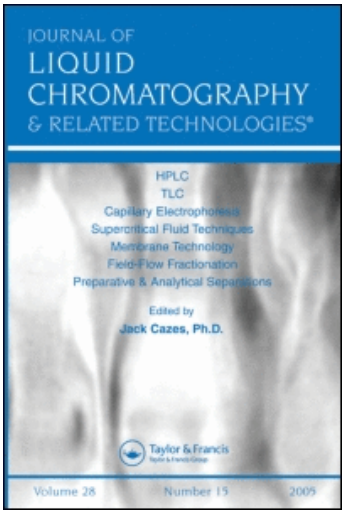

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ANALYSIS OF BAMBUTEROL HYDROCHLORIDE CHEMICAL REFERENCE SUBSTANCE AND TABLETS BY LIQUID CHROMATOGRAPHY

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ABSTRACT

A reversed-phase ion-pair liquid chromatographic method was applied to the analysis of bambuterol hydrochloride chemical reference substance (CRS) and tablets. The purity evaluation of the reference substance was performed with amperometric, UV, diode array and mass spectrometric detection. The total amount of impurities found was less then 0.1%.

For the determination of bambuterol hydrochloride in tablets the method reproducibility was 0.9% and the recovery was in the range 99.8 - 100.7%. Detection limits for conceivable degradation products were about 1 ng. The suitability of different column packing materials was investigated.

INTRODUCTION

Bambuterol hydrochloride, a new bronchodilator, is under clinical evaluation for treatment of asthma. The drug substance is the bis-dimethylcarbamate of terbutaline (Fig. 1). Clinically, bambuterol acts as a prodrug of terbutaline with 24 h duration of action and with an increased lung affinity compared to terbutaline (1, 2).

Recently, the chromatographic properties of bambuterol and some related compounds were investigated (3). In that study a reversed-phase ion-pair chromatographic method was suggested for the pharmaceutical analysis of bambuterol. Complete separation of the seven studied compounds was achieved within 12 minutes by that method. Terbutaline and the monocarbamate derivative (MONO) were included among these compounds as potential degradation products formed by hydrolysis (Fig. 1). The purpose of the present study was to investigate the applicability of the above mentioned

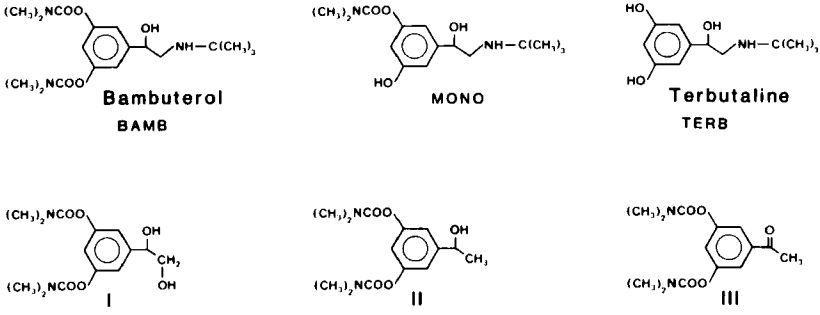

Figure 1. Structures and abbreviations of bambuterol, related degradation products (MONO, TERB) and impurities (I-III).

liquid chromatographic method to the analysis of a chemical reference substance (CRS) and of tablets. There is an important connection between these two applications as the availability of a well characterized standard substance is a prerequisite for accurate and reliable determinations by liquid chromatography.

MATERIALS AND METHODS

Chemicals and Reagents

Ammonium acetate and acetic acid were of analytical grade. The CRS batch was prepared from a part of a production batch of high purity, which was further purified by a recrystallization in acetone/water. All other chemicals and test compounds were as described in ref. 3.

