Rapid Spectrophotometrical Estimation of the Concentration of Porphyrins Existing Within Human Urine

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Abstract

In this paper we are presenting a new screening method applicable in order to spot the indicators of the presence of porphyrins within human urine that is to say the spectrophotometrical method. It does consist as a first step in recording the absorption spectrum which does exist within the visible range for spontaneous urine and then in finding out through analysis if it does or not own an absorption band situated within the interval which is distinctive for porphyrins that is to say into the one between 395 and 410 nm. Should a peak exist within this spectrum interval the presence of porphyrins within the human urine would be ascertained. Their concentration could be therefore calculated by making use of the Lambert-Beer law as well as by being aware of the mean molar extinction coefficient of porphyrins which is $55 \cdot 10^4$ M^{-1} cm^{-1} respectively of their mean molar mass which is 310.4 g/mol. This method is highly rapid (since results could be obtained in a few minutes). It does not make use of reactive substances at all and it does as well involve some much reduced costs should we compare it with the HPLC method which is currently applied in order to determine the existing levels of porphyrins within human urine. Our technique may be successfully made use of in the laboratories where are performed medical analyses in order to ascertain by its screening the presence of porphyrins in human urine as well for the cases of patients who do submit themselves under an ambulatory regime as for the ones who are effectively hospitalized.

Keywords: porphyrins, spectrophotometrical method, Lambert-Beer' law, absorption spectrum

INTRODUCTION

Porphyrins are cyclic compounds which are generated from the delta-aminolevulinic acid which does hold distinct parts in the formation of hemoglobin and of other hemo-proteins which are participating in the transportation of oxygen towards the human tissues [1].

Porphyria do represent a group of diseases the pathogenic mechanism of which does consist in the existence of a deficit of enzymes which does appear at the level of one among the stages of the formation of heme, see Fig. 1. For each of the ascertained syndromes their clinical as well as laboratory proven appearances are generated due to the progressive accumulation of porphyrins and of their predecessors which is ultimately leading towards the enzymatic blockade.

The major occurring symptoms of the diseases related to porphyrins are represented by: an occurring cutaneous sensitivity, some neural and visceral symptoms (abdominal pains, cardio-vascular ailments) as well as neurological symptoms such as paralysis and neuropathy. The increased levels of the bilynogenic porphyrin (PBG) and respectively of the delta-aminolevulinic acid (ALA) do generate abdominal pains as well as some neural and psychiatric phenomena while the increased number of porphyrins does determine the appearance of the cutanea sensitivity towards light. Therefore the deficits which are located nearby the end of the metabolic path are consequently and ultimately producing a more accentuated cutanea sensitivity but some less intense manifestations pertaining to a psychiatric condition [2-4].

Enzymatic anomalies may pertain to a genetic source (the hereditary ones) or they may be acquired (that is to say induced as consequences of various causes: alcoholism, intoxications with lead or with some organic solvents, with some administrated steroid hormones or with barbituric substances, due to some developed infections or issued from stress) [5,6]. The locations where porphyrins might accumulate are respectively plasma, erythrocytes, urine and fecal residues. The method currently made use of in order to determine the level of the amount of porphyrins accumulated in the human urine is the HPLC which does make use of the urine quantity sampled throughout a time period of 24 hours and of reactive substances. It does as well require in order to be performed a large period of time.

But in the present work in order to point out the presence of porphyrins within human urine as well as to evaluate their total concentration we do make use of the spectrophotometrical method. This latter method is quick, its required costs are low and it does not make use of reactive substances at all. While the HPLC method does require a quantity of 10 ml from the human urine sampled throughout a time period of 24 hours in order to be performed in the spectrophotometrical method only 1 ml sampled from the spontaneous urine is necessary for its applying. It is precisely in virtue of its simplicity, of its quickness and of its very much reduced costs that the spectrophotometrical method may be practiced successfully in order to perform the screening of the disease generated by the presence of porphyrins.

