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Different reactivities of amphetamines with *N*-methyl-bis(trifluoroacetamide) in heated gas chromatographic injectors

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A fast gas chromatographic mass spectrometric method has been developed earlier for the determination of amphetamine derivatives in human serum and urine. For derivatization, *N*-methyl-bis(trifluoroacetamide) (MBTFA) was used. Derivatization was performed using an on-line mode, since 1 μl of MBTFA and 1 μl sample extract, dissolved in toluene were injected simultaneously. In this study, the reactivity of the several amphetamine type analytes with MBTFA was investigated. MBTFA used for flash derivatization was applied undiluted on the one hand and diluted 4 – 4096-fold with acetonitrile on the other hand. Studying several amphetamines in the test sample spiked at the same concentrations we found that they could be divided into 3 groups based on relative target ion peak areas as a function of MBTFA dilution. Group 1, containing only primary amines showed an early increase of the relative peak areas if we increased MBTFA concentration, where group 2 (mainly N-methyl secondary amines) showed that relative peak areas started to increase intensively at higher MBTFA concentrations. Finally, MDEA as an N-ethyl secondary amine, representing group 3, showed significant increase if only slightly diluted MBTFA was used as a flash reagent. This phenomenon can be explained mainly with the less and less reactivity of amine groups in the case of groups 2 and 3, compared to group 1. These findings could help to optimise analytical methods involving flash derivatization processes.

1. Introduction

In a previous work (Hidvégi et al. 2006) we elaborated a method for the fast and sensitive gas chromatographic mass spectrometric (GC-MS) determination of certain amphetamine derivatives. This method covers the on-line trifluoroacetylation of the analytes via co-injection of *N*-methyl-bis(trifluoroacetamide) (MBTFA) with the sample extract into the gas chromatograph. During optimization, this chemical process seemed to be affected by several factors, such as the temperature of the injection port, splitless time and the amount of derivatizing reagent applied. The dependence of the analytical method on these factors may affect robustness. Therefore, the effects of variations of these factors should be studied, especially as a part of validation studies.

In this particular study we intended to focus on the dependence of the flash trifluoroacetylation on the amount of MBTFA using some amphetamine type compounds as test analytes.

2. Investigations, results and discussion

Investigations of the prepared test samples were started by injecting 1 μL of acetonitrile along with the test samples. This way we did not detect any trifluoroacetylated amphetamines. Next we applied MBTFA diluted with acetonitrile in the order of increasing MBTFA percentage. According to this we used 4096-, 1024-, 256-, 64-, 16- and 4-fold diluted and undiluted MBTFA. We made duplicate analyses at each MBTFA concentration. After the analyses, we integrated target fragment peaks of the analytes showing retention times of 5.69 min for amphetamine, 5.90 min for phentermine, 6.60 min for methamphetamine, 8.05 min for methylenedioxyamphetamine (MDA), 8.55 min for methylthioamphetamine (4-MTA), 8.77 min for methylenedioxymethamphetamine (MDMA), 9.04 min for methylenedioxyethylamphetamine (MDEA) and 9.12 min for methylamino-1-(3,4-methylenedioxyphenyl)-butane (MBDB) (for target and identifier ions see Experimental). We plotted the relative target fragment ion peak areas related to the maximal value of each analyte during the series of analyses, as a function of logarithmically transformed volumetric composition of MBTFA in the dilutions (Fig.). Plotted values are the means of the results of the two series of analyses.

We observed that we could detect trifluoroacetylated forms of the analytes already using highly diluted MBTFA. However, the relative peak areas of the target fragment ions were very different. As we applied less and less diluted MBTFA, relative peak area values increased, but again not at the same rate at different analytes. Based on the characters of the curves plotted, three groups of analytes could be determined. The first group, containing

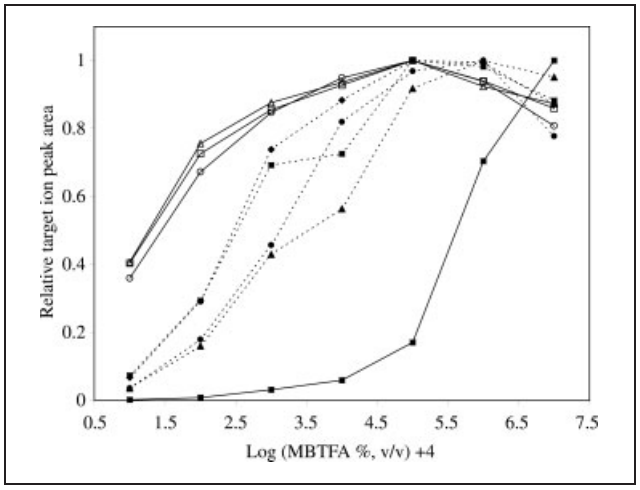

Fig.: Relative target fragment ion peak areas related to the maximal value of each analyte as a function of logarithmically transformed MBTFA dilution. Three groups of analytes are represented by different lines and symbol types (see text)

amphetamine, MDA and 4-MTA (solid lines with hollow symbols), showed quite high relative area values using very diluted MBTFA compared to other analytes. The second group, containing phentermine, methamphetamine, MDMA and MBDB showed low relative areas at lower MBTFA concentration, but then a high increase of the peaks (dashed lines with black symbols). The third group contains MDEA only, where we could find very low relative peak areas at low MBTFA concentrations and a significant increase only using slightly diluted or undiluted MBTFA (solid line with black symbol). Groups 1 and 2 showed also relative peak area decrease using 4-fold diluted or undiluted MBTFA.

