INTRODUCTION

Acetylsalicylic acid, also known as ‘aspirin’, is a very popular analgesic and antipyretic agent that has very labile properties. Hence, knowledge concerning the degradation of this drug as placed in a variety of solutions is important from the point of view of analytical procedures. This because the usage of an improper solvent for the preparation of aspirin samples could lead to its fast degradation, and, hence, introduce into such work significant analytical errors [3]. Although it can seem to the reader that the stability of acetylsalicylic acid is a well-elaborated topic, very few studies can actually be found in current literature. Most often, the stability of this agent was tested in mixtures of water and polar solvents. Gharbo and Williamson, for example, noticed its degradation in aqueous ethanol solution [3], while Chang and Whitworth [2] studied the degradation of aspirin in water-propylene glycol and water-triethylene glycol diacetate mixtures. Moreover, various substituted and non-substituted polyhydric alcohols, in the absence and presence of water, were tested by Jun and co-workers [4]. Furthermore, the hydrolysis of the acid in water-polyethylene glycol 400, water-methanol-acetic acid, phosphate buffer and freshly drawn blood and plasma was also investigated by Bakar and Niazi [1]. In addition, the stability of aspirin was also studied in mixed solvent systems (ethanol, ether, glycols and water) under ultrasonic conditions [5]. The aim of our study was, hence, to establish the most stable solvent for routine analytical procedures. For this purpose, a new analytical method which employed a new generation hybrid Q-TOF spectrometer combined with a UHPLC system, was developed.
MATERIALS AND METHODS

Materials

For our study, acetylsalicylic acid (99.5% purity) was obtained from BUFA B.V. (Uitgeest, Holland). Formic acid for LC-MS was purchased from Fluka (Steinherin, Germany). Solvents (1,4-dioxane, acetonitrile, tetrahydrofuran, propan-2-ol, water, methanol, ethanol) were purchased from Merck (Dramstadt, Germany). A stock solution of acetylsalicylic acid was prepared in methanol at a concentration of 1 mg/mL.

UHPLC-ESI-Q-TOF analysis

UHPLC-MS/MS analysis was performed with the use of a Agilent Accurate-Mass Q-TOF LC/MS G6520B system, with a dual electrospray (DESI) source, an Infinity 1290 UHPLC system and a Zorbax Extend-C18 (2.1x50mm, dp=1.8 μm) column (Agilent, Santa Clara, USA). A mixture of acetonitrile (A) and water (B), with the addition of a 0.1% solution of formic acid in both media was used as a mobile phase. The gradient elution was carried out at a constant flow of 0.4 ml/min, from 15%A (85%B) to 80%A, in a time period of 0-13 mins and with a 1 min isocratic post-time (15%A). The injection volume was 4 μl and the column temperature was maintained at 25°C. The instrument condition optimization was initiated through the proper tuning of the Q-TOF detector while in a negative mode, and with the use of an Agilent ESI-L tuning mix in a high resolution mode. Here, the following settings were applied: gas temp.: 250°C, drying gas: 10 l/min, nebulizer pressure: 40 psig, capillary voltage: 3500 V, fragmentor voltage: 110 V, skimmer voltage: 65 V, octopole voltage: 250 V. Data acquisition was performed in the auto MS/MS mode, the spectral parameters being: mass range: 50-1100 m/z and acquisition rate: 1.4 spectra/s. Furthermore, collision energy was calculated by way of auto algorithm, utilizing the formula: 4.6 V (slope)*(m/z)/100 + 4.8 V (offset). Mass 122.9858 and 1033.9881 were used as lock masses.

Quantitative analysis and degradation kinetics

The method calibration for the determination of the concentration of acetylsalicylic acid in the tested samples was performed via the use of MS detection. Herein, the extracted ion chromatograms (EIC) were set for mass 179.0349 m/z, and a calibration curve was obtained in the range: 0.4-1.83%. The obtained results were employed against signal to noise level). Herein, RSD values were in the range 0.14-1.83%. The obtained results were employed for calculating the concentration of acetylsalicylic acid at set time intervals in all the tested organic solvents and in water. The degradation profile of aspirin in all solvents by way of utilizing EIC (179.0349 m/z). The calibration of the quantitative analysis method was performed in the range 0.4-14 μg/mL. The obtained calibration curve: y = 18697.30(±456.34)x + 6971.73(±1961.48) was linear over the concentration range (r = 0.9991) and the limits of detection (LOD) and quantification (LOQ) were 0.04 μg/mL and 0.14 μg/mL, respectively (experimentally obtained against signal to noise level). Herein, RSD values were in the range 0.14-1.83%. The obtained results were employed for calculating the concentration of acetylsalicylic acid at set time intervals in all the tested organic solvents and in water. The degradation profile of aspirin in these solvents is shown in Fig. 3.

RESULTS AND DISCUSSION

In order to achieve high selectivity by way of utilizing the developed method, MS detection was employed for all quantification, while DAD detection was only used for the additional chromatographic process put in place for monitoring purposes (Fig. 1). After chromatographic separations, the analyzed compounds were identified through the use of TOF accurate MS spectra, as well as fragmentation MS/MS spectra. Acetylsalicylic acid was encountered in all analyzed samples and its molecular ion (179.0349 m/z) was detected with very good accuracy (1.22 ppm). The obtained MS/MS spectra also confirmed its structures (Fig. 2A). In all tested solvents, one main degradation product, salicylic acid (137.0242 m/z), was found - this, also with good accuracy (1.82 ppm). What is more, its MS/MS spectra were registered (Fig. 2B). The registered data from Q-TOF was further enlisted for the quantification of aspirin in all solvents by way of utilizing EIC (179.0349 m/z). The calibration of the quantitative analysis method was performed in the range 0.4-14 μg/mL. The obtained calibration curve: y = 18697.30(±456.34)x + 6971.73(±1961.48) was linear over the concentration range (r = 0.9991) and the limits of detection (LOD) and quantification (LOQ) were 0.04 μg/mL and 0.14 μg/mL, respectively (experimentally obtained against signal to noise level). Herein, RSD values were in the range 0.14-1.83%. The obtained results were employed for calculating the concentration of acetylsalicylic acid at set time intervals in all the tested organic solvents and in water. The degradation profile of aspirin in these solvents is shown in Fig. 3.
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The determined results were used to assign the kinetics reaction and the best fit was observed for the equation (1). This action suggested that the decomposition of acetylsalicylic acid in all the tested organic solvents yields an apparent second-order kinetics reaction. The rate constant values, the half-life times and the correlation coefficients of the degradation process for the analyzed compounds are presented in Table 1. In this work, 1,4-dioxane and acetonitrile turned out to be the most stable solvents, with the half-life times of 83 and 63 h, respectively. Furthermore, the fastest degradation of acetylsalicylic acid was observed in ethanol and methanol solutions, the half-life times being about 8 h in both cases. The obtained results of our work can be helpful in the process of developing new analytical methods of acetylsalicylic acid assay.

CONCLUSIONS

We established that the most stable solvents for acetylsalicylic acid were 1,4-dioxane and acetonitrile, with the half-life times of 83 and 63 h, respectively. Furthermore, the fastest degradation of acetylsalicylic acid was observed in methanol and ethanol solutions, the half-life times being about 8 h in both cases. The obtained results of our work can be helpful in the process of developing new analytical methods of acetylsalicylic acid assay.

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REFERENCES