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# Physicochemical Analysis and Thin-Layer Chromatographic Fingerprinting of Some Beta-Lactam Antibiotics

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## ABSTRACT

**Background**: The study of beta-lactam antibiotics requires spectrophotometric fingerprinting. This study describes thin layer chromatographic (TLC) fingerprinting procedures, which allow for easy and rapid separation and detection of cephalosporin beta-lactam antibiotics in complex mixtures, as well as their physicochemical analysis. **Aim**: This study aimed to use thin-layer chromatography to separate and identify cephalosporins from complicated mixtures, as well as to compare the physicochemical properties of different brands and generations of cephalosporins. **Method**: For the physicochemical analysis, different tests including weight uniformity, hardness test, disintegration, friability, and dissolution test were conducted. Each brand profile was recorded and the samples were coded CE1-CE4. **Results**: From the experiment, it was seen that for the disintegration test; CE2 had the highest disintegration time, while CE3 had the lowest disintegration time, the disintegration test of six tablets from every brand was placed in a disintegration tester filled with distilled water at 37 ± I ° C. For the hardness test; CE2 had the highest value, while CE4 had the lowest value. CE1 had the lowest friability percentage value, while CE4 had the highest percentage value. **Conclusion:** UV wavelengths at 254 nm and 366 nm can detect beta-lactam antibiotics, and color interactions with iodine vapor can reduce the detection limit. TLC analysis is cost-effective, time-saving, safe, and simple to perform, yielding good quality results, and can be applied in therapeutic drug monitoring, purity test, stability control, and assay procedures.

**Keywords**: Cephalosporins, Beta-lactam, TLC, physicochemical, friability, disintegration,

## I. INTRODUCTION

Cephalosporins are one of the most frequently used beta-lactam antibiotics (Pandey, & Cascella, 2021). Cephalosporins are beta-lactam antibiotics that work in similar way as the penicillins and have a related chemical structure. Cephalosporins and penicillins both have a four-member beta-lactam ring, but cephalosporins have an extra atom in the side ring. Modified side chains on each ring alter antimicrobial action, beta-lactamase resistance, and pharmacokinetics features of these compounds (Tooke et al., 2019). Penicillin-resistant organisms are generally cephalosporin-resistant. *Listeria* and *Pasteurella sp*., are exceptions. Cephalosporins are effective against common gram-negative bacteria such as *E. coli*, non-typeable *Haemophilus influenzae*, and *Staphylococcus aureus*, but ineffective against methicillin-resistant *S. aureus*, and *enterococci* (Huang et al., 2007). Chemical, microbiological, and immunological assays, chromatographical assays, and other physicochemical approaches are used routinely to detect cephalosporins in medications, body fluids, and food (Pauter et al., 2020).

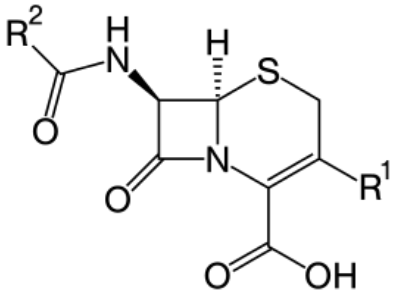


Figure 1. Basic structure of cephalosporins: (2R)-2-[(R)-{[(6R)-6-amino-6-carboxyhexanoyl] amino} (carboxy) methyl]-5-methylidene-5, 6-dihydro-2H-1, 3-thiazine-4-carboxylic acid (Hancu et al., 2013).

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The nucleus of cephalosporins can be altered to give it alternative features. They are bactericidal, although they are more resistant to -lactamases (Hancu et al., 2013). Cephalosporins hinder bacteria from producing the peptidoglycan layer in their cell walls. The peptidoglycan is essential for the bacterial cell wall's structural integrity. Penicillin-binding proteins (PBPs) aid in the last transpeptidation phase of the peptidoglycan production process (Kapoor et al., 2017). PBPs crosslink peptidoglycan precursors (muropeptides) by binding to the D-Alanine-D-Alanine sequence at the end. PBP crosslinking of peptidoglycan is irreversibly inhibited by beta-lactam antibiotics that resemble this amino acid sequence binding site (Hugonnet et al., 2016).

