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Determination of parts per million levels of trifluoroacetic acid in ceronapril bulk substance by headspace capillary gas chromatography

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ABSTRACT

A headspace capillary gas chromatographic method was developed to determine trifluoroacetic acid (TFA) in a bulk substance (ceronapril). The bulk sample is reacted in a sealed headspace vial with dimethyl sulfate in concentrated sulfuric acid to convert TFA to methyl trifluoroacetate (MTFA). A portion of the headspace of the vial containing the MTFA is chromatographed on a PoraPLOT Q 0.32 mm I.D. capillary column in the split injection mode with flame ionization detection. The limit of detection is less than 10 ppm (w/w).

INTRODUCTION

Trifluoroacetic anhydride is used as a reagent in the penultimate step in the synthesis of ceronapril (Fig. 1), a new class of angiotensin–converting enzyme inhibitor [1–3]. A sensitive analytical method was required which is capable of determining residual trifluoroacetic acid (TFA) in ceronapril bulk substance. This paper describes a headspace capillary gas chromatographic (GC) method which was developed to determine TFA down to the low ppm level. The method is based on the reaction of ceronapril with dimethyl sulfate in concentrated sulfuric acid in a sealed headspace vial, where the TFA is converted into the more volatile and more easily chromatographable derivative methyl trifluoroacetate (MTFA). A portion of the headspace gas is then chromatographed using a capillary GC system equipped with a split injector and a flame ionization detector. The limit of detection is less than 10 ppm (w/w).

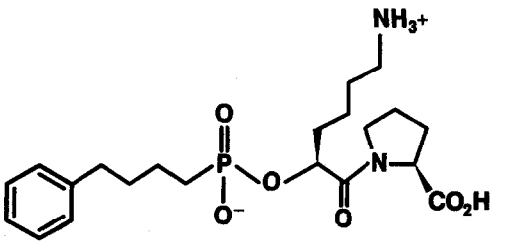

Fig. 1. Structure of ceronapril.

EXPERIMENTAL

Reagents and Chemicals

Samples of ceronapril bulk substance (powder) were obtained from the Department of Chemical Process Technology of Bristol-Myers Squibb Pharmaceutical Research Institute (New Brunswick, NJ, USA). Dimethyl sulfate (gold label, 99+%) and trifluoroacetic acid (99+%) were purchased from Aldrich (Milwaukee, WI, USA). Concentrated sulfuric acid (ACS grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA).

The derivatizing solution was prepared by adding 20 ml of dimethyl sulfate to a 100-ml volumetric flask containing about 50 ml of refrigerated con-

centrated sulfuric acid and then diluting to volume with additional refrigerated concentrated sulfuric acid. The solution was kept in a refrigerator for at least 20 min before use.

Stock TFA standard solution (0.01%, v/v) was prepared by adding 1.0 μl of TFA beneath the surface of 9 ml of cold derivatizing solution in a 10-ml volumetric flask and then diluting to volume with additional cold derivatizing solution. The solution was kept in a refrigerator until the time of use. Dilute TFA standard solution (0.001%, v/v) was prepared by diluting 1.0 ml of stock TFA standard solution to 10 ml with cold derivatizing solution. The solution was kept in a refrigerator until the time of use.

Working standard set

The working standard set consisted of a blank standard preparation and a spiked standard preparation, prepared as described below.

Blank standard preparation. A 300 ± 10-mg portion of ceronapril was weighed into a headspace vial (Hewlett-Packard No. 9301-0717). After adding 3 ml of cold derivatizing solution, the vial was capped with a Teflon-lined septum (Hewlett-Packard No. 9301-0719) and an aluminum crimp seal with pressure release (Hewlett-Packard No. 9301-0718). The ceronapril powder will float on the surface of the solution and will dissolve during the heating preceding the headspace sampling (see below). The mixture can be gently mixed to hasten the dissolution; however, it should not be allowed to come into contact with the Teflon septum to prevent contamination of the headspace sampler needle.

