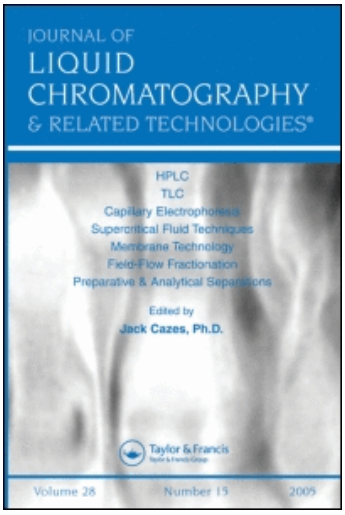

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HPLC VERSUS SFC FOR THE DETERMINATION OF SALBUTAMOL SULPHATE AND ITS IMPURITIES IN PHARMACEUTICALS

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ABSTRACT

A method to determine salbutamol sulphate and six impurities: 5-formyl-saligenin, salbutamol ketone, salbutamol bis ether, isopropyl salbutamol, desoxysalbutamol sulphate and salbutamol aldehyde, using reverse phase high performance liquid chromatography (RP-HPLC) with diode array detection (DAD) is proposed. The best separation was achieved using a gradient of 0.1 M ammonium acetate pH 3.0 and acetonitrile.

When the procedure was applied to the analysis of tablets and cough syrups, the versatility of the HPLC method was higher than one based on supercritical fluid chromatography (SFC). When using the later method the excipient difficulted the identification and quantification of some compounds in cough syrup samples.

INTRODUCTION

Salbutamol sulphate is a bronchodilator widely used for asthma treatment, which is sold under the Glaxo trade mark of Ventolin.

Until now, most of the papers published in relation to salbutamol analyses described its determination and quantification in tissues and biological fluids of animals under treatment with this drug. Usually, the methods employed were based on HPLC techniques using detectors of high sensitivity such as fluorescence[1-3] and electrochemical.[4-6]

It is known that there are some impurities which could be produced during the synthetic process or during an unsuitable storage of the drug being their analysis important. HPLC[7] or capillary electrophoresis[8] methods have been used to determine these compounds, but only two of the impurities were analysed. Recently, in a work done by our group[9] salbutamol and six impurities were separated satisfactorily in a short time by employing packed column supercritical fluid chromatography, although the method provided good results in the analysis of Ventolin tablets, some problems appeared with excipient peaks when analysing Ventolin cough syrups.

The aim of this work has been to establish the best chromatographic conditions to separate and determine salbutamol sulphate and the six related impurities using RP-HPLC with diode array detection, trying to obtain a method useful to analyse both kind of samples: tablets and cough syrups and using a sample treatment as simple as possible. For this purpose the variables affecting most the separation have been studied. The method has been applied to the analysis of Ventolin (Glaxo trade mark) tablets and cough syrups, and the results have been compared to those obtained in the SFC analysis of the same samples.

EXPERIMENTAL

Reagents

Ammonium acetate and n-propylamine were purchased from Sigma Aldrich Quimica (Madrid, Spain). Acetonitrile and methanol HPLC grade were obtained from Lab-Scan (Dublin, Ireland). Samples and drug standards were kindly supplied by Glaxo S.A. (Aranda de Duero factory, Burgos, Spain), the stock solutions were prepared in methanol. SFC grade carbon dioxide was purchased from Carburos Metálicos (Barcelona, Spain).

