RSC Advances

Graphical Abstract

Industrial Effluents
- Paper
- Textile
- Laundry
- Printing Press

Solid Phase Extraction

Conditionin

Loading/W

Elution

Evaporation

Filtration
(0.22μm PTFE filter)

UPLC-MS/MS

MB

H_3C―N ... CH_3

Cl^-　CH_3

CH_3

MV(IS)

H_3C―N ... NH_2

N^+

H_3C　Cl^-

0.68

1.24

MRM of 3 Channels ES+
TIC (MB & MV 3RAX)
Intensity: 2.21e6

100%

Intensity (%)

0

0　　　　1　　　　2

Time (min)

Quantitative Determination of Methylene Blue in Environmental Samples by Solid Phase Extraction and Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry: A Green Approach

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Abstract

Industrial effluents with dyes may contain appreciable concentration of materials with high chemical oxygen demand and suspended solids, posing adverse effects to both humans and aquatic life. Therefore, needs to be quantitatively monitored. In the present study, an analytical method based on solid–phase extraction (SPE) and ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) has been optimized for the quantitative analysis of methylene blue (MB) in the environmental samples. For the extraction of MB, a variety of solvents including formic acid proportion were studied to obtain the optimum recovery. MB extraction using SPE method was best achieved using methanol with formic acid, 1M. Chromatographic separation of MB and methylene violet 3RAX (MV 3RAX, internal standard) was accomplished on Acquity$^{®}$ BEH C$_{18}$ column using water (64.99%) with formic acid (0.01%) and acetonitrile (35%) in isocratic elution mode. Chromatographic separation for both MB and MV 3RAX were obtained in < 2 min with good resolution and superior peak symmetry. MB and MV 3RAX were quantified with electrospray ionization coupled with MS/MS in the multiple reaction monitoring mode. The good quality parameters were achieved for instance linearity (r^2>0.999), run–to–run and day–to–day precisions with relative standard deviations <4%, sensitivity with low limit of detection (LOD), 0.1 ng/mL and limit of quantification (LOQ), 0.4 ng/mL were obtained. Industrial wastewater samples (paper, textile, laundry and printing press) were analyzed, MB level was found in between 0.36 and 1.08 µg/mL, with excellent recovery rates (95 - 99%) depending on samples.

1. Introduction

Since the evolution of civilization use of dyes and pigments is growing, generating gallons of waste effluents. These waste effluents limit sunlight penetration reducing photosynthetic activity and dissolved oxygen concentration causing adverse effects on both flora and fauna. Methylene blue (MB; 3, 7-bis(dimethylamino)-phenothiazin-5-ium chloride), a cationic dye, is widely used in textiles, printing, and research laboratories. Methylene blue is a well-known photosensitizer that has been extensively used as an optical probe in many biochemical systems.[1] Federal drug and food administration (FDA) has approved MB for various testing applications.[1] However, MB poisoning can cause eye burns by direct contact and nausea, vomiting, profuse sweating, mental confusion and if ingested can cause methemoglobinemia.[2]

In order to estimate the intakes and threats to human health, it is highly essential to quantify the amounts of MB present in a variety of industrial wastewater effluents. However, due to a low level of MB concentration and high number of matrix interferences found in wastewater sample, the challenge has been to develop fast analytical technique that unambiguously determine MB in these complex matrices. For MB assays, a number of analytical methods have been reported in different matrices. Typically, an extraction and enrichment steps including liquid–liquid extraction (LLE) [3–5] and solid phase extraction (SPE) [6-8] are performed, followed by separation methods such as liquid chromatography (LC) [9-12], capillary electrophoresis (CE) [13-17] or ion chromatography (IC) coupled to either optical[18] or mass spectrometric detectors.[11] However, co-extracted substances from the wastewater sample matrix often emerge and can impede in the determination of MB. One of the most significant features of the analysis of MB is the authentication of the chromatographic peaks using selective methods, while many peaks with the same retention times as those of MB are often present in the chromatograms leading to false peak

detection. Indeed, almost all these conventional extraction and detection methods are lengthy, requires large amount of organic solvents, expensive (SPE employs significantly a smaller amount of organic solvent than LLE, but it can be comparatively expensive), use of large injection volumes or with limited sensitivity and selectivity. To sort out this problem, the coupling of more selective methods like mass spectrometry that permits the unequivocal detection of the compounds is needed. Besides, to reduce sample extraction and enrichment cost it is essential to develop economically and ecologically appealing SPE biomass materials. Globally research is going on to explore new biosorbent and modifying them to enhance their performance for the removal and recovery of metal or metalloid species, organic compounds and particulates from synthetic and wastewater samples.[19] Considering the environmental and economic factors, the use of biosorbent in SPE is essential to be exploded for developing an analytical method to identify and quantify traces of MB in wastewater sample taking benefit of the gain in sensitivity and selectivity, reduce analysis time and solvent consumption.

