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Liquid chromatographic study of the stability of 5-halogeno-2′-deoxyuridines*

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Abstract: The stability of a series of 5-halogeno-2′-deoxyuridines was investigated using liquid chromatography as the analytical technique. Characteristics and profiles of the acidic, neutral and alkaline degradation are described, together with Arrhenius relationships and activation parameters for weakly acidic media.

Keywords: *5-Halogeno-2′-deoxyuridines; stability; reversed-phase liquid chromatography.*

Introduction

Among the 5-halogenated 2′-deoxyuridine congeners (see Fig. 1) several nucleoside analogues are endowed with antiviral and/or antitumoural properties. 5-Fluoro-2′-deoxyuridine (FdUrd) is a widely used antineoplastic agent and 5-iodo-2′-deoxyuridine (IdUrd) is the first antiviral agent approved for the treatment of herpes keratitis in humans [1]. The interest in 5-chlorinated-2′-deoxyuridine analogues increased during the last years because of the discovery of 5-chloro-3′-fluoro-2′,3′-dideoxyuridine as a very selective anti-HIV agent [2]. 5-Bromo-2′-deoxyuridine (BrdUrd) has been used clinically in the radiation treatment of various tumours, as well as in monitoring DNA replication [3] and in investigating electron transfer during radiolysis of nucleosides and nucleotides [4]. 5-Bromo-2′-deoxynucleosides with a pyrimidine base moiety are further commonly incorporated in oligodeoxynucleotides for crystallographic studies. The extensive use of 5-halogenated 2′-deoxyuridine derivatives for different purposes justifies a profound study of their stability in different circumstances. The present study was undertaken to compare the stability of these compounds and yield information on the influence of different electronegative substituents in position 5 of the heterocyclic base moiety of pyrimidine nucleosides. Previous stability studies on FdUrd [5], 5-chloro-2′-deoxyuridine (CldUrd) [5], BrdUrd [5–7] and IdUrd [5, 8, 9] were performed using spectrophotometry as the analytical tool. Liquid chromatography (LC) under low [10, 11] and high pressure conditions [12] has been applied in a kinetic study of IdUrd. The pH-ranges investigated were 0–0.57 for all four 5-halogenated 2′-deoxyuridines [5] and 4–6.5 for BrdUrd [6], while the latter was also subjected to conditions of H_0 (Hammett acidity function) ranging from −0.92 to −5.82 [7]. Neutral and alkaline studies on the analogous uridine derivatives were performed [13].

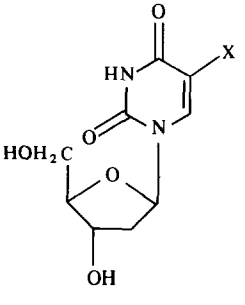

Figure 1
Structure of 5-halogeno-2′-deoxyuridines and 2′-deoxyuridine. dUrd: X = H; FdUrd: X = F; CldUrd: X = Cl; BrdUrd: X = Br; IdUrd: X = I.

Experimental

2′-Deoxyuridine (dUrd), FdUrd, BrdUrd and IdUrd were purchased from Janssen Chimica (Beerse, Belgium) and CldUrd from

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Aldrich Chemie (Brussel, Belgium). Reagents were of *pro analysi* quality (Merck, Darmstadt, Germany) and double-distilled water was used throughout.

Buffers for the calibration of pH measurements were prepared following the instructions of the European Pharmacopoeia [14]. pH was measured at room temperature with a Consort P 514 pH meter (Turnhout, Belgium) using a Schott pH electrode (Mainz, Germany). Liquid chromatography (LC) was performed with a Milton Roy pump (Laboratory Data Control, Riviera Beach, FL, USA), a Marathon autosampler (Spark Holland, Emmen, The Netherlands) equipped with a 20 μl loop, a Waters detector model 440 (Milford, MA, USA) set at 254 nm and an integrator model 3396 A (Hewlett–Packard, Avondale, PA, USA). A Waters model 990 photodiode-array detector (Milford, MA, USA) was used to record the on-line UV spectra.

