Acta Poloniae Pharmaceutica – Drug Research, Vol. 55 No. 1 pp. 9–14, 1998

ISSN 0001-6837
Polish Pharmaceutical Society

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 Ceclor 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; (6*R*)–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 –carboxamido)–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 $HClO_4$ 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

Shimadzu, SCL–10A system controller, LC–10AS solvent delivery unit, SPD–10A UV spectrophotometric detector, C–R6–A Chromatopac integrator.

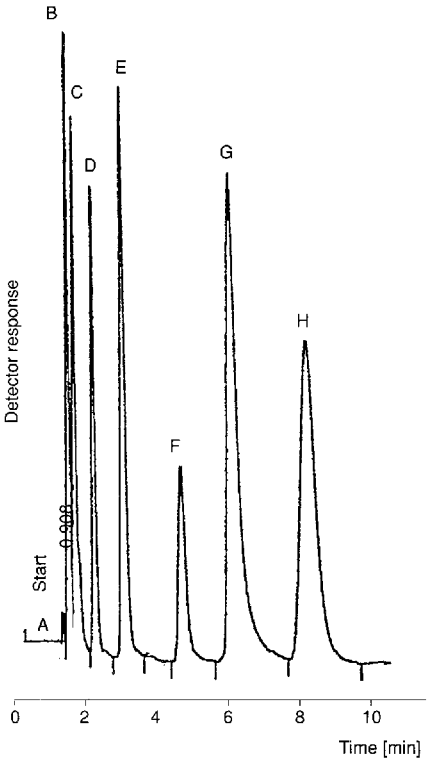

Figure 1. Chromatogram of p–hydroxyphenylglycin; A; 6–APA, B; sulbactam, C; sultamicillin tosylate, D; cefaclor, E; ampicillin, F; salicylamide, G; and cefoperazone, H at 230 nm.

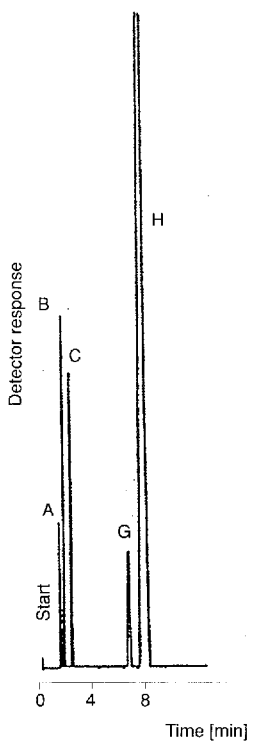

Figure 2. Chromatogram of authentic mixture of sulbactam, C; salicylamide, G; cefoperazone, H; p-hydroxyphenylglycin, A; 7–ACA, B at 230 nm.

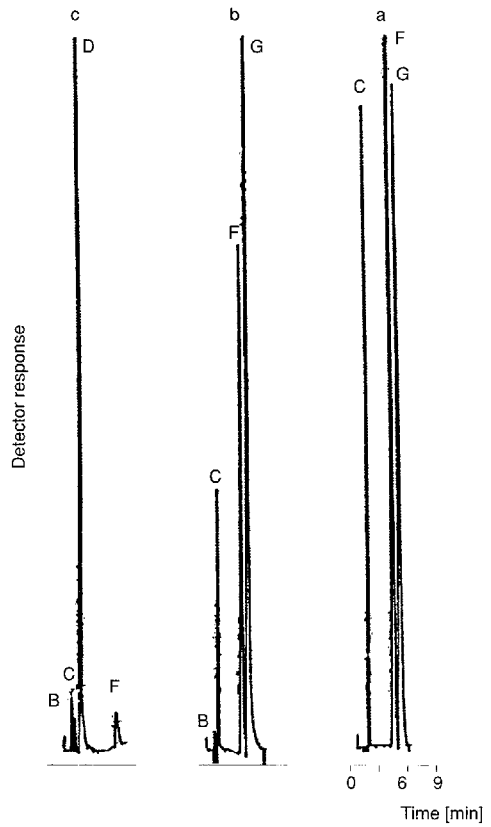

Figure 3. a – Chromatogram of authentic mixture of sulbactam, C; ampicillin, F and salicylamide, G
b – After extraction from Unasyn injection
c – After extraction from Unasyn tablets

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–Aminocephalosporanic acid, Aldrich Chem. Co.;

All the standard solutions were dissolved in the mobile phase.

