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1	High-resolution mass spectrometry and non-traditional mass defect analysis of brominated
2	historical pigments
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9	

10 GRAPHICAL ABSTRACT



13 ABSTRACT

The implementation of high-resolution mass spectrometry (HRMS) systems offers new 14 possibilities for the analysis of complex art samples such as historical oil paintings. However, these 15 multicomponent systems generate large and complex datasets that require advanced visualization 16 tools to aid interpretation, especially when no chromatographic separation is performed. In the 17 18 context of this research, it was crucial to propose a data analysis tool to identify the products generated during the synthesis, drying and aging of historical pigments. This study reports for the 19 first time a non-traditional mass defect analysis of oil paint samples containing a fugitive 20 21 brominated-organic pigment, eosin or geranium lake, by using direct infusion electrospray ionization in combination with a high-resolution Orbitrap mass spectrometer. The use of non-22 23 traditional Kendrick mass defect plots is presented in this study as a processing and visualization tool to recognize brominated species based on their specific mass defect and isotope pattern. The 24 results demonstrate that this approach could provide valuable molecular compositional 25 information on the degradation pathways of this pigment. We anticipate that mass defect analysis 26 will become highly relevant in future degradation studies of many more historical organic 27 pigments. 28

30 INTRODUCTION

The fast advances in organic chemistry during the 19th century resulted in the discovery of new compounds and chemical reactions. These discoveries not only had an impact on the quality of life but also had a direct impact in the palette of oil painters and watercolourists, due to the synthesis of new organic pigments and their rapid manufacture and commercialization as paint tubes.

35 In recent years one of these new synthetic pigments, eosin lake (historically sold as geranium lake), was identified for the first time in the oeuvre of Gauguin and van Gogh.¹ Historical geranium lake 36 recipes describe the precipitation of the eosin dye (Figure SI.1) onto aluminium or lead salts to 37 form an insoluble pigment.² The result is an aggregate of eosin molecules complexed to the metal 38 substrate.^{3,4} However, eosin lakes were originally manufactured for lithography, book and 39 wallpaper printing, and not for easel painting. Despite its brightness and beautiful hue, from purple 40 to pink, this family of brominated pigments tends to rapidly fade leading to the discoloration of 41 masterworks within an artist's lifetime^{1,5} and makes identification in the artwork very challenging. 42 This is because eosin is a photosensitizer,^{6,7} and its degradation mechanism is therefore strongly 43 affected by the presence of light and oxygen,⁸ and surrounding media. For example, the detection 44 of bromine, an element rarely found in historical pigments, by scanning electron microscopy-45 46 energy dispersive X-ray spectrometry (SEM-EDS) and macroscopic X-ray fluorescence (MA-XRF) analysis has been hypothesized to indicate the presence of eosin on van Gogh's paintings.^{9,10} 47 48 However, the identification of geranium lakes in original paintings by chromatographic techniques has relied solely on the detection of the intact eosin chromophore.^{11,12} 49

A series of recent publications focused on the detection of geranium lakes by mass spectrometry have contributed very significantly to this research topic.¹³⁻¹⁵ Nevertheless, the side-products formed during the synthesis of geranium lakes, as well as their degradation mechanisms in oil paintings, are still unclear. In addition, it has been observed that the metal used to complex the eosin could affect the lightfastness due to the molecular structure of the lake.^{4,16} However, up to now, no mass spectrometric analysis has been performed to reach a deeper understanding of the possible degradation process of the different metallic complexes.

In our previous study we proposed a breakdown technology in the field of conservation science 57 exploring direct analysis in real time (DART) and direct infusion electrospray ionization (DI-ESI) 58 coupled to high resolution mass spectrometry (HRMS) for chemical identification of these 59 brominated historical pigments in oil paints.¹⁵ However, the interpretation of HRMS datasets 60 61 obtained by direct analysis in real time and by direct infusion can be challenging due to the large amount of information generated. At the same time, geranium lake-oil paints may contain a 62 complex mixture of related brominated products associated with impurities, due to the synthesis 63 conditions and/or interaction with the binding media, and degradation products formed over time. 64 Data analysis generated by the HRMS system is simplified here using the fact that geranium lakes 65 66 have a unique mass defect (i.e., the difference between the exact and the nominal mass) and a discrete isotopic pattern, which readily distinguishes them from non-brominated species in a 67 complex mass spectrum. A large range of visualization strategies based on the resolution and mass 68 69 defect information offered by HRMS are available to mitigate the effects of the matrix interference and improve the identification of target compounds.¹⁷ 70

