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In situ degradation: a new concept for system suitability tests in monographs of the European Pharmacopoeia[1]

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Abstract

Monographs of the European Pharmacopoeia describe in the LC-test for related substances usually a system suitability test in order to ensure the adequate separation of impurities. Since the reference substances required are often not available a recent approach to avoid this problem is the generation of the required impurity by 'in situ degradation' of the active principle. This paper describes some typical applications of this technique as well as recent examples, such as the controlled degradation of cefalotin sodium, imipenem and spiramycin. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Liquid chromatography; Hydrolysis; Epimerization; Oxidation

1. Introduction

The monographs of the European Pharmacopoeia describe a system suitability test in the test for related substances by liquid chromatography, thin layer chromatography or gas chromatography. This test is applied in order to ensure an adequate performance of the chromatographic system. The resolution test is normally carried out using a mixture of the main compound of the monograph and a chemical reference substance (CRS) in such concentration that the chromatogram obtained shows peaks of similar height [1]: this is done in order to allow for the calculation of the R_s value according to the European Pharmacopoeia:

$$R_s = \frac{1.18(t_{R_b} - t_{R_a})}{b0.5a + b0.5b}$$

$$t_{R_b} > t_{R_a}$$

Preferably such products are chosen as reference substances which elute close to the main compound so that, if possible, all impurities which shall be controlled by the monograph are separated.The reference substances used in this test are usually: (i) structurally related substances, e.g. benperidol in the monograph for droperidol

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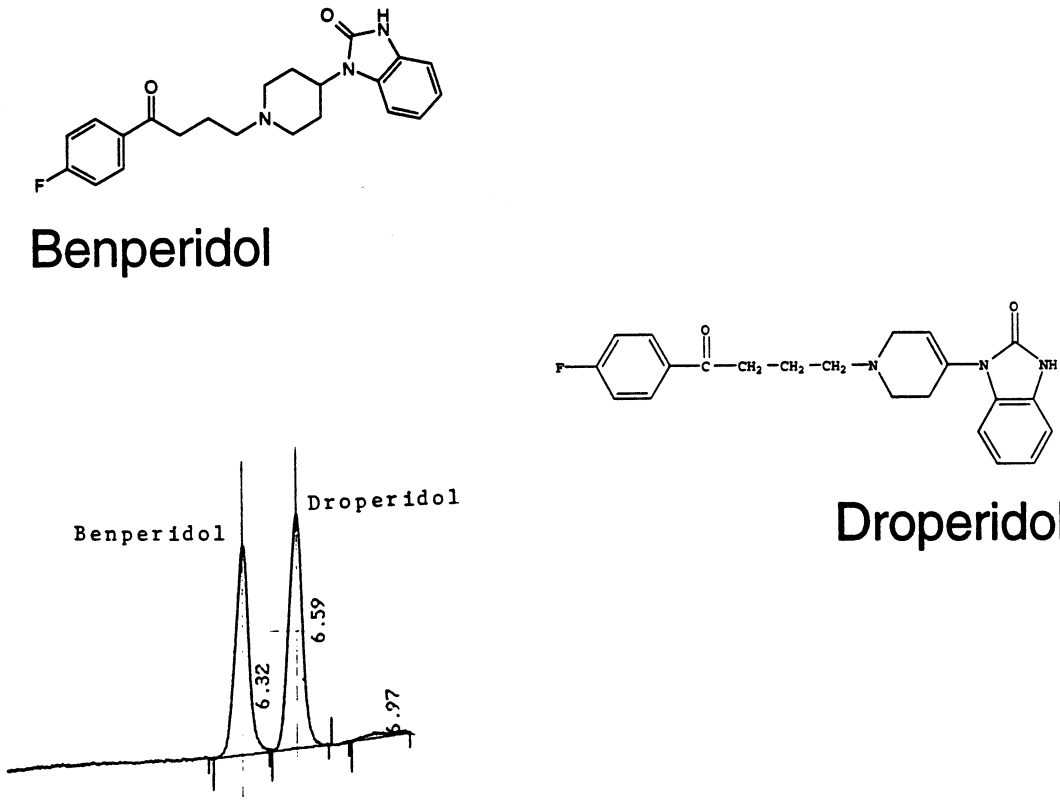


Fig. 1. Chromatographic separation of benperidol and droperidol.

(Fig. 1) [2]; (ii) by-products or degradation products, such as ceftriaxone E-isomer in the monograph for ceftriaxone sodium [3] (Fig. 2) or ciprofloxacin impurities in the monograph for ciprofloxacin HCl [4]; and (iii) performance test mixtures. These mixtures represent a mixture of the main compound with several impurities and/or degradation products and therefore ensure the suitability of the chromatographic system (Fig. 3).

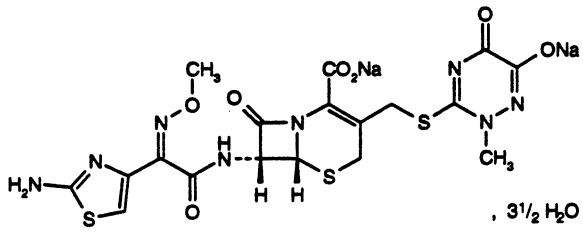
The limiting factor to this procedure, particularly in cases (ii) and (iii) is often the availability of impurities and degradation products in sufficient amounts to be established and used as reference substances. On the other hand, the idea to use related impurities or degradation products in the suitability tests is the ideal case since it allows to demonstrate the capability of the method to separate those compounds which shall be controlled by the method.