Spiked standard prepartion. A 300 ± 10-mg portion of the same ceronapril batch as used for the blank standard preparation was weighed into a second headspace vil. After adding 3 ml of dilute TFA standard solution, the vial was treated as described under *Blank standard prepartion.*

Sample preparation

A 300 ± 10-mg portion of the ceronapril sample to be analyzed was weighed into a headspace vial. The vial was then treated as described under *Blank standard preparation.*

Reagent blank preparation

A 3-ml portion of the derivatizing solution was added to a 10-ml headspace vial. The vial was then treated as described under *Blank standard preparation.*

Gas chromatography

A Hewlett-Packard Model 5890 capillary gas chromatograph, equipped with a split–splitless injection port and a flame ionization detector, was used in conjunction with a Hewlett-Packard Model 19395A headspace analyzer. The fused-capillary column used was PoraPLOT Q (Chrompack No. 7550) (10 m × 0.32 mm I.D.) with a 10-μm stationry phase film thickness. The oven temperature was maintained at 140°C for 5 min and then ballistically programmed at 70°C/min to 200°C and held there for 8 min. The injector and detector temperatures were maintained at 125 and 200°C, respectively. Injections were made in the split mode, with a split flow-rate of 10 ml/min. The split port liner was a 4 mm I.D. open tube packed with a short fused-silica-wool plug (Restek No. 20781) and deactivated with a solution of 5% dimethyldichlorosilane in toluene after packing. The helium carrier gas head pressure was maintained at 97 kPa (14 p.s.i.g.) and the flow-rate of the helium make-up gas for the flame ionization detector was 30 ml/min. The GC sensitivity was set at a range of 2^1 and an attenution of 2^1.

The headspace analyzer carrier gas flow-rate was set at 12 ml/min and the auxiliary flow-rate was set at 80 kPa. A 1.0-ml sample loop was used and the valve and sample loop temperature was 80°C. The heating bath temperature was set at 50°C and the equilibration time at 90 min. The headspace analyzer timing sequence was programmed so that probe insertion time was at 00:01 (0 min and 1 s) with a pressurizing start time of 00:03. The corresponding programmed times for the other parameters in the sequence were as follows: stop pressuring at 00:13; start vent/fill loop at 00:15; stop vent/fill loop at 00:25; start injection at 00:27; stop injection at 05:27; take out probe at 05:29.

After the system had equilibrated, the prepared headspace vials were placed in the headspace analyzer in the following order: reagent blank preparation, blank standard preparation, spiked standard preparation, reagent blank preparation and then the sample preparation of each of the samples to be analyzed. The starting of the headspace analyzer program will automatically allow the heating of the

headspace vials for 90 min prior to the first injection. A 1.0-ml portion of the headspace gas was transferred into the inlet of the capillary GC system via the headspace analyzer heated transfer line.

Quantification

The response due to the TFA spiked into the spiked standard preparation is calculated by the equation

$$A_s = A_2 - [A_1(W_2/W_1)]$$

where A_s is the area due to the TFA spiked into the spiked standard preparation, A_2 is the TFA peak area measured from the chromatogram of the spiked standard prepartion, A_1 is the TFA peak area measured from the chromatogram of the blank standard preparation, W_2 is the weight (g) of ceronapril in the spiked standard preparation and W_1 is the weight (g) of ceronapril in the blank standard preparation.

The amount of TFA in the sample to be analyzed is calculated by the equation

$$\text{TFA (ppm, w/w)} = \frac{C_s A_u V D \cdot 10^4}{A_s W}$$

where C_s is the concentration (%, v/v) of the spiked TFA in the spiked standard preparation, A_u is the TFA peak area measured from the chromatogram of the sample preparation, V is the volume of the sample preparation (3 ml), D is the density of TFA (1.48 g/ml), A_s is as above and W is the weight (g) of ceronapril in the sample preparation.

RESULTS AND DISCUSSION

The proposed method presented is based on the conversion of TFA in ceronapril into MTFA without prior extraction from ceronapril. The MTFA thus formed is equilibrated between the solution and headspace gas phase and then a portion of that gas is chromatographed in the capillary GC system. The headspace analyzer utilized allows the programming of the system for the automatic equilibration and sampling of the headspace.