A common strategy to track repeating units in polymeric materials was proposed by Kendrick in 1963.¹⁸ Kendrick mass (KM) analysis is based on arbitrarily setting the mass of the CH_2 unit to 14, instead of its IUPAC mass (m(CH_2)=14.01565). The IUPAC mass of a repeating unit can then be converted to the Kendrick mass (KM) as:

$$KM=m/z^{*}(14/14.01565)$$

76	The Kendrick mass defect (KMD) is the difference between the rounded KM and the exact KM:
77	KMD=round(KM)-KM.
78	This approach facilitates the visualization of a homologous series of compounds that share the
79	same mass defect when plotting their KMD as a function of m/z.
80	In the literature, Kendrick analysis has been extended to non-polymeric samples by selecting the
81	exact mass of an appropriate rescaling unit (R). ¹⁹ In this case, the rescaled Kendrick mass (KM(R))
82	is calculated as:
83	KM(R)=m/z*round(R)/R.
84	As before, the corresponding KMD (aka Rescaled Kendrick mass) can be calculated as:
85	KMD(R)=round(KM(R))-KM(R).
86	Recently, it has been shown that this new approach is a suitable method for the analysis and
87	chemical fingerprint of halogenated compounds. ²⁰ By using non-traditional rescaling units such as
88	-H/+Cl, CF ₂ , +F/-Cl, -HBr, the plot can act help analysts to visually separate homologous
89	(halogenated) series that are not so easy distinguishable in the original mass spectrum. ²¹⁻²³
90	In this study, a non-traditional Kendrick mass analysis, based on the concept of rescaling units,
91	has been successfully applied for first time on a series of historical brominated-organic pigments.
92	Oil paints prepared from two different metallic conformations of geranium lake, observed in van
93	Gogh paintings ¹ , aluminium (Al ³⁺) and lead (Pb ²⁺) based, were analysed by DI-ESI-HRMS with
94	the aim of detecting brominated impurities, side-products coming from the synthesis, and
95	degradation products formed during the drying and aging of the oil paint.
96	
97	MATERIAL AND METHODS

Chemicals

Eosin-Y (99% dye content) was purchased from Sigma-Aldrich. Aluminium chloride hexahydrate
(AlCl₃·6H₂O) was purchased from Fluka. Lead (II) acetate trihydrate (Pb(CH₃CO₂)₂·3H₂O) was
purchased from Merck. The bleached linseed oil (Talens) employed to prepare the paints was
diluted with turpentine (Winsor & Newton).

103

104 Synthesis of geranium lakes, oil paint samples preparation and artificial photoaging

The synthesis of the eosin-based, or geranium, lakes was carried out according to the protocol 105 proposed by Claro et al.³ Oil paint models were prepared by mixing each of the metallic lakes with 106 107 linseed oil and painting them out on glass slides; paint films of approximately 100 µm thickness were obtained in this manner. The final oil paints were kept in the dark for 4 weeks at 20 °C and 108 at environmental humidity (about 60% RH). Oil paint samples were subjected to accelerated light 109 110 aging up to 553 h in a controlled climate chamber (Model UV 200RB/20DU, Weiss Technik) equipped with a UVA (350-400 nm) lighting system (Actinic BL TL, Philips). The temperature 111 (21 °C) and relativity humidity (30%) were kept constant during the photoaging. 112

113 Studied samples

Powder samples of both metallic lakes (aluminium- or lead-eosin based) were kept in the dark until the analysis and were considered as reference materials, i.e., without the presence of linseed oil and without artificial aging. For this study, the oil paint samples corresponding to time 0 h (unaged) and 553 h (aged) were analysed to monitor the change experienced during: (1) the drying process (unaged oil paint sample) and (2) artificial aging (aged oil paint sample). Here it is important to note that unaged paint samples were also kept in the dark until the analysis.

