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Chemical Industry & Chemical Engineering Quarterly 17 (1) 25–31 (2011) CI&CEQ

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SCIENTIFIC PAPER

UDC 661.12:543.544.5

DOI 10.2298/CICEQ100422047Y

# DETERMINATION OF METOPROLOL IN PURE AND PHARMACEUTICAL DOSAGE FORMS BY SPECTROFLUOROMETRY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

*In this study, new and rapid spectrofluorometry and high performance liquid chromatography (HPLC) methods were developed for determination of metoprolol in pure and pharmaceutical dosage forms. The solvent system, wavelength of detection and chromatographic conditions were optimized in order to maximize the sensitivity of both proposed methods. The linearity was established over the concentration range of 50-4000 ng ml$^{-1}$ for spectrofluorometry and 5.0-300 ng ml$^{-1}$ for HPLC methods. The intra- and inter-day relative standard deviation (RSD) was less than 4.14 and 3.86% for spectrofluorometry and HPLC, respectively. The limit of quantitation was determined as 30 and 5.0 ng ml$^{-1}$ for spectrofluorometry and HPLC, respectively. No interference was found from tablet excipients at the selected assay conditions. The methods were applied for the quality control of commercial metoprolol dosage forms to quantify the drug and to check the formulation content uniformity.*

*Key words: metoprolol; spectrofluorometry; HPLC; validation.*

$\beta$-Blockers are clinically important drugs and are used in the treatment of disorders such as hypertension, angina pectoris and arrhythmia [1]. Metoprolol (Figure 1) has been used extensively for more than 25 years to treat such cardiovascular disorders as hypertension, arrhythmia and heart failure [2].

Several methods have been reported for determination of metoprolol including gas chromatography--mass spectrometry (GC-MS) [3-5], high performance liquid chromatography (HPLC) [6-10], LC-MS [11-13], LC-MS-MS [14] and spectrophotometry [15].

An extensive survey of literature showed that no spectrofluorometry method is reported to date for determination of metoprolol in pure and pharmaceutical dosage forms. However, the survey of literature revealed HPLC methods for determination of metoprolol. An HPLC method for metoprolol using $C_{18}$ column and mobile phase of acetonitrile-phosphate buffer-water (5:0.5:94.5 v/v/v) has been reported [2]. Another study was a reverse phase HPLC method [6] which utilized acetonitrile-phosphate buffer (30:70 v/v) as mobile phase system for metoprolol. Over the last 10 years, HPLC methods using fluorometric detection (FL) technique have been reported for the determination of metoprolol in plasma [7]. USP 2000 [16] has recommended HPLC method for analysis of metoprolol in pure and dosage forms (tablet). The method recommends the use of a mobile phase of 1-pentanesulfonic acid, sodium acetate and water at a flow rate of 1 ml min$^{-1}$.

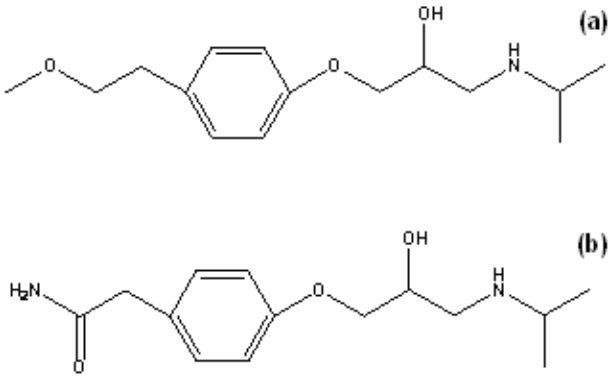


*Figure 1. Chemical structures of metoprolol (a) and atenolol (internal standard, IS) (b).*

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Paper received: 22 April, 2010
Paper revised: 20 June, 2010
Paper accepted: 23 August, 2010

This paper describes a spectrofluorometry method for determination of metoprolol and a HPLC method using a reverse phase $C_{18}$ column in the mixture of methanol-water (50:50 v/v) containing 0.1% trifluoroacetic acid (TFA) with FL detection.

