Pergamon

0731-7085(95)01277-X

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 4/5, pp. 567–574, 1995

0731-7085/95 $9.50 + 0.00

Identification of a new by-product detected in metoprolol tartrate*

MAGNUS ERICKSON,‡ KARL-ERIK KARLSSON,‡ BO LAMM,§ SAM LARSSON,‡ LARS A. SVENSSON‡ and JÖRGEN VESSMAN†‡

‡*Astra Hässle AB, Department of Analytical Chemistry, S-431 83 Mölndal, Sweden*
§*Astra Hässle AB, Department of Medicinal Chemistry, S-431 83 Mölndal, Sweden*

Abstract: A new impurity has been found in some batches of metoprolol tartrate. As the amount exceeded 0.1% it was of interest to deduce the structure. Techniques involved in solving the problem were LC, LC–MS and GC–MS. The LC systems showed that the impurity and metoprolol behaved differently to modifications of the mobile phase, indicating that there were differences in the functional groups. LC–MS was used to determine the molecular weight, which was 74 mass units higher than metoprolol. A hydrogen–deuterium shift technique using micro column LC–MS gave the information that three hydrogen atoms were bound to heteroatoms, i.e. one more than in metoprolol. This led to the conclusion that the impurity had three extra carbon and two extra oxygen atoms. It was supposedly a by-product in the synthesis. Knowledge of the synthesis steps for β-receptor blocking drugs suggested three possible structures. Two were independently synthesized and one of these was found to be identical to the impurity.

Keywords: *Drug impurity; structure elucidation; metoprolol.*

Introduction

Purity is a significant part of the quality specifications of drug substances. Developing international regulations of drug purity control within International Conference on Harmonisation (ICH) forum require that impurities exceeding the 0.1% level are examined and preferably identified. They may result from synthesis, degradation, or derive from impure starting materials. International harmonization of impurity studies are at hand [1]. New impurities in "old" drugs are discussed as well as the steps to qualify such impurities.

It may sometimes be difficult to detect impurities which are similar to the drug substance, e.g. isomers, when using single chromatographic techniques. These impurities might co-elute with the main peak which may be present in a thousand-fold excess. Although separations between most peaks of interest can be achieved, it is often a problem to find space enough in a single chromatogram to resolve all peaks within reasonable time. This has created an interest in peak purity determinations [2, 3]. The technique to discriminate co-eluting compounds based on different spectral properties may be problematic, since closely related compounds may have identical chromophores. The ordinary approach to vary the selectivity of the chromatographic system might be a more useful way to tackle those problems.

The main topic in this investigation has been to elucidate the structure of the new impurity found at levels between 0.2 and 0.3% in some batches of metoprolol tartrate of non-Astra origin, which in the following will be referred to as the external metoprolol batches.

Experimental

Equipment

An LKB model 2150 liquid chromatographic pump, a Rheodyne model 7010 injector with a 20-μl external loop, a Linear UVIS model 204 detector set at 280 nm and a Shimadzu model C-R5A integrator was used in the chromatographic systems. The column used in the LC-experiments was a LiChrospher RP-select B with 5-μm C_8 particles 125 × 4 mm (l × i.d.)

*Presented at the Fifth International Symposium on Pharmaceutical and Biomedical Analysis, Stockholm, Sweden, September 1994.

†Author to whom correspondence should be addressed.

from Merck (Darmstadt, Germany). A Finnigan Mat model TSQ 700 mass spectrometer was used for LC–MS and a Finnigan Mat model 95 was used as detector in GC–MS.

Chemicals

Metoprolol tartrate and the impurities were provided "in-house". Deuterium oxide (99.8%) was purchased from Dr Glaser AG (Basle, Switzerland) and trifluoroacetic anhydride (TFAA) p.a. from Fluka Chemie AG (Buchs, Switzerland). All other chemicals were from Merck. Acetonitrile, toluene, *n*-hexane and methylene chloride were all of HPLC grade and ammonium dihydrogen phosphate, phosphoric acid, sodium hydroxide and hydrochloric acid were of p.a. quality.

