(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
07 December 2023 (07.12.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/233333 A1

(51) International Patent Classification:
G01N 30/06 (2006.01) *H01J 49/00* (2006.01)
G01N 30/72 (2006.01)

(21) International Application Number:
PCT/IB2023/055595

(22) International Filing Date:
31 May 2023 (31.05.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/348,106 02 June 2022 (02.06.2022) US

(71) Applicant: **DH TECHNOLOGIES DEVELOPMENT PTE. LTD.** [SG/SG]; 33 Marsiling Industrial Estate Road 3, Singapore 739256 (SG).

(72) Inventors: **LE BLANC, Yves**; c/o Sciex, 71 Four Valley Drive, Concord, Ontario L4K 4V8 (CA). **HOPFGARTNER, Gérard**; 254 Route de Saconnex d'Arve, CH-1228 Plan-les-Ouates (CH). **RUSKIC, David**; Route de Saint-Julien 8, 1227 Carouge Geneva (CH).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: METHODS FOR CONTROLLED ADDUCT FORMATION IN MASS ANALYSIS

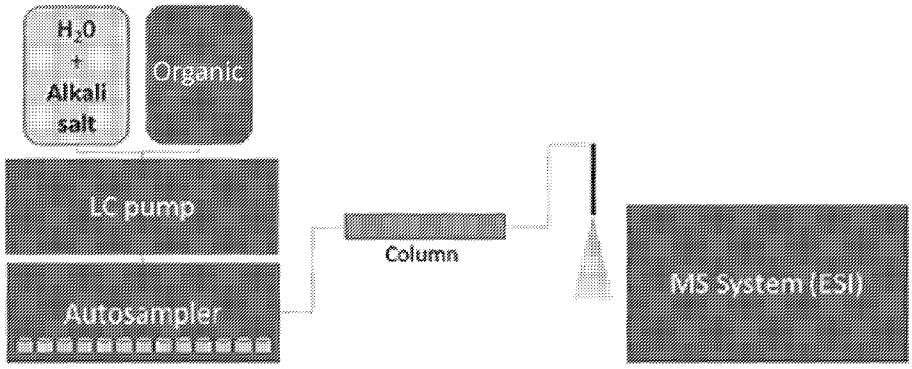


FIG. 1

(57) Abstract: Disclosed are methods and systems for preparing ionic adducts of analytes in a sample for mass analysis.

WO 2023/233333 A1

METHODS FOR CONTROLLED ADDUCT FORMATION IN MASS ANALYSIS

RELATED APPLICATIONS

[0001] The present patent application claims the priority benefit of U.S. Provisional Patent Application Ser. No. 63/348,106, filed June 2, 2022, the content of which is hereby incorporated by reference in its entirety into this disclosure.

BACKGROUND

[0002] Traditional mass analysis (e.g., mass spec, such as ESI, LC/MS) is often performed in positive ionization mode, with results predominantly reported as protonated species ($[M + xH]^{+x}$). However, other species can ionize and form adducts both as cationic species, such as metals (Group 1A and 2A (Na^{1+}, K^{1+}, Li^{1+}, Mg^{2+}, Ca^{2+}), transition metals, etc.) and as anionic species, (halides, organic anions (OAc, gluconate, citrate, lactate, etc.)) and are more frequently being identified as part of overall signals measured . The presence of such adduct species (e.g., alkali metal adducts) is usually attributed to low or trace amounts in the carrier/mobile phase(s) and common buffer systems that include ions such as Na^+ and K^+, and such adduct species formation is typically not controlled.

[0003] While the presence of alkali adducts in MS analysis has not yet been leveraged very widely (e.g., adducts yield little to no MSMS information and are avoided in such analysis), high resolution MS analysis applications more frequently account for the presence and signal attributable to adduct species (e.g., alkali adducts) to provide better estimates of analyte concentration. Further, recent complimentary applications such as Differential Mobility Spectrometry (DMS) and Electron Capture Dissociation (ECD) have shown interesting results stemming from the presence of alkali metal adducts, rather than protonated species. In DMS, for example, it has been shown that some species of adducts can increase separation between ions. . And, in some ECD applications, selecting alkali metal adduct species can provide a higher yield of radical fragments, similar to EI-spectra. Based on even these few results, there remains great potential to leverage and use adduct species in mass analysis as a way to improve separation, selective detection, and additional obtain additional complimentary analytical information.

[0004] Accordingly, there is a need for methods and systems that allow for the controlled, on-demand generation of ionic adducts and, preferably, that can avoid system contamination.

SUMMARY OF THE DISCLOSURE

[0005] In one aspect, the disclosure provides a method of performing mass analysis on a liquid sample comprising: generating an adduct of an analyte in a sample wherein the generating comprises adding a salt solution to the sample to form a diluted sample comprising the adduct of the analyte.

[0006] In one aspect, the disclosure provides a method of performing liquid chromatography/mass spec (LC/MS) comprising: generating an adduct of an analyte in a sample wherein the generating comprises adding a salt solution to the sample to form a diluted sample comprising the adduct of the analyte.

[0007] In some embodiments of the above aspects, the method further comprises loading the sample on a trap column, wherein the trap column is in fluid communication with a LC column. In some further embodiments the method comprises adding the salt solution to the sample on the trap column to generate the adduct.

[0008] In one aspect, the disclosure provides a method for generating an adduct of an analyte in a sample for liquid chromatography mass (LC/MS) analysis comprising, contacting a trap column with (i) a first volume comprising the sample, and (ii) a second volume comprising a salt solution, wherein the contacting of the first volume and second volume forms the adduct of the analyte.

[0009] In one aspect, the disclosure provides a method of performing LC/MS analysis comprising, generating an adduct of an analyte according to any one of the aspects and embodiments disclosed herein; performing LC separation of the sample comprising the adduct of an analyte; ionizing the sample comprising the adduct of an analyte; and detecting ions in the sample comprising the adduct of an analyte, or fragments thereof.

[0010] In one aspect, the disclosure provides a method of performing LC/MS analysis comprising, generating an adduct of an analyte according to any one of the aspects and embodiments disclosed herein; performing LC separation of the sample comprising the adduct of an analyte; ionizing the sample comprising the adduct of an analyte; detecting ions in the sample comprising the adduct of an analyte, or fragments thereof; acidifying the sample comprising the adduct of any analyte and performing LC separation of the acidified sample that does not comprise an adduct of an analyte; ionizing the acidified sample; and detecting ions in the acidified sample, or fragments thereof.

[0011] In embodiments of any of the aspects disclosed herein, the adduct of the analyte is an ionic adduct. In some embodiments, ionic adduct is a cationic adduct. In some embodiments, the ionic adduct is an anionic adduct. In some embodiments the salt solution and/or the ionic adduct

comprises an alkali metal, an alkaline metal, a transition metal, a halide, or an organic anion. In some further embodiments of the aspects and embodiments disclosed herein, the concentration of the salt solution is in a concentration that ranges from at least about 200 mM to up to about the saturation limit of the salt in the particular solvent/solution.

[0012] In some embodiments the methods and systems can further comprise a Differential Mobility Spectrometer (DMS) for ion selection. In some embodiments the methods and systems may further comprise Electron Capture Dissociation (ECD). In some embodiments, the methods and systems may further comprise collision-induced dissociation (CID).

[0013] In some embodiments the methods and systems can further comprise a sample handling system comprising a multi-well sample plate. In further embodiments, the sample handling system can further comprise an autoinjector.

[0014] In some embodiments, the methods and systems include an LC column comprising an ion-exchange column, a normal-phase column, a reverse-phase column, a hydrophobic interaction column, a size exclusion column, or an affinity column.

[0015] Other aspects and embodiments of the disclosure will be apparent in light of the description and illustrative examples that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIG. 1** illustrates a system suitable for generating one or more analyte adducts in accordance with some example aspects and embodiments of the disclosure.

[0017] **FIG. 2** illustrates another system suitable for generating one or more analyte adducts in accordance with some example aspects and embodiments of the disclosure.

[0018] **FIG. 3** illustrates another system suitable for generating one or more analyte adducts in accordance with some example aspects and embodiments of the disclosure.

[0019] **FIG. 4** illustrates another system suitable for generating one or more analyte adducts in accordance with some example aspects and embodiments of the disclosure.

[0020] **FIG. 5** illustrates another system suitable for generating one or more analyte adducts in accordance with some example aspects and embodiments of the disclosure.

[0021] **FIG. 6(A)-(C)** illustrates chromatogram traces of an analyte mix prepared in accordance with some example aspects and embodiments of the disclosure. (A) illustrates the analyte mix prepared in 0.1 M formic acid (aqueous water solution); (B) illustrates the analyte mix prepared in 1 M NaOAc; and (C) illustrates the analyte mix prepared in 1 M KCl. Blue

traces represent the TIC from ToF-MS analysis; the orange and pink traces represent XIC for $[M+Na]^+$ and $[M+K]^+$ adduct forms, respectively.

[0022] **FIG. 7** illustrates chromatogram traces of an analyte mix prepared in water (top frame) and the same analyte mix prepared in 1 M LiCl, in accordance with some example aspects and embodiments of the disclosure. XIC for Li adduct $[M+Li]^+$ demonstrates the increase in adduct formation following contact with the LiCl salt solution.

DETAILED DESCRIPTION

[0023] The disclosure generally provides methods and systems for the controlled formation of analyte adducts and for detection of one or more analyte adducts by mass analysis. As discussed in more detail below, the disclosure provides for generation and mass detection of analytes in protonated form, deprotonated form, and/or as ionic adducts.

[0024] The methods and systems herein include and expand upon two basic techniques that can generate ionic adducts (e.g., alkali metal-adducts) in mass spec analysis (e.g., ESI analysis). For example, the methods can comprise addition of a salt comprising an alkali metal to an LC mobile phase solvent (e.g., FIG. 1), and/or addition of such salts following LC column separation (e.g., FIG. 2). These basic approaches generate adducts of analytes present in samples, but can require adjustment of salt concentration or may require additional system features (e.g., mixers/microreactors). The methods and systems described herein further provide for additional system flexibility and allow for easy switching between generating and detecting target analytes in the protonated (or deprotonated) or adduct forms. This added flexibility provides for additional utility in leveraging new information and techniques that are provided by analysis of analyte-adducts.

[0025] In some of the aspects and embodiments of the disclosure samples can be contacted with high-concentration salt solutions to generate ionic (e.g., metal) adducts that can be used to improve ion separation (e.g., DMS separation) or to obtain alternate fragment ions (e.g., in ECD cells). As demonstrated herein, injecting standards and/or samples prepared in high concentrations (e.g., > 200 mM) of a selected salt solution, can reliably and reproducibly generate analyte adducts (e.g., as alkali metal adducts) for a wide variety of analytes. The methods can shift sample equilibriums from predominantly generating protonated ($[M+H]^+$) ions, to predominantly adduct forms (e.g., $[M+alkali]^+$) for a high proportion of analytes. Further, when samples are injected without addition of salt solution, or in acidified solution, the equilibrium can be shifted back to

favoring the protonated form over any adduct form(s). Thus, the methods provided herein allow for selective measurement and switching between protonated and adduct forms by controlling concentration of salt (or pH) added to samples, allowing for flexibility on automated systems.

