WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 2, 874-886. Research Article ISSN 2277– 7105

A DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR ESTIMATION OF FENOPROFEN IN BULK DRUG

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Article Received on
30 November 2018,

Revised on 21 Dec. 2018,
Accepted on 11 Jan. 2019

DOI: 10.20959/wjpr20192-14043

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ABSTRACT

Rapid and sensitive method was developed and validated for the estimation of fenoprofen calcium in bulk drugs. Liquid-liquid extraction with methanol and acetonitrile. The accuracy and reproducibility of the method were within acceptable. Linearity ranges from 10 to 80 microgram/ml .the run time of the sample is 10 minutes.selected wavelength of the drug is 270nm. Operating temperature at 25degree celcius (ambient) by using column –enable c18 (4.6×250mm, 5 micrometers). Limit of the developed rp-hplc method for the estimation of fenoprofen, carried out on with enable c18 (250nm×4.6, 5µm) column. Methanol and acetonitrile in the ratio (80:20v/v) used as mobile phase and flow rate 1.5 ml/min. The detection was carried at 270nm and ambient column temperature was maintained.

KEYWORDS: linearity, accuracy, precision, limit of detection, limit of quantification.

INTRODUCTION

Pharmaceutical analysis is a specialized branch of analytical chemistry derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis is essential before a quantitative analysis can be undertaken. An isolation step is usually a necessary part of both a qualitative and quantitative analysis. The result of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and the second one is the measurement of some quantity that is proportional to the amount of analytic that sample and normally completes the analysis.[1]

MATERIALS AND METHODS

The developed rp-hplc method for the estimation of fenoprofen, carried out on with enable c18 (250nm×4.6, 5µm) column. Methanol and acetonitrile in the ratio (80:20v/v) used as mobile phase and flow rate 1.5 ml/min. The detection was carried at 270nm and ambient column temperature was maintained.

Materials

Table 4: Instrument used.

S. no.	Name	Model	Manufacturer
1.	**Ph meter**	µph system 361	Systronics
2.	**Weighing balance**	Bl-220h	Schimadzu
3.	**Ultrasonitor**	Ins-014	Systronics
4.	**Hplc**	Spd-m20a	Schimadzu

Table 4: Reagents and chemicals.

S. no.	Name	Grade	Manufacturer
1.	**Methanol**	Hplc	Fisher scientific
2.	**Acetonitrile**	Hplc	Merck

Optimized chromatographic condition

Instrument used	:	hplc (schimadzu)
Operating temperature	:	ambient
Column	:	enable c18 (4.6 x 250mm, 5µm)
Mobile phase	:	methanol: acetonitrile (80:20)
Flow rate	:	1.5ml/ min
Selected wavelength	:	270 nm
Diluents	:	methanol
Injection volume	:	20 µl
Run time	:	5 min.
Retention time	:	fenoprofen

Selection of wave length (for detection)

The choice of detection wave length was based on the scanned absorption spectrum for fenoprofen. The uv-spectrum of fenoprofen was obtained by scanning the sample over the wave length range 200-400 nm. After thorough examination, the wave length 270 nm was selected for further analysis.

