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Short communication

Automated determination of flutamide by a validated flow-injection method: Application to dissolution studies of pharmaceutical tablets

Paraskevas D. Tzanavaras [a,*], Demetrius G. Themelis [b]

[a] Cosmopharm Ltd., Quality Control Department, P.O. Box 42, Korinthos 20100, Greece
[b] Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

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Abstract

The first flow-injection (FI) method for the determination of flutamide – a potent antiandrogen used for the treatment of prostate cancer – is reported. The method is based on the direct measurement of the absorbance of the analyte at 310 nm under flow conditions. Parameters affecting the determination such as detection wavelength, sample injection volume and flow rate were studied and optimized. The assay was validated (linearity, limits of detection and quantitation, accuracy, repeatability, reproducibility and selectivity) for the dissolution studies of flutamide-containing tablets during stability testing. The results were in good agreement with high performance liquid chromatography (HPLC) used as a reference method.
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1. Introduction

Flutamide (chemical structure shown in Fig. 1) is a chemical compound used to treat prostate cancer. It belongs to a class of drugs known as antiandrogens. Its action is based on blocking the effect of the male hormone, testosterone, which supplies are essential for the growth of prostate cancers. As flutamide has a structure similar to testosterone, it works by attaching itself to the receptors on the surface of the cancer cells to block and prevent the attachment of the male hormone. For some forms of prostate cancer, radiation therapy is given along with the drug [1,2].

Treatment with flutamide may cause a variety of side-effects that depend on the reaction of each organism to the medication. Most common side-effects include diarrhea, tiredness, impotence and breast fullness. A less common but more serious side-effect is liver malfunction, since flutamide can sometimes cause a change in the amount of particular chemicals produced by the liver. Although in most cases liver function returns to normal after stopping the administration of the drug, in rare cases death has been reported [1,2].

A key demand of quality control in the pharmaceutical industry is rapidity in analysis, without sacrificing accuracy, precision and reliability of the results. This an especially important feature when a lot of samples have to be analyzed in the minimum of time. A typical example is the analysis of the samples produced by dissolution control of pharmaceuticals during new drug development or routine manufacturing processes. Dissolution both as an in-process control and as a test of the finished product is very important since the *in vitro* dissolution behavior of a formulation may be related to the *in vivo* performance of a drug product [3]. In order to create the dissolution profile of a formulation (e.g. at four time intervals) 6 or 12 tablets must be processed, producing 24–48 samples. High performance liquid chromatography (HPLC) which is the most widely used analytical technique in the QC of pharmaceuticals requires two to three injections per sample. Forty-eight to 144 HPLC injections are, therefore, required to create the dissolution profile of a formulation. Based on the fact that a typical separation/detection cycle in HPLC is completed in several minutes, a more rapid but equally effective alternative analytical approach is wanted. Flow-injection (FI) analysis is an interesting and advantageous technique to the quality control of pharmaceutics. Especially when combined to spectrophotometric detection, it offers simplicity, rapidity, affordable and widely available instru-

* Corresponding author. Fax: +30 2741071685.
E-mail address: paristzanavaras@gmail.com (P.D. Tzanavaras).

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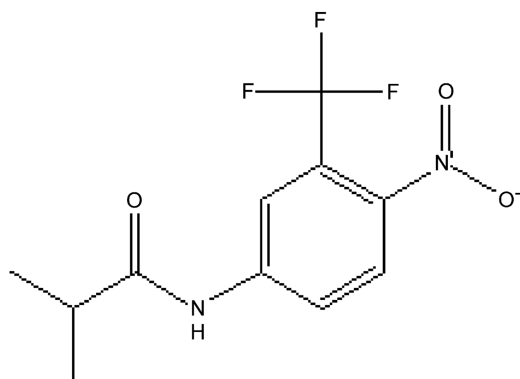

Fig. 1. Chemical structure of flutamide.

mentation and precision and accuracy that compare favorably to HPLC.

