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Fragmentation Dynamics of Drug Bioconjugates: Insights from Mass Spectrometry (Article Review)

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ديناميكيات التفتيت في المقرونات الحيوية الدوائية: رؤى مستخلصة من مطيافية الكتلة (مقال مراجعة)

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ABSTRACT

Background: Anthracyclines are chemotherapeutic medications with a broad therapeutic range. They were discovered in a pigment-producing strain of Streptomyces and are generally used as an anti-cancer medication. Doxorubicin (Dox), Daunorubicin (Dau), and epirubicin (Epi), the three most important anthracyclines, used in cancer treatment as a key class of chemotherapeutic medications and are recognized as some of the most potent antineoplastics available today. Daunorubicin is a commonly prescribed anti-cancer drug used to treat various types of leukemia. This anti-cancer molecule is frequently coupled to Gonadotropin-Releasing Hormone type III derivatives via an oxime bond, with the Serine in the fourth position substituted for Lysine (acetylated). This conjugation not only maximizes selectivity but also minimizes systemic toxicity

Materials and Methods: These bioconjugates were found to be highly selective and significantly slowed tumor development in a wide variety of tumor types. Due to their complex architectures, these peptide-drug conjugates are often confirmed using Mass Spectrometry, which may also be utilized for analytical characterization. Soft ionization technologies such as Electrospray Ionization and Matrix-Assisted Laser Desorption/Ionizationare often used to produce protonated molecules from proteins and peptides

Results: Conversely, the mass spectra of bioconjugates with daunomycin "undergo extensive fragmentation" under conventional spectrometric conditions. Daunomycin almost always produces a specific pattern of fragmentation ions. Aglycone- or daunosamine-mediated glycosidic bond breakage is a primary mechanism for fragmentation.

Conclusion: Peptide–drug conjugates (PDCs) are prodrugs in which drug molecules are covalently linked to peptides through specific linkers. The mass spectrometric analysis of daunorubicin-containing conjugates is challenging due to the loss of the sugar moiety during ionization, and their fragmentation patterns depend on technical factors such as instrument settings, solvent composition, and the structural nature of the conjugate. A novel daunorubicin–peptide conjugate offers a safe and effective cancer therapy, and elucidating its structure requires the use of complementary MS detection techniques. Ion activation remains a key concept in the analysis of peptides, proteins, and their complexes, as ionization and fragmentation methods determine fragment patterns and support the characterization of structural features and modifications.

Keywords: Anthracyclines; Doxorubicin; Daunorubicin; GnRH-III conjugates; Mass spectrometry; Fragmentation; Glycosidic bond cleavage.

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INTRODUCTION

Daunomycin bioconjugates have been extensively studied for their biological properties since they were conjugated to several cancer-specific peptides [1]. Promising new anti-cancer drugs are now being tested in the lab. reflecting the most up-to-date laboratory research [2]. Regulatory authorities require extensive analytic characterization of drug candidates, which represents a crucial phase in the drug discovery process [3]. Massive volumes of data can be acquired from Tandem Mass Spectrometry to identify PDCs structurally. These drugs cannot be analyses using ESI-MS owing to considerable spontaneous fragmentation of anthracycline-containing conjugates during ESI [4].

A tetracyclic quinoid aglycone and an O-glycosidic link are required to attach the sugar moiety to the aglycons to create daunomycin. ESI-MS analysis exhibits a different pattern for daunomycin. This secondary dissociation mechanism occurs in conjunction with the glycosidic bond cleavage as the major way for daunomycin to fragment; however, the charge can be located on either the aglycons or sugar moiety [5]. HCD-MS/MS analysis suppresses the sugar-loss fragmentation typically observed in daunomycin bioconjugates. This suppression occurs because higher-energy collisions induce alternative fragmentation pathways rather than glycosidic bond cleavage. Consequently, the resulting mass spectra contain fragments that are highly similar to those of free daunomycin. This similarity complicates data interpretation, making unambiguous assignment, purity estimation, and structural identification problematic.

