

UPLC-UV-MS method development for veterinary penicillins



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 in collaboration with Dopharma

Introduction

The assignment that came from Dopharma was to develop a UPLC-UV-MS method for analyzing four different penicillins; amoxicillin, ampicillin, phenoxymethylpenicillin and benzylpenicillin. Each of these penicillins have specific degradation products which must be baseline separated from their particular compound. Beforehand other groups also worked on this assignment, hence a method was already partially made. Therefor the focus was to optimize the separation of the degradation peak amoxy-D01 from the penicillin amoxicillin.

Plan of Action

First Part:

- Reproducing the results of the 2nd project group

Second Part:

- Optimizing the separation of the degradation amoxy-D01 from the penicillin amoxicillin by changing the gradient, column, mobile phase or the mobile phase pH.

Materials & Method

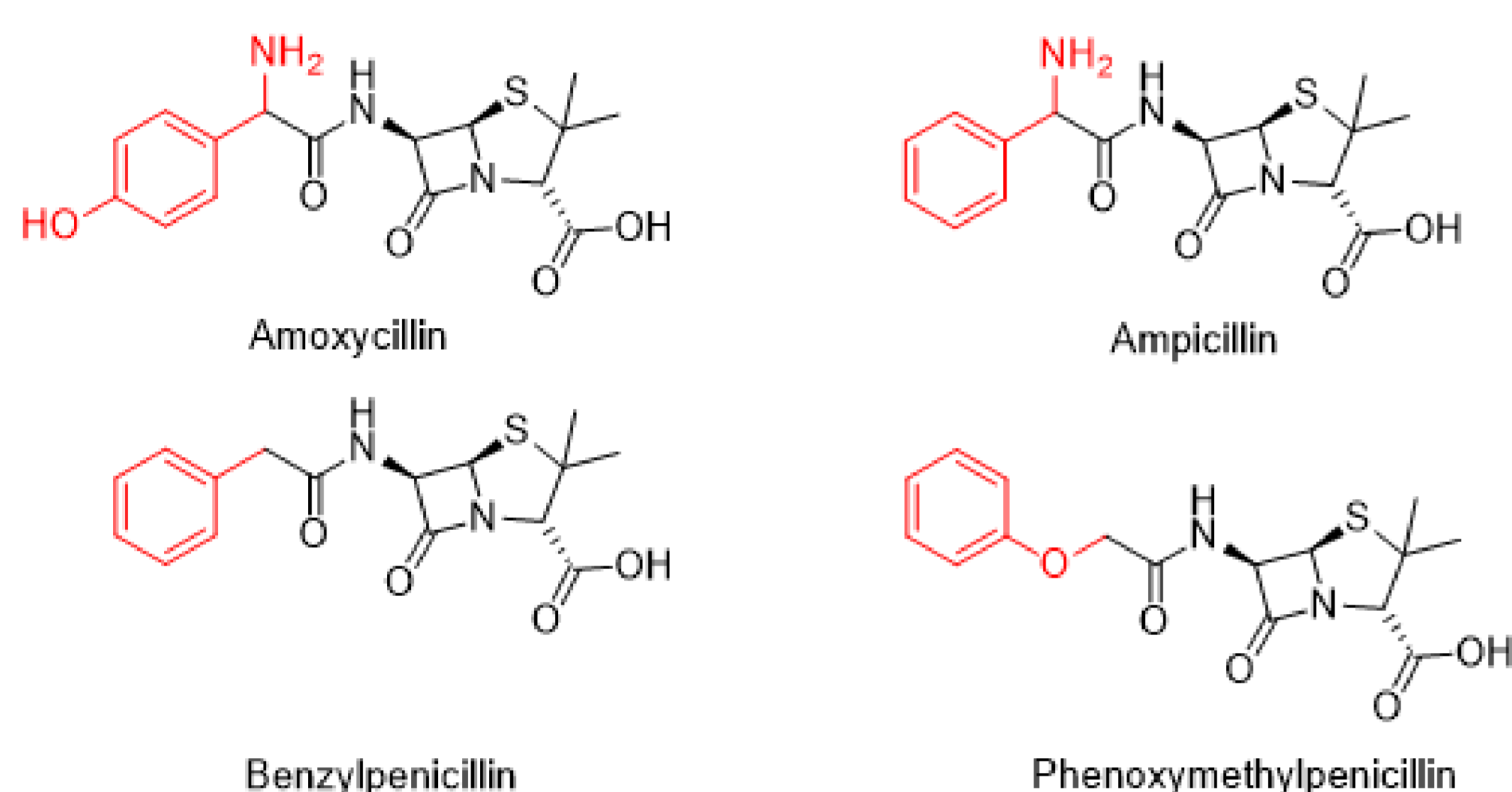


Figure 1: the four penicillins that were used during the project

The wavelength at which the penicillins were analyzed:

