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Short communication

Studies on 1-alkylamine adduct formation in electrospray ionization mass spectrometry for quantitative analysis

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ABSTRACT

We investigated the formation of 1-alkylamine adduct ions using 1,2-, 1,3-, and 1,4-cyclohexanediol (CHD) as model compounds and the relationships of the peak intensity between the protonated molecules ($[M+H]^+$), sodium adduct ions ($[M+Na]^+$), and 1-alkylamine adduct ions ($[M+A+H]^+$) of 16 model compounds using electrospray ionization mass spectrometry. When 1-octylamine was added to 1,2-, 1,3-, and 1,4-CHD solutions, the peak intensity of the 1-octylamine adduct ions ($[M+Oct+H]^+$) was higher than those of $[M+H]^+$ and $[M+Na]^+$. The highest peak intensity of $[M+Oct+H]^+$ was observed in 1,2-CHD, followed by 1,3-CHD and 1,4-CHD and this order was the same as that of $[M+Na]^+$ for CHDs in the solution without 1-octylamine. These results suggest that the mechanism of formation of $[M+Oct+H]^+$ is similar to that of $[M+Na]^+$ and that adduct formation seems to occur between 1-octylamine and two oxygen atoms in CHD in a similar manner to $[M+Na]^+$. Based on these results, 16 model compounds including CHDs were investigated with respect to the relationship between $[M+Na]^+$ and $[M+A+H]^+$. A positive correlation was observed between the peak intensities of $[M+Na]^+$ and $[M+A+H]^+$, supporting that the formation mechanism of $[M+A+H]^+$ is potentially similar to the $[M+Na]^+$ formation mechanism. These data indicate that a sensitivity enhanced quantitative analysis using $[M+A+H]^+$ could be a feasible approach for compounds generating $[M+Na]^+$.

1. Introduction

The liquid chromatography/tandem mass spectrometry (LC–MS/MS) technique is commonly used to determine drug concentrations in biological fluids from human and animal species. In particular, selected reaction monitoring (SRM) is a very powerful technique for sensitive and selective analysis and we previously reported a highly sensitive and selective quantitative analytical method using the SRM [1,2]. However, when drug concentrations are very low or the collectable sample volumes are limited, it requires higher sensitivity to clarify the pharmacokinetic profiles of the drugs. The use of mobile phase additives is a very simple way to enhance sensitivity since the adduct ion of the analyte is formed during the ionization process and one of the mobile phase additive methods for electrospray ionization (ESI) mass spectrometric analysis is alkylamine adduct ion formation. In ESI analysis with an alkali metal, such as sodium or potassium, the adduct ions are sometimes detected with a higher signal intensity than with the protonated molecules. Generally, it is difficult to use the alkali metal adduct ion for precise and accurate quantitative LC–MS/MS analysis because sodium and potassium exist everywhere (e.g. in glassware, stainless steel, and as impurities in chemicals and solvents) resulting in difficulty controlling the amount of the alkali metals present during the ionization process [3]. In contrast, the addition of alkylamines to the mobile phase as adduct forming agents has been demonstrated to be more reliable. We therefore applied the alkylamine additive method to enhance detection sensitivity in quantitative analysis [4,5]. Our results show that detection sensitivity using 1-alkylamine adduct ions could be significantly enhanced with good linearity, precision and accuracy and that the 1-alkylamine additive method could be used to determine the analyte concentrations in biological samples. Additionally the study reported by Zhao et al. evaluated the effect of alkyl ammonium (methyl- ethyl-, dimethyl-, and trimethylammonium) adduct ions on simvastatin and showed that one major molecular ion, alkylammonium adducted simvastatin, was observed in each of the alkylammonium acetate buffers with enhanced sensitivity [6]. The detection sensitivity for paclitaxel and docetaxel was also enhanced 3.7–4.8-fold using octyl ammonium adduct ions [7]. These applications demonstrate that the peak intensity of alkylamine adduct ions is significantly stronger than its protonated molecules. Furthermore, this mobile phase