[0026] Thus, methods and systems described herein that further comprise EAD/ECD and DMS can take advantage of the presence of adduct species to provide EI-like MSMS spectra or to improve separation of species, respectively. For example, typical ESI conditions allow for the detection of protonated species are detected. The flexibility in the methods disclosed herein (e.g., toggling between production of protonated species or, e.g., alkylated adducts) enhance the utility and applicability of EAD/ECD and/or DMS.

[0027] As disclosed and demonstrated below, contacting, mixing, and/or co-injection of high-concentration of salt solution with sample comprising one or more analytes can be performed under conditions to increase and control the level of ionic adduct formation. The methods can be performed directly as part of application/injection of sample solution to an LC system, or via a trap column where salt solution and sample solution may be combined.

[0028] As used herein, the "total ion current chromatogram" ("TIC") represents the summed signal intensity across the entire detected mass range versus time. The mass range can vary widely but is typically about several hundred mass-to-charge (m/z) units or more. In complex samples, the TIC chromatogram often provides limited information as multiple analytes elute simultaneously, obscuring individual species.

[0029] An "extracted-ion chromatogram" ("XIC", or alternatively EIC), as referred to herein, depicts one or more m/z values representing one or more analytes of interest that are recovered ('extracted') from the entire data set for a chromatographic run. The total intensity or base peak intensity within a mass tolerance window around a particular m/z is plotted at every point in the analysis. The size of the mass tolerance window typically depends on the mass accuracy and mass resolution of the instrument collecting the data.

[0030] FIG. 1 depicts an embodiment of the general systems and methods that fall within the scope of the disclosure. As shown in FIG. 1, an LC system comprises one or more reservoirs for the solvent(s) to be used with the LC separation (e.g., mobile phase solution and gradient solution), a pump, a sample handling platform, a chromatography column, and a mass spec system. In this embodiment, one of the solvents used in the LC separation, typically the mobile phase, further comprises an amount of salt, forming a salt solution. The sample is loaded onto the LC column for species separation and analysis on the mass spec system. This embodiment allows for direct

control of the salt concentration throughout the LC system over the course of the separation, and allows for detection of salt-derived adduct species. After the separations and detection of adduct species, the system may be used to detect protonated species, which can comprise flushing the system with solvent that does not comprise salt, prior to loading the sample onto the LC column for separation and analysis.

[0031] FIG. 2 depicts another embodiment of the general systems and methods that fall within the scope of the disclosure. As shown in FIG. 2, an LC system comprises one or more reservoirs for the solvent(s) to be used with the LC separation, a pump, a sample handling platform, a chromatography column, a salt solution and salt solution delivery system, and a mass spec system. In this embodiment, the sample is loaded onto the LC column for species separation. As the species elute from the column, a salt solution is added directly to the column eluate under conditions that allow formation of one or more adduct species. Suitably, the salt solution can be added to the column eluate in a device or a junction that is in fluidic communication with the LC system and a reservoir containing salt solution. Non-limiting examples of such a device (or junction) can include a reactor or a mixer, such as T mixers or microreactors of any geometry (e.g., arrow mixers) and cross-sectional dimension, and that may utilize active or passive modes of mixing and flow. In this embodiment, the salt solution may be added using an auto-injector or a pump that can adjust the amount, concentration, and/or flow rate of the salt solution that is added to the column eluate.

[0032] The column eluate comprising any adduct species is then analyzed on the mass spec system. This embodiment allows for direct application of salt to column eluate/fractions for each individual separation, which can help limit overall system exposure to high salt solutions. The embodiment by direct application of salt solution to the column eluate, also allows the option to alternate between mass detection of salt-derived adduct species and protonated species.

[0033] FIG. 3 depicts another embodiment of the general systems and methods that fall within the scope of the disclosure. As shown in FIG. 3, an LC system comprises one or more reservoirs for the solvent(s) to be used with the LC separation, a pump, a sample handling platform, a chromatography column, and a mass spec system. In this embodiment, a salt solution is added directly to one or more of the samples in the sample handling platform to form one or more adduct species, which may be a preparative step for the sample. The sample is then loaded onto the LC column for species separation and analysis on the mass spec system. This embodiment allows for direct control of the salt concentration at the individual sample level, which can help limit overall system exposure to high salt solutions. The embodiment also allows the option to alternate between

mass detection of salt-derived adduct species and protonated species.

[0034] FIG. 4 depicts another embodiment of the general systems and methods that fall within the scope of the disclosure. As shown in FIG. 4, an LC system comprises one or more reservoirs for the solvent(s) to be used with the LC separation, a reservoir or reservoirs for a salt solution, a pump, a sample handling platform, a chromatography column, and a mass spec system. In this embodiment, salt solution from the reservoir is added as a paired injection (e.g., a second injection) with the sample onto the column to form one or more adduct species. The paired injection of salt solution is made prior to the application of any elution gradient to the column. In this embodiment, the injection can be made as a dual injection (i.e., of sample and salt solution), providing an option for a single trigger for the start of the LC gradient (e.g., application of eluent solution) and start of the MS analysis. This embodiment allows for direct control of the salt concentration at the individual sample level and allows for mass detection of salt-derived adduct species and protonated species.

[0035] FIG. 5 depicts another embodiment of the general systems and methods that fall within the scope of the disclosure. As shown in FIG. 5, an LC system comprises one or more reservoirs for the solvent(s) to be used with the LC separation, a pump, a sample handling platform, a trap column, a chromatography column, and a mass spec system. In this embodiment, a salt solution is added directly to one or more of the samples in the sample handling platform to form one or more adduct species. Prior to LC analysis, the samples comprising the salt solution and adduct species are injected on a TRAP column that is in liquid communication with the system, e.g., using a column-switching set up such as a diverter valve. The sample is then loaded onto the LC column for species separation and analysis on the MS. This embodiment allows for direct control of the salt concentration at the individual sample level, which can help limit overall system exposure to high salt solutions. The embodiment also provides the option to alternate between mass detection of salt-derived adduct species and protonated species.

[0036] The methods can provide for contacting one or more samples - which may be unprocessed, raw, or crude samples, or may be samples that are pre-processed through one or more preparative steps - with a salt solution under conditions effective to form one or more adduct species with one or more sample components (e.g., analyte(s)). In embodiments, a sample is contacted with a salt solution having a known amount of salt under conditions to form a solution comprising an adduct. The salt solution may comprise an amount of an ionic salt that, in solution, dissociates to cations (e.g., alkali, alkaline, or transition metal ions) and anions (e.g., halides, hydroxides, organic anions, etc.). In some particular embodiments, the cation in the salt solution comprises a Group 1A

metal ion (e.g., Li+, Na+, K+, etc.) or a Group 2A metal ion (Mg2+, Ca2+, Sr2+, etc.). In some other particular embodiments, the anion in the salt solution comprises a halide (e.g., F-, Cl-, I-, Br-, etc.) or an organic anion such as, for example, acetate, lactate, gluconate, citrate, etc. The salt solutions can be provided at various concentrations, typically from at least about 200 mM to about the saturation limit of the salt in the particular solvent. In some embodiments, the concentration may range from about 200 mM to about 4 M (e.g., 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 550 mM, 600 mM, 650 mM, 700 mM, 750 mM, 800 mM, 850 mM, 900 mM, 950 mM, 1 M, 1.25 M, 1.5 M, 1.75 M, 2 M, 2.25 M, 2.5 M, 2.75 M, 3 M, 3.25 M, 3.5 M, 3.75 M, or about 4.0 M).

[0037] In embodiments in accordance with the above aspects, the disclosure comprises liquid chromatography (e.g., separation media in a column) with mass analysis of a sample (e.g., analyte adducts). The column can comprise any media generally known and available in the art such as, for example, media comprising resins, beads, or other micro or nano-structures that comprise ceramics, glasses, metals, and/or polymers, and that have functional groups or moieties that can interact with one or more target analytes that may be present in a sample. The column media can be selected based on a physical property that can distinguish and separate one or more components present in a sample (e.g., binds a target analyte(s) and does not bind sample matrix/buffer), based on molecular size, shape, charge, or hydrophobicity, or based on binding affinity to e.g., an antibody, a small molecule, or a macromolecule. In some embodiments, the column can comprise an ion exchange column (e.g., amine and/or carboxylate functional groups), a chelating column, a hydrophobic column (e.g., H.I.C.), an affinity column (e.g., immunoaffinity), a carbohydrate column, or a size exclusion column, and the like.

[0038] In some embodiments one or a plurality of solvent reservoirs are prepared each comprising a mobile phase and/or an eluent solution having a concentration of eluent that can be applied to the system in adjustable eluent concentrations (e.g., increasing or decreasing eluent concentration gradients). In some embodiments, the methods may comprise applying a gradient of eluent to the LC/column to separate one or more species (e.g., adducts, protonated, etc. species) present in sample after it is applied to the column. Some embodiments can comprise addition of salt solution to one of the solvent reservoirs. Some other embodiments can comprise addition of salt solution to the sample prior to, or after its application to the LC system (i.e., prior to or after application to the column).

[0039] The eluent in the eluent solution (or gradient buffer(s)) can comprise any molecule, or composition comprising a molecule or molecules, as a solvent or solution that, when applied to a

column/LC system, can bind (e.g., competitively bind) to the column media and elute or displace one or more components from the sample solution that are bound to the media, thereby making the eluted or displaced component(s) in the eluent volume available for detection/analysis. In some non-limiting embodiments, the eluent comprises one or more organic molecules/solvents, a non-ionic solution, an ionic (salt) solution (e.g., high concentration/high ionic strength solution), a low pH buffer solution (e.g., pH 2-6), a high pH buffer solution (e.g., pH 8-12), one or more affinity partners including for example, immunoglobulins, specific binding partners (e.g., antigen/antibody, enzyme/substrate, receptor/ligand, biotin/avidin, protein/nucleic acid, aptamers, fusion protein partners (e.g., HIS-tags/Ni-agarose, glutathione), etc.), carbohydrates (e.g., lectins/polysaccharides), and the like. In embodiments, the eluent can comprise a higher binding affinity (e.g., binding constant) for the separation media than the one or more components (target analyte(s)/analyte-adducts) in the sample.

[0040] In accordance with embodiments disclosed herein, the methods comprise the analysis of volumes of LC fractions (e.g., gradient/eluent solutions) that are analyzed and sampled over any range of eluent solution concentrations. The analysis can comprise removing a volume(e.g., ejecting the volume, injecting the volume, withdrawing the volume) from the LC separation and delivering it to a sampling interface. In some embodiments, the volume may be injected or ejected using, for example, an autosampler/injector, manual injection, a microinjector, a nanoinjector, an inkjet printer nozzle, a low pressure pump (e.g., peristaltic pump), or an acoustic droplet ejector (ADE) and the volume may be captured by any known sample receiving interface, for example, an open port interface (OPI) or an acoustic mist ionization interface.

