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Gas chromatography with mass spectrometric, atomic emission and Fourier transform infrared spectroscopic detection as complementary analytical techniques for the identification of unknown impurities in pharmaceutical analysis

Krzysztof Łaniewski*, Thomas Wännman, Gunnar Hagman
AstraZeneca, Process R&D, Department of Analytical Chemistry, SE-151 85 Södertälje, Sweden

Abstract

An example of the complementary use of GC–MS, GC–AED and GC–FT-IR is described for efficient structure elucidation of an unknown impurity in pharmaceutical analysis. None of the analytical techniques could solve the structure of the unknown impurity alone; identification was, however, straightforward by combining the available spectroscopic information. GC–MS provided information about structural fragments and molecular mass of the unknown compound. GC–AED was used for confirmation of the occurrence of the individual elements in the structure and to enable calculation of the empirical formula. GC–FT-IR gave valuable information regarding functional groups in the molecule.

Keywords: Pharmaceutical analysis; Butyl fluorohydroxypropylcarbamate; Butoxycarbonylaminofluoropropyl acetate

1. Introduction

Manufacturing of drug substances and drug products requires strict control of impurities [1,2]. Organic impurities at and above an apparent level of 0.1% in the drug substance or drug products should be identified and controlled during manufacturing. Actual and potential organic impurities may be present in the raw materials used for synthesis, formed as by-products during the manufacturing process or arise as degradation products during storage. Pharmaceutical analysis therefore involves development of validated analytical methods suitable for detection and quantification of impurities and degradation products in raw materials, intermediates, drug substances and drug products.

Gas chromatography (GC) is an attractive analytical technique for control of organic impurities, since it provides high separation efficiency, universal flame ionisation detection, rapid method development and validation as well as straightforward coupling to several spectroscopic techniques. Many drug substances unfortunately are too polar and/or labile to be ideally suitable for GC. Therefore, the widest applications of gas chromatography for control of impurities in pharmaceutical analysis can be found for raw materials and intermediates used for synthesis of drug substances. Identification of impurities can be carried out by interpretation of spectral data generated by coupling GC to mass spectrometry (MS), atomic emission detection

*Corresponding author. Tel.: +46-8-5532-5575; fax: +46-8-5532-4274.
E-mail address: krzysztof.laniewski@astrazeneca.com (K. Łaniewski).

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(AED) or Fourier transform infrared spectroscopy (FT-IR).

GC–MS [3–7] is generally the method of choice for identification of solutes that can be analysed by gas chromatography. Mass spectrometry provides molecular mass data as well as very useful structural information based on molecular fragmentation. There are large mass spectral libraries available for rapid confirmation of identity of already known compounds. Moreover, the libraries could also provide valuable information comparing the spectrum of an unknown substance to the spectrum of known compounds. However, for a number of impurities, use of GC–MS as a single technique will not provide the information needed for a reliable structure elucidation. Combining GC–MS data with structural information from other techniques such as GC–AED and GC–FT-IR then becomes a valuable tool for a correct interpretation of the unknown structure [8].

GC–AED [9–14] provides information on the occurrence of individual elements in the analysed compounds. Monitoring at different wavelengths is carried out in order to measure the atomic emission of the chosen elements, and data are recorded as element selective chromatograms. Furthermore, information on possible empirical formulae can be obtained by calibrating the instrument with standard compounds of known composition and comparing to the response of the unknown impurity.

GC–FT-IR [15–17] provides information on the functionality of a molecule in that the presence or absence of a particular functional group such as an ester, ether, carbonyl, hydroxyl, aromatic, aliphatic, amine as well as other functionality can be determined. Infrared spectrometry is also complementary to mass spectrometry in areas such as substitution of aromatics, *cis-trans* isomers and double bond or heteroatom position in rings. The spectra obtained can also be compared to available vapour phase IR spectra through library searching.

This paper describes an example of the complementary use of GC–MS, GC–AED and GC–FT-IR for efficient structure elucidation of unknown impurities in pharmaceutical analysis.

2. Experimental

2.1. Sample preparation

A sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate (Fig. 1) was dissolved in acetonitrile.

