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Development and validation of HPLC methods for the enantioselective analysis of bambuterol and albuterol

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Abstract

Suitable HPLC methods for the direct separation of bambuterol and albuterol enantiomers were developed. The enantioseparation was tested on numerous commercial chiral HPLC columns. For bambuterol the most convenient separation was determined on amylose Chiralpak AD column, and for albuterol on vancomycine Chirobiotic V column. The mobile phase compositions were systematically studied to obtain the optimal chromatographic methods. Validation of methods in selected conditions shows that the chosen methods are selective and precise with linear response of detector for both pairs of enantiomers.

Keywords: Chiral separation; Antiasthmatic drugs; Albuterol; Bambuterol; Chiral columns

1. Introduction

Drugs used for treatment of asthma can be broadly divided into bronchodilatators and prophylactic drugs. In a last few years there is increasing use of bronchodilatators, which include adrenoreceptor agonists (β_2-agonists), while anti-cholinergic drugs are not used so much [1]. There are two types of β_2-agonists, short-acting and long-acting, and each group has it's own advantage. Short-acting β_2-agonists are used individually, most commonly by inhalations, because they are easily released. Long-acting β_2-agonists are suitable for use in treatment of wasting illness. The work here presented deals with two β_2-agonist drugs: short-acting albuterol (salbutamol) and long-acting bambuterol, both with a structure of chiral benzylic alcohol.

Bambuterol, which is inactive at adrenergic receptors, is bisdimethylcarbamate of the β_2-agonist terbutaline. It is commercially available in a form of racemate by name Bambec® (AB Draco) [2]. In organism it is converted to terbutaline via oxidation and hydrolysis. Bambuterol demonstrates hydrolytic stability, an affinity for lung tissue and relative specificity for hydrolysis by butyrylcholinesterase. Studies of the pharmacological effects of terbutaline in animals have shown its relatively moderate effects on heart, circulation and CNS (central nervous system), relative to its pronounced bronchodilatator activity [3].

Albuterol is selective β_2-adrenoceptor agonist developed in 1960's. At that time it offered the significantly improved β_2-stimulation selectivity with the least side effect of any other existing bronchodilatator. Common drawback of many earlier bronchodilatators was their short duration of action, usually 1–4 h [4]. Albuterol extended duration of effectiveness to 6 h and is used to relieve bronchoconstriction in asthma as well as to prevent pre-mature labour during pregnancy. Until 1999, albuterol was on the market only in a form of a racemate, because it was assumed that (*S*)-enantiomer is biologically inactive, and it was considered to be "enantiomeric ballast". Because of that it's presence has been ignored in pharmacokinetic and pharmacodynamic studies [5]. However, recent studies [6] have shown that metabolism of albuterol in humans is stereoselective, with (*R*)-albuterol be-

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ing metabolised many times faster then it's antipode, leading to relatively higher plasma and urinary concentrations of (*S*)-albuterol. Further investigations showed that the (*R*)-isomer is exclusively responsible for the therapeutic effect and perfectly matches the human body's receptor. Although (*S*)-enantiomer shows little or no adrenoceptor activity it can still be, in some extent, bonded to targeted receptor, which can lead to competitive inhibition of pharmacodynamic activity of enantiomer at receptor and many other factors. This can explain the fact that daily dose of enantiomericaly pure (*R*)-albuterol is more than twice smaller then daily dose of racemic albuterol [7]. All the above-mentioned research has lead to FDA's approval of enantiomericaly pure albuterol (Xenoprex) in March 1999.

Several chromatographic methods have been proposed for the enantioresolution of albuterol. Separation can be achieved after chemical derivatisation with different reagents, such as with 2,3,4,6-tetra-*O*-acetyl-α-gluco-pyranosyl isothiocyanate [7] and without derivatisation using chiral stationary phases, such as chiral AGP (α-acid glycoprotein human serum), [8,9] chiral CBH (cellobiohydrolase), [10] Chirex 3022 (Pirkle stationary phase consist of (*S*)-indoline-2-carboxylic acid and (*R*)-1-(α-naphtyl) ethylamine), [11,12] Chirobiotic T (teicoplanine) [13–15] and Chiralcel OJ [16]. Despite of the such number of described method for albuterol enantioseparations, the accurate method useful for practical analysis is not distinguished. On the contrary, there are just two methods for separation of bambuterol enantiomers described in literature. Enantiomers of bambuterol are partially separated by capillary electrophoresis using cyclodextrine derivatives, [17,18] but there is no described method that uses liquid chromatography. So, we have spent exhaustively testing of the numerous commercial chiral HPLC columns in various mobile-phase conditions, in order to find simple and reliable methods useful for the enantioselective pharmaceutical analysis of albuterol and bambuterol.

