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ORIGINAL RESEARCH

Simultaneous quantitation and monitoring of rosuvastatin with NSAIDs by liquid chromatography with UV detection

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Overview and methods: A simple, accurate, and sensitive high-performance liquid chromatography-ultraviolet detection method was developed for simultaneous determination of rosuvastatin with co-administered nonsteroidal anti-inflammatory drugs (meloxicam, ibuprofen, and mefenamic acid) in active pharmaceutical ingredient (API), pharmaceutical formulations, and human serum. Isocratic separation was employed on prepacked Purospher Star C_{18} (5 µm, 25 × 0.46 cm) columns at ambient temperature. The mobile phase consisted of methanol:water:acetonitrile (80:17.5:2.5 v/v), pH adjusted to 3.0 with *o*-phosphoric acid at 1 mL min^{-1}. The drugs in the eluant were monitored at isosbestic point of drugs at 230 nm. The method was compared by programming the detector adjusting the wavelength with time to match the individual analyte's chromophore which enhanced sensitivity with linear range.

Results: Linear behavior was observed between 0.1 and 2.5 µgmL^{-1} for rosuvastatin, 0.4 and 10 µgmL^{-1} for meloxicam, 0.25 and 6.25 µgmL^{-1} for ibuprofen, and 0.15 and 3.75 µgmL^{-1} for mefenamic acid, with $r^2 > 0.998$. The relative standard deviation for inter-day precision was <2 in API, formulations, and human serum. Percent recovery for all drugs was 97.3%–100.89% in API and formulations and 99.3%–100.4% in human serum. Wavelength-programmed analysis made the method more sensitive, where 4 < limit of quantification (LOQ) < 11 and 1 < limit of detection (LOD) < 4 ngmL^{-1} for API; 6 < LOQ < 10 and 2 < LOD < 3 ngmL^{-1} for pharmaceutical formulations; and 3 < LOQ < 10 and 1 < LOD < 3 ngmL^{-1} in human serum, reduced from 9 < LOQs < 23 and 3 < LODs < 7 ngmL^{-1} for all drug analytes in API; and 4 < LOQs < 17 and 1 < LODs < 6 ngmL^{-1} in human serum recorded at isosbestic point for rosuvastatin, meloxicam, ibuprofen, and mefenamic acid, respectively. Recovery of drugs was 99.998%–104.000% in all API, formulations, and serum samples.

Conclusion: The proposed method can be used for the quantitative analysis of these drugs in raw materials, in bulk drugs, dosage formulations and in human serum and for clinical studies even when the drug is present in low amounts.

Keywords: meloxicam, ibuprofen, mefenamic acid, liquid chromatography, quantitative analysis

Introduction

Rosuvastatin (ROS) is an oral drug for lowering blood cholesterol levels and belongs to the HMG-CoA reductase inhibitors, chemically a 6-heptenoic acid – (3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid – calcium salt (Figure 1).[1–3] Subjects with hyperlipidemia and hypercholesterolemia aged older than 40 years usually suffer from anti-inflammatory disorders as well for which they are prescribed nonsteroidal anti-inflammatory drugs (NSAIDs).

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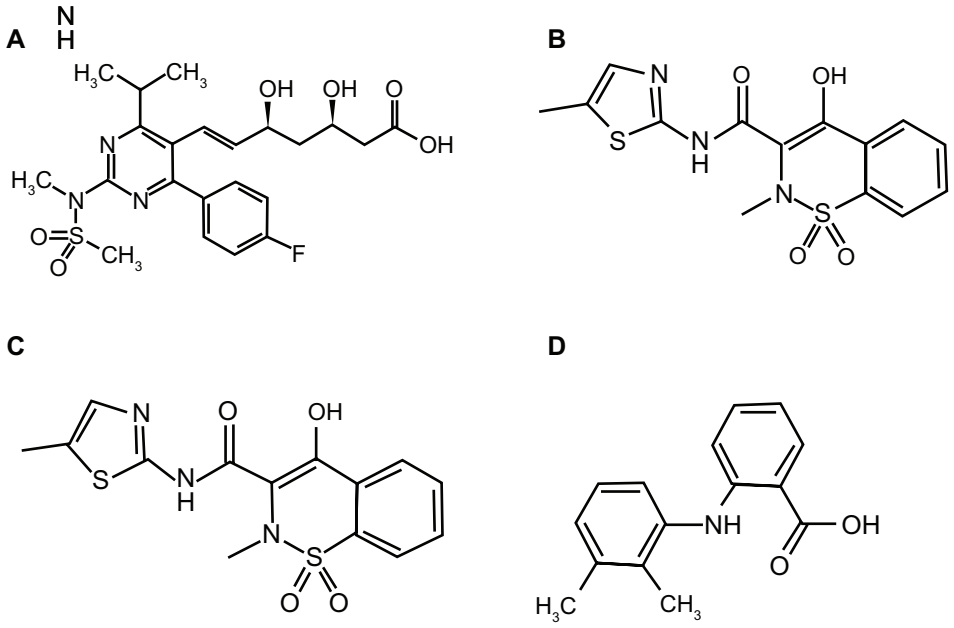

Figure 1 Chemical structures of (**A**) rosuvastatin, (**B**) meloxicam, (**C**) ibuprofen, and (**D**) mefenamic acid.

