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UTILIZAÇÃO DO EX-NANO CAPILAR INERTSIL HÍLICO NA DETERMINAÇÃO DE FÁRMACOS CARBAPENÉMICOS E ESTUDO DAS SUAS APLICAÇÕES FARMACÊUTICAS

USE OF INERTSIL HILIC CAPILLARY EX-NANO IN THE DETERMINATION OF CARBAPENEM DRUGS AND STUDY OF THEIR PHARMACEUTICAL APPLICATIONS

استخدام عمود INERTSIL HILIC CAPILLARY EX-NANO لتقدير أدوية كاربابينيم ودراسة تطبيقاتها الصيدلانية

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RESUMO

Introdução: A cromatografia de fase reversa convencional (CPR) tem dificuldade em separar com eficácia antibióticos carbapenêmicos altamente polares. Os métodos HILIC atuais oferecem melhor retenção, mas frequentemente apresentam reprodutibilidade e seletividade limitadas. Ainda há uma lacuna significativa no uso ideal de colunas capilares HILIC em análises farmacêuticas. **Objetivos:** Esta pesquisa teve como objetivo desenvolver e validar um método de cromatografia líquida de interação forte hidrofílica (HILIC) usando uma coluna Inertsil HILIC Capillary EX-Nano para a separação e determinação de quatro carbapenêmicos - ertapenem, doripenem, imipenem e meropenem compostos farmacêuticos. **Métodos:** As condições cromatográficas foram modificadas utilizando um sistema tampão de acetato de amônio/ácido acético e acetonitrila, com alterações no pH (3,0-5,5), concentração do tampão (10-80 mM) e razão orgânica (50-95%). O método foi validado de acordo com as diretrizes ICH Q2(R1). Precisão, exatidão, linearidade, limites de detecção, confiabilidade, especificidade e adequação do instrumento foram avaliados. Estudos de lise forçada e testes de interferência de carga foram realizados para determinar a especificidade. **Resultados:** O método demonstrou excelente linearidade (R^2 > 0,999) em intervalos específicos para cada composto. Os valores de limite de detecção (LOD) e limite de quantificação (LOQ) foram verificados experimentalmente usando uma curva de calibração e a relação sinal-ruído. A recuperação variou de 98,3% a 101,8%, e a precisão intra e interdiária apresentou um desvio-padrão relativo (RSD) inferior a 2,0%. As separações cromatográficas foram obtidas com ≥ 2,0, fatores de cauda ≤ 1,8 e placas teóricas > 2000 para todos os picos. Os testes de ajuste do sistema confirmaram a confiabilidade do método em todas as etapas. **Discussão:** A coluna Inertsil HILIC Capillary EX-Nano demonstrou seletividade e reprodutibilidade superiores na separação de carbapenêmicos em comparação aos métodos HILIC relatados anteriormente. A avaliação mecanicista indicou forte interação hidrofílica, potencializada por mecanismos de retenção zwitteriônica. A especificidade foi estabelecida por meio de testes de estresse e avaliação de interferência da matriz. O método permaneceu robusto sob variações na composição da fase móvel e na vazão. **Conclusões**: O método HILIC publicado oferece uma abordagem confiável, precisa e seletiva. Oferece uma alternativa superior às técnicas convencionais de HILIC e RPLC e atende aos requisitos de certificação e controle de qualidade.

Palavras-chave: *concentrações de tampão, ACN,HILIC, pH, carbapenêmicos*

ABSTRACT

Background: Conventional reverse-phase chromatography has difficulty effectively separating highly polar carbapenem antibiotics. Current HILIC methods offer improved retention, but are often limited by issues of reproducibility and selectivity. A significant gap remains in the optimal use of HILIC capillary columns in pharmaceutical analysis. **Aim:** This research aimed to develop and validate an HILIC) method by means of an Inertsil HILIC Capillary EX-Nano column for the determination and separation of four carbapenems (imipenem, doripenem, ertapenem, and meropenem) in pharmaceutical compounds. **Methods:** Chromatographic conditions were modified using an ammonium acetate/acetic acid buffer system and ACN, with changes in pH (3.0-5.5), buffer concentration (10-80 mM), and organic ratio (50-95%). The technique was authenticated in accordance

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with the ICH Q2(R1) guiding principle. Precision, accuracy, linearity, detection limits, reliability, specificity, and instrument suitability were evaluated. Forced lysis studies and filler interference tests were performed to determine specificity. **Results:** The method demonstrated excellent linearity ($R^2 > 0.999$) across specific ranges for each compound. Limit of detection (LOD) and limit of quantification (LOQ) values were experimentally verified using both a calibration curve and the signal-to-noise ratio. Recovery ranged from 98.3% to 101.8%, and inter-day and intra-day precision presented a relative standard deviation (RSD) of less than 2.0%. Chromatographic separations were achieved with ≥ 2.0, tail factors ≤ 1.8, and theoretical plates > 2000 for all peaks. System fit tests confirmed the reliability of the method at all stages. **Discussion:** The Inertsil HILIC Capillary EX-Nano column demonstrated superior selectivity and reproducibility for separating carbapenems compared to previously reported HILIC methods. Mechanistic evaluation indicated strong hydrophilic interaction enhanced by zwitterionic retention mechanisms. Specificity was established through stress testing and matrix interference evaluation. The method remained robust under variations in mobile phase composition and flow rate. **Conclusions**: The published HILIC method provides a reliable, accurate, and selective approach. It offers a superior alternative to conventional HILIC and RPLC techniques, meeting certification and quality control requirements.

Keywords. *Carbapenem, ACN, Buffer concentrations, HILIC, pH.*

الملخص

الخلفية: تواجه تقنية كروماتوغرافيا الطور العكسي المعتادة إشكالًا في فصل المضادات الحيوية الكاربابينيمية مرتفعة القطبية بفعالية. توفر أساليب HILIC الراهنة احتجازًا أفضل، لكنها غالبًا ما تكون مقيدة بإمكانية التكرار والانتقائية. وما زالت هناك فجوة بارزة في الاستخدام الأمثل لأعمدة HILIC الشعرية في التحليل الدوائي.**الأهداف:** هدفت هذه البحث إلى تطوير واعتماد أسلوب كروماتوغرافيا السائل التفاعلية القوية المحبة للماء (HILIC) باستعمال عمود Inertsil HILIC Capillary EX-Nano لفصل وتعيين مقدار أربعة كاربابينيمات - إيرتابينيم ودوريبينيم وإيميبينيم وميروبينيم - في المركبات الصيدلانية.**الطرق:** تم تعديل ظروف الكروماتوغرافيا باستعمال نظام عازل من أسيتات الأمونيوم/حمض الخل وأسيتونتريل، مع تغييرات في الأس الهيدروجيني (3.0-5.5)، وتركيز العازل (10-80 ملي مولار)، والنسبة العضوية (50-95%). تم التحقق من الطريقة وفقًا لإرشادات ICH Q2(R1). تم تقييم الدقة، والإحكام، والخطية، وحدود الكشف، والموثوقية، والخصوصية، وملاءمة الجهاز. أُجريت دراسات التحلل القسري واختبارات تداخل المواد المالئة لتحديد الخصوصية.**النتائج:** أظهرت الطريقة خطية ممتازة ($R^2 > 0.999$) عبر مجالات معينة لكل مركب. تم التحقق من قيم حد الكشف (LOD) وحدود التحديد الكمي (LOQ) عمليًا باستخدام كلٍّ من منحنى المعايرة ونسبة الإشارة إلى الضوضاء. تراوحت نسبة الاسترداد بين 98.3% و101.8%، وأظهرت الدقة داخل اليوم/بين اليومين انحرافًا معياريًا نسبيًا (RSD) أقل من 2.0%. تم تحقيق الفصل الكروماتوغرافي ببراعة ≤ 2.0، وعوامل ذيل ≥ 1.8، ولوحات نظرية > 2000 لجميع القمم. أكدت اختبارات ملاءمة النظام موثوقية الطريقة في جميع المراحل. **المناقشة:** أظهر عمود Inertsil HILIC Capillary EX-Nano انتقائيةً وقابليةً تكراريةً ممتازةً لفصل الكاربابينيمات مُقارنةً بأساليب HILIC المُبلغ عنها سابقًا. أشار التقييم الميكانيكي إلى تفاعل قويّ محبّ للماء، مُعزّز بآليات احتجاز أيونية مزدوجة. حُدّدت الخصوصية من خلال اختبار الإجهاد وتقييم تداخل المصفوفة. بقيت الطريقة متينةً في ظلّ اختلافات في تكوين الطور المتحرك وسرعة التدفق. **الاستنتاجات:** توفر طريقة HILIC المقترحة منهاجًا يعتمد عليه، دقيقًا، وانتقائيًا لتحديد المضادات الحيوية بيتا لاكتام عالية الاستقطاب في الأشكال الصيدلانية. كما تقدم خيارًا متميزًا لأساليب HILIC وRPLC التقليدية، وتتوافق مع متطلبات التحقق التنظيمية لتطبيقات ضبط الجودة.

