Research Article

SIMULTANEOUS EQUATION METHOD AND Q-ABSORPTION RATIO METHODS DEVELOPMENT AND VALIDATION OF KETOTIFEN FUMARATE AND THEOPHYLLINE IN BULK AND DOSAGE FORM

Chaudhari Narendra Kesharbhai*

Kalol Institute of Pharmacy, Kalol, Sardarpur (chikana), Mehsana, Gujarat, India

*Corresponding Author Email: nkchaudhari62@yahoo.com

Article Received on: 27/03/14 Revised on: 24/04/14 Approved for publication: 02/05/14

DOI: 10.7897/2230-8407.050586**ABSTRACT**

A simple, accurate and precise UV- Spectroscopy method for the simultaneous estimation of Ketotifen fumarate and theophylline in Bulk and Dosage form has been developed and validated. Absorbance of ketotifen fumarate and theophylline measured at 296 nm and 271 nm for simultaneous equation method and 281 nm and 271 nm for the Q-absorption ratio method respectively. The drug response with respect to absorbance was linear over the concentration range 2-16 µg/ml (n = 5) for ketotifen fumarate and 4-32 µg/ml for theophylline. The percentage recovery of ketotifen fumarate and theophylline was found to be 99.65-100.95 % and 99.68-100.47 % respectively for simultaneous equation method and 99.07-100.55 % and 99.98-100.65 % respectively for Q-absorption ratio method. The %RSD values for intra-day precision study and inter-day study were ≤ 2.0 %, confirming that the method was sufficiently precise. The system stability test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the simultaneous determination of Ketotifen fumarate and Theophylline in pharmaceutical formulation.

Keyword: Ketotifen fumarate, Theophylline, Simultaneous equation method, Q-absorption ratio method**INTRODUCTION**

Ketotifen is a second-generation noncompetitive H₁-antihistamine and mast cell stabilizer. It is most commonly sold as a salt of fumaric acid, ketotifen fumarate, and is available in two forms.¹⁻³ In its ophthalmic form, it is used to treat allergic conjunctivitis, or the itchy red eyes caused by allergies. In its oral form, it is used to prevent asthma attacks. Ketotifen relieves and prevents eye itchiness and/or irritation associated with most seasonal allergies. Besides its anti-histaminic activity, it is also a functional leukotriene antagonist and a phosphodiesterase inhibitor.⁴⁻⁶ Theophylline, also known as 1, 3-dimethylxanthine, is a methyl xanthine drug used in therapy for respiratory diseases such as COPD and asthma under a variety of brand names. As a member of the xanthine family, it bears structural and pharmacological similarity to caffeine.^{7,8} Theophylline is naturally found in cocoa beans; amounts as high as 3.7 mg/g have been reported in Criollo cocoa beans. Trace amounts of theophylline are also found in brewed tea, although brewed tea provides only about 1 mg/L, which is significantly less than a therapeutic dose.⁹⁻¹¹

MATERIAL AND METHOD

Standard Ketotifen Fumarate (Gifted by Intas Laboratories Pvt. Ltd., Ahmedabad, India) and Standard Theophylline (Gifted by Lincoln Pharmaceuticals Ltd., India) marketed formulation AZOFEN-T manufactured by Lark Laboratories (India) Ltd., Delhi, India; purchased by Dawaicartonline pharmacy, Orissa, India.

Ketotifen fumarate Standard stock solution (1000 µg/ml)

100 mg of ketotifen fumarate was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol and sonicated for about 10 minutes. Volume was made up to the mark with methanol to give a solution containing 1000 µg/ml ketotifen fumarate.

Ketotifen fumarate Working Standard solution (100 µg/ml)

From standard stock solution, take 10 ml solution and transferred to 100 ml volumetric flask. Volume was made up to the mark with methanol to give a solution containing 100 µg/ml ketotifen fumarate.

Theophylline Standard stock solution (2000 µg/ml)

200 mg of theophylline was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in methanol and sonicated for about 10 minutes. Volume was made up to the mark with methanol to give a solution containing 2000 µg/ml Theophylline.

Theophylline Working Standard solution (200 µg/ml)

From standard stock solution, take 10 ml solution and transferred to 100 ml volumetric flask. Volume was made up to the mark with methanol to give a solution containing 200 µg/ml theophylline.

