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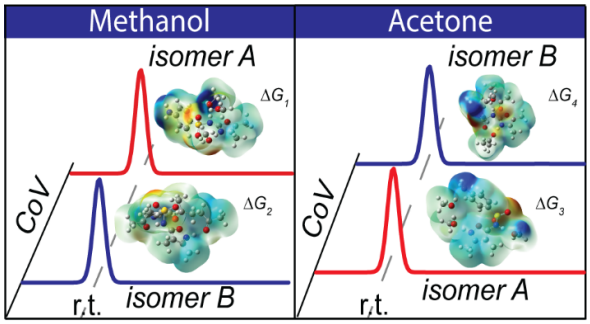
Modifier Selectivity Effect on Differential Ion Mobility Resolution of Isomeric Drugs and Multidimensional Liquid Chromatography Ion Mobility Analysis

ABSTRACT:

Cluster formation in the alternating electric field during differential ion mobility is critical for separation selectivity and is governed by two factors. One is the reduced mass and the other factor is cluster binding energy between an ion and a neutral solvent molecule (modifier). Therefore, separations of isomeric analytes using a modifier can be related to the thermochemistry of the cluster formation, as subtle changes in the molecular structure will affect its charge delocalization and the binding energy with the corresponding modifier will be different. We have examined the relation between calculated Gibbs free energies of the cluster formation and experimental ion mobility measurements (CoV dispersion plots) considering the most prominent ion-modifier interactions: charge-dipole, dipole-dipole and charge-quadrupole. In order to explain selectivity effects due to the modifier, we have selected series of positional isomers of sulfonamide drugs that were analyzed in positive and negative electrospray and the diastereoisomers ephedrine and pseudoephedrine in positive mode. The following modifiers were investigated: water, linear and branched alcohols, acetonitrile, acetone, toluene and ethyl acetate. We could demonstrate a dependence of the separation selectivity of the differential mobility on the reduced mass and Gibbs free energy of the cluster formation. These results are supported by thermochemistry calculations (DFT) and interpreted by molecular modeling. Finally, we describe differential mobility spectrometry selectivity tuning for the multidimensional LCxDMS-MS separation of sulfonamide isomers in human plasma.



Introduction

Ion mobility spectrometry (IMS), which was described by J. Cohen *et al.* in 1970[1] as plasma chromatography, separates ions in the gas phase based on their mobilities in an electric field and enables differentiation of isobaric or isomeric analytes either as a standalone system or coupled to mass spectrometry[2]. In the past two decades IMS has been used for the analysis of small molecules including amino acids, peptides fatty acids, lipids oligonucleotides, carbohydrates and other small molecules[3]. In differential ion mobility spectrometry (DMS), also known as high field asymmetric waveform ion mobility spectrometry (FAIMS), the mobility function (alpha) is based on normalized difference between the high- and low-field mobility of the gas phase ions in the alternating electric field (separation voltage SV) resulting in a radial drift[4] of the ions. To counteract the ion migration toward the electrodes a compensation voltage (CoV) is applied which can be ramped (scanning-DMS) or set to a fixed value (filter-DMS). The alpha curve can be generated by mapping the relationship between SV and CoV. Three different alpha curve shapes have been described which are analyte and modifier dependent: A-type, B-type and C-type[5]. Ions with the same low field mobility can have substantially different high-field mobility depending on the nature of the ion–molecule interactions. For quantitative analysis DMS is often combined with a triple quadrupole mass analyzer operated in selected reaction monitoring mode and DMS acts as an additional selectivity filter[6].

Although ion mobility in DMS depends on the applied electric field[7] and the type of carrier gas (nitrogen, CO_2, helium or a mixture)[8,9], there is growing interest in the application of organic solvent vapor (modifier) to the carrier gas in order to alter ion mobility[10]. Modifier assisted DMS has an additional selectivity dimension to MS and its analytical performance has been reported for different classes of analytes such as isomeric amino acids[11], steroids[12], steroisomeric drugs[13] and drugs of abuse[14].

One theoretical explanation of DMS separation is based on the association/dissociation process between ions and molecules in the gas phase. Using five piperidine analogues as model compounds and 2-propanol, 2-butanol, and cyclopentanol as modifiers Levin *et al.*[15] investigated the molecular dynamics and chemical factors influencing the association/dissociation process and proposed two DMS separation mechanisms, introduced as Core and Façade, corresponding to strong and weak attractions between ions/molecules in the gas phase.

Schneider *et al.*[16] investigated nitrogen, nitrogen with varying amounts of helium, and nitrogen with (1.5% mole ratio) 2-propanol vapor added as a clustering agent with a mixture of 70 and 24 low molecular weight compounds for positive and negative mode, respectively. They found that mixtures of inert gases improve the resolution but have limited effect on the peak capacity or selectivity. On the other hand, the addition of isopropanol enhanced the formation of clusters resulting in an increased peak capacity and separation power. The increase in mobility field dependence was consistent with a cluster formation model, also referred to as the dynamic cluster-decluster model. Further modifiers were studied on a larger set of analytes including: isopropanol, methanol, acetonitrile, acetone, and ethylacetate illustrating that interaction chemistry strongly depends on the properties of the modifier and the nature of the analyte ion [17]. Besides resolution and selectivity benefits, modifiers can also exhibit undesirable effects

such high proton affinity or arcing resulting in significant loss of analyte signal. One way to overcome these limitations is to apply multicomponent modifiers as suggested by Blagojevic *et al.*[18].

Auerbach *et al.*[19] evaluated CoV prediction for compounds using a homologous series of alcohols as modifiers. The prediction was based on linear regression of the compensation voltages versus the square root of the inverse reduced mass, proton affinity and gas phase acidity of the modifier molecules. Comprehensive calculations such as Basin-hopping and density function theory (DFT) are powerful tools for better understanding mobility behavior of analyte/modifier as a function of their structures[20]. Campbell *et al.*[20] described the modifier role in mobility behavior of symmetric and asymmetric tetraalkylammonium ions by calculating the cluster binding energy which supports experimental observations. Improved selectivity and sensitivity has also been demonstrated with non-polar modifiers which can provide the π-π interaction such as benzene, toluene, ethylbenzene, p-xylene, 1,2,4-trimethylbenzene, and naphthalene [21].

For the analysis of complex samples such as biological fluids, addition of IMS to 1D or 2D LC-MS workflows can significantly improve separation performance[22]. The combination of drift time IMS with TOFMS is particularly attractive as it matches the sampling requirement for fast analysis and collision cross section (CCS) libraries can be used in addition to mass-to-charge for compound identification or confirmation[23]. In the case of DMS[24] and FAIMS[25] coupled to TOFMS the DMS device, which acts as a filter, has to be operated in scan mode (scanning-DMS) which was demonstrated for devices with microsecond ion time residences .

In the present work we investigated different modifier effects on differential mobility behavior (dispersion plot) and separation selectivity of isomeric sulfonamide drugs as positional isomers, and stereoisomers such as ephedrine and pseudoephedrine. The following modifier classes were considered in positive and negative mode: i) polar protic with a hydroxyl functional group (homologue series of alcohols from methanol to octanol), ii) polar aprotic with keto- and ester functional groups (acetone and ethyl acetate) and iii) non-polar aromatic (toluene). Calculations using a combination of the molecular-mechanics theory (MM) and density function theory (DFT) with thermochemistry data were applied to rationalize the DMS observations. Finally, this paper describes the combination of liquid chromatography with scanning DMS and high resolution mass spectrometry for multidimensional separation (LC**x**DMS/HRMS). Different modifiers were used to tune selectivity in the DMS dimension to improve the orthogonal separation of isomeric drugs in human plasma.

Materials and Methods

The set of eight sulfonamides drugs and ephedrine isomers used in this study is listed in Table S1. (Supporting Information). A differential ion mobility device (SelexION, AB Sciex, Concord, ON, Canada) was mounted on a QqQ_{LIT} mass spectrometer (QTRAP 5500, AB Sciex, Concord, ON, Canada) and on a QqTOF mass spectrometer (TTOF 6600, Sciex, Concord, ON, Canada). Modifiers were introduced at constant concentration of 1.5 % mole ratio of the nitrogen gas. The following modifiers were studied: methanol, ethanol, 1-propanol, 2-

propanol, 1-butanol, 2-butanol, 1-hexanol, 1-octanol, acetonitrile, ethyl-acetate, acetone and toluene. Acquisition details can be found in Supporting Information. Standards and plasma samples were analyzed either by infusion or with a micro high-pressure liquid chromatography (μHPLC) system (Eksigent-Express HT-Ultra). A Turbo-V Ionspray source was used in positive and negative electrospray ionization (ESI) mode. The change in Gibbs free energy has been calculated as a difference between a product (cluster) and the sum of reactants (charged molecule and modifier). Conformers were calculated using molecular mechanics theory with MMFFaq with a maximum examined conformers of 10`000 (Spartan14). 1% of the lowest energy conformers were kept for further Semi-Empirical calculations using PM6 level of theory. All further calculations have been performed using density function theory (DFT) with the same basis set (B3LYP/6-311g**) including empirical dispersion, GD=3, and the nature of the stationary point is determined by performing frequency calculation (Gaussian 09, Revision D.01). The charge delocalization has been visualized as a heat map using GausView 6.0.16 (Semichem, Inc., KS, 2000-2016) with calculated electron density mapped with electrostatic potential using a Cubegen software (see supporting information: 1) experimental details).

Results and Discussion

The DMS behavior (CoV shift, dispersion plot) of an analyte with a given carrier gas is mostly dependent on its molecular structure, molecular weight and its physico-chemical properties, and challenges the prediction of its CoV[26]. The situation is even more complicated with the addition of modifiers where the choice of the modifier for best resolution is usually based on trial and error experiments. To explore modifier effect on ion mobility behavior and selectivity, a series of ten sulfonamide molecules, separated in five groups (A, B, C, D, E), as a positional isomers and four stereoisomers (1S,2R)-(+)-ephedrine and (1S,2S)-(+)-pseudoephedrine were selected and are shown in Table S1 (Supplemental Info).

Positional isomers of sulfonamide drugs share similar structural motifs, i.e. the p-aminuosufonyl moiety, while the rest of the molecular structure represents the heterocycle aromatic ring with different *o, m* and *p*-positions of the heteroatoms (N or O) and the functional groups (methyl- or methoxy-). Therefore, within one isomeric group, the position of the heteroatom will dictate the charge position and the charge delocalization by inductive and resonance effects. On the other hand, ephedrine and pseudoephedrine have the same charge positions and slightly less difference in inductive effects, but more importantly, conformational arrangements of the cluster around the charge are different.

In positive mode, protonation will occur on one of the nitrogen atoms as the heterocycle ring of all examined sulfonamides is the most basic structural moiety of the molecule. The protonation site was determined using DFT calculation with the basis set B3LYP/6-311g** by sampling different protomers for each heteroatom of the molecule (Supplemental Info Tables S2-S12) and the site with the lowest potential energy (PE) was selected. The attribution is further supported by product ion spectra with the presence of the fragment *m/z* 126.04 assigned to the protonated fragment containing the heterocycle ring (Supplemental Info Figure S11). Also, it is reported that the DMS can separate different protomers[5] but we have not observed two peaks for any single analyte under any modifier conditions, suggesting that only one

protonation form is predominant. When the polarity in ESI is changed to the negative mode, deprotonation takes place on the sulfonamide N-H group as the most acidic and the charge is located around this group for all sulfonamide molecules (supporting information, Tables S13-S21).

The Effect of alcohol chain lengths and modifier molecular geometry on CoV of sulfonamide drugs

When a solvent vapor is introduced in the DMS cell, the CoV shift becomes more negative depending on the SV and the intrinsic modifier physicochemical properties (molecular mass, structural geometry and the functional group), that determines its clustering ability with an ion.

Figure 1 shows dispersion plots for sulfameter (A1) in positive mode (Figure 1A) and negative mode (Figure 1B). The DMS temperature was kept constant for all experiments and set to 150°C. When pure nitrogen was used, bulk temperature and field-induced heating do not allow any ion-nitrogen clustering and both polarities exhibit C-type of differential mobility behavior (alpha curve) according to a collisional-deflection mechanism[5].

When using higher mass alcohols such as 2-propanol, 1-butanol, 1-hexanol or 1-octanol the observation of the more negative CoV is in good agreement with the proposed model for dynamic clustering-declustering mechanism. The mobility of an ion is smaller at low field than at the high field potion of the waveform up to maximum SV= 4000 V and the ion exhibits A-type behavior.

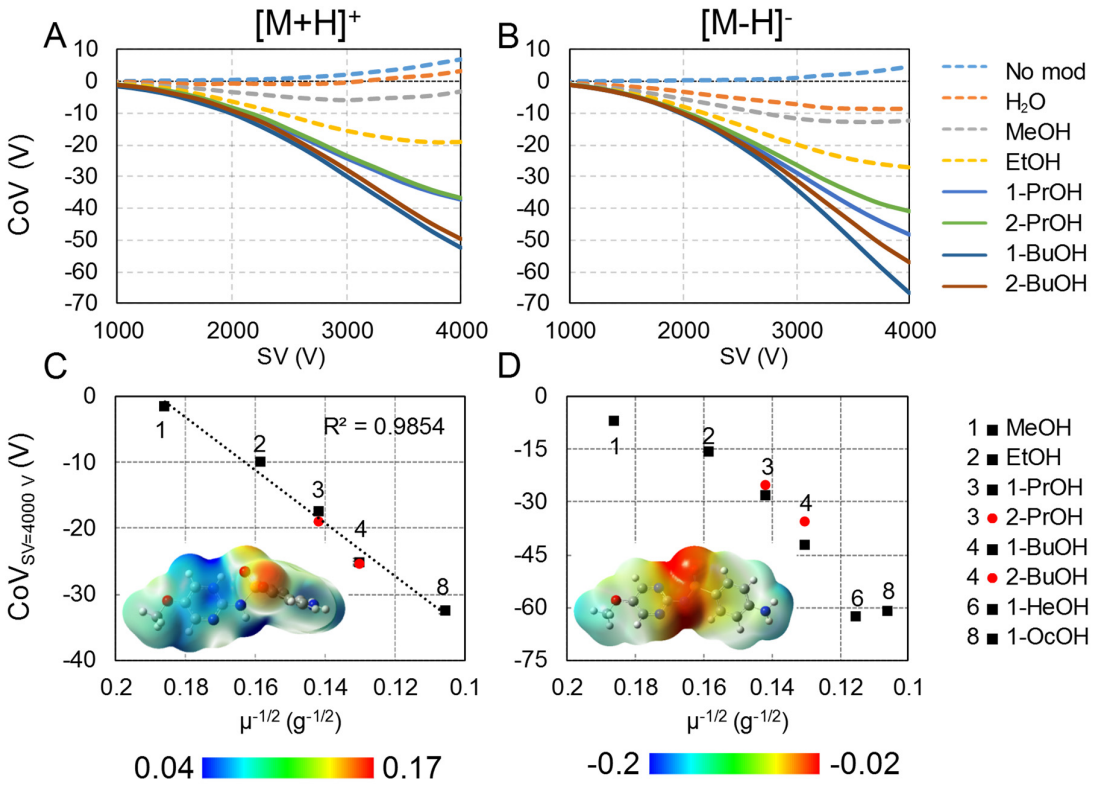


Figure 1. Dispersion plots for sulfameter A1 A) in positive and B) negative ESI mode using modifier series from water to ethanol and isomeric modifiers 1-PrOH/2-PrOH, 1-BuOH/2-BuOH. CoV vs $\mu^{-1/2}$ plots C) positive mode and D) negative mode. Triplicate analysis with CV < 0.1V . SCF electron density mapped with electrostatic potential for both, positive and negative ions. Region with high electron density (red) and low electron density (blue).

	$[A1+H\cdots L]^+$			$[A1-H\cdots L]^-$		
L	ΔH (*kJ/mol*)	ΔS (*J/mol*K*)	ΔG (*kJ/mol*)	ΔH (*kJ/mol*)	ΔS (*J/mol*K*)	ΔG (*kJ/mol*)
1-BuOH	-89.12	-173.96	-37.25	-100.56	-172.27	-49.19
2-BuOH	-84.61	-154.25	-38.63	-91.81	-165.19	-42.56

Table 1: Thermochemistry data for A1 single-solvent cluster formation in positive and negative ESI mode

When water, methanol or ethanol is introduced to the carrier gas (1.5% mole ratio), both positive and negative ions show B-type of differential mobility behavior. This is explained as a combination of dynamic clustering-declustering (A-type) and collisional-deflection (C-type). Therefore, as can be seen from dispersion plots, the CoV shift is negative until certain point where curve deflects towards positive value. Liu *et al.* showed for water and methanol that the deflection point, where for a given SV a CoV reach a minimum, can be correlated with the cluster binding energy [27]. In both polarities, the deflection point (Figure 1A and 1B) is moving towards higher SV values through the series of modifiers from water to ethanol indicating that the cluster binding energy of the ion-methanol is higher than ion-water and so on.

The comparison of CoVs at the maximum SV shows that for the negative mode, the CoVs are 20 V more negative than for positive mode for linear chain alcohols (1.5% mole ratio), which supports the idea that anion-neutral molecule interaction is stronger than the cation-neutral molecule, particularly for the A1 isomer. In order to explain the observation, we considered a single-solvent cluster and the binding energy that comes from the strongest interaction cation-dipole or anion-dipole. Although it is likely that there are larger solvent cluster forms within the DMS environment, there is no existing model that can predict how many solvent molecules are bound to an ion under DMS conditions, but it is expected that the first binding modifier molecule will give the highest contribution to the cluster binding energy. The difference in the Gibbs free energy for the cluster formation, with respect to 1-BuOH, is 11.9 *kJ/mol* more negative in the negative mode than in the positive mode (Table 1). When isomeric alcohols (1-propanol/2-propanol or 1-butanol/2-butanol) as the chemical modifier are compared, the protonated molecules show a smaller difference in CoVs of ~Δ2V, while deprotonated molecules show a more apparent difference of ~Δ10 V. According to the thermochemistry data, the difference in the Gibbs free energy between 1-BuOH and 2-BuOH in the negative mode is 6.6 *kJ/mol*, while in the positive mode is 1.4 *kJ/mol*. This indicates that the steric interaction around hydroxyl group of the branched chain alcohol will destabilize the ion-modifier cluster, and is more pronounced in negative mode. The results of the other sulfonamide isomers series (C, D, E) are in good agreement with that obtained for A (Supplemental Infos Figure S1-S2) suggesting that the observations are modifier and not analyte dependent.

Correlation of modifier reduced mass with CoV of the positional isomers

In order to investigate dependence of the CoV shift and the square root of the inverse reduced mass ($\mu^{-1/2}$), we extended the alcohol series up to 1-octanol and the experimental conditions were the same between two ESI polarities. The temperature was set at 225°C to match the b.p. of 1-octanol (b.p.=188°C). Note that the water molecule is not included because it does not show the linear fit with the alcohols in the positive mode. In addition, there are differences between water and alcohols in acidity, Van der Waals dispersion force and steric interaction due to the presence of the hydrocarbon moiety of the alcohols.

Figure 1C (positive ESI mode) shows the CoV dependence on $\mu^{-1/2}$ (calculated for a one-to-one cluster) and with increasing chain length in alcohol series (from methanol to 1-octanol) as DMS modifiers, a linear relationship between CoV and $\mu^{-1/2}$ is apparent. In addition, the CoVs between isomeric modifiers are very close, 1-PrOH/2-PrOH and 1-BuOH/2-BuOH, respectively. This confirms the finding of Auerbacher *et al.*[19] which showed CoV value can be correlated with reduced mass of the analyte-modifier series.

Figure 1D (ESI negative) illustrates CoV change with $\mu^{-1/2}$ with the same alcohol series including 1-hexanol. In this case, two "non-linear trends" of the CoVs with $\mu^{-1/2}$ are apparent. First one is with linear chain alcohols (black squares on the graph) and second one between methanol, ethanol and branched alcohols including 1-octanol (red circles on the graph). Furthermore, opposite to positive mode, the differences in CoVs between isomeric alcohols are more obvious. The non-linear trend of the CoVs with $\mu^{-1/2}$ indicates that in addition to the modifier mass, there is an effect of the steric-shield on ion-modifier molecule cluster binding energy. The same behavior was observed for all investigated isomeric groups as shown in supporting information Figures S3 and S4.

The rational explanation for above-mentioned observations can be understood as follows. Protonated aromatic ring of the sulfonamide molecule has a rigid and planar geometry enabling the formation of a cation-hydrogen bond with hydroxyl group of the alcohol. Further extension in the alcohol chain length (linear or branched) does not significantly affect the cluster stability and therefore the CoV is linearly dependent on $\mu^{-1/2}$. The negative charge is delocalized on the sulfonamide group and it is surrounded by relatively bulky groups, p-aminosulfonyl and one heterocycle ring. For a cluster which is formed between an anion and a neutral molecule, it is necessary to consider the free rotation around the S-N bond and the S-C bond. Consequently, the number of possible cluster assemblies will be smaller for branched alcohols than for normal chain alcohols.

DMS mobility inversion with modifier functional group

Effect of modifiers on resolution of isomers

The dispersion plots of the two positional isomers C1 and C2 using acetone and methanol, representing common differential mobility behavior of all model compounds, are shown in Figure 2. With acetone both isomers exhibit A-type behavior, where the C1 isomer shows higher clustering ability than C2. However, when the carrier gas composition is changed to methanol, inversion of the DMS dispersion plots is observed, where C1 now displays B-type

behavior and C2 shows higher clustering ability with methanol than C1. The two charged isomers differ by the position of the nitrogen in the heterocyclic ring resulting in different inductive and resonance effects. The different CoV for both isomers can only be explained by cluster stability.