Apparatus and Chromatographic Conditions

The following liquid chromatographic apparatus and conditions were used unless otherwise stated: pump, Waters M 510; flow-rate 1.5 ml/min; mobile phase, 6.0 mM sodium octanesulphonate in methanol-acetonitrile-50 mM phosphate buffer pH 3.0 (34 : 11 : 55); autosampler, Spectra-Physics SP 8780XR equipped with a 10 µl loop; column, Supelcosil LC-18-DB (20+50) x 4.6 mm; UV detectors, Waters M 441 operated at 214 nm or HP 1040A diode array, sampling at 0.7 Hz with 2 nm bandwidth between 200 and 400 nm; electrochemical detector, BAS LC-4B, working at 1.2 V vs Ag/AgCl; chromatographic data system, Nelson Analytical 6000. During the purity evaluations and column comparisons a 150 x 4.6 mm Supelcosil LC-18-DB column was used.

Liquid chromatography with thermospray mass spectrometric detection was performed with a VG Trio 2 mass

spectrometer. The mobile phase contained methanol-acetonitrile-50 mM acetate buffer pH 4.7 (28.5 : 9.5 : 62). Flow-rate 1.0 ml/min. The eluent was mixed with a make up flow of 0.2 M ammonium acetate at 0.15 ml/min.

The gas chromatographic investigations were performed on a HP 5840A equipped with an FID and a column packed with Carbowax 1500 on Carbopak C. Water was determined by use of a Metrohm 652 KF-coulometer. A Metrohm 636 Titroprocessor was used both for the argentometric and the two-phase titration. The latter was performed according to the method described by Johansson et al (4).

Method for Tablet Analysis

Ten bambuterol tablets were dissolved in a 50 mM phosphate buffer pH 3.0 (as in the eluent) to a final concentration of the drug substance between 0.4 and 0.5 mg/ml. The solutions were filtered through a Millipore Millex HV-filter (0.45 μm) discarding the first 10 ml. The samples were evaluated against an external standard dissolved in the same phosphate buffer.

RESULTS AND DISCUSSION

Chemical Reference Substance (CRS)

Figure 2 shows chromatograms of bambuterol hydrochloride CRS. Traces of related substances i.e. < 0.05% each, were observed and I, MONO, II and III were recognized from retention times and UV spectra. Compounds containing phenolic groups were determined with high specificity by electrochemical detection (Fig. 2b). The amount of terbutaline was below detection limit (0.04 ng). A comparison between the MONO peaks in

