

Determination of deferiprone in urine and serum using a terbium-sensitized luminescence method

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ABSTRACT: Optimized conditions, validation and practical applications of a new, rapid and specific fluorometric method for the determination of deferiprone (DFP) in urine and serum samples are reported. The proposed method, which is based on the formation of a luminescent complex with Tb³⁺ ion, is evaluated in terms of linearity, accuracy, precision, stability, recovery and limits of detection (LOD) and quantification (LOQ). Under optimum conditions (pH 7.5, [Tb³⁺] = 3×10^{-4} mol/L, temperature 0°C and excitation wavelength 295 nm), the relative intensities at 545 nm are linear, with the concentration of DFP in the range 0.072–13 mmol/L for urine and serum samples. The LOD and LOQ, respectively, are calculated to be 0.014 and 0.045 mmol/L for urine and 0.022 and 0.072 mmol/L for serum samples. The intra-day and inter-day values for the precision and accuracy of the proposed method are all < 5%, and the recovery of the method is in the range 97.1–103.8%. The method was applied to human urine and serum samples collected from patients receiving DFP. The results indicated that the method can be successfully applied to the determination of DFP in human urine and serum samples collected for clinical or biopharmaceutical investigations in which simple, rapid, cheap and specific determination methods facilitate and speed up the analytical procedure. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: terbium-sensitized; deferiprone; serum; urine; fluorometric method

Introduction

Iron overload due to chronic transfusion therapy causes organ damage; therefore, chelators of iron, such as deferiprone (DFP), should be used to remove excess iron from various parts of the body. DFP (1,2-dimethyl-3-hydroxypyridin-4-one) (with a molecular weight 139.15 g/mol) is an active iron chelator and superoxide-radical scavenger, and belongs to the new class of chelating agents, α -ketohydroxypyridines. DFP, the first oral iron chelator, is prescribed mainly in β -thalassaemia minor, Cooley's anaemia and hereditary haemochromatosis. It is also indicated in treating cancer and haemodialysis. DFP can inhibit DNA synthesis and therefore can be used as anti-HIV replication drug; in addition, it is a potent chelator of iron in the mitochondrial matrix and can be applied for treatment of Friedreich's ataxia (1–7).

To maintain a negative iron balance in overloaded patients, DFP must be administered at a high daily dosage of 75 mg/kg (8). The limited efficiency of DFP is mainly due to extensive phase II drug metabolism in the liver, where the hydroxyl group – essential for chelation and iron clearance – undergoes glucuronidation (9). Arthropathy and neutropenia are very frequent side-effects of DFP and need strict monitoring during therapy. Most of the neutropenia cases are neither very severe nor do they recur with a rechallenge with the drug. Similarly, arthropathy does not need withdrawal of the drug in the majority of the patients (10).

In a pharmacokinetic study in humans, the plasma concentration of DFP was reported to vary from 25 μ mol/L (12 min after oral administration of 3 g DFP) to 450 μ mol/L (30 min after the

same dose) (11). The maximum reported concentration of DFP excreted in urine was 306.1 ± 67.6 μ mol/L, observed 3 h after administration of a single dose of 500 mg. The average excretion rate was 0.0001 μ mol/min/kg at 24 h after drug administration. Abbas *et al.* (12) reported that 2.7% of DFP is excreted in urine and a plasma concentration of 23.2 ± 1.9 μ mol/L is obtained after oral administration of 500 mg DFP. The daily dose of DFP needed to treat iron overload is 2–3 g every 6 h (11).

Complexes of trivalent lanthanide ions, especially terbium, with appropriate ligands have attracted more attention in recent years because the specific physicochemical properties of this ion, as a result of its electronic structure, make it useful for probes and sensors in the chemical and medical sciences. The electron transition in the 4f shells is responsible for the narrow

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emission bands in the NIR to UV range, with long emission lifetimes, large Stokes' shifts (> 200 nm) and microsecond luminescence decay times (13–19).