Methods reporting the determination of flutamide in pharmaceutical formulations include batch spectrophotometry [4–10], HPLC [11–13] and electroanalysis [13–15]. Batch spectrophotometric methods are generally simple and cost-effective, but time-consuming as well. Regarding flutamide analysis, Zarapkar et al. [4] were the first to report a photometric assay for flutamide based on the development of yellow color when the drug is dissolved in HCl. However, the proposed method requires heating, while key analytical data is missing (sensitivity, selectivity, etc.). Nagaraja et al. [5] reported the use of *N*-(1-naphthyl)ethylendiamine (NEDA) in neutral and resorcinol in basic medium as chromogenic reagents for the determination of flutamide. The method is unattractive to QC of pharmaceutics since: (a) pre-reduction of the analyte using zinc in acidic medium is required, (b) reaction development involves several time-consuming steps and (c) the determination ranges are narrow requiring 100-fold dilution of the dissolution samples prior to analysis. Pre-reduction of both standards and samples is also required in methods [6–8]. Especially in the procedure proposed by Rangappa et al., apart from the chromogenic reaction, two-step solvent extraction using chloroform is involved prior to the final measurement. Additionally, the determination range achieved does not allow direct analysis of the samples [6]. Finally, an indirect method developed by Murthy et al. [9] involves a time-consuming two-step process, including oxidation of the analyte by potassium permanganate and subsequent determination of its excess by fast green FCF. Electroanalytical procedures based on direct current (DCP) [14] or differential pulse (DPP) polarography [13] are generally sensitive but employ ethanolic solutions of samples and standards which are not compatible to the aqueous dissolution medium. On the other hand, the retention times of flutamide using HPLC range between 8 and 10 min making this technique not attractive for dissolution studies, where a lot of samples have to be processed [11–13]. To the best of our knowledge no flow-injection method has been reported so far for the determination of flutamide.

The present study reports the first FI method for the determination of flutamide. The method is based on the direct measurement of the absorbance of the analyte at 310 nm under flow conditions. Optimization of parameters affecting the determination such as detection wavelength, sample injection volume and flow rate was carried out, while the method was validated thoroughly in terms of linearity, limits of detection and quantitation, accuracy, repeatability, reproducibility and selectivity. Compared to previously reported methods for the determination of flutamide, the developed FI procedure offers low cost, rapidity and no complicated procedures prior to analysis. The proposed assay was applied to the dissolution studies of flutamide-containing tablets during stability testing. Samples from dissolution experiments were analyzed directly, without any additional pretreatment. The results were compared to an in-house validated HPLC reference method and were found to be in good agreement.

2. Experimental

2.1. Reagents and chemicals

Flutamide working standard was provided by Sigma. A standard stock solution (400 mg L^{-1}) was prepared by dissolving 40.0 mg of the working standard in a 2% (w/v) aqueous sodium lauryl sulfate (SLS) solution. Working standard solutions (100–400 mg L^{-1}) were prepared by dilution of the stock in the same medium. All standard solutions were kept refrigerated (at 4 °C) and protected from light when not in use.

SLS was also provided by Sigma. A 2% (w/v) stock SLS solution was prepared by dissolution of the appropriate amount of the reagent in HPLC grade water (Merck). This solution was filtered through 0.45 μm nylon membrane filters (Whatman) and degassed ultrasonically for 30 min prior to use.

The pharmaceutical excipients used in the selectivity and accuracy studies (microcrystalline cellulose, maize starch, lactose monohydrate, sodium lauryl sulfate, silica colloidal anhydrous and magnesium stearate) were provided by domestic suppliers. The composition of the placebo mixture (all excipients except for the active ingredient) was: 200 mg g^{-1} microcrystalline cellulose, 320 mg g^{-1} maize starch, 440 mg g^{-1} lactose monohydrate, 30 mg g^{-1} sodium lauryl sulfate, 2 mg g^{-1} silica colloidal anhydrous and 8 mg g^{-1} magnesium stearate.