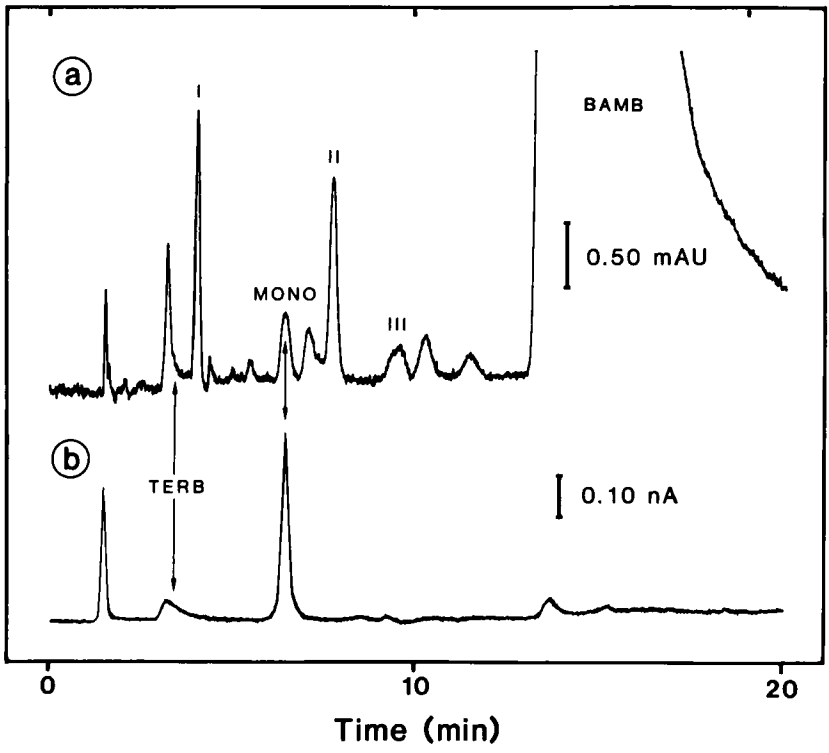

Figure 2. Chromatograms of bambuterol CRS. Conditions: mobile phase, 6.0 mM sodium octanesulphonate in methanol-acetonitrile-50 mM phosphate buffer pH 3.0 (34 : 11 : 55, v/v); flow-rate, 1.0 ml/min; column, Supelcosil LC-18-DB, 150 x 4.6 mm. The arrows for TERB indicate expected retention time; (a) UV detection at 214 nm and 60 µg injected; (b) amperometric detection at 1.2 V vs a Ag/AgCl and 10 µg injected.

figure 2a and b demonstrates the large improvement in sensitivity by the electrochemical detector compared to the UV detector at 214 nm. The identities of the above mentioned impurities were confirmed by thermospray mass spectrometry (Fig. 3). The relatively high gas phase basicity of the secondary amino group in bambuterol and MONO resulted in a dominating molecular ion (Fig. 3a-b)

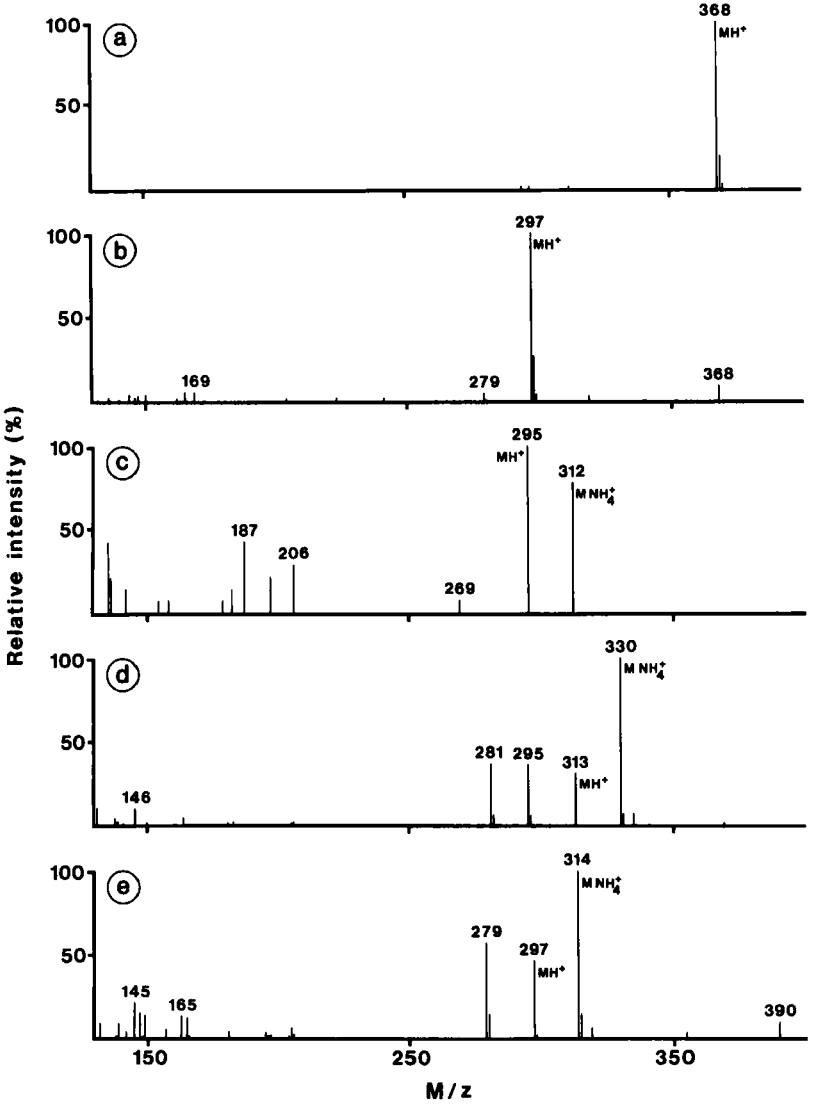

Figure 3. Thermospray mass spectra of bambuterol and impurities in bambuterol hydrochloride CRS; (a) bambuterol, 7.5 µg injected; (b) MONO, 0.01 µg injected; (c) III, 0.005 µg injected; (d) I, 0.02 µg injected; (e) II, 0.03 µg injected.

while compound III showed an additional ammonium adduct (Fig. 3c). For the alcoholic structures I and II the adduct was more prominent and the two compounds also displayed loss of water (Fig. 3d-e). The four spectra of the impurities in the CRS (Fig. 3b-e) showed conformity with the corresponding spectra of the synthesized structures.

