Journal of Visualized Experiments

Two-step pyrolysis-gas chromatography method with mass spectrometric detection for identification of tattoo ink ingredients and counterfeit products

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE59689R1
Full Title:	Two-step pyrolysis-gas chromatography method with mass spectrometric detection for identification of tattoo ink ingredients and counterfeit products
Keywords:	Organic pigments; Pyrolysis; tattoo ink; gas chromatography; polymers; analytical chemistry
Corresponding Author:	Ines Schreiver Bundesinstitut fur Risikobewertung Berlin, Berlin GERMANY
Corresponding Author's Institution:	Bundesinstitut fur Risikobewertung
Corresponding Author E-Mail:	Ines.Schreiver@bfr.bund.de
Order of Authors:	Ines Schreiver
	Christoph Hutzler
	Andreas Luch
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Berlin, Berlin, Germany

TITLE:

A Two-Step Pyrolysis-Gas Chromatography Method with Mass Spectrometric Detection for Identification of Tattoo Ink Ingredients and Counterfeit Products

AUTHORS & AFFILIATIONS:

Ines Schreiver[1], Christoph Hutzler[1] and Andreas Luch[1]

[1]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany

Corresponding Author:

Ines Schreiver (Ines.Schreiver@bfr.bund.de)

Tel: +49(30)-18412-4181

Email Addresses of Co-authors:

Christoph Hutzler (Christoph.Hutzler@bfr.bund.de)

Andreas Luch (Andreas.Luch@bfr.bund.de)

KEYWORDS:

organic pigments, pyrolysis, tattoo ink, gas chromatography, polymers, counterfeit identification

SUMMARY:

This method for two-step pyrolysis online coupled to gas chromatography with mass spectrometric detection and data evaluation protocol can be used for multi-component analysis of tattoo inks and discrimination of counterfeit products.

ABSTRACT:

Tattoo inks are complex mixtures of ingredients. Each of them possesses different chemical properties which have to be addressed upon chemical analysis. In this method for two-step pyrolysis online coupled to gas chromatography mass spectrometry (py-GC-MS) volatile compounds are analyzed during a first desorption run. In the second run, the same dried sample is pyrolyzed for analysis of non-volatile compounds such as pigments and polymers. These can be identified by their specific decomposition patterns. Additionally, this method can be used to differentiate original from counterfeit inks. Easy screening methods for data evaluation using the average mass spectra and self-made pyrolysis libraries are applied to speed up substance identification. Using specialized evaluation software for pyrolysis GS-MS data, a fast and reliable comparison of the full chromatogram can be achieved. Since GC-MS is used as separation technique, the method is limited to volatile substances upon desorption and after pyrolysis of the sample. The method can be applied for quick substance screening in market control surveys since it requires no sample preparation steps.

INTRODUCTION:

Tattoo inks are complex mixtures consisting of pigments, solvents, binders, surfactants, thickening agents, and, sometimes, preservatives[1]. The increased popularity of tattooing in the

last decades has led to the establishment of legislation addressing tattoo ink safety across Europe. In most instances, color-giving pigments and their impurities are restricted and therefore should be monitored by state laboratory market surveys to control their compliance with law.

Using the approach of online pyrolysis-gas chromatography mass spectrometry (py-GC-MS) described here, multiple ingredients can be identified simultaneously. Since volatile, semi-volatile and non-volatile compounds can be separated and analyzed within the same process, the variety of target compounds is high compared to other methods used for tattoo ink analysis. Liquid chromatography methods are mostly carried out with pigments solubilized in organic solvents[2]. Raman spectroscopy as well as Fourier-transform infrared (FT-IR) spectroscopy have been described as suitable tools for the identification of pigments and polymers but are limited with multi-ingredient mixtures since no separation technique is used in standard laboratory applications[3,4]. Laser desorption/ionization time-of-flight mass spectrometry (LDI-ToF-MS) has also been used for pigment and polymer identification[5,6]. Altogether, most methods lack the analysis of volatile compounds. The lack of suitable commercial spectral libraries is a common disadvantage of all of these methods. The identification of inorganic pigments has often been carried out with either inductively coupled plasma mass spectrometry (ICP-MS)[7,8] or energy dispersive X-ray spectroscopy (EDX)[4,9]. Also, FT-IR and Raman spectroscopy have been used for the analysis of inorganic pigments such as titanium dioxide or iron oxides in other research fields[10-13].

The goal of this study was to establish a method applicable in standard analytical laboratories with moderate financial costs to upgrade existing and common devices. Py-GC-MS as described here is a non-quantitative approach for identification of organic ingredients from mixtures. Upon identification of suspicious substances in a py-GC-MS screening, target substances can be quantified with more specialized approaches. It is especially interesting for the analysis of non-volatile and non-soluble substances like pigments and polymers.

The described method can be adapted for inks and varnishes in other fields of application. The data evaluation methods described are applicable to all pyrolysis investigations. Also, counterfeit products, mostly from Asian markets, display a potential source of risk to the consumer and a financial burden to manufacturers (personal communication at the 3rd ECTP in Regensburg, Germany, 2017). The method described here can be used to compare the characteristics of putative counterfeit inks to an original bottle, similar to published forensic approaches for car varnish identification[14].

PROTOCOL:

1. Tattoo ink preparation and sample mounting

1.1. Use a 25 mm hollow glass pyrolysis tube as a sample holder and quartz wool for sample preparation.

1.1.1. Grab the pyrolysis tube with the specialized tweezers for pyrolysis tubes (bake out for

decontamination) and insert the necessary amount of quartz wool with pointed tweezers into the tube.

1.1.2. Insert two steel sticks (bake out for decontamination) at each side of the pyrolysis tube and compress the wool into a 1–2 mm thick stopper. The stopper must be positioned at the lower third of the pyrolysis tube to achieve adequate heating during the pyrolysis process.

1.1.3. Ignite a gas burner and bake the pyrolysis tube and filling for 2–3 s from each side to remove contaminants.

NOTE: Use clean gloves and do not touch any item before handling the pyrolysis tube and wool. Use eye protection and remove all flammable liquids and items during the pyrolysis tube bake out.

1.2. The protocol can be stopped here. Store the prepared pyrolysis tubes in a clean glass dish until further use.

1.3. Shake the tattoo ink bottles vigorously for 1 min by hand to ensure homogeneity. Additionally, they can be placed in an ultrasonic water bath for 1 min. Some inks may still show sedimented pigments after conducting both steps and may need prolonged homogenization.

1.4. Obtain a 2 µL microcapillary with a diameter below the inner diameter of the pyrolysis tube. Dip the capillary tip in the ink and aspirate about 1 µL of ink by filling half of the capillary.

1.5. Insert the capillary into the pyrolysis tube and stain the quartz wool stopper with the ink. A clear color staining must be visible without adding too much ink to the sample.

1.6. Obtain a steel transport adapter for the automated injection unit and attach the prepared pyrolysis tube to it using tweezers for pyrolysis tubes.

1.6.1. Check that the pyrolysis tube is perfectly vertical and does not fall off during shaking.

1.6.2. Place the transport adapter in the tray at the desired position.

NOTE: The ink might contaminate the steel transport adapter to which the pyrolysis tube is attached; therefore, this needs more thorough cleaning afterwards.

2. Analysis of ink samples by py-GC-MS

2.1. Set up the GC-MS-system equipped with a Thermal Desorption Unit (TDU) and a pyrolyzer module on top of the Cold Injection System (CIS) according to the manufacturer's instructions. Use an inert electron impact (EI) ion source at 70 eV and helium with a purity of 99.999% as carrier gas (1 mL/min). Set the split ratio of the CIS to 1:50.

2.2. Install an HP-5MS column and a guard column for separation. Set the following analysis parameters in the instruments' control software: start the oven temperature at 50 °C, hold for 2 min, and ramp at 10 °C/min to 320 °C. Hold the final temperature for additional 5 min. Set the transfer line temperatures to 320 °C.

2.3. Run each sample in a solvent vent mode without pyrolysis to analyze for semi-volatile compounds.

2.3.1. Use the solvent vent option of the TDU/injector and ramp the TDU temperature after 0.5 min starting at 50 °C to 90 °C at a rate of 100 °C/min. The solvent vent will be shut off after 1.9 min.

2.3.2. Heat the temperature of the TDU to 320 °C at a rate of 720 °C/min for 3.5 min. The volatile compounds are captured in the CIS at -150 °C.

2.3.3. Hold the CIS temperature for 2 min and ramp to 320 °C at a 12 °C/min rate.

NOTE: Extended time between sample preparation and analysis leads to evaporation of volatile compounds since the sample holder is open. Analyze samples within a few hours after preparation if volatile compounds are being targeted.

2.4. Conduct a second run of the same sample in which the pyrolysis unit is used to investigate non-volatile compounds.

2.4.1. Use the same oven program as for the first desorption run.

2.4.2. Keep the temperature of the CIS constant at 320 °C and use a split ratio of 1:100.

2.4.3. Ramp the TDU directly from 50 °C to 320 °C at a 720 °C/min rate and keep constant for 1.6 min.

2.4.4. Program a 6 s pyrolysis at the desired temperature (here 800 °C) in the final temperature segment of the TDU.

NOTE: Make sure to use an adequate amount of sample and split ratio to prevent contamination of the column and MS. Adapt the split ratios for individual instrument set-ups if necessary.

2.5. Use a polystyrene standard to check the performance of the instrument. Run at least three polystyrene samples before and after each experiment. Insert 2 µL of a 4 mg/mL polystyrene standard in dichloromethane into the glass wool and perform a pyrolysis at 500 °C for 0.33 min.

2.6. Check if the peak area ratio of the polystyrene monomer and the dimer is between 3 and 4 and the tailing of the dimer is below 2 in the resulting chromatogram (also called a pyrogram). Keep historical data of polystyrene parameters and calibrate the pyrolysis temperature if the

peak ratio is out of range.

NOTE: Values for polystyrene monomer and dimer ratio as well as tailing should be taken from historical values of well operating systems.

3. Data evaluation approaches

NOTE: Data evaluation should be adapted depending on the individual analytical questions, e.g., the search for volatiles, non-volatile compounds, hazardous cleavage products from azo pigments, or similar.

3.1. Data evaluation for volatile compounds

3.1.1. For data evaluation of volatile compounds, start the GC-MS analysis/MS library searching software (see **Table of Materials**) and open the chromatogram of the desorption run.

3.1.2. Select commercial libraries by clicking on **Spectrum** and **Select Library.** Load the library of interest.

3.1.3. Select integration parameters and perform a library search by clicking on **Spectrum** and **Library Search Report**.

NOTE: Take special care to compare library spectra of putative identified compounds with the spectra obtained in the analysis of the ink. Sometimes good matches can be achieved despite the presence of additional molecular mass peaks in the unknown spectra. For unequivocal identification, analytical standards have to be analyzed using the set up and instrument parameters to verify retention times and spectra.