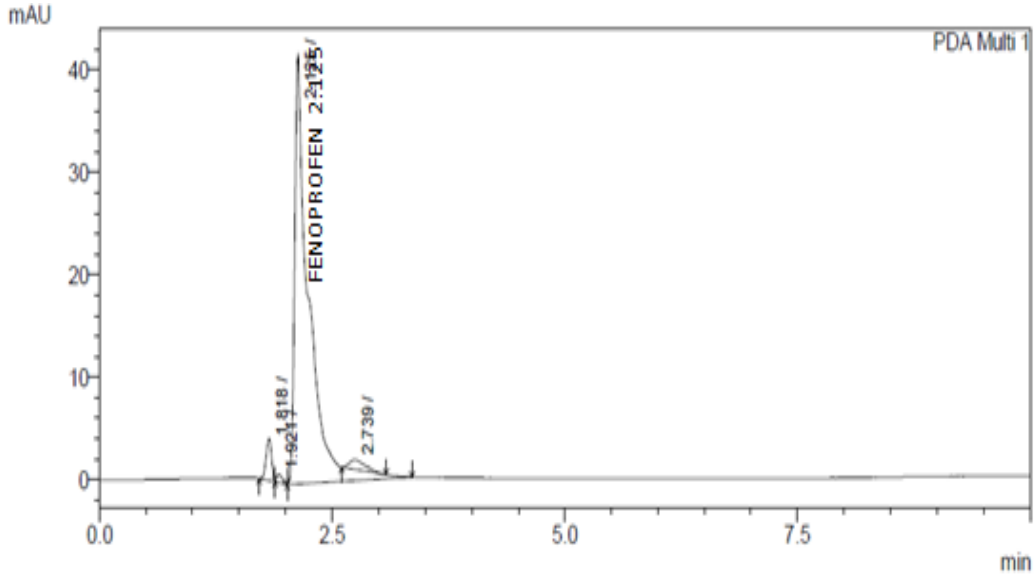

Fig. 3: Spectrum of fenoprofen.

Selection of chromatographic method

The choice of chromatographic method is based on the nature of sample, its molecular weight and solubility. The reverse phase chromatographic technique was selected for present work, due to polar nature of the drug.

After the method development, the method is validated in terms of parameters like linearity, precision, accuracy, robustness, lod and loq.

1. System suitability studies

System-suitability tests are a necessary part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (rt), number of theoretical plates (*n*), tailing factor (*t*), and peak asymmetry (as), resolution (rs) were calculated for five replicate injections of the drug. The system suitability test was performed using five replicate injections of standards before analysis of samples.

Note

Tailing factor	----	not more than 2
Theoretical plates	----	not less than 2000
Resolution	----	not less than 2

2. Linearity

From the stock solution of fenoprofen calcium take 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml, 6mg/ml, 7mg/ml, and 8mg/ml of stock solution in eight volumetric flasks with the methanol to give the following concentrations.

Fenoprofen: 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml and 80µg/ml.

The calibration graph was plotted with mean peak area on the y-axis and concentration of standard solution in the x-axis. The degree of linearity was estimated by calculating the correlation coefficient, y- intercept and slope of the regression line.

3. Precision

A) The system precision of test method was performed by injecting six portions from a standard solution on to the analytical column and the peak area data obtained then % rsd was calculated.

B) the method precision of test method was done by performing assay on six replicate determination of sample preparation at test concentration level (as per method of analysis) and the relative standard deviation of assay results was obtained.

4. Accuracy

Accuracy of the method confirmed by performing recovery studies at 50%, 100%, and 150% levels of sample concentration, in accordance with ich guidelines, by replicate analysis (n=3). Standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured.

% recovery = $[(c_t - c_u)/ c_a] \times 100$.

Where c_t is the total conc. Of the analyte found,

c_u is the conc. Of the analyte present in formulation;

c_a is the conc. Of the pure analyte added to the formulation.

Procedure

Injected the solutions of 50%, 100%, and 150% with spiked amount of standard concentration. Calculate the amount added and amount found for fenoprofen and calculate

the individual recovery and mean recovery values.

5. Robustness

For determining the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to endure such slight changes and allow routine analysis of the sample. Robustness of method was carried out with variation in flow rate, mobile phase composition and detection wavelength (± 2 nm).

6. Limit of detection and quantification

Detection and quantification limit were calculated by the method based on the standard deviation (σ) and slope of the calibration plot, using the formula

Limit of detection=σ × 3.3 S

Limit of quantization =σ × 10/ s

Where σ = the standard deviation of the response.

s = the slope of the calibration curve (of the analyte).

RESULTS AND DISCUSSION

Trail 11

Mobile phase	:	Methanol: acetonitrile (80:20 V/v)
Flow rate	:	1.5 ml min^{-1}
Operating temperature	:	25c (ambient)
Column	:	Enable c18 (4.6 x 250mm, 5µm)
Injection volume	:	20 µl
Selected wavelength	:	270 nm

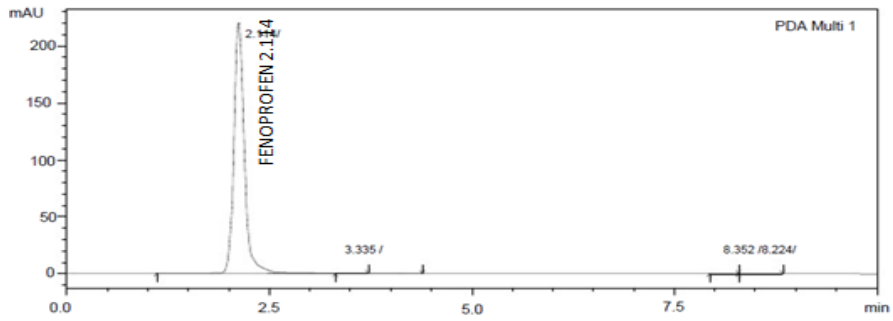

Fig. 6: 1k chromatogram for trail 11.