Lanthanide-sensitized luminescence is a highly selective method because when lanthanide ions form chelates with certain organic ligands, characteristic emission spectra are obtained. These ions, as analytical reagents, are stable and can form stable luminescent complexes with ligands; they have poor absorption in the gastrointestinal tract and cannot penetrate living cells when injected (16). According to these characteristics, terbium ions are widely employed in some applications, such as the investigation of the function of nucleic acids, immunoassays, direct determination of organic compounds and detection of analytes in chromatographic and electrophoretic methods (16–20).

High-performance liquid chromatography (7) and voltammetry (6,21,22) methods have been developed for the determination of DFP in biological fluids. Our earlier observations showed that a simple terbium-sensitized luminescence method based on the formation of a Tb^{3+} -DFP complex in aqueous solutions could be developed. The aim of this study was to develop and validate a simple and rapid method for the determination of DFP in samples of urine and serum. It is obvious that simple, cheap, rapid and selective analytical methods are highly in demand in pharmaceutical and biomedical analyses. Data related to the formation of complexes of Fe^{2+} and Fe^{3+} with DFP, which are required for the justification of the biological effects of DFP, were also investigated.

Experimental

Reagents and solutions

All chemicals and solvents were of analytical grade. Double-distilled water was used throughout this study. A 10 mmol/L solution of terbium(III) was prepared by dissolving the appropriate amount of terbium(III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$) (Acros Organics, Fairlawn, NJ, USA) in double-distilled water, which was stored in polyethylene containers to avoid memory effects of terbium adsorbed on glass vessels.

A stock solution (7.2 mmol/L) of DFP (Arasto Pharmaceutical Company, Tehran, Iran) was prepared by dissolving DFP in absolute ethanol and stored at room temperature. A 10 mmol/L solution of Tris-(hydroxymethyl) aminomethane-hydrochloric acid (Tris-HCl) buffer was prepared by dissolving the desired amount of Tris-HCl (Merck, Darmstadt, Germany) in water, finally making up the volume to 100 mL with water. Working standard solutions were prepared daily by dilution of the stock standard solutions with water.

Apparatus

Measurements of the luminescence spectra and intensity were carried out using a JASCO FP-750 spectrofluorometer (Tokyo, Japan) equipped with a 150-W xenon lamp and 1.0 cm quartz cells. The excitation and emission monochromator bandwidths were 10 nm. The excitation wavelength was set at 295 nm, and the luminescence was measured using the peak height at 545 nm. All measurements were carried out at 0.0 ± 0.1 °C, using a Peltier thermostated cell holder (Tokyo, Japan). The pH of the solutions was measured using a Metrohm Model 744 pH meter (Herisau, Switzerland).

Sample preparation

Samples of serum and 24 h urine were collected from patients taking 500–1500 mg DFP/day. The research protocol was confirmed by the

Ethics Committee of the Tabriz University of Medical Sciences, and the patients gave their written informed consent to participate in this project. Except for a 1000-fold dilution with water, no further pretreatment was required for the urine and serum samples.

Validation of the method

The specificity of the method was confirmed by analysing 10 samples of DFP at the limit of quantification (LOQ) in both the presence and absence of urine and serum matrices; then, the matrix factor for each sample was calculated using the ratio I/I_0 , where I and I_0 are the responses in the presence and absence of the matrix, respectively. As the serum and urine samples were diluted 1000 times, the matrices of the samples were very similar; therefore, most of the validation results were very close in the serum and urine samples.

Linearity data were assessed by analysing samples of urine and serum spiked with DFP, then diluted 1000-fold, in which the final concentration range was 0.072–13.0 μ mol/L. The linearity was evaluated by linear regression analysis.

The LOD and LOQ were calculated as $3S_b/m$ and $10S_b/m$, respectively, where S_b is the standard deviation of the blank and m is the slope of the calibration graph.

The precision of the method was determined by calculating intra-day and inter-day precision. For intra-day precision, six repeated analyses of urine and serum were carried out at concentrations of 0.072, 7.2, and 13.0 μ mol/L. Inter-day precision was evaluated by repeated analyses on 3 days using the same samples and then calculating the relative standard deviation (RSD).