Determination of ROS has been reported alone;[4–6] with other statins such as simvastatin and pravastatin sodium;[7] and in pharmacological, pharmacokinetic, and analytical studies in combination with atorvastatin, lovastatin, pravastatin, and simvastatin.[8] ROS has also been determined with atenolol, spironolactone, glibenclamide, and naproxen sodium;[9] pioglitazone, gliquidone, and simvastatin;[10] simvastatin, atorvastatin, pravastatin, and ceftriaxone;[11] diltiazem, atorvastatin, and simvastatin;[12] lisinopril, pravastatin, and atorvastatin;[13] captopril, atorvastatin, and simvastatin;[14] and lisinopril, captopril, and enalapril.[15]

Methods have also been developed for NSAIDs,[16–19] including ibuprofen (IBU).[20–27] In 2000, Velpandian et al[28] reported a high-performance liquid chromatography (HPLC) method for the determination of meloxicam (MEL) in biological samples. Methods for the determination of MEL in plasma using liquid chromatography (LC) have been developed by Dasandi et al[29] and Baeyens et al.[30] Liquid chromatographic method has also been reported for determining MEL in bulk drug and pharmaceutical formulation by Zawilla et al[31] and Arayne et al.[32]

Mefenamic acid (MEF) has been determined simultaneously with drotaverine HCl,[33,34] with ethamsylate,[35] and with tranexamic acid.[36] Our research group has been working for some time on the simultaneous determination of co-administered drugs[9–15] as NSAIDs with lisinopril,[37] sparfloxacin,[38] and metformin.[39–41] Sun and colleagues have worked on the simultaneous determination of NSAIDs at their absorption maxima.[42]

The present work describes a simple reverse phase (RP)-HPLC method for the simultaneous determination of ROS with IBU, MEL, and MEF in active pharmaceutical ingredient (API), dosage formulations, and human serum. A method such as this is needed for co-administration of drugs in multiple-drug therapy and, as far as the authors are aware, there is no such method reported in the literature for simultaneous determination of ROS and NSAIDs. Our present work is also unique because it includes the analysis of drugs by programming the detector adjusting the wavelength with time to match the individual analyte's chromophore which enhanced the sensitivity with linearity range. The use of a conventional ultraviolet (UV) detector in the system, without the installation of drug-sensitive or expensive detectors with isocratic elution, increased the sensitivity of the method, raising it to the level of the nanogram. The method is applicable for drug–drug interaction studies between ROS and NSAIDs as well as pharmaceutical and clinical routine analyses.

Experimental

Materials and reagents

ROS, MEL, IBU, and MEF active ingredients were kind gifts from PharmEvo (Karachi, Pakistan), Hilton Pharma Karachi (Karachi, Pakistan), Abbot Laboratories (Karachi, Pakistan),

and Pfizer Pakistan (Ltd.) (Karachi, Pakistan), respectively, while their dosage formulations, ROS (X-plended 5 mg tablets, PharmEvo), MEL (Xobix 7.5 mg tablets, Hilton Pharma Karachi), IBU (Brufen 200 mg tablets Abbot Laboratories (Karachi, Pakistan)), and MEF (Ponstan® 250 mg tablets) Pfizer Laboratories, Karachi, Pakistan were purchased from a local market. *O*-phosphoric acid, methanol, and acetonitrile (ACN) of HPLC grade were purchased from Merck (Darmstadt, Germany). Double-distilled de-ionized water was used throughout the experiments and was prepared freshly daily.

Apparatus

Two HPLC systems were used in this research: a Shimadzu 10A HPLC System and a Shimadzu 20A HPLC System (Kyoto, Japan), both with LC-20-AT HPLC pumps; SPD-20A Shimadzu UV visible detectors; CBM-102 communication Bus Modules (Shimadzu) – to record the chromatographic and integrated data; and Shimadzu Class-GC 10 software (v 5.03). Chromatographic separations were carried out on Purospher® STAR C_{18} (5 µm, 250 × 0.46 cm) (Merck Millipore) and Sapilco® C_{18} (5 µm, 250 × 0.46 cm) columns (Sigma-Aldrich, St Louis, MO) for ruggedness studies of the method. A Shimadzu UV-1800 spectrophotometer was used for the detection of the maximum drug absorption. Deionization of water was carried out using a Stedec CSW-300 deionizer (Stedec (Pvt) Ltd., Karachi, Pakistan) and sonication and degassing of mobile phase was carried out with an Elma Ultrasonic LC 30 H sonicator (Elmer, NY).

Preparation of standard solutions

Standard stock solutions of ROS, MEL, IBU, and MEF were prepared by dissolving their appropriate amounts in diluents of mobile phase and afterwards adjusted to the mark for 100 mL/min. These stock solutions were subsequently used in the preparation of working standards by further dilution. All stock solutions were kept in refrigerator at 4°C. Working solutions were made from 0.025 to 2.500 µgmL^{-1} for ROS, 0.1 to 10.0 µgmL^{-1} for MEL, 0.0625 to 6.2500 µgmL^{-1} for IBU, and 0.0375 to 3.7500 µgmL^{-1} for MEF.