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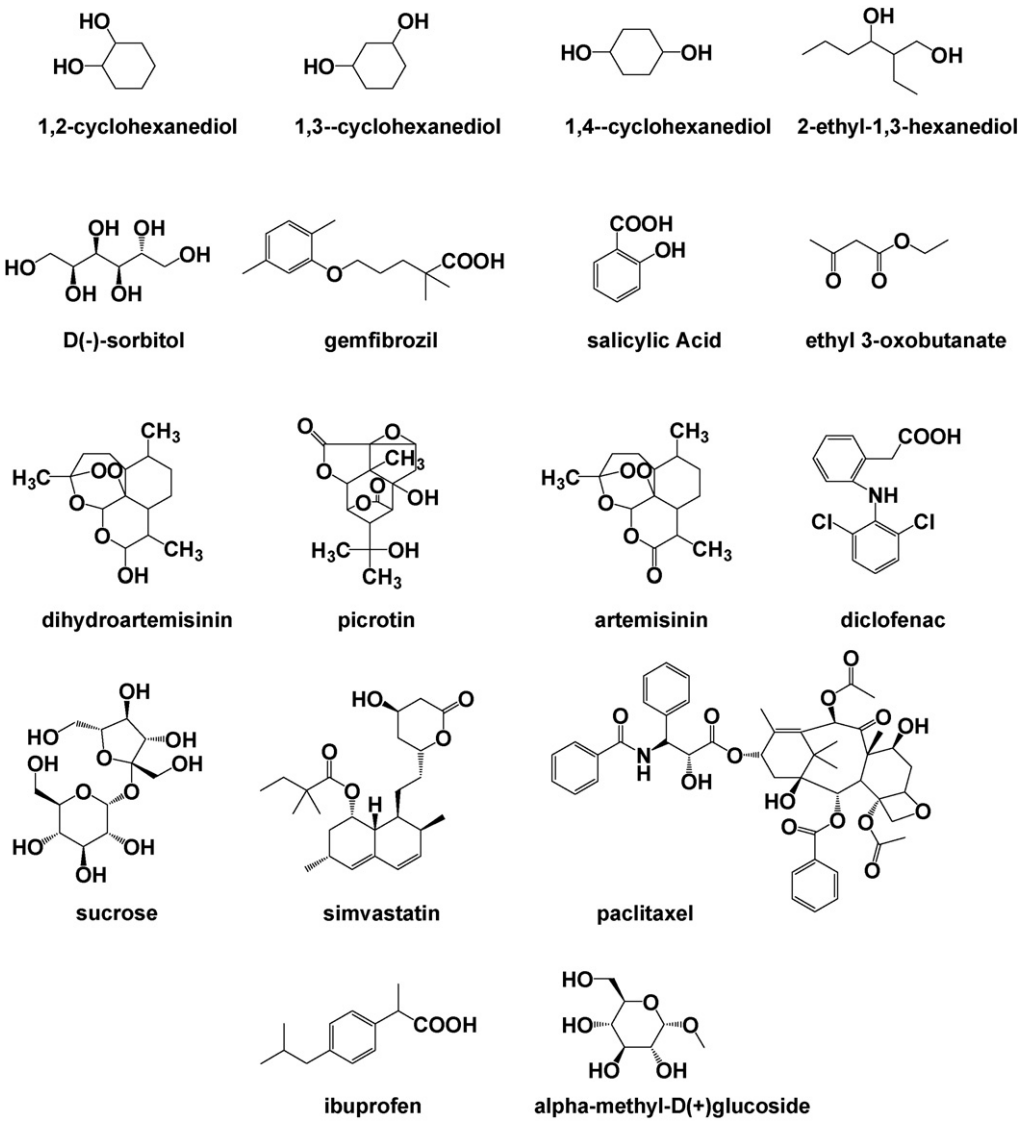

Fig. 1. Chemical structures of the model compounds.

