Development and validation of a method for the simultaneous determination of ambroxol and clenbuterol in syrup by high resolution liquid chromatography

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Introduction

The combination of ambroxol (mucolytic) and clenbuterol (bronchodilator) in a syrup have a complementary action and is designed to facilitate the elimination of excess mucus and phlegm.[1]

Due to the fact that validated analytical methods are of vital importance for the assurance of the composition of the drug as a necessary requirement for its commercialization, the aim of this work was to develop and validate an analytical procedure by HPLC for the simultaneous determination of ambroxol hydrochloride and clenbuterol. in syrups, giving rise to an analytical methodology that allows both components to be determined simultaneously, using the high-performance liquid chromatography (HPLC) technique.

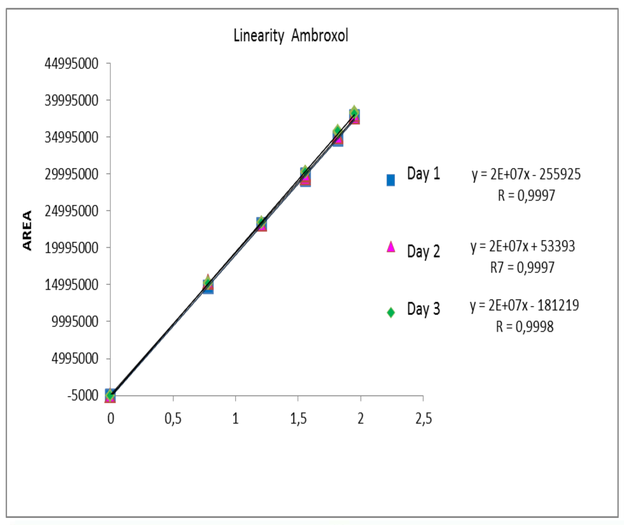
Validation of the Analytical Procedure

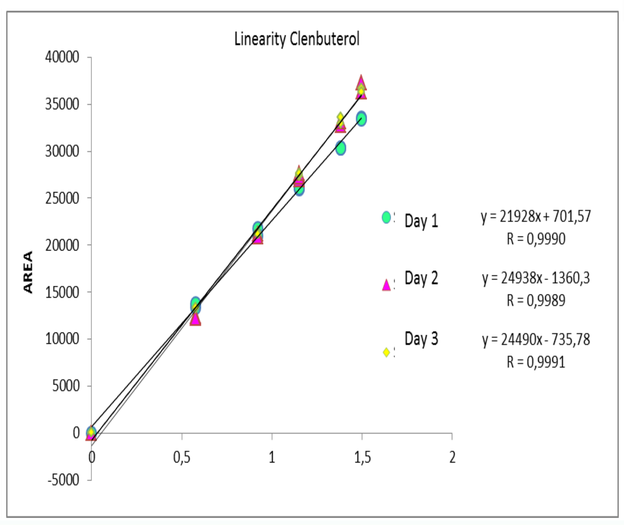
The following parameters were evaluated: linearity and range, precision and accuracy, as established by the ICH[2] and USP 42.[3]
Precision was evaluated as repeatability and intermediate precision, performed by the same analyst, same instrument, on different days.

Methodology

Mobile phase	acetonitrile, methanol, buffer / ionic pair (KH_2PO_4 and 1-octanesulfonic acid) (15:20:65)% v / v at pH 3.70
Flow	1.5 mL / min
Volume of injection	15 µL / min
Column	C18 (L1) 4.5 mm X 100 mm, 3.5 µm
wavelength	245 nm

