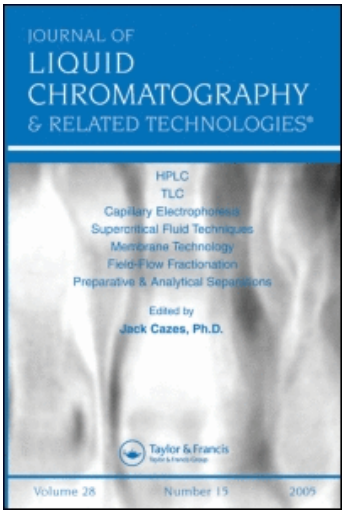

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HPLC and Chemometrically-Assisted Spectrophotometric Estimation of Two Binary Mixtures for Combined Hypertension Therapy

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Abstract: Three different methods are developed for the determination of felodipine with either (+)−metoprolol tartrate salt or ramipril. The high performance liquid chromatography (HPLC) method depends on the simultaneous separation of each drug in a reverse phase Hypersil BDS C_{18} 3 μm (150 × 4.6) column at 25°C. Elution was carried out with a mobile phase consisting of 0.015 M 1-heptanesulfonic acid sodium salt-methanol-acetonitrile (35:40:25, v/v/v, pH 2.5). Optimization of the separation in terms of mobile phase composition is crucial to the method development, which is discussed in detail. Quantitation was achieved with UV detection at 210 nm. Moreover, the resolution of the two binary mixtures separately, has also been accomplished by using a mobile phase consisting of 0.015 M sodium dihydrogen phosphate monohydrate-methanol-acetonitrile 40:30:30, v/v/v, at pH 6.5 ($\lambda = 230$ nm) for the determination of felodipine with metoprolol, and at pH 2.5 ($\lambda = 210$ nm) for felodipine with ramipril, respectively.

The other two chemometrically-assisted spectrophotometric methods that have been used were "Derivative-Ratio" and "Partial Least Squares" PLS. These approaches were successfully applied to quantify each drug in the mixture using the information of the zero order UV spectra between 210–420 nm. Both methods could determine, with linearity, in the range of 1.56–15.60 μg/mL for ramipril, 4.82–80.40 μg/mL for metoprolol, 1.61–17.69 μg/mL for felodipine. They were successfully applied to the "dose uniformity" test of the two binary combinations in commercial tablets.

Keywords: Felodipine, (+)−Metoprolol tartrate, Ramipril, HPLC, Derivative-Ratio, Partial Least Squares, Commercial tablets

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INTRODUCTION

Felodipine (FEL) belongs to the group of medicines called calcium channel blockers, which stop calcium entering into cells through holes in the cell wall.[1] It is prescribed with (+)−metoprolol tartrate salt (MET) which reduces the heart rate, or ramipril (RAM) an inhibitor which blocks the production of angiotensin II. Both combinations are used to treat high blood pressure, hypertension.[2]

Reviewing the literature revealed that no analytical method has been reported for the simultaneous determination of these drugs in binary mixtures. However various chromatographic methods have been reported for individual determination of each component separately. These methods include HPLC combined with UV,[3,4] amperometric,[5] fluorescence,[6] and chemiluminescence[7] detection.

Enantioselectivity determination of metoprolol acidic metabolite in plasma and urine had also been achieved using liquid chromatography chiral columns.[8] The screening analysis of β-blockers including MET in doping control test has been preferably done by gas chromatography mass spectrometry GC/MS after derivatization.[9,10] More recently, the use of GC/MS/MS[11] and LC/MS/MS[12] chromatography has improved the selectivity and sensitivity of the method for screening β-blockers agents in urine. However, such expensive instruments are available only in a few laboratories.

Determination of FEL (which is a dihydropyridine calcium blocker) with another β-blocker atenolol, using reverse-phase chromatography had also been reported.[13].

A literature survey reveals few analytical methods for the analysis of RAM in pharmaceutical preparation and biological fluids by radioimmunoassay,[14] GC-MS,[15] HPLC,[16] and capillary electrophoresis.[17] RAM bears a proline analogue moiety and is frequently coformulated with hydrochlorothiazide[18] or FEL.

The aim of this work is to develop simple, rapid, sensitive, and reliable HPLC and spectrophotometric procedures for the quality control of FEL combined with MET or RAM in pharmaceutical preparations.

EXPERIMENTAL

Apparatus and Analytical Conditions

The apparatus used for HPLC analysis consisted of the following LC-10Avp Shimadzu series: two Model LC-10ADvp pumps for gradient, a Model SCL-10Avp controller, a Model CTO-10ACvp programmable column oven, and a Model SIL-10ADvp programmable auto sampler with the volume injection set to 100 μL. Detection was via a Model LPD-M10Avp UV/diode

array detector operated at 210 nm. The chromatographic peaks were recorded by a HP DeskJet 940c series printer and elaborated automatically by employing a computerized Shimadzu program "CLASS-VP". Separation was achieved on a thermostatted (at 25°C) Hypersil BDS C-18 column (150 × 4.6 mm) 3 μm particle size, end capped to minimize unreacted silanol effects. The isocratic elution system consisted of aqueous 0.015 M 1-heptanesulfonic acid sodium salt-methanol-acetonitrile (35:40:25, v/v/v, pH 2.5).

