

Synthesis and Characterization of Maillard Reaction Products of Salbutamol and Terbutaline with Lactose and Development and Validation of an LC Method for the Determination of Salbutamol and Terbutaline in the Presence of These Impurities

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ABSTRACT: Being secondary amines, both salbutamol (SLB) and terbutaline (TRB) react by Maillard reaction (MR) with lactose, which is added as an inactive ingredient in tablets. The Amadori rearrangement products were synthesized, isolated, and characterized by mass spectrometry. In addition, a simple, selective, and precise reversed-phase liquid chromatography (LC) method was developed and validated for the determination of SLB and TRB in tablets, each in the presence of its MR impurity. The chromatographic separation was performed on a Symmetry® Waters C18 column (150 mm × 4.6 mm, 5 μm) using a mobile phase consisting of 0.5% aqueous phosphoric acid to acetonitrile (90:10, v/v) at a flow rate of 0.7 mL minute⁻¹. Quantitation was achieved using UV detection at 230 nm. Linearity, accuracy, and precision were found to be acceptable for the determination of SLB and TRB in the concentration range of 0.2–60 and 0.5–80 μg mL⁻¹, respectively. The proposed method was successfully applied to the determination of SLB and TRB in bulk and in their tablets.

KEYWORDS: salbutamol, terbutaline, reversed-phase liquid chromatography, lactose, Maillard reaction impurities, Amadori rearrangement

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Introduction

Salbutamol (SLB), (RS)-4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol (Fig. 1), and terbutaline (TRB), (RS)-5-[2-(tert-butylamino)-1-hydroxyethyl]benzene-1,3-diol (Fig. 2), are two well-known β₂-adrenergic receptor agonists used for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease.¹

The reaction between amino compounds and reducing sugars is known as Maillard reaction (MR), and the chemical pathways of the MR are very complicated. The majority of “small molecule” active pharmaceutical ingredient instability reactions occur via hydrolysis, oxidation, and MR.² Drugs that are secondary amines, not just primary amines, are reported to undergo MR with lactose under pharmaceutically related conditions.

Hodge presented it as a multistage reaction series in which each stage depends on a number of controlling factors such as temperature, pH, and nature of reactants.³ High temperature and high pH support a quick completion of the reaction. However, degradation products formed by MR have also been observed under less extreme conditions in pharmaceutical products containing both lactose, an excipient commonly used in solid dosage formulations,⁴ and drug substances containing amino groups, such as fluoxetine hydrochloride,⁵ aminophylline, amlodipine, acyclovir,² and baclofen.⁶

Formation of MR impurities was also reported in memantine tablets,⁷ and they were simultaneously determined with memantine in tablets using LC with charged aerosol detector.⁸

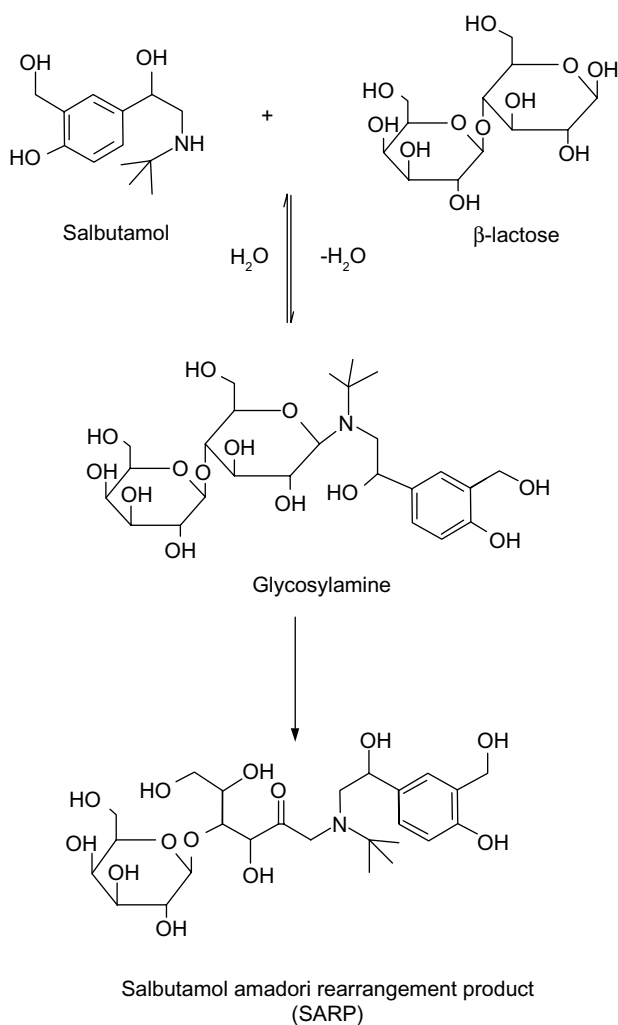


Figure 1. A schematic diagram showing MR between lactose and SLB by refluxing with triethylamine in dimethylacetamide and ethanol.

