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Original Research Article

Development and Validation of a Rapid, Simple, and Precise LC-MS/MS Method for the Quantification of Vincristine

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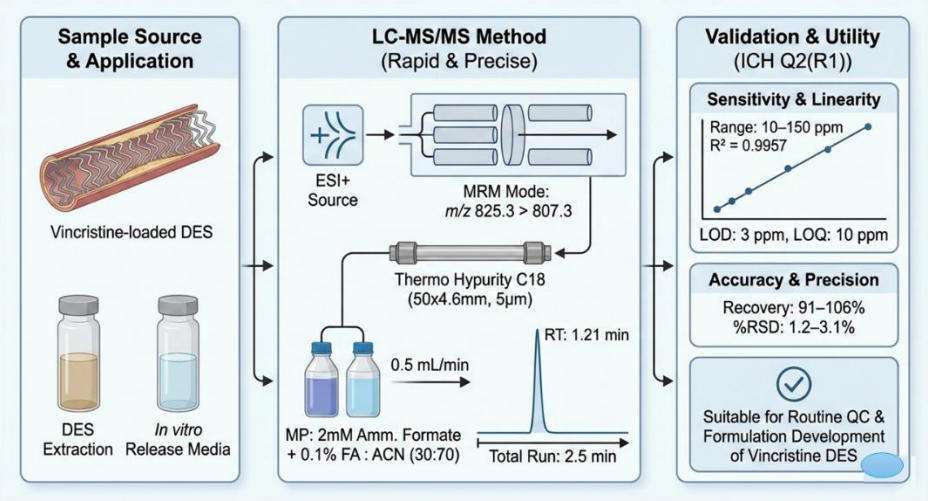
ABSTRACT

A rapid, precise, and economical LC-MS/MS method was developed and validated for the quantification of vincristine. The method utilizes electrospray ionization in positive mode with multiple reaction monitoring (MRM) for a precursor-to-product ion transition of m/z 825.3 > 807.3. Effective chromatographic separation was achieved using a Thermo Hypurity C18 column (50 mm x 4.6 mm, 5 µm). The mobile phase consisted of 2 mM ammonium formate with 0.2% formic acid in acetonitrile at a ratio of 30:70 (v/v), pumped at a flow rate of 0.5 mL/min. The retention time (RT) for vincristine was 1.21 minutes and the total run time was 2.5 minutes. The method was validated according to ICH Q2(R1) guidelines, with the Limit of Detection (LOD) and Limit of Quantitation (LOQ) determined to be 3 ppm and 10 ppm, respectively, calculated using standard regression statistics. Linearity was established over a concentration range of 10– 150 ppm (y = 698.983 x – 2606.21; R^2 = 0.995734). Accuracy (mean % recovery) across LOQ, 50%, 100%, and 150% levels was within 91–106%, and precision (relative standard deviation [%RSD]) ranged from 1.2% to 3.1% (intraday and inter-day), meeting the acceptance criteria for routine analysis. The system suitability %RSD for the peak area was 2.27%, and the RSD for RT was 0.45%. The method was successfully applied to quantify vincristine loaded on drug-eluting stents (DES) and to evaluate *in vitro* release kinetics (DES extraction and release sample testing included in validation). This method is suitable for routine quality control and formulation development of vincristine-containing DES.

GRAPHICAL ABSTRACT



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Introduction

Vincristine is a therapeutically significant vinca alkaloid that was originally isolated from *Catharanthus roseus* (Madagascar periwinkle). Since its first approval, vincristine has been extensively used in combination chemotherapy regimens for hematological malignancies, such as acute lymphoblastic leukemia (ALL), Hodgkin's lymphoma, and non-Hodgkin's lymphoma [1-4]. Its mechanism of action involves inhibition of microtubule polymerization, resulting in mitotic arrest and subsequent apoptosis of rapidly dividing cancer cells. Beyond oncology, vincristine also exhibits immunomodulatory potential, making it useful in managing thrombotic thrombocytopenic purpura (TTP) and chronic idiopathic thrombocytopenic purpura (CITP) [5]. Vincristine has also been explored for its neuromodulatory benefits in the selection of clinical conditions characterized by denervation. Despite its therapeutic importance, the clinical utility of vincristine is complicated by several analytical limitations. Published bioanalytical methods for vincristine include protein precipitation (PPT), liquid–liquid extraction (LLE), and solid-phase extraction (SPE), each with trade-offs in throughput, cleanliness, and sensitivity [6,7]. The major analytical challenges include matrix effects (ion suppression/enhancement), adsorption of vincristine to laboratory surfaces, short serum half-life (requiring sensitive assays), and low drug loads encountered in device coatings. Recent interest in localized delivery via DES has motivated the development of robust analytical methods that quantify small amounts of vincristine in polymer matrices and release media, which can support formulation development and batch release testing [7-11].

