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Porphyrrins profile by high performance liquid chromatography/electrospray ionization tandem mass spectrometry for the diagnosis of porphyria

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KEYWORDS
Porphyrias; Fluorescence; HPLC mass spectrometry (tandem mass); Polyneuropathy; Skin photosensitivity

Abstract Porphyrias are a group of inherited or acquired disorders of certain enzymes in the heme bio-synthetic pathway. Most porphyria symptoms are nonspecific and occur intermittently; resulting frequently in missed diagnosis since the disease itself is a rare one. The aim of the study is to establish a new reliable and accurate laboratory method for separation, identification and quantitation of urinary porphyrins by liquid chromatography tandem mass spectrometry (LC/MS/MS) and thereby the diagnosis of different porphyria types for the first time in Egypt. Screening by plasma fluorescence and quantitative determination of urinary porphyrins by high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC/ESI/MS/MS) of 50 clinically suspected patients revealed one case of variegate porphyria and five cases of porphyria cutanea tarda. Plasma fluorescence scanning is a simple procedure that can be used as screening test to detect porphyria patients that require quantitation of urinary porphyrins as a second step. Quantitative determination of urinary porphyrins using HPLC/ESI/MS/MS and ion
1. Introduction

Porphyrias are a group of seven inherited and acquired metabolic disorders of haem biosynthesis including aminolevulinic acid dehydratase porphyria (ADP), acute intermittent porphyria (AIP), variegate porphyria (VP), hereditary coproporphyria (HCP), porphyria cutanea tarda (PCT), erythropoietic protoporphyria (EPP) and congenital erythropoietic porphyria (CEP). Each results from a specific enzymatic alteration in the haem biosynthesis pathway. Specific patterns of accumulation of the haem precursors 5-aminolevulinic acid, porphobilinogen and porphyrins are associated with characteristic clinical features: acute neurovisceral attacks, skin lesions or both. Eight enzymes bring about haem synthesis from glycine and succinyl CoA. The biosynthetic pathway begins in the mitochondria and after three cytoplasmic stages the final step of haem formation takes place again in the mitochondria [1].

Although haem is synthesized in every human cell for respiratory and oxidation-reduction reactions, it is mostly produced in the erythropoietic cells for haemoglobin synthesis and the liver parenchymal cells for synthesis of cytochromes and haemoproteins. Control of haem production differs between these two tissues, mostly because of differences in rates of synthesis of 5-aminolevulinic acid. The first enzyme, 5-aminolevulinic acid synthase (ALAS), is coded by two genes: one erythroid specific (ALAS2 on chromosome X) and one ubiquitous (ALAS1 on chromosome 3). ALAS1 is the rate limiting enzyme in the production of haem in the liver and is controlled via negative feedback regulation by the intracellular uncommitted haem pool [2].

In erythroid cells, synthesis of haem is regulated during erythroid differentiation in response to erythropoietin. In these cells, ALAS2 synthesis is induced only during active haem synthesis. The rate is limited by iron availability and is not inhibited by haem [3]. Spleen and liver macrophages degrade haem and recycle iron after erythropagocytosis through inducible haem oxygenase 1. Porphyrins are often classified as hepatic or erythropoietic according to the organ in which haem precursors accumulate. However, a classification as acute porphyrias, cutaneous porphyrias, and rare recessive porphyrias based on clinical presentation is directly related to a simple biological diagnosis strategy and is more practical than other classifications [4].

People with autosomal-dominant acute porphyrias—aminolevulinic acid dehydratase porphyria, acute intermittent porphyria, variegate porphyria, and hereditary coproporphyria—can present with a sudden life threatening crisis. These attacks are infrequent because penetrance is low and they are difficult to diagnose because they are non-specific. Acute attacks happen in all acute porphyrias. Skin lesions never develop in acute intermittent porphyria but are the only clinical manifestation in some patients with variegate porphyria (60% of patients), and rarely (5%) develop in patients with hereditary coproporphyria [1].

Porphyric attacks begin with a prodromic phase including minor behavioural changes such as anxiety, restlessness, and insomnia [5]. Most people with acute attacks present with severe abdominal pain, but this pain might also be felt in the back or thighs. Nausea, vomiting, and constipation are common. Tachycardia, excess sweating, and hypertension, which are symptoms of increased sympathetic activity, are often present [6].

