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Study of the stability of tylosin A in aqueous solutions

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Abstract

The decomposition of the 16-membered ring macrolide antibiotic tylosin A in aqueous buffers has been investigated in the pH range 2–13, by means of a liquid chromatographic assay with ultraviolet detection at 280 nm. In acidic medium, tylosin A is converted into tylosin B, while in neutral and alkaline medium, tylosin A aldol is formed together with a number of polar decomposition products of unknown identity. The decomposition kinetics have been studied as a function of the type and concentration of the buffer, ionic strength, pH and temperature.

Keywords: Aqueous solutions; Liquid chromatography; Poly(styrene–divinylbenzene); Stability; Tylosin A

1. Introduction

Tylosin is a 16-membered ring macrolide antibiotic, produced by fermentation of Streptomyces strains [1]. It is produced as a mixture of several related substances, of which tylosin A (TA) is the main component. Desmycosin or tylosin B (TB), a precursor in the biosynthesis of TA, is also formed from TA by hydrolysis of the mycarose sugar in acidic aqueous solution [2]. Macrocin or tylosin C (TC), lactenocin, relomycin or tylosin D, demycinosyltylosin are other related substances that have been isolated from fermentation media [3–5]. In solutions for injection containing tylosin, an alkaline degradation product called tylosin aldol (TAD) was detected [6,7]. Two epimers of this product were recently isolated [8]. Structures for TA and its related substances are shown in Fig. 1.

Until now, detailed kinetic data for the decomposition of tylosin in aqueous solution have not been reported. Therefore, in this work the stability of tylosin A in aqueous buffer solutions in the pH range 2–13 was monitored, using a recently developed liquid chromatography (LC) method for tylosin A and its related substances [9]. Factors possibly affecting the degradation reaction (ionic strength, buffer and temperature) were investigated.

2. Experimental

2.1. Preparation of the solutions

A tylosin A house standard (TA-HS) was available, having a purity of 99.2% m/m on an anhydrous basis. TA-HS was dissolved in the different buffer solutions at a concentration of 1.0 mg ml^{-1} throughout the study. For most kinetic studies, potassium phosphate buffers were used in the pH range 2–13. The chemicals used for the preparation of these buffers were of analytical grade. Tripotassium hydrogen phosphate was purchased from Merck (Darmstadt, Germany), dipotassium hydrogen phosphate, potassium dihydrogen phosphate and phosphoric acid (85% aqueous solution) were from Janssen Chimica (Beerse, Belgium). In order to examine the influence of the buffer

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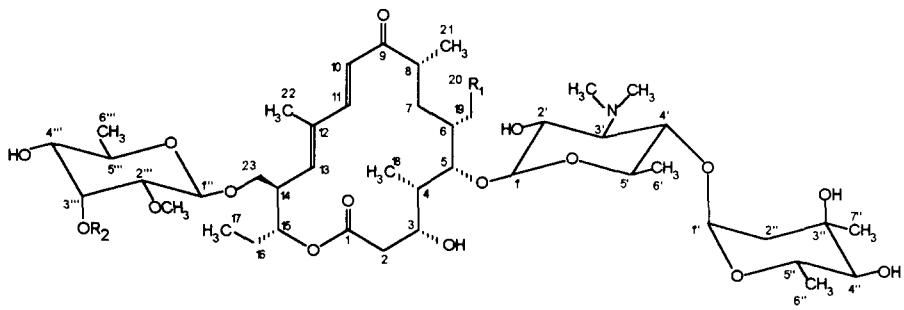
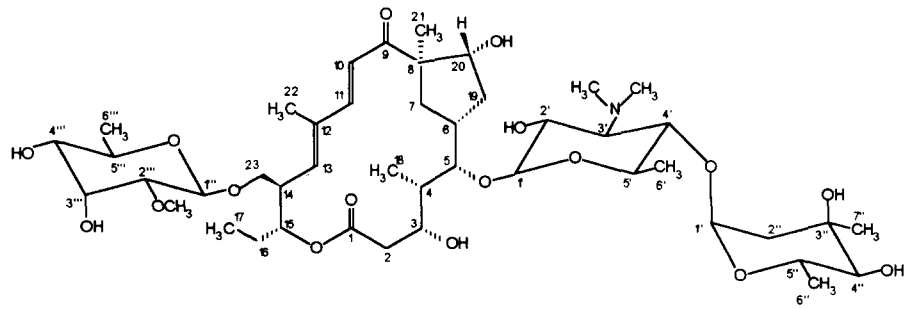