The dissolution medium consisted of a 2% (w/v) SLS aqueous solution. Doubly de-ionized water ($\kappa < 3.6$ μS) was used for this purpose. The dissolution medium was degassed under vacuum prior to use and kept at 35 °C to avoid re-aeration.

2.2. Instrumentation

The single-channeled FIA setup is depicted in Fig. 2. The hardware (pump, injection valve, autosampler and detector) of an HP1100 instrument (Agilent Technologies) was used throughout the experiments. In FI experiments the injection valve of the instrument was connected directly to the DAD detector *via* a 10-cm long/0.28 mm i.d. PTFE mixing coil, while a Hypersil BDS C_{18} column (250 mm × 4 mm/5 μm) was used instead for HPLC measurements. Data acquisition (peak height and/or peak area, signal-to-noise ratios, etc.) was performed *via* Chem Station® software.

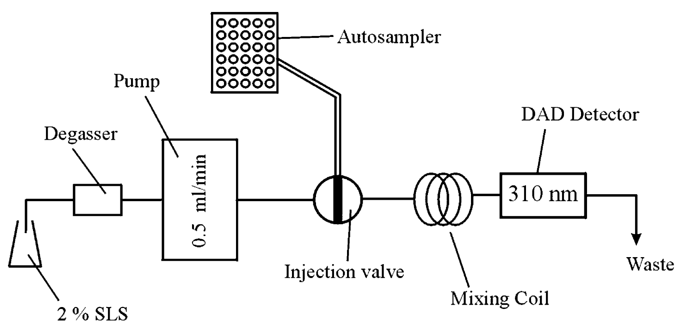

Fig. 2. Flow-injection manifold for the determination of flutamide.

Dissolution experiments were carried out using a Distek Premiere 5100 system equipped with a programmable autosampler.

A vacuum filtration system (Schleicher and Schuell) and 0.45 μm nylon membrane filters (Whatman) were used for the filtration of the carrier stream (FI) and the mobile phase (HPLC). A model 19H ultrasonic bath (Ney-ultrasonics) and a model HI190M magnetic stirrer (Hanna Instruments) equipped with Teflon coated magnets (20 mm × 3 mm) were used throughout this study.

2.3. FI procedure for aqueous solutions

Ten microliters of flutamide standards (100–400 mg L^{-1}) or samples from dissolution experiments were injected *via* the autosampler of the HP1100 instrument to the carrier stream (2% (w/v) SLS in water). The sample zone was propelled at a flow rate of 0.5 mL min^{-1} through a 10-cm long mixing coil towards the diode-array photometric detector (λ_{max} = 310 nm). Peak area was used for quantitative measurements, while each standard/sample was injected in triplicate. Sharp peaks and stable base-line were observed in all cases. The sampling rate was 30 h^{-1}.

2.4. Dissolution of flutamide-containing tablets

In each dissolution experiment, 12 flutamide-containing tablets were weighed and introduced to the dissolution apparatus in batches of six. The dissolution profile of the formulation was recorded *via* automated sampling at 10, 20, 30 and 60 min. The temperature was kept constant at 37.0 ± 0.5 °C and the volume of the dissolution medium was 1000 mL in all cases. The rotation speed of the paddles was 100 rpm, while the withdrawn aliquots were filtered in-line using 45 μm PTFE disc filters. The resulting samples were analyzed by the proposed FI procedure mentioned above without any additional pretreatment.

2.5. HPLC analysis

The results obtained by the proposed FI method were compared to an in-house-validated HPLC assay for the determination of flutamide. The mobile phase consisted of a mixture of ACN and HPLC grade water (50:50 v/v). The flow rate was 1.5 mL min^{-1}, the sample injection volume 20 μL, the column temperature 25 °C and the detection wavelength 295 nm. A Hypersil BDS C_{18} column (250 mm × 4 mm/5 μm) was used throughout the experiments. Under the above-mentioned conditions, the retention time of the analyte was 8.8 min and the duration of the separation/detection cycle 12 min. Aliquots of the samples collected from the dissolution apparatus were analyzed by HPLC without any further pretreatment. Peak area was used for quantitative measurements, while each standard/sample was injected in triplicate.