Accuracy was expressed as the percentage of deviation between the true and measured values. To assess the accuracy, replicated analyses of urine and serum samples spiked at concentrations of 0.072, 7.2 and 13.0 μ mol/L were carried out.

For evaluation of the recovery, the serum and urine samples were spiked with standard DFP solution at concentrations of 0.072, 7.2 and 13.0 μ mol/L, then the spiked samples were assessed. Subsequently, the recovery of the method was expressed as a percentage of the added concentrations.

For determination of stability at room temperature, three samples were prepared at concentrations of 0.072, 7.2 and 13.0 μ mol/L and maintained at room temperature (23 ± 2 °C) for 12, 24, 48 and 72 h. The samples were analysed and the obtained concentrations were compared with the original concentrations. For evaluation of the effect of freeze-thaw cycles on the stability of the products, samples were prepared and stored at -20 °C; the samples were analysed after 24, 48 and 72 h and the obtained concentrations were compared with the corresponding original values.

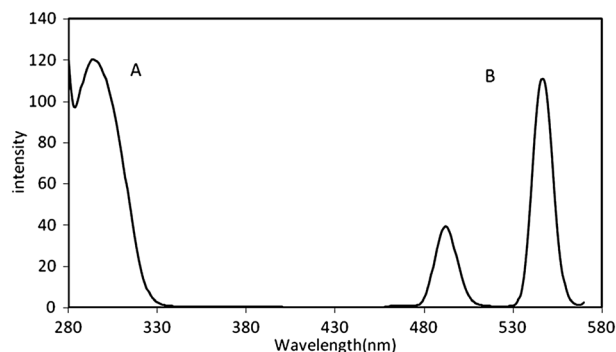


Figure 1. Luminescence excitation (A) and emission (B) spectra of: Tb^{3+} -DFP complex. Experimental conditions: $[Tb^{3+}] = 10^{-5}$ mol/L; $[DFP] = 7.2 \times 10^{-6}$ mol/L; pH = 7.5; $\lambda_{ex}/\lambda_{em} = 295/545$ nm.

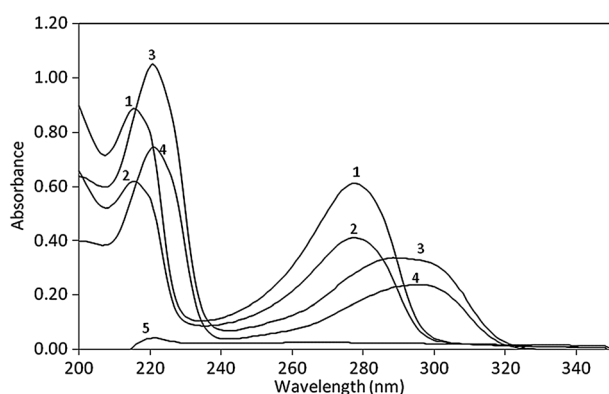


Figure 2. Absorption spectra of DFP in different systems (background correction was performed using a reference solution): DFP (1, 2); Tb^{3+} -DFP (3, 4); and Tb^{3+} (5). Conditions: $[Tb^{3+}] = 1.0 \times 10^{-4}$ mol/L; $[DFP] = 2.87 \times 10^{-5}$ mol/L (1, 3), 1.44×10^{-5} mol/L (2, 4).

Results and discussion

Fluorescence emission and excitation spectra of Tb^{3+} -DFP are shown in Fig. 1. Solutions containing only Tb^{3+} or DFP have no luminescence with excitation at 295 nm. Under the same conditions, the characteristic luminescence spectrum of Tb^{3+} -DFP was observed, with two emission peaks at 545 nm and 490 nm. These peaks are the characteristic luminescence peaks of Tb^{3+} and correspond to $^5D_4 \rightarrow ^7F_6$ and $^5D_4 \rightarrow ^7F_5$ transitions, respectively, of which the emission at 545 nm is much stronger. The luminescence intensity was proportional to the concentration of DFP.

Absorption spectra of Tb^{3+} , DFP and Tb^{3+} -DFP are shown in Fig. 2. It can be seen that after addition of Tb^{3+} into the DFP solution, a small red shift occurred in the maximal absorption peak, which is due to the formation of Tb^{3+} -DFP complex.