2. Experimental

2.1. Chemicals

Albuterol (α^1-[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol) was purchased from Sigma (Germany) and bambuterol (1-[bis(3',5'-*N*,*N*-dimethylcarbamoyloxy)phenyl)]-2-*N*-tert-butylaminoethanol) was produced by Belupo Pharmaceuticals and Cosmetics Ltd. 2-Propanol, ethanol, acetonitrile, methanol and hexane of HPLC grade were provided by Merck (Germany). Diethyl amine, triethylamine, diisopropylamine, potassium hexafluorophosphate and phosphoric acid were of p.a. purity and also provided by Merck.

2.2. Equipment's

HPLC analyses were performed on HPLC system (Thermo Separation Products, USA) consisted of vacuum degasser SCM 1000, quaternary gradient pump P4000, automatic sampler injector AS3000, diode array UV–vis detector UV3000HR and data was processed using computer program ChromQuest 2.51. UV detection was carried out at 220 nm for bambuterol and 225 nm for albuterol. Columns used for chiral analysis are: Chiralcel OD (250 mm L × 4.6 mm ID), Chiralcel OJ (250 mm L × 4.6 mm ID), Chiralcel OC (250 mm L × 4.6 mm ID), Chiralpak AD (250 mm L × 4.6 mm ID), Chiralcel OD-RH (150 mm L × 4.6 mm ID), Chiralcel OJ-R (150 mm L × 4.6 mm ID), all produced by Daicel (Japan), Chirobiotic V (250 mm L × 4.6 mm ID, Astec, USA), Whelk-O1 (250 mm L × 4.6 mm ID, Regis, USA), and Ultron ES-OVM (150 mm L × 4.6 mm ID, Shinwa, Japan). Acidity of mobile phases was controlled by digital pH-meter DELTA 345 equipped with electrode type InLab 413 (Mettler Toledo, Germany).

2.3. Sample preparation and HPLC analysis

Stock solutions were prepared by dissolving 20.0 mg of bambuterol in 10.0 ml of ethanol, or 5.0 mg of albuterol in 10.0 ml of ethanol. Samples needed for validation were prepared by dissolution of stock solutions using mobile phase.

During the chromatographic analysis the following parameters were measured:

k_1': capacity factor of the first eluted enantiomer, $(t_1-t_0)/t_0$;
k_2': capacity factor of the second eluted enantiomer, $(t_2-t_0)/t_0$;
α: selectivity factor, $\alpha = k_2'/k_1'$;
R_S: resolution factor, $R_S = 2(t_2-t_1)/(w_1 + w_2)$; w is the baseline bandwidth obtained by drawing tangents to the inflexion points of the chromatographic peak.

3. Results and discussion

The analysis for the both compounds are beginning with the screening of the wide range of chiral columns listed in Section 2. After that the investigation is continued only with columns that exhibit the possibility for convenient enantioseparations

3.1. Separation of bambuterol enantiomers on Chiralcel OD-RH column

Chiralcel OD-RH column has a 3,5-dimethylphenylcarbamoyl derivative of cellulose adsorbed on silica gel as a chiral stationary phase. It is specially modified so it can be used in reverse-phased conditions [19]. Buffer solutions of $NaClO_4$ or KPF_6 can be used as a mobile phase and in our work better results were achieved using KPF_6. Effect of pH value of mobile phase was studied in a pH range of 2.0–6.0, which is the operation range recommended by producer for this column. Experiments were performed using 100 mM KPF_6 solution and acetonitrile as organic modifier. The obtained results

Table 1
Parameters determined for separation of the bambuterol enantiomers on column Chiralcel OD-RH (150 mm L × 4.6 mm ID), flow rate 1.0 ml/min

Mobile phase	pH	k_1'	α	R_S
100 mM KPF_6/ACN = 80/20	2.0	5.70	1.08	0.36
100 mM KPF_6/ACN = 80/20	2.5	5.72	1.08	0.36
100 mM KPF_6/ACN = 80/20	3.0	5.77	1.08	0.36
100 mM KPF_6/ACN = 80/20	4.0	5.74	1.09	0.37
100 mM KPF_6/ACN = 80/20	5.0	5.76	1.09	0.36
100 mM KPF_6/ACN = 80/20	6.0	5.76	1.08	0.36
50 mM KPF_6/ACN = 80/20	6.4	5.80	1.09	0.36
100 mM KPF_6/ACN = 80/20	6.4	5.75	1.09	0.36
200 mM KPF_6/ACN = 80/20	6.4	5.70	1.05	0.34
100 mM KPF_6/ACN = 60/40	6.4	0.41	1	0
100 mM KPF_6/ACN = 80/20	6.4	5.75	1.09	0.36
100 mM KPF_6/ACN = 85/15	6.4	7.67	1.07	0.28

