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Short Communication

Example of pitfalls in the UV detection used in the resolution of racemic compounds by liquid chromatography

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ABSTRACT

The commercially available nitrile (**1**) and the epoxide (**2**) that we used as racemic compounds in previous studies each contained an impurity. Unfortunately, both impurities have a high molar absorptivity at 254 nm, the wavelength at which HPLC detection was carried out. This led us to consider the impurity as one of the enantiomers. Both compounds have been identified. The nitrile (**1**) contains 1,1,1-trifluoroacetophenone (**3**) as impurity and the expoxide (**2**) contains 4-chlorobenzophenone (**4**). The identification of these compounds is described.

INTRODUCTION

In the course of our research into the chromatographic behaviour of several chiral stationary phases of the "brush" type, compounds **1** [1,2] and **2** [1–3] (Fig. 1) were used as test compounds among other racemics. A routine purity control of both commercial products (GC for **1** and ^{1}H NMR for **2**) was carried out. A small quantity of an impurity was detected in

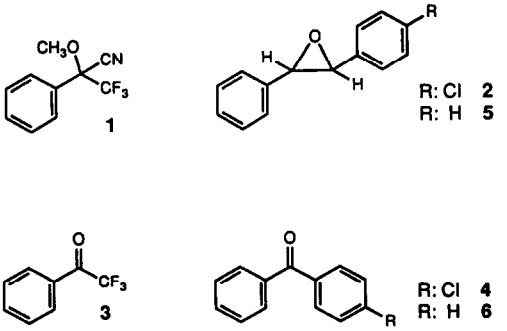

Fig. 1. Structures of racemic compounds and their impurities.

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each product, but it was considered to be negligible. Therefore the nitrile (**1**) and the epoxide (**2**) were used as received from suppliers without further purification. Unfortunately, in both cases the molar absorptivities of compounds **1** and **2** at 254 nm were much lower than those of the accompanying impurities, so racemics and impurities produced similarly important peaks. This fact led us to consider the resolution of two racemics instead of the separation of the impurity from the racemic.

When the attribution errors were discovered, an attempt was made to identify the impurities. Before finishing this study Pirkle *et al.* [4] pointed out the mistake. This identification is now reported.

EXPERIMENTAL

Rotatory power was measured with a Perkin-Elmer 241 polarimeter (Perkin-Elmer, Uberlingen, Germany). GC–MS spectra were performed in an HP 5988A apparatus (Hewlett-Packard, Palo Alto, CA, USA). The UV spectra were recorded on a Uvikon 940 spectrometer (Kontron AG Instruments, Zurich, Switzerland). The analytical liquid chromatographic experiments were carried out on a Hewlett-Packard HP 1090 liquid chromatograph equipped with a PU4020 UV detector (Philips, Cambridge, UK) and the preparative separation was carried out using a chromatograph consisting of a Gilson 302 pump and a UV 115 detector (Gilson, Villiers le Bel, France) and a Valco injection valve (Valco, Houston Instruments, TX, USA). The chiral stationary phases were packed into stainless-steel tubes (analytical, 100 × 4.6 mm I.D.; preparative, 250 × 20 mm) by the slurry method. The flow-rate of the pump was 1 ml/min in analytical experiments and 9 ml/min in the preparative separation. The detection wavelength was 254 nm. The mobile phases consisted of a mixture (95:5, v/v) of *n*-heptane and chloroform (to which 0.5% methanol was added). Gas chromatographic experiments were carried out on a Perkin-Elmer 8600 apparatus equipped with a capillary column BP1 (12 m × 0.22 mm).

Chemicals and reagents

Compound **1** was purchased from several suppliers: Aldrich (Saint-Quentin Fallavier, France), Janssen (Beerse, Belgium) and JPS (Bevaix, Switzerland). Compounds **2** and **4** were supplied by Aldrich, **3** by Fluka (Saint-Quentin Fallavier, France), **5** by Sigma (Saint-Quentin Fallavier, France) and **6** by Prolabo (Paris, France). Compounds **4** and **6** were purified by crystallization from ethanol 96% and **3** by distillation prior to use.

