HPLC DETERMINATION OF SULBACTAM, SULTAMICILLIN TOSYLATE, CEFACLOR, AMPICILLIN AND CEFOPERAZONE IN PHARMACEUTICAL PREPARATIONS

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Abstract: A simple, rapid and sensitive high performance liquid chromatography procedure is described for the determination of sulbactam, sultamicillin tosylate, cefaclor, ampicillin and cefoperazone in authentic mixtures and in different pharmaceutical formulations. The manufacture precursors: 6-aminopenicillanic acid (6APA) in ampicillin or 7-aminocephalosporanic acid (7ACA) in cefaclor and cefoperazone and the expected degradation products; phenylglycin in cefaclor and ampicillin or p-hydroxyphenylglycin in cefoperazone do not interfere with the determination. The drugs were chromatographed on a Spherisorb ODS-2 column with 25% methanol in 0.005 M tetramethylammonium (TMAH) hydroxide adjusted to pH 3.4 with 1 M phosphoric acid as mobile phase, using salicylamide as internal standard. The flow rate was 1 ml min⁻¹ at 230 nm detection. The proposed method was applied to Unasyn vials and tablets, Cefobid vials and Cefclor capsules and packets. The relative standard deviation ranged from 1.23 to 2.22%.

Keywords: Sulbactam, sultamicillin tosylate, cefaclor, ampicillin, cefoperazone, HPLC determination.

Sulbactam penicillanic acid 1,1-dioxide, generally has only weak antibacterial activity but it is an irreversible inhibitor of several beta-lactamases produced by Gram-negative bacteria. Therefore, it can enhance the activity of ampicillin; (6R)-6-[(α-D-phenylglycyl)-amino] penicillanic acid, which is an aminopenicillin with broader spectrum of activity than benzylpenicillin, and cefoperazone a third generation cephalosporin, sodium-7-[(R)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl-carboxamidin)-2-[(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate.

Sultamicillin is a prodrug of sulbactam and ampicillin; it is a double methylene ester hydrolyzing during absorption from the GIT releasing equimolar quantities of both compounds.

Cefaclor is cephalosporin antibiotic more active against Gram-negative bacteria; 3-chloro-7-[(α-D-phenylglycinamino)-3-cephem-4-carboxylic acid.

Different method were reported for the determination of these compounds including non aqueous titrimetric assay of ampicillin and other antibiotics using tetrabutylammonium hydroxide or HClO₄ as titrant (1, 2). Ampicillin was determined also in pharmaceutical samples by flow injection analysis (3). Polarographic determinations of ampicillin and cefoperazone in pharmaceutical dosage forms were also reported (4, 5).

Spectrophotometric determination of ampicillin with some nitro-compounds (6) or with picric acid (7) and in the presence of other β-lactam antibiotics was described (8, 9). First and second derivatives spectrophotometric procedure was described for the determination of sulbactam and cefoperazone in injections (10). HPLC–photolysis–electrochemical detection method was reported for the determination of cefoperazone and four penicillins (11). Different analytical procedures based on HPLC determination of cefoperazone, cefaclor, or sulbactam were reported (12–16). HPLC electrospray mass spectrometry for ampicillin was also described (17).

The proposed method was successfully employed for the determination of cefaclor, sulbactam, sultamicillin, ampicillin and cefoperazone in suspensions, vials and tablets containing any mixture of these compounds and can be recommended for the routine analysis and detection of impurities in these compounds.

EXPERIMENTAL

Apparatus and reagents


Figure 2. Chromatogram of authentic mixture of sulbactam, C: salicylamide, G: cefoperazone, H: p-hydroxyphenylglycin, A: 7-ACA, B at 230 nm.

Figure 3. a – Chromatogram of authentic mixture of sulbactam, C: ampicillin, F and salicylamide, G  
b – After extraction from Unasyn injection  
c – After extraction from Unasyn tablets

Sulbactam, 0.1 mg.ml⁻¹, Pfizer, Poland; Sultamicillin tosylate, 0.1 mg.ml⁻¹, Pfizer, Turkey; Ampicillin, 0.2 mg.ml⁻¹, Aldrich Chem. Co.; Cefoperazone, 0.2 mg.ml⁻¹ Sigma Chem. Co.; Salicylamide, 0.1 mg.ml⁻¹, Aldrich Chem. Co.; Cefaclor, 0.1 mg.ml⁻¹, Ranabaxy Lab. LTD, India; p-Hydroxyphenylglycin, and phenylglycin, Aldrich Chem. Co.; 6-Aminopenicillanic acid and 7-Aminopenicillosporanic acid, Aldrich Chem. Co.;

All the standard solutions were dissolved in the mobile phase.

