Stability-indicating high-performance liquid chromatographic assay for the simultaneous determination of dixyrazine and chlorprothixene in intravenous admixtures

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Abstract

A liquid chromatographic assay is described allowing simultaneous quantitation of dixyrazine and chlorprothixene in the presence of their degradation products. A reversed-phase system, gradient elution with a binary solvent system and diode-array detection were used. The method is used to test the stability of an admixture containing dixyrazine (0.04 mg/ml) and chlorprothixene (0.1 mg/ml) in 5% dextrose infusion. Drug stability was assayed under different physical conditions corresponding to those encountered clinically. Over a 6-h period under light protection, the mixture was stable at room temperature and at 50°C; exposure to sunlight led to a more than 50% loss of both drug substances.

Keywords: Dixyrazine; Chlorprothixene; Phenothiazine; Thioxanthene

1. Introduction

Dixyrazine and chlorprothixene are widely used in medical practice, mainly for the treatment of psychiatric disorders, but also to reduce withdrawal symptoms during detoxication of drug addicts. The drugs are frequently administered as intravenous admixtures to 5% dextrose infusions, which may prove to be beneficial to the patients and time-saving for hospital personnel. As the compatibility of the resulting admixture has not been investigated up to now, determination of stability and compatibility of the infusion admixture should be undertaken to support the safe clinical use of drugs in combination. Phenothiazines, as well as thioxanthenes, are known to readily undergo photodegradation, and the manu-

facturers, accordingly, recommend to keep their products protected from light. Nevertheless, in clinical practice, all manipulations connected with the preparation and administration of admixtures are usually not carried out under light protection. Therefore special emphasis should be paid to test the stability of the admixture when exposed to different light sources.

Photodegradation of phenothiazines is a well-investigated feature of these compounds which is documented in a large number of publications. Most of the authors used selected examples, e.g. promethazine [1], phenothiazine [2] or fluphenazine [3], to study the degradation pathways. The effect of the side chain on phenothiazine cation radical reactions has been elucidated [4]. However, such studies have not been undertaken for dixyrazine. The chromatographic behaviour of dixyrazine has been described in connection with general screening tests for drugs [5]. Dixyrazine was also included in a study aimed at

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the HPLC differentiation of 14 phenothiazine psychopharmaceuticals [6]. HPLC assays performed to determine the stability have not been reported up to now. The HPLC behaviour of chlorprothixene has been studied in a project aimed at the determination of antidepressants in plasma [7]. Irradiation of tranquillizers based upon the thioxanthene nucleus as chlorprothixene induces oxidative degradation [8]. The presence of a substituent in position 2 allows the existence of geometrical isomers about the exocyclic olefin function at position 9. Exposure to light is known to result in a rapid change in the isomeric composition which can be monitored by HPLC [8]. Isomerisation may be of considerable interest as the biological activity mainly resides in the Z-isomer [9], whereas the corresponding E-isomer has only relatively low activity.

The aim of the present study was to develop a HPLC assay permitting simultaneous quantitation of both psychopharmaceuticals in the presence of their degradation products including separation of Z-chlorprothixene from the corresponding E-isomer. The procedure can be used to study the stability of the drugs on exposure to light and elevated temperatures and the compatibility of the pharmaceutical preparations with 5% dextrose infusions.

2. Experimental

2.1. Materials

The pharmaceutical preparations were kindly donated by the manufacturers: Esucos (UCB Pharma, Vienna, Austria; lot 92C30, dixyrazine, 20 mg); Truxal 5% (Lundbeck, Kopenhagen, Denmark; lot B220C, Z-chlorprothixene, 50 mg); dextrose 5% infusion (Laevosan, Linz, Austria; lot 10324, 500 ml). Reference samples of the drug substances were obtained: Z-chlorprothixene from Sigma (Vienna, Austria), dixyrazine was donated by the manufacturer UCB Pharma.

Methanol LiChrosolv and water LiChrosolv for HPLC were obtained from Merck (Darmstadt, Germany). Sodium acetate and concentrated acetic acid for the preparation of acetate buffer pH 4.6 were of analytical grade.

2.2. Sample preparation

Admixtures were prepared adding aliquots of the content of the ampoules (200 μl Esucos and 100 μl Truxal) to 50 ml 5% dextrose infusion solution yielding a sample solution containing 0.04 mg/ml dixyrazine and 0.1 mg/ml Z-chlorprothixene. This corresponds to the therapeutically used admixture of one ampoule each added to 500 ml 5% dextrose infusions. Three admixtures were prepared and each tested in triplicate for exposure to irradiation in the Suntest and for exposure to fluorescent room lighting, respectively. One admixture each was prepared and tested in triplicate for a comparative study of the influence of natural daylight and for testing temperature stability (50°C, oven) under light protection.