Chromatographic conditions

After a number of trials by varying the parameters of solvent compositions, pH, flow rates, shortest retention times, and best resolution among the peaks of the analytes, the optimum conditions were achieved. The mobile phase, methanol:water:ACN (80:17.5:2.5 v/v/v) was filtered through a membrane filter (0.45 micron) and degassed in ultrasonic bath for 10 minutes. The analytes were isocratically eluted with the mobile phase at a flow rate of 1.0 mL/min. The mobile phase was brought to pH 3.0 by *o*-phosphoric acid against a Purospher STAR C_{18} (5 µm, 250 × 0.46 cm) column as the stationary phase. The retention times of ROS, MEL, IBU, and MEF were 3.6, 5.0, 7.8, and 11.9 minutes, respectively. Samples of 20 µL were injected for a single run through a rheodyne sample loop. The method was first developed and validated at 230 nm, the isosbestic point of drugs, then at their individual λ_{max}: 240 nm, 361 nm, 230 nm, and 282 nm for ROS, MEL, IBU, and MEF, respectively (these were found by scanning them on a UV spectrophotometer (Figure 2) by programming the UV detector for 0–4.2, 4.3–5.8, 5.9–8.8 and 8.9–13.2 minutes for ROS, MEL, IBU, and MEF, respectively, at a flow rate of 1 mL/min^{-1}).

Preparation of solutions of ROS and NSAIDs in pharmaceutical formulations

The contents of ten tablets each of ROS, MEL, IBU, and MEF were finely ground separately. An accurately weighed powdered sample containing the labeled amount of each drug was transferred to a 100 mL volumetric flask. The volume was adjusted with mobile phase and the resultant solution was sonicated for 5 minutes. A portion of the solution was then filtered through a 0.45 µm Millipore filter paper (Billerica, MA) and appropriately diluted. An aliquot (20 µL) of each solution was injected into the column.

Preparation of solutions of ROS and NSAIDs in human serum

A 3 mL sample of blood from a healthy volunteer (aged 24 years) was collected in an evacuated glass tube through an indwelling cannula placed in the forearm vein at Fatimid Foundation (Karachi, Pakistan). The volunteer was not taking any medication, a smoker, or undertaking any strenuous activity. The blood was shaken and centrifuged at 10,000 rpm for 10 minutes to separate out the plasma. A total of 9 mL ACN was added to 1 mL plasma and centrifuged at 10,000 rpm for 10 minutes to deproteinate it.[14,43] The supernatant serum thus obtained from the plasma was filtered and used for analysis and stored at –20°C. Working solutions of various concentrations – that is, 0.025–2.5 µgmL^{-1} for ROS, 0.1–10 µgmL^{-1} for MEL, 0.0625–6.25 µgmL^{-1} for IBU, and 0.0375–3.75 µgmL^{-1} for MEF – were prepared by spiking the serum with stock solutions maintaining the ratio of 1:1 (drug stock diluted by diluent:serum v/v). Triplicate injections were made for each working solution for the analysis in serum.

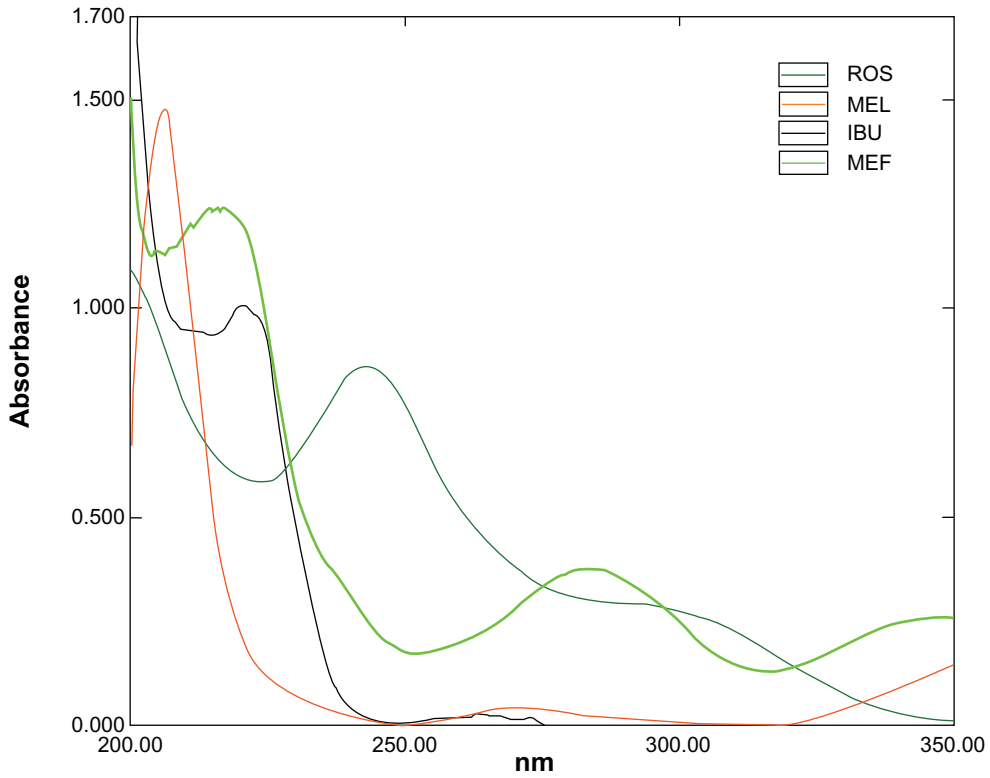

Figure 2 Representative ultraviolet spectra of rosuvastatin (ROS), ibuprofen (IBU), and mefenamic acid (MEF).

Results and discussion

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. The newly developed method for determination of ROS and NSAIDs (MEL, IBU, and MEF) described here has been validated and holds well for determination of drugs in raw materials, dosage formulations, and human serum, as the literature survey revealed that no UV or LC methods have been reported for the simultaneous determination of ROS in combination with MEL, IBU, and MEF in bulk and pharmaceutical formulations. The present paper describes our attempt to develop a rapid, more accurate, precise, 1, economical, and less time-consuming method based on RP-HPLC separation simultaneously combined with UV detection for ROS and NSAIDs as raw materials, in bulk drug samples, dosage formulations, and, especially, human serum.

