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Short communication

Possibilities for confirmatory analysis of some β-agonists using two different derivatives simultaneously

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Abstract

A previously described method for the confirmatory analysis of clenbuterol with chemical ionization GC–MS was extended to fit more β-agonists. The method has been used routinely for about one year and a large number of samples have been analysed according to the described procedure. This paper summarizes the results obtained with this method with regard to clenbuterol and furthermore discussion is focussed on applicability to β-agonists other than clenbuterol with respect to the European Community requirements for confirmatory analysis.

1. Introduction

According to EC requirements, confirmatory analysis using low resolution mass spectrometry has to be based on "..preferably at least four diagnostic ions.." [1]. For β-agonists like clenbuterol this usually is achieved by application of two different techniques, viz. electron impact (EI) and chemical ionization (CI), each yielding two diagnostic ions. Alternatively, different derivatives may be used each yielding two or more diagnostic ions. We previously described the application of two different derivatives for the confirmatory analysis of clenbuterol in urine [2]. In this paper, results on the long-term performance of this procedure are presented with emphasis on the comparability of the results with the results that were obtained by EI and CI measurements. Furthermore this method was extended to a broader range of β-agonists and concerning these compounds, attention is focused on the results obtained using a quality assurance (QA) sample spiked at 3 ng/ml. The (im)possibilities for confirmatory analysis of these other β-agonists are discussed.

2. Experimental

2.1. Materials

Part of the materials used were described elsewhere [2]. In addition, salbutamol and terbutaline were purchased from BUFA-chemie (Alphen a/d Rijn, Netherlands), mabuterol was a generous gift of Boehringer-Ingelheim (Ingelheim am Rhein, Germany) and cimaterol was a generous gift of Dr G. Kristiansen (Danish Meat Research Institute, Roskilde, Denmark). Mapenterol, clenbuterol-d_6 and salbutamol-d_6

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were custom made and bromobuterol was a generous gift of Dr L. van Ginkel [National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, Netherlands]. 2,3,4,2′,4′,5-Hexachlorobiphenyl (PCB138) was purchased from Promochem (Wesel, Germany).

2.2. Instrumentation

GC–MS analyses were carried out using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) coupled to a HP 5971A mass spectrometer operated in the chemical ionization mode using ammonia as reagent gas, adjusted according to manufacturer's instructions. Data acquisition was performed in the multiple-ion detection mode (MID). GC separation was achieved on a DB5 capillary column (J and W Scientific, Folsom, CA, USA) (30 m × 0.25 mm I.D., 0.25 μm film thickness). The injector was kept at 260°C, the interface was at 280°C and the source temperature was 200°C. The GC oven was programmed from 110°C to 280°C at 10°C/min.

2.3. Sample preparation

Aliquots of five ml of urine were prepared as described previously [2,3]. In brief, the samples were hydrolysed overnight using glucuronidase/arylsulfatase from *Helix pomatia*, pH was adjusted and the analytes were extracted using immunoaffinity chromatography. The extracts resulting from this procedure were purified using C_{18} cartridge columns. Finally, the analytes were eluted with methanol–acetonitrile (85:15, v/v).

The resulting extracts were split into two equal aliquots of one ml each. The solvent was evaporated under nitrogen. One of the aliquots was derivatized using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), obtaining the trimethylsilyl (TMS) derivatives. The other was derivatized using N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), obtaining the *tert.*-butyldimethylsilyl (tBDMS) derivatives. Subsequently the reagents were evaporated, the residues were dissolved in ethyl acetate and corresponding sample aliquots were recombined. Ethyl acetate was removed by evaporation and the residue was then dissolved in 20 μl of toluene already containing 5 ng/μl of PCB 138 as a syringe standard. Aliquots of 1 μl, corresponding to an equivalent of 0.125 ml of urine, were injected for GC–MS analysis.

3. Results and discussion

Fig. 1 shows a chromatogram of a calibration solution containing both derivatives of the analytes at 2.0 ng/μl corresponding to 8 ng/ml in urine. The difference in retention time between the TMS and tBDMS derivatives of the same compound is approximately 2 min. For terbutaline and salbutamol however, the increase in retention time for the tBDMS derivative compared to that of the TMS derivative is larger due to the formation of tri-TMS and tri-tBDMS derivatives while the other analytes only gave mono-TMS and mono-tBDMS derivatives.