The development of vincristine-coated DES presents several challenges that require meticulous attention to drug loading, release kinetics, chemical stability, and regulatory safety. [12,13] Achieving uniform drug loading is critical, necessitating accurate quantification of vincristine per stent through validated extraction and recovery studies [14]. The release kinetics must be carefully controlled, with the choice of dissolution medium, apparatus, and sink conditions that significantly impacting the results, and the medium composition (*e.g.,* PBS and surfactants) must be justified to mimic *in vivo* conditions [15,16]. Vincristine's chemical stability is another concern, as it may degrade under certain sterilization or storage conditions, highlighting the need for stability-indicating assays. The polymer matrix may also introduce challenges, potentially binding the drug or causing interference during analytical testing, requiring matrix evaluation and, if necessary, matrix-matched calibration or isotopic internal standards in LC-MS/MS analysis [11]. Furthermore, regulatory and safety considerations, including addressing local cytotoxicity, systemic exposure, and sterility, are crucial to ensuring the success of both the preclinical and clinical development stages [17].

Considering the above challenges and limitations, this study aimed to develop a rapid, economical, and robust LC-MS/MS method for the accurate quantification of vincristine, to validate the method according to ICH Q2(R1), and to demonstrate its applicability to vincristine extracted from DES and to *in vitro* release samples. Advantages over earlier work include (i) a short runtime with maintained specificity, (ii) a validated extraction for polymer matrices, and (iii) inclusion of matrix effect evaluation and stability testing relevant to DES analyses.

Materials and Methods

Reagents and chemicals

All HPLC-grade solvents and chemicals (ammonium formate, formic acid, and acetonitrile) were procured from Merck India Ltd. All chemical reagents and aqueous solvents were

purified using Millipore (0.45 μm) filters (Merck India Ltd). All the solvents and reagents used were of analytical/LC-MS grade.

LC-MS/MS instrumentation and conditions

Chromatographic analysis was performed using a UPLC instrument (Waters Corp. USA) with an Acquity model and auto-sampling system. An MS detector (Waters Corp., USA) Quattro Premier XF model triple quadrupole MS was used. Open Lab software was used for the LC-MS/MS system. The selected MRM transition for vincristine was m/z 825.3 to 807.3. The cone voltage was 35 V and the cone gas flow was 102 L h^{-1}. Chromatography was performed using a Thermo Hypurity C18 column (50 mm × 4.6 mm, 5 μm). Mobile phase: 2 mM ammonium formate with 0.2% formic acid (solvent A) and acetonitrile (solvent B) at 30:70 (v/v). A flow rate of 0.5 mL min^{-1}, and an injection volume of 10 μL (as indicated in table/figure captions) were maintained. RT was 1.21 min with a total run time of 2.5 min.

Rationale for MRM selection

Vincristine readily formed a stable protonated molecular ion $[M+H]^+$ at m/z ≈ 825.3 in positive electrospray ionization (ESI); the product ion at m/z ≈ 807.3 was the most abundant and reproducible fragment under the optimized collision energy. The selection was based on the observed abundance, stability, and minimal in-source fragmentation during tuning.

Preparation of standards and samples

Accurately weighed 10 mg of vincristine (Active Pharmaceutical Ingredient, API, Natco Pharmaceuticals Ltd, India) and dissolved in 10 mL of methanol in a volumetric flask to prepare a standard stock solution with a concentration of 1 mg/mL. Working standards were prepared by serial dilution of the stock solution in methanol to yield calibration standards at 10, 25, 50, 75, 100, 125, and 150 ppm. Quality control (QC) samples at LOQ (10 ppm) and 50%, 100%, and 150% were prepared similarly. Stock and calibration standards were prepared in methanol to ensure stability of the standard solution and reproducible instrument responses. Acetonitrile was used for DES extraction because of its superior ability to dissolve typical polymeric coatings and liberate vincristine from the polymer-metal matrix. This method includes matrix effect evaluation (see below) to confirm that solvent differences do not lead to biased quantification.

