ELSEVIER

Talanta 44 (1997) 1517–1526

Talanta

Drug impurity profiling strategies[1,2]

S. Görög *, M. Babják, G. Balogh, J. Brlik, A. Csehi, F. Dravecz, M. Gazdag, P. Horváth, A. Laukó, K. Varga

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

Received 16 August 1996; received in revised form 1 October 1996; accepted 2 October 1996

Abstract

A general scheme is set up for the estimation of the impurity profile of bulk drug substances by the complex use of chromatographic, spectroscopic and hyphenated techniques. Several examples are presented as illustrations to the scheme from the authors' laboratory involving the use of chromatographic methods such as thin-layer-(TLC), gas-(GC), analytical and preparative high-performance liquid chromatography (HPLC), spectroscopic methods such as mass spectrometry (MS) and NMR spectroscopy as well as hyphenated techniques (HPLC/diode-array UV, GC/MS and HPLC/MS). In addition to summarizing earlier work, new examples are also presented: identification of an impurity (propyl 4-[diethylcarbamoyl(methoxy)]-3-methoxy phenylglyoxylate, **II**) in propanidid (**I**) and two unsaturated impurities in allylestrenol (**VII**) by GC/MS and HPLC/diode-array UV as well as estimation of the impurity profile of mazipredone (**III**) by HPLC/MS and HPLC/diode-array UV. © 1997 Elsevier Science B.V.

Keywords: Impurities; Propanidid; Allylestrenol; Mazipredone

1. Introduction

The estimation of the impurity profiles of bulk drug substances is one of the most important fields of activity in contemporary industrial pharmaceutical analysis [2]. In general impurities present in excess of 0.1% should be identified and quantified by sufficiently selective methods but drug registration authorities are increasingly interested in impurities in the range 0.01–0.1% [3] The main reasons for the increasing interest of drug manufacturers and drug registration authorities in the impurity profiles of bulk drug substances are as follows:

a. In the course of the development of a new drug or a new technology for manufacturing an existing drug it is essential to know the structures of the impurities: by possessing this information synthetic organic chemists are often able to change the reaction conditions in such a way that the formation of the impurity can be avoided or its quantity reduced to an acceptable level.

* Corresponding author.

[1] Presented at Euroanalysis IX, Bologna, September, 1996. This paper is Part 17 in the series 'Estimation of impurity profiles of drugs and related materials' and Part 52 in the series 'Analysis of Steroids'. For Parts 16 and 51, respectively, see Ref. [1].

[2] This paper is dedicated to Prof. G.-E. Baiulescu on the occasion of his 65th birthday.

0039-9140/97/$17.00 © 1997 Elsevier Science B.V. All rights reserved.

PII S0039-9140(96)02179-0

b. Having suggested structures for the impurities, they can be synthesized and thus provide final evidence for their structures previously determined by spectroscopic methods.

c. The material synthesized (or in exceptional cases isolated by large scale preparative column chromatography) can be used as an 'impurity standard' during the development of a selective method for the quantitative determination of the impurity and the use of this method as part of the quality control testing of every batch.

d. In the case of major impurities the synthesized or isolated material can be subjected to toxicological studies thus greatly contributing to the safety of drug therapy.

e. The impurity profile of a drug substance depends on several factors such as the synthetic route, reaction conditions, source and quality of the starting materials, reagents and solvents used during the synthesis, the purification steps, conditions of crystallisation, distillation, drying and storage of the endproduct, etc. For this reason in the hands of drug authorities the impurity profile of a drug substance is a good fingerprint to indicate the level and constancy of the manufacturing process of the bulk drug substance: even minor changes of the above listed factors may cause dramatic changes in the impurity profile.

As a consequence of the above points the impurity profile of a bulk drug substance has to be checked repeatedly not only during the research and development period but in addition in all cases when changes take place in the factors listed under e. above. For this reason the estimation of impurity profiles of bulk drug substances is a frequent task, especially in industrial analytical research and quality control laboratories. Taking into account the large time and labour consuming nature of these studies it is essential to find a strategy which enables the results to be achieved within the shortest possible time with the greatest possible certainty. The aim of this study is to introduce a general scheme for the rational use of chromatographic, spectroscopic and hyphenated techniques in drug impurity profiling studies and to present several examples from the authors' laboratory as illustrations to the scheme.

2. Experimental

2.1. Instruments and methods

UV spectroscopy. Varian Cary 3 double beam instrument.

NMR spectroscopy (^{1}H and ^{13}C). Varian VXR-300 and Varian UNITYplus 500 spectrometers. Chemical shifts in the text are relative to δ_{TMS} = 0.00.