Amoxicillin: 230 nm Benzylpenicillin: 215 nm
 Ampicillin: 230 nm Mix penicillins: 220 nm
 Phenoxymethylpenicillin: 215 nm



Column:
 Acquity BEH C18, 2.1*50 mm, 1.7 μm

Mobile phase:
 %C = MiliQ with 0.1% formic acid

%D = Acetonitrile with 0.1% formic acid

Optimisation:

There were different gradients used to try to separate the amoxy-D01 from amoxicillin. Starting with 98% C or 75% C. After 4 minutes the gradient, was switched to the "normal" gradient, which continued with 95% C.

Figure 2: UPLC-UV-MS(QDA) from WATERS that is used during the project

Gradient	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.600	0.0	0.0	95.0	5.0	Initial
2	1.00	0.600	0.0	0.0	95.0	5.0	6
3	4.00	0.600	0.0	0.0	5.0	95.0	6
4	4.10	0.600	0.0	0.0	95.0	5.0	6
5	6.00	0.600	0.0	0.0	95.0	5.0	6

Figure 3: The gradient applied during the analyzes

Results

A mix of the four penicillins and amoxicillin + amoxy-D01 were analyzed with a gradient of 95% C

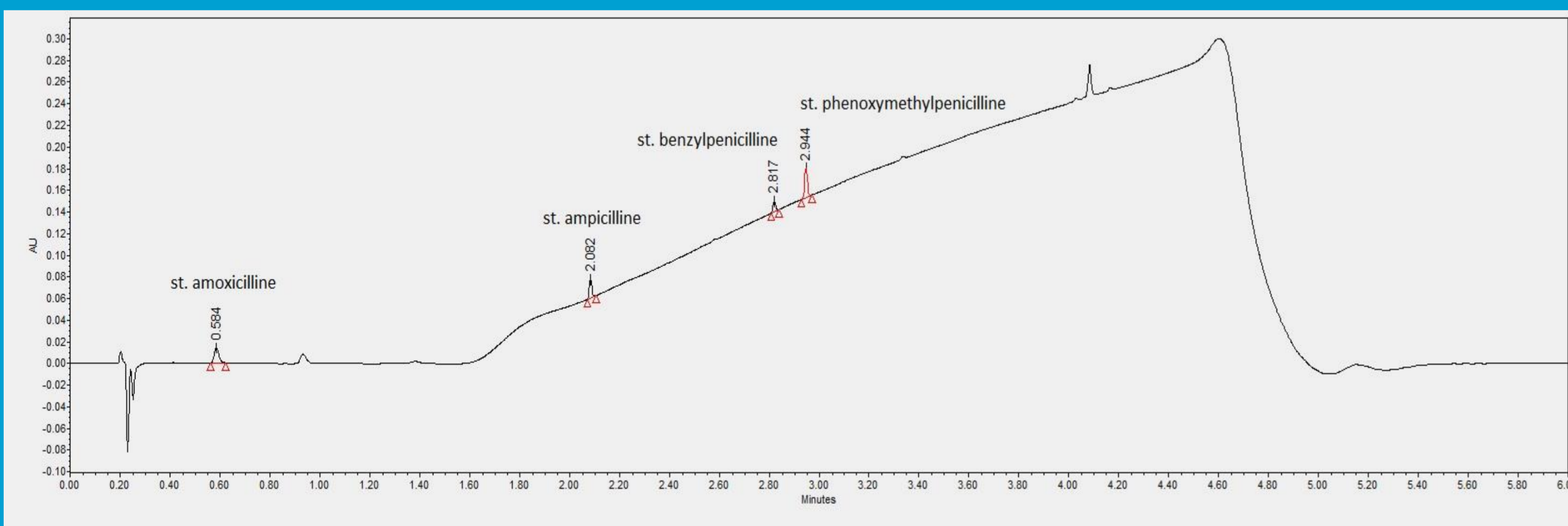


Figure 4: A mix of the four different penicillins with the 95% C gradient

In order to be able to separate amoxy-D01 from amoxicillin, different gradients were used:

