

# Reduction of Nitriles to Amines in Positive Ion Electrospray Ionization Mass Spectrometry

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## Introduction

It was observed that acetonitrile was reduced to ethylamine to form  $[M+46]^+$  adduct ions during LC/(+)ESI-MS analyses. Here, we report the phenomenon and mechanism of nitrile ( $C\equiv N$ ) reduction under (+)ESI-MS conditions that may facilitate characterization of unknown chemical entities having a nitrile functional group.

## Experimental

### Liquid chromatography:

Pump: Finnigan Model 11 Syringe Pump  
Mobile Phases:  
A:  $CH_3CN-H_2O$  (1:1) w/ 1% HCOOH and 1%  $NH_4OH$   
B:  $CH_3OH-H_2O$  (1:1) w/ 1% HCOOH and 1%  $NH_4OH$

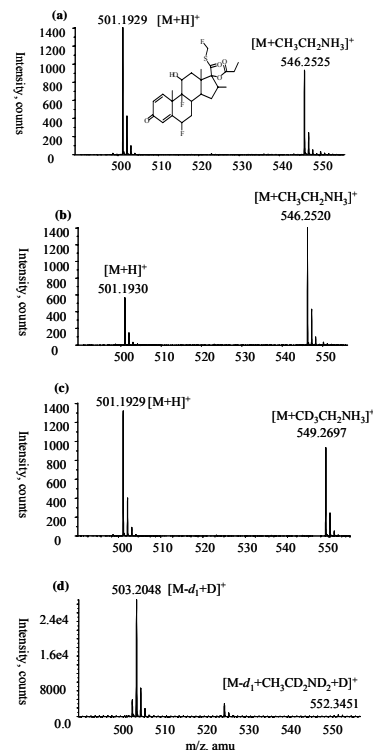
### Mass Spectrometry:

MS Systems I: Finnigan LCQ<sup>TM</sup> Mass Spectrometers  
Ion spray (IS): 4.5 kV  
Capillary temp: 200 °C  
Sheath gas: 40  
Auxiliary gas: 20  
MS System II: AB QSTAR XL LC/MS/MS System  
IS: 5.5 kV  
Curtain gas: 15  
Gas 1: 15  
Gas 2: 10

## Results and Discussion

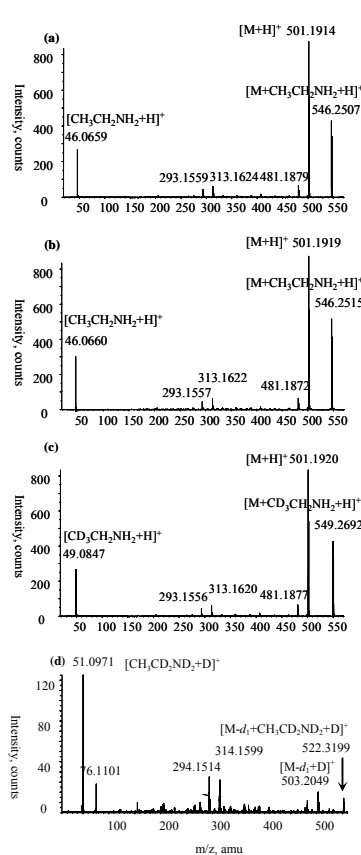
**1** An  $[M+46]^+$  adduct ion was observed for some compounds, e.g. fluticasone propionate, during LC/(+)ESI-MS analysis with  $CH_3CN-H_2O$  (1:1) as mobile phases (Figure 1a). The adduct ion was identical to that found when the HPLC mobile phase contained 0.01%  $CH_3CH_2NH_2$  (Figure 1b).

**2** The  $[M+46]^+$  ion was proposed to be an adduct ion of ethylamine, which was determined through use of deuterated agents to be derived from  $CH_3CN$  in the mobile phase (Figure 1c and 1d).



**Figure 1.** (+)ESI-MS spectra of fluticasone propionate prepared in (a)  $CH_3CN-H_2O$  (1:1), (b)  $CH_3OH-H_2O$  (1:1) containing 0.01%  $CH_3CH_2NH_2$ , (c)  $CD_3CN-H_2O$  (1:1), and (d)  $CH_3CN-D_2O$  (1:1) as mobile phase (each containing 1% formic acid and 1%  $NH_4OH$ ).

