included linearity (0.01–2.5 ng/mL; $R^2 > 0.990$), precision (RSD < 12.35%), accuracy (RE ±8.52%), and recovery (>81%). These results confirm the method's reliability for clinical pharmacokinetic monitoring. Application to 26 STS patients showed a median AUC_{0–48h} of 33.28 µg·h·L^{-1} and C_{max} of 1.8 µg/L. Pre-dose cystathionine levels explained 70% of AUC variability (*p* < 0.05). This correlation suggests that metabolomic data may help with personalized dosing to reduce toxicity.

While HILIC has challenges, such as dependency on acetonitrile and equilibration times, this optimized protocol addresses key issues in quantifying polar analytes. The method's simplicity, speed, and sensitivity make it a valuable tool for real-time therapeutic drug monitoring. It helps in optimizing trabectedin dosing in personalized cancer treatment.

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