The sequence coverage provided by the most widely used fragmentation methods, such as ETD and EThcD, often outperforms that of the HCD approach. These methods now make it possible to better characterize peptide-based samples. When used in combination with an analyzer, MALDI can provide extremely sensitive peptide sequence information in addition to ESI [6].

Daunorubicin (Dau) is a commonly prescribed anti-cancer medicine used to treat various types of leukemia. A tetracycline O-glycosidic link connects the anthraquinone aglycon group to the daunosamine sugar [7, 8]. Due to their complex architectures, peptide-drug conjugates are often confirmed using MS, which may also be utilized for analytical characterization. However, the spectrometric analysis of anthracycline-containing peptide-drug conjugates is still difficult because these conjugates undergo extensive and unpredictable fragmentation, generating spectra that complicate unambiguous structural assignment. [9] .

ESI is the most widely utilized ionization technology for peptides. Soft ionization is used to produce single or "multi-protonated" compounds from peptides. MS/MS may be used to sequence peptides and confirm modification sites in addition to measuring the molecular size and verifying chemical contents. However, the breakdown of molecules during ESI complicates the spectrometric detection of daunomycin-containing peptide conjugates. Bioconjugates containing daunomycin demonstrate significant fragmentation inside the ion source [10].

MS is a critical tool for quickly and reliably identifying the final products used in bioconjugate chemical synthesis, which usually involves a complicated chemical procedure. However, the "identity" of anthracycline and its derivatives cannot be accurately assessed because of their unusual fragmentation during MS analysis.

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To produce protonated molecules from proteins and peptides, soft ionization technologies such as ESI and MALDI are often used. Mass spectra of bio-conjugates with daunomycin, on the other hand, "undergo extensive fragmentation" under conventional spectrometric conditions. Daunomycin almost always produces a specific pattern of fragmentation ions. Glycosidic bond breakage involving either the aglycone or the daunosamine is the primary fragmentation mechanism [11].

Next, cleavage of the sugar moiety (m/z 130.0) results in an unsaturated (m/z = 528.0 – 129.0 = 399.5) aglycone or hydroxyl functionality on the side of the aglycones. New anthracycline-containing bio-conjugates are continuously being synthesized, as evidenced by the growing number of publications [12,13]. However, in most cases, the analytic data show mass spectra comprising complex mixtures of the protonated compound, adducts, and various ion fragments. The peaks observed are often erroneously allocated and misinterpreted. Therefore, the structure of the compounds remains unknown. Developing reliable analytical techniques for the characterization of bio conjugates necessitates an understanding of how morphological and instrumental variables impact the quality of mass spectra , such as drug-to-peptide ratio and linker type) and instrumental settings (including collision energy and ionization conditions) affect mass spectral quality for peptide-drug conjugates. Special attention is given to anthracycline-containing bio-conjugates, where extensive fragmentation complicates data interpretation. The article compares different MS approaches (ESI, MALDI, and HCD-MS/MS) and concludes with practical recommendations for achieving unambiguous structural identification and purity estimation.

MATERIALS AND METHODS

1. Mass Spectrometric Analysis of Anthracycline Fragmentation

When in-source fragmentation during MS analysis separates the aglycone component [16] from the sugar moiety [14,15], the resulting mass spectra show a few protonated adducts ranging from approximately 130 to 528 m/z, indicating a convoluted but interpretable pattern. The glycosidic bond can also be disrupted during conjugate synthesis under acidic conditions, leading to loss of the daunosamine moiety and a significant reduction in biological activity [17].