Variegate porphyria, hereditary coproporphyria, and porphyria cutanea tarda share the same chronic cutaneous photosensitivity. Lesions are restricted to sun-exposed areas such as the backs of the hands, face and neck; some women might also develop lesions on the legs and feet. Skin fragility is perhaps the most specific feature, in which negligible trauma is followed by superficial erosion that is soon covered by a crust [7].

Haemodialysis in patients with chronic renal failure can predispose to this disorder, but in chronic renal failure and end-stage liver disease, skin blisters resembling those of porphyria cutanea tarda and often referred to as pseudoporphyrria can develop [8].

Erythropoietic protoporphyria is an inherited disorder that is caused by partial deficiency in mitochondrial ferrochelatase. Accumulation of free protoporphyrin, mainly in erythrocytes and secondarily in other tissues (skin and liver) or biological fluids (bile and faeces), leads to painful photosensitivity and potential liver complications. The most common clinical manifestation is seasonal lifelong acute photosensitivity of sun-exposed skin. Many patients have a slight microcytic, hypochromic anemia [9].

Congenital erythropoietic porphyria (or Gunther disease) is the most frequent of the rare recessive porphyrias. Most patients have severe photosensitivity, leading to bullae, scarring, and eventually disfigurement of the light-exposed parts of the body such as hands, ears, nose, and eyelids. Ocular involvement includes chronic ulcerative keratitis and corneal scarring [10].

The aim of the study is to establish a new reliable and accurate laboratory method for separation, identification and quantitation of urinary porphyrins by liquid chromatography tandem mass spectrometry (LC/MS/MS) and thereby the diagnosis of different porphyria types for the first time in Egypt.

2. Patients and methods

Fifty cases suspected to have porphyrria were selected from patients attending Cairo University pediatrics hospital and clinical genetics department’s clinic, National Research Centre including:

- Nine patients having neurological manifestations without skin manifestations: Neurological manifestations presented
as convulsions (100%), polynephropathy (33%), spastic muscle tone (88%) and abdominal pain (22%), white matter demyelination on magnetic resonance imaging (55%).

- Eight patients having neurological manifestations with skin manifestations: Neurological manifestations presented as convulsions (75%), polynephropathy (37.5%) and abdominal pain (50%). Skin manifestations presented as dry skin (37.5%), itching attacks (62.5%) and photosensitivity (12.5%).
- Thirteen patients having erythropoietic manifestations and presented with anemia (100%), splenomegaly (30%) and hepatomegaly (46%).
- Sixteen patients having chronic liver disease and presented with hepatomegaly (100%), anemia (87.5%), lesions on sun exposed areas especially arms and legs and skin fragility (31.8%).
- Four patients having chronic renal disease and presented with abdominal pain (100%), skin lesions and blisters in sun exposed areas (50%) and high levels of creatinine and urea (100%): 25 healthy subjects of matching age and sex were also included for comparison as controls.

All patients were subjected to complete history taking and physical examination.

2.1. Plasma fluorescence scanning

For screening of porphyria using plasma by the method of Poh-Fitzpatrick [11]: 1 ml of plasma was diluted with 9 ml of saline at an excitation wavelength of 405 nm and emission wavelength of 620 nm, with the monochromator slit width set at 5 nm. The emission spectrum was then scanned from 550 to 650 nm with the excitation monochromator set at 17–30 min, 30% A, 50% B, 20% C; 30–33 min; 10% A, 60% B, 30% C; 33–34 min, 90% A, 5% B, 5%C. The PDA detector was set to scan in the wavelength range 250–600 nm and the mass detector range was 150–900 m/z. The sample injection volume was 10 μl [13].

We used ESI mode: sheath gas–nitrogen (6 l/min); the temperature and the voltage of the heated capillary were 300 °C and 25 V, respectively and tube lens offset 5 V. Helium was used as a collision gas [13]. Triple fragmentation mass spectrometry (MS3) was done on the positive mode at collision energy 45%. The masses of porphyrins and their MS3 fragments are shown in Table 1 [14].

