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Mass Spectrometric Behaviour of Thiazide-based Diuretics after Electrospray Ionisation and Collision-Induced Dissociation

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Introduction

The analysis of products based on the structure of 6-chloro-7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide (hydrochlorothiazide) has gained attention ever since the diuretic effect of thiazides was observed with chlorothiazide by Novello and Sprague in 1957[1]. The interpretation of mass spectra generated from thiazidic diuretics by electrospray ionisation and collision-induced dissociation is of paramount importance in order to identify and characterise these compounds in urine samples of high performance athletes elected for doping controls[2-3]. Therefore, mass spectral data of 21 closely related thiazides were compared and fragmentation pathways proposed, which enable the determination of generic and particular dissociation products of diuretics based on a thiazide structure. Evidence for the presence of specific functional groups in fragment ions was obtained by syntheses of ^{15}N- or deuterium-labelled analogues of thiazides and structurally related compounds. Detailed description and discussion of experiments and results is published elsewhere[4].

Experimental

Mass spectrometry

All analyses were performed on a PE Sciex API2000 triple quadrupole mass spectrometer (PE Biosystems, Foster City, California, USA) or an Agilent 1100 Series LC/MSD trap, both of which were equipped with an electrospray interface. The flow rate of the solution containing the analyte was 10 µL/min, introduced by a syringe pump into the ESI interface. All analytes were solved in a mixture of acetonitrile/water (50:50, v:v) with a concentration of 5 µg/mL. The ionization mode was negative.

Synthesis of $^{15}N_2$-ethiazide

The selective introduction of two ^{15}N-atoms into the thiazide structure was accomplished by synthesis of 4-amino-6-chlorobenzene-1,3-disulfonyl chloride (1), ist reaction with ^{15}N-labelled ammonium hydroxide (2) and subsequent condensation to $^{15}N_2$-ethiazide (3) as shown in Figure 1.

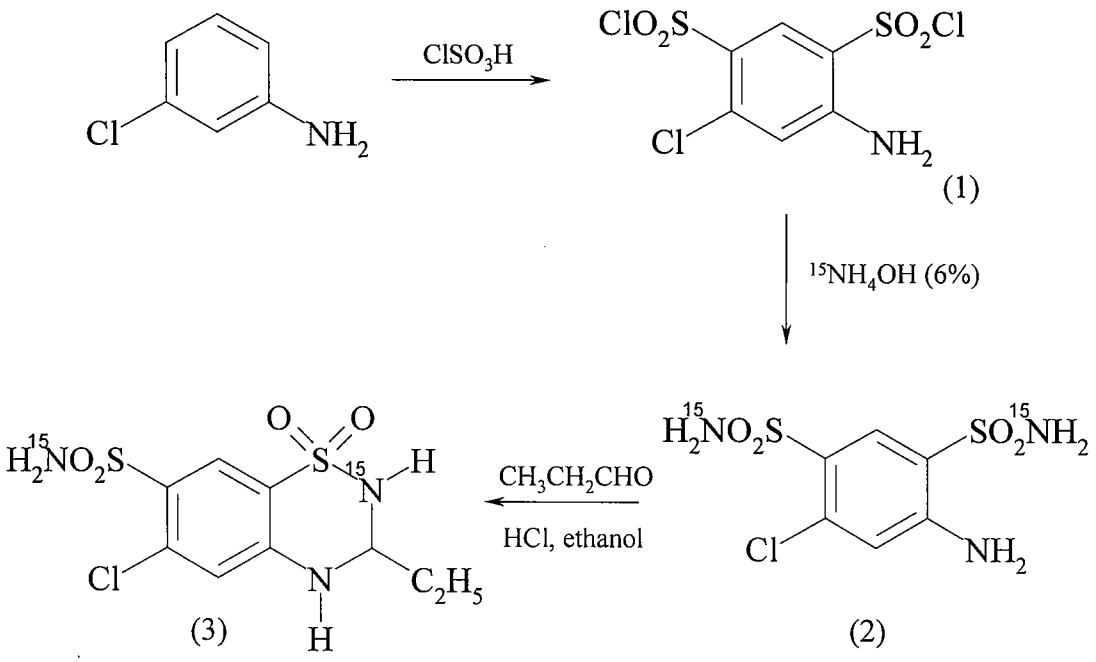

Figure 1: Synthesis of $^{15}N_2$-ethiazide.