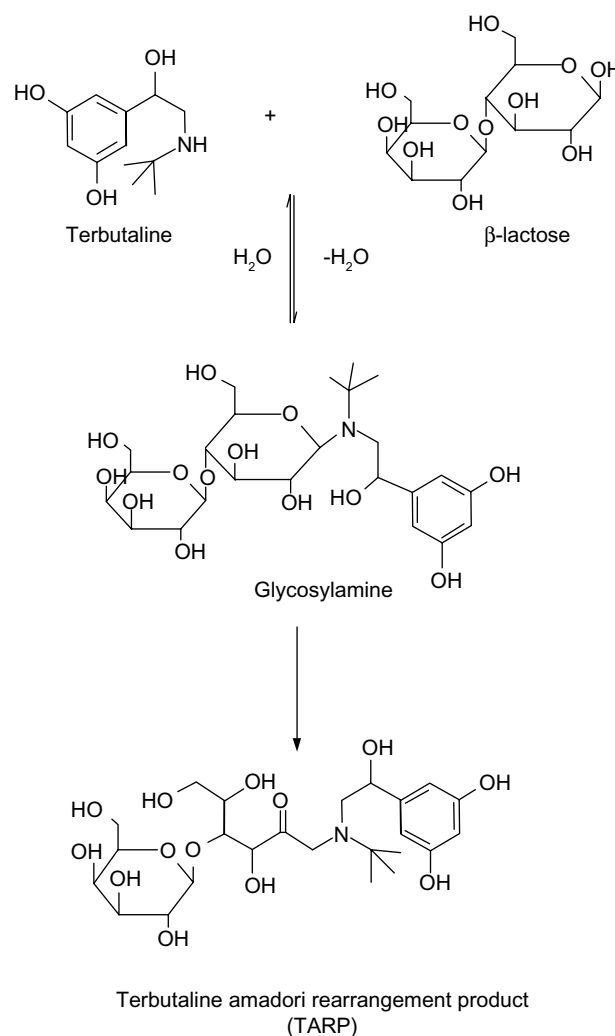


Figure 2. A schematic diagram showing MR between lactose and TRB by refluxing with triethylamine in dimethylacetamide and ethanol.

Several analytical methods have been described for the determination of SLB in pharmaceutical and biological samples. These methods include spectrophotometry,^{9–12} LC,^{13,14} LC–MS/MS,¹⁵ capillary electrophoresis,^{16–18} and TLC.¹⁹ TRB has been analyzed by spectrophotometry,^{20,21} LC,^{22–25} capillary electrophoresis,²⁶ and HPTLC.²⁷

Survey of many generic formulations of SLB and TRB revealed the presence of lactose as the most common excipient, which makes the formulation less stable than those including starch as diluent because of the possibility of MR between each of the two drugs, being secondary amines, and being lactose. Based on this knowledge, the aim of this work was to synthesize MR adducts of both SLB and TRB with lactose (SARP and TARP, respectively) and to elucidate their chemical structures by different spectroscopic techniques. The method of Wirth et al.⁵ to prepare fluoxetine adduct was adapted to SLB and TRB. In addition, a sensitive, precise, and accurate LC method was to be developed and validated for the determination of these β_2 -adrenergic agonists in tablets, each spiked with its MR impurity. A schematic diagram showing

the MR between lactose and SLB or TRB is presented in Figures 1 and 2. No reported method has described the separation of the cited drugs from their adduct impurities, which is the aim of the present investigation.

Experimental

Instrumentation. The HPLC system consisted of Shimadzu LC-20AT Liquid Chromatograph (Japan) using a Symmetry[®] Waters C18 column (150 mm \times 4.6 mm, 5 μ m). The system was equipped with a UV–visible detector (SPD-20A, Japan) and an autosampler (SIL-20A, Shimadzu, Japan). An Elma S100 ultrasonic processor model KBK 4200 (Germany) was used for the degassing of the mobile phases. Mass spectra were performed on Shimadzu GCMS-QP1000EX mass spectrophotometer (Kyoto, Japan).

Materials and reagents. Pharmaceutical grade SLB, lactose monohydrate, and ventolin tablets, nominally containing SLB equivalent to 2 mg of SLB and 139.8 mg of lactose monohydrate per tablet, were supplied by GlaxoSmithKline Company (Cairo, Egypt). Pharmaceutical grade TRB and

