Distinguishing Between Isobaric Ions Using Microdroplet Hydrogen-Deuterium Exchange Mass Spectrometry

Xiaowei Song¹,², Jia Li², Mohammad Mofidfar¹ and Richard N. Zare¹

¹ Department of Chemistry, Stanford University, Stanford, CA, USA. 94305
² Department of Chemistry, Fudan University, Shanghai, China. 200438

* Correspondence: rnz@stanford.edu

Abstract: Isobaric ions having the same mass-to-charge ratio cannot be separately identified by mass spectrometry (MS) alone, but this limitation can be overcome using hydrogen-deuterium exchange (HDX) in microdroplets. Because isobaric ions may contain a varied number of exchangeable sites and different types of functional groups, each one produces a unique MS spectral pattern after droplet spray HDX without the need for MS/MS experiments or introduction of ion mobility measurements. As an example of the power of this approach, isobaric ions in urinary metabolic profiles are identified and used to distinguish between healthy individuals and those having bladder cancer.

1. Introduction

Ambient ionization mass spectrometry (AIMS) refers to the strategy that directly analyzes the sample’s composition or target species under atmospheric and room-temperature conditions [1,2]. A wide range of chemical species can be successfully detected by AIMS such as synthetic drugs, pesticides, and endogenous metabolites including amino acids, fatty acids, nucleosides, carboxylic acids, carbohydrates, aldehydes, glycerophospholipids, etc. [3-7]. AIMS has advantages in that it is free from labor-intensive pretreatment and thus can be very useful for those on-site detection scenarios that demand quick feedback about the test result such as forensic detection of controlled drugs [8-10] and point-of-care medical emergencies. In the past, AIMS has been used to acquire the metabolic profile for different biological fluids, such as urine, saliva, serum, and extracellular vesicles [8,11-13], which are found in breast cancer, prostate cancer, cervical cancer, oral cancer, etc. [12-16].

However, AIMS has its own limitations. Unlike the combination of chromatographic separation with mass spectrometric detection, which separates most components before detection, this direct infusion mode gains the convenience of directly characterizing the sample’s profile at the cost of losing in-depth molecular resolving ability. Specifically, AIMS fails to distinguish those species that have the same molecular weight and formula (isobaric ions). Consequently, it is difficult to assign the unambiguous identity to a certain ion without further investigating and matching the MS/MS pattern. These issues pose a methodological challenge to direct infusion-based metabolomic studies.

Ion mobility mass spectrometry (IMS) provides an alternative possibility of distinguishing isobaric ions based on their different collision cross-sections. Not surprisingly, IMS can more easily achieve good performance on macromolecules (e.g., peptides and proteins) or small molecules that have obvious differences in molecular shape, size, or spatial conformation [17]. For isomers that have very intricate structural differences, the current IMS still has a very limited ability to achieve an ideal separation. To complement IMS, several reports introduced deuterium reagents into the mobility cell to conduct gas-phase hydrogen-deuterium exchange (GP-HDX) [18]. Compared to IMS, GP-HDX helps to probe more intricate structural details, such as the number of exchangeable proton sites from proteins, carbohydrates, amines, lipids, etc. [19-22] Practically, this IMS/GP-HDX combination needs a specially modified IMS setup and consumes more deuterium reagent. In recent years, the Valentine group has conducted a series of systematic HDX
studies on small molecules. This work ranges from predicting the HDX pattern of functional groups to the proof-of-concept droplet HDX study for metabolomic studies [18,20,23]. Motivated by this past work, we were inspired to integrate liquid-phase HDX with AIMS to develop a simple, robust, and cost-effective method for distinguishing isobaric ions in untargeted metabolomic studies.