The spectral homogeneity of the bambuterol peak was proved by spectral normalization using a UV diode array detector (5). Identical spectra were recorded on different parts of the peak (Fig. 4a). The ability of

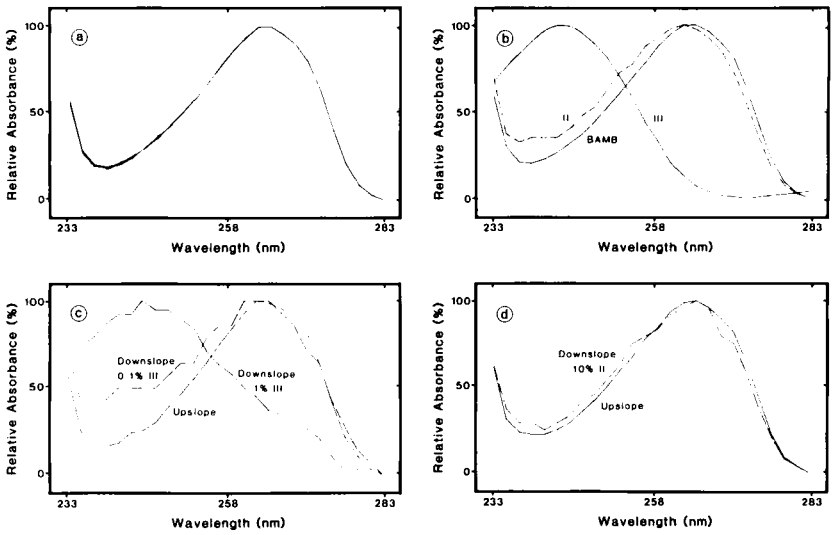

Figure 4. Normalized UV spectra from diode array detection; (a) spectral homogeneity of bambuterol peak at $h_{45\%}$ on upslope, apex ($h_{100\%}$) and on the peak downslope at $h_{67\%}$ and $h_{31\%}$; (b) spectra from resolved peaks of 3 µg II, 0.2 µg III, and 13 µg bambuterol; (c) co-elution with $\alpha = 1.05$ for two levels of III and bambuterol. Spectra taken on $h_{64\%}$ (upslope) and at peak height maximum of III, corresponding to $h_{19\%}$ and $h_{14\%}$ on downslope for 1.0% and 0.1% respectively; (d) co-elution with $\alpha = 1.03$ for II and bambuterol. Spectra taken on $h_{69\%}$ (upslope) and at peak height maximum of II, corresponding to $h_{30\%}$ (downslope).

the method to detect co-eluting impurities is largely dependent on the spectral properties of the compounds, e.g. position of absorptions bands and molar absorptivities (6). The sensitivity in the present case was investigated by adjustments of the octanesulphonate concentration in the mobile phase, so that two related substances (II and III in Fig. 1) were partly co-eluted with the bambuterol peak (Fig. 4b-d). The large spectral differences between bambuterol and III resulted in a detection limit of about 0.1% of III. A discrimination between II and bambuterol was difficult even with 10% of II in the bambuterol peak due to the spectral similarities of the compounds.

The results from the liquid chromatographic investigations of the CRS were compared with results obtained by other techniques (Table 1). A content of 99.8% was assigned to the CRS by subtracting the total amount of impurities from 100.0%. The high purity of the CRS was supported by the results from two-phase titration, 99.9%, and potentiometric titration with silver nitrate, 100.1%.

