Original Article

Development and validation of reverse phase-high performance liquid chromatographic method for simultaneous estimation of naproxen sodium and esomeprazole magnesium trihydrate

Abstract

Background: Naproxen sodium (NAP) is a non steroidal anti-inflammatory drug and Esomeprazole magnesium trihydrate (ESO) is a proton pump inhibitor. **Aim:** A high performance liquid chromatographic method was developed and validated for the quantitative determination of naproxen (NAP) sodium and esomeprazole (ESO) magnesium trihydrate. The different analytical parameters such as linearity, precision, accuracy, limit of detection and limit of quantification were determined according to the International conference harmonization (ICH) Q2B guidelines. **Materials:** Chromatography was carried out by isocratic technique on a reversed phase C18 base deactivated silanol hypersil column with mobile phase and optimized depending upon the polarity of the molecules. **Results:** The calibration curves were linear (r2 > 0.9997) over the concentrations 20-120 µg/mL for NAP sodium and 0.8-4.8 µg/mL for ESO magnesium trihydrate. The method was accurate and precise with recoveries in the range of 99.48-99.98% for the two drugs and relative standard deviation less than 2%. No chromatographic interferences from the tablet excipients were found. **Conclusion:** The proposed method was highly sensitive, precise and accurate. Hence the method was successfully applied for the reliable quantification of active pharmaceutical ingredients content in house prepared tablet formulation of NAP sodium and EOS magnesium trihydrate.

Key words:
Esomeprazole magnesium trihydrate, naproxen sodium, reverse phase-high performance liquid chromatography, validation, % relative standard deviation

Introduction

Naproxen (NAP) sodium [Figure 1a] is a non steroidal anti-inflammatory drug, whose action has been inhibition of cyclooxygenase (COX-1). It is thought to be associated with gastrointestinal and renal toxicity while inhibition of COX-2 provides anti-inflammatory activity.[1] Esomeprazole (ESO) magnesium trihydrate [Figure 1b] is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition on the H^+/K^+-ATPase in the gastric parietal cell.

The S and R isomers of omeprazole are protonated and converted in the acidic compartment of the parietal cell forming the active inhibitor, the achiral sulphonamide. By acting specifically on the proton pump, ESO blocks the final step in production, thus reducing gastric activity. NAP sodium is chemically known as 2-naphthalene acetic acid, 6-methoxy-α-methyl-sodium salt and ESO magnesium trihydrate is chemically known as 5-methoxy-2 –([S]-[(4 methoxy-3, 5-dimethyl-2-pyridyl)] methyl) sulfinyl benzimidazole, magnesium salt trihydrate.[2]

A literature survey reveals that there are a number of various analytical methods available for the quantitative individual determination of NAP sodium or combination with other drugs mainly using chromatographic methods such as liquid chromatography with different columns.[3-9] Although several high performance liquid chromatographic (HPLC) methods have been published for this combination of drugs[10-12], a trial

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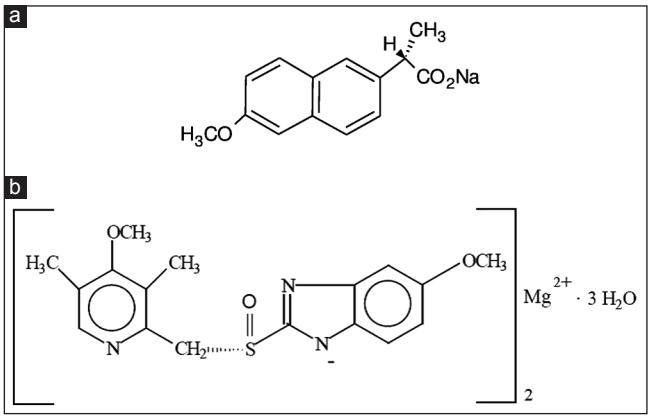

Figure 1: (a) Structure of naproxen sodium, (b) structure of esomeprazole magnesium

has been made to evaluate the same combination which could be a better method with less retention time, less percentage degradation and a cost effective mobile phase. Hence an attempt has been made to develop a simple, efficient and selective method for the determination of NAP and ESO. In this study, HPLC instrumentation with ultra violet (UV) detection which is readily available in most analytical and pharmaceutical laboratories was used.