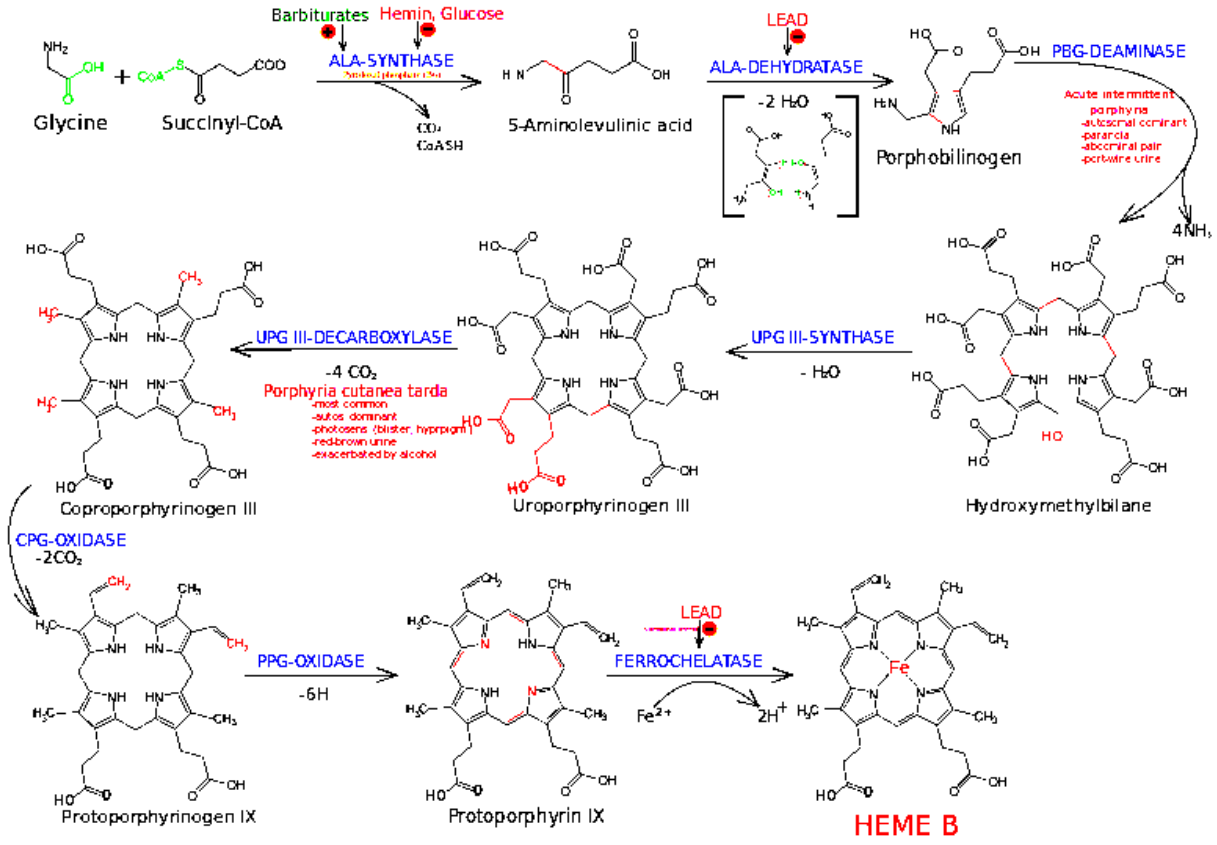

Fig.1. Heme B biosynthesis and its modulators. Major enzyme deficiences are also shown [7].

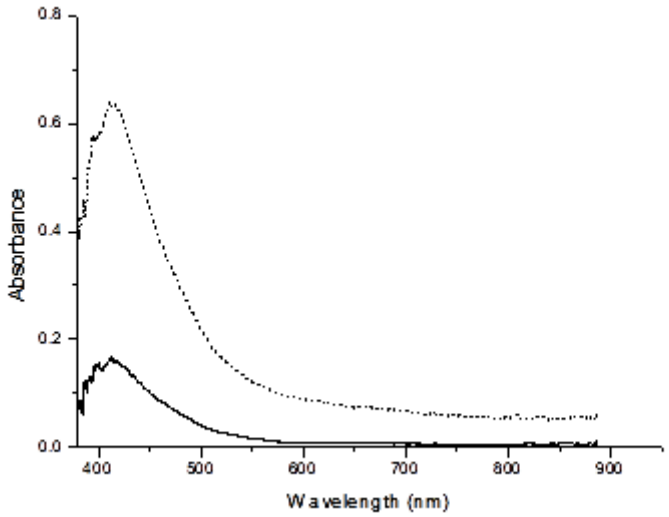

Fig. 2. Absorption spectra for: a person (1) who has ingested absinth five hours before her urine has been sampled (dash line) and a person (2) who has not at all ingested alcohol (solid line).

EXPERIMENTAL PART

In order to obtain the absorption spectra which had been acting within the visible range we have worked with a spectrophotometer of the Ocean Optics S-2000 UV-Vis type endowed with a lamp of the Tungsten-Halogen type. The cells of which we made use of have been made of quartz and their thickness has been the one of d=1 cm. The investigated biological samples have consisted in some freshly sampled urine of which 1 ml has been analyzed each time.

RESULTS AND DISCUSSIONS

In Fig. 2 we have represented only two obtained absorption spectra. Let us remark insofar these are concerned the existence of the peak at 410 nm. This fact does precisely indicate the presence of porphyrins within the analyzed samples of urine.

Between the absorbance A(λ) and the concentration c of the concerned solution a connecting relationship does exist which could be formulated in virtue of the Lambert-Beer law:

$$A(\lambda) = \varepsilon(\lambda) \cdot c \cdot d \tag{1}$$

into which for a wavelength λ, ε(λ) is the molar extinction coefficient of the concerned solution while d is the thickness of the cell (1 cm).

