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Short communication

Silylation of acetaminophen by trifluoroacetamide-based silylation agents

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ABSTRACT

In the presented report, we have described the silylation reaction between the amide group in acetaminophen and a two most popular trifluoroacetamide-based silylation reagents – BSTFA and MSTFA. Both reagents had a amide groups on structures. An investigation was made through the performance of a set of experiments, GC–MS analysis, and a theoretical study, namely interpretation of mass spectra, presentation of the resonance states of all the involved compounds and S_N2 reaction schemes, which was found to be different when BSTFA and MSTFA was applied. The negligible effect of used solvent was also described. The fragmentation of TMS-derivatives (MS spectra) was presented and it has confirmed our previous investigations with silylation of pharmaceuticals, and a general rules of fragmentation patterns. Thanks to this the structure of di-*O*,*O*-TMS-acetaminophen was proven.

1. Introduction

Silylation, as a type of derivatisation, aims to lower the polar character of the reacting compound. This reaction is performed by the replacement of labile hydrogen in the carboxyl, hydroxyl, amine or amide functional group into a non-polar fragment [1]. In most cases this group is an alkylsilyl substituent, like the trimethylsilyl (TMS) or *tert*-butyldimethylsilyl group. Silylation has great importance in analytical chemistry, mainly gas chromatography, because of the increased volatility and thermal stability of polar analytes [2,3].

Commercially available silylation reagents have differences in their reaction properties. Some of them are universal, but most react in a specific functional group [4]. The necessary information is easily available in the manufacturers' folders. The effectiveness of silylation depends on various parameters, like reaction time, temperature, the presence of a catalyst and the solvent used. In our previous studies we investigated and optimized the derivatisation reaction in these silylation reagents for various groups of pharmaceuticals [5–8]. We observed that there is a need for the optimisation of silylation reaction conditions, especially when a mixture of pharmaceuticals is targeted.

The N-based active groups in pharmaceuticals are generally less active for silylation compared to hydroxyl and carboxyl groups. All of already available silylation reagents works with —OH and —COOH groups (most silyl reagent, and dimethyl(3,3,3-trifluoropropyl)silyldiethylamine, which was developed in our laboratory [7,9]), while only the selected additionally with amine groups, for example BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) and MSTFA (*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide). Thereby, special attention should be taken for acetaminophen derivatisation, while both hydroxyl and amide groups occur in their structure.

One of the tested parameters was the solvent for silylation. In most protocols the silylation reagents are used alone, but the appropriate solvent can increase the reaction rate and speed. The role of the addition of a polar aprotic solvent is to increase the solubility of less soluble compounds, but also to increase reaction performance. The reason is that silylation is an S_N2 nucleophilic substitution reaction [10] and an aprotic polar solvent has the ability to stabilize the intermediate state (Fig. S1, Supplementary Information).

The solvents of choice for silylation reagents are pyridine, DMSO, DMF, THF, or acetonitrile. It seems that pyridine has a great ability to support silylation. Pyridinium-like compounds, which act as catalytic Lewis bases, are believed to react with silyl chlorides to form silyl pyridinium ion pairs, whose subsequent reaction with the alcohol substrate yields silyl ether products [11]. Suppliers of silylation reagents often suggest the preparation of a mixture of the reagent with pyridinium and with the addition of trimethylchlorosilane (TMCS) as a catalyst. Pyridinium as a solvent for the silylation reaction is important not only for the efficiency of the reaction, but also the stability of the analytes. The ideal example is the case of the

