Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for:

Cluster-induced desorption/ionization mass spectrometry of highlighter ink: unambiguous identification of dyes and degradation processes based on fragmentation-free desorption

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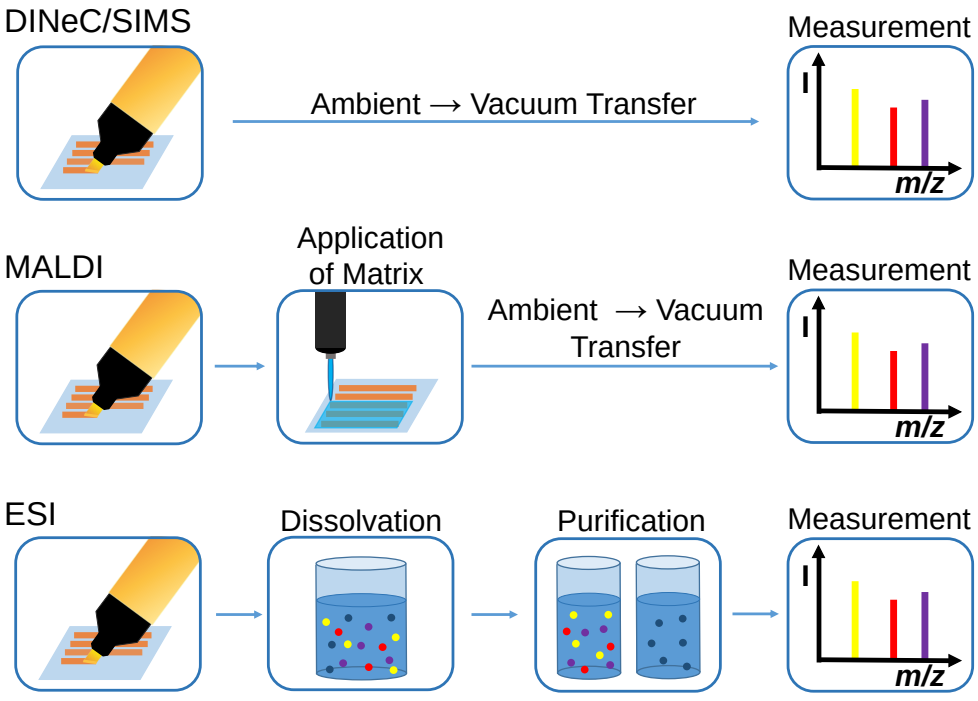

Figure S1: Schematic illustration of the workflow required for different ionization methods when applied for the investigation of inks written on paper substrates.