Mycinose Mycaminose Mycarose

	R1	R2	Mycarose	Mycinose
Tylosin A	CHO	CH_3	+	+
Tylosin B	CHO	CH_3	-	+
Tylosin C	CHO	H	+	+
Tylosin D	CH_2OH	CH_3	+	+
Lactenocin	CHO	H	-	+
OMT	CHO		-	-
DMT	CHO		+	-

+ = sugar present

- = sugar not present



Tylosin A aldol

Fig. 1. Structures of tylosin A and its related substances.

type, separate experiments were carried out at pH 4.0 using 0.05 M buffer solutions of sodium phosphate or sodium acetate, prepared by mixing suitable amounts of 0.05 M solutions of sodium acetate and acetic acid (Janssen Chimica), respectively. The ionic strength (μ) of the buffers was adjusted with potassium chloride (Janssen Chimica). Reference buffers for calibration in pH measurements were prepared according to the European Pharmacopoeia (V.6.3, 1980).

2.2. Storage of solutions

Solutions were stored in glass vials (1.5 ml), closed with aluminium crimp caps, and fitted with a rubber disc and a PTFE film. Vials were stored in incubators (60, 70 and 80 °C) and withdrawn at appropriate time intervals. Sample vials were stored briefly at −15 °C until they were analysed. During analysis, they were protected from light in order to prevent decomposition.

2.3. LC method

Degradation of TA was monitored using an LC method, recently developed for the analysis of TA and related substances [9], which enables the separation of TA from all its potential impurities and degradation products. The chromatographic system consisted of a Merck–Hitachi model L-6200 intelligent pump (Darmstadt, Germany) or a Waters M45 solvent delivery system (Milford, MA, USA), a Marathon autosampler (Spark Holland, The Netherlands) or a Merck–Hitachi 655A-40 autosampler, equipped with a 20 µl loop, a Merck–Hitachi L-4000 variable UV detector set at 280 nm and an HP 3393 A Integrator (Hewlett-Packard, Avondale, PA, USA). The 25.0 × 0.46 cm i.d. column was home-packed with 8 µm 1000 Å PLRP-S (Polymer Laboratories, Church Stretton, Shropshire, UK). The mobile phase was tetrahydrofuran–potassium phosphate buffer (pH 9.0; 0.2 M)–water (20:5:75, v/v/v). HPLC grade tetrahydrofuran was obtained from Rathburn (Walkerburn, UK). The mobile phase was degassed by ultrasonication. The flow rate was 1.0 ml min^{-1}. The column was maintained at 60 °C by immersion in a water bath, heated by means of a Julabo EM immersion heater (Seelbach, Germany).

Quantitation of TA was based on peak area maeasurements. The amount of TA remaining was calculated relative to the peak area obtained with solutions of TA-HS, dissolved in potassium phosphate buffer (pH 7.0; 0.05 M)(1.0 mg ml^{-1}). These TA-HS solutions were analysed alternately with the sample solutions. A typical chromatogram of TA-HS is shown in Fig. 2.

3. Results and discussion

3.1. Influence of pH

Figs. 3 and 4 give the concentration–pH profiles for the degradation of TA in acidic (pH 4.0–7.0) and in alkaline (pH 7.0–11.0) medium, respectively. The other experimental conditions, i.e. buffer type and concentration (0.05 M potassum phosphate), ionic strength (0.25) and temperature (60 °C) were constant. Pseudo-first order rate constants $k(h^{-1})$, calculated from the slope of the log concentration–time graphs, are reported in Table 1. A graph of log k versus pH is depicted in Fig. 5. Four linear parts (I–IV), having different slopes, can be distinguished in this profile: −1.189 for range I (pH 2–4), −0.299 for II (pH 4–7), 0.337 for III (pH 7–10) and 0.769 for IV (pH 10–12.8). The slopes differ from −1 (acidic medium) and from +1 (alkaline medium), which indicates that there is no specific acid or base catalysis in the decomposition reactions.

