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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF HYDROXYAMPHETAMINE HYDROBROMIDE AND TROPICAMIDE IN OPHTHALMIC FORMULATION

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ABSTRACT

A simple and rapid RP-HPLC method was developed and validated for the simultaneous estimation of Hydroxyamphetamine hydrobromide and Tropicamide in ophthalmic formulation using Phenomenex BDS C-18 (250 × 4.6 mm, 5 µ) column and Acetonitrile: Phosphate buffer (50 mM, pH 5.6) in the ratio of 40: 60 v/v as mobile phase with 1 mL/min flow rate and UV detection at 257 nm. Retention time for Hydroxyamphetamine hydrobromide and Tropicamide was observed to be 2.40 and 4.10 minutes respectively. The content in the formulation was found to be about 104.83 and 105.91% for Hydroxyamphetamine hydrobromide and Tropicamide respectively. The mean percentage recovery at three different levels was found to be 100.75 – 102.09% for Hydroxyamphetamine hydrobromide and 99.32 – 101.89% for Tropicamide. Intraday and Interday precision was found to be below 2% with no significant difference in reproducibility between two different analysts. Linearity (R^2) was found to be above 0.99 for Hydroxyamphetamine hydrobromide and Tropicamide in the concentration range of 40-400 µg/mL and 20-250 µg/mL respectively. All the ICH parameters Q2 (R1) were found to be well within the acceptance criteria and hence can be applied for routine analysis for Hydroxyamphetamine hydrobromide and Tropicamide in ophthalmic formulation.

KEYWORDS: Hydroxyamphetamine hydrobromide, Tropicamide, RP-HPLC, Ophthalmic formulation.

INTRODUCTION

Drugs that dilate the pupil (mydriatics) and paralyze accommodation (cycloplegics) are used topically in the examination of the eye and other ophthalmic procedures. They are also used in the management of inflammatory conditions of the eye to treat or prevent the formation of adhesions between the lens and the iris. Hydroxyamphetamine hydrobromide (HAHB), *(±)-p-(2-Aminopropyl) phenol hydrobromide*, illustrated in Fig. 1, is an indirect-acting sympathomimetic agent that causes the release of norepinephrine from adrenergic nerve terminals, resulting in Mydriasis.[1,2]

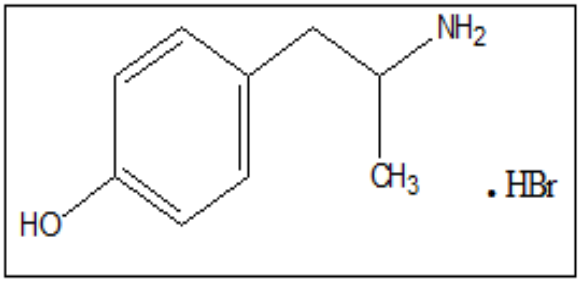


Fig. 1 Structure of Hydroxyamphetamine Hydrobromide

Tropicamide (TPC), *(RS)-N-ethyl-3-hydroxy-2-phenyl-N-(pyrid-4-ylmethyl) propionamide*,[3] illustrated in Fig.2, is a parasympatholytic agent that produces mydriasis and paralysis by blocking the sphincter muscle in the iris and the ciliary muscle. The combination of HAHB and TPC produces a synergistic effect which leads to faster recovery of patients.[4]

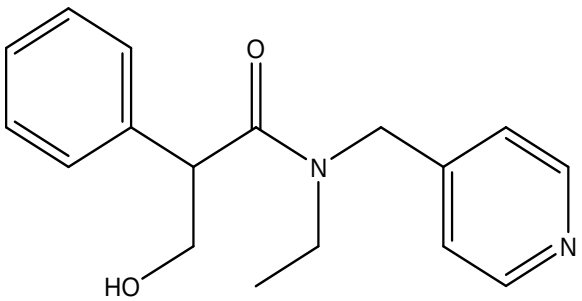


Fig. 2 Structure of Tropicamide

Literature survey had revealed that no analytical method is available for the simultaneous estimation of HAHB and TPC in ophthalmic solution. The reported analytical methods for estimation of HAHB are RP-HPLC[5], GC[6] and Amperometry[7] while those for TPC are TLC[8],

Spectrophotometry[9, 10] and RP-HPLC[11, 12] which are either alone or in combination with other drugs. Hence, there was a need to develop and validate a simple, accurate and rapid method for estimation of HAHB and TPC in pharmaceutical products.