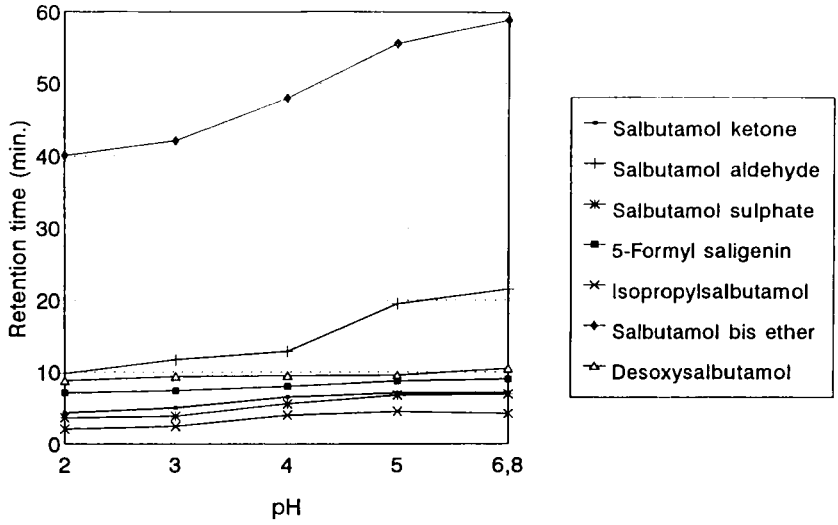

Figure 1. Variation of the retention time with the pH of the mobile phase in HPLC.

Apparatus and Chromatographic Conditions

HPLC : A Philips PU4100 liquid chromatograph (Cambridge, GB) equipped with a PU 4021 diode array detector was used. The column employed was a 200 x 2.1 mm, 5µm Hypersil ODS (Phenomenex, Torrance CA, USA). The elution was carried out employing a gradient of acetonitrile/0.1 M ammonium acetate pH=3.0. The flow-rate was set at 0.3 mL min^{-1}. and the injection volume was 20 µL.

The compounds were determined at 227 nm which was the wavelength where they presented the highest absorbance.

SFC : A Hewlett-Packard G1205A supercritical fluid chromatograph (Palo Alto, CA) with a diode array detector was used. The instrument was operated in the downstream mode, the pressure and temperature were fixed at 300 bar and 70°C respectively, the flow-rate was 1.5 mL min^{-1} and a gradient of modifier (methanol with 0.5% n-propylamine) was used. The injection volume was 5 µL (full loop). The column employed was a 250 x 4.6 mm, 5 µm Lichrosphere Diol column (Phenomenex, Torrance CA, USA).

Table 1

Gradient Employed in HPLC

Time (min)	% 0.1 M $AcNH_4$, pH 3	% Acetonitrile
0	96	4
5	96	4
8	88	12
30	88	12

Sample Treatment

To analyse Ventolin cough syrups, 1 mL of the sample was diluted to 10 mL with nanopure water and then passed through an 0.45 μm filter.

To analyse Ventolin tablets, 5 tablets of 4 mg were mixed with 20 mL of nanopure water and sonicated for 10 minutes, then the solution was centrifuged and the liquid was passed through an 0.45 μm filter.

RESULTS AND DISCUSSION

HPLC Procedure:

Effect of pH

Figure 1 shows the results obtained for a mixture of 30 μg mL^{-1} of each compound, working at ambient temperature (20°C) and using different mobile phases where the percentage of acetonitrile was fixed at 8% and the pH of the 0.1 M ammonium acetate was varied between 2 and 6.8. As can be seen, a pH increase caused an increase of the retention especially in the case of the late eluted compounds: salbutamol aldehyde and salbutamol bis ether. The selectivity of the separation did not change. Taking into account that, at pH 3, the compounds were separated and the retention time of salbutamol bis ether was the lowest, this pH was selected.

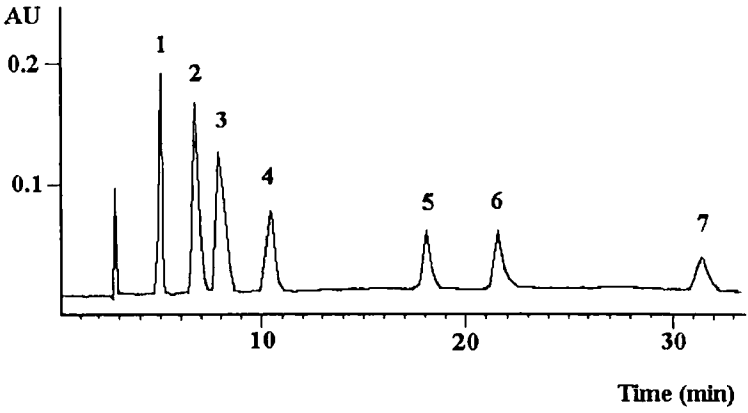

Figure 2. HPLC Chromatogram of a mixture of 30 μg mL^{-1} of the compounds. Peak labels are the same as in table 2.

