Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 721–727

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Understanding of the mass spectrometric fragmentation pathways of a few potentially genotoxic haloaniline isomers in their protonated form by collision-induced dissociation

Rex Patrick[a,*], N.S. Nagarajan[b], Hefeng Pan[c], Subhra Kanti Saha[a], Jayan Rapai[a], Venkatarao Torlikonda[a], Manoranjan Panda[a], Sulur G. Manjunatha[a], Sudhir Nambiar[a]

[a] Analytical Sciences, Pharmaceutical Development, AstraZeneca India Pvt Limited, Bangalore 560024, India
[b] Department of Chemistry, Gandhigram Rural Institute-Deemed University, Gandhigram 624 302, Tamil Nadu, India
[c] Analytical Sciences, Pharmaceutical Development, AstraZeneca, 151 85 Södertälje, Sweden

ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 15 July 2011
Accepted 19 July 2011
Available online 26 July 2011

Keywords:
CID
PGI
ESI
APCI
Ortho-effect
Haloanilines

ABSTRACT

Collision-induced dissociation (CID) mass spectra of a few haloaniline isomers, (chloroanilines, dichloroanilines, difluoroanilines, chloro-fluoroanilines and bromo-fluoroanilines) were characterized. The mass spectral behaviour of difluoroanilines was different from those of the corresponding regioisomers of the other haloanilines. For all *ortho* regioisomers except difluoroanilines, CID mass spectra resulted in hydrogen halide as well as halogen radical loss. In the case of difluoroanilines, peaks corresponding to hydrogen fluoride loss were observed during the same process. *Meta* and *para*-haloanilines have the tendency to lose either ammonia or halogen radicals. Six regioisomers of dichloroanilines were subjected to hydrogen/deuterium exchange experiments in solution to determine the CID fragmentation pathways. From the experimental results we propose two fragmentation pathways for the dicholoroanilines: (a) formation of aza-biheterocyclic intermediate and (b) via heterolytic hydrogen transfer from the charged center. The demonstrated unique characteristics in CID mass fragmentation of haloanilines may be useful in identification and differentiation of isomers as impurities during chemical process development. A good use of the ortho effect is the significant differentiation between 2-chloro-4-fluoroaniline and 4-chloro-2-fluoroaniline by CID mass spectra.

© 2011 Elsevier B.V. All rights reserved.

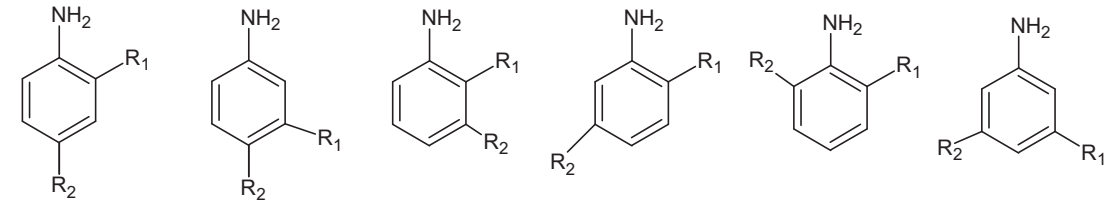
1. Introduction

Control of impurities in active pharmaceutical ingredients (API) plays a major role in pharmaceutical development. During the development and optimisation stages, different types of impurities are observed. A special class of impurities known as "potentially genotoxic impurities" or PGIs [1] and/or carcinogenic impurities have unusually high toxicity. Whether an impurity is a PGI or genotoxic is determined by Ames test [2,3]. Primary and secondary aromatic haloamines are generally not inherently genotoxic but require metabolic activation in order to generate an electrophilic species [4]. To exert their mutagenic effect, aromatic amines are activated through *N*-oxidation by cytochrome P450s to form *N*-hydroxylamines which in turn undergo N–O bond cleavage to form the nitrenium ion either directly or following conjugation of the hydroxylamine with acetate or sulfate. The nitrenium ion intermediate then forms an adduct with DNA, which results in miscoding during DNA replication. According to this mechanism the stability of the nitrenium ion is fundamental in determining the extent of that mutagenic effect [5–8] that leads to genotoxicity.

4-Chloroaniline [5] is considered to be a mutagen as per Ames test which is the starting material for chlorohexidine diacetate and proguanil, an antimalarial drug. 2,6-Dichloroaniline [9] is the starting material for the manufacture of diclofenec sodium which is an anti-inflammatory analgesic and clonidine, an antihypertensive agent and an epidural agent for refractory cancer pain. 2,4-Dichloroaniline [10], 2,4-difluoroaniline [3,5] and 4-fluoroaniline [5] are key building blocks for the manufacture of few new drugs under development. All the aforementioned compounds show Ames positive results. 2,6-Dichloroaniline was reported as a PGI in an application note [9]. Further, toxicological studies of dichloroaniline (DCA) isomers on rats indicate that these chemicals are capable of altering renal function *in vivo* and *in vitro*, and possess nephrotoxic potential [11]. Thus, it is imperative to determine the presence of these compounds during the development stages and adequately control them. In this article, the use of CID mass spectrometry in the analysis and differentiation of these haloanilines isomers is described.

