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SIMULTANEOUS ESTIMATION OF NABUMETONE AND PARACETAMOL IN PHARMACEUTICAL DOSAGE FORM BY ION PAIR CHROMATOGRAPHY

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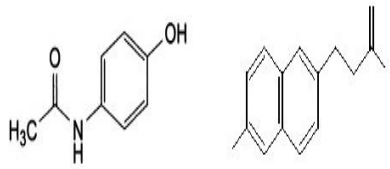
ABSTRACT: An isocratic ion pair chromatographic (IPC) method for simultaneous determination of nabumetone (NAB) and paracetamol (PCM) was developed for the application to pharmaceuticals. Isocratic ion pair chromatographic method was employed for quantitative analysis using triethylamine and tetrabutyl ammonium hydroxide sulphate (TBAH) as ion-pair reagents. The IPC assay was carried out using an Inert Sil ODS 3V (5µ,25 cm× 4.6 mm, i.d.) column. The mobile phase consisted of 0.43 g of TBAH dissolved in 1000 ml of a mixture of acetonitrile, water and triethylamine (300:700:1, v/v) adjusted with phosphoric acid to pH 6.5.
The calibration curves of NAB and PCM showed good linearity in the concentration range 2 –100 µg/ml with UV detection (239 nm) for pharmaceuticals. The correlation coefficients were better than 0.999 in both cases. The lower limits of detection (defined as asignal-to-noise ratio of about 3) were approximately 0.29 µg/ml for NAB and 2.8 µg/ml for PCM. The procedure described here is rapid, simple, selective, and is suitable for routine analysis of pharmaceuticals. Commercial dosage form of both drugs was assayed by developed method.
KEYWORDS: Ion pair chromatography (IPC), Nabumetone, Paracetamol, Ion pairing reagent, Triethylamine and Tetrabutyl ammonium hydroxide sulphate (TBAH).

INTRODUCTION

Nabumetone, 4-(6-methoxynaphthalen-2-yl) butan-2-one, is a nonsteroidal anti-inflammatory drug (NSAID) of naphtylalkanone class. The drug has proved to be effective in the treatment of rheumatoid arthritis, osteoarthritis and acute soft tissue injuries. Nabumetone is a prodrug which undergoes extensive first pass metabolism to 6-methoxy-2-naphthylacetic acid (6-MNA), the major circulating metabolite; 6-MNA is largely responsible for the therapeutic efficacy of nabumetone [1, 2, 3]. Paracetamol (PARA) is chemically N-(4-hydroxyphenyl) acetamide, It has analgesic and antipyretic activity [1, 4]. A combination of Nabumetone and Paracetamol is effective as these agents act through different analgesic mechanisms and act synergistically. The combination offers faster as well as prolonged relief from pain and inflammation [3].



A: Paracetamol **B: Nabumetone**

Figure 1: Structure of Nabumetone and Paracetamol [2, 4]

Many RP-HPLC methods [5-7] have been reported for the determination of Nabumetone and its metabolite in tablet dosage form & also in human plasma. Simultaneous estimation of Naproxen and Nabumetone was also reported by RP-HPLC in human plasma, human urine and in pharmaceutical. Paracetamol [8-15] individually and in combination with other drugs like Valdecoxib, Aceclofenac, Chlorpheniramine maleate, Dipyrone, caffeine and Cetrizine in human plasma and pharmaceuticals were reported to be estimated by UV Spectroscopy and RP-HPLC. But no method is available for simultaneous estimation of Nabumetone and Paracetamol in tablet dosage form by RP-HPLC.

One such formulation containing Nabumetone (500 mg) and Paracetamol (500 mg) could be eluted from Inertsil ODS 3V (250×4 mm; 5µm) column using a mobile phase consisting of 0.43 g of TBAH dissolved in 1000 ml of a mixture of acetonitrile, water and triethylamine (300:700:1, v/v) adjusted with phosphoric acid to pH 6.5.

EXPERIMENTAL

CHEMICALS AND REAGENTS USED:

The reference standard of Nabumetone and Paracetamol were obtained as gift samples from Ipca laboratory and Torrent Pharmaceuticals, respectively. All chemicals used were of HPLC grade of Merck. Potassium dihydrogen phosphate, Triethylamine and Ortho-phosphoric acid, Tetrabutyl ammonium hydroxide sulphate (TBAH) as having HPLC grade of Merk Limited were used for chromatographic procedure. Mili-Q Water was used to prepare solutions. Tablet dosage form manufactured by Ipca laboratory; NILTIS-P was used. Each tablet containing 500 mg of Nabumetone and 500 mg of Paracetamol were used for the study.

