

1 **Identification of phenolic compounds in strawberries by liquid**
2 **chromatography electrospray ionization mass spectroscopy**

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1 Abstract

2 Strawberry (*Fragaria x ananassa* Duch.) fruits contain phenolic compounds that have
3 antioxidant, anticancer, antiatherosclerotic and anti-neurodegenerative properties. Identification
4 of food phenolics is necessary since their nature, size, solubility, degree and position of
5 glycosylation and conjugation influence their absorption, distribution, metabolism and excretion
6 in humans. Freeze-dried whole strawberry fruit powder and strawberry fruit extracts were
7 analyzed by liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS)
8 methods. Phenolics were identified as ellagic acid (EA), EA-glycosides, ellagitannins,
9 gallotannins, anthocyanins, flavonols, catechin and coumaroyl glycosides. The anthocyanidins
10 were pelargonidin and cyanidin, found predominantly as their glucosides and rutinosides. The
11 major flavonol aglycons were quercetin and kaempferol found as their glucuronides and
12 glucosides. LC-ESI-MSⁿ methods differentiated EA from quercetin conjugates since both
13 aglycons have identical molecular weights (302 g/mol). The identification of strawberry
14 phenolics is necessary to generate standardized materials for *in vitro* and *in vivo* studies and for
15 the authentication of strawberry-based food products.

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18 *Keywords:* Strawberries; Ellagic acid; Ellagitannins; Anthocyanins; Flavonols; LCMS

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1 **1. Introduction**

2 It is now well established that a diet high in fruits and vegetables is associated with a
3 reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular and
4 neurodegenerative diseases (Halliwell, 1994). The health beneficial effects of fruits and
5 vegetables are attributed to their high levels of a wide variety of phytochemicals, of which
6 phenolics constitute the greatest proportion. Phenolic compounds contain aromatic ring (s)
7 bearing hydroxyl group (s) and can range from simple molecules to very large oligomers (Figure
8 1). They frequently occur naturally in glycosylated forms, which make them more water-soluble
9 although the higher molecular weight oligomers are more insoluble (Bravo, 1998). Phenolic
10 compounds are abundant in highly colored berry fruits, and due to their popularity and high
11 consumption, these berries serve as one of our most important dietary sources of phenolics
12 (Wiliner, Pirovani, & Guemes, 2003; Kahkonen, Hopia, & Heinonen, 2001). Berry fruits are
13 reported to contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic
14 acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins) and
15 hydrolyzable tannins (Machiex, Fleuriet & Billot, 1990).

16 Strawberry (*Fragaria x ananassa* Duch.) fruits are very popular among berries and are
17 widely consumed in fresh forms and as food-products such as preserves, jams, yogurts and ice
18 creams. Strawberry fruits are reported to have antioxidant, anticancer, anti-inflammatory and
19 anti-neurodegenerative biological properties (reviewed in Hannum, 2004). Because of the
20 reported biological properties associated with strawberry fruits, the identification of their
21 phenolic compounds is necessary for the evaluation of strawberry consumption on human health.
22 The nature, size, structure, solubility, degree and position of glycosylation, and conjugation of
23 phenolics with other compounds can influence their bioavailability, absorption, distribution,

1 metabolism and excretion in humans (Aherne, & O'Brien, 2002; Hollman, 2001). However, it is
2 also necessary to identify strawberry phenolics in the different forms in which the fruit has been
3 studied, namely, as freeze-dried whole fruits (Chung, Lee & Sung, 2002; Cao, Russel, Lischner
4 & Prior, 1998; Carlton, et al, 2001; Stoner, Kresty, Carlton, Siglin & Morse, 1999; Joseph, et al,
5 1998), and extract forms (Seeram, Momin, Bourquin, & Nair, 2001; Xue et al, 2001; Meyers,
6 Watkins, Pritts, & Liu, 2003). Due to the lack of standard methods for sample preparation,
7 extraction and analyses, there is no general consensus on a standard protocol for quantitation of
8 phenolic compounds in fruits and other foods (Naczki, & Shahidi, 2004). Quantitation of fruit
9 phenolics is also complicated by factors such as differences in fruit cultivars, growing
10 conditions, degree of ripeness, handling after harvest, etc. (Bravo, 1998). Our study is focused
11 on the identification and not quantitation of phenolic compounds in strawberry fruits as freeze-
12 dried whole strawberry fruit powder (SFP) and strawberry fruit extracts (SFE).

13 Many previous methods to identify strawberry fruit phenolics were optimized for
14 particular groups of compounds such as anthocyanins (Lopes-da-Silva, Pascual-Teresa, Rivas-
15 Gonzalo, & Santos-Buelga, 2002) or ellagitannin-based compounds (Hakkinen, Karenlampi,
16 Mykkanen, Heinonen, & Torronen, 2000; Wiliner, Pirovani, & Guemes, 2003). In addition,
17 many previous methods to identify strawberry fruit phenolics were based on spectrophotometry
18 (Meyers, Watkins, Pritts, & Liu, 2003) or high performance liquid chromatography with UV
19 detection (HPLC-UV) [also commonly referred to as photodiode array detection (HPLC-PDA)
20 or diode array detection (HPLC-DAD)] (Gil, Holcroft, & Kader, 1997; Kosar, Kafkas, Paydas,
21 & Baser, 2004; Wang & Zheng, 2001). However, HPLC-UV has its limitations since it relies on
22 the comparison of characteristic absorbance wavelength spectra, wavelength maxima (λ_{max}),
23 and chromatographic retention times (t_R) with authentic standards. On the other hand, the use of

1 HPLC with mass spectrometry (HPLC-MS) detection provides useful structural information and
2 allows for tentative compound identification when standard reference compounds are unavailable
3 and when peaks have similar t_R and similar UV-absorption spectra (Hakkinen, Karenlampi,
4 Heinonen, Mykkanen, & Torronen, 1999; Lopes-da-Silva, Pascual-Teresa, Rivas-Gonzalo, &
5 Santos-Buelga, 2002; Maata-Riihinen, Kamal-Eldin, & Torronen, 2004). In addition, tandem
6 mass spectrometric (MS^n) techniques are useful for distinguishing compounds with identical
7 molecular weights (Mullen, Yokota, Lean, & Crozier, 2003).

8 The objective of our study was to identify the phenolic compounds and generate
9 characteristic chromatographic 'fingerprints' of SFP and SFE by liquid chromatography
10 electrospray ionization mass spectrometry (LC-ESI-MS) methods. We also report on the use of
11 LC- MS^n methods to differentiate between the sugar conjugates of quercetin and ellagic acid (EA)
12 present in strawberries since both aglycons have identical molecular weights (MW 302 g/mol).
13 Our study provides useful information required for the generation of standardized strawberry
14 materials for *in vitro* and *in vivo* studies and for the authentication of strawberry-based food
15 products.

17 2. Experimental

19 2.1.1. Reagents

20 All solvents were HPLC grade and purchased from Fisher Scientific Co. (Tustin, CA).
21 EA, catechin, epicatechin and quercetin standards were purchased from Sigma Aldrich Co. (St.
22 Louis, MO). Pelargonidin and cyanidin aglycons and their respective 3-glucosides were
23 purchased from Chromadex Inc. (Santa Ana, CA).

1 2.1.2. *Strawberry Fruit Powder (SFP) and Strawberry Fruit Extract (SFE)*

2 Fresh strawberry fruits and freeze-dried whole strawberry fruit powder (SFP) were
3 provided by the California Strawberry Commission (Watsonville, CA). Strawberry fruits (975 g)
4 were separately blended (Waring Blender, New Hartford, Conn., USA) with either methanol
5 (0.1% HCl) or acetone: water (7:3, v/v) and then centrifuged. The supernatant liquids were
6 concentrated *in vacuo* (Buchi Rotavap) at low temperature (37 °C) to yield methanol and
7 aqueous acetone strawberry fruit extracts (SFE), respectively. The SFE or SFP (~1mg/mL) were
8 dissolved by sonication in water: acidic methanol (1:1, v/v), and injected directly for HPLC-MS
9 analyses.