2.3. HPLC equipment

Analyses were carried out using a Shimadzu HPLC [pumps, Shimadzu LC 10 AS; diode-array detector, Shimadzu SPD-M10A; column oven, Shimadzu CTO-10AC (20°C); rheodyne injection valve with a 20-μl loop]. Separation was achieved on a M&W-Chrom column Superspher 100 RP18 4 μm endcapped, 125×4.6 mm I.D.

2.4. Analytical conditions

HPLC mobile phase was prepared using methanol–acetate buffer pH 4.6. The mobile phase was filtered and degassed before use. Gradient elution was employed, using the program shown in Table 1.

For simultaneous detection of both drugs diode-array detection was used, wavelengths set at the respective absorption maxima (channel 1, λ=252 nm, dixyrazine; channel 2, λ=267 nm, chlorprothix-

Table 1
Gradient elution program

t (min)	Flow-rate (ml/min)	Methanol (%)
0–25	0.9	55
25–27	0.9–1.2	55–75
27–33	1.2	75
33–35	1.2	75–55
35–38	1.2–0.9	55
38–38.5	0.9	55

ene). The peak-purity index for both drug substances was investigated and found to be better than 0.9997 in chromatograms of the standard compounds as well as in the chromatograms of the stressed solutions. Base line substraction was used. For quantitation, external calibration was carried out. Standard solutions containing dixyrazine (free base) 20, 30, 40 and 50 μg/ml; and Z-chlorprothixene (free base) 40, 60, 80 and 100 μg/ml were prepared under light protection and the resulting solutions subjected to chromatography immediately after preparation. Linear response in peak areas over the range of interest were observed for both substances (regression equation expressed as conc.=slope×area+intercept; dixyrazine, slope=9.1846, intercept=0.2480; chlorprothixene, slope=1.6303, intercept=0.3086). The coefficient of correlation was found to be 0.9998 (dixyrazine) and 0.9997 (chlorprothixene), respectively. The method was validated by evaluation of the intra- and inter-day precision. The relative standard deviations (R.S.D.) on the basis of peak area ratios for 6 replicate injections were found to be between 0.93 and 1.12% (dixyrazine, 40 μg/ml) and 1.08 and 1.23% (chlorprothixene, 100 μg/ml) in the intra-day assay. The R.S.D. in the inter-day assay (3 days, $n=6$) was 1.76% for dixyrazine and 2.03% for chlorprothixene at the same amount.

2.5. Light conditions

The sample solutions (50 ml each in a volumetric flask) were exposed to electric room light (fluorescent tubes), daylight behind window glass and to forced irradiation using a Suntest CPS accelerated exposure machine (Heraeus, Hanau, Germany): xenon burner, black panel temperature, 49°C at maximum radiation intensity (95.2 W/m^2); window-glass filter; time factor, 15 (1 min Suntest ≅15 min natural sunlight).

3. Results and discussion

The HPLC assay was developed using a sample solution that was subjected to forced degradation by exposing it to artificial irradiation from a xenon source in a Suntest. This accelerated exposure machine is rated at 15 times the intensity of sunlight,

thus leading to reduced testing time. It provides radiation distribution similar to that of natural sunlight and for reproducible conditions was chosen to give a repeatable level of irradiation rather than reliance being placed on varying intensities of natural sunlight. In the stressed solution, 9 degradation products of dixyrazine and 3 of chlorprothixene were detected, most of them produced by light-initiated oxidation [1,8]. Furthermore, photoisomerisation of Z-chlorprothixene to the corresponding E-isomer was observed [8].

