Short Communication

Confirmation of the structure of by-products in the synthesis of Modafinil by liquid chromatography–mass spectrometry

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ABSTRACT

By-products formed during the synthesis of Modafinil, a new awakening drug, were analysed by liquid chromatography–mass spectrometry with thermospray interface; the working conditions were optimized, in particular the value of the tension of repeller. The obtained mass spectra, giving in three cases out of four not only molecular adducts but lower ones, furnished structural information concerning these by-products. Through comparison of spectra with standards, confirmation of their structure was achieved.

INTRODUCTION

Modafinil, (diphenylmethyl)sulphinyl-2 acetamide, a new awakening drug [1], is thermally unstable and cannot be analysed by gas chromatography (GC). So its purity was studied by high-performance liquid chromatography (HPLC) and three by-products were detected: their structures were deduced from the synthetic pathway of Modafinil. These supposed impurities were synthesized: their retention times were equal to those of Modafinil by-products, but no further proof of their structure could be given.

The present study involving thermospray HPLC–mass spectrometry (MS) was developed to confirm the structure of these impurities. After having optimized the equipment and the chromatographic separation, the tension of repeller was optimized to obtain not only molecular adducts, but lower ones, able to give structural information.

EXPERIMENTAL

Solvents

HPLC-grade acetonitrile and analytical-grade ammonium acetate used for the mobile phase were purchased from Prolabo (Paris, France).

Standards

Modafinil and impurities, the structures of which are given in Fig. 1, were synthesized in the laboratories of Laboratoire L. Lafon (Maisons-Alfort, France). These impurities are (1) (diphenylmethyl)sulphonyl-2 acetamide (sulphone), (2) (diphenylmethyl)sulphinyl-2 acetic acid (acid) and (3) methyl(diphenylmethyl)sulphinyl-2 acetate (ester).

Equipment

Analyses were carried out on a HPLC–MS system consisting of a Waters Model 600 MS solvent delivery system, a Waters Model U6K injector (Waters, Division of Millipore, Trappes, France) equipped with a 50-μl loop, a Vestec thermospray interface (Vestec, Houston, TX, USA) and a Nermag R 10-10 L quadrupolar mass spectrometer (Delsi-Nermag, Argenteuil, France).

A six-port Rheodyne valve, used as injector shunt, allowed the chromatographic column to be bypassed in order to calibrate the mass spectrometer and to optimize the working conditions of the thermospray interface. The second solvent delivery system, originally used after the chromatographic column to introduce the ionization reagent ammonium acetate, was removed in order to maintain the efficiency of the chromatographic separation, to avoid dilution of the analyte, and to increase the sensitivity. Ammonium acetate was put directly into the chromatographic mobile phase.

Chromatographic analysis and mass spectrometry

The mobile phase [acetonitrile- 0.1 M ammonium acetate (4:6, v/v)], degassed prior to use and kept under a stream of helium, was adjusted to a flow-rate of 1.00 ± 0.01 ml/min through a stainless-steel column (15 cm × 4.6 mm I.D.) packed with
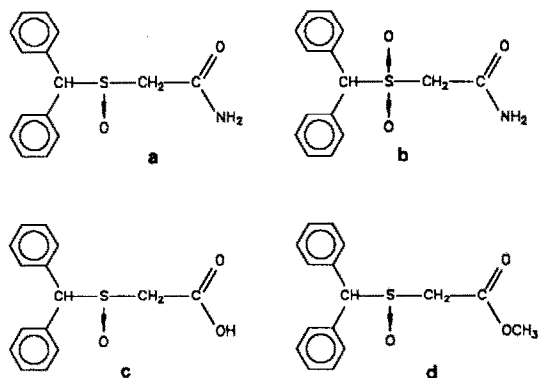

Fig. 1. Chemical formulae of Modafinil and its supposed impurities. (a) Modafinil (molecular mass 273); (b) sulphone (289); (c) acid (274); (d) ester (288).

Ultrabase C_8, 5 μm (S.F.C.C., Neuilly-Plaisance, France), maintained at a temperature of 40°C. Ammonium acetate was only used as ionization reagent for the thermospray interface and did not affect chromatographic separation.