Table 2
Parameters determined for separation of the bambuterol enantiomers on column Chiralpak AD (250 mm L × 4.6 mm ID), flow rate 1.0 ml/min

Mobile phase	k_1'	α	R_S
Hexane/ethanol = 80/20 + 0.5% DEA	0.99	1.27	2.15
Hexane/ethanol = 85/15 + 0.5% DEA	1.48	1.20	1.96
Hexane/ethanol = 90/10 + 0.5% DEA	2.60	1.28	2.65
Hexane/2-propanol = 70/30 + 0.5% DEA	0.85	1.51	3.11
Hexane/2-propanol = 75/25 + 0.5% DEA	1.11	1.54	3.90
Hexane/2-propanol = 80/20 + 0.5% DEA	1.58	1.56	4.09
Hexane/2-propanol = 85/15 + 0.5% DEA	2.53	1.58	4.00
Hexane/2-propanol = 70/30 + 0.1% DEA	0.91	1.49	3.13
Hexane/2-propanol = 70/30 + 0.3% DEA	0.87	1.52	3.25
Hexane/2-propanol = 70/30 + 0.5% DEA	0.85	1.51	3.11
Hexane/2-propanol = 70/30 + 0.1% DIPA	0.97	1.46	2.55
Hexane/2-propanol = 70/30 + 0.1% TEA	1.15	1.37	1.62

show that pH value of mobile phase has a negligible influence on retention and selectivity. A separation of bambuterol enantiomers was studied using KPF_6 solutions of different concentrations with same pH value. Retention time of bambuterol enantiomers is slightly shorter using higher concentrations of KPF_6, but increasing of ionic strength of mobile phase has no influence on enantioselectivity. Type of organic modifier in mobile phase has large influence on retention time of enantiomers and their separation. Studies were performed using mobile phases with same amount (v/v) of three different organic modifiers: methanol, ethanol and acetonitrile. It is determined that strength of elution of organic modifiers is of following order: acetonitrile > ethanol > methanol. It is also shown that relatively small changes of amount of organic modifier can have great influence on retention and enantioselectivity. However, satisfactory baseline separation of bambuterol enantiomers was not achieved on this column, Table 1.

3.2. Separation of bambuterol enantiomers on Chiralpak AD column

Chiralpak AD column has a 3,5-dimethylphenylcarbamoyl derivative of amylose adsorbed on silica gel as a chiral stationary phase and it operates under normal-phase conditions [20]. The quantity of organic modifier in mobile phase has great influence on the retention of enantiomers and their separation. Studies of separation of bambuterol enantiomers on Chiralpak AD column were performed using mobile phases with different amounts (v/v) of ethanol and 2-propanol, Table 2. Increasing of amount of alcohol in the mobile phase causes reduction of retention times of bambuterol enantiomers. Type of alcohol has a great effect on separations of bambuterol enantiomers. Although retention times of bambuterol enantiomer are longer using 10% ethanol than using 30% 2-propanol, separation is much better when 2-propanol is used.

Since tailing of peaks is observed, examinations were performed using mobile phases with different amounts (v/v) of diethylamine. Influence of diisopropylamine and triethylamine in mobile phase causes insignificant influence on retention of bambuterol enantiomers. Since amount of amine has no significant role on separation of bambuterol enantiomers, the mobile phase with the smallest quantity of amine was chosen as the best. Examination of influence of sort of amine on the retention and separation of bambuterol enantiomers has shown that sort of amine has great influence on both their retention and separation, and diethylamine was chosen as the best amine modifier.

On column Chiralpak AD for separation of bambuterol enantiomer mobile phase of following composition was used; *n*-hexane/diethylamine/2-propanol = 70/0.1/30. Analysis was performed under optimal conditions and its chromatogram is shown in Fig. 1. The performed research shows that method is robust, since small changes in mobile phase composition don't cause significant change in the separation of bambuterol enantiomers. Selectivity of method is also satisfactory because base line separation of bambuterol enantiomers is achieved. All this makes this method suitable for routine analysis for accurate determination of enantiomer composition of bambuterol samples.