Chromatographic separation of Z- and E-chlorprothixene has been reported, utilizing HPLC on Spherisorb as stationary phase and ethylacetate–methanol–ammonia as eluent [10] and TLC on silica gel as layer material [11]. HPLC based on a reversed phase (LiChrospher RP18 5 μm endcapped) has been proposed by us for testing the compatibility of chlorprothixene with 5% dextrose; here isocratic elution with methanol–acetate buffer pH 4.6 was applied [12]. Z-Chlorprothixene was well resolved from the E-isomer and all oxidation products. This system, nevertheless, proved not to be suitable to test the stability of an admixture additionally containing dixyrazine and its large number of degradation products. For simultaneous quantitation of dixyrazine and chlorprothixene in the presence of all degradation products Supersphere RP18 4 μm endcapped was considered to be best suited, leading to a marked improvement in peak shape and selectivity. In spite of the better column material, isocratic elution did not lead to satisfactory results. As the use of a longer column should be avoided with regard to the time of analysis, gradient elution was chosen, combining a solvent gradient with a flow gradient. Thus, the time for one analysis can be kept to less than 40 min. All peaks were well resolved. A chromatogram of the admixture right after preparation is shown in Fig. 1. Fig. 2 shows a chromatogram of a stressed sample solution irradiated in the Suntest for 24 min (corresponding to 6 h of natural sunlight). Most of the oxidation products of both neuroleptics show higher polarity than the drug itself and elute with retention times less than 15 min, thus not interfering with the peaks of the drug substances. The formation of 2-chlorthioxanthone (t_R=37.2 min) and phenothiazine (t_R=29.9 min) could be demonstrated by comparison with authentic material exhibiting identi-
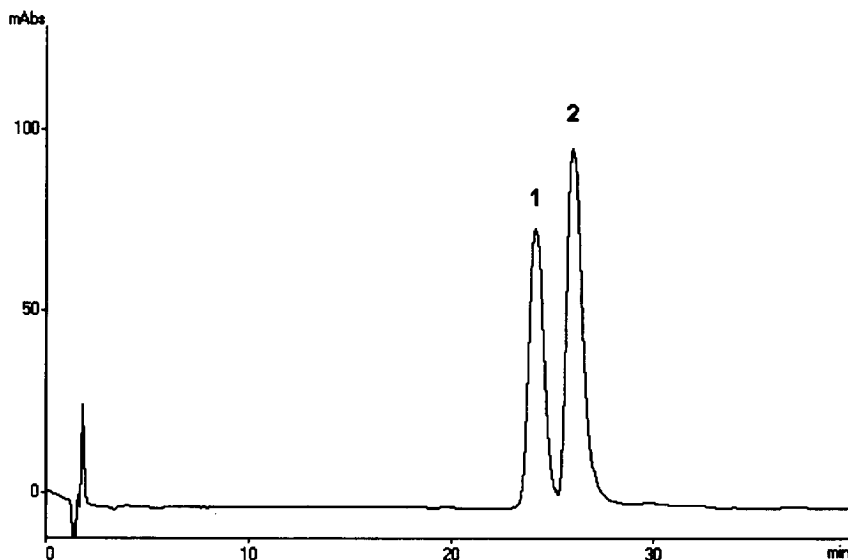
Fig. 1. Chromatogram of a freshly prepared admixture. Conditions: methanol–acetate buffer pH 4.6, gradient elution (see Section 2), detection wavelength $\lambda=252$ nm. Peaks: 1=dixyrazine, 2=Z-chlorprothixene.

cal chromatographic and UV spectroscopic behaviour. Dixyrazine sulphoxide elutes at 3.6 min, the UV spectrum recorded with the diode-array detector showed the characteristic four maxima at $\lambda=240$, 272, 296 and 341 nm according to Ref. [13]. Z-Chlorprothixene is well resolved from the E-isomer, the latter eluting at 29.2 min. The formation of the E-isomer was proved subjecting a solution of Z-chlorprothixene to forced degradation (Suntest) followed by preparative TLC (silica gel; toluene–ethanol–diethylamine, 60:40:5, v/v) of the analyte solution. The migration behaviour of the E-isomer is
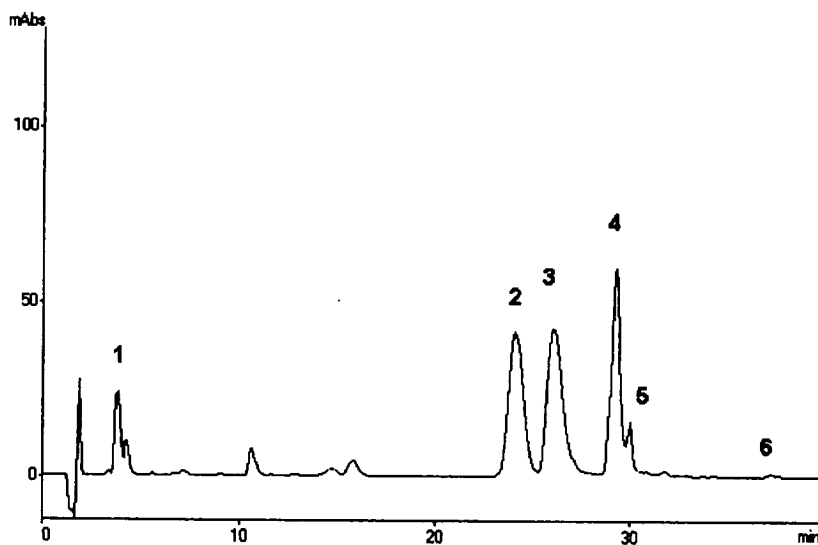

Fig. 2. Chromatogram of an admixture subjected to irradiation in the Suntest for 24 min. Conditions: methanol–acetate buffer pH 4.6, gradient elution (see Section 2), detection wavelength $\lambda=252$ nm. Peaks: 1=dixyrazine sulphoxide; 2=dixyrazine; 3=Z-chlorprothixene; 4=E-chlorprothixene; 5=phenothiazine; 6=2-chlorthioxanthone.

