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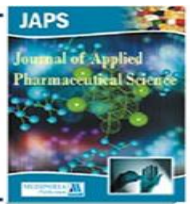

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Method Development and Validation of Levosalbutamol in Pure and Tablet Dosage Form by RP-HPLC

Narendra Nyola, Govinda Samy Jeyabalan, Garima Yadav, Rajesh Yadav, Subash Gupta and Habibullah Khalilullah

Narendra Nyola
Department of Pharmaceutical Sciences, Shridhar University, Pilani, India

Govinda Samy Jeyabalan, Garima Yadav, Rajesh Yadav, Habibullah Khalilullah
Department of Pharmaceutical Sciences, Alwar Pharmacy College, Alwar, India.

Subash Gupta
Oasis Test House Limited, Jaipur, India

For Correspondence
Narendra Nyola
Department of Pharmaceutical Sciences, Shridhar University, Pilani, India.

ABSTRACT

A simple, rapid and accurate RP-HPLC method was developed for the determination of levosalbutamol in pure and tablet dosage form by RP-HPLC method using C_{18} BDS column (Phenomenex, 250 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase consisted of Acetonitrile and buffer in the ratio of 20:80 (v/v) was used and maintain the pH 3. The flow rate was maintained at 1 mL/min and the injection volume was 20 µL . Detection wavelength with UV detector at 276 nm and run time was kept 10 min. The retention time of levosalbutamol was 5.4 min. The method was linear over the concentration range 7-12 µg/ml. The recovery was found to be 100.44± 0.27%. The validation of method was carried out utilizing ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations.

Keywords: HPLC method, Levosalbutamol, Method development.

INTRODUCTION

Levosalbutamol (LVS) is the R - enantiomer of short acting β_2-adrenergic receptor agonist of Salbutamol. Chemical it is 4[(1R)-2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol and its Molecular formula $C_{13}H_{21}NO_3$, Molecular wt. 239.311 g/mol. As a bronchodilator, it is used to treat asthma and COPD (Gilman AG and Limbird LE, 2001; Milgrom H, 2006). Literature survey reveals that, only few spectrophotometric (Dave HN *et al.*, 2007; Basavaiah K *et al.*, 2007; Arun K *et al.*, 2010; Thulasamma P *et al.*, 2011) and bio-analytical methods by HPLC was found using human plasma (Ghulam Murtaza *et al.*, 2009; McCarthy *et al.*, 1993), urine (De Groof J *et al.*, 1991) , blood (Black SB and Hansson RC, 1999) and biological fluids (Girault J *et al.*, 1991) for the quantitative estimation of Levosalbutamol sulphate in bulk and pharmaceutical formulations have been developed.

Hence an attempt has been made to develop new HPLC methods for its estimation in bulk and pharmaceutical formulation with good accuracy, simplicity and precision.

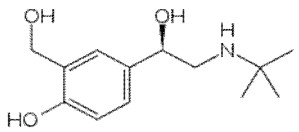

Fig. 1: Levosalbutamol.

MATERIAL AND METHOD

Instrumentation and Materials

The liquid chromatographic system consists of shimadzu 20 AT UFLC with UV-VIS detector, binary pump and rheodyne injector valve with 20 µl fixed loop. The analytes were monitored at 276 nm. Chromatographic analysis was performed on Phenomenex C_{18} BDS column having 250 mm× 4.6 mm i.d. and 5 µm particle size. Chramotagram was automatically obtained by spinchrome system software.

Reagents and Materials

All chemicals and reagents were used of AR grade.

Chromatographic Conditions

The Phenomnex C_{18} column BDS (250 x 4.6mm, 5µm) equilibrated with mobile phase Acetonitrile and buffer in the ratio of 20:80 (v/v) was used and maintain the pH 3. The flow rate was maintained at 1 mL/min. Detection wavelength with UV detector at 276 nm, and the injection volume was 20 µL and run time was kept 10 min.

Preparation of phsophate buffer solution

To prepare buffer solution 6.8 gm. of Potassium Di hydrogen phosphate (KH_2PO_4) was weighed accurately and was dissolved in 1000 mL HPLC grade water. After that its pH was adjusted to pH 3 with the help of ortho phosphoric acid.

