Determination of amikacin and ciprofloxacin by liquid chromatography with pre-column derivatization to evaluate sustained delivery of antibiotics from Drug-Eluting Biopsy Needle

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Keywords
antibacterial antibiotics
derivatization
drug delivery systems
high performance liquid chromatography

Abstract
Determination of chosen antibacterial antibiotics: amikacin and ciprofloxacin was carried out by hplc-uv after derivatization with 9-fluorenylmethyl chloroformate and in their native form by HPLC-MS/MS. Developed methods have been applied to control the kinetics of antibiotic release from polymer-based controlled drug delivery system.

1. Introduction

Amikacin and ciprofloxacin are the most commonly used antibiotics used to fight bacterial infections mainly caused by *Escherichia coli* [1]. Thanks to their efficiency these antibiotics are used to prevent possible infections in the case of a transrectal prostate biopsy. Nowadays, for this purpose, medicine willingly uses the "controlled drug delivery systems", i.e., polymer-based drug-eluting biopsy needles. This approach allows administration of antibiotics during the biopsy directly into the tissue, elimination of onerous antibiotic therapy and decrease possibility of infection complications.

Amikacin is an aminoglycoside antibiotic especially effective against Gram-Negative bacteria. Chemically amikacin consist of aminosugars (D-glucosamine, D-kanosamine) which are connected to aminocyclitol by glyosidic bonds.

Ciprofloxacin is a second generation quinolone derivative. In comparison to amikacin, ciprofloxacin is characterized by completely different chemical properties such as lower polarity and water solubility (Table 1).

Table 1
Selected properties of amikacin and ciprofloxacin (dissotiation exponents predicted from ChemAxon).

Name	Molecular formula	M/g mol^{-1}	Log P	Water solubility	pK_a	pK_b
amikacin	$C_{22}H_{43}N_5O_{13}$	585.6	–7.40	185 g L^{-1} (25 °C)	12.1	9.79
ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.3	0.28	30 g L^{-1} (20 °C)	5.76	8.68

As a result of the aforementioned differences, the chromatographic determination of amikacin and ciprofloxacin may cause problems due to their completely different retention behaviour during separation. In addition, due the lack of chromophores and high polarity, direct analysis of amikacin in its native form, especially under reversed phase conditions is difficult and the derivatization step is required [2]. Derivatization of antibiotics with amino-groups is realized mainly with: 9-fluorenylmethyl chloroformate, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, ortho-phthaldialdehyde with 3-mercaptopropionic acid, etc. However, derivatization often results in the decrease of precision and increase of costs and time of analysis [2–4]. The procedure for precolumn derivatization of amikacin and ciprofloxacin with 9-fluorenylmethyl chloroformate was proposed. Additionally obtained results were compared with direct analysis of the antibiotics in their native forms using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Proposed procedure was applied to control the kinetic of antibiotics release from polymer-based controlled drug delivery system, namely the trans-rectal biopsy needle.

2. Experimental

2.1 Reagents and chemicals

Amikacin and Ciprofloxacin were purchased from Interquim (Cuautitlán Izcalli, Mexico) and Aarti Drugs Limited (Maharashtra, India) respectively. 9-fluorenylmethyl chloroformate (≥ 99%), acetonitrile (LC-MS grade) and glycine were purchased from Sigma Aldrich. Ultrapure water was prepared using HLP5 system from Hydrolab (Wiślina, Poland). Formic acid was purchased from Merck. Boric acid, phosphoric acid, sodium hydroxide and potassium chloride were purchased from POCH (Gliwice, Poland). Borate buffer was prepared by titrating the water solution of boric acid (0.2 M) and potassium chloride (0.2 M) with sodium hydroxide to pH = 7.3. Polymer-coated biopsy needles with different concentrations of hydrophilic polymers and antibiotics were prepared in cooperation with Department of Polymer Technology (Gdańsk University of Technology, Poland).

Table 2
Multiple reaction monitoring mode parameters.

Analyte	Multiple reaction monitoring	Declustering potential/V	Collision energy/V
amikacin	586.4–163.1	81	47
	586.4–425.3	81	27
ciprofloxacin	332.3–288.1	91	27
	322.3–231.0	91	49

2.2 Instrumentation

HPLC-UV determination of antibiotic was performed using an Agilent 1200 LC system consisting of degasser, binary pump, ALS autosampler, thermostated column compartment and diode array detector (DAD) detector. The separation of antibiotics was carried out using Kinetex C18, 1.7 µm (50×2.1 mm) chromatographic column working at 40° C. As a mobile phase water (component A) and acetonitrile (component B) both with 0.5% (*v/v*) of diluted phosphoric acid (in a mass ratio 1:1 with deionized water) were used in following gradient elution: 0 min – 20% B, 15 min – 95% B, 20 min – 95% B, 20.1 min – 20% B, 24.5 min – 20% B. Flow rate of 0.3 mL min^{-1} was used and the injection volume was 2 µL.

