J Fluoresc (2009) 19:817–828
DOI 10.1007/s10895-009-0479-6

ORIGINAL PAPER

Simultaneous Determination of Labetalol and Furosemide by First-Derivative Synchronous Spectrofluorimetry

D. R. El-Wasseef · D. T. El-Sherbiny · M. A. Abu-El-Enein · S. M. El-Ashry

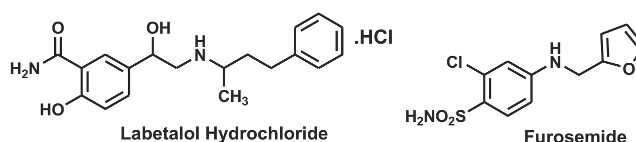
Received: 23 October 2008 / Accepted: 27 March 2009 / Published online: 1 May 2009
© Springer Science + Business Media, LLC 2009

Abstract A rapid, simple and highly sensitive first derivative synchronous spectrofluorimetric method was developed for the simultaneous analysis of a binary mixture of labetalol HCl (LBT) and furosemide (FUR) without prior separation. The method was based upon measuring the first derivative of synchronous fluorescence spectra of the two drugs at $\Delta\lambda$ =130 nm in aqueous ethanol (55% V/V). The different experimental parameters affecting the synchronous fluorescence of the studied drugs were carefully studied and optimized. The first derivative amplitude-concentration plots were rectilinear over the range of 0.10 to 1.00 μg/mL and 0.05–0.50 μg/mL with lower detection limits of 0.0149 and 7×10^{-3} μg/mL and quantification limits of 0.045 and 0.021 μg/mL for LBT and FUR, respectively. The proposed method was successfully applied for the determination of the studied drugs in synthetic mixtures. The results obtained were in good agreement with those obtained by the reference methods.

Keywords Labetalol · Furosemide · First derivative synchronous spectrofluorimetry

D. R. El-Wasseef (✉) · D. T. El-Sherbiny · M. A. Abu-El-Enein · S. M. El-Ashry
Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura,
35516 Mansoura, Egypt
e-mail: dodomum@yahoo.com

Introduction



Labetalol HCl: 5-[1-hydroxy-2-(1-methyl-3-henylpropylamino)ethyl] salicylamide hydrochloride. LBT is a non-cardiovascular β-blocker. It is reported to possess some intrinsic sympathomimetic and membrane stabilizing activity. It has-in addition-selective α_1-blocking properties which decrease peripheral vascular resistance. The ratio of α to β-blocking activity has been estimated to be about 1:3 following oral administration, and 1:7 following intravenous administration. It is used in the management of hypertension and to induce hypotension during surgery [1]. LBT is the subject of a monograph in each of the British Pharmacopoeia, BP [2] and the United States Pharmacopoeia, USP [3]. The BP recommends non aqueous titration for the raw material and spectrophotometric measurement at 302 nm for tablets and injections. The USP [3], on the other hand, recommends HPLC method for the raw material and its formulations

The therapeutic importance of LBT initiated several reports on its determination, both in formulations and in biological fluids, *viz*: spectrophotometry [4–7], spectrofluorimetry [8–13], HPLC [14–17], HPLC-MS [18–20], capillary electrophoresis [21, 22], and voltammetry [23]. LBT HCl was also determined in pharmaceuticals using an ion selective electrode sensitive to LBT with a liquid membrane [24].

Furosemide, 4-Chloro-N-furfuryl-5-sulphamoylanthranilic acid, is a potent diuretic with a rapid action. Like the other loop or high-ceiling diuretics it is used in the treatment of oedema associated with heart failure, including pulmonary oedema, and with renal and hepatic disorders and may be effective in patients unresponsive to thiazide diuretics. It is also used in high doses in the management of oliguria due to renal failure or insufficiency. Furosemide is also used in the treatment of hypertension, either alone or with other antihypertensives [1]. FUR is the subject of a monograph in each of the BP [2] and the USP [3]. The BP and USP recommend non aqueous titration for the raw material. The BP recommends spectrophotometric measurement at 271 nm for the tablets and injections, while USP [3] recommends HPLC method for the tablets and injections. There are several reports on the determination of furosemide, both in formulations and in biological fluids, *viz*: spectrophotometry [25–32], spectrofluorimetry [33–35], chemiluminescence [36, 37], enzyme immunoassay [38] gas chromatography [39–41] and HPLC [42–47].

In fluorimetric methods, high sensitivity and selectivity are generally expected. However, problems of selectivity can occur in multicomponent analysis because of the overlap of the broadband spectra observed. Synchronous fluorescence spectroscopy (SFS) has been found to have several advantages such as simple spectra, high selectivity and low interference [48]. Because of its sharp and narrow spectrum; SFS serves as a very simple, effective method of obtaining data for quantitative determination in a single measurement. SFS techniques are classified according to the scanning modes of monochromators into constant-wavelength, variety angle and constant-energy. The combination of synchronous and derivative fluorimetry enhances minor spectral features and allows more reliable identification of chemical species [49].

Recently, Derivative synchronous fluorometry (DSF) technique has been utilized for the determination of cinnarizine and domperidone in pharmaceutical preparations [50], diflunisal and salicylic acid in serum [51], acetylsalicylic acid and caffeine in pharmaceutical formulation [52] and amiloride and triamterene in urine [53]

The aim of the present work was to develop a simple, sensitive and rapid method for the simultaneous determination of LBT and FUR through first derivative synchronous fluorimetry (FDSF) based on their native fluorescence. Because of the high overlap of the emission spectra of LBT and FUR, it was difficult to analyze and determine their contents by conventional fluorimetry. Such problem was resolved by using FDSF. The synchronous spectrum at constant $\Delta\lambda$=130 nm between the emission and excitation wavelengths, has been selected as the optimum one to perform the determination. This method was applied to the determination of LBT with FUR in synthetic mixtures and in their single pharmaceutical preparations.

Experimental

Apparatus

- The fluorescence spectra and measurements were recorded using a Perkin-Elmer UK model LS45 luminescence spectrometer, equipped with a 150 W Xenon arc lamp, gratting excitation and emission monochromators for all measurements, and a Perkin-Elmer recorder. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. Derivative spectra were evaluated using FL WINLAB version 4.00.02 software
- A Consort P-901 pH-meter.

Materials and reagents

All reagents and solvents were of Analytical Reagent Grade.

- LBT and FUR pure samples were purchased from Sigma-Aldrich Chemie GmbH, Germany and were used as received.
- Absolute ethanol (Merck, Darmstadt, Germany)
- Britton Robinson buffer solution (pH3.0), prepared by adding 0.5 M sodium hydroxide solution to a 0.02 M solution of phosphoric acid, boric acid and acetic acid [2]

Standard solutions

Stock solutions of LBT and FUR were prepared by dissolving 10.0 mg and 2.5 mg of LBT and FUR in 50.0 mL ethanol to give solutions of 200.0 µg/mL and 50.0 µg/mL, respectively, which were further diluted with the same solvent as appropriate.

The standard solutions were found to be stable for one week when kept in the refrigerator.

