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Estimation of impurity profiles in drugs and related materials. Part 11 — the role of chromatographic and spectroscopic methods in the estimation of side-reactions in drug syntheses*†

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Abstract: Impurities in drugs are classified on the basis of the types of side-reactions in drug syntheses resulting in their formation. This is shown by summarizing the authors' earlier results in the field of impurity profiling of 19-nor-steroids, ethynodiol diacetate, mazipredone, pipecuronium bromide, flumecinol, enalapril, pyridinol carbamate, phenylbutazone, thymotrinan and some new results related to danazol and famotidine.

Keywords: *Impurity profiling; estimation of side-reactions; 19-nor-steroids; flumecinol; danazol; famotidine; pipecuronium bromide.*

Introduction

The estimation of impurity profiles of drug substances is one of the most important fields of activity in contemporary pharmaceutical analysis. The primary aim of impurity profiling, i.e. separation, identification or structure elucidation and quantification of all impurities present in excess of 0.1%, is to increase the safety of drug therapy by controlling the biological potency and toxicity of the impurities. The limit of 0.1% is in accordance with the general requirements of drug registration authorities. In addition, the information obtained from impurity profiling studies is of immense importance for synthetic organic chemists. On this basis, various types of side-reactions can be detected and with the aid of this information in the course of the optimization of the reaction conditions, steps can be taken in order to avoid such reactions or at least minimize their effect; thus the yield of the reaction and the quality of the product can be enhanced.

The aim of this paper is to characterize the impurities in drugs with emphasis on the products of various types of side-reactions. Examples are presented from experience accumulated in the authors' laboratory in the field of impurity profiling of drugs.

Experimental

Instruments

High-performance liquid chromatography. A Hewlett–Packard 1090A Series II chromatograph equipped with a built-in HP 1040 diode-array UV detector was used.

Semi-preparative HPLC. An ISCO Model 2350 pump was used and was attached to a model 7125 Rheodyne injector and a ISCO Model V4 variable wavelength UV detector.

Gas chromatography. A Hewlett–Packard 5890 instrument equipped with FID was used. (The columns and chromatographic conditions are specified in refs 3, 4, 9, 10, 15 and 16.)

Gas chromatography — mass spectrometry. Two coupled instruments were used: a Kratos MS-80 mass spectrometer equipped with a Carlo Erba 4200 gas chromatograph (impurity profiling of 5α-androst-2-ene-17-one); and a

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VG Trio-2 mass spectrometer equipped with a Hewlett–Packard 5890 gas chromatograph (all other cases).

Thin-layer chromatography. Usually Kieselgel 60 F_{254} (Merck 5554) aluminium sheets (20 × 20 cm) were used. The samples were applied manually. Where necessary densitometric evaluation of the spots was carried out using an Opton KM-3 densitometer.

UV spectroscopy. A Varian DMS-200 double-beam instrument was used for the spectra of the main components and the isolated impurities.

IR spectroscopy. A Nicolet 20DXC FT instrument was used with the potassium bromide disc technique.

Mass spectroscopy. Of the above two instruments, the Kratos MS-80 was used for the FAB spectra and some of the EI–CI spectra whereas the VG Trio-2 instrument was used for the other EI–CI spectra of the isolated impurities.

NMR spectroscopy. A Varian VXR-300 instrument was used.

Detection, isolation and identification of the impurities

The general methodology of impurity profiling in the authors' laboratory has been described earlier [2]; details are given in other references in this paper. Further data can be found under subsections (f) and (g) in the subsequent section.