System suitability

System suitability was determined by six replicate injections of the 100-ppm standard. The recorded parameters included RT, peak area, %RSD for area, and RT. The carryover was evaluated by injecting a blank following the highest calibration standard and by monitoring the residual signal at the analyte RT.

Linearity and range

The linearity was assessed at 10–150 ppm (LOQ = 10 ppm). Calibration plot: Peak area versus concentration; least-squares linear regression was used to derive the slope (S), intercept (C), coefficient of determination (R^2), and standard error of regression. Calibration acceptance criteria: $R^2 \geq 0.99$ and individual calculated concentrations within ±15% (±20% at LOQ).

Sensitivity (LOD and LOQ)

The LOD and LOQ were calculated from regression statistics using standard formulas and are presented in the following Equations (1) and (2):

$$\text{LOD} = \frac{3.3 \times \sigma}{S} \quad (1)$$

$$\text{LOQ} = \frac{10 \times \sigma}{S} \quad (2)$$

Where, σ is the standard deviation of the y-

intercept of the regression line and S is the slope of the calibration curve.

Accuracy and precession

Accuracy (% recovery) and precision (%RSD) were assessed at the LOQ (10 ppm) and 50%, 100%, and 150% QC levels. Each QC level was analyzed in triplicate for intra-day (repeatability) and across six days for inter-day (intermediate) precision. The acceptance criteria were mean accuracy within ±15% of nominal (±20% at LOQ) and precision (%RSD) ≤15% (≤20% at LOQ).

Solution stability

Stability of a 100-ppm working solution and QC samples was assessed under the following laboratory conditions: short-term bench-top stability at room temperature (22–25 °C) for 8 h, refrigerated stability at 4 °C for 72 h, and processed sample stability in the autosampler at 10 °C for 24 h. Freeze–thaw stability was evaluated for three cycles at −20 °C. The percentage of stability (ST%) was calculated by comparing the stored sample area responses to the freshly prepared samples.

Sample preparation — vincristine extraction from DES

Vincristine extraction from DES-coated stents was performed by placing each device in a glass vial containing 5 mL of acetonitrile, which was selected for its strong ability to dissolve polymeric coatings and compatibility with LC-MS/MS analysis. The vials were sonicated for 30 min at ambient temperature to ensure complete release of the drug from the stent surface. Following sonication, the extracts were centrifuged at 3,000 rpm for 10 min to remove insoluble particulates. The resulting supernatants were then diluted with methanol to align the analyte levels with the calibration range and subsequently filtered through 0.22 µm PTFE syringe filters prior to LC-MS/MS injection. To evaluate potential matrix effects, calibration curves were prepared both in neat methanol and in post-extraction spiked samples derived from blank stent matrices. A comparison of the two slopes enabled the assessment of ion suppression or enhancement, with deviations within ±15% indicating no significant matrix effect.

In vitro release study

Stents were incubated in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5 °C in a USP IV dissolution apparatus (Sotax) under sink conditions. Aliquots were collected at predetermined time points (*e.g.,* 1, 6, 24, 48, and 72 h), filtered (0.22 µm), diluted where necessary, and stored at 4 °C until analysis. Each time point sample was quantified against the calibration curve and corrected for dilution.

Results

Specificity and MRM selection

Full-scan MS and product ion spectra showed a predominant $[M+H]^+$ ion at m/z ≈ 825.3, and an abundant product ion at m/z ≈ 807.3. The transition 825.3 → 807.3 provided excellent sensitivity and selectivity; no interfering peaks were observed in the blank chromatograms at vincristine RT (~1.21 min). The representative spectra and chromatograms are shown in Figures 1 and 2, respectively.