Effect of Acetonitrile Percentage

The retention decreased as expected when the percentage of acetonitrile was increased. Isocratic conditions failed to provide a good separation in a short time. For optimum performance in terms of resolution and analysis time, several gradients of acetonitrile were tested. The best results were obtained with the gradient listed in Table 1. Working under these conditions the seven compounds were separated in 32 minutes (Figure 2).

Sensitivity

The detection limits obtained using as mobile phase the 0.1 M ammonium acetate pH=3/acetonitrile gradient schedule, listed in Table 1, are given in Table 2. They were calculated according to the IUPAC[10] recommendations and ranged from 0.60 μg mL^{-1} to 0.70 μg mL^{-1}, except for salbutamol bis ether, for which the detection limit was 3.50 μg mL^{-1}.

This also shows that they are similar to those obtained with SFC, but somewhat higher due to the fact that the efficiency of HPLC is lower than that of SFC and so the HPLC peaks are wider.

Table 2

Detection Limits

Compound	Detection Limit ($\mu g\ mL^{-1}$) HPLC	SFC
(1) Isopropyl salbutamol	0.6	0.5
(2) Salbutamol sulphate	0.6	0.5
(3) Salbutamol ketone	0.7	0.5
(4) 5-Formyl-saligenin	0.7	0.2
(5) Desoxysalbutamol	0.7	0.3
(6) Salbutamol aldehyde	0.7	0.5
(7) Salbutamol bis ether	3.5	1.3

Table 3

Efficiency and Resolution in the HPLC Separation

Compound	Number of Plates	Resolution
Isopropyl salbutamol	249	
Salbutamol sulphate	554	193
Salbutamol ketone	508	0.99
5-Formyl-saligenin	892	1.83
Desoxysalbutamol	1975	4.60
Salbutamol aldehyde	1263	1.73
Salbutamol bis ether	2625	3.92

SFC Procedure:

The SFC working conditions were as described in a previous paper.[9]

Application To Pharmaceutical Samples Analysis

Both SFC or HPLC methods gave a good resolution for the impurities and salbutamol sulphate at relative levels present in a typical salbutamol sample (Figure 3). The determination of 4 $\mu g\ mL^{-1}$ salbutamol bis ether and 1 $\mu g\ mL^{-1}$ of

