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Degradation of furosine during heptafluorobutyric anhydride-derivatization for gas chromatographic determination

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Abstract

To evaluate further the reported degradation of furosine during gas chromatographic (GC) determination, a set of experiments with different derivatization conditions were carried out, utilizing a pure furosine standard. The results showed that the decomposition of furosine is not a consequence of the GC separation process, but a result of the derivatization procedure applied. Depending on the derivatization conditions, distinct differences in the percentage degradation were observed. Particularly, an incorrect strong drying during the second and third evaporation steps resulted in substantial degradation (up to ca. 21%), suggesting that the isobutyl ester and the heptafluorobutyryl isobutyl ester of furosine are sensitive to dryness. This demonstrates that the GC determination of furosine as a heptafluorobutyryl isobutyl ester derivative cannot be recommended for routine analytical application. Even under optimum derivatization conditions, degradation in the range 3–5% cannot be completely avoided.

1. Introduction

In the early stages of the Maillard reaction, lysine and sugar compounds react to form derivatives such as fructoselysine, lactuloselysine or maltuloselysine. During hydrolysis with 7.8 *M* HCl these Amadori compounds form furosine [ε-N-(2-furoylmethyl)-L-lysine], an indicator of Maillard-type heat damage [1,2]. Furosine determinations are often applied in food science and nutrition, clinical research and medical biochemistry.

For furosine determination, chromatographic techniques including ion-exchange chromatography (IEC) with commonly used amino acid analysers [3,4], reversed-phase high-performance liquid chromatography (HPLC) [5–7], gas chromatography (GC) [8], respectively gas chromatography–mass spectroscopy [9] have been applied. The GC determination of furosine as the heptafluorobutyryl isobutyl ester, using nitrogen–phosphorus-selective detection (NPD), was first established by Büser and Erbersdobler [8]. The simultaneous determination of amino acids and furosine by GC, using a capillary column and NPD, allows the successful resolution of these compounds with high selectivity, linearity and sensitivity [10–12]. Resmini et al. [6] proposed that during GC analysis according to the above method [8], considerable decomposition

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of furosine may take place. They determined furosine in heat-treated milk samples after acid hydrolysis, first using an HPLC method and subsequently examined the collected furosine peak by GC. In addition to the furosine peak an unexpected lysine peak was detected.

To examine further the probability that furosine can be destroyed during GC determination, in this work the degradation of furosine under different derivatization conditions was measured, using pure furosine standard.

2. Experimental

2.1. Reagents

Furosine standard with a purity of >99% was obtained from Neosystem (Strasbourg, France). Norleucine hydrochloride, used as an internal standard, was purchased from Serva (Heidelberg, Germany) and heptafluorobutyric anhydride (HFBA), isobutanol and ethyl acetate, all of analytical-reagent grade, from Merck (Darmstadt, Germany). Isobutanol–3 *M* HCl was prepared by bubbling anhydrous HCl through two successive traps containing concentrated sulfuric acid and then into isobutanol at 0°C.

In all experiments the furosine standard was used at a concentration of 0.2 μmol/ml and the norleucine standard at a concentration of 0.25 μmol/ml.

The percentage degradation of furosine was calculated from the recovery of lysine compared with the initial lysine content in furosine.

2.2. Instrumentation

Chromatography was performed using a DANI Model 65.00 gas chromatograph equipped with a nitrogen–phosphorus detector, a programable temperature vaporizer (PTV)–injection system and a OV-1–CB fused-silica capillary column (25 m × 0.32 mm O.D. × 0.25 mm I.D.) from CS (Langerwehe, Germany). The injector was operated in the split mode with a splitting ratio of 1:20 and the injection temperature was varied form 54 to 260°C. The detector temperature was set at 280°C. The detector was provided with air (140 ml/min) and hydrogen (3 ml/min).

Nitrogen was used as the make-up gas at a flow-rate of 25 ml/min. The carrier gas (helium) flow-rate was typically 2.6 ml/min. After 2 min at 54°C, the oven temperature was programmed to 260°C at 6°C/min, the final temperature being held for about 12 min. The chromatograph was linked to a C-R5A Chromatopac integrator from Shimadzu (Duisburg, Germany), which performed data acquisition.

2.3. Derivatization

The heptafluorobutyryl isobutyl ester was prepared according to the reaction scheme summarized in Fig. 1. Additionally a possible degradation route of the furosine derivatives is shown. Compared with the method described by Büser and Erbersdobler [8], two slight modifications were introduced. First, the heating times in the oven were both increased by about 5 min, and second, no co-injection with acetic anhydride was carried out. In a set of experiments, the impact of each individual evaporation step on the percentage degradation was studied. The experiments are summarized in Table 1.