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11 2.1.3. *Liquid chromatography (LC) diode array detection (DAD) and electrospray ionization*

12 *mass spectrometry (ESI-MS) methods*

13 Samples were analyzed on a Surveyor HPLC system equipped with a diode array
14 absorbance detector (DAD), scanning from 250 to 600 nm, and an autosampler cooled to 4 °C
15 (Thermo Finnigan, San Jose, USA). A Symmetry C-18 column, 250 x 4.6 mm, i.d. 5 µm
16 (Waters, MA, USA), was used and solvent elution consisted of a gradient system over 70 min of
17 acetonitrile (ACN) and H₂O (1% formic acid) at a flow rate of 1 ml/min. The linear gradient
18 system consisted of 10% ACN in H₂O (1% formic acid) for 10 min to eventually 20% ACN in
19 H₂O (1% formic acid) at 70 min. The column was maintained at 25 °C. After passing through
20 the flow cell of the DAD, the column eluate was split and 0.2 ml/ min was directed to a LCQ
21 Advantage ion trap mass spectrometer fitted with an electrospray (ESI) interface. Analyses
22 utilized the positive ion mode ($m/z M+H^+$) for detection of anthocyanins and negative ion mode
23 ($m/z M-H^+$) for all other compounds. Preliminary analyses were carried out using full scan, data

1 dependent MS/MS scanning from m/z 250–2000. The capillary temperature was 275 °C, sheath
2 gas and auxiliary gas were 45 and 0 units/min respectively, and source voltage was 4 kV. MSⁿ
3 fragmentation was carried out with 50% energy. Zoom scan analyses were carried out to
4 determine the charge state of some of the ellagitannin-based compounds. Identities of the
5 compounds were obtained by matching their molecular ions (m/z) obtained by LC-ESI-MS and
6 LC-ESI-MSⁿ with literature data (Lopes-da-Silva, Pascual-Teresa, Rivas-Gonzalo, & Santos-
7 Buelga, 2002; Mullen, Yokota, Lean, & Crozier, 2003; Maata-Riihinen, Kamal-Eldin, &
8 Torronen, 2004).

10 3. Results and discussion

12 HPLC-DAD-ESI-MS methods were used to analyze SFP and SFE. Common solvents
13 used for extraction of phenolic compounds from foods include water, methanol, aqueous
14 acetone, ethanol and ethyl acetate (Naczki, & Shahidi, 2004). Our preliminary extractions with
15 different solvents showed that the methanol (0.1% HCl) and aqueous acetone extracts were
16 similar and yielded the most peaks in the HPLC-DAD chromatogram when monitored at 280,
17 360 and 520 nm, absorbance wavelengths typical of phenolics including hydroxycinnamic acids,
18 flavonols, flavanols and anthocyanins (Figure 1). However, because acidic methanol is also the
19 widely accepted solvent of choice for the extraction of anthocyanins (Naczki, & Shahidi, 2004),
20 the SFE discussed in our studies was generated using this solvent.

21 Figs. 2A-C show the HPLC-DAD profiles of SFP at 280, 360 and 520 nm, respectively.
22 Figs. 3A-C show the HPLC-DAD profiles of SFE at 280, 360 and 520 nm, respectively. Table 1
23 shows the identification of the phenolic compounds present in strawberry fruits labeled as peaks

1 1-22 following the elution orders in the HPLC-DAD chromatograms. Whenever available,
2 reference standards of phenolics (see Experimental Section 2.1.1), were used to substantiate the
3 identification of peaks in the SFP and SFE. Tentative identities of phenolics which were not
4 available as standard reference materials, were obtained by matching their molecular ions
5 ($M+H^+$, for anthocyanins) or ($M-H^+$, for other phenolics) obtained by LC-ESI-MS and LC-MSⁿ
6 methods with the theoretical molecular weights from literature data.

7 LC-MSⁿ methods were also used to distinguish between conjugates of quercetin and EA
8 since their aglycons produce identical molecular ions on fragmentation ($M-H^+$, m/z 301)
9 (Mullen, Yokota, Lean, & Crozier, 2003). On MS/MS analyses, the quercetin m/z 301 ion
10 further fragments to form characteristic m/z 179 and 151 ions (Fig. 4A) whereas the equivalent
11 EA m/z 301 ion yields ions at m/z 257 and 229 (Fig. 4B). The use of LC-MSⁿ methods is
12 therefore useful to differentiate between EA and quercetin aglycons that are ubiquitous to berry
13 fruits.

14 The basis for identification of the phenolic compounds grouped as hydrolyzable tannins
15 [ellagitannins (ETs), gallotannins (GTs), and EA], anthocyanins, flavonols, hydroxycinnamic
16 acid derivatives and their esters, and flavanols (including catechin) is described below.

17 ***Hydrolyzable tannins (ETs, GTs and EA).*** ETs are esters of hexahydroxydiphenic acid
18 (HHDP: 6,6'-dicarbonyl-2,2',3,3',4,4'-hexahydroxybiphenyl moiety) and a polyol usually
19 glucose or quinic acid (Quideau, & Feldman, 1996). A key feature of ETs is their ability to
20 release the bislactone, EA, which is formed from the hydrolytic release of HHDP esters groups,
21 which undergo rapid, facile and unavoidable lactonization. GTs can also be transformed to ETs
22 by oxidative C-C coupling between spatially adjacent galloyl groups to form HHDP groups. ETs
23 are known to be abundant in berry fruits and after hydrolytic conversion, are commonly detected

1 and quantified in the form of EA (Amakura, Okada, Tsuji, & Tonogai, 2000). Hence EA may be
2 considered as a chemical marker compound for hydrolyzable tannins. We have also recently
3 reported that EA may be considered as a biomarker of ETs in human bioavailability studies
4 (Seeram, Lee, & Heber, 2004).

5 Peak 9 was identified as a galloyl-bis-HHDP-glucose molecule (MS $m/z = 935.0$; MSⁿ
6 $m/z = 898, 633, 463, 301, 279, 251$) due to its key fragments in MSⁿ analyses at m/z 633 and 301
7 (Maata-Riihinen, Kamal-Eldin, & Torronen, 2004). In addition, the m/z 257 and 229 ions in the
8 MSⁿ analysis showed that an EA moiety, and not quercetin, was associated with this molecule
9 (Fig. 4B). Galloyl-bis-HHDP-glucose (Figure 1) is a basic unit of many ETs, for example,
10 sanguin H-6 and lambertianin C contain 2 and 3 units, respectively (Maata-Riihinen, Kamal-
11 Eldin, & Torronen, 2004).

12 Similarly, peaks 2, 3a, 4, 13, 14, 17 and 19 were also ascertained as being EA-based by
13 MSⁿ analyses. Peak 14 was identified as free EA from its HPLC-DAD and LC-MS data and by
14 co-elution and comparison of its t_R with a reference standard of EA. Peak 17 was identified as
15 sanguin H-6 (MS $m/z = 1869$; MSⁿ $m/z = 1567, 1265, 935, 633, 301, 257, 229$) (Figure 1) by
16 comparison of its LC-MSⁿ data with literature reports (Mullen, Yokota, Lean, & Crozier, 2003;
17 Maata-Riihinen, Kamal-Eldin, & Torronen, 2004).

18 A key tool used to aid in the identity of the ET-based compounds was the use of high-
19 resolution 'zoom scan' analyses. This technique improves resolution of the ¹²C/¹³C isotopes of
20 compounds, allowing their charge state to be determined and hence facilitating the correct
21 determination of their molecular weights (MW) (Mullen, Yokota, Lean, & Crozier, 2003).
22 Therefore, peak 17 had a (M-H⁺) ion at m/z 934, which was shown to be doubly charged by
23 zoom-scan analysis (Fig. 5A), giving MW 1870 for this compound. However, zoom scan

1 analysis of peak 9 showed a singly charged ion at m/z 935, giving MW 936, and confirming the
2 presence of a free molecule of galloyl-bis-HHDP-glucose (Fig. 5B). Similarly, peaks 2 and 3a
3 (both MS m/z = 783; MSⁿ m/z = 481, 301, 257, 229) were shown to be a singly charged ions by
4 zoom-scan analysis, giving a MW of 784 for these compounds. Peaks 2 and 3a were tentatively
5 identified as isomeric forms of an EA-based compound.

6 Peak 4 (MS m/z = 639; MSⁿ m/z = 463, 301, 257, 229), was also tentatively assigned as
7 an EA-based compound based on its LC-MSⁿ data. Peaks 13 and 19 were identified as methyl-
8 EA-pentose conjugates (MS m/z = 447; MSⁿ m/z = 315), as previously reported (Mullen,
9 Yokota, Lean, & Crozier, 2003). The significantly different t_R of these compounds in the HPLC-
10 DAD trace may possibly be attributed to isomeric forms due to differences in the point of
11 attachment of the methyl and pentose substituents.

12 ***Anthocyanins.*** Anthocyanins are the glycosides of anthocyanidins responsible for the
13 attractive colors of fruits and vegetables and have a characteristic absorption wavelength in their
14 HPLC-DAD of approximately 500-530 nm. Anthocyanins are typically observed in LC-ESI-MS
15 analyses in the positive mode ($M+H^+$). Strawberry fruit anthocyanins are reported to be based on
16 pelargonidin (Plg) and cyanidin (Cy) aglycons (Figure 1) (Lopes-da-Silva, Pascual-Teresa,
17 Rivas-Gonzalo, & Santos-Buelga, 2002). Strawberry anthocyanins were identified from their
18 HPLC-DAD chromatograms at 520 nm (Fig. 2C for SFP and Fig. 3C for SFE), by comparison
19 with reference standards when available, and from their LC-MSⁿ data. Peaks 1, 3, 5 and 6a were
20 identified as Plg-diglucoside (MS m/z = 595; MS/MS m/z = 433, 271); Cy-glucoside (MS m/z =
21 449; MSⁿ m/z = 287); Plg-glucoside (MS m/z = 433; MSⁿ m/z = 271); and Plg-rutinoside (MS
22 m/z = 579; MSⁿ m/z = 433, 271), respectively. Peak 1 was confirmed as Plg-diglucoside and not
23 Cy-rutinoside (both m/z 595) by its MS/MS data which showed the fragment for the Plg aglycon

1 at m/z 271. The MS/MS analyses of anthocyanins containing the Cy aglycon would give a
2 characteristic fragment at m/z 287. The identities of these anthocyanins in strawberries are in
3 agreement with the previous literature report (Lopes-da-Silva, Pascual-Teresa, Rivas-Gonzalo, &
4 Santos-Buelga, 2002).