Another chromatographic system, 0.015 M sodium dihydrogen phosphate monohydrate-methanol-acetonitrile 40:30:30, v/v/v, in two different pH values 6.5 and 2.5, was also considered appropriate for the individual determination of the two binary mixtures FEL-MET (230 nm) and FEL-RAM (210 nm) in tablets, respectively. The flow rate was 1 mL/min whereas the mobile phases were degassed by filtering through a Millipore HV 0.45 μm pore membrane filter. Identification of the peaks was accomplished at the convenient wavelength by using diode-array detector.

A Shimadzu UV-Vis double beam Spectrophotometer model UV-2501 PC with a 1 cm quartz cell was used under the following operating conditions: scan speed 210 nm/min, slit width 1.0 nm and sampling interval 0.1 nm. Derivative spectra were automatically obtained by UV-PC Personal Spectroscopy software.

For sample preparation, an ultrasonic bath "Bransonic 220", a pHmeter "Radiometer analytical 10 Ncheck 10", and a "Pierce Reacti-ThermTM" heating/stirring module were used.

Chemicals

The pharmaceutical company, Aventis, kindly provided pure standards felodipine and ramipril. (+)–Metoprolol tartrate salt was purchased as crystalline powders from Sigma (St. Louis, MO, USA). Stock standard solutions for each of the analytes were prepared in methanol and stored in the dark at 4°C.

Acetonitrile, methanol, and water of HPLC grade were purchased from Merck Company (Darmstadt, Germany). Different buffers were used for various chromatographic systems development. These buffers consisting of tri-sodium citrate-2-hydrate, sodium dihydrogen phosphate monohydrate, sodium acetate trihydrate and diluents 10% v/v ortho-phosphoric 85%, sulfuric acid 95–97%, acetic acid 100%, citric acid monohydrate, and ammonia solution 25% wer also obtained from Merck (Darmstadt, Germany). 1-Propanesulfonic acid sodium salt monohydrate, 1-Butanesulfonic acid sodium salt, 1-Pentanesulfonic acid sodium salt, 1-Hexanesulfonic acid sodium salt, Heptan-1-sulfonic acid sodium salt, were obtained from Sigma Aldrich (Taufkirchen, Germany).

The coated tablets, Triacor® containing felodipine 5 mg, ramipril 5 mg, and excipients iron oxide (yellow) E 172, hyprolose, hypromellose 5 cps, 50 cps &10000 cps, lactose, starch maize pregelatinized, cellulose

microcrystalline, polyoxyl 40 hydrogenated castor oil, propyl gallate, sodium aluminum silicate, sodium stearyl fumarate, color E 172 and E 171, hypromellose 5 cps, paraffin oil, macrogol 6000 was supplied from Aventis commercial source.

Logimax® was purchased from Astra Zeneca company. The tablets were labeled as containing felodipine 5 mg, (+)−metoprolol tartrate salt 50 mg and excipients silicon dioxide colloidal, ethylcellulose, hyprolose, hypromellose, sodium aluminium silicate, lactose anhydrous, cellulose microcrystalline, polyoxyl 40 hydrogenated castor oil, propyl gallate, sodium stearyl fumarate, macrogol 6000, titanium dioxide E 171, iron oxides E 172 (yellow and red brown), paraffin powder.

Standard Solutions

Stock standard solutions for FEL, RAM, and MET were prepared by separately dissolving about 20.0 mg of each drug in 100 mL methanol. Two further dilutions were made using mobile phase for HPLC and MeOH 100% or MeOH-H_2O 40:60 v/v for spectrophotometric methods. The concentration ranges of the six standard solutions for FEL, RAM, and MET were presented in Tables 1 and 3.

Content Uniformity Procedure for Tablets

Both Triacor® and Logimax® are film coated slow release enteric tablets. As most of the analytes and tablet excipients were insoluble in water and soluble in methanol, a solution containing methanol was necessary to dilute drugs and insure for tablet disintegration. Moreover in the case of Triacor®, the presence of 60% water was considered as convenient because it helps to release the drugs from the hydrophilic gel of the tablet.

The content uniformity test was according to the following procedure: The film of ten coated (individually weighed) tablets were removed with water and transferred separately into ten volumetric flasks of 250 mL. Each of them was disintegrated and then dissolved by adding 40 mL of methanol. The dispersions were vigorously shaken for 45 min on a mechanical shaker and ultrasonication followed for 30 min. The solutions were diluted to volume with methanol (Logimax®) or water (Triacor®) and left to precipitate. Filtration with acrodisc GHP (Gelman Hydrophilic Polypropylene membrane) was used to ultra clean them of particles 0.45 μm or larger. Further dilution of the filtrate was carried out with mobile phase (for HPLC method) and MeOH 100 v/v or MeOH-H_2O 40:60 v/v (for spectrophotometric methods).