Method optimization

In this study, an isocratic separation of ROS from MEL, IBU and MEF in standard solution of active pharmaceuticals, formulations and human serum was carried out using methanol:water:ACN (80:17.5:2.5, v/v/v) as the mobile phase adjusted to pH 3.0 with *o*-phosphoric acid.

Since quality control analyses, in vitro and in vivo interactions, and pharmacokinetic studies require very specific methods for the analytes, optimized conditions need to be adopted after studying various parameters selective to the drug analytes. Each drug analyte was scanned on a UV-visible spectrophotometer to detect similar absorptivity at its maxima, called its "isosbestic point," (230 nm) at the λ_{max} (240 nm, 361 nm, 230 nm, and 282 nm, for ROS, MEL, IBU, and MEF, respectively) of ROS, MEL, IBU, and MEF (spectra shown in Figure 2).

Different ratios of methanol and water were taken as the starting test solvents for the mobile phase considering peak parameters, ease of separation, and cost: 95:5, 90:10, 85:15, 80:20, and 75:25 v/v of methanol:water were tested. The ratio 80:20 v/v of methanol:water gave better peak separation and resolution, while the lower ratios gave larger retention times and broader peaks; above this ratio, the retention times were closer and merged. Further, the addition of ACN with the ratio 80:17.5:2.5 methanol:water:ACN v/v/v gave sharper and better resolved peaks.

Investigating for pH effect from 2.5 to 4.5, the peaks were well resolved and sharp when the pH was adjusted to 3.0. The peak area of each compound remarkably decreased when the pH value of the mobile phase was above 3.5, whereas there were little changes up to pH 3.2. Thus, pH 3.0 was chosen as optimal. *O*-phosphoric acid was used for pH adjustment due to its inertness towards the column packing.

The Purospher Star C_{18} (5 µm, 250 × 0.46 cm) column was found efficient for the reproducible separation of nonpolar compounds, minimizing solvent usage with typical peak symmetry. The ROS, MEL, IBU, and MEF eluted at 3.6, 5.0, 7.8, and 11.9 minutes, respectively, from the column at 1 mL/min^{-1} at isocratic condition. After the method was developed and optimized, it was validated.

Selection of detection wavelength

Each compound exhibited a different maximum UV absorbance. To detect ROS and NSAIDs sensitively, a wavelength program was performed along with the study at the isosbestic point of the wavelengths. The analysis was carried out at the isosbestic point 230 nm and the program was set at 240 nm (0–4.2 minutes), 361 nm (4.2–5.8 minutes), 230 nm (5.8–8.8 minutes), and 282 nm (8.8–13.2 minutes). For all API, pharmaceutical formulation, and human serum analyses, each drug was monitored at the isosbestic point and λ_{max} of each drug. Under these conditions, standard calibration curves showed good linearity ($r^2 = 0.999$), ranging from 0.025 to 2.500 µgmL^{-1} for ROS, 0.1–10.0 µgmL^{-1} for MEL, 0.0625–6.2500 µgmL^{-1} for IBU, and 0.0375–3.7500 µgmL^{-1} for MEF.

Method validation

The guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)[44] and USP were followed for the validation of the optimized method.[45–47]

Specificity and system suitability

The good separation of analyte drugs observed in API and human serum chromatograms (Figure 3) shows the method is specific. There is no interfering peak of any additional components like excipients, degradation products or any interfering endogenous plasma components in serum samples. Excipients were spiked in samples to observe the additional peaks due to them, but no interfering peak is noticable in the chromatogram.

The uniformity of the system operation throughout the analysis was developed by initially equilibrating the system with ten consecutive injections of the mobile phase. The system suitability studies, as per ICH parameters, were found to be symmetrical in peaks. Theoretical plates, peak symmetry factor and tailing factor, resolution, and repeatability of the system were found favorable (Table 1).

Linearity

Calibration curves drawn for ten different concentrations by linear regression analysis showed good linearity with the correlation coefficient (r^2) > 0.998 and no significant variation in slopes and intercepts over the range of 0.1–2.5 µgmL^{-1} for ROS, 0.4–10 µgmL^{-1} for MEL, 0.25–6.25 µgmL^{-1} for