Types of Impurities in Drugs

(a) *'Trivial' impurities*

These are the impurities which are characterized in drug master files and other registration documents as 'probable' or 'expected' impurities. These are on the one hand the unreacted last intermediate of the synthesis, e.g. propiophenone in the synthesis of flumecinol (see Fig. 1) [1a, 3, 4], prednisolone-21-mesylate in the synthesis of mazipredone (see Fig. 2) [5] or partially reacted derivatives if the final step of the synthesis involves the introduction of two or more groups into the molecule, e.g. ethynodiol-3-acetate in ethynodiol diacetate [6, 7], unethinylated 17-keto steroids in 17α-ethinyl-17-hydroxy steroids [8, 9]. On the other hand some impurities fall into the category that originates from a well known (e.g. isomeric) impurity of the starting material which undergoes the same reactions as the main component leading to formation of the impurity in question, e.g. the 4-trifluoromethyl analogue of flumecinol originating from an isomeric impurity (4-trifluoromethylbromobenzene) in the starting material (3-trifluoromethylbromobenzene) (see Fig. 1) [1a, 3, 4].

(b) *Impurities originating from the solvent of the reaction*

In some cases the solvent of a reaction can undergo a side-reaction with one of the reactants leading to an impurity. For example, in the course of the synthesis of pipecuronium bromide (2β,16β-bis-(4-dimethylpiperazino)-3α,17β-diacetoxy-5α-androstane dibromide) one of the key steps is the catalytic elimination of methanesulphonic acid from 3β-hydroxy-5α-androstane-17-one methanesulphonate leading to 5α-androst-2-ene-17-one. In addition to several (mainly isomeric) impurities, 3β-phenyl-5α-androstane-17-one was found as an impurity in the latter substance if the catalysts for the elimination were silica and aluminium chloride and the solvent mixture contained benzene (Friedel–Crafts type side-reaction with the solvent) [10, 11].

More typical are the reactions of the impurities of the solvents leading to unexpected impurities. An example is the reaction of the Grignard reagent in the synthesis of flumecinol (see Fig. 1) with 2-hydroxytetrahydrofuran (cyclic acetal of γ-hydroxybutyraldehyde, a common impurity in tetrahydrofuran which is used as the solvent for the Grignard reaction) [1a, 3, 4].

Drug syntheses often start with the Friedel–Crafts reaction of benzene with various acyl chlorides or anhydrides. In such cases benzene is not only the reaction partner but is used in great excess to serve also as the solvent of the reaction. For this reason even its minor impurities (e.g. toluene) can undergo the Friedel–Crafts reaction (with increased reactivity) thus leading to methylated analogues as impurities. This kind of impurity (3-trifluoromethyl-4'-methyl-α-ethylbenzhydrol) was found by capillary gas chromatography [3] and HPLC [1a] in flumecinol where the origin of this impurity is the Friedel–Crafts step of the synthesis (see Fig. 1). The reason for the presence of the 4-