The spectrofluorometry method was aimed at developing an easy and rapid assay method for metoprolol without any time consuming sample preparation steps for routine analysis. The HPLC method was attempted to demonstrate the utility of FL detection for determination of metoprolol coupled with simple and economical mobile phase and reasonable analysis time with high precision.

In both proposed methods, there is no need to extract the drug from the formulation excipient matrix thereby decreasing the error in quantitation. Formulation samples can be directly used after dissolving and filtration. The developed methods were used to determine the total drug content in commercially available tablets of metoprolol.

## EXPERIMENTAL

### Chemicals

Metoprolol tartrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Atenolol as an internal standard (IS) was kindly donated by Abdi Ibrahim Pharmaceutical Industry (Istanbul, Turkey). Problok and Beloc ZOK tablets (100 mg metoprolol tartrate) were obtained from Terra and Astrazeneca Pharmaceutical Industry (Istanbul, Turkey), respectively. All chemicals were of analytical grade. Distilled water was prepared as required by using aquaMAX™ ultra, Young instrument (Korea) ultrawater purification system.

### Systems and conditions

All fluorescence measurements were done on a Shımadsu RF-5301 PC spectrofluorometer equipped with a 150 W Xenon lamp. Experimental parameters were slit width 5.0 nm, $\lambda_{exc}$ = 276 nm and $\lambda_{em}$ = 296 nm.

A Perkin Elmer series 200 HPLC system equipped with programmable fluorescence detector and Total Chrom Chromatography Data System software were used (Perkin Elmer Life and Science, Shelton, CT, USA). The HPLC mobile phase was composed of methanol-water (50:50 v/v) containing 0.1% TFA (v/v). The mobile phase was filtered through a nylon membrane of 0.45-mm pore size. Separation was operated on a Phenomenex reversed-phase C18 column with particle size of 5 μm (250mm×4.6 mm i.d.) with a guard column (4 mm×3 mm i.d., Phenomenex) packed with the same material. The flow rate was 1 ml $min^{-1}$, and the column temperature was set at 20 °C. The eluent was monitored by fluorescence detection at 276 (excitation) and 296 nm (emission). The injection volume was 20 μl.

### Preparation of the standard and quality control solutions

The stock solution of metoprolol was prepared in methanol to a concentration of 100 μg $ml^{-1}$. Standard solutions were prepared as 50-4000 ng $ml^{-1}$ (50, 125, 250, 500, 1000, 2000, 3000 and 4000 ng $ml^{-1}$) for spectrofluorometry method.

Internal standard (IS) atenolol solution was prepared at concentration of 50 μg $ml^{-1}$. HPLC metoprolol standard solutions were prepared as 5.0-300 ng $ml^{-1}$ (5.0, 10, 25, 50, 100, 200 and 300 ng $ml^{-1}$) with constant concentration of IS (100 ng $ml^{-1}$).

The quality control (QC) samples were separately prepared at the concentrations of 100, 750 and 3500 ng $ml^{-1}$ for spectrofluorometry. Also, 7.5, 75 and 250 ng $ml^{-1}$ standard metoprolol solutions with constant concentration of IS (100 ng $ml^{-1}$) was prepared for HPLC method.

### Procedure for pharmaceutical preparations

Ten tablets of Problok and Beloc ZOK were weighed and finely powdered. A weighed quantity of the powder equivalent to one tablet was transfered into a 30 ml measuring flask in methanol. After 15 min of mechanical shaking, the solution was filtered in a 50 ml calibrated flask through Whatman no. 42 filter paper. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting them with methanol.

### Data analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated $P$ values were 0.05 or less.

## RESULTS AND DISCUSSION

### Method development and optimization

For the spectrofluorometry method, various solvent systems (water, methanol, acetonitrile) were investigated. The final decision for using methanol as the solvent was based on sensitivity, ease of preparation, suitability for drug content determination and stability studies.

In the case of HPLC, method development was focused on the optimization of column detection, sample preparation and chromatographic separation.