Anacyclines (or anthracyclines? — I assume you meant anthracyclines, as "anacyclines" is not standard) are derived via glycosidic bonding of tetracyclic rings to daunosamine sugars, producing a hydrophobic ring system useful in treating bacterial infections. Additionally, these drugs are effective against several solid tumor types. The only structural difference between Epirubicin (Epi) and other doxorubicin (Dox) 4-epimers is the orientation of the hydroxyl group on the C-4' position of the daunosamine ring. Unlike Dox, Epi is less cardiotoxic, yet its therapeutic efficacy remains comparable to that of Dox [18]. Furthermore, Epi exhibits higher lipophilicity, enhancing its cellular penetration [19]. Both Dox and Epi are conjugated with glucuronide at the C-4' hydroxyl group and excreted via bile This seems to be a confusion of two different drug classes and their metabolic pathways.

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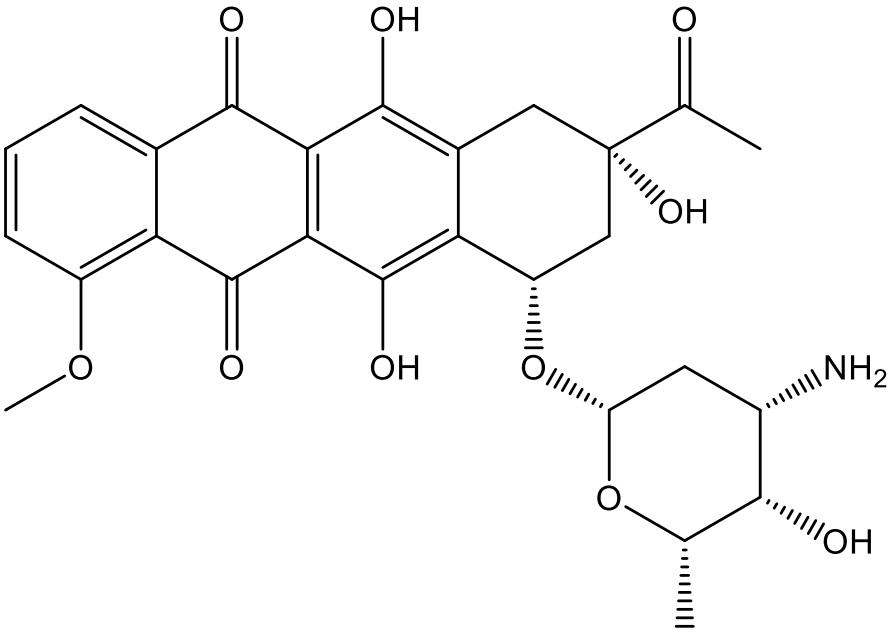
1. Dox (Doxorubicin) and Epi (Epirubicin) are anthracyclines (anticancer antibiotics).
   - They are conjugated with glucuronide primarily on the 4'-hydroxyl group (not the 4-hydroxyl, which is in a different position on the ring system).
   - Epirubicin undergoes glucuronidation more readily than doxorubicin.
   - Both are excreted significantly in bile.
2. Anacyclines is not a standard term in medicinal chemistry. It may be a misspelling of anthracyclines (the class containing doxorubicin/epirubicin) or possibly tetracyclines (antibiotics).
   - Tetracyclines are not glucuronidated on a “fourth hydroxyl” in the same way, nor are they primarily biliary-excreted as glucuronides.
3. MS in this context likely means Mass Spectrometry . a common analytical technique used to study drug metabolism (including glucuronidation and biliary excretion [20].