Previously, our group has developed a series of polymer-based ambient ionization methods [24-27], which have shown advantages in weak absorption of hydrophilic species, stronger ion intensity, and more stable signal duration [28]. Among these AIMS methods, conductive polymer spray ionization mass spectrometry (CPSI-MS) has been successfully used for salivary metabolic profiling and oral cancer diagnosis [12]. Only a few microliters of methanol-water solvent suffice to desorb and ionize a wide range of metabolites within a few seconds. In other studies, we have also shown that the transient process of microdroplet HDX can be well captured by a DESI-MS system [29]. In this study, a proportion of methanol-deuterium water is used as the desorption solvent in CPSI-MS. when the deuterium-containing solvent contacts a dried sample spot on the conductive polymer tip with a high voltage applied, the microdroplet HDX process commences and the post-HDX metabolic profile can be easily recorded. The general workflow is schematically illustrated in Figure 1.

![Figure 1. Workflow for using HDX-CPSI-MS for distinguishing between isobaric ions.](image)

2. Results and Discussion

2.1. Rapid recognition of opioid narcotics

We first selected several pairs of frequently abused opioid narcotics as model compounds for testing the feasibility of microdroplet HDX combined CPSI-MS in distinguishing isobaric ions. The first pair of opioids are codeine and hydrocodone, which have the same formula (C18H21NO3) and an m/z value ([M+H]+, 300.1594, Figure 2A). But when focusing on precise structural differences, codeine has one active proton in the 6-hydroxyl group in contrast to hydrocodone that has only carbonyl and no active proton in the carbon-6 position. Therefore, these two compounds can be very easily distinguished in the HDX-CPSI mass spectra from their differences in deuterium peak number (Figure 2B and 2C). By contrast, it is relatively difficult to distinguish these two compounds from examining the MS/MS spectra which have quite similar fragment ion patterns (Figure S1) under the same CID energy (30 V). It should be noted that a protonated ion derived from the compound with no exchangeable proton could also yield one deuterated peak under HDX-CPSI-MS analysis because of the deuterium cation dissociated from heavy water. The deuterium peak can be easily recognized from the native isotope caused by its specific mass shift around 1.0063, measured by the high-resolution mass spectrometer.
The second pair of isobaric compounds are 6-acetylmorphine and naloxone (C19H21NO4, [M+H]+ 328.1543, Figure 2D). Apart from one phenolic hydroxyl group that they both have in the 3-position, naloxone carries an extra hydroxyl group in the 14-position. So, it can yield one more deuterium peak than 6-acetylmorphine (Figure 2E and 2F). In this case, the D1 peak of naloxone also becomes the base peak instead of D0. This behavior has a simple explanation. Either one of two exchangeable proton sites from naloxone contributed to the D1 peak. For another more important reason, the hydroxyl group has a faster HDX rate compared to the phenol group [30].

The second study case raises the question whether microdroplet HDX can also distinguish isobaric ions that have the same number of exchangeable proton sites from different functional groups. So, we investigated the third pair of opioid compounds, morphine and norcodeine, which have the same formula of C17H19NO3 and protonated ion at m/z 286.1437 (Figure 2G). They both have a hydroxyl group in the 6-position, but morphine possesses one phenol group in the 3-position whereas norcodeine has one imine group in the 17-position. As a result, the D1 and D2 from norcodeine were greatly increased compared to that from morphine, although there were three deuterium peaks for both of them (Figure 2H and 2I). Norcodeine’s base peak became D1, but morphine’s base peak was still D0, involving the exchange rate difference between phenol and imine protons when the pH ranged from 4.0 to 10.0, which is predominantly catalyzed by the base. This result indicates that HDX-based isobaric ions can be distinguished according to not only the exchangeable proton number but also the functional groups they possess.
Figure 2. HDX pattern for distinguishing between three pairs of opioid drug ions. (A, D, G) High-resolution mass spectra and parent structures for three pairs of isobaric ions in mixed solutions: (A) C₁₈HNO₃; (D) C₁₉H₂₁NO₄; and (G) C₁₇H₁₉NO₃. Post-HDX mass spectra for (B) codeine (CDN); (C) hydrocodone (HCDN); (E) 6-acetyl morphine (6-AM); (F) naloxone (NLX); (H) morphine (MOR); and (I) norcodeine (NCD).