Liquid chromatography

The mobile phase buffers were prepared according to Table 1. These buffers were mixed with different amounts of acetonitrile, also listed in Table 1, and degassed by sonication. The columns were equilibrated at 1.0 ml min^{-1} for about 1 h prior to analysis. Twenty-microlitres of a 2.00 mg ml^{-1} solution of the metoprolol tartrate batches was injected into the chromatographic system. In order to see the complete separation in the systems, nine other possible impurities have been added to a solution containing metoprolol tartrate from one of the external batches. In this case the batch containing the largest amount of the new impurity was chosen.

Liquid chromatography–mass spectrometry

The molecular weight was obtained in an LC–MS system using an electrospray ionization (ESI) interface. A one meter long 50-μm i.d. fused silica capillary was packed with 5-μm octadecyl-silica particles. This was used as a column. The mobile phase was 1% acetic acid solution/acetonitrile (4 + 1) using either water or deuterium oxide [4].

Gas chromatography–mass spectrometry

The TFA derivatives made from the external metoprolol batches of interest were introduced onto a phenyl-methyl polysiloxane coated fused silica column in the GC–MS equipment with electron impact (EI) ionization. Metoprolol tartrate as well as the synthesized impurity was derivatized according to the procedure described by Ervik *et al.* [5].

Synthesis of the impurity

An independent route to the suspected impurity was performed as indicated in Fig. 1. The deduced structure of the impurity was finally proved, using the synthesized compound.

Results and Discussion

Liquid chromatographic behaviour

In the LC method originally proposed for a pharmacopoeial monograph, mobile phase number 1 was used. However, in that system, the impurity and metoprolol have the same retention time, i.e. the small impurity peak "drowns" in the signal from the main peak. The impurity was first detected in the system utilizing mobile phase number 2. This is the current proposal for an LC method in the European Pharmacopoeia [6]. This mobile phase was considered somewhat complex, so to study the chromatographic behaviour of the new impurity, a third and simpler method was set up. This system was prepared with three different concentrations of acetonitrile corresponding to the mobile phases 3a, b and c. As an example, a chromatogram run with LC

Table 1
The mobile and stationary phases used in the different chromatographic systems

Mobile phase number	Type of buffer solution	Volume of buffer (ml)	Volume of acetonitrile (ml)
1	Phosphate buffer*	750	250
2	Acetate and phosphate buffer†	1650	380
3a	Ammonium acetate buffer‡	860	140
3b	Ammonium acetate buffer‡	850	150
3c	Ammonium acetate buffer‡	830	170

*Phosphoric acid (1.78 g) and sodium dihydrogen phosphate dihydrate (15.6 g) was diluted up to 2.00 l (0.9 g octylsulphonic acid sodium salt was dissolved in the acetonitrile before adding the buffer).

†Ammonium acetate (7.8 g) was dissolved in 1620 ml of water, 4.0 ml triethyl amine, 20.0 ml glacial acetic acid and 6.0 ml phosphoric acid (85%) was added (pH 3.6).

‡Ammonium dihydrogen phosphate (11.5 g) and 10.0 ml of 1.0 M phosphoric acid was diluted up to 2.00 l (pH 3.2).