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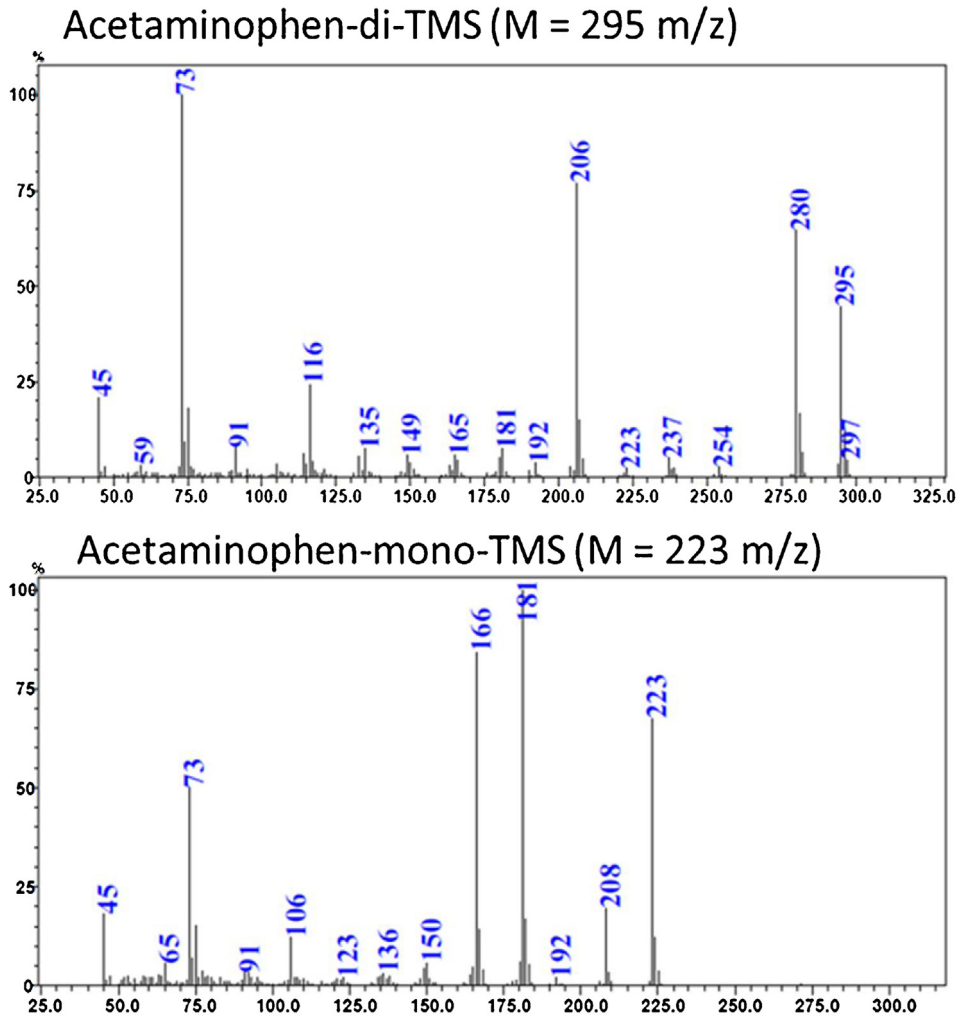

Fig. 1. The mass spectra of acetaminophen-mono-*O*-TMS and acetaminophen-di-*O,O*-TMS.

silylation of 17α-ethynylestradiol, which without the addition of pyridine into the sample can convert into estrone, because of the removal of the labile ethinyl group [7,12].

In one of our previous studies we investigated the influence of a solvent among other parameters for silylation of beta-blocker and beta-agonist pharmaceuticals [6]. BSTFA + 1% TMCS was mixed with dichloromethane, ethyl acetate, pyridine, toluene or hexane and the efficiency of the silylation of the target analytes was determined. The results showed that a mixture of the silylation reagent with ethyl acetate gave the highest efficiency, but generally the values were similar.

In the present study, we tested the trifluoroacetamide-based reaction agents (BSTFA and MSTFA) for silylation of acetaminophen. These reagents are already proven to be suitable for the acetaminophen derivatisation. Nevertheless, we present a deep study of mass spectra and fragmentation of derivatives to check the differences between those two versatile reagents.

2. Materials and methods

2.1. Chemicals

Acetaminophen (paracetamol, *N*-(4-hydroksyfenylo)acetamide, CAS 103-90-2, M = 151.17 g/mol) was purchased from Sigma-Aldrich (Germany). The stock solution of acetaminophen (1 mg/mL) was prepared in methanol, as well as a working solution (5 μg/mL). The derivatising reagents, BSTFA with 1% TMCS (trimethylchlorosilane) (hereafter BSTFA +1% TMCS) and MSTFA, were purchased from Sigma-Aldrich. BSTFA +1% TMCS was also purchased from Synthese Nord (Germany). The organic solvents (ethyl acetate, acetonitrile, acetone, methanol) were supplied by POCH (Polskie Odczynniki Chemiczne, Poland).

2.2. Methods

The derivatisation protocol was the same for all experiments. Specifically, 100 μL of working solution of acetaminophen was introduced into a 2 mL amber glass vial, and the solvent was evaporated in a nitrogen stream. 100 μL of derivatisation reagent/mixture was introduced into the vial. After vortex mixing of the sample, the vial was heated for 30 min at 60 °C in a heating block. The obtained samples were analyzed by GC/MS (Shimadzu GCMS-QP2010 SE). An Rtx-5 (30 m × 0.25 mm × 0.25 μm; Restek) capillary column was used. The injector temperature was 300 °C. The injection (1 μL of the sample) was performed by the autosampler. The temperature program was as follows: starting temperature 120 °C, 1 min in 120 °C, then a rate of 12 °C/min, to reach 270 °C. The helium was kept in a constant pressure mode of 100 kPa. The EI ion source temperature was 200 °C, while the interline temperature was 300 °C. The mass spectrometer recorded the spectra in the *m*/*z* range of 45–650 and a speed of 3 scans per second. At least two repetitions