The compounds investigated were dissolved in the buffer of appropriate pH at a concentration of 10^{-3} M and stored in a Memmert (Schwabach, Germany) oven at the temperatures indicated below. The ionic strength of all the buffers used was adjusted to 0.4 with KCl. Samples were quenched at appropriate intervals by both the addition of a neutralizing KOH or HCl solution and freezing. Each kinetic analysis was performed in duplicate, i.e. for each kinetic variable of pH and temperature two independent experiments were done. The samples were quantitatively analysed at room temperature by reversed-phase LC on a 250 × 4.6 mm column using the stationary and mobile phases mentioned in Table 1, and with a flow rate of 1.0 ml min^{-1}. Rate constants of degradation were calculated from the first order plots of disappearance of the starting material.

Results and Discussion

Hydrolysis of 5-halogeno-2'-deoxyuridines: analytical method

The stability of this series of compounds has in the past been investigated mainly by a spectrophotometric method which only yields results after subtraction of the interfering absorption of the degradation compounds [8]. Liquid chromatography on the contrary, allows for a selective quantitation of the compound under study, and was thus adopted here. Several mobile phases have been described for the analysis of 5-halogenated 2'-deoxyuridines on reversed-phase columns, most of them using simple mixtures of an organic modifier and water (FdUrd [15], BrdUrd [16], IdUrd [12, 17, 18]) or a buffer (FdUrd [19–22], BrdUrd [3], IdUrd [23]).

In this study, the pH of the mobile phase was kept constant by the addition of 5% v/v of phosphate buffer pH 5.0, and the use of reversed phases with different carbon loadings (see Table 1) made it possible to use isocratic elution with the same organic modifier (methanol) in different concentrations throughout the entire series of compounds. These analytical systems allowed to separate each nucleoside from its respective heterocyclic base moiety and from the dehalogenated uracil (U). (See Fig. 2 for representative chromatograms.) Only 5-fluorouracil (FU) could not be separated from U under these conditions. Other alternatives for this separation were the use of ion-pair chromatography on a PRP-1 column at pH 8, with cetrimide as ion pairing reagent [24] or on a C_{18} column at pH 2.1, with 0.0025 M sodium pentane- and heptanesulphonate as ion-pairing agents [25]. The latter system, applied on a Hypersil C_{18} 5 μm column, could be used to investigate whether U is formed from FU during degradation studies of FdUrd. Because of the longer retention time of FdUrd with this system (25.5 min vs 13.2 min) the conditions of Table 1 were preferred for use throughout the kinetic studies.

The elution characteristics of two other possible degradation compounds, namely dUrd and degraded 2-deoxyribose (DDR) had to be investigated. dUrd is clearly separated

Table 1
Stationary and mobile phases used for the analysis of samples from kinetic studies

Compound	Stationary phase	Mobile phase methanol–water–0.2 M phosphate buffer pH 5.0 (*x*:*y*:*z*, v/v/v)
FdUrd	Spherisorb ODS1 10 μm	(1.5:93.5:5)
CldUrd	Spherisorb ODS1 10 μm	(4:91:5)
BrdUrd	Spherisorb ODS1 10 μm	(4:91:5)
IdUrd	RSil C18 LL 10 μm	(5:90:5)