INSTRUMENTATION:

A SHIMDZU AHT HPLC system consisting of PV-980 pump, a 975 UV-Visible detector was used. The peaks were quantified by means of PC based Class-VP software.

CHROMATOGRAPHIC CONDITIONS:

The chromatographic separation was performed at 25 ºC temperature on reverse phase Inert Sil ODS 3V (5µ, 25 cm× 4.6 mm, i.d.) column. The mobile phase consisted of 0.43 g of TBAH dissolved in 1000 ml of a mixture of acetonitrile, water and triethylamine (300:700:1, v/v) adjusted with phosphoric acid to pH 6.5. The separation was carried out at detector wavelength 239 nm for 7.00 min. The injection volume of standard and sample solutions was 5µl.

PREPARATION OF STANDARD AND SAMPLE SOLUTION

The standard stock solutions of Nabumetone (100 µg/ml) and Paracetamol (100 µg/ml) were prepared by dissolving appropriate amounts of respective compounds in acetonitrile. Whereas in the preparation of sample solution, quantity of powdered tablet equivalent to 10 mg of Nabumetone or 10 mg of Paracetamol was weighed and dissolved in acetonitrile. It was further diluted in order to get solution having concentration 50 µg/ml of each drug, Nabumetone and Paracetamol.

RESULTS AND DISCUSSION

OPTIMIZATION OF ANALYTICAL CONDITIONS:

Different columns containing octyl, octadecyl, phenyl and base deactivated silane stationary phase were tried for separation and resolution. The Inertsil base deactivated silane column became more advantageous over the other columns. Individual drug solution was injected into column, both elution pattern and resolution parameters studied as a function of pH, as a function of mobile phase component and their ratio. To develop a suitable LC method for estimation of nabumetone and paracetamol in formulations, different mobile phases were employed to achieve the best separation. The selected and optimized mobile phase was Buffer: ACN: TEA (30:70:0.1) and conditions optimized were: flow rate (1.5 ml/minute), detector wavelength (239 nm). Run time was 7 min. Here the peaks were separated and showed better resolution, theoretical plate count and asymmetry was found as 1.09 & 1.30 respectively for Nabumtone and Paracetamol. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.

The typical chromatogram of two drugs assayed is

shown in Figure 2. The system suitability parameters are reported in Table 1.

METHOD VALIDATION [16]:

SYSTEM SUITABILITY:

The system suitability of the method was studied to determine the reproducibility of the chromatographic system and column performance was acceptable for the intended analytical application. Four parameters i.e. precision of peak area of five replicate injections,retention time of eluted drugs, number of theoretical plates, asymmetry factor and resolution between two peak of analytes were evaluated.

The results are shown in Table 1.

LINEARITY:

The Linearity of analytical method is its ability to obtain test results, which are directly proportional to the concentration of analyte in the test sample .The linearity of the assay method, was established by injecting test samples in the range of 2-100 µg/ml for Nabumetone and also for Paracetamol. Each solution was injected twice into HPLC and the average area at each concentration was calculated. The regression analysis was carried out from graph of peak area Vs Concentration; correlation co-efficient and Y-Intercept of plot was also evaluated. The results are shown in Table 2. Linear regression equation and correlation coefficient was found to be y = 18388 X +51580 and r = 0.9992 for Nabumetone and for Paracetamol, it was found to be y = 15649 X + 27764 and r = 0.9994; where 'y' is area of peak and 'X' is the concentration of drug solution, respectively.

ACCURACY:

The accuracy study was performed by spiking placebo with known quantity of API. The accuracy of test method was demonstrated by preparing recovery samples at the level of 80%, 100%, and 120% of target concentration. The recovery samples were prepared in triplicate at each level. The above samples were injected and the percentage recovery for amount added, were estimated. The precision of recovery at each level was determined by computing the relative standard deviation of triplicate recovery results. The result for accuracy is shown in Table 3, indicating good accuracy of the method for simultaneous determination of two drugs.