TFA is methylated with dimethyl sulfate, which is normally used in a basic medium [4]. On the other hand, dimethyl sulfate has been used in an acidic medium for the determination of halogenated acids such as trichloroacetic acid and TFA in blood, plasma and urine [5–7]. It appears that even in strongly acidic media there is sufficient ionization of the relatively strong carboxylic acids such as TFA that the proposed mechanism [4] of methylation by dimethyl sulfate can take place with nucleophilic attack of the trifluoroacetate anion on the methyl

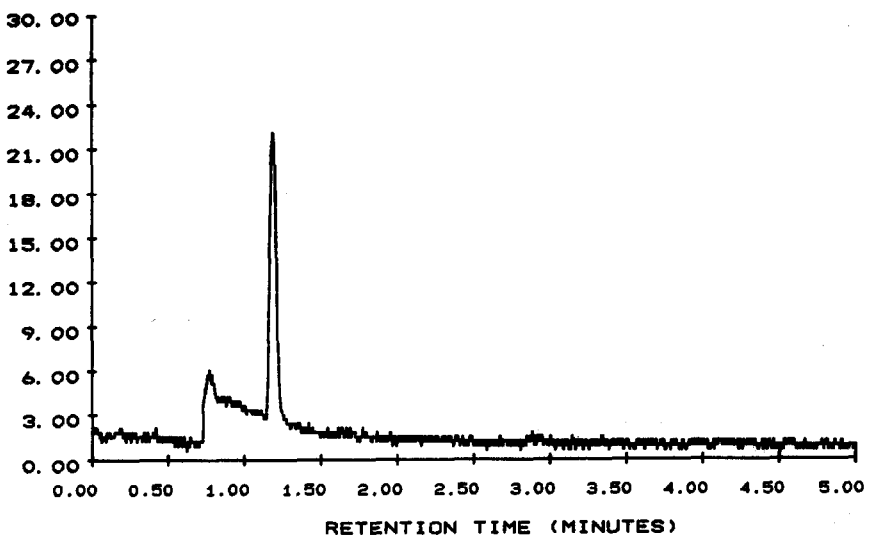

Fig. 2. Chromatogram of the reagent blank preparation.

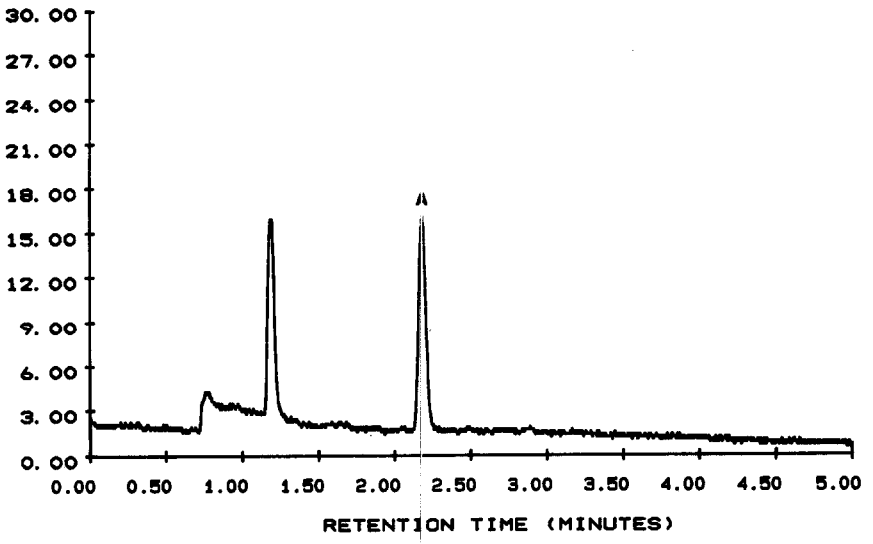

Fig. 3. Chromatogram of the spiked standard preparation corresponding to about 148 ppm (w/w). Peak A (2.2 min) is due to MTFA.

carbon of dimethyl sulfate. The reaction in concentrated sulfuric acid appears to be an ideal application for headspace GC, as the concentrated sulfuric acid will act to "salt out" the MTFA, decreasing its partition ratio and, hence, increasing the sensitivity of the method. A second advantage is that the sulfuric acid gives no solvent peak to obscure the TFA response. A third advantage is that the reaction is selective and hence gives less chemical noise as only relatively strong acids would be methylated under the strongly acidic conditions utilized here.