In addition, methiazide, d_4-methiazide, prothiazide, *n*-buthiazide, *n*-penthiazide were synthesized accordingly.

Results

The 21 thiazidic compounds investigated in the present study were divided in three groups, each of which demonstrated individual and also common fragmentation routes by ESI-MS/MS analysis. Group 1 (*3-hydrogenated and 3-alkylated 7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxides*) was represented by ethiazide and its nitrogen-labelled analogue (Figure 2), group 2 (*3-Chloroalkylated 6-chloro-7-sulfamoyl-3,4-dihydro-1,2,4-*

benzothiadiazine-1,1-dioxides) by methylclothiazide (Figure 4) and group 3 (*3-Alkylthio methyl-6-chloro-7-sulfamoyl-1,2,4-benzothiadiazine-1,1-dioxides*) by epithiazide (Figure 5).

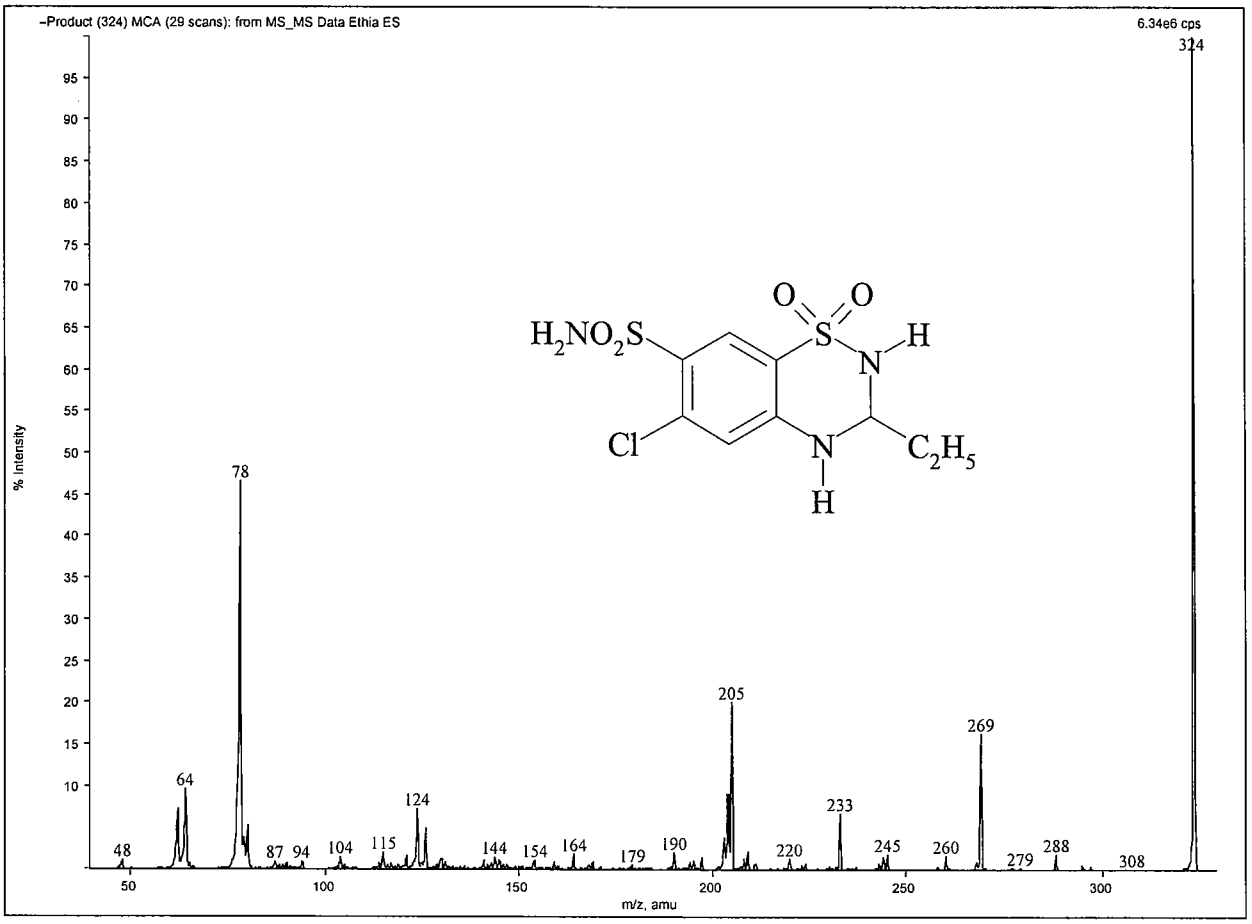

Figure 2: ESI product ion spectrum of ethiazide (mol wt = 325)

Common fragment ions for members of group 1 are m/z 269, 205, 126 an 78, the proposed structures of which are shown in Figure 3.