General procedure

Aliquots of LBT and FUR standard solutions covering the working concentration range cited in Table 1 were transferred into a series of 10.0 mL volumetric flasks. 2.0 mL of Britton Robinson buffer (pH3.0) were added to each flask. The solutions were diluted to the mark with 55% aqueous ethanol and mixed well. The synchronous fluorescence spectra of the solutions were recorded by scanning both monochromators at a constant wavelength difference $\Delta\lambda$= 130 nm with scan rate of 600 nm/min using 10 nm excitation and emission windows. The first derivative fluorescence spectra of LBT and FUR were derived from the normal synchronous spectra using FL WINLAB version 4.00.02 software. For best resolution and smoothing, number of points of 99 was used

Table 1 Performance data for the first derivative synchronous spectrofluorimetric method for the determination of LBT and FUR in pure state

Parameters	LBT	FUR
Concentration range (μg/mL)	0.100–1.000	0.050–0.500
Correlation coefficient	0.9998	0.9998
Slope	96.086	136.864
Intercept	1.397	−1.525
LOD (μg/mL)	14.9×10^{-3}	7.0×10^{-3}
LOQ (μg/mL)	0.045	0.021
$S_{y/x}$	0.634	0.426
S_a	0.433	0.291
S_b	0.698	0.937
%RSD	0.872	1.161
%Er	0.276	0.367

LOD limit of detection, *LOQ* Limit of quantification; $S_{y/x}$ = standard deviation of the residuals; S_a = standard deviation of the intercept; S_b = standard deviation of the slope, *%RSD* relative standard deviation (%RSD= SD x 100/$\bar{x}$ where SD is the standard deviation and $\bar{x}$ is the mean recovery), *%Er* percent error (%Er=RSD/$\sqrt{n}$ where n is the number of values)

for deriving the first derivative spectra. The amplitude of the first derivative spectra were estimated at 276.5 nm and 304.5 nm for LBT and FUR, respectively. A blank experiment was performed simultaneously. The derivative amplitude of the first derivative technique was plotted *vs.* final concentration of the drug (μg/mL) to get the calibration graph. Alternatively, the corresponding regression equations were derived.

Applications

Procedure for the synthetic mixture

Aliquots of LBT and FUR standard solutions in different ratios were transferred into a series of 10 mL volumetric flasks. 2.0 mLs Britton Robinson buffer (pH3.0) were added to each flask. The solutions were diluted to the mark with 55% aqueous ethanol and mixed well. The "general procedure" was then adopted and the nominal contents were determined either from the previously plotted calibration graphs or using the corresponding regression equations.

Results and discussion

FDSF spectra of LBT and FUR

Both the studied drugs exhibit native fluorescence at λ_{ex} 300 and 345 nm λ_{em} 425 and 415 nm for LBT and FUR, respectively, in water and ethanol-water media (Fig. 1). The fluorescence spectra of these drugs overlap considerably and, as a result, the conventional spectrofluorimetric method does not permit the simultaneous determination of both drugs.

Figure 2 shows synchronous spectra of LBT and FUR and a mixture of both compounds, corrected for the blank signal, and maintaining a constant interval between the emission and excitation wavelengths of 130 nm. Because of the large overlap of the spectra, the determination of LBT and FUR by synchronous spectrofluorimetry is still not feasible. This overlap was resolved by taking the first derivatives of the spectra. The technique used to choose the

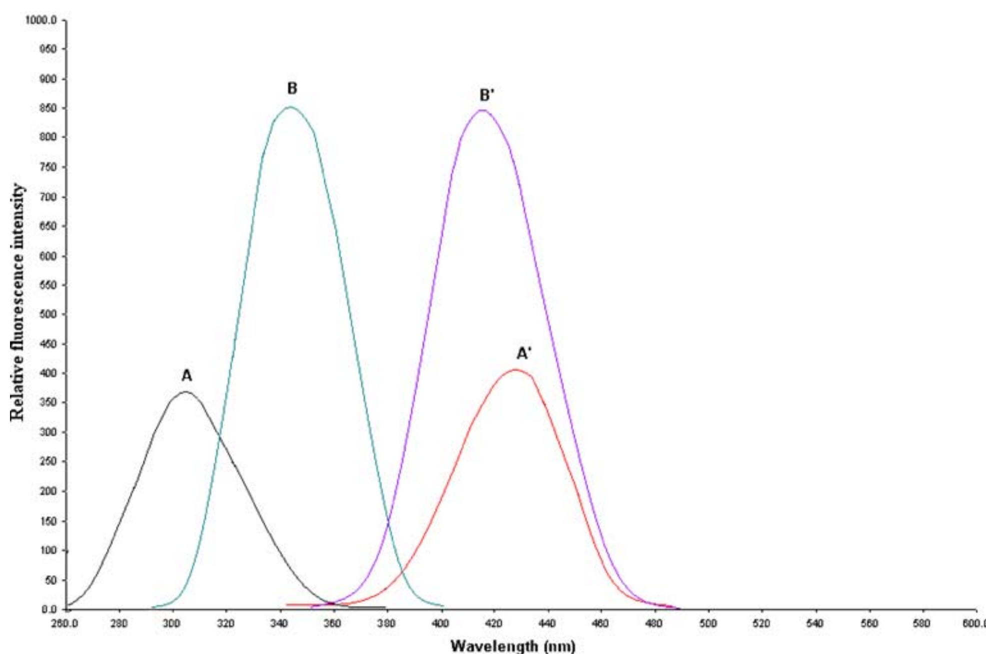


Fig. 1 Normal fluorescence spectra at pH 3.0 of LBT and FUR: (A, A') are excitation and emission spectra of LBT (0.40°μg /mL),(B, B') are excitation and emission spectra of FUR (0.40°μg/ mL)

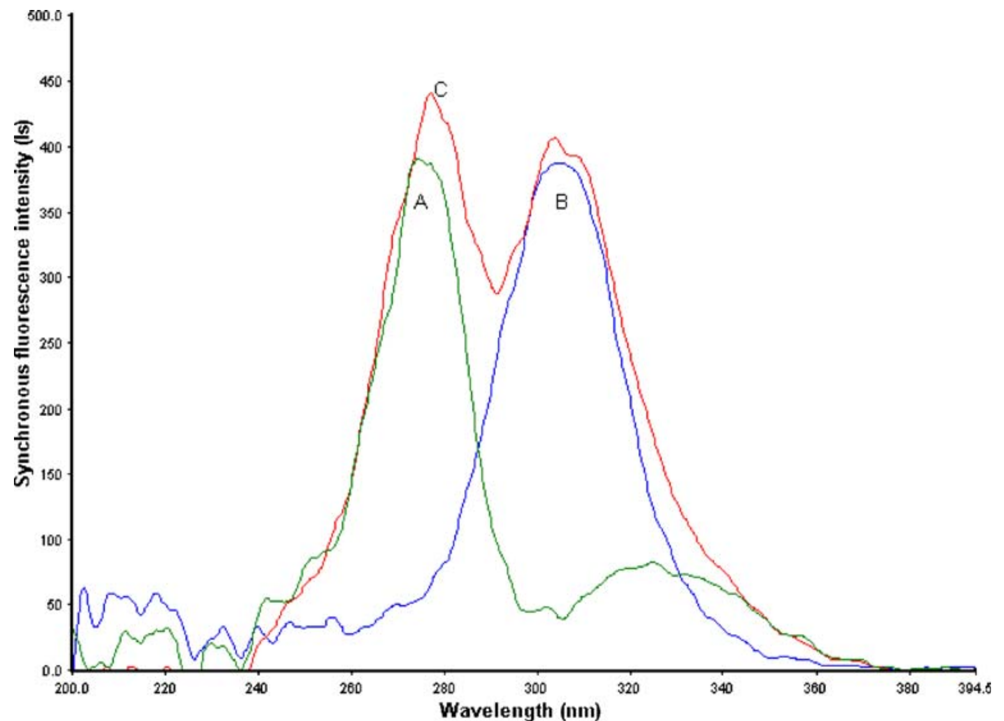


Fig. 2 Synchronous fluorescence spectra of FUR (A; 0.30°μg/mL), LBT (B; 0.60°μg/mL) & a mixture of LBT & FUR (C)