Mass spectrometry. VG-TRIO-2 and Finnigan MAT 95 SQ tandem spectrometers.

Gas chromatography. Hewlett-Packard 5890A and Gas chromatography/mass spectrometry (GC/MS). Fisons MD-800. Column for GC and GC/MS DB-5 MS (J and W) fused silica capillary (30 m × 0.32 mm × 1.0 μm). EI$^+$ 70 eV. Ion source temperature 240°C.

Propanidid. Temperatures: column (150°C for 9 min, 150–260°C at 10°C min^{-1}, 260°C for 10 min, 260–300°C at 10°C min^{-1}, 30 min at 300°C), injector and FID (250 and 300°C, respectively); split ratio 1:50.

High-performance liquid chromatography (HPLC). Hewlett-Packard 1090A equipped with an HP 1040 diode-array UV detector.

Propanidid. Column: 250 mm × 4 mm packed with LiChrospher Si-60 5 μm (Merck); eluent: hexane–methanol–2-propanol (90:8:2, v/v/v) at a flow rate of 1 ml min^{-1} at ambient temperature. Diode array detector set at 210, 282 and 320 nm or used in the spectrum scanning mode.

Allylestrenol. Column: 150 mm × 4 mm packed with Hypersil ODS 5 μm (Shandon); eluent: acetonitrile–methanol–water (64:18:18, v/v/v) at a flow rate of 1 ml min^{-1} at ambient temperature. Diode-array detector set at 210 and 240 nm or used in the spectrum scanning mode.

High-performance liquid chromatography/mass spectrometry (HPLC/MS). Waters 600/ Finnigan MAT 95 SQ.

Mazipredone. Column: 100 × 4.6 mm packed with Hypersil BDS C8 3 μm (Shandon); eluent: 25 mM ammonium acetate–methanol (65:35, v/v) at a flow rate of 1 ml min^{-1} at ambient temperature. Diode-array detector set at 240 nm or used in the spectrum scanning mode. The