A lot of factors may affect the actual run of curves such as analyte concentration, analysis parameters like splitless time, amount of glass wool etc. However, the result of classification of analytes based on the characters of curves mentioned above remains the same.

Belonging to a particular group seems to be correlated with chemical structure. Group 1 contains only primary amines where there are no substitutes along the alpha-methyl-ethylamine side chain. Group 2 contains either N-methyl secondary amines (methamphetamine, MDMA, MBDB) or phentermine as a primary amine, where alpha-methyl-ethylamine side chain is substituted. MDEA, as the only member of group 3 is an N-ethyl secondary amine. The correlation found can be explained with the higher reactivity of group 1 analytes with the derivatizing agent MBTFA. Group 2 has probably lower reactivity because of N-substitution and/or the influence of side chain substituent. Finally, the N-ethyl group of MDEA probably decreases the reactivity with MBTFA even further.

The cause of decrease of the relative peak area values of group 1 and 2 analytes after using 16-fold MBTFA dilution or less (see x-axis values higher than 4.5) is not clear, but suggests that the whole phenomenon is also affected by other factors than the reactivity of the amine moieties alone. We suppose e.g. that MBTFA concentration may influence mass transfer from the liner space towards the capillary column.

Analysis of more test substances would probably make the correlation even clearer. However, our results may help to optimize of analytical methods using on-line derivatization techniques.

3. Experimental

3.1. Chemicals

Analytes were purchased as certified reference materials form the following companies: D,L-amphetamine, D,L-methamphetamine, D,L-methylenedioxy-amphetamine (MDA), D,L-methylenedioxy-methamphetamine (MDMA), D,L-methylenedioxy-ethylamphetamine (MDEA) and D,L-methylamino-1-(3,4-methylenedioxyphenyl)-butane (MBDB) as hydrochloric salts from Lipomed (Arlesheim, Switzerland); phentermine as 1 mg/ml solution in methanol from Cerilliant® (Round Rock, TX, USA); and finally D,L methylthio-amphetamine (4-MTA) as hydrochloric salt from the National Measurement Institute (Australian Government, Pymble, NSW, Australia). Derivatization grade *N*-methyl-bis(trifluoroacetamide), approx. 98% (GC) (MBTFA) was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Other chemicals were of analytical grade.

3.2. Sample preparation

Each analyte (10 μg) was applied into a test tube in the form of methanolic stock solutions. The samples were evaporated to dryness under a mild stream of N_2 at room temperature. Then, 20 μl of 0.5 M aqueous potassium hydroxide were applied for dissolution of the residues. We performed a liquid-liquid extraction for 10 s using 100 μl of toluene. After centrifugation, 80 μl of organic phase were introduced into GC-MS vials.

3.3. Gas chromatographic-mass spectrometric (GC-MS) analysis

Instrument: Shimadzu QP5000 type GC-MS. The conditions of instrumental analysis were as follows. Capillary column: HP-1MS, length: 25 m, inner diameter: 0.2 mm, film thickness: 0.33 μm (Agilent Technologies, Wilmington, DE, USA). Carrier gas: helium (6.0, Messer, Gumpoldskirchen, Austria), head pressure: 120 kPa, split ratio: 1 : 14, splitless time: 0.1 min. Injector temperature: 270 °C. Split precision liner (Restek, Bellefonte, PA, USA) was used filled with 39 mg of silane treated glass wool (Supelco, Bellefonte, PA, USA). Oven temperature program: 90 °C for 1 min, 20°/min until 200 °C, then 30°/min until 280 °C, final time: 6 min. Interface temperature: 280 °C. Detection mode: SCAN, mass range: m/z 35–400, scanning interval: 0.2 s. Target and identifier ions for analytes were as follows, giving target ions underlined: m/z 140, 118 and 117 for amphetamine; m/z 154, 132 and 114 for phentermine; m/z 154, 118 and 110 for methamphetamine; m/z 135 and 162 for MDA; m/z 137, 164 and 277 for 4-MTA; m/z 154, 162, 135 and 110 for MDMA; m/z 168, 162, 140 and 135 for MDEA, and finally m/z 168, 176 and 135 for MBDB.

3.4. Sample application

N-Methyl-bis(trifluoroacetamide) (1 μl), either neat or diluted, then 1 μl of the sample were sucked into the same syringe and these 2 μl were injected into the gas chromatographic injector. This co-injection made the on-line derivatization possible.

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Reference

Hidvégi E, Fábián P, Hideg Zs, Somogyi G (2006) GC-MS determination of amphetamines in serum using on-line trifluoroacetylation. Forensic Sci Int 161: 119–123.