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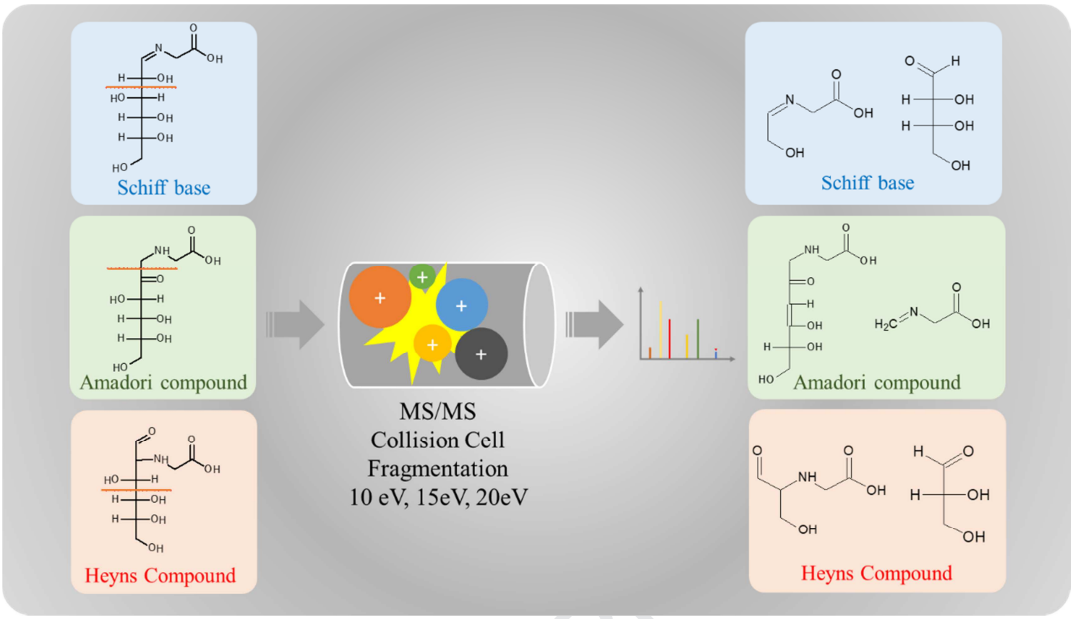

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Diagnostic MS/MS fragmentation patterns for the discrimination between Schiff bases and their Amadori or Heyns rearrangement products

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Highlights:

- The main diagnostic ion for the Schiff base during MS/MS fragmentation is generated through the retro-aldol degradation reaction yielding a diose attached to the amino acid moiety;
- The diagnostic ions of the Amadori compound result from a series of dehydrations of the intact molecule at low collision voltages, and a residual CH_2 group from the sugar attached to the amino acid moiety at the high voltage;
- The diagnostic ion of the Heyns compound forms through the retro-aldol degradation yielding a triose attached to the amino acid moiety;
- The MS/MS fragmentation patterns are consistent under both acidic and neutral conditions, but neutral condition provides more stability to the fragment ions.

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Abstract:

Schiff bases, the Amadori and Heyns rearrangement products are the most important isomeric intermediates involved in the early Maillard reaction; distinguishing between them by analytical mass spectroscopic techniques remains a challenge. Here we demonstrate that MS/MS fragmentation patterns can be used for the discrimination between glucose derived Schiff bases, Amadori, and Heyns compounds with glycine. An ESI-qTOF-MS system operated in the positive mode under both acidic and neutral conditions was employed to generate unique MS/MS fragmentation patterns of the molecules. Analysis of the MS data has indicated that acidic medium is suitable for generating characteristic and diagnostic ions. At high collision energy (20 eV), the spectrum of Schiff base was largely uninformative, whereas both Amadori and Heyns compounds undergo characteristic fragmentations with high diagnostic value. At low collision energy values (10eV), we observed formation of prominent diagnostic ions from the Schiff base precursor, as well as extensive dehydration reactions of all three molecules. Under acidic conditions, the diagnostic fragmentation pattern of the Amadori compound featured consecutive dehydration reactions. At higher values (20 eV) it underwent the α-fission at the carbonyl group and produced a prominent diagnostic ion $[AA+H+CH_2]^+$ at m/z 88. The Schiff base was found to preferentially undergo the retro-aldol degradation and produce diagnostic ions at m/z 118 $[AA+H+diose]^+$ and m/z 140 $[AA+Na+diose]^+$, together with their sugar complements at m/z 85 $[tetrose+H-2H_2O]^+$ and m/z 143 $[tetrose+Na]^+$. In the case of Heyns compound, several diagnostic ions were also detected, including the ions at m/z 154 $[M+H-2H_2O-C_2H_4O_2]^+$, m/z 170 $[AA+Na+triose]^+$ and m/z 142 $[AA+H+Furan]^+$.

Keywords: Diagnostic ions, Schiff base, Amadori compound, Heyns compound, tandem mass spectrometry (MS/MS), Collision induced fragmentations, Maillard reaction

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1. Introduction:

The Maillard reaction is a common term for a broad array of reactions that typically involve reducing sugars, such as glucose, and aliphatic amino groups of biological molecules. It has been the focus of great interest for researchers in areas as diverse as human pathology and food chemistry for more than 100 years.[1, 2] The complex reaction cascade is initiated by the formation of a Schiff base (or glycosylamine) between a reducing sugar and an amino acid. In presence of a nucleophilic catalyst, glycosylamine derived from an aldose can rearrange into a more stable 1-amino-1-deoxy-2-ketose; such reaction is termed the Amadori rearrangement. When glycosylamine is derived from a ketose sugar, it can rearrange into a 2-amino-2-deoxyaldose, or Heyns rearrangement product (HRP).[2] The breakdown of the Amadori or the Heyns rearrangement products ultimately yields a complex mixture of volatile carbocyclic and heterocyclic compounds, as well as characteristic yellow-brown oligomers and polymers.[3]

Although the initial stage of the Maillard reaction is well-documented, the analytical discrimination of the early intermediates, i.e. isomeric Schiff base and ARP (or HRP), remains a challenge. As a result, the critical role played by Schiff bases for initiating and propagating the Maillard reaction perhaps may have been undervalued. Besides rearranging into ARP and HRP, Schiff bases can undergo various transformations, such as the base-catalyzed transamination,[4-6] the intramolecular cyclization leading to decarboxylated isomeric imines via oxazolidin-5-one intermediate,[7, 8] the decarboxylative transamination,[9] the Namiki oxidative fragmentation pathway,[10, 11] and the acid-catalyzed Pictet-Spengler condensation pathway.[12, 13]

