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Research Article

DETERMINATION OF PHARMACOKINETIC PARAMETERS FOR *ATROPA BELLADONA* EXTRACT CONTAINING ATROPINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The present work was to formulate oral herbal tablets of *Atropa belladona* extract and also with pure atropine, further to determine the pharmacokinetic parameters and determine the dose frequency based on half-life.

Methods: After the quantification of the extract through high-performance thin layer chromatography, tablets were prepared with extract and pure form of atropine through direct compression technique by varying the process and formulation parameters. The pre- and post-compression parameters were evaluated for the formulated batches. *In vivo* studies using rabbit models of either sex were done and the various pharmacokinetic parameters were reported.

Results: The pharmacokinetic data obtained for pure and extract showed no significant difference in C_{max} at t_{max} of 2 hrs.

Conclusion: According to the results the *Atropa belladonna* extract and pure atropine tablets gave a $t_{1/2}$ of 3 hrs, representing that one tablet (equivalent to 0.021 mg of atropine) can be administered at every 3 hrs.

Keywords: Atropine, *Atropa belladonna*, Herbal, Pharmacokinetics, Tablets.

INTRODUCTION

Herbal medicine, the backbone of traditional medicine, has played an important role in human health and welfare for a long period. Traditional therapeutic approaches of regional significance are found in Africa, South and Central America, China, India, Tibet, Indonesia, and the Pacific Islands [1]. The considerable scientific significance and commercial potential of traditional medicines have resulted in increased international attention and global market demands for herbal medicines, especially Chinese herbal medicines [2]. Herbal medicines currently are the primary form of health care for the poor in the developing countries, and also are widely used as a supplement or substitute for conventional drugs in developed countries. These traditional medicines have a pivotal role in the treatment of various ailments and more than 50% of drugs used in Western pharmacopoeia are isolated from herbs or derived from modifications of chemicals found in plants [2,3]. Herbal medicines usually contain a complex mixture of various bioactive molecules, which make its standardization complicated, and there is little information about all compounds responsible for pharmacological activity. Several research papers have been published that claim pharmacological activity of herbal medicines but few are discussing the role of the exact phytoconstituent. Understanding the pharmacokinetic profile of such phytoconstituents is essential. Although there are research papers that deal with pharmacokinetic properties of phytoconstituents, there are a number of phytoconstituents yet to be explored for their kinetic properties. The present work is to estimate the pharmacokinetic parameters for *Atropa belladonna* which is a commonly used herb against spasms and colic – like pains in gastrointestinal and biliary tract [4].

The rationale of this project work is to formulate an oral tablet dosage form with the extract of *A. belladonna* containing atropine as their therapeutic molecule and also with synthetic commercial compound available in the market. The optimized formulations in extract and synthetic molecules were then subjected to *in vivo* studies in rabbit models to determine their pharmacokinetic parameters in two different dose levels one with low dose and other with high dose. The pharmacokinetic parameters obtained will be used for the determination of the dose frequency of specified herbal drugs to avoid their side effects and toxicity. The report may, which not only minimize cost, but also increase the efficacy, safety, quality, and better use of herbal drugs.

MATERIALS AND METHODS

Materials

A. belladonna roots were collected from the local market (Ooty), atropine from allied chemicals and pharmaceuticals and all other chemicals used were of analytical grade.

Preparation of *A. belladonna* extract

The *A. belladonna* dry roots were made into coarse powder using mixer. Extraction was done with ethanol at 70°C for 36 hrs and concentrated under vacuum. The residue was then treated with 1% HCl. The acid solution was further purified by shaking with light petroleum ether to remove impurities. Sufficient quantity of ammonia was added to make solution alkaline, and then treated with an organic solvent such as chloroform 3 times. Chloroform was removed under vacuum and the residue comprising crude alkaloid extract was dried at 40°C under vacuum to get the *A. belladonna* extract [5-7].