3. Results and discussion

3.1. Preliminary studies

Preliminary experiments had two objectives. The first was to determine the optimal detection wavelength and the second to examine the most effective way for signal processing (peak area versus peak high). It should also be mentioned that a carrier stream identical to the dissolution medium (2% (w/v) SLS aqueous solution) was used throughout this study in order to avoid potential matrix effects.

Flutamide spectra were recorded on line by the diode array detector and a typical example can be seen in Fig. 3 (400 mg L^{-1} flutamide, V = 10 μL, Q = 1.0 mL min^{-1}). A wavelength of 310 nm was selected for further studies.

Signals processing using peak area proved to be advantageous over peak height in terms or repeatability. Under the experimental conditions mentioned above, the relative standard deviations were 0.5% for peak area and 4.2% for peak height evaluation (n = 12 in both cases). For this reason peak area was selected as the preferred approach for signals evaluation.

3.2. Study of FI variables

FI variables studied were the sample injection volume and the flow rate of the carrier stream. The starting values of these variables were those mentioned in the previous section. The study was carried out at three flutamide mass concentrations, namely 100, 240 and 400 mg L^{-1}.

3.2.1. Effect of sample injection volume

Sample injection volume is a critical parameter when optimizing a FI method. As it is inversely proportional to dispersion, it affects the sensitivity, linearity and even the sampling rate

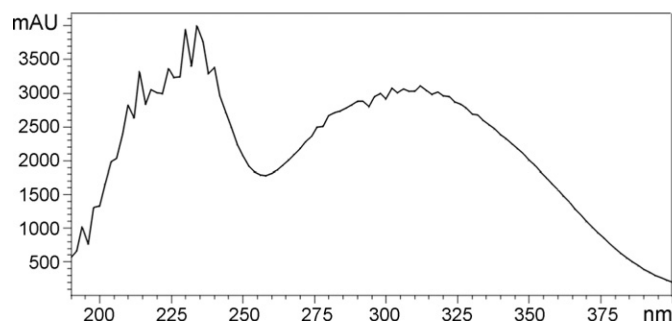

Fig. 3. UV-spectrum of flutamide under flow conditions (see text for experimental details).

of a determination by FI. As expected, variation of the sample injection volume in the range of 5–20 μL resulted in increase of the sensitivity. However, better linearity was obtained at an injection volume of 10 μL. At this value repeatability was also slightly better compared to 5 μL (R.S.D. of 0.45% versus 1.1% at 240 mg L^{-1}, $n = 12$). In all cases the sampling frequency was not affected by the sample injection volume, as it was determined by the operating characteristics of the autosampler (30 h^{-1}). A value of 10 μL was selected for further studies.

3.2.2. Effect of the flow rate

The flow rate of the carrier stream is not expected to have great impact on sample dispersion in single-channeled FI manifolds. This was confirmed experimentally, since variation of the flow rate in the range of 0.5–1.5 mL min^{-1} resulted in only slight decrease of the signals, while linearity and repeatability remained unaffected. Although flow rate has usually great impact on sampling rate, for the reasons mentioned in the previous section the latter was not affected. A value of 0.5 mL min^{-1} was selected as optimal in terms of minimum waste generation.

3.3. Validation of the FI method

The developed FI method was validated for linearity, limits of detection (LOD) and quantitation (LOQ), precision (repeatability and reproducibility), selectivity and accuracy.

3.3.1. Linearity, LOD and LOQ

The theoretically "expected" concentration of flutamide after the dissolution experiments is ca. 250 mg L^{-1} (250 mg flutamide per tablet in 1000 mL dissolution medium assuming quantitative dissolution). In order to bracket effectively the above-mentioned concentration, linearity was validated in the range of 100–400 mg L^{-1} (40–160% of the target concentration, $n = 6$). Peak area versus analyte mass concentration was found to obey the linear regression equation ($r^2 > 0.9999$):

$$A = 8.61\ (\pm 0.06) \times \gamma(\text{flutamide}) + 43.57(\pm 14.99)$$

Validation of the regression line was performed by the response factor (r.f.) test [16]. The deviation of the r.f. of each point of the calibration curve from the experimental slope must be within ±3% and is given by the equation:

$$\text{r.f.} = \frac{\text{peak area} - \text{intercept}}{\gamma[\text{flutamide}]}$$

The experimental results conformed to the above mentioned limit (±3%), since the deviations of all points of the calibration curve from the slope of the corresponding regression equation were <±2.0%.