3.3. Separation of albuterol enantiomers on Chirobiotic V column

Chirobiotic V column contains the macrocyclic antibiotic vancomycine covalently bonded to silica gel as a chiral stationary phase. It can be used both under reverse and normal-phase conditions [21]. However, according to recent literature the best results are achieved using so-called "polar organic" mobile phase [22]. Polar organic mobile phase, methanol with acid and basic modifiers, is used in this work to separate enantiomers of albuterol. The concentration of acid and base affects retention, but it is the ratio of these two components that determines the selectivity. Acetic acid and triethylamine were used as the acid/base components. It was studied how concentration of acid and base affects retention of albuterol enantiomers and how ratio of these two components affects selectivity. The obtained results shown that total amount of base and acid doesn't substantially affect on selectivity but

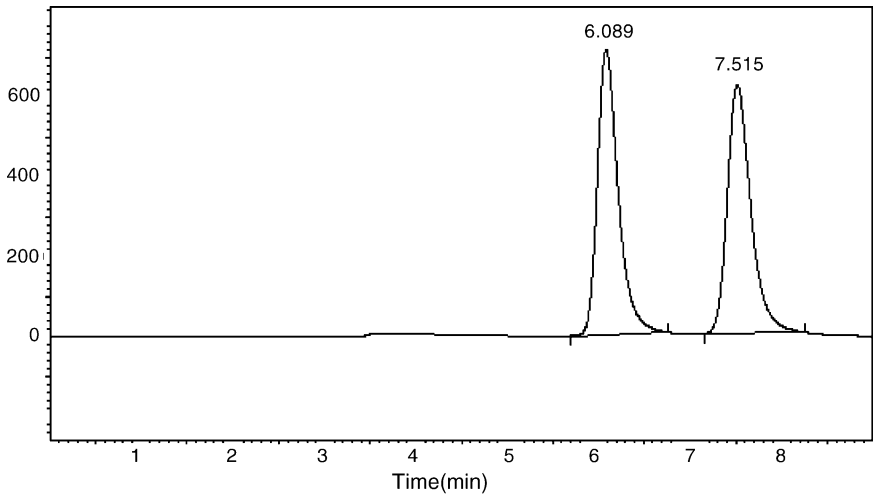

Fig. 1. Chromatogram of analysis of the bambuterol enantiomers on Chiralpak AD column under optimal conditions.

have strong affect on the peak resolution [22]. In theory total amount of base and acid should not have any affect on selectivity, but only on retention. The best selectivity is achieved with 0.02% of polar modifiers.

The results from Table 3 shown that acid/base ratio have great influence on enantioselectivity. Amount of basic modifier must be larger or equal to amount of acid modifier in order to achieve separation of albuterol enantiomers. Best separation of albuterol enantiomers is achieved when total amount of modifiers is 0.02%, but analysis time is ca. 35 min (retention times of albuterol enantiomers are 27.9 and 31.5 min). Chirobiotic V column can be used with higher mobile phase flow, in contrast to cellulose and amylose columns that can work at maximum flow of 1.0 ml/min. That is why attempt was made to work with higher mobile phase flow rate in order to shorten analysis time, without jeopardising separation of albuterol enantiomers. Separation was studied using methanol modified with 0.01% acetic acid and 0.01% of triethylamine at two different mobile phase flow rates (1.0 and 2.0 ml/min). Results show that increasing of mobile phase flow can decrease analysis time, without influence on the separation of albuterol enantiomers. On Chirobiotic V column the best separation of albuterol enantiomers is achieved with mobile phase of the following composition; methanol modified with 0.01% acetic acid and 0.01% triethylamine with flow rate of 2.0 ml/min. Analysis was performed under these conditions and its chromatogram is shown in Fig. 2.

3.4. Separation of albuterol enantiomers on Chiralcel OJ column

Chiralcel OJ column has a 4-methyl benzoate derivative of cellulose adsorbed on silica gel as a chiral stationary phase [23]. Chiral separation is carried out under normal-phase conditions and mobile phase is usually the mixture of *n*-hexane and alcohols. Addition of small amounts of amine is necessary for separation of enantiomers of compounds with basic amino group, while small amount of acid is necessary when separating acid compounds. Research of influence of the quantity and sort of alcohol in the mobile phase on retention of albuterol enantiomers and their separation was performed with mobile phases with different amounts (v/v) of two different alcohols; ethanol and 2-propanol. Increasing of alcohol quantity in the mobile phase results in decrease of retention time of albuterol enantiomers. It is shown that ethanol has a stronger elution power then 2-propanol and separation of enantiomers of albuterol is significantly better when ethanol is used, Table 4.