5 **Flavonol glycosides.** Peaks were identified as flavonol glycosides based on their HPLC-
6 DAD and LC-MSⁿ data. Peaks 12, 15 and 16 were identified as quercetin-rutinoside (MS m/z =
7 609; MSⁿ m/z = 301,179, 151), quercetin-glucoside (MS m/z = 463; MSⁿ m/z = 301,179, 151),
8 and quercetin-glucuronide (MS m/z = 477; MSⁿ m/z = 301,179, 151), respectively. The m/z 179
9 and 151 ions in the MSⁿ analysis showed that a quercetin aglycon, and not EA, was associated
10 with these compounds (Fig. 4A).

11 Peaks 20 and 21 were identified as isomers of kaempferol-glucuronide (MS m/z = 461;
12 MSⁿ m/z = 285) and (MS m/z = 461; MSⁿ m/z = 285, 179, 161), respectively (Maata-Riihinen,
13 Kamal-Eldin, & Torronen, 2004). The occurrence of the kaempferol aglycon in berry fruits is
14 uncommon but reported to be present in strawberries, artichoke, and gooseberries (Hakkinen,
15 Karenlampi, Heinonen, Mykkanen, & Torronen, 1999; Maata-Riihinen, Kamal-Eldin, &
16 Torronen, 2004).

17 There have also been other reports about the occurrence of flavonols as their
18 glucuronides in berry fruits. Quercetin-glucuronide has been reported in raspberries and
19 strawberries (Ryan, & Coffin, 1971; Maata-Riihinen, Kamal-Eldin, & Torronen, 2004), although
20 Maata-Riihinen et al (2004), only detected kaempferol-glucuronide in strawberries. The
21 pharmacokinetics of flavanols in humans is reported to differ significantly between food sources
22 depending on the type of glycosides that they contain (Manach, & Donovan, 2004). Hence, the
23 occurrence of flavanols as their glucuronides in these berry fruits is interesting since many

1 studies continue to probe the mechanisms of absorption, metabolism, biotransformation, and
2 excretion of these dietary phenolics in humans. Flavanols are usually found in foods as their
3 glycosides and are detected in biological fluids as their glucuronidated, as well as sulphated and
4 methylated forms (Manach, & Donovan, 2004). After ingestion, whether these molecules are
5 transformed by gut bacteria enzymatic and/or physiological pH action from glucosides to
6 glucuronides, or de-glycosylated then glucuronidated, or in the case of ingestion as naturally
7 occurring glucuronide forms, whether they are absorbed intact as these glucuronides, is worth
8 investigating.

9 **Flavanols.** Peak 5a was identified as catechin by comparison of its t_R (~ 9.2 min in the
10 280 nm chromatograms, Figs. 2A and 3A) with an authentic reference standard and by its LC-
11 MS data (MS m/z = 289; MS^n m/z = 245). Although catechin has the same molecular weight as
12 its isomer, epicatechin, we were able to differentiate between these two compounds due to their
13 different t_R in HPLC-DAD. A reference standard of epicatechin showed that this isomer eluted
14 at a later time than catechin using these HPLC conditions. Catechin has previously been
15 reported to be present in strawberries (Hannum, 2004).

16 **Hydroxycinnamic acid derivatives.** Peaks 6, 7 and 10 (all MS m/z = 325; MS^n m/z =
17 265, 187, 163, 145), were identified as isomeric forms of *p*-coumaroyl-glucoside (Maata-
18 Riihinen, Kamal-Eldin, & Torronen, 2004). Peak 8 (MS m/z = 355; MS^n m/z = 295, 217, 193,
19 175, 134) was identified as *p*-coumaroyl sugar ester, as previously reported (Maata-Riihinen,
20 Kamal-Eldin, & Torronen, 2004). Free hydroxycinnamic acids (Figure 1) are uncommon in
21 fruits (Machiex, Fleuriet & Billot, 1990) and are found more likely in their conjugated forms. It
22 has also been reported that *p*-coumaric acid is the common hydroxycinnamic acid aglycon found
23 in strawberries and raspberries (Maata-Riihinen, Kamal-Eldin, & Torronen, 2004).

1 **Unidentified compounds.** Peaks 11, 18 and the late-eluting peak 22, all for which LC-MS
2 data were obtained, remain without further identification.

3

4 **4. Conclusions**

5 In conclusion, we have identified the major phenolic compounds present in strawberry
6 fruits and established characteristic chromatographic profiles for whole freeze-dried strawberry
7 fruit powder (SFP) and strawberry fruit extract (SFE). Because it may be more meaningful to
8 evaluate the biological properties of strawberries as whole fruits rather than in extract forms,
9 these methods can aid in the standardization of strawberry materials for *in vitro* and *in vivo*
10 studies. The chromatographic fingerprinting of strawberries is also useful for the authentication
11 of strawberry-based food products. The information provided by our study will aid in the
12 evaluation of the importance of strawberry consumption on human health.

13

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18

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1 Figure 1: Examples of phenolic compounds found in strawberry fruits.

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3 Figure 2: HPLC-DAD profiles of freeze-dried whole strawberry fruit powder (SFP). A gradient
4 solvent elution system was used over 70 min with acetonitrile and 1% aqueous formic acid at a
5 flow rate of 1 ml/min. Detection is shown at (A) 280 nm, (B) 360 nm and (C) 520 nm. Peaks
6 were identified by comparison with reference standards when available or by the LC-ESI-MS
7 and LC-ESI-MSⁿ data. Numbering of peaks refers to their identification as shown in Table 1.

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9 Figure 3: HPLC-DAD profiles of strawberry fruit extract (SFE). A gradient solvent elution
10 system was used over 70 min with acetonitrile and 1% aqueous formic acid at a flow rate of 1
11 ml/min. Detection is shown at (A) 280 nm, (B) 360 nm and (C) 520 nm. Peaks were identified
12 by comparison with reference standards when available or by the LC-ESI-MS and LC-ESI-MSⁿ
13 data. Numbering of peaks refers to their identification as shown in Table 1.

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15 Figure 4: LC-MSⁿ trace by direct infusion of (A) quercetin (M-H m/z 301) showing its
16 characteristic fragment ions at m/z 179 and 151 and (B) ellagic acid (EA) (M-H m/z 301)
17 showing its characteristic fragment ions at m/z 257 and 229.

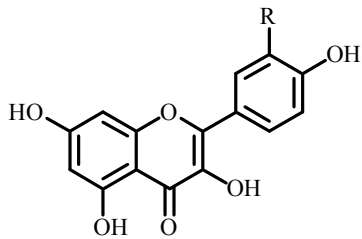
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19 Figure 5: Zoom scan mass spectra of (A) HPLC-DAD peak 17, showing the intervals between
20 the m/z 934 isotope peaks of 0.5 amu which demonstrates the doubly charged state of the ion
21 corresponding to MW 1870; (B) HPLC-DAD peak 9, showing the intervals between the m/z 935
22 isotope peaks of 1.0 amu which demonstrates the singly charged state of the ion corresponding to
23 MW 936.