MATERIAL AND METHOD

Chemicals and Reagents

Hydroxyamphetamine hydrobromide was procured by the institution and Tropicamide was obtained as a gift sample from Micro Labs, Bangalore. HPLC grade acetonitrile (Finar, Ahmedabad, India) and water were used throughout the experiment along with analytical grade Triethylamine (TEA) and Potassium dihydrogen orthophosphate (SDFCL, Mumbai).

Instrumentation

Shimadzu double beam UV-Visible Spectrophotometer model 1700 with 1 cm matched quartz cells connected to a PC running UV Probe Software 2.34 was used for measuring the absorbance, computing and storing the data of all absorption spectra.

The HPLC (Shimadzu, Japan) instrument was equipped with binary pump (LC-10 AT) and SPD- 10AVP UV detector. Sample and standard solutions were injected using Rheodyne injector of 20 µl loop and the chromatographic analysis was carried out on Phenomenex BDS C-18 column (250 × 4.6 mm, 5 µ). A computer running LC Solution software was used for data acquisition and processing.

Chromatographic conditions

The mobile phase composed of acetonitrile and potassium dihydrogen orthophosphate (50 mM, pH 5.6) in a ratio of 40:60 v/v run under isocratic elution and pumped at a flow rate of 1 mL/min with UV detection at 257 nm. Under these conditions, 10 min run time was maintained.

Preparation of mobile phase for RP-HPLC method

Potassium dihydrogen orthophosphate (50 mM) was prepared by accurately dissolving 6.8 g in 1000 mL water and the pH was adjusted to 5.6 with 1% Triethylamine (TEA). Buffer was then filtered through membrane filter (0.45 µ), mixed with Acetonitrile in the ratio 40: 60 v/v and sonicated for 10 minutes.

Preparation of Standard Stock Solutions of Hydroxyamphetamine hydrobromide and Tropicamide

Accurately weighed 40 mg of HAHB Standard and 10 mg of TPC Standard were transferred to separate clean and dry 10 mL volumetric flasks, dissolved and made up to volume with mobile phase to obtain a concentration of 4000 µg/mL and 1000 µg/mL of HAHB and TPC respectively.

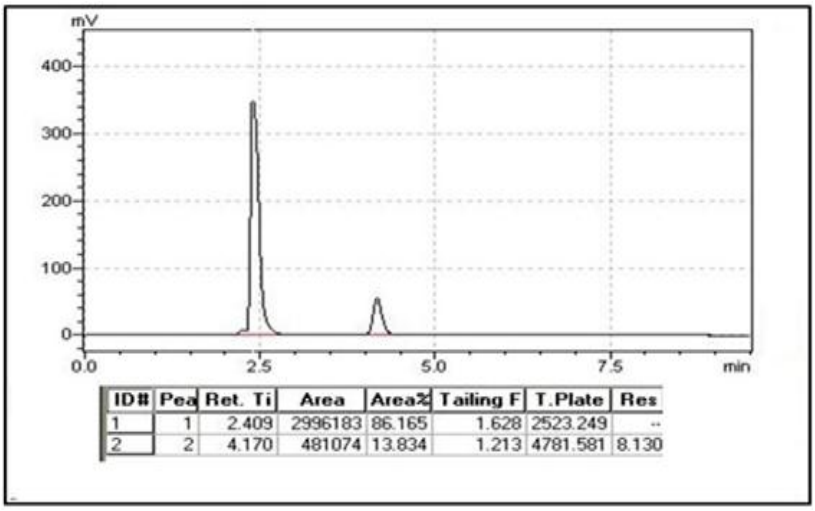
Preparation of Working Standard Solution

The standard stock solution was further diluted to get a concentration of 40 - 2000 µg/mL and 10 - 500 µg/mL of HAHB and TPC respectively. These solutions were filtered through Nylon membrane filter (0.45 µ) and sonicated for 5 minutes.