Section	Suitable	Notes
Abstract	No	Too detailed
Introduction	Partially	Only the first 1–2 sentences (general anthracycline use)
Main body — MS fragmentation of anthracyclines	Yes	Paragraph 1 (in-source fragmentation, m/z range, glycosidic bond cleavage
Main body — Glycosidic bond cleavage mechanisms	Yes	Bond disruption under acidic conditions [17]
Main body — Structure-activity relationship (SAR)	Yes	Epi vs Dox, cardiotoxicity, lipophilicity, glucuronidation, biliary excretion
Discussion / Critical analysis	Possibly	If comparing fragmentation stability vs biological activity trade-offs
Conclusion	No	Too specific



Scheme 1. Chemical structure of anthracycline

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Novel anthracycline-containing bioconjugates have recently been synthesized [21, 22]. When anthracyclines are broken down chemically, they can produce a wide range of by-products depending on how they separate. In contrast, ESI is a gentle ionization approach that yields mostly protonated molecules. In positive ion mode, ESI-MS/MS is particularly effective for examining anthracyclines (aglycones). Rapid atom bombardment has been used to characterize the mass-spectral properties of several anthracyclines and their related aglycones [23].

Due to the inability to ionise aglycones as positively charged particles, they were classified as negatively charged particles. Even if the glycosidic bond is broken, to facilitate metabolite identification, understanding the fragmentation paths of the whole anthracycline parental molecule is indispensable. Reduction of the C-13 keto group, glycosidic link breakage, and glucuronide molecule conjugation are the three main mechanisms for the metabolic breakdown of Dox [24].

It is possible to ionize all these by-products using ESI in positive ion mode. Therefore, ESI-MS/MS is the technique of choice if only a small amount of anthracycline drug material is available for structural analysis [25]. PDC synthesis relies on MS because it enables the swift and precise final product to be identified. However, the purity of anthracyclines and their derivatives cannot be accurately assessed via MS owing to atypical fragmentation.

The MS characterization of a series of anthracyclines and anthracyclinones, including adriamycin, Dau, and their modified counterparts, was made possible by the application of negative and positive fast atom bombardment (FAB) ionization procedures. The molecular ions of anthracyclines have more than one hydrogen atoms added to them. The FAB matrix is an important choice for chemical structure investigation. Molecular ions M (or M+) and "[M+H]+" predominate when surfoplane and glycerol are employed as FAB solvents. The aglycone moiety retains a large amount of charge even after cleavage of the glycosidic linkage. The tetracyclic ring aromatized, resulting in further fragmenting of the aglycone molecule. Anthracyclines could only be detected by negative FAB ionization when surfoplane was used as the FAB matrix [27].

Lachatre et al. [28] used an SPE followed by HPLC-ESI-MS to simultaneously identify four anthracycline anti-cancer medications and three metabolites in human blood. Automated SPE with chloroform/2-propanol elution, evaporation, and acetonitrile reconstitution on 5 µL serum samples (60:40, chloroform/2-propanol elution volume/volume) was performed. SIM-containing mobile phase was used for ESI-MS analysis, and then reversed-phase HPLC was performed. Darubicin and idarubicinol, Dau and daunorubicinol, Epi and Dox, Dox and aclarubicin, and idarubicin and aclarubicin had m/z 291, 321, 361, 361 and 361, respectively. These ions were used to confirm the specific analytes. All root-square deviation values were less than 15%, and extraction recoveries ranged from 71% to 100%. Limits of detection were 0.5–2.5 ng/mL, and limits of quantification were 2.5–5.0 ng/mL.