suitable wavelengths that make the measurements proportional to LBT and FUR concentrations was the "zero-crossing". Assuming that the derivative of a spectral band is equivalent to the sum of the derivatives of its individual bands, when the first-derivative spectrum of one of two components is zero the total derivative spectrum is a function only of the concentration of the other component and *vice versa*. In fact, the height h_1 (λ=276.5 nm, zero-crossing of FUR) is proportional to the LBT concentration, whereas h_2 (λ=304.5 nm, zero-crossing of LBT) is proportional to the FUR concentration, Fig. 3.

Optimization of experimental conditions

Different experimental parameters affecting the formation and stability of the fluorescence of the studied compounds

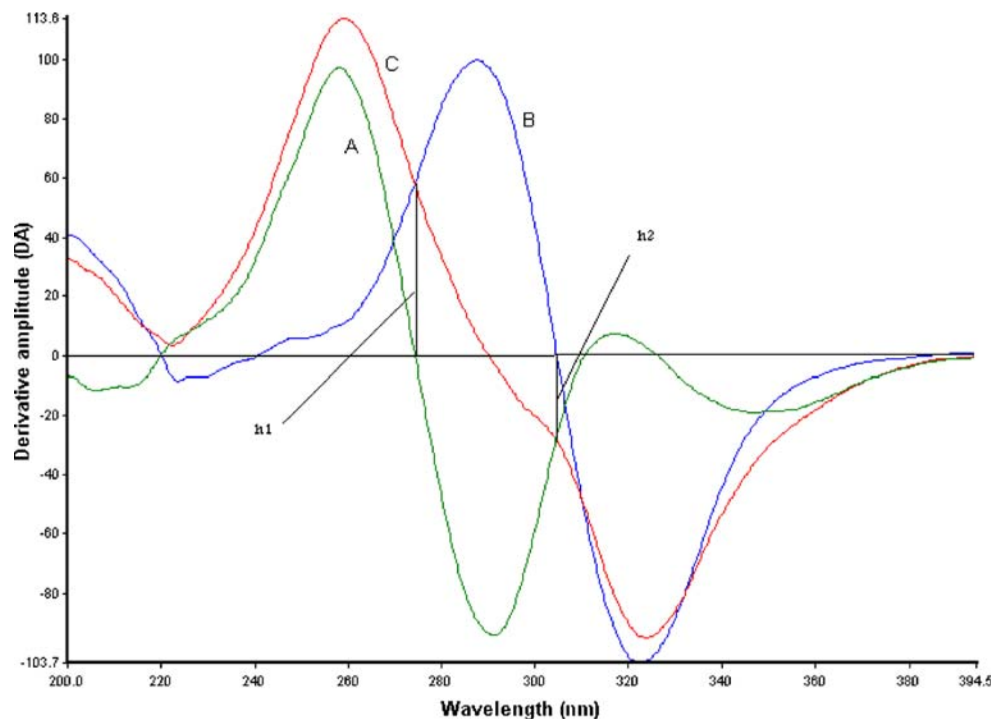


Fig. 3 First-derivative synchronous spectrum of A; FUR (0.30 μg/mL), B; LBT (0.60 μg/mL) & C; mixture of LBT & FUR

were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors included Δλ selection, pH, concentration of ethanol, and stability time .

Selection of optimum Δλ

The synchronous fluorescence spectra of LBT and FUR were recorded using different Δλ. The optimum Δλ value is very important for performing synchronous fluorescence scanning technique concerning resolution, sensitivity and features. It can directly influence spectral shape, band width and signal value. For this reason a wide range of Δλ (20 to 160 nm) was examined by obtaining the total synchronous spectrofluorimetric information available in the matrix as shown in Fig. 4. In the left side of the figure, the three-dimensional synchronous spectra of LBT, FUR, and mixture of both were represented as an isometric projection, where the synchronous spectra at stepped increments of Δλ have been recorded and plotted. In right side; the three-