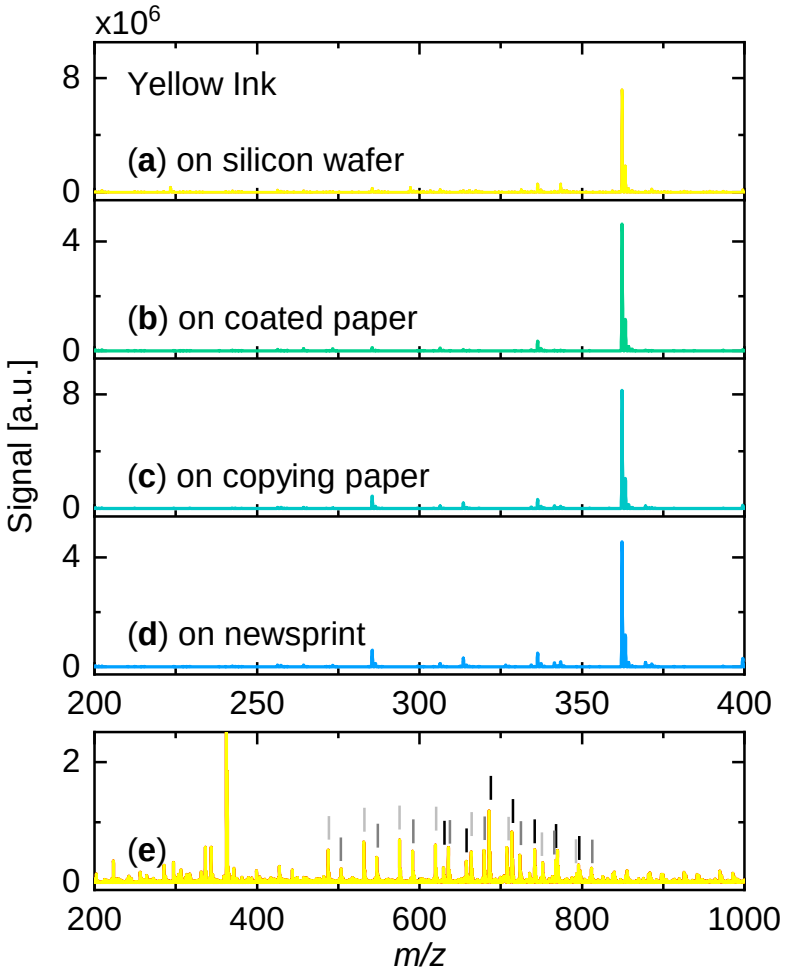

Figure S2: Positive ion mode DINeC mass spectra obtained from samples with yellow highlighter drawn on different substrates: **(a)** silicon wafer, **(b)** coated paper, **(c)** copying paper, and **(d)** newsprint. **(e)** shows the spectrum of **(a)** in an extended mass range. In addition to the peak associated with the yellow dye, three peak progressions with the peaks separated by $\Delta(m/z) = 44$ (indicated by light and dark grey lines) and $\Delta(m/z) = 28$ (black lines) are observed, which are assigned to polymer matrix in the highlighter ink.

Table S1: Ion peaks observed in the spectra shown in Figure 2 obtained by means of DINeC-MS

m/z	Intensity [a.u.]				
	(a)	**(b)**	**(c)**	**(d)**	**(e)**
471.4	35401	1170803	1192156	2198989	2518462
429.4	34588	688925	1303573	2105223	454977
362.3	7178677	3773461	494993	21871	7489

Table S2: Relative peak intensities of peaks associated with the three dye molecules of the highlighter inks investigated by means of DINeC-MS.

Color	**Substrate**	**BY40**	**BR1:1**	**BV11**
Yellow	Si	100	0	0
	paper	100	0	0
Red	Si	17	43	40
	paper	11	43	46
Purple	Si	0	15	85
	paper	0	10	90
Orange	Si	68	12	20
	Si	65	14	21
	Si	78	11	11
	Si	68	13	19
	Si	76	10	14
	paper	72	11	17
	average	**71±4**	**12 ±1**	**17±3**
Pink	Si	0	49	51
	Si	0	52	48
	Si	0	62	38
	Si	0	59	41
	Si	0	56	44
	paper	0	52	48
	average	**0 ±0**	**55 ±5**	**45±4**
Mix Orange + Pink	Si	47	29	24
	Si	27	37	36
	Si	32	35	33
	Si	53	26	21
	average	**40 ±11**	**32 ±4**	**29±6**

