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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF IDOXURIDINE AND RELATED SUBSTANCES ON CHEMICALLY BONDED OCTADECYLSILYL SILICA PACKINGS

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SUMMARY

The high-performance liquid chromatographic procedures for the assay of idoxuridine and the test for related substances described in the British Pharmacopoeia 1980 were investigated. It was found possible to optimize the composition of the eluent with respect to the elution time without loss of separation efficiency. Ten commercially available octadecylsilyl-bonded silica packings were investigated in order to find any suitable supports other than the one prescribed. The packings exhibited large differences in retention behaviour and selectivity. An impurity, which the test for related substances does not take into account, was found in some samples of idoxuridine and it has been isolated and identified as 5-bromo-2′-deoxyuridine.

INTRODUCTION

The British Pharmacopoeia 1980 (BP 80)[1] includes a monograph on idoxuridine, which describes the assay of the drug by high-performance liquid chromatography (HPLC). A test for related substances is also performed by HPLC, and the same procedures are mentioned in the monograph on idoxuridine eye-drops. The test for related substances is limited to the two degradation products 2′-deoxyuridine and 5-iodouracil, *cf.*, the main degradation routes of idoxuridine shown in Fig. 1.

The HPLC procedures for pharmacopoeial purposes involve the use of a prescribed eluent and support. Because some interfering peaks corresponding to unknowns were found in the chromatograms of some samples of this drug substance and in some preparations using a support of the prescribed nature, we have attempted

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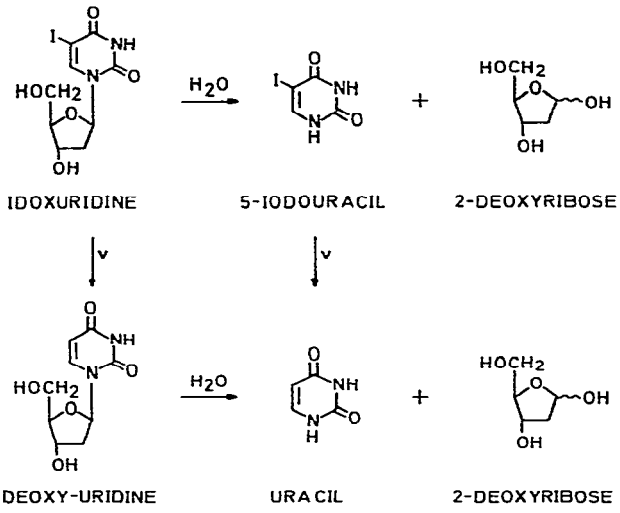

Fig. 1. The main degradation routes of idoxuridine.

to modify the official procedures in order to improve the selectivity and, if possible, the time of analysis. Several investigations over the last few years[2–7] have shown that reversed-phase packings of different origins have different selectivities and efficiencies, and that some packings are preferred for separation of certain classes of compounds. This behaviour is believed to be due primarily to differences in the relative proportion of hydrocarbon-bonded and free silanol groups, and to differences in the manufacturing processes leading to either monolayers or polymerized multilayers of the chemically bonded octadecylsilyl (ODS) phases.

For the present study ten different commercially available ODS silica packings and various eluent compositions were tested. By means of the calculated capacity factors, k', separation factors, α, and reduced plate heights, h, the possible gains in selectivity and elution time were evaluated. Investigations leading to the identification of one of the unknown peaks are also presented.

EXPERIMENTAL

Apparatus

The liquid chromatograph used consisted of an Altex Model 110 solvent metering pump, a Cecil 2012 spectrophotometer detector and a Rheodyne Model 7120 injection valve. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder, and retention times and peak areas were measured by means of a Hewlett-Packard Model 3353 A laboratory data system.

Freeze drying of collected fractions was carried out after freezing in liquid nitrogen in a Heraeus Model RVT 220 vacuum oven at ambient temperature and at a pressure less than 10 Pa using a Trivac Model S2A vacuum pump. Mass spectrometry (MS) of the freeze-dried fractions was performed on a Finnigan Model 3100D/9500 instrument using direct sample insertion and the following instrumental settings: ion source temperature 250°C; electron energy 70 eV; and multiplier potential 2.3 kV.

Chemicals

5-Bromo-2′-deoxyuridine was obtained from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade from E. Merck (Darmstadt, G.F.R.). The samples and preparations of idoxuridine were from different suppliers.