The produced concentration of the substances for the determination of Logimax® were 4 μg/mL for FEL and 40 μg/mL for MET, and for the

Table 1. Statistical parameters using PLS algorithm

		Parameter of interest[a]					
Method	Compounds	Concentration range (μg/mL)	Number of components	Q^2	r^2	RMSEE	RMSEP
PLS-OSC	MET	4.82–80.40	2	0.9919	0.9946	1.2310	3.0838
	FEL	1.61–16.08		0.9989	0.9998	0.0649	0.1028
PLS	RAM	1.56–15.60	3	0.9990	0.9980	0.1807	0.6278
	FEL	1.61–16.08		0.9997	0.9946	0.0967	0.2490

[a] $Q^2 = 1\text{-PRESS}/SS$, $RMSEP = \text{sqrt}(\Sigma(\text{obs-pred})^2/N)$, $RMSEE = \text{sqrt}(\Sigma(\hat{c}_i - c_i)^2/N)$.

$r^2 = \sum_{i=1}^{N}(\hat{c}_i - c_i)^2 / \sum_{i=1}^{N}(c_i - c_i)^2$.

Where SS is the residual sum of squares, N is the total number of calibration samples, $\hat{c}_I$ represent the estimated concentration and c_i the reference concentration, c_i represents the means of the true concentrations in the predictor set.

determination of Triacor® tablets were 4 μg/mL for FEL and 4 μg/mL for RAM, respectively.

RESULTS AND DISCUSSION

Partial Least Squares Method

The theory and application of PLS and other multivariate calibration methods in analytical chemistry have been thoroughly reported in several books and monographs.[19,20] Partial least squares is a factor analysis based method that was recently demonstrated to have a high capacity to resolve complex mixtures of components with similar spectra characteristics.

Figure 1 shows the UV absorption spectra of FEL, MET, and RAM at their nominal concentration in the tablets. A significant overlap in absorption bands was noticed. Concretely, the simultaneous determination of MET and RAM in tablets is hindered by strong spectral overlap of FEL throughout the wavelength range. PLS or derivative ratio methods were necessary for such determination due to the presence of interference. Moreover, the UV spectrum of FEL is not strongly affected by the presence of MET and RAM and conventional zero order or direct derivative methods could be applied.

The PLS procedures are designated to be full spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detract from precision. Discarding particularly noisy wavelengths can lessen this. This is quite sensible in UV spectrophotometry as the pure spectra of

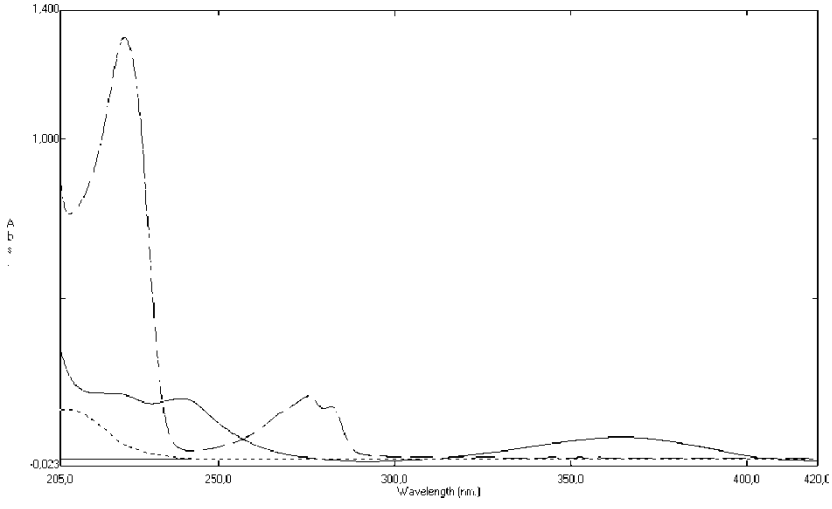

Figure 1. Zero order UV absorption spectra of 4.02 μg/mL FEL (—), 40.0 μg/mL MET (– – –), and 3.95 μg/mL RAM (----).

the analytes are often available and the positions of their bands are not usually affected by the presence of the excipients. So, one can predict which spectral region in the sample spectrum will contain the information relevant to the analyte.

The spectral data between 210 420 nm for FEL-MET and 210–300 nm for FEL-RAM, in zero order, were selected using 106 points as (X) variables and pretreated by a (OSC) filter for the analysis of the above substances. OSC (Orthogonal Signal Correction) is a PLS related filter, which removes variations from (X) that do not contain any information about (Y), i.e., it removes only so much of (X) as is unrelated (orthogonal) to (Y).

The optimum number of components to be used within the PLS algorithm is also an important parameter to achieve better performance in prediction. This allows modeling the system with the optimum amount of information, avoiding overfilling. The cross validation procedure was applied, consisting of symmetrically removing one of the training samples in turn, and using only the remaining ones for construction of the latent factors and regression. The prediction error sum of squares (PRESS) was then calculated:

$$\text{PRESS} = \sum_i \sum_m (Y_{im} - \hat{Y}_{im})^2$$

Table 1 summarizes the most relevant information of the calibration system using the crossvalidation method. Critical values of the calibration, such as the square of the correlation coefficient (r^2), the root mean square error of prediction (RMSP) and estimation (RMSEE), and Q^2, demonstrated the quality of fit of the calibration data.

In order to test the performance of the proposed method, PLS was applied to the resolution of synthetic mixtures using samples with different concentrations of FEL-MET and FEL-RAM. The %Relative Standard Deviation (RSD) and % mean recovery values have been calculated and summarized in Table 2.