The conditions of use for the thermospray interface, optimized from a direct injection of Modafinil (30 μl of a 5 mg/ml solution of Modafinil in the mobile phase) through the injector shunt, were as follows: control temperature: 128°C; vaporizer tip temperature: 250°C; tension of repeller: + 130 V and source temperature: 170°C. Mass spectra were scanned from m/z = 150 a.m.u. to m/z = 350 a.m.u.

RESULTS AND DISCUSSION

The mass spectrum of Modafinil (Fig. 2), obtained as described above, was characterized by an $[M+H]^+$ at m/z 274 (44%) and an $[M+NH_4]^+$ adduct at m/z 291 (100%). Note the presence of an ion at m/z 332 (5.5%) corresponding to M + 59. It is an $[M+(CH_3CN)NH_4]^+$ adduct resulting from reaction with a primary $(CH_3CN)NH_4{}^+$. The intensity of such an $[M+(CH_3CN)NH_4]^+$ depended on repeller voltage and source temperature [2].

Three fragments with lower m/z values gave structural information. The ion at m/z 167 (70%) was the result of cleavage from the SO group in the α position: it corresponded to the fragment $(C_6H_5)_2CH^+$. The ion at m/z 184 (6.1%) was the result of migration of the $(C_6H_5)_2CH$ group on the oxygen atom, followed by a cleavage at the S–O bound to give a secondary alcohol, $(C_6H_5)_2$ CH–OH$^+$ [3]. The fragment at m/z 225 (9.4%) resulted in a loss of SO [3]. The occurrence and the relative intensity of fragments 167–184 and 225 were closely bound to the value of the tension of repeller. The relative intensity of such fragments *versus* tension of repeller was a complex function depending on the fragment itself and on the analysed compound [4]. The importance of such optimization in thermospray LC–MS has already been pointed out [5].

The chromatographic conditions, described above, were directly derived from those previously optimized to analyse the purity of Modafinil in the Centre de Recherches du Laboratoire Lafon; only the mobile phase was different: acetonitrile–water (50:50, v/v). The addition of ammonium acetate as ionization reagent directly into the mobile phase did not modify the chromatographic separation, but the k' (capacity factor) value of impurity acid decreased to zero. To avoid this, the amount of acetonitrile in the mobile phase was reduced as described above.
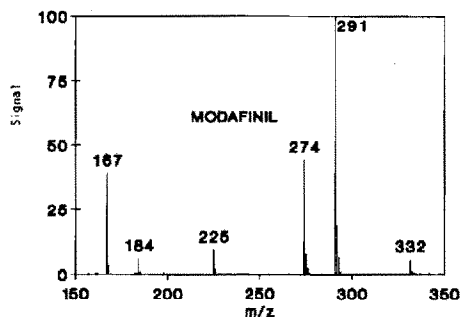

Fig. 2. Mass spectrum of Modafinil.