Merhanol; HPLC grade, Merck, Germany.
Water; double distilled.
TMAH; 10% Merck, Germany.

Authentic mixture of sulbactam, 0.1 mg.ml⁻¹ and ampicillin, 0.2 mg.ml⁻¹.

Authentic mixture of sulbactam, 0.1 mg.ml⁻¹ and cefoperazone 0.2 mg.ml⁻¹.

Pharmaceutical preparations
Unasyn tablets: from Pfizer, Turkey, labelled to contain sultamicillin tosylate equivalent to 375
Table 1. Parameters of the calibration curves obtained by regression analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulbactam</td>
<td>Y = 2.6x + 0.006</td>
<td>0.999</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Y = 2.5x + 0.006</td>
<td>0.999</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>Y = 2.4x + 0.113</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Figure 1: Chromatogram of different concentrations of cefoperazone. H = 0.1 mg/mL and the same concentration of sulbactam.

Table 2. Determination of authentic mixtures of sulbactam and ampicillin or cefoperazone using the proposed HPLC method.

<table>
<thead>
<tr>
<th>Amount added</th>
<th>Amount found</th>
<th>Recovery %</th>
<th>Amount added</th>
<th>Amount found</th>
<th>Recovery %</th>
<th>Amount added</th>
<th>Amount found</th>
<th>Recovery %</th>
<th>Amount added</th>
<th>Amount found</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.098</td>
<td>98.00</td>
<td>0.2</td>
<td>0.196</td>
<td>98.00</td>
<td>0.15</td>
<td>0.153</td>
<td>102.0</td>
<td>0.3</td>
<td>0.31</td>
<td>103.3</td>
</tr>
<tr>
<td>0.2</td>
<td>0.21</td>
<td>105.0</td>
<td>0.4</td>
<td>0.41</td>
<td>102.5</td>
<td>0.2</td>
<td>0.202</td>
<td>101.0</td>
<td>0.4</td>
<td>0.396</td>
<td>99.0</td>
</tr>
<tr>
<td>0.4</td>
<td>0.41</td>
<td>102.5</td>
<td>0.8</td>
<td>0.81</td>
<td>101.25</td>
<td>0.25</td>
<td>0.247</td>
<td>98.8</td>
<td>0.5</td>
<td>0.505</td>
<td>101.0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.493</td>
<td>98.6</td>
<td>1.0</td>
<td>0.99</td>
<td>99.0</td>
<td>0.3</td>
<td>0.305</td>
<td>101.6</td>
<td>0.6</td>
<td>0.61</td>
<td>101.6</td>
</tr>
</tbody>
</table>

Mean: 101.03  
SD: 3.3  
RSD: 3.28
Table 3. Determination of sulbactam and ampicillin in Unasyn vials

<table>
<thead>
<tr>
<th>Sulbactam</th>
<th>Amoxicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount present</td>
<td>Amount found</td>
</tr>
<tr>
<td>0.1</td>
<td>0.099</td>
</tr>
<tr>
<td>0.15</td>
<td>0.145</td>
</tr>
<tr>
<td>0.2</td>
<td>0.201</td>
</tr>
<tr>
<td>0.25</td>
<td>0.251</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td></td>
</tr>
</tbody>
</table>

Cefclor packets; labelled to contain 250 mg cefaclor, BN. p 6749 y 1, Eli Lilly, Italy.
Cefobid vial: from Institute of Biotechnology, Poland. BN. 9011194, labelled to contain 2 g cepo-
perazone. Average weight of powder in vial is 2.5 g.

Chromatographic conditions

Mobile phase: 25% methanol in 0.005 M TMAH adjusted to pH 3.4 with phosphoric acid and degassed for 10 min using ultrasonic bath.
Columns: Spherisorb ODS 2.5 μm, 120 x 4.0 mm i.d.
Detector: 230 nm, 0.1 aufs.
Flow rate: 1 mL/min−1.
Pressure: 125 psi.
Temperature: ambient.