* Corresponding author. Tel.: +91 80 2362 1212; fax: +91 80 2362 2011.
E-mail address: rex.patrick@astrazeneca.com (R. Patrick).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.07.022



1. R_1=Cl, R_2=H
2. R_1=R_2=Cl
3. R_1=R_2=F
4. R_1=F, R_2=Cl
5. R_1=Cl, R_2=F
6. R_1=Br, R_2=F
7. R_1=H, R_2=Cl
8. R_1=R_2=Cl
9. R_1=R_2=F
10. R_1=F, R_2=Cl
11. R_1=Cl, R_2=F
12. R_1=Br, R_2=F
13. R_1=F, R_2=Br
14. R_1=H, R_2=Cl
15. R_1=R_2=Cl
16. R_1=R_2=F
17. R_1=F, R_2=Cl
18. R_1=R_2=Cl
19. R_1=R_2=F
20. R_1=F, R_2=Br
21. R_1=R_2=Cl
22. R_1=Cl, R_2=F
23. R_1=R_2=Cl
24. R_1=R_2=F

Fig. 1. Haloanilines studied.

Some reports in literature provide description of distinguishing *ortho* isomers through ortho effect in mass fragmentations [12–16]. N-Acylanilines bearing a proximal halo substituent are distinguished by electron ionisation mass spectrometry techniques [17]. An ortho effect in mass spectrometric fragmentation has been observed from hydroxyphenyl carbaldehydes, ketones and alkyloxybenzoic acids with negative electrospray ionization [18–20]. It has been reported that the characterization of *ortho*-, *meta*- and *para*- isomers of haloanilines by Electron Ionization (EI) is not straight forward [21]. However CID spectra of electrospray derived positive ions of mono haloanilines allow the distinction of the *ortho* isomer [21]. Recently mass spectrometric techniques have been used to recognise and resolve representative isomeric pairs of *N*-alkyl and ring-alkyl substituted anilines using ion/molecule reactions of structurally diagnostic fragmentation ions (SDFI) [22]. In our exploration, we have studied collision-induced dissociation mass analysis of 24 haloaniline regioisomers (Fig. 1) of which, a few are considered to be PGIs.

2. Materials and methods

2.1. Reagents

All haloanilines were purchased from Sigma–Aldrich (St. Louis, USA) with the purity of 98% as per the label claim.

2.2. LC/MS/MS instrumentation

The CID mass spectra of all haloanilines, except dichloroanilines, were recorded using an Agilent Triple Quadrupole Mass Spec-

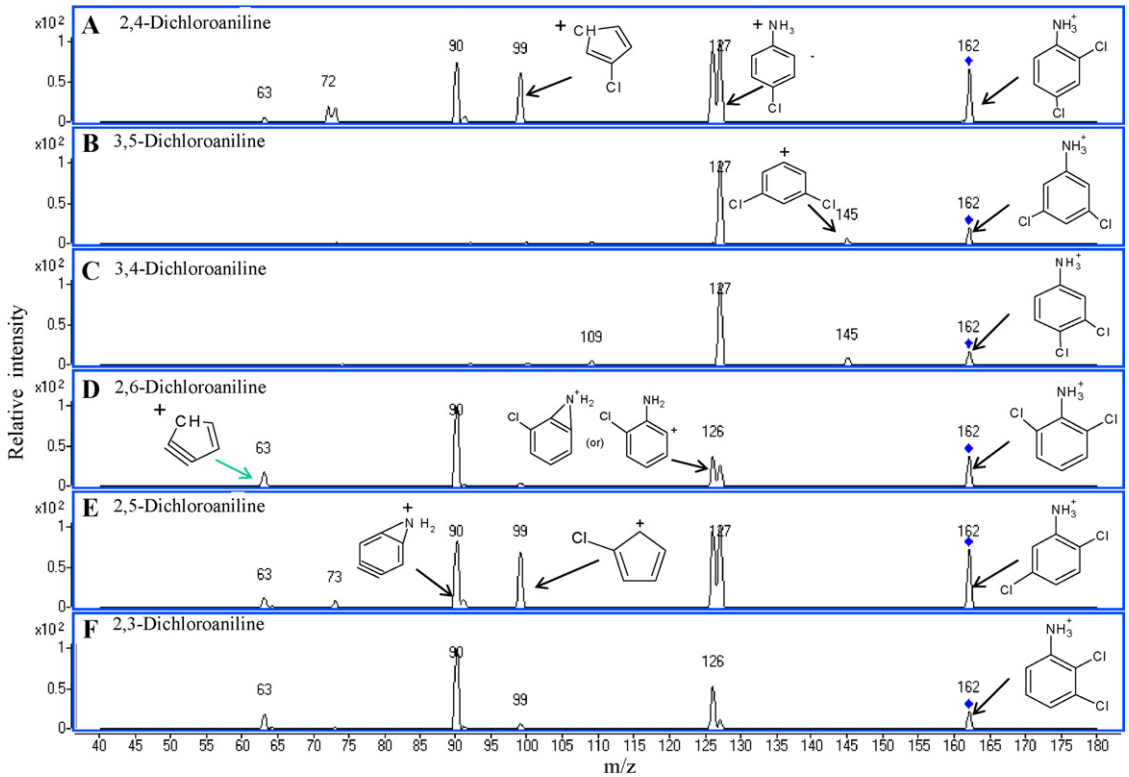


Fig. 2. CID Product ion spectra of protonated 2,4-dichloroaniline (A), 3,5-dichloroaniline (B), 3,4-dichloroaniline (C) 2,6-dichloroaniline (D), 2,5-dichloroaniline (E) and 2,3-dichloroaniline (F) obtained by MS–MS.