S. no.	Name	Retention time	Area	Sup tailing	Sup plate count
1	Fenoprofen	2.114	393345	1.25	9654

Observation: tailing is with in the limits.

Linearity

Linearity was observed over the concentration range of 10-80µg/ml fenoprofen. Correlation coefficient was found to be 0.999 for the drug which indicates that the concentration had.

Table 6.2: linearity data of fenoprofen.

S. no.	Concentration of acitretin (µg/ml)	Peak area (mau)
1	10	466836
2	20	852927
3	30	1054567
4	40	1107697
5	50	1685924
6	60	2168156
7	70	2572134
8	80	3261592

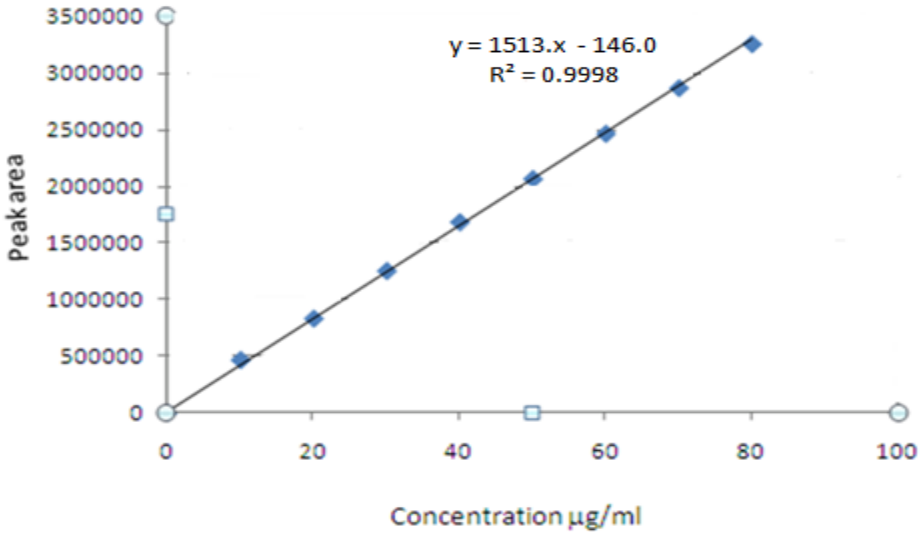

Fig. 6.11: Linearity plot for fenoprofen.

Table 6.3: Analytical performance parameters for fenoprofen.

Parameters	Fenoprofen
Linearity (µg/ml)	10-80
Correlation coefficient	0.999
Intercept	1513.x-146.0

Acceptance criteria

Correlation coefficient (r^2) should not be less than 0.999.

The correlation coefficient obtained was 0.9998 for fenoprofen.

Precision

The %rsd values of the fenoprofen of system precision and method precision was found to be 0.69 and 0.5 respectively. As the results are within the acceptance limits of less than 2%, indicates that than the proposed method has good reproducibility.

Table 6.4: Results for system precision.

S. no.	Peak area of fenoprofen
1	4915822
2	4905647
3	4873613
4	4965957
5	4896719
Mean	4911552
S.d	34177.4
%rsd	0.69

Table 6.5: Results for method precision.

S. no.	Peak area of fenoprofen
1	383417
2	382430
3	391024
4	383249
5	391013
6	390413
Mean	386924
S.d	135443.7
%rsd	0.5

Acceptance criteria

% Rsd should not be nmt 2.

The % rsd for the results was below 1, which were within the limits and hence method is precise.

Accuracy

The accuracy of the method was persistent by recovery experiments. The recovery studies were carried out by correlating method of three individual standards with each of three samples with same procedure from the formulation and injecting. Sample solution spiked with standard at different concentrations (50%, 100% and 150%) was prepared and the % recovery was calculated.

Table 6.6: % recovery offenoprofen.