[0041] In some embodiments, the methods and systems incorporate a trap column (as depicted in, e.g., FIG. 5). In such embodiments, any trap column that is known and available in the art can be incorporated into any of the methods and systems described herein, including as depicted in, e.g., FIGs. 1-4. Trap columns typically comprise short columns and contain high- capacity, typically low-efficiency, resins (e.g., ion-exchange resin). Trap columns can, among other functional features, concentrate samples, purify samples, act as specialized polisher columns, provide for formation of adducts, and provide for normalized sample retention times. Trap columns can be incorporated at various locations within the systems, including between the sampler and injector, between the injector and LC column, and between the LC column and MS system. Thus, trap columns can be employed to facilitate and provide one or more features that may be desirable in the performance of adduct formation and detection. For example, and among other features, a

trap column can provide: (i) a region in which adduct formation is performed (e.g., via mixing of sample and salt solution), and/or (ii) better reproducibility in terms of analyte retention time (RT), particularly when toggling between detecting protonated (e.g., acidified) and adduct-forms. These features can enhance aspects of the methods including, for example, allowing for consistent and reproducible RTs, which can provide additional confirmation criteria during analysis and avoids the need to perform additional RT alignment/correlations during analysis.

[0042] The methods and systems in accordance with the disclosure are operable with ion sources and/or mass spectrometers in both positive and negative ionization modes. As is known in the art, in positive mode, protonated and/or cationic adduct (e.g., alkali and alkaline) analyte molecules are generally observed in the mass spectra, while in the negative mode deprotonated and/or anionic adduct (e.g., halide, organic anion) analyte molecules can be observed.

[0043] In some embodiments, the methods can include Differential ion Mobility Spectrometry (DMS), which may also be referred to as Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) or Field Ion Spectrometry (FIS). DMS typically performs gas- phase ion sample separation and analysis by continuously transmitting ions-of-interest while filtering out unwanted/non-selected species. In accordance with the example aspects and embodiments of the disclosure, a DMS can be interfaced with a mass spectrometer (e.g., LC/MS) to take advantage of the atmospheric pressure, gas-phase, and continuous ion separation capabilities of the DMS and the detection accuracy of the MS. The combination of a DMS with an MS can enhance numerous areas of complex sample analysis, including proteomics, peptide/protein conformation, pharmacokinetics, and metabolic processes. In addition to pharmaceutical and biotech applications, DMS-based analyzers have been used for trace level explosives detection and petroleum monitoring.

[0044] A DMS separates and analyzes ions based on the mobility characteristics of the ions rather than based on the mass-to-charge ratio as in MS. Specifically in DMS, ions within a drift gas can be continuously sampled, between two parallel electrodes that generate an asymmetric electric field (S or separation field) therebetween that tends to move the ions in a direction perpendicular to the direction of the drift gas flow (i.e., toward the electrodes). The asymmetric field (S) can be generated by applying an electrical signal(s) (e.g., RF voltages) to one or more of the electrodes so as to generate an asymmetric waveform, the amplitude of which is referred to as the SV (separation voltage). Typically, the DMS is in fluid communication with a mass spectrometer in any variety of configurations that are generally known and described in the art (*see*, e.g., US Patent 8,084,736, US2019/0113478 and US2019/0086363 all of which are incorporated herein by reference).

Suitably, the DMS is configured to resolve ions (e.g., ionized isobaric species) based on their mobility through a fixed or variable electric field. As such, the DMS can comprise any ion mobility device configured to separate ions based on their mobility through a carrier or drift gas, including by way of non-limiting example, an ion mobility spectrometer, a drift-time ion mobility spectrometer, a traveling-wave ion mobility spectrometer, a differential mobility spectrometer, and a high-field asymmetric waveform ion mobility spectrometer (FAIMS) of various geometries such as parallel plate, curved electrode, spherical electrode, micromachined FAIMS, or cylindrical FAIMS device, among others.

[0045] In addition to mass analysis by MS, the analysis used in accordance with the aspects and embodiments described herein may comprise any one or more known technique that may be useful in identifying, detecting, characterizing, and/or quantifying the components that are present in a sample including, for example, ionic adducts. In some embodiments the analysis may comprise one or more spectrometers, spectrophotometers, spectrographs, or spectral analyzers, and include one or more technique(s) comprising UV-Vis, IR, near-IR, Raman, fluorescence, absorption spectroscopy (e.g., atomic absorption), emission spectroscopy (e.g., ICP-AES), EPR, and NMR.

[0046] The examples that follow illustrate some example embodiments of the aspects and embodiments described herein, and should not be construed as limiting to the scope of the disclosure or the appended claims.

Examples

[0047] *Example 1a: Formation and detection of alkali adducts.*

[0048] Using an experimental setup as generally depicted by FIG. 5, a sample comprising an array of target analytes (*e.g.*, a mixture of over 200 analytes representing a wide chemical diversity including: pesticides, peptides, pharmaceutical compounds (APIs) and drugs of abuse) was prepared in an aqueous solution of formic acid (0.1% formic acid in water). The diluted sample is injected into the Trap column and the species are subsequently separated on a C18 column. The species as detected by ESI/MS in the positive mode are detected as the protonated form ($[M+H]^+$) with little to no detection of any alkali adduct species. FIG. 6(A) depicts the TIC from ToF-MS data (blue, indicated by arrow) as a function of time. FIG. 6(A) also depicts the extracted ion chromatogram (XIC) traces for the $[M+Na]^+$ and $[M+K]^+$ adducts colored in orange and pink (respectively), also indicated by arrows.

[0049] The same analyte sample is prepared in two salt solutions, one in 1M sodium acetate (NaOAc) and the other in 1M potassium chloride (KCl). As separate column separations, each of the prepared samples are injected into the Trap column and the species are subsequently separated on the C18 column. The species are detected by ESI/MS in the positive mode for each of the samples and demonstrate that the samples include an increased amount of alkali-adduct species. FIG. 6(B) depicts the TIC from the ToF-MS data (colored blue and indicated by an arrow) as well as the XIC trace for the detected sodium adducts ([M+Na]+ colored orange, indicated by an arrow). Comparing the XIC for the [M+Na]+ adducts between FIG. 6(A) and 6(B), the total number of [M+Na]+ species increased from 2 to 54. FIG. 6(C) depicts the TIC from the ToF-MS data (colored blue and indicated by an arrow) as well as the XIC trace for the detected potassium adducts ([M+K]+ colored pink, indicated by an arrow). Comparing the XIC for the [M+K]+ adducts between FIG. 6(A) and 6(C), the total number of [M+K]+ species increased from 1 to 37.

[0050] The same experimental procedure is performed using a third salt solution, 1 M lithium chloride (LiCl). As illustrated in FIG. 7, the top panel illustrates the species detected in the aqueous sample (low/no salt), and the bottom panel illustrates the species detected in the sample containing LiCl. Each panel in FIG. 7 depicts the XIC, and when the results from the aqueous sample are adjusted to remove signal associated with isotopic overlap (three false positives), a single $[M+Li]^+$ adduct is detected. Similar to the results obtained for the Na and K salt solutions, an analysis of the XIC for the 1M LiCl sample shows that as many as 37 $[M+Li]^+$ adducts were generated and detectable.

[0051] Following the examples noted above, injection of acidified solution (following the salt-containing solution), increased the detection of species in protonated form (similar to Fig 6 (A)). This demonstrates that the method allows for switching between a desired mode of detection ($[M+H]^+$ or $[M+alkali]^+$) which can be used to take advantage of other analytical tools such as, for example, DMS or ECD which can provide additional selectivity and/or obtain orthogonal fragmentation information, respectively.

[0052] *Example 1b: Formation and detection of alkaline (Group IIa) adducts.*

[0053] Using an experimental setup as described in Example 1a, the same analyte sample is prepared as an aqueous solution, and is injected onto the Trap column and the species are subsequently separated on a C18 column. It is expected that the detected species (e.g., by ESI/MS in the positive mode) are detected as the protonated form ($[M+H]^+$) with little to no detection of any alkaline adduct species.

[0054] The same analyte sample is prepared in two salt solutions, one as a high concentration magnesium salt (e.g., ~ 1M $Mg(OAc)_2$, $MgCl_2$, $MgSO_4$) and the other as a high concentration calcium salt (e.g., $Ca(OAc)_2$, $Ca(lactate)_2$, $Ca(citrate)_2$, $Ca(gluconate)_2$). As separate column separations, each of the prepared samples are injected into the Trap column and the species are subsequently separated on the C18 column. The species are detected by ESI/MS in the positive mode for each of the samples and are expected to show an increased amount of detectable alkaline-adduct species, relative to the aqueous sample.

[0055] *Example 1c: Formation and detection of adducts in negative mode ionization.*

[0056] Using an experimental setup as described in Example 1a, the same analyte sample is prepared as an aqueous solution, and is injected onto the Trap column and the species are subsequently separated on a C18 column. In this example, however, the detection of species is performed in negative ionization mode (e.g., by ESI/MS in the negative mode) and are expected to be detected as the de-protonated form ($[M-H]^-$) with little to no detection of any adduct species, formed with the negatively charge salt ion.

[0057] The same analyte sample is prepared in two salt solutions, one as a high concentration sodium acetate (e.g., ~ 1M Na(OAc)) and the other as a high concentration potassium chloride (e.g., (e.g., ~ 1M KCl). As separate column separations, each of the prepared samples are injected into the Trap column and the species are subsequently separated on the C18 column.

[0058] The species are detected by ESI/MS in the negative mode for each of the samples and are expected to show an increased amount of detectable adduct species, as the acetate and the chloride adducts, relative to the aqueous sample.

[0059] Further description is included in Appendix A.

