THE VALIDATION OF THE UV SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF 5 FLUOROURACIL

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Abstract

In order to determine the release profile of 5 – fluorouracil (5-FU) from modern pharmaceutical dosage forms with vaginal administration during *in vitro* dissolution tests, an UV spectrophotometric assay method was developed, in acetate buffer solution (pH = 4.3). The working conditions were established with an absorbance maximum at λ = 266 nm. The method was validated by determining the following parameters: the linearity in the chosen concentration domain (1.02 to 20.40 µg/mL), the correlation coefficient r^2 = 0.9992, the detection limit (LD) was 0.59 µg/mL, the quantification limit (LQ) was 1.79 µg/mL, the precision of the method (RSD = 0.5176 %), the intermediate precision of the method (RSD = 0.5521 %) and the accuracy of the method for which we obtained a mean recovery of 99.95%. In conclusion, this assay method for 5 - FU is simple, easy to apply, sensitive, linear, precise and accurate.

Rezumat

Pentru evaluarea profilului de cedare a 5 – fluorouracilului (5-FU) din forme farmaceutice moderne cu administrare vaginală, în cadrul testelor de dizolvare *in vitro*, s-a dezvoltat o metodă de dozare spectrofotometrică în UV, în soluție tampon acetat (pH = 4,3). Au fost stabilite condițiile de lucru, cu un maxim de absorbție la λ = 266 nm. Metoda a fost validată prin determinarea următorilor parametri: liniaritatea în domeniul de concentrație ales (1,02 – 20,40 µg/mL), coeficientul de corelație r^2 = 0,9992, limita de detecție (LD) este de 0,59 µg/mL, limita de cuantificare (LQ) este de 1,79 µg/mL, precizia metodei (RSD = 0,5176 %), precizia intermediară a metodei (RSD = 0,5521 %) și acuratețea metodei pentru care s-a obținut o valoare medie a regăsirii de 99,95 %. În concluzie, această metodă de dozare a 5 – FU este simplă, ușor de aplicat, sensibilă, liniară, precisă și exactă

Keywords: 5 – fluorouracil, UV spectrophotometry, validation.

Introduction

5-Fluorouracil (5-FU) is a next-generation antiviral drug, administered as vaginal topical treatment of vaginal lesions associated with the *Human papilloma* virus [3,7], including *Condylomata acuminata* [5] and

cervical intraepithelial neoplasia [7]. From the chemical point of view, 5 - FU is a fluoropyrimidine derivative, namely 5-fluoro-1 H-pyrimidine-2, 4-dione (Fig. 1).

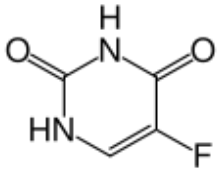

Figure 1.
The chemical structure of 5-FU

In the cervical-vaginal disease therapy, 5-FU is used in several classical pharmaceutical dosage forms such as gels, creams, suppositories, as well as in some modern pharmaceutical dosage forms such as liposomes, niozomes, nanoparticles, microemulsions and vaginal films [1, 4, 6, 8, 9, 11]. Several analytical methods have been previously reported for the determination of 5 - FU, including assays based on gas liquid chromatography, and reversed-phase high-performance liquid chromatography [2,10]. The objective of this study was to develop and validate a new method for the quantitative determination of 5 - FU by UV spectrophotometry. This method will be used to assess the *in vitro* release profile of 5 - FU in various modern pharmaceutical dosage forms with vaginal administration that are under research in our department.

Materials and Methods

Materials

5 - Fluorouracil purity > 99% (Sigma Aldrich, Germany), *sodium acetate* and *glacial acetic acid* (Merck, Germany) were used.

Solutions: The *acetate buffer solution (pH = 4.3)* was obtained by dissolving a quantity of 1.99 g $CH_3COONa \cdot 3H_2O$ and 17 mL CH_3COOH 2N in a volumetric flask of 1,000 mL; the s*tandard solution* 5 - FU in *acetate buffer* (pH = 4.3) was obtained by dissolving a quantity of 0.1020 g of 5 - FU (reference substance) in a volumetric flask of 100 mL; the *stock solution of* 5 - FU was obtained by diluting 5 mL acetate buffer standard solution (pH = 4.3) in a 50 mL volumetric flask.

Methods

The principle of the method: a stock solution of 5 - FU in acetate buffer solution pH = 4.3 was subjected to the UV spectrophotometric analysis at a wavelength of 266 nm in a 1 cm cell *versus* a blank solution consisting of acetate buffer pH = 4.3.

Validation of the method

Establishing the wavelength: a sample was prepared as previously described and was submitted to the spectrophotometric analysis, then the absorption spectrum between 190-400 nm was recorded, and compared with the acetate buffer pH=4.3 in a 1 cm cell. A peak was noticed at λ = 266nm.

Linearity: Three sets of solutions containing 5 - FU were prepared in the concentration range of 1.02 - 20.40 mg/mL. For each set we separately measured the absorbance at λ = 266 nm and calculated the mean value. The obtained data were analyzed by linear regression (the calibration curve equation for this concentration range, the correlation coefficient (r), the regression coefficient (r^2), the standard error (SE) were calculated) and afterwards the calibration curve was drawn.