Preparation of mobile phase

The mobile phase was prepared by taking 20% Acetonitrile and 80% Buffer (KH_2PO_4) at pH-3. It was filtered through 0.22µm Millipore filter and degassed under ultrasonic bath prior use. The mobile phase was pumped through the column to stabilize the column.

Preparation of stock solution

100 mg LVS drug was weighed accurately and it was dissolved in the mobile phase and after complete dissolution the volume was made up to 100 ml. The stock solution was prepared.

VALIDATION OF METHOD DEVELOPED BY RP-HPLC

Specificity

The specificity of the proposed method was determined by comparing the results obtained by running the standard solution and placebo solution with standard.

Preparation and running of Placebo

Colloidal silicon dioxide IP 2 mg, lactose IP 50 mg, magnesium stearate 2 mg, microcrystalline cellulose IP 50 mg, sodium starch glycolate IP 5 mg, starch IP 55 mg, talc IP 5 mg and titanium dioxide 1 mg were transferred to a 100 mL volumetric flask and about 20 mL mobile phase was added .This mixture was sonicated to dissolve the contents and the volume was made up to the mark with mobile phase. 4 mL of this solution was pipetted into a 10 mL volumetric flask and 6 mL of standard stock solution (1000 ppm) was added to it. The resultant solution was filtered through a 0.22µm Millipore filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The data are shown in table below.

Table. 1: Specificity.

S. No.	Peak area Before Spiking (µV*sec)*	Conc. Before spiking (µg/mL)	Peak area after spiking (µV*sec)*	Conc. After spiking (µg/mL)	% Interference
1	534.363	100.80	602.422	100.77	0.029
2	534.680	100.81	602.380	100.76	0.049
3	534.378	100.82	602.131	100.74	0.079
				Mean	0.052

Linearity and Range

Preparation of calibration curve for LVS

3, 4,5,6,7 and 8 mL of the stock solutions of LVS (1000 µg/mL) was transferred to a series of six 50 mL volumetric flasks. The volume in each flask was adjusted to 50 mL with mobile phase and mixed so as to obtain a final concentration in the range of about 60 to 160 µg/ml. The solutions were filtered through a 0.2µm Millipore filter and degassed under ultrasonic bath prior to use. The solution was injected into HPLC system. The run time was 10 min and the peak areas were measured. The calibration curve data are shown below in Fig 2.

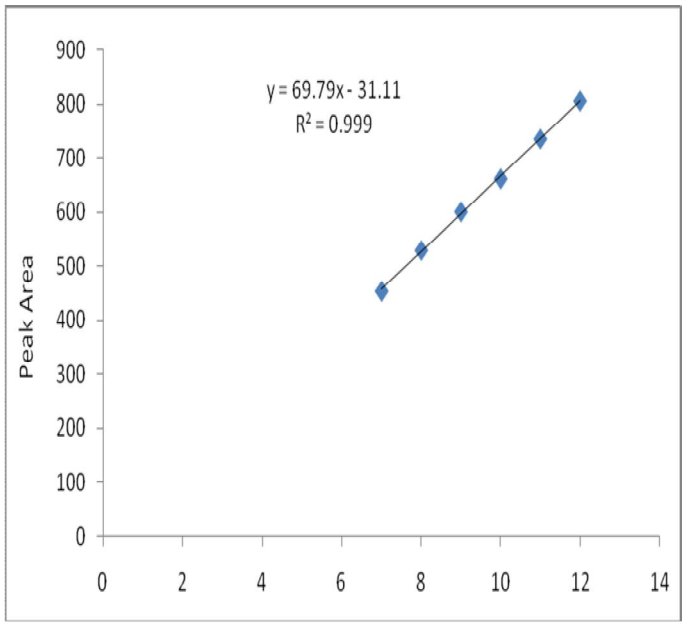

Fig. 2: Calibration curve of LVS.