Structural identification of small organic molecules have been one of the cornerstone applications of mass spectrometry over the last decades, especially with the development of the electrospray ionization in combination with a collision cell (ESI-MS/MS).[14] It can also be

considered as one of the key analytical techniques that is capable of generating large datasets in the emerging "omics" fields. Recently, the Maillard reaction has also been investigated in this regard. [15, 16] As a complex reaction cascade, hundreds and thousands of ions were detected in various Maillard reaction mixtures represented simply by their m/z values where the same molecular weight or a given elemental composition could represent different isomeric structures. The main goal of this study was to develop MS/MS based tools to distinguish the isomeric intermediates from the Maillard reaction such as Amadori compounds and Schiff bases without lengthy separation and purification steps. The MS/MS fragmentation of Amadori compounds under protonated positive ion mode has been studied extensively and systematically by LC-ESI-MS/MS.[17-19] The potential of distinguishing between proline derived Amadori and Heyns compounds,[20] or distinguishing between hexose versus pentose-derived Amadori compounds by their MS/MS fragmentation patterns have been already reported. [17] However, to the best of our knowledge, there are no published studies on the MS/MS behaviour of the Schiff bases, nor on their chemical behaviour under different collision energies. To fill this knowledge gap, we ventured to identify specific and diagnostic collision-induced dissociations (CID) of glucose/glycine derived Schiff base and ARP and of fructose derived HRP by LC-ESI-MS/MS under acidic (formic acid) and ESI-MS/MS under neutral (sodium formate) conditions using variable collision energies.

2. Material and Methods

2.1 Materials:

All reagents and chemicals were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada) and used without further purification. Synthesis and characterization of *N*-carboxymethyl-D-glucosylamine (Schiff base), 1-carboxymethylamino-1-deoxy-D-fructose

(Amadori compound), 2-carboxymethylamino-2-deoxy-D-glucose (Heyns compound), 1-[(2'-carboxy)pyrrolidinyl]-1-deoxy-D-fructose (proline Amadori compound), and *N*-[(2'-carboxy)pyrrolidinyl]-D-glucosylamine (proline Schiff base) were performed according to published procedures. [21-23] See Table S1 for ^{13}C-NMR chemical shifts and the supplementary material for more details on their preparation.

2.2 ESI/MS/MS under formic acid condition

Samples were analyzed using an Agilent 1290 Infinity II LC system coupled to the 6545 qToF - MS (Agilent Technologies, Santa Clara, USA). The LC separation was conducted on a Poreshell120 EC-C18 analytical column (Agilent Technologies; 2.7 μm × 3 mm × 100 mm) connected with a Poreshell120 EC-C18 guard column (Agilent Technologies; 2.7 μm × 3 mm × 5 mm). The elution condition was at a flow rate of 0.4 ml/min with the mobile phase as the mixture of 0.1% (V/V) aqueous formic acid (40%) with methanol (60%). The injection volume was 1 μL, and the column temperature was set to 20 °C. The samples were analyzed in positive electrospray ionization mode using product ion (MS/MS) experiment. The drying gas temperature was at 275 °C with a flow of 10 mL/min, the sheath gas temperature was at 300 °C with a flow of 12 mL/min, the pressure on the nebulizer at 45 psi, the capillary voltage at 4500 V, the fragmentor voltage at 1000 V, the skimmer voltage at 50 V, and the nozzle voltage at 2000 V. The Tandem mass spectrometry (MS/MS) data was collected by scans between m/z 50 and 1000 at a scan rate of 3 spectra/s for four different collision energies (0 V, 10 V, 15 V, and 20 V) for the ions at $[M+H]^+$ 238. The data sets were processed with MassHunter Profinder B.08.00 software (Agilent Technologies).

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115 2.3 ESI/MS/MS under sodium formate condition

116 The diluted sample solutions (1 μL) in methanol/ water (10%/ 90%) were supplied to the source
117 directly via a syringe. The analysis was on a Bruker Maxis Impact quadrupole time of flight
118 mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in positive ion mode.
119 Instrument calibration was performed using sodium formate clusters. The electrospray interphase
120 settings were as follows: nebulizer pressure, 0.6 bar; drying gas, 4 L/min; temperature, 180 °C;
121 and capillary voltage, 4500 V. The scan range was from m/z 50 to 800. Tandem mass
122 spectrometry (MS/MS) was carried out in MRM mode using 10.0 eV collision energy for the
123 ions at $[M+Na]^+$ 260. The data were analyzed using Bruker Compass Data Analysis software,
124 version 4.2.

125

126 **3. Results and discussion**

127

128 In-source fragmentations are usually observed under ESI conditions depending on the structural
129 features of the analytes (e.g. presence of basic or acidic functional groups) and on the
130 environmental conditions (e.g. solvents or calibration compound used for a specific MS system);
131 all the three analytes exhibited minor in-source fragmentations mainly under acidic conditions
132 (Figures 1-3). Under acidic analytical conditions, samples were introduced as they were eluted
133 from a reverse-phase LC column. To facilitate protonation of the samples, water/methanol
134 mixture containing 0.1% formic acid was used as the mobile phase. Schiff base, ARP, and HRP
135 showed similar retention times (1.101, 1.070 and 1.080 min respectively) as shown in Figure S1.
136 The MS spectra of these peaks indicated the occurrence of acid catalyzed hydrolysis especially
137 for the Schiff base (see Table 1 Entry 1 & Figure 1) which was identified as the most labile

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molecule compared to the ARP and HRP generating free glycine and free sugar, the latter being the most abundant peak, while the intact protonated Schiff base represented only 11.2% of the total ion intensity. Furthermore, dehydrations were also observed for ARP and HRP, especially for the former. (Table 1 Entries 5, 9 & Figures 2 & 3) The environmental conditions could play a critical role in distinguishing the Schiff base from the ARP, since the former, as indicated, can undergo hydrolysis and/or rearrange under acidic conditions to ARP and consequently undergo acid catalyzed dehydrations as shown in Table 1 (Entries 2 & 3) and Figure 1. Because of this concern, the Schiff base, ARP and HRP were also analyzed under neutral conditions in sodium formate cluster calibrated system. In this setup, samples were introduced as dilute solutions in water/methanol to the source directly via a syringe, resulting in the formation of sodiated ions (Figure 4). The extra stability gained by the analytes under neutral conditions was reflected in the intensities of molecular ions generated at $[M+Na]^+$ = 260 as shown in Figure 4 (100% relative peak intensity in all cases). Some in-source fragmentation still observed for the Schiff base. (Table 1 Entry 13 & Figure 4) However, the overall stability was greatly enhanced relative to the acidic condition, which is the most adopted MS/MS analytical conditions from literature. In this study, MS/MS under both acidic and neutral condition were investigated, and the product ions were screened elementally and reported in Table 2, the relative abundances of the product ions and their proposed structure can be found in supplementary material (Tables S2 & S3).