2.2. Distinguishing isobaric ions that are challenging to tell apart by MS/MS.

Although MS/MS dissociation still serves as the major strategy to identify the above-mentioned compounds from their fragmentation patterns (Figure S1-S3), microdroplet HDX provides an alternative method for simple and quick isobaric ion recognition. To give a better demonstration, glucose and inositol were selected as a typical case. They both exist in all varieties of biological fluids (e.g., serum, saliva, urine, etc.) and act as carbon and energy sources to maintain body functions. But these two types of metabolites were difficult to be discerned from the metabolic profile. We also investigated their MS/MS fragmentation patterns under CID (energy 25 V). As shown in Figure 3A and 3B, MS/MS experiments failed to differentiate these two metabolites based on the top 10 fragment ions. Their patterns were completely the same owing to the similarities in structure and functional groups. Fortunately, from the HDX mass spectrum, there is one more deuterium peak (D₆) in the inositol than that in glucose. Besides, the D₃ becomes the base peak instead of D₂ in glucose (Figure 3C and 3D). Thus, glucose and inositol can be readily distinguished by microdroplet HDX mass spectrometry. We presented this example to illustrate in these situations that the MS/MS pattern is quite close with each other, microdroplet HDX is a much simpler but effective strategy if possible isobaric ions happen to have a different number of exchangeable protons. Using the similar strategy, we made a careful retrospective analysis on results of our previous studies on oral cancer, and we summarize a list of isobaric ions that can be frequently detected from the CPSI-MS-based saliva and serum-based metabolic profiles for reference (Table S1).

Figure 3. HDX for isobaric ions that also share similar MS2 patterns. CID-MS/MS spectra for (A) glucose; (B) inositol; Post-HDX, high-resolution mass spectra for (C) glucose and (D) inositol.

2.3. Correcting falsely matched adduct ions
In the untargeted metabolic profiling by AIMS, the delta m/z shift often helps to indicate the type of adduct ion. However, this strategy sometimes causes a misleading judgment. We found a very interesting case from the ongoing study about serum metabolomics for oral cancer. The suspect ions were located at m/z 300.2897 and 322.2716, which may be normally assigned to a metabolite with one proton or with one sodium adduct (Figure 4A), respectively. However, when we retested a self-collected dried saliva spot sample by HDX-CPSI-MS (LTQ), it was surprisingly seen that the ion at m/z 300 yielded 4 deuterium peaks whereas the ion at m/z 322 generated 2 deuterium peaks (Figure 4B). After searching the human metabolome database (HMDB), we narrowed down our attention to the two most possible metabolites, palmitoylethanolamide and sphingosine. The former one has 4 exchangeable proton sites and the latter one has only 2 exchangeable proton sites. In this case, the pair of [M+H]+ and [M+Na]+ adduct ions seemed to derive from the same metabolite but actually not. This is probably because of their affinity differences to positively charged species. This study case illustrates that microdroplet HDX can help to reduce the chance of a false match of adduct ion pairs in AIMS-based metabolic profiling.

Figure 4. HDX-CPSI-MS for adduct ion differentiation. (A) Mass spectra of a pair of protonated and sodiated ions recorded by an LTQ mass spectrometer and (B) the distinguishing result for the pair of adduct ions, which are sphingosine (red, m/z 300, [M+H]+) and palmitoylethanolamide (blue, m/z 322, [M+Na]+).

2.4. Comparison with gas-phase HDX

The HDX process we implemented by the CPSI method is mainly happening in charged microdroplets during the travel between the conductive polymer tip and the MS inlet on the microsecond timescale. Unlike the gas-phase HDX that uses deuterium reagents in the gas phase, back exchange also exists in this liquid-phase HDX process [31,32]. However, from the point of qualitative analysis, this insufficient H/D exchange becomes an advantage for isobaric ion discrimination. Taking morphine and norcodeine as examples, we compared patterns of the two compounds’ HDX, which happened under liquid-phase and gas-phase conditions. The apparently different patterns (Figure 5A and 5B) observed from the previous study become consistent with each other during gas-phase HDX (Figure 5C and 5D). This is largely because the much faster exchange rate in the gas phase without back exchange eliminates the functional-group-dependent HDX difference in the
liquid phase. In this regard, insufficient HDX caused by back exchange in the liquid phase provides more detailed information on isobaric ion structure.