Table 3
Parameters determined for separation of the albuterol enantiomers on column Chirobiotic V (250 mm L × 4.6 mm ID), flow rate 1.0 ml/min

Mobile phase	k_1'	α	R_S
Methanol, 0.2% TEA, 0.2% AcOH	1.52	1.16	1.87
Methanol, 0.1% TEA, 0.1% AcOH	2.58	1.16	2.17
Methanol, 0.06% TEA, 0.06% AcOH	3.98	1.15	2.16
Methanol, 0.02% TEA, 0.02% AcOH	9.15	1.14	2.82
Methanol, 0.2% TEA	2.76	1.11	1.47
Methanol, 0.15% TEA, 0.05% AcOH	1.83	1.14	1.78
Methanol, 0.05% TEA, 0.15% AcOH	Eluted with dead volume without retention		
Methanol, 0.2% AcOH			

Table 4
Parameters determined for separation of the albuterol enantiomers on column Chiralcel OJ (250 mm L × 4.6 mm ID), flow rate 1.0 ml/min

Mobile phase	k_1'	α	R_S
Hexane/ethanol 90/10 + 0.05% TFA	1.90	1.38	1.67
Hexane/ethanol 92/8 + 0.05% TFA	3.12	1.39	1.97
Hexane/ethanol 95/5 + 0.05% TFA	7.86	1.48	2.62
Hexane/2-propanol 80/20 + 0.05% TFA	0.98	1.18	0.28
Hexane/2-propanol 90/10 + 0.05% TFA	4.36	1.25	0.86
Hexane/ethanol 90/10 + 0.10% TFA	1.97	1.37	1.67
Hexane/ethanol 90/10 + 0.30% TFA	2.03	1.37	1.69
Hexane/ethanol 90/10 + 0.50% TFA	2.07	1.40	1.70

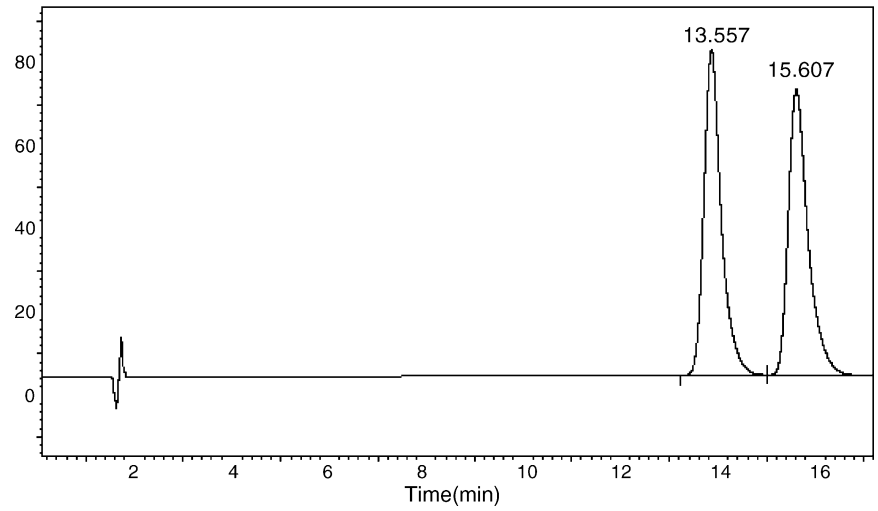

Fig. 2. Chromatogram of analysis of the albuterol enantiomers on Chirobiotic V column under optimal conditions.

Testing of influence of quantity and sort of amine in the mobile phase on retention time of albuterol enantiomers and their separation was done with mobile phases with different amounts of diethylamine (v/v). The effect of adding diisopropylamine and triethylamine as modifiers (using mobile phase *n*-hexane/amine/2-propanol = 90/*x*/10) was examined. The obtained results have shown that enantiomers of albuterol could not be separated using mobile phases with amine modifiers, although albuterol contains basic amino group. Tang has described separation of the group of drugs on Chiralcel OJ column, [16] and among them was albuterol. Although in separation of basic compounds, such as albuterol, amine is added to improve separation and peak shape, in this paper trifluoroacetic acid is added in the mobile phase to separate some basic compounds. It is discovered that enantioselectivity is enhanced when acid is added to mobile phase only for chiral secondary amines with amino group that is one atom away from chiral centre. Acid in mobile phase can form ion-pair with basic analyte and enable better separation. However, mechanism of this process is still not fully explained. It is possible that acid partially masks the silanol groups on chiral stationary phase and diminished non-chiral interactions between analyte and stationary phase. Also, ion-pair of analyte and acid probably moves through column as one molecule and polar trifluoroacetic group represents additional place for interaction with chiral stationary phase. Since, in this work albuterol was mentioned as a compound that could be separated using mobile phase modified with trifluoroacetic acid we have tested how addition of acid influences separation and retention of albuterol enantiomers. Examination were done using mobile phase of the following composition; *n*-hexane/trifluoroacetatic acid/ethanol = 90/*x*/10. It was determined that amount of acid has only slight influence on enantioselectivity and that retention time of enantiomers is longer with greater quantity of acid in mobile phase. These facts support the mecha-