Chromatography

Stainless-steel columns (150 × 4.6 mm I.D.) were packed with the bulk ODS silica packings according to a previously described procedure[8]. ODS phases available only in pre-packed columns were used as obtained from the manufacturers. The efficiency of the columns, expressed as the reduced plate height, h, measured on naphthalene when eluted by 90% methanol in water, was determined for all the packings investigated. The columns were operated at room temperature, identically in every respect, apart from intentional modifications, to the procedure for related substances described in the BP 80 monograph on idoxuridine.

The separation on a semi-preparative scale was performed with a column (250 × 8 mm I.D.) packed with ODS-Hypersil and eluted with 5% acetonitrile in water at a flow-rate of 3 ml/min.

RESULTS AND DISCUSSION

The eluent

The BP 80 procedure for testing the content of related substances in idoxuridine using a μBondapak C_{18} column eluted with methanol–water (4:96 v/v) and sulphanilamide as internal standard was published by Carr[9]. The elution time is long, *ca.* 30 min, at the prescribed flow-rate of 1.7 ml/min. Furthermore, it is increased to 1–2 h if the procedure is carried out according to the BP 80 monograph on idoxuridine eye-drops which contain preservatives such as parabens.

In order to shorten the elution time, the amount and nature of the organic solvent in the eluent were changed. Table I shows the k' values for idoxuridine, its degradation products and sulphanilamide chromatographed on a μBondapak C_{18} column with different eluents. The order of elution of sulphanilamide and 2′-deoxyuridine is reversed when the methanol (MeOH) in the eluent is replaced by acetonitrile (MeCN) or tetrahydrofuran (THF). In the last systems the nucleosides are eluted faster, whereas the retention of sulphanilamide is affected only to a minor extent. The selectivity of the systems with 5% MeCN and 2% THF is equivalent to

TABLE I

k' VALUES FOR IDOXURIDINE, RELATED SUBSTANCES AND SULPHANILAMIDE OBTAINED ON A μBONDAPAK C_{18} COLUMN ELUTED WITH WATER CONTAINING DIFFERENT ORGANIC SOLVENTS

Substance	*Methanol*		*Acetonitrile*			*Tetrahydrofuran*			
	4%	*5%*	*3%*	*4%*	*5%*	*2%*	*3%*	*4%*	*5%*
(1) Sulphanilamide	1.3	1.2	1.0	0.9	0.8	1.1	0.8	0.7	0.6
(2) 2′-Deoxyuridine	2.1	1.8	1.0	0.7	0.5	0.6	0.4	0.2	0.1
(3) 5-Iodouracil	3.3	3.0	2.0	1.7	1.5	1.6	1.3	1.1	0.9
(4) Idoxuridine	11.7	10.1	6.3	4.9	3.5	3.5	2.4	1.8	1.4

TABLE II

k', α AND h VALUES FOR IDOXURIDINE, RELATED SUBSTANCES AND SULPHANILAMIDE OBTAINED ON VARIOUS ODS SILICA COLUMNS ELUTED WITH DIFFERENT ELUENTS

Substances numbered as in Table I. V_m is the hold up volume of the column.