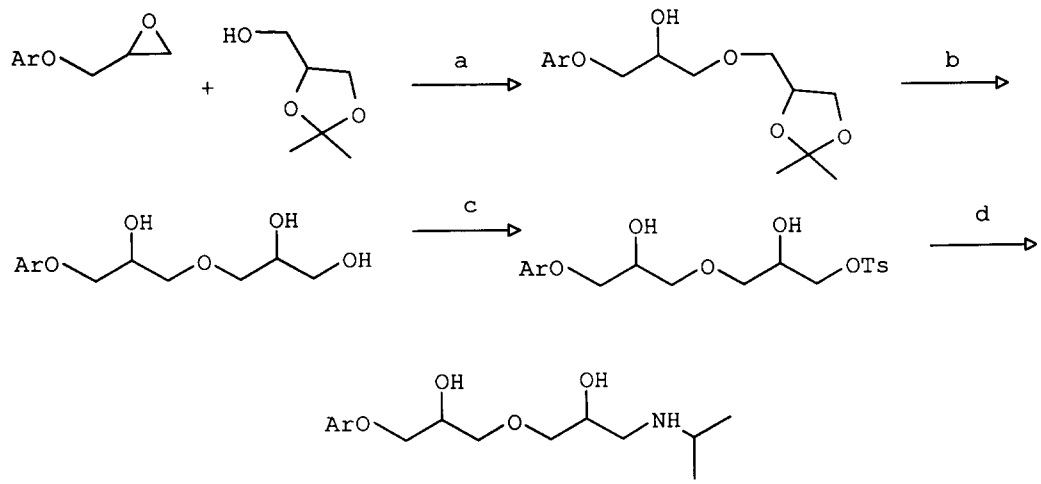

Figure 1
Synthesis scheme of the new impurity. Ar = 4-$CH_3OCH_2CH_2C_6H_4$–, Ts = 4-$CH_3C_6H_4SO_2$–. Conditions (a) Na (cat.); (b) MeOH, H_2O HCl (cat.); (c) TsCl, pyridine, 5°C, 24 h; (d) i-$PrNH_2$, i-PrOH, 70°C, 4 h.

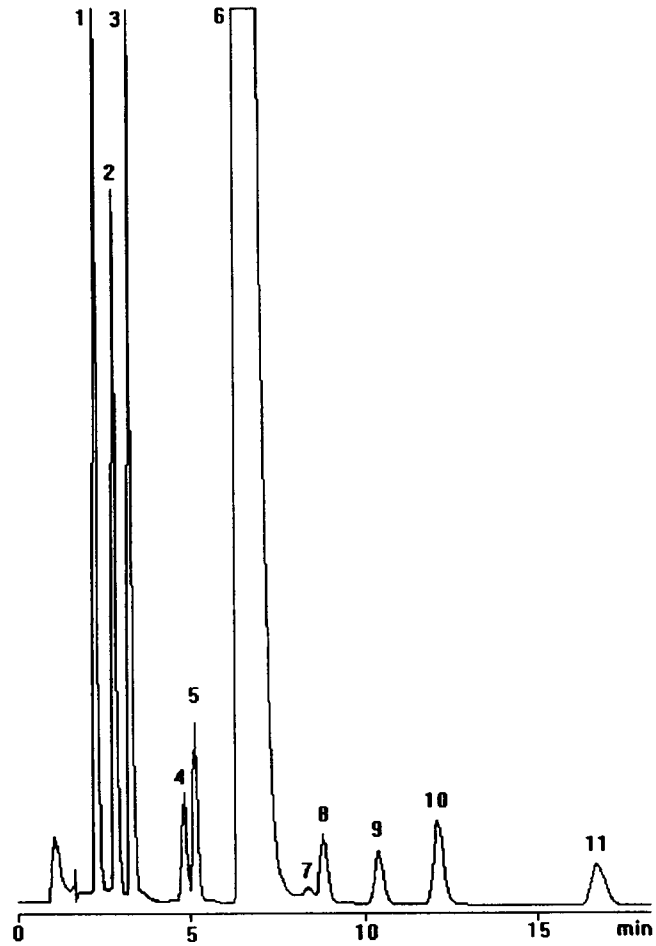

Figure 2
Chromatogram showing the separation of metoprolol (6) and the new impurity (7) as well as nine other possible impurities (1–5, 8–11), which have been added to the solution of an external metoprolol tartrate batch. Chromatographic system: as described under experimental. Stationary phase: LiChrospher RP-select B, 5-μm C_8-particles (125 × 4 mm l × i.d.). Mobile phase: 3c in Table 1.

