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HPLC/DAD-MS CHARACTERISATION OF DIVERSE DYESTUFFS FROM A CASE STUDY OF HISTORIC FABRIC

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ABSTRACT

Unknown dyestuffs from a red crimson coloured historic fabric are analysed with both High Performance Liquid Chromatography (HPLC) coupled with Diode Array Detection (DAD) and Liquid Chromotography coupled with Mass Spectrometry(LC-MS.)The dyed fabric dates back to 18th -19th centuries AD and is located in the National Museum of Beit El Omma in Egypt. the lengthwise and crosswise yarns have different colours, thus different dyes are anticipated. Chromatographic separation of the hydrolysed samples of the lengthwise and crosswise yarns is performed with H_2O: MeOH: 37% HCl (1:1:2; v/v/v; 400 µL) using a gradient elution program of HPLC/DAD with two solvents. HPLC / DAD analysis results prove that the green dye of the lengthwise yarn originates from tannin and Gallic acid from rich natural resource ie., Gall oak or Walloon oak. Meanwhile, the red dye of the crosswise yarns is produced from a synthesised material, known as Orange II (acid orange 7). LC-MS is used to analyse the same dyes samples using Electrospray Ionisation Interface (ESI) ion source operating in negative mode to confirm the given results. This remarkable combination of natural and synthetic dyes on the same fabric is rarely encountered.

KEYWORDS: Synthetic, natural, Chromatography, Crimson, Tannin, Orange II, dyes.

1. INTRODUCTION

Characterisation of colouring materials in historic dyed textile objects has become of paramount .dyestuffs with their variety of sources, shades and colours give aesthetic additions to the textile items. Natural dyes are mainly used anciently a lot in historical textiles, costumes, drapery, and vestments. At beginnings of 19th century, synthetic dyes are used as well for their first uses to not only replace the natural ones; but also they are used alongside the natural dyes. Some cases show the employment of both natural and synthetic dyes together for the same works of art when being investigated and analysed (Yurdun et al., 2012, Mantzouris et al., 2011) .The rise of the synthetic dyestuffs industry dates to the nineteenth century, when science-based innovations lead to the artificial products, and the changes bring about in the twentieth century by the challenge of novel fibres, increasing chemical and fluctuating economic conditions (Vettese, 2013; Morris, 1992).

Fourier Transform Infra-Red (FTIR) is mostly used to identify unknown dyes on historic textiles; however it encounters probably some difficulties when unusual dyes exist and no standard spectrum is available (Amin, 2017). High Performance Liquid Chromatography (HPLC) coupled with Diode Array Detection (DAD)has recently been successfully employed to identify the colouring ingredients in historic textile) (Yurdun et al., 2012, Wouters et al.,1985; Wouters and Verhecken,1989, Halpine et al., 2009; Balazsy and Eastop, 1998;Yurdun et al., 2011; Karapanagiotis and Karadag, 2015).

The natural dyes are widely used at the beginning of dyeing processes for textile, due to the ease to obtain and prepare. Natural sources, either plant, animal or mineral, for brownish/green colours greatly vary. Seeds of Golden dock (*Rumex maritmus*), Hypogymnia lichen (*Hypogymnia psychodes*), bark of Groundnut (*Alnus spp*), twigs and bark of Buckthorn (*Rhammus cathartica*), bark of Cherry (wild) *Prunus Spp*, and inner bark of Oak (*Quercus Spp*) are employed for brown colours; aided by addition of mordant at times (Green, 1991). Oak galls are rich in tannin and are used for dyeing and mordanting. They can also be used to get a brown colour. Catechu or cutch obtained from the heartwood of Acacia catechu is used to dye cotton, wool, and silk for brown colour. It is also rich in tannins and can be used to get black colour with iron mordant (Samanta and Konor, 2011). Oak Galls are the result from the puncture of the bark of the young twigs of an Oak tree by the female *Gallwasp Cynips Gallae-tinctoriae*, which lays its eggs inside. Oak Galls are good for producing blacks, greys and buffs as well as saddening other dyes. The basic composition of tannins is Gallic acid, Digallic acid, and Trigallic acid (Bohmer et al, 2002). Gall oak (*Quercus infectoria Olivier*) or walloon oak (*Quercus ithaburensis Decaisne)* as natural dyes are known since antiquity (Orska-Gawrys, 1989, Clark, 1981, Cardon, 2007). The galls used are of black colour in 13th century Seljuk carpets and 15th–20th century Ottoman textiles (Yurdun et al, 2011, Kardag and Yurdun, 2010). oak is a semi-deciduous little tree that grows to 12 m height and skirts along Asia Minor and the Mediterranean countries as well as south-eastern Europe (Fig.1). The gallnuts are used for various scopes such as dye and leather as well as ink manufacture from Sumerians to today. About Walloon oak (*Quercus ithaburensis Decaisne*), the acorn caps of Quercus ithaburensis are used for black dyeing in the Ottoman textiles (Yurdun et al, 2011, Bohmer et al, 2002). Both Gall Oak (Q. *infectoria Olivier*) and Walloon oak (Q. *ithaburensis Decaisne*) include tannin compounds such as Gallic acid, Digallic acid, and others.