Determination of wavelength for measurement for proposed methods

To determine wavelength for measurement, standard spectra of ketotifen fumarate and theophylline were scanned between 200-400 nm against methanol as blank. Absorbance maxima were obtained at 296.0 nm and at 271.0 nm for ketotifen fumarate and theophylline respectively and Iso- absorptive point was obtained at 281 nm. Overlain spectra of ketotifen fumarate and theophylline are presented in Figure.

Method A: Simultaneous Equation Method**Calibration curve for ketotifen fumarate and theophylline**

The solutions of ketotifen fumarate and theophylline ranging from 2-16 µg/ml and 4-32 µg/ml respectively were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 ml of working standard solution of ketotifen fumarate (100 µg/ml) and theophylline (200 µg/ml) into series of 10 ml volumetric

flasks and the volume was adjusted to mark with methanol separately. The absorbance of the solutions was measured at 296 nm for ketotifen fumarate and 271 nm for theophylline against methanol as blank. Calibration curve was plotted at both wavelengths and two equations were formed using the specific absorbance (absorptivity).¹²⁻¹⁵

$$C_x = (A_2 a_{Y1} - A_1 a_{Y2}) / (a_{Y1} a_{X2} - a_{Y2} a_{X1}) \text{ ----- (1)}$$

$$C_y = (A_1 a_{X2} - A_2 a_{X1}) / (a_{Y1} a_{X2} - a_{Y2} a_{X1}) \text{ ----- (2)}$$

Where, A_2 = Absorbance of Mixture at 296 nm

A_1 = Absorbance of Mixture at 271 nm

a_{X1} = absorptivity of ketotifen fumarate at 271 nm (241.05),

a_{Y1} = absorptivity of theophylline at 271 nm (534.00),

a_{X2} = absorptivity of ketotifen fumarate at 296 nm (438.54),

a_{Y2} = absorptivity of theophylline at 296 nm (27.09),

C_x is Concentration of ketotifen fumarate,

C_y is Concentration of theophylline.

Method B: Q-absorption Ratio Method

Calibration curve for ketotifen fumarate and theophylline

The solutions of ketotifen fumarate and theophylline ranging from 2-16 µg/ml and 4-32 µg/ml respectively were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 ml of working standard solution of ketotifen fumarate (100 µg/ml) and theophylline (200 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol separately. The absorbance of the solutions was measured at 296 nm for ketotifen fumarate, 271 nm for theophylline and 281 nm for ketotifen fumarate and theophylline both against methanol as blank. Calibration curve was plotted at both wavelengths and two equations were formed using the specific absorbance (absorptivity).¹⁴⁻¹⁶

$$C_x = [(Q_m - Q_y) / (Q_x - Q_y)] \cdot A_1 / a_{X1} \text{ ----- (3)}$$

$$C_y = [(Q_m - Q_x) / (Q_y - Q_x)] \cdot A_1 / a_{Y1} \text{ ----- (4)}$$

Where, $Q_m = A_2 / A_1$

$Q_X = a_{X2} / a_{X1}$, $Q_Y = a_{Y2} / a_{Y1}$

A_2 = Absorbance of Mixture at 271 nm

A_1 = Absorbance of Mixture at 281 nm

a_{X1} = absorptivity of ketotifen fumarate at 281 nm (336.99)

a_{Y1} = absorptivity of theophylline at 281 nm (359.26)

a_{X2} = absorptivity of ketotifen fumarate at 271 nm (241.05)

a_{Y2} = absorptivity of theophylline at 271 nm (534.00)

Analysis of ketotifen fumarate and theophylline in Tablet Dosage Form

Twenty tablets were weighed, their mean weight determined and finally powdered. An accurately weighed tablet powder equivalent to 200 mg of theophylline and 1 mg ketotifen fumarate were used for analysis by the proposed method which follows a ratio of 200 mg: 1 mg. Then transferred to 100 ml volumetric flask containing 50 ml methanol and sonicate for 20 minutes. The solution was filtered through 0.45 µm filter (Millifilter, MA) and the volume was adjusted up to mark with methanol. From the above solution 0.1 ml was taken into a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of ketotifen fumarate (0.1 µg/ml) and theophylline (20 µg/ml). Absorbance of this solution was measured and the analysis was repeated for three times. Estimation of ketotifen fumarate and theophylline concentration was done by simultaneous equation method and Q-Absorption ratio method.