In order to justify the DMS selectivity inversion based on the one charged analyte molecule and one modifier molecule hypothesis, we extended the calculations for cluster size of three modifier molecules. The cluster global minimum structures in Figure 2 show the most important interactions between ionized C isomers and three modifier molecules. The geometries with potential global minima have been calculated after determining the charge position and by sampling different conformers around each heteroatom (Supplemental Infos Tables S22-S27). The initial structures for the conformational search were estimated by taking into account the most prominent charge-dipole, dipole-dipole interactions.

As supported by our calculations, all ions exhibit the same type of ion/modifier interactions, cation hydrogen-heteroatom bond (N – H*O) between the protonated nitrogen atom of the heterocycle ring (N) and the oxygen atom of the modifier, and hydrogen bonding between the sulfonamide N-H and modifier heteroatom (O). In addition to cation-hydrogen bond interaction, the second ion-dipole (sulfonamide N-H and modifier dipole) interaction also depends on the charge vicinity, which affects the cluster stability. By inductive or resonance effects of N-H the protonated sulfonamide group will be more acidic compared to the neutral molecule and the proton donation ability of this group will increase, resulting in more negative ΔG. To demonstrate more stable cluster upon allosteric protonation, i.e. of the $C2(MeOH)_3$, we calculated the Gibbs free energies for the non-charged cluster formation $[C2\cdots(MeOH)_3]$ (ΔG = -25.04 *kJ/mol),* and for the charged cluster formation $[C2+H\cdots(MeOH)_3]^+$, (ΔG = -103.81 *kJ/mol)* (Supplemental Info Table S26).

Figure 2 presents the general ranking in the Gibbs free energies of the cluster formation and its correlation with dispersion plots between two isomeric molecules C1 and C2; i) acetone C1 : -113.31 *kJ/mol*, C2 : -100.75 *kJ/mol* and ii) methanol C1 : -74.85 *kJ/mol*, C2: -103.81 *kJ/mol* (enthalpy, entropy and internal energies data are provided in Supplemental Info Tables S19-S21). The thermochemistry data is in good agreement with selectivity inversion and the rationale can be understood as follows. Since the Gibbs free energy is correlated with the reaction quotient (Q) or the thermal equilibrium (K_{eq}) of the chemical interaction, one might expect that with more negative Gibbs free energy values the position of the equilibrium will be shifted towards the cluster formation.

In conclusion, for isomeric compounds, and more generally for any analyte, with a given modifier it is the thermochemistry of the cluster formation which dictates the CoV shift and therefore the selectivity between two analytes.

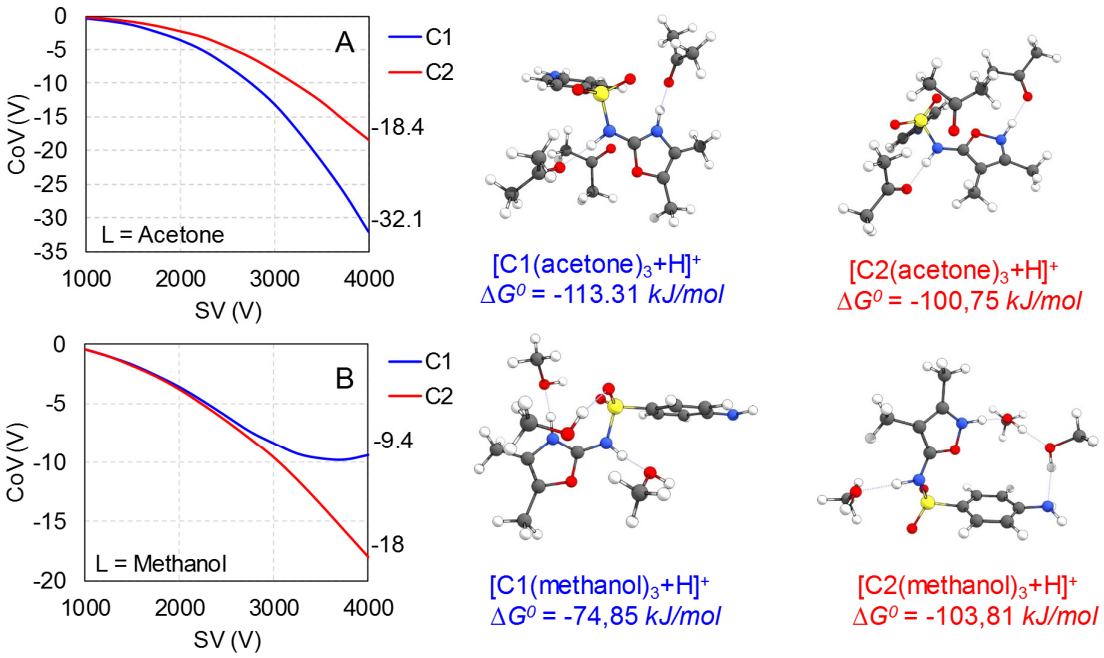


Figure 2. Dispersion plots for C1 (blue) and C2 (red) using A) acetone and B) methanol as modifiers. Three dimensional structures at the global minimum of the ion-neutral molecule cluster with Gibbs free energies, calculated at the standard conditions.

Diastereoisomers have the same positions of the functional groups but with different arrangements in the three-dimensional space, i.e. hydroxyl-group on C-1 and secondary amine on C-2. Figure 3 shows the dispersion plots for the two diastereoisomers (1S,2R)-(+)-ephedrine (Eph) and (1S,2S)-(+)-pseudoephedrine (PsEph) with toluene (1.5% mole ratio) vapor in the nitrogen carrier gas. The charge is located on the protonated nitrogen atom, where cation-quadrupole (cation-π) interaction is established with the aromatic ring of the toluene. In order to generate a cation-π interaction, aromatic ring of the toluene has to approach orthogonally to the proton. Because of the molecular size of the toluene and the nature of chemical interaction, it will sterically interact with adjacent functional groups. Therefore, the clustered isomeric ions such as Eph and PsEpH will have different dihedral angels (N-C1-C2-O). In addition, the hydrogen-bond between protonated amine and vicinal hydroxyl group will stabilize the cluster depending on the dihedral angles that determine the internal hydrogen-bond length. The global minimum structures (Figure 3) show one-solvent clusters between cation and the toluene. As can be seen, the PsEph-toluene cluster can form an internal hydrogen bond (1.98 Å, dihedral angle N-C1-C2-O = 47.32°), while the Eph-toluene complex has a greater distance between the protonated amine and the oxygen of the hydroxyl group (2.14 Å) because it has a bigger dihedral angle N-C1-C2-O = 56.98°. The Gibbs free energy for the cluster formation of PsEph-toluene cluster is ~13 *kJ/mol* more negative than Eph-toluene cluster. Similar to the previous

explanation of the positional isomers C1 and C2, the more negative Gibbs free energy correlates with the more negative CoV.

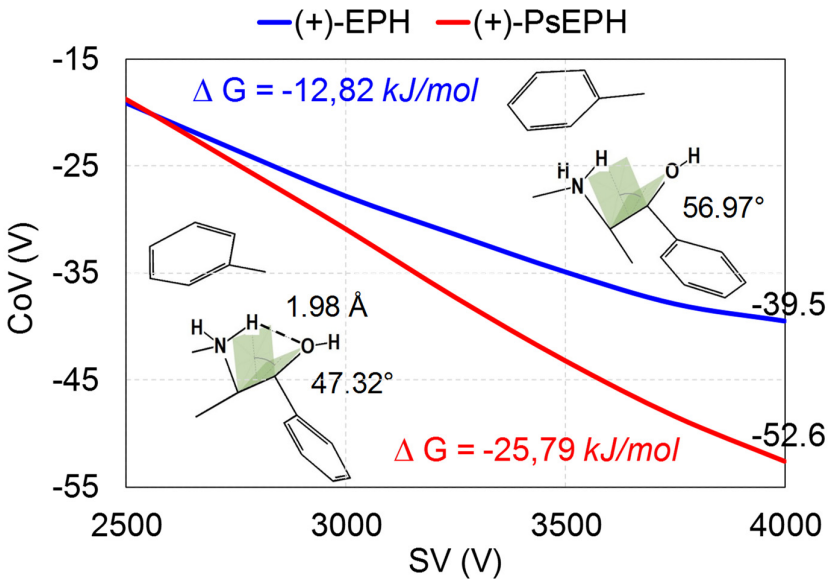


Figure 3. Dispersion plots for (+)-ephedrine and (+)-pseudoephedrine using toluene as modifier. Three dimensional structures at the global minimum of the ion-neutral molecule cluster with Gibbs free energies

DMS resolution dependence on analyte charge state and on modifier mass

In this paragraph we demonstrate an effect of the ESI polarity on DMS selectivity, as well as the relationship between the absolute CoV differences for two isomers and $\mu^{-1/2}$. The absolute difference in CoVs is an important factor for both peak capacity and resolution, but it is also important to point out that there is a slight difference in average peak width at half maximum, under different modifier conditions. Figure 4 (A and B) presents the inversion of the DMS selectivity for three isomers (A1, A2 and A3) by changing ESI polarity between positive (Fig.4 A) and negative (Fig.4 B) mode, using 1-octanol as modifier. Figures 4C and fig. 4D as well as fig. 4E and fig. 4F show relationship between $|CoV_{A1}- CoV_{A3}|$ and $\mu^{-1/2}$ and $|CoV_{A2}- CoV_{A3}|$ and $\mu^{-1/2}$ respectively with respect to the ESI polarity. Note that in the positive ESI mode the hexanol data point was not included due to the complete loss of the signal. It is interesting that all three isomers (A1, A2 and A3) individually showed a linear relationship with $\mu^{-1/2}$ in positive ESI mode, fig.1C, but here there is no correlation between absolute CoV differences of the selected isomeric pairs and $\mu^{-1/2}$ (fig.4C and fig.4E). Also, if we consider isomeric modifiers i.e. 1-BuOH and 2-BuOH, we have seen that there is almost no difference in CoVs between two isomeric modifiers for individual analytes (fig.1C), but here the difference in the $|CoV_{A1}- CoV_{A3}|$ between 1-BuOH and 2-BuOH is significant. In contrast, in the negative mode (fig.4D and fig.4F) a linear relationship between i.e. $|CoV_{A1}-$

CoV_{A3} | and $\mu^{-1/2}$ (fig.4D). is apparent, from ethanol to 1-octanol, which is different to the non-linear behavior of individual analytes in the alcohol series (fig. 1D). Regarding the positive ESI mode, since protonation takes place on the aromatic ring and because of the charge delocalization that depends on the position of the nitrogen atom within one isomeric group (i.e. A), it will highly affect cluster binding energy. Therefore, the absolute difference in CoVs is more dependent on the analyte structure than on the modifier mass and consequently the peak capacity and resolution will be affected in the same way. On the other hand, in the negative ESI mode, all isomeric analytes show the same charge position on the sulfonamide group (electron density map, Supplemental Info Figure S10) and the clustering will be similar between ethanol and octanol and the absolute difference in CoVs will linearly depend on $\mu^{-1/2}$.

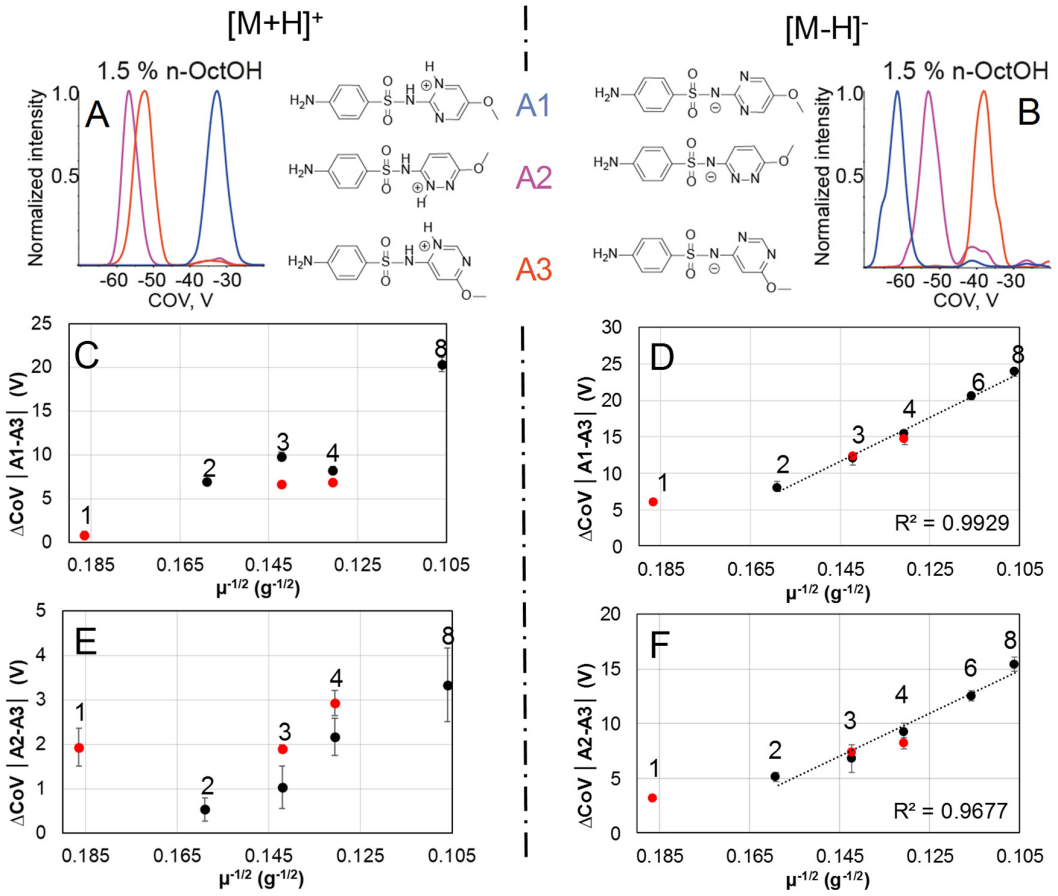


Figure 4. DMS separation of positional isomers A1, A2 and A3, A) positive and B) negative ESI polarityThe DMS ionograms are recorded at SV = 4000 V. ΔCoV|A1-A3| versus $\mu^{-1/2}$ plots, C) positive and D) negative ESI mode. ΔCoV|A2-A3| versus $\mu^{-1/2}$ plots, E) positive, and F) negative ESI mode (triplicate analysis).

The limited peak capacity of liquid chromatography and the challenge to separate isobaric analytes by mass spectrometry limits the application of LC-MS for analysis of endogenous and exogenous compounds. LC Peak capacity can be improved by applying comprehensive two-dimensional liquid chromatography systems (LCxLC) with different separation mechanisms, but at the cost of analysis time. Drift Time Ion Mobility Spectrometry has gained of interest as an additional separation dimension, in combination with high-resolution mass spectrometry, for the analysis of complex mixtures but the selectivity is restricted to the collision cross-section of the analytes. However, DMS with assistance of the different organic modifiers, that have been shown herein to improve the selectivity, shows promising application for profiling using LCxDMS/TOFMS. Therefore, such applications can substantially benefit from selectivity tuning in the mobility dimension, by changing the carrier gas composition instead of changing column packing material or mobile phase composition in the LC dimension. Further, the analysis time can be certainly improved, because the DMS full-scan rate takes up to 1 s for a DMS CoV window size of 40 V, which corresponds to one point of the chromatographic peak.

Figure 5, illustrates the LC-DMS-HRMS analysis for sulfonamide drugs in a human plasma sample for different modifiers. The separations in the DMS dimension are presented as a heat maps with different carrier gas compositions, where it shows the separation orthogonality between the LC and the DMS. The red spots represent peak position with the peak maxima. For a particular case of the A-group isomers, at a given LC condition, it is challenge to separate A1 and A2 isomers while A3 is base line separated in the LC dimension. Note that the SV values are not the same between different experiments, because SV is adjusted for the best separation under any particular set of the experiment. Also, the product ion scan of the A isomers are identical, with respect to the fragmentation profile (see supplemental info, Figure S6), which means that the product ion scan or SWATH data acquisition will not help selective analyte detection. However, as can be seen from the Figure 5, the A1 and A2 isomers are base line separated in the DMS dimension by using 2-propanol (1.5% mole ratio) as chemical modifier. For other chemical modifiers, acetone (1.5% mole ratio) does not provide separation, while acetonitrile (1.5% mole ratio) gives separation close to base line. Moreover, when a chemical modifier is employed, it can remove chemical background interferences (blue smearing line on the heat map) that could originate from LC mobile phase, sample or the DMS chemical modifier.

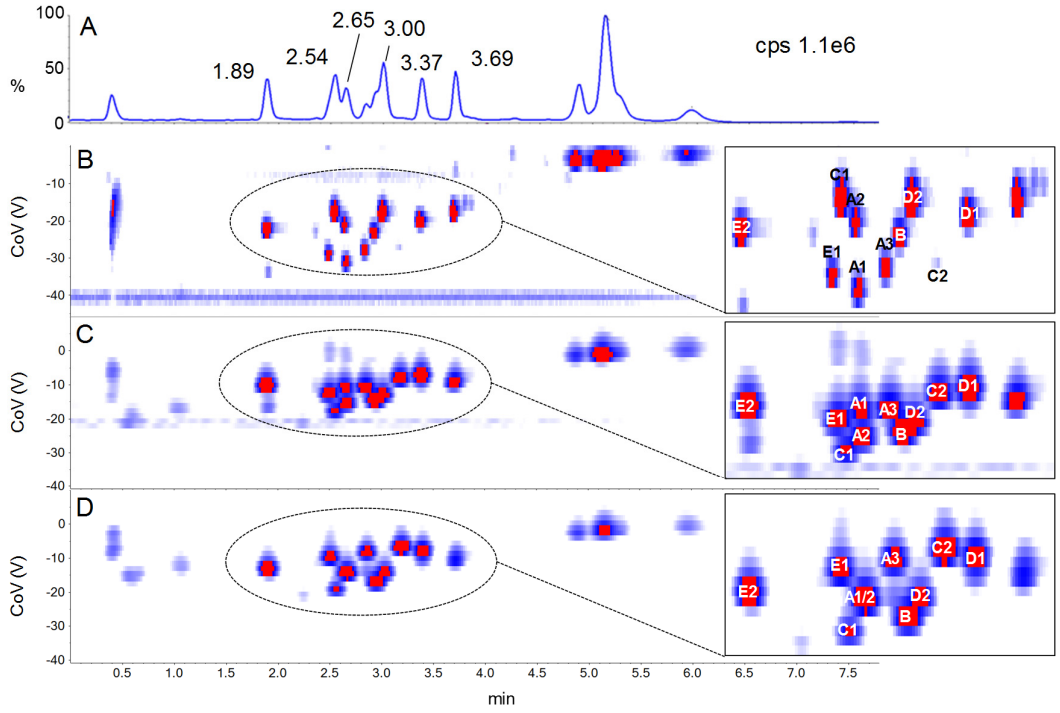


Figure 5. A) Total ion current chromatogram in human plasma. Heat maps of LCxDMS-TOFMS separations with different modifiers (1.5% mole ratio, in the nitrogen carrier gas) B) isopropranol, C) acetonitrile D) acetone. SV values were 3500V for isopropanol is, 3200 V for acetonitrile and for acetone 3250 V.

Noteworthy, beyond the DMS advantages regarding selectivity and ability for fast selectivity tuning, is evident loss in absolute signal, which is also analyte dependent, when a chemical modifier (1.5% mole ratio) is used, due to the ion solvent molecule charge transfer. However, it does not always lead to lower absolute sensitivity, in fact the DMS ability to reduce noise as above mentioned can improve *S/N* and overall sensitivity. Also, it is important to consider DMS peak capacity, which can be very different with different modifiers. Therefore, it is important to correctly optimize the DMS window scan range and the DMS step sizes, because the different modifiers have different CoV shifts, and the DMS duty cycle will be smaller as the DMS scan window size is bigger (similar to quadrupole mass analyzer). Ultimately, this study shows that ion molecule interactions in the DMS are important to consider in order to compromise between DMS resolution and the MS sensitivity. For example, 1-octanol as chemical modifier (Figure 4) shows the best DMS separation in negative ESI mode, but had the highest signal loss in both ESI polarities compared to all investigated modifiers.

Conclusions

In this study, we have investigated apolar and polar modifier effects in DMS separation in the positive and the negative mode through a set of isomeric analytes. Beside the reduced mass effect of the modifier, which has already been reported[19], there is a significant contribution of

the thermochemistry of the cluster formation between an ion and a neutral molecule. Modifiers with different functional groups, such as alcohols versus ketones or esters, display an inversion of the DMS ionograms, due to the different types of interactions with the charged analytes resulting in a change of differential mobility behavior. Regarding modifier effects on the CoVs absolute difference between isomeric analytes, two scenarios were observed with alcohols as modifier: i) in positive mode there is a high dependence on the sub-structural differences of the analytes besides the modifier size and ii) in the negative mode the absolute difference is linearly dependent on the $\mu^{-1/2}$ due to identical charge sites within the isomeric group of analytes. Toluene as a modifier showed a different type of interaction (cation-π) and its size brought a significant steric effect on the DMS resolution, which is especially the case for the separation of the (+/-) - ephedrine and pseudoephedrine diastereoisomers. These results are supported by calculations (thermochemistry) and molecular modeling.

Overall, these findings rather bring intuitive understanding of the modifier effects on the DMS separation than a quantitative assessment of each factor including $\mu^{-1/2}$ and cluster binding energy. This is an important step in the understanding of DMS behavior to avoid trial and error approach for the modifier choice in a method development. Also when using scanning DMS as an additional separation dimension to LC, different modifiers allow for orthogonal selectivity in the second dimension without the challenge of analyte refocusing from the first separation into the second separation dimension. In addition, fast DMS scanning (1s cycle time), gives enough points across the chromatographic peak for a quantitative analysis. This opens potential applications in omics for enhanced QUAL/QUANT analysis.