TABLE 1

Analysis of Bambuterol Hydrochloride CRS (149/83)

Compound (technique)	Content (%)
I, II, III and MONO (LC)	0.07
Acetone (GC)	0.04
Water (Coulometric KF titration)	0.04
Unidentified related substances (LC)	0.05
Unidentified volatile substances (GC)	0.03
Estimated sum of impurities	0.2

Tablet Analysis

By the liquid chromatographic method the tablet excipients were separated from bambuterol and its degradation products (Fig. 5). Detection limits were about 1 ng for terbutaline and 0.5 ng for MONO. The quantitative performance of the method was demonstrated by analyzing placebo tablet solutions spiked with bambuterol hydrochloride (Table 2) and with terbutaline and MONO (Table 3).

Table 2 shows that accurate and precise determinations are achieved within a concentration range corresponding to 50-130% of the target value for the analysis method described. The area normalization procedure in Table 3 resulted in a slight underestimation of MONO, emanating from a lower weight response. Accurate determination can however be done by use of weight factors or external standard technique. The overestimation of terbutaline at lower concentrations originated

TABLE 2

Recovery and Precision for Bambuterol in Spiked Placebo Tablet Solutions

Solution no	Added (µg/ml)	Found (µg/ml)	Per cent of added	Range n=3, (%)
1	240	241	100.6	0.13
2	300	301	100.4	0.07
3	400	401	100.4	0.10
4	480	482	100.0	0.48
5	602	601	99.8	0.30
3, low placebo (a)	401	403	100.6	0.02
3, high placebo (a)	400	403	100.7	0.09

(a) The low value corresponds to 50% and the high value to 150% of normal content of excipients.