The limits of detection (LD) and quantification (LQ) were calculated using the following equations:

LD = 3.3 x standard error / slope Eq. 1

LQ = 10 x standard error / slope Eq. 2

Method precision: we used the stock solution of 5 - FU to prepare three solutions with the following three concentrations: 7.14, 10.20 and 13.26 µg/mL 5 - FU. For each level of concentration, three samples were prepared and analyzed, and the absorbances of the obtained samples were measured experimentally.

Intermediate precision: the entire experiment was repeated on the following day; different reagents were used, with identical solutions and procedures as for the determination of the accuracy of the method. For each concentration, three samples were prepared and analyzed.

Method accuracy was assessed by the addition method: the stock solution of 5 – FU was used for preparing three solutions with the following three concentrations: 7.14, 10.20 and 13.26 µg/mL 5 - FU. The obtained solutions were analyzed spectrophotometrically following the described method. For each concentration, three samples were prepared and analyzed. Using the equation of the calibration line and the values of the corresponding absorbances, we calculated the concentration (expressed in µg/mL) for each sample separately. We determined the recovery, calculated as a percentage of the value of the theoretical concentration, mean recovery, as well as the domain where recovery varies.

Results and Discussion

Figure 2 presents the absorption spectrum for 5 - FU. Two peaks of absorption can be seen at λ = 211 and λ = 266 nm. For subsequent determinations we used the absorption peak at λ = 266 nm.

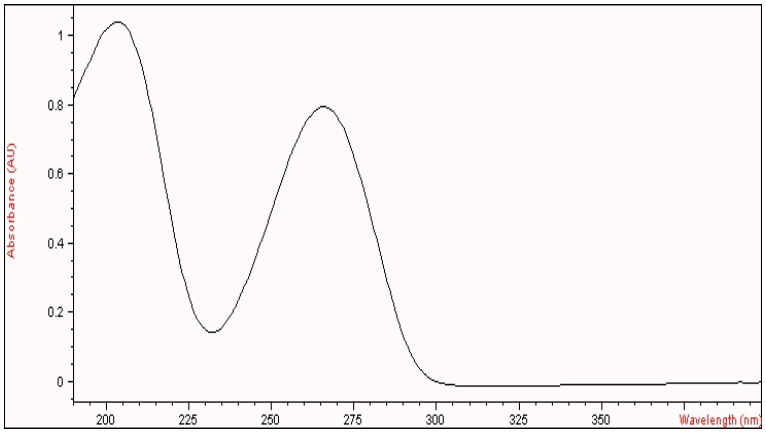

Figure 2.

Absorption spectrum of 5-fluorouracil in acetate buffer (pH = 4.3)

The statistical analysis of the data obtained in the determination of the linearity of the method is presented in Table I. The calibration curve and equation curve are presented in figure 3.

Table I.

Method linearity

5-FU µg/mL	Absorbance			
	I	II	III	Average
1.02	0.04269	0.04312	0.04289	0.04290
2.04	0.07435	0.07393	0.08392	0.07740
3.06	0.14104	0.14048	0.14049	0.14067
4.08	0.17842	0.17862	0.17822	0.17842
5.10	0.20934	0.20954	0.20890	0.20926
6.12	0.24849	0.24868	0.24953	0.24890
7.14	0.29347	0.29274	0.29192	0.29271
8.16	0.34316	0.34373	0.34376	0.34355
9.18	0.37207	0.37244	0.37299	0.37250
10.20	0.40970	0.41000	0.40919	0.40963
11.22	0.46503	0.46583	0.46549	0.46545
12.24	0.49144	0.49177	0.49057	0.49126
13.26	0.53500	0.53446	0.53440	0.53462
14.28	0.56308	0.56307	0.56198	0.56271
15.30	0.61724	0.61726	0.61608	0.61686
16.32	0.65249	0.65308	0.65238	0.65265
17.34	0.69345	0.69414	0.69369	0.69376
18.36	0.73607	0.73586	0.73634	0.73609
19.38	0.77445	0.77499	0.77406	0.77450
20.40	0.80580	0.80648	0.80668	0.80632
$r = 0.996$; $r^2 = 0.9992$; SE = 0.0070; Intercept = 0.01067; Slope = 0.0393				

SE – standard error, r – correlation coefficient, r^2 – regression coefficient

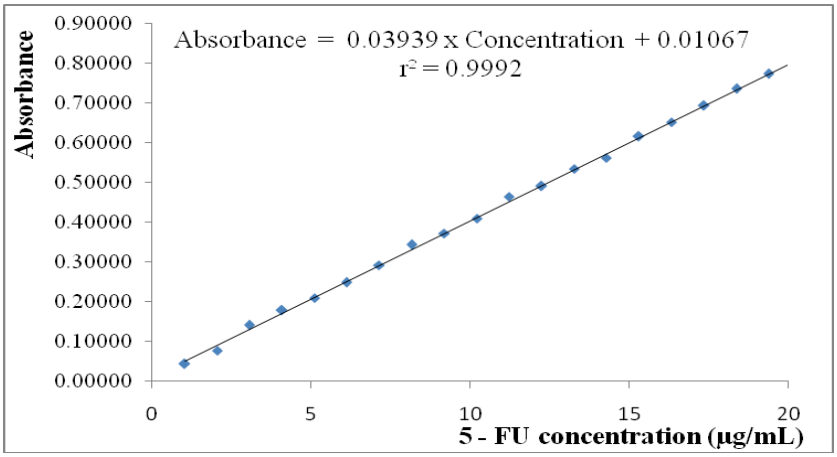

Figure 3.
The calibration curve and equation calibration for determining 5-FU through UV spectrophotometry