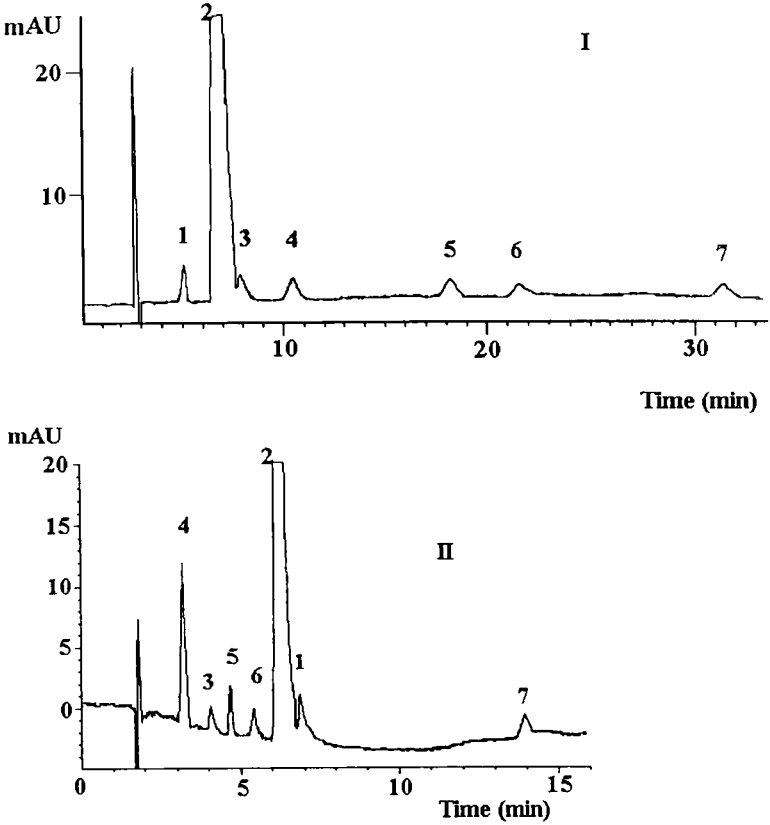

Figure 3. Chromatogram of a mixture of 1 μg mL^{-1} 5-formyl-saligenin, 1 μg mL^{-1} salbutamol ketone, 1 μg mL^{-1} desoxysalbutamol, 1 μg mL^{-1} salbutamol aldehyde, 1000 μg mL^{-1} salbutamol sulphate, 1 μg mL^{-1} isopropyl salbutamol and 3 μg mL^{-1} salbutamol bis ether.
Peak labels are the same as in Table 2.
(I) Using the HPLC method..
(II) Using the SFC method.

the other impurities in the presence of 1000 μg mL^{-1} salbutamol sulphate was possible. It should be noted that using the SFC method the efficiency and resolution of the separation was much better than using the HPLC one (Tables 3 and 4), and the analysis time was shorter in SFC than in HPLC.

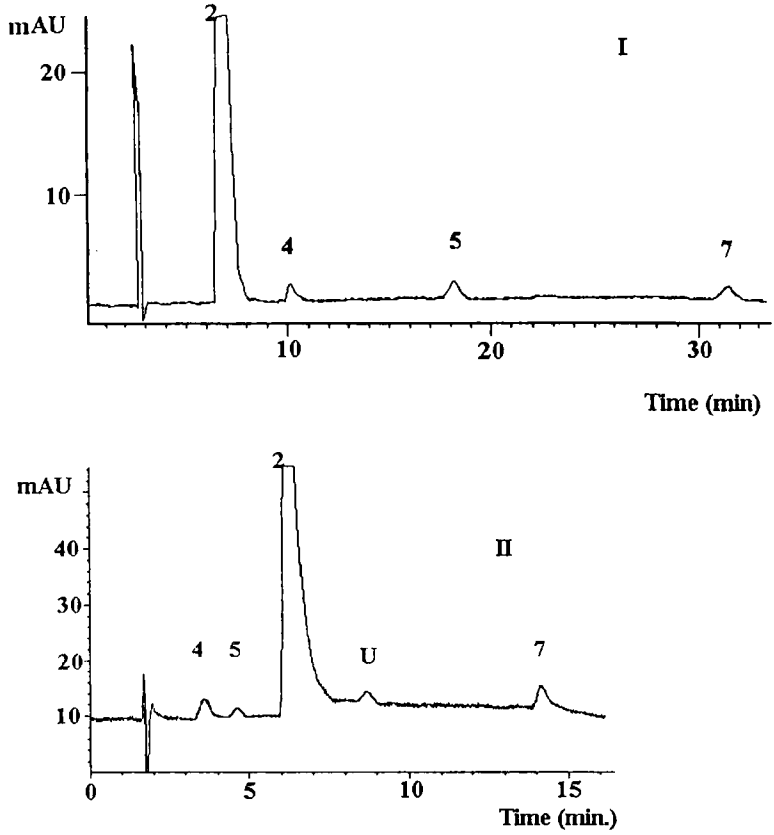

Figure 4. Chromatogram of a sample of Ventolin tablets degraded at 37°C and 70% of humidity for a year. (I) Using the HPLC method. (II) Using SFC method.(U) Unknown peak. Peak labels are the same as in table 2.