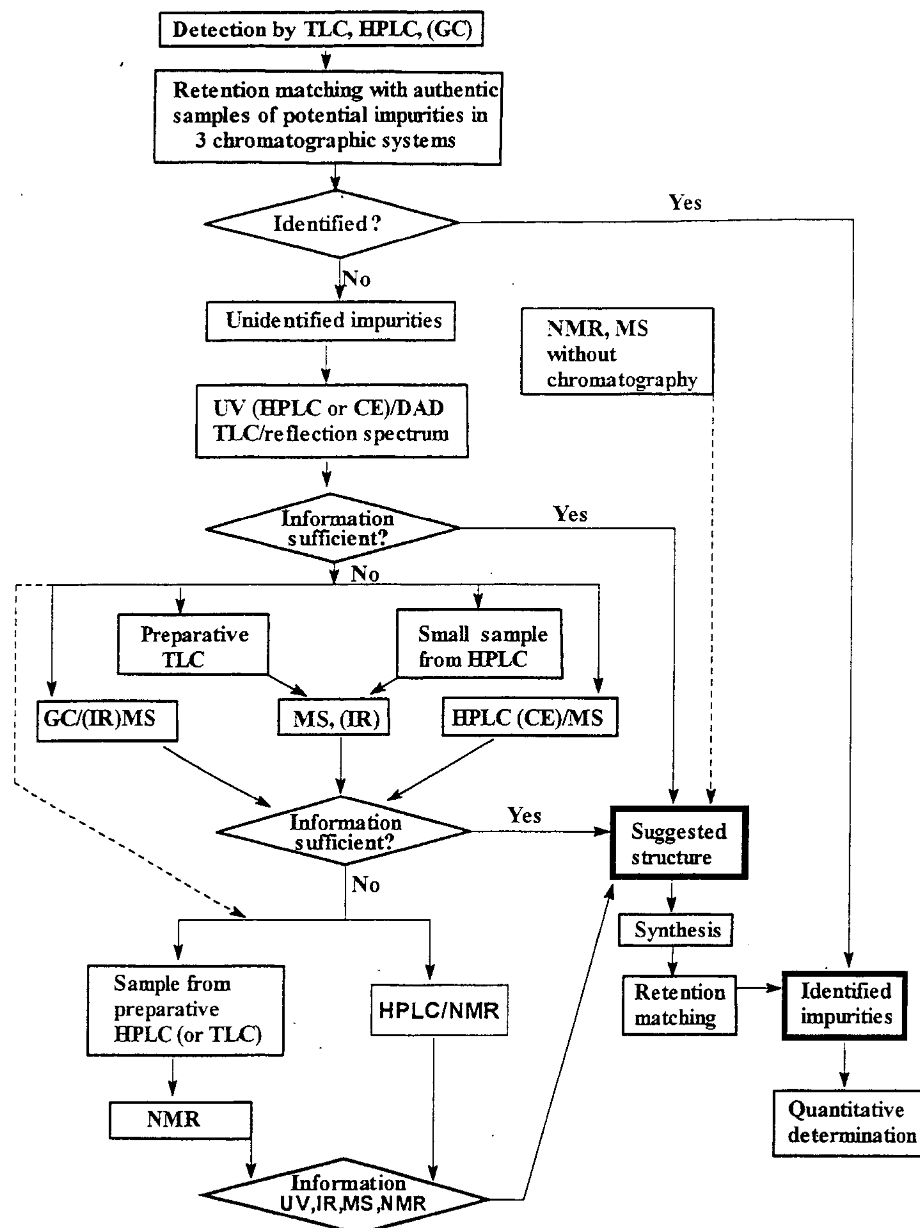


Fig. 1. A general scheme for drug impurity profiling.

mass spectrometer was used in the APCI (atmospheric pressure chemical ionisation) mode. Corona current: 4.8 μA. Temperatures: vaporizer 400°C, capillary 200°C. Sheath gas: nitrogen at 5.0 bar.

2.2. Samples

The investigated materials were laboratory or industrial samples from the Chemical Works of Gedeon Richter Ltd., Budapest.

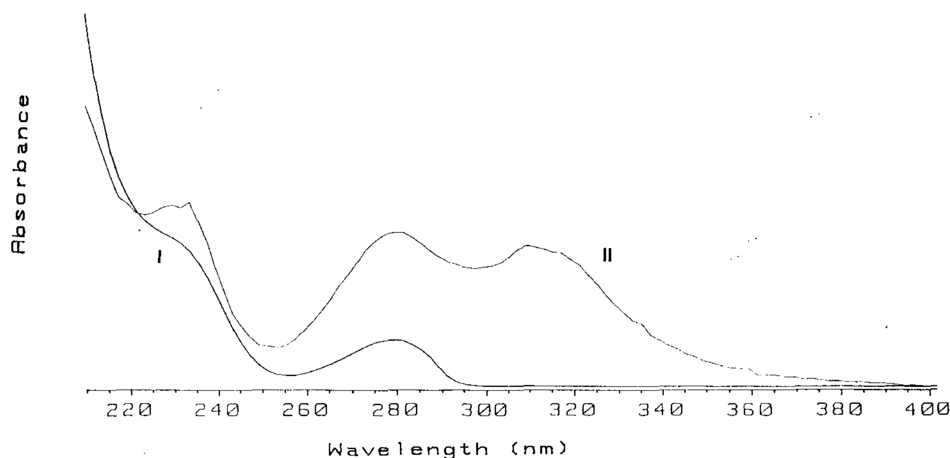


Fig. 2. Diode-array UV spectra of propanidid (**I**) and impurity **II**. See Section 2 for the HPLC conditions.

3. Results and discussion

3.1. General scheme for drug impurity profiling

As seen in Fig. 1 the procedure of impurity profiling begins with detecting the impurities on the thin-layer chromatograms, high-performance liquid chromatograms or gas chromatograms. (In the latter case caution is necessary since minor peaks originating from the thermal decomposition of the investigated drug material can easily be confused with real impurities.) The next step is to obtain as many potential impurity samples from the synthetic organic chemists as possible. These include the last intermediate of the synthesis, products of predictable side reactions and degradation reactions (if available). These samples should undergo retention matching with the previously detected impurities in the chromatographic systems where they were detected. The criterion for a positive identification is identical R_f or retention time in at least three different chromatographic systems selected in such a way that the separation mechanisms should be as different as possible. (In addition to the agreement of the retention data the TLC reflection spectra or the colour after visualization as well as the HPLC diode-array spectra, etc. should also be identical.)

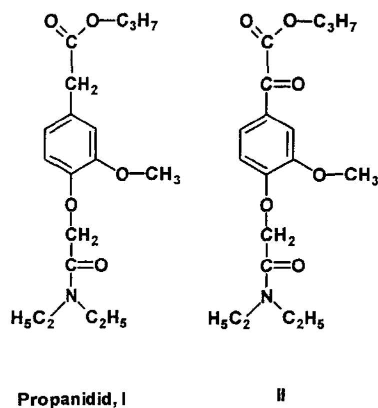
In the case of unsuccessful identification with standard samples the most reasonable way to determine the structure of the impurity starts with the investigation of the UV spectra, easily obtainable with the aid of the diode-array detector in the case of HPLC and the densitometer with reflection spectrum scanning facilities in the case of TLC. Although in the majority of cases the UV spectra of the impurities do not markedly differ from that of the main component, in some instances even minor differences are of diagnostic value and hence because of the ease of obtaining the spectra by means of the standard HPLC and TLC instrumentation, it is reasonable to try to draw as much conclusions from the UV spectra as possible. In exceptional cases (with full knowledge of the synthesis of the drug material) it is even possible to propose a structure for the impurity exclusively on the basis of the UV spectrum thus saving much time and efforts or at least the information thus obtained can be a useful complement to those obtained from the mass- and NMR spectra [4].