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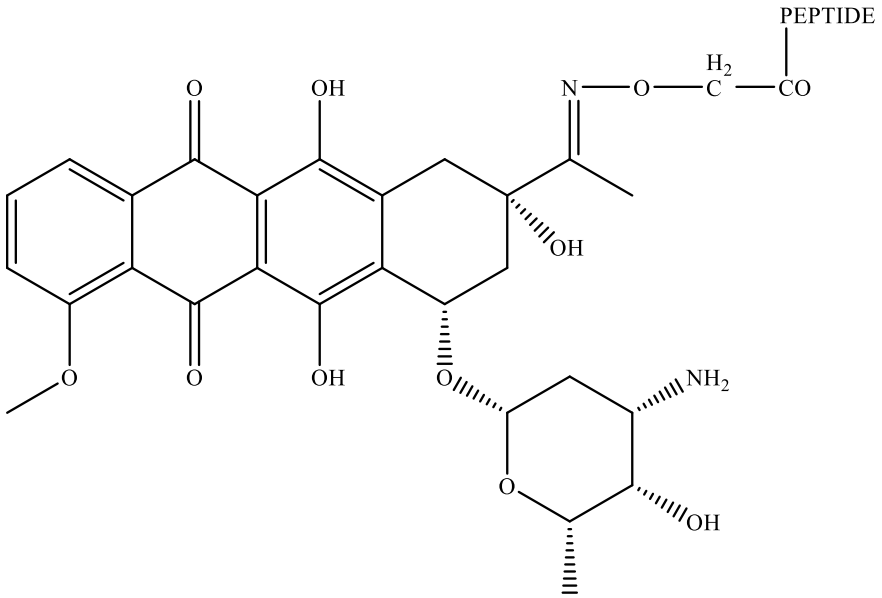
2. Structural Analysis of Daunomycin-Peptide Conjugates

Traditional cytotoxic drugs have desirable pharmacokinetics, including oral absorption, metabolic stability, and water solubility [29]. However, the quality of life of the patient suffers as a result of the adverse effects of traditional treatments. In PDCs, drug molecules are attached to the peptides via particular linkers, making them pro-drugs. In addition to being a tumour-homing agent, the peptide may also improve the physicochemical qualities of the medication. In light of all of this, PDCs appear to hold great promise as targets for personalized cancer therapy [30, 31].



Scheme 2. Chemical structure of a daunomycin-containing peptide conjugate with an oxime bond.

This strategy is used to treat a variety of cancers, including leukaemia, with Dau. Several peptide-drug conjugates containing Dau have been created and exhibit remarkable anti-cancer action both in vitro and in vivo [33, 32]. Topoisomerase II is inhibited by this anthracycline antibiotic, which is found in the nucleus and may bond with DNA [34]. An O-glycosidic link binds the aminoglycoside sugar moiety of the molecule to a tetracyclic quinoid aglycone component (daunosamine).

Daunomycin is not suitable for therapeutic use due to its many side effects, the most serious of which is cardiotoxicity. Much research has focused on the clinical drawbacks of peptide-based bio-conjugates containing daunomycin, including lack of specificity, fast clearance from circulation, and toxicity [35,36].

It is common for drugs to be coupled to targeting peptides that bind specifically to a receptor found in tumor cells, resulting in an anti-cancer action targeted at those tumor cells [37]. Tuftsin is an immunoglobulin G (IgG) Fc heavy chain tetrapeptide (TKPR) (289–292). Methotrexate is the active ingredient in pharmaceutical delivery systems that contain tuftsin derivatives. This

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branched structure might increase the stability of the bioconjugate in biological systems by serving as a drug conjugation coupling site for the amino group of lysine [38].

CKAAKN substitutes the cysteine residue for serine in the tumour-homing-peptide based on the Frizzled-receptor-specific-sequence CKAAKN, yielding SKAAKN [39, 40]. This peptide has a basic amine at the Dau binding site following the addition of a lysine residue, i.e., KSKAAKN. To increase the distance between the Dau and basic lysine amino groups, a spacer peptide (GFLG) was used in the third design, viz., GFLGKSKAAKN [40].

3. Comparison of Different Activation Modes in Tandem MS of Peptides

Yamashita and Fenn introduced ESI-MS in 1984 and it has experienced significant commercial impact over the last decade [41]. The mechanism by which ions in solution are converted into gases before MS analysis has been investigated by several authors, including Kebarle and Tang [42] and Cole [43].

MALDI has increased the scope of MS by enabling the analysis of relatively small quantities of high-molecular-mass molecules, including proteins, nucleotides, and synthetic polymers. ESI-MS is compatible with practically all mass spectrometer types, depending on the generation of highly charged ions. It has also been used to investigate drugs and their metabolites, which have low molecular masses [44].