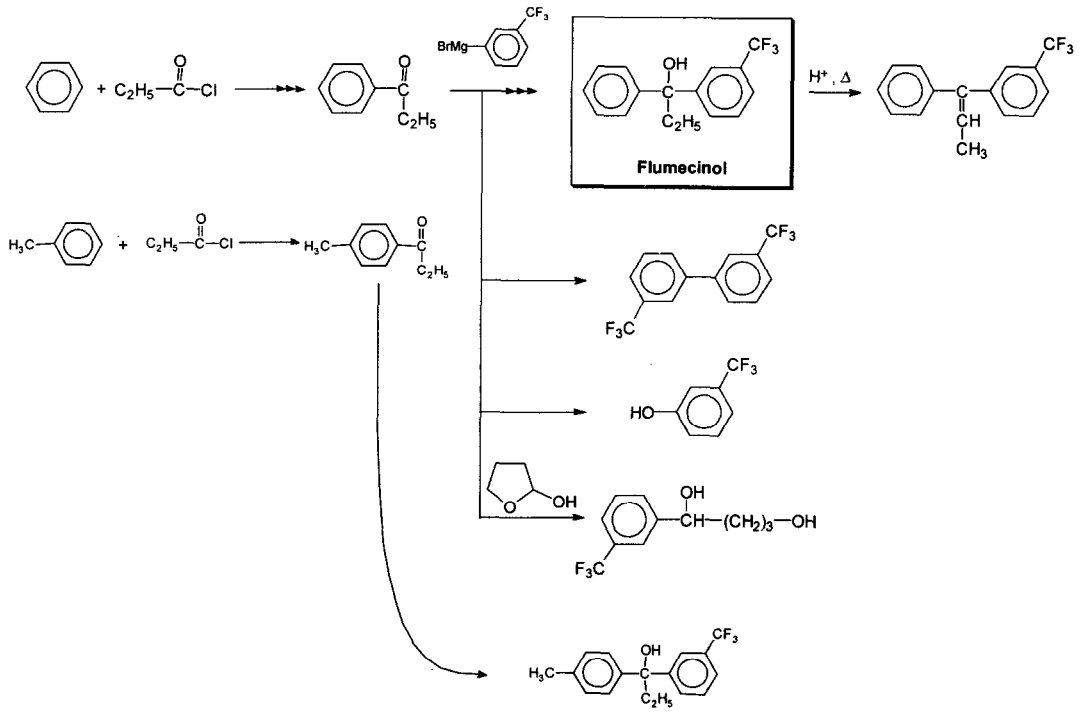


Figure 1
Formation of flumecinol and its impurities.

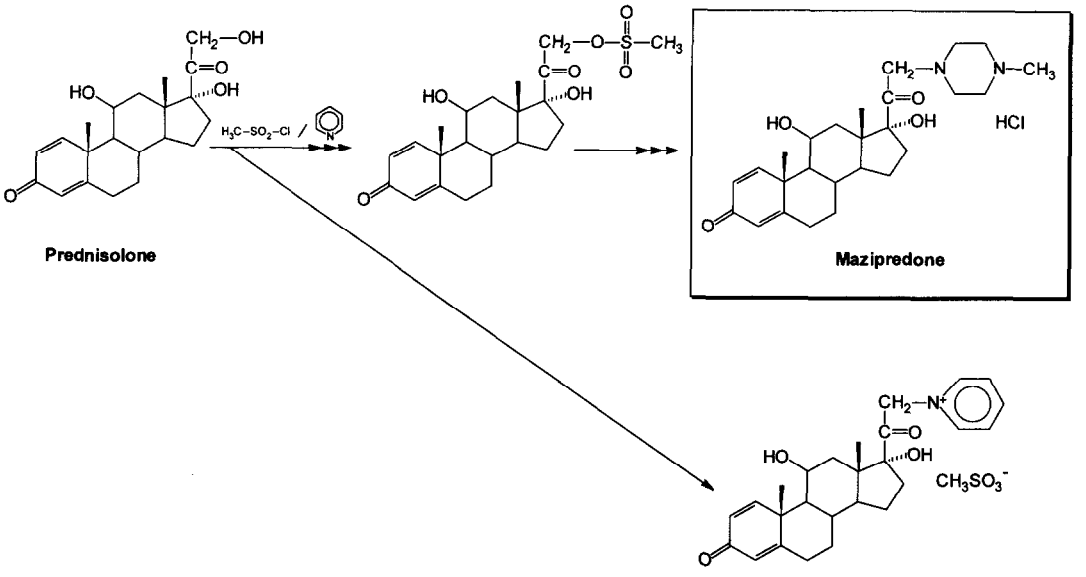


Figure 2
Formation of mazipredone and an impurity.

methylphenyl analogue as an impurity in some of the batches of crude enalapril is the same. The β-phenylethyl moiety in the molecule of enalapril is introduced by using 3-carboxy-1-phenyl-2-buten-1-one as the reagent. This is prepared by the Friedel–Crafts reaction from maleic anhydride and benzene. The presence of the toluene impurity in the latter leads to the above-mentioned impurity in enalapril [12].

(c) *Impurities originating from the catalyst*

Use of homogeneous catalysts may lead to the formation of rarely occurring impurities in which the catalyst molecule is incorporated.

An example of this is the mesylation of prednisolone catalysed by pyridine in the course of the synthesis of mazipredone (see Fig. 2) where a side-product is the quaternary 21-pyridinium derivative of prednisolone [5]. It is interesting to note that after the detection of this impurity, possessing excellent properties for the spectrophotometric assay (ϵ_{414nm} in alkaline medium = 21000), this side-reaction could be made quantitative for the photometric determination of 21-chloro, bromo and sulphonyloxy corticosteroids [13].