3.1 Diagnostic MS/MS fragmentations under acidic conditions

Under acidic conditions, the MS/MS fragmentation pattern of ARP was in accordance with those reported in the literature,[17, 19] where series of dehydration reactions lead to the formation of characteristic $[M+H-n(18)]^+$ peaks in the MS/MS spectrum under low CID voltage (10eV), whereas high CID voltage (20eV) resulted in α-fission between the C-1 and C-2 of sugar

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backbone carbon atoms.[18, 20] This fragmentation pattern, where dehydrated ion series has been observed as the most abundant peaks in the spectrum, is typical of most of the Amadori compounds of eighteen amino acids with glucose studied in the literature.[19] Under all investigated conditions reported in the literature, the cleavage of the sugar moiety during MS/MS fragmentations of Amadori compounds was only observed between C1-C2 or C5-C6 of the sugar, leaving a $-CH_2$ unit attached to an amino acid or leading to a loss of formaldehyde [$-H_2CO$] from the terminal carbon of the hexose, consistent with data reported in Table 1 (Entries 6-8 & Figure 2). Briefly, under conditions of low collision energy, dehydrated products were the most abundant peaks with 100% relative intensity for $[M+H-H_2O]^+$ (m/z 220) or $[M+H-2H_2O]^+$ (m/z 202) at 10eV or 15eV respectively. At higher collision energy values (20 eV), the glycine ARP generated $[AA+H+CH_2]^+$ (m/z 88) through α-fission as the most abundant peak, as well as the complementary sugar portion at m/z 97 (proposed as a pyrylium ion). (Scheme 1)

Contrary to the ARP, the Schiff base showed a different behaviour under MS/MS fragmentation, especially under lower energy conditions (10 and 15eV) generating through C2-C3 retro-aldol cleavage a diagnostic ion at (m/z 118) incorporating intact amino acid and two carbon moiety from the sugar in addition to its complementary 4-carbon sugar fragment at m/z 85 (see Table 1 Entries 2, 3 & Scheme 1). Overall, the Schiff base of glycine and glucose appears to be less stable during MS/MS fragmentation compared to its Amadori counterpart. Even when submitted to low collision voltage (10 eV), the Schiff base generated the most abundant peak $[AA+H]^+$ at m/z 76 representing the free glycine released during the fragmentation process. It appears that the free glycine was released from doubly dehydrated Schiff base precursor at m/z 202 leaving behind the sugar residue at m/z 145 (see Table 2). At 15 eV, the spectrum does not differ much from the one operated at 10 eV. However, at 20 eV the ion count intensity was too low ($\times 10^2$) to

provide useful information, and most of the ions could not be identified elementally. (Figure 1) This property of Schiff bases can be exploited to detect their presence in mixtures containing various amounts of Amadori products. When the ion at m/z 238 $[M+H]^+$ in five different mixtures of glycine Schiff base and its ARP (molar ratios of 1:0, 1:2, 1:1, 2:1, 0:1) was fragmented using collision energy of 20 eV where Schiff bases no longer generate any fragments, the intensity of ions generated from the ARP predominates the spectrum and its diagnostic ion at m/z 88 increased proportionately to the increased content of ARP. On the other hand, the intensity of the diagnostic ion originating from the Schiff base at m/z 118 was only detected in trace abundances as expected (see Figure S4).

Although Heyns rearrangement product is structurally different from ARP, its MS/MS fragmentation pattern was partially similar to that of ARP and partially to that of the Schiff base (see Scheme 1). Its resemblance to the behaviour of ARP was manifested through the presence of dehydration peaks and its similarity to the behaviour of the Schiff base was indicated through detection of the ion at m/z 130 (Scheme 1). From the elemental composition of the ion at m/z 130 ($C_5H_8NO_3$) it can be concluded that it incorporates intact glycine with three carbon unit originating from the sugar. Such a structure can only be generated through retro-aldol cleavage of the Schiff base of fructose with glycine at C3-C4 as shown in Scheme 1. This implies that open form of Heyns product is prone to isomerization to produce fructose/glycine Schiff base adduct as shown in Scheme 1. This diagnostic ion is generated only under acidic conditions, although its precursor ions m/z 170 was also detected under neutral conditions. Furthermore, detection of the high intensity protonated ion at m/z 118 and the same ion at m/z 140 under sodiation with common molecular formula of $C_4H_8NO_3$ indicates the predominance of the open form of the Heyns product under MS/MS analytical conditions, since this ion can be generated

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only through C2-C3 retro-aldolization of the open form of the Heyns product. This is equivalent to the process observed in the case of Schiff base shown in Scheme 1. The diagnostic ion at m/z 130 was absent from both Schiff base and the ARP and it is the most abundant ion at higher collision voltages of the HRP indicating its stability and therefore the tendency of accumulating relative to the other fragments. Its three-carbon sugar complement was detected only under sodiation condition at m/z 113. (Table 1 Entry 18; Scheme 2) Interestingly, Yuan *et al.* (2016)[20] reported a characteristic fragment ion from MS/MS of proline Heyns compound at m/z 182 formed by the loss of two water molecules and a two-carbon sugar moiety $[M+H-2H_2O-C_2H_4O_2]^+$, this fragment ion was absent from the MS/MS of its Amadori compound and can be considered as a diagnostic ion for Heyns products. The glycine equivalent of this ion was also identified in its Heyns product at m/z 142 ($C_6H_8NO_3$) (Scheme 1) along with its two-carbon split moiety at m/z 61 ($C_2H_5O_2$). The ion at m/z 142 was nearly absent from MS/MS fragmentation of glycine Schiff base and Amadori compound making it a good candidate as diagnostic ion for HRP. Furthermore, we have identified another fragment ion at m/z 154 $[M+H-3H_2O-H_2CO]^+$ that also preferentially formed only in Heyns compound, that can also be considered as a diagnostic ion. (Figure 3) To illustrate the generality of the diagnostic fragmentation patterns proposed above, another set of Amadori and Schiff base of glucose this time using proline was studied under the same conditions (see Table 1, Figures S3 and S4). The product ion from the Amadori compound at m/z 128 was identified as the diagnostic fragments ($[AA+H+CH_2]^+$) which is the equivalent to m/z 88 of glycine ARP. Proline Schiff base, on the other hand, produced the diagnostic fragment at m/z 158 equivalent to m/z 118 of glycine Schiff base (see Scheme S1). Furthermore, both proline Schiff base and ARP behaved the same way compared to glycine Schiff base and ARP in response to increased CID energy at 10, 15, and 20 eV. Both