Database publications have revealed that HPLC-ESI-MS and capillary electrophoresis-ESI-MS are reliable methods for determining the identity and concentration of pharmaceuticals and drug metabolites [45].

RESULTS AND DISCUSSION

Anacyclines are derived via the glycosidic bonding of tetracycline rings to daunosamine sugars, producing a hydrophobic ring that may be used to treat bacteria. Several types of solid tumors can be treated with these drugs. The only difference between Epi and the other Dox 4-epimers is that Epi has a differently oriented hydroxyl group on the C-4 daunosamine ring. Because Dox is less cardiotoxic, the therapeutic efficacy of the medication is unaffected [18]. Additionally, Epi has higher lipophilicity, making it more effective in penetrating cells [19]. Both Dox and Epi are conjugated with glucuronide on the fourth hydroxyl group and excreted in bile [20].

ESI or matrix-assisted laser ionization/desorption peptides use MALDI to generate their peptides and , then different digestion processes. In the case of average-sized peptides, ESI produces predominantly doubly or triply charged ions, while MALDI-peptide-ions contain just one ionizing proton [46]. The fragmentation patterns of protonated peptides can be radically different even under comparable experimental conditions (time scale, excitation, etc.) ESI versus MALDI for average-sized peptides are often overlooked in bio-conjugate analysis. Many researchers assume these techniques are interchangeable, but I disagree. The fact that ESI yields doubly or triply charged ions while MALDI yields singly charged ions means that fragmentation patterns cannot be directly compared, even under similar experimental conditions. Therefore, I

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suggest that future studies should systematically evaluate both ionization methods rather than defaulting to one. Furthermore, I encourage the field to move away from the assumption that comparable excitation conditions produce comparable fragmentation—this is clearly not the case, as the radically different patterns demonstrate. [47].

Peptide spectrometry was used to investigate alternative activation processes of 36 synthetic peptides, ranging from 4 to 16 amino acid residues, including three disulphide-linked peptides [48]. More than 80 amino acids and peptides have been identified in this collection [49, 50], including several previously unreported species, as confirmed by database comparison [51].

When the collision energy (CE) was raised, the CID of each peptide could be determined in both positive and negative ion modes. This study examined the CID properties of deprotonated peptides using a collection of 33 peptides ranging in size from 7 to 16 amino acids. The charge state as a function of CE and the impact of the precursor on peptide fragmentation were examined by HCD or CID in negative ion mode during deprotonated peptide sequencing , The impact of the precursor on peptide fragmentation was examined during deprotonated peptide sequencing because deprotonated ions follow different dissociation pathways than protonated ions, and understanding this relationship is necessary for accurate sequence identification in negative ion mode.

Proteins that have been deprotonated, such as peptides sequenced mostly in negative-ion mode, can be analyzed to reveal proteome information. Mass spectrometry using hydrogen-deuterium exchange (HDX-MS) can be utilized to examine protein structural elasticity, investigating the rearrangement of hydrogen bonds of the protein backbone using a time-dependent deuterium atom transfer between the solvent and amide groups [51].

Processing and interpretation of HDX-MS data are more difficult for the enzyme CYP3A4 because it can simultaneously interact with many ligands and delocalize inside the enzyme. It is difficult to distinguish between ligand binding and rate-enhancing variables using other allosteric research methodologies [52].

The choice of analysis, both quantitatively and qualitatively, is crucial. The UV-Vis detector is one of the most extensively used detectors. It detects light in the visible (Vis) and ultraviolet (UV) regions of the electromagnetic spectrum. It reacts with any compound that absorbs radiation in the 200–800 nm region, making it practically universally applicable. However, its low sensitivity and selectivity are substantial drawbacks [53].