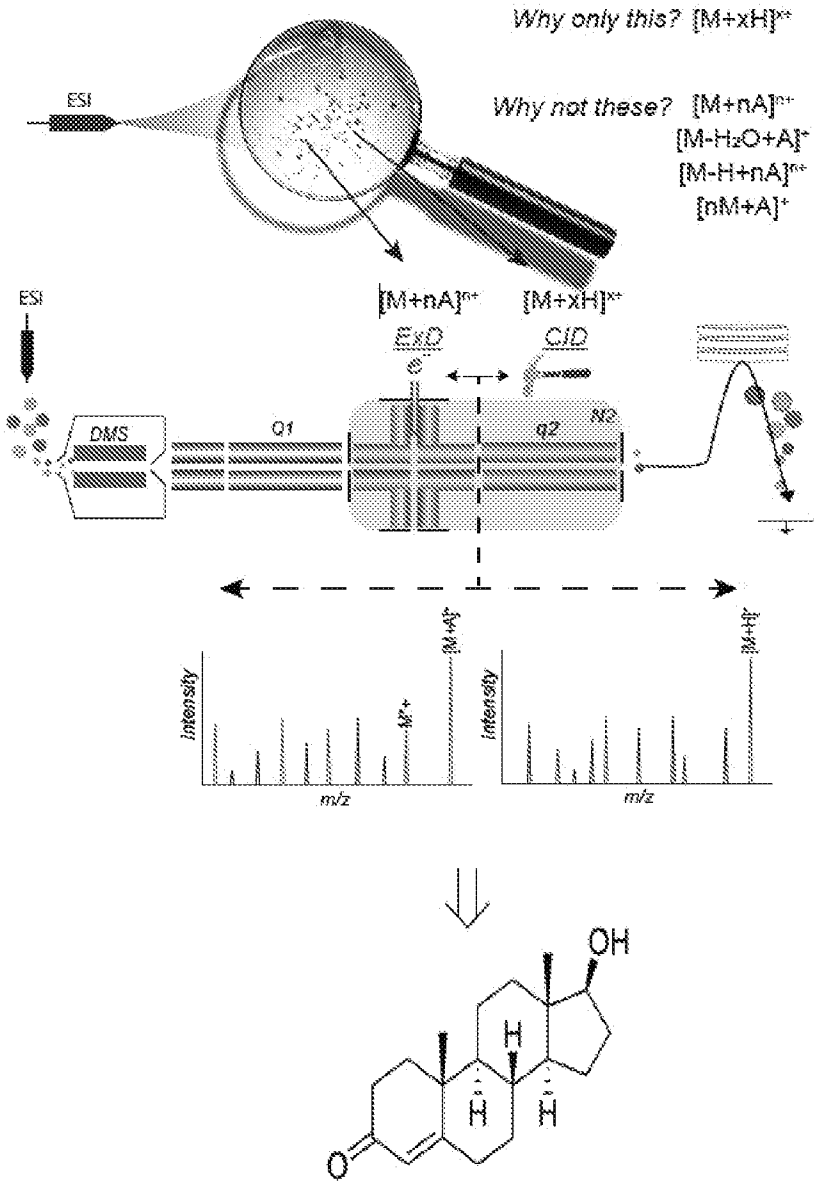
APPENDIX A

Overview

- Enhancing signal response of the alkali metal adducts vs protonated analogues
- Control of the sample matrix for reproducible adduct formation
- Electron Capture Dissociation (ECD) of the alkali metal abducts
- Combination of ECD and CID fragmentation for improved metabolite characterisation/identification

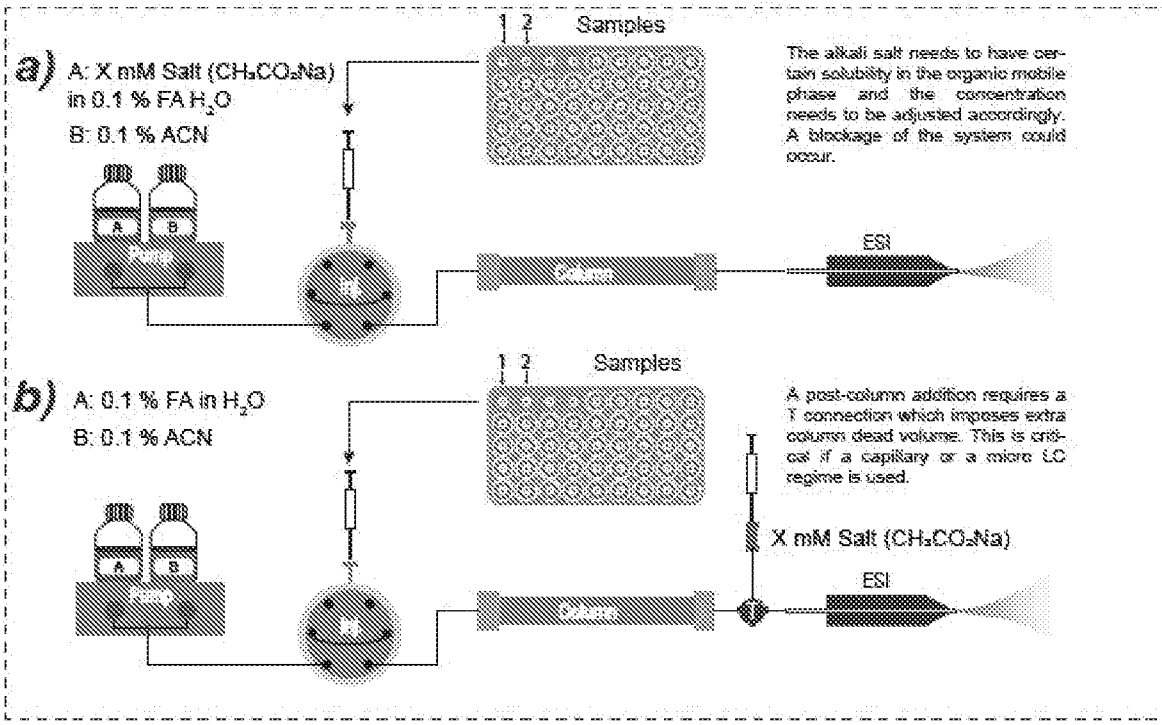
Introduction

The analyte's identification using ESI-LC-MS analysis is predominantly based on the presence of protonated $[M+xH]^{x+}$ species, since higher signal intensity (typically > 95%) and good CID fragmentation. The presence of alkali metal adducts $[M+xA]^{x+}$ in an MS spectrum is very often omitted due to low signal intensity and no fragmentation in CID. Their origin is often attributed to the trace presence of the salt in the mobile phase, glassware, and LC hardware, and the presence/absence of the alkali metal adducts is always changing. However, a complementary fragmentation approach to CID, such as Electron Capture Dissociation (ECD) has shown interesting benefits of using alkali metal adducts that provide different fragmentation which is more similar to EI. Therefore, an approach to control adduct formation in ESI is highly desirable regarding sensitivity and reproducibility.

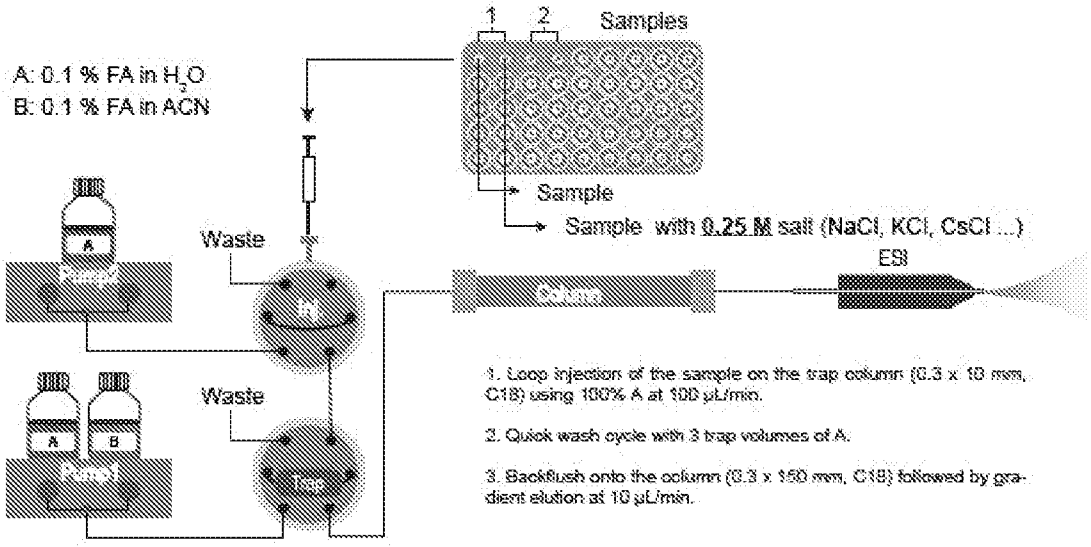


Methods

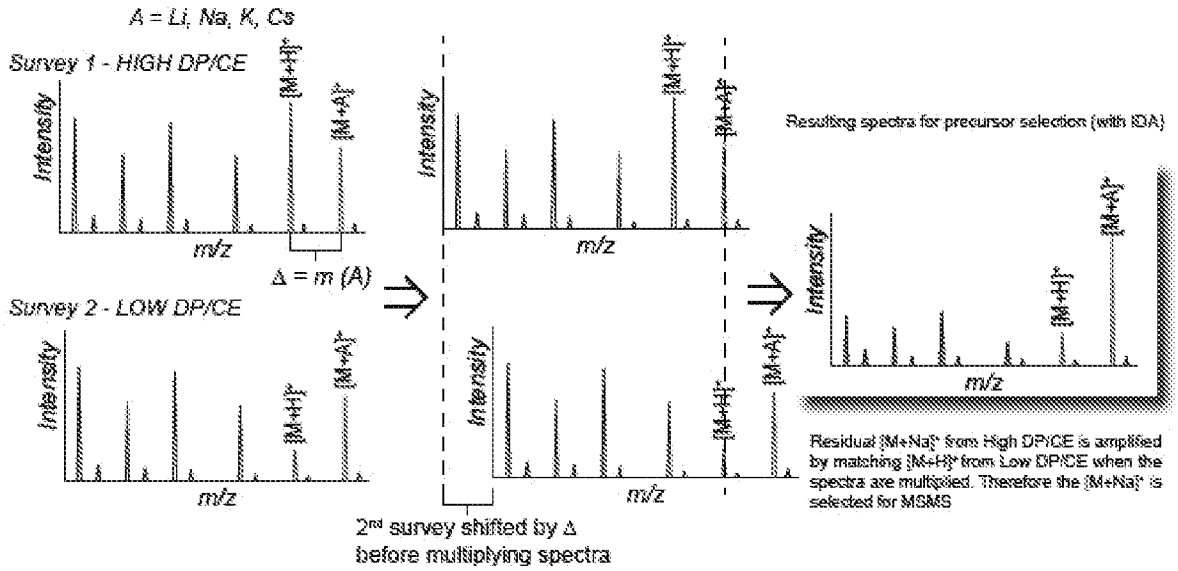
1 Salt infusion a) mixed with mobile phase or b) post-column addition