Column	*Sub-stance*	*4% Methanol*			*5% Acetonitrile*			*2% Tetrahydrofuran*		
		k'	α	h	k'	α	h	k'	α	h
10-μm μBondapak C_{18}	1	0.9	1.8		0.7	1.7		0.7	1.7	
(300 × 4 mm),	2	1.6			0.4			0.4		
prepacked,	3	2.7			1.3			1.3		
V_m = 3.7 ml	4	9.7		20	3.2		28	2.9		28
10-μm Bio-Sil ODS	1	1.5	1.7		1.0	1.5		0.9	1.6	
(250 × 4 mm),	2	2.5			0.7			0.6		
prepacked,	3	4.1			1.8			1.7		
V_m = 2.6 ml	4	14.1		10	4.0		15	3.5		15
5-μm LiChrosorb	1	1.2	1.8		0.9	2.0		0.7	4.0	
RP-18	2	2.1			0.4			0.2		
(150 × 4.6 mm),	3	3.8			1.6			1.4		
V_m = 1.9 ml	4	14.2		20	3.5		33	2.5		111
5-μm Nucleosil C_{18}	1	2.0	1.5		1.3	1.6		1.2	1.6	
(150 × 4.6 mm),	2	2.9			0.8			0.8		
V_m = 2.2 ml	3	4.1			2.0			2.0		
	4	15.9		9	4.8		33	4.3		23
5-μm ODS-Hypersil	1	1.4	1.9		1.1	1.8		0.9	1.7	
(150 × 4.6 mm),	2	2.7			0.6			0.6		
V_m = 1.9 ml	3	4.4			1.9			1.7		
	4	17.3		6	4.3		10	3.4		12
10-μm Partisil ODS	1	1.0	1.5		0.7	1.1		0.8	1.3	
(250 × 4.6 mm),	2	1.5			0.6			0.6		
V_m = 3.4 ml	3	2.3			1.2			1.3		
	4	5.4		8	2.2		19	2.3		22
10-μm Partisil ODS-2	1	2.4	1.4		1.2	2.3		0.9	6.7	
(250 × 4.6 mm),	2	3.4			0.5			0.1		
V_m = 3.4 ml	3	6.5			2.2			1.6		
	4	22.4		62	4.6		130	3.0		115
10-μm Radial Pak C_{18}	1	2.4	1.1		1.5	2.9		1.2	3.0	
(100 × 8 mm),	2	2.6			0.5			0.4		
prepacked,	3	3.1								
V_m = 3.1 ml	4	13.1		8	2.6		130	2.8		16
5-μm Spherisorb S ODS	1	1.8	1.4		1.2	1.7		1.1	1.4	
(150 × 4.6 mm),	2	2.5			0.7			0.8		
V_m = 1.9 ml	3	3.9			1.9			1.8		
	4	12.4		11	4.0		22	3.6		18
6-μm Zorbax ODS	1	1.7	1.7		1.0	2.2		0.8	3.8	
(250 × 4.6 mm),	2	2.9			0.4			0.2		
prepacked,	3	5.3			1.7			1.5		
V_m = 3.0 ml	4	21.3		20	4.0		75	3.0		75

that of the system with 4% MeOH, but the total elution time is reduced to about one third.

The effect of the pH of the mobile phase was investigated by substituting 4% MeOH in water by 4% MeOH in 0.01 *M* potassium phosphate buffers with pH varying from 3.0 to 7.0. No influence of pH on the retention of any of the substances tested was seen in this range.

Octadecylsilyl silica packing

The suitability of a number of reversed-phase ODS silica packings for the separation in question was tested. In Table II are given the k' values for idoxuridine, its degradation products and sulphanilamide obtained with the prescribed eluent as well as with the above two solvents based on MeCN and THF. The differences in selectivity and efficiency are illustrated by the separation factor, α, for sulphanilamide and 2′-deoxyuridine and by the reduced plate height, h, measured with idoxuridine.

The results demonstrate the very different characteristics of ODS silica packings of different origin. Under the present conditions the compounds chromatographed show little retention on Partisil-10 ODS, which is only partly covered with ODS groups, compared to that on the other packings tested. The greatest retention is obtained on Partisil-10 ODS-2 and Zorbax ODS, both of which are declared to be of the monolayer type and with a high coverage of ODS groups. They give good resolution between sulphanilamide and 2′-deoxyuridine but the peaks are misshapen and display severe tailing. These two columns, however, have, as do all the others, a high efficiency equivalent to a reduced plate height, h, of 4–8, when operated in the reversed-phase manner as described under Experimental.

From Table II other chromatographic systems may be selected which enable a reduction of the elution time to about one third of the original. If the order of elution and thus the eluent is to be unchanged, Partisil-10 ODS is the preferred packing. Use of 5% MeCN in water as eluent on an ODS-Hypersil column affords an effective alternative.

Unknown impurities

During this study some samples of idoxuridine were found to contain unknown impurities. The one present in largest amounts was collected from a semi-preparative scale column and the fractions were freeze-dried. An electron impact mass spectrum of the impurity was recorded and is shown in Fig. 2 together with the mass spectrum of idoxuridine. The fragments with m/e = 306/308, 190/192 and 147/149 indicate a bromine compound. The fragment with m/e = 117 (deoxyribose) and other similarities to the fragmentation of idoxuridine strongly indicate the unknown impurity to be 5-bromo-2′-deoxyuridine. This was later confirmed also chromatographically by comparison with an authentic sample of the suspected compound.