2.2. Quantitation of urinary porphyrins by high performance liquid chromatography (HPLC) mass spectrometry (tandem mass)

Coproporphyrin I dihydrochloride, Uroporphyrin I dihydrochloride, 5-carboxylporphyrin dihydrochloride, 6-carboxylporphyrin dihydrochloride and porphobilinogen used as standards were purchased from Frontier Scientific Inc. Logan, USA. Working solutions of standards were prepared by dissolving in HPLC grade methanol for construction of calibration curve.

Extraction of porphyrins from urine was done by mixing of 1 ml of urine with 40 μl of concentrated HCl and centrifuged. The supernatant was used for analysis by LC/MS/MS [12].

2.2.1. Instrumentation

2.2.1.1. Liquid chromatography tandem mass spectrometry (LC–MS–MS) system. Thermo ion trap mass spectrometer LCQ ADVANTAGE MAX coupled to a modular surveyor LC system was used for the identification of porphyrins. The LC system includes LC pump, autosampler and photodiode Array detector (PDA). The software linked to the instrument is Xcalibur, Version 1.1 (Thermo Finnigan Company).

2.2.2. High performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method

The used LC column was Inertsil ODS-2 C18, 150 mm × 2.1 mm with a Synergy C18, 4.0 mm × 2.0 mm precolumn. A ternary mobile phase and a gradient elution were used at a flow rate of 0.25 ml/min. Mobile phases used were A: 20 mM Ammonium acetate buffer, B: acetonitrile and C: methanol. The elution system was: 0–17 min, 90% A, 5% B, 5% C; 17–30 min, 30% A, 50% B, 20% C; 30–33 min; 10% A, 60% B, 30% C; 33–34 min, 90% A, 5% B, 5% C. The PDA detector was set to scan in the wavelength range 250–600 nm and the mass detector range was 150–900 m/z. The sample injection volume was 10 μl [13].

We used ESI mode: sheath gas–nitrogen (6 l/min); the temperature and the voltage of the heated capillary were 300 °C and 25 V, respectively and tube lens offset 5 V. Helium was used as a collision gas [13]. Triple fragmentation mass spectrometry (MS3) was done on the positive mode at collision energy 45%. The masses of porphyrins and their MS3 fragments are shown in Table 1 [14].

2.2.3. Quantitative determination of porphyrins profile

Calibration curve for each standard was plotted as a response (peak area) versus concentration. Using these curves, quantitation of porphyrins in urine samples (unknowns) can be processed Xcalibur, Version 1.1. software.

2.2.4. Ion mapping method

Another comparative method was applied using mass spectrometer detector without HPLC system. Infusion experiments were performed using syringe pump (Hamilton syringe, 500 μl) directly connected to the electrospray ionization (ESI) at a flow rate 10 μl/min and run time 5 min.

We used ESI mode: sheath gas–nitrogen (6 l/min); the temperature and the voltage of the heated capillary were 300 °C and 25 V, respectively and tube lens offset 5 V. Helium was used as a collision gas. Fragmentation was done on the positive mode at collision energy 45%.

The technique used was Total Ion Mapping Experiment where we get product ion scans for each parent ion, so we can determine which parent ions lost a particular fragment to yield a particular daughter ion. This technique is applied for the first time for determination of urinary porphyrins.

<table>
<thead>
<tr>
<th>Name of porphyrin</th>
<th>Molecular mass [M + 1]</th>
<th>Daughter m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphobilinogen</td>
<td>226</td>
<td>227, 210</td>
</tr>
<tr>
<td>Pentacarboxylporphyrin</td>
<td>698</td>
<td>699, 640–596</td>
</tr>
<tr>
<td>Hexacarboxylporphyrin</td>
<td>742</td>
<td>743, 639–684–699</td>
</tr>
<tr>
<td>Coproporphyrin I</td>
<td>654</td>
<td>655, 596–537</td>
</tr>
<tr>
<td>Uroporphyrin I</td>
<td>830</td>
<td>831, 727–785–767</td>
</tr>
</tbody>
</table>
3. Results

Fifty patients were included: in forty patients the age ranged between 1 month and 27 months with mean age 8.75 ± 5.76 months, in five patients the age ranged between 5 years and 12 years with mean age 9.6 ± 4.15 years and in five patients the age ranged between 31 years and 46 years with mean age of 38.4 ± 6.02 years. Twenty-five controls had age range between 2 years and 24 years with mean age 10.36 ± 6.2 years. Patients included 29 (58%) males and 21 (42%) females, while controls were 14 males and 11 females (Fig. 1). The parents of 28 (56%) patients were consanguineous compared to 13 (52%) controls (Fig. 2).