trometer (Model: G6410A, Wilmington, DE, USA) coupled with Agilent 1200 Liquid Chromatography System (76337, Waldbronn, Germany). All the samples were introduced into a multimode ionisation source which is a combination of electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) ionisation sources, through HPLC using water–acetonitrile (1:1) as mobile phase at a flow rate of 1.0 ml/min. Collision energy was kept as 20 eV, with a fragmentation voltage of 50 V, and nitrogen gas was used as collision gas. Deuterated DCAs were introduced into the mass spectrometer by infusion at a flow rate of 15 μl/min. Positive ESI was used for analysing deuterated compounds. For chloro and bromoanilines, the ^{35}Cl– or ^{79}Br–isotoplogues were selected for fragmentation in order to avoid complexities.

2.2.1. Preparation of N-deuterated dichloroaniline isomers

The *N*-deuterated dichloroanilines were prepared by dissolving each dichloroaniline in methanol-d4 followed by D_2O in the ratio 2:1 and stirred for 30 min under nitrogen atmosphere and the solvents were removed by flushing nitrogen. This procedure was repeated to ensure maximum deuterium-exchange for all exchangeable hydrogen. The residue was dissolved in a mixture of methanol-d4 and D_2O (2:1) and the solutions were directly infused into the mass spectrometer.

2.3. Computational details and systems studied

Full geometry optimization using B3LYP functional were performed with 6-31+G(d,p) basis set. The stationary points were characterized by analytical frequency calculations. All the calculations have been carried out using Gaussian 03 suite of programs [23].

3. Results and discussion

Mass spectra of many *ortho*-disubstituted aromatic compounds are generally distinguished from those of their *meta* and *para* isomers as a result of ortho effect, in which transfer of a labile hydrogen atom from a donor functional group to the ortho position of an aromatic ring takes place, via a six-membered transition state [24–26]. However, there is a growing body of evidence to support that the reaction follows a stepwise mechanism to eliminate a neutral molecule from the molecular ion [21]. Product ion spectra from the haloanilines in the study showed distinctive peak patterns among *ortho* isomer and *meta* or *para* isomers demonstrating that the effect provided by *ortho*-amine function played a significant role in the MS fragmentations. Generally, ortho effect leads to loss of a neutral molecule, although peaks corresponds to loss of a radical has also been reported [27]. In the present study, the results indicate loss of both neutral molecule and radical in the CID mass spectra of *ortho*-haloanilines.

3.1. Chloroaniline isomers

The CID mass spectrum of *ortho*-chloroaniline was different from those of *meta* and *para* isomers. The ions at *m*/*z* 93, 92, 65 are formed by the losses of chlorine radical (35 Da) or hydrogen chloride (36 Da) and hydrogen cyanide (27 Da) respectively; the *meta* and *para* isomers exhibited peaks at *m*/*z* 111 $[M+H–NH_3]^+$, *m*/*z* 93 $[M+H–Cl^•]^+$ and *m*/*z* 75 $[M+H–NH_3–HCl]^+$. Chlorine at meta position influence the removal of ammonia molecule hence the relative intensities of peak at *m*/*z* 111 in *meta* isomer is almost double that of the *para* isomer.

