Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1527–1533, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

The changing rôle of ultraviolet spectroscopy in drug analysis*

S. GÖRÖG,† M. RÉNYEI and B. HERÉNYI

Chemical Works of Gedeon Richter Ltd, P.O. Box 27, H-1475 Budapest, Hungary

Abstract: New applications in the identification of minor components in drugs by rapid scanning diode-array UV spectrophotometers as high-performance liquid chromatographic (HPLC) detectors are exemplified by (a) identification and quantification of α-chloro-4-methoxycinnamic acid methyl ester as the byproduct of the Darzens reaction between 4-methoxybenzaldehyde and chloroacetic acid methyl ester; and (b) identification of 4-androsten-17-one and 3β-phenyl-5α-androstan-17-one as impurities in 5α-androst-2-en-17-one.

The advantages of the use of derivative spectrophotometry are illustrated by the following examples: (a) determination of flumecinol (3-trifluoromethyl-α-ethylbenzhydrol) in an oily emulsion formulation; (b) determination of RGH-6148 (2-benzylthiazolidinone) in a suspension used in toxicological studies; and (c) determination of mestranol as an impurity in norethisterone.

Keywords: *Ultraviolet-visible spectrophotometry; diode-array UV detector; Darzens reaction; 5α-androstan-17-ones; derivative spectrophotometry; flumecinol; norethisterone; mestranol.*

Introduction

Ultraviolet-visible (UV–vis) spectroscopy has always had a dual rôle in drug analysis:
(1) qualitative analysis (structure elucidation of potential drugs of natural or synthetic origin, identification of impurities, degradation products, metabolites etc.);
(2) Quantitative analysis (purity check of bulk pharmaceuticals, assay of drugs in formulations, biological samples, etc.).

As a consequence of the development of highly sophisticated spectroscopic (NMR, MS) and diffractometric techniques in the last decades the importance of UV spectroscopy in qualitative drug analysis has greatly decreased. At the same time, however, the increased use of rapid-scanning diode-array UV detectors in high-performance liquid chromatography (HPLC) with their capabilities to scan the spectrum within a few milliseconds has created an entirely new situation in this field. This technique is extremely useful in the rapid, online identification of minor components in drugs after HPLC separation. After the successful application of this technique in the

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
†To whom correspondence should be addressed.

authors' laboratory, mainly for the identification of impurities in synthetic steroids [1–3], in this paper further examples are given with the emphasis on side reactions in drug syntheses.

The widespread use of quantitative chromatographic methods has also caused the importance of UV–vis spectrophotometry in the classical sense to decrease in the field of quantitative drug analysis. On the other hand, however, several new developments such as the use of UV spectrophotometers as detectors in HPLC, autoanalysers, flow injection analysers, computer-based, difference and derivative techniques, etc. have become available for the drug analysts and as a result of these, spectrophotometry is still a valuable technique in various areas of drug analysis.

After some achievements in the authors' laboratory in the field of difference spectrophotometry [4–10] in this paper some derivative spectroscopic methods are presented for the solution of various types or problems (determination of the active ingredient of a formulation, determination of an experimental drug in a suspension used in toxicological studies and direct determination of an impurity in a drug material).

Experimental

Apparatus

A Hewlett–Packard high-performance liquid chromatograph, model 1090A equipped with HP 1040 diode-array UV detector (column, LiChrosorb RP-18, 10 μm, 250 × 4 mm) and a Varian DMS-100 recording UV spectrophotometer with first and second derivative spectrum scanning capabilities were used.

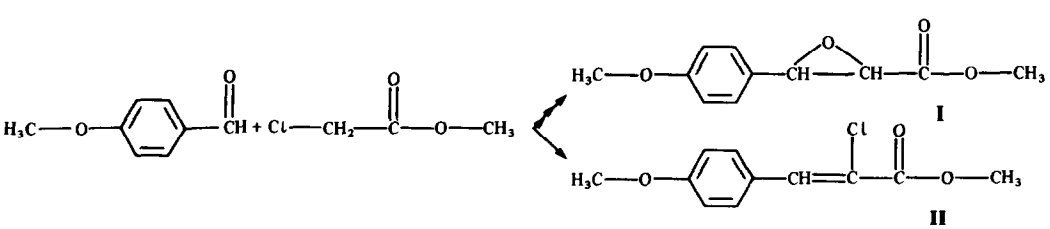
Reagents

All the chemical compounds examined were obtained from the Chemical Works of Gedeon Richter Ltd (Budapest).