Effects of pH, buffer volume, terbium concentration and temperature were optimized and pH 7.5, buffer volume 0.5 mL, terbium concentration 3×10^{-4} mol/L and temperature $0^\circ C$ were found to be the optimized conditions. Under the optimal conditions, calibration graphs for the determination of DFP were constructed. The linear range in both serum and urine was 7.2×10^{-9} – 1.3×10^{-5} mol/L.

A systematic study of the effects of a number of coexisting substances on the luminescence of the Tb^{3+} -DFP system was carried out under optimal conditions. The concentrations of the substances examined were in the range of their normal levels in biological samples, and the results obtained are shown in Table 1. These substances (existing in normal urine and serum) have no effect on the determination of DFP under the permitted 5% error limit. It should be mentioned that after the 1000-fold dilution of urine and serum, possible interferences from creatinine, urea and ions, such as bicarbonate, magnesium and phosphate, were eliminated.

Under optimal analytical conditions, calibration graphs for the determination of DFP were constructed. The linearity parameters for each medium – urine and serum – are shown in Table 2. The results of linearity evaluation indicated that this method is linear for the determination of DFP in samples with correlation coefficients of 0.999 and 0.997, respectively, for urine and serum. The detection limit, calculated as $3S_b/m$, where S_b and m are the standard deviation (SD) of the blank and the slope of the

Table 1. Effects of common interferents on the determination of 7.2 μ mol/L deferiprone

Coexisting substance	$\Delta F\%^*$	Examined concentration** of coexisting substance (mol/L)	Normal concentrations	
			Urine mol/24 h (ref)	Serum mol/L (ref)
K^+ (Cl^-)	2.33	0.0031 [430]	92×10^{-3} (23)	4.8×10^{-3} (24)
Na^+ (Cl^-)	3.1	0.11 [15277]	150×10^{-3} (23)	149.8×10^{-3} (24)
Al^{3+} (Cl^-)	−4.00	0.33×10^{-6} [0.046]	$(2-5) \times 10^{-6}$ (25)	–
Phosphate	−4.5	1.00×10^{-4} [14]	$\geq 1 \times 10^{-3}$ (23)	$(1.12-1.45) \times 10^{-3}$ (24)
Cu^{2+} (Cl^-)	−2.8	1.00×10^{-6} [0.14]	$\leq 1 \times 10^{-6}$ (24)	$(7.7-18.6) \times 10^{-6}$ (24)
Ca^{2+} (Cl^-)	−3.9	1.00×10^{-3} [139]	$(2.5-7.5) \times 10^{-3}$ (26)	$(2.1-2.5) \times 10^{-3}$ (26)
Mg^{2+} (Cl^-)	−3.5	0.5×10^{-5} [0.7]	$(5.2-6.2) \times 10^{-3}$ (23)	0.9×10^{-3} (25)
Zn^{2+} (Cl^-)	−3.6	2.50×10^{-6} [0.35]	$(2.3-15.3) \times 10^{-6}$ (24)	$(11.5-19.8) \times 10^{-5}$ (24)
Fe^{3+} (Cl^-)	−4.9	1.75×10^{-7} [0.024]	–	$(9.0-31.3) \times 10^{-6}$ (27)
Uric acid	−4.5	5.90×10^{-6} [0.82]	$(1.48-4.43) \times 10^{-3}$ (28)	$(1.8-4.2) \times 10^{-3}$ (28)
Glucose	2.50	2.33×10^{-2} [3236]	–	$(3.8-7.2) \times 10^{-3}$ (29)
HCO_3^- (Na^+)	−3.5	0.25×10^{-5} [0.35]	2×10^{-3} (23)	$(25-26) \times 10^{-3}$ (30)
Fructose	2.00	1.00×10^{-3} [139]	–	8.1×10^{-6} (31)
Urea	3.15	0.015 [2083]	0.38 (23)	$\{2.5-4\} \times 10^{-3}$ (23)
Citric acid	2.00	0.005 [694]	$(1.66-6.45) \times 10^{-3}$ (32)	0.011×10^{-3} (33)
Creatinine	2.35	0.005 [695]	0.044–0.17 (34)	$\{0.07-0.12\} \times 10^{-3}$ (34)
Dopamine	4.00	0.5×10^{-4} [7]	$(0.42-2.6) \times 10^{-6}$ (35)	–
Epinephrine	3.00	2.0×10^{-5} [2.8]	$(0.027-1.1) \times 10^{-7}$ (35)	$(0-0.49) \times 10^{-11}$ (35)
Norepinephrine	3.24	3.0×10^{-5} [4.2]	$(0.89-4.7) \times 10^{-7}$ (35)	$(0-3.6) \times 10^{-9}$ (35)