Quantification of the extract by high-performance thin layer chromatography (HPTLC)

The extracts of were quantified using standard drugs at λ_{max} of 200 nm (CAMA LINOMAT, IV, Switzerland) after development of precoated plates of 4×10 size (silica gel 60F 254,6 Merck) [5].

Preparation of samples

A value of 100 mg of the selected extracts was weighed accurately and dissolved in methanol in a volumetric flask. The flask was shaken for 30 minutes, the solution was filtered through Whatman filter paper, and the final volume was made up to 10 ml with methanol.

Preparation of standard solutions

Accurately weighed 100 mg of extracts were dissolved in 10 ml of methanol. From this 1 ml was diluted to 20 ml with methanol to produce 0.5 mg/ml. The standard drugs solution were also prepared at a concentration of 0.5 mg/ml with methanol in a similar manner and used for the analysis.

Application of standard and sample solutions

For the application of the solutions, pre-coated plates of 4×10 cm size (Silica gel 60 F 254, E.MERCK) were used. The standards and the sample solutions were applied on different tracks of the plate. A thin band of 6 mm width was applied using Linomat IV (automatic TLC applicator, CAMAG, Switzerland).

Chromatogram development and densitometric scanning

With the help of the suitable solvent system selected for the quantification of the selected extracts the plates were developed in the twin trough chamber. After the development of the chromatogram, plates were taken out, dried using hair drier and observed under ultraviolet light. The suitable solvent system selected for the quantification of Atropine was chloroform: methanol 80:20 (v/v) for the development of chromatogram.

Densitometric scanning

The developed plates were scanned using densitometer at 200 nm wavelength. The quantification of the atropine was calculated with reference to standard solution [8-10].

Compatibility studies

The infrared (IR) peak matching technique was applied to study the interaction between the drug and excipients. The accelerated temperature was applied to the physical mixture for 3 months to attenuate any physical or chemical interaction between them [11].

Formulation of tablets

Tablets of both synthetic atropine 1.2 mg and the extract 150 mg (equivalent to 1.2 mg) were compressed (Rotary tablet compressor, 10 stations, Rimek, Ahmedabad, Gujarat, India) using 12 mm standard concave punches by direct compression technique with varying the process and formulation parameters. All the powder mass was passed through BSS-80 mesh and mixed geographically for 10 minutes. Prior the compression, the powder mixture was evaluated for angle of repose, bulk density and compressibility index and drug content [10-14]. The tablets were then punched. Table 1 gives the formulae for the formulated batches.

Evaluation of pre-compression parameters

The powder mixtures were studied for angle of repose, bulk and tapped density compressibility index and drug content. The angle of repose was determined by funnel method using the below formula where "h" and "r" was the height and radius of the powder cone [15]. The bulk and tapped density were determined by taking the known quantity of slightly shaken powder mixture. These powder samples were introduced to 50 ml measuring cylinder to record the bulk density. This was then allowed to fall under its own height onto a hard surface from the height of 2.5 cm at 2 seconds interval. The tapping was continued until no further change based volume was observed. The density was calculated based on the weight of powder to volume occupied by it. The compressibility index was calculated using the below formula. Drug content was determined by analyzing the quantity of quinine extracted into phosphate buffer pH 3 from 200 mg of powder. The samples were analyzed spectroscopically at λ_{max} of 230 nm and expressed given percentage.

Table 1: The formula for the formulation of *Atropa belladonna* extract and atropine tablets

Serial number	Ingredient	Pure atropine tablets (mg)	*Atropa belladonna* extract tablets (mg)
1	Atropine	1.2 (equal to 1.00 2 mg of atropine)	-
2	*Atropa belladona* extract	-	150 (equal to 1.2 mg of atropine)
4	Starch 1500	106.8	22
5	MCC	80	20
6	Croscarmellose	10	10
7	Aerosil	2	2

MCC: Microcrystalline cellulose

Angle of repose $(\Theta)=\tan^{-1}(h/r)$

Compressibility index (%)=100×(TD-BD)/TD

Where, TD and BD are tapped density and bulk density, respectively.