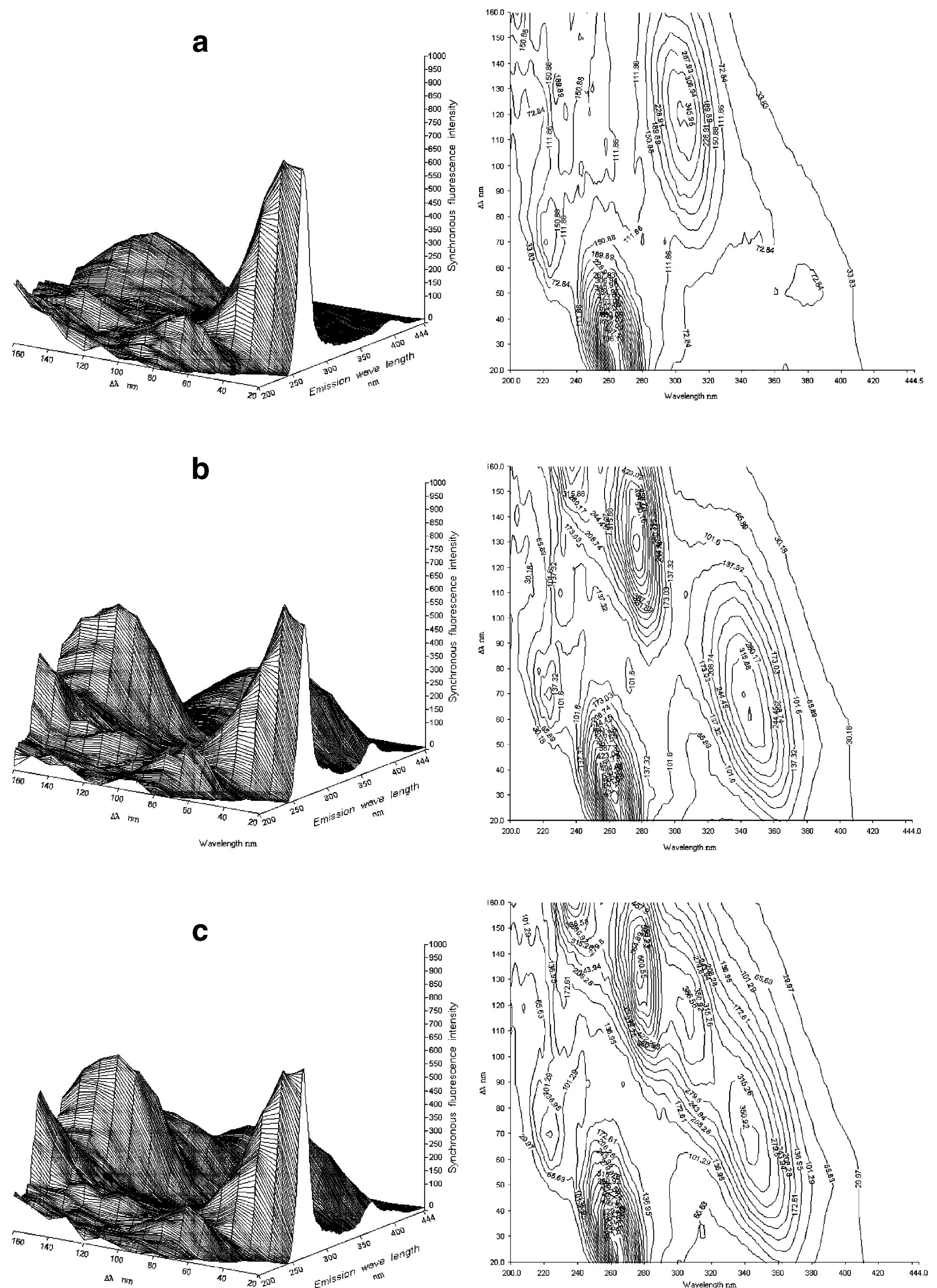


Fig. 4 Three dimensional and two dimensional (contour plots) total synchronous fluorescence spectra of LBT (A, 0.50 μg/mL), FUR (B, 0.50 μg/mL) & Mixture of LBT & FUR (C)

dimensional synchronous spectra have been transformed into contour plots in the excitation-emission plane.

When Δλ is less than 130 nm, the spectra shape is irregular and the synchronous fluorescence intensity is very weak. When Δλ is more than 130 nm, poor separation of the two peaks was obtained. Therefore, Δλ of 130 nm was chosen as the optimal one for the separation of mixture of this binary mixture, since it resulted in two distinct peaks with good shape and a minimized spectral interference caused by each compound in the mixture.

Effect of pH

The influence of pH on the normal synchronous fluorescence intensity (Is) of the studied drugs was investigated using Britton Robinson buffer covering the pH range 1.5–7.0 Fig. 5. The results showed that the native fluorescence of LBT increases as the pH of the medium increase, mean while the native fluorescence of FUR is pH dependant with the maximum fluorescence observed over a pH range of 2.5–3.5. At pH greater than 4.0 the fluorescence is negligible and a hypsochromic shift in the excitation and emission maxima was observed due to ionization of the carboxylic group (pK_a=3.9).

The volume of the Britton Robinson buffer on the synchronous fluorescence intensity of FUR and LBT was also studied using different volumes of buffer, and 2.00 ml of Britton Robinson buffer pH3.0 was chosen throughout the study, Fig. 6.

Effect of ethanol concentration

The effect of ethanol concentration was also studied. It was found that synchronous fluorescence intensity (Is) of LBT and FUR increase as the ethanol concentration increase till 50% (v/v) ethanol-water, after which the Is remain constant,

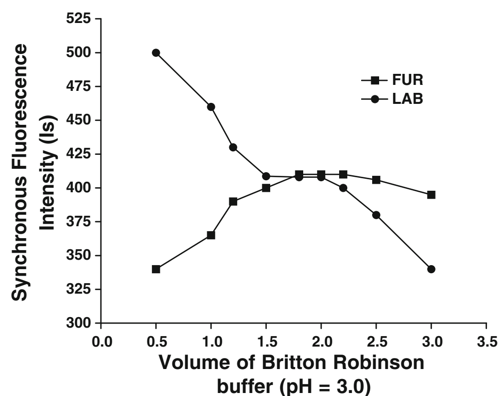


Fig. 6 Effect of volume of Britton Robinson buffer (pH=3.0) on the synchronous fluorescence spectra of FUR (0.25 μg/mL) & LBT (0.50 μg/mL) in 55% ethanol

Fig. 7. Therefore, 55% (v/v) ethanol-water was selected as the optimum concentration for determination of LBT with FUR.

Effect of time

The effect of time on the development and stability of the fluorescence of the drugs was also studied. It was found that the fluorescence was developed immediately and remained stable for more than 1 h.

Effect of ionic strength

The impact of ionic strength on the system was also studied using different concentrations of NaCl ranging from 5×10^{-3} M to 0.1 M. NaCl has been used as a counter ions to study ionic strength since sodium ions have higher adsorption over the negative charge on the molecule and chloride ions have the same effect. The results showed that there was no effect of NaCl concentration on the fluorescence intensity of the studied drugs.

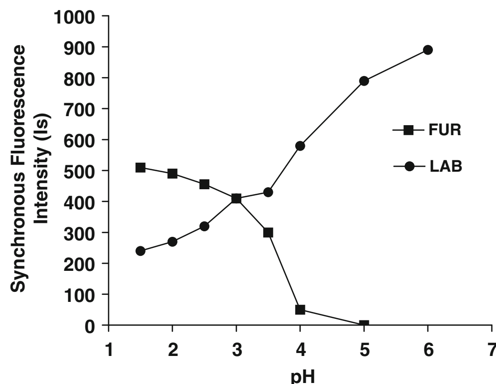


Fig. 5 Effect of the pH on the synchronous fluorescence spectra of FUR (0.25 μg/mL) & LBT (0.50 μg/mL) in 55% ethanol

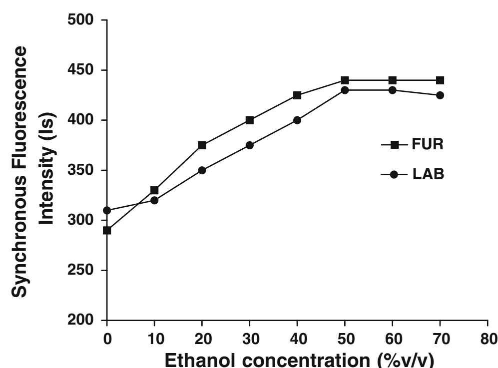


Fig. 7 Effect of ethanol concentration on the synchronous fluorescence spectra of FUR (0.25 μg/mL) and LBT (0.50 μg/mL)

Analytical performance

The derivative amplitude (DA)-concentration plots for the studied drugs by FDSF were linear over the concentration range cited in Table 1. Linear regression analysis of the data gave the following equations:

$$\mathrm{DA} = 1.397 + 96.086\,\mathrm{C}\ (\mathrm{r} = 0.9998)\ \text{for LBT at 276.5 nm}$$

$$\mathrm{DA} = -1.525 + 136.864\,\mathrm{C}\ (\mathrm{r} = 0.9998)\ \text{for FUR at 304.5 nm}$$

where C is the concentration of the drug (μg/mL) and r is correlation coefficient.