* $\Delta F\%$, percentage of changed luminescence.

** Values in brackets are ratios of drugs to coexisting substance concentrations.

Table 2. Linearity parameters of the proposed method (after 1000-fold dilution)

Sample	Data point*	Slope	Y-intercept	r^{**}	Range ($\mu\text{mol/L}$)	LOD***	LOQ***
Urine	9	25.001	3.112	0.999	0.072–13	0.014	0.045
Serum	9	25.001	3.111	0.997	0.072–13	0.022	0.072

* Data point is the number of concentrations included in the calibration graphs.

** r , regression coefficient of calibration graph. Each sample analysis was repeated three times.

*** LOD and LOQ values were calculated from calibration graphs in $\mu\text{mol/L}$ and the real LOD and LOQ were in $\mu\text{mol/L}$ (after multiplying in the dilution factor of 1000).

calibration graph, respectively, was $6.3 \times 10^{-9} \text{ mol/L}$. The LOD and LOQ for each medium were calculated from the calibration graphs.

The results of the specificity studies of the method are given in Table 3. As the results indicate, the matrix factor for each sample was approximately 1; therefore, this method is specific for the determination of DFP in urine and serum matrices.

The results for accuracy, precision and recovery of the method are given in Table 4. These results illustrate that this method is accurate and precise for the determination of DFP and can recover DFP from urine and serum matrices. As noticed above, serum and urine samples were diluted 1000 times with water, and their matrices were very similar; therefore, most of the validation data for serum and urine were very close to each other.

Details of the analytical performances of the previously reported methods and the proposed method for the determination of DFP are summarized in Table 5. Compared with the previous methods, the proposed method has a relatively lower detection limit.

Analytical applications

Determination of DFP in human urine samples. The proposed method was used to determine the DFP content of urine samples obtained from patients. For assay of DFP, the samples must be diluted appropriately (1000-fold) with water, hence the obtained data from the calibration graph were multiplied

by 1000 and reported in Table 6. It should be noted that the samples were taken from 24 h urine samples with various differences in terms of time and tablet intakes.

Determination of DFP in human serum samples. The developed method was applied to the determination of DFP in serum samples of patients. For assays of DFP in serum, the samples must be diluted appropriately (1000-fold) within the linear ranges of determination. The results obtained are shown in Table 6. The developed method can be easily carried out, as the results show, and affords good precision and accuracy when applied to real samples.

Complexes of DFP with Fe^{2+} and Fe^{3+} . It is believed that a majority portion of DFP circulating in the blood or excreted in

Table 4. Precision (intra-day and inter-day), accuracy and recovery of the method in biological fluids*

Sample	Concentration ($\mu\text{mol/L}$)	Intra-day (RSD %)	Inter-day (RSD %)	Accuracy (RSD %)	Recovery (%)
Urine	0.072	4.4	1.6	2.9	97.1
	7.2	1.4	0.9	1.8	98.2
	13	1.2	0.9	1.3	98.7
Serum	0.072	0.2	1.2	1.2	102.7
	7.2	2.4	2.0	2.0	103.7
	13	1.1	0.8	0.8	103.8

* Each analysis was repeated three times.