Acknowledgments

The authors are indebted to Yves, J.C LeBlanc, Brad Schneider and Larry Campbell (Sciex) and Ron Bonner for valuable discussions and input. The authors are grateful to Nico Ricardi and Tomasz Wesolowski (University of Geneva) for support on computational calculations. CCS values for sulfonamide drugs were kindly provided by O. Schmitz (University of Duisburg-Essen). GH would like to thank the Swiss National Sciences Foundation for the financial support: Project 206021_170779.

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Modifier Selectivity Effect on Differential Ion Mobility Resolution of Isomeric Drugs and Multidimensional Liquid Chromatography Ion Mobility Analysis

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Experimental details

Materials and Methods

Chemicals

The drug mix consisted of sulfameter (A1) > 99%, sulfamethoxypyridazine (A2) > 99%, sulfamonomethoxine (A3), sulfachlorpyridazine (B), sulfamoxole (C1) > 95%, sulfisoxazole (C2) > 99%, sulfadimethoxine (D1) > 99%, Sulfadoxine (D2), sulfadimidine (E1) > 99%, sulfisomidine (E2), D-(+)-(1S,2R)-Ephedrine ((+)-Eph) > 99%, L-(-)-(1R,2S)-Ephedrine ((-)-Eph) > 98%, (+)-(S,S)-Pseudoephedrine ((+)-PsEph) 98%, (-)-(R,R)-Pseudoephedrine ((-)-PsEph) 98% all purchased from Sigma Aldrich (Buchs, Switzerland). Following modifiers, all from Sigma Aldrich (Buchs, Switzerland),wereused: HPLC grade MeOH, ethanol (EtOH), 1-propanol (1-PrOH), 2-propanol (2-PrOH), 1-butanol (1-BuOH), 2-butanol (2-BuOH), 1-hexanol (1-HexOH), 1-octanol (1-OctOH), acetonitrile (ACN), ethyl-acetate (AcOEt), acetone and toluene.

Formic acid (FA) was provided by Merck (Darmstadt, Switzerland) and HPLC grade water was obtained using a Milli-Q gradient A10 system (Millipore, Bedford, MA).

DMS-MS/MS infusion experiment

A differential ion mobility device (SelexION, AB Sciex, Concord, ON, Canada) was mounted on a QqQ_{LIT} mass spectrometer (QTRAP 5500, AB Sciex, Concord, ON, Canada). A Turbo-V Ionspray source was used for positive and negative electrospray ionization (ESI) mode with voltages of 5 kV and 4 kV, respectively. The temperature of the source was of 250 °C, the nebulizer gas (GS1) was set to 20 psi and drying gas (GS2) at 0 psi. The DMS separation voltage (SV) was stepped from 0 to 4000 V by steps of 250 V. The compensation voltage (CoV) was ramped from -70 V to +20 V by steps of 0.25 V. The DMS resolution was switched off and the curtain gas (carrier gas) was of 10 psi. The DMS cell temperature was constant for all infusion experiments and it was of 225 °C (DT medium). Modifiers were introduced at constant concentration of 1.5 % mole ration of the nitrogen gas. Infusions of four drug mix, Mix1 (A1, B1, C1 and (+)-Eph), Mix 2 (A2, B2, C2 and (-)-Eph), Mix 3 (B3, D1 and (+)-PsEph) and Mix 4 (D2 and (-)-PsEph) (~500 ng/ml in 0.1 % FA water/MeOH (1:1, v/v)) were performed at 7 µl/min. The MRM transitions of all analytes are given in Supplemental Info in the supplemental) in both ESI polarities

µ-HPLCxDMS-MS

A micro high-pressure liquid chromatography (µHPLC) system (Eksigent-Express HT-Ultra) was used. The system was equipped with a Stack Cooler DW (Eksigent-CTC) and an autosampler (Eksigent-CTC). Mobile phase A consisted of water with 0.1 % FA, mobile phase B consisted of ACN containing 0.1 % FA. Chromatographic separation was achieved in gradient mode at a flow-rate of 30 µl/min on a Fused-Core HALO C18 column (100 x 0.5 I.D, particle size of 2.7 µm). The gradient started after 0.5 min from 5 % B to 95 % B in 7 min. and hold for 0.5 min. The injection volume was of 1 µl and column temperature was set to 25°C.

A differential ion mobility device (SelexION, Sciex, Concord, ON, Canada), was mounted on a QqTOF mass spectrometer (TTOF 6600, Sciex, Concord, ON, Canada). A µ-electrospray capillary of 50 µm (I.D.) was mounted on Duo-Ionspray source operated in positive electrospray (ESI) polarity at a voltage of 5 kV. The temperature of the source was of 200 °C, the nebulizer gas (GS1) was set to 20 psi, drying gas (GS2) of 15 psi. A calibration solution for positive APCI time-of-flight calibration was delivered with an integrated pump system after every fifth sample injections. The DMS was operated in scan mode at fixed SV=3500 V. A variable DMS window size (CoV ramp range) was adjusted with respect to the carrier gas composition; when pure nitrogen gas was used the CoV range was 20 V - -15 V; when any of the gas phase modifier was introduced at 1.5 % mole ration of the nitrogen gas in the DMS, the CoV range was -45 V – 0 V. That means 35 CoV steps by 1.5 V, that corresponds to 35 TOFMS full scans (100 *m/z* – 1000 *m/z*). The accumulation time was of 25 ms with settling time of 5 ms, which counts in total ~ 1s cycle time for exp. The DMS resolution was switched off and the curtain gas (carrier gas) was of 25 psi. The DMS cell temperature was constant for all LC x DMS - MS experiments and it was of 150 °C (DT low). Modifiers were introduced at constant concentration (1.5% mole ratio) in the nitrogen gas.

Pooled blank plasma samples (1 mL) were spiked with 20 µl of drug mix to a final concentration of 1.428 µg/mL. Protein precipitation was achieved by adding 1 mL of ACN to 500 µl spiked plasma, vortex 10 s and centrifuged for 15 min (14 000 r.p.m). 1 ml of the supernatant was evaporated and reconstituted in 1 ml of 0.1 % FA in CH_3CN/H_2O (v/v; 20/80).

Computational calculations

The change in Gibbs free energy has been calculated as a difference between a product (cluster) and the sum of reactants (charged molecule and modifier). All calculations have been performed using density function theory (DFT) with the same basis set (B3LYP/6-311g**)[1-4], and the nature of the stationary point is determined by performing frequency calculation (Gaussian 09, Revision D.01, M. J. Frisch at all, Gaussian, Inc., Wallingford CT, 2013.). After initial minimization, all heteroatom candidates have been protonated/deprotonated in order to obtain the suitable ions for the interaction with modifier. The one with lowest electronic energy has been chosen as charge position of a molecule in the electrospray for further investigation. The charge position can be critical for this investigation due to the high difference in thermochemistry and it was assumed that charge density of a molecule stays intact during the DMS clustering-decluttering mechanism. The charge delocalization has been visualized as a heat map using GausView 6.0.16 (Semichem, Inc., KS, 2000-2016) (with calculated electron density mapped with electrostatic potential using a Cubegen). Cube files were generated in a formatted checkpoint files for both, electron density and electrostatic potential. The density and potential type was taken as SCF. A value for number of the points per side in the cube was 0. Based on the hit map, a search for potential global minima of a cluster were sampled by orienting modifier's dipole for each heteroatom where the charge delocalization takes place. The conformational distribution were evaluated using Molecular Mechanics with MMFFaq theory with maximum examined conformers of 10`000 (Spartan14) and a 1% of the lowest energy conformers were kept for further Semi-Empirical calculations using PM6 level of theory. A number of the conformers with potential global minima were further trimmed down to 30 percent of the lowest energy. Then, a DFT calculation was employed for the selected conformers. Also, the procedure was repeated for different cluster number from one to three and those that had positive change in Gibbs free energy were not considered. Graphics of the molecules were made using Chemcraft 1.5.

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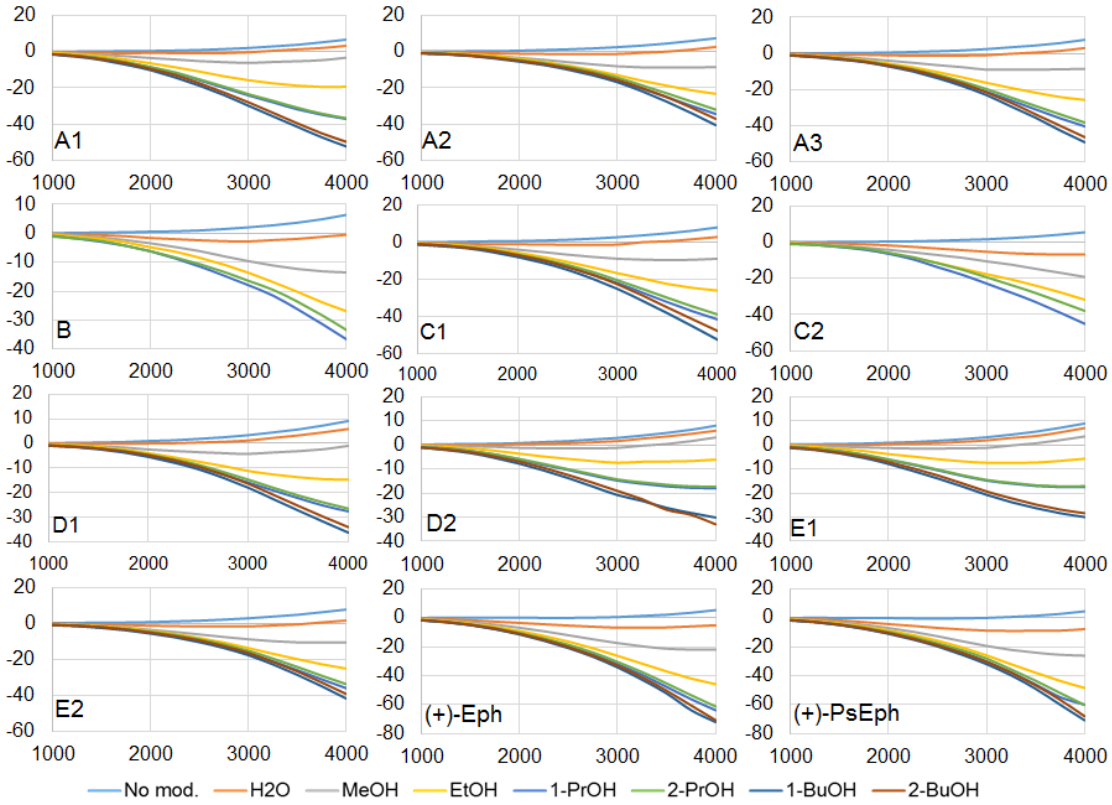


Figure S1: CoV dispersion plots of each analyte depicted on the graph (positive ESI mode), without modifier and with modifier series from water to 2-BuOH shown in the legend.

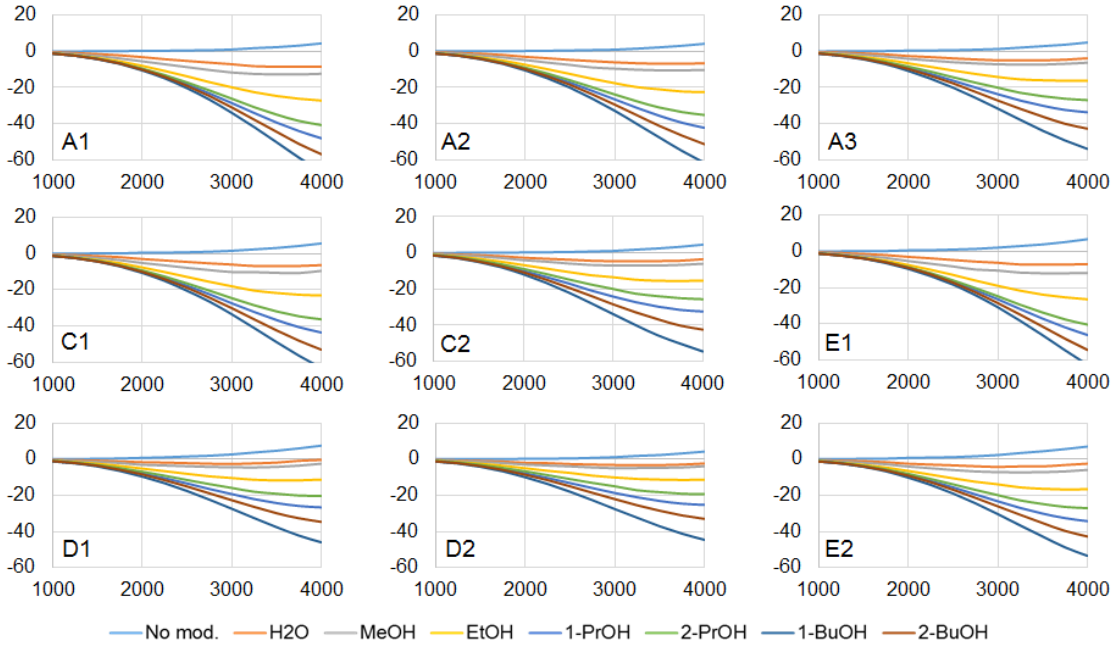


Figure S2: CoV dispersion plots of each analyte depicted on the graph (negative ESI mode), without modifier and with modifier series from water to 2-BuOH shown in the legend.

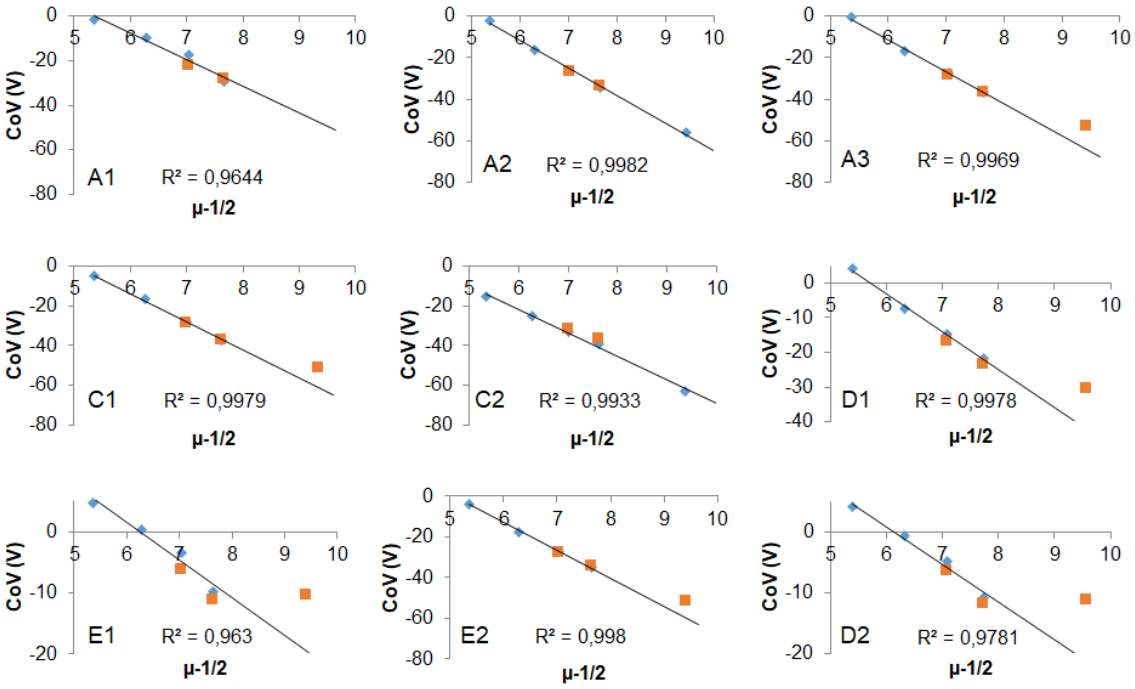


Figure S3: Linear regression of the CoV with square root of the invers reduced mass for linear alcohols (MeOH – OcOH) in positive ESI mode. Outlier points correspond to the branched alcohols (2-PrOH, 2-OcOH and OcOH).

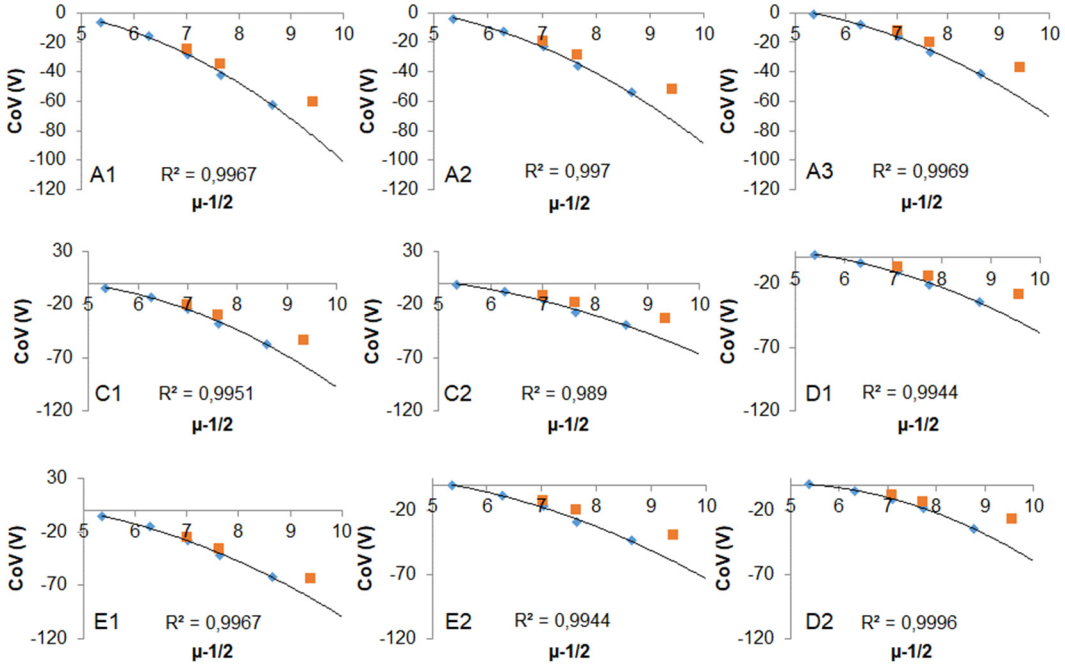


Figure S4: Polynomial regression of the CoV with square root of the invers reduced mass for linear alcohols (MeOH – HexOH) in negative ESI mode. Outlier points correspond to the branched alcohols (2-PrOH, 2-OcOH and OcOH).

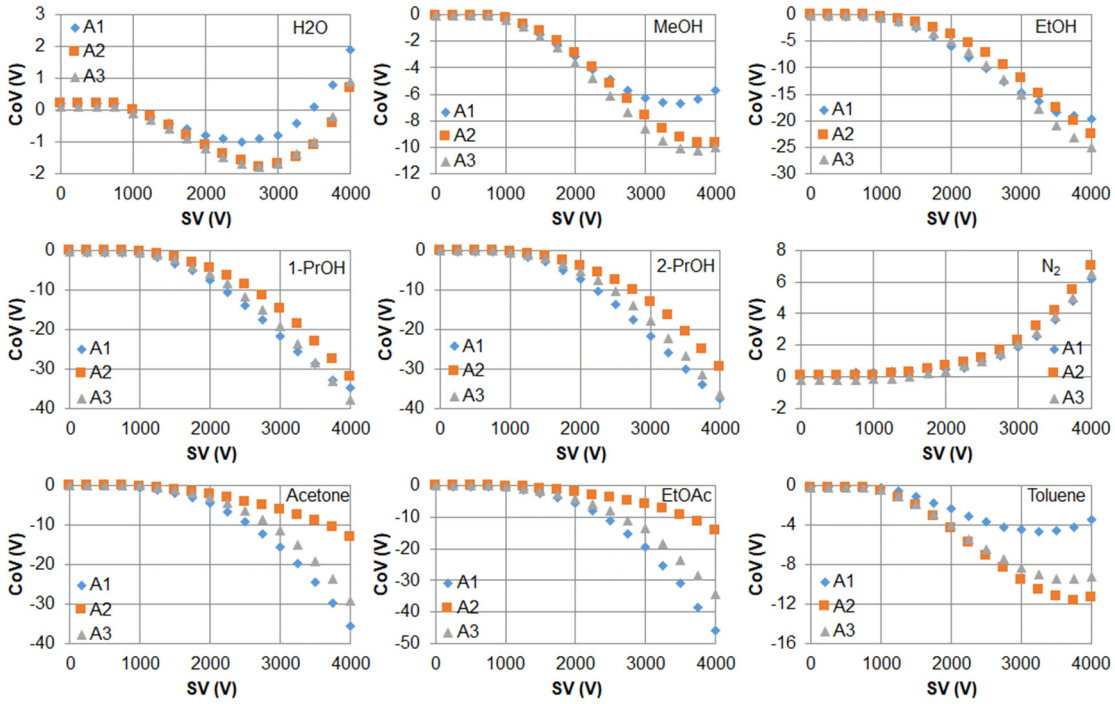


Figure S5: CoV dispersion plots of the three isomers A1, A2 and A3 with corresponding modifier depicted on the chart (positive ESI mode).