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ARPs showed dehydration as the major product ions at low CID energy values and tended to undergo α-fission and results in accumulation of the diagnostic ion at m/z 88 (glycine ARP) and m/z 128 (proline ARP) at higher energies.

3.2 Diagnostic MS/MS fragmentations under neutral condition

When the mass spectrometric run was performed in presence of sodium formate, both molecular ions and their major fragments were detected exclusively as sodium adducts, (Figure 4). There was no evidence that the Schiff base underwent the Amadori rearrangement under such conditions. Additionally, direct infusion of the analytes compared to prior LC separation also reduced the time that Schiff base spent in aqueous environment and minimized its hydrolysis. Under neutral conditions, dehydration of the sugar moiety and the dehydration of its subsequent retro-aldolization products were also not significant, thus allowing for the detection of intact triose and tetrose species at m/z 113 and 143 respectively (Scheme 2). Under acidic conditions, these molecules underwent dehydration and cyclization into furan and pyran derivatives, as shown in Scheme 1. Under neutral conditions, in the MS/MS spectrum of the Heyns compound, a peak at m/z 170 (see Scheme 2) was detected which was not observed under acidic conditions. This ion can be considered as diagnostic ion for Heyns product under sodiation condition. Taking together all the data, it seems that the retro-aldolization of C2-C3 sugar chain was the main pathway of fragmentation for all the three intermediates studied under neutral conditions; in the spectra of the Schiff base and Heyns product the respective peaks were relatively more intense, as compared to the Amadori product (Figure 4). We assume that the observed retro-aldolization of ARP at C2-C3 carbon bond occurred after retro-isomerization of ARP into Schiff

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base. (Scheme 2) Furthermore, only the spectra of the Amadori product contained peaks, albeit in low abundances, that were ascribed to its dehydration under neutral conditions.

4. Conclusion

We have demonstrated that ESI-qToF-MS/MS could be used to distinguish between glucose/glycine Schiff base and two most relevant isomers, Amadori and Heyns rearrangement products. Analysis performed under neutral conditions (using sodium formate clusters) is more suitable for identification of the intact sodiated molecular ions, due to lower hydrolysis rates of the Schiff base. On the other hand, MS/MS analysis under acidic conditions (such as in presence of formic acid) offered more informative patterns of characteristic and diagnostic ions for each molecule, thus allowing to discriminate the Schiff base from its corresponding Amadori and Heyns rearrangement products. At higher collision energy (20 eV), both Amadori and Heyns products undergo unique fragmentations that do not involve dehydration. In contrast, the Schiff base generates useful diagnostic ions under low collision energy value (10-15 eV). Loss of water from all three molecules is prominent at energy levels between 10-15 eV.

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Conflicts of interest

There are no conflicts of interests to declare

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275 **Figure Captions**

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Figures and Tables

Table 1: MS and MS/MS spectral data of Schiff base, Amadori and Heyns compounds and their significant and diagnostic fragment ions.

#	**Compound**	**Precursor ion $[M+X]^+$ (m/z)**	**CID voltage (eV)[a]**	**Significant fragment ions in MS and MS/MS spectra $[M+X]^+$ (m/z) [c]**
	Acidic conditions (ESI +ve)			
1	Glycine Schiff base	$[M+H]^+$ 238	0	238, 203
2			10	238, 220, 202, **118** [b], 85, 76
3			15	220, 202, 127, **118**, 85, 97, 76
4			20	n/a
5	Glycine Amadori	$[M+H]^+$ 238	0	238, 220
6			10	**220, 202**
7			15	220, 202, **97, 88**, 76
8			20	202, **97, 88**, 76
9	Glycine Heyns	$[M+H]^+$ 238	0	238, 220, 202
10			10	220, 202, **154, 130**
11			15 and 20	202, 156, **154, 142, 130**, 126, 118, 117, 101, 99, 97, 84, 76
12	Proline Schiff base	$[M+H]^+$ 278	0	278, 116
13			10 and 15	278, 260, 242, 232, 214, **158**, 116, **85**, 70
14			20	116, 70
15	Proline Amadori	$[M+H]^+$ 278	0	278, 260
16			10, 15 and 20	278, 260, 242, 232, 214, **128,** 116, 100, 70
	Neutral conditions (ESI +ve)			
17	Glycine Schiff base	$[M+Na]^+$ 260	0	260, 203, 143, 140
18			10	**143, 140**
19	Glycine Amadori	$[M+Na]^+$ 260	0	260
20			10	260, **242, 224**
21	Glycine Heyns	$[M+Na]^+$ 260	0	260
22			10	260, **170, 164,** 143, 140, **113, 108**

280 [a]: CID voltage at 0 represents ions found in the MS spectra without collision (fragmentation
281 occurs during ionization).
282 [b]: The characteristic ions are in bold, where they are either uniquely present in a sample or
283 significantly differ in their relative abundances.
284 [c]: Proposed structural assignments and relative abundances are reported in the supplementary
285 material (see Tables S1 and S2)

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286 **Table 2**: Elemental composition of important product ions generated for glycine/glucose Schiff
287 base, Amadori and Heyns compounds during MS/MS under acidic and neutral conditions.
288

