

2,2,2-Trifluoroethyl Chloroformate as a Rapid Derivatizing Reagent of Amino Acids for Fast Enantiomer Separation by Gas Chromatography

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Amino acids were derivatized rapidly into N(O)-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl esters for enantiomer separation by chiral phase capillary gas chromatography. All the derivatives showed considerable reduction in retention times compared with N(O)-ethoxycarbonyl ethyl ester derivatives with almost complete separation of their enantiomers.

Development in capillary columns coated with chiral stationary phases made increasingly popular the analysis of enantiomeric compounds by gas chromatography(GC).¹ The method has been most widely used for the determination of amino acid enantiomers. For an analysis of amino acids by GC, the starting free amino acids must be derivatized in order to convert them into volatile compounds which are suitable for GC analysis within a reasonable time. Preparation of the conventional N(O)-perfluoroacyl alkyl esters of amino acids takes over 1.5 h with laborious multi-step processes. Recently, a very rapid method has been reported for derivatizing amino acids in quantitative² and enantiomeric³ analysis. The amino acids are derivatized with ethyl chloroformate at room temperature within 1 min. However, this approach for the application to the amino acid enantiomer separation has drawback of relatively long retention times for elution of all protein amino acids.

In this paper, we report our novel approach employing 2,2,2-trifluoroethyl chloroformate (TFECF) as a rapid derivatizing reagent for shorter time elution of amino acid enantiomers, using Chirasil-Val capillary column (20 m x 0.25 mm i.d., film thickness: 0.15 μ m) which was prepared in our laboratory.⁴ TFECF was prepared by reacting 2,2,2-trifluoroethanol (TFE) with phosgene overnight and twice distillation (bp. 67-69°C).

Amino acid derivatives were prepared according to the following procedure:

Standard amino acid solution (100 μ l) containing 2.5 μ mol/ml of each amino acid of DL form was transferred into Reacti-Vial (Pierce, IL, USA), and TFE/pyridine (3:1, v/v) mixture (50 μ l) was added. To this solution, about 15 μ l of TFECF was added, capped tightly, and shaken vigorously the vial by hand for 10 sec. Amino acid derivatives of N(O)-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl esters (TFEC-TFET) were formed immediately. Then 30 μ l of chloroform was added to the vial to extract the derivatives into organic layer. Finally, about 1 μ l of the organic phase was injected into the GC by a microliter syringe. Also, amino acid derivatives of N(O)-ethoxycarbonyl ethyl esters (EC-Et)³ were prepared in a similar manner in comparison.

The retention times and the separation factors of TFEC-TFET have been compared with those of EC-Et as shown in Table 1 and 2, respectively. As can be seen in Table 1, all TFEC-TFET have showed reduced retention time considerably compared with that of EC-Et. This effect has been found to be remarkable in the case of Pro, Thr, Ser, His, Tyr, and Trp which showed the retention times below 0.40-fold compared with corresponding

Table 1. Retention times(min) of L-amino acid derivatives

Amino acid	Derivative		Column temp.(°C)
	EC-Et ^a	TFEC-TFET ^b	
Ala	7.21	4.21(0.58)	110
Gly	8.26	5.62(0.68)	110
Val	5.73	3.05(0.53)	130
Pro	7.62	2.91(0.38)	130
allo-Ile	8.09	3.82(0.47)	130
Ile	8.62	4.11(0.48)	130
Leu	9.66	5.10(0.53)	130
Asp	10.80	4.58(0.42)	150
Thr	13.87	4.11(0.30)	150
Glu	15.13	10.65(0.70)	150
Ser	15.36	6.17(0.40)	150
Met	17.26	9.10(0.53)	150
Phe	24.41	10.74(0.44)	150
Orn	12.75	5.97(0.47)	210
His	15.03	4.34(0.29)	210
Lys	16.93	7.06(0.42)	210
Tyr	21.16	7.15(0.34)	210
Trp	57.20	22.06(0.39)	210

a: N(O)-ethoxycarbonyl ethyl ester derivative; b: N(O)-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl ester derivative; Number in parenthesis represents relative retention time of TFEC-TFET in case when the retention time of EC-Et is taken as 1.00.