MS/MS fragmentation methods include collision-based techniques such as CID (collision-induced dissociation), in-source CID (also called SID), and HCD (higher-energy collisional dissociation), as well as electron-based techniques such as ECD (electron capture dissociation) and ETD (electron transfer dissociation). Each method generates different types of fragment ions by cleaving the peptide backbone at specific bonds. Understanding these differences is essential for top-down sequencing, where intact proteins or peptides are fragmented directly in the mass spectrometer to determine their sequence.

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Specifically, collision-based methods (CID, HCD, and in-source CID) primarily cleave the amide bond along the peptide backbone, producing b-type ions (containing the N-terminus) and y-type ions (containing the C-terminus). In contrast, electron-based methods (ECD and ETD) cleave the bond between the nitrogen and the alpha carbon (N–Cα bond), producing c-type ions (N-terminal fragments) and z-type ions (C-terminal fragments). Some methods can also produce a-type ions, which result from further loss of carbon monoxide (CO) from b ions, but this is not exclusive to SID or ETD as suggested elsewhere [54]. Therefore, using multiple complementary fragmentation methods provides richer structural information than any single method alone [54].

Many top-down proteomic techniques are complicated by the low abundance and poor signal-to-noise ratio (S/N) associated with large-molecule spectrometry, intricate data analysis of MS and MS/MS spectra, and the co-elution of proteoforms during the preliminary separation stages of liquid chromatography or capillary zone electrophoresis. Top-down sequencing of proteoforms with molecular weights greater than 50–70 kDa is difficult [55]. New mass spectrometers, such as FT-ICR, can capture short time-domain transients to maximize the spectral S/N of large proteoforms [56].

ECD involves adding a low-energy thermal electron to a peptide or protein to generate charge-reduced radicals. Its major drawback is poor efficiency (5–20%) and molecules larger than 20 kDa are not accurately fragmented. ETD uses an anionic reagent with positively charged peptides/proteins. Both ECD and ETD involve little vibrational energy redistribution, preserving non-covalent interactions. However, ETD suffers poor dissociation efficiency, especially for low charge-density precursors. EThcD combines the fragmentation advantages of ETD and HCD and is advantageous for peptide de novo MS and identification of post-translational modifications . Importantly, Daunomycin (daunorubicin) is an anthracycline, structurally analogous to doxorubicin (Dox) and epirubicin (Epi). Your earlier note about glucuronidation at the 4′-hydroxyl group is relevant to the *metabolism* of free anthracyclines, but for *peptide conjugates*, the focus shifts to fragmentation MS/MS methods (ECD, ETD, HCD, EThcD) that preserve or cleave the linker-drug moiety, Across different research groups, the consensus is that ETD and EThcD are the most effective MS/MS techniques for characterizing peptide–daunomycin conjugates. ECD is inefficient for larger peptides/proteins (>20 kDa) and has low fragmentation yield. HCD alone causes premature loss of the daunomycin moiety. EThcD combines the drug-preserving ability of ETD with the high sequence coverage of HCD, making it the preferred method for *de novo* sequencing and mapping post-translational modifications or drug attachment sites [57].

CONCLUSIONS

The mass spectrometric analysis of anthracycline-containing peptide-drug conjugates, particularly daunomycin bioconjugates, is significantly challenged by extensive in-source fragmentation due to glycosidic bond cleavage. Although soft ionization techniques such as ESI and MALDI are essential for peptide analysis, they fail to preserve the structural integrity of daunomycin conjugates, producing complex spectra that complicate unambiguous structural assignment and purity estimation. Higher-energy collisional dissociation (HCD) suppresses characteristic sugar-loss fragmentation, yet the resulting spectra still resemble those of free

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daunomycin, making differentiation between the conjugate and its drug payload problematic. Complementary activation methods, including ETD and EThcD, offer improved sequence coverage and structural characterization compared to CID or HCD alone. Therefore, future analytical protocols must account for both morphological variables (e.g., linker chemistry, drug-to-peptide ratio) and instrumental parameters (e.g., collision energy, ionization mode) to achieve reliable characterization of next-generation daunomycin-based cancer therapeutics.