2.2. Instrumentation

We used Hewlett-Packard (HP) gas chromatographs of the 6890 series equipped or coupled with:

1. flame ionisation detection (GC–FID).
2. MS-1: HP model 5973, electron ionisation, 70 eV (GC–EI-MS).
3. MS-2: HP model 5972, chemical ionisation, reagent gas: CH_4 (GC–CI-MS).
4. AED: HP model G2350A. Reagent gases: O_2 and H_2 (for C monitored at 179 nm, and N at 174 nm), O_2 (for H at 486 nm), H_2 and CH_4/N_2, 1:9 (for O at 171 nm), and H_2 (for F at 690 nm).
5. FT-IR: Perkin-Elmer model Spectrum 2000, at wave number 4000–600 cm^{-1}, resolution 8 cm^{-1}.

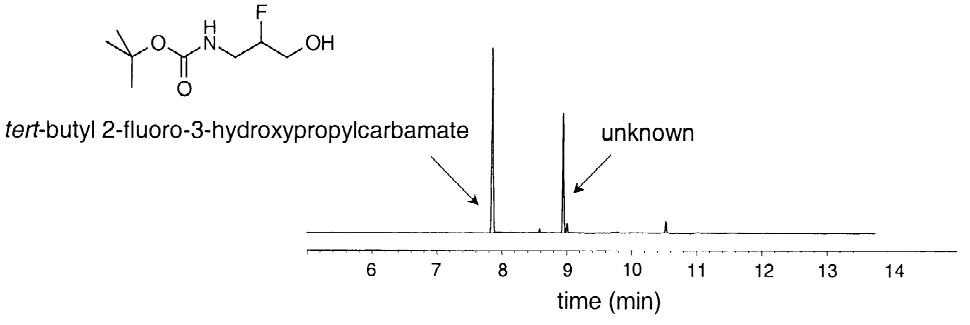

Fig. 1. GC–FID analysis of the sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate.

