Journal of Chromatography, 229 (1982) 217—221
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1189

Note

A time-saving method for the determination of the β_2 sympathomimetics terbutaline, salbutamol and fenoterol. Preliminary results

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(Received August 26th, 1981)

Fenoterol, salbutamol and terbutaline are frequently used in asthma therapy. These compounds belong to the β_2-selective sympathomimetics and as such they show few side-effects on the heart. Although these compounds have been in clinical use for approximately a decade, the first useful methods for large-scale clinical studies were only published in 1976 for salbutamol [1] and terbutaline [2,3]. Since then, especially for terbutaline, more methods have been published [4—7]. The major disadvantage of all these methods is that they are time-consuming and specific for each drug. Some procedures involve difficult extraction steps [1,5—7] or use too large samples to allow frequent patient sampling [4]. Although the method developed by Leferink et al. [2,3] is relatively simple, it does not allow more than one injection per 20—25 min into the gas chromatography—mass spectrometry (GC—MS) system due to the disturbing influence of the ion-pair extracting agent [2,3]. An increasing number of requests for analysis of the β_2-sympathomimetics in our laboratory urged us to develop an assay that could be applied to terbutaline, salbutamol and fenoterol. In this article such a method is described comprising one extraction and derivatization procedure for these compounds and a mass-fragmentographic method for quantitation.

MATERIALS AND METHODS

Chemicals

The sympathomimetics terbutaline, salbutamol and fenoterol and the internal standard d_6-terbutaline, d_3-salbutamol and d_5-fenoterol were a gift from the respective manufacturers Draco (Lund, Sweden), Glaxo (Ware, Great Britain) and Boehringer (Ingelheim, G.F.R.). Methanol and acetonitrile were of analytical quality and used without further purification. Bondelut® C-18 cartridges (Nos. 6001 and 6003) were obtained from Analytichem International (Harbor

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City, CA, U.S.A.) and Sep-Pak® C-18 cartridges were obtained from Waters Assoc. (Milford, MA, U.S.A.). The derivatizing agent, bis(trimethylsilyl)trifluoroacetamide (BSTFA), was a Pierce (Rockford, IL, U.S.A.) product. A phosphate buffer of pH 7.6 was used with a strength of 0.1 *M*.

Instrumental

A Finnigan 3200 GC—CI—MS system and a four-channel Promim® were used in the assay. The Promim channels were set according to the quasi-molecular ion masses ($M + H^+$) of the respective compounds and their standards. For terbutaline these masses were *m/z* 442 and 448, for salbutamol *m/z* 456 and 459, and for fenoterol *m/z* 592 and 597. The chemical-ionization reagent gas was ammonia at a source pressure gauge read-out of 800 μm. The source temperature was controlled at 140 ± 2°C. The gas chromatograph was equipped with a 20 m × 0.5 mm I.D. glass capillary column coated with CP-Sil 8. The inlet pressure for the carrier gas (helium) was 56 kPa. The connection between the capillary column and the mass spectrometer was a 60-cm fused silica capillary (0.20 mm I.D.) and an open split coupling. The interfacing capillary was kept at 310°C. The GC column was operated at 210°C for terbutaline and salbutamol and at 280°C for fenoterol. The injector remained at 300°C.

Sample preparation

Aqueous and serum samples were used in the development of the procedure. The aqueous samples contained 10 ng of the sympathomimetic in 1 ml of buffer solution.

Serum samples (1 ml) were spiked with 2—20 ng of the sympathomimetic and 1 ml of the buffer was added. These samples were used for recovery measurement. Two actual patient samples were used: one serum sample was obtained 2 h after intake of a 5-mg terbutaline sulphate tablet and one blood sample was from a case of salbutamol overdose. To these samples 10 ng of d_6- terbutaline and 20 ng of d_3-salbutamol were added as the respective internal standards: in addtion, 1 ml of buffer was added to each sample.

Method

Bondelut C-18 and Sep-Pak C-18 cartridges were pretreated as suggested by the manufacturers. The cartridges were washed with 4—5 ml of methanol, 2 ml of water and 0.5 ml of buffer. During the prewashings with water and buffer the columns were not allowed to run dry. After pretreatment the prepared samples were allowed to drain into the column and were then pressed through the column; subsequently the column was washed with 1 ml of water. Finally, the columns were eluted with 4 × 1 ml of methanol—acetonitrile (85:15, v/v). The first 150—200 μl (3—4 drops) of the eluate were discarded. The total elution time was 1 min/ml.

In the case of recovery studies 10 ng of the various internal standards were added to the combined eluate. The eluate was transferred into 1-ml Reacti® vials (Pierce) in 0.5-ml portions and blown to dryness under a nitrogen stream at 70°C. The dried residue was reconstituted and derivatized in 20 μl of BSTFA at 80°C for 10—15 min. Subsequently, 1 μl was injected onto the capillary column.

RESULTS AND DISCUSSION

Recovery measurements on the aqueous solution containing 10 ng of the sympathomimetic showed that the best recoveries are obtained with the small Bondelut columns (No. 6001). The recoveries were highest for terbutaline and fenoterol (90%) and slightly lower for salbutamol (82%). The recoveries on the larger Bondelut columns (No. 6003) were low (< 5%) using 4 ml of eluent. Even after elution with 12 ml of eluent only 50% was recovered. Sep-Pak cartridges showed recoveries between 70 and 75% for the three sympathomimetics. From these experiments it seemed that the sympathomimetics bind strongly to the C-18 cartridge packing. It was also clear that for this reason more eluent was required to elute the compound than recommended by the manufacturers for general extraction purposes.

