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# Estimation of impurity profiles of drugs and related materials. Part 9: HPLC investigation of flumecinol*†

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**Abstract**: Two HPLC systems have been developed for the investigation of flumecinol (3-trifluoromethyl-α-ethyl-benzhydrol). The reversed-phase system (LiChrosorb RP-18; methanol–water, 7:3, v/v) enables the isolation and identification of the impurities. The chiral system (Chiralcel OD; hexane–2-propanol, 98:2, v/v) separates the enantiomers of flumecinol and its impurities. The potential of spectral convolution in peak identification in HPLC impurity profiling is demonstrated by an example involving the identification of the 4′-methyl-analogue of flumecinol.

**Keywords**: *Impurity profiling; HPLC; chiral separation; spectral convolution; flumecinol; 4′-methylflumecinol.*

## Introduction

The impurity profile of the enzyme-inducing agent, flumecinol (Chemical Works G. Richter Ltd, Budapest, Hungary) has been determined by packed column [2] and capillary [3] gas chromatography. This paper reports on the HPLC investigation of flumecinol with the aim of confirming and supplementing the gas chromatographic results. The chiral separation of the enantiomers of the drug and its impurities was also an objective of the study.

## Experimental

A Hewlett–Packard Model 1090A high-performance liquid chromatograph equipped with a Model 1040 diode-array UV detector was used. The columns were: LiChrosorb RP-18, 10 μm, 25 × 4 mm (Merck; packed by Bioseparation Technologies, Budapest) for reversed-phase separation, (mobile phase: methanol–water, 7:3, v/v, 1 ml $min^{-1}$) and Chiralcel OD, 250 × 4.6 mm (Daicel Chemical Industries Ltd) for chiral separation, (mobile phase: *n*-hexane–2-propanol, 98:2, v/v, 1 ml $min^{-1}$). The detection wavelength was 210 nm. Injected amount: 20 μl from a 0.1% solution in the eluent. Chromatography was performed at ambient temperature.

A Varian DMS-200 UV spectrophotometer was used to record the spectra.

## Results and Discussion

*Impurity profiling using reversed-phase HPLC*

In the course of impurity profiling of flumecinol by packed column [2] and capillary [3] gas chromatography, the following impurities were separated, identified and quantified: propiophenone, 3- and 4-trifluoromethylphenol, 3,3′-bis-trifluoromethylbiphenyl, 4-trifluoromethyl-α-ethyl-benzhydrol, 3-trifluoromethyl-4′-methyl-α-ethyl-benzhydrol, 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol, 1-(3-trifluoromethyl-phenyl)-1-phenyl-propene-1.

In accordance with the general rules of impurity profiling the use of another chromatographic system with an entirely different separation mechanism was also investigated with the aim of confirming the results obtained by gas chromatography. Reversed-phase HPLC was found to be an ideal technique for this purpose. The chromatogram of a flumecinol sample is shown in Fig. 1. In this sample, the majority of the impurities listed above (and also 3-trifluoro-methylaniline which was not found by gas chromatography) could be detected. The identification of one of the impurities, 3-trifluoromethyl-phenyl-4′-

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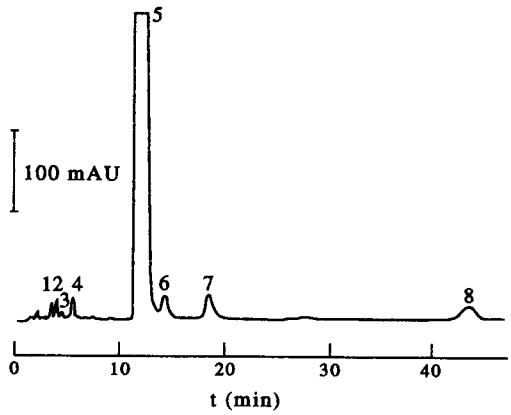


**Figure 1**
Reversed-phase HPLC separation of the impurities of flumecinol. See Experimental for details. 1: 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol; 2: 3-trifluoromethyl-aniline; 3: 3-trifluoromethyl-phenol; 4: propiophenone; 5: flumecinol (3-trifluoromethyl-α-ethyl-benzhydrol); 6: 4-trifluoromethyl-α-ethyl-benzhydrol (quantity: 0.11%); 7: 3-trifluoromethyl-4′-methyl-α-ethyl-benzhydrol (4′-methylflumencinol); and 8: 1-(3-trifluoromethyl-phenyl)-1-phenyl-propene-1.

methyl-α-ethyl-benzhydrol (peak 7) will be the subject of the section.

