Institute of Pharmaceutical Chemistry, University of Vienna, Austria

HPTLC-based stability assay for the determination of amiodarone in intravenous admixtures

H. KOPELENT-FRANK and A. SCHIMPER

Dedicated to Professor Gottfried Heinisch (University of Innsbruck, Austria) with the best wishes on the occasion of his 60th birthday

A thin layer chromatographic assay is described, allowing quantitation of amiodarone in the presence of its degradation products. The method is applied to stability testing of an admixture containing amiodarone (1 mg/ml) in 5% dextrose and 0.9% sodium chloride infusion, respectively. Drug stability was assayed under different physical conditions corresponding to those encountered clinically. For 6 hours under light protection, the mixture was stable even at elevated temperatures, however, exposure to sunlight leads to a significant decrease in drug concentration.

1. Introduction

Amiodarone [2-butylbenzofuran-3-yl 4-(2-diethylaminoethoxy)-3,5-diiodophenylketone], a class III anti-arrhythmic, is given orally or intravenously as the hydrochloride. Polysorbate 60 or 80 is used to increase its poor aqueous solubility in commercial intravenous preparations. The drug is frequently administered as intravenous admixture to 5% dextrose and 0.9% sodium chloride infusions. Therefore, stability and compatibility of the infusion admixture should be determined.

A number of analytical procedures mainly based on HPLC have been previously described; most are aimed at the determination of the drug and its metabolite in biological samples [1]. Assay procedures based on derivative UV spectrophotometry and HPLC have been developed for the determination of amiodarone hydrochloride in pharmaceutical dosage forms [2]. Only a few reports deal with investigations of the stability of the compound in infusions. Visual compatibility of amiodarone hydrochloride with drugs commonly given in continuous infusion was tested [3]. No visible changes were observed except in those mixtures containing aminophylline. Amiodarone was found to be incompatible with heparin [4]. Studies aimed at investigating the influence of the container material indicated that glass bottles and rigid PVC bottles did not influence the drug concentration whereas a substantial loss can occur in solutions stored in flexible PVC bags or infused through PVC infusion sets [5]. Precipitations observed in individual cases when amiodarone hydrochloride admixtures were dispensed in evacuated glass bottles, have been assumed to be caused by chemicals added to adjust the pH of the sterile water used for steam sterilization of the bottles [6]. Low concentrations of the drug (5 μg/ml) in water or TRIS-buffer pH 7.4 have been found to exhibit significant differences in stability depending on whether the solution is kept standing or slightly shaken [7]. HPLC on normal phase columns has been proposed as a method to test the stability of amiodarone in admixtures containing other injectable drugs [8]. The decomposition studies were made by simultaneously heating the test solution to 75 °C and exposing it to 254 nm ultraviolet light. Admixtures were tested under normal storage conditions (24 h at 24 °C, fluorescent light) in PVC and polyolefin containers, respectively. Except in admixtures containing quinidine gluconate during storage in PVC, amiodarone was considered to be sufficiently stable in all admixtures [8]. Contradictory findings were reported regarding the compatibility of amiodarone hydrochloride with 0.9% sodium chloride infusion; one study observed precipitation in an admixture containing 0.60 mg/ml [5] whereas in another investigation, which used higher concentrations (4 mg/ml), no precipitation was seen [3].

These adverse results promoted interest in further investigations. Therefore, the stability of amiodarone hydrochloride in 5% dextrose and 0.9% sodium chloride infusions should be studied. Since in clinical practice all manipulations connected with preparation and administration of admixtures usually are not carried out under light protection, special emphasis was laid upon the testing of the stability of the admixture exposed to different light sources.

Furthermore, we intended to use HPTLC as stability assay method. The advantages of HPTLC as a sensitive, simple and rapid method for drug analysis are well known. Stability assays based on HPTLC have not yet been reported for amiodarone. However, this method seems well suited in particular for application in hospital pharmacies because it is rapid, can be economically employed for routine use, is versatile and needs less system maintainance than e.g. HPLC. The system proposed should therefore offer hospital pharmacists further capabilities to solve questions of current interest, especially those considering the necessity of custom, product-specific tests in their field.

2. Investigations, results and discussion

The HPTLC based stability assay was developed using a sample solution (2.5 mg/ml) subjected to forced degradation by exposing to artificial irradiation from a xenon source in a Suntest for 4 h. This accelerated exposure machine is rated at 15 times the intensity of sunlight, thus leading to reduced testing time. It provides reproducible conditions with a radiation distribution similar to natural sunlight and yields a repeatable level of irradiation, thus avoiding the varying intensities of natural sunlight. Since TLC systems published previously were mostly developed for general drug screening [9] or separation of the drug substance from chlorotriethylamine [10], a system had to be found which allowed the separation of amiodarone from all its degradation products. A mobile phase consisting of ethyl acetate saturated with conc. ammonia/tetrahy-

drofurane (4/1, v/v) was found to be suitable with silica gel 60 as the stationary phase. In order to establish the capability of the assay for determing stability, a comparison of the remission spectra of the sample zone in a chromatogram of a degraded solution (4 h irradiation in the Suntest) with the zone of amiodarone in a freshly prepared solution on a neighbouring track was carried out. The spectra proved to be identical, therefore it can be assumed that no other degradation products are overlapping the peak of the drug substance. To ascertain that overlaying degradation products would show differences in the remission spectra, the UV absorption spectra of a solution of amiodarone immediately after preparation and after 4 h irradiation were measured and found to be significantly different. Interestingly, irradiation with ultraviolet light of a single wavelength (254 nm) did not lead to changes in the absorption spectrum [8] which points out the importance of using light sources corresponding to the full sunlight spectrum to obtain conditions comparable to daylight exposure.