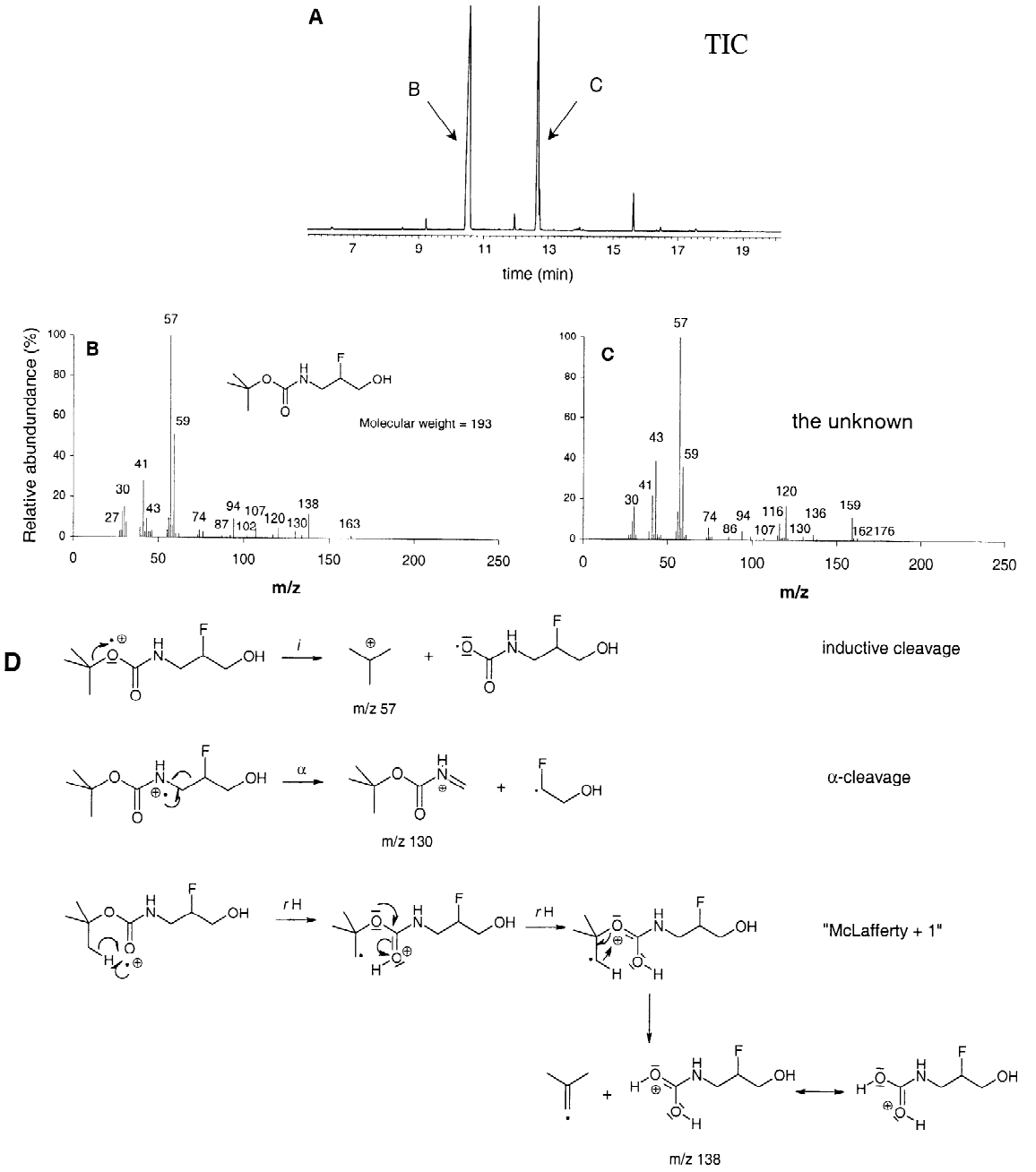

Fig. 2. GC–EI-MS analysis of the sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate. (A) Total ion current (TIC); (B) mass spectrum corresponding to *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate; (C) mass spectra of the unknown substance; (D) fragmentation pathways of the target compound in electron ionisation mode. *r*H = Rearrangement of hydrogen atoms.

2.3. GC conditions

The carrier gas was helium, 99.9999%. The following capillary columns (cross-linked 5% phenyl–methylsiloxanes) were used:

1. HP-Ultra 2, 25 m×0.32 mm, 0.52 μm film thickness (GC–FID, GC–AED).
2. HP-5MS, 30 m×0.25 mm, 0.25 μm film thickness (GC–MS).
3. HP-5, 30 m×0.52 mm, 1.5 μm film thickness (GC–FT-IR).

The oven temperature profiles were:

1. 60 °C for 2 min, 20 °C/min, and 300 °C for 5 min (GC–FID, GC–EI-MS, GC–AED).
2. 70 °C for 2 min, 20 °C/min, and 250 °C for 5 min (GC–CI-MS).
3. 80 °C for 2 min, 20 °C/min, and 300 °C for 5 min (GC–FT-IR).

Inlet: temperature 200 °C, He pressure 70 kPa (GC–FID, GC–MS and GC–AED) or 30 kPa (GC–FT-IR).

Injection volume and mode: 1 μl; split.

2.4. Determination of the empirical formula by GC–AED

tert-Butyl 2-fluoro-3-hydroxypropylcarbamate was used as a response standard and the calculations were carried out according to the following formula:

$$(E1/E2)_u = \frac{(E1/E2)_k \cdot (\mathrm{Area}E1/\mathrm{Area}E2)_u}{(\mathrm{Area}E1/\mathrm{Area}E2)_k}$$

where $(E1/E2)$ is the ratio between number of atoms of respective elements in a molecule, (Area$E1$/Area$E2$) is the peak area ratio, ''u'' denotes ''unknown'', and ''k'' stands for ''known''.

2.5. Synthesis of 3-[(tert-butoxycarbonyl)amino]-2-fluoropropyl acetate

3-[(*tert*-Butoxycarbonyl)amino]-2-fluoropropyl acetate was synthesised by mixing *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate (6.2×10^{-5} mol) with acetic anhydride (1.6×10^{-4} mol) and pyridine (approximately 0.8×10^{-4} mol). The mixture was diluted with CH_3CN to 600 μl and 1 μl injected into GC–FID and GC–MS.

3. Results and discussion

During synthesis of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate, an intermediate in the synthetic route of a drug substance in development, analysis by GC–FID showed the presence of two main components (Fig. 1). One of them was the target compound and the other a major unknown impurity. Further investigations were focused on the identification of the unknown compound using GC–MS, GC–AED and GC–FT-IR.