3.2. Fast screening for non-volatile compounds

NOTE: Identification of non-volatile compounds from pyrolysis is based on the presence of multiple specific decomposition products of the parent compound within the same pyrogram. Since pyrograms can contain up to several hundreds of compounds, manual evaluation is hardly feasible. Start with a quick data evaluation approach using the average mass spectra (AMS). This is useful for identification of the most abundant pigment or polymer within the sample. This approach is only suitable for fast screening for ink declaration fraud and for having a starting point for manual pyrogram evaluation.

3.2.1. For pyrolysis data evaluation, mark the whole chromatogram in the GC-MS evaluation software (see **Table of Materials**) with the right mouse button pressed down to obtain an AMS.

3.2.2. Create a library of obtained spectra of known reference substances of all compounds of interest, e.g., pigments and polymers, by clicking on **Spectrum** and **Edit Library** (click **Create New Library** if none is present).

3.2.3. Select **Add New Entry** and fill in all information of interest.

NOTE: Select the AMS spectra of standards or inks, and click on **Spectrum** and **NIST Search** if forwarding to another software capable of MS library searches is desired.

3.2.4. Generate an AMS of the investigated ink pyrogram and use the library search for comparison to the self-made AMS library[15]. Exclude masses from column bleed or other column noises.

NOTE: The highest match in the library search list will likely be the most abundant pigment or polymer in the inks. To verify this, compare the single characteristic decomposition products of the substance seen in the pyrolysis of the standard substance in the pyrogram of the analyzed ink (see section 3.4 and **Supplementary Table 1**).

3.3. Identification of non-volatile compounds with specialized pyrogram evaluation software

3.3.1. Convert the pyrograms of interest to the needed format as stated in the software instructions. Integrate the pyrogram in a way that a maximum of 200 compounds are found. Otherwise, data evaluation time in the pyrogram evaluation sofware increases significantly.

3.3.2. Build a folder on your computer with all pyrogram entries that should serve as a library, e.g., pigment pyrogram library for pigment identification or pyrograms of an original ink to compare it to putative counterfeit products.

3.3.3. Load the unknown pyrogram in the tab **Library Search** by clicking on **Browse**.

3.3.4. Load the library folder and select only **MS matching** and **RT matching** in the **Search options**, since the overall abundance will vary compared to pyrogram of reference pigments.

3.3.5. Click on **advanced** in the Search options. Select the parameter “use only peaks with specified MS spectra” and use a fit threshold of 850 in the pyrogram evaluation software.

3.3.6. Click on **Add** to save specified MS spectra (3–5 MS spectra of the most abundant peaks) from each pyrogram of reference pigments or polymers from the library in the advanced search options (cf. **Supplementary Table 1**). In this way only pigment related peaks are compared even in a multi-component ink with otherwise interfering high-abundant peaks.

3.3.7. Press **OK** to return to the main window.

3.3.8. Click on **Search** to start the comparison.

3.3.9. If needed, go to **Chromatogram Match** tab, select a compound, and click **Send to NIST** to forward the spectra to the MS library software and identify the compound.

NOTE: Click **send to MS options** to include the spectra in the advanced **Search options** (cf. step 3.3.6).

3.4. Manual substance identification

NOTE: If no specialized pyrogram evaluation software is available use the data evaluation by standard MS library search program and commercial library together with reported fragments (**Supplementary Table 1**) and our pyrolysis library[15]. Manual comparison of pyrogram compounds with known decomposition products is also carried out to verify the hits given in the AMS.

3.4.1. For data evaluation for non-volatile compounds, start the GC-MS analysis/MS library searching software and open the chromatogram of the pyrolysis run.

3.4.2. Select commercial and (self-made) pyrolysis libraries (e.g., our provided pyrolysis library[15]) by clicking on **Spectrum** and **Select Library.** Load all libraries of interest.

3.4.3. Integrate the pyrogram in the GC-MS evaluation software and consider all peaks with an area ≥0.2% of the total area (may be adapted in a way that a manageable amount of peaks will be integrated).

3.4.4. Start the library search by clicking on **Spectrum** and **Library Search Report**.

3.4.5. Manually compare all library matches to specific pigment and polymer decomposition products in **Supplementary Table 1** or fragments stated in literature[16-26]. For pigments, 2 to 3 decomposition products are needed to unambiguously identify the pigment used.

NOTE: All spectral matches with corresponding libraries must be carefully evaluated. Additional peaks higher than the mass peak often account for similar structures with additional side groups. If possible, reference substances should be analyzed to obtain the individual retention time in the analytical set-up.

REPRESENTATIVE RESULTS:

The method includes a two-step chromatographic approach for each sample (**Figure 1**). In the first run, the sample is dried inside the injector system at 90 °C before volatile compounds are transferred onto the column. Since the drying process is incomplete in most cases, residual solvents and volatile compounds are transferred and analyzed. In the second run, the previously dried sample is subsequently pyrolyzed to facilitate the analysis of non-volatile organic components.

[Place **Figure 1** here]

Well produced inks with highly pure ingredients and a limited number of components result in

chromatograms easy to interpret with standard libraries, since most peaks can be identified (**Figure 2**). But even in the high-quality inks, non-declared ingredients have been found. For example, propylene glycol is often found in addition to the declared glycerol (**Figure 2** and **Figure 3**).

Other substances such as formaldehyde might be added as a preservative. 1-Hydroxy-2-propanone may result as an impurity of pigment synthesis and is therefore an example of a non-intentionally added substance (NIAS).

[Place **Figure 2** here]

Inks containing multiple ingredients and impurities will result in a pyrogram that is difficult to interpret (**Figure 3**). Most peaks occurring in the second run may not be baseline separated from each other, making identification difficult, even when utilizing data deconvolution. Some substances might also result in peaks below the threshold set during data evaluation (e.g., 0.2% of total peak area). A solution to this problem might be a step-wise approach using 400 °C, 600 °C, and 800 °C in consecutive pyrolysis steps for the very same sample (see **Figure 4**).

Some pigment decomposition products may descent from multiple pigments (Supplementary **Table 1**). For example in **Figure 3** and **Figure 4**, acetyl cyanide can derive from multiple yellow or orange pigments. The degradation product 2-methoxyphenylisocyanate may also derive from the pigment Red 9, and *o*-anisidine from the pigments Red 170 and Red 9. However, due to the combination with the degradation product 4-methoxy-2-nitro-aniline and the yellow appearance of the ink, only pigments yellow 65 and 74 would be plausible as parent compounds. These two pigments are regional isomers and cannot be distinguished from another with this method. Pigment orange 13 — which was declared on the ink bottle — has not been identified. If the pigment was only present in low amounts, it might have been below the limit of detection. On the other hand, inks are often declared falsely[27].

[Place **Figure 3** here]

[Place **Figure 4** here]

A positive result for counterfeit product identification is displayed in the following example (**Figure 5**). Three “lemon yellow” inks have been purchased either from a licensed distributor of the US-based ink manufacturer, via an internet auction platform or via an Asian market place. All inks have been analyzed with the two-step py-GC-MS method. In this example, the differences in peak numbers and retention times are already visible by eye.

The chromatogram from the 1st desorption run and the pyrogram from the 2nd run of the original ink were compared against three independent acquisitions of the original ink and the two counterfeit products using pyrogram evaluation software. The software was found to be highly useful in distinguishing the different inks. The forward match factor was above 0.9 (with 1 being the perfect match) only towards pyrograms or desorption chromatograms of the same ink,

respectively.

Also, forward matches above 0.9 were only achieved with the same ink when comparing the ink to library contained pyrograms of 84 inks of various colors and manufacturers.
Alternatively, a statistical comparison as proposed by Yang et al. for car varnishes may be applied[14].

[Place **Figure 5** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic diagram of the pyrolysis workflow.

Figure 2: Representative results of the py-GC-MS analysis of a tattoo ink with only a few, pure ingredients. (**A**) 1st run: desorption for identification of volatile compounds. (**B**) 2nd run: pyrolysis for identification of less and non-volatile compounds. Declared and identified ingredients are indicated below.

Figure 3: Representative results of the py-GC-MS analysis of the yellow tattoo ink "banana cream" with many, impure ingredients. (**A**) 1st run: desorption for identification of volatile compounds. (**B**) 2nd run: pyrolysis for identification of less and non-volatile compounds. Declared and identified ingredients are indicated below.

Figure 4: Gradual pyrolysis of the yellow ink "banana cream" displayed in Figure 3. A-C) Consecutive pyrolysis runs at 400 °C, 600 °C, and 800 °C.

Figure 5: Identification of counterfeit products by py-GC-MS. Three "lemon yellow" inks from licensed distributor (**A**), an online auction platform (**B**), and an Asian market place (**C**) have been analyzed.

DISCUSSION:

Py-GC-MS is a useful screening method for a broad range of substances in tattoo inks that can also be used for the analysis of other products. Compared to other methods, py-GC-MS can be conducted with only minimal sample preparation. GC-MS devices can be found in most analytical laboratories compared to more specialized methods such as MALDI-ToF-MS and EDX.

The data evaluation of pyrograms may be challenging, since the list of possible ingredients is infinite in theory and library searches that also account for the combination of substances towards a parent compound in the library are necessary. The data evaluation methods described here allow for reliable fast screening of substances that have been added to standard pyrogram libraries. Conversely, testing for counterfeit products is a fast and straightforward approach that can be conducted without any pre-build libraries, since the identification of single substances is irrelevant.

To obtain the best possible results, the amount of ink added to pyrolysis should neither be too high nor too low. This will either result in a contamination of the pyrolysis unit, liner or column or a lack of significant peaks for proper pyrogram interpretation. Therefore, using a defined volume of ink as described in this method with adjusted split ratios is highly recommended. As shown in **Figure 3**, impurities or polymers can overload the pyrogram with peaks impairing the identification of single substances. Therefore, the detection limit for pigments is highly dependent on the corresponding mixtures. In such cases, pigments might first be separated from other ink ingredients by dilution and precipitation in alcohol-water solvents.

The limitation of the method is the analysis of organic pigments without specific cleavage sides such as quinacridones, perylenes and perinones[16-18]. Also, if a mixture of multiple pigments with the same cleavage group occurs (e.g., with azo pigments), the identification might be challenging (cf. ink displayed in **Figure 3**). In addition, the pyrolysis products must be able to enter the gas phase. Polymers like hydroxyethyl-cellulose consisting of sugar monomers that have to be chemically modified for GC-MS analysis cannot be detected by py-GC-MS. As in all other methods, only pigments with known pyrograms can be identified. However, main decomposition products can be concluded from the pigment structure, especially in the case of azo pigments. Therefore, checking for plausibility of a declared pigment can be carried out even if the pigment has never been analyzed in py-GC-MS before.

The method can be used to discriminate original inks from counterfeit products. However, a reliable sample of the original ink must be available. Since the composition of inks may change over time, inks produced in the same time range or at best from the same batch must be used for comparison. In future, py-GC-MS might be used to monitor tattoo ink ingredients and thereby reveal declaration fraud and the use of banned pigments and possible other ingredients. A further application of these methods might be the identification of counterfeit products[14].

ACKNOWLEDGMENTS:
This work was supported by the intramural research project (SFP #1323-103) at the German Federal Institute for Risk Assessment (BfR).