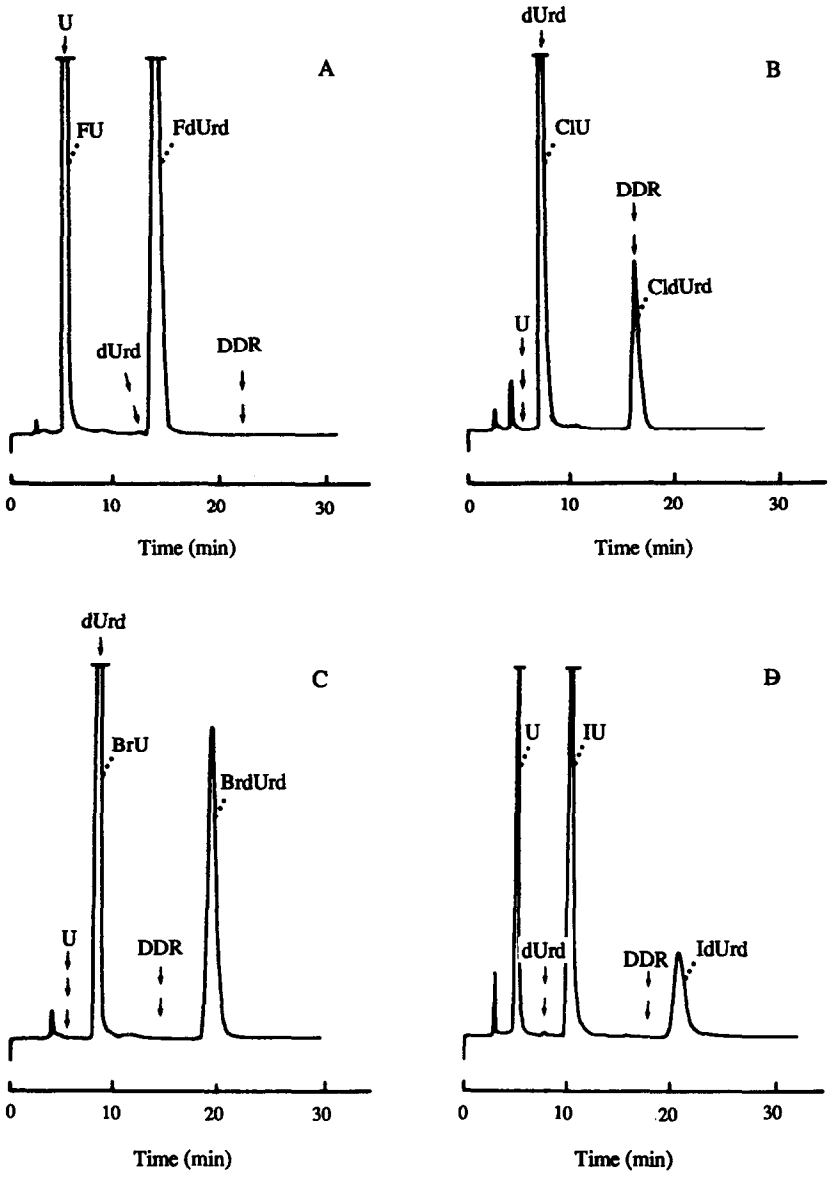

Figure 2
High-performance liquid chromatogram of a sample of FdUrd (A), CldUrd (B), BrdUrd (C) and IdUrd (D), degraded at pH 4.79 (T = 110°C) for 4 h. For chromatographic conditions see Table 1. U = uracil; FU = 5-fluorouracil; ClU = 5-chlorouracil; BrU = 5-bromouracil; IU = 5-iodouracil; dUrd = 2'-deoxyuridine; DDR = degraded 2-deoxyribose.

from all other compounds, except from 5-chlorouracil (ClU) and 5-bromouracil (BrU). The mass balance calculations discussed further, exclude however the formation of dUrd through dehalogenation of CldUrd or BrdUrd. Through acid degradation of the sugar 2-deoxyribose, a substance DDR is formed which has a strong chromophore at 261 nm that can be destroyed by treatment with mild alkali at room temperature, so that it does not interfere with spectrophotometric measurement of nucleosides [26]. LC of mixtures of the respective series of compounds spiked with DDR, showed that it could be separated from all other compounds except from CldUrd. Since DDR was not observed in samples from kinetic studies, it was deduced that its chromophore had disappeared due to the applied quenching conditions.

Hydrolysis of 5-halogeno-2'-deoxyuridines: features

The hydrolysis of the 5-halogeno-2'-deoxyuridines investigated here, was of pseudo-first

Table 2
Summary of compounds formed during degradation of 5-halogeno-2'-deoxyuridines (OHdUrd = 5-hydroxy-2'-deoxyuridine; OHU = 5-hydroxyuracil)

		Products formed during degradation of			
pH	*T* (°C)	FdUrd	CldUrd	BrdUrd	IdUrd
1.24	82	FU	ClU	BrU	IU + U + dUrd
2.85	82	FU	ClU	BrU	IU + U
4.79	82	FU	ClU	BrU	IU + U
	92	FU	ClU	BrU	IU + U
	101	FU	ClU	BrU	IU + U
	110	FU	ClU	BrU	IU + U
6.87	82	FU + dUrd	ClU	BrU + OHdUrd + OHU	IU + U + dUrd
8.86	82	FU	ClU	BrU + OHdUrd	IU + U + dUrd
11.97	82	FU + OHdUrd + dUrd	ClU + OHdUrd	BrU + OHdUrd	IU + U + dUrd

order over the entire pH-range examined (pH 1.24–11.97). The products formed during degradation under different conditions are summarized in Table 2.

