ISSN : 0974-7419 Volume 10 Issue 9

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(9) 2011 [595-603]

Performance comparison between monolithic C_{18} and conventional C_{18} particle- packed columns in the liquid chromatographic determination of ibuprofen in presence of its degradation products

E.A.Abdullah[1]*, Maha A.Hegazy[2], M.Abdelkawy[2], A.E.El Gindy[1], A.Ashour[1]

[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Misr International University, KM 28 Cairo-Ismailia Road (Ahmed Orabi District), Cairo, (EGYPT)

[2]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El Aini Street, 11562 Cairo, (EGYPT)

E-mail: nosa_ebraheem@hotmail.com

Received: 27th February, 2011 ; Accepted: 9th March, 2011

ABSTRACT

A rapid, sensitive and reproducible HPLC method using C_{18} monolithic and conventional column was developed and validated for the analysis of Ibuprofen and its degradates including; acid degradate, oxidative and thermal degradates. Chromatographic separation was achieved using a mixture of methanol: 0.05M Phosphate buffer (75:25 v/v) (pH=6.5) as a mobile phase at a flow rate of 2.0 ml/min with fluorescence detection at λex = 224 nm and λem = 290 nm on the monolithic column and flow rate 1.0 ml/min on conventional column. Calibration curves were obtained in the concentration range of 0.25-0.55 µg/ml on both monolithic and conventional column. Limit of quantitation (LOQ) = 0.00017 µg/ml and 0.0025 µg/ml for monolithic and conventional column respectively. The intra-day R.S.Ds and inter-day RSDs were all less than 2.5 %. The total run was reduced by 30% by using C_{18} monolithic column. In conclusion, by this developed method, Ibuprofen and its degradates can be determined rapidly with good precision and accuracy in pharmaceuticals.

KEYWORDS

Ibuprofen;
Degradation;
Stability;
Monolithic column;
High performance liquid chromatography.

INTRODUCTION

Monolithic columns were developed recently with potential of achieving separation faster than conventional columns. Monolithic columns consist of a single rod of silica – based material[1,2]. The porous structure of monolithic rods of silica have bimodal pore structures, that is, macro pores (2 µm) and mesopores (12 nm)[3,4]. The former allow rapid flow of the mobile phase at low pressure. While the latter create the large uniform surface on which adsorption takes place[5,6]. Monolithic columns have been used in environmental and pharmaceutical areas of application[7,8].

Quality by design (QbD) development programme uses a systematic approach that fully utilizes designed experiments and multivariate statistical tools to assemble a product and process design space where critical parameters are defined and where possible, linked to the demonstrated product safety and efficacy. Appropiate measurement systems will be required to gain greater understanding of the product and process and to establish this product and process design space.

Full Paper

An important tool in the application of QbD approach is design of experiment. DOE is statistical approach that simultaneously changes all experimental parameters to get useful information about the significance of experimental parameters and more importantly their interactions.

A comprehensive assessment of robustness during analytical method validation is a major activity in gaining the understanding of quality by design (QbD). QbD facilitates continual improvement by establishing a systematic framework to scientifically assess the impact of any proposed changes.[9]

Robustness testing is done in this work either on the conventional column or the monolithic column with the use of design of experiment (DOE), when performing a robustness test of a method; the objective is to ascertain that the method is robust to small fluctuations in the factor levels, to understand how to alter the bounds of the factors so that robustness may still be claimed.[10] With robustness testing design, it is possible to determine the sensitivity of the responses to small changes in the factors.

Where such minor changes to the factor levels have little effect on the response values, the analytical system is determined to be robust[11,12]

Ibuprofen is a potent a chiral non-steroidal anti-inflammatory drug (NSAID) used to relieve moderate pain, acute arthritis, non-rheumatic inflammation, fever and dysmenorrhea[13].

Analytical methods described for Ibuprofen includes: Liquid chromatography with fluorescence detection for determination of enantiomers in human plasma[14,15]. In dosage forms, Ibuprofen has been determined using HPLC and derivative spectroscopy[16,17] and chemometric assisted spectrophotometric methods[18]. Previous studies described a stability indicating method designed to investigate the degradation of Ibuprofen in tablet dosage form but nothing was reported on application of monolithic column technology on evaluation of stability indicating method of Ibuprofen

In this paper, a comparison is made between the performance of monolithic and conventional column in the development of a validated HPLC method for Ibuprofen in presence of its degradation products. DOE is implemented in the study of method development on the monolithic column as it is known for faster separation and short analysis time so can be used for method development steps in R&D laboratories.

EXPERIMENTAL

Materials and reagents

Ibuprofen standard was obtained from El- Kahira for pharmaceutical and chemical Industries.

HPLC grade methanol was obtained from Sigma-Aldrich (Germany).