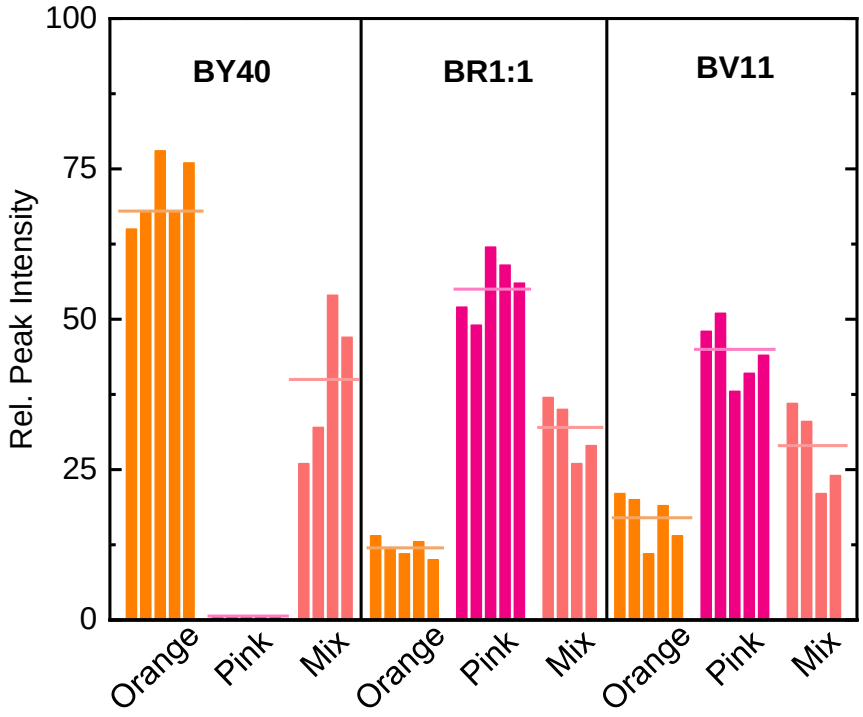

Figure S3: Relative peak intensity of the peaks associated with the three dyes Basic Yellow 40, Basic Red 1:1, and Basic Violet 11 as observed in DINeC-MS for the orange and pink highlighter inks as well as for a mixture of orange and pink highlighter ink (ratio approx. 1:1). The values of the pure inks scatter slightly around the mean value (compare also Tab. S2); the distribution for the pure inks and the mixture are clearly separated.

Table S3: Ion peaks observed in the spectra shown in Figure 4 obtained by means of DINeC-MS

m/z	Intensity [a.u.]
429.3	115943
414.3	20460
401.3	12682
399.4	21832
365.3	15223
355.3	32017
341.3	67041
327.3	220820

Table S4: Ion peaks observed in the spectra shown in Figure 5 obtained by means of DINeC-MS

m/z	Intensity [a.u.]	
	(a)	**(b)**
362.3	30399	80392
347.3	2377	3867
332.3	10306	8030
318.3	86282	40389

Table S5: Ion peaks observed in the spectra shown in Figure 6 obtained by means of DINeC-MS

m/z	Intensity [a.u.]	
	(a)-(d)	**(e)-(h)**
471.4	366334	6841730
443.3	393313	5834746
399.3	377406	3045115
355.3	315823	1054573

Table S6: Ion peaks observed in the spectra shown in Figure 7 obtained by means of DINeC-MS

m/z	Intensity [a.u.]		
	(a)	**(b)** (inset)	**(c)**
376.3	64801	52294 (8953)	1670345
362.3	28684768	6149794 (64273)	1316430
348.3	50053	71441 (8110)	1085953
334.3	172624	669177 (44314)	861559
306.3	Contamination		
264.4	112327	69156 (37615)	70667
256.3	161211	91489 (56581)	83525
242.4	64613	20702 (22030)	32460
224.4	52082	28886 (20023)	27829
200.3	241682	96139 (36716)	136424
182.3	166165	54502 (66079)	82115
170.3	248930	187977 (70029)	169699
161.3	2624	11973 (6739)	1007542
155.3	Contamination		
147.3	14421	137040 (156510)	2628727

Table S7: Ion peaks observed in the spectra shown in Figure 8 obtained by means of DINeC-MS

m/z	Intensity [a.u.]		
	(a)	**(b)** (inset)	**(c)**
485.4	337919	236890 (17636)	203644
471.4	41780428	11294170 (312748)	2136593
457.4	1519330	251161 (24148)	607563
443.4	2317717	6093131 (235756)	646205
429.3	45260	24662 (9527)	547898
415.3	173562	282014 (112150)	383407
401.3	8838	5498 (2418)	699801
387.3	14077	14498 (24115)	538323
359.3	4970	2548 (4398)	854511