Reversed-phase column ($C_{18}$) can be used for the separation of non-ionic as well as ion forming non--polar to medium polar substances while normal phase chromatography can be used for the separation of non-ionic and/or non-polar substances. The majority of the ionizable pharmaceutical compounds can be very well separated on $C_{18}$ [17]. Thus, metoprolol can be satisfactorily separated by reversed phase chromatography.

Several tests were performed for optimizing the components of mobile phase in order to achieve good chromatographic peak shape and resolution. The test results showed that the solvent system of water could improve the peak shapes of metoprolol. Good separation of target compounds and short run time were obtained using a mobile phase system of methanol-water (50:50 v/v*)* containing 0.1% TFA (v/v*)*. The retention time of metoprolol (4.7 min) was quite shorter than that studied by Fang *et al.* [2] and Chiu *et al.* [8]. On the other hand, the mobile phase in the proposed method methanol-water instead of buffered systems is used in previously reported HPLC methods [2,6,8].

## Method validation

*Specificity*. All the solutions were scanned from 250 to 350 nm at a slit width of 5.0 nm and checked for change in the emission at respective wavelengths (Fig. 2).

In a separate study, the specificity of the HPLC method was investigated by observing interferences between metoprolol, IS and excipients. The retention time of metoprolol in HPLC method was approximately 4.7 min with good peak shape (Fig. 3).

*System suitability***.** A system suitability test of the HPLC system was performed before each validation run. Five replicate injections of a system suitability/calibration standard and one injection of a control standard were made. Area % *RS*D, tailing factor and efficiency for the five suitability injections were determined. For all sample analyses, the tailing factor was ≤1.12 and efficiency ≥2750. The % *RSD* of peak area and retention time ($t_R$) for metoprolol are within 2% indicating the suitability of the system.

*Linearity***.** The calibration curve was linear between the range 50-4000 ng $ml^{-1}$ for spectrofluorometry. The calibration plot was constructed for metoprolol standard by plotting the concentration of metoprolol versus spectrum emission intensity response.

For HPLC measurements, the solutions were prepared by dilution of the stock solution of metoprolol to reach a concentration range of 5.0-300 ng $ml^{-1}$. The calibration curve was obtained by plotting the peak area ratio between metoprolol and that of IS.

The constructed calibration curves were evaluated by their correlation coefficients. The correlation coefficients ($r$) of all the calibration curves were consistently greater than 0.99.The regression equations were calculated from the calibration graphs, along with the standard deviations of the slope and intercept on the ordinate. The results are shown in Table 1. The calibration curves were as good as those reported in the other papers [3,7-9].

*Precision and accuracy***.** The precision of the spectrofluorometry and HPLC methods was determined by repeatability (intra-day) and intermediate

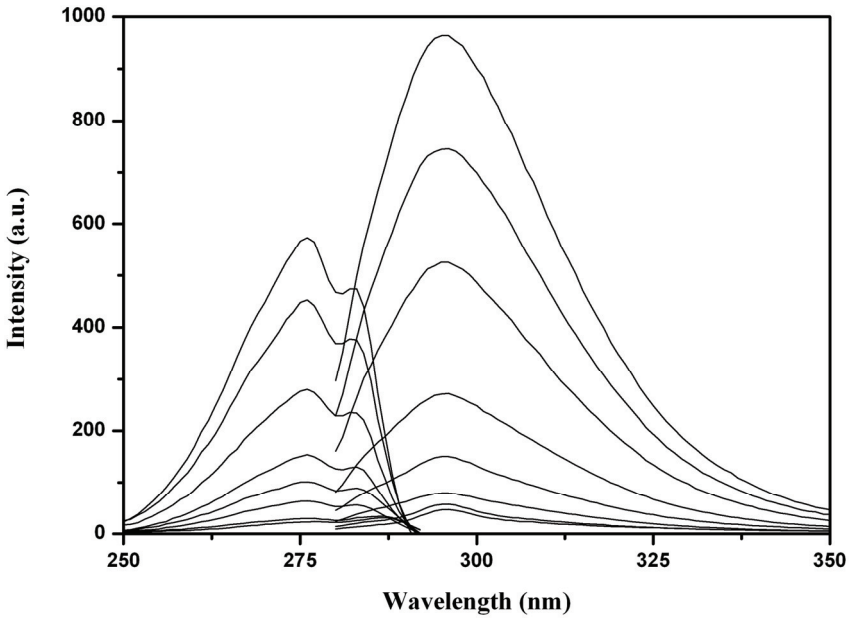


*Figure 2. The excitation and emission spectrum of metoprolol (50, 125, 250, 500, 1000, 2000, 3000 and 4000 ng $ml^{-1}$).*