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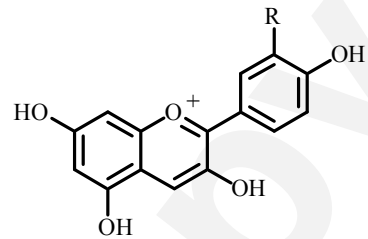
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Flavonols

Quercetin R = OH

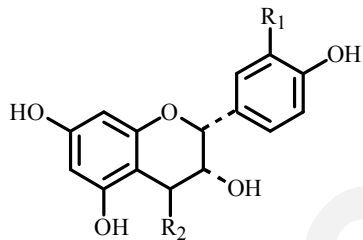
Kaempferol R = H



Anthocyanidins

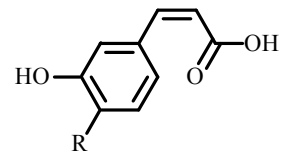
Cyanidin R = OH

Pelargonidin R = H



Flavanols

(+)-Catechin (2R, 3S) R₁ = OH, R₂ = H



Hydroxycinnamic acid

p-coumaric acid R = H

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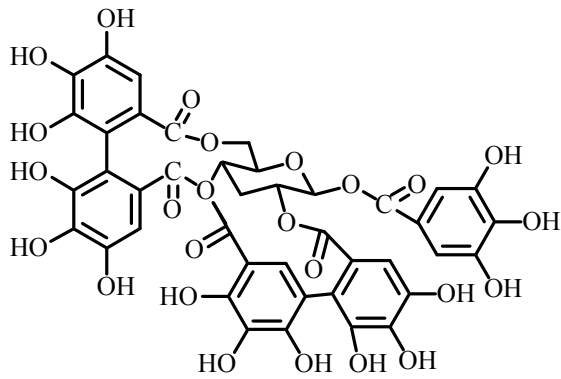
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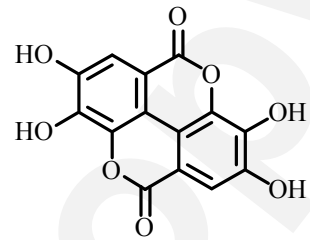
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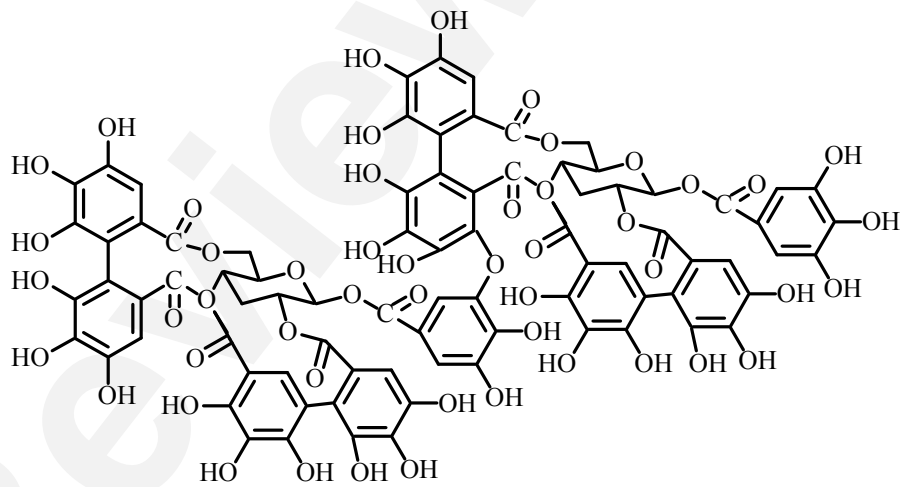
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Ellagitannin
Galloyl-bis-HHDP-glucoside