Experimental

Chemicals

The bulk drugs of NAP and ESO were obtained as gift samples from Chandra labs, Hyderabad. HPLC grade water and Acetonitrile were obtained from Rankem. Analytical grade potassium dihydrogen phosphate and dipotassium hydrogen phosphate were obtained from Fluka laboratories, Mumbai. Excipients such as polydextrose, propylene glycol, magnesium stearate, polysorbate 80 and starch were obtained from Ankit enterprises, Mumbai.

Instrumentation and analytical conditions

Chromatography was performed by using a Shimadzu liquid chromatographic HPLC system equipped with reciprocating plunger pump LC-20, manual injector and UV-detector. Data acquisition and processing were performed using spinchrom software. Separation was achieved on C_{18} column, base deactivated silanol Hypersil with a length of 250 mm, 4.6 inner diameter and pore size of 5 µ. The elution was isocratic with mobile phase of mixed phosphate buffer adjusted to pH 6.8 with ortho phosphoric acid and acetonitrile (55:45 v/v). The flow rate was 1.0 mL/min. Column temperature was maintained at ambient; the detection was monitored at a wavelength of 236 nm and injection volume was 20 µL.

Standard solution and calibration graphs for chromatographic measurements

Standard stock solution was prepared by dissolving 50 mg of NAP and 2 mg of ESO in the mobile phase to obtain concentration of 1000 µg/mL and 40 µg/mL respectively. Further dilutions with the mobile phase were done to obtain the working standard concentration in the range of 20 µg/mL to 120 µg/mL and 0.8 µg/mL to 4.8 µg/mL respectively. Quality control samples were prepared in the range of calibration curves at different concentrations (70, 90, 110 µg/mL NAP and 2.8, 3.6, 4.4 µg/mL for ESO) following the same procedure as for the calibration standard using a primary stock. The samples were analyzed with the reagent blank. All the solutions were prepared in triplicate.

Method Validation

The method was validated according to the international conference harmonization (ICH) Q2B guidelines.[13] The following validation characteristics were addressed for linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Linearity and range

Standard calibration curves were prepared with six calibrators over a concentration range of 20 µg/mL to 120 µg/mL for NAP and 0.8 µg/mL to 4.8 µg/mL for ESO. The data of peak areas versus concentrations were treated by linear least square regression analysis.

Accuracy

To study the reliability and suitability of the developed method, recovery experiments were carried out. Accuracy is the percent of analysis recovered by among from a known added amount. Data from the nine determinations over time (70, 90, 110 µg/mL NAP and 2.8, 3.6, 4.4, µg/mL for ESO) concentration levels covering the specified ranges were determined.

Precision

Precision is the degree of repeatability of an analytical method under normal conditions. Repeatability and method precision were done to the precision of the method. Aliquots of standard stock solutions of NAP and ESO (1 mL of 1000 µg/mL for NAP and 1 mL of 40 µg/mL for ESO) were transferred into a 10 mL standard flask and made up to the mark with mobile phase (100 µg/mL for NAP and 4.0 µg/mL for ESO). 20 µL of the solution was injected and the chromatograms were recorded. The procedures were repeated for 5 times. The peak areas were measured and the % relative standard deviation was calculated.

LOD and LOQ

The LOD is defined as the lowest concentrations of an analytical process can reliably differentiate from background levels. The LOQ is defined as the lowest concentrations of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as

$$LOD = \frac{3.3\sigma}{s} \text{ and } LOQ = \frac{10\sigma}{s}$$

Where σ — is the standard deviation of the lowest standard concentration and 's' is the slope of the standard curve.

Specificity

Forced degradation studies were performed to provide an indication of the stability indicating properties and specificity of the method. The degradation samples were prepared by transferring from standard stock (1 mL of 1000 μg/mL for NAP and 1 mL of 40 μg/mL for ESO) into 10 mL volumetric flasks. International degradation was attempted using heat, acid and base. After the degradation treatments were completed, the samples were allowed to equilibrate to room temperature and prepared according to precision preparation. The samples were analyzed against a freshly prepared control sample (with no degradation treatment). Degradation peaks were observed, were resolved from the active peak and did not reveal any degradation products coeluting with the active peak.