In order to test the mutual independence of the analytical signals for LBT and FUR, i.e., to show that h_1 and h_2 are independent of FUR and LBT concentrations, respectively, calibration graphs were obtained from height (h) measurements for standards containing between 0.1 and 1.0 μg/mL of LBT, in the presence of different concentrations of FUR (Fig. 8) and for standards containing between 0.05 and 0.50 μg/mL of FUR, in the presence of different concentrations of LBT (Fig. 9).

The analytical parameters for all the calibration graphs were summarized in Table 2, from which it can be deduced that the amplitude of the derivative signal of the mixture at the zero crossing point of the derivative spectrum of one of the two components is a function only of the other component, in accordance with theoretical predictions. Moreover, the values of the correlation coefficients and the low values for the intercepts indicate good linearity for all the calibration graphs obtained for the first-derivative measurements.

Statistical analysis [54] of the results obtained by the proposed and the official method for FUR [2] and a comparison fluorimetric method [13] for LBT using Student's *t*-test and variance ratio *F*-test, shows no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 3).

Analysis of synthetic mixture sample

The proposed method was applied to the simultaneous determination of LBT with FUR in synthetic mixtures containing different concentrations of both drugs in different ratios. The relative fluorescence intensities of first derivative technique were measured for both drugs.

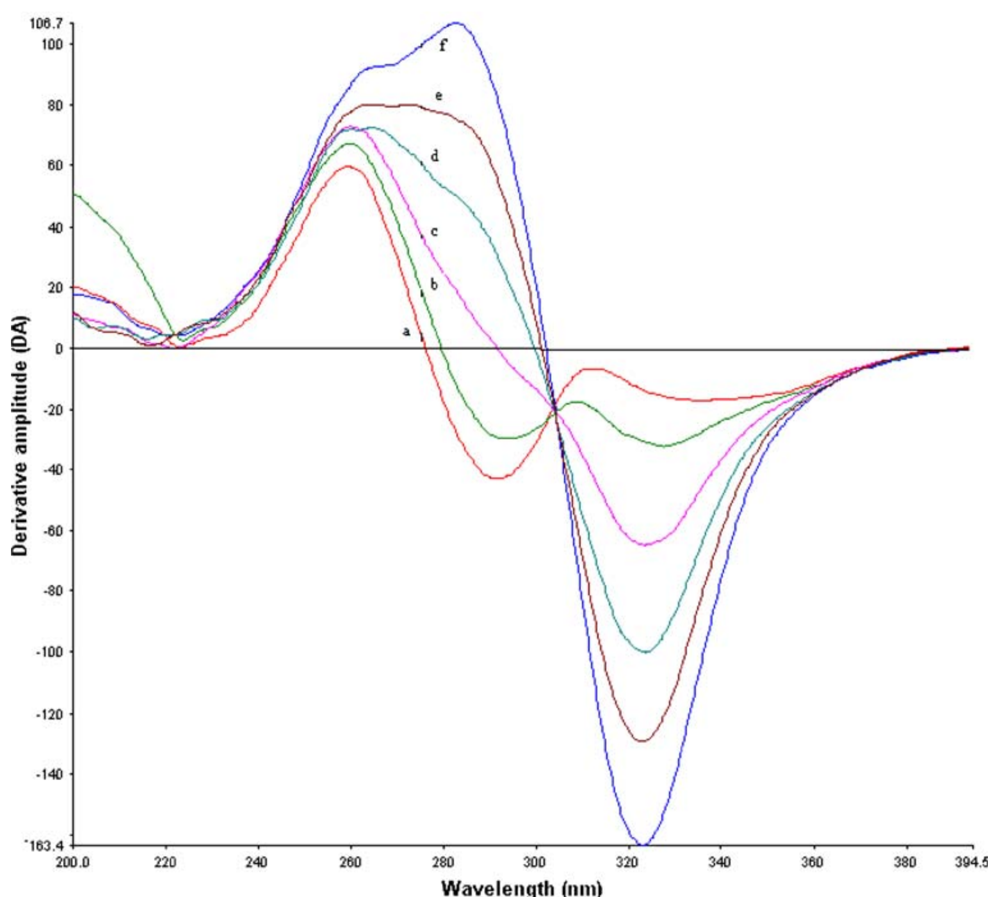


Fig. 8 First derivative synchronous spectra for calibration graph of LBT in the presence of 0.20 μg/mL of FUR. Measurement of the signal made at λ=276 nm (Zero-crossing point of FUR). LBT concentration; a = 0.10; b = 0.20, c = 0.40, d = 0.60; e = 0.80, f = 1.00 μg/mL

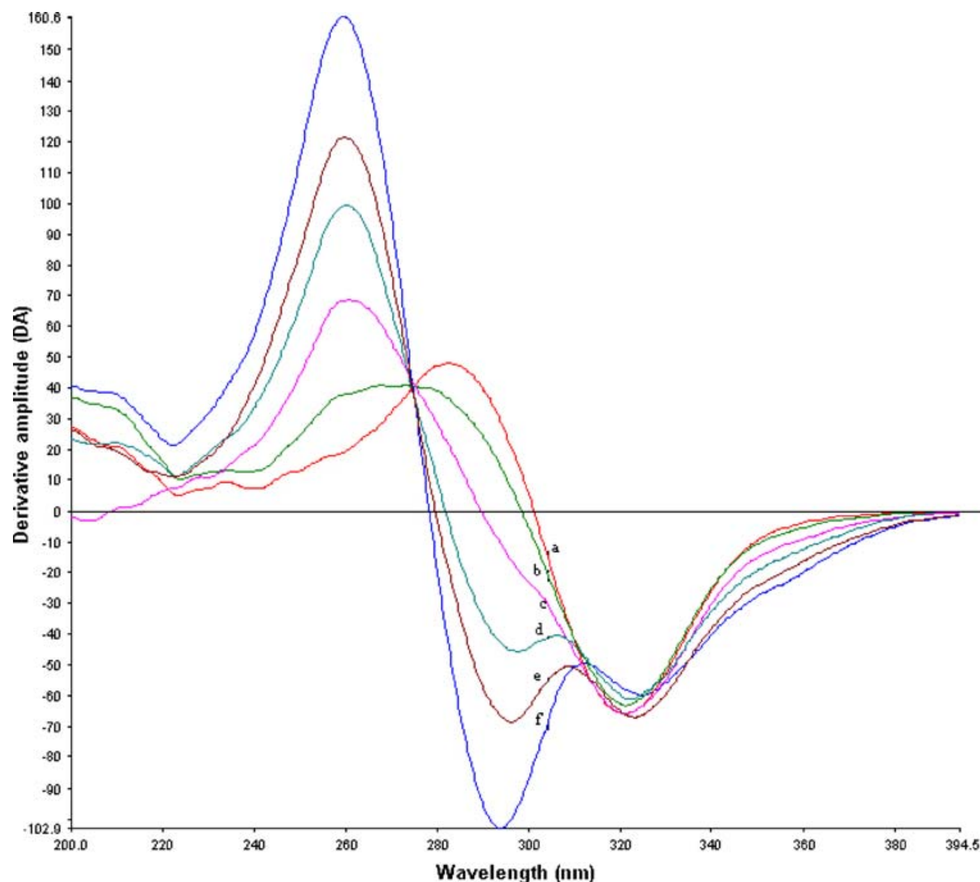


Fig. 9 First derivative synchronous spectra for calibration graph of FUR in the presence of 0.40 μg/mL of LBT. Measurement of the signal made at λ = 305°nm (Zero-crossing point of LBT). FUR concentration; a = 0.05; b = 0.10, c = 0.20, d = 0.30; e = 0.40, f = 0.500° μg/mL

The concentrations of both drugs in the synthetic mixture were calculated according to the linear regression equation of the calibration graphs. The results obtained regarding %RSD and %Er were calculated. The results indicate high accuracy of the proposed method as shown in Table 4.