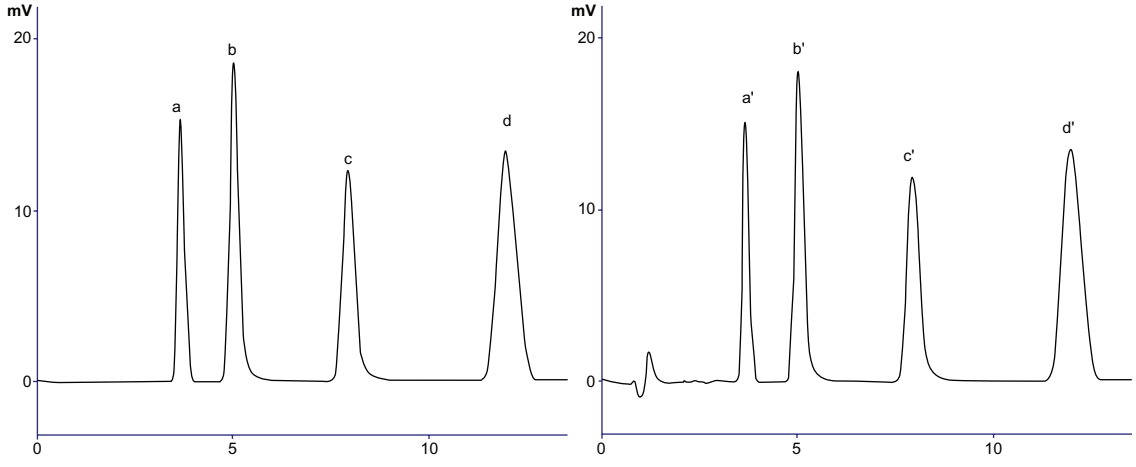

Figure 3 Representative chromatograms of rosuvastatin (a and a′), meloxicam (b and b′), ibuprofen (c and c′) and mefenamic acid (d and d′) in active pharmaceutical ingredient (left) and human serum (right), respectively.

Table 1 System suitability parameters of rosuvastatin (ROS) and nonsteroidal anti-inflammatory drugs

Analytes	t_R	k′	N	T	Rs	α
At isosbestic point						
ROS	3.52	0.54	1819	–	–	1.32
MEL	4.56	1.13	1933	0.99	3.48	2.09
IBU	7.73	2.39	2837	1.30	5.61	2.11
MEF	11.76	4.13	2669	1.57	5.36	1.73
At individual λ_{max}						
ROS	3.570	0.00	2268	1.58	–	–
MEL	4.887	0.37	2372	1.49	3.76	0.00
IBU	7.575	1.12	3100	1.46	5.69	3.04
MEF	11.334	2.17	2358	1.71	5.09	1.94

Abbreviations: α, separation factor; IBU, ibuprofen; k′, capacity factors; MEF, mefenamic acid; MEL, meloxicam; N, theoretical plates; Rs, resolution; t_R, retention time; T, tailing factor.

IBU, and 0.15–3.75 μgmL^{-1} for MEF. Results analyzed are tabulated in Table 2, with standard error, standard error of estimate, and slope showing the linear affiliation between concentration and peak areas.

Accuracy

The percentage recovery of the drugs was evaluated to be within the range of 99%–100.89% for the concentrations of 80%, 100%, and 120% of each drug. The results were found in accordance with the formulations and analytes spiked in human serum (Table 3) following the formula:

$$\text{Recovery (\%)} = [(\text{Measured concentration} - \text{Original concentration}) / \text{Spiked concentration}] \times 100 \quad (1)$$

Precision

The repeatability of the method confirmed its precision. Six concentrations were analyzed for 3 days for inter-day precision. The statistical summary comprises coefficients of variance (relative standard deviation [%RSD] <2) and recoveries (99%–100.89%; Table 4).

Detection and quantification limit

The sensitivity of the method was analyzed from the slope of the calibration curve and standard deviation was used to calculate the limits of quantification and detection; as a result LOQ was greater than 9 and less than 23, while LOD was greater than 3 and less than 7 ngmL–1 for all drug analytes in API (Table 2).

Table 2 Regression statistics of rosuvastatin (ROS) and nonsteroidal anti-inflammatory drugs

Parameter	At isosbestic point				At individual λ_{max}			
	ROS	MEL	IBU	MEF	ROS	MEL	IBU	MEF
Active pharmaceutical ingredient								
Conc (μgmL^{-1})	0.1–2.5	0.4–10	0.25–6.25	0.15–3.75	0.025–2.5	0.1–10	0.0625–6.25	0.0375–3.75
Slope	16507	51076	73944	23345	40106	16203	19563	42294
Intercept	–3405	19674	18733	9383	2614	5034	7085	2722
LOD (ngmL^{-1})	3	5	4	7	1	2	1	4
LOQ (ngmL^{-1})	9	14	13	23	4	7	3	11
r^2	0.9992	0.9997	0.9997	0.9993	0.9996	0.9993	0.998	0.9993
SEE	17062	15581	9925	21321	14954	29462	15545	27001
SE	9744	8899	5668	12177	7480	14737	8878	13506
Serum								
Conc (μgmL^{-1})	0.1–2.5	0.4–10	0.25–6.25	0.15–3.75	0.025–2.5	0.1–10	0.0625–6.25	0.0375–3.75
Slope	16497	49128	76452	23393	43243	26203	26715	33108
Intercept	–1913	35695	5656	8823	10630	30516	39485	25703
LOD (ngmL^{-1})	6	8	6	1	3	2	4	1
LOQ (ngmL^{-1})	17	23	19	4	10	6	12	3
r^2					0.999	0.998	0.998	0.999
SEE	16515	15545	10067	21508	24866	27001	30380	35709
SE	94312	8878	5749	12284	12438	13506	15196	17862
Pharmaceutical formulations (tablets)								
Conc (μgmL^{-1})	0.1–2.5	0.4–10	0.25–6.25	0.15–3.75	0.025–2.5	0.1–10	0.0625–6.25	0.0375–3.75
Slope	165591	52083	75467	234264	42977	26650	26814	33746
Intercept	–4129.2	6652.7	12297	7983	19791	28544	37886	25610
LOD (ngmL^{-1})	11	4	5	4	2	3	2	3
LOQ (ngmL^{-1})	34	11	14	11	7	10	6	9
r^2	0.9997	0.9988	0.998	0.9994	0.998	0.998	0.998	0.999
SEE	16993	20547	10040	24378	22571	26566	25012	31711
SE	9705	11735	5734	13923	10709	12604	11867	15045

Abbreviations: conc, concentration; IBU, ibuprofen; LOD, limit of detection; LOQ, limit of quantification; MEF, mefenamic acid; MEL, meloxicam; SE, standard error; SEE, standard error of estimate.