Robustness

As a part of robustness deliberate change in flow rate (0.9 mL/min and 1.1 mL/min) and wavelength (234 nm and 238 nm) were made to elevate the impact of the method.

Formulation and standard test

At the time of my doing this research work, the tablet formulations of NAP and ESO combination were not available in Indian market and it was only available in USA market. Hence 40 tablets of NAP and ESO combination were prepared in the laboratory with the help of excipients by direct compression method.

Direct compression method

Required quantity of polysorbate 80 and propylene glycol were weighed and triturated. Then weighed quantity of drugs were added and mixed well. Polydextrose was added and then the required quantities of starch and magnesium stearate were mixed. The powder was punched by using single punching machine.

Extraction of active ingredients

The tablets were accurately weighed and powdered. The amount of the drug in weighed quantity of powder was calculated based on the label claim and the active ingredients were extracted in the mobile phase. The solution was sonicated for 20 min and filtered through Whatmann No: 41 filter paper. Appropriate dilutions were made and the samples were subjected for HPLC analysis.

Results and Discussion

Method development

Method development focuses on identifying buffer type, strength and pH organic solvent and implementing small changes to optimize selectivity and enhance resolution. Initially, the efficiency was found to be 636 and 1718 for NAP sodium and ESO magnesium trihydrate by using the stationary phase with YMC C_{18} short column with mobile phase containing water: Acetonitrile in the ratio of 50:50 v/v with flow rate 1.0 mL/min. To increase the efficiency for NAP and ESO by using stationary phase YMC C_{18} short column with mobile phase containing mixed phosphate buffer adjusted to pH 6.8 and acetonitrile in the ratio of 55:45 v/v with flow rate of 1.0 mL/min but the resolution was found to be 1.790 min. Then tried with YMC C_{18} short column with mobile phase containing ammonium phosphate buffer and acetonitrile in the ratio of 60:40 v/v with flow rate of 1.5 mL/min but the efficiency was found to be 674 and 823 for NAP and ESO respectively. At this stage the stationary phase BDS Hypersil C_{18} long column and with mobile phase containing mixed phosphate buffer adjusted to pH 6.8 and acetonitrile in the ratio of 70:30 v/v with flow rate of 1.5 mL/min. The retention time of NAP and ESO were found to be 7.907 min. and 14.030 min. respectively. To reduce the retention time by using the same stationary phase and mobile phase containing water: Methanol 50:50 v/v with flow rate of 1.5 mL/min. The retention time of NAP was found to be 2.593 min. However, the retention time of ESO was found to be 14.803 min. Again to reduce the retention time of ESO by using the same stationary phase with mobile containing mixed phosphate buffer adjusted to pH 6.8 and Acetonitrile in the ratio of 55:45 v/v with flow rate of 1.0 mL/min. The retention time was found to be 3.137 min. and 4.340 min. for NAP and ESO respectively.

Using the above chromatographic conditions resulted in the development of an efficient and reproducible method for the quantitative determination of NAP and ESO in bulk and tablet dosage form. *In-vitro* quality control tests such as friability, uniformity of weight, hardness, dissolution, disintegration and assay were performed. Quality control tests for the tablets results are shown in Table 1. The optimized chromatogram is shown in Figure 2 and the method development trials results are shown in Table 2.

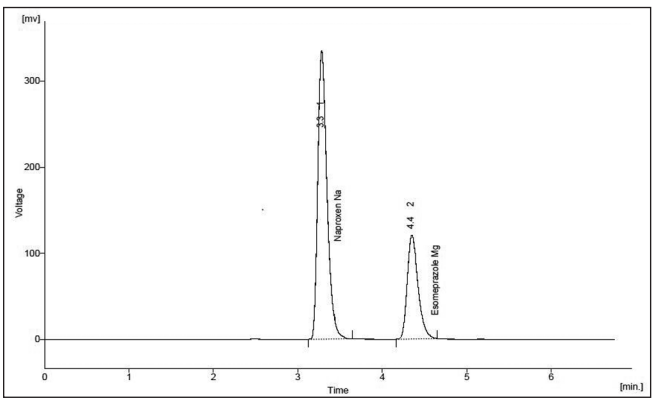

Figure 2: Optimized chromatogram

Method validation

When a method has been developed, it must be validated before practical use. By following the ICH guidelines for analytical method validation Q_2 (R_1), the system suitability test was performed and the validation characteristics were addressed.

