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Research Article

Dual Spectrophotometric Estimation of Paracetamol and Meloxicam in Bulk and its Pharmaceutical Dosage Form by Simultaneous Equation Method

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ABSTRACT

A simple and sensitive UV spectrophotometric method has been developed and subsequently validated for the simultaneous estimation of paracetamol and meloxicam in bulk and pharmaceutical formulations. Borate buffer (pH 9.0) was used as a solvent in the present investigation. Quantitative measurements were made at 256.8 - and 268.8 nm for paracetamol and meloxicam respectively. The method was validated over the range of 10 to 90 μg/ml for paracetamol and 1 to 16 μg/ml for meloxicam with a correlation coefficient (r^2) 0.999. The method was shown to be accurate and precise with inter-day and intra-day percent relative standard deviation values in the range 0.051 to 0.281 and 0.033 to 0.295 for paracetamol and 0.527 to 0.952 and 0.344 to 0.620 for meloxicam. The percent recoveries were found to be 99.95 to 99.99 for paracetmol and 99.24 to 100.45 for meloxicam. The limit of detection and limit of quantification was 0.010 and 0.036 μg/ml for paracetamol and 0.017 and 0.056μg/ml for meloxicam. The method has been successfully utilized for simultaneous estimation of paracetamol and meloxicam in tablets and can be extended for the routine analysis in bulk drugs.

Key words: Borate buffer, correlation coefficient, Paracetamol, Meloxicam.

1. INTRODUCTION

Meloxicam (MEL) is chemically designated as 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide with preferential cyclooxygenase-2(COX-2) inhibitory activity. *In vitro* studies with human tissues have confirmed high affinity of MEL for COX-2, whereas COX-1 was inhibited only at the highest concentrations[1]. The drug is indicated for the treatment of musculo-skeletal disorders and other syndromes involving pain[2] and has been used successfully in the treatment of pain associated with ankylosing spondylitis or other musculoskeletal conditions involving acute periarticular inflammation or low back pain. Combination therapy of NSAIDs has proven to be the most effective approach for pain[3]. Combination of Paracetamol (PAR), an analgesic-antipyretic drug with poor anti-inflammatory action with MEL greatly enhances the management of osteoarthritis and rheumatoid arthritis in comparison to single drug therapy either with PAR or MEL.

Numerous analytical methods, such as spectrophotometry[4,5], spectrofluorimetry[6], gas chromatography[7,8], gas liquid chromatography[9,10] high pressure liquid chromatography[11-16], reverse phase high pressure liquid chromatography[17,18], high performance liquid chromatography coupled with tandem mass spectrometry[19-20], reversed phase capillary electrochromatography[21], mass spectrometry[22] and polarography[23] have been reported either for MEL or PAR in pharmaceutical preparations or biological fluids.

In the present investigation an attempt has been made to develop accurate and precise UV spectrophotometric method for the simultaneous estimation of PAR and MEL in bulk and pharmaceutical formulations. The method is potentially suitable for drug monitoring and determination of pharmacokinetic profiles by using the proposed simultaneous equation.

2. MATRIALS AND METHODS

2.1 Instruments and Reagents

Spectrophotometric analysis was carried out on a UV-VIS double beam spectrophotometer (Systronics 2101) with a fixed slit width (2 cm) using a pair of 1 cm matched quartz cells. All weighing were performed on an electronic single

pan balance (Citizen). Calibrated borosilicate glass wares were used in the study. Reference standards of MEL and PAR were kindly provided by Alkem Pharm. Pvt. Ltd. (Daman, India) and Abicee Pharm. Pvt. Ltd. (Mancheswar, India), respectively. MEL and PAR tablets (Melodol, Aristo Pharm. Pvt. Ltd., Aurangabad, India) were procured from the local pharmacy with a labeled content of 7.5 mg MEL and 325 mg of PAR. Other chemicals and solvents were of analytical grade.

2.2 Determination of wavelength of maximum absorption

Borate buffer with varied pH was tested to establish the absorption maxima of PAR and MEL. Borate buffer with pH 8.0, 8.6, 9.0 and 10.0 was used and the buffer with pH 9.0 was selected as the best solvent for the simultaneous estimation of PAR and MEL. The concentration PAR and MEL used was 10 μg/ml. The solutions were scanned separately in the range of 200 to 400 nm against the borate buffer (pH 9.0) as a blank.