3.1. GC–MS analysis

The identity of the target compound was verified

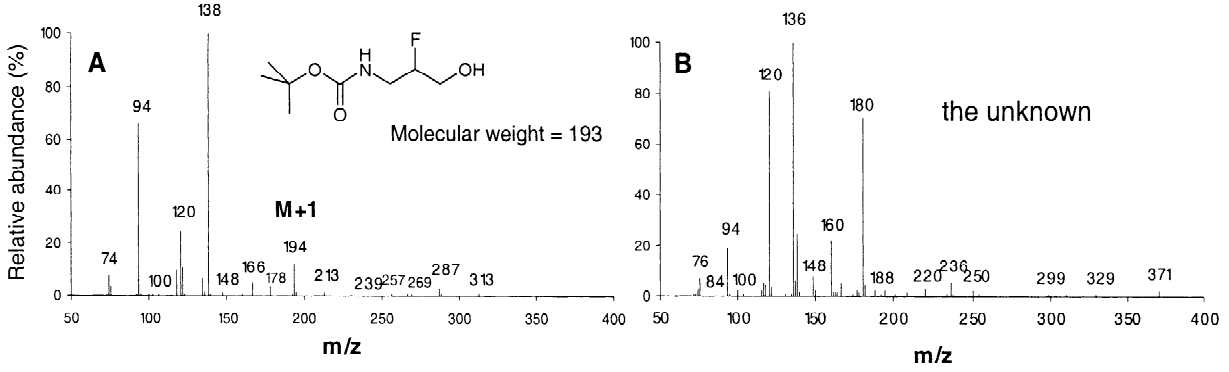

Fig. 3. Mass spectra obtained by GC–CI-MS analysis of the sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate. (A) Mass spectrum corresponding to *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate; (B) mass spectrum of the unknown substance.

by the retention time as well as the mass spectrum from the GC–EI-MS analysis (Fig. 2). The following expected fragmentation pathways were recognised in the mass spectrum. The ion m/z 57 was formed by an inductive cleavage and corresponds to a *tert*-butyl fragment. A peak at m/z 130 indicated α-cleavage and loss of fragment CHF–CH_2–OH. The formation of ion m/z 138 was caused by rearrangement of two hydrogen atoms, i.e. the 'McLaferty +1' rearrangement.

The spectrum of the unknown impurity was, however, not straightforward to interpret. Nevertheless, the following observations were made. The peak m/z 57 corresponding to the *tert*-butyl fragment of the protecting group is observed as the base peak in both mass spectra. The occurrence of several similar ions formed, together with the overall appearance of both mass spectra indicated that the unknown impurity has a structure that was related to the target compound. The molecular ion in the mass spectrum of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate is absent, and it was assumed that the same could apply for the unknown substance.

In an attempt to determine the molecular mass of the unknown impurity, the sample was analysed by GC–CI-MS. Chemical ionisation is a soft ionisation technique, which often facilitates detection of an ion corresponding to the molecular mass for compounds where the molecular ion (M) is not stable in electron ionisation mode. In the GC–CI-MS mass spectrum corresponding to the target compound, an ion $[M+H]^+$ of m/z 194 (M+1) was recorded, but the expected adduct peaks of $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ at m/z (M+29) and (M+41), respectively, often verifying the correct molecular mass, were absent (Fig. 3). Additionally, species of higher molecular mass were formed. The pattern with relatively high m/z values was also characteristic for the mass spectrum of the unknown compound. Therefore, the molecular mass of the compound could not securely be determined at this stage of the investigation. It was, however, concluded that the peaks with high m/z values in the unknown mass spectrum probably was caused by adduct formation, since the unknown impurity eluted relatively close to the target compound during GC analysis and therefore should have a molecular mass not too far from the target compound.