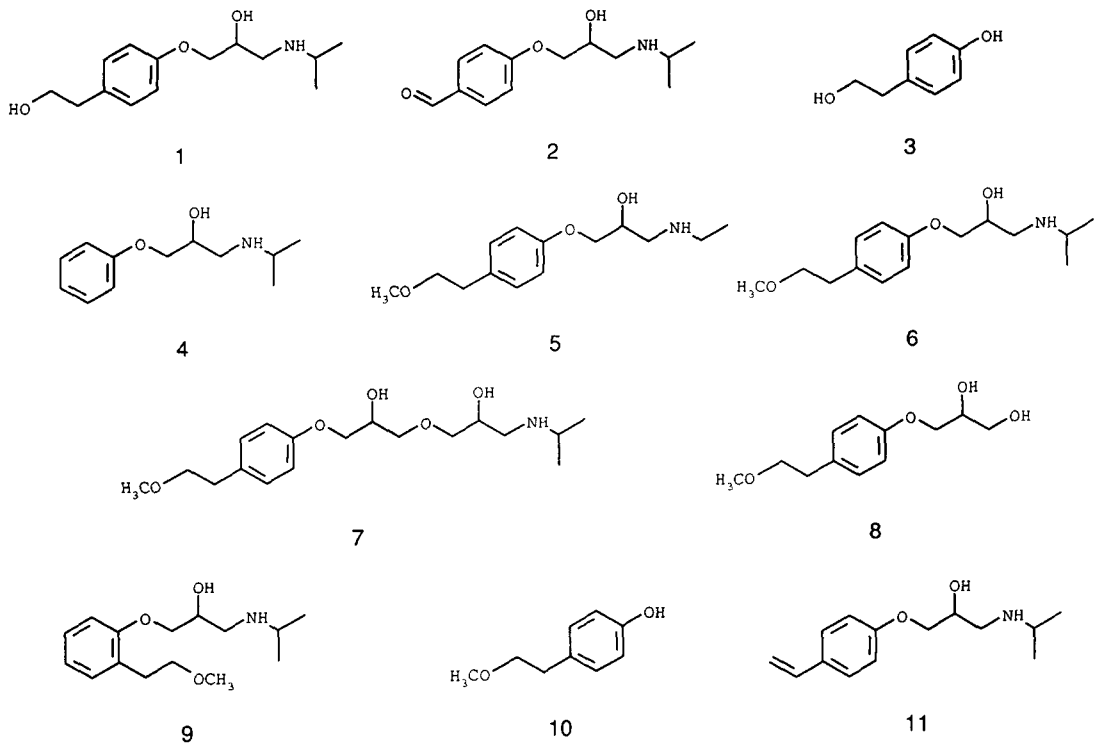

Figure 3
The structure corresponding to the chromatographic peaks are (1) 1-isopropylamino-3-[4-(2-hydroxyethyl)phenoxy]-2-propanol; (2) 4-(2-hydroxy-3-isopropylaminopropoxy)benzaldehyde; (3) 4-(2-hydroxyethyl)phenol; (4) 1-isopropylamino-3-phenoxy-2-propanol; (5) 1-ethylamino-3-[4-(2-methoxyethyl)phenoxy]-2-propanol; (6) 1-isopropylamino-3-[4-(2-methoxyethyl)phenoxy]-2-propanol (metoprolol); (7) 3-{2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]-propoxy}-1-isopropylamino-2-propanol (new impurity); (8) 3-[4-(2-methoxyethyl)phenoxyl]-1,2-propandiol; (9) 1-isopropylamino-3-[2-(2-methoxyethyl)phenoxy]-2-propanol; (10) 4-(2-methoxyethyl)phenol; (11) 1-isopropylamino-3-(4-vinylphenoxy)-2-propanol.