(d) *Consequences of an over-reaction during synthesis*

Over-reaction in the course of the synthesis of drugs may lead to impurities in their intermediates which can be precursors of impurities in the final product. As seen in Fig. 3 this is the case in the synthesis of pyridinol carbamate where the hydroxylated impurity (detected and separated by HPLC and identified with the aid of complex application of all spectroscopic methods) originates from the over-chlorination of 2,6-lutidine (easily detectable by gas chromatography) [14, 15].

Another example is the over-reduction of the 4-ene-3-keto steroids in the course of the synthesis of ethynodiol diacetate. If in addition to the main reaction (reduction of the 3-keto group) the C=C double bond at position 4 is also partly saturated this leads to the appearance of a saturated impurity in the final product [16].

(e) *Consequences of an incomplete reaction during synthesis*

In an analogous manner to that described in the previous section, incomplete reaction during synthesis may lead to impurities in the final product. Examples can be taken from the total synthesis of 19-nor-steroids. The stepwise hydrogenation of the two double bonds in the key intermediate of their synthesis (13β-methyl or ethyl-3-methoxy-1,3,5(10),8,14-gonapentaen-17β-ol) is the source of various unsaturated impurities in the final products. For example, after the saturation of the Δ^{14} double bond in the course of the isolation of the intermediate, the $\Delta^{8(9)}$ double bond easily isomerizes to the $\Delta^{9(11)}$ position and this impurity in the intermediate is the precursor of the 9(11)-dehydro impurities in ethinyloestradiol [5, 8] and mestranol [4].

If, however, a 1,4-type addition of hydrogen takes place as a side-reaction in the first hydrogenation step, a 8(14) double bond is formed which is the precursor of 8(14)-dehydronorgestrel (13-C_2H_5) and norethisterone (13-CH_3) [8].

(f) *Products of real side-reactions in the final step of the synthesis*

Side-reactions in the last step of drug synthesis may include, for example, the formation of epimeric impurities. Figure 4 shows that in the course of the ethinylation of 17-keto steroids, to obtain a variety of important 17α-ethinyl-17β-hydroxy steroidal drugs, small quantities of the epimeric 17β-ethinyl-17α-hydroxy derivatives are also formed [4, 8, 9]. Another example of the formation of epimeric impurities is the incompletely stereospecific reduction of the 3-keto group in the last but one step in the synthesis of ethynodiol diacetate leading to the 3α-acetoxy analogue

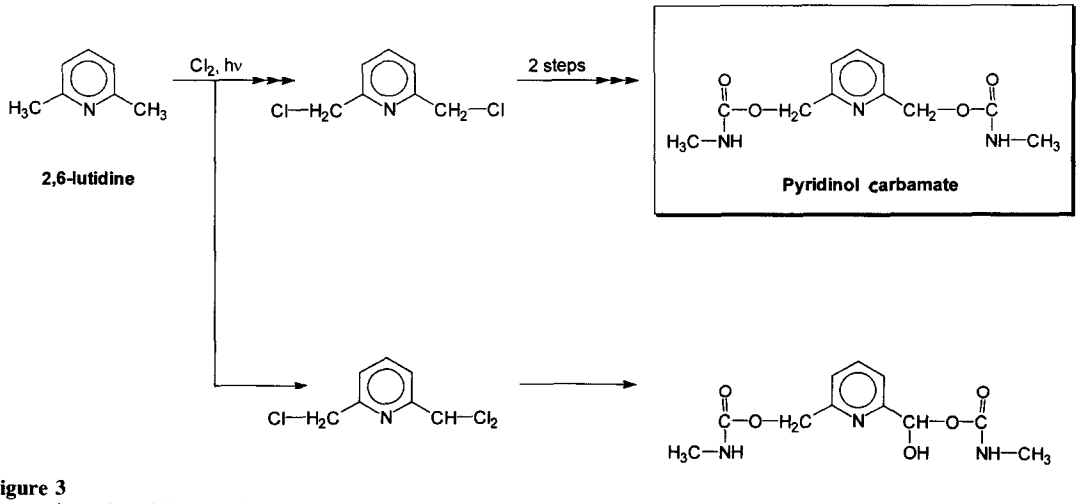


Figure 3
Formation of pyridinol carbamate and the origin of its impurity.

