Unveiling Chinese red dyes through the centuries with quadrupole time-of-flight mass spectrometry

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Introduction

Identifying organic dyestuffs in cultural heritage artifacts is of paramount importance to conservators, historians and curators as they provide useful clues on the trade routes, source of materials and the colouring processes [1]. However, it is always a challenging task due to minute quantities of sampling as well as the variation and the complexity in the dyeing processes. This paper presents the workflows on the analysis of the red dyes of the artefacts dated from mid-Qing Dynasty (1644-1912 C.E.) to 1950s collected by the Hong Kong museums.

Extraction Method

Textiles -*Silk birthday hangings*

The textile fibres were dissolved in 37% hydrochloric acid [2] and then diluted with methanol and deionized water (2:1:1) for 12 hours and filtered with 0.2μm PTFE syringe filter.

Paper -*wedding documents, in-patient registers and opera libretto*

The colourants were extracted by immersion small pieces of paper in methanol, ultra-sonicated for 10 minutes and then filtered with 0.2μm PTFE syringe filter.

Experimental Condition

Liquid chromatographic separation was performed on Waters Acquity UPLC equipped with BEH C18 column with dimensions 2.1x100mm and particles with 1.7μm in diameter. The mobile phase started with 9:1 (0.1% formic acid in water : 0.1% formic acid in acetonitrile) and changed to 1:9 in generic gradients within 12 minutes with flow rate 0.4mL/minute. Separated components were analyzed by uv-visible photo diode array (PDA) detector from 400-800nm at 1.2nm resolution. The analytes were ionised by electrospray ionization technique at +3kV and the mass spectra were acquired by the quadrupole time-of-flight mass spectrometer (qTof) Synapt G2 with leucine as lock-mass.

Analyte ions were scanned under positive mode to determine the m/z ratio, molecular formulae and then the structures. The mass spectra were obtained by integrating the total ion chromatogram at the time shortly after the absorption peaks (about 0.02-0.03 minutes) in the visible PDA spectra. The molecular formulae of the analytes were elucidated by studying the isotopic profiles of 3 isotopic peaks. Ions selected for fragmentation were broken down by collision energy 35V & 70V and detected in positive mode.

Useful hints for dye identification

- direct dyes on paper could be easily dissolved in methanol while textile dyes are always tightly bound to the fibres which requires concentrated acid (i.e. HCl) to extract [2]
- weak acids (formic or acetic acid) are added to mobile phase to facilitate protonation for positive detection
- positive potential ionization is generally used except for dye molecules contain proton donor groups such as sulphur groups [3]
- electrospray ionization is a soft ionization method that seldom cause fragmentation which is better for formula elucidation by high resolution mass spectrometer [4]
- The lag time between PDA detector and qTof is measured beforehand under the same experimental condition to determine the time of chromophores reaching the mass spectrometer
- Under positive ionization, the analytes may protonate to $M\text{-}H^+$ or lose the anions (e.g. Cl^-) while metal cations (e.g. Na^+) will be replaced by hydrogen

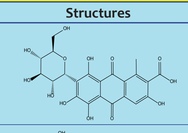
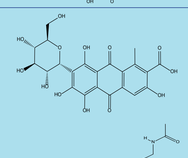
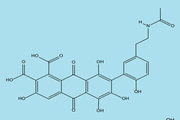
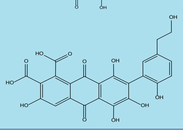
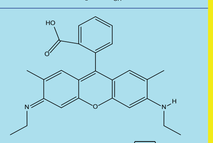
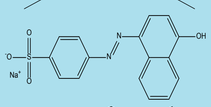
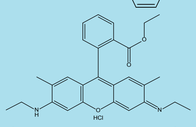
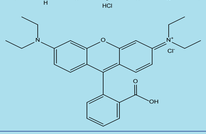
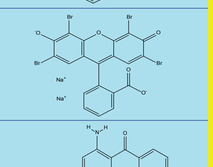
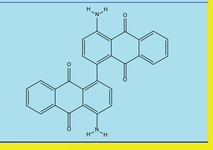
Elucidating the formulae and structures of the dyes

- The most intense peaks in the mass spectra are supposed to be the chromophores integrated from the total ion chromatograms at the measured lag time after the visible absorption at the PDA.
- Possible molecular formulae could be generated and sorted according to the isotopic profiles and detected masses. Dye molecules always comprise C, O, H and N (azo and amino groups) with mass range between 100 to 1,000. Cl and Br can be included in the second stage.
- Some formulae generated can be immediately excluded due to the unreasonable elemental ratio (e.g. very high oxygen or nitrogen ratio to carbon); many dyes are ring structures with unsaturations.
- Chemspider (http//:www.chemspider.com) is a very useful internet database to generate structures from molecular formulae. Colour index [5] or other reference materials [6] are useful for looking up synthetic dye formulae and structures.
- Tandem mass spectrometry can help to elucidate or confirm the structures by breaking up the molecules further into fragments in the second stage. It is particularly useful for distinguishing isomers [3].