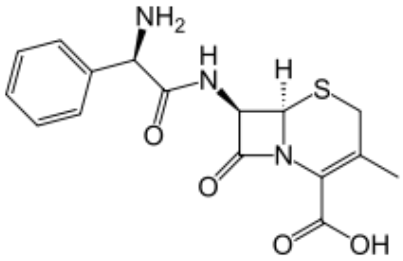

Fig 2: Structure of cefalexin – 1st-generation cephalosporin

Cefalexin is bactericidal and acts by inhibiting peptidoglycan layer synthesis (Bothara et al., 2006). Because it closely mimics d-alanyl-d-alanine, an amino group terminating on the cell wall peptidoglycan membrane, cefalexin can bind irreversibly to the active center of PBP, which is necessary for cell wall production (Fisher et al., 2005). Cefuroxime, second-generation cephalosporins retain high activity against gram-positive organisms, but less S. aureus (gram-negative) activity. Second-generation cephalosporins have very little or no activity against enterococci, Listeria, Pseudomonas, MRSA, or S epidermidis (Bui, & Preuss, 2021).

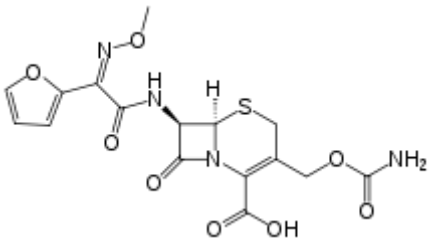

Fig 3. Structure of cefuroxime – 2nd-generation cephalosporin

Cephalosporins of the third generation (ceftriaxone, cefixime) are broad-spectrum antimicrobials that can be used in a range of clinical settings. Except for cefoperazone, these cephalosporins permeate the cerebral fluid and are used to treat bacterial meningitis. Third-generation cephalosporins are the recommended in many clinical conditions due to their evidence-based high clinical efficacy, good pharmacokinetic parameters, and low side effects frequency of various soft tissue and skin infections, pneumonia, UTIs, gonorrhea, meningitis, Lyme disease, and sepsis are all treated with third-generation cephalosporins (Klein, & Cunha, 1995).

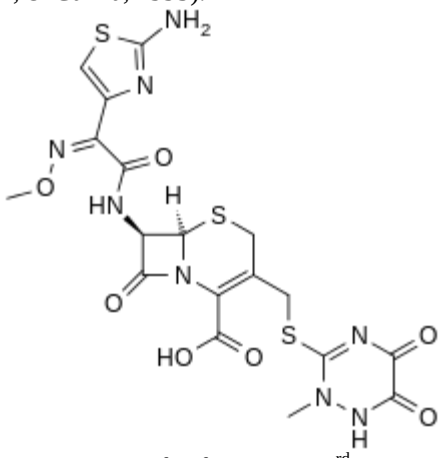

Fig 4. Structure of ceftriaxone – 3rd-generation cephalosporin

Cefepime is a 4th-generation cephalosporin that has a broad spectrum of activity against both gram-positive and gram-negative bacteria and is more effective against both than third-generation drugs. According to a meta-analysis of trial data published in 2007, those given cefepime had a higher mortality rate than those given other beta-lactam antibiotics. Soft tissue and dermatitis of bacterial origin, pneumonia, urinary tract infections, gastrointestinal infections, meningitis, and sepsis are all treated with cefepime. It is, however, normally reserved for the most serious illnesses (Yahav et al., 2007).