Procedure

A – Preparation of calibration curves: From the standard solution of each drug, volumes from 0.1 to
0.9 ml were pipetted into 10 ml volumetric flasks, then 0.1 ml of the internal standard was added to
each sample and the flasks were completed to volume with the mobile phase. 10 μl of each sample was injected into the column and all meas-
urements were repeated three times at each concentra-
tion. The calibration curve of each component was a plot of its peak area to that of the internal standard ratio vs concentration.

B – Authentic mixture: Different aliquots from the authentic mixtures stock solution–prepared in ratios claimed to be present in pharmaceutical prepara-
tions–within the range recorded in the calibration were transferred into 10 ml volumetric flask and completed as described under procedure A.

The concentrations were within the range used for the calibration curve.

C – Commercial dosage forms: The contents of 10 Unasyn or Cefobid vials, Unasyn tablets, Cefclor
capsules, or Cefclor oral suspension packets were weighed and the average weight was determined,
the contents were mixed and powdered. An accurately weighed portion of the powder was dissol-
ved in 100 ml of the mobile phase with the aid of ultrasonic bath, then filtered to 100 ml volumetric
flask and completed to volume. Different aliquots were transferred to 10 ml volumetric flasks and completed as under procedure A.
Table 6. Determination of cefaclor in Ceelex capsules and Ceelex oral suspension packets

<table>
<thead>
<tr>
<th>Conc. present</th>
<th>Conc. found</th>
<th>Recovery %</th>
<th>Conc. present</th>
<th>Conc. found</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.196</td>
<td>98.0</td>
<td>0.2</td>
<td>0.205</td>
<td>102.5</td>
</tr>
<tr>
<td>0.4</td>
<td>0.397</td>
<td>99.25</td>
<td>0.4</td>
<td>0.396</td>
<td>99.0</td>
</tr>
<tr>
<td>0.6</td>
<td>0.609</td>
<td>101.4</td>
<td>0.6</td>
<td>0.605</td>
<td>100.83</td>
</tr>
<tr>
<td>0.8</td>
<td>0.81</td>
<td>101.25</td>
<td>0.8</td>
<td>0.805</td>
<td>100.63</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>99.98</td>
<td></td>
<td></td>
<td>100.74</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>1.42</td>
<td></td>
<td></td>
<td>1.23</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The chromatogram shown in Figure 1 indicates the possibility of separation of p-hydroxyphenylglycin, sulbactam, sulmucillin tosylate, cefaclor, ampicillin, salicylamide and cefoperazone with the retention times 0.8, 1.45, 2.1, 2.8, 4.6, 5.8, and 7.9 min, respectively. These drugs were detected at 230 nm and 0.1 aufs. Salicylamide, which has suitable retention time and absorption under these conditions was used as an internal standard. Degradation products or manufacture precursors as phenylglycin, 6APA and 7-ACA, also do not interfere with the determination, but they appear as one peak at 1.1 min. Figure 2 shows authentic mixture of cefoperazone and sulbactam spiked with two expected impurities. Figure 3-a shows authentic mixture of sulbactam and ampicillin. 6APA was detected in Unasyn vials as shown in Figure 3-b and also detected in Unasyn tablets with sulbactam and ampicillin which claimed to contain sulmucillin tosylate only, Figure 3-c. The 7ACA not expected to be present in these drugs and phenylglycin shows no absorption at 230 nm and detector sensitivity 0.1 aufs.

The plots of peak area ratios Y to concentrations X in mg% were found to be linear within the concentration range 0.1–0.5, 0.1–0.9, 0.1–0.8, 0.1–1 and 0.1–1 mg% for sulbactam, sulmucillin tosylate, cefaclor, ampicillin and cefoperazone, respectively. Figure 4 shows different concentrations of cefoperazone and the same concentrations of salicylamide.

Regression analysis of the data of each component gave the slope, intercept and correlation coefficient for each calibration curve, Table 1. The validity of the listed regression equations was tested by the assay of authentic mixtures containing known quantities of sulbactam and ampicillin or cefoperazone in ratios equal to those claimed in commercial dosage forms. The results in Table 2 showed good accuracy and precision as shown from percentage recovery and relative standard deviation, 1.41–3.28%.

The proposed method was applied successfully to Unasyn vials and tablets, Cefobid vials, Ceelex capsules and oral suspension packets (Tables 3–6) without any interference from the excipients, additives or the coloring matter present in Ceelex suspensions.

Moreover, the method is highly sensitive, time saving and could be used in quality control of antibiotics in the pharmaceutical preparations containing these mixtures.

Acknowledgment

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REFERENCES


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