Fig.1.Quercus infectoria Olivier tree (left). The oak galls after puncture (right) (after Cardon, 2007).

The synthetic dyes are used widely for textile, paper, food, cosmetics, and pharmaceutical industries (Saratale et al, 2017). They are recently introduced into textile dyeing. Among all the available synthetic dyes, azo dyes are the largest group of dyes used in textile industry constituting 60-70% of all dyestuffs produced because of their ease and effectiveness of synthesis, their stability and the variety of colours (Zolliger, 1987). the first synthetic dye is "Mauve" (aniline, a basic dye) which is discovered in 1856 by William Henry Perkin while searching for a cure for malaria. It is a brilliant fuchsia type colour, but it fades easily. Alizarin (synthetic madder) is produced in1868 by Graebe and Liebermann, German chemists. It is the first synthetic substitute for a vegetable dye. while Bismarck Brown is developed in 1862by Martius and Lightfoot, as the first soluble azo dye. Biebrich Scarlet is invented in 1878, a very pure red acid dye, which looks like cochineal in brightness. The azo dyes are applied to those synthetic organic colourants that are characterised by the presence of the chromophoric azo group. They are used for dyeing all natural fibres as cotton, paper, silk, leather, and wool; and for synthetics as polyamides, polyesters, acrylics, polyolefins, viscose rayon, and cellulose acetate. There are three general classifications of azo acid dyes ,acid dyes that dye directly from the dye bath, then mordant dyes that are capable of forming metallic lakes on the fibre after being treated with metallic salts, and pre-metallised dyes(Paradey et al, 2007). Synthesised Orange II (acid orange 7), is a kind of acid dyes which is characterised by its water-solubility. It composes of sodium salts of the sulfonic or carboxylic acids. It is an anionic mono-azo dye that is made by coupling diazotised sulfanilic acid to 2-naphthol and converting the coupling product to sodium salt. It is very soluble in water while very slightly soluble in ethanol. It is orange-brown powder. It is also called 2-naphthol orange II, and acid orange 7 as well (Solis et al, 2012).

Tiny samples of historic crimson-dyed fabric, supposed to be used as a table cover or wall hanging, are selected for analysis. The historic fabric is dates back to 18th -19th centuries AD and is now located in the National Museum of Beit El Omma in Egypt.it suffers from several degradation aspects i.e., splits, stiffness, missing parts, unravelling,…etc due to poor storage conditions. Both the lengthwise and the crosswise yarns of the ground fabric have different colours; green and red, while the crimson red colour covers the whole fabric for the ground fabric and the velvet pile. It is anticipated that the historic fabric is dyed with different dyes colours, and probably sources. HPLC-DAD is used to identify the present dyes. In addition, LC/MC is used to confirm the given results.

2. MATERIALS AND METHODS

2.1 Chemicals for HPLC analysis

All reagents are at analytical grade, unless otherwise stated. High purity water is obtained by passing water through a Milli-Q treatment system (Millipore, Bedford, MA, USA) and the HPLC mobile phase is prepared using Milli-Q water. Gallic acid is obtained from Sigma (Steinheim, Germany).