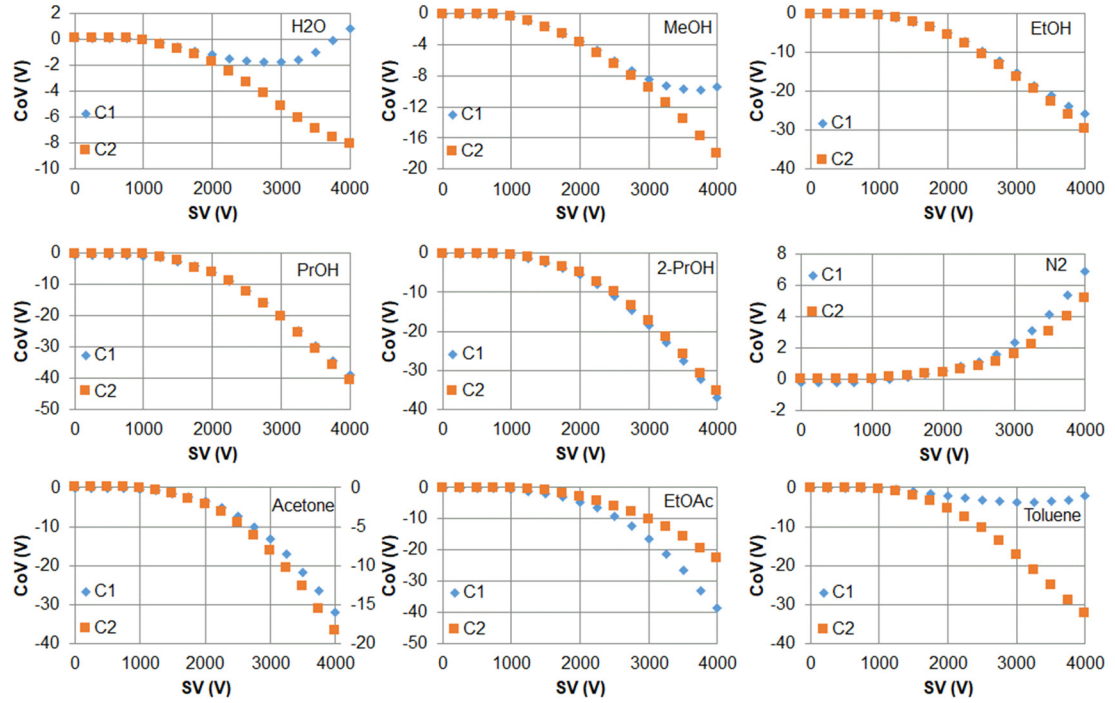


Figure S6: CoV dispersion plots of the two isomers C1 and C2 with corresponding modifier depicted on the chart (positive ESI mode).

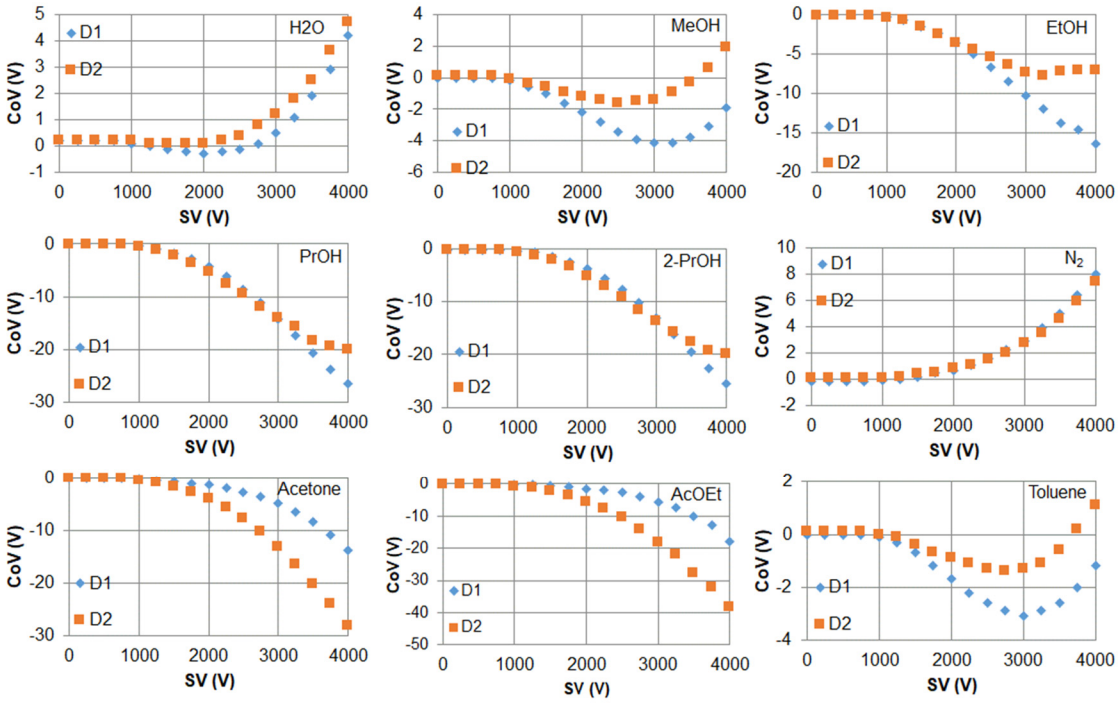


Figure S7: CoV dispersion plots of the two isomers D1 and D2 with corresponding modifier depicted on the chart (positive ESI mode).

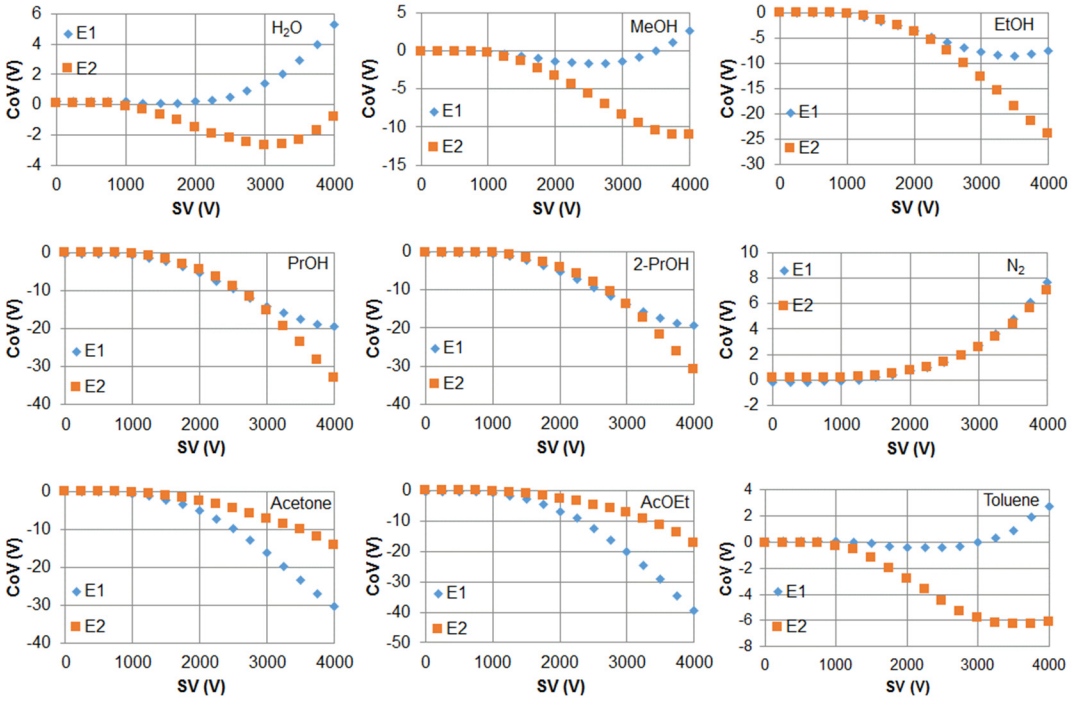


Figure S8: CoV dispersion plots of the two isomers E1 and E2 with corresponding modifier depicted on the chart (positive ESI mode).

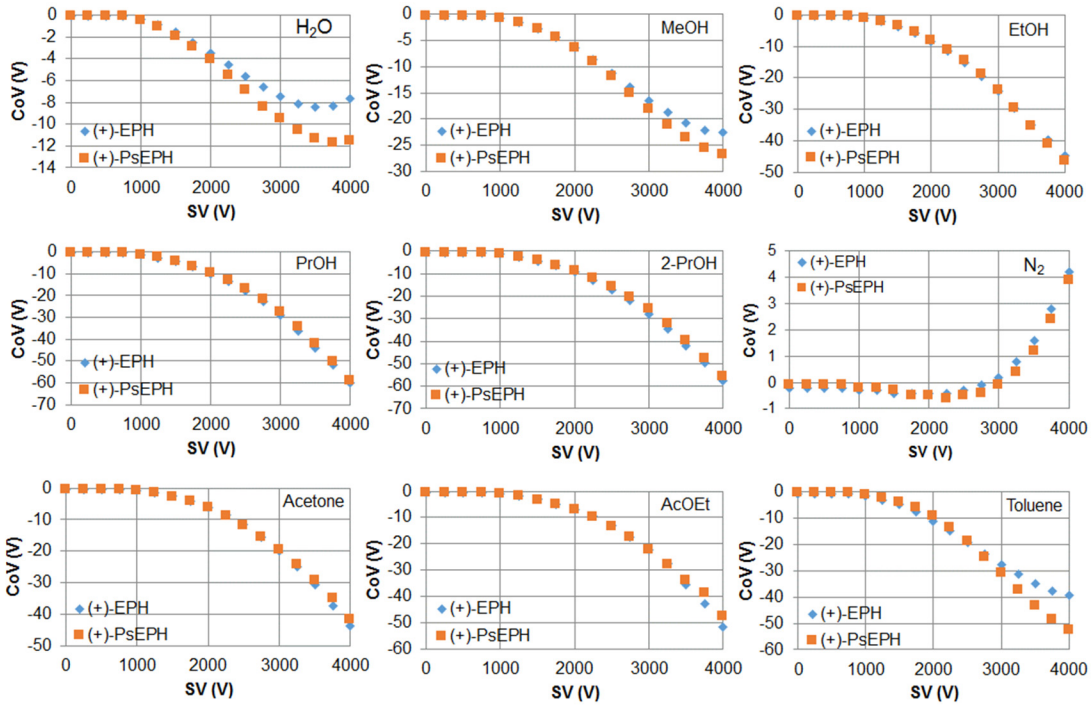
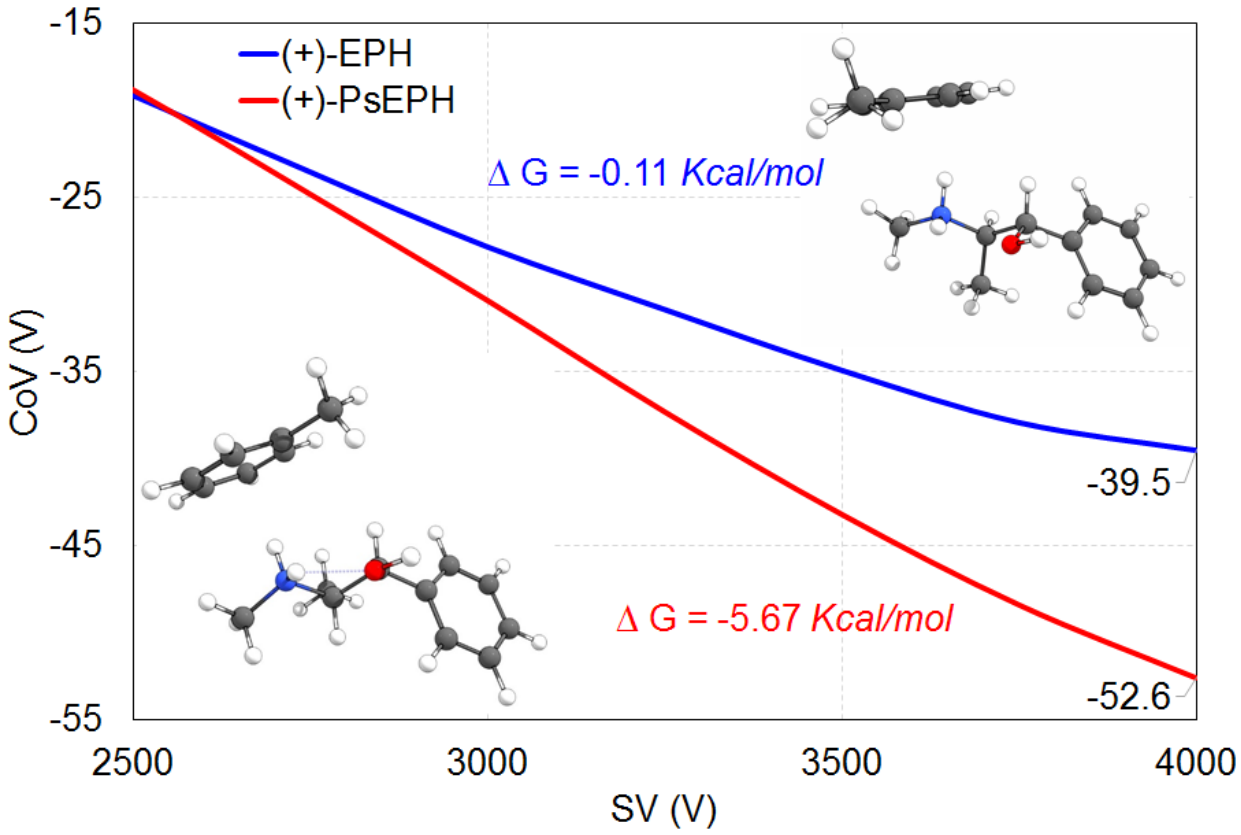


Figure S9: CoV dispersion plots of the two diasteroisomers ephedrine and pseudoephedrine with corresponding modifier depicted on the chart (positive ESI mode).



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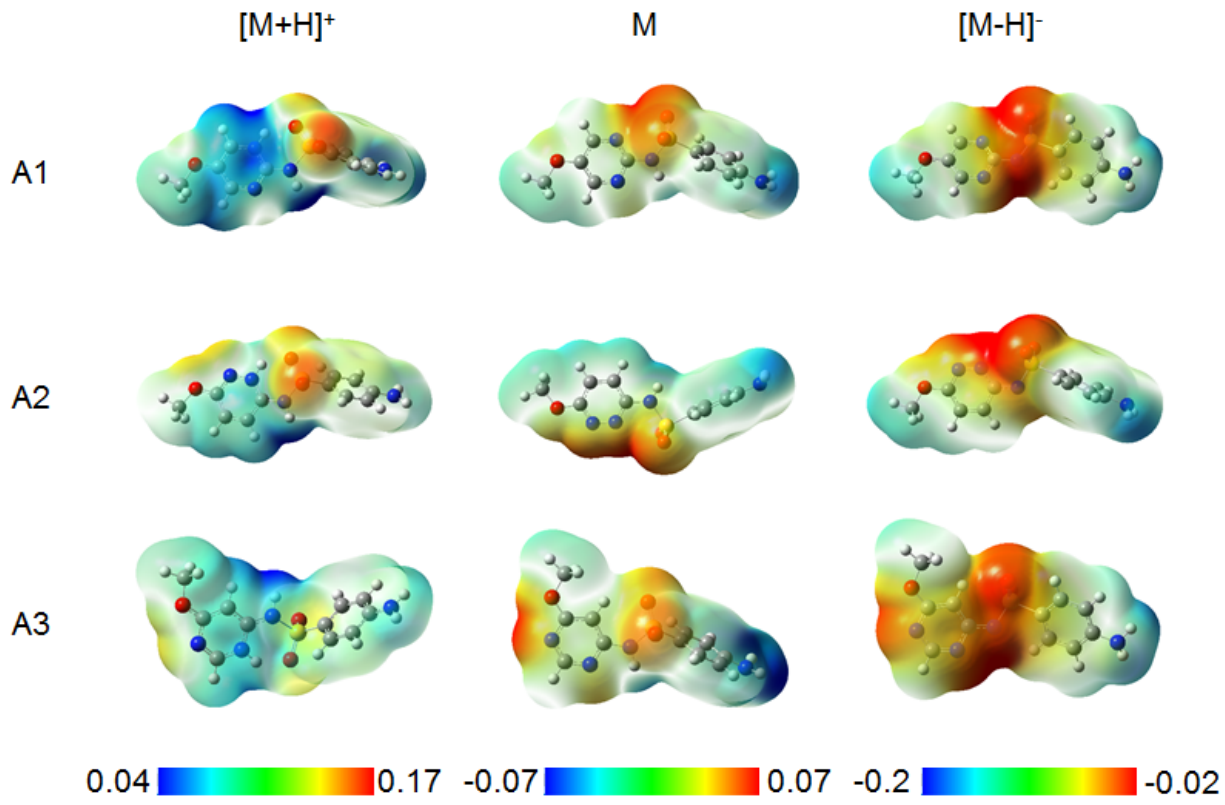

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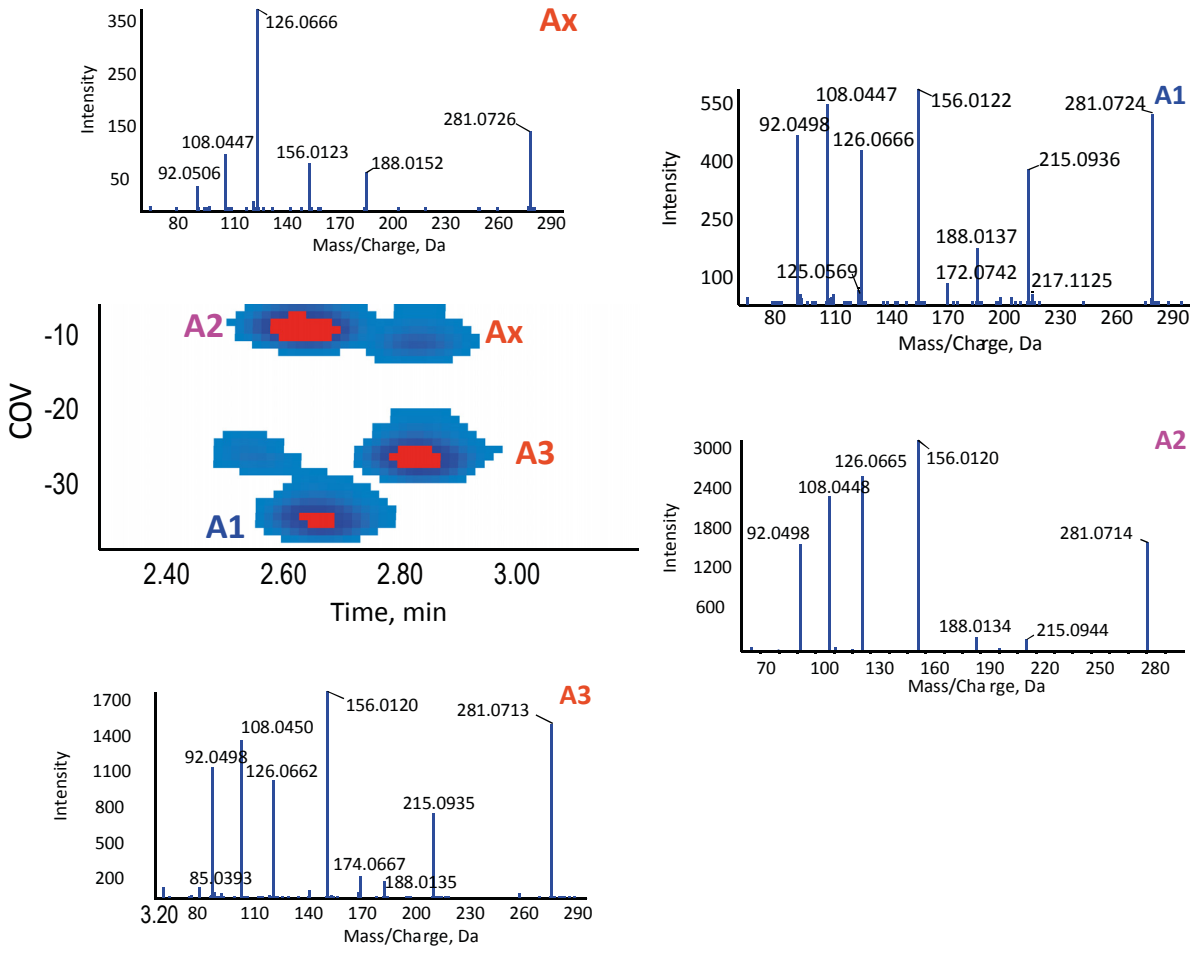


Figure S12: Heat map of the LCxDMS-MS/MS with product ion spectra (m/z 281) at CE 25. Each MS/MS spectra is labeled corresponding to the peak position on the heat map.

Table 1. Analytes investigated divided in isomeric groups, depicted as capital later, with the measured retention time, CCS and molecular weights.