2 Addition of high salt concentration to the sample and injection on column-switching setup

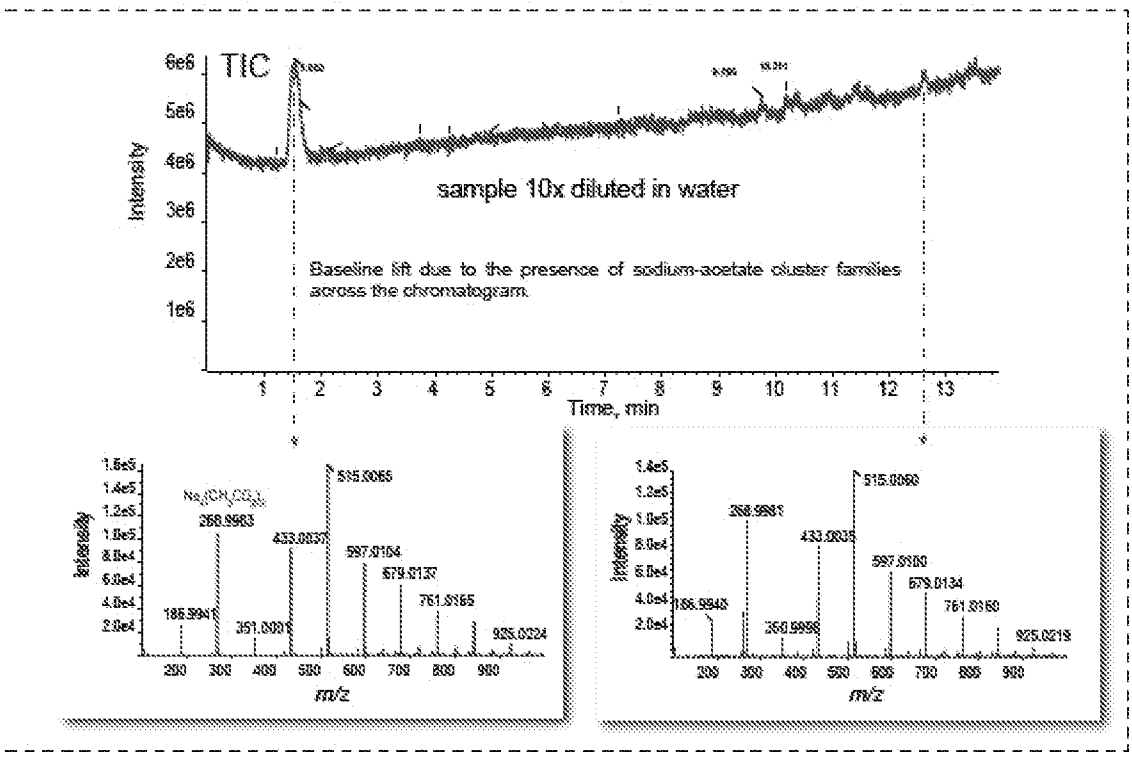


3 Information dependent acquisition (IDA) for non-targeted MS/MS of alkali metal adducts using ECD/CID fragmentation

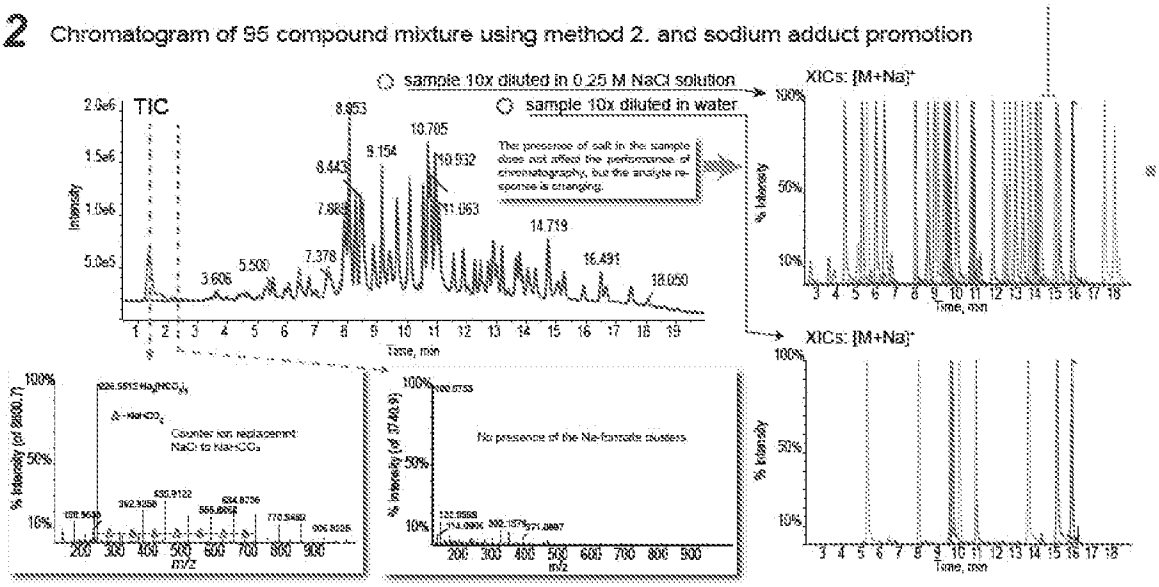


Results

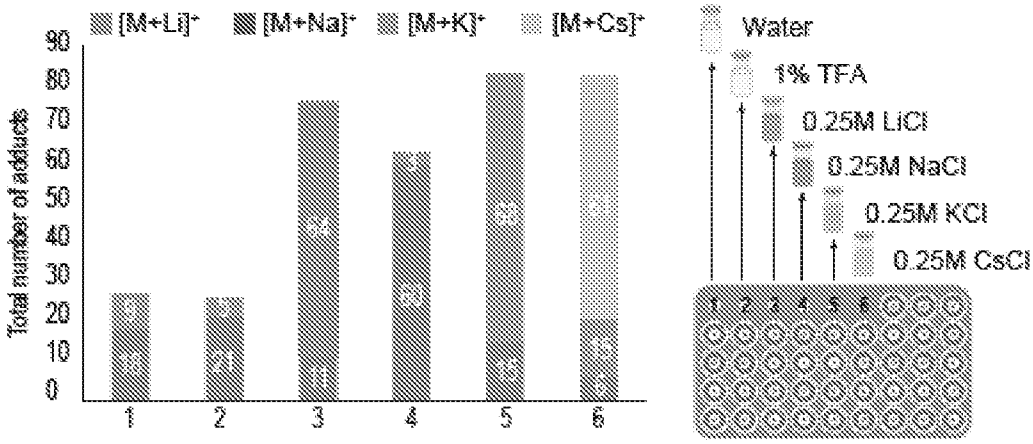
1 Chromatogram of 95 compound mixture using method 1. b)



2 Chromatogram of 95 compound mixture using method 2. and sodium adduct promotion

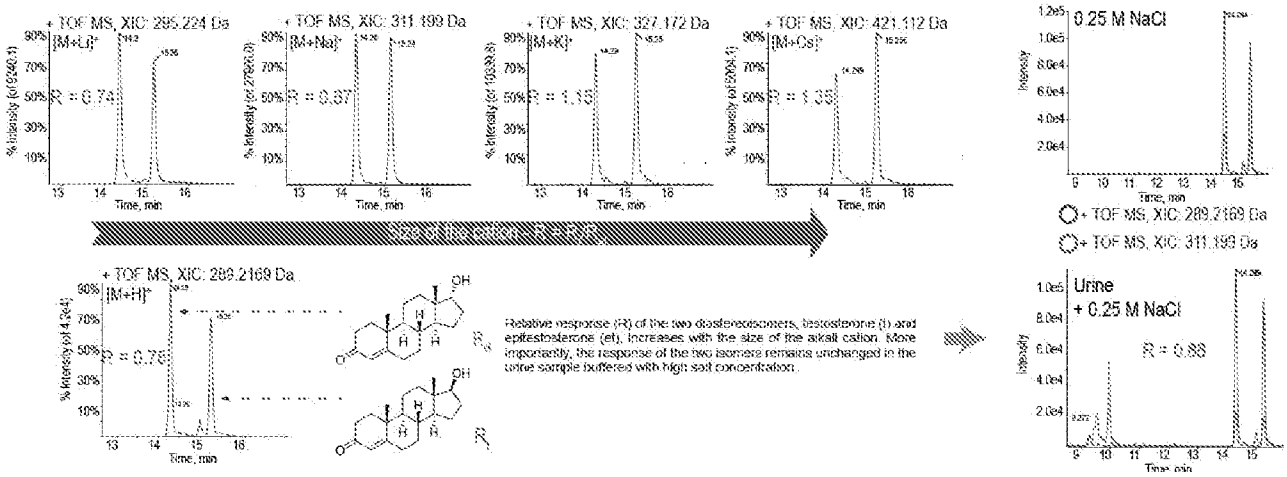


3 Adduct distribution with different alkali salt type

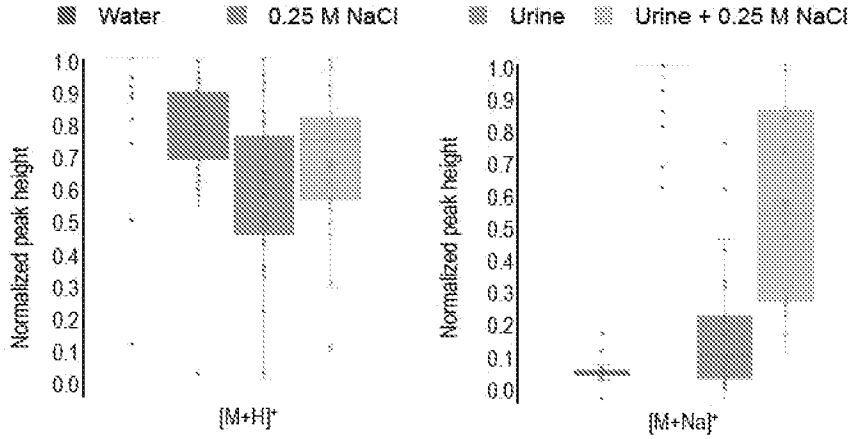


The total number of detected adducts depends on the salt concentration in the sample (85 analytes mixture 10x diluted in the corresponding solution indicated on the graph). Sodium and potassium adducts are present in all samples because of a trace amount in HPLC grade water used for salt stock solutions. Only for samples 4 and 5, the numbers of sodium and potassium adducts are higher, because of spiked salt, respectively. The batch sequence was made as indicated by the sample numbers, and lithium and cesium adducts are only present in samples 3 and 8, which means no carryover.