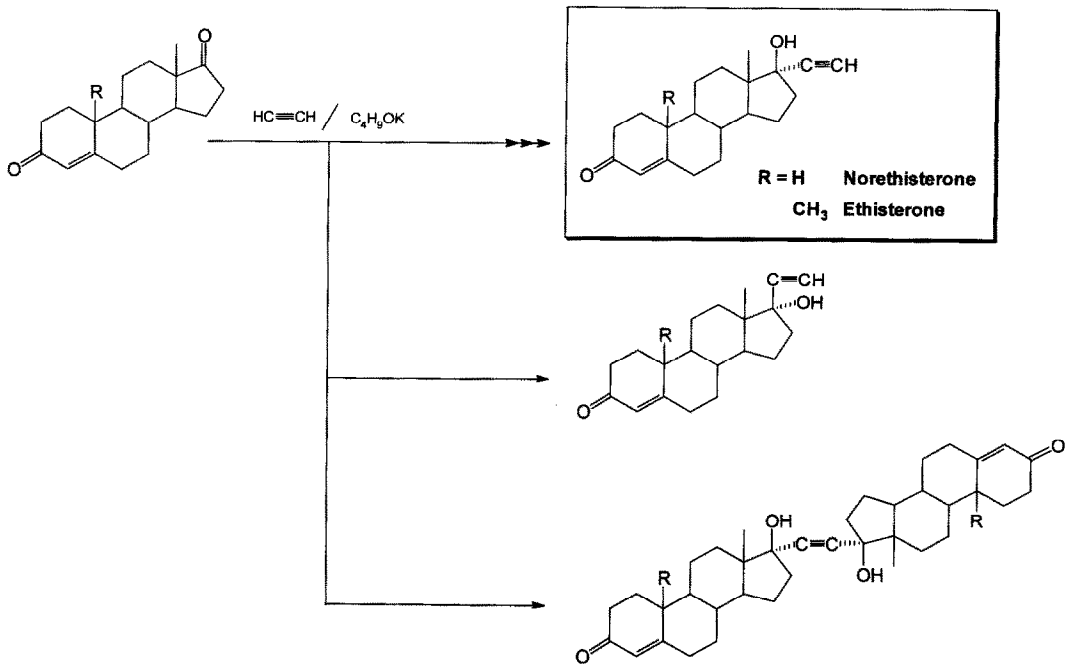


Figure 4
Ethinylation of 17-keto steroids with two side-reactions.

which can be easily separated and measured by HPLC [6, 17], this measurement being recently specified by the USP XXII [18].

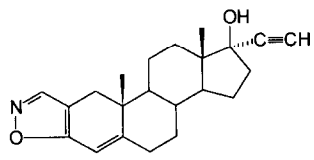
Dimerization is also a typical side-reaction in drug synthesis. As seen in Fig. 4 this reaction also takes place (in addition to the formation of epimeric derivatives) in the course of the ethinylation of 17-ketosteroids as found in the course of the impurity profiling of ethisterone, norethisterone and the acetate of the latter [8, 9]. The basis of the formation of this impurity is the reaction of the ethinyl steroids where the acetylenic hydrogen is replaced by an alkali metal atom with another molecule of the 17-keto steroid. As shown in Fig. 1 the dimerization of the Grignard reagent also leads to an impurity in the synthesis of flumecinol [1, 3, 4].