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:

1 Dirks, M. in *Tattooed skin and health* Vol. 48 *Current Problems in Dermatology.* (eds Serup, J., N. Kluger, N., Bäumler, W.). 118–127 (Karger, 2015).
2 Engel, E. et al. Establishment of an extraction method for the recovery of tattoo pigments from human skin using HPLC diode array detector technology. *Analytical Chemistry.* **78** (15), 6440-6447 (2006).
3 Poon, K. W. C., Dadour, I. R., McKinley, A. J. In situ chemical analysis of modern organic tattooing inks and pigments by micro-Raman spectroscopy. *Journal of Raman*

Spectroscopy. **39** (9), 1227-1237 (2008).
4 Timko, A. L., Miller, C. H., Johnson, F. B., Ross, V. In vitro quantitative chemical analysis of tattoo pigments. *Archives of Dermatology.* **137**, 143-147 (2004).
5 Boon, J. J., Learner, T. Analytical mass spectrometry of artists' acrylic emulsion paints by direct temperature resolved mass spectrometry and laser desorption ionisation mass spectrometry. *Journal of Analytical and Applied Pyrolysis.* **64**, 327-344 (2002).
6 Hauri, U. Inks for tattoos and permanent make-up / pigments, preservatives, aromatic amines, polyaromatic hydrocarbons and nitrosamines. *Department of Health, Kanton Basel-Stadt. Swiss National Investigation Campaign 2014.* (2014).
7 Bocca, B., Sabbioni, E., Mičetić, I., Alimonti, A., Petrucci, F. Size and metal composition characterization of nano- and microparticles in tattoo inks by a combination of analytical techniques. *Journal of Analytical Atomic Spectrometry.* **32** (3), 616-628 (2017).
8 Schreiver, I. et al. Synchrotron-based nano-XRF mapping and micro-FTIR microscopy enable to look into the fate and effects of tattoo pigments in human skin. *Scientific Reports.* **7**, 11395 (2017).
9 Taylor, C. R., Anderson, R. R., Gange, R. W., Michaud, N. A., Flotte, T. J. Light and electron microscopic analysis of tattoos treated by Q-switched ruby laser. *Journal of Investigative Dermatology.* **97**, 131-136 (1991).
10 Namduri, H., Nasrazadani, S. Quantitative analysis of iron oxides using Fourier transform infrared spectrophotometry. *Corrosion Science.* **50** (9), 2493-2497 (2008).
11 Burgio, L., Clark, R. J., Hark, R. R. Raman microscopy and x-ray fluorescence analysis of pigments on medieval and Renaissance Italian manuscript cuttings. *Proceedings of the National Academy of Sciences of the United States of America.* **107** (13), 5726-5731 (2010).
12 Manso, M. et al. Assessment of toxic metals and hazardous substances in tattoo inks using Sy-XRF, AAS and Raman spectroscopy. *Biological Trace Element Research.* **187** (2), 596-601 (2017).
13 Yakes, B. J., Michael, T. J., Perez-Gonzalez, M., Harp, B. P. Investigation of tattoo pigments by Raman spectroscopy. *Journal of Raman Spectroscopy.* **48** (5), 736-743 (2017).
14 Yang, S.-H., Shen, J. Y., Chang, M. S., Wu, G. J. Differentiation of vehicle top coating paints using pyrolysis-gas chromatography/mass spectrometry and multivariate chemometrics with statistical comparisons. *Analytical Methods.* **7**, 1527-1534 (2015).
15 Schreiver, I., Hutzler, C., Luch, A. Data from: Two-step pyrolysis-gas chromatography method with mass spectrometric detection for identification of tattoo ink ingredients and counterfeit products. *Dryad Digital Repository.* https://doi:10.5061/dryad.5060kb5061h5063 (2019).
16 Schreiver, I., Hutzler, C., Andree, S., Laux, P., Luch, A. Identification and hazard prediction of tattoo pigments by means of pyrolysis—gas chromatography/mass spectrometry. *Archives of Toxicology.* **90** (7), 1639–1650 (2016).
17 Ghelardi, E. et al. Py-GC/MS applied to the analysis of synthetic organic pigments: characterization and identification in paint samples. *Analytical and Bioanalytical Chemistry.* **407** (5), 1415-1431 (2015).
18 Russell, J., Singer, B. W., Perry, J. J., Bacon, A. The identification of synthetic organic pigments in modern paints and modern paintings using pyrolysis-gas chromatography-

mass spectrometry. *Analytical and Bioanalytical Chemistry.* **400** (5), 1473-1491 (2011).

19 Silva, M. F., Domenech-Carbo, M. T., Fuster-Lopez, L., Mecklenburg, M. F., Martin-Rey, S. Identification of additives in poly(vinylacetate) artist's paints using PY-GC-MS. *Analytical and Bioanalytical Chemistry.* **397** (1), 357-367 (2010).

20 Peris-Vincente, J., Baumer, U., Stege, H., Lutzenberger, K., Gimeno Adelantado, J. V. Characterization of commercial synthetic resins by pyrolysis-gas chromatography/mass spectrometry: application to modern art and conservation. *Analytical Chemistry.* **81**, 3180-3187 (2009).

21 Kleinert, J. C., Weschler, C. J. Pyrolysis gas chromatographic-mass spectrometric identification of polydimethylsiloxanes. *Analytical Chemistry.* **52** (8), 1245-1248 (1980).

22 Scalarone, D., Chiantore, O. Separation techniques for the analysis of artists' acrylic emulsion paints. *Journal of Separation Science.* **27** (4), 263-274 (2004).

23 Sonoda, N. Characterization of organic azo-pigments by pyrolysis-gas chromatography. *Studies in Conservation.* **44**, 195-208 (1999).

24 Chiantore, O., Scalarone, D., Learner, T. Characterization of artists' crylic emulsion paints. *International Journal of Polymer Analysis and Characterization* **8** (1), 67-82 (2003).

25 Schossler, P., Fortes, I., Figueiredo Júnior, J. C. D., Carazza, F., Souza, L. A. C. Acrylic and Vinyl Resins Identification by Pyrolysis-Gas Chromatography/Mass Spectrometry: A Study of Cases in Modern Art Conservation. *Analytical Letters.* **46** (12), 1869-1884 (2013).

26 Wallisch, K. L. Pyrolysis of random and block copolymers of ethyl acrylate and methyl methacrylate. *Journal of Applied Polymer Science.* **18**, 203-222 (1974).

27 Hauri, U. Inks for tattoos and PMU (permanent make-up) / Organic pigments, preservatives and impurities such as primary aromatic amines and nitrosamines. *State Laboratory of the Canton Basel City.* (2011).