HPLC-MS/MS determination of antibiotics in multiple reaction monitoring mode (Table 2) was performed using an Agilent 1200 Series Rapid Resolution LC system (USA) consisting of an online degasser, a binary pump, a high-performance SL autosampler and a thermostated column compartment. The system was coupled to the Q-Trap 4000 triple quadrupole mass spectrometer (AB SCIEX, USA) with electrospray ionization (ESI) source working in positive ion mode. Other parameters of ESI source were as follows: curtain gas pressure: 20 psi, source temperature: 550 °C, nebulizer gas pressure: 30 psi, heater gas pressure: 30 psi and capillary voltage: 4000 V. For separation ZORBAX Eclipse XDB-C18 1.8 µm (50×4.6 mm) chromatographic column was used. Temperature of column was maintained at 35 °C. Separation was carried out in isocratic conditions with mixture of acetonitrile and water (85:15, *v/v*) with 0.1% of formic acid as a mobile phase. Flow rate of 0.8 mL min^{-1} was used and the injection volume was 2 µL. The time of analysis was 3 minutes.

2.3 Sample preparation

Two sets of biopsy needles were prepared with two different compositions and thickness of coatings (8 needles for each set) and injection simulation test was performed. For this purpose, pork prostates obtained from a local slaughterhouse were used. Prostates were frozen and heated to approx. 37 °C on the test day. Additionally, in order to simulate the real biopsy procedure, the special biopsy gun was used. For each of 8 needles from a given series (with the same coatings),

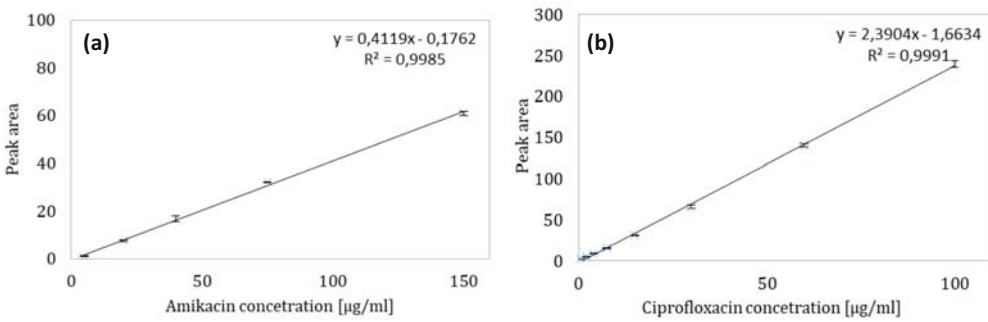

Fig. 1 Calibration curve of (a) amikacin (5–150 µg ml^{-1}), and (b) ciprofloxacin (0.5–100 µg ml^{-1}).

a different number of tissue injections were performed: 0 (without injection – reference sample), 1, 3, 5, 7, 9, 11 and 12 injections. Subsequently, each needle was immersed in 5.5 mL of deionized water in dedicated test-tube for 40 min. After that, the solution was transferred into Eppendorf vials and vortex for 5 minutes. The clarified solution was transferred to the vials and analysed by HPLC-MS/MS.

In the case of HPLC-UV analysis, the modified Chang's [4] procedure was applied. The clarified solution was mixed with acetonitrile in volume ratio 1:1. The 200 µL of this mixture was introduced in to the vial with 200 µL borate buffer (0.2 M, pH = 7.3). For derivatization, 200 µL of 9-fluorenylmethyl chloroformate acetonitrile solution (2.5 mM) was added and mixed. After 20 minutes, the 50 µL of glycine (0.1 M) was added and mixed for stopping the derivatization reaction. After all, samples were analysed by HPLC-UV.

2.4 Calibration curves

Stock solution of amikacin (10 mg mL^{-1}) and ciprofloxacin (1 mg mL^{-1}) were prepared in deionized water. Working standard solutions were prepared freshly by mixing volumes of stock solutions with water in graduated flasks. Amikacin and ciprofloxacin LC-MS/MS calibration standard solutions of 0.5, 1, 5, 15, 25, 50, 100 µg mL^{-1} were prepared. In the case of LC-UV method, calibration standards of 5, 20, 40, 75, 150 µg mL^{-1} for amikacin and 0.5, 2, 4, 7.5, 15, 30, 60, 100 µg mL^{-1} for ciprofloxacin after derivatization with 9-fluorenylmethyl chloroformate were used.

3. Results and discussion

In the case of derivatization procedure, the buffer pH (7.3 and 8.5), concentration of 9-fluorenylmethyl chloroformate (1, 2.5, 3.5, 5 and 25 mM) and time of reaction (1, 3, 5, 15, 20 and 45 minutes) were optimized (data not shown). The optimal conditions were summarized in section 2.3.