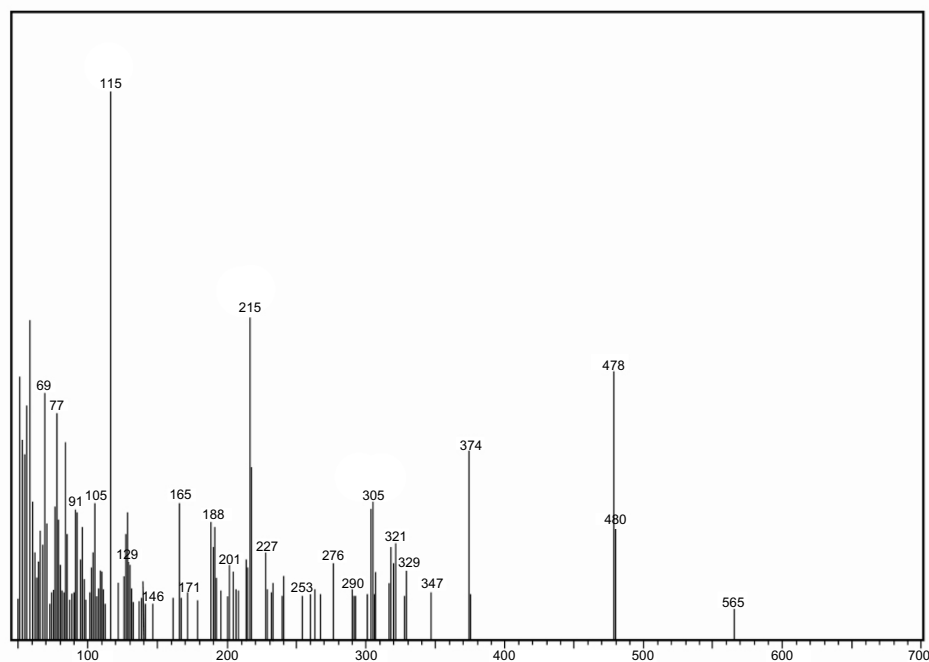


Figure 3. Mass spectrum of SARP.

Aironyl tablets, nominally containing 2.5 mg of TRB and 97.1 mg of lactose monohydrate per tablet, were supplied by SEDICO Pharmaceutical Company (6 October City, Egypt). Acetonitrile (HiPerSolv) was purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Orthophosphoric acid (85%) was obtained from VWR Chemicals (Pool, England). Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters (0.45 μm) from Teknokroma

(Barcelona, Spain) were used. *N,N*-Dimethylacetamide and triethylamine were purchased from Loba Chemie Pvt. Ltd. (Bombay, India). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Preparation of lactose-salbutamol (SARP) and lactose-terbutaline (TARP) Amadori rearrangement products. In a 100 mL round-bottom flask, 3 g of lactose monohydrate (8.325 mmol) and 1 g of SLB (4.176 mmol)

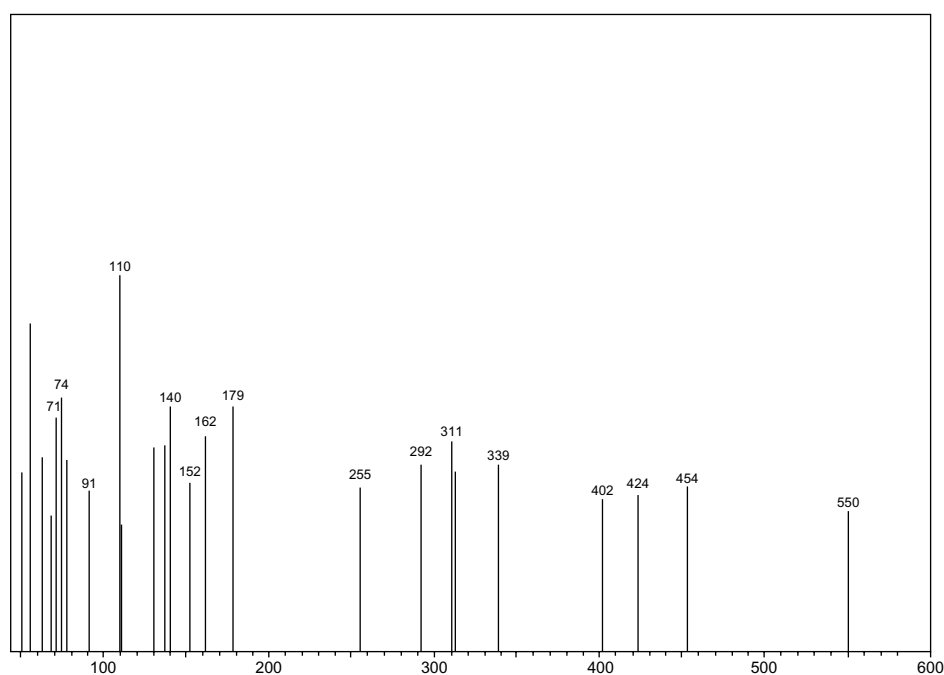


Figure 4. Mass spectrum of TARP.