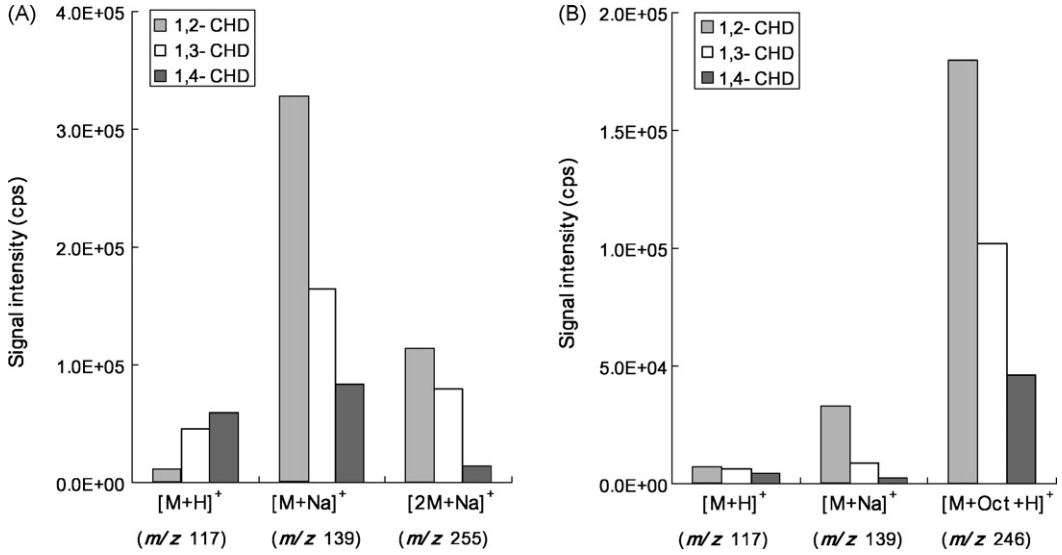

Fig. 2. Comparison of peak intensities for the CHDs. The concentration of CHD was 2 μmol/L and the infusion rate was 10 μL/min for both experiments. The composition of the CHD solutions was methanol–water–acetic acid (70:30:0.1) (A) and methanol–water–acetic acid (70:30:0.1) with 0.1 mmol/L 1-octylamine (B).