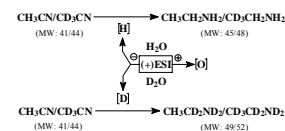
**3** The product ions of the  $[M+46]^+$  adduct further confirmed the phenomenon (Figure 2).



**Figure 2.** (+)ESI-MS/MS spectra of fluticasone propionate ethylamine adduct molecular ions at  $m/z$  546 in  $CH_3CN-CH_3CH_2NH_2$  solution (a) and  $CH_3CH_2NH_2$  containing  $CH_3OH-H_2O$  solution (b), at  $m/z$  549 in  $CD_3CN-H_2O$  solution (c), and at  $m/z$  552 in  $CH_3CN-D_2O$  solution (d).

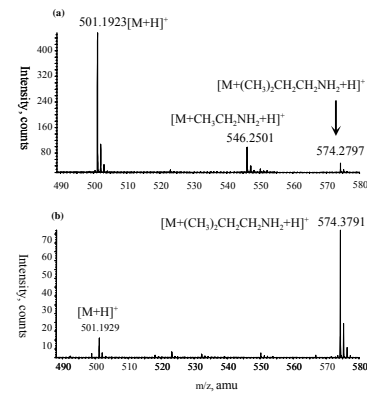
## Results and Discussion

**3** The mechanism for the formation of  $CH_3CH_2NH_2$  was proposed based on the well known conversion of water to hydrogen and oxygen through the process of electrolysis (Figure 3).



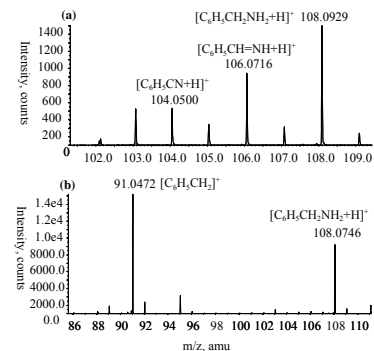
**Figure 3.** Formation of ethylamine and various deuterated ethylamines via reduction of acetonitrile or deuterated acetonitrile under (+)ESI conditions

**4** Both  $[M+46]^+$  and  $[M+74]^+$  ions were observed when fluticasone propionate was prepared in (a)  $CH_3CN-(CH_3)_2CH_2CH_2NH_2-H_2O$  (1:1:0.5) and only  $[M+74]^+$  was observed when fluticasone propionate was prepared in (b)  $CH_3OH-H_2O$  (1:1) containing 0.01%  $(CH_3)_2CH_2CH_2NH_2$  (Figure 4).



**Figure 4.** (+)ESI-MS spectra of fluticasone propionate prepared in (a)  $CH_3CN-(CH_3)_2CH_2CH_2NH_2-H_2O$  (1:1:0.5) and (b)  $CH_3OH-H_2O$  (1:1) containing 0.01%  $(CH_3)_2CH_2CH_2NH_2$ .

**5** Reduction of other nitriles was also observed. For example, benzonitrile was reduced to benzylamine and benzylamine in  $CH_3CN-H_2O$  and was completely reduced to benzylamine in  $CH_3OH-H_2O$  (Figure 5).



**Figure 5.** (+)ESI-MS spectra of benzonitrile prepared in (a)  $CH_3CN-H_2O$  (1:1) and (b)  $CH_3OH-H_2O$  (1:1) each containing 1% HCOOH and 1%  $NH_4OH$ .

## Conclusions

- Nitriles (acetonitrile, isobutyronitrile, benzonitrile) are readily reduced to their respective amines under (+)ESI-MS conditions.
- The source of the reductive hydrogen was determined to originate from electrolysis of water.
- This finding may facilitate the characterization of the nitrile-moiety in a variety of chemical entities of interest.
- Since water electrolysis occurs readily within the ESI source, other reduction products via a similar reaction mechanism with reductive hydrogen may also be anticipated.