One case (2% out of 50 suspected patients) and (12.5% out of eight patients) presented with neurological and skin manifestations was diagnosed with variegate porphyria, while five cases (10% out of 50 suspected patients) and (31.2% out of sixteen patients) presented with chronic liver disease were diagnosed to have porphyria cutanea tarda from (Tables 2 and 3).

Figs. 3 and 4 represent the fluorescence emission spectrum of normal plasma (no emission peak) and variegate porphyria patient plasma (case no. 1) showing the emission peak at 625 nm while in porphyria cutanea tarda patients the emission peak was at 620 nm.

Case no. one showed markedly increased urinary porphobilinogen and coproporphyrin levels which are shown in Figs. 5 and 6 respectively.

The other five diagnosed cases as porphyria cutanea tarda showed markedly increased urinary uroporphyrin and 7-carboxylporphyrin levels which are shown in Figs. 7 and 8 respectively.

The six urinary porphyrin concentrations were calculated using Xcalibur, Version 1.1 software. Peak area and calibration curve of coproporphyrin as an example is shown in Figs. 9 and 10.

4. Discussion

Porphyrias are a group of inherited or acquired disorders of certain enzymes in the heme bio-synthetic pathway. They are broadly classified as hepatic porphyrias and erythropoietic porphyrias, based on the site of the overproduction and accumulation of the porphyrins or their precursors [1]. Clinically they are classified as acute and non acute (cutaneous) porphyrias. They manifest with either neurological complications or skin problems or occasionally both [15]. Most symptoms found in porphyrias are nonspecific and occur intermittently; resulting in frequently missed diagnosis since the disease itself is rather rare.

Laboratory tests can mostly confirm or exclude the diagnosis of porphyria. The accumulation of porphyrin precursors or porphyrins in each disorder can differentiate each type of porphyria.

This study included 50 highly suspected cases of porphyria. Positive consanguinity was found in 56% of the studied cases.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Consanguinity</th>
<th>Clinical manifestation</th>
<th>Family history</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>✔</td>
<td>+</td>
<td>Polyneuropathy, abdominal pain, skin photosensitivity, dark urine</td>
<td>–</td>
<td>Variegate porphyria</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>✔</td>
<td>–</td>
<td>Hepatomegaly, anemia, dark urine, red conjunctiva, skin manifestations</td>
<td>–</td>
<td>Porphyria cutanea tarda</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>✔</td>
<td>–</td>
<td>Hepatomegaly, anemia, dark urine, skin manifestations</td>
<td>–</td>
<td>Porphyria cutanea tarda</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>✔</td>
<td>–</td>
<td>Hepatomegaly, anemia, dark urine, red conjunctiva, skin manifestations</td>
<td>–</td>
<td>Porphyria cutanea tarda</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>✔</td>
<td>–</td>
<td>Hepatomegaly, anemia, dark urine, skin manifestations</td>
<td>–</td>
<td>Porphyria cutanea tarda</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>✔</td>
<td>+</td>
<td>Hepatomegaly, anemia, dark urine, skin manifestations</td>
<td>–</td>
<td>Porphyria cutanea tarda</td>
</tr>
</tbody>
</table>
This clearly shows the high incidence of positive consanguinity among Egyptian population. Temtamy and Loutife [16] stated that the rate of consanguineous marriages among general population in Egypt was 33% and in another studies carried out by Hafez et al. [17] was 28.9% and by Temtamy et al. [18] was 20–40%.