Validation of the method

Linearity

The proposed method was tested for linearity, specificity, accuracy and precision. Linear regression equations were

Table 2 Statistical analysis of the determination of FUR (0.05–0.50 μg/mL) and LBT (0.10–1.00 μg/mL) in mixtures by 1st derivatives synchronous spectrofluorimetry

Compound determined	Conc. of co-existing compound μg/mL		Intercept	Slope	Correlation coefficient
FUR	LBT	0.100	−1.627	138.790	0.9998
		0.200	−1.650	139.115	0.9995
		0.400	−1.553	137.670	0.9996
		0.600	−1.420	134.101	0.9994
		0.800	−1.459	133.813	0.9997
		1.000	−1.652	136.711	0.9997
LBT	FUR	0.050	1.559	98.193	0.9993
		0.100	1.418	98.185	0.9996
		0.200	1.499	97.121	0.9998
		0.300	1.329	93.921	0.9995
		0.400	1.625	98.861	0.9997
		0.500	1.479	94.073	0.9994

Table 3 Application of the first derivative synchronous spectrofluorimetric method to the determination of LBT and FUR in pure state

Labetalol HCl				
Proposed method			**Reference method** [13]	
Amount taken (µg/mL)	**Amount found (µg/mL)**	**% Found**	**Amount taken (µg/mL)**	**% Found**
0.100	0.1009	100.900	0.500	101.570
0.200	0.1992	99.600	1.000	100.400
0.300	0.3009	100.300	1.500	99.770
0.400	0.4009	100.225	2.000	100.100
0.500	0.5073	101.460	2.500	98.960
0.600	0.5988	99.800	3.000	101.060
0.700	0.6915	98.786	3.500	99.140
0.800	0.7887	98.588	4.000	100.360
0.900	0.9029	100.322	4.500	99.500
1.000	1.009	100.900	5.000	100.480
$\bar{X}$ ± SD =100.088 ± 0.918 *t*-value = 0.159 (2.101) *F*-value = 1.250 (3.180)			100.134 ± 0.821	
Furosemide				
Proposed method			**Reference method** [2]	
Amount taken (µg/mL)	**Amount found (µg/mL)**	**% Found**	**Amount taken mg**	**% Found**
0.050	0.0510	102.000	150.00	99.210
0.100	0.1012	101.200	200.00	100.530
0.150	0.1495	99.667	250.00	102.320
0.200	0.1999	99.950		
0.250	0.2490	99.600		
0.300	0.2941	98.033		
0.350	0.3556	101.600		
0.400	0.4004	100.100		
0.450	0.4476	99.467		
0.500	0.5018	100.360		
$\bar{X}$ ± SD =100.198 ± 1.163 *t*-value = 0.700 (2.201) *F*-value = 1.802 (4.260)			100.687 ± 1.561	

Each result is the mean recovery of three separate determinations.
Values between brackets are the tabulated *t* values and *F*-values at (P= 0.05).

obtained. The regression plots showed a linear dependence of derivative amplitude values on drug concentration over the range cited in Table 1. The small values of the %RSD and %Er point out to the low scattering of the points around the calibration curve and high accuracy and precision of the proposed method.

Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2(R1) recommendations [55], below which the calibration graph is non linear ($LOQ=10\sigma/S$ where S is the slope and σ is the standard deviation of the intercept of regression line of the calibration curve). The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected ($LOD=3.3\sigma/S$). The results of LOD and LOQ of LBT and FUR by the proposed method are abridged in Table 1.

Accuracy and precision

The results of the inter-day and intra-day accuracy and precision of the method have been summarized in Table 5. The inter-day and intra-day precisions were examined by analysis of LBT in concentrations 0.20 and 0.60 µg/mL and FUR in concentrations 0.20 and 0.30 µg/mL each three times a day for three consecutive days. The precision of the proposed method was fairly high, as indicated by the low values of SD and RSD%, respectively. Also the inter-day and intra-day accuracy was proved by the low values of Er%.

Table 4 Application of first derivative synchronous spectrofluorimetric method to the determination of FUR and LBT in synthetic mixtures

Ratio FUR/LBT	FUR			
	Taken (μg/mL)	Found (μg/mL)	% Recovery	Error %
1 : 10	0.100	0.102	102.000	+ 0.710
4 : 10	0.400	0.388	97.000	−1.630
4 : 10	0.200	0.197	98.500	− 1.620
2 : 10	0.200	0.197	98.500	− 1.620
2 : 10	0.100	0.097	97.042	− 0.870
3 : 10	0.300	0.312	104.000	+ 1.020
5 : 10	0.400	0.397	99.250	− 0.850
5 : 10	0.200	0.200	100.000	0.350
LBT				
1 : 10	1.000	1.027	102.700	+ 0.820
4 : 10	1.000	1.035	103.500	+ 1.862
4 : 10	0.500	1.026	102.600	+ 1.543
2 : 10	1.000	0.495	98.900	− 0.321
2 : 10	0.500	0504	100.800	+ 0.840
3 : 10	1.000	0.992	99.200	− 0.650
5 : 10	0.800	0.80	100.000	1.234
5 : 10	0.400	0.408	102.000	+ 0.983

Robustness of the method

The robustness of the proposed method was demonstrated by the constancy of the fluorescence intensity with the deliberated changes in the experimental parameters such as pH, 3.0±0.2, volume of the buffer 2.0±0.2 mL and the ethanol concentration, 55%±5%, for the studied drugs with %RSD < $1x10^{-3}$ for all the investigated variables proves that; these minor changes that may take place during the experimental operation didn't greatly affect the fluorescence intensity of the mixture.

Application

Analysis of synthetic mixtures

The proposed method was applied to the simultaneous determination of LBT with FUR in synthetic mixtures containing different concentrations of both drugs in different ratios. The derivative amplitudes of first derivative technique were measured for both drugs. The concentrations of both drugs in the synthetic mixture were calculated

Table 5 Evaluation of the accuracy and precision data of the proposed first derivative synchronous spectrofluorimetric method for the determination of labetalol HCl and furosemide

Drug	Amount added (μg/ml)	Amount found (μg/ml) [(a)]	$\overline{X}$ ± SD	RSD%	Er%
Labetalol	Intra-day				
	0.200	0.198	99.000 ±0.610	0.616	0.356
	0.600	0.603	100.500±0.370	0.368	0.212
	Inter-day				
	0.200	0.199	99.50 0±0.827	0.831	0.480
	0.600	0.596	99.33 0±0.809	0.814	0.470
Furosemide	Intra-day				
	0.200	0.199	99.500 ±0.840	0.844	0.487
	0.300	0.304	101.330±0.920	0.908	0.524
	Inter-day				
	0.200	0.198	99.000±0.669	0.676	0.390
	0.300	0.297	99.330±0.707	0.712	0.411

[(a)] Each result is the average of three separate experiments

$\overline{x}$ = The mean recovery; *SD* Standard deviation of results

according to the linear regression equation of the calibration graphs. The results obtained regarding %RSD and %Er were calculated. The results indicate high accuracy of the proposed method as shown in Table 4.