Ratio First Derivative Spectrophotometry

The spectra for each component of the mixtures overlap sufficiently (Fig. 1) and demonstrate the resolving power of the proposed method. In order to investigate the sensitivity and stability during 36 h of FEL, MET, and RAM in 0.1 N NaOH, 0.1 N HCl, methanol, and methanolic solution 40%, their UV spectra were recorded every six hours. The results of the experiments have shown as a more suitable solvent, methanol or 40% methanolic solution.

In binary mixtures, the ratio spectra derivative spectrophotometry[21,22] consists in the differentiation of the curves resulting from the division, amplitude by amplitude, of normal spectra of the analyte of interest by an appropriate spectrum of the other component of the mixture. The

Table 2. Recovery Results for different synthetic mixtures of FEL-RAM and FEL-MET, applying HPLC, PLS and "derivative ratio" methods

Felodipine							Ramipril						
HPLC			Derivative ratio		PLS		HPLC			Derivative ratio		PLS	
Real (μg/mL)	Found (μg/mL)	Recovery (%)	Found (μg/mL)	Recovery (%)	Found (μg/mL)	Recovery (%)	Real (μg/mL)	Found (μg/mL)	Recovery (%)	Found (μg/mL)	Recovery (%)	Found (μg/mL)	Recovery (%)
3.22	3.20	99.4	3.23	100.3	3.31	103.1	15.60	15.78	101.1	15.74	100.9	15.85	101.6
8.04	7.87	97.8	8.39	104.3	7.86	97.7	7.80	7.77	99.7	7.89	101.1	8.14	104.4
8.04	8.00	99.5	8.11	100.9	7.95	98.9	15.60	15.79	101.2	15.67	100.5	15.85	101.6
16.08	15.87	98.7	15.62	97.1	16.01	99.6	7.80	7.81	100.1	7.44	95.4	7.95	101.9
16.08	15.77	98.1	16.04	99.7	16.11	100.2	15.60	15.76	101.0	15.07	96.6	15.65	100.3
Mean % Recovery		98.7		100.46		99.9			100.6		98.90		101.7
S.D		0.76		2.59		2.00			0.71		2.69		1.71
%RSD		0.76		2.58		2.01			0.70		2.72		1.68
Student's-*t* values[a]				1.84		1.59					1.73		1.68
Variance ratio F-test [b]				6.71		4.00					7.20		2.90

Felodipine							Metoprolol						
3.22	3.15	97.9	3.22	100.0	3.24	100.9	3.22	3.34	102.8	3.15	98.4	3.32	102.8
8.04	8.00	99.5	8.17	101.7	8.42	104.7	4.82	4.59	100.3	4.81	100.1	4.95	103.1
8.04	8.14	101.2	8.13	101.2	8.11	100.9	8.04	8.05	100.1	7.99	99.9	7.75	96.4
4.00	3.95	98.8	3.96	99.1	4.01	100.1	40.4	40.17	99.4	40.56	100.4	39.49	97.7
16.08	15.82	98.4	16.13	100.3	15.95	99.2	16.08	15.96	99.3	15.97	99.8	16.00	99.5
Mean %Recovery		99.16		100.46		101.1			100.38		99.72		99.9
S.D		1.28		1.02		2.12			1.42		0.77		2.99
%RSD		1.29		1.02		2.10			1.41		0.77		2.99
Student's-*t* values[a]				2.25		2.21					1.16		0.41
Variance ratio F-test[a]				1.64		4.49					2.02		8.94

[a]t critical = 2.306.
[b]F critical = 9.605, p = 0.05.

corresponding calibration graph is constructed by plotting absorption at a maximum or a minimum of the so obtained derivative spectra against analyte concentration.

In order to obtain the best spectra recoveries for FEL, MET, and RAM, it is necessary to study and optimize parameters as $\Delta\lambda$ to obtain the first derivative, smoothing function, scaling factor, and divisor standard concentration. The following values were chosen as optima:

Binary Mixture of FEL-MET

The absorption spectra of FEL solutions in MeOH were recorded in the range of 210–420 nm and smoothened with $\Delta\lambda = 4$ through the use of 17 experimental points. A division of them was followed (Fig. 2) using as divisor a sample of MET 32.16 μg/mL. Figure 3 indicates their first derivatives from the ratio spectra, which were calculated with $\Delta\lambda = 4$ and scaling factor 1. FEL can be determined by measuring the signal at different wavelengths (237.4, 245, 320, 328, 337 nm). However, it was found that sensitivity was best at 245 nm.

An analogue procedure was followed for preparing the calibration graph for MET; the absorption spectra of the drug were divided sensor by sensor, by the spectrum of 3.22 μg/mL FEL (Fig. 2) and the first derivative of the resulting spectra ratios were calculated (Fig. 3). The concentration of MET