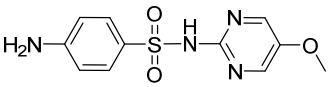
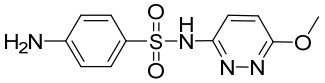
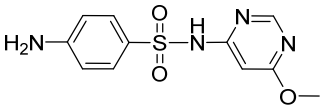
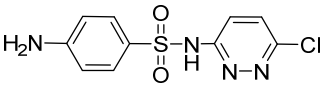
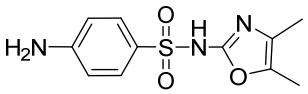
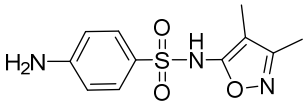
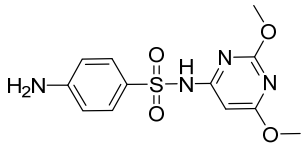
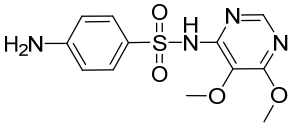
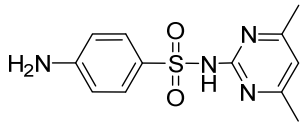
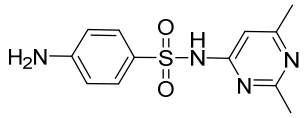
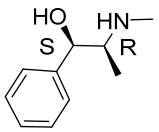
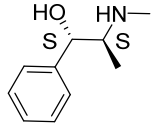
Name	Abbreviation	Molecular weight (g/mol)	Collision-cross section (Å^2)	Structure
Sulfameter	A1	280.30298	-	
Sulfamethoxypyridazine	A2	280.30298	162.6	
Sulfamonomethoxine	A3	280.30298	162.6	
Sulfachlorpyridazine	B	284.72206	-	
Sulfamoxole	C1	267.30422	161.6	
Sulfisoxazole	C2	267.30422	-	
Sulfadimethoxine	D1	310.32896	169.7	
Sulfadoxine	D2	310.32896	169.7	
Sulfadimidine	E1	278.33016	-	
Sulfisomidine	E2	278.33016	-	
(1S,2R)-(+)-Ephedrine	(+)-Eph	165.2322	-	
(S,S)-(+)-Pseudoephedrine	(+)-PsEph	165.2322	-	

Table S2 : Protomer A1 geometry optimization and thermochemistry

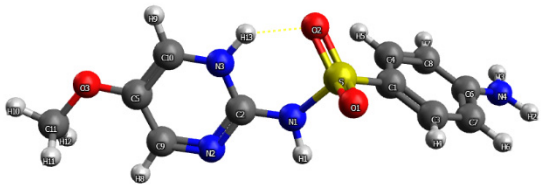
#p B3LYP/6-311g** Opt freq

Protomer A1_N3

Charge = 1 Multiplicity = 1

Stoichiometry C11H13N4O3S(1+)

Coordinates



S	0.877481000	1.204820000	-0.124198000
O	0.932589000	2.399211000	0.692079000
O	0.325663000	1.235299000	-1.485253000
O	-5.536287000	-0.467655000	-0.536047000
N	-0.259296000	0.100269000	0.676068000
N	-2.378678000	-0.582745000	1.312143000
N	-2.083620000	0.359875000	-0.804481000
N	6.010788000	-1.666529000	0.057560000
C	2.382881000	0.337164000	-0.063708000
C	-1.587052000	-0.031037000	0.396910000
C	3.344711000	0.703561000	0.891108000
C	2.637384000	-0.693528000	-0.982921000
C	-4.251227000	-0.348695000	-0.188743000
C	4.828495000	-1.008143000	0.015625000
C	4.549982000	0.037162000	0.928493000
C	3.843264000	-1.355326000	-0.942382000
C	-3.664099000	-0.745682000	1.036575000
C	-3.391374000	0.219599000	-1.111626000
C	-6.467931000	-1.039944000	0.400595000
H	-0.028501000	-0.140764000	1.634704000
H	6.739093000	-1.386448000	0.692677000
H	6.240974000	-2.366415000	-0.627475000
H	3.148677000	1.517654000	1.577194000
H	1.902949000	-0.952397000	-1.734907000
H	5.298578000	0.324045000	1.657674000
H	4.048171000	-2.143713000	-1.657231000
H	-4.259583000	-1.203880000	1.818192000
H	-3.719145000	0.563149000	-2.083114000
H	-7.428496000	-1.018789000	-0.106183000
H	-6.520540000	-0.438875000	1.312153000
H	-6.198884000	-2.073471000	0.634032000
H	-1.413270000	0.781083000	-1.465367000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.245769 (Hartree/Particle)
Thermal correction to Energy=	0.263469
Thermal correction to Enthalpy=	0.264414

Thermal correction to Gibbs Free Energy= 0.198673
Sum of electronic and zero-point Energies= -1269.576156
Sum of electronic and thermal Energies= -1269.558456
Sum of electronic and thermal Enthalpies= -1269.557511
Sum of electronic and thermal Free Energies= -1269.623252

Proto. site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N3	-1269.821925	-1269.558456	-1269.557511	0.220496394	-1269.623252
N4	-1269.821925	-1269.558457	-1269.557512	0.220496394	-1269.623253
O2	-1269.765543	-1269.500912	-1269.499968	0.230994466	-1269.568839
O3	-1269.739959	-1269.477717	-1269.476773	0.228073118	-1269.544773

Table S3: Protomer A2 geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

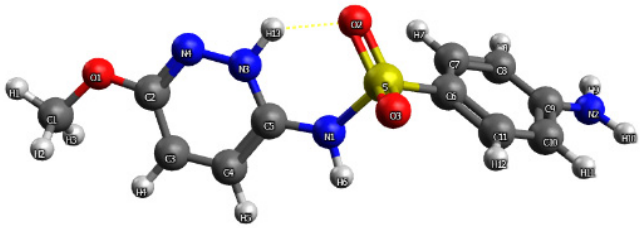
Protomer A2 N3_1

Charge = 1 Multiplicity = 1

Stoichiometry C11H13N4O3S(1+)

Coordinates

S	0.877481000	1.204820000	-0.124198000
O	0.932589000	2.399211000	0.692079000
O	0.325663000	1.235299000	-1.485253000
O	-5.536287000	-0.467655000	-0.536047000
N	-0.259296000	0.100269000	0.676068000
N	-2.378678000	-0.582745000	1.312143000
N	-2.083620000	0.359875000	-0.804481000
N	6.010788000	-1.666529000	0.057560000
C	2.382881000	0.337164000	-0.063708000
C	-1.587052000	-0.031037000	0.396910000
C	3.344711000	0.703561000	0.891108000
C	2.637384000	-0.693528000	-0.982921000
C	-4.251227000	-0.348695000	-0.188743000
C	4.828495000	-1.008143000	0.015625000
C	4.549982000	0.037162000	0.928493000
C	3.843264000	-1.355326000	-0.942382000
C	-3.664099000	-0.745682000	1.036575000
C	-3.391374000	0.219599000	-1.111626000
C	-6.467931000	-1.039944000	0.400595000
H	-0.028501000	-0.140764000	1.634704000
H	6.739093000	-1.386448000	0.692677000
H	6.240974000	-2.366415000	-0.627475000
H	3.148677000	1.517654000	1.577194000
H	1.902949000	-0.952397000	-1.734907000
H	5.298578000	0.324045000	1.657674000
H	4.048171000	-2.143713000	-1.657231000
H	-4.259583000	-1.203880000	1.818192000
H	-3.719145000	0.563149000	-2.083114000
H	-7.428496000	-1.018789000	-0.106183000
H	-6.520540000	-0.438875000	1.312153000
H	-6.198884000	-2.073471000	0.634032000
H	-1.413270000	0.781083000	-1.465367000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.245666 (Hartree/Particle)
Thermal correction to Energy=	0.263474
Thermal correction to Enthalpy=	0.264418

Thermal correction to Gibbs Free Energy= 0.198402
Sum of electronic and zero-point Energies= -1269.547641
Sum of electronic and thermal Energies= -1269.529833
Sum of electronic and thermal Enthalpies= -1269.528889
Sum of electronic and thermal Free Energies= -1269.594904

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2	-1269.732925	-1269.468789	-1269.467845	0.226560456	-1269.535394
N3_1	-1269.793306	-1269.529833	-1269.528889	0.221415395	-1269.594904
N3_2	-1269.785444	-1269.521899	-1269.520954	0.225272514	-1269.588119
N4_1	-1269.783905	-1269.520544	-1269.5196	0.225554251	-1269.586849
N4_2	-1269.792387	-1269.528516	-1269.527572	0.223129297	-1269.594098
O1	-1269.722181	-1269.460131	-1269.459187	0.22695623	-1269.526854
O3_1	-1269.746837	-1269.485474	-1269.48453	0.222525574	-1269.550876
O3_2	-1269.793306	-1269.529833	-1269.528889	0.221415395	-1269.594904

Table S4: Protomer A3 geometry optimization and thermochemistry

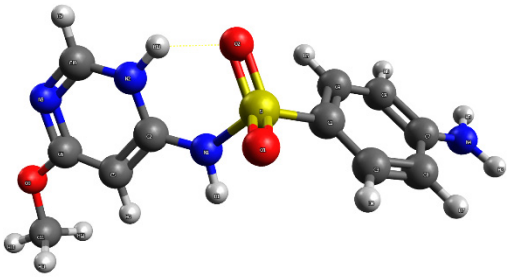
#p B3LYP/6-311g** Opt freq

Protomer A3 N2_2

Charge = 1 Multiplicity = 1

Stoichiometry C11H13N4O3S(1+)

Coordinates



S	0.818420000	-1.204497000	-0.611355000
O	0.836764000	-1.593197000	-2.004882000
O	0.432423000	-2.136386000	0.450024000
O	-5.079652000	1.310966000	0.087393000
N	-0.475081000	0.033833000	-0.484729000
N	-2.067800000	-1.233193000	0.700675000
N	-4.302057000	-0.574738000	0.977216000
N	5.724843000	1.727368000	0.784554000
C	2.252079000	-0.316232000	-0.192596000
C	-1.747181000	-0.136263000	-0.044728000
C	3.105554000	0.128775000	-1.215491000
C	2.564775000	-0.084119000	1.157198000
C	-2.765374000	0.788000000	-0.293854000
C	-4.031556000	0.534069000	0.238522000
C	4.593885000	1.056674000	0.464613000
C	4.258996000	0.806854000	-0.888353000
C	3.718520000	0.592968000	1.478856000
C	-3.331996000	-1.404305000	1.175555000
C	-4.982471000	2.530690000	-0.673949000
H	-0.325799000	0.825054000	-1.099697000
H	6.385154000	1.999914000	0.075830000
H	6.004714000	1.846747000	1.743652000
H	2.871264000	-0.082377000	-2.251172000
H	1.918977000	-0.456778000	1.942123000
H	-2.554448000	1.664877000	-0.887227000
H	4.925929000	1.140521000	-1.674669000
H	3.969735000	0.762252000	2.519410000
H	-3.499536000	-2.306546000	1.753569000
H	-5.976423000	2.966198000	-0.635905000
H	-4.712245000	2.313596000	-1.709713000
H	-4.261583000	3.211176000	-0.215277000
H	-1.327449000	-1.919306000	0.881816000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.246204 (Hartree/Particle)
Thermal correction to Energy=	0.263895
Thermal correction to Enthalpy=	0.264839

Thermal correction to Gibbs Free Energy= 0.198905
Sum of electronic and zero-point Energies= -1269.589837
Sum of electronic and thermal Energies= -1269.572147
Sum of electronic and thermal Enthalpies= -1269.571203
Sum of electronic and thermal Free Energies= -1269.637137

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2_1	-1269.826317	-1269.562309	-1269.561364	0.222468556	-1269.627693
N2_2	-1269.836042	-1269.572147	-1269.571203	0.22114372	-1269.637137
N3	-1269.82903	-1269.565239	-1269.564294	0.223296998	-1269.63087
N4	-1269.781998	-1269.516692	-1269.515748	0.229974845	-1269.584315
O2_1	-1269.836042	-1269.572147	-1269.571203	0.221133658	-1269.637134
O2_2	-1269.786732	-1269.524655	-1269.52371	0.2245514	-1269.59066
O3	-1269.757165	-1269.4947	-1269.493756	0.228401811	-1269.561854

Table S5: Protomer C1 geometry optimization and thermochemistry

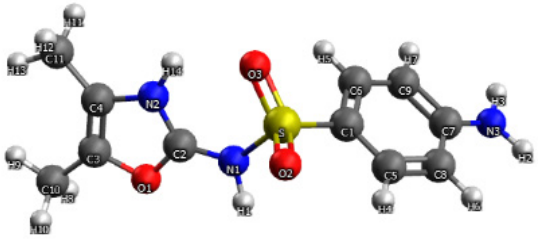
#p B3LYP/6-311g** Opt freq

Protomer C1 N2_2

Charge = 1 Multiplicity = 1

Stoichiometry C11H14N3O3S(1+)Coordinates

S	-0.515849000	1.250664000	-0.061391000
O	2.715783000	-0.821220000	-1.178112000
O	-0.573888000	2.326123000	-1.025846000
O	0.097530000	1.404720000	1.254315000
N	0.603272000	0.033262000	-0.804557000
N	2.481513000	0.254217000	0.686967000
N	-5.661077000	-1.582516000	0.228413000
C	-2.025696000	0.398399000	0.022303000
C	1.873033000	-0.143271000	-0.422945000
C	3.961168000	-0.862070000	-0.502883000
C	3.823368000	-0.192272000	0.655310000
C	-2.990904000	0.615755000	-0.974976000
C	-2.278601000	-0.478268000	1.090493000
C	-4.476174000	-0.933431000	0.161793000
C	-4.198234000	-0.042545000	-0.903516000
C	-3.487085000	-1.132306000	1.158018000
C	5.054057000	-1.582283000	-1.191251000
C	4.770874000	0.108055000	1.762442000
H	0.382511000	-0.261802000	-1.749671000
H	-6.389134000	-1.407243000	-0.443645000
H	-5.887960000	-2.174267000	1.009863000
H	-2.796984000	1.314527000	-1.778807000
H	-1.541178000	-0.620086000	1.870264000
H	-4.950068000	0.133140000	-1.663974000
H	-3.691611000	-1.798469000	1.988007000
H	4.791577000	-2.630852000	-1.354214000
H	5.960172000	-1.545324000	-0.586659000
H	5.270016000	-1.129357000	-2.162419000
H	4.417899000	-0.311882000	2.708326000
H	4.893372000	1.186869000	1.892113000
H	5.749643000	-0.319054000	1.547977000
H	2.009133000	0.806879000	1.397758000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.250045 (Hartree/Particle)
Thermal correction to Energy=	0.267988
Thermal correction to Enthalpy=	0.268932
Thermal correction to Gibbs Free Energy=	0.203050
Sum of electronic and zero-point Energies=	-1215.432935

Sum of electronic and thermal Energies= -1215.414992
Sum of electronic and thermal Enthalpies= -1215.414048
Sum of electronic and thermal Free Energies= -1215.479931

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2_1	-1215.671753	-1215.40401	-1215.403066	0.224068422	-1215.469872
N2_2	-1215.68298	-1215.414992	-1215.414048	0.220972665	-1215.479931
N3	-1215.624435	-1215.356059	-1215.355115	0.218876404	-1215.420373
O1_1	-1215.626507	-1215.360909	-1215.359965	0.240781486	-1215.431754
O1_2	-1215.634926	-1215.368541	-1215.367597	0.220060372	-1215.433208
O3	-1215.632552	-1215.366053	-1215.365109	0.226805299	-1215.432731

Table S6: Protomer C2 geometry optimization and thermochemistry

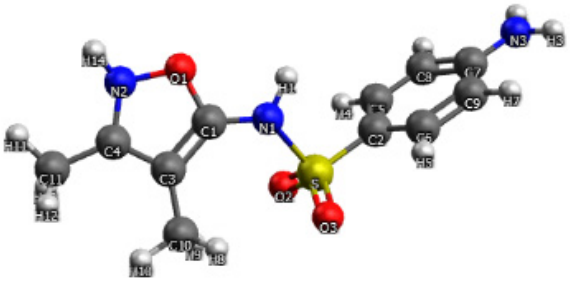
#p B3LYP/6-311g** Opt freq

Protomer C2 N2

Charge = 1 Multiplicity = 1

Stoichiometry C11H14N3O3S(1+)

S	0.310727000	1.040213000	0.139130000
O	-2.324929000	-1.881779000	-0.508543000
O	-0.197042000	1.130548000	1.496030000
O	0.138701000	2.108597000	-0.823089000
N	-0.635266000	-0.364123000	-0.568754000
N	-3.674903000	-1.905224000	-0.198170000
N	5.780043000	-1.130955000	-0.029780000
C	-1.933211000	-0.591547000	-0.372905000
C	1.920066000	0.376987000	0.079852000
C	-3.029019000	0.229034000	-0.048245000
C	-4.109615000	-0.640872000	0.001236000
C	2.407381000	-0.358388000	1.172720000
C	2.724579000	0.615491000	-1.046323000
C	4.521217000	-0.633510000	0.008368000
C	3.690827000	-0.856000000	1.134772000
C	4.007068000	0.114174000	-1.079349000
C	-3.056755000	1.710743000	0.170247000
C	-5.549980000	-0.367242000	0.261363000
H	-0.065484000	-1.152123000	-0.855840000
H	6.178739000	-1.594817000	0.769120000
H	6.401009000	-0.911572000	-0.790536000
H	1.788534000	-0.508911000	2.048445000
H	2.347656000	1.209301000	-1.869530000
H	4.076676000	-1.411639000	1.981522000
H	4.636210000	0.306064000	-1.940714000
H	-2.459872000	2.229465000	-0.581654000
H	-2.652986000	1.972301000	1.150414000
H	-4.080011000	2.082111000	0.107834000
H	-6.166513000	-1.250589000	0.091558000
H	-5.903467000	0.438221000	-0.385155000
H	-5.687100000	-0.049089000	1.298228000
H	-4.163344000	-2.652175000	-0.678445000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.248955 (Hartree/Particle)
Thermal correction to Energy=	0.267292
Thermal correction to Enthalpy=	0.268236

Thermal correction to Gibbs Free Energy= 0.201188
Sum of electronic and zero-point Energies= -1215.370707
Sum of electronic and thermal Energies= -1215.352371
Sum of electronic and thermal Enthalpies= -1215.351427
Sum of electronic and thermal Free Energies= -1215.418475

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2	-1215.619663	-1215.352371	-1215.351427	0.224880094	-1215.418475
N3	-1215.585632	-1215.316773	-1215.315829	0.229330874	-1215.384204
O1_1	-1215.555698	-1215.291004	-1215.29006	0.221120241	-1215.355987
O1_2	-1215.590148	-1215.324365	-1215.323421	0.2221298	-1215.389649

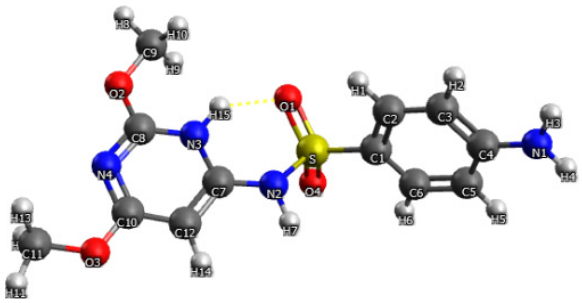
Table S7: Protomer D1 geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

Protomer D1 N3_2

Charge = 1 Multiplicity = 1

Stoichiometry C12H15N4O4S(1+)



O	-0.587077000	-1.785407000	-0.227771000
S	-1.111253000	-0.589026000	-0.902116000
C	-2.661738000	-0.118614000	-0.268641000
C	-2.980487000	-0.378297000	1.074091000
C	-4.219865000	-0.025135000	1.556774000
C	-5.176326000	0.594974000	0.714162000
N	-6.392247000	0.947146000	1.194250000
C	-4.832964000	0.841662000	-0.636760000
C	-3.593522000	0.489367000	-1.124782000
N	-0.039754000	0.674717000	-0.260461000
C	1.316575000	0.553493000	-0.106445000
N	1.826572000	-0.705879000	0.125255000
C	3.160901000	-0.890189000	0.326017000
O	3.630473000	-2.099338000	0.518395000
C	2.760976000	-3.256296000	0.490205000
N	4.019963000	0.097954000	0.340612000
C	3.549348000	1.332277000	0.130216000
O	4.376732000	2.349529000	0.135428000
C	5.793420000	2.112028000	0.361407000
C	2.190936000	1.615648000	-0.115776000
O	-1.084775000	-0.481479000	-2.347497000
H	-2.266840000	-0.870806000	1.722174000
H	-4.473164000	-0.232305000	2.589965000
H	-6.665960000	0.719818000	2.135237000
H	-7.098404000	1.330884000	0.589003000
H	-5.557991000	1.302318000	-1.297496000
H	-3.346354000	0.659902000	-2.164933000
H	-0.355822000	1.608034000	-0.498617000
H	3.421948000	-4.102433000	0.649308000
H	2.271485000	-3.346044000	-0.481586000
H	2.028197000	-3.204169000	1.298371000
H	6.244516000	3.098513000	0.312440000
H	6.190088000	1.460313000	-0.415638000
H	5.943748000	1.660171000	1.340714000
H	1.866960000	2.628323000	-0.303351000
H	1.149559000	-1.477597000	0.129426000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.278324 (Hartree/Particle)
Thermal correction to Energy=	0.298831
Thermal correction to Enthalpy=	0.299775
Thermal correction to Gibbs Free Energy=	0.227802
Sum of electronic and zero-point Energies=	-1384.132817
Sum of electronic and thermal Energies=	-1384.112310
Sum of electronic and thermal Enthalpies=	-1384.111366
Sum of electronic and thermal Free Energies=	-1384.183339

298.15	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N1	-1384.360259	-1384.059846	-1384.058902	0.247754486	-1384.13277
N3_1	-1384.396222	-1384.097556	-1384.096612	0.907271575	-1384.367115
N3_2	-1384.411141	-1384.11231	-1384.111366	-0.140539997	-1384.069464
N4	-1384.407066	-1384.10796	-1384.107016	-0.129119571	-1384.068519
O1	-1384.367115	-1384.069464	-1384.068519	0.241811169	-1384.140615

Table S8: Protomer D2 geometry optimization and thermochemistry

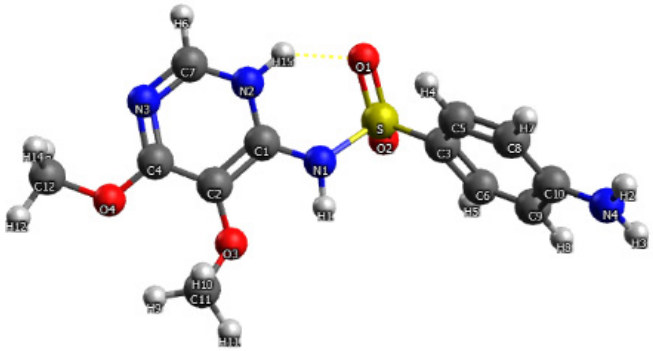
#p B3LYP/6-311g** Opt freq

Protomer D2 N2_2

Charge = 1 Multiplicity = 1

Stoichiometry C12H15N4O4S(1+)