Acidic Condition				**Neutral Condition**			
#	m/z	MF	Error (ppm)	#	m/z	MF	Error (ppm)
	Fragments derived from the adduct				*Fragments derived from the adduct*		
1	238.0914	$C_8H_{16}NO_7$	5.783	1	260.0735	$C_8H_{15}NNaO_7$	4.312
2	220.0812	$C_8H_{14}NO_6$	0.964	2	242.0631	$C_8H_{13}NNaO_6$	3.953
3	202.0710	$C_8H_{12}NO_5$	1.225	3	224.0522	$C_8H_{11}NNaO_5$	5.767
4	184.0605	$C_8H_{10}NO_4$	5.883	4	216.0842	$C_7H_{15}NNaO_5$	2.741
5	174.0757	$C_7H_{12}NO_4$	3.636	5	198.0729	$C_7H_{13}NNaO_4$	6.702
6	126.0546	$C_6H_8NO_2$	6.374	6	180.0628	$C_7H_{11}NNaO_3$	4.792
7	156.0652	$C_7H_{10}NO_3$	6.204	7	162.0516	$C_7H_9NNaO_2$	9.245
8	154.0491	$C_7H_8NO_3$	4.662				
	Fragments containing glycine				*Fragments containing glycine*		
9	76.0388	$C_2H_6NO_2$	11.224	8	98.0206	$C_2H_5NNaO_2$	12.223
10	88.0389	$C_3H_6NO_2$	8.558	9	110.0209	$C_3H_5NNaO_2$	8.163
11	118.0495	$C_4H_8NO_3$	6.93	10	140.0310	$C_4H_7NNaO_3$	9.732
12	72.0443	C_3H_6NO	8.867	11	170.0417	$C_5H_9NNaO_4$	7.219
13	130.0501	$C_5H_8NO_3$	6.291	12	108.0426	C_4H_7NNaO	0.615
14	102.0546	$C_4H_8NO_2$	8.853	13	164.0310	$C_6H_7NNaO_3$	8.308
15	84.0421	C_4H_6NO	9.981				

16	142.0495	$C_6H_8NO_3$	5.759				
	Sugar fragments				*Sugar fragments*		
17	163.0598	$C_6H_{11}O_5$	5.203	14	185.0424	$C_6H_{10}NaO_5$	1.044
18	145.0498	$C_6H_9O_4$	1.956	15	167.0309	$C_6H_8NaO_4$	6.756
19	127.0384	$C_6H_7O_3$	7.235	16	173.0415	$C_5H_{10}NaO_5$	6.317
20	109.0279	$C_6H_5O_2$	6.919	17	155.0320	$C_5H_8NaO_4$	0.183
21	97.0279	$C_5H_5O_2$	9.836	18	143.0308	$C_4H_8NaO_4$	8.588
22	99.0438	$C_5H_7O_2$	9.132	19	113.0202	$C_3H_6NaO_3$	11.181
23	117.0551	$C_5H_9O_3$	4.862				
24	101.0209	$C_4H_5O_3$	11.572				
25	85.0283	$C_4H_5O_2$	11.224				
26	69.0326	C_4H_5O	6.371				
27	61.0288	$C_2H_5O_2$	2.53				

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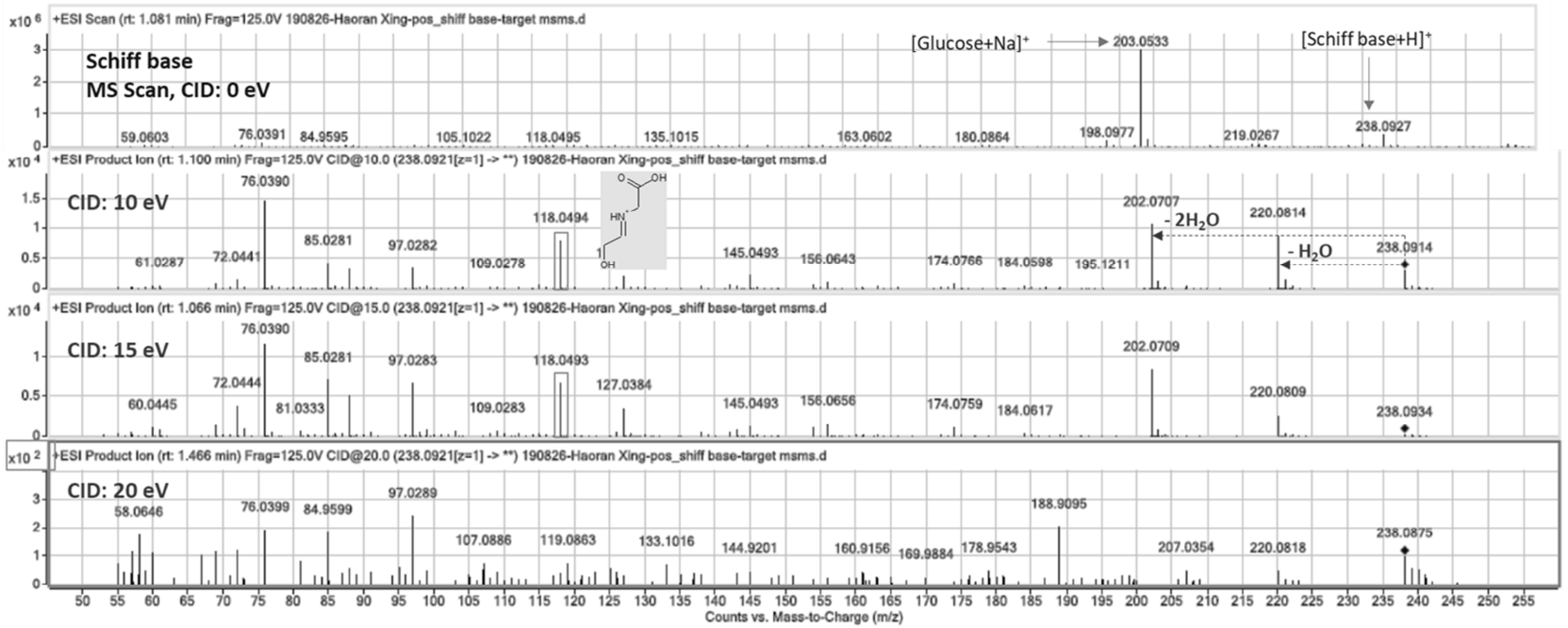

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292 Figure 1: MS and MS/MS spectra acquired at 0, 10, 15, 20 eV of glycine Schiff base under acidic condition.