% level	Amount added (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % recovery
50%	10	9.98	99.8	99.96%
	10	9.99	99.9	
	10	10.02	100.2	
100%	20	19.98	99.9	99.85%
	20	19.88	99.4	
	20	20.05	100.25	
150%	30	30.25	100.8	100.27%
	30	30.05	100.1	
	30	29.98	99.93	

Acceptance criteria

- The % recovery for each level should be between 98.85 to 100.27%.

The results obtained for recovery at 50%, 100% and 150% are within the limits. Hence the limit of detection

- The lowest concentration of the standard solution was prepared with respect to the base line noise and measured the signal to noise ratio.

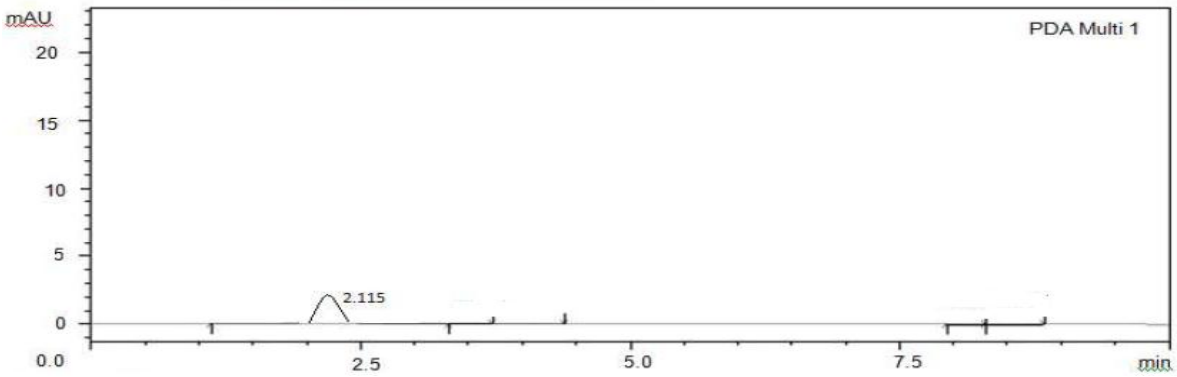

Fig. 6.34: Typical chromatogram for lod of fenoprofen.

Table 6.9: Results for lod.

Sample	Lod
Fenoprofen	2.082

Acceptance criteria

- Signal to noise ratio shall be 3 for lod solution.
- The result obtained was within the limit.

Limit of quantification

The lowest concentration of the standard solution was prepared with respect to the base line noise and measured the signal to noise ratio.

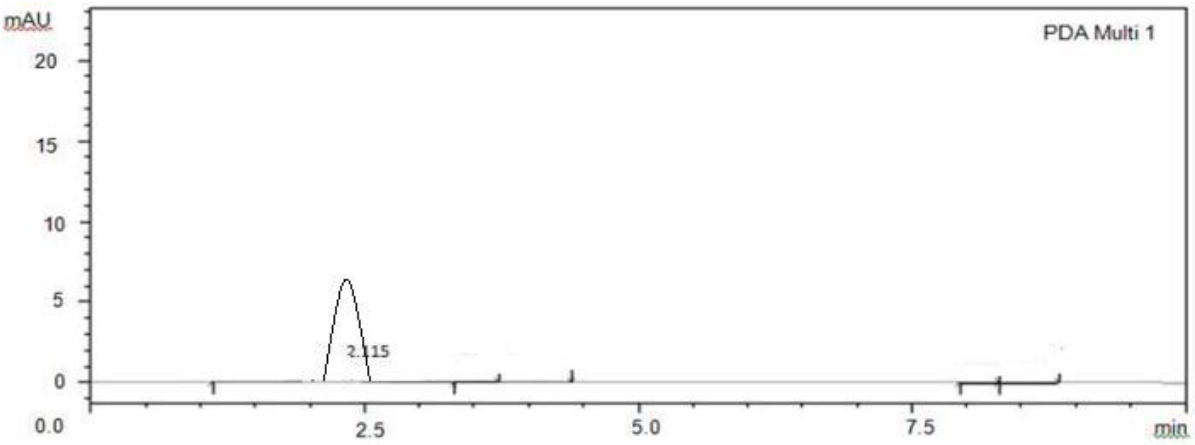

Fig. 6.35: Typical chromatogram for loq of fenoprofen.