Analysis of ketotifen fumarate and theophylline in Laboratory Mixture

ketotifen fumarate 10 mg + theophylline 20 mg were accurately weighed and dissolved in 100 ml volumetric flask in sufficient quantity of methanol, sonicate and dilute up to 100 ml with the same solvent so as to get the concentration of 100 µg/ml ketotifen fumarate and 200 µg/ml theophylline. Further take 1 ml of solution from the stock solution in to 10 ml volumetric flask and dilute up to mark with methanol. So finally get the concentration of 10 µg/ml ketotifen fumarate and 20 µg/ml theophylline. Estimation of ketotifen fumarate and theophylline concentration was done by simultaneous equation method and Q-Absorption ratio method.

Method Validation

Linearity

Linearity responses for ketotifen fumarate and theophylline were assessed in the concentration range 2-16 µg/ml and 4-32 µg/ml of standard solutions, respectively. Linearity spectra and calibration curves of ketotifen fumarate and theophylline at 296 nm and 271 nm for Simultaneous equation method and at 271 nm and 281 nm for Q-absorption ratio method are shown in Figure. Calibration curve data of ketotifen fumarate and theophylline shown in Table

Accuracy

To the pre-analysed sample a known amount of standard solution of pure drug (ketotifen fumarate and theophylline) was spiked at three different levels. These solutions were subjected to re- analysis by the proposed method. The accuracy of both the methods was determined by calculating recovery of ketotifen fumarate and theophylline by the standard addition method. The mean recoveries were 99.65-100.95 and 99.68-100.47 in Simultaneous equation method and 99.07-100.55 and 99.98-100.65 in Q-Absorption ratio method for ketotifen fumarate and theophylline, respectively. The low value of standard deviation indicates that the proposed methods are accurate. Results of recovery studies are shown in Table.

Precision

Method precision (Repeatability)

Repeatability of sample application was assessed by analyzing 8 µg/ml ketotifen fumarate and 16 µg/ml theophylline drug solutions six times. The absorbance of the each solution was measured at selected wavelengths and % R.S.D was calculated. The % RSD for absorbance values of ketotifen fumarate and theophylline for Simultaneous equation method and Q-absorption ratio method are given in Table.

Intermediate precision (System precision)

Intermediate precision of the method was determined in the terms of intra-day and inter-day variation (% RSD). Intra-day precision (% RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (% RSD) was assessed by analyzing drug solutions within the calibration range on three different days. The intra-day and inter-day precisions were determined and results of both methods which are given in Table.

Sensitivity

The sensitivity of measurement of ketotifen fumarate and theophylline by the use of proposed method was estimated in

terms of Limit of Detection (LOD) and Limit of Quantitation

Figure 1: Overlay absorption spectra of ketotifen fumarate (296 nm) and theophylline (271 nm) showing Iso-absorptive point (281 nm) in methanol

Figure 2: Calibration curve of ketotifen fumarate in Simultaneous equation method and Q-Absorbance ratio method

Figure 3: Calibration curve of theophylline in Simultaneous equation method and Q-Absorbance ratio method

Table 1: Calibration curve data of ketotifen fumarate in Simultaneous equation method and Q-Absorbance ratio method

Conc. (µg/ml)	Simultaneous equation method				Q-Absorption ratio method			
	Mean of Absorption		%RSD		Mean of Absorption		%RSD	
	296 nm	271 nm	296 nm	271 nm	281 nm	271 nm	281 nm	271 nm
2	0.0855	0.0481	0.845	0.945	0.0685	0.0481	0.854	0.945
4	0.1623	0.0979	0.786	0.738	0.1262	0.0979	0.648	0.738
6	0.2605	0.1408	0.452	0.646	0.2045	0.1408	0.745	0.646
8	0.3501	0.1849	0.567	0.765	0.2604	0.1849	0.345	0.765
10	0.4265	0.2302	0.212	0.515	0.3415	0.2302	0.524	0.515
12	0.5275	0.2839	0.345	0.375	0.4041	0.2839	0.476	0.375
14	0.6105	0.3221	0.456	0.296	0.4885	0.3221	0.625	0.296
16	0.7102	0.3829	0.325	0.625	0.5511	0.3829	0.385	0.625