The dimethyl sulfate–sulfuric acid solution is added cold to the ceronapril sample in order to avoid any evaporative loss of TFA, as the dissolution of

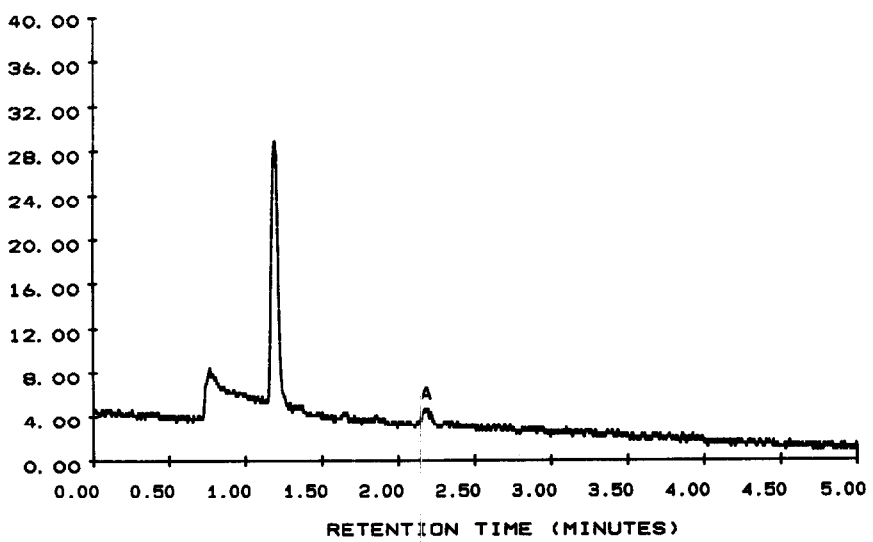

Fig. 4. Chromatogram of the sample preparation. The MTFA concentration was found to be <10 ppm (w/w) (peak A).

the ceronapril generates heat and the boiling point of TFA is only 72°C and that of MTFA is only 43°C. This reagent should be made fresh and kept refrigerated until the time of use. Some unknown non-interfering peaks were observed in the reagent blank preparation when older solutions were used. Complete reaction of the TFA was observed when the reaction mixture was heated at 50°C for 60 min. Heating the samples in the headspace analyzer for longer than 6 h showed no loss of the MTFA as long as the headspace vial was sealed airtight.

Figs. 2, 3 and 4 show chromatograms of the reagent blank preparation, spiked standard preparation and sample preparation, respectively. Note that the reagent blank is clean in the region of MTFA and the sample preparation was found to contain TFA at a level near the limit of detection.

The linearity of response was established by analyzing several 300-mg portions of a ceronapril sample spiked with various amounts of TFA and measuring the absolute peak-area response of the MTFA produced. A correlation coefficient of 0.999 and an intercept of 109 (which corresponds to about 6 ppm, w/w) were obtained (Table I).

The accuracy of the method was established by comparing the amount of TFA added to ceronapril with the amount recovered. As shown in Table II, added TFA was quantitatively recovered. Excluding the 15 ppm level, the mean recovery was 99%. This good sensitivity is achieved in spite of the split injection mode, which is required for obtaining good chromatographic peaks without cryofocusing when the headspace anlyzer is connected to the GC system through the split–splitless inlet. One approach to avoiding splitting of the sample is to connect the heated transfer line probe of the headspace sampler (which would normally enter the injection port) directly to the GC column by use of an appropriate connector (*e.g.*, Chrompack No. 4782). Another approach is to install the headspace sampler transfer line in the packed injection port of the GC system. The packed injection port is equipped with a Restek Uniliner sleeve adaptor (Restek No. 20310) and a 0.53 mm I.D. direct injection–on-column Uniliner (Restek No. 20311). In this instance a 0.53 mm I.D. column butts up to the radial taper of the head of the expansion chamber of the Uniliner. This allows the heated transfer line probe tip to enter the 0.53 mm I.D. fused-silica column so that on-column injections are achieved. The optimum carrier flow-rate of the headspace sampler necessary to sweep the sample loop and deposit the headspace sample in the injection port is about 10–20 ml/min. In spite of these high flow-rates, the utilization of the 0.53 mm I.D. capillary column affords acceptable column efficiency without having to split the sample stream. These two modes of "injection" of the headspace sample have been successfully applied in our laboratories with other methods when high sensitivity was required.

TABLE I

LINEARITY OF MTFA RESPONSE

Amount spiked (ppm, w/w)	MTFA response (area counts)
740	13196
148	2803
74	1517
14.8	224
0	0
Slope	17.7
Intercept	109
Correlation coefficient	0.999

TABLE II

RECOVERY OF TFA FROM CERONAPRIL

Added (ppm, w/w)	Found (ppm, w/w)	Recovery (%)
748	662	89
144	155	108
75	75	100
15	22	147

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