Analytical grade di-potissium hydrogen orthophosphate, orthophosphoric acid, hydrogen peroxide (30%v/v), hydrochloric acid and methanol were purchased from Adwic (Cairo, Egypt).

Double distilled water was used throughout the experiment.

Brufen® tablet was manufactured by El- Kahira for pharmaceutical and chemical Industries; it was labeled to contain 400 mg of Ibuprofen (Batch No. 82220 and 87834).

Equipment and chromatographic conditions

Chromatographic experiments were performed with a HPLC system equipped with an isocratic pump, fluroscent detector agilent 1200. Chromatographic signals were acquired and processed by Agilent LC chemstation software 1200. A chromolith® performance RP-18 e (100mm x 4.6 mm) column (Merk, Germany) and YMC- pack ODS (250 mm x 4.6 mm, 5 μm) were used for separation. The optimized mobile phase was a mixture of methanol: phosphate buffer (0.05M) (pH= 6.5) (75:25 v/v). The flow rate was set at 1.0 and 2.0 ml/min for conventional and monolithic column respectively, detector wavelength at (λex = 224 nm, λem = 290 nm). The injection volume was 20 μL.

Preparation of solutions

Standard solutions

Standard solution of Ibuprofen was prepared at 100 μg/ml using methanol as a solvent and is protected from light. Working standard solution of Ibuprofen was prepared at 1 μg/ml using methanol as solvent. Calibration solutions were prepared with appropriate aliquots of the working standard solution were diluted with the mobile phase to obtain concentration range of (0.25-0.55 μg/ml).

Analysis of dosage form

Twenty tablets of Brufen® 400 mg Ibuprofen were weighed and finely powdered. An accurately weighed powder equivalent to 10 mg Ibuprofen was placed in a 100 ml volumetric flask and diluted to volume with methanol. The solution was ultrasonicated for 30 min. and filtered. The filterate was having the concentration of 100 µg/ml. Several dilutions were made, then an aliquot was taken (0.4 µg/ml) diluted with the mobile phase and injected.

Forced degradation studies

Acidic degradation

10 mg of Ibuprofen were dissolved in 10 ml of 2 M HCl and kept at 100 °C on a boiling water bath for 90 hours.

Oxidative degradation

10 mg of Ibuprofen were dissolved in 10 ml of 10%v/v of H_2O_2 and kept at 100 °C on a boiling water bath for 90 hours.

Thermal degradation

Five tablets of Brufen® were left for 140 hours at oven at 80 °C.

Method development

Eleven experiments were done in which different factors affecting chromatographic separation. Method parameters were changed according to the following parameters: Flow rate of (3.0 ml/min) and wavelength of detection ($\lambda_{ex.}$ = 224 nm, $\lambda_{em.}$ = 290 nm), Phosphate buffer (pH= 6.5). Different ratios of methanol and buffer until the chosen system is (Methanol: Phosphate buffer) (80:20). The First design of experiment (DOE I) used a Plackett-Burman design involving change in pH, Flow rate, methanol and wavelength of excitation and emission. As for pH (-1=6.3, 0=6.5, +1=6.7), Flow rate (-1=2.8, 0=3.0, +1=3.2), methanol (-1=70, 0=80, +1=90), $\lambda_{ex.}$ (-1=223, 0=224, +1=225) and $\lambda_{em.}$ (-1=289,0=290,+1=291) as shown in TABLE 1.

Another experimental design was made to reach a better understanding of the factors influencing the chromatographic separation of Ibuprofen and the acid degradation products. So trials were done involving the change of the concentration of buffer and changing the flow rate and ratio of methanol to buffer. To ensure the resolution of this mixture, a second design of experiment (DOE II) was done. As for pH(-1=6.3, 0=6.5, +1=6.7), Flow rate (-1=2.0, 0=2.5, +1=3.0), buffer concentration (-1=0.01, 0=0.03, +1=0.05), $\lambda_{ex.}$ (-1=223, 0=224, +1=225) and $\lambda_{em.}$ (-1=289, 0=290, +1=291) as shown in TABLE 1. The experimental results of DOE I and II were computed using MODDE 9.0 trial version with respect to resolution between Ibuprofen's peak and the peaks of the acid degradation products.

Method validation

Method validation parameters studied were: Linearity, precision, accuracy and limit of quantitation and robustness.

Specificity

Different aliquots of acidic, oxidative and thermal degradates were injected.

Repeatability

The repeatability of the method was studied by preparing six replicate samples solution for Brufen® tablet. From the same powder (described under dosage form preparation) injections of each sample was done in duplicates.

Intermediate precision

Intermediate precision of the method was studied by repeating the repeatability experiment in three different days.

Linearity

Assay of linearity was studied by preparing serial dilutions from the working standard solution within the range of 0.25-0.55 µg/ml for both monolithic and conventional column which is the range covering 80-120% of the target concentration.