Table 3 Accuracy and recovery studies of rosuvastatin (ROS) and nonsteroidal anti-inflammatory drugs

Conc %	% rec					
	At isosbestic point			At individual λ_{max}		
	API	Tablet	Serum	API	Tablet	Serum
ROS						
80	99.99	100.03	99.32	99.97	97.60	97.52
100	100.03	100.03	100.49	99.98	105.73	104.09
120	100.00	100.05	99.582	99.94	100.91	100.28
Meloxicam						
80	100.02	99.88	99.89	100.01	98.43	97.69
100	100.00	100.09	100.01	100.05	96.49	96.63
120	100.02	99.97	99.971	99.95	90.99	91.20
Ibuprofen						
80	99.97	100.03	100.07	99.99	103.30	101.42
100	100.03	100.05	99.90	100.10	110.92	106.94
120	100.01	99.95	100.01	100.14	110.88	103.99
Mefenamic acid						
80	99.84	100.00	100.28	99.88	99.50	96.14
100	100.00	99.94	99.96	100.06	104.71	101.17
120	99.99	99.95	100.02	99.79	102.39	103.62

Abbreviations: API, active pharmaceutical ingredient; conc, concentration; rec, recovery.

Table 4 Inter-day precision studies of rosuvastatin and nonsteroidal anti-inflammatory drugs

Conc μgmL^{-1}	%RSD								
	API			Tablet		Serum			API at individual λ_{max}
Rosuvastatin									
0.1	0.28	0.10	0.09	0.20	0.08	0.44	0.05	0.13	0.22
0.3	0.11	0.01	0.10	0.16	0.01	0.07	0.17	0.03	0.30
0.6	0.26	0.01	0.34	1.08	0.07	0.05	0.51	0.12	0.32
0.9	0.66	0.01	0.11	0.13	0.01	0.04	0.06	0.42	0.07
1.5	0.16	0.01	0.11	0.00	0.02	0.10	0.03	0.19	0.01
2.5	0.04	0.03	0.02	0.03	0.09	0.05	0.70	0.09	0.32
Meloxicam									
0.4	0.06	0.27	0.16	0.20	0.35	0.440	0.23	0.45	0.04
1.2	0.01	0.06	0.02	0.165	0.31	0.073	0.52	0.10	0.07
2.4	0.05	0.17	0.01	1.079	0.34	0.051	0.61	0.07	0.04
3.6	0.03	0.04	0.04	0.127	0.05	0.040	0.11	0.03	0.06
6	0.01	0.94	0.01	0.001	0.04	0.099	0.03	0.10	0.02
10	0.02	0.04	0.01	0.001	0.01	0.055	0.05	0.04	0.05
Ibuprofen									
0.25	0.04	0.50	0.74	0.53	0.77	0.70	0.60	0.60	0.06
0.75	0.08	0.04	0.05	0.13	0.06	0.77	0.06	0.75	0.12
1.5	0.09	0.04	0.06	0.16	0.50	0.36	0.50	0.38	0.06
2.25	0.21	0.02	0.04	0.08	0.02	0.02	0.02	0.18	0.21
3.75	0.05	0.19	0.15	0.03	0.01	0.06	0.01	0.02	0.13
6.25	0.01	0.02	0.08	0.01	0.07	0.02	0.01	0.06	0.01
Mefenamic acid									
0.15	0.69	0.15	0.03	0.02	0.51	0.03	0.51	0.06	0.08
0.45	0.23	0.10	0.05	0.03	0.07	1.06	0.07	0.49	0.11
0.9	0.54	0.01	0.03	0.27	0.11	0.08	0.17	0.11	0.02
1.35	0.13	0.00	0.11	0.07	0.03	0.04	0.03	0.41	0.29
2.25	0.01	0.09	0.03	0.02	0.20	0.77	0.21	0.04	0.04
3.75	0.22	0.09	0.01	0.03	0.01	0.08	0.04	0.10	0.11

Abbreviations: API, active pharmaceutical ingredient; conc, concentration; %RSD, relative standard deviation.

Table 5 Robustness and ruggedness studies of rosuvastatin and nonsteroidal anti-inflammatory drugs