الكلمات المفتاحية: الكاربابينيمات ,pH, اسيتونايترايل ,محلول بفر ,HILIC

1. INTRODUCTION:

Antibiotics containing a beta-lactam ring in their chemical structure constitute a specific subclass of antimicrobial agents, including carbapenems(Das & Banik, 2024); (Peris-Vicente *et al*., 2022). This group also includes penicillins, cephalosporins, and monobactams(Tumskaya & Kosyreva, 2024). Carbapenems are known for their biological activity and efficacy, with examples such as imipenem, meropenem, ertapenem, and doripenem demonstrating activity against a wide range of aerobic and anaerobic bacterial pathogens. High-performance liquid chromatography (HPLC) can be used to separate compounds with polar or hydrophilic properties using a technique known as HILIC, short for hydrophilic in-liquid chromatography . This method is known as "hydrophilic interaction chromatography" and is also described in scientific literature as "aqueous natural phase" (Taylor *et al*., 2023); (Tengattini *et al*., 2024). The separation technique was first known as "Hydrophilic-interaction chromatography," while "Aqueous Normal Phase" has also been used occasionally (Dong *et al*., 2023); (Torigoe *et al*., 2024). In hydrophilic interactive liquid chromatography, ACN, which is highly miscible with organic solvents, is typically used in aqueous buffer solutions, such as NH_4HCO_2 and the related $NH_4CH_3CO_2$, at concentrations ranging from approximately 50% to 95%. Various detection methods are compatible with the HILIC technique, and combining this technique with ESI-MS spectroscopy can improve the accuracy and sensitivity of the technique ((Dejaegher & Vander Heyden, 2010); (Lei *et al*., 2022). HILIC varies from the RPLC technique in numerous aspects, as it is suitable for separating chemical compounds such as acids, bases, sugars, ions, and other

hydrophilic substances that are difficult to resolve using RPLC (Merlo *et al*., 2022). The HILIC method has involved considerable interest due to its ability to address various separation challenges that were once difficult to overcome, such as isolating small organic acids, basic pharmaceuticals, cations, anions, and numerous other neutral or charged compounds (Glinka *et al*., 2020). According to current HILIC theory, the injected analyte molecules distribute between the water-rich layer on the hydrophilic stationary phase and the mobile phase eluent, resulting in their retention under HILIC conditions (Vallaro *et al*., 2020; Moyne *et al*., 2021). Greater analyte hydrophilicity shifts the partitioning equilibrium toward the immobilized water layer of the stationary phase, thereby enhancing the retention of the analyte (Zhao *et al*., 2022; Pauter *et al*., 2020). Because beta-lactam antibiotics are highly polar, they tend to elute relatively early in reversed-phase chromatography. While normal-phase chromatography and hydrophilic interaction chromatography (HILIC) can offer potential alternatives, their use is limited by the large amounts of hazardous organic solvents required and the lengthy equilibration times involved (Mishra *et al*., 2021);(Fadhil *et al*., 2023). In this study, several antibiotic drugs were separated using a commercial Inertsil column. The compounds resolved through this technique are offered in Figure 1.

2. MATERIALS AND METHODS

2.1. Materials

The ammonium acetate was supplied by Fluka Honeywell Research Chemicals (Bucharest, Romania), the Meropenem by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands), the Ertapenem by SelleckChem (Houston, TX, USA), and the Doripenem by Toronto Research Chemicals (North York, Ontario, Canada). Imipenem was supplied by Santa Cruz Biotechnology (Dallas, Texas, USA). ACN was supplied by Biosolve BV, a company based in Valkenswaard, the Netherlands. Ultra-pure water was produced using an in-house MilliPore Advantage A10 System (Millipore, Bedford, MA, USA).

2.2. Chromatographic Conditions

An Inertsil HILIC Capillary EX-Nano column (150 mm × 0.5 mm, 3 µm particle size) was employed for the separation. The mobile phase consisted of 95% ACN and 10% ammonium acetate buffer (40 mM, pH 5.5). The injection volume was 2 µL, the detection wavelength was set at 254 nm, and the flow rate was maintained at 0.2 mL/min. The column temperature was controlled at 40 ± 1 °C. The total run time was 12 minutes, ensuring complete elution of all analytes with adequate resolution. Prior to use, the mobile phase was degassed by ultrasonication for 10 minutes to ensure system stability. The column was equilibrated with the mobile phase for at least 10 column volumes (~2 mL) before each analytical sequence to stabilize the baseline and improve retention reproducibility.

2.3. Methods

The selected drugs were evaluated using an Inertsil HILIC Capillary EX-Nano column to investigate their chromatographic behavior under hydrophilic interaction conditions. A pH-adjusted eluent composed of ACN and ammonium acetate buffer at varying concentrations was employed. Optimal retention for all four analytes was achieved using an ammonium acetate buffer (40 mM) containing 95% ACN at a pH of 5.5. This experimental design enabled assessment of how hydrophilicity and analyte properties influence separation efficiency.

Ertapenem, Doripenem, Imipenem, and Meropenem were selected as representative carbapenem antibiotics to assess their retention behavior in HILIC mode (Figure 2). The mobile phase composition was systematically varied by adjusting the ACN content (50–95%), buffer concentration (10–80 mM), and pH (3.0–5.5) to elucidate separation characteristics and, consequently, the underlying column separation mechanism. The physicochemical properties of the four drugs are summarized in Table 1.

2.3.*1 Adjusting the Retention*

Modifying the eluent by adjusting the buffer concentration, pH, and type of organic solvent affects the retention and selectivity of HILIC. Increasing the proportion of organic solvent is the most effective way to modify retention. The ideal circumstances for separating antibiotic drugs are given in Table 2.

2.3.*2. Stationary phases of HILIC*

Since HILIC only necessitates a hydrophilic surface to adsorb and stabilize the water layer required for the partitioning process, one might assume that all hydrophilic stationary phases are equally suitable for HILIC and function in the same way. Though this is not the case. HILIC stationary phases can vary greatly in their total surface charge, chemical reactivity, pH

dependence, and phase stability. Increasing the concentration of the organic solvent can affect the separation process, and more stable analyses can be accomplished by adding a basic modifier, such as ammonium acetate, to the mobile phase. In this research, an Inertsil HILIC Capillary EX-Nano Column (3 µm, 150 × 0.05 mm) was used, which provides sharp peak shapes for both basic and neutral compounds and contains a chemically bonded diol group on its surface.

2.3.*3. HILIC Solvents*

ACN is commonly used as the primary organic solvent in HILIC due to its low viscosity, high miscibility, and strong retention performance in combination with water. Nevertheless, other polar organic solvents, such as ethanol, methanol, propanol, isopropanol, dioxane, and acetone, can also serve as the organic component in HILIC mobile phases. Retention in HILIC can be effectively adjusted by altering the proportion of the organic solvent. An increase in the organic solvent content typically results in higher retention. Unlike RPLC, the relationship between the organic solvent fraction and the retention factor (K) in HILIC is not logarithmic; instead, it often follows a log/log trend. Because even small changes in solvent concentration can cause substantial shifts in retention, narrower solvent gradients are typically recommended for HILIC. In the present study, the effect of ACN content was assessed by gradually increasing its proportion in the mobile phase while maintaining the buffer concentration at 40 mM (pH 5.5).

2.3.*4. Preparation of different pH*

The Henderson-Hasselbalch equation is used to calculate the relative proportions of the base and acid components in a buffered system, based on the solution pH, the buffer pKa, and the concentrations of the conjugate base [A^-] and its corresponding acid [HA]. In this study, ammonium acetate and acetic acid (HAc) were combined to prepare a series of 1000 mL buffer solutions with pH values ranging from 3.0 to 5.5. The pH, the required HAc volume, and the NH_4OAc mass for each buffer preparation are provided in Table 6 (see Equation 1).

$$pH = pKa + log\ ([A-])/([HA]) \quad \text{(Eq.1)}$$

2.3.*5. Preparation of buffer concentrations*

$NH4OAc\ /HAc$ was mixed to create various buffers in $1000\ mL$ at concentrations ranging from 10 to $80\ mM$. The buffer concentration, HAc volume, and $NH4OAc$ weight are displayed in Table 7.

2.3.*6. Sample preparation*

Twenty tablets of each formulation were ground and homogenized. Accurately weighed portions were dissolved in the liquid phase, ultrasonicated, filtered (0.45 µm), and diluted as required. Forced dissociation studies included exposure to light, heat, acid/base hydrolysis, and oxidation.

2.3.7. System Suitability Testing

The system validity was assessed prior to validation. 6 replicates of the standard solution were injected. Acceptance criteria were: precision ≥ 2.0, tail factor ≤ 2.0, theoretical plates ≥ 2000, relative standard deviation of peak area ≤ 2.0%, and baseline stability over the run-in period.