System suitability

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. System suitability parameters such as retention time, tailing factor, efficiency, capacity factor and resolution were performed. The results were found to be within the United States Pharmacopeia[14] limits and the reports of analysis are shown in Table 3.

Linearity and range

For the construction of calibration curves, six calibration standard solutions were prepared over the concentration range from 20 µg/mL to 120 µg/mL for NAP and 0.8 µg/mL to 4.8 µg/mL for ESO. The results are summarized in Table 4, shows a good correlation between analytes peak area and concentration with r^2 > 0.9997 (n = 6). The calibration curves are shown in Figure 3a and b.

Accuracy

The accuracy of the method was confirmed by the recovery studies. To the pre analyzed formulation a different concentrations (70, 90, 110 µg/mL for NAP and 2.8, 3.6, 4.4 µg/mL for ESO) of the raw material were added and the amount of drug recovered was calculated. The percentage recovery was found to be 99.94, 99.98, 99.97% for NAP and 99.51, 99.53, 99.48 % for ESO respectively. The data is shown in Table 5.

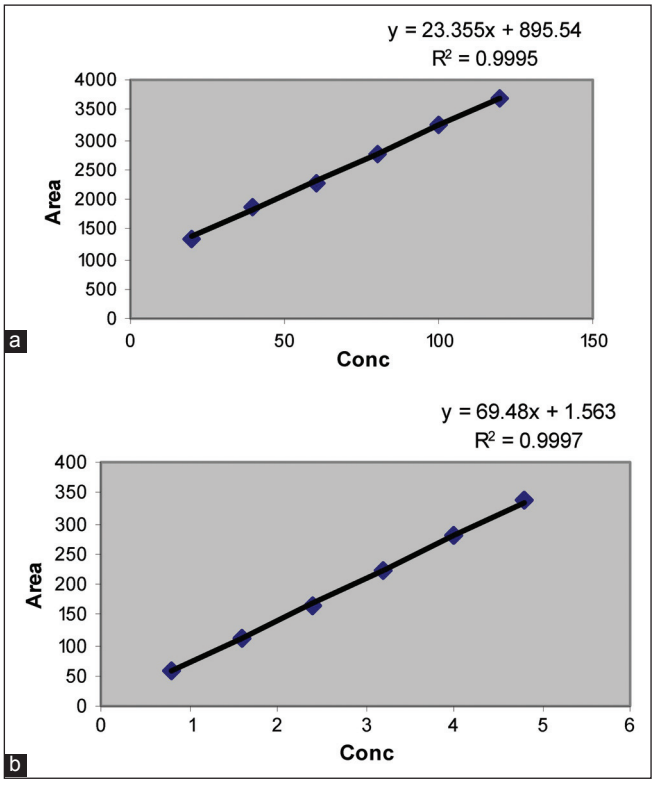

Figure 3: (a) Linearity curve of naproxen sodium, (b) linearity curve of esomeprazole magnesium

Table 1: Quality control tests for formulation

Quality control tests	Result	Limit (%)
Uniformity of weight	Upper = +0.647 Lower = −0.0048	NMT 5
Hardness test	3.3	NMT5
Friability test	0.809	NMT 1
Disintegration test	1.80	NMT 15
Dissolution test	92	80-110
Assay for NAP and ESO	100.78 99.69	99-101