To our best knowledge previous studies have reported the use of commercially available SPE cartridges. These cartridges are expensive, ecologically unsafe and lengthy. None of the previous studies have reported the use of biomass for preparing SPE cartridges. Also, the matrix used in previous researches was aquatic product. Therefore, in present research, efforts have been rendered to develop a novel two step analytical method based on solid–phase extraction using biosorbent and reversed-phased ultra-performance liquid chromatography–tandem mass spectrometry for the extraction enrichment, and quantification of MB in industrial wastewater samples as matrix. A green SPE extraction method based on (pistachio shell) biomass was developed. The overall high throughput offered by proposed method can be an advantage for this type of analysis.

2. Material and methods

2.1. Chemicals and reagents

Solvents and chemicals used in this study were of LC or analytical reagent (AR) grade. The chemical structure of the studied compounds has been shown in **Figure 1**. MB (purity 82%) and formic acid (98%) were purchased from Panreac (Barcelona, Spain). The methylene violet 3RAX (MV 3RAX; purity 90%), was used as internal standard (I.S.) and was obtained from Aldrich (Missouri, USA). Methanol, acetone, dichloromethane, ethyl acetate and acetonitrile were purchased from Merck (Darmstadt, Germany). Water was purified through a Milli–Q water purification system (Millipore Corporation, Bedford, USA).

Extraction columns (Extrelut-20) were provided by Merck (Darmstadt, Germany), coupling pieces and stopcocks were from Varian (Harbor City, USA). In the SPE method and solvent evaporation, Visiprep^{TM} and Visidry^{TM} vacuum manifolds (Supelco, Gland, Switzerland) were used, respectively. Argon of high purity (99.99%) was supplied by Speciality Gas Centre (Jeddah, Saudi Arabia). MB methanolic stock standard solution of concentration 100 µg/mL was prepared and used for further dilutions. Standard mixtures of MB with MV 3RAX (I.S.) at varied concentration levels (0.01-5.0 µg/mL) were prepared to establish the range of linearity and for the calibration curves in all systems. Standards and samples were filtered through a 0.22 µm PTFE syringe filter (Chromafil® Xtra, Macherey-Nagel, Duren, Germany) before being injected into the UPLC–MS/MS system. The MB quantification was achieved using standard addition method at varied concentration levels.

2.2. Instrumentation

The samples were analyzed using Acquity® UPLC system equipped with a quaternary pump (Waters, Milford, USA). The reversed-phase analytical column used was an

Acquity$^{®}$ BEH C_{18} column with dimension of 50 mm × 2.1 mm id, 1.7 μm particle size (Waters, Milford, USA). The best possible chromatographic separation of MB and MV 3RAX was achieved with a mobile phase consisting of 0.01% of formic acid in water (64.99%) and acetonitrile (35%) in isocratic elution mode at flow rate 300 μL/min. The sample injection volume was 5 μL. The column was washed with water/methanol (50:50, v/v) for 5 min after every 20 injections.

The UPLC system was equipped with electrospray ionization source (ESI, Z–spray) and Quattro Premier triple quadrupole mass analyzer (Micromass, Milford, MA, USA). For MB and MV 3RAX, the MS system was operated in the positive ionization mode and the data was acquired in multiple reaction monitoring (MRM). The optimized source working conditions for monitoring MB and MV 3RAX were as follows: capillary voltage, 3.5 kV; cone voltage, 68 V; source temperature, 120°C; desolvation temperature, 350°C; desolvation gas flow rate, 700 L/h cone gas flow rate, 70 L/h. Nitrogen gas of high purity (99.99%), generated using a nitrogen generator model NM30LA (Peak Scientific, Inchinnan, United Kingdom), and argon were used as cone and collision gases, respectively. An Oerlikon rotary pump, model SOGEVACSV40 BI (Cedex, France) was used to provided the primary vacuum to the MS system. The MS/MS parameters including collision energy voltages, dwell times and the precursors and daughter ions related to the selected transitions for MB and MV 3RAX are illustrated in **Table 1**. The most abundant daughter ion was monitored to estimate the MB and MV 3RAX, and the second most abundant daughter ion was monitored to authenticate MB and MV 3RAX identification. Data acquisition was performed by MassLynx V4.1 software (Waters, Milford, USA).

Fourier transform infra-red (FT-IR) spectra of samples in range 4000 – 600 cm^{-1} were recorded using a Varian 3100 spectrometer, USA. Wide angle X-ray diffraction

(WXRD) pattern of biosorbent was recorded using a Philips Xpert wide-angle X-ray diffractometer with nickel-filtered CuKα radiation (1.54056).

2.3. Sample analysis

2.3.1. Preparation of biosorbent for SPE procedure

Soild phase extraction method is a well-known practical extraction technique. It can be used to isolate compounds of interest from a variety of complex matrices, including soil, water, urine, blood, animal tissue, and beverages.[20-22] The solid-phase adsorbent is the most important part of the extraction system, and extraction recovery depends typically on the adsorbent properties. [20-22] The modification of adsorbent with different functional groups could change the extraction properties of the final adsorbent. Thus, it is essential to select the most suitable SPE adsorbent for a specific application.