	Parameters	t_R	N	T
Rosuvastatin				
pH	2.9	3.637	2068	1.76
	3	3.83	2352	1.85
	3.1	3.62	2205	1.55
Flow rate	0.9	3.95	2316	1.45
(mL/min^{-1})	1	3.83	2352	1.85
	1.1	3.71	2397	1.25
Mobile phase	78/19.5/2.5	3.64	2162	1.54
(v/v/v)	80/17.5/2.5	3.83	2352	1.85
	82/15.5/2.5	3.62	2175	1.58
Column	Purospher® STAR C_{18}	3.83	2352	1.85
	Sapilco® C_{18}	3.641	2172	1.55
System	LC-20AT	3.83	2352	1.85
	LC-10AT	3.568	2399	1.51
Meloxicam				
pH	2.9	4.99	2282	1.33
	3	5.79	2398	1.57
	3.1	4.97	2321	1.42
Flow rate	0.9	5.86	2412	1.54
(mL/min^{-1})	1	5.79	2398	1.57
	1.1	5.60	2389	1.56
Mobile phase	78/19.5/2.5	4.99	2193	0.47
(v/v/v)	80/17.5/2.5	5.79	2398	1.57
	82/15.5/2.5	4.95	2194	1.48
Column	Purospher STAR C_{18}	5.79	2398	1.57
	Sapilco C_{18}	5.002	2304	1.39
System	LC-20AT	5.79	2398	1.57
	LC-10AT	4.853	2486	1.41
Ibuprofen				
Ph	2.9	7.91	3046	1.32
	3	7.01	3100	1.56
	3.1	7.89	2986	1.35
Flow rate	0.9	7.11	3125	1.57
(mL/min^{-1})	1	7.01	3100	1.56
	1.1	6.91	3091	1.44
Mobile phase	78/19.5/2.5	7.85	2966	1.38
(v/v/v)	80/17.5/2.5	7.01	3100	1.56
	82/15.5/2.5	7.808	2978	1.27
Column	Purospher STAR C_{18}	7.01	3100	1.56
	Sapilco C_{18}	7.933	3018	1.30
System	LC-20AT	7.01	3100	1.56
	LC-10AT	7.58	3251	1.39
Mefenamic acid				
pH	2.9	11.95	2793	1.61
	3	11.34	2778	1.58
	3.1	11.99	2763	1.65
Flow rate	0.9	11.51	2784	1.51
(mL/min^{-1})	1	11.34	2778	1.58
	1.1	11.52	2764	1.41
Mobile phase	78/19.5/2.5	11.87	2743	1.67
(v/v/v)	80/17.5/2.5	11.34	2778	1.58
	82/15.5/2.5	11.75	2736	1.67
Column	Purospher STAR C_{18}	11.34	2778	1.58
	Sapilco C_{18}	11.33	2430	1.63
System	LC-20AT	11.34	2778	1.58
	LC-10AT	11.16	2893	1.77

Abbreviations: T, tailing factor; t_R, retention time; N, theoretical plates.

Ruggedness

The analysis of the method was carried out in different laboratories and with different instruments at the Research Institute of Pharmaceutical Sciences and HEC laboratory Chemistry Department, both at the University of Karachi, to determine the reproducibility of the method.[48,49] The instruments used at the two laboratories were the LC-10AT and LC-20AT, used at the Research Institute of Pharmaceutical Sciences and HEC laboratory Chemistry Department, respectively. The results obtained on different days with different drug samples show an acceptable range of variation on the two instruments. Data acquisition was compared on the Purospher STAR C_{18} (5 µm, 250 × 0.46 cm) and Sapilco C_{18} (5 µm, 250 × 0.46 cm) columns. This shows suitability of all the analytes in the method developed (Table 5).

Robustness

Robustness studies were carried out to measure the method's capacity to remain unaffected by small but deliberate variations in parameters by varying the mobile phase ratio ± 2%, flow rate ± 0.1%, and pH ± 0.1% from their optimum conditions. Changing only one parameter resulted in column and system suitability parameters that did not exceed 2%, showing that the method is reliable under normal usage (Table 5).

Determination of ROS and NSAIDs in pharmaceutical formulations

The accuracy of the proposed method was evaluated by recovery assays made on the formulation samples. Thus, known

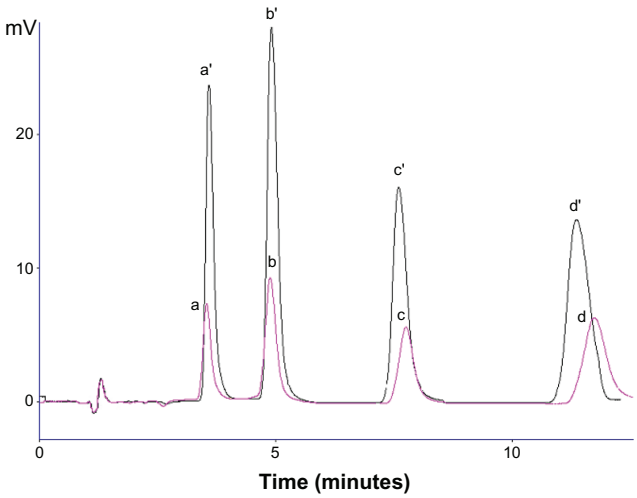

Figure 4 Representative chromatograms of rosuvastatin (a and a′), meloxicam (b and b′), ibuprofen (c and c′) and mefenamic acid (d and d′) at isosbestic point and individual wavelength maxima, respectively.