precision (inter-day). Repeatability was evaluated by analyzing quality control samples six times per day, at three different concentrations which were quality control samples. The intermediate precision was evaluated by analyzing the same samples once daily for three days. The *RSD* of the predicted concentrations from the regression equation was taken as precision. The accuracy of this analytic method was assessed as the percentage relative error. For all the concentrations studied, intra- and inter-day RSD values were ≤4.14% and for all concentrations of metoprolol the relative errors were ≤4.60%. These results were given in Table 2.

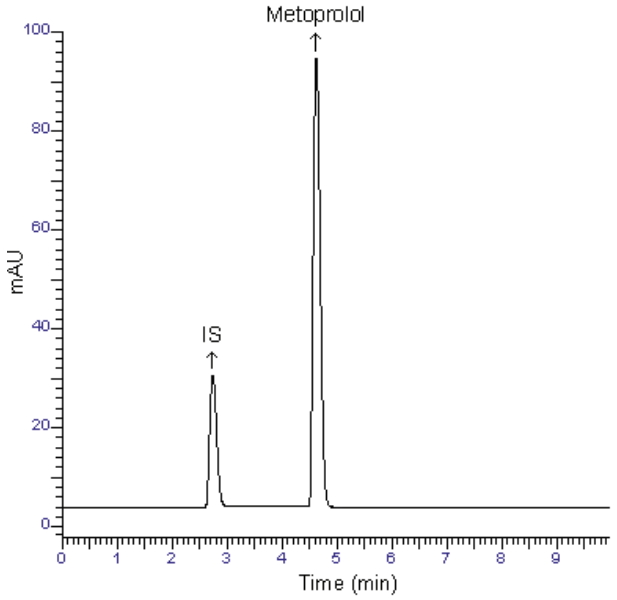


*Figure 3. HPLC chromatogram of metoprolol (200 ng ml$^{-1}$) and atenolol (IS) (100 ng ml$^{-1}$).*

In statistical comparison ($p$ > 0.05) with other methods in the literature [3,6-8,12,14], the proposed methods have indicated high accuracy and precision.

### Limits of detection (LOD) and quantitation (LOQ)

For spectrofluorometry measurements, *LOD* and *LOQ* of metoprolol were determined using calibration standards. The *LOD* and *LOQ* values were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where $S$ is the slope of the calibration curve and $\sigma$ is the standard deviation of $y$-intercept of regression equation ($n$ = 6).

For HPLC measurements, the *LOD* and *LOQ* of metoprolol were determined by injecting progressively low concentrations of the standard solution under the chromatographic conditions. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as *LOQ* [18]. The *LOD* was defined as a signal/noise ratio of 3:1. The *LOD* and *LOQ* for spectrofluorometry were 10 and 30 ng ml$^{-1}$, for HPLC 2 and 5 ng ml$^{-1}$, respectively. Among the two methods, HPLC is more sensitive than spectrofluorometry (Table 1).

### Recovery

To determine the accuracy of the spectrofluorometry and HPLC methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding known amount of pure drugs to pre-analyzed samples of commercial dosage forms. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 3.

### Stability

Stability studies indicated that the samples were stable when kept at room temperature and 4 °C refrigeration temperatures for 12 (short-term) and 48 h (long-term). The results of these stability studies are given in Table 4, where the percent ratios are within the acceptance range of 90-110%.