Evaluation of post-compression parameters

The post-compression parameters such as weight variation (n=20), drug content (n=10), friability (n=tablet to whole weight of 6.4 g), disintegration time (n=6), and hardness were determined. The test for post-compression parameters except hardness was performed as per IP 2007. The drug content was estimated for individual tablets. The hardness and friability were calculated using Monsanto hardness tester (Cadarach, Ahmedabad, Gujarat, India) and friability testing apparatus (Indian Equipment, Mumbai, Maharashtra, India). The n represents the number of tablets used for the test.

***In vitro* drug release**

The dissolution medium consisted of 900 ml of 0.1N HCl (pH 1.2), from 0 to 1 hr for the developed formulation maintained at 37°C±0.5°C. The drug release at different time intervals was measured by high-performance liquid chromatography (HPLC) (Shimadzu) at 230 nm. It was made clear that none of the ingredients used in the matrix formulations interfered with the assay. The release studies were conducted in triplicate and the mean values were plotted versus time [15,16].

Pharmacokinetic studies

Experiments were carried out on New-Zealand white rabbits of either sex 1.5±0.2 kg (mean±standard deviation [SD]). The animals were overnight fasted before treatment but had free access to water. No subject was receiving any other drug at least 2 weeks before commencement of the study and no other drug was permitted throughout the duration of the study. They were randomly assigned into 5 groups (3 animals per group). Two groups received, pure Atropine tablets, two groups received Atropine extract tablets and one group as control [17]. All experiments adhered to the Institutional Animal Ethics Committee (JSSCP/IAEC/Projects/01/2008-09).

Administration and collection of blood samples

The dose for the study taken was 0.021 mg and 0.084 mg of atropine from atropine tablets and extract tablets, respectively, calculated according to body weight and body surface area of rabbit with the help of conversion factor with respect to the absolute human dose. The rabbits received the dose orally through an intragastric tube as 0.3% w/v carboxymethylcellulose suspension. Blood samples (1 ml) were collected in heparinized tubes from the marginal ear vein at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hrs after the drug administration. The samples were centrifuged at 3500 rpm for 10 minutes to separate the plasma, and they were transferred into airtight containers and stored at −20±0.2°C until analyzed [17].

Determination of atropine in plasma

The atropine was extracted from plasma samples (1 ml) obtained from study subjects by addition of 200 µl of perchloric acid to precipitate

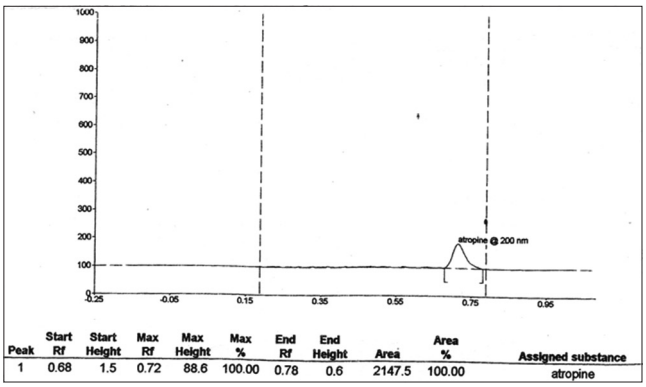
plasma proteins. The resulting solution was vortexed for 5 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant layer was separated and analyzed using Shimadzu gradient HPLC system with LC-20 AD 230V Solvent delivery system (Pump), manual injector 25 µl (Rheodyne), SPD-M20A 230V photodiode array detector, and LC solutions data station. Separation was achieved at room temperature on a phenomenex Gemini C18 (250×4.6 mm i.d., 5 µ) column. The mobile phase was a mixture of water and acetonitrile 97.5:2.5 v/v. The flow rate was 1.0 ml/minutes. The detection was done at 254 nm using SPD 20AD Diode Array Detector [18,19].

