i 1 [illegible]

which a sample can be obtained depends on the type of object. It can be a straightforward process (e.g. using loose ends on tapestries, better preserved and less faded as they are on the back) (Fig. 1). Sampling can be harder with ethnographic materials where broken pieces may be retrieved during restoration or it can be sometimes almost impossible when dealing with easel paintings. The nature of the substrate affects how the dyestuff is fixed, and in turn, this can cause variations in the dye 'fingerprint'.

Before dyes can be analysed it is usually necessary for the analyst to extract the dyestuffs from their textile substrate. This requires the use of a solvent to break the dye–mordant–fibre complex. Until recently, extraction using concentrated hydrochloric acid with methanol and water was widely used. The main advantage of this method is that the sample is completely broken down during the extraction process, thereby maximising the yield of extracted material. However, its main drawback is the chemical modification or complete degradation of the substances in the sample, which can hinder the identification of a specific dye source. In recent years, several alternative procedures have been developed using milder extraction methods,[5] still based on aqueous solutions of acids mixed with solvents. Vat dyes (indigoids from indigo, bromo-indigoids from shellfish purple) are a category apart and specifically require extraction using the polar solvents dimethyl sulfoxide or dimethylformamide.[1] Finally, paint substrates require a derivatization procedure which releases the trapped pigment from the binding medium and then de-complexes the dye from the pigment. This can be achieved using methylation with boron trifluoride/methanol.[1]

[illegible]

[illegible]

Reverse-phase LC is the most suitable technique to investigate dye sources, as the majority of the molecules with chromophores are polar and water-soluble compounds. The separation of most natural and synthetic dyes is obtained using a combination of water and an organic modifier (methanol or acetonitrile) as the eluant; elution can be performed in either isocratic or gradient modes. The pH at which elution is carried out is controlled either by the use of a constant percentage of a buffer solution, or the addition of an appropriate acid (e.g., formic acid). Recent developments in ultra-high-performance liquid chromatography (UHPLC), together with the development of columns packed with sub-2 µm particles, or core–shell technology, allow increased efficiency and sensitivity of detection, which are important requirements when analysing small or highly degraded samples.

D[illegible]

The most suitable detector is a PDA, which can identify chromophores absorbing in the UV/visible spectrum range (210–800 nm) and which the analyst can tune to specific absorption bands in order to detect each type of coloured chromophore: colourless at 254 nm, yellow at 350 nm, red at 485 nm, violet at 550 nm, to blue at 650 nm. The identification of a dye molecule is usually based on its retention time and UV-visible spectrum, while the identification of a specific dye source is based on the presence of a combination of molecular markers, and on the semi-quantitative interpretation of the chromatogram by comparing it with fresh and artificially aged reference samples. More recently, the application of the multivariate statistical techniques of principal component analysis (PCA) and partial least squares regression (PLS) have proven to be successful approaches for extracting information from large and complex data sets. When dealing with complex samples, it is often necessary for the analyst to couple the chromatographic separation of components to a MS to obtain additional information. ESI in positive and negative mode is commonly used to study natural and synthetic dyes to obtain information on their molecular mass (MS spectra) and further fragmentation patterns (MS/MS).

imp c5ro-5.9(a)op

mauve, the [illegible]

twelve biological sources, ten dye classes, dyed yarns, pigments and paints, Stud. Conserv., 2011, **56**, 231