*Table 1. Results of regression analysis of metoprolol by the proposed methods*

| Parameter | Spectrofluorometry | HPLC |
|---|---|---|
| Linearity, ng ml$^{-1}$ | 50-4000 | 5.0-300 |
| Regression equation[a] | $y = 0.2359x + 33.508$ | $y = 0.2822x + 0.4962$ |
| Standard deviation of slope | 0.01 | 0.032 |
| Standard deviation of intercept | 0.714 | 0.078 |
| Correlation coefficient | 0.999 | 0.999 |
| Standard deviation of correlation coefficient | $1.25\times10^{-3}$ | $2.48\times10^{-3}$ |
| Limit of detection, ng ml$^{-1}$ | 10 | 2.0 |
| Limit of quantitation, ng ml$^{-1}$ | 30 | 5.0 |

[a]Based on three calibration curves, $y$ = emission intensity (spectrofluorometry method) and peak area ratio (HPLC method) and $x$ = concentration in ng ml$^{-1}$

*Table 2. Precision and accuracy of metoprolol by the proposed methods*

| Added, ng ml$^{-1}$ | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| | Found±*SD*[a] | Precision (*RSD*[b] / %) | Accuracy[c], % | Found±*SD*[a] | Precision (*RSD*[b] / %) | Accuracy[c], % |
| Spectrofluorometry | | | | | | |
| 100 | 98.5±2.866 | 2.91 | -1.50 | 95.4±3.948 | 4.14 | -4.60 |
| 750 | 748.2±7.257 | 0.97 | -0.24 | 741.2±10.96 | 1.48 | -1.17 |
| 3500 | 3476.1±86.21 | 2.48 | -0,68 | 3522.5±92.99 | 2.64 | 0.64 |
| HPLC | | | | | | |
| 7.5 | 7.41±0.209 | 2.82 | -1.20 | 7.52±0.290 | 3.86 | 0.27 |
| 75 | 73.6±1.509 | 2.05 | -1.87 | 75.6±2.874 | 3.80 | 0.80 |
| 250 | 242.7±3.616 | 1.49 | -2.92 | 256.6±4.298 | 1.67 | 2.64 |

[a]Standard deviation of six replicate determinations; [b]relative standard deviation; [c]relative error = 100(found-added)/added

*Table 3. Recovery values of metoprolol in pharmaceutical preparations*

| Commercial preparation | Method | Added, ng ml$^{-1}$ | Found±*SD*[a], ng ml$^{-1}$ | Recovery, % | RSD[b], % |
|---|---|---|---|---|---|
| Problok tablet (100 ng ml$^{-1}$) | Spectrofluorometry | 100 | 97.7±2.716 | 97.7 | 2.78 |
| | | 900 | 910.8±38.16 | 101.2 | 4.19 |
| | | 3500 | 3383.0±121.1 | 99.5 | 3.58 |
| Beloc ZOK tablet (50 ng ml$^{-1}$) | HPLC | 50 | 49.1±1.320 | 98.2 | 2.69 |
| | | 150 | 149.1±6.143 | 99.4 | 4.12 |
| | | 250 | 254.0±8.865 | 101.6 | 3.49 |

[a]Standard deviation of six replicate determinations; [b]relative standard deviation

## Comparison of the methods

Spectrofluorometry and HPLC methods were applied for the determination of metoprolol in commercial tablets. The results show the high reliability and reproducibility of the two methods.

The results were statistically compared using the *F*-test. At 95 % confidence level, the calculated *F*-values do not exceed the theoretical values (Table 5). Therefore, there is no significant difference between spectrofluorometry and HPLC methods.

There is a study for determination of metoprolol by zero-order derivative spectrophotometry method in the literature [15]. In this study, the method is based on the formation of Cu (II) dithiocarbamate complex by derivatization of the secondary amino group of metoprolol with $CS_2$ and $CuCl_2$ in the presence of ammonia. The copper-bis(dithiocarbamate) complex was extracted into chloroform and the concentration of metoprolol tartrate was determined directly by spectrophotometry.

Also, the suggested spectrofluorometry and HPLC methods were compared with the HPLC method of USPXXIV [16]. There was no significant difference between the three methods with respect to mean values and standard deviations at the 95% confidence level (Table 5). Therefore, it is suggested that the two methods are equally applicable.