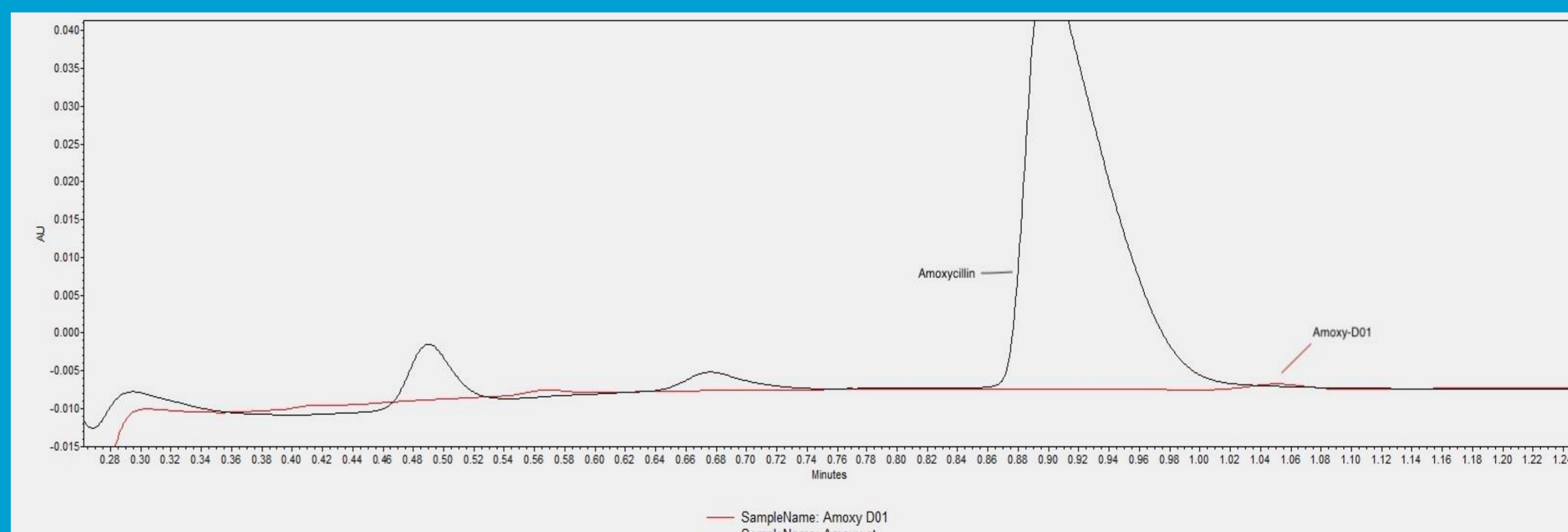


Figure 6: Amoxicillin and Amoxy-D01 with the 98% C gradient

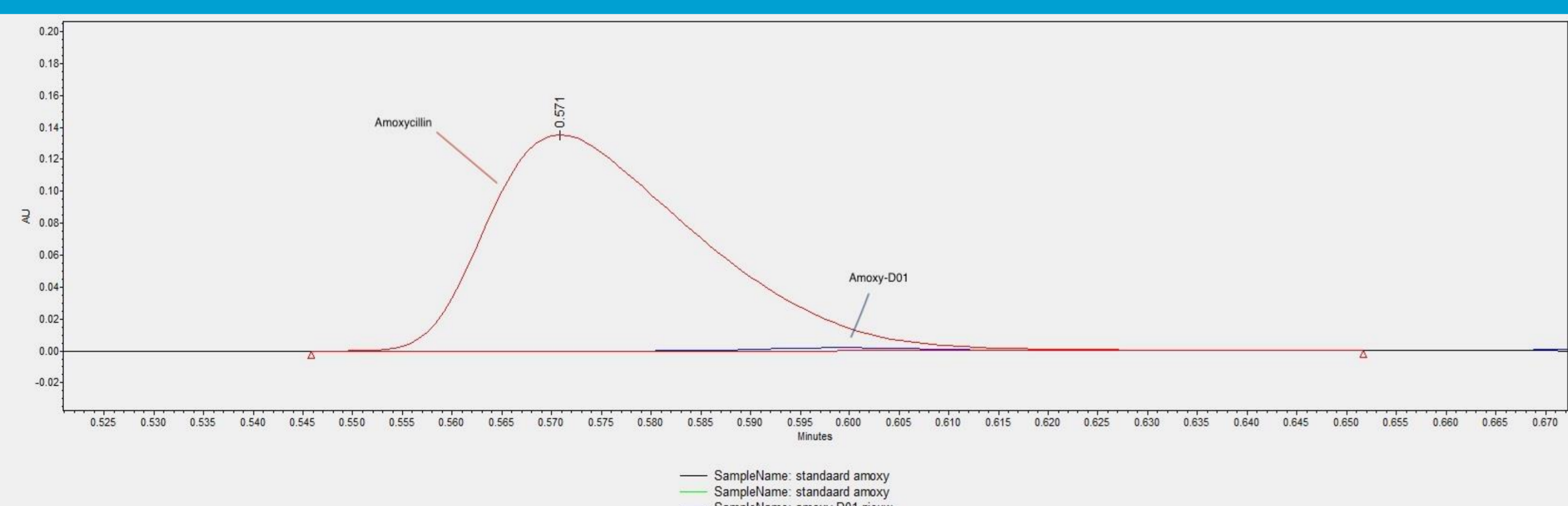


Figure 5: Amoxicillin and Amoxy-D01 with the 95% C gradient

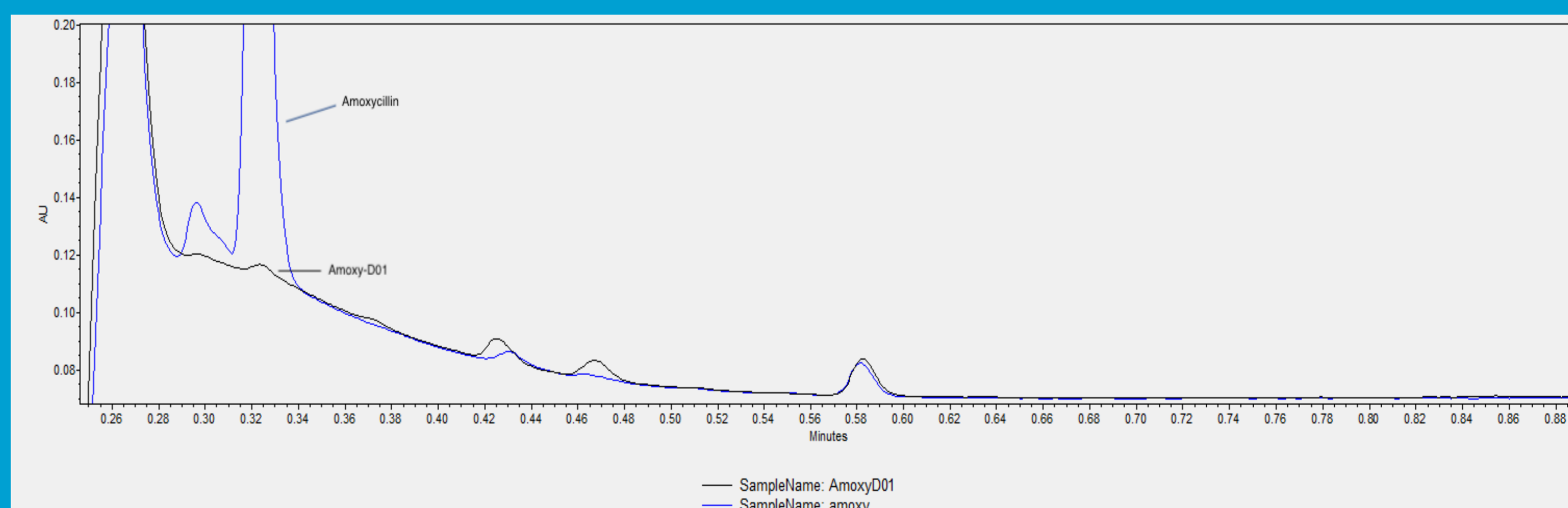


Figure 7: Amoxicillin and Amoxy-D01 with the 75% C gradient

Conclusion

The conclusion is that changing the gradient (starting with 98% C or 75%) on the Acquity BEH C18 column does not help in separating the degradation peak amoxy-D01 from the penicillin amoxicillin.

Future work

For the next project group:

- Focus on the pH from the mobile phase. The penicillins have different pKa values among which some high ones. Therefor the separation maybe could work with a mobile phase that has an pH of 9.5
- A HSS T3 C18 column could also work. This column is designed to separate more polar components.