![Diagram showing comparison of HDX in liquid and gas phases](image)

**Figure 5.** Comparison of HDX that happens in the liquid phase and the gas phase. MOR represents morphine and NCD represents norcodeine. Mass spectra for (A) morphine (MOR); (B) norcodeine (NCD) after conducting liquid-phase HDX; (C) MOR; and (D) NCD after conducting gas-phase HDX.

2.5. Creating new dimension and features for metabolic profiling

After illustrating the usage of microdroplet HDX on isobaric ion recognition, we continued investigating its practical value in AIMS-based untargeted metabolic profiling. There were 25 bladder cancer (BC) and 10 normal contrast (NC) urine samples collected for this proof-of-concept study. The post-HDX mass spectra from BC and NC samples were averaged for comparison. Most metabolites were mainly distributed within the range of \( m/z \) 50-300 under positive scan mode (Figure 6A). The deuteration peaks can be easily discerned based on the specific mass shift around 1.0063. Then we carefully checked through the mass spectra and selected the top 10 ions whose peaks have the most deuteration shifts. Urea ([2M+H]+) and creatinine ([M+H]+) were the most typical ones because their isotope peak intensity differences between two groups were so obvious to be directly read out from the average mass spectra (Figure 6B).

Because there were more HDX-generated deuterated peaks in the metabolic profiles compared to the native metabolic profile, we evaluated the performance of principal component analysis (PCA) on sample clustering according to the native and HDX metabolic profiles, respectively. In the contrast, the top 10 ions from native metabolic profiles were selected as original features. For HDX metabolic profile, extra 31 deuterium ions were selected as the newly created features apart from the original top 10 featured ions. As a result, we can clearly see from the score plots that PCA, as an unsupervised machine learning method, failed to separate cleanly the BC from NC merely based on the top 12 ions (Figure 6C) but performed well when taking the corresponding 29 deuterium ions into account (Figure 6D). These results clearly demonstrated that HDX-CPSI-MS helps to create a new dimensional feature (number of exchangeable proton sites) to enhance the
profile difference between two groups for pattern recognition. From the loading plots in Figure 6E, we learned that these newly created deuterium peaks indeed make contributions to the sample grouping.

Figure 6. HDX for statistics-free highlight of the cancer signature. (A) Average mass spectra with HDX acquired from bladder cancer and normal contrast urine samples; (B) Typical metabolite ions that have significant difference between two groups can be directly observed; (C) PCA score plots based on the original features collected from the plain metabolic profile; (D) PCA score plots based on the original features and newly generated deuterium peaks as the additional features; and (E) PCA loading plot that visualize the weight coefficient of each feature to the clustering.

2.6. Relative and Absolute Quantitation

Often in AIMS-based metabolic profiling more than one overlapped isobaric ion is present. This situation poses challenges for not only qualitative differentiation but also the quantitative estimation of each species. Consequently, we were motivated to carry out an absolute quantitation study on three pairs of mixed opioid drugs and a relative quantitation study on mixtures of glucose and inositol.

First, given the fixed total concentration at 100 μM, solutions containing different molar ratios of glucose and inositol solution (2:2, 1:3, 3:1) were prepared and tested by HDX-CPSI-MS. Given the hypothesis that two tested isobaric ions have very approximate ion efficiencies, the absolute intensity of each deuterium peak ($D_k$: $k=0-6$) in a mixed HDX pattern was first simulated by a linear combination of two pure HDX patterns according to formula (1) shown below. $N_g$ and $N_i$ denote the molar numbers for glucose and inositol.

$$Y_{D_k} = a_{D_k} N_g + b_{D_k} N_i$$
Ig and Ii denote the certain deuterium peak (Dk: k=0-6) intensity from pure glucose and pure inositol solutions, respectively. Then, the relative intensity of each deuterium peak can be normalized by the base peak according to equation (2). IBP denotes the absolute intensity of the base peak. A loss function in equation (3) was proposed as a metric to evaluate the closeness between a simulation and an actual HDX pattern. Here “n” denotes the number of deuterium peaks. The “sim” and “obs” in equation (3) refer to the simulated and observed intensities. As can be seen from Figure 7A, the simulated HDX patterns can be quite close to the actual ones. The scores of losses for three mixed samples of different molar ratios ranged from 2.8 to 5.3% (Table S2). This result indicates the feasibility of HDX-CPSI-MS for the relative quantitation of two isobaric ion, with the premise that they share a quite close ion efficiency.