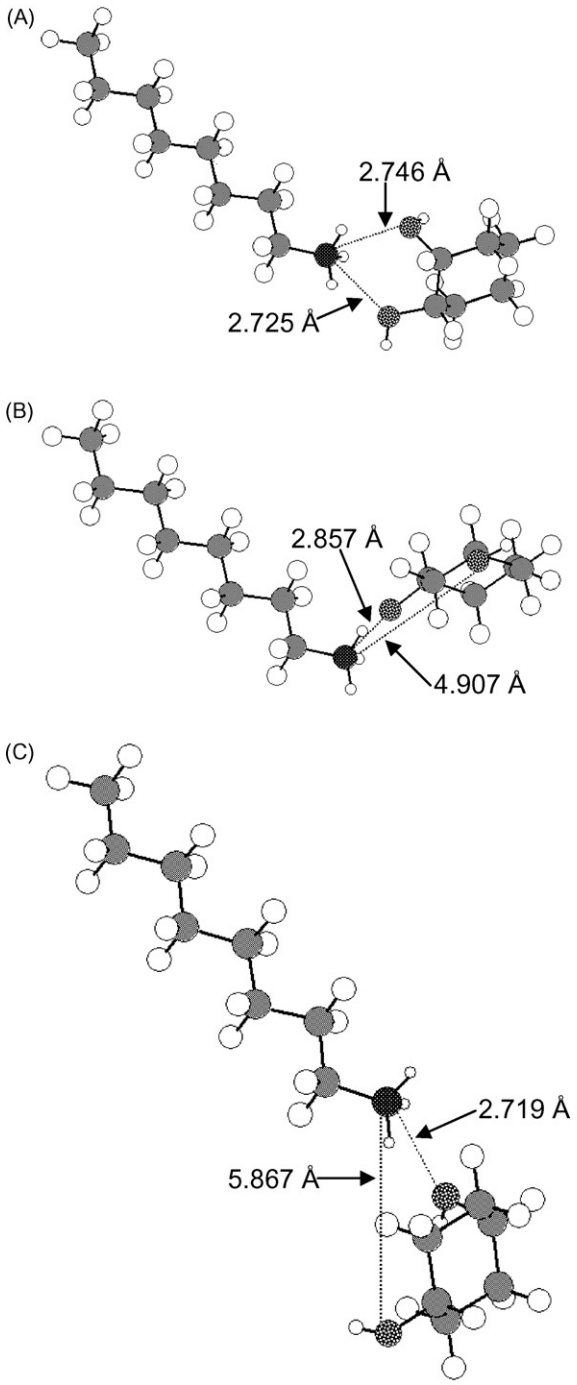

Fig. 3. Representative interaction of 1-octylamine and *cis*-1,2-CHD (A), *cis*-1,3-CHD (B), and *cis*-1,4-CHD (C).

additive approach was reviewed as one of the key factors for the enhancement of sensitivity [8]. For a better understanding of the mechanism of alkylamine adduct formation, Iyer et al. calculated the Hydropathic INTeraction (HINT) score as the parameter for interaction between taxanes and alkylamines [9]. Although this report indicates that the HINT score could potentially be used to predict alkylamine adduct formation, it is still unclear which function is contributing to the formation of the alkylamine adduct ions in ESI-MS analysis.

In this paper, cyclohexanediols (CHDs) were used as simple analyte models to evaluate alkylamine adduct formation. The peak intensities of the $[M+H]^+$, $[M+Na]^+$, and $[M+A+H]^+$ of the CHDs were compared to understand the potency of alkylamine adduct formation. Seventeen model compounds were tested to determine the correlation of peak intensity between $[M+A+H]^+$ and $[M+H]^+$ or $[M+Na]^+$ to allow the evaluation of the feasibility of quantitative analysis using alkylamine adduct ions.

2. Experimental

2.1. Materials

1,2-CHD (mixture of *cis*/*trans*), 1,3-CHD (mixture of *cis*/*trans*), 1,4-CHD (mixture of *cis*/*trans*), 2-ethyl-1,3-hexanediol, ethyl-3-oxobutanate, D-(−)-sorbitol, sucrose, simvastatin, paclitaxel, ibuprofen, diclofenac, and salicylic acid were from Wako Chemical Industry Ltd. (Osaka, Japan). Dihydroartemisinin, picrotin, and gemfibrozil were obtained from Sigma (St. Louis, MO, USA). Alpha-methyl-D(+)glucoside and artemisinin were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan) and Sigma–Aldrich Chemical Co. (Milwaukee, WI, USA), respectively. The chemical structures of these tested compounds are shown in Fig. 1. Ethylamine, 1-butylamine, 1-hexylamine, 1-octylamine, and 1-decylamine were obtained from Wako Chemical Industry Ltd. Methanol, acetic acid, and sodium acetate were purchased from Wako Chemical Industry Ltd. Milli-Q water was generated in-house using Milli-Q SP TOC system.

2.2. Instruments

An API4000 Qtrap mass spectrometer (Applied Biosystems, CA, USA) with Analyst Software Version 1.4 for data acquisition was used. This was coupled to an Agilent 1100 pump (Agilent Technologies Inc., CA, USA) and HTC-PAL system (Carrboro, NC, USA) for constant infusion. The ESI interface and mass spectrometer were optimized to 1,2-CHD using auto-tune program in Analyst software and operated under the following conditions: ionization polarity, positive; spray voltage, 5.5 kV; multiplier voltage, 2.0 kV; curtain gas flow-rate setting, 10 (N_2; 99.999% purity); nebulizer gas flow-rate setting, 20 (zero grade air; 99.999% purity).