3.2. Degradation products

In acidic medium, TB is the major degradation product of TA. Fig. 6(A) shows a chromatogram of a solution of TA at pH 4.0 stored for 216 h at 60 °C. As the specific absorbances of TB and TA at 280 nm are similar, the mass balance can be calculated as a function of time. Fig. 7 shows that the mass balance is always close to 100% when the percentages of TA and TB are combined.

In neutral medium, TA is decomposed into different products. Apart from TB, TAD and polar degradation products are detected. Fig. 6(B) shows a chromatogram of a solution of TA at pH 7.0 stored for 216 h at 60 °C. The mass balance in the neutral pH range is not 100%, because the polar degradation products of unknown identity have a lower specific ab-

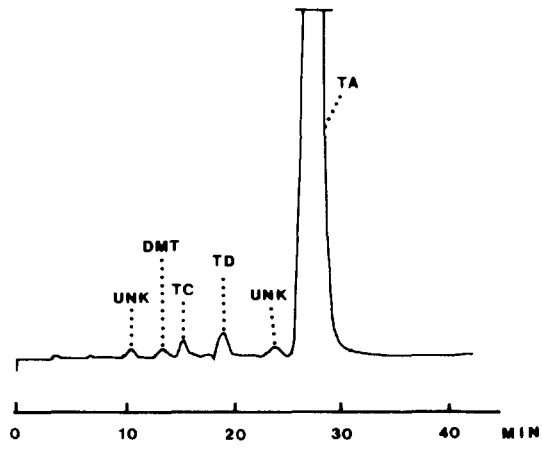


Fig. 2. Typical chromatogram of tylosin A-House Standard.

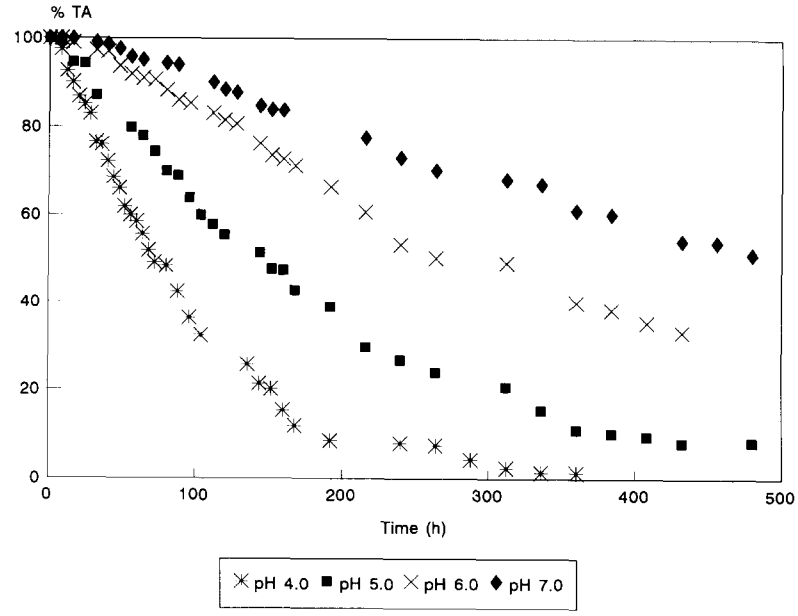


Fig. 3. Concentration–pH profile in acidic medium.