Accuracy

Accuracy and recovery of the method was studied by analyzing data obtained from standard solutions during the range portion of validation. Different concentrations covering different parts of the calibration range (0.25, 0.35, 0.40, 0.45, 0.55 µg/ml). Each concentration was injected in triplicate.

Limit of quantitation (LOQ)

The LOQ was defined as the lowest concentration

that could be determined with acceptable accuracy and precision under the stated experimental conditions.

Robustness

Robustness testing done on the monolithic column and on the conventional column. Different factors are changed including: methanol ratio, pH, Flow rate and the excitation and emission wavelengths. All are changed through a small range using Plackett-Burman design. As for pH (-1= 6.3, 0=6.5, +1=6.7), Flow rate (-1=1.8, 0=2.0, +1=2.2) (-1=0.8, 0=1.0, +1=1.2) for monolithic and conventional column respectively, Methanol (-1=70, 0=75, +1=80), $\lambda_{ex.}$ (-1=223, 0=224, +1=225) and $\lambda_{em.}$ (-1=289, 0=290, +1=291) as shown in TABLE 1.

TABLE 1 : Design of experiment (DOE) for method development (DOE I and DOE II) & method robustness of ibuprofen on monolithic and conventional column.

Exp. No.	pH	Flow rate	Methanol	Excitation wavelength	Emission wavelength
1	1	-1	-1	1	-1
2	1	1	-1	-1	1
3	1	1	1	-1	-1
4	-1	1	1	1	-1
5	1	-1	1	1	1
6	-1	1	-1	1	1
7	-1	-1	1	-1	1
8	-1	-1	-1	-1	-1
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0

RESULTS AND DISCUSSIONS

Method development

The concept of quality by design (QbD) and design of experiment (DOE) was used to reach the optimum conditions for resolution of Ibuprofen from its degradation products. The first design of experiment (DOE I) used consider the change in five factors which are pH (X_1), Flow rate (X_2), percentage of methanol (X_3) and excitation wavelength (X_4) and emission wavelength (X_5). The ranges examined were small deviations from the center points and the corresponding responses which are resolution between the adjacent peaks (Y) were observed. Experimental results were computed by MODDE 9.0 trial version. The Coefficients of the model were estimated by the multiple linear regression (MLR). The model equation for Y was as follows:

Res 2:

$$Y_1 = 3.1836 + 2.33X_1 - 0.055X_2 - 0.055X_3 + 0.055X_4 - 2.33X_5$$

Res 3:

$$Y_2 = 3.1582 + 2.728X_1 - 0.05X_2 - 0.05X_3 - 0.229X_4 - 3.006X_5$$

Res 4:

$$Y_3 = 1.358 - 0.383X_1 + 1.293X_2 + 1.293X_3 + 0.548X_4 - 1.458X_5$$

Res 5:

$$Y_4 = 1.574 + 0.799X_1 - 0.566X_2 - 0.301X_3 + 0.566X_4 - 0.534X_5$$

The maximum resolution obtained in this experimental condition range was not enough to give satisfactory resolution (resolution between the 2 critical pairs which are two peaks in the degradation product with lowest resolution). So another factor was added which is the buffer concentration, a second design of experiment (DOE II) was done considering change in six factors which are pH (X_1), Flow rate (X_2), Buffer concentration (X_3), excitation wavelength (X_4), emission wavelength (X_5) and methanol (X_6) and the corresponding responses were resolution of different peaks (Y).

Res 2:

$$Y = 3.309 - 0.198X_1 + 1.018X_2 - 1.461X_3 + 1.133X_4 + 1.466X_5 + 0.686X_6$$

Res 3:

$$Y = 0.816 + 1.123X_1 - 1.123X_2 - 1.123X_3 + 1.123X_4 + 1.123X_5 - 1.123X_6$$

Res 4:

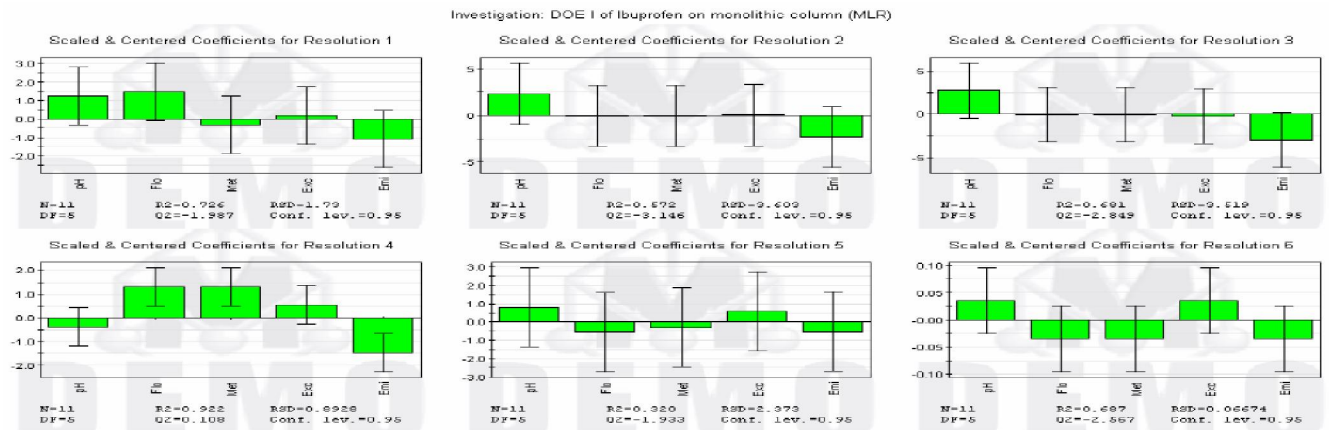
$$Y = 0.902 + 1.24X_1 - 1.24X_2 + 1.24X_3 + 1.24X_4 + 1.24X_5 - 1.24X_6$$