Validation of pre-column derivatization HPLC-UV method was performed. It consisted of estimation of linearity, limits of detection (*LOD*) and limits of

Table 3
Comparison of amikacin and ciprofloxacin content in polymer-based coatings after injection simulation test obtained by pre-column derivatization HPLC-UV and derivatization-less HPLC-MS/MS methods ($n = 3$).

Coating number	Injection number	c(amikacin)±SD/μg mL^{-1}		c(ciprofloxacin)±SD/μg mL^{-1}	
		HPLC-UV	HPLC-MS/MS	HPLC-UV	HPLC-MS/MS
1	0	68.4±6.2	60.9±1.8	48.63±0.51	45.1±1.6
	1	44.2±7.1	39.2±1.3	37.60±0.46	35.0±1.7
	3	28.7±4.8	26.23±0.67	23.85±0.47	24.0±1.6
	5	17.1±1.3	13.27±0.55	15.61±0.57	16.8±1.4
	7	20.9±2.1	17.50±0.92	25.2±2.2	23.5±1.9
	9	6.86±0.88	3.96±0.52	4.53±0.33	4.82±0.65
	11	*n.a.*	1.60±0.63	0.55±0.13	*n.a.*
	12	5.0±1.1	2.84±0.58	1.56±0.22	1.46±0.35
2	0	59.9±4.1	44.92±0.32	39.42±0.72	42.1±1.3
	1	44.5±8.3	38.9±1.5	40.83±0.84	40.0±1.9
	3	35.6±1.1	25.50±0.54	36.3±2.2	33.7±2.1
	5	42.0±4.1	34.75±0.97	35.52±0.73	37.3±2.4
	7	16.24±0.37	14.08±0.31	20.15±0.17	19.0±1.5
	9	*n.a.*	1.051±0.051	0.96±0.21	0.92±0.11
	11	9.69±0.71	11.19±0.75	13.99±0.51	16.4±1.9
	12	6.86±0.24	7.24±0.61	8.95±0.28	10.79±0.56

quantification (LOQ). Both calibration curves (Fig. 1) were linear within the studied concentration ranges. Determination coefficients were satisfactory.

Also the limits of detection (*LOD* = calibration curve intercept standard deviation multiplied by 3.3 and divided by the slope of the calibration curve) and limits of quantitation (*LOQ* = 3×*LOD*) were established. For amikacin the *LOD* = = 3.2 μg mL^{-1} and *LOQ* = 9.6 μg mL^{-1}, whereas for ciprofloxacin 0.65 and 1.95 μg mL^{-1} respectively.

Results of real-world sample analysis (with and without pre-column derivatization) are summarized in Table 3. Developed HPLC-UV method can be applied for determination of antibiotics in polymer matrix. Both HPLC-UV and HPLC-MS/MS methods produces similar results. For ciprofloxacin, HPLC-UV method involving the derivatization step gives more precise results while the accuracy remains similar. In case of amikacin results obtained with the help of HPLC-UV method are less precise than those provided by direct HPLC-MS/MS method. This is most probably caused by inconsistent derivatization reaction efficiency. On the other hand we can observe that the results obtained with the HPLC-MS/MS method are slightly lower than those produced by HPLC-UV. This phenomenon can be explained by the fact that amikacin was not retained on C18 stationary phase and eluted in a dead time together with other signal suppression causing components.

4. Conclusions

The results obtained with the developed HPLC-UV method are similar to those obtained by HPLC-MS/MS analysis of the antibiotics in their native forms. It can be used to study the kinetics of amino-antibiotic drug release from drug's controlled-delivery systems. In relation to amikacin it is slightly less sensitive than HPLC-MS/MS method but seemingly the accuracy is better. With regard to ciprofloxacin the developed method seems to be superior over the HPLC-MS/MS since the precision of the results is higher while accuracy stays the same. Also the instrumentation is simpler, cheaper and widely available.

References

[1] Sieczkowski M., Gibas A., Wasik A., Kot-Wasik A., Piechowicz L. Namieśnik J. Matuszewski M.: Drug-Eluting biopsy needle as a novel strategy for antimicrobial prophylaxis in transrectal prostate biopsy. *Technol. Cancer Res. Treat.* **16** (2017), 1038–1043.

[2] Farouk F., Azzay H.M.E., Niessen W.M.A.: Challenges in the determination of aminoglycoside determination, a review. *Anal. Chimica Acta* **890** (2015), 21–43.

[3] Baranowska I., Markowski P., Baranowski J.: Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reversed-phase high-performance liquid chromatography method. *Anal. Chimica Acta* **570** (2006), 46–58.

[4] Chang X.-J., Peng J.-D., Liu S.-P.: A simple and rapid high performance liquid chromatographic method with fluorescence detection for estimation of amikacin in plasma – application to preclinical pharmacokinetics. *J. Chin. Chem. Soc.* **57** (2010), 34–39.