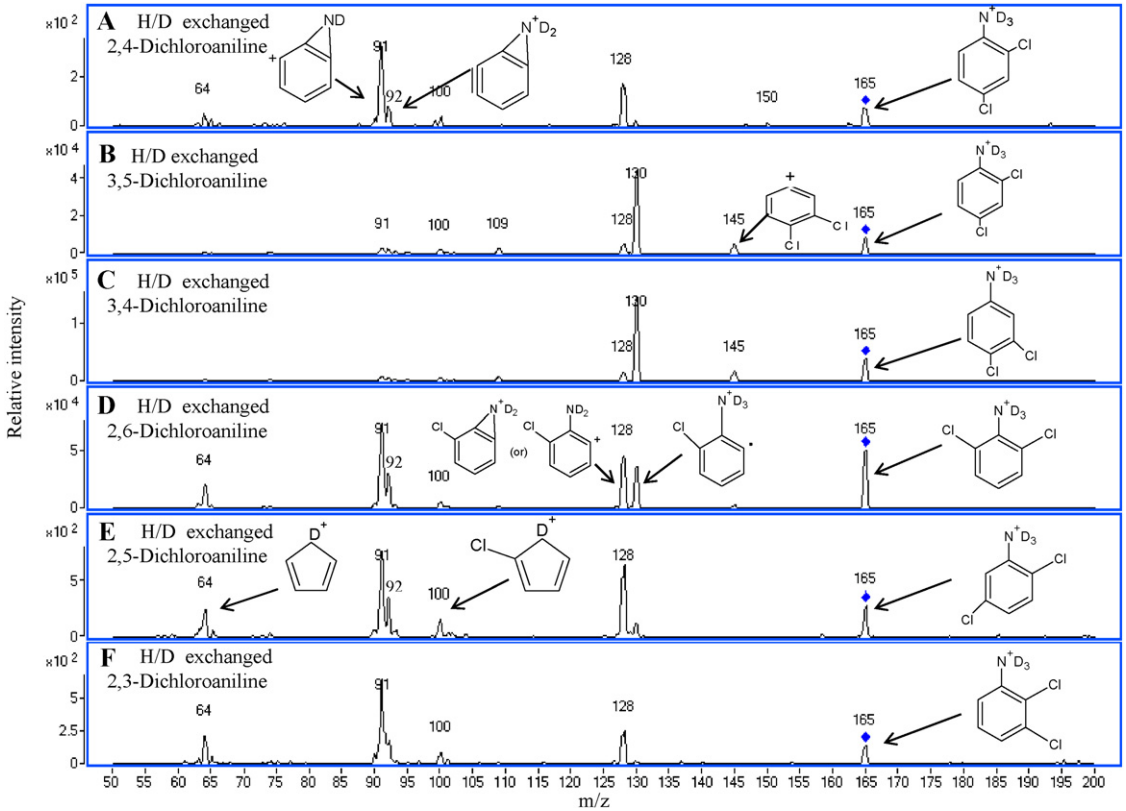


Fig. 3. CID Product ion spectra of protonated H/D exchanged 2,4-dichloroaniline (A), 3,5-dichloroaniline (B), 3,4-dichloroaniline (C) 2,6-dichloroaniline (D), 2,5-dichloroaniline (E) and 2,3-dichloroaniline (F) obtained by MS–MS.

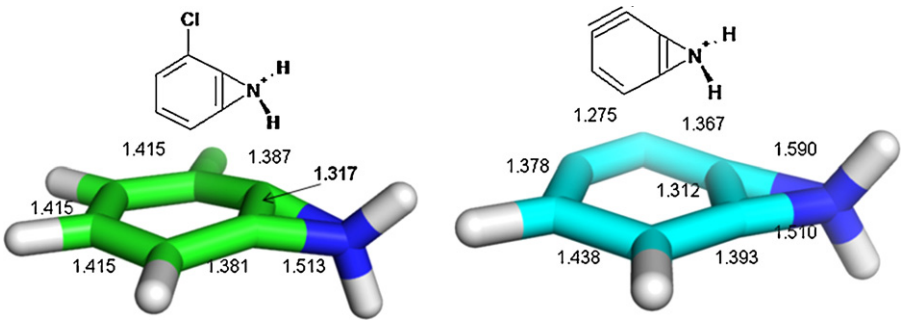


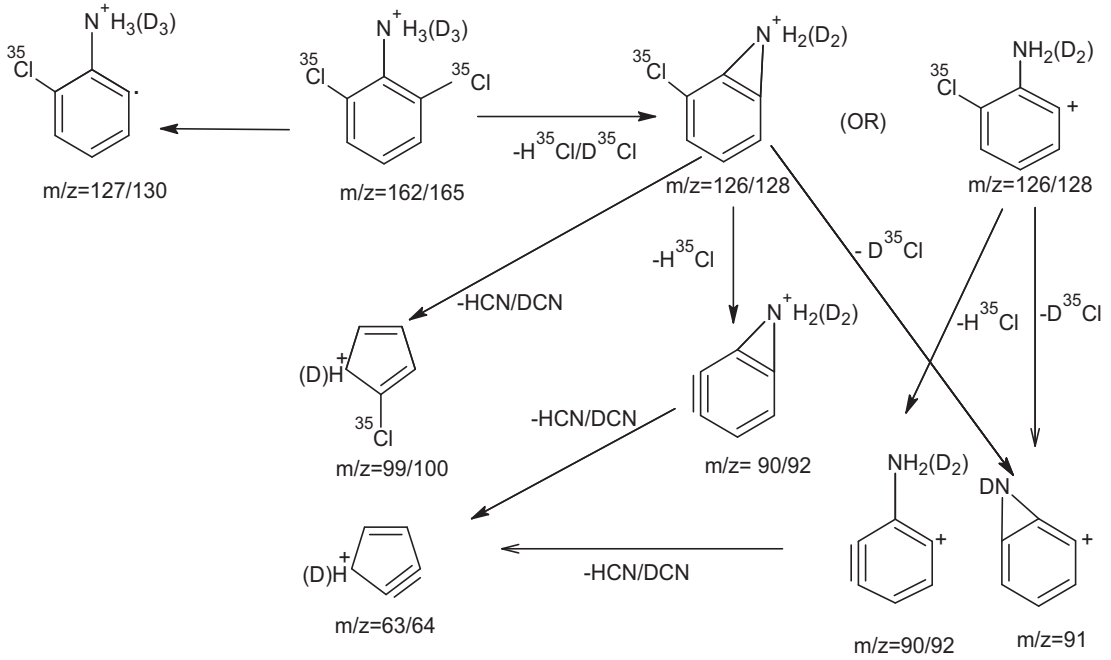
Fig. 4. B3LYP/6-31+G(d,p) optimized geometries.