FdUrd degraded into FU in the acid region, and no U was seen when using the ion-pair chromatographic system discussed above. The relative deviation obtained in mass balance calculations ranged from 7 to 10%. In neutral and alkaline media, the quantities of FU, an unknown and dUrd formed could not account for the disappearance of FdUrd, hence other products must be formed which do not absorb at 254 nm. It has been described that FU loses its ultraviolet absorbance at all pH values greater than 5.6 [27], where it is hydrolysed to urea, fluoride and an (unidentified) aldehyde [28]. The presence of fluoroacetaldehyde in FU (which is the clinically used precursor for FdUrd) must be avoided since it is thought to cause FU related cardiotoxicity [29]. Diode array analysis of the unknown peak showed a spectrum similar in shape to the spectrum of 5-hydroxyuracil but with a λ_{max} of 281 nm vs 278 nm for 5-hydroxyuracil. This value is closely similar to the value described for 5-hydroxy-2'-deoxyuridine (λ_{max} = 280 nm) [5] and this, together with the presence of 5-hydroxyuracil, suggests that the unknown might be 5-hydroxy-2'-deoxyuridine. This compound is extremely unstable in alkali [9, 13] and readily transforms into non-chromophoric products, either directly or through 5-hydroxyuracil.

CldUrd decomposed to ClU in the acid region, and mass balance calculations confirmed ClU to be the only degradation compound. In the neutral and alkaline region, the mass balance did not fit, so that, apart from small quantities of 5-hydroxy-2'-deoxyuridine at pH 11.97, products not-absorbing at 254 nm were resulting from degradation of CldUrd.

BrdUrd also decomposed to BrU in the acid region, with mass balance calculations giving a relative deviation of 3–6%. The neutral and alkaline decomposition of BrdUrd again revealed the formation of non-absorbing products mainly. Small quantities of BrU, 5-hydroxyuracil and 5-hydroxy-2'-deoxyuridine were present, as was shown by diode array analysis.

IdUrd degraded in acidic media to 5-iodouracil (IU) which was further dehalogenated to uracil. At pH 1.24 and 82°C, a small quantity of dUrd was also formed, as was confirmed by diode array analysis, probably through direct dehalogenation under the influence of light [11], which could not be entirely excluded during the experiment. This is, in our view, also the identity of the unknown discussed in the past [12]. The profiles of formation of IU and subsequent build-up of U were consistent with the spectrophotometrically determined mechanism of degradation [8]. Although IdUrd is only used in topical treatment, it must be pointed out that uracil acts as a carcinogen in rats and mice through formation of bladder calculi [30]. The degradation of IdUrd in neutral media yielded IU, U and dUrd, while these compounds were present in only minor quantities at pH 8.86 and 11.97, which again points to the formation of non-absorbing substances.

Hydrolysis of 5-halogeno-2'-deoxyuridines: pH–rate profile

The rate constants *k* obtained at pH 1.24, 2.85, 4.79, 6.87, 8.86 and 11.97 at 82°C are listed in Table 3. They represent a mean of two independent experiments unless stated other-