Mass spectrometric fragmentation of the TMS and tBDMS derivatives of clenbuterol under the CI conditions used was described previously [2]. In general, the spectra of all β-agonists used in this study almost exclusively show abundant quasi-molecular ions without any other significant fragmentation occurring. Therefore the choice of diagnostic ions is limited to the quasi-molecular ions and if applicable their isotope peaks and possibly the fragments corresponding to the loss of OH-TMS $[M + H - 90]^+$ or OH-tBDMS $[M + H - 132]^+$. Table 1 presents the diagnostic ions used. Obviously some compounds do not yield four diagnostic ions and can therefore not be identified according to the EC requirements. Nevertheless these compounds are readily detected and whenever found, additional work may be done to obtain sufficient information for confirmatory analysis. Since, compared to clenbuterol, the other β-agonists are not very frequently found, additional work is seldom required.

A number of other compounds do yield four diagnostic ions and can be identified according to EC requirements.

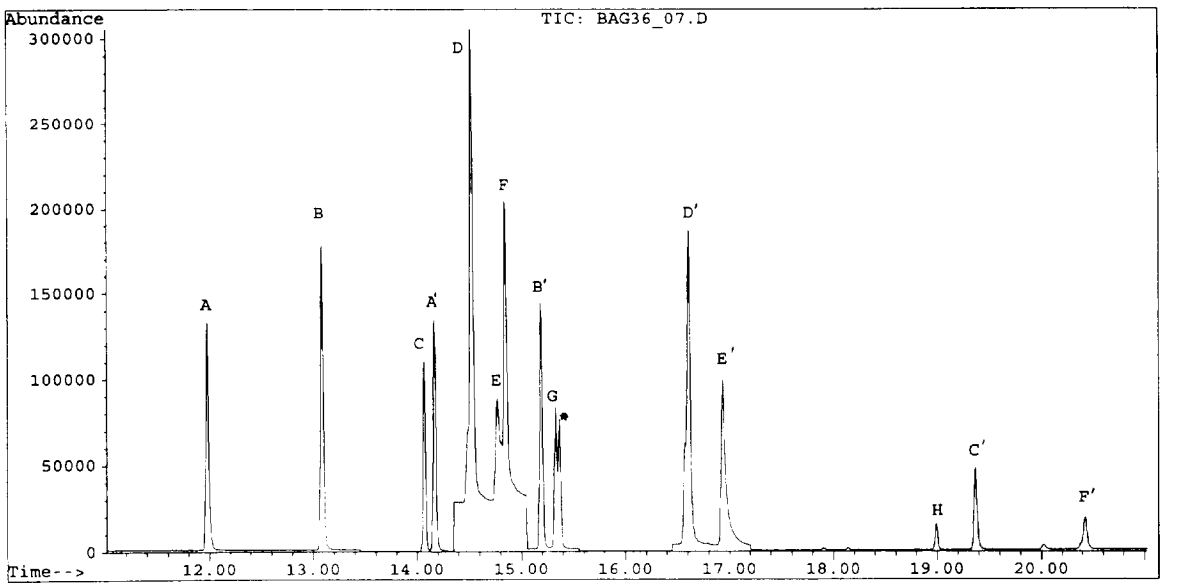

Fig. 1. Chromatogram recorded in SIM mode of a calibration solution containing 2.0 ng/μl of both TMS and tBDMS derivatives of mabuterol (A and A'), mapenterol (B and B'), terbutaline (C and C'), clenbuterol (D and D'), cimaterol (E and E') and salbutamol (F and F'). For each compound, the latter eluting peak corresponds to the tBDMS derivative. Furthermore cimaterol-di-TMS (G), an unknown (*) from the derivatization reagent and PCB138 (H) are present.

Table 2 presents the TMS/tBDMS results obtained by analysis of independently prepared QA samples spiked at 3 ng/ml. The pooled urine sample used for spiking experiments was demonstrated prior to use to contain none of the analytes in detectable amounts. The results are indicative of the reproducibility of the procedure rather than of the repeatability. Considering this, the results are rather good, especially for clenbuterol and salbutamol for which compounds isotope dilution is applied. The other compounds are quantitated using clenbuterol-d_6 as an internal standard and consequently, variability is higher but still acceptable for trace level analysis [4]. Bromobuterol was not added to the QA sample because of the limited availability of this compound, and thus no data are reported.