or 1 g of TRB (3.64 mmol) were dissolved in 50 mL of ethanol. In all, 24 mL of dimethylacetamide and 0.6 mL of triethylamine (4.305 mmol) were added. The mixture was refluxed stirring for 48 hours. The reaction mixture was cooled and filtered, and the residue was washed with 25 mL of ethanol. The filtrate was evaporated under vacuo to a thick solution. A total of 30 mL of ethyl acetate, 30 mL of 20% aqueous sodium chloride, and 10 mL of ether were added. The pH was adjusted to 11 with 50% sodium hydroxide. The layers were separated, and the bottom layer was discarded and the upper layer was set aside. To the middle layer, 30 mL of ethyl acetate and 10 mL of ether were added. The layers were separated, and the combined organic layers were evaporated under vacuo, to which were added 20 mL of 20% aqueous sodium chloride, 40 mL of water, and 60 mL of chloroform. The pH was adjusted to 1.5 with concentrated HCl. The layers were separated, and to the aqueous layer were added 6 g of NaCl, 50 mL of ethyl acetate, and 10 mL of ether. The pH was adjusted to 10.6 with 50% NaOH, and the layers were separated. The ethyl

acetate layer was evaporated in vacuum to give a residue of 1.8 g of SARP or 2.3 g of TARP.

Chromatographic conditions. Chromatographic separation was achieved on a Symmetry Waters C18 column (150 mm × 4.6 mm, 5 μm). Isocratic elution using a mobile phase consisting of 0.5% aqueous phosphoric acid and acetonitrile (90:10, *v/v*) at a flow rate of 0.7 mL minute⁻¹ was applied. Quantitation was achieved using UV detection at 230 nm. The acidic aqueous solution was filtered through 0.45 μm membrane filter and degassed for 30 minutes in an ultrasonic bath before use. Analyses were performed at ambient temperature, and the injection volume was 25 μL.

Standard solutions. Standard stock solutions of each drug (1 mg mL⁻¹) were prepared by dissolving 100 mg of the drug in methanol in a 100 mL volumetric flask and then completed to volume with methanol. Standard stock solutions, SARP and TARP impurities (1 mg mL⁻¹), were prepared by dissolving 100 mg of the prepared adduct in methanol in a 100 mL volumetric flask and then completed to volume with methanol. Then required concentrations were prepared by serial dilutions

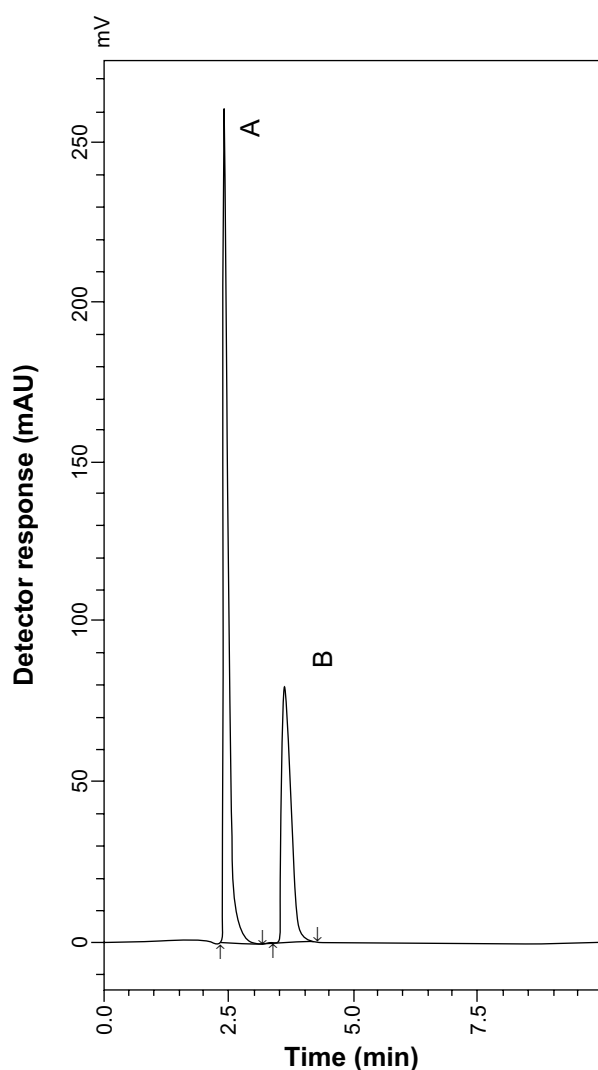


Figure 5. HPLC chromatogram of SLB sulfate (10 μg mL⁻¹) (A) and SARP (3 μg mL⁻¹) (B).