2.3. Alkylamine adduct formation of CHD

Standard solutions for 1,2-CHD, 1,3-CHD, and 1,4-CHD (concentration of 2 μmol/L) were prepared by dissolving these compounds separately in the infusion solvent consisting of methanol/water/acetic acid (70:30:0.1, v/v/v). In addition, each CHD was dissolved separately in an infusion solvent (methanol/water/acetic acid (70:30:0.1, v/v/v)) containing 0.1 mmol/L of 1-octylamine. These solutions were separately infused into the mass spectrometer at a flow rate of 10 μL/min to obtain the full ion mass spectra of each CHD. Molecular modeling simulations were performed using CS Chem3D Ultra (Version 7.0.0). The MOPAC analysis summary for Minimize Energy is as follows: Job Type, Minimize Energy to Minimum RMS Gradient of 0.100, Theory, AM1, Wave Function, Closed Shell (Restricted).

2.4. Correlation between alkylamine and sodium adduct ion formation

Seventeen model compounds, shown in Fig. 1, were dissolved separately in the infusion solvent, which was a mixture of methanol/water/acetic acid (70:30:0.1), to provide standard solutions at the concentration of 10 μmol/L for determination of the peak intensity of $[M+H]^+$. In addition, each analyte was dissolved

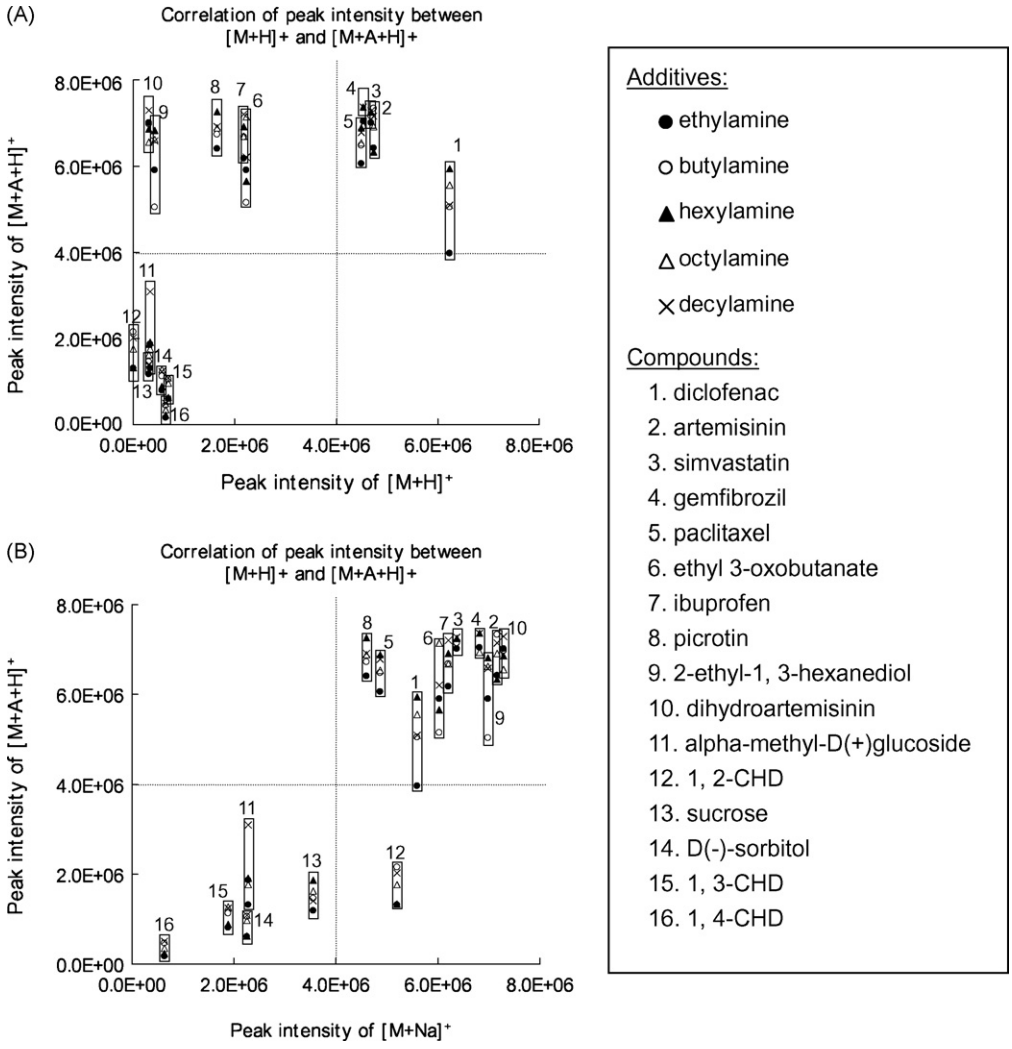

Fig. 4. Correlation of the peak intensities of $[M+H]^+$ and $[M+A+H]^+$ (A) and $[M+Na]^+$ and $[M+A+H]^+$ (B) for 17 model compounds. The concentration of analyte was 10 μmol/L and the infusion rate was 10 μL/min in all the experiments. The composition of the solutions was methanol–water–acetic acid (70:30:0.1) for $[M+H]^+$, methanol–water–acetic acid (70:30:0.1) with 0.3 mmol/L sodium acetate for $[M+Na]^+$, and methanol–water–acetic acid (70:30:0.1) with 1 mmol/L alkylamine for $[M+A+H]^+$.

in an infusion solvent containing 1 mmol/L of each of the following 1-alkylamines: ethylamine, 1-butylamine, 1-hexylamine, 1-octylamine or 1-decylamine, or 0.3 mmol/L of sodium acetate. These solutions were separately infused into the mass spectrometer at a flow rate of 10 μL/min to obtain the full ion mass spectra and the peak intensities of $[M+H]^+$, $[M+Na]^+$, and $[M+A+H]^+$ for each analyte were measured.

3. Results and discussion

3.1. Alkylamine adduct formation of the CHDs