Conclusion

New simple, sensitive and time saving method was explored for the simultaneous determination of LBT and FUR in binary mixture. The first derivative synchronous spectrofluorimetric method, by virtue of its high sensitivity, could be applied to the analysis of both drugs in their synthetic mixtures. The first derivative spectrofluorimetry technique enables the determination of either drug in the presence of the other by applying the zero-crossing technique in the spectra without prior separation steps.

References

1. Martindale: The Complete Drug Reference (2007) 35th, Electronic Version.
2. The British Pharmacopoeia (2007) Vol. I, II, Her Majesty's Stationery Office, London, Electronic Version
3. The United States Pharmacopoeia XXVII (2007). National Formulry XXII, Rockville, USP Convention Inc., Maryland, Electronic Version
4. Rahman N, Rahman H, Najmul S, Azmi H (2007) Determination of labetalol hydrochloride in drug formulations by spectrophotometry. J. Chin. Chem. Soci. 54(2):185–196
5. Rahman N, Singh M, Nasrul MD, Hoda SM, Kashif RZ, Naheed B (2005) Validation of an optimized spectrophotometric method for the selective determination of labetalol hydrochloride. Chin. J. Chem. 23(12):1611–1617. doi:10.1002/cjoc.200591611
6. Belal F, Al-Shaboury S, Al-Tamrah AS (2003) Spectrophotometric determination of labetalol in pharmaceutical preparations and spiked human urine. Farmaco. 58(4):293–299. doi:10.1016/S0014-827X(02)01291-0
7. Sastry CSP, Krishna DM (1996) Spectrophotometric methods for the determination of labetalol hydrochloride in pure and dosage forms. Mikrochim. Acta 122(1–2):87–93. doi:10.1007/BF01252409
8. El-Enany N (2007) Micellar enhanced spectrofluorometric determination of labetalol through complexation with aluminium(III): application to dosage forms and biological fluids. J. AOAC Int 90 (4):948–956
9. Llorent EJM, Atanska D, Solich P (2007) Fluorescence optosensing implemented with sequential injection analysis: a novel strategy for the determination of labetalol. Anal. Bioanal. Chem 387:2065–2069. doi:10.1007/s00216-006-1058-1
10. El-Wasseef DR, El-Ashry SM, Abu-El-Enein MA, Moustafa MAA (2006) Spectrofluorimetric determination of labetalol in pharmaceutical preparations and biological fluids. J. Food. Drug Anal 14(2):133–140
11. Abdine H, Sultan MA, Hefnawy MM, Belal F (2005) Spectrofluorometric determination of some β-blockers in tablets and human plasma using 9, 10-dimethoxyanthracene-2-sodium sulfonate. Pharmazie 60(4):265–268
12. Belal F, Al-Shaboury S, Al-Tamrah AS (2002) Spectrofluorometric determination of labetolol in pharmaceutical preparations and spiked human urine through the formation of coumarin derivative. J. Pharm. Biomed. Anal 30(4):1191–1196. doi:10.1016/S0731-7085(02)00471-5
13. Mohamed ME (1983) Fluorimetric and spectrophotometric determination of labetalol hydrochloride and its tablets. Pharmazie 38 (11):784–785
14. Umezawa H, Lee XP, Arima Y, Hasegawa C, Izawa H, Kumazawa T, Sato K (2008) Simultaneous determination of beta-blockers in human plasma using liquid chromatography-tandem mass spectrometry. Biomed. Chromatogr 22(7):702–711. doi:10.1002/bmc.987
15. Delamoye M, Duverneuil C, Paraire F, Mazancourt P, Alvarez JC (2004) Simultaneous determination of thirteen β-blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection. Forensic Sci. Int 141(1):23–31. doi:10.1016/j.forsciint.2003.12.008
16. Maurer HH, Tenberken O, Kratzsch C, Weber AA, Peters FT (2004) Screening for library-assisted identification and fully validated quantification of 22 beta-blockers in blood plasma by liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization. J. Chromatogr. A 1058(1–2):169–181
17. Sultan M, Abdine H, Zoman N, Belal F (2004) High performance liquid chromatographic method for the simultaneous determination of labetalol and hydrochlorothiazide in tablets and spiked human plasma. Sci. Pharm 72(2):143–156
18. Josefsson M, Sabanovic A (2006) Sample preparation on polymeric solid phase extraction sorbents for liquid chromatographic-tandem mass spectrometric analysis of human whole blood-a study on a number of beta-agonists and beta-antagonists. J. Chromatogr. A 1120(1–2):1–12. doi:10.1016/j.chroma.2006.03.013
19. Thevis M, Opfermann G, Schänzer W (2001) High speed determination of beta-receptor blocking agents in human urine by liquid chromatography/tandem mass spectrometry. Biomed. Chromatogr 15:393–402. doi:10.1002/bmc.87
20. Gergov M, Robson JM, Duchoslav EI, Ojanperai I (2000) Automated liquid chromatographic/tandem mass spectrometric method for screening β-blocking drugs in urine. J. Mass Spectrom 35(7):912–918. doi:10.1002/1096-9888(200007)35:7<912::AID-JMS19>3.0.CO;2-4
21. Goel TV, Nikelly JG, Simpsonl RC, Matuszewski BK (2004) Chiral separation of labetalol stereoisomers in human plasma by capillary electrophoresis. J. Chromatogr. A 1027(1–2):213–221. doi:10.1016/j.chroma.2003.08.082
22. Christine EE, Apryll MS (2003) Comprehensive strategy for chiral separations using sulfated cyclodextrins in capillary electrophoresis. Chirality 15(8):709–723. doi:10.1002/chir.10285
23. Radi A, El-sherif Z, Wassel A (2004) Voltammetric determination of labetalol in pharmaceuticals and spiked human urine. Chem. papers-Slovak academy sci 58(4):242–246
24. Gorodkiewicz E, Falkowski P, Sankiewicz A, Figaszewski Z (2003) Analytical applications of electrode sensitive to labetalol in pharmaceuticals. Cent. Eur. J. Chem 1(3):242–259. doi:10.2478/BF02476227
25. Maheshwari RK, Deswal S, Tiwari D, Ali N, Pothen B, Jain S (2007) Novel spectrophotometric estimation of furosemide using hydrotropic solubilization phenomenon. Indian J. Pharm. Sci 69 (9):822–824
26. Felipe SS, Éder T, Gomes C (2006) Spectrophotometric determination of furosemide based on its complexation with Fe(III) in ethanolic medium using a flow injection procedure anal. Lett 39 (13):2557–2567
27. Millership JS, Parker C, Donnelly D (2005) Ratio spectra derivative spectrophotometry for the determination of furosemide and spironolactone in a capsule formulation. Farmaco 60(4):333–338. doi:10.1016/j.farmac.2005.02.001
28. Semaan FS, De-Sousa RA, Cavalheiro ETG (2005) Flow injection spectrophotometric determination of furosemide in pharmaceut-