Table 3
Observed rate constants (h^{-1}) for the hydrolysis of 5-halogeno-2'-deoxyuridines at 82°C

pH	k_{obs} (h^{-1})	N	X	t	e
FdUrd					
1.24*	0.018 ± 0.00048	18	9	4	2
2.85†	0.013 ± 0.00017	18	9	3	2
4.79†	0.013 ± 0.00022	18	9	3	2
6.87†	0.0095 ± 0.00029	17	8	2	2
8.86†	0.0053 ± 0.00038	18	9	1	2
11.97†	0.026 ± 0.0011	18	9	6	2
CldUrd					
1.24*	0.028 ± 0.00099	17	8	6	2
2.85†	0.023 ± 0.00032	13	7	5	1
4.79†	0.026 ± 0.00043	16	8	6	2
6.87†	0.026 ± 0.00036	17	8	5	2
8.86†	0.020 ± 0.00074	14	7	1	2
11.97†	0.030 ± 0.00085	16	8	1	2
BrdUrd					
1.24*	0.019 ± 0.00035	17	9	4	2
2.85†	0.017 ± 0.00025	18	9	4	2
4.79†	0.020 ± 0.00074	18	9	5	2
6.87†	0.022 ± 0.00030	18	9	5	2
8.86†	0.021 ± 0.00071	18	9	1	2
11.97†	0.060 ± 0.0019	18	9	1	2
IdUrd					
1.24*	0.014 ± 0.00044	18	9	3	2
2.85†	0.011 ± 0.00014	18	9	3	2
4.79†	0.012 ± 0.00055	18	9	3	2
6.87†	0.018 ± 0.00037	16	8	4	2
8.86†	0.036 ± 0.0014	18	9	2	2
11.97†	0.080 ± 0.0018	18	9	1	2

N= total number of chromatographic observations; X = number of points on the time axis; t = number of half-lives during which tested; e = number of independent experiments.
*0.1 M glycine.HCl buffer; †0.1 M phosphate buffer.

wise. Since all buffers had the same ionic strength of 0.4 (although a primary salt effect can be expected in the basic region where these compounds are ionized (pK_a = 8.25 for IdUrd [8]), none was observed [13]) and since in the past no general acid–base catalysis was demonstrated [6, 8, 9, 13], all the data of Table 3 can be pooled for comparison. The non-unity slope in the lower pH region shows that catalysis is not of the specific acid type. On the other hand, at still lower pH values ranging from 0 to 0.57, the slope was found to be equal to −1 for FdUrd, CldUrd and BrdUrd which points to specific acid catalysis [5]. In the pH range 1–7 a mixed acid and solvent catalysed hydrolysis of the neutral compounds was observed.

The following descending order of acid stability exists: CldUrd > BrdUrd > FdUrd > IdUrd. This order does not follow the order of electronegativity of the halogen atoms (F > Cl > Br > I). This is due to the fact that the presence of an electron-attracting group results in a composite effect, namely a lowering of the concentration of protonated nucleoside and an enhancement of the rate of *N*-glycosidic bond cleavage [6].

The apparent absence of anomerization or isomerization is consistent with the previously established mechanism of acidic hydrolysis, namely a direct cleavage of the *N*-glycosidic bond without sugar ring opening [31].

The presence of a halogen atom in the base part decreases the acid stability of the *N*-glycosidic bond. Indeed, the following ratios were found when comparing the degradation rate constants of the 5-halogeno-2'-deoxyuridines with dUrd at pH 4.8 and 95°C: 9.5 for FdUrd vs dUrd, 4.7 for the pair CldUrd/dUrd, 10.8 for BrdUrd vs dUrd and 4.5 for IdUrd vs dUrd. The degradation rate constant at pH 4.8 and 95°C is 0.011 h^{-1} for dUrd [6]. Rate constants of 5-halogeno-2'-deoxyuridines under these conditions can be calculated from the Arrhenius equations determined in this

study and discussed further. The ratio found for BrdUrd is of the same range as the value of 17 described earlier for pH 4.0 [6]. At higher acidities (pH 0.04) the rate constant ratios of the different halogen derivatives compared to dUrd are 3 to 4 [5].

For each compound the highest rate of decomposition was observed at pH 11.97. The ionized form thus is considerably less stable than the acid form. In the basic pH region the solvolysis may be considered as catalysed by hydroxyl ions and solvent. The order of stability in this case does follow the order of electronegativity of the halogen atoms, with the most stable nucleoside carrying the most electronegative halogen atom. This is consistent with the previously described nucleophilic attack of hydroxyl ions on C_5 of uridine [32], followed by expulsion of the halogen. The order of stability is probably determined by the ability of the halogen to act as a leaving group.