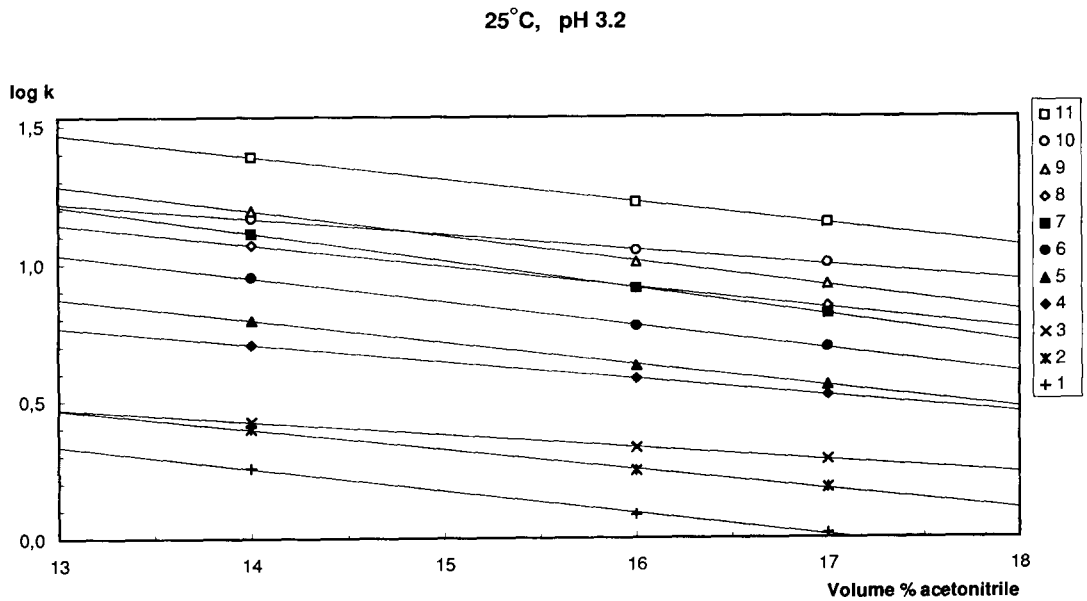

Figure 4
Log *k* values plotted against modifier concentrations. Numbers correspond to the structures in Fig. 3.

system 3c is shown in Fig. 2. The new impurity eluted on the tail of the main metoprolol peak. A satisfactory separation between metoprolol, the new impurity and nine other possible impurities in less than 20 min makes it a good alternative LC method. The structures of this impurity, as well as metoprolol and the other impurities are listed in Fig. 3. These compounds were all subject to investigation of the chromatographic behaviour. In Fig. 4 the logarithmic capacity ratios (log k) are plotted vs volume per cent of acetonitrile in the mobile phase. As can be seen in the figure, all compounds seem to give a linear relationship between the modifier concentration and log k. It can also be seen that two pairs (7 and 8 as well as 9 and 10) actually change their order of elution, even at these small changes. When introducing the independently synthesized impurity into the chromatographic systems, it behaved in the same manner to changes in the mobile phase composition and also eluted at the same retention times as the actual impurity in the external batches of metoprolol tartrate.

LC–MS

Important information on the molecular weight of the impurity was obtained when an aliquot of an external batch was introduced into a micro liquid chromatographic system connected to a mass spectrometer. The *m/z* detected for the molecular ion was 342 and hence the corresponding molecular weight would be 341 g mol^{-1}, i.e. 74 more than for metoprolol. In an additional experiment the water in the mobile phase was exchanged for deuterium oxide to show how many hydrogen atoms were connected to heteroatoms [4]. The resulting molecular ion had an *m/z* of 346 and the corresponding molecular weight would be 344 g mol^{-1}. This indicated 3 heteroatom–hydrogens in the unknown structure, which is one more than in metoprolol.

Of several possible increments of 74 mass units a three-carbon chain containing two oxygen and six hydrogen atoms was decided most probable. At least one of the hydrogens was connected to an oxygen atom. Another conclusion was that the compound probably was a by-product in the synthesis, formed by incorporation of two C_3-moieties instead of one into the end product.