2.2 *Extraction procedure*

Tiny Samples of the coloured lengthwise and crosswise yarns are subjected to analysis(Fig.2.).Dyestuff extraction is done using the previously described methods according to Wouters (Wouters, 1985, Wouters and Verhecken, 1989). For the extraction of dyestuffs, historical textile samples (0.4-1.0 mg) are hydrolysed with H_2O: MeOH :37% HCl (1:1:2; v/v/v; 400 µL) in conical glass tubes for precisely 8 min. in a water bath at 100 ^{0}C to extract the organic dyes. After rapid cooling under running cold tap water, the solution is evaporated just to dry in a water bath at 50–65 ^{0}C under a gentle stream of nitrogen. The dry residue is dissolved in 200 µL of the mixture of MeOH:H_2O (2:1; v/v) and is centrifuged at 2500 rpm for 10 min. Then, 25 µL and/or 50 µL of the supernatant is injected into the HPLC apparatus

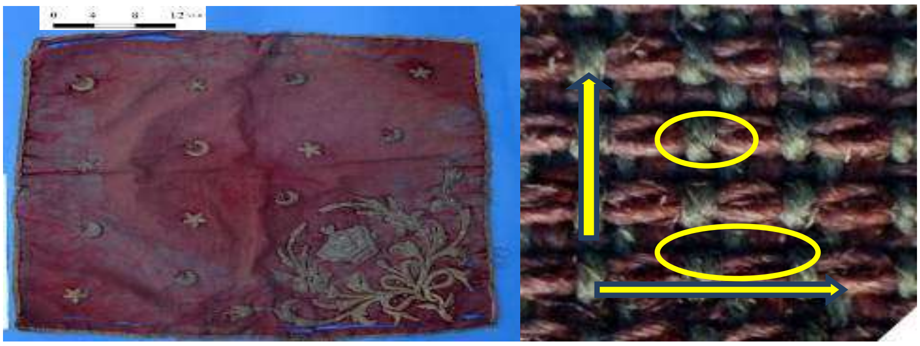

Fig 2. The historic fabric (left); microscopic image of the red crimson ground fabric showed the both the lengthwise and crosswise yarns in different colours(400x).

2.3 HPLC Instrumentation

Chromatographic experiments are performed using Agilent 1100 series system (Agilent Technologies, Hewlett-Packard, Germany) including a model G1311A gradient delivery pump with a 50 µL loop and Rheodyne valve (7725i sample injector), G1315A diode-array detector is performed by scanning from 191 to 799 nm with a resolution of 2 nm and chromatographic peaks are monitored at 255, 268, 274, 350 nm and 491 nm.G1322A vacuum degasser , G1316A thermostatic column compartment, and the data station are of Agilent Chemstation. A Nova-Pak C18 analytical column (3.9 x 150 mm, 4 µm, Part No WAT 086344; Waters, Ireland) protected by a guard column and filled with the same material is used. Analytical and guard columns are maintained at 30 ^{0}C. The HPLC gradient elution is performed using the method of Halpine and Karapanagiotis (Halpine, 1996, Karapanagiotis et al., 2009). Chromatographic separation of the hydrolysed sample is carried out using a gradient elution program that utilises two solvents: A: H_2O-0.1 % TFA and B: CH_3CN-0.1 % TFA. The flow rate is 0.5 mL/min and following elution program is applied (Table 1).

Table 1. Parameters for HPLC gradient elution

Time (min)	Flow rate (mL/min)	H_2O-0.1%TFA (v/v)	CH_3CN-0.1%TFA (v/v)
0	0.5	95	5
1	0.5	95	5
20	0.5	70	30
25	0.5	40	60
28	0.5	40	60
33	0.5	5	95
35	0.5	5	95
40	0.5	95	5

2.4 HPLC-MS analysis

The analyses of dyes are performed on a LC-MS with SIL-20AC autosampler, a LC-20AD chromatography pump and a SPD-20A single quadrupole liquid chromatograph mass spectrometer equipped with an ESI ion source (Shimadzu LC-MS-2020, Japan), operates in negative mode. Data acquisition is performed using Lab Solutions software. LC-MS separations are carried out using a Inertsil C18 (50 mm x 2.1 mm I.D. 3 µm particle size, GL Science Inc, Japan). The column is kept at controlled temperature 25 oC and used for all analyses. The mobile phase is a mixture of acetonitrile-ammonium acetate 0.1% (60:40 v/v). The flow rate is set at 0.5 mL/min. An automated injector is used while injection volume is 1 µL. The optimised parameters are as follows: The ESI probe temperature is 400 ^{0}C, and corona voltage is 4.5 kV. Nitrogen is used as drying and nebulising gas at flow rate 5 L/min, and 1.5 L/min respectively. Interface drying gas is at temperature 350 ^{0}C.