The influence of deoxygenation of the sugar on the neutral and alkaline degradation is negligible since 5-iodouridine, 5-bromouridine and 5-chlorouridine degrade to approximately the same extent as their 2'-deoxygenated counterparts [13]. This is not surprising in view of the alkaline mechanism of degradation discussed above.

The rate constant ratio for IdUrd vs dUrd at a pH of 11.97 and at 101°C was 17. The rate constant for IdUrd was calculated on the basis of our alkaline data at 82°C using the published alkaline energy of activation [9], while in a separate study (Van Schepdael, personal communication), the rate constant was found to be 0.02 h^{-1} for the degradation of dUrd at pH 11.97 and at 101°C.

Hydrolysis of 5-halogeno-2'-deoxyuridines: temperature dependence

The rate constants obtained at pH 4.79 and at three supplementary temperatures (92, 101, 110°C) are grouped in Table 4. The following Arrhenius relationships exist:

$$\text{FdUrd: } \log k = 17 - 6619 \times 1/T \quad r = 0.9778;$$
$$\text{CldUrd: } \log k = 17 - 6731 \times 1/T \quad r = 0.9869;$$
$$\text{BrdUrd: } \log k = 18 - 6967 \times 1/T \quad r = 0.9936;$$
$$\text{IdUrd: } \log k = 19 - 7476 \times 1/T \quad r = 0.9926.$$

Table 5 contains the activation parameters energy (E_a), enthalpy ($\Delta H\ddagger$) and entropy ($\Delta S\ddagger$) calculated at pH 4.79 and at 82°C for the

Table 4
Observed rate constants (h^{-1}) for the hydrolysis of 5-halogeno-2'-deoxyuridines at pH 4.79 as a function of temperature

T (°C)	k_{obs} (h^{-1})	N	X	t	e
FdUrd					
82	0.013 ± 0.00022	18	9	3	2
92	0.028 ± 0.0010	17	8	2	2
101	0.069 ± 0.0056	12	6	1	2
110	0.32 ± 0.015	15	8	3	2
CldUrd					
82	0.026 ± 0.00043	16	8	6	2
92	0.056 ± 0.0020	18	9	4	2
101	0.17 ± 0.0029	14	7	2	2
110	0.63 ± 0.024	17	8	5	2
BrdUrd					
82	0.020 ± 0.00074	18	9	5	2
92	0.054 ± 0.0017	18	9	4	2
101	0.15 ± 0.0078	18	9	2	2
110	0.56 ± 0.027	18	9	5	2
IdUrd					
82	0.012 ± 0.00055	18	9	3	2
92	0.039 ± 0.0012	17	9	3	2
101	0.10 ± 0.0053	18	9	1	2
110	0.45 ± 0.028	15	8	3	2

N = total number of chromatographic observations; X = number of points on the time axis; t = number of half-lives during which tested; e = number of independent experiments.

Table 5
Activation parameters for the hydrolysis of 5-halogeno-2'-deoxyuridines at pH 4.79 and at 82°C

	E_a (kcal mol^{-1})	$\Delta H\ddagger$ (kcal mol^{-1})	$\Delta S\ddagger$ (e.u.)
FdUrd	30.3 ± 4.6	29.6 ± 4.5	−0.49 ± 12.6
CldUrd	30.8 ± 3.6	30.1 ± 3.5	2.34 ± 9.8
BrdUrd	31.9 ± 2.6	31.2 ± 2.5	4.86 ± 7.1
IdUrd	34.2 ± 3.0	33.5 ± 2.9	10.4 ± 8.2

different compounds. The positive or near-zero activation entropies point to a unimolecular mechanism of degradation. Positive entropy values were also found by other investigators at pH 0.04 [33], pH 6.5 [6] and $H_0 = -2.20$ [7].