Chiral stationary phases

Analytical columns packed with CSP-1 (Chirachrom D1) were obtained from Interchim (Montluçon, France). The CSP used in the preparative chromatography (CSP-1p) was prepared as CSP-1 [3] but using 25–45 μm silica. CSP-2 was obtained from N-(3,5-dinitrobenzoyl)-(S)-cyclohexylalanine [5].

RESULTS AND DISCUSSION

Nitrile 1

Because of the high selectivity factors observed for this racemic compound on CSP-1 (CSP-6c in ref. 3), we studied the possibility of effecting a preparative separation using a chiral stationary phase with the same chiral selector. When a sample of **1** was subjected to preparative chromatography on CSP-1p, the two products collected had no action under polarized light. Therefore, no enantiomeric resolution had taken place. The first fraction had a UV spectrum identical to that of **1**. The UV spectrum of the second fraction was different, stable in heptane and changed as a function of time in ethanol. The rate of this evolution increased with the increased amount of water contained in the solvent.

The racemic nitrile **1** was isolated from the first fraction by evaporating the solvent, but the product in the second fraction could not be isolated in the same way. This fact, together with the evolution of the UV spectrum, seemed to point to an unstable product. However, the characterization of the compound was undertaken.

GC–MS of the two fractions collected allowed us to identify the product in the first fraction as the nitrile **1**, but three different products (*m/e* 174, 235 and 229) were detected in the second. Careful examination of the GC–MS spectrum of **1** before chromatography showed only a small quantity of the product with a molecular mass of 174, besides nitrile **1**, at a very short retention time. The other compounds seemed to be aliphatic impurities coming from the solvent used in the preparative separation. The MS spectrum of the product contaminating compound **1** seemed to correspond to 1,1,1-trifluoroacetophenone (**3**, Fig. 1). The UV spectrum of **3** in ethanol evolved in the same way as the product in the second fraction. Such evolution may be due to the hydration of the carbonyl group. This assignation was confirmed by comparing spectral (MS, UV and kinetic evolution in ethanol) and chromatographic data (HPLC and GC) with a real sample of **3**. Thus, the evolution kinetics of **3** in absolute ethanol (Merck)–ethanol 96% (100:1, v/v) is of first order relating to **3** ($k_{observed} = 0.57$/min at 24°C). The presence of this compound in **1** could be expected because it is a synthetic precursor of the nitrile [6].

Molar absorptivities of **1**, purified by distillation, and **3** in heptane were calculated (Fig. 2). Compound **1** had an ε value of 415 l mol^{-1} cm^{-1} at $\lambda_{max} = 261$ nm and **3** had an ε value of 13 190 l mol^{-1} cm^{-1} at $\lambda_{max} = 251$ nm. At 254 nm, the wavelength at which UV detection was carried out, these ε values were 322 for **1** and 12 330 for **3**. Therefore the same absorption (chromatographic peaks with the same area) could be obtained with only 1 mol of **3** in 38 mol of the nitrile, **1**, *i.e.* 2.1 g of **3** in 100 g of contaminated **1**.

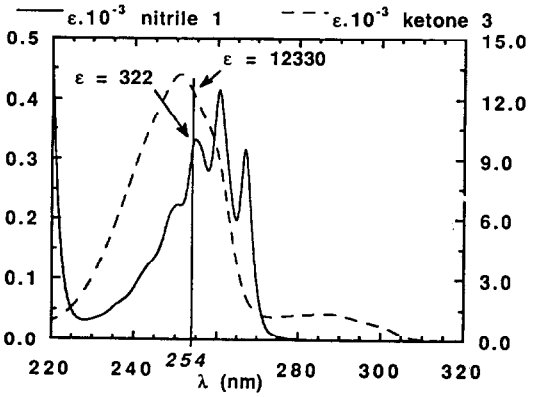

Fig. 2. UV spectra of **1** and **3** in *n*-heptane.

