lower photostability of the PR-γ-CD complex relative to that of PR-β-CD clathrate, however, ^{13}C NMR spectra indicated a similar structure of both inclusion compounds.

Experimental

1. Materials

Analytical grade of promazine hydrochloride and β-cyclodextrin, hydroxypropyl-β-cyclodextrin, heptakis (2,6-di-O-methyl)-β-cyclodextrin, heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin, γ-cyclodextrin from Sigma-Aldrich Chemical Co.

2. Spectroscopic determination of stability constants (K_c)

To seven glass vials, protected from light, containing 10 ml of promazine solution (1.5×10^{-5} mol/l) in phosphate buffer (pH = 6.47), different quantities (0–80 mg) of the selected cyclodextrin were solubilized. The UV absorption changes of promazine, resulting from the addition of cyclodextrin, were measured at $\lambda = 252$ nm (spectrophotometer UV-160 A, Shimadzu). The stability constants K_c of complexes obtained were calculated according to Scott's equation [12].

3. Kinetic studies

The solutions of promazine and promazine-cyclodextrin complexes (in phosphate buffer) were exposed to UV radiation ($\lambda_{max} = 254$ nm, distance 20 cm) in quarz cuvette cells (1 cm). The changes of promazine concentration were followed spectrophotometrically at $\lambda = 252$ nm. To determine the kinetic parameters of photodegradation of promazine a kinetic model of the first-order reaction was used.

References

1 Tarasiewicz, M.; Wołyniec, E.; Puzanowska-Tarasiewicz, H.: Pharmazie **53**, 151 (1998)
2 Misiuk, W.; Tarasiewicz, M.: Acta Polon. Pharm.-Drug Res. **54**, 115 (1997)
3 Lutka, A.: Pharmazie, in press
4 Roseboom, H.; Fresen, J. A.: Pharm. Acta Helv. **50**, 55 (1975)
5 Duchêne, D.; Wouessidjewe, D.: Acta Pharm. Technol. **36**, 1 (1990)
6 Grimm, W.; Krummen, K.: Stability Testing in the EC, Japan and the USA – Scientific and Regulatory Requirements, p. 225, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1993
7 Singh, U. V.; Aital, K. S.; Udupa, N.: Pharmazie **53**, 208 (1998)
8 Lutka, A.; Pawlaczyk, J.: Acta Polon. Pharm.-Drug. Res. **54**, 279 (1997)
9 Mielcarek, J.: J. Pharm. Biomed. Anal. **15**, 681 (1997)
10 Mielcarek, J.: Drug. Dev. Ind. Pharm. **24**, 197 (1998)
11 McCandless, R.; Yalkowsky, S. H.: J. Pharm. Sci. **87**, 1639 (1998)
12 Otagiri, M.; Uekama, K.; Ikeda, K.: Chem. Pharm. Bull. **23**, 188 (1975)

Received November 13, 1998
Accepted February 10, 1999

Dr. Anna Lutka
Department of Inorganic and Analytical Chemistry
K. Marcinkowski University of Medical Sciences
ul. Grunwaldzka 6
60-780 Poznań
Poland

Department of Medicinal Chemistry, Medical Academy, Lublin, Poland

Determination of fluoxetine hydrochloride in capsules and moclobemide in tablets by first, second and third derivative spectrophotometry

G. Misztal and R. Skibiński

Both fluoxetine and moclobemide belong to a new generation of antidepressant drugs. The literature relating to the determination of this drugs in pharmaceuticals is rather scare.

Fluoxetine hydrochloride in pharmaceuticals was determined by GC [1, 2] and HPLC [3–5]. Moclobemide was determined by an electrochemical method with ionselective electrode [6]. Although the use of derivative spectra is not new, it has only become practical in recent years, with the development of computer technology, wich allows the almost instant generation of derivative spectra. In the derivative ultraviolet (UV) – visible spectroscopy, the information contained in the spectrum is presented in a potentially more useful form, with respect to the versatility of the technique [7–9] and offers a convenient solution to a number of well definied analytical problems: removal of sample turbidity, matrix background and enhancement of spectral details [10].

Two graphical techniques – "peak-zero" and "peak-peak" – have been used. In the case of the "peak-zero"-technique the measurement was made from the maximum value of the peak to the baseline. In the "peak-peak"-technique the amplitude of the neighbouring peaks was measured.