In Figure 4 the chromatogram of a sample of Ventolin tablets which was degraded, at 37°C and 70% of humidity for a year, is shown. There is no interference of the matrix in the analysis of that kind of pharmaceutical preparations, neither using the SFC method nor using the HPLC one, and the impurities could be perfectly quantified. Nevertheless, when a sample of undegraded Ventolin syrup and spiked with the impurities at the concentration levels of 4 $\mu g\ mL^{-1}$ salbutamol bis ether and 1 $\mu g\ mL^{1}$ for the other compounds, was analysed (Figure 5), the correct determination and quantification of these compounds was only possible in the case of the HPLC method. As can be seen in Figure 5 when using the SFC method, there were some problems with the peaks

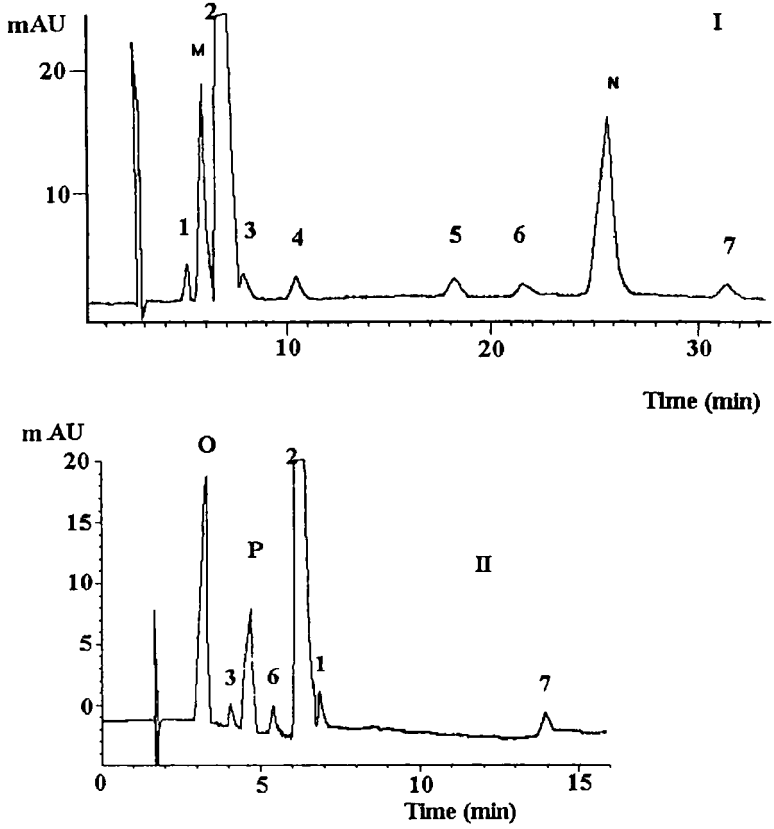

Figure 5. Chromatogram of a sample of Ventolin cough syrup spiked with the impurities at the concentration levels of figure 3. (I) Using the HPLC method, saccharine (M), benzoate (N). (II) Using the SFC method, benzoate and 5-formyl-saligenin (O), saccharine and isopropyl salbutamol (P). Peak labels are the same as in Table 2.

due to the excipient (benzoate and saccharine): 5-formyl-saligenin coeluted with benzoate and isopropylsalbutamol co-eluted with saccharine. This shortcoming could be circumvented introducing some clean-up procedures, but this would complicate sample treatment and result in a longer analysis time.

The HPLC method could be considered more useful in this particular case, because of the two kinds of pharmaceutical preparations, tablets and syrups, can be analysed and their impurities quantified without interferences, in spite of the fact that the analysis take 30 minutes.

Table 4

Efficiency and Resolution in the SFC Separation

Compound	Number of Plates	Resolution
5-Formyl-saligenin	2440	
Salbutamol ketone	3047	3.05
Desoxysalbutamol	12159	2.66
Salbutamol aldehyde	8333	3.65
Salbutamol sulphate	8216	3.10
Isopropyl salbutamol	7817	2.25
Salbutamol bis ether	10128	16.31

CONCLUSIONS

Salbutamol sulphate and six related impurities are separated, and can be quantified, in Ventolin tablets and cough syrup samples, by employing RP-HPLC and DAD detection without no interferences of the matrix.

Efficiency and resolution of the separation as well as the analysis time, were better using SFC than HPLC. Nevertheless the analysis of cough syrup samples using SFC presented some problems related to the excipient interferences, which provoked an incorrect identification and quantification of the salbutamol related impurities. Using SFC these compounds could only be analysed in tablets samples, while using HPLC the two kind of samples could be analysed perfectly.

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