4 Relative response of diastereoisomers considering the size of the cation

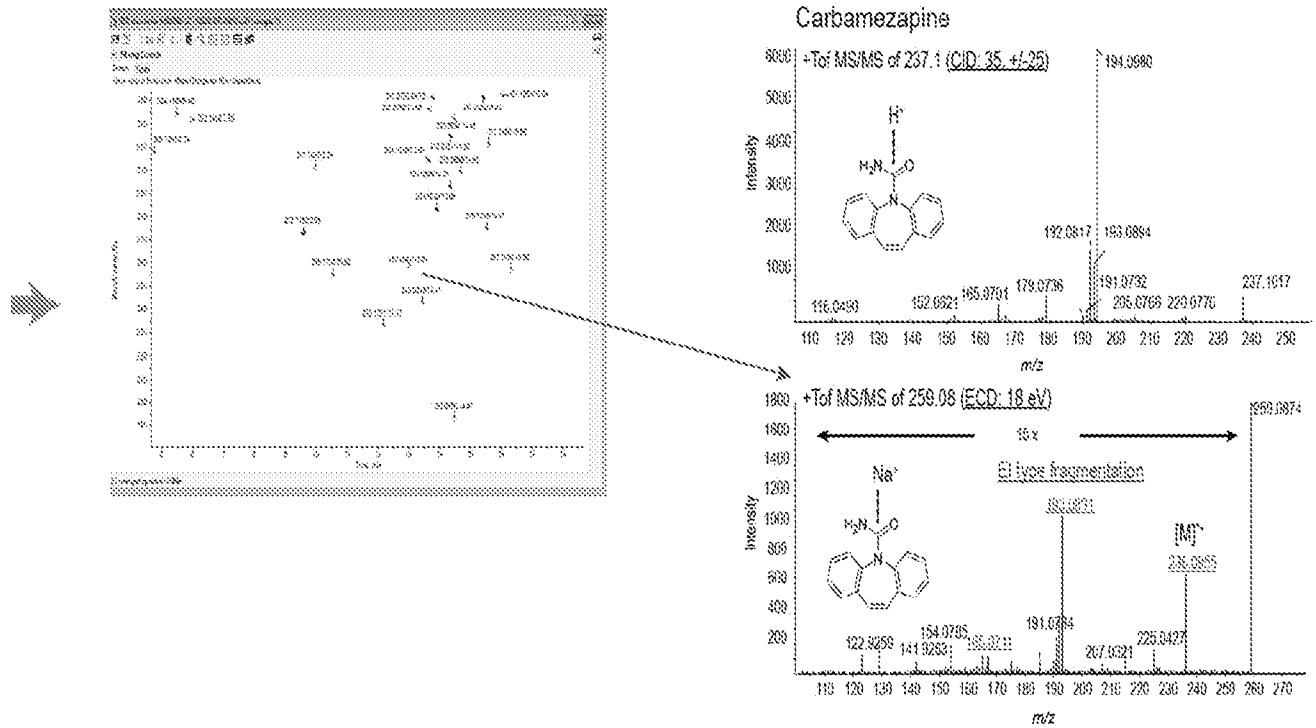


5 Analyte response with different matrices



The relative response of all analytes ([M+H]⁺ left, [M+Na]⁺ right) is normalized and compared for different sample matrix compositions: water, 0.25 M NaCl solution, urine, and 0.25 M NaCl plus urine (indicated with different colors). Considering [M+H]⁺ responses, the sample diluted in pure water has the highest median value, for 0.25 M NaCl the median is 15 % smaller than for water, and the urine shows the smallest median value. A small difference in the median values between 0.25 M NaCl and urine plus 0.25 M NaCl indicates that high excess of salt in the urine buffers the matrix effect. Regarding [M+Na]⁺, a boost in average signal response is observed with the addition of the salt.

6 Colection of MS/MS data in the single run for all Na adducts in the chromatogram - method 3.



Conclusions

- The presence of salt in the sample does not affect the performance of chromatography, but the analyte response is improved.
- The relative response of [M+Na]+ for two diastereoisomers, testosterone and epitestosterone, increases with the size of the alkali cation. This parameter can potentially be used to distinguish isomers.
- The addition of high salt concentration in the sample buffers the matrix effect and allows reproducible adduct formation.

We Claim:

1. A method of performing mass analysis on a liquid sample comprising:
generating an adduct of an analyte in a sample, wherein generating comprises adding a salt solution to the sample to form a diluted sample comprising the adduct of the analyte.

2. A method of performing liquid chromatography/mass spec (LC/MS) comprising: generating an adduct of an analyte in a sample, wherein generating comprises adding a salt solution to the sample to form a diluted sample comprising the adduct of the analyte.

3. The method according to any one of claims 1-2, wherein the adduct of the analyte is an ionic adduct.

4. The method according to any one of claims 1-3, wherein the adduct of the analyte is a metal adduct.

5. The method according any one of claims 1-4, further comprising loading the sample on a trap column, wherein the trap column is in fluid communication with a LC column.

6. The method according to claim 5, wherein adding the salt solution to the sample is performed on the trap column to generate the adduct.

7. A method for generating an adduct of an analyte in a sample for liquid chromatography mass (LC/MS) analysis comprising, contacting a trap column with (i) a first volume comprising the sample, and (ii) a second volume comprising a salt solution, wherein the contacting of the first volume and second volume forms the adduct of the analyte.

8. A method of performing LC/MS analysis comprising,
generating an adduct of an analyte according to one any of claims 1-7; performing LC separation of the sample comprising the adduct of an analyte;

ionizing the sample comprising the adduct of an analyte; and
detecting ions in the sample comprising the adduct of an analyte, or fragments thereof.

9. A method of performing LC/MS analysis comprising,
generating an adduct of an analyte according to any of claims 1-7; performing LC separation of the sample comprising the adduct of an analyte; ionizing the sample comprising the adduct of an analyte;
detecting ions in the sample comprising the adduct of an analyte, or fragments thereof;
acidifying the sample comprising the adduct of any analyte and performing LC separation of the acidified sample that does not comprise an adduct of an analyte;
ionizing the acidified sample; and
detecting ions in the acidified sample, or fragments thereof.

10. The method according to any one of claims 1-9, further comprising Differential ion Mobility Separation (DMS).

11. The method according to any of claims 1-10, further comprising Electron Capture Dissociation (ECD).

12. The method according to any of claims 1-11, wherein the salt solution comprises an alkali metal, an alkaline metal, a transition metal, a halide, or an organic anion.

13. The method according to any of claims 1-12, wherein the salt solution comprises an alkali (Group Ia) or alkaline (Group IIa) metal.

14. The method according to any of claims 1-13, wherein the concentration of the salt solution is at least 200 mM.

15. The method according to any of claims 1-14, wherein the concentration of the salt solution added to the sample is from about 500 mM to about its solvent saturation point.

16. The method according to any one of claims 1-15, further comprising a multi-well sample plate.

17. The method according to any one of claims 1-16, wherein the LC column comprises, an ion-exchange column, a normal-phase column, a reverse-phase column, a hydrophobic interaction column, a size exclusion column, and an affinity column.

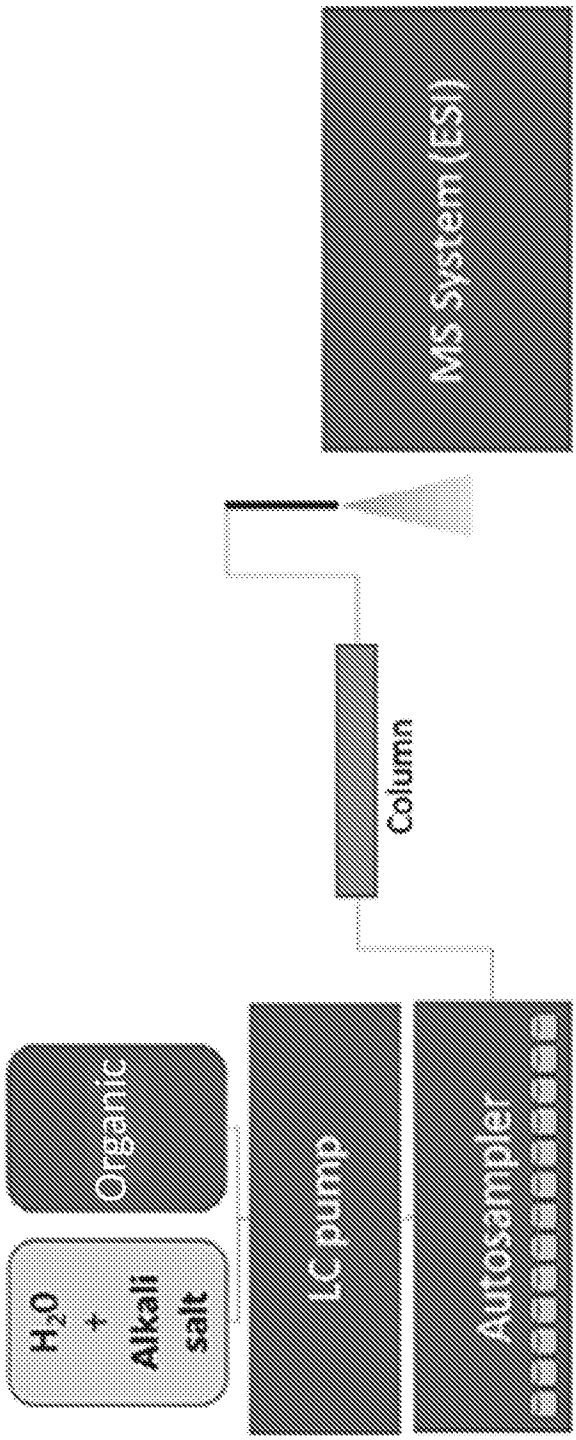

MS System(ESI)
Column
LC pump
Autosampler
Organic
H_2O + Alkali salt

FIG. 1

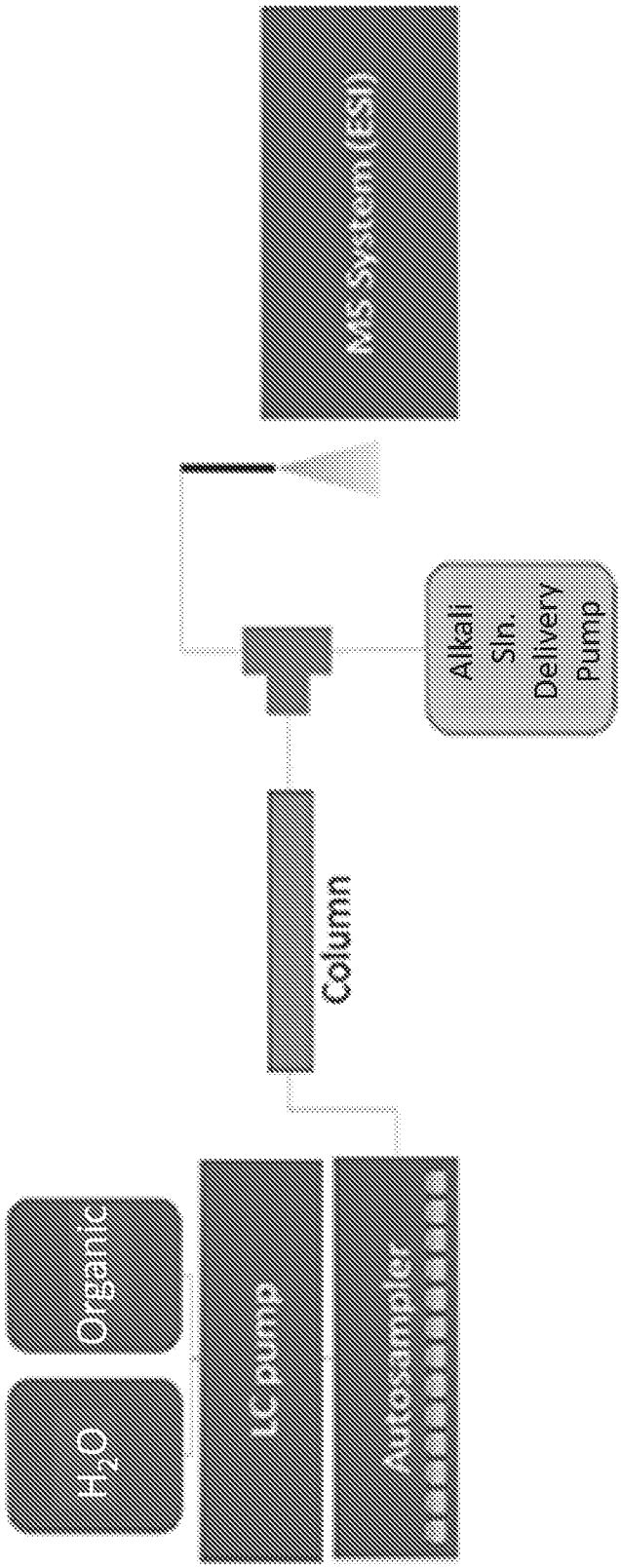

MS System (ESI)
Alkali Sln. Delivery Pump
Column
Organic
H_2O
LC pump
Autosampler