In order to avoid the problem of describing such reference substances in pharmacopoeial monographs a new approach has been developed in several recent monographs to ensure the system suitability.

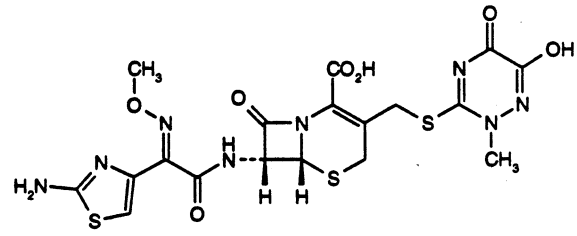
2. Results and discussion

This concept describes the generation of the 'reference substance' by in situ degradation of the active principle. In practical terms this means that a solution containing the monograph substance must be treated in such a way that a chemical reaction takes place in order to form the desired degradation product. The chemical reaction applied may be:

- hydrolysis
- epimerisation
- oxidation



Ceftriaxone sodium



Ceftriaxone E-isomer

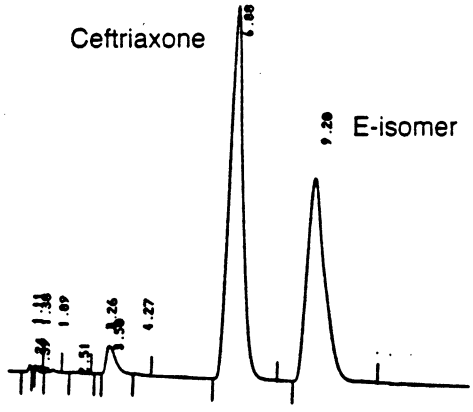


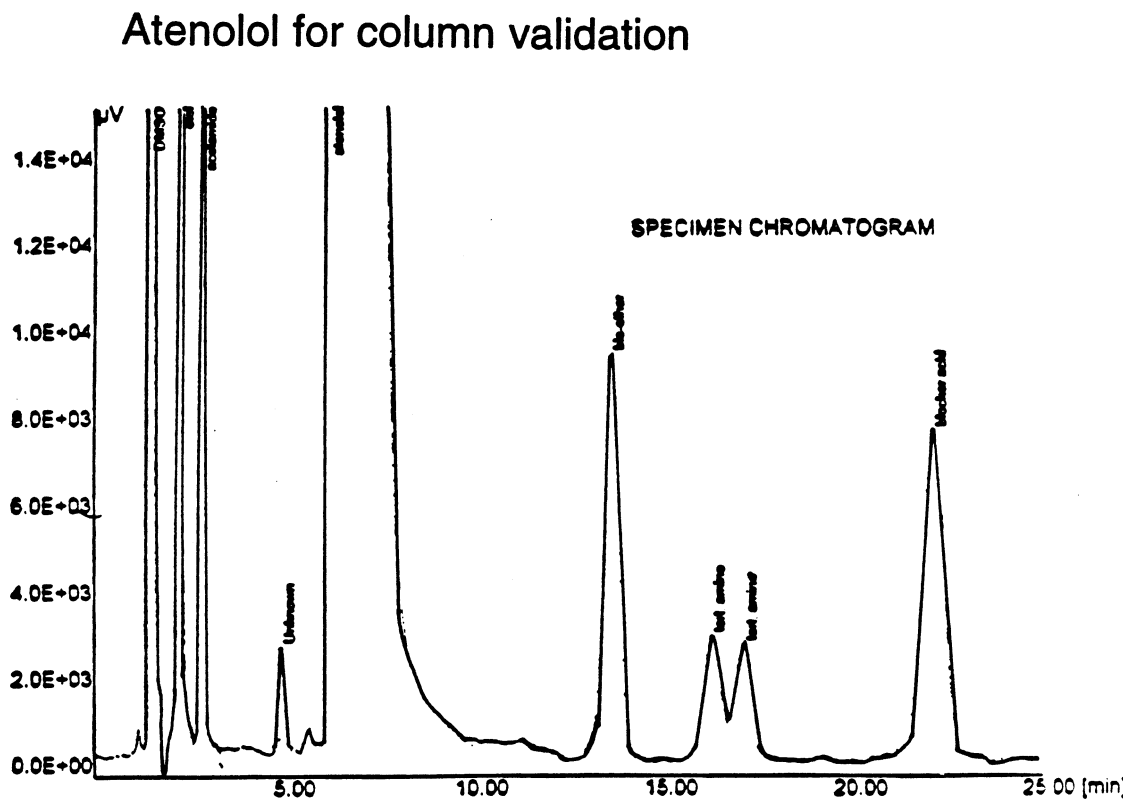
Fig. 2. Chromatographic separation of ceftriaxone and ceftriaxone E-isomer.

2.1. Hydrolysis

Hydrolysis is the most widely applicable reaction for this type of suitability test. Typical examples for this method are the hydrolysis of cefuroxime to descarbamoylcefuroxime (Fig. 4) as described in the monograph for cefuroxime sodium [5], the hydrolysis of fentanyl to the so-called fentanyl impurity D (Fig. 5) [6] or the hydrolysis of pentamidine diisetionate (Fig. 6) [7].