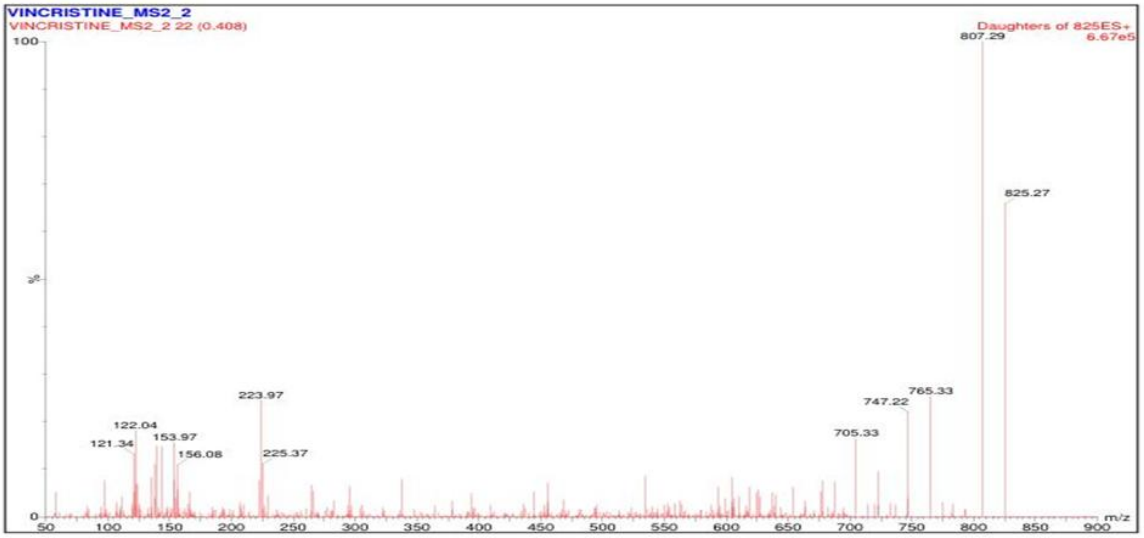

Figure 1. Molecular ion and product ions of vincristine

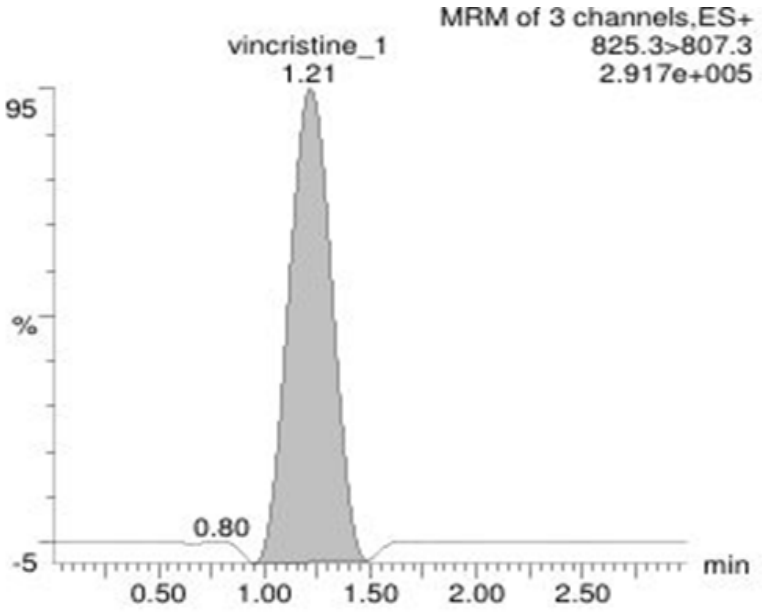

Figure 2. Optimized chromatogram of vincristine

Linearity and range

Calibration data (10–150 ppm) produced the regression:

$$y = 698.983\,x - 2606.21 (R^2 = 0.995734)$$

Slope (S) = 698.983; intercept (C) = −2606.21. Coefficient of variance (CV) across calibration points = 0.0653 (reported). The calculated concentrations for the calibration points met the acceptance criteria (±15% deviation; ≤ ±20% at the LOQ). The linearity details are listed in Table 1 and linearity plot for vincristin is shown in Figure 3.