FIG. 2

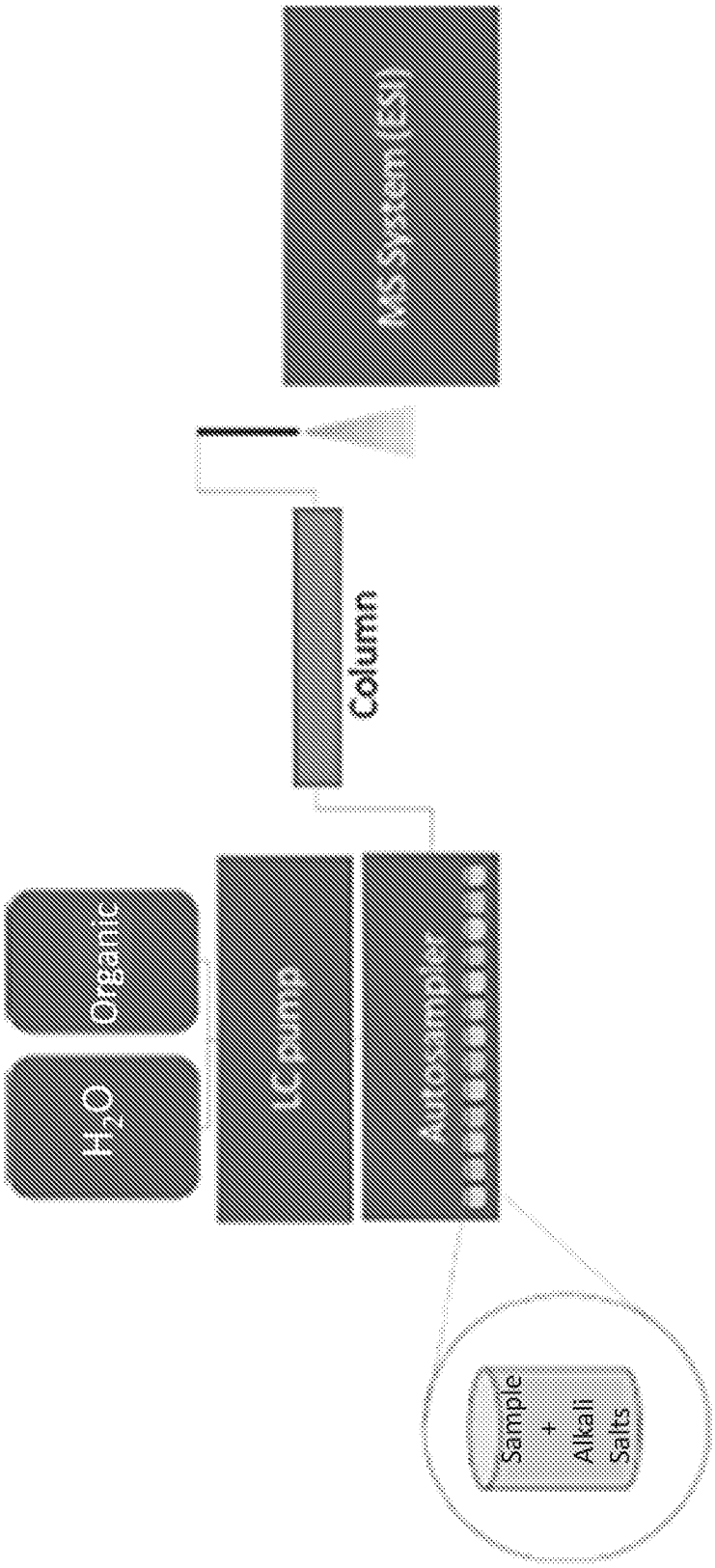

MS System (CSI)
Column
LC pump
Autosampler
Organic
H_2O
Sample
+
Alkali
Salts

FIG. 3

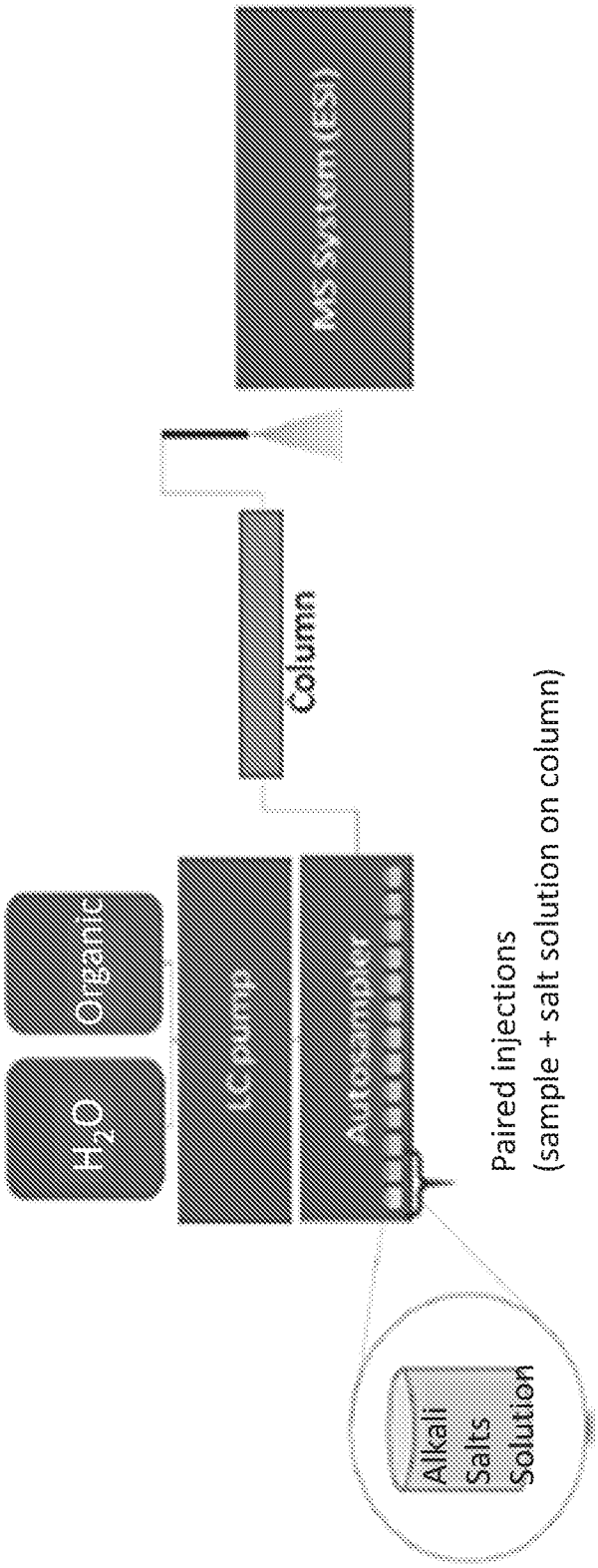

MS System (ESI)
Column
LC pump
Autosampler
Organic
H_2O
Alkali Salts Solution
Paired injections
(sample + salt solution on column)