In this study, Pistachio shells (PS), a lignocellulose biomass was used as SPE biosorbent. The PS were collected from the local dry fruits supplier (Riyadh, Saudi Arabia), washed with Milli-Q water to remove dust and dirt, dried in oven at 60°C for 24 h. The dried PS were grounded and sieved to 0.2 – 0.4 mm particle size. Biomass (PS, 1.0 g) was treated with 100 mL hydrogen peroxide (30% w/w) and the resulting mixture was kept in a water bath shaker at 50°C for 60 min with constant stirring at 100 rpm to oxidize the organic content. Afterwards, the biomass was washed several times with Milli-Q water to remove the traces of hydrogen peroxide. The oxidized biomass was again treated with alkaline (0.1M NaOH) solution in a water bath shaker at 25°C for 24 h with constant stirring at 100 rpm. The alkali treatment of lignocellulose containing biomass results in swelling, increasing its internal surface area by disrupting the lignin structure making carbohydrates in the heteromatix more accessible to binding adsorbate, and decreases both the degree of polymerization and cellulose crystallinity. [23] Considering environmental and economic aspects, lower environmental load in its life cycle, less corrosive ability, and

economic feasibility are the major merits of using NaOH as a modifying agent.[24] The

NaOH treated biomass was washed several times with Milli-Q water to achieve neutral pH.

Resultant biomass was dried in oven at 65°C for 24 h to get constant weight and the PS

biomass was kept in sealed plastic bags and stored in a desiccator to avoid the moisture

contamination.

2.3.2. Sample extraction procedure

Wastewater samples were collected using glass bottles (250 mL) from different industries

based in Saudi Arabia. The samples were filtered through Whatman filter paper (No. 42),

(Maidstone, United Kingdom) and stored at 4°C to avoid any microbial contamination. A

1.0 g of biosorbent was used to fill an empty Extrelut column (6 mL) followed by washing

with Milli-Q water (50 mL) and drying under vacuum for 10 min. The filtered wastewater

samples (20 mL) were passed through biosorbent at controlled flow rate of 1 mL/min. After

that, the biosorbent was rinsed with Milli-Q water (50 mL) and dried under vacuum for 10

min. The MB adsorbed on the Extrelut packing was eluted using methanol (50 mL) with

1M formic acid at controlled flow rate of 1 mL/min. The sample extract was evaporated to

dryness under a stream of nitrogen and the analyte was reconstituted with 1 mL of MV

3RAX (I.S.) in methanol-water (50/50, v/v). The final extract was filtered through 0.22 μm

PTFE syringe filter prior to UPLC-MS/MS analysis.

To assess the recovery rates of MB separated and analyzed using this SPE

procedure, in addition to prevent matrix effect influence on peak retention time in the

UPLC-MS/MS chromatograms, two non-spiked and three spiked samples (50%, 200%, and

500%) were analyzed by standard addition quantification method. The samples were spiked

at in the beginning of extraction process.

3. Results and discussion

3.1. Characterization of SPE biosorbent

3.1.1. FT-IR spectroscopic analysis

The major constituents of PS biomass are hemicellulose, cellulose, and lignin.[25] These constituents were confirmed from the obtained peaks in 1750–1500 cm^{-1} (**Figure 2a**) which were attributed to the stretching vibrations of keto -C=O groups (1732 cm^{-1}) and C=C stretching vibrations of aromatic rings (1597 and 1494 cm^{-1}) in lignin. The peaks in 1500-1314 cm^{-1} were observed due to bending vibrations of O−H and aliphatic deformation vibration of -CH$_3$ & - CH$_2$ groups present in cellulose and lignin. The absorption bands in 1300-1000 cm^{-1} were assigned to stretching vibration of C-O in carboxyl groups, alcohol and phenol constituents.[26] The existence of cellulose in PS biosorbent was confirmed from the relatively high intensity peak at 1026 cm^{-1} due to the stretching vibration of C-O in alcohols and phenol.[25] The obtained results proved that the cellulose constituent was left in PS biosorbent and other constituents like lignin and hemicellulose were dissolved after treatment with NaOH. The FT-IR spectra of PS biosorbent after MB biosorption was also recorded (**Figure 2a**). The reduction in the intensity of absorption bands at 3311 and 1026 cm^{-1} was observed after biosorption. This confirmed that MB biosorption was occurred through a hydrogen bonding between the acidic oxygen groups (O-H and phenolic) on PS and nitrogen atoms present in MB.

3.1.2. WXRD analysis

The WXRD patterns of PS before and after NaOH treatment are depicted in **Figure 2b**. The intensity of peak for PS biosorbent after NaOH treatment was reduced, which confirmed the amorphous nature of PS biomass was improved as lignin and hemicellulose constituents were leached out.