120 DI-ESI-HRMS analysis

121 The extraction protocol was followed as described in our previous publication.¹⁵ Solvent extract 122 was infused at 10 μ L/min and electrosprayed into an LTQ Orbitrap Velos mass spectrometer. 123 High-resolution MS1 and MS2 were acquired in positive mode (m/z 150–2000) at 30000 resolving 124 power. For tandem mass analysis, each selected analyte was isolated with a 4 m/z window for 125 0–45% collision energy (CE) for collision induced dissociation (CID) and 0–45% normalized 126 collision energy (NCE) for higher-energy collisional dissociation (HCD). The background 127 subtraction of the solvent was performed during data analysis.

128

129 RESULTS

130 *Characterization of historical geranium lakes*

Geranium lakes are constituted by an aggregate of eosin molecules complexed to an inorganic substrate. Unaged powder samples of aluminium- and lead-based eosin lakes were studied in this section as reference materials to identify the eosin:metal complex (aka lake) and to evaluate the possible formation of side-products generated during the synthesis.

The presence of a heteroatom such as Br, present not only in the lake, but also in the side-products, 135 produced a rich and specific isotopic pattern that makes the evaluation of the mass spectrum more 136 137 complicated (Figure SI.2). To simplify the identification of the native conformation of geranium lake, we selected the mass of the most abundant isotope of a single tetra-brominated eosin 138 molecule, $([C_{20}H_9O_5^{79}Br_2^{81}Br_2]^+$, abbreviated as EoBr₄), as a base unit (R= 648.7132). The 139 140 advantages of choosing the most abundant isotope, over the monoisotopic mass, for mass rescaling and the nature of its impact on point alignments in Kendrick analysis have been already discussed 141 to track brominated flame retardants.²⁴ 142

A pattern of brominated species can be visually recognized in the non-traditional Kendrick plot or 143 rescaled KMD plot of the aluminium and lead-based lakes (Figure 1). These plots show a 144 horizontal alignment of brominated species differing along the m/z axis by the repeating unit (in 145 this case a tetra-brominated eosin, EoBr₄). Because they have the same mass defect, for both Al-146 eosin and Pb-eosin lakes, the 2:1 (eosin:metal) complexes (EoBr₄-Al-EoBr₄ at m/z 1318.4069 147 $([C_{40}H_{12}^{79}Br_4^{81}Br_4O_{10}Al]^+)$ and EoBr_4-Pb-EoBr_4 at m/z 1502.3843 $([C_{40}H_{15}^{79}Br_4^{81}Br_4O_{10}Pb]^+))$. 148 can also be identified in this horizontal line. In the mass spectrum of both metallic lakes, it can 149 been seen that next to the 2:1 (eosin:metal) complex also the doubly charged tetramer form 150 151 (2[EoBr₄-M-EoBr₄]) is observed (Figure SI.3). The mass spectrum of the lead-based lake also shows a larger lead complex at m/z 1707.3408, which correspond to the doubly charged 2:2 152 (eosin:metal) complex ($[C_{40}H_{14}^{79}Br_4^{81}Br_4O_{10}Pb_2]^{2+}$) (Figure SI.4). The doubly charged species 153 154 detected in both lakes, indicate that larger structures can be formed beside the 2:1 eosin:metal complex reported up to now.^{3,4} In addition, the difference between the exact m/z of the aluminium 155 156 complex detected in this study and what is reported by Anselmi shows that eosin molecules can show different coordination modes in presence of aluminium. 157

However, other brominated species that do not contain the repeating unit (EoBr₄) appear with an oblique alignment relative to the tetra-brominated forms in Figure 1. As these species are already observed in the lake powder studied in this section, they can be considered as side-products formed during the synthesis and will be discussed in the next section.



Figure 1. Rescaled Kendrick plots of (A) aluminium-based and (B) lead-based eosin lakes using
the most abundant isotope of the repeating unit of EoBr4 (R=648.7132).