S	1.172315000	-1.241873000	0.687540000
O	0.823059000	-2.312443000	-0.252721000
O	1.182451000	-1.464412000	2.118304000
O	-2.062710000	1.727702000	0.812551000
O	-4.706858000	1.046436000	-0.006441000
N	-0.119229000	-0.066533000	0.374699000
N	-1.716577000	-1.554506000	-0.559273000
N	-3.966776000	-0.989600000	-0.789596000
N	6.059335000	1.584173000	-0.987158000
C	-1.395573000	-0.355585000	0.007968000
C	-2.421764000	0.585313000	0.189993000
C	2.605310000	-0.391828000	0.182768000
C	-3.714148000	0.203991000	-0.212294000
C	2.933387000	-0.316155000	-1.179978000
C	3.436791000	0.184468000	1.155407000
C	-2.984419000	-1.826107000	-0.941439000
C	4.082115000	0.337135000	-1.565591000
C	4.585415000	0.838168000	0.764666000
C	4.935212000	0.932315000	-0.603287000
C	-2.561331000	3.001347000	0.326043000
C	-6.054499000	0.659229000	-0.394945000
H	-0.037760000	0.805932000	0.889135000
H	6.356417000	1.582709000	-1.948341000
H	6.710430000	1.937400000	-0.306246000
H	2.303452000	-0.788747000	-1.922841000
H	3.188809000	0.095151000	2.205426000
H	-3.156644000	-2.798019000	-1.388133000
H	4.345089000	0.387212000	-2.615839000
H	5.235113000	1.274504000	1.514365000
H	-3.619329000	3.108610000	0.551990000
H	-2.386812000	3.087532000	-0.748741000
H	-1.981604000	3.750405000	0.859469000
H	-6.671402000	1.507900000	-0.114629000
H	-6.355384000	-0.236443000	0.146160000
H	-6.098304000	0.481167000	-1.468344000
H	-0.952886000	-2.232113000	-0.674180000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.278465 (Hartree/Particle)
Thermal correction to Energy=	0.299059
Thermal correction to Enthalpy=	0.300003
Thermal correction to Gibbs Free Energy=	0.227287
Sum of electronic and zero-point Energies=	-1384.114935
Sum of electronic and thermal Energies=	-1384.094340
Sum of electronic and thermal Enthalpies=	-1384.093396
Sum of electronic and thermal Free Energies=	-1384.166113

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2_1	-1384.373676	-1384.074827	-1384.073883	0.243266812	-1384.146413
N2_2	-1384.3934	-1384.09434	-1384.093396	0.243894013	-1384.166113
N3	-1384.366993	-1384.068275	-1384.067331	0.243930907	-1384.140059
N4	-1384.335879	-1384.036495	-1384.035551	0.233281905	-1384.105104
O3	-1384.328104	-1384.030494	-1384.029549	0.232493711	-1384.098867
O3_1	-1384.356934	-1384.060067	-1384.059123	0.241006205	-1384.130979
O4	-1384.312411	-1384.014511	-1384.013567	0.243404327	-1384.086138

Table S9: Protomer E1 geometry optimization and thermochemistry

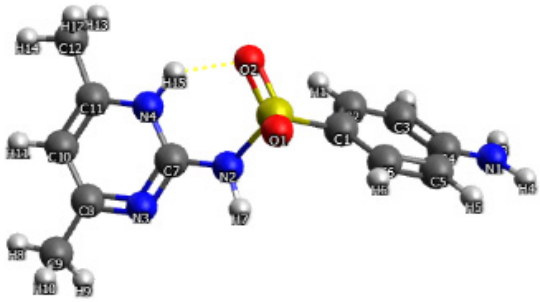
#p B3LYP/6-311g** Opt freq

Protomer E1 N4

Charge = 1 Multiplicity = 1

Stoichiometry C12H15N4O2S(1+)

O	0.662079000	-0.914459000	-2.328681000
S	0.632225000	-0.829952000	-0.883901000
C	2.159476000	-0.276823000	-0.261521000
C	2.442183000	-0.387853000	1.109243000
C	3.664275000	0.029375000	1.585336000
C	4.638042000	0.568938000	0.708130000
N	5.836671000	0.985345000	1.180733000
C	4.330800000	0.666613000	-0.670071000
C	3.109311000	0.248996000	-1.151511000
N	-0.471358000	0.506959000	-0.492504000
C	-1.785572000	0.424468000	-0.157760000
N	-2.518698000	1.527170000	-0.239409000
C	-3.806293000	1.465173000	0.108179000
C	-4.597803000	2.726721000	-0.015005000
C	-4.396266000	0.261066000	0.549844000
C	-3.619144000	-0.869258000	0.629419000
C	-4.090382000	-2.214551000	1.074140000
N	-2.307267000	-0.755931000	0.273566000
O	0.076130000	-1.913190000	-0.064887000
H	1.716461000	-0.820614000	1.785992000
H	3.890518000	-0.065139000	2.641013000
H	6.086527000	0.860863000	2.147205000
H	6.556237000	1.309997000	0.556997000
H	5.069998000	1.063403000	-1.356075000
H	2.891147000	0.304158000	-2.210451000
H	-0.214949000	1.410270000	-0.878347000
H	-5.561135000	2.657698000	0.488811000
H	-4.026691000	3.565859000	0.385760000
H	-4.773498000	2.937329000	-1.075333000
H	-5.441192000	0.222343000	0.822218000
H	-3.946315000	-2.952658000	0.279463000
H	-3.523855000	-2.552013000	1.947129000
H	-5.146562000	-2.185416000	1.334784000
H	-1.665160000	-1.557644000	0.314563000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.268026 (Hartree/Particle)
Thermal correction to Energy=	0.286740

Thermal correction to Enthalpy= 0.287684
Thermal correction to Gibbs Free Energy= 0.219097
Sum of electronic and zero-point Energies= -1233.678741
Sum of electronic and thermal Energies= -1233.660027
Sum of electronic and thermal Enthalpies= -1233.659083
Sum of electronic and thermal Free Energies= -1233.727670

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N1	-1233.89475	-1233.606686	-1233.605742	0.235388227	-1233.675923
N4	-1233.946767	-1233.660027	-1233.659083	0.230041925	-1233.72767
O1	-1233.906318	-1233.621242	-1233.620298	0.23150763	-1233.689322

Table S10: Protomer E2 geometry optimization and thermochemistry

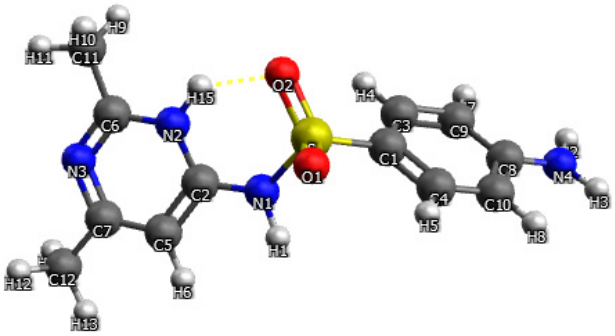
#p B3LYP/6-311g** Opt freq

Protomer E2 N2_1

Charge = 1 Multiplicity = 1

Stoichiometry C12H15N4O2S(1+)

S	-0.673990000	-0.867750000	0.863953000
O	-0.719402000	-1.017643000	2.302541000
O	-0.103516000	-1.900370000	-0.004189000
N	0.444241000	0.501870000	0.565107000
N	2.280188000	-0.707520000	-0.279552000
N	4.412003000	0.208790000	-0.559505000
N	-5.846053000	1.058872000	-1.174146000
C	-2.189052000	-0.277086000	0.250960000
C	1.754905000	0.440715000	0.222790000
C	-2.453506000	-0.317927000	-1.128078000
C	-3.151388000	0.203633000	1.154120000
C	2.622690000	1.537262000	0.333996000
C	3.595306000	-0.802318000	-0.655692000
C	3.938726000	1.387803000	-0.065968000
C	-4.654356000	0.619294000	-0.707612000
C	-3.668422000	0.124695000	-1.598395000
C	-4.365825000	0.646157000	0.678343000
C	4.053716000	-2.124404000	-1.180117000
C	4.931210000	2.500057000	0.021966000
H	0.148384000	1.362996000	1.010019000
H	-6.082671000	0.984798000	-2.149248000
H	-6.574099000	1.352656000	-0.544747000
H	-1.719457000	-0.717377000	-1.816300000
H	-2.948553000	0.202558000	2.217564000
H	2.258681000	2.474943000	0.731471000
H	-3.880835000	0.083616000	-2.660332000
H	-5.115038000	1.006080000	1.373725000
H	3.473132000	-2.416753000	-2.059908000
H	3.928230000	-2.904049000	-0.422933000
H	5.104156000	-2.052288000	-1.449979000
H	5.767058000	2.190297000	0.655427000
H	4.495953000	3.416256000	0.418888000
H	5.346808000	2.694201000	-0.970677000
H	1.648768000	-1.511187000	-0.364236000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.267840 (Hartree/Particle)
Thermal correction to Energy=	0.286585
Thermal correction to Enthalpy=	0.287529
Thermal correction to Gibbs Free Energy=	0.218963
Sum of electronic and zero-point Energies=	-1233.674314
Sum of electronic and thermal Energies=	-1233.655569
Sum of electronic and thermal Enthalpies=	-1233.654624
Sum of electronic and thermal Free Energies=	-1233.723191

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N2_1	-1233.942154	-1233.655569	-1233.654624	0.229974845	-1233.723191
N2_2	-1233.930262	-1233.64365	-1233.642706	0.234667114	-1233.712672
N3	-1233.938021	-1233.651285	-1233.650341	0.231396948	-1233.719332
N4	-1233.874607	-1233.587208	-1233.586264	0.235441892	-1233.656461
O2_1	-1233.942154	-1233.655569	-1233.654625	0.229988261	-1233.723196
O2_2	-1233.893059	-1233.608006	-1233.607062	0.235646487	-1233.67732

Table S11: Protomer ephedrine geometry optimization and thermochemistry

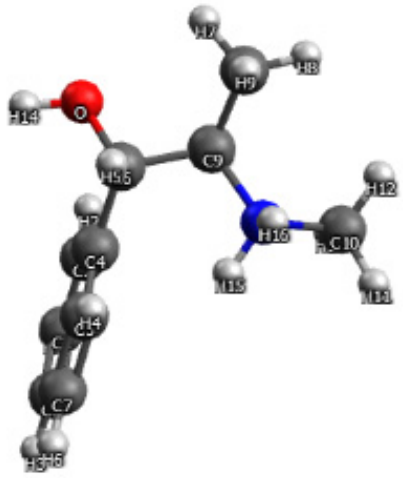
#p B3LYP/6-311g** Opt freq

Protomer Eph N_1

Charge = 1 Multiplicity = 1

Stoichiometry C10H16NO(1+)

C	-2.631415000	-0.089431000	1.233142000
C	-1.363190000	-0.621419000	1.021576000
C	-3.300673000	0.566932000	0.198984000
C	-0.751250000	-0.505578000	-0.234255000
C	-1.434501000	0.144145000	-1.270989000
C	0.647342000	-1.056700000	-0.459891000
C	-2.704050000	0.681909000	-1.053629000
C	3.157697000	-0.586087000	-0.180525000
C	1.742224000	-0.159662000	0.175953000
C	2.110738000	2.338059000	0.631320000
O	0.870564000	-2.305932000	0.164952000
N	1.490512000	1.286576000	-0.242704000
H	-3.103979000	-0.194436000	2.202563000
H	-0.856124000	-1.156942000	1.816170000
H	-4.290915000	0.973146000	0.366757000
H	-0.994616000	0.194749000	-2.263441000
H	0.834740000	-1.120163000	-1.543312000
H	-3.230877000	1.168412000	-1.865804000
H	3.275606000	-1.631519000	0.101976000
H	3.916272000	-0.008331000	0.350380000
H	3.339271000	-0.508802000	-1.257599000
H	1.585873000	-0.176021000	1.256623000
H	1.861682000	3.318552000	0.228498000
H	3.189235000	2.203092000	0.644554000
H	1.704896000	2.231464000	1.635218000
H	0.248100000	-2.952066000	-0.187758000
H	0.468202000	1.417239000	-0.270507000
H	1.817000000	1.410042000	-1.205134000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.249276 (Hartree/Particle)
Thermal correction to Energy=	0.261751
Thermal correction to Enthalpy=	0.262695
Thermal correction to Gibbs Free Energy=	0.210163
Sum of electronic and zero-point Energies=	-520.335479

Sum of electronic and thermal Energies= -520.323004
Sum of electronic and thermal Enthalpies= -520.322060
Sum of electronic and thermal Free Energies= -520.374591

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N_1	-520.5847546	-520.591434	-520.56884	0.057913107	-520.5861067
N_2	-520.323004	-520.329256	-520.309741	0.047003186	-520.323755
O_1	-520.32206	-520.328312	-520.308797	0.047003186	-520.322811
O_2	-520.374591	-520.379615	-520.366052	0.024880094	-520.37347

Table S12: Protomer pseudoephedrine geometry optimization and thermochemistry

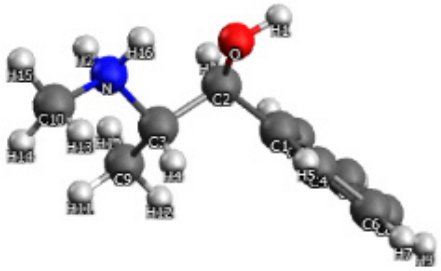
#p B3LYP/6-311g** Opt freq

Protomer PsEph N_1

Charge = 1 Multiplicity = 1

Stoichiometry C10H16NO(1+)

O	-0.922615000	-1.475758000	1.141024000
N	-2.864765000	-0.097726000	0.231606000
C	0.957548000	-0.098126000	0.345604000
C	-0.473411000	-0.146950000	0.832593000
C	-1.472936000	0.335441000	-0.241707000
C	1.415452000	-1.023594000	-0.600236000
C	1.834540000	0.870903000	0.840350000
C	2.731438000	-0.970471000	-1.048621000
C	3.149945000	0.926049000	0.384676000
C	3.598410000	0.006869000	-0.560584000
C	-1.429971000	1.828391000	-0.519325000
C	-3.912639000	-0.233856000	-0.830810000
H	-0.289264000	-1.911195000	1.723014000
H	-3.186731000	0.554034000	0.951694000
H	-0.572569000	0.492330000	1.721135000
H	-1.300723000	-0.242316000	-1.152047000
H	0.752036000	-1.799017000	-0.967520000
H	1.495495000	1.579155000	1.589627000
H	3.082193000	-1.693279000	-1.775550000
H	3.824562000	1.678376000	0.775257000
H	4.623056000	0.045350000	-0.910433000
H	-1.643713000	2.411972000	0.381488000
H	-2.131289000	2.119733000	-1.302918000
H	-0.430291000	2.099216000	-0.860278000
H	-3.578440000	-0.979796000	-1.549164000
H	-4.055457000	0.725148000	-1.323357000
H	-4.843199000	-0.556008000	-0.365963000
H	-2.701643000	-1.001486000	0.703933000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.249520 (Hartree/Particle)
Thermal correction to Energy=	0.261884
Thermal correction to Enthalpy=	0.262829
Thermal correction to Gibbs Free Energy=	0.210797
Sum of electronic and zero-point Energies=	-520.342658

Sum of electronic and thermal Energies= -520.330293
Sum of electronic and thermal Enthalpies= -520.329349
Sum of electronic and thermal Free Energies= -520.381381

Proto. Site	SCF (a.u.)	U (a.u.)	H (a.u.)	S (a.u.)	G (a.u.)
N_1	-520.592178	-520.5814218	-520.592178	-0.082289639	-520.5676433
N_2	-520.330293	-520.31947	-520.330293	-0.072705014	-520.308616
O_1	-520.329349	-520.318526	-520.329349	-0.072705014	-520.307672
O_2	-520.381381	-520.369926	-520.38138	-0.057162502	-520.364337

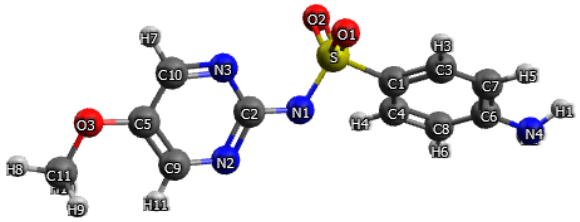
Table S13: Deprotomer A1 geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

Negative A1

Charge = -1 Multiplicity = 1

Stoichiometry C11H11N4O3S(1-)



S	0.696403000	1.038718000	-0.223810000
O	0.640652000	2.061119000	0.844356000
O	0.671490000	1.546258000	-1.613980000
O	-5.670133000	-0.024837000	0.081311000
N	-0.220882000	-0.272339000	0.016659000
N	-2.230138000	-1.362595000	0.215962000
N	-2.213244000	1.007592000	-0.149507000
N	6.174500000	-1.428115000	0.284393000
C	2.316495000	0.243871000	-0.028527000
C	-1.565147000	-0.186512000	0.023536000
C	3.279274000	0.853470000	0.766585000
C	2.637323000	-0.916379000	-0.731049000
C	-4.290023000	-0.151935000	0.072326000
C	4.895650000	-0.846389000	0.152933000
C	4.560489000	0.313754000	0.858938000
C	3.911976000	-1.459913000	-0.637318000
C	-3.557674000	-1.325955000	0.237336000
C	-3.531015000	1.003745000	-0.122655000
C	-6.414777000	-1.206834000	0.266310000
H	6.893589000	-0.755758000	0.515570000
H	6.449596000	-1.967430000	-0.525552000
H	3.005555000	1.747523000	1.313732000
H	1.872741000	-1.397336000	-1.327781000
H	5.306137000	0.792373000	1.488475000
H	4.152621000	-2.372599000	-1.176200000
H	-4.053188000	-2.282800000	0.393685000
H	-4.039969000	1.957823000	-0.261244000
H	-7.466123000	-0.915808000	0.241086000
H	-6.199030000	-1.682878000	1.233193000
H	-6.227513000	-1.940281000	-0.530543000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction= 0.218627 (Hartree/Particle)

Thermal correction to Energy=	0.235946
Thermal correction to Enthalpy=	0.236890
Thermal correction to Gibbs Free Energy=	0.170673
Sum of electronic and zero-point Energies=	-1268.658714
Sum of electronic and thermal Energies=	-1268.641395
Sum of electronic and thermal Enthalpies=	-1268.640451
Sum of electronic and thermal Free Energies=	-1268.706668

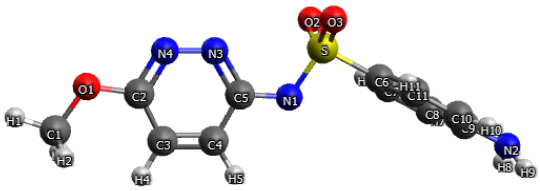
Table S14:Deprotomer A2 geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

Negative A2

Charge = -1 Multiplicity = 1

Stoichiometry C11H11N4O3S(1-)



C	-6.406570000	-1.209732000	-0.000731000
O	-5.621986000	-0.040977000	0.000128000
C	-4.246749000	-0.197546000	0.000173000
C	-3.594795000	-1.444156000	0.000521000
C	-2.223942000	-1.423682000	0.000610000
C	-1.540974000	-0.172843000	0.000381000
N	-0.187930000	-0.209687000	0.000497000
S	0.701965000	1.142304000	-0.000089000
O	0.650402000	1.913733000	-1.260349000
O	0.650756000	1.914620000	1.259644000
C	2.334730000	0.344804000	-0.000036000
C	2.966225000	0.045515000	-1.202590000
C	4.216823000	-0.564058000	-1.205163000
C	4.855047000	-0.885659000	-0.000058000
N	6.144493000	-1.458039000	-0.000024000
C	4.217012000	-0.563691000	1.205059000
C	2.966401000	0.045838000	1.202513000
N	-2.261135000	0.980320000	0.000103000
N	-3.591741000	0.944502000	-0.000025000
H	-7.445934000	-0.878017000	-0.001141000
H	-6.234539000	-1.829282000	0.891029000
H	-6.233577000	-1.828585000	-0.892784000
H	-4.135229000	-2.383369000	0.000719000
H	-1.635516000	-2.333462000	0.000860000
H	2.472845000	0.308524000	-2.130294000
H	4.708555000	-0.790498000	-2.147635000
H	6.335845000	-2.004140000	-0.829308000
H	6.335546000	-2.004748000	0.828929000
H	4.708949000	-0.789787000	2.147506000
H	2.473169000	0.309092000	2.130226000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.217854 (Hartree/Particle)
Thermal correction to Energy=	0.235218

Thermal correction to Enthalpy=	0.236162
Thermal correction to Gibbs Free Energy=	0.169804
Sum of electronic and zero-point Energies=	-1268.628819
Sum of electronic and thermal Energies=	-1268.611455
Sum of electronic and thermal Enthalpies=	-1268.610511
Sum of electronic and thermal Free Energies=	-1268.676869

Table S15: Deprotomer A3 geometry optimization and thermochemistry

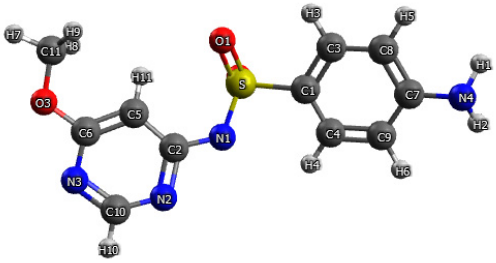
#p B3LYP/6-311g** Opt freq

Negative A3

Charge = -1 Multiplicity = 1

Stoichiometry C11H11N4O3S (1-)