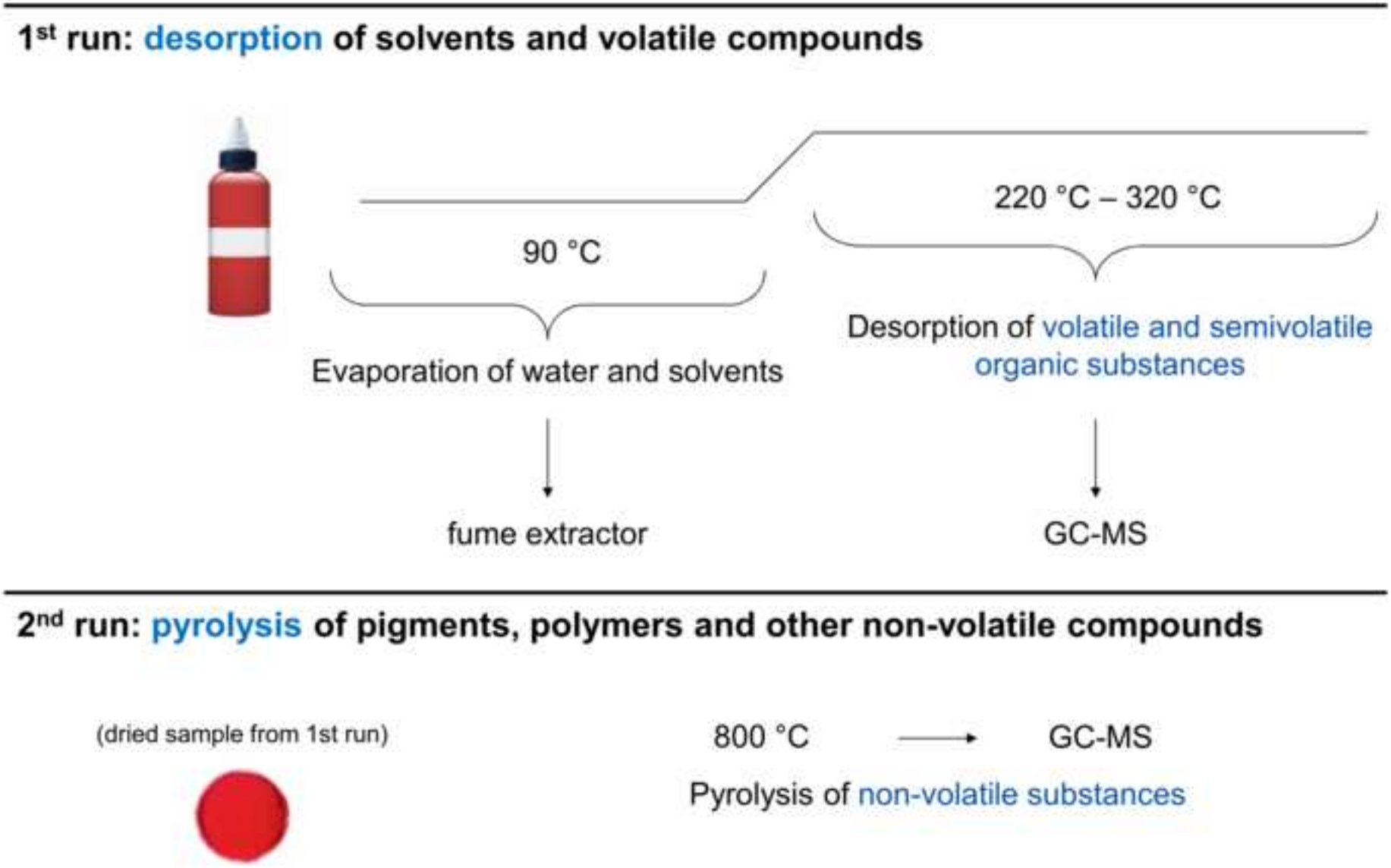
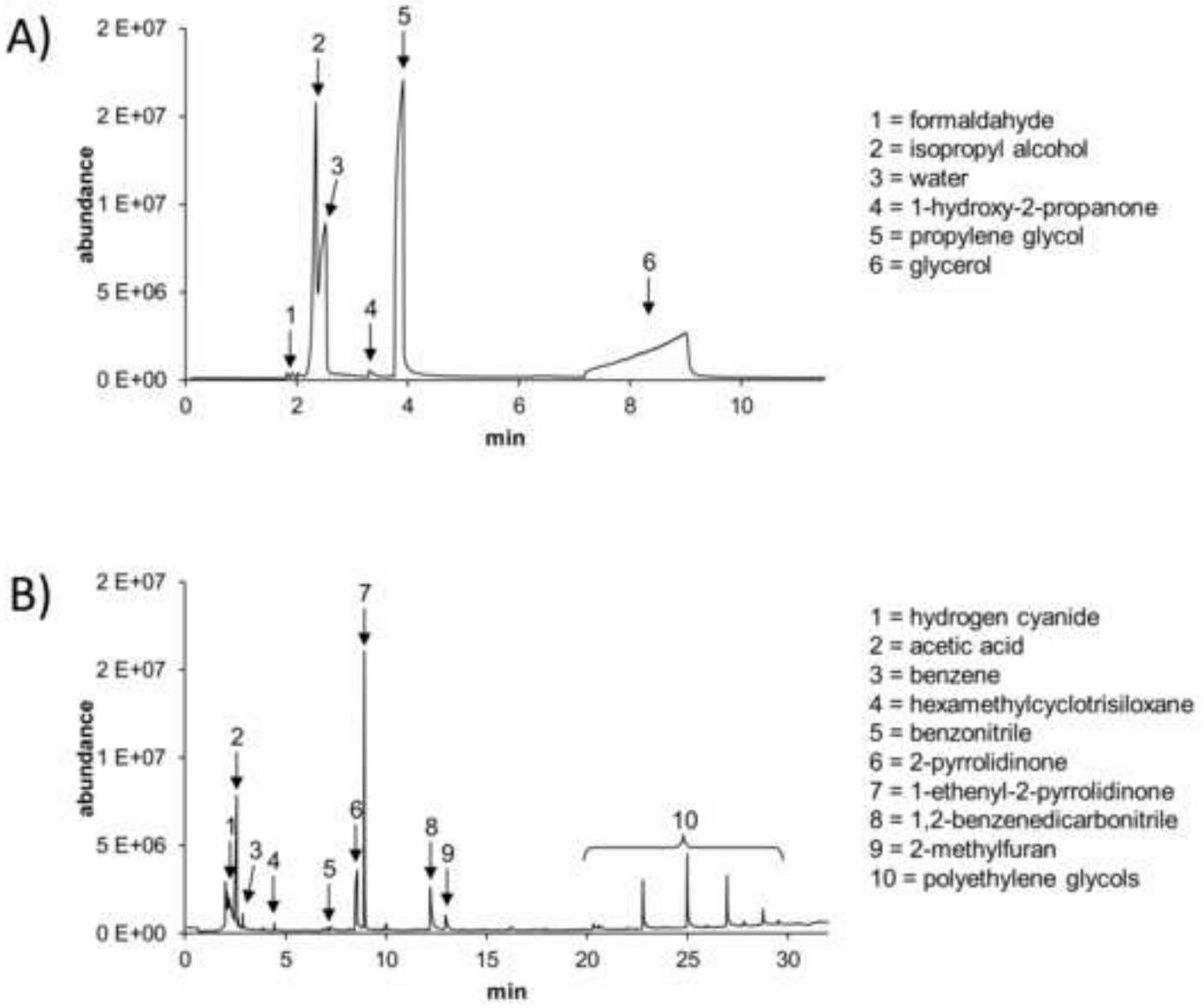
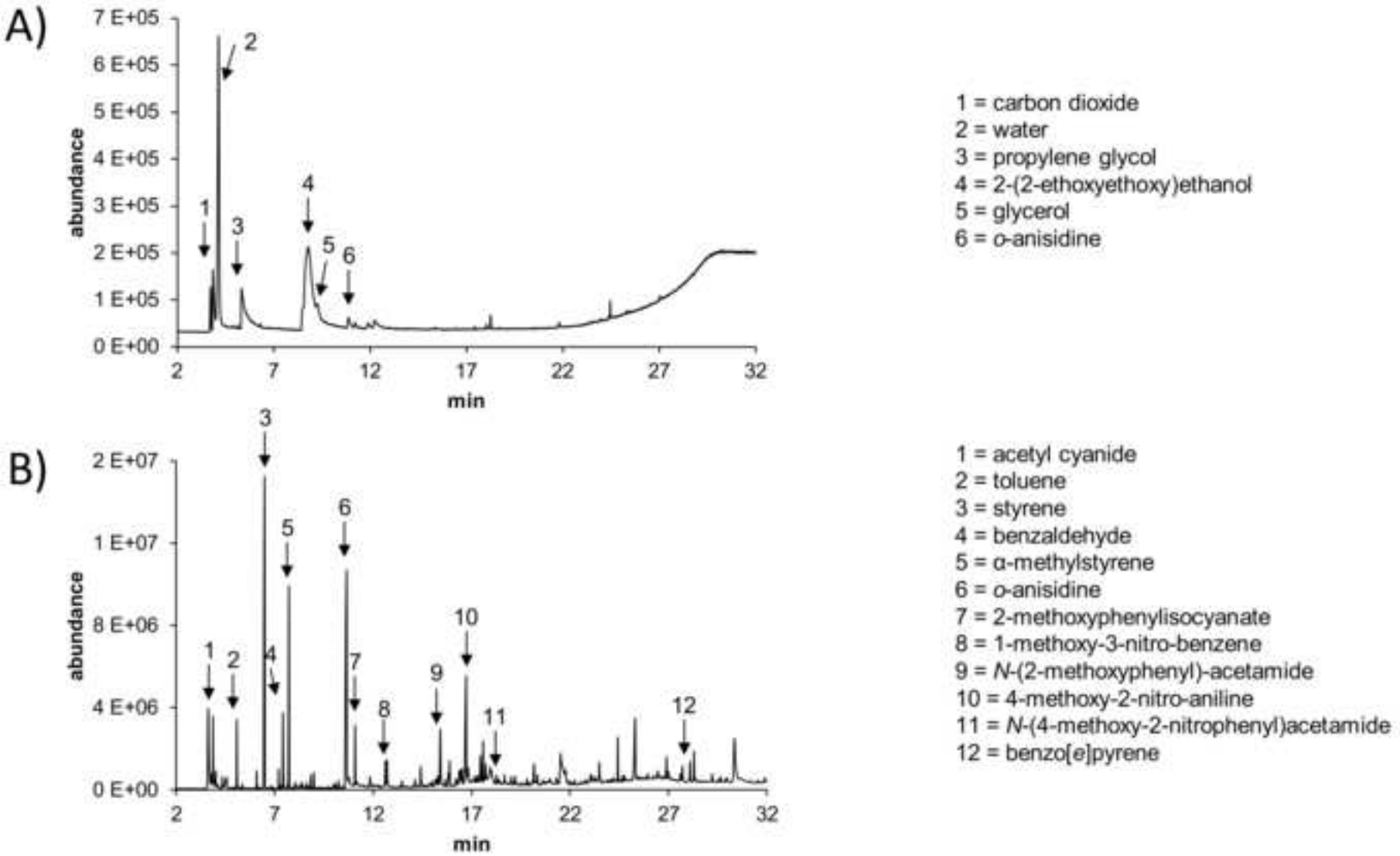


Figure 2



Declared ingredients (blue ink): water, isopropyl alcohol, glycerol, polyvinyl-pyrrolidone, C.I.74160 (Pigment Blue 15:3), TiO_2.
Identified ingredients:
1st run, desorption: water, isopropyl alcohol, glycerol. Additional: formaldehyde, propylene glycol.
2nd run, pyrolysis: polyvinyl-pyrrolidone (pyrolysis products: 2-pyrrolidinone, 1-ethenyl-2-pyrrolidinone), C.I.74160 (Pigment Blue 15) (pyrolysis products: hydrogen cyanide, benzene, benzonitrile, 1,2-benzenedicarbonitrile), cyclosiloxanes.

Figure 3



Declared ingredients (yellow ink "banana cream"): proprietary, water, TiO_2, C.I.11741 (Pigment Yellow 74), C.I.21110 (Pigment Orange 13), glycerol, isopropyl alcohol.
Identified ingredients:

1st run, desorption: water, isopropyl alcohol, glycerol, propylene glycol, 2-(2-ethoxyethoxy)ethanol, *o*-anisidine (synthesis residue)

2nd run, pyrolysis: C.I.11740 (Pigment Yellow 65) or C.I.11741 (Pigment Yellow 74) (pyrolysis products: acetyl cyanide, *o*-anisidine, 2-methoxyphenylisocyanate, 4-methoxy-2-nitro-aniline), styrene-polymere (pyrolysis products: styrene, α-methylstyrene).