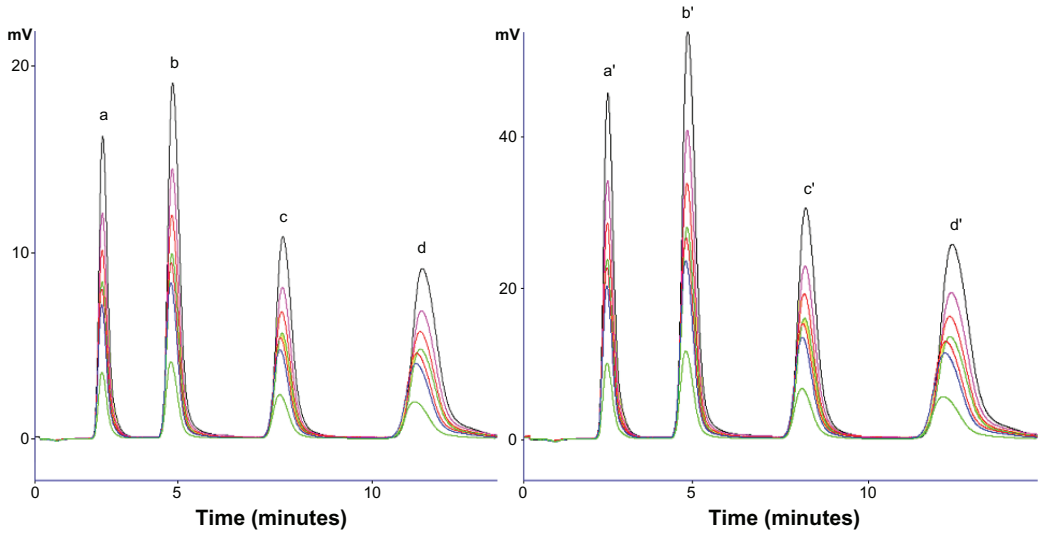

Figure 5 Representative chromatograms of comparison of linearity of rosuvastatin (a and a′), meloxicam (b and b′), ibuprofen (c and c′) and mefenamic acid (d and d′) at isosbestic point (left) and individual wavelength maxima (right), respectively.

amounts of each compound were added to the corresponding formulations at three levels of concentration (80%, 100%, and 120%; Table 3).

The precision of the proposed method was performed by adding each compound to the selected formulation on different days. The precision (%RSD) for all the studied components in different dosage forms was <2% for the inter-day assay for all drugs (Table 4).

Determination of ROS and NSAIDs in human serum

The calibration curves were prepared over the concentration range of 0.1–2.5 μgmL^{-1} for ROS, 0.4–10 μgmL^{-1} for MEL, 0.25–6.25 μgmL^{-1} for IBU, and 0.15–3.75 μgmL^{-1} for MEF in human serum by assaying in triplicate at eight different concentrations. As shown in Table 2, the calibration curves were linear over the spiked range for each compound with a good correlation coefficient ($r^2 > 0.999$). The method was sensitive to $4 < LOQs < 17$ and $1 < LODs < 6$ $ngmL^{-1}$ in serum (Figure 3).

Assessment of the inter-day accuracy and precision of the method was performed in drug-free serum samples spiked with ROS (range of 0.1–2.5 μgmL^{-1}), MEL (0.4–10.0 μgmL^{-1}), IBU (0.25–6.25 μgmL^{-1}), and MEF (0.15–3.75 μgmL^{-1}). For the inter-day precision analysis, the spiked serum was studied at the same concentration of each drug on three different days. Precision is shown as %RSD and accuracy as percent recovery value (Equation 1) and these are presented in Tables 3 and 2, respectively. Precision was observed to be <2% for all drugs spiked at all concentrations and assay accuracy was found to be 99.30%–100.89% for all compounds.

Analysis under program detector

The programming of the HPLC detector according to the λ_{max} of each analyte during simultaneous determination has been found informative and useful for routine analysis and special cases in which the sample contains analytes in amounts that can be measured in quantities as small as nanograms.

The linearity curves obtained for the same concentration range optimized for the method showed clear gaps in the respective peak heights and, thus, peak areas of the analytes. Figure 4 shows a comparison of the peak heights of the chromatograms of same concentration of analytes in human serum recorded at isosbestic point and when the detector was programmed. The calibration curves were linear, with $r^2 > 0.998$ shown in Table 2. The upper limit of the analytes for ROS 2.5 μgmL^{-1}, MEL 10 μgmL^{-1}; IBU 6.25 μgmL^{-1}, and MEF 3.75 μgmL^{-1} shifted above 100 mV from the peaks below 100 mV, so the lower limit of the concentration range shifted down from 0.1 to 0.025 μgmL^{-1} for ROS, from 0.4 to 0.1 μgmL^{-1} for MEL; from 0.25 to 0.0625 μgmL^{-1} for IBU, and from 0.15 to 0.0375 μgmL^{-1} for MEF.

The LOD values made the method sensitive for ROS, MEL, IBU, and MEF to quantifiable limits of the drugs: $4 < LOQ < 11$ and $1 < LOD < 4$ $ngmL^{-1}$ for API and $3 < LOQ < 10$ and $1 < LOD < 3$ $ngmL^{-1}$ for human serum – these can be seen in Table 2. The linear corresponding behavior of the analytes according to both measurement techniques is shown in Figure 5.

To test the accuracy and precision of this technique, the corresponding criteria of validation were applied. The %RSD < 2 and percent recoveries within the range of 97.6%–106.9% showed it to be precise and accurate (comparison

is shown in Tables 3 and 4). There were no significant differences in the results of both practices for system suitability parameters for the system shown in Table 1.

The method was found specific for the drugs from their excipients and foreign materials, even when the results were amplified. This technique was found equally possible for API, tablet formulations, and serum samples.

Conclusion

A simple and sensitive HPLC-UV detection method for the simultaneous determination of ROS and NSAIDs (MEL, IBU, and MEF) was developed. The proposed method was successfully applied to the determination of these NSAIDs in pharmaceutical formulations as well as in human plasma samples. The LODs of each compound in human plasma were as low as 1–6 $ngmL^{-1}$ at a signal-to-noise ratio of 3, which was sufficient for monitoring the plasma concentrations of each compound. In addition, the sensitivity of the proposed method increased when compared with the analysis at the programmed λ_{max} of each drug. The detection limits reached 1–3 $ngmL^{-1}$ for all the drug analytes.

The low limits of detection and quantification in pharmaceuticals and serum make the method applicable to quantification of the drug in quality control analysis, forensic medicine, clinical laboratories, and raw materials.

Disclosure

The authors declare no conflicts of interest in this work.

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