Statistics

All data were expressed as mean value±SD. Statistical analysis was performed using ANOVA test (Tukey's test). Mean differences were considered as statistically significant at a level of $p<0.05$.

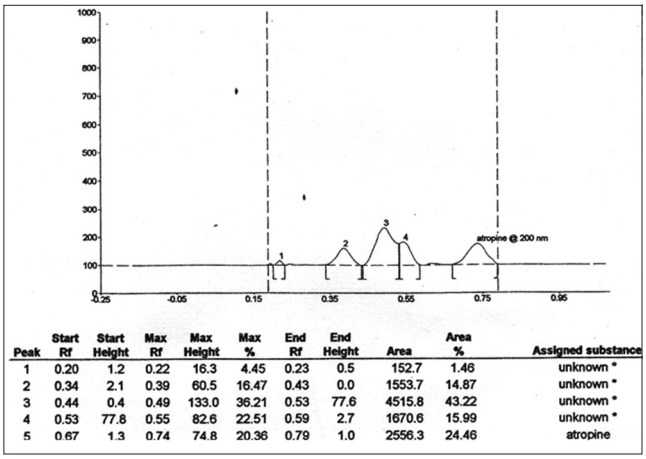
RESULTS

The extraction of Atropine from *A. belladonna* was done, and the obtained extract was quantified for the Atropine by HPTLC with comparison with the peak area obtained for 1 mg/ml methanolic solution of pure atropine. The precoated plates developed using mobile phase chloroform and methanol (80:20) v/v was scanned by densitometer at wavelength of 200 nm (Figs. 1 and 2). With the obtained peak area at R_f value of 0.74±0.01, it was estimated that 0.8±0.042% w/w of Atropine is present Table 2. The Fourier transform infrared spectra of the pure forskolin revealed the predominant bonds at wave number - OH (B) - 1064, O-H(S) - 3530, Ar C-H - 702, Hetero V-N - 1629, Ester C-O - 1728, respectively. The same results were also observed for the extract and the blend of the excipients containing pure atropine and extract confirming the presence of atropine and absence of any interaction of it with the excipients.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.68	1.5	0.72	88.6	100.00	0.78	0.6	2147.5	100.00	atropine

Fig. 1: Typical densitogram of atropine



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.20	1.2	0.22	16.3	4.45	0.23	0.5	152.7	1.46	unknown *
2	0.34	2.1	0.39	60.5	16.47	0.43	0.0	1553.7	14.87	unknown *
3	0.44	0.4	0.49	133.0	36.21	0.53	77.6	4515.8	43.22	unknown *
4	0.53	77.8	0.55	82.6	22.51	0.59	2.7	1670.6	15.99	unknown *
5	0.67	1.3	0.74	74.8	20.36	0.79	1.0	2556.3	24.46	atropine

Fig. 2: Typical densitogram of *Atropa belladonna* extract

The pure drug and the extract powder were mixed with excipients in the geometrical pattern for 10 minutes and to confirm the uniform distribution of the drug the degree of mixing was carried. The drug content was around 93.58±2.84% and 97.14±1.92% in case of pure drug and extract, respectively. Various batches of tablets were, compressed by direct compression technique for pure and extract tablets. Tables 3 and 4 displace their pre- and post-compression parameters. The angle of repose gives a qualitative assessment of internal and cohesive frictional forces. All the batches had an angle of repose around 31° indicating good flow potential. The size and shape of the particles reflect the density of the material. The density is directly proportional to the number of spherical particles present, whereas inversely to the size of the particles. As the value of compressibility is less than 15% in all the cases, the granules produced the adequate flow and stable packing. The starch 1500 is used as diluent in the formulation; croscarmellose was used as a superdisintegrant and microcrystalline cellulose (MCC) was used as the binder. The aerosil was used at concentration of 2% w/w. The hardness of all the formulations was 3.16±0.29 kg/cm^2 for pure and 4±0.86 kg/cm^2 for extract tablets. Other post-compression parameters such as average weight, friability, disintegration, and drug content were to the official limits. With the satisfactory post-compression parameters, the *in vitro* release studies were carried out in 900 ml of 0.1N HCl (pH 1.2) and it was observed that compared to extract tablets the pure tablets showed high *in vitro* drug release (Fig. 3).