Table 6.10: results for loq.

Sample	Loq
Fenoprofen	6.92

Acceptance criteria

- Signal to noise ratio shall be 10 for lod solution.
- The result obtained was within the limit.

Limit of quantification

The lowest concentration of the standard solution was prepared with respect to the base line noise and measured the signal to noise ratio.

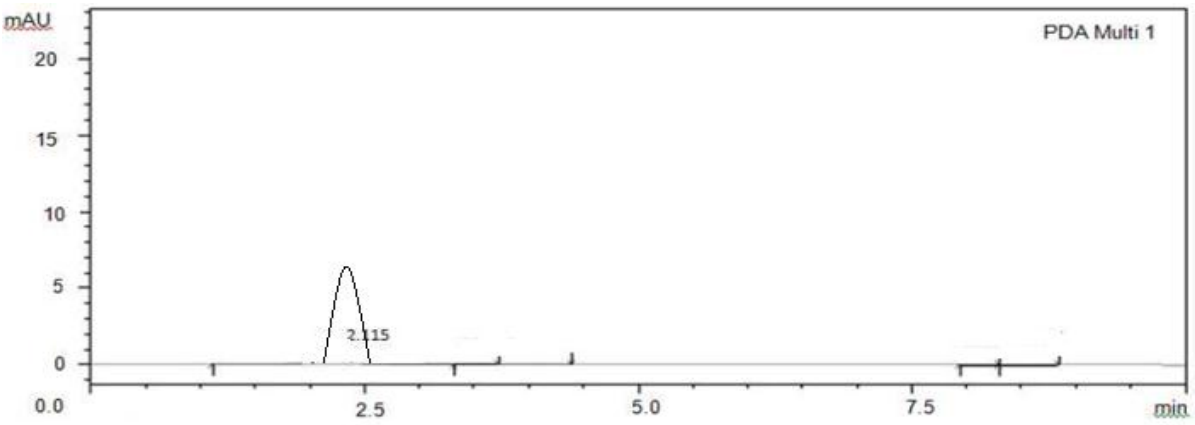

Fig. 6.35: Typical chromatogram for loq of fenoprofen.

Table 6.10: Results for loq.

Sample	Loq
Fenoprofen	6.92

Acceptance criteria

- Signal to noise ratio shall be 10 for lod solution.
- The result obtained was within the limit.

CONCLUSION

From the reported literature, there were few analytical method established for the determination of fenoprofen by rp-hplc method. However, there are no methods were reported for estimation of fenoprofen in pharmaceutical dosage forms.

The scope and objective of the present work is to develop and validate a simple rp- hplc method for estimation offenoprofen in pharmaceutical dosage form.

In rp-hplc method development, modrobes hplc spd-m20a system with pda detector and column used is enablec18 (250 x 4.6mm) column with 5-micron particle size. Injection volume of 20 µl is injected and eluted with the mobile phase selected after optimization was methanol and acetonitrile in the ratio of 80:20 v/v was found to be ideal. The flow rate was found to be optimized at 1.5 ml/min. Detection was carried out at 270 nm. Quantization was done by external standard method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of fenoprofen was found to be respectively.

The fenoprofen showed linearity in the range of 10-80 µg/mlrespectively.regression equation of fenoprofen is y = 1513.x -146.0. The correlation coefficient value for fenoprofen 0.9998 respectively which indicates excellent correlation between response factors vs. concentration of standard solutions.

Precision of the developed method was studied under system precision and method precision. %rsd of the fenoprofen were and found to be 0.69 and 0.5 respectively. Hence the %rsd values for precision were found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. %recovery was obtained as 99.85% and 100.27% for fenoprofen respectively. The %rsd value for percentage recovery was found to be within the acceptance criteria. Lod, loq values are

obtained from regression equations of fenoprofen were 2.082, 6.92 respectively. The results indicate satisfactory accuracy of method for estimation of the fenoprofen.

Hence, the chromatographic method developed for the fenoprofen said to be rapid, simple, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

ACKNOWLEDGEMENTS

Syed. rihana, dr.a.suneeta.