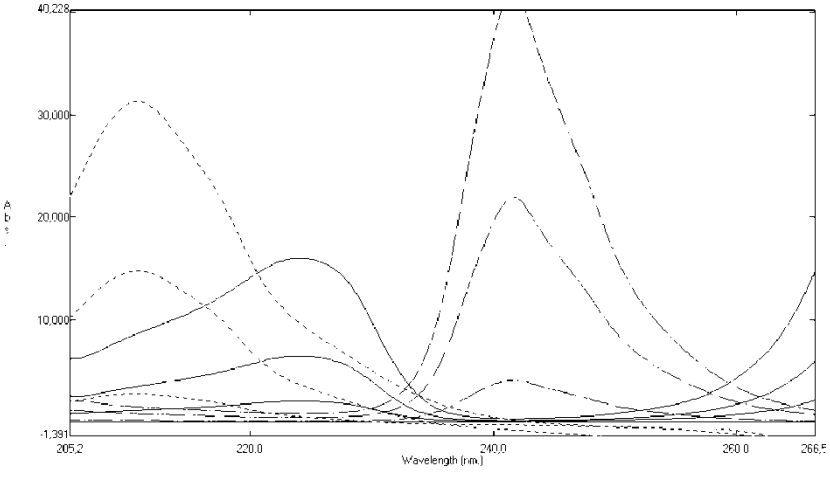

Figure 2. Ratio spectra of FEL(– – –) 1.61, 8.04, 16.08 μg/mL (32.16 μg/mL MET as divisor), MET (—) 9.64, 32.16, 80.40 μg/mL (3.22 μg/mL FEL as divisor), and RAM(----) 1.56, 7.80, 15.60 μg/mL with scaling factor 10 (3.22 μg/mL FEL as divisor).

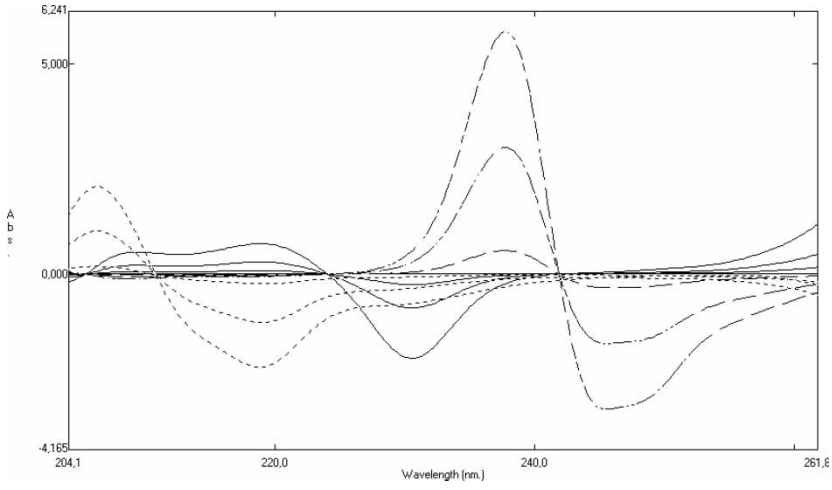

Figure 3. First derivative ratio spectra of FEL(– – –) 1.61, 8.04, 16.08 μg/mL (32.16 μg/mL MET as divisor), MET(——) 9.64, 32.16, 80.40 μg/mL (3.22 μg/mL FEL as divisor), and RAM(----) 1.56, 7.80, 15.60 μg/mL with scaling factor 10 (3.22 μg/mL FEL as divisor).

was determined by measuring the amplitude at 230.4 nm (219, 230.4, 279 nm) corresponding to a maximum point.

Binary Mixture of FEL-RAM

The stored UV absorption spectra of standard solutions of FEL in 40% MeOH were divided wavelength-by-wavelength by the spectrum of standard mixture solution of RAM (15.60 μg/mL)and smoothing using $\Delta\lambda = 4$ nm. The ratio spectra of the first derivatives, with $\Delta\lambda = 4$ nm and scaling factor 1, were calculated at 261.4 nm. Similarly for RAM the divisor was a standard solution of FEL 3.22 μg/mL, as well as the corresponding first derivative spectra, on the basis of which RAM can be quantified was 218.8 nm (Figs. 2 and 3).

Recovery studies of the described method were performed in two different series of synthetic mixtures, FEL-RAM and FEL-MET prepared by adding accurately weighed amounts of drugs. Results are presented in Table 2.

Adequate linearities were observed in both cases and the statistical parameters calculated from calibration graphs are summarized in Table 3. Limit of detection (LOD) and limit of quantitation (LOQ) values, for the ratio derivative procedure (Table 3) were calculated according to the following criterions: $\text{LOD} = 3.3 \cdot S_{y/x}/\text{m}$, $\text{LOQ} = 10 \cdot S_{y/x}/\text{m}$, respectively, where $S_{y/x}$ is the residual standard deviation and m is the calculated slope of the corresponding calibration.[23]

Table 3. Statistical parameters for calibration graphs by ratio first derivative and HPLC methods

	HPLC		
Parameter	FEL	MET	RAM
Concentration range	1.37–17.12	2.26–56.56	1.99–19.87
LOD (μg/mL)	0.10	0.45	0.09
LOQ (μg/mL)	0.31	1.36	0.29
Slope	351043.6 ± 6888	125065.7 ± 6620	156205.1 ± 2028
Intercept	−27540.3 ± 26543	−3259.7 ± 42140	13151.6 ± 11344
R	0.9999	0.9993	0.9999
	Spectrophotometric derivative ratio		
	FEL	MET	RAM	FEL
Wavelength	245 nm	230.4 nm	218.8 nm	261.4 nm
Concentration range	1.61–17.69	4.82–80.40	1.56–15.60	1.61–17.69
LOD (μg/mL)	0.28	0.28	0.61	0.62
LOQ (μg/mL)	0.87	0.85	1.84	1.87
Slope	0.22011 ± 0.0048	0.31119 ± 0.0067	0.13625 ± 0.0065	0.559751 ± 0.0263
Intercept	0.03079 ±0.0465	0.05797 ± 0.0645	0.06159 ± 0.0611	0.21315 ± 0.2552
R	0.9999	0.9999	0.9994	0.9994