3.2. Optimization of SPE method

Since there were no earlier studies into the extraction of MB from wastewater samples using SPE and UPLC–MS/MS method, preliminary studies were performed on standard solutions of MB. To find most favorable solvent for MB extraction using SPE technique various solvents such as dichloromethane, ethyl acetate, acetone and methanol were tested. The obtained results demonstrated that none of above solvents extracts MB efficiently and the obtained recovery rates were almost negligible. Thus, the several experiments were carried out using sample modifier such as formic acid proportion in each extraction solvents to enhance the recovery rate of the target analyte. Among the tested solvents, methanol at varied formic acid concentrations (0.1 - 1 M) showed comparatively higher recovery values ranging from 75 to 104% (**Figure 3**). From all the solvents tested, a methanolic solution of formic acid (1M) produced the maximum MB recovery of 104%. As a result, methanolic solution containing formic acid (1M) was chosen as the most favorable extraction solvent for MB in wastewater sample.

3.3. Optimization of chromatographic separation

The development of a method for the high throughput quantification of MB using UPLC is significant because this is the first method for the analysis of MB with this advanced technique. The UPLC method has revealed to get better the chromatographic performance of established liquid chromatography in terms of speed, resolution and sensitivity of analysis.[27, 28] A varieties of analytical column and conditions were investigated in order to optimize the chromatographic separation in terms of analysis time, peak symmetry and resolution. The Acquity® UPLC BEH C_8, C_{18} columns were tested, these columns incorporate trifunctional ligand bonding chemistries which produce superior low pH stability and ultra-low column bleed. This low pH stability is combined with the high pH stability of the 1.7 μm BEH particle to deliver the widest usable pH operating range. Besides, these new chemistries also employ new, proprietary end-capping processes which

produce the highest efficiencies, sharpest peaks and maximum MS sensitivities. An Acquity® UPLC Hydrophilic Interaction Chromatography (HILIC) column with a stationary phase having amide groups was also studied which are designed to retain and separate highly polar compounds. HILIC is an alternative of normal phase liquid chromatography (NP-LC), but the separation mechanism used in HILIC is more complicated than that in NP-LC. Like NP-LC, HILIC uses conventional polar stationary phases such as silica, amino or cyano,[29] but the mobile phase used is similar to those employed in the reversed-phase liquid chromatography mode,[29] and it can be conveniently coupled to mass spectrometry (MS), especially in the electrospray ionization (ESI) mode. A standard mixture solution of MB and MV 3RAX at 1.0 μg/mL was injected onto the column of UPLC system and the mobile phase was optimized with methanol, water and acetonitrile alone and at different proportions. The effect of the addition of formic acid (1 - 0.01%) in mobile phase was also studied. The most reliable results (resolution, high peak symmetry, reproducible and signal-to-noise ratio values) acquired for target analyte peaks was favorable when using Acquity® UPLC BEH C_{18} column and mobile phase, 65% of Milli-Q water with formic acid (0.1%, v/v) and 35% of acetonitrile in isocratic elution mode. The amount of formic acid in the mobile phase leads to the Gaussian peaks and improves the peak symmetry of the studied compounds. A fairly low mobile phase flow rate, 300 μL/min was established to be most favorable for the analysis of MB and MV 3RAX which favored efficient ionic evaporation and desolvation in the ion source (ESI) and a chromatographic peak wide enough to be defined by at least fifteen scan points in their detection. With these optimum conditions, the retention time for MB and MV 3RAX was <2 min. Among the Acquity® UPLC BEH C_8, C_{18} and HILIC columns tested for MB and MV 3RAX separation, the Acquity® UPLC BEH C_{18} column illustrated the highest capacity factor and was chosen for further analysis. As can be seen from **Figure S1**

(Supporting Information) the obtained chromatograms of MB and MV 3RAX (standard mixture, 1.0 µg/mL) shows the good resolution and high peak symmetry. An additional benefit of an UPLC method using a reversed phase column is that this type of column is usually obtainable in laboratories with UPLC systems.

3.4. Optimization of MS detection

Mass spectrometric conditions were optimized by infusing a methanolic mixture solutions of MB and MV 3RAX at concentrations levels of 5 µg/mL depending on the sensitivity of the compound. The experiments were performed in both positive and negative ionization modes. Initially, full-scan acquisitions were performed to select the precursor ion and optimum cone voltage. The studied compounds offered higher sensitivity in positive ionization mode and in concurrence with previously developed LC–MS method.[30] The ionization by ESI adding H^+ to the target compounds could not take place due to MB and MV 3RAX are already positively charged, nevertheless both compounds could be analyzed particularly due to they build up intensive $[M]^+$ ions. As a result, the positive ionization mode led to enhanced sensitivity for the studied compounds, and thus, it was chosen for further experiments.