Table 1. Linearity data for vincristine

Sr. No.	Drug	Std. Conc. (ppm)	Peak area	RT (min)	% Accuracy
1		10.000	5,016	1.22	109.0
2		25.000	12,477	1.22	86.3
3		50.000	32,070	1.21	99.2
4	Vincristine	75.000	51,457	1.21	103.1
5		100.000	72,047	1.22	106.8
6		125.000	82,948	1.22	97.9
7		150.000	99,698	1.22	97.6

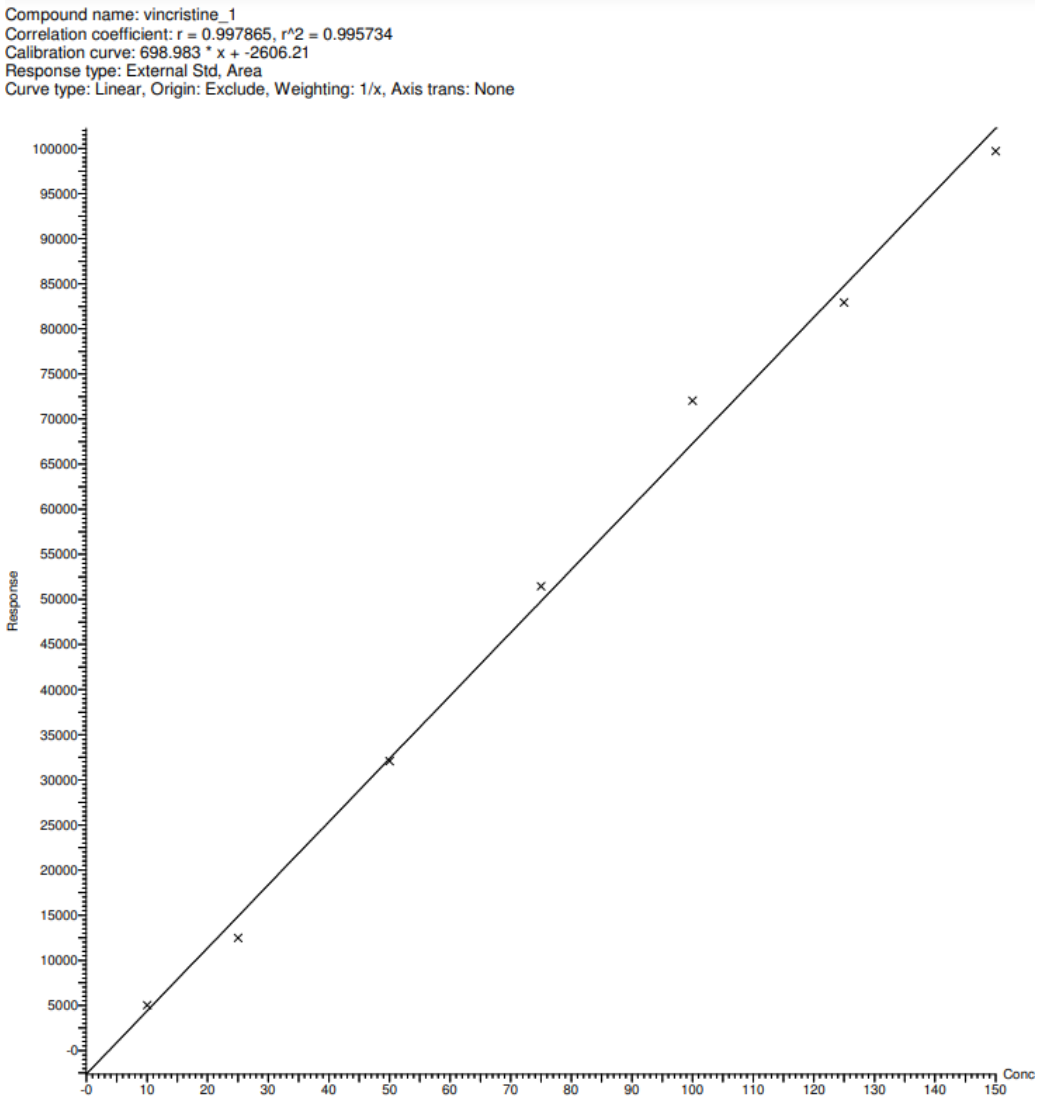

Figure 3. Linearity plot for vincristine

System suitability, carryover and RT RSD

Six replicate injections of 100 ppm produced consistent results. The system suitability data (Table 2) show a %RSD for a peak area of 2.27% and %RSD for RT of 0.45%.

