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Estimation of impurity profiles in drugs and related materials*

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Abstract: The estimation of impurity profiles in drugs and related materials has been demonstrated using combinations of chromatographic and spectroscopic techniques. Examples chosen to illustrate this approach are: (1) an examination of flumecinol and its impurities using packed column GC–mass spectrometry; (2) the estimation of 17β-ethinyl-17α-hydroxy impurities in 17α-ethinyl-17β-hydroxy steroids using capillary GC; (3) the identification of impurities in hexoestrol using HPLC–linear photodiode array detection (LDA) and off-line mass spectrometry; (4) the estimation of 9(11)-dehydromestranol in mestranol using HPLC–LDA; and (5) the estimation of an *l*-threo impurity in a *d*-threo hydroxy acid intermediate using a chiral column containing covalently bonded bovine serum albumin.

Keywords: *Gas chromatography; high-performance liquid chromatography; enantiomeric separations; 17-ethinyl steroids, hexoestrol; mestranol; flumecinol; impurity profiles.*

Introduction

The estimation of impurity profiles in drug substances and related materials has become one of the most important fields of activity in contemporary pharmaceutical analysis. In general, all impurities present in excess of 0.1% should be identified, for the following reasons: (1) on the basis of the information thus obtained synthetic organic chemists are often able to avoid the formation of the impurity in question or to develop a purification method to decrease its quantity to a tolerable level. (2) Following the structural identification of an unavoidable impurity, it may be synthesized to provide a sufficient amount for: (a) final proof of its structure; (b) its use as an "impurity standard"; (c) its use in toxicological studies.

Recently, examples have been given to demonstrate the usefulness of high performance liquid chromatography (HPLC) equipped with linear photodiode array UV-detection (LDA) for the on-line estimation of impurity profiles [1] and also the off-line coupling of HPLC with various spectroscopic techniques such as infrared (IR), mass spectrometry (MS) and nuclear magnetic resonance (NMR) for the same purpose [1, 2, 3]. The estimation of enantiomeric purity of amino acid derivatives has also been described [4].

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In this paper further examples are presented to demonstrate the above approach, which has been extended by application of an on-line GC–MS technique.

Experimental

Apparatus

High-performance liquid chromatography. A Hewlett–Packard 1090 instrument equipped with a 1040 linear photodiode array UV-detector was used. The stationary phases were purchased from Merck (Darmstadt, F.R.G.) and the columns packed by Bio Separation Technologies (Budapest, Hungary) with the exception of the chiral separations which used a Macherey-Nagel (Düren, F.R.G.) Resolvosil column.

Gas chromatography. A Hewlett–Packard 7620 was used for packed columns, and a Hewlett–Packard 5890 instrument for capillary column separation. Both were equipped with flame ionisation detectors. A Kratos MS-80 instrument was used for GC–MS and a Varian XL-400 spectrometer for NMR studies.

Chemicals

The chemicals and solvents used in this study were of analytical reagent grade and were purchased from Aldrich (Beerse, Belgium) or Reanal (Budapest, Hungary).

Methods

For chromatographic details see text and figure legends in the Results and Discussion section.

The samples analysed were from the laboratories of the Chemical Works of Gedeon Richter Ltd. (Budapest, Hungary). Where appropriate, crude materials or mother liquor products were first investigated since the relative quantities of the impurities was much higher than in the final products.

Results and Discussion

Impurity profile of flumecinol (Zixoryn®) by GC–MS

Flumecinol (Zixoryn®) is a new enzyme-inducing agent manufactured by the Chemical Works of Gedeon Richter Ltd. (Budapest, Hungary) [5]. It is a liquid with boiling point of 140°C at 2 mm Hg and for this reason GC–MS is an ideal tool for the estimation of its impurity profile. Figure 1 shows a gas chromatogram of a crude sample of flumecinol together with an equation of the final step of its synthesis and the formation of its impurities. Peak 1 (propiophenone) is one of the *starting materials* of the Grignard reaction while peak 2 is the dimerisation product of the Grignard reagent. Peak 3 is the decomposition product of flumecinol (peak 5). Peak 6, the 4-trifluoromethyl isomer of flumecinol originates from the impurity 4-trifluoromethyl bromobenzene in the starting material 3-trifluoromethyl bromobenzene. Peak 7 is 1,4-dihydroxy-1-(3-trifluoromethyl-phenyl)-butane and originates from the impurity 2-hydroxytetrahydrofuran which is present in tetrahydrofuran, the solvent used in the Grignard reaction. Hence this analysis demonstrates that the formation of the latter two impurities can be avoided by selecting starting material and solvent of higher quality for the synthesis. Similarly, with the careful selection of the reaction and distillation conditions the formation of the decomposition product, peak 3, can be dramatically reduced [6].