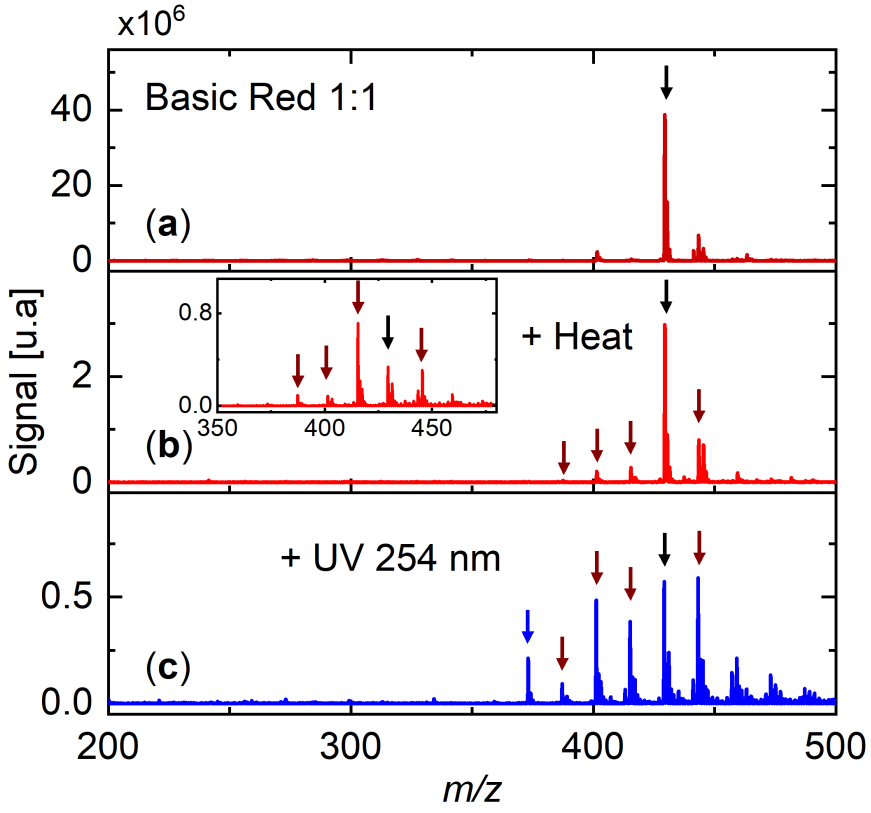

Figure S4: **(a)** DINeC mass spectrum in positive ion mode of the dye Basic Red 1:1. The black arrow indicates the peak at $m/z = 429$ associated with intact cation of the dye. **(b)** The mass spectrum obtained from the heated dye sample shows three fragment peaks indicated by brown arrows. An additional peak at $m/z = 443$, i.e., at higher m/z values when compared to the intact dye molecule, is also observed and labelled. It is attributed to a dye molecule carrying an additional methyl group, similar as observed for the Basic Yellow 40 sample after UV-light-induced decomposition (Fig. 7(c)). Further decomposition with higher thermal load (intensity of the main peak reduced to less than 2 % of the initial value) does not lead to further prominent fragment peaks. **(c)** Mass spectrum taken after UV-light induced decomposition of the red dye. An additional fragment peak is observed (labeled by a blue arrow).

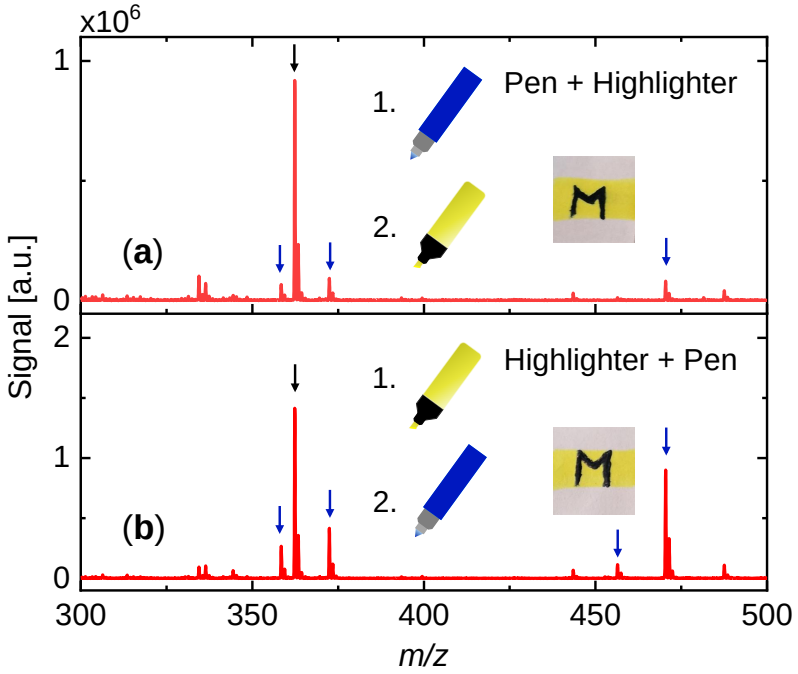

Figure S5: Positive ion mode DINeC mass spectra of **(a)** highlighter ink drawn on pen ink on a paper substrate and **(b)** strokes of pen ink drawn on the highlighter ink. Insets: Photographs of the respective samples. Black arrows indicate the peak assigned to Basic Yellow 40 from the yellow highlighter ink, blue arrows indicate the peaks assigned to the dyes used in the pen ink. If the highlighter is applied after the pen ink **(a)**, the intensity of the fluorescent dye shows highest signal intensitiy. When the order of application is reversed **(b)**, the intensity of the peaks associated with the pen ink increase in intensity. The relative intensity of the peaks is given by the intensity as measured for the pure and the completely covered ink/highlighter and highlighter/ink systems (Fig. 9) weighted by the relative area covered by the differently colored parts of the sample.