The quantification of the impurities was not among the aims of this study since this could be done with satisfactory accuracy and precision by gas chromatography [3]. It is, however, evident from Fig. 1 that, in agreement with the gas chromatographic results, the overall impurity level of the sample of flumecinol investigated is below 0.5% (impurities 2, 3, 4 and 8 spectrophotometrically are highly active compounds which would be greatly overestimated by the HPLC–area normalization method). For the sake of comparison, the quantity of impurity 6 (determined by gas chromatography) is presented in the legend of Fig. 1.

*The use of spectral convolution in the identification of one of the impurities*

The general method for the estimation of impurity profiles by HPLC starts with the visual examination of the chromatogram and the diode-array UV spectra. This is followed by recording as many other types of spectra of the separated substances as necessary (e.g. MS, IR, NMR) for the identification of the impurities. The final step is retention matching with authentic standards selected (or synthesized) on the basis of the information obtained from the above mentioned spectra [4]. In advantageous cases in which the UV spectrum is characteristic enough, it can furnish sufficient information to allow the proposed substance to be synthesized for the purpose of retention matching [2, 5, 6]. The situation is similar (but somewhat easier) when information is available about the structures of the impurities from independent GC–MS investigations. In such instances, it is necessary to determine the HPLC and GC peaks that correspond to each other.

The synthesis of the proposed impurity is often more difficult than that of the main component itself and recording the spectra mentioned above usually requires semi-preparative HPLC separation of the impurity. It is therefore essential to obtain as much information from the diode-array UV spectra as possible (comparison with the spectra of compounds having the same chromophoric system, etc.). If such compounds are not available, other solutions to the problem may be required. As an example, the usefulness of comparing the diode-array UV spectrum of one of the impurities of flumecinol with a convoluted spectrum will be demonstrated.

The problem to be solved was the identification of the HPLC peak of 3-trifluoromethyl-4′-methyl-α-ethyl-benzhydrol (the 4′-methyl analogue of flumecinol) in the chromatogram of flumecinol. This structure was proposed on the basis of the GC–MS investigation [3] carried out simultaneously with the HPLC study. Authentic standards of all the other impurities identified by GC–MS were available and hence their identification in the HPLC chromatogram could easily be made. There was only one significant, unidentified peak in the HPLC chromatogram (peak no. 7). Because of the lack of a reference standard of 4′-methylflumecinol, two possibilities had to be considered: either this peak corresponds to 4′-methylflumecinol or to another impurity not detected by GC–MS.

The molecule of flumecinol contains two non-conjugated benzene rings without chromophoric groups. In an earlier study, methyl substitution on an unsubstituted phenyl moiety in the molecule of enalapril caused a considerable bathochromic shift (from 258 to 264 nm) and this was of diagnostic value in the identification of the methyl substituted impurity in enalapril [1]. On the basis of this, a similar shift was expected in the case of the methyl substitution of flumecinol. This supposition was supported by the bathochromic shift of 5 nm in the spectrum of 1-(4-methyl-

phenyl)-propan-1-ol as compared to that of 1-phenyl-propan-1-ol (of 4′-methylflumecinol and flumecinol, respectively). The spectra are shown as curves C and B in Figs 3 and 2.

After obtaining these encouraging data, it was disappointing to compare the diode-array UV spectra of flumecinol and the peak in question (peak no. 7). As shown in Fig. 4, the positions of the bands in the two spectra are almost entirely identical.

Since, in addition to the above mentioned phenyl- and methylphenyl-carbinols, another compound containing the 3-trifluoromethyl-substituted benzene ring, 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol was available (spectrum: curves A in Figs 2 and 3) it was possible to convolute the spectra of flumecinol and its 4′-methyl homologue. As shown in Fig. 2, the convoluted spectrum of flumecinol (A + B) is in good agreement with the recorded spectrum.