## CONCLUSIONS

The proposed methods were found to be accurate, precise and easy for the determination of me-

*Table 4. Stability of metoprolol in solution (n = 6)*

| Added, ng ml$^{-1}$ | Intra-day | | | Inter-day |
|---|---|---|---|---|
| | Room temperature (12 h) | Refrigerator (4 °C, 12 h) | Room temperature (48 h) | Refrigerator (4 °C, 12 h) |
| Spectrofluorometry | | | | |
| 100 | 98.1±3.85 | 99.6±3.78 | 97.6±4.12 | 99.3±2.16 |
| 1000 | 99.2±2.27 | 100.7±3.15 | 98.5±2.47 | 99.5±1.41 |
| 4000 | 101.7±2.34 | 101.8±2.76 | 101.2±2.71 | 100.4±2.56 |
| HPLC | | | | |
| 10 | 97.6±4.12 | 99.8±1.35 | 99.1±3.26 | 97.7±4.97 |
| 100 | 98.5±2.47 | 99.6±0.46 | 99.8±2.19 | 98.6±3.06 |
| 300 | 101.2±2.71 | 100.1±0.35 | 100.6±2.39 | 102.1±3.54 |

*Table 5. Statistical comparison (F-test) of the results obtained by proposed methods*

| Commercial preparation | Method | Mean±*SD*[a], mg | *P* | *F*-test[b] |
|---|---|---|---|---|
| Problok (100 mg/tablet) | Official method (HPLC) | 100.4±0.85 | 0.321 | $F_c$ = 2.19 |
| | Spectrofluorometry | 98.9±4.642 | | $F_t$ = 3.00 |
| Beloc ZOK (100 mg/tablet) | Official method (HPLC) | 100.4±0.85 | 0.289 | $F_c$ = 2.27 |
| | HPLC | 99.7±3.378 | | $F_t$ = 3.00 |

[a]Standard deviation of six replicate determinations; [b]$F_c$: calculated *F* values, $F_t$: tabulated *F* values; Ho hypothesis: no statistically significant difference exists between two methods; $F_t > F_c$; Ho hypothesis is accepted ($P > 0.05$)

toprolol. The *LOQ* of the proposed methods are very low (25 and 5.0 ng $ml^{-1}$).

The HPLC method is found to be superior to earlier reported methods, as the mobile phase is simple to prepare and economical. The medium for dissolving of metoprolol is the same at HPLC and spectrofluorometry analysis. The sample recoveries in a formulation were in good agreement with their respective label claims. No extraction procedure is involved.

These methods can be used effectively, without separation and interference, for routine analysis of metoprolol in pure form and its formulations and can also be used for dissolution or similar studies. On the other hand, the spectrofluorometry method is also suitable for analysis of samples during accelerated stability studies, routine analysis of formulations and raw materials.

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NAUČNI RAD

# SPEKTROFLUOROMETRIJSKO I HPLC ODREĐIVANJE METOPROLOLA U ČISTOJ SUPSTANCI I FARMACEUTSKIM PREPARATIMA

*U ovom radu razvijene su nove i brze spektrofluorometrijske i HPLC metode za određivanje metoprolola u čistoj supstanci i u farmaceutskim preparatima. U cilju maksimiziranja osteljivosti obe predložene metode, optimizovani su sistem rastvarača, talasna dužina određivanja i hromatografski uslovi. Utvrđena je lineranost u opsegu koncentracije od 50 do 4000 ng ml$^{1}$ za spektrofluorometrijsko određivanje i od 5,0 do 300 ng ml$^{1}$ za HPLC metodu. Relativna standardna devijacija dnevne i višednevne preciznosti je manja od 4,14 i 3,86% za spektrofluorometrijsku HPLC metodu, respektivno. Limit detekcije za spektrofluorometrijsku, odnosno HPLC metodu je 30, odnosno 5,0 ng ml$^{1}$. Odabrani uslovi određivanja omogućavaju određivanje bez interferencija pomoćnih supstanci iz tableta. Metode su primenjene za kontrolu kvaliteta komercijalnih preparata na bazi metoprolola i proveru jednobraznosti sadržaja formulacije.*

*Ključne reči: metoprolol; spectrofluorometrija; HPLC; validacija.*