REFERENCES

1. Gurudeep chatwal and sham anand, instrumental methods of chemical analysis. Himalaya publishers, 7th edition, 1992; 2.624-2.639.
2. Skoog et al., principles of instrumental analysis. Barkhanath publishers, 8th edition, 973-995.
3. Hobart.h.willard et al., instrumental methods of analysis, cbs publications and distributors, New Delhi, 1st edition, 1986; 529-563.
4. Sethi p.d., quantitative analysis of drugs & pharmaceuticals. Cbs publishers and distributors, New Delhi, 3rd edition, 2001; 1-120.
5. Janeyulu.y&marayyah, quality assurance & quality management in pharmaceutical industry. Pharma book publishers, hyd, edition, and, 2005; 78-108.
6. Vogel's text book of quantitative chemical analysis. Published by dorling kindersley pvt.ltd. 6th edition, 289-304.
7. Lloyd r. Snyder et al., practical hplc method development. John wiley & son's publishers, 2nd edition, 350-400.
8. Knevel a.m. &.digengl f.e, Jenkins quantitative pharmaceutical chemistry, mc graw hill book co.
9. Daniel w.armstrong, bonded phase material for chromatographic separations, u.s.patent, 1985; 4539399.
10. Sastry, c.s.p., singh, n.r., reddy, methods of analysis, 1986; 316.
11. Baveja s.k. et al., 1987; 337-344.
12. Puthlis.p.vavia, p.r j.pharm. Biomed. Anal. 22, published in, 2000; 673-677.
13. Salo j.p, j.pharm. Biomed. Anal. 14, published in, 1996; 1261-1266.

14. Loyd .r Snyder, ET al.practical hplc method development, john wiley & sons publishers, Inc, New York, 2nd edition, 686-706.
15. Www.science direct.com.
16. D.helmeste et al., j.chromatogr. Published in, 1997; 195-201.
17. Interna ich of technical requirements for the registration of pharmaceuticals for human use, validation of analytical parameters; methodology adopted in, Geneva, 1996.
18. Ich guidelines q2b, validation of analytical procedure: definitions, published in March, Geneva, Switzerland, 1996.
19. Singh Gn, gupta rp. Stability of pharmaceuticals. Dept. Of pharmaceutics, institute of technology, b.h.u., Varanasi u.p. eastern pharmacist aug, 1987; 85-89.
20. Teresa i, Lucas, bishara rh, Robert hs. A stability program for the distribution of drug products. Pharmaceutical technology resource guide, 2004; 86-9.
21. Gennaro ar. Remington: the science and practice of pharmacy. Philadelphia, 12thedn, 2000; 986-90.
22. Singh s, bakshi m. Development of validated stability-indicating assay methods critical review. J pharm biomed anal, 2002; 28: 1011-40.
23. Ich harmonised tripartite guideline stability testing of new drug substances and products q1a (r2), 2003; 2: 1-24.
24. M. Debackere et al developed a high performance liquid chromatographic method to measure plasma and urine fenoprofen levels in equine biofluids is described. Liquid–liquid extraction with diethylether was used to isolate the drug from plasma and urine.
25. *Akimasashibukawa et al* developed a high-performance frontal analysis (hpfa) and high-perfon-ance liquid chromatography (hplc) were incorporated in an on-line coupled column system for the enantioselective determination of unbound fenoprofen (fp) concentrations in the state of a protein binding equilibrium following direct sample injection.
26. *Yi-fen pai et al* developed a wall-coated histidine capillary column was developed for the on-line preconcentration of nonsteroidal anti-inflammatory drugs (nsaids) in capillary electrochromatography (cec).
27. *T et al* developed the kinetics of fenoprofen release from poly[α,β-(*n*-2-hydroxyethyl-dl-aspartamide)]-fenoprofen conjugate (phea-fen) in aqueous buffer solutions (ph 10 and 1.1), simulated gastric (sgf) and intestinal fluids (sif) was studied.

28. *deiaabd el-hadyet al*developrd a new biosensor or protein label-free sensor composed of 1-butyl-3-methylimidazolium hexafluorophosphates (bmimpf$_6$)-human serum albumin (hsa) film on glassy carbon electrode (gce) was produced.
29. Alan rubin et al said because orally administered *dl*-2-(3-phenoxyphenyl)-propionic acid, fenoprofen, may be useful for maintenance anti-inflammatory/analgesic therapy in man; evaluations were begun of certain pharmacokinetic parameters related to the absorption and disposition of this compound.