A single dose study, for two concentrations of formulated extract and pure Atropine were carried out in five groups, containing three rabbits in each group, for each concentration of the formulations. The plasma samples were analyzed by reverse phase HPLC (Shimadzu UFLC LC20AD) with chromatographic conditions as Phosphate buffer 50 mM pH 3.0 and Acetonitrile (80:20 v/v) as mobile phase and phenomenex Gemini C18 (250 × 4.6 mm, i.d. 5 µ) as a stationary phase. The flow rate of mobile phase was set at 1 ml/minute and the samples were detected

Table 2: Quantification of atropine in the herbal extract by HPTLC

Serial number	Sample	R_f	Peak area	Atropine (%w/w)
1	Extract	0.74	2556.3	0.8
2	Atropine	0.72	2147.5	

HPTLC: High-performance thin layer chromatography

Table 3: Pre-compression evaluations of *Atropa belladona* extract and atropine blend

Serial number	Parameters	Unit	*Atropa belladona* extract	Pure atropine
1	Angle of repose	°(degree)	31.61±0.27	31.08±0.51
2	Bulk density	g/cm^3	0.472±0.01	0.484±0.01
3	Tapped density	g/cm^3	0.582±0.01	0.555±0.01
4	Compressibility	%	18.23±1.27	12.83±1.24
5	Drug content	%	93.58±2.84	97.14±1.92

n=6±SD. SD: Standard deviation

Table 4: Post-compression evaluations for *Atropa belladona* extract and atropine tablets

Serial number	Parameters	Unit	*Atropa belladona* extract tablets	Atropine tablets
1	Average weight	mg	200.77±2.14	200.19±1.12
2	Hardness	kg/cm^2	4±0.86	3.16±0.29
3	Friability	%	0.23±0.01	0.22±0.01
4	Disintegration	Minutes	8.81±1.03	2.50±38.0
5	Drug content	%	93.37±1.78	96.84±1.74

All the values represents the mean and SD of six observations. n=6±SD.
SD: Standard deviation

Table 5: Pharmacokinetic parameters obtained for *Atropa belladonna* extract and pure atropine tablets

Serial number	Parameters	Pure atropine tablets		*Atropa belladonna* extract tablets	
	Dose	0.021 mg	0.084 mg	0.021 mg	0.084 mg
1	C_{max} (µg/ml)	7.51±0.34	8.16±0.10	6.66±0.96	7.36±0.25
2	t_{max} (hours)	2	2	2	1.5
3	k_{eli} (hours^{-1})	0.225±0.64	0.208±0.72	0.227±0.51	0.203±0.87
4	$t_{1/2}$ (hours)	3.08±0.62	3.33±0.47	3.05±0.43	3.40±0.27
5	$AUC_{(0-24)}$ (µg.h/ml)	24.58±0.43	27.10±0.35	30.67±0.38	33.89±0.53
6	$AUC_{(0-\infty)}$ (µg.h/ml)	27.50±0.49	31.48±0.51	33.51±0.60	38.78±0.85
7	Lag time (t_0)	No	No	No	No