\[
I_{abs} = N_g \times I_g + N_i \times I_i \quad (1)
\]

\[
I_{rel}(\%) = 100 \times \frac{I_{abs}}{I_{BP}} \quad (2)
\]

\[
Loss = \frac{1}{n+1} \sum_{i=0}^{k} |I_{sim} - I_{obs}| \quad (3)
\]

We investigated another pair of isobaric ions (codeine, and hydrocodone). A series of codeine solutions (5, 10, 20, 50, 100, 200 μg/mL) was spiked with a fixed concentration of hydrocodone (35 μg/mL), and an internal standard solution (6-acetylmorphine, 50 μg/mL) to construct samples tested by HDX-CPSI-MS. To rule out interference from hydrocodone, the specific deuterium peak from codeine (D2) was selected as the quantitative ion (Figure 7B). A quantitation curve was constructed by fitting the codeine molar concentration with calibrated responses based on the ratio of D2 ion versus the internal standard ion (Dis). It was shown that the quantitation curve reflected an ideal linear relationship between the codeine’s concentration and its specific deuterated ion with a Pearson coefficient of 0.9968 (Figure 7C). It should be noted that this absolute quantitation was only suitable for one of the isobaric ions that has a unique deuterium signature. After obtaining the concentration of this isobaric ion, it is possible to estimate the concentration of the other one by the relative quantitation strategy we mentioned above.

To sum up, some of isomers or isobaric ions that are difficult to distinguish even by MS/MS experiments can be easily recognized using HDX-CPSI-MS. Microdroplet HDX provides a cost-effective alternative for distinguishing between isobaric ions which can be complementary to CID-MS/MS fragmentation-based identification and ion-mobility-based separations. In terms of Untargeted metabolomics, microdroplet HDX mass spectrometry provides an extra dimension in that it is sensitive to active exchangeable sites of each MS peak which creates additional features in the profile. This behavior makes the global metabolic pattern more recognizable by multivariate analysis or machine learning and it also helps to find the underlying intricate differences.
Figure 7. Evaluating the HDX-CPSI-MS in relative and absolute quantitation. (A) Simulated and actual HDX mass spectra collected from the mixed solution of glucose and inositol. (B) Representative HDX mass spectra of target codeine, and the internal standard (IS, 6-acetyl morphine). The interference compound, hydrocodone, was overlapped with codeine in peaks at $m/z$ 300 ($D_0$), and 301 ($D_1$). But the deuterium ion at $m/z$ 302 ($D_2$) only belongs to the target and was selected as the signature ion for quantitation; (C) Quantitation curve constructed by fitting the IS-calibrated response ($D_2/D_{is}$) versus codeine’s concentration.

3. Methods

3.1. Reagents and Materials

All model compounds were purchased from Sigma-Aldrich (St. Louis, USA) including metabolites (glucose, inositol), controlled narcotics (codeine, hydrocodone, 6-acetyl-morphine, naloxone, morphine, norcodeine), and isomers (para-/meta-/ortho-aminobenzoic acid). D2O (99.9 atom % D), methanol (99.9%), deionized ultra-filtered water, ammonium hydroxide, and glacial acetic acid (99.7%) were all obtained from Fisher Scientific. Polymethyl methacrylate (PMMA) was purchased from Titan Scientific Co. Ltd. (Shanghai, China), and multi-walled carbon nanotubes (MWCNT, ID 2-5 nm, OD < 8 nm, length 10 - 30 μm) were purchased from J&K Scientific Ltd (Beijing, China).