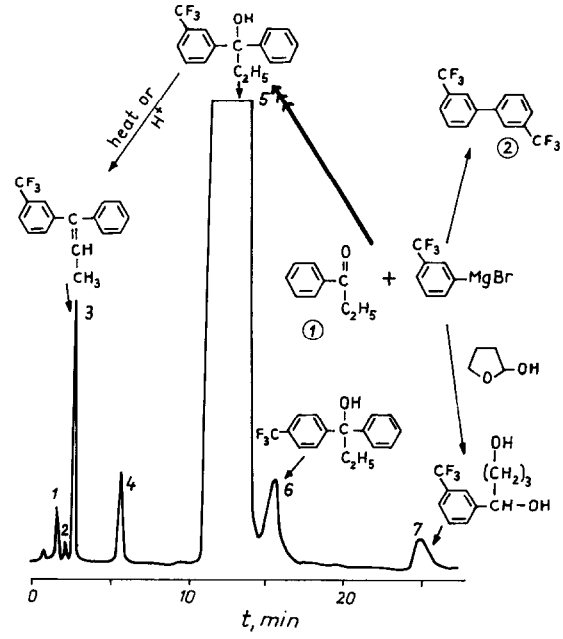

Figure 1
Gas chromatogram of a crude flumecinol sample. Key: 1. propiophenone; 2. 3,3'-bis-trifluoromethyl diphenyl; 3. 1-phenyl-1-(3-trifluoromethylphenyl)-propen-1; 4. 0.2% w/w internal standard (3-trifluoromethyl benzophenone); 5. flumecinol; 6. 4-trifluoromethyl isomer of flumecinol; 7. 1,4-dihydroxy-1-(3-trifluoromethylphenyl)-butane. 1.8 m × 4 mm glass column packed with 3% HIEFF-4BP on 80/100 mesh GasChrom Q. Column temperature 190°C, FID, injector 240°C.

Estimation of 17β-*ethinyl*-17α-*hydroxy impurities in* 17α-*ethinyl*-17β-*hydroxy steroids*

Capillary column gas chromotography can be used for the resolution of closely-related compounds and is a very useful technique to complement HPLC in the estimation of impurity profiles.

To illustrate this the determination of the epimeric 17β-ethinyl-17α-hydroxy steroid impurities in various 17α-ethinyl-androstane and estrane derivatives can be mentioned. The identification of this type of impurity in norethisterone by HPLC and spectroscopic techniques has been described in an earlier publication [1]. The separation of these epimeric impurities from the main components can be achieved by fused silica capillary gas chromatography and some examples are presented in Table 1. This method is suitable for the quantitative determination of each impurity down to the 0.1% level.

Impurity profile of hexoestrol by HPLC–LDA and other spectroscopic techniques

The on-line scan of UV spectra by HPLC–LDA has serious limitations when employed for the identification of impurities. In the majority of cases the spectra of the impurities are of value only if they show characteristic differences from that of the main component. However, even slight differences in spectra can be exploited. In the following example it is shown that in spite of the close similarity of the spectra the information obtained by HPLC–LDA is still of great value in the identification of impurities.

Table 1
Separation of the epimeric 17β-ethinyl-17α-hydroxy impurities from various 17α-ethinyl-17β-hydroxy steroid derivatives by capillary gas chromatography, 20 m long fused silica capillary column with 0.2 mm I.D. 0.33 μM film thickness; Ultra-2 Hewlett–Packard (crosslinked 5% phenyl-methyl silicone gum phase)

Main component	Retention time, min		Oven temperature	Retention time ratio
	17α-ethinyl	17β-ethinyl	°C	$r_{\beta,\alpha}$
17α-ethinyl-17β-hydroxy-4-oestrene-3-one (norethisterone)	18.05	18.67	240	1.034
17α-ethinyl-17β-hydroxy-4-androstene-3-one	18.93	19.57	250	1.034
17α-ethinyl-17β-hydroxy-4,9(11)-androstadiene-3-one	23.06	23.88	250	1.036
17α-ethinyl-2-androstene-17-ol	28.90	29.48	210	1.020

Figure 2 shows an HPLC chromatogram of a crude hexoestrol sample and Fig. 3 shows the spectra of the main component hexoestrol (peak 4) and of the impurities 1–3, as obtained by photodiode array detection. Although the spectra are very similar, the slight but characteristic differences which are present even with the moderate spectral resolution of LDA (spectral bandwidth 2 nm) are of diagnostic value in the identification of impurities 1 and 2.