2.2. Epimerisation

Most of the European Pharmacopoeia monographs on tetracyclines describe in the LC-suitability test the preparation of a solution containing the tetracycline and the corresponding epimer as reference substance. The monograph on Minocycline HCl [8] describes an in situ epimerisation to partially form 4-epiminocycline (Fig. 7) and the solution obtained allows for the calcula-



#	Name	Rt
1	DMSO	1.48
2	diol	2.24
3	acetamide	2.83
4	Unknown	4.91
5	atenolol	6.18
6	bis-ether	13.56
7	tert. amine	16.22
8	tert. amine	17.06
9	blocker acid	22.06

Fig. 3. Specimen chromatogram of atenolol for column validation CRS.

tion of the resolution between minocycline and epiminocycline.

2.3. Oxidation

The first monograph in which an 'in situ oxidation' is used to carry out the LC-suitability test is the monograph for captopril [9]. The test for related substances describes a partial iodometric oxidation of captopril to captopril-disulphide. The solution obtained contains captopril and its dimer and can be used to determine the resolution in the HPLC-test for related substances (Fig. 8). However the latter example shows how carefully the reaction conditions must be chosen.

During the establishment of captopril CRS in the European Pharmacopoeia laboratory it was shown that the initially chosen quantity of iodine

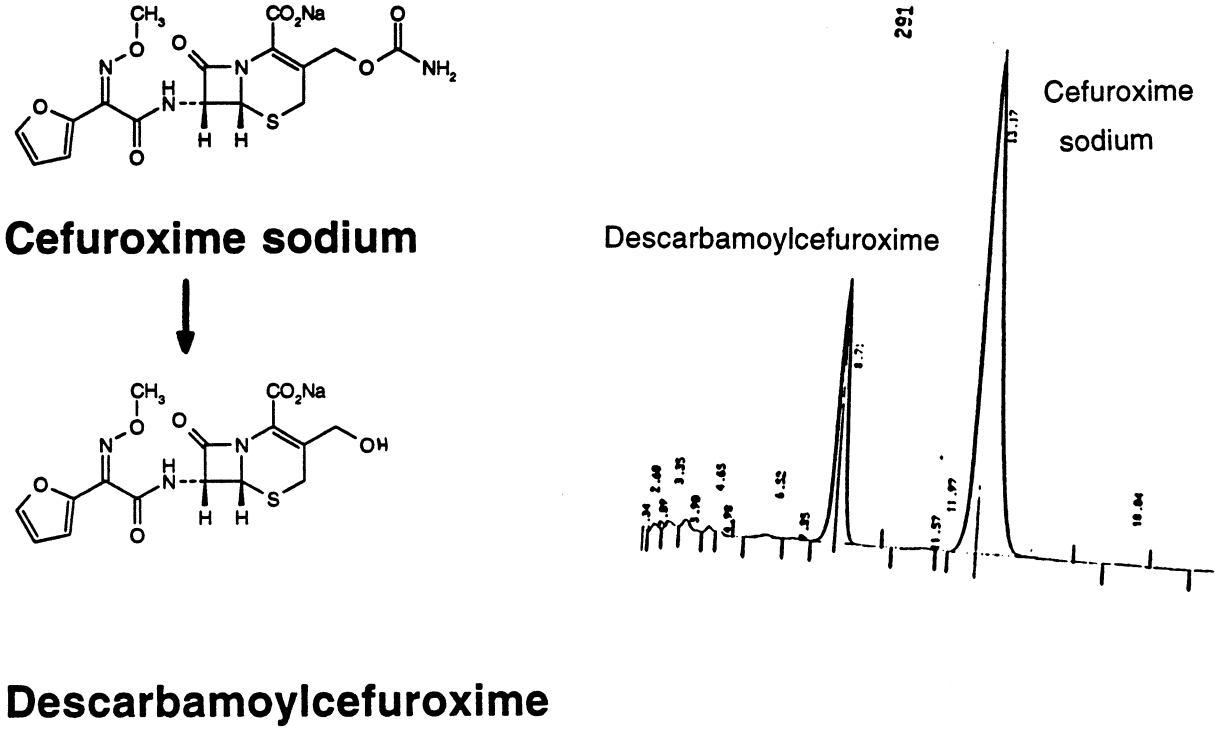


Fig. 4. Hydrolysis of cefuroxime sodium.

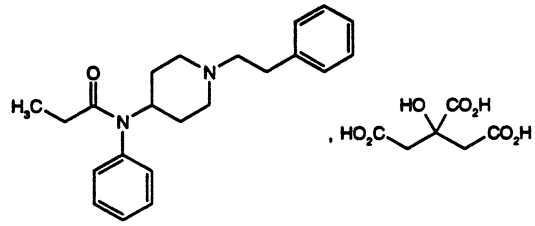
added led to an over-oxidation of captopril and only one peak due to captopril disulphide was identified in the chromatogram obtained. After modification of the reaction conditions by reducing the quantity of iodine added, the chromatogram obtained shows two peaks allowing for the calculation of the resolution.