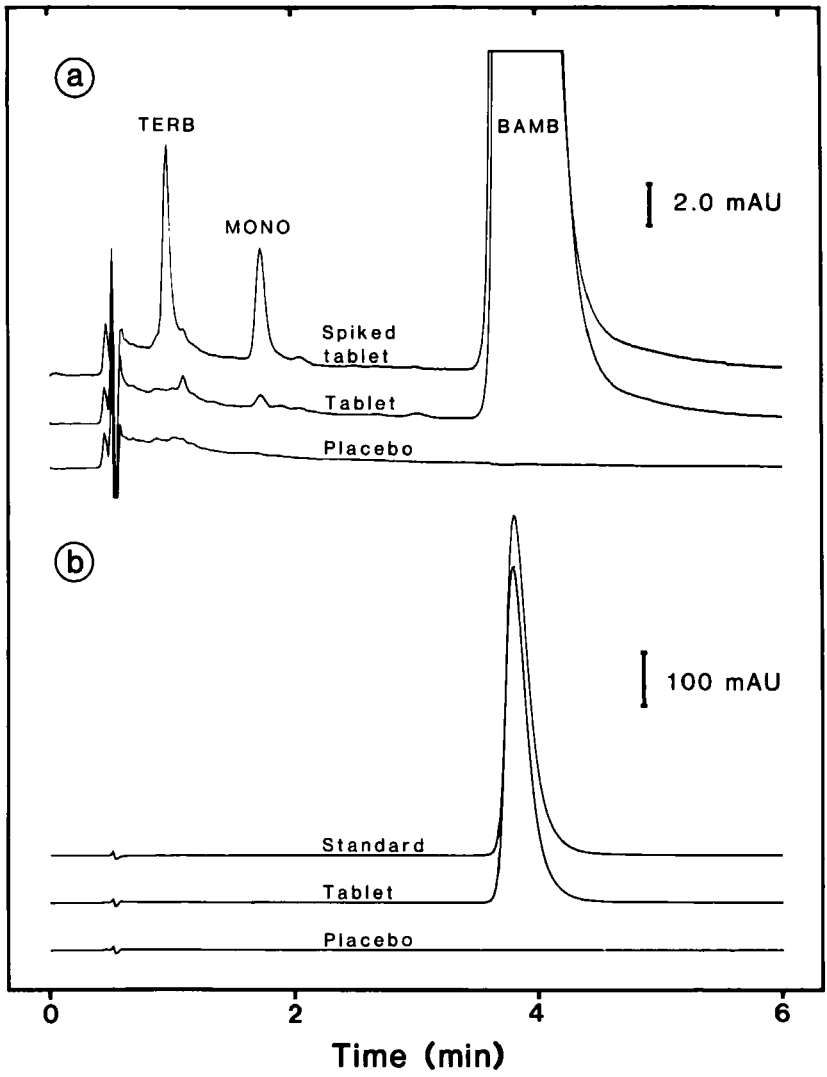

Figure 5. Typical chromatograms from analysis of Bambuterol tablets. Conditions as in figure 2 except for a (20+50) x 4.6 mm column and flow-rate 1.5 ml/min; (a) recorded with high sensitivity. Tablet sample solution spiked with 0.5% w/w of TERB and MONO; (b) low sensitivity setting.

TABLE 3

Recovery and Precision for Degradation Products in Spiked Placebo Tablet Solutions Containing 0.4 mg/ml Bambuterol Hydrochloride using the Area Normalization Procedure

Degradation product	Added (µg/ml)	Found (µg/ml)	Per cent of added	Repeatability RSD, n=6 (%)
Terbutaline	0.426	0.524	123	1.7
	2.13	2.11	99.2	1.1
	4.26	4.16	97.6	0.24
MONO	0.424	0.313	73.7	4.1
	2.12	1.53	72.3	0.53
	4.24	3.05	72.0	0.18

from small interferences from excipients and impurities in the drug substance. This effect was negligible at concentrations on or above 2 µg/ml.

The method reproducibility (between days) for tablet analysis was 0.9% (n=6, batch DME 105). Table 4 gives some examples of batch analysis and results from stability studies. It can be concluded that no chemical degradation takes place during long-term storage of the tablets.

The content uniformity of 10 tablets was measured for the batch DMK 28. The mean strength was 10.1 mg with a precision of 1.6% RSD and a range within 4.6%.

Column Packing Material

The main part of this work was performed on a single brand of C_{18}-packing material due to its high plate number and a comparatively low backpressure. Other packing materials were evaluated (Table 5). Four of the columns fulfilled the requirements in the

TABLE 4

Batch Analysis and Data from Stability Studies of Bambuterol Tablets

Batch	Declared strength (mg)	Amount found (mg)	Per cent of declared	TERB and MONO (%)
DMB 25: initial	5.0	5.05	101.0	< 0.1
DMB 25: 1 year at 30°C/75% RH	5.0	5.06	101.2	< 0.1
DMD 29	5.0	5.06	101.1	< 0.1
DMK 28	10.0	10.18	101.8	< 0.1
DMB 26	10.0	10.19	101.9	< 0.1
DME 105: initial	20.0	20.42	102.1	< 0.1
DME 105: 1 year at 30°C/75% RH	20.0	20.38	101.9	< 0.1
DMB 102	20.0	20.44	102.2	< 0.1

TABLE 5

Capacity Factors and Peak Shape Characteristics for C_{18}-column Packing Materials

Column/Supplier	k'			Nx10^{-3} (plates /m)[a]	As[b]
	BAMB	TERB	MONO		
µBondapak/Waters	4.5	0.63	1.7	21	1.2
Supelcosil DB/Supelco	6.7	0.85	2.4	50	1.4
Nucleosil/Machery-Nagel	7.3	0.95	2.7	44	1.7
Nova-Pak/Waters	7.4	0.86	2.6	48	1.5
Spherisorb S5 ODS 2/ Phase Separations	10.2	1.1	3.4	35	2.4

(a) Measured at $h_{50\%}$ on the bambuterol peak
(b) Measured at $h_{10\%}$ on the bambuterol peak

"System suitability test" for the bambuterol tablet analysis method. These requirements were:
N > 18 000 plates/m and asymmetry factor (As) < 2.0.
The capacity factors for bambuterol and the degradation products on these three columns made them useful for the analysis of the drug.

CONCLUSIONS

A liquid chromatographic method in combination with different specific and non-specific detectors has been of great value for the characterization of a chemical reference substance batch of high purity.

When applied to the analysis of tablets the method was found to be precise, accurate and stability indicating.

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