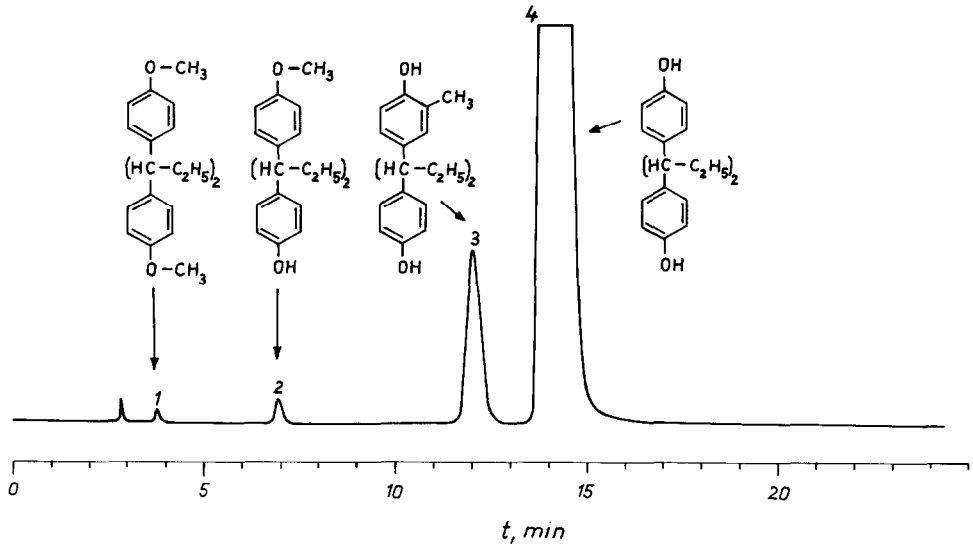

Figure 2
High-performance liquid chromatogram of a crude hexoestrol sample. Key: 1. hexoestrol dimethyl ether; 2. hexoestrol monomethyl ether; 3. 3-methyl-hexoestrol; 4. hexoestrol. Column 250 × 4 mm packed with 10-μM LiChrosorb SI-60; eluent, *n*-hexane–tetrahydrofuran (85:15, v/v) at a flow rate of 1 ml min^{-1}. Detector wavelength 280 nm.

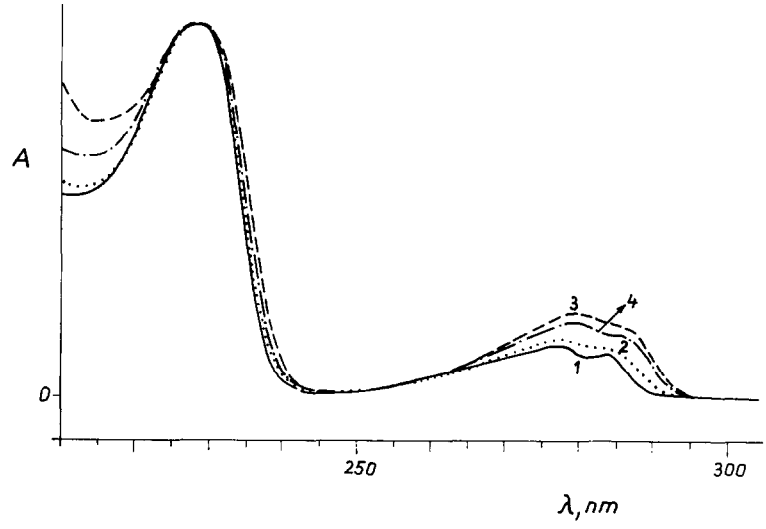

Figure 3
HPLC–LDA–UV spectra of the compounds separated in Fig. 2. For the key see Fig. 2.

In Fig. 3, the spectrum of hexoestrol has a maximum at 279 nm with a shoulder at about 286 nm due to its free phenolic chromophores. In the case of peak 1 the maximum is at 277 nm and the shoulder is transformed to a maximum at 284 nm. These changes are characteristic of the formation of phenol ethers and for this reason impurity 1 was considered to be the dimethyl ether of hexoestrol originating from the incomplete removal of the methyl groups from the phenolic hydroxyls at the end of the synthesis. The spectrum of peak 2 is intermediate between that of peaks 1 and 4, suggesting that it is the monomethyl ether of hexoestrol. These proposals have been substantiated by comparison of chromatographic retention for peaks 1 and 2 with impurity standards prepared on the basis of the preliminary information obtained by the HPLC–LDA–UV technique.