GC–MS

The TFA-derivatization of the impurity would yield an *m/e* of 629 (341 + 3 × 96) according to the reaction scheme in Fig. 5. The mass spectra in Fig. 6(a) and (b) show that the incorporation of a C_3-moiety was a correct suspicion. Some of the peaks present, e.g. *m/e* 266 and 308, are typical for the rearrangement of the β-chain in aryloxy β-receptor blocking drugs according to Garteiz and Walle [7]. Hence, the traditional β-chain is supposedly intact, suggesting that this part should be present in the new structure. Other specific fragments in the spectra are suggested in Fig. 7.

Possible route for formation of the impurity in the synthesis of metoprolol

The standard route to metoprolol is a two-step synthesis (shown in Fig. 8) starting with 4-(2-methoxyethyl)phenol and epichlorohydrin. The resulting aryloxy epoxide is then reacted with isopropylamine. Traces of water in the epichlorohydrin used in the first step might cause formation of a C_6 compound shown in Fig. 8. This would then react with 4-(2-methoxyethyl)phenol to an epoxide which then yields the final impurity after reaction with isopropylamine. Suitable purification of the aryloxy epoxide would conceivably suppress the formation of the impurity. The compound is a mixture of two diastereomers, but no separation of these were observed in any of our chromatographic systems. In Fig. 6(b), however, one could possibly suspect from the broad peak that more than one compound is present.

Consequences

As the amount of the impurity in the batches, of non-Astra origin, exceeded 0.1%

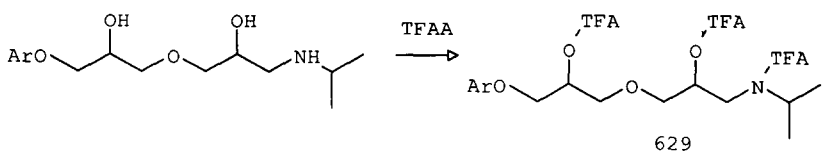

Figure 5
TFA derivatization of the impurity. Ar = 4-$CH_3OCH_2CH_2C_6H_4$-.