Recovery measurements on spiked serum samples revealed no differences between the Bondelut and Sep-Pak cartridges. Again the highest recoveries were found for terbutaline and fenoterol (95%) and a lower recovery for salbutamol (80%). However, the percentage recovery was unaffected by the concentration of the sympathomimetic. At concentrations of 2, 5 and 20 ng/ml

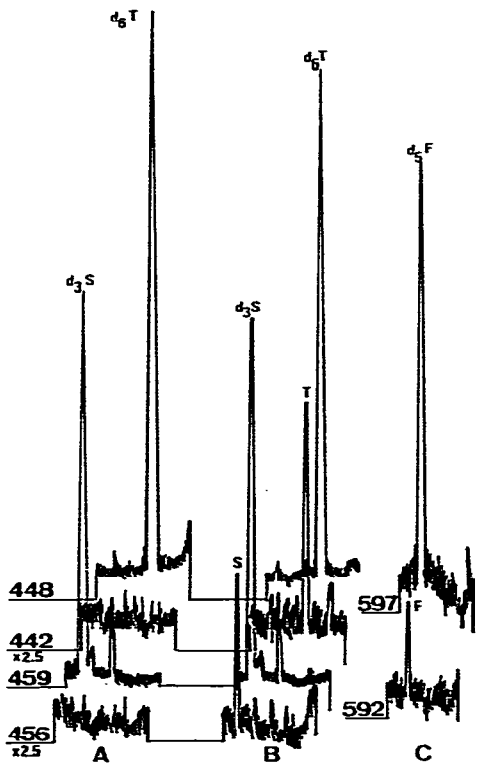

Fig. 1. Mass fragmentograms of (A) blank serum spiked with 10 ng of d_3-salbutamol and 10 ng of d_6-terbutaline, (retention times were 4.38 and 3.45 min, respectively); (B) as (A) but also spiked with 2 ng of salbutamol and 2 ng of terbutaline; (C) serum spiked with 10 ng of d_5-fenoterol and 2 ng of fenoterol (retention time 4.72 min). S = salbutamol, T = terbutaline, F = fenoterol.

the recoveries remained the same within the error in the measurement of 3% at the 20 ng/ml level to 10% at the 2 ng/ml level. Unspiked serum samples showed clean ion chromatogram recordings with no background at the retention times of the various compounds. Only in the salbutamol recordings was a major peak found at the ion trace of the internal standard (*m/z* 459). Since a capillary column was used, this coextracted impurity did not affect the accuracy of the measurement due to the large difference in retention times (Fig. 1A). Fig. 1B and C show the result of serum samples spiked with 2 ng/ml of the sympathomimetics and 10 ng of the respective internal standards and run on Sep-Pak. In this example all three sympathomimetics were added to the serum and two separate GC runs were made to obtain the results for terbutaline and salbutamol at 210°C and for fenoterol at 280°C. These data show that measurements at the 1 ng/ml level can be performed with an acceptable signal-to-noise ratio. Fig. 2A shows the recording of a serum sample taken 2 h after intake of a 5-mg terbutaline sulphate tablet. The concentration of terbutaline was calculated at 2.8 ng/ml. This value is in agreement with those obtained with the previously described method [2,3]. This experiment therefore indicates that terbutaline conjungates, which are present especially after oral intake, do not affect the measurement.

Fig. 2B represents the fragmentogram of a blood sample in a case of salbutamol overdose. The calculated concentration was 23 ng/ml of blood. The results of Fig. 2. were obtained using Bondelut (No. 6001).

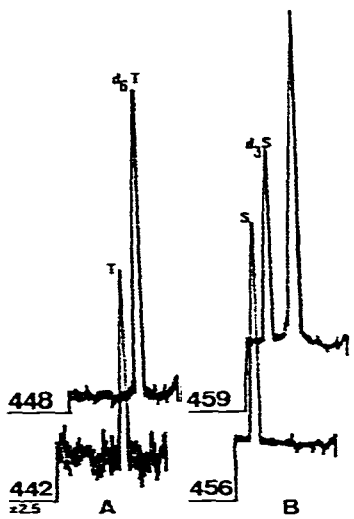

Fig. 2. (A) Patient serum sample 2 h after intake of 5 mg of terbutaline sulphate. (B) Patient blood sample after an overdose of salbutamol.

CONCLUSION

From the reported experiments it is obvious that this extraction method, using either Bondelut (No. 6001) or Sep-Pak cartridges, shows a large improvement over previously published methods in terms of time and simplicity. Futhermore, its uniformity is advantageous; in fact, on adding all three internal

standards to the biological sample routinely, one can simply screen the sympathomimetics quantitatively. One warning should be issued at this stage: although the detection limits for the three compounds are similar (around 1 ng/ml) it is not possible to measure fenoterol at the average therapeutic level. This level is <300 pg/ml as established with tritiated compounds [8]. The detection limit is sufficient, however, to measure levels in overdose cases. The therapeutic ranges for salbutamol and terbutaline are well within the range of the method.

ACKNOWLEDGEMENTS

The authors would like to thank the drug companies Boehringer, Draco and Glaxo for their generous gifts of the sympathomimetics and the deuterated standards. Part of this work has been made possible by a grant (No. 269) from the Dutch Asthma Foundation.

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