The Lambda 15 spectrophotometer enables to store the spectra's derivatives in its own computer memory and to read the value of a given derivative in any point marked on the received spectra. This makes both the "peak-zero"- and "peak-peak"-technique easy to use.

The influence of $0.1 \text{ mol} \cdot l^{-1}$ sodium hydroxide and $0.1 \text{ mol} \cdot l^{-1}$ hydrochloric acid and methanol on the absorption spectra of fluoxetine hydrochloride and moclobemide and the first, second and third derivative was studied. The best results for analytical purposes were obtained at $0.1 \text{ mol} \cdot l^{-1}$ hydrochloric acid for moclobemide and methanol for fluoxetine hydrochloride.

First, second and third derivative spectra of fluoxetine were determined in methanol in the 190–250 nm wavelength region in concentrations of 15, 20, 25, and

Table 1: Determination of fluoxetine hydrochloride in capsules

Derivative	λ (nm)	Technique	Content of fluoxetine hydrochloride in capsule (mg)	Standard deviation	Confidence interval (95%)
D1	228	P–O[x]	20.96	0.203544	20.71–21.21
D1	228–214	P–P[y]	20.87	0.323466	20.47–21.27
D2	234	P–O	20.84	0.185876	20.60–21.07
D2	222	P–O	20.80	0.206446	20.54–21.05
D2	234–222	P–P	20.85	0.220386	20.57–21.12
D3	236.7	P–O	21.49	0.507149	20.23–22.74
D3	227	P–O	20.84	0.246414	20.54–21.15
D3	236.7–227	P–P	21.21	0.409919	20.19–22.23
D3	215.5	P–O	21.03	0.243783	20.73–21.33
D3	227–215.5	P–P	20.91	0.240375	20.61–21.21

x: "peak-zero", n: number of determinations 6
y: "peak-peak"

Table 2: Determination of moclobemide in tablets

Derivative	λ (nm)	Technique	Content of moclobemide in tablet (mg)	Standard deviation	Confidence interval (95%)
D1	246.5	P–O[x]	152.41	1.66474	150.3–154.5
D1	246.5–217.8	P–P[y]	148.01	1.46508	146.2–149.8
D2	255.3	P–O	151.85	2.01316	149.3–154.4
D2	234.4	P–O	150.24	1.70034	148.1–152.4
D2	255.3–234.4	P–P	150.89	1.80148	148.7–153.1
D3	244	P–O	150.14	1.34344	148.5–151.8
D3	209.2	P–O	155.53	1.06809	154.2–156.9
D3	244–209.2	P–P	154.26	1.1842	152.8–155.7

x: "peak-zero", n: number of determinations-6
y: "peak-peak"

30 μg mol · l^{-1} fluoxetine hydrochloride and of moclobemide in 0.1 mol · l^{-1} hydrochloric acid in the 190–280 nm wavelength region in concentrations 5, 10, 15 and 20 μg · ml^{-1}. The indispensable time for the extraction of fluoxetine hydrochloride from capsules and moclobemide from tablets was 10 min. The inactive ingredients present in the capsules or tablets powder did not interfere with the assay procedure.
Tables 1 and 2 illustrate the assay results.

Experimental

1. Reagents and apparatus

Prozac® capsules (20 mg of fluoxetine hydrochloride, Eli Lilly Company, England); fluoxetine hydrochloride subst. (Sanofi-Biocom Co., Poland).
Aurorix® tablets (150 mg of moclobemide, F. Hoffmann La Roche LTD, Switzerland); moclobemide subst. (. Hoffmann La Roche LTD, Switzerland). Methanol was of analytical grade.
Spectrophotometer UV/Vis "Lambda 15" (Perkin-Elmer Co GmbH – Germany). Hand-press LQ-850 (Epson – Germany).