3.2. GC–AED analysis

The next step in the investigation was to use GC–AED in order to collect information about elemental composition. The results obtained by GC–AED analysis showed that the unknown compound contains the same heteroatoms as *tert*-butyl 2-fluoro-

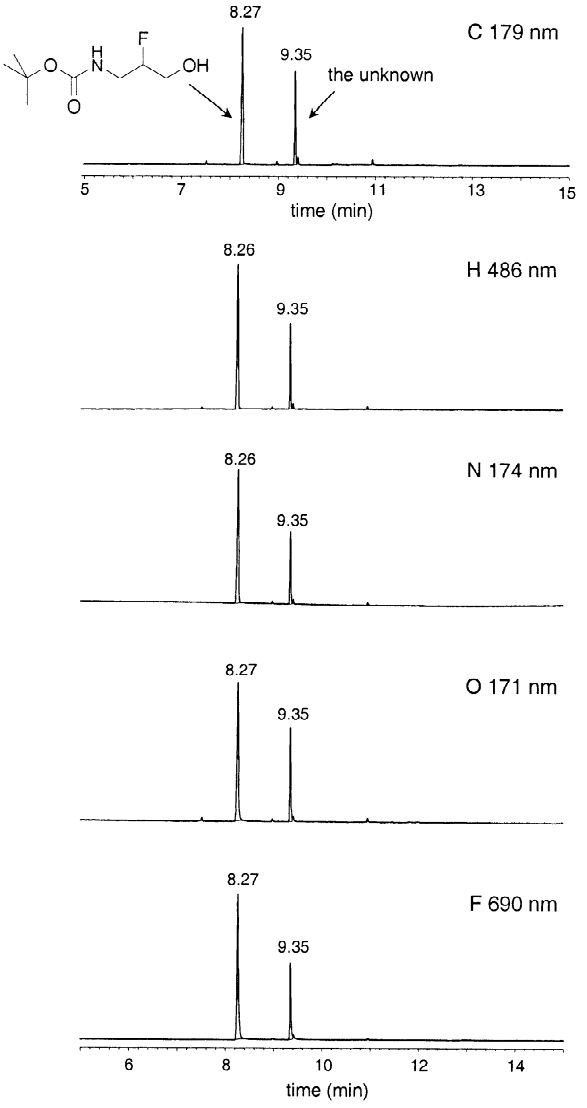

Fig. 4. GC–AED analysis of the sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate. The element selective chromatograms show the response to carbon, hydrogen, nitrogen, oxygen and fluorine, respectively.

3-hydroxypropylcarbamate (Fig. 4), i.e. carbon, hydrogen, oxygen, nitrogen and fluorine.

Determination of the empirical formula gave three theoretically possible results (Table 1). In all three cases there is only one nitrogen atom in the structure. Hence, there is only one possible correct formula, the one which gives an uneven molecular mass, i.e. 235. This molecular mass is 42 a.m.u. higher than the molecular mass of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate, and this may indicate the presence of a group of 43 a.m.u. replacing one hydrogen atom. Furthermore, it is noteworthy that the GC–AED analysis revealed that the unknown structure apparently contains four oxygen atoms compared to three oxygen atoms in the target compound.

The results from the GC–AED analysis enabled additional interpretation and understanding of the GC–MS results. The GC–CI-MS mass spectrum contains a peak at m/z 236 (Fig. 3) which might correspond to the ion of (M+1) of the unknown compound. Although, similarly to *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate, no distinct peaks of m/z (M+29) and (M+41) were observed. Furthermore, for the known compound, the most prominent fragmentation pattern produced m/z 138 presumably by elimination of isobutene from the *tert*-butyl group after H-rearrangement ([M+H]−56). This was followed by neutral loss of carbon dioxide yielding m/z 94. A similar fragmentation pathway is apparently visible in the spectrum of the unknown substance.

Table 1
Empirical formula determination of the unknown compound using *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate as a response standard in the GC–AED analysis

Compound	Empirical formula	Retention time (min)	Peak area				
			C179	H486	F690	N174	O171
tert-Butyl 2-fluoro-3-hydroxypropylcarbamate	$C_8H_{16}FNO_3$	8.3	984.6	2326.5	268.2	244.8	208.1
Unknown	?	9.4	465.1	946.2	94.8	93.4	101.5

	Peak area ratio									
	C/N	C/O	C/F	O/N	O/F	N/F	H/C	H/N	H/O	H/F
tert-Butyl 2-fluoro-3-hydroxypropylcarbamate	4.02	4.73	3.67	0.85	0.78	0.91	2.36	9.50	11.18	8.67
Unknown	4.98	4.58	4.91	1.09	1.07	0.99	2.03	10.13	9.32	9.98