2.3 Preparation of standard stock solutions

Standard stock solutions of MEL (100 μg/ml) and PAR (100 μg/ml) were prepared in borate buffer (pH 9.0). These solutions were protected from light using foil and stored at 4 ^{0}C for 10 days and were found to be stable during this period.

2.4 Linearity of the method

Aliquots of standard stock solutions of PAR and MEL were dispensed separately into 10 ml volumetric flasks and the volume of the flask was made up with borate buffer (pH 9.0), to give final concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 μg/ml for PAR and 1, 2, 4, 6, 8, 10, 12, 14, 16 μg/ml for MEL. The absorbances were measured at 256.8 nm for PAR and 268.8 nm MEL for solutions using borate buffer (pH 9.0) as a reagent blank. The linear graph was plotted taking the values of the absorbance's against the concentration.

2.5 Estimation of absorptivity coefficients

Absorptivity values of PAR and MEL were determined at the wavelengths of 256.8 nm and 268.8 nm within their linearity concentration range of 10-90 μg/ml and 1-16 μg/ml, respectively.

2.6 Simultaneous equation method

The calibration curves for PAR and MEL were prepared in the concentration range of 10-90 μg/ml and 1-16 μg/ml at their respective absorption maxima using borate buffer (pH 9.0) as a reagent blank. The absoptivity coefficients were determined for both the drugs. Two simultaneous equations were developed using absorptivity coefficient values.

$$A_1 = 419.60\ C_P + 209.99\ C_M \quad \ldots\ldots\ldots\ldots (1)$$

$$A_2 = 255.97\ C_P + 269.62\ C_M \quad \ldots\ldots\ldots\ldots (2)$$

Where C_P and C_M are the concentrations of PAR and MEL, respectively, A_1 and A_2 are the absorbances of mixture at the selected wavelengths of 256.8 nm and 268.8 nm, respectively. 419.60 and 255.97 are the absorptivity values of PAR and 209.99 and 269.62 are the absorptivity values of MEL at 256.8 and 268.8 nm respectively.

By applying Vierodt's method to equation 1 and 2, the connotation C_M and C_P can be obtained as follows:

$$C_P = A_1\,(269.62) - A_2\,(209.99) / 59381.41 \quad \ldots\ldots\ldots (3)$$

$$C_M = A_2\,(419.60) - A_1\,(255.97) / 59381.41 \quad \ldots\ldots\ldots (4)$$

2.7 Preparation of mixture solution

Powder equivalent to 10 mg each of PAR and MEL were transferred separately into 100 ml volumetric flasks and the flasks made up to volume with borate buffer (pH 9.0) to obtain a final concentration of 100 μg/ml each. Mixture of required concentration of PAR and MEL solutions were prepared by mixing appropriate quantities of the above-prepared solutions to obtain a final concentration in the ratio 10:1, 20:2 and 30:3. The absorbances of the mixture solutions were measured at 256.8 and 268.8 nm against borate buffer (pH 9.0) as a reagent blank and the content of both the drugs were estimated.

2.8 Assay of tablets

100 ml of borate buffer (pH 9.0) was added to powder of tablet equivalent to 1300 mg of PAR and 30 mg of MEL for extraction. After two hours, the suspension was filtered and the filtrate was diluted to 300 ml with borate buffer. 10 ml of this solution was transferred to a 100 ml volumetric flask and diluted with borate buffer to obtain concentration of 433.33 μg/ml PAR and 10 μg/ml MEL. The solution was further diluted to final concentrations of 43.33, 64.99 and 86.66 μg/ml PAR and 1.0, 1.5 and 2.0 μg/ml MEL. The absorbance of the prepared solution was measured at 256.8 nm for PAR and at 268.8 nm for MEL.

2.9 Validation criteria

2.9.1 Repeatability

The repeatability of the proposed method was investigated by the analysis of eight samples of PAR and MEL. The concentration of PAR and MEL used was 10 μg/ml. From the absorbance values mean concentrations, standard deviation (SD), % RSD was calculated.

2.9.2 Accuracy

The accuracy of the method was evaluated by calculating recovery of MEL and PAR by standard addition method at three-concentration levels. Different amounts of standard PAR and MEL (80%, 100%, and 120%) of the target level in tablets (within the linearity range) were added to the pre-analyzed formulations of PAR (64.99 μg/ml) and MEL (1.5 μg/ml).