Res 5:

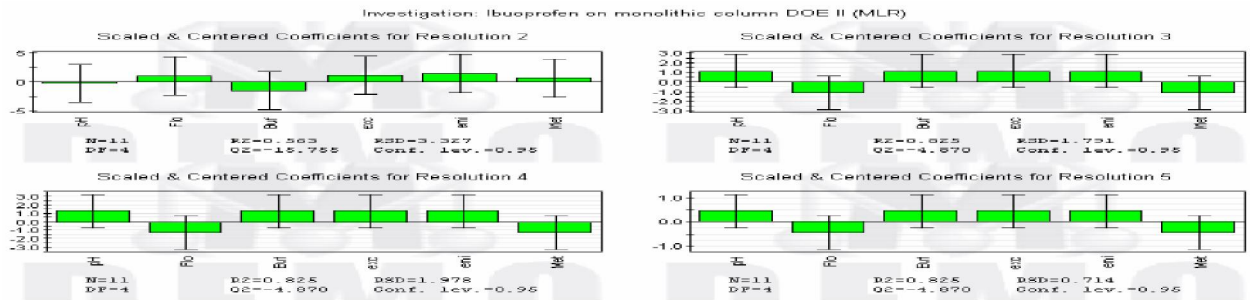
$$Y = 0.325 + 0.448X_1 - 0.448X_2 + 0.448X_3 + 0.448X_4 + 0.448X_5 - 0.448X_6$$

DOE I and II were done on monolithic column to show the effect of the studied factors. In DOE I, all factors are found to be non-significant ($P>0.05$) except flow rate and methanol. So DOE II was done to optimize these two factors until all the studied factors are found to be non-significant as shown in the Coefficient plot in Figures 1.

Also values of R^2, R^2 adj. and Q^2 for DOE I and II are shown in TABLE 2.



(a)



(b)

Figure 1 : The coefficient plot of (a) DOE I and (b) DOE II of ibuprofen on monolithic C_{18} column.

Method validation

Specificity

Aims to show the separation of Ibuprofen peak from other degradation peaks.

Figures 3 and 4 shows the separation of Ibuprofen from acidic, oxidative and thermal degradation products, furthermore, the purity of the Ibuprofen peak was further checked by the diode array detector. No impurities in the peak were detected.

Precision

Repeatability

The repeatability of the method for assay was demonstrated by preparing six samples for Brufen® tablet. The samples were analyzed according to the analytical method and the percent label claim for Ibuprofen was determined for each sample. Results are presented in TABLE 3. The RSD% values for six samples of Ibuprofen at each of the concentration of 0.25, 0.40 and 0.55 µg/ml. The results of repeatability for both conventional and monolithic column were shown in TABLE 3.

Intermediate precision

Intermediate precision of the method was demonstrated by repeating the repeatability experiment with a different day. Intermediate precision was done on both conventional and monolithic columns as shown in TABLE 3.

TABLE 2 : The summary list of DOE I and DOE II of ibuprofen on monolithic C_{18} column

		R^2	R^2 adj	Q^2	SDY	RSD	N	Reproducibility
Res 2	DOE I	0.572	0.145	-3.145	3.896	3.603	11	0.9972
	DOE II	0.563	-0.094	-15.75	3.182	3.327	11	0.999
Res 3	DOE I	0.681	0.362	-2.849	4.407	3.518	11	0.967
	DOE II	0.825	0.563	-4.869	2.708	1.791	11	1
Res 4	DOE I	0.922	0.845	0.108	2.266	0.892	11	0.990
	DOE II	0.825	0.563	-4.869	2.991	1.978	11	1
Res 5	DOE I	0.319	-0.360	-1.932	2.034	2.373	11	0.091
	DOE II	0.825	0.563	-4.869	1.079	0.714	11	1

Linearity

Assay linearity was demonstrated by preparing serial dilutions from the working standard solution, where linearity was assessed in the range of (0.25 – 0.55 µg/ml) for both monolithic and conventional column which is covering (80%-120%) of the target concentration. Linear regression analysis data were tabulated for both columns as in TABLE 4.