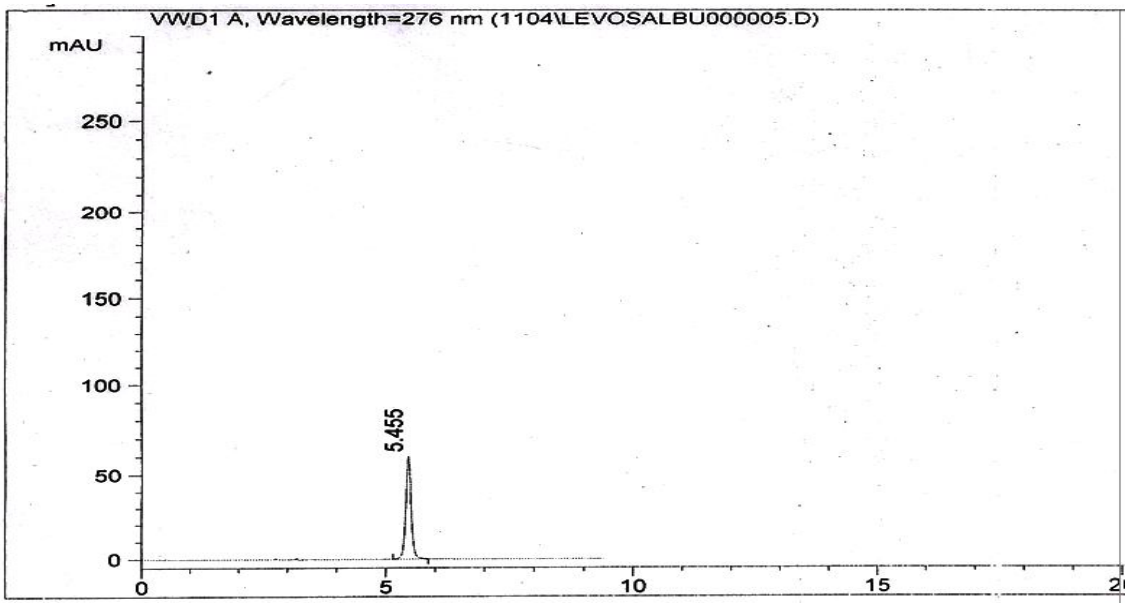

Fig. 3: Chromatogram of LVS.

Accuracy

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 80%, 100% and 120% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

Table. 2: Accuracy.

S. No.	% Recovery study	Conc. Before spiking (µg/mL)	Reference Std. added (µg/mL)*	Peak area (µV*sec)*	Conc. after spiking (µg/mL)*	% Recovery
1	80	60	40	453.874	80.10	100.13
2	100	60	60	454.140	100.56	100.56
3	120	60	80	453.982	120.80	100.67
					Mean ±SD	100.44± 0.27

*Average triplicate readings

Precision

Repeatability

Repeatability was assessed using five determinations at 100 percent of the test concentration i.e. 100 µg/mL of LVS. Data were subjected to statistical treatment for the calculation of SD and RSD. The data are shown in table no. 3.

Table. 3: precision (Repeatability).

S. No	Conc. (µg/mL)	Peak Area (µV*sec)
1	10	661.695
2	10	662.037
3	10	661.514
4	10	661.617
5	10	663.300
	Mean	662.032
	SD	0.735
	%RSD	0.111

* Mean of three determinations

Robustness

The robustness was determined by injecting triplicate injections of standard and three-sample solutions in single at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions; flow rate (±0.1 mL/min), temperature (±5 ˚C) and change in pH of buffer (±0.1). Sample solution was injected in each condition. The data are shown in table below.

Table. 4 : Robustness.

S. No	CONTROL	FLOW RATE 1.1mL/min	FLOW RATE 1.3mL/min	pH 3 - 0.1 pH	pH 6 + 0.1 ph
1	401.45	401.56	401.39	401.51	401.42
2	402.19	402.26	402.14	402.22	402.17
3	401.91	401.89	401.46	401.94	401.89
MEAN	401.85	401.90	401.66	401.89	401.83
SD	0.373	0.35	0.41	0.357	0.379
RSD	0.093	0.087	0.10	0.09	0.09

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in table below.

Table 5: System Suitability Parameter.

S.No.	Parameters	Limit	Results
1	Injection precision	RSD < 1% for n≥5	0.111 % n=5
2	Tailing factor	T ≤ 2	1.33
3	Theoretical plate	N > 2000	2088
4	Retention time	5.4	

Estimation of LVS in tablet dosage form

Twenty tablets were taken. The average weight of the 20 tablets was taken. Equivalent to 50 mg of LVS of powder (i.e. 50 mg) was taken and transferred to a 50 mL volumetric flask and about 25 mL of mobile phase was added and sonicated to dissolve drug. The volume was made up to the mark with mobile Phase. The solution was filtered through a membrane filter (0.22 µm) and

sonicated to degas. The solution prepared was injected in triplicate into the HPLC system and the observations were recorded. Column I of table shows the peak area, column II of table shows labeled quantity.