Ellagitannin
Ellagic acid



Ellagitannin
Sanguin H-6

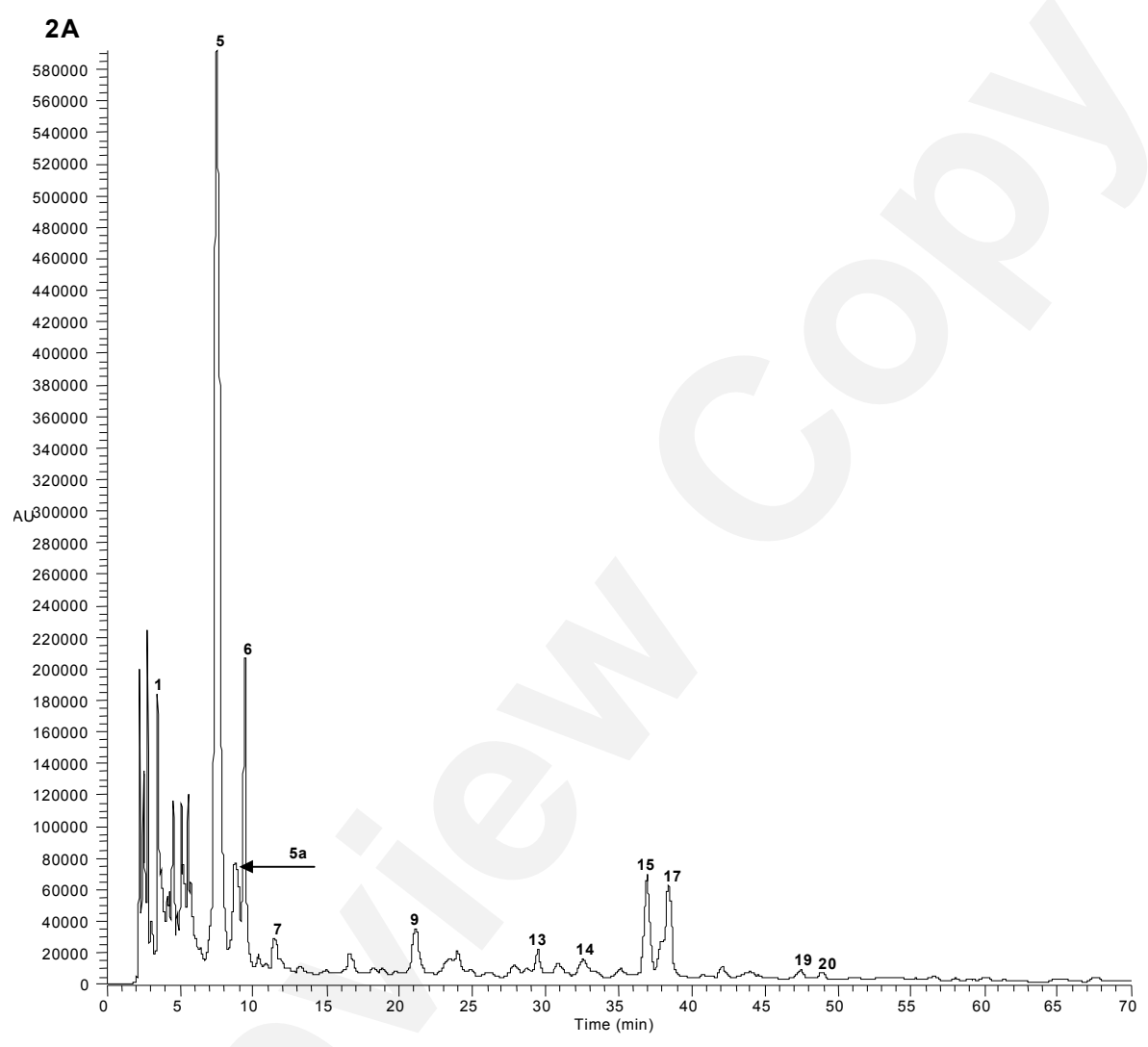
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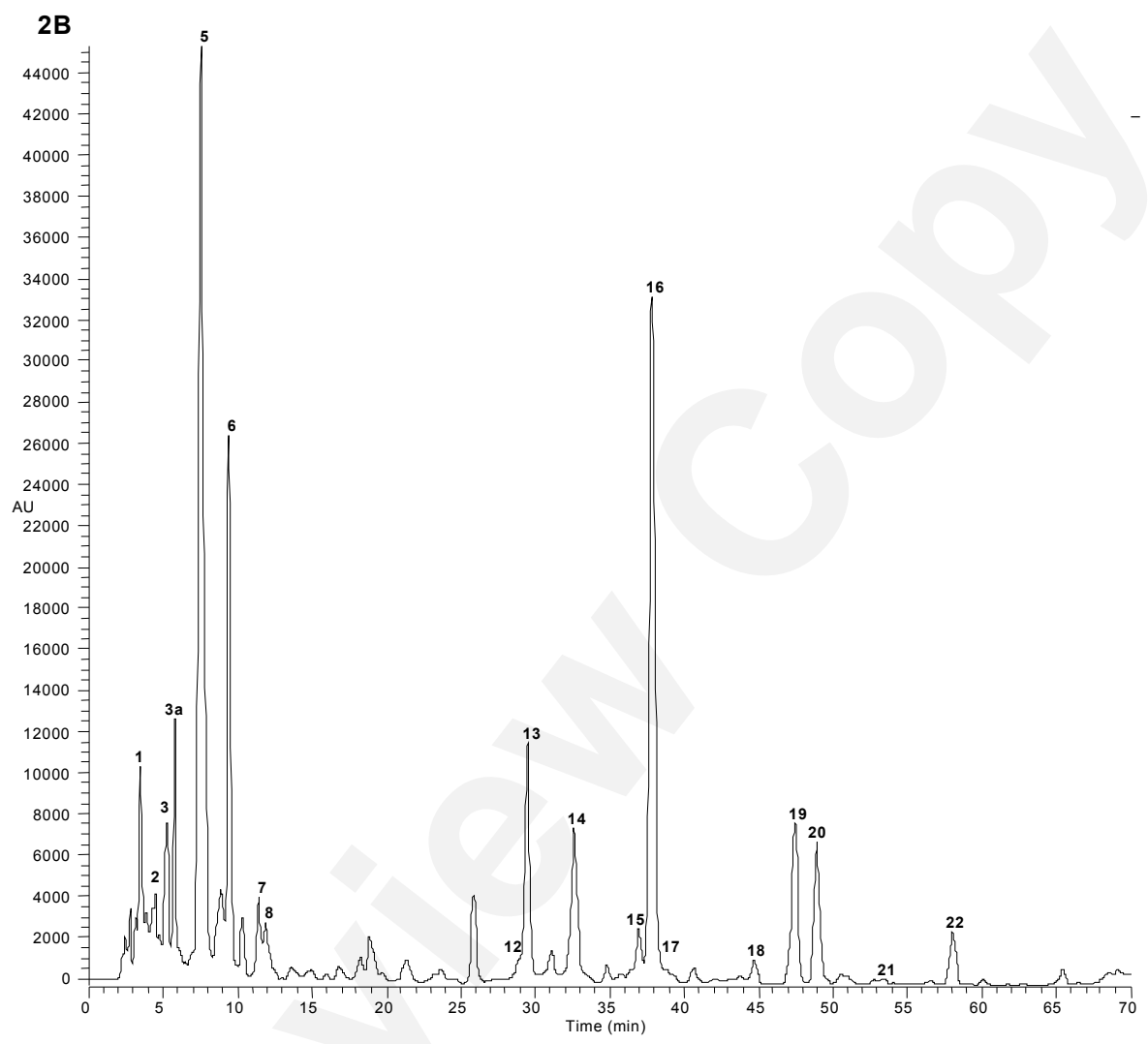
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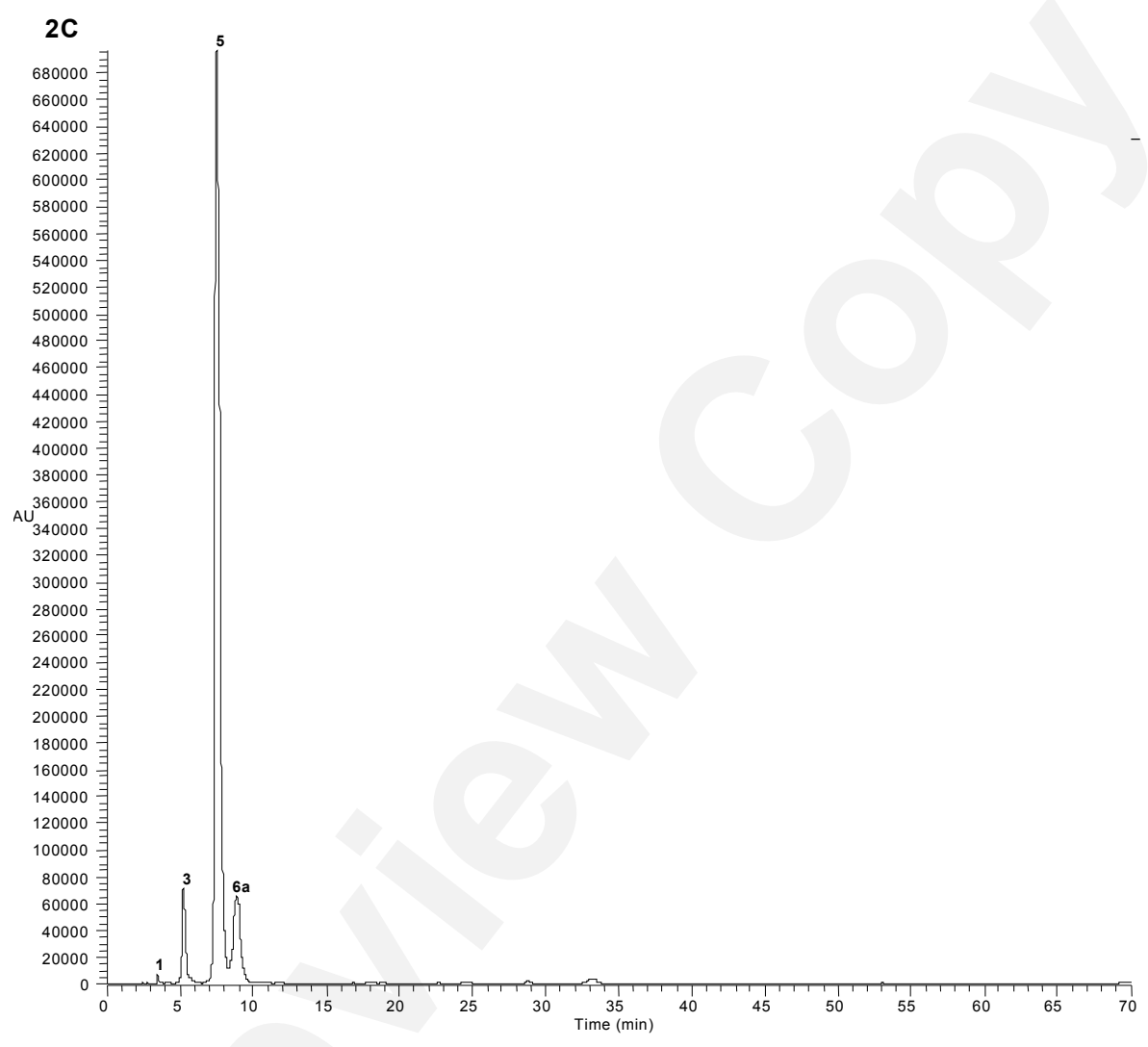
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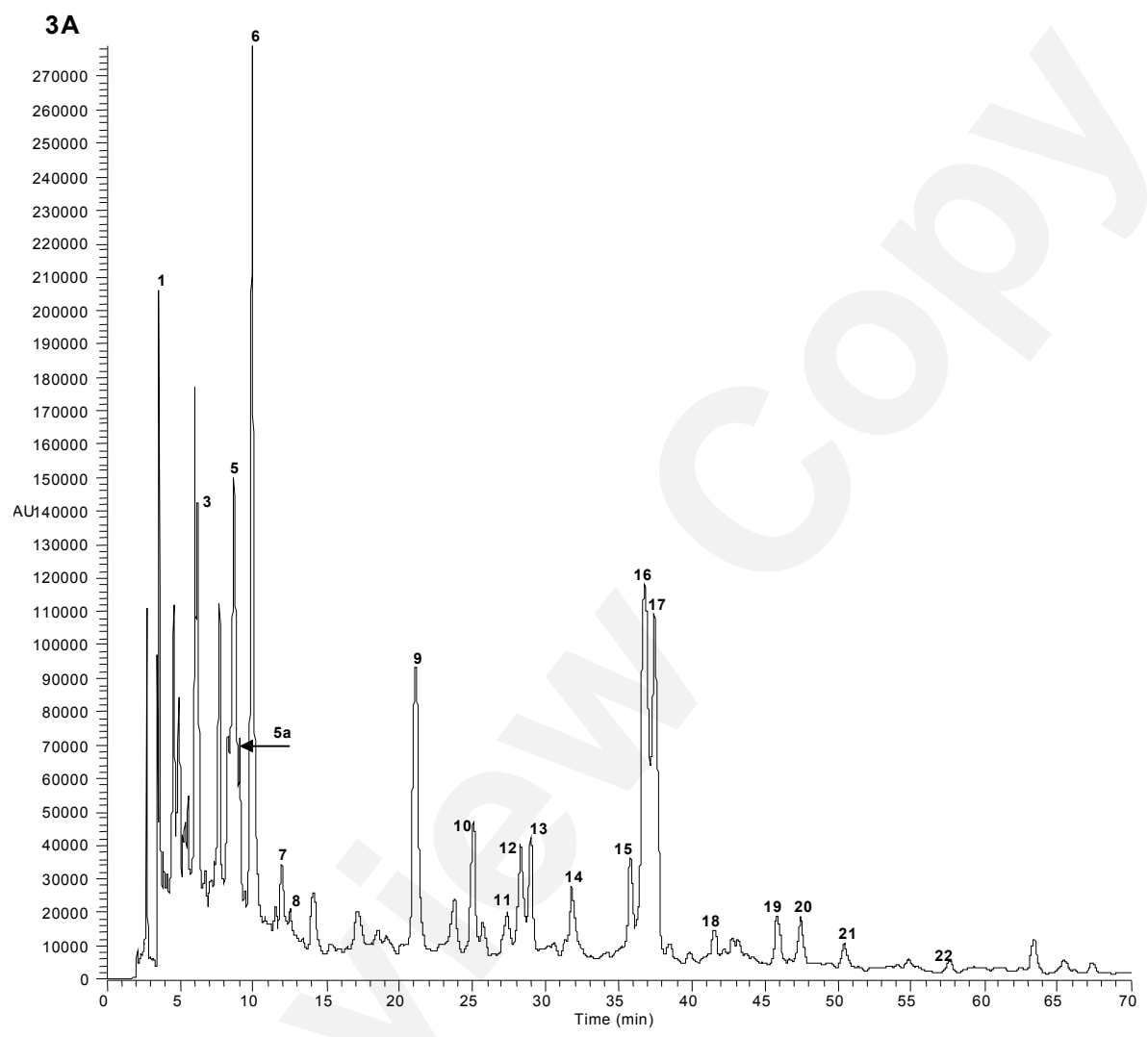
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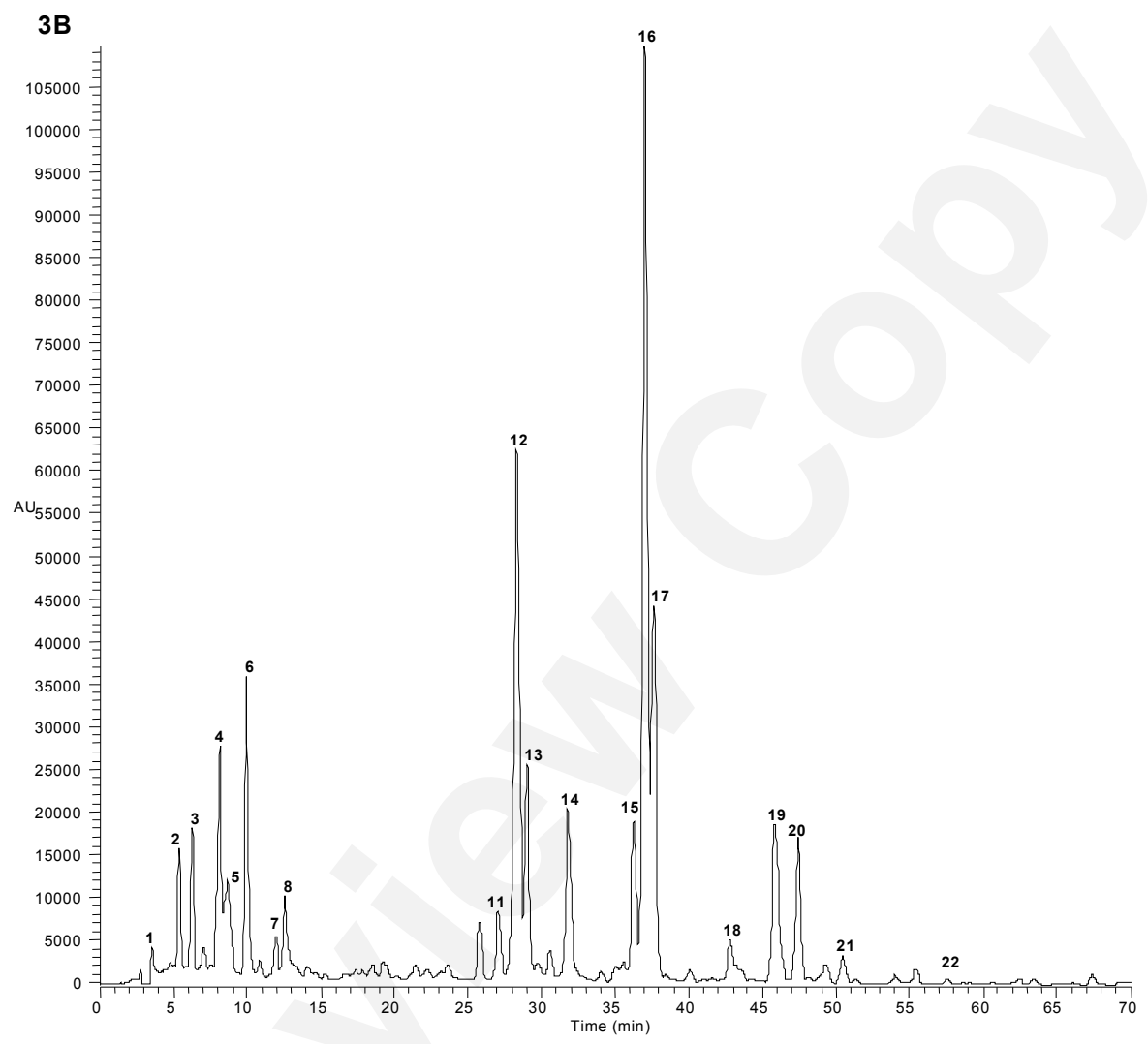