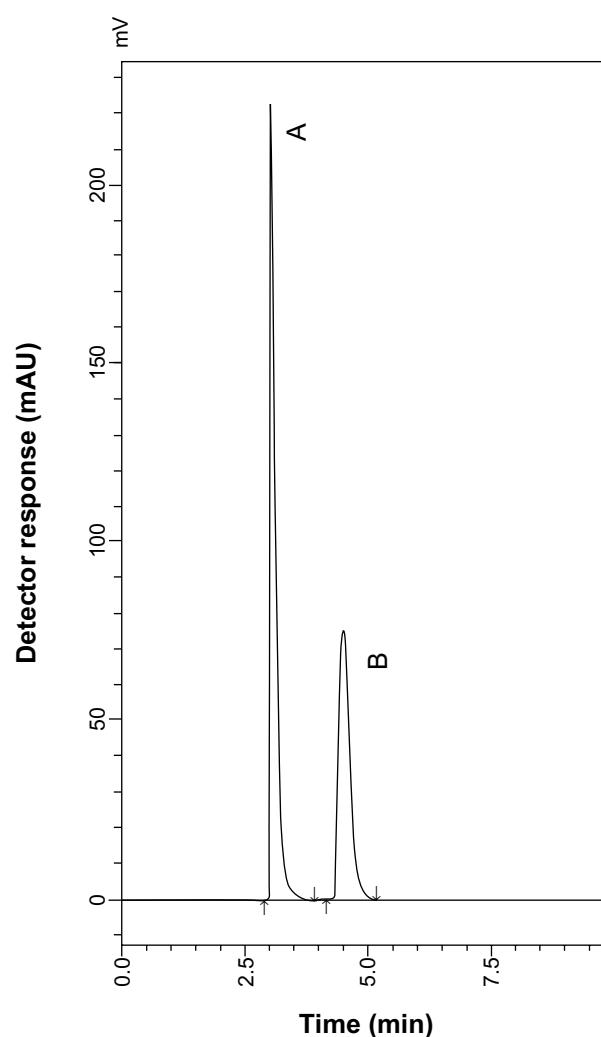


Figure 6. HPLC chromatogram of TRB sulfate (10 μg mL⁻¹) (A) and TARP (3 μg mL⁻¹) (B).

**Table 1.** System suitability tests for LC method for the determination of SLB and TRB in the presence of their MR impurities.

ITEM	SLB	TRB
N	2829	2827
T	1.03	1.05
%RSD of six injections of		
Peak area	0.78	0.93
Retention time	0.11	0.31

with methanol of these stock solutions. Laboratory-prepared mixtures were prepared by spiking standard solutions of SLB and TRB with their lactose-adducts prepared in Section 2.3.

Sample preparations. A total of 20 tablets of each drug were weighed. An accurately weighed amount of the finely powdered ventolin tablets equivalent to 24 mg of SLB base (28.8 mg SLB) and Aironyl tablets equivalent to 20 mg of TRB were separately made up to 100 mL with methanol. The solutions were sonicated for five minutes to dissolve and filtered followed by serial dilution to the required concentrations for each experiment.

Results and Discussion

Preparation and characterization of SARP and TARP.

The MR between SLB and TRB with lactose to produce the SARP or TARP, respectively, is shown in Figures 1 and 2. As reviewed in literature, preparation of Amadori rearrangement product of fluoxetine, which is a secondary amine drug, can be achieved by reaction of lactose with fluoxetine hydrochloride in *N,N*-dimethylacetamide and ethanol as solvent with triethylamine as the base.⁵ Following the same published procedure, SARP or TARP was obtained by reaction of lactose with SLB

or TRB. It was purified by extractions. Mass spectrometry confirmed the chemical structures and indicated the molecular weight to be 563 ($M + 2H^+$, m/z 565) for SARP (Fig. 3) and 549 ($M + H^+$, m/z 550) for TARP (Fig. 4).

LC method development. During the optimization cycle, various reversed-phase columns, isocratic mobile phase systems, and pH values of the buffer were attempted. To detect each of SLB and TRB and their MR impurity with sufficient peak intensity, a wavelength of 230 nm was selected. It was found that at least 90% of aqueous phase was needed to elute all peaks within 10 minutes because of relatively high polarity of the drugs and their lactose-adducts. Isocratic elution based on a mobile phase consisting of 0.5% aqueous phosphoric acid and acetonitrile (90:10, *v/v*) at a flow rate of 0.7 mL minute^{-1} was applied. The retention times were 2.46 and 3.66 minutes for SLB and SARP, respectively, as shown in Figure 5, and the retention times were 3.08 and 4.52 minutes for TRB and TARP, respectively, as shown in Figure 6.

System suitability tests. System suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method.²⁸ %RSD of peak area for six injections of a solution of 30 or 40 $\mu\text{g mL}^{-1}$ (100% concentration) for SLB or TRB, respectively, was used to test repeatability, and %RSD of retention time to test retention time reproducibility. The results of these tests for the proposed method are listed in Table 1. Drugs under investigation were well resolved from their MR impurities as shown in Figures 5 and 6. Resolution factors were found to be 2.404 between SLB and SARP and 2.403 between TRB and TARP.

Method validation. Linearity. In this study, eight concentrations were chosen for each drug. Each concentration was

Table 2. Results obtained by LC method for the determination of SLB and TRB in the presence of their MR impurities.