Analysis of Eye Drops

Accurately 0.4 mL of eye drops (Paremyd ophthalmic solution containing 1% Hydroxyamphetamine hydrobromide and 0.25% Tropicamide) was transferred to a clean and dry 10 mL volumetric flask and made up to volume with mobile phase. The solution was filtered through Nylon membrane filter (0.45 µ) and sonicated for 5 minutes.

The sample and working standard (400 and 100 µg/mL of HAHB and TPC) solutions were analyzed and the concentration of HAHB and TPC in ophthalmic solution was determined by comparing the area under curve of sample solution to that of working standard solution. The chromatogram of sample has been shown in Fig.3.



ID#	Pea	Ret. Ti	Area	Area%	Tailing F	T.Plate	Res
1	1	2.409	2996183	86.165	1.628	2523.249	--
2	2	4.170	481074	13.834	1.213	4781.581	8.130

Fig. 3 Chromatogram showing Hydroxyamphetamine hydrobromide (2.409 min) and Tropicamide (4.170 min) with Acetonitrile: Phosphate buffer (50 mM) at pH 5.6 with 40: 60 v/v.

Validation of RP-HPLC Method[13]

The developed RP-HPLC method was validated as per ICH Q2 (R1) guidelines for specificity, accuracy, precision, linearity, range, detection limit, quantitation limit and robustness.

Specificity

The chromatograms of blank, standard solutions of HAHB and TPC, sample and excipient solution were overlaid and studied to determine the specificity of the method.

Accuracy

The accuracy of the proposed method was performed by standard addition method at three different levels (50%, 100% and 150%). A known amount of standard HAHB and TPC are added to pre-analyzed sample solution and percent recoveries were determined. The results are shown in Table 1.

TABLE: 1. Data for Recovery studies at 3 different levels

Analyte	Assay (%, n=6)	Mean % Recovery (n=3)		
		50 %	100 %	150 %
HAHB	104.83	100.75	101.69	102.09
TPC	105.90	101.88	101.89	99.32

PRECISION

The precision of the method was determined by measuring % RSD of standard solutions (n=9) at different time intervals on the same day (intraday) and three consecutive days (interday) and by two different analysts (intermediate precision). The results are shown in Table 2.

TABLE: 2 Data for Precision study

Precision Parameters		HAHB (% RSD)	TPC (% RSD)	Acceptance Criteria
Intraday Precision (n=9)		1.64	1.54	NMT 2 %
Interday Precision (n=9)		1.78	1.10	
Intermediate Precision (n=6)	F-test	0.1117	0.2918	9.00 at degree of freedom (υ) 2,2 at p=0.01
	t-test	0.1140	0.1037	1.638 at degree of freedom (υ) 4 at α=0.10

Linearity and Range

Linearity of the method was determined by analyzing six sets of the mixed standard solutions in the concentration range of 40 - 2000 µg/mL for HAHB and 10 - 500 µg/mL for TPC and by plotting the graph of AUC versus concentration for HAHB and TPC.