	Number of atoms ratio									
	C/N	C/O	C/F	O/N	O/F	N/F	H/C	H/N	H/O	H/F
tert-Butyl 2-fluoro-3-hydroxypropylcarbamate	8	2.7	8	3	3	1	2	16	5.3	16
Unknown (calculated)	9.9	2.6	10.7	3.8	4.1	1.1	1.7	17.1	4.4	18.4

	Ratio of possible 'real' number of atoms in the unknown empirical formula									
	C/N	C/O	C/F	O/N	O/F	N/F	H/C	H/N	H/O	H/F
	10:1	10:4	11:1	4:1	4:1	1:1	17:10	17:1	18:4	18:1
or	11:1	11:4					19:11			

Possible empirical formulae	Molecular mass
$C_{10}H_{17}FNO_4$	234
$C_{10}H_{18}FNO_4$	235
$C_{11}H_{19}FNO_4$	248

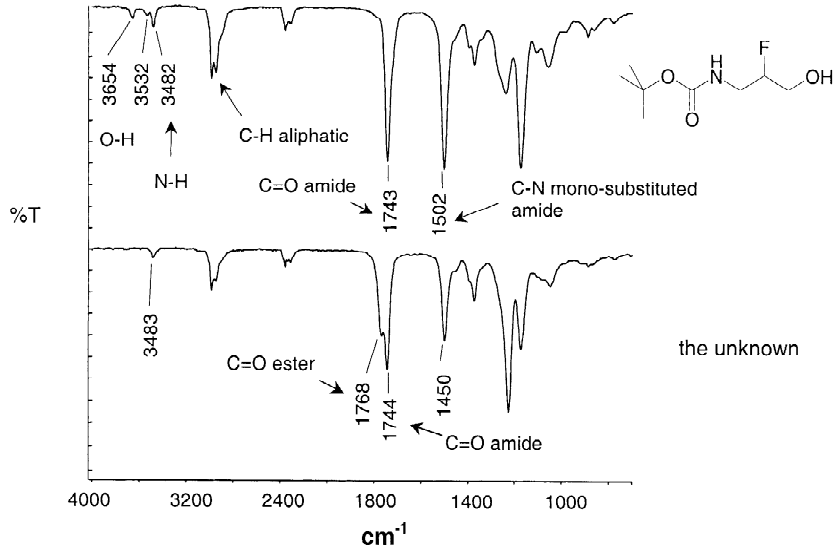

Fig. 5. IR spectra obtained by GC–FT-IR analysis of the sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate.

The loss of carbon dioxide is observed from m/z 180 to 136, and thus 180 + 56 gives m/z 236 as $[M+H]^+$ assuming intact carbamate structure in the unknown.

Moreover, an extra oxygen atom together with a mass increase of 42 a.m.u. and the larger abundance of the ion of m/z 43 in the EI mass spectrum (Fig. 2) suggested that the unknown structure contained an acetyl group. However, no source of acetyl formation in the synthetic route was anticipated and consequently no chemical rationale for the presence of this impurity in the sample could be made.

3.3. GC–FT-IR analysis

To further strengthen the acetyl hypothesis, GC–FT-IR was employed to ascertain the absence of a hydroxyl and the presence of two non-equivalent carbonyl groups, i.e. having a different adjacency, in the unknown structure (Fig. 5). The IR spectrum of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate contains distinct bands at wave number 3654 and 3532 cm^{-1} originating from an O–H bond. At 3482 and at 1743 cm^{-1}, two bands corresponding to N–H and C=O (amide), respectively, were recorded. In the IR spectrum corresponding to the GC peak for the unknown compound, the O–H band is not observed. Furthermore, in the carbonyl region two bands occur at wave numbers of 1768 and 1744 cm^{-1}. These results support the view that two carbonyl groups are present in the unknown structure, i.e. the amide C=O and the ester C=O. In addition, both IR spectra contain the C–N band of monosubstituted amide at approximately 1500 and 1450 cm^{-1}, respectively.