3. RESULTS AND DISCUSSION

3.1 Identification of components with HPLC-DAD

The same extraction procedure is followed due to the both dyes mixtures are acidic. Gallic acid standard compound spectrum is compared to the given chromatogram of the sample. It is found that both are alike (as shown in Fig. 3). It is proved that this chromatogram is for Gallic acid. It indicates that dye source produced from natural source which is oak plant; probably gall oak *Quercus infectoria Olivier* or wallen oak *Quercus ithaburensis Decaisn*e (Table 2).

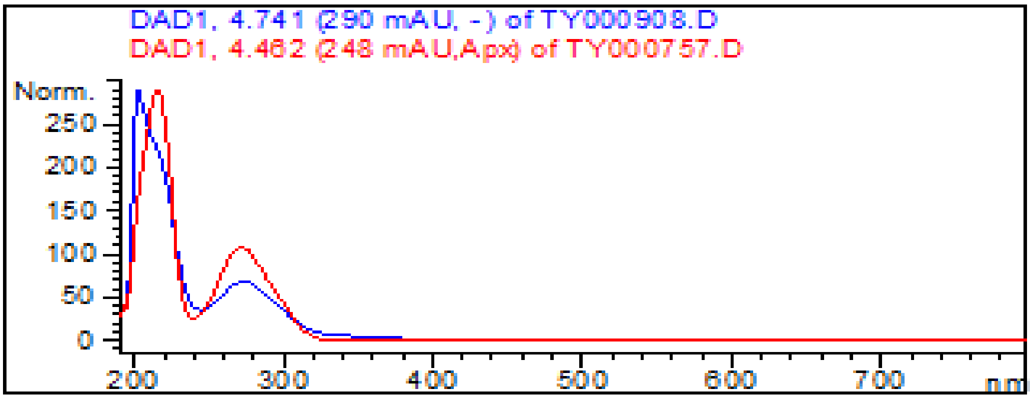

Fig.3. Gallic acid standard compound spectrum (red line) and spectrum obtained from green- brown colour samples(lengthwise yarn) (blue line).

Table. 2. The detected compounds and the identified dye source for samples.

Peak identification (min.)	Detected compounds	Identified dye source
(Rt: 4.744)	Gallic acid	Quercus infectoria Olivier or Quercus ithaburensis Decaisne
(Rt: 6.02; 8.003; 10.34)	Tanin derivative compounds	Quercus infectoria Olivier or Quercus ithaburensis Decaisne
(Rt: 25.226)	Acid orange 7=orange II (free acid)	Synthetic dye
(Rt 20.62; 26.89; 27.47)	unknown peaks	Probably synthetic isomeric dye

Dye peak (Rt: 25.226 min.) is compared to the standard synthetic orange II dye spectrum in literature (Bakheet, 2013) (Table 2., Fig.4, 5). This compound spectrum is completely similar to that of orange II.

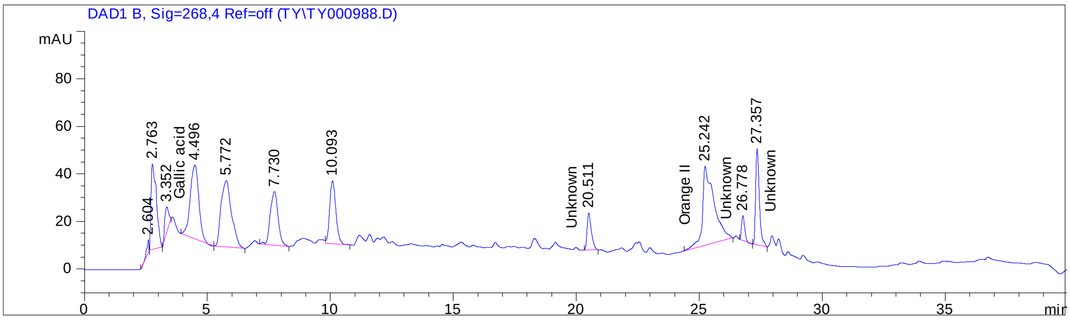

Fig.4. Peak identification for (lengthwise and crosswise dyes) Gallic acid+Orange II and unknown synthetic dyes (Rt 20.62; 26.89; 27.47).