If the information obtainable from the UV spectrum is not sufficient, the next step in the procedure of impurity profiling is usually to take the mass spectrum of the impurity. Of course the most effective way to do this is to make use of the on-line GC/MS or HPLC/MS facilities available in the majority of laboratories dealing with impurity profiling. A great benefit of these techniques is that data can be obtained simultaneously on

several impurities down to the 0.01% level. An advantage of the GC/MS method is that reliable molecular weight value is obtainable using chemical ionisation and, in addition, information on fragmentation necessary for the solution of more complicated structure elucidation problems can also be obtained using the electron impact ionisation technique. A disadvantage is that due to volatility and thermal stability problems the possibilities of this method are limited. (The use of derivatization reactions widely used in other fields of GC/MS analysis is problematic here because the side-products of the derivatization reaction can be confused with the impurities.) An advantage of the HPLC/MS method [5] is its general applicability. A disadvantage is, however, that the ionisation techniques used in association with the generally used instruments (the older thermospray and the more up-to-date electrospray and atmospheric pressure chemical ionisation (APCI) techniques) usually give only molecular weight information. To obtain the fragmentation pattern highly sophisticated HPLC/MS/MS facilities are necessary.

It is worth mentioning that capillary electrophoresis (CE) is increasingly used in drug impurity profiling as an alternative to the above chromatographic techniques [6]. This technique can also be coupled with mass spectrometry [5].

If the hyphenated GC/MS, LC/MS or CE/MS facilities are not available, mass spectra can be taken on samples obtained from preparative or—



Scheme 1.

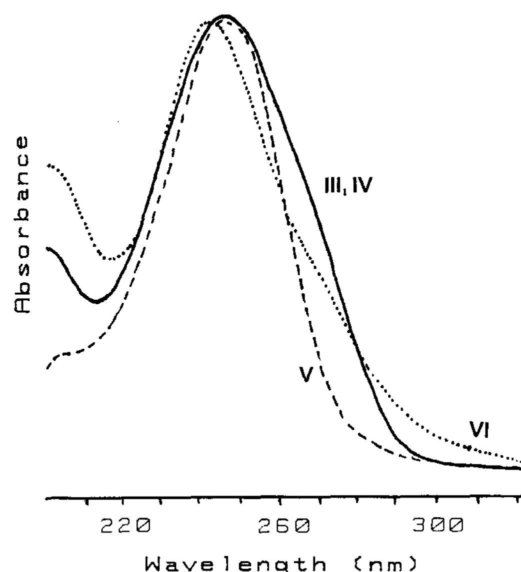
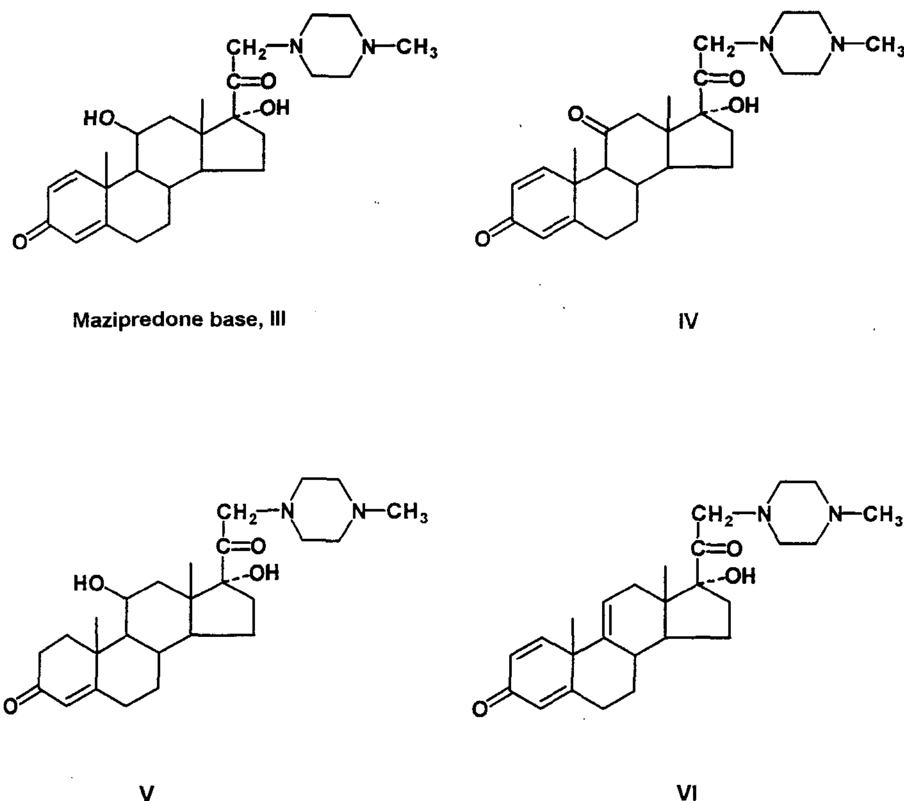


