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Determination of pefloxacin in serum by time-resolved fluorimetry

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Pefloxacin [1-ethyl-6-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid] belongs to the class of fluorinated quinolone antibiotics. It has a broad spectrum activity against many Gram-negative and some Gram-positive bacteria. The mechanism of its action is based on the inhibition of bacterial DNA gyrase, so interfering with normal bacterial replication. The most commonly used methods for the determination of pefloxacin in serum are microbiological [1], HPLC [2] and colorimetric determinations [3–6].

The present paper reports a new procedure for the assay of pefloxacin in serum by time-resolved fluorimetry. The method is based on quenching the fluorescence of europium(III) ion after the addition of serum containing pefloxacin. Since pefloxacin belongs to the class of fluorinated quinolones it may be determined by direct fluorimetry. However, the advantage of the use of time-resolved fluorimetry in the serum sample in relation to direct fluorimetry is that pefloxacin is determined directly, i.e., without preliminary preparation of the sample (deproteinization).

Europium(III) ions exhibit a strong fluorescence at 615 nm in a micellar solution containing Triton X-100, (0.1%), β-diketone (acyltrifluoroacetones) and 0.1 M acetate buffer pH 3.2 (enhacement solution). The fluoroketone forms a highly fluorescent complex with Eu(III). The surface active substance dissolves the chelating compound between Eu(III) and ketone and excludes water from the fluorescent complexes. Similar synergistic effects on Eu(III)-ketone fluorescence exhibits the Lewis base tri-n-oktyl-phosphine oxyde (TOPO).

The dose-response standard curve was constructed by plotting the ratio B/B_0, where B is the number of counts obtained with the sample, B_0 is the number of counts obtained with the solution containing only Eu(III) in enhancement solution, against the concentration of pefloxacin (2.5–250 μg/ml). The obtained dose-response standard curve was used for the determination of unknown drug concentrations in serum (Fig.).

Four serum samples were spiked with different concentrations of the drug. These samples were then used in the same procedure as for the standard curve. The results of four serum sample analysis are given in the Table.

The obtained results show that this method is simple, fast, has good accuracy and precision and can be used in routine analysis of perfloxacin in serum as an alternative to other [1–6] methods. The minimum detection limit, defined as the pefloxacin concentration corresponding to the mean fluorescence signal of 12 replicates of the zero standard plus 3 times the standard deviation is 0.08 μg/ml.

Using the time-resolved fluorescence in micellar medium no interference with serum components was seen. In contrast to previously reported procedures, time-resolved fluorimetry allows direct analysis of pefloxacin without any sample pretreatment.

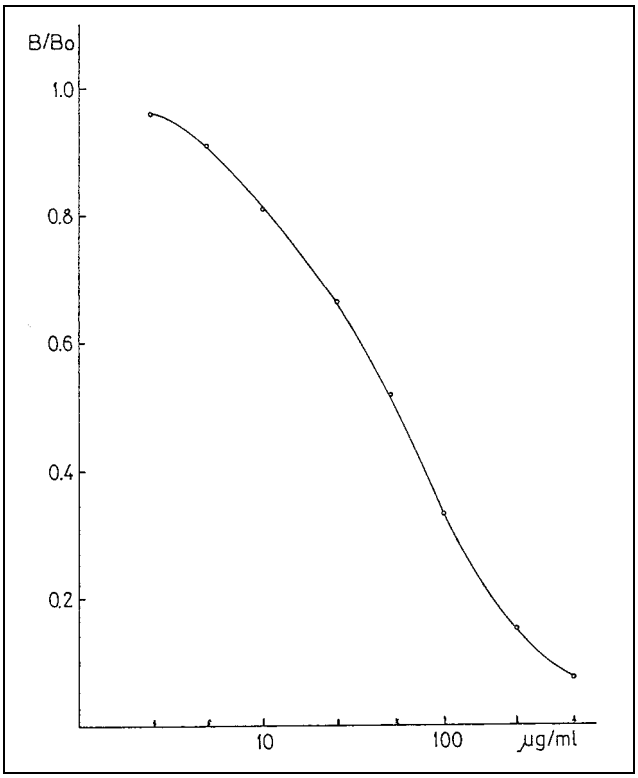

Fig. Dose-response standard curve for the time-resolved fluorimetric assay of pefloxacine (cocnentration of pefloxacin – 2.5–250 μg/ml – logarithmic values)