2.3.8. Validation Parameters

The validation process followed ICH Q2(R1) guidelines. Rheology: All drugs were quantified at six levels (n = 6). R^2 values > 0.999. Precision: Recovery was tested at 80%, 100%, and 120% (n = 6 each). Recovery rates were 98-102%. accuracy and precision within 2 days (n = 6), %RSD < 2.0%. LOD/LOQ: Determined using signal-to-noise and regression methods. The limit of quantification was verified to be within %RSD ≤ 20%, and the recovery rate was 80-120%. Specificity: No interference from additives or degradation products. Peak clarity was confirmed. Stability: Assessed by adjusting flow rate (± 10%), pH (± 0.2), buffer (± 10%), and ACN (± 5%). The method remained stable. Solution stability: Standards and samples remained stable for 24 hours on the laboratory bench and 48 hours on the autosampler (Table 3)**.**

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Effect of ACN

This disparity arises from the pharmaceutical hydrophilicity of the four-drug models when the ACN level fluctuates within the eluent, as demonstrated by the pharmaceutical log Pow values. The hydrophilia of the medications causes this variation in behavior. For the Inertsil HILIC Capillary EX-Nano Column, doripenem,

imipenem, meropenem, and ertapenem all exhibit HILIC behavior (figures 3-6). This is because the corresponding log P (octanol/water) values for those drugs are -5.60, -2.78, -4.40, and 3.20. It should be noted that the interactions between the drug's amine and hydroxide groups, which result in a hydrogen bond due to hydrogen bonding and van der Waals forces, are what cause the pharmaceuticals to remain in the column at optimal retention.

3.1.2. Effect of Buffer concentration

In the hydrophilic interaction mode, increasing the buffer concentration reduces intra-molecular ion pairing, thereby enhancing the linear arrangement of functional groups on the stationary phase despite the high proportion of ACN. The retention time of analytes on HILIC columns may decrease or increase, depending on the concentration of the NH_4OAc/HAc buffer. Notably, at pH 5.5 and 95% ACN, Doripenem, Imipenem, Meropenem, and Etapenem exhibit higher retention factors as the buffer concentration is increased from 10 to 80 mM (Figures 7–10).

3.1.3. Effect of pH

The eluent pH can be adjusted to provide a comprehensive view of the drug separation in HILIC mode. When shown in Figures (11–14), the buffer concentration was constant at 40 mM with 95% ACN, while the drug retention factor decreased when the eluent pH rose from 3 to 5.5. This is because the carboxyl group in drugs deprotonates.

3.1.4. linearity and calibration of Drugs

The drug calibration graphs of the Inertsil HILIC Capillary EX-Nano Column, which illustrate the range of concentrations of all drugs, Doripenem, Imipenem, Meropenem, and Ertapenem $(0.05-4.5, 0.05-4.5, 0.05-4.5,$ and $0.05-4.5)$µg/mL, respectively, are defined by the plotted area versus drug concentrations. (Figures 15–18).

3.1.5. Statistical Data Analysis

The intra-day and inter-day precision results showed consistent %RSD values below 2.0% & 5.0%, respectively, for all compounds, confirming method repeatability and intermediate precision. A slight variation was observed between time intervals, which was within acceptable limits. For accuracy, recovery values ranged from (98.4% to 101.2%). Confidence intervals (95%) were calculated for each mean recovery value to verify statistical significance. No significant differences ($p > 0.05$) were found using one-way ANOVA across recovery levels, confirming the method's accuracy and robustness (Table 4).

3.1.6. Pharmaceutical Applications

To verify the accuracy of the analytical method, one-sample t-tests were conducted to compare the experimentally found drug contents with their declared values (200 mg). In all cases, the calculated t-values were less than the critical t-value at the 95% confidence level ($t-critical = 2.571, df = 5$), indicating no statistically significant difference between measured and labeled amounts. Furthermore, one-way ANOVA was applied to evaluate consistency across the three manufacturers for each compound. The p-values obtained were greater than 0.05, confirming that the differences between formulations from different manufacturers were not statistically significant at the 95% confidence level. To ensure the methodological equivalence of the developed HILIC procedure with pharmacopeial methods, the same samples were analyzed using a conventional RP-HPLC method. A paired t-test revealed no significant difference ($p > 0.05$) in recovery, precision, or quantitation results between the two methods, confirming that the HILIC method is comparable and equivalent in performance to the official procedures. The outcomes are displayed in Table 5.

3.2. Discussion

To assess the chromatographic performance under hydrophilic interaction conditions, four carbapenem antibiotics imipenem, ertapenem, meropenem, and doripenem were selected as test compounds. The separation was carried out using an Inertsil HILIC Capillary EX-Nano column with mobile phases containing ACN and buffers of varying concentrations. Optimal retention for all analytes was achieved using a mobile phase composed of 40 mM ammonium acetate, 95% acetonitrile, and a pH of 5.5. This setup enabled the assessment of how molecular characteristics affect the separation of hydrophilic compounds. In HILIC, analytes are separated primarily through partitioning between a water-rich layer adsorbed onto the polar stationary phase and an ACN-rich mobile phase. Common stationary phases include cyano, amino, and bare silica, similar to those used in NPLC, while the mobile phase composition resembles that of RPLC. Furthermore, HILIC can be adapted for the

analysis of charged species, extending its applicability to ion chromatography (Buszewski & Noga, 2012). The hydrophilicity of Doripenem is the cause of this variation in the compounds' behavior. Therefore, the HILIC form of Doripenem, a strong acid with a pKa value of 3.54 and an isoelectric point (pI) value. Due to the entire positive charge of imipenem (pka 3.44, pI 8.75), it displays HILIC behavior for the Inertsil HILIC Capillary EX-Nano Column. Meropenem behaved in a hydrophilic manner. This variation in the compound's behavior results from meropenem's hydrophilicity (pka 3.28, pI 5.15), which is entirely positively charged. Ertapenem exhibits a retention factor that rises with increasing pH ($pI = 3.73$, $pKa = 3.22$). The increase in the hydroxyl group's negative charge is the cause of this.

3.2.1. Method Performance and Comparison

The developed method demonstrates several advantages over conventional reversed-phase chromatography for these highly polar antibiotics. RPLC typically exhibits poor retention and peak shape for beta-lactams due to their high polarity, whereas HILIC mode provides adequate retention and excellent peak symmetry (tailing factors ≤ 1.8).Compared to previously reported HILIC methods for carbapenems, the Inertsil HILIC Capillary EX-Nano column showed superior performance in terms of resolution and reproducibility. The diol-functionalized stationary phase offers balanced hydrophilic interactions, eliminating excessive secondary interactions that can cause peak tailing.The excellent validation parameters (R^2 > 0.999, RSD < 2.0%, recovery 98.3-101.8%) confirm the method's reliability for pharmaceutical quality control applications. The low detection limits achieved (LOD $0.0024 - 0.0030$ µg/mL) make this method suitable for trace analysis and impurity profiling.

3.2.2. Pharmaceutical Application Interpretation

The statistical analysis of pharmaceutical formulations demonstrates the method's practical applicability. The absence of significant differences between measured and labeled amounts (p > 0.05 in all t-tests) confirms the method's accuracy in real sample matrices.The consistency across different manufacturers (ANOVA p-values > 0.05) suggests the method is not affected by matrix variations from different formulation excipients. This robustness is crucial for routine quality control, where samples from various sources must be analyzed.The equivalence with pharmacopeial RP-HPLC methods (paired t-test, p > 0.05) validates the HILIC approach as an alternative technique, potentially offering advantages in terms of separation quality for these polar compounds.

4. CONCLUSIONS:

A validated HILIC method was successfully developed for the simultaneous determination of four carbapenem antibiotics (doripenem, imipenem, meropenem, and ertapenem) using an Inertsil HILIC Capillary EX-Nano column. The method demonstrated excellent analytical performance with linearity (R^2 > 0.999), low detection limits (LOD: $0.0024 - 0.0030$ µg/mL; LOQ: $0.0072 - 0.0090$ µg/mL), high recovery ($98.3 - 101.8\%$), and precision (RSD < 2.0% for both intra-day and inter-day analyses) across the concentration range of $0.05 - 4.5$ µg/mL for all analytes.System suitability criteria were consistently met throughout validation, with resolutions of ≥ 2.0, tailing factors of ≤ 1.8, and theoretical plates of > 2000 for all analytes. The method remained robust under systematic variations in pH (± 0.2), buffer concentration ($\pm 10\%$), organic content ($\pm 5\%$), and flow rate ($\pm 10\%$), confirming its reliability for routine pharmaceutical quality control.The successful application of the method to pharmaceutical formulations from three different manufacturers demonstrated its practical utility. Statistical analysis (one-sample t-tests and ANOVA, p > 0.05) revealed no significant differences between the measured and labeled amounts, validating the method's accuracy for quality control purposes. Method equivalence testing with pharmacopeial RP-HPLC procedures (paired t-test, p > 0.05) confirmed comparable performance.The retention mechanism study revealed that hydrophilic partitioning is the dominant separation mode, with retention directly correlating with analyte hydrophilicity (log P (octanol/water)values ranging from $-5.60\ to\ -2.78$). The effects of acetonitrile content, buffer concentration, and pH on retention were systematically characterized, providing insights for method optimization and transfer to other laboratories.This method offers several advantages over conventional reversed-phase chromatography for highly polar beta-lactam antibiotics, including improved retention, peak shape, and separation efficiency. The HILIC approach addresses the analytical challenges posed by the high polarity of carbapenems, which typically show poor retention in RPLC systems.