The halogenation of the C-3 alkyl side-chain results in a different fragmentation pattern than the one described for 3-hydrogenated and 3-alkylated 7-sulfamoyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxides. The mass spectra of those compounds are predominantly

characterised by the elimination of HCl and the subsequent loss of SO_2 as demonstrated with methylclothiazide in Figure 4.

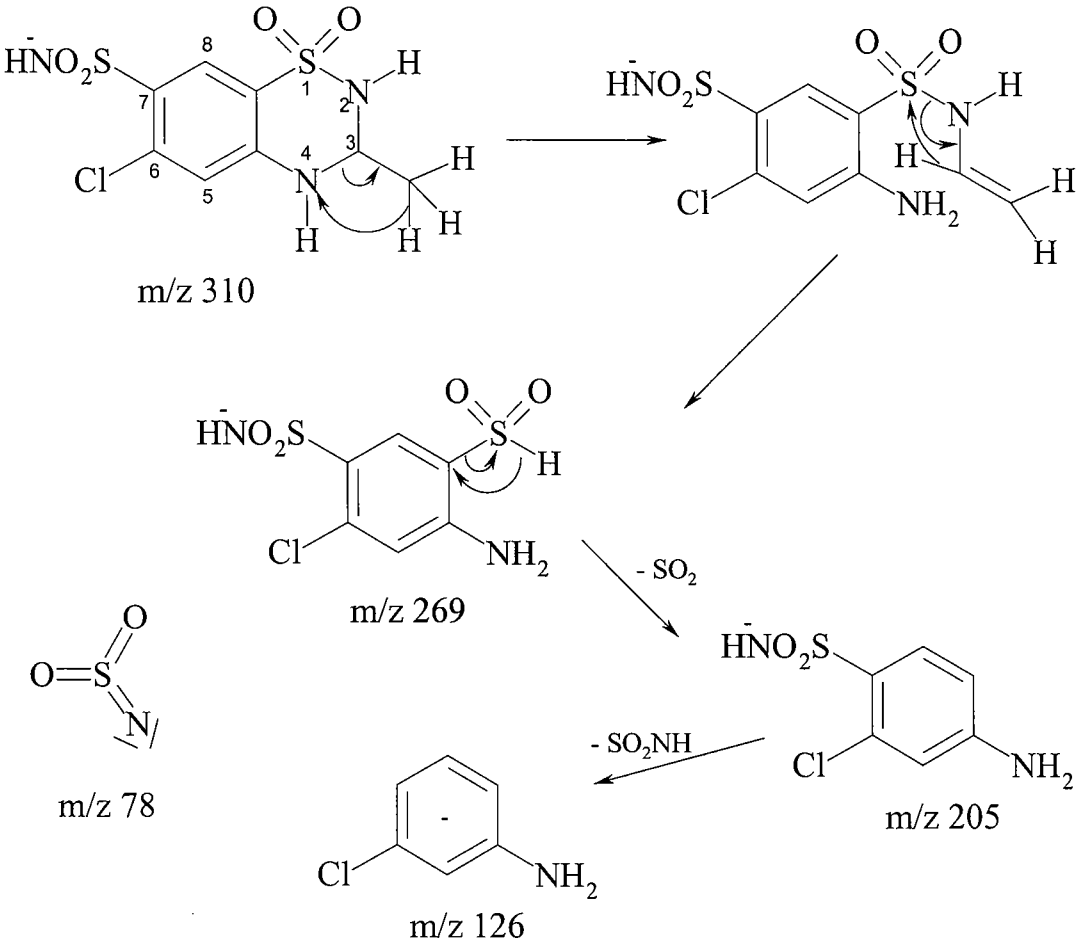

Figure 3: Proposed structures of common fragment ions of group 1.