The HPTLC method was applied to test the stability of an admixture containing amiodarone hydrochloride (1 mg/ml) in 0.9% sodium chloride or 5% dextrose infusion. For quantitation, the drug was densitometrically scanned at $\lambda = 241$ nm. External calibration was employed, calibration curves being linear in the concentration range of interest. Three different admixtures were prepared and each was tested in duplicate. As in clinical practice admixtures should be prepared immediately before administration to the patient, the study period was limited to 6 h. The admixtures were tested in the Suntest and under light protection at 50 °C. The latter was necessary to ensure that degradation of the drug in the solution subjected to a stress test in the Suntest is due to the irradiation and not to the rise in temperature during irradiation. Influence of sunlight (simulated in the Suntest) leads to a marked loss of the drug (Table 1), which is slightly different for the two types of infusions. The loss in 0.9% sodium chloride was about 20% within the test period whereas in 5% dextrose the degradation led to a 30% loss during the same time. The admixture was stable under light protection at 50 °C in both infusions (Table 2). As can be seen from Tables 1–3, the concentration found at $t = 0$ is less than expected. However, since all tests gave corresponding results and the exclusive aim of the study was to investigate stability, no further lots of the preparation were tested. Since especially sodium chloride was preciously assumed to cause incompatibilities, a test was carried out exposing the respective sample solution to artificial room light (for results see Table 3) for 6 h. This did not cause a relevant decrease in drug concentration and no precipitation was visually observable.

Table 1: Concentrations of amiodarone hydrochloride in admixtures exposed to artificial sunlight (Suntest)

Time (min)	Amiodarone in 5% dextrose (% of initial conc.)[a]	Amiodarone in 0.9% NaCl (% of initial conc.)[b]
t = 0	100	100
t = 4	94.51 ± 1.20	98.05 ± 4.46
t = 8	90.77 ± 0.64	93.00 ± 2.91
t = 12	83.34 ± 3.74	88.39 ± 3.81
t = 16	78.14 ± 3.87	82.28 ± 3.47
t = 20	72.54 ± 1.48	80.18 ± 3.98
t = 24	69.14 ± 1.35	78.46 ± 2.83

Drug concentration as % of initial concentration as mean (n = 6) ± rel. SD
[a] initial concentration 0.85 mg/ml ± 1.39%
[b] initial concentration 0.86 mg/ml ± 3.04%

Table 2: Concentrations of amiodarone hydrochloride in admixtures exposed to 50 °C under light protection

Time (min)	Amiodarone in 5% dextrose (% of initial conc.)[a]	Amiodarone in 0.9% NaCl (% of initial conc.)[b]
t = 0	100	100
t = 24	94.92 ± 3.04	100.26 ± 2.07
t = 60	93.99 ± 3.22	103.27 ± 2.51
t = 120	95.28 ± 4.09	100.03 ± 3.29
t = 360	97.40 ± 3.66	101.69 ± 3.37

Drug concentration as % of initial concentration as mean (n = 4) ± rel. SD
[a] initial concentration 0.85 mg/ml ± 2.84%
[b] initial concentration 0.82 mg/ml ± 1.12%

Table 3: Concentrations of amiodarone hydrochloride in 0.9% NaCl exposed to fluorescent room light

Time (h)	Amiodarone in 0.9% NaCl (% of initial conc.)[a]
t = 0	100
t = 1	101.90 ± 0.17
t = 2	98.29 ± 3.46
t = 3	99.97 ± 3.08
t = 4	96.86 ± 0.19
t = 5	97.19 ± 3.16
t = 6	95.42 ± 2.95

Drug concentration as % of initial concentration as mean (n = 4) ± rel. SD
[a] initial concentration 0.84 mg/ml ± 3.74%

In conclusion it could be established that without protection from sunlight the admixture is not compatible and the safe use in patients should at least be questioned. Therefore, even if the admixture is administered to the patient immediately after preparation, care should be taken to protect the solution from sunlight.

3. Experimental

3.1. Materials

Pharmaceutical preparations were Sedacoron® 150 mg Konzentrat zur Infusionsbereitung (Ebewe Arzneimittel GmbH, Unterach, Austria; lot 603463/3 ml containing 150 mg amiodarone hydrochloride and 150 mg polysorbate 60); Dextrose 5% infusion (Mayrhofer Pharmazeutika, Linz, Austria; lot 547020/250 ml); Sodium chloride 0.9% infusion (Mayrhofer Pharmazeutika, Linz, Austria; lot 605020/250 ml). A reference sample of amiodarone hydrochloride was obtained from Sigma (Vienna, Austria), Sigma A-8423, lot 16H0192.

Solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

3.2. Sample preparation

To prepare the admixtures, aliquots of 400 µl commercial drug solution were diluted to 20.00 ml with dextrose 5% and sodium chloride 0.9% infusion solution, respectively. This corresponds to the concentration of the therapeutically used infusion admixtures. The sample solutions were then transferred to 250 ml white glass infusion bottles (SGD II ISO). Three admixtures were prepared and each was tested in duplicate for exposure to irradiation in the Suntest. Two admixtures each were prepared and tested in duplicate for the comparative study of the influence of fluorescent room lighting, and for testing temperature stability (50 °C, oven) under light protection, respectively.

3.3. HPTLC equipment

HPTLC plates silica gel 60 F 254 (Merck) 10 × 10 cm with 0.25 mm thickness were used. The plates were prewashed with dichloromethane/methanol (1/1, v/v) and allowed to evaporate under light protection for 1 h before use. The samples were spotted using a Camag Nanomat III with 0.5 µl disposable glass capillaries (CV ≤ 0.6%) on both sides of the plate. The sample application was carried out under light protection. After all spots were introduced the plate was allowed to dry light protected for 1 h before being developed. Horizontal development chambers for 10 × 10 cm plates (Camag) were used.

Densitometric measurements were carried out using a Shimadzu CS 9000 Dual Wavelength Flying-Spot Scanner. Scan parameters were set as follows: photomode, reflection; scan mode zig-zag scan, swing width 3.0 mm; detection wavelength 241 nm; delta "Y" 0.02 mm; zero set mode at start.

3.4. Analytical conditions and assay

The mobile phase consisted of ethyl acetate saturated with conc. ammonia/ tetrahydrofuran (4/1, v/v). The development chamber was used in the saturation configuration, the plate was allowed to be preconditioned for 10 min. The development was made from both sides through a distance of 4.5 cm. After removing the plate, it was stored under light protection for 30 min to evaporate residual mobile phase before densitometric evaluation.

For quantitation external calibration was carried out. Standard solutions containing amiodarone hydrochloride 1.1, 1.0, 0.9, 0.8, 0.7 mg/ml were prepared under light protection. The standards were applied to each plate, the calibration curves were set up for each plate separately. Linear response in peak areas over the range of interest were observed (typical regression equation expressed as $y = \text{intercept} + \text{slope} \cdot x$; slope = 17129.94, intercept = 911.5881). The coefficient of correlation was typically found to be ≥ 0.996. The method was validated by spotting a sample solution containing amiodarone hydrochloride 1.0 mg/ml 24 times on two different plates. The relative standard deviation (R.S.D.) on the basis of peak areas was found to be 2.37%.

3.5. Light conditions

The sample solutions were exposed to forced irradiation using a Suntest CPS Accelerated Exposure Machine (Heraeus, Hanau, Germany): xenon burner, black panel temperature: 49 °C at maximum radiation intensity; window-glass filter; time factor: 15 (1 min Suntest $\cong$ 15 min natural sunlight). For the test under room lighting (fluorescent light) the samples were stored in a windowless room without daylight.

References

1 Plomp, A.; in: Florey, K. (ed.): Anal. Profiles Drug Subst., vol. 20, p. 1 (1991) and literature cited therein
2 Pietra, A. M.; Cavrini, V.; Gatti, R.; Raggi, M. A.: Pharm. Res. **5**, 709 (1988)
3 Hasegawa, G. R.; Eder, J. F.: Am. J. Hosp. Pharm. **41**, 1379 (1984)
4 Cairns, C. J.: Pharm. J. **236**, 68 (1986)
5 Weir, S. J.; Szucs Meyers, V. A.; Bengtson, K. D.; Ueda, C. T.: Am. J. Hosp. Pharm. **42**, 2679 (1985)
6 Strozyk, W. R.; Williamson, R.; Thompson, D.: Am. J. Health-Syst. Pharm. **53**, 184 (1996)
7 Andreasen, F.; Agerbaek, H.; Bjerregaard, P.; Gotzsche, H.: Eur. J. Clin. Pharmacol. **19**, 293 (1982)
8 Campbell, S.; Nolan, P. E.; Bliss, M.; Wood, R.; Mayersohn, M.: Am. J. Hosp. Pharm. **43**, 917 (1986)
9 Musumarra, G.; Scarlata, G.; Cirma, G.; Romano, G.; Palazzo, S.; Clementi, S.; Giulietti, G.: J. Chromatogr. **350**, 151 (1985)
10 European Pharmacopoeia 1997

Received September 8, 1998
Accepted December 4, 1998

Dr. Hannelore Kopelent-Frank
Department of Drug Analysis
Institute of Pharmaceutical Chemistry
Centre of Pharmacy
Althanstr. 14
1090 Vienna
Austria