Full Paper

Accuracy

Accuracy and recovery of the method for assay was demonstrated by analyzing data obtained from standard solutions during the range portion of the validation. The average percent recovery at each concentration was determined at the concentration of 0.250, 0.350, 0.400, 0.450 and 0.550 µg/ml respectively (n = 3) as in TABLE 4.

Limit of quantitation (LOQ)

The LOQ was defined as the lowest concentration that could be determined with acceptable accuracy and precision under the stated experimental conditions.

The limit of quantitation was found to be 0.0025 µg/ml of Ibuprofen on conventional column and 0.00017 µg/ml on monolithic column which shows higher sensitivity obtained on the monolithic column.

Robustness

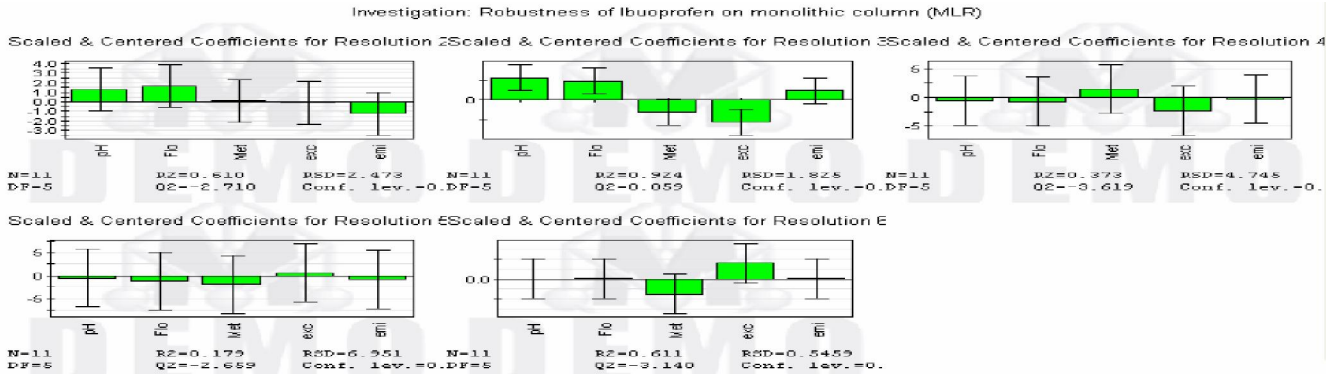
Robustness was done on monolithic and conventional columns considering five factors: pH (X_1), Flow rate (X_2), methanol (X_3), excitation wavelength (X_4) and emission wavelength (X_5). The corresponding responses is the resolution between adjacent peaks considered (Y).

TABLE 3 : Repeatability and intermediate precision of ibuprofen.

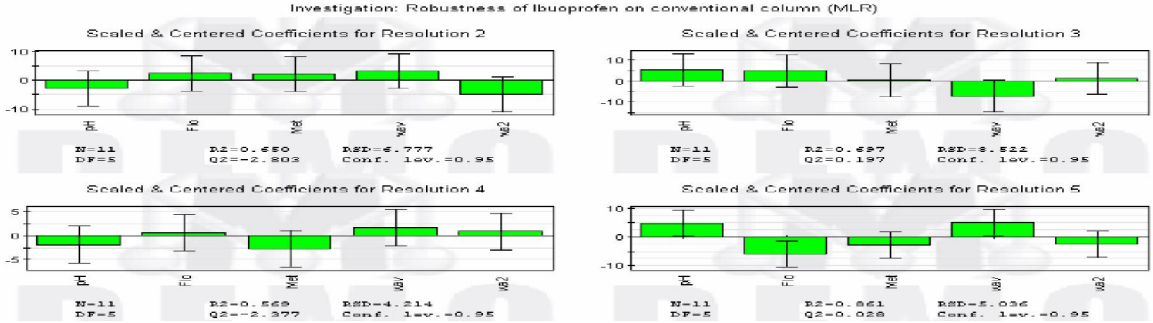
Parameter	Monolithic column	Conventional column
Repeatability	% R.S.D	% R.S.D
0.25 µg/ml	0.610	0.139
0.400 µg/ml	0.172	1.472
0.550 µg/ml	0.323	0.471
Intermediate precision	% R.S.D	% R.S.D
0.250 µg/ml	0.823	0.733
0.400 µg/ml	1.191	0.800
0.550 µg/ml	0.900	0.545

TABLE 4 : Linear regression data for analysis of ibuprofen on monolithic& conventional column

Parameter	Monolithic column	Conventional column
Linearity	0.25 – 0.55 µg/ml	0.25 – 0.55 µg/ml
Correlation coefficient (r^2)	0.9995	0.9995
Intercept coeff. ± S.E	10.297 ± 0.673	4.240 ± 0.222
Slope coeff. ± S.E	158.45 ± 1.633	55.57 ± 0.538
Standard error	0.432	0.142
Accuracy	99.43 ± 1.426	99.99 ± 1.298
Limit of quantitation (LOQ)	0.00017 µg/ml	0.0025 µg/ml



(a)



(b)

Figure 2 : The coefficient plot of robustness testing of ibuprofen on (a) monolithic C_{18} (b) conventional C_{18} columns.