Six cases were diagnosed as porphyria patients. They included one case of variegate porphyria (VP) and five cases of porphyria Cutanea Tarda (PCT). Variegate porphyria (VP) is an autosomal dominant disorder. The disease has now been reported worldwide and with the exception of South Africa there is no racial or geographic predilection [19]. The variability implicit in its name refers to the propensity of the disease to present with neurovisceral symptoms, photosensitivity or both in variable degrees. This agrees with our finding in case no. one which presented with polyneuropathy, abdominal pain and skin photosensitivity. Symptoms of VP are most common after puberty but in rare homozygous cases of these porphyrias clinical manifestations begin in childhood [20]. Our case was a six years old male.

Neurological symptoms can be severely disabling and, when the condition can not been diagnosed, may be fatal. Abdominal pain, the most frequent symptom, is usually poorly localized and may be accompanied by vomiting, constipation, distention, and rarely diarrhea. Sympathetic overactivity may result in tachycardia, hypertension, tremors, excessive sweating, urinary retention, cardiac arrhythmias, and sudden death. Acute psychiatric manifestations and seizures may occur, although these are limited to the period of the acute attack. Seizures may be due to hyponatremia, which may be exacerbated by intravenous infusion of sodium-free dextrose. Peripheral neuropathy is due to axonal degeneration, primarily affecting motor neurons, and can lead to respiratory and bulbar paralysis and death [21]. Porphyric crises are often induced by precipitating factors such as inappropriate use of porphyrogenic drugs, starving, excess of alcohol, infection and psychological stress [22]. Skin manifestations are less frequently observed in cold climate [1] which is not the case in our country. The cutaneous manifestation of VP i.e., blisters and fragility in light exposed skin areas is similar to that of porphyria cutanea tarda (PCT) [19].

Porphyria cutanea tarda is a heterogenous group of porphyria which may be either acquired or inherited. It is the most common of all the porphyrrias [1]. Five cases out of 50 (10%) studied patients were diagnosed with PCT. Typically porphyria cutanea tarda presents in adults and this was obvious in our five cases. They were all adult males with mean age of 38.4 ± 6.02 years [1]. The sporadic subtype (75% of cases) is most often identified in male patients without a family history of the disease. The familial subtype (25% of cases) has an earlier onset than does the sporadic subtype, and arises equally in both sexes. It is transmitted as an autosomal-dominant mendelian disorder of low penetrance, attributable to a family-specific uroporphyrinogen decarboxylase (UROD) gene defect.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Fluorometric screening of porphyria in plasma</th>
<th>Quantitative determination of porphyrins in urine by LC/MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluorometric screening of porphyria in plasma</td>
<td>Porphobilinogen 5 Carboxyl-porphyrin</td>
</tr>
<tr>
<td>1</td>
<td>+ ve emission peak at 625 nm 115.3 (†)</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>+ ve emission peak at 620 nm 21.3</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>+ ve emission peak at 620 nm 17.11</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>+ ve emission peak at 620 nm 2.4</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>+ ve emission peak at 620 nm 12.7</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>+ ve emission peak at 620 nm 20.4</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Reference range

0–25 µmol/L 0–8 nmol/l 0–8 nmol/l 0–9 nmol/l 0–60 nmol/l 0–200 nmol/L 0–8 nmol/L

Figure 3: Fluorescence emission spectrum of normal plasma: no porphyrin like emission is observed. (The nonzero baseline is due to scattered exciting light).

Figure 4: Fluorescence emission spectrum of plasma of VP patient: excitation, 400 nm and 625 indicates fluorescence emission maximum of 625 nm.
Our five cases were suggested to have the sporadic subtype of PCT since they had no reported family history of the disease. Cutaneous lesions on light exposed areas especially legs and arms is found in almost all patients [4]. All our cases had skin lesions on the legs or arms. Patients have increased skin fragility on minor traumas. Hepatomegaly was found in all five cases. Patients with PCT display siderosis with widely variable degrees of fatty damage and chronic inflammatory changes [1]. Anemia also was found in all five PCT cases. Anemia in these patients may be due to many factors like the abnormal serum iron and ferritin concentrations, absorption and turnover.

Figure 5  LC/MS/MS product ion spectrum of the (M + H)+ ion of porphobilinogen (m/z 227 > 210) from urine of variegate porphyria patient. the product ion is obtained by losing of NH3 group (−17 m/z).