Figure 2. Linearity of Ambroxol and Clenbuterol

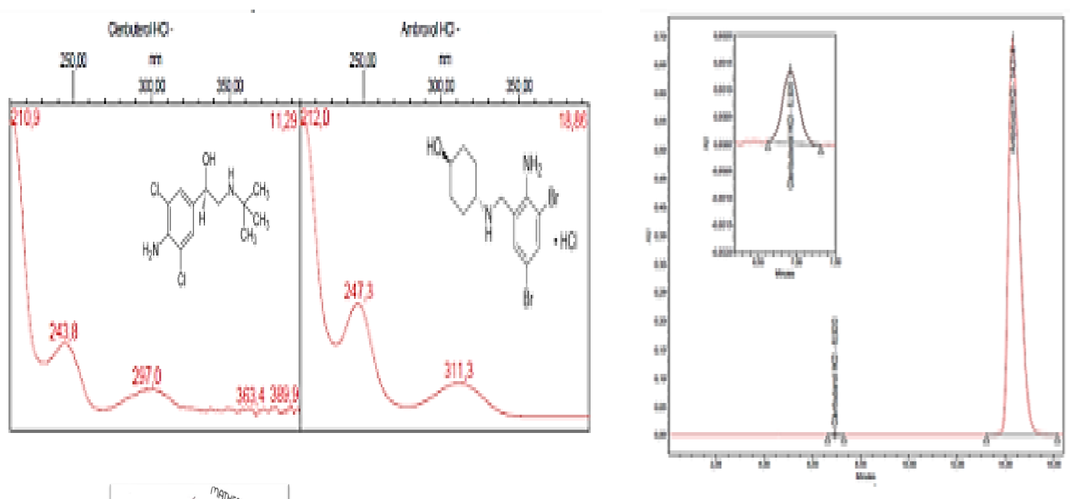


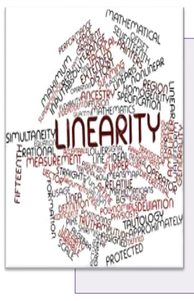


Accuracy			Precision									
	Ambroxol	Clenbuterol	Repeatability				Intermediate precision					
							Ambroxol (mg/5 mL)			Clenbuterol (mg/5 mL)		
Level	Recovery (%)/RSDR	Recovery (%)/RSDR	Replica	Ambroxol (mg/5 mL)	Clenbuterol (mg/5 mL)	Replica	Days					
							1	2	3	1	2	3
70	98.23 (0.20%)	99.06 (0.38%)	1	14.69/0.50	9.70/0.63	1	14.20	14.37	14.00	9.90	9.70	10.35
			2	14.71/0.29	9.40/0.45		14.29	14.27	13.92	10.20	9.50	10.27
100	99.16 (1.63%)	100.86 (0.61%)	3	14.68/0.07	9.27/0.13	2	14.09	14.03	13.93	10.10	9.81	10.33
			4	14.61/0.15	9.31/1.06		14.14	14.08	14.06	10.17	9.67	10.30
130	98.24 (0.35%)	99.76 (0.24%)	5	14.72/0.74	9.52/2.33	3	14.26	14.09	13.85	10.17	9.56	10.17
			6	14.54/0.32	9.36/0.09		14.21	14.09	13.86	10.23	9.71	10.44
			Mean/RSDR	14.66/0.47	9.43/1.68		14.20	14.37	14.00	9.90	9.70	10.35
RSDR= Relative standard deviation			% declared	97.33%	94.3%	Mean/ RSDR	14.20/ 0.49	14.15/ 1.01	13.94/ 0.53	10.13/ 0.75	9.66/ 0.76	10.31/ 0.05
						% declared	94.66	94.14	92.90	102.28	96.56	103.06

Results

Figure 1. Chromatogram and spectra of Ambroxol and clenbuterol





A high tendency to linearity was found between the responses of the areas by variation of the concentration for ambroxol and clenbuterol, with R values in ranges of (0.9993-0.9995) and (0.9989-0.9992), respectively.

Accuracy measured as percentage of recovery for ambroxol was found between 68.76% and 127.71% and for clenbuterol between 69.34% and 129.69% for fortified concentration levels between 70% and 130%, results that are within the limits of the acceptance criteria ranging from 98.0% to 102.0% for each level, as established in the ICH.

The results of RSDR were less than 2%, which indicates that the precision evaluated through the repeatability of the method is within the limits established for chromatographic.

References

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