The detection (LOD) and quantitation limits (LOQ) of the assay were determined based on the S/N criteria. The respective values were found to be 0.12 mg L^{-1} (S/N = 3) and 0.4 mg L^{-1} (S/N = 10), respectively.

3.3.2. Repeatability and intermediate precision

The repeatability (within-day precision) of the proposed FI method was validated by calculation of the relative standard deviations (R.S.D.s) of the peak areas from 12 consecutive injections at three flutamide standard solutions (100, 250 and 400 mg L^{-1}) at the beginning, middle and end of a working day. The calculated R.S.D.s were <0.7% in all cases.

The intermediate precision of the FI method (day-to-day precision) was validated by constructing six calibration curves (100–400 mg L^{-1} flutamide × 6 concentration levels) within a period of 30 days. The experimental results verified the day-to-day precision of the assay, since the R.S.D. of the slopes of the calibration curves was 3.9% ($n = 6$).

3.3.3. Selectivity and accuracy

The selectivity of the proposed FI against the pharmaceutical excipients used for the manufacturing of the flutamide-containing tablets was evaluated using a placebo mixture (i.e. all excipients except for the active ingredient). The composition of the placebo is described in detail in Section 2.1. The experiments were carried out by adding suitable amounts of the placebo to flutamide standard solutions (250 mg L^{-1}). The resulting suspensions were ultrasonicated for 15 min and filtered through 0.45 μm disposable syringe filters prior to analyses. Up to 1000 mg L^{-1} of the placebo (maximum concentration tested) could be tolerated by the proposed method. The criterion for interference was a relative error of >3% at the flutamide mass concentration level mentioned above. It should be noted that the above-mentioned tolerated placebo concentration is very satisfactory, since the theoretical concentration of the excipients during dissolution experiments is ca. 500 mg L^{-1}.

The accuracy of the procedure was validated by analyzing synthetic samples – containing 1000 mg L^{-1} of placebo – spiked with different amounts of flutamide (final flutamide mass concentrations of 100, 250 and 400 mg L^{-1}). The above-described ultrasonication–filtration procedure was followed prior to each synthetic sample analysis. The experimental results are shown in Table 1. The percent recoveries were satisfactory in all cases, ranging between 98.9 and 100.7%.

3.4. Analytical applications

The proposed FI method was applied to the analyses of filtrates from dissolution experiments of flutamide-containing tablets during production and stability quality control. A typical dissolution profile is depicted in Fig. 4. The experimental results covering a period of 9 months are presented in Table 2. All results were within the specifications of the finished product (percent

Table 1
Accuracy of the FI method

Synthetic sample	Placebo added (mg L^{-1})	Flutamide added (mg L^{-1})	Recovery (±S.D.) (%)
FL1	1000	100.0	99.7 (±0.6)
FL2	1000	100.0	98.9 (±0.5)
FL3	1000	250.0	100.1 (±0.8)
FL4	1000	250.0	100.7 (±0.8)
FL5	1000	400.0	99.1 (±0.7)
FL6	1000	400.0	99.3 (±0.6)

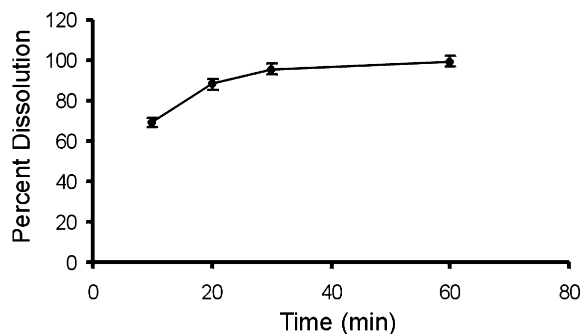

Fig. 4. Typical dissolution profile of flutamide-containing tablets (see text for experimental details).