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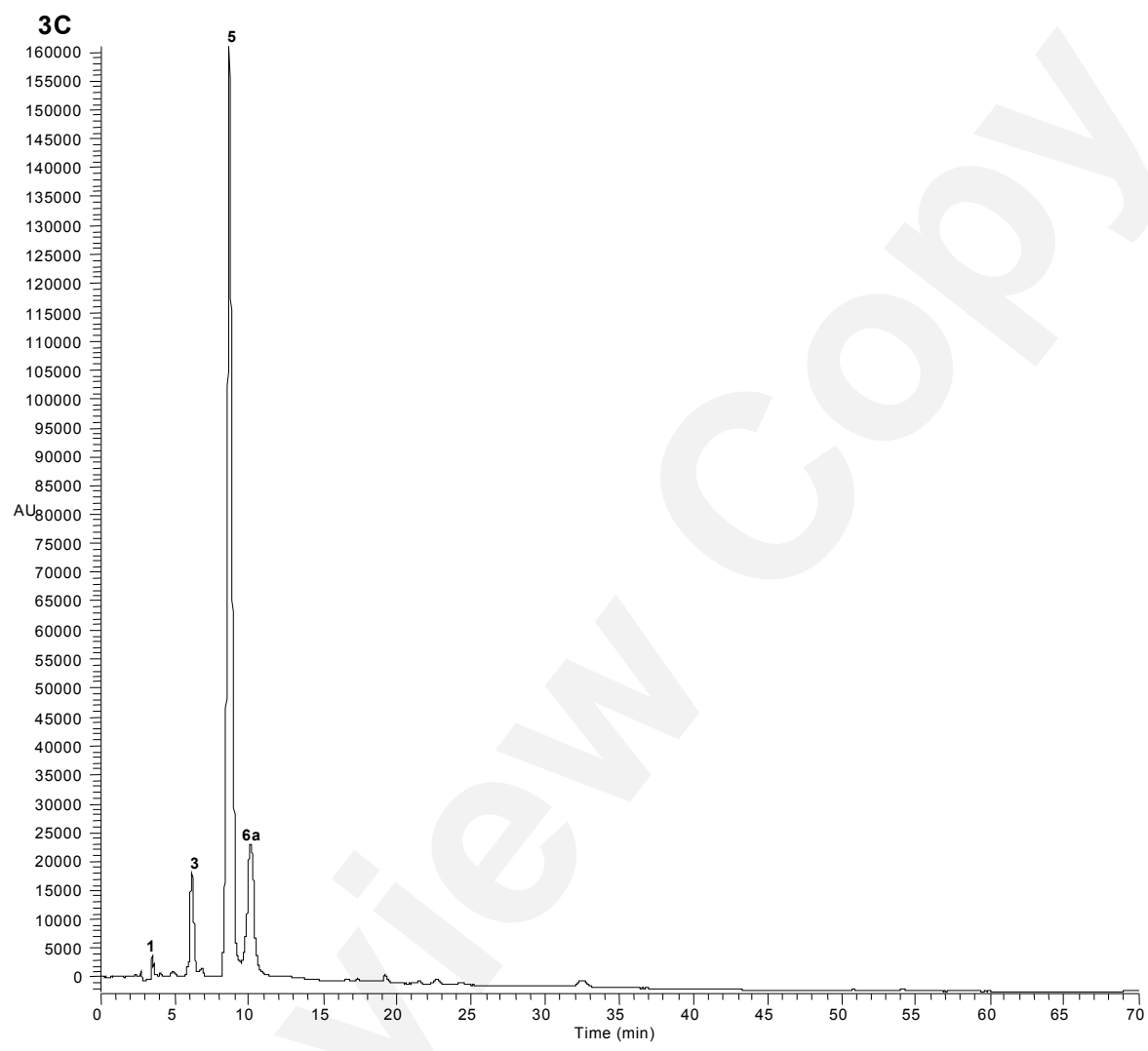