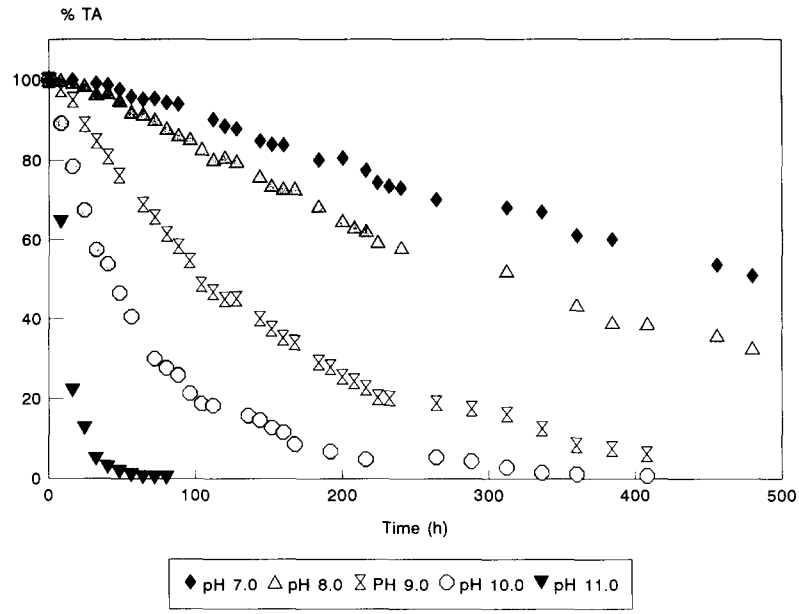


Fig. 4. Concentration–pH profile in alkaline medium.

sorbance at 280 nm than TA. The degradation of TA in alkaline medium gives a complex mixture of TAD and different polar products, as can be seen in Fig. 6(C). These polar compounds are probably hydrolysis products with an opened lactone ring.

3.3. *Influence of the buffer type and concentration*

The decomposition of TA in solutions with three different types of buffer was investigated at pH 4.0 and 70 °C. 0.05 M solutions of potassium phosphate, sodium phosphate and sodium acetate, adjusted to an ionic strength of 0.25, were used. Table 2 mentions the pseudo-first order rate constants $k(h^{-1})$ obtained with these three buffers. TA is most stable in sodium phosphate buffer. The type of anion (acetate, phosphate) has a much greater effect on the degradation rate than has the cation (K^+, Na^+). The effect of the concentration of the buffer was studied using potassium phosphate buffers (pH 4.0) at four concentration levels:

0.025, 0.05, 0.10 and 0.20 M. The temperature (70 °C) and the ionic strength (0.25) were kept constant. The results are presented in Table 3. The degradation rate increases with the buffer concentration. This means that there is general acid catalysis at pH 4.0, which can explain the deviation from slope −1 in the acidic pH range of the log k versus pH graph (see Fig. 5).

3.4. Influence of the ionic strength

Potassium chloride was added to potassium phosphate buffer (pH 4.0; 0.05 M) in order to obtain buffers with an ionic strength (μ) of 0.05, 0.15 and 0.25, respectively. The effect of μ on the degradation rate of TA was investigated at 70 °C. The pseudo-first order rate constants $k(h^{-1})$ were found to be 2.583×10^{-2}, 3.762×10^{-2} and 4.775×10^{-2}, respectively. Addition of salt to a buffered solution of TA negatively influences the stability.

Table 1
Observed rate constants (h^{-1}) for the degradation of TA as a function of pH, in 0.05 M potassium phosphate buffer with an ionic strength of 0.25 and at 60 °C

pH	$k(h^{-1})$
2.0	2.606 ± 0.271
3.0	$(3.818 \pm 0.077) \times 10^{-1}$
4.0	$(1.091 \pm 0.019) \times 10^{-2}$
5.0	$(5.857 \pm 0.118) \times 10^{-3}$
6.0	$(2.582 \pm 0.052) \times 10^{-3}$
7.0	$(1.444 \pm 0.024) \times 10^{-3}$
8.0	$(2.418 \pm 0.032) \times 10^{-3}$
9.0	$(6.504 \pm 0.128) \times 10^{-3}$
10.0	$(1.384 \pm 0.041) \times 10^{-2}$
11.0	$(9.364 \pm 0.408) \times 10^{-2}$
12.0	$(7.050 \pm 0.017) \times 10^{-1}$
12.8	1.756 ± 0.421

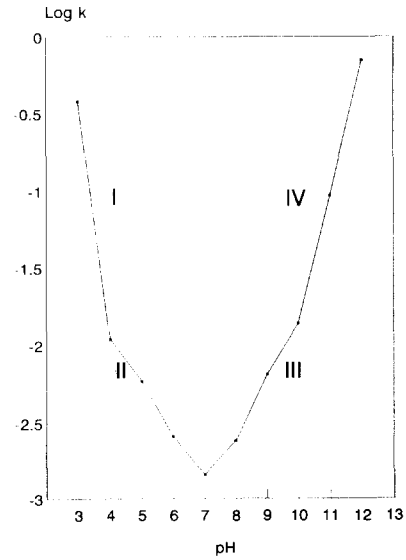


Fig. 5. Log k–pH profile for the degradation of tylosin A in 0.05 M potassium phosphate buffer with ionic strength 0.05 and at 60 °C.