Subsequent to the affirmation of precursor ions, more than two daughter ions should be chosen when using LC–MS analysis in agreement with European Union Legislation.[31] As stated by these guidelines, the first transition should be used for quantification, while the second transition should be used to authenticate the presence of target analytes during the analysis of real samples. In this study, once the precursor ions were chosen, daughter scan acquisitions were carried out at various collision energies in an attempt to select daughter ions and most favorable collision energies. Two MRM transitions were acquired for both MB and MV 3RAX, the most abundant transition was usually chosen for quantification and the other transition was selected for confirmation purposes. The MS/MS

transitions monitored in the positive ionization mode were 284>269, 284>240 at 60 V collision energy and 343>299, 343>327 at 72 V collision energy for MB and MV 3RAX, respectively. The dwell time was 0.025 s in both the cases. The individual mass spectra and fragmentation pattern for the studied dyes have been illustrated in **Figure 4**. The obtained mass transitions in the MRM mode were used for unambiguous and sensitive quantification.

3.5. Method validation

Under the optimum chromatographic and MS conditions, the proposed UPLC-MS/MS method was validated by linearity, limit of detection, limit of quantification, repeatability (run-to-run precision), reproducibility (day-to-day precision) and recovery. The linearity of the method was investigated over a wide range of MB concentration (0.01 – 5 µg/mL). The obtained peak area was plotted against concentration and the correlation coefficient (R^2) was found higher than 0.999, showed that the response was linear.

The LOD and LOQ are terms used to illustrate the minimum concentration of a compound that can be consistently calculated by an analytical procedure. The LOD as well as LOQ were estimated for a signal-to-noise ratio (S/N) equal to 3 and 10, respectively, the obtained LOD and LOQ values were 0.1 ng/mL and 0.4 ng/mL in standard, and 0.3 ng/mL and 0.9 ng/mL in sample, spiked at lowest concentration level. The LOD and LOQ values were assessed using statistical LINEST function.

The run–to–run precision (repeatability) was estimated from five replicate injections of MB standard (0.5 µg/mL) and laundry sample with known calculated concentration (0.36 µg/mL) on the same day into the UPLC-MS/MS system. These determinations were evaluated on the basis of the calculation of the relative standard deviation (%RSD) of the peak area values. The RSD values obtained for repeatability were as follows: 1.86% in the analysis of the standard and 2.52% in the analysis of laundry

sample. The day–to–day precision (reproducibility) was calculated by five replicate injections of MB standard (0.5 µg/mL) and laundry sample with known calculated concentration (0.36 µg/mL) along three consecutive days into the UPLC-MS/MS system. These finding were estimated on the basis of the calculation of the RSD of the peak area values. The RSD values obtained for reproducibility were as follows: 2.35% in the analysis of the standard and 3.98% in the analysis of laundry sample. The precisions (repeatability and reproducibility) of the proposed UPLC-MS/MS method for the analysis of low levels of MB either in standard or in real samples were found to be adequate for the quantification.

The effect of the sample matrix on the signal-to-noise ratio of the target compound has been assessed using standard addition quantification method. A total of four industrial wastewater samples were analyzed and the recovery rates for MB were found between 95 and 99%, as it has been detailed in **Table 2**. The low matrix effect can be in part due to the chromatographic conditions; the Z–configuration of the ionization source of the MS system, which prevented the entrance of neutral compounds in the MS. These results indicate that the matrix does not alter the target compound signal in this type of samples.

3.6. Application of UPLC-MS/MS method

The practical applicability of SPE and UPLC-MS/MS method was established for the analysis of MB in industrial wastewater samples. The detection of MB was not affected by the investigated sample matrices, as it was determined experimentally. The results relating to the MB concentration are presented in **Table 2**, the lowest concentration of MB (0.36 µg/mL) was found in laundry sample, however, the highest MB concentration (1.08 µg/mL) in textile samples. As an example, **Figure 5** demonstrates the chromatograms of MB and MV 3RAX (I.S.)   analyzed with the proposed UPLC–MS/MS method. The low matrix effect can be in part due to optimized green SPE procedure, chromatographic

conditions and the Z–configuration of ESI source of the MS system, which barred the entrance of neutral compounds in the MS system. These results signify that the matrix does not alter the target analyte signal in such type of samples. The analysis of blanks carried out routinely indicated that cross contamination during the analytical process did not take place. These results are a source of data on the presence of MB in industrial wastewater samples.

4. Conclusions

Highly sensitive, speedy, cost effective, ecofriendly and reliable method based on SPE and UPLC–MS/MS has been developed in order to quantify MB in industrial wastewater samples. The absence of matrix effects observed in the analysis and minimal sample pre-treatment without the loss of the target analyte offered by low cost SPE technique is a big advantage since added steps during sample preparation would increase the analysis time, solvent consumption, variability and significant losses of target analyte and therefore sensitivity. The performance of the method in terms of good sensitivity, linearity and precision, and very short analysis time achieved with this method as well as the results obtained in the analysis make possible to propose the new analytical methodology for the routine analysis of MB in industrial wastewater samples. In the future, the proposed method could be extensively applied in MB exposure estimation of humans and aquatic animals.