The intra-day precision was evaluated through replicate analysis of three standard solutions containing three times the same concentration of 40.4 μg/mL MET, 4.1 μg/mL FEL, and 4.1 μg/mL RAM. Each standard was analyzed 3 times. The repeatability of the system was fairly good as indicated by the small values of Relative Standard Deviation ($\%RSD_{RAM} = 0.60$, $\%RSD_{FEL} = 0.86$, $\%RSD_{MET} = 1.49$).

Application of HPLC Method

The developed HPLC method was applied for the simultaneous determination of FEL, MET, and RAM in binary or ternary mixtures.

Peak interference and broadening, in reversed phase liquid chromatography, of the analytes were observed and this phenomenon prompted investigation (buffers solution, pH, organic strength, and ion pairing agent). The optimization steps were performed by directly injecting solutions of each substance separately on the analytical column using different mobile phases at a flow rate of 1 mL/min.

Initially, the influence of three organic solvents (acetonitrile, methanol, tetrahydrofuran) in combination with water, on the retention time (in terms of capacity factor, k') and peaks resolution (in terms of resolution factor, R_s) of the analytes, was investigated. Methanol-H_2O (60:40 v/v) as mobile phase gives high k' values for the two active ingredients and produces peak broadening and tailing especially for MET and FEL. Acetonitrile-H_2O (60:40 v/v) produces peak broadening and tailing to MET and elute RAM with solvent front. At the same time, the presence of this solvent in quantity $>60\%$ decrease seriously the R_f factor among the three substances. About the same results have been obtained when using, as mobile phase, methanol-acetonitrile-H_2O or methanol-acetonitrile-tetrahydrofuran-H_2O. Improvement was observed by the addition of a buffer, producing aqueous buffer solutions.

The aim of the selection of the appropriate aqueous buffer solution was to obtain a sufficient retention of the peaks corresponding to MET, FEL, and RAM with good resolution between them, and also to reach a good peak symmetry as closely as possible (in terms of symmetry factor, A_s).

Tri-sodium citrate-2-hydrate (0.015 M), sodium dihydrogen phosphate monohydrate, 1-heptanesulfonic acid sodium salt, and sodium acetate trihydrate at pH 2.5, have been tested as mobile phase combined with CH_3CN-MeOH. Finally, two of them (sodium dihydrogen phosphate monohydrate and 1-heptanesulfonic acid sodium salt) presented as the most convenient.

Generally, alcyl-sulfonic acid sodium salts proved to be useful ion-pairing agents able to improve the retention time of the analytes and provide single symmetric peaks. However, approaching the chromatographic problem in a different way, the influence of the increment of atoms -C- in the carbonic chain of the alcyl-sulfonic (1-Propanesulfonic, 1-Butanesulfonic, 1-Pentanesulfonic, 1-Hexanesulfonic, 1-Heptanesulfonic) acid sodium salt,

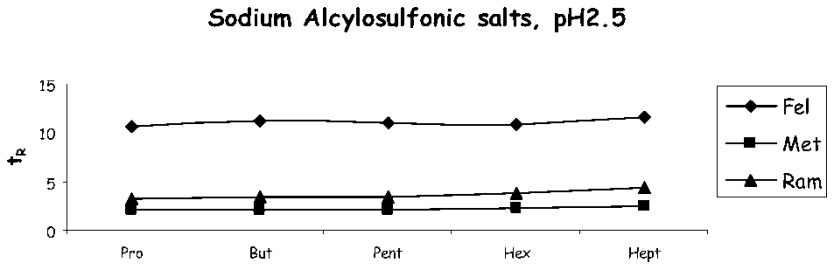

Figure 4. Influence of different sodium alkyl-sulfonic (1-Propanesulfonic, 1-Butanesulfonic, 1-Pentanesulfonic, 1-Hexanesulfonic, 1-Heptanesulfonic) acid sodium salts, to t_R of analytes. Mobile phase: 0.015 M alcyl-1-sulfonic acid sodium salt -MeOH-CH_3CN, 40 : 30 : 30 v/v/v, pH 2.5.

in a mobile phase consisting of 0.015 M alcyl-1-sulfonic acid sodium salt -MeOH-CH_3CN, (40:30:30 v/v/v pH 2.5), was investigated. According to the results, neither the increment of atoms -C- (Fig. 4) or even changes in the concentration of the buffer solution (0.010–0.030) plays an important role to the chromatographic behavior of the substances.

Changes in percentage of acetonitrile against buffer solution, in a mobile phase consisting of 40% MeOH-CH_3CN-0.015 M heptan-1-sulfonic acid sodium salt (pH = 2.5), were found to have a profound influence (especially in FEL and RAM) on the retention time (Fig. 5a) and in peak shape of the chromatographic compounds. The same influence was observed (Fig. 5b) by changing the percentage of methanol against the same buffer solution and keeping acetonitrile stable at 30%. With a higher percentage of acetonitrile or methanol the k′ decreases, the peak shape improved, and the resolution between the chromatographic peaks becomes lower. A intermediary situation was selected.