Epoxide **2**

On testing a new chiral stationary phase with N-(3,5-dinitrobenzoyl)-(*S*)-cyclohexylalanine as chiral selector (CSP-2, Fig. 3) [5], an impurity of compound **2** was detected while the partial resolution of the racemic was taking place. Epoxide **2** was easily purified by crystallization from hexane. The impurity was identified from the GC–MS spectrum of the mother liquors from the purification. Thus the major impurity of the sample was a chlorine-containing compound with a molecular mass of 216/218, which corresponds to 4-chlorobenzophenone (**4**, Fig. 1). As in the previous case, the assignation was confirmed by comparing spectral (MS and UV) and chromatographic data (HPLC and GC) with a real sample of **4**.

Molar absorptivities of **2** and **4** were also calculated (Fig. 4). Compound **2** had an ε value of 28 060 l mol^{-1} cm^{-1} at $\lambda_{max} = 234$ nm, and **4** had an ε value of 21 970 l mol^{-1} cm^{-1} at $\lambda_{max} = 255$ nm. At 254 nm, the wavelength at which UV detection was carried out, these ε values are 2180 for **2** and 21 960 for **4**.

The same kind of impurity was detected in a sample of commercially available epoxide **5**.

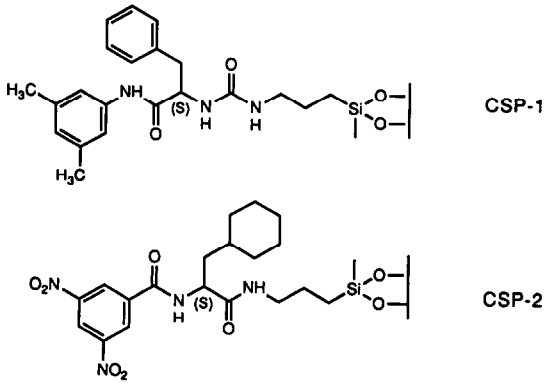

Fig. 3. Structures of stationary phases.

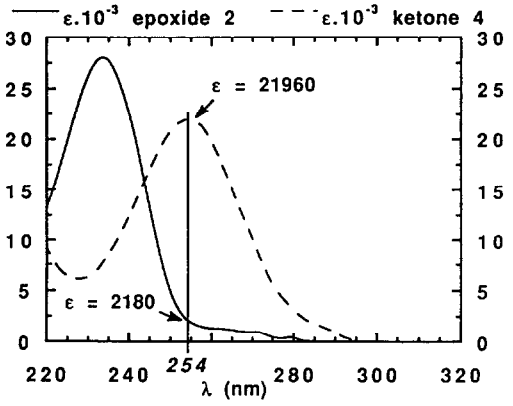

Fig. 4. UV spectra of **2** and **4** in *n*-heptane.

Thus, ketone **6**, with a high molar absorptivity, could produce important peaks in HPLC while simple UV detection is carried out.

CONCLUSIONS

Our errors show two specific examples in which a small quantity of impurity with a strong absorption in the UV range, contained in a product with a weak absorption in the UV range, could produce a peak as important as the principal product in HPLC coupled with UV detection.

Certainly, there are technical resources that provide protection against errors of interpretation in chiral liquid chromatography, such as the use of chiroptic detectors (polarimetric or circular dichroism detector) or multi-wavelength detectors (diode-array detectors) [4], but these are expensive and not available to a number of researchers. However, risks can be limited by carrying out the detection at the absorption maximum of the racemic compound. The spectra in Figs. 2 and 4 show that, in the present case, the surface of peaks belonging to compounds **1** and **2** should have been multiplied by a coefficient equal to the ratio of molar absorptivities of these compounds at the wavelength of their absorption maximum and at the wavelength at which detection was carried out, 254 nm (1.3 and 12.9, respectively), and those of the impurities **3** and **4** divided by 1.4 and 2.8 ($\varepsilon_{254\,nm}/\varepsilon_{\lambda_{max}}$), respectively, carrying out the UV detection at 261 nm for **1** and at 234 nm for **2**.

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