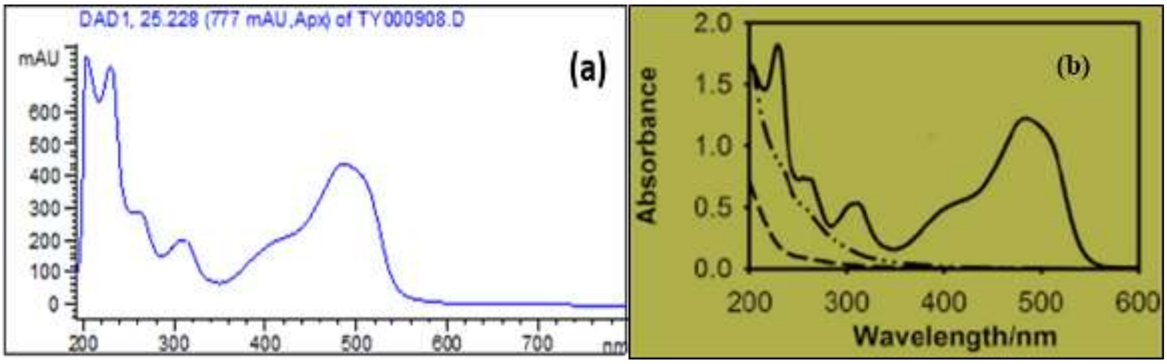

Fig. 5. Spectrum of Rt 25.226 min. from samples (a) and orange II reference spectrum from literature (Bakheet, 2013) (b).

It is found that the case study includes both natural and synthetic dyestuff sources with different colours on lengthwise and crosswise yarns of the fabric. Concerning the natural dye, Gallic acid and tannin derivatives compounds are detected (these compounds are used as dye and mordants). *Quercus infectoria Olivier* or *Quercus ithaburensis Decaisne* are proposed based upon the scheduled data (Table 2). According to literature, Quercus infectoria Olivier or Quercus ithaburensis Decaisne are used for giving green colours in Ottoman textiles (Yurdun et al.,2011, Cardon,.1981). They are rich in tannin and are used by the Romans in combination with iron to make a black dye. When they are used alone as a dye, oak give tan and light brown colours.some other unknown peaks are found and could not be identified.

3.2 *Identification of components with LC-MS*

LC-MS is used to confirm the previous results; particularly for synthetic orange II dye. Gallic acid is identified in the historical textile extract when compared to the Rt, UV and MS spectra. The deprotonated molecule ion at m/z 169 (M-H)- confirms the presence of this compound and further fragmentation shows ion at m/z 124.45 (-44, CO_2) for Gallic acid as shown in Fig 6. (Sawant et al., 2017, Mendes et al., .2011, Gao. et al., 2010).