Table 2
Dissolution stability of flutamide-containing tablets

Batch	Percent dissolution (30 min)[a]	
	FIA	HPLC
003 (production)	95.6 (±2.9)	94.6 (±1.9)
003 (3 months LTS[b])	94.2 (±3.5)	94.8 (±2.5)
003 (3 months ACS[c])	96.2 (±2.4)	95.2 (±2.2)
003 (6 months LTS)	95.3 (±2.7)	96.4 (±3.0)
003 (6 months ACS)	94.8 (±2.0)	93.9 (±2.3)
003 (9 months LTS)	94.6 (±1.8)	95.8 (±2.9)

[a] Mean percent dissolution (±S.D.) of 12 tablets per batch or test.
[b] Long term stability (at $T = 25.0\,^{\circ}\mathrm{C}$ and RH = 60%).
[c] Accelerated stability (at $T = 40.0\,^{\circ}\mathrm{C}$ and RH = 75%).

dissolution of >80% after 30 min) and in good agreement with HPLC based on the *t*-test.

4. Conclusions

The present study reports the first flow-injection method for the determination of the active pharmaceutical compound flutamide. The developed procedure is simple and effective, offering significant advantages over previously reported methods for the determination of the analyte. Thorough validation of the method yielded excellent results in terms of in all key parameters such as linearity, precision, selectivity and accuracy. Compared to an HPLC assay previously used in-house for the same purpose, the FI procedure is 6 times faster (sampling rate of $30\,h^{-1}$ versus $5\,h^{-1}$) and generates 18 times less wastes per injection (1 mL versus 18 mL, respectively). Additionally, no organic solvents are employed. The results obtained from the application of the FI method to the dissolution studies of flutamide-containing tablets were in good agreement with HPLC.

References

[1] http://www.cancerbackup.org.uk.
[2] Health Square. URL: http://www.healthsquare.com.
[3] F. Gudrun, Drug Inform. J. 35 (2001) 865–874.
[4] S.S. Zarapkar, C.D. Damle, U.P. Halkar, Indian Drugs 33 (1996) 193–194.
[5] P. Nagaraja, K.R. Sunitha, M.F. Silwadi, J. Pharm. Biomed. Anal. 23 (2000) 617–622.
[6] K.S. Rangappa, P. Nagaraja, K.C.S. Murthy, Anal. Sci. 16 (2000) 637–639.
[7] P. Nagaraja, H.R.A. Kumar, R.A. Vasantha, H.S. Yathirajan, Int. J. Pharm. 325 (2002) 113–120.
[8] G.D. Rao, Asian J. Chem. 16 (2004) 1769–1772.
[9] T.K. Murthy, M.N. Reddy, M. Dharma Reddy, D.G. Sankar, Asian J. Chem. 13 (2001) 915–918.
[10] M.N. Reddy, T.K. Murthy, M. Dharma Reddy, D.G. Sankar, Asian J. Chem. 13 (2001) 1261–1262.
[11] A. Miranda, I. Caraballo, M. Millan, Drug Develop. Ind. Pharm. 28 (2002) 413–422.
[12] H.R.N. Salgado, M. De Menezes, M.P.B. Storti, Acta Farm. Bonaerence 24 (2005) 246–249.
[13] A. Alvarez-Lueje, C. Pena, L.J. Nunez-Vergara, J.A. Squella, Electroanalysis 10 (1998) 1043–1051.
[14] A. Snycerski, J. Pharm. Biomed. Anal. 7 (1989) 1513–1518.
[15] E. Hammam, H.S. El-Desoky, K.Y. El-Baradie, A.M. Beltagi, Can. J. Chem. 82 (2004) 1386–1392.
[16] J.M. Green, Anal. Chem. 68 (1996) 305A–309A.