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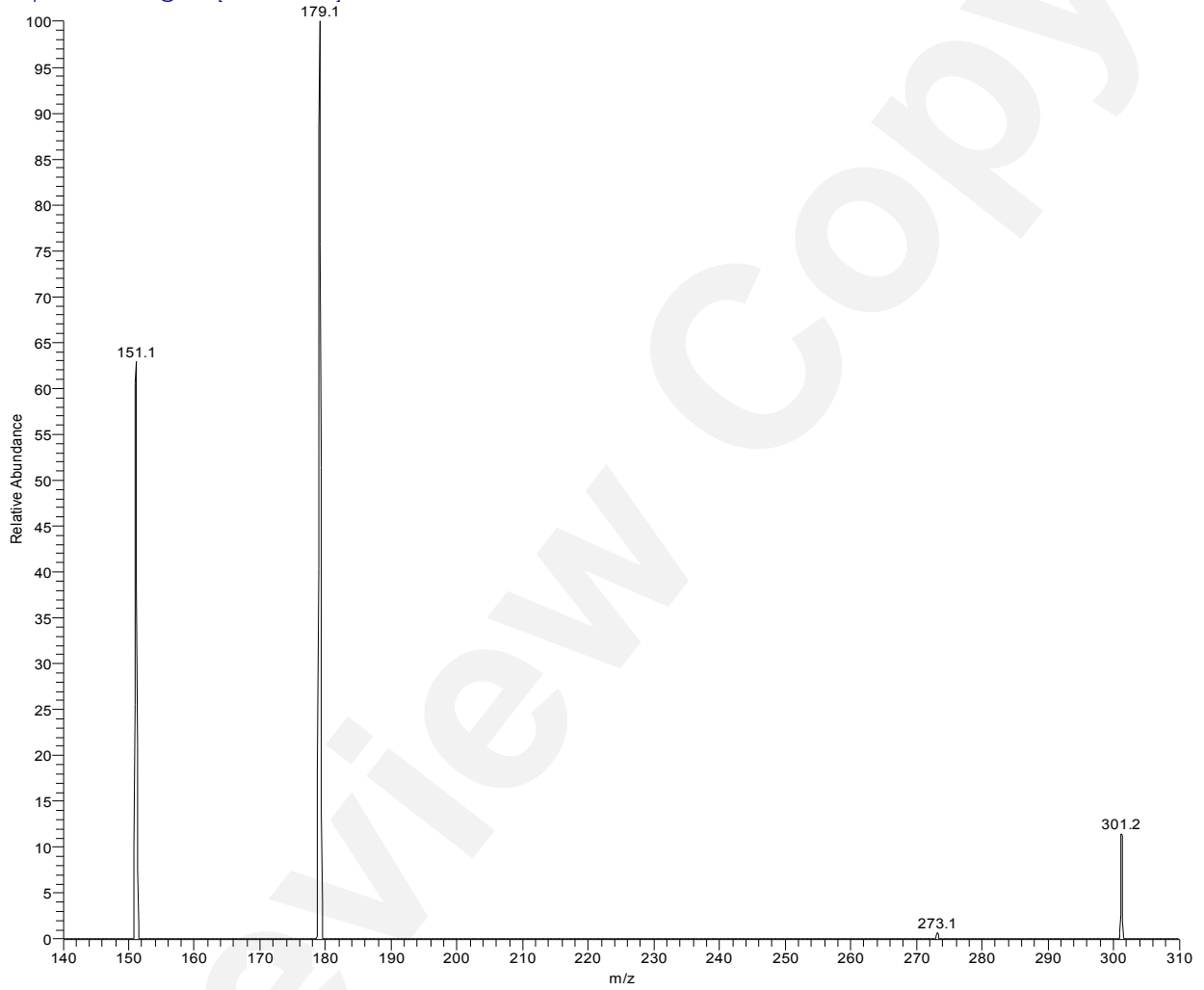
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4 **4A**

Quercetin_041105141917 #6-8 RT: 0.10-0.13 AV: 3 NL: 1.90E4
T: - p Full ms2 301.10@43.00 [120.00-1000.00]

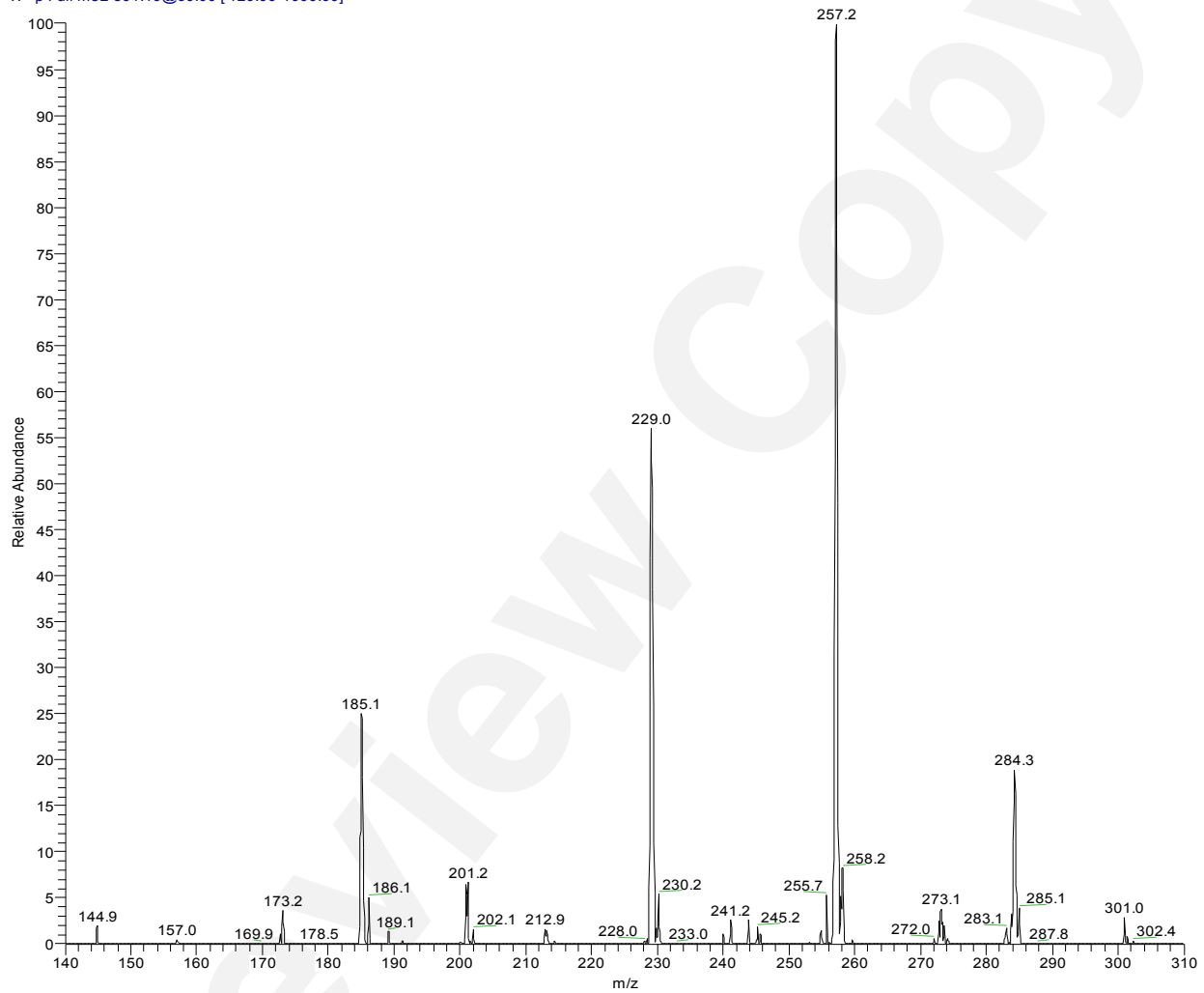


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4B

EA_041105141917 #35 RT: 0.58 AV: 1 NL: 6.77E3
T: - p Full ms2 301.10@60.00 [120.00-1000.00]



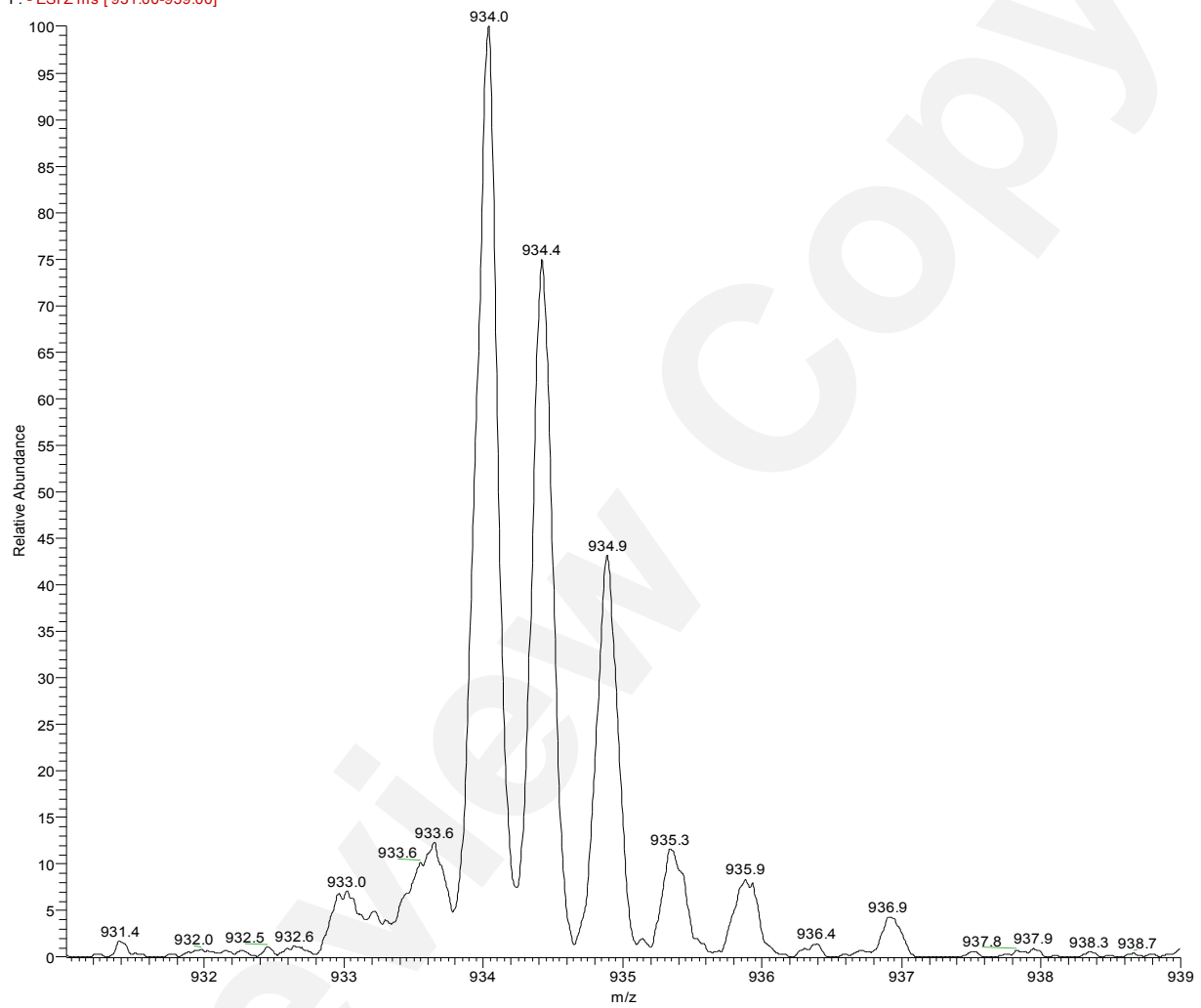
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4 **5A**