165

166 *Evaluation of side-products generated during the synthesis*

The first approach to identify in Figure 1 the brominated species that appear with an oblique alignment regarding the tetra-brominated form (EoBr₄) was to consider the monoisotopic mass of bromine (⁷⁹Br) as the base unit (R=78.9183) for Kendrick mass analysis. This aims to visually identify compounds correlated by subsequent addition or subtraction of bromine atoms. Surprisingly, the resulting rescaled or non-traditional Kendrick plot (Figure 2A and SI.5A) displays several point series with oblique alignments, indicating that the bromine atom is not the only difference between these compounds. Secondly, the scale factor -HBr (R=79.9262) was then

used as a new base unit. However, in the resulting mass defect plots, the tangential alignment 174 indicated that the loss of HBr (or dehydrobromination) did not explain the difference between 175 adjacent compounds (Figure 2B and SI.5B). The third variation consisted in setting the scale factor 176 to -Br+H (R=77.9105), obtaining the instant rotation of the mass defect plot until the series are 177 clearly aligned horizontally (Figure 2C and SI.5C). This confirms that one of the side reactions 178 179 occurring during synthesis is the loss of a radical Br, which is replaced by a H radical (debromination). The use of this base unit clearly facilitates the rapid identification of brominated 180 side-products generated during the synthesis of the aluminium- and lead-based lakes (Table SI.1 181 182 and SI.2). In Figure 2C (aluminium-based lake) two compounds, with horizontal alignment regarding the tetra-brominated form and differing by the number of bromine substituents, can be 183 identified as tri- and di-brominated eosin forms (EoBr3 and EoBr2 at m/z 568.8056 184 $([C_{20}H_{10}O_5^{79}Br_2^{81}Br]^+)$ and 490.8949 $([C_{20}H_{11}O_5^{79}Br^{81}Br]^+)$, respectively). Simultaneously, the 185 presence of other brominated species with oblique alignments regarding the tetra-, tri- or di-186 brominated forms in the unaged aluminium-based lake (Figure 2C) indicates the formation of other 187 compounds associated to these species. The clusters at m/z 584.8192 ([C₂₁H₁₂O₅⁷⁹Br₂⁸¹Br]⁺) and 188 m/z 662.7296 ([C₂₁H₁₁O₅⁷⁹Br₂⁸¹Br₂]⁺) correspond to the methylated forms of EoBr₃ and EoBr₄ 189 eosin, respectively (EoBr₃+Me and EoBr₄+Me).^{13,15} 190

191 Other two species associated to the tetra-brominated form at m/z 706.7683 ([C₂₃H₁₅O₆⁷⁹Br₂⁸¹Br₂]⁺,

192 EoBr₄-Ester) and at m/z 670.6949 ([C₂₀H₄O₅⁷⁹Br₂⁸¹Br₂Al]⁺, EoBr₄+Al) were also identified.

After applying the scale factor -Br+H (R=77.9105) to the unaged lead-based powder lake, the only compound with a horizontal alignment regarding EoBr₄ was the tri-brominated form (EoBr₃) (Figure SI.5C). In contrast to the case of the aluminium-eosin complexes discussed above, no other brominated species were visible about the detection limit of the method. Furthermore, no methylated or ester derivatives were identified in the lead-based lake. However, a single species at m/z 329.0048, not detected in the aluminium-based lake, and yet unidentified is observed in Figure SI.5C.



200

Figure 2. Rescaled Kendrick plot of the aluminium-based eosin lake using: (A) 79Br as the base unit (R=78.91833), (B) -HBr as the base unit (R=79.9262) and (C) -Br+H as the base unit (R=77.91051).

204

205 Monitoring of geranium lake markers during the drying and aging of oil paints

206 The abundance of all original (i.e., present in the powders) and newly formed species were

207 monitored in the oil paint samples during drying and artificial aging (Table SI.1 and SI.2).

As described in the previous section, by using -H+Br (R=77.9105) as the rescaling unit, three horizontally aligned species, corresponding to tetra- tri- and di-brominated eosin, were observed in the unaged aluminium-based powder lake (Figure 3A). At the moment that the aluminium-based lake was mixed with linseed oil, the mono- and non-brominated species (EoBr at m/z 412.9836 ([C₂₀H₁₂O₅Br]⁺) and fluorescein at m/z 333.0755 ([C₂₀H₁₃O₅]⁺)) became readily visible in the rescaled Kendrick plot (Figure 3B and C).