PRECISION:

Precision was determined by two ways; by System precision and Intermediate precision. System precision was demonstrated by making five replicate injections of standard solution. The peak area of analyte for replicate injections was recorded. The %RSD for the analyte peak area of these replicate injections was evaluated. The results of System precision is shown in Table 4, indicating that an acceptable precision was achieved for simultaneous determination of Nabumetone and Paracetamol, as revealed by RSD < 2.0%. The intermediate precision of test method was demonstrated by carrying out precision study at three concentration level as 80 %, 100%, 120% (i.e 40, 50, 60µg/ml). Intermediate precision study includes intra-day and inter-day analysis. The result summary of intermediate precision is shown in Table 5A and 5B.

ROBUSTNESS:

Robustness of the test method was demonstrated by carrying out system suitability under normal conditions and each of the altered conditions as follows.

Flow rate was changed by -10% and +10%; Organic phase ratio of mobile phase was changed by -5% and +5% absolute; Mobile phase pH was changed by -0.02 and +0.02, temperature changed by 5 °c. The result summary of robustness study are summarised in Table 6, result indicates that the method is robust for simultaneous determination of Nabumetone and Paracetamol.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION:

Limit of detection and limit of quantitation was established based on the residual standard deviation method. LOD and LOQ were found to be 0.29 µg/ml and 0.89 µg/ml for Nabumetone and 0.28 µg/ml and 0.87 µg/ml for Paracetamol, respectively.

SPECIFICITY:

Specificity was carried as interference from placebo; first only placebo and than injecting synthetic mixtue containing placebo and API's as tablet ratio.

INTERFERENCE FROM PLACEBO:

Interference from placebo was carried out by preparing the following specificity samples.

By preparing placebo equivalent to the sample weight in triplicate; 6 time spiking placebo with API at target concentration and by preparing sample solution as per test method. There was no peak observed in chromatogram of excipients at RT 1.75 min and 5.12 min. It indicates that there was no interfere from excepients with drug during analysis. Chromatograms obtained from the study are shown in figure 3.

CONCLUSION

The data demonstrate that the ion pair chromatographic method we have developed showed acceptable linearity, specificity, accuracy, precision and robustness in the concentration range of 2-100 µg/ml for Nabumetone and Paracetamol, as per the requirement of ICH guidelines. The method described is rapid since chromatographic run time is 7 min. The limit of quantification value for Nabumetone and Paracetamol are observed to be 0.896 and 0.870 µg/ml, respectively. The proposed method is precise, accurate, robust and does not suffer from any interference from other excipients. In conclusion, the proposed method could be routinely used for the analysis of Nabumetone and Paracetamol in pharmaceutical dosage form.

Table 1: Results of system suitability study

Nabumetone		**Paracetamol**	
Injection No.	Standard response	Injection No.	Standard response
1	978264	1	805423
2	987901	2	798562
3	985747	3	809519
4	978854	4	814531
5	971562	5	796003
Average	980465.6	Average	804807.6
SD	6517.22	SD	7642.79
% RSD	0.64	% RSD	0.94
Retention time	5.12 min	Retention time	1.75 min
Relative Retention time	3.37	Relative Retention time	-
Theoretical plates	3759.43	Theoretical plates	11057.17
Assymetry factor	1.27	Assymetry factor	1.09
Resolution	15.72	Resolution	-

TABLE 2: RESULTS OF LINEARITY STUDY

Nabumetone		Paracetamol	
Concentration (g/ml)	Area	Concentration (g/ml)	Area
02	63490	02	44698
10	238472	10	187109
20	419321	20	340005
40	789135	40	653210
50	998564	50	815423
60	1175884	60	991572
80	1514049	80	1278756
100	1870290	100	1576149

TABLE 3: RESULTS OF ACCURACY STUDY

Drug	Preanalysed conc. (g/ml)	Amount spiked* (mg)	Amount recovered* (mg)	% recovery*	% RSD
Nabumetone	40.02	40	79.84	99.80	0.85
	40.02	50	89.86	99.84	
	40.02	60	100.26	100.26	
Paracetamol	40.1	40	80.21	100.27	0.67
	40.1	50	89.75	99.72	
	40.1	60	99.73	99.73	

*Average of three experiments

TABLE 4: RESULTS OF PRECISION STUDY
RESULTS OF SYSTEM PRECISION

Nabumetone		Paracetamol	
Injection No.	Standard response	Injection No.	Standard response
1	974264	1	815423
2	986901	2	792562
3	985347	3	804519
4	978454	4	814231
5	971262	5	796203
Average	979245	Average	804587
SD	6800.97	SD	10311.67
% RSD	0.69	% RSD	1.28