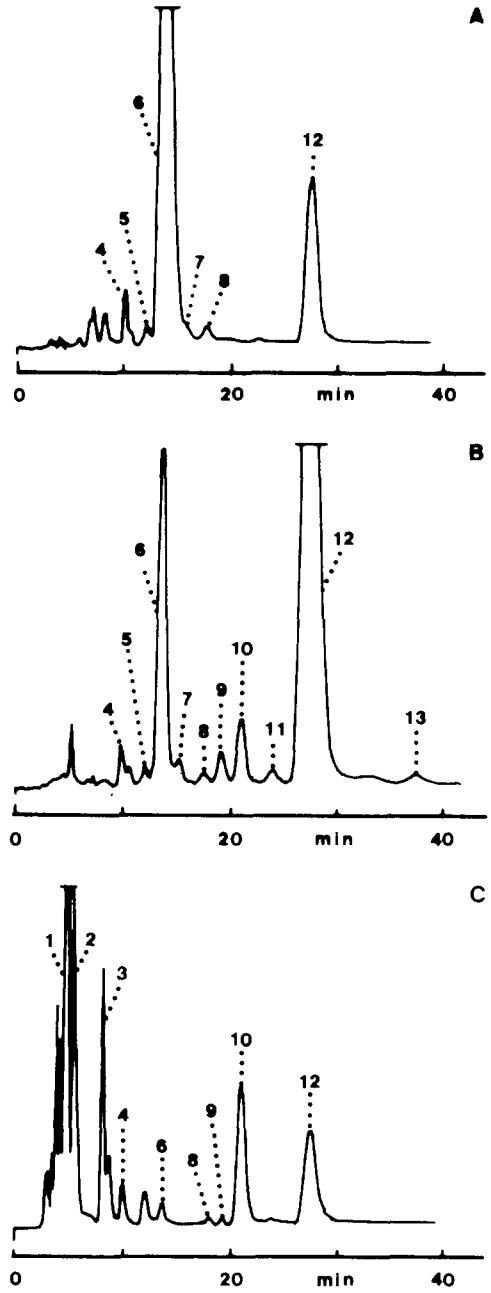


Fig. 6. Typical chromatograms obtained with solutions stored at 60 °C for 216 h at pH 4 (A), 7 (B) and 10 (C). 1–4 = unknown, 5 = DMT, 6 = TB, 7 = TC, 8 = unknown, 9 = TD, 10 = TAD, 11 = unknown, 12 = TA, 13 = isoTA.

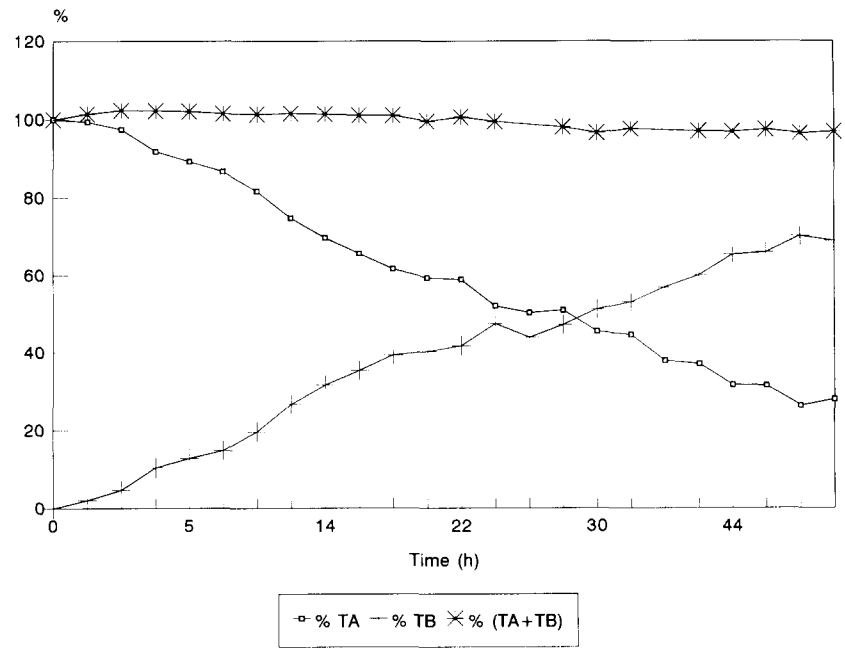


Fig. 7. Decomposition of tylosin A and formation of tylosin B at pH 4.0 and 70 °C.