3.2. Solution Preparation and Bio-sample Collection

Stock solutions of glucose and inositol were prepared in ultrapure water with concentrations set as 20 μM. For each narcotics standard, a stock solution was constructed in
methanol with a concentration at 20 μM. Urine samples of bladder cancer (BC) and healthy control (HC) volunteers were collected from the Veterans Affairs Palo Alto Healthcare System. In each case, consent was given in writing for samples acquired.

3.3. Droplet Spray Ionization

Conductive polymer spray ionization (CPSI) was employed as the ambient ionization method for the investigation of microdroplet HDX. For a CPSI experiment, the polymer substrate, which is made of PMMA and MWCNT, was cut into a triangular shape (8.0 mm wide and 10.0 mm high). Details of its step-by-step fabrication protocol can be found elsewhere [28]. For PSI and CPSI analysis, the biological fluid or compound solution (3 μL) was micropipetted onto the triangular tip of the substrate and fully dried to form a spot for analysis. A positive 4.5 kV high voltage was applied by a metal alligator clip onto the substrate that was positioned 13 mm in front of the mass spectrometer inlet. Then, methanol-H2O or methanol-D2O (5 μL, 7:3, v/v) was drop-wise loaded onto the conductive polymer tip. Driven by the strong electric field, charged microdroplets leave the conductive polymer substrate and head for the entrance to the mass spectrometer. During this process, transient HDX was taking place as captured and recorded in the mass spectrum.

3.4. Data Acquisition and Processing

An LTQ orbitrap mass spectrometer (Thermo Fisher, San Jose, CA) was employed for HDX data acquisition. For untargeted metabolic profiling, two duplicates of saliva or urine (3 μL) were first loaded onto tips of two paper or conductive polymer substrates to form dried fluid spots (DFS), respectively. After the high voltage was powered on, a droplet of methanol-H2O or methanol-D2O (7:3, v/v, 5 μL) was spiked onto the DFS to trigger the metabolic profiling without or with HDX. Mass spectra within the range of m/z 50-1000 under both polarities were recorded. The MS capillary temperature was set at 275 °C. The tube lens and capillary voltage was set at 35 V and 110 V, respectively. The number of microscans was set at 1 and the maximum injection time was set at 100 μs.

The Xcalibur software (Thermo Fisher Scientific, CA, USA) was employed for generating the average mass spectrum for each sample. Each spectrum was saved into a txt file for further processing. The in-built functions and self-programmed scripts under the MATLAB 2021 (Mathworks, MA, USA) were used for accessing txt files, total ion current normalization, and searching for deuterated peaks. SIMCA-P (Umetrics, Umea, Sweden) was used for multivariate analysis principal component analysis (PCA).

3.5. Isobaric Ion Discrimination and Assignment

Any unknown ion was first searched through HMDB (http://hmdb.ca/) and Metlin (https://metlin.scripps.edu) with the mass tolerance set at 5.0 ppm. Given the metabolites found in the libraries, we narrowed down the possibilities using knowledge of the specimens. The collision-induced dissociation (CID)-MS/MS experiment was also implemented to match the CID fragmentation pattern either with given standards or recorded MS/MS spectra in the database. For those metabolites that shared the same parent structure or very close MS/MS pattern, the proposed microdroplet HDX strategy was employed for further investigation and intricate discrimination. When the m/z mass shift of an ion falls into 1.0063 ± 0.0002, it can be considered as one deuterium replacement.

4. Conclusion

The ultrafast HDX process for active protons can be readily captured by AIMS using microdroplet HDX mass spectrometry. This new technique provides structural information about the number of exchangeable sites from a metabolite. Microdroplet HDX mass spectrometry is demonstrated to be an easy tool for isobaric ion discrimination and can be practically useful in a scenario in which metabolite candidates share the same
parent structure and similar MS/MS patterns. In untargeted metabolomic studies, the microdroplet HDX-based metabolic profiling creates a new dimension for increasing pattern differences and facilitating the direct observation of inter-group pattern difference without statistical analysis, which shows its promise as an additional tool for metabolite biomarker discovery.

Supporting Information: The supporting information provides additional information on the MS/MS spectra of opioid drugs, as well as list of isobaric ions that were frequently detected in biological fluids and successfully distinguished by HDX-CPSI-MS.