Table 2: Calibration curve data of theophylline in Simultaneous equation method and Q-Absorbance ratio method

Conc. (µg/ml)	Simultaneous equation method				Q-Absorption ratio method			
	Mean of Absorption		%RSD		Mean of Absorption		%RSD	
	296 nm	271 nm	296 nm	271 nm	281 nm	271 nm	281 nm	271 nm
4	0.0111	0.2137	0.653	0.875	0.1452	0.2137	0.784	0.875
8	0.0219	0.4276	0.855	0.798	0.2808	0.4276	0.485	0.798
12	0.0293	0.6403	0.729	0.546	0.4351	0.6403	0.759	0.546
16	0.0437	0.8539	0.549	0.741	0.5718	0.8539	0.415	0.741
20	0.0548	1.0683	0.652	0.615	0.7044	1.0683	0.245	0.615
24	0.0661	1.2812	0.365	0.413	0.8697	1.2812	0.746	0.413
28	0.0768	1.4947	0.726	0.478	1.0154	1.4947	0.255	0.478
32	0.0881	1.7093	0.435	0.592	1.1612	1.7093	0.818	0.592

Table 3: Assay Result of Marketed Formulation

Parameters	AZOFEN-T (1:200 mg)			
	Simultaneous equation method		Q-Absorption ratio method	
	ketotifen fumarate	theophylline	ketotifen fumarate	theophylline
Actual Conc. (µg/ml)	0.1	20	0.1	20
Conc. Obtained (µg/ml)	0.0993 ± 0.0005	19.8663 ± 0.0338	0.0997 ± 0.0001	19.8712 ± 0.0205
% Assay*	99.3667	99.3318	99.7067	99.3563
% RSD	0.5164	0.1702	0.1391	0.1032

*Denotes average of three determinations

Table 4: Assay Result of Laboratory Mixture (10 mg ketotifen fumarate + 20 mg theophylline)

Parameters	Simultaneous equation method		Q-Absorption ratio method	
	ketotifen fumarate	theophylline	ketotifen fumarate	theophylline
Actual Conc. (µg/ml)	10	20	10	20
Conc. Obtained (µg/ml)	9.9296 ± 0.0147	19.8523 ± 0.0488	9.9345 ± 0.0011	19.8524 ± 0.0412
%RSD	0.1486	0.2458	0.1602	0.2076
% Assay*	99.2963	99.2615	99.3450	99.2623

*Denotes average of three determinations

Table 5: Recovery data for the proposed methods

Method	Drug	Conc. Of sample Taken (µg/ml)	Conc. Of pure API spiked (µg/ml)	Total Conc. (µg/ml)	Conc. Found (µg/ml) (n = 3)	% Recovery Mean (n = 3)	%RSD
Method- A	ketotifen fumarate	5	4	9	8.96	99.65	0.2911
		5	5	10	9.98	99.87	0.3151
		5	6	11	12.11	100.95	0.1502
	theophylline	10	8	18	18.01	100.05	0.1054
		10	10	20	20.09	100.47	1.0627
		10	12	22	21.91	99.68	0.2139
Method- B	ketotifen fumarate	5	4	9	8.91	99.07	0.3901
		5	5	10	10	100.15	0.2145
		5	6	11	11.05	100.55	0.1205
	theophylline	10	8	18	17.98	99.98	0.1523
		10	10	20	20.14	100.47	0.8754
		10	12	22	22.13	100.65	0.4265

Table 6: Repeatability study of ketotifen fumarate for proposed method

Conc.(µg/ml)	Simultaneous equation method		Q-Absorption ratio method	
	296 nm	271 nm	281 nm	271 nm
8	0.3501	0.1849	0.2604	0.1849
8	0.3518	0.1835	0.2611	0.1835
8	0.3498	0.1855	0.2589	0.1855
8	0.3517	0.1851	0.2595	0.1851
8	0.3521	0.1845	0.2615	0.1845
8	0.3518	0.1832	0.2609	0.1832
Mean	0.3512	0.1844	0.2604	0.1845
SD	0.00099	0.00099	0.00101	0.00099
%RSD	0.28323	0.49661	0.38435	0.49659