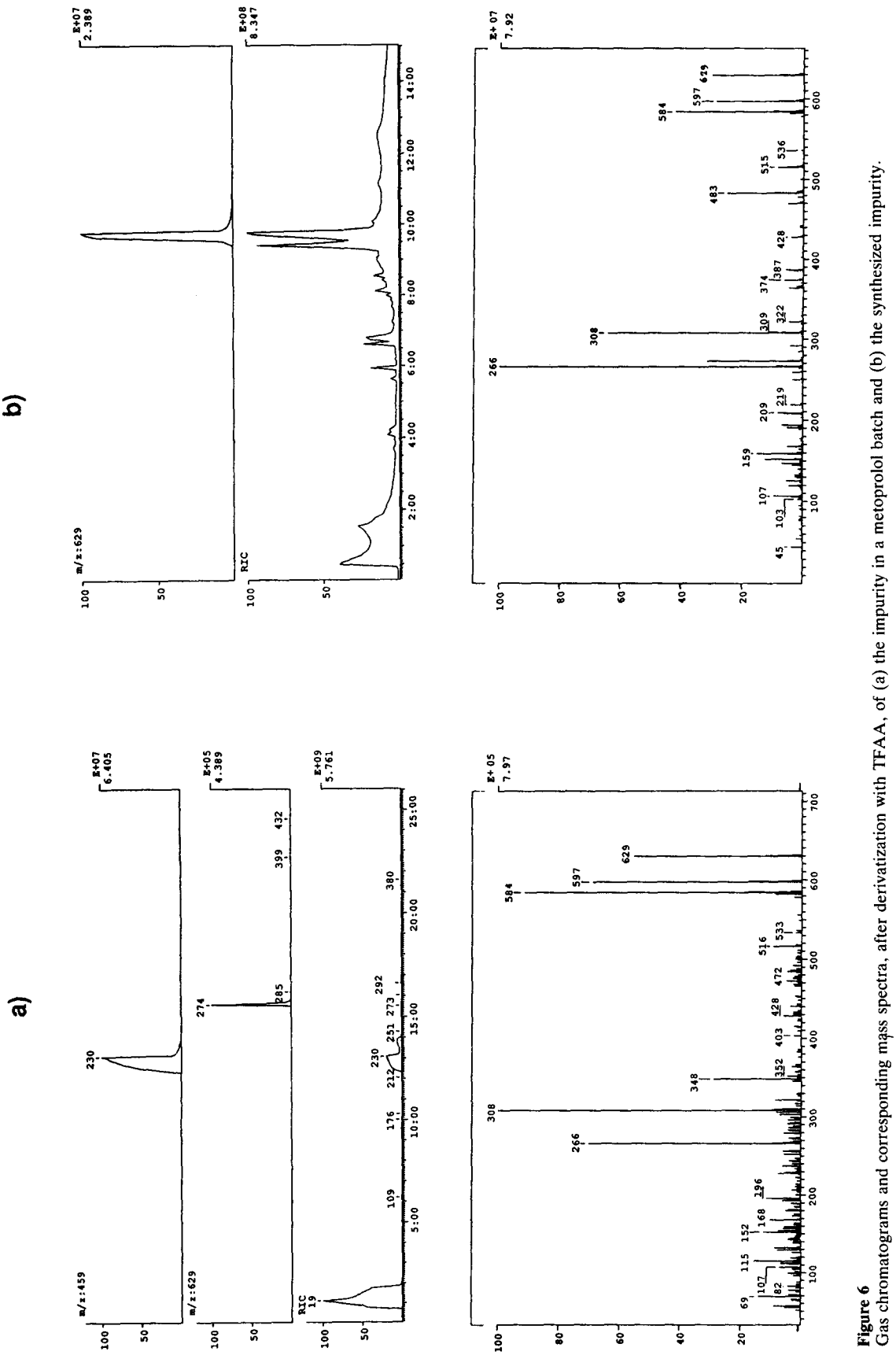

Figure 6
Gas chromatograms and corresponding mass spectra, after derivatization with TFAA, of (a) the impurity in a metoprolol batch and (b) the synthesized impurity.