ITEM	SLB	TRB
Retention time (minute)	2.46	3.08
Wavelength of detection (nm)	230	230
Linearity		
Range of linearity ($\mu\text{g mL}^{-1}$)	0.20–60	0.5–80
Regression equation	$\text{Area} = 233051C_{\mu\text{g mL}^{-1}} - 366156$	$\text{Area} = 244255C_{\mu\text{g mL}^{-1}} - 526308$
Regression coefficient (r^2)	0.9981	0.9973
LOD (ng mL^{-1})	33.71	70.96
LOQ (ng mL^{-1})	112.36	236.50
Precision		
Intra-day %RSD	0.33–0.41	0.18–1.60
Inter-day %RSD	0.85–1.64	0.28–1.36
Drug in dosage form	102.83 ± 0.34	99.33 ± 0.53
Accuracy		
Drug in bulk	99.88 ± 0.48	100.24 ± 0.75

Abbreviations: LOD: limit of detection; LOQ: limit of quantitation.

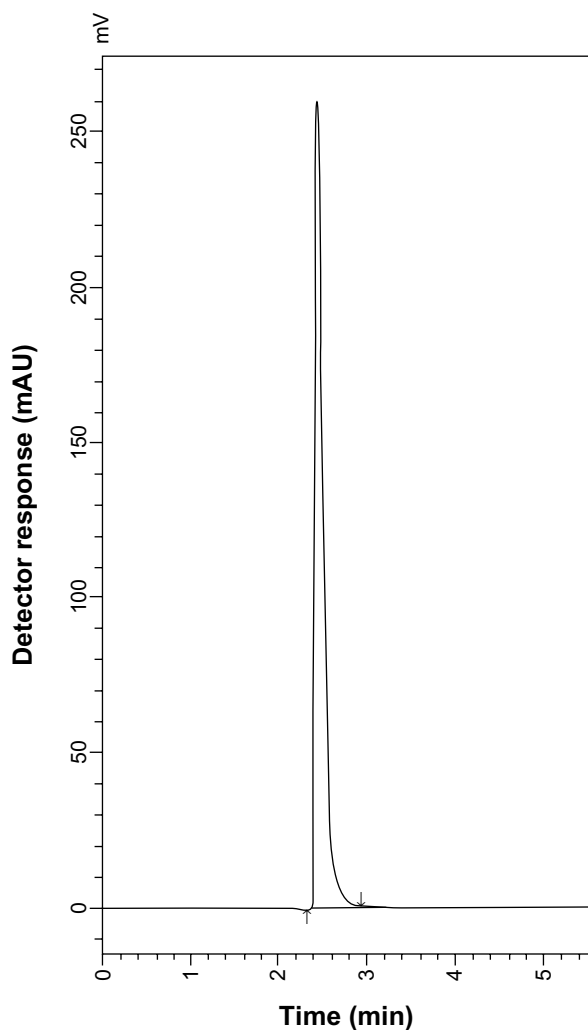


Figure 7. HPLC chromatogram of ventolin tablets containing $10 \mu\text{g mL}^{-1}$ SLB sulfate.

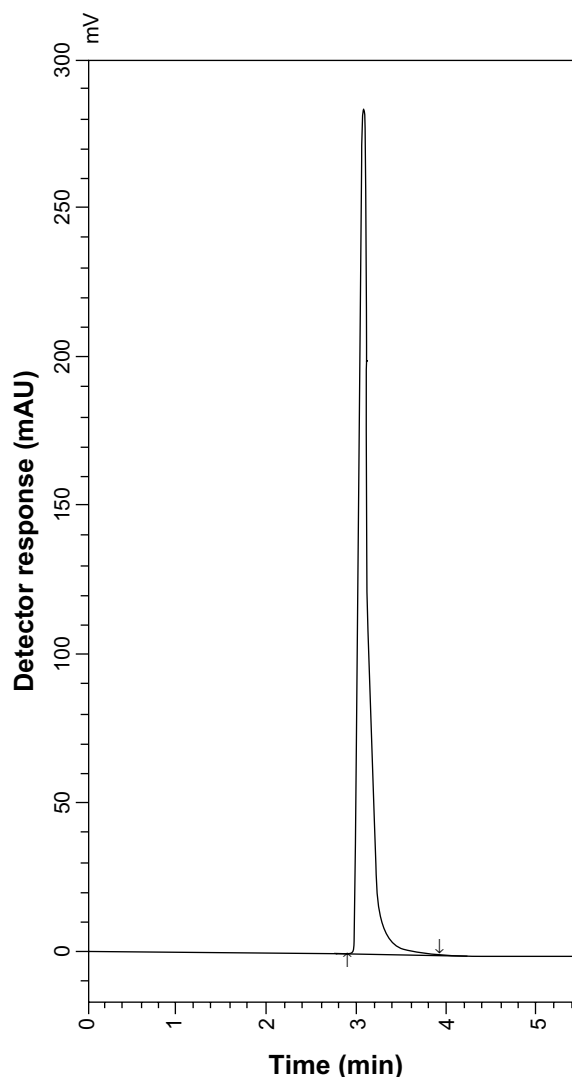


Figure 8. HPLC chromatogram of Aironyl tablets containing $10 \mu\text{g mL}^{-1}$ TRB sulfate.

repeated three times. Good linearity of the calibration curves was verified by the high correlation coefficient (Table 2).