Figure 4

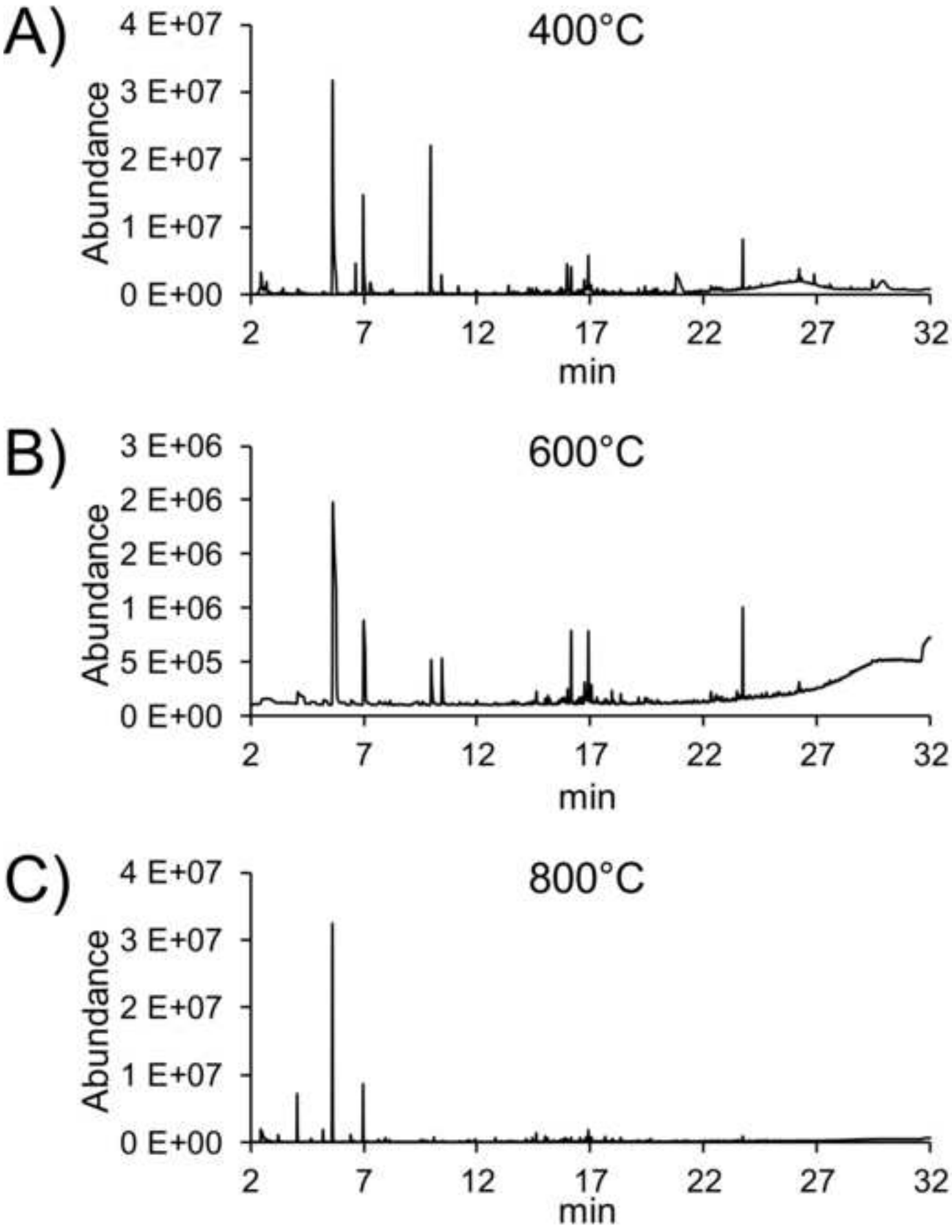
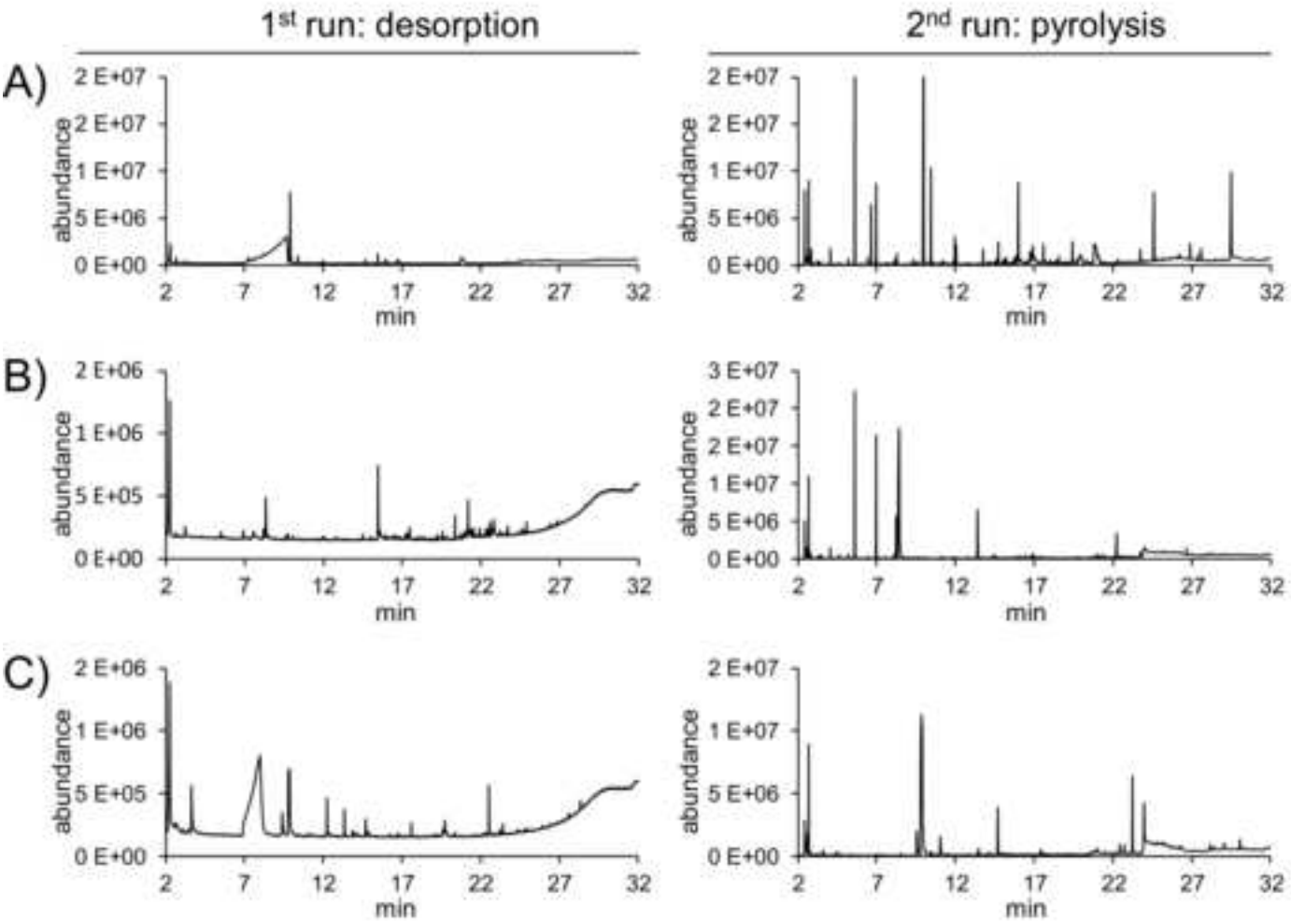


Figure 5



distibutor	ingredients (identified by NIST MS library search)
licensed	C.I.11740 (Pigment Yellow 65) or C.I. 11741 (Pigment Yellow 74)(decomposition products: acetyl cyanide, 4-methoxy-2-nitro-aniline, o-anisidine, 2-methoxy-phenylisocyanate, N,N'-bis-(4-methoxyphenyl)urea), propylene glycol, glycerol, dimethylsiloxane, acetic acid, styrene-polymere (pyrolysis products: styrene, α-methylstyrene)
online auction platform	C.I. 21095 (Pigment Yellow 14)(decomposition products: acetyl cyanide, 2-methyl-phenylisocyanate, o-toluidine, N-(2-methylphenyl)-acetamide, 3,3'-dichlorobenzidine, N,N'-bis(2-methylphenyl)-urea), ammonia, ethyl oleate, palmitic acid ethyl ester, styrene-acrylate-polymere (pyrolysis products: styrene, α-methylstyrene, methyl methacrylate), dimethylsiloxane
Asian market place	C.I. 21095 (Pigment Yellow 14) (decomposition products: acetyl cyanide, 3,4-dimethylaniline, 3,3'-dichloro-1,1'-biphenyl, 3,3'-dichlorobenzidine, 2,3-dimethylphenyl isocyanate), propylene glycol, acetic acid, glycerol, formaldehyde, polyethyleneglycole, dimethylsiloxane

Table of Materials

Click here to access/download;Table of Materials;JoVE_Materials_revision.xlsx

Name of Material/ Equipment	Company	Catalog Number
99.999% Helium carrier gas	Air Liquide, Düsseldorf, Germany	-
5975C inert XL MSD with Triple-Axis Detectors	Agilent Technologies, Waldbronn, Germany	-
7890A gas chromatograph	Agilent Technologies, Waldbronn, Germany	-
AMDIS software (Version 2.7)	The National Institute of Standards and Technology, Gaithersburg, MD, USA	-
Cold Injection System (CIS)	Gerstel, Mühlheim, Germany	-
electron impact (EI) source	Agilent Technologies, Waldbronn, Germany	-
Enhanced ChemStation (E02.02.1431)	Agilent Technologies, Waldbronn, Germany	-
J&W HP-5MS GC Column, 30 m, 0.25 mm, 0.25 µm, 5975T Column Toroid Assembly	Agilent Technologies, Waldbronn, Germany	29091S-433LTM
MassHunter Software	Agilent Technologies, Waldbronn, Germany	-
Microcapillary tube Drummond Microcaps, volume 2 µL	Sigma-Aldrich, St. Louis, MO, USA	P1549-1PAK
MS ChromSearch (Version 4.0.0.11)	Axel Semrau GmbH & Co. KG, Sprockhövel, Germany	-
NIST MS Search Program (MS Search version 2.0g)	The National Institute of Standards and Technology, Gaithersburg, MD, USA	-
NIST/EPA/NIH Mass Spectral Library (EI) mainlib & replib (Data version: NIST v11)	The National Institute of Standards and Technology, Gaithersburg, MD, USA	-
Polystyrene (average Mw ~192,000)	Sigma-Aldrich, St. Louis, MO, USA	430102-1KG
Pyrolysis tubes, tube type - quartz glass - lenght 25 mm; 100 Units	Gerstel, Mühlheim, Germany	018131-100-00
Pyrolyzer Module for TDU	Gerstel, Mühlheim, Germany	-
Quartz wool	Gerstel, Mühlheim, Germany	009970-076-00
Steel sticks	Gerstel, Mühlheim, Germany	-
Thermal Desorption Unit (TDU 2)	Gerstel, Mühlheim, Germany	-
Transport adapter	Gerstel, Mühlheim, Germany	018276-010-00
Tweezers for Pyrolysis tubes	Gerstel, Mühlheim, Germany	009970-074-00
Zebron Z-Guard Hi-Temp Guard Column, GC Cap. Column 10 m x 0.25 mm, Ea	Phenomenex Ltd. Deutschland, Aschaffenburg, Germany	7CG-G000-00-GH0

Comments/Description
can be used for GC/MS peak integration, e.g. for transfer to pyrogram evaluation software
used to generate Average Mass Spektra (AMS), can be used for peak integration and standard GC/MS library search
no Version specified, can be used for GC/MS peak integration and standard GC/MS library search
specialized pyrogram evaluation software used for MS and AMS library generation and corresponding substance search with selfmade and commercial libraries used commercial mass spectral library

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Analysis of tattoo inks and combustion products by means of pyrolysis [...]

Author(s): Schreiver, Steckler, Luch

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):

☒ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Ines Schreiver

Department: Chemical + Product Safety

Institution: Bundesinstitut für Risikobewertung

Article Title: Analysis of tattoo inks and counterfeit products by means of pyrolysis

Signature: [signature] Date: 14.01.13

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Rebuttal document

Editorial comments:

General:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Answer: All authors proofread the manuscript again.

2. Please ensure that the manuscript is formatted according to JoVE guidelines–letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.

Answer: Page size, font, text alignment, single spacing within paragraphs and spaces between paragraphs and protocol steps have been revised where necessary.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Enhanced ChemStation, MS ChromSearch

Answer: Enhanced ChemStation and MS ChromSearch were replaced throughout the text. Also other company/software names such as NIST MS have been replaced.

Protocol:
1. There is a 10 page limit for the Protocol, but there is a 2.75 page limit for filmable content. If revisions cause the highlighted portion to be more than 2.75 pages, please highlight 2.75 pages or less of the Protocol (including headers and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

Answer: The highlighted portion is about 2.75 pages.

2. For each step/substep, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

Answer: Protocol steps and substeps have been revised to better answer of how the respective step is being performed. No more than 2-3 actions and sentences are used per step.

Specific Protocol steps:
1. 2, 3: Please provide specific instructions for software steps; e.g., Click, Select.

Answer: Specific instructions for software steps have been introduced were possible. In some steps, no specific software is named anymore and for some steps multiple software is suitable. In these cases, specific instructions are impractical.

Figures:
2. Figure 1: Please include a space between numbers and units; e.g., 90 °C instead of 90°C.

Answer: Spaces are now included in Figure 1 and throughout the text.

References:
1. Please do not abbreviate journal titles.