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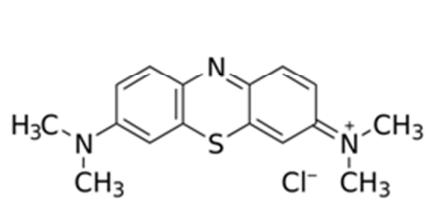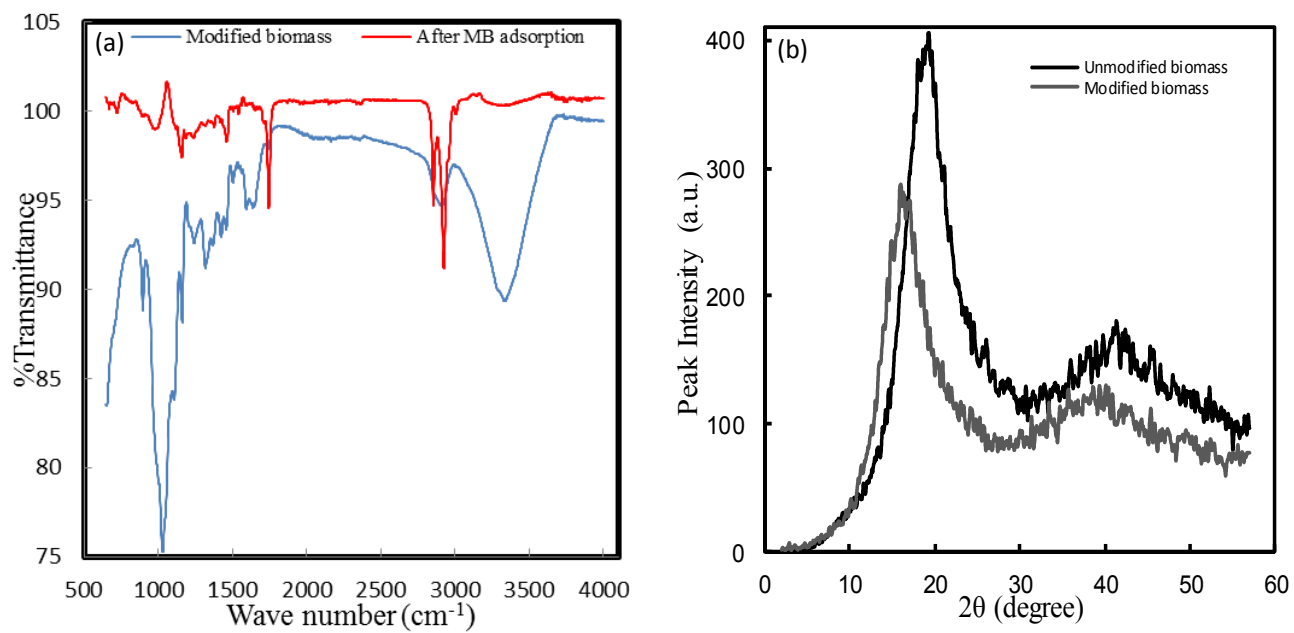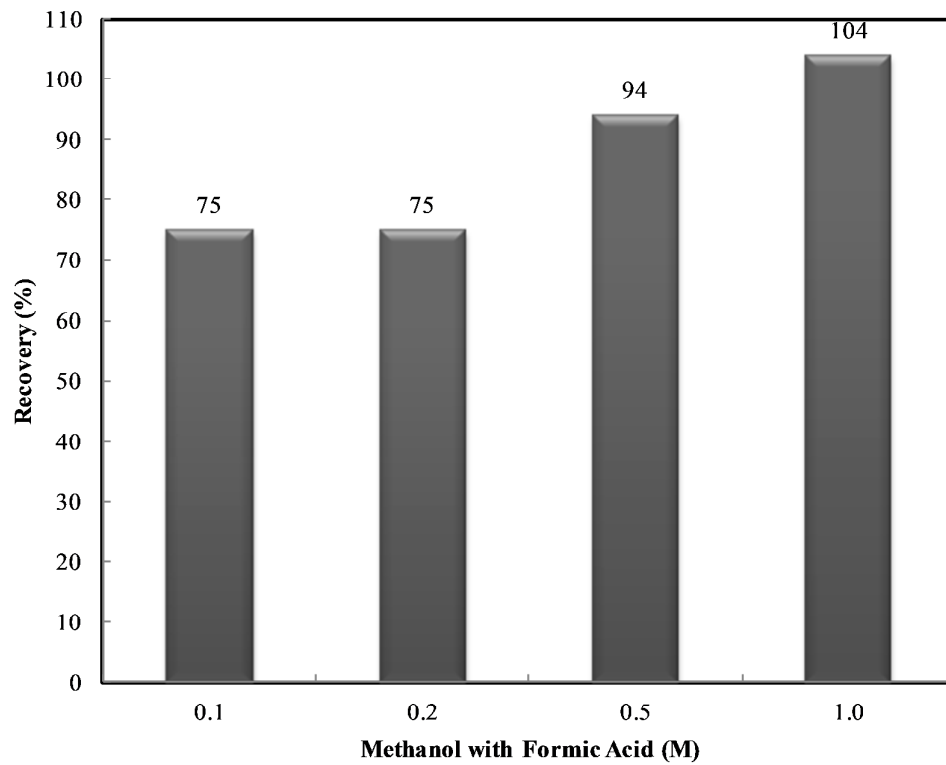