TABLE 5 : The summary list of robustness of ibuprofen on conventional and monolithic column:

		R^2	R^2 adj	Q^2	SDY	RSD	N	Reproducibility
Res 2	Monolithic	0.610	0.221	-2.709	2.802	2.473	11	0.999
	Conventional	0.649	0.299	-2.803	8.098	6.777	11	0.999
Res 3	Monolithic	0.924	0.848	0.0589	4.682	1.825	11	0.999
	Conventional	0.697	0.394	0.197	10.947	8.522	11	-0.047
Res 4	Monolithic	0.373	-0.254	-3.619	4.237	4.745	11	0.971
	Conventional	0.568	0.137	-2.377	4.536	4.214	11	0.127
Res 5	Monolithic	0.178	-0.643	-2.659	5.424	6.951	11	0.941
	Conventional	0.861	0.723	0.028	9.562	5.036	11	1

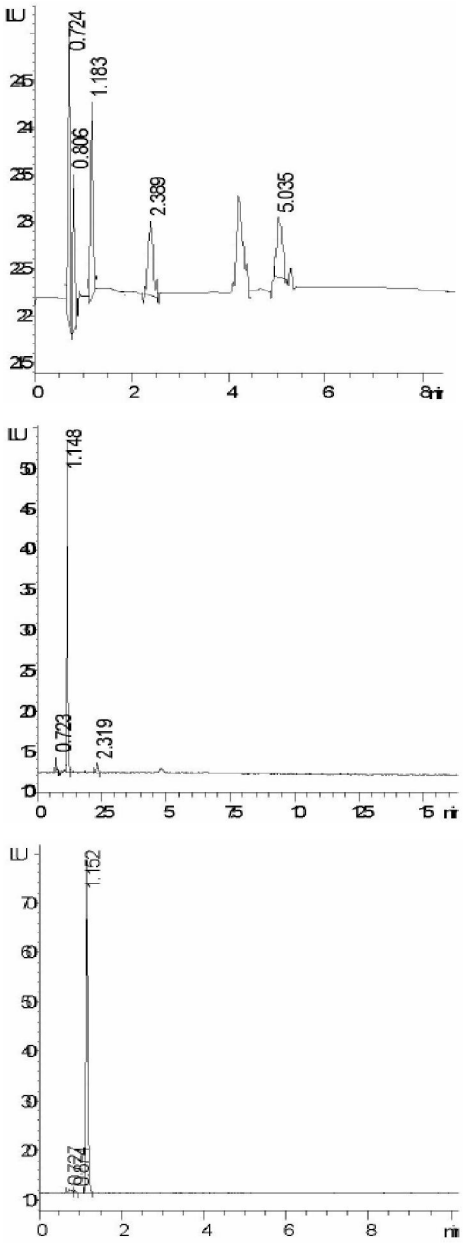


Figure 3 : HPLC chromatogram of acidic, oxidative and thermal degradation of ibuprofen under the specified chromatographic conditions using monolithic column.