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References

[1] E. De Clercq, *Meth. Find. Exptl. Clin. Pharmacology* **2**, 253–267 (1980).
[2] A. Van Aerschot, P. Herdewijn, J. Balzarini, R. Pauwels and E. De Clercq, *J. Med. Chem.* **32**, 1743–1749 (1989).
[3] D.A. Ganes and J.G. Wagner, *J. Chromatogr.* **432**, 233–242 (1988).
[4] M. Ye, *J. Liq. Chromatogr.* **14**, 3497–3511 (1991).
[5] E.R. Garrett, J.K. Seydel and A.J. Sharpen, *J. Org. Chem.* **31**, 2219–2227 (1966).
[6] R. Shapiro and S. Kang, *Biochemistry* **8**, 1806–1810 (1969).
[7] R. Shapiro and M. Danzig, *Biochemistry* **11**, 23–29 (1972).
[8] E.R. Garrett, T. Suzuki and D.J. Weber, *J. Am. Chem. Soc.* **86**, 4460–4468 (1964).
[9] E.R. Garrett, P.B. Chemburkar and T. Suzuki, *Chem. Pharm. Bull.* **15**, 1113–1130 (1965).
[10] L.J. Ravin, C.A. Simpson, A.F. Zappala and J.J. Gulesich, *J. Pharm. Sci.* **53**, 1064–1066 (1964).
[11] C.A. Simpson and A.F. Zappala, *J. Pharm. Sci.* **53**, 1201–1204 (1964).
[12] G. Szepesi, M. Gazdag and J. Mezei, *Pharmazie* **35**, 602–604 (1980).
[13] E.R. Garrett and G.J. Yakatan, *J. Pharm. Sci.* **57**, 1478–1487 (1968).
[14] *European Pharmacopoeia*, 2nd edn, V.6.3.1., Maisonneuve, Sainte-Ruffine, France (1980).
[15] J.L. Day, J. Maybaum and W. Sadée, *J. Chromatogr.* **206**, 407–409 (1981).
[16] A. Turturro, N.P. Singh and R.W. Hart, *J. Chromatogr.* **252**, 335–337 (1982).
[17] G.P.R. Carr, *J. Chromatogr.* **157**, 171–184 (1978).
[18] S.H. Hansen and M. Thomsen, *J. Chromatogr.* **209**, 77–83 (1981).
[19] F.P. LaCreta and W.M. Williams, *J. Chromatogr.* **414**, 197–201 (1987).
[20] J.A. Smith-Rogers, W.P. Tong, M.E. Duafala, M. Markman and J.R. Bertino, *J. Chromatogr.* **566**, 147–154 (1991).
[21] A.R. Buckpitt and M.R. Boyd, *Anal. Biochem.* **106**, 432–437 (1980).
[22] C.F. Gelijkens and A.P. De Leenheer, *J. Chromatogr.* **194**, 305–314 (1980).
[23] N. Belotto, V. Reiner, F. Verga, G. Canobbio, F. Lietti, P. Magnani and C. Lucarelli, *J. Chromatogr.* **572**, 327–332 (1992).
[24] G.J. Peters, I. Kraal, E. Laurensse, A. Leyva and H.M. Pinedo, *J. Chromatogr.* **307**, 464–468 (1984).
[25] L. Biondi and J.G. Nairn, *J. Liq. Chromatogr.* **8**, 1881–1892 (1985).
[26] J.K. Seydel and E.R. Garrett, *Anal. Chem.* **37**, 271–274 (1965).
[27] E.R. Garrett, H.J. Nestler and A. Somodi, *J. Org. Chem.* **33**, 3460–3468 (1968).
[28] B.C. Rudy and B.Z. Senkowski, in *Analytical Profiles of Drug Substances*, Vol. 2 (K. Florey, Ed.), pp. 221–244. Academic Press, New York (1973).
[29] L. Lemaire, M.C. Malet-Martino, S. Longo, R. Martino, M. De Forni and M. Carton, *Lancet* **337**, 560 (1991).
[30] S. Fukushima, H. Tanaka, E. Asakawa, M. Kagawa, A. Yamamoto and T. Shirai, *Cancer Res.* **52**, 1675–1680 (1992).
[31] J. Cadet and R. Téoule, *J. Am. Chem. Soc.* **96**, 6517–6519 (1974).
[32] H. Lönnberg, P. Suokas, R. Käppi and E. Darzynkiewicz, *Acta Chemica Scandinavica* **B40**, 798–805 (1986).
[33] B. Capon, *Chem. Reviews* **69**, 407–498 (1969).

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