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Short Communication

High-performance liquid chromatographic determination of mexiletine in film-coated tablets using a new polymeric stationary phase

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ABSTRACT

The optimum column liquid chromatographic separation of mexiletine and one of its possible impurities is only achievable using alkaline mobile phases. Conventional silica-based reversed phases, however, are subject to considerable stability problems under these alkaline conditions, disqualifying most HPLC columns from routine use. An Asahipak ODP-50 column containing a new polymeric phase with a separation efficiency similar to that of silica-based reversed phases was used to develop an HPLC method for mexiletine that is unaffected by stability problems. The stability of the stationary phase was verified in long-term teats and the suitability of the method for assaying mexiletine in film-coated tablets was demonstrated by determining the selectivity, linearity, accuracy and precision.

INTRODUCTION

Mexiletine [1-(1,6-dimethylphenoxy)-2-aminopropane] is an antiarrhythmic drug used in the treatment of acute and chronic ventricular arrhythmias [1–3]. A variety of gas and column liquid chromatographic techniques for assaying mexiletine, especially in plasma, have been described [4–8]. HPLC methods employ exclusively silica-based reversed stationary phases and mobile phases with added modifiers in order to improve the peak symmetry of the basic substance mexiletine (pK_a = 9.0). The problems of analysing basic substances by HPLC on silica-based reversed-phase columns are well documented [9–12]. Low efficiency, tailing peaks, irreversible adsorption and stability problems with the stationary phase are typical symptoms that can be mitigated by mobile phase buffering and/or by the addition of modifiers such as EDTA or triethylamine or by the addition of ion-pair forming agents, e.g., heptanesulphonic acid.

The development and optimization of such HPLC methods are generally difficult and require close monitoring of several parameters such as the influence of additive concentrations on the mobile phase. Furthermore, optimum separation by hydrophobic interactions of the neutral solutes with the stationary phase is only achieved when the **pH** of the mobile phase is at least 2 units above the pK_a of the basic substance to be analysed. In practice, this means that for basic drug substances with pK_a values around 9, an eluent **pH** above 11 should be used. This generally gives rise to severe stability problems

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with silica-based stationary phases, as the alkaline mobile phase attacks the silica matrix and gradually destroys the chemical structure of the silica gel.

The recently introduced polymeric phases, in which the polymer is adsorbed on silica gel, aluminium oxide or an organic support material, are much more stable in the alkaline range [13]. However, these phases with polymer coating almost always have the disadvantage of lower efficiency.

In this paper, the suitability of a new polymeric, reversed-phase column developed specially for the chromatography of basic substances is described with specific reference to the assay of mexiletine in film-coated tablets. Asahipak ODP-50 is based on a microparticulate mesoporous poly(vinyl alcohol) which has stearic acid bonded as an ester to the free alcohol groups of the matrix. The polymer is cross-linked with a molecule containing three vinyl groups. The suitability of this material for the determination of mexiletine using a mobile phase adjusted to pH 11 is demonstrated with reference to the test parameters selectivity, linearity, accuracy and precision. The stability of the phase was verified by a long-term test in which a test solution was injected continuously over several weeks and the resolution between mexiletine and the possible impurity 3,9-dimethyl-2,3-dihydrobenzo[*f*][1,4]-oxazepine (I) was evaluated in accordance with the system suitability test of the US Pharmacopeia (USP) .

EXPERIMENTAL

Chemicals

Mexiletine and I were reference substances from Boehringer Ingelheim (Ingelheim, Germany). Acetonitrile and diethylamine were HPLC-grade reagents from Merck (Darmstadt, Germany). The stationary phase for the Asahipak ODP-50HPLC column is manufactured by Asahi Chemical Industry (Kawasaki, Japan) and the columns are filled and marketed by Hewlett-Packard (Waldbronn, Germany).

Equipment

The analyses were carried out using two different chromatographic systems, a Hewlett-Packard Model 1090 liquid chromatograph and a chromatograph consisting of the following components: a Merck-Hitachi L 6000 pump, a Gilson Model 231 autosampler fitted with a Rheodyne Model 7125 injection valve and 20-μl loop and a Merck-Hitachi L 4000 variable-wavelength UV spectrophotometric detector equipped with an 8-μl cell.

The test samples were analysed on an Asahipak ODP-50 (5 μm) column (125 mm x 4.0 mm I.D.). Peak integration and data evaluation were performed on a Hewlett-Packard Model 3357 laboratory data system.