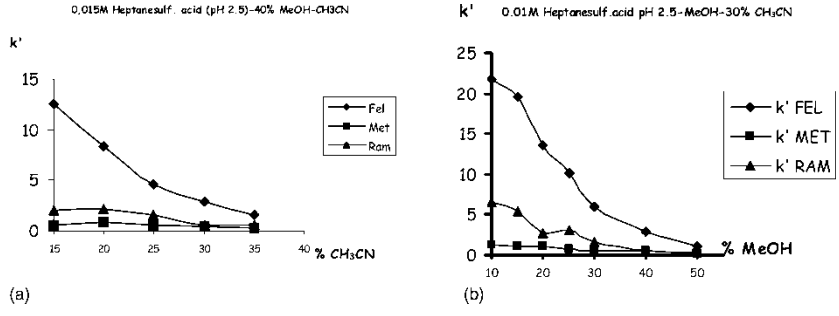

Figure 5. (a) Relationship between the capacity factor of FEL, RAM, MET and the percent of acetonitrile. Mobile phase: $CH_3(CH_2)_6SO_3Na$-40%MeOH-CH_3CN v/v/v (pH = 2.5). b) Relationship between k′ of the analytes and the percent of methanol. Mobile phase: $CH_3(CH_2)_6SO_3Na$-MeOH-30%CH_3CN v/v/v (pH = 2.5).

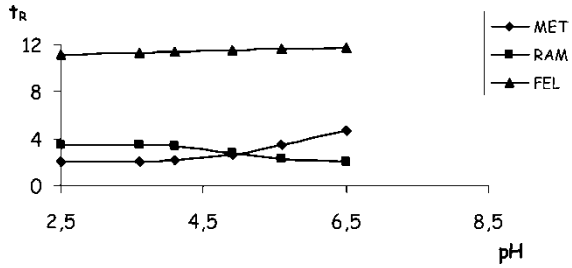

Figure 6. Influence of different pH values to t_R of FEL, RAM, MET; Mobile phase: $NaH_2PO_4 \cdot H_2O$-MeOH-CH_3CN, 40 : 30 : 30 v/v/v.

Finally, using as the most convenient, two mobile phases consisting of a) 0.015 M 1-heptanesulfonic acid sodium salt-methanol-acetonitrile 35:40:25 v/v/v and b) 0.015 M sodium dihydrogen phosphate monohydrate-methanol-acetonitrile 40:30:30 v/v/v, the influence of different pH values on peaks asymmetry factor and retention time was studied. Owing to the individual characteristics of the compounds, ionization of them enhances the elution ability of mobile phase at low pH value for bases and at high pH value for acidic compounds.

The first mobile phase has proved that changes of pH values between 3 and 7 does not effect dramatically the retention time of FEL, MET, and RAM. Moreover, the addition of sulfuric acid, in the second mobile phase, slightly increases t_R of RAM, decreases t_R of MET, and is not considered important for FEL (Fig. 6). Generally, lower pH values improve peaks width. According to the above investigation, two chromatographic systems were selected: a) 0.015 M 1-heptanesulfonic acid sodium salt-methanol-acetonitrile 35:40:25, v/v/v, pH 2.5 for the determination of MET, FEL, RAM (Fig. 7a). b) 0.015 M sodium dihydrogen phosphate monohydrate-methanol-acetonitrile 40:30:30, v/v/v, at pH 6.5 (Fig. 7b) for the determination of FEL-MET in Logimax®, and at pH 2.5 (Fig. 7c) for FEL-RAM in Triacor®. Chromatographic characteristics of the determinants are presented in Table 4.

Variation of pH of the mobile phase by ± 0.1 and its organic strength by $\pm 2\%$ did not have any significant effect on chromatographic resolution. Moreover, the above study can be used as good guidance for the simultaneous or separate determination of FEL, MET, and RAM in different matrixes, by using high performance liquid chromatography.

Statistical parameters for regression equations of the HPLC method obtained by least squares treatment of the results were given in Table 3.

For evaluation of the precision estimates, repeatability of 6 determinants at 100% of the test concentration was performed. The %RSD values were 1.80 for FEL, 1.88 for MET, and 1.93 for RAM.

In order to test the accuracy of the proposed method, HPLC was applied for the determination of synthetic mixtures containing various concentrations

