ESI or APCI to ionize pharmaceuticals: a chemometric approach

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1. Introduction

LC-MS(/MS) could evolve into an alternative to GC-MS for systematic toxicological analysis (STA). To that end, we have successfully explored the use of data directed MS/MS acquisition for small molecules. However, compounds not ionized by electrospray will inevitably be missed. Save for dedicated target analysis, the ionization technique used is nowadays chosen rather arbitrarily for small molecules. Only when ESI fails, APCI or even as yet less common approaches such as atmospheric pressure photoionization are tried. Our aim was to explore, for a number of common pharmaceuticals, often encountered in a toxicological setting, which ionization technique was most suitable and, moreover, try to find some systematic pattern.

2. Experimental

- Over 200 compounds analyzed in both ESI and APCI
  - Opiates, benzodiazepines, thiazide diuretics, amphetamins, steroids, NSAI's, β-blockers, tricyclic antidepressants, barbiturates, fibrates, antihistamins, butyrophenons, hypoglycaemic sulfonylureas, …
  - Triplicate flow injections (Waters 2790 HPLC, 1mM NH_4Ac buffer/CH_3CN, 50/50, pH 7.0), single MS (Micromass QTof®), positive ion mode
  - Standardized conditions, peak area ratio to cocaine as standard, ratios related to molar concentration
- t-test to statistically evaluate which ionization approach is the better. Delineation of 4 groups: ESI, APCI, EQ (no statistical difference), and NS (no observed signal, some acidic compounds were deliberately introduced into the data set to produce this counter-group.)
- Calculation of 76 descriptors for each molecule, based on geometry optimized (Polak-Ribiere algorithm, Hyperchem®) 3D-molecular structure. Descriptors were chosen to represent various physico-chemical characteristics of the molecule:
  - Constitutional descriptors (surface area, number of oxygen atoms, sum of Van der Waals volumes, ...)
  - Topological descriptors (polarizability, solvation connectivity index χ-2)
  - Geometrical descriptors (span, 3D Wiener index, ...)
  - Charge descriptors (nr. of acid or basic centres, weighed sum of acid ($\Sigma w_i.1/pka_i$) or basic ($\Sigma w_i.pka_i$) pka's, maximum formal charge, ...)
  - Functional group descriptors (nr. of carboxylic acid groups, nr. of acceptor atoms for hydrogen bonds, ...)
  - Empirical descriptors (hydrophilic factor, logP, logD at pH 7, ...).

  Descriptors were calculated using Hyperchem® or Dragon® 2.1 and pka's were, if unavailable through literature, approximated using Pallas® 3.0 prediction software. All results were normalized (x_i/x_{max})
- Resulting multivariate 223x76 data matrix was evaluated using PCA, Principal Component Analysis (The Unscrambler®). Data were centred and full cross validation was used.

3. Results and discussion

PCA showed clustering of the group members and allowed the reduction of the number of descriptors to those with intergroup discrimination power (25 descriptors).

In a first stage, we were able to clearly identify, descriptors separating the NS samples from the rest (nr. H donors, nr. COOH's, nr. acid pka's, weighed sum acid pka's, max. neg. formal charge, nr. Oxygens). Logically, they were associated with the physico-chemical molecular reality of lack of positive charge or (potential) presence of a negative charge.

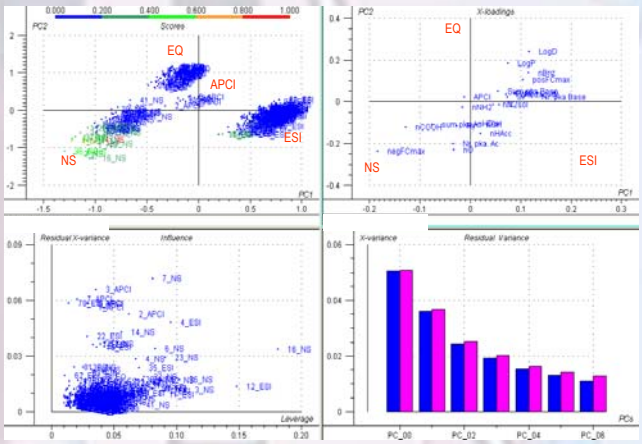

Fig. 1: Score (samples color grouped according to weighed sum pka acid) and loading plot for PC1 vs. PC2; residual variance vs. leverage and residual variance vs. PCs.

The higher the descriptor loading in the direction of a cluster, the more this descriptor influences the position of the cluster members. Taking out the NS group, in a second stage, showed clustering of the remaining groups along PC1 (EQ: large neg. PC1, ESI: large pos. PC1), although the loadings indicated that only few of our descriptors (see loadings Fig 2.) were useful for group separation. Interestingly, most other descriptors (such as logP) aligned to PC2 and rather than separating the groups, they are useful for within group evaluation of the efficacy of the particular ionization technique.

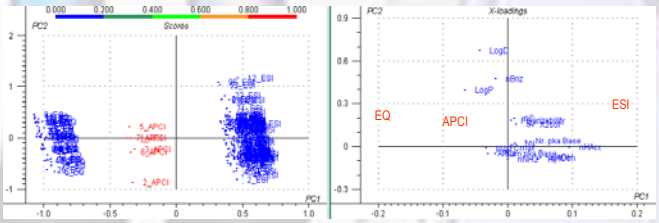

Fig. 2: Score and loading plot for PC1 vs. PC2 (without NS samples)

4. Conclusion

From the now available results, we conclude that complete modelling for classification purposes needs additional descriptors with intergroup discrimination power. Nevertheless, it seems that e.g. a large number of basic centres in the molecule (NrPkaBase pos. along PC1) favours ESI while APCI might be more appropriate with a small number of high magnitude basic centres (SumPkaBase neg. along PC1 while NrPkaBase positive). However, as the APCI cluster is closer to the EQ cluster than the ESI cluster, compounds working with APCI are expected to give at least some ESI response too in the majority of cases.

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