Results and Discussion

Detection and elucidation of a side reaction of the Darzens reaction

In advantageous situations, the UV spectrum of the impurities separated by HPLC and recorded by the diode-array UV spectrophotometric detector is of diagnostic value in the recognition of side reactions in organic syntheses. For example, in the course of the Darzens reaction taking place between 4-methoxybenzaldehyde and chloroacetic acid methyl ester (Scheme 1) an impurity (**II**) was detected, with a capacity factor (k') = 10.6 in the HPLC chromatogram of the crude reaction product [eluent, acetonitrile–water, 1:1; k' of the main component (**I**) 3.8].



Scheme 1

From the spectra shown in Fig. 1 the following conclusions can be made. The very strong bathochromic shift of the *p*-band (73 nm relative to the phenolic ether type *p*-band of **I**) under which the α-band is submerged indicates that two conjugated double bonds are attached to the phenolic ether chromophore of the molecule. For this reason formula **II** in Scheme 1 was proposed for the structure of the impurity. Without isolating the impurity, its identity has been successfully proved by means of high resolution NMR spectroscopic analysis of a sample with an impurity content of about 6%.

It is interesting to note that the above mentioned large bathochromic shift in the UV spectrum of the impurity enables it to be determined in **I** down to the 0.1% level at 309 nm. As is seen in Fig. 2, the impurity shows selective and very strong absorbance at this wavelength ($\epsilon = 3.2 \times 10^4$), and hence this can be regarded as one of the rare examples when an impurity can be determined by simple spectrophotometry in an

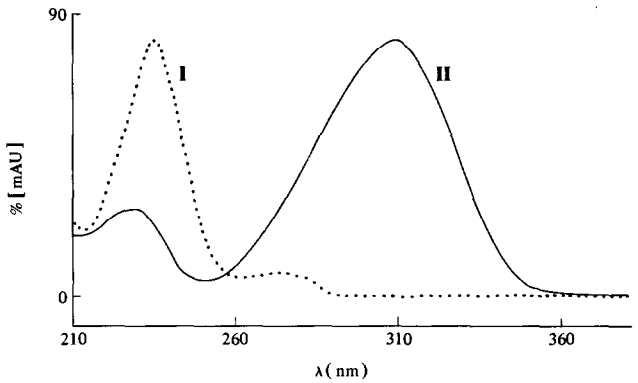


Figure 1
Spectra recorded by the diode-array UV spectrophotometric detector after HPLC separation. **I**, Main component (*trans*-(4-methoxyphenyl)-oxirane carboxylic acid methyl ester); **II**, impurity (α-chloro-4-methoxy-cinnamic acid methyl ester).

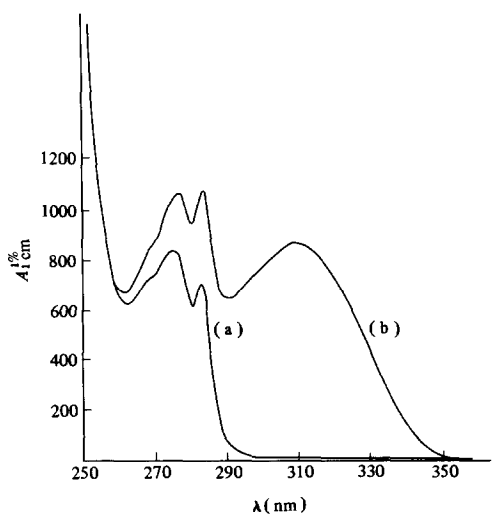


Figure 2
UV spectra without chromatographic separation. Solvent 96% ethanol (a) pure *trans*-(4-methoxy-phenyl)-oxirane carboxylic acid methyl ester (**I**); (b) **I** containing 6% of the impurity α-chloro-4-methoxy-cinnamic acid methyl ester (**II**).

absorbing organic compound without the use of chromatographic separation, chemical reaction or algebraic background correction.

Identification of two impurities in 5α-*androst-2-en-17-one*

In the course of the estimation of the impurity profile of 5α-androst-2-en-17-one [3] prepared by the catalytic splitting of methanesulphonic acid from 3β-hydroxy-5α-androstan-17-one mesylate, HPLC–diode-array spectrophotometry played an important rôle in the identification of two of the byproducts of the reaction. Two eluent systems were used: for the separation of impurity **I** acetonitrile–water–methanol (6:4:1) mixture was used (k' values for the main component and impurity **I** were 52.4 and 50.7, respectively); for the separation of impurity **II** methanol was used (k' values 2.55 and 3.81).