Method

The mobile phase was a 280:720 (v/v) mixture of acetonitrile and purified water adjusted to pH 11.0 with diethylamine with the aid of a pH meter. The flow-rate was 0.9 ml/min and the uncorrected retention times were 8.6 min for mexiletine and 11.8 min for I. Detection was performed by UV spectrophotometry at a wavelength of 264 nm.

System suitability test

A solution was prepared containing 80 mg of mexiletine and 8 mg of I in 100 ml of mobile phase. The injection volume was 20 μl. The resolution factor had to be greater than 2.5. The tailing factor of mexiletine and I was not to be greater than *ca.* 2.5 at 5% of the peak height.

RESULTS AND DISCUSSION

The Asahipak ODP-50 column material was characterized in terms of the most important test criteria. Chromatograms are presented to demonstrate the selectivity of the method, and results for linearity, accuracy and precision are reported. The stability of the column is demonstrated on the basis of a long-term test.

Selectivity of the method

A stability-indicating assay method must be specific, *i.e.*, substance-related impurities, potential decomposition products and interfering excipients must be separated from the active ingredient.

Fig. 1 shows a chromatogram of a sample of mexiletine film-coated tablets with a dosage of

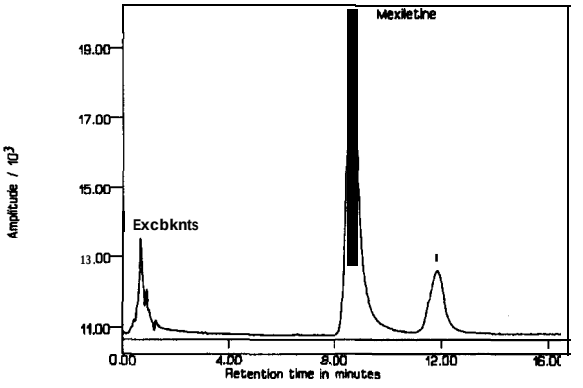


Fig. 1. Chromatogram of mexiletine with tablet matrix spiked with 1% of I.

150 mg; 1% of the possible impurity I was added to the test solution (for the structures of mexiletine and I, see Fig. 2). It is evident from the chromatogram that neither the mexiletine nor the I determination is affected by tablet excipients. Most tablet excipients are highly polar and elute with the solvent peak.

Linearity and accuracy

The linearity was determined in the mexiletine concentration range 0.30-0.70 **mg/ml.** The influence of the excipient matrix was also investigated over the entire range by adding a constant amount of excipient to the calibration solutions. The linearity of the two graphs was characterized by the following equations:

calibration without placebo:

$$y = -13.131.1 + 63.057.3x$$

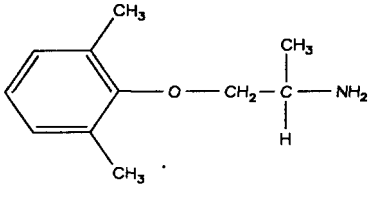
calibration with placebo:

$$y = -24.083.3 + 63.873.3x$$

where y is the absorbance and x is the mexiletine concentration **(mg/ml).** The two calibration graphs were compared statistically by means of analysis of variance **[14]** at a significance level of $p = 95\%$ using a statistics problem from SAS (Cary, NC, USA). The calculation showed that neither the slopes nor the ordinate intercepts of the regression lines diier significantly.

The accuracy of the mexiletine assay was calculated with the same data in the more restricted working range of **80–120%,** using five working concentrations. The test solutions were measured against a reference solution of mexiletine adjusted to 100%. The deviation of the measured values from the required standard is stated as the error (Table I). The deviation at the usual working concentration 0.4975 **mg/ml** (100%) is -0.527%.

Mexiletine



3,9-Dimethyl-2,3-dihydro-benzo[f][1,4]oxazepine (I)

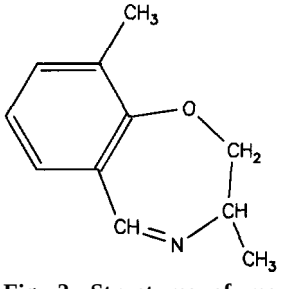


Fig. 2. Structures of mexiletine and 3,9-dimethyl-2,3-dihydrobenzo[*f*][1,4]oxazepine (I).