S	-0.377079000	-0.676156000	-0.529982000
O	-0.006100000	-1.769904000	0.405597000
O	-0.365883000	-1.057518000	-1.961756000
O	4.926967000	-0.705453000	0.397582000
N	0.311077000	0.753340000	-0.259366000
N	2.068451000	2.195843000	0.049144000
N	4.340401000	1.470755000	0.361306000
N	-6.140381000	0.572188000	0.667269000
C	-2.086665000	-0.250585000	-0.123325000
C	1.638838000	0.902056000	-0.059605000
C	-2.880950000	-1.201377000	0.508621000
C	-2.638164000	0.972483000	-0.501322000
C	2.593564000	-0.148152000	0.059941000
C	3.914369000	0.205789000	0.266054000
C	-4.794664000	0.281149000	0.371430000
C	-4.225259000	-0.938559000	0.756024000
C	-3.978679000	1.236965000	-0.251941000
C	3.363082000	2.375830000	0.247205000
C	4.589279000	-2.079586000	0.305520000
H	-6.724241000	-0.250447000	0.732811000
H	-6.552048000	1.248458000	0.038717000
H	-2.429934000	-2.137705000	0.813948000
H	-1.997969000	1.714582000	-0.960636000
H	2.262938000	-1.173185000	0.018372000
H	-4.838550000	-1.681470000	1.258689000
H	-4.401152000	2.197155000	-0.535072000
H	3.685478000	3.414609000	0.329191000
H	5.525066000	-2.625063000	0.434035000
H	4.155268000	-2.327033000	-0.669887000
H	3.883202000	-2.379360000	1.088063000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.219989 (Hartree/Particle)
Thermal correction to Energy=	0.237042

Thermal correction to Enthalpy=	0.237986
Thermal correction to Gibbs Free Energy=	0.172971
Sum of electronic and zero-point Energies=	-1268.684262
Sum of electronic and thermal Energies=	-1268.667210
Sum of electronic and thermal Enthalpies=	-1268.666265
Sum of electronic and thermal Free Energies=	-1268.731281

Table S16: Deprotomer C1 geometry optimization and thermochemistry

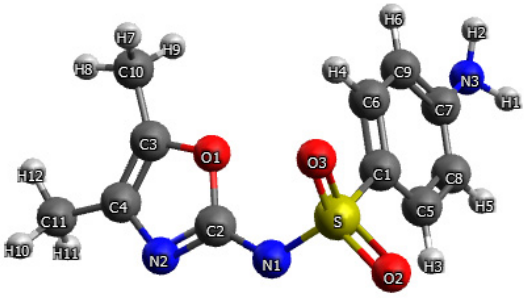
#p B3LYP/6-311g** Opt freq

Negative C1

Charge = -1 Multiplicity = 1

Stoichiometry C11H12N3O3S(1-)

S	0.370957000	-1.934622000	0.335720000
O	-1.555149000	0.289651000	0.541389000
O	1.075588000	-3.119849000	-0.179518000
O	0.045152000	-1.919325000	1.776787000
N	-0.866106000	-1.659198000	-0.648644000
N	-2.835374000	-0.438822000	-1.149899000
N	4.241743000	2.615065000	-0.524189000
C	1.542348000	-0.563164000	0.096148000
C	-1.744161000	-0.665763000	-0.448829000
C	-2.627202000	1.158657000	0.421493000
C	-3.391691000	0.708113000	-0.602415000
C	2.371438000	-0.554471000	-1.024216000
C	1.621166000	0.477399000	1.016972000
C	3.363433000	1.535657000	-0.298305000
C	3.272570000	0.484688000	-1.222175000
C	2.524421000	1.518370000	0.822555000
C	-2.686174000	2.292313000	1.377960000
C	-4.663205000	1.271266000	-1.150367000
H	5.049443000	2.363565000	-1.077940000
H	4.529740000	3.080066000	0.326147000
H	2.314546000	-1.377914000	-1.725596000
H	0.975568000	0.452562000	1.885092000
H	3.916736000	0.484991000	-2.097554000
H	2.581723000	2.327638000	1.545859000
H	-2.738938000	1.946204000	2.418140000
H	-3.569955000	2.904361000	1.181641000
H	-1.803194000	2.939626000	1.298547000
H	-5.472042000	0.535152000	-1.084230000
H	-4.548926000	1.524006000	-2.210375000
H	-4.974239000	2.172167000	-0.614318000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.223441 (Hartree/Particle)
Thermal correction to Energy=	0.240736

Thermal correction to Enthalpy=	0.241680
Thermal correction to Gibbs Free Energy=	0.176739
Sum of electronic and zero-point Energies=	-1214.518708
Sum of electronic and thermal Energies=	-1214.501414
Sum of electronic and thermal Enthalpies=	-1214.500469
Sum of electronic and thermal Free Energies=	-1214.565410

Table S17: Deprotomer C2 geometry optimization and thermochemistry

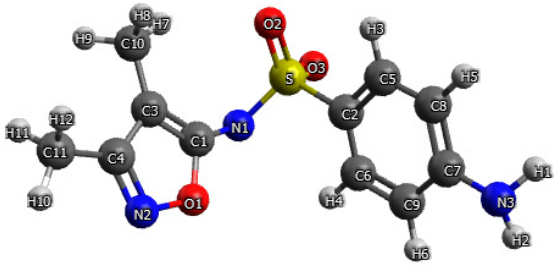
#p B3LYP/6-311g** Opt freq

Negative C2

Charge = -1 Multiplicity = 1

Stoichiometry C11H12N3O3S(1-)

S	0.152560000	1.676849000	-0.295771000
O	-1.701675000	-1.274877000	-1.264428000
O	-0.289278000	2.015779000	1.078353000
O	0.693556000	2.785362000	-1.100080000
N	-0.898085000	0.882060000	-1.190037000
N	-2.702326000	-2.096452000	-0.727265000
N	4.851001000	-2.029826000	0.509558000
C	-1.744527000	-0.037029000	-0.670482000
C	1.542762000	0.525823000	-0.038567000
C	-2.767771000	-0.043094000	0.261893000
C	-3.307623000	-1.352193000	0.172181000
C	2.277875000	0.573293000	1.143253000
C	1.911070000	-0.369912000	-1.040321000
C	3.767500000	-1.150240000	0.312578000
C	3.380003000	-0.257403000	1.319843000
C	3.010962000	-1.201941000	-0.866170000
C	-3.250508000	1.082395000	1.122006000
C	-4.451776000	-1.928379000	0.949220000
H	5.548596000	-1.668829000	1.146107000
H	5.283582000	-2.329331000	-0.353719000
H	1.965727000	1.255582000	1.924523000
H	1.310550000	-0.425286000	-1.940164000
H	3.943205000	-0.221002000	2.248777000
H	3.283095000	-1.908137000	-1.645934000
H	-2.786946000	2.017897000	0.811472000
H	-2.986079000	0.944682000	2.176857000
H	-4.341879000	1.188272000	1.064614000
H	-4.637110000	-2.954221000	0.624536000
H	-5.366780000	-1.344189000	0.802432000
H	-4.239662000	-1.933530000	2.023677000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.223368 (Hartree/Particle)
Thermal correction to Energy=	0.240775

Thermal correction to Enthalpy=	0.241719
Thermal correction to Gibbs Free Energy=	0.175648
Sum of electronic and zero-point Energies=	-1214.481614
Sum of electronic and thermal Energies=	-1214.464207
Sum of electronic and thermal Enthalpies=	-1214.463263
Sum of electronic and thermal Free Energies=	-1214.529334

Table S18: Deprotomer D1 geometry optimization and thermochemistry

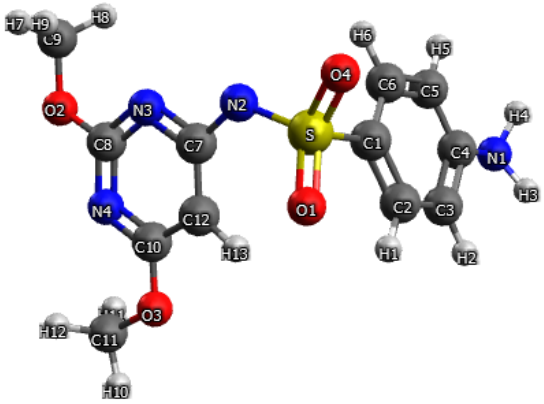
#p B3LYP/6-311g** Opt freq

Negative D1

Charge = -1 Multiplicity = 1

Stoichiometry C12H13N4O4S(1-)

O	0.979071000	-0.746307000	-2.579621000
S	1.169101000	-1.477324000	-1.303864000
C	2.221566000	-0.396116000	-0.290986000
C	2.642321000	0.840418000	-0.767619000
C	3.467722000	1.649379000	0.010053000
C	3.884450000	1.230772000	1.278417000
N	4.673461000	2.066734000	2.090598000
C	3.447679000	-0.016246000	1.752618000
C	2.627255000	-0.821044000	0.974564000
N	-0.149270000	-1.718324000	-0.409704000
C	-1.129216000	-0.795781000	-0.273532000
N	-2.091559000	-1.142865000	0.645133000
C	-3.070650000	-0.297074000	0.845202000
O	-4.035134000	-0.628749000	1.752318000
C	-3.881184000	-1.886754000	2.401634000
N	-3.286524000	0.897020000	0.279470000
C	-2.358132000	1.225052000	-0.616739000
O	-2.488947000	2.419326000	-1.262917000
C	-3.622443000	3.211454000	-0.924999000
C	-1.264602000	0.441602000	-0.948609000
O	1.883368000	-2.760032000	-1.379920000
H	2.323894000	1.153586000	-1.754115000
H	3.788418000	2.616557000	-0.367383000
H	5.238422000	2.721502000	1.567405000
H	5.240035000	1.562757000	2.758933000
H	3.754636000	-0.349482000	2.740249000
H	2.291158000	-1.782270000	1.343756000
H	-4.736366000	-1.970206000	3.075187000
H	-2.945561000	-1.934129000	2.964783000
H	-3.879153000	-2.710168000	1.682936000
H	-3.543587000	4.106899000	-1.543680000
H	-3.621290000	3.482258000	0.134249000
H	-4.556991000	2.685527000	-1.138132000
H	-0.577454000	0.748522000	-1.719737000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.252074 (Hartree/Particle)
Thermal correction to Energy=	0.271841
Thermal correction to Enthalpy=	0.272785
Thermal correction to Gibbs Free Energy=	0.201974
Sum of electronic and zero-point Energies=	-1383.226317
Sum of electronic and thermal Energies=	-1383.206549
Sum of electronic and thermal Enthalpies=	-1383.205605
Sum of electronic and thermal Free Energies=	-1383.276416

Table S19: Deprotomer D2 geometry optimization and thermochemistry

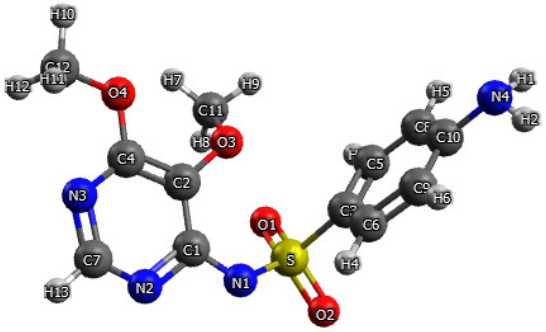
#p B3LYP/6-311g** Opt freq

Negative D2

Charge = -1 Multiplicity = 1

Stoichiometry C12H13N4O4S(1-)

S	0.869908000	1.891595000	0.427412000
O	0.503573000	1.740778000	1.855370000
O	1.696465000	3.064853000	0.100114000
O	-0.681415000	-0.752261000	0.776586000
O	-3.093108000	-1.980320000	0.453607000
N	-0.357936000	1.888462000	-0.601504000
N	-2.491626000	1.612574000	-1.357248000
N	-3.895054000	-0.261430000	-0.876429000
N	4.618633000	-2.636490000	-0.928239000
C	-1.470194000	1.123456000	-0.577245000
C	-1.683004000	-0.128324000	0.072823000
C	1.941733000	0.478181000	0.026373000
C	-2.909398000	-0.762091000	-0.136346000
C	2.635771000	-0.182024000	1.034723000
C	2.152423000	0.117503000	-1.301655000
C	-3.600482000	0.916692000	-1.447818000
C	3.532146000	-1.198994000	0.721240000
C	3.043652000	-0.901373000	-1.618429000
C	3.752510000	-1.569457000	-0.611198000
C	-0.897093000	-0.884350000	2.186105000
C	-4.335429000	-2.628025000	0.201812000
H	5.354213000	-2.768094000	-0.247055000
H	5.011152000	-2.567187000	-1.857482000
H	2.458143000	0.102855000	2.064704000
H	1.602985000	0.630152000	-2.082359000
H	-4.388616000	1.344104000	-2.067465000
H	4.064226000	-1.716558000	1.515278000
H	3.192665000	-1.186287000	-2.656500000
H	-1.770267000	-1.511436000	2.385836000
H	-0.996484000	0.100463000	2.645276000
H	-0.002723000	-1.369889000	2.579579000
H	-4.281663000	-3.574651000	0.741465000
H	-4.481432000	-2.809290000	-0.866305000
H	-5.178771000	-2.033087000	0.562641000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.251704 (Hartree/Particle)
Thermal correction to Energy=	0.271495
Thermal correction to Enthalpy=	0.272439
Thermal correction to Gibbs Free Energy=	0.202019
Sum of electronic and zero-point Energies=	-1383.192311
Sum of electronic and thermal Energies=	-1383.172521
Sum of electronic and thermal Enthalpies=	-1383.171576
Sum of electronic and thermal Free Energies=	-1383.241996

Table S20: Deprotomer E1 geometry optimization and thermochemistry

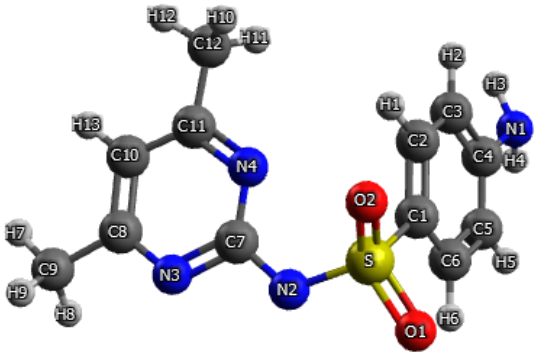
#p B3LYP/6-311g** Opt freq

Negative E1

Charge = -1 Multiplicity = 1

Stoichiometry C12H13N4O2S(1-)

O	1.084846000	-3.160056000	-0.045855000
S	0.475659000	-1.930960000	0.491332000
C	1.674003000	-0.624263000	0.090196000
C	1.842993000	0.473147000	0.929359000
C	2.764751000	1.463907000	0.606206000
C	3.523783000	1.378275000	-0.568123000
N	4.490263000	2.359759000	-0.872715000
C	3.351021000	0.264975000	-1.401451000
C	2.434249000	-0.727702000	-1.072393000
N	-0.853617000	-1.661737000	-0.396243000
C	-1.637416000	-0.586988000	-0.205198000
N	-2.779401000	-0.569682000	-0.965076000
C	-3.596742000	0.459512000	-0.825014000
C	-4.854740000	0.430368000	-1.664133000
C	-3.336153000	1.522051000	0.050993000
C	-2.145657000	1.443677000	0.773793000
C	-1.736413000	2.532264000	1.738409000
N	-1.307645000	0.421528000	0.656075000
O	0.277076000	-1.870878000	1.952590000
H	1.240413000	0.536029000	1.824728000
H	2.899163000	2.315746000	1.267891000
H	4.270866000	3.265580000	-0.480269000
H	4.669415000	2.444464000	-1.864372000
H	3.945552000	0.176190000	-2.306938000
H	2.308071000	-1.597793000	-1.704847000
H	-5.399619000	1.377150000	-1.617000000
H	-4.601465000	0.210200000	-2.704046000
H	-5.516348000	-0.370364000	-1.318722000
H	-4.016256000	2.359006000	0.156766000
H	-1.608966000	2.113113000	2.740393000
H	-0.771240000	2.951760000	1.440118000
H	-2.474783000	3.337323000	1.780976000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.241028 (Hartree/Particle)
Thermal correction to Energy=	0.259270
Thermal correction to Enthalpy=	0.260214
Thermal correction to Gibbs Free Energy=	0.192137
Sum of electronic and zero-point Energies=	-1232.755983
Sum of electronic and thermal Energies=	-1232.737742
Sum of electronic and thermal Enthalpies=	-1232.736797
Sum of electronic and thermal Free Energies=	-1232.804875

Table S21: Deprotomer E2 geometry optimization and thermochemistry

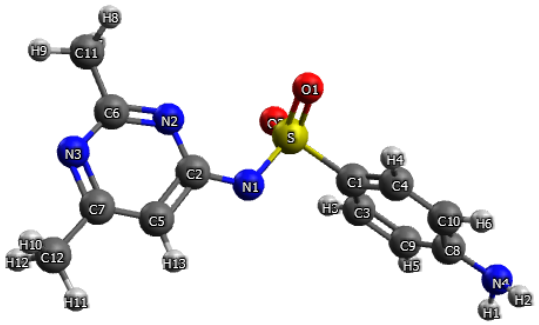
#p B3LYP/6-311g** Opt freq

Negative E2

Charge = -1 Multiplicity = 1

Stoichiometry C12H13N4O2S(1-)

S	-0.471439000	-1.091055000	0.000076000
O	-0.375729000	-1.854442000	1.261632000
O	-0.375743000	-1.854574000	-1.261412000
N	0.356250000	0.311966000	0.000016000
N	2.463622000	-0.804530000	-0.000168000
N	4.481351000	0.485892000	-0.000283000
N	-6.033705000	1.232903000	-0.000049000
C	-2.141720000	-0.379265000	0.000040000
C	1.700013000	0.318454000	-0.000036000
C	-2.786443000	-0.111626000	-1.202937000
C	-2.786124000	-0.110746000	1.202996000
C	2.358440000	1.587418000	-0.000064000
C	3.786425000	-0.656584000	-0.000210000
C	3.730609000	1.616855000	-0.000188000
C	-4.718457000	0.725330000	-0.000026000
C	-4.065316000	0.435297000	-1.205324000
C	-4.064977000	0.436215000	1.205313000
C	4.589962000	-1.936475000	0.000346000
C	4.504479000	2.911858000	-0.000184000
H	-6.254195000	1.767452000	-0.829507000
H	-6.254228000	1.767382000	0.829445000
H	-2.280465000	-0.348400000	-2.130848000
H	-2.279912000	-0.346832000	2.130954000
H	1.760143000	2.490441000	-0.000054000
H	-4.567324000	0.637738000	-2.147700000
H	-4.566708000	0.639415000	2.147675000
H	4.332092000	-2.541126000	-0.873733000
H	4.342799000	-2.533483000	0.882863000
H	5.656893000	-1.711230000	-0.006576000
H	5.154344000	2.961708000	0.879435000
H	3.842021000	3.780874000	-0.000841000
H	5.155359000	2.961066000	-0.879075000



Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.241320 (Hartree/Particle)
Thermal correction to Energy=	0.259546
Thermal correction to Enthalpy=	0.260490
Thermal correction to Gibbs Free Energy=	0.192586
Sum of electronic and zero-point Energies=	-1232.763301
Sum of electronic and thermal Energies=	-1232.745074
Sum of electronic and thermal Enthalpies=	-1232.744130
Sum of electronic and thermal Free Energies=	-1232.812035

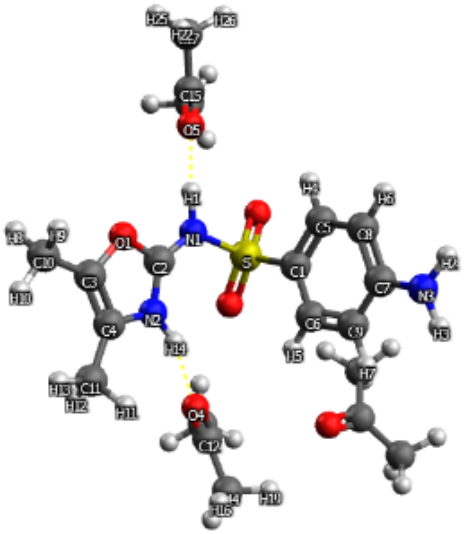
Table S22: Cluster C1-acetone geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

C1L3 acetone cluster
Charge = 1 Multiplicity = 1

Stoichiometry C20H32N3O6S(1+)

S	-0.544467000	-0.825731000	0.985438000
O	-2.927947000	1.922083000	-0.534970000
O	-1.198123000	-1.841589000	1.789631000
O	0.421549000	0.114128000	1.536509000
N	-1.896955000	0.157978000	0.494602000
N	-0.810477000	2.225043000	-0.162710000
N	1.565963000	-3.159830000	-4.021233000
C	0.089319000	-1.517287000	-0.501627000
C	-1.825665000	1.382715000	-0.035185000
C	-2.595430000	3.202004000	-1.018658000
C	-1.283328000	3.396141000	-0.798373000
C	-0.640439000	-2.516254000	-1.161383000
C	1.321120000	-1.080044000	-1.002623000
C	1.092812000	-2.634431000	-2.857578000
C	-0.146098000	-3.063848000	-2.326123000
C	1.816131000	-1.638940000	-2.165856000
C	-3.698361000	3.985344000	-1.618428000
C	-0.382679000	4.543485000	-1.093841000
H	-2.819814000	-0.333899000	0.468375000
H	1.116640000	-3.954911000	-4.442702000
H	2.496674000	-2.944699000	-4.336096000
H	-1.584039000	-2.863650000	-0.759077000
H	1.902291000	-0.337894000	-0.471075000
H	-0.709347000	-3.835468000	-2.838484000
H	2.776459000	-1.309726000	-2.546170000
H	-4.487238000	4.178136000	-0.886245000
H	-4.143190000	3.453345000	-2.463571000
H	-3.321536000	4.943939000	-1.975036000
H	0.503582000	4.206476000	-1.634424000
H	-0.044139000	5.023594000	-0.171781000
H	-0.904544000	5.286802000	-1.695734000
H	0.175458000	2.100914000	0.133996000
C	2.914071000	2.601746000	0.650780000
O	1.891736000	2.541637000	-0.022131000
C	2.908314000	2.327769000	2.131080000
C	4.228375000	2.957967000	0.012884000
H	2.011049000	1.784302000	2.419983000
H	4.070387000	3.405338000	-0.967506000