Answer: Jove Endnote Style has been used and the abbreviations of journal titles have been removed.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Answer: The Table of Materials has been revised.

Reviewer #1:

Manuscript Summary:
This manuscript described the use of pyrolysis-GC/MS for the characterization of tattoo inks for the purpose of ink quality monitoring. The method is simple and results are relevant for quality monitoring. Particularly, two-step approach generated more chemical information for the detection of tattoo ink ingredients.

Major Concerns:
none.

Minor Concerns:
Since in Line 59, authors used LDI-ToF-MS for laser desorption/ionization time-of-flight "mass spectrometry" and in Line 61, the authors used ICP-MS for inductively coupled plasma "mass spectrometry". It should be reasonable to use Py-GC/MS for pyrolysis-gas chromatography "mass spectrometry" instead of using pyrolysis online coupled to gas chromatography with "mass spectrometric detection" in line 51. Use MS for "mass spectrometry" in a consistent way through the manuscript is suggested.

Answer: Gas chromatography mass spectrometry and all similar terms are now abbreviated with GC-MS as suggested by the reviewer, including the title.

Reviewer #2:

Manuscript Summary:
The manuscript entitled "Analysis of tattoo inks and counterfeit products by means of pyrolysis online coupled to gas chromatography with mass spectrometric detection" presents a methodology for two-step pyrolysis online coupled to gas chromatography with mass spectrometric detection and data evaluation protocol can be used for multi-component analysis that can be used in the study of any kind of inks and other compounds (varnishes, organic solvents, …) and not specifically tattoo inks, as

mentioned in the last paragraph of the introduction. I understand that tattoo inks are a good sample for the validation of the methodology.

Minor Concerns:
The title suggests that a study of tattoo inks had been made using pyrolysis online coupled to gas chromatography with mass spectrometric detection but in fact emphasis is on the analytical methodology which the authors describe step by step in the form of an analytical protocol.
I would strongly recommend changing the title which has led me and probably possible readers in mistake.

> *Answer: The title has been amended including the deletion of the word "analysis" to circumvent that the protocol can be mistaken as a study report.*

Supplemental Table 1

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Table 1_new.xlsx

Legend:
* verified by standard substances;
‡ overlapping peak areas

Name	C.I.	Fragments	CAS	in library (NIST 11 mainlib or pyrolysis "pyro" library at datadryad.org)	m/z	Area [%]	Literature
P.B.15	74160	Hydrogen cyanide*	74-90-8	main	27	8	
		Benzene	71-43-2	main	78	0.63	
		2-Methylbenzonitrile	529-19-1	main	117	1.19	
		Benzonitrile*	100-47-0	main	103	10.01	Ghelardi (2014)
		1,2-Benzenedicarbonitrile*	91-15-6	main	128	51.44	Russell (2011) Sonoda (1999) Ghelardi (2014)
		Phthalimide / *o* -Cyanobenzoic acid *(similar mass spectra)*	85-41-6 / 3839-22-3	main/main	147, 104, 76	6.03	Ghelardi (2014)
P.G.7	74260	Hydrogen chloride	7647-01-0	main	36	11.42 ‡	
		Cyanogen chloride	506-77-4	main	61	11.42 ‡	
		Carbon tetrachloride	56-23-5	main	152	1.05	
		Tetrachloroethylene	127-18-4	main	164	0.62	
		Hexachlorobenzene*	118-74-1	main	282	5.18	
		Pentachlorobenzonitrile	20925-85-3	main	273	18.28	Russell (2011)
		3,4,5,6-Tetrachloro-1,2-benzenedicarbonitrile	1953-99-7	main	266	52.69	Russell (2011)
		4,5,6,7-Tetrachloro2,3-dihydro-1*H* -isoindole	-	pyro	257	2.52	
P.G.36	74265	Hydrogen bromide	10035-10-6	main	81	31.7	
		Cyanogen bromide	506-68-3	main	106	15.34	
		Bromine	7726-95-6	main	80	6.88	
		Bromobenzene	108-86-1	main	157	1.94	Russell (2011)
		3,4,5,6-Tetrachloro-1,2-benzenedicarbonitrile	1953-99-7	main	266	1.09	Russell (2011)
		1,2-Benzenedicarbonitrile [+1Br, +3Cl]	-	pyro	310, 229	3.65	
		Benzonitrile [+2Br, +3Cl]	-	-	365, 310, 284	1.4	
		1,2-Benzenedicarbonitrile [+2Br, +2Cl]	-	pyro	354, 275, 194	7.26	Russell (2011)
		Benzonitrile [+3Br, +2Cl]	-	-	409, 328, 249	1.83	
		1,2-Benzenedicarbonitrile [+3Br, +1Cl]	-	pyro	400, 319, 240	11.08	Russell (2011)
		Benzonitrile [+4Br, +1Cl]	-	-	453, 374, 293	1.71	
		1,2-Benzenedicarbonitrile [+4Br]	-	pyro	444, 363, 284	7.69	Russell (2011)
		Benzonitrile [+5Br]	-	-	497, 444, 418	1.17	
		Unknown product	-	-	464, 398, 309	1.91	
P.O.73	561170	Benzene	71-43-2	main	78	1.63	
		Styrene	100-42-5	main	104	1.88	
		α -Methylstyrene	98-83-9	main	118	4.5	
		Benzonitrile*	100-47-0	main	103	2.9	
		tert -Butylbenzene	98-06-6	main	134	2.86	Russell (2011)
		1-Methyl-4-(1-methylethenyl)-benzene	1195-32-0	main	132	2.61	
		4-Methylbenzonitrile	104-85-8	main	117	2.56	
		Naphthalene*	91-20-3	main	128	1.82	
		4-(Propan-2-yl)-benzonitrile	-	pyro	144, 129, 102	4.18	
		1-Methylisoquinoline	1721-93-3	main	143	12.92	
		4-*tert* -Butylbenzonitrile	4210-32-6	main	159, 144, 116	16.33	Russell (2011)
		Orange 73 [-5CH3 +5H]	-	pyro	331, 316	3.83	
P.R.254	56110	Benzene	71-43-2	main	78	1.17	
		Chlorobenzene*	108-90-7	main	112	10.35	Russell (2011)
		4-Chlorotoluene	106-43-4	main	126	1.53	
		Benzonitrile*	100-47-0	main	103	2.13	
		4-Chlorostyrene	1073-67-2	main	136	1.22	
		4-Chlorobenzonitrile*	623-03-0	main	137	37.23	Russell (2011)
		Napththalene	91-20-3	main	128	4.26	
		Red 254 [–HNCO]	-	pyro	313, 243, 214	4.33 ‡	Russell (2011)
		Unknown product	-	pyro	313, 177, 136	4.33 ‡	
		Red 254 [–HNCO -O]	-	-	298, 263, 228	1.19	
		Red 254 [–HNCO]	-	pyro	313, 250, 214	9.59 ‡	Russell (2011)
		Red 254 [–2C –O2 –N +H]	-	pyro	287, 252, 217	9.59 ‡	

P.Y.138	56300	Xylene*	95-47-6 / 108-38-3 / 106-42-3	main	106	6.31	
		1,2,3,4-Tetrachlorobenzene*	634-66-2	main	216	3.52	
		Pentachlorobenzene*	608-93-5	main	248	5.47	
		2,3,4,5-Tetrachlorobenzaldehyde	56962-16-4	pyro	241, 206, 99	4.99	
		Hexachlorobenzene*	118-74-1	main	282	1.19	
		Pentachlorobenzonitrile	20925-85-3	main	273	1.64	
		4,5,6,7-Tetrachloro-1,3-isobenzofurandione	117-08-8	main	286	0.9	
		3,4,5,6-Tetrachlorophthalimide	1571-13-7	main	285	2.11	
		2-(8-Aminoquinolin-2-yl)-4,5,6,7-tetrachloro-3-hydroxy-1*H*-inden-1-one	-	pyro	426, 345, 310	16	
P.R.122	73915	Benzene	71-43-2	main	78	0.71	
		Toluene	108-88-3	main	92	0.93	
		9(10*H*)-Acridanone	578-95-0	main	195	0.71	
		4-Methylacridone	68506-36-5	main	209	1.54	
		R122 [-6C]	-	pyro	268, 240, 134	10.69	
		R122 [-5C]	-	pyro	280, 251, 140	2.37	
		Quinacridone product	-	pyro	294, 147	5.5	
		Biquinoline	612-79-3 / 119-91-5	main	256, 227, 128	6.69	
		R122 [-5C +2H]	-	pyro	282, 141	19.02	
		R122 [-6C +2H]	-	pyro	270, 135	4.38	
		Unknown product	-	pyro	308, 284, 154	3.94	
		R122 [-4C +4H]	-	pyro	296, 148	15.22	
P.R.202	73907	Hydrogen chloride	7647-01-0	main	36	35.22	
		Benzene	71-43-2	main	78	0.64	
		2-Amino-9-oxo-9,10-dihydroacridine-3-carbaldehyde	-	-	238, 209, 139	1.06	
		Red 202 [-2Cl -4C +5H]	-	pyro	268, 238, 225	8.27	
		Quinacridone product	-	pyro	294, 147	2.6	
		Biquinoline	612-79-3 / 119-91-5	main	256	14.28	
		Red 202 [-Cl -2C +6H]	-	pyro	328, 293, 265	6.56	
		Unknown product	-	pyro	364, 362, 263	9.81	
		Unknown product	-	pyro	284, 255, 227	6.16	
P.V.19	73900	Benzene	71-43-2	main	78	1.83	
		2-Amino-9-oxo-9,10-dihydroacridine-3-carbaldehyde	-	pyro	238, 209, 139	7.51	
		Quinacridone product	-	pyro	294, 147	3.54	
		Violet 19 [-4C +4H]	-	pyro	268, 240, 134	34.22	
P.V.23	51319	Benzene	71-43-2	main	78	1.13	
		1,4-Dichlorobenzene	106-46-7	main	146	4.11	Ghelardi (2014)
		9*H*-Carbazole	86-74-8	main	167	7.89	Ghelardi (2014)
		9-Ethyl-9*H*-carbazole	86-28-2	main	195	1.03	
		Unknown product	-	pyro	211, 196, 183	31.55	Ghelardi (2014)
		3-Amino-9-ethylcarbazole	132-32-1	main	210	3.81	
		Unknown product	-	pyro	250, 235, 221	6.37	Ghelardi (2014)
		Unknown product	-	pyro	235, 220	2.04	
		Unknown product	-	pyro	206, 178, 103	1.94	
P.V.37	51345	Acetonitrile	75-05-8	main	41	3.19	
		Acetic acid	64-19-7	main	60	1.67	
		Benzene	71-43-2	main	78	8.37	
		Toluene	108-88-3	main	91	1.74	
		Benzonitrile*	100-47-0	main	103	7.81	Ghelardi (2014)
		Benzoic acid	65-85-0	main	122	10.49	
		Benzamide*	55-21-0	main	121	9.02	Ghelardi (2014)
		2-Phenylbenzoxazole	833-50-1	main	195	1.34	Ghelardi (2014)
		Unknown product	-	pyro	211, 104, 52	11.09	Ghelardi (2014)
		Unknown product	-	pyro	210, 105, 79	3.41	
		Unknown product	-	pyro	250, 239, 224	1.58	
		Unknown product	-	pyro	315, 105, 77	0.86	