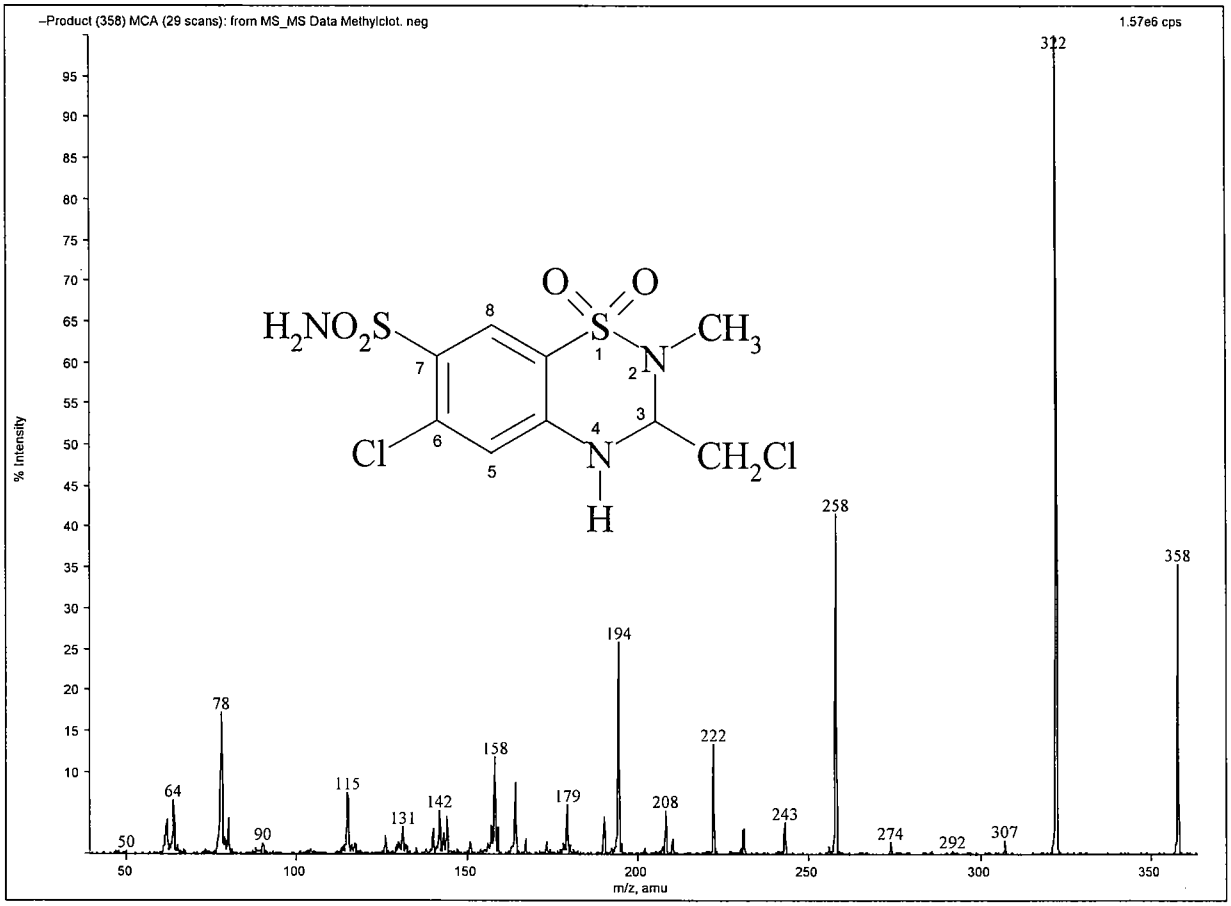

Figure 4: ESI product ion spectrum of methylclohiazide (mol wt = 359)

Epithiazide is selected as a representative of the substances belonging to group 3 (Fig. 5). These compounds contain a side-chain including a thio-ether functionality. Except those compounds bearing a 3-4-double bond (benzthiazide) or a 2-N-methyl group (polythiazide), the diuretics of this section generate the common fragments m/z 269, 205, 126 and 78. The thio-ether linkage turned out to be of great interest since it obviously represents a preferred position of dissociation. With epithiazide, benzthiazide and polythiazide, fragmentation occurs on both sides of the sulfur atom, generating characteristic fragments for this particular thiazide structure.