3.2. Dichloroaniline isomers

Our CID mass spectral analysis of *ortho*-chloroanilines substituted with one more chlorine at positions 3/4/5 or 6 (2,3; 2,4; 2,5 and 2,6-DCA) clearly showed two distinctive peaks at *m*/*z* 126 and 127 due to loss of neutral molecule HCl and loss of chlorine radical respectively. In case of 3,5-dichloroaniline and 3,4-dichloroaniline, the most intense peak was *m*/*z* 127, and peak at *m*/*z* 126 was absent in the CID mass spectra (Fig. 2) indicating that neutral loss of $H^{35}Cl$ was primarily due to the interaction between the amine function and the chlorine atom at the *ortho* position. When amine function is not in proximity with chlorine atom, as no active hydrogen is available for a neutral loss of hydrogen chloride, loss of chlorine radical or ammonia has taken place as an alternative pathway.

To confirm this observation, experiments were performed on *N*-deuterated DCA isomers ([*N*,*N*-$^2H_2$]dichloroanilines). The CID mass spectra of *N*-deuterated *ortho*-chloroanilines (2,3; 2,4; 2,5 and 2,6-DCA) showed a peak at *m*/*z* 128 corresponding to the neutral loss of $D^{35}Cl$ (37 Da) (Fig. 3). This observation confirms the ortho effect and revealed that the neutral loss of $D^{35}Cl$ may have taken place through either formation of aza-biheterocyclic intermediate or via a heterolytic hydrogen transfer from the charged center. Subsequently, in case of 2,6-DCA, loss of hydrogen cyanide leads to fragment at m/z 99 and elimination of the second chlorine atom as hydrogen chloride has resulted in the formation of fragments at *m*/*z* 90, which indicates that probably hydrogen atom of the eliminated hydrogen chloride was either from the aromatic ring or from the amine function. Studies on deuterated 2,6-DCA has resulted in the formation of a more intense ion peak of *m*/*z* 91 and relatively less intense ion peak of *m*/*z* 92 which suggests that loss of $D^{35}Cl$ and $H^{35}Cl$ respectively. The experimental results indicate the second neutral loss of H35Cl is due to the interaction of the ortho- chlorine with the hydrogen available either from the amine function or the aromatic ring. From this experimental results we have proposed two fragmentation pathways. Density functional theory (DFT) calculations suggested that the aza-biheterocyclic intermediates are energetically feasible. Two fused aziridine systems we have taken for DFT calculations are found to be stable electronically (Fig. 4). The frequency calculations suggest that both the structures lie in local minima.

From these observations, we propose two types of fragmentation pathways for DCAs: (a) formation of aza-biheterocyclic intermediate and (b) via a heterolytic hydrogen transfer from the charged center (Scheme 1). For 3,5-dichloroaniline and 3,4-dichloroanilines, ions at *m*/*z* 127 and 130 (deuterated analog) are formed by the loss of chlorine radical. A peak at *m*/*z* 145 was observed due to the neutral loss of NH_3 and ND_3 in the respective non deuterated or deuterated analogs. The differences are not enough to distinguish the 3,4 and 3,5-dichloroanilines (Figs. 2 and 3).



Scheme 1. The proposed fragmentation pathways of 2,6-DCA.