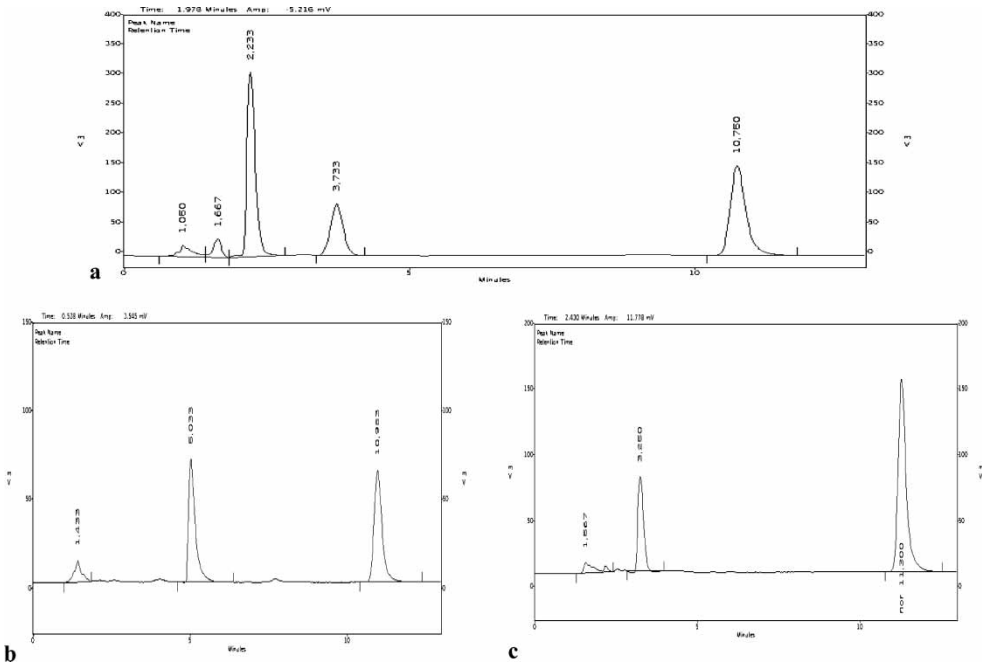

Figure 7. Typical high-performance liquid chromatogram of mixtures a) 24.24 μg/mL MET ($t_R = 2.23$) with 7.80 μg/mL RAM ($t_R = 3.73$) and 8.04 μg/mL FEL ($t_R = 10.76$); mobile phase: $CH_3(CH_2)_6SO_3Na$-MeOH-CH_3CN 35:40:25 v/v/v (pH = 2.5) at $\lambda = 210$ nm. b) 40.0 μg/mL MET ($t_R = 6.03$) with 4.0 μg/mL FEL ($t_R = 10.98$) in Logimax® tablets using mobile phase $NaH_2PO_4 \cdot H_20$-MeOH-CH_3CN, 40 : 30 : 30 v/v/v, (pH 6.5) at $\lambda = 230$ nm. c) 8.0 μg/mL RAM ($t_R = 3.25$) with 8.0 μg/mL FEL ($t_R = 11.30$) in Triacor® tablets using mobile phase $NaH_2PO_4 \cdot H_20$-MeOH-CH_3CN 40 : 30 : 30 v/v/v, (pH 2.5) at $\lambda = 210$ nm.

Table 4. Chromatographic characteristics of MET, FEL, RAM using three different mobile phases

Mobile phase	Substance	t_R(min)	k′	As	R_s
$CH_3(CH_2)_6SO_3Na$-MeOH-CH_3CN, (pH = 2.5) 35:40:25 v/v/v	MET	2.23	1.1	1.4	4.4[a] 14.9[b]
	RAM	3.73	2.5	1.1	
	FEL	10.76	9.2	1.6	
$NaH_2PO_4 \cdot H_2O$-MeOH-CH_3CN, (pH 6.5) 40:30:30 v/v/v	MET	6.03	3.2	1.7	20.3
	FEL	11.30	5.8	1.3	
$NaH_2PO_4 \cdot H_2O$-MeOH-CH_3CN, (pH 2.5) 40:30:30 v/v/v	RAM	3.26	1.0	1.2	11.9
	FEL	10.98	6.7	1.1	

[a] R_s *MET-RAM*.
[b] R_s *RAM-FEL*.

of MET-FEL-RAM. The tested mixtures were compared in respect to the amount of drug added and found. As can be observed from Table, 2 all the results are satisfactory with a mean %RSD < 1.29 and %recovery value 100 ± 1.3. Statistical analysis of the results between the HPLC and each spectrophotometric method, using student's t-test and variance ratio two tailed F-test [23], shows no significant difference in the performance of the thee methods regarding the accuracy and precision, respectively (Table 2).

Additionally, the drug content calculations for the commercial tablets have evaluated by a "Dose uniformity" test. The excellent recoveries of the samples (Table 5) suggested the high accuracy of each method.

CONCLUSIONS

Two chemometric methods in spectrophotometric analysis, PLS and derivative ratio, are proposed for the simultaneous determination of FEL with MET or RAM. These techniques were applied successfully to commercial

Table 5. Mean %recovery values of FEL, MET, RAM in "content uniformity" test

	Mean %recovery (10 tablets) ± S.D		
	HPLC	PLS	Derivative ratio
Felodipine	95.2 ± 1.4	97.4 ± 1.7	95.1 ± 1.5
Ramipril	96.4 ± 2.9	98.3 ± 2.5	96.3 ± 2.4
Felodipine	98.3 ± 2.1	99.3 ± 2.6	98.7 ± 2.8
Metoproll	96.3 ± 2.2	96.2 ± 2.4	97.6 ± 2.6

pharmaceutical enteric-coated tablets. The assay results obtained using these methods were compared with a new HPLC method; good agreement was observed.

Although the HPLC method is more specific than the chemometric methods it needs expensive equipment and materials. Spectrophotometric methods are less expensive and they do not require sophisticated instrumentation and any prior separation step. The sensitivity, simplicity, and short analysis time of the three proposed methods makes them suitable for routine analysis tests.

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