Limit: % RSD for area NMT 2.0 %

Table 7: Repeatability study of theophylline for proposed method

Conc.(µg/ml)	Simultaneous equation method		Q-Absorption ratio method	
	296 nm	271 nm	281 nm	271 nm
16	0.0437	0.8538	0.5718	0.8538
16	0.0431	0.8558	0.5702	0.8558
16	0.0434	0.8521	0.5729	0.8521
16	0.0433	0.8544	0.5731	0.8544
16	0.0431	0.8535	0.5715	0.8535
16	0.0437	0.8544	0.5712	0.8544
Mean	0.0434	0.8541	0.5717	0.8541
SD	0.00027	0.00122	0.00108	0.00122
%RSD	0.62562	0.14303	0.19011	0.14303

Limit: % RSD for area NMT 2.0 %

Table 8: Intra-day and Inter-day Precision Data for Simultaneous equation method

Intra-day and Inter-day Precision Data for ketotifen fumarate								
Conc. (µg/ml)	Intra-day precision				Inter-day precision			
	Absorbance		%RSD		Absorbance		%RSD	
	296 nm	271 nm	296 nm	271 nm	296 nm	271 nm	296 nm	271 nm
2	0.086	0.048	0.642	0.634	0.086	0.049	0.506	0.536
8	0.350	0.184	0.307	0.556	0.351	0.185	0.289	0.354
14	0.611	0.322	0.246	0.583	0.611	0.325	0.197	0.535
Intra-day and Inter-day Precision Data for theophylline								
4	0.011	0.213	0.901	0.703	0.012	0.215	0.806	0.687
16	0.043	0.853	0.703	0.217	0.044	0.855	0.651	0.186
28	0.076	1.491	0.667	0.262	0.078	1.506	0.582	0.283

Limit: % RSD for area NMT 2.0 %

Table 9: Intra-day and Inter-day Precision Data for Q-Absorption ratio method

Intra-day and Inter-day Precision Data for ketotifen fumarate								
Conc. (µg/ml)	Intra-day precision				Inter-day precision			
	Absorbance		%RSD		Absorbance		%RSD	
	281 nm	271 nm	281 nm	271 nm	281 nm	271 nm	281 nm	271 nm
2	0.068	0.048	0.448	0.634	0.070	0.049	0.464	0.536
8	0.260	0.184	0.432	0.556	0.262	0.185	0.404	0.354
14	0.489	0.322	0.287	0.583	0.490	0.325	0.262	0.535
Intra-day and Inter-day Precision Data for theophylline								
4	0.145	0.213	0.827	0.703	0.146	0.215	0.686	0.687
16	0.572	0.853	0.238	0.217	0.573	0.855	0.247	0.186
28	1.016	1.491	0.153	0.262	1.026	1.506	0.104	0.283

Limit: % RSD for area NMT 2.0 %

Table 10: Limit of detection and Limit of quantification

Parameters	Simultaneous equation method				Q-Absorption ratio method	
	ketotifen fumarate		theophylline		Ketotifen fumarate	theophylline
	At 296 nm	At 271 nm	At 296 nm	At 271 nm	At 281 nm	At 281 nm
LOD (µg/ml)	0.0117	0.0161	0.1863	0.0101	0.0152	0.0118
LOQ (µg/ml)	0.0357	0.0487	0.5647	0.0307	0.0459	0.0358

RESULT

Absorbance of ketotifen fumarate and theophylline measured at 296 nm and 271 nm for simultaneous equation method and 281 nm and 271 nm for the Q-absorption ratio method respectively. The drug response with respect to absorbance was linear over the concentration range 2-16 µg/ml (n = 5) for ketotifen fumarate and 4-32 µg/ml for theophylline. The percentage recovery of ketotifen fumarate and theophylline was found to be 99.65-100.95 % and 99.68-100.47 % respectively for simultaneous equation method and 99.07-100.55 % and 99.98-100.65 % respectively for Q-absorption ratio method. The % RSD values for intra-day precision study and inter-day study were ≤ 2.0 %, confirming that the method was sufficiently precise. The system stability test parameters were also performed and were found to be within acceptable criteria.