Figure 3 shows the convolution of the spectrum of 4′-methylflumecinol. The comparison of the convoluted spectrum with those of flumecinol in Fig. 2 indicates that the position of the bands is again almost identical. This

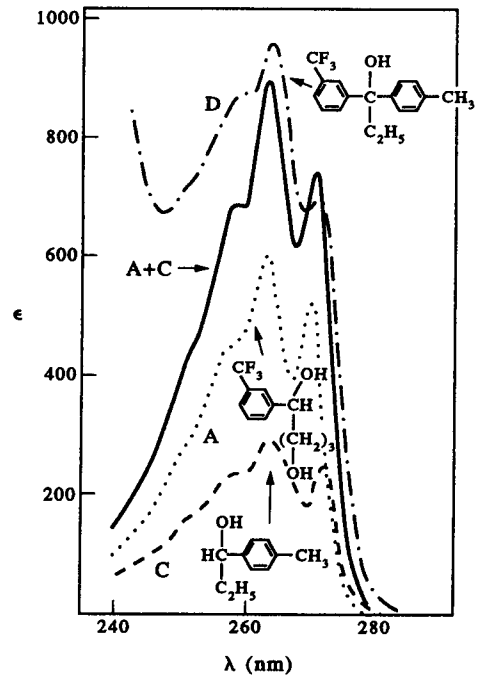


**Figure 3**
Convolution of the UV spectrum of 4′-methylflumecinol. Solvent: methanol. A: Recorded spectrum of 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol; C: recorded spectrum of 1-(4-methylphenyl)-propan-1-ol; A + C: convoluted spectrum of 4′-methylflumecinol; D: recorded spectrum of 4′-methylflumecinol.

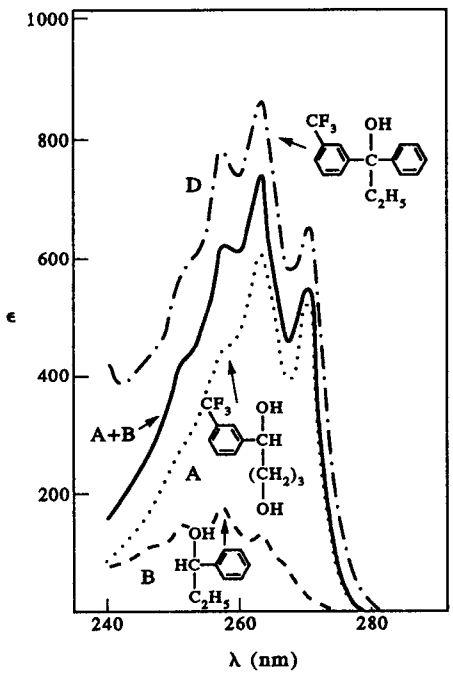


**Figure 2**
Convolution of the UV spectrum of flumecinol. Solvent: methanol. A: Recorded spectrum of 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol; B: recorded spectrum of 1-phenyl-propan-1-ol; A + B: convoluted spectrum of flumecinol; and D: recorded spectrum of flumecinol.

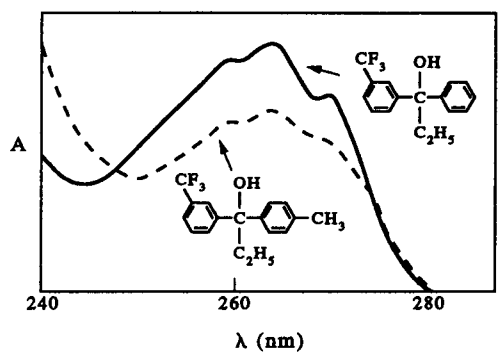


**Figure 4**
Diode-array UV spectra of flumecinol and 4′-methyl-flumecinol separated by reversed-phase HPLC.

means that the methyl substitutions causes a dramatic effect in the case of 1-phenyl-propan-1-ol but has practically no effect on the spectrum of flumecinol. The reason for this difference is the hyperchromic effect of the trifluoromethyl group. As shown in Figs 2 and 3, the contribution of the trifluoromethyl-substituted aromatic ring on the spectra of flumecinol and 4′-methylflumecinol is about 3- and 2-fold, respectively, compared to the

contribution of the unsubstituted and methyl-substituted aromatic ring.

On the basis of these considerations, the close similarity between the spectra of flumecinol and its methyl homologue cannot be regarded as a contradiction. This prompted the synthesis of 4′-methylflumecinol. As shown in Fig. 3, the agreement between the convoluted and recorded spectra is good. The retention matching of the synthesized impurity with peak 7 was successful and hence the identification of the impurity as 4′-methylflumecinol could be regarded as being proved.