The opposite case was found in the resolution test described in the monograph for pentamidine diisetionate when the method described (heating of a neutral aqueous solution) generated the hydrolysis product only in small amounts (2%) so that the R_s-calculation according to European Pharmacopoeia was not possible. A higher hydrolysis rate and thus peaks of similar height resulted when the pH of the solution was adjusted to pH 10.5–11.0 and subsequently heated under reflux for 20 min.

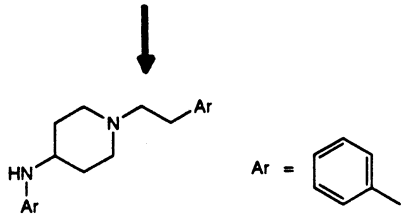
These examples demonstrate that the reaction conditions must be carefully chosen and the preconditions for a successful outcome of these tests are the following:

- solubility of the substance in a suitable medium, usually aqueous medium;
- quick and easy performable chemical reaction which leads predominantly to the desired product; and
- the reaction must be 'controlled', i.e. the reaction should not be too rapid and lead to a complete formation of the degradation product.

As a consequence the reaction conditions must



Fentanyl citrate



Impurity D

Heating of an acidic solution under reflux for 4h.

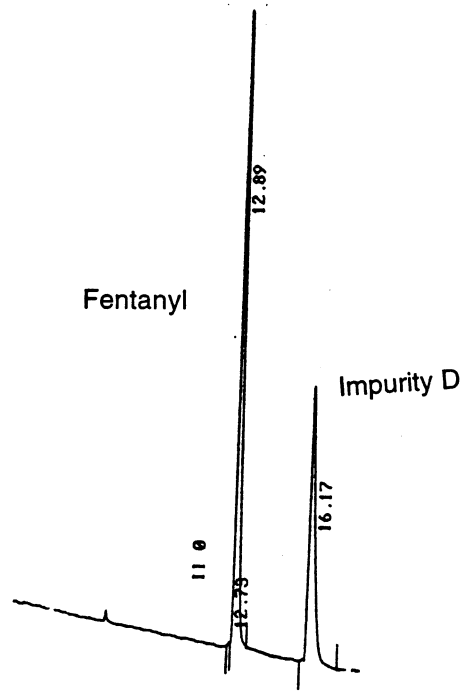
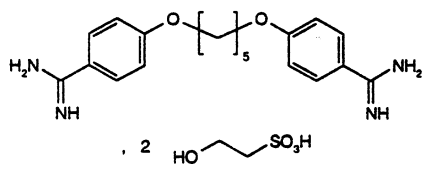
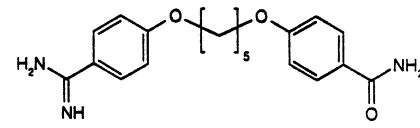


Fig. 5. Hydrolysis of fentanyl.

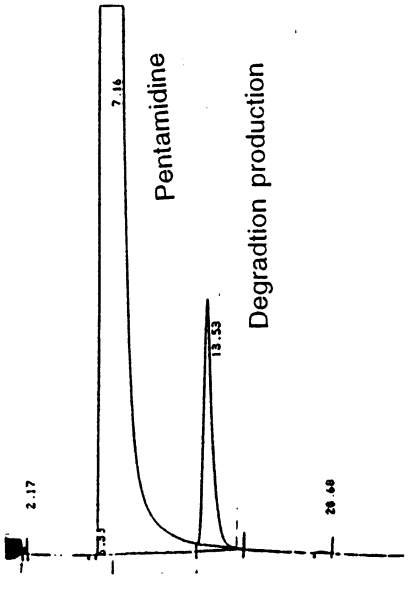


Pentamidine diisetionate

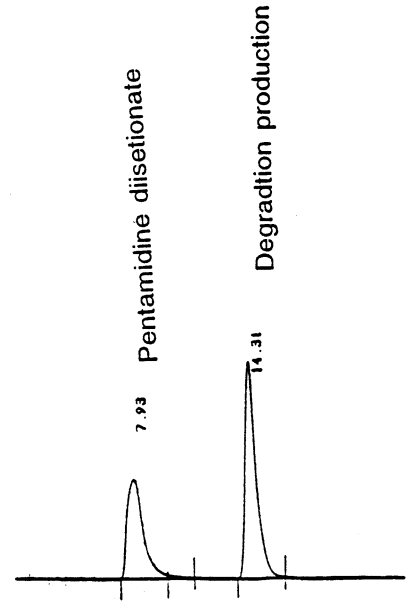


Impurity A

Boiling of an aqueous solution under reflux.



As described



Adjusted to pH 10.5-11.0,
heating under reflux for 20 min.

Fig. 6. Hydrolysis of pentamidine diisetionate.