CONCLUSION

A simple, accurate and precise Simultaneous equation and Q-Absorption ratio methods for the simultaneous estimation of Ketotifen fumarate and Theophylline in Bulk and Dosage form has been developed and validated.

REFERENCES

1. Rang HP, Dale MM, Ritter JN and Moore PK. In Pharmacology; 5th Ed; Churchill Livingstone, New York; 2003. p. 332-333.
2. Klooker TK, Braak B, Koopman KE, Welting O, Wouters MM, Van Der Heide S, Schemann M, Bischoff SC, Van Den Wijngaard RM, Boeckxstaens GE. The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. Academic Journal 2010; 59: 1213-1221.
3. Rosenfeld GC, Loose DS. In pharmacology, 4th Ed; Lippincott Williams and Wilkins; 2007. p. 216-217.
4. Golan DE, Tashjian AH, Armstrong EJ. In pharmacology, 3rd Ed; Lippincott Williams and Wilkins; 2012. p. 822.
5. Budavari S. An encyclopedia of chemicals, drugs and biological, 12th Edition; Merck and co.inc; 1996. p. 836.

6. Galindez OA and Kaufman HE, The management of allergic conjunctivitis. *Ophthalmology* 1996; 103: 1335-1336. [http://dx.doi.org/10.1016/S0161-6420\(96\)30499-5](http://dx.doi.org/10.1016/S0161-6420(96)30499-5)
7. Bartlett JD and Jaanus Sd. In clinical ocular pharmacology, 5th Ed; Butterworth helnenann Elsevier; 2008. p. 255, 258.
8. Ciprandi G, Buscaglia S and Cerqueti PM. Drug treatment of allergic conjunctivitis: a review of the evidence. *Annals of Allergy, Asthma and Immunology* 1992; 43: 154-176.
9. Apgar JL, Tarka JR, Stanly M. Methyl xanthine composition and consumption patterns of cocoa and chocolate products. In Gene A. Spiller. *Caffeine*. CRC Press; 1998. p. 171.
10. Yawn BP. Factors accounting for asthma variability: achieving optimal symptom control for individual patients. *Primary Care Respiratory Journal* 2008; 17: 138-147. <http://dx.doi.org/10.3132/pcrj.2008.00004>
11. Baur X, Sigsgaard T, Aasen TB, Burge PS, Heederik D, Henneberger P, Maestrelli P, Rooyackers J, Schlunssen V, Vandenplas O, Wilken D. The management of work-related asthma guidelines: a broader perspective. *European respiratory Journal* 2012; 124: 125-139. <http://dx.doi.org/10.1183/09059180.00004711>
12. Skoog DA, Leqary JJ. *Principle of Instrumental Analysis*, 54th Ed; Thomson Asia Pte Ltd; Singapore; 2004. p. 2-8, 580.
13. Connors AK. *Textbook of Pharmaceutical Analysis*, 3rd Ed; A Wiley-Inter sciences Publication; Singapore; 1999. p. 616.
14. Sharma BK. *Instrumental Method of Chemical Analysis*, 21th Ed; Goel Publishing Housing, Krishna Prakashan Ltd; 2002. p. 3, 10.
15. Jeffery GH, Bassett J, Mendham J, Denney RC. *Vogel's Textbook of Quantitative Chemical Analysis*, 5th Edition, Adison Wesley Longman LTD; 1996. p. 216-20.
16. Beckett AH, Stenlake JB. *Practical Pharmaceutical Chemistry*, 4th Ed; CBS publishers and distributors; New Delhi; 2004. p. 275-337.

Cite this article as:

Chaudhari Narendra Kesharbhaj. Simultaneous equation method and q-absorption ratio methods development and validation of ketotifen fumarate and theophylline in bulk and dosage form. *Int. Res. J. Pharm.* 2014; 5(5):412-417 <http://dx.doi.org/10.7897/2230-8407.050586>

Source of support: Nil, Conflict of interest: None Declared