Fig. 3. Diode-array UV spectra of mazipredone (**III**) and impurities **IV**, **V**, **VI**. See Section 2 for the HPLC conditions.

due to the high sensitivity of mass spectrometry—analytical TLC or HPLC.

In the majority of cases the information from the mass spectrum (together with the previously taken UV spectrum) is sufficient to propose a structure for the impurity. If not, NMR spectroscopy is the ultimate method for elucidating the structure of the impurity. Due to the limited sensitivity of this method NMR spectra are usually obtained on samples from preparative HPLC (or TLC) separation. Quite recently on-line HPLC/NMR instruments became commercially available [7]. This new hyphenated technique will certainly have a bright future in various fields, among them in drug impurity profiling.

The next step in the impurity profiling is the synthesis of the material with the proposed structure. The retention and spectral matching of the synthesized material (impurity standard) with the impurity in question is carried out as outlined above. In the case of successful matching the procedure of impurity profiling is terminated by working out selective quantitative methods for the determination of the impurity making use of the impurity standard.



Scheme 2.

The possibilities of spectroscopic techniques in drug impurity profiling without chromatographic separation are also worth mentioning. Spectra obtained by using high-resolution, highly sensitive NMR spectrometers and mass spectrometers with FAB or electrospray/APCI facilities are suitable to provide a fingerprint-like picture regarding the purity of the sample. In addition to this, in advantageous cases (especially if there is only one major impurity in the sample), conclusions can be drawn from the minor NMR signals regarding the structure of the impurity. Making use of the above mentioned MS facilities the molecular weight of the impurity can be obtained, if it differs from that of the main component, and this can also be a useful information in the course of the impurity profiling procedure. It has to be emphasized, however, that investigations of this type by no means substitute for the systematic chromatographic analysis in the search for the structures of the impurities.

3.2. Practical examples from the authors' laboratory

Characteristic examples where the UV spectra obtained by the HPLC diode-array UV detector were of diagnostic value are the structure elucidation of 3,17α-diethinyl-13-ethyl-3,5-gonadien-17-ol (an impurity in norgestrel with the characteristic *trans*-dienine UV chromophore) [1], identification of 9(11)-dehydro derivatives as impurities in oestradiol [8], ethinyloestradiol [9] and mestranol [10], where the difference between the spectra of the phenol-type main component and its α,β-unsaturated impurity is remarkable and on the basis of the UV spectra even to differenciate between the three regioisomers (Δ^6, $\Delta^{8(9)}$ and

$\Delta^{9(11)}$) was also possible. The discrimination between 4-ene-3-keto- and 1-ene-3-ketosteroids was also possible on the basis of the rather large difference between their UV maxima (228 and 237 nm in the apolar eluent of the normal-phase HPLC system used in this study) [11]. However, even minor differences can be of diagnostic value such as in the case of the identification of 8(14)-dehydronorgestrel as an impurity in norgestrel where the slight hypsochromic shift (2 nm) of the band of the α,β-unsaturated 3-keto group together with the remarkable band broadening is characteristic of 'through-space' conjugation with the $\Delta^{8(14)}$ double bond [9]. Another example of this type is the identification of 6α- and β- as well as 10β-hydroxy derivatives as impurities in norgestrel [11].

