3.2. d-1H-Indole-2,3-dione (d-isatin) (**2**)

d-Isonitrosoacetanilide (**1**) (8.4 g, 0.05 mol) was carefully added in small portions to D_2SO_4 (0.15 mol, 8 ml, 99.5%) under stirring and careful control of the reaction temperature which was kept below 70 °C. Upon completion of addition, the reaction mixture was poured on D_2O-ice (100 ml). The red precipitate was filtered, washed with D_2O (3×50 ml) and dried (in vacuo, P_2O_5, 72 h). Yield: 3.0 g (41%), red-orange precipitate, m.p. 193–197 °C. ESI-MS: m/z (%) = 150.2 ($[d_4\text{-M-H}]^-$, 100), 149.4 ($[d_3\text{-M-H}]^-$, 23), 148.4 ($[d_2\text{-M-H}]^-$, <2). IR (KBr): ν = 3432, 3185, 1721, 1604, 1166, 591 cm^{-1}. UV δ_{max} (CH_3OH) 208, 241, 295 nm. ^1H NMR (DMSO-d_6): δ = 6.92 (d, J = 2.8 Hz, H-7), 7.10 (d, J = 2.8 Hz, H-5).

3.3. d-Indolo[2,1-b]-quinazoline (d-tryptanthrin) (**3**)

d-Isatin (**2**) (3 g, 0.02 mol) in D_2O (60 ml) was brought to ebullition, and $KMnO_4$ (3 g, 0.02 mol) was added in small portions and under stirring. While the reaction mixture was kept boiling, SO_2 gas (generated from Na_2SO_3 (50 g) and H_2SO_4 (50%, 150 ml) was bubbled through the solution until a precipitate appeared. The mixture was heated for additional 1–2 min, then cooled to RT. The green-brown precipitate was filtered off, washed with D_2O (3×50 ml) and dried (in vacuo, P_2O_5, 48 h). d-Tryptanthrin was extracted with $CHCl_3$ and recrystallised from CH_3OH. Yield: 540 mg (21.8%), yellow needles, m.p. 268–270 °C. MS (ESI): m/z (%) = 257.2 ($[d_8\text{-M+H}]^+$, 100), 256.3 ($[d_7\text{-M+H}]^+$, 26.6), 255.3 ($[d_6\text{-M+H}]^+$, 2.4). IR (KBr): ν = 3400, 1721, 1684, 1586, 1462, 1351, 1215, 1184, 1160, 1110, 1036, 925, 758, 690, 474 cm^{-1}. UV δ_{max} (CH_3OH) 254, 380, 387 nm. ^1H NMR ($CDCl_3$): δ = 7,45 (s, H-8), 7,69 (s, H-2), 8.05 (s, H-4), 8.65 (s, C-10).

References

1 Danz, H.; Stoyanova, S.; Wippich, P.; Brattström, A.; Hamburger, M.: Planta Med. **67**, 411 (2001)
2 Danz, H.; Stoyanova, S.; Thomet O. A. R.; Simon, H. U.; Dannhardt, G.; Ulbrich, H.; Hamburger, M.: Planta Med. in press
3 Ishihara, T.; Kohno, K.; Ushio, S.; Iwaki, K.; Ikeda, M.; Kurimoto, M.: Eur. J. Pharmacol. **407**, 197 (2000)
4 Danz, H.; Baumann, D.; Hamburger, M.: Planta Med. **68**, 152 (2002)
5 Di Mari, S. J.; Supple, J. H.; Rapoport, H.: J. Amer. Chem. Soc. **88**, 1226 (1966)
6 Rasku, S.; Wähälä, K.; Koskimies, J.; Hase, T.: Tetrahedron **55,** 3445 (1999)
7 Rasku, S.; Wähälä, K.: J. Labelled Cpd. Radiopharm. **43,** 849 (2000)
8 Wähälä, K.; Mäkelä, T.; Bäckström, R.; Brunow, G.; Hase, T.: J. Chem. Soc. Perkin Trans. I, 95 (1986)
9 Sandmeyer, T.: Helv. Chim. Acta **2**, 234 (1919)
10 Friedländer, P.; Roschdestwensky, N.: Chem. Ber. **48**, 1841 (1915)

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Pulse polarographic determination of levofloxacin in tablets

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Levofloxacin (**1**), (−)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate, is a quinolone antimicrobial agent which exhibits broad-spectrum *in vitro* bactericidal activities against gram-positive and gram-negative aerobes. **1** is the pure (−)-(*S*)- enantiomer of the racemic drug substance ofloxacin [1].

A few number of studies have been reported for the determination of **1** including first-derivative fluorescence spectroscopy [2], spectrofluorimetry [3], terbium-sensitized luminescence [4] and HPLC [5–9].