The diode-array UV spectra are shown in Fig. 3. In the spectrum of impurity **II** [curve (c)] the partially fused peaks with maxima at 292 and 257 nm (with shoulders at 252 and 265 nm) are the n-π^* band of the 17-keto group and the α-band typical of an unsubstituted phenyl chromophore. On the basis of this, the impurity was likely to be 3-phenyl-5α-androstan-17-one, product of a side reaction between 3β-hydroxy-5α-androstan-17-one mesylate and benzene present in the reaction mixture. It is worth mentioning that the intensity of these bands is very weak; nevertheless the spectrum was of good quality and it provided very useful information for the elucidation of the structure of impurity **II** which was later confirmed, after preparative HPLC separation, by MS and NMR spectrometry. By means of the latter technique the β-configuration of the 3-phenyl group was also established.

From the comparison of the spectra of the main component and impurity **I** [Fig. 3, curves (a) and (b)], in spite of their strong similarity important conclusions can be drawn: both contain the band of the 17-keto group, and the bathochromic shift of the short wavelength band due to the isolated double bond indicates that unlike that of the main component where the double bond is between two tertiary carbon atoms it is likely that in impurity **I** it connects a tertiary and quaternary carbon atom. The 4-androsten-17-one structure was later confirmed by other spectroscopic techniques and by chromatographic retention time, matching that of an authentic sample.

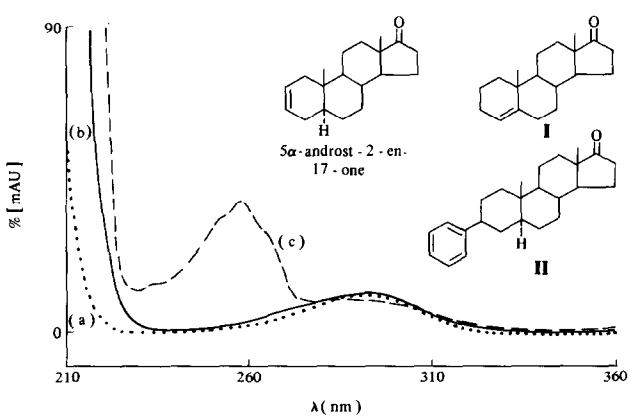


Figure 3
Spectra of 5α-androst-2-en-17-one [curve (a)] and two of its impurities, **I** (androst-4-en-17-one) [curve (b)], **II** (3β-phenyl-5α-androstan-17-one) [curve (c)].

Second derivative spectrophotometric method for the determination of flumecinol in Zixoryn^R oily emulsion

Figure 4 shows the spectrum of flumecinol, a new enzyme-inducing agent of the Chemical Works of Gedeon Richter Ltd [curve (a)] the spectrum of its oily emulsion formulation [curve (b)] and that of the placebo emulsion [curve (c)]. Because of the presence of strongly absorbing components (mainly the preservative sodium benzoate) in the emulsion, the flumecinol which exhibits a weak benzenoid spectrum cannot be assayed by simple spectrophotometry.* However, the fine structure of the benzenoid band with its narrow spectral bandwidths provides the basis for the assay of flumecinol by second derivative spectrophotometry in the presence of components with broad spectral bands [12], such as sodium benzoate in this case. As seen from the similarity of curves (a) and (b) in Fig. 5, the background can be greatly eliminated in such a way. The best results were obtained when the amplitude between the maximum and minimum of the second derivative curve at 267 and 264 nm was selected for the assay ($^2D_{267,264}$ according to the nomenclature suggested by Fasanmade and Fell [13]. Using this value, a rectilinear calibration curve was obtained in the 3–60 mg/100 ml range (r = 0.998, intercept negligible). The method was used successfully for the assay of flumecinol in the emulsion after a 25-fold dilution with 96% ethanol (recovery 101.1%, RSD = 0.8%).

The use of derivative spectroscopy in toxicological studies

Several successful attempts have been made to solve the background absorption problems in the determination of drugs and other exogenous materials in biological samples by using derivative spectrophotometry [14], e.g. determination of paraquat [15], paracetamol [16], nitrofurantoin [17], etc.