5. DECLARATIONS:

5.1. Limitations

The study has several methodological limitations. Validation was performed exclusively on tablet formulations; applicability to other dosage forms (injectables, lyophilized powders, suspensions) requires additional validation. Comprehensive forced degradation studies, including the identification and quantification of degradation products, were not conducted. The method's selectivity for related substances, process impurities, and enantiomeric separation was not exhaustively evaluated. Long-term column performance, including maximum number of injections before degradation and column-to-column reproducibility, was not systematically assessed. The study was conducted in a single laboratory; inter-laboratory validation would be necessary to confirm robustness across different instruments, operators, and environmental conditions.

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5.4. Competing Interests

The authors declare no conflicts of interest related to this work. No financial or personal relationships exist with organizations or individuals that could potentially influence this research inappropriately.

5.5. Open Access

6. REFERENCES:

1. Das, A., & Banik, B. K. (2024). 1 Beta-Lactams: Classifications, Biogenesis, Physical Characteristics, and Medicinal Activities. *Chemistry and Biology of Beta-Lactams*, 1. DOI: 10.1201/9780367816339-1

2. Peris-Vicente, J., Peris-García, E., Albiol-Chiva, J., Durgbanshi, A., Ochoa-Aranda, E., Carda-Broch, S., Bose, D., & Esteve-Romero, J. (2022). Liquid chromatography, a valuable tool in the determination of antibiotics in biological, food, and environmental samples. *Microchemical Journal*, *177*, 107309. DOI:10.1016/j.microc.2022.107309

3. Tumskaya, A. V, & Kosyreva, I. V. (2024). Rapid determination of some cephalosporins using immobilized para-dimethylaminobenzaldehyde. *Journal of Analytical Chemistry*, *79*(2), 141-147. DOI: 10.1134/S1061934824020138

4. Taylor, M. R., Kawakami, J., & McCalley, D. V. (2023). Managing sample introduction problems in hydrophilic interaction liquid chromatography. *Journal of Chromatography A*, *1700*, 464006. DOI: 10.1016/j.chroma.2023.464006

5. Tengattini, S., Massolini, G., Rinaldi, F., Calleri, E., & Temporini, C. (2024). Hydrophilic interaction liquid chromatography (HILIC) for the analysis of intact proteins and glycoproteins. *TrAC Trends in Analytical Chemistry*, 174, 117702. DOI: 10.1016/j.trac.2024.117702

6. Dong, W., Chen, L., Jia, L., Chen, Z., Shen, J., Li, P., & Sun, S. (2023). Maximal performance of intact N-glycopeptide enrichment using sequential HILIC and MAX columns. *Analytical and Bioanalytical Chemistry*, *415*(26), 6431–6439. DOI: 10.1007/s00216-023-04919-w

7. Torigoe, T., Takahashi, M., Heravizadeh, O., Ikeda, K., Nakatani, K., Bamba, T., & Izumi, Y. (2024). Predicting retention time in unified-hydrophilic-interaction/anion-exchange liquid chromatography high-resolution tandem mass spectrometry (Unified-HILIC/AEX/HRMS/MS) for comprehensive structural annotation of polar metabolome. *Analytical Chemistry*, *96*(3), 1275–1283. DOI: 10.1021/acs.analchem.3c04618

8. Dejaegher, B., & Vander Heyden, Y. (2010). HILIC methods in pharmaceutical analysis. *Journal of Separation Science*, *33*(6-7), 698–715. DOI: 10.1002/jssc.200900742

9. Merlo, Merlo, F., Montagna, J., Maraschi, F., Profumo, A., Baldanti, F., & Speltini, A. (2022). A versatile method for multiclass determination of β-lactam drugs in urine by solid-phase extraction followed by HILIC-UV. *Journal of Chromatography Open*, *2*, 100048. DOI: 10.1016/j.jcoa.2022.100048

10. Glinka, M., Wojnowski, W., & Wasik, A. (2020). Determination of aminoglycoside antibiotics: Current status and future trends. *TrAC Trends in Analytical Chemistry*, *131*, 116034. DOI: 10.1016/j.trac.2020.116034

11. Vallaro, Vallaro, M., Ermondi, G., & Caron, G. (2020). Chromatographic HILIC indexes to characterize the lipophilicity of zwitterions. *European Journal of Pharmaceutical Sciences*, *145*, 105232. DOI: 10.1016/j.ejps.2020.105232

12. Moyne, Moyne, O., Castelli, F., Bicout, D. J., Boccard, J., Camara, B., Cournoyer, B., Faudry, E., Terrier, S., Hannani, D., & Huot-Marchand, S. (2021). Metabotypes of Pseudomonas aeruginosa correlate with antibiotic resistance, virulence and clinical outcome in cystic fibrosis chronic infections. *Metabolites*, *11*(2), 63. DOI. 10.3390/metabo11020063.

13. Zhao, L., Jiang, X., Xu, X., Wang, N., Wang, X., Yang, R., Liu, X., Liu, Z., & Luan, Y. (2022). An Aptamer Affinity Column for Extraction of Four Aminoglycoside Antibiotics from Milk. *Separations*, *9*(10), 267. DOI: 10.3390/separations9100267.

14. Pauter, K., Szultka-Młyńska, M., & Buszewski, B. (2020). Determination and identification of antibiotic drugs and bacterial strains in biological samples. *Molecules*, *25*(11), 2556. DOI: 10.3390/molecules25112556

15. Lei, X., Zhang, B., Zhang, Y., Huang, T., Tang, F., & Wu, X. (2022). In situ photoinitiated fabrication of phosphorylcholine-functionalized polyhedral oligomeric silsesquioxane hybrid monolithic column for mixed-mode capillary electrochromatography. *Analyst*, *147*(10), 2253–2263. DOI: 10.1039/D2AN00195K

16. Mishra, H., Behera, A., Sankar Kar, S., Pattnaik, G., Kanhar, S., & Dash, S. (2021). Analytical strategies for the detection and quantification of nano-formulated antibiotics: *Updates and perspectives*. DOI: 10.9734/jpri/2021/v33i59A34282

17. Fadhil, A. K., Rasheed, A. S., & Hassan, M. J. M. (2023). A review of recent advances in the estimation of pharmaceutical products using hydrophilic interaction chromatography (HILIC) technology. *Egyptian Journal*

of Chemistry, *66*(1), 399–417. DOI:10.21608/EJCHEM.2022.121396.5443

18. Buszewski, B., & Noga, S. (2012). Hydrophilic interaction liquid chromatography (HILIC) a powerful separation technique. *Analytical and Bioanalytical Chemistry*, *402*(1), 231–247. DOI: 10.1007/s00216-011-5308-5