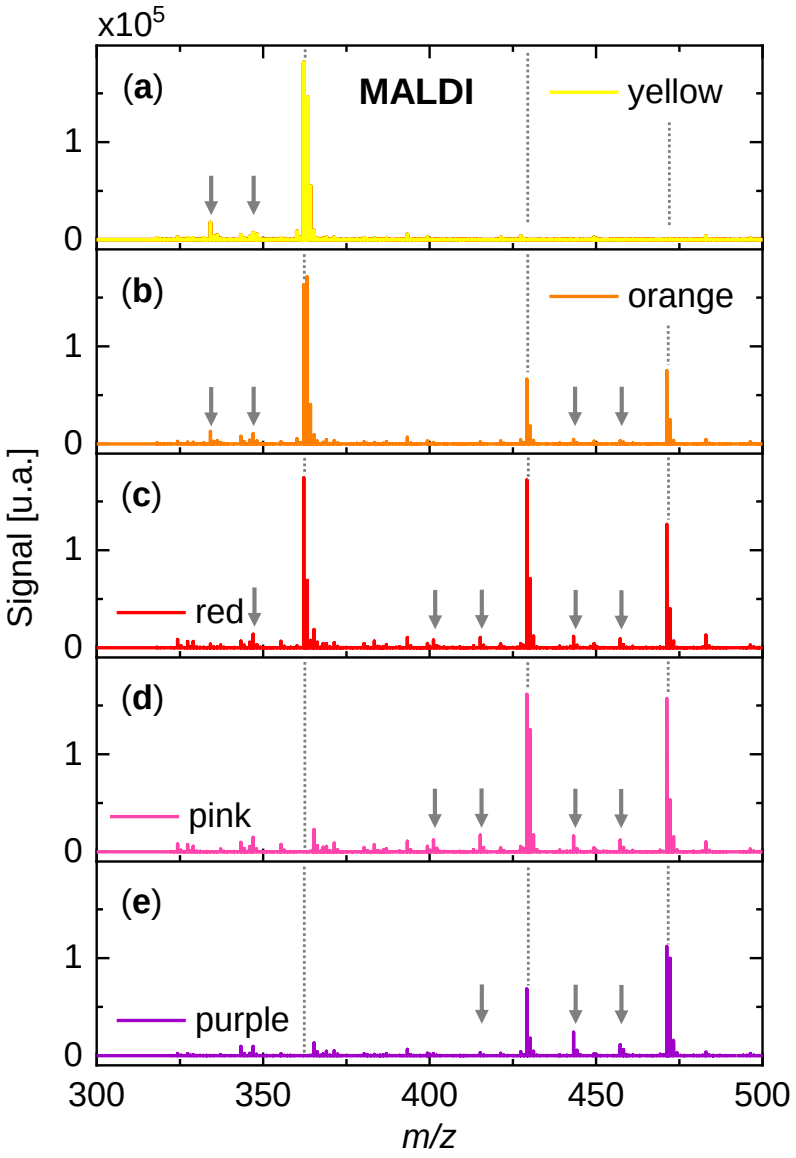

Figure S6: Positive ion mode MALDI mass spectra from **(a)** yellow, **(b)** orange, **(c)** red, **(d)** pink, and **(e)** purple colored highlighter ink applied to paper substrates. Three major peaks which are assigned to the dye molecules are observed at $m/z = 362$, $m/z = 429$, and $m/z = 471$ in most of the spectra but with varying relative intensity. Arrows indicate fragmentation peaks at $m/z = 334$ and $m/z = 348$ (yellow dye), $m/z = 401$ and $m/z = 415$ (red dye), and $m/z = 443$ and $m/z = 457$ (violet dye), which are also observed in DINeC spectra measured after dye degradation. The measurements were performed on a Bruker timsTOF fleX time-of-flight apparatus; 2,5-dihydroxybenzoic acid (DHB) was used as matrix. DHB was dissolved in an acetonitrile/water (90:10) mixture and sprayed (nozzle temperature 60 °C) onto the highlighter ink written on copying paper.