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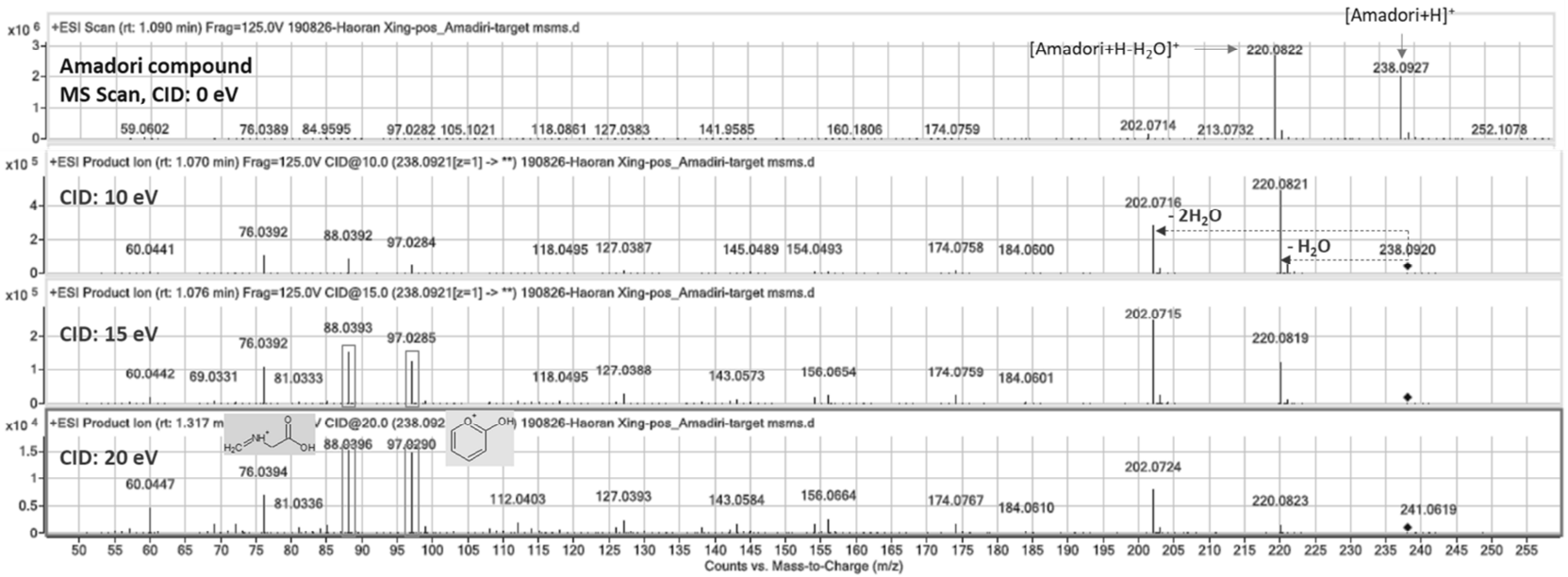

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295 Figure 2: MS and MS/MS spectra acquired at 0, 10, 15, 20 eV of glycine Amadori compound under acidic condition.

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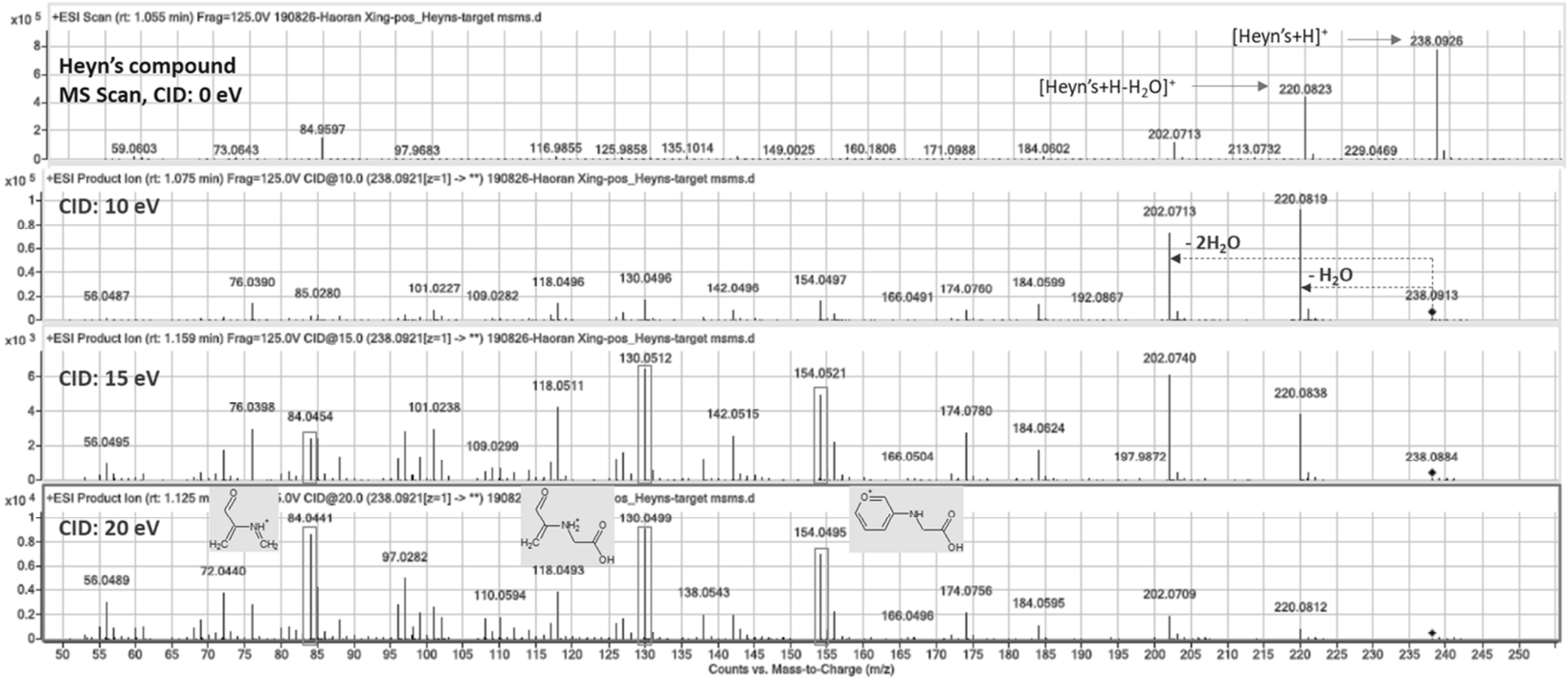

Figure 3: MS and MS/MS spectra acquired at 0, 10, 15, 20 eV of glycine Heyns compound under acidic condition; the diagnostic ions are indicated in the graph.