2.9.3 Precision

Precision of the proposed method was demonstrated by variation studies. Analyzing the three different concentrations of each drug for three times on the same day assessed intra-day precision. Inter-day precision was determined by analyzing the three different concentrations of each drug over three consecutive days.

2.9.4 Ruggedness

Ruggedness of the proposed method was determined by carrying out the experiment on different instruments by different analyst under similar environmental conditions.

3. RESULTS AND DISCUSSION

The proposed method was found to be simple, precise, accurate and sensitive. High percentage recovery showed that the method was free from interference of excipients used in the formulation. Values of limit of detection (LOD) and limit of quantitation (LOQ) showed that the proposed method was sensitive enough to analyze the drug in bulk as well as in its pharmaceutical formulation. Hence the proposed method renders suitable for routine analysis in quality-control laboratories.

The present study was carried out to develop a simple, accurate and sensitive UV spectrophotometric method for the simultaneous estimation of PAR and MEL in tablets. Figure 1 and 2 shows the absorption maxima of PAR and ML at pH 9.0. The absorption spectra showed the characteristic absorption maxima at 256.8 nm for PAR and 268.8 nm for MEL. The absorption maxima of PAR and MEL at different pH have been presented in table 1. The result revealed that borate buffer (pH 9.0) was found to be a better solvent for the spectral analysis of PAR and MEL. From the optical characteristics of the proposed method, it is evident that PAR and MEL obey linearity within the concentration range of 10-90 μg/ml and 1-16 μg/ml, with a correlation coefficient (r^2) 0.999 (Table 2, Fig.3 and 4) at 256.8 nm and 268.8 nm respectively. Absorptive values of PAR and MEL were determined at the wavelengths of 256.8 nm and 268.8 nm within their linearity concentration range of 10-90 μg/ml and 1-16 μg/ml, respectively and have been presented in table 3. Percentage recovery values of pure drug from the analyzed formulations were in the range 99.95 to 99.99 for PAR and 99.24 to 100.45 for MEL (Table 4). The high percentage of recovery of the pure drugs shows accuracy of the proposed method. The percent recovery values of PAR and MEL in the mixture were found to be in the range 99.78 ± 0.32 to 99.98 ± 0.147 and 99.27 ± 0.918 to 99.78 ± 0.897, respectively. The results obtained from analysis of mixture of different concentrations of PAR and MEL has been presented in table 5. The values of percent recovery of individual drugs in the mixture and low values of standard deviation further indicate the accuracy of the proposed method. The results of assay of tablets are given in table 6. The labeled amount of drug and that obtained from tablet assay were almost equal. The results of repeatability studies for PAR and MEL have been presented in table 7. The absorbance of PAR remained within the range of 0.419-0.421 and that of MEL in the range of 0.210-0.212. The intra and inter day precision result (data not shown) showed that the amount of PAR and MEL found in the sample on the same day and after three consecutive days remained same in both the cases. The changes in concentration were very negligible. The results of ruggedness are presented in table 8 which shows that the amount of PAR determined by analyst 1and 2 was 9.983 and 9.980 μg/ml respectively. Similarly in case of MEL the amount found was 3.973 and 3. 963 μg/ml by analysts 1 and 2 respectively. The optical characteristics of the proposed method have been presented in table 9.

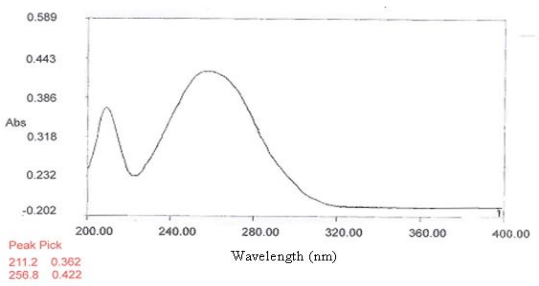

Fig. 1 Spectra of Paracetamol.