TABLE 5: A- RESULTS OF INTERMEDIATE PRECISION FOR NABUMETONE

Conc. (g/ml)	Intra-day (n=3)	% RSD	Inter-day (n=3)	% RSD
40	40.31 ± 0.475	1.17	40.82 ± 0.358	0.87
50	50.23 ± 0.271	0.54	50.28 ± 0.426	0.74
60	60.90 ± 0.427	0.70	60.98 ± 0.522	0.93

B- RESULTS OF INTERMEDIATE PRECISION FOR PARACETMOL

Conc. (g/ml)	Intra-day (n=3)	% RSD	Inter-day (n=3)	% RSD
40	40.34 ± 0.330	0.82	40.08 ± 0.373	0.93
50	50.05 ± 0.262	0.52	50.12 ± 0.266	0.53
60	61.23 ± 0.272	0.44	61.02 ± 0.372	0.60

TABLE 6: RESULT SUMMARY OF ROBUSTNESS STUDY

Condition	Nabumetone			Paracetamol		
	RSD of replicate injection	Tailing Factor	Theoretical plates	RSD of replicate injection	Tailing Factor	Theoretical Plates
Normal	0.06	1.09	11057	0.08	1.27	3759
Flow rate 1.3 ml/min	0.17	1.1	11326	0.17	1.41	4002
Flow rate 1.7 ml/min	0.08	1.03	10865	0.25	1.22	3425
Mobile phase (28:72:0.1)	0.09	1.02	10798	0.62	1.24	3503
Mobile phase (32:68:0.1)	0.86	1.13	11162	0.41	1.34	3698
Mobile phase pH -0.02	0.42	1.09	11236	0.1	1.27	3825
Mobile phase pH +0.02	0.31	1.17	11023	0.77	1.29	3702
Temp 20 °c	0.12	1.21	11204	0.67	1.49	3941
Temp 30 °c	0.99	1.01	10962	0.74	1.1	3320

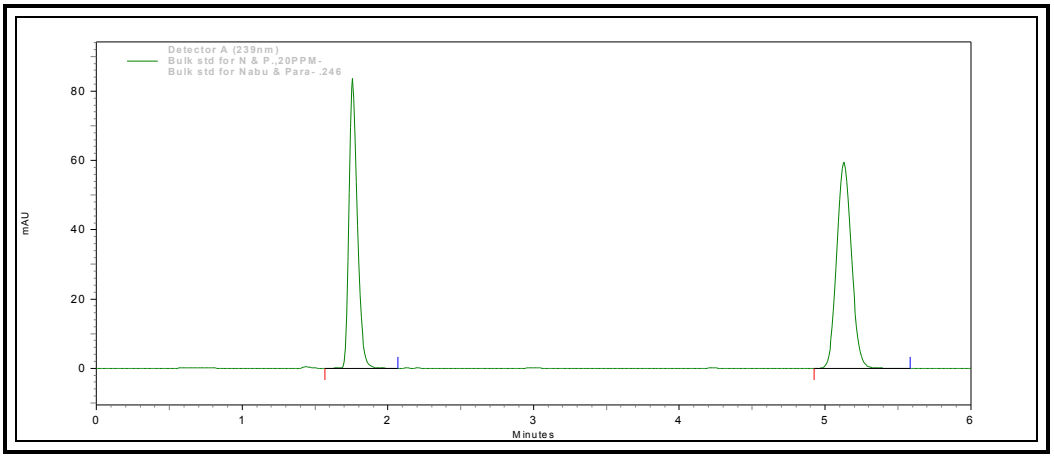
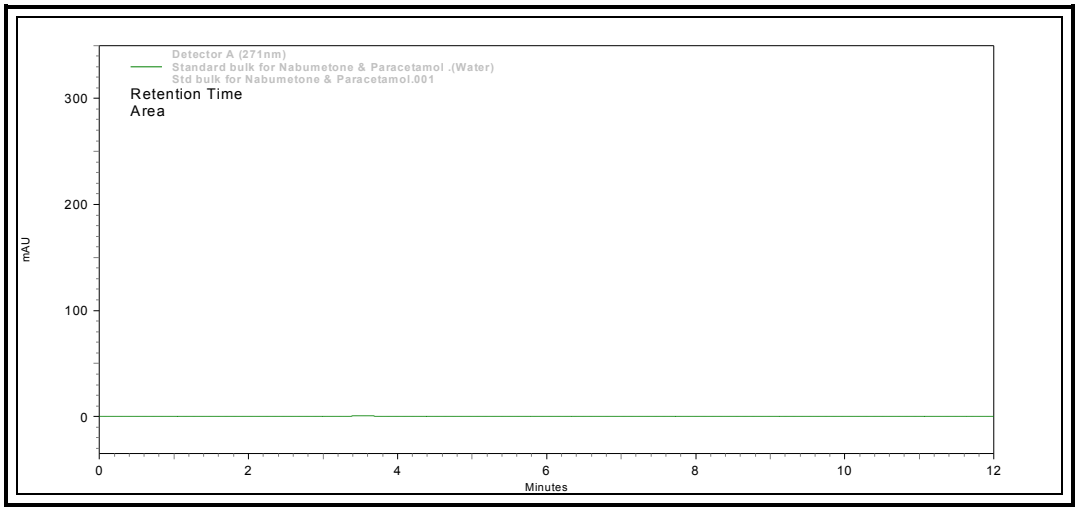