The formation of a positional isomer was found in the course of the impurity profiling of danazol. The reaction starts from ethisterone, and the isoxazole ring is formed by reacting it with ethyl formate and hydroxylamine. In addition to unreacted ethisterone (trivial impurity) another impurity was found in the HPLC tests at a retention time of 11.5 min (main component 15.9 min) (column: 10-μm LiChrosorb RP-18; 250 × 4 mm i.d.; eluent: methanol–water (7:3, v/v) at 1 ml min^{-1}; λ = 240, 254, 284 nm). The diode-array UV spectrum (band width 2 nm) showed a characteristic difference from that of danazol (λ_{max}: 254 and 286 nm, respectively). Comparison of the mass spectra of danazol and the impurity indicated identical molecular weights and rings B, C and D. The exact structure of the isomeric impurity was based on the comparison of the NMR spectra taken on a sample isolated by semi-preparative HPLC (Davisil Silica column, 250 × 21.2 mm i.d., hexane–2-propanol–tetrahydrofuran (95.5:4:0.5, v/v/v) with those of danazol (Hetcor, Cosy, Ineptl, homo-Noe spectra). The final proof was the synthesis of the impurity with the proposed structure and retention matching. The structures of danazol and isodanazol are shown in Scheme 1.

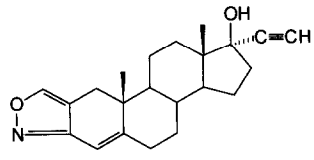
Further examples of side-reactions in the final steps of drug synthesis are the formation of the *Z* and *E* isomers of 17α-ethinyl-4-oestrene-3β,17-diol-3-acetate-17-(3′-acetoxy-2′-butenoate) as impurities forming during the final acetylation step in the synthesis of ethynodiol diacetate [7, 12] and the formation of 3-trifluoromethylphenol during the synthesis of flumecinol [1, 3] (see Fig. 1).

(g) *Products of the transformation of drugs during their preparation or isolation*

After their formation some drug molecules can further react with the excess of the reagent, another reaction product, the solvent, atmos-

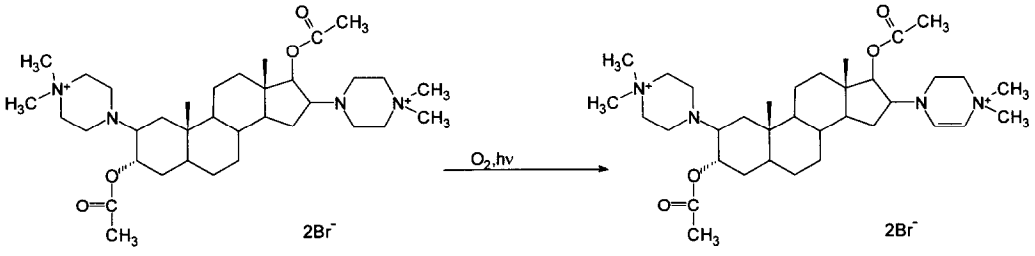


Danazol



Isodanazol

Scheme 1



Pipecuronium bromide

Figure 5
Pipecuronium bromide and its dehydrogenation reaction.

pheric oxygen, etc., leading to further impurities. A well known example of the last type is phenylbutazone which is very sensitive to oxidation and the formation of its 4-hydroxy derivative may occur during the isolation from the reaction mixture. It is worth mentioning that the autoxidation may also take place on the TLC plate thus causing uncertainties in the detection of this impurity; HPLC, however, under anaerobic conditions is an ideal tool for the separation, detection and quantitation of 4-hydroxy-phenylbutazone in phenylbutazone [19].

Another example of the formation of an oxidation (dehydrogenation) product of a drug during the synthesis or isolation (drying, etc.) is the formation of the impurity shown in Fig. 5. The separation, structure elucidation and quantification of this impurity in pipecuronium bromide required the complex application of several chromatographic and spectroscopic techniques [12, 20].

As seen in Fig. 1, one of the impurities of flumecinol: its dehydration product, also belongs to this group [1a, 3, 4].

The change of the configuration at (one of) the chiral centre(s) of drug molecules during the synthesis often leads to enantiomeric (or diastereomeric) impurities. As an example, the case of thymotrinan (L-Arg-L-Lys-L-Asp) is mentioned where the detection and identification of an impurity (L-Arg-L-Lys-D-Asp) revealed the formation of a diastereomeric impurity during the synthesis [21, 22]. Although the presence of enantiomeric impurities in chiral drugs is usually not the consequence of side-reactions but that of incomplete separation of the enantiomers; for this reason their detection does not belong to the topic of this paper but it is still noteworthy that chiral HPLC has proved to be suitable among others for the determination of the optical purity of (+)- and (−)-flumecinol [1a].