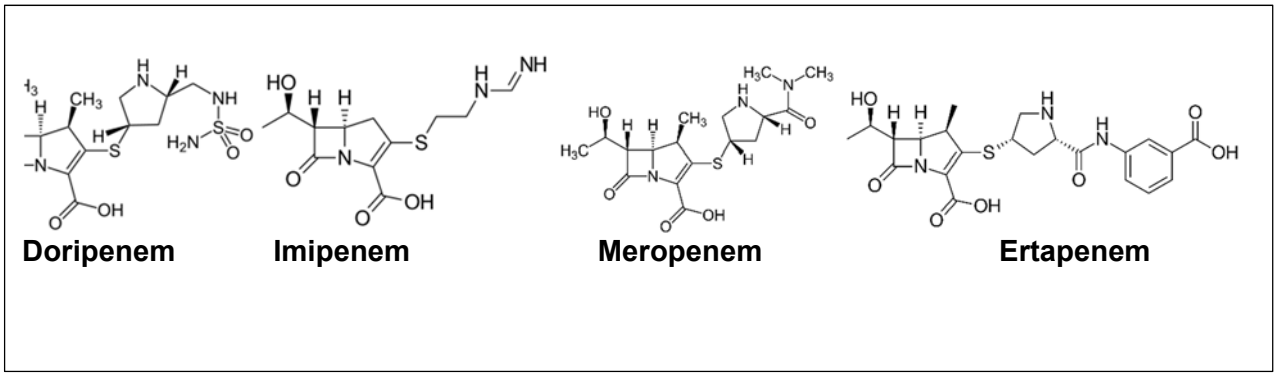

Figure 1. *shows the pharmaceutical drugs that were used in the study [1]*

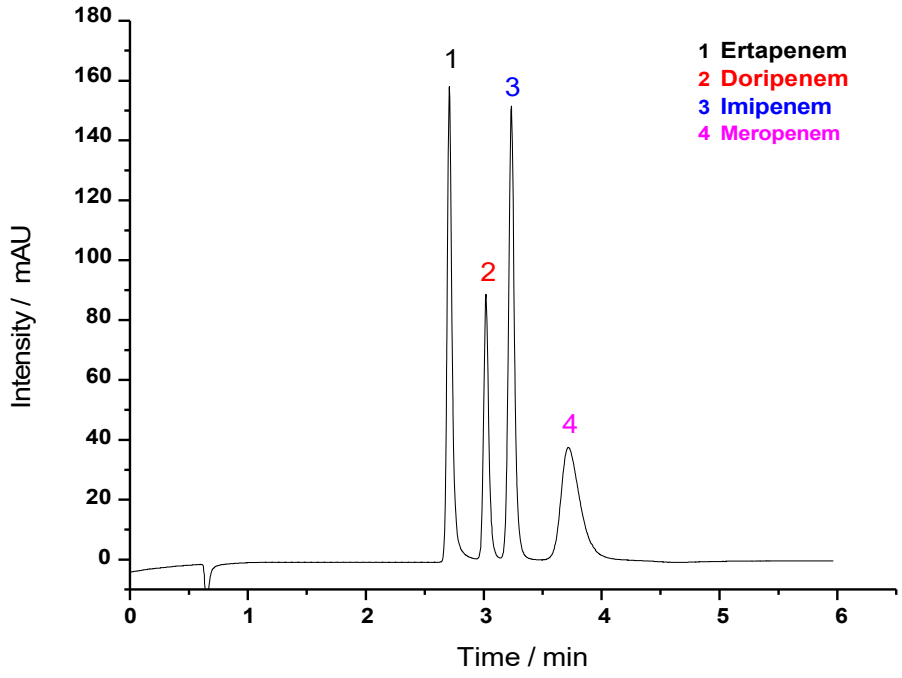

Figure 2. *Preliminary chromatograms for Imipenem, Meropenem, Ertapenem, and Doripenem mg/kg Inertsil HILIC Capillary EX-Nano Column*