Chromatograms of impure samples of idoxuridine using one of the above alternative systems are shown in Fig. 3. Other impurities than the one identified were present, especially in preparations, but in amounts too small for isolation and identification by this technique.

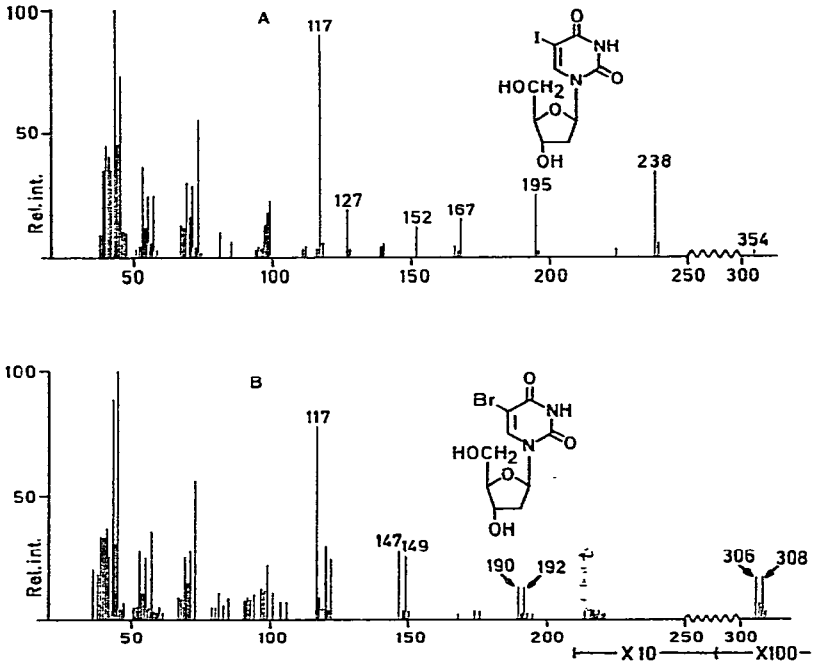

Fig. 2. Mass spectra (electron impact) of idoxuridine (A) and unknown impurity (found to be 5-bromo-2′-deoxyuridine) (B).

Sample preparation

The dissolution in water of even small amounts of idoxuridine takes a long time (12–24 h). The rate of dissolution cannot be increased by heating as the idoxuridine becomes partially hydrolyzed (Fig. 1). However, if the sample is dissolved in a

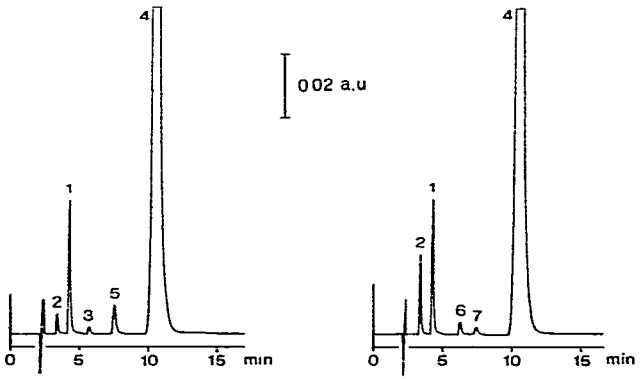

Fig. 3. Chromatograms of two commercial samples containing unknown impurities obtained on an 5-μm ODS-Hypersil column (150 × 4.6 mm) eluted with 5% acetonitrile in water, flow-rate 1 ml/min, with detection at 254 nm. Peaks: 1 = sulphanilamide; 2 = 2′-deoxyuridine; 3 = 5-iodouracil; 4 = idoxuridine; 5 = 5-bromo-2′-deoxyuridine (0.6%); 6 and 7 = unknowns.

small volume of dimethyl sulphoxide and the solution then diluted with water, sample preparation may be performed within a few minutes, thus minimizing the risk of biased results and reducing the overall time of analysis.

CONCLUSION

In official monographs, HPLC procedures may be specified in various degrees of detail. If the prescriptions involve a fixed composition of the eluent and a non-specific reference to a support (*e.g.*, ODS silica) difficulties may be expected due to the known differences in selectivity of packings of different origin. Consequently the specific brand of support which was used in the development of the procedure should be stated in the monograph. Better still, all brands which have been found suitable in a comparative study, such as the present, could be mentioned as validated possibilities.

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