3. Results and discussion

Typical chromatograms, one for a sample with a high percentage degradation of furosine and one for a sample with a low percentage degradation are shown in Fig. 2. Between the norleucine peak (retention time 18 min), used as an internal standard, and the furosine peak (retention time 36 min), a lysine peak (retention time 26 min) is recognizable in both instances.

As a pure furosine standard (purity >99%) was used and confirmed by other chromatographic techniques [7], lysine can only be a decomposition product of furosine. Repeated injections of the same volumes from the same sample solution revealed that the percentage degradation did not differ. Hence, the main

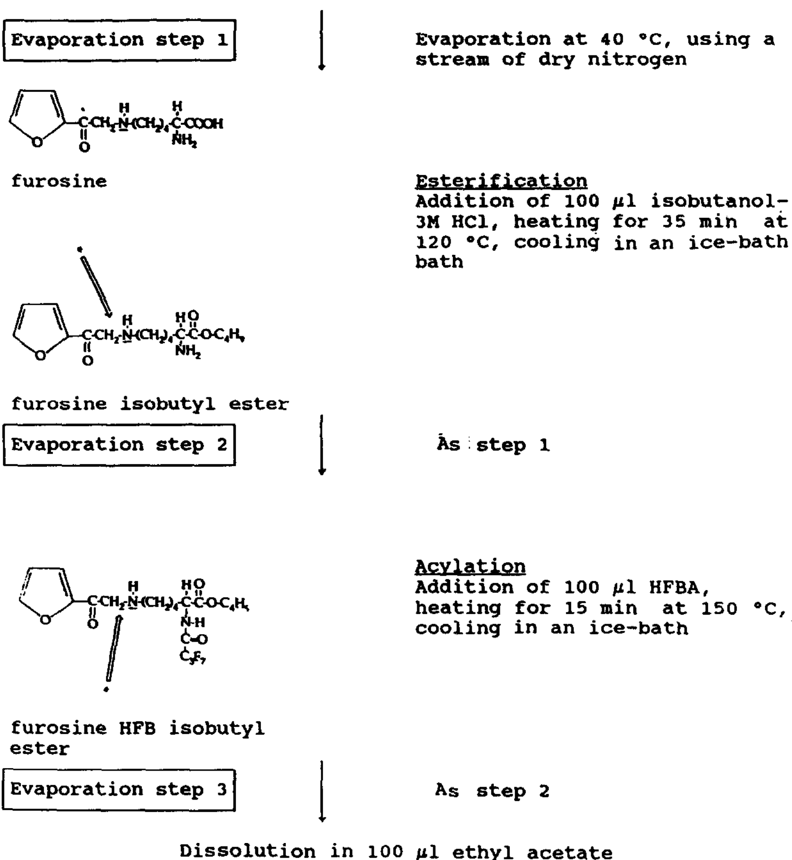

Fig. 1. Derivatization procedure.

Table 1
Experimental conditions

Experiment	Extent of dryness in different evaporation steps		
	Step 1	Step 2	Step 3
A	Dry	Dry	Close to dryness
B	Dry	Close to dryness	Dry
C	Dry	Close to dryness	Close to dryness
D	Dry	Moist	Close to dryness

Close to dryness means that after the evaporation step concerned, a very slight residual moisture remained behind in the screw-capped glass tube.