Table 3. Specificity of the method for determination of DFP in biological fluids

Sample No.	Matrix factor* in serum	Matrix factor in urine
1	1.085	0.955
2	1.055	0.975
3	1.075	0.925
4	1.045	0.940
5	1.095	0.945
6	1.090	0.965
7	1.065	0.920
8	1.050	0.955
9	1.085	0.950
10	1.090	0.900

* Matrix factor, response in the presence of matrix/response in the absence of matrix. Each analysis was repeated six times.

Table 5. Comparison of proposed method with other methods used for determination of deferiprone

Method	Linear range (mol/L)	Detection limit (mol/L)	Reference
HPLC	3.5×10^{-6} – 1.43×10^{-5}	3.5×10^{-7}	(7)
Voltammetry	9.9×10^{-5} – 5.34×10^{-4}	1.9×10^{-5}	(21)
Cyclic voltammetry	3.0×10^{-5} – 1.0×10^{-3}	1.43×10^{-5}	(6)
	5.0×10^{-5} – 1.0×10^{-3}	5.25×10^{-7}	(22)
Terbium sensitized fluorescence	7.2×10^{-9} – 1.3×10^{-5}	6.3×10^{-9}	This work

Table 6. Determination of deferiprone in urine and serum samples of patients receiving DFP

Sample No.	Prescribed dose (mg/day)	Urine**		Serum**	
		Detected amount (μmol/L)	RSD (%)*	Detected amount (μmol/L)	RSD (%)*
1	500	96	2.8	81	3.5
2	1000	879	1.5	336	1.8
3	1000	871	2.0	167	2.3
4	1500	1546	2.9	370	3.2
5	1000	463	1.3	232	1.6
6	1000	863	3.0	140	2.4
7	1500	623	3.6	200	3.1

* The separate samples were prepared from urine and serum samples of a patient and the RSD values were calculated using mean and SD of four measurements.

** The calculated data from the calibration graphs were multiplied by 1000, since the samples were diluted 1000 times.

Table 7. Details of the stability of the proposed method for three concentrations investigated

	Nominal concentration (μmol/L)	Found concentration (μmol/L)			
		12 h	24 h	48 h	72 h
Room temperature	0.072	0.071	0.070	0.070	0.068
	7.20	7.24	7.30	7.20	7.16
	13.00	13.04	12.65	12.85	12.75
−20 °C	0.072	–	0.074	0.071	0.072
	7.20	–	7.34	7.28	7.21
	13.00	–	13.24	13.18	12.90

* Each analysis was repeated three times.

urine is in the chelated form with iron. The complex-formation constants of Fe^{2+} and Fe^{3+} with DFP are not available from the literature. However, this information is required to evaluate the iron-chelating efficiency of DFP and the possibility of replacing the Fe^{2+} and Fe^{3+} ions with Tb^{3+} to form luminescent complexes. To provide this information, the stoichiometries of the complexes between Fe^{2+} and Fe^{3+} with DFP were investigated using Job's method (36). Equal concentrations of Tb^{3+} and DFP were used, and the ratio of the molar fractions of Tb^{3+} and DFP was varied; the luminescence intensity of the complex was then recorded. The maximum intensity was obtained at a mole fraction of 0.32 for Fe^{2+} and 0.26 for Fe^{3+} . Thus, the stoichiometries of Fe^{2+} :DFP and Fe^{3+} :DFP were detected to be 1:2 and 1:3. The complex-formation constants were calculated using a method described elsewhere (36) and were found to be 1.7×10^8 for Fe^{2+} -DFP and 3.9×10^{12} for Fe^{3+} -DFP complexes. By comparing the complex-formation constants of Tb^{3+} -DFP (i.e. 1.6×10^{16}) and Fe^{3+} -DFP, and because both complexes have the same stoichiometry, the replacement of Fe^{3+} by Tb^{3+} could be easily justified. This is also confirmed by the interference result of Fe^{3+} reported in Table 1.

Stability results

The stability data for the proposed method are shown in Table 7. There were no significant differences between the original and

final concentrations, indicating that the proposed method is stable in the short-term at room temperature and at -20°C . Therefore, this method can be applied successfully to samples stored either at room temperature or in a freezer.