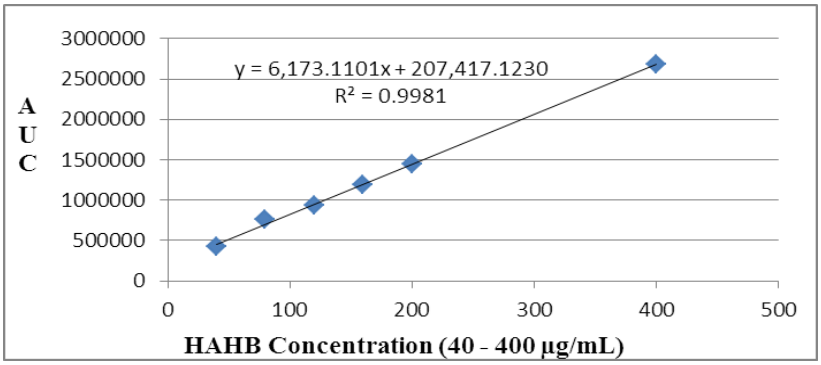


Fig: 4. Linearity curve for HAHB

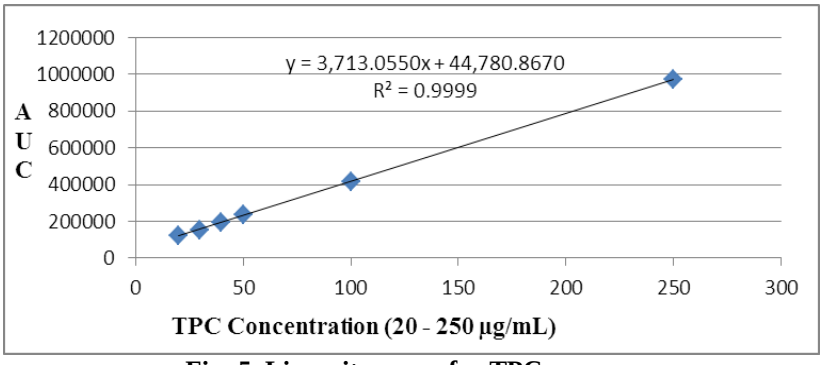


Fig: 5. Linearity curve for TPC

Detection and Quantitation limit (DL and QL)

The DL and QL were determined by formulae method after taking mean of slopes and standard deviation of intercepts from the calibration curves.

$$DL = \frac{3.3 \times Standard\,Deviation\,of\,Intercept}{Mean\,of\,Slope} \quad QL = \frac{10 \times Standard\,Deviation\,of\,Intercept}{Mean\,of\,Slope}$$

Robustness

The robustness of the developed system was determined by % RSD of Retention volume, Resolution, Theoretical plates and Tailing factor on flow rate (± 3%), organic phase (± 3%) and pH (± 1%).

TABLE: 3 Data for Robustness study

Analyte	Parameters	Flow Rate	Org Phase	pH
HAHB (% RSD, n=9)	Retention Volume (mL)	1.06	0.30	0.42
	Theoretical Plates	1.85	1.00	1.58
	Tailing Factor (min)	0.92	0.84	0.85
TPC (% RSD, n=9)	Retention Volume (mL)	0.91	1.85	1.51
	Theoretical Plates	1.82	1.35	1.84
	Tailing Factor (min)	1.99	1.15	1.66
MEAN RESOLUTION (min, n=9)		8.44	8.68	8.65

System suitability

The working standard solution was injected six times successively and parameters like retention time, tailing factor, theoretical plates and resolution were studied.

RESULTS AND DISCUSSION

The Standard solutions of HAHB and TPC were scanned in the range of 200 – 400 nm using UV-Visible Spectrophotometer where maximum absorbance for HAHB was observed at 270 nm and for TPC at 257 nm. The concentration of TPC in the formulation is very low and to improve its sensitivity and detection, the analysis was carried out at 257 nm.

The pKa of HAHB[14] and TPC[15] was reported to be 9.6 and 5.2 respectively. Hence, different mobile phase combinations were tried in the pH range 3 - 4 and 6 - 7.5 along with varying molarity of buffer to resolve HAHB and TPC. Optimum resolution between HAHB and TPC was found in the mobile phase ratio of ACN: Phosphate buffer (50 mM, pH 5.6).

40: 60 v/v on Phenomenex BDS C-18 column (250 × 4.6 mm, 5 µ) with a flow rate of
1 mL/min.

The retention time was found to be 2.40 min and 4.17 min for HAHB and TPC respectively with resolution 8.13 min and theoretical plates 2523 for HAHB and 4781 for TPC. The tailing factor was found to be 1.62 and 1.21 min for HAHB and TPC respectively.

The method was applied for analysis of Paremyd ophthalmic solution containing 1% HAHB and 0.25% TPC. The concentration in the formulation was found to be about 104.83% and 105.90% w/v for HAHB and TPC respectively which were within the acceptance limits.

The developed method was validated. Accuracy was determined by standard addition method and mean percentage recoveries was found to be 100.75 – 102.09 for HAHB and 99.32 – 101.89% for TPC (Table 1).

Relative standard deviation was used to analyze precision of the developed method for inter and intraday studies and found to be less than 2%. Reproducibility was performed by analyzing three sets of sample by two different analysts and compared by F-test and t-test. The calculated F and t-test values were found to be less than the tabulated values which showed no significant difference in the results and precision between the two analysts. (Table 2).