Table 1
Diagnostic ions for the GC–MS analysis of clenbuterol, salbutamol, cimaterol, terbutaline, bromobuterol, mabuterol and mapenterol using TMS and tBDMS derivatives

Compound	Diagnostic ions	
	TMS derivative	tBDMS derivative
Clenbuterol	349, 351	391, 393
Salbutamol	456	582
Cimaterol	292	334
Terbutaline	442	568
Bromobuterol	437, 439, 441	479, 481, 483
Mabuterol	383, 385	425, 427
Mapenterol	397, 399	439, 441

Table 2
Results of the analysis of some β-agonists in a QA sample spiked at 3 ng/ml with a mix of β-agonists

Compound	TMS derivative		tBDMS derivative	
	Mean (ng/ml)	C.V. (%)	Mean (ng/ml)	C.V. (%)
Clenbuterol	3.1	17	3.2	13
Salbutamol	3.0	15	3.3	18
Cimaterol	3.0	24	3.2	20
Terbutaline	2.9	33	3.1	27
Mabuterol	3.1	30	2.8	27
Mapenterol	3.1	26	2.9	22

The number of replicates is 68 except for cimaterol ($n = 49$).

Bromobuterol is readily detected and considering its similarity with clenbuterol, performance may be expected to be comparable.

Assuming a signal-to-noise ratio of ten as the minimum for confirmatory analysis, the identity of clenbuterol can be confirmed down to approximately 0.2 ng/ml in urine. For mabuterol and mapenterol the limit of detection is approximately 0.4 ng/ml. The other compounds can not be identified according to EC requirements but applying the same criterium of a signal-to-noise ratio of ten, these compounds can be detected down to 0.2 ng/ml for salbutamol and 0.4 ng/ml for cimaterol and terbutaline. For bromobuterol no exact data are available.

An incurred sample with clenbuterol was also analysed within several series of samples. This resulted in a mean clenbuterol content of this urine sample of 1.50 ng/ml (C.V. = 15%, $n = 13$) for the TMS derivative and 1.52 ng/ml (C.V. = 17%, $n = 13$) for the tBDMS derivative. Again the results for both derivatives prove to be highly comparable.

Fig. 2 presents a comparison of the results for clenbuterol obtained with this method and with the previously used EI/CI method. The agreement between the quantitative data obtained with both methods is good, the coefficient of correlation being 0.953. Even more important, conclusions regarding the presence or absence of clenbuterol drawn from the EI/CI method are the same as for the TMS/tBDMS method. Especially this feature is important because β-agonists are banned substances within the EC and therefore detection and identification of the analytes is equally or even more important than accurate quantitation.

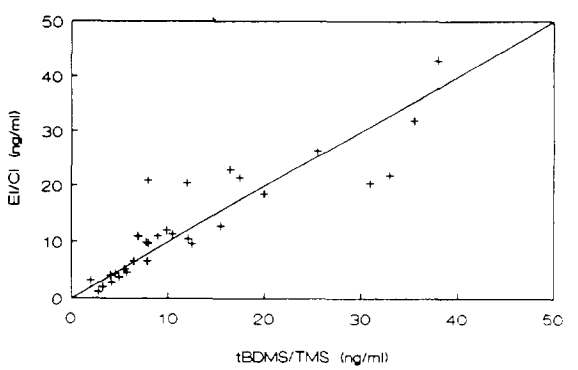

Fig. 2. Correlation graph of the mean result of the EI and CI analysis versus the mean result of the TMS and tBDMS derivative analysed in the CI mode. The correlation coefficient is 0.953. Results are expressed in ng/ml in urine.

4. Conclusions

The application of two different derivatives and simultaneous detection of these derivatives yields confirmatory information according to EC requirements for clenbuterol, bromobuterol, mabuterol and mapenterol. For salbutamol, cimaterol and terbutaline the demand of four diagnostic ions is not met; however, these compounds are readily detected when present. For the latter compounds, additional work has to be carried out to obtain enough information for unambiguous identification according to EC requirements. Quantitative results are comparable for both derivatives.

This method is considered to be a time- and cost-saving alternative for confirmatory analysis of several β-agonists using EI and CI analysis. The information obtained is comparable to EI/CI analysis and leads to identical conclusions regarding the presence or absence of the targeted β-agonists.

References

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