The aim of this study was to investigate the optimum polarographic conditions for the determination of **1** based on the reduction of the quinoline ring and to apply the method to pharmaceutical preparations. The optimum polarographic parameters were elucidated and **1** was determined using direct current (DC), differential pulse (DP), superimposed constant amplitude pulse (SCAP) and superimposed increasing amplitude pulse (SIAP) polarographic techniques in tablets. The experiments were conducted in the aqueous supporting electrolyte containing 0.2 mol/l KCl 10% v/v MeOH and 0.2 mol/l buffer solution.

A two step polarogram was obtained at pH values higher than 8 and lower than 5. The morphology of this curves dramatically changes depending on small changes in pH. A single step perfect polarogram was obtained at pH values between 5 and 8. Quantitative determination of **1** is not possible outside of this range. In spite of low limiting current, at pH between 5-8, suitable curves were obtained in the view of quantitative evaluation. Therefore pH 7.12 has been chosen as a working value. The stability of **1** in pH 7.12 buffer solution was examined. The prepared solutions give the same polarograms during a week time.

The process was found to be irreversible according to the criteria of Birke et al. [10], and the control of the polarographic current was diffusional at pH 7.12. The effect of temperature was investigated in the range of 15–45 °C. The variation of limiting current was found to be 1.15 μA/°C. The variation confirms that the polarographic current is diffusion controlled [11].

The calibration studies were performed using DC, DP, SCAP and SIAP polarographic techniques. The polarograms are demonstrated in the Fig.

The variation of **1** concentration was investigated in the range of $1 \times 10^{-4} - 5 \times 10^{-4}$ mol/l. The equations were calculated measuring the current at the maximum of the waves or peaks.

At −1340 mV for DC
$[i_{lim}(\mu A) = 1812.3\ C(mol/l) - 0.048\ r = 0.9998]$
At −1300 mV for DP;

$[i_{lim}(\mu A) = 1167.8\ C(mol/l) - 0.032\ r = 0.9997]$
At − 1360 mV for SIAP;

$[i_{lim}(\mu A) = 3630.6\ C(mol/l) - 0.017\ r = 0.9996]$
At the peak maximum for SCAP;

$[i_{lim}(\mu A) = 3570.6\ C(mol/l) - 0.112\ r = 0.9994]$

Table: Assay results of 1 in tablets[a]

	DCP		DPP		SCAPP		SIAPP		UV
	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	Filtered	Unfiltered	
Mean[b] (mg)	482	489	488	497	485	490	487	497	491
SD	0.14	0.17	0.15	0.18	0.17	0.18	0.16	0.20	0.12
CL ($p = 0.05$)	±0.2	±0.3	±0.3	±0.3	±0.2	±0.3	±0.3	±0.4	±0.2
F-test of significance	2.37	2.93	2.84	3.22	3.76	3.98	3.65	4.02	4.12 (table)
t-test of significance	1.47	1.29	1.78	1.96	1.70	187	1.76	2.07	2.14 (table)

[a]Each tablet contains 500 mg of **1**
[b]Each value is the average of eight determination
SD: standard deviation
CL: confidence limit

The detection limit (LOD) and limit of quantification (LOQ) were calculated to be 3×10^{-5} mol/l (S/N = 3) and 1×10^{-5} mol/l (S/N = 10), respectively.

The methods were applied to pharmaceutical preparations of **1**. The determination was performed with filtered and unfiltered solutions. The drug was also analysed by UV-spectrophotometry for comparison. The results were statistically evaluated using F and t tests and these are demonstrated in the Table. A high reproducibility was observed and insignificant differences were found between the polarographic techniques and UV-spectrophotometry at the 95% probability level. These results confirm the suggestions regarding the validity of the polarographic method in both filtered and unfiltered solutions [12].

It is concluded that the results of the polarographic techniques seem to be analytically acceptable according to official requirements. Therefore, it can be suggested for the routine analysis of **1** in the field of pharmaceutical control.