Table: Results of analysis of serum samples (n = 7) with the addition of various amounts of pefloxacin

Serum sample	Taken (μg/ml)	Found[a] (μg/ml)	Recovery (%)	Rsd (%)
1	8	7.7	96.3	3.9
2	12	11.7	97.5	2.9
3	16	16.3	101.9	2.7
4	20	26.6	103.0	2.4

[a] Average of seven determinations

Experimental

1. Apparatus

Fluorescence measurements were made with a LKB (Wallac Arcus, Finland) time-resolved fluorimeter. Fluorescence was measured by means of single-photon-counting time-resolved fluorometry using a xenon flash lamp (1000 Hz) for a total measuring time of 1 s with a 400 μs delay time and 400 μs counting time per flash. Excitation wavelength was at 340 nm with the emission at 615 nm. Fluorescence of europium(III) was measured in a solution containing 15 μmol/l 2-naphtoyltrifluoroacetone, 50 μmol/l tri-n-octyl-phosphine oxyde (TOPO), 0.1% Triton X-100 and 0.1 M acetate buffer pH 3.2 (enhancement solution).

A Corning (USA) model 250 pH-meter equipped with a combined Ross (Orion, USA) electrode was used for pH measurements.

2. Reagents

All reagents and solvents were of analytical reagent grade, unless otherwise stated. Pefloxacin methanesulfonate dihydrate was from Rhone Puolenc, (France), purity 99.6%. The enhancement solution was purchased from Delfia (Wallac Oy, Finland). Europium(III) nitrate solution was prepared by dissolving europium(III)-oxide (99.9%, Sigma, USA) in nitric acid (Merck, FRG). For preparation of all standard solutions doubly distilled water (conductivity less than 10 μS) was used.

3. Analytical procedure

The 25 μl of human pool serum samples containing pefloxacin in the concentration range of 2.5–250 μg/ml, were added to 250 μl of a solution

containing 15 μmol/l β-diketone, 50 μmol/l tri-n-octyl-phosphine oxyde (TOPO), 0.1% Triton X-100, and 0.1 nmol/l europium (III) in 0.1 M acetate buffer pH 3.2. The obtained solution was mixed well and the fluorescence was measured at 615 nm. The dose-response standard curve was constructed by plotting the ratio B/Bo against the concentration of pefloxacin. The obtained curve was used for the determination of unknown drug concentrations in serum.

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References

1 Gonzales, J. P.; Henwood, J. M.: Drugs **37**, 628 (1989)
2 Clan, C. Y.: Lam, H. W.; French, G. L.: J. Antimicrob. Chemother. **23**, 597 (1989)
3 Kuchekar, B. S.; Shetty, R. S.: J. Inst. Chem. (India) **65**, 185 (1993)
4 Avdahanalu, A. B.; Pantutu, A. R.; Anjaneyulu, Y.: East Pharm. **37**, 123 (1994)
5 Kuchehar, B. S.: Shetty, R. S.: East Pharm. **37**, 171 (1994)
6 Jelikić-Stankov, M.; Veselinović, D.; Malešev, D.; Radović, Z.: J. Pharm. Biomed. Anal. **7**, 1571 (1989)

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Studies on formulation and evaluation of oral osmotic pumps of nimesulide

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The purpose of this communication is to report the findings of an attempt to develop an oral osmotic pump (OP) of nimesulide (NE). The elementary osmotic pump (EOP) consists of an osmotic core having drug surrounded by a semipermeable (SP) membrane and an orifice [1]. When the EOP reaches the gastro intestinal tract (GIT), the core imbibes GI fluid at a constant rate determined by membrane permeability and osmotic pressure inside the core. For a system at constant internal volume, the EOP delivers, in any time interval, a volume of saturated drug solution equal to the volume of solvent uptake. The drug delivery rate remains contant as long as excess solid is present inside the device.