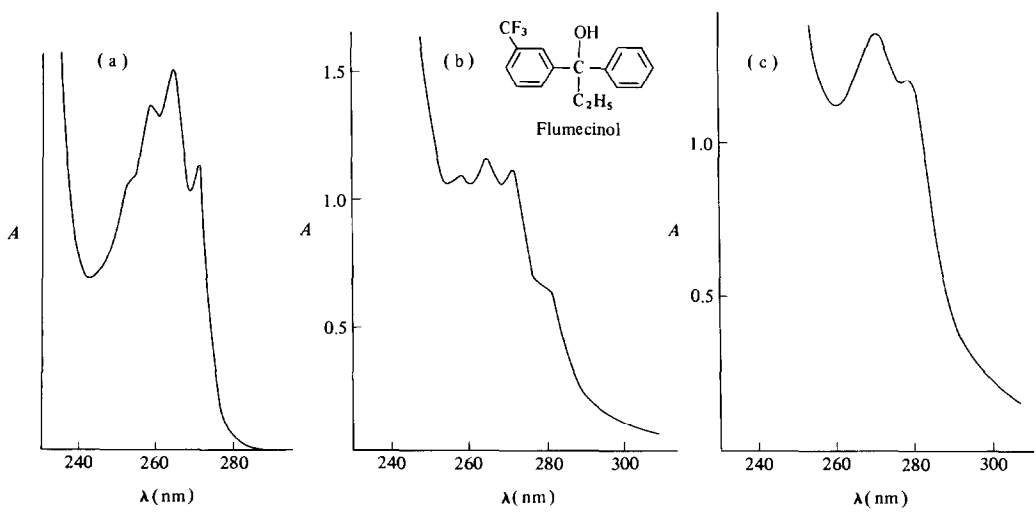


Figure 4
(a) Spectrum of pure flumecinol (0.0376 g/100 ml in 96% ethanol). (b) Spectrum of Zixoryn emulsion (1%, w/v, flumecinol content) after 50-fold dilution with 96% ethanol. (c) Spectrum of the placebo emulsion after 25-fold dilution with 96% ethanol.

*Flumecinol and other pharmacologically active α-ethyl benzhydrol derivatives can be determined by gas chromatography and spectrophotometry after acid-catalysed water elimination [2, 7, 11].

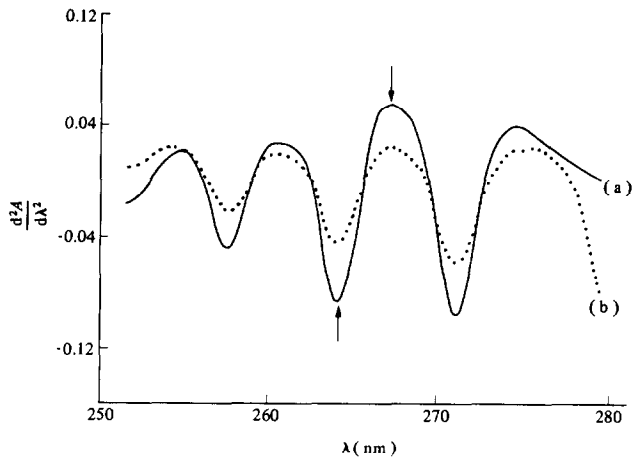


Figure 5
Second derivative spectra of the solutions of Figs 4(a) and 4(b). (a) Pure flumecinol; (b) zixoryn emulsion.

The determination of drugs or potential drugs in feed-stuffs used in toxicological studies is also an important task. Here we shortly report on the determination of the experimental drug RGH-6148 (2-benzylthiazolidinone) in a suspension containing high quantities of Tween-80 which causes serious background problems. The second derivative spectrum of a chloroform extract of the suspension in the 250–280 nm range is very similar to that of flumecinol. The selection of $^2D_{262,264}$ as the basis for the assay, enables the active ingredient to be determined at concentrations down to 1 mg RGH-6148 ml^{-1} suspension with similar accuracy and precision as those described for the Zixoryn^R emulsion.

Determination of mestranol as an impurity in norethisterone

Progestogenic steroids are often administered together with low doses of oestrogens and, as a consequence, the determination of the latter is difficult. Various spectrophotometric approaches to this problem have been proposed. These are based on the measurement of the absorbance of the phenolic band around 280 nm and the elimination of the background interference by reduction of the keto group of the gestogens by potassium borohydride [18, 19] or sodium borohydride [20], and using various algebraic background correction procedures. More recently, such formulations have been successfully assayed by derivative [21, 22] and difference-derivative [23] spectrophotometry.

Here we report on the use of second derivative spectrophotometry for the determination of mestranol (17α-ethynyl-1,3,5(10)-oestratriene-3,17-diol-3-methyl ether) as an impurity in norethisterone (17α-ethynyl-17-hydroxy-4-oestrene-3-one). This assay requires a very sensitive and selective method of analysis because of the very low level of mestranol (usually <0.3%).