icals by the bleaching of a permanganate carrier solution. J. Flow Inj. anal 22(1):34–38
29. Basavaiah K, Chandrashekar U, Nagegowda P (2005) Rapid titrimetric and spectrophotometric determination of frusemide (furosemide) in formulations using bromate-bromide mixture and methyl orange. Indian J. Chem. Tech 12(1):149–155
30. Shah J, Jan MR, Khan MA (2005) Determination of furosemide by simple diazotization method in pharmaceutical preparations. J. Chin. Chem. Soc 52(2):347–352
31. Iara LTD, Jorge LSM, Graciliano DN (2005) Furosemide determination by first-derivative spectrophotometric method. Anal. Lett 38(7):1159–1166
32. Ines TM, Stefanie P, Silvia Q, Pablo R (2002) Simultaneous determination of amiloride and furosemide in pharmaceutical formulations by first digital derivative spectrophotometry. Int. J. Pharm 249(1–2):117–126. doi:10.1016/S0378-5173(02)00482-9
33. Felipe SS, Paulo AN, Éder TGC (2008) Flow-based fluorimetric determination of furosemide in pharmaceutical formulations and biological samples: use of micelar media to improve sensitivity. Anal. Lett 41(1):66–79. doi:10.1080/00032710701746782
34. Llorent EJM, Ortega PB, Molina AD (2005) Multicommuted flow-through fluorescence optosensor for determination of furosemide and triamterene. Anal. Bioanal. Chem 383(5):797–803. doi:10.1007/s00216-005-0079-5
35. Luis ML, Fraga JMG, Jiménez AI, Jiménez F, Hernández O, Arias JJ (2004) Application of PLS regression to fluorimetric data for the determination of furosemide and triamterene in pharmaceutical preparations and triamterene in urine. Talanta 62(2):307–316. doi:10.1016/j.talanta.2003.07.010
36. Juan X, Xinghu J, Shaohong Z, Xinping A, Zhike H (2005) Investigation of RuBPS–Ce(IV) chemiluminescence reaction and its application in determination of two diuretics. Anal. Chim. Acta 541(1–2):191–196. doi:10.1016/j.aca.2005.02.040
37. Rao Y, Xinrong Z, Guoan L, Willy RGB (1999) Chemiluminescence flow-injection determination of furosemide based on a rhodamine 6G sensitized cerium(IV) method. Anal. Chim. Acta 396(2–3):273–277. doi:10.1016/S0003-2670(99)00425-0
38. Nagata S, Kurosawa M, Kuwajima MA (2007) Direct enzyme immunoassay for the measurement of furosemide in Horse Plasma. J. Vet. Med. Sci 69(3):305–307. doi:10.1292/jvms.69.305
39. Morra V, Davit P, Capra P, Vincenti M, Di-Stilo A, Botrè F (2006) Fast gas chromatographic/mass spectrometric determination of diuretics and masking agents in human urine: development and validation of a productive screening protocol for antidoping analysis. J. Chromatogr. A 1135(1):219–229. doi:10.1016/j.chroma.2006.09.034
40. Brunelli C, Bicchi C, Di-Stilo A, Salomone A, Vincenti M (2006) High-speed gas chromatography in doping control: fast-GC and fast-GC/MS determination of β-adrenoceptor ligands and diuretics. J. Separation Sci 29(18):2765–2771. doi:10.1002/jssc.200500387
41. Margalho C, De-Boer D, Gallardo E, Barroso M, Vieira DN (2005) Determination of furosemide in whole blood Using SPE and GC-EI-MS. J. Anal. Toxicol 29(5):309–313
42. Galaon T, Udrescu S, Sora I, David V, Medvedovici A (2007) High-throughput liquid-chromatography method with fluorescence detection for reciprocal determination of furosemide or norfloxacin in human plasma. Biomed. Chromatogr 21(1):40–47. doi:10.1002/bmc.715
43. Dragica Z, Trajce S (2006) Sample preparation and RPHPLC determination of diuretics in human body fluids. Acta Pharm 56 (1):115–142
44. Carolina GG, Carlos VPR, Cgloria GM, Rolando RH, Ricardo GR (2005) Method validation for the determination of furosemide in plasma by liquid- liquid extraction and high-performance liquid chromatography with fluorescence detection. J. Chil. Chem. Soc 50(2):479–482
45. Felipe SS, Alvaro JSN, Fernando ML, Éder TGC (2005) Rapid HPLC-DAD determination of furosemide in tablets using a short home-made column. Anal. Lett 38(10):1651–1658. doi:10.1081/AL-200065813
46. Vanessa M, Regina LMM (2005) Liquid chromatographic screening test for some diuretics of doping interest in human urine. J. Liq. Chromatogr. A 28(17):2753–2768. doi:10.1080/10826070500225036
47. El-Saharty YS (2003) Simultaneous high-performance liquid chromatographic assay of furosemide and propranolol HCl and its application in a pharmacokinetic study. J. Pharm. Biomed. Anal 33(4):699–709. doi:10.1016/S0731-7085(03)00229-2
48. Chen GZ, Huang XZ, Xu JG, Zheng ZZ, Wang ZB (1990) The methods of fluorescence analysis, 2nd edn. Science, Beijing
49. Patra D, Mishra AK (2002) Recent developments in multicomponent synchronous fluorescence scan analysis. Trends Analyt. Chem 21(12):787–798. doi:10.1016/S0165-9936(02)01201-3
50. Walash MI, Belal F, El-Enany N, Abdelal AA (2008) Second-derivative synchronous fluorometric method for the simultaneous determination of cinnarizine and domperidone in pharmaceutical preparations. J. Fluoresc 18(1):61–74. doi:10.1007/s10895-007-0238-5
51. Pulgarín JAM, Molina AA, López PF, Robles IS (2007) Direct determination of closely overlapping drug mixtures of diflunisal and salicylic acid in serum by means of derivative matrix isopotential synchronous fluorescence spectrometry. Anal. Chim. Acta 583(1):55–62. doi:10.1016/j.aca.2006.10.009
52. Mohammad MK, Chi WJ, Hyun SL, Seikh MA, Sang HL, Jong HC, Seung OJ, Ajoy KD (2006) Simultaneous determination of acetylsalicylic acid and caffeine in pharmaceutical formulation by first derivative synchronous fluorimetric method. J. Fluoresc 16:713–721. doi:10.1007/s10895-006-0115-7
53. Pulgarín JA, Molina AA, López PF (2001) Simultaneous direct determination of amiloride and triamterene in urine using isopotential fluorometry. Anal. Biochem 292(1):59–68. doi:10.1006/abio.2001.5064
54. Miller JC, Miller JN (2005) Statistics and chemometrics for analytical chemistry, 5th edn. Prentice Hall, U.K
55. International conference on harmonization of technical requirements for regression of pharmaceutical for human use. (2005) Validation of analytical procedures: text and methodology Q2 (R1). http://www.ich.org/LOB/media/MEDIA417.pdf