Acknowledgments: The work was funded by US Air Force Office of Scientific Research through Basic Research Initiative (AFOSR FA9550-12-1-0400), and National Natural Science Foundation of China (81903575).

Conflicts of Interest: The authors declare no potential conflicts of interest.

REFERENCE
1. Feider CL, Krieger A, DeHoog RJ, Eberlin LS (2019) Ambient Ionization Mass Spectrometry: Recent Developments and Applications. Anal Chem 91: 4266-4290.
2. Zoltan Takats JMW, Bogdan Gologan, R. Graham Cooks (2004) Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. Science 306: 471-473.
3. Q Wang, Bhattrai M, Zhao P, Alnsour T, Held M, et al. (2020) Fast and Sensitive Detection of Oligosaccharides Using Desalting Paper Spray Mass Spectrometry (DPS-MS). J Am Soc Mass Spectrom 31: 2226-2235.
4. Yang Q, Wang H, Maas JD, Chappell WJ, Manicke NE, et al. (2012) Paper spray ionization devices for direct, biomedical analysis using mass spectrometry. Int J Mass Spectrom 312: 201-207.
5. Su Y, Wang H, Liu J, Wei P, Cooks RG, et al. (2013) Quantitative paper spray mass spectrometry analysis of drugs of abuse. Analyst 138: 4443-4447.
6. Li Z, Li Y, Zhan L, Meng L, Huang X, et al. (2021) Point-of-Care Test Paper for Exhaled Breath Aldehyde Analysis via Mass Spectrometry. Anal Chem 93: 9158-9165.
7. Chiu K-Y, Wang Q, Gunawardena HP, Held M, Faik A, et al. (2021) Desalting paper spay mass spectrometry (DPS-MS) for rapid detection of glycans and glycoconjugates. International Journal of Mass Spectrometry 469: 116688.
8. Rossini EL, Kulyk DS, Ansu-Gyeabourh E, Saha B, Pezza HR, et al. (2020) Direct Analysis of Doping Agents in Raw Urine Using Hydrophobic Paper Spray Mass Spectrometry. J Am Soc Mass Spectrom 31: 1212-1222.
9. Yao YN, Di D, Yuan ZC, Wu L, Hu B (2020) Schirmer Paper Noninvasive Microsampling for Direct Mass Spectrometry Analysis of Human Tears. Anal Chem 92: 6207-6212.
10. Wang T, Zheng Y, Wang X, Austin DE, Zhang Z (2017) Sub-ppt Mass Spectrometric Detection of Therapeutic Drugs in Complex Biological Matrices Using Polystyrene-Microsphere-Coated Paper Spray. Anal Chem 89: 7988-7995.
11. Chamberlain CA, Hatch M, Garrett TJ (2021) Extracellular Vesicle Analysis by Paper Spray Ionization Mass Spectrometry. Metabolites 11.
12. Xiaowei Song XY, Rahul Narayanan, Vishnu Shankar, Sathiayaraj Ethiraj, Xiang Wang, Ning Duan, Yan-Hong Ni, Qingang Hu, Richard N. Zare (2020) Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proc Natl Acad Sci 117: 16167-16173.
13. Li C, Li K, Xu X, Qi W, Hu X, et al. (2021) A pilot study for colorectal carcinoma screening by instant metabolomic profiles using conductive polymer spray ionization mass spectrometry. Biochim Biophys Acta Mol Basis Dis 1867: 166210.
14. Mendes TPP, Pereira I, de Lima LAS, Morais CLM, Neves A, et al. (2020) Paper Spray Ionization Mass Spectrometry as a Potential Tool for Early Diagnosis of Cervical Cancer. J Am Soc Mass Spectrom.
15. Mahmud I, Pinto FG, Rubio VY, Lee B, Pavlovich CP, et al. (2021) Rapid Diagnosis of Prostate Cancer Disease Progression Using Paper Spray Ionization Mass Spectrometry. Anal Chem 93: 7774-7780.