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Figure 3. Rescaled Kendrick plot of the aluminium-based eosin lake using -Br+H as the base unit
(R=77.9105) of: (A) unaged powder lake, (B) unaged oil paint and (C) aged oil paint.

By monitoring the abundance in the oil paints of these four brominated species, plus fluorescein (in the graph as EoBr₀) (Figure 4), it is clear that the debromination process, already detected during the synthesis, continues to take place during the drying and aging of the oil paints. As it can

be observed in Figure 4, the abundance of the tetra-brominated species decreased, while all other debrominated species increased during the drying and photoaging of the oil paint. It is interesting to note here that mono-brominated and non-brominated eosin (or fluorescein) were not detected in the unaged lake powder, indicating that these species already begin to be generated during the drying process of the oil, and their abundance further increases under irradiation with light. These results are consistent with our previous study, where we described the generation of debrominated species during the artificial aging of oil paint samples by DART-MS.¹⁵





Figure 4. Abundance ratio of brominated products identified in the aluminium-based eosin lakein powder lake, unaged oil paint and aged oil paint.

232

In addition, the Kendrick plots of the oil paint samples (Figure 3B and C) show two new clusters, at m/z 504.9106 ([C₂₁H₁₃O₅⁷⁹Br⁸¹Br]⁺) and m/z 686.6701 ([C₂₀H₄O₆⁷⁹Br₂⁸¹Br₂Al]⁺), associated with the methylated form of EoBr₂ (EoBr₂+Me) and with the oxidation form of the EoBr₄+Al (EoBr₄+Al+O).The visual differences observed between the Kendrick plots of the powder pigment (Figure 3A) and the oil paint samples (Figure 3B-C) can be confirmed by monitoring the abundance of the exact m/z during drying and aging. For example, the abundance of the di- and tri-methyl derivatives ($EoBr_2+Me$ and $EoBr_3+Me$) slightly increases when the lake powder is mixed with linseed oil (Figure 5A). However, the abundance of $EoBr_4+Me$ is substantially reduced after being in contact with the oil (Figure 5A). These trends can be linked to the abundance of their non-methylated species during drying and aging in Figure 4, where the di- and tri-brominated forms increased but the tetra-brominated form decreased.

However, the ester derivative form of EoBr₄ and the two fragments containing aluminium
(EoBr₄+Al and EoBr₄+Al+O) clearly increase during the drying and aging of the oil paints (Figure
5B) probably due to the breakdown of the 2:1 (eosin:aluminium) complex and its further oxidation
in presence of linseed oil.



248

Figure 5. Abundance ratio of geranium aluminium-based lake markers identified in unaged powder lake, unaged oil paint and aged oil paint. (A) methyl derivatives, (B) markers containing aluminium and (C) fragments after chromophore breakdown.

Figures 3B and C also indicate the presence of two new compounds in the oil paints with oblique 253 alignments regarding the di- and mon-brominated forms. The form at m/z 430.8875 254 $([C_{15}H_{11}O_5^{79}Br^{81}Br]^+)$ corresponds to the fragmentation of the di-brominated eosin chromophore 255 and further methylation of the carboxylic group (Figure SI.6).^{13,25} The second eosin fragment is 256 257 observed at m/z 329.0048 and might be associated to the fragmentation of the mono-brominated eosin ($[C_{16}H_8O_3^{79}Br]^+$). The extraction and further monitoring of the abundance associated with 258 these two fragments indicates that they form only when the lake pigment is mixed with linseed oil 259 260 and increase with the aging (Figure 5C).

When -H+Br (R=77.9105) was used as the rescaling unit for the lead-based oil paints, none of the side-products detected in the aluminium lakes were visible in the Kendrick plots of the lead-based samples (Figure 6). Only the species at m/z 430.875 and at 329.0048, associated with the fragmentation of the chromophore, were visible in the Kendrick plot of the oil paints. The tracking of these fragments also showed an increase in abundance during the aging of the oil paint (Figure SI.7A).

The cluster observed at m/z 914.6973 in Figure 1B corresponding to $[C_{22}H_{11}O_7^{79}Br_2^{81}Br_2Pb]^+$ (EoBr₄+Pb) has been also tracked during the drying and aging (Figure SI.7B). The decrease of the abundance of this specie confirms that it is an intermediate form generated during the synthesis of the lead-base geranium lake, and not a breakdown product of the eosin:lead complex.