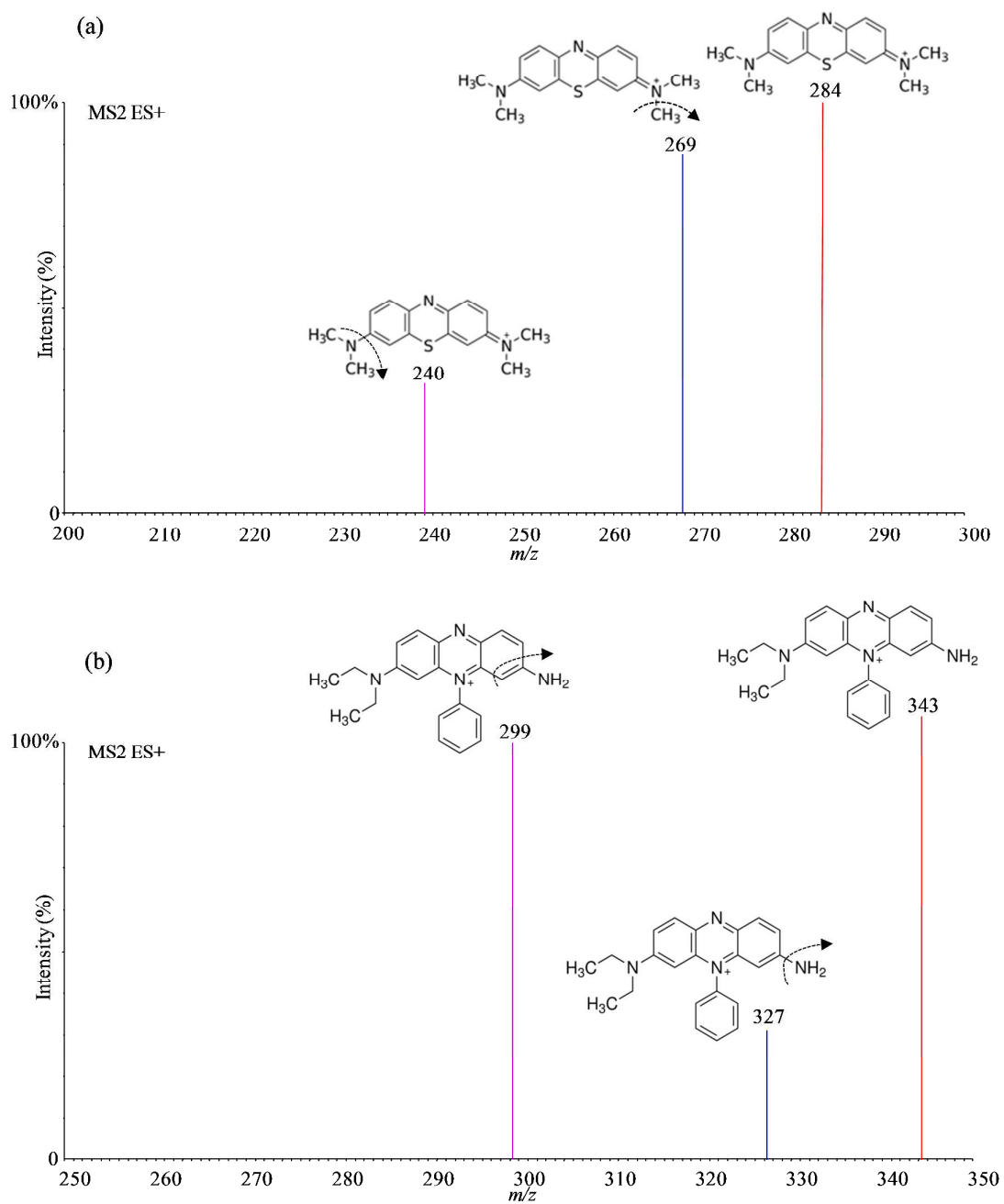Methylene blue
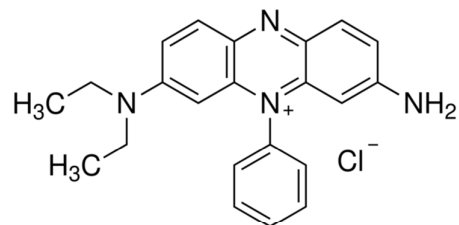
Methylene violet 3RAX (I.S.)
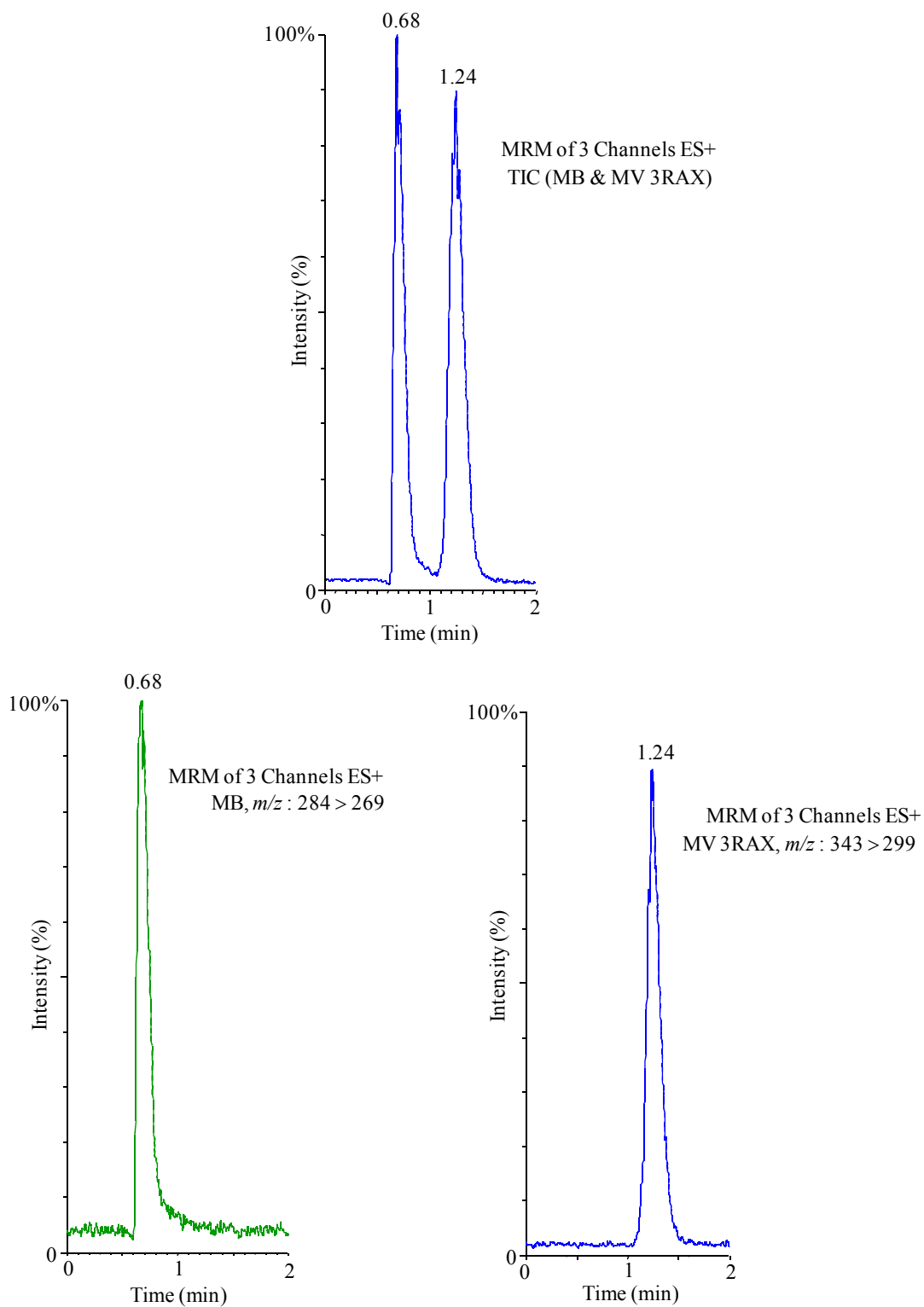
Figure 1. Chemical structures of dyes studied.