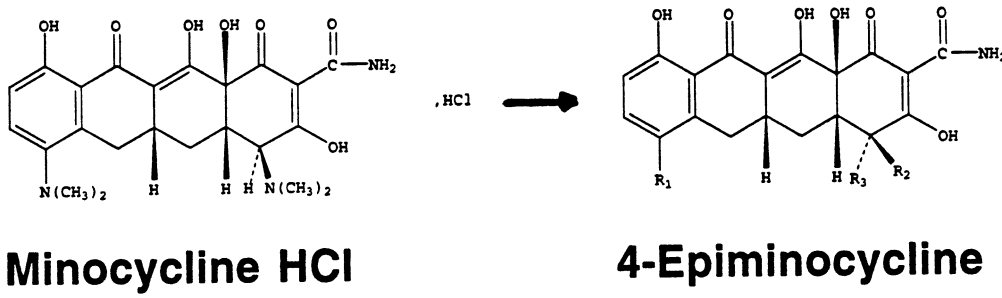


Fig. 7. Epimerisation of minocycline.

be chosen in such a way that the reference solution obtained contains approximately equal concentrations of the main compound and the degraadation product. Three further examples of recent investigations carried out in the European Pharmacopoeia laboratory may illustrate the applicability of this technique.

2.3.1. Monograph for cefalotin sodium

The initial version of this monograph described the use of cefalotin sodium and desacetoxycefalotin CRS in the system suitability test of the LC-test for related substances. Since the impurity described was not available in sufficient amounts to be established as CRS, a method was developed to generate in situ the hydrolysis by-product of cefalotin, namely desacetylcefalotin by alkaline hydrolysis. The following procedure was applied to optimise and validate the method.

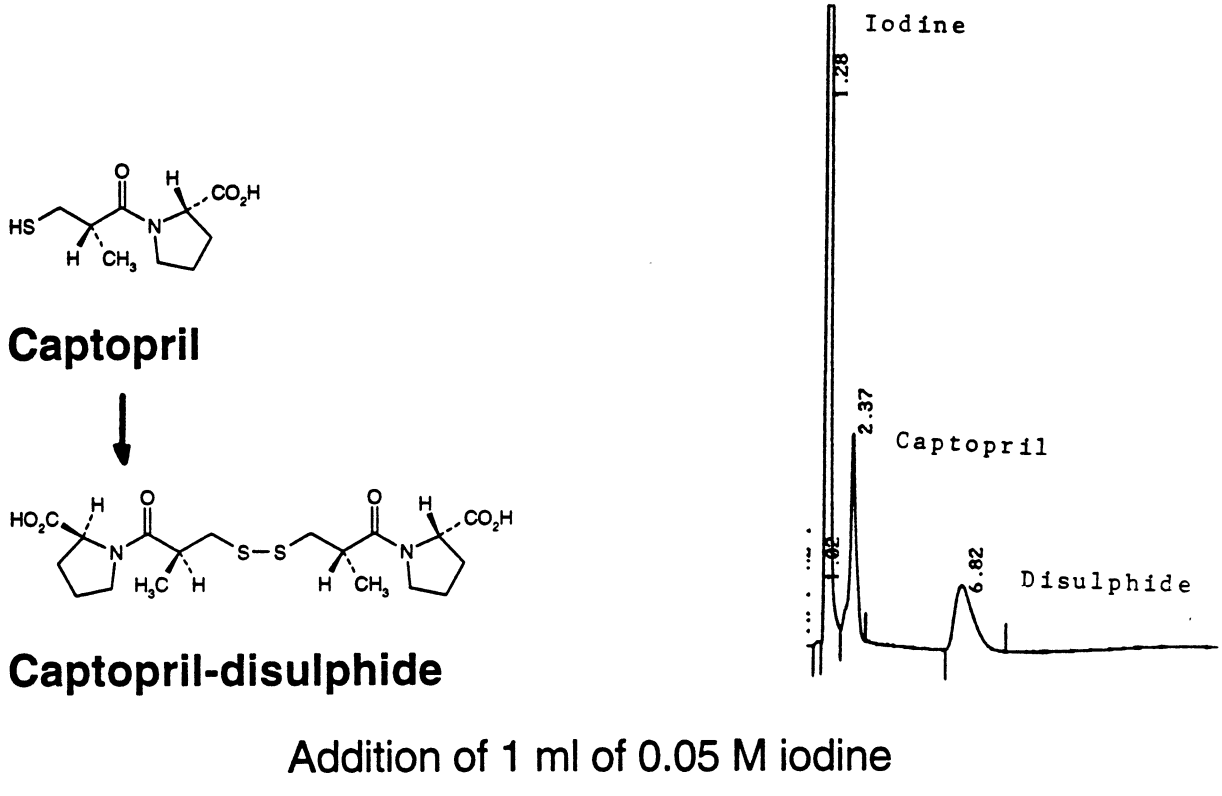


Fig. 8. Oxidation of captopril.

Cefalotin sodium was dissolved in the mobile phase (17 g of sodium acetate in 790 ml of water, 0.6 ml of glacial acetic acid, adjusted to pH 5.8–6.0 with dilute sodium hydroxide solution or glacial acetic acid, 150 ml of acetonitrile, 70 ml of ethanol) and heated in a water bath under different conditions. For comparison of the retention times solutions of desacetylcefalotin and desacetoxycefalotin were also injected. The best conditions to generate desacetylcefalotin from cefalotin and to obtain peaks of similar height was to heat the solution for 10 min at 90°C. The R_s-values obtained were as follows:

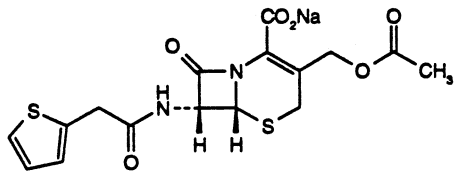
R_s-value	Found	Limit
R_s (desacetoxycefalotin-cefalotin)	9.0	6.0
R_s (desacetylcefalotin-cefalotin)	14.2	9.0

The chromatogram obtained (Fig. 9) allows for the calculation of the resolution. Subsequently an interlaboratory study including five laboratories was conducted in order to assign an assay value to the candidate CRS of cefalotin sodium. In this context the participants were asked to follow exactly the same protocol which described the in situ degradation of cefalotin under the above mentioned conditions. All of these laboratories successfully carried out the system suitability test and obtained the resolution required.