In the following examples the diode-array UV spectra together with the off-line mass spectra afforded sufficient evidence for the structure elucidation of various impurities. In an impurity of oestradiol (4-chlorooestradiol) the mass spectrum indicated the presence of a chlorine atom in the phenolic ring while its position was established on the basis of the UV spectrum [8]. Another example is the identification of propyl 4-[diethylcarbamoyl(methoxy)]-3-methoxy phenylglyoxylate (**II**) impurity (oxidative degradation product) in propanidid (**I**). The molecule peak of **II** from a GC/MS scan was at m/z 351 ($t_R = 34.6$ min) while the molecular weight of **I** is 337 ($t_R = 30.5$ min). In the normal phase high-performance liquid chromatogram of propanidid **I** appears at 11.9 min, while the peak of **II** is at 16.4 min. It is evident from the diode-array UV spectra of **I** and **II** in Fig. 2 and Scheme 1 that the reason for the difference of 14 units between the molecular weights of **I** and **II** is that in the latter the active methylene group of **I** between the phenolic ring and the propyloxycarbonyl group is oxidized leading to a highly conjugated ketone derivative .

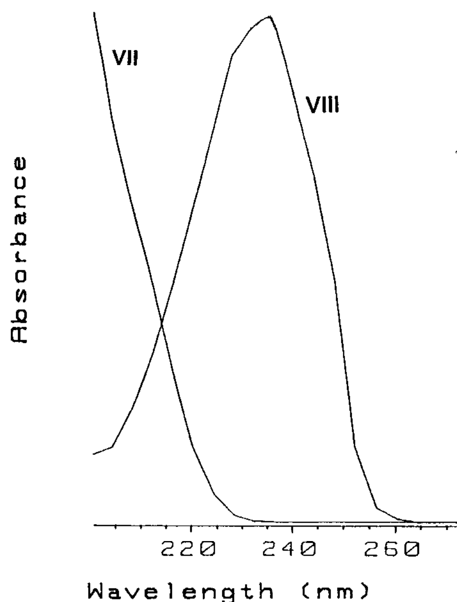
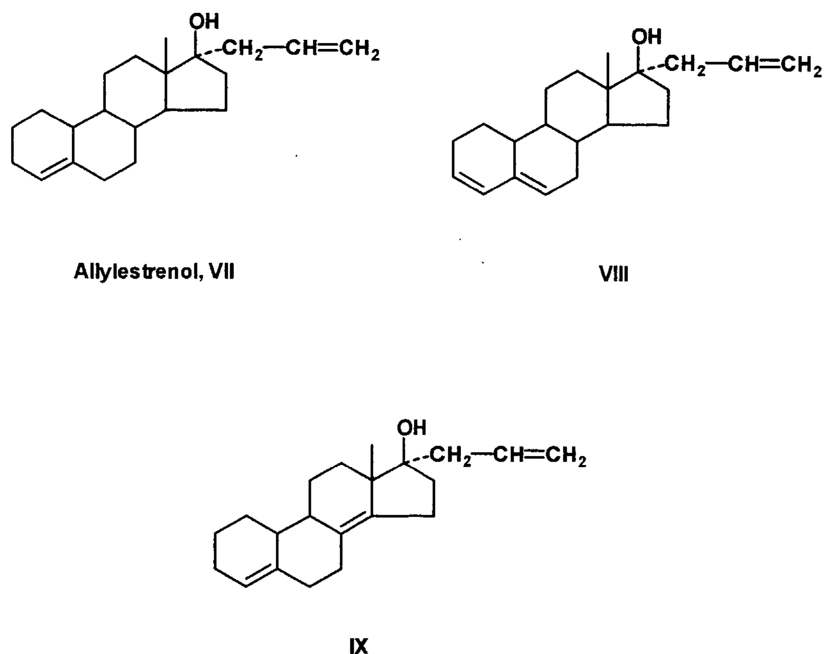


Fig. 4. Diode-array UV spectra of allylestrenol (**VII**) and impurity **VIII**. See Section 2 for the HPLC conditions.

In the following the potential of the LC/MS technique supplemented with information obtained from diode-array UV spectra will be demonstrated on the example of the impurity profiling of mazipredone (21-deoxy-21-*N*-methylpiperazinyl-prednisolone hydrochloride, **III**), the water-soluble prednisolone derivative of the Chemical Works of Gedeon Richter Ltd., Budapest. Under the HPLC conditions described in Section 2 the retention time of mazipredone (**III**) is 30.7 min. In addition to minor trivial impurities such as prednisolone (retention time 24.9 min) and prednisolone-21-mesylate, the last intermediate of the synthesis (retention time 48.5 min) we report on the identification of three main impurities present in the range 0.1–0.4%. It is to be noted that under the APCI conditions described in Section 2 only the molecular weights of the protonated compounds are obtainable. The molecular weight of **III** is 442 ($MH^+ = 443$). The molecular weight of 440 of impurity **IV** eluting at 28.2 min indicates that the 11-hydroxyl group in **III** is replaced by a 11-ketone group.This is supported by the full identity of the diode-array UV spectra of **III** and **IV** [12] (Fig. 3 and Scheme 2). Impurity V (29.9 min) has a molecular weight of 444. It can be seen from its UV spectrum that the additional hydrogen atoms are at the 1 and 2 positions: the absorption maxima of 1,4-diene-3-keto and 4-ene-3-keto steroids are known to be at nearly the same wavelength but the bandwidth of the latter is much smaller [12–15]. The molecular