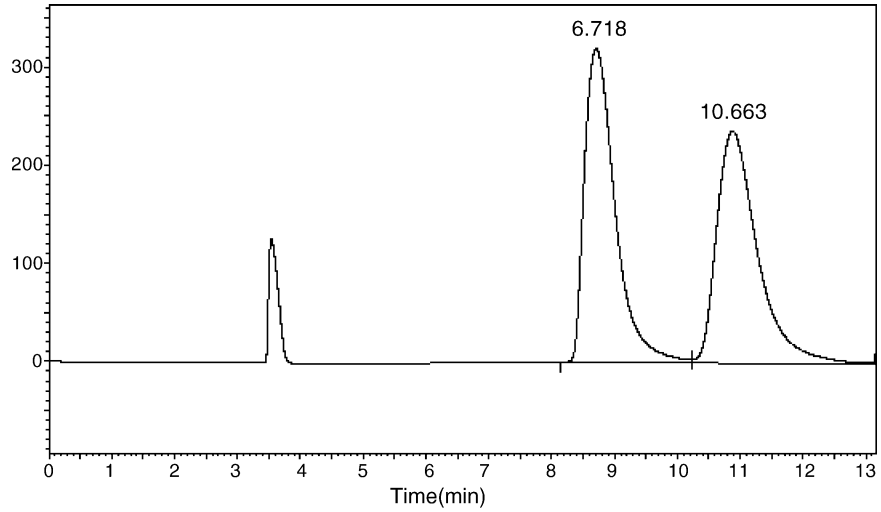

Fig. 3. Chromatogram of analysis of the albuterol enantiomers on Chiralcel OJ column under optimal conditions.

Table 5
Accuracy of method for determination of bambuterol enantiomes

Added concentration (mg/ml)		Found concentration (mg/ml)		Recovery (%)	
1. Enantiomer	2. Enantiomer	1. Enantiomer	2. Enantiomer	1. Enantiomer	2. Enantiomer
0.004	0.004	0.003781	0.038974	94.52	97.44
0.008	0.008	0.007883	0.008532	98.54	106.65
0.012	0.012	0.011680	0.011800	97.33	98.33
0.016	0.016	0.015178	0.015041	94.86	94.01
0.020	0.020	0.019592	0.019481	97.96	97.40

nism, which include the forming of ion-pair between basic analyte and trifluoroacetic acid. Finally, on Chiralcel OJ column the best separation of albuterol enantiomers is achieved using mobile phase; *n*-hexane/trifluoroacetic acid/ethanol = 90/0.05/10. Chromatogram of the analysis done under those conditions is represented in Fig. 3.

3.5. Validation of method for analysis of bambuterol enantiomers

Separation of bambuterol enantiomers is achieved on two columns: on Chiralcel OD-RH column separation was poor, and on Chiralpak AD column it was very good. That is why we decided to validate method on Chiralpak AD column. During development of method on Chiralpak AD column it was shown that method is selective and robust, because enantiomers have baseline separation (R_S = 3.13) and small changes in mobile phase composition don't effect separation significantly. Detection limit, determined as signal/noise ratio ∼3/1, for both bambuterol enantiomers is 0.002 mg/ml. Quantification limit for both bambuterol enantiomers is 0.004 mg/ml, and it was confirmed by determining relative standard deviation (R.S.D.) of multiple injections (5) of bambuterol solution at previously determined quantifica-