Figure 2: Typical chromatogram of two drugs assayed; Retention time 1.75 for Paracetamol and 5.12 for Nabumetone.



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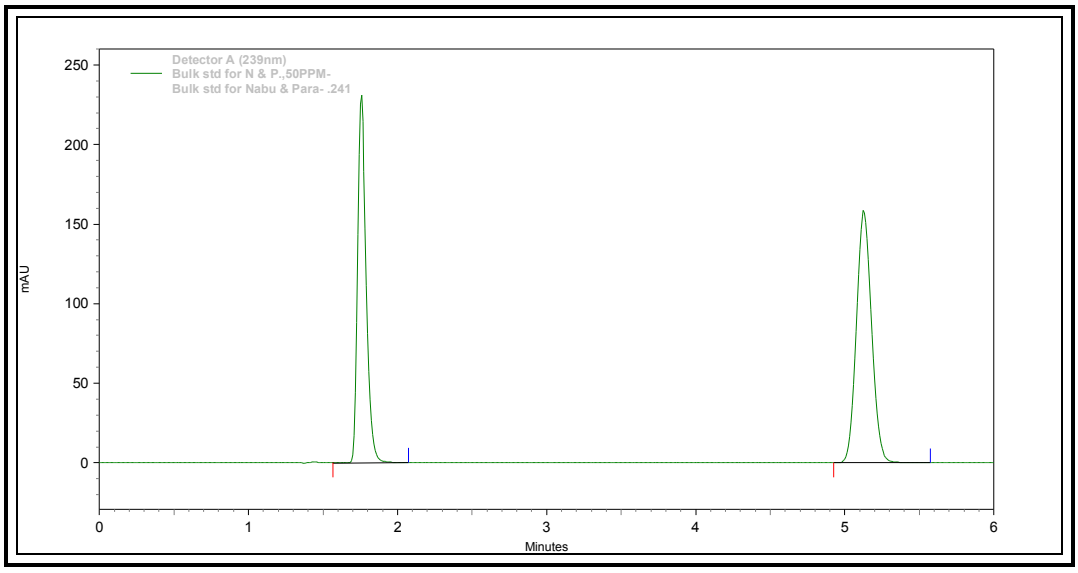

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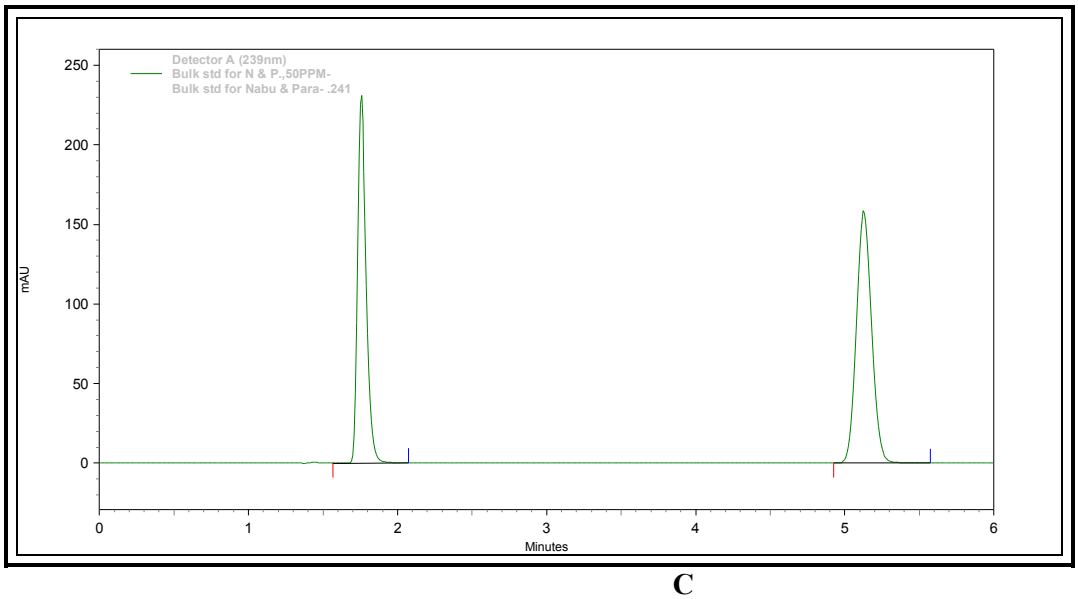