   The total ionic current, obtained after injection into the chromatographic col-
umn of 30 μl of a Modafinil solution (8 mg/ml in mobile phase) (Fig. 3a), showed
three peaks of impurity at retention time 1 min 36 s (impurity 1), 5 min 36 s (impurity
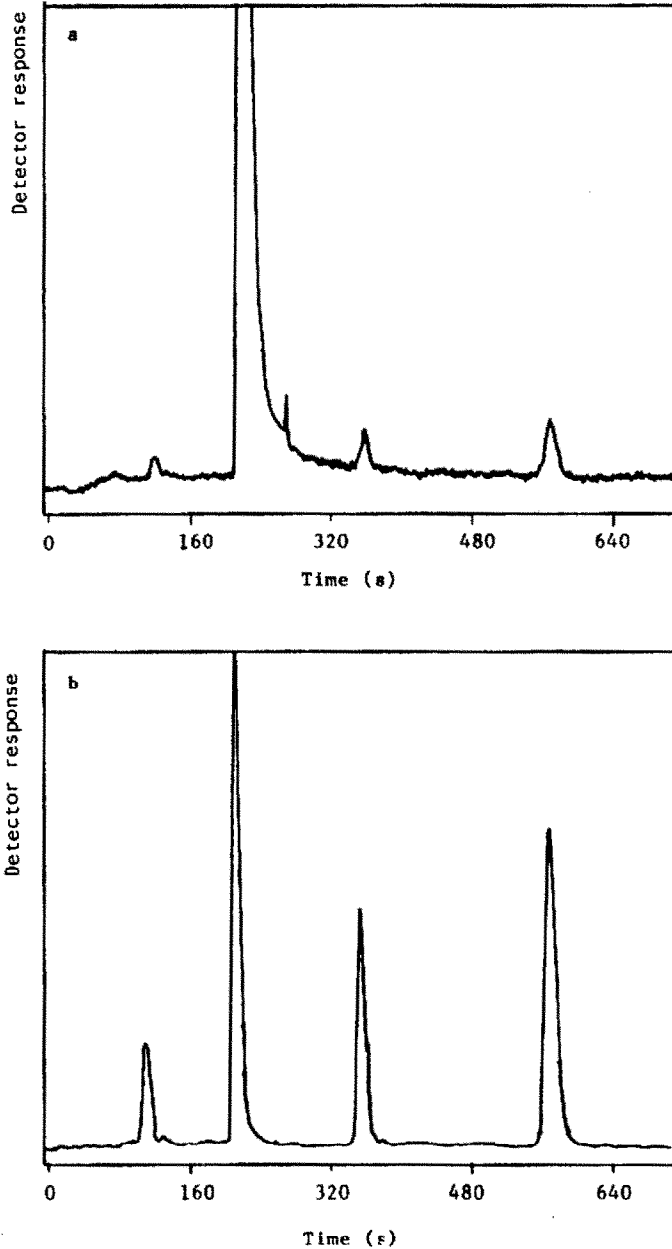2) and 9 min (impurity 3). The peak close to 3 min was an artefact due to perturba-

Fig. 3. Reconstructed chromatogram of Modafinil. Conditions: see text. (a) Modafinil solutions (240 μg
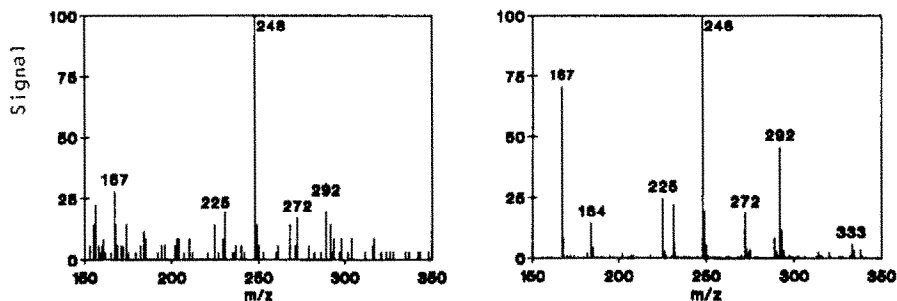injected). (b) Modafinil spiked with impurities.

Fig. 4. Mass spectra of impurity 1 (left) and acid (right).

tions created in thermospray regulation by the large amount of Modafinil to be vaporized. Its mass spectrum was exactly the same as that of Modafinil. Fig. 3b shows the total ionic current obtained after an injection of Modafinil (12.5 μg) spiked with acid (12.5 μg), sulphone (4 μg) and ester (7.5 μg). Retention times were, respectively, 1 min 48 s, 3 min 23 s and 5 min 39 s for acid, sulphone and ester. These values are quite similar to those of, respectively impurity 1, impurity 2 and impurity 3.

On the mass spectra of impurity 1 and acid (Fig. 4), the ions at m/z 167, 184 and 225, already described for Modafinil, appeared together with ions at m/z 231, 248 (100% on both spectra), 272 and 292. Despite the fact that the impurity 1 spectrum was largely noisy (because of the very small amount of this impurity), the two spectra were rather similar: the ion at m/z 292 corresponded to the $[M + NH_4]^+$ adduct. The ion $[M + H]^+$ at m/z 275, present in the acid spectrum (3.36%), was masked by the noise in the impurity 1 spectrum. In the same way, the $[M + (CH_3CN)NH_4]^+$ adduct appeared at m/z 333 only on the acid spectrum. Fragments at m/z 231 and 248 (100% in both cases) were due to decarboxylation of $[M + H]^+$ and the $[M + NH_4]^+$ adduct.