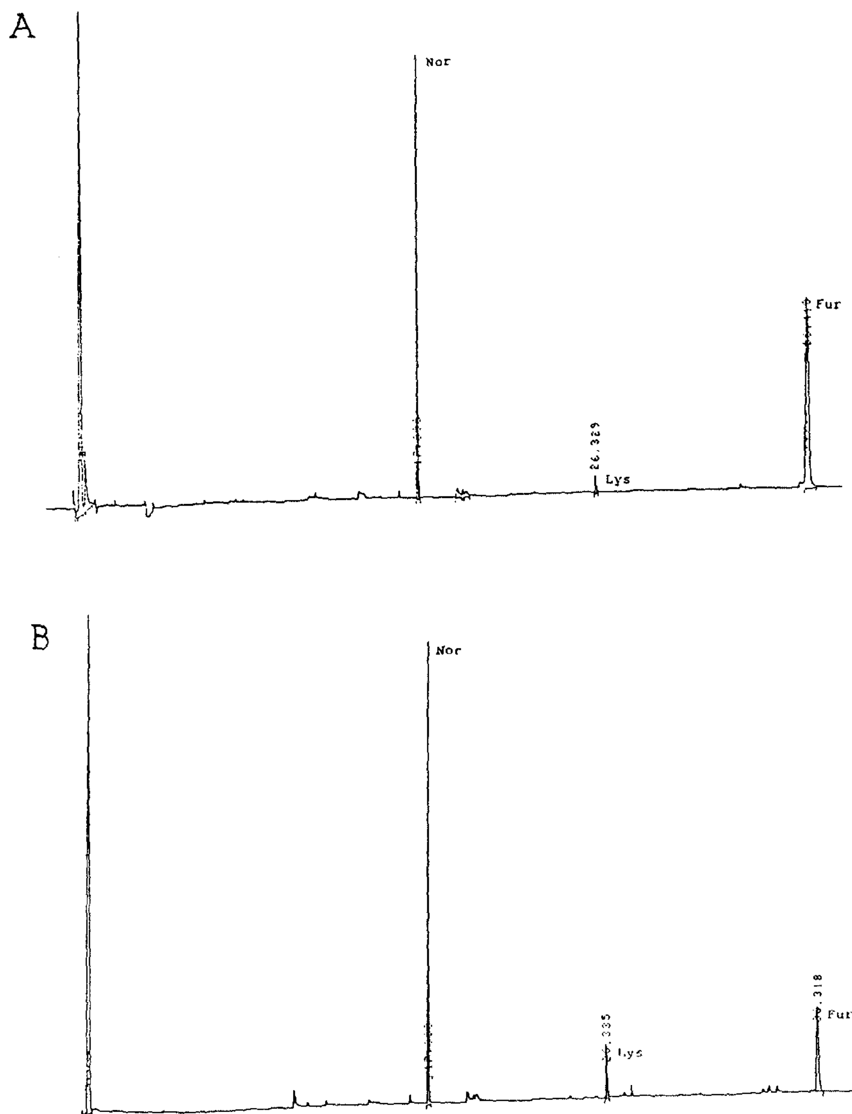

Fig. 2. Typical chromatograms for (A) a low and (B) a high percentage degradation of furosine. The HFB derivatives were prepared according to Fig. 1. Peaks: Nor = norleucine; Lys = lysine; Fur = furosine. Numbers at peaks are retention times in min.

cause of the different degradation rates lies in the derivatization procedure.

In Fig. 3, the percentage degradation under different derivatization conditions (see Table 1) is compared. Each column represents the mean + standard deviation of six to ten determinations.

Evaporation of the underivatized furosine (step 1, Fig. 1) to dryness did not lead to an increased degradation as compared with an evaporation performed close to dryness, suggesting that furosine itself is stable against dryness. Therefore, in subsequent experiments, evaporation in step one was always carried out to dryness.

Different results were obtained when in step 2 (experiment A) or in step 3 (experiment B) excess solvent was evaporated to complete dry-

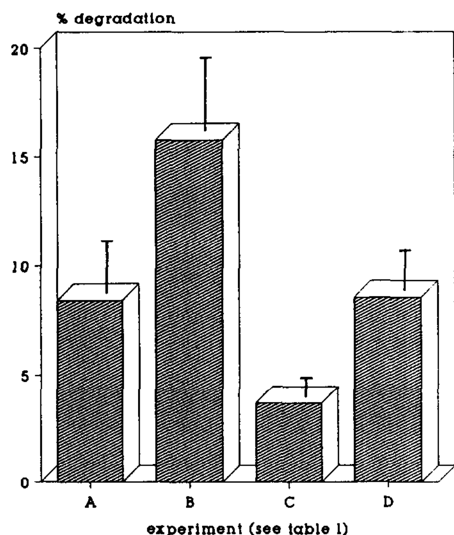

Fig. 3. Percentage degradation of furosine under different derivatization conditions.

ness. Then the percentage degradation was high, ranging from 5.3 to 11.3% for step 2 and from 11.9 to 20.3% for step 3. These results suggest that the HFB isobutyl ester of furosine is very sensitive to dryness. Experiments A and B show that a low percentage degradation of furosine is obtainable only when in evaporation steps 2 and 3 evaporation is carried out not too far, i.e., close but not completely to dryness.

The third evaporation step is especially critical, because the excess HFBA and also the HFB isobutyl ester are relatively volatile and hence the optimum extent of drying can easily be missed. Moreover, when a large batch of samples are derivatized simultaneously, the correct degree of dryness seems difficult to achieve for all samples.

In experiment D, the effect of an insufficient evaporation in step 2 was tested. In this instance, unremoved water, which is formed during esterification, can react with HFBA to form HFBA acid. Possibly this contamination leads to the poor furosine response, with a simultaneous increase in the variability. Unexpectedly, the percentage degradation also increased. Similar observations were made by Moodie et al. [13] and described for arginine.

Careful evaporation in steps 2 and 3 close to dryness (experiment C) yielded the best results, but a percentage degradation ranging from 3.2 to 4.6% has to be accepted.

In conclusions, these experiments have demonstrated that the GC determination of furosine as the HFB isobutyl ester derivative is affected by technical difficulties. For this reason, this method may be useful for comparative and confirmative assays, but is unsuitable for routine analytical applications.

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