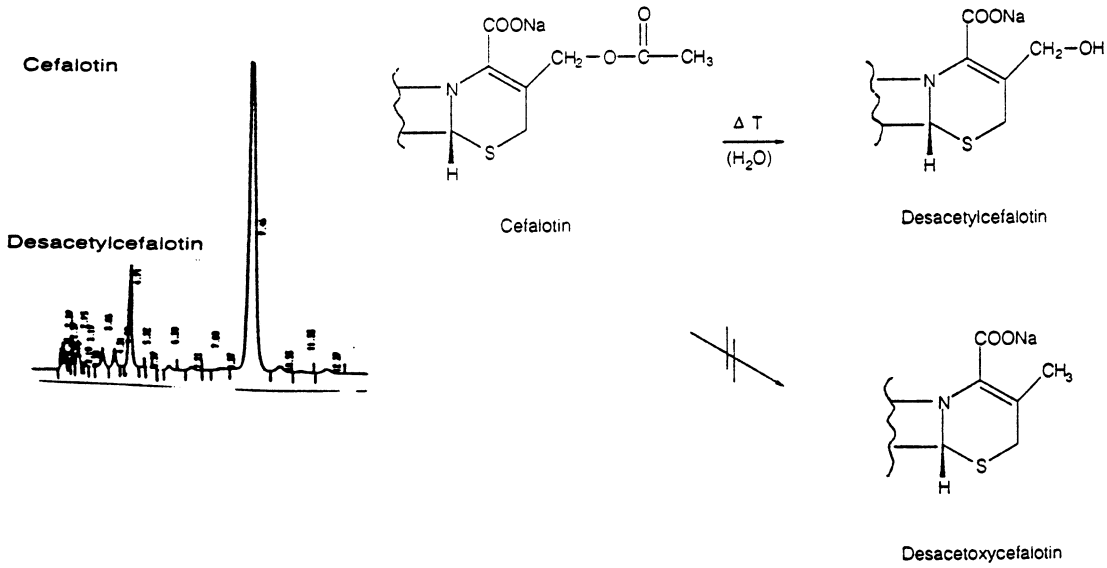
Following these examinations the monograph for cefalotin sodium was revised accordingly [10].

2.3.2. Monograph for imipenem

The draft monograph for imipenem described in the LC-assay a system suitability test using a reference solution containing 0.4 mg/ml of



Cefalotine sodium



Heating of a solution (pH 6.0) at 90°C for 10 min.

Fig. 9. Hydrolysis of cefalotin sodium.

imipenem. Since imipenem always contains a certain amount of thienamycin, the chromatogram obtained shall show a peak due to imipenem at a retention time of about 9 min and a peak due to thienamycin with a relative retention time of 0.8. The resolution between the two peaks is supposed to be at least 3.5.

On the other hand, as the content of thienamycin in imipenem is limited to at most 1%, the determination of the resolution according to European Pharmacopoeia is not possible. For this reason it was attempted to develop a more suitable test by in situ degradation.

A degradation of imipenem is observed in acidic solution mainly by opening of the β lactam ring and in alkaline medium by formation of thienamycin and of a 6-amido-*N*-formyldihydropyrrole-derivative [11]. Heating of a solution of imipenem in strong acid (pH 1) or strong alkaline (pH 14) medium resulted in a complete degradation of imipenem.