In the full ion mass spectrum for 1,2-, 1,3-, and 1,4-CHD, the peak intensity of the $[M+H]^+$ of 1,4-CHD at m/z 117 was the highest followed by those of 1,3-CHD and 1,2-CHD; however, the reverse was observed for the peak intensity of $[M+Na]^+$ at m/z 139 and $[2M+Na]^+$ at m/z 255 (1,2-CHD > 1,3-CHD > 1,4-CHD). Additionally, the peak intensities of $[M+Na]^+$ were higher than those of $[M+H]^+$ and $[2M+Na]^+$ for each CHD (Fig. 2A). These results suggest that $[M+Na]^+$ was the most stable ion of the CHDs in the ESI and the stability of $[M+Na]^+$ is possibly dependent on the distance between the sodium and two oxygen atoms in the CHD. It has been reported that the alkali metal adduct ions of organic compounds having an oxygen functional moiety were detected using various ionization sources: fast atom bombardment, matrix-assisted laser desorption, and electrospray. The binding site of sodium was postulated to be two neighboring oxygen functional groups of the analyte [10,11]. When 1-octylamine was added to each CHD solution, peaks corresponding to $[M+H]^+$, $[M+Na]^+$, and $[M+Oct+H]^+$ were observed at m/z 117, 139, and 246, respectively, while the peaks of $[2M+Na]^+$ disappeared. It is considered that the disappearance of the $[2M+Na]^+$ peak was due to the complete suppression of multimer formation by the primary amine in the ESI [12]. In addition, it has been reported that the formation of $[M+Na]^+$ was suppressed by alkylamine adduct formation [4,7]. In fact, as shown in Fig. 2B, the peak intensities of $[M+Oct+H]^+$ were higher than that of $[M+Na]^+$. This observation suggests that competition exist between the formation of $[M+Oct+H]^+$ and $[M+Na]^+$. Furthermore, the order of the sensitivity of $[M+Oct+H]^+$ with the CHDs (1,2-CHD > 1,3-CHD > 1,4-CHD) is the same as that with $[M+Na]^+$, indicating that the stability of $[M+Oct+H]^+$ for CHDs is also dependent on the distance between 1-octylamine and the two oxygen functional groups. To support these observations, molecular modeling simulation of 1-octylamine adducts of CHDs was performed and the minimized interaction configurations between 1-octylamine and *cis*-1,2-, 1,3-, and 1,4-CHD are shown in Fig. 3. Although the dis-