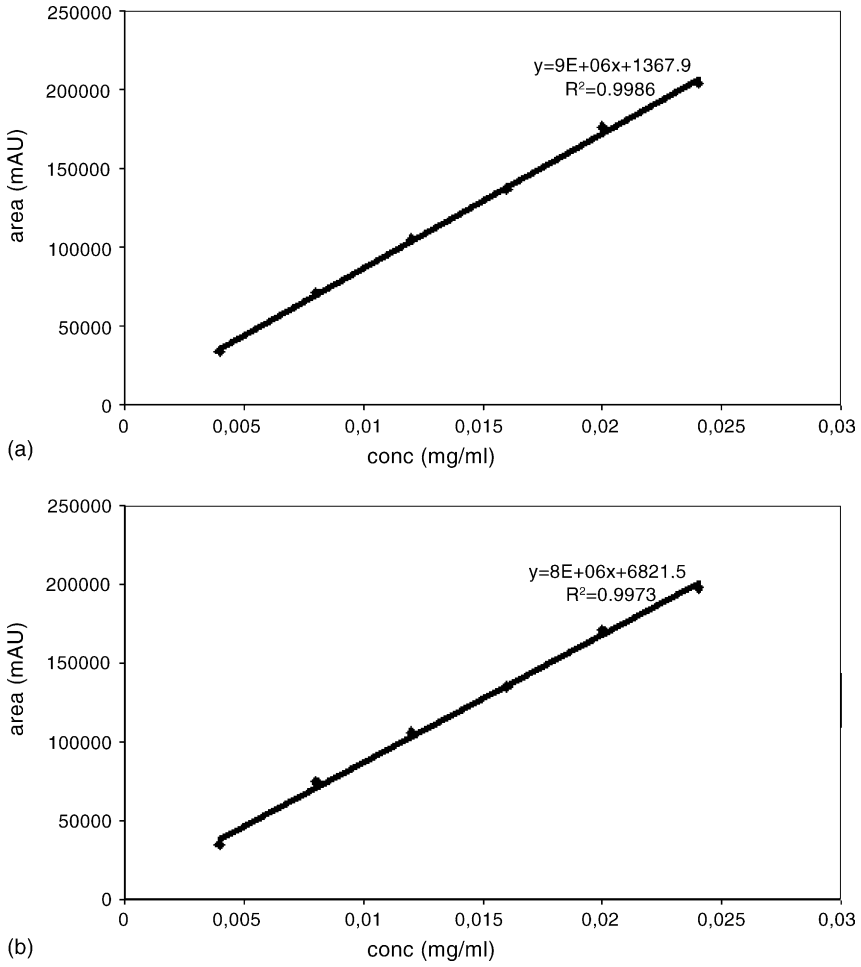

Fig. 4. Linearity of method for bambuterol determination; (a) for first eluting enantiomer, (b) for second eluting enantiomer.

Table 6
Accuracy of method for determination of albuterol enantiomers

Added concentration (mg/ml)		Found concentration (mg/ml)		Recovery (%)	
1. Enantiomer	2. Enantiomer	1. Enantiomer	2. Enantiomer	1. Enantiomer	2. Enantiomer
0.003	0.003	0.003441	0.003478	114.69	115.94
0.006	0.006	0.005911	0.005809	98.51	96.82
0.009	0.009	0.008956	0.008744	99.51	97.16
0.012	0.012	0.011770	0.011845	98.09	98.70
0.015	0.015	0.014745	0.014603	98.30	97.35

tion limit. R.S.D. of five injections at QL was 1.29% for first eluting enantiomer, and 1.21% for second eluting enantiomer. Accuracy of method was determined on five concentration levels of bambuterol from QL −0.040 mg/ml. Solutions of known concentrations of bambuterol were prepared, obtained parameters are given in Table 5. Results show that method is accurate in examined range since recovery is within preset limits (95–105%, R.S.D < 5%).

Same samples were used for determination of linearity of detector response. Equation of regression line is calculated and results are presented by Fig. 4. Coefficient of correlation between concentration and detector response (0.9986 for the first eluting enantiomer and 0.9973 for the second eluting enantiomer) show that method is linear in examined range.

3.6. Validation of method for analysis of albuterol enantiomers

Separation of albuterol enantiomers was examined on the wide range of columns of which two; Chiralcel OJ and Chirobiotic V, have shown good separation of albuterol enantiomers. However, considering better peak shape on Chirobiotic V column this method was chosen for validation. Method is selective and robust, what was shown during