Estimating the linearity of the results as seen in figure 4, there is a linear correlation between the theoretically introduced concentration and the calculated concentration, using the calibration curve equation, the slope of this being very close to the unity (slope = 0.9999) and the intercept having the value of $7x10^{-6}$. The correlation coefficient has the value $r^2 = 0.9992$. In conclusion, in the considered concentration domain we considered, between the values of the calculated and the theoretical concentrations, there is a very good correlation.

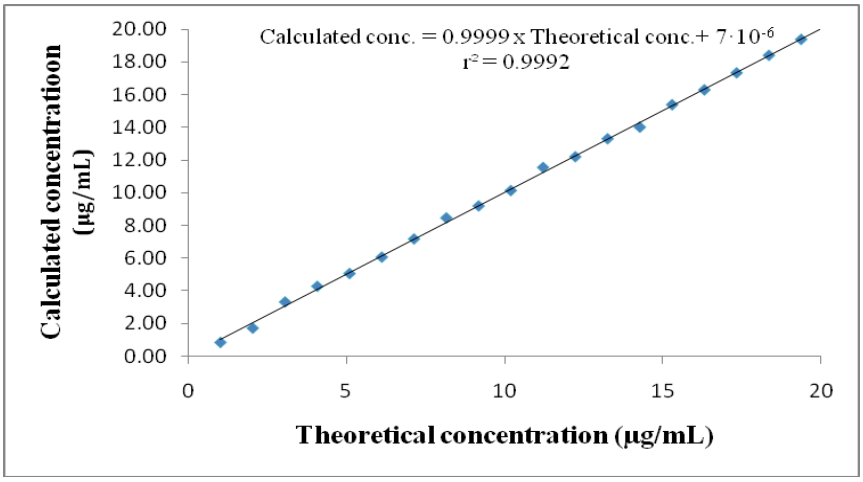

Figure 4.
Linearity results for the assay of 5-FU through UV spectrophotometry

The values of the detection and quantification limits are the following: LD = 0.59 µg/mL and LQ = 1.79 µg/mL.

The experimental values obtained in the study of precision and intermediate precision of the assay method of 5 - FU through UV spectrophotometry are presented in Table II.

Table II.

Precision and accuracy of the assay method of 5-FU through UV spectrophotometry

5 – FU concentration (µg/mL)	Method precision		Intermediate precision		Method accuracy	
7.14	Absorbance	Recovery (%)	Absorbance	Recovery (%)	Absorbance	Recovery (%)
	0.2934	100.56	0.2924	100.14	0.2932	100.42
	0.2927	100.28	0.2928	100.28	0.2928	100.28
	0.2919	100.00	0.2930	100.42	0.2920	100.00
10.20	0.4097	99.31	0.4099	99.41	0.4096	99.31
	0.4100	99.41	0.4096	99.31	0.4099	99.41
	0.4091	99.22	0.4084	99.02	0.4093	99.22
13.26	0.5350	100.38	0.5341	100.23	0.5340	100.23
	0.5344	100.30	0.5349	100.38	0.5346	100.30
	0.5344	100.30	0.5352	100.45	0.5351	100.38
Statistical data	Mean = 99.97 % SD = 0.5175 RSD = 0.5176 %		Mean = 99.96 % SD = 0.5519 RSD = 0.5521 %		Mean = 99.95 % Minim = 99.22 % Maxim = 100.42 %	

SD – standard deviation, RSD – relative standard deviation

According to the experimental data and after their statistical processing, it is clear that on a range of 30% compared to the value of interest (10 µg/mL), the method is precise, as the relative standard deviation (RSD) is below the required limit (5%).

Conclusions

In this study we presented the results obtained in the development and validation of an assay method of 5 - FU in sodium acetate buffer solution (pH = 4.3) by UV spectrophotometry. The method was validated by determining the following parameters: linearity in the chosen concentration interval (1.02 - 20.40 µg/mL), r = 0.9996, r^2 = 0.9992, LD = 0.59 µg/mL, LQ = 1.79 µg/mL, precision of the method (RSD = 0.5176%), intermediate precision of the method (RSD = 0.5521%) method accuracy for which we obtained a mean recovery of 99.95 %.

In conclusion, this method for the assay of 5 - FU is simple, easy to apply, sensitive, linear, precise and accurate, and can be used in future research for the quantitative evaluation of 5 - FU in modern pharmaceutical dosage forms with vaginal administration during *in vitro* dissolution tests.

Acknowledgments

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