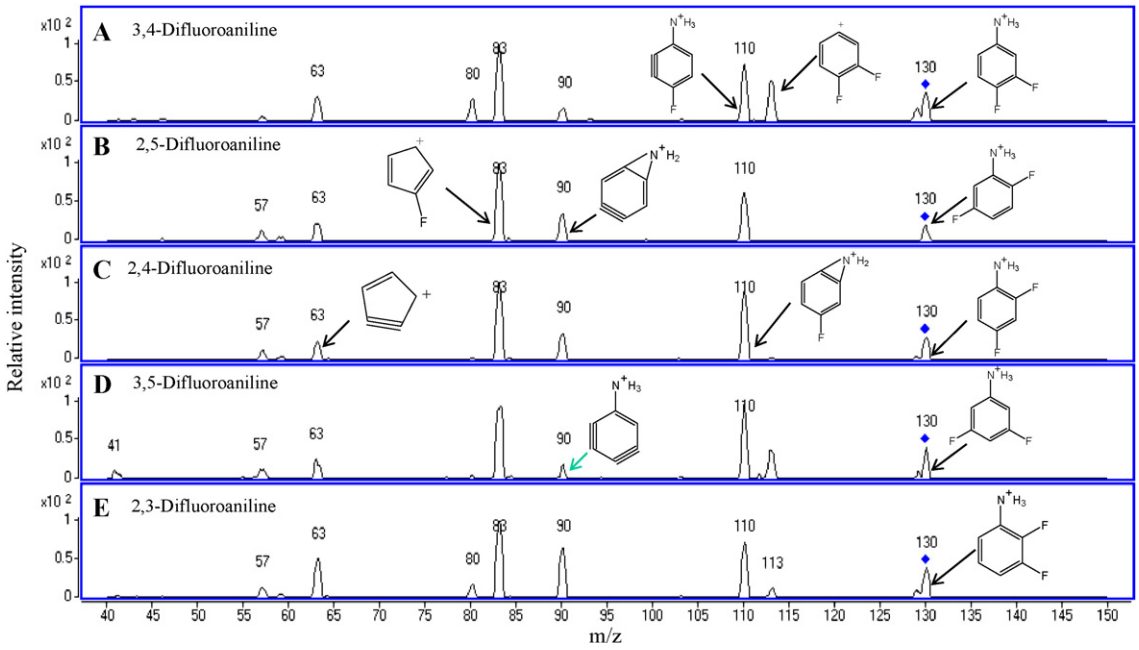


Fig. 5. CID Product ion spectra of protonated 3,4-difluoroaniline (A), 2,5-difluoroaniline (B), 2,4-difluoroaniline (C), 3,5-difluoroaniline (D), and 2,3-difluoroaniline (E) obtained by MS–MS.

3.3. *Difluoroaniline isomers*

The only reported mutagen in the difluoroaniline category we have studied is 2,4-difluoroaniline. The difluoroanilines (DFA) isomers, (2,3; 2,4; 2,5; 3,4 and 3,5-DFA) showed a peak at *m*/*z* 110 due to loss of HF (20 Da) (Fig. 5). However, no radical cations were observed for these fluorine isomers, which may be due to high electronegativity of fluorine atom. For *meta*-difluoroanilines (3,4 and 3,5-DFA), since no active hydrogen atom was available, the electronegative fluorine has taken a hydrogen atom from the aromatic ring leading to the loss of neutral HF molecule. A peak at *m*/*z* 113 represented a formal neutral loss of NH_3 (for

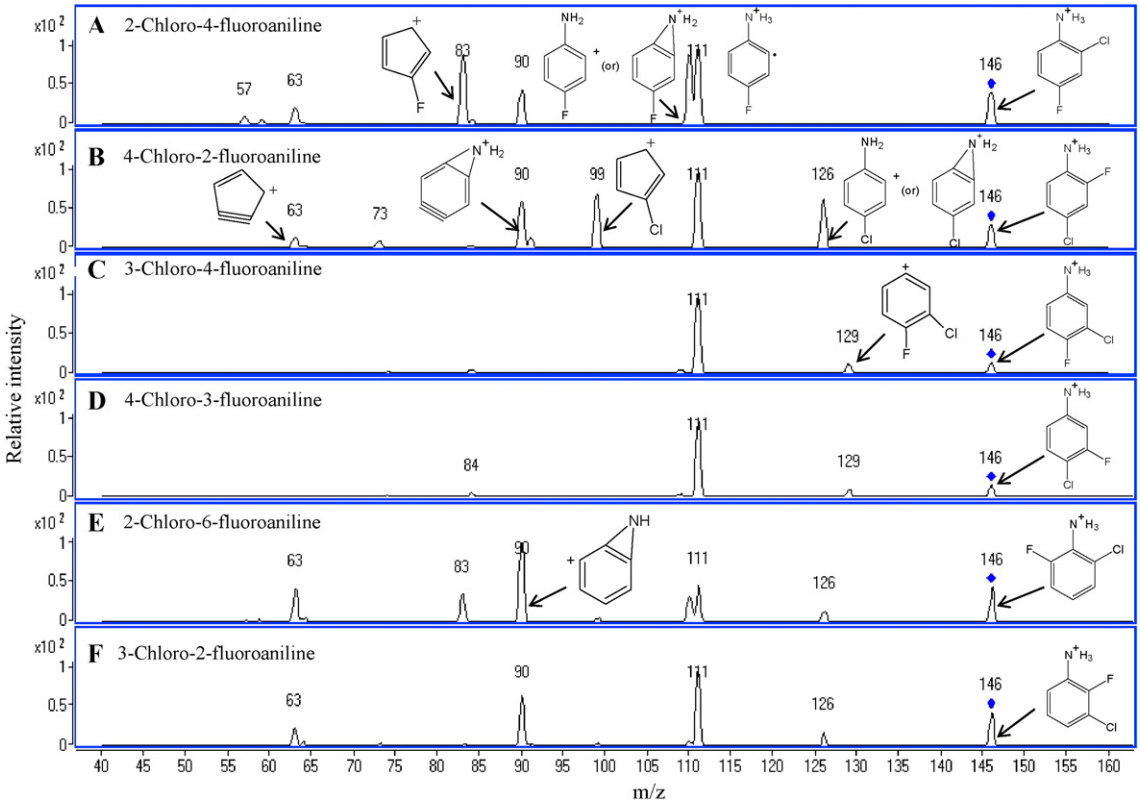


Fig. 6. CID Product ion spectra of protonated 2-chloro-4-fluoroaniline (A), 4-chloro-2-fluoroaniline (B), 3-chloro-4-fluoroaniline (C) 4-chloro-3-fluoroaniline (D), 2-chloro-6-fluoroaniline (E) and 3-chloro-2-fluoroaniline (F) by MS–MS.

all the *meta*-DFA isomers (3,4 and 3,5-DFA) as well 2,3-DFA. Two base peaks at *m*/*z* 90 and 83 in the CID mass spectra of all DFA isomers, represent the loss of second neutral HF and HCN molecules respectively. Except the difference in relative intensities of ion at *m*/*z* 110 for 2,4-DFA and 2,5-DFA, CID mass spectra may not able to provide much help to differentiate the 2,4-DFA from the other isomer.