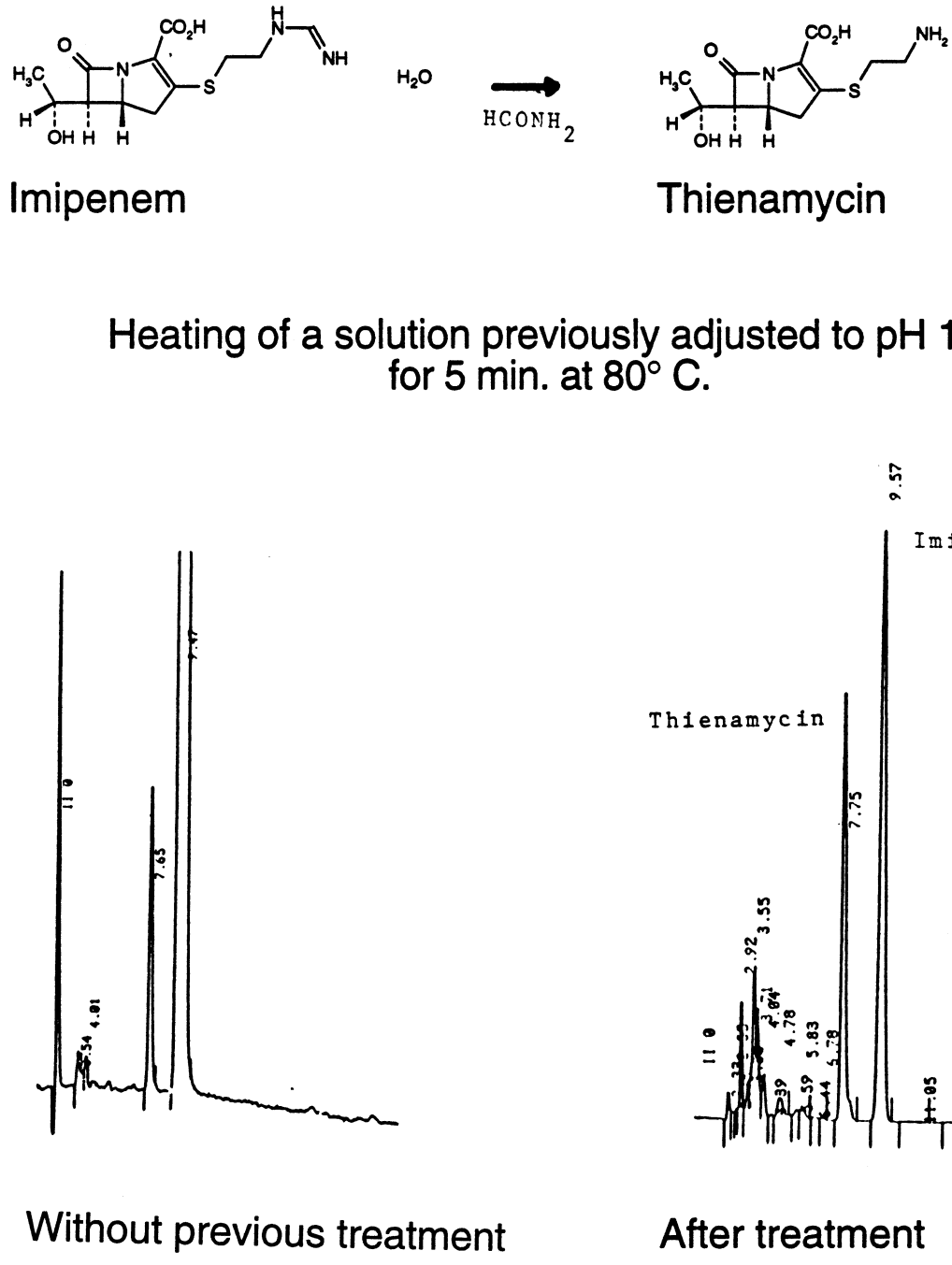


Fig. 10. Hydrolysis of imipenem.

Adjustment of the solution to pH 10 followed by heating to 80°C for 5 min led to a considerable formation of thienamycin without complete degradation of imipenem and thus allowing for the calculation of the resolution (Fig. 10). The method was validated in a collaborative trial and

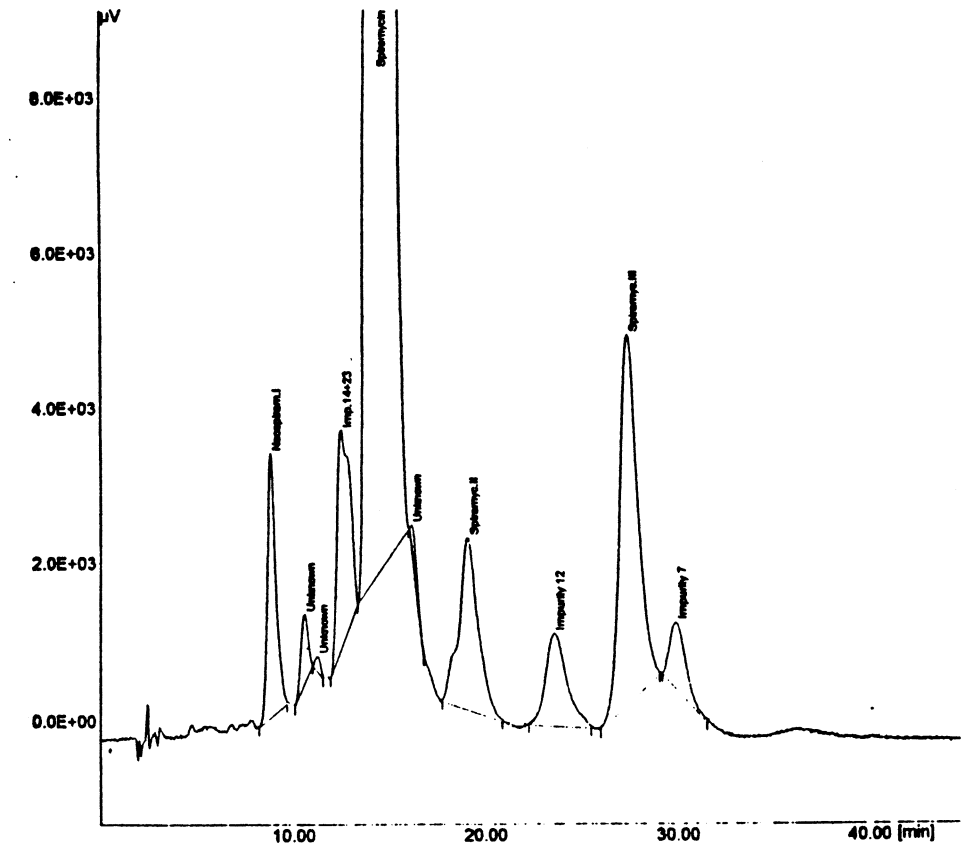


Fig. 11. Chromatogram of spiramycin spiked with impurities.

the monograph for imipenem was revised accordingly.