n=3±SD. SD: Standard deviation

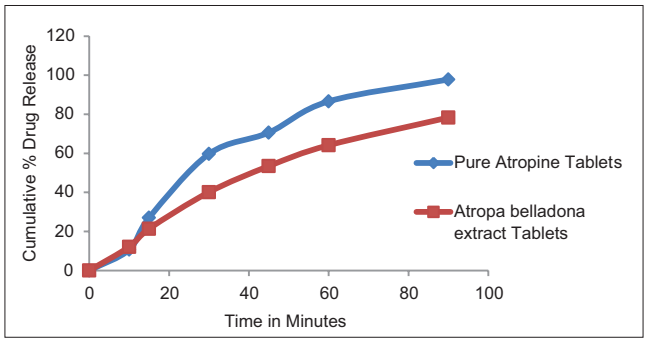


Fig. 3: Cumulative percentage drug release of *Atropa belladonna* and atropine tablets

by LC solutions as the data station at 230 nm. The quantification of the chromatogram was performed using peak area ratios (response factor) of the drug to the internal standard. Table 5 provides the various PK parameters estimated by PK1 and PK2 software. The pharmacokinetic data obtained for pure and extract shows no significant difference in C_{max} at t_{max} of 2 hrs. The *A. belladonna* extract and pure atropine tablets gave a $t_{1/2}$ of 3 hrs, representing that one tablet (equivalent to 0.021 mg of atropine) can be administered at every 3 hrs.

DISCUSSION

Extraction was done with ethanol at 70°C for 36 hrs and concentrated under vacuum until complete removal of ethanol to get a syrupy residue. The residue was then treated with 1% HCl followed by ammonia and treating with chloroform 3 times and dried at 40°C under vacuum to get the *A. belladonna* extract. Through the HPTLC studies it was found that about 0.8% of atropine was present [18]. The IR peak matching technique was, applied for the study of drug polymer interactions between the selected blend. The results were positive for the further study as all the ingredients were compatible with each other as well as with the extract and the pure atropine. The preparation of tablets involves two basic techniques: (a) Direct compression and (b) wet compression. The techniques have their own advantages and disadvantages. The limitation of wet granulation is its cost. It is an expensive process because of labor, time, equipment, energy and space requirements, and loss of material during various stages of processing. Stability may be major concern for moisture sensitive or thermolabile drugs; an inherent limitation of wet granulation is that any incompatibility between formulation components made us to opt for direct compression. The leeway of the technique was minimized loss of mixing quantity for moisture sensitive material and improved disintegration since powder particles are not bonded together by a binder. The excipients, in this study, are commonly used to formulate tablets. The starch 1500 was used as diluent, croscarmellose as superdisintegrant, aerosil as a glidant and lubricant. In the process of product development, some of the chemicals were added, omitted or varied in concentrations. Starch 1500 is unique pharmaceutical excipients combining several properties in a single product. It is particularly effective with moisture sensitive active low dose applications and exhibits synergy, enhancing the functionality of other commonly used excipients. It could serve as excipients for direct compression, owing to their low elastic recovery and excellent dilution potential [17]. The MCC is used as binder in the formulation as some researchers reported that MCC can sustain the release of the drug, to overcome this croscarmellose was used as the superdisintegrant at concentration of 5% w/w. The formulations provided the satisfactory post-compression results. The aerosil was used at concentration of 2% w/w which can nullify the use of additional lubricant as it can serve both as glidant and lubricant. With the hardness of 3-4 kg/cm^2, the other post-compression parameters such as average weight, friability, disintegration, and drug content were found to be optimum. As the hardness increased the disintegration time prolonged, the increase in hardness may be due to inbuilt nature of the extract.

The pharmacokinetic parameters for both the extract and the pure formulation were evaluated directly after oral administration of them into the stomach of rabbits. The study was carried to evaluate the difference in the PK parameters between the two formulations. The PK study of the extract formulation was to nullify the effect of subjects [20-22].

As herbal medicines are not restricted to a particular ailment, and these medicines are used with increasing frequency to improve health, it is essential to know the pharmacokinetic profile of the aliment being treated in detail.