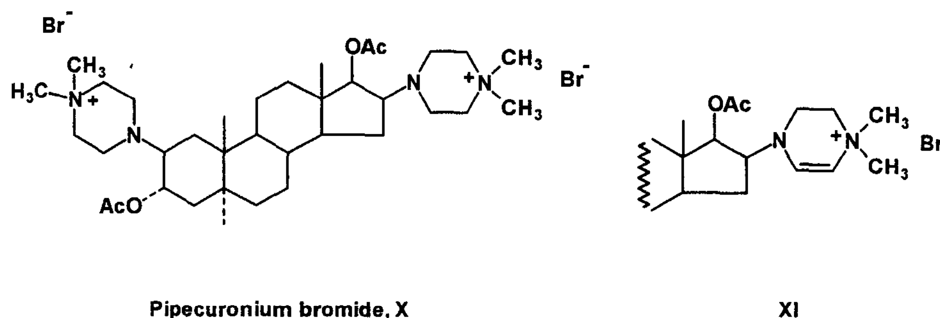


Scheme 3.

weight of impurity **VI** (retention time 41.2 min) is 424. This indicates the loss of one molecule of water from the parent compound: either Δ^{16} or $\Delta^{9(11)}$ derivative. The hypsochromic shift observable in Fig. 3 is characteristic of the latter [12], the structure of which was proved by synthesis, retention and spectral matching. It is noteworthy that the characteristic molecular weights of the protonated forms of **IV–VI** (441, 445 and 425) could be estimated by direct APCI investigation of the contaminated sample of mazipredone without any chromatographic separation.

An example of the use of the GC/MS method is the impurity profiling of flumecinol (3-trifluoromethyl-α-ethyl-benzhydrol) [10,16,17]. This technique has also been successfully used (usually combined with other methods) in the impurity profiling of steroids, e.g. the identification of 3-deoxonorgestrel impurity in norgestrel [1], several impurities in 2-androstene-17-one (intermediate in the synthesis of pipecuronium bromide) [18] and in bulk cholesterol [19].

A recent example for the use of GC/MS supplemented by other spectroscopic techniques is the estimation of impurities **VIII–IX** in allyloestrenol (**VII**). The gas chromatographic retention times were as follows: **VII** 7.2 min, **VIII** 7.9 min, **IX** 6.3 min. The molecular weight of **VII** is 300 whereas those of **VIII** and **IX**, obtained from the GC/MS scans, is 298 indicating the presence of one additional double bond. The location of this in the structures of **VIII** and **IX** was estimated on the basis of the fragmentation pattern in the mass spectra and from NMR spectra obtained on samples isolated by preparative HPLC. The location of the additional double bond at the 8(14) position in **IX** is supported by a base peak at m/z 214 which is due to the cleavage of the C_{13}–C_{17} and C_{15}–C_{16} bonds (β-cleavage related to the 8(14) double bond). Further evidence is furnished by the NMR data. The chemical shifts (in $CDCl_3$) of the protons and carbons in the environment of the two bonds in the steroid nucleus are as follows. ^{1}H NMR: 1.09 (3 H, s, H-18), 5.44 (1 H, m, H-4); ^{13}C NMR: 21.7 (q, C-18), 120.1 (d, C-4), 130.8 (s,C-8), 136.2 (s,C-14), 140.0 (s,C-5). The base peak in the mass spectrum of **VIII** is the molecule peak. The intense fragment ion at m/z



Scheme 4.

199 (88%) is due to the cleavage of the C_{13}–C_{17} and C_{14}–C_{15} double bonds. NMR chemical shifts (in DMSO-d_6) supporting the 3,5-diene structure are as follows. ^{1}H NMR: 0.81 (3 H, s, H-18), 5.42 (1 H, m, H-6), 5.64 (1 H, m, H-3), 5.95 (1 H, m, H-4); ^{13}C NMR: 14.4 (q, C-18), 122.7 (d, C-6), 126.4 (d, C-3), 129.5 (d, C-4), 136.2 (s, C-5). In the case of **VIII** the HPLC/diode-array UV spectral scan (see Fig. 4 and Scheme 3) also contributed to the structure of this conjugated *trans*-diene type impurity; (retention times: **VII** 12.8 min, **VIII** 8.1, **IX** 10.0 min).