Table. 6: Estimation of LVS in Tablet Dosage Form.

S. No.	Peak area (µV*sec)	Labeled quantity in Tab. (mg/tab.)	Quantity found in (mg/tab.)	Percent of drug
1	664.192	400	413.48	103.37
2	664.750	400	413.53	103.38
3	665.193	400	413.24	103.31
			Mean	103.35

RESULT & DISCUSSION

Specificity

The mean percent interference was found to be 0.052, which is shown in column V. The comparison of the data of the drug solution before spiking and the spiked drug solution revealed that there was no significant interference of placebo with the recovery of LVS, inferring that the method was specific.

Linearity and Range

The method was found to be linear. In the linearity study, regression equation and coefficient of correlation for LVS was found to be $y = 69.793x + 31.117$, $r^2 = 0.9992$.

Accuracy

The mean recovery was found to be 100.44%. The limit for mean recovery is 90-110%. Thus the method was found to be accurate.

Precision

Repeatability

The repeatability study which was conducted on the solution having the concentration of about 10 µg/mL for LVS (n = 5) showed a RSD of 0.111% for LVS. It was concluded that the analytical technique showed good repeatability.

Intermediate precision

The intra-day and inter-day precision study, which were conducted, showed a RSD of 0.483% for LVS intraday analysis thus the data showed that the RSD was below 2% inferring that the analytical technique had a good intraday and Interday precision.

Robustness

This method is robust for the analysis of LVS within the specified range of deviations in the experimental conditions.

Assay

The percent content of LVS in tablet was found to be 103.35%.

CONCLUSION

The developed method was validated as per ICH guideline and was found to be within the prescribed limit. It concludes that the developed methods are simple, accurate, sensitive and precise and suitable for both authentic and pharmaceutical dosage form.

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REFERENCES

Arun K., Mishra, Manoj Kumar, Amrita Mishra, Anurag Verma, Pronobesh Chattopadhyay. Validated UV spectroscopic method for estimation of salbutamol from tablet formulations. Arch of App Sci. 2010; 2(3): 207-211.

Basavaiah K., Somashekar BC., Ramakrishna V. Rapid titrimetric and spectrophotometric methods for salbutamol sulphate in pharmaceuticals using N-bromosuccinimide. Acta Pharm. 2007; 57(1): 87-98.

Black SB, Hansson RC. Determination of Salbutamol and detection of other β-agonists in human posmortem whole blood and urine by GC-MS-SIM. J Anal Toxicol. 1999; 23:113-118.

Dave HN, Mashru RC, Thakkar AR. Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. Anal Chem Acta. 2007; 597(1): 113-120.

De Groof J, Degroodt JM, Wyhowski de Bukanshi B, Beernaert H. Salbutamol identification in liver and urine by high performance thin layer chromatography and densitometer. Lebensm Unters Forsch. 1991; 193(2): 126-129.

Mc Carthy PT, Atwal S, Sykes AP, Ayres JG. Measurement of terbutaline and salbutamol in plasma by high performance liquid chromatography with fluorescence detection. Biomed Chromatogr. 1993; 7(1): 25-28.

Milgrom H. LVS in the treatment of asthma. Expert Opin Pharmacother. 2006; 7(12): 1659-1668.

Thulasamma P, Kishore Kumar R, Venkateswarulu. Development of new spectrophotometric methods for the estimation of LVS in tablet dosage forms. Analytical chemistry. 2009; 8(4).

Ghulam Murtaza, Mahmood Ahmad, Muhammad Asadullah Madni, Muhammad Waheed Asghar. A new reverse phase HPLC method with fluorescent detection for the determination of Salbutamol sulphate in human plasma. *Bull Chem Soc Ethiop*. 2009; 23(1): 1-8.

Gilman AG and Limbird LE. Goodman and Gilman's the pharmacological Basis of Therapeutics, New York and Mc Graw Hill, 2001; 11: 720

ICH, Harmonised Tripartite Guideline. Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, 1994; 1-5.