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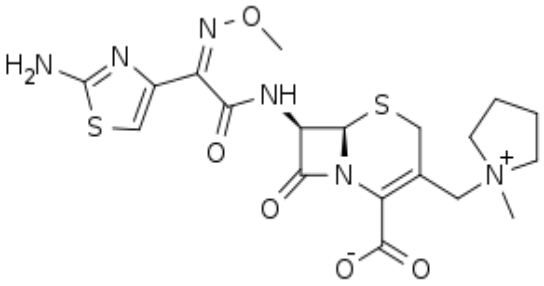

Fig 5. Structure of cefepime - fourth-generation cephalosporin

Ceftaroline is a 5th-generation cephalosporin that is commonly used in bacterial infections that are resistant to penicillin medicines, such as methicillin-resistant S. aureus and Streptococcus species (Ishikawa & Nobuyuki, 2003; Kanafani & Corey, 2009). The acetate version of ceftaroline fosamil is used clinically but biotransformed into the active ceftaroline (Yukihiro & Junko, 2008).

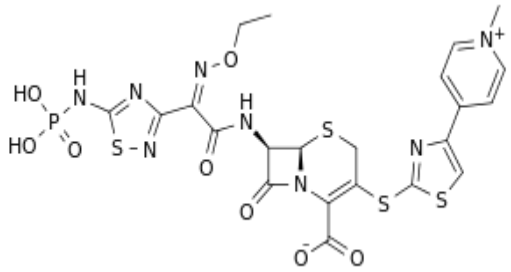

Fig 6. Structure of ceftaroline – 5th-generation cephalosporin

Apart from antibacterial action, cephalosporins are distinguished by their pharmacokinetics. The plasma half-life of most cephalosporins has been reported to be about 30 - 120 minutes. Only ceftriaxone has an 8-hour half-life, implying that longer dosing intervals are required. Cephalosporins are eliminated by the kidneys. Cefoperazone and ceftriaxone, on the other hand, are eliminated in the bile by up to 30%. Cefalotin, cefacetril, and cefotaxime are all metabolized in some way. Cefotaxime's primary metabolite is the only one with antibacterial action (Graninger, 1983).

Beta-lactam antibiotics are determined using a variety of analytical procedures, including high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). Due to its great sensitivity and separation efficiency, HPLC is the established first choice analytical method for the analysis of beta-lactams, despite being expensive and requiring specialist equipment (Hancu et al., 2013). Thin Layer Chromatography is a cost-effective and less difficult chromatographic process used to successfully screen medicinal compounds (Hancu et al., 2013). TLC is a separation procedure that identifies the presence of beta-lactam antibiotics (Fried & Sherma, 1999). It was employed in the 7th edition of the European Pharmacopoeia to separate beta-lactam antibiotics from their specific impurities, but the procedures provided are only applicable to one antibiotic and its structure-related impurities, making them less appropriate for identifying complicated combinations (European Pharmacopoeia, 2010). The rising resistance of some bacterial strains, as well as the major instability of beta-lactam medications, necessitate the use of low-cost, easily available analytical methodologies for rapid determination and quantification in various dosage formulations. The study aimed to evaluate and compare the physicochemical profiles of different brands of cefuroxime axetil and cefixime tablets using the TLC fingerprint technique.

## II. METHOD

**Reagents and Apparatus**

Acetic acid, ammonia, distilled water, ethanol, ethyl acetate, iodine crystals, methanol, and sodium hydroxide. Disintegrating apparatus, Dissolution apparatus, Monsanto hardness tester, Roche fribilator, Analytical weighing balance (Ohaus, USA), Calibrated measuring cylinders, Beakers, Spatula, Mortar and pestle, Funnel, Filter papers, Volumetric flasks, Hot air oven, Chromatoplates (TLC plates), Chromatographic chambers, Capillary tubes, Iodine tank, UV lamp, UV-Visible Spectrophotometer, Test tubes, Test tube rack, Fume cupboard, Aluminum foil.

**Uniformity testing;** With an analytical weighing balance, 10 tablets from each of the four brands were weighed individually. We calculated the average weights for each brand as well as the percentage (%) variation from the mean.

**Hardness testing;** A tablet hardness tester was used to assess crushing strength. Each brand had 10 tablets randomly picked, and the pressure at which each tablet was crushed was recorded.

**Friability testing;** 10 tablets of every brand were weighed and abrasion was applied for 4 minutes using a Roche friabilator at 25 rev/min. The tablet

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also was weighed and compared to its original weight to determine its percentage friability.