CONCLUSION

The pharmacokinetic data obtained for the pure and the extract formulations had no significant difference which was confirmed by application of one-way ANOVA followed by Tukey's test. The pharmacokinetic data obtained for pure and extract shows no significant difference in C_{max} at t_{max} of 2 hrs. The *A. belladonna* extract tablet gave a $t_{1/2}$ of 3 hrs, representing that one tablet (equivalent to 0.021 mg of atropine) can be administered at every 3 hrs to the selected animal model. As it is seen, the extract is equivalent in potency further studies are needed to report the advantages of extract compared to pure form.

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REFERENCES

1. Evans WC. Trease and Evans Pharmacogonasy. 16th ed. Philadelphia, PA: Saunders Elsevier; 2009. p. 25-60.
2. De Smet PA. Herbal remedies. N Engl J Med 2002;347(25):2046-56.
3. Robinson M, Zhang X. The World Medicines Situation. Geneva, Switzerland: World Health Organization; 2011.
4. Sivasankari B, Pitchaimani S, Anandharaj M. A study on traditional medicinal plants of Uthapuram, Madurai District, Tamil Nadu, South India. Asian Pac J Trop Biomed 2013;3(12):975-9.
5. Gottumukkala V, Subbaraju T. Estimation of hyoscyamine in *Atropa*

Belladonna plant by HPLC and HPTLC. Pharm Times 2005;12:18-22.
6. Raghuwanshi AS, Jain UK. RP-HPLC method development for estimation of atropine sulphate in bulk drug. Orient J Chem 2009;25:621-4.
7. Ong ES. Extraction methods and chemical standardization of botanicals and herbal preparations. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812(1-2):23-33.
8. Cieri UR. Determination of atropine (hyoscyamine) sulfate in commercial products by liquid chromatography with UV absorbance and fluorescence detection: Multilaboratory study. J AOAC Int 2003;86(6):1128-34.
9. Dräger B. Analysis of tropane and related alkaloids. J Chromatogr A 2002;978(1-2):1-35.
10. Soares LA, Ortega GG, Petrovick PR, Schmidt PC. Optimization of tablets containing a high dose of spray-dried plant extract: A technical note. AAPS PharmSciTech 2005;6(3):E367-71.
11. Liberman HA, Lachman L, Schwartz JB. Pharmaceutical Dosage Forms; Tablets. 9th ed., Vol. 1-2. New York: Marcel Dekker., Inc.; 1990. p. 110-50.
12. Ansel C. Pharmaceutical Dosage Forms and Drug Delivery Systems. 8th ed. New Delhi. Wolters Kluwer Health (India) Pvt. Ltd.; 2005. p. 228-33.
13. Leon L, Lieberman HA. Pharmaceutical Dosage Forms - Tablets. 2nd ed. New York: Marcel Dekker; 1987. p. 123-42.
14. Aulton ME. Pharmaceutics: The Science of Dosage Form Design. London, England: Churchill Livingston; 1988. p. 30-61.
15. The United States Pharmacopoeia. 24th ed., Vol. 19. Rockville, MD: National Formulary USP Conventional Inc.; 2000. p. 546-7.
16. India. Indian Pharmacopoeia Committee. Pharmacopoeia of India. New Delhi: Ministry of Health and Family Welfare, Government of India, Controller of Publications; 1996.
17. Ghosh MN. Fundamentals of Experimental Pharmacology. 3rd ed. Kolkata, India: Hilton; 2005. p. 192-3.
18. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical Formulations. 1st ed. New Delhi: CBS Publishers and Distributors; 2000. p. 1-200.
19. Sharma V, Gulati A, Ravindranath S, Kumar V. A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC. J Food Compos Anal 2005;18(6):583-94.
20. Shargel L, Wu-Pong S. Applied Biopharmaceutics and Pharmacokinetics. 5th ed. New York: McGraw Hill; 1998. p. 161-84.
21. Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and Application. 2nd ed. Philadelphia, PA: Lea and Febizer. 1989. p. 89-90.
22. Shobha RH. Textbook of Biopharmaceutics and Pharmacokinetics. Bangalore: Prism Book PVT Ltd.; 2000. p. 84-95.