The final example has been taken from the impurity profiling of famotidine. Figure 6 shows the reaction used at the Chemical Works of Gedeon Richter, Budapest, Hungary for the preparation of this drug [23]. The impurity in question was detected by HPLC using a 250 × 4 mm i.d. column packed with LiChrosorb SI-100 and an eluent of chloroform–methanol–acetic acid–ammonium hydroxide (90:15:6:2.4, v/v/v/v) at 1 ml min^{-1}; λ = 287 nm. Retention times: impurity, 13 min; famotidine, 24 min. In the course of the TLC separation (benzene–ethyl acetate–methanol–water–ammonium hdyroxide, 50:25:20:4:1, v/v/v/v/v) the R_f values were: impurity, 0.70; famotidine, 0.57.

The diode-array UV spectra of famotidine and the impurity were similar, indicating that the differences do not affect the thiazole ring and its environment. The same was found from the mass spectrum and NMR spectra taken on

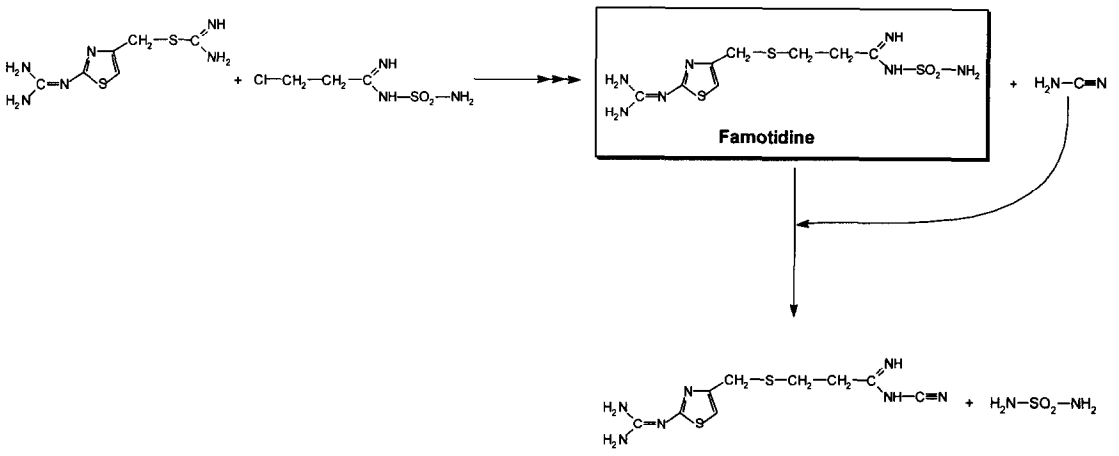


Figure 6
The final step of the synthesis of famotidine with the formation of an impurity.

a sample isolated by semi-preparative HPLC (column packing material and eluent as above using a 250 × 16 mm i.d. column). The molecular weight of 283 obtained from the FAB mass spectrum and the other spectroscopic data, especially the lack of the sulphamide bands in the IR spectrum and the appearance of the strong nh—C≡N band at 2176 cm^{-1} provide evidence for the structure given in Fig. 6.

It is also evident from the reaction scheme in Fig. 6 that the reason for the formation of this impurity is the reaction of famotidine with cyanamide which is also formed in the course of the reaction. This was proved by reacting famotidine with an excess of cyanamide; the impurity was obtained with good yield. The final evidence for the given structure was obtained by an indepedent synthesis of the impurity followed by retention matching.

Conclusions

In the studies summarized in this paper the conclusions were drawn by the organic chemists in the group of workers rather than by the analytical chemists who carried out the investigations. From these conclusions, the side-reactions could be either eliminated or their extent reduced to a tolerable level, thus ensuring the level of the impurities to be below 0.1%.

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