**Disintegration testing;** six (6) tablets from each brand were employed for the test using the disintegrating apparatus in which the water was at 37$^{o}$C. The disintegration time was taken to be the number of the particle that remained in the system basket after the analysis, i.e., a measure of the ability of the tablet to break down into smaller granules or particles.

**Stock solution preparation;**

One liter (1000ml) of 50% methanol was prepared by mixing 500ml of methanol in 500ml distilled water. The stock solution was prepared by dissolving an amount equivalent to 0.2g of each drug sample in 100ml of the medium

**Chromatoplate Preparation;**

The Chromatoplate (TLC Plate) was scaled and appropriately labeled after being activated in the oven. Silica gel was employed as the stationary phase. The drug samples then were spotted using capillary tubes on the appropriate indicated locations on the chromatoplate's origin mark and allowed to dry before being placed in the solvent systems. The mobile phase consisted of methanol, ammonia, and acetic acid. The colors/fluorescence of each sample was monitored and recorded for the solvent systems after the generated chromatoplate was evaluated under UV radiation at wavelengths of 254nm and 366nm. Iodine vapor was used to visualize the spots. The drug samples' retardation factor (Rf) values were then computed and recorded. Retardation factor = distance traveled by sample/distance traveled by solvent. Two generations of cephalosporins were used; second generation (cefixime), and third generation (cefuroxime).

## III. RESULTS

Table 1. Weight Uniformity (%Deviation)

| CODE | CE1 | CE2 | CE3 | CE4 |
|---|---|---|---|---|
| **1** | 1.98 | 1.94 | 0.76 | 1.03 |
| **2** | 1.28 | 0 | 2.31 | 0.47 |
| **3** | 1.51 | 0.91 | 0.94 | 0.76 |
| **4** | 1.58 | 0.59 | 0.63 | 0.68 |
| **5** | 2.45 | 1.84 | 1.8 | 0.31 |
| **6** | 1.56 | 0.37 | 0.44 | 0.12 |
| **7** | 0.4 | 1.68 | 0.24 | 1.18 |
| **8** | 0.45 | 1.36 | 0.81 | 0.65 |
| **9** | 0.13 | 1.98 | 2.49 | 0.4 |
| **10** | 1.34 | 1.62 | 1.77 | 0.47 |
| Mean ± SD | 1.27±0.74 | 1.23±0.71 | 1.22±0.80 | 0.61±0.32 |

SD – Standard Deviation: BP Standard; for tablets above 250mg = 5% deviation

Table 2. Hardness Test (kgf**) -** BP Standard; 4kgf – 15kgf

| CODE | CE1 | CE2 | CE3 | CE4 |
|---|---|---|---|---|
| 1 | 15 | 14 | 13.5 | 13.4 |
| 2 | 14.2 | 10.2 | 13 | 13 |
| 3 | 15 | 15 | 14 | 13.9 |
| 4 | 14 | 12 | 14 | 13.5 |
| 5 | 13.3 | 13.6 | 14.1 | 13 |
| 6 | 13 | 14.5 | 14.3 | 13 |
| 7 | 9.9 | 14 | 13.3 | 12 |
| 8 | 10 | 13.2 | 13.8 | 13.8 |
| 9 | 13 | 14.9 | 13.2 | 14 |
| 10 | 9 | 12.3 | 14 | 13.9 |
| Mean ± SD | 12.64±2.21 | 13.37±1.50 | 13.72±0.44 | 13.35±0.62 |

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Table 3. Disintegration Test

| CODE | CE1 | CE2 | CE3 | CE4 |
|---|---|---|---|---|
| **1** | 0:26 | 3:32 | 0:15 | 0:28 |
| **2** | 0:28 | 3:51 | 0:17 | 0:31 |
| **3** | 0:29 | 4:10 | 0:19 | 0:34 |
| **4** | 0:33 | 4:33 | 0:20 | 0:37 |
| **5** | 0:38 | 4:54 | 0:22 | 0:39 |
| **6** | 0:44 | 5:18 | 0:25 | 0:41 |
| Mean ± SD | 0.02±0.01 | 0.18±0.07 | 0.01±0.01 | 0.02±0.01 |