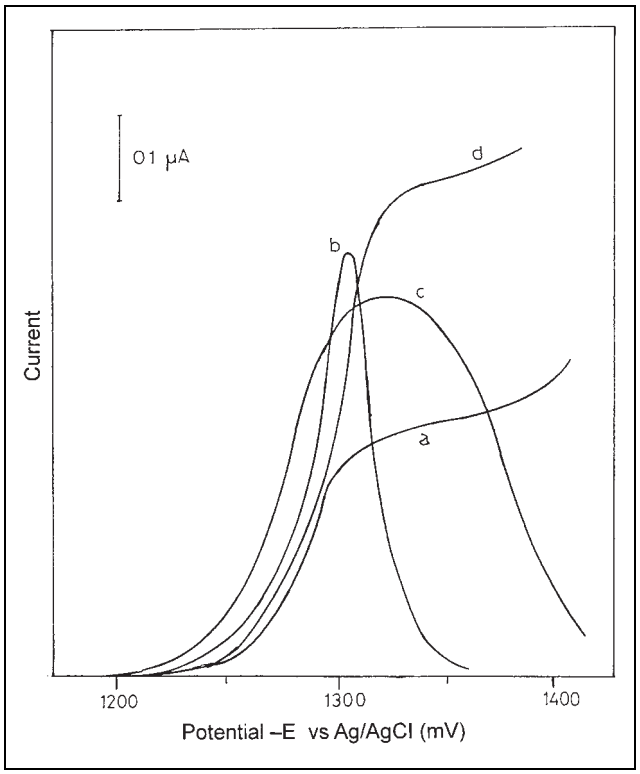

Fig: DC(a), DP(b), SCAP(c) and SIAP(D) polarograms of **1** (5×10^{-4} mol/l) at pH 7.12

Experimental

1. Apparatus and chemicals

A polarographic system comprising of Polaropulse Model PRG-5; the electrodes dual function EGMA type cell stand for polarography and voltammetry, with dropping Hg as working, Pt wire as auxiliary and saturated Ag/AgCl as reference electrodes were used (all Tacussel). The polarograms were recorded by a Model SE 790 X-Y recorder (BBC Goertz Metrawatt). A model WTW Multiline P4 Universal pH-meter cabled WTW Sen-Tix 97 T pH electrode (Germany) was adjusted the pH of the solutions. Spectrophotometric studies were done using a Model UV-2401 PC (Shimadzu). Standard **1** (99.8%) and tablets (Cravit®) were kindly supplied from Fako Ilac. A. S. (Istanbul, Turkey). The other chemicals were of analytical grade (E. Merck).

2. Procedures

2.1. Preparation of the stock solution

1×10^{-3} mol/l aqueous solution of **1** in methanol (10% v/v) was prepared and dilutions were made from this solution. The final concentration of the supporting electrolyte consisting of 10% v/v MeOH, 0.2 mol/l KCl and 0.2 mol/l phosphate buffer. (pH 7.12). The pH of the buffers were adjusted by 2 mol/l KOH or 2 mol/l HCl solutions.

2.2. Polarographic procedure

Ten ml supporting electrolyte containing 5×10^{-4} mol/l **1** was put into the polarographic cell and purified N_2 was passed through the solution for 10 min. 5×10^{-4} mol/l **1** was employed to investigate the effect of pH on the limiting current and the other polarographic parameters. The polarographic examinations were carried out by scanning cathodically in the range of 0 mV and −2000 mV against saturated Ag/AgCl reference electrode potential. The optimum conditions were: initial potential 0 mV, potential rate $10\ mV \cdot s^{-1}$, pressure applied to the mercury reservoir $1000\ dyne \cdot cm^{-2}$, pulse height 50 mV and drop time of 0.8 s.

2.3. Application to the tablets

Twenty tablets were separately weighed and the average weight of a tablet was calculated. The tablets were powdered in a mortar. A sample equivalent to one tablet was weighed and transferred to a 100 ml calibrated flask. 1 ml MeOH was added and the volume was made up with bidistilled water. Following vigorous shaking, half of the solution was filtered. The first 10 ml portion was discarded and the remaining solution was employed for the determination of **1** using the techniques mentioned above. The spectrophotometric assays were carried out dissolving the active material in 10% v/v MeOH. The calibration equation was found to be $A = 23408.4\ C(mol/l) + 0.013$ ($r = 0.9999$) at 288 nm under the mentioned conditions.

References

1 Tanaka, M.; Kurata, T.; Fujisawa, C.; Ohshima, Y.; Aoki, H.; Okazaki, O.; Hakusui, H.: Antimicrob. Agents Chemother. **37**, 2173 (1993)
2 Gong, Q. J.; Qiao, J. L.; Du, L. M.; Dong, C.; Jin, W. J.: Talanta **53**, 359 (2000)
3 Gonzales, J. A. O.; Mochon, M. C.; De la Rosa, F. J. B.: Talanta **52**, 1149 (2000)
4 Ocana, J. A.; Callejon, M.; Barragan, F. J.: Analyst **125**, 1851 (2000)
5 Wong, F. A.; Juzwin, S. J.; Flor, S. C.: J. Pharm. Biomed. Anal. **15**, 765 (1997)
6 Hansen, E.; Bucher, M.; Jakob, W.; Lemberger, P.; Kees, F.: Intensive Care Med. **27**, 371 (2001)