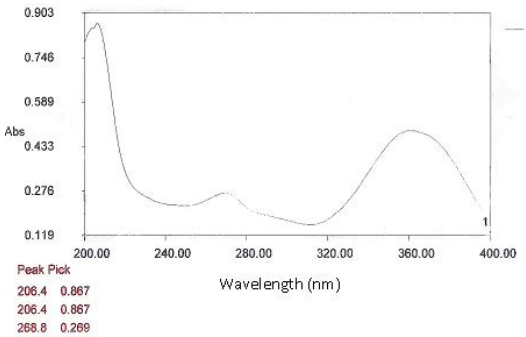

Fig. 2 Spectra of Meloxicam.

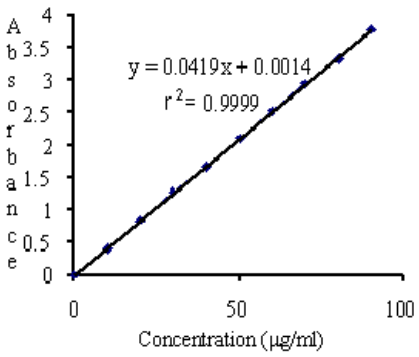

Fig. 3 Linearity of Paracetamol at 256. 8 nm

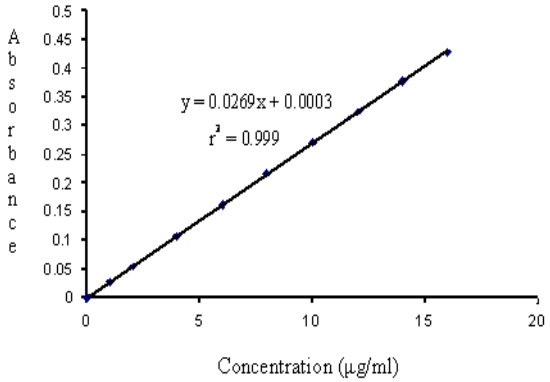

Fig. 4 Linearity of Meloxiacm at 268.8 nm

Table 1 Selection of solvent pH

Solvent	Paracetamol (λ_{max})	Absorbance	Meloxicam (λ_{max})	Absorbance
Borate buffer (pH 8.0)	257.0	0.372	270	0.163
Borate buffer (pH 8.6)	257.6	0.398	269.6	0.177
Borate buffer (pH 9.0)	256.8	0.420	268.8	0.211
Borate buffer (pH 10.0)	256.0	0.402	269	0.192

Table 2 Linearity table of PAR and MEL.

PAR (µg/ml)	Abs at 256.8 nm	Abs 268.8 nm	MEL (µg/ml)	ABS at 256.8 nm	ABS at 268.8 nm
10	0.420	0.256	1	0.021	0.027
20	0.840	0.512	2	0.042	0.054
30	1.259	0.770	4	0.084	0.108
40	1.68	1.021	6	0.125	0.161
50	2.099	1.281	8	0.165	0.216
60	2.52	1.528	10	0.211	0.270
70	2.939	1.79	12	0.251	0.325
80	3.331	2.062	14	0.296	0.378
90	3.787	2.300	16	0.338	0.428

Table 3 Absorptivity coefficients of PAR and MEL. Values in parentheses indicate ±SD.

Substance	PAR		MEL	
Wavelength (nm)	256.8	268.8	256.8	268.8
Absorptive coefficient	419.60 (±1.25)	255.97 (±0.88)	209.99 (±1.09)	269.62 (±1.03)
% RSD	0.297	0.342	0.519	0.382

Table 4 Recovery studies of pure drugs. Values in parentheses indicate ±SD.

Formulation (%)	Amount taken (µg/ml)		Standard added (µg/ml)		Recovery		% RSD	
	PAR	MEL	PAR	MEL	PAR	MEL	PAR	MEL
80	64.99	1.5	51.99	1.2	99.99 (±0.009)	99.59 (±1.17)	0.009	1.18
100	64.99	1.5	64.99	1.5	99.98 (±0.054)	100.45 (±0.921)	0.054	0.916
120	64.99	1.5	77.98	1.8	99.95 (±0.049)	99.24 (±0.837)	0.049	0.843

Table 5 Analysis of mixture of different concentrations of PAR and MEL.