SB_ZS_102904 #2104-2150 RT: 42.09-42.84 AV: 9 SM: 15B NL: 6.71E3
F: -ESI Z ms [931.00-939.00]



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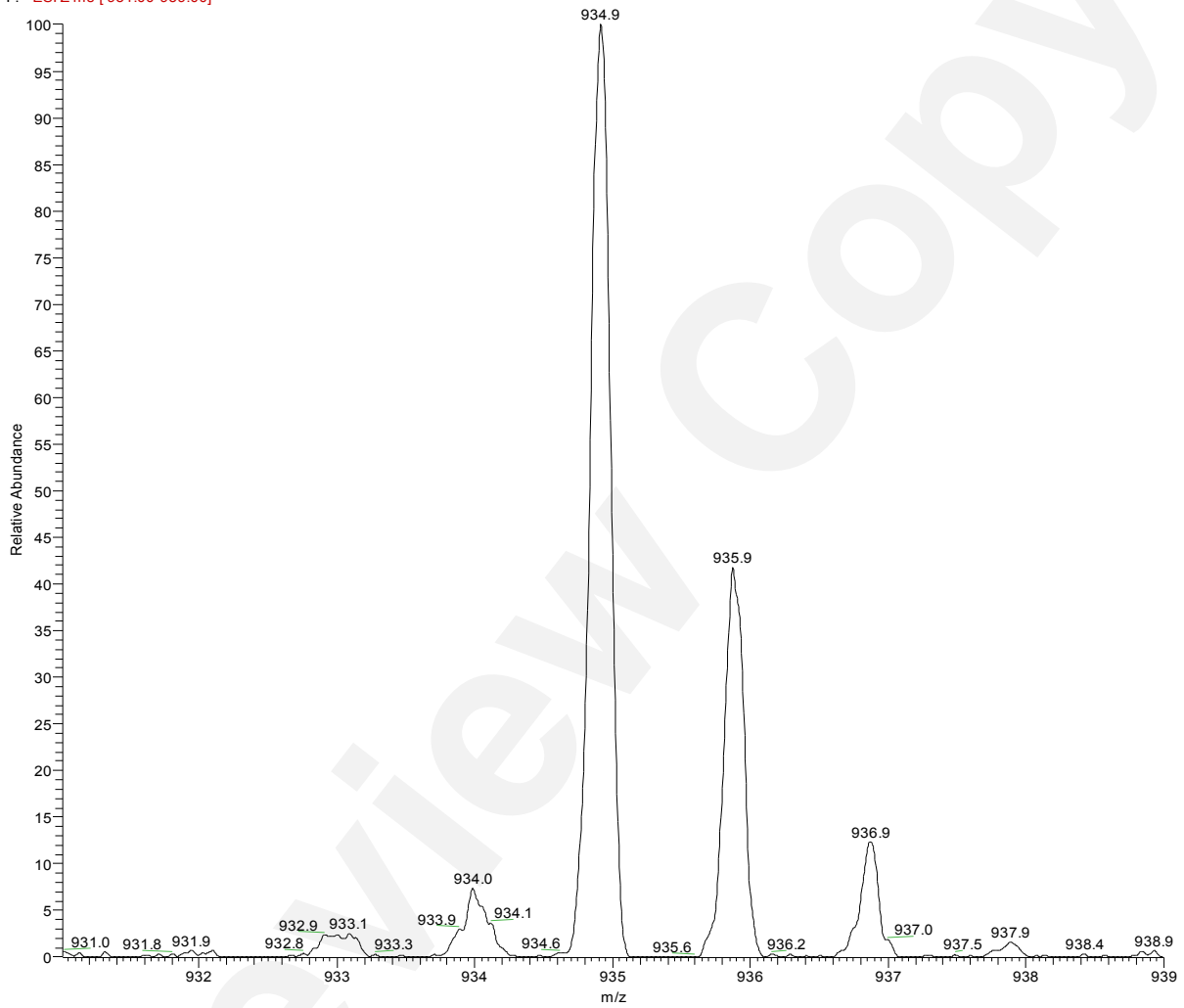
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4 **5B**

SB_ZS_102904 #1177-1218 RT: 24.62-25.37 AV: 9 SM: 15G NL: 1.86E4
F: -ESI Z ms [931.00-939.00]



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- 1 Table 1. Identification of phenolic compounds in strawberry fruits by using their HPLC-DAD, LC-MS and LC-MSⁿ data*.
- 2 Identification were aided by comparison with reference standards where available and by correlation with previous literature reports.

Peak #	t _R (min)	λ _{max} (nm)	MW	MS	Ion (+/-)	MS ⁿ	Tentative ID
1	3.20	275, 520	594	595	+	433, 271	Plg-diglucoside
2	5.37	235, 350	784	783	-	481, 301, 257, 229	GT/ET
3	6.16	275, 520	448	449	+	287	Cy-glucoside
3a	6.20	235, 350	784	783	-	481, 301, 257, 229	GT/ET
4	8.13	235, 350	640	639	-	463, 301, 257, 229	GT/ET
5	9.12	275, 520	432	433	+	271	Plg-glucoside
5a	9.20	280	290	289	-	245	Catechin
6	9.95	235, 310	326	325	-	265, 187, 163, 145	<i>p</i> -coumaroyl-glucoside
6a	10.13	310, 505	578	579	+	433, 271	Plg-rutinoside
7	11.95	235, 310	326	325	-	265, 187, 163, 145	<i>p</i> -coumaroyl-glucoside
8	12.55	235, 330	356	355	-	295, 217, 193, 175, 134	<i>p</i> -coumaroyl-ester
9	21.3	235, 250	936	935	-	898, 633, 463, 301, 279, 251	galloyl-HHDP-glucoside
10	25.10	240, 325	326	325	-	265, 187, 163, 145	<i>p</i> -coumaroyl-glucoside
11	27.07	265, 350	596	595	-	300, 271, 179	unknown
12	28.32	255, 355	610	609	-	301, 179, 151	Q-rutinoside
13	29.02	250, 370	448	447	-	315	methyl-EA-pentose
14	31.80	250, 370	302	301	-	257, 229	EA
15	36.27	285	464	463	-	301, 179, 151	Q-glucoside
16	37.03	265, 355	478	477	-	301, 179, 151	Q-glucuronide
17	37.63	260, 345	1870	1869	-	1567, 1265, 935, 633, 301, 257, 229	Sanguiin-H6
18	42.80	250	594	593	-	285	unknown
19	45.85	260, 345	448	447	-	315	methyl-EA-pentose
20	47.43	265, 345	462	461	-	285	K-glucuronide
21	50.47	325	462	461	-	285, 179, 161	K-glucuronide
22	55.43	265, 345	490	489	-	285	unknown

- 3 *Peak numbers and retention times (t_R) refer to HPLC chromatograms in Figs. 2 and 3. Peaks 3/3a; 5/5a and 6/6a co-elute. GT, gallotannin; ET,
- 4 ellagitannin; Q, quercetin; K, kaempferol; Plg, pelargonidin; Cy, cyanidin; EA, ellagic acid; HHDP, hexahydroxydiphenoyl
- 5