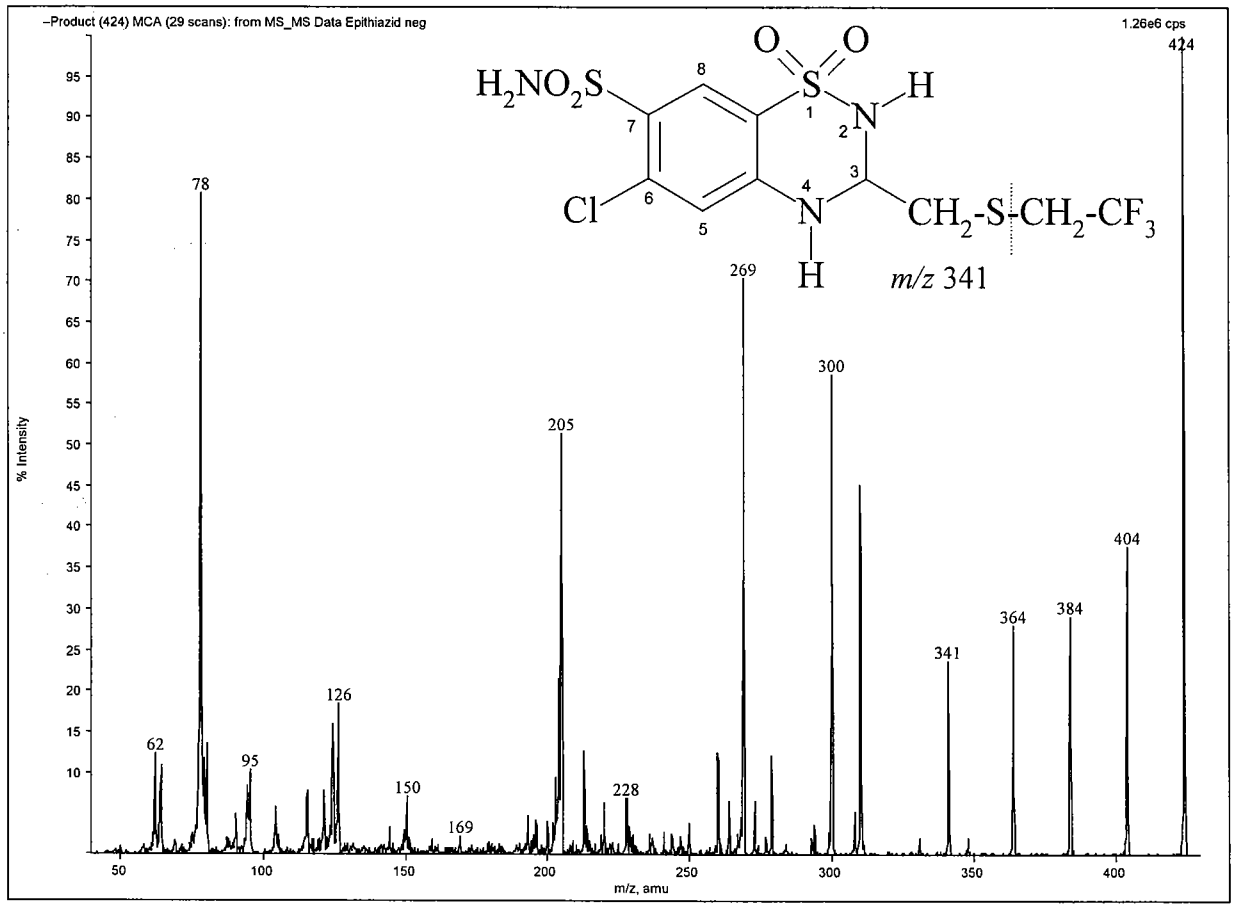

Figure 5: ESI product ion spectrum of epithiazide (mol wt = 425)

Conclusion

Negative electrospray ionisation followed by collision-induced dissociation enables the characterisation of thiazide-based diuretics. Specific funtional groups give rise to characteristic fragmentation routes, which indicate the principal structure of the analyte, and aditional individual fragments as well as the quasimolecular ion allow unambiguous determinations of thiazidic diuretics.

Acknowledgement

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References

[1] Novello, F. C.; Sprague J. M. *J. Am. Chem. Soc.* **1957**, *79*, 2028-2029.

[2] IOC List of Classes of Prohibited Substances and Methods of Doping, Lausanne, 2002

[3] Thieme, D.; Grosse, J.; Lang, R.; Mueller, R. K.; Wahl, A. *J. Chromatogr. B* **2001**, *757*, 49-57

[4] Thevis, M.; Schmickler, H.; Schänzer, W. *Anal. Chem.* **2002**, *74*, 3802-3808