Methanol; 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

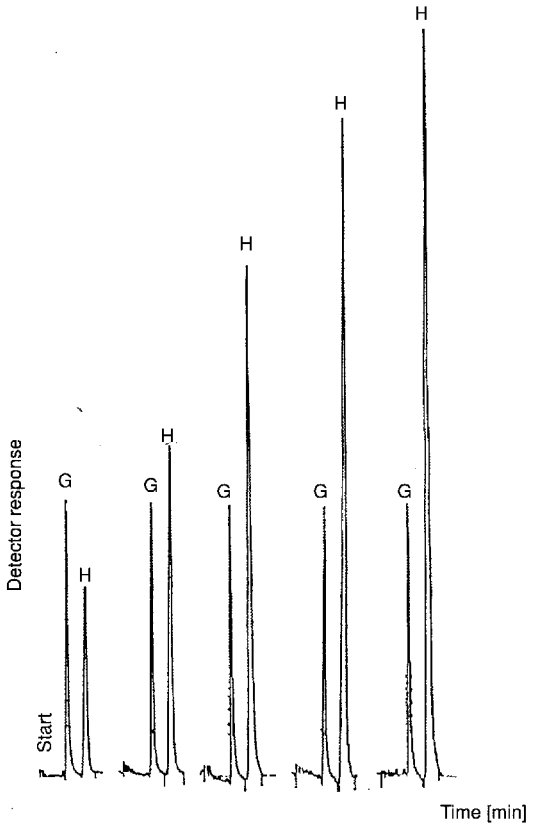

Figure 4. Chromatogram of different concentrations of cefoperazone, H; 0.1–1 mg% and the same concentration of salicylamide, G – 0.1 mg% at 230 nm.

mg sultamicillin base, BN 317–63404, average weight of one tablet 757 mg.

Unasyn injection: 1.5 g vial, Pfizer, Poland, labelled to contain sulbactam sodium equivalent to 0.5 g sulbactam and ampicillin sodium equivalent to 1.0 g ampicillin BN 2010596. Average weight of powder in vial 1.9 g.

Ceclor capsules: from Eli Lilly, Italy, labelled to contain 500 mg cefaclor, BN 220040. Average weight 590 mg.

Table 1. Parameters of the calibration curves obtained by regression analysis

Compound	Regression equation	Correlation coefficient
Sulbactam	Y = 0.95 x – 0.009	0.990
Sultamicillin	Y = 2.6 x + 0.026	0.999
Ampicillin	Y = 1.56 x + 0.006	0.997
Cefoperazone	Y = 4.27 x + 0.002	0.997
Cefaclor	Y = 2.115 x + 0.113	0.999

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

Sulbactam			Ampicillin			Sulbactam			Cefoperazone		
Amount added	Amount found	Recovery %	Amount added	Amount found	Recovery %	Amount added	Amount found	Recovery %	Amount added	Amount found	Recovery %
0.1	0.098	98.00	0.2	0.196	98.00	0.15	0.153	102.0	0.3	0.31	103.3
0.2	0.21	105.0	0.4	0.41	102.5	0.2	0.202	101.0	0.4	0.396	99.0
0.4	0.41	102.5	0.8	0.81	101.25	0.25	0.247	98.8	0.5	0.505	101.0
0.5	0.493	98.6	1.0	0.99	99.0	0.3	0.305	101.6	0.6	0.61	101.6
Mean		101.03			100.19			100.85			101.23
SD		3.3			2.05			1.43			1.77
RSD		3.28			2.05			1.41			1.75

Table 3. Determination of sulbactam and ampicillin in Unasyn vials

Sulbactam			Ampicillin		
Amount present	Amount found	Recovery %	Amount present	Amount found	Recovery %
0.1	0.099	99.0	0.2	0.197	98.5
0.15	0.145	96.67	0.3	0.29	96.7
0.2	0.201	100.5	0.4	0.398	99.5
0.25	0.251	100.4	0.5	0.51	102.0
Mean		99.14			99.18
SD		1.78			2.21
RSD		1.8			2.22

Ceclor 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 cefoperazone. 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 psig.
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 measurements were repeated three times at each concentration. 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 preparations–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, Ceclor capsules, or Ceclor 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 dissolved 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 4. Determination of sultamicillin tosylate in Unasyn tablets

Amount present	Amount found	Recovery %
0.1	0.0977	97.7
0.15	0.151	100.67
0.2	0.201	100.50
0.25	0.251	100.40
0.3	0.298	99.33
Mean		99.72
SD		1.25
RSD		1.26

Table 5. Determination of cefoperazone in Cefobid vial

Amount present	Amount found	Recovery %
0.2	0.205	102.5
0.4	0.394	98.5
0.6	0.608	101.33
0.8	0.819	102.38
1.0	0.988	98.8
Mean		100.7
SD		1.93
RSD		1.91

Table 6. Determination of cefaclor in Ceclor capsules and Ceclor oral suspension packets

Ceclor capsules			Ceclor oral suspension		
Conc. present	Conc. found	Recovery %	Conc. present	Conc. found	Recovery %
0.2	0.196	98.0	0.2	0.205	102.5
0.4	0.397	99.25	0.4	0.396	99.0
0.6	0.609	101.4	0.6	0.605	100.83
0.8	0.81	101.25	0.8	0.805	100.63
Mean		99.98			100.74
RSD		1.42			1.23

RESULTS AND DISCUSSION

The chromatogram shown in Figure 1 indicates the possibility of separation of p–hydroxyphenylglycin, sulbactam, sultamicillin 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 sultamicillin 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, sultamicillin 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, Ceclor capsules and oral suspension packets (Tables 3–6) without any interference from the excipients, additives or the coloring matter present in Ceclor 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

This research was supoorted by Drug Institute, Warsaw, Poland.

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Received: 31.07.1997