HPLC Monitoring of Flutamide Drug Used in the Prostate Cancer Treatment

M. Filip, V. Coman, V. Avram and I. Coman

Abstract: Flutamide (α,α,α-trifluoro-2-methyl-4′-nitro-m-propionotoluidide) is a non-steroidal compound with anti-androgenic properties. It is a chemotherapeutic drug used in the treatment of prostate cancer, its effects being responsible for the decreasing of the Prostate Specific Antigen (PSA) level in blood. The efficiency of the treatment with flutamide requires its optimal stability in blood, after that it is metabolised in liver. In this paper is presented a HPLC method elaborated for the determination of flutamide and their metabolites in blood in order to correlate its serum level with PSA values of patients with prostate cancer under the hormonotherapy. The HPLC chromatograms of studied samples were performed using a JASCO-980 liquid chromatograph with ultraviolet detection. The maximum of absorbance was determined on the basis of UV–Vis spectra recorded in the range of 190-600 nm with an UNICAM UV-4 spectrometer. The solutions of flutamide and of serum samples were prepared in acetonitrile. The compounds were eluted on a Nucleosil 120 C18 column with acetonitrile-water (60:40, v/v) as mobile phase and detected at 295 nm. The chromatograms and calibration curves were registered and processed by means of Borwin Soft. The obtained results demonstrated the utility of HPLC method in the monitoring of patients with prostate cancer under flutamide treatment.

Keywords: HPLC monitoring, flutamide drug, prostate cancer.

1. INTRODUCTION

Flutamide (α,α,α-trifluoro-2-methyl-4′-nitro-m-propionotoluidide) is a non-steroidal compound with anti-androgenic properties that acts in tissues via inhibition of androgen uptake and binding, forming inactive complexes with nuclear androgen receptor [1]. This medicine is used in the treatment of prostate cancer, having responsible effects for the decreasing of Prostate Specific Antigen (PSA) level in blood. The efficiency of flutamide treatment requires its optimal stability in blood, after that it is metabolised in liver [2]. The patients with prostate cancer under chronic intake of flutamide can become refractory to the therapy. This situation is called hormonoresistence and it is diagnosed observing a peak of PSA values requiring a second-line hormonotherapy [1,3].

The flutamide and their metabolites have been investigated by high performance liquid chromatography (HPLC) [4-8], thin layer chromatography [10], differential scanning calorimetry [10], infra-red spectroscopy [10], liquid chromatography-tandem mass spectrometry [11], photochemistry [12], spectrophotometric [13], voltammetric [14] and electroanalytical methods [9].

The aim of this paper is the HPLC monitoring of flutamide drug and their metabolites in blood in order to correlate the serum level of flutamide with PSA values of patients with prostate cancer under hormonotherapy.

2. EXPERIMENTAL

2.1. Equipment

The chromatograms of studied samples were performed using a high performance liquid chromatograph (JASCO-980). This HPLC system is equipped with a pump (Model PU-980), a low-pressure gradient unit (Model LG-980-02), an in-line degasser (Model DG-980-50) and an UV-Vis detector (Model UV-970/975). The maximum of absorbance of flutamide was determined with an UV-Vis spectrometer (UNICAM UV-4) equipped with photomultiplier detection.

2.2. Materials

Flutamide capsules (USP), 125 mg/capsule, were purchased from Salutus Pharma GmbH, Barleben, Germany [15]. Double distillated water was prepared in laboratory. Methanol and acetonitrile were purchased from E. Merck (Darmstadt, Germany). The serum samples were obtained by centrifugation of the blood samples prelevated from the patients with prostate cancer which were administrated one dose of flutamide (250 mg/dose) at each 8 hours: Patient A (78 years old) – localized prostatic cancer intraglandular (T2b), without limphnode (N0) or bone (M0) metastases; PSA – 10.3 ng/mL. Patient B (75 years old) – infiltrative prostatic cancer (T4a), with limphnode (N2) and bone (M1) metastases; PSA – 27 ng/mL.
methastases; PSA = 106 ng/mL. Both patients had normal weight and hepato-renal functions and also agressive histopathologic forms, Gleasson index value being 8. The therapeutic decision for the two patients was the same, namely bilateral radical orchidectomy and anti-androgenes drugs (flutamide, 3 x 250 mg/day).