Table 2
Influence of artificial room lighting on drug concentration[a]

t (h)	Esucos (dixyrazine)			Truxal (Z-chlorprothixene)		
	Admixture			Admixture		
	1	2	3	1	2	3
0	41.87	39.99	42.35	102.49	100.26	104.66
	(1.1%)	(0.5%)	(0.5%)	(1.3%)	(1.3%)	(1.8%)
2	40.68	39.42	41.50	99.39	99.58	103.19
	(1.9%)	(1.2%)	(0.6%)	(2.9%)	(3.2%)	(0.2%)
4	41.19	38.91	41.47	101.77	99.31	104.83
	(2.1%)	(1.3%)	(0.7%)	(1.5%)	(3.3%)	(0.4%)
6	41.26	38.65	41.17	101.60	99.47	104.57
	(2.0%)	(1.2%)	(0.9%)	(2.3%)	(2.4%)	(1.1%)
Diff. % after 6 h	−1.5%	−3.3%	−2.8%	−0.9%	−0.8%	−0.1%

[a] The concentrations are give in μg/ml as the mean of three replicates; values in parenthesis represent the R.S.D. in %.

known to lead to a lower R_F value than the Z-isomer [11]. Both substances eluted from the sorbent of the respective TLC zones showed identical GC–MS and UV data corresponding to those given in the literature [14]. The structures of the other degradation products of minor quantities have not been investigated.

The method is applied to test the stability of an admixture containing dixyrazine (0.04 mg/ml) and chlorprothixene (0.1 mg/ml) in 5% dextrose infusion. For quantitation, the drugs were monitored at the respective absorption maxima of $\lambda=252$ nm (dixyrazine), and $\lambda=267$ nm (chlorprothixene). External calibration was employed, calibration curves being linear in the concentration range of interest. Three different admixtures were prepared and each tested in triplicate. As 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, at fluorescent room lighting, natural daylight and also under light protection at 50°C, the latter necessary to ensure that degradation of the drugs in the solution subjected to stress in the Suntest is due to the irradiation and not to elevated temperature. The admixture can be assumed to be stable under light protection at 50°C (after 6 h the loss was found to be: dixyrazine, −3.3%, Z-chlorprothixene, −2.3%). No isomerisation of Z-chlorprothixene was observed with solutions stored in the dark. Exposure to artificial room light (see Table 2 for results) did not cause a relevant decrease in drug concentration. Influence of sunlight (simulated in the Suntest) leads to marked loss of both drugs (see Table 3). Comparison with the drug concentration

Table 3
Influence of sunlight simulated by irradiation in the Suntest on drug concentration[a]

t (min)	Esucos (dixyrazine)			Truxal (Z-chlorprothixene)		
	Admixture			Admixture		
	1	2	3	1	2	3
0	39.23	42.10	42.56	99.57	98.24	108.63
	(1.0%)	(2.4%)	(5.0%)	(2.7%)	(2.6%)	(5.1%)
4	29.53	31.51	31.02	52.59	50.92	52.55
	(1.5%)	(1.2%)	(2.0%)	(1.6%)	(1.5%)	(2.1%)
24	16.70	18.16	18.88	44.50	42.16	45.01
	(2.2%)	(2.4%)	(3.2%)	(1.5%)	(1.3%)	(1.5%)
Diff. %	−57.4%	−56.9%	−55.7%	−55.3%	−57.1%	−58.6%

[a] The concentrations are given in μg/ml as the mean of three replicates; values in parenthesis represent the R.S.D. in %.

found in an admixture exposed for 6 h to natural daylight (dixyrazine, −42.5%; Z-chlorprothixene, −55.1%) showed good correlation, the smaller loss being due to the fact that the tests were carried out on a day with varying intensity of sunlight. Accordingly, the simulated irradiation from a xenon source proved to be a satisfying test system.

In conclusion, it was shown that without light protection the admixture is not compatible and safe use with patients should at least be doubted. As the biological activity of chlorprothixene resides mainly in the Z-isomer formation of the E-isomer leads to a marked loss of therapeutic activity. Of significance is the fact that most of the loss of both psychopharmaceutics occurs during the first 1–2 h. Therefore, even if the admixture is administered to the patient immediately after preparation, utmost care should be taken to protect the solution from daylight.

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