FIG. 4

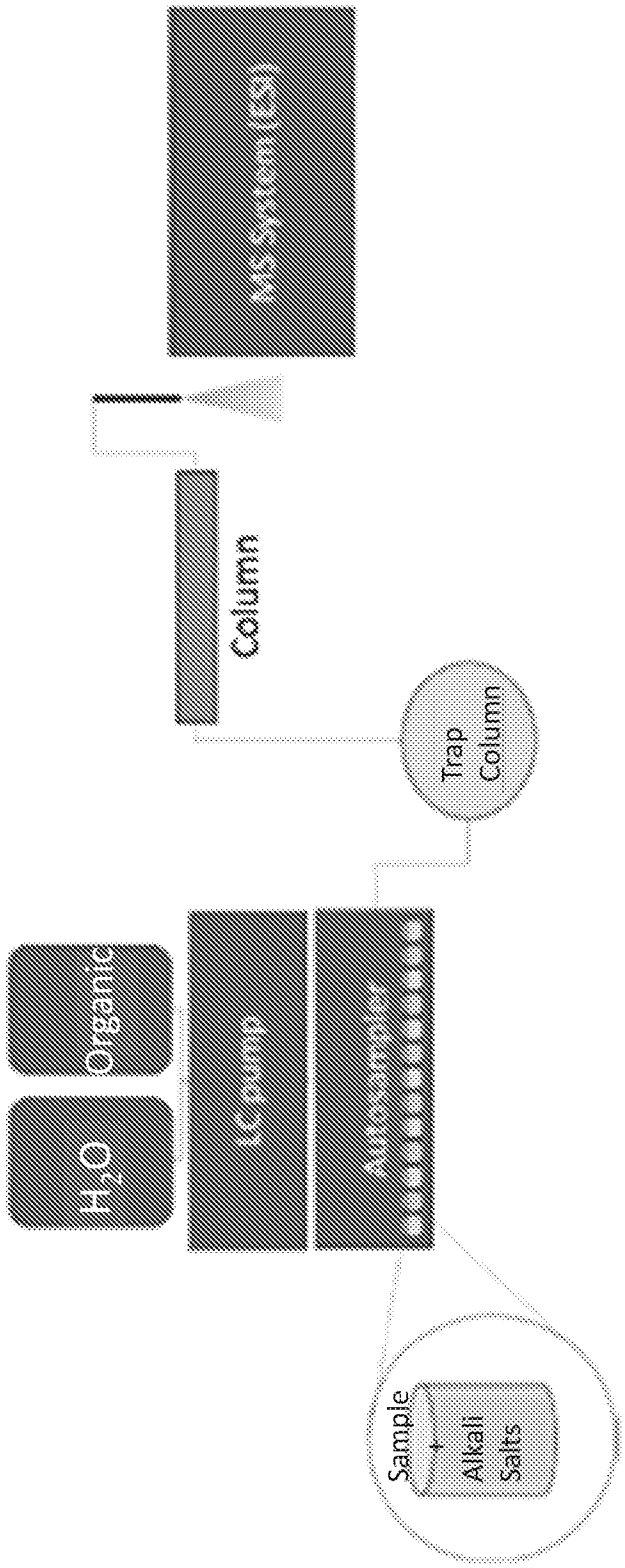

MS System (QE)
Column
Trap Column
Organic
H_2O
Sample
+
Alkali Salts

FIG. 5

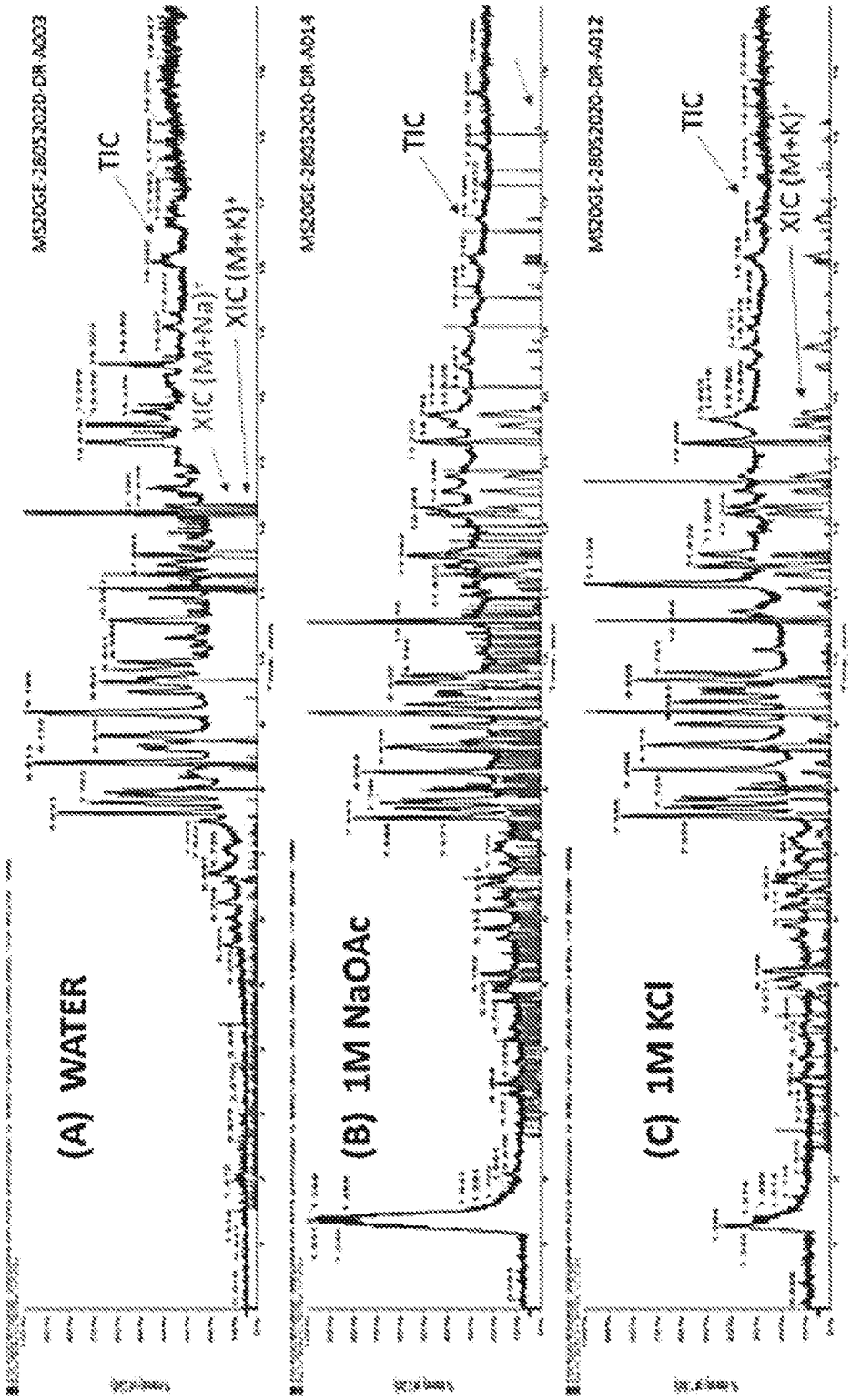


FIG. 6

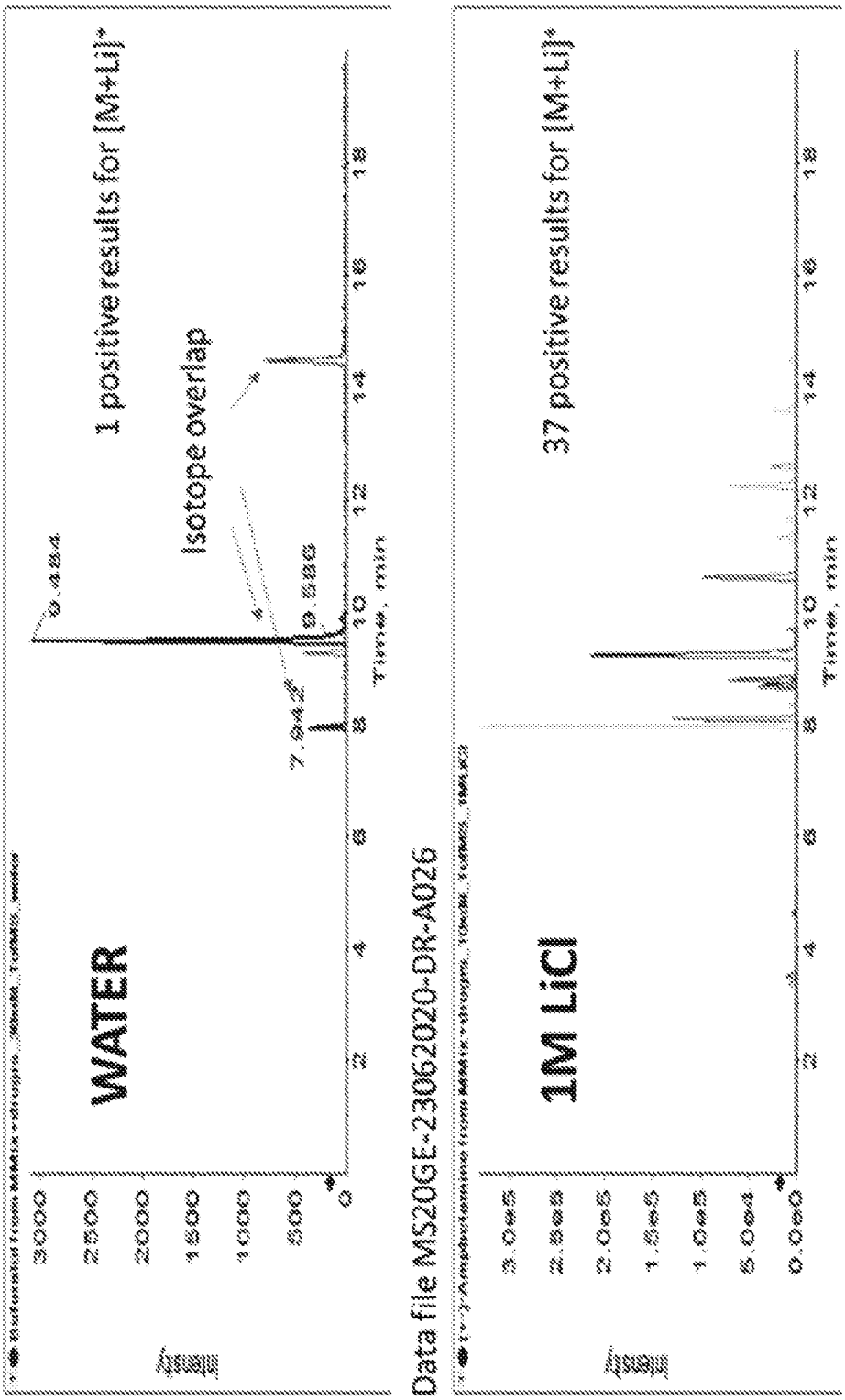

WATER
1 positive results for [M+Li]+
Isotope overlap
9.484
9.886
7.642
Time, min
Data file MS20GE-23062020-DR-A026
1M LiCl
37 positive results for [M+Li]+
Time, min

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/055595

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N30/06 G01N30/72
ADD. H01J49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N H01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TANG YANG ET AL: "Characterization of Isomeric Glycans by Reversed Phase Liquid Chromatography-Electronic Excitation Dissociation Tandem Mass Spectrometry", JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, ELSEVIER SCIENCE INC, US, vol. 29, no. 6, 13 April 2018 (2018-04-13) , pages 1295-1307, XP036885951, ISSN: 1044-0305, DOI: 10.1007/S13361-018-1943-9 [retrieved on 2018-04-13] page 1299, left-hand column, lines 6-35 abstract page 1297, right-hand column, last paragraph page 1297, left-hand column, paragraph 2 page 1296, left-hand column, last paragraph ----- -/--	1-17

[x] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2023	19/09/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Marembert, Vincent

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/055595

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	H. GALLART-AYALA ET AL: "Recent advances in mass spectrometry analysis of phenolic endocrine disruptors and related compounds", MASS SPECTROMETRY REVIEWS, vol. 29, no. 5, 1 September 2010 (2010-09-01), pages 776-805, XP055001862, ISSN: 0277-7037, DOI: 10.1002/mas.20234 page 794, left-hand column, line 31 - right-hand column, line 21	1,2,7-9
A	ZOLLNER P ET AL: "Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography-atmospheric pressure ionisation mass spectrometry", JOURNAL OF CHROMATOGRAPHY A, ELSEVIER, AMSTERDAM, NL, vol. 1136, no. 2, 15 December 2006 (2006-12-15), pages 123-169, XP024967150, ISSN: 0021-9673, DOI: 10.1016/J.CHROMA.2006.09.055 [retrieved on 2006-12-15] page 152, right-hand column, last paragraph	1,2,7-9
A	MORRISON KELSEY A ET AL: "Enhanced Mixture Separations of Metal Adducted Tetrasaccharides Using Frequency Encoded Ion Mobility Separations and Tandem Mass Spectrometry", JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, ELSEVIER SCIENCE INC, US, vol. 28, no. 4, 28 October 2016 (2016-10-28), pages 664-677, XP036199319, ISSN: 1044-0305, DOI: 10.1007/S13361-016-1505-Y [retrieved on 2016-10-28] page 667, right-hand column, last paragraph - page 668, left-hand column, paragraph first	1,2,7-9

1