By monitoring the brominated forms, it is also clear that the lead-based lake follows the samedebromination pattern as the aluminium-based lake (Figure SI.8).





Figure 6. Rescaled Kendrick plot of the lead-based eosin lake using -Br+H as the base unit
(R=77.9105) of: (A) unaged powder lake, (B) unaged oil paint and (C) aged oil paint

277 Monitoring of geranium lake complex during the drying and aging of oil paints

278 The 2:1 (eosin:aluminium) complex (EoBr₄-Al-EoBr₄ at m/z1318.4069 $([C_{40}H_{12}^{79}Br_4^{81}Br_4O_{10}Al]^+)$ was monitored along the drying and aging of the oil paints. Figure 7 279 shows a decrease in abundance of the complex, indicating the breakdown of the eosin-aluminium 280 281 bonds. Nevertheless, an increase in the abundance of the species derived from the 2:1 complex takes place during aging (Figure 7). These species are formed by the debromination of the 2:1 282 complex ($[C_{40}H_{13}^{79}Br_4^{81}Br_3O_{10}Al]^+$ at m/z 1240.4971), oxidation ($[C_{40}H_{12}^{79}Br_4^{81}Br_4O_{11}Al]^+$ at m/z 283 1334.3775) or both mechanisms ($[C_{40}H_{13}^{79}Br_4^{81}Br_3O_{11}Al]^+$ at m/z 1254.4699) (Figure SI.9 and 284 Table SI.1). Interestingly, no 2:1 (eosin:lead) complex or derivatives were detected once the 285 powder was mixed with the oil. 286



Figure 7. Abundance ratio of the metallic complex (eosin: aluminium) and derivatives identifiedin powder lake, unaged oil paint and aged oil paint.

287

Finally, MS/MS analysis carried out to confirm the different stability between the aluminium- and 291 lead-based lakes revealed a significantly different fragmentation pattern for both metallic 292 293 complexes, visible even with very low product ion abundance. The aluminium complex shows a fragmentation peak at m/z 672 which likely corresponds to the fragment EoBr₄-Al 294 $([C_{20}H_5O_5^{79}Br_2^{81}Br_2A1]^+)$ already detected in the powder lake. In the case of the lead complex two 295 fragmentation peaks (at m/z 639 and 676) are present. As the intensities of the obtained fragment 296 ions are relatively low, additional experiments could be performed to optimize the fragmentation 297 conditions of these species. These results indicate the difference in fragmentation pattern between 298 aluminium and lead-based lakes, where both complexes open in different ways (Figure SI.10). The 299 slightly different behaviour among aluminium- and lead-based lakes observed during the aging of 300 the oil paints can be linked to the different conformations of the two lake complexes.¹⁶ 301

303 DISCUSSION

The information obtained by mass defect analysis of HRMS data allows one to propose a degradation scheme of the main molecular changes that geranium lakes undergo during aging (Scheme 1).

Although it is the breakdown of the chromophore moiety that causes the total discoloration of the eosin, we confirm here that geranium lakes present a tendency to lose bromine when the lake is in contact with linseed oil in the presence of light and oxygen. Previous studies have proposed a radical-mediated start of the reaction followed by oxidative debromination of the eosin molecule and the production of high energetic ${}^{1}O_{2}$. The latter species will break the eosin chromophore. Two breakdown products of the chromophore have been detected in this study (Scheme 1 and Tables SI.1 and SI.2), however any smaller fragments due to further cleavage remain unidentified.