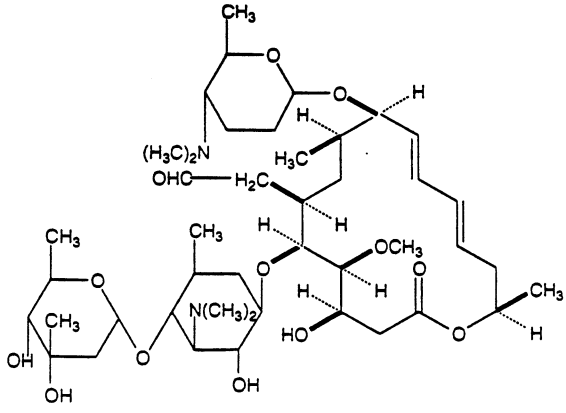
2.3.3. Monograph for spiramycin

The last example of this series describes some examinations which were carried out in order to revise the monograph for spiramycin which belongs to the group of makrolide antibiotics. The present monograph still describes a microbiological assay and it is the policy of the European Pharmacopoeia Commission to replace this type of assay by physico-chemical methods, e.g. HPLC, wherever possible.

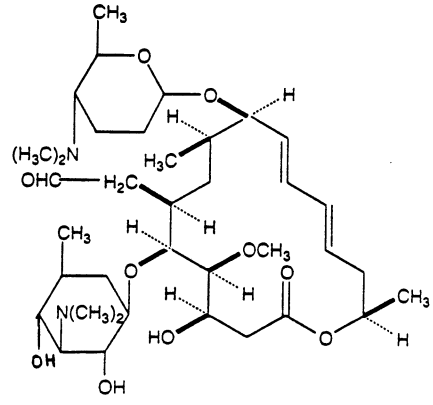
Spiramycin is not a homogeneous substance

but consists mainly of three compounds: spiramycin I, II and III and other related substances, spiramycin I representing the most important component.

Two HPLC-methods proposed were tested in the European Pharmacopoeia laboratory but none of them described a useful system suitability test.



Spiramycin I



Neospiramycin I

Fig. 12. Structural formulas of spiramycin 1 and neospiramycin I.

Fig. 11 shows the chromatogram of a solution containing spiramycin spiked with the impurities to be controlled by the method. The impurities themselves are not available in sufficient amounts to be established and used as reference substances. It was therefore again attempted to generate one of the impurities by in situ degradation. An impurity which is to be controlled by the monograph and which may be obtained by acid hydrolysis is neospiramycin I [12] (Fig. 12). This substance elutes close to spiramycin I and represents one of the impurities which shall be controlled in the purity analysis. Employing different reaction conditions the best results were obtained when a solution of 1 mg of spiramycin/ml in the mobile phase (pH 2.2) was heated for 30 min at 60°C. The chromatogram obtained (Fig. 13) shows neospiramycin I eluting at $Rt = 14.4$ min, the resolution found was 6.3.

3. Conclusion

The technique presented here, called 'in situ degradation' is a very useful method to carry out system suitability tests in HPLC. Two essential prerequisites for this test are: (i) the existence of a suitable trigger in the molecule, and (ii) an easy performable reaction which does not require specialised knowledge of synthetic chemistry by the user. Under these conditions this method is a useful approach for resolution tests for pharmacopoeial purposes avoiding impurities as reference substances.

Acknowledgements

Thanks are due to the technicians of the European Pharmacopoeia Laboratory for the practical work and E. Polland for the preparation of the manuscript.

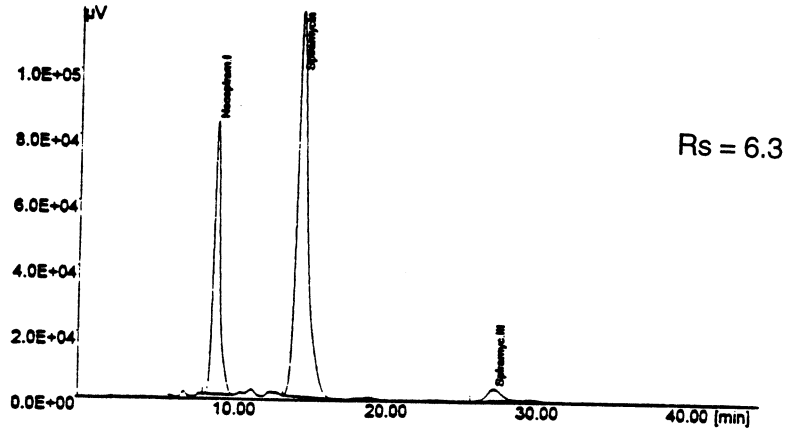


Fig. 13. Chromatogram of a partially degraded solution of spiramycin I.

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