DETERMINATION OF DESFERRIOXAMINE-B METHANE SULPHONATE IN URINE

J. DE WAEL AND J. E. PLOEM
Biochemical Laboratory and Medical Department, University Hospital, Utrecht (The Netherlands)
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SUMMARY

A modification of the method of Keberle for the determination of desferrioxamine-B methane sulphonate in urine is described.

In the treatment of iron overload, a specific iron-binding chelating agent, desferrioxamine-B[1,2], has been available since 1961. Desferrioxamine-B has molecular weight of 561. The stability constant of the desferrioxamine-B–iron complex (Fig. 1) is about

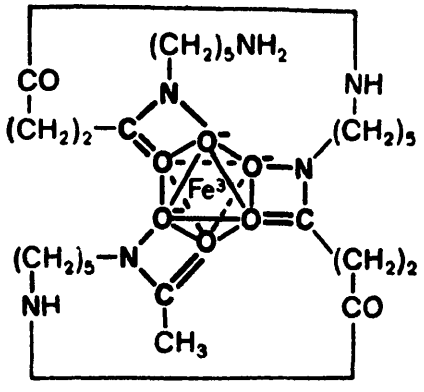

Fig. 1. Chemical structure of desferrioxamine-B–iron complex. In this compound the iron ion occupies the center of an octahedron formed by six oxygen atoms from three molecules of trihydroxamic acid.

10^{31} (cf. [3]). The methane sulphonate of desferrioxamine-B (= Desferal = DFOM; M = 657) readily dissolves in water. One hundred parts by weight of DFOM can bind 8.5 parts by weight of Fe^{3+}.

A simple method of determining DFOM in urine is of clinical importance. We used a method described by Keberle[4] as a starting point.

Principle

The DFOM present in the urine is rapidly converted, in an acid medium in the presence of trivalent iron, into the DFOM–iron complex—a substance with a red-

brown colour (maximum absorbance at 430 mμ). After neutralization, the urine is saturated with sodium chloride. The DFOM–iron complex can then be quantitatively extracted with benzyl alcohol, and spectrophotometrically determined.

REAGENTS

Only analytical grade reagents are used, *viz:*
Benzyl alcohol
$FeCl_3 \cdot 6\ H_2O$: 300 mg in 10 ml distilled water
Sodium citrate: 300 mg in 10 ml distilled water
Sodium chloride
Hydrochloric acid, about 2 *N*
NaOH, about 1 *N*
NaOH, about 0.1 *N*
Ethanol, about 96%.

METHOD

Ten ml urine is brought to pH 2 with 2 *N* HCl and allowed to react for 5 min with 0.3 ml $FeCl_3$ solution. It is then allowed to react for 1 min with 0.3 ml sodium citrate solution, whereupon it is neutralized with NaOH (1 *N* and 0.1 *N*) to pH 7–8, and shaken for 1 min with 3 g NaCl and 10 ml benzyl alcohol. After centrifugation for

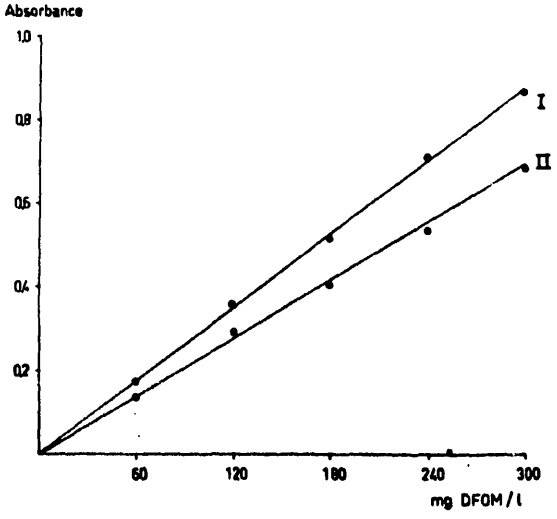

Fig. 2. Absorbance of DFOM–iron complex. I: with addition of sodium citrate; II: without addition of sodium citrate.

5 min at about 3000 rev./min, 4 ml of supernatant is pipetted off, and ethanol is added up to 10 ml. Absorbance is determined against a mixture of ethanol and benzyl alcohol (6 : 4 v/v) at 430 mμ wave length in microcuvettes of 2 cm light path. To prevent evaporation, the microcuvettes must be covered. Absorbance found: *a*.

For determination of absorbance in a urine blank, the procedure is the same, but without $FeCl_3$. Absorbance found: *b*. The difference *(a—b)* is read from the standard curve.

DFOM standard curve: 30 mg DFOM (dried to constant weight at 70°) is dis-

solved in distilled water and filled up to 100 ml. From this solution, samples of 2, 4, 6, 8 and 10 ml are diluted with distilled water to 10 ml. The DFOM concentration in these solutions is determined (Fig. 2; curve I).

RESULTS

The reproducibility of results is presented in Table I. Table II shows that recovery varies from 97 to 100%. The influence of storage time and pH on the DFOM concentration in urine is indicated in Table III, which shows that DFOM is broken down when left at a pH below 4 for 60 hours.

The DFOM concentration of the urine was determined in a normal subject following intramuscular injection of 1000 mg DFOM (Table IV).

TABLE I

REPRODUCIBILITY OF RESULTS

Number of determinations	*Initial DFOM concentration mg/l*	*Concentration of DFOM found, mg/l*		*S.D. mg/l*	*S.D. %*
		mean	*range*		
10	180	181	178–186	3	1.7

TABLE II

RECOVERY

DFOM added to urine mg/l	*DFOM concentration found mg/l*	*Recovery %*
60	58	97
90	89	99
120	120	100
150	149	99
180	178	99

TABLE III

INFLUENCE OF TIME AND pH ON URINARY DFOM CONCENTRATION

DFOM added to urine mg/l	*Left in darkness for 60 h at pH*	*DFOM recovered mg/l*
185	2	158
185	3	170
185	4	179
185	5	183
185	7	181

TABLE IV

URINARY DFOM EXCRETION AFTER 1000 mg DFOM INTRAMUSCULARLY IN A NORMAL CONTROL SUBJECT

Hours after intramuscular injection of 1000 mg DFOM	*Urinary DFOM*	
	mg/l	*mg*
0–3	1150	173
3–6	320	30
6–9	150	17
9–12	20	16
12–24	10	3
24–48	—	—

DISCUSSION

The method described differs from the original method of Keberle in two respects:

(1) We use a larger quantity of iron chloride. This guarantees an excess of iron in most circumstances.

(2) Sodium citrate is added to prevent precipitation of iron hydroxide.

When sodium citrate is omitted, results are much lower (Fig. 2, curve II) and reproducibility is rather poor. Therefore, the addition of sodium citrate is essential.

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