Accuracy. Accuracy of the results was calculated by percentage recovery of five different concentrations of SLB and TRB standard solutions spiked with different quantities of SARP and TARP impurities in standard solutions ($3\text{--}5$ and $3\text{--}7 \mu\text{g mL}^{-1}$), respectively ($10\text{--}30\%$) (w/w). Triplicate sample preparations were made at each concentration level. The results obtained including the mean of the recoveries and standard deviation are displayed in Table 2 indicating good accuracy of the proposed method.

Precision. For SLB and TRB, repeatability of the method was assessed by analyzing a standard solution containing 30 and $40 \mu\text{g mL}^{-1}$, respectively ($n = 6$). In addition, intra-day and inter-day precision (using three different concentrations in triplicates for three consecutive days) was also carried out. The three concentrations applied are ($24\text{--}30\text{--}36 \mu\text{g mL}^{-1}$) for SLB and ($32\text{--}40\text{--}48 \mu\text{g mL}^{-1}$) for TRB, representing $80\text{--}100\text{--}120\%$, respectively. The values of %RSD of repeatability and intra-day and inter-day

precision were all found to be less than 2% . The results are displayed in Tables 1 and 2.

Specificity. Good resolution and absence of interference from any of the MR impurities are shown in Figures 5 and 6. Also, the chromatograms of the pharmaceutical formulation samples were checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention times of each of the examined drugs after extraction of the active ingredient (Figs. 7 and 8). In addition, the chromatograms of SLB and TRB in the sample solution were found to be identical to the chromatogram obtained by the standard solutions.

Limit of detection (LOD) and limit of quantitation (LOQ). Limit of detection that represents the concentration of analyte at S/N ratio of 3 and limit of quantification at which S/N ratio is 10 were determined experimentally for the proposed methods, and the results are given in Table 2.

Robustness. Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of



the mobile phase was changed from 0.7 to 0.6 and 0.8 mL minute⁻¹. The organic strength was varied by $\pm 2\%$. It was found that these variations did not affect the chromatographic resolution significantly when the proposed LC method was applied for the determination of SLB and TRB, each in the presence of its Maillard impurity.

Conclusion

The MR products of SLB and TRB with lactose were synthesized, and their chemical structures were elucidated. The proposed LC method has the advantages of simplicity, precision, accuracy, and convenience for the separation and quantitation of SLB and TRB. In addition, it is suitable for the simultaneous determination of their binary mixtures with their MR impurities. Hence, the proposed LC method can be used for the quality control of the cited drugs in ordinary laboratories.

Author Contributions

Conceived and designed the experiments: MF, AE, EE. Analyzed the data: MF, AE, EE. Wrote the first draft of the manuscript: MF, AE, EE. Contributed to the writing of the manuscript: MF, AE, EE. Agree with manuscript results and conclusions: MF, AE, EE. Jointly developed the structure and arguments for the paper: MF, AE, EE. Made critical revisions and approved final version: MF, AE, EE. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