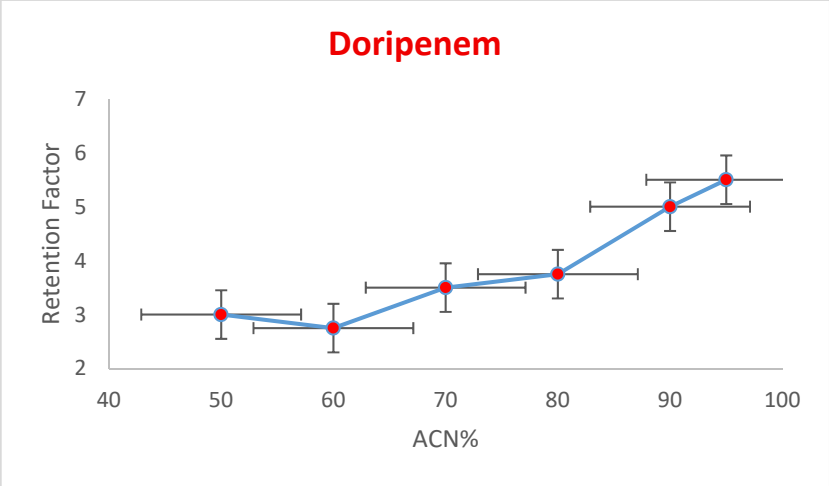

Figure 3. *Effect of ACN on the behavior of Doripenem*

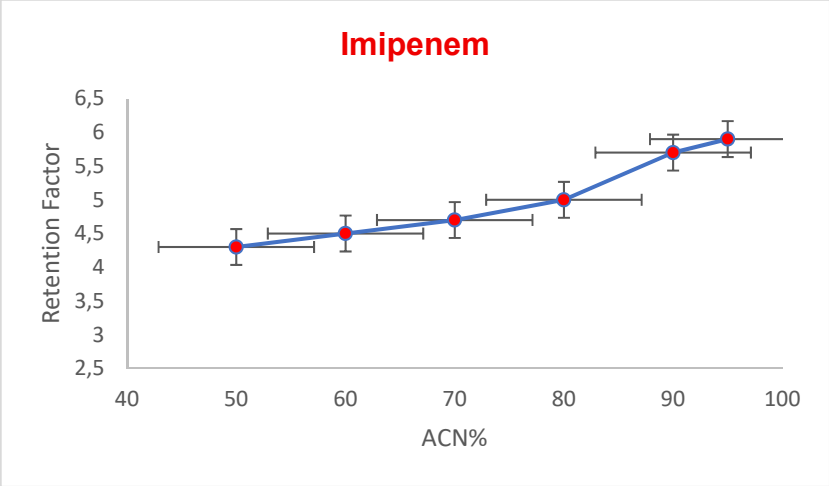

Figure 4. *Effect of ACN on the behavior of Imipenem*

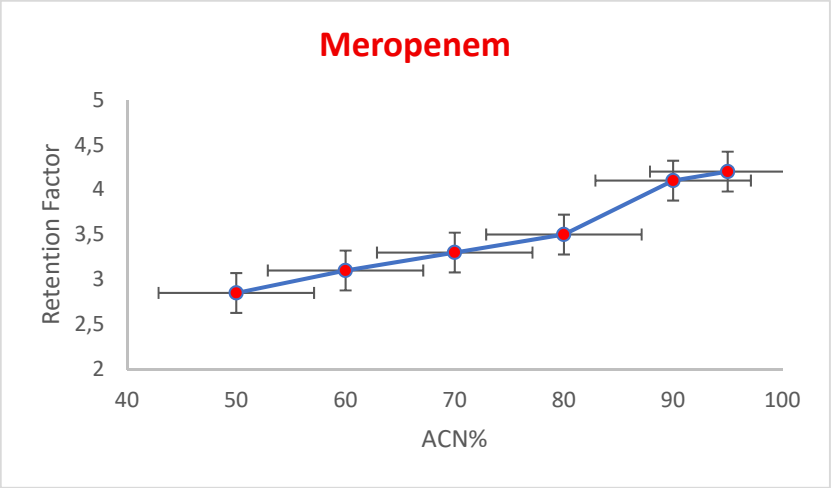

Figure 5. *Effect of ACN on the behavior of Meropenem*

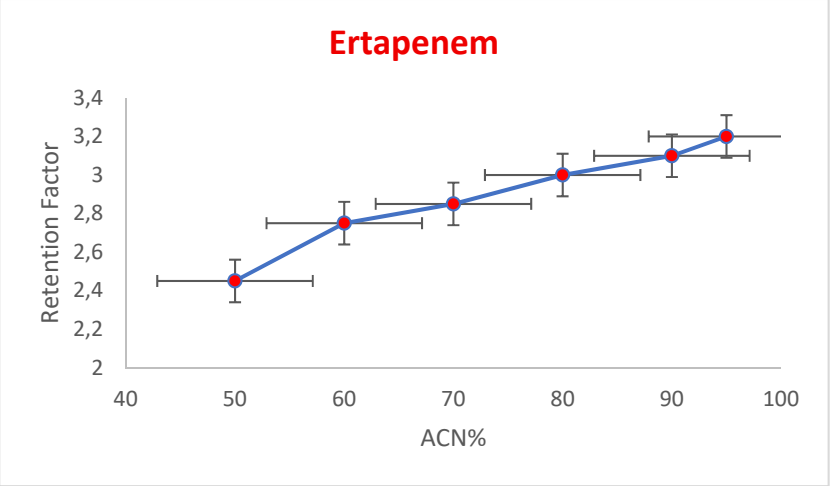

Figure 6. *Effect of ACN on the behavior of Ertapenem*

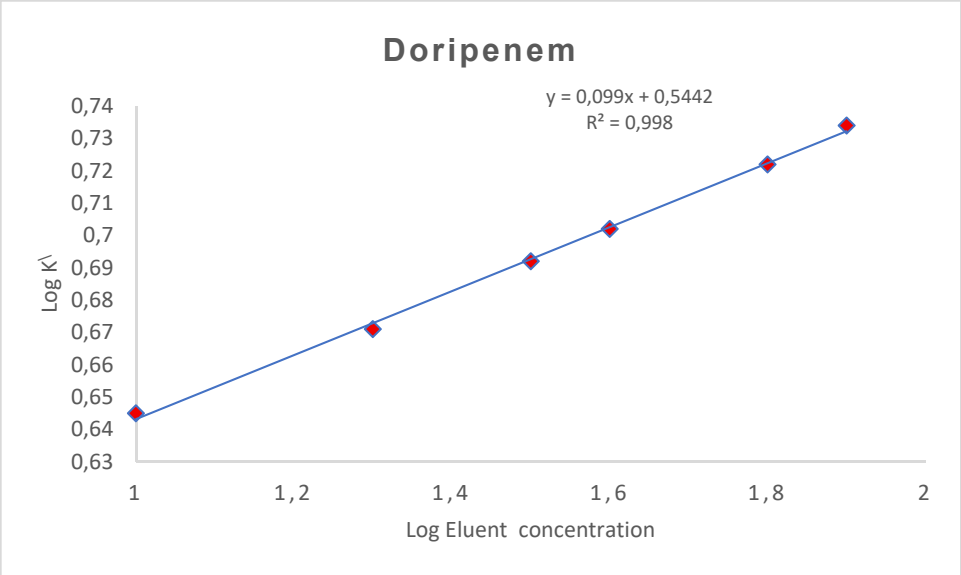

Figure 7. *Effect of buffer concentration on the behavior of Doripenem*

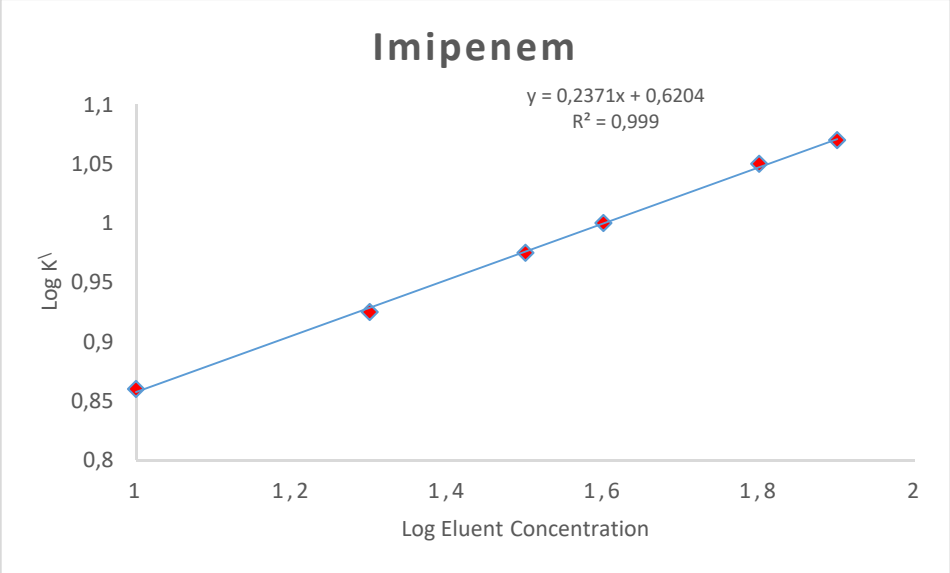