Linearity (R^2) was found to be 0.9981 and 0.9999 in the concentration range of 40 - 400 µg/mL and 20 - 250 µg/mL for HAHB and TPC respectively (Fig.4,5). The DL and QL were found to be 6.649 and 20.148 µg/mL for HAHB and 2.419 and 7.330 µg/mL for TPC respectively.

The developed method was validated for robustness by varying flow rate (± 3%), organic phase (± 3%) and pH (± 1%). The percentage Relative Standard Deviation was found to be less than 2 for Retention volume, theoretical plates and tailing factor while resolution was found to be more than 1.5 min (Table 3).

The theoretical plates were found to be 2523 and 4781 for HAHB and TPC respectively. The tailing factor was 1.62 and 1.21 minutes for HAHB and TPC respectively with a resolution of 8.13 minutes.

Thus, the developed and validated RP-HPLC method was found to be accurate, precise, robust and rugged for simultaneous estimation of Hydroxyamphetamine hydrobromide and Tropicamide in Ophthalmic solution.

CONCLUSION

The developed and validated RP-HPLC method with a run time of 10 minutes can be considered to be cost

effective and time saving and hence can be applied for routine analysis of Hydroxyamphetamine hydrobromide and Tropicamide in combination.

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REFERENCES

1. United States Pharmacopoeia 30. Rockville: United States Pharmacopoeial Convention; 2005. p. 2310
2. Martindale – The Complete Drug Reference. 36th ed. London: Pharmaceutical press, 2009; 2322.
3. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Published by the Indian Pharmacopoeia Commission Ghaziabad, 2010; 2270.
4. Martindale – The Complete Drug Reference. 36th ed. London: Pharmaceutical press, 2009; 1886.
5. Kazuaki Shimosato, Masafumi Tomita and Iwao Ijiri. Rapid determination of p-hydroxylated methamphetamine metabolites by column liquid chromatography – electrochemistry. J Chromatogr, 1986; 377: 279-86.
6. E.M. Sellers, S.Cheung, H.Nolte, S.V. Otton, R.F. Tyndale, RH.Wu. Simultaneous gas chromatographic determination of methamphetamine, amphetamine and their p-hydroxylated metabolites in plasma and urine. J Chromatogr B, 1997; 690: 77-87.
7. Mumtaz. H. Shah and James. T. Stewart. Amperometric determination of Hydroxyamphetamine hydrobromide in a flowing stream at the glassy-carbon electrode. J Pharm Biomed Anal, 1984; 2: 555-59.
8. Alani SD. The ophthalmic rod: A new ophthalmic drug delivery system I. Graefes Arch Clin Exp Ophthalmol, 1990; 228: 297-301.
9. Pohloudek-Fabini R, Martin E, Gallasch V. Contribution to the stability of tropicamide solutions. Pharmazie, 1982; 37: 184-7.
10. Dubey BK, Upadhyayr R, Tiwari AK, Shukla IC. Assay of some ophthalmic drugs with ammonium metavanadate reagent. J Indian Chem Soc, 2004; 81: 511-4.
11. Amanlou M, Asmardi G, Andalibi PC, Javadi N, Khodadady F, Omarny ZB. Determination of tropicamide in pharmaceutical formulations using high performance liquid chromatography. J Chromatogr A, 2005; 1088: 136-9.
12. Stefanowicz Z, Stefanowicz J, Mulas K. Determination of tropicamide and its major impurity in raw material by the HPLC-DAD analysis and identification of this impurity using the off-line HPLC-FT-IR coupling. J Pharm Biomed Anal, 2009; 49: 214-20.
13. International Conference on Harmonization, Q2 (R1), Harmonised tripartite guidelines, Validation of analytical procedures: text and methodology, Geneva, November, 2005.
14. Leffler EB, Spencer HM, Burger A. Dissociation Constant of Adrenergic Amines. J Amer Chem Soc., 1951; 73(6): 2611.
15. Blessel KW, Rudy BC, Senkowski BZ. Tropicamide. Florey KF, editor. Anal Profile Drug Subst. Elsevier, 1974: 3: 565-80.