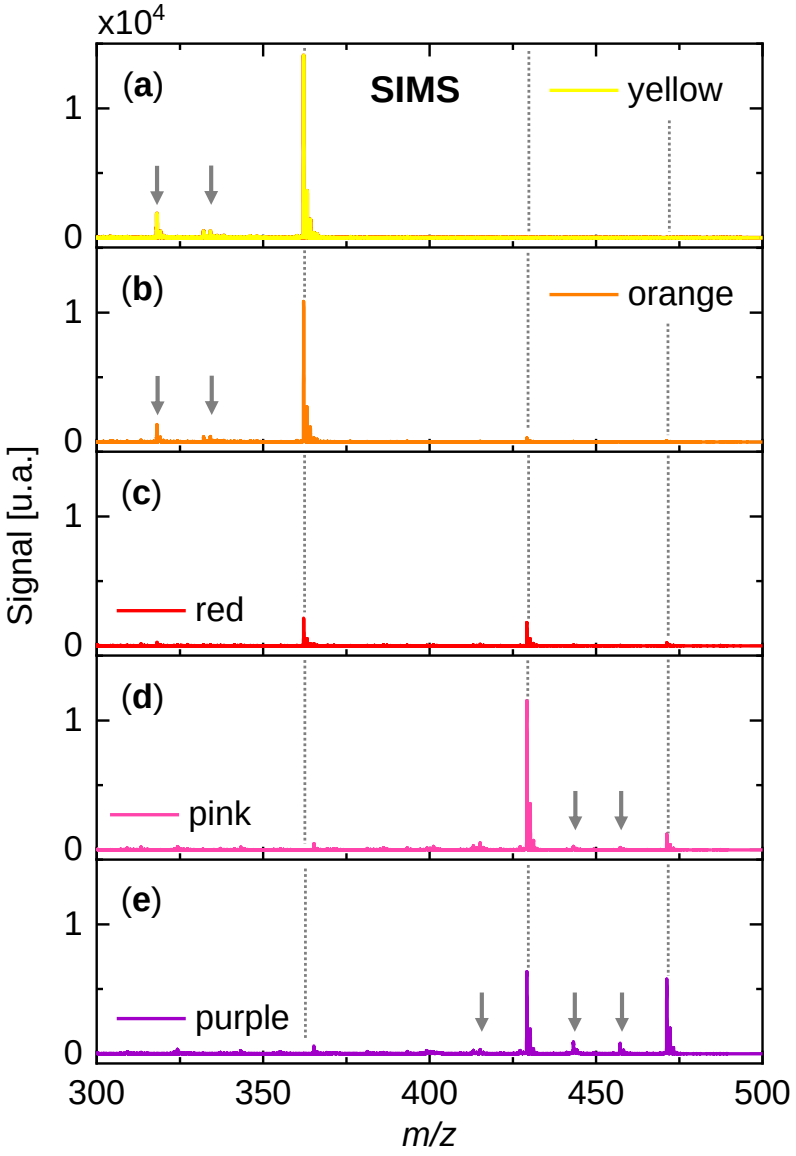

Figure S7: Positive ion mode SIMS mass spectra from **(a)** yellow, **(b)** orange, **(c)** red, **(d)** pink, and **(e)** purple colored highlighter ink applied to Si substrates. Three major peaks which are assigned to the dye molecules are observed at $m/z = 362$, $m/z = 429$, and $m/z = 471$ in most of the spectra but with varying relative intensity. Arrows indicate fragmentation peaks at $m/z = 318$ and $m/z = 334$ (yellow dye), $m/z = 415$ (red dye), and $m/z = 443$ and $m/z = 457$ (violet dye), which are also observed in DINeC spectra measured after dye degradation. The measurements were performed with a M6 Hybrid SIMS instrument (IONToF GmbH, Münster, Germany). As primary ions, 20 keV $Ar_{1500}{}^{+}$-clusters were used at a cycle time of 150 μs resulting in a primary ion current of $I = 0.07$ pA. The ion beam was rasterized with 128 $\times$ 128 pixels on an area of 100 $\times$ 100 μm^2. Total ion dose was 10^{12} ions/cm^2 for all measurements. The obtained mass resolution (FWHM) at $m/z = 362.2$ was $m/\Delta m > 3.500$ for all spectra.