Figure 8. *Effect of buffer concentration on the behavior of Imipenem*

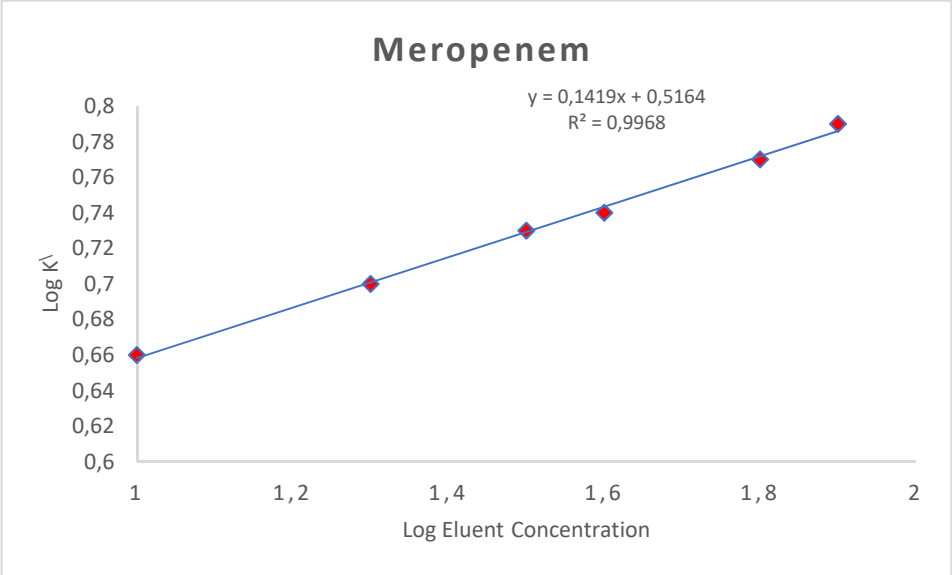

Figure 9. *Effect of buffer concentration on the behavior of Meropenem*

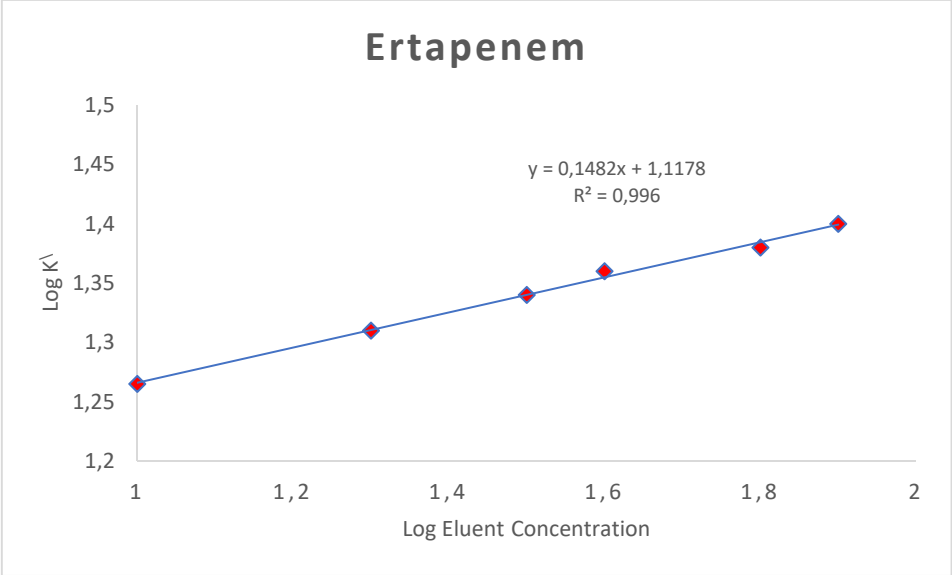

Figure 10. *Effect of buffer concentration on the behavior of Ertapenem*

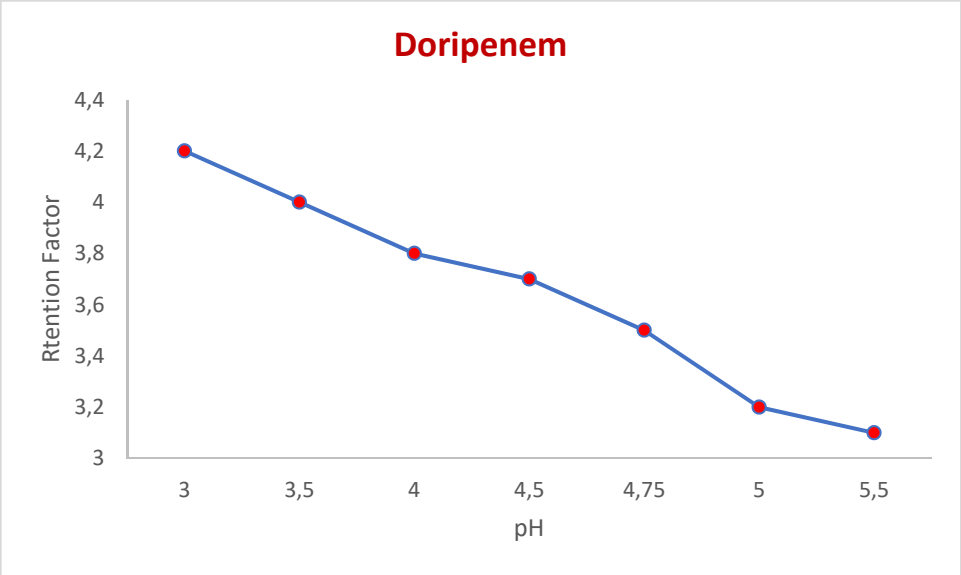

Figure 11. *Effect of pH on the behavior of Doripenem*

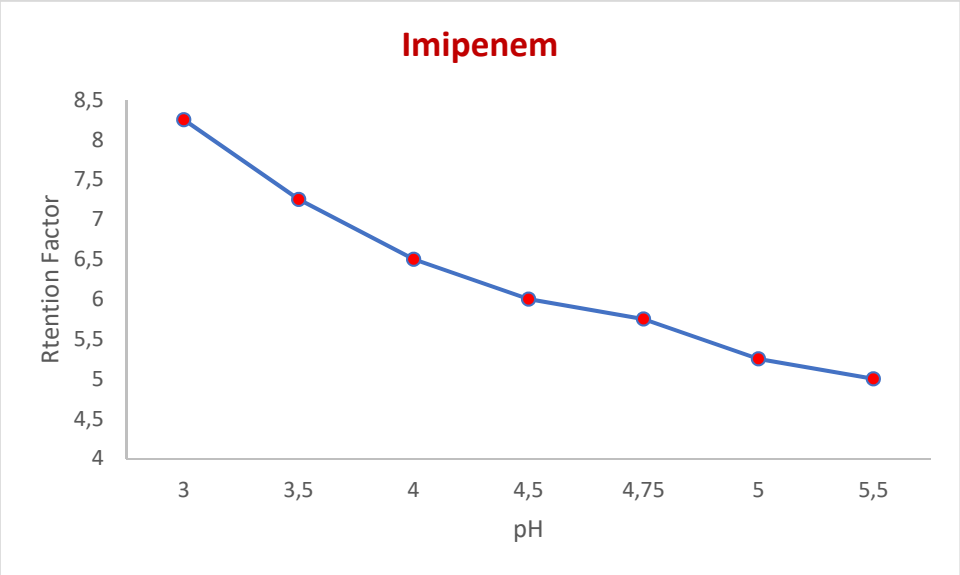

Figure 12. *Effect of pH on the behavior of imipenem*

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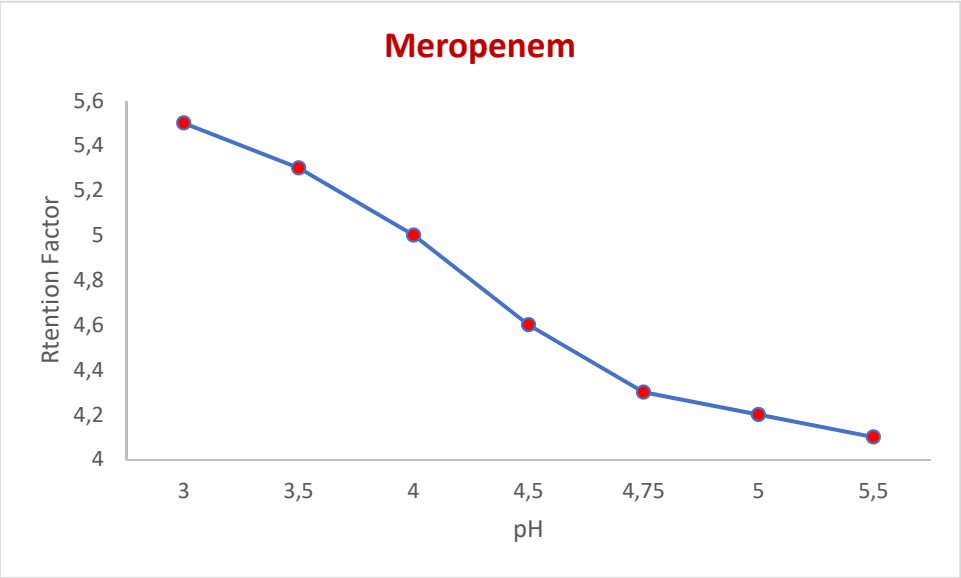