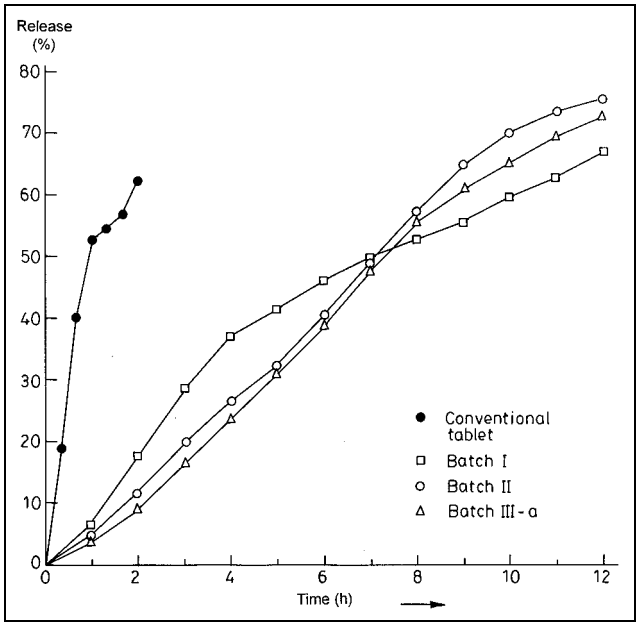

Fig.: Cumulative percent release profiles of EOP's of NE in comparison to conventional tablets

NE, a potent NSAID, is a poorly water soluble (~0.01 mg/ml) drug [2] and is associated with problems of frequent administration and GI disturbances when given in conventional formulations. Generally, EOP is well suited for drugs of intermediate water solubility [3]. However, by use of buffers along with the drug, it is possible to modulate the drug's solubility and thus to formulate EOP of poorly water soluble drugs [4]. Based on the above facts, this study was aimed towards the develop-

Table: Formula, data (mean ±SD) for different parameters and time to release 30% ($t_{30\%}$) and 60% ($t_{60\%}$) NE from different formulations

Ingredients (quantity in mg/tab)	Batch. No. of formulations				
	I	II	III-a	III-b	III-c
Nimesulide	100	100	100	100	100
SBC	–	50	20	20	20
Coated SBC	–	–	80	80	80
DSP	100	100	–	–	–
NaCl	200	200	–	–	–
KCl	–	–	300	300	300
SLS	12	15	15	15	15
Talc	4	5	5	5	5
PVP	8	9	10	10	10
Coating nature	SP	SP	SP	SP*	MP
Hardness kg/cm^2 (n = 3)	8.00 ± 0.72	8.21 ± 0.96	8.46 ± 0.09	8.46 ± 0.09	8.46 ± 0.09
Thickness before coating, mm (n = 10)	4.26 ± 0.98	4.61 ± 1.21	5.01 ± 0.68	5.01 ± 0.68	5.01 ± 0.68
Weight variation, mg (n = 20)	430 ± 1.96	491 ± 0.96	550 ± 1.21	552 ± 0.96	549 ± 1.09
Coating thickness, μm (n = 3)	200 ± 0.46	215 ± 0.72	208 ± 1.21	208 ± 0.98	209 ± 1.21
Content of active ingredient, mg (n = 5)	102.48 ± 1.08	101.72 ± 2.18	99.92 ± 0.96	99.92 ± 0.96	99.92 ± 0.96
Orifice diameter, μm (n = 5)	320.67 ± 0.43	321.00 ± 0.42	320.21 ± 0.58	320.19 ± 1.21	–
$t_{30\%}$ (h)	3.2	4.6	4.8	6.8	2.6
$t_{60\%}$ (h)	10.2	8.4	8.8	**	5.7

SBC: Sodium bicarbonate; Coated SBC: Sodium bicarbonate coated with 10% w/w cellulose acetate phthalate in acetone; DSP: Disodium hydrogen phosphate dihydrate; SLS: Sodium lauryl sulphate; PVP: Poly vinyl pyrolidone; SP: Semipermeable (coating with 2% w/w cellulose acetate in acetone); SP* Semipermeable (coating with 2% w/w ethyl cellulose in ethanol); MP: Microporous (coating with 2% w/w solution of cellulose acetate/sorbitol/PEG 400 (10 : 7.5 : 1) by parts dissolved in acetone; ** Not achieved