The identification of impurity 3 was a more delicate task. From the identity of its spectrum and that of the main component, it is evident that it has intact phenolic hydroxyls. The possibility that it may be a stereoisomeric impurity can be excluded. For this reason a few micrograms of impurity 3 collected by HPLC were investigated by mass spectrometry. A weak molecular ion peak at m/z 284 (M + 14) and strong fragment ions at 135 and 149 indicated that impurity 3 is hexoestrol methylated at the aromatic nucleus. To obtain further evidence for this and to estimate the position of the methyl group ^{1}H-NMR investigation was carried out. The high resolution instrument used in this study enabled all conclusions to be drawn from the spectrum of the crude material in deuterochloroform at 50°C without any preliminary separation of the impurity. The singlet at 2.24 ppm is that of the aromatic methyl group and from the signals of the aromatic protons (6.59 ppm dd J_o = 8 Hz, J_n = 3 Hz; 6.73 ppm d J_o = 8 Hz; 6.85 ppm dd J_n = 3 Hz) it was concluded that the methyl group is in the vicinity of the hydroxyl group.

Estimation of 9(11)*dehydro-mestranol in mestranol*

In a recent paper the isolation and identification of 9(11)dehydro ethinyloestradiol as the main impurity in ethinyloestradiol was described [1]. In this example, the main impurity of the methyl ether of ethinyloestradiol (mestranol) was similarly found to be the 9(11)dehydro derivative of mestranol.

This example demonstrates the usefulness of information based on the LDA–UV spectrum for the quantitative determination of the impurity. Figure 4 shows the HPLC chromatogram of a mestranol sample containing 0.5% of the 9(11)dehydro impurity scanned at 280 nm (maximum of mestranol) and at 261 nm (maximum of the impurity). Peak area linearity studies showed that injection of 20 μg of mestranol spiked with 0–2% of the 9(11)dehydro impurity produced a calibration slope that was 4 times higher at 261 nm than that at 280 nm. The intercept was negligible, with $r > 0.99$ in both cases.

Measurement at 261 nm favourably influenced both the precision and the sensitivity of the measurement. The relative standard deviation (RSD) of the measurement of 0.5% 9(11)dehydro mestranol in mestranol was 1.1% at 280 nm and 0.6% at 261 nm (n = 8) while the detection limits (S/N = 3) were 0.026% and 0.004%, respectively.

Estimation of optical purity using covalently bound protein as the stationary phase

The estimation of the optical purity of chiral drugs and the intermediates in their production is an important branch of impurity profiling. Several methods are available based on the formation of covalently bound diastereomeric derivatives, or adducts using

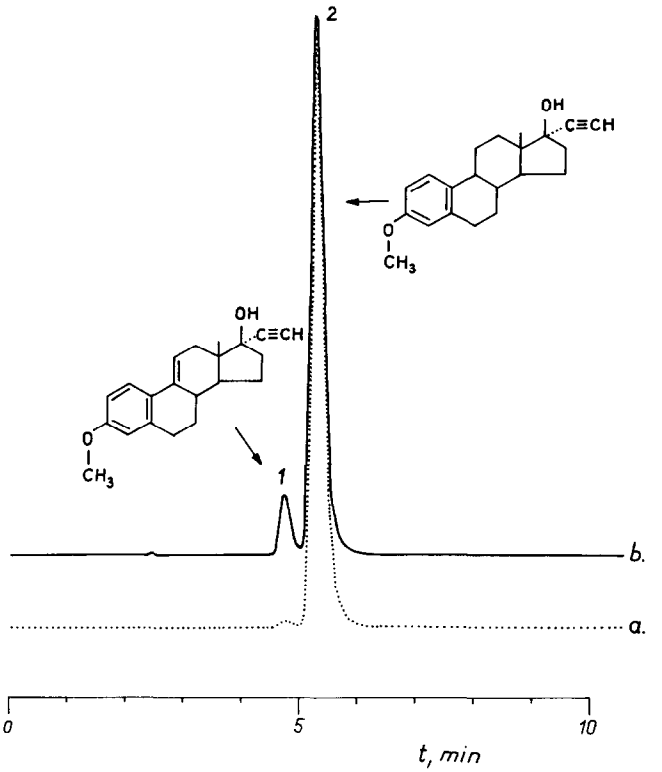

Figure 4
High-performance liquid chromatogram of mestranol (peak 2) containing 0.5% of 9,11-dehydro mestranol (peak 1) as the impurity. Key: curve a, chromatogram scanned at 280 nm; b, chromatogram scanned at 261 nm. Column: 250 × 4 mm packed with 10-μM LiChrosorb RP-18. Eluent: methanol–water (9:1, v/v) at a flow rate of 1 ml min^{-1}.

achiral stationary phases, or on the separation of the underivatized enantiomers using chiral stationary phases [7]. All these methods have their own advantages and disadvantages and none of them seem to be of general applicability, so that for each particular problem the most suitable method has to be found. Here we report on some preliminary experiences with the use of a column containing covalently bound protein (bovine serum albumin) as the chiral stationary phase.