Mixture (µg/ml)		Concentration (µg/ml)		% Amount found (µg/ml)		% RSD	
PAR	MEL	PAR	MEL	PAR	MEL	PAR	MEL
10	1	9.978	0.997	99.78 (±0.32)	99.78 (±0.897)	0.320	0.899
20	2	19.997	1.994	99.98 (±0.147)	99.72 (±1.09)	0.147	1.09
30	3	29.991	2.978	99.97 (±0.033)	99.27 (±0.918)	0.033	0.925

Table 6 Results of analysis of tablets.

Formulation	Amount taken (mg)		Amount obtained (mg)		% Drug found		% RSD	
	PAR	MEL	PAR	MEL	PAR	MEL	PAR	MEL
Melodol	1300	30	1299.66 (±0.020)	30.13 (±0.829)	99.97	100.45	0.020	0.825

Table 7 Results of repeatability studies

Sample No.	Concentration (µg/ml)		Absorbance		Statistical analysis
	PAR	MEL	PAR	MEL	
1	10	10	0.420	0.210	**PAR Mean:0.4201 S.D: 0.00064 %R.S.D:0.152**
2	10	10	0.421	0.212	
3	10	10	0.420	0.211	
4	10	10	0.420	0.211	
5	10	10	0.419	0.210	**MEL Mean: 0.2108 S.D: 0.00083 %R.S.D:0.395**
6	10	10	0.421	0.211	
7	10	10	0.420	0.211	
8	10	10	0.420	0.211	

Table 8 Determination of ruggedness parameters

Drug	Analyst-1		Analyst-2	
	PAR	MEL	PAR	MEL
Concentration taken (µg/ml)	10	4	10	4
Concentration found (µg/ml)	9.983	3.973	9.980	3.963
% Found	99.83	99.32	99.80	99.09
SD	±0.335	±0.724	±0.295	±0.545
% RSD	0.336	0.729	0.296	0.550

*Each value is average of three determinations

Table 9 Optical characteristics of proposed method

Parameters	PAR	MEL
Absorption maximum (nm)	256.8	268.8
Beer's law limit (µg/ml)	10-90	1-16
Absorptivity	419.60	269.62
Sandell's sensitivity (µg/cm 2/0.001)	0.0238	0.0370
Molar absorptivity ($mole^{-1}cm^{-1}$)	6.3426×103	9.4744×103
Correlation coefficient (r^2)	0.9999	0.9999
Regression equations (y*)	0.0419x+0.0014	0.0269x+0.0003
Slope (a)	0.0419	0.0269
Intercept (b)	0.0014	0.0003
% Range of error		
0.05 Confidence limits	±0.044	±0.057
0.01 Confidence limits	±0.057	±0.075
Limit of detection (LOD)	0.010	0.017
Limit of quantitation (LOQ)	0.036	0.056

*y = ax + bx = concentration. Values in parentheses indicate ±SD.

4. CONCLUSION

The results of the above studies indicate that this method is very simple, accurate and the commonly used excipients and additives present in the formulations do not interfere in the proposed method. The system suitability parameters also reveal that the values were within the specified limits.

