Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011: 3 (5) 28-33
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Development and validation of RP-HPLC method for determination of marker in polyherbal marketed Kankasava formulations

Manisha K. Gharate[1]and Veena S. Kasture[2]

[1]*M.G.V'S Pharmacy College, Panchavati, Nasik, Maharashtra, India*
[2] *Pinnacle Biomedical Research Institute, Bhopal, India*

ABSTRACT

A simple, precise, accurate RP -HPLC method was developed for the quantitative estimation of atropine in Kankasava polyherbal branded formulations. The separation was achieved with a column RP C- 18 (250 mm X 4.6 mm X 5 micron) using mobile phase mixture of Methanol &10 mmol dihydrogen phosphate buffer (the pH -2.5 adjusted with orthophosphoric acid) in a ratio of 50:50 v/v at a flow rate of 1 ml/min, &analysis was screened with UV detector at 254 nm. The retention time for standard atropine sulphate was found to be 4.0667 minutes. Calibration curve was linear over concentration range 20-100 µg mL^{-1} Linearity was found to be r^2 =0.998.The proposed method was validated for linearity, accuracy, precision and LOD, LOQ. The active content of atropine in Kankasava formulation was varying and also compared with label claim.

Keywords:-Atropine sulphate, Kankasava, HPLCmethod, Validation parameters.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the implements in the acceptance of the Ayurveda or Siddha formulation is the lack of quality control profile. (I)

Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and modern analytical techniques are accepted to help in circumventing this problem. (II)

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is

gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization.HPLC has preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs because of its simplicity, sensitivity, accuracy, suitability for thorough screening etc.(III)

Kankasava is a fermented polyherbal product prepared with Kanaka (dhattura), &other ingredients. It is used in Chronic Bronchitis, Asthmatic Cough and Breathlessness. *Datura metel* (Family:Solaneace),popularly known as dhattura, is a valuable plant drug in ayurvedic and modern system of medicine & was used as mydriatic &anti asthmatic in the Middle Ages. Tropane alkaloid namely hyoscyamine &Atropine are responsible for the activity. These are toxic compound. The tropane alkaloid atropine is the racemic form of hyoscyamine, which occurs in *Atropa belladonna*, Datura species, *Hyoscyamus niger* and other extracts. The plant leaves contain about 0.3 - 0.7 % alkaloids. (IV)

MATERIALS AND METHODS

Chromatographic conditions &instrumentation:-
Chromatographic separation was performed with YOUNGLIN HPLC (model no.ACME-9000) equipped with isocratic pump and manual injector (20 µl) (Revodyne type).Autopro-3000 chromatographic software was used for data acquisition. A RP-C18 (250 mm X 4.6 mm X 5 micron) column was used for analysis. Mobile phase comprising of Methanol &10 mmol dihydrogen phosphate buffer (the pH -2.5 adjusted with orthophosphoric acid) in ratio of 50: 50v/v was filtered through 0.45µm membrane filter (Millipore) and degassed by sonication. Throughout the run a flow rate 1 ml/ min was maintained. Column effluent was monitored at 254 nm with variable wavelength UV detector.

Preparation of standard solutions:-
Standard Atropine sulphate was procured from market& stock solution (600ppm) was prepared in methanol. From the stock solution series of different concentrations (20-100 µg/ml) was prepared in mobile phase.

Preparation of sample solution from marketed Kanakasva formulations:-
Three brands of Kankasava have been selected of reputed companies from local market and coded to avoid conflict of interest as A,B,C. Randomly two batches from each brand were used for the study and named as A_1, A_2,B_1, B_2, C_1, C_2.

10 ml of sample from each brand was pipetted out and treated with dil.HCL. Resinous matter was separated by filtration &resulting solution was further purified by shaking out with petroleum ether (40-60^{0}c) several times. The purified acidic solution thus obtained was made alkaline with dil. Ammonia solution and extracted with chloroform successively. The combined chloroform layer was removed by distillation under reduced pressure. Residue was diluted to 2.0 ml with mobile phase. From that 20 µl was injected.

Validation experiments were performed to demonstrate system suitability, linearity, precision, accuracy study and robustness as per ICH guidelines.