We also show that the metal used to complex the lake (Scheme 1A for aluminium and 1B for lead) influences the reactivity occurring during the aging, which may be correlated with the literature describing the different conformations and reactivity of geranium lakes.⁴

The methyl and ester derivatives, mostly detected in the aluminium lake (Scheme 1A), could be 317 produced during the synthesis due to the interaction with the solvent.^{13,26,27} The photochemical 318 319 behaviour of eosin in different environments has been deeply investigated showing that its complex protolytic system may easily react with the surrounding media.²⁸⁻³⁰ As a result, the 320 321 formation of methylated and ester intermediates observed in this study is explained by the higher 322 acidity of the carboxylic group compared to the phenolic group due to the presence of bromine groups. The negative charge of the basic form better stabilizes in the carboxylate than in the 323 phenolate, keeping the chromophore moiety intact.²⁶ However, since the monitoring of these 324 325 compounds indicates that the abundance increases with aging, their formation can also be

associated with the interaction with the oil matrix.³¹ This proves that the reaction pathways involved in the discoloration of geranium lakes are much more than those described in the literature. The data obtained here suggests that esterification can also occur due to interaction with the oil in presence of light. In this case, the anchoring of the eosin to the metal through the carboxylic group will determine the chemoselective methylation and esterification reactions. This hypothesis confirms the different reactivity observed in the two metallic conformations studied here.

Finally, Kendrick mass analysis allowed visual detection of larger complexes that have not been described before in the literature, such as 2:2 eosin:metal complexes (in the case of the lead-based lakes) and oxidation products (in the aluminium-based lakes). These products indicate that the lakes not only undergo the breaking of bonds but also their functional groups can react to form bigger clusters.



339 Scheme 1. Tentative fragmentation pathways of: (A) aluminium- and (B) lead-based geranium
340 lakes. See Table SI.1 and SI.2 for more information about the compounds.

341

342 CONCLUSIONS

Non-traditional Kendrick mass analysis has been evaluated for the first time as a visualization tool to identify species associated with fugitive geranium lakes in oil paint samples. The approach proposed here is based on the detection of brominated products, either produced during the synthesis of the eosin pigment or formed as degradation products during the aging process of eosincontaining oil paint samples.

The combination of HRMS and Kendrick mass analysis revealed a variety of previously 348 unreported brominated compounds, underscoring the complexity of the synthesis, drying and 349 discoloration process of geranium lake pigments. Despite the possible differences found between 350 the two geranium lakes studied here, Kendrick mass analysis has shown that the use of the most 351 abundant isotope of a single tetra-brominated eosin as a base unit (R = 648.7132) facilitated the 352 353 visualization of the metallic complex and the side-products generated during the synthesis. In the case of evaluating the degradation mechanism of geranium lakes over time, the use of a non-354 traditional Kendrick scale factor -Br+H (R=77.9105) eases the identification of the main pathways 355 356 and facilitates tracking of the brominated markers in complex oil paint samples. The visible changes observed in the Kendrick plots can be confirmed by extracting the masses of these clusters 357 and, monitoring their abundance during drying and aging. This allows us to track for first time not 358 only the molecular changes associated to the base unit (R = 648.7132), but also to monitor the 359 abundance of eosin-metal complexes during aging. 360

The results indicate that two different types of conformation, with different fragmentation patterns, could be spotted as a function of the metal used during the synthesis. Despite the possible differences found between the two metallic lakes, Kendrick mass analysis has shown that for both eosin-metal complexes, the loss of bromine begins to take place during the drying of the (linseed) oil and increases during the artificial light aging. In addition, Kendrick mass analysis facilitated the identification of other brominated species, either formed by the esterification of the carboxylate group or by fragmenting the chromophore.

The methodology developed in this study is expected to facilitate the characterization by HRMS of historical brominated pigments in a more complex matrix such as a painting. Historical paintings are highly complex mixtures containing not only pigments and oil, but preparation layers, a

371	mixture of different (organic and inorganic) pigments, waxes, and varnishes, among other
372	materials added for conservation and restoration. Thus, Kendrick mass defect analysis will be used
373	to track specific markers in samples taken from historical paintings; and the results will improve
374	our knowledge on the possible reactions that take place during the lifetime of the painting.
375	
376	ASSOCIATED CONTENT
377	Supporting information
378	The Supporting Information is available free of charge on the ACS Publications website.
379	Additional information on chemical structures of eosin, DI-ESI-HRMS spectra of unaged powder
380	lakes, rescaled Kendrick plot of the lead-based eosin lake, tentative structure of the identified
381	fragment, abundance ratio plots, DI-ESI-HRMS spectrum indicating the 2:1 complex, MS-MS
382	spectra.
383	
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