16. Huang YC, Chung HH, Dutkiewicz EP, Chen CL, Hsieh HY, et al. (2020) Predicting Breast Cancer by Paper Spray Ion Mobility Spectrometry Mass Spectrometry and Machine Learning. Anal Chem 92: 1653-1657.
17. Burnum-Johnson KE, Zheng X, Dodds JN, Ash J, Fourches D, et al. (2019) Ion Mobility Spectrometry and the Omics: Distinguishing Isomers, Molecular Classes and Contaminant Ions in Complex Samples. Trends Anal Chem 116: 292-299.
18. Maleki H, Maurer MM, Ronaghi N, Valentine SJ (2017) Ion Mobility, Hydrogen/Deuterium Exchange, and Isotope Scrambling: Tools to Aid Compound Identification in Omics Mixtures. Anal Chem 89: 6399-6407.
19. Upal SS, Beasley SE, Scian M, Guttmann M (2017) Gas-Phase Hydrogen/Deuterium Exchange for Distinguishing Isomeric Carbohydrate Ions. Anal Chem 89: 4737-4742.
20. Kelly K, Bell S, Maleki H, Valentine S (2019) Synthetic Small Molecule Characterization and Isomer Discrimination Using Gas-Phase Hydrogen-Deuterium Exchange IMS-MS. Anal Chem 91: 6259-6265.
21. Wu F, Huang Y, Yu F, Li Z, Ding CF (2020) Effect of Transition-Metal Ions on the Conformation of Encephalin Investigated by Hydrogen/Deuterium Exchange and Theoretical Calculations. J Phys Chem B 124: 101-109.
22. Trabjerg E, Nazari ZE, Rand KD (2018) Conformational analysis of complex protein states by hydrogen/deuterium exchange mass spectrometry (HDX-MS): Challenges and emerging solutions. TrAC Trends in Analytical Chemistry 106: 125-138.
23. Majuta SN, Li C, Jayasundara K, Kiani Karanji A, Attanayake K, et al. (2019) Rapid Solution-Phase Hydrogen/Deuterium Exchange for Metabolite Compound Identification. J Am Soc Mass Spectrom 30: 1102-1114.
24. Narayanan R, Song X, Chen H, Zare RN (2020) Teflon Spray Ionization Mass Spectrometry. J Am Soc Mass Spectrom 31: 234-239.
25. Dulay MT, Boeser CL, Walker KL, Feider C, Zare RN (2021) Polymer substrate with surface solvent reservoir for polymer-spray mass spectrometric analysis of hydrophilic drugs. Talanta Open 4: 100048.
26. Dulay MT, Zare RN (2017) Polymer-spray mass spectrometric detection and quantitation of hydrophilic compounds and some narcotics. Rapid Commun Mass Spectrom 31: 1651-1658.
27. Song X, Chen H, Zare RN (2020) Coulometry-assisted quantitation in spray ionization mass spectrometry. J Mass Spectrom 56: e4628.
28. Song X, Chen H, Zare RN (2018) Conductive Polymer Spray Ionization Mass Spectrometry for Biofluid Analysis. Anal Chem 90: 12878-12885.
29. Song X, Zang Q, Zare RN (2021) Hydrogen-Deuterium Exchange Desorption Electrospray Ionization Mass Spectrometry Visualizes an Acidic Tumor Microenvironment. Anal Chem 93: 10411-10417.
30. Hamuro Y (2021) Tutorial: Chemistry of Hydrogen/Deuterium Exchange Mass Spectrometry. J Am Soc Mass Spectrom 32: 133-151.
31. O. Tara Liyanage MRB, Emvia I.Calixte, Touradj Solouki, Kevin L.Shuford, Elyssia S. Gallagher (2019) Characterization of Electrospray Ionization (ESI) Parameters on In-ESI Hydrogen/Deuterium Exchange of Carbohydrate-Metal Ion Adducts. J Am Soc Mass Spectrom 30: 235-247.
32. Yury Kostyukevich TA, Alexander Zherebker, Arif Ahmed, Sunghwan Kim, Eugene Nikolaev (2018) Hydrogen/deuterium exchange in mass spectrometry. Mass Spec Rev 37: 811–853.