It was found that in addition to the enantiomeric separation of several more or less polar compounds such as amino acids, their derivatives, sulphoxides, coumarin derivatives etc. achieved by Allenmark [8], this type of chiral stationary phase can be used with some degree of success for the enantiomeric separation of other materials including less polar compounds. For example in the case of flumecinol which is prepared and administered as the racemate, the separation of the enantiomers was acceptable for their detection ($\alpha = 1.23$), but the resolution was very poor ($R = 0.7$) precluding the possibility of using this method for quantitative purposes. The mobile phase was 0.1 M aqueous phosphate buffer, pH 5.9, *n*-propanol, (97:3, v/v).

In the following example, however, the resolution of the *d*-threo and *l*-threo forms of the hydroxyacid I was much better ($\alpha = 1.76$; $R = 2.0$) and hence this method was suitable for the determination of the *l*-threo impurity in the *d*-threo derivative down to

the 1% impurity level. The *d*-threo derivative is an intermediate in the synthesis of diltiazeme. Figure 5 shows the chromatogram of the 95:5 mixture of the *d*-threo and *l*-threo forms.

Conclusions

The estimation of impurity profiles in drugs and related materials can be approached in three general ways: (a) On-line method. On-line coupling of chromatographic separations with spectroscopic techniques provides the simplest and most powerful tool for the estimation of impurity profiles. Of the methods based on gas chromatography, GC–MS is most widespread, but the importance of GC–IR and GC–IR–MS is also increasing. The application of these methods, however, is restricted to those instances when the drug and its impurities are volatile enough for gas chromatography without derivatization as demonstrated in this paper by the examples of flumecinol and 17-ethinyl steroids. HPLC–MS will certainly be an even more powerful method with wider applicability, but in the present state of its development, this technique is not generally used for impurity profiling. HPLC–LDA–UV is restricted to those cases where the UV spectra of the impurities and those of the main component show characteristic differences as demonstrated with mestranol and hexoestrol. Even in these examples however, this method is only a very powerful first screening method.

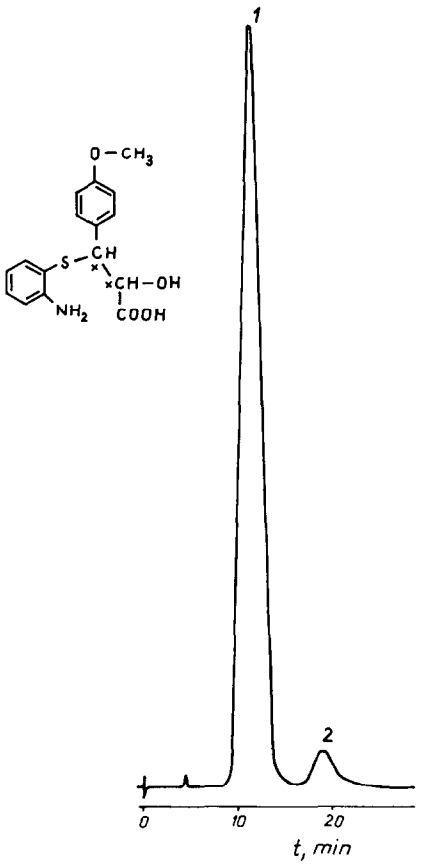

Figure 5
High performance liquid chromatogram of the *d*-threo form of the hydroxyacid I (peak 1) spiked with 5% of the *l*-threo form (peak 2). Column: 150 × 4 mm packed with Resolvosil. Eluent: 0.1 M aqueous phosphate buffer, pH 6.8, *n*-propanol (97:3, v/v); 1 ml min^{-1}. λ = 234 nm.

(b) Separation on the macro scale by preparative HPLC or TLC, isolation of the individual impurities and determination of their structure by appropriate spectroscopic techniques. This produces excellent results but is time-consuming and labour intensive.

(c) A hybrid method where the on-line HPLC–LDA–UV method is combined with chromatographic retention matching with impurity standards prepared on the basis of the preliminary information obtained from the LDA–UV data followed, if necessary, by off-line MS, NMR and IR investigation, as shown in the case of hexoestrol.

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