Table 2
Observed rate constants $k(h^{-1})$ for the degradation of TA as a function of buffer type (pH 4.0; 0.05 M) with an ionic strength of 0.25 and at 70 °C

Buffer type	$k(h^{-1}) \times 10^2$
Potassium phosphate	4.775 ± 0.122
Sodium phosphate	2.367 ± 0.052
Sodium acetate	17.807 ± 0.128

Table 3
Influence of the concentration of potassium phosphate buffer pH 4.0 ($\mu = 0.25$) on the degradation rate of TA at 70 °C

Buffer concentration (M)	$k(h^{-1}) \times 10^2$
0.025	1.911 ± 0.122
0.05	4.775 ± 0.122
0.10	6.604 ± 0.126
0.20	10.951 ± 0.177

Table 4
Pseudo-first order rate constants (h^{-1}) and Arrhenius parameters for the decomposition of TA as a function of temperature, in 0.05 M potassium phosphate buffer at pH 4.0 and pH 9.0

Temperature (°C)	$k \times 10^2$ (h^{-1}) at pH 4.0	$k \times 10^2$ (h^{-1}) at pH 9.0
60	1.091 ± 0.019	0.650 ± 0.0128
70	2.379 ± 0.041	1.760 ± 0.0744
80	5.580 ± 0.120	4.437 ± 0.0937
Slope ($-E_{obs}/R$)	− 9585 ± 408	− 11233 ± 51.6
E_{obs}(kJ mol^{-1})	79.6 ± 3.4	93.3 ± 0.42

3.5. *Influence of the temperature*

The decomposition rate of TA in acidic and in alkaline solution was determined at 60, 70 and 80 °C, using 0.05 M potassium phosphate buffers with $\mu = 0.05$. Table 4 gives the pseudo-first order rate constants $k(h^{-1})$ observed in this temperature range, at pH 4.0 and pH 9.0, respectively. Plotting of the natural logarithm of the rate constant k versus $1/T$ gave a straight line. The apparent activation energies at pH 4.0 and pH 9.0 were calculated from the slope as shown in Table 4.

4. Conclusion

The previously developed LC method for analysis of tylosin is suitable for monitoring the stability of TA in solution in the pH range 2.0–13.0. In acidic medium, TB is the major degradation product. At neutral and alkaline pH, TAD is formed together with polar degradation products of unknown identity. The rate of decomposition of TA is largely dependent on pH, buffer type and concentration, as well as on the ionic strength. TA solutions are most stable at about pH 7. The effect of the temperature can be described by Arrhenius plots at both acidic and alkaline pH, and the apparent activation energies can be calculated.

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References

[1] J.M. McGuire, W.S. Boniece, C.E. Higgins, W.M. Stark, J. Westhead and R.N. Wolfe, Antibiot. Chemother., 11 (1961) 320–327.
[2] R.L. Hamill, M.E. Haney, M. Stamper and P.F. Wiley, Antibiot. Chemother., 11 (1961) 328–334.
[3] R.L. Hamill and W.M. Stark, J. Antibiot., 17 (1964) 133–139.
[4] H.A. Whaley, E.L. Patterson, A.C. Dornbush, E.J. Backus and N. Bohonos, Antimicrob. Agents Chemother., (1963) 45–48.
[5] H.A. Kirst, G.H. Wild, R.H. Baltz, E.T. Seno. R.L. Hamill, J.W. Paschal and D.E. Dorman, J. Antibiot., 36 (1983) 376–382.
[6] B.J. Fish and G.P.R. Carr, J. Chromatogr., 353 (1986) 39–50.
[7] J.H. Kennedy, US Patent 4,581,346, 1986.
[8] J. Paesen, W. Cypers, R. Busson, E. Roets and J. Hoogmartens, J. Chromatogr., 699 (1995) 99–106.
[9] J. Paesen, P. Claeys, W. Cypers, E. Roets and J. Hoogmartens, J. Chromatogr., 699 (1995) 93–97.