P.O.5	12075	1,3-Dinitrobenzene	99-65-0	main	168	2.35	Russell (2011)
		2-Naphthol	135-19-3	main	144	4.61	Russell (2011) Sonoda (1999)
		2,4-Dinitroaniline	97-02-9	main	183	16.93	Russell (2011) Sonoda (1999)
		Unknown product	-	pyro	290, 263, 244	18.09	
		1-(2,4-Dinitrophenoxy)-naphthalene	3761-15-7	main	310	2.77	
		Unknown product	-	pyro	275, 229, 217	2.29	
		Unknown product	-	pyro	290, 265, 244	9.06	
		Orange 5 [-2O]	-	pyro	306, 232, 128	6.14	
		Orange 5 [-2O +2H]	-	pyro	308, 216, 187	5.93	
		Orange 5	3468-63-1	pyro	338	10.53	
P.R.4	12085	2-Naphthol	135-19-3	main	144	2.88	Russell (2011) Scalarone (2004) Sonoda (1999)
		2-Chloro-4-nitroaniline	121-87-9	main	172	5.99	Russell (2011) Scalarone (2004) Sonoda (1999)
		Unknown product	-	pyro	299, 218, 189	5.69	
		Unknown product	-	pyro	290, 263, 244	2.38	
		Red 4 [-2O +2H]	-	pyro	297, 223, 187	2.67	
		Pigment Red 1	6410-10-2	main	293, 143, 115	4.49	
		Red 4	2814-77-9	pyro	327, 143, 115	68.54	Russell (2011)
P.Y.14	21095	Acetonitrile	75-05-8	main	41	3.56	
		Acetyl cyanide	631-57-2	main	69	4.16	Russell (2011)
		2-Methylphenylisocyanate	614-68-6	main	133	10.17	Russell (2011)
		o-Toluidine	95-53-4	main	107	22.21	Russell (2011)
		3,5-Dimethylphenylisocyanate	54132-75-1	main	147	1.73	
		2,4-Dimethylaniline	95-68-1	main	121	1.21	
		N-(2-Methylphenyl)-acetamide	120-66-1	main	163	2.08	
		N,N'-Bis(2-methylphenyl)-urea	617-07-2 (3-methyl isomer: 620-50-8)	main (3-methyl isomer	240	2.99	
		4,4'-Diisocyanato-3,3'-dichloro-1,1'-biphenyl	-	pyro	306, 304, 241	14.94 ‡	Russell (2011)
		4-Amino-4'-isocyanato-3,3'-dichloro-1,1'-biphenyl	-	pyro	280, 278, 215	14.94 ‡	Russell (2011)
		3,3'-Dichlorobenzidine	91-94-1	main	252	14.94 ‡	
		2,2',5,5'-Tetrachlorobenzidine	15721-02-5	main	320	0.37	
P.Y.83	21108	Acetyl cyanide	631-57-2	main	69	2.37	Russell (2011) Sonoda (1999)
		4-Chloro-2,5-dimethoxyphenyl isocyanate	55440-55-6	main	213	5.77	Russell (2011)
		4-Chloro-2,5-dimethoxyaniline	6358-64-1	main	187	21.12	Russell (2011) Sonoda (1999)
		N-(4-Chloro-2,5-dimethoxyphenyl)acetamide	6938-75-6	pyro	229, 187, 172	5.89	
		3,3'-Dichlorobiphenyl-4-amine	926223-12-3	pyro	237, 167, 139	4.61	
		3,3'-Dichlorobenzidine	91-94-1	main	252	12.5	
		4,4'-Diisocyanato-3,3'-dichloro-1,1'-biphenyl	-	pyro	306, 304, 241	4.41	Russell (2011)
		Unknown product	-	pyro	293, 250, 180	2.38	
		Unknown product	-	pyro	295, 293, 250	2.1	
		Unknown product	-	pyro	294, 252, 154	2.15	
		Unknown product	-	pyro	399, 308, 277	3.65	
P.O.13	21110	Acetonitrile	75-05-8	main	41	2.45	
		Benzene	71-43-2	main	78	1.88	
		Phenylisocyanate	103-71-9	main	119	6.22	Sonoda (1999)
		Aniline*	62-53-3	main	93	2.22	Sonoda (1999)
		3-Methyl-1-phenyl-2-pyrazolin-5-one	89-25-8	main	174	5.61	Sonoda (1999)
		3,3'-Dichloro-1,1'-biphenyl*	2050-67-1	main	223	2.69	Sonoda (1999)
		3,3'-Dichlorobiphenyl-4-amine	926223-12-3	pyro	237, 167, 139	2.02	
		3,3'-Dichlorobenzidine	91-94-1	main	252	0.68	
		Orange 13 [-2 Phenylisocyanate]	-	pyro	394, 359, 77	1.84	
P.O.16	21160	Acetonitrile	75-05-8	main	41	1.9	
		Acetyl cyanide	631-57-2	main	69	3.32	Russell (2011)
		Phenylisocyanate	103-71-9	main	119	2.58	
		Aniline*	62-53-3	main	93	18.45	Russell (2011)
		Acetanilide	103-84-4	main	135	15.95	
		4-Aminobiphenyl-3,3'-diol	-	pyro	202, 110, 93	1.13	
		Unknown product	-	pyro	255, 212, 169	2	Russell (2011)
		N,N'-Diphenylurea	102-07-8	main	212	3.23	
		4,4'-Diisocyanato-3,3,-dimethoxy-1,1'-biphenyl	91-97-4	main	296	18.18	Russell (2011)
		Unknown product	-	pyro	311, 268, 238	0.78	
		Unknown product	-	pyro	312, 286, 270	1	

P.O.34	21115	Benzene	71-43-2	main	78	1.01	
		4-Methylphenylisocyanate	622-58-2	main	133	6.12	
		p-Toluidine	106-49-0	main	107	1.65	Russell (2011)
		3,3'-Dichloro-1,1'-biphenyl*	2050-67-1	main	223	10.87	
		2,4-Dihydro-5-methyl-2-(4-methylphenyl)-3H-pyrazol-3-one	86-92-0	main	188	43.45	
		3,3'-Dichlorobenzidine	91-94-1	main	252	1.16	
		3,3'-Dichlorobiphenyl-4-amine	926223-12-3	pyro	238, 167, 139	8.57	
		Unknown product	-	pyro	408, 373, 262	5.98	
		Unknown product	-	pyro	408, 373, 358	6.63	
		Unknown product	-	pyro	408, 373, 358	1.74	
		Unknown product	-	pyro	423, 388	2.55	
P.Y.1	11680	Benzene	71-43-2	main	78	1.42	
		Phenylisocyanate	103-71-9	main	119	1.15	Russell (2011) Sonoda (1999)
		Aniline*	62-53-3	main	93	23.37	Russell (2011) Sonoda (1999)
		Benzonitrile	100-47-0	main	103	7.8	
		p-Toluidine	106-49-0	main	107	2.64	
		4-Methylbenzonitrile	104-85-8	main	117	1.11	
		5-Methylbenzofurazan	20304-86-3	main	134	2.93	Russell (2011)
		Acetanilide	103-84-4	main	135	3.82	
		3-Nitrotoluene	99-08-1	main	137	2.02	Russell (2011) Sonoda (1999)
		2,5-Dimethylbenzoxazole	5676-58-4	main	147	1.41	
		Acetanilide	103-84-4	main	135	4.91	
		Unknown product	-	pyro	175, 147, 104	0.64	
		Unknown product	-	pyro	175, 160, 131	1.64	
		4-Methyl-2-nitroaniline	89-62-3	main	152	3.75	Sonoda (1999)
		6-Methyl-1*H*-benzotriazole	136-85-6	main	133	3.38	
		N,N'-Diphenylurea	102-07-8	main	212	2.31	
		Unknown product	-	pyro	264, 117, 90	2.78	
P.Y.3	11710	Acetyl cyanide	631-57-2	main	69	2.39	Russell (2011) Sonoda (1999)
		2- or	95-51-2	main	127	23.82	Russell (2011)
		4-Chloroaniline	106-47-8	main	127	23.82	Scalarone (2004) Sonoda (1999)
		2-Chlorophenylisocyanate	3320-83-0	main	153	5.43	Russell (2011) Scalarone (2004) Sonoda (1999)
		5-Chlorobenzofurazan	19155-86-3	main	152	3.33	Russell (2011)
		Unknown product	-	pyro	194, 153, 124	1	
		6-Chloro-2-methyl-4H-3,1-benzoxazin-4-one	7033-50-3	main	195, 180, 151	3.45	
		4-Chloro-2-nitroaniline	89-63-4	main	172	7.21	Scalarone (2004) Sonoda (1999)
		Unknown product	-	pyro	280, 245, 127	5.52	
		Unknown product	-	pyro	331, 296, 177	1.81	
		Unknown product	-	pyro	320, 283, 137	3.88	
P.Y.74 /P.Y.65	11741 / 11740	Acetyl cyanide	631-57-2	main	69	3.41	Sonoda (1999)
		o-Anisidine	90-04-0	main	123	18.76	Russell (2011) Scalarone (2004) Sonoda (1999)
		2-Methoxyphenylisocyanate	700-87-8	main	149	13.39	Russell (2011) Scalarone (2004) Sonoda (1999)
		Unknown product	-	pyro	194, 164, 77	3.7	Russell (2011)
		2-Methoxy-4-nitroaniline	97-52-9	main	168	10.8	Russell (2011) Scalarone (2004) Sonoda (1999)
		N-(4-Nitrophenyl)-formamide	16135-31-2	pyro	166, 138, 92	2.86	
		Unknown product	-	pyro	232, 201, 110	0.98	
		Unknown product	-	pyro	239, 210, 168	1.96	
		Unknown product	-	pyro	235, 193, 147	2.31	Russell (2011)
		N,N'-Bis-(2-methoxyphenyl)urea	1226-63-7	main	272, 123, 108	3.87	
P.Y.97	11767	Acetyl cyanide	631-57-2	main	69	1.75	Russell (2011) Sonoda (1999)
		Aniline*	62-53-3	main	93	11.74	Sonoda (1999)
		Acetanilide	103-84-4	main	135	3.16	
		4-Chloro-2,5-dimethoxyphenylisocyanate	55440-55-6	main	213	1.08	Russell (2011)
		4-Chloro-2,5-dimethoxyaniline	6358-64-1	main	187	26.44	Russell (2011) Sonoda (1999)
		N-(4-Chloro-2,5-dimethoxyphenyl)acetamide	6938-75-6	pyro	229, 187, 172	3.5	
		Unknown product	-	pyro	296, 281, 265	1.28	
		2,5-Dimethoxy-*N*-phenylbenzenesulfonamide	-	pyro	293, 198, 122	3.73	
		3-Amino-2,5-dimethoxy-*N*-phenylbenzenesulfonamide	-	pyro	308, 216, 168	21.71	Russell (2011)
		Unknown product	-	pyro	350, 168, 152	0.98	
P.Y.151	13980	Acetone	67-64-1	main	58	3.13	
		Benzene	71-43-2	main	78	1.62	Russell (2011)
		Aniline*	62-53-3	main	93	11.67	Russell (2011)
		Benzonitrile*	100-47-0	main	103	5.22	
		3-Aminobenzonitrile	2237-30-1	main	118	3.18	
		2-Methyl-4*H*-3,1-benzoxazin-4-one	525-76-8	main	161	3.4	Russell (2011)
		4(1*H*)-Quinazolinone	491-36-1	main	146	6.32	
		5-Amino-1,3-dihydro-2*H*-1,3-benzimidazol-2-one	-	main	149	1.39	
		Unknown product	-	pyro	239, 119, 92	3.68	
		Unknown product	-	pyro	238, 120, 92	3.75	