Precision

The precision of the assay of mexiletine **film**-coated tablets were assessed by analysing the results obtained by two technicians each carrying out three measurements on each of five preparations of the sample. Different HPLC systems and two Asahipak ODP-50 columns were used. The

TABLE I

ACCURACY OF MEXILETINE DETERMINATION IN THE CONCENTRATION RANGE 80–120% IN 150-mg FILM-COATED TABLETS

Theoretical value (mg/ml)	Measured value (mg/ml) (mean, n = 6)	Deviation (%)
0.398	0.397	-0.25
0.448	0.447	-0.22
0.498	0.495	-0.50
0.547	0.546	-0.18
0.597	0.604	+1.17

TABLE II

PRECISION OF MEXILETINE DETERMINATION IN 150-mg FILM-COATED TABLETS

Number of technicians, n = 2; number of sample preparations, m = 5; number of multiple measurements, l = 3.

Technician No.	Preparation No.	Measurement (mg)			Mean value (mg)
		1	2	3	
1	1	150.6	151.3	149.9	150.6
	2	149.9	148.3	150.3	149.5
	3	149.0	149.4	150.0	149.5
	4	149.0	149.1	150.6	149.6
	5	148.2	149.3	148.7	148.7
2	1	143.3	143.0	144.1	143.5
	2	144.8	144.2	145.4	144.8
	3	148.0	147.8	148.5	148.1
	4	142.3	142.9	142.3	142.5
	5	145.4	145.4	145.9	145.6
				Total:	147.2

S.D. of ruggedness: 3.6437, R.S.D. 2.47%.
S.D. of method reproducibility: 1.6737, R.S.D. = 1.14%.
S.D. of system reproducibility: 0.6314, R.S.D. = 0.43%.

stationary phases of the columns originated from different batches, and therefore the result for precision also includes the reproducibility of the stationary phase. Table II presents the data for precision with the appropriate evaluation. The data were statistically evaluated by means of hierarchical analysis of variance [14].

The system reproducibility is a measure of the reproducibility of the injection and chromatographic separation, and the method reproducibility additionally includes the sample preparation and ruggedness of the various components of the apparatus and human error of the individual technicians.

Stability of chromatography

The Asahipak ODP-50 column was subjected to a long-term test in which solutions for the system suitability test and test solutions of mexiletine film-coated tablets were injected alternately over several weeks. The chromatographic system was characterised by means of the system suitability test of the USP XXII by determining the resolution factor between mexiletine and I and the tailing factor of both substances at 5% of the peak height. Table III gives the minimum and maximum values and the relative standard deviation (R.S.D.) of the system suitability test for both substances.

To determine the influence of the matrix on the reproducibility of the method, the test solution spiked with 10% of I was also injected 120 times. The integrator units and retention times of mexiletine and I were determined and the R.S.D. was calculated (Table IV).

After about 600 injections the peaks of both substances showed a slight shoulder. This shoulder developed because of shrinkage of the packed column bed in the column which produced a dead volume, and which was rectified by replenishing the stationary phase in the column. A subsequent check on the chromatographic separation revealed that both the tailing factor and resolution data were in conformity with the initial values.

TABLE III

SYSTEM SUITABILITY TEST OF USP XXII

No. of injections: n = 120.

Substance	Tailing factor	Resolution	Retention time (min)
Mexiletine	F_{min} = 2.0 F_{max} = 2.3 R.S.D. = 3.8%	R_{min} = 3.7 R_{max} = 5.0 R.S.D. = 10.2%	t_{min} = 8.7 t_{max} = 8.8 R.S.D. = 0.3%
I	F_{min} = 1.4 F_{max} = 1.5 R.S.D. = 3.1%		t_{min} = 11.6 t_{max} = 12.2 R.S.D. = 0.4%

TABLE IV

INFLUENCE OF THE MATRIX ON THE REPRODUCIBILITY OF THE METHOD

No. of injections, n = 120.

substance	Integrator units	Retention time (min)
Test solution (mexiletine)	A_{min} = 639456 A_{max} = 716231 R.S.D. = 1.1%	t_{min} = 8.6 t_{max} = 8.8 R.S.D. = 1.1%
I	A_{min} = 891599 A_{max} = 921527 R.S.D. = 0.5%	t_{min} = 11.7 t_{max} = 12.3 R.S.D. = 1.2%

CONCLUSIONS

The results of long-term testing demonstrate that the Asahipak ODP-50 columns are free from stability problems even when using mobile phases with a pH above 10. Asahipak ODP-50 is therefore superior to conventional silica-based reversed-phase columns when using alkaline mobile phases. Because of its good separation efficiency, Asahipak ODP-50 is suitable for further applications, particularly the determination of substances with basic groups, as the selectivity range in the alkaline environment can be considerably extended for such substances.

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