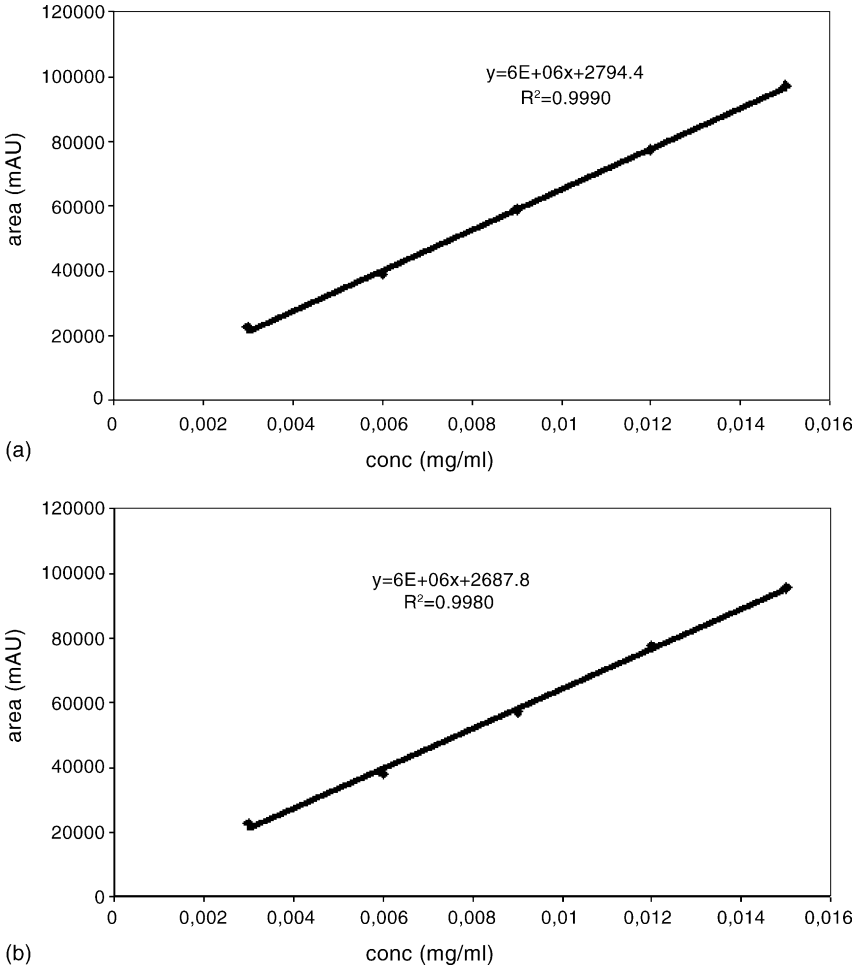

Fig. 5. Linearity of method for albuterol determination; (a) for first eluting enantiomer, (b) for second eluting enantiomer.

method development. Enantiomers show baseline separation (R_S = 2.52) and the little changes in mobile phase composition don't have big effect on separation of enantiomers.

Signal/noise ratio ~3/1 was used for estimation of DL and signal/noise ratio ~10/1 for estimation of QL. QL was confirmed by determination of relative standard deviation (R.S.D.) of multiple injections (5) of albuterol solution at pre-determined QL. DL for both enantiomers of albuterol is 0.0015 mg/ml, and QL is 0.0030 mg/ml (R.S.D. of five injections at QL is 1.91% for the first eluting enantiomer and 2.13% for the second eluting enantiomer). Accuracy of method was examined on five concentration levels, from QL = 0.0030 mg/ml of albuterol. In Table 6 values for added and found concentrations, and results for recovery are given. Results show that method is accurate in examined range since recovery is within preset limits (95–105%). Only exception of preset values is noticed at lowest examined concentration.

Same samples were used for determination of linearity of detector response. Equation of regression line is calculated and results are shown in Fig. 5. Coefficient of correlation between concentration and detector response (0.9986 for the first eluting enantiomer and 0.9973 for the second eluting enantiomer) show that method is linear in examined range.

4. Conclusion

Simple HPLC methods for separation of bambuterol and albuterol enantiomers were developed. Method on Chiralpak AD column, using mobile phase *n*-hexane/diethylamine/2-propanol = 70/0.1/30, was determined as the most convenient for separation of bambuterol enantiomers. Method is linear and accurate, with QL = 0.004 mg/ml for both enantiomers of bambuterol. Method on Chirobiotic V column, using methanol modified with 0.01% acetic acid and 0.01% triethylamine as a mobile phase, was determined as the most convenient for separation of albuterol enantiomers. Method is linear and accurate, with QL = 0.003 mg/ml for both enantiomers of albuterol. The both methods are accurate and suitable for daily direct enantioselective analysis of bambuterol and albuterol. The proposed methods are very robust and offers the advantageous opportunity for control the enantiomeric composition and purity of a large number of samples.

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