ESO – Esomeprazole; NAP – Naproxen; NMT – Not more than

Table 2: Method development trails

Trail no.	Mobile phase ratio	Flow rate (mL/min)	Column	Results
1	Water: Acetonitrile (50:50)	1 mL/min	YMC C_{18}	Efficiency is less
2	Mixed phosphate buffer: Acetonitrile (55:45)	1 mL/min	YMC C_{18}	Resolution is less
3	Ammonium phosphate buffer: Acetonitrile (60:40)	1.5 mL/min	YMC C_{18}	Efficiency is less
4	Mixed phosphate buffer: Acetonitrile (70:30)	1.5 mL/min	BDS hypersil C_{18}	Retention time is more
5	Water: Methanol (50:50)	1.5 mL/min	BDS hypersil C_{18}	Retention time is more
6	Mixed phosphate buffer: Acetonitrile (55:45)	1 mL/min	BDS hypersil C_{18}	Optimized chromatogram

BDS – Base deactivated silanol

Table 3: System suitability parameters

Parameters	NAP	ESO
Retention time	3.137	4.340
Tailing factor	1.571	1.370
Efficiency	2653	7666
Capacity factor	0.383	0.467
Resolution	—	5.447
% RSD	0.25	0.11

NAP – Naproxen; ESO – Esomeprazole; RSD – Relative standard deviation

Table 4: Linearity

Parameters	NAP	ESO
Concentration range	20-120 µg/mL	0.8-4.8 µg/mL
R^2	0.9995	0.9997
Regression equation	Y = 23.30X + 895.54	Y = 69.48X + 1.563
Slope	23.35	69.48
Intercept	895.54	1.563
LOD	0.445	0.207
LOQ	1.362	0.633

LOD – Limit of detection; LOQ – Limit of quantification; NAP – Naproxen; ESO – Esomeprazole

Precision and intermediate precision

The standard NAP and ESO solution of 100 µg/mL and 4 µg/mL were selected for analysis. The % RSD values for precision were found to be 0.25 and 0.11 for NAP and ESO respectively. The % RSD[15] values for intermediate precision were found to be 0.76 and 0.21 for NAP and ESO respectively. The low % RSD values (not more than 2%) showed that the proposed method was precise. The results are shown in Table 6a and b.

Robustness

Robustness was performed by changing the flow rate and by changing the wavelength. It showed that there was no change in the values even after making deliberate changes in the analytical procedure. The results are shown in the Table 7.