Linearity:- Working dilutions of standard Atropine sulphate in the range of 20-100 µg/ml were prepared by taking suitable aliquots of working standard solutions in different 10ml of volumetric flasks and diluting upto the mark with mobile phase.20µl was injected from it each time into the column at flow rate of 1ml/min.

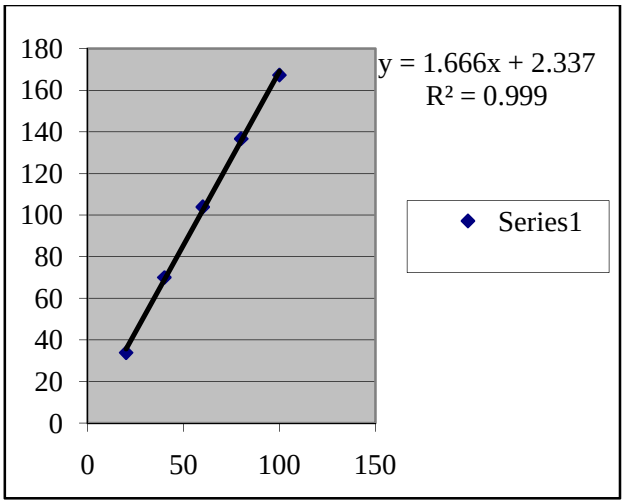
The standard in the eluate was monitored at 254 nm and corresponding chromatograms were obtained. From these chromatograms peak area were calculated and a plot of concentration over peak area was constructed.

The regression of the plot was computed by least square regression method. The experiment was performed three times and the mean was used for the calculations.

Precision and Accuracy:-
Precision of analytical method was studied by multiple injecting of homogenous sample. The intra-day and inter-day precision was used to study the variability of the method. Accuracy of the method was studied using the method of standard addition. Standard Atropine sulphate solutions were added to the unknown formulation of Kankasava. The percent recovery was determined at four different levels 0%, 80% and 100% and 120%. Atropine sulphate content was determined and the percent recovery was calculated.

Robustness & Ruggedness:-
Robustness was studied by changing parameters like flow rate. For the study of ruggedness 60 µg/ml standard Atropine sulphate was injected by two analysts.

Figure I:-Linearity Graph of Peak Area vs. Concentration.



RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for analysis of atropine from polyherbal Kankasava formulations. In order to achieve optimum separation of component peaks, mixture of methanol and 10 mmol dihydrogen phosphate buffer

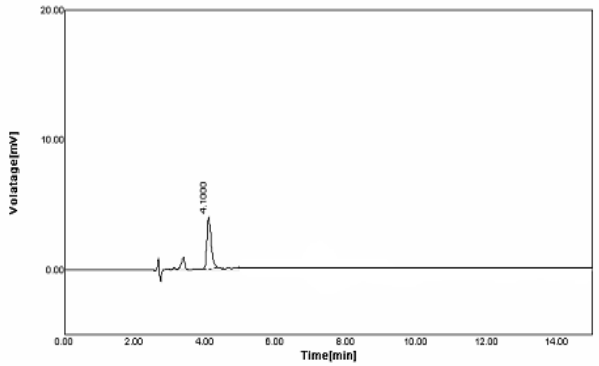
(the pH -2.5 adjusted with orthophosphoric acid) in ratio of 50: 50v/v was selected as chromatographic and resolved with no tailing.

A good linearity was achieved in the concentration range of 20– 100µg/ml for the standard atropine sulphate. (Figure I)

The regression equations and correlation coefficient for the standard were y = 1.666x + 2.337, R^2 = 0.999.

The retention time obtained for Atropine sulphate was 4.055 min. (Figure.II)

Figure II: - HPLC chromatograph of standard Atropine sulphate.



Validation summary of the method is shown in Table I.

Table I: - Method validation summary

parameters	standard
Linearity range [µg/ ml]	20-100 µg/ ml
Slope (m)a)	1.666
Intercept(c) a)	2.337
Correlation coefficient (R)	0.999
LOD [µg/ ml]	6.16 µg/ ml
LOQ [µg /ml]	18.67 µg /ml

a) of the equation y = mx + c, where y is peak area, m is the slope, x is the concentration, and c is the intercept.