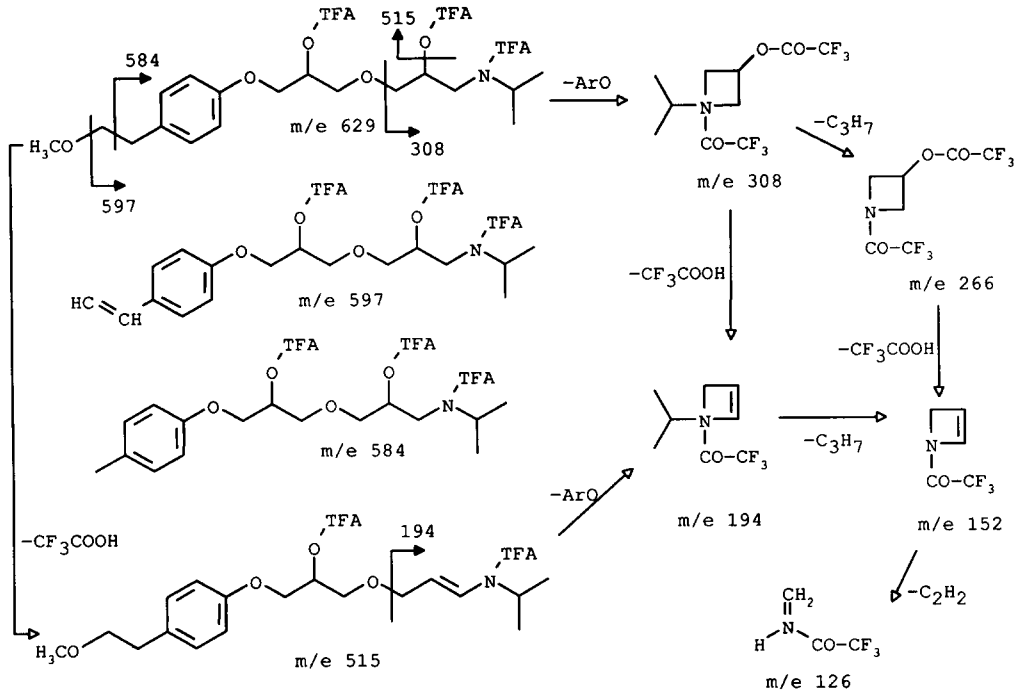

Figure 7
Proposed structures for some fragments in the GC–mass spectra. Ar = 4-$CH_3OCH_2CH_2C_6H_4$-.

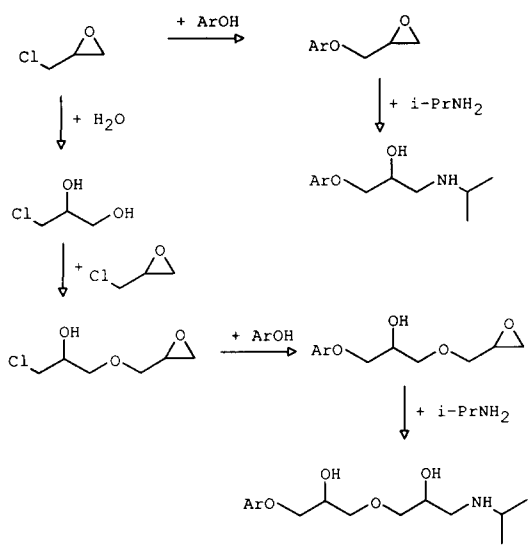

Figure 8
Possible route for formation of the impurity in the synthesis of metoprolol. Ar = 4-$CH_3OCH_2CH_2C_6H_4$-.

and as it did not separate in the earlier method it was clearly of interest to investigate if it had been present in older Astra-batches of metoprolol tartrate. Examination of current production batches from Astra revealed that this impurity is usually not detectable (<0.01%). In one batch, however, 0.04% was detected. Moreover, the impurity was not detected upon LC-analysis of batches used in toxicological and early clinical studies. This means that this impurity is not qualified in the spirit of the proposed ICH guideline on impurities.

A peak purity test with diode array detection would certainly not reveal the presence of this impurity as the chromophore is identical to that of metoprolol itself. A test of peak purity with MS detection might be more successful [3], although the molecular weight of the impurity might have to be known in advance in order to detect it at such low concentrations as 0.1%. Deliberately changing the chromatographic system to vary the selectivity of the separation, seems to us the best strategy to detect this type of related substances in a bulk drug substance.

Acknowledgement — We wish to thank Magnar Ervik for the help with running the GC–MS equipment.

References

[1] International Conference on Harmonisation, *Impurities in New Drug Substances*, **III/5442/94**, 3–14 (1994).
[2] J.B. Castledine, A.F. Fell, R. Modin and B. Sellberg, *J. Pharm. Biomed. Anal.* **9**, 619–624 (1991).

[3] D. Lincoln, A.F. Fell, N.H. Anderson and D. England, *J. Pharm. Biomed. Anal.* **10**, 837–844 (1992).
[4] K.-E. Karlsson, J. Chromatogr. **647**, 31–38 (1993).
[5] M. Ervik, K. Kylberg-Hanssen and L. Johansson, *J. Chromatogr.* **381**, 168–174 (1986).
[6] *Pharmeuropa* **5**, 298–300 (1993).
[7] D.A. Garteiz and T. Walle, *J. Pharm. Sci.* **61**, 1728–1731 (1972).

[*Received for review 13 October 1994*]