Figure 2. Fourier transform infra-red (FT-IR) spectra (a), and Wide angle X-ray diffraction (WXRD) pattern (b) of PS biomass.

Figure 3. Effect of sample modifier (formic acid) on MB extraction using SPE method.

Figure 4. MS/MS spectra of MB precursor ions, *m/z* 284 (a), and MV 3RAX, *m/z* 343 (b) with the proposed fragmentation.

Figure 5. UPLC-MS/MS chromatograms of MB & MV 3RAX, TIC (a), MB, *m/z*
284→269 (b), MV 3RAX, *m/z* 343→299 (c) from textile samples.

Table 1

MRM parameters used with the MS/MS instrument*

Analyte	Precursor ion (*m/z*)	Quantification		Confirmation	
		Collision energy (V)	Daughter ion (*m/z*)	Collision energy (V)	Daughter ion (*m/z*)
MB	284	60	269	60	240
MV 3RAX	343	72	299	72	327

* Dwell time was 0.025 s in all cases

Table 2

MB concentration levels and recovery rates in wastewater samples

Sample	MB (μg/mL $\pm$ s*)	Recovery (%)
Paper	0.54 $\pm$ 0.02	96
Textile	1.08 $\pm$ 0.04	99
Laundry	0.36 $\pm$ 0.01	95
Printing press	0.83 $\pm$ 0.03	98

*Standard deviation obtained from addition standard calibration curve