Table 2. System suitability (100 ppm vincristine; six injections)

Injection	Peak area	RT (min)
1	67,925.227	1.13
2	71,573.547	1.14
3	70,300.789	1.13
4	69,099.352	1.14
5	71,093.172	1.14
6	72,156.234	1.14
Mean	70,358.0535	1.135
S.D	1,597.34	0.00516
%RSD	2.27	0.45

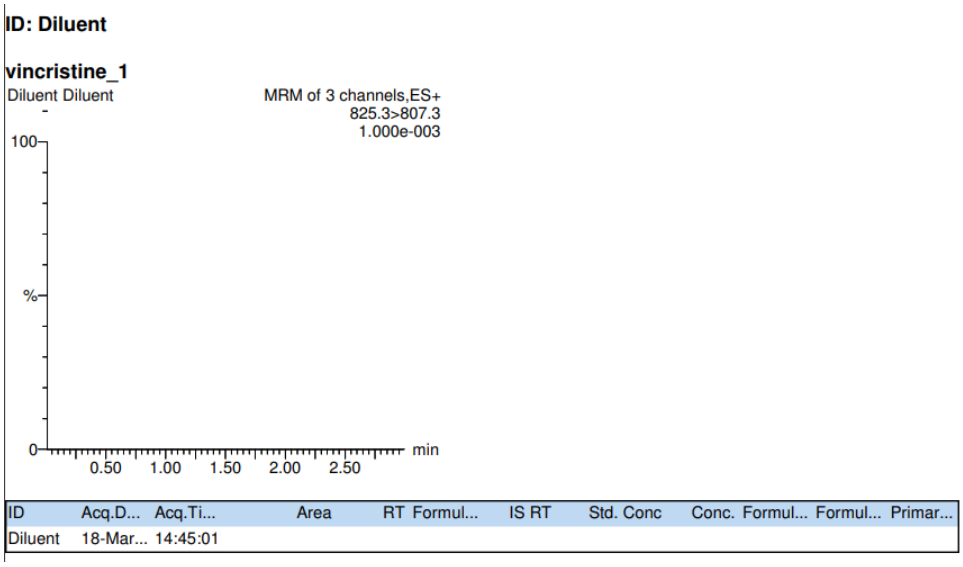

Figure 4. Chromatogram of the solvent after injecting vincristine sample

No carryover was observed in blank injections following the highest calibration standard (Figure 4).

Sensitivity (LOD and LOQ)

Using regression statistics and the equations in the methods section, LOD = 3 ppm and LOQ = 10 ppm. The LOQ level (10 ppm) was confirmed by precision and accuracy testing (Table 3).

Accuracy and precision (intraday and inter-day)

Accuracy and precision were evaluated at the LOQ and 50%, 100%, and 150% QC levels. The QC level data are summarized in Table 4. The mean % recoveries (accuracy) were within 91–106% across levels; intraday %RSD values were ≤2.0%, and inter-day %RSD across six days was ≤3.1% for area (reported as 3.07% area RSD in inter-day precision; see Table 5, Figure 4). These results met ICH Q2(R1) acceptance criteria.

Solution stability

The short-term bench-top stability, refrigerated stability, and freeze–thaw stability met the acceptance criteria (±15% of the initial response). Autosampler stability of processed samples at 10 °C for 24 h was acceptable.

Table 3. LOQ (10 ppm) precision/accuracy (six replicates)

Replicate	Peak Area	Calculated conc. (ppm)
1	4,620	10.338
2	4,947	10.807
3	5,008	10.893
4	4,837	10.648
5	4,786	10.576
6	4,511	10.182
Mean	—	10.57
%RSD	—	3.6%

Table 4. Accuracy and method precision (QC levels)

Level	Rep	Peak area	Calculated Conc. (ppm)	%Recovery
50%	1	32,998	50.94	101.9
50%	2	33,266	51.32	102.6
50%	3	34,769	53.47	106.9
100%	1	69,353	102.95	102.95
100%	2	68,737	102.07	102.07
100%	3	68,879	102.27	102.27
150%	1	105,850	155.16	103.4*
150%	2	105,457	154.60	103.1*
150%	3	102,957	151.02	100.7*