Conclusions

In this study, a previously developed spectrofluorometric method was validated and applied for the determination of trace amounts of DFP in urine and serum samples. The results illustrated that this method was specific, linear, accurate and precise for the determination of DFP in urine and serum. These characteristics and the obtained LOD and LOQ values proved the reliability and applicability of the proposed method. The method was successfully applied for the determination of DFP in urine and serum samples obtained from patients who were routinely receiving DFP. In addition to the higher specificity and lower LOD and LOQ values of this method in comparison with the HPLC and voltammetric methods, its simplicity and the elimination of pretreatment, because of the non-interference between DFP and biological matrices, could be considered as its main advantages, especially for routine analysis of DFP in biological samples, in therapeutic drug monitoring and in pharmacokinetic studies.

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References

1. Cappellini MD. Exjade (deferiprone, ICL670) in the treatment of chronic iron overload associated with blood transfusion. *Ther Clin Risk Manag* 2007;3:291–9.
2. Debebe Z, Ammosova T, Jerebtsova M, Kurantsin-Mills J, Niu X, Charles S, Richardson DR, Ray PE, Gordeuk VR, Nekhai S. Iron chelators ICL670 and 311 inhibit HIV-1 transcription. *Virology* 2007;367:324–33.
3. Goncalves S, Paupe V, Dassa EP, Rustin P. Deferiprone targets aconitase: implication for Friedreich's ataxia. *BMC Neurol* 2008;8:20–25.
4. Wanless IR, Sweeney G, Dhillon AP, Guido M, Piga A, Galanella R, Gamberini MR, Schwartz E, Cohen AR. Lack of progressive hepatic

- fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent β -thalassemia. *Blood* 2002;100:1566–9.
5. Olivieri NF, Brittenham GM. Long-term trials of deferiprone in Cooley's anemia. *Ann NY Acad Sci* 1998;80:217–22.
6. Yadegari H, Jabbari A, Heli H, Moosavi-Movahedi AA, Majidi S. Electrochemistry of deferiprone as an orally active iron chelator and HIV-1 replication inhibitor and its determination. *J Braz Chem Soc* 2008a;19:1017–22.
7. Goddard JG, Kontoghiorghe GJ. Development of an HPLC method for measuring orally administered 1-substituted 2-alkyl-3-hydroxypyrid-4-one iron chelators in biological fluids. *Clin Chem* 1990;36:5–8.
8. Balfour JAB, Foster RH. Deferiprone: a review of its clinical potential in iron overload in β -thalassemia major and other transfusion-dependent disease. *Drugs* 1999;58:553–78.
9. Kalinowski DS, Richardson DR. The evolution of iron chelators for the treatment of iron overload disease and cancer. *Pharmacol Rev* 2005;57:547–83.
10. Choudhry VP, Pati HP, Saxena A, Malaviya AN. Deferiprone, efficacy and safety. *Ind J Pediatr* 2004;71:213–16.
11. Kontoghiorghe GJ, Goodard JG, Bartlett AN, Sheppard L. Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. *Clin Pharmacol Ther* 1990;48:255–61.
12. Abbas M, Nawaz R, Shahid M, Nawaz M, Alim M, Asi MR. Evaluation of urinary excretion and renal clearance of deferiprone, creatine, iron and zinc in human. *Asian J Chem* 2009;21:4583–92.
13. Dossing A. Luminescence from lanthanide³⁺ ions in solution. *Eur J Inorg Chem* 2005;8:1425–34.
14. Ndao AS, Buzady A, Erotyak J, Hornyak I. Sensitized luminescence of trivalent lanthanide complexes Eu³⁺/quinadine acid and Eu³⁺/1,4-dihydro-oxo-chinoline-3-carboxylic acid. *J Fluoresc* 2008;18:649–54.
15. Leonard JP, Gunnlaugsson T. Luminescent Eu(III) and Tb(III) complexes: developing lanthanide luminescent-based devices. *J Fluoresc* 2005;15:585–95.
16. Gomez-Hens A, Aguilar-caballeros MP. Terbium-sensitized luminescence: a selective and versatile analytical approach. *Anal Chem* 2002;21:131–41.
17. Ioannou PC, Rusakova NV, Andrikopoulou DA, Glynnou KM, Tzompanski GM, Rusakova NV. Spectrofluorimetric determination of anthranilic acid derivatives based on terbium sensitized fluorescence. *Analyst* 1998;123:2839–43.
18. Yegorova AV, Scripinets YV, Duerkop A, Karasyov AA, Antonovich VP, Wolfbeis OS. Sensitive luminescent determination of DNA using the terbium(III)–difloxacin complex. *Anal Chim Acta* 2007;584:260–67.
19. Miao Y, Liu J, Hou F, Jiang C. Determination of adenosine disodium triphosphate (ATP) using norfloxacin–Tb as a fluorescence probe by spectrofluorimetry. *J Lumin* 2006;116:67–72.
20. Yu F, Li L, Chen F. Determination of adenosine disodium triphosphate using prulifloxacin–terbium(III) as a fluorescence probe by spectrofluorimetry. *Anal Chim Acta* 2007;610:257–62.
21. Hajjizadeh M, Jabbari A, Heli H, Moosavi-Movahedi AA, Shafiee A, Karimian K. Electrocatalytic oxidation and determination of deferiprone and deferiprone on a nickel oxyhydroxide-modified electrode. *Anal Biochem* 2008;373:337–48.
22. Yadegari H, Jabbari A, Heli H, Moosavi-Movahedi AA, Karimian K, Khodadadi A. Electrocatalytic oxidation of deferiprone and its determination on a carbon nanotube-modified glassy carbon electrode. *Electrochim Acta* 2008b;53:2907–16.
23. Guyton AC, Hall JE. *Textbook of medical physiology*. Philadelphia: Saunders, 2000.
24. McPherson RA, Pincus MR. *Henry's clinical diagnosis and management by laboratory methods*. Philadelphia: Saunders, 2006.
25. Blotcky AJ, Hobson D, Leffler JA, Rack EP, Recker RR. Determination of trace aluminum in urine by neutron activation analysis. *Anal Chem* 1976;48:1084–8.
26. Sullivan JF, Blotcky AJ, Jetton MM, Hahn HK, Burch RE. Serum levels of selenium, calcium, copper magnesium, manganese and zinc in various human diseases. *J Nutr* 1979;9:1432–7.
27. Doherty GM, Lowney GK, Mason JE. *The Washington manual of surgery*. St. Louis: Lippincott, Williams & Wilkins, 2002.
28. Dugdale DC. Medline Plus, 12 May 2009a: <http://www.nlm.nih.gov/medlineplus/ency/article/003482.htm> [accessed 12 November 2010].
29. Dugdale DC. Medline Plus, 7 May 2009b: <http://www.nlm.nih.gov/medlineplus/ency/article/003476.htm> [accessed 12 November 2010].
30. Shah SN, Abramowitz M, Hostetter TH, Melamed ML. Serum bicarbonate levels and the progression of kidney disease: a cohort study. *Am J Kidney Dis* 2009;54:270–77.
31. Kawasaki T, Akanuma H, Yamanouchi T. Increased fructose concentrations in blood and urine in patients with diabetes. *Diabetes Care* 2002;25:353–7.
32. Dugdale DC. Medline Plus, 7 August 2009c: <http://www.nlm.nih.gov/medlineplus/ency/article/003618.htm> [accessed 9 November 2010].
33. Pincus JB, Natelson S, Lugovoy JK. Response of citric acid levels of normal adults and children to intramuscular injection of epinephrine. *J Clin Invest* 1949;28:741.
34. Dugdale DC. Medline Plus, 7 August 2009d: <http://www.nlm.nih.gov/medlineplus/ency/article/003475.htm> [accessed 12 November 2010].
35. Vorvick L. Medline Plus, 14 March 2009: <http://www.nlm.nih.gov/medlineplus/ency/article/003561.htm> [accessed 12 November 2010].
36. Bryce M, Talens-Alesson FI. A three-component job method for the study of complexation and its effect on co-adsorption of pairs of organic compounds: application to the study of adsorptive micellar flocculation. *Coll Surf A Physicochem Eng Aspects* 2006;274:85–90.