Should we be aware of the value held by the maximum point reached to by the absorbance A(λ) as well as of the one held by the mean molar extinction coefficient of the porphyrins $\varepsilon(\lambda)= 55 \cdot 10^4\ M^{-1}\ cm^{-1}$ (see Fig. 3).

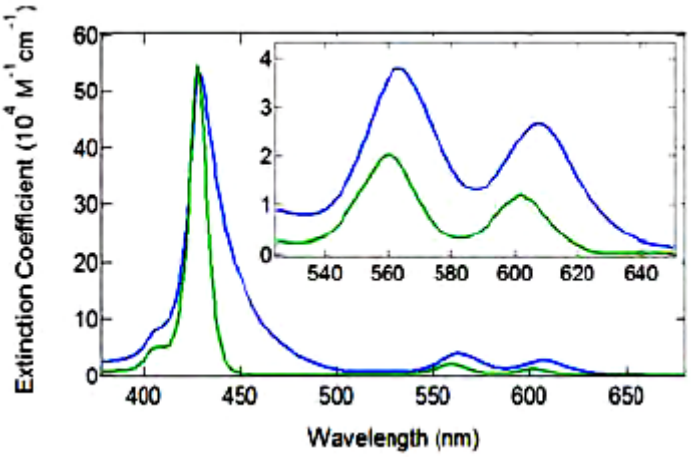

Fig. 3. Molar extinction coefficients for a porphyrin monomer (green) and for a dimer (blue) in solution [8].

We would be therefore able to calculate the molar concentration for the porphyrins which do exist within the urine sample through the Eq. 1. We could then take advantage from knowing the value of the mean molecular mass held by the porphyrins which is the one of 310.4 g/mol [9] and so we would finally be able to deduce the value of the concentration held by the existing porphyrins from the concerned urine sample which should be expressed in μg/l. In the case of the person (1) in her urine the concentration held by the existing porphyrins has resulted as being equal to 357.24 μg/l. In respect to the case of the reference person (2) (who has not at all ingested alcohol and for whom the value of the concentration

held by the existing porphyrins from the concerned urine sample has been of 93.68 μg/l) the former value is considerably larger. This fact could be explained through the occurred circumstance that the person (1) had ingested absinth five hours before her urine had been sampled. In the respective cases of a patient who had ingested some sulphonamides a few days before the biological sample had been collected (327.89 μg/l) and of a patient suffering from dehydration (248.32 μg/l) the values of the concentrations held by the existing porphyrins from the concerned urine sample have as well been some increased ones. In order to certainly obtain some results which should be accurately relevant for the concerned patient he should be warned to avoid the consumption of alcohol as well as the excessive ingestion of liquids at least two weeks before the moment when the biological product should be collected. The same recommendation should as well concern the interruption of the administrating of whatever medicine drug which might interfere with the test to be made upon the level of existing porphyrins for each of the patients.

To verify whether our method is reproducible, the concentration of porphyrins in the same sample was repeated ten times (n=10). The obtained results are presented in the table 1.

Table 1. DETERMINATION OF THE CONCENTRATION OF PORPHYRINS FROM THE SAMPLE (1) BY SPECTROPHOTOMETRICAL METHOD

No.	Absorbance at 410 nm	Concentration X of porphyrins (μg/L)	Statistic
1	0.633	357.24	$\bar{X} = 357.294$ (μg/L)
2	0.640	361.19	
3	0.628	354.42	$s = \sqrt{\frac{\sum_1^{10}(\bar{X}-x_i)^2}{n-1}} = 2{,}71$(μg/L)
4	0.639	360.62	
5	0.630	355.54	
6	0.636	358.93	$s_{\bar{X}} = \sqrt{\frac{\sum_1^{10}(\bar{X}-x_i)^2}{n(n-1)}} = 0{,}859$ (μg/L)
7	0.627	353.85	
8	0.638	360.06	
9	0.631	356.11	
10	0.629	354.98	

CONCLUSIONS

In this work we have presented the modalities through which the spectrophotometrical method could be made use of in order to identify the presence of porphyrins within the human urine and respectively through which the total concentrations of porphyrins that do respectively exist in the human urine could be estimated by making use of the Lambert-Beer law. The HPLC method does require a quantity of 10 ml from the human urine sampled throughout a time period of 24 hours while when applying the spectrophotometrical method only 1 ml sampled from the spontaneous urine is necessary. This new method is highly rapid and reproducible. It does not make use of reactive substances at all and it would as well involve some much reduced costs should we compare it with the HPLC method. This is precisely the reason why the method we have presented here is highly appropriate to be made use of in order to unveil through its screening the existence of porphyrins within the human urine.

The statistical interpretation of the experimental results shows that the method is not affected by systematic analysis errors, so it can be used in the analysis laboratories to quickly have an approximate information of this medical parameter.

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