P.Y.154	11781	Acetone	67-64-1	main	58	3.47	
		(Trifluoromethyl)benzene	98-08-8	main	146	7.78	Russell (2011)
		2-(Trifluoromethyl)aniline	88-17-5	main	161	20.54	Russell (2011)
		2-(trifluoromethyl)phenylisocyanate	2285-12-3	main	187	10.22	Russell (2011)
		3-Trifluoromethylphenylacetone	21906-39-8	main	202	7.1	
		5-Amino-2-cyanobenzotrifluoride	654-70-6	main	186, 166, 138	9.98	
		Unknown product	-	pyro	228, 186, 138	2.57	Russell (2011)
		Unknown product	-	pyro	228, 213, 193	6.58	
		Unknown product	-	-	305, 285, 258	0.87	
		N-(2,3(1*H*)-Dihydro-2-oxo-5-benzimidazolyl)-acetamide	91085-68-6	main	191, 149, 121	1.88	
		Unknown product	-	pyro	191, 175, 149	12.48	
P.R.9	12460	Chloromethane	74-87-3	main	50	2.7	
		1,4-Dichlorobenzene	106-46-7	main	147	3.45	Russell (2011) Sonoda (1999)
		o-Anisidine	90-04-0	main	123	7.15	Russell (2011)
		2-Methoxyphenylisocyanate	700-87-8	main	149	1.69	Russell (2011) Sonoda (1999)
		2-Aminophenol	95-55-6	main	109	2.9	
		2,5-Dichloroaniline*	95-82-9	main	162	33.85	Russell (2011) Sonoda (1999) Sonoda (1999)
		2-Naphthol	135-19-3	main	144	1.24	Sonoda (1999)
		Naphthol AS [+OCH3+NH2]	-	pyro	307, 235, 117	1.51	
		Unknown product	-	pyro	252, 189, 126	1.75	
		Unknown product	-	pyro	261, 233, 204	4.4	
		Naphthol AS-DL	135-62-6	main	293	5.03	
		Unknown product	-	pyro	318, 196, 168	3.37	
		Unknown product	-	pyro	369, 315, 280	7.03	
		Red 9 [-OCH2]	-	pyro	437, 369, 315	4.94	
P.R.22	12315	Aniline*	62-53-3	main	93	15.75	Russell (2011)
		Benzonitrile*	100-47-0	main	103	1.03	Russell (2011)
		4-Nitrotoluene	99-99-0	main	137	3.14	Russell (2011)
		2-Naphthol	135-19-3	main	144	1.54	
		2-Methyl-5-nitrophenol	5428-54-6	main	153	1.08	
		2-Methyl-5-nitroaniline	99-55-8	main	152	18.19	Russell (2011)
		Unknown product	-	pyro	228, 211, 180	1.1	
		Unknown product	-	pyro	266, 231, 202	1.02	
		Naphthol AS	92-77-3	main	263	29.4	
		Unknown product	-	pyro	288, 196, 168	1.95	
		Unknown product	-	pyro	398, 306, 260	8.87	
P.R.112	12370	2-Methylphenylisocyanate	614-68-6	main	133	2.1	Russell (2011) Scalarone (2004)
		o-Toluidine	95-53-4	main	107	3.47	Russell (2011) Scalarone (2004) Sonoda (1999)
		2,4,5-Trichlorobenzene	120-82-1	main	180	4.74	Russell (2011) Scalarone (2004) Sonoda (1999)
		2-Naphthol	135-19-3	main	144	1.3	
		2,4,5-Trichloroaniline	636-30-6	main	195	50.73	Russell (2011) Scalarone (2004) Sonoda (1999)
		Unknown product	-	pyro	375, 305, 152	1.86	
		Naphthol AS [+CH3] (C.I.37520)	135-61-5	main	277	6.98	Russell (2011)
P.R.170	12475	Aniline*	62-53-3	main	93	2.14	
(+P.R.210)	12477	Benzonitrile*	100-47-0	main	103	1.72	
		Indene	95-13-6	main	116	0.99	
		o-Toluidine	95-53-4	main	106	0.71	
		o-Anisidine	90-04-0	main	123	2.64	
		Naphthalene*	91-20-3	main	128	1	
		Formanilide	103-70-8	main	121	1.05	
		2-Ethoxyaniline*	94-70-2	main	137	5.19	Russell (2011) Sonoda (1999)
		2-Aminophenol	95-55-6	main	109	1.73	
		Indole	120-72-9	main	117	0.73	
		Benzamide*	55-21-0	main	121	0.6	
		2-Ethoxyphenylisocyanate	5395-71-1	main	163	1.66	Russell (2011)
		4-Aminobenzonitrile	873-74-5	main	118	4.53	
		2-Naphthol	135-19-3	main	144	3.06	Sonoda (1999)
		4-Aminobenzoic acid	150-13-0	main	137	0.59	
		4-Aminobenzamide	2835-68-9	main	136	2.94	
		Unknown product	-	pyro	245, 226, 214	3.32	
		Unknown product	-	pyro	261, 233, 204	9.05	
		Unknown product	-	pyro	289, 274, 261	2.43	
		Naphthol AS [+OCH2CH2]	-	pyro	307, 171, 137	3.45	Russell (2011)
		Unknown product	-	pyro	362, 345, 333	0.84	
		Unknown product	-	pyro	362, 333, 214	3.8	
		Naphthol AS-DL	135-62-6	main	293	2.59	only with P.R.210

P.R.5	12490	Benzene	71-43-2	main	78	1.4	
		Naphthalene*	91-20-3	main	128	11.52	
		5-Chloro-2,4-dimethoxyaniline	97-50-7	main	187	2.4	Russell (2011) Scalarone (2004)
		1-Methoxyphenyl-4-sulfonic acid diethylamide	-	pyro	243, 228, 171	8.33	Russell (2011) Scalarone (2004)
		Unknown product	-	main	218, 189, 109	2.55	
		2-Methoxyaniline-5-sulfonic acid diethylamide	97-35-8	main	258	8.66	Russell (2011) Scalarone (2004)
		Binaphthyl sulfone	32390-26-4	main	318	5.8	
P.R.146	12485	Aniline*	62-53-3	main	93	4.4	
		2-Naphthol	135-19-3	main	144	0.84	Sonoda (1999)
		4-Chloro-2,5-dimethoxyaniline	6358-64-1	main	187	15.78	Sonoda (1999)
		4-Methoxy-*N*-phenylbenzamide	7465-88-5	pyro	227, 135, 92	6.59	Sonoda (1999)
		3-Amino-4-methoxybenzanilide	120-35-4	main	242	1.7	
		Naphthol AS [+2OCH3+Cl]	92-72-8	main	357	5.56	
		Unknown product	-	pyro	382, 196, 168	10.58	
		Unknown product	-	pyro	396, 210, 182	2.57	
P.Brown 25	12510	1,4-Dichlorobenzene	106-46-7	main	146	3.24	Russell (2011)
		2,5-Dichloroaniline*	95-82-9	main	161	44.48	Russell (2011)
		2-Naphthol	135-19-3	main	144	1.35	
		Unknown product	-	pyro	307, 235, 117	5.22	Russell (2011)
		5-Amino-1,3-dihydro-2H-1,3-benzimidazol-2-one	-	main	149	4.38	
		Unknown product	-	pyro	288, 218, 189	8.92	
		Unknown product	-	pyro	252, 189, 94	4.99	
		Unknown product	-	pyro	396, 326, 261	5.49	
P.O.43	71105	C5H10	-	main	70	3.91	
		C6H12	-	main	84	2.76	
		Benzene	71-43-2	main	78	1.32	
		Octadecanenitrile	638-65-3	main	265	1.17	
		Unknown product	-	pyro	319, 122, 55	8.38	
		Unknown product	-	pyro	242, 214, 121	12.41	
		Unknown product	-	pyro	270, 241, 214	3.07	
		Unknown product	-	pyro	267, 239, 136	7.62	
		Unknown product	-	pyro	295, 266, 239	3.42	
P.R.177	65300	Benzene	71-43-2	main	78	0.6	
		4-Biphenylamine	92-67-1	main	169	1.49	
		9*H*-Fluoren-9-one	486-25-9	main	180	1	
		5*H*-Indeno[1,2-*b*]pyridine	244-99-5	main	167	2.4	
		9,10-Anthracenedione	84-65-1	main	208	1.85	
		2-Amino-9-fluorenone / 9(10H)-Acridone	3096-57-9 / 578-95-0	main	195	13.05	
		1-Amino-9,10-anthracenedione	82-45-1	main	223	69.75	
P.R.179	71130	Acetanhydride	108-24-7	main	43, 42, 29	14.23 ‡	
		Isocyanatomethane	624-83-9	main	57, 56, 28	14.23 ‡	
		1-Dodecanol	112-53-8	main	168, 140, 125	2.62	
		1-Tetradecanol	112-72-1	main	196, 168, 154	0.78	
		Unknown product	-	pyro	211, 183, 166	33.03	
		Unknown product	-	pyro	252, 126, 113	1.55	
		Unknown product	-	pyro	277 ,139, 125	4.51	
Pigment V1 / Rhc	45170	Benzene	71-43-2	main	78	0.52	
		Unknown product	-	pyro	357, 342, 280	1.31	
		Unknown product	-	pyro	326, 316, 239	4.52	
		Rhodamine B cation [-COOH -2CH2CH3 +3H]	-	pyro	344, 267, 223	12.83	
		Rhodamine B cation [-*N*-ethylethanamine +H]	-	-	372, 344, 267	6.87	
		Rhodamine B cation [-*N*-ethylethanamine +H]	-	pyro	372, 295, 251	18.81	
		Rhodamine B cation [-COOH +H]	-	pyro	400, 323, 279	23.79	
		Unknown product	-	-	369, 326, 298	2.61	
		Rhodamine B cation [-COOH]	-	pyro	399, 354, 326	3.59	
		Rhodamine B cation	-	-	444, 415, 371	1.2	
		Unknown product	-	pyro	472, 443, 323	3.92	
P.R.88	73312	Hydrogen chloride	7647-01-0	main	36	11.88	

[#] o-Cyanobenzoic acid and phthalimide have similar mass spectra
* Verified with standard substance
[‡] Overlapping peak areas