** At 150%, the calculated concentration is expressed relative to the nominal 150 ppm (values within ±15% are accepted).*

Table 5. Inter-day precision (100% level; six days)

Day	Peak area	RT (min)	Calculated conc. (ppm)
Day 1	72,247	1.13	100.906
Day 2	78,070	1.14	106.125
Day 3	76,177	1.13	104.304
Day 4	77,969	1.14	102.586
Day 5	75,509	1.14	105.438
Day 6	78,419	1.14	106.959
%RSD	—	—	3.07

Matrix effects and carryover testing

Matrix effects were evaluated by comparing calibration slopes generated in a solvent (methanol) with post-extraction spiked calibration slopes prepared in DES extract blanks. The slopes were within ±10% of each other, indicating no significant ion suppression or enhancement in the extraction matrix under the validated conditions. Carryover testing (blank after the highest calibration standard) showed no detectable analyte above the instrument noise (no carryover).

Application to vincristine-coated DES and in vitro release

The validated method was used to quantify vincristine extracted from DES (extraction procedure in methods). Representative chromatograms for the extracted DES and for day-3 release samples are shown in Figures 5-7. Using the calibration equation, a measured peak area of

81,557.492 corresponded to an extracted vincristine concentration of 120.40 ppm (interpreted in context of sample dilution and expressed as µg per stent when converted; authors note units must be consistently reported depending on stent mass and dilution factor). In an *in vitro* release experiment, extrapolation gave a vincristine release of 10.9 µg on day 3 based on calculations from the linear regression equation from the calibration curve (Figure 5).

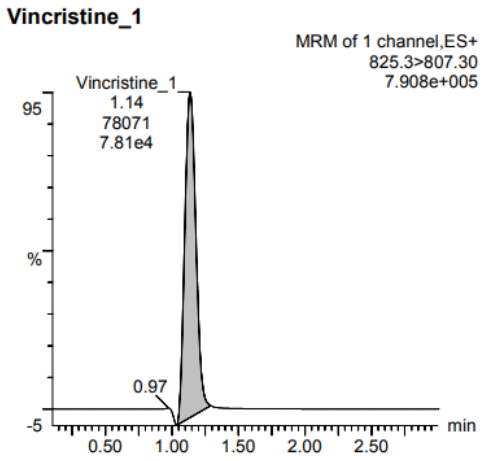

Figure 5. Day-2 inter-day precision for vincristine

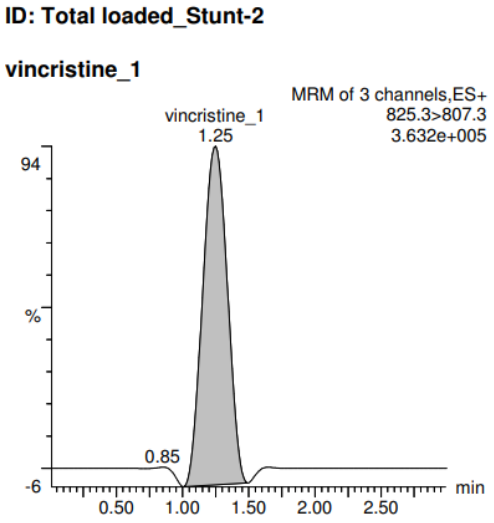

Figure 6. Chromatogram showing the amount of vincristine loaded on the DES

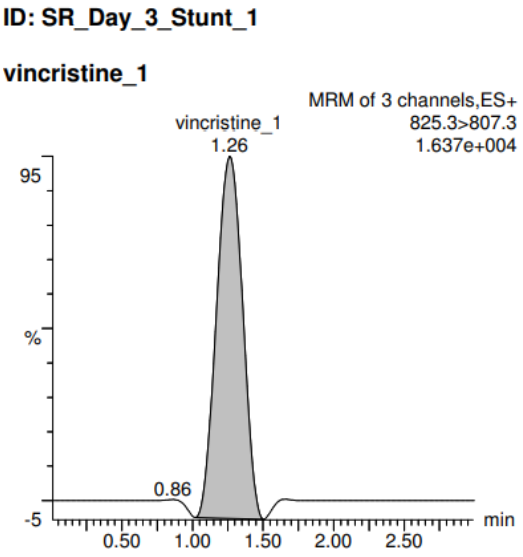

Figure 7. Chromatogram showing the amount of vincristine released on the 3rd day in the release kinetics study of DES