REFERENCES

- Liu S., Adrenergic agents. In: *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*. John M. Beale, Jr., John H. Block eds. 12th ed., Chapter 16. Philadelphia, PA: Lippincott Williams & Wilkins, a Wolters Kluwer business; 2011:519–57.
- Bharate SS, Bharate SB, Bajaj AN. Incompatibilities of pharmaceutical excipients with active pharmaceutical ingredients: a comprehensive review. *J Excipients Food Chem*. 2010;1:3–26.
- Hodge JE. Dehydrated foods, chemistry of browning reactions in model systems. *J Agric Food Chem*. 1953;1:928–43.
- Wade A, Weller PJ, eds. *Handbook of Pharmaceutical Excipients*. 2nd ed. Washington/London: American Association/The Pharmaceutical Press; 2000.
- Wirth DD, Baertschi SW, Johnson RA, et al. Maillard reaction of lactose and fluoxetine hydrochloride, a secondary amine. *J Pharm Sci*. 1998;87:31–9.
- Monajjemzadeh F, Hassanzadeh D, Valizadeh H, et al. Assessment of feasibility of Maillard reaction between baclofen and lactose by liquid chromatography and tandem mass spectrometry, application to pre formulation studies. *AAPS Pharm-SciTech*. 2009;10:649–59.
- Stable pharmaceutical composition of memantine and process of preparation Thereof, IP.com Prior Art Database, IPCOM/000168000. 2008. <http://ip.com/IPCOM/000168000>. Accessed August 31, 2012.
- Rystov L, Chadwick R, Krock K, Wang T. Simultaneous determination of Maillard reaction impurities in memantine tablets using HPLC with charged aerosol detector. *J Pharm Biomed Anal*. 2011;56:887–94.
- Bakry RS, Razak OA, El Walily AM, Belal SF. Spectrophotometric determination of salbutamol sulphate using chlorinated quinones in the presence or absence of acetaldehyde. *J Pharm Biomed Anal*. 1996;14:357–62.
- Dol I, Knochen M. Flow-injection spectrophotometric determination of salbutamol with 4-aminoantipyrine. *Talanta*. 2004;64:1233–6.
- El-Enany N, Belal F, Rizk M. A simple kinetic spectrophotometric method for the determination of salbutamol in dosage forms. *Chem Anal*. 2004;49:587–99.
- Habib IH, Hassouna MEM, Zaki GA. Simultaneous spectrophotometric determination of salbutamol and bromhexine in tablets. *Il Farmaco*. 2005;60:249–54.
- Jain DK, Patel PN, Kushwaha A, Raghuvanshi RS, Jain N. Simultaneous determination of salbutamol sulphate and doxophylline in tablets by reverse phase liquid chromatography. *Der Pharmacia Lettre*. 2011;3(4):56–62.
- Martis EA, Gangrade DM. Reverse phase isocratic HPLC method for simultaneous estimation of salbutamol sulphate and beclomethasone dipropionate in rotacaps formulation dosage forms. *Int J Pharm Pharm Sci*. 2011;3:64–7.
- Wu J, Ding C, Ge Q, Li Z, Zhou Z, Zhi X. Simultaneous determination of ipratropium and salbutamol in rat plasma by LC–MS/MS and its application to a pharmacokinetic study. *J Chromatogr B Anal Technol Biomed Life Sci*. 2011;879:3475–83.
- Ekiert E, Garcia-Ruiz C, Garcia MA, Marinam ML. Rapid determination of salbutamol in pharmaceutical preparations by chiral capillary electrophoresis. *Electrophoresis*. 2003;24:2680–6.
- Servais A, Fillet M, Mol R, et al. On-line coupling of cyclodextrin mediated nonaqueous capillary electrophoresis to mass spectrometry for the determination of salbutamol enantiomers in urine. *J Pharm Biomed Anal*. 2006;40:752–7.
- Felix FS, Quintino MSM, Carvalho AZ, Coelho LHG, do Lago CL, Angnes L. Determination of salbutamol in syrups by capillary electrophoresis with contactless conductivity detection (CE–C4D). *J Pharm Biomed Anal*. 2006;40:1288–92.
- Dave HN, Mashru RC, Patel AK. Thin layer chromatography method for the determination of ternary mixture containing salbutamol sulphate, bromhexine hydrochloride and etofylline. *J Pharm Sci Res*. 2010;2:143–8.
- Gangwal S, Trivedi, P. Simultaneous determination of terbutaline sulphate, bromhexine hydrochloride and guaiphenesin in three-component tablet formulation by UV spectrophotometry. *Indian J Pharm Sci*. 1999;61:128–30.
- Smith AA, Manavalan R, Sridhar K. Spectrophotometric estimation of terbutaline sulphate in pharmaceutical dosage forms. *Int Res J Pharm*. 2010;1:213–9.
- Herring VL, Johnson JA. Simple method for determination of terbutaline plasma concentration by high-performance liquid chromatography. *J Chromatogr B*. 2000;741:307–12.
- Kim KH, Kim HJ, Kim J, Shin SD. Determination of terbutaline enantiomers in human urine by coupled achiral–chiral high-performance liquid chromatography with fluorescence detection. *J Chromatogr B*. 2001;751:69–77.
- Daraghme N, Al-Omari MM, Sara Z, Badwan AA, Jaber AMY. Determination of terbutaline sulfate and its degradation products in pharmaceutical formulations using LC. *J Pharm Biomed Anal*. 2002;29:927–37.
- Althanyan MS, Assi KH, Clark BJ, Hanaee J. Microemulsion high performance liquid chromatography (MELC) method for the determination of terbutaline in pharmaceutical preparation. *J Pharm Biomed Anal*. 2011;55:397–402.
- Li S, Wang J, Zhao S. Determination of terbutaline sulfate by capillary electrophoresis with chemiluminescence detection. *J Chromatogr B*. 2009;877:155–8.
- Faiyazuddin Md, Rauf A, Ahmad N, et al. A validated HPTLC method for determination of terbutaline sulfate in biological samples: application to pharmacokinetic study. *Saudi Pharm J*. 2011;19:185–91.
- The United States Pharmacopoeia (USP 30), National Formulary (NF 25), Rockville, MD; 2007.