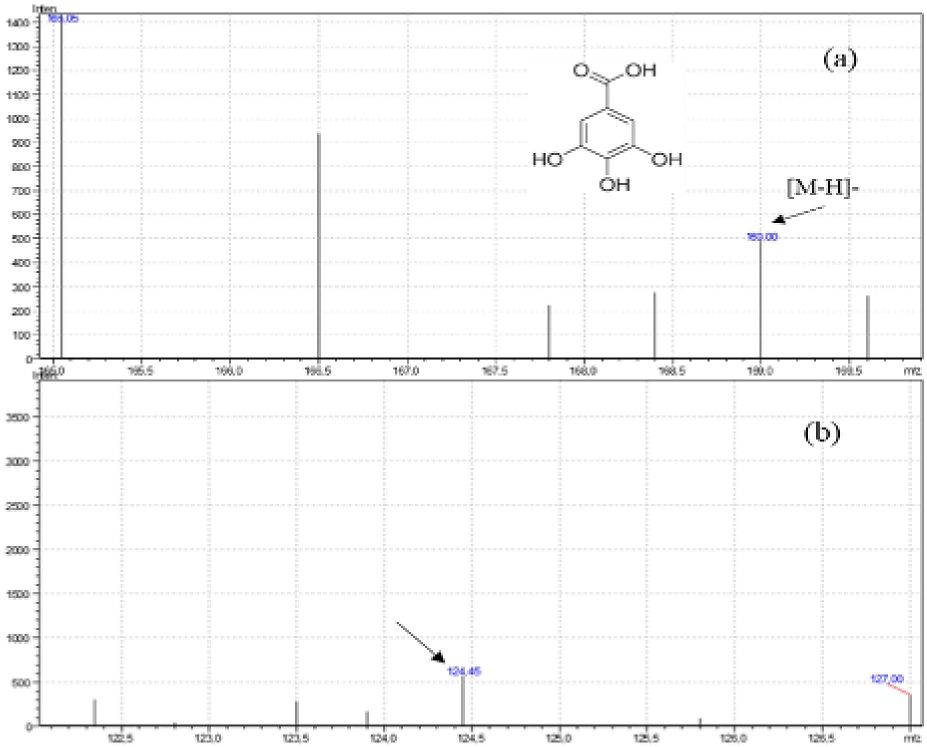

Fig. 6. Chemical structure and ESI/MS spectra of Gallic acid in negative-ion mode (a, b)

Synthetic dye orange II (acid orange 7) presents the ion at m/z 327 [M-Na]-, the ion at m/z 247.35 (-80, SO_3), corresponding to the loss of SO_3 group and the ion at m/z 170.70 (-156, $C_{10}H_7ON_2$) corresponding index in formulas to the N=N bond cleavage (Fig. 7) (Souto et al., 2010).Other synthetic dye peaks (Rt 20.62; 26.89; 27.47) are not identified with LC-MS. This compound peaks spectra are completely similar. Therefore, they presumably are the isomers of the unknown substance, could be a product of degradation process of the case study or a sign of a particular staining (fig.8).

Other synthetic dye peaks (Rt 20.62; 26.89; 27.47) are not identified with LC-MS. This compound peaks spectra are completely similar. Therefore, three unknown peaks are found as the isomers of the unknown substance.