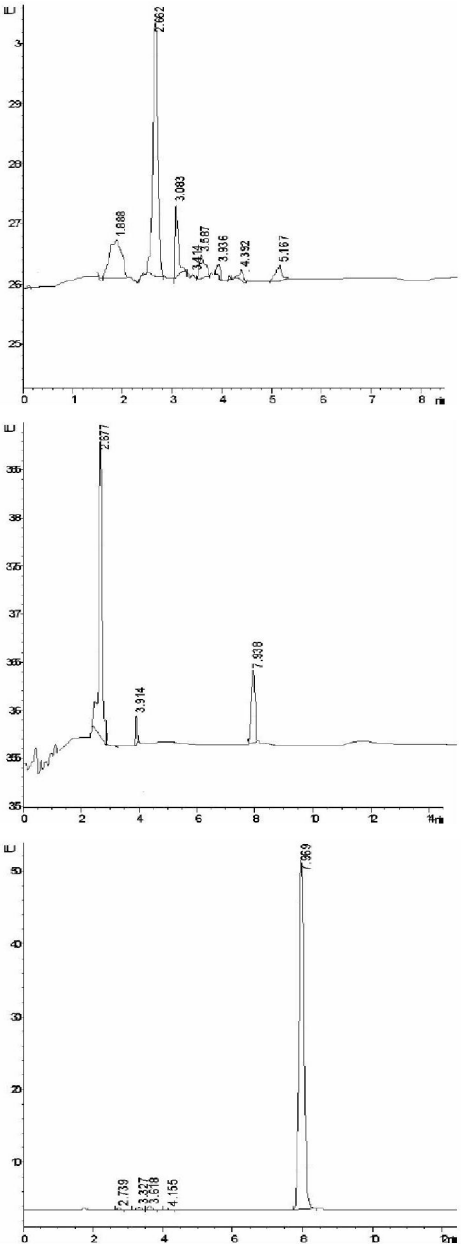


Figure 4 : HPLC chromatogram of acidic, oxidative and thermal degradation of ibuprofen under the specified chromatographic conditions using conventional column.

Full Paper

Monolithic column

Res 2:

$$Y_1 = 2.566 + 1.29X_1 + 1.65X_2 + 0.065X_3 - 0.089X_4 - 1.265X_5$$

Res 3:

$$Y_2 = 4 + 2.82X_1 + 2.39X_2 - 1.55X_3 - 2.82X_4 + 1.163X_5$$

Res 4:

$$Y_3 = 5.367 - 0.544X_1 - 0.746X_2 + 1.474X_3 - 2.296X_4 - 0.279X_5$$

Res 5:

$$Y_4 = 5.126 - 0.553X_1 - 1.218X_2 - 1.943X_3 + 0.55X_4 - 0.84X_5$$

Conventional column

Res 2:

$$Y_1 = 5.11 - 2.95X_1 + 2.43X_2 + 2.16X_3 + 3.13X_4 - 4.92X_5$$

Res 3:

$$Y_2 = 10.39 + 5.35X_1 + 4.93X_2 + 0.406X_3 - 7.074X_4 + 1.141X_5$$

Res 4:

$$Y_3 = 2.772 - 1.876X_1 + 0.661X_2 - 2.651X_3 + 1.679X_4 + 0.904X_5$$

Res 5:

$$Y_4 = 4.512 + 4.801X_1 - 5.866X_2 - 2.86X_3 + 5.139X_4 - 2.52X_5$$

Where Y is the resolution between different peaks of the acidic degradation product. This DOE was done for robustness testing on both monolithic and conventional columns. All factors are found to be non- significant (P>0.05) as shown in Coefficient plot in Figure 2. Values of R^2, R^2 adj. and Q^2 which are best indicators for model fitness are shown in TABLE 5.

Chromatography

Complete separation of Ibuprofen from its degradation products on conventional and monolithic column can be seen in Figures 3 and 4. Relevant chromatographic data obtained are reported . These results show that the developed method meets the separation and system suitability requirements.

For comparison, the chromatographic data in TABLE 6 was obtained on conventional C18 particle-packed column using the same mobile phase, with chromatogram shown in Figure 5, although the length of the monolithic column

Was shorter than that of the conventional column (10 Vs 15 cm) and flow rate was higher (2.0 ml/min versus 1.0 ml/min). Separation efficiency was comparable for both columns judged from the number of theoretical plates (N) and resolution (Rs).

TABLE 6 : Chromatographic data for the separation of ibuprofen and its degradation products on a monolithic column and a conventional particle- packed column.

Parameter	Monolithic column	Conventional column
Resolution (R_s)	3.94	21.53
Relative retention time(α)	7.58	11.14
Capacity factor($K^{'}$)	0.64	1.76
Tailing factor (t)	0.83	0.87
Number of theoretical plates (N)	2076	22542

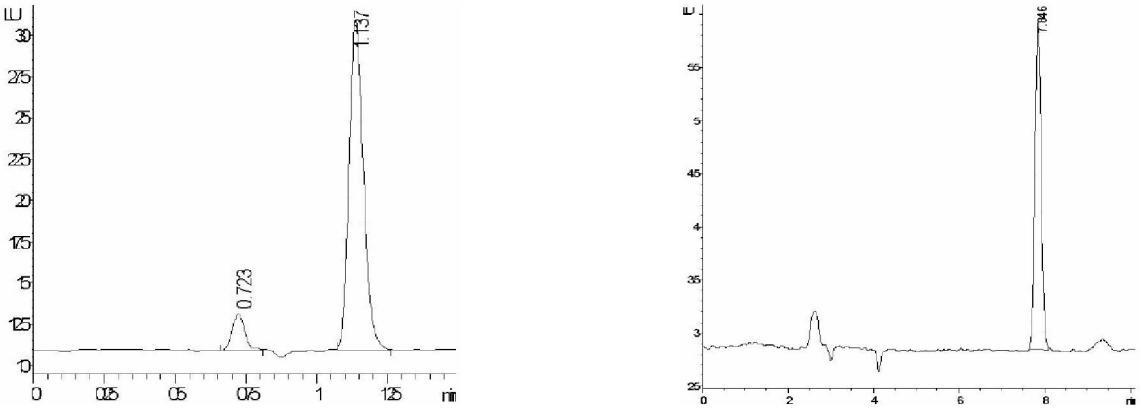


Figure 5 : HPLC chromatogram of standard ibuprofen using monolithic and conventional C_{18} column.