BP Standard; Uncoated tablet = 5-30 minutes, Coated tablet = 1-2 hours

Table 4. Friability Test

| CODE | BEFORE | AFTER | %FRIABILITY |
|---|---|---|---|
| **CE1** | 10.261 | 10.234 | 0.26 |
| **CE2** | 6.737 | 6.716 | 0.31 |
| **CE3** | 9.802 | 9.773 | 0.30 |
| **CE4** | 9.866 | 9.832 | 0.34 |

BP Standard; 0.1%-0.5%

Table 5: Retardation Factor Values for the Drug Samples

| CODE | $R_f$ | TOTAL | AVERAGE $R_F$ |
|---|---|---|---|
| **CE1** | 0.70 | 0.70 | 0.70 |
| **CE2** | 0.65 | 0.65 | 0.65 |
| **CE3** | 0.69 | 0.69 | 0.69 |
| **CE4** | 0.64 | 0.64 | 0.64 |

## IV. DISCUSSION

From the experiment conducted, it can be seen that for the disintegration test; CE2 had the highest disintegration time, while CE3 had the lowest disintegration time, the disintegration test of six tablets of each brand of the drugs were placed in a disintegration tester filled with distilled water at 37 ± I ° C. For the hardness test; CE2 had the highest value, while CE4 had the lowest value.

For friability; CE1 had the lowest percentage value, while CE4 had the highest percentage value. The uniformity of weight standards is applied to tablets and capsules, that are supplied in unit dose forms because they are subject to more variations than comparable preparations supplied in multi-dose forms. For tablets with an average weight above 250 mg, the percentage deviation from the average weight permissible in the official compendium (BP, 2008) is ± 5%. The beta-lactam antibiotics used for the study which comprised four cephalosporins passed the test for uniformity of weight. The most common stationary phase for thin layer chromatographic study of beta-lactam antibiotics is a cellulose gel, however, reversed-phase or cellulose plates have also been utilized. Silica gel surface bears silicon hydroxide (Si-OH) groups capable of polar substances with hydrogen bonding. Polar mobile phases were utilized for the detection and separation of cephalosporins. To

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prevent the beta-lactam ring on the silica gel from disintegrating, acetic acid was added to all of the mobile phases utilized as solvent systems for chromatoplate development. The substance zones contain more iodine than the polar substance-free silica gel layer adjacent to it.

The solvent system (50:25:25 percent v/v ratio of Methanol, Ammonia, and Acetic acid) was seen to produce fine and noticeable spots in the test samples. It was discovered that the presence of methanol as one of the solvent system's components is critical for the appropriate development of the drug samples (beta-lactam antibiotics) and that the presence of ammonia considerably reduces tailing for the various drug samples.

The absorbance measurement on the spectrophotometer for all of the drug samples was observed at various wavelengths ranging from 250nm to 430nm. A sharp increase in absorbance (other than the initial reading and in some cases above the initial reading) was observed for the different drug samples at a wavelength of 350nm. This is the wavelength at which the maximum absorbance of all beta-lactam antibiotics was measured.

## V. CONCLUSION

The beta-lactam antibiotics used in the experiment can be separated by TLC, using silica gel as a stationary phase with a suitable mobile phase as developed in this study. This enables the distinction of the beta-lactam antibiotics from other complex mixtures. UV wavelengths of 254 nm and 366 nm can be used to detect beta-lactam antibiotics, and color reactions (especially iodine reactions) under iodine vapor can lower the detection limit. The study of beta-lactam antibiotics requires spectrophotometric fingerprinting since maximum absorption occurs at a wavelength of 350nm. The identification techniques used are cost-effective, time-efficient, safe, reproducible, and simple to use, with good results, and can be used in preparatory and explorative analytical testing, quality assurance studies, therapeutic drug surveillance, purity test, and stability control, as well as beta-lactam antibiotic assay procedures.

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