Figure 6 shows the second derivative curves of a methanolic solution of norethisterone (10 mg ml^{-1}) spiked with mestranol (5–100 μg ml^{-1}), 10 ml of which had been treated with 80 mg of sodium borohydride. A rectilinear calibration graph was derived from these curves in which $^2D_{291,287}$ was plotted against the concentration of the added

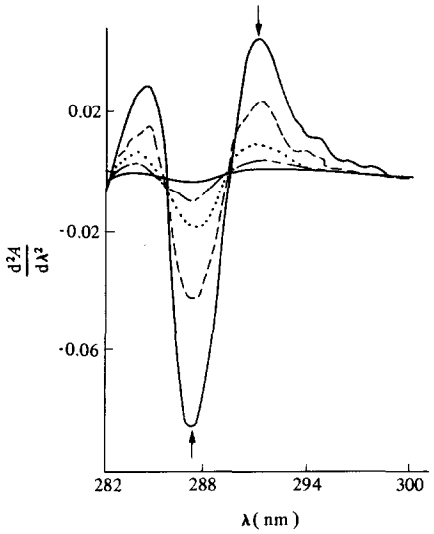


Figure 6
Second derivative spectra of 1 g norethisterone/100 ml of methanol, spiked with 0.05, 0.1, 0.2, 0.5 and 1% of mestranol, after reduction with sodium borohydride.

mestranol (r = 0.999, intercept negligible). This rapid method is accurate and precise enough for the routine determination of mestranol in norethisterone; for example, in a sample containing 0.21% of mestranol, a concentration of 0.22 ± 0.015% was found (n = 7).

References

[1] S. Görög and B. Herényi, *J. Chromatogr.* **400**, 177–186 (1987).
[2] S. Görög, A. Laukó and B. Herényi, *J. Pharm. Biomed. Anal.* **6**, 697–705 (1988).
[3] S. Görög, A. Laukó, B. Herényi, A. Georgakis, É. Csizér, G. Balogh, Gy. Gálik, S. Mahó and Z. Tuba, *Chromatographia* **22**, 316–320 (1989).
[4] S. Görög, *J. Pharm. Sci.* **57**, 1737–1741 (1968).
[5] S. Görög and É. Csizér, *Z. Anal. Chem.* **254**, 119–121 (1971).
[6] S. Görög, *Analyst* **96**, 437–442 (1971).
[7] S. Görög, A. Laukó, M. Rényei and B. Hegedüs, *J. Pharm. Biomed. Anal.* **1**, 497–506 (1983).
[8] K. Görög and S. Görög, *J. Pharm. Biomed. Anal.* **6**, 109–114 (1988).
[9] S. Görög, *Zbl. Pharm.* **116**, 259–264 (1977).
[10] S. Görög, M. Fütö and A. Laukó, *Acta Pharm. Hung.* **46**, 113–119 (1976).
[11] S. Görög, M. Rényei and A. Laukó, *J. Pharm. Biomed. Anal.* **1**, 39–46 (1983).
[12] A. F. Fell, in *Practical Absorption Spectrometry* (A. Knowles and C. Burgess, Eds), pp. 178–184. Chapman and Hall, London (1984).
[13] A. A. Fasanmade and A. F. Fell, *Analyst* **110**, 1117–1124 (1985).
[14] T. C. O'Haver, *Clin. Chem.* **25**, 1548–1553 (1979).
[15] A. F. Fell, D. R. Jarvie and M. J. Stewart, *Clin. Chem.* **27**, 286–292 (1981).
[16] B. Dingeon, M. A. Charvin, M. T. Quenard and H. Thome, *Clin. Chem.* **34**, 1119–1121 (1988).
[17] M. Poulou and P. Macheras, *Int. J. Pharm.* **34**, 29–34 (1986).
[18] M. Legrand, V. Delaroff and R. Smolik, *J. Pharm. Pharmac.* **10**, 683–686 (1958).
[19] R. A. Bastow, *J. Pharm. Pharmacol.* **19**, 41–44 (1967).
[20] S. Görög and É. Csizér, *Acta Chim. Hung.* **65**, 41–49 (1970).
[21] P. Corti, E. Lencioni and G. F. Sciarra, *Boll. Chim. Farm.* **122**, 281–288 (1983).
[22] F. A. El-Yazbi, *Anal. Letters* **18**, 2127–2142 (1985).
[23] M. A. Korany, F. A. El-Yazbi, O. Abdel-Razak and M. A. Elsayed, *Farm. Weekbl. Sci. Ed.* **7**, 163–166 (1985).

[*Received for review 16 May 1989; revised manuscript received 18 May 1989*]