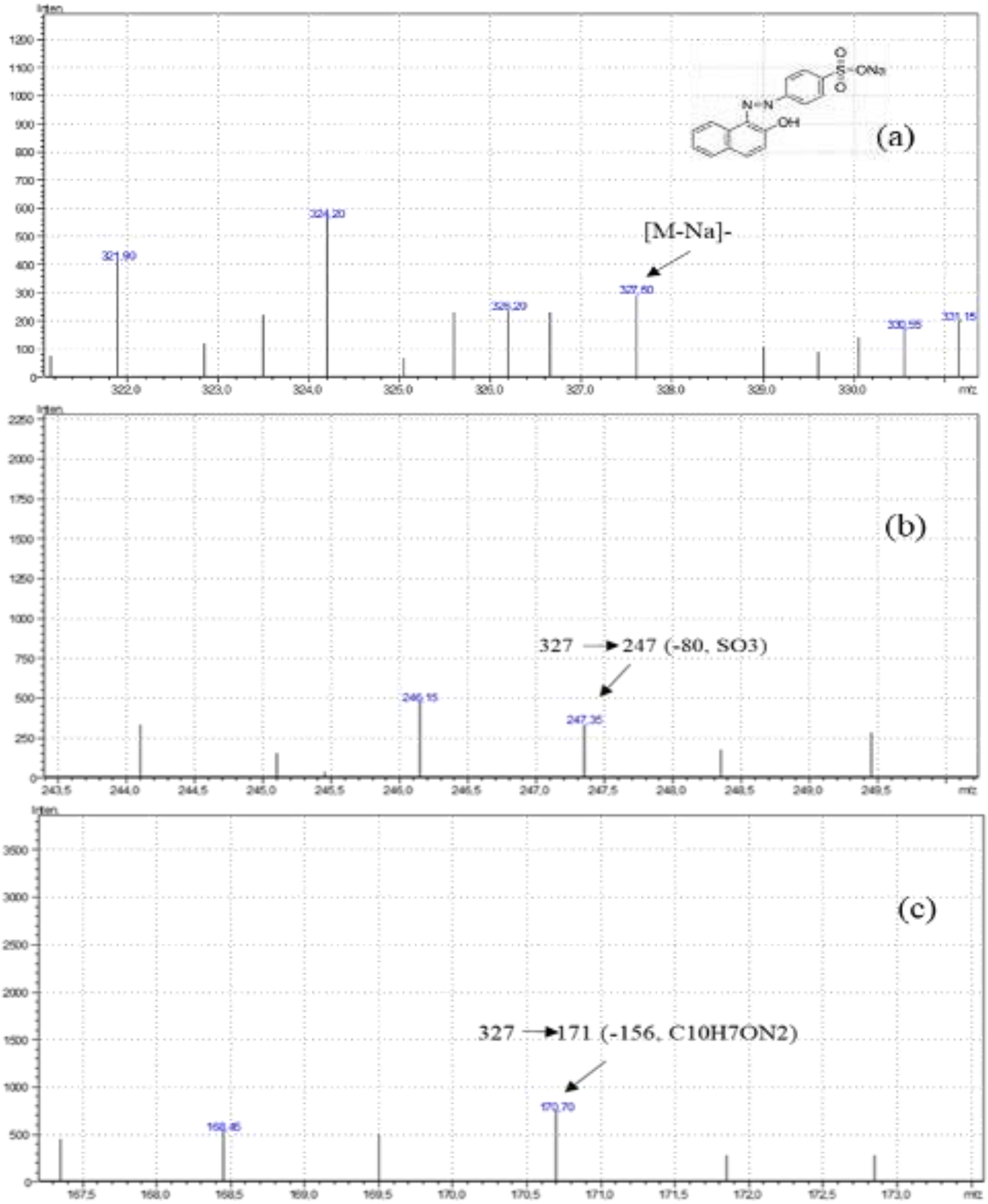

Fig. 7. Chemical structure and product ions of precursor ion of orange II (a, b, c)

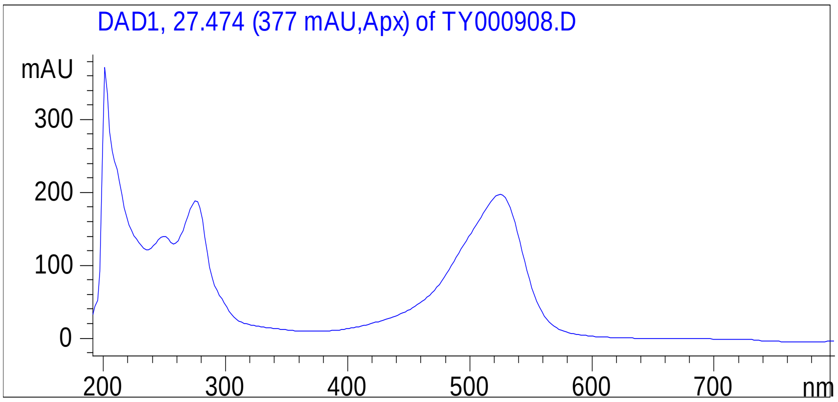

Fig.8. Unknown synthetic dye three peaks are noticed and not identified with LC/MS (Rt 20.62; 26.89; 27.47) have same spectrum.

4. CONCLUSION

Combination of instrumental analytical techniques is applied in order to analyse dye extracts of historical art object; a historic stored fabric from the National Museum of Beit El Umma collection, Cairo, Egypt from 18-19th century. In this study, fibres of red crimson and green- brown colours are selected for analysis. Some difficulties are encountered to identify the dye source; particularly if more than one dye is anticipated which give different colours under microscopic investigation. Both HPLC-DAD and LC/MS are used on the extracted samples of yarns to finally give plant origin–Gallic acid and tannin derivatives as natural source of dye which give greenish to brownish colour; while synthesised orange II as synthetic source is given for the other red yarn; in addition to unknown peaks (isomeric compound) are found and could not identified by both instruments probably due to degradation. Presence of the synthetic and natural on the same historic fabric is noteworthy to be rarely seen in historical objects.it attributes probably to the manufacturer wish to experiment with synthetic dyes in its first form in 19th century beside the widely used natural ones. This is accordingly corresponded to the date of the studied fabric as 18-19th century.

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