The differences between the convoluted and recorded spectra in Figs 2 and 3 in the short wavelength region of the spectra deserves a short discussion. As shown, the position of the principal bands, and even the fine structures, are very close to each other. However, the position of the minimum between the low wavelength and high wavelength benzenoid bands and especially the absorbance at the minimum are different occurring at higher wavelength with higher intensity in the recorded spectra. The reason for this is the interaction of the two aromatic non-conjugated rings which are separated only by a single carbon atom. This interaction, which affects only the short wavelength benzenoid band, can easily be seen if the spectra of toluene and diphenylmethane are compared. The long wavelength benzenoid bands with their complicated fine structures are very similar: the principal band of toluene in methanol is at 261.5 nm ($\epsilon$ = 240). The same band in the spectrum of diphenylmethane is at the same wavelength ($\epsilon$ = 520; $\epsilon$/number of aromatic rings = 260). In contrast to this, the minimum between the benzenoid bands is at 228 nm in the spectrum of toluene ($\epsilon$ = 14) while the minimum in the spectrum of diphenylmethane appears at 238 nm ($\epsilon$ = 230).

*Chiral separation of the enantiomers of flumecinol and its impurities*

The separation of the enantiomers of several racemic α-ethyl-benzhydrol derivatives (among them flumecinol) using Chiral AGP column has been described by Görög and Herényi [7]. Even better separation can be achieved using the Chiralcel OD column containing cellulose tris-(3,5-dimethyl-phenylcarbamate) coated on macroporous silica and this has been successfully used for the determination of the enantiomers of flumecinol in biological samples

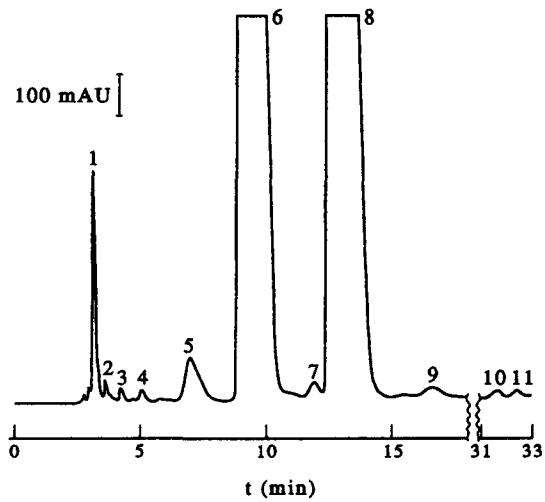


**Figure 5**
Chiral separation of the enantiomers and impurities of flumecinol. See Experimental for details. 1: 3,3′-bis-trifluoromethylbiphenyl; 2 and 3: *Z* and *E* isomers of 1-(3-trifluoromethyl-phenyl)-1-phenyl-propene-1; 4: propiophenone; 5: 3-trifluoromethyl-4′-methyl-α-ethyl-benzhydrol (4′-methylflumecinol); 6 and 8: (+) and (−)-flumecinol (3-trifluoromethyl-α-ethyl-benzhydrol); 7 and 9: (+) and (−)-4-trifluoromethyl-α-ethyl-benzhydrol; and 10 and 11: (+) and (−)-1-(3-trifluoromethyl-phenyl)-butan-1,4-diol.

(personal communication from Fujimoto Pharmaceutical Corporation, Osaka, Japan). The extremely high efficiency of this column enables the simultaneous separation of the enantiomers of flumecinol and the impurities. The chromatogram of a flumecinol sample is shown in Fig. 5.

In this system, which is suitable for both impurity profiling and chiral separation the enantiomers of 1-(3-trifluoromethyl-phenyl)-butan-1,4-diol, described in detail in ref. 2 and also mentioned in this paper (peak 1 in Fig. 1; curves A in Fig. 2 and 3) can also be separated if a stepped gradient system is used starting at 28 min by increasing the proportion of 2-propanol from the original 2 to 8% at a rate of 6% $min^{-1}$. The *Z* and *E* isomers of 1-(3-trifluoromethyl-phenyl)-1-phenyl-propene-1 are also separated.

It is interesting to note that, in contrast to the excellent separation of the enantiomers of flumecinol, its 4′-methyl analogue is eluted as a single peak even if its retention time is increased by decreasing the proportion of 2-propanol in the eluent. The reason for this is probably the lower degree of asymmetry in the molecule of the 4′-methyl derivative: it contains two monosubstituted phenyl rings while

flumecinol contains one bare and one mono-substituted ring.

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