C

Figure 3: Chromatogram obtained from the specificity study;
(A)Chromatogram of placebo solution, (B) Chromatogram of API by spiking placebo,
(C) Chromatogram of sample solution.

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REFERENCES

1. Current Index of Medical Specialties, Update Prescribers Handbook published by CMP Medica India Pvt. Ltd., Bangalore, Jul-Oct 2007, 117-119,263.

2. Merck Index, Merck Research Laboratories, division of Merck and company NJ, USA, 13th ed., 2001, 1088.

3. United State Pharmacopoeia 26, NF 21, 2003, 1259.

4. Indian Pharmacopoeia (4th ed.), vol.-2, Govt. of India, Ministry of Health and Family Welfare, Controller of Publications, New Delhi, 1996, 554.

5. Kobylinska, K., Barlinska, M. and Kobylinska, M., Analysis of nabumetone in human plasma by HPLC. Application to single dose pharmacokinetic studies, J Pharm Biomed Anal, 32 (2003), p. 323-328.

6. Mikami E., Goto T., Ohno T., Matsumoto H. and Nishida M., Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography, J Pharm Biomed Anal. 2000 Oct; 23 (5), p. 917-925.

7. Idrees F. and Al-Momani, Determination of Nabumetone and Its Major Metabolite in Plasma and Tablet Formulations by Reverse-Phase HPLC, Analytical Letters, 30(14) Nov. 1997, p. 2485 – 2492.

8. Celema, C., Allue, J.A., Prunonosa, J., Peraire, C. and R. Obach, Simultaneous determination of Paracetamol and Chlorpheniramine in human plasma by liquid chromatography tandem mass spectrometery, Journal of Chromatography Analysis. 2000, 870(1-2), p. 77-86.

9 Nagaralli, B.S., Seetharamappa, J., Gowda B.G. and Melwanki, M.B., Liquid chromatographic determination of ceterizine hydrochloride and paracetamol in human plasma and pharmaceutical formulations, Journal of Chromatography B, 2003, 798 (1), p. 49–54.

10. Eswaraselvam A, Simultaneous estimation of paracetamol and ibuprofen by RP-HPLC by using same mobile phase for assay and dissolution in tablet dosage form, I.P.C. Abstracts. 2004, p. 83.

11. Subramanian, G., Simultaneous RP-HPLC estimation of Tizanidine, Diclofenac potassium and Paracetamol in tablets. Indian J. Pharm. Sci., 2004, 66(5), p. 694-696.

12. Momin M.Y., Yeole P.G., Puranik M.P. and Wadher S.J., RP-HPLC estimation of Aceclofenac and Paracetamol in tablet dosage form, Indian J. Pharm. Sci., 2006, 68 (3), p. 387-389.

13. Bhavsar, A.S., Talele, G.S., Fursule, R.A. and Surana, S.J., RP-HPLC estimation of paracetamol and valdecoxib in combined dosage form, 2006, 68(5), p. 675-677.

14. Nagulwar, V., Dhurvey, Y., Despande S., Upadhye K., Bakhle, S. and Wadetwar R. UV Spectroscopic estimation of paracetamol and valdecoxib in tablet dosage form, Indian J. Pharm. Sci., 2006, 68 (5), p. 639-640.

15. Mahaparale, P.R., Sangshetti, J.N. and Kuchekar, B.S., UV Spectroscopic estimation of Aceclofenac and Paracetamol in tablet dosage form, Indian J. Pharm. Sci., 2006, 69 (2), p. 289-292.

16. Text on validation of analytical procedure, Q2B in ICH Harmonized Triplicate Guidelines, Nov.2005.

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