2.3. Sample preparation

The PolySpher™ RP18-Cat (100 mg) cartridges were used for the preparation of serum samples for injection in HPLC. After the cartridge conditioning with acetonitrile and double distilled water, the serum was introduced into the cartridge and eluted with acetonitrile. The obtained eluate was passed through a special syringe filter (PTFE 0.45 µm, TRACER) to avoid the presence of some possible solid particles.

3. RESULTS AND DISCUSSION

For the HPLC registerings it was necessary to establish the optimum range of the UV detection. Maximum of absorbance of flutamide was determined on the basis of UV–Vis spectra recorded in the range of 190-600 nm. These spectra were processed with the Vision Soft. The UV-Vis spectrum of flutamide presented in Figure 1 shows the maxima of absorbance at 205, 227, 296 nm.

![Figure 1. The UV-Vis spectrum of flutamide (c = 10^-4M in methanol).](image1)

All HPLC chromatograms were registered at 295 nm. The solutions of flutamide and serum samples prepared in acetonitrile were manually injected into the liquid chromatograph with a 100 µL Hamilton Rheodyne syringe through a valve of 20 µL loop volume. The compounds were eluted on a Nucleosil 120 C18 column (5µ, 25 x 0.4 cm) with the acetonitrile-water (60:40, v/v) mobile phase at a flow rate of 1 mL/min. The chromatograms and the calibration curves of studied samples were registered and processed by means of Borwin Soft.

In Figure 2 and 3 are presented the HPLC chromatogram and the calibration curve of flutamide drug respectively.

![Figure 2. The HPLC chromatogram of flutamide from capsule.](image2)

![Figure 3. The calibration curve of flutamide from capsule.](image3)

The calibration curve was obtained by plotting peak area versus a known, injected amount of compound, using 8 points in the 0.0125 - 0.6250 mg/mL range.

In Figure 4 are presented the HPLC chromatograms of serum samples prelevated during 24 hours from the patient A. The chromatograms of samples 2-4 put in evidence some metabolites that have been formed at the patient under treatment. According to the literature data [4,16] the three metabolites of flutamide (metabolite I, II and III respectively) have the retention times before that of flutamide. The metabolite I (2-hydroxy-flutamide) is identified in the HPLC chromatograms of biological samples as the first peak before that of flutamide [4]. A comparative study of the HPLC chromatograms indicates the presence of flutamide after 4 hours of the intake of the first dose, when the flutamide is accumulated in blood (Sample 2).

<table>
<thead>
<tr>
<th>Table 1. HPLC parameters of flutamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Retention time</td>
</tr>
<tr>
<td>Detection limit</td>
</tr>
<tr>
<td>Regression factor</td>
</tr>
</tbody>
</table>

In Figure 4 are presented the HPLC chromatograms of serum samples prelevated during 24 hours from the patient A. The chromatograms of samples 2-4 put in evidence some metabolites that have been formed at the patient under treatment. According to the literature data [4,16] the three metabolites of flutamide (metabolite I, II and III respectively) have the retention times before that of flutamide. The metabolite I (2-hydroxy-flutamide) is identified in the HPLC chromatograms of biological samples as the first peak before that of flutamide [4]. A comparative study of the HPLC chromatograms indicates the presence of flutamide after 4 hours of the intake of the first dose, when the flutamide is accumulated in blood (Sample 2).
The selected cases are different by the tumour cell mass that is reduced at the patient A in comparison with the patient B. Although the therapeutic doses administered have been the same, the found again flutamide quantities are different, that suggest the flutamide pharmacokinetics is strong correlated to the tumour cell mass.

In Figure 5 it can be observed a non-complete elimination of flutamide at 8 hours from administration. In the rhythm of 3 x 250 mg/day intake it can appear a flutamide accumulation (Sample 4) that during long time administrations can become efficient in the blocking of the androgen receptors without a supplementary increase of an every day dose.

The presented results suggest the necessity to extend the study over a greater number of patients and a prolonged monitoring of the flutamide treatment. The periodic dosage of serum flutamide and/or of its metabolites should permit a particularization of doses for a better therapeutic efficiency.

4. CONCLUSION

The obtained results show the possibility to apply the HPLC method to the monitoring of flutamide treatment at the patients with prostate cancer in order to establish and to increase the efficiency of this drug.

5. ACKNOWLEDGMENTS

The results presented in this paper come and are financially supported from the project CEEX 63 / 2005-2008, program VIASAN, in the frame of the Romanian Program of Excellence Research.

6. REFERENCES


[15] ***, Flutamide capsules UPS (Prospect), 125mg, Rx only, Manufactured by Salutas Pharma GmbH Barleben, Germany.