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REFERENCES

1. Tacca MD, Colucci R. Clin Drug Invest. Clin Drug Invest 2002;22(12):799-818. [Google Scholar]
2. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--differences and similarities.. N Engl J Med 1991 Jun;324(24):1716-1725. PubMed PMID: 2034249. doi: 10.1056/NEJM199106133242407. [Google Scholar]
3. Beaver WT. Combination analgesics. Am J Med 1984;1984(3):3-38. [Google Scholar]
4. Reddy MN, Murthy TK. Indian J Pharm Sci 2001;2001(63):245-247. [Google Scholar]
5. Seedher N, Garg A. Indian J Pharm Sci 2005;2005(65):655-658. [Google Scholar]
6. Taha EA, Salama NN, Fattah LEA. Elham Anwer Taha - Nahla Nour Salama - Laila El-Sayed Abdel Fattah. Chemical & Pharmaceutical Bulletin - Vol. 54 - Issue 5 2006;2006(5):653-658. Available from: http://joi.jlc.jst.go.jp/JST.JSTAGE/cpb/54.653?from=PubMed PubMed PMID: 16651760. [Google Scholar]
7. Kinsella HC, Smith S. et al, Journal of Pharmacy and Pharmacology. 1979;31(1):153-156. [Google Scholar]
8. Murray S, Boobis AR. Combined assay for phenacetin and paracetamol in plasma using capillary column gas chromatography-negative-ion mass spectrometry.. J Chromatogr 1991 Aug;568(2):341-350. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+103-90-2 PubMed PMID: 1783640. [Google Scholar]
9. Prescott LF. J Pharm Pharmacol . Gas-liquid chromatographic estimation of paracetamol.. J Pharm Pharmacol 1971;23(10):807-808. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+103-90-2 PubMed PMID: 4399923. [Google Scholar]
10. Huggett A, Andrews P. Chromatogr J. Rapid micro-method for the measurement of paracetamol in blood plasma or serum using gas-liquid chromatography with flame-ionisation detection.. J Chromatogr 1981;209(1):67-76. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+103-90-2 PubMed PMID: 7228941. [Google Scholar]
11. Dasndi B, Shivaprakash.LC determination and pharmacokinetics of meloxicam. J Pharm Biomed Anal 2002 2002;28(5):999-1004. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0731708500200064X doi: 10.1016/S0731-7085(02)00064-X. [Google Scholar]
12. Ji HY, Lee HW. Chromatogr B Analyt Technol Biomed Life Sci;2005(826):1-2. [Google Scholar]
13. Medvedovici A, Albu F, Georgita C, Mircioiu C, David V. A non-extracting procedure for the determination of meloxicam in plasma samples by HPLC-diode array detection.. Arzneimittelforschung 2005;55(6):326-331. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+71125-38-7 PubMed PMID: 16032972. doi: 10.1055/s-0031-1296867. [Google Scholar]
14. Velpandian T, Jaiswal J. Development and validation of a new high-performance liquid chromatographic estimation method of meloxicam in biological samples. J Chromatogr B Biomed Sci Appl 2000;2000(738):431-436. Available from: http://linkinghub.elsevier.com/retrieve/pii/S03783479900537X doi: 10.1016/S0378-4347(99)00537-X. [Google Scholar]
15. Adriaenssens PI, Prescott LF. High performance liquid chromatographic estimation of paracetamol metabolites in plasma.. Br J Clin Pharmacol 1978;6(1):87-88. Available from: http://europepmc.org/abstract/MED/666951 PubMed PMID: 666951. [Google Scholar]
16. Gotelli GR, Kabra PM, Marton LJ. Determination of acetaminophen and phenacetin in plasma by high-pressure liquid chromatography.. Clin Chem 1977;23(6):957-959. Available from: http://www.clinchem.org/cgi/pmidlookup?view=long&pmid=870259 PubMed PMID: 870259. [Google Scholar]
17. Arayne MS, Sultana N. Pak J Pharm Sci 2005 2005;18(1):58-62. [Google Scholar]
18. Meatherall R, Ford D. Isocratic liquid chromatographic determination of theophylline,

acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum.. Ther Drug Monit 1988;10(1):101-115. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+58-08-2 PubMed PMID: 3376176. [Google Scholar]

19. Rigato HM, Mendes GD. Meloxicam determination in human plasma by high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) in Brazilian bioequivalence studies. Int J Clin Pharmacol Ther 2006;2006(44):489-498. Available from: http://www.dustri.com/article_response_page.html?artId=1228&doi=10.5414/CPP44489&L=0 doi: 10.5414/CPP44489. [Google Scholar]
20. Yuan Y, Chen X. Chromatogr B Analyt Technol Biomed Life Sci;2007(852):1-2. [Google Scholar]
21. Pucci V, Mandrioli R, Raggi MA, Fanali S. Reversed-phase capillary electrochromatography for the simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine in analgesic tablets. Electrophoresis 2004;25(45):615-621. Available from: http://doi.wiley.com/10.1002/elps.200305631 PubMed PMID: 14981689. doi: 10.1002/elps.200305631. [Google Scholar]
22. Baty JD, Robinson PR, Wharton J. A method for the estimation of acetanilide, paracetamol and phenacetin in plasma and urine using mass fragmentography.. Biomed Mass Spectrom 1976;3(2):60-63. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+103-90-2 PubMed PMID: 5162. [Google Scholar]
23. Altinöz S, Nemutlu E, Kir S. Polarographic behaviour of meloxicam and its determination in tablet preparations and spiked plasma.. Farmaco 2002;57(6):463-468. Available from: http://www.scholaruniverse.com/ncbi-linkout?id=12088061 PubMed PMID: 12088061. [Google Scholar].