3.4. Chloro-fluoroaniline isomers

No data is available for mutagenic potency of chloro-fluoroanilines (CFA) and bromo-fluoroanilines except 4-bromoaniline [5] which was found to be non-mutagenic by Ames test and structure–activity relationship (SAR) analysis. In the case of CFAs with chlorine atom in *ortho* positions, we have observed the loss of HCl and chlorine radical. In case of 2-chloro-6-fluoroaniline which has both the fluorine and chlorine atoms positioned in the two *ortho* positions, competitive elimination may be expected between the chlorine and fluorine atoms, however elimination of HCl favours due to the reason that C–F bond is comparatively stronger than the C–Cl bond. In the case of 4-chloro-3-fluoroaniline and 3-chloro-4-fluoroaniline in which both the ortho positions are unoccupied, the loss of either ammonia or chlorine radical was predominant. 2-chloro-4-fluoroaniline and 4-chloro-2-fluoroaniline can be distinguished each other by the presence of distinct ion peak at *m*/*z* 83 which corresponds to fluoro cyclopentadiene ion present in 2-chloro-4-fluoroaniline spectra (Fig. 6A) and ion peak at *m*/*z* 99 observed in 4-chloro-2-fluoroaniline spectra (Fig. 6B) which corresponds to chloro cyclopentadiene ion. Neutral loss of HF was observed only in 4-chloro-2-fluoroaniline which corresponds to peak at *m*/*z* 126, this unique ion peak can be used to differentiate both the CFA isomers (Fig. 6).

3.5. Bromo-fluoroaniline isomers

Four bromo-fluoroanilines (BFA) (2-bromo-4-fluoroaniline, 3-bromo-4-fluoroaniline, 4-bromo-3-fluoroaniline and 5-bromo-2-fluoroaniline) were analyzed and their CID mass spectra were recorded. As expected, due to ortho effect only 2-bromo-4-fluoroaniline received assistance from neighbouring amine function leading to the loss of both HBr and bromine radical, and the rest of BFA isomers demonstrated only the elimination of bromine radical. With the ortho effect provided by the amine function, more fragments due to loss of HF and HCN were reported for 5-bromo-2-fluoroaniline. Elimination of bromine radical was predominant over the loss of HBr since bromine radical was the most stable radical among the haloanilines we have investigated.

4. Conclusions

In CID mass spectral studies of haloanilines, it has been observed that the ortho effect provided by neighbouring amine function leads to loss of both a neutral molecule and a radical with the exception of the fluoroanilines. In fluoroanilines we have observed peaks corresponds neutral loss, due to the strong electronegativity of fluorine atom. As the result of ortho effect, an aza-biheterocyclic intermediate may be generated. The H/D exchange experimental data support the proposed fragmentation pathways. With the strong ortho effect, more fragments were possible for *ortho*-haloanilines than the meta or para isomers under equivalent experimental conditions. Initial elimination of ammonia occurred in case of *meta* and *para* haloanilines. Based on these findings, it can be concluded that protonated haloanilines follow a similar fragmentation pathway with the exception of DFA and *ortho*-haloanilines can be distinguished from their *meta* or *para* isomers using CID experiments. Hence the understanding of the mass spectrometric fragmentation of these potential genotoxic compounds can facilitate the identification and differentiation of haloaniline isomers as minor impurities in chemical process development.

Acknowledgements

Sincere thanks are extended to Dr. Anthony Bristow, AstraZeneca, Macclesfield, UK Dr. Debasis Hazra, and Dr. Sharmistha, AstraZeneca, Bangalore, India for their linguistic advice.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.07.022.