Table 2. Separation factors(SF) and resolutions(R) of amino acid derivatives

Amino acid	Derivative				Column temp.(°C)
	EC-Et ^a		TFEC-TFET ^b		
	SF	R	SF	R	
Ala	1.088	4.23	1.085	2.81	110
Val	1.057	2.51	1.054	1.63	130
Pro	1.000	*	1.000	*	130
allo-Ile	1.000	*	1.069	2.09	130
Ile	1.054	2.84	1.052	1.57	130
Leu	1.091	3.80	1.085	3.16	130
Asp	1.030	1.55	1.025	1.06	150
Thr	1.043	1.72	1.057	2.08	150
Glu	1.071	3.48	1.078	3.13	150
Ser	1.055	2.46	1.042	1.98	150
Met	1.068	3.11	1.065	2.95	150
Phe	1.052	2.11	1.045	1.81	150
Orn	1.036	1.70	1.029	1.32	210
His	1.025	1.12	1.020	0.53	210
Lys	1.030	1.50	1.022	1.02	210
Tyr	1.025	1.03	1.018	0.61	210
Trp	1.027	0.94	1.020	0.75	210

a: N(O)-ethoxycarbonyl ethyl ester derivative; b: N(O)-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl ester derivative; *:Not separated.

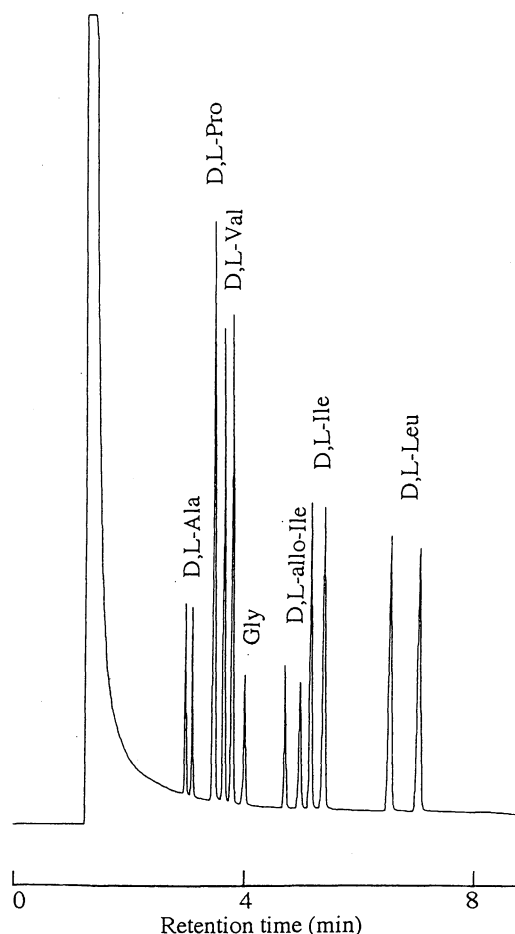


Figure 1. Gas chromatogram of N(O)-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl esters of amino acid enantiomeric mixture. Carrier gas: He, 1.0 kg/cm²; Column temp.: 120°C, isothermal. For each amino acid enantiomeric pair, the D-enantiomer eluted faster.

EC-Et. The reduction of retention time was generally remarkable in amino acids with three reactive functional groups. Especially, His showed reduced its retention time by 0.29 fold. As presented in Table 2, however, the separation factor of TFEC-TFET proved to be not appreciably improved. Most TFEC-TFET have decreased the separation factor slightly, except allo-Ile, Thr, and Glu which more or less increased the separation factor compared with that of EC-Et. Although Pro showed no separation in either

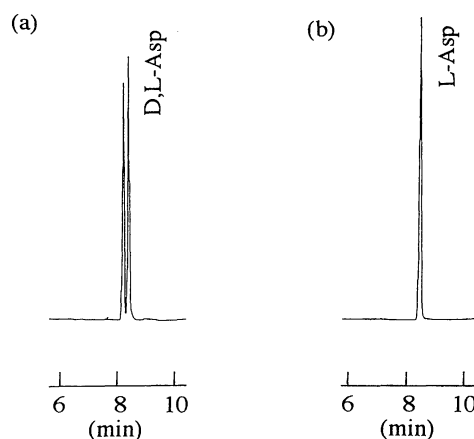


Figure 2. Gas chromatograms of N-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl esters of amino acids prepared from (a) D,L-Asp and (b) L-Asp. Column temp.: 130°C, isothermal. For other conditions, see Fig. 1.

side of the derivatives, allo-Ile, which was not able to be separated in EC-Et, showed complete separation in TFEC-TFET. TFEC-TFET of amino acids except Pro, His, Tyr, and Trp have shown excellent separation of their enantiomers with resolution of over 1.00. Fig. 1 shows a typical GC of TFEC-TFET of amino acid enantiomers. The D,L-amino acids were able to be separated into their enantiomeric pairs completely except Pro. In addition, racemization has been examined for Asp which is known as one of the most easily racemized amino acid. Fig. 2 shows two GCs of TFEC-TFET of (a) D,L-Asp and (b) L-Asp, respectively. As easily recognized from the two GCs, Asp showed no appreciable racemization.

In conclusion, TFEC-TFET of amino acids have been proved to serve in speeding up the GC enantiomeric analysis not only in derivatization step but also in GC run.

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References and Notes

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