Figure 6  LC/MS/MS product ion spectrum of the (M + H)+ ion of coproporphyrin (m/z 655 > 596) from urine of VP patient. The product ion is obtained from benzylic cleavage and loss of one acetyl group (−59 m/z) another product m/z 53 obtained by losing a single propionyl group and three methyl groups (−118 m/z).
Figure 7  LC/MS/MS product ion spectrum of the (M + H)+ ion of uroporphyrin from urine of PCT patient (m/z 831 > 727) the product ion is obtained from loss of acetyl group and a carboxyl group. Another major product (767) is obtained by loss of H2O followed by subsequent loss of protonated carboxylic group (−64 m/z) another product (785) is produced from benzylic cleavage of protonated carboxyl group (−46 m/z).

Figure 8  LC/MS/MS product ion spectrum of the (M + H)+ ion of hepta-carboxylporphyrin from PCT patient. (m/z 787 > 683) the product ion is obtained from loss of acetyl group and a carboxyl group.
Hemosiderosis is a contributing factor, it is seen in 80% of liver biopsy specimens from patients with PCT. This may be more exaggerated in cirrhotic patients [22].

Dark urine color was a sign in 32% of the whole group and 100% in the 6 diagnosed patients. It is due to excessive excretion of porphyrinogens which are oxidized to porphyrins upon exposure to light. PCT is often triggered by exposure to environmental factors, alcohol consumption, estrogens, exposure to pesticides, iron overload, and viral infections [23,24].

Red conjunctiva is found in two patients diagnosed as PCT in this group. Red conjunctiva is expected in those cases due to many factors, such as the increased capillary fragility and the photosensitivity. Rare ocular complications have been reported in porphyria cutanea tarda, such as ocular pain and photophobia [10].

As regards the fluorometric screening for porphyria in plasma of the six diagnosed cases, case one showed an emission peak at 625 nm. This peak establishes the diagnosis of variegate porphyria. Hift et al. [6] demonstrated that for diagnosis of the type of acute porphyria in the proband, plasma fluorescence emission spectroscopy is a first-line test because a peak at 624–626 nm establishes the diagnosis of variegate porphyria.

Also cases 2, 3, 4, 5 and 6 showed emission peak at 620 nm which indicates the diagnosis of porphyria but does not distinguish the type of porphyria. Plasma fluorescent spectrum is the best initial test for diagnosis of cutaneous porphyrias, differentiating between variegate porphyria and porphyria cutanea tarda [25,26].

As regards the quantitative determination of urinary porphyrins by LC/MS/MS, case one showed high level of porphobilinogen and coproporphyrin. High level of porphobilinogen indicates the presence of acute attack of porphyria. Examination of urine for excess porphobilinogen is the essential first-line test for patients with a suspected attack of acute porphyria [2]. Urinary coproporphyrin and porphobilinogen become markedly elevated during acute attack of variegate porphyria.

**Figure 9** Chromatograms of coproporphyrin detection using HPLC–MS/MS technique. The upper chromatogram is representative for the TIC of coproporphyrin showing an integrated peak at RT 21.52 min. The lower chromatogram is representative for the mass spectrum of coproporphyrin at the same RT, showing the product masses (m/z 537 & 523) resulted from the fragmentation process of the precursor mass of coproporphyrin (m/z 655). the lower one shows PDA chromatogram of coproporphyrin showing maximum intensity at wavelength 395 nm.


5. Conclusion

Porphyrias are a group of inherited or acquired disorders of certain enzymes in the heme bio-synthetic pathway. Most porphyria symptoms are non-specific and occur intermittently; resulting frequently in missed diagnosis since the disease itself is rather rare. Plasma fluorescence scanning is a simple procedure that can be used as screening test to detect porphyria patients that require quantitation of urinary porphyrins as a second step. Quantitative determination of urinary porphyrins using HPLC/ESI/MS/MS and ion mapping techniques are applicable for the differential diagnosis of porphyria types, since each type has a characteristic porphyrins excretion pattern. Quantitative determination of urinary porphyrins using HPLC/ESI/MS/MS is a modification for the method Stoev et al. and ion mapping technique is a new technique invented by the research team at the Biochemical Genetics department.

The authors declare that there is no conflict of interest.

References


Figure 10 calibration curve of coproporphyrin showing the equation used for quantitation of porphyrins in urine samples.