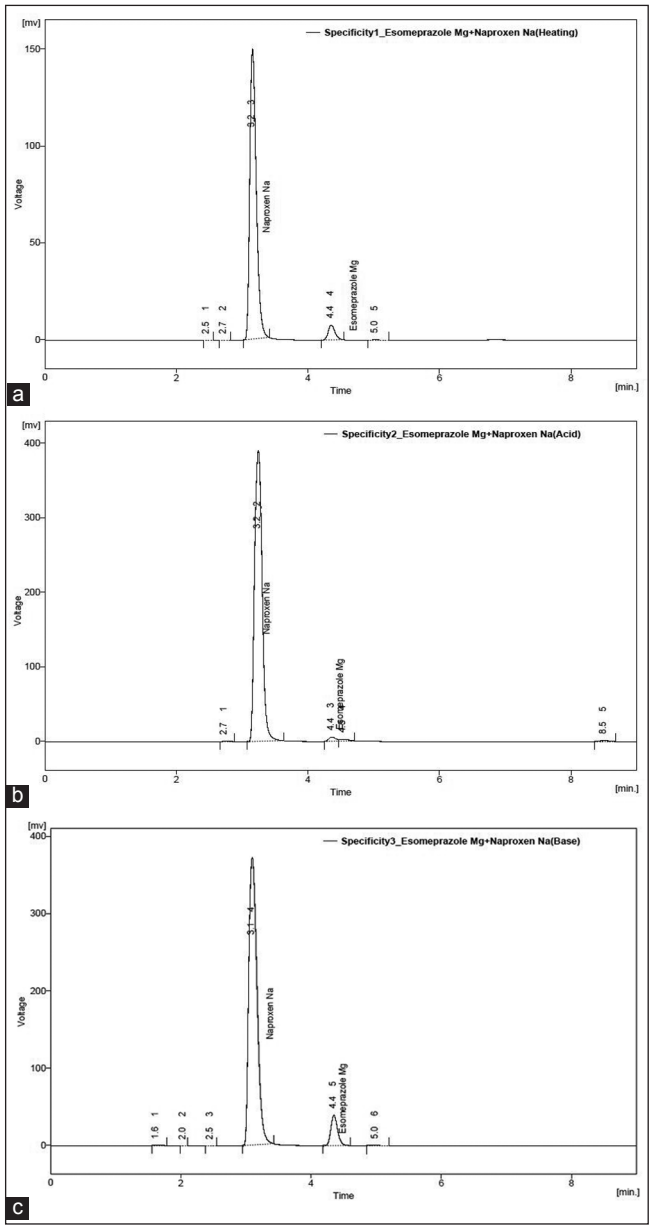

Figure 4: (a) Chromatogram for specificity (heating), (b) chromatogram for specificity (acid), (c) chromatogram for specificity (base)

Specificity

Specificity was performed by treating the standard (NAP and ESO) with acid, base and heat. 20 µl of each treated standard was injected into the chromatographic system and recorded the degradation peaks. Degradation product should not interfere with the analytes and there was no change in the retention time. The % degradation was calculated by using peak area. It was found to be within the limit (Not more than 10%). Hence the method was found to be specific. The results were shown in Table 8. The chromatograms are shown in Figure 4 a-c.

Table 5: Accuracy

Drugs	Concentration µg/ml	Average peak area	Recovery (%)	SD	RSD (%)
NAP	90	3282.24	99.94	0.0208	0.6933
	110	3609.86	99.98		
	130	4265.60	99.97		
ESO	3.6	256.84	99.51	0.0251	0.8366
	4.4	313.96	99.53		
	5.2	370.85	99.48		

NAP – Naproxen; ESO – Esomeprazole; RSD – Relative standard deviation; SD – Standard deviation

Table 6a: Precision

S. no	Naproxen sodium		Esomeprazole magnesium	
	Rt	Peak area	Rt	Peak area
1	3.137	3261.28	4.34	285.68
2	3.137	3261.12	4.34	285.74
3	3.12	3266.70	4.34	287.93
4	3.127	3287.75	4.35	288.63
5	3.137	3268.62	4.33	289.73
Average	3.1316	3269.1	4.34	287.54
SD	0.0077	10.938	0.0049	1.7921
% RSD	0.25	0.33	0.11	0.62

SD – Standard deviation; RSD – Relative standard deviation

Table 6b: Intermediate precision

S. no	Naproxen sodium		Esomeprazole magnesium	
	Rt	Peak area	Rt	Peak area
1	3.13	3280.59	4.367	280.60
2	3.13	3281.61	4.347	286.96
3	3.143	3283.82	4.357	286.37
4	3.177	3281.40	4.367	283.40
5	3.177	3283.70	4.367	284.10
Average	3.1514	3282.22	4.361	284.29
SD	0.0239	1.45424	0.0089	2.5455
% RSD	0.76	0.04	0.21	0.90

SD – Standard deviation; RSD – Relative standard deviation

Table 7: Robustness

Parameter	Retention time for NAP	Retention time for ESO
Flow rate		
0.9 mL/min	3.472	4.820
1.1 mL/min	2.867	3.967
Wavelength		
234 nm	3.140	4.347
238 nm	3.123	4.347

NAP – Naproxen; ESO – Esomeprazole

Table 8: Specificity

Drugs	Stress conditions	Degradation (%)
NAP	Acid	0.167
	Base	0.086
	Heat	0.009
ESO	Acid	2.438
	Base	2.640
	Heat	6.502

NAP – Naproxen; ESO – Esomeprazole

Conclusion

The HPLC method developed for NAP and ESO shows good precision and accuracy. The low % RSD values in the recovery studies for the method shows that there are no interferences due to excipients and for formulation. Hence it is concluded that the developed method is simple, precise and rapid for the analysis of combination of NAP and ESO in pure and in inhouse prepared tablet dosage form. Hence the developed method can be adopted for the routine analysis of combination of NAP and ESO in pure and in house prepared tablet dosage form.

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