7 Sunderland, J.; Tobin, C. M.; Hedges, A. J.; MacGowan, A. P.; White, L. O.: J. Antimicrob. Chemother. **47**, 271 (2001)
8 Wright, D. H.; Herman, V. K.; Konstantinides, F. N.; Rotschafer, J. C.: J. Chromatogr. B **709**, 97 (1998)
9 Böttcher, S.; Baum, H. v.; Hoppe-Tichy, T.; Benz, C.; Sonntag, H. G.: J. Pharm. Biomed. Anal. **25**, 197 (2001)
10 Birke, R. L., Kim, M. H., Strassfeld, M.: Anal. Chem. **53**, 852 (1981)
11 Bauer, H. H., Christian, G. D., O'Reilly, J. E.: "Instrumental Analysis". Allyn and Bacon Inc., Boston, 1978, p.56
12 Volke, J.: Bioelectrochem. Bioenerg. **10**, 7 (1983)

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Separation of racemic drugs on chiral resorcinarene-bonded HPLC-columns

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Chiral separations are becoming more important for drug quality control. Because existing methods are often not sufficient to separate the enantiomers, the development of new selective stationary phases for HPLC-separations is of continued interest.

Calixarenes are macrocycles made up of phenolic units coupled to aldehydes [1]. Among them, resorcinarenes are known as host molecules for various compounds [2–5]. Relatively few papers have dealt with calix[4]arenes as immobilized [6] or solved [7–8] chiral selectors in CE. Healy et al. [9] described the use of silica-bonded calix[4]arenes modified at the lower rim with ephedrin units for chiral separations in HPLC.

To our knowledge, this is the first time that chiral resorcinarenes have been employed in HPLC. We tested eight stationary phases with different bound resorcinarenes. Chiral compounds were chosen that have broad variety in the structures around the chiral centers. Among them, basic, acidic and neutral compounds were tested due to the potential ability of complex formation of resorcinarenes with neutral as well as charged analytes [2–5]. Methanol (MeOH) or acetonitrile (MeCN)/20 mM NaH_2PO_4 (pH = 3.5) mixtures were used in the reversed-phase mode. Normal-phase separations were achieved with eluents containing hexane/isopropanol (Hex/IPA) and addition of 0.1% triethylamine (TEA) or 0.1% acetic acid (AA), respectively.

Two of the resorcinarene phases containing L-phenylalanine ethyl ester (RES-Phe) and S-1-(2-naphthyl)-ethylamine (RES-Naph) were most appropriate to separate a variety of compounds of pharmaceutical interest (Fig. 1).

Separations on the RES-Naph-phase were achieved in normal-phase systems, indicating that the main retention mechanism is due to interactions between polar structures of analytes and the stationary phase. Beside π-basic naphthyl substituents, resorcinarene possesses secondary amino and phenolic groups. Obviously, these substituents are involved mainly in a three point chiral recognition with the

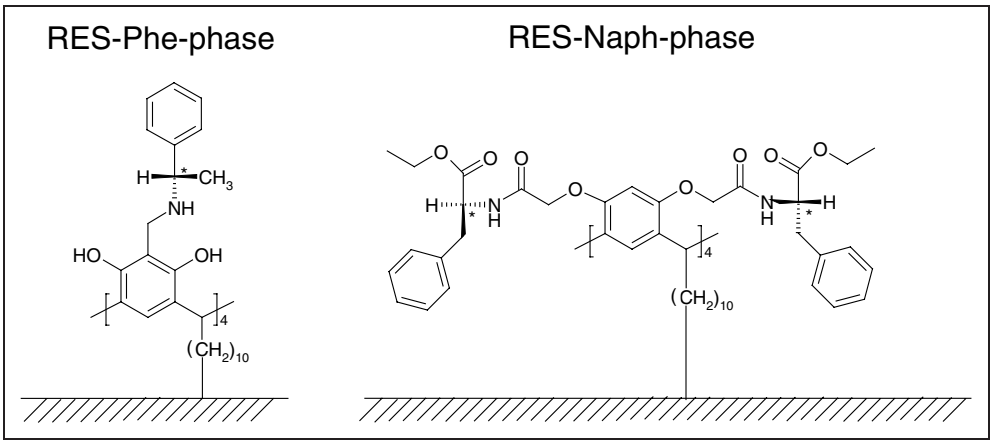

Fig. 1: Structures of investigated chiral resorcinarenephases
RES-Phe: L-phenylalanin ethyl ester linked with resorc[4]arene
RES-Naph: S-1-(2-naphthyl)-ethylamine linked with resorc[4]arene