Some examples for the complex use of chromatographic and spectroscopic techniques including NMR spectroscopy are, for example, the identification of two isomeric impurities (Z and E ethynodiol-3-acetate-17-(3′-acetoxy-2′-butenoate) in ethynodiol diacetate [20], epimeric 17α-hydroxy-17β-ethinyl and ethinyl-bridged dimeric derivatives in norethisterone [9,17], impurities with a methyl group attached to the aromatic ring of the main component in hexoestrol [10] and enalapril [20], isodanazol in danazol [21], 3-[2-(diaminomethyleneamino)thiazol-4-ylmethylthio]-*N*-cyano-propionamidine in famotidine [17], 2,5-bis-[(*N*′-cyano-*N*″-methyl)guanidino-ethyl-thiomethyl]-4-methylimidazole and 1,8-bis-[(*N*′-cyano-*N*″-methyl)guanidino]-3,6-dithiaoctane in cimetidine [22] as well as 3,17α-diethinyl-13-ethyl-3,5-gonadien-17-ol in norgestrel [1].

The last example merits special attention. NMR spectroscopy not only played a predominant role in the structure elucidation of 2′-dehydropipecuronium bromide (**XI**) impurity in pipecuronium bromide (**X**) but (due to the absence of signals in the ^{1}H-NMR spectrum of the latter in the 5.2–6.3 ppm range) the estimation of the doublets of the vinylic protons at 5.26 and 6.29 ppm enabled the impurity to be detected and even quantitated (using the internal standard method) down to the level of 0.5% in **X** without chromatographic separation [20] (Scheme 4).

References

[1] P. Horváth, G. Balogh, J. Brlik et al., J. Pharm. Biomed. Anal., in press.
[2] J.C. Berridge, J. Pharm. Biomed. Anal., 14 (1995) 7.
[3] Note for Guidance on Impurities in New Drug Substances, (Guidelines prepared within the International Conference on Harmonisation (ICH) process.) The European Agency for the Evaluation of Medicinal Products, London, 1995.
[4] S. Görög, Ultraviolet-Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, Boca Raton, FL, 1995, pp. 135–153.
[5] W.M.A. Niessen and J. van der Greef, Liquid Chromatography—Mass Spectrometry, Marcel Dekker, New York, 1992.
[6] K.D. Altria, J. Chromatogr., 646 (1993) 245.
[7] J.C. Lindon, R.D. Farrant, P.N. Sanderson et al., Magn. Res. Chem. 33 (1995) 857.
[8] S. Görög, J. Brlik, A. Csehi et al., Anal. Methods Instrum., 2 (1995) 154.
[9] S. Görög and B. Herényi, J. Chromatogr., 400 (1987) 177.

[10] S. Görög, A. Laukó and B. Herényi, J. Pharm. Biomed. Anal., 6 (1988) 697.
[11] S. Görög, M. Bihari, É. Csizér, F. Dravecz, M. Gazdag and B. Herényi, J. Pharm. Biomed. Anal., 14 (1995) 82.
[12] L.L. Engel (Ed.), Physical Properties of the Steroid Hormones, Pergamon Press, Oxford, 1963, pp. 85–93.
[13] S. Görög and Gy. Szász, Analysis of Steroid Hormone Drugs, Elsevier, Amsterdam, 1978, pp. 176–177.
[14] S. Görög, Quantitative Analysis of Steroid Hormones, Elsevier, Amsterdam, 1983, pp. 24–25.
[15] S. Görög (Ed.), Steroid Analysis in the Pharmaceutical Industry, Ellis Horwood, Chichester, 1989, pp. 6–7, 226–227.
[16] A. Laukó, É. Csizér and S. Görög, Proc. 13th Int. Symp. on Capillary Chromatography, Vol. II (P. Sandra, Ed.), Huethig, 1991, pp. 1548–1556.
[17] S. Görög, G. Balogh, A. Csehi et al., J. Pharm. Biomed. Anal., 11 (1993) 1219.
[18] S. Görög, A. Laukó, B. Herényi et al., Chromatographia, 26 (1988) 316.
[19] A. Laukó, É. Csizér and S. Görög, Analyst, 118 (1993) 609.
[20] S. Görög, G. Balogh and M. Gazdag, J. Pharm. Biomed. Anal., 9 (1991) 829.
[21] G. Balogh, É. Csizér, Gy. G. Ferenczy et al., Pharm. Res., 12 (1995) 295.
[22] Zs. Halmos, Cs. Szántay Jr., J. Brlik et al., J. Pharm. Biomed. Anal., in press.