TABLE 7 : Application of the proposed HPLC method on monolithic and conventional C_{18} column.

Parameter	Monolithic column	Conventional column
Mean	100.06	99.01
% R.S.D	1.225	0.652

Analysis of ibuprofen in Brufen® tablets

The application of the method for the determination of Ibuprofen in Brufen® tablets. The assay results are listed in TABLE 7. The contents of Ibuprofen were all within the recommended limits.

CONCLUSIONS

An HPLC method for determination of Ibuprofen

in presence of its degradation products was developed and validated on a monolithic C_{18} and conventional C_{18} column. The analysis was much shorter while the separation efficiency remained equivalent to that on a conventional C18 particle-packed column also monolithic columns were found to perform the separation with sufficient resolution and better peak symmetry as compared to the conventional column. When higher flow rates were applied on monolithic column, there was some minor slow loss in resolution.

The separation efficiency of monolithic column was found to decrease slowly when the flow rate was increased, in contrast to traditional particulate column. This could be explained by improved mass transfer of monolithic over conventional column at high flow rates.

The method is applicable for rapid quantitation of Ibuprofen and its degradation products by applying QbD and DOE concepts. Furthermore; the method could be useful for stability testing for Ibuprofen formulations. A clear advantage of the monolithic column is the ability of using high flow rates regardless of back pressure. Monolithic columns have been shown as an excellent alternative to conventional silica based columns.

REFERENCES

[1] N.Tanaka, H.Kobayashi; Anal.Bioanal.Chem., **376**, 298-301 **(2003)**.

[2] N.Wu, J.Dempsey, P.M.Yehl, A.Dovletoglou, D.Ellison, J.Wyvratt; Anal.Chimica Acta, **523(2)**, 149-156 **(2004)**.

[3] L.Nováková, L.Matysová, D.Solichová, M.A.Koupparis, P.Solich; J.Chromatogr.B, **813(1-2)**, 191-197 **(2004)**.

[4] W.Markus, D.Armin, K.Stephan; J.Chromatogr.B, **832(2)**, 313-316 **(2006)**.

[5] S.El Deeb, H.Wätzig; Turk J.Chem., **30**, 543-552 **(2006)**.

[6] A.Zarghi, A.Shafaati, S.M.Foroutan, H.Movahed; Sci.Pharm., **76**, 439-450 **(2008)**.

[7] J.Liu, J.Sun, W.Zhang, K.Gao, Z.He; J.Pharm. Biomed.Anal., **46**, 405-409 **(2008)**.

[8] H.Y.Aboul Enein, M.M.Hefnawy; Anal.Letters, **36(11)**, 2527-2538 **(2003)**.

[9] M.Kiendrebeogo, L.Choisnard, C.E.Lamien, A.Meda, D.Wouessidjewe, O.G.Nacoulma; Afr.J. Trad., **3(1)**, 115-128 **(2006)**.

[10] N.Sultana, M.Saeed Arayne, N.Shafi; Pak.J.Pharm. Sci., **20(4)**, 279-284 **(2007)**.

[11] R.Petkovska, C.Cornett, A.Dimitrovska; Maced.J. Chem.Eng., **27(1)**, 53-64 **(2008)**.

[12] W.R.G.Baeyens, G.Van der Weken, E.Smet, A.M.García-Campãna, J.P.Remon; J.Pharm. Biomed.Anal., **32**, 913-920 **(2003)**.

[13] C.S.Sean, Martindale; 'The Complete Drug Reference', 33rd Edition, The Pharmaceutical Press, London, **(2006)**.

[14] R.Canaparo, E.Muntoni, G.P.Zara, C.D.Pepa, E.Berno, M.Costa, M.Eandi; Biomed.Chromatogr., **14**, 219-226 **(2000)**.

[15] R.S.Raghunadha, I.S.Chandiran, K.N.Jayaveera, R.D.Kotesare; Arch.Apll.Sci.Res., **2**, 101-111 **(2010)**.

[16] S.R.Matkovic, G.M.Valle, L.E.Briand; Latin Am. App.Research, **35**, 189-195 **(2005)**.

[17] R.Kirtawade, C.Seervi, P.Salve, K.Patil, A.Kulkarni, P.Dhabale; Arch.Pharm.Sci&Res., **2**, 271-273 **(2010)**.

[18] S.H.Wafaa; Am.J.Applied Sci., **5**, 1005-1012 **(2005)**.