References

[1] EU Guideline of Limits of Genotoxic Impurities, http://www.ema.europa.eu/pdfs/human/swp/519902en.pdf. Q&A supplement, http://www.ema.europa.eu/pdfs/human/swp/43199407en.pdf.
[2] D. Kirkland, M. Aardema, L. Henderson, L. Muller, Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens I. Sensitivity, specificity and relative predictivity, Mutat. Res. 584 (2005) 1–256.
[3] T.R. Naven, S. Louise-May, N. Greene, The computational prediction of genotoxicity, Expert Opin. Drug Metab. Toxicol. 6 (2010) 797–807.
[4] K.T. Chung, L. Kirkovsky, A. Kirkovsky, W.P. Purcell, Review of mutagenicity of monocyclic aromatic amines: quantitative structure–activity relationships, Mutat. Res. 387 (1997) 1–16.
[5] J. Bentzien, R.E. Hickey, A.R. Kemper, L.M. Brewer, D.J. Dyekjaer, P.S. East, M. Whittaker, An in silico method for predicting ames activities of primary aromatic amines by calculating the stabilities of nitrenium ions, J. Chem. Inf. Model. 50 (2010) 274–297.
[6] R. Benigni, A. Giuliani, R. Franke, A. Gruska, Quantitative structure–activity relationships of mutagenic and carcinogenic aromatic amines, Chem. Rev. 100 (2000) 3697–3714.
[7] D. Wild, A novel pathway to the ultimate mutagens of aromatic amino and nitro compounds, Environ. Health Perspect. 88 (1990) 27–31.
[8] J.D. Snodin, Genotoxic impurities from structural alerts to qualification, Org. Process Res. Dev. 14 (2010) 960–976.
[9] G. Vanhoenacker, F. David, P. Sandra, Analysis of Potential Genotoxic Arylamine and Aminopyridine Impurities in Active Pharmaceutical Ingredients, Agilent application note, 2010, Publication Number 5990-5732EN.
[10] R. Shoji, M. Kawakami, Prediction of genotoxicity of various environmental pollutants by artificial neural network simulation, Mol. Div. 10 (2006) 101–108.
[11] H.-H. Lo, I.P. Brown, O.G. Rankin, Acute nephrotoxicity induced by isomeric dichloroanilines in Fischer 344 rats, Toxicology 63 (1990) 215–231.
[12] P. Nagi Reddy, R. Srikanth, N. Venkateswarlu, R. Nageswara Rao, R. Srinivas, Electrospray ionization tandem mass spectrometric study of three isomeric substituted aromatic sulfonic acids; differentiation via ortho effects, Rapid Commun. Mass Spectrom. 19 (2005) 72–76.
[13] A.J. Glemza, L.K. Mardis, A.A. Chaudhry, K.M. Gilson, F.G. Payne, Competition between intra- and intermolecular hydrogen bonding: effect on para/ortho adsorptive selectivity for substituted phenols, Ind. Eng. Chem. Res. 39 (2000) 463–472.
[14] B.L.A. Moraes, A.A. Sabino, C.E. Meurer, N.M. Eberlin, Absolute configuration assignment of ortho, meta, or para isomers by mass spectrometry, J. Am. Soc. Mass Spectrom. 16 (2005) 431–436.
[15] J. Yinon, Mass spectrometry of explosives: Nitro compounds, nitrate esters, and nitramines, Mass Spectrom. Rev. 1 (1982) 257–307.
[16] L.L. da Rocha, R. Sparrapan, R. Augusti, M.N. Eberlin, Direct assignment of positional isomers by mass spectrometry: *ortho, meta* and *para* acyl and amidyl anilines and phenols and derivatives, J.Mass Spectrom. 39 (2004) 1176–1181.
[17] B.F. Jariwala, M. Figus, B.A. Attygalle, Ortho effect in electron ionization mass spectrometry of *N*-acylanilines bearing a proximal halo substituent, J.Am. Soc. Mass Spectrom. 19 (2008) 1114–1118.
[18] B.A. Attygalle, J. Ruzicka, D. Varughese, J. Sayed, An unprecedented ortho effect in mass spectrometric fragmentation of even-electron negative ions from hydroxyphenyl carbaldehydes and ketones, Tetrahedron Lett. 47 (2006) 4601–4603.
[19] B.A. Attygalle, J. Ruzicka, D. Varughese, J. Bialecki, S. Jafri, Low-energy collision-induced fragmentation of negative ions derived from ortho-, meta-, and para-hydroxyphenyl carbaldehydes, ketones, and related compounds, J. Mass Spectrom. 42 (2007) 1207–1217.
[20] B.A. Attygalle, B.J. Bialecki, U. Nishshanka, S.C. Weisbecker, J. Ruzicka, Loss of benzene to generate an enolate anion by a site-specific double-hydrogen transfer during CID fragmentation of O-alkyl ethers of ortho hydroxybenzoic acids, J. Mass Spectrom. 43 (2008) 1224–1234.

[21] B.A. Attygalle, J. Ruzicka, D. Varughese, J. Sayed, B.F. Jariwala, Unequivocal identification of *ortho* isomers of some 1.2-disubstituted benzenes by collision-induced dissociation mass spectra of electrospray-generated positive and negative ions, in: Proceedings of the 54th ASMS Conference on Mass Spectrometry and Allied Topics, 2006, http://www.asms.org/ASMS06PDF/A063783.pdf.
[22] M. Benassi, E.Y. Corilo, D. Uria, R. Augusti, N.M. Eberlin, Recognition and resolution of isomeric alkyl anilines by mass spectrometry, J. Am Soc. Mass Spectrom. 20 (2009) 269–277.
[23] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Carmi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford, CT, 2004.
[24] H. Schwarz, Some newer aspects of mass spectrometric ortho effects, Top. Curr. Chem. 73 (1978) 231–263.
[25] F.W. McLafferty, F. Turecek, Interpretation of Mass Spectra, 4th ed., University Science Books, Mill Valley, CA, 1993.
[26] A. Barkow, S. Pilotek, H.F. Grützmacher, A mechanistic study, Eur. J. Mass Spectrom. 5 (1995) 525–537.
[27] G.W. Sovocool, R.K. Mitchum, Use of the 'ortho effect' for chlorinated biphenyl and brominated biphenyl isomer identification, Biomed. Environ. Mass Spectrom. 14 (1987) 579–582.