H	2.941075000	3.287669000	2.660262000
H	3.799294000	1.769099000	2.422188000
H	4.792693000	2.025890000	-0.101623000
H	4.816520000	3.623079000	0.649893000
C	-4.984578000	-1.783714000	1.128904000
O	-4.291578000	-1.182122000	0.318202000
C	-4.579762000	-1.930513000	2.573702000
C	-6.295488000	-2.390626000	0.703746000
H	-3.498789000	-1.851989000	2.684741000
H	-6.547128000	-2.082621000	-0.309529000
H	-4.937099000	-2.871305000	2.996694000
H	-5.053683000	-1.122733000	3.144351000
H	-7.093028000	-2.108907000	1.397335000
H	-6.216936000	-3.482471000	0.745145000
C	4.861551000	-1.259753000	1.023114000
O	4.380859000	-0.305446000	0.439714000
C	4.040924000	-2.104300000	1.972967000
C	6.309387000	-1.655365000	0.832873000
H	3.852734000	-3.081766000	1.515569000
H	6.748809000	-1.099439000	0.005953000
H	3.088799000	-1.620421000	2.185820000
H	4.585687000	-2.291850000	2.902305000
H	6.403265000	-2.730685000	0.657369000
H	6.867835000	-1.436870000	1.749573000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.503533 (Hartree/Particle)
Thermal correction to Energy=	0.542961
Thermal correction to Enthalpy=	0.543905
Thermal correction to Gibbs Free Energy=	0.421493
Sum of electronic and zero-point Energies=	-1794.877468
Sum of electronic and thermal Energies=	-1794.838041
Sum of electronic and thermal Enthalpies=	-1794.837097
Sum of electronic and thermal Free Energies=	-1794.959508

Table S23: Cluster C1-methanol geometry optimization and thermochemistry

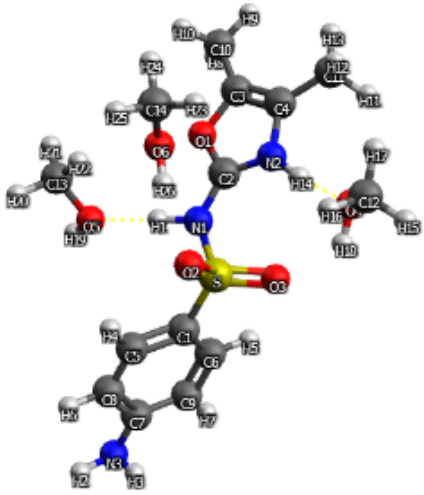
#p B3LYP/6-311g** Opt freq

C1-methanol cluster

Charge = 1 Multiplicity = 1

Stoichiometry C14H26N3O6S(1+)

S	-0.786429000	-0.893279000	0.378826000
O	1.864083000	1.517441000	-1.223115000
O	-0.544139000	-0.700853000	1.806746000
O	-0.312195000	-2.107253000	-0.282909000
N	0.024839000	0.393947000	-0.463037000
N	2.245090000	-0.566682000	-0.757080000
N	-6.469182000	0.073684000	-0.861365000
C	-2.463067000	-0.583689000	0.001503000
C	1.337010000	0.393429000	-0.765814000
C	3.216601000	1.263364000	-1.516855000
C	3.460494000	-0.027543000	-1.230541000
C	-3.262845000	0.108040000	0.920641000
C	-2.999010000	-1.060993000	-1.203265000
C	-5.160562000	-0.150996000	-0.573197000
C	-4.595540000	0.320065000	0.634668000
C	-4.330771000	-0.845749000	-1.485292000
C	4.002751000	2.401117000	-2.043001000
C	4.687711000	-0.863019000	-1.338182000
H	-0.396757000	1.338865000	-0.312945000
H	-7.088148000	0.450311000	-0.163113000
H	-6.899856000	-0.368318000	-1.656155000
H	-2.841381000	0.455246000	1.854509000
H	-2.378480000	-1.614120000	-1.896811000
H	-5.220606000	0.843913000	1.348696000
H	-4.751390000	-1.222157000	-2.410615000
H	3.570203000	2.782974000	-2.971672000
H	5.025090000	2.082976000	-2.247235000
H	4.037043000	3.221687000	-1.321287000
H	4.499960000	-1.755374000	-1.940210000
H	5.029469000	-1.189322000	-0.352104000
H	5.493077000	-0.297112000	-1.805273000
H	2.154775000	-1.547175000	-0.401012000
H	2.625864000	-4.834178000	1.234048000
C	2.723176000	-3.746138000	1.262938000
H	2.213016000	-3.357089000	2.149387000
H	3.781747000	-3.494411000	1.318563000
O	2.210897000	-3.161486000	0.056602000



H	1.264675000	-3.346709000	-0.015243000
H	-1.731222000	3.291234000	-0.339806000
O	-1.100994000	2.791722000	0.188299000
C	-0.375633000	3.684003000	1.058909000
H	-1.052109000	4.154499000	1.776929000
H	0.148481000	4.453604000	0.485551000
H	0.350080000	3.067410000	1.587477000
H	3.145206000	0.185593000	3.322368000
C	2.694843000	1.163691000	3.116467000
H	3.478344000	1.837719000	2.769204000
H	2.282360000	1.564748000	4.048851000
O	1.711061000	1.090792000	2.084596000
H	0.997555000	0.504615000	2.371446000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.409429 (Hartree/Particle)
Thermal correction to Energy=	0.441282
Thermal correction to Enthalpy=	0.442226
Thermal correction to Gibbs Free Energy=	0.341296
Sum of electronic and zero-point Energies=	-1562.610127
Sum of electronic and thermal Energies=	-1562.578274
Sum of electronic and thermal Enthalpies=	-1562.577330
Sum of electronic and thermal Free Energies=	-1562.678260

Table S24: Cluster C2-acetone geometry optimization and thermochemistry

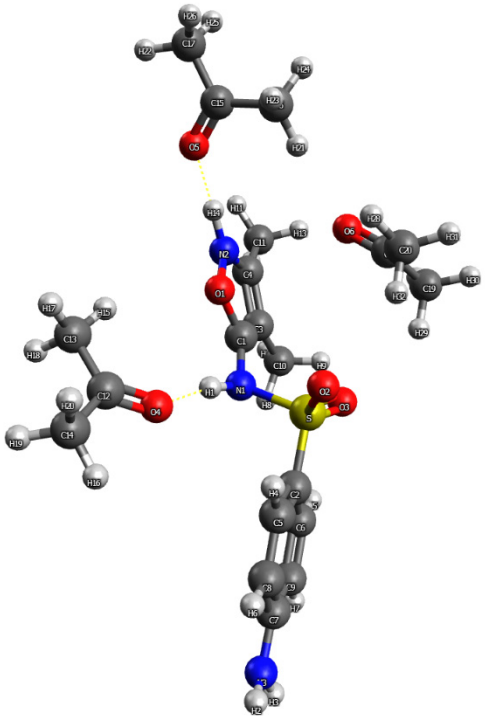
#p B3LYP/6-311g** Opt freq

C2-acetone cluster

Charge = 1 Multiplicity = 1

Stoichiometry C20H32N3O6S(1+)

S	-1.550790000	-0.930014000	0.500307000
O	1.421392000	0.741552000	-0.668836000
O	-1.149965000	-0.453726000	1.819679000
O	-1.151981000	-2.258513000	0.045982000
N	-0.829378000	0.164818000	-0.688266000
N	2.579702000	0.288416000	-1.231197000
N	-7.350771000	-0.088726000	-0.284372000
C	0.441831000	-0.107126000	-1.100556000
C	-3.272194000	-0.673914000	0.264418000
C	0.977856000	-1.091372000	-1.904662000
C	2.361528000	-0.813086000	-1.927952000
C	-3.941736000	0.282502000	1.034989000
C	-3.959984000	-1.440885000	-0.682808000
C	-6.015857000	-0.294132000	-0.089980000
C	-5.299184000	0.467095000	0.858974000
C	-5.316513000	-1.254182000	-0.855137000
C	0.261965000	-2.185384000	-2.627319000
C	3.476160000	-1.556279000	-2.571743000
H	-0.991844000	1.146708000	-0.412703000
H	-7.872526000	0.460324000	0.378299000
H	-7.882547000	-0.747367000	-0.828691000
H	-3.400902000	0.863066000	1.770352000
H	-3.436932000	-2.195477000	-1.255988000
H	-5.823218000	1.201031000	1.460752000
H	-5.853992000	-1.855368000	-1.579724000
H	-0.769912000	-1.893062000	-2.823112000
H	0.234701000	-3.100362000	-2.033182000
H	0.746594000	-2.400374000	-3.581881000
H	4.365554000	-0.933536000	-2.668586000
H	3.181691000	-1.915239000	-3.558668000
H	3.719615000	-2.418865000	-1.945311000
H	3.463544000	0.770741000	-0.920505000
C	-1.334633000	3.977140000	0.089815000
O	-1.601569000	2.787995000	0.121917000
C	-0.060524000	4.491217000	-0.539001000
C	-2.275794000	5.000146000	0.675459000
H	0.642282000	3.675760000	-0.704925000



H	-3.231468000	4.538789000	0.917673000
H	0.399915000	5.265740000	0.079373000
H	-0.302214000	4.956029000	-1.501288000
H	-2.421740000	5.836846000	-0.013039000
H	-1.833740000	5.414401000	1.588158000
C	5.686602000	1.367986000	0.311747000
O	4.898180000	1.475006000	-0.622252000
C	5.396885000	0.499736000	1.506542000
C	6.984516000	2.127421000	0.290700000
H	4.600553000	-0.216375000	1.300113000
H	6.988502000	2.852454000	-0.521100000
H	5.087019000	1.150350000	2.333293000
H	6.298241000	-0.019758000	1.840464000
H	7.808978000	1.420042000	0.147842000
H	7.160380000	2.623321000	1.249111000
C	2.276641000	-2.527769000	1.658502000
O	2.965979000	-1.969506000	0.820070000
C	1.666286000	-3.884938000	1.399473000
C	1.951541000	-1.875040000	2.981608000
H	2.118124000	-4.340354000	0.518934000
H	2.512865000	-0.949099000	3.099252000
H	0.592500000	-3.756078000	1.224976000
H	1.780846000	-4.544447000	2.263113000
H	2.165743000	-2.550882000	3.813861000
H	0.880511000	-1.647593000	3.014169000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.503018 (Hartree/Particle)
Thermal correction to Energy=	0.542462
Thermal correction to Enthalpy=	0.543406
Thermal correction to Gibbs Free Energy=	0.420495
Sum of electronic and zero-point Energies=	-1794.821946
Sum of electronic and thermal Energies=	-1794.782502
Sum of electronic and thermal Enthalpies=	-1794.781558
Sum of electronic and thermal Free Energies=	-1794.904469

Table S25: Cluster C2-methanol geometry optimization and thermochemistry

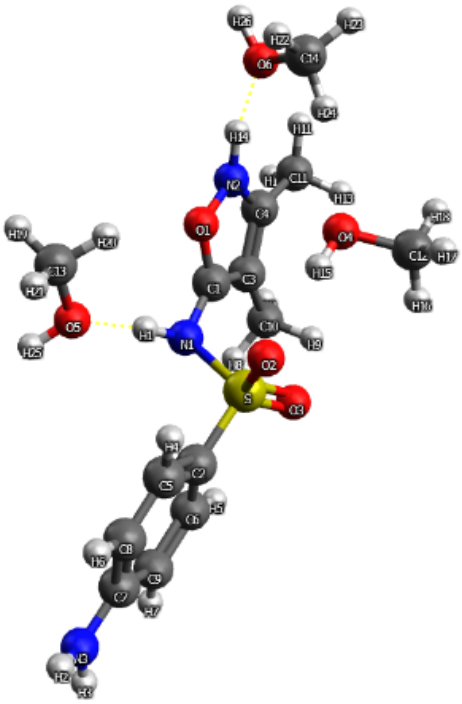
#p B3LYP/6-311g** Opt freq

C2-methanol cluster

Charge = 1 Multiplicity = 1

Stoichiometry C14H26N3O6S(1+)

S	-1.151916000	0.694070000	-1.101475000
O	0.945830000	-0.719702000	0.608518000
O	-0.264745000	-0.116745000	-1.929407000
O	-1.725387000	1.940889000	-1.575428000
N	-0.185567000	1.249065000	0.251460000
N	2.124907000	-1.122419000	1.192491000
N	-5.399359000	-2.727950000	1.183387000
C	0.904802000	0.622405000	0.732116000
C	-2.400938000	-0.330478000	-0.411584000
C	2.034732000	1.093026000	1.396344000
C	2.796382000	-0.057435000	1.624888000
C	-2.249319000	-1.721314000	-0.414468000
C	-3.559170000	0.260950000	0.108352000
C	-4.424283000	-1.943676000	0.644027000
C	-3.249860000	-2.516945000	0.107896000
C	-4.556317000	-0.536475000	0.630815000
C	2.396076000	2.505802000	1.737422000
C	4.148415000	-0.204262000	2.231349000
H	-0.314880000	2.256692000	0.454517000
H	-5.378863000	-3.724061000	1.041567000
H	-6.295034000	-2.329711000	1.411536000
H	-1.362663000	-2.169269000	-0.843183000
H	-3.683504000	1.335873000	0.077518000
H	-3.139558000	-3.595339000	0.096028000
H	-5.457465000	-0.080464000	1.024664000
H	1.680942000	3.207831000	1.308557000
H	3.386465000	2.759431000	1.350802000
H	2.412016000	2.662640000	2.819836000
H	4.831649000	0.531033000	1.802096000
H	4.546645000	-1.203214000	2.054292000
H	4.102384000	-0.028087000	3.309461000
H	2.489551000	-2.048155000	0.859727000
H	1.673598000	-0.424199000	-2.046332000
O	2.613946000	-0.430846000	-1.817511000
C	3.307482000	0.371795000	-2.772347000
H	2.957975000	1.410869000	-2.764423000
H	3.208556000	-0.022750000	-3.789747000



H	4.363963000	0.362041000	-2.500805000
C	-0.932845000	4.893741000	-0.311887000
H	-2.003449000	5.101978000	-0.248264000
H	-0.734568000	4.384175000	-1.251601000
H	-0.366444000	5.827174000	-0.266685000
C	3.627780000	-3.576986000	-1.016910000
H	2.978148000	-4.344941000	-1.444277000
H	4.674386000	-3.865039000	-1.138900000
H	3.440731000	-2.624584000	-1.510971000
O	-0.518281000	3.996235000	0.739946000
H	-0.782679000	4.364414000	1.588847000
O	3.323072000	-3.369315000	0.382100000
H	3.369788000	-4.205345000	0.856160000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.408930 (Hartree/Particle)
Thermal correction to Energy=	0.440952
Thermal correction to Enthalpy=	0.441896
Thermal correction to Gibbs Free Energy=	0.340501
Sum of electronic and zero-point Energies=	-1562.556272
Sum of electronic and thermal Energies=	-1562.524249
Sum of electronic and thermal Enthalpies=	-1562.523305
Sum of electronic and thermal Free Energies=	-1562.624700

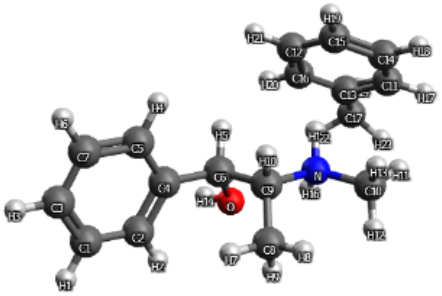
Table S26: Cluster Ephedrine-toluene geometry optimization and thermochemistry

#p B3LYP/6-311g** Opt freq

Ephedrine-toluene cluster

Charge = 1 Multiplicity = 1

Stoichiometry C17H24NO(1+)



C	-4.845387000	-0.274625000	0.914986000
C	-3.496594000	0.027172000	1.091716000
C	-5.270982000	-0.937245000	-0.233917000
C	-2.562184000	-0.340574000	0.119466000
C	-2.993622000	-1.017730000	-1.025525000
C	-1.088742000	-0.023604000	0.282733000
C	-4.342854000	-1.308458000	-1.205237000
C	-1.413363000	2.526243000	0.036349000
C	-0.658437000	1.285816000	-0.421156000
C	1.511944000	2.688243000	-0.542839000
O	-0.673879000	0.161747000	1.638617000
N	0.839227000	1.417602000	-0.129906000
H	-5.562867000	0.007622000	1.676350000
H	-3.170032000	0.542009000	1.987476000
H	-6.320150000	-1.171581000	-0.368555000
H	-2.274285000	-1.332166000	-1.775968000
H	-0.510206000	-0.842056000	-0.167422000
H	-4.667539000	-1.835310000	-2.094581000
H	-2.478261000	2.365857000	-0.129306000
H	-1.126236000	3.411089000	-0.532482000
H	-1.265531000	2.715737000	1.101424000
H	-0.745667000	1.155902000	-1.501754000
H	2.578090000	2.579510000	-0.357111000
H	1.112218000	3.516006000	0.035955000
H	1.336500000	2.851026000	-1.604931000
H	-0.911072000	-0.615646000	2.156553000
H	1.330253000	0.626645000	-0.573561000
H	0.933217000	1.263025000	0.881708000
H	5.103955000	0.686125000	0.086606000
C	4.286157000	0.029711000	-0.192926000
C	2.239198000	-1.696279000	-0.946259000
C	3.740994000	-0.834768000	0.767014000
C	3.820832000	0.034785000	-1.507991000
C	2.790442000	-0.827136000	-1.892093000
C	2.707054000	-1.692749000	0.368885000
H	4.280271000	0.690314000	-2.239529000
H	2.453496000	-0.852978000	-2.922204000

H	2.284704000	-2.386137000	1.088595000
H	1.469098000	-2.400723000	-1.241631000
C	4.295264000	-0.884412000	2.169186000
H	3.540651000	-1.203223000	2.890942000
H	4.685793000	0.085451000	2.483934000
H	5.121729000	-1.600042000	2.225542000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.378389 (Hartree/Particle)
Thermal correction to Energy=	0.398926
Thermal correction to Enthalpy=	0.399870
Thermal correction to Gibbs Free Energy=	0.324973
Sum of electronic and zero-point Energies=	-791.861639
Sum of electronic and thermal Energies=	-791.841103
Sum of electronic and thermal Enthalpies=	-791.840159
Sum of electronic and thermal Free Energies=	-791.915055

Table S27: Cluster Pseudoephedrine-toluene geometry optimization and thermochemistry

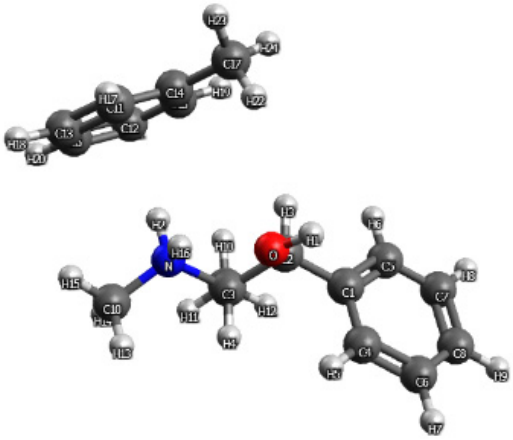
#p B3LYP/6-311g** Opt freq

PsEph toluen cluster

Charge = 1 Multiplicity = 1

Stoichiometry C17H24NO(1+)

O	0.589412000	-0.336187000	-1.594582000
N	-0.909864000	1.415285000	-0.440518000
C	2.521565000	-0.241011000	-0.075477000
C	1.023953000	-0.107946000	-0.243596000
C	0.524644000	1.317553000	0.072213000
C	3.393777000	0.375790000	-0.981245000
C	3.047728000	-0.986483000	0.982844000
C	4.770412000	0.253901000	-0.820899000
C	4.426716000	-1.102729000	1.145088000
C	5.288583000	-0.481454000	0.244782000
C	0.609311000	1.697943000	1.541673000
C	-1.378992000	2.793884000	-0.778430000
H	1.010029000	-1.131822000	-1.939032000
H	-1.556405000	0.987967000	0.238876000
H	0.519876000	-0.815139000	0.428137000
H	1.091603000	2.016504000	-0.546184000
H	2.996998000	0.932447000	-1.823406000
H	2.381065000	-1.484529000	1.679584000
H	5.439372000	0.729565000	-1.528205000
H	4.825685000	-1.684314000	1.967523000
H	6.360939000	-0.575531000	0.367502000
H	0.012890000	1.024968000	2.163488000
H	0.278346000	2.722991000	1.716766000
H	1.646866000	1.629330000	1.869428000
H	-0.730552000	3.203146000	-1.550986000
H	-1.341918000	3.417355000	0.111930000
H	-2.402588000	2.730265000	-1.141235000
H	-0.940429000	0.816399000	-1.277331000
H	-3.906685000	-1.096816000	-1.986325000
C	-3.698018000	-0.802051000	-0.962983000
C	-3.229883000	-0.062174000	1.678759000
C	-4.275691000	0.358864000	-0.452019000
C	-2.882539000	-1.618639000	-0.164952000
C	-2.658369000	-1.229241000	1.161347000
C	-4.042755000	0.736811000	0.872955000
H	-4.932803000	0.952795000	-1.077702000
H	-2.060637000	-1.862739000	1.808626000



H	-4.518380000	1.620732000	1.282021000
H	-3.072964000	0.198830000	2.719625000
C	-2.283817000	-2.886440000	-0.722932000
H	-1.510092000	-2.661539000	-1.463965000
H	-3.042883000	-3.491949000	-1.223631000
H	-1.833330000	-3.495875000	0.062013000

Thermochemistry:

Temperature 298.150 Kelvin. Pressure 1.00000 Atm.

Zero-point correction=	0.378020 (Hartree/Particle)
Thermal correction to Energy=	0.398679
Thermal correction to Enthalpy=	0.399623
Thermal correction to Gibbs Free Energy=	0.325128
Sum of electronic and zero-point Energies=	-791.866369
Sum of electronic and thermal Energies=	-791.845710
Sum of electronic and thermal Enthalpies=	-791.844765
Sum of electronic and thermal Free Energies=	-791.919261