Discussion

The method met the ICH Q2(R1) validation criteria for specificity, linearity, accuracy, and precision. The selected MRM transition m/z 825.3 → 807.3 provided sensitive and selective detection. A short chromatographic run time (2.5 min) supported high throughput without compromising the peak shape or selectivity. The reported LOD (3 ppm) and LOQ (10 ppm) values demonstrated suitable sensitivity for DES analyses and release-kinetic studies. System suitability data confirmed a stable instrument performance (%RSD area = 2.27%; %RSD RT = 0.45%). Matrix evaluation confirmed no significant ion suppression or enhancement for the validated extraction protocol, and extraction in acetonitrile ensured the efficient recovery of vincristine from polymeric coatings. Stability testing conditions and results support routine handling and sample storage practices. Vincristine-coated DES for Renal Denervation (RDN) for treating drug-resistant hypertension deploys stents within the renal artery and elutes vincristine outward into the vessel wall and adventitia to reach sympathetic nerve fibers

located several millimeters from the lumen [18-20]. Engineering challenges include achieving sufficient perivascular tissue penetration and sustained release (likely days to weeks), while avoiding an initial burst that could cause local vascular necrosis or systemic exposure [21]. Controlled-release strategies (biodegradable polymer matrices, multilayer coatings, and nanoparticle encapsulation) are established DES approaches that can be adapted to vincristine to tune the release kinetics and minimize burst release [22-25]. Quantification of vincristine in DES and quantification in plasma or tissue (local PK) and *in vitro* release testing is readily performed with validated LC-MS(/MS) assays that achieve ng/mL sensitivity, but with limitations [16]. Major safety concerns include endothelial or medial toxicity, delayed endothelialization and thrombosis, systemic neurotoxicity or myelosuppression from leakage, and renal dysfunction due to excessive denervation necessitating narrow-window dose optimization and the need for rapid, simple, and low quantification [12,26-30]. In summary, vincristine-coated DES for RDN is scientifically plausible with mechanistic neurotoxicity through DES delivery capability but remains conceptual and requires controlled-release engineering and validated LC-MS/MS analytical methods for quantification of *in vitro* release kinetics, pharmacokinetics, and tissue concentrations. The validated method supported specificity by the absence of interfering peaks at vincristine RT in blank chromatograms, ensuring reliability in complex matrices. The application of the method to drug-eluting stents demonstrated its robustness in quantifying both the total vincristine-coated DES and the cumulative amount released in *in vitro* pharmacokinetic studies. The extraction procedure enabled the complete recovery of vincristine from the stent surface, supporting accurate drug load determination. Evaluation of release kinetics confirmed that the method could sensitively detect vincristine at various time points, even at lower concentrations typical of sustained release phases. Collectively, the results demonstrate that the developed LC-MS/MS method is not only analytically valid, but also highly practical for pharmaceutical development, DES characterization, and routine quality control settings. The limitations and challenges associated with vincristine-eluting stents, together with other critical analytical considerations, were systematically incorporated into the method development and validation strategy to ensure that an LC-MS/MS assay is suitable for accurate and reliable quantification.

Conclusion

A rapid and robust LC-MS/MS method for vincristine quantification was developed and validated according to ICH Q2(R1). The method demonstrated specificity (selected MRM transition), sensitivity (LOD 3 ppm, LOQ 10 ppm), linearity (10–150 ppm, R^2 = 0.995734), accuracy (mean recoveries within acceptance limits), and precision (%RSD intra-day ≤2.0%, inter-day ≤3.1%). The extraction procedure for DES in acetonitrile produced reproducible recovery, and the method was successfully applied to quantify vincristine-coated DES and evaluate release kinetics. This method is suitable for routine QC and formulation studies; adaptations for biological matrices should include matrix-matched validation. Accordingly, these limitations and analytical challenges were incorporated into the overall method development and validation framework, enabling a rigorously tailored LC-MS/MS assay to quantify vincristine for this novel DES application.

Conflict of Interest

The authors declared no conflicts of interest in this work.

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