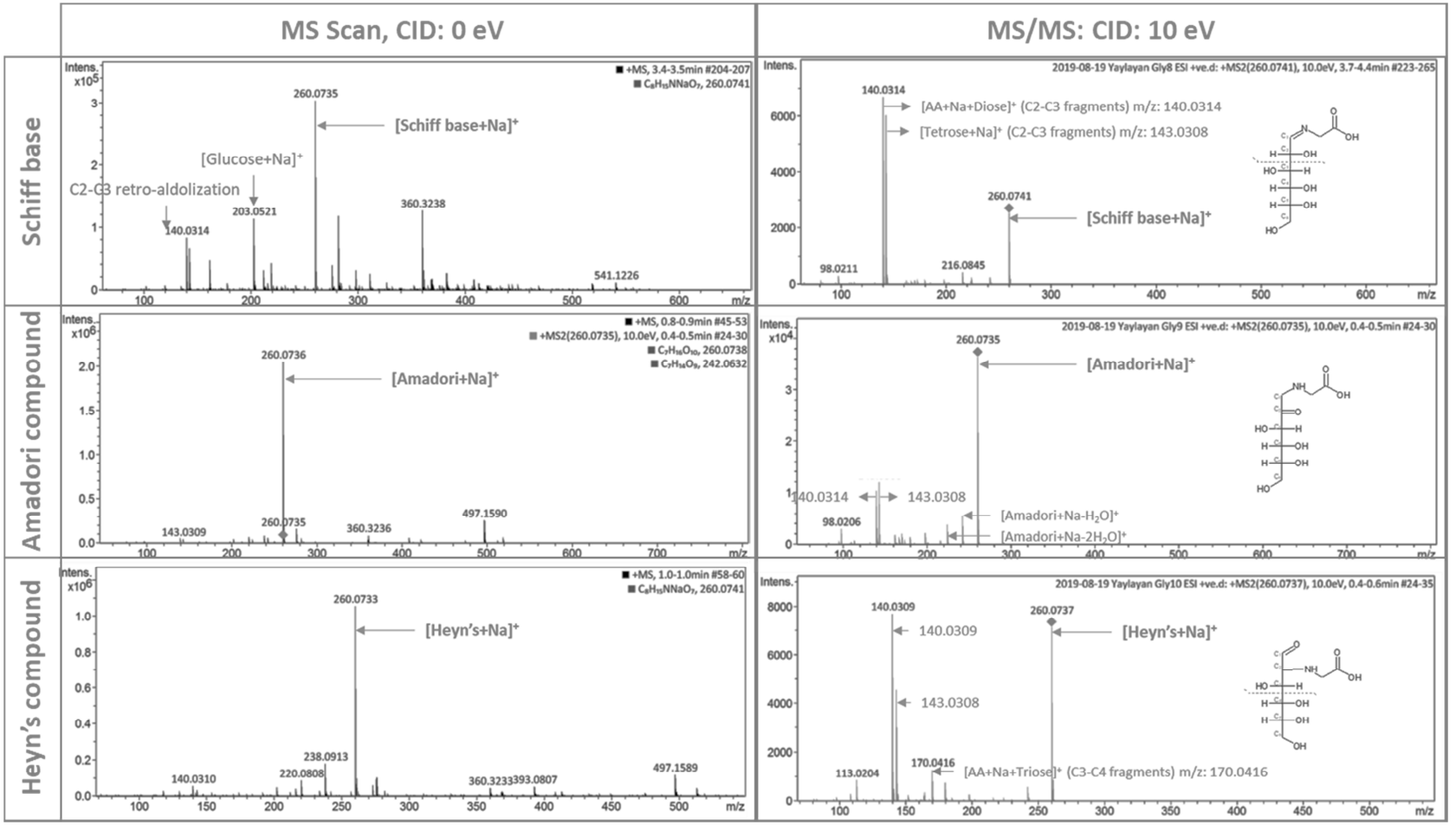

Figure 4: MS and MS/MS spectra at 0 and 10eV of glycine Schiff base, Amadori, and Heyns compound under neutral condition; the diagnostic ions are indicated in the graph.

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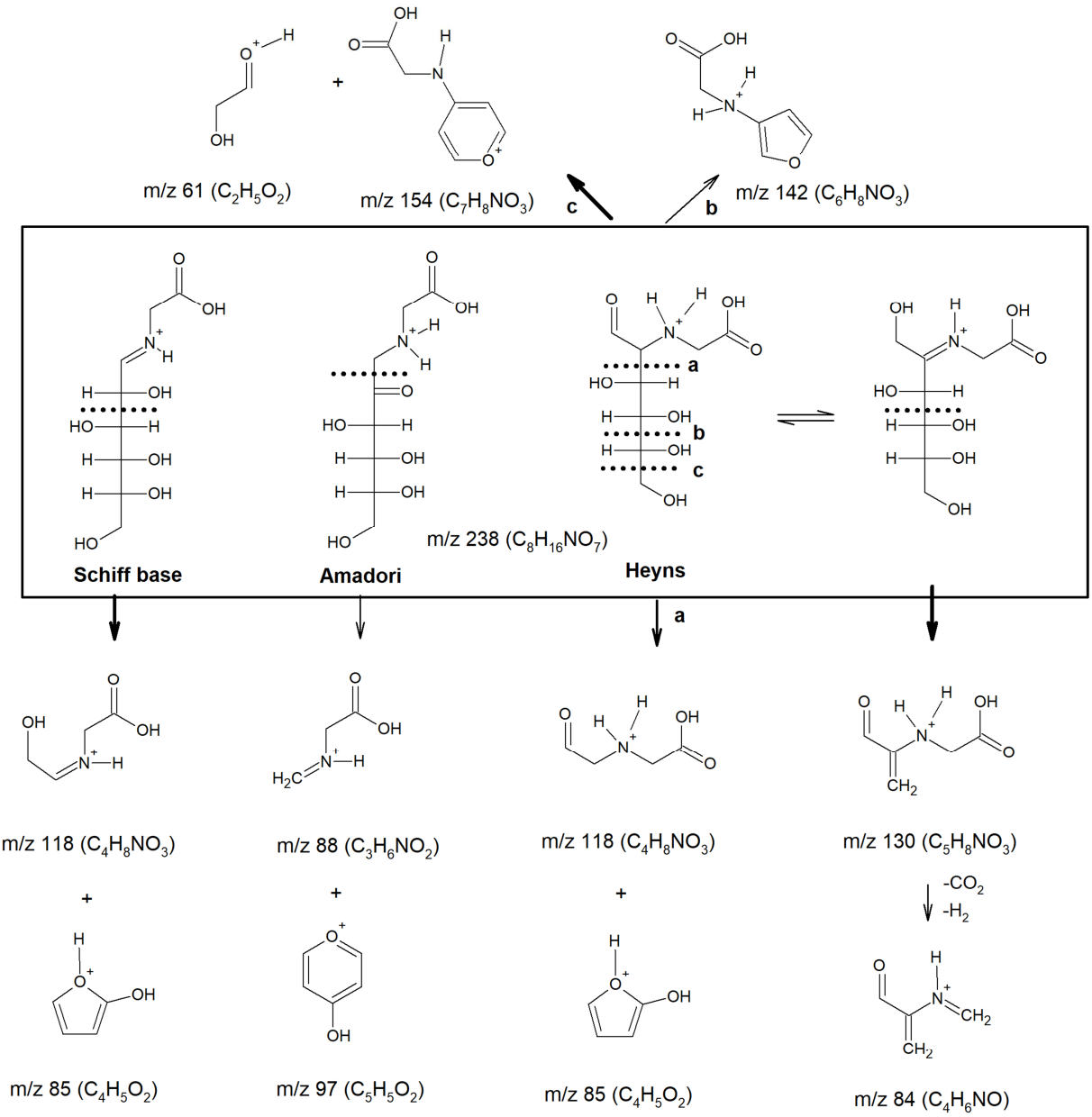