When Atropine sulphate solutions of concentration40, 60, 80 µg/ml were analyzed by proposed method for finding out intra day &inter day variations, low coefficient of variation was observed. The % RSD for intra-day and inter-day precision for atropine sulphate were 1.10%&1.06%. Precision results are shown in Table II.

Table II. Precision study Results (n=3)

Conc. (µg/ ml)	Mean Amount found **(n=3)**	% Amount found	%RSD	Conc. (µg/ ml)	Mean Amount found	% Amount found **(n=9)**	%RSD
40	43.41	108.52	0.51	40	43.68	109.19	1.50
60	66.03	110.05	1.04	60	65.59	109.32	0.73
80	79.59	99.48	1.63	80	79.91	99.89	1.05

High Recovery values obtained from different concentrations of drug by proposed method indicates the method is accurate. (Table III)

Table III. Recovery study Results (n=3)

Level	Std. (µg/ ml)	Preanalysed sample in (µg/ ml)	Amount found	% Amount Recvd	SD	%RSD
0%	0	105	103.82	98.87	1.75	1.65
80%	84	105	151.85	80.34	0.59	0.73
100%	105	105	203.17	96.75	0.13	0.13
120%	126	105	232.44	101.50	0.63	0.62

Deliberate changes in method have not much affected peak tailing, theoretical plates. This indicates robustness of method. Changing the analyst also do not cause any change in the response indicating the Ruggedness of method. (Table IV).

Table IV:-Robustness, Ruggedness of method.

Sr.No.	parameters	Theoretical plates	Tailing Factor	Peak Area	Retention Time	Conc. Found (µg/ ml)
1	Flow Rate 1.0ml/min.	5116.2	1.5833	114.9155	4.0500	60
2.	Flow Rate 1.1ml/min.	5143.7	1.5000	110.7929	3.8167	60
3.	Flow Rate 0.8ml/min.	5263.5	1.5714	104.6125	4.8333	60
4.	Analyst 01	5116.2	1.5833	104.9155	4.0500	60
5	Analyst 02	5116.2	1.5833	114.9155	4.0500	60

System suitability parameters were studied such as RSD of six replicates of standard atropine solution and calculated parameters were within acceptance criteria. (Table V)

Table V:-system suitability Parameters

parameters	standard
Linearity range [µg/ ml]	20-100 µg/ ml
Theoretical plates	3509.66
Tailing Factor	1.525

Atropine content in branded Kankasava formulations was calculated by linear regression method. Formulation of Brand A with batch A_1 contains 114.47% &batch A_2 contain102.71% of label amount. Formulations of brand B with batch B_1contain 122.22%&batch B_2 contain 56.49% of

the labeled claim. Formulation of brand C does not have the atropine content claimed on the label. (Table V)

Table V: - Assay results

Sr.No.	Sample code	RT	Peak area	Conc.(µg./ml) found	% Label Claim
1	A_1	4.11	293.04	114.47	114.47%
2	A_2	4.03	173.44	102.71	102.71%
3	B_1	4.08	15.34	7.81	122.22%
4	B_2	4.00	8.43	3.66	56.49%
5	C_1	4.08	197.2	116.97	-----
6	C_2	4.10	177.94	105.41	--------

CONCLUSION

A simple, rapid and accurate HPLC method for the quantitation of atropine in Marketed Kankasava formulation was developed. All the brands of Kankasava showed different amount of active content and it was compared with label claim. It was observed that all formulations shows varying amount of active principle and also different than the label claim. This indicates the necessity of standardization of polyherbal formulation. The method was validated to track the active principles in the complex polyherbal formulations. The method could be extended for the marker-based standardization of other herbal product containing atropine. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and herbal formulations.

REFERENCES

[1] M.S. Bagul, M.Rajani M. *Indian Drugs*, **2005**, 42, 15-19;
[2] J Natalie J. Lazerowych and P. Peter, *Drug information journal*, **1998,** 32; 497-512.
[3] L. Snyder, J. Kirkland, J. Glajch. *A Wiley Interscience publication*, **1997**.
[4] Ayurvedic Pharmacopoeia of India. **2001,** New Delhi: Government of India, Ministry of Health and Family Welfare, Department of Health, 2nd edition, 3(01) 142-144;.
[5] ICH, Q2A, **1994,**Validation of Analytical Procedure: Methodology, In. Proc. Int. Con.Harmonization, Geneva, 1994