Wedding document

Conclusion

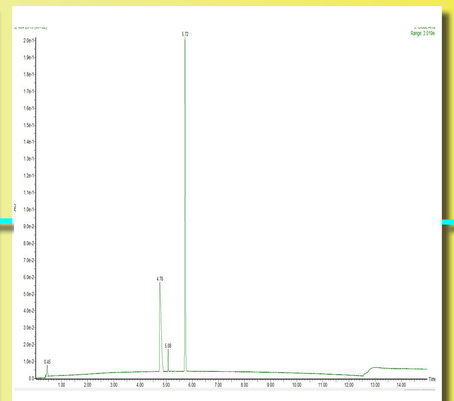
LC-PDA-qTof is a power analytical method for the identification of both natural and synthetic organic colourants from artefacts with simple sample preparation. Nine different red dyes: carminic acid, laccaic acid A & B [7], rhodamine B, 6G & 575, 2-naphthol orange, eosin Y [8] and cromophtal red A3B were identified from collections of Hong Kong museums dating from the mid-Qing dynasty to 1950s. Results not only show the trend of red dyes used in the Pearl River Delta region, but also testify the transition from natural to synthetic materials in the dyeing industry through the centuries in Guangdong region.

Artefacts	Date	λmax (nm)	m/z	Dyes	Structures
Silk birthday hanging	1878 Qing dynasty	494	493.0977 $C_{22}H_{21}O_{13}^+$	Carminic acid $C_{22}H_{20}O_{13}$	
		493	493.0981 $C_{22}H_{21}O_{13}^+$	Carminic acid $C_{22}H_{20}O_{13}$	
Silk birthday hanging	1789 Qing dynasty	487	538.0988 $C_{26}H_{20}NO_{12}^+$	Laccaic acid A $C_{26}H_{19}NO_{12}$	
		487	497.0716 $C_{24}H_{17}O_{12}^+$	Laccaic acid B $C_{24}H_{16}O_{12}$	
Chinese wedding documents	1940-50	542	415.2013 $C_{26}H_{27}N_2O_3^+$	Rhodamine 575 $C_{26}H_{26}N_2O_3$	
		485	329.0593 $C_{16}H_{13}N_2O_4S^+$	2-naphthol orange $C_{16}H_{11}N_2O_4S\ Na$	
		558	443.2323 $C_{28}H_{31}N_2O_3^+$	Rhodamine 6G $C_{28}H_{31}N_2O_3Cl$	
		558	443.2323 $C_{28}H_{31}N_2O_3^+$	Rhodamine B $C_{28}H_{31}N_2O_3Cl$	
Grid-lines on in-patient registers	1911 to 1945	528	644.7183 $C_{20}H_9O_5Br_4^+$	Eosin Y $C_{20}H_6O_5Br_4Na_2$	
Grid-lines on a Opera libretto	1950	507	445.1207 $C_{28}H_{17}N_2O_4^+$	Cromophtal red A3B $C_{28}H_{16}N_2O_4$	

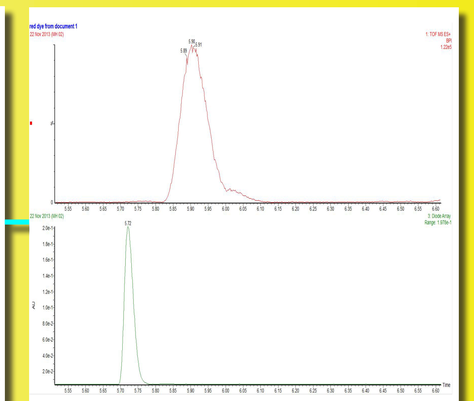
Results

Extraction

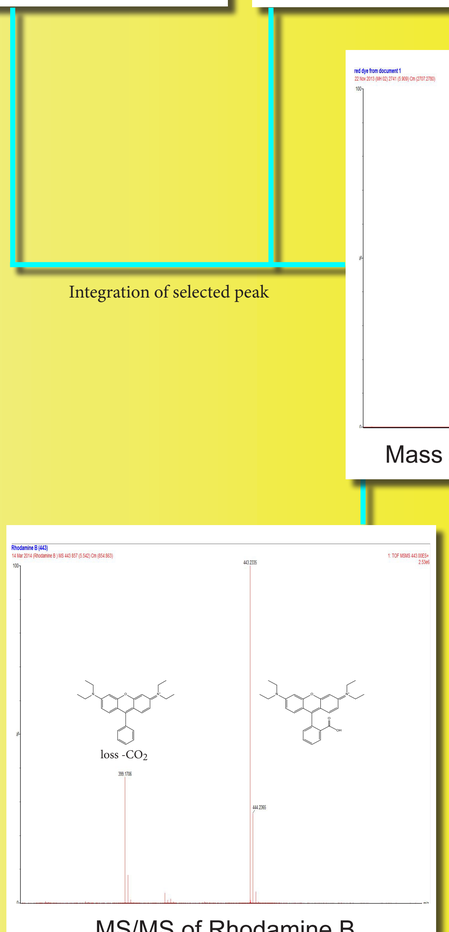
Liquid chromatography



Visible spectrum by PDA



Total ion chromatogram by qTof

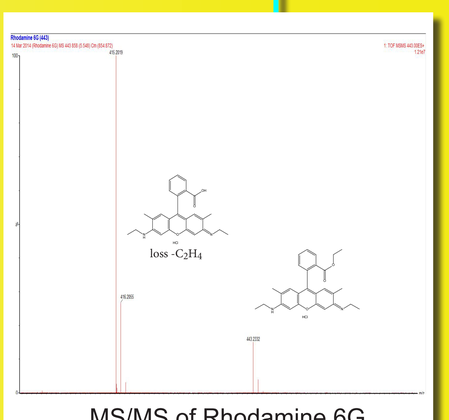


Integration of selected peak

Mass spectrum of chromophore

Silk birthday hanging

MS/MS of Rhodamine B



MS/MS of Rhodamine 6G

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