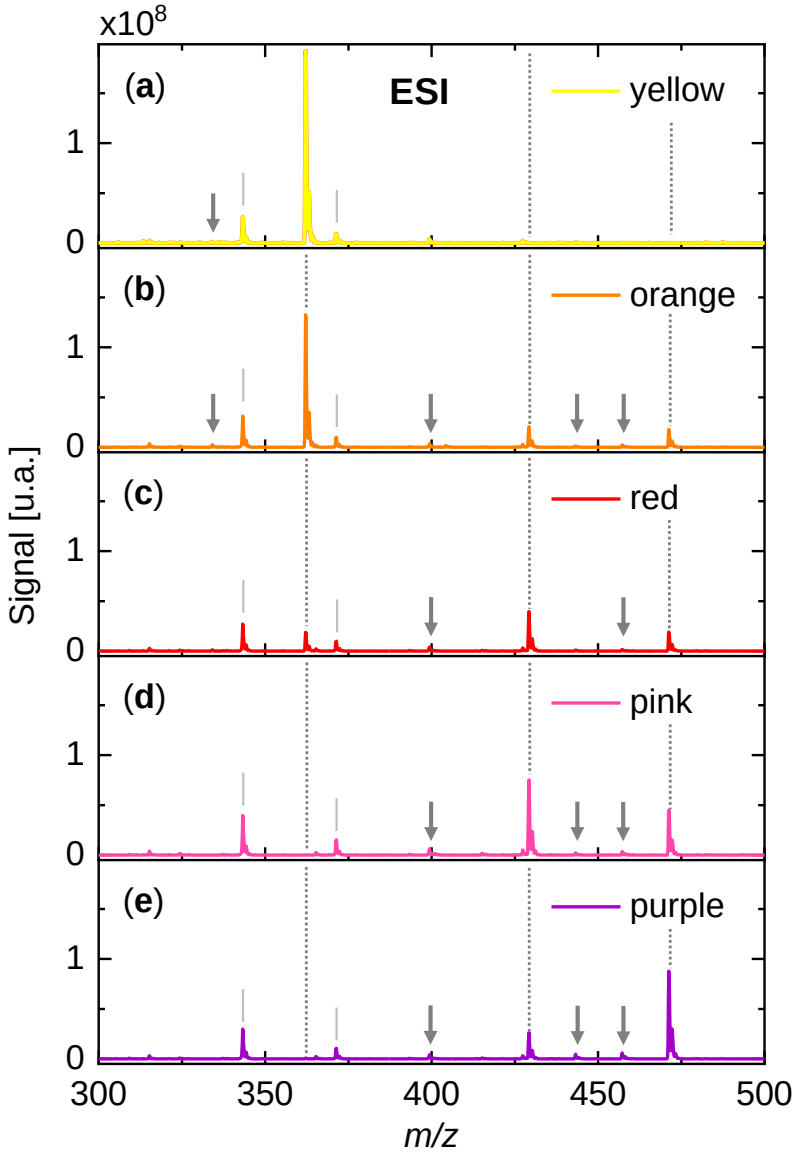

Figure S8: Positive ion mode ESI mass spectra from **(a)** yellow, **(b)** orange, **(c)** red, **(d)** pink, and **(e)** purple colored highlighter ink. For the measurements, which were performed on a Bruker amaZon speed mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), a dot of ink was dissolved in a mixture of acetonitrile/water (50:50) containing 0.1 Vol% formic acid. Three major peaks which are assigned to the dye molecules are observed at $m/z = 362$, $m/z = 429$, and $m/z = 471$ in most of the spectra but with varying relative intensity. Arrows indicate fragment peaks at $m/z = 334$ (yellow dye), $m/z = 399$ (red dye), and $m/z = 443$ and $m/z = 457$ (violet dye), which are also observed in DINeC mass spectra measured after dye degradation. Gray lines indicate additional peaks which are not attributed to dye fragments, they are also observed in DINeC mass spectra of the highlighter inks. They are tentatively assigned to additional compounds of the highlighter ink (Fig. 2, Tab. S1).