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

المقدمة: تُعدّ الأنثراسيكلينات من الأدوية الكيميائية واسعة المجال العلاجي. وقد جرى اكتشافها في سلالة منتِجة للأصباغ من بكتيريا **Streptomyces**، وتُستخدم بشكل عام كأدوية مضادة للسرطان. وتُعدّ **دوكسوروبيسين (Dox)** و**داونوروبيسين (Dau)** و**إبيروبيسين (Epi)**من أهم الأنثراسيكلينات المستخدمة في علاج السرطان، وهي من أكثر العوامل المضادة للأورام فعالية المتاحة اليوم. ويُعدّ **الداونوروبيسين** دواءً شائع الاستخدام في علاج عدة أنواع من اللوكيميا. وغالبًا ما تُربط هذه الجزيئة المضادة للسرطان بمشتقات **GnRH–III**عبر رابطة أوكسيمية، بحيث يُستبدل الحمض الأميني **سيرين** في الموضع الرابع بـ **ليزين** .**(Ac)** وتُسهم هذه الاقترانات في تعزيز الانتقائية وتقليل السُمّية الجهازية.

طرق العمل: لقد تبيّن أن هذه المتقارنات الحيوية شديدة الانتقائية، وقادرة على إبطاء نمو الأورام بشكل ملحوظ في مجموعة واسعة من أنواعها. ونظرًا لبنيتها الجزيئية المعقّدة، يجري عادةً تأكيد هوية هذه المتقارنات بين الببتيدات والأدوية باستخدام **مطياف الكتلة(MS)** ، والذي يمكن الاستفادة منه أيضًا في أغراض التوصيف التحليلي. وتُستخدم تقنيات التأيّن اللطيف مثل **الرش الإلكتروني (ESI)** و**الامتصاص/الاستشراب بالليزر بمساعدة المصفوفة (MALDI)** غالبًا لإنتاج الجزيئات البروتونية من البروتينات والببتيدات.

النتائج : من ناحية أخرى، فإن أطياف الكتلة للمتقارنات الحيوية المحتوية على داونوميسين **تخضع لعمليات تفتّت واسعة** تحت ظروف المطيافية التقليدية. ويُظهر الداونوميسين غالبًا نمطًا مميّزًا من شوارد التفتّت. ويُعدّ **انقسام الرابطة الغليكوزيدية** بوساطة الجزء اللاألكالي (aglycone)أو بواسطة **الدونوزامين** آليةً رئيسية لحدوث هذا التفتّت.

الاستنتاجات: عدّ المتقارنات الدوائية الببتيدية (PDCs) طلائع دوائية ترتبط فيها الجزيئات الدوائية بالببتيدات عبر وصلات تساهمية. ويُواجه تحليل متقارنات الداونوروبيسين باستخدام مطيافية الكتلة تحديات بسبب فقدان الجزء السكّري أثناء التأيّن، كما تعتمد أنماط التفتّت على عوامل تقنية مثل إعدادات الجهاز والمذيب وبنية المتقارن. ويوفّر المتقارن الجديد للداونوروبيسين مع الببتيد خيارًا علاجيًا آمنًا وفعّالًا للسرطان، ويتطلّب توضيح بنيته استخدام تقنيات كشف متعددة. ويظلّ تنشيط الأيونات محورًا أساسيًا في تحليل الببتيدات والبروتينات، إذ تحدّد تقنيات التأيّن والتفتّت المستخدمة أنماط الشوارد وتساعد في توصيف البنى والتعديلات المختلفة.

الكلمات المفتاحية: الأنثراسيكلينات؛ دوكسوروبيسين؛ داونوروبيسين؛ متقارناتGnRH–III ؛ مطيافية الكتلة؛ التفتّت؛ انقسام الرابطة الغليكوزيدية.