tances from the nitrogen atom in 1-octylamine to one of the oxygen atoms in 1,2-, 1,3-, and 1,4-CHD are almost the same (approximately 2.7 Å), the distances to another oxygen atom is shortest for *cis*-1,2-CHD, followed by *cis*-1,3-CHD and *cis*-1,4-CHD reflecting the results of the peak intensity order for $[M+Oct+H]^+$. It is also calculated that the distance from the nitrogen atom to the oxygen atoms in *trans*-1,2-CHD (2.856 and 3.637 Å) are still shorter than in *trans*-1,3-CHD (3.084 and 3.932 Å) and *trans*-1,4-CHD (2.722 and 6.086 Å). These molecular modeling simulation results support that alkylamine adduct formation depends on the interaction between 1-octylamine and two oxygen functional groups.

3.2. Correlation between alkylamine and sodium adduct ion formation

Although the molecular modeling results support the correlation between the $[M+Na]^+$ and $[M+A+H]^+$for the CHDs, this modeling approach cannot be extrapolated to larger molecules due to the complexity of the computations. Therefore, 17 model compounds were tested to evaluate whether $[M+Na]^+$ and $[M+A+H]^+$ formation for a variety of compounds is correlated. Results from the determination of peak intensity of $[M+H]^+$, $[M+Na]^+$, and $[M+A+H]^+$ for each analyte show that all tested compounds formed $[M+H]^+$, $[M+Na]^+$, and $[M+A+H]^+$ except for salicylic acid. Salicylic acid is a highly acidic compound due to the inductive effect of the hydroxy moiety and neither the $[M+Na]^+$ nor the $[M+A+H]^+$ were detected. For the other 16 compounds, as shown in Fig. 4, the peak intensities of $[M+H]^+$ vs. $[M+A+H]^+$ and $[M+Na]^+$ vs. $[M+A+H]^+$ were plotted. In Fig. 4A, the peak intensities of $[M+A+H]^+$ for ethyl 3-oxobutanate, ibuprofen, picrotin, 2-ethyl-1,3-hexanol, and dihydroartemisinin (compound number 6 through 10) are seen to be relatively high (>4.0E06 cps) whereas the peak intensities of $[M+H]^+$ are lower (<2.3E06 cps). These results indicate that there is no correlation between the peak intensity of $[M+H]^+$ and $[M+A+H]^+$, suggesting that the formation mechanism of $[M+A+H]^+$ is different from that of $[M+H]^+$. With the correlation between $[M+Na]^+$ and $[M+A+H]^+$ (Fig. 4B), the plots for the compound number 6 through 10 shifted to higher peak intensities of $[M+Na]^+$ (>4.0E06 cps). By this shift, a positive correlation between $[M+Na]^+$ and $[M+A+H]^+$ is seen, supporting that the formation mechanism of $[M+A+H]^+$ is similar to that of $[M+Na]^+$.

4. Conclusion

The present study shows the interaction between octylamine and the CHDs and the necessity of two oxygen functional group to form the $[M+Oct+H]^+$ of the CHDs. Furthermore, positive correlation between the peak intensity of $[M+A+H]^+$ and $[M+Na]^+$ for 16 model compounds was observed demonstrating that the formation mechanism of $[M+A+H]^+$ is potentially similar to that of $[M+Na]^+$. These data support that this sensitivity enhanced quantitative analytical method using $[M+A+H]^+$ could be a feasible approach for compounds generating $[M+Na]^+$.

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