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310 **Scheme 1**: Proposed diagnostic collision-induced fragmentations of the glycine Schiff base and
311 Amadori or Heyns rearrangement products under acidic conditions. The characteristic loss of
312 water molecules from ARP is not shown.

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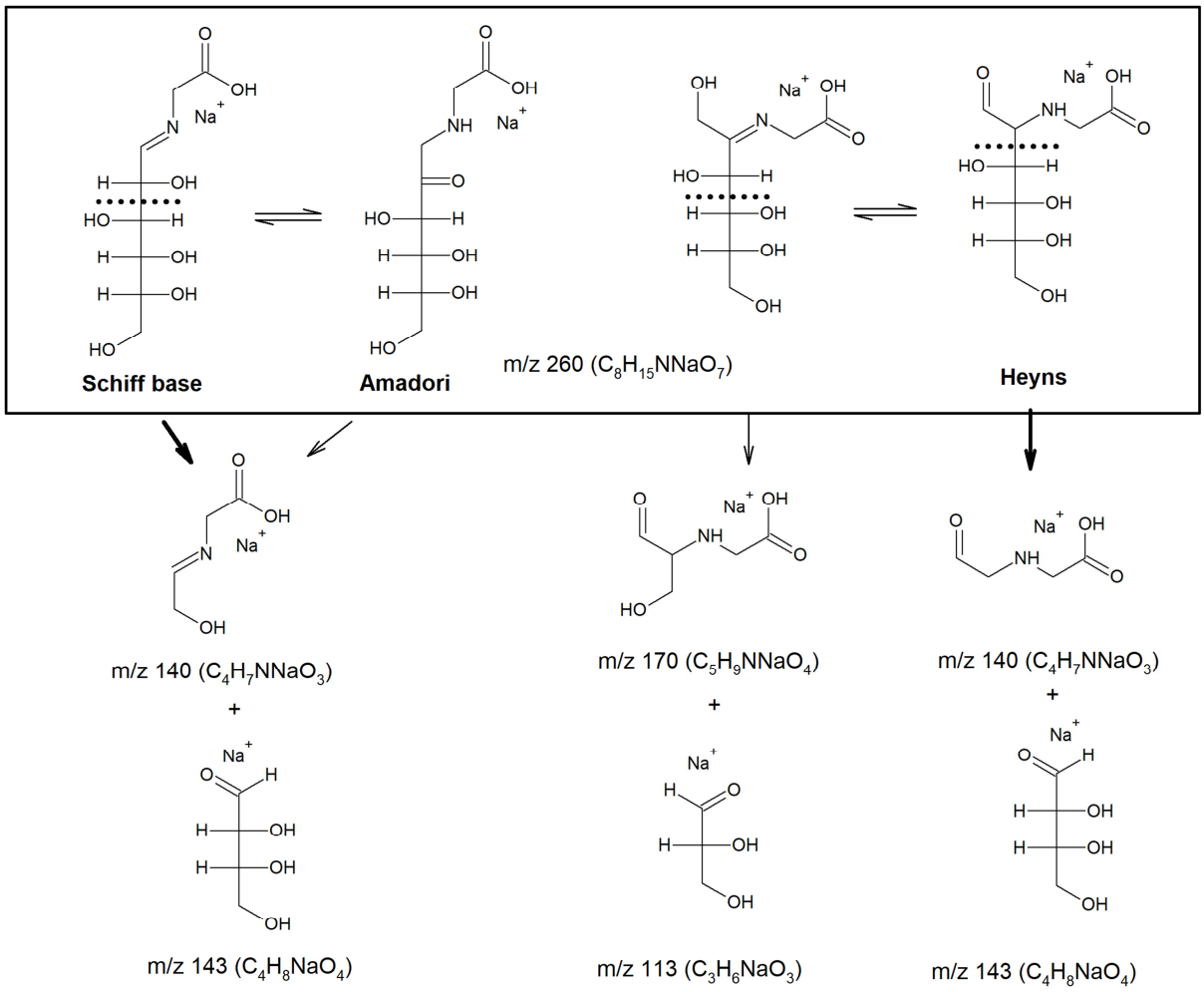

Scheme 2: Proposed diagnostic collision-induced fragmentations of the glycine Schiff base and Amadori or Heyns rearrangement products under neutral sodiation conditions.

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Highlights:

- The main diagnostic ion for the Schiff base during MS/MS fragmentation is generated through the retro-aldol degradation reaction yielding a diose attached to the amino acid moiety;
- The diagnostic ions of the Amadori compound result from a series of dehydrations of the intact molecule at low collision voltages, and a residual CH_2 group from the sugar attached to the amino acid moiety at the high voltage;
- The diagnostic ion of the Heyns compound forms through the retro-aldol degradation yielding a triose attached to the amino acid moiety;
- The MS/MS fragmentation patterns are consistent under both acidic and neutral conditions, but neutral condition provides more stability to the fragment ions.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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