3.4. Analysis of a reference substance

In order to finally verify the structure of the unknown impurity, a pure sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate was acetylated using acetic anhydride and pyridine. The GC–EI-MS analysis of this sample detected a compound with the same retention time and mass spectrum as the investigated unknown substance (Fig. 6).

4. Conclusions

1. The unknown compound was identified as 3-[(*tert*-butoxycarbonyl)amino]-2-fluoropropyl acetate.
2. None of the analytical techniques employed could solve the analytical problem alone. GC–MS provided information about structural fragments and molecular mass of the compound. GC–AED confirmed the occurrence of the individual heteroatoms in the structure and enabled calcula-

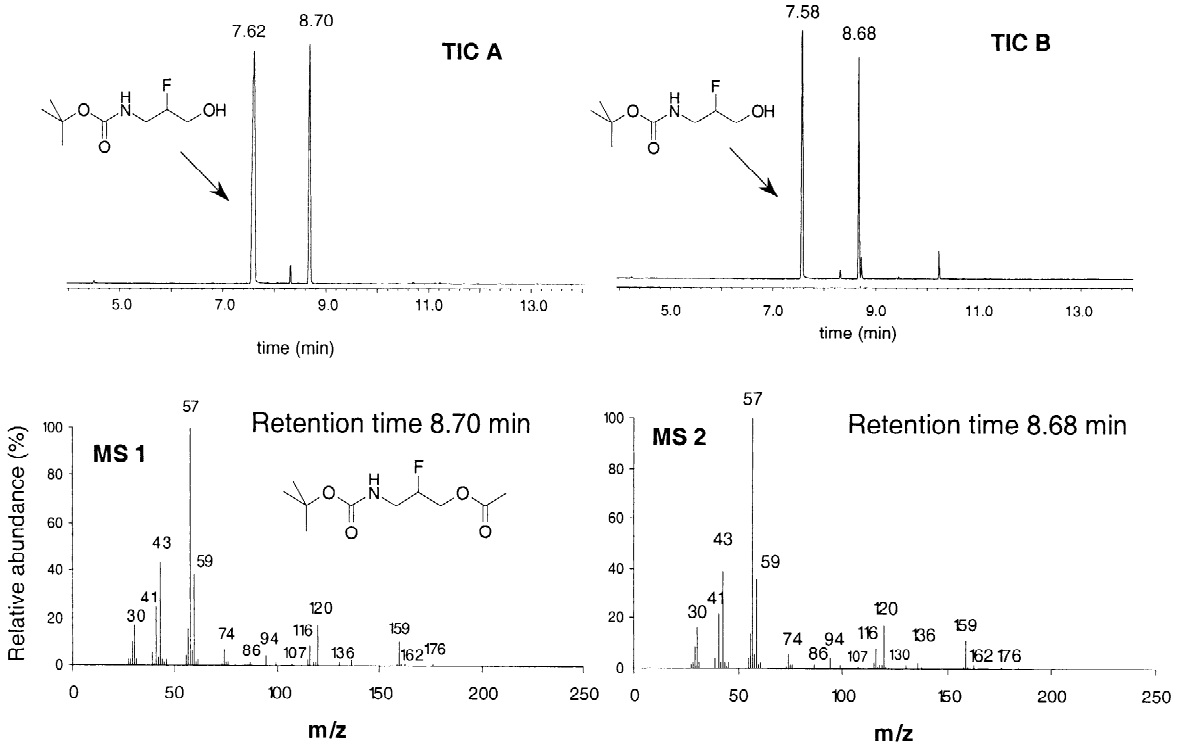

Fig. 6. (TIC A) GC–MS analysis of a sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate subjected to acetylation with acetic anhydride. Since the reaction was not completed at the time when the extract was taken for analysis, both the major substrate and the product could be detected. (TIC B) GC–MS analysis of the original sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate containing the unknown impurity. (MS 1) Mass spectrum of acetylated *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate. (MS 2) Mass spectrum of the investigated unknown compound detected during the analysis of the original sample of *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate.

tion of the empirical formula. GC–FT-IR gave valuable results regarding functional groups in the molecule.

3. This investigation demonstrates how GC–MS, GC–AED and GC–FT-IR can complement each other in identification of unknown organic compounds.

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