The mass spectra of impurity 2 and sulphone (Fig. 5) were identical. They were characterized by an $[M + NH_4]^+$ adduct at m/z 307 (100% in both cases) and an $[M + (CH_3CN)NH_4]^+$ adduct at m/z 348. The two spectra did not exhibit any important fragmentation at lower values: on the sulphone spectrum, the fragment at m/z 167 had relative abundance of only 0.34%. Whatever the working conditions of thermospray, no additional fragment occurred.

The mass spectra of impurity 3 and ester (Fig. 6) were quite similar. They gave the same kind of information as the Modafinil spectrum: three fragments at m/z 167,
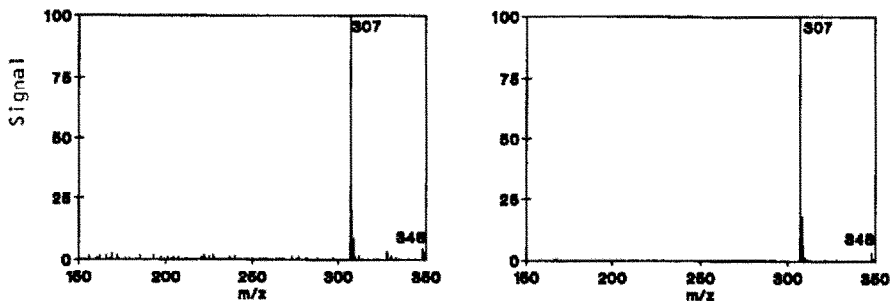

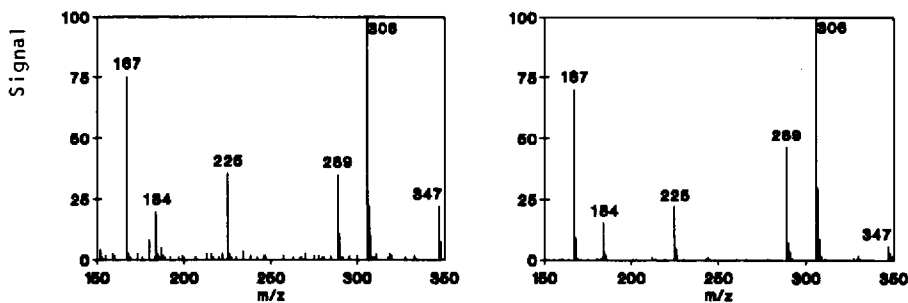Fig. 5. Mass spectra of impurity 2 (left) and sulphone (right).

Fig. 6. Mass spectra of impurity 3 (left) and ester (right).

184 and 225, an $[M + H]^+$ at m/z 289, an $[M + NH_4]^+$ adduct at m/z 347; their relative intensities exhibited the same kind of dependency on repeller voltage.

The results described above show the importance of identification of adducts for determining molecular mass. But, for a given set of thermospray working conditions, the presence of these adducts seemed to be bounded to molecular structure: all four studied compounds gave [M + 18] and [M + 59] adducts but sulphone did not exhibit an [M + 1] ion.

The most important result was to demonstrate the ability of thermospray HPLC–MS to obtain fragments lower than the molecular adduct and giving structural information. On first approximation, their relative intensity, related to the tension of the repeller, increased when the relative intensity of molecular and bigger adducts decreased [4].

On the other hand, under the conditions described above for the thermospray interface regulation, the mass spectra were rigorously reproducible from day to day. But any minor change in these conditions could drastically modify the profiles of spectra [4], some of the fragments or of the adducts being able to disappear from the spectra.

CONCLUSIONS

The results described above show the total similarity between impurity 1 and acid, impurity 2 and sulphone and impurity 3 and ester and confirm the structure of the impurities occurring in the synthesis of Modafinil. But the most important result of this work is probably the demonstration that the technique of LC–MS with thermospray interface is able to provide not only molecular mass information but also structural information if optimization of thermospray parameters can be achieved.

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