2. Determination of fluoxetine hydrochloride in Prozac®-capsules

From of the methanolic solution containing 0.1 mg · ml^{-1} fluoxetine hydrochloride volumes from 0.5 to 3.0 ml were pipetted into 10.0 ml measuring flasks and made with CH_3OH up to 10.0 ml. Then the first, second, and third derivative spectra were recorded against a reagent blank (CH_3OH).
The contents of ten capsules were weighed and powdered. An accurately weighed portion of the powder (each equivalent to the weight of 2 mg of fluoxetine hydrochloride) 0.02883 g (after declaration) was extracted with CH_3OH in 10 ml flasks by the means of a reciprocating shaker for 10 min and filtered. An extract volume of 1 ml was transferred into a 10 ml flask and made with CH_3OH up to 10.0 ml. The fluoxetine hydrochloride content was determined from the first-derivative spectrum by measuring the first-derivative signal at the "peak-zero" point (228 nm) and at the "peak-peak" point (228–214 nm); from the second-derivative spectrum by measuring the second-derivative signal at the "peak-zero" points (222 nm, 234 nm) and at the "peak-peak" point (234–222 nm) and from the third-derivative spectrum by measuring the third-derivative signal at the "peak-zero" points (215.5 nm, 227 nm, 236.7 nm) and at the "peak-peak" points (227–215.5 nm), (236.7–227 nm). This procedure was repeated six times. The values were compared with an appropriate calibration graph.

3. Determination of moclobemide in Aurorix®-tablets

From the acidic solution containing 0.1 mg · ml^{-1} moclobemide, volumes from 0.5 to 2.0 ml were pipetted into 10.0 ml measuring flasks and made with 0.1 mol · l^{-1} HCl up to 10.0 ml. Then the first, second, and third derivative spectra were recorded against a reagent blank (0.1 mol · l^{-1} HCl).
Ten tablets were weighed and powdered. An accurately weighed portion of the powder (each equivalent to the weight of 15 mg moclobemide), 0.0412 g (according to declaration) was extracted with 0.1 mol · l^{-1} HCl in 10 ml flasks by the means of a reciprocating shaker for 10 and filtered. An extract volume of 1.0 ml was transferred into a 100.0 ml flask and made with 0.1 mol · l^{-1} HCl up to 100.0 ml. The moclobemide content was determined from the first-derivative spectrum by measuring the first-derivative signal at the "peak-zero" point (246.5 nm) and at the "peak-peak" points (246.5–217.8 nm); from the second-derivative spectrum by measuring the second-derivative signal at the "peak-zero" points 234.4 nm, 255.3 nm and at the "peak-peak" point (255.3–234.4 nm) and from the third-derivative signals at the "peak-zero" points 209.2 nm, 244 nm and at the "peak-peak" points (244.0–209.2 nm). This procedure was repeated six times.

Acknowledgements: The authors are grateful for the gifts of fluoxetine hydrochloride subst. (Sanofi-Biocom Co., Poland), moclobemide subst. and Aurorix® tablets – (F. Hoffmann La Roche LTD, Switzerland) and to the companies mentioned for their help in various aspects of this investigation. The authors thank Elżbieta Kędziera for typing the manuscript.

References

1 Sane, R. T.; Jani, A. B.; Ghadge, J. K.; Vaidya, A. J.; Kotwal, S. S.: Indian Drugs **29**, 237 (1992)
2 Reghuveer, S.; Avadhanuln, A. B.; Pantulu, A. R. R.: Indian Drugs **30**, 83 (1993)
3 Parodi, B.; Caviglioli, G.; Bachi, A.; Cafaggi, S.; Romussi, G.: Pharmazie **48**, 678 (1993)
4 Pramar, Y.; Das Gupta, V.; Bethea, Ch.: Drug Dev. Ind. Pharm. **18**, 257 (1992)
5 Misztal, G.; Paw, B.: Acta Pol. Pharm. Drug Research **53**, 177 (1996)
6 Stefan, R. J.: Anal. Chim. Acta **350**, 105 (1997)
7 O'Haver, T. C.; Green, G. L.: Anal. Chem. **48**, 312 (1976)
8 Fell, A. F.; Smith, G.: Anal. Proc. **19**, 28 (1982)
9 O'Haver, T. C.: Anal. Chem. **51**, 91A (1979)
10 Cottrell, C. T.: Anal. Proc. **19**, 43 (1982)

Received August 24, 1998
Accepted January 11, 1999

Dr. habil. Genowefa Misztal
Str. Chodźki 6
20-093 Lublin
Poland