Figure 13. *Effect of pH on the behavior of Meropenem*

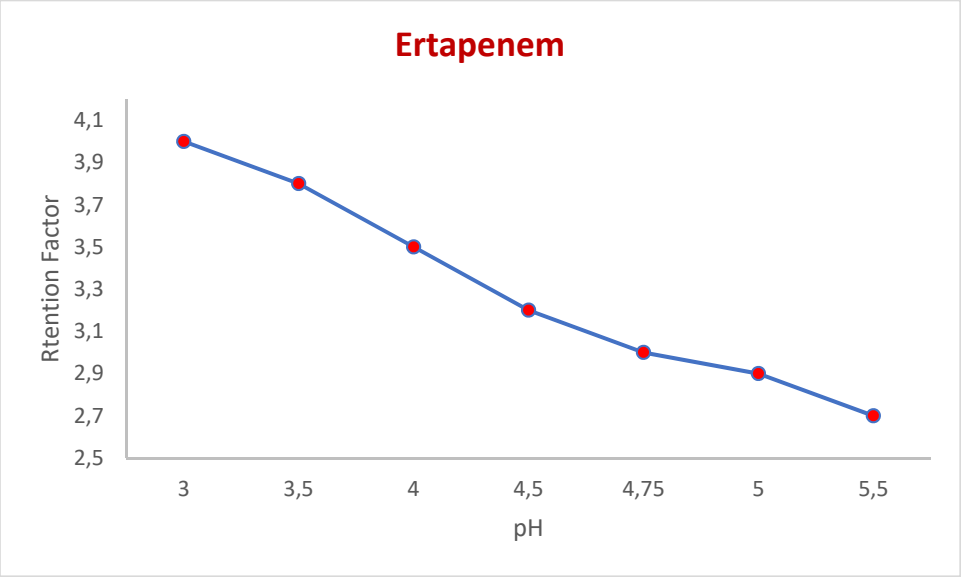

Figure 14*. Effect of pH on the behavior of Ertapenem*

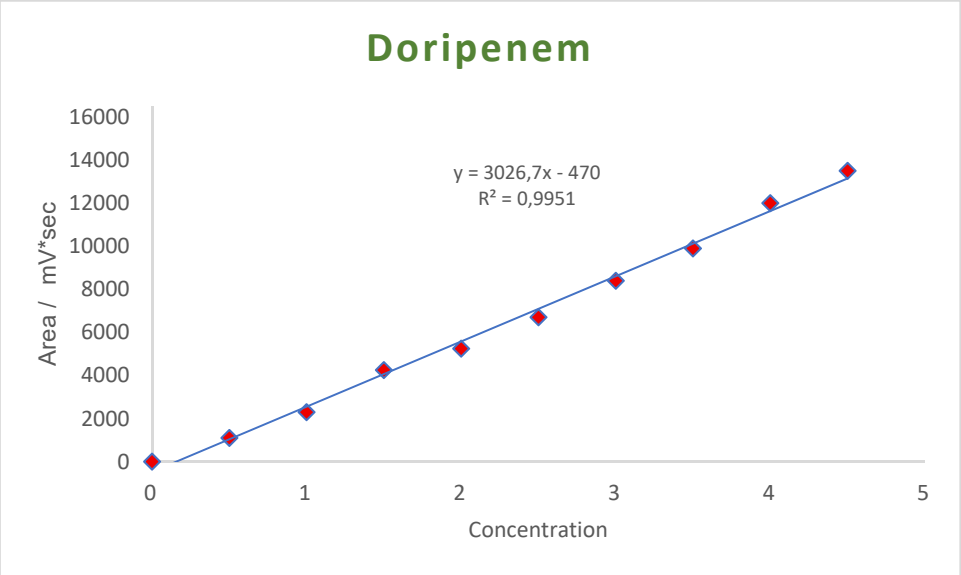

Figure 15. *Calibration graph of Doripenem.*

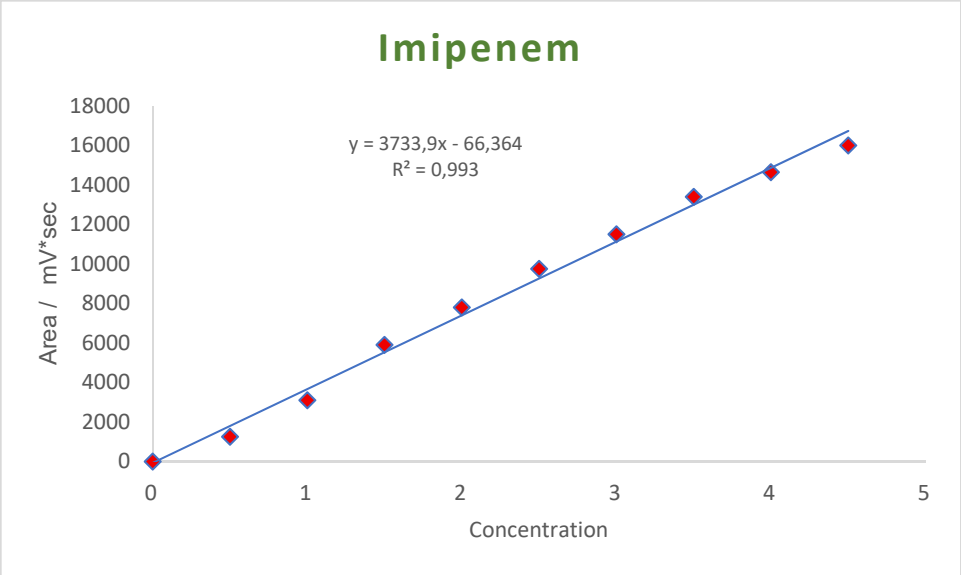

Figure 16. *Calibration graph of imipenem.*

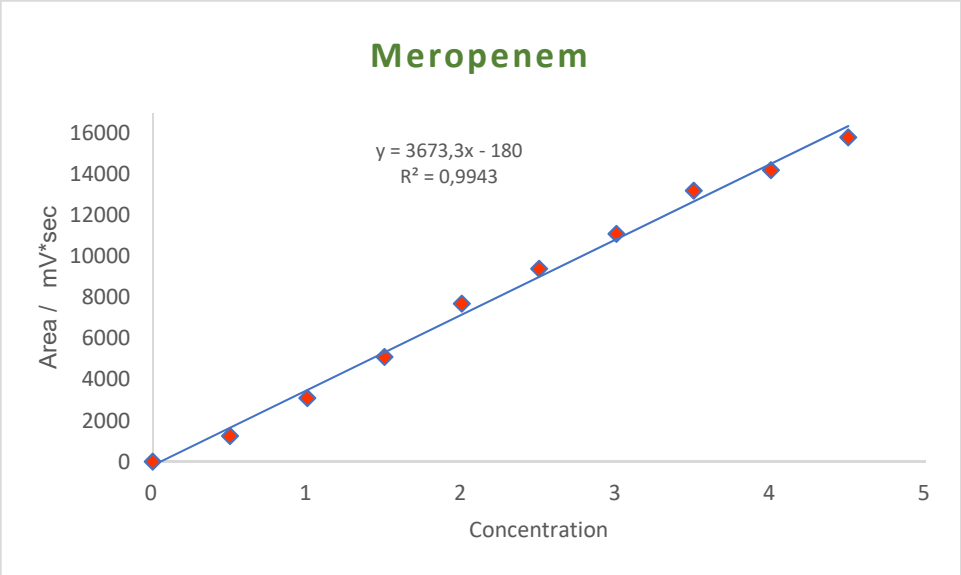

Figure 17. *Calibration graph of Meropenem*

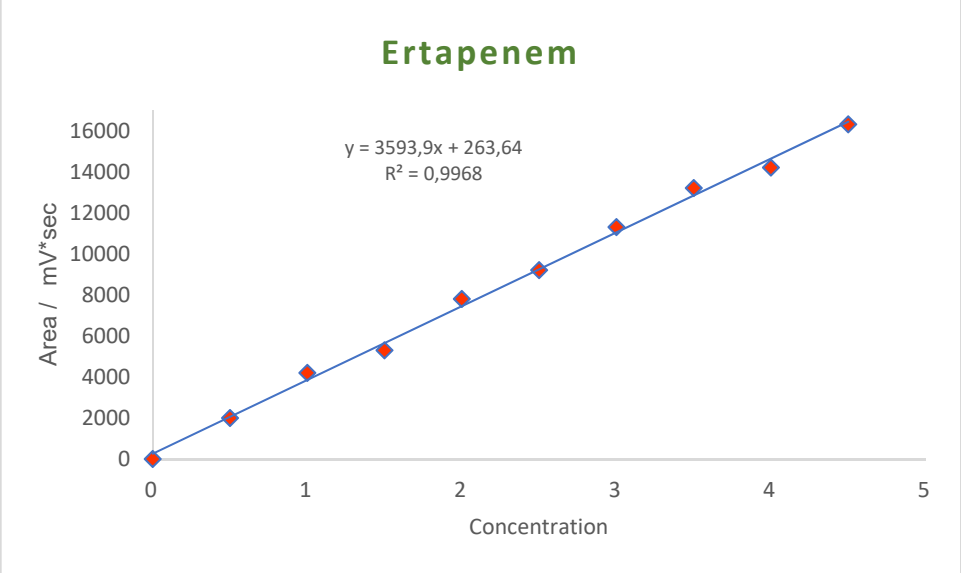

Figure 18. *Calibration graph of Ertapenem*

Table 1*. Physical and chemical characteristics of the four antibioticdrugs studied*

$\boldsymbol{Pharmaceuticals}$	$\boldsymbol{pI}$ **	$\boldsymbol{pKa}$	$\boldsymbol{logPow}$ *
Doripenem	6.55	3.54	$\mathbf{-5.60}$
Imipenem	8.75	3.44	$\mathbf{-2.78}$
Meropenem	5.15	3.28	$\mathbf{-4.40}$
Ertapenem	$\mathbf{3.73}$	$\mathbf{3.22}$	$\mathbf{-3.20}$

***logPow**:
$At\ equilibrium, the\ concentrations\ of\ an\ unionized\ chemical\ in\ the\ two\ phases\ of\ immiscible\ solvents\ (water\ \&\ n-octanol)\ are\ divided\ by\ the\ partition\ coefficient.$
***** Isoelectric point (pI):***$The\ pH\ at\ which\ a\ specific\ molecule\ has\ no\ net\ electrical\ charge\ is\ known\ as\ the\ isoelectric\ point. The\ pH\ of\ the\ molecule's\ surroundings\ influences\ its\ net\ charge, which\ can\ increase\ or\ decrease\ depending\ on\ whether\ protons\ are\ lost\ or\ gained.$

Table 2*. shows the optimal conditions used to separate antibiotic drugs.*

Column	**Inertsil HILIC Capillary EX-Nano Column**
pH	$3-5.5$
Temperature of the column	$40co$
Flow rate	$0.2\ ml/min$
Detection	$254\ nm$
Eluent	$A)\ CH3CN \quad B)10mM\ NH4OAc\ in\ H2O$

Table 3*. Results of the analytical features of drugs.*

$\boldsymbol{Drugs}$	$\boldsymbol{LOD}$ ($\mu g/mL$)	$\boldsymbol{LOQ}$ ($\mu g/mL$)	$\boldsymbol{S/N\ at\ LOD}$	$\boldsymbol{S/N\ at\ LOQ}$	$\boldsymbol{RSD}$ (%)	$\boldsymbol{Recovery}$ (%)
$\boldsymbol{Ertapenem}$	0.0029	0.0087	3.1	10.2	1.8	98.7
$\boldsymbol{Doripenem}$	0.0024	0.0072	3.3	10.4	1.6	99.1
$\boldsymbol{Imipenem}$	0.0030	0.0090	3.0	10.3	1.7	98.3
$\boldsymbol{Meropenem}$	0.0027	0.0081	3.2	10.5	1.9	97.9

Table 4. The suggested methods of precision and accuracy of drugs

Compound (µg/mL)	**Taken (µg/mL)**	**Found (µg/mL)**	**Recovery (%)**	**95% CI**	**Inter-day RSD (%)**	**Intra-day RSD (%)**	**Statistical test (P-*valu*)**
Etapenem	10.0	9.93	99.3	$98.-99.7$	1.4	1.1	0.125
Doripenem	10.0	9.89	98.9	$98.-99.3$	1.3	1.0	0.198
Imipenem	10.0	100.2	100.2	$99.-100.8$	1.5	1.3	0.109
Meropenem	10.0	99.7	99.7	$99.-100.1$	1.2	1.0	0.165

Table 5. *Application in pharmaceutical drugs.*

Name of pharmaceutical	Manufacturer	Stated conc. (mg)	Found direct. (mg)	Rec %	RSD n=6 %	Erel %	Anova p-value	t-test P-value	p>0.5
Doripenem	LDP-spain	**200**	**201**	**100.5**	**1.11**	**0.50**	**0.375**	**0.191**	**0.254**
	Pharma-international-Jordan	**200**	**203**	**101.5**	**1.29**	**1.50**			
	Bravn-India	**200**	**199**	**99.5**	**1.03**	**−0.50**			
Imipenem	Bravn-India	**200**	**193**	**96.5**	**1.11**	**−3.50**	**0.288**	**0.134**	**0.203**
	LDP-spain	**200**	**196**	**98.0**	**0.88**	**−2.00**			
	Pharma-international-Jordan	**200**	**199**	**99.5**	**1.02**	**−0.50**			
Ertapenem	Bravn-India	**200**	**205**	**102.5**	**1.21**	**2.50**	**0.441**	**0.160**	**0.219**
	LDP-spain	**200**	**195**	**97.5**	**1.00**	**−2.50**			
	Pharma-international-Jordan	**200**	**197**	**98.5**	**0.81**	**−1.50**			
Meropenem	Bravn-India	**200**	**204**	**100.2**	**1.03**	**2.00**	**0.397**	**0.213**	**0.186**
	LDP-spain	**200**	**198**	**99.0**	**1.01**	**−1.00**			
	Pharma-international-Jordan	**200**	**201**	**100.5**	**0.81**	**0.50**			

Table 6. Preparation buffer range (3-5.5).

$\boldsymbol{Buffer\ (pH)}$	$\boldsymbol{HAc}$ (μ$\boldsymbol{L}$) $\boldsymbol{17.3\ M}$	$\boldsymbol{Weight\ of\ NH4OAc\ (g)}$
3.00	2247.6	0.095
4.00	1941.9	0.822
4.50	1463.8	1.959
4.75	1143.8	2.722
5.00	823.8	3.484
5.50	345.7	4.621

Table 7. Preparation buffer range (10-80 mM) at pH 5.5

$\boldsymbol{Buffer\ concentration\ (mM)}$	$\boldsymbol{HAc}$ (μ$\boldsymbol{L}$) $\boldsymbol{17.3\ M}$	$\boldsymbol{Weight\ of\ NH4OAc\ (g)}$
10	285.7	0.680
20	572.4	1.361
30	858.1	2.041
40	1143.8	2.722
50	1429.5	3.402
60	1715.2	4.082
70	2001.9	4.763
80	2287.6	5.443